Volume 47 July 1980 Number 2

PROCEEDINGS

of

The Helminthological Society of Washington

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

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

Subscription $18.00 a Volume; Foreign, $19.00

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Proc. Helminthol. Soc. Wash.
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Redescription of *Diandrya composita* Darrah, 1930 (Cestoda: Anoplocephalidae) from Nearctic Marmots (Rodentia: Sciuridae) and the Relationships of the Genus *Diandrya* emend.

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ABSTRACT: According to the original description of *Diandrya composita* Darrah, 1930 (the type and only species of the Nearctic genus *Diandrya* Darrah, 1930), this cestode would possess a combination of organs, the "interproglottidal glands" and "pedunculated prostate glands," otherwise unknown in any member of the Anoplocephalidae. Neither of these organs is present in *D. composita*. This species is redescribed and the generic diagnosis is emended accordingly. *Diandrya composita* has its closest affinities with cestodes of the genus *Andrya* Railliet, 1893, from which it differs significantly only in reduplication of the reproductive organs.

The monotypic genus *Diandrya* Darrah, 1930 was established for a cestode from the yellow-bellied marmot, *Marmota flaviventris* (Audubon and Bachman), in Wyoming. This cestode, *D. composita* Darrah, 1930, has been found to occur widely in western North America in five of the six Nearctic species of the genus *Marmota* (vide Rausch and Rausch, 1971).

On the basis of the original generic diagnosis, Spasskii (1951, p. 420) considered *Diandrya* to have some morphologic characteristics in common with the genera *Moniezia* Blanchard, 1891 and *Andrya* Railliet, 1893. However, the study of specimens of *D. composita* has shown that the affinities of the genus have been obscured by errors in the original interpretation of certain anatomic details. The present paper provides a redescription of this cestode and emends accordingly the diagnosis of the genus *Diandrya*.

Materials and Methods

The cestodes were routinely fixed in a hot solution of 10% formalin, stained in acetic carmine or acid hematoxylin, processed by standard methods, and mounted permanently. Superficial tissues were removed from selected strobilae before mounting to facilitate study of organs. Thick transverse sections as well as transverse and frontal sections prepared by the paraffin-embedding method also were studied. Of some hundreds of *D. composita* collected during the period 1949–1974, 110 specimens were used in the present study. These cestodes are listed below by host and general locality, with numbers of specimens indicated in parentheses. The holotype of *D. composita* (USNM Helm. Coll. No. 30263) also was examined.

Marmota caligata (Eschscholtz): (Alaska) Talkeetna Mountains (37); Chugach Mountains (15); Alaska Range (6); Kenai Peninsula (16); Hinchenbrook Island (1); (Alberta, Canada) Gorge Creek (2); (Washington) Mt. Rainier (2). *Marmota broweri* Hall and Gilmore: (Alaska) central and eastern Brooks Range (22). *Marmota olympus* (Merriam): (Washington) Olympic Peninsula (7). *Marmota vancouverensis* Swarth: (British Columbia) Vancouver Island (2).

Diandrya composita was compared with *Andrya rhopalocephala* (Riehm,

1881), the type species of the genus *Andrya*. The material studied included cotypes of *A. rhopalocephala* (USNM Helm. Coll. Nos. 1379, 1484, and 1485 (cf. Stiles, 1896, p. 158)) as well as specimens collected recently from rabbits, *Oryctolagus cuniculus* (L.), in Spain and from hares, *Lepus europaeus* Pallas, in Switzerland and Hungary. Also compared were cestodes of the genus *Moniezia*, representing the subgenera *Moniezia* Skriabin and Shul'ts, 1937 and *Blanchardiezia* Skriabin and Shul'ts, 1937, from wild ungulates of various species examined in northwestern North America.

Results

Numbers of *D. composita* found in individual marmots ranged up to 587. The wide variation observed in size of strobila and in state of development of the cestodes in some marmots indicated that acquisition of infective cysticercoids was more or less continuous after the rodents emerged from hibernation free of intestinal helminths. Because of age-related differences in size of fully developed (i.e., with gravid segments) strobilae, mean dimensions of organs have little significance and are not included in the following redescription. Measurements are in micrometers unless otherwise stated.

Diandrya composita Darrah, 1930 (Figs. 1, 2)

REDESCRIPTION: Strobila 46 to 625 mm long, with 108 to 817 segments. Maximum width 4 to 11 mm, attained in early gravid segments. Strobila attenuated anteriorly; margins otherwise essentially parallel and slightly serrate. All segments wider than long, with relative length increasing posteriad. Length/width ratio of mature segments (at level of first filling of seminal receptacle) 1:11 to 1:6; of gravid segments, 1:4 to 1:2. Scolex globular, distinctly set off from neck, 740 to 1.3 mm wide by 520 to 1 mm long. Suckers 360 to 600 in greater diameter. Neck 450 to 1 mm long and 550 to 800 wide. *Anlagen* of male genital ducts visible in first segments. Genital pores bilateral, situated posterior to middle of segmental margin in mature segments; farther posterior in gravid segments. Genital ducts passing dorsally across longitudinal excretory canals. Ventral canals 100 to 170 in diameter, connected across posterior margin of segment by transverse duct 50 to 140 in diameter; dorsal canals 12 to 40 in diameter, usually poral to ventral canals. Cirrus sac thick-walled, claviform, and somewhat curved; directed mediad, overlapping or sometimes extending mediad beyond, ventral excretory canals bilaterally. Size of cirrus sac increasing posteriad from area of mature segments with enlargement of internal seminal vesicle; maximum dimensions 370 to 700 long by 160 to 220 in diameter, attained in early gravid segments. Cirrus spinose, about 250 long when fully extended, with diameters of about 40 and 20 at proximal and distal ends, respectively. Internal seminal vesicle at first tubular, enlarging proximally in early mature segments with first appearance of spermatozoa; elongate-ellipsoidal in early gravid segments, attaining maximum dimensions of 190 to 450 long by 130 to 170 in diameter. Well developed retractor muscle, 8 to 40 in diameter and consisting of about 10 fibers, arising from proximal end of cirrus sac, extending anteromediad and fusing with fibers of internal (circular) layer of muscle anterolateral to ovary. External seminal vesicle first visible as aggregation of cells situated dorsally at proximal end of primordial cirrus sac;

cavity and ducts becoming visible with further development in immature segments. In mature and early post-mature segments, external seminal vesicle elongate, with long axis directed anteromediad; walls of vesicle provided with abundant glandular ("prostate") cells. External seminal vesicle enlarging posteriad, attaining maximum dimensions of 370 to 530 long by 130 to 410 in diameter in early gravid segments. Short afferent duct extending from distal end of external seminal vesicle, forming loop ventral to proximal end of cirrus sac and entering latter at apex. Vas deferens originating near anterior margin of ovary, formed by junction of 2 to 7 major ducts resulting from confluence of numerous vasa efferentia. Vas deferens extending directly laterad, forming reflex bend dorsal to external seminal vesicle and entering latter at anterior surface of proximal end. Testes subspherical, 287 to 327 per segment (av. 10 segments: 308), arranged 2–3 deep in dorsal layer extending across segment anterior to and between female genital organs; in some strobilae confined between ventral longitudinal excretory canals, sometimes overlapping canals, or with 1 to 8 testes disposed poral to canals bilaterally. Testes appearing before female genital organs in early immature segments and persisting in pregravid segments after disappearance of ovaries and vitelline glands. Testes 24 to 65 in diameter in mature segments, increasing to 97 to 129 in early gravid segments and thereafter disappearing. Vagina thick-walled with numerous glandular cells, about 50 in diameter in mature segments, extending mediad from genital atrium posterior to cirrus sac; enlarging just medial to ventral excretory canal, forming elongate seminal receptacle, latter extending mediad near posterior margin of segment and passing dorsally across poral side of ovary. Seminal receptacle attaining maximum dimensions of 630 to 800 long by 190 to 370 in post-mature segments. Short seminal duct arising from proximal end of seminal receptacle, joining oviduct just poral of Mehlis' gland. Ovary wider than long, situated bilaterally near posterior margin of segment; maximum dimensions of 360 to 650 wide by 250 to 400 long attained in post-mature segments. Fully developed ova, about 20 in diameter, abundant in ovary immediately before filling of uterus; ovaries disappearing in post-mature segments following expulsion of ova. Oviduct relatively long, extending porad to near proximal end of seminal receptacle, joining seminal duct, and then turning mediad to Mehlis' gland. Rudimentary ovaries or supernumerary masses of ovarian tissue frequently present near posterior margin of segment, between fully developed organs; sometimes associated with masses of vitelline cells or rudimentary vitelline glands and small, vesicular structures apparently representing rudimentary seminal receptacula. Vitelline gland entire to coarsely lobed, rounded to reniform, situated bilaterally dorsal to ovaries at posterior margin of segment, attaining maximum dimensions of 130 to 390 wide by 110 to 180 long in post-mature segments; persisting somewhat longer than ovaries. Vitelline duct extending anterolaterad to Mehlis' gland. As many as 7 rudimentary vitelline glands or masses of vitelline cells present in most segments of some strobilae, 30 to 170 in greater diameter, situated near posterior margin of segment between normally developed female genital organs. Such rudimentary organs persisting longer than functional female organs. Uterus first visible anterolateral to ovary, arising bilaterally as extensions from uterine duct, thereafter spreading ventrally as thin, cellular layer; early-stage uteri becoming confluent near midline of segment and extending laterad beyond ventral excretory canals. Uterus becoming reticulate posteriad, receiving

ova in early post-mature segments. Terminal gravid segments filled by uterus, in which reticular structure not evident but trabeculae visible when eggs lacking. Eggs subspherical to spherical, 71 to 90 by 68 to 83 (av. 81 by 75). Embryo 19 to 21 in greater diameter; embryonic hooks about 10 long. Cirrus sac and seminal receptacle persisting at posterior margin of gravid segments.

TYPE HOST: *Marmota flaviventris* (Audubon and Bachman); occurring in other Nearctic species of *Marmota* excepting *M. monax* (L.).

TYPE LOCALITY: Marquette Basin, Carter Mountain, south of Cody, Wyoming.

MATERIAL DEPOSITED: An entire mounted specimen of *D. composita* demonstrating the morphologic characteristics described above has been deposited in the USNM Helm. Coll. No. 75730.

The diagnosis of the genus *Diandrya* is here emended to accommodate the morphologic characteristics of the type species as redescribed.

Diandrya Darrah, 1930, emended

DIAGNOSIS: Monieziinae Spasskii, 1951. Strobila large, ribbonlike, with numerous segments. All segments wider than long. Scolex unarmed. Excretory system simple, with dorsal and ventral canals bilaterally. Genital pores bilateral. Two sets of genital organs in each segment. Genital ducts passing dorsal to longitudinal excretory canals. Vagina opening in genital atrium posterior to orifice of male duct. External seminal vesicle present. Testes numerous, distributed across segment anterior to and between female genital organs; may be poral to ventral excretory canals. Female genital organs situated posteriorly in lateral thirds of segmental width. Uterus reticulate, of bilateral origin, and filling gravid segments. Eggs with pyriform apparatus. Parasites of *Marmota* spp. (Rodentia: Sciuridae).

TYPE AND ONLY SPECIES: *Diandrya composita* Darrah, 1930.

Discussion

Diandrya composita was described from a small series of specimens, of which none was intact. The present redescription more adequately defines the range of nonsignificant morphologic variation of the species and corrects some errors in the original interpretation of anatomic details.

As originally described, *D. composita* would possess a combination of organs, the "interproglottidal glands," and the "pedunculated prostate glands," that otherwise is unknown in any cestodes of the family Anoplocephalidae. Interproglottidal glands occur in members of two of the three subgenera of *Moniezia,* and a pedunculated prostate gland has been considered characteristic of the genus *Andrya* s. str. (Spasskii, 1951). In reality, neither organ is present in *D. composita.*

The rudiments of supernumerary female genital organs so often present in *D. composita* were taken to be interproglottidal glands by Darrah (1930), who remarked (p. 254) that they ". . . are very inconstant in both size and number. They appear as globular, or small compact masses of spherical follicles along the posterior margin of the proglottids between the ovaries." These rudimentary organs are identifiable as genital tissue from both their form and microscopic structure, and when present they occur always in a row in the parenchyma of the

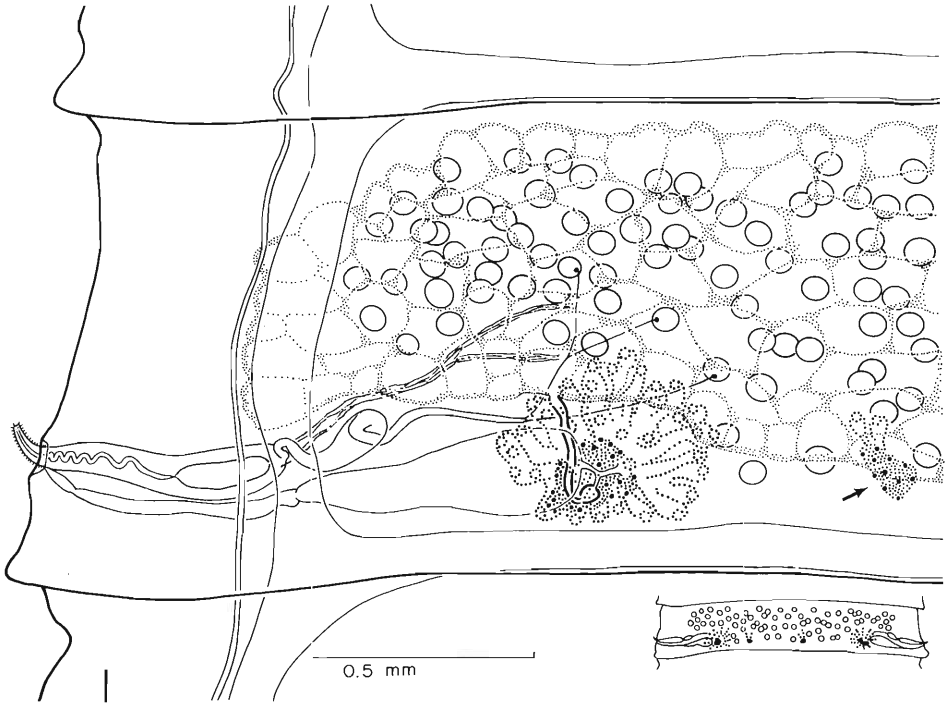
segment at the level of the functional female organs, rather than superficially at the anterior margin of the segment in parallel, dorsal and ventral rows, as do the interproglottidal glands in cestodes of the subgenus *Moniezia* (e.g., *M.* (*M.*) *expansa* (Rud., 1810)). In *D. composita,* the rudimentary organs persisted after the functional ovaries and vitelline glands had degenerated and disappeared, perhaps indicating that the former are not subject to the same degree of hormonal influence. In the absence of the "interproglottidal glands," the morphologic similarity of *Diandrya* to *Moniezia* is much diminished. However, that the uterus of *Diandrya* is reticulate permits retention of the genus in the subfamily Moniezinae.

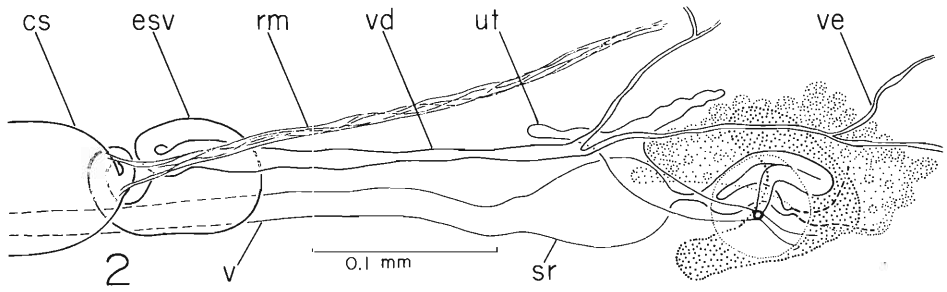
The concept of a pedunculated prostate gland in certain cestodes now included in the family Anoplocephalidae had its origin with Riehm (1881), who believed such an organ to exist in *Taenia rhopalocephala* Riehm, 1881 (=*Andrya rhopalocephala* (Riehm, 1881), type species of the genus *Andrya* Railliet, 1893) and *T. rhopaliocephala* Riehm, 1881 (=*A. cuniculi* (Blanchard, 1891)), both described from leporids in Germany. With reference to his interpretation of the structure of the male genital duct in *T. rhopalocephala,* Riehm (1881, p. 9) stated that "Das Vas deferens . . . ist unschwer zu erkennen. Bevor dieses in den Cirrhusbeutel eintritt, nimmt es noch einen Gang auf, welcher an dem Excretionskanal entlang zieht und mit einer kleinen, ovalen bis spindelförmigen Blase blind endigt." Riehm considered the content of this vesicle to be the product of the glandular cells covering its surface. Compared with that of *T. rhopalocephala,* the prostate gland in the second species (*rhopaliocephala*) was stated (p. 20) to have a shorter peduncle. The pedunculated prostate gland as conceived by Riehm was portrayed in his figures (1881, Taf. VI, figs. 1 and 3).

With publication of Riehm's dissertation, the grounds were established for perpetuation of the concept of a pedunculated, blindly ending prostatic vesicle in cestodes in the family Anoplocephalidae. The uniqueness of such a structure in the Cestoda was noted already in 1897 by Braun (p. 1408), who remarked "Sind auch diese Angaben gewiss nicht erschöpfende, so dürften sie doch so viel beweisen, dass in der That bei wenigen Taenien am männlichen Leitungsapparat Drüsen vorkommen, die man mit Recht Prostatadrüsen nennen kann; freilich weichen dieselben von den Prostatadrüsen anderer Plathelminthen ab." Such a gland was subsequently reported to occur in additional species of *Andrya,* as well as in *Diandrya.*

In describing *Diandrya composita,* Darrah (1930) considered the published work of Stiles (1896), Douthitt (1915), and Baer (1927) concerning the morphologic characteristics of *Andrya* spp. Stiles (1896) had examined some of Riehm's original material, but its condition was so poor (p. 155) that he was ". . . unable to enter into a detailed study of the organs." Baer's (1927, p. 31) descriptions of the two species from leporids were evidently based on the data published by Riehm (1881). Douthitt (1915) described two additional species, *A. primordialis* Douthitt, 1915 and *A. communis* Douthitt, 1915 (=*A. primordialis*), which he placed in a "*rhopalocephala*-group" characterized in part (p. 367) by the presence of a ". . . pedunculated prostate gland opening into the vas deferens near the ventral excretory vessel." The existence of the latter organ has been generally accepted until only recently (cf. Spasskii, 1951; Yamaguti, 1959; etc.).

From the study of cestodes identified as *A. rhopalocephala* and *A. cuniculi*





Figures 1, 2. ***Diandrya composita.*** **1. Relationships of organs, ventral view. Arrow indicates rudiments of supernumerary ovary and vitelline gland. 2. Details of genital ducts, dorsal view. Abbreviations: cs = cirrus sac; esv = external seminal vesicle; rm = retractor muscle; sr = seminal receptacle; ut = early-stage uterus; v = vagina; vd = vas deferens; ve = vas efferens.**

(now considered to be a synonym of the former (cf. Sugár et al., 1978)), I determined (Rausch, 1976) that neither possessed a pedunculated prostate gland such as was described by Riehm and concluded that the typical structure of the male genital duct in these cestodes had been misinterpreted. At the same time (p. 523) it was noted that the structure of the male genital duct in *Diandrya composita* had been similarly misinterpreted. A figure of the "prostate gland" of *D. composita* published by Joyeux and Baer (1961, p. 394) showed a duct entering at the proximal end. However, the origin of the figure was not given, and the organ was described in the text (p. 395) as consisting of a blindly ending vesicle from which

an efferent duct entered the vas deferens: "Chez *Diandrya* ainsi que chez certaines espèces du genre *Andrya*, les cellules prostatiques déversent leur contenu dans la portion dilatée en cul-de-sac d'un canalicule qui vient se jeter dans le canal déférent à l'endroit où celui-ci pénètre dans la poche du cirre. Une 'prostate' sous la forme décrite ne s'observe que rarement chez les Cestodes. On peut même se demander quel en est le rôle, puisque dans un même genre (*Andrya*) quelques-unes des espèces seulement en sont pourvues." In any case, it is evident that the structure of the male genital duct is fundamentally identical in cestodes of the genera *Andrya* (sensu Rausch, 1976) and *Diandrya*.

The relationships of the female genital ducts in *D. composita* also were misinterpreted by Darrah (1930), who stated (p. 254) that "There is no oviduct, the eggs passing directly into the uterus when the ovary breaks down." In this species, the uterine duct (=oviduct of Darrah) arises bilaterally and extends somewhat anterolaterad to the margin of the ovary, where the early-stage uterus is first visible (Fig. 2). When in early post-mature segments the uterus has become a well-defined reticulum, it rather abruptly fills with ova, after which the ovaries degenerate and soon disappear. The process is like that in *Andrya* spp. and other anoplocephalids, such as *Anoplocephaloides* spp. (Rausch, 1976).

The genus *Diandrya* appears to be a derivative of *Andrya*, from which it differs only in the doubling of the reproductive organs. The rudiments of supernumerary genital organs frequently present in this cestode probably had their origin with the process that led to reduplication of the functional organs. The diploid number of chromosomes in *D. composita* is 10 (V. R. Rausch, unpublished). Although the chromosomes of cestodes of the genus *Andrya* have not been studied, the low number in *D. composita* indicates that it is not autopolyploid. *Diandrya* evidently arose in Nearctic marmots during the early Pleistocene, after the trans-Beringian dispersal of *Marmota* to the Palaearctic.

Acknowledgments

Marmots or cestodes from marmots were provided by Dr. M. L. Johnson, University of Puget Sound Museum of Natural History, Tacoma; Dr. I. McT. Cowan and Mr. D. G. King, University of British Columbia, Vancouver; Dr. John C. Holmes, University of Alberta, Edmonton; and Dr. Allen McIntosh, Animal Parasitology Institute, Beltsville. Specimens of *Andrya rhopalocephala* were provided by Dr. F. Simón Vicente, Centro de Edafologia y Biologia Aplicada, Salamanca; Dr. J. M. Tarazona, Departamento Higiene y Sanidad Animal, Madrid; Dr. M. Graber, École Nationale Vétérinaire, Lyon; Dr. B. Hörning, Universität Bern; Dr. T. Kubelej, Âllatorvostudományi Egyetem, Budapest; and Dr. J. R. Lichtenfels, Animal Parasitology Institute, Beltsville. The figures were prepared by Ms. V. R. Rausch, who also determined the diploid number of chromosomes in *D. composita*. These contributions are gratefully acknowledged.

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Echinobothrium euzeti, a New Cestode from the Spiral Valve of a Chilean Elasmobranch

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ABSTRACT: A new cestode, *Echinobothrium euzeti* sp. n. is described from the skate *Psammobatis lima* (Poeppig, 1835) taken in coastal waters off Constitución, Chile. The species is distinguished from others in the genus by attaining a size of 5.5 cm, rostellar armature of 25 large hooks flanked by six to seven hooklets per side, 100 to 107 spines per row on the cephalic peduncle, 37 to 42 testes per mature segment, and smooth ovarian lobes.

While examining the spiral valves of elasmobranchs taken in Chilean coastal waters during the summers of 1977–1978, several new cestodes were discovered. Among them were specimens of *Echinobothrium* having distinctive features indicating that it is new. The scolex is very characteristic of other species but the long cephalic peduncle and size of the worms makes the species one of the largest in the genus. The scolices were flattened at the time of collecting to facilitate display of the armature prior to fixation in 10% formalin. Specimens were stained in hematoxylin, dehydrated, and mounted in Canada balsam. Measurements made from specimens subjected to pressure will not be completely accurate but the characters upon which the species is recognized, hook and spine numbers and dimensions, testes number, and form of the ovary are unaffected. These features and other details have been verified by comparison with a smaller unflattened specimen. Illustrations were made with the aid of a drawing tube. Measurements are expressed as length by width and are in micrometers unless otherwise indicated.

HOST: *Psammobatis lima* (Poeppig, 1835); Rajidae.

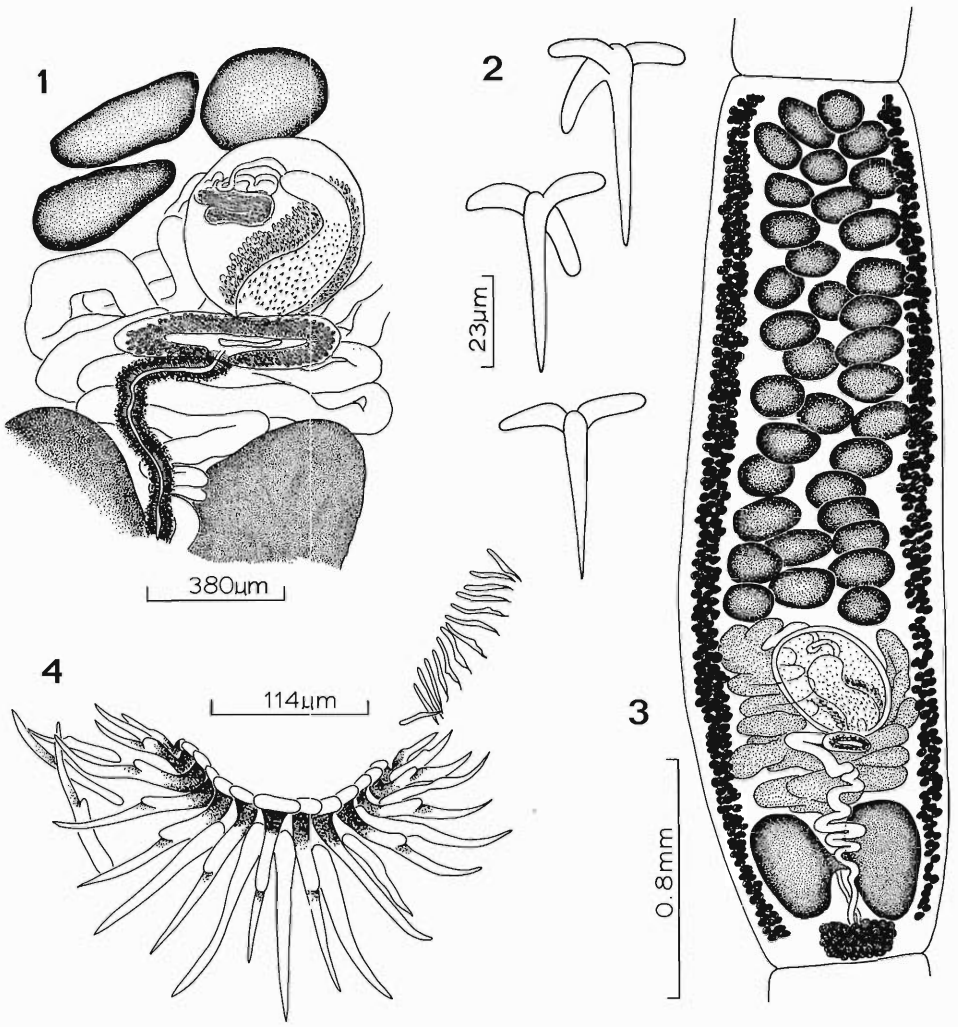
LOCALITY: Coastal waters off Constitución, Chile; 35°10′N, 72°30′W.

SITE: Spiral valve.

TYPE SPECIMENS: USNM Helm. Coll. Nos. 75773 (holotype); paratype, 75774.

Echinobothrium euzeti sp. n.
(Figs. 1–4)

DESCRIPTION (based upon 4 specimens, 2 complete and 2 without scolices): Apolytic, acraspedote worms up to 5.5 cm long by 900 wide. Strobila consists of 26 to 34 segments; 26 segments contain formed reproductive organs in largest specimen. Scolex proper 1,000 to 1,040 by 640 to 860, bothridia patelliform, slightly notched on posterior margin; covered with spines, 716 long with transverse bases up to 11.4 long, arranged in longitudinal bands. Rostellum smooth. Armature consists of 25 large apical hooks per group, 13 anterior and 12 posterior. Hooks decrease in size from center to margins of group. Hooks of anterior row 55 to 133 in greatest dimension; bases transversely flattened, up to 38 wide, bent at right angle proximally and possess distinct tuberosity at mid-shaft (Fig. 4). Hooks of posterior row 49 to 139 long, slightly curved, bases rounded, handles



Figures 1–4. ***Echinobothrium euzeti*** **sp. n. 1. Detail of reproductive terminalia. 2. Spines from anterior and posterior regions of cephalic peduncle. 3. Mature segment. 4. Rostellar armature.**

straight, also with tuberosity at midshaft though less pronounced. Each group of apical hooks flanked laterally by 6 to 7 small hooks (total of 13–14 per side), 30 to 34 long, each with tiny falciform point distally and tuberosity at midlength. Cephalic peduncle up to 6.5 mm long by 289 wide, armed with eight longitudinal rows of 100 to 107 spines each; bases triradiate, blades 40 to 56 long, width across transverse processes 32 to 44, ventral processes 11 to 23. Longest spines in middle of rows. Mature segments 1,120 to 2,960 by 440 to 880. Testes subspherical 160 to 260 by 80 to 100; number varies with maturity, 37 to 42 in mature segments, 28 to 32 in gravid segments. Testes confined to median field anterior to cirrus pouch. Cirrus pouch ovoid, 288 to 440 by 168 to 296, containing tubular pars prostatica and armed cirrus surrounded by diffuse gland cells. Ovarian lobes

smooth, compact, 200 to 408 by 168 to 280. Ootype forms a compact oval mass up to 304 wide immediately posterior to ovary. Gravid uterus coils between ovarian lobes and lateral to cirrus pouch. Vagina short, sinuous, ascends to genital atrium in midline. Genital pore large, orifice transversely elongate, immediately preovarian. Eggs thin-shelled, collapsed in whole mounts; oncospheres about 20 in diameter. Vitelline follicles form wide lateral bands extending entire length of segment.

The species is named in honor of Dr. Louis Euzet of the Université des Sciences et Techniques du Languedoc, France for his contributions to cestode taxonomy.

Remarks

Of the species of *Echinobothrium* that have been described only two, *E. longicolle* of Southwell (1925) and *E.mathiasi* of Euzet (1951), have 50 or more spines per row on the cephalic peduncle. *Echinobothrium euzeti* is easily distinguished from *E. mathiasi* in having more spines per row on the cephalic peduncle (100–107 vs. 58–60), number of apical and lateral hooks (25 + 7 per side vs. 26 + 4 per side), greater number of testes (37–42 vs. 25–30), and smooth versus lobed ovary. Both *E. euzeti* and *E. mathiasi* have two forms of large hooks: (1) hooks with curved and expanded bases (Fig. 4) which alternate with; (2) hooks lacking modified bases. *Echinobothrium euzeti* is distinct from *E. longicolle* in number (180 or more per row) and form of the spines on the cephalic peduncle (triradiate base vs. irregular base), scolex hooks (about 20 per group), testes number (26–30), and form of the ovary (''radiating club-shaped acini'').

Acknowledgment

Collecting trips by Dr. Juan Carvajal G. were supported by Grant No. 1/77 from the Direccion de Investigacion, Universidad Catolica de Chile.

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Southwell, T. 1925. A monograph on the Tetraphyllidea. Liverpool School Trop. Med. Memoir, N.S., No. 2. Liverpool Univ. Press. 368 pp.

Editor's Note

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.

Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 168–178

Caryoaustralus gen. n. and *Tholophyllaeus* gen. n. (Lytocestidae) and Other Caryophyllid Cestodes from *Tandanus* spp. (Siluriformes) in Australia

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ABSTRACT: *Caryoaustralus sprenti* gen. n., sp. n. and *Tholophyllaeus johnstoni* gen. n., sp. n. (Lytocestidae) are described from *Tandanus ater* and *T. glencoensis* in Queensland, Australia. *Caryoaustralus* has the common gonopore in the anterior half of the body, annular vitellaria, and a choanocampanulate scolex. *Tholophyllaeus*, also monogonoporate, has a tholate scolex, annular vitellaria, and lacks postovarian vitellaria. *Notolytocestus minor* Johnston and Muirhead, 1950, originally described from a single specimen, is redescribed. New information on the variation and hosts of *Balanotaenia bancrofti* Johnston, 1924 (Balanotaeniidae) is presented. Twenty-nine illustrations complement the text.

The Australian caryophyllid fauna consists of four speices from three families (Johnston, 1924; Johnston and Muirhead, 1950): *Balanotaenia bancrofti* Johnston, 1924 (Balanotaeniidae); *Notolytocestus major* Johnston and Muirhead, 1950, and *N. minor* Johnston and Muirhead, 1950 (Lytocestidae); and *Biacetabulum tandani* Johnston and Muirhead, 1950 (Caryophyllaeidae), inadequately described from a single specimen and of questionable generic and family status. All species were described from the freshwater catfish, *Tandanus tandanus* Mitchell, in South Australia. This paper reports of two new genera and species from the Lytocestidae from two other species of *Tandanus* in North Queensland.

Materials and Methods

Worms were placed either in 10% formalin or Carnoy's fluid after removal from freshly caught fish; most tapeworms were alive when fixed. All specimens were stained in Semichon's carmine; serial sections were prepared from material overstained for 48 hr. Drawings were prepared with the aid of a microprojector.

Examples of hosts have been deposited as follows: *Tandanus glencoensis* (Rendahl, 1922) USNM 217464; *T. ater* (Perugia, 1894) USNM 217463, AMNH 36518, BMNH 1977.6.15.1 to 3.

Order Caryophyllidea Van Beneden (in Carus, 1863)
Family Lytocestidae Wardle and McLeod, 1952
Caryoaustralus gen. n.

DIAGNOSIS: Lytocestidae. Scolex choanocampanulate. Single gonopore, in anterior half of worm. Ovary H-shaped. Uterus not extending anterior of cirrus, primarily postovarian. Annual vitellaria. Postovarian vitellaria present. External seminal vesicle absent. Seminal receptacle present.

TYPE SPECIES: *Caryoaustralus sprenti*.

Remarks

By having the gonopore in the anterior half of the body the new genus differs from all other Lytocestidae. It may be separated further from other monogono-

porate genera as follows: from *Djombangia* Bovien, 1926, *Stocksia* Woodland, 1937, *Notolytocestus* Johnston and Muirhead, 1950, *Crecentovitus* Murhar, 1963, and *Tholophyllaeus* gen. n., all of whom lack postovarian vitellaria, so conspicuous in the new genus; and from the following genera on the basis of scolex type: tholate (domelike) of *Lytocestoides* Baylis, 1928, cuneifimbriate (wedgelike with anterior margin of small fingerlike projections) of *Khawia* Hsu, 1935, and bulbate (swollen, knob- or bulblike) of *Atractolytocestus* Anthony, 1958; all of these are in strong contrast to the choanocampanulate scolex (with terminal funnel, bell-like) of *Caryoaustralus*.

Caryoaustralus superficially resembles *Wenyonia* Woodland, 1923 (Caryophyllaeidae; Fig. 9), both having the cirrus in the anterior half of the body. *Wenyonia*, however, has two gonopores, lateral preovarian vitellaria, a primarily preovarian uterus, and less important, a strongly follicular ovary. *Pliovitellaria* Fischthal, 1951 (Fig. 8), also of the Caryophyllaeidae, has the gonopore near the midpoint of the body, but the scolex is clavoloculate, there is an external seminal vesicle and the uterus is primarily preovarian. Although both of these genera are in another family and differ in basic characteristics it is interesting to note the convergence in gonopore position; convergence is especially pronounced also in the body shape of *Wenyonia*.

The new genus is named *Caryo-* from the order name, and *-australus*, from the country of its origin; it is masculine in gender.

Caryoaustralus sprenti sp. n.
Sprent's caryophyllid
(Figs. 1–7)

DIAGNOSIS (except as indicated, means based on measurements from 3 mature worms from 3 fish; ranges in parentheses): With characters of genus, worms 5.4 (4.8–6.4) mm long by 0.52 (0.39–0.65) mm wide at common gonopore. Scolex aloculate. Neck present. Testes number 54–59 (N = 2), begin anterior of vitellaria 0.53–0.68 mm from scolex apex, extend to ovary; larger than vitellaria. Internal seminal vesicle present. Gonopore at anterior level of, or between, arms of ovary. Vitellaria begin 0.78 (0.60–1.0) mm from scolex apex, extend to posterior tip of worm. Pre- and postovarian vitellaria continuous. Previtelline distance contained 7.2 (4.9–8.5) times in worm length; 14.8 (11.7–20.5) percent of length. Postgonopore distance 3.1 (2.3–20.5) mm, contained 1.8 (1.4–2.1) times in worm length; 57.5 (47.8–70) percent of length. Ovary nonfollicular, compact, 0.22 (0.18–0.25) mm long, H-shaped, ovarian bridge preequitorial. Uterine glands prominent, postovarian. Seminal receptacle approximately 85 μm in diameter (N = 1). Intrauterine eggs (N = 5, from holotype) 32.5–35.0 μm wide, 45–50 long, nonembryonated.

MATERIAL STUDIED: Whole mounts: 6.

TYPE HOST: *Tandanus ater* (Perugia, 1844) (Siluriformes: Plotosidae).

OTHER HOSTS: *Tandanus glencoensis* (Rendahl, 1922).

TYPE LOCALITY: Australia, Queensland, Annan River, Helenvale near Cooktown.

HOLOTYPE: U.S. National Museum Helminth Coll. No. 75485.

PARATYPE (1): South Australian Museum No. V1899.

SUPPLEMENTARY MATERIAL: From *T. glencoensis*, Queensland, Ross River

at Townsville, includes immature worms: (2) USNM Helminth Coll. No. 75486; (1) South Australian Museum (Adelaide) No. V1900; and (1) in author's collection, JSM No. X65.2.2.

Remarks

Despite the fact that sections were not made because of the limited amount of material the presence of inner longitudinal muscles internal to the vitellaria was verified from study of whole mounts, thus establishing the lytocestide nature of this species.

The terminal funnellike structure was everted on some worms (Fig. 6) and up to 150 μm deep (Fig. 5) in others; it lacks the specialized musculature of an acetabular sucker. The presence or absence of an operculum could not be established on intrauterine eggs.

Anomalies include one individual with only three testes (Fig. 2).

This species occurred in mixed infections with the other three species reported in this paper.

The species is named after Professor J. F. A. Sprent, University of Queensland, to honor his many contributions to helminthology.

***Tholophyllaeus* gen. n.**

DIAGNOSIS: Lytocestidae. Scolex tholate, aloculate. Ejaculatory duct and uterovaginal canal join to form single gonopore. Ovary H-shaped. Uterus not extending anterior of cirrus, primarily postovarian. Preovarian vitellaria in lateral and median position. Postovarian vitellaria absent. External seminal vesicle absent. Seminal receptacle absent.

TYPE SPECIES: *Tholophyllaeus johnstoni*.

Remarks

By being monogonoporate *Tholophyllaeus* is similar to *Caryophyllaeides* Nybelin, 1922, *Balanotaenia* Johnston, 1924, *Djombangia* Bovien, 1926, *Lytocestoides* Baylis, 1928, *Khawia* Hsu, 1935, *Stocksia* Woodland, 1937, *Bothrioscolex* Szidat, 1937, *Notolytocestus* Johnston and Muirhead, 1950, *Atractolytocestus* Anthony, 1958, *Crecentovitus* Murhar, 1963, and the new genus *Caryoaustralus*, described above. It is readily separated from *Caryophyllaeides*, *Lytocestoides*, *Khawia*, *Bothrioscolex*, *Atractolytocestus*, and *Caryoaustralus*, all of which have postovarian vitellaria and scolex types quite unlike those present in *Tholophyllaeus*. *Djombangia* and *Notolytocestus* have the uterus extending far anterior of the cirrus unlike the condition in the new genus. *Stocksia* has preovarian vitellaria

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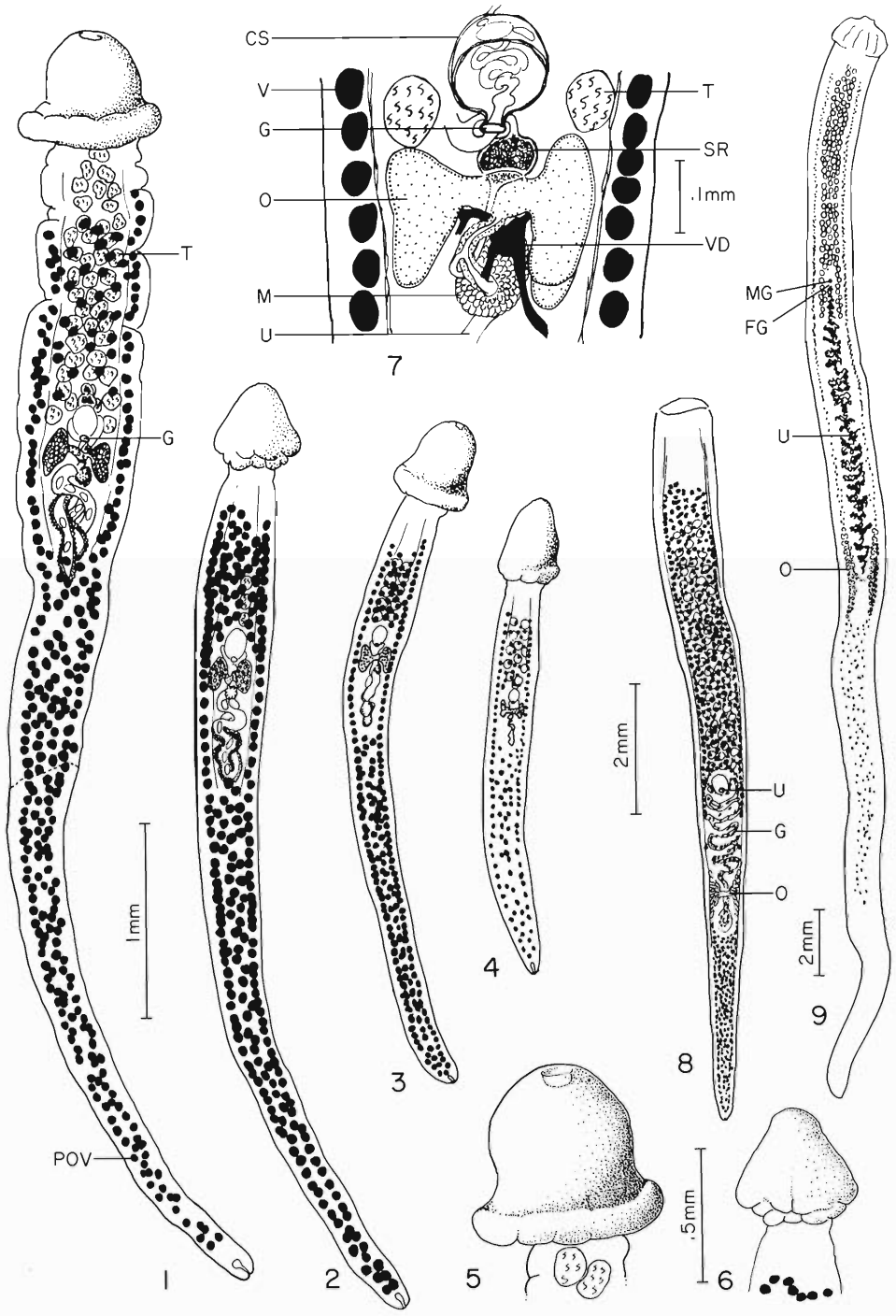
Figures 1–9. ***Caryaustralus* gen. n., except as indicated. 1. Holotype, dotted line across postovarian region indicates union of broken parts. 2. Mature worm with three testes. 3–4. Immature worms. 5. Scolex showing terminal funnel. 6. Scolex extended. 7. Detail of cirrus and ovary region. 8. *Pliovitellaria wisconsinensis* Fischthal, 1951 from *Notemigonus crysoleucus* (Raf) in Arkansas (USA) (courtesy of G. Hoffman). 9. *Wenyonia virilis* from *Synodontis schall* (Bloch-Schneider), Egypt (courtesy of O. Amin). Abbreviations (Figs. 1–29): CS, cirrus sac; E, egg; EP, excretory pore; G, gonopore; ILM, inner logitudinal muscles; M, Mehlis' gland; O, ovary; POV, postovarian vitellarium; SR, seminal receptacle; T, testis; U, uterus; V, vitellarium; VD, vitelline duct; VDF, vas deferens.**



in lateral rows only while *Balanotaenia* has a well-defined scolex with a folded margin; both of these characteristics are in marked contrast to the annularly arranged vitellaria and tholate (domelike) scolex of *Tholophyllaeus*. *Crecentovitus* has a filiform scolex, vitellaria in lateral rows, uterus primarily preovarian and a strongly follicular ovary with the shape of an inverted letter "A" although the posterior arms are not joined. *Tholophyllaeus,* on the other hand, has a tholate scolex, annularly arranged vitellaria, uterus primarily postovarian and an H-shaped, nonfollicular ovary. Although the original description (Murhar, 1963) and the key in Schmidt (1970) described the ovary of *Crecentovitus* as U-shaped, no doubt referring to the pattern formed by the follicles only, this designation should be corrected to "modified H-shaped" or possibly "inverted A-shaped" because there is a distinct ovarian commissure, as clearly illustrated by Murhar (1963) and verified from study of whole mounts. A true U-shaped ovary occurs in *Spartoides wardi* Hunter, 1929 (Capingentidae).

The new genus is named *Thol-* (*tholus* L., dome) which refers to the shape of the scolex, and *-phyllaeus,* from the suffix of *Caryophyllaeus,* first genus described in the order; it is masculine in gender.

Tholophyllaeus johnstoni sp. n.
Johnston's caryophyllid
(Figs. 20–23, 26–29)

DIAGNOSIS (means based on measurements from 20 mature worms from 2 fish; ranges in parentheses): With characters of genus, worms 3.2 (2.5–4.0) mm long by 0.47 (0.28–0.78) mm wide at common gonopore; clavate in general shape. Inner longitudinal muscles (ILM) small fascicles in single row; outer longitudinal muscles (OLM) absent. Testes number 80 (58–104), begin behind anterior vitellaria 0.71 (0.48–1.13) mm from scolex apex, extend to vas deferens or occasionally to cirrus sac; larger than vitellaria. Internal seminal vesicle absent. Cirrus sac strongly muscular, 0.161 (0.120–0.200) mm in diameter. Gonopore between arms of ovary. Vitellaria begin 0.60 (0.43–1) mm from scolex apex, extend occasionally to ovary. Previtelline distance contained 5.6 (3.5–7.9) times in worm length, represents 18.8 (14.8–28.0) percent of length. Postgonopore distance 0.63 (0.48–0.80) mm, contained 5 (4–6.4) times in worm length, 20 (15.7–24.5) percent of length. Ovary reticulolobate, 0.26 (0.20–0.31) mm long, H-shaped. Ova 38.3 (36.8–41.4) by 30.1 (25.3–32.2) μm (N = 15, from 2 worms, measured in water); operculate, 7–9 vitelline cells per ovum; not embryonated when shed.

MATERIAL STUDIED: Whole mounts: 53. Sectioned: 4.

TYPE HOST: *Tandanus glencoensis* (Rendahl, 1922) (Siluriformes; Plotosidae).

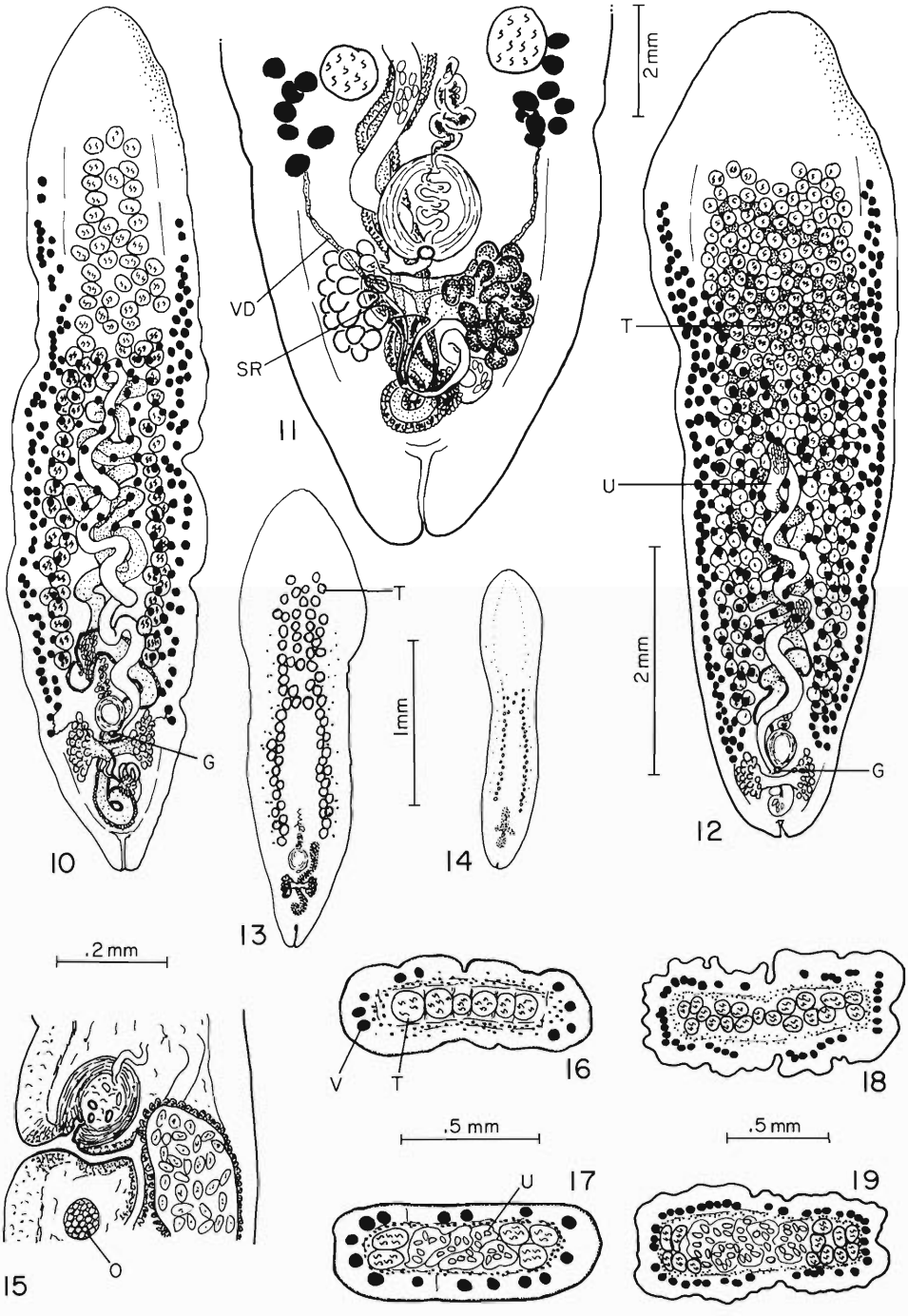
TYPE LOCALITY: Australia, Queensland, Ross River near Townsville.

OTHER HOST AND LOCALITIES: *Tandanus ater* (Perugia, 1894) from Ross River at Townsville; Annan River, 2 mi upstream of Helenvale, near Cooktown; Fossilbrook Creek, Fossilbrook between Almsden and Mt. Surprise.

HOLOTYPE: USNM Helm. Coll. No. 75481.

PARATYPES (6): From same host and locality as holotypes. Two each to USNM Helm. Coll. No. 75482, British Museum (Natural History) Helm. Coll. No. 1979.8.31.3 and 4; South Australian Museum (Adelaide) No. V1893, V1894.

SUPPLEMENTARY MATERIAL: Five whole mounts: (2) USNM Helm. Coll. No. 75483; (1) British Museum Helm. Coll. No. 1979.8.31.5; (2) South Australian



Figures 10–19. *Notolytocestus minor* **Johnston and Muirhead, 1950, except as indicated. 10. Mature worms (same scale as 13–14). 11. Details of posterior end. 12.** *N. major* **from** *Tandanus tandanus* **(courtesy M. Angel). 13–14. Immature worms. 15. Section showing single gonopore. 16. Section through preuterine region. 17. Section through miduterine region. 18.** *N. major*, **section through preuterine region. 19.** *N. major*, **section through miduterine region.**

Museum No. V1895, V1896; (2) Sections: USNM Helm. Coll. No. 75484 and South Australian Museum No. V1897, V1898. Other specimens in senior author's collection, JSM No. X65 series.

Remarks

The body shape is much like that of *Hunterella,* a monotypic genus found in deep mucosal pits in North American catostomid fish. Although the scolex too is somewhat like that of *Hunterella,* in *Tholophyllaeus* it is flattened dorsoventrally and is evidentally motile as shown by some variations in shape (Figs. 21, 22). Unlike *Hunterella* this species had the scolex so securely attached that it was often broken off in the process of removing specimens from the host mucosa.

The vitelline duct does not make a postovarian loop, a condition consistent with the absence of postovarian vitellaria. Ovarian shape does not vary a great deal (Figs. 24, 26, 27). The cirrus sac is the most muscular of any of the Australian caryophyllids.

Redescription

Notolytocestus minor Johnston and Muirhead, 1950
Australian minor caryophyllid
(Figs. 10, 11, 13–17)

DIAGNOSIS (means based on 15 mature worms from 3 fish; ranges in parentheses): Worms 3.9 (2.8–5.9) mm long by 0.65 (0.43–0.95) mm wide at common gonopore; clavate in general shape. Scolex tholate (i.e., domelike), aloculate. Neck absent. Inner longitudinal muscles small fascicles randomly arranged; outer longitudinal muscles absent. Testes number 120 (93–145), begin anterior to vitellaria, 0.57 (0.33–0.98) mm from scolex apex, extend on either side of uterus to level of vas deferens, rarely to cirrus sac; larger than vitellaria. External and internal seminal vesicle absent. Cirrus sac strongly muscular, 0.20 (0.14–0.38) in diameter. Gonopore between arms of ovary. Vitellaria begin 0.80 (0.53–1.18) mm from scolex apex, extend to ovary; postovarian vitellaria absent. Uterine glands on the proximal third of uterus. Previtelline distance contained in length of worm 5 (3.8–6.4) times, represents 20 (15.7–23.2) percent of worm length. Postgonopore distance 0.61 (0.40–0.88) mm, contained in length of worm 6.6 (5–10.3) times, represents 15.4 (9.8–20) percent of worm length. Ovary follicular, 0.30 (0.18–0.48) mm long, dumbbell shaped. Seminal receptacle absent. Ova 32.4 (32.2–34.5) by 22.2 (20.7–23) μm (N = 15 from 1 worm, measured free in water), operculate; not embryonated when shed.

MATERIAL STUDIED: Whole mounts: 29. Sectioned: 2.

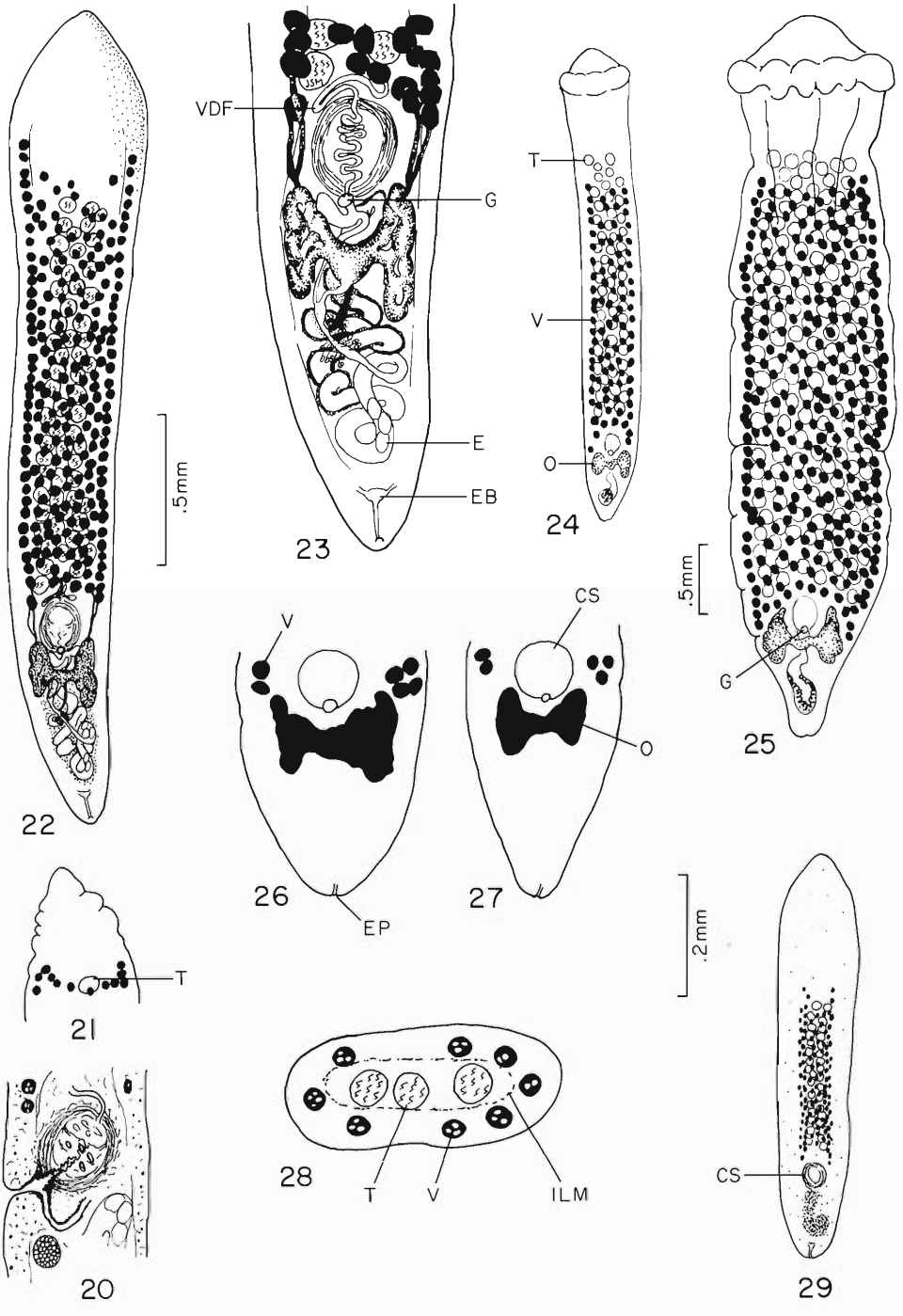
TYPE HOST: *Tandanus tandanus* Mitchell (Siluriformes; Plotosidae).

TYPE LOCALITY: Australia, South Australia, Murray River at Murray Bridge.

OTHER HOSTS AND LOCALITIES: Queensland, *T. glencoensis* (Rendahl, 1922), Ross River at Townsville; *T. ater* (Perugia, 1894), Annan River, 2 mi upstream of Helenvale, near Cooktown.

HOLOTYPE: South Australian Museum No. E. 715.

SUPPLEMENTARY MATERIAL: Seven whole mounts: from *T. glencoensis* and *T. ater* at each depository. (2) USNM Helm. Coll. No. 75487, 75488; (2) British Museum (Natural History) Helm. Coll. No. 1979.8.31.1 and 2; (3) South Austra-



Figures 20–29. ***Tholophyllaeus*** **gen. n., except as indicated. 20. Section showing single gonopore. 21. Scolex variation. 22. Holotype. 23. Posterior end. 24.** ***Balanotaenia bancrofti*** **from** ***Tandanus glencoensis,*** **Ross R., 90 testes. 25.** ***B. bancrofti*** **from** ***T. ater,*** **Ross R., 303 testes. 26–27. Ovary variation. 28. Section through midbody. 29. Immature worm.**

lian Museum No. V1890 to V1892. Other specimens in senior author's collection, JSM No. X65 series.

Remarks

Numerous specimens referable to this species were found in *T. glencoensis* and *T. ater,* enabling us to make the redescription; the original description by Johnston and Muirhead (1950) was based on a single gravid worm. This holotype specimen (No. E. 715 from the South Australia Museum) was studied by one of us (D.B.) and found to differ somewhat from the original description. For example, the size of the worm was 5.05 mm long by 1.44 mm wide compared with the original report of 6.5 by 1.7 mm. Furthermore, the testes ranged in size from 90 to 101 μm compared with 76 in the 1950 paper. Unfortunately, the massive uterus and poor condition of the worm prevented making an accurate testes count. It is our impression, however, that the holotype specimen contains more testes than any of our specimens but that such a difference may be within the limits of intraspecific variation.

Our material generally agrees with the original description in size, cirrus sac dimensions, distribution of organs, and even testes size, which in our material ranged from 75 to 125 μm, depending on the size of the specimen. Eggs of our material (N = 15, free in water, from 1 fish) were 32.2–34.5 μm long by 20.7–23.0 wide, slightly smaller than the 39 by 20 μm reported by Johnston and Muirhead (1950), however, they presented average measurements from intrauterine eggs which tend to be larger than free eggs.

Comparisons of our material with *N. major* (Figs. 12, 18, 19) from *T. tandanus* in the Murray River showed well-defined difference, especially in size, with some *N. major* being as wide as *N. minor* is long. Testes are difficult to count accurately but a specimen 6 mm long and just beginning to produce eggs had approximately 410 testes. Thirty eggs (15 from 2 worms) dissected from *N. major* ranged from 41.4 to 46 μm long and 25.3–29.0 wide, considerably larger than those from *N. minor*. Differences were evident also in comparisons between cross sections with *N. minor* (Figs. 16, 17) having larger and fewer testes and vitellaria than at comparable levels in *N. major* (Figs. 18, 19). No intermediates between these two species were found. Until additional specimens of *N. minor* can be found in *T. tandanus* from the type locality in order to clarify testes number we feel it is prudent to refer our material to that species rather than describe a third in the genus *Notolytocestus*.

Family Balanotaeniidae Mackiewicz and Blair, 1978
Balanotaenia bancrofti Johnston, 1924
Bancroft's caryophyllid
(Figs. 24, 25)

The commonest species found was *B. bancrofti* from *T. ater* at Ross River, Townsville; Annan River near Cooktown; Hann River crossing of Laura-Coen Road; Wenlock River, Crossing of Iron Range Track; and Fossilbrook Creek, Fossilbrook (between Almsden and Mt. Surprise); from *T. glencoensis* at Ross River, Townsville. Though originally reported from *T. tandanus* in Queensland, Johnston and Muirhead (1950) subsequently reported it from the same host in

New South Wales, Victoria, and South Australia. This species is thus the most widely distributed one in Australia.

Our material indicates that this widely distributed species may also be the most variable yet found in Australia. For example, Johnston reported that mature worms ranged from 2.5 to 4.7 mm, yet mature specimens from *T. tandanus* in South Australia (Murray R.) were from 5.7 to 6.5 mm ($N = 5$), and those from *T. ater* (Ross R., Fig. 25) were from 2.3 to 4.8 ($N = 17, \bar{x} = 3.9$), while from *T. glencoensis* (Ross R., Fig. 25), they were from 2.2 to 4.0 mm long ($N = 17, \bar{x} = 3.0$). Two mature specimens from *T. ater* of the Annan River were 2.7 and 5.5 mm long; one had 165 testes. Along with these size differences were conspicuous differences in testes numbers. Unfortunately the original description simply stated that the testes "are very numerous" but counts of material from various hosts show that there is continuous variation with 54–90 testes ($\bar{x} = 83.4$; $N = 12$) from material from *T. glencoensis;* 105–303 ($\bar{x} = 148$; $N = 14$) from *T. ater;* and 407–519 ($\bar{x} = 463$; $N = 2$) from *T. tandanus* in South Australia. This variation is also evident in egg size with material from *T. glencoensis* having eggs 37.8 by 27.6 μm ($N = 10$); from *T. ater,* 40.1–44.7 by 27.6–32.2 μm ($N = 10$); and from *T. tandanus,* 42.7–47 by 29.9–32.2 μm ($N = 15$). These figures are near the range reported by Johnston (1924), ".03 to .042 by .025 to .03 mm."

Although the samples are not large there is an indication that here we have either an excellent example of host influence on parasite phenotype or an example of a species complex with several closely related and integrating species occurring in different hosts. Certainly the presence of worms of two sizes, correlated with host (*T. ater* and *T. glencoensis*) in the same river (Ross) argues strongly for host influence. Sections of worms from both hosts revealed no significant differences between the worms. And still larger specimens in the Annan R. indicates that there is variation in size depending on the river. The largest specimens occur in still another host and river system in South Australia. Until we are able to conduct experimental studies or study the chromosomes of forms from each host we prefer to regard *B. bancrofti* as a highly variable species in the newly erected family Balanotaeniidae (Mackiewicz and Blair, 1978). It is easily separated from *B. newguinensis* Mackiewicz and Blair, 1978, which is a minute species (0.85–1.14 mm long) and has testes starting at same level as vitellaria, as well as other differences.

MATERIAL STUDIED: Whole mounts: 29 from *T. ater,* 36 *T. glencoensis,* 5 *T. tandanus.* Sectioned: 3 *T. ater,* 4 *T. glencoensis,* 4 *T. tandanus.*

MATERIAL DEPOSITED: USNM Helm. Coll. No. 75489, 75490 (4); British Museum (Natural History) Helm. Coll. No. 1979.8.31.6 to 9 (4); South Australian Museum Coll. No. V1901 to V1909 (9).

Acknowledgments

It is a pleasure to acknowledge the generous cooperation of Dr. Madeline Angel of the South Australian Museum for providing comparative material of *B. bancrofti* and *N. major.* We thank Mr. D. C. Lee, Curator of Arachnids and Helminths of the South Australian Museum (Adelaide) for loan of holotypes, and Ms. M. Norma Feinberg of the American Museum of Natural History for assistance in fish identification. Collections from Hann, Annan, and Wenlock rivers

were supported by an Australian Biological Resources study grant to James Cook University.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 179–185

The Life Cycle and Description of *Psilostomum magniovum* n. sp. (Trematoda: Psilostomidae)

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ABSTRACT: *Psilostomum magniovum* differs from *P. brevicolle* (Creplin, 1829) in the shape of the suckers and sucker ratios and in the size of its large eggs; the latter characteristic also separates it from other species in the genus. *Psilostomum magniovum* also differs from *P. brevicolle* in the species of first intermediate host and in the morphology of its rediae and cercariae. Cercariae from *Littorina scutulata* Gould were used to infect mussels, *Mytilus edulis* (L.); the resulting metacercariae were fed to newly hatched chicks. Adults, obtained after 8 days, resembled specimens recovered from three species of diving ducks: *Bucephala islandica* (Gmelin), *Melanitta nigra* (L.), and *M. perspicillata* (L.).

The name of the family has been given either as Psilostomidae or Psilostomatidae with various authors and dates. Looss (1900), in discussing the similarity of the generic name, *Psilostomum,* with that of the molluscan group, *Psilostomata,* gave the subfamily and family names of the trematode as Psilostominae and Psilostomidae. Odhner (1913) overlooked Looss' names and indicated wrongly, that the family Psilostomidae was new.

The type species for the genus is listed by Yamaguti (1971) as *P. platyurum* (Mühling, 1896) but Loos-Frank (1968) supported her synonymy of *P. platyurum* with *P. brevicolle* (Creplin, 1829) with measurements and observations on worms of two age groups from experimental infections of oystercatchers and gulls. She showed that the body form and arrangement of testes could change with age, and the form of "*platyurum* merged with *brevicolle.*" The type species, therefore, should be *P. brevicolle.*

Other species listed by Yamaguti (1971) include three fish parasites, two being mistakenly reported from fish-eating birds. *Psilostomum chilkai* Chatterji, 1958 was already listed in brackets and transferred by Yamaguti to *Hamacreadium* in the section on fish trematodes. *Psilostomum lineatum* Linton, 1928 and *P. plicatum* Linton, 1928 were synonyms of *Podocotyle olssoni* and *Bianium plicatum* as Odhner (1928) and Stunkard (1931) have stated, respectively. Oshmarin (1964) erected a new genus for *Psilostomum lineatum* but it does not even belong to the family due to its trilobed ovary and elongate body. Reimer (1964) suggested that *P. progeneticum* Wisniewski, 1932 be placed in the family Allocreadiidae. Another species listed is *P. reflexae* of Feldman, 1941, actually an echinostome renamed by Beaver (1943) as *Protoechinostoma mucronisertulatum.* Two species have descriptions based on single specimens: *P. fulicae* Ricci and Carrescia, 1961 was immature, and *P. arvicolae* Schult's and Dobrova, 1934, described from a mammal, lacked an oral sucker and could well be an echinostome according to Mettrick (1956). There remain only four species which can be considered valid in the genus: *P. anserinum* Oshmarin, 1963, *P. borealis* Ryzhikov, 1963, *P. brevicolle* (Crepl., 1829), Braun, 1902, and *P. cygnei* Southwell and Kirshner, 1937.

In a survey of the parasites of the snail, *Littorina scutulata* Gould, one of nine digenetic trematodes has been observed sporadically and in low prevalence since

1957. In 1977, enough cercariae were obtained to infect mussels, and the mussels subsequently provided metacercariae to infect chicks. The adults closely resembled specimens previously collected from the intestines of diving ducks. Although the life cycle and stages were similar to *P. brevicolle* as described by Loos-Frank (1968), distinct morphological differences warranted the description of a new species. The marine life cycle is believed to be the first reported from North America for the family Psilostomidae Looss, 1900.

Collections

Six infections with the psilostome rediae and cercariae were found in 1,707 snails from four localities between 1957 and 1978. Snails were examined from June to October and were infected at Garrison Bay, Washington; Boundary Bay, Coal Harbour, and Thetis Island, British Columbia; a fifth locality Bolinas Lagoon, California was reported to me by John M. Boss (pers. comm.) in 1969.

Metacercariae were found in low numbers in mussels, *Mytilus edulis* L. at Thetis Island in August 1977. These as well as experimentally recovered metacercariae were studied and will be described later.

A total of 46 adults and six immature worms were collected from the intestines of three species of diving ducks between 1972 and 1978. In addition, 441 specimens of *P. brevicolle* from five avian host species were available from the collections of Dr. Brigitte Loos-Frank; 20 were selected for measuremens.

Methods of Study

Stages in the life cycles including experimentally recovered metacercariae and adults were observed alive, and measured either alive or from preserved whole mounts. The trematodes were heat killed in seawater (for rediae, cercariae, and metacercariae), or saline (for adults), fixed in AFA (alcohol-formalin-acetic acid), stained with Semichon's acetocarmine, and mounted in permount. Twenty-four worms collected from one frozen bird were prepared for permanent mounts in the same manner. Dr. Loos-Frank's specimens of *P. brevicolle* were mostly fixed and preserved in 70% ethanol or "demke" (formalin, acetic acid, glycerine, ethanol, and water). These were stained and mounted in similar manner to the author's specimens. Measurements are given in micrometers with the mean in parentheses. Figures were drawn with the aid of a camera lucida.

Experimental Results

Twenty-five mussels infected naturally with psilostome metacercariae were fed to three newly hatched chicks and 8 days later, two worms (1 mature, 1 immature) were recovered from the intestines of two chicks.

Mussels from Spanish Bank, B.C. were used after repeated sampling and showed no psilostome trematode infections. Twenty-five were examined prior to experiments; 25 were used as controls. Although three successful infections of mussels with cercariae were carried out with all controls being negative, in only one experiment were all mussels dissected out completely. In this experiment, 21 were negative and four contained 15 metacercariae loosely attached to the gills, mantle, and digestive gland after 8 days of infection. These metacercariae were fed to one newly hatched chick and 8 days later, seven mature worms were found in the posterior fourth of the intestine.

Table 1. Comparisons of mean measurements (μm) of *P. magniovum* and *P. brevicolle* from A—diving ducks, B—chicks or gulls.

	P. magniovum		*P. brevicolle*	
	A—Natural—19†	B—Experimental—5	A—Natural—20	B—Experimental—6
Length	2,055	1,579	2,146	1,450
Width	490	499	381	306
Oral sucker	177 × 184	189 × 186	232	233 × 212
Pharynx	162 × 147	163 × 136	147	159 × 156
Ventral Sucker	210 × 251	218 × 249	228	168 × 208
Sucker ratio*	1:0.8:1.3	1:0.7:1.3	1:0.6:1	1:0.7:0.9
Ovary	109 × 104	106 × 104	115	108 × 102
Anterior testis	287 × 267	251 × 312	214	231 × 208
Posterior testis	313 × 249	319 × 265	203	253 × 176
Eggs (mean and No. measured)	122 × 80 (84)	126 × 76 (9)	104 × 62 (84)	103 × 63

* Measurements for *P. brevicolle* "B" are taken from Loos-Frank (1968) except for my calculated sucker ratios. Sucker ratios, with the oral sucker as 1 include the transverse diameter of the pharynx, and the ventral sucker.
† Numbers of specimens or eggs measured.

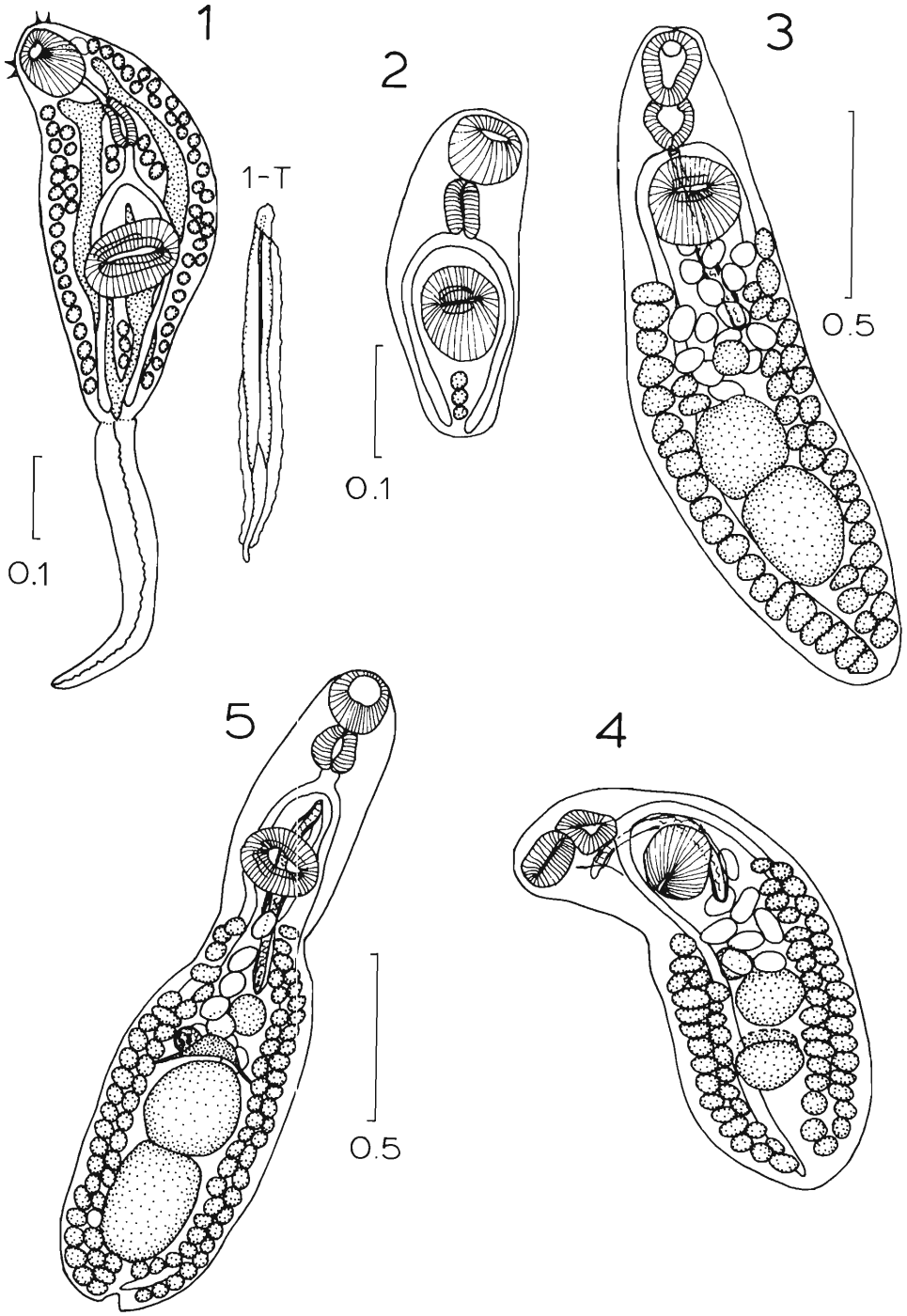
Although the occurrence of *P. magniovum* was rare compared to other trematodes that infect the host snail in the intertidal area, the development in mussels and birds (1 week in each experimental host) was very rapid. Ingestion of the metacercariae by the definitive host resulted in settlement in the posterior fourth of the intestine and rapid growth of the hindbody and maturity of the gonads.

Comparisons of the nine specimens recovered from chicks and 45 from diving ducks showed both groups to belong to the same species. Measurements of experimental and natural infections are found in Table 1.

Stages in the Life Cycle

1. Rediae. Rediae orange colored, cylindrical with tapered ends, no procrusculi, found in the digestive gland of host. Ten measured alive, 564–1268 (963) by 228–365 (302), with 7–18 (10) tailed cercariae present, plus germ balls, and developing cercariae.

2. Cercariae (Fig. 1). Cercariae swim with bodies curled, tails lashing in vertical, zigzag manner; no phototaxis observed. Bodies white, sensory papillae around oral sucker. Cystogenous gland cells over entire body, 4 ducts terminating at anterior edge of oral sucker. Excretory bladder with anterior accessory horns, arms forming continuous tube dorsal to oral sucker and pharynx, V-shaped, ending posteriorly as small sac at dorsal excretory pore. Flame cells difficult to observe, at least 16 on each side. Measurements on 10 preserved specimens: body length, 343–513 (414); body width, 182–256 (224); tail length, 343–598 (455); tail width, 46–63 (59); fin fold on almost entire tail, tiny knob at tail tip, excretory tubule bifurcating towards tail end (Fig. 1—T). Tegument thick, unspined. Oral sucker subterminal, 65–97 (82) by 74–95 (85), prepharynx lacking to 55, pharynx well-developed, longer than wide, 49–68 (59) by 42–58 (46). Ceca dividing immediately after short esophagus, extending posteriorly to end of body. Ventral sucker in posterior half of body, 68–91 (80) by 97–130 (117), with distinctive



Figures 1–5. Stages of *P. magniovum*, scale in millimeters. 1. Cercaria, preserved, with excretory bladder drawn in freehand. T—tail showing ventral fin fold and knobby tip. 2. Metacercaria, excysted and preserved. 3. Adult from chick, from experimentally recovered metacercaria. 4. Adult from chick, from naturally recovered metacercaria, scale as in Figure 3. 5. Adult from the goldeneye, natural infection.

sphincter muscles. Ratio of oral, pharynx, and ventral suckers 1:0.5:1.3. Gonadal anlagen tandem, posterior to ventral sucker.

3. Metacercariae (Fig. 2). Only 3 metacercariae from natural infections of mussels studied. Cyst 274–291 by 251–296 in diameter, with granular excretory network; prominent ventral sucker visible through thin, single-layered wall; subspherical. Excysted metacercariae about the size of cercarial bodies, 302–376 by 160–199 in length and width. Oral sucker, 87–97 by 65–91; pharynx, 71–84 by 35–47; ventral sucker, 110–134 by 103–142.

4. Adults (Figs. 3–5). Description of *Psilostomum magniovum* sp. n. based on measurements of 19 worms: elongate worms with broad hindbodies covered with thick almost scaly teguments. Length, 1,430–2,665 (2,055), width at testes, 309–650 (490). Oral sucker terminal to subterminal, oval in shape, length and width, 154–234 (184) by 108–239 (184), pharynx longer than wide, 114–217 (162) by 114–199 (147). Esophagus lacking; ceca reaching to posterior end of body. Forebody, 16–33% (24%) in length, in relaxed specimens, narrower than hindbody. Ventral sucker with sphincter muscles, transversely oval, 137–285 (210) by 182–313 (251), about ⅓ wider than oral sucker. Ratio of oral sucker to pharynx to ventral sucker: 1:0.8:1.3. Testes broadly oval, tandem, anterior, 199–376 (287) by 182–343 (267); posterior, 205–410 (313) by 171–291 (249). Space from posterior testis to posterior edge of body, 114–239 (191). Seminal vesicle within cirrus sac extending to ovary, elongate, joining cirrus at level of ventral sucker, cirrus protrusible; genital pore median, directly posterior to pharynx. Distance of ovary from anterior testis, 28–256 (138), ovary small, round, 65–142 (109) by 63–142 (104), often obscured by eggs. Seminal receptacle prominent, common vitelline duct, Mehlis' gland located directly posterior to ovary. Laurer's canal not seen. Uterus restricted to level between ventral sucker and testes; eggs large, 91–148 (122) by 57–97 (86), numbering 2–19 (11). Vitellaria with large follicles overlapping ceca, testicular edges, distributed from the posterior edge of ventral sucker to posterior edge of body, usually distributed some distance posterior to ventral sucker.

LOCATION: Posterior fourth of intestine.

TYPE HOST: *Bucephala islandica* (Gmelin), Mount Vernon, Washington, USA. Twenty-three specimens were recovered from the type host in 1972. A single specimen was found in another goldeneye and other hosts included *Melanitta nigra* (L.) with one specimen, and *Melanitta perspicillata* (L.) with 21 specimens, all birds collected freshly killed from Vancouver in 1978.

The type specimen, No. 75862 is deposited in the National Parasite Collection, Beltsville, Maryland.

Discussion

The life cycle pattern of *P. magniovum* is similar to that of *P. brevicolle*. Both appear to have brief adult life spans, the shortness of which may contribute to the considerable speciation in the family which is in turn due to isolation from other populations over a period of time, as suggested by Wright (1971).

The morphology of both species is similar but the differences are in the shapes of the suckers, sucker ratios, and egg sizes. These characteristics are all subject to variation due to fixation and the age and condition of the worms when fixed, and must be considered together if differentiating between the two species. Adults

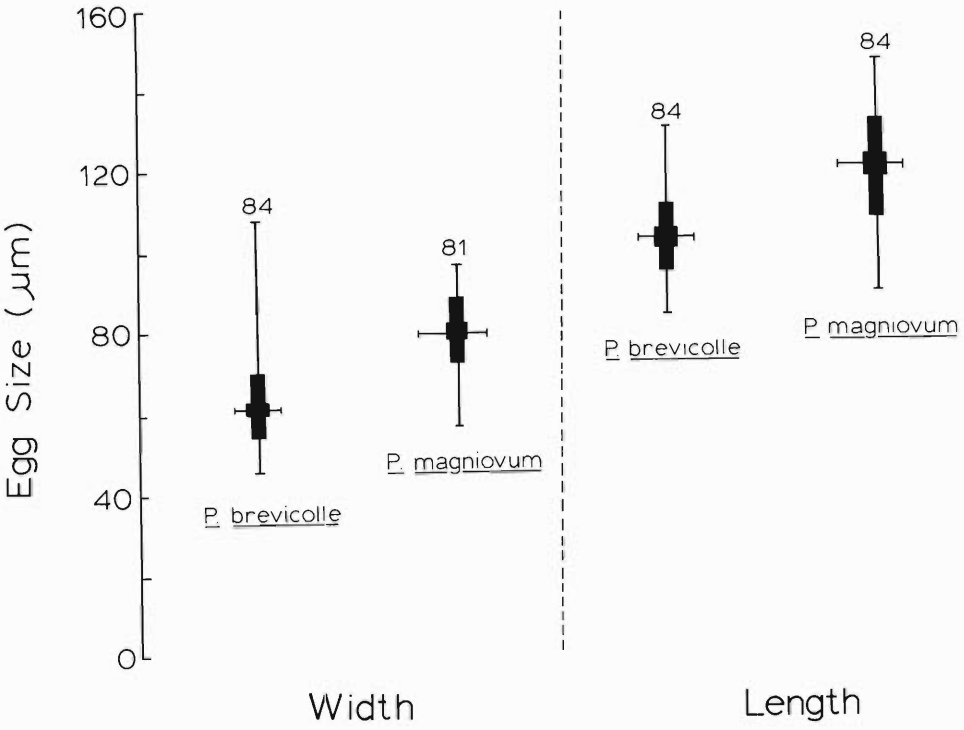


Figure 6. Egg sizes of *P. brevicolle* and *P. magniovum*. Numbers above the vertical lines indicate number of eggs measured; the vertical line is the range; the horizontal line the mean; the long bar represents one standard deviation on either side of the mean, the short bar, the 95% confidence interval of the mean.

of the two species appeared similar in body size (Table 1) but the shapes of the suckers is uniformly different: elongate or oval oral sucker and transversely oval ventral sucker for *P. magniovum,* and globose oral sucker and spherical ventral sucker for *P. brevicolle*. The ventral sucker in *P. brevicolle* has its greatest dimensions dorsoventrally, with support from the surrounding tissue so that it appears almost pedunculate. In addition, its sphincter muscles are powerfully developed; most specimens in Dr. Loos-Frank's collections are laterally oriented with the ventral suckers in lateral view. When the sucker ratios in experimental infections of 5–6 days were compared, *P. magniovum* had a ventral sucker that was one-third wider than the oral sucker (Table 1); however, *P. brevicolle* had a ventral sucker that is almost equal in size to the oral sucker. The greatest contrast then is in the size and shape of the ventral sucker—one-third larger than the oral sucker in *P. magniovum,* and equal but dorsoventrally thick in *P. brevicolle*.

Comparisons of egg sizes of the two species are presented in Figure 6. While there is some overlap of egg ranges, the mean of lengths and widths of the two species is fairly distinct. Eggs of *P. magniovum* when measured alive were even larger than those in the preserved state and 27 were 125–154 (141) by 77–85 (82), which are the largest eggs of all the species described in the genus.

Loos-Frank's (1968) study of *P. brevicolle* indicated that its body form could change from broad to elongate, and broadly oval, contiguous testes became elongately oval with intesticular space. Specimens of *P. magniovum* from three species of diving ducks showed no differences in body form, i.e., they have broad hindbodies with contiguous testes. Various aspects of the life cycles differ: the species of the first intermediate host and the larval morphology which includes no procrusculi in the rediae, and accessory arms of the excretory bladder in the cercariae of *P. magniovum*.

Besides *P. brevicolle,* only *P. anserinum* Oshmarin, 1963 and *P. borealis* Ryzhikov, 1963 can be compared with the new species. *Psilostomum anserinum* has a long pharynx that is larger than the oral sucker and a large globular vesicle; *P. borealis* has a ventral sucker three times the size of the oral sucker. Both species have much smaller eggs than *P. magniovum,* 93–112 × 58–70.

Acknowledgments

This study was conducted in the laboratory of Dr. J. R. Adams, Department of Zoology, University of British Columbia. I am grateful for the use of specimens from Dr. Loos-Frank, and the computer analysis of egg sizes by Dr. M. J. Kennedy.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 186–191

Response of Lambs to Challenge Infections After Repeated Inoculations with *Fasciola hepatica* Cysts

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ABSTRACT: Four groups of lambs 8 months old were used in an experiment to study response of sheep to repeated infections with *Fasciola hepatica*. Groups 1, 2, and 3 were inoculated with 20 cysts each weekday for 5 weeks, and Group 4 was maintained as uninoculated controls. Group 3 was necropsied 13 weeks after the last inoculation, and Groups 1 and 2 were treated 2 weeks later with albendazole to terminate the infections. Group 1 was challenged with 500 cysts per lamb 4 days posttreatment, and Group 2 was challenged with 500 cysts per lamb 30 days posttreatment. Group 4 was inoculated with 500 cysts per lamb on the day that Group 1 was challenged. Groups 1, 2, and 4 were necropsied 14 weeks postchallenge. No significant differences in worm numbers were found between groups, though the challenged controls, Group 4, had more worms. The only possible indication of acquired resistance was the presence of significantly smaller worms in the repeatedly infected lambs.

Sheep are generally considered unable to develop resistance to reinfection with *Fasciola hepatica* (Sinclair, 1967; Smithers, 1976), but various manifestations of resistance such as retarded rate of development (Rushton, 1977; Campbell et al., 1978), smaller worm size (Sinclair, 1975), and immunological responses (Movsesijan and Jovanović, 1975) have been reported. Ross (1967) reported a smaller percentage challenge take of infections after single and double intramuscular implantations of 6-week-old flukes in sheep, and Tsvetaeva et al. (1965) reported a smaller percentage challenge take of infections in sheep doubly infected and in sheep reinfected after anthelmintic removal of the first infection than in sheep infected one time. Sinclair (1975) found more flukes in challenge control sheep than in sheep challenged after a preliminary single infection and in sheep previously infected five times, though variation in worm numbers prevented concluding that the differences were significant. Meek and Morris (1979) concluded that sheep subjected to single infections that were terminated after 7 and 14 weeks and sheep doubly inoculated 7 weeks apart and then treated with a fasciolicide did not develop any resistance. Apparently, no study has been conducted in which sheep have been subjected to small daily inoculations of cysts for a period of weeks, as would likely occur in grazing animals. The following experiment was conducted to determine the response of sheep to such small daily inoculations of *F. hepatica* for a period of 5 weeks.

Materials and Methods

Forty-four Polled Dorset lambs 8 months old were tested. They had been raised in stilt pens in a barn and were maintained therein during the test. An ad lib ration of pelleted alfalfa hay was provided.

The deails of the experiment are presented in Table 1. The cysts for inoculations were examined for viability by observation for metacercarial movement or flickering of flame cells, then placed on filter paper in gelatin capsules and administered by a balling gun. Three groups of lambs were given daily inoculations, 20 cysts per lamb per day, on Monday through Friday for 5 weeks for a total of 500

Table 1. Plan of experiment.

Group no.	No. lambs	Preliminary inoculations	Treatment	Challenge and control inoculations	Necropsy
1	12	20 cysts each weekday for 5 weeks (500 cysts)	Albendazole 20 mg/kg 15 weeks after last inoculation	500 cysts in one dose 4 days posttreatment. 2 lambs killed	14 weeks post-challenge
2	12	20 cysts each weekday for 5 weeks (500 cysts)	Albendazole 20 mg/kg 15 weeks after last inoculation	500 cysts in one dose 30 days posttreatment. 2 lambs killed	14 weeks post-challenge
3	10	20 cysts each weekday for 5 weeks (500 cysts)	None	None	13 weeks after last inoculation
4	10	None	None	500 cysts on day of challenge of Group 1	14 weeks post-inoculation

cysts per lamb. Lambs of Group 3 (serially infected only) were necropsied 13 weeks after the last inoculation (week 18). Lambs of Groups 1 and 2 (serially infected) were given albendazole at 20 mg/kg 15 weeks after the last inoculation (week 20) (albendazole previously has been shown to be 100% effective in removing *F. hepatica* adults from sheep (Knight and Colglazier, 1977)), and lambs of Group 4 (challenge controls) were each inoculated with 500 cysts in a single dose. Two lambs of Group 1 (serially infected and challenged) were necropsied 4 days posttreatment to ascertain the effectiveness of the anthelmintic treatment, and the other 10 lambs were each inoculated with 500 cysts in a single dose. Two lambs of Group 2 (serially infected and challenged) were necropsied 30 days posttreatment (week 24), and the other 10 lambs were each inoculated with 500 cysts in a single dose. The delay between treatment and challenge of Group 2 was to determine whether the challenge infection might be affected by either resolution of liver damage or formation of scar tissue during this period. Lambs of Groups 1 and 2 were necropsied 14 weeks postchallenge (week 34), and lambs of Group 4 were necropsied 14 weeks postinoculation (week 38).

Blood samples were drawn with vacuum tubes from all experimental lambs on the day of first inoculation and every 2 weeks thereafter. Packed cell volumes (PCV) were determined with heparinized microhematocrit tubes, and sera were separated by centrifugation of clotted blood samples and then refrigerated or frozen at −18°C until used for enzyme studies. Activities of gamma-glutamyl transpeptidase (GGT) were determined with a Clinicard Analyzer Model 368.[1]

Flukes were recovered by slitting the bile ducts and slicing the livers. The flukes were counted and preserved in formalin. All intact flukes from Groups 1,

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

Table 2. Average numbers (ranges) and lengths of *Fasciola hepatica* recovered at necropsy of experimentally infected lambs.

Group no.	No. lambs	Average no. flukes (range)*	Average length ± SD (mm)*
1	10	75 (14–166)a	20.2 ± 0.06a
2	10	61 (19–106)a	20.3 ± 0.07a
3	10	62 (15–90)a	(Not measured)
4	8	89 (21–159)a	21.8 ± 0.01

* Means within a column followed by a common letter do not differ significantly ($P \leq 0.01$).

2, and 4 were measured. Blood data, worm counts, and worm measurements were subjected to analysis of variance for significance, and Kramer's modification (1956) of Duncan's multiple range test was used to separate treatment means.

Results and Discussion

No flukes were recovered from the four serially infected lambs of Groups 1 and 2 necropsied posttreatment. Two challenge-control lambs (Group 4) died approximately 1 month before the group was to be necropsied. One of these showed massive liver pathology with few immature flukes present; the other was not examined because of excessive deterioration.

Average numbers of flukes recovered at necropsy and average lengths of flukes are presented in Table 2. Though the challenge-controls (Group 4) had more flukes than the other groups, the differences are not significant. Flukes from the challenge-controls (Group 4) were significantly larger than those from the serially infected and challenged lambs (Groups 1 and 2), but whether the difference of 1.5 and 1.6 mm reflects more than statistical significance is conjectural.

PCV for all groups, though variable, were above the normal value of 34.9% (Schalm, 1965) until about 10–13 weeks after initial inoculations of Groups 1, 2, and 3 (Fig. 1). At this time, these groups had PCV dropping below the normal value, whereas PCV were significantly higher for the uninfected Group 4. PCV for Groups 1, 2, and 3 reached a low 9–11 weeks after the last inoculation (week 14–16). The PCV increase at this time coincided with entry of the flukes into the bile ducts. In Groups 1 and 4, PCV increased after challenge and inoculation, respectively, and then decreased in both groups beginning 6 weeks postchallenge in Group 1, and 6 weeks postinoculation in Group 4. PCV in Group 2, which was uninfected for 30 days, began to drop about 4 weeks postchallenge. Final PCV for all lambs in Groups 1, 2, and 4 were comparable, having dropped about 28% from preinfection levels.

Boray (1969) reported a drop in PCV with anemia in sheep infected with 200–700 flukes after about 12 weeks. The results reported herein similarly show such drops in sheep infected with 500 cysts approximately 12 weeks after multiple inoculations (Groups 1, 2, and 3), after single challenge inoculations following treatment (Groups 1 and 2), and after a single inoculation (Group 4).

Increases in PCV in Groups 1 and 2 after challenge and in Group 4 after inoculation, before the onset of depression of PCV at about 12 weeks postinfection, may reflect erratic variation dependent upon the state of excitement, stress, and water balance in the lambs.

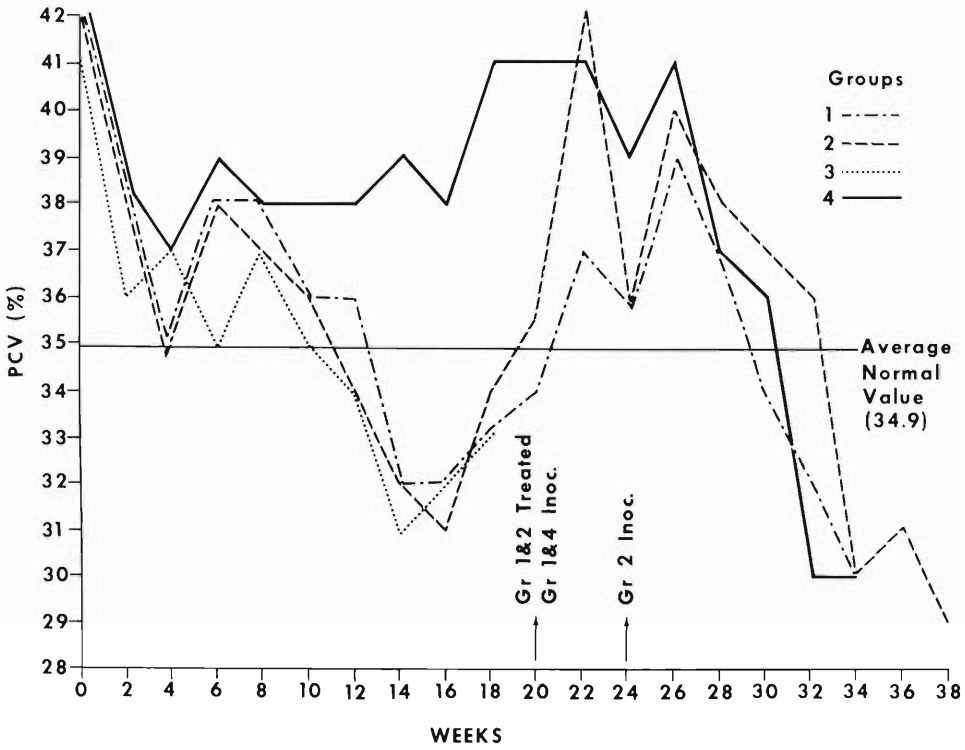

Figure 1. Average percentage packed cell volumes (PCV) in lambs experimentally infected with *Fasciola hepatica*.

GGT activity in Groups 1, 2, and 3 began to rise about 6 weeks after the first inoculation, peaked at about 12 weeks, and then declined (Fig. 2). GGT activity in the challenge controls (Group 4) followed essentially the same pattern postinfection; however, GGT activity rose earlier after 2 weeks and peaked after about 10 weeks. Serially infected Groups 1 and 2 had higher activity levels after challenge infections than before challenge, but they did not approach the levels of the first peaks. Activity level of the challenge controls (Group 4) was significantly higher 10 weeks postinoculation than was that of the serially infected Groups 1 and 2, 10 and 6 weeks postchallenge, respectively. The activity levels of Groups 1, 2, and 3 after daily inoculations peaked over a period of 2–4 weeks whereas that of Group 4 peaked sharply after the single inoculation and then declined. This action of Group 4 may be the result of a shorter time span for entry of flukes into the bile ducts of Group 4 than into those of the other groups infected over a period of 5 weeks. The lower GGT peak after challenge infections may reflect a manifestation of resistance. Anderson et al. (1978) found upon reinfection of cattle with *F. hepatica* that the GGT activity did not increase. They ascribed this result to resistance and reduced liver damage but did not indicate the numbers of flukes recovered at necropsy. Albert et al. (1961) found that GGT was in the bile canaliculi in rats; the rise in GGT activity in sheep has been shown to coincide with time of entry into bile ducts (Knight, 1978; Rew et al., 1978). Possibly, cells

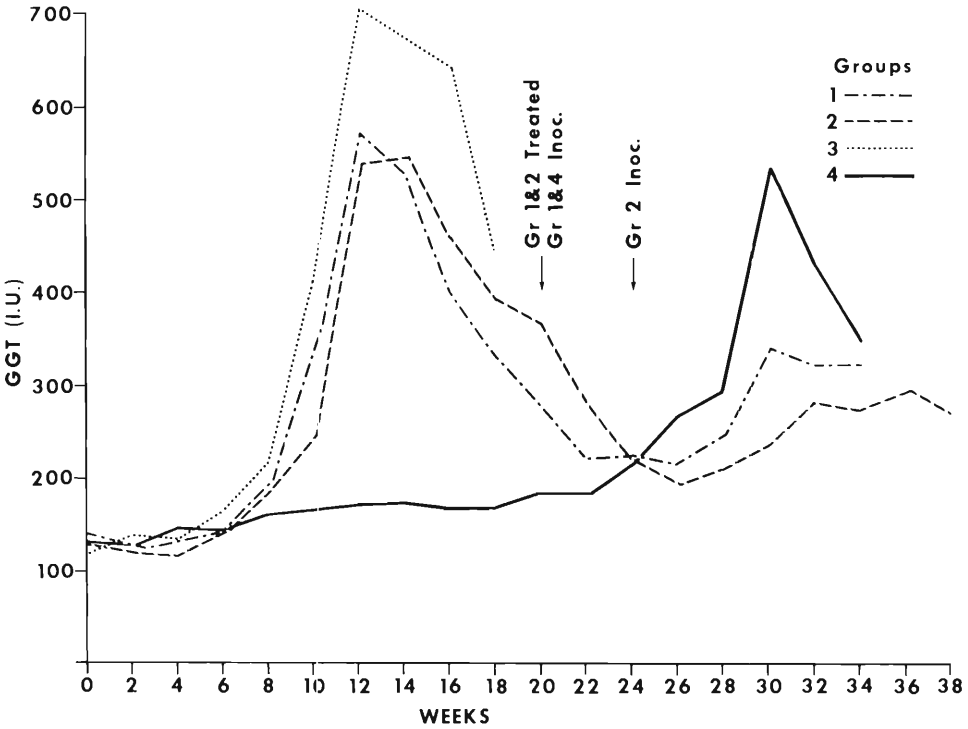

Figure 2. Average levels of gamma-glutamyl transpeptidase activity (I.U.) in lambs experimentally infected with *Fasciola hepatica*.

damaged during the first GGT peak had not regenerated so that there were fewer GGT-containing cells to be damaged upon challenge infection, or there may have been less cellular damage upon challenge reinfection.

One challenge-control lamb in Group 4 had high levels of GGT activity (300–650 I.U.) from the day of initial infection. Because these were so aberrant, they were not considered in the averages for Group 4. Upon necropsy, this animal had only 21 flukes whereas the others of Group 4 had from 66 to 159. Whether there was any meaningful correlation between the high initial GGT activity and the low numbers of flukes in this animal is unknown.

The results of this present study agree with those of Sinclair (1975). More flukes were recovered from the challenge controls than from lambs previously infected, but the differences were not significant. The length of the flukes was significantly smaller in the previously infected lambs of Sinclair and in this study, but the difference was greater in this study. No indication of resistance to challenge infection was found other than differences in worm length.

The results of the present study in which lambs were given repeated daily inoculations with *F. hepatica* cysts confirm the apparent inability of lambs to develop significant resistance to reinfection (Sinclair, 1967; Smithers, 1976; Meek and Morris, 1979).

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Editor's Acknowledgement

In addition to members of the Editorial Board I wish to thank the following persons for their valuable help in reviewing manuscripts for Volume 47 of the *Proceedings*—EDITOR

Roy C. Anderson, Harvey D. Blankespoor, Daniel R. Brooks, John I. Bruce, Wilbur L. Bullock, R. L. Calentine, Ronald A. Campbell, Murray D. Dailey, Dominic DeGiusti, David Doran, Victor H. Dropkin, R. P. Esser, Jacob H. Fischthal, Harold C. Gibbs, Douglas E. Gill, John E. Hall, Donald Heyneman, Harry W. Huizinga, Leo A. Jachowski, Irving G. Kagan, Kenneth C. Kates, Betty June Myers, Gilbert F. Otto, D. J. Raski, Larry S. Roberts, Gerald D. Schmidt, Harley G. Sheffield, Everett L. Schiller, M. A. Stirewalt, Sam R. Telford, Jr., Ralph E. Thorson, Martin J. Ulmer, Eugene C. Weinbach and Carolyn A. West.

Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 192–217

Taxonomy and Biology of North American Species of *Goezia* (Nematoda: Anisakidae) from Fishes, including Three New Species

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ABSTRACT: Three new species of *Goezia* from fishes in North America are described and supplemental data for *G. minuta* and several unidentified adults and larvae are presented. Males, especially their caudal papillae, are necessary to identify most species. For the new species, *G. pelagia* sp. n. from *Rachycentron canadum* and *Chaetodipterus faber* in the northern Gulf of Mexico possesses 12–19 preanal, two para-anal, and four postanal pairs of papillae; *G. kliksi* sp. n. from *Pogonias cromis* in Lake Borgne, Louisiana, has 10–16 preanal, two para-anal, and five postanal pairs of papillae, and *G. sinamora* sp. n. from *Tilapia aurea*, *Micropterus salmoides*, and *Morone saxatilis* in freshwater habitats in Florida possesses 13–16 preanal, two para-anal, and three postanal pairs of papillae. Records on several unidentified females without corresponding males and other assorted specimens are included to reveal a more complete understanding of hosts and localities for species of *Goezia*. Characteristics provided in a table distinguish the 18 nominal species parasitizing both fishes and aquatic reptiles throughout the world. We also provide observations on pathology, attachment, and life histories of selected species. Whereas most species of *Goezia* cause conspicuous lesions in fishes, few infected fishes are actually diseased. Also, those diseased fishes are often components of recently established host-parasite relationships.

In spite of reviews of the genus *Goezia* Zeder, 1800 by Railliet and Henry (1915), Dollfus (1935), Sprent (1978), and others, the species of this distinctive genus are incompletely known; all members have three characteristically overhung lips and plicated cuticular annulations, each possessing spines along its rear border. Herein, we describe and discuss the North American species from fishes. Previously, *G. lacerticola* Deardorff and Overstreet (1979) was reported from the American alligator in Florida which brought to three the number of species infecting crocodilians throughout the world. With the exception of *G. lacerticola*, fishes comprised the hosts for all North American reports. Two nominal species in the genus have been reported from the digestive tract of marine or estuarine fishes in North America: *G. minuta* Chandler, 1935 from a single specimen in *Bagre marinus* in Texas (Chandler, 1935) and more than one species misidentified as *G. annulata* (Molin, 1859b) from various fishes along the northeastern Atlantic seaboard (Linton, 1901, 1905; MacCallum, 1921). Rogers (1970), Ware (1971), and Gaines and Rogers (1972) reported extensive mortalities among stocked populations of the striped bass, *Morone saxatilis*, in freshwater lakes of central Florida and attributed the numberous deaths to *Goezia* sp. Other specimens also have been reported to cause extensive host-response (MacCallum, 1921).

Although *Goezia* spp. can detrimentally affect some sports and commercial fisheries, the complete life cycle of a species has not been established. Few biological data exist. Consequently, in addition to describing three new species from freshwater, estuarine, and marine hosts in North American waters and presenting supplemental data on others, we provide observations on loose and attached worms.

Materials and Methods

Most specimens we collected were removed from hosts, fixed in glacial acetic acid, stored in a solution of 5 parts glycerin and 95 parts 70% ethyl alcohol, and examined in glycerin after evaporation of the alcohol. A few others were obtained from hosts fixed in formalin and transferred to 40% isopropyl or 10% ethyl alcohol. Methods used with some loaned material are unknown; several had been permanently mounted on slides. Measurements locating the position of the nerve ring are from the anterior extremity of the worm to the center of the nerve ring. We calculated the spicule ratio as the length of the left spicule to that of the right one. Sections of attached and entire worms were stained with hematoxylin and eosin, Mason's trichome method, or other special methods (Luna, 1968) after the material had been fixed initially in buffered 10% formalin. Portions of three specimens were postfixed in osmium tetraoxide, embedded in epoxy resin, sectioned with an ultramicrotome, and stained with toluidine blue. All measurements are in micrometers unless stated otherwise, and figures were drawn with the aid of a drawing tube.

Goezia Zeder

Goezia Zeder, 1800 (type-species *Culcullanus ascaroides* Goeze, 1782).
Cochlus Zeder, 1803 (type-species *Cochlus armatus* Zeder, 1803 = *G. ascaroides*).
Prionoderma Rudolphi, 1808 (type-species *P. ascaroides*).
Lecanocephalus Diesing, 1839 (type-species *L. spinulosus* Diesing, 1839).
Pseudogoezia Mozgovoi, 1951 (as subgenus of *Goezia*).

DIAGNOSIS: Body stout, reaching greatest width near midbody. Cuticle with conspicuous plicated rings; rings more compact near anterior and posterior ends of body, with maximal separation near midbody, possessing posteriorly directed spines attached to rear border; spines in rows commencing immediately posterior to cephalic constriction, close together in anterior region, longest and separated by greatest distance near midbody, present or absent in males dorsally at base of digitiform process. Lips approximately equal in size, broader than long, with prominent angulated overhang; dorsal lip with two double papillae; subventral lips each with one lateral amphid, papilla, and double papilla; pulp pedunculate, short, slightly narrower proximally, with anterior lobes bluntly rounded. Dentigerous ridges and interlabia lacking. Ventriculus nearly spherical; ventricular appendage like narrow cylinder or saclike, with septum dividing appendage into two equal longitudinal pouches; intestinal cecum shorter than ventricular appendage. Excretory system with duct extending within left lateral cord; excretory pore located near level of nerve ring. Spicules similar, alate, equal or unequal in length. Gubernaculum absent. Caudal papillae occasionally inconspicuous. Vulva usually anterior to midbody. Uteri didelphic, opisthodelphic. Ovaries and oviducts sinuous. Tail conical; tip of tail with digitiform process, terminating with or without spinous structures. Parasites of fishes and aquatic reptiles.

TYPE SPECIES: *Goezia ascaroides* (Goeze, 1782).

Remarks

Goezia was erected by Zeder (1800) for *G. ascaroides* Goeze, 1782. Railliet and Henry (1915) reviewed the genus and added *G. spinulosa* (Diesing, 1839), *G.*

kollari (Molin, 1859a) and *G. annulata* (Molin, 1859b); those latter species were transferred from the genus *Lecanocephalus* Diesing, 1839. Baylis (1920), unaware of a similar action by Travassos (1920), proposed Goeziinae as a subfamily of Ascaridae and recognized a close relationship of *Goezia* to *Contracaecum* Railliet and Henry, 1912 because members of both possessed a ventricular appendage and an intestinal cecum. Later, Yorke and Maplestone (1926) considered Goeziinae in the family Heterocheilidae. Dollfus (1935) critically reviewed the history of the genus and differentiated the six known species. Subsequently, Skrjabin and Karochin (1945) erected Goeziidae with *Goezia* as the only genus, a view supported by Hartwich (1954). Mozgovoi (1951) proposed the subgenus *Pseudogoezia* for *G. sigalasi* Stefanski, 1938 and *G. fluviatilis* Johnston and Mawson, 1940 because each possessed a double ventricular appendage. Both Hartwich (1957) and Rasheed (1965), however, doubted the validity of the subgenus *Pseudogoezia*, a view we support because all species of *Goezia* probably possess a bicylindrical ventricular appendage.

Mozgovoi (1951) considered *Neogoezia* Kreis, 1937 a junior synonym of *Goezia*. Hartwich (1957) and Yamaguti (1961) emphasized that *Neogoezia* should not be placed in Goeziidae since its members lacked characteristic features. We also agree with that action, even through Rai (1967, 1971) also considered *Neogoezia* a synonym of *Goezia*. Until the ascaridoid classification is critically reviewed, we follow Hartwich (1974 rather than 1975), and recognize *Goezia* in the family Anisakidae.

Rasheed (1965) listed 11 species in the genus and suggested that *G. onchorynchi* Fujita, 1940 be regarded a species *incertae sedis* until adult specimens are described. Table 1 updates that list and provides a means for comparisons with species we describe.

Goezia pelagia sp. n.
(Figs. 1–7, 37–38)

DESCRIPTION: Body reaching greatest width about ⅓ body length from posterior end. Spines longest and separated by greatest distance toward end of anterior ¼ of body. Esophagus clavate, 10–17% of body length. Ventriculus narrower than widest level of esophagus, generally broader than long. Nerve ring located within anterior 22–32% of esophagus. Lateral cords salient, T-shaped anteriorly, taller than wide. Excretory system apparently similar to that in specimens from *Tilapia aurea* described later. Excretory pore slightly anterior to or at level of nerve ring. Tail conical, with digitiform process; process usually with circlet of spinous structures. Phasmids conspicuous near base of digitiform process.

MALE (based on 4 mature specimens for postcloacal papillae and 17 for most other described characters): Body 3.4–12.0 mm long by 0.4–1.0 mm at greatest

→

Figures 1–7. *Goezia pelagia*. **1. Anterior end of female including intestinal cecum and ventricular appendage, lateral view. 2. Posterior end of male showing postanal papillae and conspicuous phasmids, ventral view. 3. Atypical digitiform process of female tail, lateral view. 4. Digitiform process of female tail, lateral view. 5. *En face*. 6. Posterior end of male, ventral view. 7. Female tail, lateral view.**

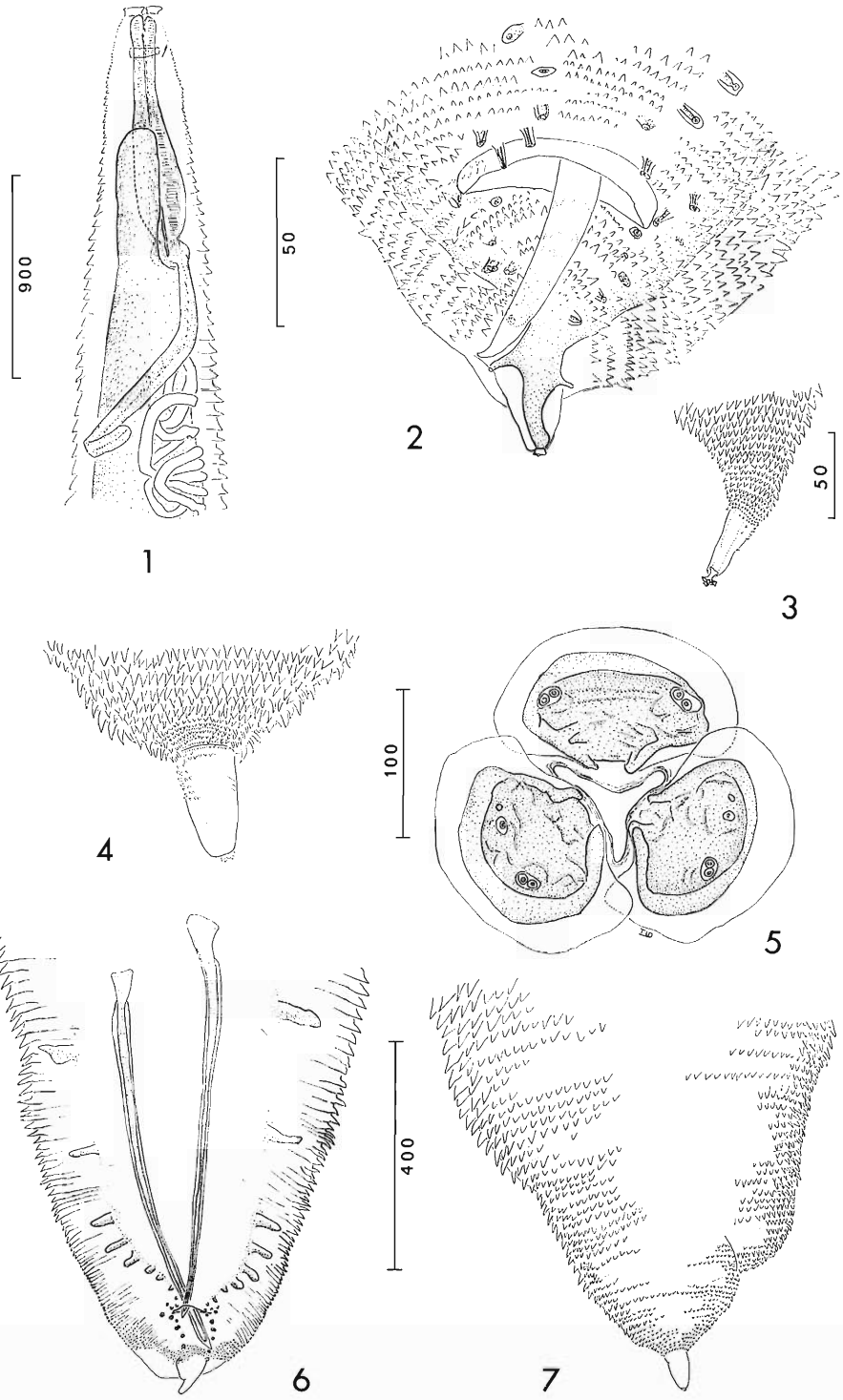


Table 1. Nominal species of *Goezia* parasitizing fishes and aquatic reptiles of the world.

Species	Type host	Type locality	Number of anal papillae pairs			Length ratio IC:VA
			pre	post	para	
G. annulata (Molin, 1859)	*Morone labrax*[a]	Trieste, Italy[b]	—	—	—	—
[d]*G. ascaroides* (Goeze, 1782)	*Silurus glanis*[a]	Europe[e]	—	—	—	—
G. fluviatilis Johnston and Mawson, 1940	[f,g]	Tailem Bend, South Australia	5	3[h]	3	1:3.9
G. gavialidis Maplestone, 1930	*Gavialis gargeticus*	Calcutta, India		Male unknown		1:1–7.5
G. holmesi Sprent, 1978	*Crocodylus porosus*[a]	Liverpool River, Northern Australia	5	3	5	1:2.8–8.6
G. intermedia Rasheed, 1965	*Cichla ocellaris*	Georgetown, British Guiana	22–23	4	0	1:4–7
[i]*G. kliksi* sp. n.	*Pogonias cromis*	Lake Borgne, Louisiana	10–16	5	2	1:1.9–3.3
G. kollari (Molin, 1859)	*Chrysophrys aurata*	Europe	—	—	—	—
[i]*G. lacerticola* Deardorff and Overstreet, 1979	*Alligator mississippiensis*	Lake Apopka, Florida	22–26	4[j]	2	1:2.1–4.6
[d,i]*G. minuta* Chandler, 1935	*Bagre marinus*[a]	Galveston Bay, Texas	16	4[b]	2[j]	1:4.4
G. nankingensis Hsü, 1933	[f,k]	Nankin, Peoples Republic of China	6–7	3–4	3	—
[l]*G. oncorhynchi* Fujita, 1940	*Oncorhynchus keta*	Mashike, Japan				Adults unknown
[i]*G. pelagia* sp. n.	*Rachycentron canadum*	Off Alabama Point in Gulf of Mexico	12–19	4[j]	2	1:2.0–4.4
G. pseudoascaroides Rehana and Bilqees, 1972	*Mustacembelus pancalus*	Kalri Lake, W. Pakistan	29	2	0	—
G. sigalasi Stefanski, 1938	*Trachinus draco*	Aquarium in Brazil	9[m]	2	0	1:3.3–3.7
[i]*G. sinamora* sp. n.	*Tilapia aurea*	Lake Parker, Florida	13–16	3[j]	2	1:1.6–5.0
[d]*G. spinulosa* (Diesing, 1839)	*Arapaima gigas*[a]	Brazil	13[n]	6	2	1:1.5–5.3
G. tricirrata Osmanov, 1940	*Onos tricirrata*[a]	Black Sea	—	—	—	1:2.1–2.5

[a] Other hosts listed in same or subsequent reports.
[b] See our text for data on other material.
[c] See discussion in text.
[d] Based entirely on holotype.
[e] Additional localities listed in subsequent reports.
[f] No type host designated.
[g] Hosts: *Nannoperca australis*, *Tandanus tandanus*, *Morgunda adspera*, *McCullochella macquariensis*, *Percalates colonorum*, and *Plectroplites ambiguus*.
[h] Four postanal papillae illustrated.
[i] Indicates confirmation by authors.
[j] Double papillae present.
[k] Hosts: *Psephurus gladium*, *Leiocassis longirostris*, and *Parasilurus asotus*.
[l] Based on larval specimen.
[m] Ten preanal papillae illustrated.
[n] Numerical values from Santos et al. (1979). We estimated proportional data by comparing largest and smallest values in their ranges.

Table 1. Continued.

Species	Spicules: % of body length	Spicules: Length ratio left:right	Body length in mm	Esophagus as % of body length	Important reference
G. annulata (Molin, 1859)	—	—	3–4[b]	—	Molin (1859b)[c]
[d]*G. ascaroides* (Goeze, 1782)	—	—	18.4	8.1	Dollfus (1935), Khan and Yaseen (1964), Rasheed (1965)
G. fluviatilis Johnston and Mawson, 1940	10–22	1:1.0	2–6	13.6–17.0	Johnston and Mawson (1940)
G. gavialidis Maplestone, 1930	—	—	6.6	11.3	Maplestone (1930)
G. holmesi Sprent, 1978	7–12	1:1.0	2.6–6.7	10.4–14.2	Sprent (1978)
G. intermedia Radheed, 1965	3–4	1:1.1	9–15.5	9.6–10.9	Rasheed (1965)
[i]*G. kliksi* sp. n.	8–14	1:1.0–1.2	6–14	8–10	This paper
G. kollari (Molin, 1859)	—	—	8–11	—	Dollfus (1935), Molin (1859a)
[i]*G. lacerticola* Deardorff and Overstreet (1979)	8–16	1:0.8–1.0	3.9–8.2	7–15	Deardorff and Overstreet (1979)
[d,i]*G. minuta* Chandler, 1935	11	—	3.1[b]	10	Chandler (1935), this paper
G. nankingensis Hsü, 1933	—	1:1.0	5.5–9.3	7.6–8.2	Hsü (1933)
[i]*G. oncorhynchi* Fujita, 1940					Fujita (1940)
[i]*G. pelagia* sp. n.	6–10	1:0.9–1.1	3.4–14.5	10–17	This paper
G. pseudoascaroides Rehana and Bilqees, 1972	—	—	3.6–7.8	11–15	Bilqees et al. (1972)
G. sigalasi Stefanski, 1938	10–20	1:0.83	2.8–6.1	13.7–20.8	Stefanski (1938)
[i]*G. sinamora* sp. n.	4–20	1:0.7–1.3	1.4–16.0	8–18	This paper
[d]*G. spinulosa* (Diesing, 1839)	3	1:1.0	16.3–24.8	3.1–8.7	Baylis (1927), Freitas and Lent (1946), Rasheed (1965), Santos et al. (1979)
G. tricirrata Osmasnov, 1940	11	1:1.0	6–7	12.5–21.0	Osmanov (1940), Pogorel'tseva (1952), Dolgikh and Naidenova (1968)

width; ratio of greatest width to length 1:8–13. Cuticular spines absent dorsally from base of digitiform process to immediately anterior to spicules and ventrally near mucron. Lips 31–48 long by 74–125 wide. Nerve ring 192–309 from anterior extremity, 14–36 in breadth. Esophagus 0.4–1.0 mm long by 116–271 wide. Ventriculus 41–105 long by 67–210 wide; ventricular appendage 0.8–2.2 mm long by 49–117 wide. Intestinal cecum 327–961 long by 92–203 wide; ratio of cecal to appendage lengths 1:1.4–3.8; ratio of cecal to esophageal lengths 1:1.0–2.0. Spicules 6–10% of body length, equal in length in 7 of 16 specimens; right spicule 800–980 long averaging 877, 9–24 wide; left spicule 800–980 long averaging 861, 12–24 wide, longer than right one in 2 specimens; spicule ratio 1:0.9–1.1. Caudal papillae 18–25 pairs, preanal pairs 12–19 in J-shaped pattern, becoming closer together and more medial when approaching anus except for posterior 3 extending laterally; postanal pairs 4, with 3rd pair from posterior end double and slightly lateral to others (one apparent atypical specimen with 4 single papillae); paraanal pairs 2. Posterior end of worm flexed ventrad; tail 84–120 long including digitiform process 21–48 long, usually terminating with 4–6 minute spinous structures; structures occasionally absent.

FEMALE (based on 29 mature specimens): Body 3.6–14.5 mm long by 0.3–1.3 mm at greatest width; ratio of greatest width to length 1:7.0–16.0. Lips 26–78 long by 81–142 wide. Nerve ring 108–364 from anterior extremity, 21–49 in breadth. Esophagus 0.5–1.3 mm long by 79–346 wide. Ventriculus 50–129 long by 67–216 wide; ventricular appendage 0.6–2.3 mm long by 41–135 wide. Intestinal cecum 247–788 long by 101–409 wide; ratio of cecal to appendage lengths 1:2.2–4.4; ratio of cecal to esophageal lengths 1:1.4–3.0. Vulva without salient lips, opening 1.2–7.0 mm or 29–55% of body length from anterior extremity. Ovaries and oviducts directed posteriad, nearly reaching tail, usually occupying posterior ⅓ of body. Eggs with smooth thin shell, spherical, 25–35 in diameter. Tail 108–284 long including stout digitiform process 14–111 long, usually terminating with 3–6 minute spinous structures; structures occasionally with 3 clove-like projections or absent.

TYPE HOST: *Rachycentron canadum* (Linnaeus), cobia (Rachycentridae).

OTHER HOSTS: *Chaetodipterus faber* (Broussonet), Atlantic spadefish (Ephippidae); nonparatypes from *Ophicthus* sp. being described by Böhlke and Caruso (Academy of Natural Sciences of Philadelphia No. 143071).

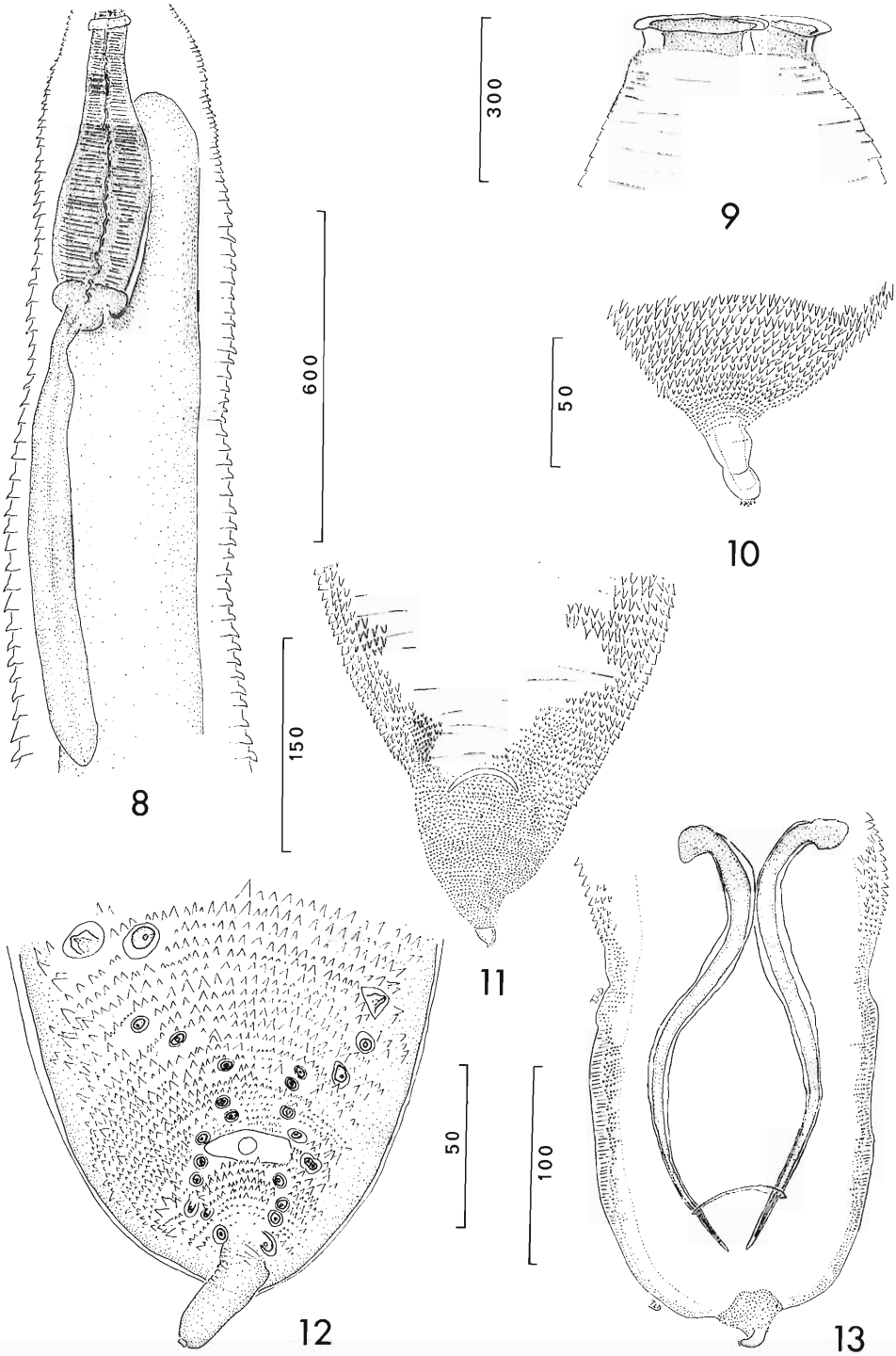
SITES OF INFECTION: Embedded in wall and free in lumen of stomach.

LOCALITIES: Offshore from Alabama Point, Alabama (type locality); Mississippi Sound, Mississippi; and Louisiana in Gulf of Mexico.

SPECIMENS DEPOSITED: Holotype, male, USNM Helm. Coll. No. 75680; allotype, female, No. 75681; paratypes, No. 75682 (pair), British Museum (Natural History) Reg. No. 1980.81–82.

ETYMOLOGY: The Latin *pelagia* refers to the habitat of the host.

COMPARISONS: Of the North American species, *Goezia pelagia* has an arrangement of preanal papillae with the most prominent recurvature posteriorly. By possessing four postanal papillae, it closely resembles *G. minuta, G. intermedia, G. nankingensis,* and *G. lacerticola*. It differs from the first three in having the third pair of postanal papillae from the posterior end doubled. In addition, *G. pelagia* differs from *G. minuta* by having more conspicuous phasmids and lacking a double para-anal papilla and differs from *G. intermedia* and *G. nankingensis* in the number of pre- and para-anal papillae (for actual values of characters,



Figures 8–13. ***Goezia minuta*** **from catfish. 8. Anterior end including intestinal cecum and ventricular appendage. 9. Lateral view of ventral lips. 10. Digitiform process of female tail, lateral view. 11. Female tail, ventral view. 12. Posterior end of male showing postanal papillae, holotype, ventral view. 13. Posterior end of male, ventral view.**

consult Table 1). *Goezia lacerticola* has a doubled third pair of postanal papillae, but *G. pelagia* differs from it most conspicuously by having a more extensive projection of the somatic musculature and lateral cords into the pseudocoel (Figs. 37–38 and Deardorff and Overstreet, 1979). *Goezia lacerticola* has been reported from an alligator in a freshwater lake only.

Goezia minuta Chandler
(Figs. 8–19)

Goezia minuta Chandler, 1935.

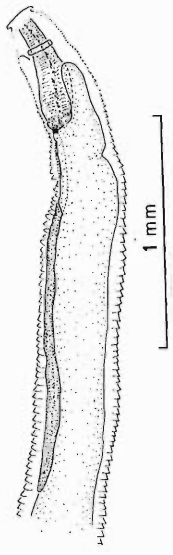
REDESCRIPTION (based on specimens from *Bagre marinus* and *Arius felis*; measurements of holotype in parentheses): Body reaching greatest width about ⅓ body length from posterior end. Spines longest and separated by greatest distance toward end of anterior ¼ of body. Esophagus clavate, 15–17% (10%) of body length. Ventriculus narrower than widest level of esophagus, generally broader than long. Nerve ring located within anterior 30–35% of esophagus. Excretory pore not visible. Tail conical, with digitiform process; process with circlet of spinous structures. Phasmids usually inconspicuous.

MALE (based on 3 specimens for most characters): Body 2.9–3.6 (3.1) mm long by 379–457 (300) at greatest width; ratio of greatest width to length 1:7.8–7.9 (10.1). Cuticular spines absent dorsally from base of digitiform process to just anteriad of cloacal papillae. Lips 24–36 (36) long by 77-90 (79) wide. Nerve ring 156–195 (132) from anterior extremity, 36 (92) in breadth. Esophagus 463–550 (321) long by 166–185 (92) wide. Ventriculus 60–74 (43) long by 86–120 (24) wide; ventricular appendage 321–346 (850) long by 80–111 wide. Intestinal cecum 290–364 (192) long by 55–117 (154) wide; ratio of cecal to ventricular appendage lengths 1:0.9–1.1 (4.4); ratio of cecal to esophageal lengths 1:1.5–1.6 (1.6). Spicules equal, 6–17% (11.1) of body length, right spicule 220–494 (346), left spicule 220–494 (broken); spicule ratio 1:1.0. Caudal papillae difficult to discern (preanal pairs 16; para-anal pairs 2, lower papilla on left side double; postanal pairs 4). Posterior end of body flexed ventrad; tail 155–160 (69) long including digitiform process 43 (26) long, terminating with 6 minute spinous structures.

FEMALE (based on 3 mature specimens): Body 2.0–3.6 mm long by 370–543 at greatest width; ratio of greatest width to length 1:5.4–6.6. Lips 26–31 long by 57–72 wide. Nerve ring 103 from anterior extremity, 37 in breadth. Esophagus 339–587 long by 93–160 wide. Ventriculus 36–55 long by 86–117 wide; ventricular appendage 438–918 long by 24–80 wide. Intestinal cecum 142–376 long by 79–105 wide; ratio of cecal to ventricular appendage lengths 1:2.4–3.0; ratio of cecal to esophageal lengths 1:1.5–2.3. Vulva without salient lips, opening 525–898 or 26–37% of body length from anterior extremity. Ovaries rarely extending beyond anterior level of vulva, nearly reaching tail, usually occupying posterior ⅓ of body. Eggs with smooth thin shell, spherical, 21–35 in diameter. Tail 96–247 long

→

Figures 14–19. ***Goezia minuta*** **from inshore lizardfish. 14. Anterior end including intestinal cecum and ventricular appendage. 15. Cross section through posterior portion of ventricular appendage. 16.** ***En face.*** **17. Posterior end of male, ventral view; poor quality of worm may have obscured one pair of postanal papillae. 18. Posterior end of male, lateral view. 19. Female tail, lateral. i: intestine; lc: lateral cord; sv: seminal vesicle; va: ventricular appendage.**



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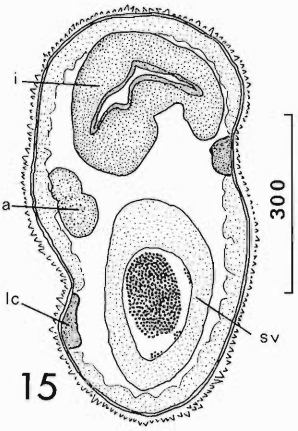

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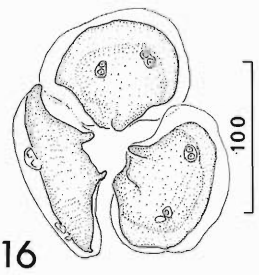

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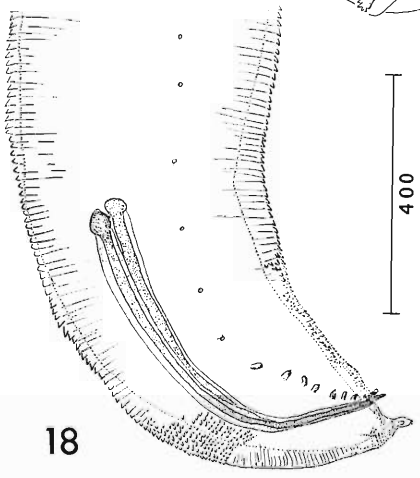

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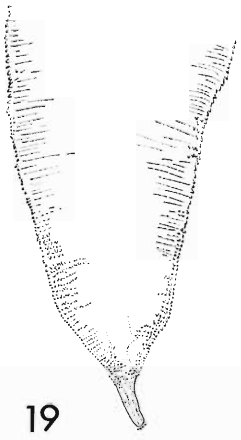

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including stout digitiform process 31–41 long, terminating with 6 minute spinous structures.

TYPE HOST: *Bagre marinus* (Mitchill), gafftopsail catfish (Ariidae).

OTHER HOSTS: *Arius felis* (Linnaeus), sea catfish (Ariidae); *Synodus foetens* (Linnaeus), inshore lizardfish (Synodontidae).

SITES OF INFECTION: Embedded in wall and free in lumen of stomach.

LOCALITIES: Galveston Bay, Texas (type locality); Gulf of Mexico offshore from Empire, Louisiana (sea catfish); Buttonwood Canal, Everglades National Park, Florida (inshore lizardfish).

SPECIMENS DEPOSITED: Holotype, male, USNM Helm. Coll. No. 39542; other material, USNM Helm. Coll. No. 75687, 3 slides (gafftopsail catfish); No. 75688 (sea catfish); No. 75686, 1 pair and 1 tail mount (lizardfish); Queensland Museum, Brisbane, Australia. SC4041, 2 pairs (lizardfish).

COMPARISONS: The arrangement and number of pairs of caudal papillae, 13–16 preanal, two para-anal (the lower papilla on the left side is double on the holotype), and four postanal (note discussion below about specimens from lizardfish), distinguishes this species.

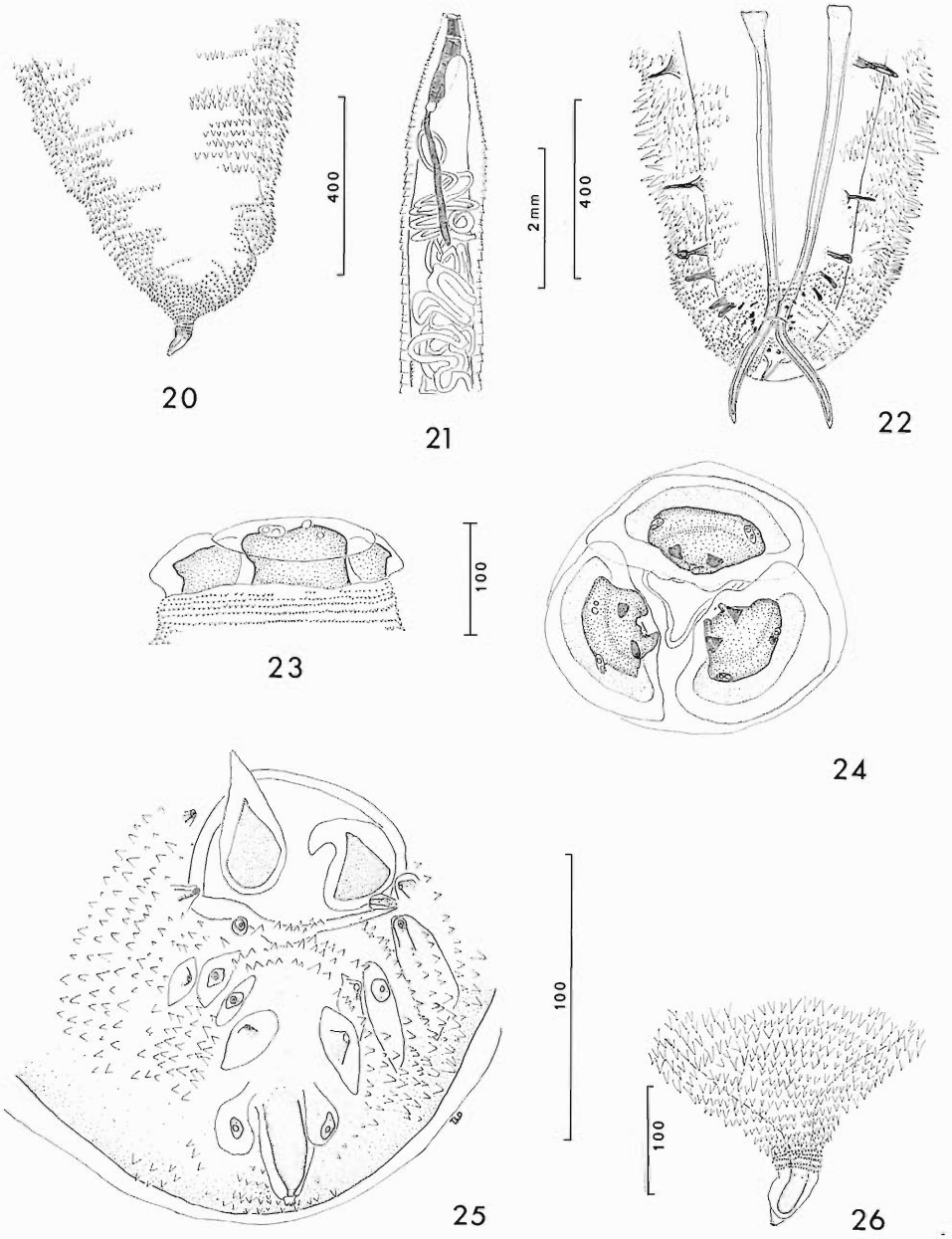
Remarks

The incomplete original description (Chandler, 1935), based entirely on the holotype from Texas, did not include the number or distribution of the characteristic caudal papillae. Chandler's illustration of the posterior end of the male is a dorsal view and shows the length of the spicules unequal rather than equal as stated in the description. The right spicule of the holotype is broken. Some specimens used in the redescription were provided by Pence and were in poor condition. After several attempts, we have been unable to collect additional specimens from marine catfishes.

We, however, critically examined 10 female (3.0–7.0 mm long) and six male (3.6–7.0 mm long) specimens from *Synodus foetens* that were fixed in situ with formalin, followed by storage in 40% isopropyl alcohol. Most are representative specimens of those reported by Overstreet (1968). Many features of those worms measured longer than the counterparts in shorter catfish material, but we nevertheless consider them conspecific and provide the following values and Figures 14–19 in case further investigation shows our identification to be wrong or variation to be host-induced. Some specimens had a different ratio of body width to length (1:6–16), ratio of cecal to ventricular appendage lengths (1:2.6–8.0), and ratio of cecal to esophageal lengths (1:1.7–3.0). The vulva opened 33–63% of the body length from the anterior extremity. The actual constant or variable number of postanal papillae could not be confirmed because of the moderate quality of the specimens. Nevertheless, there appears to be three or four pairs of such papillae. The number of preanal papillar pairs (17–20) and the spicule measurements (13–19% of body length; ratio of left to right one 1:0.9–1.0) compared well with values for catfish worms.

Goezia kliksi sp. n.
(Figs. 20–26)

DESCRIPTION: Body reaching greatest width about ⅓ body length from posterior end. Spines longest and separated by greatest distance toward end of an-



Figures 20–26. *Goezia kliksi*. **20. Female tail, allotype, ventral view. 21. Anterior end including intestinal cecum and ventricular appendage, holotype. 22. Posterior end of male, paratype, ventral view. 23. Lateral view of ventral lips. 24.** ***En face*****. 25. Posterior end of male showing postanal papillae, ventral view. 26. Digitiform process of female tail, allotype, lateral view.**

terior ¼ of body. Esophagus clavate, 8–10% of body length. Ventriculus narrower than widest level of esophagus, generally broader than long. Nerve ring located within anterior 24–31% of esophagus. Excretory pore not visible. Tail conical, with digitiform process; process with circlet of spinous structures. Phasmids usually conspicuous near base of digitiform process.

MALE (based on 4 mature specimens and 1 tail fragment for papillae and 5 for most described characters): Body 6.0–14.0 mm long by 0.9–1.5 mm at greatest width; ratio of greatest width to length 1:7–11. Cuticular spines absent dorsally from base of digitiform process to posterior extremity of intestine. Lips 37–61 long by 123–160 wide. Nerve ring 309 from anterior extremity, 31–43 in breadth. Esophagus 0.8–1.3 mm long by 154–339 wide. Ventriculus 135–142 long by 154–228 wide; ventricular appendage 1.3–1.8 mm long by 49–92 wide. Intestinal cecum 472–835 long by 123–339 wide; ratio of cecal to appendage lengths 1:1.9–3.3; ratio of cecal to esophageal lengths 1:1.4–1.6. Spicules 8–14% body length, equal in length in 3 of 5 specimens; right spicule 0.8–1.2 mm long by 24–36 wide, longer than left one in 2 specimens; left spicule 0.8–1.2 mm long by 24–36 wide; spicule ratio 1:1.0–1.2. Caudal papillae 17–23 pairs, becoming closer and more medial when approaching anus; preanal pairs 10–16, 1 specimen with a double papilla at number 12 from posterior end; postanal pairs 5; para-anal pairs 2. Posterior end of worm flexed ventrad; tail 105–129 long including digitiform process 36–43 long, usually terminating with 4–6 minute spinous structures.

FEMALE (based on 1 mature specimen): Body 8.0 mm long by 1.0 mm at greatest width; ratio of greatest width to length 1:8. Lips 122 wide. Esophagus 0.8 mm long by 247 wide. Ventriculus 92 long by 154 wide; ventricular appendage 1.2 mm long by 61 wide. Intestinal cecum 537 long by 61 wide; ratio of cecal to appendage lengths 1:2.2; ratio of cecal to esophageal lengths 1:1.5. Vulva not visible. Ovaries directed posteriad, nearly reaching tail, occupying posterior ⅓ of body. Eggs with smooth thin shell, spherical, 25–35 in diameter. Tail 315 long including stout digitiform process 74 long terminating with apparently 6 spinous structures.

TYPE HOST: *Pogonias cromis* (Linnaeus), black drum (Sciaenidae).

SITES OF INFECTION: Embedded in wall and free in lumen of stomach.

TYPE LOCALITY: Lake Borgne, Louisiana.

SPECIMENS DEPOSITED: Holotype, male, USNM Helm. Coll. No. 75689; allotype, female, No. 75690; paratypes, 1 entire male No. 75691, glycerin jelly mount of tail No. 75691.

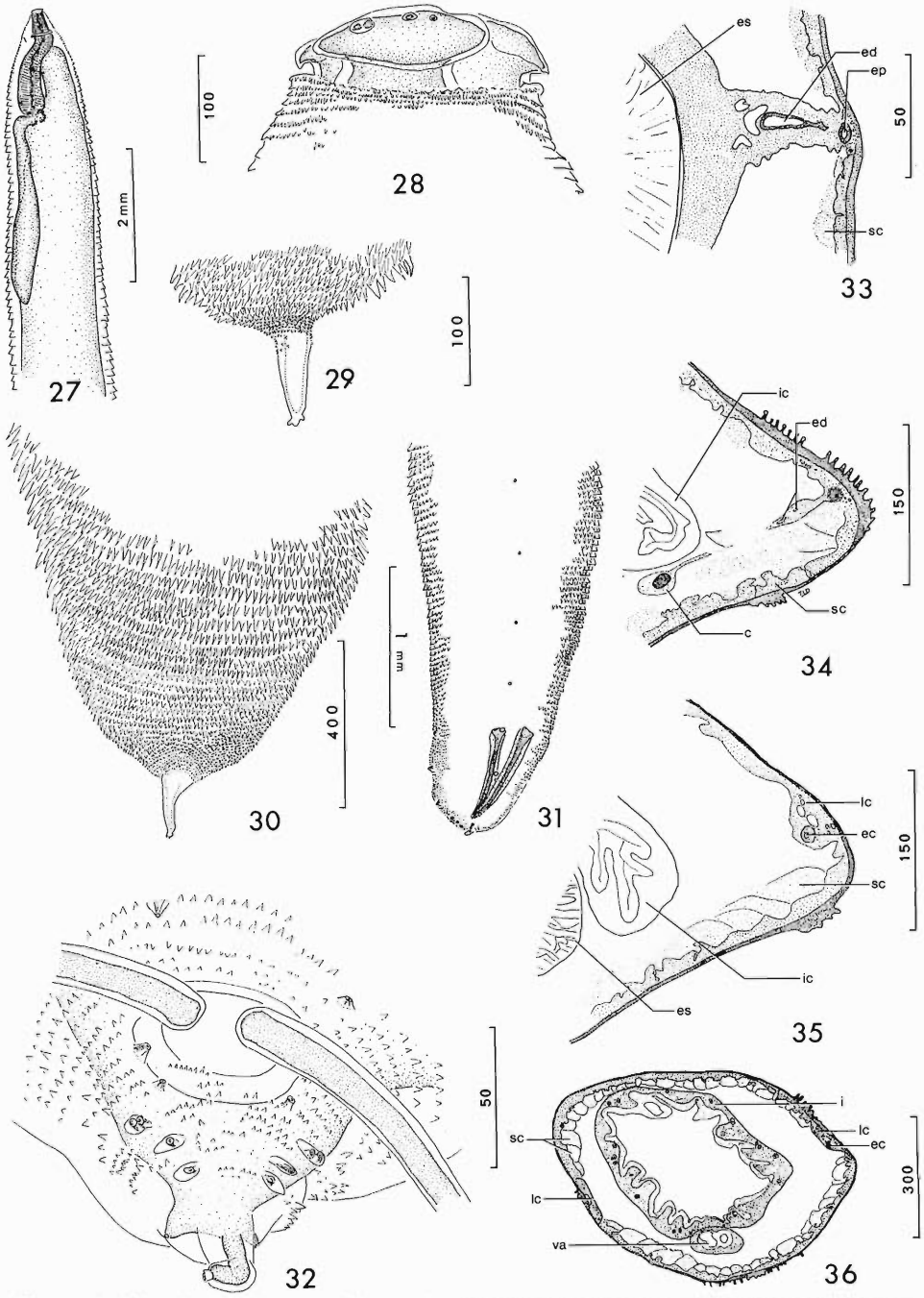
ETYMOLOGY: The specific name *kliksi* honors Dr. Michael Kliks, presently of Wisconsin Medical College, for his aid and his interest in our study and the genus *Goezia*.

COMPARISONS: The primary distinguishing characteristic of *Goezia kliksi* is the presence of five single pairs of postanal papillae. The species is most similar to *G. pelagia,* which has four pairs of postanal papillae, one of them doubled, and a more arcuate arrangement of the most posterior three preanal papillar pairs.

Goezia sinamora sp. n.
(Figs. 27–36)

Goezia sp. Rogers, 1970; Ware, 1971; Gaines and Rogers, 1972.

DESCRIPTION: Body reaching greatest width about midbody. Spines longest and separated by greatest distance toward end of anterior ¼ of body. Esophagus clavate, 8–18% of body length. Ventriculus narrower than widest portion of esophagus, generally broader than long. Nerve ring located within anterior 17–29% of esophagus. Lateral cords short and inconspicuous in cross section. Excretory pore immediately anterior to or at level of nerve ring; excretory system



Figures 27–36. *Goezia sinamora*. **27. Anterior end including intestinal cecum and ventricular appendage. 28. Lateral view of ventral lip. 29. Digitiform process of female tail, ventral view. 30. Female tail, ventral view. 31. Posterior end of male, lateral view. 32. Posterior end of male showing postanal papillae, ventral view. Figures 33–35. Cross sections showing excretory system. 33. Excretory duct in ventral cord and excretory "pore" immediately before opening. 34. Excretory canal in left lateral cord at level of intestinal cecum (Fig. 35) and ventricular appendage (Fig. 36). c: excretory cell; ec: excretory canal; ed: excretory duct; ep: excretory pore; es: esophagus; i: intestine; ic: intestinal cecum; lc: lateral cord; sc: somatic muscle cell; and va: ventricular appendage.**

with duct extending mesially from pore to single cell body; cell body free in pseudocoel, located adjacent to intestinal cecum; excretory canal joining excretory duct at approximately duct midpoint, extending posteriorly along left lateral cord to at least beyond midbody (posterior of worm not sectioned), approximately 12 in diameter along entire length. Tail conical, with digitiform process; process with or without spinous structures. Phasmids conspicuous near base of digitiform process.

MALE (based on 5 mature specimens for postanal papillae and on 19 for most other characters): Body 4.4–13 mm long by 0.5–1.5 mm at greatest width; ratio of greatest width to length 1:5.8–9.1. Cuticular spines absent dorsally from base of digitiform process to middle of retracted spicules. Lips 26–67 long by 166 wide. Nerve ring 203–284 from anterior extremity, 16–78 in breadth. Esophagus 0.6–1.1 mm long by 166–346 wide. Ventriculus 61–141 long by 67–236 wide; ventricular appendage 0.8–2.6 mm long by 43–250 wide. Intestinal cecum 296–819 long by 191–401 wide; ratio of cecal to ventricular appendage lengths 1:1.6–5.0; ratio of cecal to esophageal lengths 1:1.4–2.2. Spicules 4–20% of body length; right spicule 0.5–1.0 mm long by 12–37 wide; left spicule 0.5–1.1 mm long by 12–37 wide; spicule ratio 1:0.7–1.3. Caudal papillae 18–21 pairs; preanal pairs 13–16; para-anal pairs 2; postanal pairs 3, with 3rd pair from posterior end double. Tail flexed ventrad, 90–362 long including digitiform process 24–92 long, terminating with or without circlet of minute spinous structures.

FEMALE (based on 14 mature specimens): Body 5.7–16.0 mm long by 0.7–1.8 mm at greatest width; ratio of greatest width to length 1:6.0–11.0. Lips 31–61 long by 120–166 wide. Nerve ring 143–321 from anterior extremity, 36–67 in breadth. Esophagus 0.8–1.4 mm long by 185–395 wide. Ventriculus 61–267 wide; ventricular appendage 1.5–3.2 mm long by 43–267 wide. Intestinal cecum 0.3–1.0 mm long by 247–583 wide; ratio of cecal to ventricular appendage lengths 1:1.9–3.3; ratio of cecal to esophageal lengths 1:0.7–3.2. Vulva without salient lips, opening 3.0–3.4 mm or 42–43% of body length from anterior extremity. Eggs nearly spherical, 31–69 in diameter. Tail 154–300 long including digitiform process 37–129 long, terminating with circlet of 6 minute spinous structures.

TYPE HOST: *Tilapia aurea* (Steindachner), blue tilapia (Cichlidae).

OTHER HOSTS: *Micropterus salmoides* (Lacépède), largemouth bass (Centrarchidae); *Morone saxatilis* (Walbaum), striped bass (Percichthyidae).

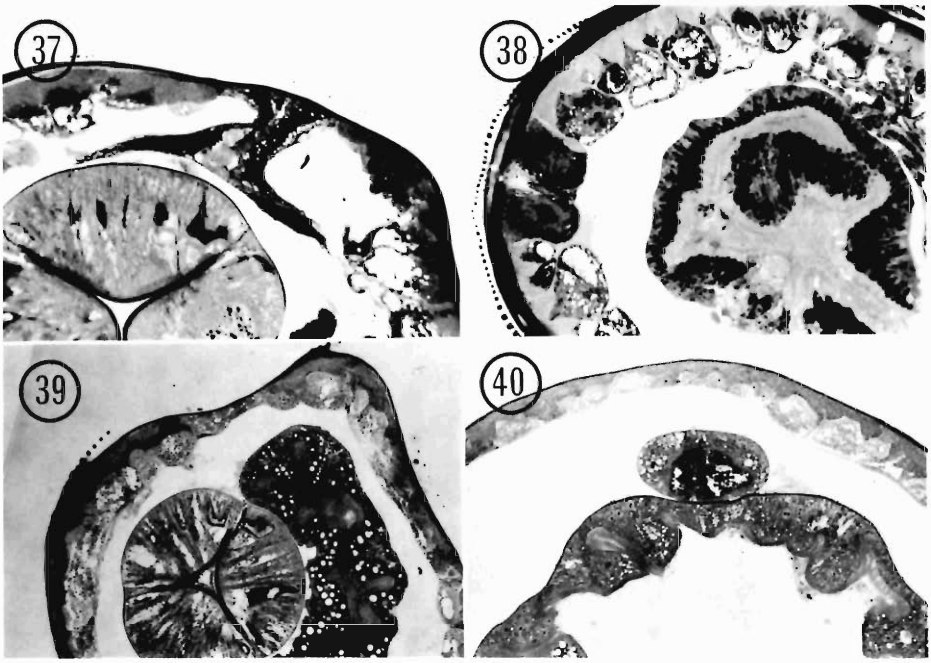
SITES OF INFECTION: Embedded in wall and free in lumen of stomach.

LOCALITIES: Lake Parker, Polk County, Florida (type); Busch Gardens, Tampa, Florida.

SPECIMENS DEPOSITED: Holotype, male, USNM Helm. Coll. No. 75683; allotype, female, No. 75684; paratypes, No. 75685 (pair).

ETYMOLOGY: The Greek *sinamora* means "injurious" and refers to the ability of the species to harm some hosts.

COMPARISONS: The primary diagnostic feature of this species is the combination of 13–16 preanal, two para-anal, and three postanal pairs of papillae with the third pair from the posterior end doubled. On the basis of the number of preanal papillae, *Goezia sinamora* most closely resembles *G. minuta, G. kliksi,* and *G. pelagia,* all American species. *Goezia sinamora* differs from *G. minuta* by possessing a pair of doubled postanal papillae and from *G. kliksi* and *G. pelagia* in number of postanal papillae. Based on presence of the single specifi-



Figures 37–40. Figures 37–38. 37. Cross sections of *Goezia pelagia*. Esophageal level immediately posterior to nerve ring showing lateral cord with included nerve tissue. 38. Intestinal level immediately below level of ventricular appendage. Note tall lateral cords and tall somatic musculature; intestine is typically irregular. Figures 39–40. Cross sections of *G. sinamora*. 39. Esophageal level immediately posterior to nerve ring with intestinal cecum shown in figure. Note the nonprojecting lateral cords. 40. Intestinal level showing distal portion of ventricular appendage. Note short somatic cells relative to the tall ones in Figure 38. (Lateral cord not shown.)

cally located pair of double postanal papillae, *G. sinamora* most closely resembles *G. pelagia*; however, it additionally differs from that species by having shorter lateral cords, shorter somatic musculature, and a more circular gut lumen in cross section (Figs. 37–40). This latter feature, however, may be a transitory condition.

Remarks

Although numerous specimens were obtained from fish in the fresh water of Lake Parker, the parasite was suspected by Gaines and Rogers (1972) as probably introduced from larvae in marine herring used to feed hatchery-reared striped bass. The worm occurred only in Lake Hollingsworth, Lake Parker, Lake Hunter, and Lake Bentley; those lakes were all stocked with striped bass from Richloan State Hatchery. According to Ware (personal communication) who was involved in the work, a misunderstanding occurred. Rather than being fed blueback herring, *Alosa aestivalis,* from the St. John's River system, the hatchery-reared striped bass were fed, and were probably infected from eating gizzard shad, *Dorosoma cepedianum,* caught directly from Lake Parker. Consequently, rather than being introduced into the four lakes, the nematode probably was already established in and restricted to those lakes.

According to Rogers (personal communication), *G. sinamora* commonly in-

fected striped bass as well as several other fishes in the lakes. In striped bass, a few individual worms passed through the stomach and encysted in the body cavity. Rogers, however, noticed that in introduced *Tilapia aurea,* the worm penetrated through the intestine causing extensive lesions in addition to forming nodules in the stomach. Descriptions of lesions in the stomach appear in our later section on biology.

A male and female specimen deposited by MacCallum on 1 July 1911 as *Lecanocephalus annulatus* from *Morone saxatilis* (*Roccus lineatus*) in the United States National Museum (USNM Helm. Coll. No. 34538) appeared to be *G. sinamora*. MacCallum's specimens were similar by having two para-anal papillae and four postanal papillae, with the third pair double; a ratio of cecal to ventricular appendage lengths of 1.5; spicule lengths 11–15% of the total body length, which is 6.0–6.5 mm; and an esophagus 12–13% of the body length. Because of the brittle condition of the deposited male, we could confirm only 11 preanal papillae. If the specimen is *G. sinamora,* the range of the species would extend at least from fresh water of Florida to New York.

By today's standards, *Goezia annulata* was inadequately described by Molin (1859a) from *Morone labrax* (=*Dicentrarchus l.*) offshore from Trieste, Italy; additional reports by Stossich (1887, 1898), Sonsino (1890, 1891), and others reported *G. annulata* from the type and other hosts in the Mediterranean Sea, but still did not characterize specimens for modern comparisons. Linton (1901, 1905) and MacCallum (1921) reported *G. annulata* from various marine fishes in the North Atlantic. Possibly none of these American reports from different regions refers to *G. annulata* because, based on material of poor condition from the USNM, there appears to be more than one species reported as that synonym and the genus is more speciose than previously assumed. Also, very few ascaridoids occur in both the western North Atlantic and the Mediterranean Sea, and those infect pelagic fishes. At least some of the material reported by MacCallum (1921) as *Lecanocephalus annulatus* appears to be *G. sinamora* and probably so are other worms from the striped bass identified as *L. annulatus* by MacCallum (USNM 34538, 35452, and 35453) and by Linton (USNM 6628) and as *Goezia* sp. by Beckland (USNM 71347). The specimens deposited by MacCallum, according to measurements of features other than spicules and hosts, correspond to his report (1921). Linton (1901) illustrated the anterior and posterior ends of a specimen, but deposited the middle portion. Even though we doubt *G. annulata* occurs in North America, not all worms identified as such can be referred to *G. sinamora* either. At least, males from *Urophycis tenuis* (USNM 35454) and *Haemulon album* (USNM 35456) cannot. Specific identification of two females from *Centropristis striata* listed as *G. annulata* (USNM 34588) could not be made without corresponding males.

Supplemental data for *G. spinulosa,* the only other species reported from the Americas, were recently reported by Santos et al. (1979) using specimens from *Arapaima gigas,* the type host collected in Brazil. This species has caused taxonomic confusion (see Rasheed, 1965). Freitas and Lent (1946) provided supplemental data on specimens from *Astronotus ocellatus* in Ceará, Brazil, which may or may not be conspecific. We examined specimens (USNM 36939) from that same host and locality, and they are unlike any of our material. They also differ from the material described by Freitas and Lent (1946); for example, in a pair we

examined, the intestinal cecum and ventricular appendage measured 31–43 μm and 1.6–1.7 mm compared with 0.4–0.7 mm and 2.2–2.9 mm for worms described with the same approximate lengths.

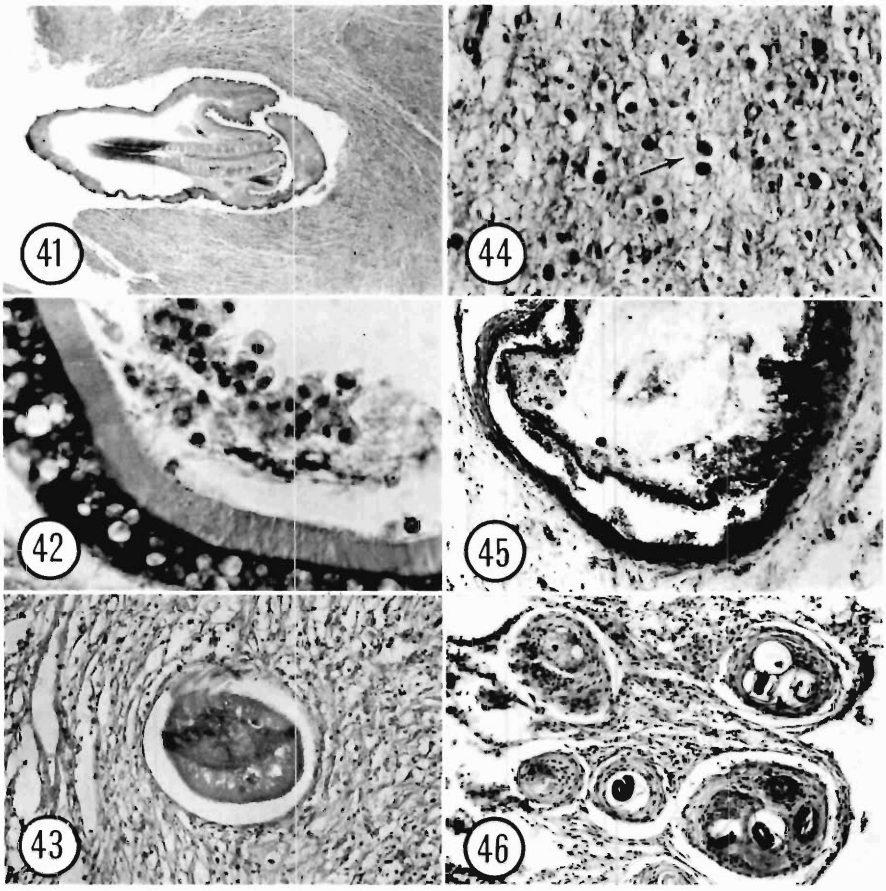
Observations and Discussion of Taxonomic and Biological Aspects of *Goezia* spp.

Differentiating most species of *Goezia* remains problematical. Several features such as ratios dealing with lengths of the intestinal cecum, ventricular appendage, esophagus, spicules, and total body may be helpful, but they are usually used in conjunction with the number and arrangement of caudal papillae in the males. As an example, we cannot identify female specimens found in *Ophicthyes gomesi* and *Micropogonias undulatus* from Davis Bayou, Mississippi, *Epinephalus nigritis* from Southwest Pass of the Mississippi River, Louisiana, and *Sciaenops ocellata* from a pond in Palacios, Texas, because no concurrent males were collected. However, we list the hosts to provide a more complete record of hosts and localities in which species of this genus occur. Unfortunately, not even male papillae have been adequately assessed. Observing all the papillae on an individual usually presents difficulty except on well-fixed specimens, and males are scarce; females often outnumber males in a nodule. Descriptions for papillae in most of the species we report have been based on few specimens, but as more specimens were examined, the number of papillae and the position of double papillae remained consistent.

The shape and length of cuticular spines appeared to vary with age, position, and individual. Consequently, we do not consider those means reliable to differentiate the species we examined. Spinous structures on the tail's tip may be characteristic, but, for some species, certain individuals do not possess them and on those that do the number and shape may vary. Observations of additional well-fixed specimens of all species will probably provide additional characters for differentiation. This is especially true for cross sections of the somatic musculature, lateral cords, alimentary tract, and other features (e.g., see Figs. 37–40). The consistency of those characters for *G. pelagia* as figured held strong for four of five examined specimens. In the fifth, the hypodermal and muscle cells were somewhat shorter, but not as short as the two sectioned specimens of *G. sinamora*.

Species of *Goezia* attach firmly to the stomach wall of hosts; however, evidence of worms associated with food in some hosts also containing lesions suggests that at least some species can detach, leave their site of attachment for various periods, and return to the same site. Presumably those species feed on both the host and its partially digested food.

Goezia sinamora, a species implicated in mortality of striped bass by Gaines and Rogers (1972), occurs within fibrous nodules. The worm in Figure 41 is one of six large healthy specimens from a 10-mm-wide by 7–mm-deep nodule in the stomach of *Tilapia aurea*. That individual had its anterior portion retracted within its trunk, with the spiny cuticular rings projected into the nodule. Perhaps the retracted position allows for both feeding and attachment. While the median part of the trunk remains stationary, the uncontorted portion with lips and esophagus may thrust into host tissue, rasp off this nodular tissue, and accumulate that tissue and host exudate. However, that was the only sectioned retracted specimen, and it did not have conspicuous protractor or retractor muscles. In any



Figures 41–46. Sections through nodule in stomach of *Tilapia aurea* caused by *Goezia sinamora*. 41. Anterior of worm retracted within its trunk. Note lips and esophagus of worm and loose connective tissue of nodule. Harris' hematoxylin and eosin (H and E), ×30. 42. Host cells in intestine of nematode. H and E, ×560. 43. Larval specimen burrowed into nodule from central cavity. H and E, ×172. 44. Region of minimal inflammation adjacent to a region of intensive response not shown; note granular cells with arrow pointing to one. McManus' method for glycogen with hematoxylin (PASH), ×135. 45. Degenerating dead worm with PAS-positive substance within worm and between worm and collagenous capsule. Gomori's trichrome method, ×133. 46. Tubercles with embryonated eggs deep within nodule. H and E, ×134.

event, intestinal contents were primarily exudate, rich with red blood cells and chronic inflammatory cells (Fig. 42); necrotic tissue, some infiltrated with bacteria, also occurred.

The figured worm (Fig. 42) has probably been attached for a long period since the nodule was thick. Connective tissue laden with collagen (as demonstrated by Gomori's trichrome method) incorporated a considerable chronic inflammatory infiltrate including eosinophils. Some regions along exposed lumenal surfaces of the nodule were necrotic and massed with bacteria. Intensely inflamed mucosal tissue immediately adjacent to the nodule sloughed into the nodule.

A juvenile form of the same species 106 mm in diameter at a midbody cross

section burrowed into the nodular tissue from the cavity (Fig. 43). It fed on exudate and elicited no additional inflammatory response. The typically low grade inflammation of repairing tissue was characterized in some areas by granular cells (Fig. 44; see Chaicharn and Bullock, 1967).

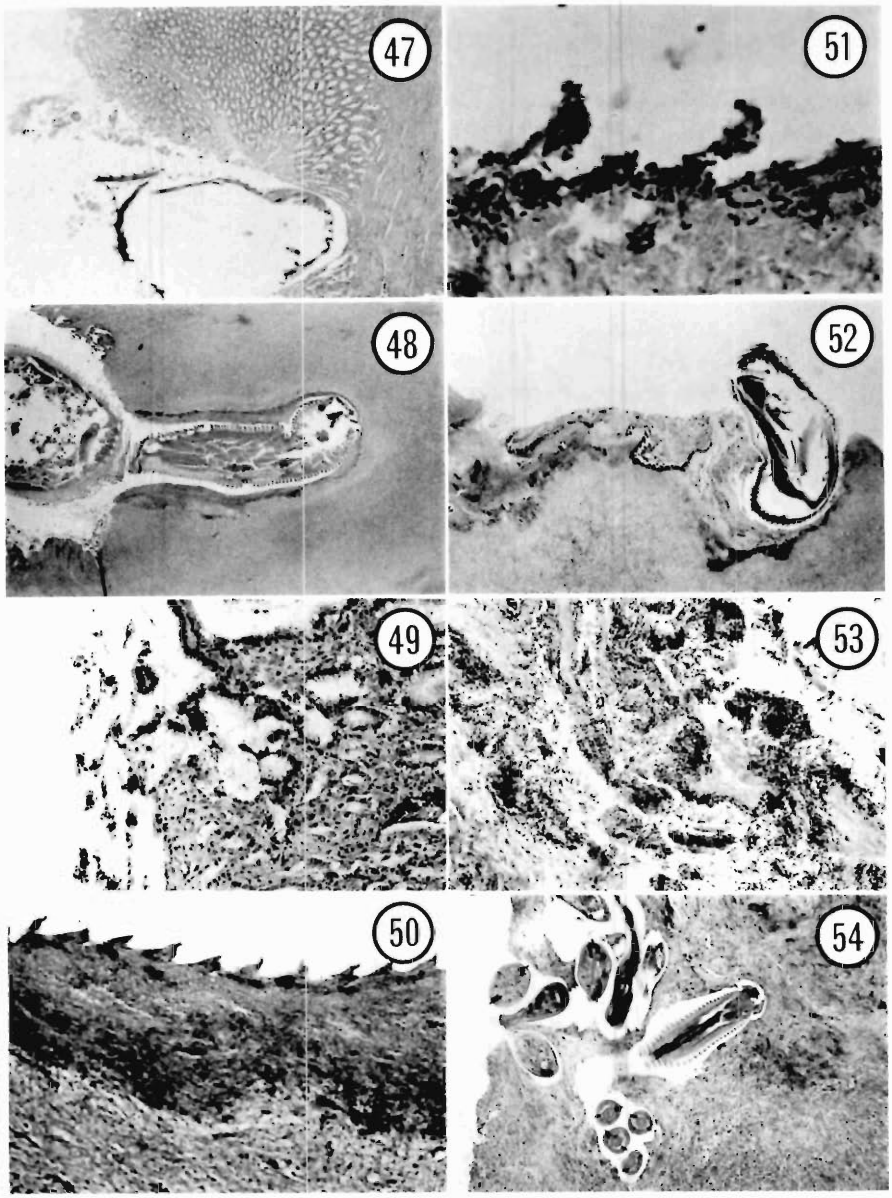
Deep within the nodular tissue occurred two dead specimens being degraded by host cells. They were encapsulated by epithelioid fibroblasts distinctly demarcated from the densely collagenous matrix. The space between the dense layer and the worm and also within the worm itself was partially filled by a PASH (periodic acid Schiff technique with hematoxylin) positive substance (Fig. 45) possibly similar to that reported from a proboscis and degenerated proboscis of the acanthocephalan *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 by Chaicharn and Bullock (1967). Those authors characterized that substance and assumed it was secreted by fibroblasts lining the capsule. Also distant from the living worms were numerous epithelioid tubercles encasing foreign matter, some of which appeared to be embryonated eggs of *Goezia sinamora* (Fig. 46).

Ware (1971) and Gaines and Rogers (1972) reported on the same extensive infections and mortalities of striped bass which presumably resulted from *G. sinamora* in four lakes in Florida, one the locality of our material. They reported a rapid decrease in the physical condition (coefficients indicated as K-factors) of most infected fish to values between 1.8 and 1.4. Death became impending at those values, whereas infected fish with K-factors between 1.8 and 2.0 usually survived. Healthy fish exhibited values above 2.0. Those authors also reported hemorrhagic ulcerlike depressions in the striped bass which presumably became scarified. Some individuals penetrated through the stomach wall into the body cavity, and the bacterium *Aeromonas* sp. was cultured from at least one stomach.

If *G. sinamora* indeed caused the mortalities, perhaps the reason was because the introduced *Morone saxatilis* was a poorly receptive host. Similarly, *Tilapia aurea* was an introduced species, and it also evoked a considerable response, including lesions in the intestine. Moreover, MacCallum (1921) suggested that in some cases the swollen irritating "nests" in *M. saxatilis* caused by the same or similar species of *Goezia* restricted the stomach's cavity such that the fish could not ingest enough food to survive.

Host response to species of *Goezia* varies among both identical and different hosts, and we describe four nodules from cobia with *G. pelagia* to illustrate some of this variation in terms of a progressive condition. We observed neither severe damage nor penetration into the body cavity as reported for *G. sinamora*. In the first case, the illustrated nonretracted worm (Fig. 47) penetrated the mucosa and nearly reached the muscularis. Some hemorrhaging occurred without an appreciable leucocytic inflammatory response, and slight autolysis of the gastric glands occurred. Consequently, postmortem migration by the worm was possible, but questionable because near the worm's anterior end a thin epithelioid lining had become established between the worm and the gastric glands. Also, considerable necrotic debris had sloughed into the nodular cavity, and some was being engulfed by giant cells and macrophages. That material plus exudate occupied the worm's intestine.

An extensive vascularized, dense, collagenous capsule characterized the second case (Fig. 48). The worm penetrated deep into the muscularis and evoked a chronic inflammatory response extending about 1 mm from the capsule. Eosin-



Figures 47–54. Sectioned *Goezia pelagia* and associated nodules in the stomach of *Rachycentron canadum*. **47.** Anterior of worm penetrating through mucosa and nearing muscularis; note absence of fibrotic nodule and inflammatory response. H and E, ×29. **48.** Another worm with associated nodule; note narrow dense collagenous capsular layer adjacent to anterior of worm. H and E, ×13. **49.** Degenerating mucosa at margin of nodule near trunk of worm. H and E, ×137. **50.** Bacterial laden, plicated lining of capsule where cuticular rings of worm inserted. H and E, ×132. **51.** Similar region as in Figure 50, but in different nodule. Bacteria in photo restricted to plications; however, large nests of bacteria occurred elsewhere in capsule. Taylor's bacteria method, ×558. **52.** Nodule with thicker and more irregular layer separating worm from portion with loose connective tissue. H and E, ×16. **53.** Close-up of irregular layer showing less restricted localization of bacteria than in previous nodules. Taylor's bacteria method, ×136. **54.** A few individuals of the related *Thynnascaris inquies* burrowing into nodule of *G. pelagia*; note one worm entering the capsular layer. H and E, ×51.

ophils were not abundant in any of the sections. Mucosa still remained in contact with the midportion of the worm, but it was inflamed, degenerating, laden with bacteria, and sloughing (Fig. 49). The exposed wall lining the capsule had plications depicting a mirror image of the worm's cuticular rings which had fit there before the tissue was fixed (Fig. 50). Some regions of the wall were heavily infiltrated by both coccoid and rod-shaped bacteria.

The third case portrayed a more extensive collagenous capsule, and focal masses of bacteria involved most of it (Fig. 51, also see Fig. 53). The capsule extended into and separated the worm from the mucosal tissue. Tissue internal to the capsule had an abundance of inflammatory cells.

In the last case, the collagen-rich irregular region was thicker than in previous cases (Fig. 52). Much of it, however, was necrotic and bacteria occurred focally in large numbers throughout (Fig. 53). This tissue was continually sloughing into the cavity. Taylor's method for bacteria demonstrated primarily gram-negative rods, but coccoid-shaped and gram-positive rod forms as well as fungi also occurred. Although the number of bacteria was enormous, few individuals invaded beyond the irregular capsule. Eggs of *G. pelagia* which had undergone few divisions occurred deep in the capsule. A degenerated worm was situated near the base of the nodule, and it had scavenging cells attached. It, however, lacked an associated chronic inflammatory response.

A group of several specimens of *Thynnascaris inquies* (Linton, 1901) also occurred in the nodule (Fig. 54). These relatively small ascaridoid worms appeared to be more active burrowers than *G. pelagia,* and they did occasionally invade the muscularis. They, however, were not observed to pass through the capsule surrounding *G. pelagia* or to induce capsular formation about themselves.

Probably the four cases from cobia progress from a recent invasion to a well-established nodule. Even though representing different stages of penetration and repair, the leucocytic response was always restricted to near the capsule and no damage to the hosts was apparent beyond the nodules except in the mucosa near the nodule margins.

Many invading helminths evoke extensive responses. Caryophyllids cause lesions that differ according to the type of scolex (Mackiewicz, 1972). Several of these cestodes not possessing loculi or bothridia cause nodules, and some of these elicit an extensive inflammatory infiltration. Piscine reactions to acanthocephalans also vary according to the species of worm and its sex (Chaicharn and Bullock, 1967). The female, but not male, of *Octospinifer macilentus* Van Cleave, 1919 provides a comparative example. Its penetration resulted in a nodule without a capsule. Chaicharn and Bullock observed neither inflammation nor bacteria; granular cells occurred abundantly among the collagen. For fish, information on tissue response toward nematodes is sparse (Williams, 1967). Hauck and May (1977) and Iversen and Kelley (1974) described the response to larval and adult ascaridoids, respectively. In both cases the inflammation was much more intense than we report, but the latter of those may be a response to mechanical injury in addition to or separate from the worms. *Contracaecum* spp. penetrating the stomachs of a sea lion or pelican (Liu and Edward, 1971) and larval anisakids within ulcerated nodules in stomachs of marine mammals almost always evoke an extensive inflammatory response (e.g., Vik, 1964; Young and Lowe, 1969).

The above examples urge us to speculate that *Goezia* spp. in most hosts seem

to demonstrate well-adapted host-parasite relationships. The fibrotic nodules remain for a considerable time, but inflammation is restricted to near the worm and subsides with time. The nodule usually prevents the worm from penetrating through the serosal tissue, and the capsule acts as a barrier to bacterial invasion. The capsule's continuous growth and degeneration probably provides a constant source of food for the worm. Introduced hosts seem more susceptible to disease than natural hosts.

Overstreet (1968) reported ecological aspects of *G. minuta* from the inshore lizardfish that passed through Buttonwood Canal, Florida. Herein we report additional data and rework some of those presented originally. The worms caused a fibrotic nodule in the stomach wall grossly similar to those described above; they were usually located near the pyloric ceca and filled with mucus. Fish fixed immediately upon capture had worms attached within the cyst, free in the stomach, and attached to or encased in partially digested food items. Probably they ate the host's partially digested prey as well as the host's exudate. On occasion, as many as 10 worms occurred within a cyst. Monthly samples were taken for 2 years, and overall, 49.8% of the fish harbored an average of 3.0 worms each. Twice as many fish had infections in 1963 as in 1964, and the highest monthly average was 5.5 worms per infected fish in January 1963. Fish as short as 37 mm FL long were examined, but worms occurred in fish only 80 mm and longer. Considering those fish between 10 and 30 cm long, more had concurrent infections with *G. minuta* and two hemiurid digeneans than expected by chance alone. Possibly products from *G. minuta* or the host's response to the nematode was advantageous for the other worms. In any event, considering the relatively low density of *Synodus foetens* in the immediate region (Roessler, 1970), the relatively high prevalence of fish infected with *G. minuta* deserves note. *Goezia minuta* was not evident in several other examined fishes from that locality in the Everglades National Park or in lizardfish from nearby Biscayne Bay (Overstreet, unpublished data).

An experimental life cycle for a species of *Goezia* has not been established. Similarities among cycles for other piscine ascaridoids, however, suggest that it is basically the same as proposed for species of *Thynnascaris* Dollfus, 1933 (see Norris and Overstreet, 1976). A crustacean may or may not be necessary depending on the species, and paratenic hosts are often critical in maintaining a worm population. These could be fishes or invertebrates. Freitas and Lent (1946) reported what they believed to be a second-stage larva of *G. spinulosa* in the copepod *Diaptomus* sp. It had a cuticle devoid of spines. Larval specimens have been observed also in fish (e.g., Johnston and Mawson, 1951; Sprent, 1978), sometimes in a degenerate state. Gaines and Rogers (1972) reported them from the mesentery of some striped bass, but that location may reflect that the bass is an abnormal host. However, Jackson et al. (1978) also retrieved 20 specimens from the viscera of a striped bass in a Washington, D.C., fish market. According to Bier (personal communication), these were probably fourth-stage larvae with some gonadal development. We found in washings from spotted seatrout viscera a third-stage larva that had cuticular spines. Only one of several hundred seatrout examined had the infection. The 1.7 mm long worm had a ventricular appendage 29 times longer than the intestinal cecum and over three times that of the esoph-

agus. Third-stage larvae also penetrate into nodules occupied by adult worms (Fig. 43), but these probably ultimately mature or die in the nodules.

Acknowledgments

We gratefully acknowledge the assistance of the following persons: Dr. M. Kliks at Wisconsin Medical College for his interest in this project and donation of several specimens; Dr. D. B. Pence at Texas Tech University for specimens of *G. minuta*; Dr. J. F. A. Sprent at the University of Queensland, Australia, for loan of specimens of *G. minuta*; Dr. C. R. Gilbert at Florida State Museum in Gainesville for loan of and permission to remove stomachs from specimens of *Synodus foetens*; Fishery Biologist Ms. K. J. Foote at Kissimmee Basin Wetlands, Florida, and Dr. W. A. Rogers at Auburn University Agricultural Experiment Station for specimens of *G. sinamora*; Dr. J. R. Lichtenfels for specimens deposited at the U.S. National Museum Helminthological Collection; Mr. R. L. Colura at Texas Parks and Wildlife Service for the specimen from *Sciaenops ocellata*; Dr. E. D. Stoddard at Florida Department of Agriculture and Consumer Services and Mr. J. Olsen of Busch Gardens in Tampa, Florida, for specimens from the hybrid bass; Dr. E. W. Cake, Mr. R. R. Lukens, Mr. T. E. Mattis (all GCRL), Mr. L. A. Covington of Ocean Springs, Mississippi, and Mr. F. Crooke of Pensacola, Florida, for their aid in collecting fish; and Mrs. R. Buxton, Mr. R. G. Palmer, Mrs. L. B. O'Toole, and Ms. T. A. Miller (all GCRL) for technical assistance.

This study was conducted in part in cooperation with the U.S. Department of Commerce, NOAA, National Marine Fisheries Service, under PL 88-309 Project No. 2-325-R and HEW/Public Health Service, Food and Drug Administration Contract No. 223-76-2141.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 218–223

Howardula apioni sp. n. (Allantonematidae: Nematoda), a Parasite of *Apion carduorum* Kirby (Curculionidae: Coleoptera) in Southern France

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ABSTRACT: *Howardula apioni* sp. n. (Allantonematidae: Tylenchida) is described as a parasite of the weevil, *Apion carduorum* Kirby (Curculionidae: Coleoptera) in Southern France. The host is a pest of artichoke and the eggs, larvae, and pupae occur in the stems of this plant. Third-stage juvenile nematodes leave the adult beetle and enter the plant tissues where they molt twice and mate. The infective stage females enter the beetle larvae and are carried through the pupae and into the adult host. Most nematode development occurs during the diapause of the adult beetle. The rate of infection varied from 2–13% over an 8-year period.

Entomogenous nematodes of the genus *Howardula* have been recorded from insects belonging to the orders Diptera, Coleoptera, and Thysanoptera (Poinar, 1975). The effect of some *Howardula* species on their hosts has given them the rank of biological control agents that could be produced under laboratory conditions (Poinar, 1979).

During an investigation of the biology of the weevil, *Apion carduorum* in Southern France, a species of *Howardula* was found to be one of the most common enemies of this insect. Since *A. carduorum* develops on young artichoke plants (*Cynara scolymus* L.) which are extensively cultivated in certain areas along the Mediterranean, it is regarded as a pest. Thus any natural means of control would be of interest from a practical standpoint.

The present paper describes this nematode parasite, outlines its biology, and compares it with previously described members of *Howardula*.

Materials and Methods

In order to obtain the free-living stages of the nematode, infected adults of *Apion carduorum* Kirby were collected from artichoke plants from November to April and placed on stems of the same plant in laboratory cages. During their routine feeding and ovipositional behavior, they liberated nematode juveniles from their alimentary tract which matured to males and infective stage females in the plant tissues. These free-living stages were removed after macerating the stems in water and were heat killed in hot (80°C) water. The mature parasitic females and other stages found in the homocoel of beetles were removed and killed in hot (80°C) 0.9% saline. All specimens were then fixed in a mixture of formol (4%), acetic acid (3%), picric acid (0.2%), and glycerine (1%) and processed to glycerin by the Seinhorst evaporation method. All measurements were made with specimens fixed in the above manner.

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Results

SYSTEMATICS: The species of *Howardula* found parasitizing *A. carduorum* was determined as being new to science and a description follows below. In the quantitative portion of the description, the value following the character represents the average whereas the numbers in parentheses represent the range of the character. All measurements are given in micrometers unless otherwise specified.

Howardula apioni sp. n.
(Figs. 1, 2)

Tylenchida (Filipjev): Allantonematidae (Pereira), *Howardula* Cobb.

FIRST-STAGE JUVENILE (N = 10), (Figs. 1A, H): Elongate, length, 275 (240–340); head rounded, bearing a faint tylenchoid stylet approximately 7 μm in length; pharyngeal lumen slightly cuticularized from the base of the stylet to the junction with the subventral gland openings; nerve ring, excretory pore, and anus visible; genital primordium composed of 1 large medial and 2 smaller polar cells; tail rounded.

SECOND-STAGE JUVENILE (N = 10), (Fig. 1B): Elongate form with head and tail rounded; length, 420 (380–500); genital primordium with many cells; sexual differentiation occurs at the end of the stage.

THIRD-STAGE JUVENILE (N = 10), (Figs. 1D, E, I): Length nearly equal to that of adult female, 660 (590–710); male, 670 (620–730); gonad well-developed; head and tail rounded. This stage leaves the host and molts twice in succession in plant tissues to reach the adult stage.

The fourth-stage juveniles were very similar to those of the preceding stage and molted quickly to the adult stage.

FREE-LIVING FEMALE (Figs. 1E, F, G): Slightly longer and more slender than the 3rd-stage juvenile that leaves the host; usually retains the 2 thin cuticles of the 3rd- and 4th-stage juveniles; body straight or curved ventrally when heat killed; cuticle with fine transverse striations approximately 1 μm apart, head rounded, without a cephalic constriction; lips, amphids, and stylet guide not visible; stylet well-developed, with a large lumen, anterior part conical with a ventral opening, posterior part cylindrical with a diameter of 1 μm, slightly less than the anterior portion; the dorsal "knob" is slightly anterior to the 2 subventral; pharyngeal lumen wide, strongly cuticularized after the stylet but becoming difficult to follow after reaching the subventral gland openings; dorsal gland opening very minute and distinct only in living material; all 3 pharyngeal glands contain distinct nuclei, approximately 20 μm from each other; ovary composed of several cells, oviduct elongate, lined with small cells; sometimes both ovary and oviduct are reflexed (rare in Allantonematidae); uterus small in nonmated forms, but greatly extended after copulation; vagina leads anteriorly before joining the uterus at the point of a small constriction; vulva faintly visible, in posterior ⅓ of body; lacking lips; anus present, tail tip rounded.

QUANTITATIVE CHARACTERS (N = 10): Length, 683 (615–770); greatest width, 20 (17–23); length of stylet, 13 (12–14); distance from head to dorsal pharyngeal gland opening, 52 (45–57); nerve ring, 98 (93–104); hemizonid, 104 (100–110); excretory pore, 73 (68–78). Distance from vulva to anus, 22 (18–27); length

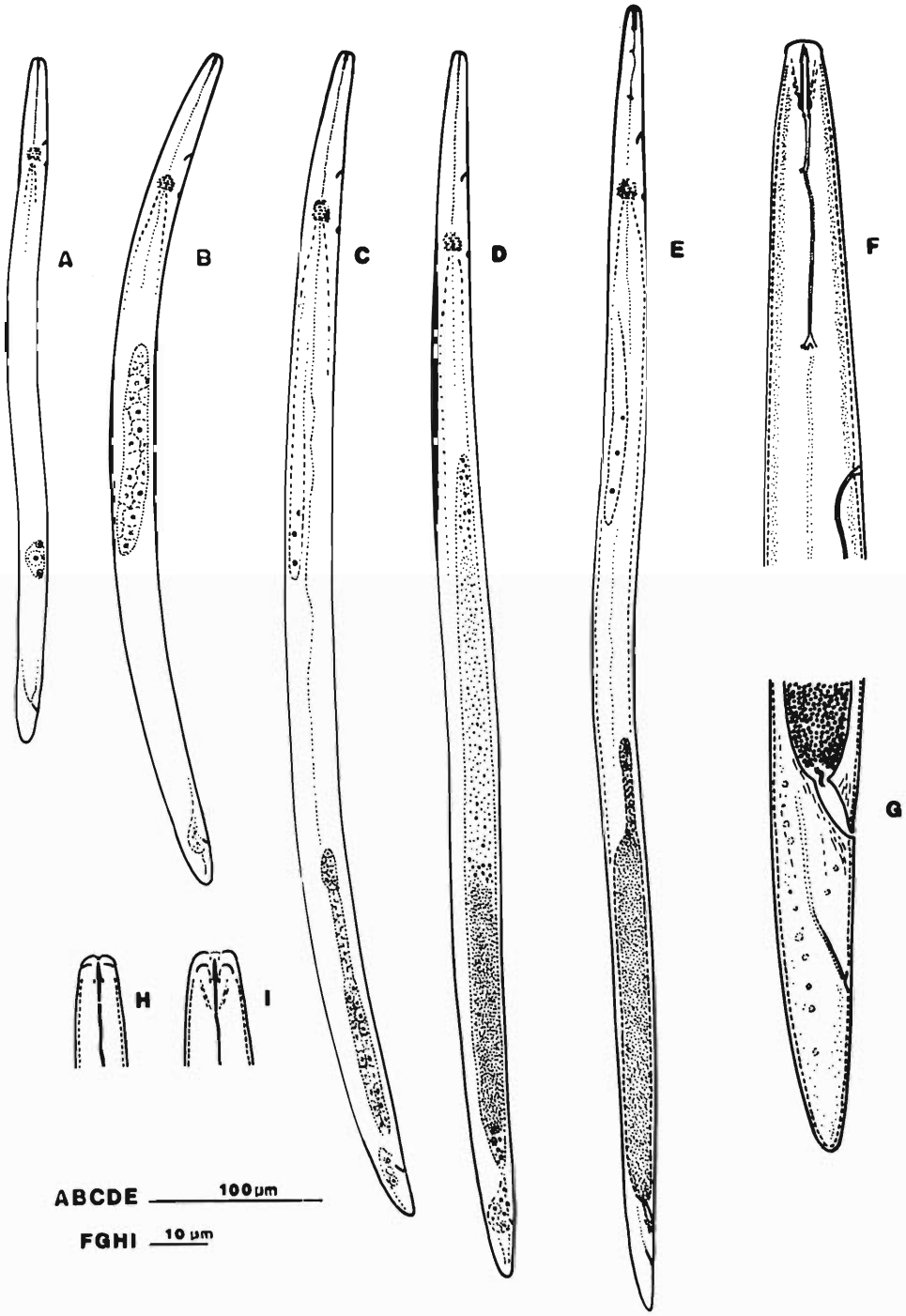

Figure 1. *Howardula apioni* **sp. n. A. First-stage juvenile. B. Second-stage juvenile. C. Third-stage juvenile female. D. Third-stage juvenile male. E. Free-living infective stage female. F. Anterior part of infective stage female. G. Posterior of infective stage female. H. Head of first-stage juvenile. I. Head of third-stage juvenile female.**

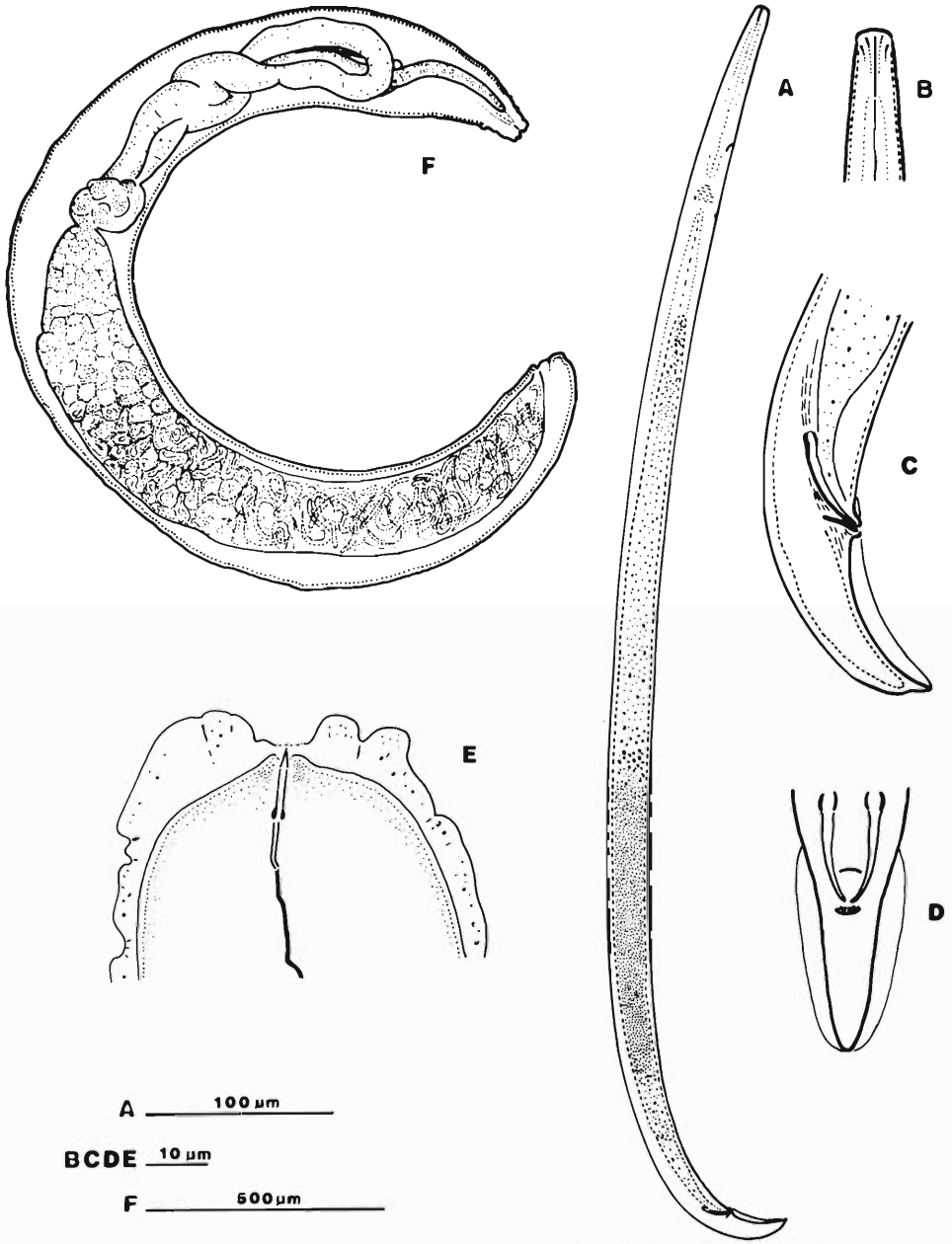

Figure 2. *Howardula apioni* **sp. n. A. Free-living male. B. Head of male. C. Lateral view of male tail. D. Ventral view of male tail. E. Head of mature parasitic female. F. Mature parasitic female.**

of tail, 31 (24–34); a = 35 (29–41); b = 2.46 (2.03–3.35); c = 22.4 (19.2–26.8); ratio of length to distance from head to hemizonid, 6.55 (6.15–7.10).

MALES (Figs. 2A, B, C, D): Cuticle smooth; body straight or curved ventrally after heat-killing; lateral cords distinct, about 4 µm wide; head flatter than in female; stylet degenerate, 9–10 µm; pharyngeal lumen indistinct; pharyngeal

glands not visible; gonad well-developed, tip reflexed; diameter of the spermatozoa, ±1.5; spicules paired, separate, equal, slightly curved; gubernaculum small, curved backwards; bursa peloderan, open; tail curved, sometimes terminated with a minute mucron.

QUANTITATIVE CHARACTERS (N = 10): Length, 712 (650–750); greatest width, 20 (16–24); distance from head to nerve ring, 93 (84–89); hemizonid, 111 (99–117); excretory pore, 78 (73–85); length of tail, 32 (30–42); length of gonad, 456 (350–522); length of spicules, 21 (19–26); length of gubernaculum, 7 (6–8); ratio of total length to distance from head to hemizonid, 6.5 (6.1–6.7); ratio of total length to length of gonad, 1.6 (1.4–2.0); a = 37 (31–43); c = 19.9 (16.4–22.0).

MATURE PARASITIC FEMALE (N = 8) (Figs. 2E, F): Length, 2.477 (2.040–2.860) mm; greatest width, 222 (172–286) μm; cylindrical or sausage-shaped, fixed striations every 2–3 μm, but often smooth near the head and tail; head rounded with oral opening often indented; stylet similar to that of the infective stage female; pharyngeal lumen visible just to the beginning of the subventral pharyngeal glands; excretory pore, hemizonid, and nerve ring not distinct; pharynx and intestine regressive; anus present; gonad well-developed, reflexed several times, the tip often reaching the stylet; spermatheca indistinct; gravid female containing eggs and hatched juveniles (ovoviviparous); vulva distinct in posterior ⅓ of body, without lips; tail rounded, variable in form.

TYPE HOST: *Apion carduorum* Kirby (Curculionidae: Coleoptera).

TYPE LOCALITY: Opio, Alpes-Maritimes, France.

TYPE SPECIMENS: Holotype (free-living female) and allotype (male) deposited at the Laboratoire des Vers, Museum National d'Histoire Naturelle, Paris.

DIAGNOSIS: There are presently 13 species described in the genus *Howardula*. The presence of a bursa separates *H. apioni* from *H. aoronymphium* Welch (1959), *H. medecassa* Remillet and Van Waerebeke (1975), *H. acris* Remillet and Van Waerebeke (1976), and *H. benigna* Cobb (1921). The species *H. dubium* Christie (1938) and *H. phyllotreta* Oldham (1933) are known only from females, which differ in size from the present species. The gravid females of both *H. acarinorum* Wachek (1955) and *H. husseyi* Richardson et al. (1977) are oviparous and the former possesses a spermatheca, whereas the latter has a protruding stylet region. The positions of the gland openings are different from those of *H. apioni* in the infective-stage females of *H. truncati* Remillet and Van Waerebeke (1975) and *H. husseyi* Richardson et al. (1977).

The infective-stage females of *H. oscinella* Goodey (1930) lack knobs on their stylets and the spicules and gubernaculum are almost half the length of those of *H. apioni*.

The lengths of the infective stage females of *H. dominicki* Elsey (1977) and *H. colaspidis* Elsey (1979) are smaller than the present species and the stylets are longer (20–21 vs. 12–13). The gravid female of *H. aptini* (Sharga, 1932) is swollen and not sausage shaped like *H. apioni*.

BIOLOGICAL OBSERVATIONS: Third-stage juveniles of *H. apioni* penetrate the intestinal wall and emerge from the anus of adult *Apion* weevils feeding, mating, or ovipositing on the stems of artichoke plants. The nematodes enter the plant tissue, molt twice to reach the adult stage and mate. This period of maturation is completed in the laboratory in 1–2 weeks at 20–22°C. The infective-stage females then search out and penetrate the larval stages of the host. Probably the

first or second stage of *Apion* is preferred yet under laboratory conditions, all three larval stages as well as the pupal stage of the host were infected. In nature, all stages of the insect except the adult remain in the plant tissues. It is after the adult weevils leave the field for their diapause when the mature females of *Howardula* begin their development. At the end of its diapause, the adult weevil usually contained a range of juvenile stages.

Over a period of 8 years, the rate of parasitism varied between 2 and 13%, depending on the time and place of sampling.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 224–227

Pterygodermatites sp. (Nematoda: Rictulariidae) from Primates in the Topeka, Kansas Zoo[1]

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ABSTRACT: Nematodes of the family Rictulariidae were found in the small intestines of a tamarin, *Sanguinus oedipus* and a white-handed gibbon, *Hylobates lar lar* from the Topeka, Kansas zoo. The gibbon had been in the zoo over 7 years indicating this is a locally cycling parasite. Comparison with described primate *Pterygodermatites* indicates they are similar to but not exactly in conformity with *P. alphi* as described from a number of different simians. This is a new genus record occurring in the white-handed gibbon, *Hylobates lar lar*.

A search of the literature revealed only three species of the genus *Rictularia* reported from primates. *Rictularia alphi* Lubimov, 1933, was found in primates of the Moscow Zoo (Quentin, 1969). These hosts originated in South America, Southeast Asia, and Africa. *Rictularia lemuri* Chaubaud and Brygoo, 1956 was described from a Madagascar primate, *Microcebus murinus murinus*. *Rictularia nycticebi* (Mönnig, 1920) was discovered in the small intestine of a loris, *Loris tardigradus*, which died in the Amsterdam Zoo, although the animal originated in Java. He described the species on eight females and no males are known. Quentin (1969) carried *P. nycticebi* under the same subgenus (*Multipectines*) as *P. alphi* but did not reduce it to synonymy. Without the benefit of *P. nycticebi* males we would not choose to synonymize either. Skrjabin et al. (1967) in their review of the genus *Rictularia* make no mention of *R. lemuri* of Chabaud and Brygoo (1956). Quentin (1969) in his "Essay on the Classification of the Rictularid Nematodes" does list it in materials studied and in one table comparing prevulvar and total spines but nowhere else in the paper. This may be because *R. lemuri* was described on the basis of only three juvenile females and no males.

The genus *Rictularia* has been divided by Quentin (1969) into two separate genera, *Pterygodermatites* Wëdl, 1861 and *Rictularia* Froelich, 1882, based on buccal teeth and position and shape of the mouth opening. It appears that all the primate forms so far named should fall under *Pterygodermatites* since they have the generic characters state by Lichtenfels (1970): a dorsally inclined but not totally dorsal buccal cavity and three buccal teeth; between 29 and 56 prevulvar cuticular processes.

Materials and Methods

In April 1972 40 nematodes from the small intestine of a tamarin, *Saguinus oedipus*, were collected when the animal was necropsied at the Topeka, Kansas Zoo. The origin of the animal was not given. The worms were tentatively iden-

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Table 1. Comparison of sexually mature females of primate *Pterygodermatites* species.

Worm recovery	*P. alphi** 2788‡	Gibbon† 21 females	Data from	Tamarin† 27 females	Data from
Length	9.5–37 mm§	8.0–23	(16)	6.3–18.5	(22)
Max. diameter	0.5–0.6	0.38–0.7	(18)	0.3–0.95	(24)
Diameter at vulva	0.5–0.6	0.22–0.51	(19)	0.21–0.55	(19)
Esophagus length	6.5	2.6–4.0	(21)	2.5–4.6	(17)
Ant. esophagus	0.7	0.31–0.5	(10)	0.36–0.78	(21)
Post. esophagus	5.8	2.30–3.45	(12)	2.0–3.90	(15)
Nerve ring from apex	—	0.22–0.357	(11)	0.292–0.630	(15)
Cervical papillae from apex	0.97	0.480–0.580	(5)	0.455–0.68	(10)
Total spines and combs	96–98	87–94	(15)	90–92	(7)
Prevulvar combs	43	41–44	(18)	40–43	(12)
Postvulvar combs and spines	53–55	50–52	(14)	48–50	(8)
Anus to tail	0.159–0.26	0.13–0.23	(17)	0.06–0.28	(15)
Egg size	0.029 × 0.037	0.033 × 0.042		0.028 × 0.040	
Vulva to posterior esophagus	4.8				

* Skrjabin et al. reference.
† Specimens deposited: USNM coll. numbers to be assigned.
‡ Worms not divided as to sex.
§ All measurements in mm.

tified as *Rictularia* sp. and filed in our collection. In the vial were five immature females, 27 mature (egg-bearing) females, and eight males.

In March 1977 a white-handed gibbon, *Hylobates lar lar,* originating in Southeast Asia died in the Topeka Zoo. A necropsy was performed, and the cause of death was determined to be intestinal intussusception and chronic parasitism. The nematodes found in the jejunum and ileum were tentatively identified as *Rictularia* sp. There were 40 nematodes in this host also, 21 mature females, 16 immature females, and three males.

Results

In comparing our material with *P. alphi* we found one character deviation, and that in the three males from the gibbon. These had only one precloacal ventral fan while males of *P. alphi* have one or two as did the males from the tamarin. Fans in other species of the group have been shown to be variable (Chen, 1936; Tiner, 1948). There were also some variations in prevulvar comb numbers. Tables 1 and 2 indicated the ranges of our measurements. Because cockroaches are common inhabitants of zoos, we examined about 20 caught in the primate room but failed to find spirurid nematode larvae.

Discussion

There do not seem to be sufficient differences to warrant assignment to a new species, but we designate the worms we found to *Pterygodermatites* sp. with the white-handed gibbon, *Hylobates lar lar,* being a new host record for this genus and perhaps other species of the genus.

It seems conceivable that *Pterygodermatites* sp. cycle within zoos, rather than being imported with the animals as suggested by Lubimov (1933). Skrjabin et al.

Table 2. Comparison of three known primate *Pterygodermatites* males.

	Tamarin	Gibbon	*P. alphi*
Number	8	3	N.G.*
Length	4.0–6.9†	5.5–6.6	9–12
Max. diameter	0.23–0.52	0.42–0.48	0.67–0.73
Buccal cavity			
Depth	0.013–0.024	0.026–0.031	0.026
Width	0.024–0.053	0.037–0.048	0.037
Esophagus length	1.8–3.4	2.1–2.6	4.2
Ant. muscular	0.26–0.49	0.35–0.42	0.45–0.50
Ant. spine no.	9–12	8–9	N.G.
Post. glandular	1.6–2.9	1.7–2.3	3.7–3.8
Post. spine no.	35–39	37	N.G.
Nerve ring from ant.	0.16–0.54	0.24–0.27	N.G.
No. spines ant. to N.R.	6	6	N.G.
Cervical papillae to ant.	0.34–0.6	0.46	N.G.
No. spines to cervical papillae	10	10	8–10
Total number spines and combs	64–69	66–68	70
Cloaca to tail	0.077–0.14	0.18–0.19	0.22
Spicules	Equal	Equal	Nearly equal
Length	0.09–0.11	0.09–0.12	0.09–0.10
Accessory piece	No	No	N.G.
Papillae	Not visible	Not visible	10–11 pairs
Preanal	Not visible	Not visible	3 pairs
Postanal	Not visible	Not visible	7–8 pairs
Ant. to 1st spine	0.037–0.07	0.048–0.058	0.08
Post. to last spine	0.211–0.50	0.74	0.56
Fans	1–2	1	1–2

* N.G. = Not given.
† All measurements in mm.

(1967, 1971 translation) questioned Lubimov's "importation hypothesis," as we do. We support the view that these nematodes may cycle locally because our white-handed gibbon had been in the Topeka Zoo for almost 8 years and yet we found 16 of 37 females to be juveniles. To further support this concept a recent report (Yue and Jordan, 1979) found *Rictularia* sp. in a golden lion marmoset born in the Oklahoma City Zoo. They too failed to find spirurids in trapped zoo cockroaches or in trapped house mice in the zoo but they successfully infected laboratory raised *Blatella germanica* and infected golden hamsters with them. Oswald (1958) was able to cycle *Rictularia coloradensis* through German Cockroaches and *Peromyscus leucopus*.

Thus far *Pterygodermatites* sp. has been found in *Cebus, Macaca, Saguinus, Cercopithicus, Loris,* and now *P.* sp. in *Hylobates*. Very often these animals are housed in the same zoo building, so the undetermined intermediate host has access to several genera and species of primate hosts. Apparently *Pterygodermatites* sp. is not very host specific. This is supported by Lubimov (1933), who found it in four different genera of primates.

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All papers are sent to at least two reviewers usually within a day or two after I receive them. When authors provide two copies with the original, as requested, I send both out for simultaneous review. This, of course, greatly hastens the review process, which ordinarily takes two to four weeks. Most papers are returned to authors for some revision. Revised manuscripts may be accepted as received or after further review. Sometimes the additional review leads to a further revision. Material for the January issue is sent to Allen Press about October 1 and that for the July issue about April 1.

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The Editor

Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 228–234

Activation of Male *Nippostrongylus brasiliensis* by Female Pheromone

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ABSTRACT: Males of a mouse-adapted strain of *Nippostrongylus brasiliensis* were activated by in vitro exposure to female pheromone during a spot plate bioassay. Pheromone that was prepared as an incubate elicited a 78% male response within 10 minutes at a 15 female-hour dosage. The male's response was dosage-dependent. The mean duration of activation of responding males at the 15 female-hour dosage was 6.1 minutes and decreased with decreasing concentration. Similar results were obtained from 10- or 20-minute observation periods. Exposure of male helminths to pheromone that was obtained from aqueous gel filtration of macerated females yielded comparable male responses, but a decreased mean duration of activation.

Pheromones, released by one sex of various nematode species, are reported to alter the behavior of the opposite, responding sex. Jones (1966) stated that the female pheromone of *Pelodera teres* served to activate and attract the male worm during bioassay. Green (1966) and Greet et al. (1968) reported that the orientation of *Heterodera rostochiensis* and *H. schachtii* males to their females was klinokinetic and klinotaxic.

A two-state model for swimming and orientation behavior was proposed for *Panagrellus silusiae* (Samoiloff et al., 1973; Samoiloff et al., 1974). *Panagrellus silusiae* exhibited a linear path of travel with minimal sweeping by the anterior and posterior ends when in the unstimulated state. Chemical or physical stimulation caused activation with the worm showing rapid alterations in orientation with maximal sweeping motions.

Studies of the pheromone communicative system of *Nippostrongylus brasiliensis* have used locomotion in a chemical gradient chamber as a bioassay device (Bone et al., 1977). Since this bioassay technique furnishes data after a standard response time, the distance traveled is determined by the percentage of the tested helminths that respond and their rate of response or activation within the defined response period. Other studies of pheromonal attraction in various nematodes also have typically used movement of one sex in a linear or radial gradient as a bioassay device. However, all of thoese techniques generally require long diffusion periods for gradient formation and response time, and also rather large pheromone dosages.

Thus, the degree of locomotor stimulation or activation of a responding helminth should serve as a suitable bioassay parameter for determination of certain aspects of the pheromone system. This activation bioassay may enable a quantitative reduction in the pheromone that is required for dosage-response testing and provide greater bioassay sensitivity and rapidity. Additionally, an awareness of the kinetic effects of the pheromones may contribute to our increased understanding of the overall behavioral function of nematode chemocommunicative schemes.

Materials and Methods

Mouse-adapted *Nippostrongylus brasiliensis* was maintained as previously reported (Bone et al., 1977). Helminths were taken from the host at 6 days postinfection and sexed to provide female pheromone sources and male responders.

Female pheromone was prepared as an incubate by maintaining helminths in Tyrode's solution at 37°C for 4 hours (Bone et al., 1978). Incubation volume was adjusted according to the numbers of available females to give final concentrations of 15, 10, 5, 1.2, 1.05, 0.9, 0.6, 0.45, 0.3, 0.24, 0.12, 0.08 female-hours/μl (1 female/1 hour/1 μl = 1 female-hour). Alternatively, gel filtration of macerated females on a Sephadex G-25-80 (Pharmacia) column was used to assess the effects of the pheromone fractions that elute at Kav 0.64 and 1.0 (Bone et al., 1979). Briefly, females were macerated in cold glucose-free Tyrode's solution in a manual tissue grinder. After centrifugation at 4,000 rpm for 10 minutes, the supernatant was applied to a 0.7 × 27-cm matrix in a glass column. All procedures were performed at 4°C. Pheromone concentrations were calculated as female-equivalents (1 female-equivalent = 1 macerated female). Dosages consisted of 0.1, 0.2, 0.4, 0.8, 1.25, 1.75, 3, and 5 female-equivalents.

Single male *N. brasiliensis* were placed in the wells of prewarmed spot plates (No. 1 size) in 10 μl of Tyrode's solution and equilibrated for 5 minutes at 37°C. After equilibration, a 40-μl aliquot of the above female pheromone dosages from incubation or fractionation was added to each spot well. Males were observed sequentially at 2-minute intervals for 20 minutes and the number of activated responders was recorded for each interval. Activation was considered as the male's thrashing appearance in which full body extension and rapid environmental sweeping by the anterior and posterior ends were evident. Body coiling and probing movements were absent.

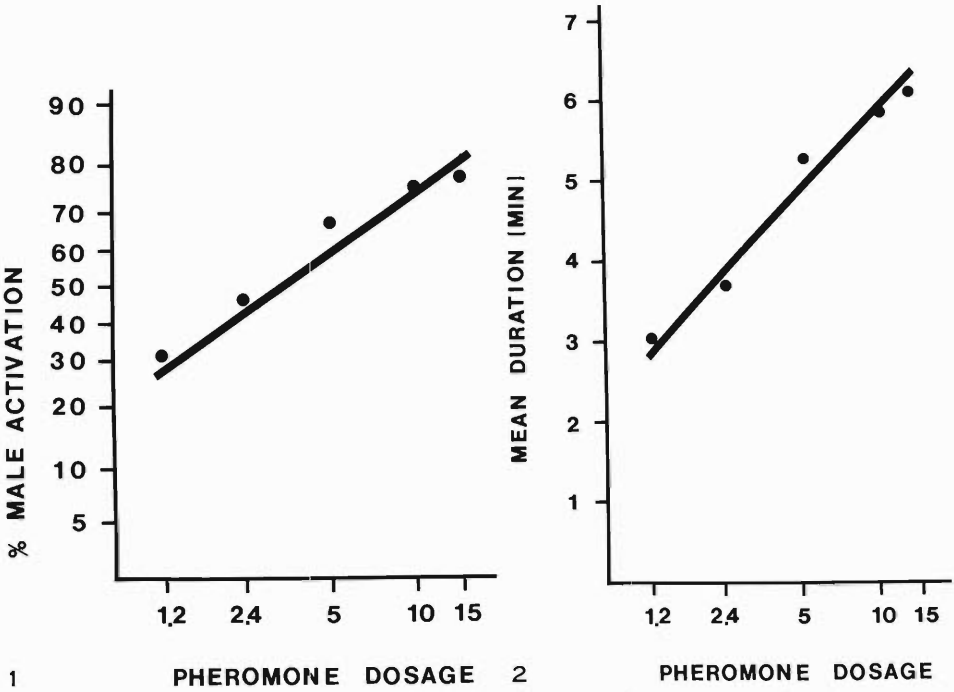
Controls consisted of Tyrode's solution alone or male incubate at 1.2 male-hours/μl. One hundred replicates were obtained for the controls and each female-hour or female-equivalent dosage.

Data were compiled as the total percent activation at 10 or 20 minutes after pheromone exposure and mean duration of the consecutive 2-minute periods of activation for the responding males. Data were tested by analysis of variance or linear regression. The 0.05 probability level was considered statistically significant.

Results

The probit percent activation of male *N. brasiliensis* to female pheromone that was prepared as an incubate solution is shown in Figure 1. The male response increased linearly ($F^5_{499} = 3.79$) as the female dosage was increased from 1.2 female-hours/μl to 15 female-hours/μl. At the highest tested dosage (15 female-hours/μl), 78% of the tested males exhibited the activated response. The male response to female dosages below 1.2 female-hours/μl was not dosage-dependent and did not differ significantly from the controls. Neither control differed significantly from zero. The Tyrode's solution and male-incubate controls elicited 12% and 17% male responses, respectively.

The mean duration of the male's activation increased also with increasing female pheromone dosage that was obtained as incubate (Fig. 2). At 1.2 female-



1 2

Figures 1, 2. 1. Probit percent male activation in 10 minutes after exposure to female-hour pheromone dosages from incubation. 2. Mean duration of male activation in minutes after exposure to female-hour pheromone dosages from incubation.

hours/μl male activation in those males that responded endured for a 3.3-minute interval while males remained excited for a 6.1-minute period at a 15 female-hour/μl dosage. The mean duration of the male's responses to five or greater female-hour dosages was significantly different from the controls. Males that were ex-

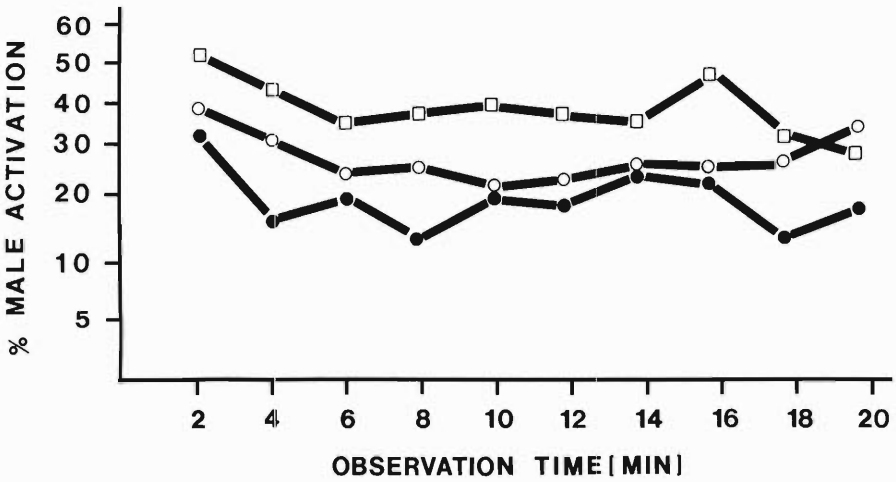
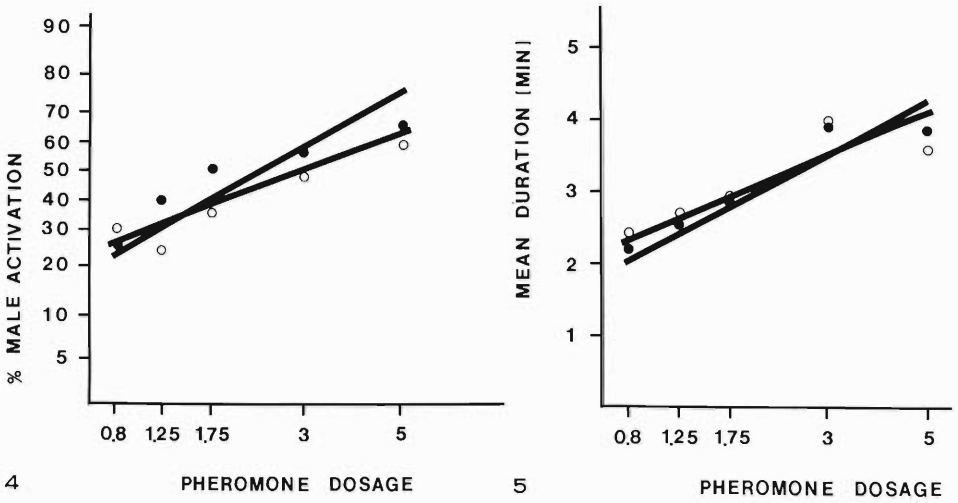


Figure 3. Probit percent male activation during the 2-minute observation periods after exposure to female-hour pheromone dosages of 10 (□), 5 (○), and 2.5 (●) from incubation.



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Figures 4, 5. 4. Probit percent male activation in 10 minutes after exposure to female-equivalent pheromone at Kav 0.64 (●) and 1.0 (○) elutions from gel filtration of macerates. 5. Mean duration of male activation after exposure to female-equivalents at Kav 0.64 (●) and 1.0 (○) elutions from gel filtration of macerates.

posed and responded to Tyrode's solution only exhibited an activation duration of 2.2 minutes. Most of these responses took place at the initial observation period. Thus, control responders were activated at a single observation interval only while rapid locomotion was exhibited for over three consecutive periods when males were exposed to the highest tested pheromone concentration at 15 female-hours/μl.

The percent of male activation throughout the timed bioassay period is shown in Figure 3 for selected female pheromone dosages from incubation. The initial observation period showed male responses that were slightly greater than subsequent examinations. This effect may result from the physical addition of the test solutions to the wells of the spot plates since a similar event occurred with both the Tyrode's and male controls.

Little difference was observed in the probit percent of the male's response for any given pheromone dosage at any of the 2-minute intervals if the initial observation period is excluded. However, the percentage of male activation increased with increased female dosage at most individual observation periods (Fig. 3). Analysis of data that was taken after 10 or 20 minutes of bioassay observation revealed no significant differences. Thus, bioassay for a 20-minute period presents no advantage over a 10-minute examination.

The dosage-dependency of the males activation became less obvious after 20 minutes of pheromone exposure as shown in Figure 3. This effect was even more evident at lower dosages of female-hour pheromone, which are not given by Figure 3.

Male *N. brasiliensis* were activated also by pheromone dosages that were prepared as female-equivalents through aqueous maceration, gel filtration, and assay of the Kav 0.64 and 1.0 elutions ($F^5_{499} = 3.87, 4.03$) (Figure 4). No significant difference was found in the responses of the males to the two elution regions

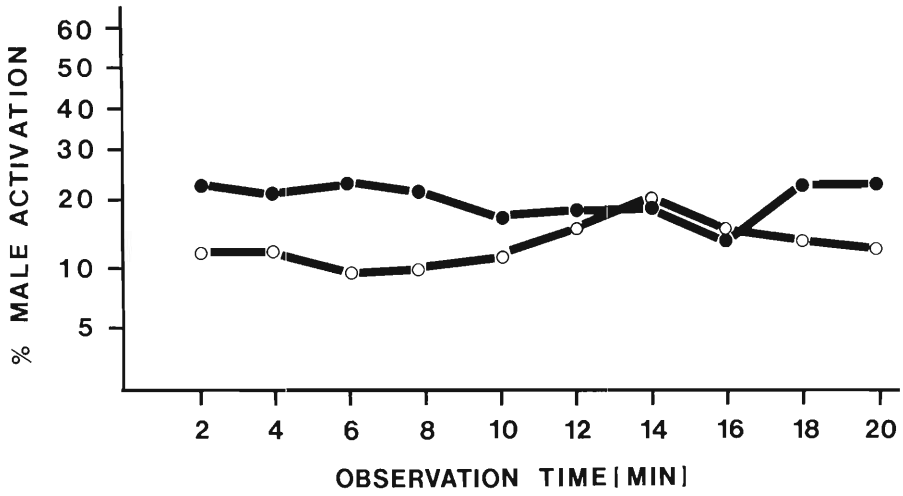

Figure 6. Probit percent male activation during 2-minute observation periods after exposure to 1.75 female-equivalent dosages at Kav 0.64 (●) and 1.0 (○) from gel filtration of macerates.

that have pheromone activity. The slope of the regression of the male's response to incubate and both elution regions was similar. The highest tested dosages of Kav 0.64 and 1.0 pheromone at five female-equivalents gave male responses of 67 and 58%, respectively, that were significantly different from the control.

The mean duration of the activated male's response at Kav 0.64 and 1.0 elutions increased also with increasing dosages of female-equivalents (Figure 5). The duration of activation by males in response to either pheromone fraction was virtually identical. However, the duration of the male's response to the female-equivalents differed from that to female incubate (Figs. 2, 5). The longest mean duration of activation was 4 minutes which represented a one-third decrease when compared to the effects of incubate. Although the mean duration of male activation to female-hours or female-equivalents was similar at lower pheromone dosages, regardless of source, the higher female-equivalent dosages failed to demonstrate as lengthy a male response to pheromone.

The percent activation of males during the bioassay period is shown in Figure 6 for 1.75 female-equivalents of Kav 0.64 and 1.0 pheromone sources. As previously indicated for incubate pheromone, bioassay responses after 10 minutes of observation were similar to those after a 20-minute period.

Discussion

The activation of male *Nippostrongylus brasiliensis* by female pheromone is similar to effects that are reported for other examined nematodes. Samoiloff et al. (1973) proposed that in premating attraction *Panagrellus* began activated behavior upon sensing the attractant and oriented itself to the chemical gradient. However, this activated behavioral state may be induced also by a range of chemical and physical stimuli (Samoiloff et al., 1973). Green (1971) stated that "stimuli seem to initiate but not to control the rate of movement, this is apparently determined by other factors." Thus, activation of male nematodes is not neces-

sarily specific for sexual attraction, but is involved in mate location, based on the present and above studies.

Although comparable data were not obtained for *N. brasiliensis,* other nematodes have revealed differences in their rate of movement. Epstein et al. (1976) reported that normal and mutant *Caenorhabditis elegans* showed marked differences in their movement rate to secretions of the bacterium *Escherichia coli.* Somers et al. (1977) found that the time for movement of male *Rhabditis pellio* toward a female pheromone source increased with increasing male age. Continuation of research from this perspective in *Nippostrongylus* may provide an additional refinement for future bioassay development.

Based on this study, a single female *N. brasiliensis* apparently produces sufficient pheromone within about 1 hour to activate about one-third of the exposed males in the 50-μl bioassay volume. Lower concentrations were erratic in their effect on male activation and were probably subthreshold, except for the more highly responsive males.

Sensory adaptation or habituation of males may also result from exposure to female pheromone. Bioassay of female-equivalents indicated a decrease in the mean duration of the male's response when compared to the less concentrated female-hour incubate. Previous findings with *N. brasiliensis* have shown that pheromone pre-exposure or excessive concentrations reduce male responsiveness in an in vitro bioassay that employed male movement in a test chamber (Bone and Shorey, 1977; Bone et al., 1978). This suppressive effect may prove an unavoidable artifact in both or all bioassay designs. Exposure of male helminths to pulsed sources of pheromone at low concentrations might circumvent this bioassay artifact and represent a more natural gradient of pheromone diffusion.

Additional investigation of the male's activation by female pheromone may lead to several advances in the study of nematode chemocommunication. More refined, sensitive bioassay techniques that employ male activation would represent a distinct improvement over present methodology, but the specificity of the male response may require highly purified pheromone which is obtained through techniques such as high-performance liquid chromatography. Additionally, the results of this study may establish a background for electrophysiological determination of somatic activity and behavioral considerations of the chemical communicative scheme of *N. brasiliensis.*

Acknowledgments

Part of this study was conducted at the Division of Toxicology and Physiology, Department of Entomology, University of California–Riverside. The technical assistance of S. Reed and P. Blomquist is appreciated. This research was supported by grants AI-14194 and AI-16763 from NIH, NIAID.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 234

70th Anniversary

The Helminthological Society of Washington

The first meeting of the Helminthological Society of Washington was held on Saturday, October 8, 1910, in the Zoological Division of the Hygienic Laboratory, Public Health and Marine Hospital Service (the predecessor of the National Institutes of Health).

In commemoration of that event, the Society has planned a 70th Anniversary Dinner to be held Wednesday evening, October 8, 1980. The program will highlight the Society's history and honor members who, in the past, have contributed greatly to the advancement of the Society.

All members and friends of the Society are invited but reservations will be necessary. If you do not live in the Washington-Baltimore area and think that you would be able to attend, contact either of the following for additional information:

Ms. Nancy D. Pacheco
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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 235–246

Eimeria necatrix in the Chicken: Response of the Host Jejunum to Infection and Subsequent Absorption of Methionine and Glucose

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ABSTRACT: Jejunal villi of chickens generally maintained their structural integrity regardless of the severity of infection with *Eimeria necatrix*. Only during the severest infection was villar height reduced to a value below measurements of uninoculated controls. Subvillar thickness increased with severity of infection, possibly because of the increasing numbers of parasites present in the host tissue. Morphologic alterations accompanying severe infection included elongation of the crypts of Lieberkühn, inflammation and edema of the lamina propria at the location of the second-generation schizonts, increased cellularity of the lamina propria, increased numbers of goblet cells, and patches of disrupted microvilli. A few abnormal villi were seen in the infected mucosa of freshly killed birds at 5 days postinoculation. These villi were translucent, highly vascularized, and often one-third taller than the other villi. The remainder of the villi exhibited a morphology similar to villi seen in uninfected mucosa.

Neither the reaction of the host tissue nor the severity of the infection correlated with changes in the rate of methionine or glucose absorption. The in vitro absorption of methionine in chickens inoculated with 10,000 or 25,000 sporulated oocysts of *E. necatrix*/bird (mild infection) at day 5 postinfection was less than the absorption measured in uninoculated controls. The absorption rate in chickens inoculated with 37,500 to 50,000 sporulated oocysts (moderate infection) was sometimes above that in uninoculated controls. The absorption rate in chickens inoculated with 100,000, 200,000, or 400,000 sporulated oocysts (severe infection) was less than that of controls. Changes were in the mediated rather than the diffusion component of methionine absorption.

Michael (1973) and Ryley (1975) reported that villous atrophy during *Eimeria* infection resulted in a reduction of the number of functional epithelial cells that ultimately reduced the surface area for absorption. Ruff et al. (1976) saw severely damaged villi and decreased methionine absorption during *Eimeria acervulina* infection, that corresponded to the region of the intestine parasitized. The developmental stages of *E. acervulina* occur in the absorptive epithelial cells; thus, destruction to absorptive surfaces is not surprising. The relationship between malabsorption and changes in the mucosal surface is less clear with species such as *Eimeria necatrix* which does not complete its life cycle in the epithelial cells lining the villi. Although *E. necatrix* does not produce severe disruption of the epithelium (Smith, 1975; Witlock and Ruff, 1977) it does decrease methionine absorption (Ruff, 1974).

The present report examined changes in mucosal architecture of the intestine with increasing doses of sporulated *E. necatrix* oocysts, and attempted to correlate changes in the mucosal morphology with changes in methionine absorption.

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[3] Reprint requests to this author.

Materials and Methods

Birds

White Leghorn cockerels, 52 to 53 days old at the time of inoculation with *E. necatrix* oocysts, were used in Exps. 1, 2, and 3. Broiler cockerels (Cobb[4]), 35 days old, were used in Exps. 4 and 5. Broiler cockerels (Hubbard), 14 days old, were used in Exp. 6. All birds were raised in wire-floored batteries with constant lighting. Feed and water were available ad libitum.

Experimental design

Birds were given various dosages of sporulated oocysts and killed at the days postinoculation (PI) indicated for the individual experiments. Intestinal lesions were scored based on the criteria of Johnson and Reid (1970). To maintain uniform intestinal damage within a dose level, tissue was taken only from those birds exhibiting lesion scores consistent with a particular dose. These were scores of 1, 2, 3, and 4 for the 10,000, 25,000, 50,000, and 100,000 sporulated oocysts per bird groups, respectively. Mortality of up to 20% was seen in birds given 100,000 or more oocysts. Five to 15 birds were used for each group.

In Exps. 1 and 2, birds were inoculated with 10,000, 25,000, 50,000, 100,000, 200,000, or 400,000 (Exp. 2 only) sporulated oocysts/bird. Birds were killed at day 5 PI, and tissue removed for absorption and histological studies.

In Exps. 4, 5, and 6, groups of birds were inoculated with 10,000, 25,000, 50,000, or 100,000 sporulated oocysts/bird. Additional groups were inoculated with 37,500 or 200,000 sporulated oocysts/bird (Exps. 4 and 5) and 75,000 sporulated oocysts/bird (Exp. 6). In these three experiments, birds were killed on day 5 PI, and tissue was removed for absorption studies.

In Exp. 6, birds were inoculated with 100,000 sporulated oocysts/bird. Two inoculated birds and one uninoculated control were killed at 24-hr intervals through day 5 PI and subsequently on days 7, 10, 14, 21, and 28 PI. Tissue was removed for histological and electron microscopic examinations.

Tissue

For all experiments, tissues were taken from the jejunum, midpoint between the duodenal loop and the yolk sac diverticulum. In Exp. 1, tissue for examination by light microscopy was processed as described by Fletcher (1975) with Lillie's buffered formalin as a fixative and acid fuchsin and toluidine blue as the stain. Tissue for scanning electron microscopy was prepared as described by Witlock et al. (1975). Tissue for transmission electron microscopy was fixed in Karnovsky's paraformaldehyde glutaraldehyde fixative (Karnovsky, 1965) and stained with 3% uranyl acetate for 5 min in an oven at 60°C followed by lead citrate (Reynolds, 1963) for 5 min at room temperature.

In Exps. 2 and 3, 1-cm lengths of intestine were removed from each bird and immediately fixed in warm (40°C) 10% neutral buffered formalin. Sections were cut and stained with hematoxylin-eosin. Longitudinal rather than cross sections

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

were used to achieve reasonable orientation of the villi (Pout et al., 1971). Nine to 10 sections from the infected area were examined from each bird. The villar height (from the top of the intact villus to the mouth of the crypts of Lieberkühn), the subvillar thickness (from the mouth of the crypts of Lieberkühn to the serosa), and the total intestinal thickness (from the top of the intact villus to the serosa) were measured.

Substrate absorption

In Exps. 2–5, absorption was measured in rings of inverted intestine according to the method described by Ruff (1974). In Exps. 2 and 3, absorption was measured in rings of tissue from individual birds. In Exps. 4 and 5, rings from four birds were pooled as one sample. The buffer system used in all incubations was Krebs-Henseleit buffered saline (KH, pH 7.2) which had been gassed with 95% O_2:5% CO_2. Each sample was preincubated in KH containing 5 mM of glucose as an energy source (KHG) at 40.0 ± 0.5°C for 10 min then incubated for 10 min in KH containing the desired concentration of C^{14}-labelled methionine. Concentrations of methionine (L-form) used were: 10 mM, Exps. 2, 3, and 6; 5 mM, Exp. 4; 0.2 mM, Exp. 5. After incubation, these samples were washed three times in cold (4°C) KH and extracted overnight in 70% ethanol. The radioactivity in all aliquots of the ethanol extract was measured, the tissue dried overnight at 95°C and weighed, and the uptake of methionine expressed as μmoles/g dry weight of intestinal tissue/10 min.

In Exp. 5, the diffusion component of methionine absorption was measured with the inhibition of the uptake of 0.2 mM C^{14}-labelled methionine by 20 mM of unlabelled methionine (Ruff et al., 1976). This inhibitor:substrate ratio gives complete inhibition of the mediated part of methionine absorption by the jejunum (Ruff, 1974).

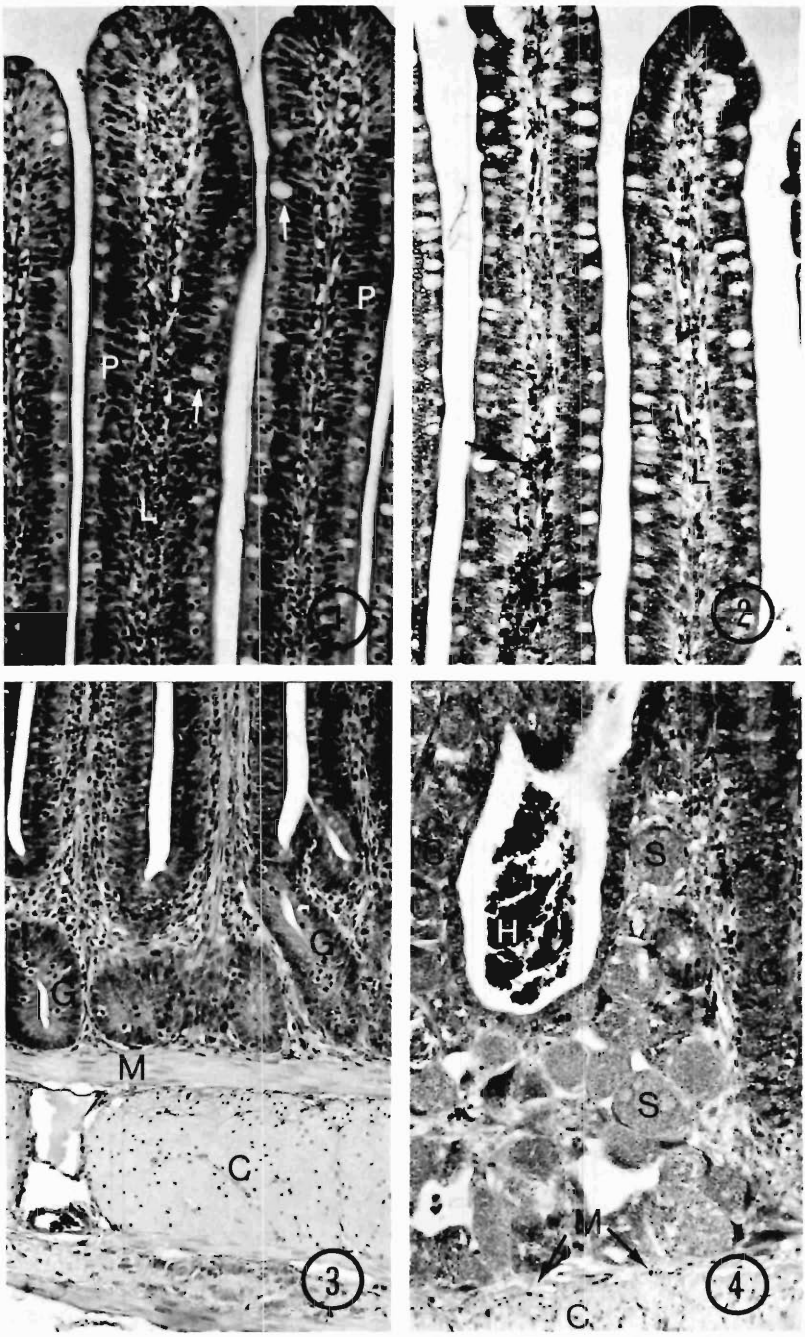
In Exp. 6, the absorption of 10 mM of glucose or 10 mM of methionine was measured by the method of Ruff (1978) with gassed (95% O_2:5% CO_2) KH and H^3-dextran as a nonabsorbed marker. Absorption was measured on four replicate samples, each containing three discs (each 38 mm^2), one disc from each of three birds. Tissue was preincubated for 5 min in KHG, incubated with C^{14}-labelled substrate and H^3-dextran for 4 min, then extracted overnight in 70% ethanol. The labelled substrate in the ethanol extract was counted and corrected for nonabsorbed incubation media, and absorption was expressed as nmoles substrate/mm^2 tissue/4 min.

Results

Histopathology

The appearance of tissue removed from uninoculated control birds was similar, regardless of the day examined. Villi (Fig. 1) were composed of an outer layer of principal epithelial cells (P) surrounding a central core of lamina propria (L). Goblet cells were interspersed in the epithelial layer. A well-defined brush border was evident with electron microscopy. Crypts of Lieberkühn opened into the intestinal lumen at the base of villi (Fig. 3). The crypts were composed of a single layer of epithelial cells interspersed with basal granular cells. Below the crypts were several muscle layers.

There were no readily apparent histopathologic alterations in the epithelial cells



Figures 1–4. All ×416. The uninoculated and infected villi are continuous with their respective submucosal region. 1. Villi from normal uninoculated controls. Note the relative number of goblet cells (arrows) scattered within the principal epithelial cell layer (P) and the absence of red blood cells within the lamina propria (L). 2. Villi from chickens inoculated with 100,000 sporulated *E. necatrix* oocysts (day 5 postinoculation). Note the increase in the number of the lightly stained goblet cells and the red blood cells originated from hemorrhaging of the submucosa. 3. Submucosa from normal uninoculated controls. Note the depth of the crypts of Lieberkühn (G) and the extent of the muscularis mucosa (M)

on the villi other than a marked increase in the number of goblet cells at day 5 PI (Fig. 2). Pathological changes were more pronounced in the region of the lamina propria below the openings of the crypts of Lieberkühn. Elongated crypts of Lieberkühn and increased cellularity of the lamina propria were found throughout the infected intestinal region, but were most pronounced in the vicinity of the second-generation schizonts (Fig. 4). Numbers of lymphocytes and other leukocytes increased in the lamina propria and an increase in the width of the villi directly above colonies of schizonts resulted. Distention of the blood vessels in the muscle layers was evident throughout the infected region of the intestine. Mature schizonts were grouped into colonies causing erosion of the muscularis mucosa. When extremely large numbers of second-generation schizonts were present in a colony, the individual schizonts extended partly up into the villus. Hemorrhage was confined to the vicinity of the schizonts, and in some instances, the lumen of the crypts of Lieberkühn were filled with blood.

Transmission electron microscopy of the epithelial cells from infected chickens showed that the microvilli of some epithelial cells were stunted and lacked the normal parallel orientation (Figs. 5, 6). These patches of abnormal microvilli were confined to a few villi that seemed to overlie the clusters of second-generation schizonts.

Distended elongate villi could be seen (Fig. 7) when the blood and mucous adhering to the mucosa were removed. These villi were relatively few in number and were translucent, highly vascularized, and often one-third taller than the other villi. With the exception of the abnormal villi, the rest of the infected mucosa was similar to uninfected mucosa.

Second-generation schizonts were still present in the tissue at days 7 and 10 PI. At days 14, 21, and 28 PI, parasites were not found. At day 7 PI, the epithelial cells along the base of villi and at the mouths of the crypts of Lieberkühn had lost their single layer configuration and appeared highly vacuolated. Erosion of the muscularis mucosa was evident below the damage produced by second-generation schizonts rupturing from the host tissue. Damaged epithelium was found progressively higher on the villar surface at days 10 and 14 PI but was no longer present by day 21 PI.

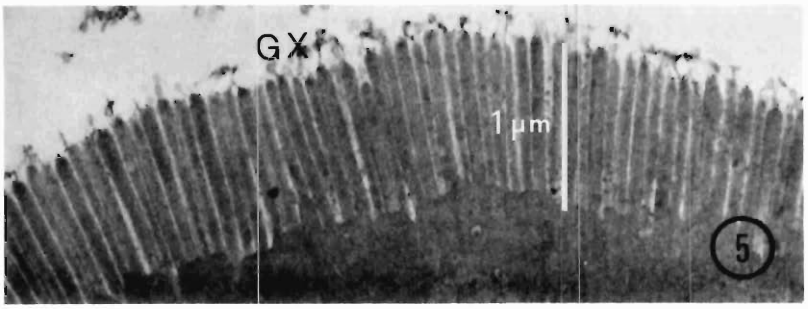
The healing process began immediately after mature second-generation schizonts ruptured from the intestine. At day 7 PI, fibrosis occurred within the previously eroded muscularis mucosa which by day 21 PI was fully healed. The damaged area of the epithelium, originally located in the crypts at day 7 PI, was found progressively higher on the villi at days 10 and 14 PI and was no longer present by day 21 PI.

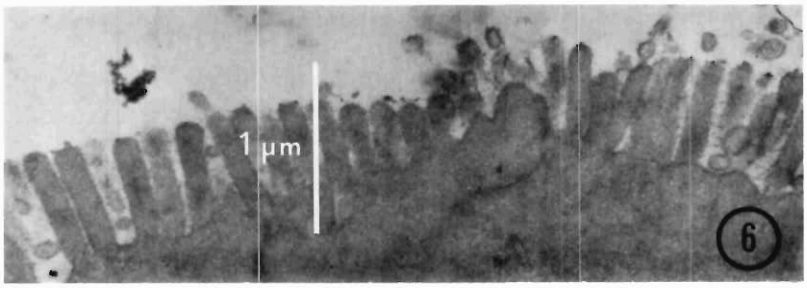
Measurements

Total mucosal thickness at day 5 PI was greater in inoculated birds than in uninoculated controls with a maximum thickness at 100,000 sporulated oocysts/

←

and circular muscles (C). 4. Submucosa from chickens inoculaed with 100,000 sporulated *E. necatrix* oocysts (day 5 PI) showing second-generation schizonts (S) grouped into a colony. Note elongation or distention of crypts of Lieberkühn (G) by hemorrhage (H); red blood cells (arrow) in vicinity of schizonts; erosion of muscularis mucosa (M); and increased cellularity of circular muscles (C).





Figures 5–7. 5. Microvilli from normal uninoculated controls. Note extent of glycocalyx (GX). ×22,500. 6. Microvilli from chickens inoculated with 100,000 sporulated *E. necatrix* oocysts (5 days postinoculation). Note reduction in height and increase in width of microvilli and the absence of a glycocalyx. Destruction of microvilli were found on 4–14 days PI, but were most pronounced on 10–14 days PI. ×22,600. 7. Scanning electron micrographs from chickens inoculated with 100,000 sporulated *E. necatrix* oocysts (5 days PI). Note the elongate, damaged villus and the normal appearance of the surface of undamaged villi. ×200. Close-up of the damaged villus pictured in Figure 7. Note the large numbers of red blood cells. ×3,000.

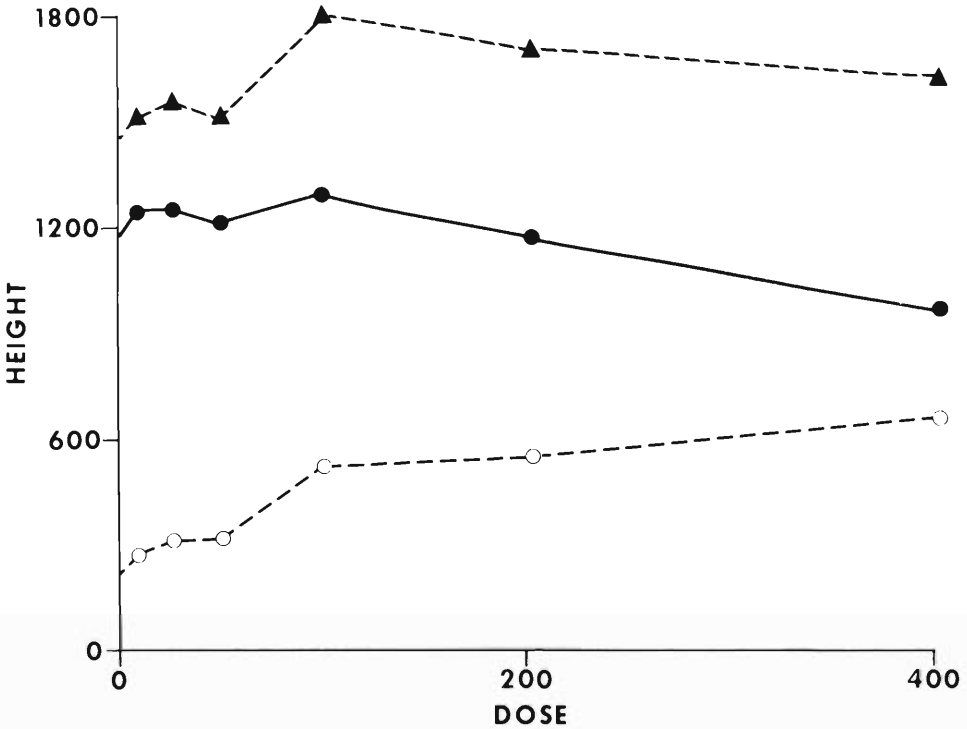


Figure 8. Biometric components of villar height (●), subvillar thickness (○), and total intestinal thickness (▲) of the jejunum at day 5 postinoculation of uninfected Leghorns and Leghorns inoculated with various doses of sporulated *E. necatrix* oocysts. Each point is the mean of 90 to 100 measurements from each of three to five birds. Height = micrometers; dose = sporulated oocysts in thousands.

bird (Fig. 8). The villi height was significantly reduced with 400,000 sporulated oocysts/bird; all other dosages showed no significant differences from the uninoculated control. A marked change was seen in subvillar thickness, which increased with inoculation dosage from a minimum value of 245 μm in uninoculated controls to a maximum of 656 μm in chickens infected with 400,000 sporulated oocysts.

Substrate absorption

In White Leghorns given doses of 10,000 and 25,000 sporulated oocysts/bird, absorption of methionine was significantly less than that in uninoculated controls (Fig. 9). When the inoculation dose was increased to 50,000 oocysts, absorption was significantly greater than that measured at other inoculation doses. In Exp. 2, this increase was significantly less than the rate measured in uninoculated controls (10.3 and 11.9 μmoles/g dry tissue weight/10 min, respectively), whereas in Exps. 3 and 4, the rate in infected birds was equal to or greater than that of the uninoculated controls. As oocyst dose levels were increased further, the absorption rate again decreased.

Broilers given 37,500 and 50,000 sporulated oocysts/bird also absorbed more methionine than uninoculated controls (Fig. 9, Exp. 4; Fig. 10). The mediated

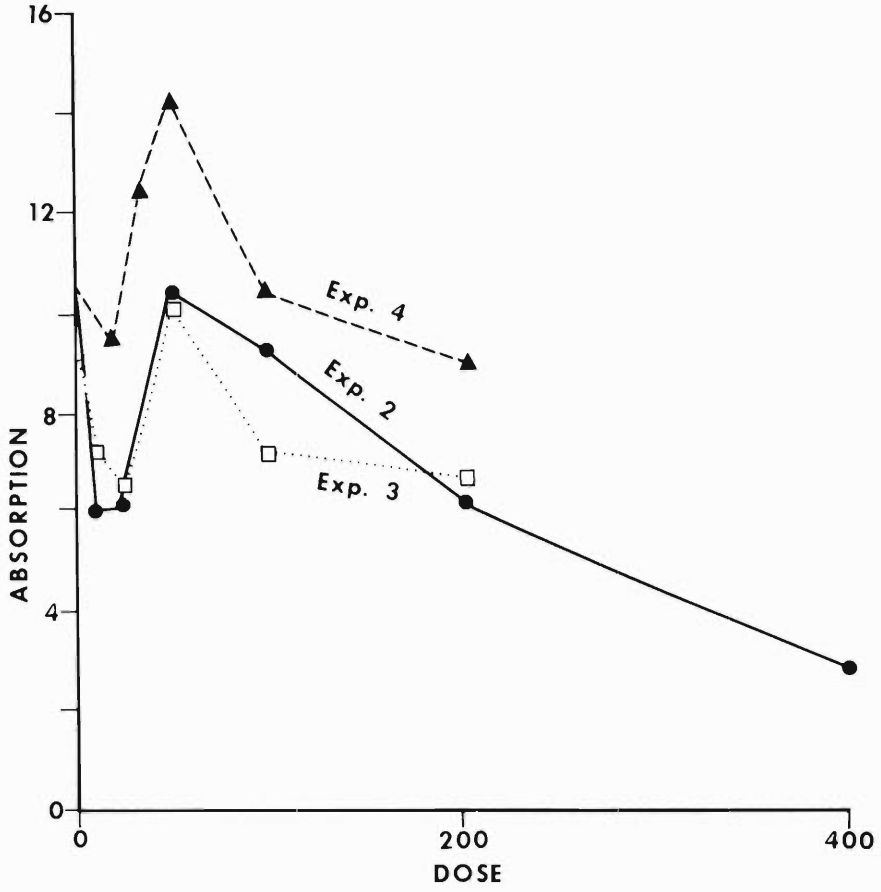


Figure 9. Absorption of 10 mM (Exps. 2, 4) or 5 mM (Exp. 3) of methionine by the jejunum of Leghorns (Exps. 2, 3) or broilers (Exp. 4) inoculated 5 days previously with various dosages of sporulated *E. necatrix* oocysts. Each point is the mean of 3 (Exps. 2, 3) or 4 (Exp. 4) replicates. Absorption = μmoles/g dry tissue weight/10 min; dose = sporulated oocysts in thousands.

component of methionine absorption also followed this pattern. Absorption due to diffusion alone remained relatively constant throughout despite severity of infection, varying from 0.13 μmoles/g of dry tissue weight/10 min for uninoculated controls to 0.17 μmoles for birds infected with 50,000 sporulated oocysts (Fig. 10).

Effects of inoculation dose on glucose and methionine absorption were similar when the double isotope method was used and absorption was expressed as nmoles/mm²/4 min (Exp. 6). Absorption of both substrates decreased with 10,000 and 25,000 sporulated oocysts/bird and then increased with 50,000 oocysts (Fig. 11).

Discussion

The experiments on absorption and host tissue response indicate that reduction of the epithelial cell surface alone does not adequately explain nutrient malab-

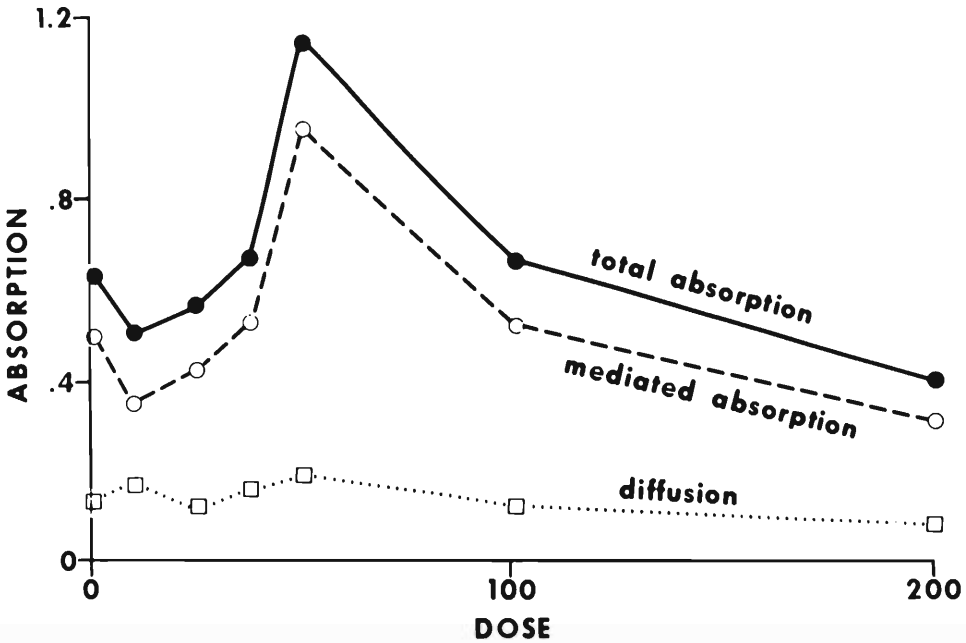


Figure 10. Absorption (Exp. 5) at 5 days postinoculation of 0.2 mM of methionine in the jejunum of uninoculated broilers and broilers inoculated with various doses of sporulated *E. necatrix* oocysts showing the mediated and diffusion components. Each point is the mean of four replicates, four rings/replicate, each ring from a different bird. Absorption = μmoles/g dry tissue weight/10 min; dose = sporulated oocysts in thousands.

sorption seen during *E. necatrix* infection. In these studies, three of the responses to infection differ markedly from the responses reported with other coccidial species. First, essentially no shortening or stunting of the villi was found except with the highest inoculation dose (400,000 sporulated oocysts/bird). Second, the infection (50,000 sporulated oocysts/bird) sometimes increased substrate absorption in the region where the parasite was found. Third, the malabsorption was not directly related to severity of infection; i.e., increasing the inoculation dose did not increase the malabsorption in many cases.

Previous studies (Smith, 1975; Witlock and Ruff, 1977) have suggested that changes in the mucosal surface during *E. necatrix* infection are much less severe than might be expected on the basis of the gross appearance of the intestine. Extensive damage to the villar surface in the jejunum was not evident in the present study, rather, villi maintained their structural integrity even when infections were severe. For example, with 200,000 sporulated oocysts/bird, no change in villar height was detected (Fig. 8) even though there was malabsorption (Fig. 9), +4 lesions, and sometimes death. Only a dose of 400,000 oocysts reduced villar height to a value below that for uninoculated controls. The decrease in the percentage of villar height of the total mucosal thickness with increasing severity of *E. necatrix* is related to increasing subvillar thickness rather than decreasing villar height (Figs. 4, 10). Mechanical pressure or edema due to the location and size of the second-generation schizonts of *E. necatrix* (Fig. 4) may contribute to

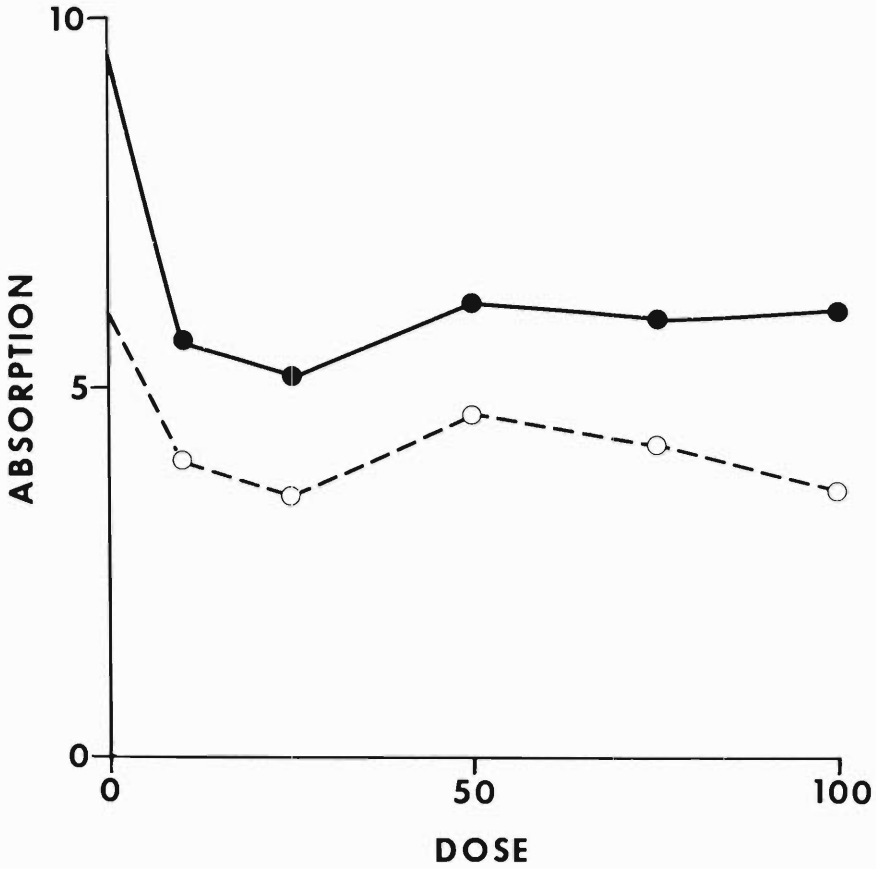


Figure 11. Absorption (Exp. 6) at 5 days postinoculation of 10 mM of glucose (●) or methionine (○) in the jejunum of uninoculated broilers and broilers inoculated with various doses of sporulated *E. necatrix* oocysts. Each point is the mean of four replicates, three birds/replicate. Absorption = nmoles/mm² tissue/4 min; dose = sporulated oocysts in thousands.

the subvillar thickness. The changes in the lamina propria observed with light microscopy were in agreement with those described by Stockdale and Fernando (1975).

Various malabsortive diseases are accompanied by changes in the mucosal architecture of the intestine. Shiner and Doniach (1960) coined the term "villous atrophy" to describe the response of the human intestine to steatorrhea. Similar conditions, with increase in villar width, flattening of the mucosal surface, or loss of villar pattern have been reported in cases of coccidiosis in man (Brandborg et al., 1970; French et al., 1974) and lambs (Pout, 1969, 1974). Pout (1967), Fernando and McCraw (1973), Joyner et al. (1975), Ruff et al. (1976), and Turk (1978) reported short, stubby, and sometimes fused villi in the duodenum of chickens infected with *Eimeria acervulina*. Villar height was reduced by nearly two-thirds during *E. acervulina* infection (Fernando and McCraw, 1973). An increase in subvillar thickness has been seen in *E. acervulina* infection (Pout, 1967; Fernando and McCraw, 1973; Joyner et al., 1975; Turk, 1978). However, Pout (1967) and

Fernando and McCraw (1973) found that the decrease in villar height/total intestinal thickness ratio during *E. acervulina* infection was due predominantly to a reduction in villar height rather than an increase in subvillar thickness.

The few abnormal villi (Fig. 7) in the *E. necatrix* infected mucosa may account for a large portion of the blood loss during the acute phase of infection. This blood loss would be in addition to the hemorrhage directly into the crypts as described by Davies et al. (1963). These villi, seen with the dissecting microscope and scanning electron microscopy, are probably located above second-generation schizonts because light microscopy showed that hemorrhage is profuse in villi associated with developing second-generation schizonts but absent in regions devoid of schizonts. Although these abnormal villi increase in number with increasing severity of infection (i.e., increasing infective oocysts doses), they are less numerous than the serosal white opacities that show the location of schizont colonies.

The failure of malabsorption to correlate with increasing severity of *E. necatrix* infection differs from the absorption of oleic acid (Turk and Stephens, 1967) and methionine and glucose (Ruff, 1978) in *E. acervulina* infection where malabsorption increases with severity of infection. No explanation is offered for the peak in absorption seen at 50,000 oocysts when compared with the peak in absorption at other inoculation doses. This peak was seen, however, in all five transport experiments, in both single and double isotope experiments.

The relative lack of villar stunting with *E. necatrix* (Fig. 7) suggests that other factors must cause malabsorption with this species. Two changes noted were an increase in the number of goblet cells (Figs. 1, 2) and changes in the microvilli (Figs. 5, 6). Michael (1973) previously reported that the brush border microvilli were reduced in size and number with *E. acervulina* and *E. necatrix* infection. Other organisms such as *Salmonella typhimurium* can cause a disruption and even complete disappearance of the microvilli (Bayer et al., 1977). It is doubtful, however, that these two changes account for all of the malabsorption that occurs.

Acknowledgments

The authors express appreciation to Wayne I. Anderson, Pricilla Carter, Pamela Smith, Emma Harrison, Joyce Johnson, and Gary Wilkins for their technical assistance.

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Proc. Helminthol. Soc. Wash.
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An Analysis of the Community and Population Dynamics of the Helminths of *Sigmodon hispidus* (Rodentia: Cricetidae) from Three Central Texas Vegetational Regions[1]

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ABSTRACT: During the period August 1974 to October 1975, an ecological study of the helminth parasites of *Sigmodon hispidus* was conducted in the area of San Marcos, Texas. A total of 355 *S. hispidus* were examined, 95% of which were infected. Twelve species of helminths were recovered, including four species of cestodes, three species of trematodes, and five species of nematodes. Parasitism in *S. hispidus* was found to vary significantly with the season of the year, and with such host-related factors as habitat, size, and sex.

Cotton rats (*Sigmodon hispidus*) are distributed throughout Central America and the southern United States and are usually the most numerous mammals where they occur (Hall and Kelson, 1959). They may be found within their range wherever there is sufficient thick, screening vegetation to provide protection and concealment (Bunn, 1941), and they may competitively exclude other rodent species from their home range (Raun and Wilks, 1964; Terman, 1974). As a result of their wide range and relatively dense populations, cotton rats have often been the subject of helminthological surveys and their helminth fauna is relatively well known. The rats themselves have also been the subject of a considerable number of general ecological studies. However, the effects of ecological factors upon the helminth fauna of the cotton rat have received little attention. Harkema and Kartman (1948) included discussion of differences in mean density of parasites over the four seasons in their study of North Carolina cotton rat helminths. Coggins and McDaniel (1975) reported the effects of season and host sex on helminth population dynamics in North Carolina, basing their findings on 130 cotton rats collected over a 2-year period. Layne (1968) studied the effects of habitat upon infections of *Capillaria hepatica* in Florida mammals, including *S. hispidus*. Kinsella (1974) reported differences in the helminth fauna of cotton rats collected from several habitats in Florida, and included a checklist of the helminths of *S. hispidus*. Mollhagan (1978) investigated the effects of five western Texas habitats upon primarily the helminthofaunal similarity between the habitats but used relatively small sample sizes and did not investigate the effects of season or of the length or age of the hosts and, as a consequence, was not able to demonstrate the effects of host-related factors.

The purpose of this study was to obtain a checklist of the helminths of *Sigmodon hispidus* in the area of San Marcos, Texas, as well as to investigate the effects of such factors as the season of collection, and the sex, size, and habitat of the host upon the incidence, mean density, and diversity of the helminth infections.

[1] Portion of a thesis submitted by James L. Martin to the Graduate School of SWTSU in partial fulfillment of the requirements for the degree of Master of Science (copies available from University Microfilms).

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Materials and Methods

During the period August 1974 through October 1975, 355 *S. hispidus* were collected from three collecting stations representing three different vegetational regions (Gould, 1969) near San Marcos, Texas. The Edwards Plateau vegetational region lies to the west of San Marcos and is characterized by highly dissected limestone hills with shallow soils and with a savannahlike flora dominated by Ashe juniper and live oak trees. The Blackland Prairies vegetational region lies to the east of San Marcos and consists of lightly rolling hills to flat grasslands with mesquite trees in heavy clay areas and post oak in lower areas. The Post Oak Savannah vegetational region is about 40 km east of San Marcos and exhibits gently rolling to hilly topography with red sandy clays and an overstory of blackjack oak but with few grasses except in lowland areas. The soils of the Edwards Plateau and Blackland Prairies regions are limy while those of the Post Oak Savannah are acid.

We attempted to collect 10 cotton rats monthly from each of the three stations for an expected monthly total of 30 animals. However, population lows resulted in low monthly totals in April (14) and May (23). The animals were captured in Sherman live traps and were brought to the laboratory and etherized immediately prior to examination. Necropsies were conducted under a binocular dissecting microscope, and the average examination was 52 minutes in duration.

Standard body length was utilized in this study as an indicator of host age. Cotton rats may be separated by length into four age groups according to information reported in Chipman (1965) and Jiminez (1971): less than 40 days of age (48 to 145 mm), 41 to 100 days (146 to 165 mm), 101 to 200 days (166 to 185 mm), and greater than 200 days (greater than 186 mm).

The data obtained in this study were analyzed with computer programs of the BioDat series written in DEC-10 BASIC by D.G.H., as well as with programs of the Statistical Package for the Social Sciences (Nie et al., 1975). Fluctuations due to sampling variability were minimized by the use of a moving average whenever mean density or incidence of infection were plotted against host body length or month of collection. This technique frequently produces a more readily interpretable graph than does a plot of raw data. Helminth diversity values were calculated using the (log base 2) Shannon Index, as described by Zar (1974). The parasitofaunal resemblances between the three areas sampled in this study were determined using the dissimilarity statistic (z) of Preston (1962) as modified by Peters (1968).

We will avoid the use of potentially ambiguous ecological terms such as "intensity" or "worm burden," and will adapt terms from the literature of quantitative general ecology as follows: (1) density—the number of helminths in one host; (2) mean density—the mean number of helminths in a specified set of hosts examined; (3) incidence—the percentage of a specified set of hosts observed to be infected.

Results and Discussion

During the course of this study, 12 species of helminths were collected, and 10 were identified to genus or species (Table 1). Of the 355 cotton rats collected, only 17 were uninfected and 95% were infected with one or more species of helminths. The modal number of species per host was three with the maximum

Table 1. Checklist and summary statistics of the identified helminths collected from *Sigmodon hispidus*.

Parasite	USNM no.	Incidence	Mean density	Mean per inf. host	Maximum density	SE of mean
CESTODES		77.5	5.3	6.9	159	0.57
Monoecocestus sigmodontis	75394	63.0	4.2	6.1	158	0.55
Raillietina bakeri	75395	12.4	0.6	5.1	24	0.14
Hydatigera taeniaeformis	75396	16.1	0.5	2.9	26	0.10
TREMATODES		17.2	4.1	23.6	121	0.75
Brachylaima thompsoni	75390	14.7	3.6	24.8	96	0.74
Zonorchis komareki	75391	4.2	0.4	10.0	42	0.17
NEMATODES		88.5	33.2	37.6	387	2.45
Hassalstrongylus aduncus	75397	75.0	25.0	33.0	324	1.94
Strongyloides sigmodontis	75398	48.7	5.4	11.1	173	0.72
Physaloptera bispiculata	75399	6.7	0.3	4.7	14	0.13
Syphacia sigmodontis	75400	8.0	2.5	34.2	142	0.69
Gongylonema sp.	75401	—	—	1.0	1	—
OVERALL		95.2	42.8	44.8	389	2.61

being six species. Approximately 15,000 helminths were collected, with a mean density of 43. Variability in the helminth community was observed to associate with variation in the season of collection, and such host-related factors as habitat, size, and sex.

Habitat of the host

There was an inverse relationship between the species richness (number of species) and the helminth diversity index of each of the three collecting stations. This relationship is reflected in the distribution of rare species among habitats. The Post Oak Savannah collecting station, while exhibiting the lowest helminth diversity value (1.49), produced the greatest number of helminth species. However, evenness was lowest there at 0.43, and only five of the species occurred in more than 10% of the hosts examined. Conversely, the Blackland Prairies collecting station exhibited the greatest helminth diversity (2.21), while producing only seven helminth species. The evenness was highest there at 0.79, and five of the species occurred in more than 10% of the hosts examined. The Edwards Plateau collecting station was intermediate with regard to number of species encountered (8), helminth diversity (1.79), and evenness (0.58).

The dissimilarity statistic (z) estimates the degree of dissimilarity between habitats, with zero indicating that all species are common to all habitats and unity indicating no species in common. The Edwards Plateau and Blackland Prairies had nearly identical species composition ($z = 0.07$) and can be considered to represent the same helminthofaunal community, but each of these stations were distinct to some degree from the Post Oak station ($z > 0.29$). Direct observation of the biotic and especially the abiotic factors at the stations also indicated the similar nature of the Edwards Plateau and Blackland Prairies stations and the dissimilarity between these stations and the Post Oak Savannah station. This is consistent with a report by Mollhagan (1978) who reported greatest dissimilarity between the xeric and mesic localities in his western Texas study and further

Table 2. Distribution of mature (scrotal) male cotton rats among species richness categories vs. that of mature (perforate and pregnant) females. Calculated chi-square = 11.41, and critical value at alpha(2)0.05 and 4 df = 9.49.

Number of species		≤1	2	3	4	≥5	Totals
MALES	freq. obs.	18	43	44	15	5	
	freq. exp.	12.0	37.7	49.2	21.8	4.3	125
	χ^2	(3.0)	(0.75)	(0.55)	(2.12)	(0.11)	(6.53)
FEMALES	freq. obs.	10	45	71	36	5	
	freq. exp.	16	50.3	65.8	29.2	5.7	167
	χ^2	(2.25)	(0.56)	(0.4)	(1.58)	(0.09)	(4.88)
TOTALS	freq.	28	88	115	51	10	292
	χ^2	(5.25)	(1.31)	(0.95)	(3.7)	(0.2)	(11.41)

supports the popular contention that similar habitats produce similar helminth communities.

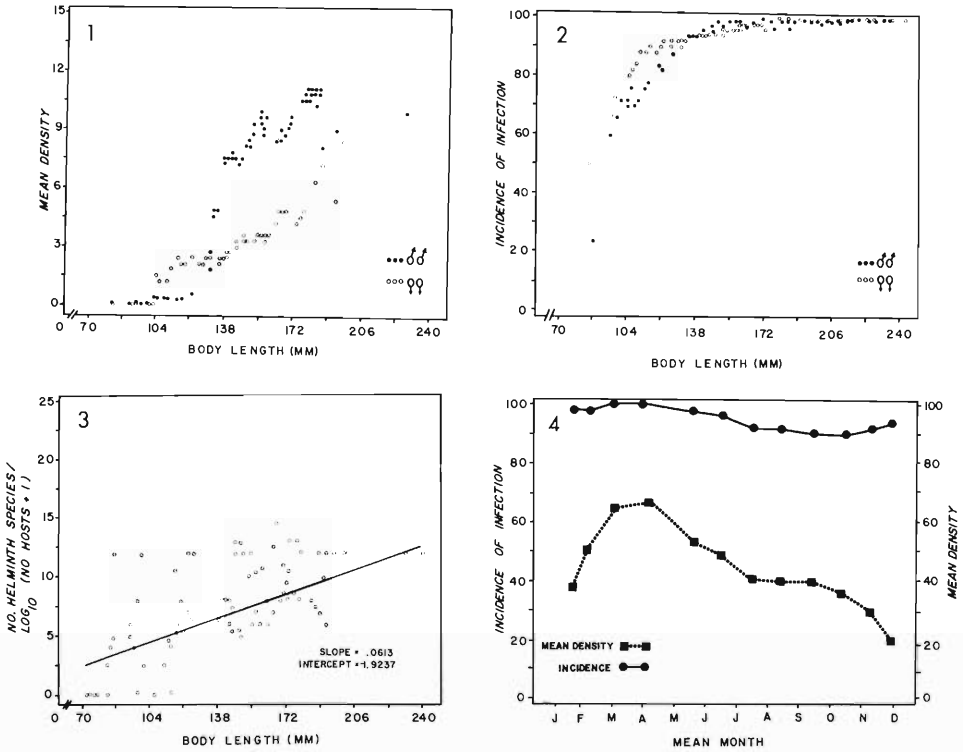
The effects of habitat were also reflected in the habitat preferences of individual helminth species. Of the 12 species of helminths recovered, six occurred at only one or two of the collecting stations. Three of the six species were encountered only at the Post Oak Savannah station and occurred only once in collections. The nematode *Syphacia sigmodontis* failed to occur at the Edwards Plateau station. The trematode *Zonorchis komareki* failed to occur at the Blackland Prairies station and occurred only once at the Post Oak Savannah station. The trematode *Brachylaima thompsoni* failed to occur at the Post Oak Savannah station. Of the remaining helminths, each occurred in all three collecting stations. However, the degree to which each habitat was preferred by these common species, as indicated by mean density, did vary considerably.

Sex of the host

While scrotal males (mature males with testes descended to the scrotum) had about the same number of species as did perforate females (mature females with perforate vaginas) and pregnant females, the two sexes differed significantly ($P < 0.05$) with regard to how the individual hosts were distributed among the species-richness categories (Table 2).

The mature males had a higher overall mean density of 59.7 while the female mean density was 33.5. The significance of this difference was tested by first transforming the number of helminths in each host to log(f + 1) and recalculating the means. The difference between the means of the log data was then tested by a *t* test assuming equal variances and the *t* value (5.2) was very highly significant ($P < 0.001$). When mean density was plotted against host body length (Fig. 1), males consistently exhibited a greater mean density than females of the same length in all upper length classes, but there appeared to be no difference between males and females with body lengths less than 140 mm.

The observation that females in upper length classes tended to have a lower density than males of the same size is difficult to reconcile with the fact that females are generally older than males of the same length class (Chipman, 1965; Jiminez, 1971). It would be expected that older individuals would acquire a greater number of helminths regardless of sex.



Figures 1–4. 1. Mean density of total helminths as a function of the length and sex of *S. hispidus* (33-mm moving average). 2. Incidence of total helminths as a function of the length and sex of *S. hispidus* (33-mm moving average). 3. Number of helminth species per log of the number of hosts examined as a function of the body length of *S. hispidus*. 4. Seasonal distribution of incidence and mean density of total helminths of *S. hispidus* (3-month moving average).

One factor which could contribute to a lower mean density in older females is their territoriality. Fleharty and Mares (1973) stated: "Our data indicates that if *Sigmodon hispidus* is territorial, territoriality is more pronounced in females than males . . . ." It would be expected that the exclusion of other possibly infected cotton rats of either sex from the home range of females would decrease the probability of parasite transfer.

Another factor which may contribute to the lower mean density in females is the reproductive physiology of the host. Noble and Noble (1976) have suggested that male sex hormones may have a beneficial effect upon the helminth fauna of the host. The critical host length of 140 mm where males begin to show higher mean parasite density than females corresponds closely to the mean length of perforate females (142 mm) and the mean length of scrotal males (152 mm). Odum (1955) indicated that breeding usually begins in cotton rats at an age of 40 to 50 days, which would correspond to a body length of 140 to 150 mm. Thus, the observed differences in mean parasite density between male and female cotton rats is apparently associated with some aspect of sexual maturity.

When mean parasite density was plotted against host body length for each of the nine abundant helminth species, the tendency for lower density to occur in

female cotton rats was demonstrated for all but one species (not figured). Only in the case of *Zonorchis komareki* was mean density consistently greater in females.

Size of the host

The mean density (Fig. 1), incidence (Fig. 2), and number of helminth species (Fig. 3) increased with host body length when calculated for overall data.

When mean density was regressed against body length using raw data, a significant linear increase for female cotton rats ($P < 0.05$) and a highly significant increase for male cotton rats ($P < 0.01$) were detected, indicating that an increase in body length is normally accompanied by a corresponding increase in mean density of overall infection (Fig. 1). Since the body length of *Sigmodon hispidus* increases significantly with increasing age (Chipman, 1965), the observed increases in mean density as body length increased can be attributed to the accumulated probability of hosts acquiring infections as their ages increase. The increased probability of older hosts acquiring infections could result from a number of factors, including increased time of exposure, increased surface area exposed to penetration, and increased volume of ingested material as a host ages. In addition, older cotton rats tend to be less neophobic and more aggressive than subordinates. This may occur to the point that dominants may aggressively exclude subdominants from preferred items (Summerlin and Wolfe, 1973) which may also be the preferred transport hosts for the helminths. Thus, the establishment of ranks of dominance could also enhance the probability of older hosts acquiring infections. As a consequence of this age-related increase in mean density, one may expect that as the age of a group of cotton rats increases, increasing numbers of helminths will be found in progressively fewer hosts. This results in an age-related overdispersion of helminths in the host population with the relatively fewer older hosts containing the heaviest infections.

When mean density was plotted against host body length for each of the nine abundant helminth species recovered (not figured), a very highly significant linear increase ($P < 0.001$) was observed for the cestodes *Monoecocestus sigmodontis*, *Hydatigera taeniaeformis*, and the nematodes *Strongyloides sigmodontis* and *Hassalstrongylus aduncus*. The nematode *Physaloptera bispiculata* did not occur in the 67 cotton rats having body lengths less than 135 mm. When mean density was plotted against the body lengths of those cotton rats infected with this nematode, density increased with increasing body length. This increase was not, however, statistically significant ($P > 0.05$). The mean density of the cestode *Raillietina bakeri* peaked in lower length classes for male cotton rats and exhibited a very highly significant linear decrease ($P < 0.001$) with increasing body length. The trematodes *Brachylaima thompsoni* and *Zonorchis komareki*, and the nematode *Syphacia sigmodontis* did not appear to be significantly affected by factors associated with increasing host body length ($P > 0.05$).

The negative linear correlation between host body length and mean density of the cestode *Raillietina bakeri* appears to be the result of two factors. First, this cestode only occurred commonly in samples during the peak of the cotton rat reproductive cycle when the population consisted primarily of immature individuals. Second, the establishment of a single *R. bakeri* appeared to prevent secondary infections with this cestode. The establishment of this cestode in popu-

lations of primarily immature individuals and the lack of secondary infections could result in the observed linear decrease in mean density.

The distributions of the trematodes *Brachylaima thompsoni* and *Zonorchis komareki* and the nematode *Syphacia sigmodontis* among hosts were highly overdispersed, with variance/mean ratios of 49.8, 22.6, and 62.3, respectively, even when using only the data from the station in which each species occurred most abundantly. This observation, coupled with the lack of an increase in mean density in the upper host-length classes suggests that heavy infections involving these species are the result of rare single pulses rather than an accumulative process.

Incidence of overall infection, when plotted against a moving average of host body length (Fig. 2), increased linearly from the smallest length class to approximately 130 mm, above which an asymptotic level was attained with the remaining length classes varying at about a 99% incidence. This trend was evident for both male and female cotton rats.

The number of helminth species observed in each host-length class was divided by the log of the number of hosts examined in that length class to help correct for differences in sample size at different length classes. This is because the number of species found within a sample of hosts increases approximately with the logarithm of sample size. The number of helminth species, when corrected for sample size, exhibited a very highly significant linear increase ($P < 0.001$) with increasing body length when determined over all hosts (Fig. 3).

Season of collection

Data from the three monthly collecting stations were pooled to analyze the effects of season on the helminth fauna of *S. hispidus*. Analysis of seasonal changes in the number of helminths, number of hosts infected, and number of species (all corrected for slight differences in sample size) by use of chi-square goodness of fit tests indicated very highly significant ($P < 0.001$) deviations from uniformity.

When overall incidence and mean density were plotted as 3-month moving averages (Fig. 4), incidence of infection remained relatively constant over the year while mean density peaked in the months of March through May and descended to the lowest levels in the months of December and January.

Several factors are believed to have contributed to the spring peak in mean density, one of which could be the reproductive cycle of *S. hispidus*. No pregnant females were encountered in collections during the months of January and February and, consequently, a seasonal peak in the mean age of the collected hosts was observed during March through May. Since mean helminth density appears to be a linear function of host body length for the cotton rat, an increase in the mean age of the host population would result in a corresponding increase in the density of helminth infection. Another factor possibly influencing this trend is the adverse weather conditions existing in the months prior to this peak. Cotton rats are well known for their characteristic huddling behavior during periods of cold weather (Fleharty and Mares, 1973) and nesting material, which is generally ignored during summer months, is extensively used during winter months. This would provide conditions ideal for the development of an epizootic, particularly for *Hassalstrongylus aduncus*, which can be transferred through nesting material (Scott and Blynn, 1952).

The summer decline in mean density was attributed to two factors, the first of which is the removal of superinfected hosts. While cotton rats can live for a year or more, this rarely occurs in nature. Layne (1974) found that the average period of residence of cotton rats in his study plots was 2.9 months. Since individual hosts during March were at least 3 months of age, and since these hosts contained the bulk of the helminth individuals when the immature rats began to appear in traps, the death of these older hosts would substantially deplete the helminth populations. Secondly, the increasing proportion of uninfected or lightly infected young cotton rats during this interval would effectively dilute the overall mean density of infection.

When mean density was plotted against a 3-month moving average for each of the nine abundant helminth species, most species exhibited peaks in the spring, but a second type of seasonal cycle became evident with peak mean densities occurring in the early fall. This was demonstrated by the cesote *Raillietina bakeri*, the trematode *Brachylaima thompsoni*, and the nematode *Strongyloides sigmodontis*.

The peak mean densities of the two abundant cestodes encountered in this study, *Raillietina bakeri* and *Monoecocestus sigmodontis*, occurred at markedly different times of the year. In addition, these two cestodes were never encountered in the same host, although both were collected at all stations and their seasonal distributions overlapped. This is consistent with observations by Hughins (1951). Melvin (1952) reported that the establishment of even a single *M. sigmodontis* produced a complete resistance to reinfection. It would appear that this premunition is equally effective against *R. bakeri* and that the establishment of even a single individual of either species is sufficient to prevent secondary infection with the same or the other species. However, the fall peak in mean density of *R. bakeri* is also consistent with observations from North Carolina (Harkema and Kartman, 1948; Coggins and McDaniel, 1975) where *M. sigmodontis* was not observed. These facts suggest that while competitive exclusion may occur during periods of overlap in the seasonal distributions of these cestodes, it is probably not entirely responsible for the radical phase difference between the peak mean densities.

The peak mean densities of the two abundant trematodes, *B. thompsoni* and *Z. komareki*, also occurred at different times of the year, with one occurring in the spring and the other in the fall. Also, the seasonal peaks of the two abundant nematodes, *H. aduncus* and *Strongyloides sigmodontis*, occurred at different times of the year.

As indicated above, species composition varied considerably over the year and helminths inhabiting similar locations in the host tended to reach peak mean densities at different times of the year. Overall mean density also varied considerably over the year (Fig. 4). However, the seasonal distribution of helminth diversity (no figure) and incidence (Fig. 4) appeared to remain relatively constant. Davis and Huffman (1975), in their study of the helminths of the mosquitofish, *Gambusia affinis*, reported similar observations. We are unable to propose a mechanism that would act to keep incidence and diversity seasonally constant, while allowing the total mean density and the mean density of individual species to vary radically on a seasonal basis.

Acknowledgment

We gratefully acknowledge the generous assistance of Dr. Maurice D. Little of Tulane University in identification of some of the nematodes.

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47(2), 1980, p. 256–259

Research Note

Scanning Electron Microscopy of Scolices of Some Cestodes from Elasmobranchs

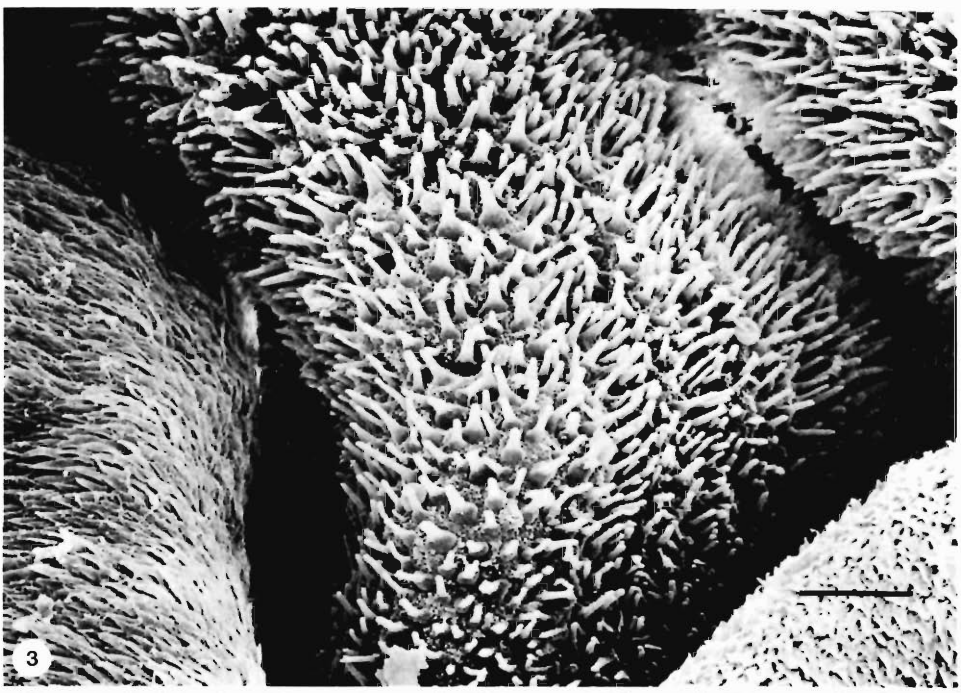
Observations of the surface morphology of cestodes of the orders Cyclophyllidea and Pseudophyllidea by scanning electron microscopy (SEM) have been reported (Berger and Mettrick, 1971, Trans. Am. Microsc. Soc. 90:393–403; Whittaker and Giammara, 1972, A Three-D Look at Life, American Education Publications, Columbus, Ohio, 35 pp; Ubelaker et al., 1973, J. Parasitol. 59:667–671; Andersen, 1975, Int. J. Parasitol. 5:295–300 and 5:487–493; Whittaker, 1975, Bioscience 25:819). A review of the literature, however, has revealed that other than a preliminary report by Whittaker (1976, Biol. Digest 3:11–20), no detailed observations by SEM have been reported for tetraphyllidean cestodes.

Since the scanning electron microscope provides a combination of increased depth of field, resolution, and magnification, we decided to employ it in the examination of the microtopography of the relatively irregular surfaces of the scolices of the tetraphyllideans *Orygmatobothrium musteli* (Van Beneden, 1850) and *Rhinebothrium ditesticulum* Appy and Dailey, 1977.

Specimens of *Orygmatobothrium musteli* from *Mustelus mento* were collected at Anna Pink Bay, Chili and identified by the second author, while specimens of *Rhinebothrium ditesticulum* from *Urolophus halleri* were collected at Anaheim Bay, Seal Beach, California by Dr. M. Dailey of California State University, Long Beach. The cestode specimens were also identified by Dr. Dailey. Several species of *Mustelus* including *M. mento* as well as *Galeus canis* and *Syllium canicula* have been previously recorded as hosts for *O. musteli* (Beneden, 1850, Mem. Acad. R. Sci. Belg. 25:1–204; Carvajal, 1974, J. Parasitol. 60:29–34). So far, *Urolophus halleri* is the only host from which *R. ditesticulum* has been reported (Appy and Dailey, 1977, Bull. S. Calif. Acad. Sci. 76:116–127). All cestodes had been fixed in alchol-formalin-acetic acid (AFA) and stored in 70% ethanol. Specimens prepared for scanning electron microscopy, as described by Allison et al. (1972, J. Parasitol. 58:414–416), were transferred to 5% glycerine–95% ethanol from which the alcohol was allowed to evaporate and cleared in 96.6% glycerol–0.05% potassium chloride–3.5% distilled water, 24 to 48 hr prior to examination. The scolices with a few attached anterior segments were mounted on metal specimen stubs with Ducco cement, outgassed in a vacuum evaporator for approximately 1.5 hr, coated with gold-palladium (200 Å or less), and examined with a Cambridge Stereoscan Mark II.

Regarding *Orygmatobothrium musteli,* the highly folded or crenulated condition of the bothridial margins is very apparent in Figure 1. The margins and outer bothridial surfaces are densely covered with minute, wedge-shaped spines which can be more clearly seen in Figure 2 (above and below center) and in Figure 3 (left and lower right). Spines on the bothridial margins are directed away from the depressions, while those on the outer surface are more or less at right angles to the surface.

The adherent surfaces of the bothridia are thickly covered with relatively minute microtriche-like structures (Figs. 1, 2). At higher magnification (Figs. 3, 4), each structure can be seen to consist of a relatively short, broad base and an



Figures 1–3. Scanning electron micrographs of the scolex of *Orygmatobothrium musteli*. 1. Apical view of scolex showing highly folded margins, adherent and outer surfaces of bothridia. Rectangle indicates region from which Fig. 2 was obtained. Bar equals 118 μ. 2. Region of bothridial margin with dense covering of spines and adherent surface with microtriche-like structures. Rectangle indicates region from which Figure 3 was obtained. Bar equals 16.8 μm. 3. Enlargement of microtriche-like structures on portion of bothridial adherent surface (center, upper right) and spines of bothridial margin (lower left, lower right). Bar equals 4.4 μm.

Figure 4. Scanning electron micrograph of *O. musteli* revealing microtriche-like structures on bothridial adherent surface. Microvilli can ben seen near bases of some microtriche-like structures (arrows, lower right). Bar equals 0.9 μm.

elongate, tapering shaft which are not exactly uniform in size. Circumscribing each shaft and running parallel to its long axis, are numerous ridges bearing rows of minute conical elevations or bosses, suggesting an ear-of-corn appearance (Fig. 4). The bosses are more uniformly distributed near the distal ends of the shafts.

Scattered over the adherent surfaces among the microtriche-like structures are numerous smaller structures (Fig. 3) which at higher manification (Fig. 4) appear cylindrical and resemble microvilli.

Figure 5 illustrates the features of the adherent surface of one of the bothridial lobes of *R. ditesticulum*. Along the medial wall of some of the loculi of the upper and lower rows can be seen minute circular depressions or pits arranged two per loculus. That these pits are revealed by SEM is considered significant, since no mention of them was made by Appy and Dailey (op. cit.) in their description of the cestode. The various minute spherules scattered in the loculi are probably debris.

With the aid of SEM, the microtriche-like structures occurring on the bothridial adherent surfaces of *O. musteli* (Figs. 3, 4) are herein described for the first time. Under oil immersion (1,000×) with light microscopy, these structures are very similar in appearance to the spines which occur on the outer surfaces and margins of the bothridia. The attenuated, straight shafts of the microtriche-like structures are clearly visible; however, the shorter and broader bases are only seldom ob-

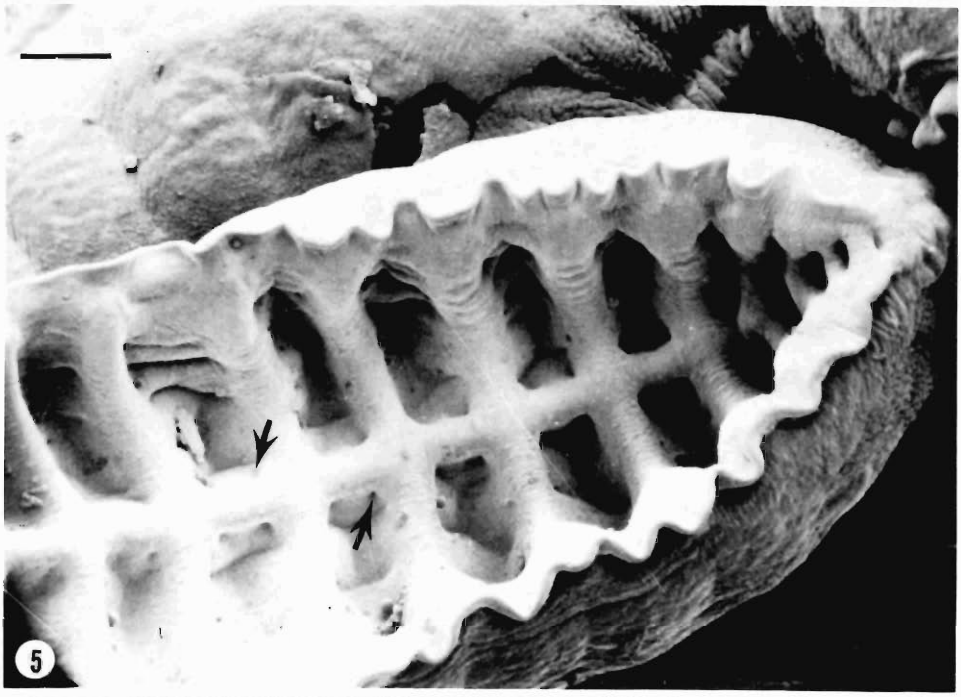


Figure 5. Part of adherent surface of bothridial lobe of *R. ditesticulum* showing pits (arrows) along longitudinal septum. Bar equals 100 μm.

servable with light microscopy and could be overlooked completely in the absence of evidence for their presence. Since the bases of these structures were not observed in their entirety, it was not possible to determine their lengths. However, measurements of the distances between the presumed proximal regions of bases to the tips of shafts of 10 of the structures reveals that they have an approximate average length of 4.3 μm (4–4.5 range). Although relatively long, these structures could indeed be modified microtriches; the distal shaft and bulbous base are indicative of such. Studies of these structures with transmission electron microscopy should provide additional information on their structure.

The minute depressions or pits along the longitudinal septum in the bothridial loculi of *R. ditesticulum* (Fig. 5) are similar in appearance to the single frontal pit on the apex of the scolices of adult *Diphyllobothrium latum* and *D. dendriticum* as described by Andersen (op. cit. 5:487–493). Additional studies are necessary before any functions for these pits of *R. ditesticulum* can be suggested.

The authors wish to express their sincere appreciation to Ray Schweri, Celanese Coatings, Louisville, Kentucky, Bill Renda and Charles Miller, University of Louisville, for their very valuable technical assistance. Appreciation is also extended to Coats-Welter Corporation for suggestions in specimen preparation.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 260–262

Research Note

The Capuchin Monkey (*Cebus apella*) as an Experimental Host for *Schistosoma intercalatum*

Schistosoma intercalatum, although reported from man by Fisher in 1934 (Trans. R. Soc. Trop. Med. Hyg. 28:277–306) has remained somewhat of a parasitologic enigma until recent years when survey work in endemic areas of Africa and compilations of information (Decroocq, 1969, Thesis Fac. Med., Univ. of Nancy, France; van Wijk, 1975, Meppel, Krips Repro B. V. 155 pp.; Wright, 1972, Trans. R. Soc. Trop. Med. Hyg. 66:28–64) revealed it to be quite common and responsible for schistosome disease in man. Characterization of this species, morphologically similar to *S. haematobium,* has been neglected but reports by Decroocq, van Wijk, and Wright (loc. cit.) have attested to its biologic identity as well as its pathologic potential. Efforts to demonstrate significant differences between *S. intercalatum* and *S. haematobium* have been concerned with general biology including snail host- and definitive host-parasite relationships, staining features of the eggs and adult parasite surface characteristics as visualized by electron microscopy (Kuntz, Tulloch, Davidson, and Huang, 1976, J. Parasitol. 63:63–69; Kuntz, Tulloch, Huang, and Davidson, 1977, J. Parasitol. 63:401–406). Of particular importance have been attempts to evaluate the bladder carcinogenic potential of *S. intercalatum* in comparison with that which has been demonstrated for *S. haematobium.* Affinity of *S. haematobium* for the urinary bladder and rather common occurrence of severe pathology has been demonstrated for a number of species of nonhuman primates (Cheever, Kuntz, Moore, Bryan, and Brown, 1976, Am. J. Pathol. 84:673–678). In similar studies several species of primates have been exposed to infection by *S. intercalatum.*

This report presents the basic biology of *S. intercalatum* (Cameroon strain) in the South American capuchin monkey (*Cebus apella*) procured from a primate importer (Primate Imports, Port Washington, New York). They were maintained in a quarantine facility for microbiologic and parasitologic monitoring until they became accustomed to regular monkey chow (Purina). Cercariae for infection were pooled from 15+ *Bulinus wrighti,* counted on glass coverslips and applied to clipped, water-cleansed abdominal skin. Capuchins were anesthetized with Sernylan (phencyclidine hydrochloride) at time of exposure. Hosts were necropsied at death or after various periods of infection. Parasites were recovered from viscera by perfusion and small forceps. Eggs in tissues were counted after digestion in 2.5% KOH (Cheever, 1970, Bull. W.H.O. 43:601–603). Selected tissue samples for histpathologic evaluations were fixed in 10% buffered formalin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Basic biologic information relative to susceptibility and distribution of schistosomes in pertinent viscera is presented in Table 1. Tissue and total body egg counts are given in Table 2. The greatest recovery of parasites occurred in hosts examined at 9 to 16 weeks postinfection; only three schistosomes were found in the bladder (Ca 90). There were marked individual differences in total body egg counts. Eggs occurred rarely in the urogenital system except in two hosts exposed to 1,000 parasites each. All hosts had eggs in the liver but the infection, judged by presence of granulomas, ranged from very light to moderate in intensity. Hosts

Table 1. Returns and distribution of *Schistosoma intercalatum* from capuchin monkeys.

Host no./sex	Duration (weeks) D or S*	Lungs			Liver			Portal veins			Stomach			S. Int.			L. Int.			Pancreas			Total worms recovered			Total	% Recovery
		Prs	♂♂	♀♀	Prs	♂♂	♀♀	Prs	♂♂	♀♀	Prs	♂♂	♀♀	Prs	♂♂	♀♀	Prs	♂♂	♀♀	Prs	♂♂	♀♀	Prs	♂♂	♀♀		
Hosts exposed to 500 cercariae																											
69 X 25♀	9/D	0	0	0	26	1	0	10	7	0	0	0	0	74	13	0	41	0	0	0	0	0	151	21	0	323	64.6
Ca 90 ♀	10/D	0	0	0	19	0	3	35	0	0	2	0	0	41	0	0	19	0	1	0	0	0	117	0	5	238†	47.6
Ca 177 ♂	15/S	0	0	0	4	0	3	10	0	0	0	0	0	17	0	0	66	0	0	0	0	0	97	0	3	197	39.4
Ca 174 ♀	25/S	0	0	0	7	0	2	3	0	0	1	0	0	25	0	1	31	0	1	0	0	0	67	0	4	138	27.6
Ca 175 ♀	34/S	0	0	0	1	0	13	0	0	0	0	0	0	5	0	1	1	0	0	1	0	0	8	0	14	30	6.0
Ca 91 ♀	52/S	1	0	0	0	3	20	6	1	0	0	1	0	2	7	3	10	6	0	0	0	0	19	18	23	79	15.8
Ca 89 ♂	52/S	2	0	0	7	0	0	7	0	0	1	0	0	37	1	0	23	0	0	0	0	0	77	1	0	155	31.0
Ca 178 ♂	67/S	0	0	0	2	9	0	11	0	0	0	0	0	9	7	0	44	1	0	0	0	0	66	17	0	149	29.8
Ca 176 ♂	89/S	0	0	0	3	0	20	0	0	0	0	0	0	5	0	0	3	0	0	0	0	0	11	0	20	42	8.4
Hosts exposed to 1,000 cercariae																											
Ca 35 ♀	16/D	0	1	0	2	0	0	41	38	0	6	0	0	42	6	0	72	8	0	9	0	0	175	53	0	403‡	40.3
Ca 34 ♀	16/D	0	0	0	2	3	46	4	0	0	0	0	0	0	0	0	132	0	0	0	0	0	138	3	46	325	32.5

* Host died or was sacrificed.
† Includes 1 pr, 1 ♀ from urinary bladder.
‡ Includes 3 pr from spleen.

Table 2. ***Schistosoma intercalatum*** **egg deposits (eggs/organ 10^3) in capuchin monkeys.**

Host no./sex	Duration (weeks) D or S*	Lungs	Liver	Stomach	S. Int.	L. Int.	Pancreas	Spleen	Urogenital	Mesentery S. Int.	Mesentery L. Int.	Total body count
Hosts exposed to 500 cercariae												
69X 25 ♀	9/D	10.0	3,565.9	47.2	2,488.5	92.1	84.0	0.1	0	12.3	1.9	6,302.0
Ca 90 ♀	10/D	45.0	146.4	5.1	331.4	191.8	12.8	0	0	0	12.6	745.1
Ca 177 ♂	15/S	0.9	8.6	1.4	130.5	504.2	36.7	0	0	18.3	54.4	755.0
Ca 174 ♀	25/D	1.1	23.4	3.6	238.0	466.6	4.8	0	0	1.5	20.0	759.0
Ca 175 ♀	34/S	0	0	0.1	8.6	24.2	0.7	0	0	0.1	0	33.7
Ca 91 ♀	52/S	0.1	9.5	0.1	85.2	146.1	2.1	0	0	0.6	7.7	252.3
Ca 89 ♀	52/S	0	4.0	6.0	1,083.0	33.0	1.2	0	0	1.5	4.0	1,132.7
Ca 178 ♂	67/S	0.4	10.8	0.5	57.2	941.7	14.3	0	0	3.4	10.2	1,038.5
Ca 176 ♂	89/S	0	3.0	0	8.8	9.1	0.4	0	0	1.5	1.2	24.0
Hosts exposed to 1,000 cercariae												
Ca 35 ♀	16/D	20.7	902.2	32.6	22.3	254.0	29.7	1.1	3.8	130.0	172.0	1,568.4
Ca 34 ♀	16/D	2.1	230.9	2.4	24.9	267.6	6.9	0.1	22.6	3.4	7.0	567.9

* Host died or was sacrificed.

with greater numbers of eggs in the pancreas showed moderate numbers of large granulomas. There were few to moderate eggs and granulomas in the mucosa and submucosa at different levels of the small and large intestine. Large numbers of eggs occurred in the colon and rectum of several hosts and in one (Ca 90), the entire colon was intensely hemorrhagic and the animal had apparently died of blood loss. Two monkeys had macroscopic serosal lesions and greatly enlarged, egg-laden lymph nodes. A third showed a bilharzioma (6 mm) in the supporting mesentery. Only one monkey (Ca 90) having very few eggs in micro section demonstrated minimal involvement of the bladder mucosa.

The capuchin monkey obviously is a very satisfactory primate for work in experimental schistosomiasis intercalata even though, in contrast with infections in earlier investigations in the cynomolgus macaque (*Macaca fascicularis*) (Cheever, Kuntz, Moore, and Huang, 1976, Cancer Res. 36:2928–2931), there was minimal pathologic involvement of the urinary bladder. As other studies on definitive host parasitism for members of the *S. haematobium* complex have shown, there is a marked individuality, i.e., susceptibility, tissue egg deposits and pathology, in the basic parameters for judging the usefulness of a given host for multidisciplinary investigations.

This study was supported by the World Health Organization, Geneva, Switzerland; by the United States–Japan Cooperative Medical Science Program, administered by NIAID; as well as by grants AI/CA 14999 from NIAID and CA 13208 from National Cancer Institute. The authors wish to acknowledge Dr. C. A. Wright, British Museum (Natural History), for original stocks of *S. intercalatum,* and Dr. Allen W. Cheever, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, Maryland, for assistance in histopathologic evaluations.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 263

Research Note

Host and Locality records for *Plagioporus sinitsini* (Trematoda: Opecoelidae)

Plagioporus sinitsini Mueller, 1934 has been reported as parasitizing *Catostomus commersoni* Lacépède in New York (Mueller, 1934, Trans. Am. Microsc. Soc. 53:231–236), *Notropis cornutus* (Mitchill) in Wisconsin (Fischthal, 1950, Trans. Wis. Acad. Sci. Arts Lett. 40:87–113), and *N. cornutus, N. heterolepus* Eigenmann and Eigenmann, *N. volucellus* (Cope), *N. rubellus* (Agassiz), *C. commersoni, Hypentelium nigricans* (LeSueur), *Pimephales notatus* (Raf.), *Nocomis biguttatus* (Kirtland), and *N. micropogon* (Cope) in Michigan (Dobrovolny, 1939, Trans. Am. Microsc. Soc. 58:121–155). This communicaton reports the species from *Moxostoma macrolepidotum* (LeSueur) (additional host record), *H. nigricans* and *C. commersoni* in Wisconsin, and from *H. nigricans* in Missouri (additional distribution record).

Three species of catostomid fishes were collected from the Red Cedar River, southern Barron County, Wisconsin, monthly from June 1977 to August 1978 (*M. macrolepidotum* were not collected in September or December 1977). Collection data are given in Williams, 1979, Iowa State J. Res. 53:305–310; 53:311–316; 1980, Rep. Wis. Flora Fauna 16:18–21. *H. nigricans* were collected from Huzzah Creek, Crawford Co., Missouri in June 1978. Trematodes were preserved in cold AFA or hot or cold formalin and stained in Mayer's paracarmine.

Twenty-four percent of 221 *H. nigricans,* 29% of 234 *C. commersoni,* and 27% of 54 *M. macrolepidotum* from Wisconsin were parasitized with *P. sinitsini,* whereas all three *H. nigricans* from Missouri were parasitized. Host burdens are as follows (mean, range): *H. nigricans* (23, 7–64); *C. commersoni* (17, 3–41); *M. macrolepidotum* (26, 5–89); and *H. nigricans* (Missouri) (42, 27–113). Parasitism occurred only during spring and early summer (April through June), possibly as a result of *C. commersoni* and *M. macrolepidotum* eating *Goniobasis livescens* Menke (intermediate host of *P. sinitsini*) which occurs in spawning areas (riffles) but is not found in sandy-bottomed pool habitats. Why *H. nigricans,* an inhabitant of riffle areas, is not parasitized during other times of the year, is not known. None of 58 *C. commersoni,* 29 *M. macrolepidotum,* two *M. anisurum* (Raf.), 19 *M. erythrurum* (Raf.) or 24 *H. nigricans,* collected from Iowa rivers, April through November (localities given in Williams, 1979, Proc. Helminthol. Soc. Wash. 46:272–274) were parasitized by *P. sinitsini,* probably because Iowa rivers are often turbid and possess a shifting sand-silt bottom; conditions not conducive to the survival of the gastropod intermediate host.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 264–266

Research Note

A Conspicuous *Philometra* sp. (Nematoda: Philometridae) from the Oculo-orbits of Centrarchid Fishes[1]

Eight bluegills (*Lepomis macrochirus*) and one warmouth (*L. gulosus*) were retained for examination from an undetermined number of centrarchid fishes collected on 13 March 1978 by seining the shoreline of Lake Sidney Lanier, Georgia. These fishes, ranging in total length from 4.8–10.1 cm, were held because of their exophthalmic condition (Fig. 1) and/or the presence of a red-colored nematode visible about their eyes (Fig. 2). The fishes were fixed in 10% formalin, and were later dissected. The orbital, cranial, branchial, abdominal, and fin regions were examined for nematodes. Nematodes were cleared and studied (standard light and phase contrast microscopy) in glycerine.

Twenty female nematodes all conforming to Wellborn's (1970, Ph.D. Dissertation, Auburn University, Auburn, Alabama) description of a *Philometra* sp. were collected from the fishes. The species is currently being formally described (W. A. Rogers, Auburn University, pers. comm.). Individuals ranged from approximately 300 to 720 mm long ($\bar{x}$ = 680 mm), with those longer than 520 mm being gravid. The nematodes were found only in the fishes' oculo-orbits, and some orbits contained two worms. In fishes with an orbit containing two nematodes, the remaining orbit always contained at least one worm. Exophthalmia was most prominent in smaller fishes, and in fishes with two nematodes per orbit. Voucher specimens have been deposited in the National Parasite Collection as USNM Helminthological Collection Number 75832.

Wellborn (loc. cit.) collected *Philometra* sp. from *L. macrochirus, L. microlophus,* and *Micropterus salmoides*. A record alluding to the same *Philometra* sp. (Cochran, 1979, Georgia Acad. Sci. 37:199–204) adds *M. punctulatus* as a host, and this report adds *L. gulosus*. All of these hosts are members of the Centrarchidae, and with the overlap in diets of many members of this family (Scott and Crossman, 1973, Freshwater Fishes of Canada, Fish. Res. Bd. Can. Bull. 184.) new hosts will no doubt be reported in the future for this copepod-vectored (Wellborn, loc. cit.) nematode. Crites (1975, Abstr. No. 247, 50th Annu. Meet. Am. Soc. Parasitol.) has reported the experimental infection of *Aplodinotus grunniens* (family Sciaenidae) with an oculo-orbital *Philometra* sp., however, whether the report represents further information on the species under consideration here is unknown.

We thank Dr. W. R. Whitworth and his class for assistance in the acquisition and subsequent relinquishing of the study material, Ms. D. Jezek for assistance in the literature search, and Drs. L. R. Penner and C. W. Schaefer for their critical reviews of the manuscript.

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[1] Scientific Contribution No. 820, Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Connecticut 06268.

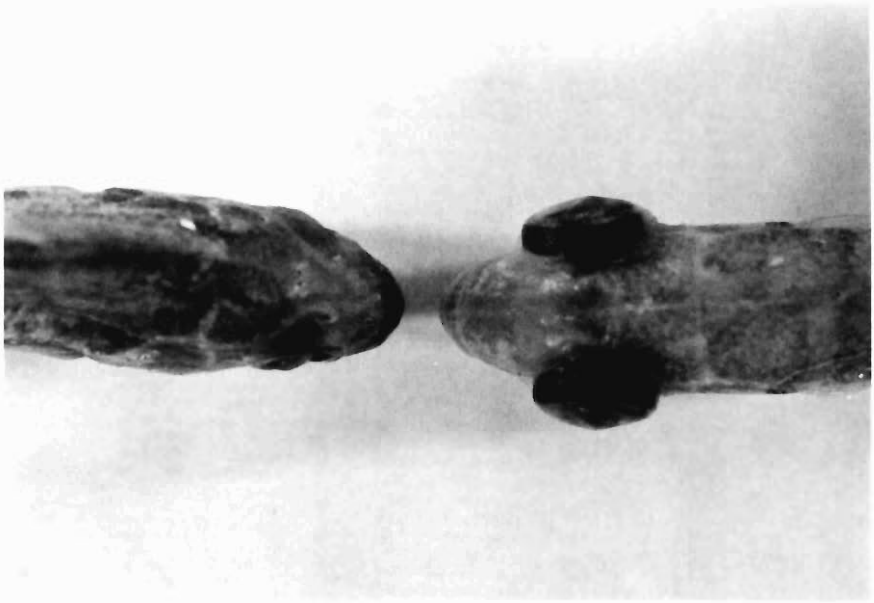

Figure 1. Uninfected (left) and infected (right) bluegills. Note the exophthalmic condition of the infected fish.

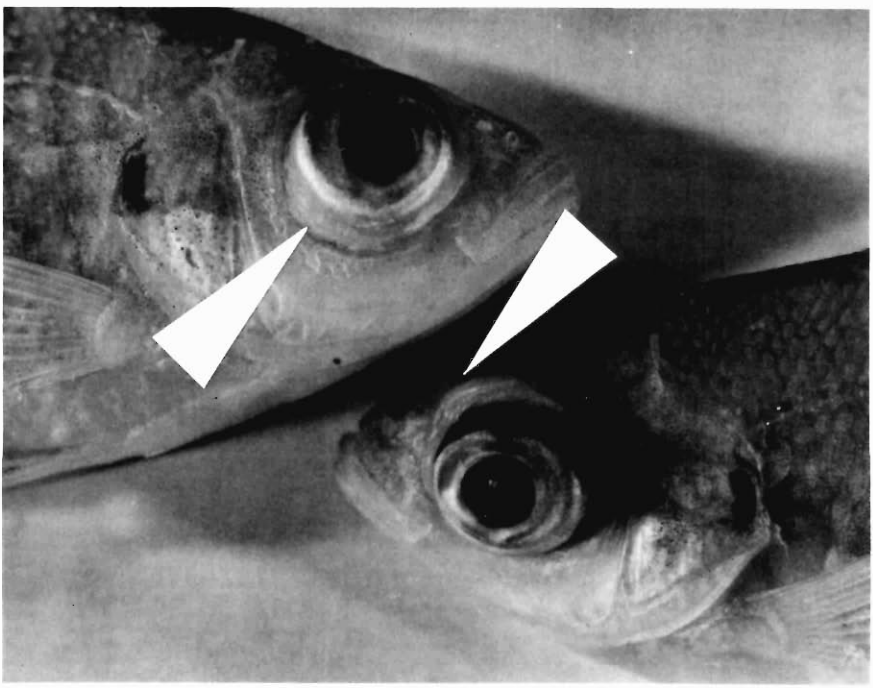

Figure 2. Typical positions of *Philometra* sp. (arrows) about the orbits of two bluegills.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 266–267

Research Note

Gongylonema pulchrum in Cervids of Northwestern California

There have been a number of reports of *Gongylonema pulchrum* (Molin, 1857) in free-living white-tailed deer (*Odocoileus virginianus*) of eastern North America, and there is one report of this parasite from a mule deer (*O. hemionus*) in the National Zoological Park, Washington, D.C. (Walker and Becklund, 1970, Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus,* in the United States and Canada. Index-catalogue of Medical and Veterinary Zoology Special Publ. No. 1, U.S. Gov. Printing Office; Prestwood et al., 1970, J. Parasitol. 56:123–127; Pursglove, 1977, Proc. Helminthol. Soc. Wash. 44:107–108). To our knowledge this parasite has not been reported from free-living black-tailed deer (*O. h. columbianus*) or wapiti (*Cervus elaphus*) from western North America.

In 1973, two female *Gongylonema* sp. were found in the esophagous of a 5½-year-old male black-tailed deer shot on 20 April near the town of Kneeland in Humboldt County, California. An additional four female *Gongylonema* sp. were found in a Roosevelt wapiti (*C. e. roosevelti*) taken during a hunt in November 1973 near Orick, also in Humboldt County.

Later, *Gongylonema* sp. were recovered from all eight esophagi donated by hunters between 13 November and 1 December 1976 at Orick during another wapiti hunt. A representative sample of male and female worms were identified as *Gongylonema pulchrum* by Dr. A. K. Prestwood, The University of Georgia. A total of 63 nematodes, 47 females and 16 males, were recovered from the eight wapiti; the infections ranged from three to 21 parasites per animal. Representative specimens were deposited in the National Parasite Collection, Beltsville, Maryland, as USNM Helm. Coll. Nos. 75758 and 75759.

Although found in stags (*C. elaphus*) of Yugoslavia (Petrovic et al., 1967, Congr. Biol. Gibier 7:449–453) and in *C. elaphus maral* of Iran (Asadov, 1960, Trudy Inst. Zool. Az. SSR 21:97–108; Asadov and Ialiev, 1971, Izv. Akad. Nauk

Az. SSR Ser. Biol. Nauk 3:91–94), this is the first known report of *Gongylonema pulchrum* in North America wapiti and the first known report in any free-living cervids of western North America.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 267–268

Research Note

New Host and Locality for *Kathlania leptura* (Rudolphi) (Nematoda: Oxyurata: Kathlanidae)

Marine testudines are known to host two species of kathlanid nematodes, *Kathlania leptura* (Rudolphi, 1819) Travassos, 1918 and *Tonaudia tonaudia* (Lane, 1914) Travassos, 1918. Each species has been reported infrequently but exhibits a wide geographic distribution. Both *K. leptura* and *T. tonaudia* have been reported from Ceylon and from the Mediterranean Sea near Egypt, and *K. leptura* has been reported also from the coast of Brazil (Skrjabin, Shikhobalova, and Lagodovskaya, 1964, *in* Skrjabin (ed.) Essentials of Nematodology, vol. 13, part 3, Academy of Sciences of the USSR, English translation, 1976, TT75-50011, U.S. Department of Commerce, NTIS, Springfield, Virginia 22151). This study reports *K. leptura* from the east African coast for the first time.

On 30 October 1975 a specimen of *Lepidochelys olivacea* (Eschscholtz, 1829) was found floating dead, belly up, about 8 km SW of Zanzibar Town, Zanzibar (approximately 39°8′E and 6°16′S). The turtle, evidently an adult female, had a straight carapace length of 64 cm. The specimen was deposited in the museum of the East African Marine Fisheries Organization of Zanzibar as No. JFLo9. Cause of death was not determined, but may have been by concussion from dynamite explosions, used illegally but frequently for "fishing." The gastrointestinal tract was examined by JF and found to contain a variety of items in various stages of digestion; details of that study will be published elsewhere. Several hundred nematodes were also found.

The nematodes occurred throughout the intestinal tract and the stomach. They were separated according to site of occurrence (stomach, upper intestine, lower intestine, rectum) and fixed with 10% formalin. Upon receiving the specimens, DRB transferred them to 70% glycerin-alcohol for permanent storage. Nematodes were examined as temporary whole mounts cleared with lacto-phenol. Representative specimens have been deposited as USNM Helm. Coll. No. 75757 (males and females).

Despite the widespread occurrence of specimens throughout the gastrointestinal tract, our collection contains only one species. Two hundred fifty-five of 324 examined specimens occurred in the posterior half of the gut, usual site of infec-

tion for kathlanid nematodes. Presumably, postmortem wandering accounts for their observed distribution. Of those 324 worms examined, 280 (86.4%) were mature and only 44 (13.6%) immature. The ratio of male worms to female worms was 118:206 or approximately 36:64%. The nematodes were identified as *Kathlania leptura* on the basis of the following traits. General: body up to 13 mm long in males, 15 mm in females, with finely tapering tail. Mouth with three well-defined lips each divided into a main lobe and 4–7 accessory lobes; buccal capsule present. Excretory pore located immediately anterior to level of esophageal bulb. Precloacal sucker lacking chitinous rim. Males: 11 pairs of lateral caudal papillae plus single medial papilla immediately precloacal. Lateral papillae arranged in groups as follows: 3 pairs posterolateral to cloaca, 2 pairs lateral to cloaca, 3 pairs immediately anterolateral to cloaca, 3 lateral pairs evenly spaced from level of precloacal sucker to slightly anterior to level of gubernaculum. Gubernaculum protruding, complex, V-shaped ventrally and ending posteriorly in 2 horns from which thin cuticular membranes extend anteriorly, surrounding the spicules. Spicules up to 550 μm long by 60 μm wide, similar and equal, with broad asymmetrical wings which are wider ventrally than dorsally. Females: Vulva postequatorial, with up to 15 ventral transverse crests immediately postvulvar. Eggs 90 μm long by 45 μm wide.

Kathlania leptura has been reported in *Chelonia mydas* (L.), *Caretta caretta* (L.), and *Thalassochelys* sp. (=either *Caretta caretta* or *Lepidochelys olivacea*) (Skrjabin et al., 1964, loc. cit.). Thus, *Lepidochelys olivacea* has never been listed as host explicitly and this report represents a new host record. However, *L. olivacea* is far more common than *Chelonia mydas* in the vicinity of Ceylon (Deraniyagala, 1939, Tetrapod Reptiles of Ceylon, vol. 1, Colombo Museum, Colombo) so it is possible that the report of *K. leptura* from *C. mydas* in Ceylon (Lane, 1914, Ind. J. Med. Res. 2:655) actually referred to specimens collected in *L. olivacea* incorrectly identified as *C. mydas*. This mistake in host identification has not been uncommon in the region (see Theobold, 1868, Catalogue of Reptiles in the Museum of the Asiatic Society of Bengal, extra number of J. Asiat. Soc., 88 pp.).

We express appreciation to the East African Wildlife Society, the East African Marine Fisheries Research Organization, and the governments of Zanzibar and Tanzania for support to JF. Mr. B. Benbow found the turtle. Funding in part was provided through a fellowship from the Friends of the National Zoo (FONZ) to DRB, and to JF by a grant from the Smithsonian Scholarly Studies Program to Dr. J. F. Eisenberg.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 269

Research Note

Heartworm (*Chinesocerca tonkinensis*) in a Wild Great Blue Heron (*Ardea herodias*) in Iowa

On 9 November 1977, the carcass of a great blue heron was submitted for necropsy. The wounded, cachectic heron had been found in the Des Moines River valley on 27 October 1977, and had been held at an animal shelter until its death on 8 November. It was not known if this heron was a local bird or one in migration from the north.

Besides cachexia, the principal lesion seen at necropsy was chronic degenerative arthritis of traumatic origin (old gunshot wounds) of both scapulohumeral joints. In addition, one immature ascarid (*Porrocaecum* sp.) in the ventriculus and numerous unidentified cestodes in the small intestine were found. In the chamber of the right heart and in the pulmonary artery were four filarioid nematodes identified as *Chinesocerca tonkinensis* Chow, 1939. The nematodes were characterized as follows, with all measurements in mm. Male: 28 long, 0.28 wide, muscular esophagus 0.36 long, glandular esophagus 2.40 long, left spicule 0.20 (0.19–0.21) long (measured around "dog leg"), right spicule 0.13 (0.127–0.134) long. Female: 37 long, 0.41 wide, muscular esophagus 0.40 long, glandular esophagus 4.80 long, vulva 2.55 from anterior end.

This report constitutes a new host record and new geographic record for *C. tonkinensis*. Previous records include *Ardeola bacchus* (Chinese pond heron) in French Indochina (Chow, 1939, Ann. Parasitol. 17:21–31), *Botaurus stellaris* (great bittern) in USSR (Feizullaev, 1963, Izv. Akad. Nauk Az. SSR Ser. Biol. Med. 2:61–68; Sonin, 1963, Trudy Gelmintol. Lab. Akad. Nauk SSR 13:227–249), and *Ardea cinerea* (gray heron) in USSR (Shigin, 1957, Trudy Darvinskogo Gos. Zapov. 4:258).

No descriptions of microfilariae from *C. tonkinensis* have been reported. Microfilariae removed in a fixed state from the uteri of the female *C. tonkinensis* were 4–5 μm wide and approximately 170 μm long. No microfilariae were found in histological sections of lung, liver, spleen, kidney, and gastrointestinal tract.

The specimens (3 male and 1 broken female) have been deposited as USNM Helm. Coll. No. 75322.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 270–272

Research Note

Inoculum Size and pH Affecting Growth of *Naegleria*

It has been proposed that a threshold inoculum is necessary for growth initiation of *Naegleria fowleri* (DeJonckheere, 1977, Appl. Environ. Microbiol. 33:751–757) and *N. gruberi* (Fulton, 1974, Exp. Cell Res. 88:365–370) in broth cultures. This idea originated to explain inconsistent ameba growth in cultures inoculated with less than 10^4 amebae/ml. If a certain ameba concentration is necessary to initiate in vitro axenic growth, cloning from one or few amebae would be difficult at best. The first part of this study examines the necessity of a threshold inoculum.

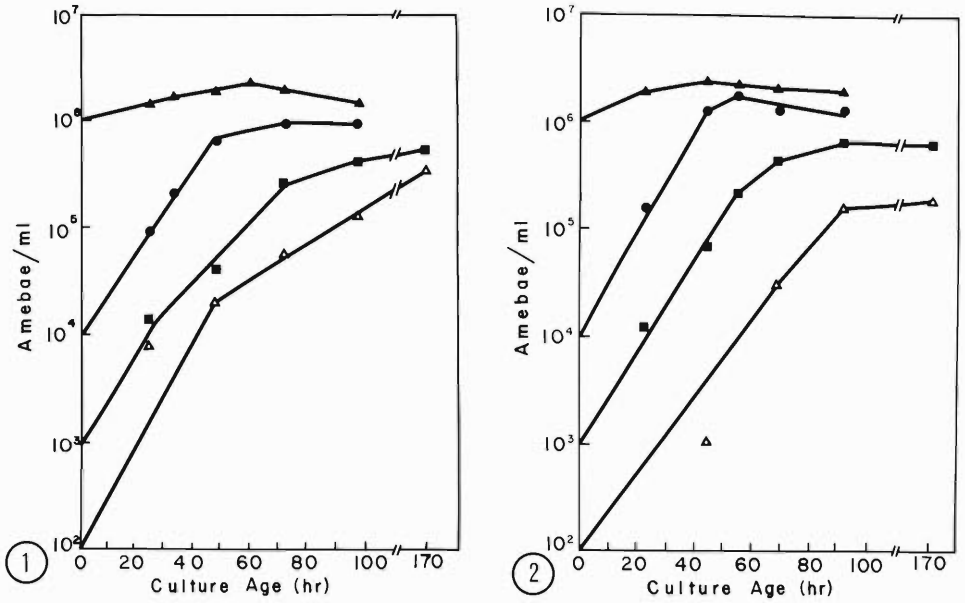
Previously, we have reported that the pH of the culture medium increased by as much as 2 pH units during cultivation of *N. fowleri* (Weik and John, 1977, J. Parasitol. 63:868–871) and *N. gruberi* (Weik and John, 1977, J. Protozool. 24:196–200). Specific ameba counting procedures and growth conditions used in the present study were also described in these reports. It was not certain whether the pH increase we observed was detrimental to optimal ameba growth. Cerva (1978, Folia Parasitol. (Praha) 25:1–8) reported good growth at pH 7 for *Naegleria* species and little or no growth at pH 7.9 and pH 8.1 for *N. fowleri* and *N. gruberi*, respectively. In this study we cultivated amebae with and without pH increases to determine its effect upon optimal ameba growth.

Naegleria fowleri (LEE strain) was grown in Nelson's medium at 37°C. *Naegleria gruberi* (EGB strain) was grown in Band and Balamuth's hemin medium (1974, Appl. Microbiol. 28:64–65) at 28°C without serum. Amebae were cultured in 25 ml of medium in 125-ml Erlenmeyer flasks in a gyrotory shaker (New Brunswick) at 100 rpm. Amebae were counted in a Coulter counter.

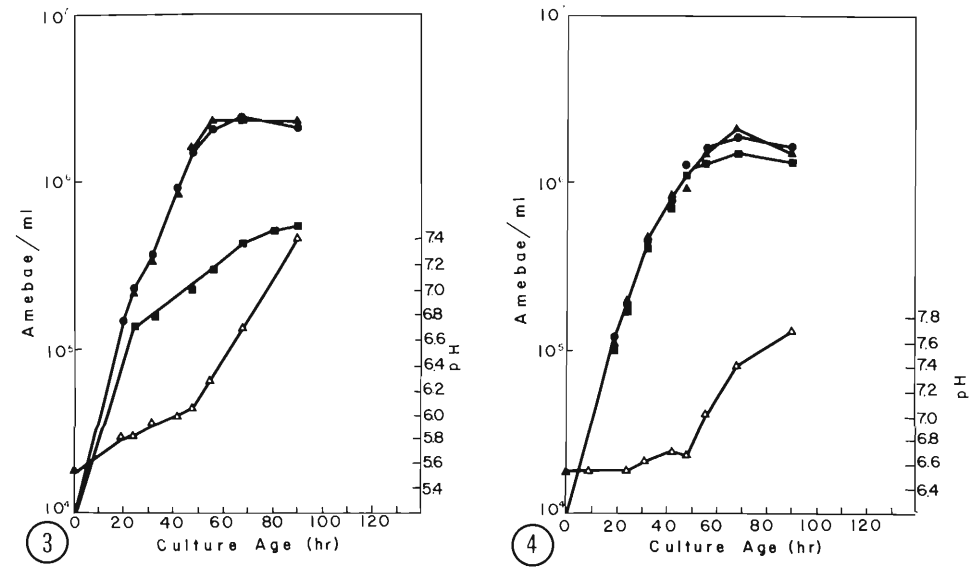
Flasks were inoculated with 10^2, 10^3, 10^4, and 10^6 amebae/ml and growth recorded at 12- to 24-hr intervals. In all experiments the culture medium was allowed to equilibrate for 48 hr after autoclaving prior to addition of serum, hemin, and inoculum.

Flasks inoculated with 10^2 and 10^3 *N. fowleri* amebae/ml yielded 3.6×10^5 and 5.5×10^5 amebae/ml, respectively at 168-hr culture age (Fig. 1). Flasks inoculated with 10^4 and 10^6 *N. fowleri* amebae/ml yielded 9.6×10^5 and 2.3×10^6 amebae/ml, respectively at 100-hr culture age (Fig. 1). Flasks inoculated with 10^2 and 10^3 *N. gruberi* amebae/ml yielded 2.2×10^5 and 6.4×10^5 amebae/ml, respectively at 168-hr culture age (Fig. 2). Flasks inoculated with 10^4 and 10^6 *N. gruberi* amebae/ml yielded 1.7×10^6 and 2.3×10^6 amebae/ml, respectively at 100-hr culture age (Fig. 2).

These results suggest that threshold inoculum levels do not exist and that cloning could be accomplished by further dilution of ameba culture. Cultures of both species inoculated with 10^2 amebae/ml grew three logarithmic units (about 10 generations) before entering stationary growth phase while cultures inoculated with 10^6 amebae/ml grew only one generation. These results indicate that maximum population density of about 3×10^6 amebae/ml was inherent under these culture conditions. Although a greater number of generations occurred at lower inoculum levels, the population density did not reach maximum levels indicating



Figures 1, 2. 1. Effect of inoculum size on growth of *N. fowleri*. (△) 10^2 amebae/ml; (■) 10^3 amebae/ml; (●) 10^4 amebae/ml; (▲) 10^6 amebae/ml. 2. Effect of inoculum size on growth of *N. gruberi*. (△) 10^2 amebae/ml; (■) 10^3 amebae/ml; (●) 10^4 amebae/ml; (▲) 10^6 amebae/ml.



Figures 3, 4. 3. Effect of pH on growth of *N. fowleri*. (▲) growth with 2 mM phosphate buffer, pH nonadjusted; (●) growth with 2 mM phosphate buffer, pH readjusted to 5.5 with HCl at intervals; (■) growth with 20 mM phosphate buffer, pH nonadjusted; (△) pH during growth with 2 mM phosphate buffer. 4. Effect of pH on growth of *N. gruberi*. (▲) growth with 2 mM phosphate buffer, pH nonadjusted; (●) growth with 2 mM phosphate buffer, pH readjusted to 5.5 with HCl at intervals; (■) growth with 20 mM phosphate buffer, pH nonadjusted; (△) pH during growth with 2 mM phosphate buffer.

that certain growth factors in the culture medium were limiting. No lag phase at any inoculum level was observed.

For pH studies, flasks were inoculated with 10^4 amebae/ml and pH monitored at 12-hr intervals. In one group of flasks, normal pH changes were recorded while the pH was maintained in another group of flasks by addition of sufficient 1 N HCl at 12-hr intervals. Amebae growth was measured in normal growth medium which contained 2 mM phosphate buffer and also in medium containing 20 mM phosphate buffer. This concentration was selected since the pH increase of the culture medium would be limited to under 0.2 pH units, a negligible amount.

The pH of Nelson's culture medium for *N. fowleri* increased about two units during 96 hr of growth (Fig. 3) while Band and Balamuth's culture medium for *N. gruberi* rose about one unit (Fig. 4). When sufficient HCl was added at 12-hr intervals during growth to maintain a constant pH, no change in growth for *N. fowleri* (Fig. 3) or *N. gruberi* (Fig. 4) was detected. Increasing the phosphate buffer from 2 to 20 mM limited the pH increase but also limited *N. fowleri* growth (Fig. 3). These results suggest that *N. fowleri* is sensitive to high phosphate concentrations and that for optimal growth the standard conditions should be used. However, 20 mM phosphate buffer did not affect *N. gruberi* growth (Fig. 4) and the pH of the culture medium during growth was nearly constant.

In conclusion a minimum inoculum size required to initiate growth of *Naegleria* was not found. Cultures inoculated with 10^6 amebae/ml showed that there was a maximum population density attainable under these culture conditions. The pH increases of the culture medium did not limit ameba growth.

This investigation was supported by Research Grant 771019 from A. H. Robbins Co., Richmond, Virginia.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 273–274

Research Note

Helminth Parasites of the Small-Mouthed Salamander, *Ambystoma texanum* Matthes, 1855 from Williamson County, Illinois

Little information on helminths of salamanders from southern Illinois is available. Landewe (1963, M.Sc. Thesis, Southern Illinois Univ. at Carbondale) examined 200 salamanders of 11 different species, but failed to publish his results. Dyer and Brandon (1973, Trans. Ill. State Acad. Sci. 66:23–29) found four species of helminths in 380 cave dwelling salamanders of three sympatric species from Saline County, and Dyer, Brandon, and Price (1980, Proc. Helminthol. Soc. Wash. 47:95–99) found four species of helminths in 417 dusky salamanders, *Desmognathus fuscus,* from Pope County.

The only published reports of helminths from the small-mouthed salamander, *Ambystoma texanum,* are those of Harwood (1932, Proc. U.S. Natl. Mus. 81:1–71) who examined seven specimens from the vicinity of Houston, Texas, and an annotated record of parasites of the Ambystomoidea presented in an abstract by Walton (1942, J. Parasitol. 28:29). Landewe (1963, M.Sc. Thesis, Southern Illinois Univ. at Carbondale) found *Neodiplostomum* sp. metacercaria, *Gorgoderina bilobata* and *Pseudopisthodiscus* sp. (Trematoda), and *Cosmocercoides dukae* and *Rhabdias* sp. (Nematoda) in the 61 specimens he examined.

Small-mouthed salamanders are found throughout southern Illinois, but their nocturnal and fossorial habits limits the collection of large numbers of specimens to breeding periods in late February through March. Their food consists of earthworms, slugs, and various arthropods (Smith, 1961, Bull. Ill. Nat. Hist. Surv. 28:1–298).

Salamanders were collected on the Crab Orchard National Wildlife Refuge, Williamson County, Illinois, on 13 March 1979 and 20 March 1979. Within 48 hr of their capture all specimens were anesthetized, pithed, and examined. Trematodes were fixed in 10% buffered formalin, stained in Harris' hematoxylin, cleared in beechwood creosote, and mounted in Canada balsam. Nematodes were killed in 70% ethanol, cleared in glycerine, and studied in temporary mounts.

Five species of helminths were removed from 54 of the 57 salamanders examined. Three specimens were not infected. Parasites recovered are listed in Table 1. To the best of our knowledge this is the first published report of *Diplostomulum ambystomae, Gorgoderina bilobata,* and *Rhabdias* sp. from *Ambystoma texanum.* Representative specimens of all parasites found have been deposited in the Southern Illinois University at Carbondale Zoological Museum Parasitology Collection (*Diplostomulum ambystomae* No. 94.A.O; *Gorgoderina bilobata* No. 96.A.O and 97.A.O; *Brachycoelium* sp. No. 95.A.O; *Rhabdias* sp. adults No. 98.A.O; *Rhabdias* sp. larvae No. 99.A.O; *Cosmocercoides dukae* No. 100.A.O).

Rankin and Hughes (1973, Trans. Am. Microsc. Soc. 57:61–66) reported 78 of 110 specimens of *Ambystoma opacum* and one of 17 specimens of *A. maculatum* infected with *D. ambystomae.* They noted that heavily infected specimens of *A. opacum* appeared bloated and sluggish. We observed no such symptoms, but the

Table 1. Helminth parasites of 57 small-mouthed salamanders, *Ambystoma texanum*, from Williamson County, Illinois.

Parasite	Site of infection	Number infected	Percent infected	Average no. per infected host	Range
Diplostomulum ambystomae	Body cavity	12	21	19	1–107
Gorgoderina bilobata	Urinary bladder	32	56	4	1–16
Brachycoelium sp.	Intestine	1	2	1	1
Cosmocercoides dukae	Intestine	3	5	1	1–2
Rhabdias sp. larvae	Body cavity	41	72	48	1–162
Rhabdias sp. adults	Lungs	44	77	12	1–28

animals we examined were not as heavily infected as those examined by Rankin and Hughes. Walton (op. cit.) reported *Diplostomulum* sp. from *A. jeffersonianum, A. maculatum, A. punctatum, A. tigrinum,* and larval ambystomids. Landewe (op. cit.) identified metacercaria he removed from the body cavities of *A. texanum* as *Neodiplostomum* sp. Upon examination of his material we found it to be similar to ours and to the description of *D. ambystomae* by Rankin and Hughes (op. cit.).

Gorgoderina bilobata, Brachycoelium, and *Cosmocercoides dukae* are common parasites of several species of salamanders (Rankin, 1937, J. Parasitol. 23:29–42; Dyer and Brandon, op. cit.). The small numbers of *Brachycoelium* sp. and *Cosmocercoides dukae,* the only intestinal parasites found, may be due to the early spring sampling dates. Mid-spring or summer sampling may increase the incidence of intestinal parasites. Since our only specimen of *Brachycoelium* was poorly preserved we did not attempt a specific identification.

Rhabdias sp. was the most frequently found parasite. Except for three salamanders both larvae and adults were found in the same animals. Landewe (op. cit.) found *Rhabdias* sp. in the lungs and body cavities of seven of 61 small-mouthed salamanders, three of 19 spotted salamanders, *Ambystoma maculatum,* and one of 18 marbled salamanders, *A. opacum*. The only other report of *Rhabdias* from the Ambystomoidea is that of Lehmann (1960, J. Parasitol. 46:10) who reported the lungs of one of 29 *Dicamptodon ensatus* infected.

We would like to thank Dr. R. Brandon for his help in collecting the salamanders and Mr. Ross Adams for his permission to collect on the Crab Orchard National Wildlife Refuge.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 275–276

Research Note

A Survey of the Helminth Fauna of *Cnemidophorus murinus* from the Island of Curacao

A collection of lizards identified as *Cnemidophorus murinus* Peters and Denoso-Barros, 1970, was made during the first week of July 1978 on the island of Curacao in the Netherlands Antilles by one of us (FHW). An extensive literature search revealed that only the avian fauna of this island had been previously surveyed for helminth parasites. Furthermore, no parasitic fauna had been reported from this reptilian host from any location.

Postmortem examination of 18 specimens revealed two species of intestinal parasitic nematodes. Three hosts were infected with a total of five spiruid nematodes of the genus *Physaloptera*. While only one male specimen was recovered, distinctive arrangement of male caudal papillae allows probable diagnosis of these specimens of *Physaloptera retusa* (Rudolphi, 1819). This species has been identified from a variety of North and South American speices of the genus *Cnemidophorus,* although many North American reports remain suspect (Specian, unpublished data). Total length of male (15.33 mm) and of spicules (right spicule 0.178 mm, left spicule 0.261 mm) fit into the range reported by Caballero y Caballero and Vogelsang (1947, Rev. Med. Vet. Parasitol. Maracay 6:1–10) for *P. retusa* from *Cnemidophorus lemniscatus lemniscatus* from the island of Orchilla, Venezuela. Although the remaining four specimens were immature females, they revealed cephalic characteristics consistent with the genus *Physaloptera* and specifically *P. retusa* (as described by Caballero y Caballero and Vogelsang, 1947, loc. cit.); the incomplete development of reproductive organs, however, made it impossible to discern the number or origin of the uteri.

A second nematode species was recovered from the colon of five of the 18 hosts. These oxyurid nematodes were identified as members of the genus *Pharyngodon*. Although five mature females were recovered, the absence of any male specimens precludes a specific identification. In spite of this, several species may be ruled out based upon the characteristics of the females. Total length was 4.27–5.53 mm (av. 4.90 mm). Width was 0.56–0.70 mm (av. 0.63 mm). Vulva was in posterior half of body 1.96–2.10 mm (av. 2.04 mm) from posterior end. Tail was subulate, measuring 0.315–0.340 mm (av. 0.325 mm) to anus, and lacks spines. Dimensions of thick-shelled eggs were 105–115 μm (av. 109 μm) long by 50–55 μm (av. 51 μm) wide. Following the key to the genus *Pharyngodon* presented by Specian and Ubelaker (1974, Proc. Helminthol. Soc. Wash. 41:46–51), these specimens fall into the group with a subulate tail, lacking spines. Of this group, no species has been previously reported from the region of the Caribbean Sea. Furthermore, dimensions of the eggs are much smaller than most other members of the genus.

The present report represents new host and distribution records for *Physaloptera retusa* and *Pharyngodon* sp. and as such helps extend our understanding of the geographical distribution of reptilian parasites on the islands of the Caribbean.

We wish to extend our gratitude to Douglas Arnold, School of Medicine, University of Kentucky, Lexington, for identification of lizards and to Dr. Ingvar

Kristensen, Director, Carmabi Biological Institute, for his aid in collection and preparation of lizards.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 276–277

Research Note

The Helminth Parasites of the Brown Water Snake, *Nerodia taxispilota*, from Kinchafoonee Creek, Georgia

Several workers have surveyed the helminths of the North American water snake genus *Nerodia*[1] (Gibson and Rabalais, 1973, Am. Midl. Nat. 89:239–241; Rabalais, 1969, Proc. Helminthol. Soc. Wash. 36:184–187; Anderson, 1935, Ohio J. Sci. 35:78–80). However, little work has been done on the helminths of *Nerodia taxispilota* (Holbrook). Byrd and Roudabush (1939, J. Parasitol. 25:461–473) described *Leptophyllum ovalis* from the small intestine of a captive specimen but expressed uncertainty as to whether the infection was a natural one or acquired in captivity. Collins (1969, J. Elisha Mitchell Sci. Soc. 85:141–144) reported finding nine species of helminths recovered from 16 *N. taxispilota* from North Carolina.

Nerodia taxispilota, unlike the other members of the genus, rarely feed on amphibians but prefer a diet of fishes (Camp, Sprewell, and Powders, J. Herpetol., in press; Brown, 1979, Brimleyana 1:113–124; Mushinsky and Hebrard, 1977, Herpetologica 33:162–166; Bowers, 1966, Herpetologica 22:225–229; Clark, 1949, J. Tenn. Acad. Sci. 24:244–261; Van Hyning, 1932, Copeia 1932: 37). Collins (op. cit.) suggested that *N. taxispilota* may harbor a less diverse helminth fauna than do other *Nerodia* species. This study provides additional information on those helminth species parasitic in this snake and is the first survey of the helminths of *N. taxispilota* in Georgia.

Twenty-five *N. taxispilota* were collected during May and June 1978 along Kinchafoonee Creek between Preston, Webster County, Georgia, and Albany, Dougherty County, Georgia. The stomachs of 15 other specimens were taken in April 1977. Snakes were collected for food analyses and, therefore, sacrificed in

[1] My generic usage follows that of Rossman and Eberle, 1977, Herpetologica 33:34–43.

Table 1. Helminths found in 25 *N. taxispilota* from Kinchafoonee Creek, Georgia.

Helminth	Percent infection	No./snake $\bar{x}$	No./snake Range	Location in host
Cestoda				
Proteocephalus perspicua (LaRue, 1911)	72.0	1.5	1–5	small intestine, colon
Plerocercoid	8.0	2.5	1–4	intestinal mesenteries
Trematoda				
Ochetosoma aniarum (Leidy, 1891)	4.0	1	—	esophagus
*Ochetosoma wardi** (Byrd, 1936)	16.0	14	1–30	esophagus
Ochetosoma sp.	4.0	40	—	esophagus
Pneumatophilus variabilus (Leidy, 1856)	4.0	1	—	lung
Nematoda				
Camallanus sp.*	4.0	2	—	colon
Spinitectus sp.*	4.0	1	—	colon
Spiroxys sp.	4.0	1	—	intestinal mesenteries
Terranova sp. (subgenus *Saurnema*)	2.5†	1	—	stomach
Unidentified Spiruroidea	4.0	3	—	small intestine
Unidentified larva	4.0	1	—	pericardial mesenteries

* New host record.
† Based on 40 total stomachs examined.

the field and immediately injected with 10% formalin to terminate digestion. All helminths found were stained with Semichon's acetocarmine and mounted in permount.

A total of 10 species of helminths were found in 23 of the snakes collected in 1978: one Cestoda, four Trematoda, five Nematoda (Table 1). One of the 15 stomachs collected in 1977 contained an additional nematode species.

The water snakes examined were most commonly infected with *Proteocephalus perspicua* (LaRue, 1911). *Ochetosoma aniarum* (Leidy, 1891) was seen in only one snake, possibly reflecting this species' use of amphibians as intermediate hosts (Byrd, 1935, Trans. Am. Microsc. Soc. 54:196–225; Talbot, 1933, Parasitology 25:518–545). The unidentified *Ochetosoma* species appeared very similar to *O. aniarum* but differed in measurements of the oral and ventral suckers; therefore, it was not assigned to species. *Ochetosoma wardi* (Byrd, 1936) represents a new host record for *N. taxispilota* as do *Camallanus* sp. and *Spinitectus* sp. *Spinitectus* sp. represents a new record for the genus *Nerodia*. *Terranova* sp. was reported by Collins (op. cit.) but was not identified to subgenus. The two unidentified nematodes were damaged and could not be identified with certainty. Helminth specimens were deposited in the USNM Helminthological Collection under collection number 75459–75470.

Thanks are extended to Dr. C. F. Dixon and W. A. Rogers for aid in identifying some of the helminths. I would further like to thank Dr. Rogers for critically reviewing this manuscript.

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Proc. Helminthol. Soc. Wash.
47(2), 1980, p. 278

Research Note

Helminths of Anurans from NW Wisconsin

From March through August 1979, four *Bufo americanus* Holbrook (13–15 cm in length), 412 *R. clamitans* Latreille (15–21 cm), 22 *R. pipiens* Shreber (14–23 cm), and five *R. sylvatica* LeCorte (15–21 cm) were collected from the Red Cedar River or five adjacent ponds (S. Barron Co. and N. Dunn Co.) and scrutinized for cestodes, adult trematodes, and nematodes. This is the first report of the parasites of Wisconsin anurans.

Platyhelminthes were preserved in 10% formalin and stained in Mayer's paracarmine; nematodes were preserved in steaming 70% isopropanol and examined in 1,2,3-propantriol-phenol solution.

Anurans were parasitized by each helminth species as follows (host: parasite: incidence in %): *R. clamitans: Glypthelmins quieta* (Stafford): 40.5; *Gorgoderina attenuata* Stafford: 13.8; *G. simplex* (Looss): 4.3; *Haematoloechus longiplexus* Stafford: 1.2; *H. parviplexus* (Irwin): 8.3; *H. varioplexus* Stafford: 25.2; *Haematoloechus* sp.: 0.2; *Halipegus eccentricus* Thomas: 43.2; *Loxogenes arcanum* (Nickerson): 1.9; *Megalodiscus temperatus* (Stafford): 0.4; *Mesocestoides* sp. tetrathyridia: 1.6; *Proteocephalus saphenus* (Osler) 0.4; *Cosmocercoides dukae* (Holl): 28.3; *Oswaldocruzia pipiens* Walton: 1.4; *R. pipiens: Cephalogonimus* sp.: 9.0; *G. quieta:* 22.7; *G. attenuata:* 4.5; *H. varioplexus:* 13.6; *H. eccentricus:* 4.5; *M. temperatus:* 4.5; *Mesocestoides tetrathyridia:* 4.5; *C. dukae:* 54.5; *O. pipiens:* 4.5; *Rhabdias ranae* Walton: 4.5; *R. sylvatica: H. medioplexus* (Stafford): 20; *R. ranae:* 20; *B. americanus: R. bufonis* (Schrank): 20. Only *H. eccentricus* were obtained during all months.

Haliplegus eccentricus was identified by examining snails for cercariae. Fifty-eight percent of 516 *Physa* sp. were parasitized; of 127 *Helisoma trivolvis* (Say), 13 *H. anceps* (Menke), and five *H. campanulata* (Say), none was parasitized.

Specimens as follows were deposited in the USNM Helm. Coll.: *P. saphenus* (75444); *H. parviplexus* (75445); *H. longiplexus* (75446); *H. varioplexus* (75447); *H. medioplexus* (75448); *M. temperatus* (75449); *G. quieta* (75450); *H. eccentricus* (75451); *G. attenuata* (75452); *G. simplex* (75453); *L. arcanum* (75454); *O. pipiens* (75455); *C. dukae* (75456); *R. ranae* (75457); and *R. bufonis* (75458).

Drs. W. F. Font, Jr., D. D. Wittrock, and M. J. Ulmer provided assistance.

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MINUTES

Five Hundred Twenty-Fifth Through Five Hundred Thirty-Second Meetings

525th Meeting: Animal Parasitology Institute. USDA, Beltsville, Maryland, 12 October 1979. The recipient of the 1979 Anniversary Award was Dr. Everett E. Wehr. The award was presented by Anniversary Awards Committee Chairman Dr. Sherman S. Hendrix to Dr. John S. Andrews on Dr. Wehr's behalf since Dr. Wehr was unable to attend the meeting. The retirement of Dr. Frank D. Enzie, Chairman of the Animal Parasitology Institute was announced. A moment of silence was observed in memory of Drs. Robert Rubin and James McDaniel, recently deceased. Editor of the Proceedings, Dr. James A. Haley, was recommended for a second 5-year term by the Executive Committee. The following slate of officers for the 1979–80 year was presented: J. Ralph Lichtenfels (President), Nancy D. Pacheco (Vice President), Sherman S. Hendrix (corresponding Secretary-Treasurer), Milford N. Lunde (Recording Secretary). The meeting was turned over to Dr. Harry Herlich who presided over the following program of papers: "Suggestions on the Cause of Mortality in *Eimeria tenella* Infection," Dale R. Witlock; "Development of Diamfenetide as a Controlled Release Prophylactic Fasciolicide in Sheep," Robert S. Rew; "Clinical Chemistry of Anaplasmosis," Patricia C. Allen; "A New Classification of the Ancylostomatoides" (Poster presentation), J. Ralph Lichtenfels.

526th Meeting: Naval Medical Research Institute, Bethesda, Maryland, 9 November 1979. President Fayer read a letter of thanks for the Anniversary Award from Mrs. Everett E. Wehr. Dr. Lichtenfels announced 2 new publications: a journal, "Systematic Parasitology" and a new translation of Vol. 1 "Trematodes of Animals and Man" by K. I. Skrjabin. The slate of officers presented at 525th meeting were elected. Dr. Wilton Vannier presided over the following papers: "Interaction of *Plasmodium berghei* Sporozoites with Peritoneal Macrophages and Rat Kupffer Cells," Harry D. Danforth; "Biochemistry and Immunology of Exoerythrocytic Malaria," Michael R. Hollingdale; "Hybridomas, The State of the Art," Paul A. Coulis; "Navy Medical Education at the Gorgas Memorial Laboratory," Timothy T. Palmer.

527th Meeting: Nematology Laboratory Plant Protection Institute, USDA and the Division of Veterinary Research Bureau of Veterinary Medicine, FDA Beltsville Agricultural Research Center, Beltsville, Maryland, 6 December 1979. Officers elected at the 526th meeting were installed. The following papers were presented, Drs. Raymond V. Rebois and Kendall G. Powers presiding: "Ultrastructure of a Feeding Peg and Tube Associated with *Rotylenchulus reniformis* Parasitism in Cotton," R. V. Rebois; "The Control of Nematodes on Strawberry," D. Babineau; "Adverse Reactions Associated with Diethylcarbamazine (DEC) Therapy in Dogs Infected with *Dirofilaria immitis*," E. L. Parbouni; "Gross and Microscopic Lesions Associated with Adverse Reactions to DEC Therapy in Dogs with Circulating *D. immitis* Microfilariae." The incoming President, J. R. Lichtenfels, announced the following appointments for 1980: Exec-

utive Committee, F. W. Douvres, B. J. Myers; Archivist, D. R. Lincicome; Custodian of Back Issues, E. A. Steck; Librarian, P. A. Pilitt; Asst. Corresponding Secretary-Treasurer, R. V. Rebois; Awards Committee, M. D. Ruff.

528th Meeting: National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, 11 January 1980. President J. Ralph Lichtenfels announced that the Society would celebrate its 70th anniversary in 1980 and an anniversary dinner is being planned for the occasion. Dr. R. Fayer announced that an NIH postdoctoral fellowship in immunoparasitology was available with Dr. Schad at the University of Pennsylvania School of Veterinary Medicine. A moment of silence was observed for Dr. T. W. M. Cameron who passed away on New Years Day. Dr. Franklin A. Neva presided over the scientific portion of the program. The following papers were presented: "The Influence of Chagasic Serum upon the Interaction of *Trypanosoma cruzi* with Vertebrate Cells," G. A. Schumunis; "Hybridoma Antibody Against Surface Antigen on Malaria Gametes," Joan Rener; "Acquired Resistance to the Trematode *Ribieroa marini* in the Snail *Biomphalaria glabrata*," John Sullivan; "Immune Clearance of *Dirofilaria immitis* Microfilariae in the Dog," Gary J. Weil. Following the scientific portion of the program, Dr. David Lincicome, Archivist, gave an illustrated talk entitled "Taking Stock in Ourselves," on the history of the Proceedings from Volume 1 in 1934 to the present.

529th Meeting: Department of Zoology and the Oxford Laboratory, NOAA University of Maryland, College Park, Maryland, 8 February 1980. The Secretary-Treasurer, S. S. Hendrix, reported that expenses for 1979 were down $2600 due mostly to a change in format of the Proceedings and that interest income for 1979 was up. Dr. Leo Jackowski presided over the following papers from the University of Maryland: "Phototactic and Geotactic Responses of the Marine Cercariae, *Renicola thaidus* and *Cercariae parvicaudata*, "J. S. Pondick; "Some Copepods of Sword Fishes and Sharks From the Western North Atlantic," G. W. Benz. The following papers were presented from the Oxford Laboratories, NOAA and presided over by Dr. Aaron Rosenfield: "An Ultrastructural Study of *Paramoeba perniciosa* and its Interactions with Hemocytes in the Blue Crab," J. E. Bodammer; "Common Parasitic Eukaryotes and Prokaryotes found in Molluscan Shellfish of the Chesapeake Bay," S. V. Otto.

530th Meeting: Walter Reed Army Institute of Research, Washington, D.C., 7 March 1980. It was announced that the Anniversary Dinner will be held 8 October 1980. This date is exactly 70 years from the date of the first meeting of the Society held at the Zoological Division, Bureau of Animal Industry. Maj. L. D. Hendricks presided over the following papers: "*Mansonella ozzardi* in Columbia," Lawrence Lightner; "Identification of *Leishmania* spp. Using Radiorespirometry," Joan Jackson; "Cell Surface Characteristics of *Trypanosoma rhodesiense* Antigenic Variants," Peter Jackson; "Research in African Trypanosomiasis with Emphasis on the Tsetse Fly as a Vector," John B. Gringrich.

531st Meeting: The Johns Hopkins University, Baltimore, Maryland, 11 April 1980. President Lichtenfels named L. A. Jackowski, K. D. Murrell and W. R. Nickle as members of the Committee on Honorary and Life Members. Dr. L. E. Rozeboom presided over the scientific portion of the program. The following

papers were presented; "The Epidemiology of Avian Malaria in Captive African Penguins (*Spheniscus demersus*) at the Baltimore Zoo," J. Beier; "Immunologic Study of Excretory/Secretory Products of Microfilariae of *Onchocerca volvulus*," K. Ojodu; "Choline Acetyltransferase of *Schistosoma mansoni* and Mouse Brain: Evidence for Isotopic Exchange Between Acetyl CoA and Acetylcholine in the Absence of Choline," C. Molineaux; "Mucoid Enteritis of Rabbits," B. Silverman; "A Study of the Immune Response to *Nippostrongylus brasiliensis* in Mast Cell-deficient Mice," D. A. Levy.

532nd Meeting: The University of Pennsylvania New Bolton Center, Kennett Square, Pennsylvania, 10 May 1980. Those present were reminded of the 70th anniversary dinner to be held October 8, 1980. Dr. Bernard Bezubik was elected to Honorary Membership in the Society and Dr. John S. Andrews was elected to Life Membership. Dr. Harry Herlich, representative to the American Society of Parasitologists, presented a proposed change in the ASP Constitution to allow ASP council members to screen names of candidates for office proposed by the nominating committee. Members of the ASP present voted against the proposed change. The following papers were presented, presided over by Dr. Gerhard A. Schad: "Genetic Control of Acquired Resistance in Murine Cutaneous Leishmaniasis," Louis DeTolla; "Immune Cell Interactions in Cutaneous Leishmaniasis in Mice," Phillip Scott; "Antibody Formation in Hamsters Infected with *Leishmania donovani*," Sergio Levy; "*Litomosoides carinii*: Comparisons of Microfilaremias of *Mastomys natalensis* with Old Infections, New Infections and Transfused Microfilariae After Treatment with Diethylcarbamazine," David J. Weiner; "Immunoregulatory Mechanisms in Schistosomiasis," Barbara Doughty.

The following new members were elected at the meetings indicated: *525th*: G. P. Agarwal, Paul C. Beaver, John C. Beier, David G. Casdorf, Kathleen B. Coy, Harry D. Danforth, Robert D'Antonio, Russell E. Ingham, Susan Kuntz, Kenneth L. Tiekotter, Paul K. Wellner. *526th*: Eugene M. Burreson, Gerald W. Esch. *528th*: John W. Crane, Willard O. Granath, Jr., Michael R. Hollingdale, Gilbert M. Myers. *529th*: Thomas McDonald. (Reinstatement of lapsed membership; Bilgees Mujib.) *530th*: Stephen T. Jaronski. *531st*: William J. Bacho, Jr., George W. Benz, Joseph W. Camp, Dean R. Furbish, Robert L. Price. *532nd*: David Blair, Paul A. Catalano, Eric P. Hoberg, and James A. Sauders.

MILFORD N. LUNDE
Recording Secretary

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Date of publication, 25 August 1980

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PRINTED BY ALLEN PRESS, INC., LAWRENCE, KANSAS 66044, U.S.A.