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CONTENTS

(*Continued on Back Cover*)

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PROCEEDINGS OF THE
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Digenetic Trematodes of Marine Fishes from Ghana: Family Opecoelidae[1]

JACOB H. FISCHTHAL AND J. D. THOMAS[2]

ABSTRACT: Seven new and two previously described species of digenetic trematodes in the family Opecoelidae are reported from marine fishes from Ghana. A new genus *Pedunculotrema* is erected for two new species, *P. ghanensis* (type) from liognathid and *P. capecoastensis* from pomadasyid fishes. This genus is closest to *Plagioporus* Stafford, 1904, and *Pseudoplagioporus* Yamaguti, 1938, differing significantly in having the acetabulum stalked; it also is close to *Podocotyloides* Yamaguti, 1934, differing in having a postoral circular muscle ring, diagonal testes with their levels overlapping, the seminal vesicle tripartite, and the ovary opposite the anterior testis. Other new species are: *Poracanthium ghanensis* from polynemid, carangid and rhinobatid fishes; *Pseudopecoelus ghanensis* from a sciaenid fish; *Podocotyle temensis* from a serranid fish; *Podocotyloides chloroscombri* from a carangid fish; *Plagioporus gerridis* from a liognathid fish. Previously described species are: *Pseudopecoelus tortugae* von Wicklen, 1946, from trichiurid and sciaenid fishes; *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902, from clinid, pomacentrid, sciaenid, lutjanid, gobiid and clupeid fishes.

The trematodes of this report were killed in hot water, transferred immediately to Lavdowsky's FAA fixative for 24 hr, and then stored in 70% alcohol plus 3% glycerine; whole mounts were stained with Mayer's carmalum and mounted in permount. Specimens have been deposited in the U. S. National Museum Helminthological Collection as noted. All measurements are in microns

Poracanthium ghanensis sp. n. (Figs. 1–2, 15–17)

HOSTS: *Galeoides decadactylus* (Bloch) (type), *Pentanemus quinquarius* (L.), threadfins (Polynemidae); *Caranx hippos* (L.), jack or horse mackerel (Carangidae); *Rhinobatus albomaculatus* Norman, white-spotted guitarfish (Rhinobatidae).

HABITATS: Stomach (*R. albomaculatus*); small intestine (others).

LOCALITIES: Cape Coast, Tema; Ghana.

DATES: 12 January, 3 February 1966 (*G. decadactylus*, Cape Coast); 12, 19 January 1966 (*P. quinquarius*, Cape Coast).

SPECIMENS: USNM Helm. Coll. No. 70663 (holotype, from *G. decadactylus*); No. 70664 (paratypes, *G. decadactylus*); No. 70665 (paratype, *P. quinquarius*); No. 70666 (paratype, *C. hippos*); No. 70667 (paratypes, *R. albomaculatus*).

DIAGNOSIS (based on 90 specimens, 16 adults measured): Body elongate, narrow, unspined, extremities round, with stalk bearing acetabulum, 1,870–2,420 by 190–310 at level of stalk. Forebody 310–405 long; hindbody 1,345–1,895 long; forebody–hindbody length ratio 1:3.3–5.8. Preoral space usually present, up to 10 long. Stalk variable in length depending on state of contraction or extension, projecting 158–220 from body in relaxed specimens, 120–175 wide at base, center at anterior 18–26% of body length. Oral sucker subterminal ventral, slightly longer than wide, 107–121 by 98–114. Acetabulum at distal end of stalk, transversely elongate, 100–127 by 123–149, bearing four simple digitiform papillae on anterior margin of trans-

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verse slitlike opening and three on posterior. Sucker length ratio 1:0.92–1.11, width ratio 1:1.12–1.46. Prepharynx 29–51 long; pharynx 95–112 by 65–80; esophagus 99–167 long; cecal bifurcation overlapping region of acetabular stalk; ceca narrow, following contour of gonads but may slightly overlap them dorsally, uniting near posterior extremity, rectum short, anus terminal.

Testes two, smooth, longitudinally elongate, tandem, 10–138 apart, lying posterior to midbody length; anterior testis 136–165 by 109–133, lying 460–770 posterior to acetabular stalk; posterior testis 138–189 by 98–136; posttesticular space 550–750 long. Vas efferens emerging from anterodorsal part of each testis. Cirrus sac absent. Seminal vesicle intercecal, bipartite; proximal part saccular, cell lined, elongate, straight to slightly curved, 162–242 by 35–58, commencing 160–350 posterior to acetabular stalk, 190–340 anterior to ovary, distances more or less than half distance between stalk and ovary; distal part tubular, thick walled, muscular, elongate, making posterior loop after leaving proximal part, then extending anteriorly with much undulations. Pars prostatica straight, with thick cellular lining, 102–150 by 20–27, commencing short distance posterior to acetabular stalk or entirely dorsal to it. Ejaculatory duct somewhat thick walled, muscular, about same length as pars prostatica, opening into tubular genital atrium. Latter surrounded by large, very thick walled, muscular, partially spined genital lobe lying sinistrally at level of posterior part of pharynx or anterior part of esophagus; lobe 56–87 by 58–90, spines primarily covering anterior, anterolateral, anterodorsal and anteroventral parts of lobe, in thin band posterolaterally and posteriorly, longest spines anteriorly placed. Genital pore in middle of unspined posteroventral part of lobe. Posteromedian to genital lobe is a large genital pit with very thick glandular walls, band of spines surrounding opening, posterior spines of genital lobe lining anterior border of opening into genital pit.

Ovary pyriform, partially vesicular, diagonally oriented anterodextrally to posteromedianly or posterosinistrally, intercecal, may be notched posteriorly presenting appearance of lobing, 116–148 by 87–106, lying 345–530 posterior to acetabular stalk and 12–109 pretesticular, in tandem with testes. Oviduct emerging from anterior tip of ovary. Uterus short, intercecal, coiling only between ovary and proximal part of seminal vesicle, ascending with slight undulations ventral to seminal vesicle and dorsal to acetabular stalk, gland cells along entire length. Metraterm thick walled, muscular, commencing at level of pars prostatica, opening into genital atrium close to but independent of ejaculatory duct. Seminal receptacle absent; proximal coils of uterus filled with sperm. Vitelline follicles extending from ovarian level to posterior extremity, filling posttesticular space, in lateral fields more anteriorly, may be interrupted on both sides opposite testes or be entirely uninterrupted, all variations from these extremes occurring. Vitelline reservoir dorsal, anterodorsal or anterolateral to ovary, usually longitudinally elongate but may be transversely elongate, 51–90 by 39–51. Eggs relatively few, usually collapsed, yellow, operculate, some with very small anopercular knob, 32 measuring 42–49 by 26–33.

Excretory bladder unbranched, tubular, extending anteriorly dorsal to testes, terminating dorsal to ovary; pore terminal.

Discussion: Two (in four hosts), five (in three), and eight (in two) worms, respectively, were recovered from nine *G. decadactylus* from Tema, and two, eight, and 13 worms, respectively, from three of 21 examined from Cape Coast. One and 25 worms, respectively, were found in two of 12 *P. quinquarius* from Cape Coast. One specimen was found in *C. hippos* from Tema. Two specimens were obtained from one *R. albomaculatus* from Tema; we believe this selachian to be an accidental host, having ingested the teleost harboring this trematode. The type and only species in the genus, *P. furcatum* (Stossich, 1883) Dollfus, 1948, was reported from mullid and soleid fishes from the Adriatic Sea at Triest and the Mediterranean Sea at Algeria. *P. furcatum* differs from our species in possessing a much larger sucker length ratio, a rounder pharynx, a shorter esophagus, a very short space between the acetabulum and ovary, a larger and round ovary, contiguous gonads, vitelline follicles which are confluent between the gonads, and a different distribution of spines on the genital lobe. No mention was made of a genital pit for *P. furcatum*. We believe that the description of a

seminal receptacle for *P. furcatum* by Dollfus (1948) is in error. In view of our description of *P. ghanensis*, the generic diagnosis given by Yamaguti (1958) needs emendation.

Poracanthium Dollfus, 1948 emend.

DIAGNOSIS: Opecoelidae. Body elongate, unspined. Oral sucker subterminal ventral. Acetabulum on stalk, with digitiform papillae on both sides of aperture, lying near anterior extremity. Prepharynx, pharynx, and esophagus present. Ceca uniting near posterior extremity, rectum short, anus terminal. Testes tandem, in middle third or posterior half of body. Cirrus sac absent. Seminal vesicle bipartite, proximal part saccular, distal tubular. Pars prostatica elongate. Ejaculatory duct and metraterm opening into tubular genital atrium surrounded by sinistrally placed genital lobe lying at pharyngeal-esophageal level. Genital lobe partly spined. Genital pore on unspined part of genital lobe. Genital pit probably present, with very thick glandular walls, lying posteromedian to genital lobe, opening spined. Ovary pretesticular, postacetabular. Uterus intercecal, extending from ovary to level of pars prostatica. Seminal receptacle absent; proximal coils of uterus with sperm. Vitelline follicles extending from ovary to posterior extremity, filling posttesticular space. Eggs operculate, thin shelled. Excretory bladder unbranched, tubular, extending to ovarian level. Parasites of marine fishes.

TYPE SPECIES: *P. furcatum* (Stossich, 1883) Dollfus, 1948.

Pseudopecoelus tortugae von Wicklen, 1946

SYNONYM: *Cymbephallus fimbriatus* of Manter, 1934, *nec* Linton, 1934.

HOSTS: *Trichiurus lepturus* L., horse-tail or ribbon fish (Trichiuridae); *Larimus peli* Bleeker (Sciaenidae).

HABITAT: Pyloric ceca.

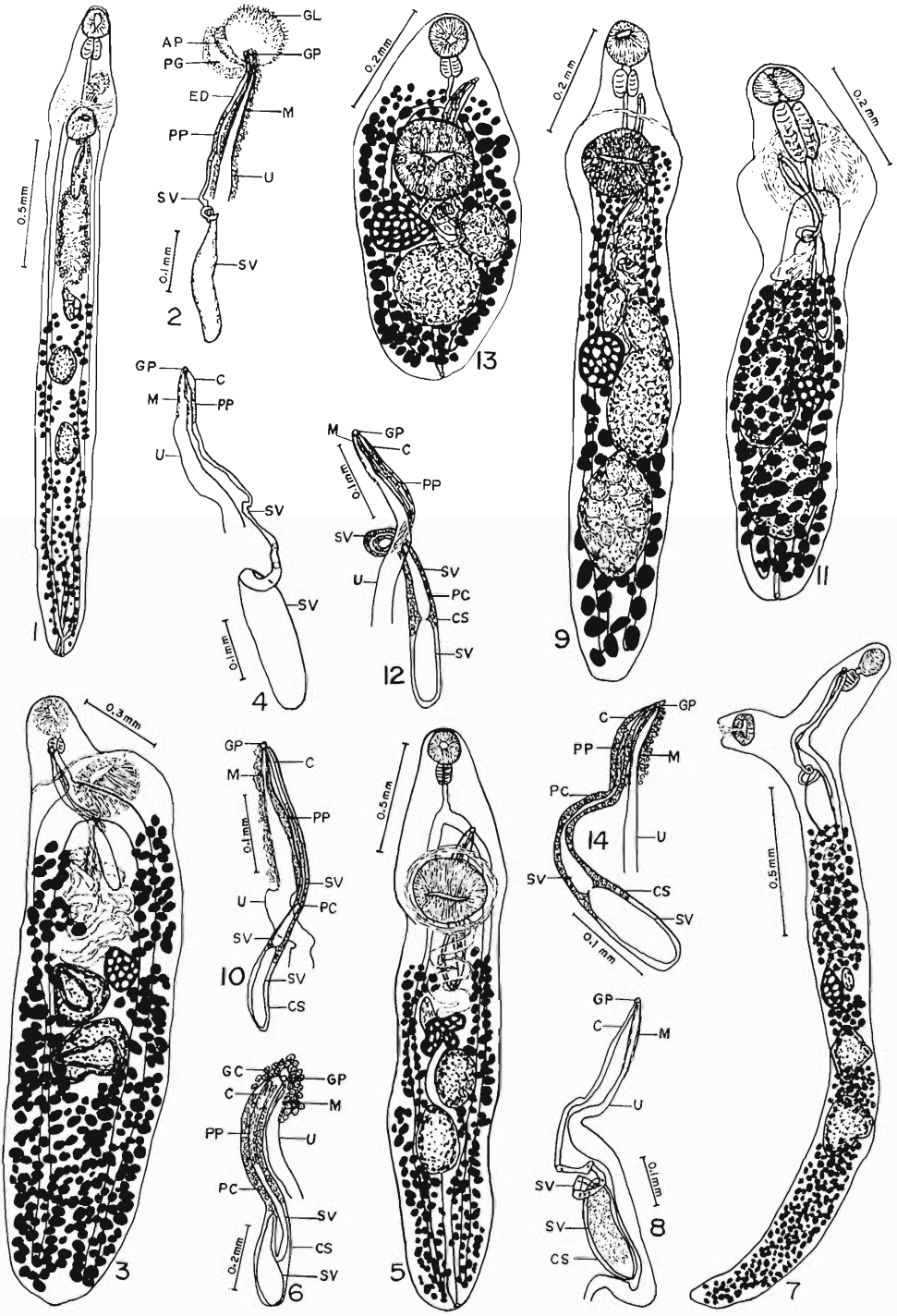
LOCALITY: Cape Coast, Ghana.

DATES: 6, 8 December 1965 (*T. lepturus*); 8 December 1965, 7 February 1966 (*L. peli*).

SPECIMENS: USNM Helm. Coll. No. 70668 (from *T. lepturus*); No. 70669 (*L. peli*).

MEASUREMENTS and some pertinent data (based on 13 adults, six from *T. lepturus* and two from *L. peli* measured): Body 1,816–2,660 by 385–540; forebody 278–425 long, hindbody 1,498–2,070 long, forebody–hindbody length ratio 1:3.8–7.0; suckers round to somewhat longitudinally or transversely elongate, oral sucker 100–128 by 104–131, acetabulum 167–206 by 165–220, sucker length ratio 1:1.50–1.78, width ratio 1:1.26–1.94; prepharynx 12–24 long; pharynx transversely elongate, 65–77 by 77–97; esophagus 85–181 long; ceca terminating 56–175 from posterior extremity; anterior testis usually longitudinally elongate but may be transversely elongate, 158–300 by 163–260, lying 370–725 postacetabular; posterior testis usually transversely elongate but may be longitudinally elongate, 157–360 by 167–320, lying 535–980 postacetabular; posttesticular space 575–840 long; seminal vesicle commencing 198–280 postacetabular; genital pore sinistral to posterior half of pharynx; ovary dextral, round to somewhat longitudinally or transversely elongate, 103–150 by 109–140, lying 265–595 postacetabular; anterior limits of vitellaria at posterior part of acetabulum or slightly postacetabular; eggs yellow-brown, operculate, 24 measuring 46–56 by 28–36; excretory bladder conspicuously cell lined, extending anteriorly dorsal to gonads to ovarian level; excretory pore subterminal dorsal, 4–19 from posterior extremity.

MEASUREMENTS and some pertinent data on four adults (USNM Helm. Coll. No. 39368) collected by Siddiqi and Cable (1960) from *Apogon maculatus* (Poey) (Apogonidae) from Puerto Rico: Body 1,013–1,192 by 310–335; forebody 191–227 long, hindbody 675–844 long, forebody–hindbody length ratio 1:3.4–4.4; oral sucker slightly transversely elongate, 70–80 by 82–87; acetabulum round to slightly longitudinally or transversely elongate, 140–157 by 140–160; sucker length ratio 1:1.93–2.0, width ratio 1:1.61–1.85; prepharynx 20–22 long (in two); pharynx transversely elongate, 50–54 by 58–69; esophagus 60–77 long; ceca terminating 80–123 from posterior extremity; testes transversely elongate; anterior testis 76–108 by 114–167; lying 210–320 postacetabular; posterior testis 85–121 by 123–157, lying 312–425 postacetabular; posttesticular space 157–305 long; seminal vesicle commencing 110–121 postacetabular; genital pore sinistral, at level of posterior part of pharynx or anterior part of esophagus; ovary transversely elongate, filling intercecal space or nearly so, 47–75 by 90–131, lying 210–260 postacetabular; anterior limits of



vitellaria at posterior part of acetabulum or slightly postacetabular; eggs yellowish, operculate, 12 measuring 46–56 by 25–35; excretory bladder conspicuously cell lined, extending anteriorly dorsal to gonads to ovarian level; excretory pore dorsal, 51–90 from posterior extremity.

DISCUSSION: Our collection consists of one, two, and eight adult worms, respectively, from three of 13 *T. lepturus* examined, and one each from two of 30 *L. peli.* We are presenting further details of this species because Manter's (1934) description is based on a single specimen from a macrurid fish from Florida. Through the courtesy of Dr. Mary Hanson Pritchard, University of Nebraska, we were able to examine the only specimen (immature but well developed) of *P. tortugae* in the Harold W. Manter collection. Siddiqi and Cable (1960), without description, presented an illustration of a whole mount specimen, noting that their material was in close agreement with the original description of *P. tortugae* except in body size and sucker ratio. Their specimens differ further in having a differently shaped pharynx, an ovary considerably transversely elongate and filling the intercecal space or nearly so, and an excretory pore distinctly dorsal in position and relatively far removed from the posterior extremity. Manter's immature specimen shows the excretory bladder also extending to the ovarian level, but the pore is just subterminal dorsal; this condition is more like that found in our specimens. The shape of the pharynx and egg size in our specimens is similar to Siddiqi and Cable's material and unlike Manter's. In spite of the differences cited above for the three collections, we feel that they probably represent a single species. The differences may be host influenced or may represent genetic population variations. For example, our two worms from *Larimus peli* are 1,816 and 2,220 long, respectively, and have sucker length ratios of 1:1.78 and 1:1.74 and width ratios of 1:1.94 and 1:1.78, respectively; the six measured from *T. lepturus* are 2,050–2,660 long and have sucker length ratios of 1:1.50–1.68 and width ratios of 1:1.26–1.76.

Pseudopecoelus ghanensis sp. n. (Figs. 3–4)

HOST: *Cynoscion macrognathus* (Bleeker), large-mouth weakfish (Sciaenidae).

HABITAT: Small intestine.

LOCALITY: Tema, Cape Coast; Ghana.

DATE: 8 December 1965 (Cape Coast).

SPECIMENS: USNM Helm. Coll. No. 70670 (holotype); No. 70671 (paratype).

DIAGNOSIS (based on two adult worms): Body elongate, robust, unspined, with protuberance bearing acetabulum, extremities round, 1,000–2,500 by 295–575 at ovarian level. Forebody conical, 140–235 long; hindbody much wider than forebody, 735–1,720 long; forebody–hindbody length ratio 1:5.3–7.3. Oral sucker subterminal ventral, longitudinally elongate, 87–125 by 73–120; acetabulum transversely elongate, aperture a transverse slit, without papillae, 125–205 by 140–230, center at level of anterior one-seventh to one-fifth body length. Sucker length ratio 1:1.44–1.64, width ratio 1:1.92–1.93. Prepharynx 21 long (in larger specimen); pharynx nearly round, 48–68 by 56–66; esophagus 120 long (in larger specimen); cecal bifurcation dorsal to acetabulum; ceca terminating blindly 72–80 from posterior extremity.

Testes two, somewhat lobed to notched, longitudinally to transversely elongate, intercecal but may overlap ceca dorsally, oblique, contiguous, lying in posterior part of middle third of body or extending slightly into posterior third; anterior testis sinistromedian, 140–240 by 181–

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Figures 1–14. 1. ***Poracanthium ghanensis,*** **holotype, ventral view.** 2. **Same. Terminal genitalia, holotype, ventral view.** 3. ***Pseudopecoelus ghanensis,*** **holotype, dorsal view.** 4. **Same. Terminal genitalia, holotype, dorsal view.** 5. ***Podocotyle temensis,*** **holotype, ventral view.** 6. **Same. Terminal genitalia, holotype, ventral view.** 7. ***Podocotyloides chloroscombri,*** **holotype, sinistrolateral view.** 8. **Same. Terminal genitalia, holotype, sinistrolateral view.** 9. ***Pedunculotrema ghanensis,*** **holotype, ventral view.** 10. **Same. Terminal genitalia, holotype, ventral view.** 11. ***Pedunculotrema capecoastensis,*** **holotype, dorsal view.** 12. **Same. Terminal genitalia, holotype, dorsal view.** 13. ***Plagioporus gerridis,*** **holotype, ventral view.** 14. **Same. Terminal genitalia, holotype, ventral view.**

Abbreviations: AP, genital pit aperture; C, cirrus; CS, cirrus sac; ED, ejaculatory duct; GC, gland cells; GL, genital lobe; GP, genital pore; M, metraterm; PC, prostate cells; PG, genital pit cavity; PP, pars prostatica; SV, seminal vesicle; U, uterus.

206, lying 191–465 postacetabular; posterior testis median to dextromedian, 176–230 by 152–285, lying 298–670 postacetabular; posttesticular space 257–820 long. Cirrus sac absent. Seminal vesicle bipartite; proximal part saccular, straight, entirely postacetabular, commencing 75–222 postacetabular or approximately halfway between ovary and acetabulum; anterior part tubular, narrow, sinuous. Pars prostatica preacetabular, cell lined, 42 by 33 (in larger specimen). Cirrus short, muscular, opening into shallow genital atrium. Genital pore at sinistral part of pharynx.

Ovary anterodextral to and contiguous with anterior testis, smooth, diagonally or transversely oval, 80–157 by 95–145, lying 167–445 postacetabular. Oviduct emerging from anteriormost margin of ovary. Ootype complex anteromedian to ovary and anterior testis. Uterus short, intercecal, coiling between ovary-anterior testis and acetabulum. Metraterm thick walled, approximately same length as pars prostatica. Vitelline follicles large, round to oval in shape, usually extending from posterior part of acetabulum (or one field more posteriorly) to posterior extremity, filling posttesticular space, in lateral fields anteriorly and completely or partially surrounding ceca. Vitelline reservoir dorsomedian to ovary. Eggs yellowish, operculate, usually collapsed in mounted specimens, seven measuring 50–59 by 33–38.

Excretory bladder unbranched, tubular, extending anteriorly to ovary; pore terminal.

Discussion: From Cape Coast, a single specimen was recovered from one of 10 *C. macrognathus* examined. Ten small-mouth weakfish, *C. brachygnathus* (Bleeker) from the same area were negative. Our new species appears closest to *P. scorpaenae* (Manter, 1947) Overstreet, 1969, from scorpaenid fishes from Florida, *P. barkeri* Hanson, 1950, from holocentrid fishes from Bermuda, Bimini, Puerto Rico, Jamaica, and Curaçao, *P. umbrinae* Manter and Van Cleave, 1951, from sciaenid fishes from California, and *P. manteri* Sogandares and Hutton, 1959, from a sciaenid fish from Florida. The latter two species differ significantly from the present form in that the seminal vesicle commences dorsal or only slightly posterior to the acetabulum, and the vitelline follicles extend preacetabularly. *P. umbrinae* differs further in having a lobed ovary and the forebody approximately the same length as the posttesticular space, while *P. manteri* has a transversely elongate oral sucker, an esophagus the same length as the pharynx, and smooth testes. *P. barkeri* differs in having the forebody approximately the same length as the posttesticular space, a larger sucker ratio, an esophagus shorter than the pharynx, and smooth testes. *P. scorpaenae* differs in having a larger sucker ratio, the pharynx as long as or longer than the oral sucker, the testes tandem, and the seminal vesicle entirely tubular and sinuous.

Podocotyle temensis sp. n. (Figs. 5–6)

Host: *Epinepheles goreensis* (Cuvier and Valenciennes), sea perch or grouper (Serranidae).

Habitat: Small intestine.

Locality: Tema, Ghana.

Date: 18 December 1964.

Specimens: USNM Helm. Coll. No. 70672 (holotype); No. 70673 (paratypes).

Diagnosis (based on 57 adults from a single fish; 12 measured): Body elongate, narrow, unspined, extremities round, with body fold around acetabulum in ventral view, protuberant in lateral view, 2,495–3,370 long, widest at acetabular level. Forebody conical, 500–660 long; hindbody 1,620–2,400 by 435–520; forebody–hindbody length ratio 1:2.9–3.9. Oral sucker subterminal ventral, usually transversely elongate but may be round or longitudinally elongate, 155–198 by 165–192; preoral space 5–15 long; acetabulum transversely elongate, without papillae, aperture a transverse slit, 295–350 by 310–375; sucker length ratio 1:1.77–2.0, width ratio 1:1.77–2.09. Prepharynx dorsal to oral sucker, 14–26 long; pharynx overlapping oral sucker dorsally, longitudinally elongate, 110–130 by 95–122; esophagus muscular, 95–135 long; cecal bifurcation 80–170 preacetabular; ceca narrow, conspicuously cell lined, terminating blindly near posterior extremity.

Testes two, smooth, slightly diagonal, intercecal, 16–165 apart, occupying middle third of hindbody, longitudinally elongate; anterior testis sinistromedian, 210–290 by 190–235, lying 407–635 postacetabular; posterior testis dextromedian, longer than anterior testis, 290–385 by 175–235, lying 690–1,020 postacetabular; posttesticular space 618–995 long. Cirrus

sac usually slightly sigmoid shaped, commencing medianly or dextrally 265–350 postacetabular, latter representing approximately 57–81 per cent of distance between acetabulum and ovary. Seminal vesicle bipartite, saccular posteriorly, tubular anteriorly, latter part with posteriorly directed loop at proximal end; saccular part 235–335 by 75–110, usually lying entirely postacetabular. Pars prostatica well developed, conspicuously cell lined, 157–220 by 27–39. Cirrus muscular, short, lying inverted. Prostate cells filling available space in cirrus sac. Genital pore sinistral, postbifurcal, ventral to cecum or extracecal, lying 20–75 preacetabular.

Ovary distinctly 4-lobed, sometimes 5-lobed, dextromedian, pretesticular, usually contiguous with anterior testis, overlapping level of latter 5–60, transversely elongate, 150–205 by 180–245, lying 380–470 postacetabular. Ootype complex anterodorsal to ovary. Seminal receptacle dorsolateral to ovary, extending anteriorly almost to level of proximal end of cirrus sac, 205–275 by 65–93. Laurer's canal present. Uterus relatively short, between ovary and acetabulum, may overlap ceca ventrally, ventral to seminal receptacle and cirrus sac. Metraterm muscular, commencing dorsal to anterior part of acetabulum, surrounded by mass of gland cells. Vitelline follicles large, smooth, anterior extent 90–190 postacetabular, posttesticularly usually separated into two fields by excretory bladder, dorsal, lateral and ventral to ceca anteriorly, uninterrupted, intrude into intertesticular space but not confluent. Vitelline reservoir median, anterodorsal to ovary. Eggs large, yellow-brown, operculate, some with knob at anopercular end, 20 uncollapsed eggs measuring 60–67 by 36–45.

Excretory bladder cell lined, unbranched, tubular, anterior end round, extending anteriorly to ovarian level or slightly preovarian, sinistrodorsal to posterior testis, intertesticular, dextrodorsal to anterior testis, dorsal to ovary, posteriorly narrowing abruptly to duct opening terminally or subterminal ventral.

Discussion: In having the testes separated by the excretory bladder our new species is similar to three other species previously included in the genus *Podocotyle* (Dujardin, 1845) but now allocated to the new genus *Allopodocotyle* by Pritchard (1966a), namely, *A. tamame* (Yamaguti, 1942), *A. serrani* (Yamaguti, 1952), and *A. plectopomi* (Manter, 1963). Species of *Allopodocotyle* are differentiated from those of *Podocotyle* in having a smooth rather than lobed ovary. Our form appears closest to *A. serrani*, resembling it further in the postacetabular extent of the cirrus sac, postbifurcal genital pore, and distribution of the vitellaria. However, it differs further in having a narrower body, longer esophagus, testes more nearly tandem, seminal vesicle with a loop (rather than being straight), and distinct pars prostatica. In addition to the intertesticular passage of the excretory bladder, *P. temensis* differs from the 17 species allocated to *Podocotyle* by Pritchard (1966a) in having a postbifurcal genital pore.

Podocotyloides chloroscombri sp. n. (Figs. 7–8, 18)

Host: *Chloroscombrus chrysurus* (L.), bumper (Carangidae).

Habitat: Small intestine.

Locality: Cape Coast, Ghana.

Date: 16 December 1965.

Holotype: USNM Helm. Coll. No. 70674.

Diagnosis (based on single specimen from one of 28 fish examined; mounted in sinistrolateral view so that measurements are length by depth): Body elongate, slender, anterior extremity round, tapering to blunt point posteriorly, unspined, with acetabular stalk, 2,575 by 190. Forebody 410 long, hindbody 2,015 long. Oral sucker nearly terminal, 111 by 104; acetabulum 105 by 85, retracted into stalk, latter 225 by 150 wide; sucker length ratio 1:0.95. Prepharynx 15 long; pharynx relatively large, anterior end truncate, posterior end round, 85 by 65; esophagus 90 long; cecal bifurcation just anterior to acetabular stalk; ceca narrow, terminating blindly near posterior extremity.

Gonads tandem, from midbody length posteriorly, anterior testis 46 postovarian, testes 40 apart. Testes two, slightly lobed, intercecal, extending from dorsal to ventral body surfaces; anterior testis 190 by 143, lying 860 posterior to acetabular stalk; posterior testis 222 by 167, lying 1,090 posterior to acetabular stalk; posttesticular space 725 long. Cirrus sac commencing 250 posterior to acetabular stalk or two-fifths distance between stalk and ovary; bipartite, proximal part saccular (222 by 94) and

lying entirely posterior to stalk, narrowing abruptly to longer tubular distal part, latter with loop at its proximal end but sinuous anteriorly. Seminal vesicle filling cirrus sac, devoid of sperm at base. Cirrus sac protruded through genital pore. Genital pore at sinistral part of pharynx.

Ovary smooth, elongate, filling ventral half of body depth, 150 by 87, lying 635 posterior to acetabular stalk. Oviduct emerging from anterior margin of ovary. Seminal receptacle dorsal, Mehlis' gland anterodorsal to ovary. Uterus containing 61 eggs in single file, extensively coiled between Mehlis' gland and cirrus sac, remainder sinuous in ascent to genital pore. Metraterm short, thick walled, without muscular sphincter. Vitelline follicles large, round to oval in shape, extending from 165 posterior to acetabular stalk to posterior extremity, overlapping level of base of cirrus sac, completely interrupted at levels of both testes but only dorsally, ventrally and sinistrally at ovary, confluent medianly dorsal to cirrus sac, uterus, and between gonads, filling posttesticular space. Vitelline reservoir dorsal to ovary. Eggs yellow-brown, operculate, eight measuring 48–56 by 29–36.

Excretory bladder unbranched, tubular, extending anteriorly to ovary; pore terminal.

DISCUSSION: Pritchard (1966a) emended the genus *Podocotyloides* Yamaguti, 1934, and recognized five species therein from Indo-Pacific fishes; she (1966b) described a sixth species from Hawaii. Our new species appears closest to *P. parupenei* (Manter, 1963) Pritchard, 1966 (syn. *Podocotyle p.* M.) from a mullid fish from Fiji. The latter differs in having the acetabulum twice as large as the oral sucker, an acetabular protuberance rather than a stalk, a very short esophagus (27–32 long), a unipartite cirrus sac, and considerably larger eggs (72–88 by 38–57).

Pedunculotrema gen. n.

DIAGNOSIS: Opecoelidae. Body small, unspined. Oral sucker subterminal ventral to nearly terminal. Postoral circular muscle ring present. Acetabulum larger than oral sucker, stalked, preequatorial. Prepharynx and esophagus short. Pharynx well developed. Ceca extending to near posterior extremity. Testes oblique, in middle to posterior part of hindbody, anterior testis submedian, posterior testis median, contiguous, levels overlapping. Cirrus sac long, bipartite, commencing postacetabular, containing tripartite seminal vesicle, pars prostatica, and protrusible cirrus. Genital pore sinistral, at posterior pharyngeal or anterior esophageal level. Ovary smooth, opposite anterior testis, smaller than either testis. Seminal receptacle present. Uterus mostly intercecal, usually overlapping anterior part of anterior testis, sometimes ovary. Vitellaria follicular, commencing at level of cecal bifurcation, acetabulum or postacetabularly, filling posttesticular space, lateral anteriorly, confluent dorsally throughout length of fields. Eggs large, operculate. Excretory bladder unbranched, tubular, extending to ovary-anterior testis level. Parasitic in marine fishes.

TYPE SPECIES: *Pedunculotrema ghanensis* sp. n.

DISCUSSION: This new genus appears closest to *Plagioporus* Stafford, 1904, and *Pseudoplagioporus* Yamaguti, 1938, but differs significantly in having the acetabulum stalked. It also appears close to *Podocotyloides* Yamaguti, 1934, as emended by Pritchard (1966a), but differs significantly in having a postoral muscle ring, diagonal testes with their levels overlapping, the seminal vesicle tripartite, and the ovary opposite the anterior testis. The name *Pedunculotrema* is from *pedunculus*, stalk, and *trema*, hole, referring to the presence of a stalk bearing the acetabulum.

Pedunculotrema ghanensis sp. n. (Figs. 9–10, 19)

HOST: *Gerres melanopterus* Bleeker, mojarra (Lognathidae).

HABITAT: Small intestine.

LOCALITY: Cape Coast, Ghana.

DATE: 12 January 1966.

SPECIMENS: USNM Helm. Coll. No. 70675 (holotype); No. 70676 (paratype).

DIAGNOSIS (based on three adults from one of two fish examined; one worm mounted in ventral view and two in lateral view so that measurements are length by width by depth): Body elongate, narrow, unspined, extremities round, with body fold around acetabulum in ventral view, with short stalk bearing acetabulum in lateral view, 1,176–1,411 long, widest at acetabular level. Forebody conical, 197–242

long; hindbody 860–1,035 by 185 by 111; forebody–hindbody length ratio 1:4.3–4.4. Oral sucker subterminal ventral, nearly round, 76–100 by 82 by 104; acetabulum nearly round, aperture a transverse slit, without papillae, 121–134 by 129 by 128. Sucker length ratio 1:1.34–1.39, width ratio 1:1.57. Postoral circular muscle ring narrow. Prepharynx 17–24 long; pharynx round to longitudinally elongate, 61–87 by 58 by 68; esophagus 53–73 long; cecal bifurcation at anterior margin of acetabulum; ceca terminating blindly 84–127 from posterior extremity.

Testes two, diagonal, longitudinally elongate, contiguous, levels overlapping, overlapping ceca ventrally, occupying middle half of hindbody; anterior testis sinistromedian, constricted at ovarian level in two worms, smooth, 222–310 by 124 by 138–147, lying 177–195 postacetabular; posterior testis smooth, oval, anterior end blunt pointed, median, 213–310 by 145 by 145–148, lying 425–435 postacetabular; posttesticular space 208–290 long. Cirrus sac sinuous, bipartite, posterior part saccular, anterior part tubular, commencing dextromedianly 120–188 postacetabular, latter representing 68–97 per cent of distance between acetabulum and anterior testis and 51–74 per cent of distance between acetabulum and ovary. Seminal vesicle tripartite; saccular part of cirrus sac containing two saccular parts terminating dorsal to acetabulum, posterior sac 85–132 by 32 by 43, anterior sac 72–124 by 23 by 39; remainder of seminal vesicle tubular, sinuous. Pars prostatica short, at anterior margin of acetabulum. Cirrus muscular. Genital pore sinistral, at posteroventral part of pharynx or just lateral to it.

Ovary smooth, dextral to midlength of anterior testis, longitudinally oval, 104–133 by 77 by 80–99, lying 233–255 postacetabular and 56–60 posterior to level of anterior margin of anterior testis. Ootype complex anteromedian to ovary. Seminal receptacle large, median, longitudinally elongate, overlapping anteromedian parts of ovary and anterior testis dorsally, entering uterine field, 100–133 by 65 by 56–62. Uterus relatively short, coils few, preovarian, overlapping anterior part of anterior testis. Metraterm long, slightly muscular, surrounded by gland cells, commencing dorsal to acetabulum. Vitelline follicles large, smooth, commencing at cecal bifurcation, filling posttesticular space, lateral anteriorly, confluent dorsally throughout length of fields. Eggs large, yellow-brown, operculate, partially collapsed, five measuring 58–60 by 31–38.

Excretory bladder unbranched, tubular, extending anteriorly to ovary-anterior testis level; pore terminal.

Pedunculotrema capecoastensis sp. n. (Figs. 11–12, 20)

HOST: *Pomadasys jubelini* (Cuvier and Valenciennes), burro (Pomadasyidae).

HABITAT: Small intestine.

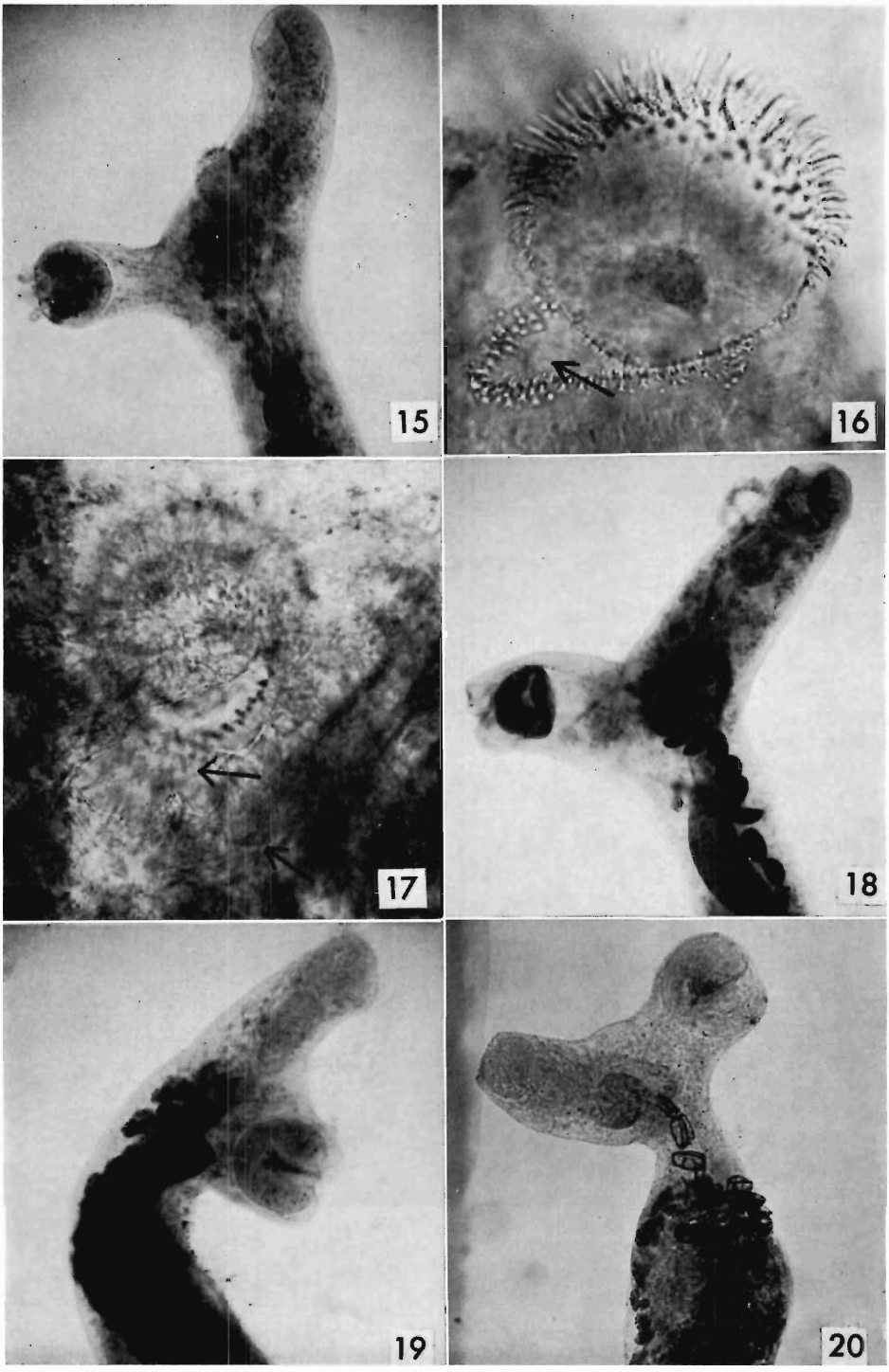
LOCALITY: Cape Coast, Ghana.

DATE: 25 March 1966.

SPECIMENS: USNM Helm. Coll. No. 70677 (holotype); No. 70678 (paratypes).

DIAGNOSIS (based on five adults from one of seven fish examined; measurements are length by width by depth): Body elongate, narrow, unspined, anterior extremity nearly truncate, posterior round, with body fold around acetabulum in ventral view, with stalk (190–218 long, 145–190 wide) bearing acetabulum in lateral view, 830–1,075 long, widest at acetabular level. Forebody narrowing slightly anteriorly, 165–220 long; hindbody 602–674 by 200–220 by 215–245; forebody–hindbody length ratio 1:3.1–3.8. Oral sucker terminal or nearly so, transversely elongate, truncate posteriorly, 73–102 by 93 by 91–105; acetabulum longitudinally elongate, aperture a transverse slit, without papillae, 145–160 by 151 by 134–150. Sucker length ratio 1:1.54–2.19. Postoral circular muscle ring narrow. Prepharynx short, up to 15 long; pharynx longer than oral sucker, oval, 92–126 by 75 by 70–78; esophagus short, up to 21 long; cecal bifurcation at acetabular level or slightly preacetabular; ceca terminating blindly 53–108 from posterior extremity.

Testes two, smooth, diagonal, longitudinally elongate, contiguous, sometimes overlapping, overlapping ceca ventrally, extending posteriorly from midlength of hindbody; anterior testis sinistromedian, 165–184 by 131 by 105–140, lying 170–234 postacetabular; posterior testis median, 181–247 by 122 by 105–169, lying 270–380 postacetabular; posttesticular space 98–160 long. Cirrus sac bipartite, posterior part saccular, anterior part tubular, sinuous, latter part with posterior loop or simple shallow



U-shaped bend at acetabular level or just postacetabular, commencing 117–238 postacetabular. Seminal vesicle tripartite; saccular part of cirrus sac containing two saccular parts of seminal vesicle, posterior sac 123–169 by 38–41 by 34–39; remainder of seminal vesicle tubular, sinuous. Pars prostatica relatively long, lying dorsal to acetabulum, may extend preacetabularly. Cirrus muscular, shorter than pars prostatica. Genital pore sinistral to posterior part of pharynx or anterior part of esophagus.

Ovary smooth, dextral to and contiguous with anterior testis, usually separated from posterior testis but may be contiguous with it, longitudinally elongate, 70–87 by 53–56 by 68–75, lying 185–270 postacetabular, 15–70 posterior to level of anterior margin of anterior testis. Ootype complex dorsomedian to ovary and anterior testis. Seminal receptacle large, dorsal or dorsomedian to ovary, may overlap anterior testis dorsally, longitudinally elongate, 70–133 by 48 by 59–63. Uterus relatively short, entirely preovarian to slightly overlapping latter, may overlap anterior part of anterior testis. Metraterm short, slightly muscular. Vitelline follicles large, smooth, commencing 20–123 postacetabular, filling posttesticular space, lateral anteriorly, confluent dorsally throughout length of fields. Eggs large, yellow-brown, operculate, partially collapsed, six measuring 53–59 by 29–33.

Excretory bladder unbranched, tubular, extending anteriorly to ovary-anterior testis level; pore subterminal dorsal.

Discussion: This new species differs from *P. ghanensis* in the shape of the oral sucker, relative size of the pharynx, and the anterior limits of the vitellaria being postacetabular.

Plagioporus gerridis sp. n. (Figs. 13–14)

Host: *Gerres nigri* Günther, mojarra (Liognathidae).

Habitat: Small intestine.

Locality: Cape Coast, Ghana.

Date: 17 February 1966.

Specimens: USNM Helm. Coll. No. 70679 (holotype); No. 70680 (paratypes).

Diagnosis (based on seven adults from one of three fish examined; five measured): Body elongate, robust, unspined, extremities round, with body fold anterior to acetabulum in some but absent in others, 550–815 by 177–300 at gonadal level. Forebody conical, 148–195 long; hindbody 290–460 long; forebody–hindbody length ratio 1:1.7–2.4. Oral sucker subterminal ventral, nearly round, 73–94 by 70–87; acetabulum tending to be flat at anterior, posterior and lateral surfaces, corners round, aperture a transverse slit, muscle fibers extending from surface of acetabulum onto body proper, 110–160 by 116–160. Sucker length ratio 1:1.51–1.88, width ratio 1:1.66–1.90. Postoral circular muscle ring narrow. Prepharynx 12 long (in one); pharynx nearly round, 53–58 by 48–58; esophagus 30–63 long; cecal bifurcation just anterior or dorsal to acetabulum; ceca terminating posttesticularly near posterior extremity.

Testes two, smooth, diagonal, contiguous, sometimes overlapping slightly, overlapping ceca ventrally, in anterior to middle two-thirds of hindbody. Anterior testis sinistromedian, round to longitudinally elongate, 123–145 by 102–133, overlapping acetabulum up to 18 to lying up to 89 postacetabular; posterior testis median, round to longitudinally or transversely elongate, 114–161 by 111–160, lying 53–182 postacetabular; posttesticular space 90–140 long. Cirrus sac sinuous, usually with dextral U-shaped bend dorsal to acetabulum, commencing 31–121 postacetabular, usually median between ovary and anterior testis, sometimes ventrolateral to ovary, overlapping gonads ventrally at its position. Seminal vesicle bipartite; posterior part saccular, overlapping posterior part of acetabulum, 87–141 by 27–60; anterior part tubular, sinuous. Pars prostatica relatively long, commencing dorsal to anterior part of

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Figures 15–20. 15. *Poracanthium ghanensis*, paratype, anterior part of body in sinistrolateral view, showing genital lobe, acetabular stalk, and acetabulum with papillae. 16. Same. Paratype, showing spined genital lobe and genital pit aperture (arrow) in ventral view. 17. Same. Paratype, showing genital pit cavity (upper arrow) and thick glandular lining (lower arrow, pointing to outer membranous margin) in ventral view. 18. *Podocotyloides chloroscombri*, holotype, anterior part of body in sinistrolateral view, showing acetabular stalk. 19. *Pedunculotrema ghanensis*, paratype, anterior part of body in dextrolateral view, showing acetabular stalk. 20. *Pedunculotrema capecoastensis*, paratype, dextrolateral view, showing acetabular stalk.

acetabulum or entirely preacetabular. Cirrus muscular, shorter than pars prostatica except when protruded through genital pore. Latter sinistral, at level of pharynx or anterior part of esophagus.

Ovary smooth, dextral to anterior testis, usually separated from anterior testis by uterus but may be contiguous with it, contiguous with posterior testis, usually longitudinally elongate, 77–109 by 58–121, lying 5–78 postacetabular, anterior margin 8–25 posterior to anterior margin of anterior testis in six worms and 11 anterior in one. Ootype complex anterodorsal to ovary. Seminal receptacle large, dorsal to ovary, longitudinally elongate, 63–141 by 40–62. Uterus short, coils few, may overlap all gonads. Metraterm short, slightly muscular, surrounded by gland cells. Vitelline follicles large, smooth, commencing at cecal bifurcation or slightly anteriorly, filling posttesticular space, lateral anteriorly, confluent dorsally throughout length of fields. Vitelline reservoir dorsomedian between and overlapping ovary and anterior testis. Eggs large, yellow-brown, operculate, partially collapsed, 11 measuring 53–64 by 30–39.

Excretory bladder unbranched, tubular, extending anteriorly to ovary-anterior testis level; pore terminal.

DISCUSSION: In having the ovary opposite the anterior testis our species appears closest to *Plagioporus triangulogenitalis* Belouss, 1958, from a cyprinid fish, and *P. glomeratus* Roitman, 1963, from cyprinid, salmonid and thymallid fishes; both species are from the maritime region of eastern Siberia. In the key to the subgenus *Plagioporus* (Stafford, 1904) given by Skrjabin and Koval (1958) our form keyed to *P. (P.) calotomi* (Yamaguti, 1934) Yamaguti, 1938, from scarid fishes from Japan. Our new species differs significantly from the three species listed above in the postacetabular extension of the cirrus sac, the presence of a bipartite seminal vesicle, and the vitellaria being confluent dorsally throughout the length of the fields.

Helicometra fasciata (Rudolphi, 1819) Odhner, 1902

HOSTS: *Labrisomus nuchipinnis* (Quoy and Gaimard), hairy blenny (Clinidae); *Glyphisodon saxatilis* (L.), sergeant-major (Pomacentridae); *Larimus peli* Bleeker (Sciaenidae); *Lutjanus modestus* Bleeker, red snapper (Lutjanidae); *Bathygobius soporator* (Cuvier and Valenciennes), goby or mapo (Gobiidae); *Ilisha melanota* Derscheid, long-finned herring (Clupeidae).

HABITATS: Small intestine, pyloric ceca.

LOCALITIES: Cape Coast, Elmina, Tema; Ghana.

DATES: 11 January (*B. soporator*, Elmina), 19 January, 7 February 1966 (four of five infected *L. peli*, Cape Coast).

SPECIMENS: USNM Helm. Coll. No. 70681 (from *L. nuchipinnis*); No. 70682 (*G. saxatilis*); No. 70683 (*L. peli*); No. 70684 (*L. modestus*); No. 70685 (*B. soporator*); No. 70686 (*I. melanota*).

DISCUSSION: The four infected (with one, one, two and three worms, respectively) *Larimus peli* of the 30 examined from Cape Coast were the only hosts to harbor worms in the pyloric ceca (none in the small intestine); the flukes were pinkish in color, and were readily visible through the cecum wall. Four *L. nuchipinnis* harbored one, three, five and eight worms, respectively; one *G. saxatilis* with one worm; one *L. peli* from Tema with five worms; one *L. modestus* with six worms; two *B. soporator* of four examined with three and 12 worms, respectively; and one *I. melanota* with one worm. This species has a wide distribution, having been recorded from the Mediterranean, North Atlantic, South-West Africa, Mexican Pacific, Japan, Caribbean, Tasmania, and New Caledonia.

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Resistance Potential of Certain Breeds of Domestic Fowl Exposed to *Raillietina tetragona* Infections. I. Contribution to the Biology of *Raillietina tetragona* (Molin, 1858)[1]

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ABSTRACT: *Tetramorium simillimum*, a new intermediate host for the poultry tapeworm, *Raillietina tetragona*, is reported from India. Mature cysticercoids ranging in number from 1–13 were found in 4.8% of the ants examined. Measurements of live cysticercoids differed from those previously reported. Cysticercoids administered to 1-day-old and 15-day-old White Leghorn chickens matured in 73.7% of the birds. The prepatent period of 12–18 days and the number of worms developing varied with the host's age and the infecting dose.

Worldwide in distribution, *Raillietina tetragona* is one of the commonest tapeworms infecting domestic fowl. But its biology and pathogenicity remain, to a large extent, unexplored. Previous investigators have reported that ants are the chief vectors. Following the work of Jones and Horsfall (1935) with *Tetramorium caespitum* and *Pheidole* sp. other reports from the United States were those of Horsfall (1938) for *P. vinelandica*, and Harkema (1943) for *P. vinelandica, P. dentata* and *Pheidole* sp. Sawada (1952a, b, 1953) reported that *T. caespitum jacoti* and *P. fervida*, and the beetles *Onthophagus ater* and *O. viduus*, act as vectors of *R. tetragona* in Japan. In North India, Chand (1964a, b), Saran (1966) and Mathur and Pande (1969) have shown that five species of ants, *P. fossulata, P. rhombinoda, Monomorium salmonis indicum, M. floricola* and *Monomorium* sp., serve as vectors. The present report deals with a new vector and the prepatent period of *R. tetragona* in White Leghorn chickens of two age groups.

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Materials and Methods

From July 1968 to February 1969, collections were made from the vicinity of 19 poultry houses in Trivandrum, Kerala. Seven species of ants were identified: *Tetramorium simillimum* (F. Smith), *Monomorium destructor* (Jerdon), *Solenopsis geminata* (Fabricius), *Pheidologeton* sp., *Pheidole* sp., *Paratrechina longicornis* (Latreille), and *Triglyphothrix striatidens* (Emery). Ants were dissected under magnification and cysticercoids recovered were transferred to physiological saline. Identification was based on larval morphology, and confirmed by recovery of adult worms after feeding cysticercoids to clean chickens.

Sixty-seven one-day-old and 15-day-old White Leghorn chickens were brought to the Laboratory from the local Government Poultry Farm. They were wing-banded and kept in brooders under parasite-free conditions. Twenty-eight one-day-old chicks received one cysticercoid each and one 10 cysticercoids. Twenty-two 15-day-old chickens received one cysticercoid each, five 10 cysticercoids each and one 5 cysticercoids. Five one-day and five 15-day-old birds were kept as controls. Cysticercoids

Table 1. Measurements (in microns) of cysticercoids of *R. tetragona* given by various authors.

Authors	Entire cysticercoid			Suckers				Rostellum			
	Length	Width	Thickness of cyst wall	Length	Width	Row of hooks	Width of hook zone	Diameter	Row of hooks	No. of hooks	Size of hooks
Horsfall (1938)	289–306	161–166	—	103	59	—	—	25	1	100	6–8
Sawada (1952c)	371–431	238–267	—	88–100	48–56	8–12	12–16	32–40	1	100	6–8
Saran (1966)	347–530	262–350	10–14	100–103	55–60	8–10	—	20–25	1	74	5–6
Present authors (1970)	250–363	150–238	25–32	75–100	35–80	8–12	12	25–38	1	100	6–8

were administered in a few drops of physiological saline with a sterilized glass dropper. On the tenth day after infection, the birds were isolated in individual cages and from that day onwards to the time of autopsy, the feces of each bird was collected daily, at intervals of three hours from 6 AM to 9 PM and examined for ripe proglottids. The experimental birds were sacrificed three to four weeks after the appearance of segments in feces and the adult worms recovered.

Results

Of 3,297 ants belonging to seven species examined, only the workers of *Tetramorium simillimum* were positive for the cysticercoids of *R. tetragona*. *T. simillimum* could be collected from the vicinity of only three of the 19 poultry houses examined. Of 1,700 ants of this species dissected, 82 of them (4.8%) were positive yielding a total of 179 cysticercoids. The number of cysticercoids harbored by individual ants ranged from 1–13. Measurements of live cysticercoids are given in Table 1.

Adult tapeworms were recovered from 42 of 57 (73.7%) chickens of the two age groups. Of 115 cysticercoids administered, 73 (63.5%) developed to the adult stage.

The average prepatent period, irrespective of the host's age was 14.6 days. It ranged from 12–18 days. We noted that the prepatent period varied in accordance with the age of the chickens. It averaged 15.5 days and 14.1 days in one-day-old and 15-day-old chickens respectively. In one-day-old chickens infected with a single cysticercoid, the percentage of development was 60.7 and in 15-day-old chickens it was 81.8. In 15-day-old chickens which received 5–10 cysticercoids each, the average prepatent period was 14.7 days and the percentage of cysticercoids developed to adult stage was 56.4. No worm was recovered from control birds.

Discussion

T. simillimum is reported for the first time as an intermediate host for *R. tetragona*. This is also the first record of a species of the genus *Tetramorium* involved in the life cycle of this tapeworm in India. Differences in the frequency of occurrence of cysticercoids in various species of ant populations have been noted by various workers. Thus Sawada (1952b) reported 2.06–2.3% of infection in *P. fervida* and 0.86% in *T. caespitum jacoti*; Chand (1964a, b) 1.4–2% in *P. fossulata* and *M. salmonis indicum*; and Saran (1966) 2.8–4.4% in *Monomorium* sp., *M. floricola* and *P. rhombinoda*. Our data indicate a higher incidence of cysticercoids in natural populations of *T. simillimum*.

The cysticercoids obtained from *T. simillimum* are considerably smaller than those studied by Sawada (1952b, c) and Saran (1966) —(Table 1). The size difference may be due to their development in a different intermediate host.

Jones and Horsfall (1935) and Sawada (1952a, b) recovered adult worms of *R. tetragona* about three weeks after infection. But Sawada later reported (1955) that nearly 25 days were required for the maturity of the worm in chickens. Chand (1964a, b) found the minimum prepatent period for this worm to be 15 days. According to Saran (1966) it was 21 and 15 days in 9- and 20-day-old chickens, respectively. Mathur (personal communication) on the other hand, noted this to be 19–21 days in 6–7-week-old chickens and 16 days in 10–11-week-old chickens. Our findings indicated that the prepatent period of *R. tetragona*

ranged from 12–18 days irrespective of the age of the chickens and dose of infection, the average being 14.6 days. With single cysticercoid infections, the average prepatent period in 1-day-old chickens was longer than that in 15-day-old chickens. We also noted that the prepatent period was longer in single worm infection than in infections with more worms in 15-day-old chickens. The earlier attainment of maturity of the worms in 15-day-old chickens may be attributed to certain favorable biotic and physico-chemical conditions prevailing in the host's gut.

We have noted that 63.5% of the cysticercoids in our experimental birds developed to maturity as against 33% and 31.3% of the cysticercoids studied by Chand (1964a) and Mathur and Pande (1969), respectively. In our studies with single cysticercoid infection, the percentage of development was higher in 15-day-old chickens than in 1-day-old chickens. Humninen (1935) has suggested that the smaller percentage of development of *Hymenolepis fraterna* in younger mice is due to the mechanical disadvantage, both on account of the reduced length and small size of the villi of their small intestine. It is not certain whether such an explanation is tenable for our findings.

In the present study, the majority of the birds were infected with a single cysticercoid. Since there was no crowding of worms, which might affect their growth rate and maturity, this procedure helped to determine accurately the prepatent period and the capacity for segment production of individual worms. Our studies have shown that cysticercoids are not affected by the triturating process in the gizzard of the bird, and that the method of administration of larvae in the gelatine capsules employed by previous workers is unnecessary for protection of cysticercoids.

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Resistance Potential of Certain Breeds of Domestic Fowl Exposed to *Raillietina tetragona* Infections. II. Studies on the Periodicity of Segment Discharge by the Domestic Fowl Infected with *Raillietina tetragona* (Molin, 1858)[1]

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ABSTRACT: Observations on 38 White Leghorn chickens of two age groups, infected with cysticercoids of *Raillietina tetragona* indicate that there is no well-defined or distinctive pattern of segment discharge by the host birds. There appears to be no correlation between segment discharge and the feeding time of the birds. The capacity for segment production per worm per day was greater in single worm infection than in infection with several worms. A wide range of variation observed in segment expulsion per worm suggests that segment count may not be taken as an index of worm burden.

It is fairly well established that diet, feeding time and feeding habits of the host animals have decisive influence on the growth of intestinal parasites. In the case of tapeworms, growth is reflected in the process of proglottization. A cyclical phenomenon in the release of ripe proglottids by the host birds has been reported by Wetzel (1932), Levine (1938) and Abdou (1958) for *Davainea proglottina*; Wetzel (1934), Reid et al. (1938) and Dutt et al. (1961) for *Raillietina cesticillus* and Sawada (1960) for *R. kashiwarensis*, *R. cesticillus* and *R. echinobothrida*. The present communication deals with the results of a study, primarily planned, to throw light on the periodicity of segment discharge by White Leghorn chickens infected with cysticercoids of *R. tetragona*.

Materials and Methods

Parasite-free White Leghorn chickens, 1-day-old and 15-day-old were infected with cysticercoids of *R. tetragona*. With the first appearance of ripe segments, feces of individual birds were collected six times a day, at an interval of three hours between 6 AM and 9 PM for segment counts. After collecting data for 3–4 weeks, the birds were autopsied and the worms recovered.

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Two series of experiments were conducted; in the first series three experiments were designed to obtain data on the daily periodicity in the release of ripe proglottids by host birds and capacity of individual worms for segment production.

EXPERIMENT I. One cysticercoid given to each of the ten one-day-old chickens.

EXPERIMENT II. One cysticercoid given to each of the five 15-day-old chickens.

EXPERIMENT III. Ten cysticercoids given to each of the four 15-day-old chickens.

In the second series, two experiments were carried out to determine whether or not segment production and discharge are related to the feeding time of the host.

EXPERIMENT IV. Seven 1-day-old chickens, infected with one cysticercoid each, were allowed to feed continuously and segment counts made for 1 week. They were then divided into three groups, A, B and C and feed was given only at a particular time of the day for each group; 6 AM to 9 AM, 3 PM to 6 PM, and 6 PM to 9 PM, respectively. Restricted feeding was continued for 2 weeks and later returned to continuous feeding for 1 week before autopsy.

EXPERIMENT V. Nine 15-day-old chickens, infected with one cysticercoid each, were maintained on a continuous diet and segment count was made for 1 week. They were then divided into three groups and subjected to the same treatment as outlined in the previous experiment, except for the limitation of restricted feeding for only 1 week.

Control groups (infected) were fed continuously throughout the experimental period.

Results

In the first experiment five birds passed the maximum percentage of segments between 6 AM and 9 AM, four birds between 3 PM and 6 PM and the remaining one between 12 noon and 3 PM. We also determined that a single worm produced a daily average of 19.04 segments the first week, 21.92 the second week and 23.4 the third week. In the second experiment all the birds discharged the maximum percentage of segments between 3 PM and 6 PM and the daily average segment production by individual worms was found to be 26.52, 27.72, and 27.52 for three consecutive weeks. Worms developed in 15-day-old chickens produced more segments than those in 1-day-old chickens. In the third experiment, two birds discharged the maximum percentage of segments between 3 PM and 6 PM and the rest between noon and 3 PM. In this case the segment production by individual worms, on an average, was 15.4, 24.05, and 21.47 for three consecutive weeks. In the fourth and fifth experiments the peak periods of segment discharge were very irregular during the periods of both continuous and restricted feeding of the host birds.

Discussion

Characteristic peak periods of segment expulsion, mostly confined to the latter half of the day, by the host birds infected with various species of poultry tapeworms have been reported by Wetzel (1932, 1934), Reid et al. (1938), Abdou (1958), Sawada (1960), Chand (1964a, b), and Saran (1966). Our findings tend to show that although there was no characteristic pattern of segment discharge, most of the segments were discharged in the evening by 11 of 19 of our experimental birds. Our studies further indicated that the peak period of segment discharge as well as the total number of segments shed were never constant for a given group of birds maintained on continuous or alternately on continuous and restricted feeding. It was also noted that the peak periods were not interchangeable with the feeding time of the host birds, contrary to the observation made by Dutt et al. (1961).

In birds with single worm infection we found that a worm has the capacity to produce a maximum number of 67 segments a day. By contrast, in birds with several worms, the maximum number of segments produced per worm a day was only 47. This fall in segment production in the latter instance may be due to competition of worms for available nutrients.

Complete cessation of segment discharge during the early morning hours and between 9 PM and 12 noon was reported by Wetzel (1932) and Dutt et al. (1961), respectively. However, our experimental birds discharged segments throughout the day with a marked fall in their number during the night and this observation is more or less in agreement with the results obtained by Sawada (1960) in the case of *R. echinobothrida*. Levine (1938) suggested that the segment discharge might be correlated with the feeding activity of the host bird, which in turn might be influenced by daylight. He attributed the occurrence of the peak period between 2 PM and 4 PM in the winter, to the shortened days and consequent changes in the feeding time of the birds.

Proglottid or egg count was the method followed by Stoll (1936), Levine (1938) and Reid (1942) to determine the approximate number of worms harbored. But Hager (1941) found no correlation between the oncosphere count and the number of adult *Hymenolepis diminuta* present in rats. Abdou (1958) and Sawada (1960) doubt the suitability and reliability of such a method. We also found that the method of segment count to determine the worm burden is inadequate because of the wide variation in segment production by individual worms.

In our studies with single worm infection, it has been possible to obtain more precise data on the number of segments shed by individual worms and periodicity in the discharge of ripe proglottids. When the worm burden is heavier,

the data collected would be less accurate because of the possibility that each worm might have different peak periods of nutrient absorption, segment production and discharge. The observed fluctuations in the peak periods of segment discharge may be ascribed to the inherent metabolic variability of individual worms and physiological variants including availability of nutrients in the gut, feeding habit and feeding time of the host birds.

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Parasites of the Clapper Rail, *Rallus longirostris* Boddaert. II. Some Trematodes and Cestodes from *Spartina* Marshes of the Eastern United States[1, 2]

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ABSTRACT: During a parasitologic survey of 146 clapper rails, *Rallus longirostris*, 26 trematode and two cestode species representing 14 families were encountered. The birds examined were collected in *Spartina* salt marshes from ten Atlantic and Gulf coast states. The trematode family Microphallidae constituted the largest group (11 species) and was present in the greatest numbers. Twenty-four of the 28 species found were new host records for the clapper rail. Ecological and life cycle data on several of the trematodes is given.

Although there are many reports concerning various aspects of the biology of *Rallus longirostris*, relatively little is known of its parasites. In January 1964 a study of the taxonomy, incidence and ecology of the parasites of clapper rails was initiated. This report, the second of a series, deals with the trematodes and cestodes collected in 146 birds from *Spartina* salt marsh habitats along the Gulf and Atlantic coasts of the southeastern United States. Data on the helminth parasites of 34 clapper rails collected from mangrove habitats in Florida will be given in future reports.

Previously four trematodes, *Athesmia heterolecithodes* (Braun, 1899); *Levinseniella byrdi* Heard, 1968; *Probolocoryphe glandulosa* (Coil, 1955) and *Maritrema prosthometra* Deblock and Heard, 1969, have been recorded from this host (Byrd et al., 1967; Heard, 1968; Heard and Sikora, 1969; Deblock and Heard, 1969). There have been no previous reports of cestodes from clapper rails.

Materials and Methods

Birds were collected with a shotgun or manually (immature birds) from the coastal regions of Louisiana (Jefferson Parish), Mississippi (Jackson County), Alabama (Mobile County), Georgia (Chatham County), South Carolina (Charleston County), North Carolina (Carteret County), Virginia (Accomac County), Maryland (Worchester County), New Jersey (Cape May County), and New York (Nassau County), and examined within 8 hr following collection. Parasites were fixed and preserved for study by various standard techniques. Suspected intermediate hosts from the several collecting areas were examined for clues as to possible intermediate hosts for the parasites encountered.

Results

Twenty-six trematode and two cestode species occurred in 146 clapper rails collected from *Spartina* marshes on the coast of the eastern United States. With the exception of the four species mentioned previously, these constitute new host records for *Rallus longirostris*. Table 1 gives: (1) the number and kinds of parasites encountered, (2) the number of hosts examined, (3) the collecting areas, (4) site of infections, and (5) the number of infected hosts for a given parasite from each collecting area. Ecological and life history data are incorporated in the Observations and Discussion.

Observations and Discussion

Trematoda

Microphallidae Travassos, 1920

The group of trematodes most frequently encountered were members of this family. These small cosmopolitan distomes are common para-

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Table 1. Incidence of trematode and cestode infections in 146 Clapper Rails from *Spartina* marshes on the Gulf and Atlantic Coasts of the United States.

	Localities										Total number of hosts infected
	La.	Miss.	Ala.	Ga.	S.C.	N.C.	Va.	Md.	N.J.	N.Y.	
Number of hosts examined—	10	10	10	40	5	30	10	10	20	1	146
Trematoda											
Ascocotyle pachycystis (1)*	–	–	1	–	–	–	–	–	–	–	1
Athesmia heterolecithodes (4)	1	–	4	1	–	2	–	–	–	–	8
Carneophallus choanophallus (1)	1	3	1	–	–	–	–	–	–	–	5
Carneophallus turgidus (1)	–	–	–	15	1	–	1	7	1	–	25
Echinochasmus schwartzi (1)	–	–	3	14	3	4	1	2	1	1	29
Echinostoma attenuatum (1)	–	1	–	–	–	–	–	–	–	–	1
Gynaecotyla adunca (1, 2)	5	–	–	34	5	26	4	5	–	–	79
Himasthla quissetensis (1, 2)	–	–	–	–	–	8	2	1	4	–	15
Levinseniella carteretensis (5)	–	–	–	–	–	1	–	–	–	–	1
Levinseniella byrdi (5)	–	–	1	17	3	12	4	5	12	1	55
Lyperosomum sinuosum (6)	2	–	4	5	1	–	–	–	–	–	12
Maritrema sp. (near *M. prolixum*) (1)	–	–	1	4	1	–	1	5	–	–	12
Maritrema prosthometra (1)	7	9	7	39	5	25	10	10	20	1	133
Microphallus sp. (1)	–	–	1	–	–	–	–	–	–	–	1
Notocotylus regis (5)	–	1	–	–	–	–	–	–	–	–	1
Notocotylus sp. (5)	1	3	–	11	–	–	–	1	2	1	19
Odhneria raminellae (5)	–	–	–	–	1	2	4	9	4	–	20
Ophthalmophagus sp. (7)	3	3	2	4	–	–	–	–	–	–	12
Parorchis acanthus (3)	6	4	6	16	2	16	10	9	15	–	84
Parvatrema sp. I (1)	–	1	1	–	–	–	–	–	–	–	2
Parvatrema sp. II (immature) (1)	–	–	–	2	–	1	–	–	–	–	3
Phagicola diminuta (1, 5)	–	–	–	2	–	1	–	–	–	–	3
Phagicola sp. (1)	–	–	–	–	–	–	1	5	–	–	6
Prosthogonimus ovatus (8)	–	–	–	1	2	2	1	1	3	–	10
Schistosomatidae (unident.) (10)	–	–	–	1	–	–	–	–	–	–	1
Tanaisia fedtschenkoi (9)	3	1	5	7	–	1	–	–	1	–	18
Cestoda											
Cyclustera sp. (1)	7	4	5	14	2	8	–	–	–	1	41
Ophryocotyle proteus (1)	–	–	–	5	2	12	2	3	4	–	28

* The numbers in parenthesis indicate the locations in the host: (1) small intestine, (2) rectum, (3) cloaca, (4) liver, (5) ceca, (6) pancreas, (7) nares, (8) bursa, (9) kidney, (10) blood vessels.

sites of marine shore birds, but have also been reported from all other vertebrate classes (Yamaguti, 1958). Eleven species representing three subfamilies are reported here.

Maritrema prosthometra Deblock and Heard, 1969, the most common and widely distributed microphallid species encountered, infected 88% of the rails examined and occurred in each collecting area. On several occasions more than 3,000 specimens were recovered from a single rail. I have found the precociously developed metacercariae of this species in the fiddler crabs, *Uca minax* (LeConte), *U. pugnax* (Smith) and *U. rapax* (Smith) from salt marsh areas of the Atlantic and Gulf coasts.

In Carteret County, North Carolina, birds collected during the winter were negative or only lightly infected with *M. prosthometra*, whereas birds examined during warmer months were usually heavily infected. This seasonal fluctuation in the degree of infection probably is due to the availability of the fiddler crab intermediate hosts. During the colder months these crabs are inactive and remain buried in the salt marsh substrate (Teal, 1958) not readily accessible to clapper rails. I found raccoons, *Procyon lotor* L.; willets, *Catoptrophorus s. semipalmatus* (Gmelin); and seaside sparrows, *Ammospiza maritima macgillivraii* (Audubon) from coastal Georgia also infected with this species.

Maritrema sp. (near *M. prolixum* Caballero and Montero-Gei, 1961) was found occasionally in clapper rails. Specimens from rails were almost always immature or nongravid, suggesting that this bird is not a normal definitive host. This trematode has been reported from South Carolina coastal raccoons as *Maritrema* sp. and *Maritreminoides* sp. by Harkema and Miller in 1962 and 1964, respectively. Also I have found this form to be a common parasite of raccoons from Georgia salt marshes. *Maritrema* sp. apparently represents an undescribed spe-

cies, and its description along with notes on its life cycle is now in progress.

During the investigation specimens of *Probolocoryphe glandulosa* (Coil, 1955) were found in rails collected from Alabama, constituting the first report of the species in the continental United States (Heard and Sikora, 1969). The metacercariae of *P. glandulosa* were found encysted in the thoracic muscles and digestive glands of the fiddler crab, *Uca rapax*, from Dauphin Island, Alabama. Fully developed metacercariae closely resembled adult worms, and in a few instances possessed partially developed eggs (Heard, in press).

A cecal trematode identified as *Odhneria raminellae* (Dery, 1958) was found in six of the 10 collecting areas, with the highest incidence of infection occurring in the middle Atlantic states. The species was originally described by Dery (1958) from red-breasted mergansers, *Mergus serrator* L., in Connecticut. Snapping shrimps *Alpheus heterochaelis* Say, harbor the metacercaria, which is not precociously developed and apparently requires several days of development in the definitive host before producing eggs (Heard, in press).

Gynaecotyla adunca (Linton, 1905) was the second most abundant and widely occurring microphallid encountered. It was not uncommon to find more than 1,000 specimens in a single rail. The original description of *G. adunca* was based on nongravid specimens from the toad fish, *Opsanus tau* (L.) at Beaufort, North Carolina (Linton, 1905). It has since been reported in several species of marine birds (Hunter, 1952; Hunter and Quay, 1953; Cable, Connor, and Balling, 1960; Hutton, 1964; Ehrhardt, Harkema, and Miller, 1966). Harkema and Miller (1962, 1964) found it a common parasite of coastal raccoons from the southeastern United States. The mud snail, *Nassarius obsoletus* (Say), and the crustaceans, *Uca pugilator* (Bosc), *U. pugnax, U. minax,* and *Talorchestia megalophthalma* (Bate), have been reported as the intermediate hosts (Hunter, 1952; Hunter and Vernberg, 1953; Hutton, 1964).

Levinseniella byrdi Heard, 1968 was widely distributed, occurring in birds from both the Atlantic and Gulf coasts. Preliminary observations indicate that *L. byrdi* utilizes the marsh periwinkle, *Littorina irrorata* (Say), as its snail host. A talitrid amphipid, *Orchestia grillus* Bosc, serves as the crustacean intermediate host (Heard, in press).

A single specimen of *Levinseniella carteretensis* Coil and Heard, 1966 occurred in an immature clapper rail collected near Beaufort, North Carolina. Coil and Heard (1966) described *L. carteretensis* from Wilson's plovers, taken from the same general locality.

Specimens of *Carneophallus* Cable and Kuns, 1951, a genus synonymized with *Microphallus* Ward, 1901 by Capron, Deblock and Biguet (1957), occurred in birds from the Atlantic and Gulf coasts. Initially these were thought to represent a single species, *Carneophallus turgidus* Leigh, 1958; however, the specimens collected from Gulf coast localities appear to be *C. choanophallus* Bridgman, 1969. The metacercariae of both *C. turgidus* and *C. choanophallus* develop in palaemonid shrimps, but they utilize different snail hosts (Bridgman, 1969; Heard, in press).

Due to their similar morphology and very small size, species of the genus *Microphallus* have been one of the most difficult groups of trematodes to distinguish taxonomically (Stunkard, 1960).

Specimens of *Microphallus*, whose specific identity has not yet been determined, were collected in rails from Dauphin Island, Alabama. Precociously developed metacercariae of *Microphallus* sp. resembling the form from clapper rails have been reported by Sogandares-Bernal and Hutton (1959) in juvenile commercial shrimp, *Penaeus duorarum* Burkenroad, from Tampa Bay, Florida.

Three specimens of *M. papillorobustus* (Rankin, 1940) were recovered from a clapper rail collected on the south shore of Long Island, New York. This fluke was originally found in several species of shore birds from Massachusetts (Rankin, 1940) and since has been reported from Eurasia (Deblock and Tran Van Ky, 1966).

Dicrocoeliidae Odhner, 1911

Athesmia heterolecithodes (Braun, 1899), reported in numerous species of birds from both the old and new world (Freitas, 1962; Byrd et al., 1967; and Lumsden and Zischke, 1962), was recorded in rails from six localities (Byrd et al., 1967).

Lyperosomum sinuosum Travassos, 1917,

originally described from a yellow-crowned night heron, *Nyctanassa violacea violacea* (L.) in Brazil was found in seven localities. This report represents the first record of *L. sinuosum* from an avian host in the United States. Harkema and Miller (1962, 1964) encountered the species in raccoons from coastal South Carolina.

Prosthogonimidae Nicoll, 1924

A species of *Prosthogonimus* Luhe, 1899, tentatively identified as *P. ovatus* (Rudolphi, 1803) occurred in the bursa Fabricii and oviducts of clapper rails from several localities on the Atlantic and Gulf coasts. Hunter and Quay (1953) reported *P. ovatus* in the seaside sparrow from a *Spartina* marsh area in North Carolina.

Renicolidae Dollfus, 1939

Several specimens of *Renicola hydranassa* Lumsden and Zischke, 1963 were found in the kidneys of a bird from Alabama. This species was originally described from the Louisiana heron, *Hydranassa tricolor* (Müller) in Louisiana. The present report is the second record of the species and extends its range eastward to Alabama.

Eucotylidae Skrjabin, 1924

Tanaisia fedtschenkoi Skrjabin, 1924, a cosmopolitan species from birds associated with aquatic habitats (Byrd and Denton, 1950), was recorded from several localities on the Gulf and Atlantic coasts.

Heterophyidae Odhner, 1914

Several specimens in the genus *Ascocotyle* Looss, 1899, closely resembling *A. pachycystis* Schroeder and Leigh, 1965, were found in a rail from Dauphin Island, Alabama. Schroeder and Leigh (1965) reported the snail *Littoridinops tenuipes* (Couper) and the fish *Cyprinodon variegatus* Lacépède as the intermediate hosts, and raccoons as the final host of this species.

Phagicola diminuta (Stunkard and Haviland, 1924), a common parasite of coastal herons and raccoons, occurred in clapper rails from Georgia and North Carolina. O'Hara (1961) described the cercaria and certain other aspects of the life cycle. The snails, *Littoridinops tenuipes, Onobops jacksoni* (Bartsch) and *Hydrobia* sp., were found in this study to serve as the molluscan first intermediate hosts (Heard, unpublished data). The metacercaria develops in the gills of brackish water cyprinodont fishes (O'Hara, 1961; Stunkard and Uzmann, 1955). Sogandares-Bernal and Lumsden (1963) consider *P. diminuta* a synonym of *Ascocotyle angrense* Travassos, 1916.

An undescribed species of *Phagicola* was found in rails from Virginia and Maryland. The adults occur in pairs, deeply buried in the mucosa of the small intestine and if numerous, may cause serious damage (Heard, unpublished data).

Echinostomatidae Poche, 1926

Echinochasmus schwartzi Price, 1931 had the highest incidence and widest distribution, however, the worms were small and usually nongravid, indicating the clapper rail to be an unfavorable host. Price (1931) described *E. schwartzi* from the muskrat, *Ondatra zibethicus* L., in Maryland. Byrd and Reiber (1942) and Penn (1942) later reported it from the same host in Louisiana. Recently, Abram (1969) found three of 75 muskrats from brackish marshes in Maryland harboring this species. A dog from New Jersey has been reported as a host for *E. schwartzi* by Burrows and Lillis (1965). Lillis and Nigrelli (1965) reported the metacercaria of this species in the gills of the killifish, *Fundulus heteroclitus* (L.). During the present study *Hydrobia* sp. was found to serve as the gastropod host. When fed to a golden hamster, *E. schwartzi* metacercariae from experimentally infected lab reared *F. heteroclitus* developed into gravid adults in 15 days (Heard, unpublished data).

Himasthla quissetensis (Miller and Northrup, 1926) was found only in immature birds from the middle Atlantic states. Previously it has been recorded from the night heron, *Nycticorax nycticorax* L., and gulls, *Larus* spp. (Stunkard, 1932; Ehrhardt, Harkema, and Miller, 1966). Stunkard (1938) reported the mud snail, *Nassarius obsoletus* and several species of marine bivalves as its first and second intermediate hosts, respectively.

Six specimens of *Echinostoma attenuatum* Lumsden and Zischke, 1963 were found in a

clapper rail at Ocean Springs, Mississippi. This echinostome was originally described from the king rail, *Rallus elegans* Audubon, in southern Louisiana. The clapper rail probably acquired the infection while feeding in nearby freshwater areas.

Philophthalmidae Travassos, 1918

Parorchis acanthus (Nicoll, 1906) a cosmopolitan parasite of marine birds, occurred in clapper rails in all but one of the collecting areas. Various snail hosts for this species have been reported from Europe, Hawaii, Australia, and both the Atlantic and Pacific coasts of the continental United States (Lebour, 1914; Stunkard and Cable, 1932; Angel, 1954; Oguri and Chu, 1955; Holliman, 1961). During the present study the periwinkle, *Littorina irrorata*, was found to serve as a molluscan host of this trematode.

Cyclocoelidae Kossack, 1911

The presence of *Ophthalmophagus* Stossich, 1902 in clapper rails constitutes the second report of this genus from North America and the first from a maritime host. Redington and Ulmer (1964) reported *O. singularis* Stossich, 1902 from the sora rail, *Porzana carolina* (L.), in Iowa. Further study is needed to identify the species from the clapper rail.

Notocotylidae Lühe, 1909

A single specimen of *Notocotylus regis* Harwood, 1939, known from the sora rail in Louisiana (Lumsden and Zischke, 1963), occurred with *Echinostoma attenuatum* in a clapper rail collected at Ocean Springs, Mississippi.

A widely distributed and apparently undescribed species of *Notocotylus* Diesing, 1839, resembling *N. prozanae* Harwood, 1939, also occurred in clapper rails. This species could be confused with a species of *Paramonostomum* Lühe, 1909, since the ventral glands are extremely difficult to detect with standard staining procedures.

Gymnophallidae Morosov, 1955

Adults of an unidentified species of *Parvatrema* Cable, 1953 infected clapper rails from the Gulf coasts of Mississippi and Alabama. The cercaria of this species may be one of several gymnophallid larvae Holliman (1961) reported from the northwest coast of Florida.

Immature specimens of another species of *Parvatrema* were found occasionally in rails from the Georgia and North Carolina coasts. Preliminary studies indicate the first intermediate host to be the brackish water clam, *Cyrenoida floridana* Dall. The metacercarial stage occurs in the kidney of the melampid marsh snails, *Melampus bidentatus* Say and *Detracia floridana* (Pfeiffer).

Schistosomatidae Poche, 1907

One immature male schistosome was found in a rail from Chatham County, Georgia, but the specimen was unsuitable for positive identification.

Cestoda

Dilepididae Railliet and Henry, 1909

An undescribed cestode in the genus *Cyclustera* Fuhrmann, 1901 was common in clapper rails and was found also in a red breasted merganser and a glossy ibis from Georgia and North Carolina, respectively. The larva occurs in the viscera of brackish water fishes, *Cyprinodon variegatus* and *Fundulus heteroclitus* (Heard, unpublished data).

Davaineidae Fuhrmann, 1907

Ophryocotyle proteus Friis, 1870 occurred frequently in chicks and immature rails from the Atlantic coast, and often in relatively large numbers. On one occasion over 200 specimens were recovered from a single rail chick. Previous to this study, *O. proteus* has been reported three times in the United States (Hunter and Quay, 1953; Yamaguti, 1959; Ehrhardt, Harkema, and Miller, 1967).

Acknowledgments

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vice, NASA (Wallop's Island Station), Hempstead Township (N. Y.), Duke University Marine Laboratory, and the Marine Institutes of the Universities of Georgia and Miami also provided facilities for the study. Special thanks are due Drs. E. L. Bousfield and J. P. E. Morrison for identifying the amphipod and molluscan intermediate hosts, and Mr. W. W. Becklund, Animal Disease and Parasite Research Division, USDA, Beltsville, Maryland, for the loan of specimens from the U. S. National Museum Helminthological Collection.

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Revision of the Genus *Pratylenchoides* Winslow, 1958 (Nematoda: Tylenchoidea)

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ABSTRACT: The history of the genus *Pratylenchoides* Winslow, 1958 is presented and an emended diagnosis of the genus given. The three nominal species are redescribed and illustrated from type specimens, and four new species are proposed. A key to the species is presented. The morphology, geographical distribution and biology of *Pratylenchoides* are discussed in relation to the most closely related taxon, *Radopholus* Thorne, 1949.

The genus *Pratylenchoides* Winslow, 1958 was proposed for the nematode described by Goodey (1932, 1940) as *Anguillulina obtusa* (Bastian, 1865) Goodey, 1932 and placed in the subfamily Pratylenchinae. *Pratylenchoides* was distinguished by Winslow from *Pratylenchus* Filipjev, 1934 by its didelphic character, and from *Radopholus* Thorne, 1949 by the short oblique overlapping esophageal glands.

A second species was described in this genus, *Pratylenchoides guevarai* Tobar Jiménez, 1963 as differing from the type in having a tail terminus without annulation and the esophageal glands overlapping the intestine, ventrally.

Tarjan and Weischer (1965) synonymized the genera *Zygotylenchus* Siddiqi, 1963 and *Mesotylus* de Guiran, 1964 with *Pratylenchoides*. They also considered *Zygotylenchus browni* Siddiqi, 1963 and *Mesotylus gallicus* de Guiran, 1964 synonyms of *Pratylenchoides guevarai* Tobar Jiménez, 1963 and transferred *Mesotylus taomasinae* de Guiran, 1964 to *Pratylenchoides taomasinae* (de Guiran, 1964) Tarjan and Weischer, 1965.

Pratylenchoides gadeai Arias, Jiménez and Lopez, 1965 was described from Spain. Braun and Loof described *Pratylenchoides laticauda* Braun and Loof, 1966 and considered *Pratylenchoides* a genus distinct from *Zygotylenchus* (Syn. *Mesotylus*). They pointed out the different type of esophagus, absence of a deirid and lack of sexual dimorphism in the esophagus of *Zygotylenchus*. *Pratylenchoides taomasinae* (de Guiran, 1964) now became *Zygotylenchus taomasinae* (de Guiran, 1964) Braun and Loof, 1966, and *Pratylenchoides guevarai* Tobar, 1963 now became *Z. guevarai* (Tobar, 1963) Braun and Loof, 1966. They also transferred the species *Pratylenchoides gadeai* Arias, Jiménez and Lopez, 1965 to the genus *Tylenchorhynchus* Cobb, 1913.

Pratylenchoides maritinus Bor and s'Jacob, 1966 was described as differing from the other two species in this genus by the long dorsal esophageal overlap of the intestine, the low lip region, shape of the female tail, the great size difference between females and males and by the median bulb, which is as strongly developed in the males as in the females. They were in agreement with Braun and Loof (1966) that the following do not belong in *Pratylenchoides*: *P. guevarai* Tobar, 1963; *P. taomasinae* (de Guiran, 1964) Tarjan and Weischer, 1965; and *P. gadeai* Arias, Jiménez and Lopez, 1965.

In 1967, de Guiran and Siddiqi differentiated *Pratylenchoides* from *Zygotylenchus* by the structure, position and length of the esophageal glands overlapping the intestine.

The present study was undertaken to diagnose the genus and differentiate it from the genera *Radopholus* and *Zygotylenchus* and to identify the numerous specimens in the University of California at Riverside collection.

Type material of all the described species has been available for study, as well as that in the closely related genera, *Radopholus* and *Zygotylenchus*. Over 1,500 specimens of *Pratylenchoides*, in permanently mounted glycerine slides, from many areas of the world, have been used in this study. Fresh specimens in water and 2% formalin have been examined for the proposed new species.

Morphology

The genus *Pratylenchoides* resembles the genus *Radopholus* Thorne, 1949, as reported

by Sher (1968), with the differences noted in the generic diagnosis and discussion.

Genus *Pratylenchoides* Winslow, 1958

DIAGNOSIS EMENDED: Radopholinae. Labial framework and stylet well-developed. Sexual dimorphism present in anterior part of body, male with slightly higher rounded lip region, stylet and esophagus not as developed as female. Deirids present. Esophageal glands overlapping intestine ventrally, laterally and dorsally, greatest development dorsally with at least one esophageal gland nucleus above the esophageal intestinal valve. Ovaries paired. Phasmids in posterior portion of tail. Caudal alae enveloping tail, gubernaculum not projecting from cloaca.

TYPE SPECIES: *Pratylenchoides crenicauda* Winslow, 1958.

The genus *Pratylenchoides* can be distinguished from the most closely related genus *Radopholus* by the characters: presence of deirids; esophagus with at least one esophageal nucleus at or above the level of the esophageal intestinal valve; less sexual dimorphism; and the gubernaculum not projecting from the body. In addition, *Pratylenchoides* shows the following: phasmids in the posterior portion of the tail; no longitudinal striations on the lip region; usually round sperm in the spermatheca; and different geographical distribution. *Pratylenchoides* can be easily distinguished from *Zygotylenchus* by the dorsal esophageal glands, which overlap the intestine, and by the presence of deirids.

Key to the species of *Pratylenchoides*

1. Female tail almost pointed, stylet 18 μ or shorter ..... *maritimus*
   Female tail rounded, stylet 19 μ or longer 2
2. Female tail with 28 or more annules ..... *crenicauda*
   Female tail with 28 or less annules 3
3. Sperm in spermatheca and testis rod-like ..... *bacilisemenus* sp. n.
   Sperm in spermatheca and testis irregularly rounded or elongated (spindle-shaped) 4
4. Female tail terminus usually without striae, occasionally one to two striae; stylet 21 μ or less ..... *leiocauda* sp. n.
   Female tail terminus striated; stylet 21 μ or longer 5
5. Female esophageal glands overlapping intestine over two times the body width ..... *ritteri* sp. n.
   Female esophageal glands overlapping intestine one-and-a-half or less times the body width 6
6. Lateral field with six incisures; lateral canals conspicuous ..... *laticauda*
   Lateral field usually with four incisures; lateral canals inconspicuous ..... *variabilis* sp. n.

Pratylenchoides crenicauda Winslow, 1958 (Fig. 1A–C, E)

Anguillulina obtusa (Bastian, 1865) Goodey, 1932, pp. 63–65; Goodey, 1940, pp. 34–36.

Rotylenchus obtusus Filipjev and Schuurmans Stekhoven, 1941, p. 219.

Pratylenchoides crenicauda Winslow, 1958, pp. 136–138; Jairajpuri, 1964, p. 339; Tarjan and Weischer, 1965, pp. 433–439.

MEASUREMENTS: 15 ♀ ♀ topotypes: L = 0.64 mm (0.53–0.86); a = 25 (21–29); b = 4.6 (4.1–5.2); b′ = 4.2 (3.5–5.2); c = 15 (13–18); V = 58 (56–62); stylet = 22 μ (20–23).

4 ♂ ♂ topotypes: L = 0.66 mm (0.61–0.72); b′ = 5.5 (5.2–5.7); stylet = 21 μ (20–21); gubernaculum = 6 μ (4–7); spicules = 22 μ (20–24).

FEMALE: Lip region flattened, slightly or not set off, three, sometimes four annules. Stylet knobs broadly rounded sloping posteriorly. Esophageal glands overlap intestine about one body width (0.6–1.2). Spermatheca usually inconspicuous or not seen, or with irregularly rounded sperm. Lateral canals present. Lateral field with six, sometimes four incisures, incompletely areolated on tail. Tail tapering with 28–36 annules, annulation conspicuous around terminus.

MALE: Lip region slightly higher and narrower than female, three to five annules. Esophagus and esophageal glands not as well developed as in female, overlapping intestine less than one body width. Sperm elongated, spindle shape.

TYPE HABITAT AND LOCALITY: Turf, Rothamsted Experimental Station, Harpenden, Herts, England.

DIAGNOSIS: *Pratylenchoides crenicauda* can be identified by the shape and large number

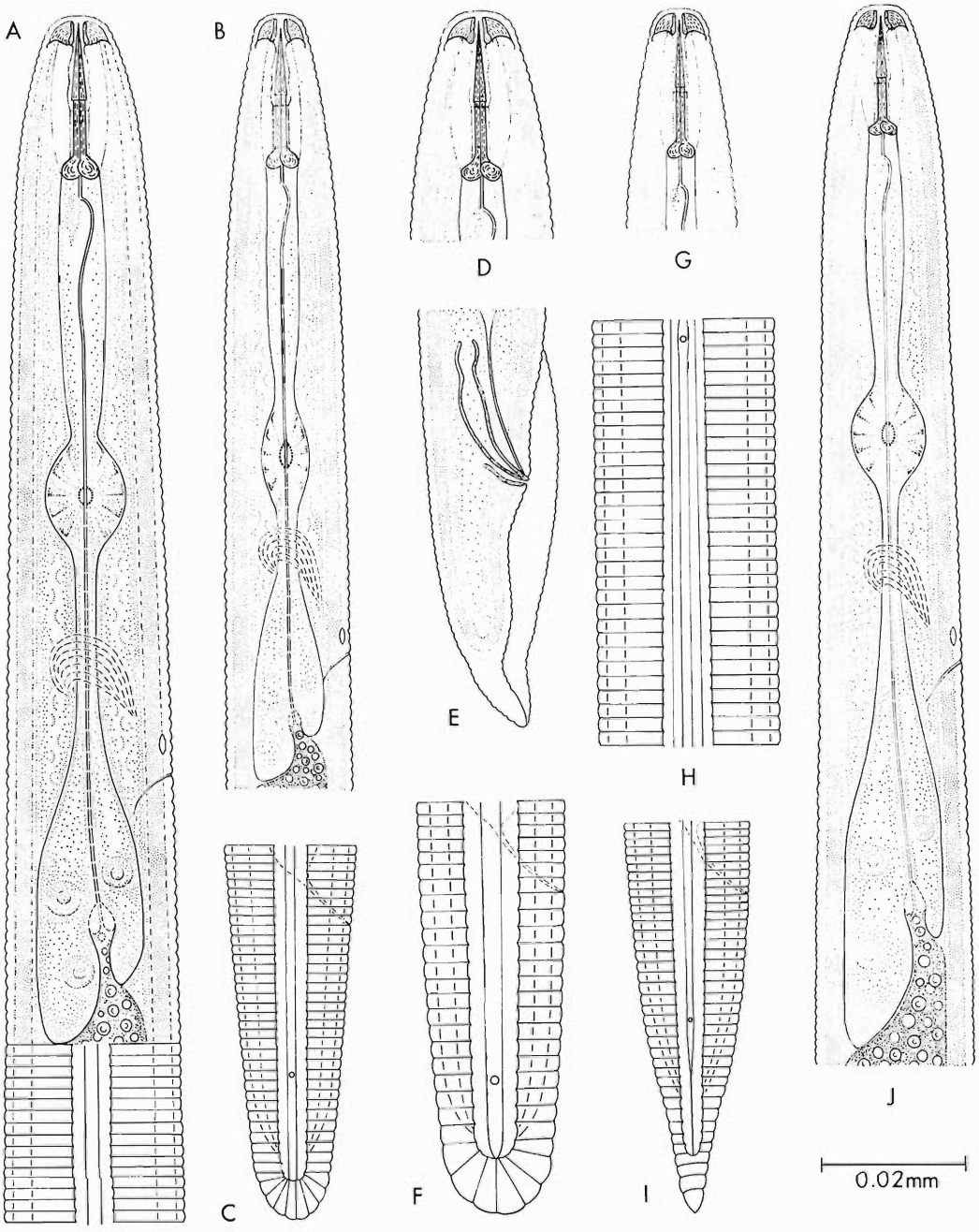


Figure 1. ***Pratylenchoides crenicauda.*** **A. Female, anterior region. B. Male, anterior region. C. Female, posterior region. E. Male, posterior region.** ***Pratylenchoides laticauda.*** **D. Female, stylet region. F. Female, posterior region. G. Male, stylet region. H. Female, surface view in region of esophageal glands.** ***Pratylenchoides maritimus.*** **I. Female, posterior region. J. Female, anterior region.**

of annules on the female tail, short esophageal overlap of the intestine, males with elongated, spindle-shaped sperm and the scarcity of males.

The description of *P. crenicauda* is based on topotypes and specimens collected from cherry soil, East Sutton, England in March 1962. Additional specimens have been identified from grass soil, Münster, Germany; grass soil, Mellissant and Leiderdorp, The Netherlands; grass soil, Budapest, Hungary; sandy soil, Vistula Riverbank, Wyszogrod, Poland; and meadow soil, Gulmarg, Srinagar, Kashmir, India. In all the populations studied, males were scarce or not present.

Pratylenchoides laticauda Braun and Loof, 1966 (Fig. 1D, F–H)

Pratylenchoides laticauda Braun and Loof, 1966, pp. 241–244.

MEASUREMENTS: 7 ♀ topotypes: L = 0.81 mm (0.76–0.83); a = 28 (26–29); b = 4.7 (4.1–5.3); b′ = 4.2 (3.8–4.5); c = 17 (14–18); V = 59 (58–61); stylet = 24 μ (23–24).

3 ♂ topotypes: L = 0.77 mm (0.75–0.80); a = 28 (24–32); b = 5.8 (5.3–6.2); b′ = 4.9 (4.7–5.0); c = 15 (14–17); gubernaculum = 9 μ (8–10); spicules = 26 μ (24–28); stylet = 21 μ (20–22).

FEMALE: Lip region slightly or not set off, three or four annules. Stylet knobs broadly rounded, sloping posteriorly. Esophageal glands with short overlap of intestine (less than one body width). Spermatheca rounded, with irregularly rounded sperm. Lateral canals conspicuous. Lateral field with six incisures. Tail with 18–26 annules, broadly rounded, coarse irregular stria at terminus.

MALE: Lip region narrower, higher and more conoid. Esophagus and esophageal glands not as well-developed as in female. Sperm irregularly rounded.

TYPE HOST AND LOCALITY: Roots of *Monarda mollis*, Huis ter Heide, The Netherlands.

DIAGNOSIS: *P. laticauda* can be distinguished from the type species by the female tail, which has fewer annules and a broader tail terminus.

A more complete description, illustrations and measurements of *P. laticauda* is given in the original description by Braun and Loof (1966).

Specimens of *P. laticauda* have been identified from the following habitats and localities: soil, Athens, Greece; and carob tree soil, Bari, Italy.

Pratylenchoides maritimus Bor and s'Jacob, 1966 (Fig. 1 I–J)

Pratylenchoides maritimus Bor and s'Jacob, 1966, pp. 463–465.

MEASUREMENTS: 5 ♀ paratypes: L = 0.54 mm (0.51–0.61); a = 24 (20–26); b = 4.3 (4.0–4.5); b′ = 3.5 (3.3–3.7); c = 13.2 (12–15); V = 59 (58–61); stylet = 17 μ (16–18).

5 ♂ ♂ (after Bor and s'Jacob, 1966): L = 0.39 mm (0.31–0.42); a = 25 (24–28); b = 3.9 (3.5–4.0); b′ = 3.4 (3.3–3.5); c = 9.8 (7.8–11.1); gubernaculum = 5 μ (4–5); spicules = 17 μ (16–17); stylet = 15 μ.

FEMALE: Lip region flattened anteriorly, not set off, two or three annules. Stylet knobs rounded, sloping posteriorly. Esophageal glands overlapping intestine about one body width. Spermatheca round with irregularly rounded sperm. Lateral canals present. Lateral field with four incisures. Tail with 28–36 annules, tapering to almost pointed terminus, often with coarse irregular striations at terminus.

MALE (after Bor and s'Jacob, 1966): Shorter and slenderer than female with shorter stylet (15 μ). Median bulb as strongly developed as female.

TYPE HOST AND LOCALITY: Soil around plant roots, Boschplaat, Island of Terschelling, The Netherlands.

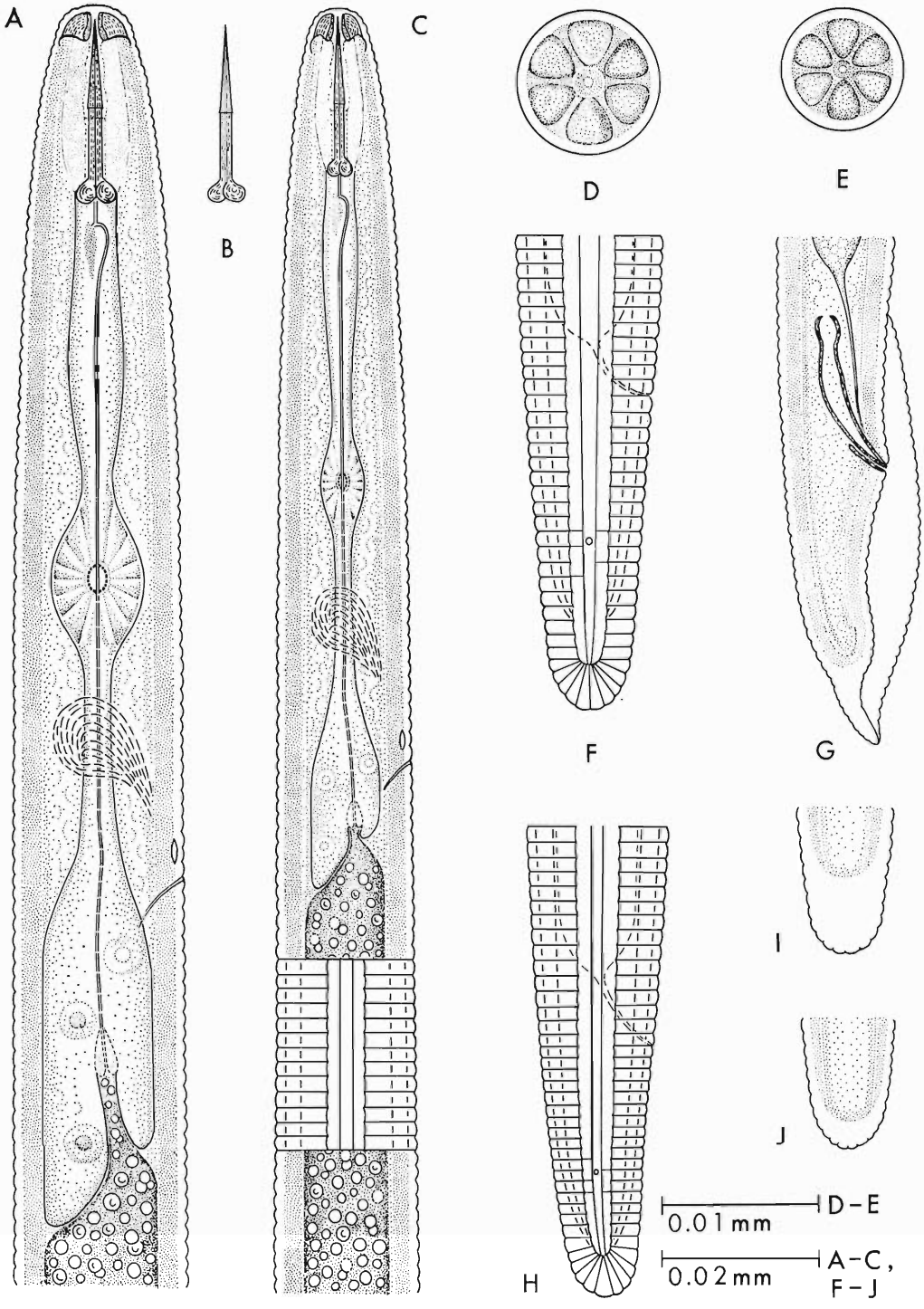
DIAGNOSIS: *P. maritimus* can be distinguished from the two preceding species by the short stylet, longer overlapping esophageal glands and the tapering, almost pointed, female tail.

A more complete description, illustrations and measurements of *P. maritimus* are given in the original description by Bor and s'Jacob (1966).

Specimens of *Pratylenchoides maritimus* (5 ♀ paratypes) have been received from the authors of this species and are deposited in the Nematode Collection of the Department of Nematology, University of California, Riverside.

Pratylenchoides variabilis sp. n. (Fig. 2)

MEASUREMENTS: 20 ♀ paratypes: L = 0.58 mm (0.50–0.66); a = 30 (26–32); b = 3.9 (3.5–



4.4); b′ = 3.4 (3.1–4.4); c = 16 (13–19); V = 58 (56–61); stylet = 22 μ (21–24).

10 ♂ paratypes: L = 0.53 mm (0.46–0.60); a = 29 (25–33); b = 5.2 (4.6–6.0); b′ = 4.4 (3.1–5.7); c = 13 (12–15); gubernaculum = 7 μ (6–9); spicules = 22 μ (20–25); stylet = 20 μ (19–21).

FEMALE (holotype): L = 0.58 mm; a = 29; b = 5.0; b′ = 4.4; c = 17; stylet = 21 μ; O = 10; m = 50; V = 55. Lip region not set off, three annules. Stylet knobs broadly rounded, sloping posteriorly. Esophageal glands overlap intestine one body width. Spermatheca round with irregularly rounded sperm. Lateral canals absent. Lateral field with four incisures, incompletely areolated on tail. Tail with 24 annules, terminus rounded with conspicuous irregular striae.

MALE (allotype): L = 0.53 mm; a = 29; b = 5.4; b′ = 4.5; c = 13; c′ = 2.7; stylet = 20 μ; gubernaculum = 7 μ; spicules = 22 μ. Lip region narrower, higher than holotype with four annules. Esophagus and esophageal glands smaller than holotype. Sperm irregularly rounded to elongate or spindle shaped.

HOLOTYPE: Female collected by S. A. Sher, 27 January 1962. U.C.R. Nematode Collection, Riverside, California.

ALLOTYPE: Male, same data as holotype.

PARATYPES: 54 ♀ ♀, 33 ♂ ♂, 4 juveniles same data as holotype distributed as follows: 5 ♀ ♀, 2 ♂, Department of Nematology, University of California, Davis; 39 ♀ ♀, 28 ♂ ♂, 4 juveniles, Department of Nematology, University of California, Riverside; 4 ♀ ♀, 2 ♂ ♂, USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland; and 6 ♀ ♀, 1 ♂ Plantenziektenkundige Dienst, Wageningen, The Netherlands.

TYPE HABITAT AND LOCALITY: Soil around unknown grass and weeds Twin Peaks, San Francisco, California.

DIAGNOSIS: *P. variabilis* can be distinguished from the closely related species *P. crenicauda* by fewer annules on the female tail, usually longer dorsal esophageal gland overlapping the intestine and smaller body size.

The paratypes have two to four annules on the lip region. The esophageal glands overlap the intestine slightly less than one body width to about one-and-a-half body widths. Some of the specimens are without sperm in the spermatheca. A few specimens have six lateral incisures, and in two specimens inconspicuous lateral canals were seen in the posterior part of the body. The female tail has 21–27 annules.

Additional specimens, identified as *P. variabilis*, have been collected from soil around unknown plants in Marin County, northern California.

Pratylenchoides leiocauda sp. n. (Fig. 3)

MEASUREMENTS: 15 ♀ paratypes: L = 0.60 mm (0.53–0.67); a = 31 (22–37); b= 4.8 (3.6–5.6); b′ = 3.8 (2.9–4.1); c = 19 (17–22); V = 59 (55–62); stylet = 20 μ (19–21).

11 ♂ paratypes: L = 0.57 mm (0.47–0.72); a = 30 (28–33); b = 5.5 (4.8–6.8); b′ = 5.0 (3.8–6.0); c = 14 (12–16); stylet = 17 μ (15–19); gubernaculum = 7 μ (6–8); spicules = 21 μ (18–23).

FEMALE (holotype): L = 0.54 mm; a = 31; b = 4.6; b′ = 3.9; c = 20; V = 57; stylet = 20 μ. Lip region flattened anteriorly, three annules. Stylet knobs broadly rounded, sloping slightly posteriorly. Esophageal glands overlapping intestine one and a third times the body width. Spermatheca round with irregularly rounded sperm. Lateral canals absent. Lateral field with six incisures to four incisures in posterior part of body. Tail with 19 annules, without striations around terminus.

MALE (allotype): L = 0.62 mm; a = 32; b = 6.2; b′ = 5.4; c = 17; stylet = 19 μ; gubernaculum = 8 μ; spicules = 22 μ. Lip region narrower and higher than holotype, hemispherical with four annules. Esophagus and esophageal glands not as well developed as holotype, esophageal nuclei not visible. Sperm in posterior part of testis elongated, spindle-shaped.

HOLOTYPE: Female collected by S. A. Sher, 23 October 1963, catalog number 11, U.C.R. Nematode Collection, Riverside, California.

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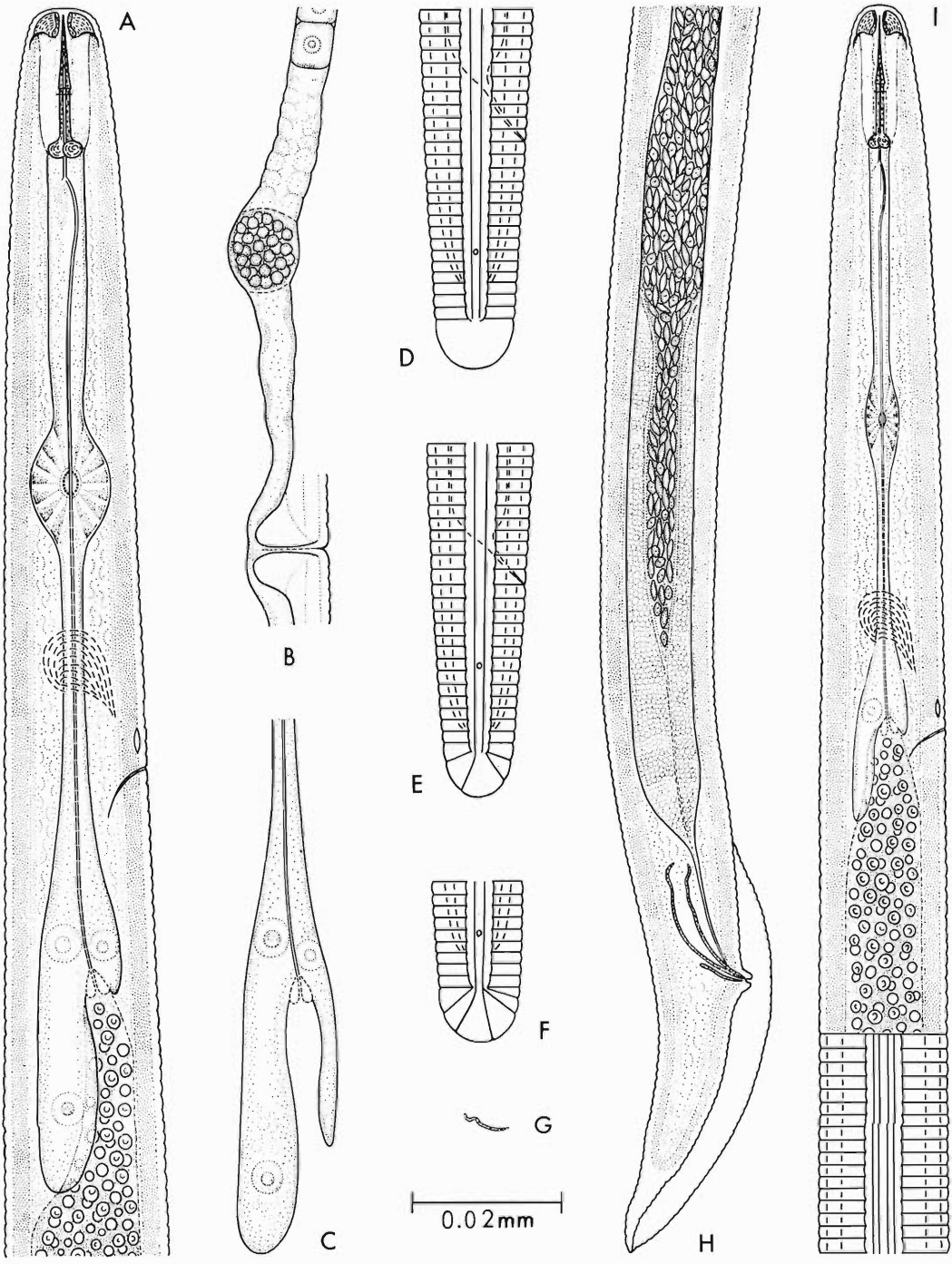
Figure 2. ***Pratylenchoides variabilis*** **sp. n. A. Female, anterior region. B. Female, stylet. C. Male, anterior region. D. Female, face view. E. Male, face view. F, H. Female, posterior region. G. Male, posterior region. I. Female, tail terminus. J. Juvenile, tail terminus.**



ALLOTYPE: Male, same data as holotype, catalog number 12.

PARATYPES: 45♀♀, 29♂♂, 6 juveniles distributed as follows: 6♀♀, 2♂♂, Department of Nematology, University of California, Davis; 27♀♀, 19♂♂, 6 juveniles, Department of Nematology, University of California, Riverside; 3♀♀, 4♂♂, USDA Nematode Collection, Nematode Investigations, Beltsville, Maryland; 3♀♀, 2♂♂, Nematology Department, Rothamsted Experimental Station, Harpenden, England; and 6♀♀, 2♂♂, Labatoire des Nematodes, Antibes, France.

TYPE HABITAT AND LOCALITY: Soil around unknown grass and weeds, St. Honorat Island, Antibes, France.

DIAGNOSIS: *Pratylenchoides leiocauda* can be distinguished from the closely related *P. laticauda* by the usual absence of striations around the female tail terminus, spindle-shaped sperm in the posterior part of the testis, smaller body size, usually shorter stylet, and absence of lateral canals.

The female lip region has three or four annules and the stylet knobs vary from broadly rounded to broadly rounded and sloping posteriorly. Ventral lobe of the esophageal gland short or elongated (Fig. 3C) and the esophageal glands overlap intestine one to two times the body width in the female. The lateral field usually has six incisures but some specimens have four. The female tail has 16–21 annules, and some specimens have one to three striations around the terminus (Fig. 3 E–F). The gubernaculum sometimes has the distal portion bent (Fig. 3G).

Pratylenchoides ritteri sp. n. (Fig. 4)

MEASUREMENTS: 14♀ paratypes: L = 0.78 mm (0.65–0.93); a = 29 (25–31); b = 5.6 (4.8–6.7); b′ = 4.0 (3.1–5.5); c = 16 (13–17); V = 57 (54–61); stylet = 23 μ (21–24).

10♂ paratypes: L = 0.71 mm (0.61–0.90); a = 31 (28–35); b = 7.1 (5.4–8.6); b′ = 5.7 (4.8–6.2); c = 14 (12–15); stylet = 20 μ (18–21); gubernaculum = 7 μ (6–9); spicules = 26 μ (23–28).

FEMALE (holotype): L = 0.88 mm; a = 30; b = 5.8; b′ = 3.9; c = 17; V = 56; stylet = 23 μ. Lip region flattened anteriorly, four annules. Stylet knobs broad, flattened anteriorly. Esophageal glands conspicuous, almost filling body cavity with the posterior glands granular in texture, overlapping intestine two body widths. Spermatheca round with round sperm. Lateral canals present. Lateral field with four incisures, incompletely areolated on tail. Tail with 24 annules, coarse striations around terminus.

MALE (allotype): L = 0.90 mm; a = 30; b = 6.0; b′ = 4.9; c = 14; stylet = 21 μ; gubernaculum = 7 μ; spicules = 26 μ. Lip region narrower and higher, basal plate extending further into the body than in holotype. Esophagus and esophageal glands not as well-developed as in holotype, esophageal nuclei not visible. Caudal alae narrow.

HOLOTYPE: Female collected by S. A. Sher, 12 October 1963, catalog number 13, U.C.R. Nematode Collection, Riverside, California.

ALLOTYPE: Male, same data as holotype, catalog number 14.

PARATYPES: 119♀♀, 99♂♂, 9 juveniles, same data as holotype distributed as follows: 4♀♀, 5♂♂, Department of Nematology, University of California, Davis; 102♀♀, 84♂♂, 9 juveniles, Department of Nematology, University of California, Riverside; 4♀♀, 4♂♂, USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland; 3♀♀, 4♂♂, Nematology Department, Rothamsted Experimental Station, Harpenden, England; 3♀♀, 4♂♂, Plantenziektenkundige Dienst, Wageningen, The Netherlands; and 6♀♀, 2♂♂, Labatoire des Nematodes, Antibes, France.

TYPE HABITAT AND LOCALITY: Soil around unknown grass and weeds, Cape Antibes, France.

DIAGNOSIS: *Pratylenchoides ritteri* can be distinguished from the closely related species *P. laticauda* and *P. variabilis* by the larger esophageal glands, and the longer overlap of the intestine and the usually more flattened anterior surface of the female stylet knobs.

The female lip region has three or four annules; stylet knobs are broadly rounded to flat-

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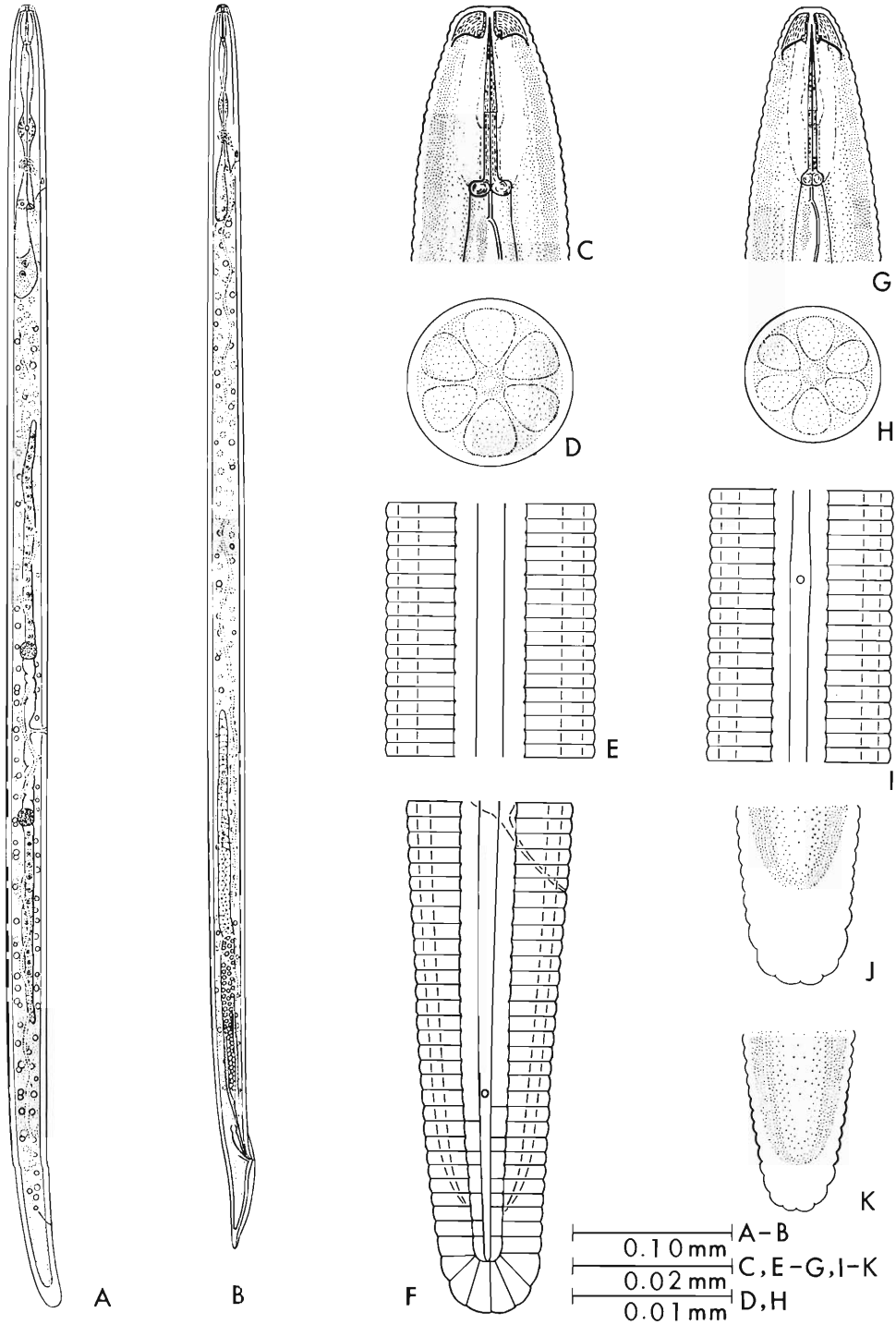
Figure 3. ***Pratylenchoides leiocauda*** **sp. n. A. Female, anterior region. B. Female, anterior ovary in region of spermatheca. C. Female, esophageal glands. D–F. Female, posterior regions. G. Male, gubernaculum. H. Male, posterior region. I. Male, anterior region.**



tened anteriorly, and slightly sloping posteriorly. The female esophageal glands overlap the intestine two- to three-and-a-half times the body width, the male esophageal glands overlap the intestine about one body width (0.8–1.2). The lateral field usually has four incisures, a few specimens have six incisures. The female tail has 21–28 annules. The sperm in the posterior portion of the testis is irregularly rounded to angular in shape.

Additional specimens collected by the author and identified as *P. ritteri* are from the following habitats and localities in southern France: unknown grass and weed soil, Digne, and sandy soil around pine trees, Juan-Les-Pins.

Pratylenchoides bacilisemenus sp. n. (Fig. 5)

MEASUREMENTS: 11 ♀ paratypes: L = 0.66 mm (0.60–0.74); a = 30 (26–34); b = 4.7 (4.3–5.1); b′ = 3.7 (3.5–4.1); c = 16 (15–18); V = 58 (55–61); stylet = 22 μ (21–23).

11 ♂ paratypes: L = 0.62 mm (0.55–0.67); a = 30 (26–33); b = 5.5 (5.1–6.1); b′ = 4.8 (4.3–5.1); c = 16 (14–19); stylet = 21 μ (20–22); gubernaculum = 7 μ (6–8); spicules = 25 μ (22–27).

FEMALE (holotype): L = 0.66 mm; a = 27; b = 4.5; b′ = 3.7; c = 15; V = 57; stylet = 22 μ. Lip region slightly rounded, three annules. Stylet knobs rounded, sloping posteriorly. Esophageal glands enlarged, overlapping intestine one-and-a-half times the body width. Spermatheca round with rod-like sperm. Lateral canals present. Lateral field with four incisures. Tail with 21 annules, tapering to striated terminus.

MALE (allotype): L = 0.57 mm; a = 31; b = 5.0; b′ = 4.7; c = 15; stylet = 21 μ; gubernaculum = 6 μ; spicules = 26 μ. Lip region slightly narrower than holotype, hemispherical, four annules. Esophagus and esophageal glands not as well-developed as in female, overlapping intestine less than one body width, nuclei inconspicuous.

HOLOTYPE: Female, collected by S. A. Sher, 6 January 1963, catalog number 15, U.C.R. Nematode Collection, Riverside, California.

ALLOTYPE: Male, same data as holotype, catalog number 14.

PARATYPES: 26 ♀ ♀, 66 ♂ ♂, 8 juveniles, same data as holotype distributed as follows: 1 ♀ ♀, 6 ♂ ♂, 1 juvenile, Department of Nematology, University of California, Davis; 21 ♀ ♀, 48 ♂ ♂, 5 juveniles, Department of Nematology, Riverside, California; 2 ♀ ♀, 3 ♂ ♂, 2 juveniles, USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland; 1 ♀, 6 ♂ ♂, Nematology Department, Rothamsted Experimental Station, Harpenden, England; and 1 ♀, 3 ♂ ♂, Plantenziektenkundige Dienst, Wageningen, The Netherlands.

TYPE HABITAT AND LOCALITY: Soil around unknown grass, Twin Peaks, San Francisco, California.

DIAGNOSIS: *P. bacilisemenus* can be distinguished from the closely related species *P. ritteri* and *P. variabilis* by the rod-shaped sperm found in the testis and spermatheca. It can be further distinguished from *P. ritteri* by the rounder lip region shape and the usually smaller body size, and from *P. variabilis* by the longer overlap of the esophageal glands.

The paratypes have three or four annules on the lip region. The female esophageal glands overlap the intestine one-and-a-third to two times the body width. Some of the females have inconspicuous spermatheca without sperm. The female tail has 18 to 23 annules.

Additional specimens of *P. bacilisemenus* have been collected by the author near the type locality (Twin Peaks, San Francisco), on three separate occasions, from soil around wild strawberry and grass.

Pratylenchoides specimens, probably representing additional speciation, have been examined from the following locations: Toronto, South Dakota; Newport, Oregon; Tok and Delta, Alaska; Sevier County, Utah; Hammond, Louisiana; Taos, New Mexico; Santa Barbara, Fillmore, Victorville, Desert Springs, Pear Blossom, Orange County and San Diego County in southern California; Mikve and Givat Rambam,

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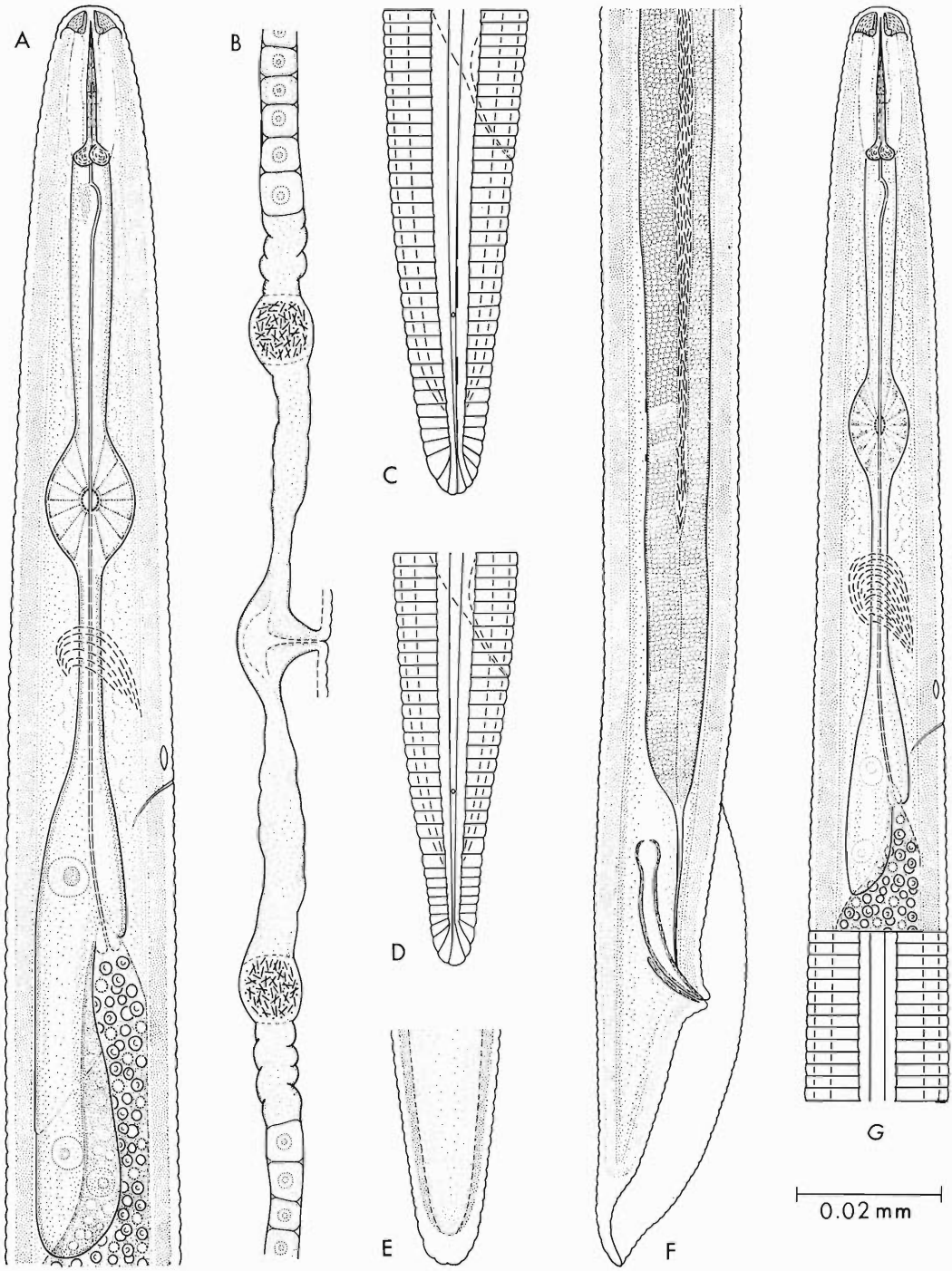
Figure 4. ***Pratylenchoides ritteri*** **sp. n. A. Female. B. Male. C. Female, stylet region. D. Female, face view. E. Female, surface at center of body. F. Female, posterior region. G. Male, stylet region. H. Male, face view. I. Female, surface view in region of deirid. J. Female, tail terminus. K. Juvenile, tail terminus.**



Israel; East Taieri, New Zealand; and Omase, Nara Prefecture, Japan. These are not described in this paper because of insufficient or poorly preserved specimens, or both.

Discussion

After an examination of the type specimens, the author agrees with Braun and Loof (1966) that *Zygotylenchus toamasinae* (de Guiran, 1964), *Zygotylenchus guevarai* (Tobar, 1963) and *Tylenchorhynchus gadeai* (Arias, Jiménez and López, 1965) do not belong in the genus *Pratylenchoides*.

The genus *Pratylenchoides* is most closely related to the genus *Radopholus*, and the species of *Pratylenchoides* with elongated esophageal glands overlapping the intestine (*P. ritteri, P. leiocauda* and *P. bacilisemenus*) are especially similar in appearance to species of *Radopholus*. *P. bacilisemenus* also has rod-like sperm, which occur in most species of *Radopholus*. The migratory endoparasitic root habitat of *Pratylenchoides* is the same as that of species of *Radopholus*, although observations have not been made on all the species and one, *P. laticauda*, has been shown to be a pathogen of plants (Hijink and van Rossen, 1968).

Pratylenchoides differs from the genus *Radopholus* in having deirids; less reduction of the stylet and esophagus in the male; usually broadly rounded stylet knobs, sloping distally; a more variable type of overlapping esophageal glands with at least one esophageal nucleus above the esophageal intestinal valve; gubernaculum not protruding from the cloaca; and usually a lower position of the phasmids on the tail.

The genus *Pratylenchoides* appears to be almost worldwide in distribution with no known area of large speciation, whereas the genus *Radopholus* is apparently native to Australia (Sher, 1968) where it has many species. In addition, the one widely distributed species of *Radopholus*, the very important plant pest *R. similis* (Cobb, 1893), is usually found in the warmer areas of the world. The genus *Pratylenchoides* is in the cooler, more temperate areas and is not known to have any important, widely distributed plant pathogenic species, although the type species, *P. crenicauda*, has been identified from widely separated areas of the world.

Acknowledgments

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Figure. 5. ***Pratylenchoides bacilisemenus*** **sp. n. A. Female, anterior region. B. Female, central portion of ovaries. C–D. Female, posterior regions. E. Juvenile, tail terminus. F. Male, posterior region. G. Male, anterior region.**

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Development, Migration, and Survival on Pasture of Gastrointestinal Nematodes of Cattle: Summer Contamination

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ABSTRACT: Development, migration, and survival of infective larvae were studied following deposition of fecal pats containing eggs of *Ostertagia ostertagi*, *Cooperia punctata* or *Oesophagostomum radiatum* on pasture at Beltsville, Maryland, at the beginning of summer (24 June 1968). Initial and peak recovery of larvae from the pats occurred 0.5–1, and 1–2 weeks later, respectively. Final recovery of larvae from them occurred 8 weeks after deposition for *O. radiatum* and 9.5 weeks for *C. punctata* and *O. ostertagi*. Initial recovery of larvae from the herbage occurred at 2 weeks for all species; peak recovery, at 2 (*O. radiatum*), 3 (*C. punctata*), or 4 (*O. ostertagi*) weeks; and final recovery, at 8 (*O. radiatum*), 20.6 (*C. punctata*), or 24 (*O. ostertagi*) weeks. The percentage of development for *O. ostertagi* was considerably higher than that of the other species, but its migration onto the herbage was not as great as it was in previous trials begun in May. The high temperatures and high evaporation rates of summer were probably responsible for the death of most larvae (97% of the peak recovery) by 1 September 1968. Eighty-five to 88% of the larvae recovered, per unit weight of herbage, was within 13 cm of the pats. This finding is an important reason for not overstocking pastures because, under normal stocking, cattle tend to avoid the forage around feces.

The most recent reviews of some of the factors affecting development and survival of the free-living stages of parasites of ruminants on pasture are given by Levine (1963), Crofton (1963), and Kates (1965).

Development and survival of gastrointestinal nematode parasites of cattle on pasture at Beltsville, Maryland, were reported by Goldberg (1968). In that investigation, egg-containing fecal pats were deposited on clean pasture at various times of the year, and at intervals, the pats and surrounding herbage were examined for infective larvae. The trial begun in summer involved only *Haemonchus contortus*. The present study concerns development and survival of *Ostertagia ostertagi*, *Cooperia punctata*, and *Oesophagostomum radiatum* in summer. Development of these parasites in late spring, and survival in summer, had been studied in the trial begun 2 June 1961. In other trials begun in summer (Goldberg and Rubin, 1956), spring, and autumn (Goldberg and Lucker, 1959 and 1963), calves were used to test the

Table 1. Average weather for Beltsville, Maryland, during 1951–1960 and deviations therefrom during the critical period of the study.

	Temperature (C)		Precipitation (cm.)		Evaporation (cm.)	
	Av. 1951–1960	Deviation 1968	Av. 1951–1960	Deviation 1968	Av. 1951–1960	Deviation 1968
June	21.2	−0.2	11.1	3.1	16.7	1.8
July	24.2	−0.1	9.9	4.4	19.0	3.0
Aug.	23.1	1.2	12.5	−6.7	15.8	1.0

Table 2. Larvae recovered from the standard cultures and peak recovery from the fecal pats and herbage in percentage of the recovery from the standard cultures.

	Ostertagia ostertagi	*Cooperia punctata*	*Oesophagostomum radiatum*
Av. no. larvae from standard cultures	31,525	16,900	10,075
% from pats	36	1	5
% from herbage	3	0.3	0.3

development and survival of larvae of the above-mentioned parasites.

The present study also concerns lateral migration of the larvae after development under nearly natural conditions. There have been few studies of lateral migration of larvae, and to the author's knowledge all involve larvae cultured in the laboratory (Dinaburg, 1944; Furman, 1944; Tarshis, 1958), or developed outside under artificial conditions (Rose, 1963). Such studies are not strictly comparable with the present one because the larvae in question may have had greater vitality than most larvae developing under nearly natural conditions. In the case of the first three authors mentioned above, the larvae were developed under more or less ideal conditions by being well aerated and sheltered from desiccation, strong sunlight, and numerous kinds of organisms of the natural environment. In addition, migration was favored because the larvae were suspended in water when deposited on the herbage or ground. Water is necessary for migration, but it is often not available to larvae under natural conditions before they have been adversely affected by the environment. Rose did not mix the feces with a culture medium, but the developing larvae were sheltered.

Materials and Methods

The procedure followed was essentially as described previously (Goldberg, 1968). Single batches of feces containing *O. ostertagi*, *C. punctata* or *O. radiatum* eggs were collected, and the following day (24 June 1968) they were mixed separately, and 300-g pats having the consistency of normal cattle feces were prepared. The pats were nearly hemispherical and approximately 11 cm in diameter and 5 cm high. Two pats from each batch were cultured in the laboratory in sphagnum with amounts of water considered to be close to optimum for development, and at average temperatures (approximately 24 C) slightly above optimum (21 C) (Goldberg, 1968). Two weeks later, the larvae were recovered from these cultures, hereinafter referred to as "standard cultures" since the number of larvae recovered from them provided an index of the potential for production of infective larvae from the eggs in the pats. The remaining pats were placed on permanent grass pasture so that they would be exposed to nearly natural conditions. The pats were spaced 1 meter apart on a clean, level area that had not been grazed by domestic animals for at least 2 years. The grass was 10 cm high at the time the pats were deposited and had grown to 19 cm one month later, and to 43 cm in 2 months, the last-mentioned height mainly reflecting the length of the slender grass spikes. The weight of the herbage per unit area had doubled in 1 month, and had increased to 2.5 times the original weight in 2 months.

Semiweekly for the first week and weekly thereafter through the larval developmental period and to the time few larvae still survived, and at longer intervals thereafter, pats and the surrounding herbage were brought into the laboratory and examined for infective larvae, using the Baermann apparatus to recover them. The pats were also examined for eggs until none remained. The herbage was clipped between 0 and 2.5 cm of the ground. The latter limit was considered adequate for this study because cattle do not normally graze below it. The herbage within a radius of 13 cm from the pat was weighed and examined separately from that 13 to 25 cm from the pat.

The data on development and persistence of larvae in the pats, and infectiousness of the herbage were examined in relation to the

Table 3. Interval in weeks between the deposition of fecal pats on pasture, 24 June 1968, and initial, peak, and final recovery of infective larvae.

	Ostertagia ostertagi			*Cooperia punctata*			*Oesophagostomum radiatum*		
Recovery	First	Peak	Last	First	Peak	Last	First	Peak	Last
From pats	1	2	9.5	0.5	1	9.5	1	1	8
From herbage	2	4	24	2	3	20.6	2	2	8

weather. Monthly precipitation and evaporation, and average monthly temperatures at the Agricultural Research Center, Beltsville, Maryland, during the present study and for 1951–60 were obtained from U. S. Weather Bureau records. The 1951–60 averages and deviations therefrom during the larval developmental period and to the time few larvae still survived are given in Table 1. During the period of larval development, June and July, precipitation was somewhat above normal. During August, the temperature was above normal and precipitation below normal. Evaporation was above normal in all 3 months.

Results and Discussion

Within 1 week of exposure on pasture, eggs were no longer recovered from the feces; all had either hatched or been destroyed. The peak of development of infective larvae occurred during June, when the average temperature was 21.0 C, precipitation 14.2 cm, and evaporation 18.5 cm. Peak infectiousness of the herbage occurred in July, when the average temperature was 24.1 C, precipitation 14.3 cm, and evaporation 22.0 cm. Larvae were no longer recovered by the 11th pat and 16th herbage collections.

The average numbers of larvae recovered from the standard cultures and the peak recovery from the herbage and fecal pats, expressed as a percentage of the number recovered from the standard cultures are given in Table 2. Few infective *C. punctata* and *O. radiatum* larvae were produced in the pats, and few of any species succeeded in migrating onto the herbage. The percentage of development for *O. ostertagi* was considerably higher than that of the other species, but its migration onto the herbage was not as great as it was in previous trials begun in May.

The interval in weeks between deposition of the fecal pats on pasture and the initial, peak, and final recoveries of infective larvae of each species from the pats and herbage is given in Table 3. The interval between the peak numbers of larvae in the feces and on the herbage was 2 weeks for *O. ostertagi* and *C. punctata*, and 1 week for *O. radiatum*. Recovery of *O. ostertagi* from the herbage exceeded 1% of the recovery from the standard cultures for about 3 weeks; recovery of *C. punctata* and *O. radiatum* exceeded 0.2% for about 11 and 5 days, respectively. The high temperatures and high evaporation rates of summer were probably responsible for the death of most larvae (97% of the peak recovery) by 1 September. At that time, recovery of each species was less than 0.1% of the recovery from the standard cultures.

There was little lateral migration of larvae. Eighty-five to 88% of the larvae recovered, per

Table 4. Comparison of recovery of larvae between 0–13 and 13–25 cm from the fecal pats.

	Period	Distance from pats (cm.)	Larvae recovered (No.)	Wt. of herbage (gm.)	Larvae/kg. of herbage	% of larvae/kg. of herbage
Ostertagia ostertagi	7-18-68 to 12-9-68	0 to 13	2,153	1,903	1,131	85
		13 to 25	591	2,995	197	15
Cooperia punctata	7-18-68 to 11-15-68	0 to 13	113	1,791	63.1	87
		13 to 25	22	2,373	9.3	13
Oesophagostomum radiatum	7-18-68 to 8-19-68	0 to 13	34	1,064	32.0	88
		13 to 25	6	1,391	4.3	12

unit weight of herbage, was within 13 cm of the pats (Table 4). This finding is an important reason for avoiding overstocking of pastures because, under normal stocking, cattle tend to avoid the forage around feces. Dryness of the environment may have been responsible for poor lateral migration. By their own efforts, the larvae can only migrate in a film of water.

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A New Species of *Gonocerca* Manter, 1925 (Trematoda: Hemiuridae) from the Eastern Pacific

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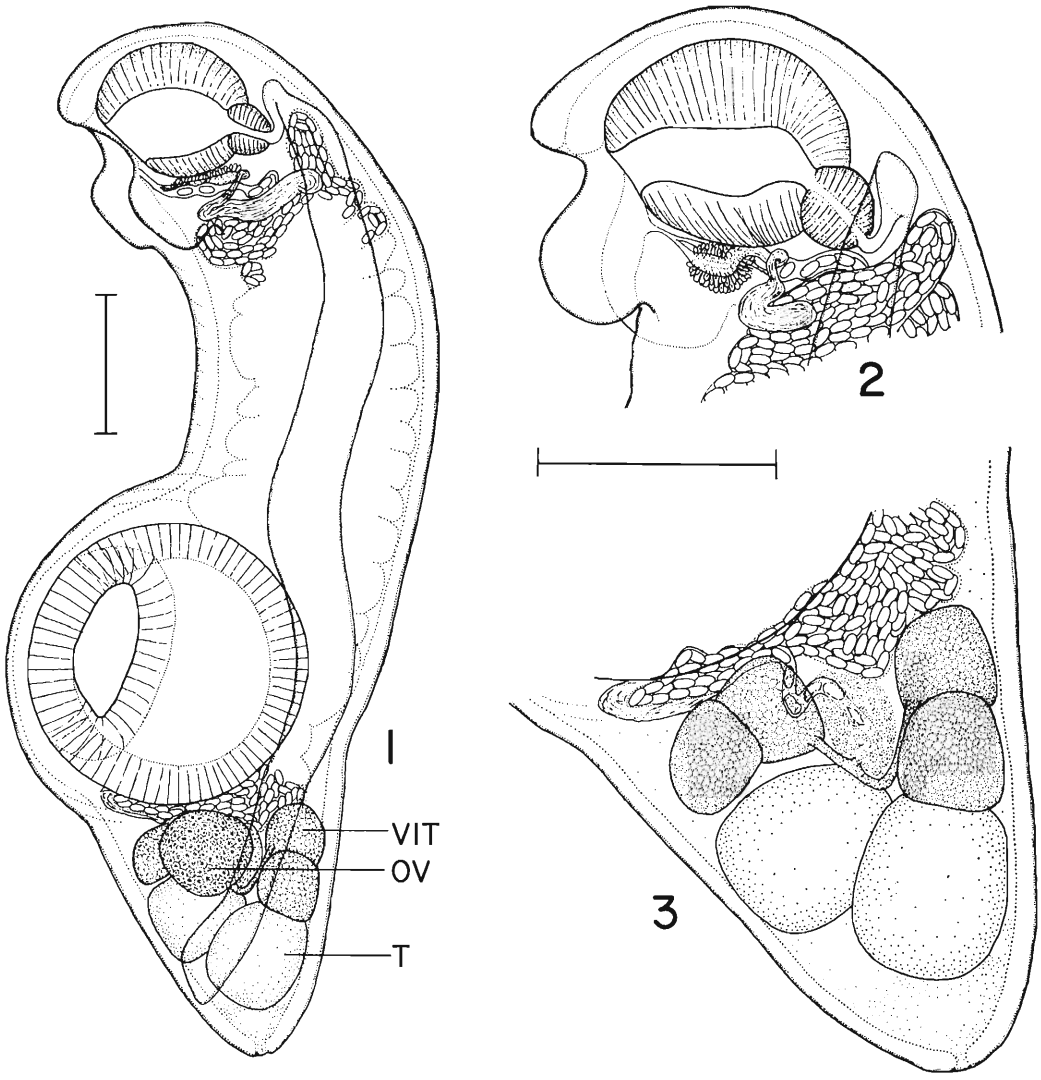
ABSTRACT: *Gonocerca oregonensis* sp. n., distinguished from all previously described *Gonocerca* by possessing fleshy lobes on either side of the oral sucker, was collected from depths of 1,530–2,850 meters off Oregon, USA. Fish hosts include *Chalinura filifera, C. pectoralis, Parabassogigas grandis,* and unidentified macrourids.

A new species of *Gonocerca* Manter, 1925, is the first representative of the genus from the Eastern Pacific. Twelve specimens were removed from the stomachs of several species of deep-water fish collected off Oregon. The fish hosts, captured with a deep-sea otter trawl, were preserved in 10% formalin by the method described by Eagle and McCauley (1965), and were not examined for their parasites until some months later. The parasites were stained with Mayer's Carmalum or Van Cleave's combination stain, and mounted for study.

Egg measurements are in microns while others are in millimeters. Measurements for the holotype are followed by the range in parentheses.

We acknowledge the support of the National Science Foundation (Grant GB-5136). Mr. Rodney J. Eagle prepared the slides and identified the hosts.



Figures 1–3. ***Gonocerca oregonensis*** **sp. n. 1. Whole mount of holotype from unidentified macrourid. 2. Anterior end of paratype from unidentified macrourid. 3. Posterior end of holotype, ovary and ceca excluded. All scales are 0.5 mm long. All drawings made with a** ***camera lucida.*** **(OV—ovary, T—testis, VIT—vitellaria).**

Gonocerca oregonensis sp. n. (Figs. 1–3)

HOSTS: *Chalinura filifera* Gilbert, 1896 (1 of 16); *C. pectoralis* (Gilbert, 1891) (1 of 5); *Parabassogigas grandis* (Günther, 1877) (1 of 7); and unidentified macrourids (6 of 57).

HABITAT: Stomach.

LOCATION: Pacific Ocean 90–155 km off Oregon in depths of 1,530 to 2,850 meters.

HOLOTYPE: USNM Helm. Coll. No. 70802.

DIAGNOSIS (based on 12 specimens, 9 measured. Measurements are length by depth): Hemiuridae, *Gonocerca.* Body subcylindrical anteriorly, fusiform posteriorly, robust, without

tail, 3.88 (1.33–4.90) by 1.42 (0.49–1.68). Oral sucker subterminal, posteroventrally directed, 0.52 (0.2–0.56) by 0.53 (0.22–0.55); surmounted by short preoral lip. Forebody bearing paired fleshy lobes posteroventral to oral sucker. Pharynx rounded, partly overlapping oral sucker, 0.18 (0.09–0.18) by 0.17 (0.09–0.21); prepharynx absent; esophagus short; ceca mildly inflated, extending to posterior extremity. Acetabulum postequatorial, subspherical, 1.07 (0.40–1.20) in diameter; sucker ratio 1:2.28 (1.95–2.54).

Testes diagonal, at posterior extremity, anterior testis 0.38 (0.16–0.41) by 0.39 (0.14–0.52), posterior testis 0.39 (0.15–0.41) by 0.34 (0.11–0.43). Seminal vesicle tubular, posteroventral to pharynx, posterior portion slightly inflated. Prostatic vesicle short, surrounded with prostate gland cells, touching oral sucker. Genital sinus short. Genital pore median, posterior to ventral margin of oral sucker, between two oral lobes.

Ovary between anterior testis and acetabulum, overlapping former and almost touching latter, subspherical, 0.33 (0.14–0.42) by 0.33 (0.14–0.41). Vitellaria divided into two compact masses, bi-lobed (unlobed to 4-lobed in some paratypes), lying dorsolateral to ovary. Vitelline ducts short. Seminal receptacle small, tubular, lying ventral to ovary and acetabulum. Uterus without descending limb, winding forward between ovary and oral sucker, filling all available space; terminating in weakly developed metraterm that enters genital sinus; uterus filled with small eggs 53–64 by 20–30.

Excretory pore terminal, arms uniting dorsal to oral sucker.

Discussion: Seven species have now been placed in the widely distributed genus *Gonocerca: G. phycidis* Manter, 1925 from Maine, Florida, and New Zealand; *G. crassa* Manter, 1934, from Florida, Japan, and Iceland; *G. kobayashi* (Layman, 1930) Manter, 1934, from the Sea of Japan; *G. macroformis* Wolfgang and Myers, 1954, from Newfoundland; and *G. lobata* Byrd, 1963, and *G. trematomus* Byrd, 1963, from Antarctica. The finding of *G. oregonensis* in deep water off Oregon supports Manter's (1954) conclusion that the genus is widespread in cold waters. Our new species differs from the above species in possessing the paired fleshy oral lobes and in possessing proportionately larger suckers. Although the vitellaria are bilobed in the holotype, there is considerable variation within the species. In two cases the left vitellarium was 4 lobed and in most other cases both vitellaria were entire.

Although the seminal receptacle is generally thought to be absent in this genus (Manter, 1934; Rees, 1953), Yamaguti (1938) stated that it is small and formed by a partial dilatation of the Laurer's canal in *G. crassa*, and subsequently included this information in his generic diagnosis (Yamaguti, 1958). The seminal receptacle is quite apparent in our specimens but we could not observe the Laurer's canal.

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Neoechinorhynchus chelonos, a New Species of Acanthocephalan Parasite of Turtles[1]

GERALD D. SCHMIDT,[2] GERALD W. ESCH,[3] J. WHITFIELD GIBBONS[4]

ABSTRACT: *Neoechinorhynchus chelonos* sp. n. is described from females from *Pseudemys scripta* in South Carolina. It is most similar to *N. magnapapillatus* Johnson, 1969, differing from it in the shape of the egg and having a longer terminal papilla. *Neoechinorhynchus constrictus* Little and Hopkins, 1968, is probably a junior synonym of *N. pseudemydis* Cable and Hopp, 1954.

Large numbers of acanthocephalans were recovered in conjunction with studies on the yellow-bellied turtle, *Pseudemys s. scripta*, on the U. S. Atomic Energy Commission's Savannah River Plant near Aiken, South Carolina. A description of the habitat is given by Clark and Gibbons (1969). A new species of *Neoechinorhynchus* Hamann, 1892, was recovered from a turtle sample collected at Gus' Pond in Aiken County, South Carolina. The specimens were relaxed in tap water, fixed in AFA, and stained with Semichon's carmine for study. All measurements are in microns unless otherwise stated.

Neoechinorhynchus chelonos sp. n. (Figs. 1–4)

Description

Trunk (Fig. 1) long, slender, curved ventrally. Giant hypodermal nuclei arranged five dorsal (occasionally lateral), one ventral. Proboscis spheroid, neck short. Right lemniscus binucleate, slightly longer than uninucleate left one. Dorso-ventral lacunar canals conspicuous.

FEMALE (10 gravid specimens): 17.0–30.0 mm long, 520–600 maximum width near anterior end. Proboscis (Fig. 2) 120–165 long, 180–200 wide. Proboscis hooks in three circles of six each. Hooks in anterior circle powerful, with well-developed roots and each with long anterior manubrium. Lateral hooks 70–90 long with manubrium about 35 long; others in anterior circle 60–80 long with manubrium about 10 long. Hooks of middle circle similar, spiniform with small roots and each with long, slender, anteriorly-directed manubrium; hooks 48–55 long, manubrium about 32 long. Hooks of basal circle similar, spiniform, with very small roots and no anterior manubrium; 44–48 long. Neck 50–90 long, 160–200 greatest width. Right lemniscus 1.0–1.2 mm long, left lemniscus 0.8–1.0 mm long. Posterior end (Fig. 3) slightly expanded bilaterally, with dorso-posterior papilla 200–320 in length. Pseudocoel extends into papilla. Mouth of uterine bell 650–920 from ventro-subterminal genital pore.

Mature eggs (Fig. 4) (measured in utero) 46–50 long, 20–22 greatest width. Outer membrane with equatorial constriction and ornamentation, as follows: ends rounded, with rod-like thickenings extending to middle membrane; sides with longitudinal thickenings each extending its entire length to middle membrane. Middle and inner membranes very thin, closely adhering to acanthor. Acanthor 30–36 long, 15–18 wide, with diffuse medullary nuclei and extremely minute spines.

MALE: unknown.

HOST: Yellow-bellied turtle, *Pseudemys s. scripta* (Schoepff), (Chelonia).

LOCATION: small intestine.

LOCALITY: Aiken County, South Carolina, USA.

TYPE SPECIMENS: USNM Helm. Coll. holo-

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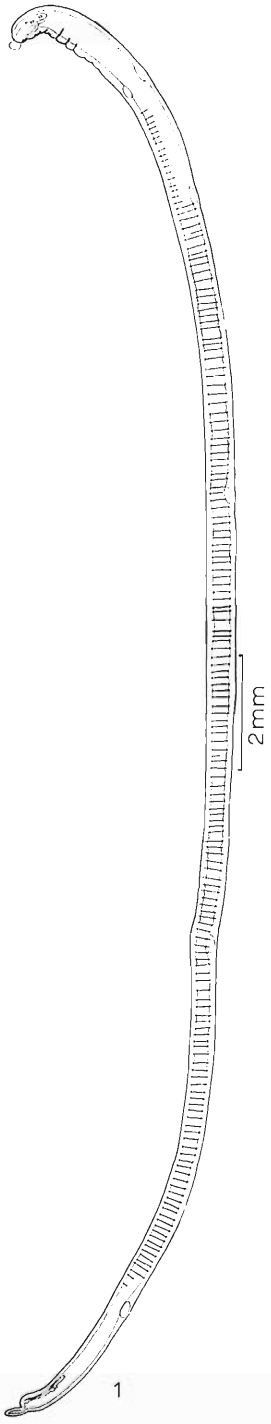
Figures 1–4. ***Neoechinorhynchus chelonos*** **sp. n. from turtles in South Carolina. 1. Holotype female, lateral view, eggs omitted. 2. Proboscis, lateral view. 3. Posterior end of holotype female, lateral view. 4. Mature egg, longitudinal and end views.**



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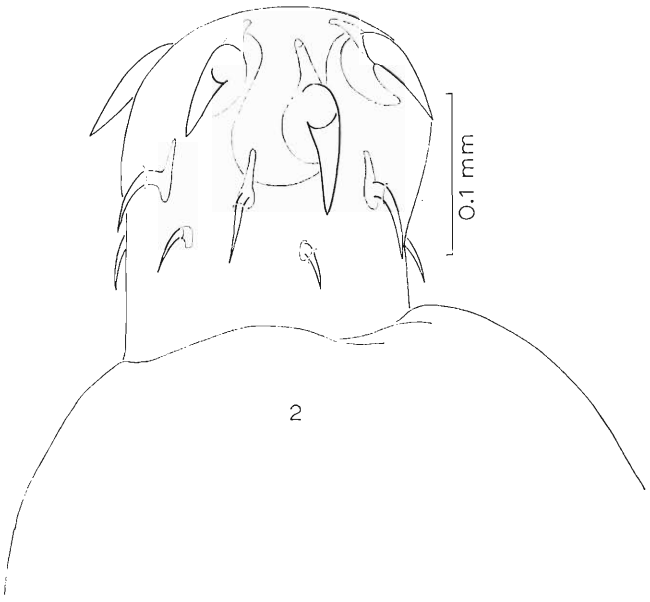

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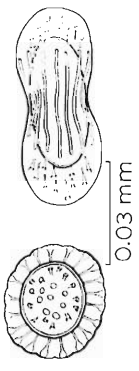

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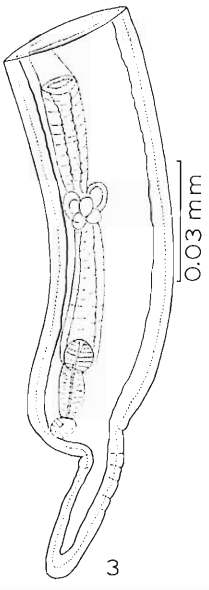

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type female No. 70708; paratype females No. 70709.

Remarks

Neoechinorhynchus chelonos sp. n. is morphologically most similar to *N. magnapapillatus* Johnson, 1969, in the possession of a very long papilla on the posterior end, and in proboscis armature. The terminal papilla of *N. magnapapillatus* (also a parasite of *P. scripta* in North Carolina) is reported as 106–205 long while it measures 200–320 long in our species. More importantly, the egg of *N. chelonos* is quite different in structure from that of *N. magnapapillatus* and indeed from any acanthocephalan egg so far described. It is noteworthy that the egg membranes of *Neoechinorhynchus* spp. from turtles are extremely variable, while these membranes are remarkably uniform in those species from fishes.

All of our specimens were collected in association with *Neoechinorhynchus pseudemydis* Cable and Hopp, 1954. No taxonomic characters could be found to separate the male worms into two species so, like Johnson (1969), we must defer the description of the male until an infection by the single species is discovered or until the life cycle has been experimentally determined.

Neoechinorhynchus constrictus Little and Hopkins, 1968, seems very close to *N. pseudemydis* Cable and Hopp, 1954. Both are reported from *Pseudemys scripta elegans* in Texas. Two points of difference were indicated by Little and Hopkins: (1) eggs of *N. constrictus* are 30–39 μ long compared to 42–54 in *N. pseudemydis*; (2) the posterior end of *N. constrictus* is constricted but that of *N. pseudemydis* is not.

Examination of a paratype female of *N. constrictus*, (USNM No. 62974), in the course of this study revealed that the eggs of that specimen, at least, were slightly immature. While most fell into the range stated by Little and Hopkins, several were found that measured 42 μ, the lower limit described for eggs of *N. pseudemydis*. The presence of numerous ovarian balls and the lack of eggs in the uterus in the paratype of *N. constrictus* supports the theory that the eggs were immature. In all species of Acanthocephala, one must exercise extreme care in describing eggs, for they vary considerably in appearance and size according to the state of maturity. The eggs of *N. constrictus* closely resemble those of *N. pseudemydis* in appearance. The constricted posterior end of the trunk, with two rounded, terminal lobes, is also identical in both species. Such a condition was clearly described and illustrated for *N. pseudemydis* by Fisher (1960) who redescribed the species under the direction of Dr. Cable. Several dozen specimens determined by us to be *N. pseudemydis* are identical in this regard to *N. constrictus*. This evidence strongly suggests that *N. constrictus* and *N. pseudemydis* are conspecific.

Acknowledgments

Thanks are due to Mr. Charles A. Johnson and Mr. Willard W. Becklund for the loan of specimens for comparative purposes, and to Mr. David B. Clark for assistance in obtaining the turtle sample. We also wish to thank Dr. George Lauff, Director, W. K. Kellogg Biological Station, Hickory Corners, Michigan, for providing facilities during the summer of 1968. Parts of this study were conducted under contract AT (38-1)-310 between the University of Georgia and the U. S. Atomic Energy Commission.

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Free-living Marine Nematodes from Biscayne Bay, Florida, III. Eurystominidae: *Pareurystomina bissonettei* sp. n. from Biscayne Bay and Other Locations

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ABSTRACT: *Pareurystomina bissonettei* sp. n. is described from sandy localities in Biscayne Bay, Florida, Baileys Bay, Bermuda and Woods Hole, Massachusetts. It is distinguished from other species in the genus by the shape and shorter length of its tail. The tail is obtusely conoid in the anterior half, then abruptly tapered and spicate posteriorly. The number of transverse rows of stomatal denticles is variable between specimens. Flattened cervical setae are regarded as characteristic of the genus *Pareurystomina* Micoletzky, 1930.

Specimens of an undescribed species of *Pareurystomina* Micoletzky, 1930 were encountered in collections from Bermuda, Florida and Massachusetts submitted to the Nematology Section, Entomology Research Institute, Ottawa, for identification. A discussion on the occurrence of flattened cervical setae within this genus and the related genus *Eurystomina* Filipjev, 1918 is based on comparative observations of the new species, herein named *P. bissonettei* sp. n., with specimens of *Pareurystomina* and *Eurystomina* in the Canadian National Collection of Nematodes and on information derived from the literature. All measurements given in the following description are in microns unless otherwise indicated.

Pareurystomina bissonettei sp. n.[1] (Figs. 1–7)

Holotype ♂ $\frac{36\ 260\ 1410\ M\ 4762}{40\ 60\ 58\ 61\ 53}$ 4877

Allotype ♀ $\frac{41\ 275\ 1306\ 3815\ 5328}{47\ 68\ 67\ 80\ 55}$ 5434, V = 70%.

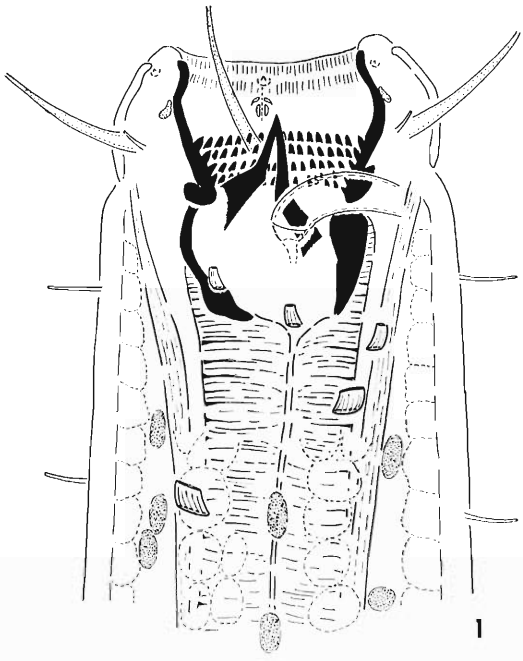
Body elongate, filiform, tapering little at extremities. Cuticle smooth, without either external and internal striations or punctations. Subcuticular vesiculation (Trabekula-Struktur in German literature) present, arranged in four paired rows along edges of lateral and medial chords. Eight longitudinal rows of hypodermal pore complexes present; pore apertures minute, circular in outline, about 1–1.5 in diameter; widely spaced along edges of lateral and medial chords, 45–85 apart. Hypodermal gland cells 10–14 in diameter; interspersed within the rows of vesiculated cells. Body with numerous small, 5–6 circular to oval cells (nuclei?). Much larger, 25–30 long, circular to longitudinally elliptical gland-like cells occur along the entire body length.

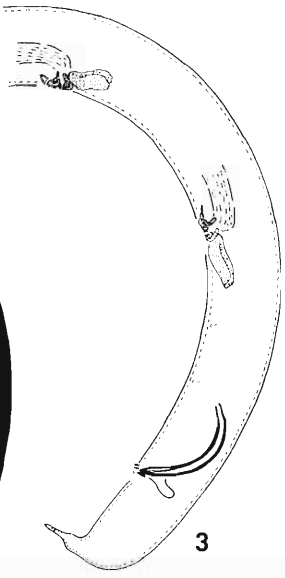
Maximum width in male 60 (55–65), in female 77 (63–85). Head 41 (36–47) wide, with six labial papillae and 10 cephalic setae 21.3 (18–23) + 4.2 (3–5) in length. Cervical setae 5–9 long, flattened, and with lense-shaped bases, 2.2 wide by 1 long; superficially arranged in three transverse circles, especially noted on dorsal and ventral surfaces; those on lateral surface not so clearly in line, or with additional setae present. Cervical setae extend 80–100 from anterior extremity. Flattened setae appear again on tail, especially noted on male. Somatic setae sparse, very fine, 3–6 long.

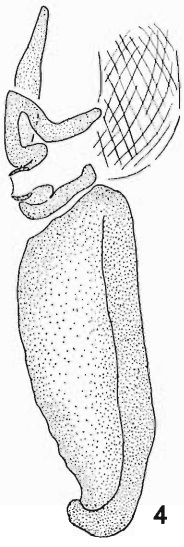
Amphid aperture transversely elongated, reniform, 18–20 by 5–6 in male, 12–15 by 4 in female, 11 by 3.5 in young females (4.12–4.19 mm long) and 9 by 3.5 in juveniles (3.44–3.62 mm long).

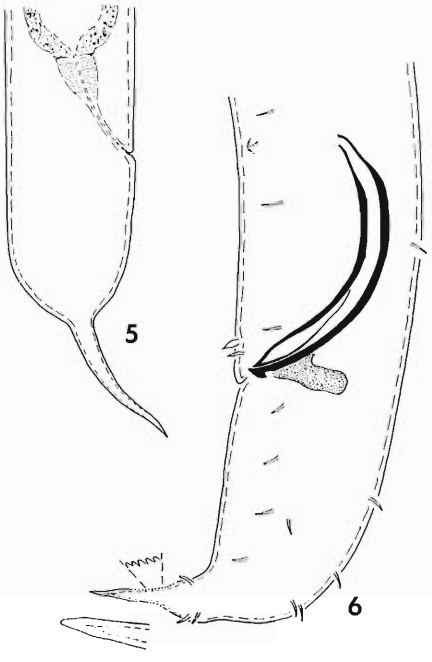
Vestibule with numerous, short (5) longitudinal striations. Stoma 37.7 (30–47) deep, divided into two portions by a transverse band of cuticle at base of anterior chamber. Posterior region of anterior chamber with one complete circle of denticles plus one to four, progressively shorter, partial segments. Fewest rows of denticles (one) on stomatal wall in vicinity

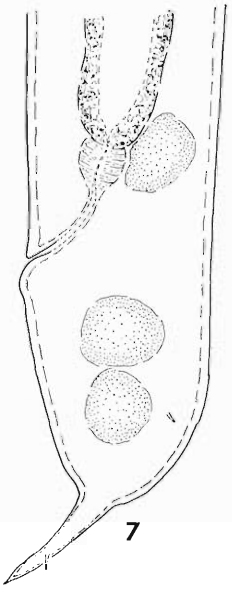
[1] Named in honor of my former technician, Mr. Raymond Bissonette, in recognition of the time he devoted to the initial phases of this study.











of subventral tooth, greatest number (up to five) at point opposite tooth. Right subventral wall of posterior stomatal chamber with large tooth; tooth projecting anterior to rows of denticles, base of tooth wide. Excretory system not detected. Esophagus elongate-conoid, 1.02–1.50 mm long (1.02–1.21 mm Massachusetts; 1.19–1.41 mm Florida; 1.35–1.50 mm Bermuda). Eight somato-esophageal muscle bands extend from anterior portion of esophagus to body wall at base of cephalic extremity. Tail 81–144 long (1.5–2.5 anal body diameters); obtusely conoid in anterior half; abruptly tapered and spicate in posterior extremity. Spicate portion of male tail with transverse rows of papilloid projections; these not occuring on females or juveniles. Anal body diameter 56.6 (51–67). Spinneret system lacking.

Male 4.82–5.76 mm long; diorchic, gonads outstretched. Spicules 93 (89–95) (chord 73 (70–77)), proximal extremity somewhat narrowed, distal extremity with hook-like processes. Gubernaculum with paired, dorsally directed apophyses, 20.5 (16–24) long. Two characteristic preanal supplements located approximately 165 and 300 preanally, respectively. Each supplement composed of central, cup-shaped portion surrounding distal extremity of prominent gland duct. Apophyses associated anteriorly and posteriorly with each central portion; anterior members reduced in size, 15 long, posterior members well developed, 34–40 and 41–42 long for anterior and posterior supplement, respectively. Two pairs of stout, 5 long, setae occur immediately in front of cloacal aperture. In addition to setae and preanal supplements, two subventral pairs of papillae (or innervated processes) occur, one at level of proximal extremity of spicules, the other midway between first pair of papillae and posteriormost preanal supplement.

Female 4.51–6.24 mm long; $V = 67$ (65–72)%; didelphic, amphidelphic, ovaries reflexed. Egg 182–210 long by 71–75 wide; not more than one mature egg present per uterus.

Young females 4.12–4.19 mm long; $V = 65$–66%. Esophagus 0.98–1.01 mm long. Maximum body width 56–58. Head 31 wide. Cephalic setae 17.5 plus 3 long. Amphid 11 wide by 3 long. Stoma 28–29 deep. Tail 99–100 long. Anal body diameter, 41–44.

Juveniles 3.44–3.62 mm long. Esophagus 0.82–0.83 mm long. Maximum width 44–50. Head 28–29 wide. Cephalic setae 15 plus 3 long. Amphid 9 wide by 3.5 long. Stoma 27–28 deep. Tail 92–96 long. Anal body diameter 42–44.

HOLOTYPE SPECIMEN: Male; in Canadian National Collection of Nematodes, Entomology Research Institute, Ottawa, Collection No. 5039, Type slide No. 203.

ALLOTYPE SPECIMEN: Female on Type slide No. 203. Other data as for holotype.

PARATYPE SPECIMENS: Six males, four females, two young females and two juveniles on Type slides Nos. 203a to 203h. Canadian National Collection of Nematodes, Collection Nos. 4823, 5039, 5990, 5991, and 5992.

TYPE LOCALITY AND COLLECTION DATA: Two males and three females extracted from sediment washed from the calcareous alga, *Halimeda* sp., located off the northwest shore of Key Biscayne. Collected 24 February 1965, by B. E. Hopper. Type locality, Biscayne Bay, Miami, Florida, USA.

OTHER LOCALITIES: Beach on the grounds of the Institute of Marine Sciences, Virginia Key, Miami (one male and two juveniles collected by S. P. Meyers, 8 October 1965); sandy sediment within a bed of turtle grass, *Thalassia testudinum* König, located offshore from the beach at Virginia Key (Site A of Hopper and Meyers, 1967) (two young females collected by B. E. Hopper, 22 January 1965); coarse and fine sand in vicinity of *Thalassia* beds, Baileys Bay, Bermuda (one male and one female collected by Bruce Coull, June and September 1967, respectively); sand and gravel, Nobska Beach, Woods Hole, Massachusetts (three males and one female collected by D. J. Zinn, 1 August 1967).

DIFFERENTIAL DIAGNOSIS: *Pareurystomina*

←

Figures 1–7. ***Pareurystomina bissonettei*** **sp. n. 1. Head, left lateral view. 2. Left spicule and gubernaculum. 3. Posterior extremity of male showing relationship of spicules and gubernaculum to preanal supplements. 4. Enlargement of posteriormost preanal supplement. 5. Tail of juvenile. 6. Tail of male showing papilloid projections and tail terminus. 7. Tail of female showing three of the gland-like cells which occur along the entire body length.**

bissonettei sp. n. differs from other species in the genus by the shape and length of its tail. The tail is obtusely conoid in the anterior portion, then abruptly tapered and spicate posteriorly. The spicate portion of the male tail bears numerous transverse rows of papilloid projections. The longitudinal rows of vesiculated cells are also considered unique for this species. The pronounced asymmetry of the winglike apophyses associated with the preanal supplements further serves to distinguish the new species from those species in which males are known.

Remarks

On the occurrence of "Flattened Cervical Setae." Wieser (1959), in describing *Eurystomina repanda* and *Pareurystomina pugetensis* was the first to recognize the existence of cervical setae which were enlarged "so as to form flaplike appendages." Chitwood (1960) regarded flattened cervical setae to be unique and in his key used their presence to separate *P. pugetensis* from the remaining species. Unfortunately, Chitwood failed to notice that his own species, *P. atypica* Chitwood, 1960, likewise possessed flattened cervical setae. Their presence on *P. atypica* was determined by an examination of a paratype female loaned to the author from the Nematology Department, University of California at Davis, California. Furthermore, all species of *Pareurystomina* that I have examined to date have flattened cervical setae. Thus, this feature can no longer be regarded as specific in nature but must be considered at least on the generic level. That *E. repanda* also possesses this characteristic would cause one to consider the feature more at a family level. However, all species of *Eurystomina* do not possess flattened cervical setae, at least not as prominently flattened as in *Pareurystomina* spp. and *E. repanda*. *E. americana* and *E. minutisculae* may have a tendency towards flatness but they are not comparable to the better known examples.

Acknowledgments

I wish to express my appreciation to Drs. S. P. Meyers, Louisiana State University, Bruce Coull, Duke University Marine Laboratory, and D. J. Zinn, University of Rhode Island, for their efforts in procuring specimen materials used in this study. A special token of appreciation is given to my former technician, Mr. Raymond Bissonette, to acknowledge the time and effort he devoted to the initial phases of this study above and beyond that required of his normal duties. Thanks are also due to Fr. R. W. Timm, University of California, for making available for study the paratype specimen of *P. atypica* Chitwood, 1960.

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Parasites of the Pika (*Ochotona princeps*) in Two Counties in South-central Montana, with New Host Records

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Contribution from the Veterinary Research Laboratory, Agricultural Experiment Station, Montana State University, Bozeman, Montana: Paper No. 740, Journal Series.

ABSTRACT: Three genera of Protozoa, three cestodes, five nematodes and three arthropods were recovered from 54 pikas (*Ochotona princeps*) collected in Gallatin and Park Counties, Montana from 1962–1967. Twenty and four-tenths per cent were infested with *Dermacentor andersoni*; 25.9% harbored *Ctenophyllus terribilis*, and 81.5% were parasitized with *Trombicula microti*. One or more genera of internal parasites were found in 87.0% of the hosts. Specific rates of infection were: *Sarcocystis* sp., 3.7%; *Eimeria* spp., 7.4%; *Isospora* sp., 1.9%; *Hydatigera* sp., 3.7%; taeniid cysticercus, 3.7%; *Schizorchis ochotonae*, 25.9%; *Graphidiella ochotonae*, 48.1%; *Murielus harpespiculus*, 44.4%; *Cephaluris coloradensis*, 35.2%; *Labiostomum coloradensis*, 18.5%; and filaroid nematode, 7.4%. The *Eimeria* spp., *Hydatigera* sp., taeniid cysticercus and filaroid nematode are new records for North America.

This report is based upon the parasites recovered at necropsy from 54 pikas (*Ochotona princeps*) which were collected at ten sites in Gallatin and Park Counties, Montana, from August 1962 to July 1967. The area surveyed forms part of the eastern slope of the northern Rocky Mountain system in south-central Montana, and comprises an area of about 1,000 square miles on the headwaters of the Yellowstone and Missouri Rivers. The localities studied varied in elevation from 6,000–11,100 feet above sea level, with the majority of the animals originating between the 7,500 and 9,000-foot levels. Included in the area surveyed were contiguous mountain chains representing portions of the Gallatin and Madison ranges in Gallatin County, and the Crazy Mountains in Park County, an isolated system of extinct volcanic peaks lying approximately 60 miles northeast of the principal area under investigation.

Although a previous regional survey of pika parasites in California was undertaken by Severaid (1955), published reports of his findings are not currently available. Existing records of pika nematodes include the description of *Murielus harpespiculus*, an intestinal trichostrongylid from *Ochotona princeps* at Jackson, Wyoming (Dikmans, 1939). Intestinal helminths reported from *Ochotona princeps figginsi* near Crested Butte, Gunnison County, Colorado were: *Schizorchis ochotonae* Hansen, 1948; *Graphidiella ochotonae* Olsen, 1948; *Cephaluris coloradensis* Olsen, 1949; and *Labiostomum coloradensis* Leiby, 1961. Jellison (1941) redescribed the two major fleas of the pika, *Amphalius necopinus* (Jordan, 1925) and *Ctenophyllus terribilis* (Rothschild, 1903) and discussed their geographic distribution in North America.

Recent Russian work on pika parasites included a study of Machurl'skii (1949) on *Eimeria* spp. of *Ochotona* which was not available to the authors. A survey of the helminths of *O. alpina* in the Baikal region was reported by Spasskii and Ryzhikov (1951). Gvozdev (1961; 1962; 1964) reported *Hydatigera* sp., *Trichostrongylus colubriformis* (Giles, 1892), *Nematodirus aspinosus* Schulz, 1929, and *Trichocephalus* sp. as new host records for *Ochotona* spp.; *Hasstilesia ochotonae* and *Murielus tjanschaniensis* were described as new species. Gvozdev (1962) also compiled a geographic host list of *Ochotona* parasites. Although the literature cited here is by no means complete, it represents the material pertinent to this survey.

Materials and Methods

The pikas were collected primarily by shooting at close range with .22 caliber ammunition.

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In one instance a Havahart Animal Trap was used. Upon recovery, the animals were placed in pint-sized plastic bags to prevent loss of ectoparasites while in transit to the laboratory. A majority of the hosts were necropsied within 24 hr after collection; the remainder were refrigerated and examined within 72 hr. The necropsy protocol was as follows: ectoparasites were collected with a fine-tipped brush and fixed in hot 70% alcohol. The animal was then opened by a ventral incision. The component parts of the digestive system were opened individually, scraped and the contents washed through 40- and 80-mesh screens. The parasites present on the screens were then collected and fixed for later examination. The heart, lungs, and kidneys were dissected and pressed between glass plates for examination. The liver, exposed musculature, mesenteries, and urinary bladder were examined grossly, and any abnormalities were checked microscopically. Qualitative fecal examinations were performed on most animals using a zinc sulfate flotation technique.

Nematodes were killed and fixed in hot 70% alcohol containing 5% glycerine. Representative specimens were cleared in glycerine and mounted in glycerine jelly for microscopic examination. Cestodes were fixed in AFA (alcohol-formalin-acetic acid) or 10% formalin, and stained with Schneider's aceto-carmine or Delafield's hematoxylin. The ectoparasites were dehydrated and mounted in Diaphane after clearing in 2% sodium hydroxide. In some cases, specific organs were examined for embedded parasites by sectioning representative tissues at 6 μ. Giemsa-stained smears were used occasionally to check tissues for Protozoa.

Results

Table 1 lists the rates of infection with the three genera of Protozoa, three cestodes, five nematodes, and three arthropods recovered from *O. princeps* during the study.

A filaroid nematode was found under the pleura and associated tissues surrounding the lung in 7.4% of the hosts. Examination of worms removed from the connective tissue capsule in which they were situated indicated that they fall into the *Litomosa–Litomosoides* complex within the family Dipetalonematidae Wehr, 1935. Specimens have been submitted to Dr. Jack H. Esslinger of the Tulane University School of Public Health and Tropical Medicine for further study.

A species of *Sarcocystis* was found in association with the musculature and connective tissue in the peritoneal cavity of 3.7% of the animals necropsied. Mature cysts measured 0.765–0.855 mm in length and 0.510–0.660 mm in width. Spores were typically banana-shaped, and averaged 12.55 μ in length (range 11.0–14.0) by 3.23 μ in width (range 2.5–5.0). Although it was not possible to identify this organism specifically, it was similar morphologically to *S. leporum* Crawley, 1914 (= *S. cuniculi* Brumpt, 1913).

Routine fecal examinations revealed coccidial oocysts in 9.3% of the pikas. There appeared to be six *Eimeria* spp. and one *Isopora* sp. in the material studied. Sporulated oocysts ranged in length from 15.0–42.0 μ and showed various morphological features which were distinctive for each type. The systematics of these coccidia will be considered elsewhere.

Comparative parasite incidence and intensity data for the two-county area are also summarized in Table 1. The prevalence of gastrointestinal helminths was similar in the two areas, except for *M. harpespiculus*, which occurred in 51.1% of the Gallatin County animals, compared with 11.1% of the Park County hosts. Of the ectoparasites, *D. andersoni* and *C. terribilis* were found only on pikas collected in Gallatin County. Because of the small number of animals examined, these apparent differences in distribution probably should be regarded with caution.

Discussion

The filaroid nematode, taeniid cysticercus, *Hydatigera* sp. and *Eimeria* spp. reported from the pika in Montana are new North American records. Four specimens of the cysticercus and three *Hydatigera* strobilocerci were administered *per os* in gelatin capsules to a captive six-month-old red fox (*Vulpes fulva*) in an effort to obtain adult worms for specific identification. Since these inoculations failed to produce adult cestodes, and a final determination apparently has not been made on the Russian material (Gvozdev, 1962), no further attempts have been made to assign specific names to these larval tapeworms, pending the availability of adult specimens.

Table 1. Incidence of parasites of *Ochotona princeps* in two counties in south-central Montana.

Parasite	Habitat	Per cent infected		Mean number of parasites (Range)	
		Park Co.[1]	Gallatin Co.[2]	Park Co.[1]	Gallatin Co.[2]
Sarcocystis sp.	Muscular and connective tissue	11.1	2.2	—	—
Eimeria spp.	Intestine	0.0	8.9	—	—
Isospora sp.	Intestine	0.0	2.2	—	—
Hydatigera sp.	Peritoneal cavity	0.0	4.4	—	2.5 (1–4)
Taeniid cysticercus	Peritoneal cavity	0.0	4.4	—	3.5 (1–6)
Schizorchis ochotonae	Small intestine	33.3	24.4	1.7 (1–3)	3.4 (1–8)
Graphidiella ochotonae	Stomach	55.6	46.7	6.4 (1–14)	4.9 (1–15)
Murielus harpespiculus	Small intestine	11.1	51.1	1.0 (1)	67.1 (1–198)
Cephaluris coloradensis	Caecum and large intestine	22.2	37.8	9.3 (2–29)	9.4 (1–48)
Labiostomum coloradensis	Caecum and large intestine	11.1	20.0	—	—
Filaroid nematode	Pleura	0.0	8.9	—	—
Ctenophyllus terribilis	External body surface	0.0	31.1	—	—
Trombicula microti	External ear	89.0	80.0	—	—
Dermacentor andersoni	External body surface	0.0	24.4	—	—

[1] Yellowstone Drainage; nine animals examined.
[2] Gallatin Drainage; forty-five animals examined.

The *Sarcocystis* sp. reported here is similar morphologically to *S. leporum* reported from the cottontail rabbit in Minnesota by Erickson (1946). Previous reports of *Sarcocystis* sp. in the pika have originated from widely scattered areas in the northern Rocky Mountain system in Alaska (Rausch, 1961), Alberta (Lubinsky, 1957), and Utah (Grundmann, personal communication).

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The authors wish to extend acknowledgment to Drs. William L. Jellison and J. M. Brennan of the U. S. Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana for their identification of the ectoparasites collected during this survey; to Dr. Jack H. Esslinger of the Tulane University School of Medicine, New Orleans, Louisiana, for his examination of the filarial worms; to Dr. J. K. Frenkel of the University of Kansas School of Medicine, Kansas City, Kansas, for examining the *Sarcocystis* material; and to Dmitry N. Berkoff, Department of Modern Languages, Montana State University, for his translation of Russian literature.

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The Influence of Seawater Media on Growth and Encystment of *Acanthamoeba polyphaga*

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ABSTRACT: A new isolate of *Acanthamoeba polyphaga* was established in culture from cysts of the amoebae discovered in nonsterile distilled water. In vitro cultivation on distilled water-agar and seawater-agar media showed that the amoebae would grow in both freshwater and marine environments. Direct subculture from freshwater medium to full strength high salinity seawater (35‰) delayed growth and encystment, and cyst morphology was abnormal. Although *A. polyphaga* adapted and grew on media designed to approximate the marine environment, the cysts were not characteristic of the genus *Acanthamoeba*. Rapid growth was restored after amoebae were maintained on seawater-agar through 4–5 generations, and when they were returned to distilled water-agar. Abnormal cyst morphology was retained on seawater-agar, but returned to normal upon subculture to distilled water-agar. The new strain, designated OX-1, was not pathogenic for mice after intranasal inoculation of 100–5,000 amoebae per animal.

Small free-living amoebae of the genus *Acanthamoeba* are well-known inhabitants of freshwater and soil, and their specific taxonomic characters have been carefully reviewed by Page (1967a). The discovery of a new species, *A. gigantea*, in seawater (Schmoller, 1964) showed that the habitat of these highly adaptive amoebae also includes the marine environment. Of the known species of *Acanthamoeba*, *A. polyphaga* Puschkarew, 1913, probably is the most universally distributed and the most frequently isolated (Page, 1967a). A new strain of *A. polyphaga*, herein designated OX-1, was isolated in our laboratory from a carboy of distilled water. The presence of *A. polyphaga* as a laboratory contaminant prompted a study to determine whether they would grow on culture media used to maintain amoebae from marine shellfish and seawater. Earlier studies (Chatton, 1913; Hollande, 1921; Wolff, 1927) showed that some species of freeliving freshwater amoebae reversibly or irreversibly lost their capacity to produce cysts on media containing high concentrations of sodium chloride. The present study was designed to test the influence of salt concentration on the growth response of *A. polyphaga*, and to determine whether or not significant changes in morphology could lead to incorrect species identifications. The importance of standardized culture methods for growing and identifying different species of *Acanthamoeba* has already been stressed by Culbertson, et al. (1965), and by Page (1967b). Further evidence for the influence of culture conditions on cyst morphology is documented in this report.

Strain OX-1 was tested for pathogenicity by intranasal inoculation into mice because of the known pathogenicity of some strains of *Acanthamoeba* in mammalian tissue culture cells (Jahnes, et al., 1957), and in experimental animals (Culbertson, et al., 1959).

Materials and Methods

Acanthamoeba polyphaga (OX-1) was isolated from nonsterile distilled water. Samples of 5 ml of the water were transferred to 60 mm plastic dishes, *Pseudomonas fluorescens* was added as a food source, and incubation was at room temperature (22–25 C). Actively dividing amoebae were transferred with a wire loop to 60 mm dishes containing 1.5% nonnutrient agar (Difco)[1] in distilled water. Stock cultures were maintained by subculturing small blocks of agar from parent cultures on fresh plates streaked with bacteria. After 3 months of maintenance a low-nutrient agar was substituted for the nonnutrient agar, and *Aerobacter aerogenes* was substituted for *P. fluorescens*. The low-nutrient agar (MYA) contained 0.1 g maltose extract,

[1] Trade names do not imply endorsement of commercial products.

0.1 g yeast extract, and 15 g Difco agar in 1.0 liter of distilled water.

PREPARATION OF EXPERIMENTAL MEDIA: The low-nutrient agar described above was made in distilled water (DWA), low salinity seawater (LSA), and high salinity seawater (HSA). Low salinity seawater (10–13‰) was obtained from the Tred Avon River, Oxford, Maryland, and high salinity seawater (32–35‰) was obtained from Chincoteague Bay, Franklin City, Virginia. Salinities were calculated from specific gravity readings. Distilled water and seawater were filtered through 0.45 μ Millipore filters and final media were sterilized by autoclaving. Other media of known salt concentrations were prepared with 1.0, 2.0 and 3.5% sodium chloride dissolved in water containing maltose extract, yeast extract, and agar (MYA). Bacteria-free axenic cultures of *A. polyphaga* were established in Neff's liquid medium (Neff, 1957) by washing agar blocks from stock cultures in three changes of sterile distilled water containing 500 units penicillin and 250 μg streptomycin sulfate per ml of water. After three washings by centrifugation at 80 g, one drop of the sediment containing the amoebae was added to Neff medium in screw-capped test tubes. Cultures were maintained in an upright position at room temperature.

CULTURE METHODS: Survival, growth, and morphologic appearance of *A. polyphaga* were recorded from cultures on DWA, LSA, and HSA. Amoebae were established on LSA by transferring small blocks of agar from DWA to LSA, and on HSA by transferring them from DWA or LSA to HSA. Permanent stocks were maintained on all three types of media, and short-term or one generation experiments were performed with agar containing known amounts of sodium chloride. Observations of all cultures were made with a Leitz inverted microscope, and the number of days required for new populations to grow and encyst was recorded. Cysts from experimental cultures were photographed to document changes in their morphology, and abnormal cysts from HSA were re-cultured on DWA to test the stability of the change and the viability of amoebae. Cysts from DWA and HSA were transferred to glass cover-slips and stained by the hematoxylin procedure of Mitchell (1966) for measurment with an ocular micrometer. Dehydration experiments with cysts were made by allowing DWA cultures to evaporate slowly to dryness at room temperature and rehydrating them with distilled water after 5 to 67 weeks (Table 1).

Table 1. Time required for excystment of *Acanthamoeba polyphaga* after rehydration of dried cultures.

Number weeks dehydrated	5	7	13	18	31	40	50	55	57	67
Number days for excystment	1	1	1	1	2	5	5	5	6	6

PHOTOMICROGRAPHIC TECHNIQUES: Phase contrast photomicrographs of living trophozoites and cysts were made with a Zeiss photomicroscope and Kodak Plux X film. Small blocks of agar were transferred to clean glass slides and covered with 11 × 22 mm coverslips moistened with a small drop of distilled water or seawater, and photographed at 400× magnification. Amoebae grown in liquid Neff's medium were transferred to 22 × 40 mm coverglasses, placed in a moist chamber for 30 min to allow them to attach, and photographed on a glass depression slide after sealing the coverglass with melted paraffin-vaseline.

IDENTIFICATION AND CHARACTERIZATION OF *A. polyphaga*: To confirm the specific identification of the amoebae used in the present study, pure cultures of related species, *A. castellanii* (Neff strain), *A. palestinensis*, and *A. astronyxis* were obtained in Neff's medium and on agar plates. The three known species (numbers 1501/1, 1547/1, and 1534/1 of the Culture Collection of Algae and Protozoa, The Botany School, Cambridge, England) were supplied through the courtesy of Dr. Joe L. Griffin, Armed Forces Institute of Pathology, Washington, D. C. The morphology of the three known species was compared with that of *A. polyphaga* under ordinary bright field and phase contrast microscopy, and nuclear morphology was studied after staining with hematoxylin (Mitchell, 1966).

Experiments to test for pathogenicity of the new strain in laboratory mice were conducted by Dr. Clyde G. Culbertson and Mr. Paul Ensminger, Eli Lilly Laboratories, Indianapolis, Indiana. Laboratory mice were inoculated intranasally with 100, 200, 500, 1,000, or 5,000 amoebae per animal, and observations were made for symptoms of disease. Mice were sacri-

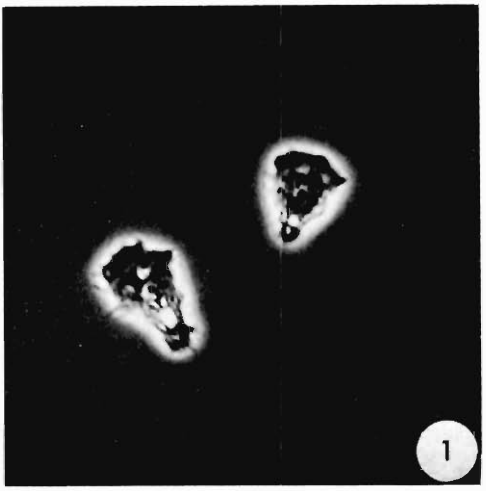


Figure 1. Living trophozoite of *Acanthamoeba polyphaga* in Neff's liquid medium. Phase contrast, ×560.

ficed 6 weeks after inoculation and examined histologically for lesions in the nose, brain, and lung.

Results

Growth characteristics of *A. polyphaga* in liquid medium and on distilled water-agar

In Neff's liquid medium and on agar made with distilled water (DWA), the amoebae grew as previously reported by Neff (1957) for *A. castellanii*, and by Page (1967a) for *A. castellanii, A. polyphaga, A. palestinensis*, and *A. astronyxis*. Taxonomic criteria for trophozoites and cysts of *A. palestinensis* and *A. astronyxis* as reviewed by Page (1967a) were adequate to distinguish them from *A. castellanii* and *A. polyphaga*. Trophozoites of *A. polyphaga* and *A. castellanii* usually were triangular and slightly longer than broad (Fig. 1). Cysts of both species were polygonal or stellate and their membranes were wrinkled (Figs. 2, 3), but these two features were distinct and more pronounced in *A. castellanii* (Fig. 3) than in *A. polyphaga* (Fig. 2). Cysts of both species were nearly identical after fixation and staining, largely because of protoplasmic contraction and pseudoinflation of cyst membranes. Encystment of the two species was complete in five to seven days on agar media, but much slower and less synchronous in liquid medium. In the latter, small numbers of amoebae frequently were present when most of the population was encysted.

Table 2. Response of *Acanthamoeba polyphaga* to growth on distilled water, seawater, and sodium chloride-agar media*.

Medium	Growth	Appearance of cyst wall	Comment
Distilled water	+	Normal-Wrinkled	Typical morphology
Seawater (10–13‰)	+	Normal-Wrinkled	Marked polymorphism
Seawater (32–35‰)	+	Abnormal-Smooth	Oval, vermiform amoeba
1.0% NaCl	+	Normal-Wrinkled	Marked polymorphism
2.0% NaCl	±	Abnormal-Smooth	Oval, vacuolated
3.5% NaCl	—	—	Degenerate

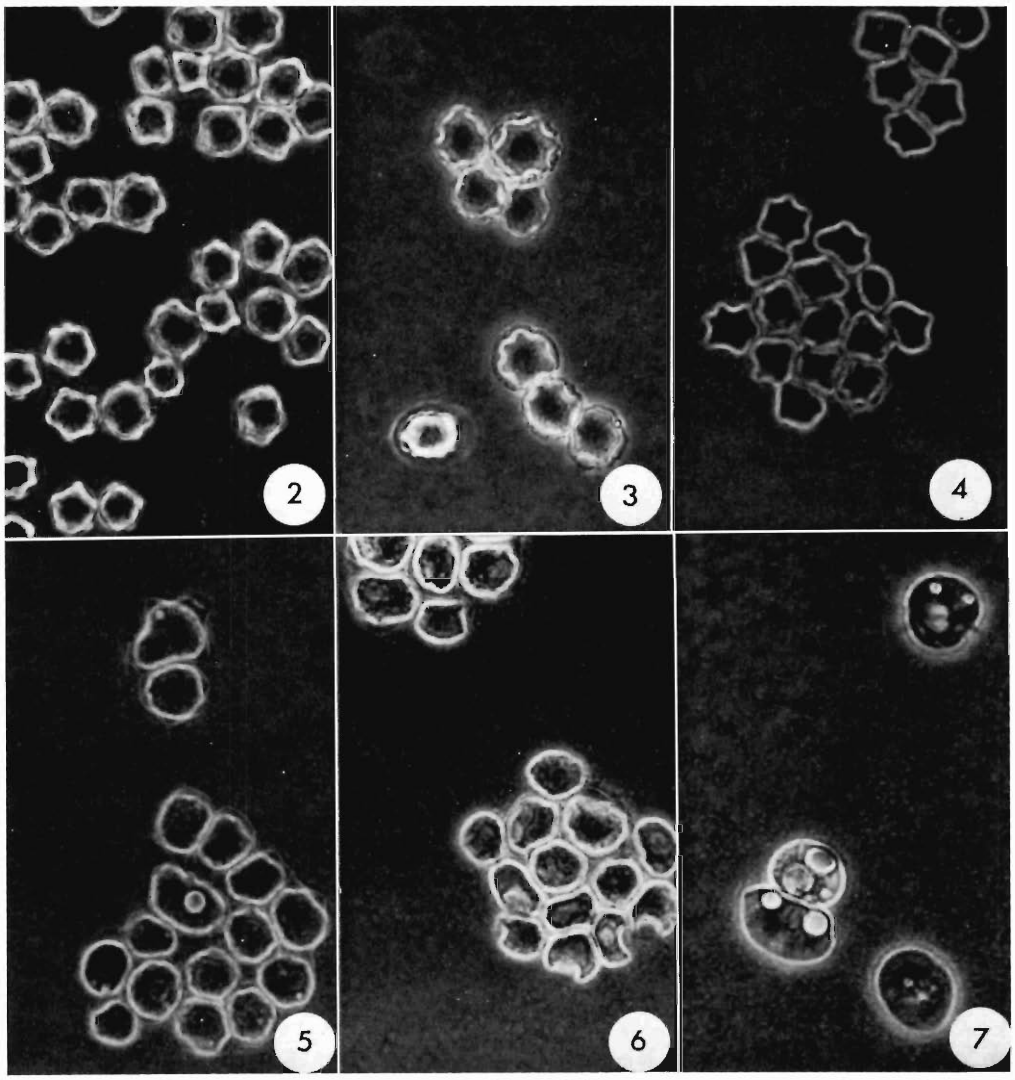
* 1.5% agar media streaked with *Aerobacter aerogenes*.

Bacterial growth on non-nutrient agar was sparse and populations of amoebae were correspondingly small. Routine maintenance on the low-nutrient agar, containing maltose extract and yeast extract, provided a satisfactory medium for balanced populations of both bacteria and amoebae. Immature cysts (precysts) of *A. polyphaga* were spherical and had a functional contractile vacuole, while mature cysts were wrinkled and polymorphic (Page, 1967a). The wrinkled cyst wall characteristic of the genus *Acanthamoeba* appeared regularly in both solid and liquid media prepared in distilled water (Table 2).

Growth of *A. polyphaga* on agar containing seawater or sodium chloride

Amoebae grew on agar media containing the nutrients provided naturally in low salinity seawater (LSA) and on agar made with one per cent sodium chloride (Table 2). Although amoebae and cysts were of essentially the same morphology on DWA and LSA, encystment was slower on LSA and small numbers of amoebae were present for up to 10 days. Cyst morphology of *A. polyphaga* was more variable with one per cent sodium chloride than with LSA of comparable salt concentrations (Fig. 4), but the specific taxonomic features were unmistakable.

Amoebae transferred directly from DWA to HSA did not show obvious growth until 7–10 days after subculture, and the period required for encystment of the entire population was



Figures 2–7. Living cysts grown on agar made with distilled water, seawater, or sodium chloride solution. Phase contrast ×560. 2. *A. polyphaga* after 20 days growth on distilled water agar. 3. *A. castellanii* after 16 days growth on distilled water agar (note stellate endocyst and wrinkled ectocyst). 4. *A. polyphaga* after 8 days growth on 1.0% sodium chloride agar (note polymorphic stellate endocyst). 5. *A. polyphaga* after 8 days growth on high salinity 35‰ seawater agar (note loss of stellate endocyst and distinct vacuole). 6. *A. polyphaga* after 10 days on 35‰ seawater agar (note oval or comma-shaped endocysts, and smooth ectocyst). 7. *A. polyphaga* after 10 days growth on 2.0% sodium chloride agar (note vacuolated oval endocyst, and smooth ectocyst).

extended to 20–25 days. Because of the lag period unchecked bacterial growth occasionally inhibited the growth of amoebae. Although *A. polyphaga* adjusted to HSA (approx. 35‰), growth did not occur when medium was made in 3.5% sodium chloride solution. In HSA medium, cysts were spherical or oval and some contained a vacuole (Figs. 5, 6). The typical

stellate endocyst and wrinkled cyst wall was markedly reduced or absent on HSA medium, and the abnormal endocysts were comma-shaped (Fig. 6). Although growth did not occur on 3.5% sodium chloride medium, moderate growth was obtained with 2.0% sodium chloride. Cysts produced on the lower concentration were large, smooth, and vacuolated (Fig. 7), and did not resemble cysts from any of the other media. Cysts that were produced on HSA or 2.0% sodium chloride agar medium did not resemble cysts of any recognized species of *Acanthamoeba*.

The significance of the extended period of time required for amoebae to grow and encyst upon transfer to HSA was tested in two ways: (1) maintaining amoebae through several successive generations on HSA; and (2) subculturing amoebae from LSA to HSA to reduce the drastic change in tonicity between DWA and HSA. Growth and encystment occurred without a lag period under both of these conditions. Thus, the extended period required for growth and encystment of *A. polyphaga* on HSA was temporary and did not persist after the initial period of adjustment. Other experiments showed that a lag period did not occur when amoebae were transferred back from HSA to DWA, and that normal cyst morphology was restored on DWA. The abnormal morphology of cysts grown on HSA remained constant upon serial cultivation in this medium.

Resistance of cysts of *A. polyphaga* to dehydration

Agar cultures of *A. polyphaga* on DWA that were dried at room temperature for periods up to 67 weeks produced new populations of amoebae after rehydration with sterile distilled water (Table 1). The new populations appeared within 24–48 hr in cultures that had been dried for less than one month, and after five days in older cultures. The viability of cysts that were dried for over one year before rehydration might explain the frequent recovery of *A. polyphaga* as a laboratory contaminant.

Influence of culture media and age on cyst measurements

The largest living cysts on DWA or HSA media usually were immature (precysts), and showed residual protoplasmic streaming or cytolysis. The smallest were produced by amoebae that had undergone a second period of encystment on the same culture plate. The double encystment cycle was observed more often on HSA than on LSA or DWA.

Table 3. Measurements of cysts of *Acanthamoeba polyphaga grown on distilled water and seawater** agar media.**

Length in microns				Width in microns		
Range	Mean	S.E.	(Number)	Range	Mean	S.E.
8-Day Culture on Distilled Water						
10.0–20.0	14.5	2.23	(20)	8.0–15.0	10.2	1.56
8-Day Culture on High Salinity Seawater						
8.0–13.0	9.7	1.12	(20)	6.0–12.0	7.7	1.34
13-Day Culture on High Salinity Seawater						
11.0–14.0	11.2	0.67	(20)	9.0–14.0	10.1	1.12

* Fixed in Schaudinn's solution and stained with hematoxylin.
** Total salinity—34‰.

The variability in size of the cysts of *A. polyphaga* grown on DWA and HSA media was reduced by measuring stained cysts that were fixed and dehydrated. Smooth spherical or oval cysts from HSA cultures yielded more uniform measurements than did wrinkled polymorphic cysts from DWA cultures, and were several microns smaller than DWA cysts of the same age (Table 3). The influence of age on cyst measurements was shown by the standard error of mean values (Table 3); 8-day-old cysts from HSA cultures had a larger S.E. than 13-day-old cysts grown on HSA. Measurements of *A. polyphaga* (OX-1) grown on DWA were in the same size range reported by Page (1967a) for this species.

Response of mice to infection with *A. polyphaga* (OX-1)

Laboratory mice inoculated intranasally with 100 to 5,000 amoebae per animal did not show overt signs of disease, and pathologic lesions were not found in tissue sections of nose, brain, or lung. The new strain of *A. polyphaga* was not pathogenic for mice under the conditions tested.

Discussion

The finding of *A. polyphaga* as a contaminant in the laboratory was consistent with earlier discoveries of *A. polyphaga* and *A. castellanii* in dust (Wells, 1911), rainwater (Pusch-

karew, 1913), cultures of soil yeasts (Castellani, 1930), and cultures of soil bacteria (Shinn and Hadley, 1936; Hewitt, 1937). *A. polyphaga* is morphologically similar to *A. castellanii* Douglas 1930, but differs from it in several significant features (Page, 1967a). The present study documents the ability of *A. polyphaga* to grow in freshwater and seawater media, and illustrates the loss of critical diagnostic features when grown on seawater or sodium chloride-agar media. Furthermore, better growth and more uniform cyst morphology was obtained on seawater media than on simple sodium chloride media. This suggests that media prepared in complex salt solutions as used by Neff (1957), and Page (1967b), are preferable to media prepared in sodium chloride solution. The study clearly demonstrates the requirement for a comprehensive and standardized approach to the in vitro cultivation of freshwater and marine amoebae. The classical taxonomic study by Schaeffer (1926), which utilized differences in the ability of free-living amoebae to adjust to concentrated or diluted seawater, unfortunately, did not include data on cyst stages. Descriptions of new species in the future, especially marine species, should be based on results of a broad experimental approach. The marine species, *A. gigantea*, should be restudied in freshwater media to confirm its identity.

Although the cyst morphology of *A. polyphaga* was altered after cultivation on HSA media, it was normal after subculture to LSA or DWA media. Hollande (1921) on the other hand, described an irreversible loss of cyst forming ability by *Vahlkampfia cruciata* cultured on agar media containing 3–4% sodium chloride. Wolff (1927) studied free-living hartmannellid amoebae under the same conditions and found that the loss of cyst forming ability was restored after subculture on low chloride media. In contrast to vahlkampfid and hartmannellid amoebae, *A. polyphaga* encysted regularly on experimental seawater and sodium chloride-agar media.

Further studies of the marine environment should extend the growing list of habitats of the acanthamoebae, i.e., tissue culture cells (Jahnes et al., 1957); experimental animals (Culbertson et al., 1959); plant sap (Troll, 1965); seawater (Schmoller, 1964; Griffin, personal communication); soil and water (Page, 1967a); and domestic animals (McConnell, et al., 1968). The universal distribution of these amoebae in nature suggests that they may also have unrecognized host-parasite relationships in marine plants and animals. The role of amoebae as serious pathogens in marine hosts has already been established, i.e., diatoms parasitized by *Amoeba biddulphiae* (Zuelzer, 1927), foraminiferans by *Vahlkampfia discorbini* (Le Calvez, 1940), and blue crabs by *Paramoeba perniciosa* (Sprague, et al., 1969; Sawyer, 1969).

New studies on the influence of natural environment and experimental culture media on morphology should answer several important questions: (1) Are some of the recognized species of marine amoebae actually well-known freshwater forms whose morphologic features have been altered by their ionic and nutritional environment? (2) Are some of the marine amoebae for which cyst stages are unknown capable of producing cysts when natural waters are diluted by rainfall during wet seasons? (3) Are the experimentally induced effects of modified culture media merely temporary physiological changes which do not persist after long-term growth and maintenance in vitro? The increasing interest on the influence of nutrition and environment on growth and morphogenesis should provide new information on the stability of critical taxonomic features among freshwater and marine protozoa.

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Report of the Brayton H. Ransom Memorial Trust Fund

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Urinary Bladder Involvement in the Langur (*Presbytis*) Infected with *Schistosoma haematobium* (Bilharz, 1852) Weinland, 1858[1]

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ABSTRACT: The langur (*Presbytis cristatus sondaicus* Robinson and Kloss, 1919), an Asiatic primate, has been included in a broad scope program to evaluate different nonhuman primates as models for experimental schistosomiasis haematobia. Preliminary observations have indicated that this monkey maintains its infection well, and may serve as a host in which *S. haematobium* (Iran strain) gives rise to involvement of the urogenital system in a manner somewhat comparable to that reported for man.

The schistosomiases constitute one of the more serious parasite infections in many populations. With an estimated worldwide prevalence of no less than 200,000,000 cases, there has been much effort and time expended on investigations directed to a better understanding of the basic biology of a parasite which is on the increase. Due to varied circumstances, including availability of study materials, more attention has been given to *Schistosoma japonicum* and *S. mansoni* than to *S. haematobium*. In man infected with *S. haematobium* there may be a predeliction for residence by parasites in the vesical plexus and variable involvement of the urogenital system, whereas with *S. japonicum* and *S. mansoni*, the parasites are more closely associated with the liver and different levels of the intestinal tract. Details on the involvement of the urogenital organs in an experimental system are essentially lacking since there has been no suitable model in which these conditions could be developed. As a consequence, a broad scope program has been instituted with a search for biomedical models acceptable for investigations with *S. haematobium*.

There are scattered references to the use of nonhuman primates in schistosome research (Edwards and McCullough, 1954; Jordan et al., 1967; Kuntz and Malakatis, 1955; Standen, 1949; Webbe and Jordan, 1966). However, there is limited information on the use of these mammals, especially the Asiatic primates, for *S. haematobium*. The present report is based upon observations of urinary bladder involvement in the langur (*Presbytis cristatus sondaicus* Robinson and Kloss, 1919), one of a series of primates being evaluated for use in experimental schistosomiasis haematobia.

Materials and Methods

Current investigations involve several schistosomes, but emphasis is given to the use of the Iran strain of *S. haematobium* which is maintained in *Bulinus truncatus rohlfsi* (Clessin) of Ghana (West Africa) origin. Stock *Bulinus* are maintained in 2–5 gallon capacity glass aquaria in constant light at 75–80 F, and fed fresh lettuce as well as supplemental food (Cerophyl, 4 parts; wheat germ, 2; Glandex fish food, 2; and dried milk, 1 part). Syrian hamsters (*Mesocricetus auratus*), exposed to 300 cercariae and sacrificed at 18–24 weeks, serve as a source of eggs for continuation of the parasite cycle.

Primates were obtained from an import dealer and kept several weeks prior to use. Hosts were anesthetized with Sernylan (phencyclidine hydrochloride) and, after immobilization, were taped ventral side up on a table top. Pooled cercariae (188–192 snails) were counted in drops of water on 18 × 18 mm coverslips. Cercarial suspensions on coverslips were placed on shaved and water cleansed

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Table 1. Distribution of worms and eggs in organs of langur (*Presbytis*) exposed to 1,000 cercariae of *Schistosoma haematobium* (Iran).

Organs	Worms recovered		Measurements (mm)*		Egg deposits (eggs/gram)
	Actual	Percentage return	Males	Females	
Lungs	0	0	0	0	63
Liver	26 prs + 12 ♂♂	43.9	4.0–6.0	6.0–14.0	100
Spleen	0	0	0	0	500
Hepatic portal veins	9 prs + 5 ♂♂	15.7	6.0–8.0	8.0–16.0	0
Stomach	0	0	0	0	38
Small intestine	4 prs + 2 ♂♂	6.8	4.0–7.0	6.0–11.0	166
Large intestine	15 prs + 1♂	21.2	4.0–7.0	6.0–10.0	64
Cecum	0	0	0	0	30
Urinary bladder	9 prs	12.3	10.0–12.0	6.0–17.0	no digest

* Worms fixed in hot 10% buffered formalin.

belly skin for 30 min. Hosts were maintained in separate cages and fed on a standardized (Southwest Foundation) primate chow.

Primate cages with hosts under study were placed on a stand provided with screened cover collection trays to obtain urine and fecal samples. Samples were collected twice weekly beginning at the 50th day after infection. Urine sediments were examined for eggs, and 1 g of stool specimen was processed by the Stoll technic (Stoll, 1923). All viscera were examined separately shortly after death of hosts, and some organs were perfused to enhance worm recovery. Pathology samples were taken at random as well as from areas in organs with obvious parasite involvement. Egg deposition in tissues was determined by KOH digestion (4%, 12–24 hr) (Cheever, 1968) of weighed samples of organ systems.

Results

S. haematobium was obtained from two experimentally infected langurs. One hundred and forty-three immature worms were recovered from the liver of an adult female which succumbed 3½ weeks after exposure to 2,000 cercariae. Formalin fixed parasites measured from 0.3–1.8 mm, with the majority in the range of 1.0–1.6 mm. Although the cause of death was not apparent, it was assumed, judged upon observations at autopsy, that it was not associated with the schistosomes. There was some fluid in the abdominal cavity and several of the mesenteric lymph nodes were enlarged, but otherwise the visceral organs appeared normal. Preinfection stools indicated the presence of *Entamoeba coli* and light infections with *Trichuris* and an unidentified trichostrongylid.

The situation was entirely different in a second adult female which was exposed to 1,000 cercariae and died 17 weeks later. This animal showed pronounced schistosomiasis haematobia, the infection leading to death. A total of 146 worms, including 63 pairs, was recovered, with the majority consisting of medium size parasites in the liver (Table 1). The sex ratio was approximately four males to three females. Nine pairs of mature worms were found in the vesical plexus with several in vessels near the urethral entrance, and others in the wall of the urinary bladder. Most of the worms along the intestinal tract were located in vessels adjacent to but not in the walls of the intestine.

Upon gross examination there were minute white spots indicating the presence of eggs in as well as on the surface of the liver and the spleen. There were small (1–2 mm) hemorrhagic spots in the walls of the small intestine associated with egg deposits and internal lesions. Scattered nodules of varying size and consistency and enclosing numerous eggs occurred on the walls of the descending colon and in contiguous mesenteries. Several pairs of worms were removed from nearby vessels. Other egg deposits accompanied internal lesions, and areas of hemorrhage were scattered along the walls of the rectum to the anus.

The greatest pathological damage was associated with the urinary bladder. Externally, the bladder showed mild diffuse hemorrhage, appeared swollen, and there were hardened tissues with egg deposits. There was marked involvement of the inner walls (Fig. 1). With more than ⅔ of the organ affected, its urine retention capacity was greatly reduced. There were several large (10 × 14 mm), elevated

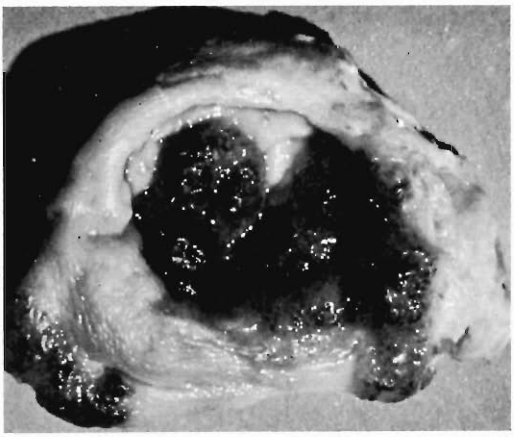

Figure 1. Inner surface of urinary bladder of *Presbytis* infected with *Schistosoma haematobium*.

(2–4 mm) plaques or hemorrhagic areas with protrusions into the bladder. Many minute petechiae at the site of micro lesions in the less thickened part of the bladder wall allowed for obvious loss of blood. Viable and nonviable eggs were present in large numbers in snips taken at random from polypoid folds. Preliminary pathology examination of section of bladder showed numerous eggs and surrounding inflammatory reaction.

The host was emaciated, had lost blood in excreta, and suffered with diarrhea for 3 weeks prior to death. Preinfection stools revealed presence of *E. coli*, *Trichuris* and a trichostrongylid. Schistosome egg passage was detected in stools 76 days after date of infection but, unfortunately, only five samples, all containing eggs, were taken prior to death. Egg sampling for urine had not been instituted at the time of death, although it seems likely eggs must have been present in urine. Digests with KOH revealed the presence of eggs in the major visceral organs.

Discussion

Even though there has been random experimental infection of nonhuman primates, and some have been found naturally infected (Nelson et al., 1962; DePaoli, 1965; Swellengrebel and Rijpstra, 1965), little attention has been given to the potential use of these mammals as models in which serious involvement of the urogenital system may be evaluated. A number of species of nonhuman primates has been exposed to infection by *S. mansoni* (Sadun et al., 1966; Jordan et al., 1967), but the list of primates used for *S. haematobium* is definitely limited (Webbe and Jordan, 1966). Nelson et al. (1962) reported natural infections of nonhuman primates in Kenya, and DePaoli (1965) found a natural infection of *S. haematobium* in a chimpanzee imported from West Africa. One of the first reports of bladder involvement is that described by Edwards and McCullough (1954) for a West African baboon infected with West African *S. haematobium*. Vogel (1967) has shown marked involvement of the urogenital system in a series of mangabeys (*Cercocebus*) and chimpanzee (*Pan*) infected with *S. haematobium*, with passage of numerous eggs in feces and urine.

Our demonstration of marked pathological involvement of the urinary bladder in the langur is similar to that seen in baboons in Tanzania by Jordan et al. (1967). Observations in the present study, plus available information on *S. haematobium* in nonhuman primates, indicate that some probably can be used for critical evaluation of host-parasites relationships in schistosomiasis haematobia. However, a compilation of data, plus experience with several species of nonhuman primates, also indicates the desired studies with *S. haematobium*, i.e. predictable involvement of urogenital system, will depend upon more efficient management of the parasite in the laboratory, as well as on a better understanding of the biology of this parasite in relation to numbers of worms involved and duration of infections.

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Baylisascaris procyonis (Stefanski and Zarnowski, 1951) from the Kinkajou, *Potos flavus*, in Colombia

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ABSTRACT: Specimens of *Baylisascaris procyonis* are described from the kinkajou, *Potos flavus*, and compared with others from the raccoon, *Procyon lotor*, from California. This new host record extends the geographical range of the worm into South America.

While in Colombia, South America, during 1966 and 1967, Dr. M. D. Little collected several specimens of *Baylisascaris procyonis* from the kinkajou, *Potos flavus*. This ascaridid has previously been reported only from the raccoon, *Procyon lotor*, in North America and Europe. Additional reports of *Ascaris columnaris* or *Ascaris* sp. from the raccoon in North America have been listed by Sprent (1968; also see Leigh, 1940; Babero and Shepperson, 1958), but Sprent (1968) suggested that these records probably apply to *B. procyonis*. Hartwich (1962) redescribed *B. procyonis* from raccoons in Europe.

Methods and Materials

All worms used in this study except one specimen of *B. columnaris*, which was fixed in

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formalin, were fixed in glacial acetic acid, stored in a solution of 19 parts 70% ethyl alcohol and 1 part glycerine, and cleared by evaporating the alcohol from the alcohol-glycerine solution. Thin cross-sections were made with a razor blade. All measurements are in millimeters; figures in parentheses apply to a single representative male or female. Drawings and some measurements were made with the aid of a camera lucida.

Results

Baylisascaris procyonis (Stefański and Żarnowski, 1951) Sprent, 1968

MALE (based on 6 specimens): Body 57–99 (99) long; width, 0.69–1.14 (0.88) at base of esophagus, maximum 0.88–1.47 (1.46) near midbody. Subventral lips 0.17–0.26 (0.24) long, 0.30–0.42 (0.40) wide, including both; dorsal lip 0.17–0.27 (0.24) long, 0.20–0.32 (0.32) wide. Excretory pore and cervical papillae 0.67–0.98 (0.98) and 0.72–1.22 (1.18, 1.22), respectively, from anterior end. Esophagus 2.23–4.15 (4.15) long or 3.5–4.7 (4.1)% of body length; width 0.18–0.23 (0.23) to 0.34–0.49 (0.39). Middle of nerve ring 0.51–0.95 (0.95) from anterior end. Left spicule 0.38–0.57 (0.57) long; right spicule 0.39–0.52 (0.52) long. Tail 0.35–0.52 (0.52) long. Precloacal papillae 36–50 (37, 43) on each side. Roughened pre- and postcloacal areas 0.03–0.06 (0.06) and 0.08–0.12 (0.11) long, respectively.

FEMALE (based on 7 specimens): Body 87–160 (160) long; width 0.78–1.16 (1.07) at base of esophagus, maximum 1.15–1.93 (1.90) near midbody. Subventral lips 0.19–0.29 (0.29) long, 0.36–0.49 (0.49) wide, including both; dorsal lip 0.18–0.30 (0.24) long, 0.23–0.34 (0.31) wide. Excretory pore and cervical papillae 0.72–1.04 (1.04) and 0.87–1.37 (1.27, 1.30), respectively, from anterior end. Esophagus 2.96–4.54 (4.54) long or 2.7–3.5 (2.8)% of body length; width 0.21–0.30 (0.23) to 0.39–0.59 (0.49). Middle of nerve ring 0.63–0.97 (0.97) from anterior end. Vulva 31.4–49.5 (49.5) from anterior end or 30–36 (31)% of body length. Vagina 1.85–2.20 (1.85) long; undivided portion of uterus 0.48–1.75 (1.56) long. Tail 0.77–1.24 (1.24) long. Phasmids 0.21–0.40 (0.40) from tip of tail. Eggs 0.066–0.080 long by 0.051–0.068 wide.

HOST: *Potos flavus*, kinkajou (Procyonidae).

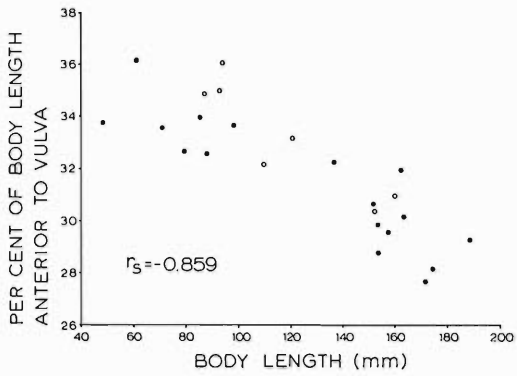


Figure 1. Per cent of body length anterior to vulva as a function of total body length of *Baylisascaris procyonis* from *Potos flavus* (o) and *Procyon lotor* (•).

SITE: Small intestine.

LOCALITY: Meneaderes, Depto. de Cauca, Colombia.

SPECIMENS DEPOSITED: Male and female, USNM Helm. Coll. No. 70788.

ADDITIONAL SPECIMENS. MALE (based on 13 specimens): Body 57–119 long. Esophagus 3.9–5.2% of body length. Spicules 0.38–0.62 long. Tail 0.36–0.59 long. Precloacal papillae 28 to 49 per side.

FEMALE (based on 17 specimens): Body 49–189 long. Esophagus 2.9–5.6% of body length. Vulva 28–36% of body length from anterior end. Tail 0.54–1.22 long.

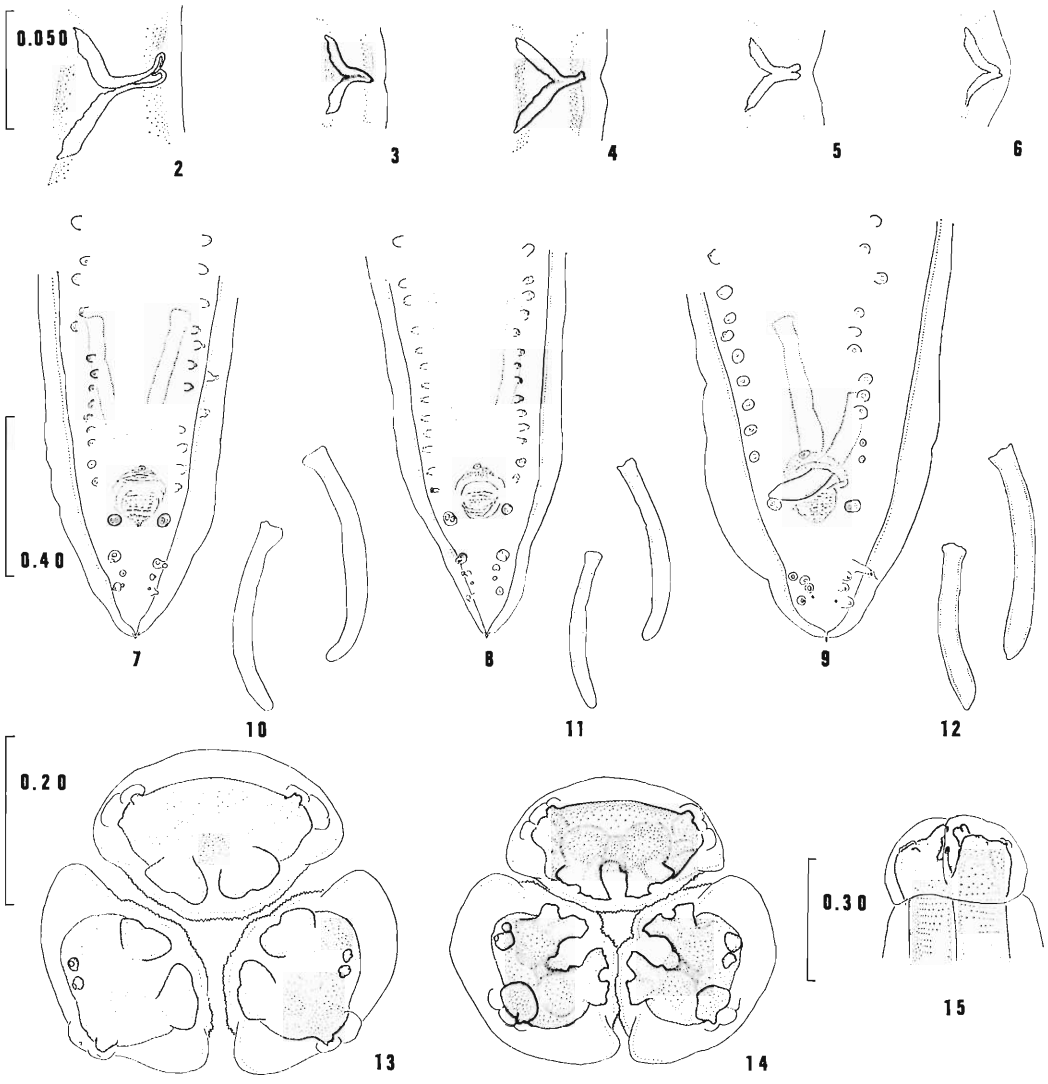
HOST: *Procyon lotor*, raccoon (Procyonidae).

SITE: Small intestine.

LOCALITY: Pacific Palisades area of Los Angeles, California.

The relative position of the vulva in my specimens depends on the length of the worm. A negative Spearman's rank correlation of $r_s = -0.859$ ($P < 0.001$) exists between the length of the worm and length of body anterior to the vulva (Fig. 1).

The vestigial cervical alae have hard, more or less V-shaped "supports" which continue throughout the length of the body and are either fused or separated (Figs. 2–6), and are much reduced in size posterior to the esophagus. The cuticle lateral to the supports is smooth, concave, or convex, both at the level of the nerve ring and the base of the esophagus



Figures 2–15. *Baylisascaris procyonis* from *Potos flavus* (2, 3, 7, 10, 14, 15) and *Procyon lotor* (4, 8, 11); *B. columnaris* from *Mephitis mephitis* (5, 6, 9, 12, 13). (Scales apply to all figures to their right). 2–6. Cuticles in lateral field at level of base of esophagus. 7–9. Ventral aspects of posterior portion of males. 10–12. Lateral aspects of spicules. 13–14. *En face* aspects. 15. Lateral aspect of cephalic region.

as shown in Figures 2–6. Even though the worm illustrated in Figure 6 was fixed in 1% formalin, the topography is not necessarily due to the fixative.

In some male specimens from the kinkajou (Fig. 7), but not in any from the raccoon, the posterior margin of the rugose pericloacal area was pointed. Small postcloacal papillae (phasmids), that in males from the raccoon are consistently located between and somewhat medial to the two posterior-most pairs of larger single papillae (Fig. 8), are, in the kinkajou, usually located at the same level as, and medial to, the most posterior pair (Fig. 7).

A single male worm, identical to those from the kinkajou, was obtained from a bushy-tailed olingo, *Bassaricyon gabbii,* after it was fed infective eggs from worms of the kinkajou. It is not known if this infection was natural or experimental.

Three male specimens of *B. columnaris* were examined from individuals of *Mephitis mephitis* collected in Michigan and Louisiana. In these, the dorsal portion of the spicules was wider (Figs. 9, 12) and the divided apical processes of the labial pulp were more rounded (Fig. 13) than in specimens of *B. procyonis* (Figs. 7, 8, 10, 11, 14). Also, the first pair of postcloacal papillae following the double papillae of the pericloacal level (Fig. 9) was not united as in *B. procyonis* (Figs. 7, 8).

Discussion

I do not consider the differences between my specimens from the kinkajou and the raccoon, such as the irregularity of the posterior margin of the rugose pericloacal area and the location of the postcloacal papillae, to be of specific magnitude. Neither do I consider of specific magnitude the difference in the relative location of the vulva between my specimens and those described from raccoons exhibited or raised in Europe. Hartwich (1962) cited a range of 23–27% of the body anterior to the vulva in European specimens as compared to 28–36% in my specimens.

Acknowledgments

I would like to thank Dr. Lawrence R. Ash for specimens from *Procyon lotor* and Dr. M. D. Little for specimens from *Potos flavus* and for reading the manuscript. Thanks also go to Dr. Paul C. Beaver who read the manuscript and Miss Rina Girard who translated the German literature. The work was supported by Public Health Service Postdoctoral Fellowship No. 1 F02 AI-36, 100-01 and Tulane University International Center for Medical Research and Training, Grant TW-00143 from NIAID, United States Public Health Service.

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Ogmogaster trilineatus sp. n. (Trematoda: Notocotylidae) from the Fin Whale, *Balaenoptera physalus* L.

ROBERT L. RAUSCH[1] AND DALE W. RICE[2]

ABSTRACT: *Ogmogaster trilineatus* sp. n. (Trematoda: Notocotylidae), described from the fin whale, *Balaenoptera physalus* L., from waters off California, is distinguished from the four previously described species of *Ogmogaster* by the number of ventral ridges and by the arrangement and dimensions of internal organs.

Since 1959, the Marine Mammal Biological Laboratory (MMBL) of the Bureau of Commercial Fisheries has conducted studies on the life history, ecology, and population dynamics of the larger cetaceans in the eastern North Pacific. In the course of these studies, Rice and his assistants have examined 677 fin whales, *Balaenoptera physalus* L., brought to whaling stations in Richmond, California. All of the whales were killed by commercial whalers operating within 200 km of San Francisco. The viscera of these whales were routinely examined for helminths.

A series of 15 trematodes of the genus *Ogmogaster* Jägerskiöld, 1891, was collected from a fin whale (MMBL field No. 1969-169) on 31 August 1969 by Robert Strawn. These trematodes, distinguished by the presence of only three ventral ridges, were recognized by Rice as differing from the known species of *Ogmogaster*. A second series of 10 was obtained from another fin whale (MMBL field No. 1969-176) on 15 September 1969. These were studied by Rausch, who prepared the description and made necessary comparisons.

Materials and Methods

After removal from the host, the trematodes were placed in fresh water for about a half-hour, then fixed in 10% formalin solution. Specimens stained in Semichon's acetic carmine, Harris' hematoxylin, and in a 1% aqueous solution of methyl green-pyronin were dehydrated in ethanol, cleared in terpineol, and mounted entire. Transverse sections were prepared, and one specimen was dissected for study of the eggs.

Results

The following description is based upon 14 specimens. All measurements are in millimeters.

Ogmogaster trilineatus sp. n. (Figs. 1–4)

DESCRIPTION: Body oval, slightly arched dorsad, 2.9–3.9 long by 1.26–2.40 in maximum width, near middle (av. 3.25 by 1.74). Margins of body thin, sometimes slightly crenulated. Tegument aspinose, with faint, longitudinal striations. Three parallel, longitudinal ridges on ventral surface, 0.107–0.300 apart, about 0.178 in maximum width, up to 0.300 high, attenuated anteriorly and posteriorly. Mesial ridge extending from genital pore posteriad to level of ends of ceca; shorter lateral ridges extending posteriad about to level of posterior margins of testes. Ridges provided throughout with numerous glandular cells arranged in parallel, transverse rows. Oral sucker terminal to subterminal, 0.200–0.321 in transverse diameter by 0.207–0.307 long (av. 0.262 by 0.270). Esophagus about 0.180 long. Ceca slightly sinuous, without lateral diverticula, extending posteriad dorsally, passing medial to vitelline

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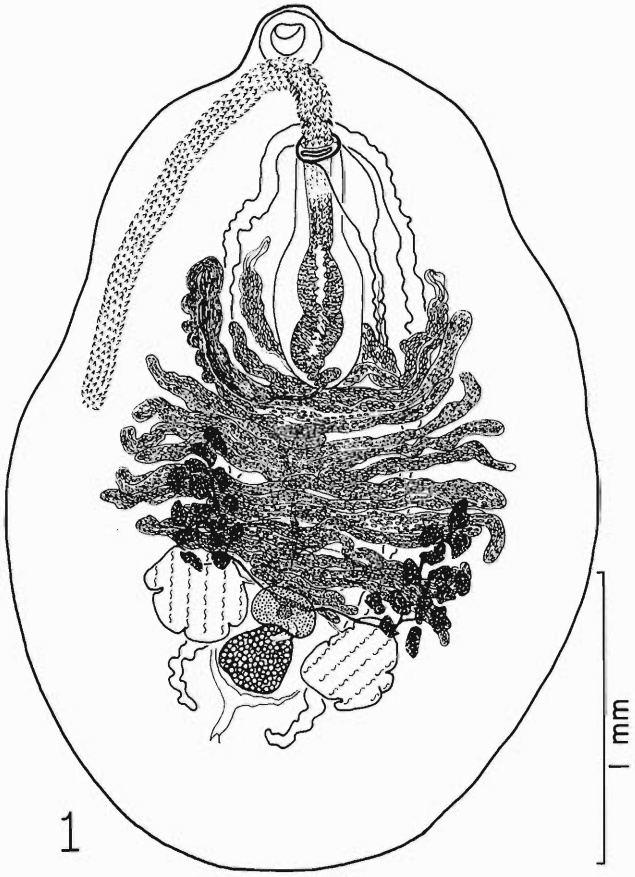
Figures 1–4. ***Ogmogaster trilineatus*** **sp. n., from the fin whale. 1. Entire specimen, ventral view. 2. Disposition of ventral ridges. 3. Arrangement of intestinal ceca. 4. Egg, with polar filaments.**



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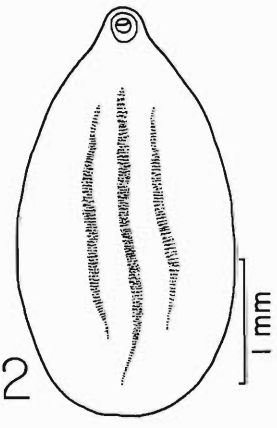

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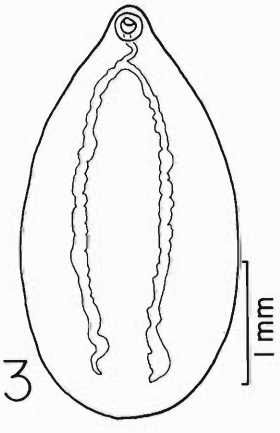

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follicles and across medial margins of testes, and terminating slightly beyond posterior margins of testes. Rounded to slightly lobed testes equal to subequal in size, situated bilaterally in posterior ⅓ of body; left testis 0.257–0.621 long by 0.200–0.407 wide (av. 0.415 by 0.288); right, 0.314–0.585 long by 0.207–0.407 wide (av. 0.393 by 0.287). Vasa efferentia running mediad, joining just anterior to Mehlis' gland. Vas deferens dorsal to uterus, 0.024–0.040 in diameter, extending anteriad near midline, and enlarging dextral to end of cirrus sac, forming external seminal vesicle. Latter somewhat undulating, 0.024–0.130 in diameter, extending anteriad dextrally to level of middle of cirrus sac, then turning abruptly posteriad to proximal end of cirrus sac, entering latter after narrowing. Elongate-piriform cirrus sac on midline, extending through approximately ⅛ of length of body, 0.750–1.606 long by 0.285–0.382 in maximum diameter (av. 1.125 by 0.329); thick-walled pars prostatica, occupying about ½ length of cirrus sac (when cirrus extruded), 0.214–0.535 long by 0.135–0.285 in diameter. Cirrus sac opening into genital atrium on midline immediately posterior to cecal bifurcation. Everted cirrus about ½ as long as body, 0.107–0.135 in diameter (av. 0.119), and covered by thick spines 0.016–0.027 long. Rounded ovary, 0.221–0.357 long by 0.193–0.293 wide (av. 0.260 by 0.237), situated intercecally between and slightly posterior to testes. Mehlis' gland subspherical, 0.107–0.285 long by 0.178–0.335 wide (av. 0.171 by 0.232), on midline adjacent to anterior surface of ovary. Laurer's canal present. Vitellaria ventral, in two lateral groups; left group with 15 or 16 follicles; right, with 14–18. Vitellaria slightly overlapping anterior margins of testes, and extending anteriad about to middle of body. Vitelline ducts arising at level of anterior margins of testes and running mediad to Mehlis' gland. Uterus ventral, extending anteriad from level of Mehlis' gland, there enlarging to form uterine seminal receptacle, and forming numerous transverse loops overlapping ceca bilaterally. Anterior loops directed anteriad, extending about to middle of cirrus sac; uterus terminating in thick-walled metraterm running anteriad to left of cirrus sac and opening in genital atrium just posterior to opening of cirrus sac. Eggs 0.030–0.034 long by 0.010–0.014 wide (av. 0.032 by 0.012), possessing at each end single polar filament 0.147–0.275 long (av. 0.205); length of egg with filaments 0.360–0.560 (av. 0.436).

HOST: Fin whale, *Balaenoptera physalus* Linnaeus.

HABITAT: Rectum.

TYPE LOCALITY: North Pacific Ocean, off San Francisco, California, lat. 37°33′ N, long. 124°06′ W.

SPECIMENS: Holotype, USNM Helm. Coll. No. 70785; paratype, No. 70786.

Differential characters

Three species of *Ogmogaster* Jägerskiöld, 1891, were recognized by Rausch and Fay (1966), viz., *O. plicatus* (Creplin, 1829), *O. antarcticus* Johnston, 1931, and *O. pentalineatus* Rausch and Fay, 1966. *O. delamurei* Treshchev, 1966, which is clearly identical with *O. pentalineatus*, was described from gray whales, *Eschrichtius robustus* (Lilljeborg) [syn. *E. gibbosus* (Erxleben)], from the Chukchi Sea. Treshchev's paper appeared in a volume which was sent to press on 17 March 1966, while the description of *O. pentalineatus* was published in February, 1966. Consequently, on grounds of priority, the latter name is applicable to this species. The recently described *O. grandis* Skriabin, 1969, very similar morphologically to *O. plicatus*, was collected from fin whales from antarctic waters. Skriabin (1969) found it also in the sei whale, *Balaenoptera borealis* Lesson, and in the blue whale, *B. musculus* Linnaeus.

The species of *Ogmogaster*, excluding *O. grandis*, can be readily differentiated by the numbers of ventral ridges: *O. plicatus*, 18–28; *O. antarcticus*, 11–17; *O. pentalineatus*, 5 (an incomplete sixth was observed in one specimen); *O. trilineatus* sp. n., 3 (Rausch and Fay, 1966; Treshchev, 1966). According to Skriabin (1969), *O. plicatus* has 15 to 17 ventral ridges, and *O. grandis* has 19–25. In *O. trilineatus*, the internal organs are arranged more compactly, leaving the margins of the body free; in the other species, the uterus and other structures extend nearly to the lateral and posterior margins of the body. *O. trilineatus* is further distinguished by the following characters.

O. trilineatus differs from *O. plicatus* (and from *O. grandis*) in having a much smaller body, a relatively short cirrus sac (extending through at least half the length of the body in

O. plicatus), smooth or weakly-lobed testes (*O. plicatus*: deeply lobed), and the uterus arranged in transverse loops (*O. plicatus*: reticulate).

Compared with *O. antarcticus*, *O. trilineatus* has a smaller body, testes and ovary of different form (both deeply lobed in *O. antarcticus*), and different uterine arrangement (*O. antarcticus*: reticulate). *O. antarcticus* is thicker, much more muscular, and often exhibits well defined, regular crenulations along the lateral and posterior margins of the body.

The new species is similar in size to *O. pentalineatus*. The latter's range in length of the body was given as 1.3–3.5 by Rausch and Fay (1966), and as 3.0–4.5 by Treshchev (1966). However, specimens subsequently obtained by one of us (DWR) from the type host measured as much as 5.6 long. *O. trilineatus* has a thinner, less muscular body, but the ventral ridges are both wider and higher than those of *O. pentalineatus*. *O. trilineatus* differs in having a cirrus sac of different shape and of relatively greater diameter, a shorter and thicker cirrus with larger spines, testes and ovary of different form (both lobed in *O. pentalineatus*), more compactly arranged vitelline follicles, and larger eggs with much longer polar filaments. The well defined lateral sacculations on the ceca of our specimens of *O. pentalineatus* were not observed in the specimens studied by Treshchev.

Discussion

Published records suggest that trematodes of the genus *Ogmogaster* usually occur in the small intestine of the host. For the examination of the fin whales considered here, the intestine was slit open at three or more randomly selected points and the surface of the mucosa scrutinized. No specimens of *Ogmogaster* were found. While working with gray whales in the winter of 1966–67, it was found that *Ogmogaster* spp. commonly occurred in the rectum. Thereafter, beginning with the 1967 whaling season, that portion of the rectum exposed after the blubber was flensed from the carcass was routinely examined.

Trematodes of the genus *Ogmogaster* were found in the rectum of 16 (30%) of 54 fin whales examined during 1967, 1968, and 1969. Three species were represented among specimens collected from 14 of these whales (Table 1). Such trematodes were found in the rectum of 53 (38%) of 139 gray whales: 7—*O. pentalineatus* only; 22—*O. antarcticus* only; 24—both species present (Rice and Wolman, 1970). None of these trematodes has been found in sei whales, *Balaenoptera borealis*, nor in sperm whales, *Physeter catodon* Linnaeus, taken off central California.

Table 1. Occurrence of *Ogmogaster* spp. in 14 fin whales.

Field No.	Date collected	*O. plicatus*	*O. antarcticus*	*O. trilineatus*
1967-173	2 Aug	×		
1967-192	2 Sep	×		
1968-151	7 Jul	×		
1968-156	31 Jul	×		
1968-159	1 Aug	×	×	
1968-169	14 Aug	×		
1969-169	31 Aug			×
1969-170	31 Aug	×		
1969-173	9 Sep	×	×	
1969-176	15 Sep		×	×
1969-181	28 Sep	×		
1969-183	5 Oct	×		
1969-184	7 Oct		×	
1969-185	8 Oct	×	×	

Acknowledgments

Robert Strawn of the Marine Mammal Biological Laboratory collected the specimens of *O. trilineatus*; other biologists who assisted in the collection of *Ogmogaster* spp. are Bernard Lenheim and Allen Wolman of the Marine Mammal Biological Laboratory, and Toshio Kasuya of the Ocean Research Institute, Tokyo. John Caito and Charles Caito of the Del Monte Fishing Company of San Francisco kindly permitted us to work at their whaling station. At the Arctic Health Research Center, Mrs. V. R. Rausch prepared the drawings, and Mr. G. C. Kelley provided photographic assistance. The contributions of these persons are gratefully acknowledged.

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RESEARCH NOTE

Current Knowledge of the Gid Bladder Worm, *Coenurus cerebralis* (= *Taenia multiceps*), in North American Domestic Sheep, *Ovis aries*

A revised checklist on parasites of domestic animals in the United States and Canada (Becklund, 1964, Am. J. Vet. Res. 25: 1380–1416), omitted the gid bladder worm of sheep, *Coenurus cerebralis* (Batsch, 1786) Rudolphi, 1808, or gid tapeworm of carnivores, *Taenia multiceps* Leske, 1780 (= *Multiceps multiceps*). This parasite, which is of medical importance, was deliberately omitted because of the dearth of available information during the last 40 years. Inquiries have since been received for general information on the gid bladder worm in North America as well as on its current incidence and geographic distribution. In an attempt to determine the status of this parasite in sheep, the literature was studied, specimens of sheep and rabbit origin in the National Parasite Collection were examined, and animal disease workers were solicited for information. This report summarizes (1) the pertinent literature, (2) the results of my observations and incidental observations of others, and contains (3) a discussion with some conclusions.

In 1905, Ransom (Bureau Animal Indust., U. S. Dept. Agr. Bull. No. 66, 23 p.) reported on specimens of *Coenurus cerebralis* collected from the brain of sheep in Montana. Although infections in sheep in North America were reported earlier, Ransom's work was the first well documented report. The certain, probable, and doubtful occurrences of larval and adult *Taenia multiceps* and *T. serialis* (Gervais, 1847) Baillet, 1863, of carnivores and the latter's larval stage *Coenurus serialis* Gervais, 1847, of rabbits, with synonymy and the findings of the earlier workers, were reviewed by Hall (1910, Bureau Animal Indust., U. S. Dept. Agr., Bull. No. 125, Pt. 1, 68 p.). Between 1909 and 1921, Hall wrote numerous articles on *C. cerebralis*, including Farmer's Bulletin 1150 (1920, issued in 1921, U. S. Dept. Agr., 53 p.) and its replacement 1330 (1923) of which there are several later revisions by other authors. The revisions of this bulletin continued to list *C. cerebralis* as a parasite of sheep in the United States, but did not include any new information on its occurrence.

Not all workers have concurred with Hall's (1910, loc. cit.) opinion that *Multiceps* is a separate genus from *Taenia*, and that *Coenurus serialis* is distinct from *C. multiceps*. Cameron (1926, J. Helm. 4: 13–22) did not consider the retention of the genus *Multiceps* justified simply because it has a coenurus larval stage. Clapham (1942, J. Helm. 20: 31–40) synonymized *M. serialis* with *M. multiceps*. She considered that hook size and conformation, intermediate hosts, and infection sites, vary in *M. multiceps* and that this species consists of "two or more races," namely, "ungulate" in which the coenuri are in the central nervous system, and "rodent" in which the coenuri are in the intermuscular connective tissues. More recently, Rausch and Williamson (1959, J. Parasit. 45: 395–403) identified adult cestodes from wolves in Alaska as *Taenia multiceps*, and Esch and Self (1965, J. Parasit. 51: 932–937) studied specimens collected from carnivores and rabbits in Oklahoma and concluded that they were *T. multiceps* and "that the analysis by Clapham (1942) is essentially correct."

To confirm the past existence and determine

the structure of *Coenurus cerebralis* in sheep, 10 coenuri from the brain of 10 sheep were studied. These coenuri were collected between 1904 and 1911 in Colorado, Montana (8 sheep), and New York. Hooks that appeared fully developed were examined from 13 scolices from the 10 coenuri. Each rostellum was removed from its scolex intact, cleared in pheno-alcohol solution, the number of hooks counted and then separated and flattened on a slide, and two or three small, as well as large, hooks drawn with the aid of a camera lucida. Similarly studied for comparison purposes were specimens of *C. serialis* from rabbits. Hooks were examined from 26 scolices that were removed from 14 coenuri collected from muscular, connective, subcutaneous, pleuoperitoneal, and undetermined tissues of 15 different rabbits in Arizona, California, Colorado, Maine, Montana (2 rabbits), Nebraska, Oklahoma, Ontario, Oregon, New York, Texas (2 rabbits), and Wyoming.

Specimens of *C. cerebralis* had very thin smooth semitransparent walls with patches of scolices that mainly protrude internally. The number of hooks on a scolex varied from 26 to 32; most scolices had 28. The lengths of 33 small hooks ranged from 85 to 118 μ (mean 106.7), and the lengths of 30 large hooks ranged from 142 to 168 μ (mean 155.8). Hook conformation was relatively uniform with the main difference between hooks from one scolex, different scolices, and different coenuri involving the angle between the guard and blade and curvature of the blade. Some anomalous hooks were observed and these were not included in measurements. Some scolices contained oval-shaped accessory hook material, varying in size and in number of pieces, and resembling the primordial hooks in very immature scolices. This material was situated beside the handles, or within the circle formed by the handles, of both normal and anomalous hooks.

Specimens of *C. serialis* were similar to those of *C. cerebralis*, but with opaque thicker walls, frequently with attached host tissues, with some having connecting outgrowths to daughter bladders, and scolices with generally larger necks making them more prominent. The number of hooks on a scolex varied from 22, besides accessory hook material, to 36; most scolices had 30. The lengths of 62 small hooks ranged from 83 to 115 μ (mean 96.7) and the lengths of 63 large hooks ranged from 125 to 157 μ (mean 143.6). The conformation of the hooks varied more than those of *C. cerebralis*, and many scolices contained immature and anomalous hooks. The lengths and conformation of the handles varied considerably, and it was often difficult to determine definitively if the handles of some hooks had attained full size. The oval-shaped accessory hook material, heretofore mentioned, was also present.

Numerous animal disease workers (from Alberta, British Columbia, California, Colorado, Illinois, Kansas, Michigan, Montana, Nebraska, New Mexico, Oregon, Utah, Wyoming, and in Federal regulatory agencies, including meat inspection) were contacted for current information on the occurrence of *C. cerebralis*. None of the workers contacted had ever encountered this parasite and many replied they had never known anyone who had done so. Dr. Wilber W. Clark (USDA) reported that the brains and spinal cords of sheep from many areas were examined during a survey for scrapie without encountering a coenurus. Moreover, for seven years, (1951–1958) I necropsied numerous sheep and cattle suspected of suffering from clinical parasitism in western and southeastern states without ever finding a coenurus. The only existing specimen from sheep, other than those in the National Parasite Collection, was reported in a letter received from Dr. William D. Lindquist. He found it in 1948 in a museum collection at Michigan State University. The origin and collection date of the specimen is unknown. Thus, coenuri in collections confirm the existence of *C. cerebralis* over 40 years ago, but no recent report of its occurrence in the United States is obtainable. Consequently, one can now only discuss the facts at hand and speculate on the current presence or absence of this parasite in relation to changes during the last 40 years in sheep management practices and predator control programs.

Scolices of *C. cerebralis* that I examined could not be distinguished from those of *C. serialis* by the number, size, and conformation of the hooks. Although not definitive for identification purposes, *C. serialis* had thicker bladder walls, larger scolex necks, either more undeveloped or anomalous hooks or both, more accessory hook material, and more anomalous scolices, than *C. cerebralis*. Also *C. serialis* had outgrowths to daughter bladders while *C. cerebralis* did not. Most of the hook anomalies and

accessory hook material observed closely resembled that figured for *Echinococcus* by Lubinsky (1959, Can. J. Zool. 37: 793–801). The most extremely anomalous scolices were found in coenuri removed from a Montana rabbit. Abnormalities included single scolices with up to ten suckers as well as six ill-defined scolices on a common base with each having two to ten suckers and hooks of various sizes and shapes. The hook and sucker arrangements in some of the abnormal scolices closely resembled those photographed by Henry (1934, Ann. Parasit. 12: 384–389).

The differences in the walls of the coenuri of sheep and those of rabbits were observed by the early workers and it is still difficult to judge their taxonomic importance. According to Hall (1910, loc. cit.), the daughter bladders and capsule around the coenuri of rabbits were reported even before the name *Coenurus serialis* was proposed, and some of the early workers did not consider the presence of daughter bladders sufficiently important to recognize *C. serialis* as distinct from *C. cerebralis*. Ransom (1905, loc. cit.) described the American *C. cerebralis* as being spherical, about one inch in diameter, without daughter bladders, with a thin semitransparent wall with the surface having white dots, the scolices, numbering over 100. This description accurately describes my own observations on *C. cerebralis*. Clapham (1942, loc. cit.) surmised her "ungulate race," which develops in the brain, does not have a capsule produced by the host, whereas, her "rodent race," which develops in the intermuscular connective tissues, is surrounded by a capsule formed by the host. Esch (1964, J. Elisha Mitchell Sci. Soc. 80: 114–120; 1967, Parasitology 57: 175–179) found that the presence or absence of the host's capsule depends on the location of the coenuri within the host. Thus, the presence or absence of a host capsule appears to be of little taxonomic value, while the presence or absence of daughter bladders may be important.

Esch's (1964, 1967, loc. cit.) findings that material of rabbit origin will infect mice and develop successfully in both the central nervous system and intermuscular connective tissues lends credence to the proposal that there is only one species in this country as concluded by Esch and Self (1965, loc. cit.). However, I am reluctant to accept the belief that the form from sheep and that of the rabbit are conspecific because: (1) Esch and Self's specimens were all collected in Oklahoma and none were from sheep; (2) the origin of Clapham's specimens of *Multiceps multiceps* (cerebral form) consisting of "three separate and unrelated coenuri and taking at random 225 scolices" is not clear; and (3) most importantly, if the rabbit form will develop in the central nervous system and intermuscular connective tissues of mice, it seems logical to assume that the sheep form should occasionally at least develop in both locations as well, but there is nothing to indicate this has ever happened. In 1910, Hall (Bureau Animal Indust., U. S. Dept. Agr., Circular 159, 7 p.) responded to objections to his belief that the sheep form only develops in the central nervous system as follows: "The idea that well-developed coenurus forms should occur outside of the central nervous system with such frequency as the objections call for, and go undetected in abattoir inspection and postmortem inspection of other sorts, is not in the least tenable." Sixty years have passed since 1910, during which time we have had increasingly stricter meat inspection laws, and nothing has been revealed that would alter Hall's statement. Southwell (1930, The fauna of British India, including Ceylon and Burma, Cestoda, v. 2, 262 p.) summarized the results of feeding experiments by various authors using *T. multiceps* and *T. serialis* in different hosts. He recognized both species based on host and body location sites. Unfortunately, American workers did not try infecting rabbits and sheep with each cestode species. In a revision of the genus *Taenia,* Verster (1969, Onderstepoort J. Vet. Res. 36: 3–58) not only recognizes *T. multiceps* and *T. serialis*, but includes subspecies of the latter.

If *C. cerebralis* still exists as an undetected parasite in the brains of sheep in the United States it must have a very low incidence and limited geographic distribution and has escaped detection only because (1) sheep brains are not routinely examined in abattoirs, (2) signs produced by cerebral coenurosis are common to other more prevalent diseases of sheep to which the symptoms would be mistakenly attributed, and (3) necropsies sufficient in depth to detect coenurosis are not normally done unless the affected animals are of considerable economic importance. The evidence strongly suggests that

C. cerebralis no longer exists in the United States. This is not surprising, since this parasite was never common nor widely distributed. Its disappearance under modern conditions is very plausible. Considerable changes during the last 40 years have been made in sheep management and predator control practices that would greatly affect the survival of this parasite. Sheep are no longer herded on open range to the extent they were previously and are now mostly maintained under fence where feral dogs and large carnivores are controlled, if not eradicated. Moreover, many farm and ranch dogs are given teniacides, and sheep offal and diseased carcasses are usually buried or destroyed. Hence, this parasite is probably now extinct.

From the foregoing it is concluded that: (1) *Coenurus cerebralis* occurred in the brains of sheep in the United States over 40 years ago; (2) it was never found developed in any other body parts than the brain; (3) the incidence and geographic distribution of this parasite was very limited; and (4) modern sheep management and predator control practices in the United States have probably made this parasite extinct.

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RESEARCH NOTE

Two Cestodes of Amphibians from Ethiopia

Baerietta janicki (Hilmy, 1936) Douglas, 1958 (Nematotaeniidae): Three of 10 *Rana angolensis* (Bocage) (Ranidae) collected from the Taffo River (18 km northeast of Addis Ababa), Shoa Province, on 22 November 1968 yielded a total of 15 worms; 3 of 13 collected from the Sebeta River (22 km west of Addis Ababa), Shoa Province, on 20, 27 December 1968 harbored 2, 5, and 7 worms, respectively; and 10 of 24 collected 31 December 1968 and 5 January 1969 from the Kebena River (in Addis Ababa), Shoa Province, were infected with a total of 104 worms. One of 3 *Rana porosissima* Steindachner (identified by Dr. Robert F. Inger, Field Museum of Natural History, Chicago) collected from the Sebeta River on 27 December 1968 harbored three worms. Both *Xenopus clivii* Peracca (Pipidae) collected from the Sebeta River on 27 December 1968 were infected with 20 and 30 worms, respectively, whereas two from the Kebena River collected 31 December 1968 were negative. Both *Ptychadena mascareniensis* (Dum. and Bibr.) (Ranidae) collected from the Arussi Mountains (2 km from Assala), Arussi Province, on 29 November 1968 were infected with a total of eight worms. One of three *Bufo regularis* Reuss (Bufonidae) collected on roads in Addis Ababa on 18 March 1969 had a single worm. All collection sites are at an elevation of 2,000 meters or higher. Specimens deposited: USNM Helm. Coll. No. 70803. Our specimens fit very closely the description of specimens from *Bufo regularis* from Rhodesia tentatively identified by Mettrick (1963, Proc. Zool. Soc. London 141: 239–250) as *B. janicki*. Mettrick transferred to this species Joyeux's (1924, Ann. Parasit. 2: 232–235) *Baerietta jaegerskioeldi* (Janicki, 1928) Hsü, 1935 (syn. *Cylindrotaenia americana* Baer, 1924, *nec* Jewell, 1916) from *Rana aequiplicata* Werner and *Arthroleptis ogoensis* Boulenger (Ranidae) from Mozambique, noting that his material and Joyeux's ". . . seem to be much more closely related to *B. janicki* than to *B. jaegerskioeldi* . . ." *B. janicki* was originally described from *Hyperolius (= Rappia) concolor* (Hallowell) and *H. fulvovittatus* Cope (Rhacophoridae) from Liberia.

Cephalochlamys namaquensis (Cohn, 1906) Blanchard, 1908 (Cephalochlamydidae): One of 24 *Rana angolensis* collected from the Kebena River on 31 December 1968 and 5 January 1969 harbored a single worm; 3 of 13 collected from the Sebeta River on 20, 27 December 1968 had 1, 2, and 15 worms, respectively; and 10 collected from the Taffo River on 22 November 1968 were all negative. Both *Xenopus clivii* collected from the Kebena River on 31 December 1968 were infected, yielding a total of 10 worms, whereas two from the Sebeta River collected 27 December 1968 were negative. Only one *R. angolensis* from the Kebena River was doubly infected with one *C. namaquensis* and two *Baerietta janicki*, while one from the Sebeta River possessed two specimens of each species. Specimens deposited: USNM Helm. Coll. No. 70804. Thurston (1967, Parasitology 57: 187–200) and Dollfus (1968, Bull. Mus. Natl. Hist. Nat., 2 Sér. 39: 1192–1201. 1967) have reviewed the genus; the former author elucidated the life cycle of *C. namaquensis*. This species has been reported from *Xenopus laevis* (Daudin) from South-West Africa, South Africa, Rhodesia, Congo (Kinshasa), and Uganda; from *Xenopus muelleri* (Peters) from Congo, Kenya, Uganda, and Nigeria; from *Rana angolensis* from Rhodesia; and from the urodele *Pleurodeles poireti* (Gervais) (Salamandridae) from either Tunisia or Algeria. Dollfus (*loc. cit.*) described and illustrated specimens from *Rana occipitalis* Günther from Gabon as a probable new species of *Cephalochlamys* Blanchard, 1908.

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Periplaneta americana (L.) as Intermediate Host of *Moniliformis moniliformis* (Bremser) in Honolulu, Hawaii

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ABSTRACT: The average infection rate of *Periplaneta americana* by *Moniliformis moniliformis* cystacanths in Honolulu, Hawaii, was 35% with a maximum infection of 397 cystacanths per host. Data on melanization of cystacanths is presented. A rapid method for the recovery of cystacanths from *P. americana* is described.

The American cockroach, *Periplaneta americana* (L.) is the normal intermediate host for the acanthocephalan *Moniliformis moniliformis* (Bremser) (= *M. dubius* (Meyer)) which requires a mammalian definitive host, usually *Rattus* spp. The biology of *M. moniliformis* is documented by Moore (1946) but relatively little information is available concerning the abundance of cystacanths within *P. americana*. Roth and Willis (1957) compiled reports indicating that most infection rates varied between 3 and 18%; however, Moore (1946) stated that 80–90% of the American cockroaches found in the Houston Zoological Gardens were infected. Available literature indicates the maximum number of cystacanths per host is "over 100" (Chandler, 1940). During a mark, release, and recapture study in Honolulu (Schaefer, 1969), a mottled pattern was observed through the ventral integument of the abdomen of several *P. americana*. This pattern was the result of heavy infestations by *M. moniliformis* cysta-

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Table 1. Infection rates of *Periplaneta americana* (L.) by *Moniliformis moniliformis* (Bremser) in four locations in Honolulu, Hawaii, with maximum number of cystacanths per host, and data on melanized cystacanths.

Location	Host sex	No. host examined	Infection rates (%)	Maximum no. cystacanths	Av. no. cystacanths	No. hosts with melanized cystacanths (A)	Percent melanized cystacanths in (A)
College Walk	♂	23	57	25	12	4	22
	♀	34	50	112	23	5	4
Iolani Ave.	♂	27	55	69	17	7	26
	♀	38	31	301	39	8	7
Harbor Area	♂	23	56	225	60	10	9
	♀	25	16	3	2	0	–
Poola St.	♂	32	6	5	5	2	67
	♀	26	11	5	2	1	71
Total	♂	105	41	–	–	–	–
	♀	123	29	–	–	–	–
Overall total	♂ + ♀	228	35	–	26	37	(11)

canths. This finding led to a quantitative survey of *P. americana* in Honolulu to determine the extent of parasitization by *M. moniliformis*.

Materials and Methods

Periplaneta americana were captured in natural populations using the trapping methods previously described (Schaefer, 1969). Four locations within Honolulu (indicated in Table 1) were selected and a random sample of at least 23 males and 25 females from each location was examined.

The wings, legs, lateral margins of the pronotum, and head were removed. The remaining thorax and abdomen of each cockroach was minced with a scalpel and washed into a 30-ml flat-bottomed, wide mouthed vial using 20 ml of tap water. The vials were corked and agitated for a few seconds. After a short period of time, some of the minced tissues floated to the surface. The floating tissues were decanted and additional water was added. This process was repeated six to eight times. The tissues that consistently precipitated consisted of fatbody, Malpighian tubules, ganglionic material, testes, and *M. moniliformis* cystacanths. These tissues and cystacanths were washed into an embryological watch dish with a small amount of water and then examined under a dissecting microscope. The number of melanized and nonmelanized cystacanths from each infected host was recorded.

Three additional cockroaches were selected from the Iolani Avenue population because they were observed to be heavily infected and examined for cystacanths. Specimen number one was completely dissected under a dissecting microscope while the other two were processed as described above.

Results

A total of 228 adult *P. americana* were examined from four Honolulu localities between 31 October 1968 and 10 July 1969. The percentage of infection was assumed to be constant during this period. The results of these examinations are tabulated in Table 1. Forty-one per cent of the males and 29% of the females were infected with *M. moniliformis* cystacanths. For those cockroaches from the random samples, the maximum number of cystacanths per host was 301.

The findings from the additional examinations of *P. americana* selected from the Iolani Avenue populations are presented in Table 2. Note that the maximum number of cystacanths per host was 397.

In addition to the normal, apparently infective, cystacanths, 37 cockroaches (16% of the total examined) contained remains of cystacanths or slightly shrivelled and melanized

Table 2. Total number and percent of melanized cystacanths recovered from three *Periplaneta americana* selected from the Iolani Avenue population, Honolulu, Hawaii.

Cockroach	Date collected	Total no. of cystacanths recovered	Total no. of melanized cystacanths	Percent melanized cystacanths
1	21 IV 68	352	210	60
2	12 XII 68	397	42	11
3	27 I 69	250	53	21
Total		999	305	(30)

cystacanths. In those randomly picked hosts with some abnormal cystacanths, about 11% of the cystacanths were melanized. In the selected hosts, one specimen had over 60% melanized. In no case did melanized cystacanths evert their praesoma after being placed in tap water whereas nonmelanized, apparently infective, cystacanths usually everted their praesoma.

Cockroach number 1 (Table 2) was so heavily infected that a few cystacanths had lodged within the proximal half of the mesocoxae and metacoxae, within fat-body and muscle tissue associated with the legs, and within the longitudinal and circular muscles. The mottled pattern referred to in the introduction was found to be due to the presence of numerous cystacanths in the subcuticular muscles of the abdomen.

Based upon the examination of the freshly recovered cystacanths, the degree of melanization appeared directly correlated with the degree of deformation (shrivelling). Lightly melanized cystacanths were usually quite normal morphologically whereas heavily melanized ones were usually greatly deformed. Darkly melanized cystacanths appeared brownish and very similar in color to the host's cuticle. Melanized cystacanths were invariably covered by a layer of transparent to greyish material which was not observed surrounding the apparently infective cystacanths.

Discussion

One area examined (Poola Street) had considerably fewer infected hosts than the other areas. This area is about 6 miles from the business district of Honolulu. It has been developed into residential housing within the last decade. It was the driest area and appeared to be devoid of sources of readily available rat food. The other areas were similar to each other and located within ½ mile of the business district of Honolulu. The percentages of infection for those areas were comparable. In general, the males were more heavily infected than the females from the same area. The total difference in the infection rates between the sexes appears significant ($P = 0.1$) but unexplainable. This difference in infection rates should be investigated further. Unfortunately, the rats within these areas were not examined for adult *M. moniliformis*. The percentage of cockroaches infected (and the degree of infection) may serve as a reliable indicator of the relative presence of infected rats in the same area.

Salt (1963) tentatively concluded that "Acanthocephala do not elicit a defence reaction in their normal intermediate hosts but are encapsulated and sometimes destroyed in unusual hosts." Poinar (1969) suggested that both normal and unusual intermediate hosts react to the invasion of larval acanthocephalans through hemocytic encapsulation. The degree to which the host reacts may be a direct reflection of the compatibility of the host-parasite relationship. The results of this study seem to verify Poinar's suggestion. The apparent lack of infectiveness of melanized cystacanths, the presence of a whitish tissue layer surrounding melanized cystacanths, and the relatively high percentage of melanized cystacanths present in hosts containing any such cystacanths, would seem to indicate a defensive reaction by the host.

These observations support those of DeGiusti (1949) who observed an acanthocephalan enveloped by syncytial layers of amoeboid cells. DeGiusti stated that these layers checked the growth of the parasite and caused its death, after which encapsulation and melanization occurred. In the present study, the sequence of encapsulation, melanization, and death of *M. moniliformis* cystacanths remains uncertain, but the evidence presented tends to support that sequence described by DeGiusti.

Acknowledgments

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The Genus *Amphimerus* Barker, 1911 (Trematoda: Opisthorchiidae) in Colombia with the Description of a New Species[1]

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ABSTRACT: *Amphimerus neotropicalis* Caballero, Montero Gei, and Caballero, 1939 and *A. guayaquilensis* (Rodríguez, Gómez Lince, and Montalvan, 1949) are reported for the first time from Colombia, and a new marsupial host is reported for each species. *Amphimerus minimus* sp. n. is described from the four-eyed opossum (*Philander opossum* L.) of Colombia, and reports of opisthorchiid-like eggs from humans in Colombia and Ecuador are mentioned. *A. guayaquilensis* is recognized as a distinct species, and *A. parviovatus* Negrão de Sousa Franco, 1967, is placed in synonymy with it.

As far as can be determined, the genus *Amphimerus* has not been reported from Colombian mammals. Seven species of the genus known from Latin American mammals are: *A. guayaquilensis* (Rodríguez, Gómez Lince, and Montalvan, 1949) from dogs of Ecuador; the same species was reported by Caballero, Grocott, and Zerecero (1953) from the cat and the common opossum (*Didelphis marsupialis* L.) from Panama; Calero, Ortíz, and De Sousa (1955) also reported this species from Panamanian cats; *A. pricei* (Foster, 1939) from the woolly opossom (*Caluromys derbianus* Waterhouse = *Philander laniger* Goldman) in Panama; *A. caudalitestis* Caballero, Grocott, and Zerecero, 1953, from the water opossum (*Chironectes minimus* (Zimmermann) = *C. panamensis* Goldman) of Panama; *A. neotropicalis* Caballero, Montero Gei, and Caballero, 1963, from the four-eyed opossum (*Philander opossum* L.) in Costa Rica; *A. pseudofelineus minutus* Artigas and Pérez, 1964, from the common opossum (*Didelphis marsupialis* L. = *D. aurita* Wied) in Brazil; *A. lancea* (Diesing, 1850) Barker, 1911, from a fresh-water dolphin in Brazil; and *A. parciovatus* Negrão de Sousa Franco, 1967, from the common opossum in Brazil.

The present paper reports two of the above species as present in Colombia and describes a previously unknown species.

The species of *Amphimerus* are of particular interest because of their close relationship to the genus *Opisthorchis* Blanchard, 1895, and because of their as yet unassessed potential for human infection. Rodríguez et al. (1949) made fecal examinations of 245 persons in a lowland area of Ecuador and found 18 of them passing opisthorchiid eggs. They also found several dogs in the same area passing similar eggs, and upon sacrificing the dogs they found the worms which they described as *Opisthorchis guayaquilensis*. They believed it was the eggs of this species that had been seen in human feces. Their species has since been regarded as a representative of *Amphimerus*.

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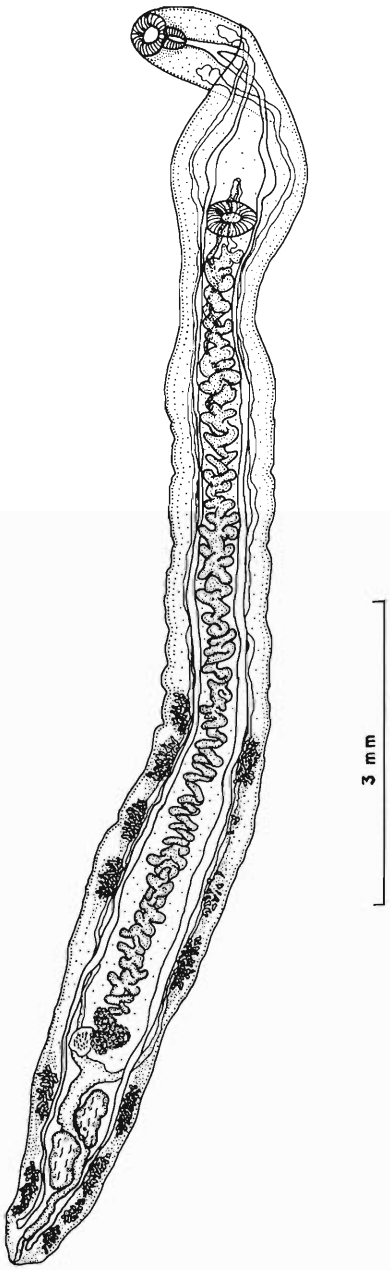

FIGURE 1

Figure 1. ***Amphimerus neotropicalis*** **Caballero, Montero Gei, and Caballero, 1963, ventral view.**

In Colombia, Restrepo (1962) reported finding opisthorchiid-like eggs in the feces of 6 out of 176 persons residing on the Amazon River.

Materials and Methods

Host livers were teased apart with dissecting needles, and then washed in tap water. The trematodes were collected by sedimentation in finger bowls. They were killed on a slide with gentle heat, fixed under a cover glass in alcohol-formalin-acetic acid solution, then stained with Mayer's carmalum and cleared in methyl salicylate. Measurements listed are in millimeters unless otherwise noted. In the diagnosis measurements in parentheses represent means. All drawings were made with the aid of a camera lucida.

Family Opisthorchiidae Braun, 1901

Subfamily Opisthorchiinae Looss, 1899

Amphimerus neotropicalis Caballero, Montero Gei, and Caballero, 1963 (Fig. 1)

HOSTS: *Philander opossum* L. and *Didelphis marsupialis* L.

LOCATION: Bile duct and gall bladder.

LOCALITIES: Buga and Villa Carmelo, Valle, Colombia.

Caballero, Montero Gei, and Caballero (1963) described this species from three specimens from the pancreatic ducts of the four-eyed opossum (*Philander opossum*) in Costa Rica.

Present material consists of nine specimens from the common opossum and three from the four-eyed opossum. In both of these infections the worms were found in the bile duct and gall bladder.

This species is elongate with the body expanded in the vicinity of the acetabulum. The ovary is three-lobed, and there is a considerable gap between the anterior and posterior vitelline fields in the region of the ovary. In the present specimens there are no characters that differ significantly from the description by Caballero et al.

The present paper reports one new host for the species, new locations within the host, and extends the known range to Colombia.

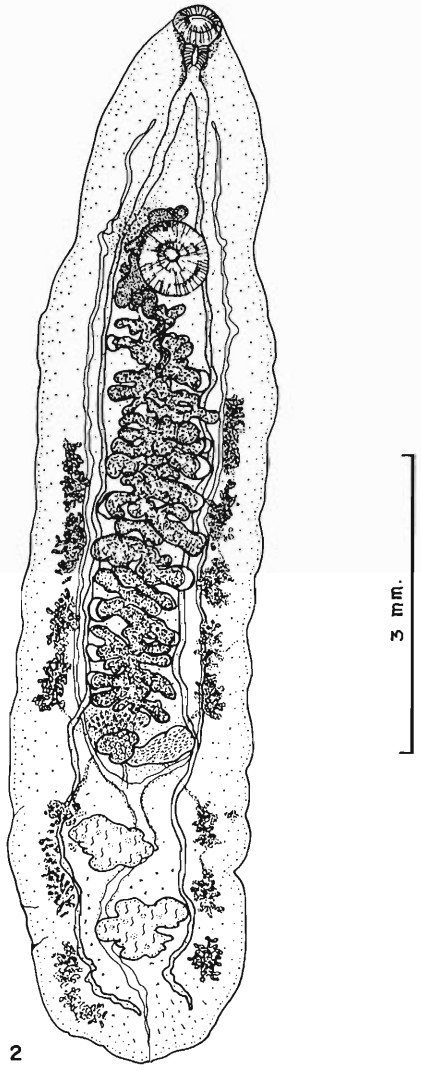

FIGURE 2

Figure 2. *Amphimerus guayaquilensis* (Rodríguez, Gómez Lince, and Montalvan, 1949), ventral view.

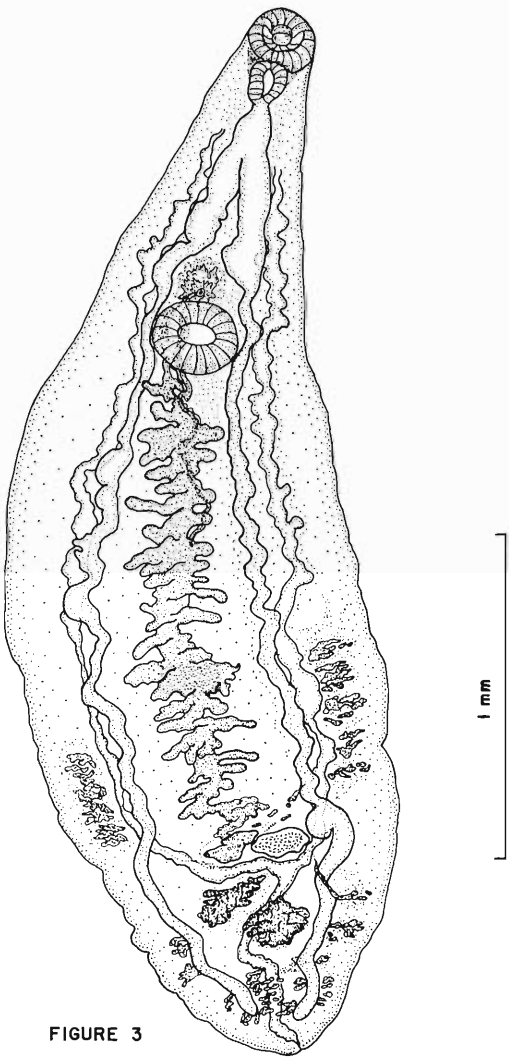

FIGURE 3

Figure 3. *Amphimerus minimus* sp. n., ventral view of paratype.

Amphimerus guayaquilensis (Rodríguez, Gómez Lince, and Montalvan, 1949) (Fig. 2)

HOSTS: *Didelphis marsupialis* L., and *Philander opossum* L.

LOCATION: Bile duct.

LOCALITIES: Buenaventura, Valle, and Turbo, Antioquia, Colombia.

This species was described from dogs in Ecuador, and has been reported from cats and the common opossum in Panama (Caballero, Grocott, and Zerecero, 1953).

In the present study, four of the worms were recovered from a common opossum, and 31 from a four-eyed opossum. This material has been compared with specimens of *A. guayaquilensis* obtained from a domestic cat in Colon

Province, Panama. Although the Colombian specimens are more robust than are those from Panama, the two forms are regarded as conspecific.

The present paper reports the species for the first time in Colombia and presents a new host record.

Amphimerus minimus sp. n. (Fig. 3)

HOST: *Philander opossum* L.
LOCATION: Bile duct and gall bladder.
LOCALITY: Buga, Valle, Colombia.
HOLOTYPE: USNM Helm. Coll. No. 70805.
PARATYPES: Author's collection.

Five specimens were found in a mixed infection with three specimens of *A. neotropicalis*.

DIAGNOSIS (based on three specimens): Body 3.37–3.67 (3.48) by 0.82–1.12 (0.98). Cuticle without spines. Oral sucker 0.18–0.24 (0.20) by 0.16–0.20 (0.18). Pharynx 0.13–0.15 (0.14) by 0.09–0.14 (0.11). Esophagus 0.14–0.16 (0.15) by 0.04–0.11 (0.06). Ceca extend to near posterior end of body; vary from 0.02–0.13 wide. Testes lobate, laterally elongate; anterior testis 0.10–0.14 (0.12) by 0.15–0.24 (0.20). Posterior testis 0.10–0.16 (0.12) by 0.16–0.21 (0.20). Seminal vesicle elongate 0.58–0.98 (0.82) by 0.01–0.02 (0.01). Genital pore immediately anterior to acetabulum, surrounded by glandular cells. Ovary lobate, laterally elongate; 0.05–0.09 (0.06) by 0.09–0.26 (0.17). Seminal receptacle sinuous, laterally elongate; 0.07–0.09 (0.08) by 0.18–0.19 (0.18). Ootype and Mehlis' gland immediately anterior to ovary; Laurer's canal present. Vitelline glands in two fields: extracecal anterior to ovary; extra and intercecal in posterior region behind testes. Uterus intercecal, extending from ovary to genital pore. Eggs 10–15 (14) by 17–31 (29) μ. Excretory ducts reaching to near pharynx.

Discussion

This species clearly belongs to the genus *Amphimerus* because of the distribution of the reproductive organs and the separation of the vitelline glands in two fields lying anterior and posterior to the ovary. The species differs from others in the genus by being much smaller, by the lobate and laterally elongate testes and ovary, and by the intercecal extension of the vitellaria posteriorly.

The validity of some species in this genus is in question. Artigas and Perez (1964) considered both *A. pricei* and *A. guayaquilensis* to be synonyms of *A. pseudofelineus* (Ward, 1901) Barker, 1911. They collected specimens of *Amphimerus* from an opossum (*Didelphis aurita*) in Brazil and since the specimens were similar to *A. pricei*, but smaller, they named this form *A. pseudofelineus minutus*. Since little is known concerning speciation in this genus, the assignment of subspecific names must be regarded as speculative.

A. guayaquilensis is herein considered as distinct from *A. pseudofelineus*. In the former species the vitelline follicles extend to near the posterior end of the body, well beyond the posterior testis. In *A. pseudofelineus* the vitellaria reach the posterior testis but do not extend beyond it.

The form described by Negrão de Sousa Franco (1967) from the common opossum of Brazil under the name of *A. parciovatus* must be regarded as a synonym of *A. guayaquilensis*. The size and general distribution of reproductive organs in these two forms are closely comparable. Negrão de Sousa Franco states that *A. parciovatus* can be distinguished from *A. guayaquilensis* because in the former the oral sucker is larger than the acetabulum. The present series indicates that the suckers are subequal, and that slight differences in the relative size of these structures should not be used as specific characters.

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Two Oviduct Flukes from Reptiles in Indiana: *Telorchis compactus* sp. n. and a Previously Described Species

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ABSTRACT: *Telorchis compactus* sp. n. from *Emydoidea blandingi*, differs from other species in the genus by living in the oviducts and having a compact body with the cirrus sac not extending posterior to the ventral sucker. The second species, from *Natrix sipedon*, is identified as *Zeugorchis natricis* Holl and Allison, 1935, but differs from accounts of that species in features which seem likely to vary with age of the worms.

Two species of trematodes from the oviducts of reptiles are among parasites sent to our laboratory from time to time. One, from a turtle, is represented by eight specimens received as wholemounts, well stained with paracarmine but imperfectly dehydrated and cleared. Reprocessing them yielded excellent preparations. The collector is identified only by the initials D. C. S. and date 12/8/40 on the slide labels.

The second species, from a water snake, is represented by a vial of trematodes which Mr. Jan Felix fixed and preserved in AFA in July 1961. Wholemounts of 15 were stained with Semichon's carmine; six were used for serial sections stained with hematoxylin and eosin. Drawings were made by microprojection. Measurements are in microns unless otherwise stated; averages are in parentheses.

Description and Discussion of Species

The turtle oviduct fluke is a new species which is closest to trematodes heretofore reported only from the digestive system of reptiles and rarely urodeles. Predominantly parasites of turtles, they have been variously assigned to *Telorchis* Lühe, 1899, and *Cercorchis* Lühe, 1900, depending on whether both are recognized as valid genera. The species at hand is placed in *Telorchis* for reasons discussed after the following description.

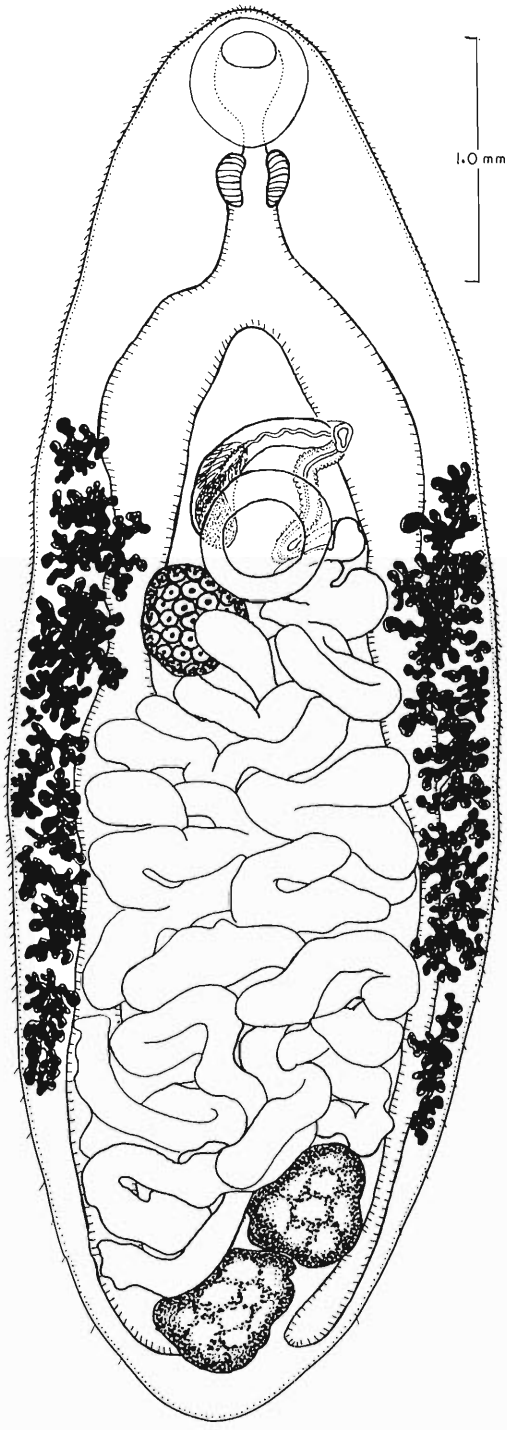
Telorchis compactus sp. n. (Fig. 1)

Host and locality: *Emydoidea blandingi* (Holbrook); northwestern Indiana.

Site: Oviducts.

Type specimens: USNM Helm. Coll. No. 70757 holotype and No. 70758 paratypes.

Description (based on seven specimens; the eighth showed amphitypy with the position of structures to right and left of midline the reverse of that given below): Plagiorchioidea; Telorchiinae. Body 5.0–6.4 (5.7) mm long, 1.9–2.5 (2.3) mm in maximum width at decidedly postequatorial level, tapering more acutely toward anterior than posterior end. Tegument spinose. Oral sucker subspherical, 402–509 (469) in diameter. Ventral sucker 1.5–2.1 (1.77) mm from anterior end, 509–610 (547) in diameter; sucker ratio 1:1.113–1.276. Prepharynx short; pharynx 208–255 (229) long, 208–328 (243) wide. Esophagus about as long as pharynx, bifurcating about midway between suckers; ceca terminating near posterior end of



body. Genital pore anterolateral to ventral sucker, ventral to left cecum. Testes intercecal, at posterior end of body, usually diagonal but occasionally almost in tandem or side-by-side, ovoid to irregular or broadly lobate in outline; right testis 442–529 (494) by 362–616 (447); left testis 295–482 (386) by 302–583 (457). Cirrus sac 576–791 (694) long, arcuate, curving posterodorsally to right from genital pore to end above side of ventral sucker without reaching posterior margin of that sucker. Seminal vesicle saccate, prostate well-developed, cirrus unarmed. Ovary subcircular to irregular or bilobed in outline, near posterodextral edge of ventral sucker, 429–623 (533) by 275–415 (356). Seminal receptacle absent, sperm stored in tubular proximal portion of uterus. Vitellaria in lateral fields beginning at level varying from intestinal bifurcation to ventral sucker, ending about one-fourth of body length from posterior end, usually distinctly shorter on right than left; follicles not in clusters or entering intercecal space but partially overlapping ceca. Uterus fills intercecal space between ventral sucker and posterior testis, partially overlapping ceca; pattern of uterine coils irregular but those of descending limb tending to be more to right, ascending to left. Metraterm muscular, almost as long as cirrus sac. Eggs numerous, 30 to 39 (33.7) by 16.5 to 22.5 (19.0).

The shape and extent of the cirrus sac alone distinguish this species from all others with which it may be congeneric. In them, the sac extends posterior to the ventral sucker, usually far into the hindbody. It ends only a short distance posterior to that sucker in *Telorchis hagmanni* described by Lent and Freitas (1937) but in that species the sac contains a long, tubular, conspicuously coiled seminal vesicle, a seminal receptable is present, and the vitellaria do not approach the level of the ventral sucker.

The genus *Telorchis* has occasioned dispute since 1899 when Lühe and Looss independently proposed a genus of that name in papers bearing the same date of publication, but with Lühe's appearing first by one day. Moreover,

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Figure 1. ***Telorchis compactus*** **sp. n. from oviducts of** ***Emydoidea blandingi.*** **Ventral view drawn from holotype except anterior end which was damaged.**

the coincidence extended to their concepts of the genus, which differed only in breadth and the species selected as types. Lühe had a more inclusive concept and designated *Telorchis clava* (Diesing, 1850) as type, whereas Looss excluded that species and named *T. linstowi* (Stossich, 1890) as type. Then Lühe (1900) defended the inclusion of *T. clava* but was constrained by what had happened to state that "es eventuell möglich wäre, die Gattung *Telorchis* Lhe. (nec Lss.) in zwei Untergattungen zu zerlegen: *Telorchis* Lhe. s. str. mit. *Tel. clava* als einziger Art und *Cercorchis* n. subg. mit *Tel. (Cerc.) linstowi* als typischer Art." Apparently Lühe never formally elevated *Cercorchis* to generic rank but he later (1909) used the name in that sense.

Subsequently workers have divided on the matter, some recognizing two genera and others following Stunkard (1915) in considering *Cercorchis* to be a synonym of *Telorchis*. Those recognizing both as valid genera have sequestered in *Cercorchis* all species except. *T. clava*. A parasite of anacondas in South America and poorly known until redescribed recently by Caubisens Poumarau (1968), *T. clava* is indeed the least typical species of the group. It differs from all others by having much more extensive uterine coils and vitellaria, and from most of them in lacking an esophagus and having a relatively short cirrus sac with a tubular, coiled seminal vesicle, a seminal receptable joining the oviduct, and an irregular uterine pattern which does not form a distinct descending limb of short, transverse loops on one side and a similar ascending one on the other. Certain species have one or two of those features, but only *T. hagmanni* shows all of them. That resemblance and the fact that the host of *T. hagmanni* is a Brazilian turtle suggest for that species and *T. clava* an affinity that is not expressed either by the concept of a single genus, i.e., *Telorchis*, or by placing *T. clava* in that genus and all others in *Cercorchis*.

If the criteria used to distinguish genera in other groups were applied to these trematodes, at least one new genus would be created. However, we believe that such action is inadvisable without evidence from life history studies that convergence has prompted an unnatural grouping of these trematodes into one or two genera. Until such evidence is forthcoming, we prefer

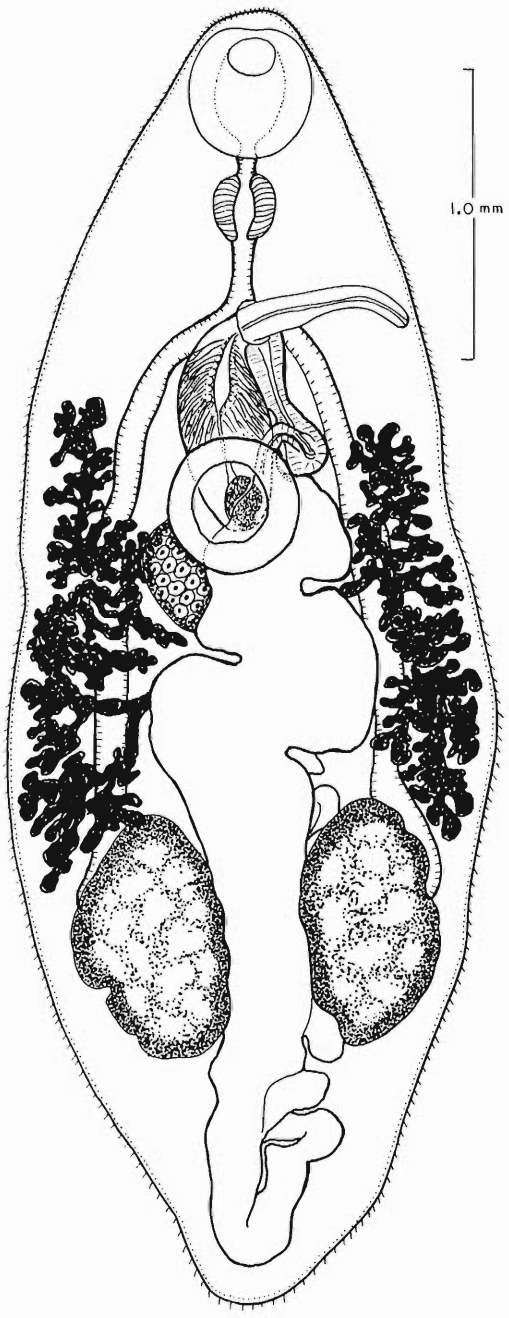

Figure 2. ***Zeugorchis natricis*** **from oviduct of** ***Natrix sipedon.*** **Ventral view.**

to follow most recent workers in treating *Cercorchis* as a junior synonym of *Telorchis*.

An insufficient number of genera may have been proposed for trematodes of the subfamily Telorchiinae but the opposite seems to hold for the Ochetosomatinae which includes the oviduct fluke from the common water snake. Our specimens are identified as *Zeugorchis natricis* although they differ from the descriptions of that species as given first by Holl and Allison (1935) and later by Canavan (1937) who was unaware of the earlier paper. The following description is based on our material and extends the range of certain specific features.

Zeugorchis natricis Holl and Allison, 1935

Syn: *Lechriorchis secundus* Canavan, 1937

(Fig. 2)

HOST AND LOCALITY: *Natrix sipedon* L., Wabash County, north central Indiana.

SITE: Oviducts.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 70759.

DESCRIPTION (based on 15 specimens): Plagiorchioidea; Ochetosomatinae. Body 4.0–5.4 (4.6) mm long, 1.52–1.72 (1.61) mm in maximum width somewhat postequatorial; anterior end usually with short preoral extension, posterior end more acute. Entire tegument spinose. Oral sucker subspherical 335–496 (451 by 427) in diameter. Ventral sucker 1.36–1.58 (1.46) mm from anterior end, 375–539 (459 by 443) in diameter; sucker ratio 1:0.095–1.167. Prepharynx short; pharynx 228–268 (248) long, 168–208 (187) wide; esophagus about as long as pharynx, bifurcating a little closer to ventral than oral sucker; ceca terminating about ⅛ body length from posterior end. Genital pore mid ventral, at bifurcation. Testes ovoid to irregular or with shallow indentations, between ends of ceca which at most extend only a short distance posterior to one or rarely both testes. Right testis 780–980 (868) by 420–580 (485); left testis 620–940 (808) by 420–560 (484). Cirrus sac stout, 500–740 (639) long, ending from level shown in Figure 2 to posterior edge of ventral sucker or slightly beyond; containing rather small, C-shaped seminal vesicle, massive prostate, and long, unarmed cirrus. Ovary ellipsoidal, entire, immediately posterodextral to ventral sucker, 320–400 (368) by 280–340 (307). Seminal receptacle absent. Vitellaria in uninterrupted lateral fields from slightly anterior to acetabular level to that of testes, overlapping ceca dorsally and ventrally, sometimes extending into intercecal space but never confluent. Uterus with a narrow, convoluted limb descending almost to posterior end of body and turning to become wide, less tortuous ascending limb. Metraterm well-developed, not closely adherent to cirrus sac. Ova numerous, 34.5–43.5 (39.9) by 16.5–24.0 (20.2). Excretory system not observed.

In our specimens the body and suckers are smaller and the uterus is less distended with eggs, but the testes are much larger and farther posterior than previously described for *Zeugorchis natricis* (verified by examination of deposited specimens). From the ecology and distribution of *Natrix sipedon*, that common water snake seems unlikely to harbor oviduct flukes of different species in Pennsylvania and Indiana. Instead, we believe that age of the worms when taken probably accounts for the above differences which may well be accentuated if eggs are not laid but retained in the uterus as in such trematodes as the turtle lung fluke, *Heronimus mollis* (Leidy, 1856), and the catfish ovarian fluke, *Acetodextra amiuri* (Stafford, 1904), which migrate from the host to release their eggs. The oviducts of reptiles would seem to be a poor habitat for trematode eggs to be laid and escape gradually in body discharges. Our material did not seem to have been fixed under pressure which could account for the larger body and sucker size in other specimens but not their smaller testes. Very likely, our material was taken at or near the peak of reproductive activity when the testes, but not other structures, had attained their maximum size, whereas previously described worms were older and larger but had the reduced testes indicative of senescence.

The order to which the host belongs more than the habitat in that host is reflected by the two oviduct flukes reported here. They belong not only to genera in which other species are intestinal parasites, but also to different subfamilies: the Telorchiinae containing *T. compactus* and other parasites mostly in turtles; and the Ochetosomatinae with *Z. natricis* and many other species predominantly in snakes.

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The Transmission of *Parafilaroides decorus* (Nematoda: Metastrongyloidea) in the California Sea Lion (*Zalophus californianus*)[1]

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ABSTRACT: *Parafilaroides decorus* (Dougherty and Herman, 1947) was found in 10 of 14 California sea lions (*Zalophus californianus* Lesson, 1828) examined over a 9 month period. Eight species of mollusc, one species of copepod, and a teleost fish (*Girella nigricans*) from contaminated pools at the San Nicolas Island rookery were examined for third-stage larvae. Larvae were found only in *Girella nigricans*. *G. nigricans* collected from an uncontaminated area were fed sea lion excrement containing first-stage larvae and the larval development was followed over a 36 day period. The first moult occurred in the intestinal mucosa and sub-mucosa 12–15 days post-infection; the second occurred in the intestinal serosa and mesenteric adipose tissue 25–36 days post-infection. Infected fish were fed to a young uninfected California sea lion. First-stage larvae appeared in its feces 21 days later.

This is the first mode of transmission of a member of the Pseudaliidae in marine mammals to be elucidated, and the first metastrongyloid life cycle wherein a vertebrate intermediate host has been found.

Each spring along the Southern California coast, as well as in amusement parks and research facilities throughout the world, large numbers of wild and captive California sea lions (*Zalophus californianus* Lesson, 1828) die from lungworm infection. This high mortality rate in valuable show and research animals has prompted this study.

[1] This study was supported by the Naval Undersea Research and Development Center under contract #N60530-68-C-0833.

Materials and Methods

Adult worms and first-stage larvae for identification and morphological studies were recovered from 10 infected California sea lions examined over a nine-month period (22 March 1968 to 12 November 1968). Lungs and trachea were removed intact. Lungs were minced and soaked in flasks with sea water. Adult worms settled to the bottom of the flask and were collected. First-stage larvae, abun-

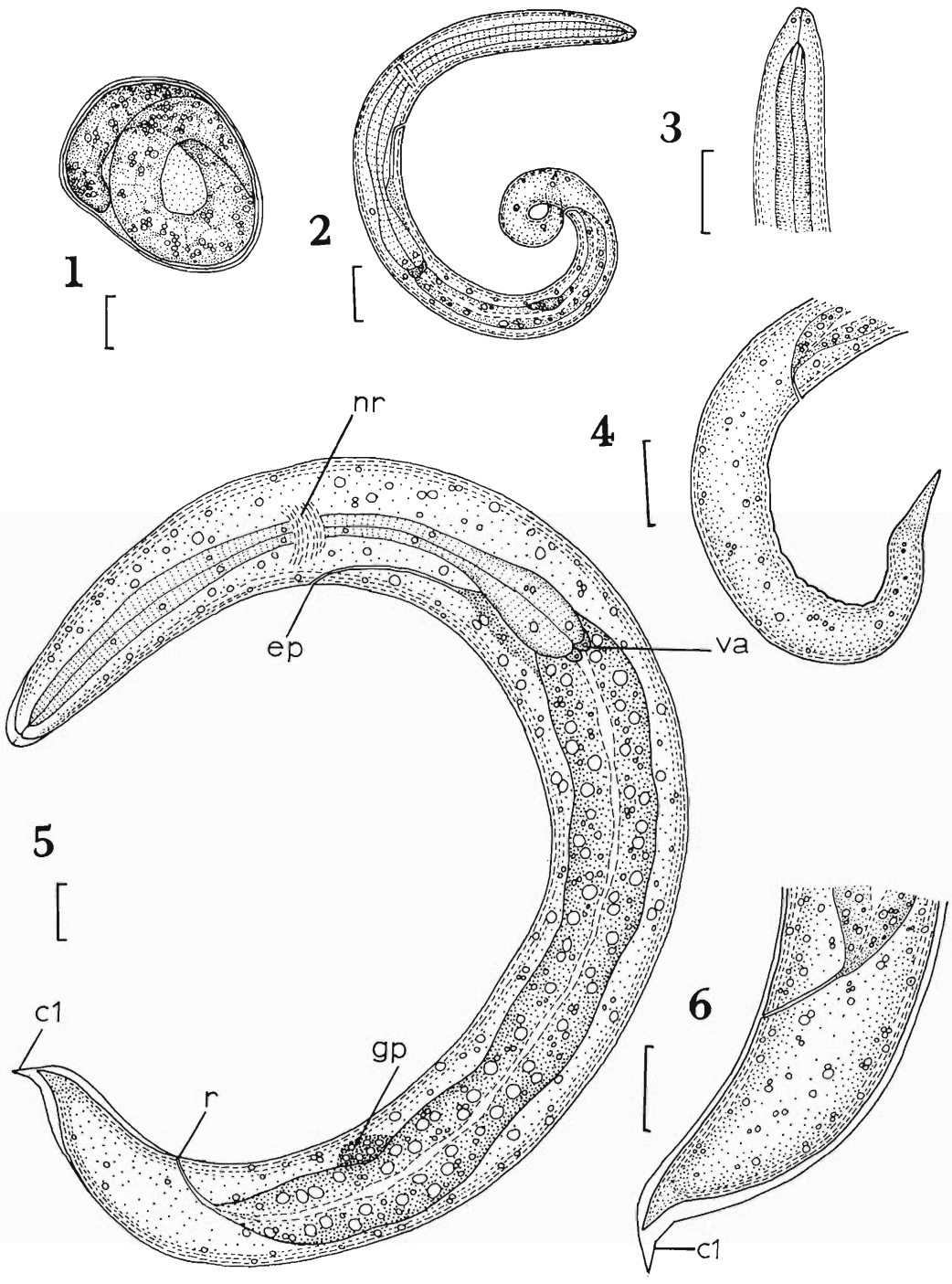

Table 1. Measurements of larval stages of *P. decorus*.

Stage	1		2	3	
Number examined	10		1	10	
Length	240–279	(260)	341	329–384	(352)
Width	13–18	(15)	25	18–22	(20)
Esophagus length	116–123	(119)	123	108–125	(117)
Nerve ring—head	64–72	(67)	64	54–61	(56)
Excretory pore—head	68–75	(73)	69	66–72	(69)
Genital primordium—head	169–190	(182)	238	245–315	(270)

dant in the mucus of the bronchi and trachea, were also collected.

Two trips were made to the sea lion rookery on San Nicolas Island, California to collect various possible intermediate hosts of *P. decorus* for examination. Molluscs, copepods and fishes were collected from clean pools and pools contaminated with feces.

Molluscs from contaminated pools were identified, minced and Baermannized in acidulated pepsin solution at 37 C (Cable, 1958). The Baerman fluid was examined for larvae. Molluscs from uncontaminated pools were identified, isolated in battery jars with filtered sea water, and kept in a cold water bath (16 C). Mucus from bronchial scrapings and fresh fecal material, both containing first-stage *P. decorus* larvae, were added to each jar. Snails were exposed to larvae for 24 hr, and were examined for infection 30 days later following pepsin digestion.

Copepods from contaminated pools were squashed under coverslips and examined for larvae under the compound microscope. Copepods from uncontaminated pools were placed in culture dishes and exposed to feces and mucus containing first-stage larvae. Exposed copepods were similarly examined for larvae 24, 48, and 72 hr later.

Girella nigricans, the "opaleye," a member of the Girellidae or "nibbler" family, were collected from contaminated pools. This fish is an omnivorous scavenger that feeds actively on fecal material. One experimentally infected fish was examined every 24 hr for the first six days and one every three days thereafter. The digestive tract was removed and placed in sea water. Small pieces of the intestinal wall just behind the pyloric ceca were pressed between slides and examined under stereoscopic and compound microscopes for *P. decorus* larvae. When larvae were found, the first 15 mm of intestine was cut into three segments, of which one was fixed in Bouin's fixative for sectioning, one was digested in pepsin and one teased apart in sea water.

Larvae were immobilized by mild heat, and covered with a vaseline-ringed coverslip for study. Permanent whole mounts were made in glycerine or glycerine jelly. Tissues for sectioning were fixed in Bouins fluid, embedded in paraffin, cut at 8 μ and stained with hematoxylin-eosin. Sections were mounted in Piccolyte resin. Measurements are in microns unless otherwise indicated; ranges are followed by averages.

Results

Incidence of *P. decorus* in sea lions

Fourteen immature sea lions of undetermined sex, 3 months to 2 years old, were examined over a 9 month period. All were taken in southern California: six at Point Mugu, seven at San Nicolas Island, and one at Seal Beach. Infections, four of which were massive, were found in 10 of 14 (71%) sea lions examined. In the massive infections the lung surface was covered with small yellowish pustules each of which yielded worms when opened.

Examination of possible intermediate hosts

All sea lions examined were infected with the lung mite *Orthohalarachne diminuata* (Doetschman, 1944) Newell, 1947. No larvae

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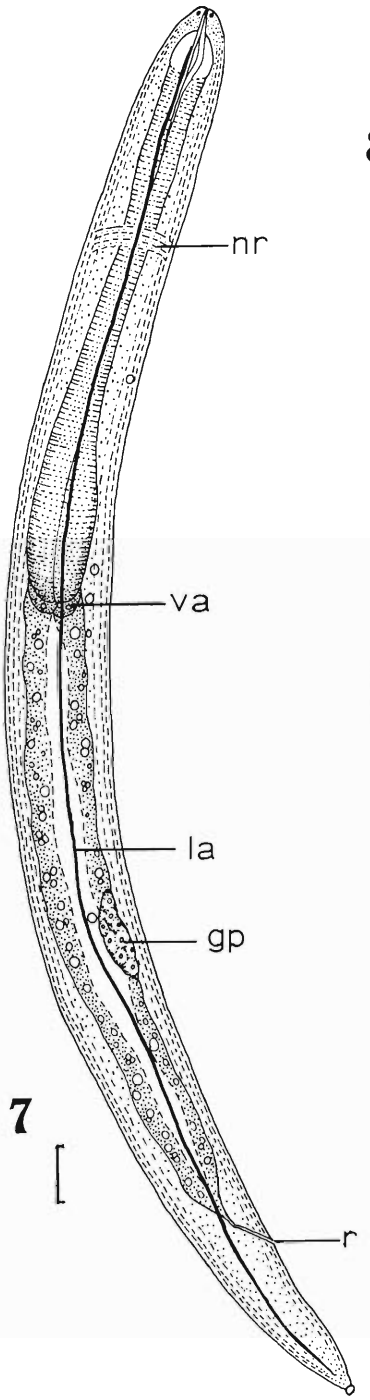
Figures 1–6. ***Parafilaroides decorus.*** **1. Egg. 2–4. First-stage larva. 2. Lateral aspect. 3. Anterior extremity, dorsal aspect. 4. Posterior region, showing spike-like tail. 5–6. Second-stage larva. 5. Lateral aspect. 6. Caudal extremity.**

Scales: Bars = 12 μ (Figs. 1–4); 10 μ (Figs. 5–11); 20 μ (Figs. 12–17).

Abbreviations: C_1, cuticle of first stage. C_2, cuticle of second stage; ep, excretory pore; gp, genital primordium; la, lateral alae; nr, nerve ring; r, anus; va, esophagointestinal valve.



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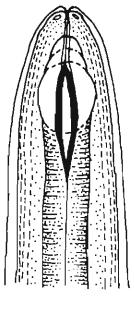

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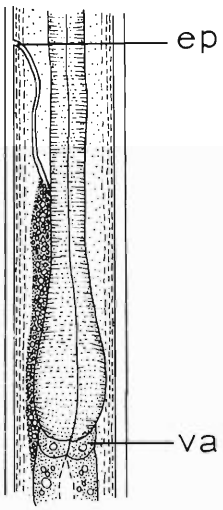

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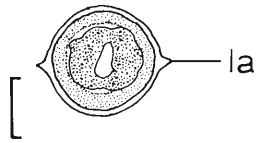

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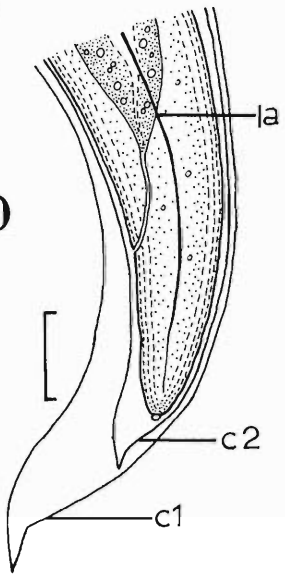

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were found in 50 mites examined although they occur together with first-stage larvae in the mucus of the bronchioles and trachea.

Two hundred and fifty specimens of each of the following mollusc-species from contaminated pools were examined: *Littorina planaxis* (periwinkle), L. *scutulata* (periwinkle, *Tegula funebralis* (turbin snail), *T. brunnea* (turbin snail), *Acamaea* sp. (limpet), *Mytilus edulis* (bay mussel), *M. californianus* (California sea-mussel), *Haliotis cracherodii* (black abalone). Examination revealed no infections. Ten specimens of each species, exposed experimentally to first-stage larvae from infected sea lions were likewise negative.

Larvae were not found in 55 *Trigriopus californianus* (splash pool copepod) collected from contaminated pools. First-stage larvae of *P. decorus* ingested by clean copepods collected from uncontaminated pools died within 48 hr after ingestion.

All of 12 *G. nigricans* from contaminated pools contained numerous larval nematodes, surrounded by a dark red pigment, and located under the intestinal serosa and in the mesenteric fat. They had the characteristics typical of infective larvae of other metastrogyloids, e.g. *Perostrongylus pridhami* (Anderson, 1962), *Trilobostrongylus bioccai* Anderson, 1963. They became active in 15–20 seconds when exposed to artificial digestion at 37 C.

Experimental infection of *Girella nigricans*

Sixteen *G. nigricans* were collected from uncontaminated pools at San Clemente, California, where sea lions are not common. Ten others previously examined from these pools contained no larvae similar to those found in the specimens from San Nicolas Island. The test fish were fed fecal material from a sea lion previously determined by fecal examination to be heavily infected; all became infected.

Twenty-four hours after infection, first-stage larvae were still in the lumen of the intestine, usually near the mucosa (Fig. 12). By 3 days, larvae had penetrated deep into the mucosa and submucosa (Fig. 13). Five to 12 days after infection they were found immediately under the longitudinal muscle layer of the intestine (Fig. 14). The first moult apparently took place 12–15 days after infection. By 18 days, second-stage larvae were found in the longitudinal muscle layer and beneath the circular muscles (Fig. 15); 12–21 days after infection, some had been walled off by host response and appeared dead (Fig. 16); after 21 days, surviving larvae were found either in the serosa or in the mesenteric adipose tissue. The second moult took place 24–36 days after infection; only one third-stage larva was found at 24 days, while approximately half of the larvae found on day 30 had completed the second moult. By 36 days only third-stage larvae were found, and all were in either the serosal lining or the mesenteric fat (Fig. 17).

To investigate the possibility that fish may serve as transport hosts, 20 pieces of intestine, each estimated to contain approximately 200 third-stage larvae were fed to each of 10 previously unexposed *G. nigricans* and *Fundulus parvipinnis* Girard. The fish were examined after 14 days, but no larvae were found.

Experimental transmission to sea lion

Fecal samples from captured sea lions waiting shipment at *Sea Lions International*, Santa Barbara, California were examined for *P. decorus* over a seven day period. Two uninfected yearlings were selected and transferred to the Marine Bioscience Facility at Point Mugu, California, where they were examined daily for *P. decorus* larvae for an additional 14 days. None was found. One animal was fed experimentally infected *G. nigricans*, while the control received noninfected synthetic food.

The control animal died from undetermined causes on day 12. A thorough necropsy revealed no larval or adult *P. decorus*. No changes in activity were noted in either animal following the feedings. The experimentally infected sea lion began to pass larvae 21 days after infection.

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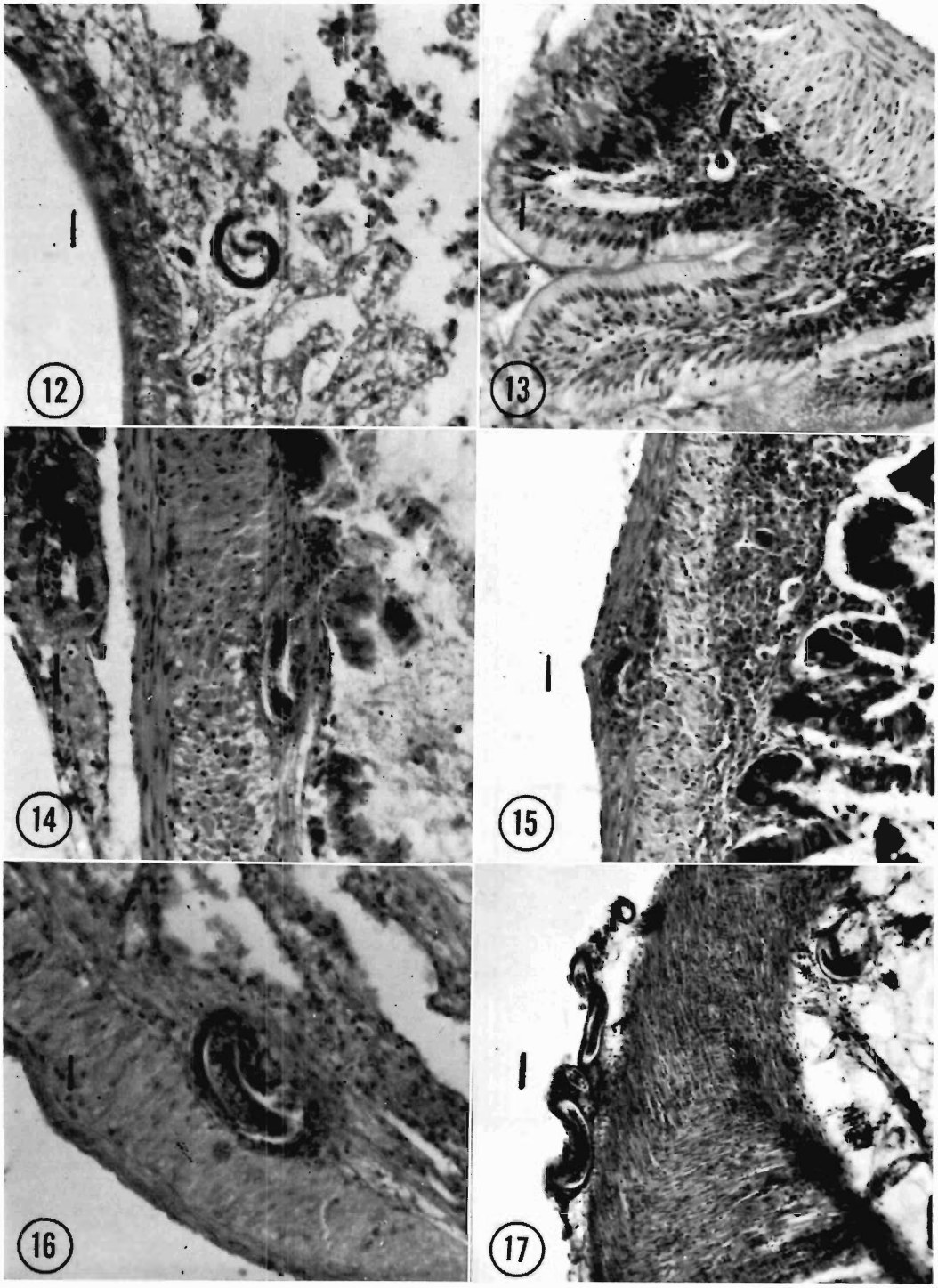
Figures 7–11. ***Parafilaroides decorus*, third-stage larva. 7. Lateral aspect. 8. Cephalic region, dorsal aspect. 9. Posterior of esophagus, excretory pore, duct and excretory sinus. 10. Caudal region, lateral aspect. 11. Cross section.**



Studies of the egg and larvae (Table 1)

EGG (Fig. 1). Twenty eggs removed from two female worms measured 49–68 (57) long by 39–49 (42) wide. The egg capsule is similar to that described by Anderson (1962) for *Perostrongylus pridhami* and *Filaroides martis* in that it consists of two delicate layers, an outer chorion and an inner vitelline membrane.

FIRST-STAGE LARVA (Figs. 2–4). The first-stage larva has a sharp spike-like tail surrounded by loose cuticle. The buccal cavity is a narrow tubular channel connecting the oral opening to the anterior end of the esophagus. A well-developed esophagointestinal valve is present.

SECOND-STAGE LARVA (Figs. 5–6). The second-stage larva is longer and thicker and its body is more granular than the first-stage larva. The genital primordium is larger; the tail pointed but not spikelike; the excretory duct more clearly defined than in the preceding stage; and the first-stage cuticle is retained after the moult.

THIRD-STAGE LARVA (Figs. 7–11). The third-stage larva is slender and serpentine with lateral alae which extend nearly the entire length of the body, beginning approximately 9 μ from the cephalic extremity and extending to within 5 μ of the caudal extremity. The tail is blunt and has a terminal knob; both first-and second-stage larval cuticles are usually retained; the stoma is narrow, leading into a dilated buccal-area followed by the thickened walls of the anterior esophagus. The excretory duct leads to a fusiform excretory sinus at the level of the esophageal glands. The esophagus, intestine, and genital primordium are similar in morphology to those of the first two larval stages.

Discussion

Transmission of *P. decorus* to sea lions at the San Nicolas Island rookery takes place by their ingestion of infected opaleye from contaminated pools. A paratenic host is apparently not required, as sea lions feed directly on the opaleye. A rookery situation is probably ideal for transmission. In these areas there are a high concentration of sea lions, resultant heavy contamination of pools with feces, and an abundance of coprophagous fish to serve as intermediate hosts. Under these conditions sea lions can acquire massive infections of this lungworm.

Animals for exhibition are usually caught at or near rookery areas and bring the infection into captivity with them. Those with heavy infections usually succumb when stressed during rigorous training and show schedules while being maintained on a set diet.

This is the first metastrongyloid life cycle wherein a vertebrate has been demonstrated to act as the first intermediate host. This finding suggests that other metastrongyloids of marine mammals may have a similar life cycle.

Acknowledgments

The author wishes to express his appreciation to Dr. John Simpson, Dr. Sam Ridgway and Mr. Bill Gilmartin of the Naval Undersea Research and Development Center, Marine Bioscience Facility, Point Mugu, California for their invaluable help throughout this study. I am grateful to Dr. Leo Margolis, Fisheries Research Board of Canada and Dr. Roy C. Anderson, University of Guelph for reviewing the manuscript and contributing helpful suggestions. Also, sincere thanks to Mr. Dwight Mudry and Mrs. Lorraine Peterson for their technical assistance.

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———. 1963. Further studies on the taxonomy

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Figures 12–17. ***Parafilaroides decorus.*** **Photomicrographs showing location of larvae in *Girella*. 12–14. First-stage larva. 12. In intestinal lumen 24 hours post-infection. 13. In intestinal mucosa 72 hr post-infection. 14. In intestinal circular muscle layer 5 days post-infection. 15–16. Second-stage larva. 15. In longitudinal muscle layer 18 days post-infection. 16. In process of encapsulation in mucosa 21 days post-infection. 17. Third-stage larvae in serosa and mesenteric fat 36 days post-infection.**

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RESEARCH NOTE

In vitro and in vivo Excystation of *Clinostomum marginatum* (Trematoda) Metacercariae

Incidental to studies on the development of metacercariae of *Clinostomum marginatum* from frogs in the chick and on the chorioallantois (Fried and Foley, 1970, J. Parasit. 56: 332–335), observations on in vitro and in vivo excystation were made and are reported herein.

In in vitro excystation studies 10 cysts were placed in petri dishes containing either 15 ml of 1% pepsin 1-10,000 (Nutritional Biochemicals Co., Cleveland, Ohio) in Ringer's (Paul, 1960, Cell and Tissue Culture, Williams, Baltimore) adjusted to pH 2.3 with HCl, 0.5% pepsin-Ringer's-HCl-pH 2.3, 0.1% pepsin-Ringer's-HCl-pH 2.3, Ringer's-HCl-pH 2.3, 0.1% pepsin-Ringer's, or Ringer's and maintained at 40 C for up to 2 hr. Table 1 summarizes the data and reveals that 100% excystation occurred within 30 min in either 1% or 0.5% pepsin-Ringer's-HCl-pH 2.3.

In in vivo excystation studies 5 cysts were fed in 1 to 2 ml of Ringer's to each of five day-old unfed chicks which were necropsied 10 to 60 min later. Table 2 summarizes the data and reveals that excysted metacercariae were present in the proventriculus and the esophagus within 10 min and in the mouth cavity within 20. Excysted metacercariae were not recovered in the gizzard, trachea, or duodenum and those in the esophagus were migrating anteriorly along the mucosal surface. Although some excystation may have resulted from cyst damage in the mouth cavity, most metacercariae probably excysted in the proventriculus where acid-pepsin is present.

Table 1. Summary of in vitro excystation of *C. marginatum* in pepsin-Ringer's-HCl or Ringer's-HCl at 40 C (ten cysts/solution).

Time in min.	1% pepsin pH 2.3	0.5% pepsin pH 2.3	0.1% pepsin pH 2.3	Ringer's pH 2.3
10	1	0	1	2
20	5	4	0	0
30	4	6	5	0
40	–	–	0	0
50	–	–	0	0
60	–	–	1	0
120	–	–	1	6
Total	10	10	8	8

Zero excysted in 0.1% pepsin in Ringer's (unadjusted).
Zero excysted in Ringer's (unadjusted).

Table 2. Summary of observations on excysted metacercariae in chicks each fed 5 cysts of *C. marginatum*.

Host	No. of min. post-exposure	Mouth cavity	Esophagus	Crop	Proventriculus
A	10	0	1	1	2
B	20	1	1	0	2
C	30	1	2	0	2
D	45	0	0	2	0
E	60	2	0	0	2

Hemenway (1948, Iowa Acad. Sci. 55: 375–381) reported excystation in *Clinostomum* sp. metacercariae at 37 C in acidified pepsin for 1 hr followed by neutral trypsin for 10 to 15 min. In the present study rapid and efficient excystation in vitro in acidified pepsin and the absence of excysted metacercariae posterior to the chick's proventriculus indicate that neutral trypsin is not necessary for excystation in *C. marginatum*.

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The Labidocarpid Bat-mites of the United States (Acarina: Listrophoridea)[1]

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ABSTRACT: Five species of three genera of labidocarpid bat-mites are recorded from the United States. Descriptions are given of two new species. The new species and type hosts are *Olabidocarpus whitakeri* sp. n. from *Myotis austroriparus mumfordi* Rice and *Olabidocarpus lawrencei* sp. n. from *Tadarida brasiliensis mexicana* (Saussure). *Dentocarpus macrotrichus* Dusbabek and Cruz is recorded for the first time from the Nearctic realm from *Tadarida brasiliensis mexicana* and a redescription of the genus *Olabidocarpus* Lawrence is given to accommodate *O. lawrencei* sp. n.

The first records of bat-mites belonging to the family Labidocarpidae from the United States were by Pinichpongse (1963b) and McDaniel and Lawrence (1964). Pinichpongse (1963b) described a new species, *Alabidocarpus longipidus*,[2] from the Yuma bat, *Myotis yumanensis saturatus* Miller from California. McDaniel and Lawrence (1964) described a new species, *Olabidocarpus americanus* McDaniel and Lawrence, from the yellow bat, *Lasiurus intermedius* H. Allen from Texas.

Five species of Labidocarpidae, associated with four hosts, have been collected. These five mite species, two of which are new, represent three genera from the United States.

Genus *Alabidocarpus* Ewing

Alabidocarpus Ewing, 1920. A Manual of External Parasites, p. 188. Pinichpongse, 1963. Acarologia, 52(2): 266 (complete bibliographical synonymy). This genus has a world-wide distribution with all previously known species having been recorded from bats of the families Rhinolophidae, Vespertilionidae, and Phyllostomatidae. The United States material is from the family Vespertilionidae, subfamily Vespertilioninae.

Alabidocarpus calcaratus Lawrence

Alabidocarpus calcaratus Lawrence, 1952. Parasitology, 34(1): 136–143. Zumpt, 1961. The Arthropod Parasites of Vertebrates in Africa South of the Sahara. South African Institute for Medical Research, 11(1): 312. Pinichpongse, 1963b. Acarologia, 5(2): 272. *Alabidocarpus longipilus* Pinichpongse, 1963b. Acarologia, 5(2): 275.

United States material examined: From *Myotis yumanensis saturatus*, Lucern, Lake County, California, holotype female of *A. longipilus* Pinichpongse. Additional material studied to establish synonymy: from *Myotis tricolor*, Town Bush Cave, Pietermaritzburg, South Africa, holotype of *A. calcaratus* Lawrence. From *Myotis myotis nigricans* 4.5 km N 2 km E Jalapa, 680 m Nueva Segovia, Nicaragua, 7 females, 10 males, 5 copulatory females.

Remarks: *Alabidocarpus calcaratus* is a distinctive species due to the presence of a pair of short, incrassate lanceolate spines located between the epimera of legs IV. *Alabidocarpus furmani* Pinichponges, the only other *Alabidocarpus* species recorded from the Western Hemisphere, has been recorded from bats of the family Phyllostomidae, subfamily Glossophaginae, from the West Indies, Nicaragua, Venezuela, and Peru.

Genus *Dentocarpus* Dusbabek and Cruz

Dentocarpus Dusbabek and Cruz, 1966, Poeyana, series A (31): 2.

Type species: *Dentocarpus silvai* Dusbabek and Cruz, 1966.

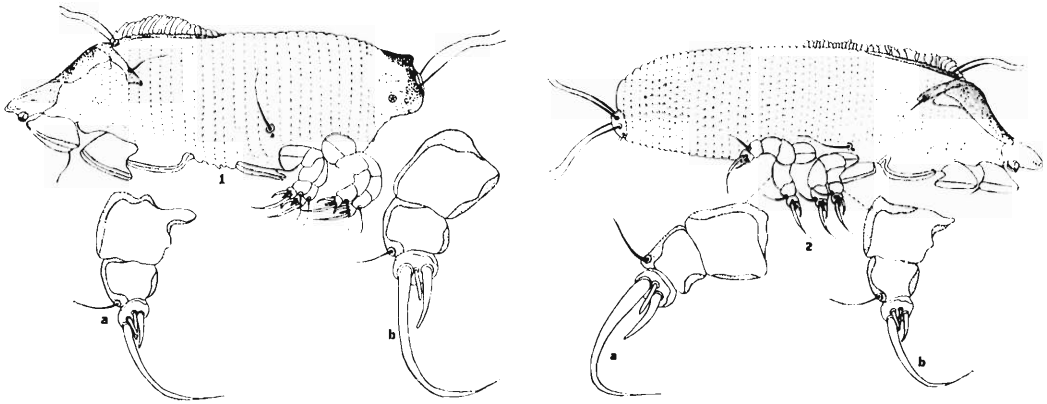
Dentocarpus macrotrichus Dusbabek and Cruz

Dentocarpus macrotrichus Dusbabek and Cruz, 1966. Poeyana, series A (31): 7.

United States material examined: From

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as journal series No. 947. Accepted for publication.

[2] McDaniel states in "The labidocarpid batmites of Nicaragua," Acarologia (In Press), that *Alabidocarpus longipilus* Pinichpongse is a synonym of *A. calcaratus* Lawrence.



Figures 1, 2. *Olabidocarpus whitakeri* sp. n. 1. Holotype male. (a. enlarged view of leg III; b, enlarged view of leg IV.) 2. Allotype female. (a, enlarged view of leg III; b, enlarged view of leg IV.)

Tadarida brasiliensis mexicana, Kleberg County, Kingsville, Texas, 4 females, 1 male, 1 nymph. Host a female, collected 26 June 1963 by B. McDaniel.

REMARKS: *Dentocarpus macrotrichus* was described from *Tadarida brasiliensis muscula* from Cuba. This record extends the known distribution of both the genus *Dentocarpus* and species *D. microtrichus* to include the Nearctic realm of the Western Hemisphere. *D. silvai*, the only other species within the genus *Dentocarpus*, was recorded from a host of the genus *Molossus* from Cuba.

Genus *Olabidocarpus* Lawrence

Olabidocarpus Lawrence, 1948. J. Parasitol., 34: 375. Pinichpongse, 1963. Acarologia, 5(3): 402. McDaniel and Lawrence, 1964. Acarologia, 6(1): 163.

This genus appears to have a world-wide distribution. At present, records show that of the five described species, two are from Africa, *O. cristatus* (Lawrence), *O. tanganyikensis* (Pinichpongse); one from Europe, *O. belsorum* (Eyndhoven) (the type of the genus *Olabidocarpus*), one from the Uniteed States, *O. americanus* McDaniel and Lawrence; and *O. aitkeni* Pinichpongse from Trinidad.

REMARKS: The discovery of two new species of the genus *Olabidocarpus*, one from *Myotis austroriparius mumfordi* firmly establishes this genus as present on bats occurring in the Nearctic realm of the Western Hemisphere.

Olabidocarpus whitakeri sp. n. (Figs. 1–3)

MALE: Body laterally compressed, having numerous fine annulations, (27+ in number); skin unsclerotized except for gnathosoma, anterior propodosomal plates, coxal apodemes and opisthosomal region. Legs I and II highly modified, of usual labidocarpid type, i.e., with plates flaplike, dilated distally, fitting around hair of host. Legs III and IV each with four segments, those of leg III somewhat thicker than those of IV. Legs III with main single claw tapering to a point, shorter than main claw of Leg IV, longer than accessory spurs; three accessory spurs, shorter than main claw of leg IV, outer accessory spur thicker than two inner spurs; all have furrowed inner surface, knife-like, with saw-toothed apex. Keel-like chitinous bar anterior to legs III, forming a v-shaped structure that aids in clasping host's hair. Prominent setae located on posterior side at distal portion of tibiae. Legs IV with main claw thicker at base than base of main claw of legs III, with two accessory spurs. Inner spurs similar in structure to inner spur of legs III, with outer surface furrowed, curved, and apex bluntly rounded. Outer accessory spur much longer than inner accessory spur, curved, with apex bluntly rounded, inner surface with fine furrowed surface. Prominent setae located on posterior side at distal portion of tibiae similar to same setae on legs III. Chelicerae small occupying the whole of the gnathosomal region,

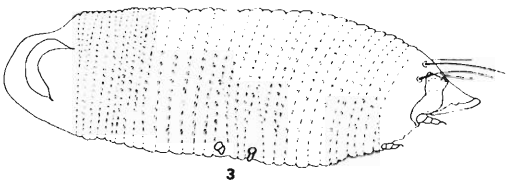
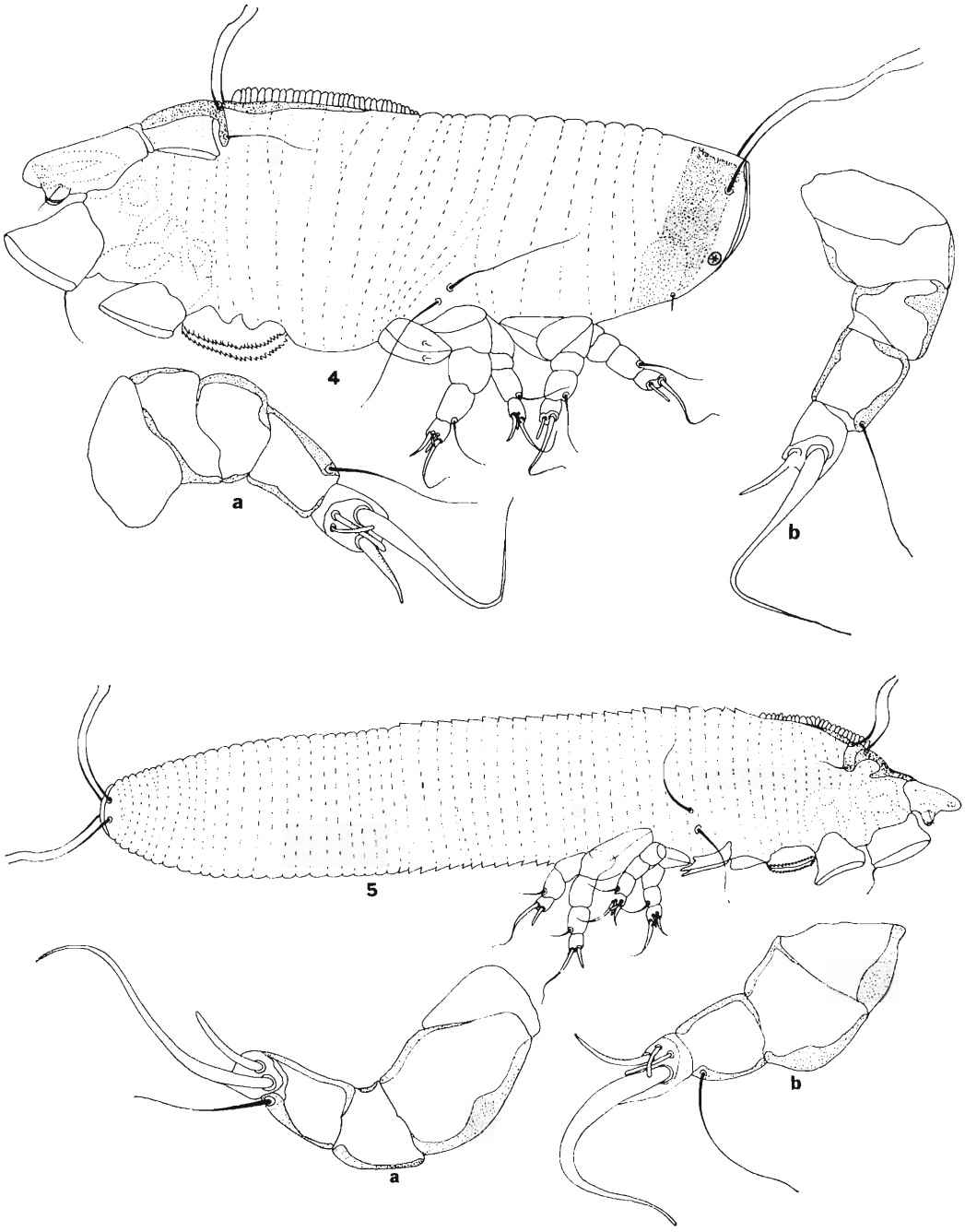


Figure 3. ***Olabidocarpus whitakeri*** **sp. n., Copulatory female.**

terminating in a single tooth at apex. Gnathosoma produced into a sharp point. Propodosomal plate elongate, with two lateral unisetose projections. Median propodosomal plate projections consisting of a pair of long narrow rodlike plates separated from main propodosomal plate, with a pair of setae at anterior apex, these plates separated along midline by a manelike crest composed of large annulations which extend backwards to posterior region of plate. Setae associated with propodosomal plate and its rodlike median plate with large setae extending beyond body. Two pairs of unequal lateral setae placed in proximity of coxa of leg III, dorsal pair much larger and longer than ventral pair, their length reaching to apodemes and coxae of legs IV. Ventral lateral setae small, length less than or equal to the diameter of dorsal setae's base. Ventrolateral setae's base similar in structure to dorsolateral and propodosomal setae except smaller. Anal region of body with pair of anal suckers, two pairs of anal setae, dorsal pair large, longer than all other body setae; ventral pair small, similar in structure and size to setae on tarsus of legs III and IV; both setae located at most posterior region of opisthosomal region. Opisthosomal region sclerotized over entire dorsal area of opisthosoma. Opisthosomal plate covering posterodorsal region of opisthosoma with lateral projection extending to ventral anal setae. Other body setae as shown in Fig. 1. Length 251 μ, width 83 μ, (measured between second and third pair of legs).

FEMALE: Body laterally compressed, numerous fine annulations (53+ in number); skin unsclerotized except for gnathosoma, propodosomal plates, and coxal apodemes of all legs. Legs I and II modified as in male. Legs III and IV with same type of main tarsal claws and accessory spurs as male, and with same setal association. Gnathosoma similar to male, not as pointed at anterior apex, chelicerae similar to male, longer, with single tooth at apex. Propodosomal plate similar to male except larger, with similar lateral projections and paired median rodlike plates. This median plate saw-toothed dorsally. Propodosomal plate setae at apex of lateral projections and anterior apex of median serrated plates. Median plates separated by a manelike annulated crest composed of large annulations which extend backwards to coxa of legs IV. Lateral setae similar to male's, the dorsal pair larger and much longer than ventral pair. Length of ventral setae equal to or less than diameter of dorsal setae. Apex of abdomen blunt except at ventral region where anal setae are located, this area forming a rounded knob. Two pairs of anal setae, dorsal pair longer than ventral pair. Opisthosomal region without sclerotization and sclerotized anal plate. A pair of setae smaller than anal setae located on venter below rounded knob which contains large anal setae. Other body setae as shown in Fig. 2. Length 344 μ, width 97 μ, (measured between II and III pair of legs).

COPULATORY FEMALE: Body elongated, without indication of developing female. Thirty-four or more body annulations, similar in structure to those of male and female. Gnathosoma well developed, elongated, pointed at apex. Chelicerae reduced. Propodosomal plate reduced, without narrow rodlike paired plates, with two pairs of setae similar to those of male and female. These are located in same area as those located on anterior apex of rodlike plates of male and female. Setae associated with lateral projection of propodosomal plate of adults absent. Lateral setae absent. Legs I developed similarly to legs III of adult, with four segments and a main claw. Legs II similar in structure to legs I, much reduced, segments not delineated, apparently with three segments, main claw present. Keel-like chitinous region, similar to keel between legs II and III of adults, present. Two pairs of papillae, located in middle region of copulatory tritonymph, small, apparently lacking spines. These two pairs of papillae, when compared with other copulatory tritonymphs, would be located in approproximately the same region as the third and fourth pair when four papillae are found. Opisthosomal dorsal region without annula-



Figures 4, 5. ***Olabidocarpus lawrencei*** **sp. n. 4. Holotype male. (a, enlarged view of leg III; b, enlarged view of leg IV.) 5. Allotype female. (a, enlarged view of leg III; b, enlarged view of leg IV.)**

tions. This copulatory tritonymph is still attached to the male, making the study of the opisthosomal area difficult, even under the high oil immersion lens. However, there appears to be a winglike process clasping the sclerotized region of the male. This process has been included in the drawing but its true shape is not certain due to the difficulty in studying this region. Length, 232 μ, width, 77 μ, (measured at site of first papillae).

LARVA: Not available for study.

TYPE MATERIAL: Holotype male, collection number, JOW-5211 with attached copulatory tritonymph collected by J. O. Whitaker and R. E. Mumford from *Myotis austroiparius mumfordi*, Lawrence County, Donneheues Cave, Indiana, 20 August 1969, host number JOW-5211. Allotype female, collection number, JOW-5211, with same data as holotype, together with five female paratypes, collection numbers: JOW-5209, 2 females; JOW-5210, 1 female; JOW-5211, 2 females. Holotype (with copulatory tritonymph), allotype, and single paratype female all from host JOW-5211, in United States National Museum, one female paratype, JOW-5209 in collection of John O. Whitaker, Jr., Department of Life Sciences, Indiana State University, Terre Haute, Indiana. Remaining material in collection of the senior author.

Olabidocarpus whitakeri sp. n. is distinguished from all other members of the genus *Olabidocarpus* by the very small size of the ventrolateral setae, type and structure of annulations contained in annulated crest, and serration of dorsal region of median propodosomal plate. This species is named in honor of its collector, Dr. John O. Whitaker, Jr., Associate Professor of Life Science, Department of Life Sciences, Indiana State University.

Olabidocarpus lawrencei sp. n.
(Figs. 4 & 5)

MALE: Body laterally compressed, having numerous fine annulations (27+ in number); skin sclerotized except for gnathosoma, anterior propodosomal plates, coxal apodemes and opisthosomal region. Legs I and II highly modified, of usual labidocarpid type, i.e., with plates flaplike, dilated distally, fitting around hair of host. Legs III and IV each with four segments, those of leg III somewhat thicker than those of IV. Legs III with single main claw, ending in a fine hairlike point, much longer and thicker than three accessory spurs. Inner accessory spur larger than other two, knifelike with furrowed curved outer surface, apex pointed. Base of this accessory spur approximately as thick as base of main claw. The other two accessory spurs equal in size and shape, located dorsally from base of main claw and inner accessory spur and on outer margin of leg; apex rounded with a blunted appearance. Each of these spurs with their inner surface having very fine furrowed inner surfaces; without keel-like clasping structure seen in *O. whitakeri* and *O. americanus*. Prominent setae located on posterior side of distal portion of tibia. One pair of setae on tarsus adjacent to and above pseudoarticulation on dorsal surface. Legs IV with main claw longer than main claw of legs III, thicker at base than base of main claw of legs III, with one accessory spur. Spur similar in structure to outer spur of legs III, with outer surface furrowed curved and apex ending in a fine point. Prominent seta similar in structure as same seta on legs III located on posterior side of distal portion of tibia. A small pair of setae associated with and enclosed within the apodemes of legs III. Chelicerae well developed occupying whole of the gnathosomal region, terminating in two digits bearing teeth. Ventral portion of gnathosoma with two flaplike structures used to clasp hair, beset with a pair of setae. Propodosomal plate elongate, with two lateral projections, each beset with a single setae. Median propodosomal plates consisting of a pair of long narrow rod-like plates separated from main propodosomal plate, with a pair of setae at anterior apex. These plates separated along midline by a manelike crest composed of large annulations which extend backwards to approximately level of apodemes of legs III. Median propodosomal plates distinctly separated from main propodosomal plate with lateral projections. Setae associated with propodosomal plate and median projections with large base. Body with two pairs of lateral setae; these equal in size and length, located near apodemes of legs III; base of seta large, similar to those of propodosomal plate setae. Anal region of body with a pair of anal suckers. Two pairs of anal setae, dorsal pair longer than opisthosomal region of body. Ventral anals small located below anal suckers. Opisthosomal

region sclerotized forming an opisthosomal plate. Anal suckers not associated with plate, anal setae located on margin of posterior section of opisthosomal plate. Other body setae as shown in Fig. 4. Length, 325 μ, width, 103 μ, (measured between second and third pair of legs).

FEMALE: Body laterally compressed, having numerous fine annulations (63+ in number); skin sclerotized except for gnathosoma, propodosomal plates and coxal apodemes of all legs. Legs I and II modified as in male. Legs III and IV with same type of main tarsal claws and accessory as male and with same setal association. Gnathosoma similar to that of male, chelicerae similar to that of male, larger, with same dentition as male chelicerae. Propodosomal plate similar to that of male, larger, with same setiferous lateral projections and paired median rodlike plates; these separated by a manelike crest composed of large annulations which extend to region between legs II and III. Lateral setae similar to male. Abdomen very long, apex rounded, without a rounded knob-like region. Two pairs of anal setae, longer than lateral and propodosomal plate setae. A keel-like chitinous structure located between legs II and III, closer to legs II, v-shaped, well developed and modified for clasping, present in female but absent or not well developed on male. Other body setae as shown in Fig. 5. Length 530 μ, width 100 μ, (measured between legs II and III).

COPULATORY FEMALE: Not seen.

LARVA: Not seen.

TYPE MATERIAL: Holotype male, collection number, BMD-1001 collected by B. McDaniel from *Tadarida brasiliensis mexicana*, Kleberg County, Kingsville, Texas under tile of Texas A & I Union building, 26 June 1963, host-female (discarded). Allotype female, collection number, BMD-1002 from same host as holotype. Holotype and allotype in collection of senior author.

Olabidocarpus lawrencei sp. n. is distinguished from all other members of the genus *Olabidocarpus* by the presence of only a single accessory spur associated with main claw of legs IV. The genus *Olabidocarpus* was erected by Dr. R. F. Lawrence, a renowned authority of this group of mites; thus, it is fitting that this new species herein described be named in honor of him in recognition of his contribution to the study of listrophorid mites.

REMARKS: The discovery of a species of *Olabidocarpus* with only a single accessory spur associated with the main claw of leg IV broadens the generic concept of the genus. This genus was characterized by McDaniel and Lawrence (1964) as containing three and two accessory spurs on legs III and IV. The generic characterization must now be revised as follows to accommodate *O. lawrencei* which contains only a single accessory spur on leg IV.

Olabidocarpus Lawrence

Tarsus of legs III with three accessory spurs, tarsus of legs IV with either one or two accessory spurs, if only a single spur present, always with a distinct annulated crest between propodosomal median plates.

Acknowledgments

I express my appreciation to Dr. J. O. Whitaker, Jr., Department of Life Sciences, Indiana State University, for data concerning hosts and for collecting host and parasite material.

Key to Genera and Species of Labidocarpidae in the United States

1. Propodosomal setae minute, barely exceeding length of base ..... *Alabidocarpus*
   One species presently recorded from the United States, *A. calcaratus* Lawrence.
   Propodosomal setae well developed, extending beyond body region in all species 2
2. Propodosomal region with a distinct annulated crest on dorsal edge between median narrow rodlike plates 3
   Propodosomal region without a distinct annulated crest, propodosomal plate divided into two parts, anterior part bifid, posterior part with lateral arms bearing setae ..... *Dentocarpus* Dusbabek and Cruz
   One species known from the United States, *D. macrotrichus* Dusbabek and Cruz.
3. Leg IV with only a single spur associated with main claw .....

---------- *Olabidocarpus lawrencei* sp. n.

Leg IV with two spurs associated with main claw ---------- 4

4. Ventrolateral setae small in length less than or equal to the diameter of dorsolateral setae's base ----------
---------- *Olabidocarpus whitakeri* sp. n.

Ventrolateral setae much longer than diameter of setae's base ----------
Olabidocarpus americanus McDaniel and Lawrence

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RESEARCH NOTE

Ochetosomatid Trematodes from Snakes in North Dakota and Illinois

In the course of parasitological examination of amphibians and reptiles from different habitats in North Dakota, 20 flukes of the genus *Lechriorchis* were found in the lungs of 2 of 10 western hognose snakes, *Heterodon nasicus* Baird and Girard, collected during the summers of 1968 and 1969 in Ward County, north central North Dakota. These specimens agree with the description of *L. tygarti* Talbot 1933 from the lungs of garter snakes, *Thamnophis sauritus* (Linné) and *T. sirtalis* (Linné) and show no significant differences in the values established except for the length of the eggs. Eggs measure 45–53 μ and 33–42 μ long for *L. tygarti* and the North Dakota specimens respectively. A review of the data recorded for other species of this genus did not reveal such a wide range in egg length. Since there have been very few records on the occurrence of *Lechriorchis* in snakes subsequent to the descriptions of new species further data are necessary to ascertain whether or not egg size is influenced by the species of host.

Fantham and Porter (1934, Proc. Zool. Soc. London, 123: 867–898) reported 2 "*Lechriorchis* near *tygarti*" from the lungs of a garter snake, *T. ordinoides* (Baird and Girard), collected in southern Saskatchewan. Comments on taxonomic differences were not given in this report.

This represents the first report of *Lechriorchis* from North Dakota. The host, *H. nasicus*, represents a new host record.

The Helminthological Collection, Department of Zoology, Southern Illinois University, contains, among other helminths, several specimens of ochetosomatid trematodes collected from snakes by Lorraine P. Moran in various counties of southern Illinois.

Measurements of 15 sexually mature specimens from the lungs of an eastern hognose snake, *H. platyrhinos* Latreille, collected during 1960 in Williamson County, fall within the range of measurements of *Ochetosoma elongatum* (Pratt, 1903) as given by Dubois and Mahon (1959, Bull. Soc. Neuchâtel Sci. Nat. 82: 191–229).

A single specimen from the mouth of a gray rat snake, *Elaphe obsoleta spiloides* Duméril, Bibron, and Duméril, collected on 8 May 1966 and a single specimen from the esophagus of *Lampropeltus* sp. collected on 19 October 1964 in Williamson County were identified as *O. kansense* (Crow, 1913).

Two specimens from the mouth of *H. platyrhinos* collected on 10 May 1966 in Jackson County were identified as *O. ellipticum* (Pratt, 1903).

All of the above mentioned parasites collected in southern Illinois represent new locality records. The host, *E. o. spiloides*, represents a new host record for *O. kansense*.

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Effect of Management Systems on the Growth of Lambs and Development of Internal Parasitism. IV. Field Trials with Lambs on Drylot and Pasture Involving Medication with Thiabendazole and Purified Micronized Phenothiazine

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ABSTRACT: Observations were made for the fourth grazing season (April through September of 1962) of the effect of management systems, including medication, on the development of internal parasitism in lambs. The systems included drylot confinement (Band 1), separate grazing of lambs and their dams on "clean" pasture (Band 2), and rotation among separate, contaminated pastures at approximately biweekly intervals (Bands 3 and 4). Purified phenothiazine (average particle size 2–3 μ) was used therapeutically in Band 3 and thiabendazole in Band 4. Lambs of Bands 1, 2, and 3 had free access to a 1:9 phenothiazine-mineral mixture, while lambs of Band 4 had free access only to the unmedicated mineral mixture. Antemortem data, including hematocrit determination (PCV) and parasite egg counts, were obtained from 20 lambs from each band. Worm counts, obtained at necropsy from 10 lambs of each band, showed that lambs in drylot remained virtually parasite-free and made excellent weight gains. Lambs grazed separately from their dams remained practically parasite-free except for *Strongyloides papillosus*. Lambs grazed on contaminated pastures developed clinical parasitism despite the treatment regimens employed. During the period of clinical parasitism in Bands 3 and 4 (phase 2), PCV were significantly higher for Band 1 than for the others. The mean hematocrit values were lowest for Band 4; in Bands 2 and 3, the values were about equal and at a level intermediate between Bands 1 and 4. Band 1 lambs made significantly greater weight gains than those in Bands 2, 3, and 4. However, rather severe drought during the grazing season adversely affected forage growth and undoubtedly contributed to the poor weight gains for animals in Bands 2, 3, and 4. As used in these trials, thiabendazole was less effective than phenothiazine against the major pathogen, *Haemonchus contortus*, but more effective than the latter drug against *S. papillosus*. Thiabendazole seemed to be superior also against *Trichostrongylus* spp. and *Nematodirus* spp., but the data on these parasites were too limited to permit more than provisional comparisons.

Previous reports (Lindahl et al., 1963, 1970; Colglazier et al., 1968) on the effect of different management systems for the control of internal parasitism in lambs emphasized the importance of good husbandry practices coupled with the judicious use of antiparasitic chemicals. For many years, phenothiazine was the unquestioned drug of choice for the treatment of parasitism in sheep; but because certain *Haemonchus* populations were less responsive than expected to commonly used commercial phenothiazine products (Drudge et al., 1957a, 1957b; Leland et al., 1957; Enzie et al., 1960; Levine and Garrigus, 1961), the need for alternative methods of chemical control seemed incontrovertible. Thiabendazole (Brown et al., 1961) showed considerable promise in this regard since it apparently combined a wide margin of safety with broad spectrum activity against the common gastrointestinal helminths of sheep (Cuckler, 1961).

It seemed desirable, therefore, to compare the net usefulness of thiabendazole and purified, micronized phenothiazine when used under similar conditions for the control of parasitic gastroenteritis in sheep. Observations were continued on the development of internal parasitism in lambs confined in drylot. The trials were conducted at the Agricultural Research Center, Beltsville, Maryland, during the 1962 grazing season.

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Materials and Methods

The general plan was similar to that followed in previous studies (Lindahl et al., 1963, 1970; Colglazier et al., 1968) and involved three basic management systems: drylot confinement (Band 1); grazing lambs on "clean" pastures separately from their dams (Band 2); and grazing lambs with their dams on contaminated pastures until weaning (Bands 3 and 4). In the present study, purified phenothiazine[5] was used for animals in Bands 1, 2, and 3; thiabendazole[6] was used in Band 4.

During the pretrial period, all lambs were creep-fed with pellets containing 65% alfalfa meal, 30% ground barley, and 5% soybean oil meal.

On 14 April, when the lamb crop averaged about 60 days of age, 312 lambs and their dams were randomly assigned within age, breed, and sex into four approximately equal bands. Each band contained lambs of the following breeds and strains: Hampshire, Merino, Shropshire, Targhee, Columbia-Southdale, and crossbreds (2-, 3-, and 4-way crosses involving the Hampshire, Shropshire, Southdown, and Merino breeds).

Four days before the bands were placed on their respective regimes, therapeutic doses of 30 grams purified phenothiazine were given to all mature ewes of Bands 1, 2, and 3. Band 4 ewes were given thiabendazole at the rate of 50 mg/kg body weight, a level approximating the standard dosage. Ewes weighing less than 50 kg were given a drench containing 2.5 g thiabendazole. Ewes weighing 50 to 60 kg were given 3.0 g; and those weighing over 60 kg were given 3.5 g. No other therapeutic treatments were given until after all lambs were weaned. A 1:9 phenothiazine-mineral mixture was available free-choice at all times to lambs and dams of Bands 1, 2, and 3. Only the basic mineral mixture containing 65 parts iodized salt, 20 parts dicalcium phosphate, and 5 parts magnesium carbonate was provided for the lambs and dams of Band 4.

[5] The phenothiazine used in all therapeutic and free-choice regimens was a wettable (wetting agent, 1% lecithin by weight), purified (99.9%) product with an average particle size of about 2–3 μ, and was supplied through the courtesy of Atomic Basic Chemicals, Inc., Pittsburgh, Pennsylvania. Information on particle size, as determined by the Fisher Sub-Sieve Sizer, and purity, as determined by the Association of Official Agricultural Chemists method, was also provided by the company.

[6] Thibenzole: Merck & Co., Rahway, New Jersey.

Therapeutic treatments were administered to lambs of the different bands on the dates shown and at the levels indicated in Tables 1a and 1b. Dosing was carried out when the *Haemonchus* egg count for the band averaged at least 1000 EPG, as determined from the surveillance samples.

Lambs in Band 1 were kept in a barn or drylot at all times. From 14 April until the lambs were weaned at approximately 120 days of age, the ewes were separated from the lambs at 8 AM daily. The ewes were then allowed to graze from 8 AM to 4 PM when they were returned to their lambs until the following morning. The lambs were creep-fed pellets, *ad lib.*, and a limited amount of hay until they were weaned. After weaning, only enough feed was given to allow for a moderate rate of gain.

Until weaned, the lambs of Band 2 were grazed alternately between two pastures (approximately 1 hectare) that had not been used by sheep since they were renovated (plowed and reseeded) at the end of the previous grazing season. After weaning, the lambs were rotated among four pastures (1.94 to 2.9 hectares), three of which had been used for 2-week periods by the "clean" pasture band during the latter part of the previous grazing season (Lindahl et al., 1970). The lambs were allowed to nurse in drylot from 4 PM to 8 AM daily until weaned at approximately 120 days of age.

Band 3 lambs and their dams grazed the same four pastures and in the same sequence as the Band 3 animals the preceding year (Lindahl et al., 1970). The sheep were rotated every 2 weeks among the contaminated pastures; and they were not returned to a previously grazed pasture within less than 4 weeks. When required, the lambs were dosed with purified phenothiazine; both N.F. and purified phenothiazine had been used the previous year.

Band 4 lambs and their dams also grazed the same four pastures and in the same sequence as the Band 4 animals the previous year. Again, they were rotated every 2 weeks among the contaminated pastures and were not returned to a previously grazed pasture within less than 4 weeks. When required, the lambs were dosed with the thiabendazole; purified phenothiazine had been used for animals in this band during the preceding year.

Table 1a. Therapeutic treatments administered to lambs.

Date	Band	Treatment	Rate
1962			
3 July	4	thiabendazole	50 mg/kg[1]
31 July	3	2–3 μ purified phenothiazine	10–20 g[2]
	4	thiabendazole	75 mg/kg
28 August	3	2–3 μ purified phenothiazine	10–20 g
	4	thiabendazole	75 mg/kg
19 September	4	thiabendazole	100 mg/kg

[1] Approximate.
[2] Lambs under 22.7 kg were given 10 grams; all others were given 20 grams.

All lambs were weighed biweekly beginning on 14 April. Surveillance studies were initiated on 2 May and included biweekly determination of packed red-cell volume (PCV) and number of parasite eggs per gram of feces (EPG) from 15 crossbred, 3 Targhee, and 2 Hampshire lambs from each band, using lambs of both sexes. Two crossbred lambs from each band were slaughtered on 6 June, 3 July, 1 August, 29 August, and 26 September (40 lambs in all) for necropsy worm counts. Procedures employed were identical to those described previously (Lindahl et al., 1963, 1970; Colglazier et al., 1968).

All lambs were weaned by 3 July. At this time, some lambs were removed from each band for other studies. The remaining ewe and ram lambs of each band were separated but continued on the experimental procedures.

The biweekly PCV and EPG determinations from the surveillance lambs, and the necropsy worm counts, were subjected to least squares analysis of variance (Harvey, 1960). Analysis of variance of biweekly weight gain data was applied to the surveillance lambs and to all other lambs that remained on experiment during the entire test period. The data were grouped for analysis into phase 1 (pretreatment) and phase 2 (treatment). Each phase contained several periods as shown in Table 2.

The total rainfall and temperatures at the experimental area during this study are given by periods in Table 2. Official Weather Bureau Station equipment and techniques were employed.

Results

The average levels of parasitism acquired by the lambs of Bands 1, 2, 3, and 4 were adjudged insignificant, light, light, and moderate, respectively, on the basis of PCV, EPG, levels of therapeutic treatment required, and necropsy data (Tables 1 and 2, Figs. 1–2). Clinical coccidiosis did not occur in any lamb, although coccidia were always found when routine fecal examinations were made.

Table 1b. Grams of thiabendazole administered to Band 4 lambs in therapeutic drenches at each of three dose rates.

Weight range (kilograms)	50 mg/kg	75 mg/kg	100 mg/kg
	Grams	Grams	Grams
Under 20	1.0	1.5	2.0
20 to 25	—	1.9	—
20 to 30	1.5	—	3.0
25 to 30	—	2.3	—
30 to 40	2.0	—	4.0
30 to 35	—	2.6	—
35 to 40	—	3.0	—
40 to 50	2.5	—	5.0
40 to 45	—	3.4	—
45 to 50	—	3.8	—
50 to 60	3.0	—	6.0

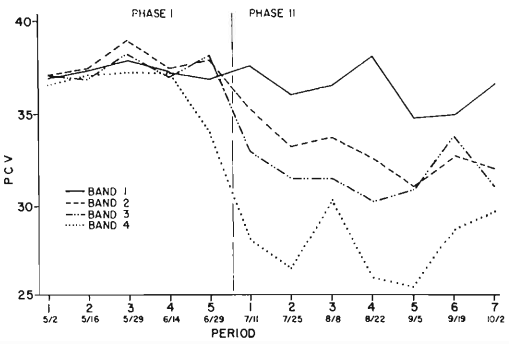

Figure 1. Comparison of packed red-cell volumes by bands.

Table 2. The total rainfall and temperature by experimental periods during the surveillance studies.

Phase	Period	Dates	Rainfall Cm	Temperature C Mean	Temperature C Range
1	1	14 April–2 May[1]	1.96	13.4	–2.2–31.1
	2	3 May–16 May	0.66	15.6	–0.6–34.4
	3	17 May–29 May	3.15	21.1	11.1–35.0
	4	30 May–14 June	4.37	21.7	11.1–31.7
	5	15 June–27 June	6.22	22.8	12.2–35.0
2	1	28 June–11 July	0.86	22.2	11.1–34.4
	2	12 July–25 July	3.20	22.8	12.8–32.2
	3	26 July–8 Aug.	0.30	23.3	10.0–33.9
	4	9 Aug.–22 Aug.	0.00	22.8	9.4–37.2
	5	23 Aug.–5 Sept.	1.88	23.3	12.8–36.6
	6	6–19 Sept.	2.54	18.9	5.5–33.3
	7	20 Sept.–2 Oct.	2.87	13.3	0.6–24.4

[1] Initial surveillance samples obtained 2 May.

The major pathogenic parasite was *Haemonchus contortus* (Table 3). No *Haemonchus* were found in any of the necropsied animals from Band 1, and only a few *Haemonchus* were recovered from lambs in Band 2 during the latter part of the test period. Band 3 lambs acquired moderate numbers of *Haemonchus* by early July, and heavier infections became established by the last of August. Band 4 lambs acquired moderate numbers of *Haemonchus* early in June and heavy infections early in July (Table 3). The largest number of *Haemonchus* was recovered from the Band 4 lambs necropsied on 1 August despite two treatments in July

Table 3. Average number of parasites recovered from necropsied surveillance lambs.

Slaughter and treatments dates	Band	No. of lambs	*Haemonchus contortus*	*Strongyloides papillosus*	Other[1]	Total
1962						
	1	2	0	0	4	4
6 June	2	2	0	25	108	133
	3	2	55	0	358	413
	4	2	320	0	1,566	1,886
3 July	4-T[2]					
	1	2	0	25	62	87
3 July	2	2	0	50	822	872
	3	2	560	175	756	1,491
	4	2[4]	1,280	975	1,198	3,453
31 July	3-P[3] 4-T					
	1	2	0	100	40	140
1 August	2	2	0	1,450	311	1,761
	3	2[4]	550	950	465	1,965
	4	2[4]	6,540	25	393	6,953
28 August	3-P 4-T					
	1	2	0	75	10	85
29 August	2	2	65	1,425	231	1,721
	3	2[4]	1,210	1,450	754	3,414
	4	2[4]	1,370	0	62	1,432
19 September	4-T					
	1	2	0	425	30	455
26 September	2	2	50	2,325	239	2,614
	3	2	90	900	305	1,295
	4	2	860	0	3	863
	1	10	0	625	146	721
Totals	2	10	115	5,275	1,711	7,101
	3	10	2,465	3,475	2,638	8,578
	4	10	10,370	1,000	3,222	14,592

[1] *Trichostrongylus* spp., *Ostertagia* spp., *Nematodirus* spp., *Trichuris* spp., and *Oesophagostomum venulosum*.
[2] Thiabendazole treatments.
[3] Phenothiazine treatments.
[4] Necropsied lambs untreated on 3 and 31 July, and 28 August when other lambs were dosed.

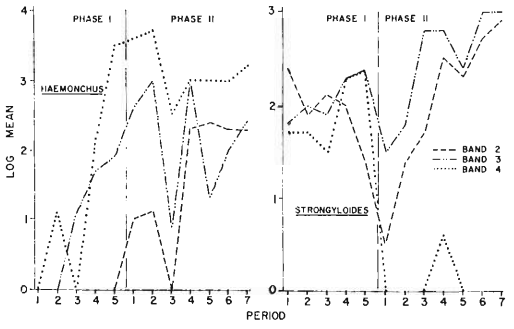

Figure 2. Logarithmic means of fecal egg counts for Bands 2, 3, and 4.

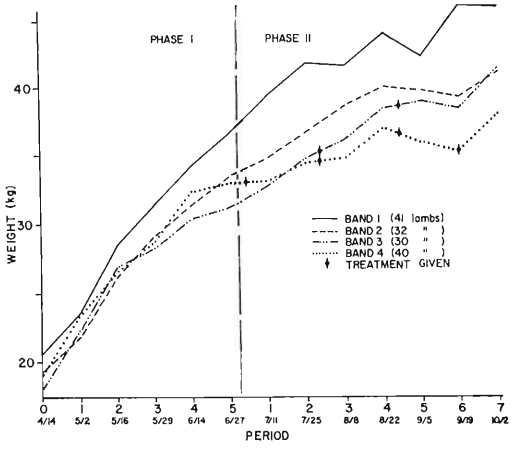

Figure 3. Comparison of lamb weights by bands.

with thiabendazole. Only a few *Strongyloides* were found in Band 1 lambs, and the numbers in Band 4 lambs were significantly lower than those in lambs from Bands 2 and 3.

Other nematodes recovered from the four bands includes *Nematodirus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Trichuris* spp., and *Oesophagostomum venulosum*. It is doubtful that these parasites were present in numbers sufficient to influence weight gains or packed red-cell volumes.

There were no significant differences between bands in PCV during phase 1, but differences were highly significant ($P < 0.01$) during phase 2 (Fig. 1). During the latter period, PCV were significantly higher ($P < 0.01$) for Band 1 than for the other bands. Band x period interactions were significant ($P < 0.05$) during phase 1 and highly significant ($P < 0.01$) during phase 2. Breed effects were highly significant ($P < 0.01$) in Bands 2, 3, and 4 during phase 2, the crossbred lambs having higher PCV values than the Hampshire and Targhee lambs.

In phase 1, no *H. contortus* eggs were found in fecal samples from lambs of Bands 1 and 2, but *Haemonchus* egg counts were significantly higher ($P < 0.05$) in Band 4 than in Band 3 (Fig. 2). During phase 2, differences in *Haemonchus* eggs counts were highly significant ($P < 0.01$) between all bands, the counts generally increased progressively from Band 1 to Band 4. The period effect and Band x period interactions were highly significant ($P < 0.01$). The fecal egg count data for *Haemonchus* roughly paralleled the necropsy worm data obtained from the surveillance lambs.

There were no significant differences between bands in *S. papillosus* egg counts during phase 1 (Fig. 2), but highly significant differences were noted during phase 2. In the latter phase, the *Strongyloides* egg counts for Bands 1 and 4 were essentially nil, but the counts in Bands 2 and 3 were comparatively high. The differences between Band 1 and Bands 2 or 3 were highly significant ($P < 0.01$), as were those between Band 4 and the latter two bands. Differences between Bands 2 and 3, however, were not significant. Egg counts for other parasite species were too low to permit analysis of variance.

The weight comparisons by bands are shown in Fig. 3. There were no significant differences between bands in phase 1, but differences were highly significant ($P < 0.01$) in phase 2. Band 1 lambs were significantly ($P < 0.01$) heavier than those in Bands 2, 3, and 4. Also, lambs in Bands 2 and 3 were significantly heavier than those in Band 4 ($P < 0.05$), but the weight differences between lambs in Bands 2 and 3 were not significant. The average weights generally paralleled the PCV and EPG values in Bands 2, 3, and 4 during phase 2 (Figs. 1–3).

Differences in mortality between bands were insignificant. No lambs were lost from Bands 1 and 4 during phase 2, and only 1 lamb each died in Bands 2 and 3.

Discussion

The management system employed for lambs in drylot effectively controlled parasitism. This finding agrees with results obtained in previous studies (Lewis et al., 1960; Hinds et al., 1961; Tiwari et al., 1963; Lindahl et al., 1963, 1970; Colglazier et al., 1968). Except for small numbers of *Strongyloides papillosus* and a few other parasites, the animals of this band remained practically parasite-free.

Providing free-choice phenothiazine and grazing lambs on relatively "clean" reseeded pastures separate from their dams (Band 2) also controlled parasitism. The animals of this band acquired only a few *Haemonchus* by the latter part of August, and only small numbers of this species were present in September. The buildup of *Strongyloides* and other parasites was minimal also.

The rotation of lambs with their dams on the same pastures (Bands 3 and 4) until the lambs were weaned resulted in a greater buildup of parasites, especially *Haemonchus*. The increment occurred more rapidly in Band 4 than in Band 3. Similar findings have been reported by other workers (Levine et al., 1956, 1958, 1960; Lewis, 1963; Michel, 1964; Zimmerman, 1965; Cameron and Gibbs, 1966; Gibson and Everett, 1968) as well as in our earlier studies (Lindahl et al., 1963, 1970; Colglazier et al., 1968).

Lambs in Bands 3 and 4 developed clinical haemonchosis, as evidenced by PCV and necropsy worm counts (Table 3, Fig. 1). The signs occurred first in early July among lambs of Band 4; at this time, the PCV had decreased to 28% and remained at comparatively low levels during the entire second phase. Clinical haemonchosis developed later in Band 3 lambs and was not as severe.

The occurrence of parasitism in lambs of Band 4 necessitated the therapeutic dosing of these animals early in the grazing season. Thiabendazole was first given in early July at the generally recommended dose level of about 50 mg per kilogram of body weight. Because the response was unsatisfactory, the dosage was increased to 75 mg/kg in late July and late August, and to 100 mg/kg in early September. The poor control of haemonchosis in this band, using thiabendazole at dose levels equal to or exceeding those commonly recommended, was noteworthy because climatic conditions during the grazing season were not especially favorable for the survival of infective larvae (Gordon, 1958: Crofton, 1963). Under these circumstances of reduced exposure to reinfection, one would expect parasite control with antiparasitic chemicals to be somewhat more effective rather than less so. Nevertheless, therapeutic dosing of these animals with thiabendazole undoubtedly prevented more serious reductions in the PCV values and probably minimized other effects of haemonchosis in the band. This was our first indication that *Haemonchus* populations might differ in their susceptibility to thiabendazole.

The Band 3 lambs were given only two therapeutic doses of phenothiazine during the grazing season, the first in late July and another in late August. The later development and lesser severity of clinical haemonchosis in this band, as compared with lambs in Band 4, was probably ascribable in part to the low-level phenothiazine mixture which was not available to lambs in Band 4. Under the conditions that prevailed, phenothiazine was superior to thiabendazole for the control of haemonchosis. Thiabendazole, on the other hand, seemed superior to phenothiazine against limited numbers of other gastrointestinal nematodes and was clearly more effective than the former against *Strongyloides* (Table 3, Fig. 2).

Lambs in Band 1 gained weight more rapidly than did those in the other bands. This fact was so despite a planned limitation of available feed for Band 1 lambs to permit only a moderate rate of gain. A rather severe drought during the grazing season had an adverse effect on forage growth and undoubtedly contributed to the poor weight gains by lambs in the three pasture groups (Fig. 3). This judgment is supported by the data on weight gains for lambs in Band 2; relatively poor growth was attained despite reasonably effective parasite control.

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Schizogony in *Toxoplasma gondii*: An Electron Microscope Study

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Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Methesda, Maryland

ABSTRACT. Schizonts of *Toxoplasma gondii* develop within vacuoles in the intestinal epithelial cells of the cat. The schizont is surrounded by a pellicle consisting of an inner and an outer membrane. There is abundant endoplasmic reticulum and ribosomes but organelles characteristic of endodyogeny are absent in the two-nucleate stage. After two or more nuclear divisions merozoites begin to form adjacent to the nuclei in a manner similar to that of endodyogeny. However, schizogony differs from endodyogeny by formation of more than two offspring per parent cell. It also differs from schizogony in other coccidia by the formation of merozoites adjacent to the nucleus rather than at the schizont surface. The adaptability of *T. gondii* to survive in a variety of hosts may be related to its unusual method of reproduction.

Nicolle and Manceaux (1909) and later workers reported that *Toxoplasma gondii* divided by binary fission. However, Goldman et al. (1958) recognized that division was accomplished by a unique method which they termed endodyogeny. Electron microscope studies by Ludvik (1958), Gavin et al. (1962), Ogino and Yoneda (1966), Wildführ (1964), van der Zypen and Piekarski (1967), and Sheffield and Melton (1968) have confirmed the findings of Goldman et al. and have suggested that endodyogeny may be the only type of division taking place in *T. gondii*.

Hutchison et al. (1970) and Frenkel et al. (1970) have reported the presence of schizogonic stages of *T. gondii* in the intestinal epithelium of cats. These stages appeared to be similar to those seen in *Eimeria* and *Isospora* infections. The present work depicts the fine structure of the developing schizont and describes a type of division unlike that previously seen in schizogony and having some of the characteristics of endodyogeny.

Materials and Methods

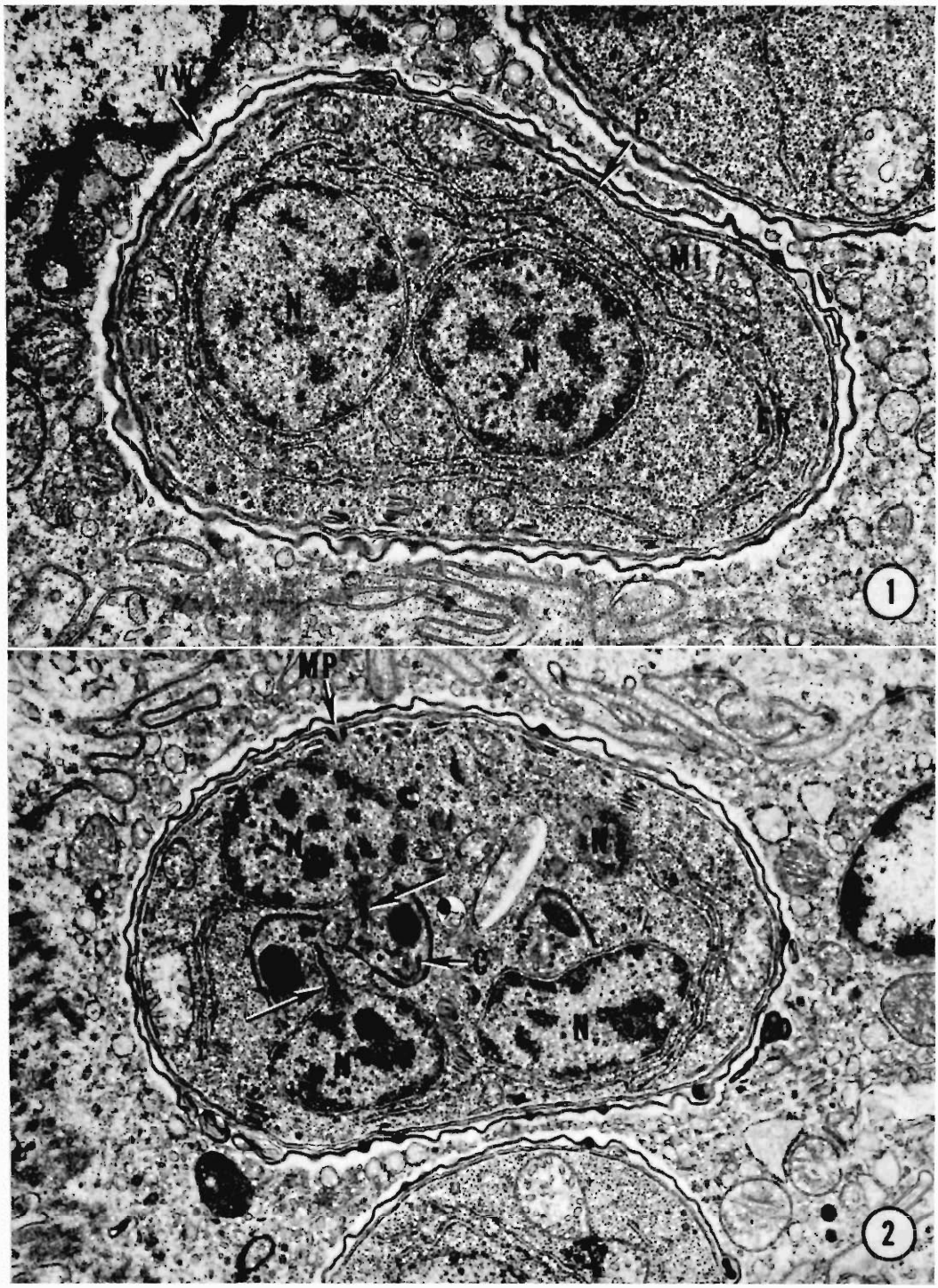
Precautions were taken to obtain kittens which were coccidia-free. A pregnant queen which had been born, raised and bred in a closed breeding colony at the NIH Animal Center was obtained and housed in an isolation cage where she delivered and nursed four kittens until they were killed. Numerous examinations of her feces did not reveal any *Isospora* oocysts. No stages of *I. felis* or *I. rivolta* were seen in histological sections of small intestine from the kittens.

Two of these kittens were infected two days after birth by feeding them emulsified mouse brains which contained cysts of *T. gondii* (strain C-56). Seven days after feeding, they were anesthetized and perfused via the descending aorta with physiological buffered saline followed by 5% glutaraldehyde in phosphate buffer at pH 7.2. The small intestine of each kitten was removed, cut into 6 equal lengths and then stored in the glutaraldehyde fixative at 4 C until needed. A portion from each length of intestine was prepared for histological examination to determine which areas were suitable for electron microscopy. Portions of heavily infected segments were then post-fixed for 2 hours with 2% OsO_4 in phosphate buffer, dehydrated and embedded in Epon. Thin sections were subsequently cut and stained with lead citrate and uranyl acetate prior to examination with the electron microscope.

Results

Histological sections of small intestine from kittens revealed various endogenous stages of *Toxoplasma gondii*. An area of heavy infection was found in the lower small intestine of one animal and a portion of that tissue was processed for electron microscopy.

An early schizont stage is shown in Figure 1. The organism is somewhat oval-shaped and lies within a vacuole near the apical edge of the host epithelial cell. An unusually thick vacuolar



wall separates the parasite from the host cell cytoplasm. The organism has the typical two-layered pellicle seen in other stages of *T. gondii* as well as other sporozoans but no subpellicular fibrils have been seen. There is an abundance of ribosomes, both free and attached to the membranes of the endoplasmic reticulum. The endoplasmic reticulum is more highly developed than in proliferative forms or sporozoites. Several mitochondria with typical tubular cristae are present. The nuclei are each surrounded by a nuclear envelope and have scattered clumps of chromatin around the periphery and in the central area. A conoid (not illustrated) is present at the anterior end. In the two-nucleate stage there are no structures in the parasite which resemble the membranes and organelles typically seen during endodyogeny.

Formation of merozoites may proceed after the second nuclear division. Three developing merozoites are seen adjacent to 3 of the 4 nuclei shown in Figure 2. Each has a cone-shaped, thickened membrane which represents the inner membrane of the mature merozoite. A well-formed conoid is seen in the central merozoite and is probably present in the others although out of plane of this section. The round dense body within the membrane is the precursor of the paired organelle of the mature merozoite. The polar areas (arrows) of 2 nuclei can be seen extending into the forming merozoites. The cytoplasm of the schizont contains many ribosomes, rough surfaced endoplasmic reticulum, mitochondria and a Golgi adjunct. The outer and inner membranes are also present and associated with them is a micropore.

It has not been determined whether more than two merozoites can form from a single nucleus (as in endodyogeny) nor how many nuclei are present in the schizont prior to the initiation of merozoite formation.

In Figure 3, a section of a schizont passes through eleven forming merozoites. Two of the merozoites are completely separated from the remainder at this level of section. Separation of merozoites appears to take place by invagination of the outer membrane of the schizont and, perhaps, vesicle formation between the merozoites with subsequent fusion as occurs in endodyogeny. The outer and inner membrane complex remains intact except where a merozoite lies at the outer edge of the schizont in which case the schizont inner membrane is absent and the two-layer complex is made up of the schizont outer membrane and the inner membrane of the forming merozoite.

At completion of schizogony, the merozoites lie free in the vacuole and are still surrounded by the thick vacuolar wall. Sections of 24 mature merozoites are seen in Figure 4.

Discussion

After the reports of Hutchison et al. (1970) and Frenkel et al. (1970) demonstrating endogenous stages of *Toxoplasma gondii* in cat intestine it became obvious that *T. gondii* could not divide exclusively by endodyogeny. Schizogony was reported in *T. gondii* by Gavin et al. (1962) as one of several means by which the organism could divide. However. Sheffield and Melton (1968) demonstrated that the rosettes which Gavin et al. had interpreted as schizonts were actually the products of repeated divisions by endodyogeny with delayed separation of the offspring. Sheffield and Melton (1968) also compared schizogony in *Eimeria* and *Plasmodium* with endodyogeny and concluded that the processes were similar with the exceptions of there being only two offspring in endodyogeny and the site of their formation was internal rather than at the surface of the parent cell.

Schizogony in *T. gondii*, as described here, begins with one or more nuclear divisions followed by formation of merozoites. Merozoite formation is initiated by the development of anterior and organelles within a cone-shaped

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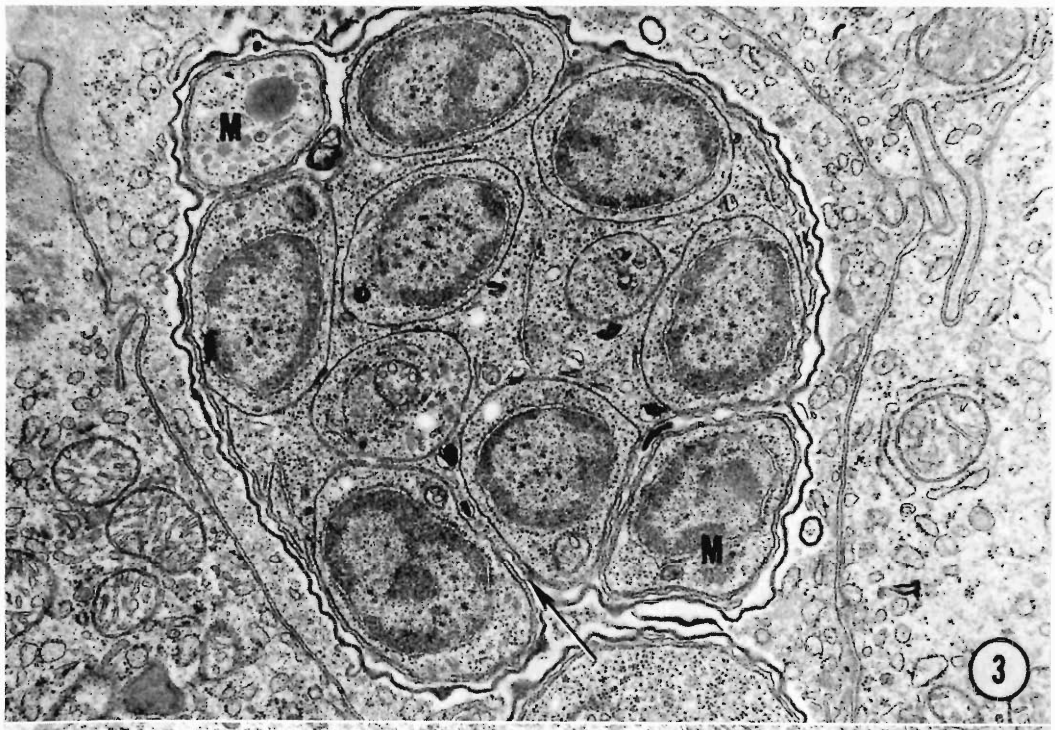
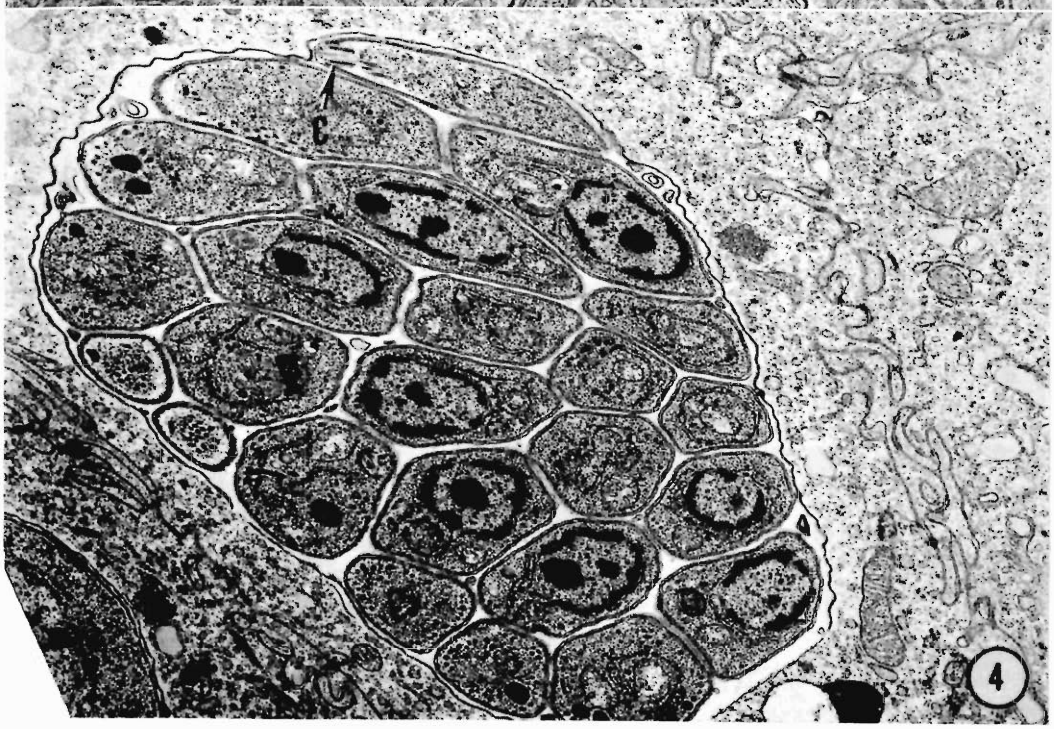
Figures 1–2. Electron micrographs of schizonts of *Toxoplasma gondii*. 1. Young schizont. 20,200×. 2. Dividing schizont. Note polar regions (arrows) of 2 nuclei extending into the forming merozoites. 16,500×.

Abbreviations for all figures: Conoid (C), endoplasmic reticulum (ER), merozoite (M), mitochrondrion (MI), micropore (MP), nucleus (N), pellicle (P), vacuolar wall (VW).





membrane near the nucleus. Elongation of the membrane and inclusion of a nucleus results in immature merozoites which have only an inner membrane and are all surrounded by the schizont membrane complex. Invagination of the schizont outer membrane and new membrane formation completes the merozoite development and separation.

Schizogony in *T. gondii* differs from endodyogeny as seen in the proliferative or cyst forms by simultaneously having more than two offspring formed within the parent organism and by having two or possibly more nuclear divisions prior to the formation of the offspring.

Formation of merozoites in *Eimeria bovis* was reported by Sheffield and Hammond (1967). The developing merozoites form at the surface of the schizont and later appear as buds. Development of the merozoites and regression of the schizont cytoplasm terminates with the separation of merozoites at the posterior end from the schizont residuum. Similar results were reported by Colley (1968) in *E. nieschulzi* and by Senaud and Cerna (1969) in *E. magna* and *E. tenella*.

Scholtyseck (1965) described a somewhat different method of division by schizogony in *E. perforans* and *E. stiedae*. In these species, merozoites separated by the formation of concentric rings of endoplasmic reticulum which isolated the merozoites from each other. Development of the conoid or other anterior end organelles was not reported.

Schmidt et al. (1967) observed merozoite formation in *Isospora* sp. and noted that buds develop at the schizont surface similar to sporozoite formation in *Plasmodium gallinaceum*.

The recognition of a type of division by schizogony which is different from those previously recorded in the literature may have some bearing on our understanding of the lack of host specificity of *T. gondii*. In cell cultures, experimental animals and humans endodyogeny is the only verified form of division. The present study has shown that endodyogeny may be a variation of schizogony as it occurs in the cat. Perhaps the ability to modify its normal pattern of division and instead repeatedly multiply by endodyogeny in abnormal hosts may account for the successful adaptation and pathogenicity of this parasite.

Acknowledgments

The assistance of Marjorie L. Melton and Ned M. Etherington is gratefully acknowledged.

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Figures 3–4. Electron micrographs of schizonts of *Toxoplasma gondii*, cont'd. 3. Late schizont. Note 2 separated merozoites and the invagination of the schizont membrane (arrow). 17,700×. 4. Mature merozoites. 10,750×.

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Announcement—New Editor

Dr. Harley G. Sheffield, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, USA, Zip Code 20014, has been elected EDITOR of the *Proceedings of the Helminthological Society of Washington* starting with Volume 38 (1971).

Effective immediately, all manuscripts and any other correspondence on Editorial Matters on Volume 38 (1971) and beyond should be addressed to Doctor Sheffield.

I wish to thank all the contributors to the Proceedings, and the staff of Allen Press for their cooperation. To my colleagues on the Editorial Board and the many others who graciously reviewed manuscripts, my deep appreciation. I am sure that you all will give Doctor Sheffield the same cooperation that you have given me in the past.

FRANCIS G. TROMBA
Immediate Past Editor

60th Anniversary Banquet

The Helminthological Society of Washington will hold a banquet commemorating its 60th anniversary during the Second International Congress of Parasitology in Washington, D. C.

The banquet is scheduled for Tuesday, September 8, 1970 at 6:00 p.m. and will feature a distinguished parasitologist as guest speaker.

All participants in the Congress are invited to attend. Tickets will be available at the Registration Desk.

Monoecocestus giganticus sp. n. (Cestoda: Anoplocephalidae) from the Porcupine *Erethizon dorsatum* L. (Rodentia)

GARY A. BUHLER

Department of Biological Sciences, University of Northern Colorado, Greeley, Colorado

ABSTRACT: *Monoecocestus giganticus* sp. n. (Cyclophyllidea: Anoplocephalidae) is described from the intestine of the porcupine *Erethizon dorsatum* L. in Colorado. It differs from other species of the genus in the size of strobila, width of ovary, number and size of testes, and lack of a functional vagina.

Five adult cestodes were recovered from the small intestine of a porcupine, *Erethizon dorsatum* L., collected in Larimer County, Colorado in August 1968. The helminths were relaxed in tap water, fixed in alcohol-formalin-acetic acid, stained in Semichon's Carmine, and mounted in Canada Balsam. Several mature proglottids were embedded in paraffin and sectioned. Drawings were made by use of a camera lucida. All measurements are in microns unless otherwise indicated. The following description is based on three of these specimens.

Monoecocestus giganticus sp. n. (Figs. 1–4)

DESCRIPTION: Scolex (Figs. 1–2) rounded, unarmed, 506–570 wide. Suckers unarmed, 217–294 in diameter. Neck 315–455 long.

Strobila (Fig. 3) wide, 139–203 mm long, 11.5 mm greatest width, with numberous calcareous corpuscles. Mature proglottids 0.575–0.920 mm long, 4.5–8 mm wide. Holotype with 200 proglottids. Genital atrium slightly postequatorial, regularly alternating, unarmed. Male duct dorsal to osmoregulatory canals. Reproductive systems protandrous. Dorsal osmoregulatory canal about 50 wide, ventral about 35 wide in mature segments. Ventral canal with one simple cross-anastomosis near posterior margin.

MALE GENITALIA: Twenty to 40 testes, 90–130 in diameter, are arranged in posterior lateral fields, separated by ovary, extending to osmoregulatory canals. Cirrus pouch (Fig. 4) heavily muscularized, 450–700 long by 175–220 wide in mature segments, extending to within 100 of ventral osmoregulatory duct. Extended cirrus about 600 long, covered with minute deciduous spines. Cirrus pouch first appears about 30 proglottids behind the neck, with testes appearing about 35 proglottids behind the neck. Vas deferens becomes greatly distended by the 90th proglottid.

FEMALE GENITALIA: Ovary posterior, slightly poral, lobate, transversely elongated, 1.5–2.0 mm wide, 450–575 long in mature proglottids. Vitellarium compact, posterior to ovary, 460–736 wide. Vagina present only as vestigial tube which completely disappears in early mature proglottids. Seminal receptacle slightly poral, fills to maximum diameter of 210, and is completely lost in early gravid proglottids. Ovary and vitellarium appear about 48 proglottids behind neck and reach maximum size within the next 48 proglottids. Uterus appears as anterior, transverse, branched tube which later expands to form reticular egg-filled sack. Fully formed eggs present in 173rd proglottid. Eggs in uterus are coupled as pairs, measure 56–77 in diameter, and contain embryos 21–24 in diameter. These egg pairs dissociate when placed in tap water.

TYPE HOST: Porcupine, *Erethizon dorsatum* L. (Rodentia: Erethizontidae)

LOCATION: Small intestine.

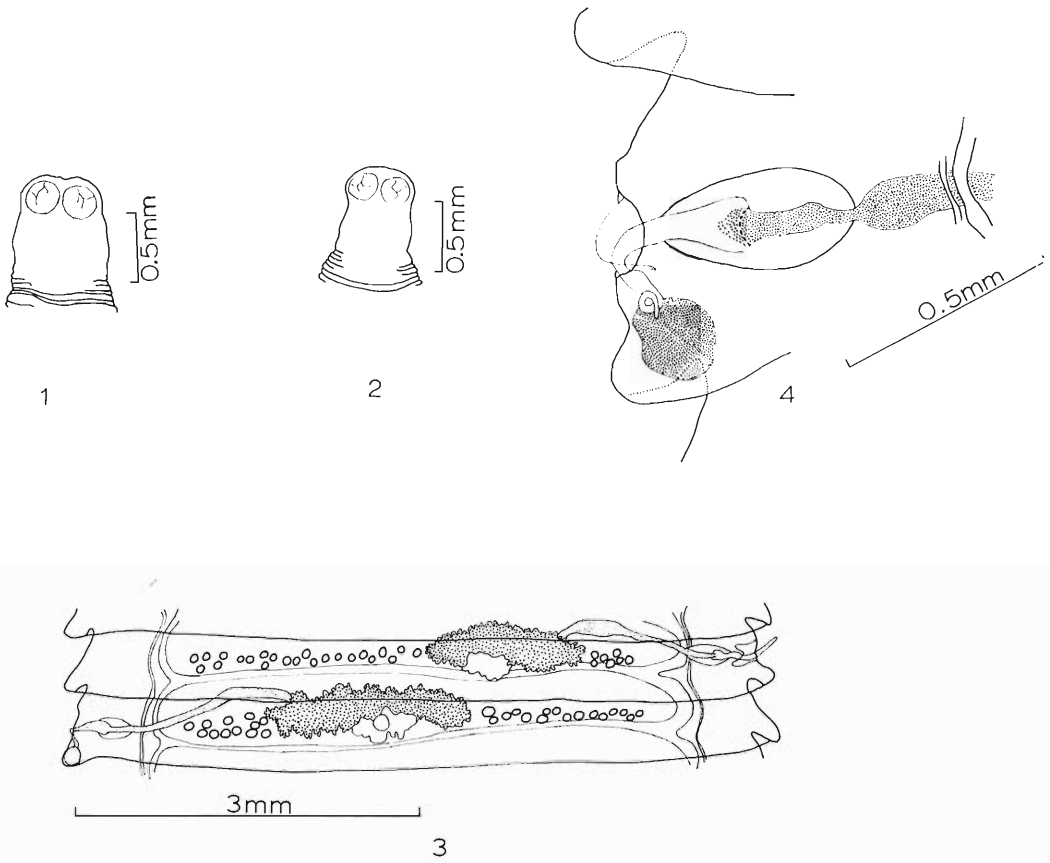
LOCALITY: Larimer County, Colorado, USA.

TYPE SPECIMENS: USNM Helm. Coll. No. 63229 (holotype) (three slides), No. 63230 (paratypes).

Remarks

Eleven species of *Monoecocestus* have been previously described and are tabulated by Rêgo 1961. Spasskii (1951) gives descriptions for seven species of *Monoecocestus*.

M. giganticus differs from the other two spe-



Figures 1–4. ***Monoecocestus giganticus*** **sp. n. 1, 2. Scoleces of two specimens. 3. Mature proglottids, dorsal view. Note seminal receptacle dorsal to vitellarium shown on posterior proglottid. 4. Drawing of cirrus involved in hypodermic impregnation, ventral view, note deposit of sperms at distal end of cirrus.**

cies found in the porcupine in the length of the strobila (33 mm in *M. americanus*, 20 mm in *M. erethizontis* and 139–203 mm in present material), the number of testes (70 in *M. americanus*, 60–100 in *M. erethizontis* and 20–40 in *M. giganticus*), in the size of the oncosphere (16 μ in diameter in *M. americanus* and *M. erethizontis* and 21–24 μ in the present material), and in the lack of a functional vagina in present material. *M. giganticus* most closely resembles *M. rheiphilus* from which it differs principally in host (the South American bird *Pterocnemia pennata* hosts *M. rheiphilus*), location of testes (*M. rheiphilus* with testes in continuous posterior band while testes of present material are separated into lateral fields by ovary), size of testes (45–50 μ in *M. rheiphilus*, 90–130 μ in *M. giganticus*), and the presence of a functional vagina which opens anterior to cirrus pore in *M. rheiphilus*.

M. hydrochoeri, which has a functional vagina in young proglottids only, differs from *M. giganticus* in strobila length, number of testes and size of oncospheres. *M. hydrochoeri* is 65 mm long, has 170–190 testes and oncospheres which are 17 μ in diameter.

M. giganticus differs from all other described species of the genus *Monoecocestus* in at least two major characteristics; thus it appears to represent a previously unknown species.

Sectioned mature proglottids of *M. giganticus* showed no sign of vaginal tissue. The vestigial vagina present in immature proglottids does not open to the outside at any time, thus fertilization apparently takes place solely by hypodermic impregnation. Mature self-impregnating proglottids are observed in all specimens (Fig. 4). Immature proglottids apparently are hypodermically inseminated by mature proglottids, as the seminal receptacle fills before testes or cirrus are developed.

The calcareous corpuscles are so numerous in early mature proglottids that they render this section of the strobila nearly opaque. As the proglottids mature the corpuscles become less numerous, until in late gravid proglottids they are almost absent.

Acknowledgments

I would like to thank Dr. Gerald D. Schmidt, University of Northern Colorado, for his help and comments which made this work possible.

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MINUTES

Four Hundred Forty-fifth Through Four Hundred Fifty-second Meetings

445th Meeting: Officers Club, National Naval Medical Center, Bethesda, Maryland, 22 October 1969. At this dinner meeting, the recipients of the Anniversary Awards were announced to be Dr. Benjamin Schwartz and Dr. Willard H. Wright. Dr. Lloyd E. Rozeboom delivered the anniversary address entitled "Entomological esotericims with possible applications to parasitological problems."

446th Meeting: Conference House, Beltsville Parasitological Laboratory, Beltsville, Maryland, 19 November 1969. Papers presented: "*Eimeria tenella*: From sporozoites to oocysts in cell culture," by D. J. Doran; "Influence of medium NCTC 135, serum, and bovine embryonic trachea and kidney cell-line cultures on the development of *Oesophagostomum radiatum* to fourth-stage larvae, in vitro," by F. W. Douvres; "Protease and esterase enzymes in the excretory gland of *Stephanurus dentatus*," by R. D. Romanowski; "*Klossiella equi*, sporadic sporozoan parasite of the equine kidney," by D. Thompson.

447th Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, 12 December 1969. Slate of officers for 1970 presented: A. James Haley (President), E. J. L. Soulsby (Vice-President), R. S. Isenstein (Recording Secretary), E. M. Buhrer (Corresponding Secretary-Treasurer). These were approved by acclamation. Papers presented: "Nature of the immune response in pigeons infected with *Trichomonas gallinae*," by R. M. Kocan; "Biology and taxonomy of a new species of amoeba in the blood of the blue crab," by T. Sawyer and V. Sprague; "New data on the biology of *Simulium innocens* (Diptera: Simuliidae), a new vector of *Leucocytozoon* of Canada geese," by I. B. Tarshis; "Cross-resistance to *Plasmodium circumflexum* and remarks on taxonomy of some *Plasmodium* spp. from avian hosts," by C. M. Herman. Installation of new officers completed.

448th Meeting: Wilson Hall, National Institutes of Health, Bethesda, Maryland, 21 January 1970. Papers presented: "New stages in the life cycle of *Toxoplasma gondii*," by H. G. Sheffield and M. L. Melton; "Transmission of human toxoplasmosis by blood transfusion," by M. N. Lunde and S. Siegel; "Resistance of mice with chronic *Besnoitia jellisoni* infections to viral challenge," by A. H. Gelderman and M. N. Lunde; "Reactions to microfilaria of *Dirofilaria imitis* or *Brugia pahangi* in immune dogs," by G. Pacheco and M. M. Wong; "Prophylactic and therapeutic activity of chlorinated lincomycin analogs against *Plasmodium gallinaceum* in chickens," by K. G. Powers.

449th Meeting: Sternberg Auditorium, Walter Reed Army Institute of Research, Washington, D. C., 18 February 1970. Treasurer's report and Auditing Committee's report given. Papers presented: "Some aspects of the carbohydrate metabolism of *Plasmodium knowlesi*," by L. W. Scheibel; "*Plasmodium falciparum* in Aotus monkey," by B. T. Wellde and M. R. Zimmerman; "Experimental schistosomiasis in owl monkeys," by D. G. Erickson; "Comparison of in vitro leukocytic and passive cutaneous anaphylaxis with homologous and heterologous antigens in rabbit trichinosis and schistosomiasis," by E. J. Colwell; "The distribution, clinical nature, and diagnosis of filariasis in the Republic of South Vietnam," by T. J. Sullivan.

450th Meeting: Conference House, Beltsville Parasitological Laboratory, Beltsville, Maryland, 20 March 1970, sponsored by the U. S. Bureau of Commercial Fisheries Biological Laboratory. Papers presented: "Oyster diseases and parasites and their effects on the industry," by A. Rosenfield; "The early life of the Chesapeake Bay oyster," by W. N. Shaw; "Histological and histochemical aspects of hyperparasitized microphallid metacercaria," by J. A. Couch; "Epizootiology of bacterial infection in larval bivalve molluscs," by H. S. Tubiash; "In-

fluence of sea water media on growth of free-living soil amoebae (Acanthamoeba)," by T. K. Sawyer.

451st Meeting: The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, 24 April 1970. Papers presented: "Immunological studies of pulmonary acariasis in Rhesus monkeys," by C. S. Kim and F. B. Bang; "Cross insemination sterilization of *Aedes polynesiensis* females by *Aedes albopictus* males," by S. R. Ali; "Histochemical localization of the biogenic amines and 5-hydroxytryptamine levels in *S. mansoni*," by J. Bennett; "Preliminary studies of the effects of hycanthone on *Schistosoma mansoni* infections in laboratory animals," by S. H. Rogers; "Weight loss and other changes in *Schistosoma mansoni* induced by subcurative doses of certain antischistosomal compounds," by R. W. Lennox; Some preliminary observations on the effects of an antischistosomal nitrofuran (SQ 18,506) on *Biomphalaria glabrata*," by J. G. Bourgeois.

452nd Meeting: University of Pennsylvania's New Bolton Center, Kennett Square, Pennsylvania, 23 May 1970. Papers presented: "Granuloma formation to the egg of *Capillaria hepatica*," by G. B. Solomon; "Cell mediated immunity responses in cutaneous leishmaniasis of the guinea pig," by T. M. Blewett; "Immunoglobulin class of the antibody response to *Haemonchus contortus* infection in sheep," by V. M. Varela-Diaz; "Reaginic antibody response to ascariasis in the guinea pig," by C. Dobson; "Immune response to larval cestodes in the mouse," by D. J. Morseth. Cocktails were served in Allam House, courtesy of the School of Veterinary Medicine, after which members and guests enjoyed dinner served in Alumni House.

The following were elected to membership at the meetings indicated: *446th*: S. M. Ali, W. A. Dillon, M. D. Clarke, H. J. P. Mathews, D. R. Mudry, R. D. Wright, J. E. Zapotosky. *447th*: R. A. Bram, R. A. McDaniel, J. E. McCauley. *448th*: N. M. Antony, V. Powders. *449th*: W. E. Noonan, E. A. Steck, J. C. Williams. *450th*: O. S. Carter, D. M. Hammond, R. J. Lacey, T. A. Miller, P. C. Stromberg, V. E. Thatcher, M. S. Wolfe. *452nd*: R. C. Bagley, J. V. Ernst, C. A. Guerrero, R. W. Gore, A. Kheiri, A. Rosenfield, P. W. Schaefer.

ROBERT S. ISENSTEIN
Recording Secretary

INDEX TO VOLUME 37

MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

(Massachusetts through Venezuela; first half in January issue)

Massachusetts
Boyd, Elizabeth M.
Castillo, Jessica M.
Hung, Chia-ling
Hurley, F. J.
Riser, N. W.
Rohde, R. A.
Stoffolano, J. G., Jr.
Michigan
Clark, D. T.
Cook, Alice R.
DeGiusti, D.
Dieter, C. E.
Dikmans, G.
Duncan, Bryan
Gee, R. J.
Peters, L. E., Jr.
Schneider, C. R.
Minnesota
Ballard, N. B.
MacDonald, D. H.
Palmer, T. T.
Vande Vusse, F. J.
Mississippi
Overstreet, R. M.
Owens, V. H.
Ward, J. W.
Wellborn, T. L., Jr.
Missouri
Dropkin, Victor H.
Montana
Canaris, A. G.
Worley, D. E.
Nebraska
Janovy, J., Jr.
Kerr, E. D.
Manter, H. W.
Nickol, B. B.
Pritchard, Mary H.
Nevada
Babero, B. B.
New Hampshire
Bullock, W. L.
Munson, D. A.
New Jersey
Balasubramanian, M.
Berger, H.
Coleman, E.
Cuckler, A. C.
Doscher, Mary Ehlers
Goble, F. C.
Green, D. F.
Haegele, C. L.
Ingalls, J. W., Jr.
Jenkins, W. R.
Kantor, S.
Katz, F. F.
Myers, R. F.
Panitz, E.
Pankavich, J. A.
Petriello, R. P.
Rohrbacher, G. H., Jr.
Stoll, N. R.
Wiest, L. M., Jr.
New Mexico
Allen, R. W.
Hopper, F. A., Jr.
Massey, C. L.
Riffle, J. W.
Samson, K. S.
Wilson, G. I.
New York
Braun, A. J.
Draggan, S.
Dunn, R. A.
Fischthal, J. H.
Granek, I.
Jackson, G. J.
Krupa, P. L.
Mackiewicz, J. S.
Mai, W. F.
Mueller, J. F.
Schroeder, P.
Stunkard, H. W.
Tiner, J. D.
White, L. V.
North Carolina
Barker, K. R.
Cort, W. W.
Harkema, R.
Hunter, Wanda S.
Johnson, C. A. III
Lapp, N. A.
McDaniel, J. S.
Miller, G. C.
Reid, W. A., Jr.
Shepperson, Jacqueline R.
Triantaphyllou, Hedwig H.
North Dakota
Larson, O. R.
Leiby, P. D.
Ohio
Crites, J. L.
Etges, F. J.
Harwood, P. D.
Watson, D. E.
Wharton, G. W.
Rabalais, F. C.
Zapotosky, J. E.
Oklahoma
Self, J. T.
Smith, P. E.
Oregon
Crook, J. R.
Jensen, H. J.
Knapp, S. E.
Lucker, J. T.
Macy, R. W.
Millemann, R. E.
Porter, C. A.
Pratt, I.
Smithson, Harriet R.
Pennsylvania
Barron, C. N.
Blewett, Theodosia M.
Cheng, T. C.
Fried, B.
Graham, G. L.
Hendrix, S. S.
Leventhal, Ruth
Martin, H. M.
Meinkoth, N. A.
Morseth, D. J.
Muncey, D. W.
Ogren, R. E.
Price, C. E.
Rellosa, F. R.
Schneider, C. M.
Solomon, G. B.
Soulsby, E. J. L.
Sylk, S. R.
Theodorides, V. J.
Uricchio, W. A.
Williams, R. R.
Puerto Rico
Acholonu, A. D.
Ayala, A.
Oliver-Gonzales, J.
Roman, J.
Rhode Island
Zinn, D. J.
South Carolina
Graham, T. W.
Rau, G. J.
South Dakota
Greichus, A.
Hugghins, E. J.
Johnson, A. D.
McDaniel, B., Jr.
Tennessee
Cosgrove, G. E.
Texas
Ehrenford, F. A.
Eni, E. U.
Hopkins, S. H.
Khan, Sekender A.
Kuntz, R. E.
Little, J. W.
Meade, T. G.
Moore, D. V.
Morrison, E. O.
Myers, Betty June
Read, C. P.
Redington, B. C.
Santmyer, P. H.
Smith, W. N.
Thames, W. H., Jr.
Turco, C. P.
Ubelaker, J. E.
Utah
Grundmann, A. W.
Havertz, D. S.
Nyberg, P. A.
Virginia
Fisher, J. E.
Freund, Frances E.
Greene, R. E.
Hargis, W. J., Jr.
Hutton, R. F.
Lawler, A. R.
Miller, L. I.
Osborne, H. S.
Reardon, Lucy V.
Seidenberg, A. J.
Sonenshine, D.
Tilton, Beverly E.
Washington
Becker, C. Dale
Chitwood, B. G.
Martin, G. W.
Saunders, J. L.
Senger, C. M.
West Virginia
Hall, J. E.
Hoffman, G. L.
Putz, R. E.
Wisconsin
Thorne, G.
Todd, A. C.
Willers, W. B.
Wyoming
Buhler, G. A.
Honess, R. F.
Kingston, N.
Argentina
Gonzalez, Stella M.
Australia
Sprent, J. F. A.
Belgium
Coomans, A.
Brazil
Franco, E.
Lordello, L. G. E.
Canada
Anderson, R. C.
Anderson, R. V.
Baker, A. D.
Bosher, J. E.
Ching, Hilda Lei
Christie, J. R.
Hopper, B. E.
Lewis, P. D., Jr.
Mettrick, D.
Mountain, W. B.
Orchard, W. R.
Sanwal, K. C.
Webster, W. A.
Wu, Liang-Yu
Canal Zone
Burke, J. C.
Chile
Tagle, Isaias V.
Costa Rica
Brenes M., R. R.
Salas, F., L. A.
Cyprus
Philis, J.
Denmark
Yeates, G. W.
Egypt
Diab, K.
Ibrahim, I. K. A.
Oteifa, B. A.
Shafiee, M. F.
France
Dollfus, R. Ph.
Vitiello, P.
Germany
Rohde, K.
Ghana
Gt. Brit. & N. Ireland
Franklin, Mary T.
Lee, D. L.
Siddiqi, M. R.
Williams, I. C.
Winslow, R. D.
Greece
Himonas, C. A.
India
Chakrabarti, K. K.
Jairajpuri, M. S.
Kahn, Shahid H.
Khan, Abrar M.
Mahajan, R.
Premvati, Mrs. G.
Saxena, S. K.
Schad, G. A.
Israel
Gershon, D.
Minz, G.
Paperna, I.
Wertheim, Guta
Italy
Lamberti, F.
Ivory Coast
Germani, G.
Luc, M.
Merny, G.
Jamaica
Japan
Ichinohe, M.
Inatomi, S.
Mamiya, Y.
Yamaguti, S.
Yokogawa, M.
Yokoo, T.
Malaysia
Lie Kian-Joe
Mexico
Briseño, C. H.
Caballero y C., E.
Téliz, D.
Vázquez, José T.
Netherlands
Dorsman, W.
Nigeria
Caveness, F. E.
Rothstein, N.
Simaren, J. O.
Pakistan
Timm, R. W.
Peru
Gonzales-Mugaburu, L.
Philippines
DeJesus, Z.
Velasquez, Carmen C.
Portugal
Bravo Lima, M.
South Africa
Heyns, J.
Kruger, S. P.
Spain
Jiménez-Millán, F.
Switzerland
Baer, J. G.
Dubois, G.
Kreis, H.
Taiwan
Chiu, Jui-Kuang
Thailand
Ratanaworabhan, S.
U.S.S.R.
Ershov, V. S.
Venezuela
Dao/D., F.
Nasir, P.

VOLUME 37 JULY 1970 NUMBER 2

CONTENTS

(*Continued from Front Cover*)

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