Volume 64 January 1997 Number 1

JOURNAL

of

The Helminthological Society of Washington

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

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

CONTENTS

(Continued on Outside Back Cover)

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE SOCIETY meets in October, November, February, April and May for the presentation and discussion of papers in any and all branches of parasitology or related sciences. All interested persons are invited to attend.

Persons interested in membership in the Helminthological Society of Washington may obtain application blanks in recent issues of THE JOURNAL. A year's subscription to the Journal is included in the annual dues of $25.00 domestic and $28.00 foreign.

OFFICERS OF THE SOCIETY FOR 1997

President: ELLEN ANDERSEN
Vice President: ERIC P. HOBERG
Corresponding Secretary-Treasurer: HARLEY G. SHEFFIELD
Recording Secretary: W. PATRICK CARNEY
Archivist/Librarian: PATRICIA A. PILITT
Custodian of Back Issues: J. RALPH LICHTENFELS
Representative to the American Society of Parasitologists: ERIC P. HOBERG
Executive Committee Members-at-Large: WILLIAM P. WERGIN, 1997
WILLIS A. REID, JR., 1997
KEVIN BAIRD, 1998
JOHN H. CROSS, 1998
Immediate Past President: SUSAN FRICKE-MEYER

THE JOURNAL OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE JOURNAL is published semiannually at Lawrence, Kansas by the Helminthological Society of Washington. Papers need not be presented at a meeting to be published in the Journal.

MANUSCRIPTS should be sent to the EDITOR, Sherman S. Hendrix, Department of Biology, Gettysburg College, Gettysburg, PA 17325. email: shendrix@cc.gettysburg.edu. Manuscripts must be typewritten, double spaced, and in finished form. Consult recent issues of the Journal for format and style. The original and two copies are required. Photocopies of drawings may be submitted for review purposes but glossy prints of halftones are required; originals will be requested after acceptance of the manuscript. Papers are accepted with the understanding that they will be published only in the Journal.

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

AUTHORS' CONTRIBUTIONS to publication costs (currently $50/pg for members, $100 for non-members) will be billed by Allen Press and are payable to the SOCIETY.

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

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

ISSN 1049-233X

♾ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 1–8

Digenetic Trematodes of Marine Fishes from the Kuwaiti Coast of the Arabian Gulf: Family Monorchiidae Odhner, 1911

O. SEY[1] AND F. M. NAHHAS[2,3]

[1] Department of Zoology, Faculty of Science, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait and
[2] Department of Biological Sciences, University of the Pacific, Stockton, California 95211

ABSTRACT: Two species of monorchiids are described from marine fishes of the Kuwaiti coast of the Arabian Gulf: *Proctotrematoides kuwaiti* sp. n. from *Synaptura orientalis* and *Pseudorhombus arsius* differs from all others in the genus by the absence of a prepharynx and esophagus and by the arrangement and position of the vitelline follicles. *Opisthodiplomonorchis elongatus* Madhavi, 1974, is reported from *Polydactylus sextarius* and *Plectorhynchus* sp., both new locality records and the latter a new host record. *Opisthodiplomonorchis* differs from all monorchiid genera with diagonal or tandem testes by the following combination of characteristics: vitellaria in 2 lateral groups of pre- and postovarian follicles, multilobed ovary, tandem testes at the posterior extremity, and unipartite seminal vesicle and terminal organ. Keys to the species of *Proctotrematoides* and monorchiids with 2 testes are included. *Pseudomonorcheides* Zhukov, 1983, nec *Pseudomonorcheides* Wang, 1982, is renamed *Zhukovtrema.*

KEY WORDS: digenetic trematodes, Monorchiidae, *Proctotrematoides, Opisthodiplomonorchis, Zhukovtrema,* marine fishes, *Synaptura orientalis, Pseudorhombus arsius, Polydactylus sextarius, Plectorhynchus* sp., Arabian Gulf, Kuwait.

During the course of a survey of helminth parasites of Kuwaiti coast fishes carried out by the first author between October 1992 and December 1995, a collection of digenetic trematodes was obtained that included several species of monorchiids, 2 of which are described in this paper. Four previous reports on adult digenea from the Kuwaiti coast have been published (see Al-Yamani and Nahhas, 1981; Abdul-Salam and Khalil, 1987; Abdul-Salam et al., 1990; Abdul-Salam and Sreelatha, 1993). No monorchiids were reported in any of these studies. Monorchiids have been recorded, however, from fishes of other parts of the Arabian Gulf. Saoud et al. (1986, 1988) listed 3 species from Qatari and adjacent waters: *Monorcheides* sp. from *Gnathodon speciosus, Proctotrema* sp. from *Liza macrolepis* and *Velamugil seheli*, and *Paraproctotrema qatarensis* from *Plectorhynchus pictus*; El-Naffar et al. (1992) listed *Lasiotocus* sp. from *Plectorhynchus cinctus* from the United Arab Emirates.

Materials and Methods

Ten oriental soles, *Synaptura orientalis* (Bloch and Schneider, 1801) (family Soleidae), 33 large-toothed flounders, *Pseudorhombus arsius* (Hamilton and Buchanan, 1822) (family Bothidae), 4 6-threads threadfins, *Polydactylus sextarius* (Bloch and Schneider, 1801) (Polynemidae), and 4 of an unidentified grunt, *Plectorhynchus* sp. (family Pomadasydae), obtained from the local fish market, were examined and found to harbor monorchiids. The digeneans were washed in saline, fixed in cold AFA under slight coverglass pressure, rinsed in 70% ethanol, stained with alum carmine, destained in diluted HCl, dehydrated in ascending concentrations of ethanol, cleared in clove oil, and mounted in Canada balsam.

All measurements are expressed in micrometers, with the range followed by measurements of the holotype in parentheses. Sucker ratio was calculated from the mean of the length and the width and is expressed with the oral sucker taken as 1. Drawings of the adult worms were prepared by microprojection and details filled in through microscopic observations; those of the terminal reproductive structures are free-hand sketches. Prevalence, mean intensity, abundance, and collection dates are listed in Table 1.

The holotype is deposited in the National Reference Collection (NRC), Department of Zoology, Kuwait University, with vouchers in the United States National Parasite Collection (USNPC), Beltsville, Maryland, and the Natural History Museum BM(NH), London. Fishes were identified using Kuronuma and Abe (1972).

Results

Proctotrematoides kuwaiti sp. n. (Figs. 1, 2)

DESCRIPTION (based on 15 gravid and 2 immature specimens): Body 1,350–2,100 (2,045) long by 425–525 (523) wide at acetabular level, rounded at both ends. Cuticle spinose, spines extending to level of posterior margin of ventral

[3] Corresponding author.

Table 1. Monorchiids found in 4 species of marine fish from Kuwait.

Host Monorchiid	% prevalence	Mean intensity	Abundance	Collection dates
Synaptura orientalis				
Proctotrematoides kuwaiti	30	10.0	3.00	5 February 1994 3 March 1995 28 March 1995
Pseudorhombus arsius				
Proctotrematoides kuwaiti	9	4.6	0.40	26 April 1995 18 October 1995 10 November 1995
Polydactylus sextarius				
Opisthodiplomonorchis elongatus	50	4.5	2.20	15 October 1993 5 October 1995
Plectorhynchus sp.				
Opisthodiplomonorchis elongatus	25	3.5	0.75	29 July 1993

sucker becoming sparse posteriorly. Eye spot pigments lateral to pharynx, often diffuse, difficult to observe in some specimens. Oral sucker cup-shaped, subterminal, 150–205 (200) long by 150–250 (220) wide, with weakly developed postoral circular muscle. Ventral sucker globular 125–200 (165) in diameter, slightly anterior to midbody. Sucker ratio 1:0.80–1.00 (1:0.82). Prepharynx absent; pharynx transversely elongate, 80–150 (135) long by 125–200 (170) wide; esophagus absent; cecal bifurcation 300–350 (318) anterior to ventral sucker; ceca wide, thick-walled, extending to near posterior extremity. Testis single, smooth, 180–340 (340) long by 200–325 (318) wide, median, equatorial or slightly postequatorial. Cirrus sac thick-walled, 275–450 (450) by 60–170 (170) at base, dextral, extending posteriorly to near midovarian level or ovario-testicular junction, containing internal spherical seminal vesicle 125–138 (138) in diameter, short prostatic duct and long spiny cirrus, 110–250 (205) long by 25–35 (30) wide, spines measuring 10–15 in length; prostate cells numerous, surrounding part of anterior region of seminal vesicle, all of prostatic duct and part of cirrus. Ovary smooth, 100–148 (148) in diameter, dextral, submedian, sometimes overlapping right cecum and often contiguous with anterior level of testis. Vitellaria 8–10 follicles (8 on right, 10 on left), relatively large, mostly extracecal, extending in 2 longitudinal columns from near level of intestinal bifurcation to anterior level of ovary; vitelline ducts entering vitelline reservoir dorsally at ovario-testicular junction. Seminal receptacle absent; proximal part of uterus serving as long, often sinuous, uterine seminal receptacle; Laurer's canal not seen; uterine coils extending posteriorly filling practically all posttesticular space, overlapping ceca laterally, entering bipartite terminal organ near junction of its anterior and posterior parts. Terminal organ thick-walled, sinistral, intercecal, one-half to two-thirds length of cirrus sac; its posterior part containing spherical vesicle, same size as or slightly smaller than seminal vesicle, with long needle-like spines and gland cells; its anterior part containing fewer and smaller spines. Genital atrium consisting of thick spiny posterior part, into which metraterm and cirrus open, and anterior shallow thin-walled part; genital pore median to submedian, about midway between ventral sucker and intestinal bifurcation. Eggs numerous, operculated, 25–30 by 16–20; eggs not seen in posterior part of terminal organ. Excretory vesicle slender, thin-walled, extending to near intestinal bifurcation.

HOSTS: *Synaptura orientalis* (Bloch and Schneider) (Soleidae) (type host); *Pseudorhombus arsius* (Hamilton and Buchanan) (Bothidae).

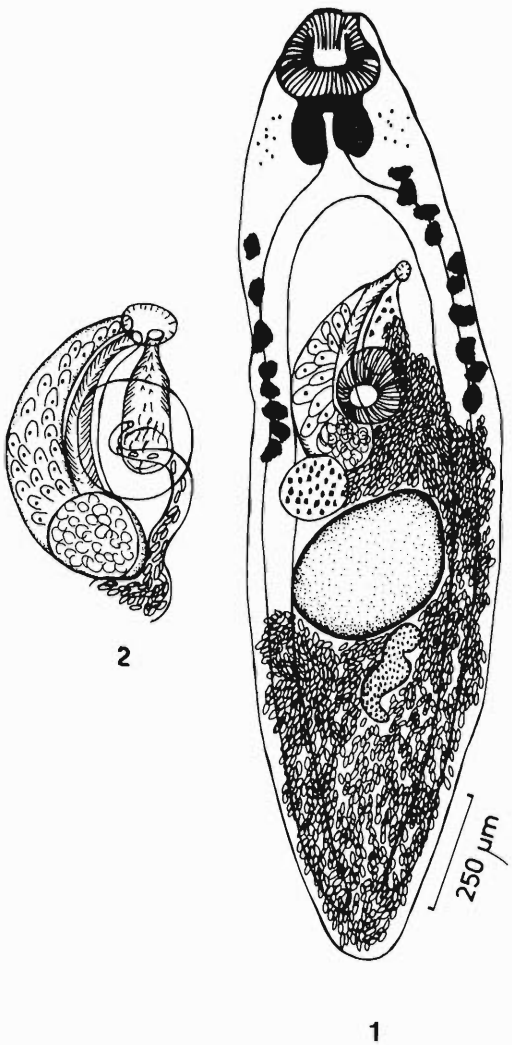
SITE: Intestine.

HOLOTYPE: NRC No. 14 (Kuwait University).

PARATYPES: USNPC No. 86780; BM(NH) No. 1996.7.26.1.

ETYMOLOGY: The species is named after the State of Kuwait.

REMARKS: These specimens were referred to the genus *Proctotrematoides* on the basis of the



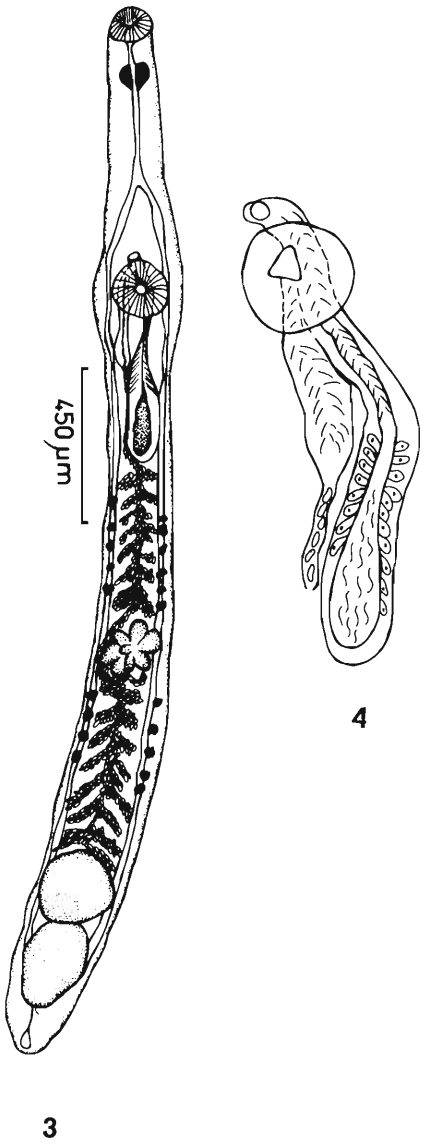
Figures 1, 2. *Proctotrematoides kuwaiti* sp. n. from *Synaptura orientalis*. 1. Holotype, ventral view. 2. Terminal parts of male and female reproductive structures, sketch.

terminal parts of the male and female reproductive organs including spines in the cirrus, metraterm and genital atrium, a spherical ovary, distribution of the vitellaria, and a long excretory vesicle that extends to near the intestinal bifurcation. This triple spination of the terminal reproductive structures is also characteristic of the genus *Genolopa* Linton, 1910. *Proctotrematoides* was named by Yamaguti (1938) for *P. pisodontophidis* to describe several specimens recovered from the intestine of *Pisodontophis cancrivora* from the Inland Sea of Japan. He distinguished *Proctotrematoides* from *Proctotrema* and *Paraproctotrema* by the character of the ovary (entire), the vitellaria (follicular, extending from the anterior level of the ventral sucker to the level of posterior end of testis), the possession of a muscular spiny atrial pouch, bipartite terminal organ with the uterus entering it at its spiny anterior part, and a long tubular excretory vesicle extending to near intestinal bifurcation. Manter (1942), characterizing *Genolopa* as having spines in the cirrus, also the anterior part of the terminal organ and the genital atrium, considered *Proctotrematoides* a synonym. Thomas (1959) accepted *Proctotrematoides* on the basis of an atrial diverticle and presence of a long tubular excretory vesicle. Yamaguti (1971), characterizing the 2 genera, incorrectly stated that a seminal receptacle is present in *Genolopa* but absent in *Proctotrematoides*. Such a structure is lacking in both. In their review of the family Monorchiidae, Manter and Pritchard (1961) accepted, with reluctance, Thomas's recommendation pointing to the probable variability of an atrial diverticle. We agree with Manter and Pritchard that this structure is unreliable as a generic characteristic; and atrial diverticle was difficult to observe in a few of our specimens. It was equally difficult, sometimes, to determine whether spines in the genital atrium were those of the atrium itself or the extended spiny cirrus.

When the type species of *Genolopa* and *Proctotrematoides* are considered, the chief differences between them are the distribution of the vitellaria and the length of the excretory vesicle. In *Genolopa*, the vitellaria consist of few follicles, usually in 2 clusters, 1 on each side near the ovarian zone, and the excretory vesicle is a short sac-like structure restricted to the posterior end of the body. In *Proctotrematoides*, the vitelline follicles extend longitudinally in lateral fields between the ventral sucker and the testes, and the excretory vesicle is a long tube extending to near the intestinal bifurcation. Both genera share the common characteristics of a cirrus sac containing a unipartite seminal vesicle, a distinct prostatic duct, a spiny cirrus, and a bipartite terminal organ that is entered by the uterus at some point between its spiny anterior portion and its aspinose posterior part. Not all species included in the 2 genera meet these criteria. Equally confusing is the relationship of *Proctotrematoides* and *Genolopa* to *Proctotrema, Paraproctotrema*, and *Lasiotocus* (see Thomas,

1959; Manter and Pritchard, 1961; Durio and Manter, 1968; Yamaguti, 1971). More than 60 species have been described in the 5 genera; many show overlapping generic characteristics, and several lack information essential for generic characterization. Some of the species in *Proctotrematoides* are also an example of this confusion, as evident in the discussion that follows.

In addition to the type species *P. pisodontophidis* Yamaguti, 1938, 7 others have been referred to this genus: *P. ophichthi* Fischthal and Thomas, 1969, from *Ophichthus* (*Pisodontophis*) *semicinctus* (Ophichthyidae) from Ghana; *P. stromateusi* Gupta and Ahmad, 1976, from *Stromateus cinereus* (Stromateidae) from the Puri Coast, Orissa, Bay of Bengal; *P. diacanthi* Zaidi and Khan, 1977, from *Epinephelus diacanthus* (Serranidae) from the Arabian Sea; *P. thapari* Ahmad, 1980, from *Stromateus cinereus* from the Arabian Sea, off the Bombay coast; *P. indicum* Ahmad and Gupta, 1985, from *S. cinereus* from the Puri coast, Orissa, Bay of Bengal; *P. gymnothoraci* Shen, 1990, from *Gymnothorax* sp. (Muraeinidae) from Hainan Island, China; and the new species *P. kuwaiti* from *Synaptura orientalis* (Soleidae) and *Pseudorhombus arsius* (Bothidae) from the Kuwaiti coast of the Arabian Gulf. *Proctotrematoides stromateusi* and *P. thapari* are mentioned in abstracts (see Gupta and Ahmad, 1976; Ahmad, 1980) but were never followed by a complete description, at least under these names. *Proctotrematoides stromateusi* was recovered from the same host species and locality as *P. indicum* Ahmad and Gupta, 1985, and is probably a synonym. *Proctotrematoides indicum* has a 3-lobed ovary and its excretory vesicle extends only to the anterior level of the testis but shares all other characteristics of *Proctotrematoides*. *Proctotrematoides diacanthi* Zaidi and Khan, 1977 is inadequately described and its figure does not show clearly the male and female terminal reproductive structures; it shows, however, a long tubular excretory vesicle and vitelline distribution characteristic of *Proctotrematoides*. For the time being, these species are retained in *Proctotrematoides* until the original material or new specimens are studied and the taxonomic problems associated with the 5 genera are resolved. We have been unable to obtain the literature on *P. gymnothoracis* Shen, 1990, but an anonymous reviewer of this manuscript suggested it was probably incorrectly assigned to the genus.



Figures 3, 4. ***Opisthodiplomonorchis elongatus*** **Madhavi, 1974, from** ***Polydactylus sextarius*****. 1. Dorsal view. 2. Terminal parts of male and female reproductive structures, sketch.**

Based on a review of the literature, a key to 5 species of *Proctotrematoides* is proposed.

Opisthodiplomonorchis elongatus Madhavi, 1974 (Figs. 3, 4)

REDESCRIPTION (based on 12 specimens): Body elongated, 1,910–3,650 long by 180–250

in greatest width at level midway between ovary and anterior testis. Forebody 410–780; hindbody 1,400–2,275. Cuticle spinose, spines extending to level of ovary becoming sparse posteriorly. Eye spot pigments absent. Oral sucker terminal, 55–100 long by 67–110 wide. Ventral sucker spherical, 100–180 in diameter, near junction of anterior and midbody thirds. Sucker ratio 1:1.3–1.6. Prepharynx about same length as pharynx; pharynx 40–58 long by 40–63 wide; esophagus 4–5.5 times the length of pharynx; cecal bifurcation about two-thirds distance from pharynx to ventral sucker; ceca narrow, extending to posterior end of posterior testis. Testes smooth or slightly irregular, subequal, tandem, contiguous, in posterior fifth of body; anterior testis 120–210 long by 130–220 wide; posterior testis 150–240 long by 80–180 wide. Cirrus sac dextral, 440–675 long by 53–90 in greatest width, extending posteriorly almost half-way between ventral sucker and ovary, containing ovoid seminal vesicle in posterior third of cirrus, prostatic duct in midthird, surrounded by prostate cells, and spiny cirrus in anterior third, spines 7–12 in length. Ovary consisting of 7–10 lobes, 135–230 long by 135–250 wide in posterior half of body about midway between posterior end of cirrus sac and anterior testis. Seminal receptacle lacking. Laurer's canal not seen. Uterine coils winding from side to side in space between anterior testis and ovary, and between ovary and posterior tip of cirrus sac, joining spiny terminal organ at its posterior end; terminal organ unipartite, spiny, spines 7–10 in length. Vitelline follicles in 2 lateral groups of 4–6 follicles each, anterior and posterior to ovary; in a few specimens 1 or 2 follicles are seen lateral to the ovary. Genital atrium shallow, aspinose, median, anterior to ventral sucker; genital pore immediately preacetabular, median or slightly submedian. Eggs operculated, without filament, 12–17 by 8–13. Excretory vesicle tubular extending anteriorly to midlevel of ventral sucker.

HOSTS: *Polydactylus sextarius* (Bloch and Schneider) (Polynemidae); *Plectorhynchus* sp. (Pomadasydae).

SITE: Intestine.

DEPOSITED SPECIMEN: NRC No. 15 (Kuwait University); USNPC No. 86781; BM(NH) No. 1996.7.26.2.

REMARKS: Madhavi (1974) described this species from the intestine of *Psettodes erumei* (Bloch) (Psettodidae) (type host) and *Polynemus* (*Polydactylus*) *sextarius* Bloch (Polynemidae) from the Waltair coast, Bay of Bengal. Our redescription adds little to Madhavi's original account. Our specimens are somewhat smaller and narrower (1,910–3,650 by 180–250 compared to 3,340–4,800 by 314–320); all other measurements overlap. She described the ovary as multilobed; ours have 7–10 lobes. Madhavi did not describe the excretory vesicle; our specimens show a tubular structure extending anteriorly to midlevel of the ventral sucker.

Twenty-two genera of monorchiids with 2 testes are known to date: 8 with symmetrical testes (*Monorcheides* Odhner, 1905; *Paramonorcheides* Yamaguti, 1938; *Diplomonorchis* Hopkins, 1941; *Paleorchis* Szidat, 1943; *Diplomonorcheides* Thomas, 1959; *Hysterorchis* Durio and Manter, 1968; *Pseudomonorcheides* Wang, 1982, nec *Pseudomonorcheides* Zhukov, 1983; and *Pseudomonorcheides* Zhukov, 1983, nec *Pseudomonorcheides* Wang, 1982) and 14 with diagonal or tandem testes (*Ancylocoelium* Nicoll, 1912; *Physochoerus* (Rud., 1819) Poche, 1926; *Triganodistomum* Simer, 1926; *Postmonorcheides* Szidat, 1950; *Diplolasiotocus* Yamaguti, 1952; *Cestrahelmins* Fischthal, 1957; *Diplohurleytrema* Nahhas and Cable, 1964; *Timonia* Bartoli and Prévot, 1966; *Paratimonia* Prévot and Bartoli, 1967; *Pseudopaleorchis* Kamegai, 1970; *Neopaleorchis* Schell, 1973; *Opisthodiplomonorchis* Madhavi, 1974; *Anapaleorchis* Fujio and Kifune, 1991; and *Neolasiotocus* Ahmad, 1991).

Thomas (1959) included *Achoerus* Wlasenko, 1931, undoubtedly by mistake, in this group. Szidat (1950) considered *Monorcheides* and *Paramonorcheides* synonyms. Manter and Pritchard (1961, p. 483) regarded *Triganodistomum* as "a close relative, if not a synonym of *Lissorchis* Magath, 1916 (Family Lissorchiidae)." Overstreet (1969) synonymized *Diplomonorcheides* with *Diplomonorchis*. *Pseudomonorcheides* Zhukov, 1983, is preoccupied; therefore, a new name, *Zhukovtrema,* is proposed.

Based on a review of the literature, a key is presented to distinguish among 21 of the 22 genera (*Physochoerus* is excluded because of limited and inadequate information).

Zhukov (1983) gave the following diagnosis of his genus (translated from Russian).

Zhukovtrema gen. n.

GENERIC DIAGNOSIS: Monorchiidae, Monorchiinae. Body ovoid. Cuticle spinose. Oral suck-

er subterminal. Prepharynx absent or very short; pharynx muscular; esophagus short, bifurcating at the junction of anterior and midbody third; ceca not reaching posterior end of body. Ventral sucker posterior to midbody. Testes 2, elongated, symmetrical, mostly in posterior half of body. Cirrus sac with internal seminal vesicle and spiny cirrus, anterolateral and dextral to acetabulum. Ovary 3-lobed, anterior to right testis; seminal receptacle absent; terminal organ (Looss organ) bipartite, anterior part spiny, posterior part muscular; uterus extensive, coils extending posteriorly and occupying space between testes and anteriorly on both sides surrounding oral sucker and pharynx; Vitellaria in 2 symmetrical groups of 6–8 large follicles each in midbody at the level of the gonads and overlapping ceca. Genital pore about midway between ventral sucker and intestinal bifurcation. Eggs small. Excretory vesicle (?); uterus joining terminal organ (?). Parasite of marine fish.

SYNONYM: *Pseudomonorcheides* Zhukov, 1983, nec *Pseudomonorcheides* Wang, 1982. Type species *Z. caballeroi* (Zhukov, 1983) in *Syacium* sp., Bay of Campeche, Gulf of Mexico.

Key to Species of *Proctotrematoides*

1a. Esophagus at least twice the length of the pharynx 2
1b. Esophagus shorter than pharynx or absent 3
2a. Ovary entire; seminal vesicle small, spherical, occupying base of cirrus sac ..... *P. pisodontophidis*
2b. Ovary trilobed; seminal vesicle cylindrical occupying three-fourths the length of cirrus sac ..... *P. indicum*
3a. Vitelline follicles relatively small and numerous, extending from level of posterior end of cirrus sac some distance posterior to testis ..... *P. ophichthi*
3b. Vitelline follicles 8–10 on each side, extending from anterior level of acetabulum to gonads 4
4a. Prepharynx as long as pharynx; esophagus absent; vitelline follicles extending from anterior level of genital atrium to testicular level ..... *P. diacanthi*
4b. Prepharynx and esophagus absent; vitelline follicles extending from level of intestinal bifurcation to ovario-testicular level ..... *P. kuwaiti*

Key to Genera of Monorchiidae with Two Testes

1a. Genital pore marginal or submarginal 2
1b. Genital pore median or submedian 6
2a. Testes symmetrical or subsymmetrical; ovary entire ..... *Paleorchis*
2b. Testes diagonal or tandem; ovary entire or lobed 3
3a. Ovary entire; esophagus 40–60% of body length, with cuticular lining anteriorly, epithelial posteriorly; ceca not extending posterior to ovary ..... *Pseudopaleorchis*
3b. Ovary lobed; esophagus short or moderately long, without cuticular lining; ceca extending beyond ovary 4
4a. Ceca reaching to near posterior extremity; vitellaria extending laterally from level of cirrus sac to level of posterior testis ..... *Triganodistomum*
4b. Ceca extending to anterior level of posterior testis; vitellaria not extensive 5
5a. Vitellaria in 2 lateral compact clusters in the acetabulo-ovarian zone ..... *Anapaleorchis*
5b. Vitellaria mainly postcecal, confluent dorsal to testes ..... *Neopaleorchis*
6a. Testes symmetrical or subsymmetrical 7
6b. Testes tandem or oblique 13
7a. Ovary entire; terminal organ bipartite, with a sphincter near its anterior end, joined by uterus just posterior to sphincter; cirrus sac chiefly postacetabular ..... *Diplomonorcheides*
7b. Ovary lobed, rarely entire; terminal organ unipartite or bipartite; cirrus sac variable in extent in relation to ventral sucker 8
8a. Body usually elongate 9
8b. Body ovoid or pyriform 10
9a. Testes elongate, near posterior end of body; seminal vesicle bipartite, terminal organ cylindrical, unipartite, unspined, joined by uterus at its posterior end; vitellaria extensive, extending from midesophageal level to anterior level of testes; eggs with filament ..... *Hysterorchis*
9b. Testes elongate, chiefly in midbody third; seminal vesicle saccular; terminal organ with a sphincter, joined by uterus at its anterior end just below sphincter; vitellaria in lateral fields extending from intestinal bifurcation to near anterior level of testes; eggs without filament ..... *Paramonorcheides*
10a. Ceca short not reaching testicular level; testes spherical, in midbody third; vitellaria in 2 lateral clusters of 6–7 follicles each in pharyngeal region ..... *Pseudomonorcheides*
10b. Ceca extending posteriorly to testes or beyond; vitellaria not reaching pharynx 11
11a. Body ovoid, almost spherical; ventral sucker at or slightly posterior to midbody; uterus extending anteriorly and laterally to oral sucker ..... *Zhukovtrema*
11b. Body pyriform; ventral sucker anterior to midbody; uterus not extending to oral sucker 12
12a. Testes elongate, chiefly in posterior body third; vitellaria in 2 clusters of few follicles each between cecal bifurcation and anterior level of ovary; terminal organ unipartite, spiny, joined by uterus at its posterior end ..... *Monorcheides*

12b. Testes ovoid, near midbody; vitelline follicles chiefly in gonadal zone ..... *Diplomonorchis*
13a. Ceca short, not reaching ventral sucker, M-shaped or inverted V ..... *Ancylocoelium*
13b. Ceca long, extending posterior to ventral sucker 14
14a. Eggs with filament; seminal vesicle bipartite; terminal organ unipartite 15
14b. Eggs without filament; seminal vesicle bipartite or unipartite; terminal organ bipartite or unipartite 16
15a. Esophagus 2–4 times length of pharynx; ventral sucker at junction of anterior and midbody thirds; seminal receptacle present ..... *Diplohurleytrema*
15b. Espophagus 8–10 times length of pharynx; ventral sucker in midbody; no seminal receptacle ..... *Diplolasiotiocus*
16a. Testes tandem 17
16b. Testes diagonal 18
17a. Testes at posterior extremity; terminal organ spiny, unipartite, joined by uterus at its base; vitellaria in 2 lateral groups anterior and posterior to multilobed ovary ..... *Opisthodiplomonorchis*
17b. Testes removed from posterior extremity by some distance; terminal organ bipartite, joined by uterus at junction of aspinose posterior part and anterior spiny metraterm ..... *Neolasitocus*
18a. Terminal organ unipartite 19
18b. Terminal organ bipartite 20
19a. Body ovoid to linguiform; esophagus short or absent; testes juxtaposed, overlapping in median line, near posterior end of body; uterus not extending posterior to testes ..... *Postmonorcheides*
19b. Body enlarged anteriorly, spoon-shaped; esophagus very long, almost 30% of body length; testes contiguous but not overlapping, in midbody third; uterus extending to posterior end of body ..... *Cestrahelmins*
20a. Ovary trilobed; vitellaria as 2 compact, intercecal masses, one anterior to ovary, the other to anterior testis ..... *Paratimonia*
20b. Ovary entire; vitellaria in 2 clusters of 9 follicles each, overlapping ceca and extending between posterior end of cirrus sac and level of anterior testis ..... *Timonia*

Acknowledgments

The authors thank Mrs. Sharon Jaman of the Department of Zoology, Kuwait University, Miss Shaza Mardini and Mr. Boun Rasaphangthong of the Department of Biological Sciences, University of the Pacific, for their technical assistance.

Literature Cited

Abdul-Salam, J., and L. F. Khalil. 1987. Two new digeneans from the needlefish *Ablennes hians* in Kuwait and the description of a new genus and species *Neohaplosplanchnus ablennis* (Haplosplanchnidae). Systematic Parasitology 10:149–158.

———, **and B. Sreelatha.** 1993. A survey of didymozoid trematodes of the barracuda *Sphyraena obtusata* from Kuwait Bay. International Journal of Parasitology 23:665–669.

———, ———, **and M. Farah.** 1990. *Gonapodasmius epinepheli* n.sp. (Didymozoidae) from the grouper *Epinephelus tauvina* from the Arabian Gulf. Systematic Parasitology 17:67–74.

Ahmad, J. 1980. A new species of the genus *Proctotrematoides* (Digenea: Monorchiidae) from the small intestine of a marine fish, *Stromateus cinereus* from the Arabian Sea, off the Bombay coast. Indian Journal of Parasitology 3(supplement, Abstracts of the Third National Congress of Parasitology):10–11.

Al-Yamani, F. Y., and F. M. Nahhas. 1981. Digenetic trematodes of marine fishes from the Kuwaiti coast of the Arabian Gulf. Kuwaiti Institute for Scientific Research Serial, Kuwait Bulletin of Marine Science (3):1–22.

Durio, W. O., and H. W. Manter. 1968. Some digenetic trematodes of marine fishes of New Caledonia. Part I. Bucephalidae, Monorchiidae, and some smaller families. Proceedings Helminthological Society of Washington 35:143–153.

El-Naffar, M. K. I., A. Gobashy, S. G. El-Etreby, and M. M. Kardousha. 1992. General survey of helminth parasite genera of Arabian Gulf fishes (coast of United Arab Emirates). Arab Gulf Journal Scientific Research 10:99–110.

Gupta, V., and J. Ahmad. 1976. On a new trematode, *Proctotrematoides stromateusi* n.sp. from the intestine of a marine fish, *Stromateus cinereus* (Bleeker) from Puri, Orissa. Proceedings of the Indian Science Congress (Waltair) 63:205–206.

Kuronoma, K., and Y. Abe. 1972. Fishes of Kuwait. Kuwait Institute for Scientific Research, Kuwait. 123 pp.

Madhavi, R. 1974. Digenetic trematodes from marine fishes of Waltair coast, Bay of Bengal. Family Monorchiidae. Rivista di Parassitiologia 35:87–98.

Manter, H. W. 1942. Monorchiidae (Trematoda) from fishes of Tortugas, Florida. Transactions of the American Microscopical Society 61:349–360.

———, **and M. H. Pritchard.** 1961. Studies on digenetic trematodes of Hawaiian fishes: Families Monorchiidae and Haploporidae. Journal of Parasitology 51:16–20.

Overstreet, R. M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. Tulane Studies in Zoology and Botany 15:119–176.

Saoud, M. F. A., M. M. Ramadan, and K. S. R. Al-Kawari. 1986. Helminth parasites of fishes from the Arabian Gulf. I. Preliminary general survey of fishes mainly from Qatari waters. (Families and genera designation.) Qatar University Science Bulletin 6:199–229.

———, ———, **and** ———. 1988. Helminth parasites of fishes from the Arabian Gulf. VI. On three species of digenetic trematodes: *Paraproctotrema*

qatarensis n.sp. and *Prosorchis breviformis* Srivastava, 1936. Rivista di Parassitologia 5:79–85.

Szidat, L. 1950. Los parasitos del robalo (*Elginops maclovinus* Cuv. and Val.) Trab. 1 Cong. Nac. Pesquerias Maritimas (Mar del Plata) 2:235–270.

Thomas, J. D. 1959. Trematodes of Ghanian sub-littoral fishes. I. The family Monorchiidae. Journal of Parasitology 45:95–113.

Yamaguti, S. 1938. Studies on helminth fauna of Japan. 24. Trematodes of fishes, V. Japanese Journal of Zoology 8:15–74.

———. 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vols. I and II. Keigaku Publishing, Tokyo. 1,074 pp.

Zhukov, E. V. 1983. New representatives of the fauna of trematodes from the fishes of the Gulf of Mexico. Parazitologia 17:112–117.

Editor's Correction
Ransom Fund Support

Acknowledgment of the Brayton H. Ransom Fund page charge support was inadvertently omitted from the article on Neotropical Monogenoidea by D. C. Kritsky, W. A. Boeger, and M. Jégu on pages 153–175 in the July, 1996 issue of the *Journal*. Further, I would like to acknowledge the support of page charges that the Ransom Fund has provided to authors in the past.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 9–16

Description of *Heteroplectanum oliveri* sp. n. (Monogenea: Diplectanidae) and Comments on the Helminth Fauna of *Kyphosus elegans* (Perciformes: Kyphosidae) from Chamela Bay, México

VIRGINIA LEÓN-RÈGAGNON,[1] GERARDO PÉREZ-PONCE DE LEÓN, AND LUIS GARCÍA-PRIETO
Laboratorio de Helmintología, Instituto de Biología, UNAM, Ap. Postal 70-153,
C.P. 04510 Del. Coyoacán México D.F., México

ABSTRACT: *Heteroplectanum oliveri* sp. n. (Monogenea: Monopisthocotylea: Diplectanidae) is described from *Kyphosus elegans* Peters, 1869 (Perciformes: Kyphosidae) in Chamela Bay, Jalisco, México. It differs from other species of the genus in the structure of the cirrus complex, in having a spined cirrus and a strongly sclerotized genital atrium. Eight additional helminth species are reported in *K. elegans*: *Heteroplectanum nenue* (Yamaguti, 1968) Rakotofiringa, Oliver and Lambert, 1987, *H. kyphosi* (Yamaguti, 1968) Oliver, 1987, *Neobivagina aniversaria* Bravo-Hollis, 1979, *Deontacylix ovalis* Linton, 1910, *Opisthadena dimidia* Linton, 1910, *Jeancadenatia dohenyi* Winter, 1956, *Filisoma bucerium* Van Cleave, 1940, and *Ascarophis girellae* Yamaguti, 1935, and Anguillicolidae Yamaguti, 1935 (larvae). Chamela Bay is a new locality for all helminth species, except for *N. aniversaria*. *Kyphosus elegans* is a new host for *D. ovalis*, *Ascarophis girellae*, and Anguilicollidae larvae. Taxonomic problems associated with these helminths are discussed, and the importance of the *Kyphosus* host–parasite system as a coevolving unit is stressed.

KEY WORDS: *Heteroplectanum oliveri* sp. n., *H. nenue*, *H. kyphosi*, *Neobivagina aniversaria*, *Deontacylix ovalis*, *Opisthadena dimidia*, *Jeancadenatia dohenyi*, *Filisoma bucerium*, *Ascarophis girellae*, Anguilicollidae, *Kyphosus elegans*, México.

We have been collecting helminths from marine and brackish water fishes from Chamela Bay, on the west coast of México, since 1992. During this survey, 18 specimens of *Kyphosus elegans* Peters, 1869, were collected and analyzed for helminths. *Kyphosus elegans*, regionally called *chopa*, is a tropical reef fish with herbivorous feeding habits and some commercial importance; its geographical distribution comprises the Pacific coast of the Americas, between the Gulf of California and the Galapagos Islands (Castro-Aguirre, 1978). Manter (1949, 1965) first recognized the genus *Kyphosus* Lacépedè as a host with considerable parasitological interest and suggested it as an excellent model to study the origin and dispersal routes of both hosts and parasites. Many collections of *Kyphosus* and their parasites have been made in the eastern Pacific (Van Cleave, 1940; Winter, 1956; Lamothe, 1961; Bravo-Hollis, 1965, 1979), Caribbean Sea (Sierra, 1984), and Gulf of México (Linton, 1910; Manter, 1947, 1949; Van Cleave and Manter, 1948; Overstreet, 1969). In this paper, we describe a new species of monogenean, characterize the helminth fauna of *K. elegans* from Chamela Bay, and address questions to be answered by a long-term survey related to the historical ecology (Brooks, 1985; Brooks and McLennan, 1991, 1993) and biogeography of this host–parasite system.

[1]Corresponding author (e-mail: vleon@servidor.unam.mx).

Materials and Methods

A total of 18 fishes were collected in Chamela Bay using gill nets, in August 1993, February and May 1995, and January 1996. This bay is located on the west coast of México, in the state of Jalisco, 19°30′–19°32′N, 105°06′W. Fish were examined no more 4 hr after capture; gills and viscera were obtained from each host and analyzed for helminths using a stereomicroscope.

Once collected, most monogeneans and digeneans were killed with boiling water and fixed under slight coverglass pressure using Bouin's fluid. Acanthocephalans were kept in distilled water at 4°C for 12 hr and fixed in 70% ethanol. Nematodes were killed with 70% boiling alcohol. Monogeneans, digeneans, and acanthocephalans were stained with Delafield and Van Cleave's hematoxylin, dehydrated in a graded alcohol series, cleared with methyl salicylate, and mounted in Canada balsam. Nematodes were mounted as semipermanent slides using lactophenol as a clearing agent. Measurements are expressed in micrometers; average is indicated with a range, in parentheses. Drawings were made using a camera lucida. Specimens were deposited in the Colección Nacional de Helmintos

(CNHE), México, and in the United States National Parasite Collection (USNPC), Beltsville, Maryland.

Results

Heteroplectanum oliveri sp. n. (Figs. 1–3)

DESCRIPTION: The following description is based on 48 specimens collected from *Kyphosus elegans*. Body slender, 895 (788–1,062) long by 148 (125–175) wide. Haptor clearly differentiated from the rest of the body, 253 (246–267) wide. Anterior region shows 3 pairs of cephalic organs and 2 pairs of eyespots.

Opisthaptor provided with 2 pairs of lateral anchors. The dorsal pair points outward, 58 (51–75) long by 10 (9–12) wide at level of root; the ventral pair points inward, has a bifurcated root, and is 64 (51–75) long by 27 (24–30) wide at level of root. There is a pair of lateral bars 59 (54–66) long by 19 (18–21) wide and a central bar with blunt ends, 221 (216–225) long by 11 (6–15) wide (Fig. 2). Seven marginal hooklets are present. There are 2 squamodiscs (1 ventral and 1 dorsal). They are composed of 63–65 radiating rows of rodlets in its anterior region and semicircular lines of tiny scales in the posterior region (Fig. 2). Mouth opening in prohaptor, at level of lateral head lobes. Pharynx 38 (24–51) long by 37 (33–48) wide. Esophagus inconspicuous; ceca simple, terminating separately midway between testis and opisthaptor. Testis ovoid, postequatorial, 146 (126–162) long by 83 (69–114) wide. Vas deferens arises from anterior end of testis and extends forward to form a tubular seminal vesicle. Cirrus sac muscular, immediately postbifurcal, divided in 2 portions; 1 anterior and proximal, bulbose, 82 (76–88) long by 34 (27–45) wide, distal portion strongly cuticularized, funnel-shaped, with a spur in its proximal region, 66 (60–75) long by 28 (27–30) maximum width. Cirrus spined, 115 (105–123) long (Fig. 3). Ovary immediately anterior to testis, lateraly elongated, 35 (27–45) long by 60 (45–75) wide; oviduct embraces right cecum. Mehlis gland directly anterior to ovary. Uterus intercecal, running forward to female genital pore, situated posterior and right of the male genital pore. Sclerotized genital atrium. Vagina situated on the left side of body, running forward and reaching the level of male genital pore. Vitelline follicles extending in lateral fields from the bifurcation to the end of intestinal ceca.

TYPE HOST: *Kyphosus elegans* Peters, 1869.

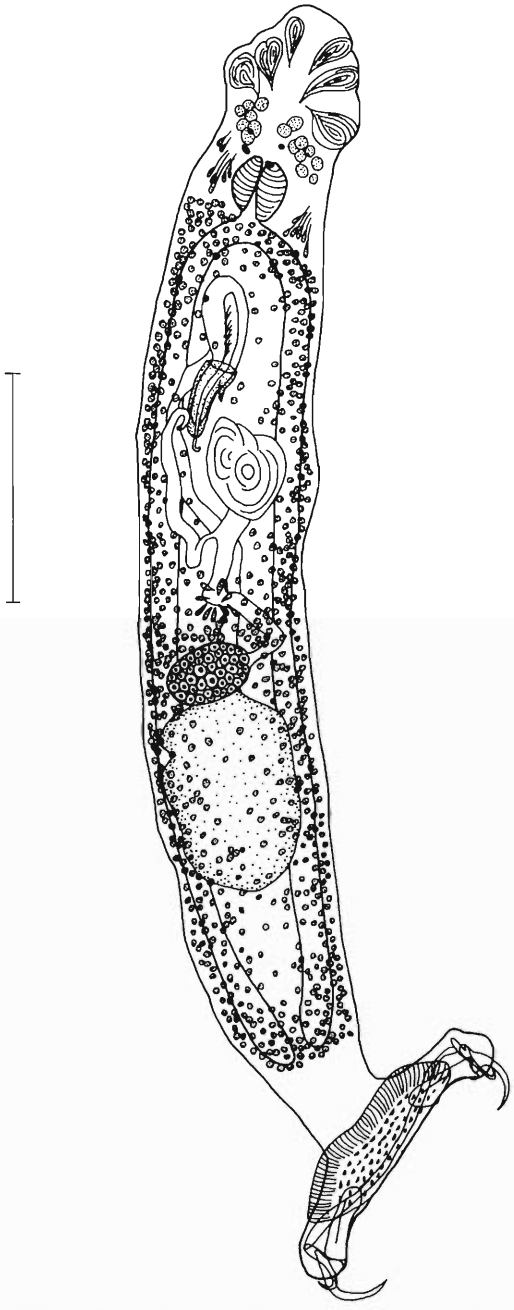
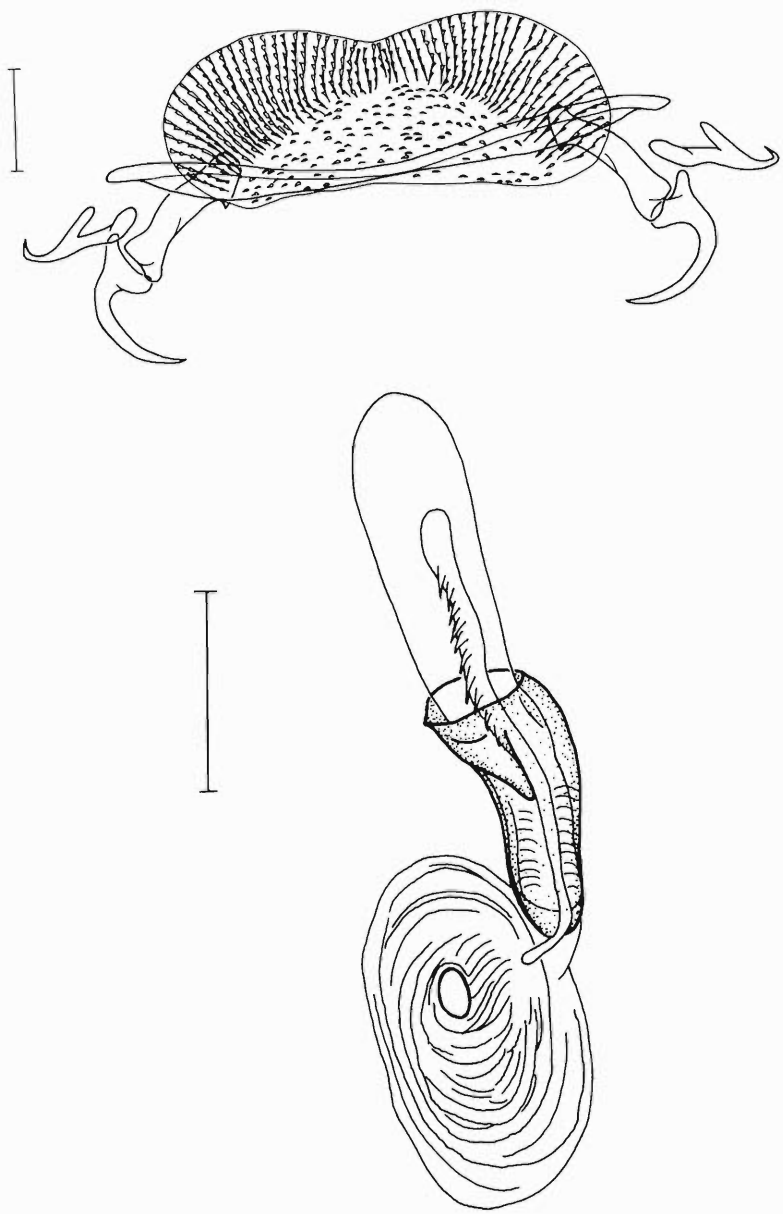


Figure 1. Holotype of *Heteroplectanum oliveri* sp. n. from *Kyphosus elegans* in Chamela Bay, Jalisco, México. Dorsal view. Scale bar = 0.2 mm.



Figures 2, 3. ***Heteroplectanum oliveri*** **sp. n. 2. Anchor/bar complex and squamodisc. 3. Genital complex. Scale bars = 0.05 mm.**

TYPE LOCALITY: Chamela Bay, Jalisco, México.

SITE OF INFECTION: Gills.

ACCESSION NUMBERS: Holotype CNHE 2728; paratypes CNHE 2729 and USNPC 84878.

ETYMOLOGY: The new species is named in honor of Dr. Guy Oliver, for his wide contribution to the knowledge of this group of monogeneans.

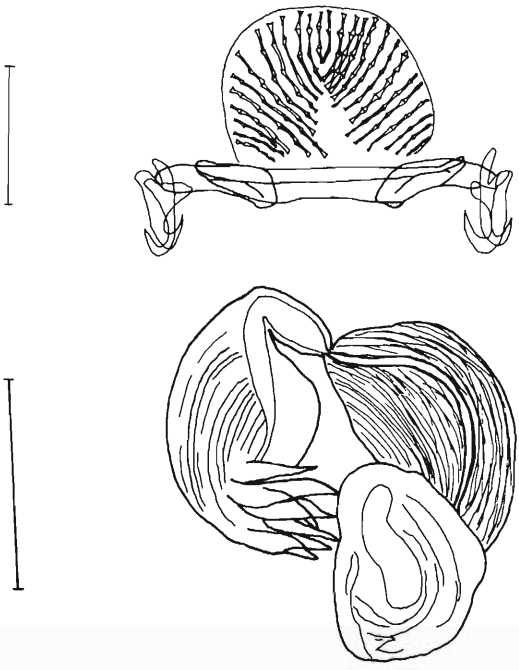
REMARKS: The structure of the squamodisc, with central rows in the form of a V, the shape of the central haptoral bar, and the distribution of the cephalic organs in 3 pairs identify these specimens as members of the genus *Hetroplectanum* Rakotofiringa, Oliver, and Lambert, 1987.

This genus was erected to include the species *H. nenuoides* and *H. serrulopenis,* described in *Rhabdosargus sarba* Försskal and *Polyamblyodon gibbosum* Pellegrin (Sparidae), *H. tamatavense* in *P. gibbosum*, and *H. parastromatei* in *Parastromateus niger* Bloch (Carangidae) from Madagascar. Rakotofiringa et al. (1987) also transfered the species *Diplectanum nenue* and *D. diplobulbus* described by Yamaguti (1968) in *Kyphosus cinerascens* (Forskal) from Hawaii to this genus. Oliver (1987) additionally transferred *D. spiculare* Yamaguti, 1968, *D. kyphosi* Yamaguti, 1968, and *D. yamagutii* Oliver, 1983, the 3 of them described in *K. cinerascens* in Hawaii (Yamaguti, 1968; Oliver, 1983).

The new species differs from *H. nenuoides, H. parastomatei, H. diplobulbus*, and *H. nenue* in the structure of the squamodisc, which is transversely elongated in *H. oliveri* and bears a higher number of sclerified ridges; in the scales posterior to the squamodisc, and in the structure of the cirrus complex, which is constituted in 2 parts, 1 muscular and proximal and 1 sclerified and distal. The new species differs from *H. kyphosi, H. tamatavense, H. yamaguti*, and *H. spiculare* in the structure of the cirrus complex, which is formed by a sinuous sclerified piece in its distal end in the former species and a large spicule in *H. spiculare,* whereas in *H. oliveri* it is funnel-shaped.

The new species most closely resembles *H. serrulopenis* because of the squamodisc structure, the structure of the cirrus sac, which is divided into 2 regions—the proximal part bulbose and the distal part sclerotized—and the spined character of the cirrus, but it differs from this species in the shape of the distal part of the cirrus sac, which in *H. oliveri* is more developed and bears a conspicuous spur in its proximal end. The distal part of the uterus is also sclerotized in *H. serrulopenis* but not so markedly as in *H. oliveri* (Oliver, pers. comm.). In addition, the general size of the body and organs of *H. oliveri* are smaller. These characters do not vary among the 48 specimens examined; thus, we think that the differences between *H. serrulopenis* and *H. oliveri* could not be considered a result of intraspecific variation.

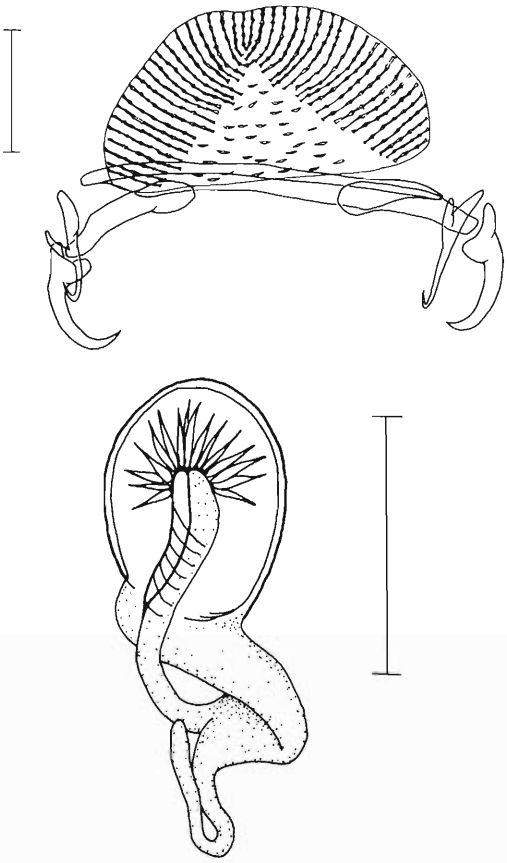
Heteroplectanum serrulopenis was described in 2 species of fishes from the family Sparidae in Madagascar; it is difficult to conceive the presence of the same species parasitizing a nonrelated host species along the Pacific coast of México, considering that *H. oliveri* is highly specific to *K. elegans.* The finding of 2 of the Hawaiian species sympatric with *H. oliveri* suggests that the new species could be the sister species of one of those originally described from Hawaii. The high resemblance to *H. serrulopenis* would then be the result of convergent evolution. The phylogenetic and biogeographical study of this group of monogeneans has interesting aspects that deserve further attention.



Figures 4, 5. ***Heteroplectanum nenue*** **(Yamaguti, 1968) Rakotofiringa, Oliver, and Lambert, 1987. 4. Anchor/bar complex and squamodisc. 5. Genital complex. Scale bars = 0.05 mm.**

Heteroplectanum nenue (Yamaguti, 1968) Rakotofiringa, Oliver, and Lambert, 1987 (Figs. 4, 5)

Yamaguti (1968) originally described this species as *Diplectanum nenue* Yamaguti, 1968, as a parasite of *Kyphosus cinerascens* in Hawaii, and later it was transferred to the genus *Heteroplectanum* Rakotofiringa, Oliver, and Lambert, 1987. This species differs from *H. oliveri* in the number of rows forming the squamodisc (13 vs. 63–65 in the new species) (Fig. 4) and in the structure of the male genitalia, which is formed by a muscular elongate proximal part, an ejaculatory bulb, and a bulbose distal zone ending



Figures 6, 7. ***Heteroplectanum kyphosi*** **(Yamaguti, 1968) Oliver, 1987. 6. Anchor/bar complex and squamodisc. 7. Genital complex. Scale bars = 0.05 mm.**

with several foliaceous projections. This report represents a new host and locality record.

SITE OF INFECTION: Gills.

ACCESSION NUMBER: CNHE 2730.

Heteroplectanum kyphosi (Yamaguti, 1968) Oliver, 1987 (Figs. 6, 7)

Heteroplectanum kyphosi, originally described as *Acleotrema kyphosi* Yamaguti, 1968, in *Kyphosus cinerascens* in Hawaii (Yamaguti, 1968), differs from *H. oliveri* and *H. nenue* in the number of rows forming the squamodisc (20 in *H. kyphosi* vs. 63–65 in *H. oliveri* and 13 in *H. nenue*) (Fig. 6); it also differs in the structure of the cirrus complex, which is formed by a sinuous sclerotized piece and lacks a spined cirrus (Fig. 7). This represents a new host and locality record for *H. kyphosi.*

SITE OF INFECTION: Gills.

ACCESSION NUMBER: CNHE 2731.

Neobivagina aniversaria Bravo-Hollis, 1979

The monogenean *Neobivagina aniversaria* was originally described from *Kyphosus* sp. (Bravo-Hollis, 1979) and later reported from *Sectator ocyurus* (Bravo-Hollis, 1981) in the same locality as our specimens. We have examined 114 different fish species in this locality and confirmed that *N. aniversaria* preferentially parasitizes members of the Kyphosidae, although it has also been occasionally collected from *Lutjanus guttatus* and *Prioporus punctatus.*

SITE OF INFECTION: Gills.

ACCESSION NUMBERS: CNHE 2732; USNPC 84879.

Deontacylix ovalis Linton, 1910

Deontacylix ovalis is a sanguinicolid digenean that lives in the vascular system of fish and was previously described from *K. sectatrix* and *K. incisor* in Florida by Linton (1910) and Manter (1947). This report represents a new host and locality record.

SITE OF INFECTION: Blood vessels.

ACCESSION NUMBER: CNHE 2733.

Opisthadena dimidia Linton, 1910

The genus *Opisthadena* Linton, 1910, comprises 9 species distributed mainly in tropical marine fish all over the world. The taxonomy of the genus is difficult because the characters that have been used to distinguish species show great intraspecific variation. Our specimens resemble most *O. dimidia* Linton, 1910, but differ from the description by Linton (1910) in having a wider distance between testes and between the ovary and testes. The 5 pairs of oral papillae described by Manter (1947) were not observed in a constant number but varied from 3 to 5 pairs. The number of papillae was used in the erection of *O. cheni* Martin, 1978, as a useful character (Martin, 1978). We question the validity of this trait as a taxonomic character, because in our observation of type specimens of *O. dimidia* (USNPC 8489), *O. bodegensis* Johnson and Copsey, 1957 (USNPC 37338), and *O. cortesi* Bravo, 1956 (CNHE 219-25), we noticed that the number of papillae vary greatly among specimens of the same species. The observation of

the types of *O. kyphosi* Yamaguti, 1970 (USNPC 63790), showed that the use of the presence or absence of the oral papillae, in contrast, is a useful character to differentiate species. Taxonomic revision and phylogenetic analysis of this genus is necessary to support or refute the validity of the present classification. *Opisthadena dimidia* is a specialist parasite of fishes of the genus *Kyphosus* along the Pacific and Atlantic coasts of tropical America, and the related species *O. kyphosi* and *O. cheni* are typical of fishes of the same family (Kyphosidae) in Hawaii and California, respectively. This makes phylogenetic analysis of the genus an important one, because it may be a significant part of any biogeographical analysis of the genus *Kyphosus* and its helminths. León-Règagnon et al. (1996) address these subjects. This report represents a new locality record for *Opisthadena dimidia.*

SITE OF INFECTION: Stomach.

ACCESSION NUMBERS: CNHE 2631, 2632; USNPC 84875.

Jeancadenatia dohenyi Winter, 1956

The genus *Jeancadenatia* was erected by Dollfus (1946) for *J. brumpti* Dollfus, 1946, from *Kyphosus sectatrix* in Africa. Subsequently, 2 additional species have been described, *J. dohenyi* Winter, 1956, from *K. elegans* from Nayarit State, on the Pacific coast of México, and *J. pacifica* Yamaguti, 1970, from *K. cinerascens* from Hawaii. In its original diagnosis, this genus differs from the related *Cadenatella* Dollfus, 1946, and *Enenterum* Linton, 1910, in body length, number of preoral lobes, and accessory suckers. When Winter (1956) described *J. dohenyi*, he emended the generic diagnosis, because the new species bore only 2 accessory suckers instead of the "many" that Dollfus (1946) stated. Later, Yamaguti (1970) included *J. pacifica* in this genus because of the resemblance in internal structures, although the species has only 8 rather than 10 preoral lobes. We examined the type specimen of *J. dohenyi* (CNHE 215-9) and observed that our specimens are identical to those described by Winter, bearing 10 oral lobes and 2 accesory suckers. This report represents a new locality for *J. dohenyi.*

The differences among species of the genera *Enenterum, Cadenatella,* and *Jeancadenatia* are not pronounced, leading Nahhas and Cable (1964) to declare *Jeancadenatia* a synonym of *Cadenatella.* In addition to that, Gibson and Bray (1982) and Bray (1986) have included several genera of opecoelids and lepocreadids within the family Enenteridae based on genital structures, although their specimens lack the preoral lobes that are diagnostic of the family. A thorough study of the phylogenetic relationships among the genera of this family will be necessary to provide a stable classification.

SITE OF INFECTION: Intestine.

ACCESSION NUMBERS: CNHE 2734; USNPC 84976.

Filisoma bucerium Van Cleave, 1940

The acanthocephalan *Filisoma bucerium* was originally described by Van Cleave (1940) from *K. elegans* from Isla Socorro, México. Our specimens, found in the same host and geographic zone, show the typical features of this species, 16 rows of 38–40 hooks in the proboscis. The hooks of the middorsal row are modified, being heavy and blunt. This report represents a new locality for *F. bucerium.*

The genus *Filisoma* Van Cleave, 1940, comprises 5 species, 2 of which were described from freshwater fish: *F. indicum* Van Cleave, 1928, in India and *F. microcanthi* Harada, 1938, in Japan. A third species, *F. rizalinum* Tubangi and Masilungan, 1946, was described from the same host as *F. microcanthi*, but from Manila Bay, in the Philippine Islands. The other 2 species were found in New World fishes of the genus *Kyphosus*: *F. bucerium* Van Cleave, 1940, from *K. elegans* in the Pacific Ocean and *F. fidum* Van Cleave and Manter, 1948, from *K. sectatrix* in Florida (Van Cleave and Manter, 1948). *Filisoma bucerium* has also been found in *Caranx hippos* from Oaxaca State, on the Pacific coast of México (Salgado, 1978), but those specimens were much smaller than those in *Kyphosus* spp. The specificity shown by the species of this genus to fishes of the genus *Kyphosus* and their restricted geographical distribution also provide an interesting host–parasite system for zoogeographical studies.

SITE OF INFECTION: Intestine.

ACCESSION NUMBERS: CNHE 2735; USNPC 84877.

Ascarophis girellae (Yamaguti, 1935) Campana, 1955

Our specimens of *Ascarophis* show the lateral lips, transversely striated cuticle, postequatorial vulva, and filamented eggs that characterize the

genus. Caballero (1975) described *Ascarophis ayalai* from *Arius liropus* collected in coastal lagoons of Nayarit and Sonora, on the Pacific coast of México. The specimens from Chamela Bay differ from *A. ayalai* in the structure of the male spicules. In *A. ayalai*, the shorter spicule is "unciform" (claw-like) and the larger is L-shaped. In our specimens, the longer spicule is slender, with a flat dilatation near the distal end, and the shorter is broad and curved. Our specimens most closely resemble *A. girellae* (Yamaguti, 1935) Campana, 1955, which was originally described as *Rhabdochona girellae* in *Girella punctata* from Japan (Yamaguti, 1935). They share the shape and size of the spicules and the distribution of caudal papillae of males: 3 preanal, 1 adanal, and 5 postanal pairs (all subventral). In addition, there are 5 pairs of small lateral papillae that are postnatal. This report represents a new host and locality record.

Kyphosid and girellid fish are thought to be closely related, and girellids have been shifted back and forth from the families Kyphosidae and Girellidae (Martin, 1978). This is the second parasite known to be shared between the host genera *Kyphosus* and *Girella*. *Opisthadena cheni* was originally described from *Girella nigricans* in California (Martin, 1978), although this digenean genus is common in *Kyphosus* species (Linton, 1910; Manter, 1947; Yamaguti, 1970). The finding of *Ascarophis girellae* in *Kyphosus elegans* supports the hypothesized relationship between the host genera.

HABITAT: Stomach.

ACCESSION NUMBER: CNHE 2736.

Anguilicollidae gen. sp. Yamaguti, 1935 (Larvae)

We collected larval nematodes belonging to an undetermined species in the family Anguilicollidae.

SITE OF INFECTION: Intestine.

Discussion

Of the 10 helminth species recorded here, 7 are common parasites of the genus *Kyphosus* (*H. oliveri, H. nenue, H. kyphosi, D. ovalis, O. dimidia, J. dohenyi*, and *F. bucerium*) and *N. aniversaria* parasitizes preferably members of the family Kyphosidae, being previously reported from *K. elegans* and *Sectator ocyurus*. Van Cleave and Manter (1948) and Manter (1949, 1965) have considered the genus *Kyphosus* an excellent host–parasite system for zoogeographical studies and proposed an origin center and dispersal routes for *Kyphosus* species based on the zoogeographical distribution of their helminth parasites. Manter (1965) proposed an Indo-Pacific origin of this fish genus with secondary dispersion to the Americas via the South Pacific Ocean and via the Eastern Pacific Ocean to the Caribbean Sea. Manter did not have the methodological tools to test *Kyphosus* evolutionary and biogeographical history that are available today in phylogenetic systematics and historical ecology, as described by Brooks (1981, 1990) and Brooks and McLennan (1991, 1993). Although the phylogeny of the genus *Opisthadena* (León-Règagnon et al., 1996) does not support Manter's view of progressive dispersion from the western to the eastern Pacific (it rather supports the notion of an ancient circum-Pacific distribution of the group), our records of species of the genus *Heteroplectanum*, which has been reported in the western as well as in the eastern Pacific, and the genera *Deontacylix* and *Filisoma*, also reported in the Caribbean Sea, suggest that phylogenetic studies of such groups and others highly specific to kyphosids as *Jeancadenatia, Cadenatella*, and *Enenterum* could provide decisive information on the evolutionary history of this host–parasite system.

Acknowledgments

We gratefully acknowledge the help of Claudia Aranda, Elizabeth Castillo, Fernando García, Maribel Garzón, Agustín Jiménez, Berenit Mendoza, Griselda Pulido, and Coral Rosas in field collections and specimen processing. We also thank Dr. Daniel R. Brooks, Scott Monks, and 2 anonymous reviewers for the careful revision of the manuscript and Dr. Ralph Lichtenfels (USNPC) for the loan of specimens. Special thanks to Dr. Guy Oliver, who kindly sent us all the literature related with diplectanids and gave us advice on the taxonomic situation of the new species. This study was supported by funds from PADEP-UNAM proy. 03333-1994 to V.L.-R. and from PAPIIT-UNAM proy. IN201593 to G.P.-P.L.

Literature Cited

Bravo-Hollis, M. 1965. Helmintos de peces de aguas mexicanas del Pacífico. XXIV. Descripción de *Opisthadena cortesi* n. sp. (Trematoda). Anales del Instituto de Biología UNAM Mexico 36(Serie Zoología):141–145.

———. 1981. Helmintos de peces del Pacífico Mex-

icano XXXVII. Sobre seis especies conocidas de monogéneos del Suborden Microcotylinea Lebedev, 1978. Anales del Instituto de Biología UNAM México 52(Serie Zoología):1–12.

Bray, R. A. 1986. Some helminth parasites of marine fishes of South Africa: families Enenteridae, Opistholebetidae, and Pleorchiidae (Digenea). Journal of Natural History 20:471–488.

Brooks, D. R. 1981. Hennig's parasitological method: a proposed solution. Systematic Zoology 30:229–249.

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

———. 1990. Parsimony analysis in historical biogeography and coevolution: methodological and theoretical update. Systematic Zoology 39:14–30.

———, **and D. McLennan.** 1991. Phylogeny, Ecology and Behavior. A Research Program in Comparative Biology. The University of Chicago Press, Chicago. 434 pp.

———, **and** ———. 1993. Parascript. Parasites and the Language of Evolution. Smithsonian Institution Press, Washington, D.C. 429 pp.

Caballero, R. G. 1975. Contribution à la connaisance des Nématodes de Poissons marins du Mexique II. Sur une nouvelle espèce de Rhabdochonidae. Bulletin du Muséum National d'Histoire Naturelle de Paris 3 (301, Zoologie):211.

Castro-Aguirre, J. L. 1978. Catálogo sistemático de los peces marinos que penetran a las aguas continentales de México con aspectos zoogeográficos y ecológicos. Dirección General del Instituto Nacional de Pesca México. Serie Científica 19. 298 pp.

Dollfus, R. P. 1946. Sur trois especes de distomes, dont une a 17 ventouses (*Enenterum* (*Jeancadenatia*) *brumpti* n. sp.) parasites du poisson marin *Kyphosus sectatrix* (L.). Annales de Parasitologie Humaine et Comparée 21:119–128.

Gibson, D. I., and R. A. Bray. 1982. A study and reorganization of *Plagioporus* Stafford, 1904 (Digenea:Opecoelidae) and related genera, with special reference to forms from european Atlantic waters. Journal of Natural History 16:529–559.

Lamothe, A. R. 1961. Estudio de los tremátodos digéneos de peces del Golfo de California, México. Anales del Instituto de Biología UNAM México 32(Serie Zoología):219–233.

León-Règagnon, V., G. Pérez-Ponce de León, and D. R. Brooks. 1996. Phylogenetic analysis of *Opisthadena* Linton, 1910 (Digenea: Hemiuridae: Bunocotylinae). Journal of Parasitology 82:1005–1010.

Linton, E. 1910. Helminth fauna of the Dry Tortugas. II. Trematodes. Carnegie Institution Publications 133:1–98 + 28 plates.

Manter, H. W. 1947. The digenetic trematodes of marine fishes of Tortugas, Florida. American Midland Naturalist 38:257–416.

———. 1949. An additional trematode from Tortugas Florida, and a new name for *Opisthoporus* Manter, 1947, preoccupied. American Midland Naturalist 41:432–435.

———. 1965. Parasites of fishes as biological indicators of recent and ancient conditions. Pages 59–71 *in* Host–Parasite Relationships. Proceedings of the XXVI Annual Biology Colloquium, 23–24 April. Oregon State University Press, Corvallis.

Martin, W. E. 1978. Digenetic trematodes of the marine fish, *Girella nigricans* (Ayers), from Southern California with the description of two new species. Proceedings of the Helminthological Society of Washington 45:175–181.

Nahhas, F. M., and R. M. Cable. 1964. Digenetic and aspidogastrid trematodes from marine fishes of Curaçao and Jamaica. Tulane Studies in Zoology 11:169–227.

Oliver, G. 1983. *Diplectanum yamagutii* sp. n. (Monogenea: Monopisthocotylea: Diplectanidae), parasite de *Kyphosus cinearascens* (Forsskal) à Hawaii. Zoologica Scripta 12:91–93.

———. 1987. Les Diplectanidae Bychowsky, 1957 (Monogenea: Monopisthocotylea: Dactylogyridea). Systèmatique. Biologie. Ontogénie. Ecologie. Essai de phylogenèse. Ph.D. Thesis. Université de Perpignan, Perpignan, France. 433pp.

Overstreet, R. M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. Tulane Studies in Zoology and Botany 15:119–176.

Rakotofiringa, S. L., G. Oliver, and A. Lambert. 1987. *Heteroplectanum* n. gen., un nouveau genre de Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea), parasite de Téléostéens marins de Madagascar. Bulletin du Museum National Histoire d'Naturelle Paris, 4th Ser. 9:145–157.

Salgado, M. G. 1978. Acantocéfalos de peces. V. Redescripción de cuatro especies de paleacantocéfalos parásitos de peces de México. Anales del Instituto de Biología UNAM México 49(Serie Zoología):49–70.

Sierra, R. N. A. 1984. Descripción taxonómica de algunos tremátodos parásitos de peces marinos de la zona del Caribe Mexicano. Tesis. Facultad de Ciencias, Universidad Nacional Autonoma de México, México D.F. 89 pp.

Van Cleave, H. J. 1940. The Acanthocephala collected by the Allan Hancock Pacific Expedition, 1934. University of Southern California Publications. Allan Hancock Pacific Expeditions 2:501–527.

———, **and H. W. Manter.** 1948. A new species of the acanthocephalan genus *Filisoma* from the Dry Tortugas, Florida. Journal of Parasitology 33:487–490.

Winter, H. A. 1956. Tremátodos de peces marinos de aguas mexicanas. XII. Dos géneros de digéneos (Lepocreadiidae), incluyendo una nueva especie procedente de *Kyphosus elegans* (Peters) de las Islas Tres Marías, en el Oceano Pacífico. Anales del Instituto de Biología UNAM México 27(Serie Zoología):403–413.

Yamaguti, S. 1935. Studies on the helminth fauna of Japan. Part 9. Nematodes of fishes I. Japanese Journal of Zoology 6:337–386.

———. 1968. Monogenetic Trematodes of Hawaiian fishes. University of Hawaii Press, Honolulu. 287 pp.

———. 1970. Digenetic Trematodes of Hawaiian Fishes. Keigaku Publishing, Tokyo. 436 pp.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 17–24

Gyrodactylus rafinesqueii sp. n. (Monogenea) from *Etheostoma rafinesquei* (Percidae) in Kentucky, with a Review of the Taxonomy and Host Specificity of Species of *Gyrodactylus* from Etheostomatid Fishes in North America

GORDON K. WEDDLE,[1,3] AND DAVID K. CONE[2]

[1] Department of Biology, Campbellsville College, Campbellsville, Kentucky 42718 and
[2] Department of Biology, Saint Mary's University, Halifax, Nova Scotia, Canada B3H 3C3

ABSTRACT: *Gyrodactylus rafinesqueii* sp. n. (Monogenea) is described from the trunk surfaces, fins, and gonopodium of the Kentucky snubnose darter (*Etheostoma rafinesquei*) from Russell Creek, Kentucky. The species has moderately sized hamuli (56–65 μm long), a ventral bar with prominent (14–18 μm long) anterolateral processes and a tapered membrane, and a relatively large, slender marginal hook sickle (8.0–8.5 μm long). An illustrated comparison of the sclerites of *G. rafinesqueii* sp. n. to those of related species known from etheostomatid fishes (*G. bretinae* Wellborn, 1967; *G. etheostomae* Wellborn and Rogers, 1967; *G. nigrum* Rogers, 1975; *G. percinae* Rogers and Wellborn, 1965) is presented. *Gyrodactylus rafinesqueii* sp. n. resembles most closely *G. percinae* but has larger marginal hook sickles and a dorsal bar devoid of a distinct medial notch. Within various rivers studied in Kentucky, *G. rafinesqueii* sp. n. parasitized *E. rafinesquei*, *E. flavum*, and *E. simoterum*, all three of which are species of darters classified in the subgenus *Nanostoma/Ulocentra*. *Gyrodactylus rafinesqueii* sp. n. did not parasitize species of darter of other subgenera living syntopically in the same habitat. In contrast, *G. etheostomae* parasitized hosts (*E. barrenense*, *E. caeruleum*, *E. spectabile*, and *E. stigmaeum*) of 3 subgenera and thus has a much broader host specificity. Field collections revealed that *G. rafinesqueii* sp. n. and *G. etheostomae* can co-occur within the same stretch of river but that they do not share hosts. Both apparently are dependent on darters, for neither parasite was found on cyprinid fishes sampled at the same sites. A key to species of *Gyrodactylus* from etheostomatid fishes and preliminary thoughts on the evolutionary history of gyrodactylids on these fishes are included.

KEY WORDS: Monogenea, *Gyrodactylus rafinesqueii*, *Gyrodactylus etheostomae*, darter fishes, Kentucky, North America.

Four species of *Gyrodactylus* Nordmann, 1832, have been described from the body surfaces and fins of etheostomatid fishes in North America. They are *G. percinae* Rogers and Wellborn, 1965, from the blackbanded darter (*Percina nigrofasciata*) in Alabama (Rogers and Wellborn, 1965); *G. bretinae* Wellborn, 1967, from the speckled darter (*Etheostoma stigmaeum*) in Arkansas (Wellborn, 1967), *G. nigrum* Rogers, 1975, from the johnny darter (*E. nigrum*) in Alabama, and *G. etheostomae* Wellborn and Rogers, 1967, from the orangebelly darter (*E. radiosum*) in Arkansas (Wellborn and Rogers, 1967), the mud darter (*E. asprigene*) in North Dakota (Kritsky and Leiby, 1971), the Iowa darter (*E. exile*) in Ontario (Molnar et al., 1974), the rainbow darter (*E. caeruleum*) in Kentucky (Kozel and Whittaker, 1982), and the johnny darter, (*E. nigrum*) in Lake Ontario (Hanek and Fernando, 1971; Dechtiar and Christie, 1988) and in Lake Huron (Dechtiar et al., 1988).

The present study describes *Gyrodactylus rafinesqueii* sp. n. from the Kentucky snubnose darter (*E. rafinesquei*) and examines the host specificity of the parasite among species of darters living syntopically at selected sites in Kentucky streams. The study compares taxonomically *G. rafinesqueii* sp. n. and the preceding species.

Materials and Methods

Parasites studied originated from host fishes sampled from stream sites in 7 Kentucky counties and 2 drainage basins: 3 and 13 April 1992, Brush Creek, Green County; 21 February 1992, Marrowbone Creek, Cumberland County; 9 December 1992 and 15 April 1993, Middle Pitman Creek, Taylor County; and 9 and 17 April 1993, Russell Creek, Adair County; 27 December 1994, Trammel Fork of Drakes Creek, Allen County; 28 December 1994, Whipporwill Creek of Red River, Logan County, and Elk Fork of Red River, Todd County (Fig. 1). Sites 1, 2, and 4 (Fig. 1) were in the Cumberland River Drainage. Sites 3 and 5–7 were in the Green River Drainage. At Sites 4, 6, and

[3] Corresponding author (e-mail: cone@husky1.stmarys.ca).

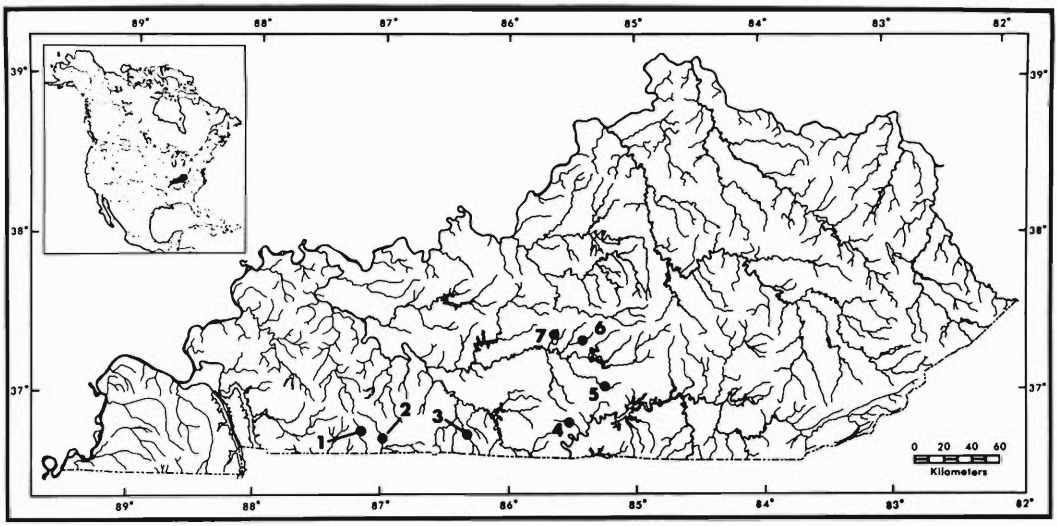


Figure 1. Map of Kentucky showing the location of 7 sampling sites. Site 1, Elk Fork, Todd County, 87°07′27″W, 36°44′02″N. Site 2, Whipporwill Creek, Logan County, 86°58′54″W, 36°45′41″N. Site 3, Trammel Fork, Allen County, 86°16′16″W, 36°44′23″N. Site 4, Marrowbone Creek, Cumberland County, 85°32′46″W, 36°50′37″N. Site 5, Russell Creek, Adair County, 85°10′53″W, 37°03′18″N. Site 6, Middle Pitman Creek, Taylor County, 85°24′04″W, 37°22′20″N. Site 7, Brush Creek, Green County, 85°35′46″W, 37°24′15″N. Modified with permission from Burr, B. M., and M. L. Warren, Jr. 1968. A distributional atlas of Kentucky fishes. Ky. Nature Preserves Comm., Sci. and Tech. Series A, 398 pp.

7 (Fig. 1), fish were preserved in 10% formalin. The sampling at Russell Creek (Site 5, Fig. 1) was much more extensive. Fishes were collected for about 6 hr using various-sized seines. Each species of fish captured was kept in a separate bag until 10–15 individuals were obtained. The fish were then placed in jars with a 1:4,000 formalin solution. The parasites were allowed to settle and then pipetted into small jars containing 5% formalin; the fish were removed and fixed in 10% formalin. The same protocol was followed at Sites 1–3 (Fig. 1), but collections were limited to darters.

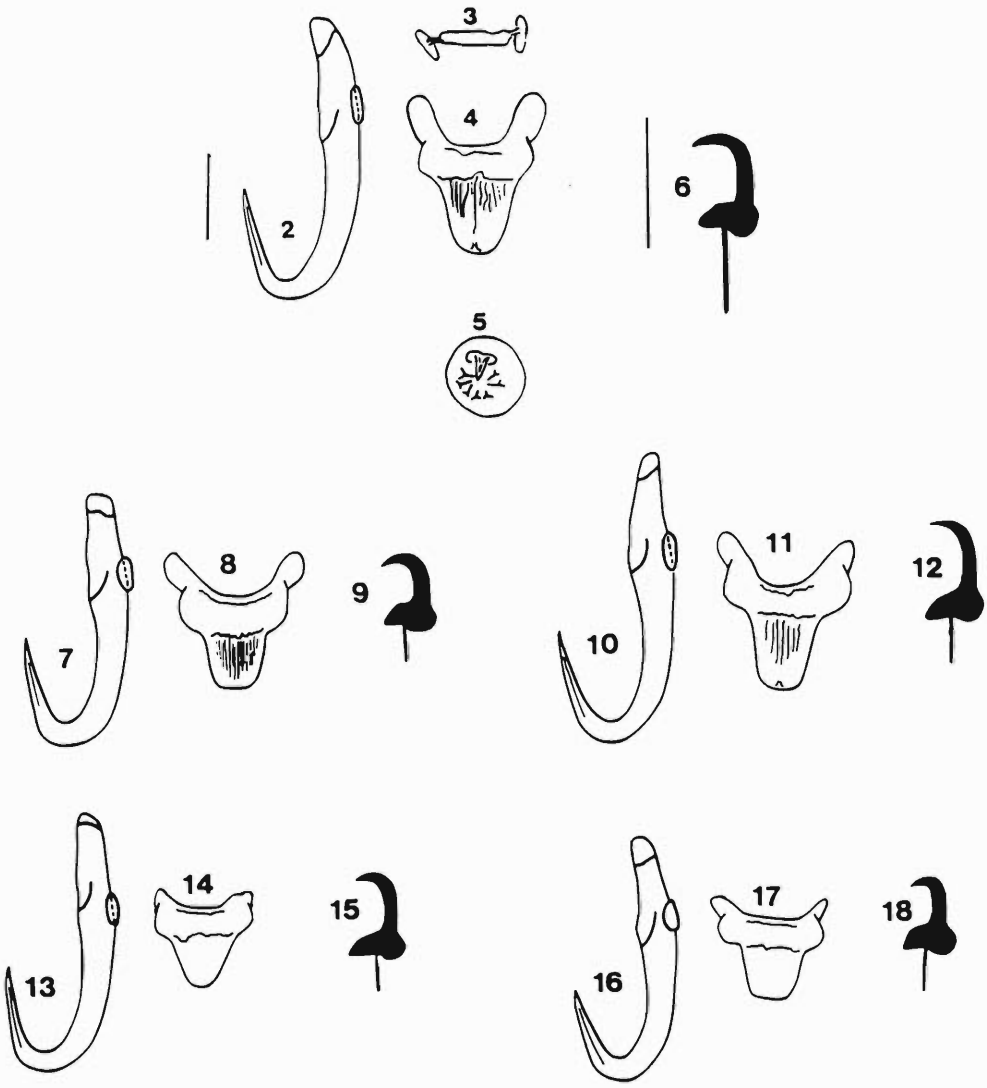
Preserved parasites were mounted unstained in a 50% solution of glycerine–water and allowed to clear for over 1 yr. Cleared specimens were studied microscopically and relevant morphometric features were determined from drawings prepared by means of an optical drawing tube. Photographs of the marginal hooks were used as an important reference when preparing the final drawings of the marginal hook sickles. Permanent slides were prepared by soaking the slide overnight in tapwater and then removing the coverslip. The specimen (which usually remained adhered to either the slide or the coverslip) was dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Unless stated otherwise, all measurements are presented in micrometers. Those of the holotype are followed in parentheses by the mean, ±1 standard deviation, range, and number of measurements determined for of those of the paratypes.

Type and voucher specimens of 4 species of *Gyrodactylus* housed in the United States National Parasite Collection (USNPC), Beltsville, Maryland, were examined. These included *G. bretinae* (holotype USNPC 61626 and paratype USNPC 61627), *G. etheostomae* (holotype USNPC 60879, paratype USNPC 60880, and a voucher specimen USNPC 71659), *G. nigrum* (holotype USNPC 71232 and paratype USNPC 71233), and *G. percinae* (holotype USNPC 61027).

Results

Gyrodactylus rafinesqueii sp. n. (Figs. 2–6)

DESCRIPTION: Flattened specimen 460 (mean = 410, ± 61 [SD], range = 320–460, n = 8) long, 95 (86, ±6.5, 80–95, n = 8) wide at midbody. Pharynx 33 (29, ±3.1, 26–33, n = 4) long, 21 (25.5, ±3.8, 21–30, n = 4) wide. Penis 13 (13.4, ±0.7, 13–14, n = 2) in diameter, with 1 large spine and a row of 6 small spines. Developing embryo encased within thin-walled, stretchable, bipolar "shell." Hamuli 60 (61.2, ±2.7, 56–65, n = 9) long; root 23 (19.6, ±2.5, 16–23, n = 9), shaft 42 (43, ±1.7, 41–46, n = 9), point 24 (25.6, ±2.0, 23–28, n = 9). Ventral bar 8 (6.2, ±0.8, 5–8, n = 10) long, 23 (24.6, ±1.6, 22–26, n = 10) wide, with anterolateral processes 10 (10.8, ±0.9, 14–18, n = 10) long. Ventral bar membrane 18 (16.2, ±1.1, 14–18, n = 10) long. Dorsal bar tubular. Marginal hook 28.5 (29.5, ±1.8, 27–32, n = 6) long; sickle 8.5



Figures 2–18. Taxonomically important features of species of *Gyrodactylus* Nordmann, 1832 (Monogenea), known from darter fishes of North America. All structures except the marginal hook sickles are drawn to the same scale, bar = 20μm. Marginal hook sickles are all drawn to the scale of the bar = 10 μm. 2–6. *Gyrodactylus rafinesqueii* sp. n. on the body surface of *Etheostoma rafinesquei*. 2. Sclerotized hamulus (holotype USNPC). 3. Dorsal bar (voucher specimen). 4. Ventral bar (holotype USNPC). 5. Penis (holotype USNPC). 6. Lateral view of marginal hook (holotype USNPC 86709). 7–9. *Gyrodactylus percinae* Rogers and Wellborn, 1965. 7. Hamulus (holotype USNPC 61027). 8. Ventral bar (holotype USNPC 61027). 9. Marginal hook sickle (holotype USNPC 61027). 10–12. *Gyrodactylus bretinae* Wellborn, 1967. 10. Hamulus (paratype USNPC 61627). 11. Ventral bar (paratype USNPC 61627). 12. (paratype USNPC 61627). 13–15. *Gyrodactylus nigrum* Rogers, 1975. 13. Hamulus (paratype USNPC 71233). 14. Ventral bar (paratype USNPC 71233). 15. Marginal hook (paratype USNPC 71233). 16–18. *Gyrodactylus etheostomae* Wellborn and Rogers, 1967. 16. Hamulus (paratype USNPC 60880). 17. Ventral bar (paratype USNPC 60880). 18. Marginal hook sickle (paratype USNPC 60880).

(8.1, ±0.2, 8.0–8.5, n = 7) long, 4.0 (4.3, ±0.2, 4.0–4.5, n = 7) wide proximally, 5.5 (4.7, ±0.5, 4.0–5.5, n = 7) wide distally; handle 21.5 (21.9, ±1.6, 20–24.5, n = 6) long; filament 8.5 (8.5, ±0.0, n = 5) long.

TYPE HOST: Kentucky snubnose darter (*Etheostoma rafinesquei* Burr and Page, 1982) (Percidae; Etheostomatini). Other known hosts include the Tennessee snubnose darter (*E. simoterum*) and the saffron darter (*E. flavum*).

SITES ON HOST: Principally, the base and membranes of fins and the gonopodium of females.

TYPE LOCALITY: Holotype and paratype specimens from Middle Pitman Creek, Kentucky Highway 210 (85°24′04″W, 37°22′20″N), Taylor County, Kentucky 15 April 1993). Other specimens studied were from Russell Creek, Adair County (January and April 1993), and Brush Creek, Green County (April 1992), both in Kentucky.

SPECIMENS STUDIED: Ten. Holotype and paratype specimens are deposited in the USNPC No. 86709, Beltsville, Maryland.

PREVALENCE AND INTENSITY OF INFECTION: As part of another study on the ecology of *E. rafinesquei* in Middle Pitman Creek, Kentucky, the prevalence and intensity of *G. rafinesqueii* sp. n. was noted on monthly samples collected from August 1987 to July 1988. Parasites were absent or rare in the months of May to October. However, the parasite was relatively common from November to April, with prevalence being 86% (n = 36 examined) in winter (December to February) and 91% (n = 33 examined) in spring (March to May). Mean intensity was the greatest during winter and spring at 13.2 and 10 parasites, respectively.

HOST SPECIFICITY: At Russell Creek, *G. rafinesqueii* sp. n., *G. etheostomae*, and *G. campostoma* Wellborn, 1967, were collected. The results reveal that, in spite of co-existing in the same stream reach, *G. rafinesqueii* sp. n. parasitized only *E. rafinesquei* whereas *G. etheostomae* parasitized *E. caeruleum* and *E. stigmaeum*. *Gyrodactylus campostoma* parasitized only *Campostoma oligolepis*. Seven species of fish at the site, including 3 darters (22 *E. bellum*, 9 *E. blennioides*, and 8 *E. flabellum*) and 4 cyprinids (2 *Cyprinella spilopterus*, 32 *Luxilus chrysocephalus*, 16 *Lythrurus ardens*, and 17 *Pimephales notatus*), were devoid of the parasites.

At Middle Pitman Creek, *G. rafinesqueii* sp. n. occurred only on *E. rafinesquei*, whereas *G. etheostomae* occurred on *E. caeruleum* and *E. spectabile*.

At Marrowbone Creek, *G. rafinesqueii* sp. n. parasitized *E. simoterum*. Gyrodactylid parasites were not found on the darters *E. blennioides*, *E. rufilineatum*, and *E. spectabile* nor on the cyprinids *Notropis telescopis* and *N. boops*.

At Whipporwill Creek, *G. rafinesqueii* sp. n. parasitized *E. simoterum* and *E. flavum*.

At Trammel Fork, *G. etheostomae* parasitized *E. caeruleum* and *E. barrenense*.

At Brush Creek, *G. rafinesqueii* sp. n. parasitized *E. rafinesquie* whereas *G. etheostomae* parasitized *E. caeruleum*.

At Elk Creek, *G. rafinesqueii* sp. n. parasitized *E. flavum*.

ETYMOLOGY: This species is named after the host on which it was first collected.

COMMENTS: *Gyrodactylus rafinesqueii* sp. n. resembles most closely *G. percinae* Rogers and Wellborn, 1965, a species described from the fins and body surface of the blackbanded darter (*P. nigrofasciata*) from Moore's Mill Creek, Lee County, Alabama. Both species are of medium body size for gyrodactylids and have similarly shaped hamuli (Figs. 2, 7). The ventral bar has prominent anterolateral projections and a similarly proportioned membrane (Figs. 4, 8). However, *G. rafinesqueii* sp. n. has relatively large sickles compared to those of *G. percinae* (Figs. 6, 9) and does not possess a medial notch in the dorsal bar, a feature that is considered diagnostic for *G. percinae* (Rogers and Wellborn, 1965).

Discussion

During the present study, we examined type material of *G. bretinae*, *G. etheostomae*, *G. nigrum*, and *G. percinae*. We concluded that all 4 represent valid taxa but that existing descriptions are lacking in taxonomically important details of the hamulus and/or the marginal hook sickle. To help address this problem, we provide a detailed comparison of *G. rafinesqueii* sp. n. with these related species.

Gyrodactylus percinae was described from the fins and body of the blackbanded darter (*Percina nigrofasciata*) in Moore's Mill Creek, Alabama (Rogers and Wellborn, 1965), but has not been reported in any subsequent parasite surveys. Study of the type material revealed that the original species description is accurate. Important diagnostic features include the relatively

short, robust hamuli (Fig. 7) and a ventral bar with distinct anterolateral projections and a blunt membrane (Fig. 8). We supplement the description by providing important details on the size and shape of the marginal hook sickle (Fig. 9).

Gyrodactylus bretinae was described from the fins and body of the speckled darter (*E. stigmaeum*) at the National Fish Hatchery, Corning, Arkansas (Wellborn, 1967). This species has also not been reported in surveys published since the description. We examined the holotype and paratype specimens and, in spite of the specimens having dried significantly since deposition, the sclerites are visible in lateral view. We concur with Wellborn's (1967) description with respect to the size and depicted shapes of the ventral and dorsal bars and the penis and its terminal spines. The hamulus as it is originally described is slightly exaggerated in overall thickness, our interpretation of these sclerites of the types being that they are thinner (Fig. 10). Furthermore, as originally described, the sickle of the marginal hook is also too thick. The ventral bar has distinct anterolateral processes (Fig. 11). The type specimens reveal that the shaft and point of the sickle are very slender (Fig. 12).

Gyrodactylus nigrum (Figs. 13–15) was described from johnny darter (*E. nigrum*) in Cubahatchee Creek, Alabama (Rogers, 1975). As with *G. percinae* and *G. bretinae*, it has not been reported subsequently in the literature. However, we believe the species identified as *G. etheostomae* from johnny darter in the Bay of Quinte, Lake Ontario, by Hanek and Fernando (1971) was in fact *G. nigrum*. We believe this because our study of the type specimens showed that *G. nigrum* has marginal hooks with a relatively long, slender sickle (Fig. 15) not depicted accurately in the original species description. Drawings provided by Hanek and Fernando (1971) of specimens collected from johnny darter and identified as *G. etheostomae* show long, thin sickles characteristic of *G. nigrum* (Fig. 15).

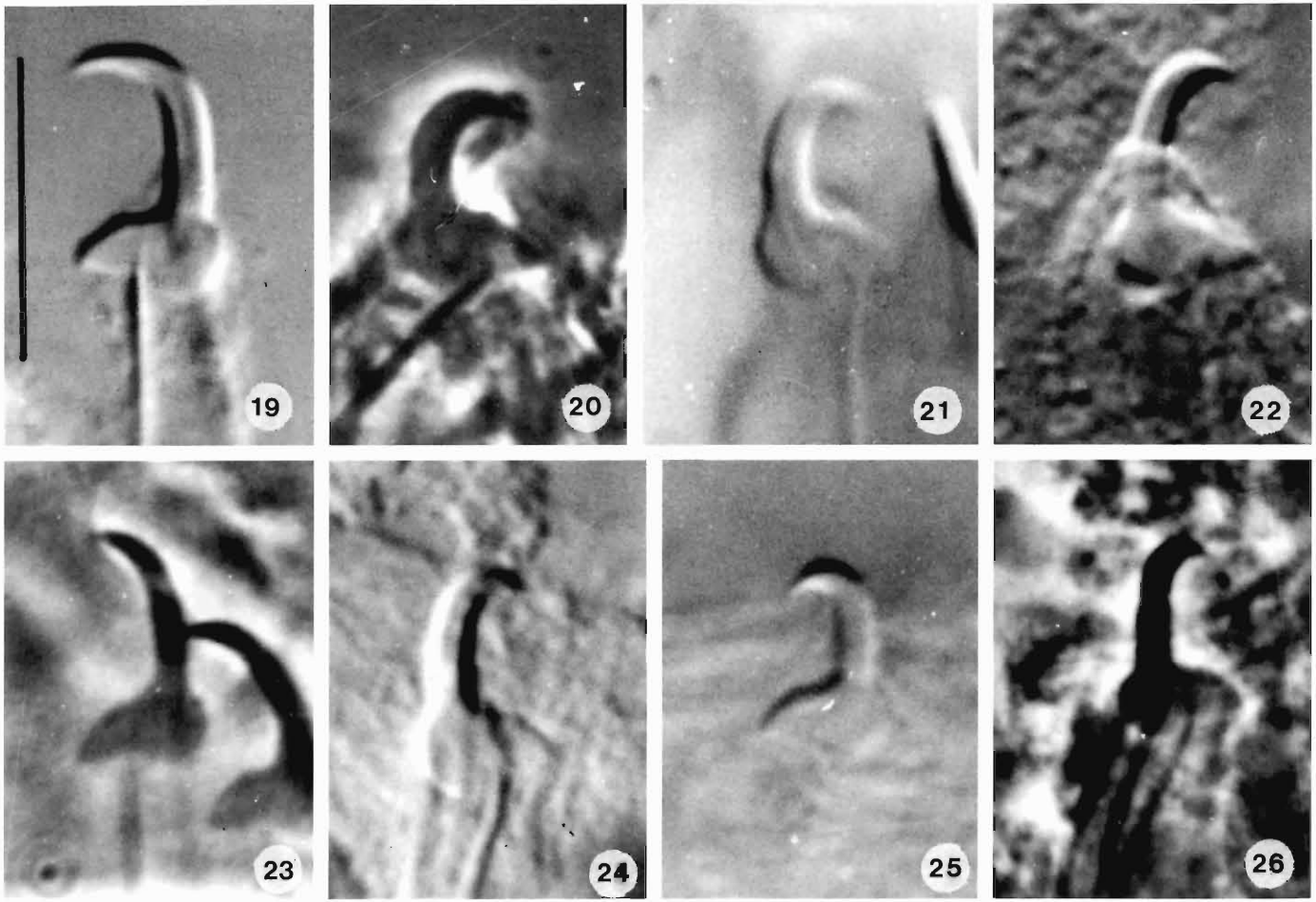
Gyrodactylus etheostomae is the most commonly reported species of *Gyrodactylus* from etheostomatid fishes. It was originally described from the orangebelly darter (*G. radiosum*) in Mammoth Spring, Arkansas (Wellborn and Rogers, 1967) and, as detailed in the introduction, has subsequently been reported from 4 other species of darter throughout the center of the continent (Hanek and Fernando, 1971; Kritsky and Leiby, 1971; Molnar et al., 1974; Kozel and Whittaker, 1982; Dechtiar and Christie, 1988; Dechtiar et al., 1988). The original species description (Wellborn and Rogers, 1967) and the subsequent redescription (Kritsky and Leiby, 1971) present important features of the haptoral and penal sclerites (Figs. 16, 17). We supplement this information by providing a detailed lateral view of the marginal hook sickle (Fig. 18).

In addition to providing a key to known species of *Gyrodactylus* from darters, we have provided photographs of the marginal hook sickle of type species (Figs. 19–26). These types are deteriorating because they were originally mounted in glycerine jelly. In spite of the less than ideal quality of the photographs, it will be important for future studies to have some accurate record of their morphology.

Key to Known Species of *Gyrodactylus* from Darters

1. Anterolateral processes of ventral bar prominent, greater than 8 μm in length 2
   Anterolateral processes of ventral bar not prominent, less than 5 μm in length 4
2. Marginal hook sickle stout and compact and relatively short in length (6–7 μm) (Fig. 9) ..... *G. percinae*
   Marginal hook sickle delicate and 8.0–8.5 μm long 3
3. Marginal hook sickle with normally recurved point (Fig. 6) ..... *G. rafinesqueii*
   Marginal hook sickle with an open, only slightly recurved point (Fig. 12) ..... *G. bretinae*
4. Marginal hook sickle with short stout shaft (Fig. 18) ..... *G. etheostomae*
   Marginal hook sickle with long delicate shaft (Fig. 15) ..... *G. nigrum*

Species of *Gyrodactylus* that parasitize darters appear to be host-specific toward this group of fishes. This was evident in Russell Creek, where *G. rafinesqueii* sp. n. and *G. etheostomae* occurred only on darters and not on any of the 4 cyprinids that shared the habitat. Similarly, *G. rafinesqueii* sp. n. occurred on darters but did not occur on cyprinids in Marrowbone Creek. However, our samples indicate that the degree of host specificity varies with species of *Gyrodactylus*. *Gyrodactylus rafinesqueii* sp. n., for example, appears to be host-specific toward a group of closely related species (Page, 1981) that are collectively known as snubnose darters and assigned by various authors to one or another of 2 subgeneric names, *Nanostoma* (Page,



Figures 19–26. Photomicrographs of marginal hook sickles of type specimens of species of *Gyrodactylus* described from etheostomatini fishes. Scale bar = 10 μm. 19. *G. rafinesqueii* sp. n. Nomarski interference contrasts. 20. *G. percinae*, embryo (holotype USNPC 61027), Nomarski interference contrast. 21. *G. percinae* (holotype, phase contrast). 22. *G. bretinae* (holotype USNPC 61626). 23. *G. bretinae*, embryo (holotype). 24. *G. nigrum* (holotype USNPC 71232). 25. *G. etheostomae* (voucher specimens, USNPC 71659).

1981) or *Ulocentra* (Bailey and Etnier, 1988). Regardless of the controversy over the taxonomy of this group of fishes, we did not collect *G. rafinesqueii* sp. n. from any species other than those that are indisputably snubnose darters.

Gyrodactylus etheostomae appears to be less specific than *G. rafinesqueii* sp. n. In our samples, *G. etheostomae* parasitized 4 species of darters that are currently classified in 3 subgenera (*Oligocephalus*, *Doration*, and *Nanostoma*/*Ulocentra*; Page, 1981; Bailey and Etnier, 1988). *Gyrodactylus etheostomae* has previously been reported from 4 other species of darter and 2 additional subgenera, *Boleichthyes* and *Boleosoma* (Wellborn and Rogers, 1967; Kritsky and Leiby, 1971; Molnar et al., 1974).

The occurrence of *G. etheostomae* on *E. barrenense* at Trammel Fork may be interpreted as further evidence that this species is less host-specific than *G. rafinesqueii* sp. n., but more importantly it demonstrates that darters of the subgenus *Nanostoma*/*Ulocentra* can support populations of *Gyrodactylus* other than *G. rafinesqueii* sp. n. *Etheostoma barrenense* is unquestionably a sister species of *E. rafinesquei* (Page and Burr, 1982), the type host of *G. rafinesqueii* sp. n. However, *G. rafinesqueii* sp. n. was not collected at Trammel Fork. We cannot exclude the possibility that the occurrence of *G. etheostomae* on *E. barrenense* was incidental or transient. Nevertheless, *G. etheostomae* has a much broader host specificity than *G. rafinesqueii* sp. n.

The observed differences in host specificity are not the result of host-specific differences in habitat. All species of darters parasitized by *G. rafinesqueii* sp. n. have been collected with 1 or more of the species that are parasitized by *G. etheostomae* (Kuehne and Barbour, 1983; Page, 1983). Unpublished microhabitat data from our Russell Creek site indicate significant microhabitat overlaps among *E. bellum*, *E. caeruleum*, *E. rafinesquei*, and *E. stigmaeum*. The absence of gyrodactylids from darters of the subgenera *Catonotus* (*E. flavellare* at Russell Creek), *Nothonotus* (*E. bellum* at Russell Creek and *E. rufilineatum* at Marrowbone Creek), and *Etheostoma* (*E. blennioides* at Russell Creek and Marrowbone Creek) further supports the conclusion that host–parasite relationships are narrowly constrained.

It is tempting to conclude that the narrow host specificity of *G. rafinesqueii* sp. n. for snubnose darters is a result of coevolutionary history. However, the phylogeny of snubnose darters is significantly uncertain to allow such a conclusion. Additional sampling from sites where other species of snubnose darter occur syntopically with darters that are known hosts of *G. etheostomae* and with darters of the subgenus *Etheostoma* (see Bailey and Etnier [1988] and Page [1981] for a justification) should yield valuable information regarding the ecology of the parasites as well as the phylogeny of snubnose darters. That host-specificity among taxa of Monogenea can be used as taxonomic indicators in parasitized fishes has been recently demonstrated by Lambert and El Gharbi (1995).

Acknowledgments

We thank Roger Farmer, Richard K. Kessler, Sean Schlobohm, and John R. Weddle for invaluable assistance in the field. Conversations with Noel M. Burkhead, Brooks M. Burr, and Larry M. Page regarding phylogeny of snubnose darters are appreciated. We thank Brooks M. Burr and Melvin L. Warren, Jr., for graciously allowing the use and modification of their drainage map of Kentucky. Financial support was provided to the senior author by the Professional Development Committee and Science Division of Campbellsville College and to the junior author by an NSERC Operating Grant. Drs. R. Arthur and Z. Kabata provided helpful discussion of certain rules of Zoological Nomenclature.

Literature Cited

Bailey, R. M., and D. A. Etnier. 1988. Comments on the subgenera of darters (Percidae) with descriptions of two new species from southcentral United States. Miscellaneous Publications of the University of Michigan Museum of Zoology 175: 1–48.

Dechtiar, A. O., and W. J. Christie. 1988. Survey of the parasite fauna of Lake Ontario fishes, 1961–1971. Pages 66–95 *in* S. J. Nepszy, ed. Parasites of Fishes in the Canadian Waters of the Great Lakes. Great Lakes Fishery Commission Technical Report No. 51.

———, **J. J. Collins, and J. A. Reckahn.** 1988. Survey of the parasite fauna of Lake Huron fishes, 1961 to 1971. Pages 19–48 *in* S. J. Nepszy, ed. Parasites of Fishes in the Canadian Waters of the Great Lakes. Great Lakes Fishery Commission Technical Report No. 51.

Hanek, G., and C. H. Fernando. 1971. Monogenetic trematodes from the Bay of Quinte area, Ontario. II. Genus *Gyrodactylus* Nordmann, 1832. Canadian Journal of Zoology 49:1331–1341.

Kozel, T. R., and F. H. Whittaker. 1982. Ectopar-

asites of the rainbow darter, *Etheostoma caeruleum* Storer, from Harrods, Creek, Oldham County, Kentucky. Proceedings of the Helminthological Society of Washington 49:138–139.

Kritsky, D. C., and P. D. Leiby. 1971. Studies on helminths of North Dakota. 1. Two new monogenetic trematodes of the genus *Gyrodactylus* from percid fishes and a redescription of *G. etheostomae* Wellborn and Rogers, 1967. Proceedings of the Helminthological Society of Washington 38:200–202.

Kuehne, R. A., and R. W. Barbour. 1983. The American Darters. University of Kentucky Press, Lexington. 201 pp.

Lambert, A., and S. El Gharbi. 1995. Monogenean host specificity as biological and taxonomic indicators for fish. Biological Conservation 72:227–235.

Molnar, K., G. Hanek, and C. H. Fernando. 1974. Parasites of fishes from Laurel Creek, Ontario. Journal of Fish Biology 6:717–728.

Page, L. M. 1981. The genera and subgenera of darters (Percidae, Etheostomatini). Occasonal Papers of the Museum of Natural History, University of Kansas 101:1–69.

———. 1983. Handbook of Darters. T. F. H. Publ., Neptune City, New Jersey. 271 pp.

———, **and B. M. Burr.** 1982. Three new species of darters (Percidae, *Etheostoma*) of the subgenus *Nanostoma* from Kentucky and Tennessee. Occasional Papers of the Museum of Natural History, University of Kansas 101:1–20.

Rogers, W. A. 1975. Four new species of *Gyrodactylus* from fishes of Alabama. Journal of Parasitology 61:51–53.

———, **and T. L. Wellborn.** 1965. Studies on *Gyrodactylus* (Trematoda: Monogenea) with descriptions of five new species from the south-eastern U.S. Journal of Parasitology 51:977–982.

Wellborn, T. L. 1967. Four new species of *Gyrodactylus* (Trematoda: Monogenea) from southeastern U.S. Proceedings of the Helminthological Society of Washington 34:55–59.

———, **and W. A. Rogers.** 1967. Five new species of *Gyrodactylus* (Trematoda: Monogenea) from the southeastern U.S. Journal of Parasitology 53: 10–14.

New Page Charges

Due to increasing costs of publication of the *Journal,* the Executive Committee was forced to raise the page charges to $50. per page for members and $100. per page for non-members of the Society. The page charges will be effective with manuscripts accepted after November 1, 1996.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 25–54

Neotropical Monogenoidea. 29. Ancyrocephalinae (Dactylogyridae) of Piranha and Their Relatives (Teleostei, Serrasalmidae) from Brazil: Species of *Amphithecium* Boeger and Kritsky, 1988, *Heterothecium* gen. n. and *Pithanothecium* gen. n.

DELANE C. KRITSKY,[1] WALTER A. BOEGER,[2] AND MICHEL JÉGU[3]

[1] College of Health Professions, Idaho State University, Pocatello, Idaho 83209 (e-mail: kritdela@isu.edu),
[2] Departamento de Zoologia, Universidade Federal do Paraná, Caixa Postal 19020, Curitiba, Paraná 81530, Brazil, and Conselho Nacional de Desenvolvimento Cientifíco e Technológico (CNPq) (e-mail: wboeger@bio.ufpr.br), and
[3] Antenne ORSTOM, Laboratoire d'Ichtyologie, MNHN, 43 rue Cuvier 75231 Paris Cedex, France (e-mail: jegu@mnhn.fr)

ABSTRACT: Fifteen species (9 new) of *Amphithecium*, 2 new species of *Heterothecium*, and 2 species of *Pithanothecium* are described and/or reported from the gills of 14 species of Serrasalmidae from the Brazilian Amazon: *Amphithecium calycinum* Boeger and Kritsky, 1988, *A. brachycirrum* Boeger and Kritsky, 1988, *A. camelum* Boeger and Kritsky, 1988, and *A. catalaoensis* Boeger and Kritsky, 1988, from *Pygocentrus nattereri*; *Amphithecium diclonophallum* sp. n. from *Pristobrycon* sp., *Serrasalmus compressus*, *S. elongatus*, *S. gouldingi*, *S. rhombeus*, and *Serrasalmus* sp. (2 of Jégu); *Amphithecium falcatum* Boeger and Kritsky, 1988, from *Pristobrycon* sp., *Pygocentrus nattereri*, *Serrasalmus compressus*, *S. elongatus*, *S. gouldingi*, *S. manuelli*, *S. rhombeus*, *S. spilopleura*, *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. (2n = 58); *Amphithecium junki* Boeger and Kritsky, 1988, from *Pygocentrus nattereri* and *Serrasalmus rhombeus*; *Amphithecium microphallum* sp. n. from *Pygocentrus nattereri* and *Serrasalmus* sp. (2n = 58); *Amphithecium minutum* sp. n. from *Pristobrycon eigenmanni*, *Pristobrycon* sp., *Serrasalmus gouldingi*, and *S. spilopleura*; *Amphithecium muricatum* sp. n. from *Pristobrycon eigenmanni*, *Serrasalmus rhombeus*, and *Serrasalmus* sp. (2 of Jégu); *Amphithecium pretiosum* sp. n. from *Pristobrycon* sp., *Serrasalmus gouldingi*, and *S. manuelli*; *Amphithecium prodotum* sp. n. from *Catoprion mento* and *Pristobrycon striolatus*; *Amphithecium speirocamarotum* sp. n. from *Serrasalmus elongatus*; *Amphithecium unguiculum* sp. n. from *Serrasalmus spilopleura*; *Amphithecium verecundum* sp. n. from *Pristobrycon eigenmanni* and *Serrasalmus* sp. (2 of Jégu); *Heterothecium globatum* sp. n. from *Serrasalmus gouldingi*; *Heterothecium dicrophallum* sp. n. from *Catoprion mento*; *Pithanothecium piranhus* (Mizelle and Price, 1965) comb. n. from *Catoprion mento*, *Pristobrycon striolatus*, *Pygocentrus nattereri*, and *Pygopristis denticulata*; and *Pithanothecium amazonensis* (Mizelle and Price, 1965) comb. n. from *Catoprion mento*, *Pristobrycon striolatus*, and *Pygopristis denticulata*. The diagnosis of *Amphithecium* is emended, and 2 new genera are proposed. *Heterothecium* gen. n. characterized by species having a sinistrodorsal vaginal pore, a sclerotized vaginal vestibule, a male copulatory organ with 2 rami, and simple distal termination of the articulation process of the accessory piece. Characters distinguishing *Pithanothecium* gen. n. include presence of a sclerotized vaginal vestibule opening on the dextrolateral surface of the trunk and a distally blunt articulation process of the accessory piece extending past the tip of the distal rod. *Cleidodiscus piranhus* Mizelle and Price, 1965, and *C. amazonensis* Mizelle and Price, 1965, are transferred to *Pithanothecium*.

KEY WORDS: Monogenoidea, Dactylogyridae, Ancyrocephalinae, *Amphithecium*, *Heterothecium* gen. n., *Pithanothecium* gen. n., *Amphithecium brachycirrum*, *Amphithecium calycinum*, *Amphithecium camelum*, *Amphithecium catalaoensis*, *Amphithecium diclonophallum* sp. n., *Amphithecium falcatum*, *Amphithecium junki*, *Amphithecium microphallum* sp. n., *Amphithecium minutum* sp. n., *Amphithecium muricatum* sp. n., *Amphithecium pretiosum* sp. n., *Amphithecium prodotum* sp. n., *Amphithecium speirocamarotum* sp. n., *Amphithecium unguiculum* sp. n., *Amphithecium verecundum* sp. n., *Heterothecium dicrophallum* sp. n., *Heterothecium globatum* sp. n., *Pithanothecium amazonensis* comb. n., *Pithanothecium piranhus* comb. n., Serrasalmidae, *Catoprion mento*, *Pristobrycon eigenmanni*, *Pristobrycon striolatus*, *Pristobrycon* sp., *Pygocentrus nattereri*, *Pygopristis denticulata*, *Serrasalmus compressus*, *Serrasalmus elongatus*, *Serrasalmus gouldingi*, *Serrasalmus manuelli*, *Serrasalmus rhombeus*, *Serrasalmus spilopleura*, *Serrasalmus* sp., Amazon Basin, Brazil.

This paper represents the second of 4 contributions dealing with Ancyrocephalinae from the gills of Amazonian Serrasalmidae (see Kritsky et al., 1996, in press a, b). It includes 15 species of *Amphithecium* Boeger and Kritsky, 1988, 2 of *Heterothecium* gen. n., and 2 of *Pithanothecium* gen. n.

Materials and Methods

Methods of collection of hosts and their parasites and of mounting, illustration, and measurement of helminths are as described by Kritsky et al. (1986, 1996). All measurements are in micrometers; the mean is followed by the range and number of specimens measured in parentheses; length of the accessory piece is that of the distal rod. Numbering (distribution) of hook pairs follows that recommended by Mizelle (1936; see Mizelle and Price, 1963). Type and voucher specimens of helminths are deposited in the parasite collections of Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil; the United States National Museum (USNPC), Beltsville, Maryland; and the University of Nebraska State Museum (HWML), as indicated in the respective descriptions or accounts of species. For comparative purposes, the following specimens were examined: *Cleidodiscus amazonensis* Mizelle and Price, 1965, holotype (USNPC 60462), paratype (HWML 21289), and *C. piranhus* Mizelle and Price, 1965, holotype (USNPC 60463), paratype (HWML 21290).

Presumed undescribed hosts have been provisionally identified by M.J. as *Pristobrycon* sp., *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. (2n = 58). Representative specimens of provisionally identified host taxa are deposited in the ichthyology collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil.

Taxonomic Account

Class Monogenoidea Bychowsky, 1937
Order Dactylogyridea Bychowsky, 1937
Dactylogyridae Bychowsky, 1933
Ancyrocephalinae Bychowsky, 1937
Amphithecium Boeger and Kritsky, 1988

EMENDED DIAGNOSIS: Body fusiform or flattened dorsoventrally; comprising cephalic region, trunk, peduncle, haptor. Tegument thin, smooth, scaled or papillate. Two terminal, 2 bilateral cephalic lobes; head organs, unicellular cephalic glands present. Eyes 4, anterior pair infrequently absent; granules elongate ovate. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; intestinal ceca 2, confluent posterior to testis, lacking diverticula. Gonads intercecal, overlapping; testis dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle a sigmoid dilation of the vas deferens. Two prostatic reservoirs; prostates comprising 2 bilateral glandular areas lying dorsal to anterior portions of ceca. Genital pore midventral near level of cecal bifurcation. Copulatory complex comprising an accessory piece articulating to base of tubular copulatory organ by variable, flexible proximal articulation process. Two bilateral vaginae, nonsclerotized, dilated; each looping respective intestinal cecum, opening on dorsolateral surfaces; seminal receptacle usually absent. Haptor subhexagonal, with pairs of dorsal and ventral anchor/bar complexes, 7 pairs of similar hooks with ancyrocephaline distribution. Hooks similar; each with delicate point, truncate protruding thumb, expanded shank comprising 2 subunits; proximal subunit variable in length between hook pairs. FH loop extending to union of shank subunits. Parasites of gills of Serrasalmidae.

TYPE SPECIES: *Amphithecium calycinum* Boeger and Kritsky, 1988, from *Pygocentrus nattereri*.

OTHER SPECIES: *Amphithecium brachycirrum* Boeger and Kritsky, 1988, from *Pygocentrus nattereri*; *A. camelum* Boeger and Kritsky, 1988, from *P. nattereri*; *A. catalaoensis* Boeger and Kritsky, 1988, from *P. nattereri*; *A. diclonophallum* sp. n. from *Pristobrycon* sp., *Serrasalmus elongatus*, *S. gouldingi*, *S. rhombeus*, and *Serrasalmus* sp. (2 of Jégu); *A. falcatum* Boeger and Kritsky, 1968, from *Pristobrycon* sp., *Pygocentrus nattereri*, *S. elongatus*, *S. gouldingi*, *S. manuelli*, *S. rhombeus*, *S. spilopleura*, *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. (2n = 58); *A. junki* Boeger and Kritsky, 1988, from *Pygocentrus nattereri* and *S. rhombeus*; *A. microphallum* sp. n. from *P. nattereri* and *Serrasalmus* sp. (2n = 58); *A. minutum* sp. n. from *Pristobrycon eigenmanni*, *Pristobrycon* sp., *S. gouldingi*, and *S. spilopleura*; *A. muricatum* sp. n. from *P. eigenmanni*, *S. rhombeus*, and *Serrasalmus* sp. (2 of Jégu); *A. pretiosum* sp. n. from *Pristobrycon* sp., *Serrasalmus gouldingi*, and *S. manuelli*; *A. prodotum* sp. n. from *Catoprion mento* and *P. striolatus*; *A. speirocamarotum* sp. n. from *S. elongatus*; *A. unguiculum* sp. n. from *S. spilopleura*; and *A. verecundum* sp. n. from *P. eigenmanni* and *Serrasalmus* sp. (2 of Jégu).

REMARKS: Boeger and Kritsky (1988) characterized *Amphithecium* by specimens possessing bilateral nonsclerotized vaginae opening dorsolaterally, a biramous copulatory organ, overlapping gonads, an accessory piece articulated to the base of the copulatory organ, and hook shanks comprising 2 subunits. Of these, the features of the vaginae apparently represent the only synapomorphies. In their phylogenetic hypothesis, Boeger and Kritsky (1988) considered double vaginae to be a synapomorphy for the clade containing *Amphithecium*, *Notothe-*

cium, and *Notozothecium* with the single vaginal branches of members of the latter 2 genera being derived. However, these authors indicated that consideration of bilateral vaginae a synapomorphy for the clade of *Amphithecium* species was equally parsimonious. One other ancyrocephaline genus, *Calpidothecioides*, is characterized by members with double vaginae (Kritsky et al., in press a). In species of *Calpidothecioides*, the dextral vagina opens middorsally, and the sinistral branch opens on the left margin of the body.

Amphithecium calycinum Boeger and Kritsky, 1988 (Figs. 1–9)

RECORDS: *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989); Furo do Catalão, Manaus, Amazonas (26, 27 November 1984).

PREVIOUS RECORDS: *Pygocentrus nattereri* (type host): Furo do Catalão, Manaus, Amazonas; Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas; Rio Pacaás-Novos, Guajará-Mirim, Rondônia; Rio Mamoré, Surpresa, Rondônia; Rio Guaporé, Surpresa, Rondônia (type locality); Rio Guaporé, Costa Marques, Rondônia (all Boeger and Kritsky, 1988).

SPECIMENS STUDIED: Forty-three vouchers, USNPC 85786, 85787.

MEASUREMENTS: Body length 287 (231–319; n = 9), greatest width 101 (80–122; n = 9); haptoral length 56 (43–63; n = 9), width 76 (64–85; n = 8); pharyngeal diameter 17 (16–19; n = 9); ventral anchor length 29 (27–31; n = 24), base width 13 (11–14; n = 20); dorsal anchor length 30 (29–33; n = 23), base width 13 (12–14; n = 16); ventral bar 28 (26–30; n = 5), dorsal bar 24 (23–26; n = 5) long; hook pair 1—16–17 (n = 11), pairs 2, 6—17 (15–20; n = 31), pairs 3, 4, 7—23 (21–25; n = 50), pair 5—13–14 (n = 16) long; copulatory organ length 32 (26–35; n = 23), accessory piece length 21 (17–24; n = 19); testis 61 (51–72; n = 6) long, 26 (23–31; n = 6) wide; germarium 62 (48–91; n = 8) long, 25 (19–28; n = 8) wide.

REMARKS: *Amphithecium calycinum* was adequately described as the type species for the genus by Boeger and Kritsky (1988). Our specimens do not differ significantly in morphology and size from those originally reported. The species is apparently restricted to *Pygocentrus nattereri* and is distinguished by having a loosely coiled or twisted primary ramus and broad secondary ramus of the copulatory organ.

Amphithecium brachycirrum Boeger and Kritsky, 1988 (Figs. 10–19)

RECORDS: *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989); Furo do Catalão, Manaus, Amazonas (26, 27 November 1984).

PREVIOUS RECORDS: *Pygocentrus nattereri* (type host): Furo do Catalão, Manaus, Amazonas (type locality); Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas; Rio Pacaás-Novos, Guajará-Mirim, Rondônia; Rio Mamoré, Surpresa, Rondônia; Rio Guaporé, Surpresa, Rondônia; Rio Guaporé, Costa Marques, Rondônia (all Boeger and Kritsky, 1988).

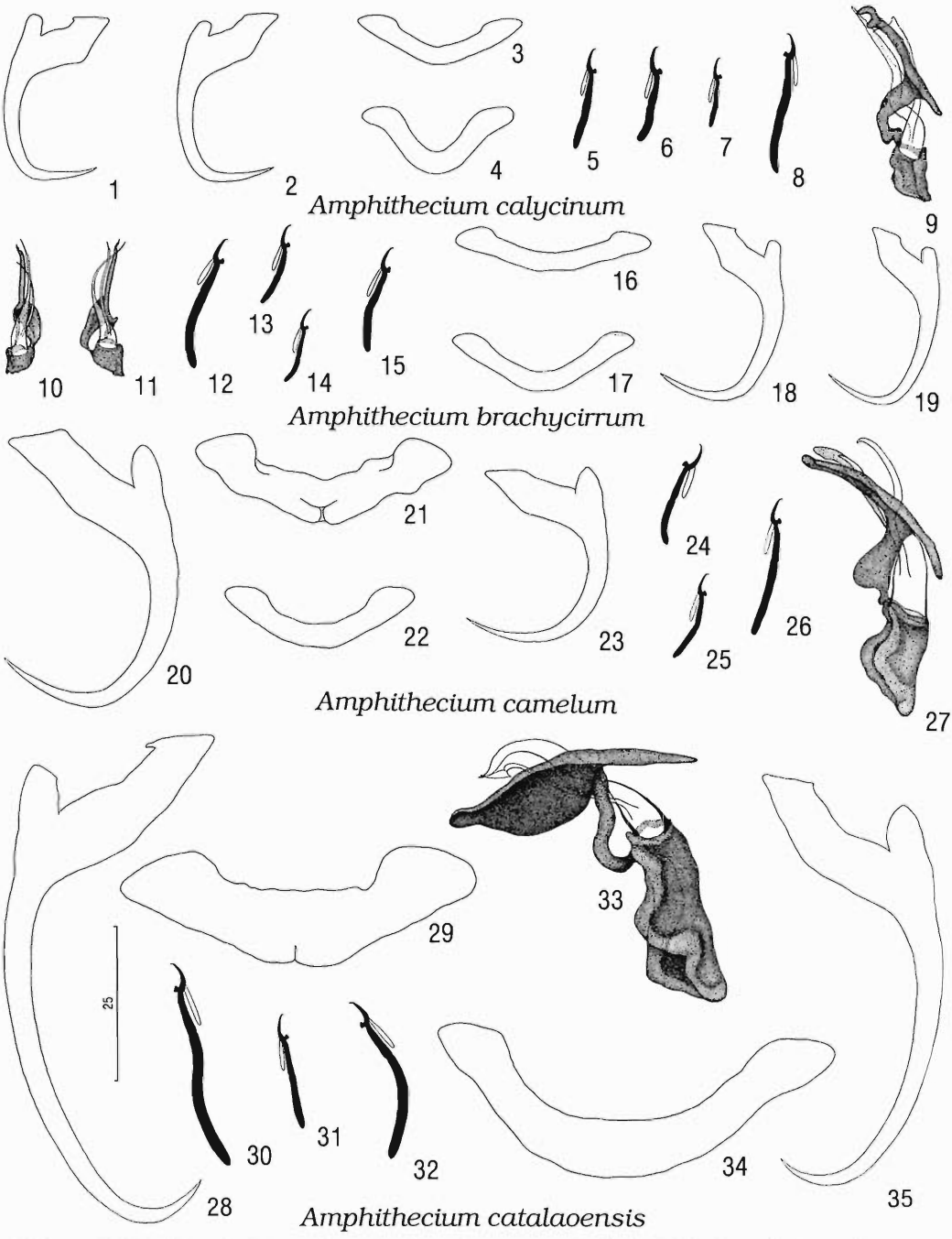
SPECIMENS STUDIED: Twenty-three vouchers, USNPC 85788, 85789.

MEASUREMENTS: Body length 260 (248–272; n = 2), greatest width 112 (n = 2); haptoral length 49 (46–52; n = 2), width 69 (62–75; n = 2); pharyngeal diameter 17 (n = 2); ventral anchor length 28 (26–31; n = 20), base width 12 (11–14; n = 19); dorsal anchor length 28 (26–30; n = 13), base width 12 (10–13; n = 8); ventral bar 30 (27–32; n = 2), dorsal bar 27 (25–29; n = 2) long; hook pair 1—15 (14–16; n = 5), pairs 2, 6—18 (17–20; n = 22), pairs 3, 4, 7—22 (21–24; n = 37), pair 5—13–14 (n = 11) long; copulatory organ length 21 (20–24; n = 13), accessory piece length 16 (15–17; n = 12); testis 53 (n = 1) long, 29 (n = 1) wide; germarium 63 (54–72; n = 2) long, 26 (24–28; n = 2) wide.

REMARKS: *Amphithecium brachycirrum* is apparently restricted to *Pygocentrus nattereri*. Our specimens do not differ significantly from the original description except that the distal rod of the accessory piece extends from a level of the base to the tip of the primary ramus of the copulatory organ. Boeger and Kritsky (1988) missed the proximal extension of the distal rod from its submedial twist. In some specimens, the proximal extension is difficult to observe when it lies over or below the articulation process of the accessory piece.

Amphithecium camelum Boeger and Kritsky, 1988 (Figs. 20–27)

RECORDS: *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989); Furo do Catalão, Manaus,



Figures 1–35. Sclerotized structures of *Amphithecium* spp. 1–9. *Amphithecium calycinum* Boeger and Kritsky, 1988. 1. Ventral anchor. 2. Dorsal anchor. 3. Ventral bar. 4. Dorsal bar. 5. Hook pair 2. 6. Hook pair 1. 7. Hook pair 5. 8. Hook pair 7. 9. Copulatory complex (ventral view). 10–19. *Amphithecium brachycirrum* Boeger and Kritsky, 1968. 10, 11. Copulatory complexes (dorsal views). 12. Hook pair 7. 13. Hook pair 1. 14. Hook pair 5. 15. Hook pair 2. 16. Ventral bar. 17. Dorsal bar. 18. Ventral anchor. 19. Dorsal anchor. 20–27. *Amphithecium camelum* Boeger and Kritsky, 1968. 20. Ventral anchor. 21. Ventral bar. 22. Dorsal bar. 23. Dorsal anchor. 24. Hook pair 1. 25. Hook pair 5. 26. Hook pair 7. 27. Copulatory complex (ventral view). 28–35. *Amphithecium catalaoensis* Boeger and Kritsky, 1968. 28. Ventral anchor.

Amazonas (26, 27 November 1984); Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas (25 November 1984).

PREVIOUS RECORDS: *Pygocentrus nattereri* (type host): Furo do Catalão, Manaus, Amazonas; Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas (type locality); Rio Pacaás-Novos, Guajará-Mirim, Rondônia; Rio Mamoré, Surpresa, Rondônia; Rio Guaporé, Surpresa, Rondônia; Rio Guaporé, Costa Marques, Rondônia (all Boeger and Kritsky, 1988).

SPECIMENS STUDIED: Nineteen vouchers, USNPC 85790, 85791, 85792.

MEASUREMENTS: Body length 456 (387–489; n = 8), greatest width 180 (100–218; n = 9); haptoral length 67 (56–75; n = 9), width 90 (67–103; n = 9); pharyngeal diameter 25 (22–26; n = 9); ventral anchor length 46–47 (n = 7), base width 23 (21–26; n = 7); dorsal anchor length 30 (29–31; n = 5), base width 19 (15–20; n = 5); ventral bar 42 (39–45; n = 7), dorsal bar 34 (31–37; n = 8) long; hook pairs 1, 5—19 (17–21; n = 8), pairs 2, 6—22 (20–23; n = 9), pairs 3, 7—24 (22–26; n = 8), pair 4—28–29 (n = 4) long; copulatory organ 48 (44–55; n = 9) long, accessory piece 33 (31–38; n = 8) long; testis 106 (79–147; n = 4) long, 48 (36–74; n = 4) wide; germarium 123 (98–155; n = 8) long, 56 (33–80; n = 7) wide.

REMARKS: *Amphithecium camelum* is known only from *Pygocentrus nattereri*. Present specimens do not differ significantly from the original description. Boeger and Kritsky (1988) reported 2 forms of this species from distant locations within the Amazon Basin based on comparative morphology of the copulatory organ. The preceding specimens are included in *Amphithecium camelum* forma amazonas, because both rami of the copulatory organ terminate acutely.

Amphithecium catalaoensis Boeger and Kritsky, 1988 (Figs. 28–35)

RECORD: *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989).

PREVIOUS RECORD: *Pygocentrus nattereri* (type host): Furo do Catalão, Manaus, Amazonas (type locality) (Boeger and Kritsky, 1988).

SPECIMENS STUDIED: One voucher, USNPC 85793.

MEASUREMENTS: Ventral anchor length 79 (n = 1), base width 31 (n = 1); dorsal anchor length 71 (n = 1), base width 26 (n = 1); ventral bar 60 (n = 1), dorsal bar 65 (n = 1) long; hook pairs 1, 6—24–25 (n = 3), pair 2—28 (n = 2), pair 3—32–33 (n = 2), pair 4—35 (n = 1), pair 5—20–21 (n = 2), pair 7—30 (n = 1) long; copulatory organ length 56 (n = 1), accessory piece length 41 (n = 1).

REMARKS: Only a single specimen was found on *Pygocentrus nattereri* from central Amazonia. It was similar to those collected by Boeger and Kritsky (1988) from this host in the Furo do Catalão.

Boeger and Kritsky (1988) found only a few specimens of *Amphithecium catalaoensis* from a single location (among 6) within the Amazon Basin. They suggested that *Pygocentrus nattereri* was not a required host or that the parasite originated from the black waters of the Rio Negro. Lago Tapaná is the lower lake of the Rio Uatumã and is characterized by black water during the annual low-water period of the Amazon Basin. During high-water periods, however, the lake contains a mixture of white and black water as a result of back flooding from the main Amazon. These periodic hydrochemical features of Lago Tapaná are similar to those occurring within the Furo do Catalão where *A. catalaoensis* was originally collected. Because *A. catalaoensis* had a low prevalence and intensity on *P. nattereri* in both the Furo do Catalão and Lago Tapaná during respective studies and has not been collected from habitats characterized by either black or white water, it appears that the parasite may be suited to locations in the Amazon Basin where periodic mixing of water types occurs.

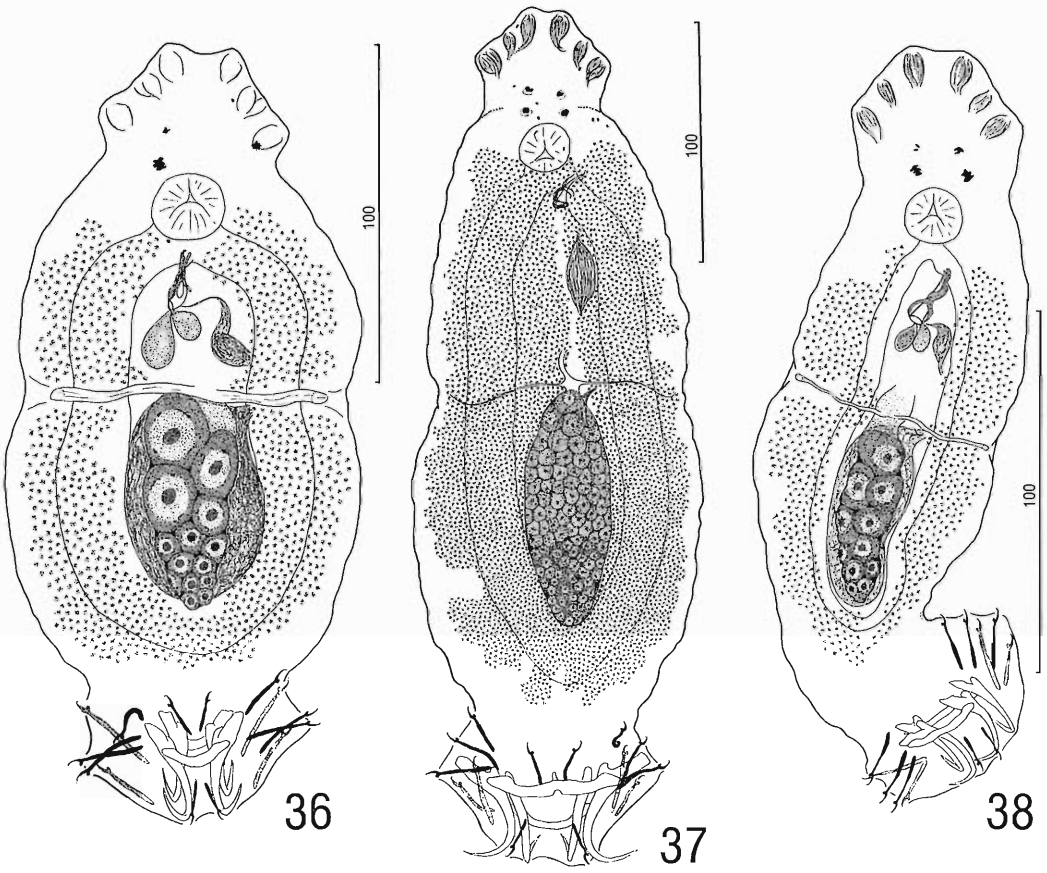
Amphithecium diclonophallum sp. n. (Figs. 36, 39–47)

TYPE HOST AND LOCALITY: *Serrasalmus rhombeus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

OTHER RECORDS: *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus compressus*: Rio Solimões

←

29. Ventral bar. 30. Hook pair 7. 31. Hook pair 5. 32. Hook pair 2. 33. Copulatory complex (ventral view). 34. Dorsal bar. 35. Dorsal anchor. All drawings are to the 25-μm scale.



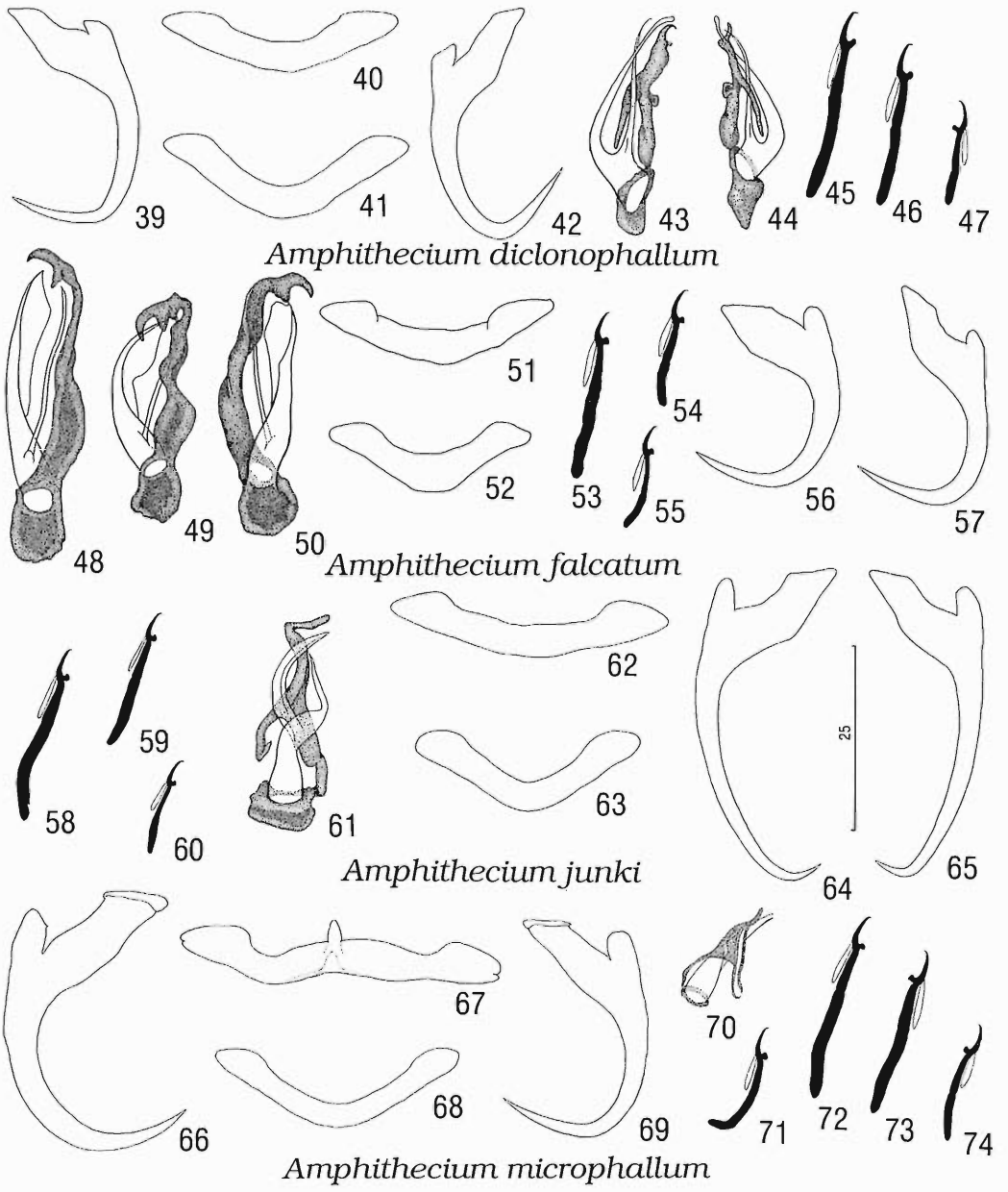
Figures 36–38. Whole-mount illustrations of *Amphithecium* spp. (composite, ventral views). 36. *Amphithecium diclonophallum* sp. n. (from *Serrasalmus rhombeus*). 37. *Amphithecium microphallum* sp. n. (from *Pygocentrus nattereri*). 38. *Amphithecium minutum* sp. n. (from *Serrasalmus spilopleura*). All drawings are to respective 100-μm scales.

near Ilha da Marchantaria, Manaus, Amazonas (28 October 1993). *Serrasalmus elongatus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus rhombeus*: Rio Uatumã, Amazonas (no date). *Serrasalmus* sp. (2 of Jégu): Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989); Santa Luzia, Rio Uatumã, Amazonas (20 September 1985).

SPECIMENS STUDIED: Holotype, INPA PLH 241; 11 paratypes, INPA PLH 242, USNPC 85794, 85795, HWML 38592 from *S. rhombeus*. 1 voucher from *Pristobrycon* sp., USNPC 85801; 2 vouchers from *S. compressus*, USNPC 85800; 5 vouchers from *S. elongatus*, USNPC 85799; 9 vouchers from *S. gouldingi*, USNPC 85802; 5 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 85796, 85797, 85798.

COMPARATIVE MEASUREMENTS: Table 1.

DESCRIPTION: Body broad, fusiform, slightly constricted near midlength; greatest width near midlength. Tegument smooth. Cephalic lobes moderately developed. Eyes 4, equidistant; posterior pair larger than anterior pair; 1 or both members of each pair infrequently absent; accessory granules uncommon in cephalic, anterior trunk regions. Pharynx spherical. Peduncle broad. Anchors similar; each with well-differentiated roots, evenly curved shaft, elongate point. Bars similar; each broadly V- or U-shaped, with small terminal enlargements. Copulatory organ with 2 subequal rami; primary ramus with small terminal bulb, secondary ramus acute; base with small proximal flap. Articula-



Figures 39–74. Sclerotized structures of *Amphithecium* spp. 39–47. *Amphithecium diclonophallum* sp. n. (from *Serrasalmus rhombeus*). 39. Ventral anchor. 40. Ventral bar. 41. Dorsal bar. 42. Dorsal anchor. 43. Copulatory complex (dorsal view). 44. Copulatory complex (ventral view). 45. Hook pair 7. 46. Hook pair 4. 47. Hook pair 5. 48–57. *Amphithecium falcatum* Boeger and Kritsky, 1968 (from *Serrasalmus spilopleura*). 48, 49. Copulatory complexes (dorsal views). 50. Copulatory complex (ventral view). 51. Ventral bar. 52. Dorsal bar. 53. Hook pair 3. 54. Hook pair 1. 55. Hook pair 5. 56. Ventral anchor. 57. Dorsal anchor. 58–65. *Amphithecium junki* Boeger and Kritsky, 1968 (from *Pygocentrus nattereri*). 58. Hook pair 7. 59. Hook pair 1. 60. Hook pair 5. 61. Copulatory complex (dorsal view). 62. Ventral bar. 63. Dorsal bar. 64. Ventral anchor. 65. Dorsal anchor. 66–74. *Amphithecium microphallum* sp. n. (from *Pygocentrus nattereri*). 66. Ventral anchor. 67. Ventral bar. 68. Dorsal bar. 69. Dorsal anchor. 70. Copulatory complex (ventral view). 71. Hook pair 1. 72. Hook pair 7. 73. Hook pair 3. 74. Hook pair 5. All drawings are to the 25-μm scale.

Table 1. Comparative measurements (in micrometers) of *Amphithecium diclonophallum* sp. n., from 6 serrasalmid hosts.

	Pristobrycon sp.	*N*	*Serrasalmus compressus*	*N*	*Serrasalmus elongatus*	*N*	*Serrasalmus gouldingi*	*N*	*Serrasalmus rhombeus*	*N*	*Serrasalmus* sp. (2 of Jégu)	*N*
Body												
Length	—	—	—	—	—	—	246	1	233 (224–239)	4	—	—
Width	—	—	—	—	—	—	117	1	101 (94–109)	4	—	—
Haptor												
Length	—	—	—	—	—	—	54	1	56 (52–63)	3	—	—
Width	—	—	—	—	—	—	76	1	76 (70–81)	3	—	—
Pharynx												
Diameter	—	—	—	—	—	—	19	1	17 (16–19)	4	—	—
Copulatory organ												
Length	34	1	32 (30–34)	2	34 (33–36)	3	35 (33–38)	7	30 (26–32)	7	30–31	5
Accessory piece												
Length	24	1	20 (18–22)	2	21–22	4	22 (20–23)	7	20 (18–22)	6	18 (16–20)	5
Dorsal anchor												
Length	—	—	33	1	36 (33–38)	5	35 (29–37)	6	33 (32–35)	6	33 (32–35)	5
Base width	—	—	15	1	13 (12–14)	4	14 (11–16)	3	14 (13–15)	4	14 (13–15)	4
Ventral anchor												
Length	32	1	34	2	34 (33–35)	5	33 (31–34)	7	32 (31–34)	8	32 (31–33)	5
Base width	13	1	13 (12–14)	2	14–15	5	13–14	6	13–14	6	15 (14–16)	5
Bar length												
Ventral	—	—	—	—	—	—	34 (33–35)	2	31 (29–32)	3	32	1
Dorsal	—	—	—	—	—	—	31	2	29 (28–30)	3	27	1
Hook lengths												
Pair 1	17	1	17	1	17–18	5	17 (14–19)	7	17–18	4	17 (16–18)	4
Pair 2	21	1	19	1	19–20	5	20 (19–21)	8	20 (19–21)	4	19 (18–20)	4
Pair 3	25	1	23	1	23–24	5	23 (22–25)	7	22 (21–23)	8	23 (22–25)	5
Pair 4	—	—	26	1	26–27	5	26 (23–28)	5	26 (25–28)	8	27 (25–29)	4
Pair 5	—	—	15	1	15–16	4	15–16	5	15–16	5	16–17	3
Pair 6	—	—	21	2	19–20	5	19 (16–20)	5	20 (19–21)	5	19–20	3
Pair 7	—	—	25 (24–26)	2	26–27	4	26 (20–29)	6	26 (25–28)	6	26 (24–27)	4
Germarium												
Length	—	—	—	—	—	—	47	1	48 (41–61)	4	—	—
Width	—	—	—	—	—	—	36	1	27 (24–31)	4	—	—
Testis												
Length	—	—	—	—	—	—	55	1	52 (48–60)	3	—	—
Width	—	—	—	—	—	—	31	1	28 (24–31)	3	—	—

tion process of accessory piece with short subterminal flap, distal rod robust with hooked end. Testis ovate. Germarium conical; oviduct, ootype, uterus not observed; vitellaria dense throughout trunk, absent in regions of reproductive organs.

REMARKS: This species resembles *Amphithecium speirocamarotum* sp. n. in the comparative morphology of the accessory piece. *Amphithecium diclonophallum* is distinct in possessing anchors with short shafts, a bulbous termination of the primary ramus of the copulatory organ, and an elongate acute secondary ramus of the copulatory organ. The specific name is from Greek (*di* ["two"] + *klon* ["branch"] + *phallos* ["penis"]) and refers to the copulatory organ.

Amphithecium falcatum Boeger and Kritsky, 1988 (Figs. 48–57)

RECORDS: *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná,

near Santana, Amazonas (3 November 1989); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus compressus*: Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (27, 28 October 1993). *Serrasalmus elongatus*: Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus manuelli*: Kaikuta, Rio Xingu, Pará (10 October 1992). *Serrasalmus rhombeus*: Rio Capucapú at its confluence with Rio Jatapú, Cachoeira das Garças, Amazonas (31 October 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989). *Serrasalmus spilopleura*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989). *Serrasalmus* sp. (2 of Jégu): Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989). *Serrasalmus* sp. (2n = 58): Furo do Catalão, near Manaus, Amazonas (5 January 1989); Ilha do Careiro, near Manaus, Amazonas.

PREVIOUS RECORDS: *Pygocentrus nattereri* (type host): Furo do Catalão, Manaus, Amazonas (type locality); Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas; Rio Pacaás-Novos, Guajará-Mirim, Rondônia; Rio Mamoré, Surpresa, Rondônia; Rio Guaporé, Surpresa, Rondônia; Rio Guaporé, Costa Marques, Rondônia (all Boeger and Kritsky, 1988).

SPECIMENS STUDIED: Two vouchers from *Pristobrycon* sp., USNPC 85812; 16 vouchers from *Pygocentrus nattereri*, USNPC 85808, 85809; 12 vouchers from *Serrasalmus compressus*, USNPC 85815; 15 vouchers from *S. elongatus*, USNPC 85807; 37 vouchers from *S. gouldingi*, USNPC 85811; 3 vouchers from *S. manuelli*, USNPC 85810; 28 vouchers from *S. rhombeus*, USNPC 85804, 85805, 85806; 21 vouchers from *S. spilopleura*, USNPC 85803; 14 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 85816, 85817; 50 vouchers from *Serrasalmus* sp. (2n = 58), USNPC 85813, 85814.

COMPARATIVE MEASUREMENTS: Table 2.

REMARKS: *Amphithecium falcatum* is known from 10 species of *Pygocentrus*, *Pristobrycon*, and *Serrasalmus*. Specimens from respective hosts showed minimal variation in morphology and size. *Amphithecium falcatum* resembles *A. unguiculum* in having the distal rod of the accessory piece incorporated into the proximal articulation process. However, *A. falcatum* has a terminal hook of the distal rod of the accessory piece, whereas that of *A. unguiculum* is C-shaped.

Amphithecium junki Boeger and Kritsky, 1988 (Figs. 58–65)

RECORDS: *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989); Furo do Catalão, Manaus, Amazonas (26, 27 November 1984); Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas (25 November 1984). *Serrasalmus rhombeus*: Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas (26 November 1984).

PREVIOUS RECORDS: *Pygocentrus nattereri* (type host): Furo do Catalão, Manaus, Amazonas (type locality); Ilha da Marchantaria, Rio Solimões, Manaus, Amazonas; Rio Pacaás-Novos, Guajará-Mirim, Rondônia; Rio Mamoré, Surpresa, Rondônia; Rio Guaporé, Surpresa, Rondônia; Rio Guaporé, Costa Marques, Rondônia (all Boeger and Kritsky, 1988).

SPECIMENS STUDIED: Twenty-one vouchers from *P. nattereri*, USNPC 85819, 85820, 85821; 1 voucher from *S. rhombeus*, USNPC 85818.

COMPARATIVE MEASUREMENTS (dimensions of the specimen from *S. rhombeus* follow those of *P. nattereri* in brackets): Body length 280 (n = 1), greatest width 112 (n = 1); haptoral length 66 (n = 1), width 85 (n = 1); pharyngeal diameter 15 (n = 1); ventral anchor length 43 (40–45; n = 17) [41 (40–43; n = 2)], base width 16 (15–17; n = 12) [17 (n = 2)]; dorsal anchor length 43 (39–45; n = 11) [41 (40–42; n = 2)], base width 16 (13–17; n = 7) [15 (14–16; n = 2)]; ventral bar 34 (n = 1), dorsal bar 32 (n = 1) long; hook pairs 1, 2, 6—21 (17–23; n = 39) [20–21 (n = 2)], pairs 3, 4, 7—26 (22–28; n = 43) [26–27 (n = 5)], pair 5—14–15 (n = 11) [15 (14–16; n = 2)] long; copulatory organ length 27 (24–29; n = 14) [28 (n = 1)], accessory piece length 22 (20–27; n = 10) [24 (n = 1)]; testis 64 (n = 1) long, 28 (n = 1) wide; germarium 66 (n = 1) long, 25 (n = 1) wide.

REMARKS: *Amphithecium junki* normally occurs on *Pygocentrus nattereri*. The specimen from *Serrasalmus rhombeus* is probably accidental.

Table 2. Comparative measurements (in micrometers) of *Amphithecium falcatum* Boeger and Kritsky, 1988, from 10 serrasalmid hosts.

	Pristobrycon sp.	*N*	*Pygocentrus nattereri*	*N*	*Serrasalmus compressus*	*N*	*Serrasalmus elongatus*	*N*	*Serrasalmus gouldingi*	*N*
Body										
Length	—	—	249 (222–275)	2	—	—	249 (217–285)	3	325 (257–373)	17
Width	—	—	113 (111–115)	2	—	—	78 (64–86)	3	121 (96–152)	19
Haptor										
Length	—	—	45–46	2	—	—	54 (50–59)	3	65 (55–78)	18
Width	—	—	67 (66–69)	2	—	—	74 (72–79)	3	92 (78–107)	18
Pharynx										
Diameter	—	—	18	2	—	—	16 (14–17)	3	21 (18–23)	19
Copulatory organ										
Length	44	1	40 (38–48)	12	37 (35–39)	3	39 (38–41)	6	43 (40–48)	12
Accessory piece										
Length	34	1	32 (28–41)	12	30 (27–32)	3	33 (31–36)	5	36 (34–39)	12
Dorsal anchor										
Length	36 (35–37)	2	31(28–36)	14	31 (30–32)	3	31 (29–34)	9	37 (33–40)	12
Base width	14 (12–16)	2	14 (12–16)	9	14	2	13 (12–14)	8	15 (13–16)	9
Ventral anchor										
Length	31	2	28 (24–31)	15	27 (26–28)	4	27 (24–28)	10	31 (28–34)	15
Base width	18 (16–19)	2	15 (14–16)	14	15	2	14–15	10	16 (14–19)	11
Bar length										
Ventral	—	—	26	1	—	—	28 (26–30)	3	30 (27–34)	11
Dorsal	—	—	22	1	—	—	25 (23–26)	3	28 (24–34)	13
Hook lengths										
Pair 1	17	1	17 (16–18)	8	16	3	16–17	8	18 (16–19)	8
Pair 2	—	—	20 (18–22)	10	21 (20–22)	2	21 (19–22)	7	22 (21–25)	7
Pair 3	24	1	24 (23–26)	12	24 (22–25)	3	24 (23–25)	9	26 (25–28)	11
Pair 4	26	1	25 (24–27)	11	25 (23–27)	3	25 (24–26)	6	27 (25–28)	12
Pair 5	—	—	14–15	10	14	2	14–15	4	15 (14–16)	6
Pair 6	—	—	18 (16–19)	10	18	2	18 (17–19)	8	19 (18–21)	12
Pair 7	27	1	23 (21–25)	9	21–22	3	22 (21–24)	4	26 (24–27)	10
Germarium										
Length	—	—	59 (57–60)	2	—	—	53 (41–72)	3	66 (51–88)	18
Width	—	—	31 (28–33)	2	—	—	23 (18–27)	3	32 (28–35)	18
Testis										
Length	—	—	66 (56–75)	2	—	—	47 (41–55)	3	68 (54–88)	14
Width	—	—	37 (35–39)	2	—	—	23 (16–31)	3	34 (26–45)	14

Amphithecium microphallum sp. n. (Figs. 37, 66–74)

TYPE HOST AND LOCALITY: *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989).

OTHER RECORD: *Serrasalmus* sp. (2n = 58): Furo do Catalão, Manaus, Amazonas (30 January 1991).

SPECIMENS STUDIED: Holotype, INPA PLH 236; 17 paratypes, INPA PLH 237, USNPC 85822, HWML 38593 from *Pygocentrus nattereri*; 2 vouchers from *Serrasalmus* sp. (2n = 58), USNPC 85823.

COMPARATIVE MEASUREMENTS: Measurements of specimens from *Serrasalmus* sp. (2n = 58) are in brackets following those of the type series.

DESCRIPTION: Body 343 (289–401; n = 9) long, robust, fusiform, slightly flattened dorsoventrally, with inconspicuous constriction near midlength; greatest width 122 (94–146; n = 9) usually in posterior trunk. Tegument smooth.

Table 2. Extended.

Serrasalmus manuelli	*N*	*Serrasalmus rhombeus*	*N*	*Serrasalmus spilopleura*	*N*	*Serrasalmus* sp. (2 of Jégu)	*N*	*Serrasalmus* sp. (2n = 58)	*N*
—	—	266 (208–345)	6	253 (219–294)	7	—	—	215 (190–261)	9
—	—	103 (88–110)	5	94 (79–108)	8	—	—	87 (61–102)	9
—	—	53 (41–61)	6	52 (43–62)	7	—	—	48 (42–57)	7
—	—	82 (75–96)	5	80 (71–95)	6	—	—	73 (70–78)	6
—	—	18 (17–19)	6	18 (16–20)	8	—	—	18 (15–24)	9
43	2	42 (38–45)	16	34 (29–42)	9	40 (35–43)	11	40 (35–44)	19
32 (30–35)	2	34 (31–37)	12	29 (26–32)	6	32 (29–35)	10	32 (27–37)	17
35 (32–36)	3	34 (28–36)	14	31 (28–33)	12	33 (29–37)	11	32 (29–36)	26
14–15	2	15 (13–16)	8	13 (12–14)	9	14 (10–16)	7	14 (11–15)	14
29	3	29 (24–31)	13	27 (25–29)	13	28 (26–30)	12	28 (25–30)	27
15 (14–16)	3	16 (15–17)	11	14–15	12	16 (15–18)	12	15 (14–16)	23
—	—	29 (28–30)	3	28 (26–29)	5	—	—	29 (25–38)	7
—	—	24 (22–28)	5	24 (22–26)	6	—	—	26 (19–33)	6
16	1	17 (16–18)	8	16 (15–17)	11	16–17	5	17 (16–18)	17
21–22	3	21 (20–22)	7	22 (20–23)	9	21 (20–22)	9	21 (18–25)	19
26–27	2	26 (24–28)	10	24 (23–26)	12	24–25	7	26 (23–29)	21
26 (25–28)	3	27 (25–28)	8	26 (25–27)	9	26 (24–27)	8	26 (25–29)	27
14	1	14–15	4	14 (13–15)	8	15 (14–16)	6	15 (14–16)	16
19 (18–21)	3	19 (18–21)	13	18 (17–19)	6	18 (17–20)	7	19 (17–20)	17
25 (24–27)	3	25 (24–26)	11	23 (22–24)	9	24 (22–25)	8	23 (21–27)	18
—	—	51 (39–66)	6	46 (35–60)	6	—	—	42 (31–58)	9
—	—	24 (20–29)	6	29 (22–32)	6	—	—	24 (18–28)	9
—	—	50 (39–62)	5	59 (46–68)	5	—	—	45 (35–55)	5
—	—	26 (18–31)	5	30 (25–35)	5	—	—	27 (20–31)	5

Cephalic region narrow in comparison to trunk, directed anteromedially from trunk; lobes moderately developed. Eyes 4, equidistant; posterior members larger; accessory granules usually numerous in cephalic, anterior trunk regions. Pharynx spherical, 21 (19–24; n = 9) in diameter. Peduncle broad; haptor 64 (52–73; n = 9) long, 92 (80–99; n = 9) wide. Anchors similar; each with elongate slightly depressed superficial root, prominent deep root, curved shaft, elongate point; shaft, point of ventral anchor forming even arc; ventral anchor 37 (35–40; n = 9) [36–37 (n = 4)] long, base 21 (18–22; n = 8) [21–22 (n = 4)] wide; dorsal anchor 32 (31–33; n = 8) [33 (32–34; n = 3)] long, base 16 (14–17; n = 6) [18 (n = 1)] wide. Ventral bar 42 (38–44; n = 8) long, wavy or straight, with enlarged terminations, short anteromedial projection, posterodorsal V-shaped indentation. Dorsal bar 34 (33–36; n = 7) long, broadly U-shaped, with small terminal enlargements. Hook pairs 1, 5—18 (16–20; n = 8) [16–17 (n = 4)], pairs 2, 3, 6—22 (21–24; n = 22) [23 (22–25; n = 6)], pairs 4, 7—26 (23–28; n = 17) [26 (24–28; n

= 6)] long. Copulatory organ 17 (16–18; n = 4) [16–17 (n = 2)] long, delicate, tubular, tapered; base lacking proximal flap. Accessory piece 13 (12–15; n = 5) [13–14 (n = 2)] long, with double distal rod blunt terminally. Gonads elongate ovate; testis 56 (47–61; n = 3) long, 24–25 (n = 3) wide; prostatic reservoirs not observed. Germarium 77 (57–92; n = 8) long, 29 (26–32) wide; oviduct, ootype not observed; vaginae with delicate narrow lateral canals, vaginal apertures uncertain. Vitellaria dense throughout trunk except absent along midline.

REMARKS: *Amphithecium microphallum* is unique in having an anteromedial process and a posterodorsal indentation on the ventral bar. The specific name is from Greek (*mikros* ["small"] + *phallos* ["penis"]) and refers to the comparatively small copulatory complex.

Amphithecium minutum sp. n. (Figs. 38, 78–85)

TYPE HOST AND LOCALITY: *Serrasalmus spilopleura*: Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (14 September 1984).

OTHER RECORDS: *Pristobrycon eigenmanni*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Nazaré, Rio Uatumã, Amazonas (17 September 1985); Santa Luzia, Rio Uatumã, Amazonas (20 September 1985); Rio Negro near Manaus, Amazonas (28 December 1988). *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus spilopleura*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 246; 17 paratypes, INPA PLH 247, USNPC 85824, 85825, HWML 38594 from *S. spilopleura*; 27 vouchers from *P. eigenmanni*, USNPC 85826, 85827, 85828, 85829; 3 vouchers from *Pristobrycon* sp., USNPC 85830; 12 vouchers from *S. gouldingi*, USNPC 85831.

COMPARATIVE MEASUREMENTS: Table 3.

DESCRIPTION: Body fusiform, constricted near midlength; greatest width in anterior or posterior trunk. Tegument smooth or with scaled annulations. Cephalic lobes moderately developed. Eyes 4, equidistant; posterior pair larger than anterior pair; 1 or both members of anterior pair frequently absent; accessory granules uncommon in cephalic, anterior trunk regions. Pharynx spherical. Peduncle broad. Anchors similar; each with well-developed roots, evenly curved shaft, elongate point. Bars similar; each broadly V- or U-shaped, with small terminal enlargements. Copulatory organ tapered, conical, frequently sigmoid; base with prominent proximal flap. Articulation process of accessory piece bowed; distal rod uniting with base of copulatory organ, distally variable, blunt. Testis subovate. Germarium conical; oviduct short; ootype, uterus not observed; vaginae slightly expanded immediately proximal to vaginal openings. Vitellaria dense throughout trunk, absent in regions of reproductive organs.

REMARKS: *Amphithecium minutum* differs from congeneric species by having both the articulation process and distal rod of the accessory piece articulating to the base of the copulatory organ. The specific name is from Latin (*minuta* ["small"]) and refers to the small size of this helminth.

Amphithecium muricatum sp. n. (Figs. 75, 86–94)

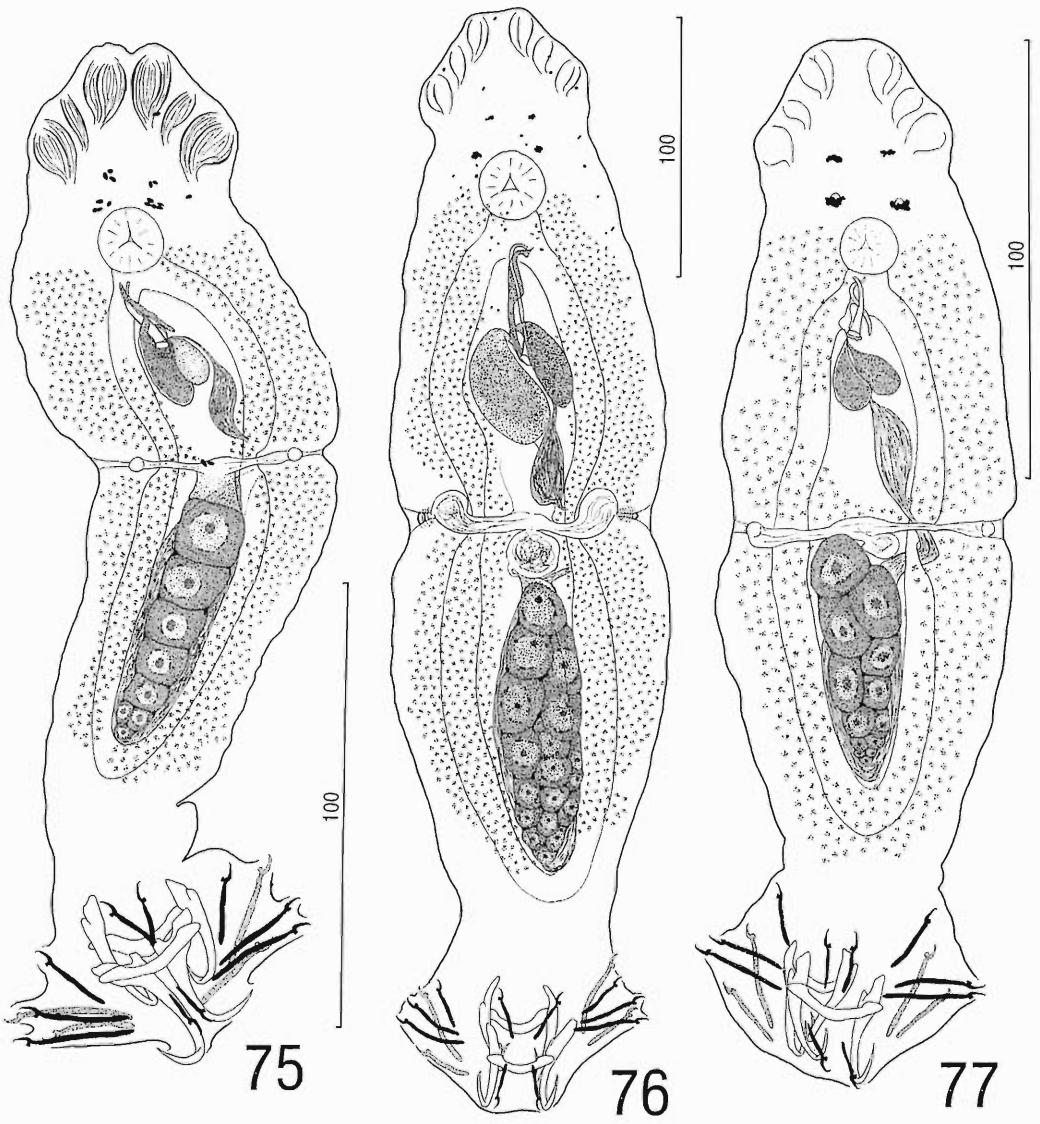
TYPE HOST AND LOCALITY: *Pristobrycon eigenmanni*: Nazaré, Rio Uatumã, Amazonas (17 September 1985).

OTHER RECORDS: *Pristobrycon eigenmanni*: Santa Luzia, Rio Uatumã, Amazonas (20 September 1985). *Serrasalmus rhombeus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Pitinga, Igarapé Água Branca, Rio Uatumã, Amazonas (15 September 1985). *Serrasalmus* sp. (2 of Jégu): Nazaré, Rio Uatumã, Amazonas (17 September 1985).

SPECIMENS STUDIED: Holotype, INPA PLH 238; 18 paratypes, INPA PLH 239, PLH 240, USNPC 85832, 85833, HWML 38595 from *Pristobrycon eigenmanni*; 16 vouchers from *Serrasalmus rhombeus*, USNPC 85835, 85836; 1 voucher from *Serrasalmus* sp. (2 of Jégu), USNPC 85834.

COMPARATIVE MEASUREMENTS: Table 4.

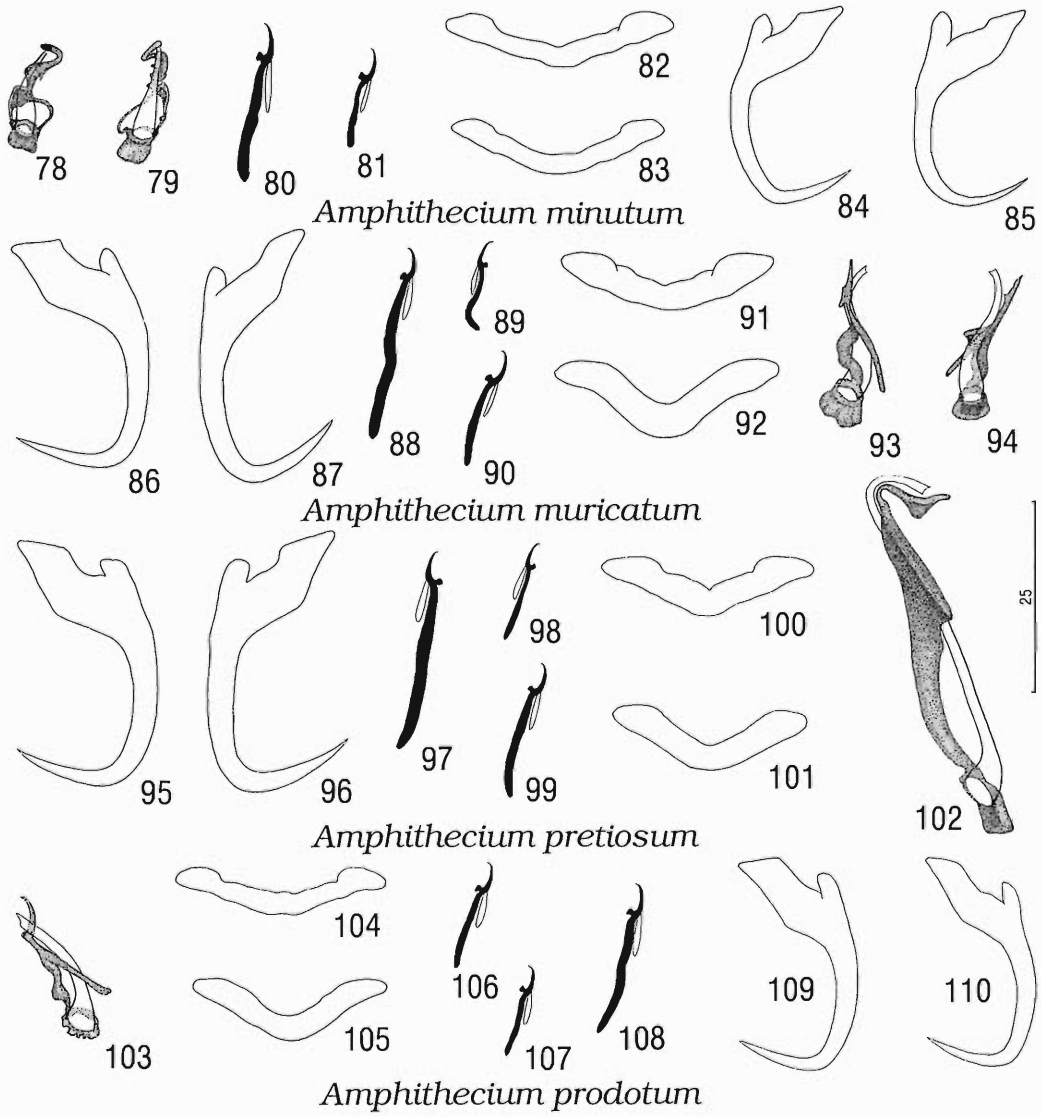
DESCRIPTION: Body fusiform; trunk constricted near midlength, tapered posteriorly; greatest width usually in anterior trunk. Tegument smooth or with scaled annulations. Cephalic lobes moderately developed. Eyes 4, equidistant, comprised of few loosely associated granules; posterior members larger, farther apart than anterior pair; accessory granules in cephalic, anterior trunk regions. Pharynx spherical. Peduncle moderate to narrow. Anchors similar; each with well-differentiated slightly depressed



Figures 75–77. Whole-mount illustrations of *Amphithecium* spp. (composite, ventral views). 75. *Amphithecium muricatum* sp. n. (from *Pristobrycon eigenmanni*). 76. *Amphithecium pretiosum* sp. n. (from *Serrasalmus gouldingi*). 77. *Amphithecium prodotum* sp. n. (from *Pristobrycon striolatus*). All drawings are to respective 100-μm scales.

superficial root, short deep root, gently curved shaft, elongate point. Ventral bar broadly V-shaped, with enlarged terminations; dorsal bar bent at midlength, with ends directed laterally. Copulatory organ sigmoid, tapered; base with short proximal flap. Distal rod of accessory piece straight, terminally pointed, with subterminal expansion. Testis elongate ovate; prostatic reservoirs large. Germarium conical; oviduct short; ootype, uterus not observed. Vitellaria throughout trunk except absent in regions of reproductive organs.

REMARKS: The haptoral armaments of *Amphithecium muricatum*, *A. minutum*, and *A. prodotum* are similar. *Amphithecium muricatum* differs from *A. minutum* by having a free proximal end of the distal rod of the accessory piece (articulated with base of copulatory organ in *A.*



Figures 78–110. Sclerotized structures of *Amphithecium* spp. 78–85. *Amphithecium minutum* sp. n. (from *Serrasalmus spilopleura*). 78. Copulatory complex (ventral view). 79. Copulatory complex (dorsal view). 80. Hook pair 7. 81. Hook pair 1. 82. Ventral bar. 83. Dorsal bar. 84. Ventral anchor. 85. Dorsal anchor. 86–94. *Amphithecium muricatum* sp. n. (from *Pristobrycon eigenmanni*). 86. Ventral anchor. 87. Dorsal anchor. 88. Hook pair 7. 89. Hook pair 5. 90. Hook pair 1. 91. Ventral bar. 92. Dorsal bar. 93. Copulatory complex (ventral view). 94. Copulatory complex (dorsal view). 95–102. *Amphithecium pretiosum* sp. n. (from *Serrasalmus gouldingi*). 95. Ventral anchor. 96. Dorsal anchor. 97. Hook pair 7. 98. Hook pair 5. 99. Hook pair 2. 100. Ventral bar. 101. Dorsal bar. 102. Copulatory complex (ventral view). 103–110. *Amphithecium prodotum* sp. n. (from *Pristobrycon striolatus*). 103. Copulatory complex (ventral view). 104. Ventral bar. 105. Dorsal bar. 106. Hook pair 1. 107. Hook pair 5. 108. Hook pair 7. 109. Ventral anchor. 110. Dorsal anchor. All drawings are to the 25-μm scale.

Table 3. Comparative measurements (in micrometers) of *Amphithecium minutum* sp. n., from 4 serrasalmid hosts.

	Pristobrycon eigenmanni	*N*	*Pristobrycon* sp.	*N*	*Serrasalmus gouldingi*	*N*	*Serrasalmus spilopleura*	*N*
Body								
Length	209 (170–245)	11	—	—	305 (273–329)	6	226 (195–271)	9
Width	58 (45–72)	14	—	—	87 (73–96)	6	74 (61–92)	9
Haptor								
Length	51 (46–59)	13	—	—	63 (55–78)	5	49 (44–59)	9
Width	60 (45–67)	13	—	—	83 (71–107)	5	65 (57–74)	9
Pharynx								
Diameter	13 (11–15)	13	—	—	16–17	6	15 (14–16)	9
Copulatory organ								
Length	18 (16–20)	8	19–20	2	19 (18–21)	6	19 (15–20)	6
Accessory piece								
Length	16 (14–17)	8	16–17	3	17 (16–18)	6	15 (12–17)	6
Dorsal anchor								
Length	31 (28–32)	9	30 (29–31)	3	31 (30–32)	5	27 (25–29)	8
Base width	12 (11–14)	9	12–13	3	11 (10–13)	3	11 (9–13)	7
Ventral anchor								
Length	32 (28–34)	12	30 (29–31)	3	30 (29–31)	5	26 (23–27)	8
Base width	12 (11–13)	12	12–13	3	12–13	5	11 (10–12)	8
Bar length								
Ventral	28 (26–30)	11	—	—	30-31	6	29 (26-30)	5
Dorsal	27 (24–28)	11	—	—	28 (25–29)	6	27 (24–29)	5
Hook lengths								
Pair 1	14 (13–15)	6	13	1	13–14	3	12–13	5
Pair 2	19 (17–20)	10	19 (18–20)	2	20 (19–21)	3	19–20	6
Pair 3	22 (20–23)	11	22–23	3	22–23	3	21 (20–22)	7
Pair 4	24 (21–25)	10	23 (22–24)	3	22 (21–23)	4	22 (21–23)	7
Pair 5	13 (12–14)	9	13 (12–14)	2	13	4	12–13	5
Pair 6	17 (16–18)	10	17–18	3	16–17	4	15–16	8
Pair 7	24 (22–26)	9	25 (23–26)	3	25 (24–26)	5	23 (22–25)	8
Germarium								
Length	38 (34–42)	6	—	—	48 (32–59)	6	44 (41–46)	4
Width	12 (10–13)	5	—	—	21 (17–23)	5	19–20	4
Testis								
Length	35 (31–42)	3	—	—	59 (51–71)	4	48 (47–49)	3
Width	13 (12–15)	3	—	—	23 (20–26)	3	20 (16–22)	3

minutum) and by lacking enlarged ends on the dorsal bar. The distal rod of the accessory piece of *A. prodotum* has an indistinct distal hook (rod lacking hook in *A. muricatum*). The specific name is from Latin (*muricatus* ["pointed"]) and refers to the copulatory organ.

Amphithecium pretiosum sp. n. (Figs. 76, 95–102)

TYPE HOST AND LOCALITY: *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

OTHER RECORDS: *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus gouldingi*: Rio Uatumã, C. Miriti, Amazonas (26 September 1985). *Serrasalmus manuelli*: Kaikuta, Rio Xingu, Pará (10 October 1992).

SPECIMENS STUDIED: Holotype, INPA PLH 243; 45 paratypes, INPA PLH 244, PLH 245, USNPC 85838, 85839, HWML 38596 from *S. gouldingi*; 2 vouchers from *Pristobrycon* sp., USNPC 85840; 50 vouchers from *S. manuelli*, USNPC 85837.

Table 4. Comparative measurements (in micrometers) of *Amphithecium muricatum* sp. n., from 3 serrasalmid hosts.

	Pristobrycon eigenmanni	*N*	*Serrasalmus rhombeus*	*N*	*Serrasalmus* sp. (2 of Jégu)	*N*
Body						
Length	219 (185–288)	6	241 (217–264)	9	—	—
Width	57 (49–62)	7	92 (69–115)	9	—	—
Haptor						
Length	58 (51–66)	6	55 (49–61)	9	—	—
Width	58 (48–69)	6	77 (75–85)	8	—	—
Pharynx						
Diameter	12 (10–14)	7	16 (15–19)	9	—	—
Copulatory organ						
Length	21 (19–24)	8	18 (16–19)	6	21	1
Accessory piece						
Length	17 (16–19)	9	15 (14–16)	6	17	1
Dorsal anchor						
Length	34 (32–36)	7	35 (33–38)	6	32	1
Base width	12 (11–13)	6	13 (12–14)	6	13	1
Ventral anchor						
Length	32 (30–33)	7	33 (31–35)	5	30–31	2
Base width	13 (12–15)	7	14 (13–15)	5	13	2
Bar length						
Ventral	24–25	3	31 (29–33)	6	—	—
Dorsal	23–24	3	30 (27–33)	7	—	—
Hook lengths						
Pair 1	17 (16–18)	9	17 (16–18)	5	17	1
Pair 2	19 (18–21)	6	19–20	4	—	—
Pair 3	23 (21–24)	6	22 (21–23)	5	26	1
Pair 4	25 (21–27)	6	25 (23–27)	5	25	1
Pair 5	14	5	14–15	3	15	1
Pair 6	18 (16–19)	6	17 (16–19)	6	19	1
Pair 7	28 (26–29)	7	26 (25–28)	4	29	1
Germarium						
Length	41 (30–54)	5	52 (38–65)	9	—	—
Width	12 (11–13)	5	23 (13–31)	9	—	—
Testis						
Length	42 (32–47)	4	57 (48–65)	7	—	—
Width	11 (10–13)	4	26 (18–31)	7	—	—

COMPARATIVE MEASUREMENTS: Table 5.

DESCRIPTION: Body fusiform, with constriction at midlength; greatest width usually in anterior trunk. Tegument smooth or with scaled annulations. Cephalic lobes moderately developed. Eyes 4; posterior members larger, farther apart than anterior pair; accessory granules in cephalic, anterior trunk regions. Pharynx spherical. Peduncle broad. Anchors similar; each with well-differentiated depressed roots, straight to slightly arcuate shaft, elongate point. Ventral bar broadly V-shaped, with enlarged terminations; dorsal bar broadly U-shaped, with slightly enlarged ends. Copulatory organ frequently recurved distally; base with short proximal flap. Articulation process of accessory piece elongate; distal rod with small subterminal keel, blunt. Gonads elongate ovate. Vaginae encircled subterminally by muscle fibers; seminal receptacle a spherical expansion of posterior wall of vaginae, with short connecting duct arising from posterodextral wall to anterior germarial duct (oviduct); vitellaria throughout trunk except ab-

Table 5. Comparative measurements (in micrometers) of *Amphithecium pretiosum* sp. n., from 3 serrasalmid hosts.

	Pristobrycon sp.	*N*	*Serrasalmus gouldingi*	*N*	*Serrasalmus manuelli*	*N*
Body						
Length	—	—	352 (298–428)	20	237	1
Width	—	—	106 (92–132)	19	85	1
Haptor						
Length	—	—	64 (51–79)	18	68	1
Width	—	—	88 (71–99)	20	70	1
Pharynx						
Diameter	—	—	19 (18–23)	20	14	1
Copulatory organ						
Length	55–56	2	55 (48–66)	22	64 (54–78)	35
Accessory piece						
Length	47–48	2	46 (41–58)	23	55 (45–66)	33
Dorsal anchor						
Length	33	1	36 (34–38)	17	38 (36–40)	17
Base width	13	1	14 (13–16)	13	16 (15–18)	13
Ventral anchor						
Length	32	1	34 (32–36)	22	35 (33–37)	20
Base width	12	1	14 (13–17)	20	15 (14–18)	19
Bar length						
Ventral	—	—	29 (28–31)	16	—	—
Dorsal	—	—	27 (25–29)	15	—	—
Hook lengths						
Pair 1	16	1	18 (17–19)	12	18 (17–19)	7
Pair 2	19	1	19 (18–21)	13	19 (18–20)	10
Pair 3	23	1	24 (23–25)	17	24 (22–26)	8
Pair 4	23	1	27 (26–28)	17	27 (25–29)	12
Pair 5	—	—	15 (14–16)	14	15 (14–16)	11
Pair 6	18	1	19 (18–21)	18	20 (19–21)	7
Pair 7	26	1	28 (26–30)	19	29 (28–31)	7
Germarium						
Length	—	—	65 (40-84)	17	42	1
Width	—	—	26 (19–35)	17	17	1
Egg						
Length	—	—	44 (42–47)	2	—	—
Width	—	—	33 (30–35)	2	—	—
Testis						
Length	—	—	69 (45–77)	11	41	1
Width	—	—	31 (23–39)	11	21	1

sent in regions of reproductive organs. Egg subovate, with short proximal filament.

REMARKS: *Amphithecium pretiosum* possesses circular muscle fibers around the vaginal ducts and a small subterminal keel on the distal rod of the accessory piece, which differentiate it from all other congeneric species. The specific name is from Latin (*pretiosus* ["of great value"]).

Amphithecium prodotum sp. n. (Figs. 77, 103–110)

TYPE HOST AND LOCALITY: *Pristobrycon striolatus*: Rio Capucapú at its confluence with Rio Jatapú, Cachoeira das Garças, Amazonas (31 October 1989).

OTHER RECORDS: *Catoprion mento*: Balbina, Rio Uatumã, Amazonas (20 September 1985);

Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989). *Pristobrycon striolatus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Santa Luzia, Rio Uatumã, Amazonas (20 September 1985); Lago Samaumá, Rio Uatumã, Amazonas (25 September 1985); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (26 November 1984).

SPECIMENS STUDIED: Holotype, INPA PLH 263; 28 paratypes, INPA PLH 329, PLH 330, USNPC 85841, 85842, 85843, 85844, HWML 38597 from *P. striolatus*; 25 vouchers, USNPC 85845, 85846, 85847 from *C. mento*.

COMPARATIVE MEASUREMENTS: Dimensions of specimens from *C. mento* follow those of *P. striolatus* in brackets.

DESCRIPTION: Body fusiform, with slight constriction near midlength; length 248 (202–349; n = 15) [245 (219–280; n = 5)], greatest width 70 (60–93; n = 16) [65 (52–73; n = 5)] in anterior or posterior trunk. Tegument smooth. Cephalic lobes moderately developed. Eyes 4; posterior members with lens, larger, slightly farther apart than anterior pair; accessory granules absent or few in cephalic, anterior trunk regions. Pharynx spherical, 15 (13–17; n = 17) [14 (13–16; n = 4)] in diameter. Peduncle broad; haptor 50 (44–68; n = 16) [49 (43–59; n = 5)] long, 65 (58–75; n = 15) [63 (57–68; n = 4)] wide. Anchors similar; each with elongate slightly depressed superficial root, prominent deep root, slightly curved shaft, long point; ventral anchor 29 (25–31; n = 10) [30 (29–32; n = 10)] long, base 12 (11–13; n = 8) [12 (11–14; n = 7)] wide; dorsal anchor 30 (26–33; n = 9) [31 (30–33; n = 6)] long, base 13 (11–14; n = 8) [14 (12–15; n = 6)] wide. Ventral bar 27 (25–29; n = 11) [28 (27–29; n = 3)] long, slightly bent at midlength, with enlarged terminations; dorsal bar 24 (22–26; n = 14) [24–25 (n = 3)] long, broadly U-shaped, ends directed laterally. Hook pair 1—15 (14–16; n = 8) [16 (14–17; n = 8)], pairs 2, 6—17 (16–19; n = 13) [19 (17–20; n = 11)], pair 3—20 (19–22; n = 7) [21 (19–23; n = 8)], pairs 4, 7—23 (21–26; n = 14) [25 (22–27; n = 19)], pair 5—13 (n = 4) [13–14 (n = 4)] long. Copulatory organ 22 (20–24; n = 6) [21 (20–23; n = 8)] long, rapidly tapered to broad tube; base with sclerotized margin. Distal rod of accessory piece 19 (18–21; n = 6) [19 (17–22; n = 8)] long, straight, with terminal hook, indistinct thumb. Gonads pyriform to subovate; testis 40 (35–44; n = 5) [44 (41–46; n = 2)] long, 20 (18–21; n = 5) [20 (16–24; n = 2)] wide; germarium 44 (34–62; n = 6) [50 (41–64; n = 3)] long, 18 (15–21; n = 6) [20 (18–23; n = 3)] wide. Ootype, oviduct, uterus not observed; vaginae slightly distended; seminal receptacle small; vitellaria dense throughout trunk except absent in areas of reproductive organs.

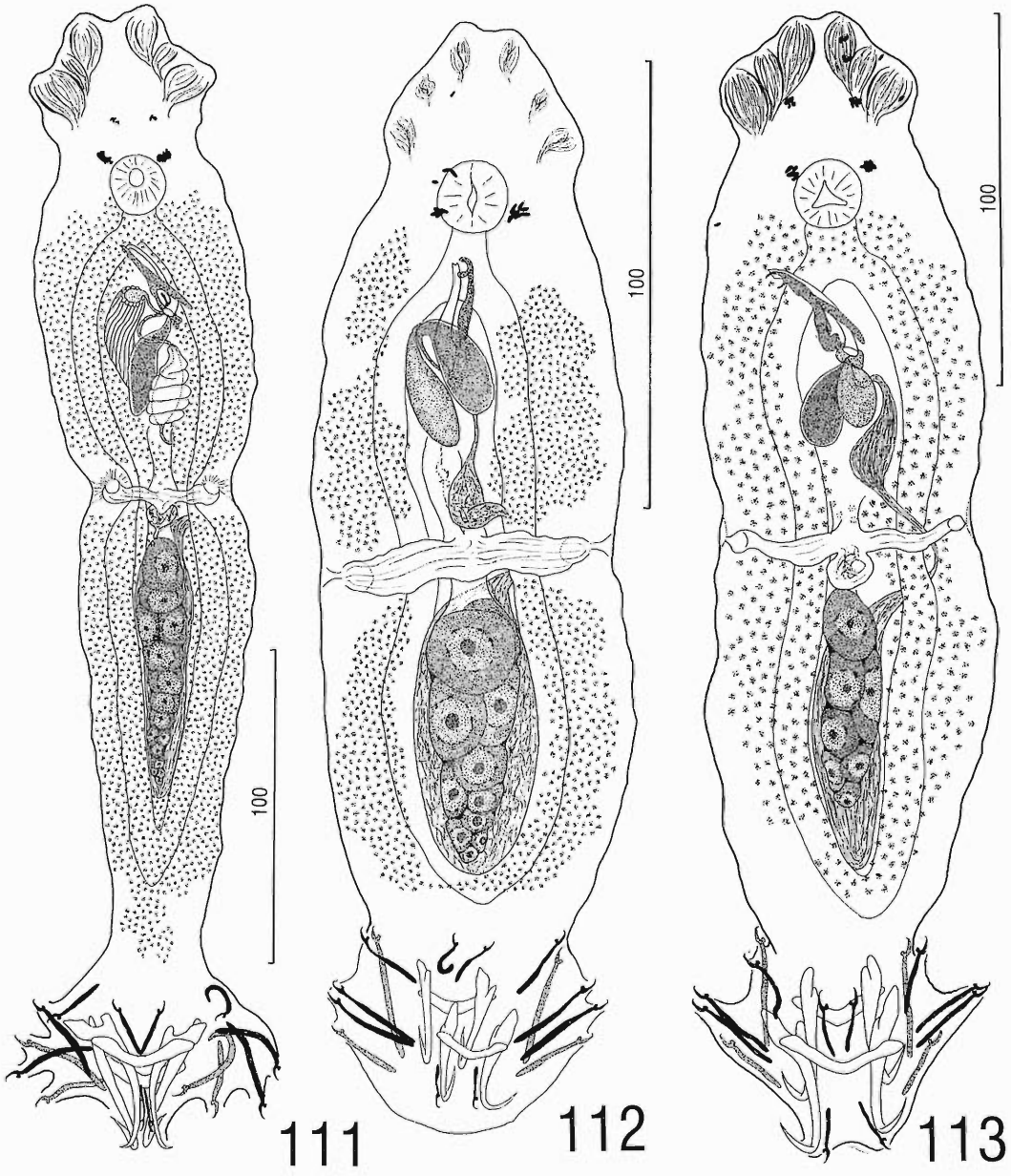
REMARKS: *Amphithecium prodotum* resembles *A. muricatum* and *A. minutum* in comparative morphology of the haptoral armament. Features distinguishing it from these species are presented in the remarks for the latter 2 species. The specific name is from Greek (*prodotos* ["betrayed"]).

Amphithecium speirocamarotum sp. n. (Figs. 111, 114–121)

TYPE HOST AND LOCALITY: *Serrasalmus elongatus*: Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (26 November 1984).

SPECIMENS STUDIED: Holotype, INPA PLH 250; 13 paratypes, INPA PLH 251, USNPC 85848, HWML 38598.

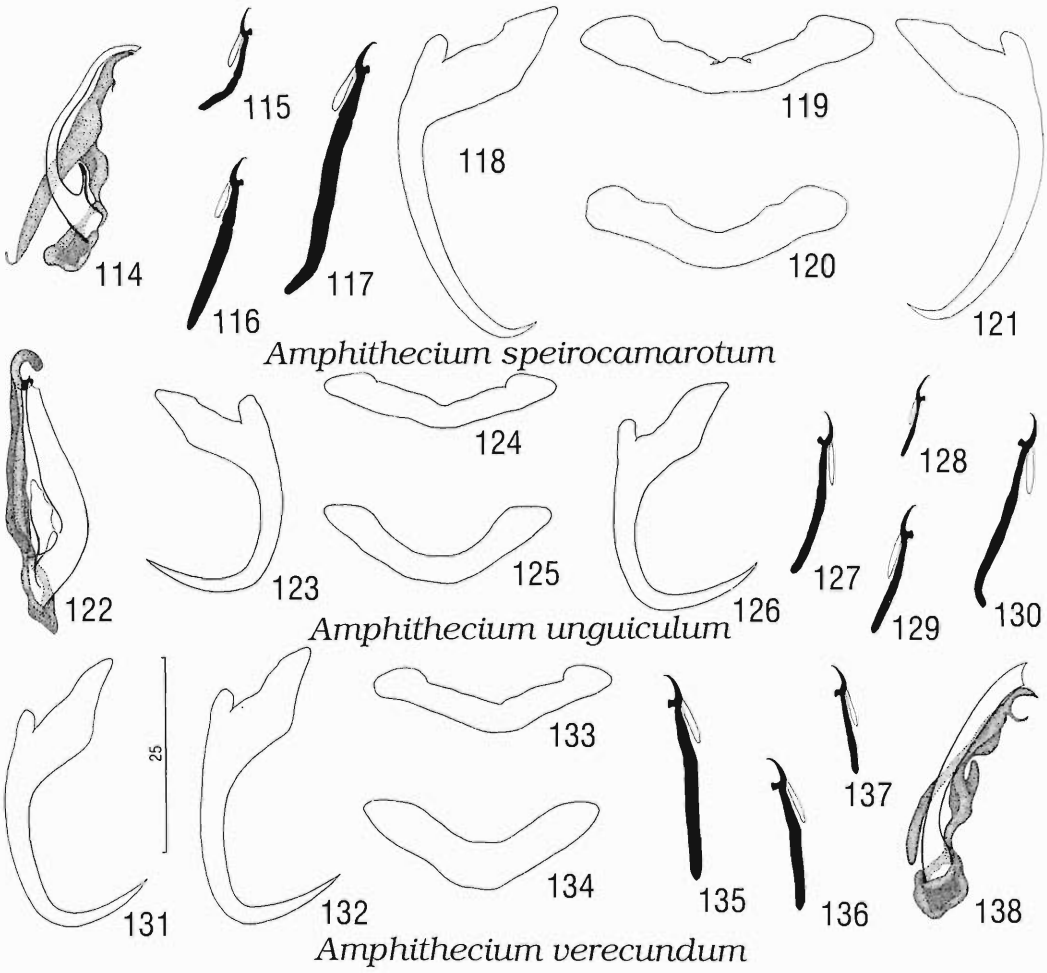
DESCRIPTION: Body 338 (290–364; n = 8) long, slender, constricted near midlength; posterior trunk tapered posteriorly; greatest width 75 (64–89; n = 9) in anterior trunk. Cephalic lobes well developed. Tegument smooth. Eyes 4 or anterior members absent; posterior members larger, slightly farther apart than anterior pair (when present); accessory granules absent or few in cephalic, anterior trunk regions. Pharynx spherical, 16 (15–18; n = 9) in diameter. Peduncle narrow; haptor 65 (60–72; n = 8) long, 87 (69–104; n = 8) wide. Anchors similar; each with elongate slightly depressed superficial root, prominent deep root, curved elongate shaft, short point; ventral anchor 47 (45–48; n = 5) long, base 15 (14–16; n = 4) wide; dorsal anchor 42 (39–44; n = 5) long, base 16 (15–18; n = 3) wide. Ventral bar 37 (35–40; n = 4) long, bent at midlength, with enlarged terminations; dorsal bar 34 (32–35; n = 5) long, broadly U-shaped, with terminal enlargements, ends developed laterally. Hook pairs 1, 2, 6—24 (21–28; n = 11), pair 3—28 (22–33; n = 3), pairs 4, 7—34 (31–38; n = 8), pair 5—16 (14–17; n = 2) long. Copulatory organ 30–31 (n = 3) long, with 2 rami; primary ramus recurved distally; short secondary ramus heavily sclerotized, blind; base with proximal flap. Distal rod of accessory piece 33 (32–35; n = 3) long, straight, with slightly recurved pointed tip, short thumb. Gonads pyr-



Figures 111–113. Whole-mount illustrations of *Amphithecium* spp. (composite, ventral views). 111. *Amphithecium speirocamarotum* sp. n. 112. *Amphithecium unguiculum* sp. n. 113. *Amphithecium verecundum* sp. n. (from *Pristobrycon eigenmanni*). All drawings are to respective 100-μm scales.

iform. Testis 59 (52–68; $n = 6$) long, 21 (14–25; $n = 6$) wide; seminal vesicle with externally coiled wall; wall of dextral prostatic reservoir with spiraled muscles. Germarium 67 (57–91; $n = 8$) long, 22 (16–28; $n = 8$) wide; ootype not observed; vaginae distended slightly; seminal receptacle small. Vitellaria dense throughout trunk except absent in areas of reproductive organs.

REMARKS: *Amphithecium speirocamarotum* is identified readily by the seminal vesicle with an externally coiled wall, the heavily sclerotized reduced secondary ramus of the copulatory or-



Figures 114–138. Sclerotized structures of *Amphithecium* spp. 114–121. *Amphithecium speirocamarotum* sp. n. 114. Copulatory complex (dorsal view). 115. Hook pair 5. 116. Hook pair 2. 117. Hook pair 7. 118. Ventral anchor. 119. Ventral bar. 120. Dorsal bar. 121. Dorsal anchor. 122–130. *Amphithecium unguiculum* sp. n. 122. Copulatory complex (ventral view). 123. Ventral anchor. 124. Ventral bar. 125. Dorsal bar. 126. Dorsal anchor. 127. Hook pair 2. 128. Hook pair 5. 129. Hook pair 1. 130. Hook pair 7. 131–138. *Amphithecium verecundum* sp. n. (from *Pristobrycon eigenmanni*). 131. Ventral anchor. 132. Dorsal anchor. 133. Ventral bar. 134. Dorsal bar. 135. Hook pair 7. 136. Hook pair 2. 137. Hook pair 5. 138. Copulatory complex (dorsal view). All drawings are to the 25-μm scale.

gan, the spiraled muscles in the wall of the dextral prostatic reservoir, and the noticeably tapered peduncle. The specific name is from Greek (*speira* ["anything wrapped round"] + *kamarotos* ["vaulted"]) and refers to the wall of the seminal vesicle.

Amphithecium unguiculum sp. n. (Figs. 112, 122–130)

TYPE HOST AND LOCALITY: *Serrasalmus spilopleura*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989).

OTHER RECORD: *Serrasalmus spilopleura*: Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas (14 September 1984).

SPECIMENS STUDIED: Holotype, INPA PLH 252; 26 paratypes, INPA PLH 253, PLH 254, USNPC 85849, 85850, HWML 38599.

DESCRIPTION: Body 240 (201–298; n = 10) long, fusiform, slightly constricted near midlength; greatest width 81 (56–104; n = 11) in anterior or posterior trunk. Tegument smooth or with scaled annulations. Cephalic lobes moder-

ately developed. Eyes 2, 4, or absent, anterior pair usually absent; each eye comprised of few, frequently dissociated granules; accessory granules common in cephalic, anterior trunk regions. Pharynx spherical, 16 (15–17; n = 11) in diameter. Peduncle broad; haptor 50 (41–67; n = 10) long, 69 (60–81; n = 10) wide. Anchors similar; each with well-developed roots, curved shaft, elongate point; ventral anchor 29 (28–30; n = 13) long, base width 12 (10–14; n = 13); dorsal anchor 31 (30–33; n = 12) long, base width 13 (11–16; n = 12). Bars similar, broadly U- or V-shaped, with slightly enlarged ends; ventral bar 27 (24–28; n = 9) long; dorsal bar 26 (24–28; n = 9) long. Hook pairs 1, 6—18 (16–19; n = 23); pair 2—20 (18–23; n = 9); pairs 3, 4—25 (23–28; n = 24); pair 5—13–14 (n = 10); pair 7—28 (25–31; n = 15) long. Copulatory organ 35 (33–36; n = 9) long, with proximal bend, submedial dilation, 2 rami; primary ramus broad; secondary ramus flattened, blind; base with sclerotized margin, short proximal flap. Accessory piece 29 (27–32; n = 13) long, blunt, with rod incorporated into articulation process, C-shaped terminally. Testis 53 (29–75; n = 8) long, 23 (14–31; n = 8) wide, subovate. Germarium conical, 46 (27–73; n = 10) long, 20 (12–28; n = 10) wide; oviduct short; ootype not observed; vaginae dilated; vitellaria in bilateral fields of anterior, posterior trunk, absent in regions of reproductive organs.

REMARKS: This species resembles *Amphithecium falcatum* by lacking a free distal rod of the accessory piece and in the general morphology of haptoral sclerites. *Amphithecium unguiculum* differs from *A. falcatum* in the comparative morphology of the copulatory organ. The secondary ramus is reduced and flattened and the primary ramus is inflated in *A. unguiculum*, whereas the primary ramus is flattened and the secondary ramus fine and elongate in *A. falcatum*. The specific name is from Latin (*unguiculus* ["a small talon or claw"]) and refers to the end of the accessory piece.

Amphithecium verecundum sp. n. (Figs. 113, 131–138)

TYPE HOST AND LOCALITY: *Pristobrycon eigenmanni*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

OTHER RECORDS: *Pristobrycon eigenmanni*: Nazaré, Rio Uatumã, Amazonas (17 September 1985); Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus* sp. (2 of Jégu): Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 248; 16 paratypes, INPA PLH 249, USNPC 85851, 85852, 85853, HWML 38600 from *Pristobrycon eigenmanni*; 3 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 85854.

COMPARATIVE MEASUREMENTS: Measurements of specimens from *Serrasalmus* sp. (2 of Jégu) are in brackets following those of the type series.

DESCRIPTION: Body fusiform, with slight narrowing at midlength; length 267 (205–310; n = 10), greatest width 73 (59–84; n = 10) in anterior or posterior trunk. Tegument smooth or with scaled annulations. Cephalic lobes moderately developed. Eyes 4, equidistant; posterior members larger, farther apart than anterior pair; few accessory granules in cephalic, anterior trunk regions. Pharynx spherical, 16 (12–20; n = 11) in diameter. Peduncle broad; haptor 59 (51–78; n = 10) long, 70 (55–77; n = 9) wide. Anchors similar; each with well-developed slightly depressed superficial root, short deep root, straight shaft, elongate point; ventral anchor 35 (34–36; n = 6) [34–35 (n = 3)] long, base 12 (11–14; n = 6) [13 (11–14; n = 3)] wide; dorsal anchor 36 (35–37; n = 6) [35 (33–36; n = 3)] long, base 13–14 (n = 6) [13 (12–14; n = 3)] wide. Ventral bar 28 (24–30; n = 10) long, broadly V-shaped, with enlarged terminations; dorsal bar 28 (27–29; n = 6) long, broadly U-shaped. Hook pairs 1, 2—20 (19–21; n = 11) [19 (18–20; n = 5)], pair 3—24 (23–28; n = 5) [21 (20–22; n = 3)], pairs 4, 7—27 (25–29; n = 12) [25 (23–26; n = 5)], pair 5—15 (14–17; n = 5) [19 (n = 1)], pair 6—21 (18–23; n = 6) [19–20 (n = 2)] long. Copulatory organ 31 (30–34; n = 4) [32 (31–33; n = 2)] long, arcuate, with slight narrowing distal to base; base with short proximal flap. Distal rod of accessory piece 26 (25–29; n = 5) [24 (22–27; n = 20] long, with distal C-shaped hook, lower arm of hook delicate. Testis fusiform, 47 (38–56; n = 3) long, 24 (23–25; n = 3) wide. Germarium irregular, 50 (42–57; n = 6) long, 17 (12–24) wide; oviduct, ootype not observed; vaginae conspicuous; seminal receptacle small or absent; vitellaria throughout trunk except absent in regions of reproductive organs.

REMARKS: The copulatory complex of this species resembles that of *Amphithecium prodo-*

tum by having a single ramus of the copulatory organ and a hook-like termination of the accessory piece. It differs from this species by the lower arm of the C-shaped hook being relatively long and delicate. The species name is from Latin (*verecundus* ["unassuming"]).

Heterothecium gen. n.

DIAGNOSIS: Body fusiform, comprising cephalic region, trunk, peduncle, haptor. Tegument thin, smooth or with scaled annulations. Two terminal, 2 bilateral cephalic lobes; head organs, unicellular cephalic glands present. Four eyes; granules elongate ovate. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; intestinal ceca 2, confluent posterior to testis, lacking diverticula. Gonads intercecal, overlapping; testis dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle sigmoid, a dilation of vas deferens. Two prostatic reservoirs saccate. Genital pore midventral near level of cecal bifurcation. Copulatory complex comprising articulated copulatory organ, accessory piece; copulatory organ tubular, with 2 subequal rami opening terminally; distal rod of accessory piece, proximal articulation process present. Vagina of soft tissue; vaginal pore sinistrodorsal; vaginal vestibule lightly sclerotized. Seminal receptacle small or absent. Haptor subhexagonal; with pairs of dorsal and ventral anchor/bar complexes, 7 pairs of similar hooks with ancyrocephaline distribution. Each hook with delicate point, truncate protruding thumb, expanded shank comprising 2 subunits; proximal subunit variable in length between hook pairs; FH loop extending to union of shank subunits. Ventral bar lacking anteromedial projection. Parasites of gills of serrasalmid fishes.

TYPE SPECIES: *Heterothecium globatum* sp. n. from *Serrasalmus gouldingi*.

OTHER SPECIES: *Heterothecium dicrophallum* sp. n. from *Catoprion mento*.

REMARKS: *Heterothecium* is characterized by the combined presence in its member species of a sinistrodorsal vaginal pore, a sclerotized vaginal vestibule, and a male copulatory organ with 2 rami, and absence of development of the distal end on the articulation process of the accessory piece. *Pithanothecium*, its apparent sister taxon, includes species possessing a dextrolateral vaginal aperture and a blunt articulation process extending past the distal rod of the accessory piece. The generic name is from Greek (*hetero* ["different"] + *theke* ["a small case"]).

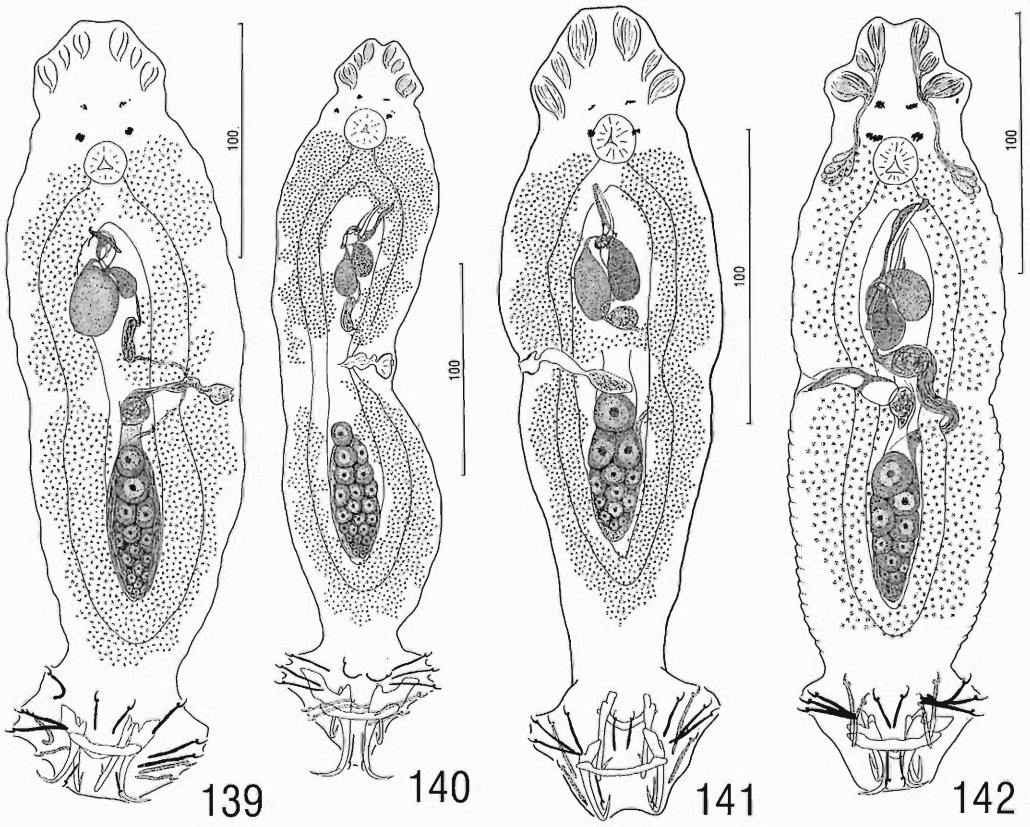
Heterothecium globatum sp. n. (Figs. 139, 143–152)

TYPE HOST AND LOCALITY: *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

OTHER RECORD: *Serrasalmus gouldingi*: C. Miriti, Rio Uatumã, Amazonas (20 September 1985).

SPECIMENS STUDIED: Holotype, INPA PLH 303; 22 paratypes, INPA PLH 304, PLH 305, USNPC 85869, 85870, HWML 38601.

DESCRIPTION: Body fusiform, 326 (310–361; $n = 7$) long; greatest width 89 (75–103; $n = 9$) usually in anterior trunk. Tegument frequently with scaled annulations. Cephalic lobes moderately developed. Eyes 4; posterior pair larger, farther apart than anterior pair; accessory granules usually absent, occasionally in cephalic, anterior trunk regions. Pharynx spherical, 17 (16–19; $n = 9$) in diameter. Peduncle broad; haptor 59 (52–67; $n = 9$) long, 78 (69–87; $n = 9$) wide. Anchors similar; each with elongate depressed superficial root, prominent deep root, slightly curved shaft, elongate point. Ventral anchor 34 (33–36; $n = 12$) long, base 13 (12–14; $n = 9$) wide; dorsal anchor 29 (28–31; $n = 11$) long, base 11 (9–12; $n = 7$) wide. Ventral bar 31 (29–32; $n = 8$) long, with indistinct bend at midlength, enlarged ends; dorsal bar 24 (23–25; $n = 8$) long, broadly V-shaped, with slightly enlarged ends. Hook pairs 1, 5—14–15 ($n = 17$), pair 2—17 (16–19; $n = 8$), pair 3—20 (19–21; $n = 10$), pair 4—22 (21–23; $n = 9$), pair 6—16 (15–17; $n = 5$), pair 7—19 (16–20; $n = 9$) long. Copulatory organ 28 (24–34; $n = 9$) long; primary ramus arced with small bulbous end; secondary ramus straight with broad termination; base with sclerotized margin, short proximal flap. Distal rod of accessory piece 20 (17–23; $n = 8$) long, terminally acute; articulation process twisted. Gonads subovate; testis 61 (55–66; $n = 3$) long, 27 (25–29; $n = 3$) wide; germarium 63 (51–73; $n = 4$) long, 21 (16–25; $n = 4$) wide. Seminal vesicle lying to left of midline, a short dilated dextroventral loop of vas deferens. Oviduct, ootype, uterus not observed; seminal receptacle small near midlength, apparently representing proximal dilation of vagina; vaginal pore irregular, vestibule lightly sclerotized; vitel-



Figures 139–142. Whole-mount illustrations of *Heterothecium* spp. and *Pithanothecium* spp. (composite, ventral views). 139. *Heterothecium globatum* sp. n. 140. *Heterothecium dicrophallum* sp. n. 141. *Pithanothecium piranhus* (Mizelle and Price, 1965) comb. n. (from *Pygopristis denticulata*). 142. *Pithanothecium amazonensis* (Mizelle and Price, 1965) comb. n. (from *Pristobrycon striolatus*). All drawings are to respective 100-μm scales.

laria limited to trunk, absent in regions of reproductive organs.

REMARKS: This species differs from *Heterothecium dicrophallum* in the comparative morphology of the copulatory complexes and by having a V-shaped dorsal bar (U-shaped in *H. dicrophallum*) and anchors of similar size (dorsal anchor about ½ length of ventral anchor in *H. dicrophallum*). The specific name is from Latin (*globatus* ["to make into a ball"]) and refers to the termination of the primary ramus of the copulatory organ.

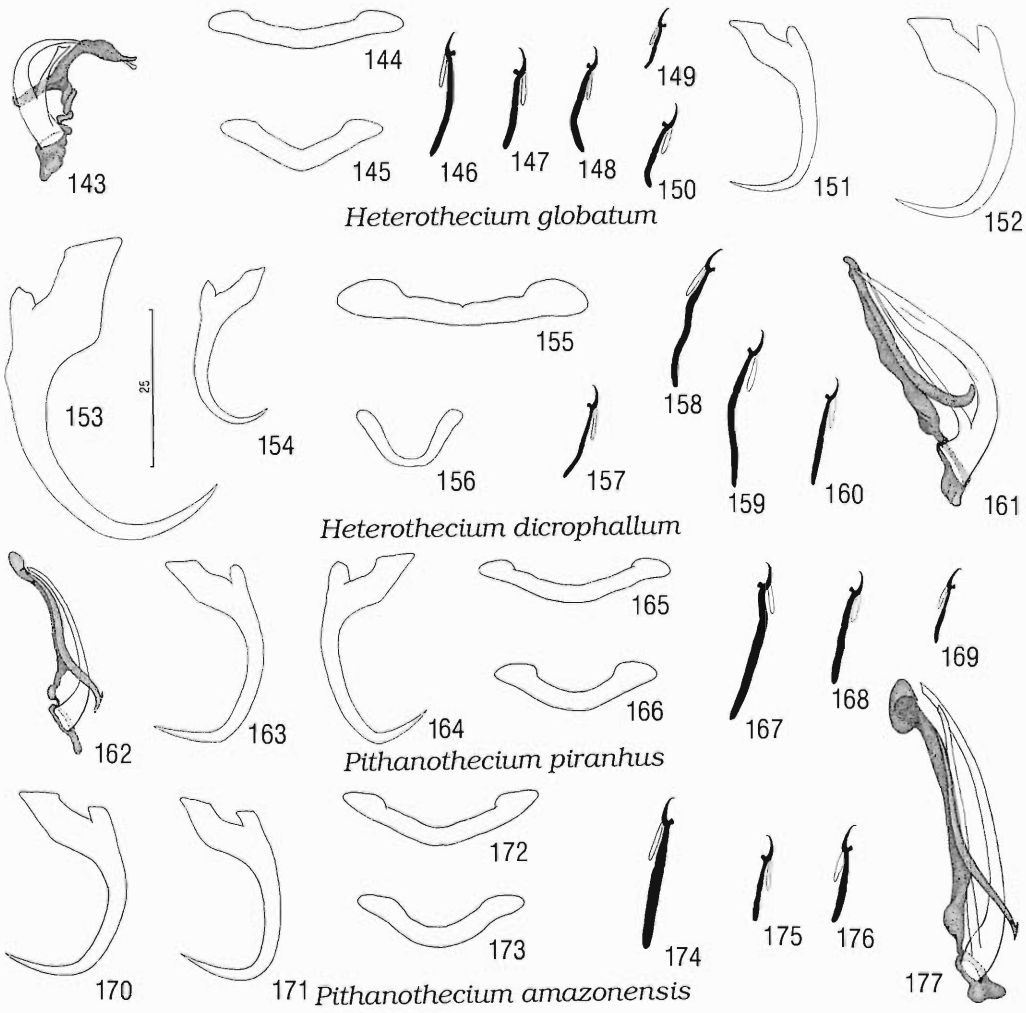
Heterothecium dicrophallum sp. n. (Figs. 140, 153–161)

TYPE HOST AND LOCALITY: *Catoprion mento*: Balbina, Rio Uatumã, Amazonas (20 September 1985).

OTHER RECORD: *Catoprion mento*: Rio Uatumã, Lago Tapaná, near Santana, Amazonas (3 November 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 306; 22 paratypes, INPA PLH 307, PLH 331, USNPC 85871, 85872, HWML 38602.

DESCRIPTION: Body 356 (318–386; n = 10) long, fusiform, with slight to obvious constriction near midlength; greatest width 76 (60–93; n = 8) in anterior or posterior trunk. Tegument smooth. Cephalic lobes moderately developed. Eyes 4, poorly organized; posterior pair larger, farther apart than anterior pair; granules variable in size; accessory granules usually present in cephalic, anterior trunk regions. Pharynx spherical, 20 (18–22; n = 8) in diameter. Peduncle broad; haptor 71 (64–77; n = 9) long, 81 (79–85; n = 8) wide. Ventral anchor 47 (46–49; n = 8) long,



Figures 143–177. Sclerotized structures of *Heterothecium* spp. and *Pithanothecium* spp. 143–152. *Heterothecium globatum* sp. n. 143. Copulatory complex (dorsal view). 144. Ventral bar. 145. Dorsal bar. 146. Hook pair 4. 147. Hook pair 2. 148. Hook pair 7. 149. Hook pair 5. 150. Hook pair 1. 151. Dorsal anchor. 152. Ventral anchor. 153–161. *Heterothecium dicrophallum* sp. n. 153. Ventral anchor. 154. Dorsal anchor. 155. Ventral bar. 156. Dorsal bar. 157. Hook pair 5. 158. Hook pair 2. 159. Hook pair 7. 160. Hook pair 1. 161. Copulatory complex (ventral view). 162–169. *Pithanothecium piranhus* (Mizelle and Price, 1965) comb. n. (from *Pygopristis denticulata*). 162. Copulatory complex (ventral view). 163. Ventral anchor. 164. Dorsal anchor. 165. Ventral bar. 166. Dorsal bar. 167. Hook pair 7. 168. Hook pair 2. 169. Hook pair 5. 170–177. *Pithanothecium amazonensis* (Mizelle and Price, 1965) comb. n. (from *Pygopristis denticulata*). 170. Ventral anchor. 171. Dorsal anchor. 172. Ventral bar. 173. Dorsal bar. 174. Hook pair 7. 175. Hook pair 5. 176. Hook pair 2. 177. Copulatory complex (ventral view). All drawings are to the 25-μm scale.

with depressed superficial root, short deep root, prominent ventral hump on base, evenly curved shaft, elongate point; base 20 (17–21; n = 6) wide. Dorsal anchor 25 (24–27; n = 7) long, with well-developed roots, evenly curved shaft, point; base 9–10 (n = 5) wide. Ventral bar 41 (40–42; n = 5) long, straight to slightly bent, with enlarged terminations; dorsal bar 20 (18–23; n = 5) long, U-shaped, with slightly enlarged ends. Hook pair 1—19–20 (n = 5), pair 2—26 (24–27; n = 4), pair 3—27 (25–29; n = 7), pairs 4, 7—30 (28–32; n = 11), pair 5—17–18 (n = 4), pair 6—21 (20–22; n = 6) long. Copulatory organ 42 (36–50; n = 10) long; pri-

mary ramus broad, expanded distally; secondary ramus slender, pointed; base with sclerotized margin, short proximal flap. Distal rod of accessory piece 32 (26–35; n = 9) long, curved, with terminal stout hook. Gonads subovate; testis 62 (61–63; n = 2) long, 21 (16–26; n = 2) wide; germarium 57 (46–75; n = 8) long, 21 (16–26; n = 7) wide. Seminal vesicle lying to left of midline, a short dilated dextroventral loop of vas deferens. Oviduct, ootype, uterus, seminal receptacle not observed. Vagina slightly dilated; vestibule lightly sclerotized. Vitellaria limited to trunk, absent in regions of reproductive organs and near body midlength.

REMARKS: Characters differentiating *Heterothecium dicrophallum* from *H. globatum* are presented in the Remarks section for the latter species. The specific name is from Greek (*dikroos* ["forked"] + *phallos* ["the penis"]).

Pithanothecium gen. n.

DIAGNOSIS: Body fusiform, comprising cephalic region, trunk, peduncle, haptor. Tegument thin, with scaled annulations. Two terminal, 2 bilateral cephalic lobes; head organs present; unicellular cephalic glands lying dorsolateral to pharynx. Eyes 4; granules elongate ovate. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; 2 intestinal ceca confluent posterior to gonads, lacking diverticula. Gonads intercecal, overlapping; testis dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle lying near or slightly sinistral to body midline, a sigmoid dilation of vas deferens. Two saccate prostatic reservoirs; prostates comprising glandular areas lying dorsal to anterior portions of ceca. Genital pore midventral at level of cecal bifurcation. Copulatory complex comprising articulated copulatory organ, accessory piece; copulatory organ tubular with 1 or 2 subequal rami opening terminally; accessory piece with distal rod, proximal articulation process extending distal to tip of rod as small blunt flap. Vagina nonsclerotized; vaginal aperture simple, dextrolateral near body midlength; vaginal vestibule present, with sclerotized wall. Seminal receptacle lying on midline anterior to germarium. Haptor subhexagonal; with dorsal and ventral anchor/bar complexes, 7 pairs of similar hooks with ancyrocephaline distribution. Anchors similar, unmodified. Ventral bar lacking anteromedial projection. Hook with truncate protruding thumb, delicate point, expanded shank comprising 2 subunits; proximal subunit variable in length between hook pairs. FH loop extending to union of shank subunits. Parasites of gills of serrasalmid fishes.

TYPE SPECIES: *Pithanothecium piranhus* (Mizelle and Price, 1965) comb. n. from *Catoprion mento*, *Pristobrycon striolatus*, *Pygocentrus nattereri* (type host), and *Pygopristis denticulata*.

OTHER SPECIES: *Pithanothecium amazonensis* (Mizelle and Price, 1965) comb. n. from *Catoprion mento*, *Pristobrycon striolatus*, *Pygocentrus nattereri* (type host), and *Pygopristis denticulata*.

REMARKS: Features distinguishing *Pithanothecium* from other genera in the complex of ancyrocephaline species infesting serrasalmids include presence of a sclerotized vaginal vestibule opening on the dextrolateral surface of the trunk and the distally blunt articulation process of the accessory piece extending past the tip of the distal rod. It is separated from *Heterothecium*, its apparent sister genus in position of the vaginal aperture (dextrolateral in *Pithanothecium*; sinistrolateral in *Heterothecium*). The generic name is from Greek (*pithanos* ["probable"] + *theke* ["a small case"]).

Pithanothecium piranhus (Mizelle and Price, 1965) comb. n. (Figs. 141, 162–169)

SYNONYM: *Cleidodiscus piranhus* Mizelle and Price, 1965.

RECORDS: *Catoprion mento*: Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Pristobrycon striolatus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Pygocentrus nattereri*: Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989). *Pygopristis denticulata*: Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989); Rio Xingu, Parana Maxipaná, Pará (17, 18 October 1992); Rio Araguari, Lago Comprido, Amapá (15 August 1992).

PREVIOUS RECORD: *Pygocentrus nattereri* (type host): Amazon River (type locality). The original host was obtained from Steinhart Aquarium, San Francisco, California (Mizelle and Price, 1965).

SPECIMENS STUDIED: Holotype, paratype, voucher from *Pygocentrus nattereri*, USNPC

Table 6. Comparative measurements (in micrometers) of *Pithanothecium piranhus* (Mizelle and Price, 1965) comb. n., from 4 serrasalmid hosts.

	Catoprion mento	*N*	*Pristobrycon striolatus*	*N*	*Pygocentrus nattereri*	*N**	*Pygopristis denticulata*	*N*
Body								
Length	277 (252–303)	3	250 (236–270)	6	—	—	250 (187–285)	12
Width	89 (83–92)	3	66 (62–72)	6	—	—	74 (61–83)	12
Haptor								
Length	59–60	3	49 (44–59)	6	—	—	54 (45–69)	11
Width	81 (70–93)	3	70 (59–75)	6	—	—	70 (63–78)	11
Pharynx								
Diameter	17 (16–18)	3	16–17	7	—	—	16 (13–17)	12
Copulatory organ								
Length	32 (30–35)	7	32–33	2	31	1	30 (24–32)	27
Accessory piece								
Length	29 (25–32)	8	29–30	2	28	1	28 (24–30)	29
Dorsal anchor								
Length	34 (32–37)	9	32	2	33 (32–35)	2	31 (29–33)	23
Base width	14 (12–16)	5	13–14	2	12 (11–14)	2	14 (12–15)	21
Ventral anchor								
Length	32 (31–34)	9	30	1	30	2	29 (27–31)	26
Base width	12 (10–14)	7	12	1	10	2	12 (10–14)	25
Bar length								
Dorsal	28–29	3	26 (25–27)	6	—	—	27 (26–28)	9
Ventral	33 (32–34)	3	30 (28–31)	6	—	—	31 (29–32)	9
Hook lengths								
Pair 1	18	3	19	1	17–18	2	17 (16–19)	17
Pair 2	20 (19–21)	7	20	1	19	2	19 (17–20)	14
Pair 3	23 (22–25)	6	23 (22–24)	2	22	2	21 (20–23)	19
Pair 4	26 (25–27)	7	25	1	25–26	2	24–25	14
Pair 5	13–14	3	—	—	13	1	13 (12–14)	8
Pair 6	19 (18–20)	5	19–20	2	18–19	2	18 (16–19)	10
Pair 7	28 (27–29)	7	27–28	2	26–27	2	25 (24–28)	19
Germarium								
Length	40 (39–43)	3	39	1	—	—	46 (33–56)	7
Width	17 (16–19)	3	19	1	—	—	15 (13–17)	7
Testis								
Length	37 (36–39)	3	40 (38–43)	2	—	—	41 (30–50)	5
Width	20 (18–21)	3	16 (15–17)	2	—	—	16 (10–21)	5

* Measurements of the holotype and paratype are not included.

60463, HWML 21290, USNPC 85863, respectively; 14 vouchers from *Catoprion mento*, USNPC 85864, 85865; 9 vouchers from *Pristobrycon striolatus*, USNPC 85862; 47 vouchers from *Pygopristis denticulata*, USNPC 85866, 85867, 85868.

COMPARATIVE MEASUREMENTS: Table 6.

REDESCRIPTION: Greatest body width usually in anterior trunk. Tegumental annulations, scales poorly developed, peduncular, absent in most specimens. Cephalic lobes moderately to poorly developed; cephalic glands not observed. Eyes equidistant or anterior pair closer together, smaller than posterior pair; accessory granules absent to numerous in cephalic, anterior trunk regions. Pharynx spherical. Peduncle broad. Each anchor with well-developed roots, slightly depressed superficial root, evenly curved shaft, elongate point. Ventral bar rod-shaped, slightly bent near midlength, with terminal enlargements; dorsal bar broadly U-shaped, with enlarged ends. Copulatory organ arcuate, tapered;

base with small proximal flap. Distal rod of accessory piece sigmoid; terminal flap of articulation process globose. Gonads subovate to pyriform; oviduct, ootype not observed; wall of vaginal vestibule thickened; seminal receptacle ovate to pyriform; vitellaria distributed throughout trunk except absent in regions of reproductive organs.

REMARKS: This species has not been reported since its original description from *Pygocentrus nattereri* by Mizelle and Price (1965), who assigned it to *Cleidodiscus* on the basis of the basally articulated copulatory organ and accessory piece. Beverley-Burton and Suriano (1980) redefined *Cleidodiscus* and provided a redescription of the type species, *C. robustus*, but refrained from commenting on the generic status of the many other described species then assigned to the genus. With the exceptions of *C. brachus* and *C. venardi* (see Beverley-Burton, 1984), other described species of *Cleidodiscus* have been generally considered incertae sedis or have been reassigned within the Ancyrocephalinae.

Kritsky and Thatcher (1983) suggested that the monogenoideans described by Mizelle and Price (1965) from *Pygocentrus nattereri* were members of undefined Neotropical genera. Our rediscovery of *Cleidodiscus piranhus* confirms that it should be reassigned, for which we propose it as the type species of *Pithanothecium* gen. n. Mizelle and Price (1965) considered the vagina to be absent in their specimens. Although vaginae cannot be seen in the unstained holotype and paratype, comparative morphology of haptoral and copulatory sclerites confirms the conspecificity of our specimens. This species differs from *Pithanothecium amazonensis*, its only congenitor, by possessing a delicate vaginal tube and vestibule, a single ramus of the copulatory organ, and a small terminal flap of the articulation process of the accessory piece and in the comparative morphology of the haptoral armament.

Pithanothecium amazonensis (Mizelle and Price, 1965) comb. n. (Figs. 142, 170–177)

SYNONYM: *Cleidodiscus amazonensis* Mizelle and Price, 1965.

RECORDS: *Catoprion mento*: Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Balbina, Rio Uatumã, Amazonas (20 September 1985). *Pristobrycon striolatus*: Rio Capucapú at its confluence with Rio Jatapú, Cachoeira das Garças, Amazonas (31 October 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Lago Samaumá, Rio Uatumã, Amazonas (25 September 1985). *Pygopristis denticulata*: Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989); Rio Araguari, Lago Comprido, Amapá (15 August 1992).

PREVIOUS RECORD: *Pygocentrus nattereri* (type host): Amazon River (type locality). The original host was obtained from Steinhart Aquarium, San Francisco, California (Mizelle and Price, 1965).

SPECIMENS STUDIED: Holotype, paratype from *Pygocentrus nattereri*, USNPC 60462, HWML 21289, respectively; 14 vouchers from *Catoprion mento*, USNPC 85859, 85860, 85861; 7 vouchers from *Pristobrycon striolatus*, USNPC 85855, 85856, 85965; 17 vouchers from *Pygopristis denticulata*, USNPC 85857, 85858.

COMPARATIVE MEASUREMENTS: Table 7.

REDESCRIPTION: Body slightly constricted near midlength; greatest width in anterior or posterior trunk. Scaled tegumental annulations in posterior trunk, peduncle. Cephalic lobes moderately developed. Anterior eyes slightly closer together, smaller than posterior pair; eye granules variable in size; accessory granules few or absent in cephalic, anterior trunk regions. Pharynx spherical. Peduncle broad. Each anchor with well-developed roots (superficial root slightly depressed), straight or slightly curved shaft, elongate point. Ventral bar rod-shaped, usually bent at midlength, with terminal enlargements; dorsal bar broadly U-shaped. Copulatory organ with 2 subequal rami with terminal openings; base with small proximal flap. Distal rod of accessory piece sigmoid; terminal flap of articulation process spatulate, with recurved end. Gonads subovate. Oviduct elongate; ootype, uterus not observed; vaginal vestibule extending to near midline, with sclerotized rib, ring; vaginal tube short to nonexistent; seminal receptacle small, pyriform, adjacent or slightly posterior to proximal end of vaginal vestibule; vitellaria in trunk, absent in regions of reproductive organs.

REMARKS: *Pithanothecium amazonensis* was originally described from *Pygocentrus nattereri* and placed in *Cleidodiscus* by Mizelle and Price (1965). Boeger and Kritsky (1988) provided an

Table 7. Comparative measurements (in micrometers) of *Pithanothecium amazonensis* (Mizelle and Price, 1965) comb. n., from 3 serrasalmid hosts.

	Catoprion mento	*N*	*Pristobrycon striolatus*	*N*	*Pygopristis denticulata*	*N*
Body						
Length	305 (246–336)	6	300 (268–330)	4	272 (247–291)	7
Width	98 (80–114)	6	103 (89–121)	4	101 (92–112)	8
Haptor						
Length	70 (55–75)	6	68 (59–75)	3	65 (53–72)	7
Width	85 (66–102)	6	81 (70-92)	3	83 (80–85)	7
Pharynx						
Diameter	18 (15–19)	6	18 (16–21)	4	18 (16–20)	8
Copulatory organ						
Length	56 (51–61)	8	51 (47–57)	3	54 (49–60)	8
Accessory piece						
Length	49 (47–53)	8	40 (32–57)	4	47 (40–51)	7
Dorsal anchor						
Length	37 (36–38)	7	38	2	33 (31–36)	5
Base width	14 (12–17)	2	16 (14–17)	2	13 (12–14)	4
Ventral anchor						
Length	34 (32–35)	7	36–37	2	31 (30–32)	6
Base width	16 (15–17)	5	13–14	2	14–15	6
Bar length						
Dorsal	32 (30–33)	3	31 (30–32)	4	30 (29–32)	7
Ventral	38 (37–39)	4	37 (35–38)	4	35 (32–36)	6
Hook lengths						
Pair 1	19–20	3	19	2	18 (17–20)	5
Pair 2	26 (25–29)	5	21	2	20 (19–21)	5
Pair 3	30 (29–32)	5	24–25	2	23 (21–25)	6
Pair 4	32 (30–34)	7	25 (24–27)	2	26 (24–27)	7
Pair 5	16 (15–17)	5	16	1	16–17	3
Pair 6	22–23	4	20–21	2	19 (18–20)	3
Pair 7	30 (28–33)	6	28–29	3	26 (24–28)	5
Germarium						
Length	50 (43–57)	5	54 (45–68)	3	53 (47–60)	6
Width	26 (23–30)	5	21 (20–23)	3	22 (21–26)	6
Testis						
Length	55 (51–64)	5	61 (47–74)	2	54 (51–55)	4
Width	26 (21–33)	5	18 (17–19)	2	24 (17–28)	4

illustration of the copulatory complex from the holotype of *C. amazonensis* but suggested that the type host may have been originally misidentified. Records of dactylogyrids collected from serrasalmid hosts, including *P. nattereri*, during the present study, supports this assertion and suggests that Mizelle and Price (1965) had a specimen of *Pygopristis denticulata* before them. Only 1 specimen of 1 (*Pithanothecium piranhus*) of 5 ancyrocephaline species collected and described by Mizelle and Price (1965) was recovered from *P. nattereri* during the present study. However, 4 of their species, including *C. amazonensis*, *C. piranhus*, *C. serrasalmus*, and *Urocleidus crescentis*, were regularly encountered on *P. denticulata* (nobis, Kritsky et al., in press a). Their fifth species, *Urocleidus orthus*, was apparently not collected, although the possibility exists that *U. orthus* may be a synonym of a species of *Calpidothecioides* described from *P. denticulata* by Kritsky et al. in press a).

Pithanothecium piranhus and *P. amazonensis* also occur on *Catoprion mento* and *Pristobrycon striolatus*. However, it is unlikely that these host

taxa represent the original fish examined by Mizelle and Price (1965), because 3 of the ancyrocephaline species described by these authors do not occur on these fishes.

Discussion

The Monogenoidea are frequently cited to have a comparatively high host specificity (Llewellyn, 1957; Rhode, 1993). Bychowsky (1957) reported that 711 (74.2%) of 958 known species of Monogenoidea occurred on a single fish species and 806 (84.1%) on species of a single host genus. In a summary of surveys conducted worldwide by various authors, Rohde (1978) found 537 (90.9%) of 591 marine Monogenoidea to occur on members of a single host genus within specific geographic localities. Rohde (1978) related this high host specificity to tendencies for K-strategies of ecological selection. Among other traits, monogenoideans generally produce significantly fewer eggs per individual than members of most other parasitic groups, have a direct life cycle with larval stages actively seeking an appropriate host (Kearn, 1967), and possess complex attachment structures that are frequently specialized to specific sites on hosts (Kearn, 1976).

Since 1984, we have examined 20 species of Serrasalmidae from the Brazilian Amazon for gill parasites (see Boeger and Kritsky, 1988; Kritsky et al., 1992, 1996, in press a, b; Van Every and Kritsky, 1992). While diversity of Dactylogyridae on these hosts has been extremely high (about 100 species have been identified), many exhibit low host specificity: *Amphithecium falcatum* occurs on 10 host species; *Notothecium aegidatum* (=*Enallothecium aegidatum*) on 9 host species; *Notozothecium teinodendrum* on 7 host species; *Anacanthorus jegui*, *A. sciponophallus*, *A. mesocondylus*, and *Amphithecium diclonophallum* on 6 host species; and *Notozothecium minor*, *Mymarothecium galeolum*, and *Anacanthorus serrasalmi* on 5 host species each (nobis; Van Every and Kritsky, 1992; Kritsky et al., 1996, in press b). In addition, 2 ancyrocephaline species to be described later (see Kritsky et al. in press b) occur on 8 and 5 hosts, respectively. Of 48 known species of Ancyrocephalinae from serrasalmids, only 21 (43.8%) are known from a single host species, but 23 (47.9%) occur on hosts of 2 or more genera. Expanded studies will undoubtedly show host specificity of these worms to be even lower because our collections included relatively small numbers of host specimens, all were from a relatively limited geographic area, and many species of serrasalmid hosts have yet to be examined for these parasites in the Neotropical region.

High species diversity is a well-documented phenomenon for a variety of animal and plant groups in the neotropics, and hypotheses have been proposed to explain maintenance of diversity levels and speciation within the region (see Bush, 1994). Although mechanisms of speciation in Neotropical river systems have been discussed less frequently than those for terrestrial systems, the monogenoideans undoubtedly have been exposed and probably responded to the same geologic and paleoecologic events affecting speciation of their fish hosts. Jégu (1992) and Jégu and dos Santos (1993) have suggested that variations in sea level during the glacial and interglacial periods of the Quaternary may have provided many vicariant opportunities for speciation of some fishes including the Serrasalmidae within the Amazon River system. Such reoccurring speciation opportunities coupled with coevolutionary scenarios associated with speciation rates (Brooks, 1979) could explain the high diversity and host occurrences of Dactylogyridae on their Neotropical hosts.

Acknowledgments

The authors are grateful to E. Belmont-Jégu and M. Martins for assistance in the field and to J. R. Lichtenfels (USNPC) and M. H. Pritchard (HWML) for allowing us to examine type and voucher specimens in their care. The Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil, provided accommodations during visits of the senior author to the Amazon Basin. Financial support for this study was provided in part by ISU-FRC (632), ORSTOM, and CNPq. Support of the Brayton H. Ransom Trust Fund for page charges is gratefully acknowledged.

Literature Cited

Beverley-Burton, M. 1984. Monogenea and Turbellaria. Pages 5–203 *in* L. Margolis and Z. Kabata, eds. Guide to the Parasites of Fishes of Canada. Part 1. Canadian Special Publication of Fisheries and Aquatic Sciences 74. Department of Fisheries and Oceans, Ottawa, Canada.

———, **and D. M. Suriano.** 1980. *Cleidodiscus robustus* Mueller, 1934 (Monogenea: Ancyrocephal-

inae) from *Lepomis gibbosus* L. (Pisces: Centrarchidae) in Ontario, Canada: anatomy and systematic position. Canadian Journal of Zoology 58: 654–660.

Boeger, W. A., and D. C. Kritsky. 1988. Neotropical Monogenea. 12. Dactylogyridae from *Serrasalmus nattereri* (Cypriniformes, Serrasalmidae) and aspects of their morphologic variation and distribution in the Brazilian Amazon. Proceedings of the Helminthological Society of Washington 55: 188–213.

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

Bush, M. B. 1994. Amazonian speciation: a necessarily complex model. Journal of Biogeography 21:5–17.

Bychowsky, B. E. 1957. Monogenetic Trematodes, Their Systematics and Phylogeny. The Academy of Sciences of the USSR, Moscow. 509 pp. Translated from Russian by P. C. Oustinoff (W. J. Hargis, Jr., ed.), AIBS, Washington, D.C.

Jégu, M. 1992. Influência das alternaçues climáticas do Quaternário sobre a distribuição e evolução dos peixes na Amazônia. Proceedings of the 10th Congresso Latino-Americano de Genética, 21–25 April 1992, Rio de Janeiro. Revista Brasileira de Genética 15(supplement 1):234–237.

———, **and G. M. dos Santos.** 1993. Quaternary variation of sea level and present aquatic refuges in central and eastern Amazonia. Page 43 *in* Resumos. International Symposium on the Quaternary of Amazonia, Manaus, Brazil.

Kearn, G. C. 1967. Experiments on host-finding and host-specificity in the monogenean skin parasite *Entobdella soleae*. Parasitology 57:585–605.

———. 1976. Body surface of fishes. Pages 185–208 (*in*) C. R. Kennedy, ed. Ecological Aspects of Parasitology. North-Holland, Amsterdam.

Kritsky, D. C., W. A. Boeger, and M. Jégu. 1996. Neotropical Monogenoidea. 28. Ancyrocephalinae (Dactylogyridae) of piranha and their relatives (Teleostei, Serrasalmidae) from Brazil and French Guiana: species of *Notozothecium* Boeger and Kritsky, 1988, and *Mymarothecium* gen. n. Journal of the Helminthological Society of Washington. 63:153–175.

———, ———, **and** ———. In press a. Neotropical Monogenoidea. 30. Ancyrocephalinae (Dactylogyridae) of piranha and their relatives (Teleostei, Serrasalmidae) from Brazil: species of *Calpidothecioides* gen. n., *Calpidothecium* gen. n., *Odothecium* gen. n., and *Notothecioides* gen. n. Journal of the Helminthological Society of Washington.

———, ———, **and** ———. In press b. Neotropical Monogenoidea. 31. Ancyrocephalinae (Dactylogyridae) of piranha and their relatives (Teleostei, Serrasalmidae) from Brazil: species of *Notothecium* Boeger and Kritsky, 1988, and *Enallothecium* gen. n. Journal of the Helminthological Society of Washington.

———, ———, **and L. R. Van Every.** 1992. Neotropical Monogenoidea. 17. *Anacanthorus* Mizelle and Price, 1965 (Dactylogyridae, Anacanthorinae) from characoid fishes of the central Amazon. Journal of the Helminthological Society of Washington 59:25–51.

———, **and V. E. Thatcher.** 1983. Neotropical Monogenea. 5. Five new species from the aruanã, *Osteoglossum bicirrosum* Vandelli, a freshwater teleost from Brazil, with the proposal of *Gonocleithrum* n. gen. (Dactylogyridae: Ancyrocephalinae). Proceedings of the Biological Society of Washington 96:581–597.

———, ———, **and W. A. Boeger.** 1986. Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). Proceedings of the Helminthological Society of Washington 53:1–37.

Llewellyn, J. 1957. Host-specificity in monogenetic trematodes. Pages 199–212 *in* First Symposium on Host Specificity among Parasites of Vertebrates. Paul Attinger, S.A., Neuchâtel.

Mizelle, J. D. 1936. New species of trematodes from the gills of Illinois fishes. American Midland Naturalist 17:785–806.

———, **and C. E. Price.** 1963. Additional haptoral hooks in the genus *Dactylogyrus*. Journal of Parasitology 49:1028–1029.

———, **and** ———. 1965. Studies on monogenetic trematodes. XXVIII. Gill parasites of the piranha with the proposal of *Anacanthorus* gen. n. Journal of Parasitology 51:30–36.

Rohde, K. 1978. Latitudinal differences in host-specificity of marine Monogenea and Digenea. Marine Biology 47:125–134.

———. 1993. Ecology of Marine Parasites. CAB International, Wallingford, U.K. 298 pp.

Van Every, L. R., and D. C. Kritsky. 1992. Neotropical Monogenoidea. 18. *Anacanthorus* Mizelle and Price, 1965 (Dactylogyridae, Anacanthorinae) of piranha (Characoidea, Serrasalmidae) from the central Amazon, their phylogeny, and aspects of host–parasite coevolution. Journal of the Helminthological Society of Washington 59:52–75.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 55–58

Oochoristica jonnesi sp. n. (Cyclophyllidea: Linstowiidae) from the House Gecko, *Hemidactylus mabouia* (Sauria: Gekkonidae), from Cameroon

CHARLES R. BURSEY,[1] CHRIS T. MCALLISTER,[2] AND PAUL S. FREED[3]

[1] Pennsylvania State University, Shenango Campus, 147 Shenango Avenue, Sharon, Pennsylvania 16146,
[2] Department of Biology, Texas Wesleyan University, 1201 Wesleyan, Fort Worth, Texas 76105, and
[3] Section of Herpetology, Houston Zoological Gardens, 1513 North MacGregor, Houston, Texas 77030

ABSTRACT: Six specimens of *Oochoristica jonnesi* sp. n. were recovered from the small intestines of 4 of 14 (29%) house geckos, *Hemidactylus mabouia*, from Cameroon. *Oochoristica jonnesi* sp. n. has few characteristics in common with African species of *Oochoristica*; rather, it belongs to that group of species possessing fewer than 25 testes in a single cluster and circular suckers, namely, *O. junkea*, *O. lygosomae*, *O. lygosomatis*, *O. novaezelandae*, and *O. sobolevi*. The new species can be readily differentiated from the other species of this group by number of lobules of the ovary or number of osmoregulatory canals.

KEY WORDS: Cestoda, *Oochoristica jonnesi* sp. n., Sauria, *Hemidactylus mabouia*.

Only 5 species of the cestode genus *Oochoristica* have been reported previously from Africa. *Oochoristica crassiceps* Baylis, 1920 (synonyms, *O. sigmoides* Moghe, 1926; *O. fusca* Meggitt, 1927), was described from 2 specimens taken from the stripe-bellied sand snake, *Psammophis subtaeniatus* Peters, collected in Mombasa, Kenya. *Oochoristica theileri* Fuhrmann, 1924, was described from 40 specimens found in a single spiny agamid lizard, *Agama hispida* Linnaeus, from Pretoria, South Africa. *Oochoristica truncata* (Krabbe, 1879) Zschokke, 1905, was originally described from specimens harbored by the steppe agama, *Trapelus sanguinolentus* (Pallas), and the European legless lizard, *Ophisaurus apodus* (Pallas), from Turkestan, but was relegated to synonymy with *Oochoristica tuberculata* (Rudolphi, 1819) Lühe, 1898, by Baer (1927). *Oochoristica agamae* Baylis, 1919, a species from agamas from Mozambique, was synonymized with *Oochoristica ameivae* (Beddard, 1914) Baer, 1924, a species from South American reptiles, by Hughes (1940). Spasskii (1951) considered the unification of *O. agamae* and *O. ameivae* to be improper and, based on anatomical and ecological features, united *O. agamae* with *O. truncata* assuming the name *Oochoristica truncata* (synonyms, *O. agamae*; *O. africana* Malan, 1939; *O. a. ookispensis* Malan, 1939, *O. ameivae* sensu Fantham and Porter, 1960). *Oochoristica ubelakeri* Bursey, McAllister, Freed, and Freed, 1994, was described from 5 specimens found in a single South African rock agama, *Agama atra knobeli* Boulenger and Power, from Namibia. The purpose of this paper is to describe a new species of *Oochoristica* that was found in the small intestines of house geckos, *Hemidactylis mabouia* (Moreau de Jonnès), from Cameroon, Africa.

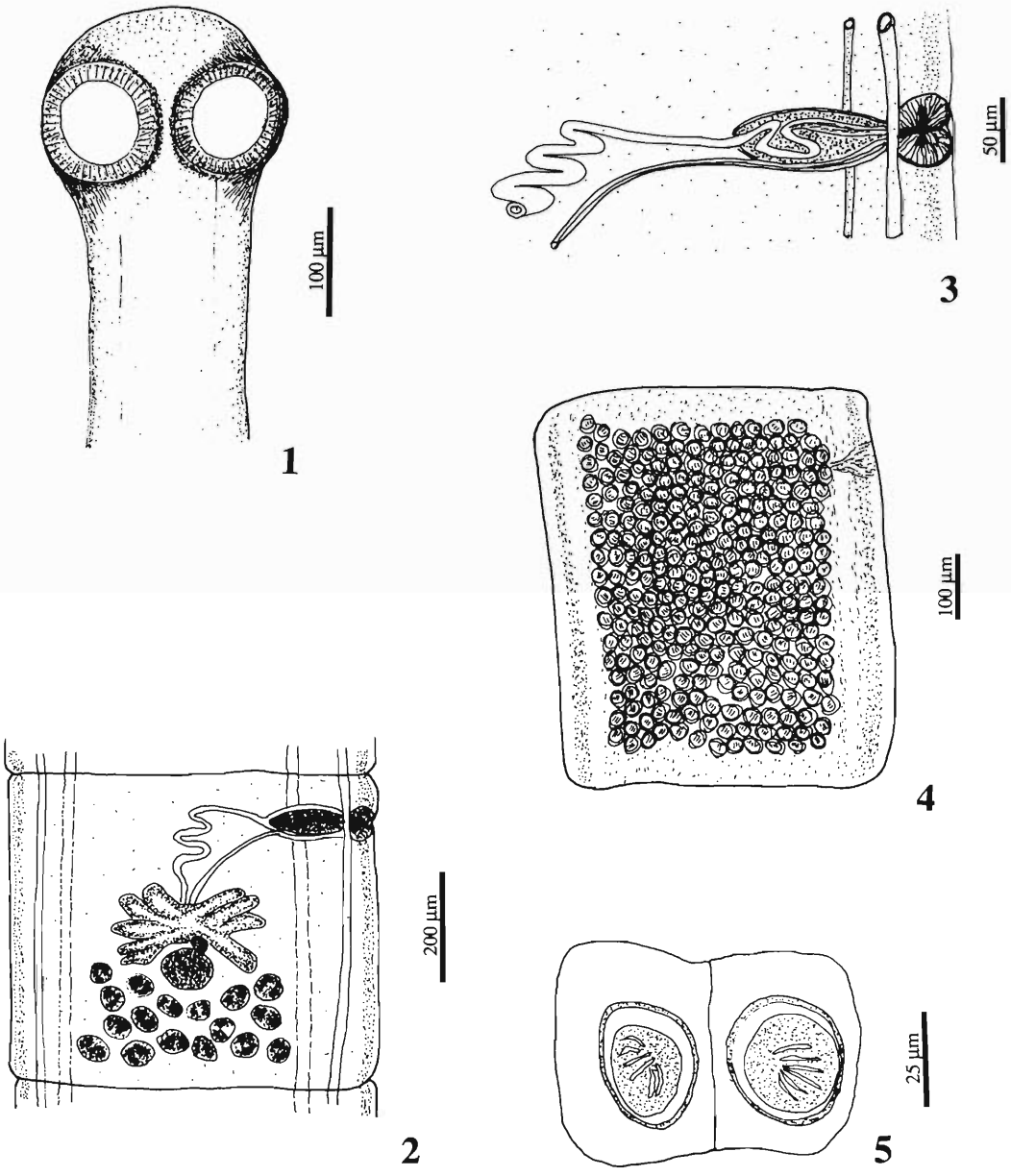
Materials and Methods

During March 1991, 14 adult *Hemidactylus mabouia* were collected by hand by P.S.F. from buildings in Douala, Cameroon. The lizards were killed with an intraperitoneal overdose of sodium pentobarbital (Nembutal®), immediately fixed in 10% formalin, and then transferred to 70% ethanol for storage. The entire gastrointestinal tract of each lizard was excised in 1994 by C.T.M. and examined for helminths. A total of 6 mature cestodes was discovered in the small intestines of 4 lizards. Each cestode was stained with acetocarmine, dehydrated in an alcohol series, cleared in xylene, mounted in damar, and examined by light microscopy. Measurements are given in micrometers unless otherwise noted. Drawings were made with the aid of a microprojector.

Results

Oochoristica jonnesi sp. n. (Figs. 1–5)

DESCRIPTION (based on 6 specimens): Total length 31 (mean) (26–34 [range]) mm; maximum width of strobila, 1.40 mm; strobila widest in midregion, tapering toward both ends. Proglottid number in mature worms 168 (150–175): 54 (40–60) immature proglottids wider than long, 0.65 mm (0.33–0.84) × 0.08 mm (0.05–0.10); 44 (40–50) mature proglottids wider than long, 1.20 mm (1.00–1.40) × 0.28 mm (0.13–0.33); 47 (40–50) gravid proglottids wider than



Figures 1–5. ***Oochoristica jonnesi*** **sp. n. 1. Scolex and neck. 2. Mature proglottid. 3. Genital atrium and cirrus sac. 4. Gravid proglottid. 5. Egg in uterine capsule. Figures 1 and 2 from type specimen; Figures 3–5 from paratype specimen.**

long 0.88 mm (0.76–1.02 wide) × 0.80 mm (0.64–1.02 long); terminal proglottids slightly longer than wide 0.88 mm (0.82–0.92 wide) × 0.94 mm (0.84–1.00 long). Scolex 240 (225–250) wide × 185 (160–200) long, with 4 circular suckers, 116 (110–120) diameter. Neck 252 (230–360) wide × 680 (500–750) long. Osmoregulatory system of 4 longitudinal canals visible throughout length of strobila. Genital pores irregularly alternating, situated in anterior quarter of proglottid; genital atrium 52 (45–55) deep, 72 (65–75) wide. Cirrus sac length 167 (150–

Table 1. Comparative measurements of various structures in species of *Oochoristica* with circular suckers and fewer than 25 testes in 1 cluster.*

	O. junkea	*O. lygosomae*	*O. lygosomatis*	*O. novaezealandae*	*O. sobolevi*	*O. jonnesi* sp. n
Locality	India	Sri Lanka	Java	New Zealand	Ukraine	Cameroon
Proglottid number	—	35–45	45	20	57–52	150–175
Length (mm)	50–53	8–15	8–11	20	10–15	26–34
Width, maximum (mm)	0.40	0.60	0.30–0.35	1.12	1.20	1.40
Scolex, width	225	260–310	220–240	240–260	200–250	225–250
Sucker diameter	125–130	140	100–120	80–100	85–100	110–120
Osmoregulatory canals	2 Pairs	1 Pair	1 Pair	2 Pairs	1 Pair	2 Pairs
Testes number	23	13–18	14–16	12–15	18–23	14–24
Cirrus sac, length	162	175	125	80–90	100–120	150–175
Ovary, width	180	236	—	130–160	170–200	200–300
Ovary, lobule number	None	5–7	—	None	Many	3–5
Egg	46–50	27 × 19	37–40	34–60	34–50	30–40 × 40–51
Oncosphere	25	22	20–25	30–40	22–32	17–22 × 23–29
Hook, length	9–10	11	12–13	15–20	15–16	9–13
Reference	Johri, 1950	Burt, 1993	Baylis, 1929	Schmidt and Allison, 1983	Spasskii, 1951	This paper

* Measurements are given in micrometers unless otherwise stated.

175 [N = 12]), width 52 (45–55). Genital ducts pass between the osmoregulatory canals. Ovary bilobed and situated in center of proglottid; each lobe subdivided into 3–5 lobules; ovary width 270 (200–300 [N = 12]); ovary length 110 (80–130); spheroid vitelline gland situated on midline directly behind ovary 160 (100–200 [N = 12]) in diameter; ootype and Mehlis' gland complex between ovary and vitelline gland. Testes posterior to ovary and vitelline gland in 1 cluster, numbering 20 (14–24 [N = 18]) in each proglottid; testes measure 38 (22–50) × 48 (34–57); do not occur lateral to osmoregulatory canals. In gravid proglottids, uterine capsules, 54 (51–57; N = 24), each containing a single egg, fill entire proglottid; eggs 36 (30–40) × 46 (40–51; N = 30); oncosphere 19 (17–22) × 27 (23–29; N = 30); oncosphere hook lengths 11 (9–13; N = 30). Genital atrium, cirrus sac, and vagina visible in gravid proglottids. On average, 350 eggs in terminal proglottid (N = 5), eggs not occurring lateral to excretory ducts.

TYPE HOST: *Hemidactylus mabouia* (Moreau de Jonnès, 1818).

TYPE LOCALITY: Douala, Cameroon (4°00′S, 9°30′E).

SITE OF INFECTION: Small intestine.

PREVALENCE: Four of 14 (29%) lizards were infected

TYPE SPECIMENS: Holotype: USNM Helm. Coll. No. 85925; paratypes: No. 85926.

ETYMOLOGY: Named in honor of Alexandre Moreau de Jonnès, 1778–1870, French herpetologist, who described the host species.

Discussion

Oochoristica jonnesi sp. n. has few characteristics in common with the 5 species of *Oochoristica* described previously from African reptiles (see table 1 of Bursey et al., 1994). It belongs to a group of species possessing fewer than 25 testes in 1 cluster and circular suckers, namely, *O. junkea* Johri, 1950, *O. lygosomae* Burt, 1933, *O. lygosomatis* Skinker, 1935, *O. novaezelandae* Schmidt and Allison, 1985, and *O. sobolevi* (Spasskii, 1948) Spasskii, 1951. Comparisons of selected measurements of *Oochoristica jonnesi* sp. n with these 5 species are presented in Table 1. The 6 species of this group are harbored by lizards: *O. jonnesi* sp. n. and *O. junkea* in geckonids; *O. lygosomae*, *O. lygosomatis*, and *O. novaezelandae* in scincids; and *O. sobolevi* in a lacertid. Four biogeographical realms are represented: *O. jonnesi* sp. n., Ethiopian; *O. junkea*, *O. lygosomae*, and *O. lygosomatis*, Oriental; *O. novaezelandae*, Australian; and *O. sobolevi*, Palearctic. Several anatomical characters can be used to separate the species: *O. lygosomae*, *O. lygosomatis*, and *O. sobolevi* have a single pair of longitudinal osmoregulatory canals and the genital apparatus lies dorsal to these canals; *O. jonnesi* sp. n., *O. junkea*, and *O. novaezelandae* have 2 pairs of longitudinal osmoregulatory canals and the genital ducts lie

between these canals. The ovaries of *O. junkea* and *O. novaezelandae* are not subdivided into lobules; the ovary of *O. jonnesi* sp. n. has 3–5 lobules.

A question must be raised concerning the assignment of *Oochoristica lygosomae*, *O. lygosomatis*, and *O. sobolevi* to the genus *Oochoristica*. Each lacks the double pair of osmoregulatory canals mentioned in the genus diagnosis given by Spasskii (1951); the presence of a single pair of osmoregulatory system should place these species in another genus, at least. Furthermore, there is a question of the validity of *O. lygosomatis*. This species was originally described by Baylis (1929) as *Oochoristica parva*. Subsequently, Skinker (1935) and Baer (1935) independently found the specific name to be preoccupied and renamed Baylis's material *O. lygosomatis* and *O. baylisi*, respectively; priority by publication date belonging to *O. lygosomatis*. Loewen (1940) relegated *O. lygosomatis* to synonymity with *O. lygosomae*; however, Hughes (1940) and Spasskii (1951) retained both species and indicated that union of these species was impossible until the similarity of the genital apparatus could be shown. *Oochoristica lygosomae* has multiple sperm ducts (Burt, 1933) not seen in other cestodes, a difference that caused Spasskii (1951) to suggest that this species belongs in a different family. Thus, it would be most appropriate to compare *Oochoristica jonnesi* sp. n. with *O. junkea* and *O. novaezelandae* only; different host families, different biogeographical realms, and significant anatomical differences in ovaries separate these three species.

Acknowledgments

We thank the Namibia Department of Agriculture and Nature Conservation in Windhoek and the South African Cape Province Permit Office for their continued support and collecting and export permits (issued to P.S.F.). We also thank J. Furman and K. Neitman for assistance in the field.

Literature Cited

Baer, J. G. 1927. Monographie des Cestodes de la famille des Anoplocephalidae. Bulletin Biologique de France et de Belgique, Supplément X. 241 pp.

———. 1935. Etude de quelques helminths de Lémuriens. Revue Suisse de Zoologie 42:275–291.

Baylis, H. A. 1929. Some new parasitic nematodes and cestodes from Java. Parasitology 21:256–265.

Bursey, C. R., C. T. McAllister, F. S. Freed, and D. A. Freed. 1994. *Oochoristica ubelaker* n. sp. (Cyclophyllidea: Linstowiidae) from the South African Rock Agama, *Agama atra knobeli*. Transactions of the American Microscopical Society 113:400–405.

Burt, D. R. R. 1933. *Oochoristica lygosomae*, sp. nov.—a cestode from the lizard *Lygosoma punctatum*. Spolia Zeylanica 18:1–7.

Hughes, C. R. 1940. The genus *Oochoristica* Lühe 1898. American Midland Naturalist 23:368–381.

Johri, L. N. 1950. Report on cestodes collected in India and Burma. Indian Journal of Helminthology 2:23–34.

Loewen, S. L. 1940. On some reptilian cestodes of the genus *Oochoristica* (Anoplocephalidae). Transactions of the American Microscopical Society 59:511–518.

Schmidt, G. D., and B. Allison. 1985. *Oochoristica novaezealandae* n. sp. (Cestoda: Anoplocephalidae) from a New Zealand skink, *Leiolopisma nigriplantare maccanni* Hardy, 1977. New Zealand Journal of Zoology 112:137–139.

Skinker, M. S. 1935. A new species of *Oochoristica* from a skunk. Journal of the Washington Academy of Science 25:59–65.

Spasskii, A. A. 1951. Essentials of Cestodology. Anoplocephalate Tapeworms of Domestic and Wild Animals. Vol. 1. The Academy of Sciences of the USSR, Moscow. Translated by The Israel Program for Scientific Translations, Jerusalem, Israel, 1961, 783 pp.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 59–95

Ultrastructure of the Lesion Nematode, *Pratylenchus penetrans* (Nemata: Pratylenchidae)*

BURTON Y. ENDO,[1] ULRICH ZUNKE,[2] AND WILLIAM P. WERGIN[1]

[1] U.S. Department of Agriculture, Agricultural Research Service, Plant Sciences Institute, Nematology Laboratory, Beltsville, Maryland 20705-2350 and

[2] Universtät Hamburg, Institut für Angewandte Botanik, Marseiller Str. 7, 20355, Hamburg, Germany

ABSTRACT: Various stages of a lesion nematode, *Pratylenchus penetrans* (Cobb, 1917) Sher and Allen, 1953, were observed with transmission electron microscopy and low-temperature scanning electron microscopy (LTSEM) to elucidate the structural anatomy of the esophagus, intestine, and reproductive system. The lumen of the esophagus is circular through the procorpus and triradiate in the metacorpus where it is part of the metacorpus pump valve. A pair of esophageal lumen branches terminate as quadriradiate valves in the subventral gland ampullae. The central lumen extends posteriad to become part of the esophago-intestinal valve. The enlarged intestinal lumen is delineated by scattered evaginated membranes of the epithelial cells. The lumen may be occluded during nonfeeding periods or when the intestine becomes compressed by the reproductive organs. The testis contains spermatocytes with membrane-bound nuclei that transform into amoeboid spermatids with electron-opaque, nonmembrane-bound nuclei surrounded by fibrous bodies. Spermatozoa with irregular clumps of nonmembrane-bound chromatin surrounded by mitochondria as well as residual fibrous bodies were found in seminal vesicles and vas deferens of males and in spermathecae of female gonads. The ultrastructure of the male and female reproductive organs is compared to similar features observed with light microscopy and LTSEM.

KEY WORDS: anatomy, esophagus, fine structure, gonad, lesion nematode, oocyte, *Pratylenchus penetrans*, sperm, ultrastructure.

Lesion nematodes (*Pratylenchus* spp.) are recognized worldwide as one of the major deterrents to crop production. These nematodes are migratory ecto- and endoparasites that cause severe root damage on a wide range of crops while feeding primarily in the cortex and secondarily on root hairs as an ectoparasite (Dropkin, 1989; Zunke, 1990a). The mode of penetration, disease symptoms, and pathogenesis of *Pratylenchus* spp., either as lone parasite or in conjunction with other pathogens, have been reviewed previously (Dropkin, 1989). The ecto- and endoparasitic feeding behavior of *P. penetrans* (Cobb, 1917) Sher and Allen, 1953, on roots in culture was observed with the use of video-enhanced contrast light microscopy. Feeding activities were separated into 4 phases consisting of stylet probing, cell penetration, salivation, and food ingestion (Zunke, 1987, 1990a, 1990b; Zunke and Institut für den Wissenschaftlichen Film, 1988; Zunke and Perry, 1992). Parasitized cortical cells had hypertrophied nuclei and protoplast separation from cell walls. Neighboring cells were also affected by components of salivation, which penetrated adjacent cell walls through plasmodesmata (Zunke, 1990b). In a related ultrastructural study of the root pathology of *P. penetrans*, plant cells that had been fed upon showed an increase in tannins, degeneration of mitochondria, numerous ribosomes, and no internal membrane structure (Townshend et al., 1989). Histological studies showed that nematode feeding was associated with polyphenolic oxidase production and tannin deposition (Townshend and Stobbs, 1981). Studies on the ultrastructure of the esophageal region, particularly on the secretory granules, are lacking for *Pratylenchus* spp. Major ultrastructural studies on the anterior region of *Pratylenchus* have emphasized the tacto- and chemosensory anatomy of the sensilla in the anterior cephalic region (De Grisse, 1977; Trett and Perry, 1985). Similar ultrastructural studies have been conducted on related tylenchid nematodes such as *Ditylenchus dipsaci* (Kühn, 1957) Filijev, 1936, *Heterodera* spp., and *Meloidogyne* spp., with emphasis on sensory systems (Yuen, 1967; Baldwin and Hirschmann, 1973, 1975; Wergin and Endo, 1976; De Grisse, 1977; Endo and Wergin, 1977,

* Mention of a trade name, warranty, proprietary product, or vendor does not constitute a guarantee of a product and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

1988; Endo, 1980; Coomans and De Grisse, 1981) and on feeding activity related to the esophageal glands (Bird, 1967, 1968; Bird and Saurer, 1967; Yuen, 1968; Rumpenhorst, 1984; Wyss et al., 1984; Atkinson et al., 1988; Atkinson and Harris, 1989; Hussey, 1989; Hussey and Mims, 1990; Hussey et al., 1990; Zunke and Perry, 1992; Wyss, 1992; Endo, 1993; Davis et al., 1994). Studies on embryogenesis and postembryogenesis in several *Pratylenchus* spp. indicated that 2 gonads developed to the fourth molt, after which the posterior gonad deteriorated (Roman and Hirschmann, 1969). Ultrastructural studies of the reproductive system of plant parasitic nematodes were reviewed in a comprehensive study of spermatogenesis and sperm ultrastructure in cyst nematodes, including *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975, *G. virginiae* (Miller and Gray, 1968) Behrens, 1975, *Heterodera schachtii* Schmidt, 1871, and *H. avenae* Wollenweber, 1924 (Shepherd and Kempton, 1973). Fine structure of developing sperm of *Ekphymatodera thomasoni* Baldwin et al., 1989, was compared with other Heteroderinae as part of a study to recognize diversity and phytogenetically informative characters within the subfamily (Cares and Baldwin, 1994a, b). Ultrastructural observations were made on the male copulatory organs of *P. penetrans* (Wen and Chen, 1976; Mai et al., 1977).

Transmission and scanning electron micrographs and their interpretations can be a foundation for subsequent biological and molecular approaches to studies of nematode feeding processes and their disruption. The identification and labeling of secretory granules, as determined with other species (Atkinson et al., 1988; Davis et al., 1994; Goverse et al., 1994), may provide ways of understanding the processes of the digestive system and the manner in which stylet secretions interact with host cells. In addition, ultrastructure of characters of lesion nematodes can contribute to studies on phylogenetic relationships among plant-parasitic nematodes.

In this study, we examined the ultrastructural anatomy of *P. penetrans* at various stages of development. Emphasis was on the alimentary canal in relation to the stylet, esophagus, and intestine and on the structure of male and female gonads.

Materials and Methods

Infective and parasitic stages of *P. penetrans* were obtained from root cultures of corn (*Zea mays* L. 'Iochief') grown in Gamborg's B-5 medium without cytokinins or auxins (Gamborg et al., 1976). Adults and juveniles were collected from infected root pieces that were incubated in water. The samples were prepared for electron microscopy as previously described (Endo and Wergin, 1973; Wergin and Endo, 1976). Nematodes embedded in 2% water agar slices or in infected root were fixed in buffered 3% glutaraldehyde (0.05 M phosphate buffer, pH 6.8) at 22°C for 1.5 hr, washed for 1 hr in 6 changes of buffer, postfixed in buffered 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, and infiltrated with a low-viscosity embedding medium (Spurr, 1969). Silver-gray sections were cut on an ultramicrotome with a diamond knife and mounted on uncoated 75- × -300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 or 400T electron microscope operating at 60 kV with a 20-μm objective aperture.

For low-temperature scanning electron microscopy (LTSEM), samples of *P. penetrans* were obtained from the cultures already above and prepared as previously described (Wergin et al., 1993). Specimens were placed on the surface of a flat specimen holder or in a 1-mm^3 vertical chamber formed by slots traversing the 2 halves of a closed, hinged, 24-carat gold holder. The specimen holders containing the suspensions were rapidly plunge-frozen in liquid nitrogen. The holders containing the samples were then mounted onto a Denton complementary freeze-etch specimen cap that was used to fracture the samples by lifting and rotating the fracture arm of the cap by 180 degrees. A standard flat holder containing the specimens was attached to the cryo-transfer arm and inserted into the prechamber of an Oxford CT 1500 Cryotrans System mounted on a Hitachi S-4000 field emission scanning electron microscope to perform low-temperature manipulations and observations. The specimens were either sputter-coated with platinum in the prechamber and inserted onto the cryostage of the microscope or etched and coated in the prechamber and moved to the cryostage for observation. Accelerating voltages of 10 kV were used to observe and record images onto Polaroid Type 55 P/N film.

Results

Line drawings of major anatomical regions of the adult stage of male and female specimens of *Pratylenchus penetrans* are shown in Figure 1. The anterior region of the lesion nematode is characterized by a robust stylet (Fig. 2) supported by extensive protractor muscles that extend from the cephalic framework and body wall to the stylet basal knobs with lateral contact to the stomatal wall (Figs. 1–5). The lip region shows a distinct boundary with the body cuticle delineating a sharp depression (Figs. 4, 5) between annules 3 and 4. The anterior sensory organs of nematodes consist of circular arrangements of sensilla arranged in a hexaradiate pattern and comprised of 6 inner labial, 6 outer la-

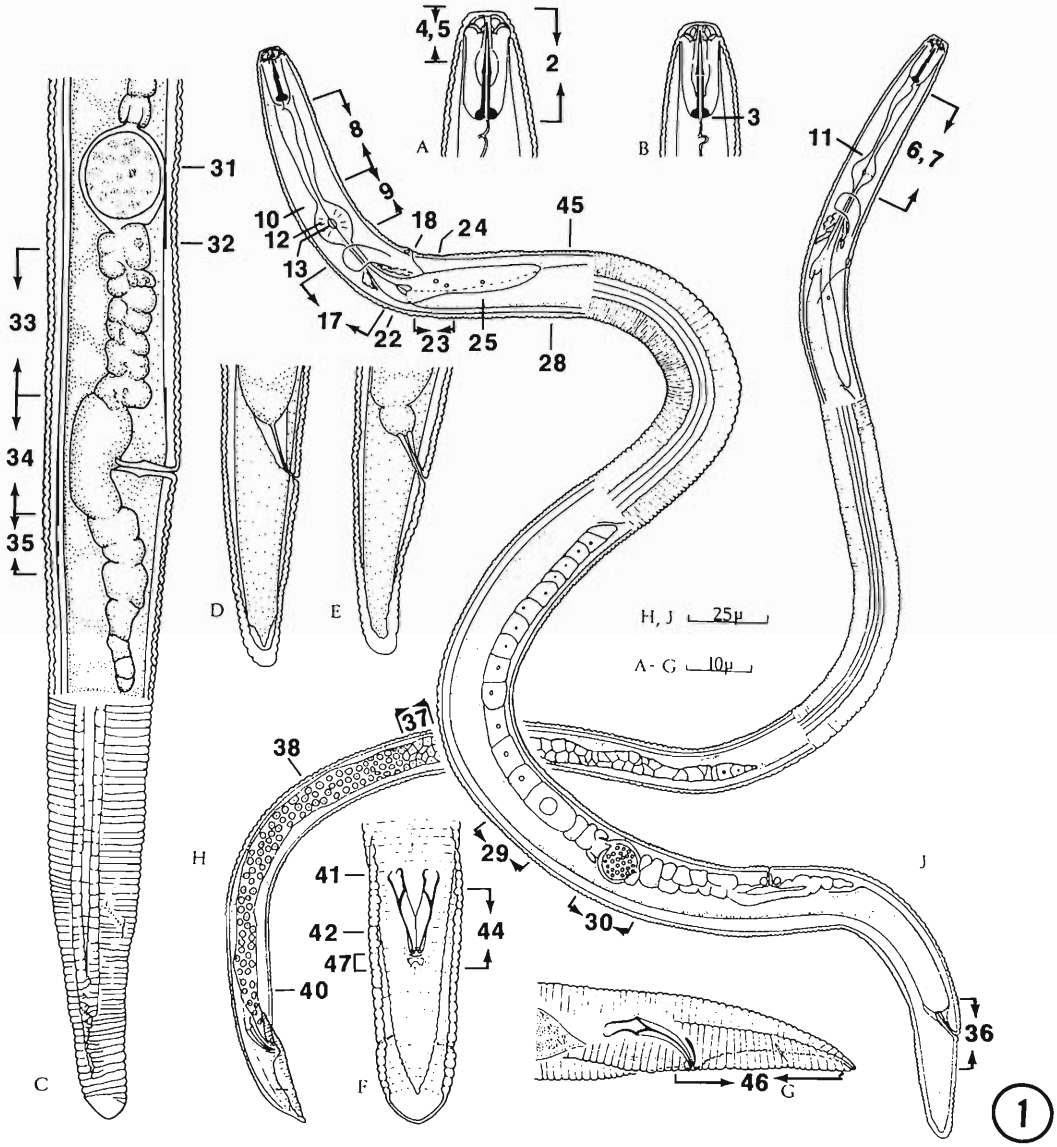


Figure 1. ***Pratylenchus penetrans*. A. Female head. B. Male head. C. Female vulva region and tail. D, E. Female tail tips. F, G. Male tails in ventral view (F) and lateral view (G). H. Male. J. Female. (A–G, topotypes, courtesy M. W. Allen). Reprinted with permission from D. C. M. Corbett. 1973. C.I.H. Descriptions of Plant-Parasitic Nematodes, Set 2, No. 25, © Commonwealth Agricultural Bureaux. Numbers indicate the approximate locations of figures used to describe various anatomical regions of *P. penetrans*.**

bial, and 4 cephalic sensilla. The amphidial receptors consist of 7 cilia that extend anteriad through the amphidial canal.

The esophageal lumen extends from the stylet base as a tubular system to the triradiate pump lumen (Fig. 1). It then continues as a triradiate cuticularized canal to the esophago-intestinal valve consisting of appressed unlined membranes of a pair of enlarged cells. The cuticularized lumen wall of the esophagus branches just posteriad to the stylet knobs, and the dorsal branch terminates as a quadriradiate valve shown obliquely in the dorsal gland ampulla (Fig. 6). The dorsal gland ampulla and the

slightly narrow elongated dorsal gland extension of the procorpus is filled with small, electron-opaque granules (Figs. 6, 7). The ampulla and adjoining dorsal gland extension follow a sinuous pathway through the procorpus adjacent to the lumen of the esophagus (Figs. 6, 7). In contrast to the dorsal gland extension shown in Figures 6 and 7, the dorsal gland extension in Figure 8 is greatly expanded and filled with numerous secretory granules exhibiting various electron densities and occupying a major part of the procorpus (Figs. 8, 9). Differentiation of a distinct dorsal gland ampulla is absent.

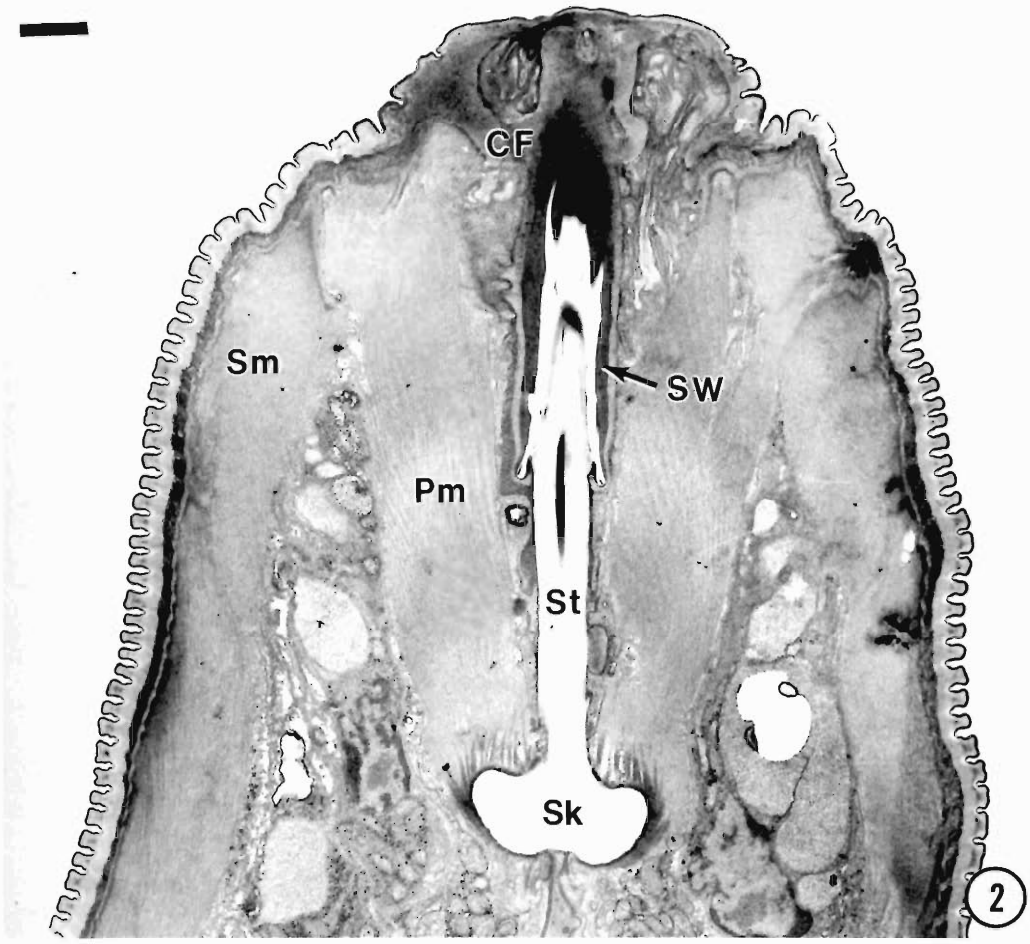
As the dorsal gland extension traverses the anterior region of the metacorpus, the extension appears constricted. It is surrounded by sphincter muscles (Fig. 10) that probably control the anterior flow and accumulation of secretory granules (Fig. 9) originating in the dorsal gland. Slightly posteriad to the sphincter muscles are parallel arrays of membranes associated with anterior muscles of the metacorpus (Fig. 11). At the midregion of the metacorpus, the walls become thickened and form the triradiate valve of the metacorpus pump (Figs. 12, 13). Hexaradiate bands of muscles are connected by hemidesmosomes to adradial positions of the triradiate valve wall (Fig. 13). Muscle elements extend centrifugally and longitudinally to the basal lamellae of the metacorpus where they are anchored. The triradiate appearance of the lumen wall of the central metacorpus is indicative of the relaxed state of the pump muscles. Contraction of these muscles would open the valve and allow passage of food from the stylet to the posteriad region of the esophagus and the intestine. Slightly posteriad from the central metacorpus pump valve, the cuticle lumen wall branches twice ventrally and posteriad to form cuticular tubes (Figs. 14–16) that terminate as quadriradiate valves within ampullae of subventral glands. The triradiate metacorpus valve lies parallel and central to the dorsal and subventral gland extensions that continue through the metacorpus sphincter muscles (not illustrated). The isthmus of the esophagus is enclosed by the nerve ring (Figs. 17, 18). The nerve processes forming the lateroventral commissure originate at the base of the nerve ring, slightly anterior to the level of the outlet of the secretory-excretory gland (Figs. 17, 19). In longitudinal view, the esophago-intestinal valve is preceded by the triradiate lumen of the esophagus surrounded by supporting membranes and terminating as a large cavity surrounded by epithelial cells of the intestine (Figs. 20, 21). The valve consists of 2 large nucleated cells. The lumen aperture forms by separation of adjacent nonlined membranes of the cells (Figs. 21, 22). Lateral membrane junctions limit loss of lumen contents. The dorsal gland extension widens abruptly posteriad from the isthmus to become part of the gland body (Figs. 23, 24). The esophago-intestinal valve and the dorsal gland nucleus lie in about

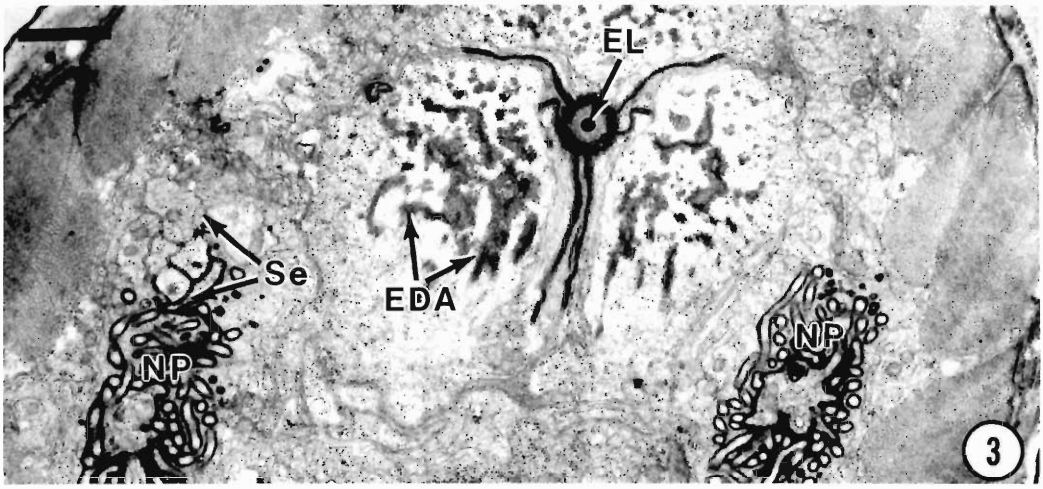
→

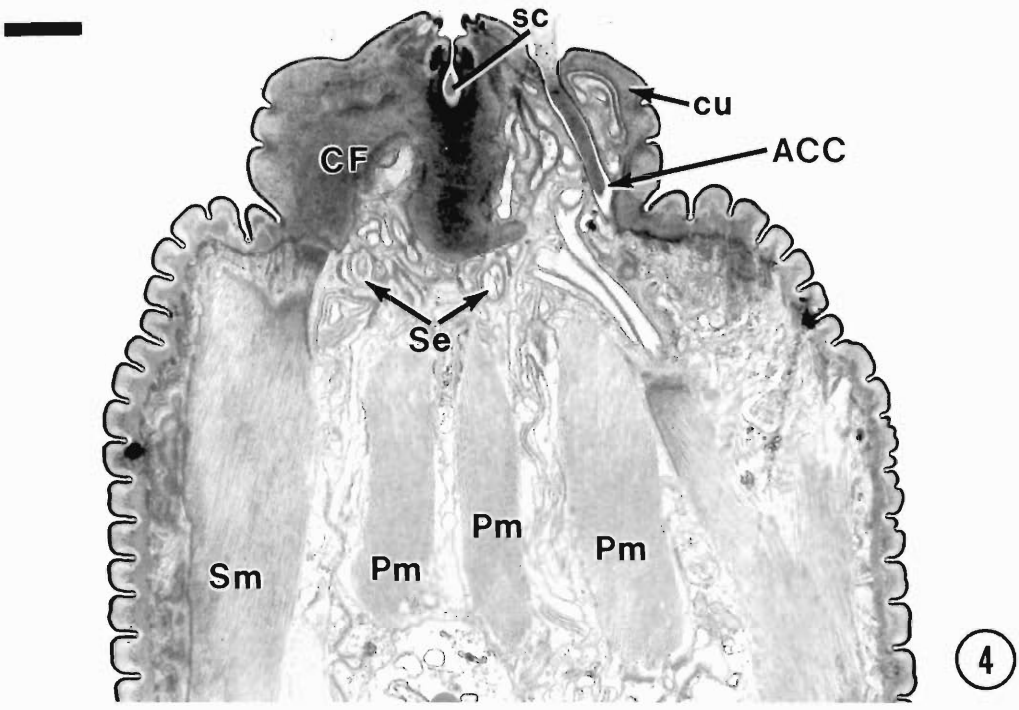
Figures 2, 3. Sections through stylet region of *Pratylenchus penetrans*. 2. Longitudinal tangential view of a retracted stylet (St). Protractor muscles (Pm) extend from the base and anterior surfaces of the stylet knobs to attachment sites at the stomatal wall (SW), cephalic framework (CF), and lateral body somatic muscle elements. Sk, stylet knob. 3. Cross-section slightly below stylet knobs shows electron-opaque accumulations (EDA) associated with electron-lucent filaments that are continuous with the main protractor muscle elements. Portions of the amphidial gland sensilla (Se) and a pair of microvillus nerve processes (NP) are also apparent. EL, esophageal lumen. Scale bars = 1.0 μm.

Figures 4, 5. Oblique sections through stoma and cephalic regions of an adult male *P. penetrans*. 4. Stomatal cavity in relation to body cuticle (cu), cephalic framework (CF), and anterior sensilla (Se). ACC, amphidial cuticular channel; Pm, protractor muscle; sc, stomatal cuticle; Sm, somatic muscle. 5. Section through blades (CFB) of cephalic framework and thick region of the stomatal wall (SW) shows portions of the amphidial sensilla (ASe), cephalic receptors (CR), and accessory receptors (AR). Pm, protractor muscles; Sm, somatic muscles; St, stylet. Scale bars = 1.0 μm.

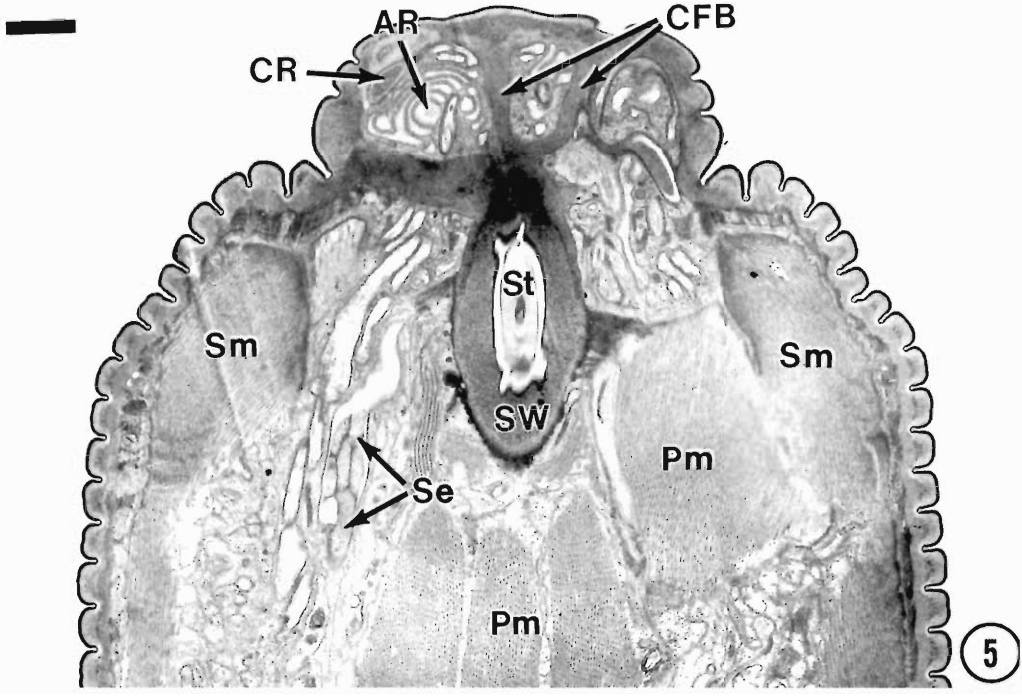
Figures 6, 7. Longitudinal sections through procorpus and metacorpus of *P. penetrans*. 6. Dorsal gland extension (Dx) and ampulla (DA) containing small secretory granules (SG) lie medially in anterior region of the procorpus. Numerous mitochondria (Mc) extend posteriad from the base of the stylet knobs shown in Figure 2 to the supporting cells of the procorpus. 7. Section posteriad to that shown in Figure 6. Transversely oriented dorsal gland extension (Dx) is folded and contains strongly to moderate electron-opaque granules. The dorsal gland extension and adjacent tubular cuticularized wall (EL) of the esophageal lumen enter the metacorpus (m) shown in a submedial tangential section. Scale bars = 1.0 μm.



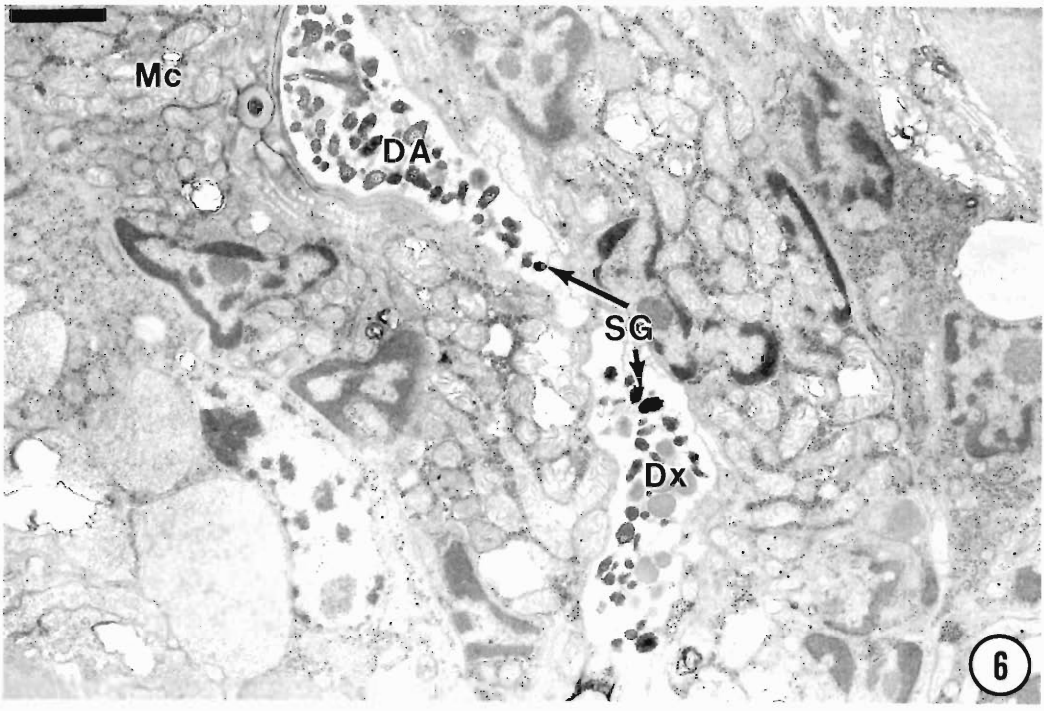


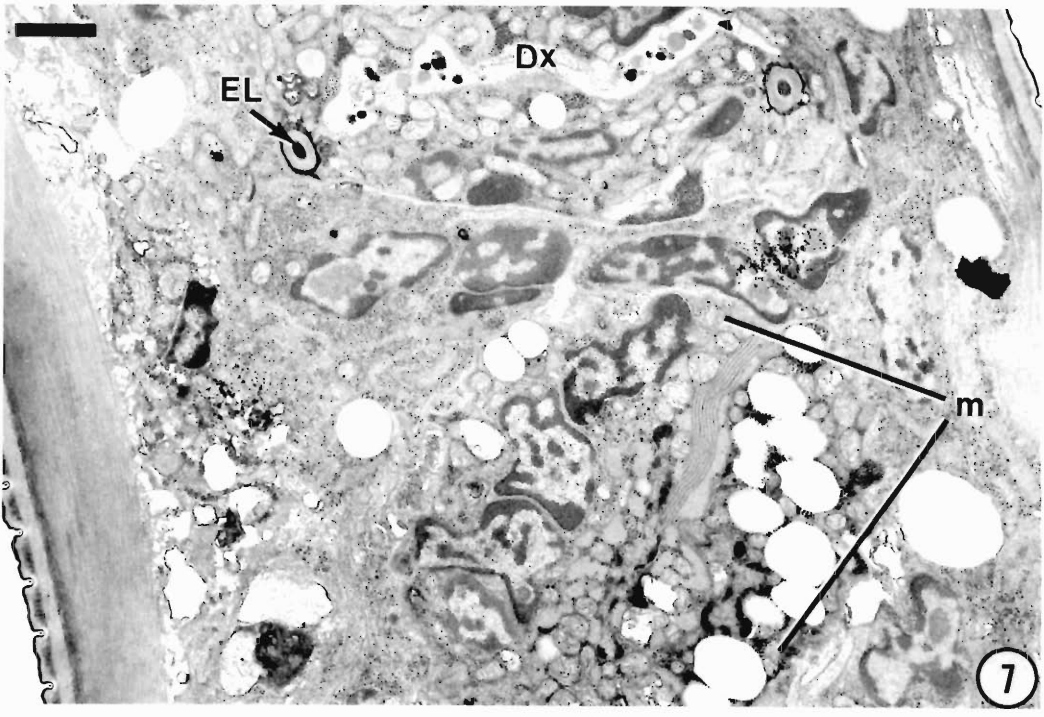


4



5





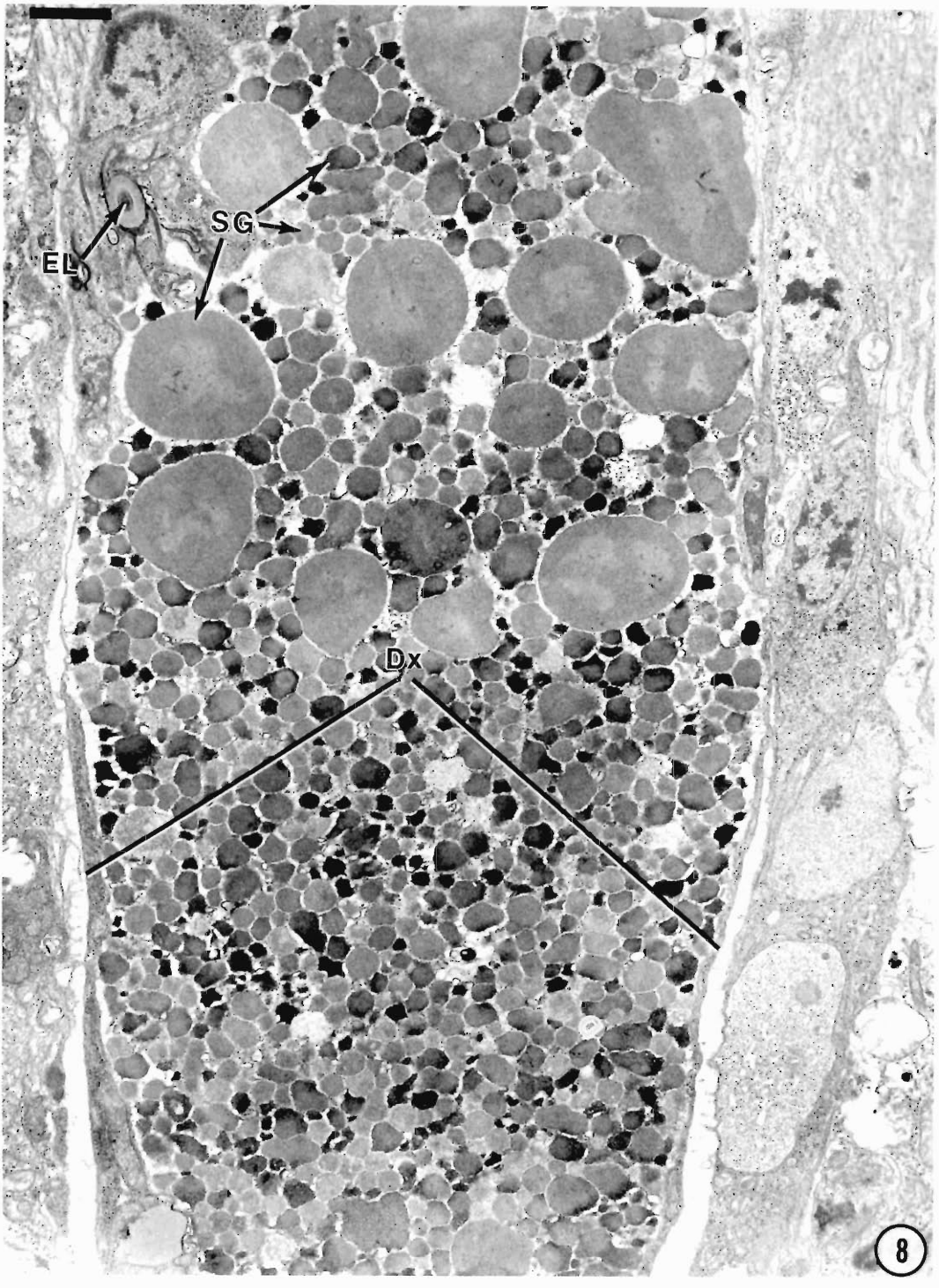


Figure 8. Longitudinal section of adult female of *P. penetrans* showing expanded dorsal gland extension (Dx) within the procorpus of the esophagus. Secretory granules (SG) are small to large in size and light to moderate in electron density. EL, esophageal lumen. Scale bar = 1.0 μm.

the same cross-sectional plane (Fig. 22). Within the dorsal gland, small electron-opaque secretory granules are assembled by Golgi complexes. The secretory granules appear as electron-opaque clusters of condensation products in various stages of enlargement and dissipation (Fig. 23). The prominent dorsal gland nucleus is surrounded by Golgi complexes and their secretory products (Fig. 24).

The subventral glands (Figs. 25, 26), which are adjacent and posteriad to the dorsal gland, terminate in a narrow region between the intestinal epithelium, the ventral nerve cord, and the somatic musculature. The subventral gland nuclei, which tend to be smaller than the dorsal gland nucleus, frequently have convoluted membranes (Fig. 26). Golgi complexes surround the nuclei and give rise to electron-opaque secretory granules that form smaller, less compact clusters than those observed in dorsal glands (Figs. 25, 26).

The secretory-excretory gland terminus is a tubular invagination of the body cuticle (Fig. 19) that extends into the elongated gland. The central lumen of the secretory-excretory gland is delineated by an electron-opaque wall that is surrounded by membranous vesicles and tubules (Fig. 20). The secretory-excretory gland body extends posteriad between the borders of the esophageal glands and intestinal epithelium.

The intestinal lumen occasionally appears occluded with a membrane complex (Fig. 27), especially when the body space is shared with reproductive organs that compress the intestines. However, in the region beyond the esophagointestinal valve, the lumen is broad and clear of ingested materials (Figs. 20, 21). In regions where the intestinal lumen is filled with contents, the lumen wall is greatly distended (Fig. 28). The filamentous or vesicular membranous invaginations along the inner wall of the lumen appear to be sections through widely dispersed intestinal microvilli. The intestinal lumen is formed by paired epithelial cells that are joined laterally by membrane junctions (Fig. 28).

Adjacent and parallel to the intestine is the ovary or testis. Beyond the germinal zone of the ovary, primary and secondary oocytes are formed. Enlarged oocytes (Fig. 29) can pass through the oviduct (Fig. 29) into a spermatheca (Figs. 1, 30). The spermatozoa within the spermatheca (Fig. 30) are similar in morphology to

---

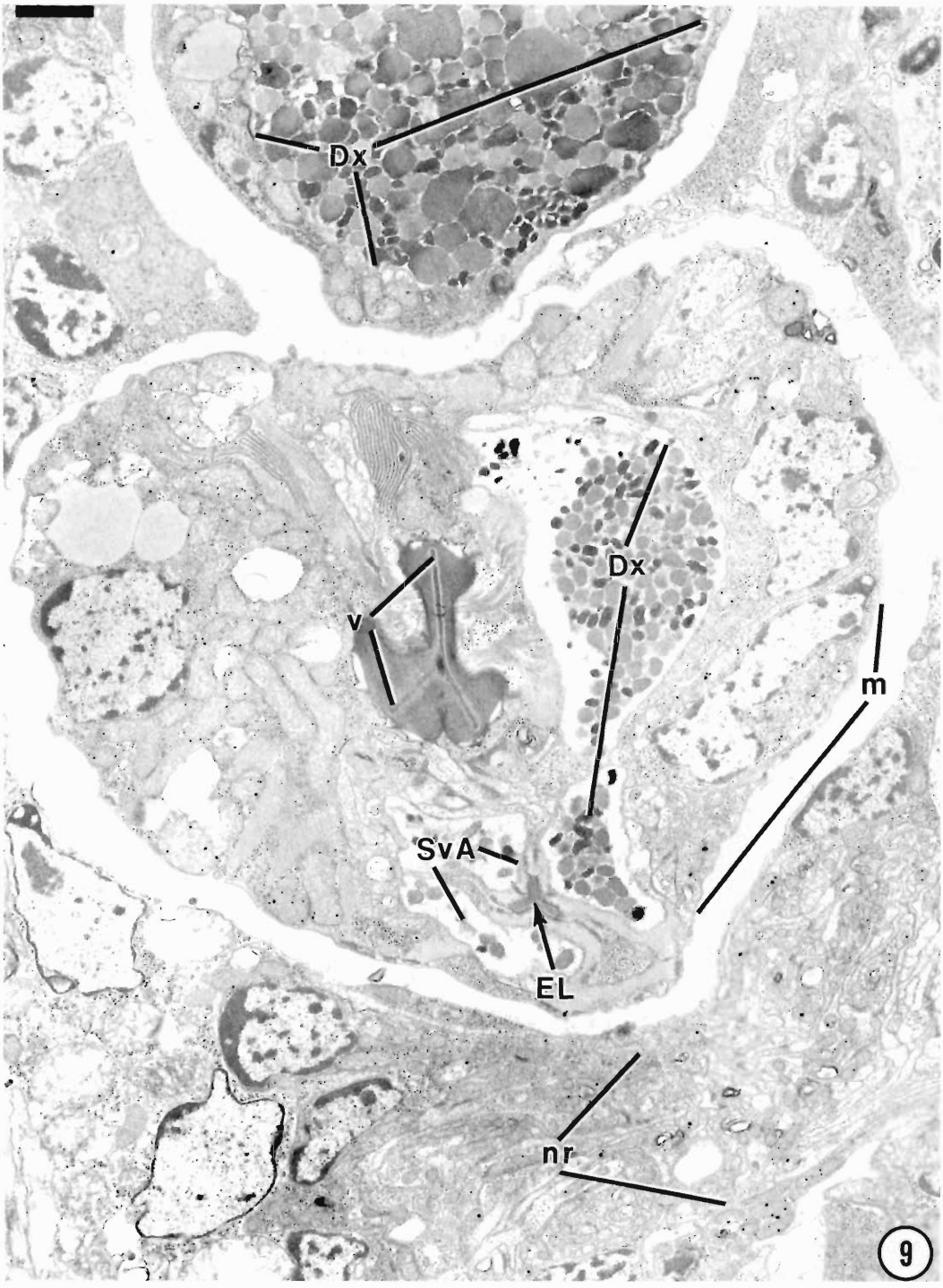
→

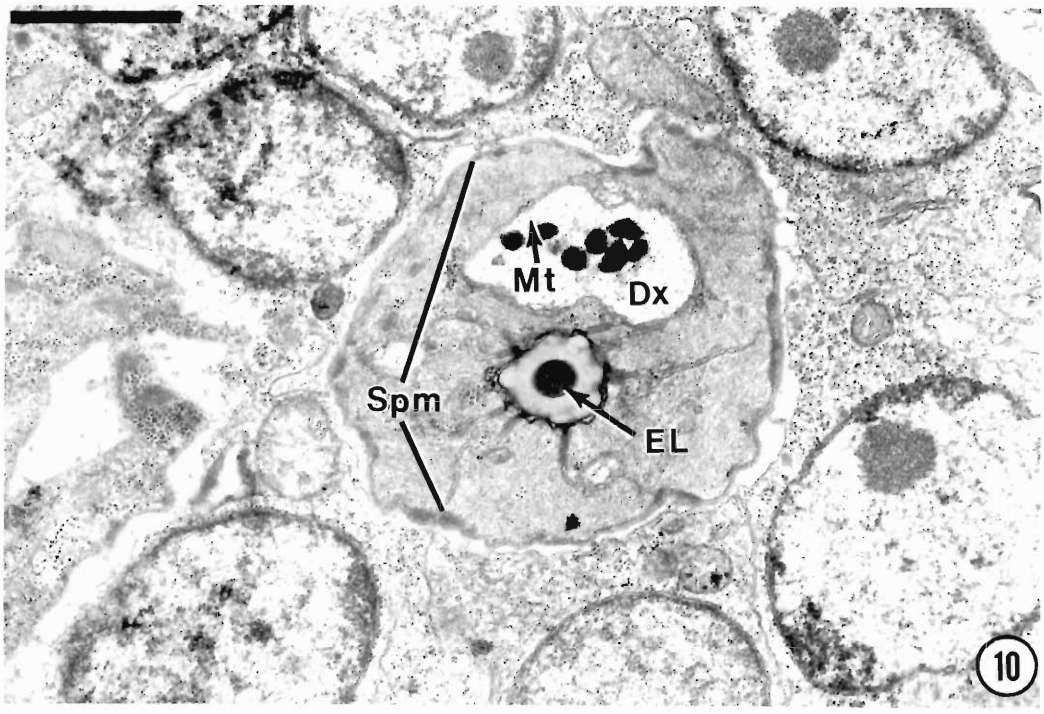
Figure 9. Section through the metacorpus of adult female of *P. penetrans* with an oblique view of the triradiate lumen of a sclerotized valve (v) of metacorpus (m) and parts of the dorsal (Dx) and subventral gland extensions. Secretory granules in the dorsal gland extension within the metacorpus appear uniformly small compared to the wide range of sizes that are found in the gland extension of the procorpus of the specimen in Figure 8. Secretory granules in the subventral gland ampullae (SvA) are also small and moderately electron-dense. nr, nerve ring. Scale bar = 1.0 μm.

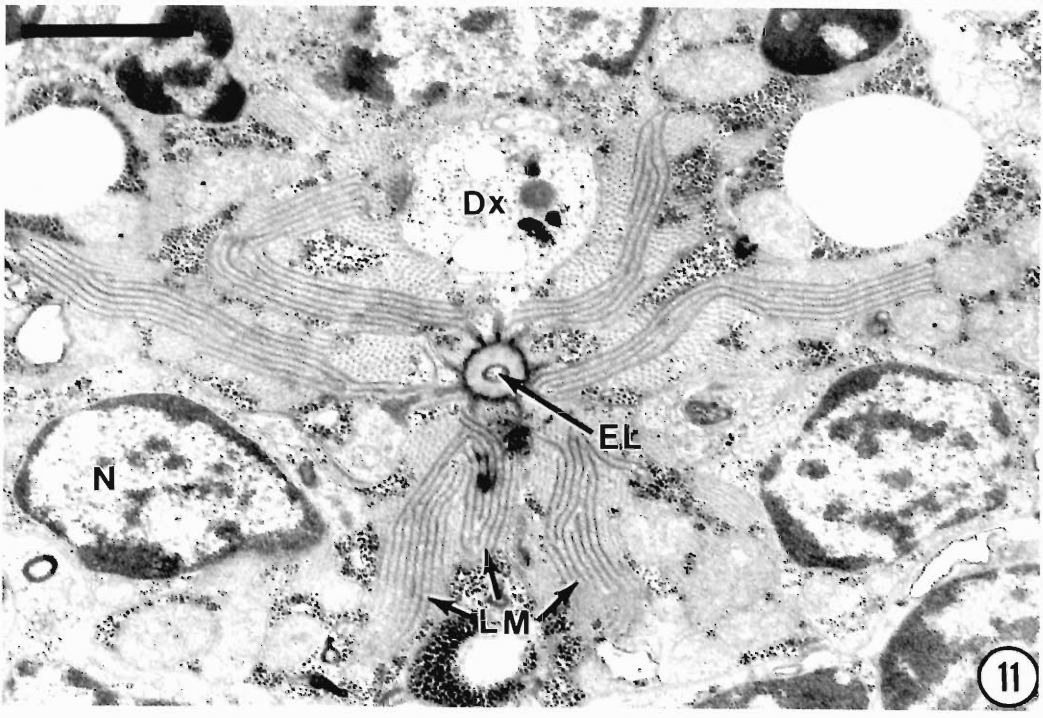
Figures 10, 11. Cross-sections through the anterior regions of the metacorpus of *P. penetrans*. 10. Adult male sphincter muscles (Spm) surround a narrow region of the dorsal gland extension (Dx) containing a few secretory granules and closely assembled microtubules (Mt). EL, esophageal lumen. 11. Section posteriad from the sphincter near the main body of the metacorpus. Laminar membrane (LM) complexes are associated with the muscle system of the anterior metacorpus. Dx, dorsal gland extension; EL, esophageal lumen; N, nucleus. Scale bars = 1.0 μm.

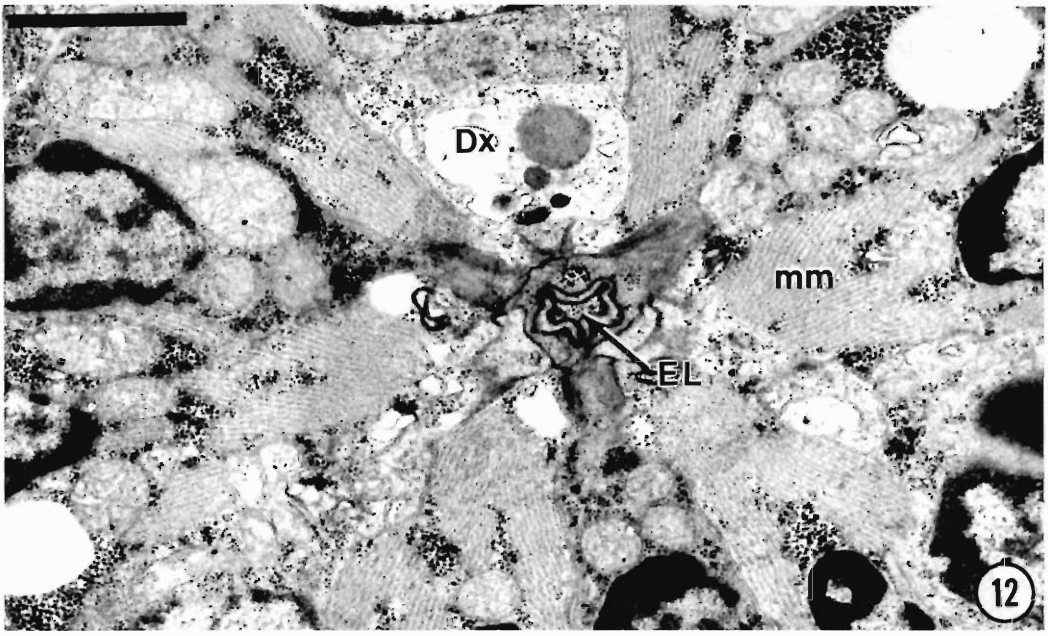
Figures 12, 13. Median cross-sections of metacorpus in *P. penetrans* show protractor muscle in relation to the esophageal pump valve and supporting basal lamina. 12. Cuticle of the esophageal lumen (EL) showing transition from circular to modified triradiate shape of the lumen wall. Dx, dorsal gland extension; mm, metacorpus muscles. 13. Metacorpus valve (mv) with triradiate thick sclerotized walls of the lumen. Metacorpus pump muscles (mpm) are attached centripetally to adradial walls of the valve and centrifugally to the metacorpus wall via hemidesmosomes (hd). cuR, cuticular ridges; Dx, dorsal gland extension. Scale bars = 1.0 μm.

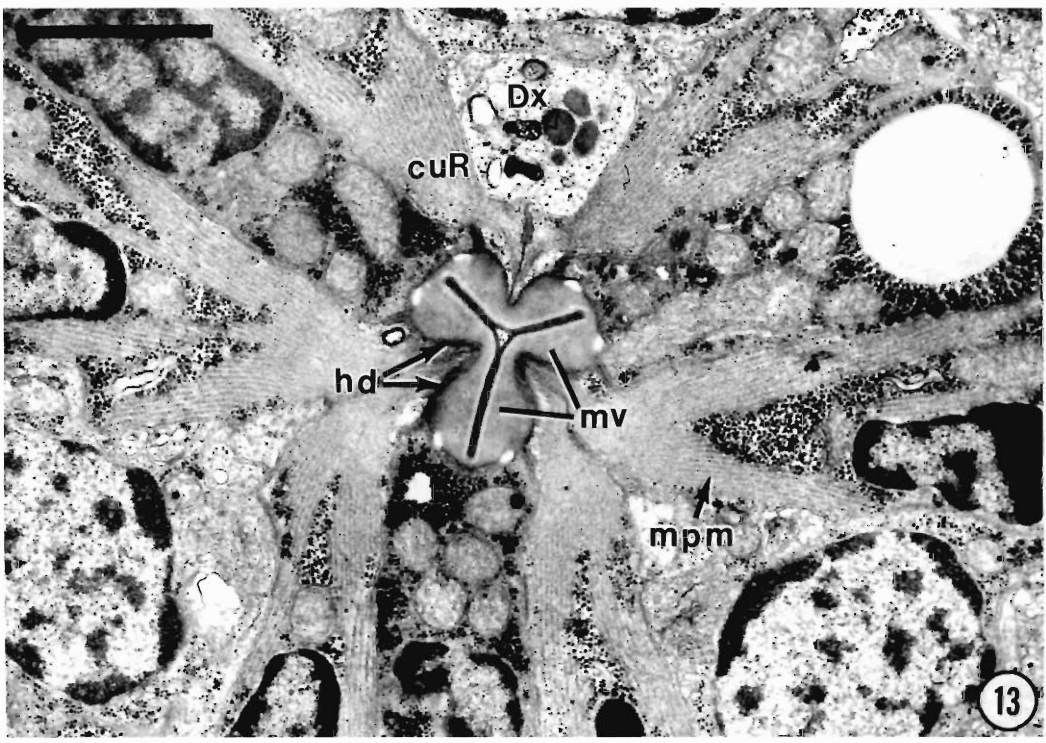
Figures 14–16. Cross-sections showing dorsal gland extension (Dx) and components of subventral gland valve of *P. penetrans*. 14. Expanded lumen of esophagus (EL) shows one lateral wall with opening into cuticularized base (→) of a subventral gland valve (Svv). mpm, metacorpus pump muscle. 15. Closed triradiate esophageal lumen (EL) flanked subventrally by cuticular bases (cb) of valves leading to subventral gland ampullae. Dx, dorsal gland extension. 16. Quadriradiate-shaped membrane terminals of subventral valves (Svv) posteriad from region illustrated in Figure 15. Dx, dorsal gland extension. Scale bars = 1.0 μm.

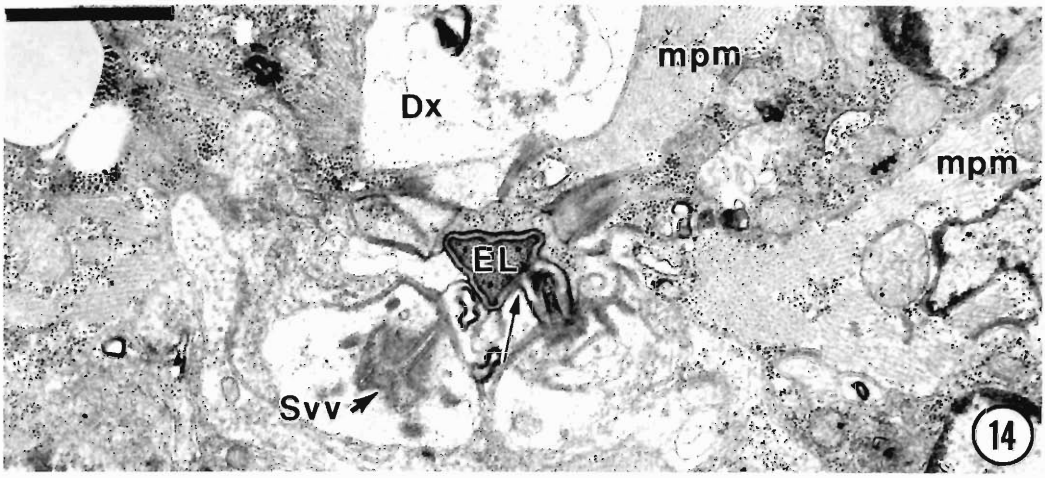


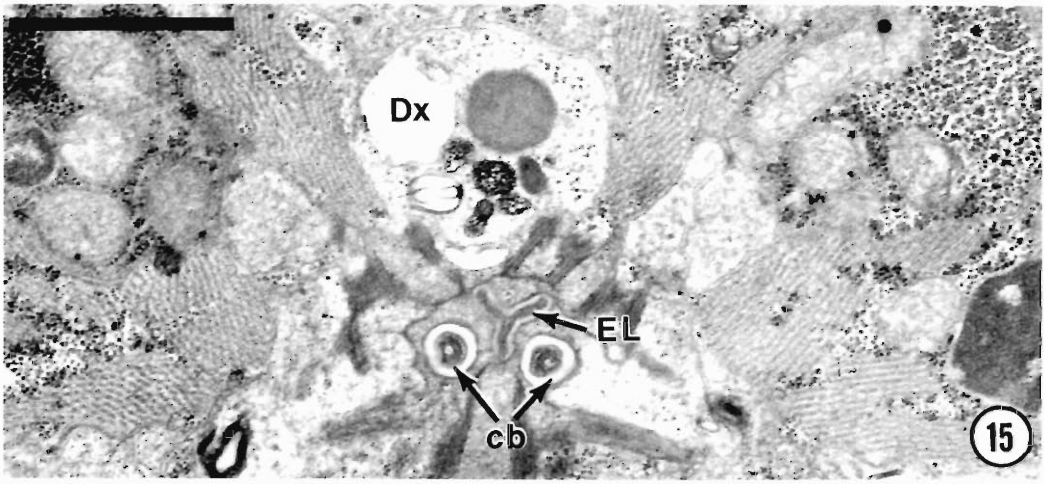


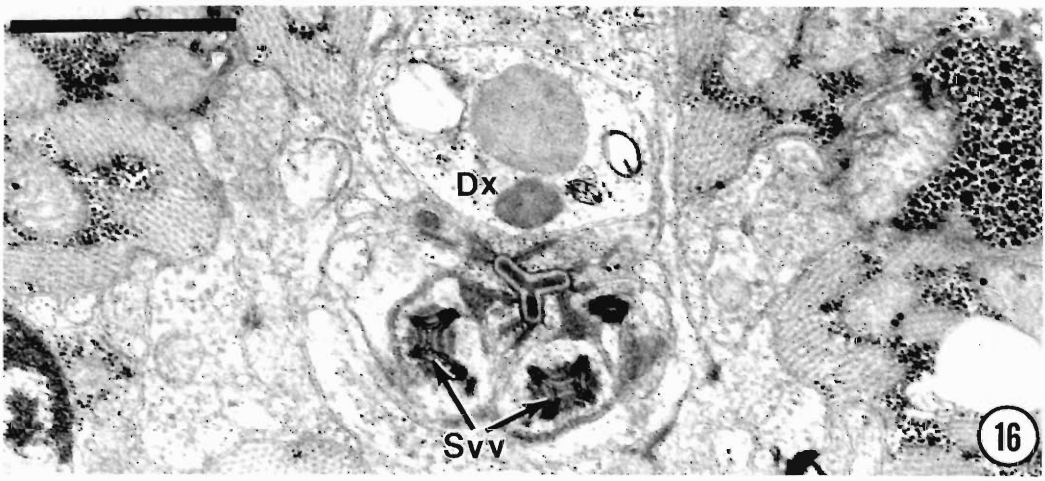












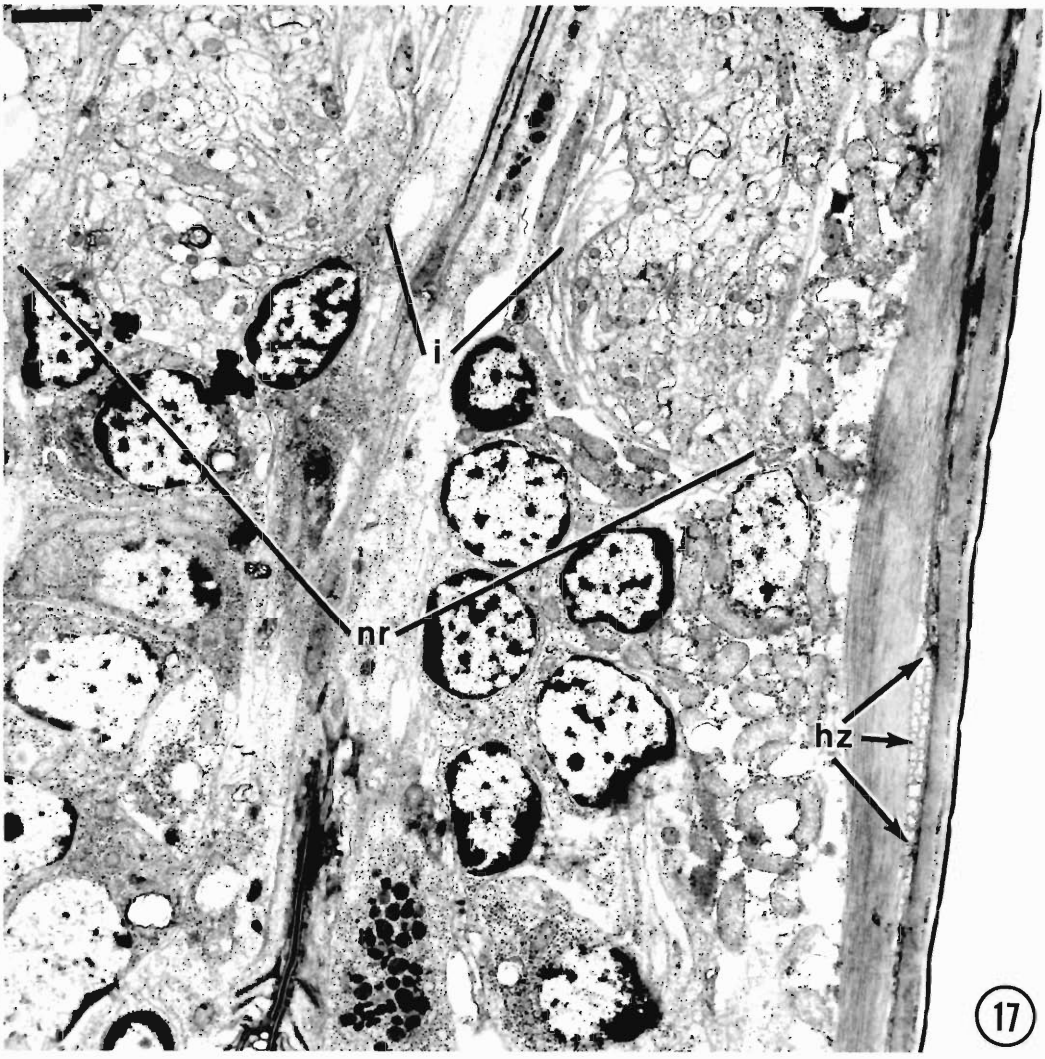


Figure 17. Longitudinal section through the isthmus (i) of the esophagus of adult male of *P. penetrans*. The nerve ring (nr) is located close to the metacorpus base and lies anteriad the base of the esophago-intestinal valve. A ventrolateral commissure of nerve fibers appears between the somatic muscles and the cuticle to form the hemizonid (hz). The hemizonid is located ventrolaterally midway between the base of the nerve ring and esophago-intestinal valve. Scale bar = 1.0 μm.

sperm observed in the vas deferens of a male specimen (Fig. 1). Each spermatozoon has irregular masses of chromatin consisting of nuclear material not bounded by a nuclear envelope, mitochondria, and small clusters of fibrous bodies (Figs. 30, 40). Fertilization occurs in the spermatheca where numerous sperm accumulate. In *P. penetrans*, stain reactions were not available to show this sequence of events. An oocyte within the spermatheca region of the uterus (Fig. 31) is filled with electron-transparent lipid granules and electron-opaque protein bodies and bounded by a unit membrane with regions of electron-opaque deposits. Columnar cells extend from the spermatheca to the fluid-filled region opposite the vaginal canal (Figs. 30, 32, 33). These cells are characterized by a dense concentration of ribosomes, mitochondria, and Golgi complexes and their secretory granules of various sizes and shapes. The cells have a centrip-

etal orientation; their bases are joined by membrane junctions. A central lumen, which is lined with the apices of these cells, provides the passage for eggs and spermatozoa (Fig. 32). The basal membranes of the columnar cells, which are highly plicated, apparently allow for expansion of the lumen during egg passage (Figs. 30, 32, 33). The lumen of this region of the uterus is readily identified by the electron-opaque membrane junctions that connect the basal portions of adjacent cells (Fig. 32). The lumen of the columnar region is continuous with the fluid-filled uterus that opens into the cuticle-lined vagina (Fig. 34). A postvulval uterine sac occurs posteriad to the central uterus (Fig. 35). In longitudinal section, the vaginal cuticle forms a flat contoured channel that initially appears convoluted near the vulva and is continuous with the body cuticle (Fig. 34). A group of muscles is attached by hemidesmosomes to the flat region of the vaginal cuticle in lateral view. Muscle filaments are primarily tangential, but some horizontal orientations also occur. Two pairs of striated muscles with longitudinal to tangential orientations are attached by hemidesmosomes to the anterior and posterior cuticle walls of the vulva. The conformation and position of the muscles, which lie adjacent to the vaginal canal and the walls of the vulva, appear to have a direct relationship with the egg-laying process. The rectum and the anus are located subterminally (Fig. 36).

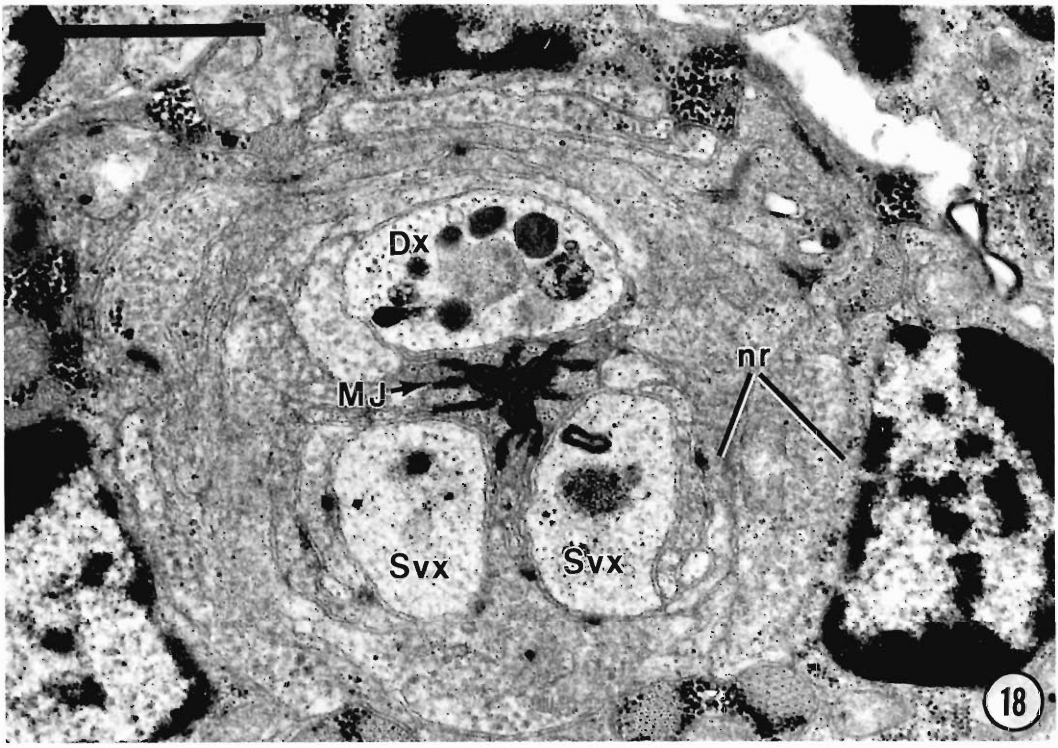
The male gonad consists of a single testis and vas deferens. The germinal zone of the testis gives rise to spermatogonial cells that develop into primary and secondary spermatocytes. The transition from spermatocytes to spermatids involves the reduction division of chromosomes from diploid to haploid within a short region of the testis. During this transition, the nuclear membrane of the spermatocyte (Fig. 37) disappears. After meiosis, the haploid chromosome complement appears as electron-opaque chromatin within an amoeboid cell (Fig. 38). Spermatid cell membranes evaginate to form filopodia that interdigitate with boundaries of adjacent spermatids to form distinctive membrane complexes. Large clusters of fibrous bodies surround the central nucleus and later become dispersed throughout the cytoplasm (Figs. 38, 39). Some fibrous bodies persist in the spermatozoa as shown in a section through a spermatheca (Fig. 30). Nuclei of spermatozoa, which tend to be irregular, are surrounded by clusters of mitochondria (Figs. 30, 40). The nonnuclear regions of the sperm are generally electron-translucent; they frequently have remnants of fibrous bodies similar to those occurring in spermatids. As sperm become concentrated in the vas deferens, their outer boundaries become ovoid to elliptical. Their membrane surfaces form pseudopodia or evaginate into filopodia that appear as tubules or small vesicles (Fig. 40).

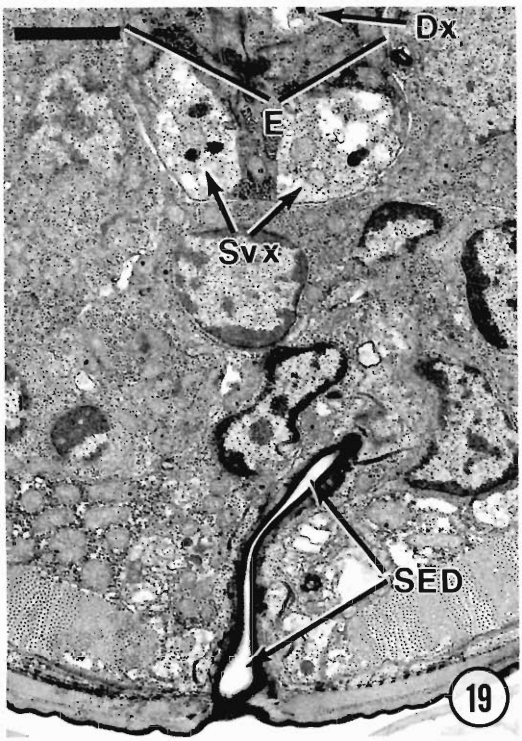
The ultrastructure of the male copulatory organs of *P. penetrans* has been described in detail by Wen and Chen (1976). Additional micrographs of the spicules and their related structures are shown as a corollary to other organs described in this paper. The base of the spicule shaft is supported by protractor and retractor muscles (Figs. 41, 42). The elongated arms of the spicules (Figs. 42–44) extend downward and centrad to enter the cloaca (Fig. 44). A pair of sensilla is located at the posterior lip of the clo-

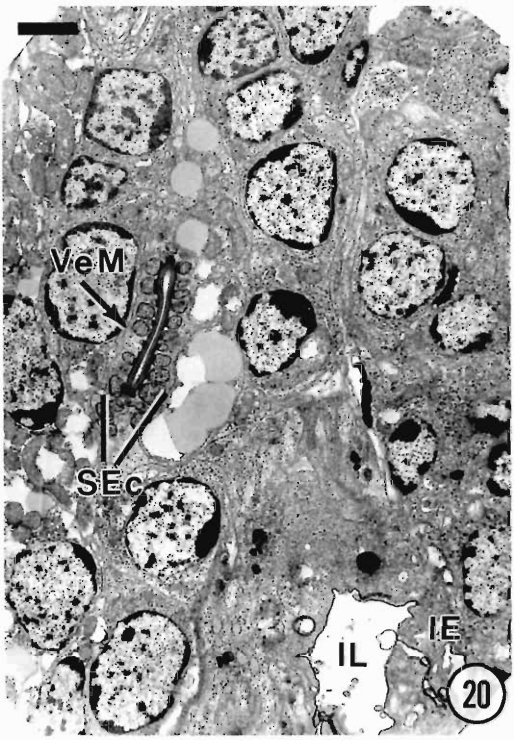
→

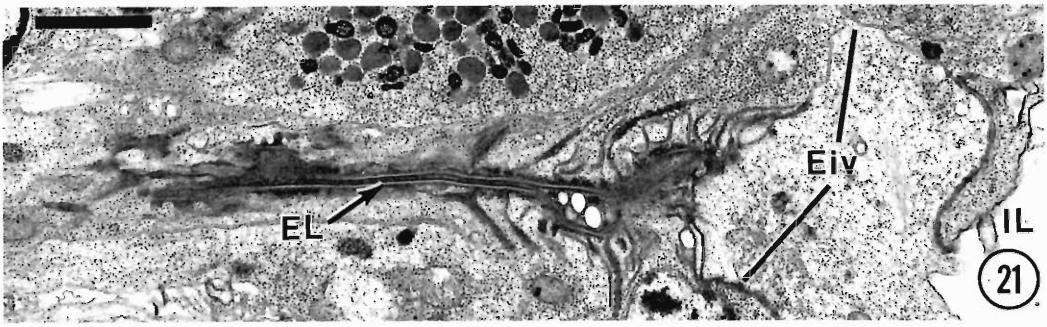
Figures 18–20. Sections through the isthmus and secretory-excretory gland of *P. penetrans*. 18. Triradiate wall of esophagus. Membrane junctions (MJ) interact with limiting membranes of dorsal (Dx) and subventral gland extensions (Svx). The section includes a part of the nerve ring (nr). 19. A cross-section of an adult male posteriad from the nerve ring shows narrow region of esophagus (E) and the cuticle-lined duct (SED) of the secretory-excretory gland. Svx, subventral gland extension. 20. Longitudinal section of an adult male shows a portion of the cuticle-lined duct within the secretory-excretory canal (SEc). Vesiculate membranes (VeM) occur in the space between the duct wall and the limiting membrane of the secretory-excretory gland. IE, intestinal epithelium; IL, intestinal lumen. Scale bars = 1.0 μm.

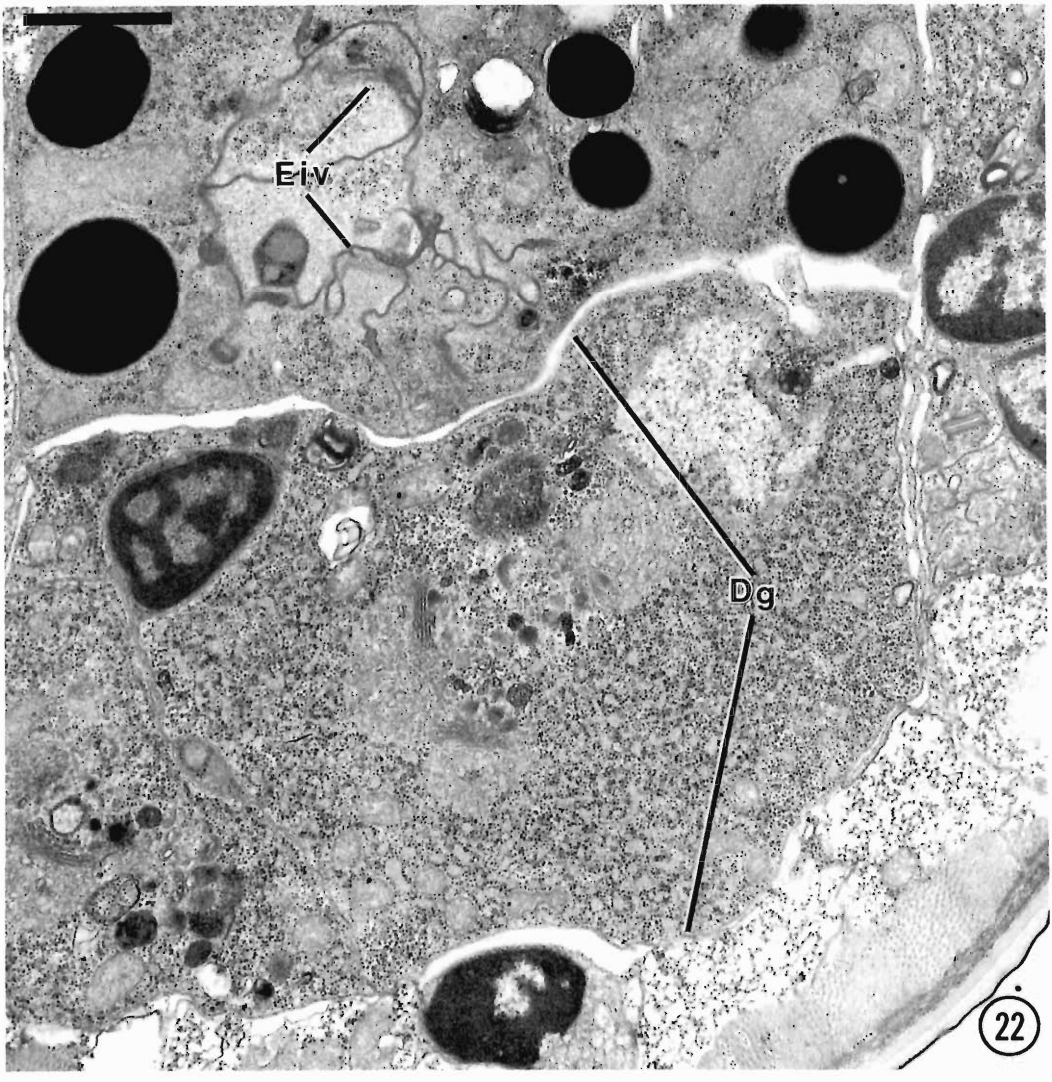
Figures 21, 22. Sections through esophago-intestinal regions of *P. penetrans*. 21. Longitudinal section of the esophago-intestinal valve of an adult male with a membrane supported terminus of cuticle-lined esophageal lumen (EL) and continuity with unlined nonmuscular cells comprising the esophago-intestinal valve (Eiv). The posterior boundary of the valve adjoins or leads into an enlarged vacuolate intestinal lumen (IL). 22. Cross-section of esophago-intestinal valve (Eiv) is shown adjacent to the expanded region of the dorsal esophageal gland (Dg). Scale bars = 1.0 μm.











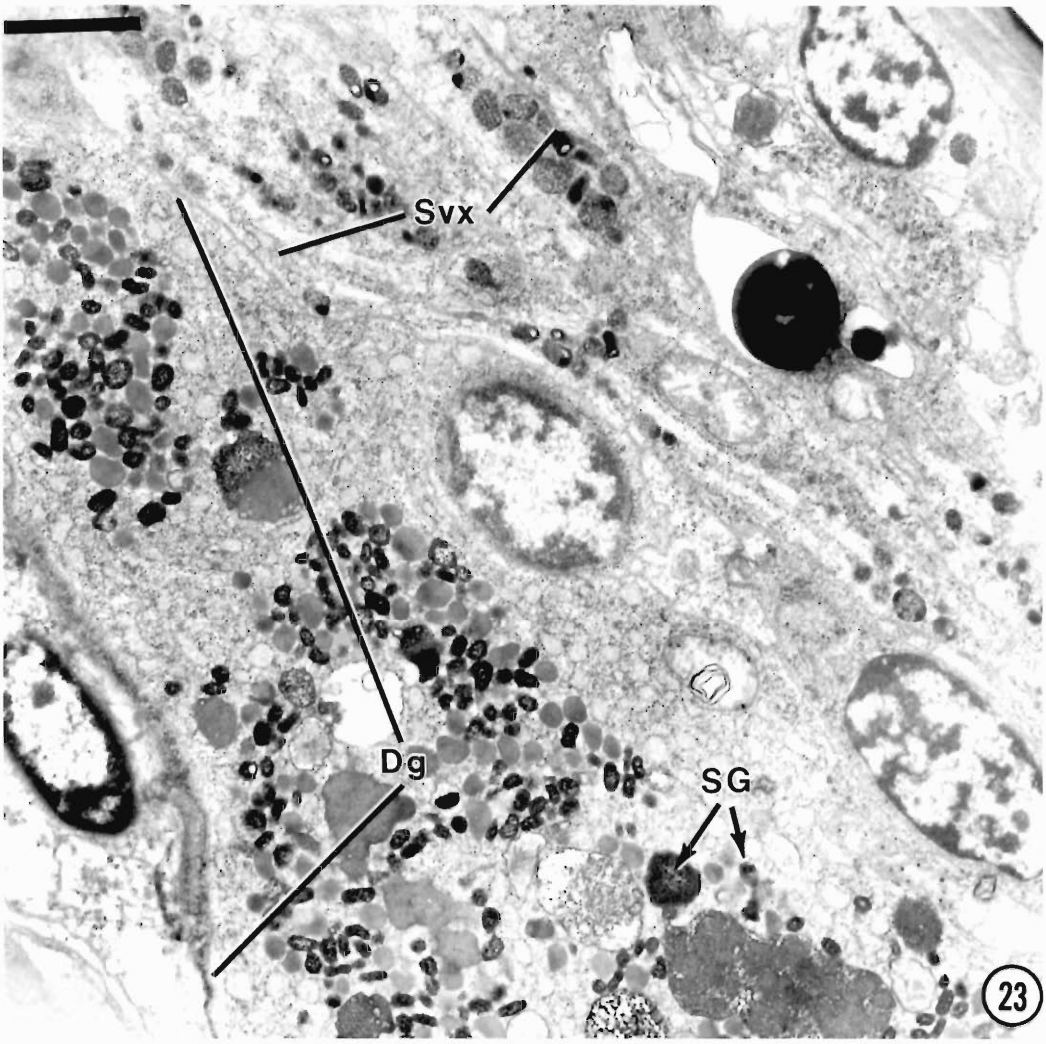


Figure 23. Longitudinal section of anterior region of the dorsal gland (Dg) of adult male of *P. penetrans*. Cytoplasm filled with dense clusters of electron-opaque secretory granules (SG) that appear to condense into larger granules. Svx, subventral gland extension. Scale bar = 1.0 μm.

aca (Fig. 44). Copulatory caudal alae extend from the cloaca to the tail terminus (Fig. 46).

Low-temperature cryofixation and scanning electron microscopy of specimens helps to verify structure–function relationships. Freeze-fractured images of the intestinal region (Fig. 45) corroborate the presence of the large lumen of the central region of the intestine as observed by transmission electron microscopy of glutaraldehyde-fixed specimens (Fig. 28). Within the lumen of the intestine, microvilli-like membrane invaginations are prominent but lack the uniform microvilli arrangement observed in other species. In addition to the membrane invaginations of the lumen wall, a few evaginations appear to extend into the intestinal epithelial cells. However, these evaginations could result from the slightly folded boundaries of the lumen. The secretory-excretory gland duct appears tubular in the anterior region of the gland cell. In chemically fixed specimens, the duct walls usually appear collapsed (Fig. 26). However, with the cryofixed specimens observed with LTSEM, the secretory-excretory

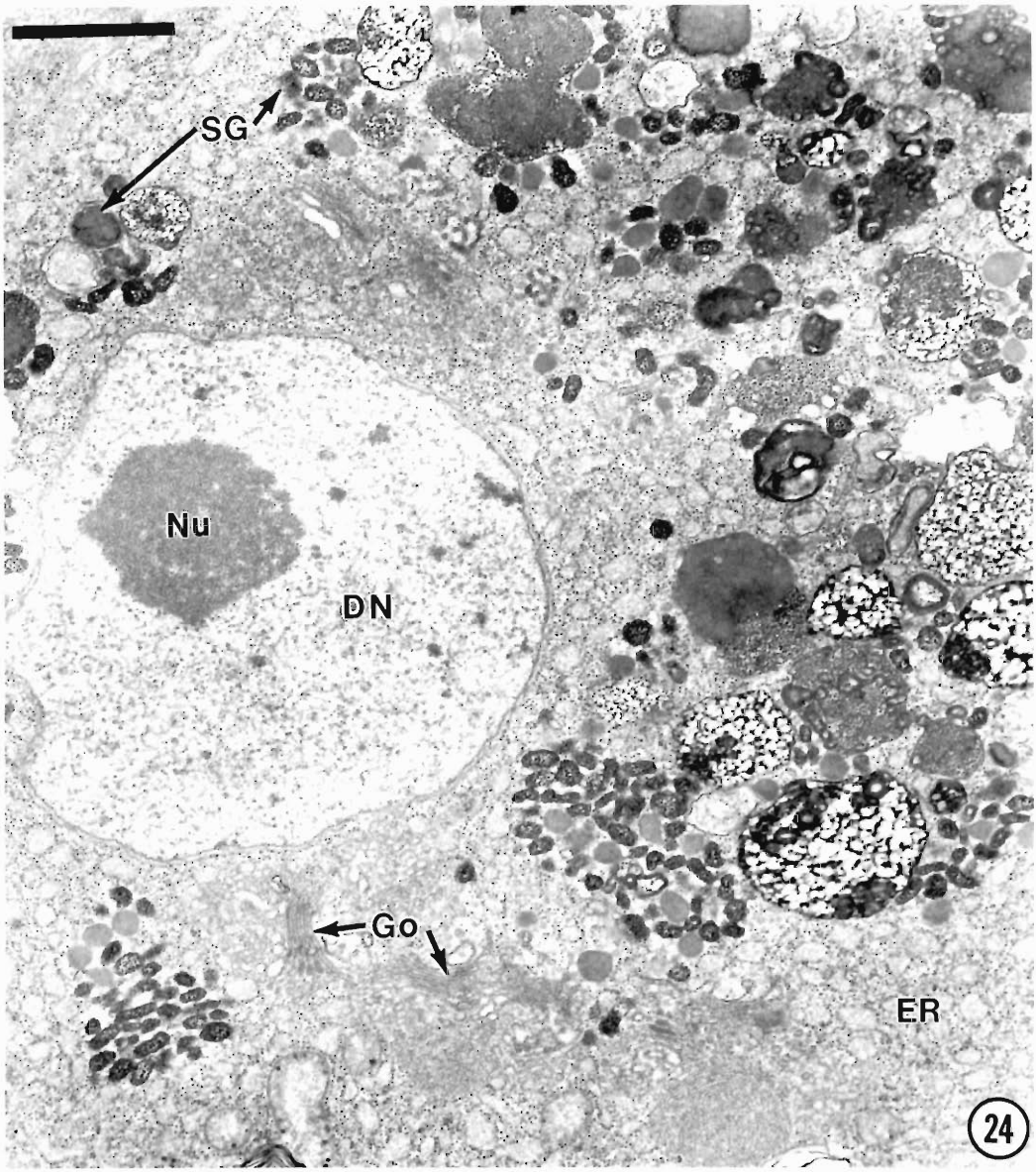


Figure 24. Longitudinal section of the midregion of dorsal gland shown in Figure 23. The dorsal gland nucleus (DN) is surrounded by numerous Golgi bodies (Go). The Golgi complexes receive newly synthesized protein and lipids from the endoplasmic reticulum (ER) and transfer them to plasma membranes, lysosomes and secretory granules (SG). Large secretory granules may form by condensation or aggregation of smaller secretory granules. Nu, nucleolus. Scale bar = 1.0 μm.

duct is cylindrical in shape. The LTSEM images of the tail region of a male specimen (Figs. 46, 47) can be compared to the thin-section images viewed in the transmission electron microscope (Fig. 44) and the micrographs by Wen and Chen (1976). The posterior lips of the cloaca and their embedded sensilla suggest a possible role in mating.

Discussion

The anterior sensory anatomy of *P. penetrans* was compared to that of other species of *Pra-*

tylenchus in an extensive study conducted with electron and scanning electron microscopy (Trett and Perry, 1985). The observations in our study are consistent with those made in other *Pratylenchus* spp. (Trett and Perry, 1985) as well as other species of Tylenchida (Baldwin and Hirschmann, 1973, 1975; De Grisse et al., 1974; McLaren, 1976; Wergin and Endo, 1976; Endo and Wergin, 1977; Endo, 1980). The protractor and anterior somatic muscles structurally interact with the cephalic framework, stylet knobs, and mitochondria-rich sarcoplasm of the protractor muscles. Similar complexity of tylenchid stylets was shown in ultrastructural studies of *Criconemoides curvatum* Raski, 1952 (Wen and Chen, 1972; Mai et al., 1977), *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 (Baldwin and Hirschmann, 1976), and *Heterodera glycines* Ichinohe, 1952 (Baldwin and Hirschmann, 1976; Endo, 1983).

The narrow, sinuous pathway of the dorsal gland extension, shown within the procorpus of Figures 5 and 7, illustrates a juvenile stage of a nematode or one that is not actively feeding. The gland extension of the nematode, shown in Figures 8 and 9, is enlarged and filled with numerous secretory granules whose presence is an indication of a very active host–parasite interaction. The ultrastructure and sometimes the chemical nature of secretory granules have been observed and studied in *Ditylenchus dipsaci* (Yuen, 1968), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Bird and Saurer, 1967; Bird, 1968), *Heterodera schachtii* (Wyss et al., 1984), *Meloidogyne incognita* (Hussey, 1989; Hussey and Mims, 1990), and *Heterodera glycines* (Endo, 1993). The extensive change in the size of the dorsal gland extension and the associated ampullae of *P. penetrans*, as observed in our study, suggests that this species should also be considered for applying monoclonal body technology that is used in other species (Atkinson et al., 1988; Atkinson and Harris, 1989; Hussey, 1989; Davis et al., 1994).

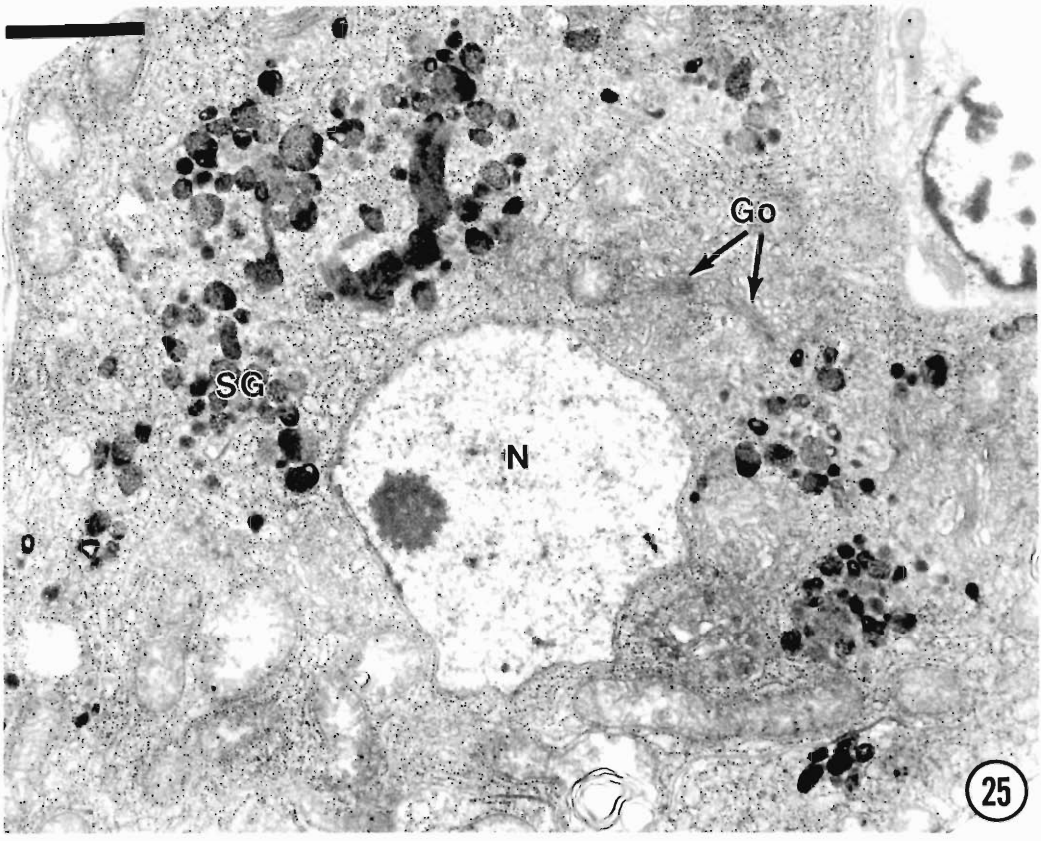
The accumulation of secretory granules in the gland extensions is controlled by sphincter muscles at the anterior and posterior ends of the metacorpus. This muscle function of *Pratylenchus penetrans* was previously shown on film with the aid of video-enhanced light microscopy (Zunke and Institut für den Wissenschaftlichen Film, 1988). This observation is consistent with results found in other tylenchid species, including *Hexatylus viviparus* Goodey, 1952, *Aphelenchoides blastophthorus* Franklin, 1952, *Heterodera glycines*, and *Meloidogyne incognita* (Shepherd and Clark, 1976; Shepherd et al., 1980; Endo, 1984, 1987; Endo and Wergin, 1988). Secretory granules synthesized in the dorsal gland and confined within the dorsal gland extension of the metacorpus were relatively small and appeared uniform in size. However, granules of the same gland extension in the procorpus were greatly enlarged and varied widely in electron opacity. These cellular changes may be related to biochemical reactions that can lead to a better understanding of nematode feeding and host responses.

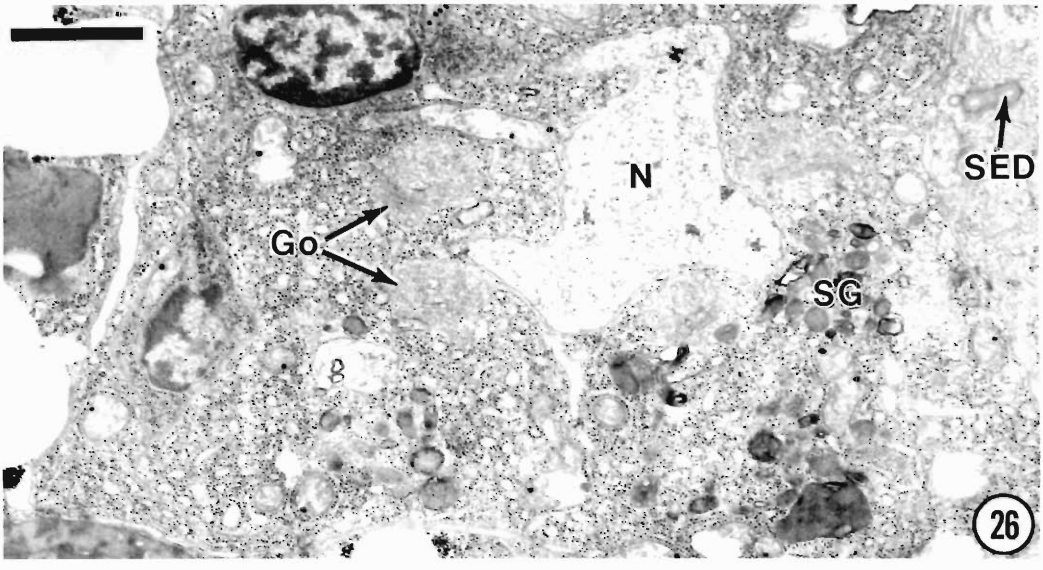
In transverse section, the short thick-walled metacorpus valve and pump muscles of *P. penetrans* appear similar to those of second-stage juveniles of *H. glycines* (Endo, 1984) but differ from juveniles of *Meloidogyne incognita* in which the metacorpus valves are thinner and more elongate (Endo and Wergin, 1988). The sphincter muscles at each end of the metacorpus

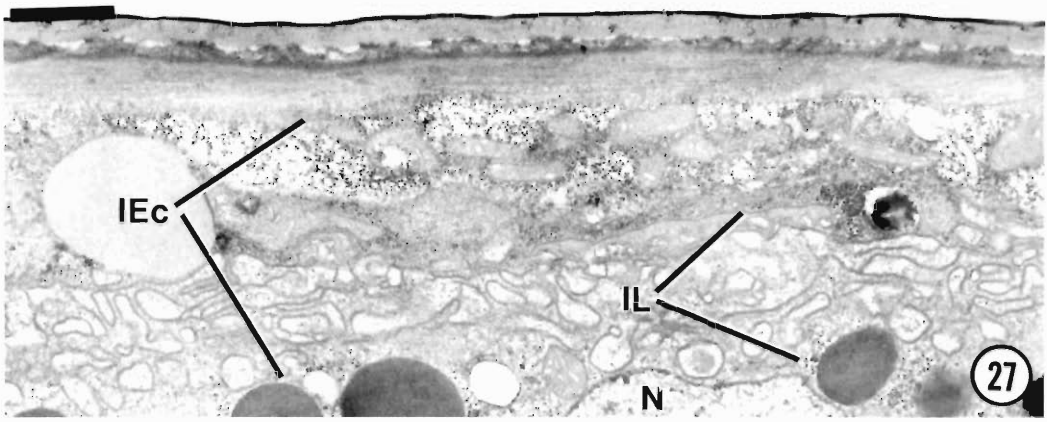
→

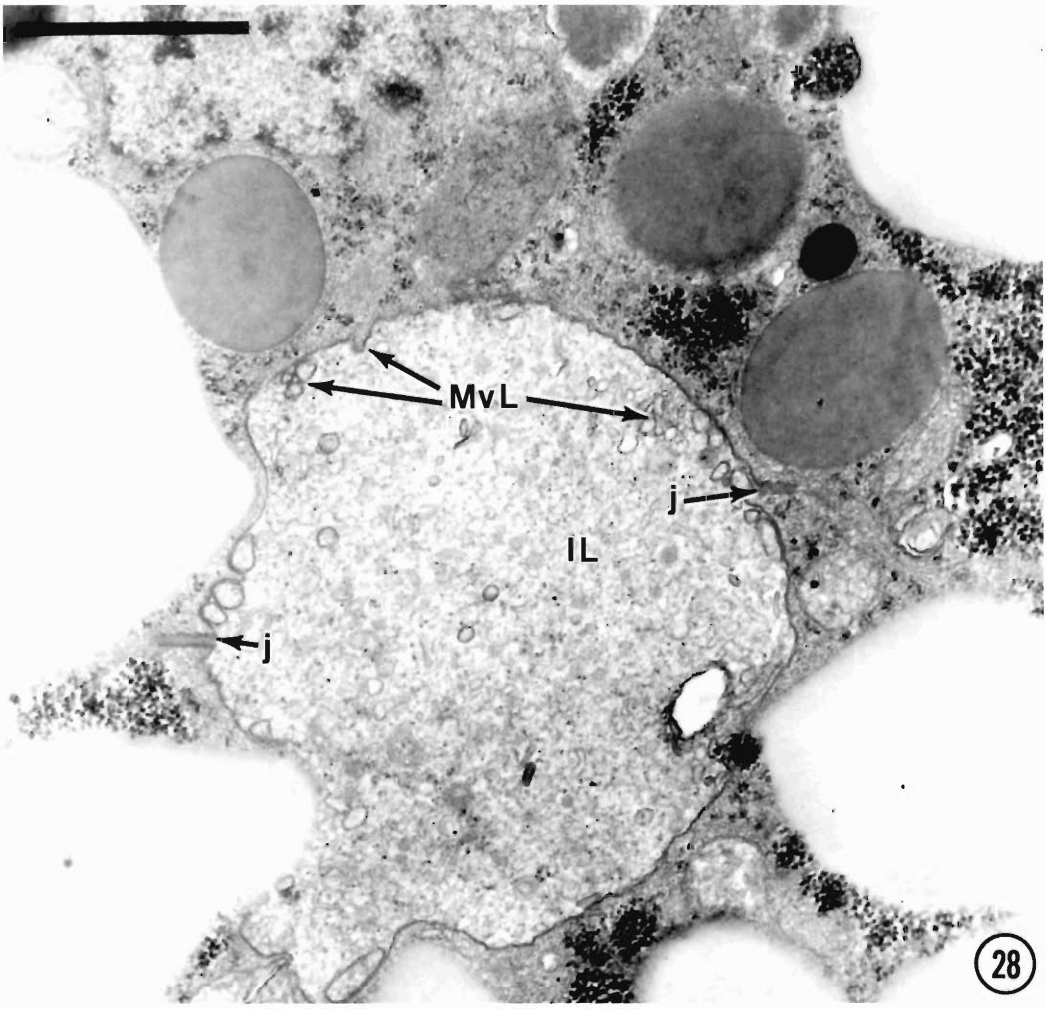
Figures 25, 26. Sections of subventral glands of *P. penetrans*. 25. Longitudinal section of a subventral gland of an adult male with Golgi bodies and numerous, small, moderately electron-opaque secretory granules (SG), some of which appear to condense into larger moderately dense granules. In contrast to the secretory granule formation and accumulation that occurs in the dorsal glands (Figs. 23, 24), the subventral glands have fewer, less electron-opaque and enlarged secretory granules. Go, Golgi; N, nucleus. 26. A cross-section of a subventral gland having an accumulation of secretory granules (SG) and convoluted nuclear (N) envelope. This gland is located at a narrow terminal region of a subventral gland adjacent to the intestinal epithelium. Go, Golgi; SED, secretory-excretory duct. Scale bars = 1.0 μm.

Figures 27, 28. Sections through intestinal regions of *P. penetrans*. 27. Longitudinal section of intestinal lumen (IL) partially occluded by evaginations of supporting membranes of the intestinal epithelial cells (IEc). N, nucleus. 28. Cross-section of an intestinal lumen (IL). Lumen wall is distended by microvilli-like membrane invaginations (MvL). Cell junctions (j) at boundary of lumen wall indicate bi-layered arrangement of cells comprising the intestinal epithelium. Scale bars = 1.0 μm.









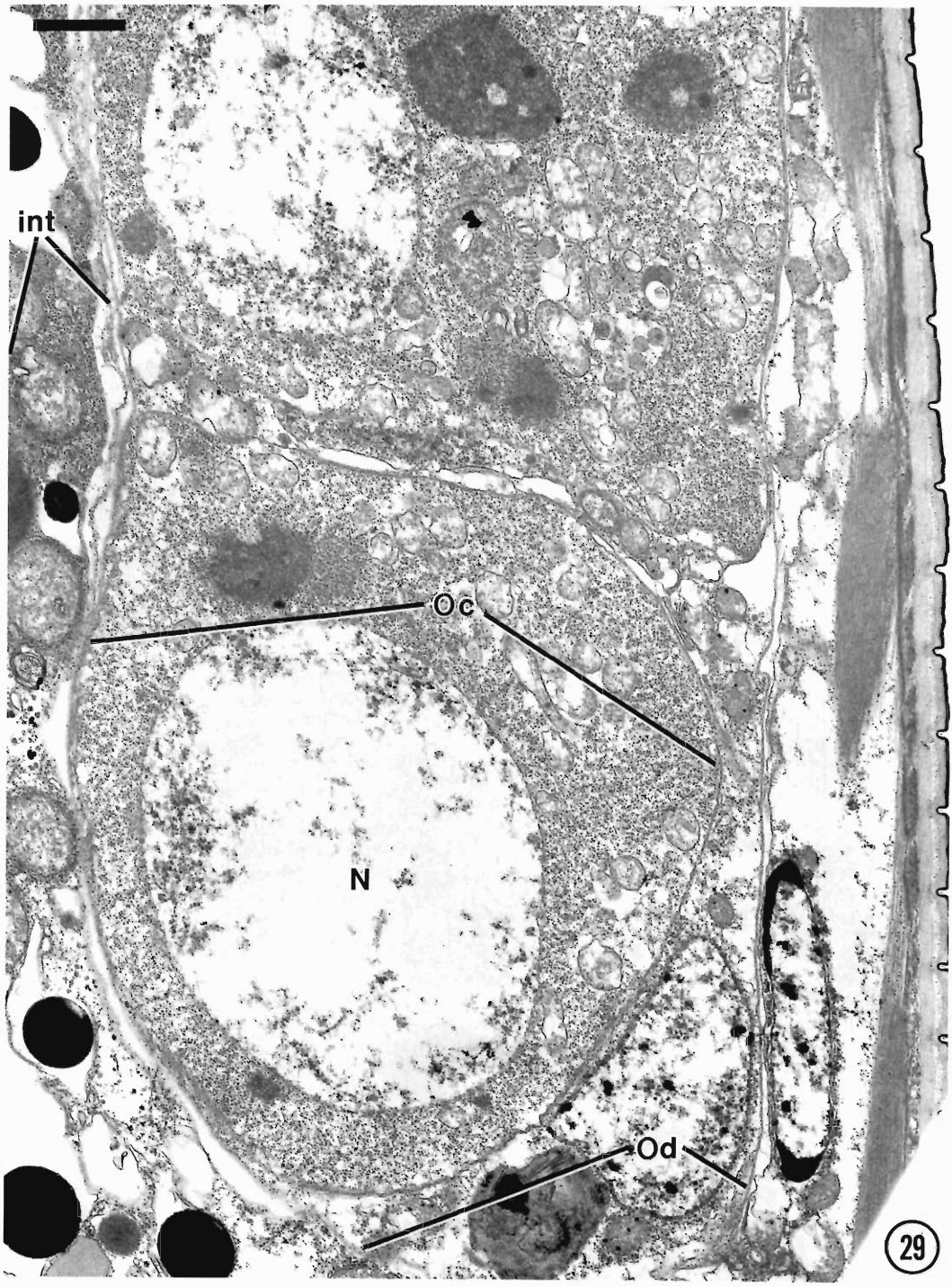


Figure 29. Longitudinal section of an adult female of *P. penetrans* showing oocytes (Oc) prior to their entry into the oviduct (Od), which may be occupied in actively reproducing females. int, intestine; N, nucleus. Scale bar = 1.0 μm.

are multidirectional internally and collar-shaped externally and appear to be in a position to open or close channels that lead from the dorsal and subventral glands (Endo, 1984). This functional role of sphincter muscles was demonstrated with video-enhanced light microscopy in which secretory materials from esophageal glands of *H. schachtii* flowed through ducts or was restricted from movement during feeding and quiescent periods (Wyss and Zunke, 1986). The asymmetrical appearance of the metacorpus and the adjoining organs of *P. penetrans*, which we observed with transmission electron microscopy, is consistent with the images that were obtained using LTSEM.

The dorsal and subventral gland extensions are part of the isthmus of the esophagus and are surrounded by the nerve ring. The dorsal gland cytoplasm contains numerous Golgi complexes that are involved with the formation and accumulation of secretory granules. More granules accumulate and condense in the dorsal gland than in the subventral gland. Future observations should correlate secretory activity with feeding and development as in the sedentary endoparasitic taxa, *Heterodera* and *Meloidogyne* (Wyss et al., 1985; Wyss and Zunke, 1986; Hussey and Mims, 1990).

In the juvenile stage, the intestinal lumen of *Pratylenchus penetrans* appeared occluded with membrane folds of the intestinal epithelium. However, in advanced stages of nematode development, the intestinal lumen appeared to have a few vesicles and tubular invaginations of the intestinal wall which are probably parts of intestinal microvilli. This observation contrasts with that of juveniles of *Globodera rostochiensis* (Wisse and Daems, 1968) and *Heterodera glycines* (Endo, 1988), in which intestinal lumina are lined with microvilli having enteric coatings. The microvilli of the anterior region of the vermiform *Aphelenchoides blastophthorus* are short and bulbous, whereas the microvilli of

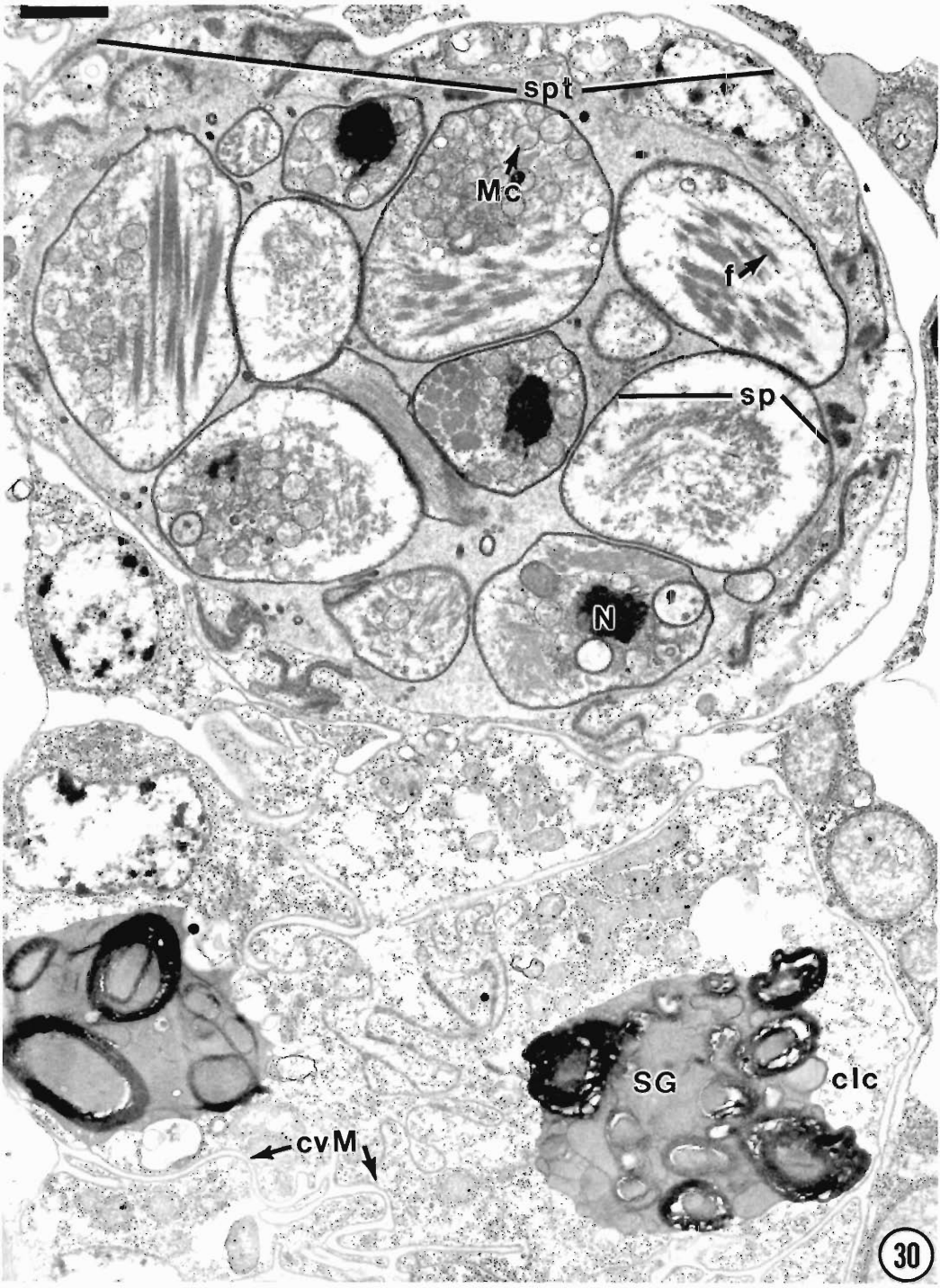
→

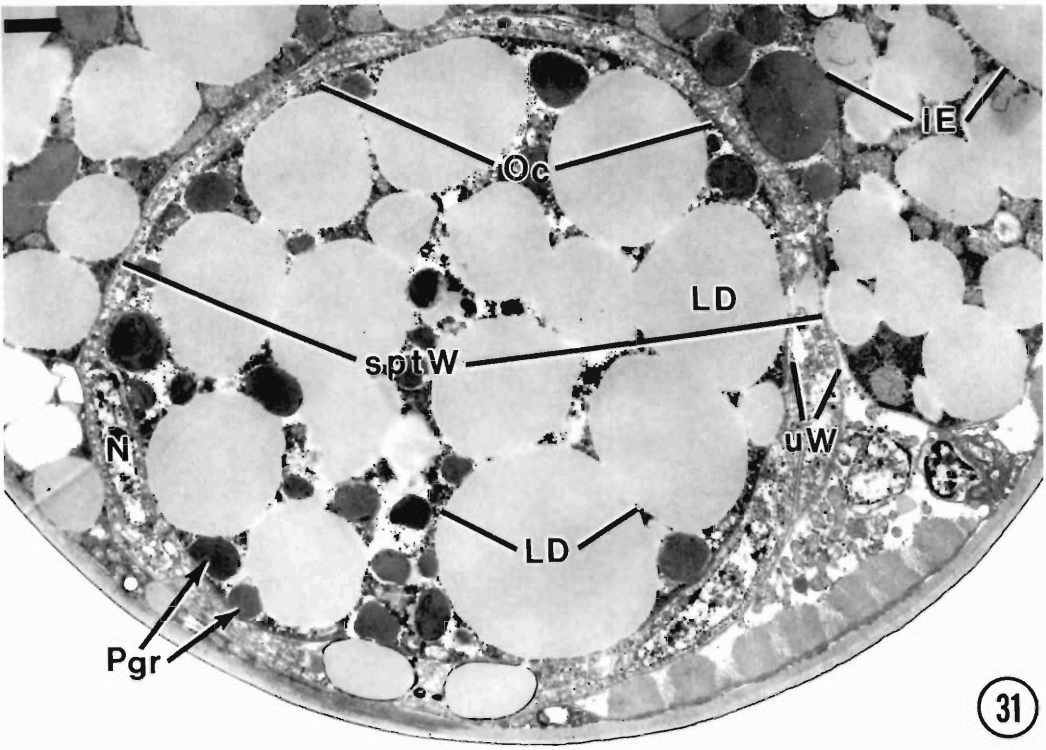
Figure 30. Longitudinal section of an adult female of *P. penetrans* showing a cluster of spermatozoa (sp) within the spermatheca (spt). Sperm cells have dense chromatin surrounded by mitochondria (Mc) and residual strands of fibrillar bundles (f) observed in spermatids of the male gonad. Convoluted membranes (cvM) of the region posteriad to the spermatheca are components of the columnar cells (clc) of the uterus. Large electron-opaque secretory bodies (SG) probably contribute to the outermost egg membranes as eggs pass through the uterus. N, nucleus. Scale bar = 1.0 μm.

Figures 31, 32. Spermatheca and uterus of *P. penetrans*. 31. Cross-section of an oocyte in or near the spermatheca. Oocyte (Oc) contains lipid droplets (LD) and protein granules (Pgr). Spermathecal wall (sptW) encloses the oocyte. IE, intestinal epithelium; N, cell nucleus of the spermatheca wall. 32. Cross-section of columnar secretory cells of the female gonad. Large glandular cells have centrad orientation with adjacent lateral membranes joined into membrane junctions (MJ). The lumen (L) is formed by the basal membranes of the columnar cells. Secretory granules (SG) occur in various stages of assembly, accumulation, and condensation into large electron-opaque secretory granules that are probably destined to be deposited on eggs passing through the columella of the uterus. Scale bars = 1.0 μm.

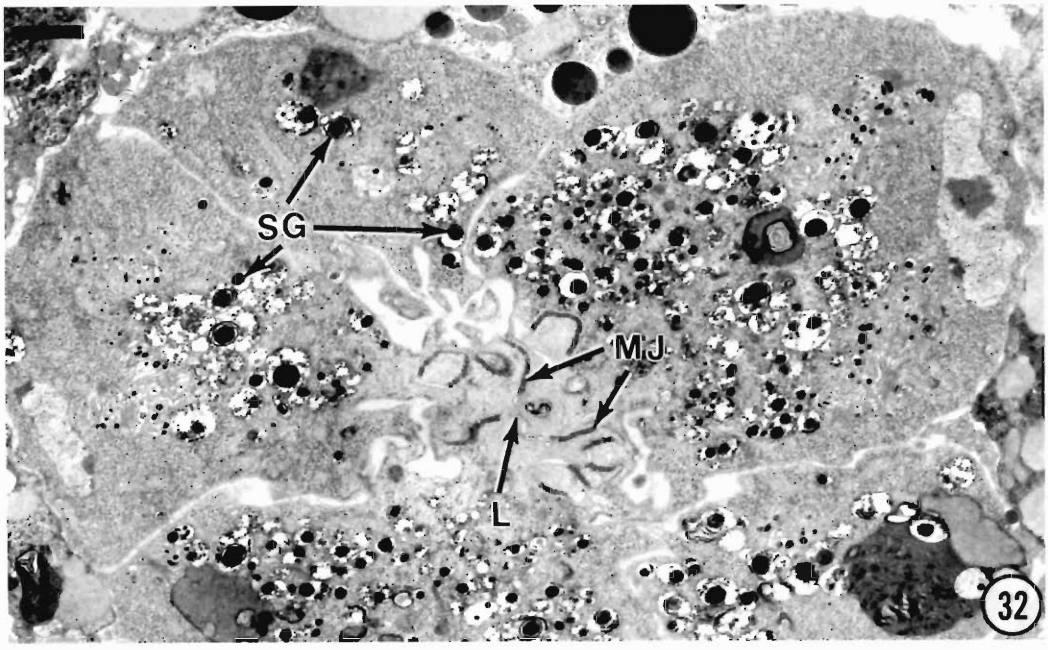
Figures 33–36. Longitudinal sections through the uterus and vaginal regions of *P. penetrans*. 33. Columnar cells (clc) lie posteriad to the spermatheca (Fig. 30) and just anteriad from the terminal vestibule region of the uterus described in Figure 34. cvM, convoluted membrane; Mc, mitochondria; MJ, membrane junctions. 34. Terminal region of uterus is filled with electron-opaque material. The vaginal duct is lined by a thick cuticularized vaginal wall supported by lateral bands of muscles (M). Dilator muscles (dm) attached to the outer region of the vaginal cuticle by hemidesmosomes show anteriad and posteriad orientation of muscle elements. The vaginal wall extends from inside the uterus (u) to the body cuticle. 35. Longitudinal section of an extension of the uterus in Figure 34. The nonfunctional region of the uterus appears as a lipid-filled postvulval uterine branch (pub) with a terminal cap cell nucleus (cCN). 36. Tail region with convoluted membranes of the rectum (r). The cuticular wall forming the anal canal is continuous with the body cuticle (cu). an, anus. Scale bars = 1.0 μm.

Figure 37. Longitudinal view of male gonad of *P. penetrans* showing transition of spermatocytes to spermatids. Spermatocytes (spc) in testis are recognized as cells with nuclei limited by nuclear envelopes. During transition from spermatocyte to spermatid (smt), reduction division occurs, the nuclear envelope disappears, and the chromatin of the nucleus (N) becomes concentrated into a sphere surrounded by fibrillar bodies (fb). Spermatids become amoeboid and have membrane evaginations that appear as filopodia (fp). Nu, nucleolus. Scale bar = 1.0 μm.

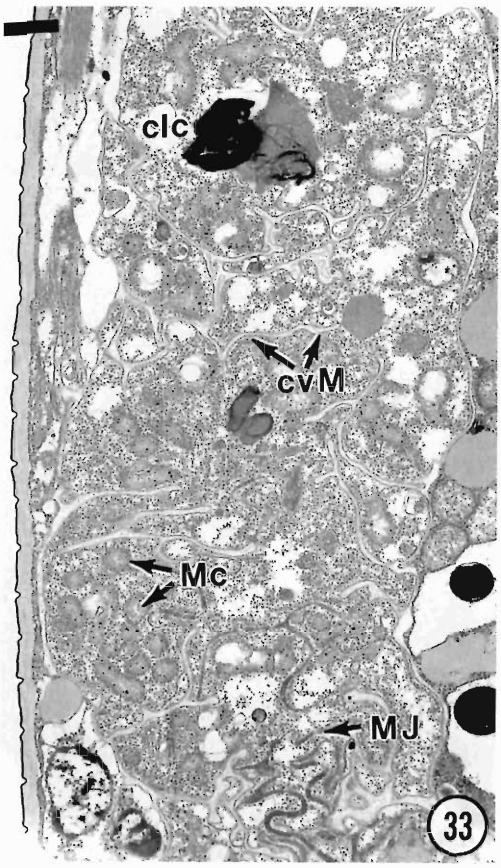


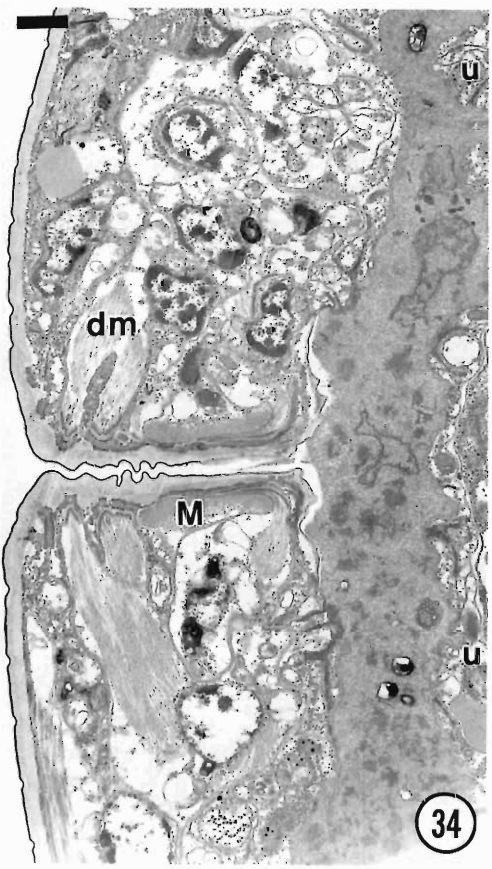


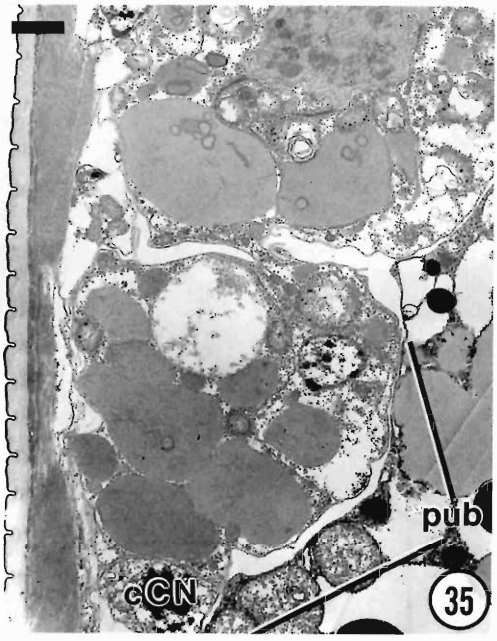
31

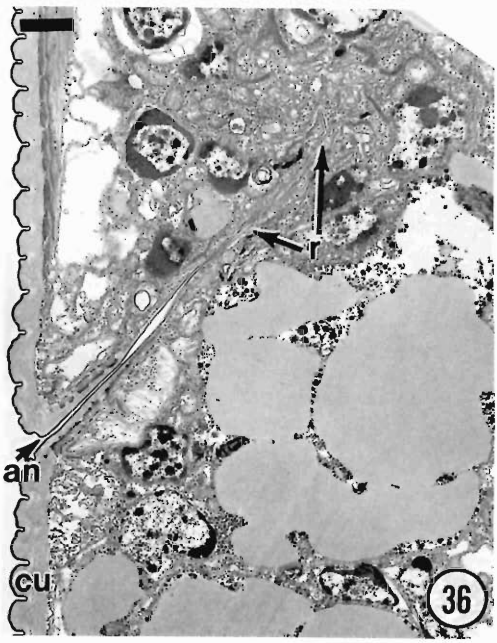


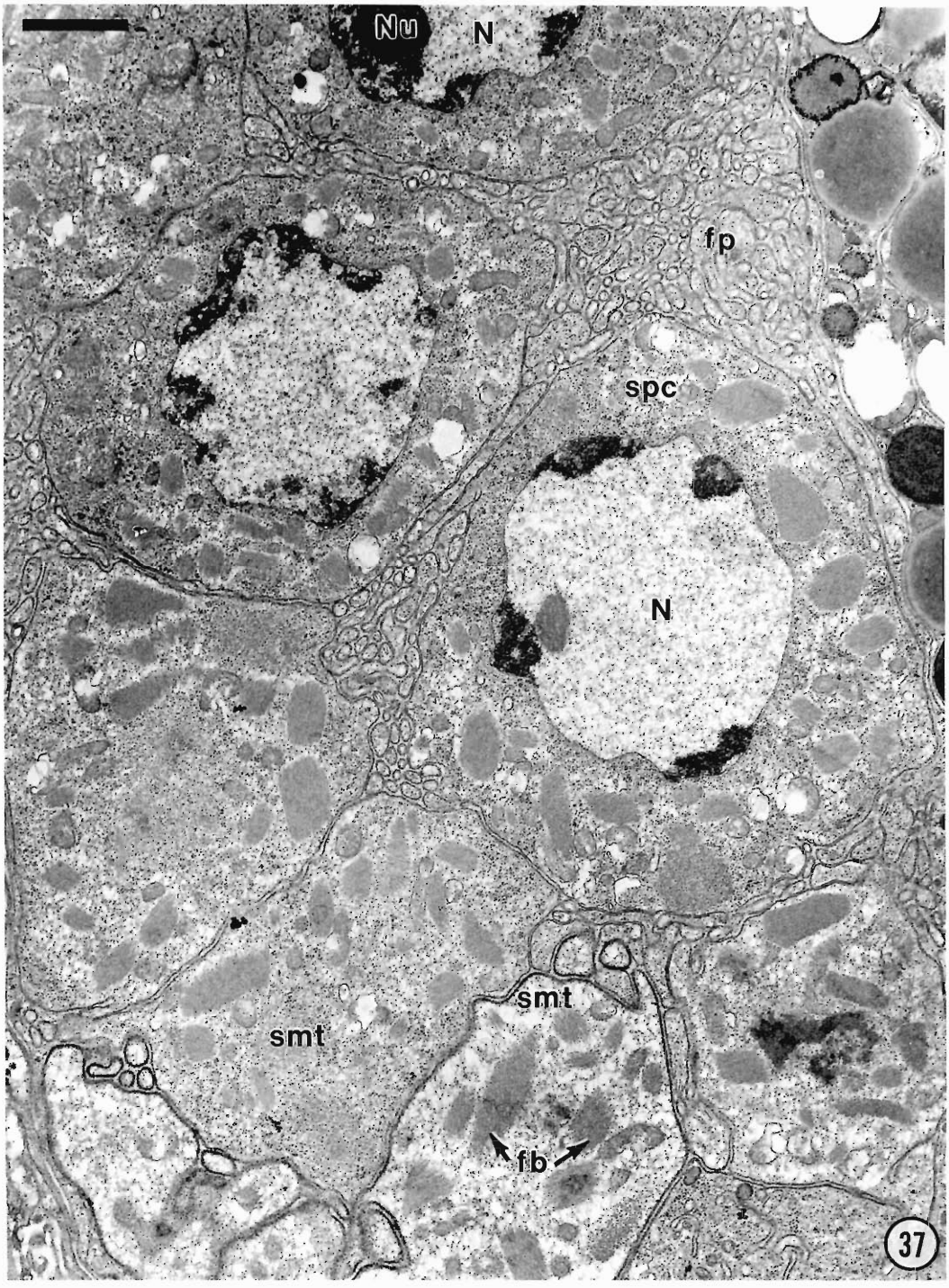
32











the midintestinal region are bottle-shaped (Shepherd et al., 1980).

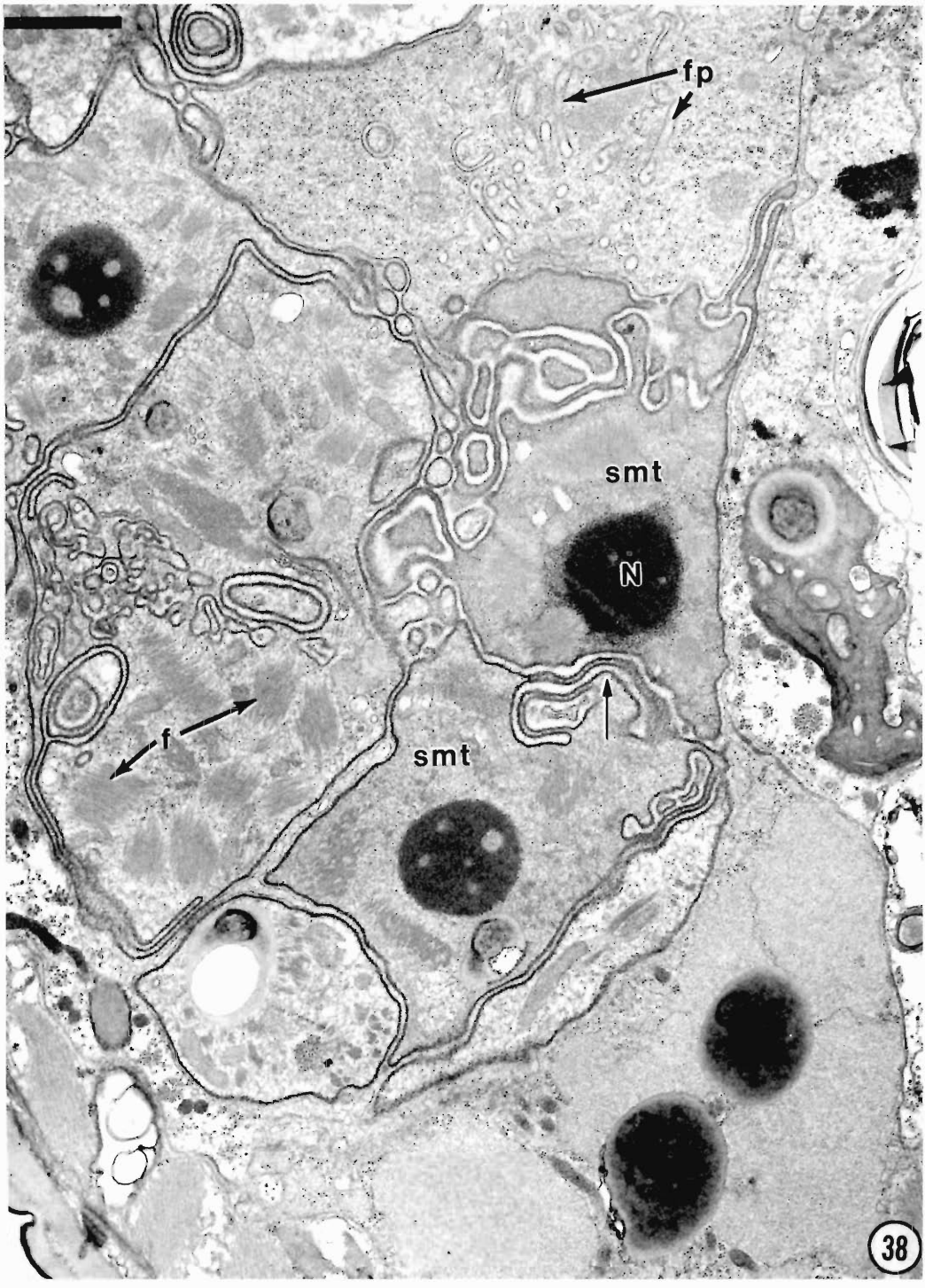
Roman and Hirschmann (1969) studied the postembryonic development of gonads in several species of *Pratylenchus*. Most species, including *P. penetrans*, had the amphidelphic type of development in which 2 gonads developed up to the fourth stage, followed by deterioration of the posterior gonad. Thus, the reproductive system appears monodelphic. However, the only true monodelphic type observed was in *P. scribneri* Steiner, 1943. The authors concluded that all of the species, except *P. scribneri*, are potentially amphidelphic, that is, capable of developing a posterior gonad, which in some cases can be maintained in the adult stage. The present study of *P. penetrans* concurs with the monodelphic feature of the gonad; that is, the adult female gonad contains oocytes, an oviduct, spermatheca, columnar cells, a uterus, a vagina, a vulva opening, and a short postvulval uterine branch. According to Bird and Bird (1991), fertilization occurs near the junction between the oviduct and the uterus, where a spermatheca may or may not occur. Because *Pratylenchus penetrans* is an amphimictic species having a spermatheca, fertilization probably takes place as the oocyte passes through the sperm-filled spermatheca. Roman and Triantaphyllou (1969) studied the maturation and fertilization of *P. penetrans*, *P. vulnus* Allen and Jensen, 1951, and *P. coffeae* (Zimmerman, 1898) Filipjev and Schurrmans Stekhoven, 1941. Oocytes in the spermatheca contained a small number of bivalent chromosomes at prometaphase I. One spermatozoon entered each oocyte, which then rapidly completed the first division. At telophase I, the chromosomes that eventually formed the first polar body nucleus were discrete and were used to determine haploid chromosome numbers. A second maturation division followed rapidly, and the sperm pronucleus was formed, which in turn fused with the egg pronucleus to form the zygote nucleus. Actual fusion of the pronuclei was observed in nondeposited eggs of *P. penetrans* and in laid eggs of *P. coffeae*. According to Delves et al. (1986), the primary oocyte of *Dirofilaria immitis* (Leidy, 1956) Railliet and Henry, 1911, completes meiosis only after fertilization within the seminal vesicle by an entire male gamete. After meiosis I and II occurs in the oocyte and the 2 polar bodies are extruded, the haploid chromosome complement of the female unites with that of the male to reestablish the diploid number in the zygote. The spermatozoa of *P. penetrans* resembled those of *Heterodera* spp. (Shepherd et al., 1973), which were described as aflagellate, amoeboid, and lacking a nuclear membrane. The fibrillar bodies that are prominent in the spermatids appear as fibrillar residues in the clear region of spermatozoa. Ultrastructural studies of sperm development in the longidorid species, *Xiphinema theresiae* Stocker and Kruger, 1988, revealed the complexity of sperm morphology among a wide range of genera (Foor, 1970, 1983). In *X. theresiae*, the nonflagellated, slightly elongated spermatozoa are not polarized into head and tail regions. In cross-section, they have tightly packed chromosomes surrounded by perinuclear mitochondria without clear cristae, as well as bundles of microfilaments and membrane evaginations. Membrane

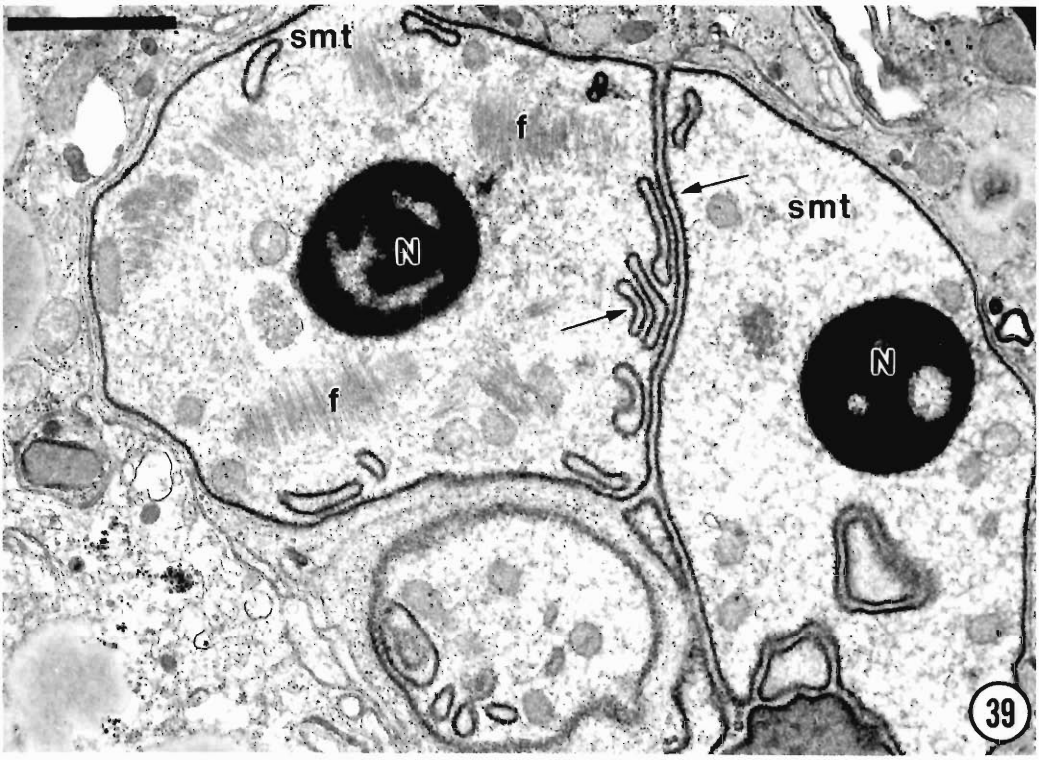
→

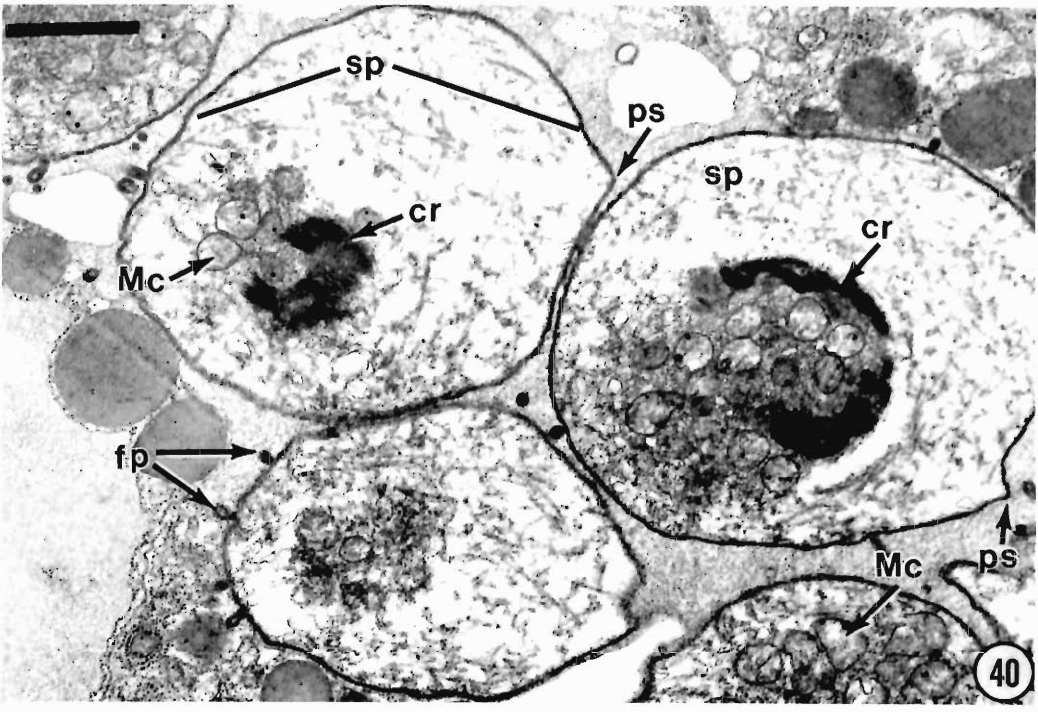
Figure 38. Section posteriad from that shown in Figure 37 illustrating maturing stage of spermatids (smt). Dense, electron-opaque chromatin of nuclei (N) is surrounded by dense clusters of fibrillar protein-like material (f). Spermatid wall membranes show convolutions that form pseudopodia and filopodia (fp). Closely packed spermatids (smt) have membrane boundaries that interdigitate with each other (→). Scale bar = 1.0 μm.

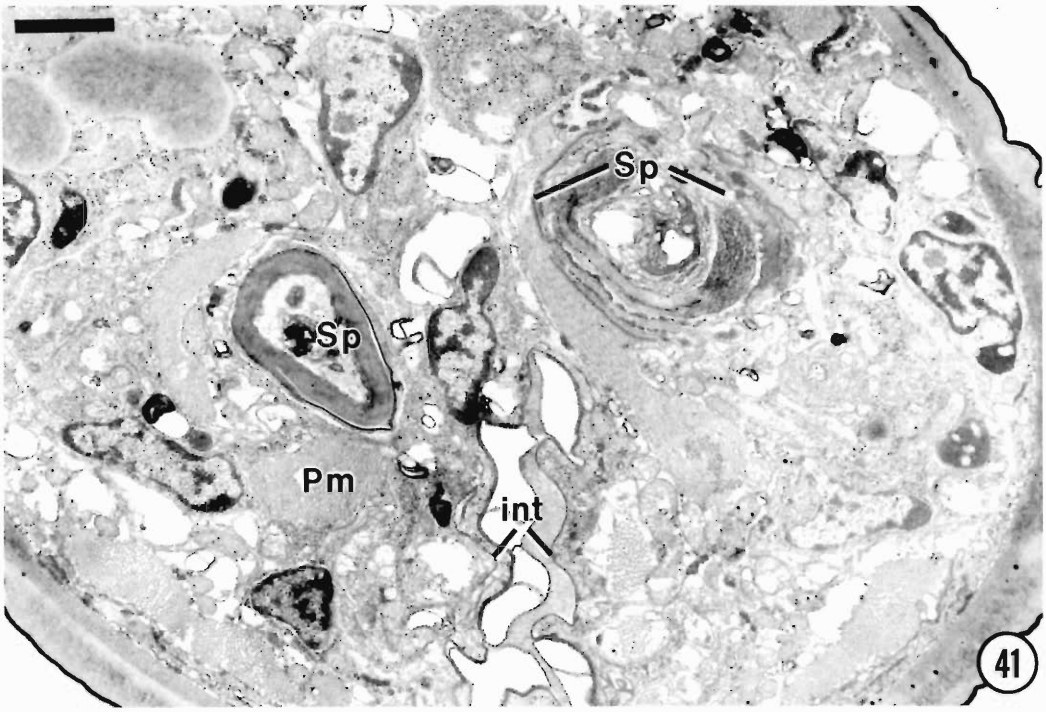
Figures 39, 40. Spermatids and spermatozoa in male gonad of *P. penetrans*. 39. Enlarged view of spermatids (smt) with electron-opaque spheroid nuclei (N). Seminal fluid is present between the tightly arranged spermatids in the seminal vesicle. Limiting membranes of spermatids have electron-opaque depositions (arrow). f, fibrillar body. 40. Gonad showing sperm (sp) within vas deferens. The nonmembrane-bound nuclei contain irregular clumps of chromatin (cr) surrounded by mitochondria (Mc). fp, filopodia; ps, pseudopodium. Scale bars = 1.0 μm.

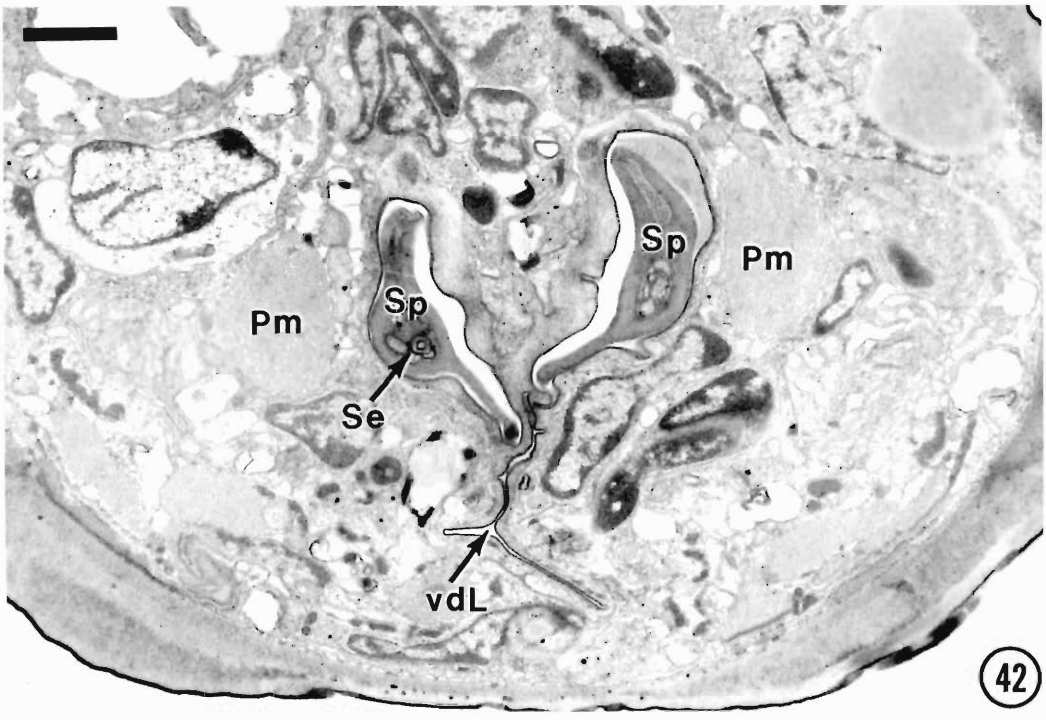
Figures 41, 42. Cross-sections of tail region of male *P. penetrans*. 41. Section through base of spicules (Sp) that border terminal region of intestine (int) and related spicule protractor (Pm) muscles. 42. Sensilla (Se) components occur within curved arms of spicules (Sp) as they extend near the narrow lumen of the vas deferens (vdL). Pm, protractor muscles. Scale bars = 1.0 μm.

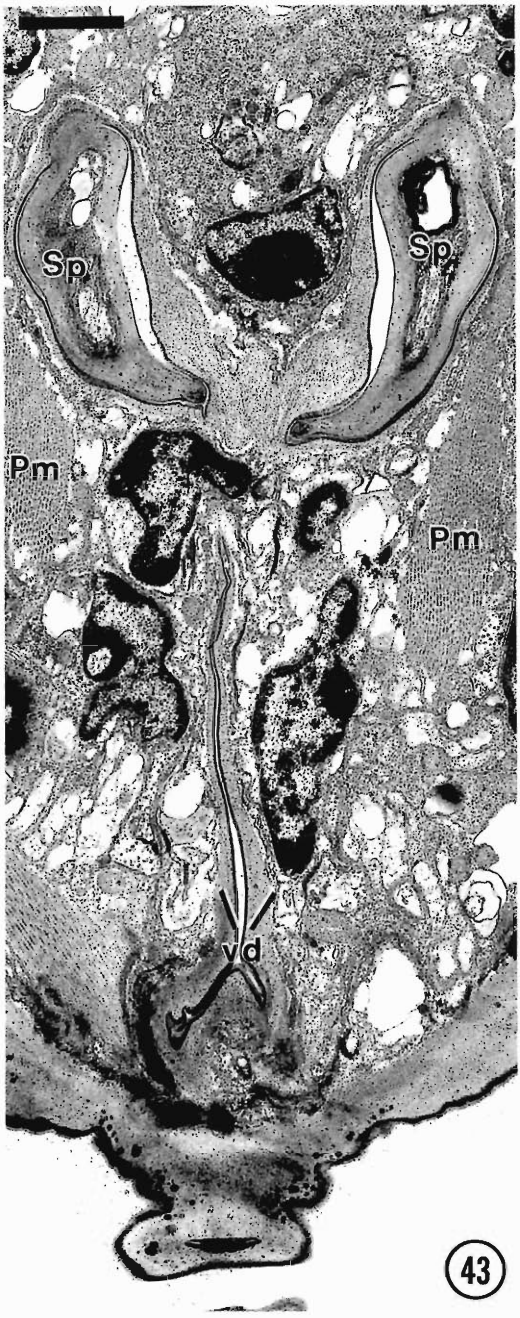


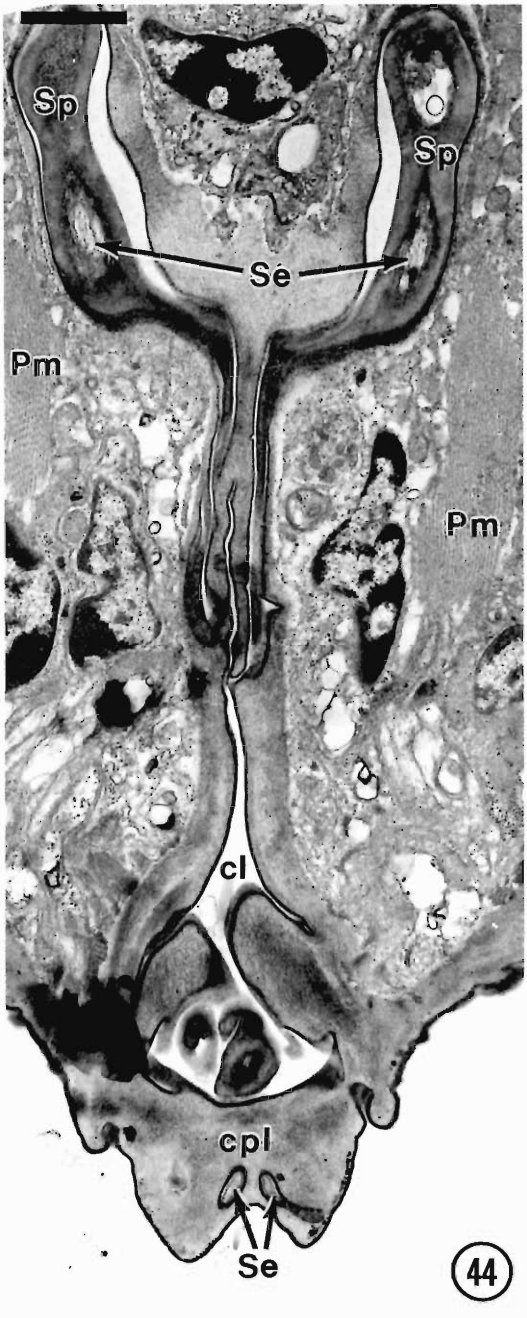




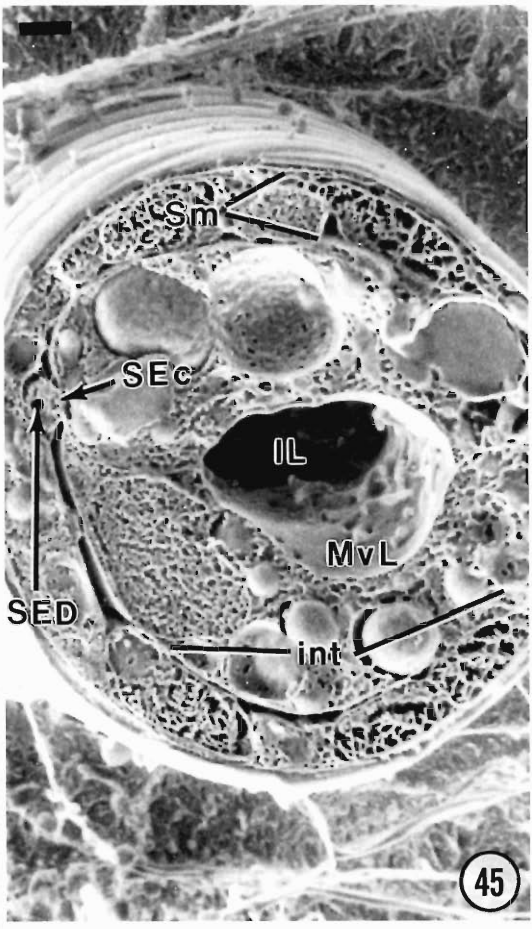


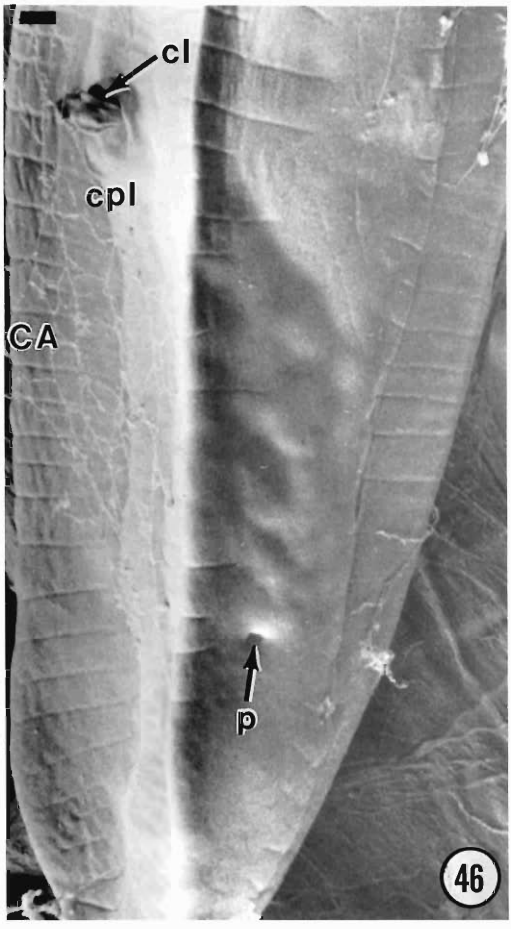


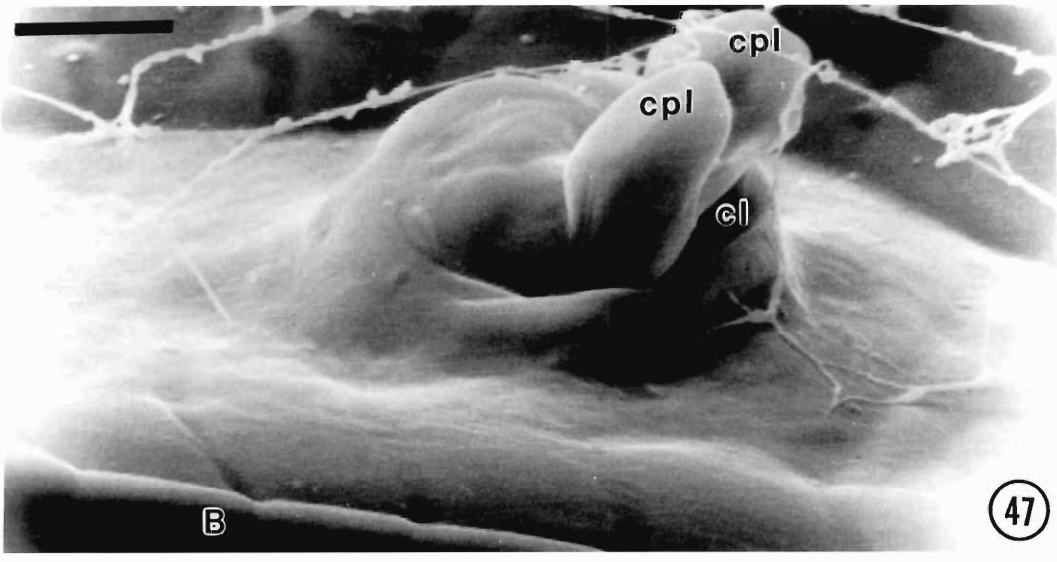




Figures 43, 44. Tangential sections showing spicule and cloacal regions of *P. penetrans*. **43.** Tangential orientation of tail region of specimen flanked by a pair of caudal alae (not shown). Vas deferens (vd) appears as an elongated slit that extends posteriad to the cloacal region. Vas deferens appears closed, is lined with cuticle and has a branched terminal zone. A pair of protractor muscles (Pm) extend posteriad from the dorsolateral region of the head or manubrium of the spicules (sp) and are assumed to be attached postanally on the body wall. **44.** Tangential section of same specimen illustrated in Figure 43 shows the retracted spicules (Sp) with sensilla (Se), protractor muscles (Pm), and part of the pathway for the extensible spicules. The cloaca (cl) is bordered terminally by posterior lips (cpl) that contain sensilla (Se). Scale bars = 1.0 μm.







evaginations are pseudopodia (Kruger, 1991). Posteriad from the spermatheca of *P. penetrans*, columnar cells, also termed tricolumella, were reported to have a secretory function in that material they produce appeared to be deposited on the surface of the eggs. This concept is plausible considering the numerous secretory granules that occur in columnar cells of *P. penetrans*.

During oviposition, it is apparent that the large bands of dilator muscles attached to the cuticularized wall of the vagina play a significant role in the egg-laying process. In film, muscle movement was clearly visible near the opening of the vulva. This action occurred many hours before actual egg laying. The vulva was opened and closed by the dilator muscles of the vagina in a nonrhythmic manner and appeared to open more widely as egg-laying time approached (Zunke and Institut für den Wissenschaftlichen Film, 1988). The tooth-like cuticularized wall of the vagina (Fig. 34) may provide a degree of protection for the nematode by preventing the entry of foreign organisms into the vaginal canal. Similar tooth-like projections on the cuticular wall of the vagina were reported by Mai et al. (1977).

Study of the internal body structure of *P. penetrans* with LTSEM provides another means of observing nematode morphology that tends to verify the presence of chemically fixed structures observed with transmission electron microscopy. For example, the distorted image of the metacorpus from an adult *P. penetrans* observed with transmission electron microscopy is consistent with the image of cryofixed and freeze-fracture images obtained with LTSEM. Furthermore, the intestinal lumen of *P. penetrans* observed with transmission electron microscopy appeared disproportionately large within the body cavity in this and other species. However, this observation was also verified using LTSEM. This latter technology also enabled one to visualize the irregular membranous lining of the intestinal lumen in 3 dimensions (unpubl. obs.). Our observations of *P. penetrans* with LTSEM are consistent with those for *P. agilis* and *Steinernema carpocapsae* (Filipjev, 1934) (=*S. bibionis*, Bovien, 1937) in which the LTSEM was used to show surface and freeze-fractured images of these species (Wergin et al., 1993).

This ultrastructural overview of the lesion nematode indicates that there are many gaps in our knowledge of nematode developmental processes. Changes occurring in the esophageal glands that relate to feeding and the host–parasite interaction should be investigated. Because the lesion nematode is a pathogen by itself as well as a member of disease complexes with fungal pathogens, further studies on its feeding habits and host responses should yield information pertinent to disease management in crop plants.

Acknowledgments

The authors express appreciation to Sharon Ochs for technical support in specimen preparation for transmission electron microscopy, photographic processing, and preparing of final plates; Eric Erbe for preparation and recording of specimens observed with LTSEM; Naeema Latif for the maintenance and extraction of *Pratylenchus penetrans* used in the study; and Daniela Müller for technical support in Germany.

Literature Cited

Atkinson, H. J., and P. D. Harris. 1989. Changes in nematode antigens recognized by monoclonal antibodies during early infections of soya bean with the cyst nematode *Heterodera glycines*. Parasitology 98:479–487.

———, ———, **E. J. Halk, C. Novitski, J. Leighton-Sands, P. Nolan, and P. C. Fox.** 1988. Monoclonal antibodies to the soya bean cyst nem-

←

Figures 45–47. Low-temperature scanning electron micrographs of the intestine and tail region of *P. penetrans*. 45. Freeze-fracture and etched surface of intestinal region shows large intestinal lumen (IL) lined with microvilli-like invaginations (MvL) that are derived from supporting cells. Secretory-excretory cell (SEc) lying between the intestine (int) and somatic muscles (Sm) has a distinct tubular duct (SED). 46. Surface view of tail region of a male specimen showing the cloacal opening (cl) and its posterior lip (cpl) flanked on either side by caudal alae (CA) formed by body cuticle. The pore (p) of one of the paired phasmids is visible near the edge of the caudal ala and approximately a body-width anteriad from the tail terminus. 47. Enlargement of cloacal region of Figure 46 showing posterior lips of the cloaca (cpl) and opening (cl) where spicules emerge. B, bursa; CA, caudal alae. Scale bars = 1.0 μm.

atode, *Heterodera glycines*. Annals of Applied Biology 112:459–469.

Baldwin, J. G., and H. Hirschmann. 1973. Fine structure of cephalic sense organs in *Meloidogyne incognita* males. Journal of Nematology 5:285–302.

———, **and** ———. 1975. Fine structure of cephalic sense organs in *Heterodera glycines* males. Journal of Nematology 7:40–53.

———, **and** ———. 1976. Comparative fine structure of the stomatal region of males of *Meloidogyne incognita* and *Heterodera glycines*. Journal of Nematology 8:1–17.

Bird, A. F. 1967. Changes associated with parasitism in nematodes. I. Morphology and physiology of preparasitic and parasitic larvae of *Meloidogyne javanica*. Journal of Parasitology 53:768–776.

———. 1968. Changes associated with parasitism in nematodes. IV. Cytochemical studies on the ampulla of the dorsal esophageal gland of *Meloidogyne javanica* and on exudations from the buccal stylet. Journal of Parasitology 54:879–890.

———, **and J. Bird.** 1991. The Structure of Nematodes, 2nd ed. Academic Press, New York. 316 pp.

———, **and W. Saurer.** 1967. Changes associated with parasitism in nematodes. II. Histochemical and microspectrophotometric analyses of preparasitic and parasitic larvae of *Meloidogyne javanica*. Journal of Parasitology 53:1262–1269.

Cares, J. E., and J. G. Baldwin. 1994a. Comparative fine structure of sperm of *Verutus volvingentis* and *Meloidogyne floridensis* (Heteroderinae, Nematoda). Canadian Journal of Zoology 72:1481–1491.

———, **and** ———. 1994b. Fine structure of sperm of *Ekphymatodera thomasoni* (Heteroderinae, Nemata). Journal of Nematology 26:375–383.

Coomans, A., and A. T. De Grisse. 1981. Sensory structures. Pages 127–174 *in* B. M. Zuckerman and R. A. Rohde, eds. Parasitic Nematodes. Vol. 3. Academic Press, New York.

Davis, E. L., R. Allen, and R. S. Hussey. 1994. Developmental expression of esophageal gland antigens and their detection in stylet secretions of *Meloidogyne incognita*. Fundamental and Applied Nematology 17:255–262.

De Grisse, A. T. 1977. The ultrastructure of the nerves in the head of 22 species of plant parasitic nematodes belonging to 19 genera (Nematoda: Tylenchida). D.Sc. Thesis, University of Gent, Belgium. (In Dutch.)

———, **P. L. Lippens, and A. Coomans.** 1974. The cephalic sensory system of *Rotylenchus robustus* and a comparison with some other Tylenchids. Nematologica 20:88–95.

Delves, C. J., R. E. Howells, and R. J. Post. 1986. Gametogenesis and fertilization in *Dirofilaria immitis* (Nematoda: Filarioidea). Parasitology 92: 181–197.

Dropkin, V. H. 1989. Introduction to Plant Nematology. John Wiley & Sons, New York. 304 pp.

Endo, B. Y. 1980. Ultrastructure of the anterior neurosensory organs of the larvae of the soybean cyst nematode, *Heterodera glycines*. Journal of Ultrastructure Research 72:349–366.

———. 1983. Ultrastructure of the stomatal region of the juvenile stage of the soybean cyst nematode, *Heterodera glycines*. Proceedings of the Helminthological of Washington 50:43–61.

———. 1984. Ultrastructure of the esophagus of larvae of the soybean cyst nematode, *Heterodera glycines*. Proceedings of the Helminthological Society of Washington 51:1–24.

———. 1987. Ultrastructure of esophageal gland secretory granules in juveniles of *Heterodera glycines*. Journal of Nematology 19:469–483.

———. 1988. Ultrastructure of the intestine of second and third juvenile stages of the soybean cyst nematode, *Heterodera glycines*. Proceedings of the Helminthological Society of Washington 55: 117–131.

———. 1993. Ultrastructure of subventral gland secretory granules in parasitic juveniles of the soybean cyst nematode, *Heterodera glycines*. Journal of the Helminthological Society of Washington 60:22–34.

———, **and W. P. Wergin.** 1973. Ultrastructural investigation of clover roots during early stages of infection by root-knot nematode, *Meloidogyne incognita*. Protoplasma 78:365–379.

———, **and** ———. 1977. Ultrastructure of anterior sensory organs of the root-knot nematode, *Meloidogyne incognita*. Journal of Ultrastructure Research 59:231–249.

———, **and** ———. 1988. Ultrastructure of the second-stage juvenile of the root-knot nematode, *Meloidogyne incognita*. Proceedings of the Helminthological Society of Washington 55:286–316.

Foor, W. E. 1970. Spermatozoan morphology and zygote formation in nematodes. Biology of Reproduction Supplement 2:177–202.

———. 1983. Nematoda 2. Pages 221–256 *in* K. G. and R. G. Adiyodi, eds. Reproductive Biology of Invertebrates. Vol. II. Spermatogenesis and sperm function. John Wiley & Sons, New York.

Gamborg, O. L., T. Murashige, T. A. Thorpe, and I. K. Vasil. 1976. Plant tissue culture media. In Vitro 12:473–478.

Goverse, A., E. L. Davis, and R. S. Hussey. 1994. Monoclonal antibodies to the esophageal glands and stylet secretions of *Heterodera glycines*. Journal of Nematology 26:251–259.

Hussey, R. S. 1989. Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species. Journal of Nematology 21:392–398.

———, **and C. W. Mims.** 1990. Ultrastructure of esophageal glands and their secretory granules in the root-knot nematode *Meloidogyne incognita*. Protoplasma 156:9–18.

———, **O. R. Paguio, and F. Seabury.** 1990. Localization and purification of a secretory protein from the esophageal glands of *Meloidogyne incognita* with a monoclonal antibody. Phytopathology 80:709–714.

Kruger, J. C. de W. 1991. Ultrastructure of sperm development in the plant-parasitic nematode *Xiph-*

inema theresiae. Journal of Morphology 210:163–174.

Mai, W. F., J. R. Bloom, and T. A. Chen. 1977. Biology and Ecology of the Plant-Parasitic Nematode *Pratylenchus penetrans.* Bulletin 815. Pennsylvania State University. Agricultural Experiment Station. University Park, Pennsylvania. 64 pp.

McLaren, D. J. 1976. Nematode sense organs. Pages 195–265 *in* B. Dawes, ed. Advances in Parasitology. Vol. 14. Academic Press, New York.

Roman, J., and H. Hirschmann. 1969. Embryogenesis and postembryogenesis in species of *Pratylenchus* (Nematoda: Tylenchidae). Proceedings of the Helminthological Society of Washington 36: 164–174.

———, **and A. C. Triantaphyllou.** 1969. Gametogenesis and reproduction of seven species of *Pratylenchus.* Journal of Nematology 1:357–362.

Rumpenhorst, H. J. 1984. Intracellular feeding tubes associated with sedentary plant-parasitic nematodes. Nematologica 30:77–85.

Shepherd, A. M., and S. A. Clark. 1976. Structure of the anterior alimentary tract of the passively feeding nematode, *Hexatylus viviparus* (Neotylenchidae: Tylenchida). Nematologica 22:332–342.

———, ———, **and A. Kempton.** 1973. Spermatogenesis and sperm ultrastructure in some cyst nematodes, *Heterodera* spp. Nematologica 19: 551–560.

———, ———, **and J. Hooper.** 1980. Structure of the anterior alimentary tract of *Aphelenchoides blastophthorus* (Nematoda: Tylenchida, Aphelenchina). Nematologica 26:313–357.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26:31–43.

Townshend, J. L., and L. Stobbs. 1981. Histopathology and histochemistry of lesions caused by *Pratylenchus penetrans* in roots of forage legumes. Canadian Journal of Plant Pathology 3: 123–128.

———, ———, **and R. Carter.** 1989. Ultrastructural pathology of cells affected by *Pratylenchus penetrans* in alfalfa roots. Journal of Nematology 21:530–539.

Trett, M. W., and R. N. Perry. 1985. Functional and evolutionary implications of the anterior sensory anatomy of species of root-lesion nematode (genus *Pratylenchus*). Revue de Nématologie 8:341–355.

Wen, G. Y., and T. A. Chen. 1972. Ultrastructure of the feeding apparatus of *Criconemoides curvatum.* Phytopathology 62:501. (Abstract.)

———, **and** ———. 1976. Ultrastructure of the spicules of *Pratylenchus penetrans.* Journal of Nematology 8:69–74.

Wergin, W. P., and B. Y. Endo. 1976. Ultrastructure of a neurosensory organ in a root-knot nematode. Journal of Ultrastructure Research 56:258–276.

———, **R. M. Sayre, and E. F. Erbe.** 1993. Use of low temperature scanning electron microscopy to observe frozen hydrated specimens of nematodes. Journal of Nematology 25:214–226.

Wisse, E., and W. T. Daems. 1968. Electron microscopic observations on second-stage larvae of the potato root eelworm *Heterodera rostochiensis.* Journal of Ultrastructure Research 24:210–231.

Wyss, U. 1992. Observations on the feeding behavior of *Heterodera schachtii* throughout development, including events during molting. Fundamental and Applied Nematology 15:75–89.

———, **C. Stender, and H. Lehmann.** 1984. Ultrastructure of feeding sites of the cyst nematode *Heterodera schachtii* Schmidt in roots of susceptible and resistant *Raphanus sativus* L. oleiformis Pers. cultivars. Physiological Plant Pathology 25: 21–37.

———, **and U. Zunke.** 1986. Observations on the behavior of second stage juveniles of *Heterodera schachtii* inside host roots. Revue de Nématologie 9:153–165.

———, ———, **and Institut für den Wissenschaftlichen Film.** 1985. *Heterodera schachtii* (Nematoda). Behavior inside Roots (rape). Film E 2904. Encyclopaedia Cinematographica, Göttingen.

Yuen, P. H. 1967. Electron microscopical studies on *Ditylenchus dipsaci* (Kühn) I. Stomatal region. Canadian Journal of Zoology 45:1019–1033.

———. 1968. Electron microscopical studies of *Ditylenchus dipsaci.* II. Oesophagus. Nematologica 14:385–394.

Zunke, U. 1987. Contrast Enhancement—Nematological Applications. Pages 65–75 *in* O. Van Laere and L. de Vael, eds. Advanced Techniques in Microscopy. Applications in Biological and Agricultural Research. Flanders Technology International Symposium, 1987. Agricultural Research Center, Ghent Belgium. 1988.

———. 1990a. Ectoparasitic feeding behavior of the root lesion nematode, *Pratylenchus penetrans,* on root hairs of different host plants. Revue de Nématologie 13:331–337.

———. 1990b. Observations on the invasion and endoparasitic behavior of the root lesion nematode *Pratylenchus penetrans.* Journal of Nematology 22:309–320.

———, **and Institut für den Wissenschaftlichen Film.** 1988. Behaviour of the Root Lesion Nematode *Pratylenchus penetrans.* Film C 1676. Institut für den Wissenschaftlichen Film. Göttingen, West Germany.

———, **and R. N. Perry.** 1992. Evolutionary implications of different feeding strategies of plant parasitic nematodes. Pages 128–132 *in* F. J. Gommers and P. W. Th. Maas, eds. Proceedings of the 2nd International Congress of Nematology (SICN), Veldhoven, 1990.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 96–101

Nematodes of Yellow Perch from Saginaw Bay, Lake Huron, with Emphasis on *Eustrongylides tubifex* (Dioctophymatidae) and *Philometra cylindracea* (Philometridae)

JENNIFER L. ROSINSKI,[1] PATRICK M. MUZZALL,[1,3] AND ROBERT C. HAAS[2]

[1] Department of Zoology, Michigan State University, East Lansing, Michigan 48824 and
[2] Michigan Department of Natural Resources, Mt. Clemens Fisheries Station, Mt. Clemens, Michigan 48045

ABSTRACT: Two hundred forty yellow perch, *Perca flavescens* (Mitchill), collected from 4 locations in Saginaw Bay, Lake Huron, Michigan, in May and September/October 1992, were examined for nematodes. A total of 6 nematodes species (*Eustrongylides tubifex* (Nitzsch, 1819) Jägerskiöld, 1909; *Philometra cylindracea* (Ward and Magath, 1917) Van Cleave and Mueller, 1934; *Dichelyne cotylophora* (Ward and Magath, 1917) Petter, 1974; *Raphidascaris* sp. Railliet and Henry, 1915; *Camallanus oxycephalus* Ward and Magath, 1917; and an unidentified gravid female nematode) infected yellow perch; *E. tubifex* and *P. cylindracea* were most common. Prevalences and mean intensities varied with month and location of yellow perch collection. Yellow perch from The Black Hole, which is the most eutrophic location, had a significantly higher mean intensity of *E. tubifex* than fish from other locations. Prevalence and intensity of *E. tubifex* increased in larger and older yellow perch. The mean intensity of *P. cylindracea* did not vary significantly with location. Occurrence of *E. tubifex* and *P. cylindracea* is influenced by the distribution of intermediate and/or paratenic hosts, the feeding habits of the perch, and the life histories of the nematodes.

KEY WORDS: nematodes, *Eustrongylides tubifex, Philometra cylindracea,* yellow perch, *Perca flavescens,* Saginaw Bay, Lake Huron.

Bangham (1955) and Dechtiar et al. (1988) surveyed the parasite fauna of Lake Huron fishes including yellow perch, *Perca flavescens*, but their studies did not include Saginaw Bay. They found 5 and 4 species of nematodes in yellow perch, respectively. Except for Allison* (1966) and Salz (1989), who reported only on the occurrence of *Eustrongylides tubifex,* investigations of the parasitic nematode fauna of yellow perch from Saginaw Bay do not exist. Yellow perch are the most important hosts of *E. tubifex* in Lakes Huron and Erie (Allison, 1966; Cooper et al., 1978; Crites, 1982). It is also thought that reduced yellow perch growth and fish mortality may result from infection with *E. tubifex* and *Philometra cylindracea* (see Allison, 1966; Crites, 1982; Salz, 1989). This paper reports on the occurrence and distribution of *E. tubifex* and *P. cylindracea* in yellow perch from 4 locations in Saginaw Bay.

Materials and Methods

A total of 240 yellow perch was collected by otter trawl in May and September/October 1992 from 4 locations in inner Saginaw Bay, Lake Huron, Michigan. Saginaw Bay is the southwestern extension of Lake Huron located in east-central Michigan. The inner bay is enriched with domestic, agricultural, and industrial inputs from the Saginaw River (Michigan Department of Natural Resources, 1988). It is a large, shallow, eutrophic bay that serves as a major fish spawning and nursery area and as a refuge and food source for many birds (Dolan et al., 1986; Michigan Department of Natural Resources, 1988).

The 4 collection locations (latitude, longitude/mean depth [m]) in the bay were The Black Hole (43°48′00″, 83°50′00″/7.5), North Island (43°53′00″, 83°26′00″/4.6), Au Gres (44°00′00″, 83°40′30″/10.1), and Fish Point (43°43′00″, 83°33′30″/5.9). The Black Hole is closest to the mouth of the Saginaw River, making it the most eutrophic location. Because of their close vicinity to the outer bay, North Island and Au Gres exhibit higher water quality and well mixed outer bay characteristics. Fish Point has less organic sediments and is less eutrophic than The Black Hole (Salz 1989).

Yellow perch were frozen in the field. Thirty yellow perch from each location in each month were measured (total length in millimeters) and sexed at necropsy. Scale samples were taken from the left side below the lateral line near the pectoral fin of each fish for age determination. Eyes, gonads, kidneys, spleen, liver, gall bladder, esophagus, gastrointestinal tract, heart, body cavity, and right or left side of musculature were examined. Nematodes were preserved in 70% alcohol and later cleared in glycerin for identification. The September/October collections are referred to as September.

The terms prevalence and mean intensity follow the definitions of Margolis et al. (1982). Mean intensities

[3] Corresponding author.

* Allison (1966) identified the nematode as *Philometra cylindracea*, but it is now known that the nematode he studied was *Eustrongylides tubifex* (R. Haas, Michigan Department of Natural Resources, pers. comm.).

Table 1. Prevalence and mean intensity of *Eustrongylides tubifex* and *Philometra cylindracea* in 240 yellow perch from Saginaw Bay, Lake Huron, 1992. Thirty fish in each month from each location were examined.

Location	Month	*Eustrongylides tubifex*		*Philometra cylindracea*	
		No. infected (%)	Mean intensity ± SD (maximum)	No. infected (%)	Mean intensity ± SD (maximum)
The Black Hole	May	26 (87)	6.0 ± 4.9 (13)	9 (30)	1.0 (1)
	September	27 (90)	12.7 ± 10.6 (48)	3 (10)	1.3 ± 0.6 (2)
North Island	May	27 (90)	4.3 ± 4.4 (20)	8 (27)	1.4 ± 0.5 (2)
	September	21 (70)	4.5 ± 3.1 (11)	11 (37)	4.4 ± 5.7 (19)
Au Gres	May	23 (77)	4.8 ± 6.4 (32)	9 (30)	1.1 ± 0.3 (2)
	September	26 (87)	5.9 ± 4.6 (18)	3 (10)	1.0 (1)
Fish Point	May	25 (83)	5.3 ± 3.9 (17)	10 (33)	1.4 ± 0.7 (3)
	September	18 (60)	5.7 ± 5.0 (19)	4 (13)	2.3 ± 1.5 (4)

are followed by ±standard deviation (SD). Chi-square analyses were performed to determine whether the prevalence of fish infected with a nematode species was independent of month, location, and fish age, length, or sex. Fish length classes were arbitrarily established. Intensity data for each species were rank transformed (Potvin and Roff, 1993) to correct for nonnormality. Multiway analysis of variance (ANOVA) was used to examine the effects of fish size and age, month, and location on nematode intensity. This determined whether individual factors had a significant effect on mean intensity of each nematode species and also whether interaction of factors significantly affected mean intensity. All tests were performed at a significance level of $P \leq 0.05$.

Voucher specimens of the following nematodes (U.S. National Parasite (USNPC) Collection No.) have been deposited at the USNPC: *Eustrongylides tubifex* (86802), *Philometra cylindracea* (86803), *Dichelyne cotylophora* (86804), and *Camallanus oxycephalus* (86805).

Results

Nematodes: general

A total of 6 nematode species was found in yellow perch from Saginaw Bay in 1992: *Eustrongylides tubifex, Philometra cylindracea, Dichelyne cotylophora, Raphidascaris* sp., *Camallanus oxycephalus,* and a single, gravid female nematode occurred in the wall of the intestine; the latter was not identified because the anterior end was missing. Of the 240 yellow perch examined, 215 (90%) were infected with at least 1 nematode, and 50 (21%) were concurrently infected with *E. tubifex* and *P. cylindracea.* The numbers (percentages) of fish infected with at least 1 nematode at each location in May and September, respectively, were The Black Hole, 28 (93%) and 28 (93%); North Island, 29 (97%) and 25 (83%); Au Gres, 28 (93%) and 28 (93%); and Fish Point, 27 (90%) and 22 (73%). No significant difference in prevalence or mean intensity of each nematode species was found between male and female perch.

Eustrongylides tubifex

Third- and fourth-stage larvae were found encapsulated and free in the mesentery, muscles, liver, and gonads and free in the body cavity of perch. Capsules were somewhat round and flattened, yellow-pink or yellow-white. When dissected, a cloudy exudate was released with the larval nematode. Out of 1,208 *E. tubifex* recovered, 127 (11%) were unencapsulated.

Prevalence of *E. tubifex* did not vary significantly with month (chi-square = 2.1; df = 1) or location (chi-square = 5.4; df = 3) (Table 1). Although mean intensity of *E. tubifex* did not vary significantly between months (ANOVA $F = 2.4$; df = 1, 145), it was consistently higher in fish collected in September (Table 1). Perch from The Black Hole had a significantly higher mean intensity (Fig. 1) than the other 3 locations (ANOVA $F = 5.0$; df = 3, 145). Mean intensity of *E. tubifex* in fish from the other locations did not significantly differ.

A significant difference in mean intensity of *E. tubifex* was detected in perch of different age classes (ANOVA $F = 7.5$; df = 10, 228). Age 0 perch were uninfected, and a trend of increasing mean intensity with host age was apparent (Fig. 2). A significant difference in mean intensity of *E. tubifex* among fish length classes was also found (ANOVA $F = 5.5$; df = 10, 282). Fish less than 100 mm in length had a significantly lower mean intensity than other length classes (Fig. 3). Intensity of *E. tubifex* increased with increasing fish length in classes 110 mm

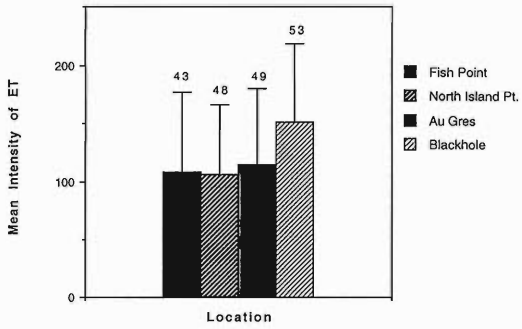


Figure 1. Mean intensity of *Eustrongylides tubifex* (ET) in yellow perch from 4 locations in inner Saginaw Bay, Lake Huron, 1992. Intensity data were rank transformed. Bars represent ±SD. Number of infected fish is indicated above each location.

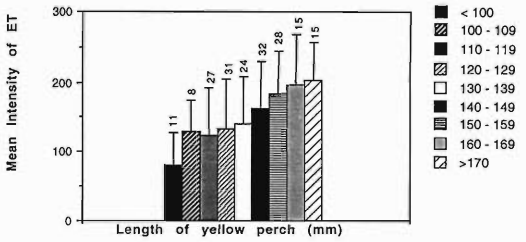


Figure 3. Mean intensity of *Eustrongylides tubifex* (ET) in yellow perch of different length (mm) classes. Intensity data were rank-transformed. Bars represent ±SD. Number of infected fish is indicated above each length class.

and larger. There was no significant difference in fish classes 100–139 mm or between 140 mm and larger.

Philometra cylindracea

Mature and gravid but not larvigerous *P. cylindracea* were found free in the body cavity, testes, mesentery, and heart of perch. There were no significant differences in the prevalence of *P. cylindracea* between months or locations (chi-square = 13.4; df = 7) (Table 1), nor in mean intensity among months or locations in Saginaw Bay.

Fish in age classes 0 and 5 had significantly higher mean intensities of *P. cylindracea* than all other age classes (ANOVA F = 2.0; df = 10, 47) (Fig. 4). No significant difference in mean intensity of this nematode was detected in other age classes. There was no significant difference in infection of yellow perch between length classes (ANOVA F = 0.4; df = 2, 35).

Other nematodes

Fourth-stage larval *D. cotylophora* were found in the stomach and small intestine of perch in May from Au Gres and North Island. Fish from The Black Hole and Fish Point were uninfected during this month. In September, gravid and a few fourth-stage larval *D. cotylophora* were found in the small intestine and stomach. Prevalence did not differ significantly between months but was significantly different among locations, being higher at Au Gres (chi-square = 29.6; df = 3). Mean intensities significantly differed between months (ANOVA F = 133.2; df = 1, 27) and among locations (ANOVA F = 78.7; df = 3, 27). There was no significant difference in either prevalence or mean intensity between Au Gres and North Island in May and no significant difference among locations in September. Differences in May data are due to uninfected fish at The Black Hole and Fish Point.

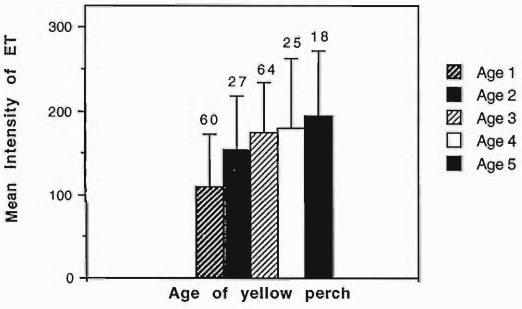


Figure 2. Mean intensity of *Eustrongylides tubifex* (ET) among age classes of yellow perch from Saginaw Bay, Lake Huron, 1992. Intensity data were rank-transformed. Bars represent ±SD. Number of infected fish is indicated above each age class.

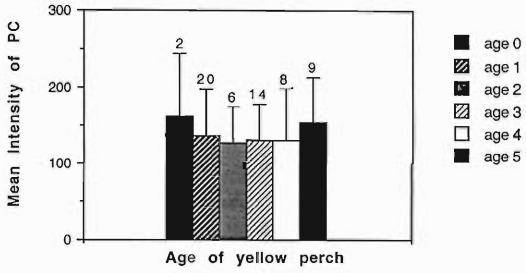


Figure 4. Mean intensity of *Philometra cylindracea* (PC) among age classes of yellow perch from Saginaw Bay, Lake Huron, 1992. Intensity data were rank-transformed. Bars represent ±SD. Number of infected fish is indicated above each age class.

Gravid *C. oxycephalus* were found in the intestine in May at North Island only. Fourth-stage larval *Raphidascaris* sp. were found free in the liver and encapsulated in the liver, mesentery, and intestinal wall of perch from all locations. Prevalence was significantly higher in May than in September (chi-square = 34.9; df = 1), but there was no significant difference among locations (chi-square = 3.1; df = 3). Mean intensity did not significantly differ between months (ANOVA F = 0.2; d = 1, 56) or among locations (ANOVA F = 0.6; df = 3, 56).

Discussion

Based on the present study and studies by Bangham (1955) and Dechtiar et al. (1988), the nematode faunas of yellow perch from Saginaw Bay and Lake Huron proper are similar. Bangham (1955) found 5 nematode species in yellow perch from South Bay, Lake Huron proper, and Manitoulin Island. *Dichelyne cotylophora* and *Philometra cylindracea* were common to both the present study and Bangham (1955). Dechtiar et al. (1988) found 4 nematode species in yellow perch from Lake Huron proper with *Eustrongylides tubifex*, *P. cylindracea*, and *D.* (*Cucullanellus*) *cotylophora* common to both studies.

In Saginaw Bay, large amounts of organic sediments at The Black Hole support an abundance of benthic invertebrates. Schneider et al. (1969) found oligochaetes concentrated in this area, and Brinkhurst (1967) reported that areas around The Black Hole contained the highest percentages of tubificid oligochaetes in Saginaw Bay. Tubificid oligochaetes serve as the intermediate host for *Eustrongylides tubifex* (Karmanova, 1968; Measures, 1988a, b). In past studies, fish from localities with an abundance of tubificids had higher prevalences and mean intensities of *Eustrongylides* spp. because oligochaetes make up a larger portion of the fish diet (Kaeding, 1981; Crites, 1982; Hirshfield et al., 1983; Measures, 1988b). At The Black Hole location, the significantly higher mean intensity of *E. tubifex* can be attributed to the abundance of tubificid oligochaetes at that location. However, in the present study few oligochaetes were found in perch guts by one of us (J.L.R.), and Haas and Schaeffer (1992) did not report the presence of tubificids in perch stomachs. Perhaps tubificids break down quickly in perch guts (although it seems as though Haas and Schaeffer accounted for this by immediately freezing fish with liquid nitrogen). It may also be possible that another invertebrate species serves as an intermediate or paratenic host for *E. tubifex* in Saginaw Bay. If another invertebrate is functioning in this role, results indicate that it is a pollution-tolerant organism that has a distribution similar to that of tubificids. The likely prospects are common food items in the diets of yellow perch from Saginaw Bay such as chironomid larvae and harpacticoid copepods.

Yellow perch exhibit age–size differences in feeding (Cooper et al., 1978; Crites, 1982; Haas and Schaeffer, 1992) that are reflected in the infection patterns of *E. tubifex* and *P. cylindracea* in different age–size classes of yellow perch. Larval *E. tubifex* have a long life span and can be transmitted from one fish to another before being transmitted to the definitive piscivorous bird host (Cooper et al., 1978). The increase in prevalence and mean intensity of *E. tubifex* with yellow perch age and length may reflect an increase in piscivory as well as an accumulation of worms over time. Piscivory by large yellow perch was observed in the present study as well as by Haas and Schaeffer (1992) and, at times, was extensive.

Feeding activity of yellow perch may also explain infection patterns with *P. cylindracea*. Yellow perch are the definitive hosts, and copepods act as intermediate hosts for *P. cylindracea* (Molnar and Fernando, 1975; Crites, 1982). Yellow perch are initially planktivores, and zooplankton remains an important food item in age classes 1 and 2 (Haas and Schaeffer, 1992) and maybe even throughout their lives (Crites, 1982). This is reflected in the significantly higher mean intensity of *P. cylindracea* in age class O fish. Age class 5 yellow perch also had a significantly higher mean intensity of *P. cylindracea*. This may indicate that large Saginaw Bay yellow perch consume an increased volume of copepods or that *P. cylindracea* can be transferred from one fish to another so that intensity increases with piscivory. Although transmission of *P. cylindracea* from one fish to another has not been demonstrated, it has been shown with *P. obturans* in pike, *Esox lucius*; perch, *Perca fluviatilis*; and rudd, *Sarcodinius erythrophthalmus* (see Molnar, 1976; Moravec and Dykova, 1978).

Philometra cylindracea has a 1-yr life cycle and becomes larvigerous in June or July in Lake

Erie (Crites 1982). This nematode declines rapidly in late June through July due to natural senescence of spent worms (Molnar and Fernando, 1975; Crites, 1982). The lack of larvigerous worms in the present study may be due to collection time prior to development of larvae within the nematodes. Abundance of *P. cylindracea* increases in September and early October as yellow perch ingest copepods infected with the new generation of larvae (Crites, 1982). The higher mean intensity, although not significant, of *P. cylindracea* in Saginaw Bay yellow perch from September may be directly related to the appearance of this new generation.

The high prevalence and mean intensity of *Eustrongylides tubifex* indicate that this nematode is well established in yellow perch from inner Saginaw Bay, Lake Huron. Although yellow perch have lower prevalence and mean intensity of *Philometra cylindracea,* it is also present in yellow perch throughout inner Saginaw Bay. Haas and Schaeffer (1992) reported that yellow perch in Saginaw Bay experience slow growth, energy depletion, and high natural mortality and suggested that this is probably due to the lack of benthic invertebrates on which to feed. Alternatively, *E. tubifex* and *P. cylindracea* may play a role in this reduced yellow perch growth and high mortality as suggested by Allison (1966), Crites (1982), and Salz (1989).

Acknowledgments

We thank J. Hodge and L. Shubel, Michigan Department of Natural Resources, Mount Clemens, Michigan, for collecting the yellow perch for this study and K. Nelson for technical assistance. K. Koster aged the yellow perch. This project was supported in part by the Department of Zoology, Michigan State University.

Literature Cited

Allison, L. N. 1966. The redworm (*Philometra cylindracea*) of yellow perch (*Perca flavescens*) in Michigan waters of the Great Lakes. Michigan Department of Conservation Research and Development Report No. 53, Institute for Fisheries Report No. 1712.

Bangham, R. V. 1955. Studies on fish parasites of Lake Huron and Manitoulin Island. American Midland Naturalist 53:184–194.

Brinkhurst, R. O. 1967. The distribution of aquatic oligochaetes in Saginaw Bay, Lake Huron. Limnology and Oceanography 12:137–143.

Cooper, C. L., J. L. Crites, and D. J. Sprinkle-Fastkie. 1978. Population biology and behavior of larval *Eustrongylides tubifex* (Nematoda: Dioctophymatida) in poikilothermous hosts. Journal of Parasitology 64:102–107.

Crites, J. L. 1982. Impact of the nematode parasite *Eustrongylides tubifex* on yellow perch in Lake Erie. U.S. Department of Commerce Commercial Fisheries Research and Development Project No. 3-298-D.

Dechtiar, A. O., J. J. Collins, and J. A. Reckahn. 1988. Survey of parasitic fauna of Lake Huron fishes, 1961 to 1971 *In* S. J. Nepszy, ed. Parasites of Fishes in the Canadian Waters of the Great Lakes. Great Lakes Fishery Commission Technical Report No. 51:19–48.

Dolan, D. M., N. D. Warry, R. Rossman, and T. B. Reynoldson, eds. 1986. Lake Huron 1980 Intensive Survey: Summary Report, Report to the Suveillance Work Group. Windsor, Ontario. 133 pp.

Haas, R. C., and J. S. Schaeffer. 1992. Predator–prey and competitive interactions among walleye, yellow perch, and other forage fishes in Saginaw Bay, Lake Huron. Michigan Department of Natural Resources. Fisheries Division. Research Report No. 1984.

Hirshfield, M. F., R. P. Morin, and D. J. Hepner. 1983. Increased prevalence of larval *Eustrongylides* (Nematoda) in the mummichog, *Fundulus heteroclitus* (L.), from the discharge canal of a power plant in Chesapeake Bay. Journal of Fish Biology 23:136–142.

Kaeding, L. R. 1981. Observations on *Eustrongylides* sp. infection of brown and rainbow trout in the Firehole River, Yellowstone National Park. Proceedings of the Helminthological Society of Washington 48:98–101.

Karmanova, E. M. 1968. Dioctophymidea of Animals and Man and Diseases Caused by Them. Fundamentals of Nematology, Vol. 20. Academy of Science of the USSR. Translated and published for U.S. Department of Agriculture. Amerind Publishing, New Delhi, 1985. 383 pp.

Margolis, L., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad. 1982. The use of ecological terms in parasitology. Journal of Parasitology 68:131–133.

Measures, L. N. 1988a. The development of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in oligochaetes. Journal of Parasitology 74: 296–304.

———. 1988b. Epizootiology, pathology, and description of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in fish. Canadian Journal of Zoology 68:2212–2222.

Michigan Department of Natural Resources. 1988. Michigan Department of Natural Resources Remedial Action Plan for Saginaw River and Saginaw Bay. Area of Concern. Michigan Department of Natural Resources, Great Lakes and Environmental Assessment Section. September 1988. 588 pp.

Molnar, K. 1976. Data on the developmental cycle of *Philometra obturans* (Prenant, 1886) (Nematoda: Philometridae). Acta Veterinaria Academiae Scientiarum Hungaricae 26:183–188.

———, **and C. H. Fernando.** 1975. Morphology and development of *Philometra cylindracea* (Ward and Magath, 1916) (Nematoda: Philometridae). Journal of Helminthology 49:19–24.

Moravec, F., and I. Dykova. 1978. On the biology of the nematode *Philometra obturans* (Prenant 1886) in the fishpond system of Macha Lake, Czechoslovakia. Folia Parasitologica (Praha) 25: 231–240.

Potvin, C., and D. A. Roff. 1993. Distribution-free and robust statistical methods: viable alternatives to parametric statistics. Ecology 74:1617–1628.

Salz, R. J. 1989. Factors influencing growth and survival of yellow perch from Saginaw Bay, Lake Huron. Michigan Department of Natural Resources, Fisheries Division, Fisheries Research Report No. 1964.

Schneider, J. C., F. C. Hooper, and A. M. Beeton. 1969. The distribution and abundance of benthic fauna in Saginaw Bay, Lake Huron. Proceedings of the 12th Conference of Great Lakes Research 1969:80–90. International Association of Great Lakes Research.

1997 Meeting Schedule

15 January 1997	Armed Forces Institute of Pathology, Washington, D.C.
19 March 1997	Uniformed Services University of the Health Sciences, Bethesda, MD
3 May 1997	New Bolton Center, University of Pennsylvania, Kennett Square, PA
October 1997	To be Announced
November 1997	To be Announced

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 102–105

Cosmocercoides variabilis (Nematoda: Cosmocercoidea) Populations in the Eastern American Toad, *Bufo a. americanus* (Salienta: Bufonidae), from Western West Virginia

JAMES E. JOY[1] AND CAROLE A. BUNTEN
Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755

ABSTRACT: *Cosmocercoides variabilis* was recovered from the small and large intestines of *Bufo a. americanus* in western West Virginia. Toads were collected from 2 different sites during the April breeding seasons of 1994 and 1995 (Cabell Co.) and 1993 and 1995 (Wayne Co.). Prevalence was 100% in both host sexes from Cabell Co. (25 of 25 females; 48 of 48 males). Prevalences in Wayne Co. toads were 87.5% for females (14 of 16) and 57.1% for males (28 of 49). A total of 3,114 *C. variabilis* were collected: 2,878 from 73 Cabell Co. hosts and 236 from 42 Wayne Co. hosts. The 3,114 nematodes were distributed as 1,852 females, 1,196 males, and 66 juveniles. Specific mean intensities, and numbers and sex of nematodes collected by host sex and collection site, are reported.

KEY WORDS: *Cosmocercoides*, *Bufo*, nematode sex ratios, West Virginia.

Cosmocercoides variabilis (Harwood, 1930) Travassos, 1931, a nematode parasite of toads, was considered a synonym of the molluscan parasite *C. dukae* (Holl, 1928) Travassos, 1931, by Ogren (1953, 1959), who presumed that amphibians acquired *C. dukae* infections by ingesting infected molluscs. More recently, however, Vanderburgh and Anderson (1987a) demonstrated that these 2 species of *Cosmocercoides* are distinct and that *C. variabilis* is an amphibian parasite. There are few data on infections of *C. variabilis* in natural toad populations and no data on this nematode species from toad populations in West Virginia. The purpose of this study was to investigate *C. variabilis* infections in breeding populations of *Bufo a. americanus* Holbrook, 1836, in 2 western West Virginia locations.

Materials and Methods

Sample areas and procedures

West Virginia collection sites, separated by a distance of 35 km, were located in Cabell Co. (Green Bottom Wildlife Management Area, 38°35′11″ N, 82°15′39″ W, elevation 550 ft) and Wayne Co. (Beech Fork Lake Lower Bowen Campground, 38°18′19″N, 82°20′49″W, elevation 600 ft). Toads were collected by hand during the April breeding seasons of 1994 and 1995 at the former site and 1993 and 1995 at the latter site. Host sample sizes by sex, site, and year of collection are given in Table 1. Toads were brought to the laboratory and necropsied within 24 hr of capture. Prior to necropsy, each toad was weighed to the nearest 0.1 g. Each toad was killed by pithing, and the small and large intestines were removed for examination. Every *Cosmocercoides variabilis* encountered in a toad was kept, sexed with a stereomicroscope, and counted. In those few instances where sex was questionable, the worms were cleared in lactophenol and sexed using a compound microscope. When sex could not be determined, the individual was considered a juvenile. Voucher specimens of *Cosmocercoides variabilis* have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland, under accession numbers USNPC No. 85430 (male) and USNPC No. 85431 (female), respectively. References to prevalence and mean intensity follow the definitions of Margolis et al. (1982).

Statistical analyses

Toad weights, recorded by sex and collection site, were compared by an unpaired, 2-tailed Student's *t*-test. Data on mean *C. variabilis* intensities were analyzed for statistical significance by the Wilcoxon rank-sums test using a statistical computer package (SAS Institute Inc., 1989; NPAR1WAY, SAS Institute, Cary, North Carolina). Nematode sex ratios were compared by a chi-square test. Levels of statistical significance for each test are shown in the appropriate tables.

[1] Corresponding author.

Table 1. Sample sizes and mean weights, by sex, for *Bufo a. americanus* collected at Beech Fork (BF) and Green Bottom (GB), West Virginia. Toad weights for both collection years at each site combined for statistical comparisons.

Site	Year	Sample size ♀♀	Sample size ♂♂	Mean (±1 SD) toad weight in grams ♀♀	Mean (±1 SD) toad weight in grams ♂♂
BF	1993	9	34	59.9 (19.1)	34.8 (6.5)
	1995	7	15	58.9 (11.5)	30.6 (5.9)
BF	Combined	16	49	59.5 (15.7)*‡	33.5 (6.4)*§
GB	1994	22	28	44.9 (10.0)	29.0 (5.6)
	1995	3	20	49.4 (8.4)	29.2 (5.2)
GB	Combined	25	48	45.5 (9.8)†‡	29.1 (5.5)†§

* BF female weight vs. male weight: $t = 9.49$; 63 df; $P < 0.001$.

† GB female weight vs. male weight: $t = 9.18$; 71 df; $P < 0.001$.

‡ BF female weight vs. GB female weight: $t = 3.52$; 39 df; $P < 0.05$.

§ BF male weight vs. GB male weight: $t = 3.57$; 95 df; $P < 0.001$.

Table 2. Two-way comparisons of mean intensities for *Cosmocercoides variabilis* infections in *Bufo a. americanus* calculated by the Wilcoxon ranked-sums test. Comparisons 1 and 2 are for the same host sex between sites, whereas comparisons 3 and 4 are for different host sexes within sites. BF = Beech Fork; GB = Green Bottom; $\bar{x}$ = mean intensity; Obs. = Σ observed ranked scores; Exp. = Σ expected ranked scores under the null hypothesis that distribution of nematodes in the 2 populations being compared are similar. H_0 rejected if $P > 0.05$.

	Site	Host sex (*n*)	$\bar{x}$	Obs.	Exp.	Z-score	$P > Z$
1.	BF	♀ (14)	8.93	121	280	−4.667	0.0001
	GB	♀ (25)	52.64	660	500		
2.	BF	♂ (28)	3.96	446	1,078	−6.808	0.0001
	GB	♂ (48)	32.67	2,480	1,848		
3.	BF	♀ (14)	8.93	356	301	1.477	0.1396
	BF	♂ (28)	3.96	547	602		
4.	GB	♀ (25)	52.64	1,222	925	3.448	0.0006
	GB	♂ (48)	32.67	1,479	1,776		

Results

Cosmocercoides variabilis was removed from the small and large intestines of 115 (39 females and 76 males) *Bufo a. americanus* in western West Virginia. All toads from Green Bottom (25 females and 48 males) were infected, but prevalences for Beech Fork toads were 87.5% (14 of 16 females) and 57.1% (28 of 49 males). Both female and male toads at Green Bottom had significantly lower body weights and significantly higher intensities of infection than their counterparts at the Beech Fork site (Tables 1, 2). Female hosts had higher intensities of infection than males at both collection sites, but the difference was significant only at Green Bottom (Table 2).

Of the 3,048 *Cosmocercoides variabilis* adults recovered during the course of this study, 1,852 were females, yielding a highly significant female-biased sex ratio of 1.55:1.00 (Table 3). This female-biased sex ratio was consistent in female and male hosts at the different collection sites, even though intensities of infection between host sexes and between sites were different (Table 3).

Discussion

High prevalences of *C. variabilis* observed in the present study (e.g., 100% in both sexes of Green Bottom toads; 87.5% and 57.1% for female and male toads, respectively, at Beech Fork) were not surprising. Vanderburgh and Anderson (1987b) reported high prevalences (e.g., 85% and 89% for late April and early May, respectively) for this nematode species in breeding populations of *B. a. americanus* from Ontario, whereas *C. dukae* (=*C. variabilis*?) was found in 75% of 24 *B. a. americanus* examined from central Ohio (J. C. McGraw, pers. comm.). Harwood (1930) reported *Oxysomatium variabilis* (=*C. variabilis*) in 38 of 44 (86.5%) *B. valliceps* from Texas.

Vanderburgh and Anderson (1987b) reported mean intensities of 3.5 and 3.8 for adult *C. variabilis* in late April and early May, respectively, and a mean intensity of 9.2 mature nematodes in summer and fall collections. Mean intensities of *C. variabilis* in our Beech Fork sample (Table 2) are not dissimilar to mean values reported in toads from Ontario. The considerably higher mean intensities in Green Bottom toads (Table 2) cannot easily be explained, although it is plausible that toads in populations where infection is high would be more likely to come in contact with more infective nematode larvae.

Data on parasitic infection by amphibian host sex do not often appear in the literature, and when they do, as Aho (1990) has pointed out, the influence of host gender on parasitic community structure is variable. His point is well taken. Mean intensities of *C. variabilis* infection

Table 3. Total numbers and sex ratios observed for *Cosmocercoides variabilis* in *Bufo a. americanus* populations from Beech Fork (BF) and Green Bottom (GB), West Virginia. ∞ = juveniles

Site	Host sex (*n*)	*Cosmocercoides variabilis* ♀♀	♂♂	∞	♀♀:♂♂	χ^2	*P*
BF	♀ (14)	70	40	15	1.75:1.00	8.18	<0.005
	♂ (28)	60	39	12	1.54:1.00	4.46	<0.05
GB	♀ (25)	786	513	17	1.53:1.00	57.37	<0.001
	♂ (48)	936	604	22	1.55:1.00	71.57	<0.001
Totals	(115)	1,852	1,196	66	1.55:1.00	141.19	<0.001

between female and male hosts at Beech Fork were not significantly different, whereas mean intensities were significantly different between sexes at Green Bottom (Table 2). Goldberg and Bursey (1991) reported different prevalences by gender in *Bufo punctatus* from Arizona, but no significant differences in prevalences by host gender were found in 3 toad species from New Mexico (Goldberg et al., 1995). There were no significant differences in prevalences by host sex in the present study at either Green Bottom (100% for both host sexes) or Beech Fork (87.5% and 57.1% for female and male toads, respectively; chi-square [Yates's correction] = 3.624, 1 df, $P = 0.0595$).

In warm-blooded hosts, the number of female nematodes typically exceeds that of males (Roche and Patrzek, 1966). Evaluation of nematode sex ratios in amphibian hosts has generally been ignored, although Muzzall (1990) and Joy et al. (1993) have provided some insights on this aspect of amphibian nematode biology. In the present study, statistically significant female-biased sex ratios of *C. variabilis* were quite consistent between host sexes, between collection sites, and even between hosts that showed significant differences in mean intensities (Table 3). This last point is of interest because Roche and Patrzek (1966) noted that female-biased sex ratios are "... often found in infections with a scanty number of worms."

In summary, our findings relative to prevalences and mean intensities corroborate those of other investigators, whereas our data on infections by sex of host and on nematode sex ratios are new. Still, the relationships between host sex and parasitic nematode infection rates in amphibians are not well understood. Future investigators, using appropriate sample sizes, could add appreciably to our knowledge of amphibian/nematode associations by segregating female from male hosts in their necropsy protocols and data analyses.

Acknowledgments

We extend our appreciation to James McGraw, who kindly provided us with raw data from his dissertation research; to Stuart Thomas, who assisted us with our statistical analyses; and to the West Virginia Department of Natural Resources, for allowing us to collect toads under permit numbers 63-1993, 22-1994, and 29-1995.

Literature Cited

Aho, J. M. 1990. Helminth communities of amphibians and reptiles: comparative approaches to understanding patterns and processes. Pages 157–195 *in* G. W. Esch, A. O. Bush, and J. M. Aho, eds. Parasite Communities: Patterns and Processes. Chapman and Hall, New York.

Goldberg, S. R., and C. R. Bursey. 1991. Helminths of the red-spotted toad, *Bufo punctatus* (Anura: Bufonidae), from southern Arizona. Journal of the Helminthological Society of Washington 58:267–269.

———, ———, **and I. Ramos.** 1995. The component parasite community of three sympatric toad species, *Bufo cognatus*, *Bufo debilis* (Bufonidae), and *Spea multiplicata* (Pelobatidae) from New Mexico. Journal of the Helminthological Society of Washington 62:57–61.

Harwood, P. D. 1930. A new species of *Oxysomatium* (Nematoda) with some remarks on the genera *Oxysomatium* and *Aplectana*, and observations on the life history. Journal of Parasitology 17:61–73.

Joy, J. E., T. K. Pauley, and M. L. Little. 1993. Prevalence and intensity of *Thelandros magnavulvaris* and *Omeia papillocauda* (Nematoda) in two species of desmognathine salamanders from West Virginia. Journal of the Helminthological Society of Washington 60:93–95.

Margolis, L. G., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad. 1982. The use of ecological terms in parasitology (report of an *ad hoc* committee of the American Society of Parasitologists). Journal of Parasitology 68:131–133.

Muzzall, P. M. 1990. Endoparasites of the red-backed salamander, *Plethodon c. cinereus*, from

southwestern Michigan. Proceedings of the Helminthological Society of Washington 57:165–167.

Ogren, R. E. 1953. A contribution to the life cycle of *Cosmocercoides* in snails (Nematoda: Cosmocercidae). Transactions of the American Microscopical Society 72:87–91.

———. 1959. The nematode *Cosmocercoides dukae* as a parasite of the slug. Proceedings of the Pennsylvania Academy of Science 33:236–241.

Roche, M., and D. Patrzek. 1966. The female to male ratio (FMR) in hookworm. Journal of Parasitology 52:117–121.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6 ed. Vol. 2. SAS Institute, Cary, North Carolina. 846 pp.

Vanderburgh, D. J., and R. C. Anderson. 1987a. The relationship between nematodes of the genus *Cosmocercoides* Wilkie, 1930 (Nematoda: Cosmocercoidea) in toads (*Bufo americanus*) and slugs (*Deroceras laeve*). Canadian Journal of Zoology 65:1650–1661.

———, **and** ———. 1987b. Preliminary observations on seasonal changes in prevalence and intensity of *Cosmocercoides variabilis* (Nematoda: Cosmocercoidea) in *Bufo americanus* (Amphibia). Canadian Journal of Zoology 65:1666–1667.

Application to the International Commission on Zoological Nomenclature

Case 2932 *Haplotrema* Looss, 1899 (Digenea): proposed designation of *H. loossi* Price, 1934 as the type species

The purpose of this application is to designate the nominal species *Haplotrema loossi* Price, 1934, a spirorchid parasite of marine turtles, as the type species of the blood fluke genus *Haplotrema* Looss, 1899. At present the type species is *Distoma constrictum* Leared, 1862, but this is due to a misidentification and the genus was based on material later named *H. loossi.* The name *H. mistroides* (Monticelli, 1896) is a senior subjective synonym of *H. loossi.*

Thomas R. Platt
Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556
David Blair
Department of Zoology, James Cook University, Townsville, Queensland 4811, Australia

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 106–112

Vigisospirura potekhina hugoti subsp. n. (Nematoda: Spirocercidae) from *Meles meles* (Carnivora: Mustelidae) in Spain

JORDI TORRES,[1] CARLOS FELIU,[1,2] AND JORDI MIQUEL[1]

[1] Parasitology Laboratory, Department of Sanitary Microbiology and Parasitology, Pharmacy Faculty, University of Barcelona, Avda. Diagonal sn, 08028 Barcelona, Spain and

[2] Institut de Salut Pública, Campus Universitari de Bellvitge, Barcelona, Spain

ABSTRACT: *Vigisospirura potekhina hugoti* subsp. n. from the stomach and esophagus of *Meles meles* (Carnivora: Mustelidae) collected in Spain is described. Morphologically, the new subspecies is distinguished by the presence of a small projection (like a knob) at the end of the male and by the size of its spicules. A key for *Vigisospirura* species based on morphological and morphometric characteristics as well as chorology and host specificity is proposed. A scanning electron microscope study of the several structures is presented.

KEY WORDS: *Vigisospirura potekhina hugoti* subsp. n., Nematoda, Spirocercidae, *Meles meles*, Carnivora, Mustelidae, Spain.

Nematodes of the genus *Vigisospirura* Petrow et Potekhina, 1953, were collected from the stomach and esophagus of several eurasian badgers, *Meles meles* (Linnaeus, 1758), on the Iberian Peninsula. The specimens differed from the valid species known to date belonging to this genus. Chabaud (1959) and Wong *et al.* (1980) have considered only 5 valid species: (a) *Vigisospirura potekhina* (Petrow et Potekhina, 1953) Chabaud, 1959 (=*V. skrjabini* Petrow et Potekhina, 1953, not Tschernikova, 1934); (b) *V. grimaldiae* (Seurat, 1915) Chabaud, 1959 (=*Habronema grimaldiae* Seurat, 1915); (c) *V. skrjabini* (Tschernikova, 1934) Chabaud, 1959 (=*H. skrjabini* Tschernikova, 1934); (d) *V. whitei* (Monnig, 1931) Chabaud, 1959 (=*H. whitei* Monnig, 1931); and (e) *V. itascensis* (Chandler, 1954) Wong *et al.*, 1980 (=*Chlamydoprocta itascensis* Chandler, 1954). The aim of this study is to describe a new subspecies, *Vigisospirura potekhina hugoti,* and to present a key to the representatives of the genus *Vigisospirura.*

Material and Methods

Seventy-eight specimens of *Meles meles* from different localities in 20 provinces of the Iberian Peninsula were examined. These provinces are Asturias (AST), Barcelona (B), Burgos (BU), Cáceres (CC), Cantabria (CAN), Ciudad Real (CR), Girona (GI), Granada (GR), Guadalajara (GU), Jaén (J), La Coruña (C), León (LE), Lleida (L), Navarra (NA), Palencia (P), Salamanca (SA), Soria (SO), Tarragona (T), Valladolid (VA), and Zaragoza (Z) (Fig. 1). Some hosts were sent frozen to our laboratory; however, most of them came from the National Museum of Natural Sciences Collection (MNCN) in Madrid, where they had been preserved in 70% ethanol or 4% formaldehyde solutions.

Nematodes obtained were preserved in 70% ethanol. Some were mounted on slides in lactophenol and used in light microscopy studies. Only 9 adult males and 2 gravid females fixed in good extension were useful for measurements. Some broken male and female specimens were used to study, respectively, the caudal and cephalic regions by means of scanning electron microscopy (SEM). These specimens were prepared following the general methodology of Prokopic and Hulínská (1983), Sanmartín *et al.* (1992), and Miquel *et al.* (1995).

The specimens of *Vigisospirura* were found in 5 of the 78 eurasian badgers examined (prevalence 6.4%). Fifty-eight individual worms were collected (11 adults [9 males and 2 females], several preadults and some broken adults). Mean intensity was 11.6 (1–42) and abundance was 0.74.

Description

Vigisospirura potekhina hugoti subsp. n. (Figs. 2–10)

Medium sized worms. Cuticle with transverse striations separated from each other about 11 μm. Lateral alae absent. Buccal cavity with thick walls expands anteriorly to form ring surrounding oral opening (without buccal teeth) and outer elevated cuticular shield. Oral opening with prominent rectangular to oval cephalic shield from which delicate lateral lips and dorsoventral and median lobes arise (Figs. 2, 7). Fourteen cephalic papillae are present: 6 small inner papillae and 4 pairs of prominent submedian outer papillae (Figs. 2, 7). Amphids are located laterally near outer edge of cephalic shield (Figs. 2, 7).

MALE (holotype) (Figs. 3–5, 8–10): Body length 13.7 mm; maximum body width 260 μm. Buccal cavity 30 μm in length and in width. Total length of esophagus 7.0 mm (51.4% of

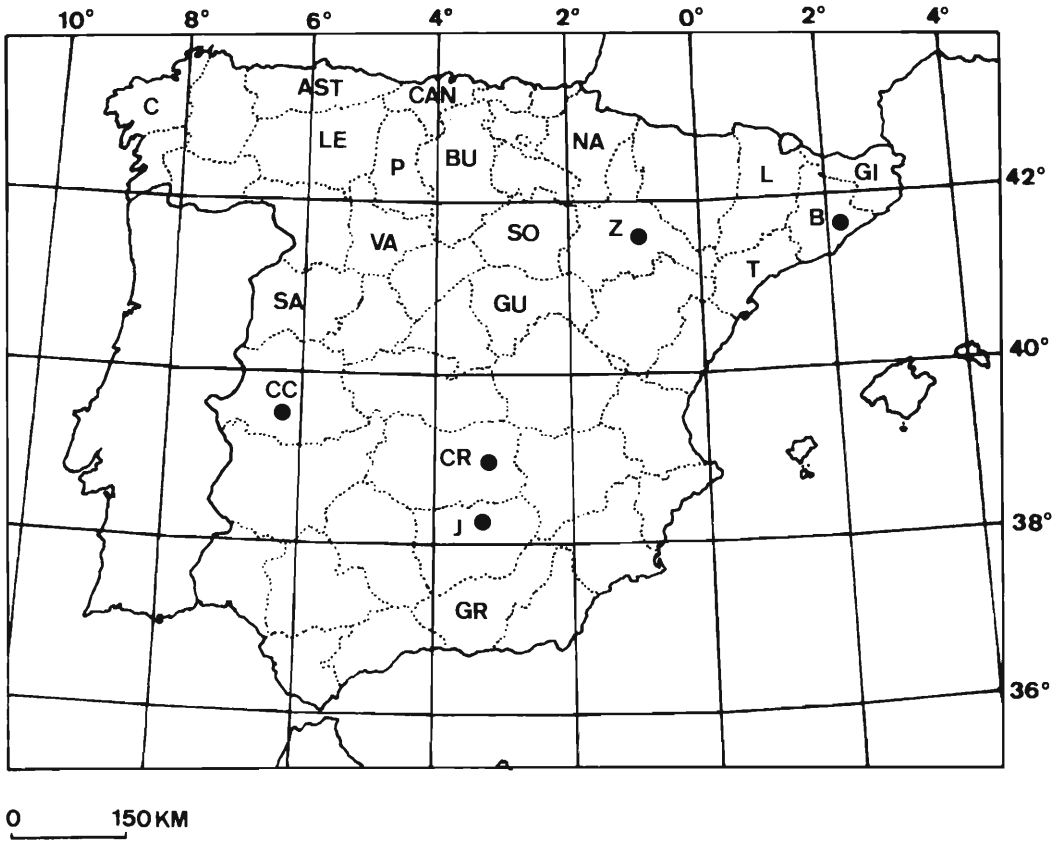
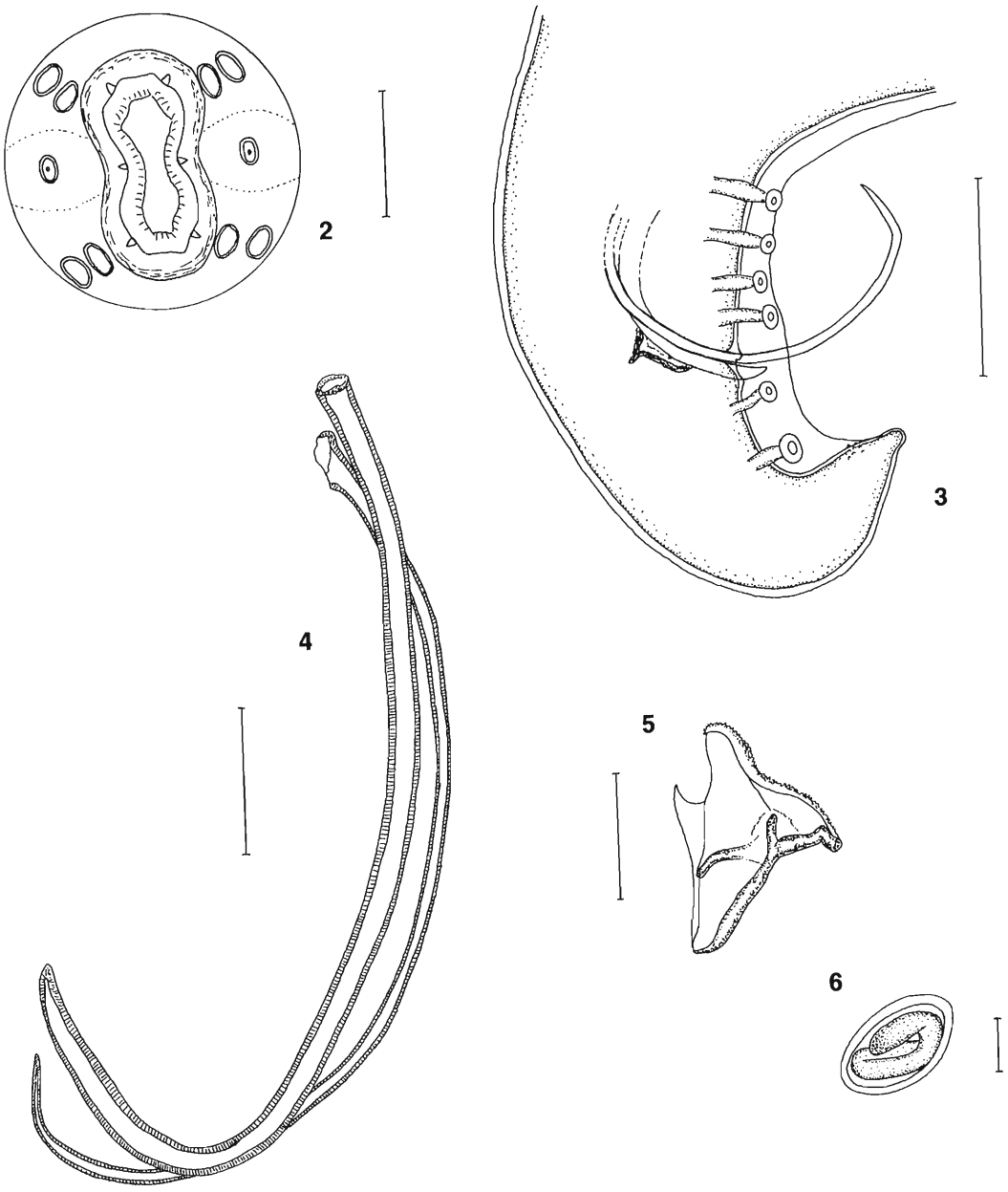


Figure 1. Provinces where *Vigisospirura potekhina hugoti* subsp. n. has been detected (●).

body length); muscular part 395 μm and glandular part 6.6 mm. Deirids, nerve ring, and excretory pore located at 231, 295, and 480 μm from the cephalic extremity, respectively. Spicules are simple, slender and arcuate in the distal end (Figs. 3, 4); right spicule 880 μm long and 13 μm in maximum width proximally; left spicule 980 μm long and 20 μm in maximum width proximally. Right spicule not attenuated distally; left spicule with an attenuated rounded apex (Fig. 4). Gubernaculum alate and complex (Fig. 5); the more chitinized part is boot-shaped on lateral view, 72 μm in length and in maximum width. Caudal end of body arcuate or coiled in the larger specimens (Fig. 3). Caudal papillae, including 6 pairs of long digitiform papillae (4 precloacal [CLP1, CLP2, CLP3, and CLP4] and 2 postcloacal pairs [CLP5 and CLP6]) and a flat median papilla, immediately anterior to anus (Figs. 3, 8, 9). Sensory area present near caudal extremity on ventral surface of tail, with 4 pairs of tiny papillae (CLP7, CLP8, CLP9, and CLP10) and phasmids (Fig. 10). Tail 235 μm in length. Caudal extremity ending with a small projection (Fig. 3).

FEMALE (allotype) (Figs. 2, 6, 7): Body length 20.9 mm; maximum body width 415 μm. Buccal cavity 30 μm in length and 23 μm in width. Total length of esophagus 9.5 mm (45.3% of body length); muscular part 470 μm and glandular part 9.0 mm. Deirids, nerve ring, and excretory pore located at 260, 290, and 355 μm from the cephalic extremity, respectively. Vulva located at 10.7 mm from the anterior extremity (51.3% of body length). Opisthodelphic. Eggs oval with smooth thick shell, 46–57 × 26–33 μm in size. Eggs in advanced uterine positions (near vagina) contain fully embryonated first-stage larvae (Fig. 6) and are bigger than unembryonated ones in other uterine positions. Tail 250 μm long. Phasmids located near apex of the



Figures 2–6. ***Vigisospirura potekhina hugoti*** **subsp. n.: details of male and female specimens. 2. Cephalic extremity in apical view. 3. Lateral view of the male's caudal extremity. 4. Spicules. 5. Lateroventral view of the gubernaculum. 6. Embryonated egg. Scale bars: 2, 6 = 20 μm; 3 = 200 μm; 4 = 100 μm; 5 = 50 μm.**

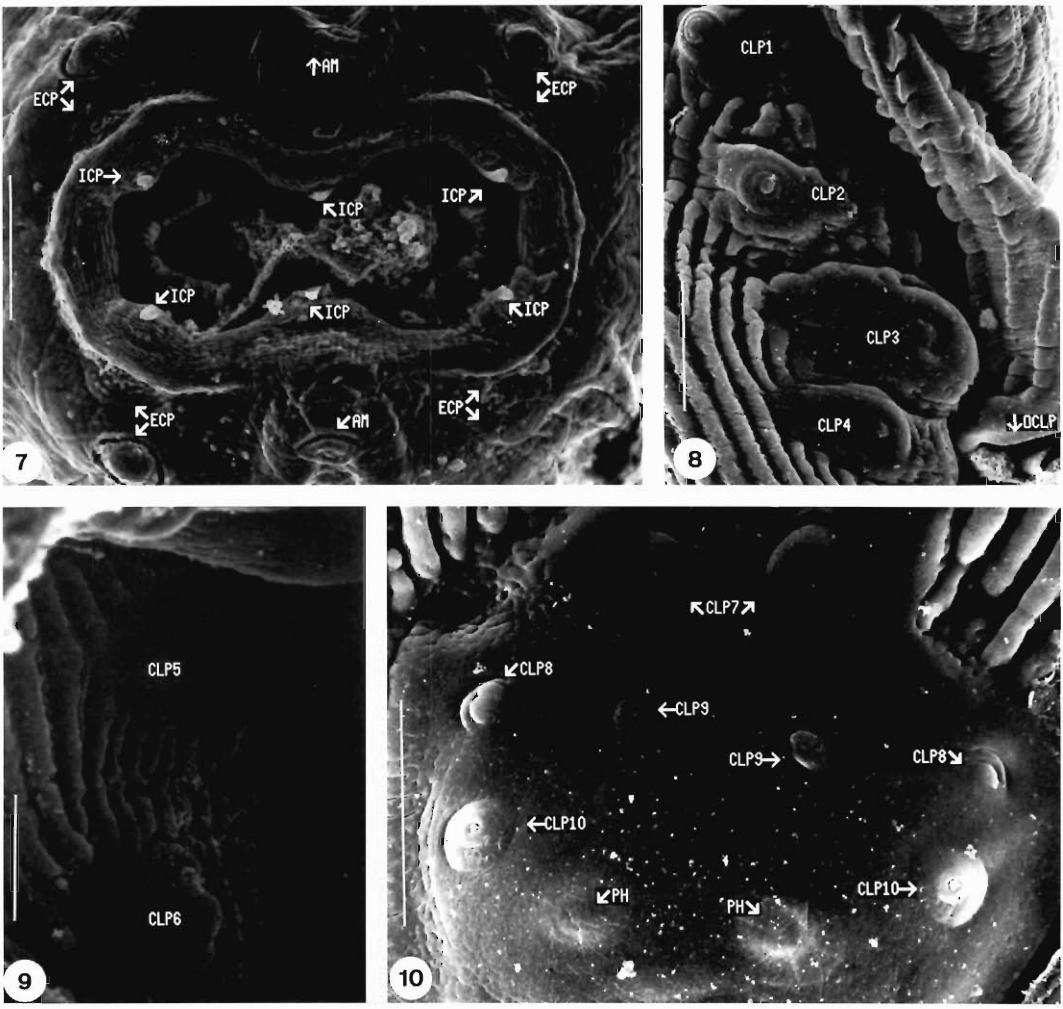
tail. Anus, broad in shape, located before 2 caudal prominent bulges.

HOST: *Meles meles* (Linnaeus, 1758).

SITES IN HOST: Stomach and esophagus.

TYPE LOCALITY: Virgen de la Cabeza (province of Jaén).

LOCATIONS: Provinces of Barcelona (B), Cáceres (CC), Ciudad Real (CR), Jaén (J), and Za-



Figures 7–10. Scanning electron microscopic observations of *Vigisospirura potekhina hugoti* subsp. n. 7. Cephalic extremity of the female, apical view. 8. Precloacal papillae. 9. Postcloacal papillae. 10. Phasmids and last postcloacal papillae group. Scale bars: 7 = 10 μm; 8–10 = 20 μm. AM, amphids; CLP, cloacal papillae; ECP, external cephalic papillae; ICP, internal cephalic papillae; OCLP, odd cloacal papilla; PH, phasmids.

ragoza (Z). All biotopes located between 38th and 42nd parallels (Fig. 1).

DATE OF COLLECTION: 21 August 1979 (holotype).

SPECIMENS DEPOSITED: National Museum of Natural Sciences in Madrid. Holotype, allotype, and 1 paratype (Male), MNCN 11.02/9, and 1 paratype (Male), MNCN 11.02/10. Several paratypes in Department of Sanitary Microbiology and Parasitology, University of Barcelona.

ETYMOLOGY: The new subspecies, *Vigisospirura potekhina hugoti*, is dedicated to Dr. Jean Pierre Hugot from the Muséum National d'Histoire Naturelle of Paris (France).

Discussion

To our knowledge, species belonging to the genus *Vigisospirura* have not been found in Central or Western Europe to date. The 5 species belonging to the genus and accepted by Chabaud (1959) and Wong *et al.* (1980) show different distributions. *Vigisospirura itascensis* is only found in North America, *V. skrjabini* in Vladivostok, Russia, *V. potekhina* in North America

Table 1. Measurements (in micrometers) of *Vigisospirura potekhina hugoti* subsp. n.

	Males (n = 8 paratypes)			Female (n = 1 paratype)
	Maximum	Minimum	Mean ± σ	Value
Body length	19,762	11,847	15,869.0 ± 2,904.2	21,512
Maximum body width	440	227	333.7 ± 70.8	750
Depth buccal capsule	34	26	28.6 ± 2.7	33
Width buccal capsule	26	18	22.3 ± 2.5	31
Deirids*	295	206	251.6 ± 29.9	385
Nerve ring*	373	266	316.5 ± 37.3	341
Excretory pore*	468	367	417.9 ± 35.5	514
Esophagus				
Total length	8,824	5,863	7,612.2 ± 1,198.3	8,968
Muscular length	534	372	461.7 ± 63.9	493
Glandular length	8,299	5,491	7,150.2 ± 1,137.3	8,475
Body length (%)	51.7	44.6	48.2 ± 1.9	41.7
Right spicule	917	855	884.7 ± 23.3	—
Left spicule	990	928	957.6 ± 20.9	—
Gubernaculum				
Length	82	72	76.3 ± 3.7	—
Maximum width	82	59	68.8 ± 7.8	—
Vulva*				10,934
Body length (%)	—	—	—	50.8
Tail	283	202	230.0 ± 27.4	326
Eggs (length)	—	—	—	46–57
Eggs (width)	—	—	—	26–33

* Distance from anterior extremity.

and the Tadzhikistan Republic (southwestern Asia), *V. grimaldiae* in Northern Africa, and *V. whitei* in Southern Africa. On the other hand, these species have been isolated as parasites of the families Canidae (*V. skrjabini, V. grimaldiae*, and *V. potekhina*); Felidae (*V. skrjabini* and *V. potekhina*); Viverridae (*V. whitei*), and Mustelidae (*V. itascensis* from *Mephitis mephitis* [Mephitinae] and *V. potekhina* from *Meles meles* [Melinae]).

The species can be divided into 2 groups according to the presence (*V. whitei, V. skrjabini*, and *V. itascensis*) or absence (*V. grimaldiae* and *V. potekhina*) of prominent cervical lateral alae. Furthermore, *V. potekhina hugoti* (without lateral alae) can be differentiated from the first group of species according to some ecological characteristics (geographical distribution and specificity). Thus, the species that show higher affinity with our specimens are *V. potekhina* and *V. grimaldiae*.

The Iberian specimens resembles *V. grimaldiae* in the small projection present at the caudal extremity of males (Fig. 3). This could be related to the fact that *V. grimaldiae* is the species most closely located to the Iberian Peninsula. However, *Vigisospirura potekhina hugoti* can be differentiated from it by the different sizes of its spicules. *V. grimaldiae* male spicules are 1,450 × 17 μm and 1,260 × 10 μm (spicular relationship 1:1.15); *V. potekhina hugoti* are 955 × 20 μm and 885 × 13 μm (spicular relationship 1: 1.07). Furthermore, *V. grimaldiae* shows a high specificity and only parasitizes *Vulpes vulpes* (Carnivora: Canidae) in its geographical distribution (Chabaud, 1959; Bernard, 1968).

The main differences between *V. potekhina* and the new subspecies are in the male sexual structures (spicule size and gubernaculum morphology). Regardless of size of helminths and host infected, size of spicules has been constant in the individuals studied (within a narrow range) (Table 1). The 2 spicules of *V. potekhina hugoti* (Table 2) are larger and more homogeneous in size than those of *V. potekhina* (right spicule: 556 μm; left spicule: 684 μm; ratio 1:

Table 2. Comparative morphometry (in micrometers) of *Vigisospirura potekhina hugoti* subsp. n. and *V. potekhina*.

	Males				Females		
Species:	*V. potekhina*		*V. p. hugoti*		*V. potekhina*		*V. p. hugoti*
Host:	*Lynx rufus*		*Meles meles*		*L. rufus*		*M. meles*
Chorology:	Tadzhikistan and U.S.A.		Spain		Tadzhikistan and U.S.A.		Spain
Reference:	Wong et al. (1980)		Present study		Wong et al. (op cit.)		Present study
Sample:	n = 10		n = 8 paratypes		n = 10		n = 1 paratype
	Range	Mean	Range	Mean	Range	Mean	Value
Body length	12,300–24,600	18,900	11,847–19,762	15,869.0	24,900–44,100	32,400	21,512
Maximum body width	300–550	411	227–440	333.7	400–850	656	750
Depth buccal capsule	40–55	48	26–34	28.6	50–72	57	33
Width buccal capsule	22–42	27	18–26	22.3	30–70	42	31
Deirids*	240–330	290	206–295	251.6	260–430	363	385
Nerve ring*	290–460	384	266–373	316.5	440–500	450	341
Excretory pore*	330–510	460	367–468	417.9	370–690	537	514
Esophagus							
Total length	7,400–11,900	9,500	5,863–8,824	7,612.2	9,800–16,000	12,000	8,968
Muscular length	420–630	517	372–534	461.7	460–770	639	493
Glandular length	6,900–11,300	9,000	5,491–8,299	7,150.2	9,400–15,300	11,300	8,475
Body length** (%)	48.4–60.1	50.3	44.6–51.7	48.2	36.3–39.4	37.0	41.7
Right spicule	370–660	556	855–917	884.7	—	—	—
Left spicule	510–790	684	928–990	957.6	—	—	—
Ratio of spicules**	1:1.23		1:1.07		—	—	—
Gubernaculum							
Length	65–90	68	72–82	76.3	—	—	—
Maximum width	20–50	29	59–82	68.8	—	—	—
Vulva*					10,800–22,600	16,500	10,934
Body length** (%)	—	—	—	—	43.3–51.2	50.9	50.8
Tail	350–540	432	202–283	230.0	270–400	336	326
Eggs (length)	—	—	—	—	45–58	51	46–57
Eggs (width)	—	—	—	—	28–31	29	26–33

* Distance from anterior extremity.

** Value calculated by us.

1.23). The gubernaculum of the new subspecies shows a characteristic morphology (Fig. 5); it is alate and wider than that of *V. potekhina*.

One of the hosts of *V. potekhina* is *Meles meles*; there is but a single report of this parasite, and it comes from Asia. No reports of this parasite exist in Europe to date, but there are several extensive studies on the helminth fauna of this mustelid on the whole continent (Hancox, 1980 [data collection]; Loos-Frank and Zeyhle, 1982; Brglez, 1988]). Other helminth faunistic studies are being performed on most wild peninsular carnivores and no species of the genus *Vigisospirura* are being found. The number of hosts studied to date is up to 1,426 individuals (206 canids, 875 mustelids, 68 felids, and 277 viverrids) (Miquel, 1993; Motjé, 1995).

Although the spicules of our specimens differ clearly from those of *V. potekhina* in size, the creation of a new species does not appear to be justified. There is no marked morphological difference between the Iberian specimens and *V. potekhina*. On the basis of the relictual characteristics of the Iberian Peninsula, the origin of the new subspecies could be the same as that of other parasites endemic to the peninsula (Hugot and Feliu, 1990). With the present distribution of *V. potekhina* in the holarctic region, the new subspecies may have originated in its main host (*M. meles*) as a consequence of the peculiar

paleobiogeographic characteristics of the Iberian Peninsula.

Key to the Species of *Vigisospirura*

1—(5) With prominent cervical lateral alae 2
2—(1) Parasitizing Canidae and Felidae in the Soviet Union ..... *V. skrjabini*
3—(2) Parasitizing Mephitinae in the United States ..... *V. itascensis*
4—(3) Parasitizing Viverridae in South Africa ..... *V. whitei*
5—(1) Without cervical lateral alae 6
6—(5) Right and left spicules >1,250 μm; ratio of spicules 1:1.15; tail of male ends in a small projection; parasitizing Canidae in North Africa ..... *V. grimaldiae*
7—(6) Right and left spicules <1,250 μm; tail of male ends with or without small tail projection 8
8—(7) Right and left spicules <800 μm; ratio of spicules 1:>1.2; without small tail projection; parasitizing Felidae and Melinae (experimentally also Canidae) in the United States and Tadzhikistan Republic ..... *V. potekhina potekhina*
9—(8) Right and left spicules between 850 and 1,000 μm; ratio of spicules 1:<1.1; with small tail projection; parasitizing only *Meles meles* in the Iberian Peninsula. ..... *V. potekhina hugoti* subsp. n.

Acknowledgments

The study was partially supported by the "Comissionat per Universitats i Recerca de la Generalitat de Catalunya" (GRQ 94-105) and Spanish DGICYT project PB 92-0517-CO2-02. We thank the "Serveis Científico-Tècnics" of the University of Barcelona for his aid and support in the preparation of the SEM micrographs.

Literature Cited

Bernard, J. 1968. Cas de parasitisme intense chez un renard saharien. Archives Institute Pasteur de Tunis 45:153–168.

Brglez, J. 1988. Some endohelminths in badgers, *Meles meles* L., in Slovenia. Zbornik Biotehniske Fakultete Univerze Edvarda Kardelja v Ljubljani, Veterinarstvo 25:251–257.

Chabaud, A. G. 1959. Sur la systématique des nématodes proche de *Spirocerca lupi* (Rud. 1809). Parassitologia 1:129–135.

Hancox, M. 1980. Parasites and infectious diseases of the Eurasian badger (*Meles meles* L.): a review. Mammal Review 10:151–162.

Hugot, J. P., and C. Feliu. 1990. Description de *Syphabulea mascomai* n. sp. et analyse du genre *Syphabulea*. Systematic Parasitology 17:219–230.

Loos-Frank, B., and E. Zeyhle. 1982. The intestinal helminths of the red fox and some other carnivores in southwest Germany. Zeitschrift für Parasitenkunde 67:99–113.

Miquel, J. 1993. Contribución al conocimiento de la helmintofauna de los Carnívoros silvestres de Cataluña. Ph.D. Thesis, University of Barcelona, Barcelona.

———, **J. C. Casanova, F. Tenora, C. Feliu, and J. Torres.** 1995. A scanning electron microscope (SEM) study of some Rictulariidae (Hall, 1915) from Iberian mammals. Helminthologia 32:3–14.

Motjé, M. 1995. Contribución al conocimiento de la helmintofauna de la familia Mustelidae (Carnivora) en la Península Ibérica. Ph.D. Thesis, University of Barcelona, Barcelona.

Prokopic, J., and D. Hulínská. 1983. Scanning electron microscopic study of the superficial cuticle of the nematodes *Heligmosomum costellatum* (Dujardin, 1845) and *H. mixtum* Schulz, 1954. Folia Parasitologica 30:27–29.

Sanmartín, M. L., F. Alvarez, H. Gijón-Botella, R. Iglesias, J. Estevez, and R. López-Román. 1992. A scanning electron microscope study of *Toxocara genettae* Warren, 1972 (Ascaridae), with data of morphometric variation. Folia Parasitologica 39:255–367.

Wong, P. L., T. Watson, and R. C. Anderson. 1980. *Vigisospirura potekhina* (Petrow and Potekhina, 1953) (Nematoda: Spiruroidea) from the bobcat, *Lynx rufus* (Schreber) in the southeastern USA. Canadian Journal of Zoology 58:1612–1615.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 113–119

Triodontophorus burchelli sp. n. and *Triodontophorus hartmannae* sp. n. (Nematoda: Strongylidae) from the Burchell's, Hartmann's, and Cape Mountain Zebras in Southern Africa

R. C. KRECEK,[1] V. A. KHARCHENKO,[2] G. M. DVOJNOS,[2] F. S. MALAN,[3] AND T. E. KRECEK[1]

[1] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa,

[2] Department of Parasitology, Institute of Zoology, Khmel'nytsky Street 15, Kiev-30, 252601 Ukraine, and

[3] Hoechst Research Farm, P.O. Box 124, Malelane 1320, South Africa

ABSTRACT: *Triodontophorus burchelli* sp. n. is described from the ventral colons of 44 Burchell's zebras, *Equus burchelli antiquorum*, in Kruger National Park in South Africa and Etosha National Park, Namibia. *Triodontophorus hartmannae* sp. n. is described from the ventral colons of 21 Hartmann's mountain zebras, *Equus zebra hartmannae*, in Etosha National Park, Namib-Naukluft Park, and the farm "Kelpie," Namibia, as well as from 13 Cape mountain zebras, *Equus zebra zebra* in Cape Province, South Africa. *Triodontophorus burchelli* sp. n. is distinguished from other members of the genus by generally greater total body length, the absence of serration on upper edges of teeth, and the shape of the gubernaculum. *Triodontophorus hartmannae* sp. n. is differentiated from other species by a denticulation with medium serration.

KEY WORDS: *Triodontophorus burchelli* sp. n., *Triodontophorus hartmannae* sp. n., Strongylidae, new species, Nematoda, taxonomy, Burchell's zebra, Hartmann's mountain zebra, Cape mountain zebra, Namibia, South Africa.

Equids harbor at least 107 known species of helminths, of which 69 are strongyles (Lichtenfels, 1975). Like the other equine strongyles, *Triodontophorus* attaches to the intestinal mucosa, which causes irritation, loss of blood, and ulcers (Levine, 1980). Parasitological investigations were carried out on 3 species and subspecies of equids in southern Africa including Burchell's zebras (*Equus burchelli antiquorum*), Hartmann's mountain zebras (*Equus zebra hartmannae*), and Cape mountain zebras (*Equus zebra zebra*) (Scialdo et al., 1982; Scialdo-Krecek, 1983a, b; Scialdo-Krecek et al., 1983; Krecek et al., 1994). Two new *Habronema*, 1 new *Cylicodontophorus*, and 1 new *Cylicostephanus* species recovered in these studies were previously described (Scialdo-Krecek and Malan, 1984; Krecek, 1989; Scialdo-Krecek, 1983b), and this paper reports on 2 previously unknown *Triodontophorus* spp.

Materials and Methods

Adult worms were recovered from 44 Burchell's zebras (*Equus burchelli antiquorum* H. Smith, 1841) in Kruger National Park (KNP), South Africa, and Etosha National Park (ENP), Namibia; 21 Hartmann's mountain zebras (*Equus zebra hartmannae* Matschie, 1898) in ENP, Namib-Naukluft Park, and the farm "Kelpie" in Namibia; and 13 Cape mountain zebras (*Equus zebra zebra*) in Mountain Zebra National Park, South Africa. The zebras were processed for parasitological studies and the nematodes were killed in Lugol's iodine and fixed in 10% formaldehyde (Malan et al., 1981a, b). The specimens were cleared in lactophenol and examined with a Nikon Optiphot light microscope fitted with disc interference contrast. *En face* sections of some of the specimens were cut in the region of the buccal capsule and mounted in lactophenol to study the structures of the head region. For scanning electron microscopy (SEM), the formalinized nematodes were dehydrated in ethanol and critical-point dried in liquid CO_2. The dried nematodes were oriented onto a stub bearing adhesive and coated with gold/palladium. They were examined by SEM at 5–20 kV. Type specimens are deposited in the United States National Parasite Collection at Beltsville, Maryland, U.S.A., and Onderstepoort Helminthological Collection, Onderstepoort Veterinary Institute, Onderstepoort, South Africa.

Results

GENERAL: Strongylida, Strongylina, Strongylidae, Strongylinae, *Triodontophorus*. Medium-sized worms. Buccal capsule subglobular with 3 large esophageal teeth protruding into buccal cavity to about ½ its depth. Anterior rim of buccal capsule surrounded by 6 platelike structures that give appearance of the capsule being thickened anteriorly. Mouth collar well developed with peripheral edge rounded or depressed as a ridge. Externo-labial papillae small, conical or slender. Cephalic papillae not prominent. External leaf-crown (ELC) of numerous slender elements protrude from buccal collar. In-

ternal leaf-crown (ILC) of oval plates of same number as ELC elements. Dorsal gutter extends to anterior edge of buccal capsule. Each of 3 esophageal teeth composed of 2 plates joined at an angle medially.

MALE: Well-developed dermal collar on genital cone. Bursa with finely denticulated margin and closed ventrally.

FEMALE: Vulva close or up to 3.0 mm from the anus. Uteri parallel.

Triodontophorus burchelli sp. n. (Figs. 1–9, Table 1)

This new species was recovered from 11 of the ventral colons of 44 Burchell's zebras in KNP and ENP in moderate numbers (1–2,500) and most closely resembles *Triodontophorus brevicauda*. Hence, measurements of *T. brevicauda* are included in Table 1 with this new *Triodontophorus* species.

GENERAL: Mouth collar flattened with a rather acute erect edge around outside perimeter. Cephalic papillae are not prominent while externolabial papillae long. Buccal capsule subglobular, wider posteriorly. Teeth have no serration of upper edges. Esophagus moderately long, the nerve ring distinct and situated just above the middle of the esophagus.

DESCRIPTION: Dimensions given as range (mean in micrometers ±1 standard deviation) unless otherwise indicated.

MALES (13 specimens): Length 12.9–18.0 (15.5 ± 1.4) mm. Width 556–858 (650 ± 81.7). Length of buccal capsule 120–165 (144 ± 15.5). Width of buccal capsule 120–153 (140 ± 10.4). Number of elements of ELC 60. Esophagus 0.8–1.2 (1.0 ± 0.1) mm long and 165–213 (183 ± 14.7) wide. Distance of excretory pore from base of buccal capsule 510–788 (664 ± 70.9) and from anterior end 684–928 (810 ± 66.1). Distance anterior end from nerve ring 464–754 (554 ± 74.1) and from deirids 638–904 (791 ± 72). Dorsal ray 464–754 (571 ± 106) long. Genital cone 224–314 (282 ± 25.9) long and 305–399 (345 ± 48.3) wide. Spicule length 1.5–2.2 (1.7 ± 0.1) mm and gubernaculum length 224–285 (245 ± 21.6).

Dorsal lobe of the male bursa is long, narrow, and demarcated from the lateral lobes. Dorsal ray divided along its length into 2 small branches. Externodorsal ray is thicker than the lateral rays and posterolateral and mediolateral rays arise together while the externolateral arises separately. All 3 laterals are of equivalent length and thickness. Ventral rays each arise independently and the prebursal papillae are evident. Gubernaculum is pistol-shaped and the genital cone is slightly elongated with hair-like genital appendages at the cone's tip. Spicule tips are hooked and sharply pointed distally.

FEMALES (11 specimens): Length 14.2–19.2 (16.3 ± 1.6) mm. Width 499–939 (758 ± 12.5). Length of buccal capsule 114–180 (140 ± 18.9). Width of buccal capsule 129–210 (149 ± 7.3). Number of elements of ELC 61. Esophagus 0.9–1.2 (1.0 ± 0.1) mm long and 177–210 (194 ± 11.5) wide. Distance of excretory pore from base of buccal capsule 650–824 (715 ± 62.6) and from anterior end 0.79–1.0 (0.86 ± 0.074) mm. Distance of nerve ring from anterior end 464–661 (539 ± 69.6) and of cervical papillae 742–962 (843 ± 69.8). Distance from vulva to tip of tail 592–812 (667 ± 71) and from anus to tip of tail 157–202 (178 ± 14.8). Egg 66–102 (85.4 ± 10.9) long and 33–57 (45.8 ± 7.4) wide.

TYPE SPECIMENS: One female holotype and 1 male allotype; 1 male paratype (T-2168), Onderstepoort Helminthological Collection, Onderstepoort Veterinary Institute, Onderstepoort, South Africa.

Two male and 2 female paratypes (USNPC No. 84404), U.S. National Parasite Collection, U.S. Department of Agriculture (USDA), Beltsville, Maryland, U.S.A.

TYPE HOST AND TYPE LOCALITY: *Equus burchelli antiquorum* H. Smith, 1841. KNP, South Africa (25°12′–24°24′S, 31°36′–32°2′E).

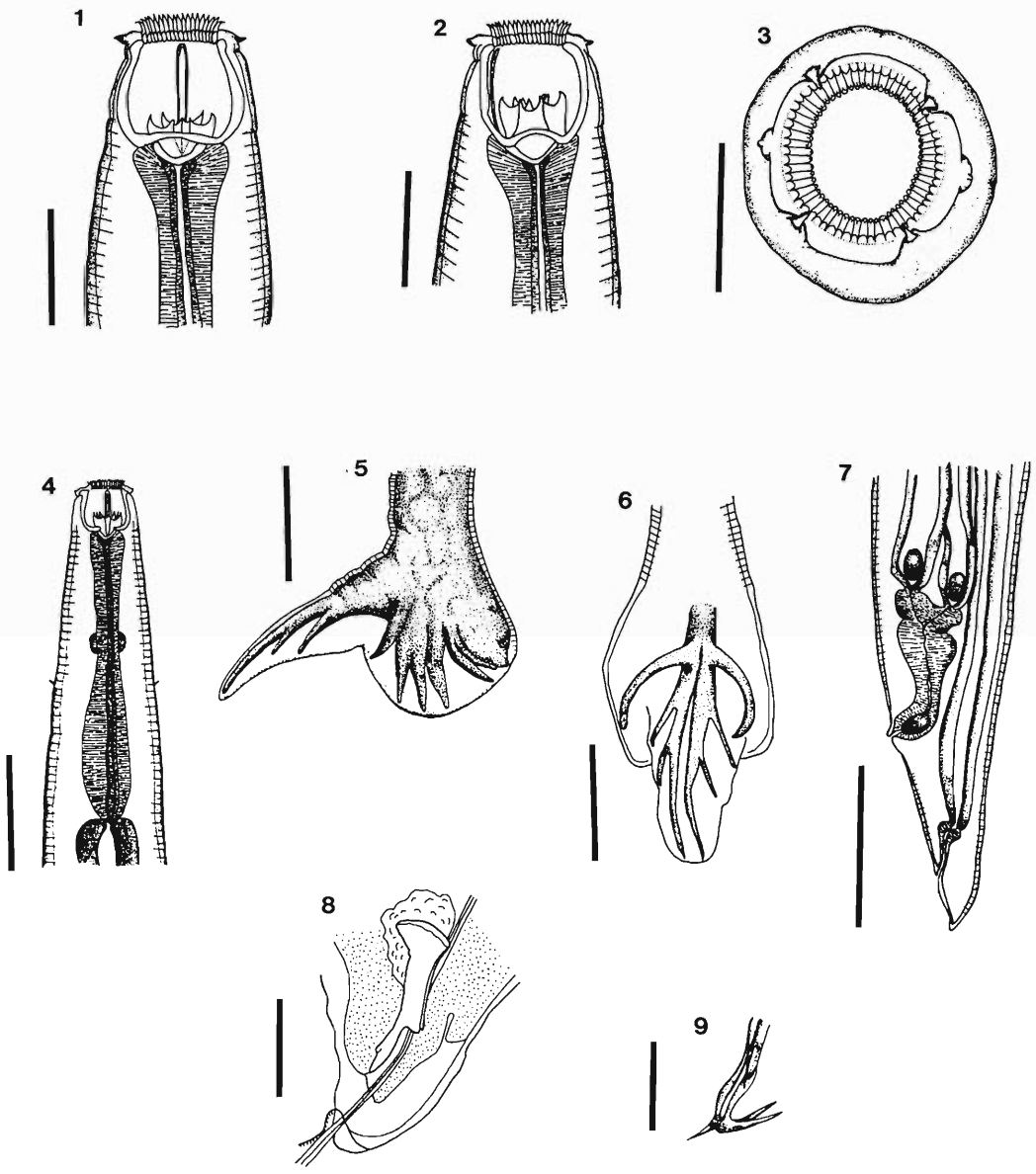
HABITAT IN HOST: Ventral colon.

ETYMOLOGY: This species is named after the host, Burchell's zebra.

Triodontophorus hartmannae sp. n. (Figs. 10–17, Table 1)

This species was recovered from all of the ventral colons of 21 Hartmann's mountain zebras in ENP, Namib-Naukluft Park, and "Kelpie" farm, Namibia, in numbers of 2–1, 865 and from 8 of 13 Cape mountain zebras, South Africa, in numbers up to 889.

GENERAL: Mouth collar flattened with a rather acute erect edge around outside perimeter. Cephalic papillae not prominent whereas externolabial papillae are long. Buccal capsule subglobular, and slightly wider posteriorly. Teeth have medium serration of upper edges and protrude well into the buccal capsule. Esophagus mod-



Figures 1–9. Drawing tube illustrations of *Triodontophorus burchelli* sp. n. 1. Buccal capsule, dorsal view of the head. Scale bar = 100 μm. 2. Buccal capsule, lateral view of the head. Scale bar = 100 μm. 3. *En face* view of head. Scale bar = 50 μm. 4. Anterior end dorsal view. Scale bar = 500 μm. 5. Male tail, lateral view. Scale bar = 500 μm. 6. Male tail, dorsal view. Scale bar = 500 μm. 7. Female tail, lateral view. Scale bar = 500 μm. 8. Genital cone of male with gubernaculum, lateral view. Scale bar = 200 μm. 9. Fused spicule tips of male. Scale bar = 50 μm.

erately long, the nerve ring distinct and situated just above the middle of the esophagus.

DESCRIPTION: Dimensions given as range (mean in micrometers ±1 standard deviation) unless otherwise indicated.

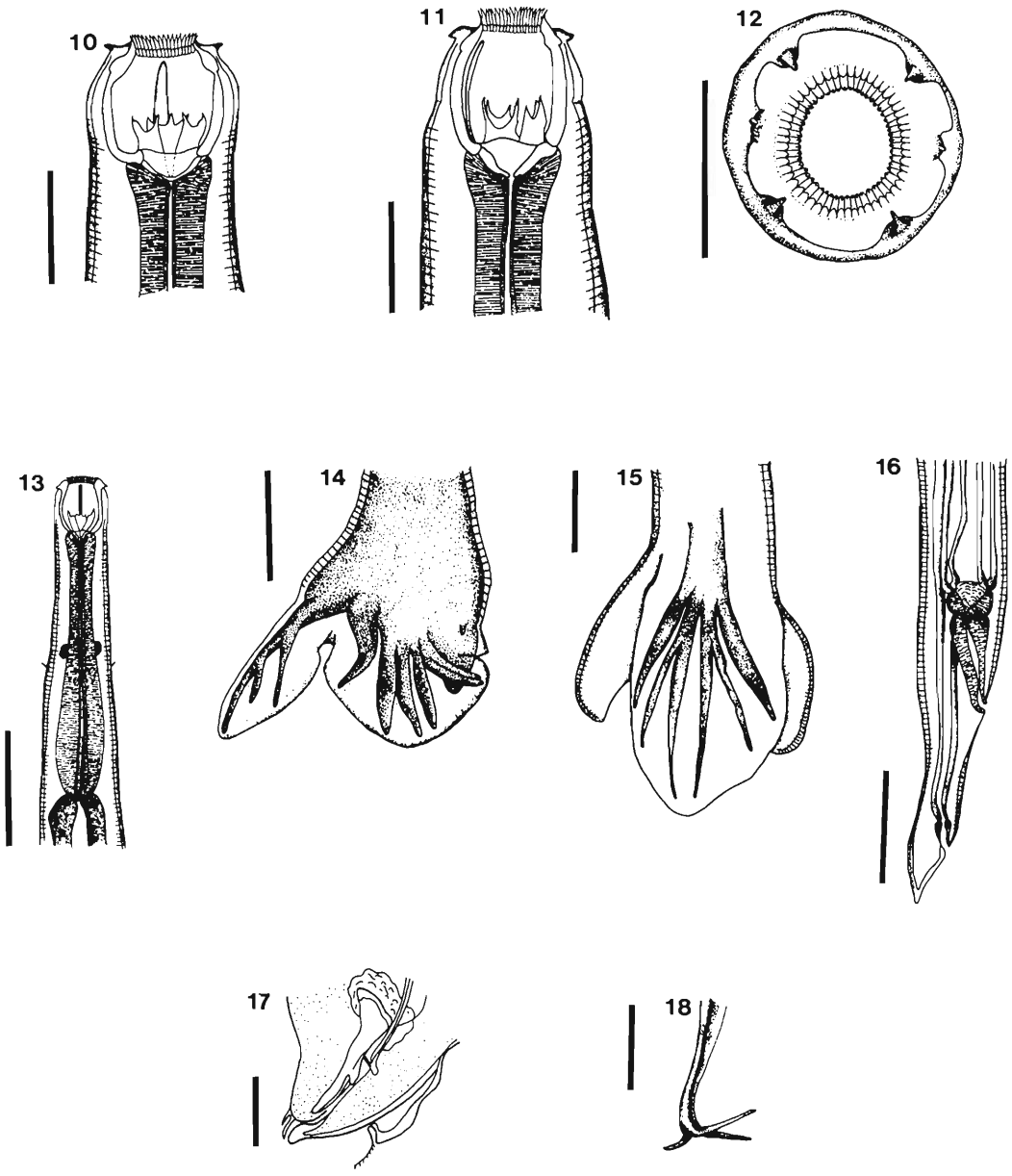
MALES (10 specimens): Length 12.6–17.3 (15.1 ± 1.2) mm. Width 557–696 (610 ± 53.6). Length of buccal capsule 180–204 (190 ± 8.4). Width of buccal capsule 135–183 (157 ± 12.8). Number of elements of ELC 55–73. Esophagus

Table 1. Principal measurements of *Triodontophorus burchelli* sp. n., *T. brevicauda, T. hartmannae* sp. n., *T. nipponicus,* and *T. minor* (all measurements in micrometers unless otherwise stated).

	T. burchelli		*T. brevicauda*		*T. hartmannae*		*T. nipponicus*		*T. minor*	
	13 ♂	11 ♀	1 ♂	3 ♀	10 ♂	9 ♀	3 ♂	3 ♀	1 ♂	3 ♀
Total length (mm)	12.9–18.0	14.2–19.2	10.4	15.1–17.6	12.6–17.3	12.5–16.8	11.2–13.1	13.1–14.8	12.4	11.8–13.6
Width	556–858	499–939	660	820–900	557–696	510–777	600–700	700–720	600	700–720
Buccal capsule										
Length	120–165	114–180	192	224–232	180–204	165–204	112–120	128–136	128	144–168
Width	120–153	129–210	—	—	135–183	135–177	160–180	178–184	176	192–208
Number of elements in external leaf-crown	60	61	*	*	55–73	67–72	*	*	*	*
Esophagus										
Length (mm)	0.8–1.2	0.9–1.2	1.0	1.1–1.2	0.7–1.2	1.0–1.2	0.9-1.0	1.0–1.1	1.0	1.0–1.2
Width	165–213	177–210	200	220–340	150–219	180–240	200–208	216–256	136	200–220
Distance of excretory pore from base of buccal capsule	510–788	650–824	640	576–608	626–731	336–731	472–640	536–704	576	416–600
Dorsal ray										
Length	464–754	—	672	—	441–580	—	416–544	—	544	—
Genital cone										
Length	224–314	—	**	—	224–336	—	184–280	—	320	—
Width	305–399	—	**	—	299–598	—	208–248	—	256	—
Spicule length (mm)	1.5–2.2	—	2.24	—	0.9–1.1	—	0.9–1.0	—	1.2	—
Gubernaculum length	224–285	—	—	—	224–280	—	—	—	—	—
Distance from vulva to tip of tail (mm)	—	592–812	—	256–360	—	661–916	—	616–776	—	592–680
Distance from anus to tip of tail (mm)	—	157–202	—	96–152	—	157–252	—	160–176	—	120–144
Egg										
Length	—	66–102	—	56–64	—	75–93	—	64–80	—	64–76
Width	—	33–57	—	32	—	39–48	—	36–40	—	40–52
Type of denticulation	No serration		No serration		Medium serration		Strong serration		Fine serration	
Externo-labial papillae	Short, conical		Short, broad, conical		Long		Long, narrow, pointed		Long, narrow, pointed with short tips	

* Not possible to cut heads in borrowed specimens.

** Could not roll borrowed specimens—old and fragile.



Figures 10–18. Drawing tube illustrations of *Triodontophorus hartmannae* sp. n. 10. Buccal capsule, dorsal view of the head. Scale bar = 100 μm. 11. Buccal capsule, lateral view of the head. Scale bar = 100 μm. 12. *En face* view of head. Scale bar = 50 μm. 13. Anterior end dorsal view. Scale bar = 500 μm. 14. Male tail, lateral view. Scale bar = 500 μm. 15. Male tail, dorsal view. Scale bar = 500 μm. 16. Female tail, lateral view. Scale bar = 500 μm. 17. Genital cone of male with gubernaculum, lateral view. Scale bar = 200 μm. 18. Fused spicule tips of male. Scale bar = 50 μm.

0.7–1.2 (1.0 ± 0.1) mm long and 150–219 (197 ± 22.5) wide. Distance of excretory pore from base of buccal capsule 626–731 (660 ± 37.9) and from anterior end 812–940 (850 ± 42.8). Distance anterior end from nerve ring 534–626 (578 ± 25.5) and from deirids 777–870 (822 ± 36.4). Dorsal ray 441–580 (512 ± 41.6) long. Genital cone 224–336 (289 ± 33.9) long and

299–598 (308.3 ± 117.6) wide. Spicule length 0.9–1.1 (1.1 ± 0.07) mm and gubernaculum length 224–280 (243 ± 15.2).

Dorsal lobe of the male bursa, is short and demarcated from the lateral lobes. Dorsal ray divided along its length into 2 branches. Externodorsal ray is thicker than the lateral rays and posterolateral and mediolateral rays arise together while the externolateral arises separately. Ventral rays arise together and prebursal papillae are evident. Gubernaculum is slender, pistol-shaped and the genital cone slightly rounded with finger-like genital appendages. Spicule tips are hooked and splayed, and distally tapered to a rounded point.

FEMALES (9 specimens): Length 12.5–16.8 (14.6 ± 1.6) mm. Width 510–777 (625 ± 80.9). Length of buccal capsule 165–204 (186 ± 14.4). Width of buccal capsule 135–177 (160 ± 15.8). Number of elements of ELC 67–72. Esophagus 1.0–1.2 (1.1 ± 0.069) mm long and 180–240 (207 ± 16.9) wide. Distance of excretory pore from base of buccal capsule 336–731 (546 ± 138) and from anterior end 568–940 (749 ± 13.8) mm. Distance of nerve ring from anterior end 487–661 (563 ± 53.2) and of cervical papillae 556–893 (710 ± 135). Distance from vulva to tip of tail 661–916 (803 ± 93) and anus to tip of tail 157–252 (212 ± 30.8). Eggs 75–93 (81 ± 6.2) long and 39–48 (42 ± 3.8) wide.

TYPE SPECIMENS: One female holotype and 1 male allotype; 2 female and 3 male paratypes (T-2167), Onderstepoort Helminthological Collection, Onderstepoort Veterinary Institute, Onderstepoort, South Africa.

Two male and 2 female paratypes (USNPC No. 84405), U.S. National Parasite Collection, USDA, Beltsville, Maryland, U.S.A.

TYPE HOST AND TYPE LOCALITY: *Equus zebra hartmannae* Matschie, 1898. Farm "Kelpie," Namibia (22°43′S, 16°43′E).

HABITAT IN HOST: Ventral colon.

ETYMOLOGY: This species is named after the host, Hartmann's mountain zebra.

For the purpose of comparison, measurements of *Triodontophorus brevicauda, Triodontophorus nipponicus*, and *Triodontophorus minor* are included with those for the 2 new species in Table 1.

Discussion

Nematodes that agreed with the generic description of *Triodontophorus* according to Lichtenfels (1975) but could not be assigned to a known species are therefore designated *Triodontophorus burchelli* sp. n. and *Triodontophorus hartmannae* sp. n.

According to Lichtenfels (1975), the species of *Triodontophorus* are *T. brevicauda* Boulenger, 1916; *T. brochotribulatus* Martinez Gomez, 1966; *T. minor* (Looss, 1900); *T. nipponicus* Yamaguti, 1943; *T. popovi* Ershov, 1931; *T. serratus* (Looss, 1900); and *T. tenuicollis* Boulenger, 1916. Other authors, Dvojnos and Kharchenko (1985), considered *T. brochotribulatus* and *T. popovi* synonyms as well as *T. nipponicus* and *T. tenuicollis.*

Triodontophorus burchelli sp. n. bears the closest resemblance to *T. brevicauda*. The new species can be distinguished from *T. brevicauda* in the male by a greater total body length, greater distances of the vulva to the tip of tail (592–812 μm as compared to 256–360 μm) and anus to tip of tail (with 157–202 μm as compared to 96–152 μm). *Triodontophorus burchelli* also differs with an absence of serration on upper edges of teeth and a more slender gubernaculum.

All the remaining species have some degree of serration of the denticulation that differs from the smooth teeth of *T. burchelli* sp. n. This characteristic is salient for each species even while the degree of teeth serration varies within a species (Dvojnos and Kharchenko, 1985). The description of *T. minor* by Skrjabin and Erschov, 1933, *in* Popova, 1964 (English translation), is similar in many characteristics to this new species. These are the absence of serration of teeth, the long median lobe of the dorsal ray, and the spicules length as well as the female vulva and anus to tip of tail distances. The most apparent difference is the number of elements of the external leaf-crowns which they report as 50.

Triodontophorus burchelli sp. n. is characterized by a greater body length, the absence of serration on the upper edges of the teeth, greater vulva to tip of tail (519–812 μm) length and shape of gubernaculum when compared with *T. brevicauda.*

Triodontophorus hartmannae sp. n. differs from *T. nipponicus* Yamaguti, 1943, both in the longer distance of the vulva to the tip of the tail (661–916 μm as compared to 616–776 μm) and less pointed serration of the teeth. *Triodontophorus hartmannae* sp. n. has shorter spicules (0.9–1.1 mm) than *T. minor* according to Theiler (1923) and Diaz-Ungria (1965), who reported a

spicule length of 1.7 mm. Additionally, only 1 type specimen of a male *T. minor* from a horse was available from the U.S. National Parasite Collection, and its spicules measured 1.2 mm. Spicules of males of this species from the collection of the Institute of Zoology, Ukrainian National Academy, were measured and were 1.2–1.4 mm in length. According to Theiler (1923), the ELC elements for *T. minor* number 50, whereas Diaz-Ungria (1965) reported 44–50, far fewer than the 61–73 of the new species *T. hartmannae* sp. n.

Triodontophorus hartmannae sp. n. is most closely related to *T. nipponicus* Yamaguti, 1943, and *T. minor* (Looss, 1900) in all features except denticulation. The medium serration of denticulation of the new species ranges between the fine serration of *T. minor* and the coarse serration of *T. nipponicus*.

Triodontophorus hartmannae sp. n. is characterized by medium serration of the teeth.

Acknowledgments

We thank Dr. J. R. Lichtenfels for comments regarding specimens of the 2 new species described in this paper and Prof. J. D. F. Boomker for comments on the manuscript. Financial support for these studies originated in South Africa from the University of Pretoria, Foundation for Research Development, Hoechst, and the Fritz Visser Agricultural Bursary and in the Ukraine from the International Science Foundation. This study forms part of the Wildlife Research Programme at the Faculty of Veterinary Science, University of Pretoria.

Literature Cited

Diaz-Ungria, C. 1965. Nuevos estudios sobre nematodes de los equidos en Venezuela. Boletín del Instituto de Investigaciones Veterinarias Caracas 13:68–95.

Dvojnos, G. M., and V. A. Kharchenko. 1985. A contribution to the fauna and systematics of the helminth genus *Triodontophorus* (Nematoda, Strongylidae). Vestnik Zoologi 2:10–16. (In Russian.)

Krecek, R. C. 1989. *Habronema malani* sp. n. and *Habronema tomasi* sp. n. (Nematoda: Habronematidae) from the Burchell's and Hartmann's mountain zebras in Southern Africa. Proceedings of the Helminthological Society of Washington 56:183–191.

Krecek, R. C., R. K. Reinecke, N. P. J. Kriek, I. G. Horak, and F. S. Malan. 1994. Helminth parasites of Cape mountain zebras from Cape Province, South Africa. Journal of Wildlife Diseases 30:277–280.

Levine, N. D. 1980. Nematode Parasites of Animals and Man. Burgess Publishing, Minneapolis, Minnesota. 477 pp.

Lichtenfels, J. R. 1975. Helminths of domestic equids. Illustrated keys to genera and species with emphasis on North American forms. Proceedings of the Helminthological Society of Washington (Special Issue) 42:1–92.

Malan, F. S., R. K. Reinecke, and R. C. Scialdo. 1981a. Recovery of helminths postmortem from equines. I. Parasites in arteries, subperitoneum, liver and lungs. Onderstepoort Journal of Veterinary Research 48:141–143.

———, ———, **and** ———. 1981b. Recovery of helminths postmortem from equines. II. Helminths and larvae of *Gasterophilus* in the gastro-intestinal tract and oestrids from the sinuses. Onderstepoort Journal of Veterinary Research 48:145–147.

Popova, T. I. 1955. Strongyloids of animals and man. Osnovy Nematodologii, Vol. V. Akad. Nauk SSSR, Moscow. 241 pp. (In Russian; English translation, 1964, Israel Program for Scientific Translations, Jerusalem. Also available from the National Technical Information Service, U.S. Department of Commerce, Springfield, Virginia, U.S.A.).

Scialdo, R. C., R. K. Reinecke, and V. de Vos. 1982. Seasonal incidence of helminths in the Burchell's zebra. Onderstepoort Journal of Veterinary Research 49:127–130.

Scialdo-Krecek, R. C. 1983a. Studies on the parasites of zebras. I. Nematodes of the Burchell's zebra in the Kruger National Park. Onderstepoort Journal of Veterinary Research 50:111–114.

———. 1983b. Studies on the parasites of zebra. II. *Cylicostephanus longiconus* n. sp. (Nematoda: Strongylidae) from the mountain zebra, *Equus zebra hartmannae* (Matschie, 1898). Onderstepoort Journal of Veterinary Research 50:169–172.

———, **R. K. Reinecke, and H. C. Biggs.** 1983. Studies on the parasites of zebras. III. Nematodes of the mountain zebra from the farm "Kelpie" and the Namib-Naukluft Park, South West Africa/Namibia. Onderstepoort Journal of Veterinary Research 50:283–290.

———, **and F. S. Malan.** 1984. Studies on the parasites of zebras. IV. *Cylicodontophorus reineckei* n. sp. (Nematoda: Strongylidae) from the Burchell's zebra, *Equus burchelli antiquorum* H. Smith, 1841 and the mountain zebra, *Equus zebra hartmannae* Matschie, 1898. Onderstepoort Journal of Veterinary Research 51:257–262.

Theiler, G. 1923. The strongylids and other nematodes parasitic in the intestinal tract of South African equines. Reports of the Director of Veterinary Education and Research, Union of South Africa 9–10:601–773.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 120–127

Key Characters for the Microscopical Identification of *Cylicocyclus nassatus* and *Cylicocyclus ashworthi* (Nematoda: Cyathostominae) of the Horse, *Equus caballus*

J. R. LICHTENFELS,[1] V. A. KHARCHENKO,[2] C. SOMMER,[3] AND M. ITO[4]

[1] Biosystematics and National Parasite Collection Unit, Agricultural Research Service, U.S. Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, Maryland (e-mail: rlichten@ggpl.arsusda.gov),
[2] Department of Parasitology, Institute of Zoology, Kiev, Ukraine,
[3] Department of Veterinary Microbiology, Royal Veterinary and Agricultural University, Frederiksberg C, Denmark, and
[4] Central Institute for Experimental Animals, Nogawa 1430, Miyamae, Kawasaki 213, Japan

ABSTRACT: Current efforts to develop improved control methods for nematode parasites of horses have been hampered by difficulties in identifying some nematodes of the genus *Cylicocyclus*. Structural characteristics of several species of *Cylicocyclus* parasitic in domesticated horses, *Equus caballus*, are described that facilitate the identification. Key microscopical characteristics are described for *C. nassatus* and *C. ashworthi* including characteristics useful for separating them from the similar species *C. triramosus*, *C. leptostomus*, and *C. radiatus*. *Cylicocyclus nassatus* is characterized by a cuticular shelf on the inner surface of the buccal capsule, a dorsal gutter that is as long as 50% of the buccal capsule depth, 20 elements in the external leaf crown (ELC) that each have a sharp tip that broadens quickly to form a parallel-sided leaf, and lateral papillae that produce a tall, narrow cuticular extension of the mouth collar. Both *C. nassatus* and *C. ashworthi* have a short, rounded dorsal bursal lobe in which the proximal branch (of 3 on each side) overlaps 75–80% of the middle branch and a female tail that is slightly longer than the vulva to anus distance. *Cylicocyclus ashworthi* can be distinguished from *C. nassatus* by the absence of a shelf on the inner surface of the buccal capsule, by its much shorter dorsal gutter that is wider than long, and by its 25–29 ELC elements that taper gradually throughout their length and lateral papillae that produce a short, broad extension in the cuticle of the mouth collar. Three other species of the genus, *C. leptostomus*, *C. radiatus*, and *C. triramosus*, all have males with elongate dorsal bursal lobes in which the proximal branch overlaps less than 50% of the middle branch and females with a tail that is much shorter (*C. triramosus*) or slightly shorter (*C. leptostomus* and *C. radiatus*) than the vulva to anus distance. *Cylicocyclus leptostomus* can be distinguished by its small buccal capsule, *C. radiatus* by its large buccal capsule without a dorsal gutter, and *C. triramosus* by its extremely short dorsal gutter and ventral and dorsal notches in the mouth collar. Our study of paratypes of *C. ashworthi* and *C. matumurai* resulted in synonomizing the latter with the former.

Strongyloid nematodes of the subfamily Cyathostominae cause significant morbidity in domesticated horses. More than 40 species occur, sometimes in large numbers, in the large intestine, and resistance to antiparasitic drugs is common among these nematodes. Considerable research is underway around the world to develop alternate or improved control strategies. This research requires accurate identification of the nematode species. Modern identification manuals and classifications of the Cyathostominae exist (Lichtenfels, 1975, 1980; Hartwich, 1986), but problems in identifying several species of the genus *Cylicocyclus* Ihle, 1922, persist. *Cylicocyclus nassatus* (Looss, 1900) Chaves, 1930, is one of the most common nematodes in the ventral colon of horses. Over the years several similar species have been described: *C. triramosus* (Yorke and Macfie, 1920) Chaves, 1930; *C. ashworthi* (LeRoux, 1924) McIntosh, 1933; and *C. matumurai* (Yamaguti, 1942) Lichtenfels, 1975, have been recognized in the recent literature. In addition, a subspecies, *C. nassatus parvum* (Yorke and Macfie, 1918), has been listed by most recent authors (Lichtenfels, 1975; Hartwich, 1986) as a synonym of *C. nassatus*. The present report of a study of specimens (including types of most species; Table 1) concludes that, of the preceding species, only *C. nassatus* and *C. ashworthi* occur commonly in domesticated horses and provides information necessary for their identification based on light microscopy of cephalic characteristics. Information is also presented that facilitates the identification of *C. radiatus* (Looss, 1900) Chaves, 1930, and *C. leptostomus* (Kotlan, 1920) Chaves, 1930, and the distinction of these species from *C. nassatus* and *C. ashworthi*. A redescription of *C. triramosus*,

Table 1. Number and sex of type and voucher specimens studied.

Species	USNPC No.*	Number and sex studied	Type	Collector
Cylicocyclus ashworthi	149†	5 males, 5 females	Paratypes	P. L. LeRoux
	31544‡	1 male	—	W. L. Threlkeld
	33344‡	1 male, 1 female	—	Van Volkenberg
	33345	1 male, 1 female	—	Van Volkenberg
	33346	1 male	—	Van Volkenberg
	50860‡	3 females	—	M. Tubangui
	69887§	3 males, 6 females	—	B. J. Torbert
	70387§	3 males, 1 female	—	C. Sommer
	79151§	3 males, 4 females	—	S. L. Eduardo
	82941§	10 males, 10 females	—	O. Slocombe
	83890§	4 males	—	G. M. Dvojnos
	83405§	1 male	—	M. Ito
	85068§	1 male	—	J. Monrad
	85076§	1 female	—	J. Monrad
	85080	1 male	—	J. Monrad
	85092	12 males, 3 females	—	J. Monrad
	85101	1 male	—	J. Monrad
	85120	1 female	—	J. Monrad
	85191	2 males, 1 female	—	J. Monrad
	85226	1 female	—	J. Monrad
	85280	1 female	—	J. Monrad
	86419	1 male, 1 female	—	J. R. Georgi
	86420	1 male, 1 female		J. R. Georgi
	86421	1 male, 1 female		J. R. Georgi
	86422	3 males, 4 females		J. R. Georgi
Cylicocyclus leptostomus	58489	4 males, 5 females	—	A. O. Foster
	85137	1 female	—	J. Monrad
	85178	1 female	—	J. Monrad
	85190	4 females	—	J. Monrad
	85281	2 males	—	J. R. Georgi
	86423	1 male, 1 female	—	J. R. Georgi
	86424	1 male, 2 females	—	J. R. Georgi
Cylicocyclus matumurai	22565‖	1 male, 2 females	Paratypes	S. Yamaguti
Cylicocyclus nassatus	9602	14 males, 41 females	Paratypes	A. Looss
	31544‡	3 males	—	W. L. Threlkeld
	32422‡	4 males, 15 females	—	H. C. Clark
	33342	1 male	—	Van Volkenberg
	33343‡	3 females	—	Van Volkenberg
	33344‡	3 males, 1 female	—	Van Volkenberg
	50860‡	4 males, 3 females	—	M. Tubangui
	58396	5 males, 5 females	—	A. O. Foster
	83405	4 males, 2 females	—	M. Ito
	83405	6 males, 3 females	—	M. Ito
	85179	3 males, 1 female	—	J. Monrad
	85189	9 males, 15 females	—	J. Monrad
	85225	1 female	—	J. Monrad
	86425	2 males, 2 females	—	J. R. Georgi
	86426	5 males, 5 females	—	J. R. Georgi
	86427	2 males, 2 females	—	J. R. Georgi
	86428	3 males, 3 females	—	J. R. Georgi
	86429	3 males, 3 females	—	J. R. Georgi
	86430	23 males, 16 females	—	J. R. Georgi
Cylicocyclus triramosus	78995	2 males, 2 females	—	R. C. Krecek

* U.S. National Parasite Collection Number, Beltsville, Maryland 20705.
† British Museum Natural History Collection Number, London.
‡ Redetermined: originally identified as *C. nassatus parvum.*
§ Redetermined: originally identified as *C. triramosus.*
‖ Meguro Parasitology Museum Collection Number, Tokyo.

believed to be a species encountered only in South African zebras, will be provided separately (V.A. Kharchenko 1996, pers. comm.), but key identifying features are described herein. The status of *C. nassatus parvum* remains uncertain (species indeterminata) with type specimens unavailable.

Materials and Methods

Specimens studied are listed in Table 1. Scientific names follow those used by Lichtenfels (1975).

Specimens were studied in temporary wet mounts, cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) with the aid of interference contrast light microscopy.

Measurements listed in Table 2 are from previous reports and in micrometers unless indicated otherwise.

Results

The identification of species of *Cylicocyclus*, like other Cyathostominae, can be made solely on the basis of cephalic characters (Lichtenfels, 1975). The results presented here are intended to provide a description of characters useful for identifying *C. nassatus* (Figs. 1–8) and *C. ashworthi* (Figs. 9–16). In addition, characters useful for distinguishing these species from *C. leptostomus*, *C. radiatus*, and *C. triramosus* are described (Table 2). Characteristics of the dorsal ray (Figs. 8, 16) of the male copulatory bursa and the ratio of female tail length to vulva to anus distance and vagina length (Figs. 7, 15) provide additional characters useful for separating these species.

The most distinctive characteristic of *C. nassatus* (Figs. 1–8) is the cuticular shelf-like projection that rings the internal walls of the buccal capsule at about midway in its depth (Figs. 1, 2). No other species of the genus *Cylicocyclus* has such a feature, and it can be seen with × 400 magnification even in most poor specimens. Other supplemental identifying features of the cephalic region include a dorsal gutter that is one-half as long as the depth of the buccal capsule (Figs. 2, 6); lateral cephalic papillae that protrude through the mouth collar sufficiently to produce a pointed, steeply sided projection of the mouth collar (Fig. 5); submedian cephalic papillae with elongate banana-shaped tips (Fig. 3); elements of the external leaf crown (ELC) that have tips that broaden quickly in the anterior one-fourth of their length and then taper more gradually toward their base (Fig. 3); and elements of the internal leaf crown (ILC) (Figs. 1, 2) that are shorter than the thickness of the ring at the base of the buccal capsule.

Cylicocyclus ashworthi (Figs. 9–16) is similar to *C. nassatus* grossly, but it lacks a cuticular buccal capsule shelf and can be separated from its co-inhabitant of the ventral colon by differences in numerous cephalic characteristics. Characteristic features of *C. ashworthi*, in addition to lacking the cuticular buccal capsule shelf, include a dorsal gutter that is less than one-third as long as the depth of the buccal capsule (Fig. 14); lateral cephalic papillae that protrude through the mouth collar to form a broad, rounded projection of the mouth collar (Fig. 13); submedian cephalic papillae with tapering, pointed tips (Fig. 14); ELC elements with uniformly, gradually tapering tips for more than one-half of their length (Fig. 11); and ILC elements that are longer (Figs. 9, 10) than the thickness of the ring at the base of the buccal capsule.

A study of paratypes of *C. ashworthi* found no differences between them and other specimens identified and described herein as *C. ashworthi*. Many lots of specimens previously identified as *C. triramosus* from horses, *E. caballus*, have been redetermined as *C. ashworthi* (Table 1).

En face counts of ELC elements found specimens collected in Japan fell into 2 groups: 1 with 20 and another with 26–28 ELC elements. Specimens with 20 ELC elements also had the characteristics already described for *C. nassatus*. Specimens with 26–28 ELC elements had the characteristics described herein for *C. ashworthi*.

A single male and 2 female paratypes of *Cylicocyclus matumauri* were found to have all the characteristics of *C. ashworthi*.

The dorsal bursal rays of both *C. nassatus* and *C. ashworthi* have 3 branches on each side (Figs. 8, 16). The middle and distal branches of the dorsal ray of *C. ashworthi* frequently have various, variable accessory branches (Fig. 16). However, about 30–50% of paratype males of *C. nassatus* also have such accessory branches, and this is not a reliable character for identifying the species. Accessory branches were also present on the branches of dorsal rays of *C. triramosus* and *C. leptostomus*.

Discussion

Although the most distinctive characteristic of the species *C. nassatus* is the cuticular shelf in the lining of the buccal capsule, this structure

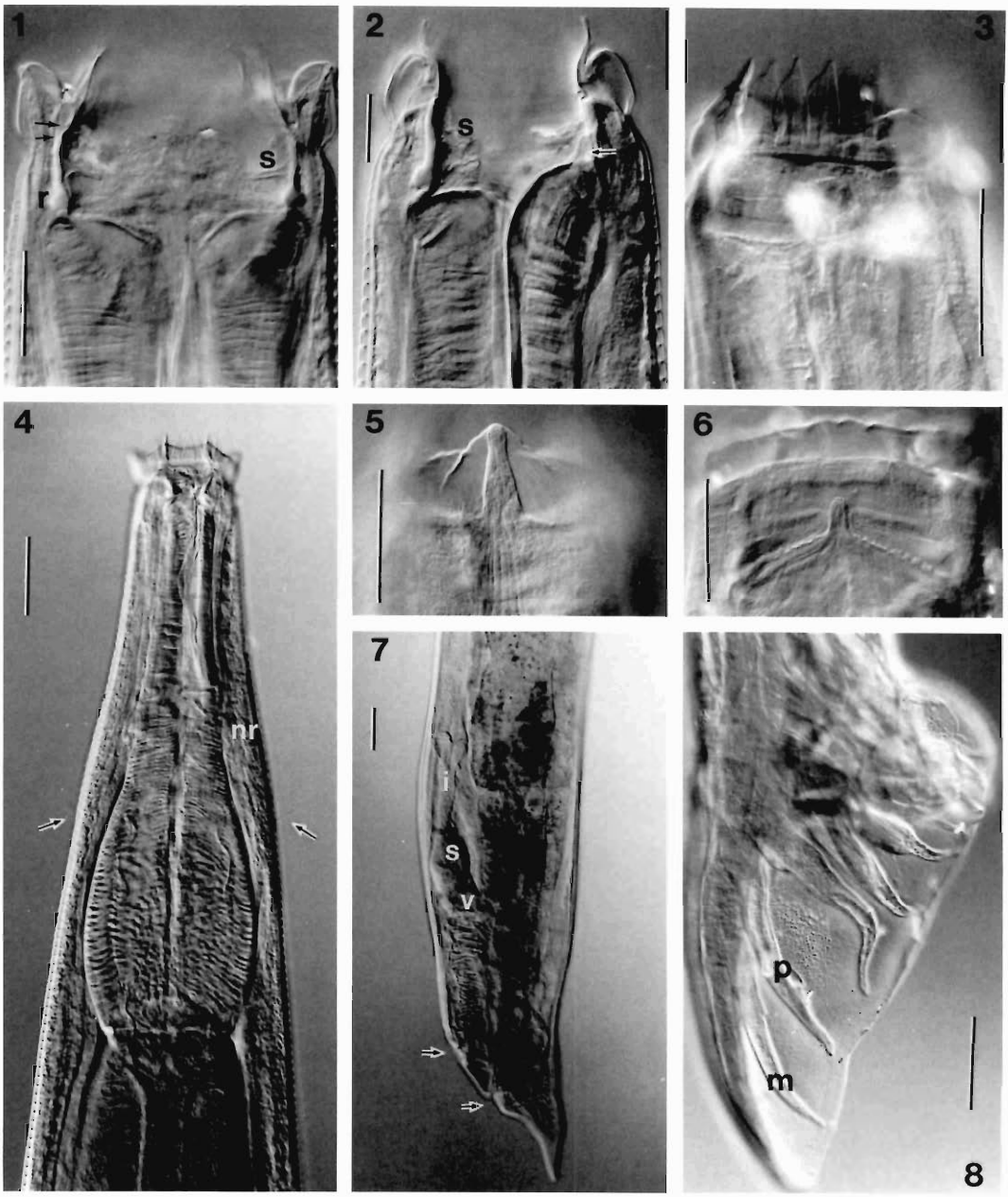
Table 2. Morphometrics of key characteristics of *Cylicocyclus* spp.*

	C. nassatus	*C. ashworthi*	*C. triramosus*	*C. leptostomus*	*C. radiatus*
Host:	*E. caballus*	*E. caballus*	*E. burchelli antiquorum*	*E. caballus*	*E. caballus*
Distribution:	Cosmopolitan	Cosmopolitan	South Africa	Cosmopolitan	Cosmopolitan
Prevalence:	Common	Common	Common	Common	Rare
Site:	Ventral colon	Ventral colon	Ventral colon	Cecum, colon	Colon
Body length ♂/♀ (in mm)	8–10[1]	7.4–8.6[2]	12.3[3]	6[4]	11[1]
	9–14[1]	8.5–9.5†	12.3[3]	7–8[4]	13–14[1]
Buccal capsule width/depth	10–135†	65–100†	90–110[3]	36[4]	112[4]
	35–47†	19–23	38[3]	18[4]	52[4]
Dorsal gutter shape/% of depth of buccal capsule	Taller than wide >50%	Wider than tall 35%	Taller than wide 16–22%‡	Taller than wide 20%	Absent[1]
Number ELC elements	19-20[B]	25–29[B]	30[3]	24–26[B]	26[1]
Distal tip of proximal branch of dorsal ray	Overlaps 80% of middle branch	Overlaps 75% of middle branch	Overlaps <50% of middle branch	Overlaps none of middle branch	Overlaps 20% of middle branch
Dorsal bursal ray shape	Short, rounded	Short, rounded	Slightly elongated	Elongate	Elongate
Vagina length	514–806†	311–349†	712–851‡	300–400[4]	600–750[4]
Vulva to anus	139–188†	79–131†	290–360[4]	80–90[4]	250–280[4]
Female tail length	180–243†	112–146†	160–200[4]	64–70[4]	200–250[4]

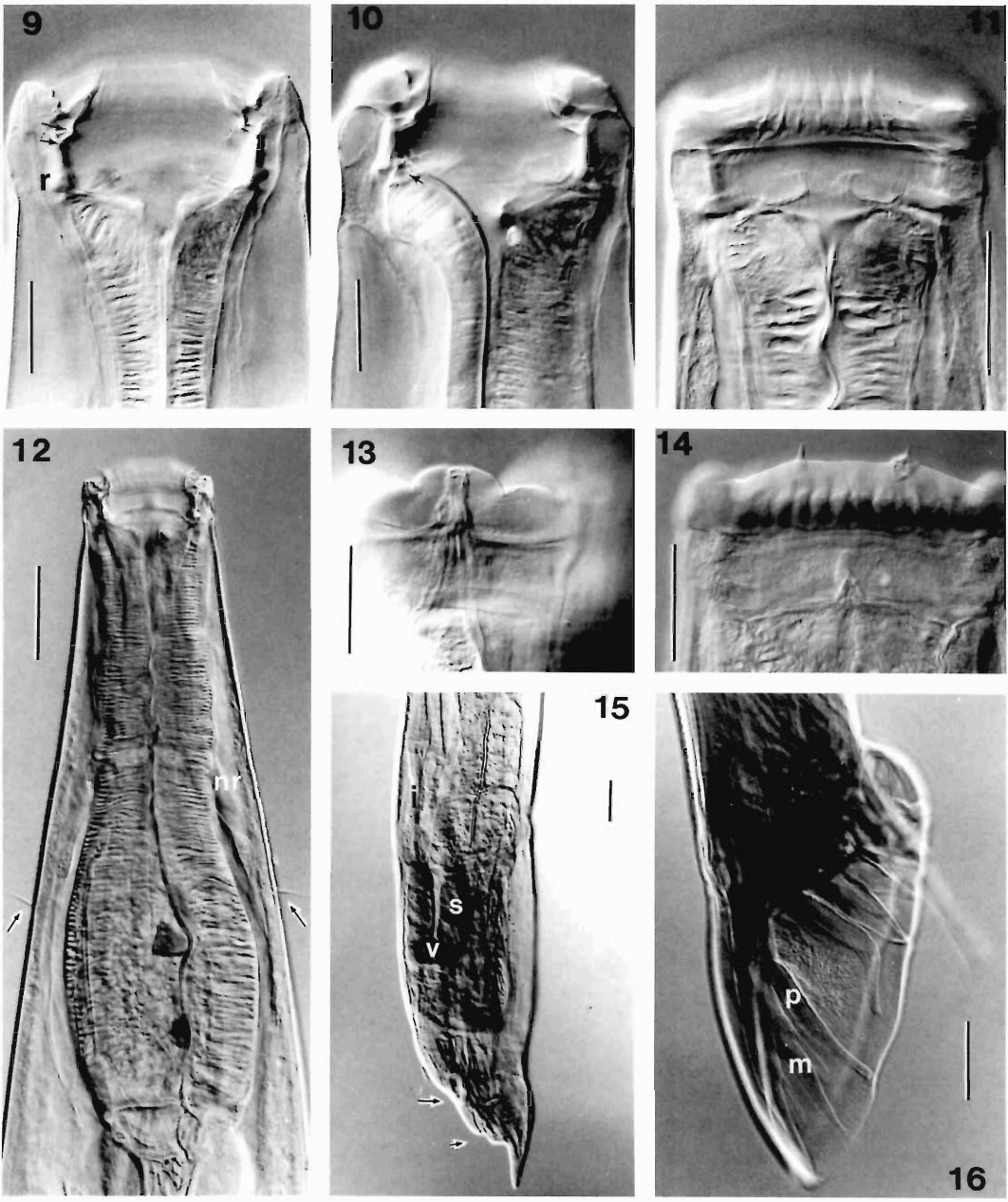
* [B]Braide and Georgi; [1]Looss, 1900; [2]LeRoux, 1924; [3]Yorke and Macfie, 1920; [4]Theiler, 1923.

† Measurements of 5 female paratypes, original.

‡ Measurements of 2 female specimens.



Figures 1–8. *Cylicocyclus nassatus*, **photomicrographs. Scale bars, Figs. 1–3, 5, 6, = 50 μm; Figs. 4, 7, 8 = 100 μm. 1. Buccal capsule, dorsoventral view, showing cuticular shelf on inner lining (s), tall, lateral papillae, ring-like thickening at base of buccal capsule (r) and short ILC element (between arrows). 2. Buccal capsule, lateral view, showing cuticular shelf (s) and dorsal gutter (arrow). 3. Mouth collar, dorsoventral view, showing several elements of ELC and tip of submedian papilla. 4. Esophageal region, dorsoventral view, showing position of nerve ring (nr), cervical papillae (arrows) and shape of esophagus. 5. Lateral papilla protruding through mouth collar. 6. Dorsal gutter. 7. Female tail showing anus and vulva (arrows) and ovejectors, including ventibule (v), sphincters (s) and infundibulae (i). 8. Male copulatory bursa showing position and shape of bursal rays, especially the proximal (p) and medial (m) branches of the dorsal ray.**



Figures 9–16. ***Cylicocyclus ashworthi*, photomicrographs. Scale bars, Figs. 9–11, 13, 14, = 50 μm; Figs. 12, 15, 16 = 100 μm. 9 Buccal capsule, dorsoventral view, showing tall, lateral papillae, ring-like thickening at base of buccal capsule (r) and short ILC element (between arrows). 10. Buccal capsule, lateral view, showing dorsal gutter (arrow). 11. Mouth collar, dorsoventral view, showing several elements of ELC. 12. Esophageal region, dorsoventral view, showing position of nerve ring (nr), cervical papillae (arrows) and shape of esophagus. 13. Lateral papilla protruding through mouth collar. 14. Dorsal gutter and submedian papilla. 15. Female tail showing anus and vulva (arrows) and ovejectors, including ventibule (v), sphincters (s) and infundibulae (i). 16. Male copulatory bursa showing position and shape of bursal rays, especially the proximal (p) and medial (m) branches of the dorsal ray.**

was not emphasized by Looss (1900, 1902) in his description of the species. Looss (1902) did describe the cuticular shelf but believed it to be a variable structure. We suspected that perhaps the reason Looss (1902) believed it to be variable might be the possible inclusion of specimens later described as *C. nassatus parvum* (which lack the cuticular buccal shelf) within his series of specimens of *C. nassatus*. Looss (1902) mentioned that his type series included smaller perfectly mature specimens, "not more than 8 millimeters in the male and 9 millimeters in the female," measurements typical of the smaller *C. nassatus parvum* later described by Yorke and Macfie (1918). However, we examined all of the 55 paratypes of *C. nassatus* available to us without finding a single specimen that fit the description of *C. nassatus parvum*. Every paratype of *C. nassatus* fit the characterization of this species presented herein. In the description of *C. nassatus parvum*, the authors did not mention or figure a cuticular shelf in the lining of the buccal capsule. Unfortunately, the types of *C. nassatus parvum* were not found either at the British Museum or at the International Institute of Parasitology, St. Albans, England. Among voucher specimens identified as *C. nassatus parvum* (Table 1), we found specimens that fit the characterization of either *C. nassatus* or *C. ashworthi* presented in this report. In the absence of type specimens of *C. nassatus parvum*, we must consider this subspecies to be unidentifiable, or a species indeterminata.

We now propose to recognize the name *C. ashworthi* for the common species from domesticated horses, previously reported either as *C. ashworthi* or as *C. triramosus* by numerous authors worldwide. Braide and Georgi (1974), the only North American workers to correctly identify this species, reported 25–29 ELC elements in *C. ashworthi*. Lichtenfels (1975) listed *C. ashworthi* as a synonym of *C. nassatus* following several earlier workers and *C. triramosus* as a species reported in Puerto Rico but not known to occur in North America because no specimens were available for study. Hartwich (1986) synonymized *C. ashworthi* with *C. triramosus* after studying syntypes of *C. ashworthi*. Other authors have reported *C. ashworthi* from horses in Brazil (Lanfredi and Honer, 1984) and *C. triramosus* from horses in the United States (Torbert et al., 1986), the Ukraine (Dvojnos and Kharchenko, 1990), and the Philippine Islands (Antiporda and Eduardo, 1990) and from Burchell's zebra (Scialdo-Krecek, 1983). However, one of us (J.R.L.) has studied paratypes of *C. ashworthi* and specimens collected by Scialdo-Krecek (1983) from a South African zebra, *Equus burchelli*, the type host of *C. triramosus*, and have concluded (with V. A. Kharchenko 1996, pers. comm.) that the latter is a distinct species that appears, as suggested by Theiler (1923), to occur only in South African zebras. *Cylicocyclus triramosus* is distinguished by an extremely short dorsal gutter and ventral and dorsal notches in the mouth collar. It will be redescribed separately (Kharchenko et al., 1997). We now can confirm that only the *C. triramosus* from South African zebras should be considered to represent that species. All available lots of "*C. triramosus*" from *E. caballus* examined in this study (Table 1) have been redetermined as *C. ashworthi*.

It appears that the morphological characteristics of *C. ashworthi* and *C. matumurai* are so similar that they should be considered synonyms. Hartwich (1986) did not study *C. matumurai*, but followed Lichtenfels (1975) in recognizing this species. However, Lichtenfels (1975) did not study *C. matumurai* but recognized it as a species of the genus that had not been reported in North America. After studying a single male and 2 female paratypes of *C. matumurai*, we believe it is a synonym of *C. ashworthi*. Yamaguti (1942) presented an excellent en face drawing that shows 26 ELC elements and other characteristics of *C. ashworthi* described herein are also present in *C. matumurai*.

Other species of *Cylicocyclus* of horses that might be confused with *C. ashworthi* or *C. nassatus* include the rare species *C. radiatus* and the common species *C. leptostomus*. Of these species, *C. radiatus* is the only one lacking a dorsal gutter and *C. leptostomus* has a small buccal capsule (Table 2). Males of both *C. radiatus* and *C. leptostomus* have elongate dorsal bursal rays in which there is a considerable distance between the proximal and middle branches of the dorsal ray so that only the tip of the proximal branch overlaps the origin of the middle branch. In contrast, in *C. ashworthi* and *C. nassatus* males, the dorsal bursal ray is short and rounded and most of the length of the proximal branch overlaps the origin of the middle branch (Table 2). Females of *C. leptostomus* and *C. radiatus* can be separated from those of *C. nas-*

satus and *C. ashworthi* because their tails are slightly shorter than their vulva to anus distance (Table 2). In many older females, however, tail structure is distorted and cephalic characters are more useful for species determinations.

Acknowledgments

We thank Patricia A. Pilitt for assistance in preparing the nematodes for study and for assembling the data for publication. We thank Judith T. Holland for literature searching and assistance in preparing the manuscript. Specimens were loaned for study by Lotfi F. Khalil, International Institute of Parasitology, Commonwealth Agricultural Bureaux, St. Albans, England, and by Shunya Kamegai, Meguro Parasitological Museum, Tokyo.

Literature Cited

Antiporda, L. R. D., and S. L. Eduardo. 1990. Prevalence and relative abundance of helminth parasites in Philippine horses. Philippine Journal of Veterinary Medicine 27:21–23.

Braide, E. I., and J. R. Georgi. 1974. Numbers of external leaf crown elements of 18 species of equine cyathostomes. Cornell Veterinarian 64: 233–239.

Dvojnos, G. M., and V. A. Kharchenko. 1990. Morphology and differential diagnostics of parasitic larvae of some Strongylidae (Nematoda) of horses. Angewandte Parasitologie 31:15–28.

Hartwich, G. 1986. Zum *Strongylus tetracanthus*—Problem und zur Systematik der Cyathostominea (Nematoda: Strongyloidea). Mitteilungen Zoologishen Museum in Berlin. 62:61–102.

Lanfredi, R. M., and M. R. Honer. 1984. Uma chave ilustrada para a identificacao dos generos e especies dos pequenos estrongylideos (subfamilia Cyathostominae: Nematoda) em cavalos da baixada fluminense. Pesquisa Veterinaria Brasileira 4: 67–72.

LeRoux, P. L. 1924. Helminths collected from equines in Edinburgh and in London. Journal of Helminthology 2:111–134.

Lichtenfels, J. R. 1975. Helminths of domestic equids. Proceedings of the Helminthological Society of Washington 42(special issue):1–92.

———. 1980. Commonwealth Institute of Helminthology Keys to the Nematode Parasites of Vertebrates. No. 7. Pages 1–41 *in* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. Keys to Genera of the Superfamily Strongyloidea. Commonwealth Agricultural Bureaux, Farnham Royal, U.K.

Looss, A. 1900. Notizen zur Helminthologie Egyptens. III. Die Sclerostomen der Pferde und Esel in Egypten. Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten 1. Abt 27:150–160, 184–192.

———. 1902. The Schlerostomidae of horses and donkeys in Egypt. Records Egypt Government School of Medicine. 25–139.

Scialdo-Krecek, R. C. 1983. Studies on the parasites of zebras 1. Nematodes of the Burchell's zebra in the Kruger National Park. Onderstepoort Journal of Veterinary Research 50:111–114.

Theiler, G. 1923. The strongylids and other nematodes parasitic in the intestinal tract of South African equines. Report on Veterinary Research in the Union of South Africa 9–10:601–773.

Torbert, B. J., T. R. Klei, J. R. Lichtenfels, and M. R. Chapman. 1986. A survey in Louisiana of intestinal helminths of ponies with little exposure to anthelmintics. Journal of Parasitology 72:926–930.

Yamaguti, S. 1942. Studies of the Helminth Fauna of Japan. Part 41. Mammalian Nematodes, III. Kyoto, Japan. 33 pp. (Issued May 30.)

Yorke, W., and J. W. S. Macfie. 1918. Strongylidae in horses. III. *Cylicostomum nassatum*, Looss. var. *parvum*. Annals of Tropical Medicine and Parasitology 11:411–416.

———, and ———. 1920. Strongylidae in horses: XIII. *Cylicostomum triramosum* sp. n. Annals of Tropical Medicine and Parasitology 14:175–179.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 128–136

Morphological Variation of the Corona Radiata in *Oesophagostomum dentatum*, *O. quadrispinulatum*, and *O. radiatum* (Nematoda: Strongyloidea)

BIRGER NEUHAUS,[1,2,4] JOSÉ BRESCIANI,[2] CHARLOTTE M. CHRISTENSEN,[1] AND CHRISTIAN SOMMER[3]

[1] Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark,

[2] Department of Ecology and Molecular Biology, Royal Veterinary and Agricultural University, Zoology Section, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark, and

[3] Danish Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen, Denmark

ABSTRACT: The anterior end of 76 adult and of several juvenile *Oesophagostomum dentatum*, of 75 adult *O. quadrispinulatum*, and of 70 adult *O. radiatum* (Strongylida, Nematoda) was investigated by scanning electron and light microscopy. Both an external and an internal ring of buccal leaves (corona radiata externa and interna) are present in *O. dentatum* and *O. quadrispinulatum*, whereas a single ring of buccal leaves occurs in *O. radiatum*. Remnants of external buccal leaves indicate that the single ring of leaves found in the latter species is homologous to the corona radiata interna of *O. dentatum* and *O. quadrispinulatum*. The number of buccal leaves of the corona radiata varies remarkably in adults of all 3 species. There are 9–12 external leaves in *O. dentatum*, 9–11 external leaves in *O. quadrispinulatum*, and 30–40 internal leaves in *O. radiatum*. Nine leaves are most common in both *O. dentatum* and *O. quadrispinulatum*, but the former species shows a higher frequency of individuals with more than 9 leaves. In *O. radiatum*, buccal leaves usually occur in even numbers and very rarely in odd numbers. Small, regularly arranged protuberances outside the ring of buccal leaves may indicate reduced leaves of the corona radiata externa. Juveniles of *O. dentatum* do not possess buccal leaves, but a thin cuticular velum in the fourth stage and neither a corona nor a velum in the second and first stage.

KEY WORDS: *Oesophagostomum dentatum*, *Oesophagostomum quadrispinulatum*, *Oesophagostomum radiatum*, corona radiata, polymorphism.

Species of *Oesophagostomum* parasitize the alimentary tract of livestock and humans around the world (Murrell, 1986; Skryabin et al., 1992; Blotkamp et al., 1993; Roepstorff and Nansen, 1994). The anatomy of members of this taxon has been described by light microscopy (Goodey, 1924a; Chitwood, 1931; Blotkamp et al., 1993). Few observations by scanning electron microscopy (SEM) are available (Gibbons, 1986; Stewart and Gasbarre, 1989), and only 2 studies report on transmission electron microscopy (TEM) findings (Neuhaus et al., 1997a, b).

At its mouth opening, a member of the genus *Oesophagostomum* typically possesses a corona radiata externa and interna composed of a variable number of buccal leaves that are of taxonomic value (Chabaud and Durette-Desset, 1974; Lichtenfels, 1980; Skryabin et al., 1992; Hartwich, 1994). Polymorphism has been reported for the number of buccal leaves of *O. asperum* Raillet and Henry, 1913, *O. bifurcum* (Creplin, 1849), *O. brevicaudum* Schwartz and Alicata, 1930, *O. columbianum* (Curtice, 1890), *O. dentatum* (Rudolphi, 1803), *O. radiatum* (Rudolphi, 1803), *O. sikae* Cameron and Parnell, 1933, and *O. venulosum* (Rudolphi, 1809) (cf. Rudolphi, 1803; Goodey, 1924b; Schwartz and Alicata, 1930; Blotkamp et al., 1993; Hartwich, 1994). However, no information is available about how commonly a given number of leaves occurs within a population. The object of this study is to provide data about the frequency distribution of the number of external buccal leaves in *O. dentatum* and *O. quadrispinulatum* (Marcone, 1901) and of internal leaves in *O. radiatum*.

[4] Corresponding author and present address: Museum für Naturkunde, Institut für Systematische Zoologie, Invalidenstr. 43, D-10115 Berlin, Germany (e-mail: birger=neuhaus@museum.hu-berlin.de).

Materials and Methods

Adult specimens and fourth-stage juveniles of *O. dentatum* and adult specimens of *O. quadrispinula-*

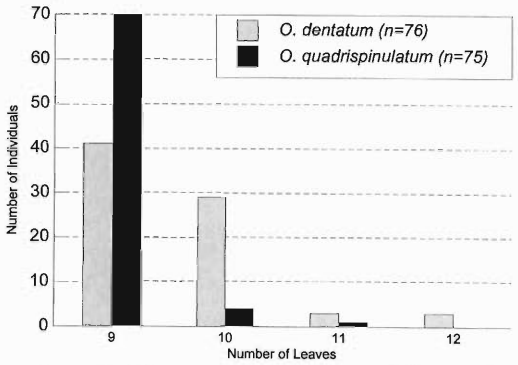


Figure 1. Frequency distribution of the number of leaves in the corona radiata externa of *Oesophagostomum dentatum* (n = 76) and *O. quadrispinulatum* (n = 75).

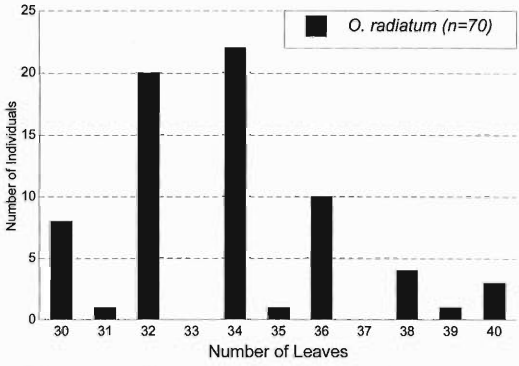


Figure 2. Frequency distribution of the number of leaves in the corona radiata interna of *Oesophagostomum radiatum* (n = 70).

tum were obtained from 2 experimentally infected pigs. Adult *O. radiatum* were collected from an experimentally infected calf. All specimens for SEM were taken at random from the nematodes sampled and were further treated for SEM and light microscopy as described by Neuhaus et al. (1997a). A subsample of about 100 nematodes from each of the infected pigs was studied by light microscopy to ensure that only a single species of *Oesophagostomum* occurred in each pig. The following morphological characters also used by Haupt (1966), Taffs (1967), Kendall et al. (1977), Poelvoorde (1978), and Hartwich (1994) were checked to discriminate *O. dentatum* from *O. quadrispinulatum*: shape of buccal capsule and of pharynx, length of spicules, and distances between tail and anus as well as between anus and vulva. Specimens of *O. radiatum* were identified according to the key by Hartwich (1994).

Eggs of *O. dentatum* were cultivated through to the third juvenile stage on agar plates as described in Neuhaus et al. (1997a). Approximately 10 specimens of each juvenile stage were studied by light microscopy and SEM, respectively.

Results

Adult specimens of *Oesophagostomum dentatum* and *O. quadrispinulatum* possess a characteristic lobed or bilobed head region (Figs. 3–5), whereas *O. radiatum* appears to show a trilobed head region (Figs. 14–16); the anteriormost lobe consists of the mouth collar, which is significantly enlarged in this species. In fixed material, alae of *O. radiatum* are very prominent in comparison with *O. dentatum* and *O. quadrispinulatum* (Figs. 3, 4, 15). The number of buccal leaves of the corona radiata at the anterior mouth opening of adult *Oesophagostomum* is difficult to observe with the light microscope (Figs. 5, 14) but can be readily seen in frontal view of clean specimens using SEM (Figs. 6–8, 13, 18–22). The number of leaves of the corona radiata interna in *O. dentatum* and *O. quadrispinulatum* is extrapolated from the observed number of internal leaves at the base of each external leaf.

Adult *Oesophagostomum dentatum* (n = 76)

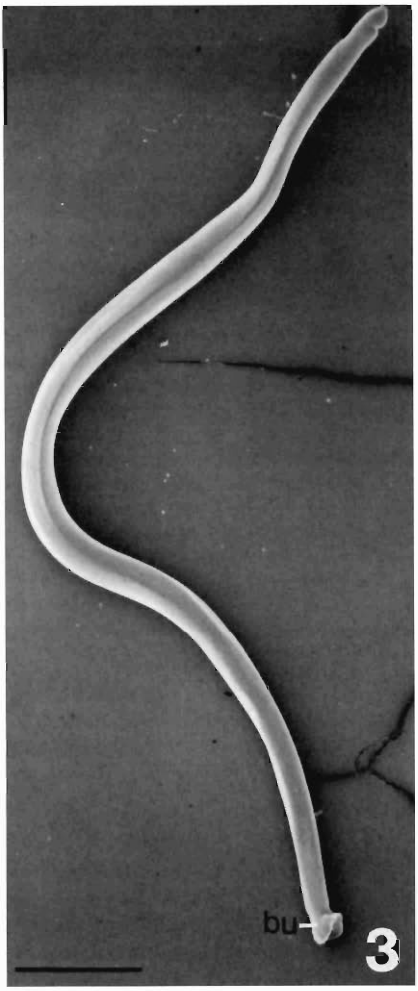
A variable number of 9–12 elements of the corona radiata externa (external buccal leaves) surrounds the mouth opening of adult *O. dentatum*. Numbers of 9 or 10 leaves (Figs. 6, 7) are most common (Fig. 1). The triangular leaves of the corona radiata may close the mouth opening almost completely (Fig. 8). At the base of each external leaf, 2 considerably smaller, triangular, tooth-like internal leaves are found (elements of the corona radiata interna) (Fig. 7; Fig. 12 for *O. quadrispinulatum*). Bacteria often occur in the buccal lumen (Fig. 6).

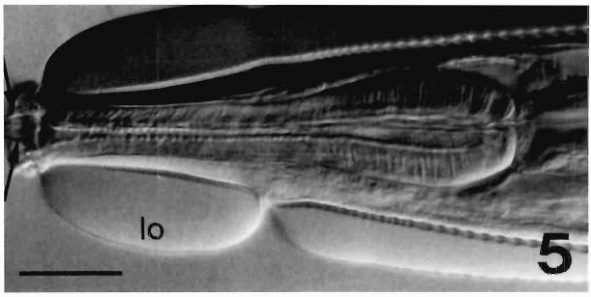
Juvenile *O. dentatum*

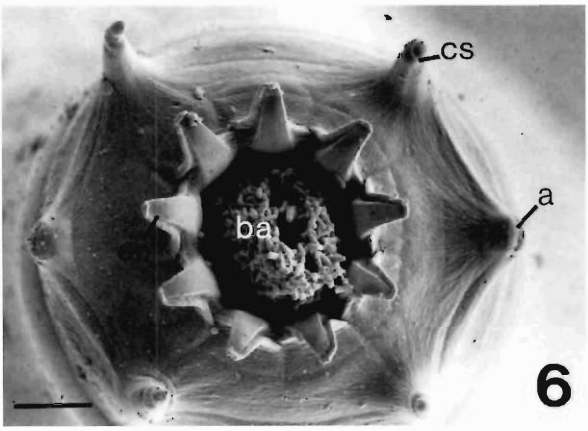
A corona radiata is missing completely in all juvenile stages (Figs. 9–11). A thin cuticular velum covers the mouth opening partly in the fourth stage (Fig. 11) but is missing in the first and second stages (Figs. 9, 10). The anterior end of the free-living third stage was not available for SEM studies, because this stage is ensheathed by the cuticle of the second juvenile stage.

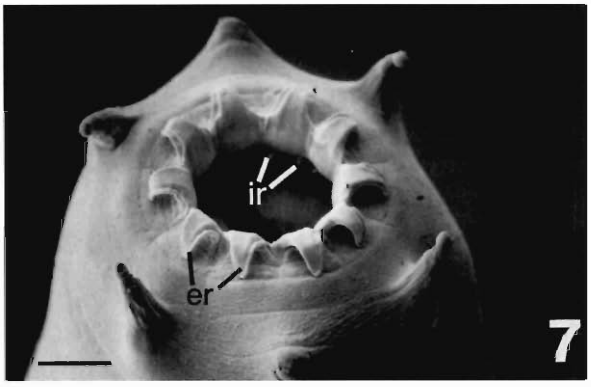
Adult *Oesophagostomum quadrispinulatum* (n = 75)

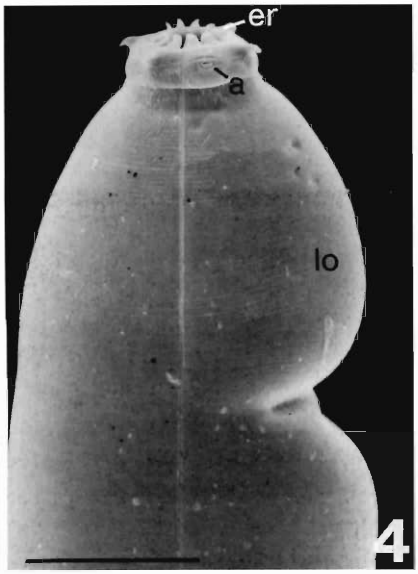
Adult *O. quadrispinulatum* exhibit 9–11 triangular buccal leaves in the corona radiata ex-

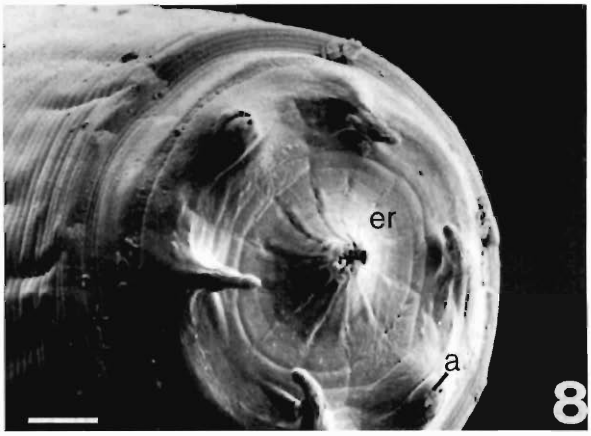












terna. Almost all specimens possess 9 leaves (Fig. 13), whereas few individuals show 10 or 11 leaves (Fig. 1). The corona radiata interna is arranged as in *O. dentatum* (Fig. 12). Shape and morphology of the buccal leaves are similar to *O. dentatum*.

Adult *Oesophagostomum radiatum* (n = 70)

Adult *O. radiatum* possess a single ring of buccal leaves (Figs. 18–22), which agree in shape, morphology, and size with the leaves of the corona radiata interna of *O. dentatum* and *O. quadrispinulatum*. The leaves are usually arranged in pairs (Figs. 17–19, 22) but appear very seldom in odd numbers (Figs. 2, 20, 21). They are not able to close the mouth opening even partly (Figs. 16, 19, 21). Occasionally, individual leaves are smaller than the neighboring leaves (Figs. 19–21). The number of buccal elements varies between 30 and 40, with 32 and 34 leaves being most common (Fig. 2).

Outside the ring of buccal leaves, a ring of small, regularly arranged protuberances is found (Figs. 17–19, 22). These projections are always located between neighboring pairs of buccal leaves.

Discussion

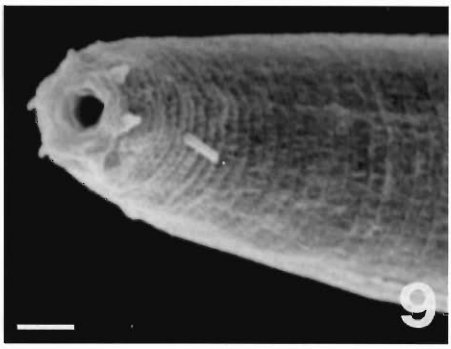
At their anterior end, many species of *Oesophagostomum* possess both a corona radiata externa and interna composed of several to many buccal leaves. At the base of each element of the corona externa, 2 leaves of the corona interna appear (e.g. Chabaud and Durette-Desset, 1974; Lichtenfels, 1980; this paper). Our findings reveal, in agreement with earlier observations (Goodey, 1924a, b; Stewart and Gasbarre, 1989), 2 coronae radiatae for both *O. dentatum* and *O. quadrispinulatum* and a single ring of buccal leaves for *O. radiatum*. Shape and size of the buccal leaves of the latter species has lead other investigators to the conclusions that the buccal leaves of *O. radiatum* represent elements of the corona radiata interna, but a corona externa is missing (e.g. Goodey, 1924b; Travassos and Vogelsang, 1932). The ring of regularly arranged, small protuberances outside the corona radiata of *O. radiatum* confirms the aforementioned assumption; the protuberances are interpreted as remnants of the corona radiata externa.

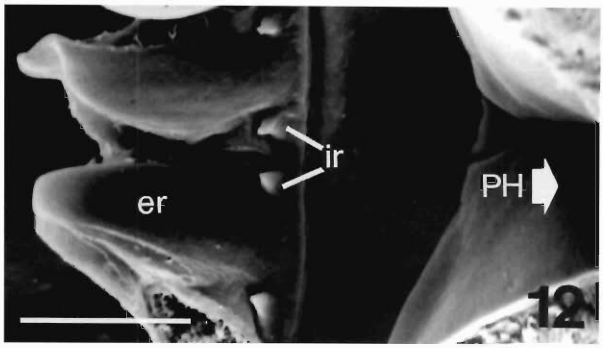
The arrangement and number of the elements of the coronae radiatae differ considerably in the taxon *Oesophagostomum*. The following patterns have been found: (1) 6–8 external leaves, no corona radiata interna (e.g., *O. oldi* Goodey, 1924, *O. mwanzae* Daubney, 1924, *O. eurycephalum* Goodey, 1924, *O. simpsoni* Goodey, 1924 [cf. Goodey, 1924c]); (2) no corona radiata externa, 38–45 leaves of the corona interna (e.g., *O. radiatum* [but compare our results], *O. curvatum* Maplestone, 1931, *O. sikae* Cameron and Parnell, 1933, and *O. traguli* (Chandler, 1931) [cf. Goodey, 1924b; Chabaud and Durette-Dusset, 1974]); (3) 30–40 external leaves, 60–80 internal leaves (e.g., *O. pachycephalum* Molin, 1861, *O. stephanostomum* Stossich, 1904, *O. ventri* Thornton, 1924 [cf. Glen and Brooks, 1985]); and (4) 9–24 external leaves and 18–48 internal leaves in the remaining species of *Oesophagostomum* (cf. Chabaud and Durette-Desset, 1974; Lichtenfels, 1980). From these light microscopical investigations, it remains open whether internal leaves have not been overlooked in the first group of taxa because of their small size. Remnants of external leaves are almost invisible in the second group of *Oesophagostomum* species as has been revealed by our observations of *O. radiatum*. We therefore suppose that the corona radiata externa has been reduced not only in *O. radiatum* but also in *O. curvatum*, *O. sikae*, and *O. traguli*.

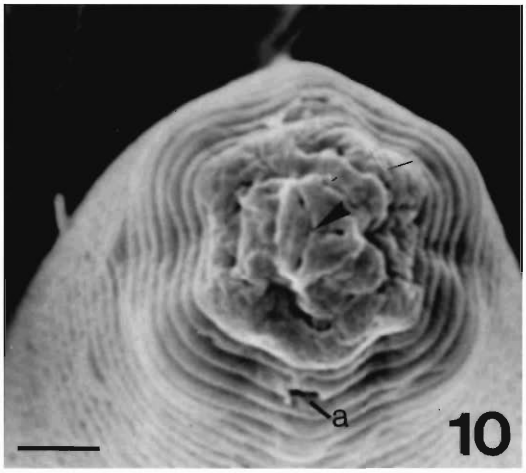
It has been assumed that 6–8 external leaves represent the original condition for *Oesophagostomum* retained only in few species, where-

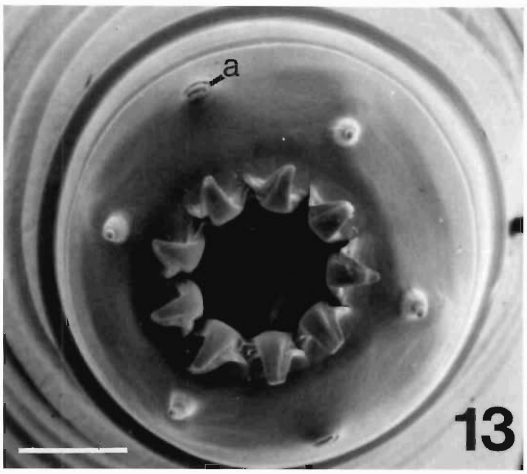
←

Figures 3–8. Adult *Oesophagostomum dentatum*. 3, 4. SEM of male worm in lateral view. 4. Higher magnification of lobed head region with prominent mouth collar. 5. Differential interference contrast microphoto of anterior end in lateral view. Arrows mark leaves of corona radiata externa. 6–8. SEM frontal view of specimen with 9 external leaves and bacteria in buccal cavity (6), with 10 external leaves (7), or with 12 external leaves in closed position (8). Abbreviations: a, amphid; ba, bacteria; bu, bursa; cs, cephalic sensillum; er, leaf of corona radiata externa; ir, leaf of corona radiata interna; lo, lobe of head region. Scale bar in 3 -1 mm, in 4 and 5 -100 μm, and in 6–8-10 μm.









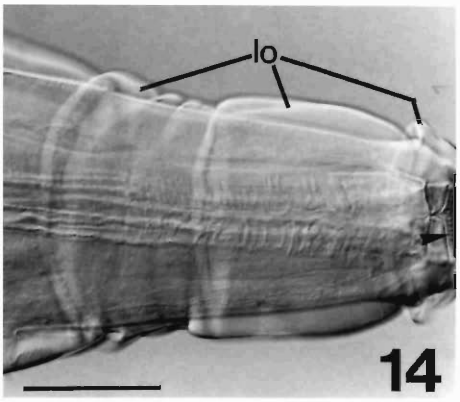


Figure 9–14. ***Oesophagostomum*** **species. 9–11. Juvenile** ***O. dentatum*****. 9. SEM of juvenile stage 2 with open mouth opening. 10. SEM of juvenile stage 3 showing cuticle of previous stage with collapsed mouth opening. 11. SEM of juvenile stage 4 with velum partly covering mouth opening. 12, 13. Adult** ***O. quadrispinulatum*****. 12. SEM view into opened buccal cavity with leaves of corona radiata externa and interna. Arrow points toward pharynx. 13. SEM frontal view. 14. Differential interference contrast photo of anterior end of adult** ***O. radiatum*** **with trilobed head region. Arrowhead marks leaves of corona radiata. Abbreviations: a, amphid; er, leaf of corona radiata externa; ir, leaf of corona radiata interna; lo, lobe of head region; PH, pharynx; ve, velum. Scale bar in 9 and 10-2 μm, in 11 and 12-10 μm, in 13-20 μm, and in 14-100 μm.**

Table 1. Polymorphism in the corona radiata externa and interna of various species of *Oesophagostomum*.

Species	Number of external leaves	Number of internal leaves	Reference
O. asperum	10–14	32–40	Hartwich, 1994*
O. bifurcum	14–16	28–32	Blotkamp et al., 1993
O. brevicaudum	14–16	28–32	Schwartz and Alicata, 1930
O. columbianum	20–26	40–52	Hartwich, 1994*
O. dentatum	10–12	?†	Rudolphi, 1803
	9	18	Goodey, 1924a; Hartwich, 1994*
	9–12	18–24	This paper
O. quadrispinulatum	9	18	Stewart and Gasbarre, 1989; Hartwich, 1994*
	9–11	18–22	This paper
O. radiatum	No corona	32–40	Hartwich, 1994*
	Reduced corona	30–40	This paper
O. sikae	No corona	36–38	Hartwich, 1994
O. venulosum	16–20	32–40	Hartwich, 1994*

* After different authors, maximal variation summarized by Hartwich (1994).
† ? = not mentioned.

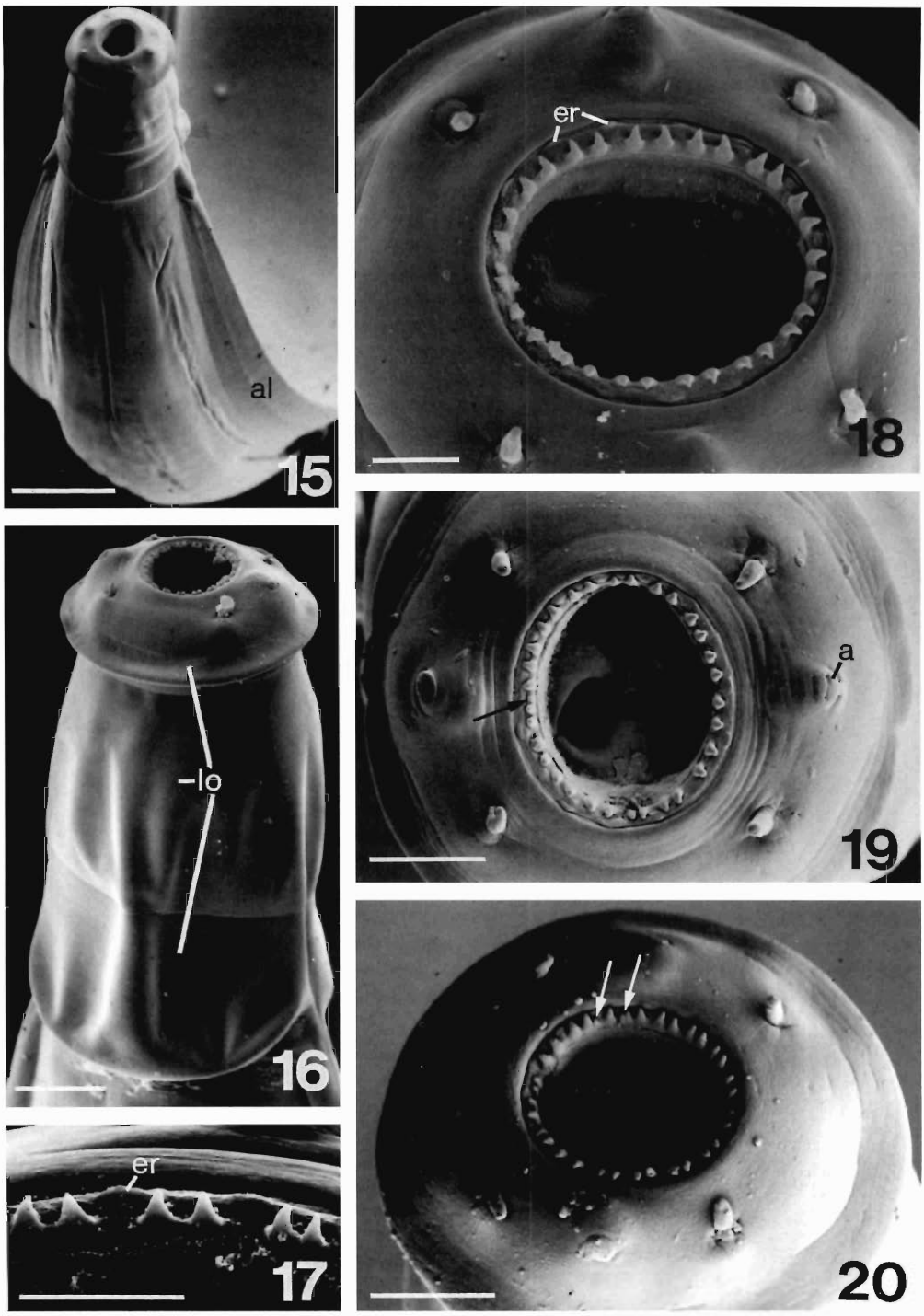
as more than 8 external leaves (i.e., 9–24 leaves) are apomorphic (Chabaud and Durette-Desset, 1974; Glen and Brooks, 1985). The phylogenetic hypothesis on the evolution of the taxon *Oesophagostomum* presented by Glen and Brooks (1985, Fig. 3) suggests that the combination 30–40 external leaves and 60–80 internal leaves has developed from a condition with 9–24 external and 18–48 internal leaves and represents an autapomorphic character of *O. pachycephalum* + *O. stephanostomum* + *O. ventri*.

Previous taxonomic or morphological investigations usually mention the number of buccal leaves for different species of *Oesophagostomum* but have only occasionally checked a larger number of specimens for polymorphism (Table 1). Data about the frequency of a given number of leaves are missing entirely. In our material, the frequency distribution of the number of buccal leaves differs considerably between *O. dentatum* and *O. quadrispinulatum*, the latter species expressing by far less variation in the number of leaves. The reason for such differences is unknown, and there is no apparent functional necessity for varying the number of buccal leaves. Our observations and a brief literature review (Table 1) suggest that polymorphism is a common character in the corona radiata of species of *Oesophagostomum*. We assume that such a polymorphism reflects the genetic potential of the species. But, the extent to which polymorphism is expressed in *O. dentatum* and *O. quadrispinulatum* (i.e., the frequency with which a certain number of leaves occurs) may be either species-specific or may depend on environmental influences during the ontogeny. In the latter case, the unfolding of the nematode morphotype may be less adversely influenced and hence the variability less pronounced under more optimal developmental conditions in the gut environment. Future investigations should be aware of polymorphism in the corona radiata of *Oesophagostomum* and, when appropriate, examine a larger number of individuals.

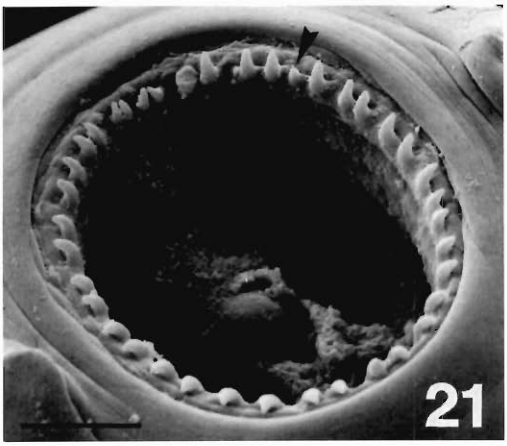
Polymorphism in the corona radiata of strongylid nematodes has also been reported for 18 species of the Cyathostominae (Braide and Georgi, 1974). A limited number of specimens (up to 18) per species was studied. Variation was either little or exceptionally large in few species but moderate in most species. No reasons for polymorphism were specified by Braide and Georgi (1974).

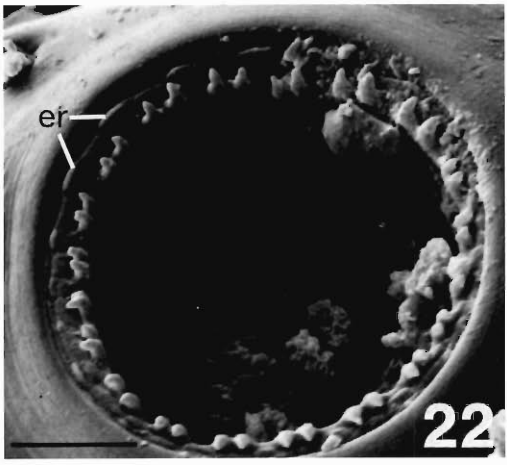
Acknowledgments

We appreciate the steady and encouraging interest and discussions with Professors Peter Nansen and Flemming Frandsen. The technical assistance by Bodil W. Jørgensen and Leif S. Jensen is gratefully acknowledged. We are indebted to the Danish National Research Foundation for financial support to the Danish Centre for Experimental Parasitology.



Figures 15–20. SEM of *Oesophagostomum radiatum*. **15.** Anterior end with trilobed head region and prominent lateral alae in trunk region. **16.** Trilobed head region at higher magnification. **17.** Arrangement of leaves of corona radiata in pairs. **18–20.** Frontal view of specimen with 30 leaves (18), 32 leaves (19), or 35 leaves (20). Arrows in 19 and 20 mark smaller leaves. Abbreviations: a, amphid; al, lateral ala; er,





Figures 21, 22. SEM frontal view of *Oesophagostomum radiatum* with 39 leaves (21) or 40 leaves (22). Arrowhead in 21 marks small leaf. Abbreviation: er, remnant of leaf of corona radiata externa. Scale bar in 21 and 22-10 μm.

Literature Cited

Blotkamp, J., H. P. Krepel, V. Kumar, S. Baeta, J. M. van't Noordende, and A. M. Polderman. 1993. Observations on the morphology of adults and larval stages of *Oesophagostomum* sp. isolated from man in northern Togo and Ghana. Journal of Helminthology 67:49–61.

Braide, E. I., and J. R. Georgi. 1974. Numbers of external leaf crown elements of 18 species of equine cyathostomes. Cornell Veterinarian 64: 233–239.

Chabaud, A. G., and M.-C. Durette-Desset. 1974. Description d'un nouveau nématode oesophagostome, parasite d'*Hyemoschus* au Gabon, et remarques sur le genre *Oesophagostomum*. Bulletin du Muséum National d'Histoire Naturelle 104:1415–1424.

Chitwood, B. G. 1931. A comparative histological study of certain nematodes. Zeitschrift für Morphologie und Ökologie der Tiere 23:237–284.

Gibbons, L. M. 1986. SEM Guide to the Morphology of Nematode Parasites of Vertebrates. Commonwealth Agricultural Bureaux International, Farnham Royal, Slough, U.K. 199 pp.

Glen, D. R., and D. R. Brooks. 1985. Phylogenetic relationships of some strongylate nematodes of primates. Proceedings of the Helminthological Society of Washington 52:227–236.

Goodey, T. 1924a. The anatomy of *Oesophagostomum dentatum* (Rud.) a nematode parasite of the pig, with observations on the structure and biology of the free-living larvae. Journal of Helminthology 2:1–14.

———. 1924b. Oesophagostomes of goats, sheep and cattle. Journal of Helminthology 2:97–110.

———. 1924c. Some new members of the genus *Oesophagostomum* from the roan antelope and the wart hog. Journal of Helminthology 2:135–148.

Hartwich, G. 1994. II. Strongylida: Strongyloidea und Ancylostomatoidea. *In* F. Dahl: Die Tierwelt Deutschlands, 68. Teil. G. Fischer, Jena. 157 pp.

Haupt, W. 1966. Ein Beitrag zur Morphologie der Knötchenwürmer des Hausschweines, ihrer Eier sowie der dritten invasionstüchtigen Larvenstadien. Archiv für experimentelle Veterinärmedizin 20:701–711.

Kendall, S. B., A. J. Small, and L. P. Phipps. 1977. *Oesophagostomum* species in pigs in England. I. *Oesophagostomum quadrispinulatum*: description and life-history. Journal of Comparative Pathology 87:223–229.

Lichtenfels, J. R. 1980. Keys to genera of the superfamily Strongyloidea. *In* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. Vol. 7. Commonwealth Agricultural Bureaux International, Farnham Royal, Slough, U.K. 41 pp.

Murrell, K. D. 1986. Epidemiology, pathogenesis, and control of major swine helminth parasites. Veterinary Clinics of North America: Food Animal Practice 2:439–453.

Neuhaus B., J. Bresciani, C. M. Christensen, and F. Frandsen. 1997a. Ultrastructure and development of the body cuticle of *Oesophagostomum dentatum* (Strongylida, Nematoda). Journal of Parasitology. (In press.)

———, ———, **and F. Frandsen.** 1997b. Ultrastructure of the pharyngeal cuticle and lectin la-

←

remnant of leaf of corona radiata externa; lo, lobe of head region. Scale bar in 15-100 μm, in 16, 19, and 20-20 μm, and in 17 and 18-10 μm.

belling with wheat germ agglutinin-gold conjugate indicating chitin in the pharyngeal cuticle of *Oesophagostomum dentatum* (Strongylida, Nematoda). Acta Zoologica. (In press.)

Poelvoorde, J. 1978. Oesophagostomosis in sows. Zentralblatt für Veterinärmedizin, Serie B 25: 835–840.

Roepstorff, A., and P. Nansen. 1994. Epidemiology and control of helminth infections in pigs under intensive and non-intensive production systems. Veterinary Parasitology 54:69–85.

Rudolphi, K. A. 1803. Neue Beobachtungen über die Eingeweidewürmer. Archiv für Zoologie und Zootomie 3(2):1–32.

Schwartz, B., and J. E. Alicata. 1930. Two new species of nodular worms (*Oesophagostomum*) parasitic in the intestine of domestic swine. Journal of Agricultural Research 40:517–522.

Skryabin, K. I., N. P. Shikhobalova, R. S. Schulz, T. I. Popova, S. N. Boev, and S. L. Delyamure. 1992. Key to Parasitic Nematodes. Vol. 3. Strongylata. E. J. Brill, Leiden, New York. 912 pp.

Stewart, T. B., and L. C. Gasbarre. 1989. The veterinary importance of nodular worms (*Oesophagostomum* spp). Parasitology Today 5:209–213.

Taffs, L. F. 1967. *Oesophagostomum quadrispinulatum* in pigs in England. The Veterinary Record 80:182–183.

Travassos, L., and E. Vogelsang. 1932. Pesquizas helminthologicas realisadas em Hamburgo. X. Contribuição ao conhecimento das especies de *Oesophagostomum* dos primatas. Memorias do Instituto Oswaldo Cruz 26:251–328.

Obituary Notice

ROBERT TRAUB

26 November 1916–21 December 1996

Elected to Membership 11 December 1946

Honorary Member 1996

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 137–141

Microfilariae in the Free-Ranging Florida Panther (*Felis concolor coryi*)

MARNIE G. LAMM,[1] MELODY E. ROELKE,[2,5] ELLIS C. GREINER,[3,6] AND CHRISTINE K. STEIBLE[4]

[1] College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610 (e-mail: v968908@student.health.ufl.edu),

[2] Florida Game and Fresh Water Fish Commission, Gainesville, Florida 32610,

[3] Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610 (e-mail: greiner.vetmed3@mail.health.ufl.edu.), and

[4] Department of Statistics, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611

ABSTRACT: Blood samples from Florida panthers (*Felis concolor coryi*) collected from 1986 to 1993 during the months of December through May were screened for the presence of microfilariae (mff) by the Difil® filter test. Thirty-five of 47 (74.5%) panthers older than 2 yr of age were positive with microfilaremias ranging from 10 to 7,380 mff/ml of whole blood. No panthers that were 6 mo of age or less (n = 10) were microfilariae-positive, and only 20% of the panthers in the 1-yr class (n = 5) were positive. A representative number of microfilariae (n = 40) from each of 7 freshly collected positive blood samples was measured and morphological characteristics were noted. The average length of microfilariae processed by the modified Knott's technique was 320 μm (273–370 μm) with a width of 4–5 μm. Of the 280 microfilariae measured, 202 (72.14%) had tapered heads and straight tails with an average length of 319 μm (276–368 μm), 61 (21.79%) had blunt heads and straight tails and averaged 323 μm (274-366 μm), 16 (5.71%) had tapered heads and button-hooked tails with an average length of 320 μm (290–368 μm), and 1 (0.35%) had a blunt head and button-hooked tail and measured 320 μm. The finding of no significant difference ($P > 0.05$) between length measurements due to differences in head and tail shape leads us to believe that all microfilariae were of 1 species. Based on microfilarial length measurements, review of necropsy reports, and comparison with bobcat microfilariae, the most likely filarial species infecting the Florida panther is *Dirofilaria striata* (Molin, 1858).

KEY WORDS: Florida panther, *Felis concolor coryi*, *Dirofilaria striata*, microfilariae, morphology, prevalence, bobcat, *Lynx rufus*.

Filarial nematodes have been reported in a number of exotic felid species. *Dirofilaria repens* has been reported in the lion (*Panthera leo*) (Nelson et al., 1962). *Dirofilaria immitis* has been found in jaguars (*Felis onca*), tigers (*Felis sondiacus* and *Felis tigris*), wild cats (*Felis bangsi costariensis*), jagouarundi (*Felis yagouarundi*) (Otto, 1974), a bengal tiger (*Panthera tigris*) (Kennedy and Patton, 1981), and bobcats (*Lynx rufus*) from Florida (Levine, 1980; Forrester, 1992). Of 22 free-ranging mountain lions (*Felis concolor*) from California, 1 was seropositive for antibodies against the somatic antigen of *D. immitis* yet was seronegative for cuticular antigen and no circulating microfilariae (mff) were found in the whole blood (Paul-Murphy et al., 1994). *Dirofilaria striata* was first reported in Brazilian pumas (*Felis concolor* and *F. macroura*) (Raillet and Henry, 1911) and since has been found in the ocelot (*Felis pardalis*), the margay (*Felis tigrina*) (Anderson and Diaz-Ungria, 1959), and the bobcat (Orihel and Ash, 1964; Miller and Harkema, 1968; Roelke, unpubl. 1985). Forrester et al. (1985) reported adult *D. striata* in mff+ adult Florida panthers.

The Florida panther is an endangered subspecies of cougar that inhabits pockets of habitat in the Big Cypress and Everglades ecosystems of southern Florida (U.S.A.). A population of less than 40 adult animals remains in the wild (Belden, 1986). Since veterinary involvement in the Florida Panther Recovery Program began in 1983, an intensive health evaluation and monitoring program has been in effect. The protocol includes the collection, screening, and storage of various biological samples each time an animal is immobilized. In the past, the presence of mff has been noted either by buffy coat analysis, by membrane filtration, or in reports from clinical laboratories. Adult *D. striata* were found in 4 out of the 7 panthers examined from 1973 to 1983, 3 of which were mff+ (Forrester et al.,

[5] Present address: Tanzania National Parks, Arusha, Tanzania, Africa.

[6] Corresponding author.

1985). It was assumed that all microfilariae from these animals were representative of *D. striata* because no other adult filariids were detected.

Adult *D. striata* were found in 5 additional microfilaremic panthers at necropsy (Roelke, Nayer, and Vickery, unpub. data 1985). According to necropsy reports, 1–3 adult filariids were present singularly in the fascia between muscle bundles in the distal extremities. No reports of any filarial species other than *D. striata* were found in review of Florida panther necropsy reports (n = 41) from 1980 to September 1994. This study was undertaken to determine the prevalence and microfilaremia of *Dirofilaria* sp. in the Florida panther population and to characterize and measure the mff in order to confirm the identity of the species of filariid present.

Materials and Methods

From 1986 to 1993, a total of 83 blood samples representing 47 individual panthers and 3 bobcats was screened for the presence of mff. Samples were collected during the field capture season in the months of December through May from free-ranging panthers in the Big Cypress and Everglades ecosystems. All animals were anesthetized with ketamine hydrochloride (Ketastat®, Bristol Laboratories, Syracuse, New York, U.S.A.) or a combination of tiletamine/zolazepam (Telazol®, A. H. Robins Co., Richmond, Virginia, U.S.A.). Blood was drawn prior to administration of fluids, vaccinations, and prophylactic injections of Ivermectin (Ivomec®, Merck & Company, Incorporated, Rahway, New Jersey, U.S.A.) at a dose of 200 mcg/kg. Blood samples were taken directly from the saphenous vein into sterile 3-ml tubes containing ethylenediaminetetraacetic acid (EDTA) via a vacutainer and butterfly apparatus. The sample was kept cool in an insulated container for the 2–8-hr transition from field to lab.

One milliliter of EDTA preserved whole blood from each of the 17 samples collected in 1992 and 5 samples from 1993 was processed using the Difil® (EVSCO Pharmaceuticals, Buena, New Jersey, U.S.A.) procedure described by Howland and Todd (1977). In addition, 58 whole blood samples preserved in EDTA that had been collected from free-ranging panthers from 1986 to 1991 and stored in a 1:10 ratio of blood (1 ml) to 2% formalin (9 ml) at 4°C were screened for mff. From the 10-ml sample, 5 ml were passed through a Difil filter. Numbers of mff were determined for all positive samples (except FP#51 in which high numbers of mff/field made accurate counting impossible) by counting all individual mff on the filter at a viewing magnification of ×200 to obtain a mff/1 ml value. When no mff were found, the remaining 5 ml were tested in the same manner to confirm that the entire sample was indeed negative. In all cases, the Difil filter holder was washed and dried well between samples in order to avoid cross-contamination leading to false-positive results.

Of the 17 samples screened in 1992, 7 samples with high numbers of mff were chosen for morphological study of individual microfilariae. All blood samples were kept in EDTA-coated blood tubes for the 2–8 hr between time of collection and time of microfilarial analysis. A 1-ml aliquot was processed using a modified Knott's procedure (Knott, 1939). Microfilariae were measured immediately after processing in order to minimize possible effects on length or width of microfilariae due to storage in 2% formalin. Measurements and morphological assessments were made with a calibrated ocular micrometer at a magnification of ×400. Length, head shape, tail shape, and body shape were recorded for 40 mff/sample. Head shape was considered tapered if the width decreased upon successive measurements anteriorly (Fig. 1); if the sides remained parallel, the head was categorized as blunt (Fig. 2). Although many posterior ends were curved, only those that had a distinct hook at the tip were labeled as such (Fig. 1, 4).

Data were analyzed using SAS (SAS Institute Inc., 1989). Comparisons of total lengths of the microfilariae were made between panthers and bobcats and between morphological types using ANOVA with a split-plot design where felid type was the whole-plot factor and morphological type was the sub-plot factor. Three morphological types (tapered head and straight tail, blunt head and straight tail, and tapered head and button-hooked tail) were included in this analysis. Microfilariae with blunt head and button-hooked tails were not included due to low prevalence (1 found in panthers and 3 found in bobcats). Confidence limits were not calculated for percentage of prevalence of mff in panthers because the number examined represented nearly all of the extant population of panthers, thus rendering confidence limit calculations moot.

Results and Discussion

Thirty-five (74.5%) of 47 adult (>2 yr of age) Florida panthers sampled from 1986 to 1993 were mff+ at some point in their lives and all except for 2, which had low counts of 20 and 330 mff/ml, remained positive on subsequent tests. Because panthers were usually sampled at 2-yr intervals, the average age when animals become mff+ is not precisely known. None of the 5 panthers tested at 6 mo of age were mff+, 2 of 10 (20%) tested positive in the 1-yr-old class, and 15 of 23 (65%) of panthers were test positive at 2–4 yr of age. Of the 23 panthers that were 10 yr of age or older, 22 (96%) were mff+.

Counts ranged from 10 to 7,380 mff/ml of whole blood. Microfilarial counts showed fluctuations with no apparent trends when comparisons were made between animals or over periodic sampling of individual panthers. Administration of ivermectin at the time of capture seemed to have no effect on the long-term microfilarial intensities. Because the panther-capture interval was normally every 2 yr, it was

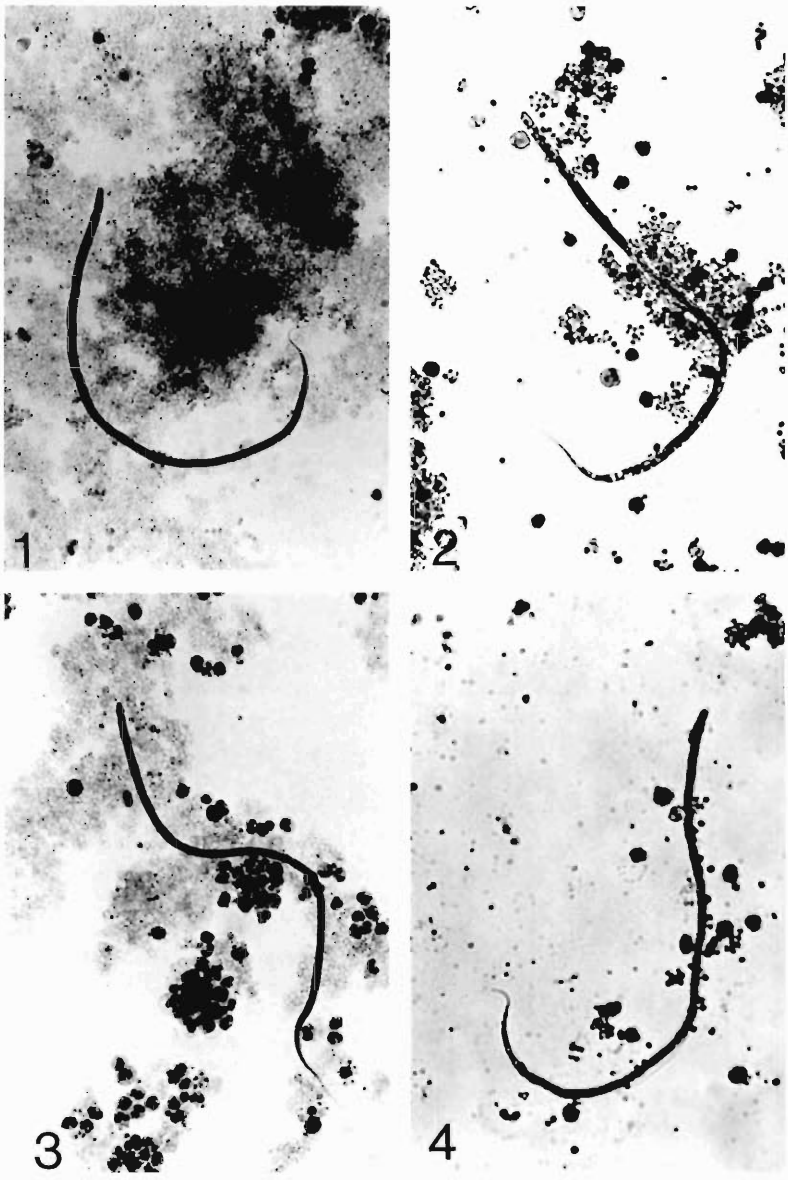


Figure 1. Microfilaria from panther blood with tapered head and hooked tail. Total length = 332 μm.
Figure 2. Microfilaria from panther blood with blunt head and straight tail. Total length = 319 μm.
Figure 3. Microfilaria from panther blood with tapered head and straight tail. Total length = 329 μm.
Figure 4. Microfilaria from panther blood with blunt head and hooked tail. Total length = 323 μm.

impossible to assess the short-term effect of this anthelmintic on microfilaremias in the Florida panther.

Panther microfilariae were of 4 morphological types (Fig. 1–4): blunt head, straight tail (BS); blunt head, button-hooked tail (BH); tapered head, straight tail (TS); and tapered head, button-hooked tail (TH). Microfilariae with tapered heads and straight tails were the most prevalent (72.1%), followed by those with blunt heads and straight tails (21.8%). The next most common were mff with tapered heads and button-hooked

tails (5.7%) and, lastly, those with blunt heads and button-hooked tails (0.4%). Body shapes were not used as criteria for distinguishing filarial types because shape appeared to be a highly variable parameter.

The average lengths of the mff from panthers measured in fresh blood samples (n = 280) was 320 μm (273–370 μm). The widths ranged from 4 to 5 μm. All of the mff measured remained in their original whole blood sample for a period of 2–8 hr before analysis. Courtney and Garber (1983) found, in measurement of *D. immitis* and *D. reconditum* mff collected from dogs and stored in EDTA for 8 hr, that there was not a significant change in microfilarial width or length. In an earlier study, Sawyer et al. (1963) determined that the width of *D. reconditum* stored in blood for 4–6 hours increased an average of 0.5 μm above the average of those measured immediately. Both authors concluded that there was no change in morphological features during this time.

Because bobcats and panthers inhabit the same habitat and *D. striata* has been reported in the bobcat, a screening of 3 bobcat blood samples and comparison of mff between the 2 species was performed. Of the 3 bobcats examined, all had high microfilaremias. The average length of bobcat mff measured (n = 120) was 333 μm (297–363 μm) and the average width was 5 μm (4–6 μm). A majority of the mff (72.5%) had tapered heads and straight tails, 20% had blunt heads and straight tails, 5% had tapered heads and hooked tails, and 2.5% had blunt heads and button-hooked tails. This distribution of morphologic types is similar to that of the panther (see Table 1). Neither felid type, morphological type, nor the interaction between felid type and morphological type had a significant effect on microfilarial length (P = 0.3375, 0.3572, and 0.4178, respectively).

Knott's procedure was the chosen method for studying morphological features because it caused less deformation and better delineation of the worms than did the Difil test. Jackson (1977) noted that the filtration step of the Difil test procedure seemed to cause shrinkage in length as compared to those measurements obtained from a Knott's preparation and that the filter membrane would often trap the mff midway through and obscure observation of the tail. The Difil method was used to obtain microfilarial counts because it was faster and was deemed a more sensitive detection method by House and Glover (1974), who reported that the Difil test is 97.5% accurate whereas the Knott's procedure is only 89% accurate.

Table 1. Distribution and average lengths of Florida panther and Bobcat microfilariae based on morphology.

Head/tail	N	%	$\bar{x}$ (μm)	SD	Range (μm)
Tapered/straight					
Panther	202	72.14%	319	7.16	276–368
Bobcat	87	72.5%	333	6.16	297–363
Blunt/straight					
Panther	61	21.79%	323	8.25	274–366
Bobcat	24	20.0%	326	6.14	299–350
Tapered/button-hooked					
Panther	16	5.71%	320	8.52	290–368
Bobcat	6	5.0%	338	6.17	311–350
Blunt/button-hooked					
Panther	1	0.35%	320	0	320
Bobcat	3	2.5%	339	7.02	322–354

From review of necropsy data and the morphological data presented here, it can be inferred that *D. striata* is the only species of filariid present in the Florida panther. Comparison of the microfilarial length values with those published for various *Dirofilaria* spp. shows that they are most similar to *D. striata*, although references regarding measurements of this filariid in felids are few and reported microfilarial lengths vary significantly depending on processing methods. Anderson and Diaz (1959) isolated unsheathed mff from the uterus of adults with a length of 235–270 μm and a width of 5 μm. The measurements of formalin-fixed *D. striata* from bobcats reported by Orihel and Ash (1964) averaged 348 μm (327–371 μm) long by 4–5 μm wide, and those measured from hematoxylin-stained thick blood films were 230–240 μm. Redington et al. (1977) reported finding similar dimensions though no values were given. The finding of such a high prevalence of mff in adult Florida panthers warrants definitive microfilarial identification as well as continued screening of whole blood samples and investigation into the subtle health effects that such high numbers of circulating mff may pose.

Acknowledgments

The authors thank N. G. Keeling, A. J. Anderson, R. Ball, R. T. McBride, D. S. Maehr, E.

D. Land, J. C. Roof, J. W. McCown, D. K. Jansen, O. L. Bass, V. L. Gibaldi, T. S. Ruth, and S. H. Parker for their cooperation in collection and/or preparation of biological samples and M. Dunbar for supplying the 1993 blood samples and for access to panther medical records. Grateful acknowledgment goes to D. J. Forrester, C. H. Courtney, and M. G. Spalding for their expert advice in the editing of this manuscript. We wish to extend a special acknowledgment of and dedication to C. M. Glass for assistance with data collection and laboratory analysis as well as expert advice and support. This paper is published as Florida Agricultural Experiment Stations Journal Series No. R-05017.

Literature Cited

Anderson, R. C., and C. Diaz-Ungria. 1959. Nematodes de Venezuela, VI. *Dirofilaria striata* en felinos suramericanos, con comentarios sobre las Dirofilaria en carnivoros. Boletin Venezolano de Laboratorio Clinico 4:3–15.

Belden, R. C. 1986. Florida panther recovery plan implementation—a 1983 progress report. Pages 159–172 *in* S. D. Miller and D. D. Everett, eds. Cats of the World: Biology, Conservation and Management. Proceedings of the Second International Cat Symposium, Caesar Kleberg Wildlife Research Institute, Kingsville, Texas.

Courtney, C. H., and R. Garber. 1983. Effects of anticoagulant, time, and storage temperature on the morphology of microfilariae of *Dirofilaria immitis* and *Dipetalonema reconditum*. Proceedings of the Heartworm Symposium 83:5–7.

Forrester, D. J. 1992. Parasites and Diseases of Wild Mammals in Florida. University Press of Florida, Gainesville. 459 pp.

———, **J. A. Conti, and R. C. Belden.** 1985. Parasites of the Florida panther (*Felis concolor coryi*). Proceedings of the Helminthological Society of Washington 52:95–97.

House, C., and F. Glover. 1974. Evaluation of and improved filter test for microfilariae detection. Proceedings of the Heartworm Symposium 74: 19–20.

Howland, T. P., and K. S. Todd. 1977. An evaluation of contamination of the Difil® test for *Dirofilaria immitis* microfilariae. Proceedings of the Heartworm Symposium 77:31–44.

Jackson, R. F. 1977. Studies of the filter techniques for the detection and identification of canine microfilaria. Proceedings of the Heartworm Symposium 77:38–44.

Kennedy, S., and S. Patton. 1981. Heartworms in a bengal tiger. Journal of Zoo Animal Medicine 12: 20–22.

Knott, J. 1939. A method for making microfilarial surveys on day blood. Royal Society of Tropical Medicine and Hygiene 33:191–196.

Levine, N. D. 1980. Nematode Parasites of Domestic Animals and of Man. 2nd ed. Burgess, Minneapolis, Minnesota. 477 pp.

Miller, G. C., and R. Harkema. 1968. Helminths of some wild mammals in the southeastern United States. Proceedings of the Helminthological Society of Washington 35:118–125.

Nelson, G. S., R. B. Heisch, and M. Furlong. 1962. Studies in filariasis in East Africa. II. Filarial infections in man, animals, and mosquitoes on the Kenya coast. Transactions of the Royal Society of Tropical Medicine and Hygiene 56:202–217.

Orihel, T. C., and L. R. Ash. 1964. Occurrence of *Dirofilaria striata* in the bobcat (*Lynx rufus*) in Louisiana with observations on its larval development. Journal of Parasitology 50:590–591.

Otto, G. F. 1974. Occurrence of the heartworm in unusual locations in unusual hosts. Proceedings of the Heartworm Symposium 74:6–13.

Paul-Murphy, J., T. Work, D. Hunter, E. McFie, and D. Fjelline. 1994. Serologic survey and serum biochemical reference ranges of the free-ranging mountain lion (*Felis concolor*) in California. Journal of Wildlife Diseases 30(2):205–215.

Raillet, A., and A. Henry. 1911. Sur une filarie peritoneale des porcins. Bulletin of the Society of Exotic Pathology 4:386–389.

Redington, B. C., R. F. Jackson, W. G. Seymour, and G. F. Otto. 1977. The various microfilariae found in dogs in the United States. Proceedings of the Heartworm Symposium 77:14–21.

SAS Institute Inc. 1989. SAS/STAT® Users Guide, Version 6, 4th ed. Vol. 2. SAS Institute Inc., Cary, North Carolina. 846 pp.

Sawyer, T. K., P. P. Weinstein, and J. Block. 1963. Canine filariasis—the influence of the method of treatment on measurements of microfilariae in blood samples. American Journal of Veterinary Research 24:395–400.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 142–147

Fessisentis acutulus (Van Cleave, 1931) comb. n. (Acanthocephala: Fessisentidae): A Parasite of Caudate Amphibians in North America, with Comments on the Single North American Report of *A. ranae* (Schrank, 1788)

DONALD F. MCALPINE

Natural Sciences Department, New Brunswick Museum, 277 Douglas Avenue, Saint John, New Brunswick, E2K 1E5 and Biology Department, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E1

ABSTRACT: Based on anatomical grounds, specimens in the type series of the acanthocephalan *Acanthocephalus acutulus* Van Cleave, 1931, from the red-spotted newt, *Notophthalmus viridescens* (Rafinesque, 1820), belong to the genus *Fessisentis*. This conforms with knowledge of the host–parasite relationships within the genus *Fessisentis*. The single North American report for *A. ranae* (Schrank, 1788) clearly is not this species and probably is *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936.

KEY WORDS: Acanthocephala, Amphibia, *Acanthocephalus acutulus*, *Acanthocephalus dirus*, *Acanthocephalus ranae*, *Fessisentis acutulus*.

With the exception of several members of the genus *Fessisentis* Van Cleave, 1931, acanthocephalans have been infrequently reported as parasitic in North American amphibians. In the earliest report, Stiles and Hassall (1894) recorded *Echinorhychus* sp. from *Notophthalmus viridescens* (Rafinesque, 1820), the red-spotted newt, collected in Maryland. Van Cleave (1915) identified the 7 specimens in this collection as *Acanthocephalus ranae* (Schrank, 1788), a species typical of European amphibians. Subsequently, a series of 12 worms collected by Holl (1932) from the red-spotted newt in North Carolina were described by Van Cleave (1931a) as *A. acutulus*.

Acanthocephalus acutulus has been rarely collected since its original description. Rankin (1937) reported the species in the North Carolina salamanders *Ambystoma opacum*, *Desmognathus fuscus*, *Plethodon glutinosus*, and *N. viridescens*. Nickol (1969) collected a single immature specimen from *Plethodon glutinosus* in Louisiana, and Dyer and Brandon (1973) found 5 specimens in a single *Cryptobranchus alleganiensis* in Missouri. *Acanthocephalus ranae* has not been reported in North America since the publication of Van Cleave (1915).

Here I review the status of these specimens and resolve several problems of systematics and biogeography presented by these reports.

Materials and Methods

The following specimens were borrowed and examined.

i. *Acanthocephalus ranae* (Schrank, 1788): Two mature males and 1 gravid female, of the 7 specimens reported by Van Cleave (1915) (USNPC No. 6322).

ii. *Acanthocephalus acutulus* Van Cleave, 1931: Eleven of the 12 worms in the type series, mounted on 10 slides (USNPC No. 81541). One of these specimens was cross-sectioned and stained in celestin blue B, Mayer's hematoxylin, and eosin for examination of the muscular structure of the proboscis receptacle.

iii. *Acanthocephalus acutulus* Van Cleave, 1931: Single specimen reported by Nickol (1969) (collection of Dr. Brent Nickol, University of Nebraska).

iv. *Acanthocephalus ranae* (Schrank, 1788): Single gravid female from *Rana esculenta* Linnaeus, 1758 (USNPC No. 81866).

Attempts to locate specimens of *A. acutulus* reported by Rankin (1937) and those collected by Holl (1932) additional to the type series were unsuccessful. Specimens of Dyer and Brandon (1973) were accidentally destroyed (W. G. Dyer, pers. comm.).

Results and Discussion

Acanthocephalus ranae (Schrank, 1788)

Two of Van Cleave's specimens are fragmented, including the single female that is also overstained. Van Cleave (1931b) noted that his identification of *A. ranae* was based on "rather unsatisfactory material." These specimens differ from European *A. ranae* (Schrank, 1788) and the closely related *A. falcatus* (Frolich, 1789) examined by Grabda-Kazubska (1962). *Acanthocephalus ranae* (Schrank, 1788) rarely possesses 12 hook rows, and then only in males. Of the 4

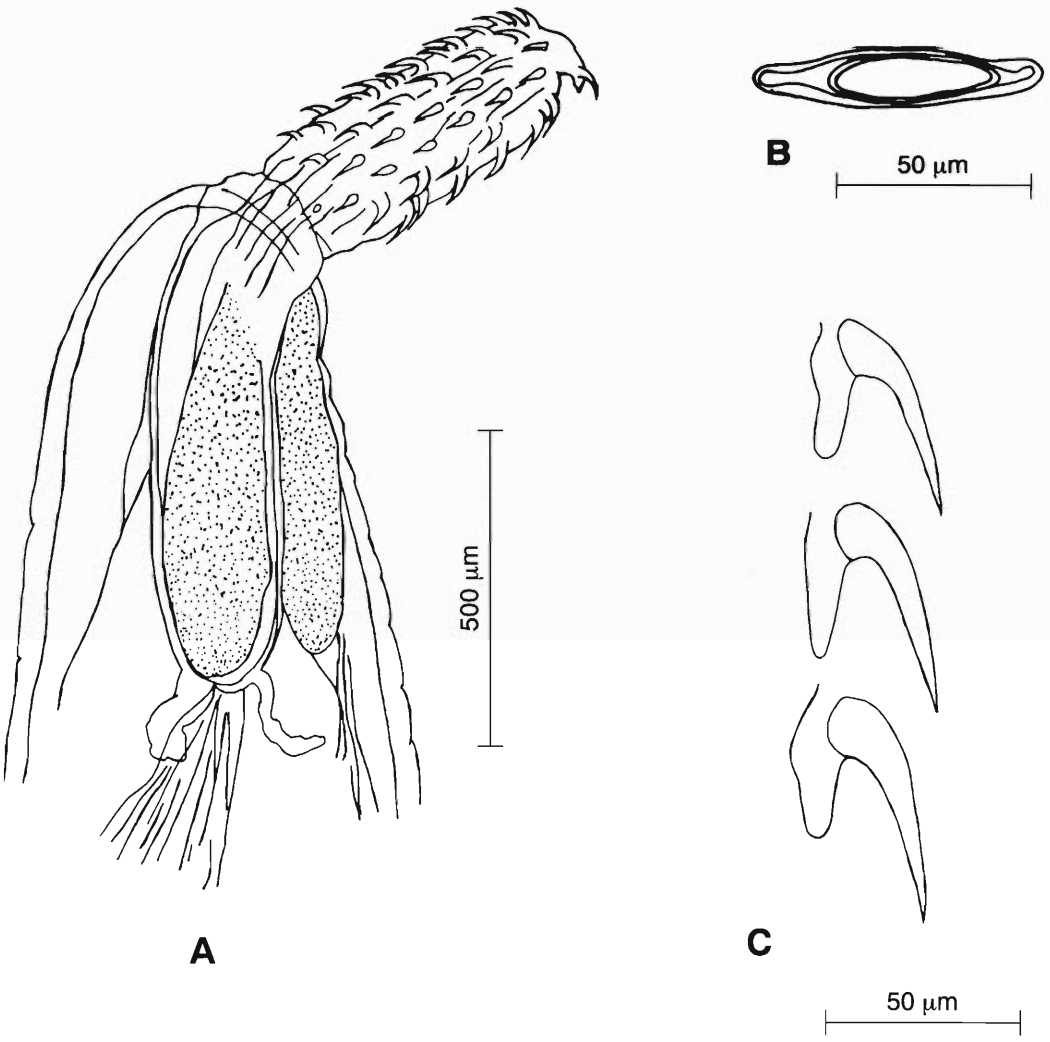


Figure 1. Details of specimens identified as *Acanthocephalus ranae* by Van Cleave (1915). A. Anterior of male. B. Egg. C. Sequence of proboscis hooks from midproboscis in male.

worms that Van Cleave (1915) noted had a protruded proboscis and easily counted rows of hooks, 3 were males and 1 was a female. However, Van Cleave (1915) reported that all worms had 12 hook rows. Grabda-Kazubska (1962) found that *A. ranae* never had 6 or 7 hooks/row, the upper limit being 5–6 for males and 6 for females, with <10% of worms reaching these maxima. All 4 of Van Cleave's (1915) worms noted above have 6 or 7 hooks/row. Grabda-Kazubska (1962) also observed that there is a well-formed neck present in *A. ranae* and that the lemnisci are longer than the proboscis receptacle and projected away from it in this species (see fig. 2 in Grabda-Kazubska, 1962). These features are not visible in the worms available (Fig. 1A), nor are they visible in the illustration of the worm provided in Van Cleave (1915). The neck is readily visible in the single Old World specimen of *A. ranae* examined.

The Van Cleave (1915) specimens are distinguished from *A. falcatus*, a European amphibian parasite closely related to *A. ranae* and long confused with it, by the following: the eggs present in the single existing Van Cleave (1915) female are unlike those illustrated for *A. falcatus*

Table 1. Morphometrics of specimens identified as *Acanthocephalus ranae* by Van Cleave (1915) compared with ranges and means for *A. dirus* as reported by Amin (1984). Figures in parentheses represent means. Body length and width are shown in millimeters. All other measurements are in micrometers.

	A. ranae, USNPC No. 6322			*A. dirus*, Amin (1984)			
	♂	♂	♀	♂		♀	
Body length	2.63	3.46	6.44	2.20–6.00	(3.41)	2.40–20.00	(8.65)
Body width	0.59	0.56	0.93	0.32–1.50	(0.58)	0.32–1.44	(0.76)
Proboscis length	465	—	688	310–742	(520)	460–882	(647)
Proboscis width	175	—	280	98–240	(156)	140–392	(216)
Proboscis receptacle length	635	830	800	364–1,300	(697)	350–1,680	(852)
Proboscis receptacle width	175	200	215	126–308	(190)	140–392	(216)
No. hook rows	12	—	12	11–20	(13.7)	12–19	(14.9)
No. hooks/row	6–7	—	6–7	6–13	(9.1)	8–14	(9.8)
Lemnisci length	525	682.5	—	196–1,078	(588)	280–1,526	(765)
Leminisci width	104	125	—	42–322	(137)	5–364	(163)
Anterior testes length	465	500	—	308–1,008	(622)	—	—
Anterior testes width	215	225	—	168–686	(322)	—	—
Posterior testes length	390	515	—	210–924	(617)	—	—
Posterior testes width	210	215	—	168–644	(322)	—	—
No. cement glands	6	6	—	0–12	(5.67)	—	—
Cement gland length	225	350	—	98–588	(231)	—	—
Cement gland width	175	175	—	84–420	(177)	—	—

by Grabda-Kazubska (1962), but are identical to *A. dirus* (Van Cleave, 1931) (=*A. parksidei* Amin 1975, Fig. 1B); in *A. falcatus*, the roots of the proboscis hooks are considerably shorter than the spines and weakly formed (see fig. 3 in Grabda-Kazubska 1962), a feature not present in the worms of Van Cleave (1915) (Fig. 1C).

Table 1 compares Van Cleave's material with the redescription of *A. dirus* of Amin (1984), an expanded diagnosis synonmizing *A. jacksoni* Bullock, 1962, and *A. parksidei* Amin, 1975. The measurements for *A. ranae* of Van Cleave (1915) fall within the range of variation for *A. dirus* described by Amin (1984).

Acanthocephalus acutulus Van Cleave (1931)

With the exception of 4 specimens, the type series was originally mounted in glycerin by Holl. In 1948, 17 yr after Van Cleave described *A. acutulus*, the glycerin-mounted material was demounted, stained, and remounted in balsam. In the process, many of the specimens were damaged. Two worms are now reduced to poorly stained posterior fragments. Five whole mounts include 2 with retracted proboscides. The remaining specimens are uninformative. In total, 7 worms have features assignable to the genus *Fessisentis*.

The proboscis receptacles of these 7 specimens have a thickened posterior end where several prominent nuclei are present, a defining character of the Fessisentidae (Fig. 2A, B). No mature males in the type series are available, and the form of the testes could therefore not be documented. The form of the female reproductive system agrees with that illustrated for the genus (Fig. 2C; see Van Cleave, 1931b; Amin, 1980). Serial sections of the proboscis receptacle sac show the distinctive muscular structure of the genus *Fessisentis* (Fig. 2D; Nickol, 1972; Buckner and Nickol, 1978).

Using the key to the genus *Fessisentis* provided by Amin (1980), and the measurements presented in Van Cleave (1931a), these specimens can be assigned to *Fessisentis friedi* Nickol, 1972. However, the range for hooks/row reported by Van Cleave (1931a) (9–12 hooks in 18–24 rows) is higher than that reported by Fried and Koplin (1967) for a variable population of *F. friedi* in Pennsylvania. Other measurements, such as the total length given by Van Cleave (1931a) of 3.5–5 mm, as well as measurements I have taken from the remaining specimens, are at the lower limit or below the ranges reported for the species by these authors and by Haley and Bullock (1953)

Acanthocephalus acutulus has been reported on 3 occasions since the original description of Van Cleave (1931a). Unfortunately, only the single specimen of *A. acutulus* reported by Nickol (1969) is still available for examination. This worm is not a member of the genus *Fessisentis*.

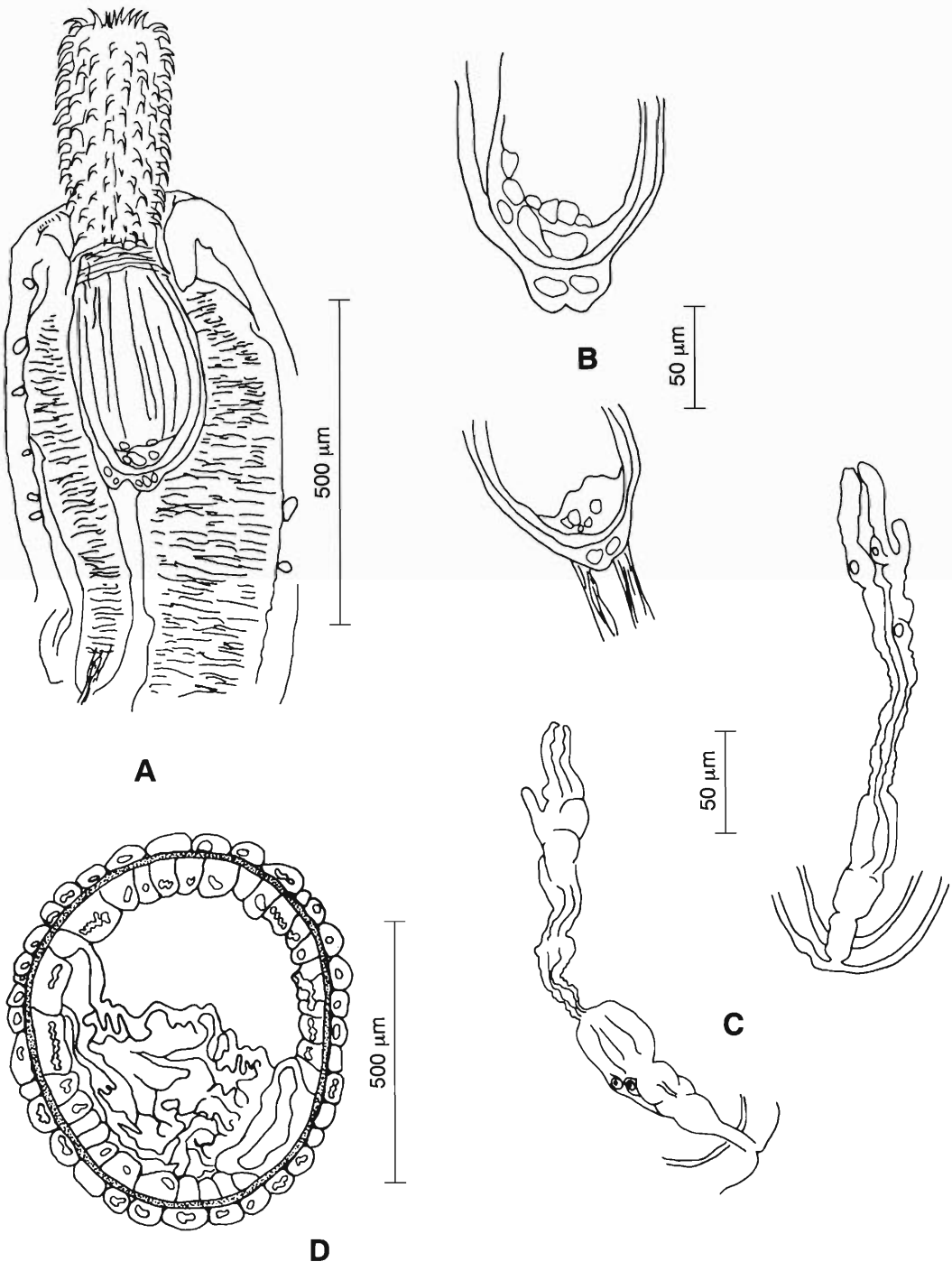


Figure 2. Details of specimens from type series of *Acanthocephalus acutulus* Van Cleave, 1931. A. Anterior of specimen No. 2308.8. This specimen is illustrated as figure 2 in the original description. B. Posterior end of proboscis receptacles in Nos. 2308.1 (top) and 2308.9. C. Female reproductive system in Nos. 2308.1 and 2308.9. D. Serial section (7 µm) through the proboscis receptacle of No. 2308.9.

The structure of the proboscis receptacle is readily visible, the specimen lacking a nuclear pouch. Although the specimen is clearly of the genus *Acanthocephalus*, the worm is immature. The 23 longitudinal hook rows of 10–11 alternating hooks/row (B. Nickol, pers. comm.) are outside the range in hook rows (11–20) provided by Amin (1984) for *A. dirus*.

Although the foregoing resolves the anomalous report of *A. ranae* in North America, identification of the material as *A. dirus* is unsatisfactory. *Acanthocephalus dirus* is the most variable and widespread member of the genus from North American freshwater fishes (Amin, 1984), and various populations may not be conspecific (B. Nickol, pers. comm.). Although Amin (1984) synonymized *A. jacksoni* and *A. parksidei*, he recognized this problem, suggesting that the resulting extreme variation within the species was sufficient reason to call for a reappraisal of the systematics of the genus from North American freshwater fishes. However, until there is a better understanding of the systematics of *A. dirus*, I believe the Maryland specimens from newts originally identified as *A. ranae* should be considered *A. dirus*. The record of *A. acutulus* reported by Nickol (1969), although certainly *Acanthocephalus*, does not appear to be *A. dirus* and cannot be assigned to species at the moment. Material identified by Rankin (1937) and Dyer and Brandon (1973) as *A. acutulus* is unavailable and can at best now be assigned to Acanthocephala sp.

It is clear that *A. acutulus* is a member of the genus *Fessisentis*. Although the material resembles *F. friedi*, it should be noted that the body lengths of all of the 5 females remaining in the series fall at the extreme lower end or below the overall range cited by Amin (1980) for *F. friedi* and well below the range reported for females by Fried and Koplin (1967). Fried and Koplin (1967) note that females are usually larger than males.

Additionally, monthly prevalences in the red-spotted newt that ranged from 50 to 100% (Holl, 1932) suggest that these *Fessisentis* infections were not accidental. This conforms with knowledge of the host–parasite relationships within the genus, several members of which are typically parasites of amphibians (Nickol, 1972). *Fessisentis necturorum* Nickol, 1967, and *F. vancleavei* (Hughes and Moore, 1943), only known as parasites of amphibians, and *F. fessus* Van Cleave, 1931, usually a fish parasite, have been recorded at similarly high prevalences in amphibian hosts. One would not expect such high prevalences in an atypical host where the infection is accidental. However, *Fessisentis friedi* has been recorded only once from a caudate amphibian and is normally a parasite of fish (McAlpine, 1996).

Until additional Acanthocephala from North American amphibians have been collected, particularly from newts, it will not be possible to resolve satisfactorily the status of *Fessisentis* described by Van Cleave from *N. viridescens*. At present, these specimens should be referred to as *Fessisentis acutulus* (Van Cleave, 1931).

Acknowledgments

I am grateful to Drs. Omar Amin and Michael Burt for comments on an earlier draft of the manuscript. Dr. Brent Nickol was particularly helpful with comments and discussion on the status of *A. dirus*. Drs. Ralph Litchtenfels and Brent Nickol kindly loaned specimens under their care for my examination, and Dr. Litchenfels was most gracious in allowing sectioning of a specimen from the type series for *A. acutulus*. I also thank Dr. Carl Bursey, who sectioned this delicate material on my behalf.

Literature Cited

Amin, O. M. 1980. *Fessisentis tichigenensis* sp. nov. (Acanthocephala: Fessisentidae) from Wisconsin fishes with a key to species. Journal of Parasitology 66:1039–1045.

———. 1984. Variability and redescription of *Acanthocephalus dirus* (Acanthocephala: Echinorhynchidae) from freshwater fishes in North America. Proceedings of the Helminthological Society of Washington 51:225–237.

Buckner, R. L., and B. B. Nickol. 1978. Redescription of *Fessisentis vancleavei* (Hughes and Moore 1943) Nickol 1972 (Acanthocephala: Fessisentidae). Journal of Parasitology 64:635–637.

Dyer, W. G., and R. A. Brandon. 1973. Helminths from salamanders in Oklahoma and Missouri. Herpetologica 29:371–373.

Fried, B., and R. S. Koplin. 1967. Morphological variation of *Fessisentis vancleavi* Haley and Bullock 1953 (Acanthocephala) from the white sucker, *Catostomus commersoni* (Lacepede). Proceedings of the Pennsylvania Academy of Science 40: 53–58.

Grabda-Kazubska, B. 1962. On the validity of the species *Acanthocephalus falcatus* (Frolich, 1789). Acta Parasitologica Polonica 10:377–394.

Haley, A. J., and W. L. Bullock. 1953. A new species of Acanthocephala from the sunfish, *Lepomis gibbosus* (Linnaeus), with a redescription of the

family Fessisentidae Van Cleave, 1931. American Midland Naturalist 50:202–205.

Holl, F. J. 1932. The ecology of certain fishes and amphibians with special reference to their helminths and linguatilid parasites. Ecological Monographs 2:84–107.

McAlpine, D. F. 1996. Acanthocephala parasitic in North American amphibians: a review with new records. Alytes 14. In press.

Nickol, B. B. 1969. Acanthocephala of Louisiana caudata with notes on the life-history of *Centrorhynchus conspectus*. American Midland Naturalist 81: 262–265.

———. 1972. *Fessisentis*, a genus of Acanthocephalans parasitic in North American poikilotherms. Journal of Parasitology 58:282–289.

Rankin, J. S. 1937. An ecological study of the parasites of some North Carolina salamanders. Ecological Monographs 7:170–269.

Stiles, C. W. and A. Hassall. 1894. A preliminary catalogue of the parasites contained in the collections of the United States Bureau of Animal Industry, United States Army Medical Museum, Biology Department of the University of Pennsylvania (coll.Leidy) and in the coll. Stiles and coll. Hassall. Veterinary Magazine 1:245–354.

Van Cleave, H. J. 1915. Acanthocephala in North American Amphibia. Journal of Parasitology 1: 175–178.

———. 1931a. Acanthocephala in North American Amphibia II. A new species of the genus *Acanthocephalus*. Transactions of the American Microscopical Society 50:46–47.

———. 1931b. New Acanthocephala from fishes of Mississippi and a taxonomic reconsideration of forms with unusual numbers of cement glands. Transactions of the American Microscopical Society 50:348–363.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 148–153

Searching for Enzymatic Targets of Antiparasitic Drugs in *Trichinella spiralis* Larvae

ELŻBIETA WANDURSKA-NOWAK, KRYSTYNA BOCZOŃ, AND EDWARD HADAŚ
Department of Biology and Medical Parasitology, Karol Marcinkowski University of Medical Sciences, 61-701 Poznań, Fredry 10, Poland

ABSTRACT: We examined the influence of closantel and benzothiazole derivatives on selected enzymes of bioenergetic metabolism of *Trichinella* larvae (oxidases, mtATPase, and malic enzyme (ME)) on the ability of larvae to invade a new host after *in vitro* incubation with these drugs. We observed their strong influence on the motility of *T. spiralis* larvae due to a paralyzing effect. Benzothiazole derivatives CGP 20376 and CGP 20308 presented the strongest inhibitory effect on the ability of *T. spiralis* larvae to invade the new host. Closantel inhibited the *Trichinella* oxidases, with no influence on the activity of ME. On the other hand, both CGPs inhibited ME but in higher concentrations (IC_{50} = 95 and 67 μM, respectively). Closantel, CGP 20376, and CGP 21835 also changed the shape of the curve of ME saturation with substrate from "double sigmoidicity" characteristic for allosteric enzymes to a hyperbolic characteristic for monomeric enzymes.

KEY WORDS: *Trichinella spiralis*, succinate- and NADH-oxidases, mtATPase, malic enzyme (ME), closantel, benzothiazole derivatives (Ciba-Geigy Products [CGP]).

Because trichinellosis constitutes an epidemiological problem as an important zoonotic parasitosis widespread on all continents, the search for new drugs acting in *Trichinella* larvae in defined targets is suitable and important. Some suitable targets of anthelmintics may be located in the pathways of larval energy metabolism (Vanden Bossche, 1990).

The bioenergetic metabolism of *Trichinella* has been the object of studies in our laboratory for some years (Boczoń, 1985). There have been, to date, limited attempts to evaluate the role of the enzymes of bioenergetic metabolism in *Trichinella* as a possible target for anthelmintics from the group of benzimidazoles and levamizole (Boczoń, 1976; Boczoń *et al.,* 1984; Criado *et al.,* 1990; Boczoń *et al.,* 1991; Jimenez-Gonzales *et al.,* 1991). The purpose of this research was to determine whether selected enzymes of the electron transport chain (succinate- and NADH-oxidases), mtATPase (ATP hydrolase, EC 3.6.1.4), and mitochondrial malic enzyme/(ME; L-malate : $NADP^+$ oxidoreductase [oxaloacetate-decarboxylating], EC 1.1.1.40) may potentially constitute targets for anthelmintics. Bearing in mind the fact that many drugs with recently elucidated modes of action seemed to be multimodal-action drugs, we undertook the investigation of the influence on *T. spiralis* larvae of 2 groups of drugs: closantel (salicylanilide), a well-known drug against *Fasciola hepatica*, and benzothiazole derivatives (Ciba-Geigy Products [CGP]) with antifilarial activity.

It has been suggested that a good chemotherapeutic effect could be achieved if the target enzyme is a regulatory one (Rew and Fetterer, 1986; Bryant and Behm, 1989). One of them is ME situated precisely at the key branchpoint of the metabolic pathways in helminths, a main source of reductive power for oxidative phosphorylation in mitochondria. The regulatory function of this enzyme has been already proven in *Ascaris suum* (Langsperger and Harris, 1976; Barrett, 1981; Bryant, 1982) and *Hymenolepis diminuta* (Barrett, 1981; McKelvey and Fioravanti, 1985) and suggested in *Trichinella* larvae (Boczoń, 1986). The tetrameric structure of NAD-dependent ME in *Ascaris* has already been elucidated (Allen and Harris, 1981). Conformation was based on the results of crystallographic studies of this enzyme (Clancy *et al.,* 1992). It is worthy to note that the tetrameric structure has also been demonstrated in the case of NADP-dependent ME from rat liver (Baker *et al.,* 1987). In helminths, ME is expected to be a target with a good specificity because its metabolic role in mammalian tissues is completely different than in helminths.

The choice of closantel was determined by the suggestion that it may act as an uncoupler of oxidative phosphorylation. Vanden Bossche *et al.,* (1979) proved in *in vivo* and *in vitro* a distinct impairment of oxidative phosphorylation by closantel and concluded that this agent may be an ionophore translocating proton inside the

Fasciola hepatica mitochondria. Recently, Boczoń *et al.* (1993) showed in their *in vivo* studies that, on the one hand, there is a reversible, inhibiting influence of closantel on the activity of mitochondrial NADP-specific ME in *Trichinella* larvae. On the other hand, they observed a simultaneous strong disintegration of outer membranes of the parasite's mitochondria, their osmotic swelling, and an increase in the number of elongated mitochondria. The last finding suggests uncoupling of oxidative phosphorylation in the mitochondria of *Trichinella* larvae, after *in vivo* administration of closantel.

The lack of a safe and reliable chemotherapeutic agent against larvae and adult worms of filariae prompted the World Health Organization to encourage investigation that use *Trichinella* as a suitable model for replacing filariae. The inhibitory influence of the drugs from the CGP group on the energy metabolism has already been demonstrated on the following parasites: adults of *Litomosoides carinii* (Benten *et al.*, 1987; Franz *et al.*, 1987; Davies *et al.*, 1989), adults and larvae of *Onchocerca volvulus* (Strote, 1989; Köhler *et al.*, 1992), adults of *Brugia malayi* (Benten *et al.*, 1987), and muscles of *Ascaris suum* (Davies *et al.*, 1989). Recently, a report on the efficacy of CGP 20376 against *B. malayi* microfilariae and infective-stage larvae was published (Green *et al.*, 1995).

Material and Methods

In this study, *Trichinella spiralis* strain MSUS/PO/60/ISS3 larvae isolated from Wistar rats were used. *In vitro* study was performed in triplicate in 1-ml culture plates in 0.9% NaCl to evaluate the effect of the drug (concentrations ranging from 10 μM to 1 mM) on *T. spiralis* larvae motility. Appropriate dilutions of CGPs in dimethyl sulfoxide (DMSO) were added to each culture plate (control larvae were incubated in a medium consisting of 0.5% DMSO), and observations were performed at 2 time intervals: after 1.5 and 24 hr of incubation. Mortality was determined on the basis of motility absence (larvae considered dead did not regain motility after washing out the drug). To confirm their death, biological testing was performed on the ability of *T. spiralis* larvae, treated *in vitro* for 24 hr with the appropriate drugs, to invade a new host in a group of 6 mice. The intensity of the infection in 1 g of muscles was estimated after digestion of the muscle tissue of those mice in pepsin/HCl solution.

A 15% homogenate from purified larvae, obtained in a medium consisting of 0.25 M sucrose, 0.03 M Tris-HCl (pH 7.3), and 0.5% BSA, was submitted to differential centrifugation for 6 min at 1,000 g and for 12 min at 12,000 g. The sediment after the second centrifugation, washed twice, was used as a fresh mitochondrial fraction for estimation of oxidases, mt-ATPase, and ME. The activity of succinate- and NADH-oxidases was performed by the method described by Estabrook (1967). Polarographic measurements were performed with the use of a Yellow Spring Co. oxygraph equipped with a Clark oxygen microelectrode. Respiration rate (QO_2) with substrate—5 mM succinate and 1 mM NADH—was measured at +30°C in a medium consisting of 0.125 M KCl, 0.02 M Tris-HCl (pH 7.3), 0.005 M KH_2PO_4, 0.003 M $MgCl_2$, and 0.0001 M EGTA. The activity of mt-ATPase was measured by the colorimetric micromethod described by Muszbek *et al.* (1977). The ATPase hydrolase activity was measured without the ATP regenerating system. Incubation with the drug was performed at +37°C for 25 min in a medium consisting of 0.05 M Tris-HCl (pH 7.3), 0.075 M KCl, 0.0004 M ATP, and 0.0002 M $MgCl_2$. The activity of ME in mitochondrial preparations frozen and thawed 3 times (to destroy mitochondrial membranes) was determined spectrophotometrically in the direction of malate decarboxylation according to the method described by Körting and Barrett (1977). The activity of ME was measured in a medium consisting of 0.06 M Tris-HCl (pH 7.3), 0.002 M $MnCl_2$, 0.0004 M NADP, and 0.008 M malate. Closantel and drugs from the CGP group were incubated before the estimation of the appropriate enzymatic activity with mitochondrial preparation for 10 min at +30°C.

The content of protein in the preparations was determined using the colorimetric method of Lowry *et al.* (1951).

Results and Discussion

As a starting point of our investigation, we checked *in vitro* the drugs' influence on the mortality of *T. spiralis* larvae. The lack of motility (larvae look like strongly coiled spirals or have strongly coiled ends of the body close to natural pores) due to a paralyzing effect of the drugs after 24 hr of incubation at room temperature was similar to the observations in *Onchocerca volvulus* (Comley *et al.*, 1989). To prove the mortality or just loss of ability to invade the next host (in the case of impairment of the generative system), biological testing with the use of a group of mice infected by the larvae incubated previously for 24 hr with appropriate drug was conducted. A statistically significant decrease in the number of new generation larvae (counted in skeletal muscles and in the diaphragm after digestion in artificial gastric juice) by 1 mM drugs was reached in the case of CGP 20376 and CGP 20308 (Fig. 1). The strongest effect on the ability of *Trichinella* larvae to invade the new host (mice) after incubation with a drug was shown by benzothiazole CGP 20308 (reduction of the new generation of the larvae in mice mus-

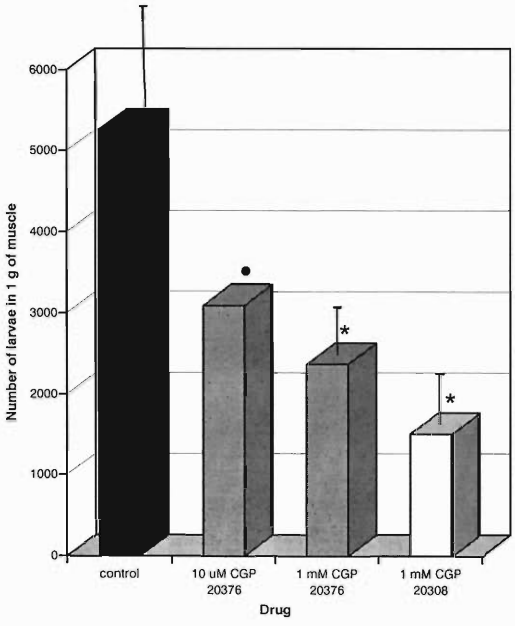


Figure 1. Intensity of the *Trichinella spiralis* infection in mice infected by *Trichinella* larvae after 24 hr of incubation with CGP 20376 and CGP 20308. * $p \leq 0.03$ in Wilcoxon test; • number of experiments = 3.

cle to 30% of the controls). A similar reduction of the number of *Trichinella* larvae was observed after *in vivo* treatment with thiabendazole in experimental trichinellosis (Kociçcka, 1971). It is worth mentioning that in parallel preliminary investigations of the influence of closantel (not presented here) the neuromuscular paralysis of *T. spiralis* larvae was the result of action of a 10 times higher (i.e., 10 mM) concentration of the drug.

Bearing in mind that, in the experiments performed up to date on other helminths, salicylanilides (e.g., closantel) and CGPs influenced the bioenergetic metabolism, in the next step of our investigations we checked *in vitro* their inhibitory effect on oxidases and mitochondrial ME. The lowest IC_{50} value was obtained for closantel and both oxidases (Table 1). CGPs do not inhibit oxidases in concentrations between 25 and 100 μM. On the other hand, the influence of closantel on ME was weak (maximal inhibition by 50 μM concentration of the drug = 40%). In the case of both CGPs, where biological testing exhibited the best activity against *T. spiralis* larvae, the influence on ME was stronger than that of closantel. As presented in Table 1, the strongest inhibitory effect on ME was noted in the case of CGP 20308, but the IC_{50} value was higher than that for oxidases (6.7×10^{-5} M).

Comparing the CGPs' concentrations effective in inhibition of *Trichinella* larvae motility and those inhibiting a particular enzyme (i.e., ME), one must remember that the effectiveness of the drug in the first situation is strongly dependent on the rate of drug penetration across the cuticule. Some authors assumed that the concentration of the drugs used in the *in vitro* experiment should be even 1,000 times higher than that of IC_{50} for a particular target enzyme. In our experiments on the *in vitro* inhibition of motility of *Trichinella* larvae, CGP 20308 concentration was just 15 times higher (1 mM) than IC_{50} for ME (about 67 μM).

The preceding results clearly show that closantel had the strongest influence, as was suggested for other helminths, on two enzymes of the electron transport chain, that is, on the bioenergetic metabolism of *T. spiralis*. The IC_{50} values were low enough to consider those enzymes

Table 1. Comparison of the effect of closantel, CGP 20376, CGP 20308, and CGP 21835 on the succinate-oxidases (SOX), NADH-oxidases (NOX), and NADP-specific malic enzyme (ME) in mitochondria from *Trichinella spiralis* larvae.*

Drug	SOX (IC_{50} [μM] ± SD)	NOX (IC_{50} [μM] ± SD)	ME (IC_{50} [μM] ± SD)
Closantel	$1.6 \pm 0.5_{(8)}$	$4.2 \pm 0.8_{(8)}$	50 μM caused 40% of inhibition $_{(24)}$
CGP 20376	50 μM caused 23% of inhibition	50 μM caused no inhibition	$95 \pm 24_{(7)}$
CGP 20308	n.d.	n.d.	$67 \pm 17_{(5)}$
CGP 21835	n.d.	n.d.	50 μM caused no inhibition

* IC_{50} = concentration of the drug expressed in μM causing 50% inhibition of the activity of enzyme; ( ) = number of measurements; n.d. = not determined; control activity of SOX (measured before addition of the drug) = $14.1 \pm 2.1_{(8)}$ nmoles O_2/min/mg of protein; control activity of NOX (measured before addition of the drug) = $6.8 \pm 1.5_{(8)}$ nmoles O_2/min/mg of protein; control activity of ME (measured before addition of the drug) = $57.1 \pm 31.0_{(6)}$ nmoles NADP/min/mg of protein.

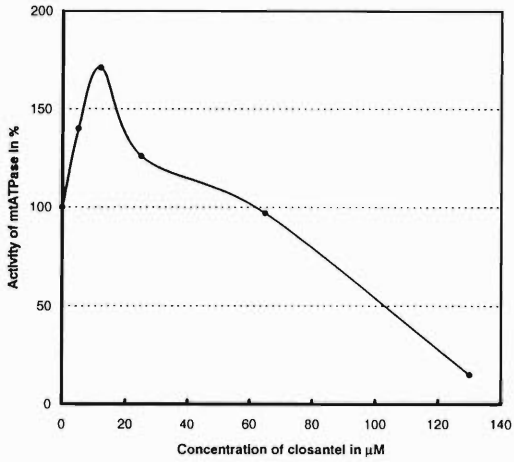


Figure 2. The influence of closantel on activity of the mtATPase in *Trichinella spiralis* mitochondria. 100% = control activity of mtATPase (measured before addition of the drug) = 26 nmoles Pi/min/mg of protein. Each point is the mean of 3 experiments.

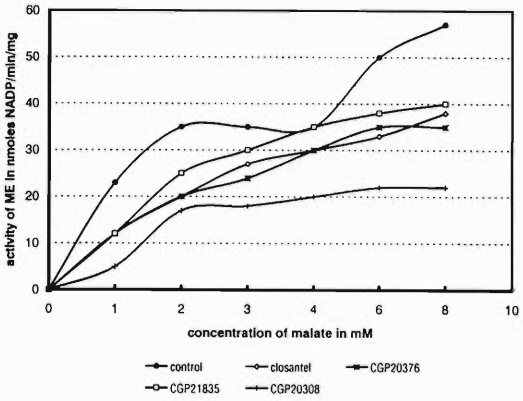


Figure 3. Substrate (malate) saturation curves of NADP-specific ME in mitochondrial fraction from *Trichinella spiralis* larvae after incubation with 100 µM CGP 20376, CGP 20308, and closantel (time of incubation 10 min, temperature +30°C). Each point is the mean of 3 experiments.

as potential targets for the drug. To prove its uncoupling activity, we performed a series of experiments with the influence of closantel on mtATPase of *T. spiralis* larvae (Fig. 2). The activity of this enzyme was previously shown to be slightly enhanced by mebendazole, thiabendazole, and levamisole (Boczoń *et al.*, 1991). The last authors concluded that, despite the fact that *Trichinella* mtATPase seemed to be more sensitive to anthelmintics than rat liver mtATPase, their effect on the parasite's mtATPase might be secondary. A weak stimulation (70%) of the activity of mtATPase hydrolase by closantel was observed in these investigations, but it is worthy to note that 2,4-dinitrophenol (used as a positive control) stimulated this enzyme only in a similar degree (100%). The activity of this enzyme was measured in properly isolated mitochondria, which was proved by relatively low activity of mtATP hydrolase (26 nmoles of P_i/min/mg of protein) and 90% inhibition by oligomycin in a dose of 1–2 ng/mg of protein (unpubl. data). The fact of slight stimulation of *Trichinella* mtATPase by drugs, as explained in our previous paper (Boczoń *et al.*, 1991), was probably a result of their "dual" effect on energy conservation. The preceding results may suggest that for closantel a bioenergetic metabolism of *Trichinella* might be a target, but additional experiments on the energy transduction process may elucidate this problem.

The curve of saturation with substrate (malate) of control ME in *T. spiralis* larvae presented in Figure 3 indicates the irregularities: double sigmoidicity, characteristic for allosteric enzymes with "frozen" subunits (Kurganov, 1975). The well-known fact of a 4-subunit structure of the ME from both mammalian tissue and *Ascaris* justifies the assumption that characteristics of association–dissociation allosteric systems could be applied for *Trichinella* ME. The plots of initial velocity against substrate concentration in "frozen" associating enzyme systems (oligomeric enzyme forms) similar to that type are characterized by rather complex shape, that is, an intermediate plateau in the curve. After incubation of *Trichinella* ME with 100 µM closantel, CGP 21835, and CGP 20376, this curve changed the shape to a hyperbolic curve, similar to the one characteristic for monomeric enzymes. Apart from the weak inhibition of *Trichinella* ME by closantel, CGP 20376, and CGP 21835 ($IC_{50} > 5 \times 10^{-5}$ M), the clear change in the shape of the curve of saturation with the substrate may be evidence of the influence of these drugs on the enzyme in such a way that it loses its subunit structure. Repetitions of these intriguing results after purification of *Trichinella* ME are planned in our laboratory.

Acknowledgment

We thank Ciba-Geigy Ltd. for providing all CGP compounds synthesized in the Research Department of Ciba-Geigy, Basel, Switzerland.

Literature Cited

Allen, B. L., and B. H. Harris. 1981. Purification of malic enzyme from *Ascaris suum* using NAD-agarose. Molecular and Biochemical Parasitology 2: 367–372.

Baker, P. J., D. H. Thomas, C. H. Barton, D. W. Rice, and E. Bailey. 1987. Crystallization of an NADP-dependent malic enzyme from rat liver. Journal of Molecular Biology 193:233–235.

Barrett, 1981. Metabolic Regulation. *In* Biochemistry of Parasitic Helminths. McMillan Publishers, London. 262 pp.

Benten, W. P. M., M. Franz, H. Zahner, and H. P. Striebel. 1987. Early alterations of the fine structures in adult *Brugia malayi* and *Litomosoides carinii* after *in vivo* treatment with CGP 20376. Tropical Medicine and Parasitology 38:61.

Boczoń, K. 1976. Bioenergetics of *Trichinella spiralis* larvae and effect of some anthelmintics on succinate dehydrogenase of *Trichinella spiralis* mitochondria. Pages 589–597 *in* H. Vanden Bossche, ed. Biochemistry of Parasites and Host–Parasite Relationships. Elsevier Biomedical Press, Amsterdam.

———. 1985. Bioenergetics of *Trichinella spiralis* and *Trichinella pseudospiralis*. Wiadomości Parazytologiczne 31:237–251.

———. 1986. The role of malic enzyme in the carbohydrate metabolism of *Trichinella spiralis* and *Trichinella pseudospiralis*. International Journal for Parasitology 16:435–440.

———, **N. Casado, F. Rodriguez-Caabeiro, A. Criado-Fornelio, M. BIotna, and A. Ramisz.** 1993. The *in vivo* influence of closantel on *Trichinella spiralis* malic enzyme and ultrastructure of mitochondria. Pages 67–72 *in* W. C. Campbell, E. Pozio, and F. Bruschi, eds. Proceedings of the 8th International Conference on Trichinellosis. Instituto Superiore di Sanita Press, Rome, Italy.

———, **W. Olba, and M. Olaszek.** 1984. The influence of some anthelmintics on the bioenergetic metabolism of *Trichinella spiralis* and *Trichinella pseudospiralis*. Biochemical Pharmacology 33: 2523–2525.

———, **Z. Uszyńska, F. Rodriguez-Caabeiro, A. Criado-Fornelio, and C. Armas-Serra.** 1991. The effect of some anthelmintics on *Trichinella spiralis* and *Trichinella pseudospiralis* mitochondrial energy-generating pathways. Research and Reviews in Parasitology 51:61–63.

Bryant, C. 1982. Pathways of energy metabolism in helminths. Pages 122–124 *in* D. F. Metrick and S. S. Desser, eds. Parasites—Their World and Ours. Elsevier Biomedical Press, Amsterdam.

———, **and C. Behm.** 1989. Energy Metabolism. Pages 55–56 *in* C. Bryant and C. A. Behm, eds. Biochemical Adaptation in Parasites. Chapman and Hall, London, New York.

Clancy, L. L., G. S. J. Rao, B. C. Finzel, S. W. Muchmore, D. R. Holland, K. D. Watenpough, H. M. Krishnamurthy, R. M. Sweet, P. F. Cook, B. G. Harris, and H. M. Einspahr. 1992. Crystallization of the NAD-dependent malic enzyme from the parasitic nematode *Ascaris suum*. Journal of Molecular Biology 226:565–569.

Comley, J. C., S. Townson, M. J. Rees, and A. Dobinson. 1989. The further application of MTT-formazan colorimetry to studies on filarial worm viability. Tropical Medicine and Parasitology 40: 311–316.

Criado-Fornelio, A., C. Armas-Serra, A. Jimenez-Gonzalez, N. Casado-Escribano, and F. Rodriguez-Caabeiro. 1990. Biochemical effects of luxabendazole on *Trichinella spiralis*. Parasitology Researches 76:518–520.

Davies, K. P., H. Zahner, and P. Köhler. 1989. *Litomosoides carinii*: mode of action *in vitro* of benzothiazole and amoscanate derivatives with antifilarial activity. Experimental Parasitology 68: 382–391.

Estabrook, R. W. 1967. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. Pages 41–47 *in* R. W. Estabrook and M. E. Pullman, eds. Methods in Enzymology. Vol. X. Academic Press, New York and London.

Franz, M., H. Zahner, H. P. Striebel, and L. Langenstrassen. 1987. Morphological alterations in female *Litomosoides carinii* after treatment with two benzothiazole derivatives *in vivo*. Tropical Medicine and Parasitology 38:1–7.

Green, D. F., K. L. Yates, and J. A. Yates. 1995. Filaricidal activity of CGP 20376 against *Brugia malayi* microfilariae, larvae and adults. Journal of the Helminthological Society of Washington 62: 217–222.

Jimenez-Gonzales, A., C. Armas-Serra, A. Criado-Fornelio, N. Casado-Escribano, F. Rodriguez-Caabeiro, and J. C. Diez. 1991. Preliminary characterization and interaction of tubulin from *Trichinella spiralis* larvae with benzimidazole derivatives. Veterinary Parasitology 39:89–99.

Kocięcka, W. 1971. Behaviour of *Trichinella spiralis* larvae with animals treated by thiabendazole and hydrocortisone. Wiadomości Parazytologiczne 17: 625–639.

Köhler, P., K. P. Davies, and H. Zahner. 1992. Activity, mechanism of action and pharmacokinetics of 2-tert-butylbenzothiazole and CGP 6140 (amocarzine) antifilarial drugs. Acta Tropica 51:195–211.

Körting, W. and J. Barrett. 1977. Carbohydrate catabolism in the plerocercoids of *Schistocephalus solidus*. International Journal for Parasitology 7: 411–417.

Kurganov, B. J. 1975. Regulatory properties of slowly equilibrating association–dissociation enzyme system. Pages 29–42 *in* T. Kaleti, ed. Proceedings of the 9th FEBS Meeting. Akademiai Kiado, Budapest, Hungary.

Langsperger, W. J., and B. G. Harris. 1976. NAD-malic enzyme. Regulatory properties of malic en-

zyme from the muscle tissue of *Ascaris suum*. Journal of Biological Chemistry 251:3599–3602.

Lowry, O., N. J. Rosenbrough, A. L. Farr, and J. R. Randal. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265–275.

McKelvey, J. R., and C. F. Fioravanti. 1985. Intramitochondrial localization of fumarate reductase, NADPH-NAD transhydrogenase, malic enzyme and fumarase in adult *Hymenolepis diminuta*. Molecular and Biochemical Parasitology 17: 253–263.

Muszbek, L., T. Szabo, and L. Fesus. 1977. A highly sensitive method for the measurement of ATPase activity. Analytical Biochemistry 77:286–288.

Rew, R. S., and R. H. Fetterer. 1986. Mode of action of antinematodal drugs. Pages 321–337 *in* W. C. Campbell and R. S. Rew, eds. Chemotherapy of Parasitic Diseases. Plenum Press, New York.

Strote, G. 1989. Electron microscopic studies on the effects of CGP 6140 and CGP 20376 on microfilariae and third stage larvae of *Onchocerca volvulus*. Tropical Medicine and Parasitology 40: 304–310.

Vanden Bossche, H. 1990. Studies of the mode of action of anthelmintic drugs: tools to investigate the biochemical pecularities of helminths. Annales de Parasitologie Humaine et Comparee 65(supplement I): 99–102.

———, **H. Verhoeven, O. Vanparijs, H. Lauwers, and D. Thienpont.** 1979. Closantel, a new antiparasitic hydrogen ionophore. Archives Internationales de Physiologie et de Biochimie 87:851–852.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 154–156

Research Note

Absence of Hematozoa from Ferruginous Pygmy-Owls (*Glaucidium brasilianum*) in Southern Texas

GLENN A. PROUDFOOT AND ANDREW A. RADOMSKI
Caesar Kleberg Wildlife Research Institute, Campus Box 218, Texas A&M University–Kingsville, Kingsville, Texas 78363

ABSTRACT: Blood smears were examined from 63 (14 females, 45 males, 4 nestlings) ferruginous pygmy-owls (*Glaucidium brasilianum*) captured during 1994–1996 in southern Texas. Of these, no hematozoa were observed. Absence of hematozoans may be a result of low vector abundance, low and chronic infections below levels of detection, an overdispersion of hematozoa masking the actual prevalence rates, or an innate ability of pygmy-owls to avoid blood-parasite infections.

KEY WORDS: Hematozoa, ferruginous pygmy-owl, *Glaucidium brasilianum*, Texas.

No hematozoa data are available for ferruginous pygmy-owls (*Glaucidium brasilianum*, Gmelin) in North America. Therefore, in conjunction with natural history studies (Proudfoot, 1996) on the threatened ferruginous pygmy-owl in southern Texas, blood from 63 owls (14 females, 45 males, 4 nestlings) was collected to determine the prevalence of hematozoa. This represents about 10% of the population (Proudfoot, unpubl. data).

Hematozoa are probably pathogenic in their natural host, although little is known about the physiological, behavioral, and ecological costs (Ewald, 1983; Atkinson and Van Riper, 1991). Hematozoa in owls may cause marginal anemia, neonatal bacterial diarrhea, and septicemia (Hunter et al., 1987). Although subclinical, the attritional effect of blood parasites may reduce survivability and recruitment or have no effect on the host (Davidar and Morton 1993). Understanding the factors affecting population dynamics of endangered or threatened species is critical for the conservation of these species.

The exoerythrocytic stage of *Haemoproteus* sp. target capillary endothelial cells, fibroblasts, and muscle tissue, while the gametocytes are within the peripheral blood (Couch, 1952). Because gametocytes are the infective stage for vectors, transmission is the culmination of a number of factors including survival, reproduction, and development within the vector, vector behavior, and the vector–bird association (Allan and Mahrt, 1989). Vectors for *Haemoproteus* spp. and *Leucocytozoon* spp. include the hippoboscid flies and ceratopogonid flies (*Culicoides* spp.) (Atkinson, 1991). Although we did not determine abundance of ornithophilic flies, hippoboscids were collected from adult pygmy-owls and nestlings and have been reported within the ecoregion of this study (Stabler, 1960).

Bennett et al. (1982) reported *G. brasilianum* as a host to *Haemoproteus* sp., *H. glaucidiumi* (Jorg, 1931), *Leucocytozoon* sp., and *L. lutzi* (Carini, 1920) in its southern range. A comprehensive study of avian hematozoa in Sao Paulo State, Brazil (Woodworth-Lynas et al., 1989) reported 8% of 121 species (32 families) infected. The only *G. brasilianum* observed was negative. Prevalence of hematozoa in 9 species of autumnal migrant raptors was reported at 75% (88/118) in the central U.S. flyway (Taft et al., 1996). The most common hematozoa were *L. toddi* (Sambon, 1908) and *Haemoproteus* sp. Incidence of hematozoa in 17 avian species in Texas was reported at 2.3% (Couch, 1952). The majority of these were *Haemoproteus* sp. infecting English sparrows, *Passer domesticus* (12/123), mourning doves, *Zenaida macroura* (201/213), and American kestrels, *Falco sparverius* (7/8).

Research was conducted within a 29,000-ha live oak (*Quercus virginiana*)–honey mesquite (*Prosopis glandulosa*) forest on the Norias Division of the King Ranch, Kenedy County, Texas (26°37′30″–26°51′30″N, 97°27′30″–97°43′30″W). The climate is subtropical with 68 cm mean annual precipitation and 24°C mean annual temperature (National Oceanic and Atmospheric Administration, 1995). Owls were collected from 10 March 1994 to 22 March 1996. Mean annual precipitation was 42.7, 92.6, and 10.16 cm in 1994, 1995, and January to June 1996, respectively.

Nylon mist nets and baited bow nets were used to capture adult ferruginous pygmy-owls from 10 March 1994 to 22 March 1996. Forty-one (65%) owls were captured during the spring months (January to June). Samples were collected 1 hr before sunset to 1 hr after sunset. Body mass was determined using a 300 g ± 3% pesola scale (Pesola Precision Scales, Switzerland). Wing chord, tail length, and total body length using a flexible ruler and measured for tarsus length with a dial caliper (505-101 Milutoyo). Owls were fitted with U.S. Fish and Wildlife Service aluminum leg bands and released after blood collection. Similar measurements were taken from 4 nestlings (4–7 days before fledgling) from 1 active nest box on 14 June 1995 between 0800 and 1000 hours.

To avoid injury and reduce stress, pygmy-owls were secured in 13- × -3.8-cm tubes and blood sample protocol followed Bennett (1970). Kwik-stop® (Gimborn-Rich Health, Atlanta, Georgia) or silver nitrate was applied to stop the bleeding. Thin blood smears were separated into 2 sets of 126 slides. One set of slides was viewed at Caesar Kleberg Wildlife Research Institute, and the other was sent to Dr. Gordon Bennett of the International Reference Center for Avian Haematozoa for verification. Slides were stained and examined as described by Bennett (1970).

Neither laboratory observed hematozoa. These results suggest this ferruginous pygmy-owl population is not affected by blood parasites. Possible causes for negative findings involve the host–parasite interaction: (1) the inability of pygmy-owls to maintain an infection, (2) infection rates are too low for observing blood parasites, (3) infection is highly virulent and lethal, and (4) overdispersion is occurring and the number of birds observed was insufficient to detect hematozoans. However, parasitemia has been reported in the southern range. This study was conducted continuously over 2 yr and all season, including wet periods. No nestling mortality nor abrupt perturbations in population occurred and an estimated 8–9% of the population was sampled, including 4 nestlings (Proudfoot, unpubl. data).

Other causes for the negative findings may involve vector–host interactions: (1) low vector abundance and prevalence or (2) pygmy-owls have an innate ability to avoid blood-parasite infections. However, hippoboscids were observed on adult pygmy-owls and collected from nestlings. And the ferruginous pygmy-owl is ecologically and behaviorally similar to other small strigiformes.

Ecological and physiological data remains limited on the ferruginous pygmy-owl. This information may aid ferruginous pygmy-owl management by directing resources toward demographic studies and other areas of research including vector ecology and immunological studies of the ferruginous pygmy owl.

We dedicate this manuscript to the memory of Gordon F. Bennett. We thank F. Chavez-Ramirez, R. T. Kazmaier, F. S. Guthery, S. A. Smith, and 2 anonymous reviewers for commenting on this manuscript. We thank M. Garvin, R. Rosenfield, and G. Jacobs for their assistance in conducting literature searches. Funding was provided by King Ranch Inc., Exxon Corp., Texas Wildlife Association, and the National Fish and Wildlife Foundation. This manuscript is Welder Wildlife Foundation No. 479.

Literature Cited

Allan, R. A., and J. L. Mahrt. 1989. Influence of transmission period on primary and relapse patterns of infection of *Leucocytozoon* spp. and *Haemoproteus mansoni*. American Midland Naturalist 121:341–349.

Atkinson, C. T. 1991. Vectors, epizootiology, and pathogenicity of avian species of *Haemoproteus* (Haemosporina: Haemoproteidae). Bulletin of the Society of Vector Ecology 16:109–126.

———, **and C. Van Riper III.** 1991. Pathogenicity and epizootiology of avian hematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. Pages 19–48 *in* J. E. Loye and M. Zuk, eds. Bird–Parasite Interactions—Ecology, Evolution, and Behavior. Oxford University Press, Oxford.

Bennett, G. F. 1970. Simple techniques for making avian blood smears. Canadian Journal of Zoology 48:585–586.

———, **M. Whiteway, and C. Woodworth-Lynas.** 1982. Host parasite catalog of avian hematozoa. Memorial University, Newfoundland Occasional Papers in Biology, No. 5.

Couch, A. B. 1952. Blood parasite of some common Texas birds. Field and Laboratory 20:146–154.

Davidar, P., and E. S. Morton. 1993. Living with parasites: prevalence of a blood parasite and its effect on survivorship in the purple martin. Auk 110:109–116.

Ewald, P. W. 1983. Host–parasite relations, vectors, and the evolution of disease severity. Annual Review of Ecological Systems 14:465–485.

Hunter, B. D., K. McKeever, L. McKeever, and G. Crawshaw. 1987. Disease susceptibility in owls. Pages 67–70 *in* R. W. Nero, R. J. Clark, R. J. Knapton, and R. H. Hamre, eds. Biology and Con-

servation of Northern Forest Owls. U.S. Forest Service General Technical Report RM-142. Rocky Mountain Forest and Range Experimental Station. Fort Collins, Colorado.

National Oceanic and Atmospheric Administration. November 1995. Climatological Data, Texas. Vol. 100. Asheville, North Carolina.

Proudfoot, G. A. 1996. Natural history of the cactus ferruginous pygmy-owls. M.S. Thesis, Texas A&M University–Kingsville, Kingsville. 84 pp.

Stabler, R. M. 1960. A parasitological survey of fifty-one eastern white-winged doves. Journal of Parasitology 47:309–311.

Taft, S. J., R. N. Rosenfield, and D. L. Evans. 1996. Hematozoa in autumnal migrant raptors from the Hawk Ridge Nature Reserve, Duluth, Minnesota. Journal of Helminthological Society of Washington 63:141–143.

Woodworth-Lynas, C. B., J. R. Caines, and G. F. Bennett. 1989. Prevalence of avian hematozoa in Sao Paulo State, Brazil. Memorial Instituto Oswaldo Cruz, 84:515–526.

J. Helminthol. Soc. Wash.
64(1), 1997 pp. 157–159

Research Note

Prevalence of Larval Trematodes in *Helisoma trivolvis* (Gastropoda) from a Farm Pond in Northampton County, Pennsylvania with Special Emphasis on *Echinostoma trivolvis* (Trematoda) Cercariae

KATHARINE A. SCHMIDT AND BERNARD FRIED[1]
Department of Biology, Lafayette College, Easton, Pennsylvania 18042

ABSTRACT: Occurrence of larval trematodes and seasonal prevalence of *Echinostoma trivolvis* in *Helisoma trivolvis* snails from a farm pond in Northampton County, Pennsylvania, were investigated from 24 May to 31 October 1995. Of 1,841 *H. trivolvis* snails (7–20 mm shell diameter), 589 were infected based on snail isolation data. Prevalence data showed that 457 (24.8%) released cercariae of *Echinostoma trivolvis*, 52 (2.8%) released cercariae of *Zygocotyle lunata*, 46 (2.5%) released an unidentified species of armatae cercariae, 26 (1.4%) released the psilostome cercariae of *Ribeiroia* sp., 5 (0.3%) released 2 unidentified species of brevifurcate-apharyngeate cercariae, and 3 (0.2%) released the cystophorous cercariae of *Halipegus occidualis*. The percentage increase in prevalence of *E. trivolvis* was greater than 2-fold in the July versus June collections. Previous reports on larval trematode infections in *H. trivolvis* are discussed.

KEY WORDS: *Helisoma trivolvis*, Gastropoda, Trematoda, *Echinostoma trivolvis*, seasonal prevalence, cercariae, larval trematodes.

Helisoma trivolvis (Say, 1816) is a ubiquitous planorbid snail in North America and is infected with a variety of larval trematodes (Friesen, 1981). Rosen et al. (1994) reported prevalence of 3 species of digenetic trematodes, *Echinostoma trivolvis* (Cort, 1914), *Cephalogonimus vesicaudus* Nickerson, 1912, and *Spirorchis scripta* Stunkard, 1923, in *H. trivolvis* at Owsley Fork Reservoir in Kentucky. They tested the prediction that autogenic species of trematodes (those that complete their life cycles in hosts living almost exclusively within the pond) would be more prevalent than allogenic species (those that complete their life cycles in hosts that are not always present at the pond). They found that, contrary to their hypothesis, the allogenic species *E. trivolvis* was the most prevalent species.

Echinostoma trivolvis uses *H. trivolvis* as its first and second intermediate hosts (Kanev et al., 1995). This snail has been collected from a farm pond in Northampton County, Pennsylvania, by one of us (B.F.) for more than 20 yr to obtain larval stages of *E. trivolvis* for laboratory studies on this echinostome. Other species of larval trematodes were also observed, but no records of the species, their relative abundance, or the seasonal prevalence of *E. trivolvis* were kept. The purpose of this study was to determine which species were present at the study site, calculate the overall abundance of all species found, and observe the pattern of *E. trivolvis* larval prevalence in the snail population during a 6-mo period.

Helisoma trivolvis snails were collected biweekly from a farm pond 4 mi north of Bath, Pennsylvania, and 1 mi northwest of Klecknersville, Pennsylvania, at 75°27′15″West, 40°47′20″North. Snails were collected from 24 May to 31 October 1995 ($\bar{x}$ = 184 per collection; range 59–380) and were taken from the perimeter of the pond, no more than 1.5 m from the edge. The snails were isolated to determine infection with larval trematodes within 48 hr of collection by placing them individually in Stender dishes containing 5 ml of artificial spring water prepared according to Ulmer (1970). Two 50-watt bulbs were placed approximately 30 cm from the dishes to maintain the snails at 28–29°C. Each dish was examined up to 4 hr after snail isolation for cercariae. Live cercariae were examined unstained or stained with 0.01% neutral red and some were also fixed in cold neutral-buffered formalin and mounted in glycerin jelly to aid in specific or generic identification. To approximate the number of infections missed by the isolation procedure, 20% of the isolated negative snails were crushed and examined for larval trematodes.

Voucher specimens have been deposited in the University of Nebraska State Museum, Harold W. Manter Laboratory, Lincoln, Nebraska (HWML 39074–39079).

[1] Corresponding author (e-mail: friedb@lafvax.lafayette.edu).

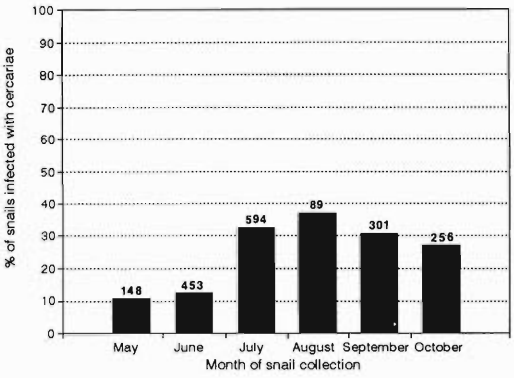


Figure 1. Percentage of snails infected with *Echinostoma trivolvis* from May to October 1995. The number over the bar equals the sample size for that month.

A total of 1,841 *Helisoma trivolvis* snails ranging in shell diameter from 7 to 20 mm was collected, and 7 species of larval trematodes were found.

Cercarial infections in snails based on isolation were as follows: 457 (24.8%) with the echinostome cercaria, *Echinostoma trivolvis*; 52 (2.8%) with the amphistome cercaria, *Zygocotyle lunata* (Diesing, 1836); 46 (2.5%) with an unidentified species of armatae cercariae; 26 (1.4%) with the psilostome cercaria, *Ribeiroia* sp.; 3 (0.2%) with the cystophorous cercaria, *Halipegus occidualis* Stafford, 1905; 3 (0.2%) with brevifurcate-apharyngeate cercariae with tail finfolds; and 2 (0.1%) with brevifurcate-apharyngeate cercariae without tail finfolds.

The percentage of infection of the most prevalent trematode, *E. trivolvis* was calculated on a monthly basis (Fig. 1). A greater than 2-fold increase in prevalence was observed from June (12.5%) to July (32.6%). A slight decrease in prevalence was observed in September (30.9%) and October (27.1%) compared to August (37.1%).

Necropsies of 250 snails that were negative based on isolation showed that 92 (36.8%) harbored larval trematodes. No double infection was found in any snail based on both isolation and necropsy data, probably due to the low prevalence of larval trematodes other than *E. trivolvis*.

Rosen et al. (1994) recorded the prevalence of *Echinostoma trivolvis*, *Cephalogonimus vesicaudus*, and *Spirorchis scripta* from *Helisoma trivolvis* snails in a reservoir in Kentucky. We found a greater diversity of larval trematodes in a single location and sharing the same snail host than in the aforementioned study. As in Rosen et al. (1994), *E. trivolvis* was the most prevalent species in the farm pond in Northampton County, Pennsylvania. However, we did not observe the midsummer decline in this species that was reported by Rosen et al. (1994). The increased prevalence of *E. trivolvis* infections from June to July can probably be explained by the development of infections to patency in late spring to early summer. The decreased prevalence of *E. trivolvis* in the fall was possibly due to death of infected snails and/or loss of the infection. Rosen et al. (1994) suspected that the decreased prevalence in *E. trivolvis* and *C. vesicaudus* was due to the entry of large numbers of uninfected snails in the population. We have no evidence to confirm either suggestion as the reason for the decreased prevalence of *E. trivolvis*.

The necropsy data on purported uninfected snails reflect the fact that some infections were not yet patent when the snails were isolated or that cercariae were not released on the day of isolation. Cercarial release from snails, as shown by Schmidt and Fried (1996) for *E. trivolvis* from *H. trivolvis*, did not always occur on a daily basis.

Observations on the brevifurcate-apharyngeate cercariae from the 5 snails infected with this larval type suggested the presence of 2 different schistosome-like species. The cercaria with finfolds was probably a turtle blood fluke and the cercaria without finfolds was possibly an avian or mammalian schistosome. Rosen et al. (1994) noted the presence of cercariae of the turtle blood fluke, *S. scripta* from *H. trivolvis* in Kentucky.

The cercaria of *Ribeiroia* sp. may be *R. thomasi*, a species previously described as *Psilostomum ondatrae* by Beaver (1939) from the snail *H. antrosum percarinatum*. Previous reports on cercariae of *Zygocotyle lunata* in *Helisoma* snails include those of Willey (1936) on this species in *H. antrosum* and Fried (1970) on this species in *H. trivolvis*. *Halipegus occidualis* larval infections have been reported from *H. anceps* snails by Goater et al. (1989).

We have no idea what the species of the armatae cercaria is. According to Schell (1985), armatae cercariae occur in the families Plagiorchidae, Auridistomidae, Cephalogonimidae, Telorchiidae, and Ochetosomatidae. Rosen et al.

(1994) noted the presence of *C. vesicaudus* (Cephalogonimidae) cercariae in *H. trivolvis* from Kentucky. Acholonu (1968) reported the occurrence of xiphidiocercariae in 4 (4.3%) of 94 *H. trivolvis* collected in Northern Colorado.

Acknowledgments

We thank Dr. Eric Wetzel, Department of Biology, Wabash College, Crawfordsville, Indiana, for his advice on armatae cercariae. We thank Professor Ivan Kanev, Institute of Parasitology, Bulgarian Academy of Sciences, Sofia, Bulgaria, for confirming the identity of *Ribeiroia* sp. based on examination of cercariae fixed in neutral-buffered formalin.

Literature Cited

Acholonu, A. D. 1968. Studies on the freshwater cercariae of Northern Colorado. Proceedings of the Helminthological Society of Washington 35:259–271.

Beaver, P. C. 1939. The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda: Psilostomidae). Journal of Parasitology 25:383–393.

Fried, B. 1970. Infectivity, growth, development, excystation, and transplantation of *Zygocotyle lunata* (Trematoda) in the chick. Journal of Parasitology 56:44–47.

Friesen, M. K. 1981. *Helisoma trivolvis* (Say). Pages 23–30 *in* S. G. Lawrence, ed. Manual for the Culture of Selected Freshwater Invertebrates. Canadian Special Publication in Fish and Aquatic Sciences, Ottawa, Canada.

Goater, T. M., A. W. Shostak, and J. A. Williams. 1989. A mark–recapture study of trematode parasitism in overwintered *Helisoma anceps* (Pulmonata) with special reference to *Halipegus occidualis* (Hemiuridae). Journal of Parasitology 75: 553–560.

Kanev, I., B. Fried, V. Dimitrov, and V. Radev. 1995. Redescription of *Echinostoma trivolvis* (Cort, 1914) (Trematoda: Echinostomatidae) with a discussion on its identity. Systematic Parasitology 32:61–70.

Rosen, R. B., J. M. Ilagan, J. S. Law, M. Asuncion, M. E. Denton, and M. L. San. 1994. Seasonal prevalence of three species of digenetic trematodes in the snail *Helisoma trivolvis* at Owsley Fork Reservoir, Kentucky. Transactions of the Kentucky Academy of Science 55:32–35.

Schell, S. C. 1985. Handbook of Trematodes of North America, North of Mexico. University Press of Idaho, Moscow. 263 pp.

Schmidt, K. A., and B. Fried. 1996. Emergence of cercariae of *Echinostoma trivolvis* from *Helisoma trivolvis* under various conditions. Journal of Parasitology 82:674–676.

Ulmer, M. J. 1970. Notes on rearing of snails in the laboratory. Pages 143–144 *in* A. J. MacInnis and M. Voge, eds. Experiments and Techniques in Parasitology. W. H. Freeman, San Francisco.

Willey, C. H. 1936. The morphology of the amphistome cercaria *C. poconensis* Willey, 1930, from the snail, *Helisoma antrosa*. Journal of Parasitology 22:68–75.

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Journal is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues of $25.00 domestic or $28.00 foreign are payable on notification of election. Send this completed form to:

Dr. Harley G. Sheffield
11831 Enid Drive
Potomac, MD 20854

Print name _____ Date of birth _____

Mailing address: _____

_____

_____

Degree and year received: _____

Present position: _____

Field of interest: _____

_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
(a member)

ANNIVERSARY AWARD RECIPIENTS

*Edna M. Buhrer	1960	Kenneth C. Kates	1978
*Mildred A. Doss	1961	*Everett E. Wehr	1979
*Allen McIntosh	1962	*O. Wilford Olsen	1980
*Jesse R. Christie	1964	*Frank D. Enzie	1981
*Gilbert F. Otto	1965	Lloyd E. Rozeboom	1982
*George R. LaRue	1966	*Leon Jacobs	1983
*William W. Cort	1966	Harley G. Sheffield	1984
*Gerard Dikmans	1967	A. Morgan Golden	1985
*Benjamin Schwartz	1969	Louis S. Diamond	1986
*Willard H. Wright	1969	Everett L. Schiller	1987
*Aurel O. Foster	1970	Milford N. Lunde	1988
Carlton M. Herman	1971	J. Ralph Lichtenfels	1989
*May Belle Chitwood	1972	A. James Haley	1990
*Elvio H. Sadun	1973	Francis G. Tromba	1991
E. J. Lawson Soulsby	1974	Thomas K. Sawyer	1992
David R. Lincicome	1975	Ralph P. Eckerlin	1993
Margaret A. Stirewalt	1975	Willis A. Reid, Jr.	1994
*Leo A. Jachowski, Jr.	1976	Gerhard A. Schad	1995
*Horace W. Stunkard	1977	Franklin A. Neva, M.D.	1996

HONORARY MEMBERS

*George R. LaRue	1959	E. J. Lawson Soulsby	1990
*Vladimir S. Ershov	1962	Roy C. Anderson	1991
*Norman R. Stoll	1976	Louis Euzet	1992
*Horace W. Stunkard	1977	John C. Holmes	1993
*Justus F. Mueller	1978	Purnomo	1994
John F. A. Sprent	1979	Naftale Katz	1995
Bernard Bezubik	1980	*Robert Traub	1996
Hugh M. Gordon	1981		

CHARTER MEMBERS 1910

*W. E. Chambers	*Philip E. Garrison	*Maurice C. Hall	*Charles A. Pfender
*Nathan A. Cobb	*Joseph Goldberger	*Albert Hassall	*Brayton H. Ransom
*Howard Crawley	*Henry W. Graybill	*George F. Leonard	*Charles W. Stiles
*Winthrop D. Foster			

LIFE MEMBERS

*Maurice C. Hall	1931	*Mildred A. Doss	1977
*Albert Hassall	1931	*Everett E. Wehr	1977
*Charles W. Stiles	1931	Marion M. Farr	1979
*Paul Bartsch	1937	John T. Lucker, Jr.	1979
*Henry E. Ewing	1945	George W. Luttermoser	1979
*William W. Cort	1952	*John S. Andrews	1980
*Gerard Dikmans	1953	*Leo A. Jachowski, Jr.	1981
*Jesse R. Christie	1956	Kenneth C. Kates	1981
*Gotthold Steiner	1956	Francis G. Tromba	1983
*Emmett W. Price	1956	A. James Haley	1984
*Eloise B. Cram	1956	*Leon Jacobs	1985
*Gerald Thorne	1961	*Paul C. Beaver	1986
*Allen McIntosh	1963	*Raymond M. Cable	1986
*Edna M. Buhrer	1963	Harry Herlich	1987
*Benjamin G. Chitwood	1968	Glenn L. Hoffman	1988
*Aurel O. Foster	1972	Robert E. Kuntz	1988
*Gilbert F. Otto	1972	Raymond V. Rebois	1988
*Theodor von Brand	1975	Frank W. Douvres	1989
*May Belle Chitwood	1975	Thomas K. Sawyer	1989
Carlton M. Herman	1975	*J. Allen Scott	1990
Lloyd E. Rozeboom	1975	Judith H. Shaw	1990
*Albert L. Taylor	1975	Milford N. Lunde	1991
David R. Lincicome	1976	Everett L. Schiller	1991
Margaret A. Stirewalt	1976	Harley G. Sheffield	1991
*Willard H. Wright	1976	Louis S. Diamond	1994
*Benjamin Schwartz	1976	Mary Hanson Pritchard	1994

*Deceased.

CONTENTS

(*Continued from Front Cover*)

Date of publication, 3 February 1997

* * *

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