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Activity of Praziquantel Against *Anoplocephala perfoliata* (Cestoda) in Horses

EUGENE T. LYONS, SHARON C. TOLLIVER, J. HAROLD DRUDGE,
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ABSTRACT: Activity of praziquantel was evaluated against the tapeworm, *Anoplocephala perfoliata*, by a modified (24-hr) critical test method in 19 infected horses. The injectable formulation of the drug was administered by stomach tube (ST) or intraorally (IO). Removals were 89% to 100% (average of 98%) at 1.0 mg/kg (ST) ($N = 6$ horses), 100% at 1.0 mg/kg (IO) ($N = 2$ horses), and 82% to 100% (average of 91%) at 0.75 mg/kg (IO) ($N = 11$ horses). Toxicosis was not evident in any of the horses after treatment.

KEY WORDS: praziquantel, efficacy, tapeworm, *Anoplocephala perfoliata*, horses.

Anoplocephala perfoliata has been found in over 50% of necropsied Thoroughbreds in Kentucky in recent years (Drudge and Lyons, 1986). This species of tapeworm is generally located in the cecum around the ileo–cecal junction. Various detrimental effects attributed to this parasite include ulceration of the mucosa, edema, inflammation and blockage of the ileo–cecal opening, rupture of the cecum, intussuception of the ileum and cecum, and ileal hyperplasia and hypertrophy (Beroza et al., 1986; Drudge and Lyons, 1986; Edwards, 1986; Owen et al., 1989).

Currently, there are no drugs on the market in the U.S.A. labelled for removal of tapeworms in horses. Reports have indicated that pyrantel pamoate, which is commercially available as a nematocide, is efficacious, but activity is variable against *A. perfoliata* at the single (6.6 mg base/kg) and double (13.2 mg base/kg) dose rates (Lyons et al., 1974, 1986, 1989; Slocombe, 1979).

The purpose of the present study was to determine the efficacy of praziquantel against natural infections of *A. perfoliata* in horses.

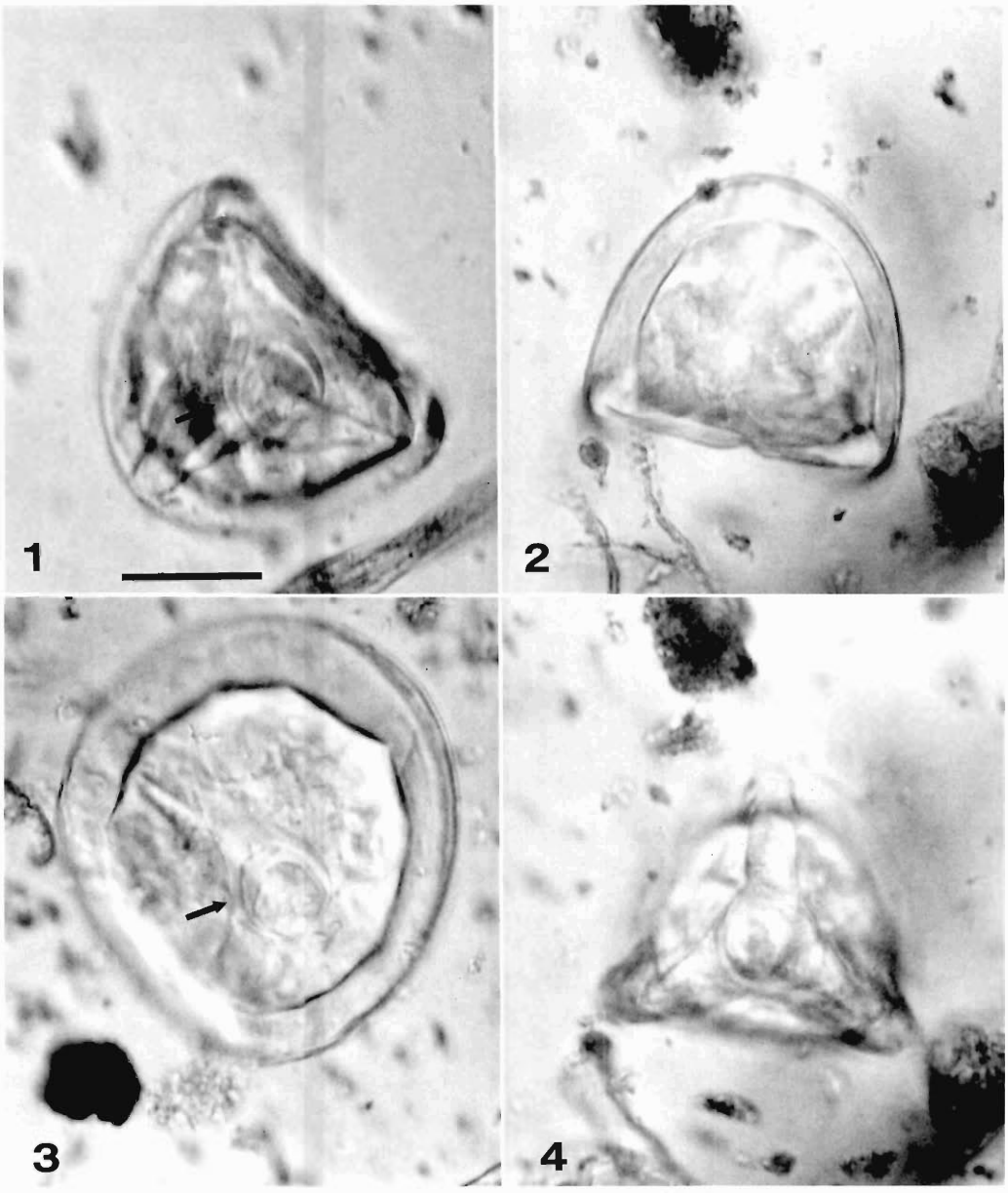
Materials and Methods

Techniques are deficient in detecting tapeworm eggs (Figs. 1–4) in feces of horses. For example, in 2 studies (Lyons et al., 1983, 1984) on dead Thoroughbreds, *A. perfoliata* eggs were found in feces by flotation with saturated NaCl in 3% of the horses (prevalence = 54%), and with $ZnSO_4$ in 7% of the horses (prevalence = 53%). In the present study, feces were not examined for *A. perfoliata* eggs before treatment of the horses because of the poor correlation with actual infections of tapeworms. However, it was presumed that, based on several recent prevalence studies in dead Thoroughbreds in Kentucky, about 50% of the test horses would be infected with *A. perfoliata* (Drudge and Lyons, 1986). Data (mostly on Thoroughbreds) indicated no apparent differences in prevalence of *A. perfoliata* by sex or age of the animals (Lyons et al., 1983, 1984).

For the present investigation, 35 horses, 19 of which were infected with *A. perfoliata* at necropsy, were treated with praziquantel, 1 at a time as each was acquired, between 15 February 1988 and 2 October 1990, in critical tests. The horses were cull animals, donated by local farms because of physical problems, including leg deformities—several were wobblers. The majority of the horses were Thoroughbreds ($N = 32$); others were Standardbreds ($N = 2$) and mixed lighthorse-type ($N = 1$). Sexes included 17 males (intact), 4 geldings, and 14 females. Ages varied from <1 to 23 years; (<1 year = 2 horses; 1 = 18; 2 = 3; 3 = 1; 5 = 1; 7 = 2; 13 = 1; 14 = 1; 17/18 = 3; 20 = 2; 23 = 1).

The critical test method (Hall, 1917; Hall et al., 1919; Moskey and Harwood, 1941; Drudge and Lyons, 1977) is commonly used to determine efficacy of compounds against internal parasites of animals, particularly horses. Each animal serves as its own control. Typically, a count is made of parasites passed in the feces for several days after treatment and of those remaining inside the animal at necropsy to obtain the total number present at the time of treatment. From these data, efficacies are calculated.

In the present investigation, a modification (Todd and Brown, 1952; Lyons et al., 1986, 1989) of the basic critical test was used. Equids were euthanatized at 24 hr after treatment. Feces were not examined during this short posttreatment period. Details of this critical test modification have been described in 2 publications on evaluation of activity of pyrantel pamoate on *A. perfoliata* (Lyons et al., 1986, 1989). In these publications, mention is made that 1 advantage of this quick test is that horses are examined soon enough (24 hr) after treatment so the tapeworms, affected by the drug, generally have had time to be moved posteriorly from their normal location in the intestine, but generally have not yet been passed in the feces. This allows more accurate finding of intact specimens (particularly with scolices still attached) which tend to disintegrate soon after being affected by a drug. The normal location of *A. perfoliata* is in the cecum, but it is occasionally found in the small intestine and ventral colon. At necropsy, all specimens recovered from the small intestine and



Figures 1–4. Eggs of *Anoplocephala* sp. (probably *Anoplocephala perfoliata*) recovered from feces of horses by concentrated sugar flotation. Various views show the shape of the eggs as cupped (Figs. 1, 2), round (Fig. 3), and triradiate (Fig. 4). Arrows denote the oncospheres in Figures 1 and 3. Scale bar = 30 μm for all photos.

cecum were considered not removed by the drug. Tapeworms found in other portions of the intestines—ventral colon (except for attached specimens), dorsal colon, small colon, and rectum—were considered removed by the drug. A total of all tapeworms found at necropsy 24 hr after treatment is the basis for calculation of efficacy.

Praziquantel injectable formulation (Mobay), currently marketed as a cestocide for dogs and cats, was given once by stomach tube (ST) at 1.0 mg/kg to 7 horses, intraorally (IO) at 1.0 mg/kg to 6 horses, and IO at 0.75 mg/kg to 22 horses. Specific doses of drug for each horse were removed from the stock bottle with a plastic syringe and needle. The drug was expelled from the syringe (after the needle was removed) into a funnel attached to a stomach tube or IO directly into the mouth. Both the stomach tube and funnel were rinsed with about 300 ml of water to complete admin-

Table 1. Data on *Anoplocephala perfoliata* recovered at necropsy from 19 infected horses treated once with praziquantel.*

	Drug							
	Dose rate	Route or method of administration	No. of specimens—range (mean)				Clearance‡	
No. horses†	(mg/kg)		Remaining	Removed	Total	% removal	≥90%	100%
6	1.0	ST	0–1 (0.17)	5–21 (9.7)	5–21 (9.8)	89–100 (98)	83	83
2	1.0	IO	0 (—)	6–7 (6.5)	6–7 (6.5)	100–100 (100)	100	100
11	0.75	IO	0–95 (12.7)	1–936 (131.6)	1–1,031 (144.3)	82–100 (91)	91	73

* Injectable formulation.
† Examination at necropsy was 24 hours posttreatment.
‡ Refers to the percentage of infected horses that had ≥90% removal and 100% removal.
ST = stomach tube; IO = intraorally.

istration of the drug. Efficacies of the 2 dose rates were compared statistically (*t*-test) for significance (Snedecor and Cochran, 1980). The injectable formulation was not administered parenterally. It was given only by ST or IO because of ease of administration and also to preclude adverse reactions (Campbell, 1972) sometimes associated with substances injected parenterally.

All horses were observed visually at varying intervals for toxicosis during the posttreatment period.

Results and Discussion

At necropsy, 19 (54%) of the 35 treated horses were found to be infected with *A. perfoliata.* Efficacy of praziquantel against *A. perfoliata* is presented (Table 1). Toxicosis was not evident in the horses after treatment.

For the 1.0 mg/kg dose rate (N = 8 horses), removal was 89% to 100% (average 98%) when the drug was given by ST (N = 6), and was 100% when administered IO (N = 2). Of the 6 horses treated by ST, removals were 100%, except for 1 horse that had 1 of 9 specimens remaining in the cecum. Praziquantel at the 0.75 mg/kg dose rate IO (N = 11) removed 82% to 100% (average 91%) of *A. perfoliata.* There was 100% removal in all but 3 of the infected horses, for which efficacies were 82%, 90%, and 91%.

Both dose rates had overall efficacious removal of *A. perfoliata.* There was no significant difference ($P < 0.05$) between the 2 dose rates. Possibly, this was because not enough tests were done to ascertain the validity of the small difference (8%) between means in the observed activities. Research should be pursued further to determine if even lower dose rates than 0.75 mg/kg of praziquantel are effective against *A. perfoliata.*

Prevalence of *A. perfoliata,* while 54% in all horses examined, was 59% in the 32 Thoroughbreds. The prevalence of this tapeworm species for these Thoroughbreds was similar to that (50% to 60%) found for over 1,000 necropsied Thoroughbreds in Kentucky in recent years (Lyons et al., 1983, 1984, 1986, 1987, 1989).

Acknowledgments

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Slide Available

Dr. Herman Zaiman has generously made available several copies of a 35-mm color transparency of *Hymenolepis nana* cysticercoids in mouse villi. The slide was prepared from a specimen donated by the late Dr. M. Yoeli and was photographed by Dr. B. Gueft. Members and other interested scientists who wish to obtain a copy should contact the Corresponding Secretary, David Chitwood, USDA, Nematology Laboratory, Building 467, 10300 Baltimore Boulevard, Beltsville, MD 20705-2350, USA.

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A New Digenetic Trematode, *Gibsonia borealis* sp. n. (Lepocreadiidae: Lepidapedinae), Parasitic in the Rattail *Macrourus berglax* from the Flemish Cap off Newfoundland

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ABSTRACT: *Gibsonia borealis* sp. n. is described from the macrourid fish, *Macrourus berglax* Lacépède, 1802, trawled from depths of 425–521 m in the North Atlantic Ocean east of Newfoundland, Canada. The second species in the genus, it is distinguished from the type species by its longer excretory vesicle, irregularly shaped gonads, long, unipartite external seminal vesicle, and single genital pore with shallow atrium. Affinities of the genus *Gibsonia* are discussed, and the genus is considered a member of the subfamily Lepidapedinae Yamaguti, 1958. The genus is now known to have a bipolar distribution in the Atlantic Ocean above latitudes 46° north and south in rattail fishes of the genus *Macrourus*.

KEY WORDS: Digenea, Lepocreadiidae, *Gibsonia*, fish parasites, taxonomy.

During investigations of the hydrology and fauna of waters contrasting the Flemish Cap with the Grand Bank off Newfoundland, a sample of 18 specimens of *Macrourus berglax* Lacépède, 1802, from Flemish Cap stations yielded 3 new helminth species that were not found in a sample of 28 *M. berglax* from the Grand Bank. Two of the helminth species, a monogenean and a cestode, were described earlier (Campbell et al., 1982). The third species, a lepocreadiid digenetic trematode of the genus *Gibsonia* Gaevskaya and Rodyuk, 1988, is described herein.

Materials and Methods

Fish were collected by Gulf of Mexico shrimp trawl at depths of 425–521 m from the southeastern Grand Bank off Newfoundland and the Flemish Cap. All hosts were moribund or freshly dead when examined. Trematodes from the intestine were observed alive, fixed in AFA at room temperature, and preserved in 70% ethanol. No coverglass pressure was applied during fixation because the worms were well relaxed when discovered. Whole-mount preparations were stained with Mayer's paracarmine and mounted in Canada balsam. Two specimens were embedded in paraffin, serially sectioned at 8–10 μm, and stained with Harris' hematoxylin and eosin. The fish were identified by Dr. Richard L. Haedrich of Memorial University of Newfoundland. Measurements are in micrometers unless otherwise stated and include the range followed by the mean in parentheses. All measurements are from whole mounted specimens and are expressed as length by width.

Results

Gibsonia borealis sp. n. (Figs. 1–5)

TYPE HOST: *Macrourus berglax* Lacépède (Macrouridae).

LOCALITIES: Flemish Cap: 46°31′N, 45°52′W (2 hosts, 10 worms), depth 425–464 m; 46°43′N, 44°08′W (1 host, 1 worm), depth 514–521 m.

PREVALENCE: 16.6% (3 of 18) from Flemish Cap; 0 of 28 from the Grand Bank; 6.5% (3 of 46) of all *M. berglax* sampled.

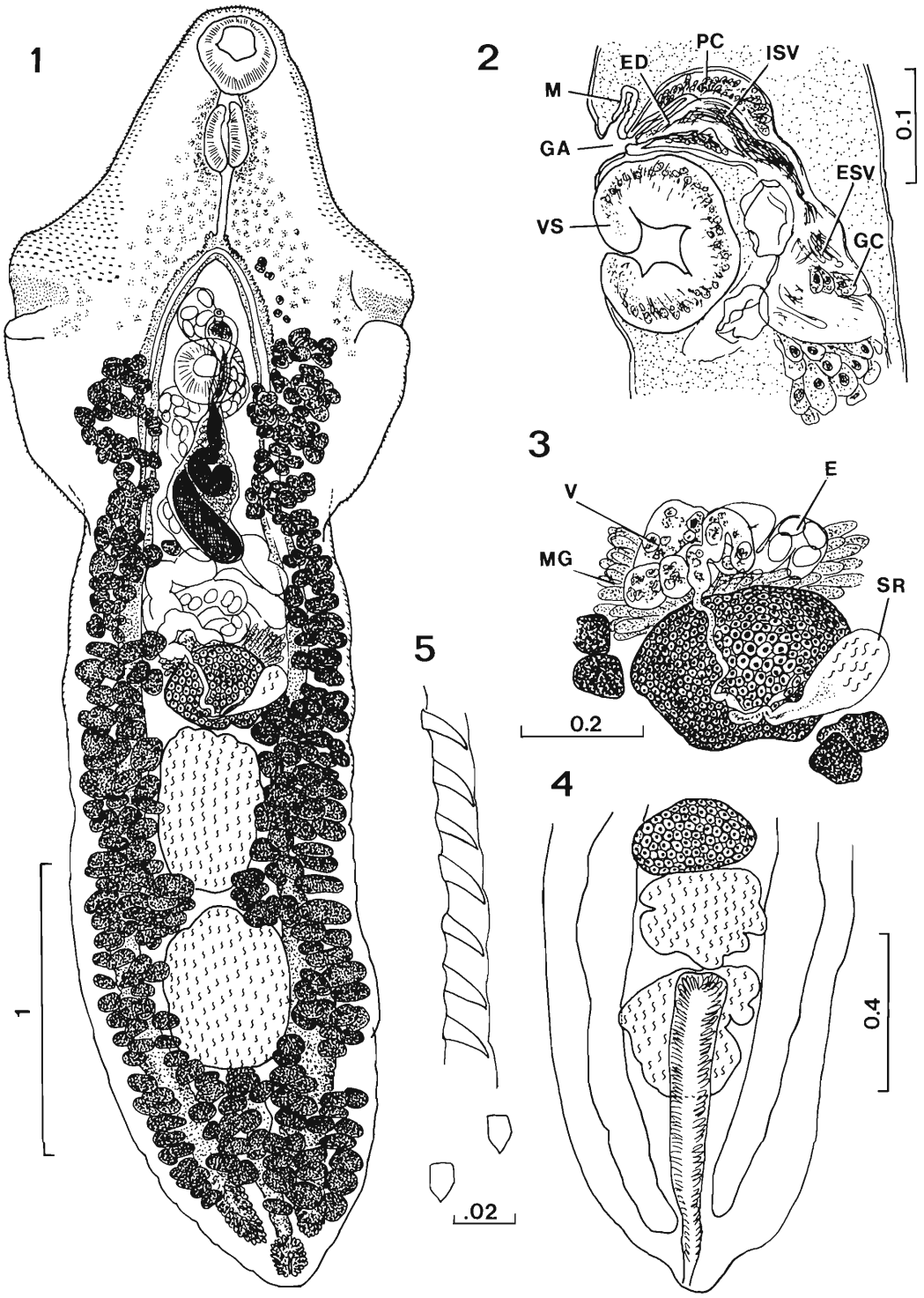
INTENSITY: 1–7 per host.

MEAN INTENSITY: 3.6%.

SPECIMENS: USNM 81927 (holotype), 2 paratypes (USNM 81298); British Museum (NH) 1991, 5.7.1–3 (2 paratypes and sections).

DESCRIPTION (based on 11 specimens): Lepocreadiidae; Lepidapedinae. Body elongate 1.9–4.4 mm long, having a broadly triangular forebody and becoming narrower in the hindbody region posterior to the ventral sucker. Maximum forebody dimensions 920–1900 long by 840–1,340 wide with conspicuous lateral marginal folds 57–220 by 84–277. Margins of forebody capable of assuming a cuplike shape. Hindbody, 880–2,700 by 540–960, tapering posteriorly. Tegument spinose, spines aligned in horizontal rows on forebody, diminishing in number at ovarian level, sparse in posttesticular region. Oral sucker subterminal, 163–272 in diameter, preoral space negligible, oral lobe absent. Prepharynx, 76–136, leading to elongate pharynx, 152–240 by 65–152. Prepharynx and pharynx surrounded by gland cells. Esophagus straight, 106–264 by 19–56, bifurcating about midway between pharynx and ventral sucker. Ceca, 34–160 wide, often with dilatations, gradually enlarging posteriorly, terminating blindly near posterior extremity. Ventral sucker, 144–240, in posterior third of forebody. Sucker ratio 1:0.80–0.96.

Two testes, 156–568 by 228–496, lie in tan-



Figures 1–5. ***Gibsonia borealis*** **sp. n. 1. Holotype, dorsal mount. 2. Sagittal section showing terminal genitalia and genital atrium. 3. Female reproductive organs. 4. Posterior extremity showing extent of excretory vesicle. 5. Tegumental spines. Scale lines are in millimeters. Abbreviations: E, egg; ED, ejaculatory duct; ESV, external seminal vesicle; GA, genital atrium; GC, gland cell; ISV, internal seminal vesicle; M, metraterm; MG, Mehlis' gland; PC, prostatic cells; SR, seminal receptacle; V, vitelline cells; VS, ventral sucker.**

dem within intercecal space of middle third of hindbody, separated by small space or not, margins irregularly indented. External seminal vesicle, 179–576 by 76–248, surrounded by gland cells enclosed by delicate membrane, beginning near junction of fore- and hindbody as a distended tube then forming single coil before continuing anteriorly to join cirrus sac. Cirrus sac pyriform, 87–240 by 68–112, median, extending to slightly anterior to ventral sucker, occupied for most of its length by a tube expanded proximally into an internal seminal vesicle surrounded by gland cells and narrowing distally to become short pars prostatica leading to thick walled ejaculatory duct that can be everted into the shallow genital atrium. Genital pore single, immediately preacetabular, median or slightly sinistral.

Ovary, 114–320 by 175–384, directly pretesticular, subspherical to irregular in shape, margin smooth or irregularly indented. Mehlis' gland, 336–360 by 240–400, ventral, occupying most of intercecal space over anterior half of ovary. Seminal receptacle elongated, lying posterodorsal to ovary on right side. Laurer's canal not observed. Oviduct originates on posterodorsal surface of ovary and extends anteriorly to Mehlis' gland. Uterus entirely preovarian, forming several loose coils in intercecal space, ascending anteriorly above external seminal vesicle, ventral sucker, and cirrus sac; terminating in narrow metraterm entering genital atrium just anterior to cirrus sac. Vitelline follicles large, forming dense lateral bands extending from anterior or posterior level of ventral sucker to posterior extremity; follicles often surround ceca, confluent in posttesticular space, sometimes occupy intertesticular space. Eggs oval, 57–68 (60.8) by 36–42 (38.5), delicate, unembryonated, operculum not observed.

Excretory pore terminal, leading to simple expanded tubular vesicle reaching to posterior margin of anterior testis or ovary.

Discussion

Gibsonia is a monotypic genus created by Gaevskaya and Rodyuk (1988) for *G. hastata* from *Macrurus carinatus* (sic) collected north of the Falkland Islands in the South Atlantic Ocean at a depth of 500 to 650 meters. According to the generic diagnosis, *Gibsonia* differs from other lepocreadiids in possessing a lanceolate body divided into 2 distinctly shaped regions ("fore- and hindbody"), possession of papilla-like structures on the lateral forebody, and large numbers of gland cells in a thickened body wall in the forebody region. They considered *Gibsonia* most similar to *Lepidapedon* but differentiated from it by the possession of separate genital pores. Only 6.3% of 239 *M. carinatus* examined over several years contained the worms.

Gibsonia borealis sp. n. is the second species in the genus and the first record of a species of the genus in the Northern Hemisphere. Its host is also a member of the genus *Macrourus,* the rough-head grenadier, *M. berglax.* Eleven *G. borealis* were found in 3 of 18 grenadiers from the Flemish Cap off Newfoundland, but none of the 28 fish examined from the Grand Bank contained these trematodes. Evidence of a restricted distribution of this parasite to fish from the Flemish Cap is further supported by Zubchenko (1981; 30 fish) and Houston and Haedrich (1986; 191 fish) who also did not find *G. borealis* in their samples from the Grand Bank. *Gibsonia borealis* may be limited to shallower depths of the Flemish Cap as indicated by its presence in fish taken from 400 to 500 m and its absence in fish taken from 1,200 to 1,400 m. This species serves as 1 among several examples where parasite faunas provide contrast in deep-sea hosts over contiguous geographical regions in the northwest Atlantic (Zubchenko, 1981; Campbell, 1990).

Gibsonia borealis is very similar to *G. hastata* but differs in a number of ways. Spines are present over the entire body of *G. borealis,* but are not present posterior to the level of the anterior testis in *G. hastata.* No lobe was observed in the wall of the oral sucker of *G. borealis,* and the pharynx is about twice as long as wide instead of rounded as in *G. hastata.* The ovary of *G. hastata* is spherical, but the ovary of *G. borealis* varies from subspherical to triangular and is more often asymmetrical with irregular indentations on the posterior margin. The testes of *G. hastata* are described as round or with slightly irregular margins, but testes of *G. borealis* are very irregular and often with deep indentations. The external seminal vesicle of *G. borealis* is more developed, coiled, and extends posterior to the junction of the fore- and hindbody, whereas in *G. hastata* the external seminal vesicle is smaller, bipartite, and does not extend posterior to the junction of the fore- and hindbody. The presence of a single genital pore was confirmed by sections in *G. borealis,* but separate genital pores are described for *G. hastata* (confirmed by personal communication). However, sections of *G. hastata* were not made but should be done because

the possession of separate genital pores is 1 of the few features unique to the genus. There are no papillae in the marginal lappetlike folds of *G. borealis,* and the body spines do not project as figured by Gaevskaya and Rodyuk (1988). Finally, the excretory vesicle extends to the anterior testis or beyond in *G. borealis* but only to the posterior testis in *G. hastata.*

Gibsonia possesses characters that are clearly intermediate between the lepocreadiid subfamilies and raises questions about the characters used to define them. Specimens of *G. borealis* were observed alive and fixed without coverglass pressure, so it can be said with certainty that the forebody margins do not unite posteriorly to form a scoop-shape as in *Diploproctodeum* Park, 1938, and *Caecobiporum* Mamaev, 1970. Serial sections clearly show the absence of ani in *G. borealis,* a feature in agreement with *Diplocreadium,* but unlike *Diplocreadium* the genital pore is preacetabular instead of postacetabular. *Gibsonia* lacks the anterior cecal extensions of *Caecobiporum,* and the esophagus is long like *Diploproctodeum,* not short like other lepocreadiid genera with a laterally expanded forebody (*Diplocreadium* Park, 1939, *Bianium* Stunkard, 1930, and *Caecobiporum*). The ovary is smooth, like *Bianium,* not lobed. Features inconsistent with the genera of the Diploproctodaeinae are forebody shape, the absence of ani, length of esophagus, position of the genital pore, ovarian shape, and extent of vitellaria. Therefore, resemblances of *Gibsonia* to members of the subfamily Diploproctodaeinae Park, 1939, are only superficial.

If body shape was considered of secondary importance, then *Gibsonia* would agree with *Lepidapedon* in all other respects. This is especially true of the male terminal genitalia that Shimazu and Shimura (1984) consider a bipartite cirrus sac and Bray and Gibson (1989) note as an important distinction for the subfamily Lepidapedinae. Except for the laterally expanded forebody, both species of *Gibsonia* strongly resemble *Lepidapedon lebouri* Manter, 1934, from macrourids off the coast of Florida. Furthermore, it should be noted that *L. nicolli* Manter, 1934, is widest anterior to the ventral sucker. Features of *Gibsonia* showing strong affinities to *Lepidapedon* are: the absence of ani or a uroproct; form of the prepharynx, pharynx, esophagus, and ceca; disposition and form of the gonads; distribution of the vitellaria, preovarian position of the uterus; preacetabular genital pore; presence of a seminal receptacle; an elongated external seminal vesicle with gland cells enclosed by a delicate membranous sac that is apparently continuous with the wall of the cirrus sac; a cirrus sac enclosing an internal seminal vesicle, prostatic cells and short ejaculatory duct; a shallow genital atrium; I-shaped excretory vesicle extending to the anterior testis (see *L. lebouri,* etc.), and macrourid hosts. These common characteristics and hosts indicate a closer relationship to *Lepidapedon* and the Lepidapedinae Yamaguti, 1958, than to the Diploproctodaeinae.

Acknowledgments

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Demonstration that *Sarcocystis montanaensis* has a Speckled Kingsnake–Prairie Vole Life Cycle

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ABSTRACT: Sporulated oocysts and free sporocysts of a *Sarcocystis* species were obtained from the feces of a naturally infected speckled kingsnake (*Lampropeltis getula holbrooki*) collected in northwestern Arkansas. Twenty sporocysts were 11.7 × 8.9 μm. Sporocysts lacked a Stieda body, had a granular sporocyst residuum, and contained 4 sporozoites. Sporocysts were inoculated orally into laboratory mice (*Mus musculus*), white-footed mice (*Peromyscus leucopus*), and prairie voles (*Microtus ochrogaster*). Sarcocysts were found only in prairie voles. Precystic stages occurred in the liver. Severe gross and microscopic lesions were observed in the livers of voles examined 7 and 8 days postinoculation (PI) of 5,000 sporocysts. Sarcocysts were present in the tongues of voles examined 117 days PI. Sarcocysts were thin-walled, divided into compartments by septa, and had osmiophilic, electron-dense, knoblike projections on the primary cyst wall. Metrocytes divided by endodyogeny and were present both centrally within groups of compartmentalized bradyzoites and at the periphery of the sarcocysts. Sarcocysts of the parasite examined in this study were similar to those of *Sarcocystis montanaensis* Dubey, 1983, and a *Sarcocystis* species Lindsay, Upton, Blagburn, Toivio-Kinnucan, McAllister, and Trauth, 1991, with a southern copperhead–prairie vole life cycle. It was concluded that the parasite was *S. montanaensis* and that it may use several species of snakes as definitive hosts.

KEY WORDS: *Sarcocystis montanaensis,* sarcocyst, prairie vole, *Microtus ochrogaster,* speckled kingsnake, *Lampropeltis getula holbrooki,* transmission, life cycle, pathogenesis, ultrastructure.

Little is known about the life cycles and pathogenicity of *Sarcocystis* species utilizing snakes as definitive hosts. Dubey et al. (1989) listed 10 species of *Sarcocystis* that had snakes as definitive hosts; 9 had rodent intermediate hosts and 1 had a lizard intermediate host. The present study was undertaken because we are interested in defining the host range and pathogenicity of snake-transmitted *Sarcocystis* species that occur in the United States. We herein report intermediate host transmission studies and sarcocyst ultrastructure of a *Sarcocystis* species isolated from a speckled kingsnake. The results indicate that this parasite is *Sarcocystis montanaensis* Dubey, 1983, and that at least 2 species of snakes are definitive hosts for the parasite.

Materials and Methods

Source and preparation of inocula

Feces containing sporulated oocysts and free sporocysts were collected from a naturally infected female speckled kingsnake (*Lampropeltis getula holbrooki*) (Arkansas State University Museum of Zoology [ASUMZ 13094], State University, Arkansas) collected in mid-May 1989 from Benton County, Arkansas (36°18′N, 94°33′W). Feces were placed in 2.5% (w/v) potassium dichromate solution for 8 days before they were used in transmission studies. Feces were washed free of potassium dichromate solution by centrifugation in phosphate-buffered saline (pH 7.2) (PBS), sporocysts were counted in a hemacytometer, and stored at 4°C in PBS for 6 days prior to use for inoculations into experimental intermediate hosts.

Inoculation and examination of rodents

Two experiments were conducted. In experiment 1, sporocysts were inoculated orally into 2 ICR laboratory mice (*Mus musculus*) (2,000 or 3,500 sporocysts/mouse), 2 white-footed mice (*Peromyscus leucopus*) (600 or 1,200 sporocysts/white-footed mouse), and 2 prairie voles (*Microtus ochrogaster*) (1,200 or 2,400 sporocysts/vole). All experimental hosts were obtained from commercial sources or were from laboratory colonies maintained at Kansas State University. The laboratory mice were killed 115 days postinoculation (PI), the white-footed mice were killed 110 days PI, and the voles were killed 117 days PI and examined at necropsy. Squash preparations were made of the brain, tongue, diaphragm, and heart of each animal and examined with Nomarski interference-contrast microscopy for sarcocysts. Portions of tissues were also fixed in 10% (v/v) neutral buffered formalin solution for his-

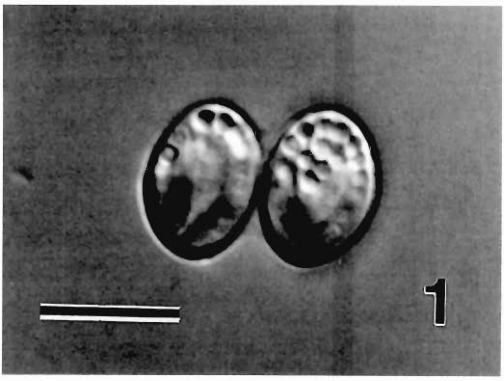


Figure 1. Nomarski interference-contrast photomicrograph of an oocyst of *Sarcocystis montanaensis* from the feces of a naturally infected speckled kingsnake. Bar = 10 μm.

tologic examination. Portions of tissues positive for sarcocysts were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for transmission electron microscopy (TEM).

In experiment 2, 2 voles were inoculated orally with 12,000 sporocysts and killed 3 or 5 days PI, and 3 voles were inoculated orally with 5,000 sporocysts and killed 7, 8, or 15 days PI. Portions of the lungs, liver, spleen, pancreas, kidneys, and adrenal glands were removed from the voles and processed for histologic examination to detect precystic stages. Portions of the liver from each vole were also fixed and processed for TEM. Impression smears were made from each liver, air dried, and fixed in 100% methanol.

Histologic processing and transmission electron microscopy

Tissues fixed in 10% neutral buffered formalin were embedded in paraffin, sectioned at 8 μm, and duplicate sections were stained with Giemsa or hematoxylin and eosin. The impression smears were stained with Diff-Quick® blood stain (Giemsa type) (Scientific Products, McGraw Park, Illinois).

Tissues in glutaraldehyde solution were postfixed in 1% osmium tetroxide, dehydrated in ethanols, embedded in Spurr's plastic, sectioned, and stained with uranyl acetate and lead citrate. Thin sections were examined in a Philips 301 transmission electron microscope operating at 60 kV.

Measurements of developmental stages and sarcocyst structures are expressed in micrometers.

Comparison to *Sarcocystis montanaensis* Dubey, 1983, and *Sarcocystis* species (Lindsay et al., 1991)

The sarcocysts observed in this study were structurally similar to *S. montanaensis* Dubey, 1983, described from meadow voles (*Microtus pennsylvanicus*) and long-tailed voles (*Microtus longicaudatus*) from Montana, U.S.A. (Dubey, 1983a) and a *Sarcocystis* species that has a southern copperhead (*Agkistrodon contortrix contortrix*)–prairie vole cycle (Lindsay et al., 1991); therefore, we reexamined our original photomicrographs of these parasites.

Results

Description of fecal stages of *Sarcocystis* species from *Lampropeltis getula holbrooki*

Fully sporulated oocysts and free sporocysts were present in the feces of the speckled kingsnake (Fig. 1). Twenty sporocysts were 11.2–12.6 × 8.6–9.2 (mean, 11.7 × 8.9). The shape index was 1.2–1.4 (mean, 1.3). The sporocyst wall was about 0.5 thick, lacked a Stieda body, and enclosed 4 sporozoites and a granular sporocyst residuum.

Transmission studies

Sarcocysts were not demonstrated in laboratory mice examined 115 days PI or in white-footed mice examined 110 days PI. Sarcocysts were observed in impression smears of the tongues from both voles examined 117 days PI. Grossly visible sarcocysts were not observed.

Lesions and developmental stages seen in voles

Tissue sections from laboratory mice and white-footed mice in experiment 1 were not examined. No lesions were seen in the tissues from voles in experiment 1 (both examined 117 days PI).

In experiment 2, no gross or microscopic lesions were seen in the voles examined 3 or 15 days PI. Gross lesions consisting of multiple 1–2-mm areas of white discoloration were observed on the serosal and cut surfaces of the livers of voles examined 7 and 8 days PI. Voles examined 5, 7, and 8 days PI had microscopic lesions in the liver (Fig. 2) but not the other tissues examined. Multifocal small discrete areas of coagulative necrosis of hepatocytes and portal infiltrates of mononuclear cells were observed in the vole examined 5 days PI. Lesions were more severe in the voles examined 7 and 8 days PI. They included coalescing areas of coagulative hepatocellular necrosis with moderate infiltration of the portal regions and occasionally central veins with mononuclear cells.

Precystic developmental stages were identified only in the livers of the voles examined 5, 7, and 8 days PI. Schizonts were few in number and were within hepatocytes. Schizonts were not present directly in lesions but were in areas of the liver that had not undergone necrosis (Fig. 2). A single immature schizont was observed 5 days PI; it was 14 × 11 and had a bilobed nucleus. Mature schizonts were seen 7 and 8 days PI. They were 15–25 × 14–24 (mean, 21.5 ×

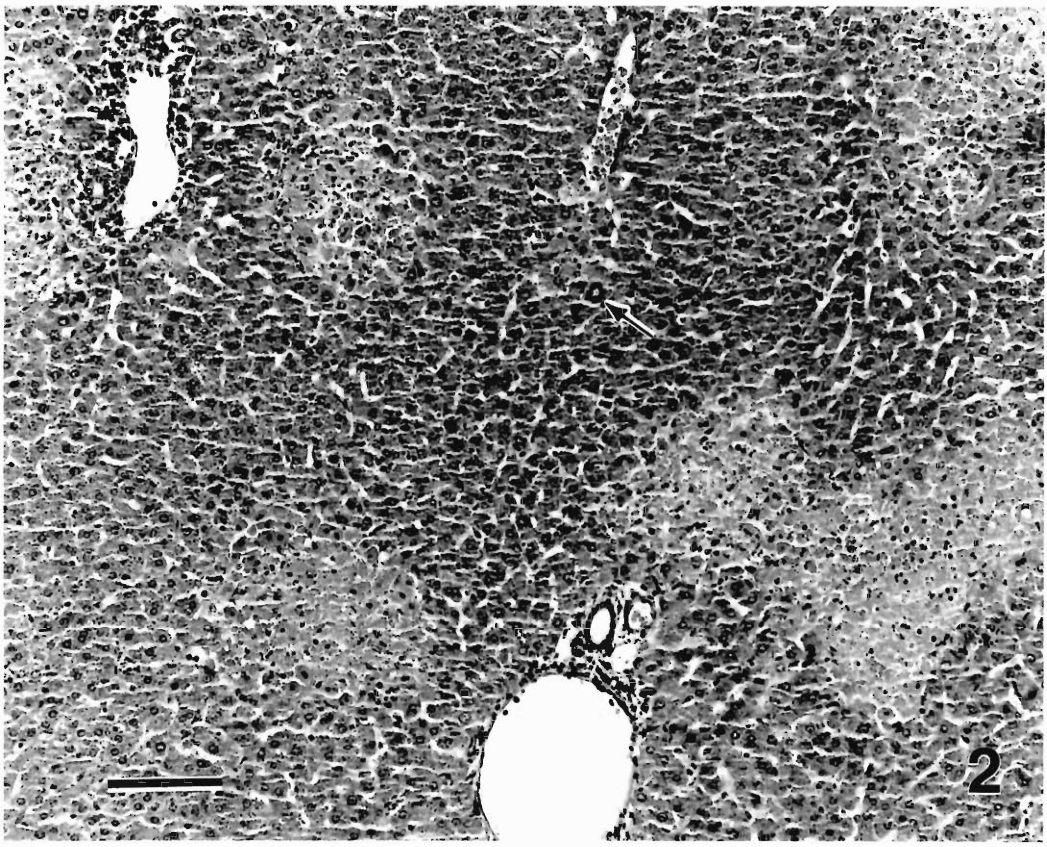


Figure 2. Photomicrograph of lesions caused by *Sarcocystis montanaensis* in the liver of prairie vole orally inoculated 7 days previously with sporocysts isolated from a speckled kingsnake. Hematoxylin and eosin stain. Note the areas of necrosis and a single schizont (arrow). Bar = 100 μm.

18.8; $N = 11$) and contained from 30 to 34 radially arranged merozoites and a residuum. A single 23 × 22 schizont with 33 radially arranged merozoites was seen in the impression smears from the vole examined 7 days PI. Both intracellular and extracellular merozoites were present in impression smears from voles examined 7 and 8 days PI. The merozoites were 7–11 × 2–3 (mean, 8.6 × 2.3; $N = 24$). Merozoites usually occurred singly in monocyte-like cells; one merozoite was observed in a polymorphonuclear neutrophil.

Schizonts were not observed with TEM. A few extracellular merozoites were seen in hepatic capillaries in the vole examined 7 days PI. They were structurally consistent with merozoites of other *Sarcocystis* species.

Sarcocysts were observed in tissue sections from both voles examined 117 days PI. Sarcocysts were thin-walled and measured 45–84 (mean, 67.9; $N = 7$) in diameter. All were seen in the tongues with the exception of a single sarcocyst seen in the diaphragm of 1 vole.

Transmission electron microscopy revealed that the sarcocyst wall could be classified as a type 1 sarcocyst wall using the classification of Dubey et al. (1989). The primary cyst wall was composed of the parasitophorous vacuole (PV) membrane. The PV membrane was ornamented with numerous knoblike structures (Fig. 3). The knoblike structures were 0.1–0.2 and had an electron-dense upper portion and a lower portion that was composed only of the unit membrane of the PV. Because of the plane of section, some knoblike structures of some sarcocysts appeared to blend together to form flattened projections (Fig. 4) or there appeared to be holes in the primary cyst wall (Fig. 5). The entire sarcocyst wall was 0.5–1.1 thick and was composed of the primary cyst wall and the underlying electron-dense

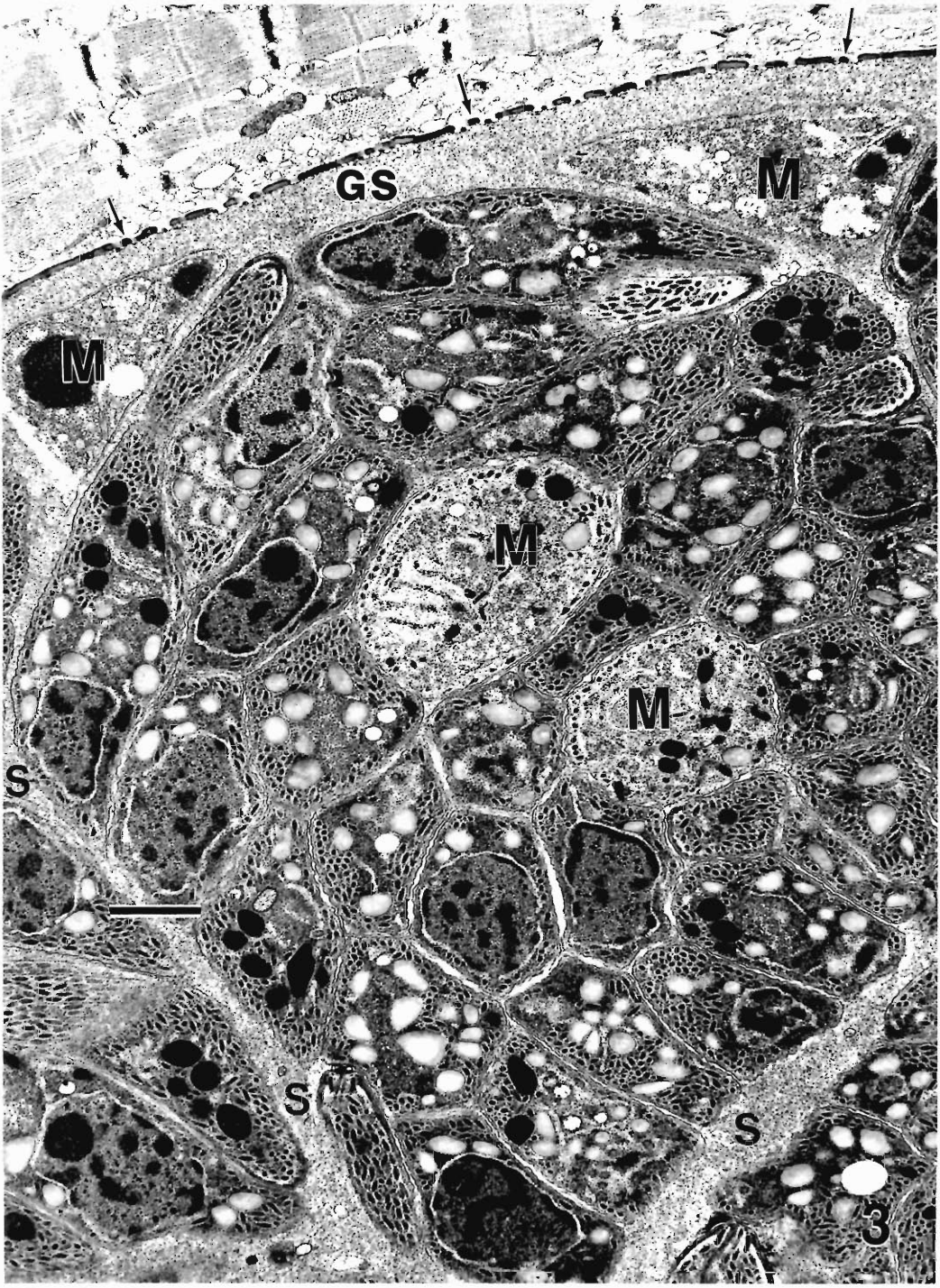
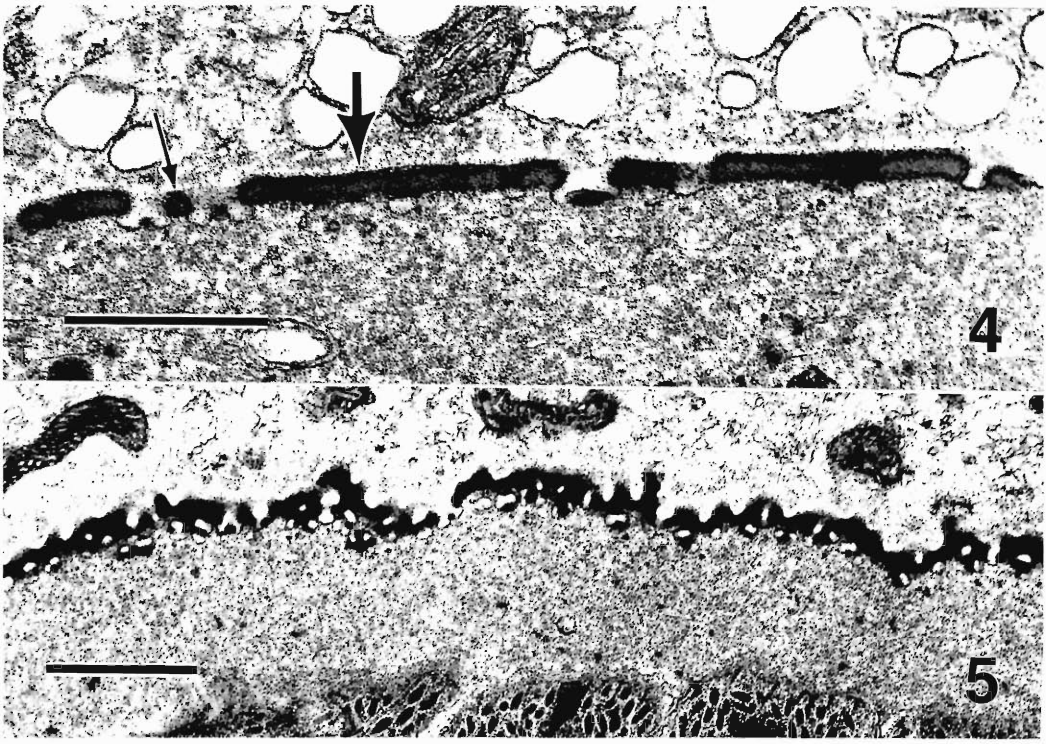


Figure 3. Transmission electron micrograph of a portion of a sarcocyst in the tongue of a prairie vole orally inoculated with sporocysts from a speckled kingsnake. Note the thin primary sarcocyst wall (arrows) that contains numerous knoblike projections, the underlying ground substance (GS), and septa (S) that divide the sarcocyst into compartments. Metrocytes (M) are present both centrally within groups of bradyzoites and at the periphery of the sarcocyst. Bar = 1.0 μm.



Figures 4, 5. Transmission electron micrographs of the sarcocyst wall of *Sarcocystis montanaensis* in prairie voles orally inoculated with sporocysts from a speckled kingsnake. 4. Note how the knoblike projections (small arrow) appear to be fused and give the primary sarcocyst wall a flattened appearance (large arrow) in areas. Bar = 0.5 μm. 5. Portion of the sarcocyst wall that appears to have holes in the primary cyst wall due to the angle of sectioning. Bar = 0.5 μm.

ground substance. The ground substance formed septa that divided the sarcocyst into compartments (Fig. 3). Metrocytes were present both at the periphery of the sarcocyst and centrally within groups of bradyzoites (Fig. 3). Metrocytes divided by endodyogeny. Bradyzoites contained all the organelles typical of this developmental stage and had micronemes that extended to the posterior of the parasite.

Comparison with *Sarcocystis montanaensis* and *Sarcocystis* species

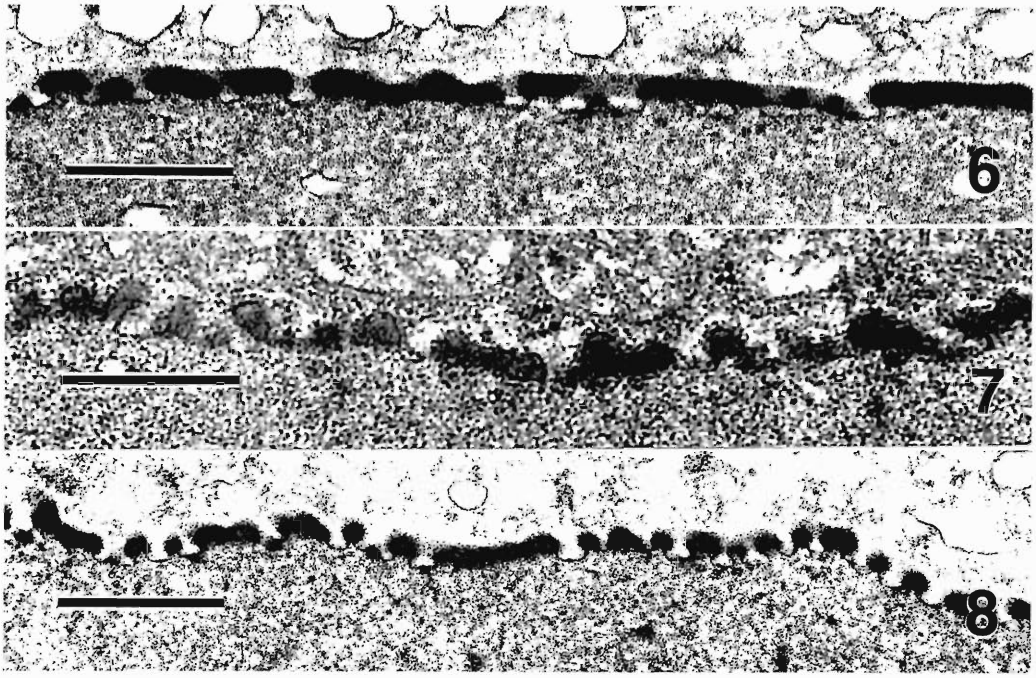
No differences were observed in the structure of the sarcocyst walls of the parasites examined using TEM (Figs. 6–8); therefore, we concluded that all were *S. montanaensis*. We were able to identify metrocytes at the periphery of some *S. montanaensis* sarcocysts in naturally infected meadow voles and *S. montanaensis* sarcocysts in prairie voles inoculated with sporocysts from a southern copperhead, but did not observe centrally located metrocytes in these cases.

Discussion

The hepatic necrosis observed in the voles in this study was severe, appeared to be specific, and associated with the presence of schizonts. Hepatic necrosis was not observed in voles examined 3 and 15 days PI that did not have demonstrable hepatic schizonts. Direct destruction of hepatocytes by parasites did not appear to be the cause of the hepatic necrosis because so few schizonts were observed. The hepatic necrosis may be due to a host reaction to merozoites or to metabolites released from mature schizonts.

The presence of a thin sarcocyst wall in the *S. montanaensis* sarcocysts examined in our study distinguishes it from a thick-walled species of *Sarcocystis* that use snakes as definitive hosts and rodents as intermediate hosts (see Beaver and Maleckar, 1981; Matuschka, 1986; Mehlhorn and Matuschka, 1986; Matuschka et al., 1987; Munday and Mason, 1980).

The sarcocyst wall of *S. idahoensis* Bledsoe, 1980, is thin (<1.0 μm) and may contain villar-



Figures 6–8. Comparison of the sarcocyst walls of *Sarcocystis montanaensis* in voles. 6. Sarcocyst in a prairie vole orally inoculated with sporocysts isolated from a speckled kingsnake. Bar = 0.5 μm. 7. Sarcocyst in a naturally infected meadow vole. Bar = 0.5 μm. 8. Sarcocyst in a prairie vole orally inoculated with sporocysts isolated from a southern copperhead. Bar = 0.5 μm.

like processes (Bledsoe, 1980a; Dubey, 1983b). This species has a gopher snake (*Pituophis catenifer*) (syn. *Pituophis melanoleucus*)–deer mouse (*Peromyscus maniculatus*) life cycle (Bledsoe, 1980a, b). The ultrastructure of *S. idahoensis* sarcocysts has not been examined. The presence of villar-like projections and our inability to infect white-footed mice (*P. leucopus*) demonstrate that *S. montanaensis* and *S. idahoensis* are distinct species.

The ultrastructure of *S. montanaensis* sarcocysts seen in the present study closely resembles *S. crotali* Enzeroth, Chobotar, and Scholtyseck, 1985, that has a Mojave rattlesnake (*Crotalus scutulatus scutulatus*)–laboratory mouse (*M. musculus*) life cycle (Enzeroth et al., 1985). However, electron-dense projections of the *S. crotali* sarcocyst wall also have an electron-dense layer underlying the projections. This, and our inability to infect laboratory mice with sporocysts, indicate that the 2 are separate species.

The structure of the sarcocysts of *S. montanaensis* observed in naturally infected meadow and long-tailed voles and *S. montanaensis* in prairie voles inoculated with sporocysts from southern copperheads or speckled kingsnakes are similar, and the sporocysts isolated from southern copperheads (11.0 × 8.5) and speckled kingsnakes (11.7 × 8.9) are also similar. Minor differences such as sarcocyst size and positioning of metrocytes may be due to sarcocyst age (Dubey et al., 1989; Cawthorn and Speer, 1990). Sporocyst structure usually is not of importance in identifying *Sarcocystis* species (Dubey et al., 1989). The finding of posteriorly located micronemes in bradyzoites in all cases examined is a strong indication that the parasites are all the same species and is a major reason for our conclusion that the parasites are all *S. montanaensis.*

Acknowledgments

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1991 Professor of the Year

Helminthological Society of Washington member Harvy D. Blankespoor, Professor of Biology at Hope College, was named the 1991 Professor of the Year by the Council for Advancement and Support of Education. The award is to recognize undergraduate faculty members for their commitment to teaching, their contribution to students' lives, and their service to the teaching profession. With the award goes a $10,000 cash stipend from the Carnegie Foundation.

(Excerpted from the Chronicle of Higher Education)

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Superimposed Infections in Golden Hamsters Infected with *Echinostoma caproni* and *Echinostoma trivolvis* (Digenea: Echinostomatidae)

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ABSTRACT: Intra- and interspecific competition were studied in *Echinostoma caproni* and *Echinostoma trivolvis* infections in golden hamsters. The *E. caproni, E. trivolvis*/hamster model had a high level of compatibility using the criterion of superimposing 5 age classes of parasites. It was possible to establish 5 age classes of both species in the hamster. Intraspecific competition occurred in *E. caproni* with the 7- and 14-day-old age classes and in *E. trivolvis* with the 21-, 28-, and 35-day-old classes. The locations of the worms in concurrent infections suggested that competition between the 2 species did not occur. Worms of both species were found in clusters or in close association with each other.

KEY WORDS: Trematoda, *Echinostoma caproni, Echinostoma trivolvis,* hamster, primary infection, challenge infection, site selection.

Widespread interest involving all aspects of infections with 37-collar-spined *Echinostoma* spp. has resulted in the compilation of extensive information. Some confusion, however, exists regarding the classification of the species used in these studies. In accord with the work of Kanev (1985), our study uses the names *Echinostoma caproni* and *E. trivolvis* for 2 related species previously referred to as *E. liei* and *E. revolutum,* respectively.

Infectivity, growth, and development of *E. caproni* and *E. trivolvis* in the golden hamster were studied by Isaacson et al. (1989) and Franco et al. (1986), respectively. Clinical and pathological effects of *E. trivolvis* in golden hamsters have also been reported (Huffman et al., 1986). Huffman et al. (1988) investigated single and concurrent infections of the golden hamster with *E. trivolvis* and *E. caproni.* The establishment, survival, and fecundity in *E. caproni* infections in NMRI mice were reported by Odaibo et al. (1988).

Holmes (1990) suggested 3 major selective forces for niche restriction in intestinal helminths: specialization, reproductive efficiency, and competition. Some authors (Behnke, 1987; Christensen et al., 1987) appeared to suggest that niche restrictions are side effects of immune mechanisms, and are not related to any selective forces acting on niches as such.

The selective factor most frequently invoked in analysis of niche occupation is competition. Intraspecific competition is a major force extending niche width, with interspecific competition a major force restricting niche overlap. Negative interactions can be revealed by reductions in establishment, growth, maturation, or

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Table 1. Percent recovery, mean number, and total number of worms recovered from superimposed infections with *E. caproni*. Four hamsters were killed at each day postinfection (PI).

	Age class—days PI				
Day PI	7	14	21	28	35
7	26				
14	8	10			
21	13	19	30		
28	10	20	21	28	
35	17	14	20	28	34
Total no. of worms	74	63	71	56	34
Mean ± SE/host	3.7 ± 1.4	3.9 ± 1.9	5.9 ± 1.8	7.0 ± 0.92	8.5 ± 0.80
Percent recovery	37	39	59	70	85

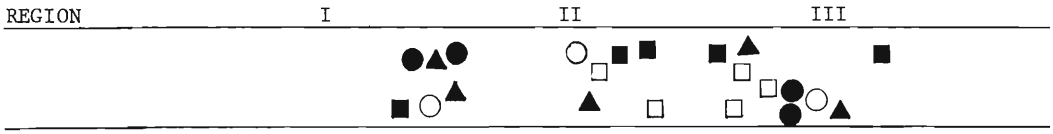

Figure 1. General distribution of the 5 age classes of *E. caproni* in the small intestine on day 7 (■), day 14 (□), day 21 (○), day 28 (●), and day 35 (▲) postinfection. Symbols represent the areas in which various age classes of parasites were recovered. Average length of the small intestine was 38 cm.

reproduction; displacement of 1 species is also common (Dobson, 1985).

Dobson (1985) noted that the extent of competition is markedly affected by intensity of the interacting species. With relatively low populations, neither intra- nor interspecific competition is likely to be significant; with relatively high populations, both are likely to be significant.

The purpose of this study was to provide evidence for: 1) the involvement of immunological reactions in superimposed *E. caproni* and *E. trivolvis* infections; 2) the site selection of 5 age classes of *E. caproni* and *E. trivolvis;* 3) the occurrence of intraspecific competition between the age classes of *E. caproni* and *E. trivolvis;* 4) the occurrence of interspecific competition between an established infection of *E. caproni* and a challenge infection with *E. trivolvis;* and 5) the occurrence of interspecific competition between an established infection of *E. trivolvis* and a challenge infection with *E. caproni.*

Materials and Methods

An outbred strain of golden hamsters (*Mesocricetus auratus*) was used in all studies. Metacercarial cysts of *E. trivolvis* and *E. caproni* were obtained from the kidney and pericardial cavities of laboratory-infected *Biomphalaria glabrata.* The metacercariae were preselected for viability.

To evaluate the occurrence of superimposed infections with either *E. trivolvis* or *E. caproni* the following protocol was used. Twenty adult male hamsters were each infected per os with 10 cysts of *E. caproni.* On day 7 postinfection (PI) 4 hamsters were killed and examined. The remaining hamsters were each infected with 10 cysts. Feces were checked for eggs on day 9 following the initial infection to confirm infection in the animals. On day 14 PI, 4 hamsters were killed and examined, the remaining 12 hamsters were again each infected with 10 cysts. This protocol was repeated on days 21, 28, and 35 PI. Twelve additional hamsters were infected and used as controls. Three animals were killed on days 14, 21, 28, and 35 PI to provide size and location data for the various age classes. It was possible to distinguish each age class based on size (length) and maturation of worms. The small intestine was then divided into 3 equal regions (I, II, and III). Worm location and percent recovery were recorded from all animals.

To evaluate the effect of a primary *E. trivolvis* infection on subsequent challenge with *E. caproni,* 15 golden hamsters were each infected with 10 metacercarial cysts of *E. trivolvis.* Feces were checked on day 9 PI. On day 14 PI the 10 infected hamsters were each infected with 10 cysts of *E. caproni.* The remaining 5 infected hamsters were designated as controls to compare parasite location. An additional 5 hamsters were each infected with 10 cysts of *E. caproni.* All animals were killed on day 28 after the primary infection. The small intestine was removed and measured from the pyloric valve to the ileocecal valve. The number of worms and their locations in the small intestine were recorded. The same protocol was used to evaluate the effect of a primary *E. caproni* infection on subsequent challenge with *E. trivolvis.*

Table 2. Percent recovery, mean number, and total number of worms recovered from superimposed infections with *E. trivolvis.* Four hamsters were killed at each day postinfection (PI).

	Age class—days PI				
Day PI	7	14	21	28	35
7	26				
14	26	36			
21	26	30	37		
28	25	33	38	31	
35	25	33	38	31	27
Total no. of worms	128	132	113	62	27
Mean ± SE/host	6.4 ± 1.3	8.3 ± 1.8	9.4 ± 0.66	7.8 ± 2.0	6.8 ± 0.95
Percent recovery	64	83	94	78	68

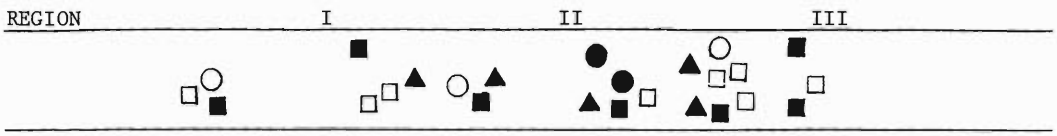

Figure 2. General distribution of 5 age classes of *E. trivolvis* in the small intestine on day 7 (■), day 14 (□), day 21 (○), day 28 (●), and day 35 (▲) postinfection. Symbol represent the areas in which various age classes of parasites were recovered. Average length of the small intestine was 38 cm.

Results

Superimposed infections with *E. caproni*

Percent recovery, average number, and total number of worms recovered from superimposed infections with *E. caproni* are summarized in Table 1. Twenty-six of 40 (65%) 7-day-old parasites were present when not challenged by another age class. Clustering, the presence of more than 2 parasites at a site, occurred in *E. caproni* infections. It was possible to establish 5 different age classes in the hamster. Age class distinction was made based on the size and maturation of the worms. Figure 1 depicts the distribution of the 5 age classes within the intestine. Parasites of each age class were found in close association with one another. Site selection occurred based on the preference for the lower two-thirds of the intestine by the parasites. Seven- and 35-day-old *E. caproni* appeared to occupy the same region of the intestine.

No unthriftiness or diarrhea were observed in infected hamsters. At necropsy, ballooning of the small intestine and cecum occurred in hamsters infected with heavy worm burdens. Enlarged lymphatic nodules occurred along the length of the intestine, along with increased vascularization.

Superimposed infections with *E. trivolvis*

Percent recovery, average number, and total number of worms from superimposed infections are summarized in Table 2. The day 21 age class was the dominant age class in the *E. trivolvis* superimposed infections. Day 35 worms occurred in about the same percentage as day 7 worms. It was possible to establish 5 different age classes in the hamster. Figure 2 depicts the distribution of the 5 age classes within the intestine. Parasites of each age class were found in close association with one another. In *E. trivolvis* the 35-day-old age class occupied about half the total intestinal section occupied by the 7-day-old worms.

No unthriftiness or diarrhea were observed in infected hamsters. At necropsy, ballooning of the small intestine and cecum occurred in hamsters infected with heavy worm burdens. Enlarged lymphatic nodules occurred along the length of the intestine, along with increased vascularization.

The effect of a primary *Echinostoma caproni* infection on subsequent challenge with *Echinostoma trivolvis*

No resistance could be demonstrated when a challenge was made 14 days following the primary infection. The primary infection did not appear to be reciprocally influenced by the challenge infection. As illustrated in Figure 3, worms of both species were found closely associated with one another. Table 3 reports the average number of worms recovered and the percent infectivity for this experiment. A difference was noted in the percent infectivity for the 2 species in the concurrent infection. Greater infectivity occurred in the single infection.

The effect of a primary *Echinostoma trivolvis* infection on subsequent challenge with *Echinostoma caproni*

No resistance could be demonstrated when a challenge was made 14 days following the pri-

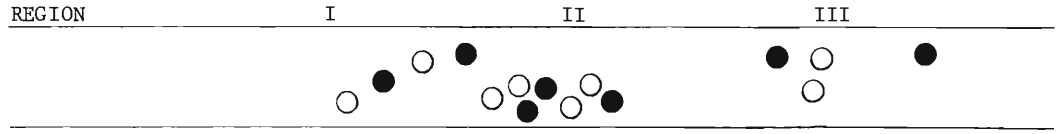

Figure 3. General distribution of parasites from a primary *E. caproni* infection followed by a challenge with *E. trivolvis*. *E. caproni* day 28 (●), *E. trivolvis* day 14 (○). Symbols represent the areas in which the two age classes of parasites were recovered. Average length of the small intestine was 38 cm.

Table 3. The average number of worms and infectivity in hamsters infected with *E. caproni* followed by an *E. trivolvis* infection. Results from single infections are also listed.

	E. caproni/E. trivolvis	*E. caproni*	*E. trivolvis*
Mean no. of worms recovered ± SE	5.7 ± 1.7/4.8 ± 1.1	7.6 ± 0.54	6.2 ± 1.1
Percent infectivity	57/48	76	62

mary infection. The primary infection did not appear to be reciprocally influenced by the challenge infection. As illustrated in Figure 4, worms of both species were found in close association with one another. A difference was noted in the percent infectivity for the 2 species in the concurrent infection. The infectivity was similar when the 2 species occurred singly. Table 4 reports the average number of worms recovered and the percent infectivity.

Discussion

The susceptibility to a superimposed *E. caproni* and *E. trivolvis* infection in hamsters in this study differs from the previous findings by Sirag et al. (1980) and Christensen et al. (1986). Their studies involved the resistance of mice to superimposed infections. Christensen et al. (1990) reported on the establishment, survival, and fecundity in *E. caproni* infections in hamsters. They reported that the hamster has a limited capacity to expel primary infections and to mount a regulatory response to superimposed challenge worm establishment. In our study, a cellular immune response occurred. Lymphatic nodules were enlarged and increased vascularization was present in the small intestine. A humoral response may be lacking as evidenced by the survival of the multiple age classes of parasite. This agrees with the study of Mabus et al. (1988) in which no antibody was detected in the serum of golden hamsters infected with *E. trivolvis*.

The results from the present study show a high level of compatibility in the *E. caproni, E. trivolvis*/hamster model. It was possible to establish 5 age classes for both *E. caproni* and *E. trivolvis* in the golden hamsters. The model also is characterized by the ability to superimpose 1 species on the other after establishing 1 infection. In the superimposed infections with *E. caproni,* 26 of 40 (65%) of the 7-day-old worms survived when unchallenged by other age classes, whereas a range of 8–17 (37%) 7-day-old worms survived when other age classes were present. The number of 7-day-old survivors of *E. trivolvis* was the same, 26%, whether challenged or not.

The survival of the 14-day-old worm class also was reduced in the multi-age class infections with *E. caproni,* but unaffected in the study using *E. trivolvis.* Percent recoveries for the 28- and 35-day-old age classes in *E. caproni* were similar. The percent recovery for *E. trivolvis* decreased from a high of 94% for day 21 to 78% for day 28 and 68% for day 35.

A difference between the species occurs as they mature. The 7-and 35-day-old *E. caproni* occupy the same region of the intestine. The young and old worms do not differ in habitat selection. However, in *E. trivolvis* the 35-day-old age class occupies about half the total intestinal section occupied by the 7-day-old worms. Thus, this species may become more specific in habitat selection as it matures.

The results seem to indicate that intraspecific competition occurred in *E. caproni* with the 7- and 14-day-old age classes and in *E. trivolvis* with the 21-, 28-, and 35-day-old age classes. According to Kisielowska (1970), parasites within the same host organism may interact negatively resulting in a decrease in the number of surviving parasites. Indirect effects of the immunologically mediated inflammatory reaction or worm crowding may be possible mechanisms for the results obtained.

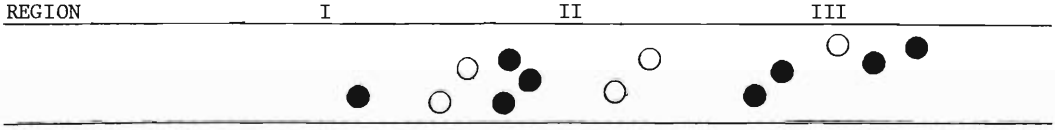

Figure 4. General distribution of parasites from a primary *E. trivolvis* infection followed by a challenge with *E. caproni. E. trivolvis* day 28 (○), *E. caproni* day 14 (●). Symbols represent the areas in which the two age classes of parasites were recovered. Average length of the small intestine was 38 cm.

Table 4. The average number of worms and percent infectivity in hamsters infected with *E. trivolvis* followed by an *E. caproni* infection. Results from single infections with each parasite are also listed.

	E. trivolvis/E. caproni	*E. caproni*	*E. trivolvis*
Mean no. of worms recovered ± SE	4.6 ± 1.1/6.7 ± 0.94	6.1 ± 1.3	4.8 ± 0.89
Percent infectivity	46/67	61	48

Huffman et al. (1988) infected golden hamsters simultaneously with *E. caproni* and *E. trivolvis*. In that study, concurrent infections with *E. trivolvis* differed from single ones in that there was a tendency for the parasites to move from the ileum to the jejunum. The number of *E. caproni* recovered in concurrent infections was markedly reduced. Fried and Gainsburg (1980) reported a reduced recovery of *Notocotylus* sp. in the presence of *Zygocotyle lunata*. Holmes (1961) found that worm distribution in the host gut was affected by concurrent infections with *Moniliformis dubius* and *Hymenolepis diminuta*.

In the present concurrent study the parasites were not administered simultaneously but at an interval of 2 weeks between primary and challenge infections. This study is in agreement with Huffman et al. (1988) in that worms of both species were found in clusters or in close association with each other. The general findings from the present study indicate that this model is useful for elucidating aspects of intra- and interspecific interactions.

Acknowledgments

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MEETING NOTICES

The Second International Symposium on Monogenea will be held in Montpellier, France, 5–8 July 1993. For information contact:

Dr. Alain Lambert
Laboratoire de Parasitologie Comparée C. C. 105
Université des Sciences et Techniques de Languedoc
Place E. Bataillon–34095
Montpellier Cédex 05, FRANCE

The Twenty-first International Nematology Symposium will be held in Albufeira, Portugal, 12–17 April 1992. For information contact:

Secretary 21st International Nematology Symposium
% Departmento de Zoologia
Universidade de Coimbra
3049 Coimbra Codex, PORTUGAL

The Eighth International Conference on Trichinellosis will be held in Herceg Novi, Montenegro, Yugoslavia, 22–26 September 1992. For information contact:

Dr. Kosta Cuperlovic
INEP
Banatska 31B
11080 Zemun, YUGOSLAVIA

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Zygocotyle lunata: Laboratory Maintenance in Snails and Mice

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ABSTRACT: A reliable method for laboratory maintenance of all stages of *Zygocotyle lunata* (Digenea) is described which permits further detailed studies of relatively poorly known stages of the life history. The experimental infection of the snail host, *Helisoma anceps,* was achieved by the feeding of incubated eggs of the fluke, rather than exposure to hatched miracidia. Techniques for obtaining maximum numbers of eggs, incubating eggs, and storage of encysted metacercariae also are described.

KEY WORDS: *Zygocotyle lunata, Helisoma anceps,* laboratory infection, Digenea.

The recent monograph of Sey (1991) on the biology of amphistome flukes demonstrates that since the comprehensive investigation of Willey (1941), only a few experimental studies have been published concerning the cecal worm, *Zygocotyle lunata* (Diesing, 1836) Stunkard, 1916. Most of these studies involve aspects of adult development in mammalian and avian hosts (Bacha, 1964; Fried, 1970; Joyner and McDaniel, 1970; Fried and Nelson, 1978; Fried and Gainsburg, 1979; Huffman, et al., 1991). Other studies, such as Fried and Wilson (1981) and Fried et al. (1978) are concerned with the encysted metacercaria. Consequently, there are few data on the free-living and intramolluscan stages of *Z. lunata,* except for the original life history study of Willey (1941).

In studying a population of *Helisoma anceps* infected with *Z. lunata* in 1989, it became apparent why so few recent studies have dealt with pre-adult stages; Willey's (1941) account of the ease with which snails could be infected by miracidia could not be confirmed by the writer, an experience shared by Drs. B. Fried, W. J. Bacha, and J. E. Huffman (pers. comm.). All of their work has originated with field-collected, naturally infected snails.

The present study was undertaken to determine whether snails could be infected in the laboratory, and whether maintenance of *Z. lunata* could be improved to make this trematode a more accessible system for experimental and classroom study.

Materials and Methods

Helisoma anceps, collected in 1989 from 2 ponds adjacent to the 10th and 18th holes of the Greencrest Golf Club, Butler Co., Ohio, were infected with an amphistome cercaria species which rapidly encysted on the surface of bowls in which infected snails were kept. Encysted metacercariae were given to Balb C mice by stomach intubation. Adult worms that developed in the cecum and colon were identified as *Zygocotyle lunata.*

Fingerbowls and petri dishes used to collect encysted metacercariae were stored in aerated tap water in a refrigerator at 8° to 10°C for up to 6 mo. Eggs of *Z. lunata* were obtained by placing gravid adults in 0.95% saline in depression dishes kept at either room temperature (23°–26°) or 37°C for several hours. After rinsing 3 times in aerated, dechlorinated tap water, eggs were incubated at room temperature for at least 28 days. Single worms of 5 age groups were similarly isolated to determine changes in fecundity over their life span of 225 days in mice.

Snails were maintained in 40-liter aerated aquaria and fed lettuce. Numerous offspring were thus produced for experimental infection. Exposed snails were kept in 11-cm fingerbowls at room temperature and fed lettuce. Water was added or replaced as required, usually weekly.

Snails were exposed singly or in groups of 2 to 5 to 1 to 10 miracidia/snail in small glass vessels. Other snails were exposed to *Z. lunata* eggs that had been incubated for 28 to 48 days, among which some miracidia appeared ready to hatch. Exposure to eggs continued for at least a week or more, since both snails and eggs were transferred to fingerbowls to be maintained until patency was observed.

To determine miracidial longevity, incubated eggs containing motile miracidia were illuminated with a 60-W incandescent lamp for 1 hr. All hatched miracidia were then transferred to a depression dish and observed microscopically until no motility was seen. The number of motile miracidia was recorded hourly.

Results

Infected *Helisoma anceps* (either field- or laboratory-infected) usually survived for more than 4 mo, providing a constant source of encysted metacercariae to infect mice. Mice necropsied after 35 days postinfection (PI) provided gravid adult worms from which large numbers of eggs were emitted. Eggs incubated at room temperature developed and began hatching on day 26, and continued to hatch until day 48. Hatching rates increased to a peak on days 35 to 40, then

Table 1. Oviposition by *Zygocotyle lunata* maintained at 37°C for 4 hr.

Worm age (days PI)	Number	Mean number of eggs/worm (±1 SD)
15–35	10	24 (±5.2)
36–70	10	76 (±11.1)
71–105	10	122 (±14.8)
106–140	10	84 (±12.4)
141–225	10	55 (±29.8)

* Worms older than 100 days produced more defective eggs and showed progressive gonadal hypertrophy as age increased.

declined gradually until day 48, after which no further hatching was observed. Based on counts of unhatched eggs in 10 dishes incubated for 50 days, hatching rates varied from 0 to 80%, usually about 50%. Among these groups of eggs, it was noted that eggs from adults maintained at room temperature had higher hatching rates and fewer abnormalities than eggs from worms kept at 37°C during oviposition. The rate of oviposition was dependent upon age of adult worms; maximum productivity was observed in 75- to 105-day-old worms (Table 1).

To test posthatching survival of miracidia, fully incubated eggs were allowed to hatch for 1 hr, then isolated and observed hourly. By 4 hr, more than 50% were dead (nonmotile), and none survived more than 8 hr (Table 2).

A series of 24 exposures of laboratory-reared *H. anceps* was performed, using from 1 to 10 newly hatched miracidia/snail. Snails ranged from 4 to 12 mm in diameter, and were exposed in groups of 1 to 5 in depression dishes with 2 ml of water. Miracidial penetration was rarely seen, but no miracidia were present after 4 to 5 hr. Exposed snails were observed for cercarial emergence for 100 days or more, unless death occurred. Dissections of all snails, living, moribund, or dead, revealed a single infection of daughter rediae on day 26 PI in a total of 56 exposed snails.

Table 3. Infection of *Helisoma anceps* exposed en masse to embryonated eggs of *Zygocotyle lunata*.

Group	Number and size of snails	Pre-patent mortality	Number infected (%)
A	15 adults (10–13 mm)	0	2/13%
B	4 adults (10–12 mm)	2	1/50%
C	8 adults (10–12 mm)	2	1/17%
D	3 adults (10–11 mm)	0	2/67%
E	6 adults (10–15 mm)	1	1/20%
F	13 adults (10–12 mm)	7	1/16%
G	55 young (2–3 mm)	0	9/17%
H	24 young (3–5 mm)	3	4/19%
I	12 young (3–6 mm)	4	2/25%
J	10 young (4–8 mm)	4	2/33%

Table 3 shows the results of exposing groups of snails to fully embryonated eggs. Ingestion of eggs was verified by observation of feces in which both hatched and unhatched eggs were seen. Some unhatched eggs contained partly embryonated, motile miracidia. At least 1 snail in each of the 10 exposed groups became patent using this exposure method. Cercarial emergence began as early as day 46 PI, but more often occurred after day 50; in 1 case, patency was not reached until day 84 PI. Infection rates are based on the number of patent snails as a percentage of surviving snails in each group.

Discussion

Few digenean parasites are routinely maintained and studied in all developmental stages. Consequently, the present state of knowledge of this large and diverse group of helminths is based on only a few species, usually on only those stages

Table 2. Longevity of *Zygocotyle lunata* miracidia.

Group	Number	Posthatching survival (hr)							
		1	2	3	4	5	6	7	8
A	6	5	3	3	1	1	1	0	—
B	10	8	8	5	4	2	1	1	0
C	12	11	9	6	5	3	0	—	—
D	9	9	7	5	4	3	2	1	0
E	15	12	9	9	8	4	1	0	—
F	18	14	12	12	9	7	5	1	1
G	7	6	5	3	3	1	0	—	—
H	14	13	10	7	6	4	1	1	0
Survival (%)	91	79/87%	65/71%	53/58%	44/49%	30/33%	17/19%	4/4%	1/1%

convenient to obtain, or having medical or veterinary importance.

Having identified a wild population of *Helisoma anceps* infected with *Zygocotyle lunata,* initial attempts to infect laboratory mice were highly successful, as were efforts to obtain and incubate eggs from gravid adults (Table 1). However, attempts to infect snails with hatched miracidia largely failed. Willey (1941) described methods of snail infection with *Z. lunata* and emphasized that miracidia hatched from incubated eggs "easily infected" helisome snails, a view perpetuated by Sey (1991). An alternative method of snail exposure described by Willey involved embryonated eggs. When I used this approach, a fairly high degree of success was obtained (Table 3). The explanation for poor results with hatched miracidia in the present study is probably due to the relatively short survival time of hatched *Z. lunata* miracidia (Table 2). Conversely, embryonation time for these eggs varies greatly, so that hatching occurs over a period of nearly 3 weeks (days 26 to 48 PI). This allows for a prolonged period of exposure when snails are placed in contact with unhatched embryonated eggs, most probably when snails ingest them. This inference is based on the dramatic difference in snail infection rates obtained with the 2 methods used in the present study. Regrettably, this method makes it difficult to measure the exact time required for snails to reach patency.

Optimum results in embryonating *Z. lunata* eggs were obtained by using adults 3 to 4 mo old (Table 1). While keeping adults at 37°C stimulates more rapid and copious egg production, it was noted that eggs obtained at this temperature were often abnormal in appearance and had lower hatching rates compared with eggs obtained at room temperature.

With the information gained in this study, it is now possible to begin critical experiments on various aspects of the growth, maturation, and longevity of all stages in the development of *Z. lunata* in both the snail and mammalian host, and to investigate further the relationships between this parasite and its hosts.

Acknowledgments

Appreciation is expressed to Mr. Wallace R. Horton for his permission to collect snails on his property, and to Dr. John B. Burch for confirming the identity of snails used in this study.

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Neotropical Monogenoidea. 17. *Anacanthorus* Mizelle and Price, 1965 (Dactylogyridae, Anacanthorinae) from Characoid Fishes of the Central Amazon

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ABSTRACT: The diagnosis of *Anacanthorus* Mizelle and Price, 1965, is emended and 35 new species are described from characoid fishes: *A. andersoni* sp. n., *A. carinatus* sp. n., *A. chaunophallus* sp. n., *A. chelophorus* sp. n., *A. cornutus* sp. n., *A. glyptophallus* sp. n., *A. lygophallus* sp. n., *A. nanus* sp. n., and *A. pithophallus* sp. n. from *Triportheus angulatus* (Spix); *A. calophallus* sp. n., *A. formosus* sp. n., *A. furculus* sp. n., *A. pelorophallus* sp. n., and *A. strongylophallus* sp. n. from *T. elongatus* (Guenther); *A. alatus* sp. n., *A. bellus* sp. n., *A. quinqueramus* sp. n., and *A. ramulosus* sp. n. from *T. albus* Cope and *T. elongatus*; *A. tricornis* sp. n. from *T. angulatus* and *T. elongatus*; *A. acuminatus* sp. n. and *A. euryphallus* sp. n. from *T. angulatus, T. elongatus,* and *T. albus*; *A. beleophallus* sp. n., *A. mastigophallus* sp. n., and *A. palamophallus* sp. n. from *Pristobrycon eigenmanni* (Norman); *A. xaniophallus* sp. n. from *P. eigenmanni* and *Pristobrycon* sp.; *A. catoprioni* sp. n. from *Catoprion mento* (Cuvier); *A. dipelecinus* sp. n. from *Roeboides myersii* (Gill); *A. hoplophallus* sp. n., *A. pedanophallus* sp. n., *A. spinatus* sp. n., and *A. stagmophallus* sp. n. from *Myleus rubripinnus* (Mueller and Troschel); *A. lepyrophallus* sp. n. from *Serrasalmus elongatus* Kner, *Serrasalmus* sp. (1 of Jégu), and *Serrasalmus* sp. (2n = 58); *A. periphallus* sp. n. from *Serrasalmus* sp. (2n = 58) and *Serrasalmus* sp. (1 of Jégu); *A. paraspathulatus* sp. n. from *Mylossoma duriventris* (Cuvier); and *A. stachophallus* sp. n. (syn., *Anacanthorus* sp. of Boeger and Kritsky, 1988) from *Pygocentrus nattereri* Kner. New host records for *A. jegui* Van Every and Kritsky, 1992, from *S. spilopleura* Kner, *Serrasalmus* sp. (2 of Jégu), *Serrasalmus* sp. (2n = 58), *Pristobrycon eigenmanni,* and *Pristobrycon* sp. are reported. All described species of *Anacanthorus* lack a vagina with the finding that the structure originally described as the vagina in *A. cuticulovaginus* Kritsky, Thatcher, and Kayton, 1979, is a sclerotized portion of the distal uterus. *Anacanthorus spathulatus* Kritsky, Thatcher, and Kayton, 1979, is chosen from multiple original spellings as the correct spelling for the species.

KEY WORDS: Brazil, taxonomy, Monogenoidea, Dactylogyridae, Anacanthorinae, *Anacanthorus, Anacanthorus jegui, Anacanthorus cuticulovaginus, Anacanthorus spathulatus, Anacanthorus acuminatus* sp. n., *Anacanthorus alatus* sp. n., *Anacanthorus andersoni* sp. n., *Anacanthorus beleophallus* sp. n., *Anacanthorus bellus* sp. n., *Anacanthorus calophallus* sp. n., *Anacanthorus carinatus* sp. n., *Anacanthorus catoprioni* sp. n., *Anacanthorus chaunophallus* sp. n., *Anacanthorus chelophorus* sp. n., *Anacanthorus cornutus* sp. n., *Anacanthorus dipelecinus* sp. n., *Anacanthorus euryphallus* sp. n., *Anacanthorus formosus* sp. n., *Anacanthorus furculus* sp. n., *Anacanthorus glyptophallus* sp. n., *Anacanthorus hoplophallus* sp. n., *Anacanthorus lepyrophallus* sp. n., *Anacanthorus lygophallus* sp. n., *Anacanthorus mastigophallus* sp. n., *Anacanthorus nanus* sp. n., *Anacanthorus palamophallus* sp. n., *Anacanthorus paraspathulatus* sp. n., *Anacanthorus pedanophallus* sp. n., *Anacanthorus pelorophallus* sp. n., *Anacanthorus periphallus* sp. n., *Anacanthorus pithophallus* sp. n., *Anacanthorus quinqueramus* sp. n., *Anacanthorus ramulosus* sp. n., *Anacanthorus spinatus* sp. n., *Anacanthorus stachophallus* sp. n., *Anacanthorus stagmophallus* sp. n., *Anacanthorus strongylophallus* sp. n., *Anacanthorus tricornis* sp. n., *Anacanthorus xaniophallus* sp. n., *Triportheus angulatus, Triportheus elongatus, Triportheus albus, Pristobrycon eigenmanni, Pristobrycon* sp., *Catoprion mento, Roeboides myersii, Myleus rubripinnus, Serrasalmus spilopleura, Serrasalmus elongatus, Serrasalmus* sp., *Mylossoma duriventris, Pygocentrus nattereri.*

Anacanthorus was proposed by Mizelle and Price (1965) for 3 species from the gills of the red-breasted piranha, *Pygocentrus nattereri* Kner (formerly *Serrasalmus nattereri*). The genus was included in the Ancyrocephalinae (Dactylogyridae) and characterized by monogenoideans lacking anchors and possessing 7 pairs of haptoral hooks and 2 pairs (1 dorsal, 1 ventral) of 4A's. Price (1967) proposed the Anacanthorinae (Dactylogyridae) for the genus based primarily on the original generic characters. The Anacanthorinae was accepted by Kritsky and Thatcher (1976) who added the monotypic *Anacanthoroides* and a third diagnostic feature to define the subfamily, i.e., presence of a modified distal uterus.

At present, 62 species of *Anacanthorus,* including those of this and the following paper, have been described (see Appendix in Van Every

and Kritsky, 1992). These species apparently form a monophyletic subgroup of Dactylogyridae (see Kritsky and Boeger, 1989), parasitic exclusively on characoid fishes (Cypriniformes) from the Neotropical Region. Individual species of *Anacanthorus* show varying ability to infest closely related host species. Further, subgroups within *Anacanthorus,* based on general morphology of the copulatory complex, appear to express high host specificity to familial groups within the Characoidea. Because of these traits, species of *Anacanthorus* may provide valuable models for study of phylogeny, coevolution, and biogeography. The purpose of this study was to provide descriptions of new species so that the latter would be available for phylogenetic and coevolutionary analysis (e.g., Van Every and Kritsky, 1992).

Materials and Methods

Fish hosts were collected by hook-and-line, seine, or throw net from locations in the central Amazon during 1979–1989. Methods of parasite collection, preparation of helminths for study, measurement, and illustration are those of Kritsky et al. (1986). Measurements, all in micrometers, represent straight-line distances between extreme points and are expressed as a mean followed by the range and number of specimens measured in parentheses; body length includes that of the haptor. Numbering of hook pairs follows that recommended by Mizelle (1936) (see Mizelle and Price, 1963). Type and voucher specimens are deposited in the helminth collections of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (IOC); the U.S. National Museum, Beltsville, Maryland (USNM); the University of Nebraska State Museum, Lincoln, Nebraska (HWML); and the Zoological Institute, U.S.S.R. Academy of Sciences, Leningrad (ZIAC), as indicated in the respective descriptions. Museum numbers have not been received from IOC and ZIAC for publication. For comparative purposes, the following specimens were examined: Holotype, *Anacanthorus anacanthorus* Mizelle and Price, 1965 (USNM 60459); holotype, *A. brazilensis* Mizelle and Price, 1965 (USNM 60460); holotype, 9 paratypes, *A. cuticulovaginus* Kritsky and Thatcher, 1974 (USNM 72837, 74021); holotype, 9 paratypes, *A. jegui* Van Every and Kritsky, 1992 (INPA PA338, USNM 81751, HWML 33383); holotype, *A. neotropicalis* Mizelle and Price, 1965 (USNM 60461); 17 vouchers, *A. spathulatus* Kritsky, Thatcher, and Kayton, 1979 (USNM 81798); 4 vouchers, *Anacanthorus* sp. of Boeger and Kritsky (1988) (=*A. stachophallus* sp. n.) (USNM 79197, HWML 23371).

Some hosts have been provisionally identified as *Serrasalmus* sp. (1 of Jégu), *Serrasalmus* sp. (2 of Jégu), *Serrasalmus* sp. (2n = 58), and *Pristobrycon* sp. by M. Jégu, ORSTOM, INPA, Manaus, Amazonas, Brazil, and may represent undescribed species. Specimens of each undescribed form are available through the ichthyology collection, INPA, Manaus, Amazonas, Brazil.

Results

Anacanthorus Mizelle and Price, 1965

EMENDED DIAGNOSIS: Dactylogyridae, Anacanthorinae (see Kritsky and Boeger, 1989). Body fusiform, divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth; peduncular region may be corrugated. Four cephalic lobes, 2 terminal, 2 bilateral; head organs present; cephalic glands comprising 2 bilateral groups of cells lying posterolateral, dorsal to pharynx. Eyes present. Pharynx muscular, glandular; esophagus present; intenstinal ceca 2, confluent posterior to testis, lacking diverticula. Gonads tandem or slightly overlapping, intercecal; common genital pore midventral. Testis postovarian; vas deferens expanding into fusiform seminal vesicle, with anterior loop prior to entry into cirral base, looping left cecum or not. Sclerotized cirrus present; accessory piece present or absent. Ovary near midlength; oviduct short; uterus well developed, with terminal region consisting of variably sclerotized, thickened internal wall; vagina absent. Two bilateral bands of vitellaria coextensive with intestinal ceca; vitelline commissure anterior to ovary, ventral. Haptor bilobed, armed with 7 pairs (4 ventral, 3 dorsal) of hooks, 2 pairs (1 dorsal, 1 ventral) of 4A's. Anchors, bars absent. Parasites of Neotropical characoid fishes.

TYPE SPECIES: *Anacanthorus anacanthorus* Mizelle and Price, 1965.

TYPE HOST: *Pygocentrus nattereri* Kner, Serrasalmidae.

REMARKS: General body features of *Anacanthorus* species are provided in the whole-mount illustrations by Van Every and Kritsky (1992; Figs. 2–9, 38–42). Available diagnoses of *Anacanthorus* do not include a description of the course of the vas deferens in relation to the intestinal ceca (Mizelle and Price, 1965; Kritsky et al., 1979). However, descriptions of *Anacanthorus* species have been provided in which the vas deferens was not mentioned (Mizelle and Price, 1965; Mizelle and Kritsky, 1969), in which the vas deferens was suggested to loop the left intestinal cecum (Kritsky et al., 1979; Boeger and Kritsky, 1988), and in which clear indication of it looping the left cecum is provided (Kritsky and Thatcher, 1974; Kritsky et al., 1979; Boeger and Kritsky, 1988). Further, Van Every and Kritsky (1992) suggest that the vas deferens does not loop the intestinal cecum in 13 species of *Anacanthorus* infesting Serrasalmidae in Brazil, and we could

not determine with confidence the course of the vas deferens in any of the 36 species of *Anacanthorus* studied herein. From new slide preparations, we confirm that the vas deferens does not loop the intestine in *A. spathulatus* Kritsky, Thatcher, and Kayton, 1979, although a sinistral loop of the duct occurs ventral to the left cecum immediately proximal to the seminal vesicle. Although the course of the vas deferens may be diagnostic at the generic level, this character will have limited value until its states are determined in all *Anacanthorus* species. Thus, we recognize *Anacanthorus* for Neotropical species characterized by 1) possessing a bilobed hapter armed with 7 pairs of hooks and 2 pairs (1 dorsal, 1 ventral) of 4A's, 2) having tandem or slightly overlapping gonads (testis postovarian), 3) lacking a vagina, haptoral anchors, and bars, and 4) having a modified (thickened or sclerotized) distal uterine wall.

Anacanthorus cuticulovaginus Kritsky and Thatcher, 1974, is the only species in the genus reported to possess a vagina. However, our examination of the type specimens of this species has confirmed that the structure described as the vagina by the original authors is a sclerotized portion of the distal uterus similar to what we report in *A. quinqueramus* sp. n. While *cuticulovaginus* becomes an "inappropriate name" sensu the ICZN Art. 18, which cannot be rejected, these findings suggest that the absence of a vagina is also diagnostic for the genus.

Kritsky et al. (1979) described *Anacanthorus spathulatus* from the environs of Manaus, Amazonas, Brazil. In their paper, which the authors did not have an opportunity to review in galley, the specific name occurs in 2 forms. Correspondence in Kritsky's files and type specimen museum labels both clearly indicate that *spathulatus* was intended for this species.

Anacanthorus acuminatus sp. n. (Figs. 1–3)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORDS: *Triportheus elongatus* (Guenther), Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989) and Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (6 January 1989); *T. albus* Cope, Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA347; paratypes, USNM 81662, 81663, 81664, 81665, HWML 33340.

DESCRIPTION (based on 14 sepcimens): Body 239 (150–310; $N = 10$) long, robust, fusiform, tapered toward both ends from level of ovary or body midlength; greatest width 66 (54–78; $N = 10$). Cephalic lobes large, well developed; anterior lobes fused along midline. Two eyes; granules variable, usually moderate in size, subovate; few accessory granules scattered in cephalic, anterior trunk regions of adults, numerous throughout body in young specimens. Pharynx subspherical, 15 (13–17; $N = 9$) in diameter. Haptor 38 (26–52; $N = 10$) long, 52 (48–59; $N = 10$) wide. Hooks similar; each with truncate slightly depressed thumb, slightly expanded shank with small proximal enlargement; hook 17–18 ($N = 19$) long; filamentous hooklet (FH) loop about 0.5 shank length. 4A's similar, each 7–8 ($N = 4$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 28 (23–32; $N = 4$) long, 21 (16–25; $N = 4$) wide; ovary 33 (26–41; $N = 5$) long, 22 (15–25; $N = 5$) wide. Cirrus, accessory piece articulated by copulatory ligament 4 (3–6; $N = 13$) long. Cirrus 34 (30–37; $N = 13$) long, sigmoid, with base lacking flange. Accessory piece 25 (23–28; $N = 14$) long, with short basal, 3 distal branches; 2 distal branches acuminate.

REMARKS: *Anacanthorus acuminatus* may be confused with *A. glyptophallus* sp. n. but lacks the second accessory piece and a cork-screw portion of the vas deferens distal to the seminal vesicle characteristic of the latter species. The specific name is from Latin (*acuminatus* = taper-pointed) and refers to the distal branches of the accessory piece.

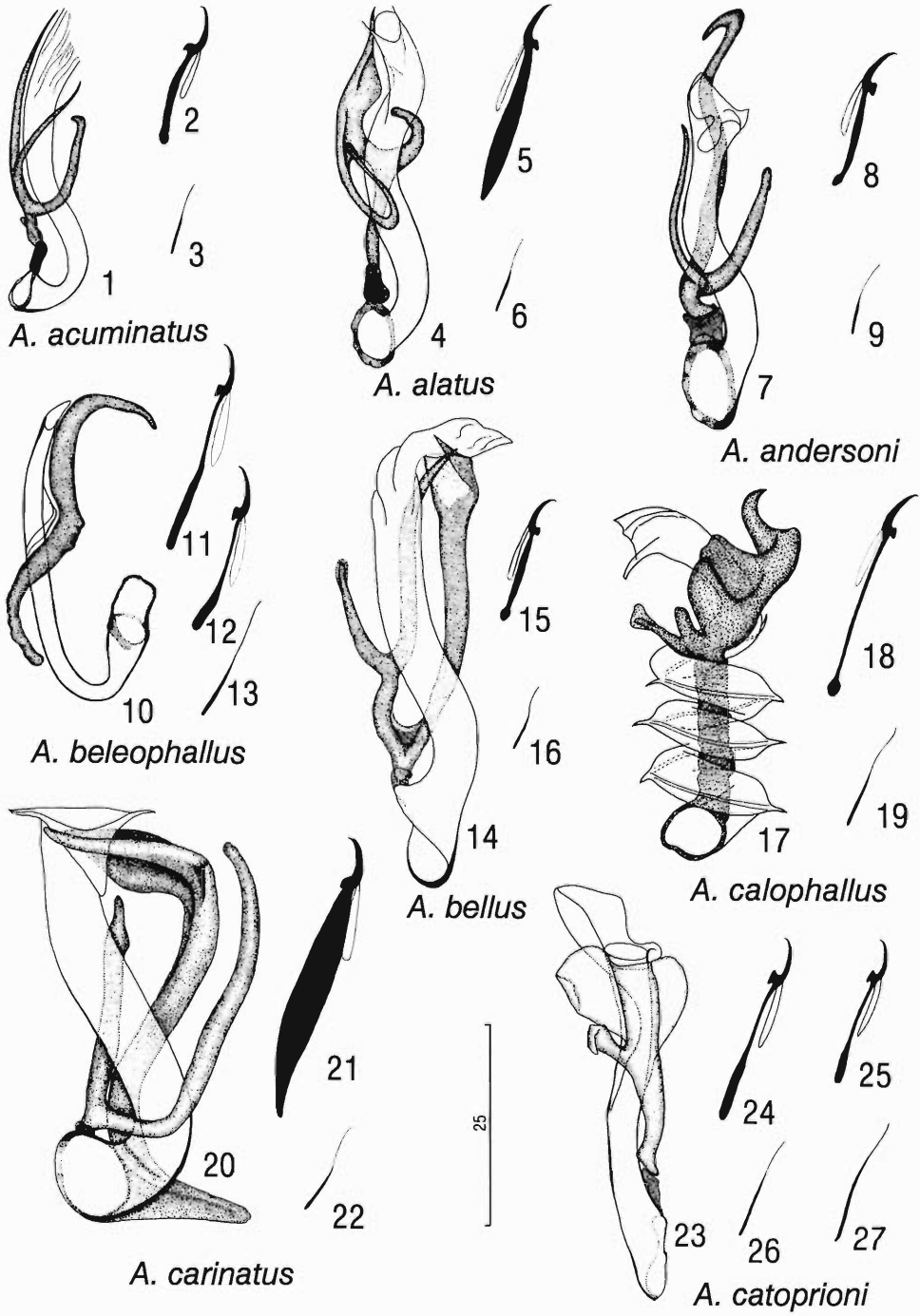
Anacanthorus alatus sp. n. (Figs. 4–6)

TYPE HOST AND LOCALITY: *Triportheus albus* Cope; Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORD: *Triportheus elongatus* (Guenther), Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (6 January 1989).

TYPE SPECIMENS: Holotype, INPA PA348; paratypes, USNM 81666, 81667, HWML 33341.

DESCRIPTION (based on 6 specimens): Body 316 (261–356; $N = 4$) long, robust, fusiform, tapered posteriorly from level of gonads; greatest width 93 (81–113; $N = 4$) in anterior trunk. Ce-



Figures 1–27. Sclerotized parts of *Anacanthorus* spp. 1–3. *Anacanthorus acuminatus*. 1. Copulatory complex. 2. Hook. 3. 4A. 4–6. *Anacanthorus alatus*. 4. Copulatory complex. 5. Hook. 6. 4A. 7–9. *Anacanthorus andersoni*. 7. Copulatory complex. 8. Hook. 9. 4A. 10–13. *Anacanthorus beleophallus*. 10. Copulatory complex. 11. Hook pairs 3, 4. 12. Hook pairs 1, 2, 5–7. 13. 4A. 14–16. *Anacanthorus bellus*. 14. Copulatory complex. 15. Hook. 16. 4A. 17–19. *Anacanthorus calophallus*. 17. Copulatory complex. 18. Hook. 19. 4A. 20–22. *Anacanthorus carinatus*. 20. Copulatory complex. 21. Hook. 22. 4A. 23–27. *Anacanthorus catoprioni*. 23. Copulatory complex. 24. Hook pairs 3, 4. 25. Hook pairs 1, 2, 5–7. 26. 4A (ventral). 27. 4A (dorsal). All figures are drawn to the 25-μm scale.

phalic lobes large, well developed; anterior lobes fused along midline. Four eyes equidistant; members of anterior pair absent or poorly developed, smaller than those of posterior pair; granules small, variable in shape; accessory granules absent or few in cephalic, anterior trunk regions. Pharynx subspherical, 26 (24–28; N = 4) in diameter. Haptor 51 (45–57; N = 4) long, 64 (62–68; N = 4) wide. Hooks similar; each with truncate slightly depressed thumb, conspicuously expanded shank gently tapered toward both ends; hook pairs 1–6: 26 (25–28; N = 16), pair 7: 31 (30–32; N = 6) long; FH loop about 0.5 shank length. 4A's similar; each 10 (N = 2) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 33–34 (N = 2) long, 28 (24–33; N = 2) wide; ovary 28 (N = 1) long, 30 (N = 1) wide. Cirrus, accessory piece articulated by copulatory ligament 5–6 (N = 5) long. Cirrus 50 (47–54; N = 5) long, sigmoid, with slight terminal flare, base lacking flange. Accessory piece 40 (36–43; N = 5) long, with long basal, 3 distal branches; medial distal branch looping posteriorly; distal branch comprising 2 acute lobes.

REMARKS: A single specimen of *Anacanthorus alatus* was recovered from *Triportheus elongatus*. This worm did not differ in morphology of the copulatory complex or haptoral armament from those taken from *T. albus*. However, hooks of the specimen from *T. elongatus* were slightly larger (pairs 1–6: 33 [32–34; N = 4], pair 7: 38 [N = 2]).

Anacanthorus alatus resembles *A. cornutus* and *A. lygophallus* spp. n. based on morphology of the hooks and accessory piece. These species differ by the distal branch of the accessory piece in *A. alatus* apparently representing a fusion of 2 branches (branches are clearly separated in *A. cornutus* or single in *A. lygophallus*). The specific name is from Latin (*alatus* = winged) and refers to the distal branch of the accessory piece.

Anacanthorus andersoni sp. n. (Figs. 7–9)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

TYPE SPECIMENS: Holotype, INPA PA349; paratypes, USNM 81668, HWML 33342.

DESCRIPTION (based on 4 specimens): Body 421 (355–460; N = 3) long, robust, with irregular margins; greatest width 194 (177–222; N = 3) in anterior trunk. Cephalic lobes large, well developed; terminal lobes confluent. Four eyes; members of posterior pair larger, slightly closer together than those of anterior pair; granules small, subspherical to ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subovate, greatest width 28 (24–32; N = 3). Haptor 59 (57–62; N = 3) long, 90 (80–100; N = 3) wide. Hooks similar; each with truncate slightly depressed thumb, slightly expanded shank with small proximal enlargement; hook pairs 19–20 (N = 9) long; FH loop about 0.5 shank length. 4A's similar; each 8–9 (N = 2) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 56 (43–70; N = 2) long, 37 (27–48; N = 2) wide; ovary 72 (68–77; N = 3) long, 55 (49–61; N = 3) wide. Cirrus, accessory piece articulated by copulatory ligament 5–6 (N = 4) long. Cirrus 44 (35–59; N = 4) long, sigmoid, with terminal flare, large base lacking flange. Accessory piece 36 (32–39; N = 3) long, with short basal, 3 distal branches; longest distal branch terminally recurved; slender lateral branch with sigmoid origin.

REMARKS: This species is similar to *Anacanthorus chaunophallus* sp. n. but differs from it by having slightly expanded hook shanks and an elongate recurved primary branch of the accessory piece (reduced in *A. chaunophallus*). The remaining 2 branches of the accessory piece in *A. andersoni* are comparatively shorter than those of *A. chaunophallus*. This species is named for Dr. Robert C. Anderson, Idaho State University, in appreciation of his assistance during field excursions.

Anacanthorus beleophallus sp. n. (Figs. 10–13)

TYPE HOST AND LOCALITY: *Pristobrycon eigenmanni* (Norman); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

TYPE SPECIMENS: Holotype, INPA PA350; paratypes, USNM 81669, HWML 33343.

DESCRIPTION (based on 5 specimens): Body 352 (289–389; N = 3) long, fusiform, tapered toward each end from midlength; greatest width 82 (53–113; N = 3). Cephalic lobes poorly developed. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules small, ovate to elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 22 (N = 1) in diameter. Haptor 60 (56–64; N = 3) long, 65 (57–

71; $N = 3$) wide. Hooks similar; each with truncate slightly depressed thumb, variable proximal enlargement of shank; hook pairs 1, 2, 5, 7: 23 (21–26; $N = 12$), pairs 3, 4, 6: 26 (24–28; $N = 9$) long; FH loop about 0.5 shank length. 4A's similar, each 14–15 ($N = 3$) long, proximally expanded about 0.5 length. Gonads indistinct. Cirrus, accessory piece nonarticulated. Cirrus 38 (36–40; $N = 5$) long, J- or C-shaped, with submedial fold on shaft, base with flange. Accessory piece 37 (35–39; $N = 5$) long, with sickle-shaped distal tip; submedial (muscle) articulation point (see Van Every and Kritsky, 1992) slightly elevated.

REMARKS: The submedial cirral fold of *Anacanthorus beleophallus* is apparently homologous to the cirral "feather" characterizing a group of *Anacanthorus* species infesting serrasalmid hosts in the environs of Manaus, Brazil (compare Figs. 10, 60, 118, 122). Van Every and Kritsky (1992) consider *A. beleophallus* as the sister to this group of species (see fig. 70 in Van Every and Kritsky, 1992). It is easily distinguished from this species group by possessing a submedial fold on the cirrus and a sickle-shaped termination of the accessory piece. The specific name is from Greek (*beleos* = a dart + *phallos* = penis).

Anacanthorus bellus sp. n. (Figs. 14–16)

TYPE HOST AND LOCALITY: *Triportheus albus* Cope; Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORDS: *Triportheus elongatus* (Guenther), Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989) and Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (6 January 1989); *Triportheus* sp., Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (1 November 1984).

TYPE SPECIMENS: Holotype, INPA PA351; paratypes, USNM 81670, 81671, 81672, 81673, HWML 33344.

DESCRIPTION (based on 24 specimens): Body 311 (233–389; $N = 12$) long, robust, fusiform; greatest width 82 (59–110; $N = 16$) near trunk midlength. Cephalic lobes large, well developed; anterior lobes fused along midline. Four eyes equidistant; members of posterior pair larger than those of anterior pair, 1 or both members of anterior pair poorly developed or occasionally absent; granules small, variable in shape; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 17 (14–21; $N = 17$) in diameter. Haptor 40 (33–50; $N = 12$) long, 49 (40–57; $N = 11$) wide. Hooks similar, each with truncate slightly depressed thumb, shank slightly expanded with small proximal enlargement; hook pairs 17 (16–18; $N = 36$) long; FH loop about 0.6 shank length. 4A's similar; each 8 ($N = 5$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 44 (30–53; $N = 9$) long, 34 (28–40; $N = 7$) wide; ovary 46 (33–67; $N = 8$) long, 30 (20–39; $N = 7$) wide. Cirrus, accessory piece articulated by copulatory ligament 5 (3–6; $N = 19$) long. Cirrus 56 (52–62; $N = 24$) long, sigmoid, with subterminal folds, large base lacking flange. Accessory piece 38 (35–42; $N = 24$) long, with 3 branches; longest branch terminally spathulate.

REMARKS: Based on the proximal portion of the accessory piece, the branches of the accessory piece, and haptoral hooks, *Anacanthorus bellus* resembles *A. chelophorus* and *A. tricornis* spp. n. These species are easily differentiated by the comparative morphology of the cirral tip (with subterminal folds in *A. bellus*; flared in *A. chelophorus* and *a. tricornis*) and of the termination of the first branch of the accessory piece (long, spathulate in *A. bellus*; long, slightly flared in *A. chelophorus*; short, pointed in *A. tricornis*). The specific name is from Latin (*bellus* = neat, charming, handsome).

Anacanthorus calophallus sp. n. (Figs. 17–19)

TYPE HOST AND LOCALITY: *Triportheus elongatus* (Guenther); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (22 November 1983, 6 January 1989).

OTHER RECORD: *Triportheus elongatus* (Guenther), Manaus Fish Market, Manaus, Amazonas, Brazil (10 March 1979).

TYPE SPECIMENS: Holotype, INPA PA352; paratypes, USNM 81674, 81675, HWML 33345.

DESCRIPTION (based on 11 specimens): Body 384 (288–476; $N = 7$) long, robust, fusiform; greatest width 114 (64–164; $N = 6$) at various levels along trunk, usually at level of copulatory complex. Cephalic lobes large, well developed. Two eyes; granules small, subovate to spherical; accessory granules absent or rare in cephalic, anterior trunk regions of adults, numerous throughout body in young specimens. Pharynx subspherical, 29 (22–36; $N = 7$) in diameter. Haptor 53 (47–60; $N = 6$) long, 77 (69–89; $N = 6$) wide.

Hooks similar; each with truncate thumb, slender shank with prominent proximal enlargement; hook 27 (26–29; N = 29) long; FH loop about 0.4 shank length. Dorsal 4A: 12–13 (N = 3) long, ventral 4A: 9 (N = 1) long; each proximally expanded about 0.5 length; dorsal 4A comparatively more robust. Gonads slightly overlapping; testis 79 (69–102; N = 5) long, 49 (39–59; N = 4) wide; ovary 52–53 (N = 2) long, 40 (33–46; N = 2) wide. Cirrus, accessory piece articulated; copulatory ligament apparently absent. Cirrus 41 (36–48; N = 8) long, comprising small base lacking flange, tight clockwise coil of 3.5 rings; outer surface of coil with keel; ring diameter 17 (16–20; N = 9). Accessory piece 34 (31–43; N = 10) long, comprising proximal shaft lying within cirral coil, terminal branches or plates, most distal with recurved point.

REMARKS: Relatively few species of *Anacanthorus* have been described with a coiled cirrus: *A. strongylophallus, A. mastigophallus, A. furculus* spp. n., and *A. spiralocirrus* Kritsky, Thatcher, and Kayton, 1979. The cirral coils of *A. spiralocirrus* and *A. mastigophallus* are counterclockwise and clearly represent different evolutionary states developed independently within the group. The clockwise coil of *A. calophallus* apparently represents a similar state to that found in *A. strongylophallus* and *A. furculus*. Based on hook structure, *A. calophallus* most closely resembles *A. furculus*, from which it differs by possessing a lateral keel along the cirral coil and by lacking a secondary accessory piece. The specific name is from Greek (*kalos* = beautiful + *phallos* = penis).

Anacanthorus carinatus sp. n. (Figs. 20–22)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

TYPE SPECIMENS: Holotype, INPA PA353; paratypes, USNM 81676, HWML 33346.

DESCRIPTION (based on 29 specimens): Body 564 (384–707; N = 20) long, robust, fusiform, gently tapered toward both ends from midlength; greatest width 130 (92–159; N = 23). Cephalic lobes large, well developed; anterior pair fused along midline. Four eyes equidistant; members of posterior pair larger than those of anterior pair; anterior pair absent or poorly developed; granules small to moderately large, variable in shape; accessory granules scattered in cephalic, trunk regions. Pharynx subspherical or subovate, 30 (25–36; N = 24) in greatest width. Haptor 62 (45–71; N = 20) long, 89 (75–103; N = 18) wide. Hooks similar; each with depressed truncate thumb, conspicuously inflated shank tapering toward both bends; hook pairs variable in length, 34 (29–37; N = 54) long; FH loop about 0.3 shank length. 4A's similar; each 9–10 (N = 4) long, proximally expanded about 0.5 length. Gonads slighly overlapping; testis 68 (57–94; N = 17) long, 38 (26–53; N = 17) wide; ovary 69 (50–89; N = 16) long, 42 (30–59; N = 16) wide. Egg 63 (N = 1) long, 34 (N = 1) wide, subovate, with tapered anterior end, small proximal filament. Cirrus, accessory piece articulated by copulatory ligament 2–3 (N = 14) long. Cirrus 57 (53–64; N = 21) long, sigmoid, with subterminal spine or flare, large base with flange developed anteriorly to form secondary accessory piece; secondary accessory piece 57 (52–64; N = 15) long, with distal fingers supporting cirral tip. Primary accessory piece 42 (37–47; N = 14) long, with 2 blunt branches arising from short base.

REMARKS: Based on structure of the primary accessory piece, *Anacanthorus carinatus* resembles *A. euryphallus* sp. n. However, *A. euryphallus* lacks inflated hook shanks and a secondary accessory piece. *Anacanthorus carinatus* possesses hooks similar to those of *A. alatus, A. cornutus, A. lygophallus,* and *A. strongylophallus* spp. n., but is the only species in the genus with a secondary accessory piece developed from the cirral base. The specific name is from Latin (*carinatus* = keel formed) and refers to the development of the posterior margin of the cirral base.

Anacanthorus catoprioni sp. n. (Figs. 23–27)

TYPE HOST AND LOCALITY: *Catoprion mento* (Cuvier); Balbina, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (20 September 1985).

OTHER RECORD: *Catoprioni mento* (Cuvier), Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA354; paratypes, USNM 81677, 81678, HWML 33347.

DESCRIPTION (based on 32 specimens): Body 294 (233–431; N = 16) long, fusiform, gently tapered toward both ends; greatest width 78 (59–111; N = 15) near midlength. Cephalic lobes moderately developed; anterior lobes fused along midline. Four eyes equidistant; members of pos-

terior pair larger than those of anterior pair; granules variable in size; ovate to elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 19 (14–26; $N = 13$) in diameter. Haptor 45 (34–60; $N = 12$) long, 75 (58–89; $N = 10$) wide. Hooks similar; each with truncate depressed thumb, shank proximally inflated 0.25 (pairs 1, 7), 0.3 (pairs 2, 5, 6), or 0.5 (pairs 3, 4) length; hook pairs 1, 2, 5–7: 19 (17–21; $N = 54$), pairs 3, 4: 25 (23–27; $N = 27$) long; FH loop about 0.5 shank length. 4A's similar; each proximally expanded about 0.5 length; ventral 4A: 11 (8–14; $N = 11$), dorsal 4A: 13 (11–14; $N = 7$) long. Gonads slightly overlapping; testis 33 (28–41; $N = 6$) long, 21 (19–25; $N = 6$) wide; ovary 31 (27–39; $N = 11$) long, 22 (17–28; $N = 8$) wide. Cirrus, accessory piece articulated; copulatory ligament absent. Cirrus 48 (41–54; $N = 21$) long, sigmoid, with terminal flare, base lacking flange. Accessory piece 29 (27–32; $N = 18$) long, with submedial truncate branch, subterminal flap.

REMARKS: Based on hook morphology, *Anacanthorus catoprioni* is related to the *Anacanthorus* complex infesting other piranha hosts (Boeger and Kritsky, 1988; Van Every and Kritsky, 1992; this study). However, it differs from these species by possessing a sigmoid cirrus and an accessory piece articulated to the cirral base. This species is named for its host.

Anacanthorus chaunophallus sp. n. (Figs. 28–30)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA355; paratypes, USNM 81679, HWML 33348.

DESCRIPTION (based on 4 specimens): Body 341 (303–370; $N = 3$) long, slender, fusiform, gently tapered toward both ends; greatest width 70 (51–80; $N = 3$) near midlength. Cephalic lobes well developed; anterior lobes fused along midline. Four eyes equidistant; members of anterior pair infrequently absent, usually poorly developed, smaller than those of posterior pair; granules small, subspherical to elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 22 (19–24; $N = 3$) in diameter. Haptor 48 (46–50; $N = 3$) long, 56 (54–58; $N = 3$) wide. Hooks similar; each with broad truncate thumb, small proximal enlargement of slender shank; hook pairs 23 (22–24; $N = 10$) long; FH loop about 0.6 shank length. 4A's similar; each 10–11 ($N = 3$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 37 (35–38; $N = 2$) long, 21 (17–24; $N = 2$) wide; ovary 39 (38–40; $N = 2$) long, 24 (20–27; $N = 2$) wide. Cirrus, accessory piece articulated by copulatory ligament 9–10 ($N = 4$) long. Cirrus 56 (53–59; $N = 4$) long, sigmoid, with terminal flare, lateral subterminal flaps, large base lacking flange. Accessory piece 37 (36–38; $N = 4$) long, with 3 branches; medial branch flattened distally.

REMARKS: *Anacanthorus chaunophallus* is similar to *A. bellus* sp. n. It differs from this species by the proximal branch of the accessory piece being reduced and by hooks having slender shanks. The specific name is from Greek (*chaunos* = bloated + *phallos* = penis).

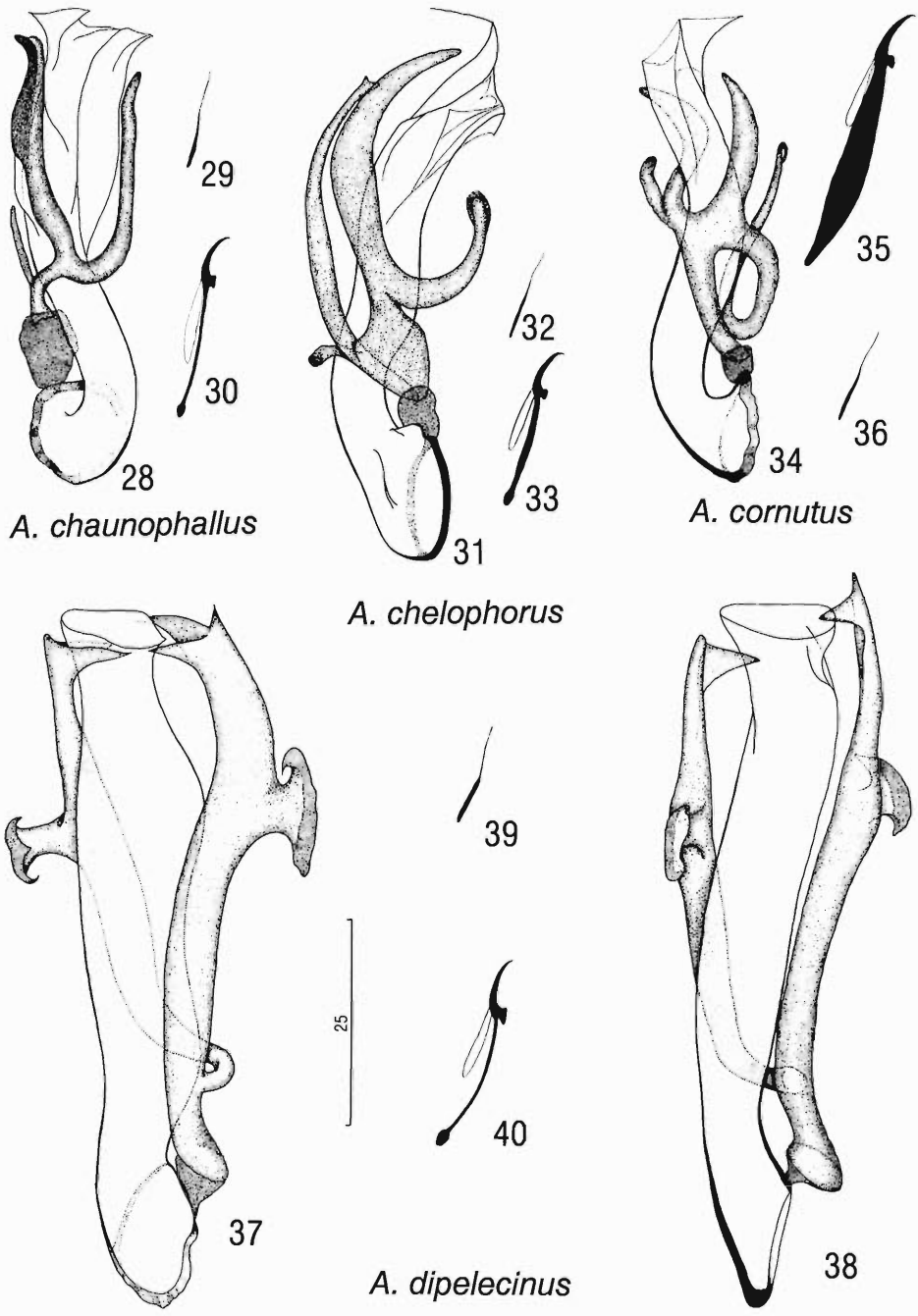
Anacanthorus chelophorus sp. n. (Figs. 31–33)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

OTHER RECORDS: *Triportheus angulatus* (Spix), Furo do Catalão near Manaus, Amazonas, Brazil; *Triportheus* sp., Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (22 November 1983).

TYPE SPECIMENS: Holotype, INPA PA356; paratypes, USNM 81680, 81681, 81682, HWML 33349.

DESCRIPTION (based on 15 specimens): Body 412 (309–578; $N = 10$) long, variable, delicate to robust, fusiform, with short to elongate peduncle; greatest width 101 (62–129; $N = 11$) at various levels along trunk. Cephalic lobes large, well developed; anterior lobes frequently fused along midline. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules small, subovate to elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 21 (17–24; $N = 9$) in diameter. Haptor 50 (44–59; $N = 9$) long, 67 (53–87; $N = 9$) wide. Hooks similar; each with truncate slightly depressed thumb, slightly expanded shank with small proximal enlargement; hook pairs 19 (18–20; $N = 24$) long; FH loop about 0.5 shank length. 4A's similar; each 8–9 ($N = 3$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 51 (37–67; $N = 8$) long, 28 (23–33; $N = 8$) wide; ovary 55 (36–83; $N = 8$) long, 31 (23–47;



Figures 28–40. Sclerotized parts of *Anacanthorus* spp., continued. 28–30. *Anacanthorus chaunophallus*. 28. Copulatory complex. 29. 4A. 30. Hook. 31–33. *Anacanthorus chelophorus*. 31. Copulatory complex. 32. 4A. 33. Hook. 34–36. *Anacanthorus cornutus*. 34. Copulatory complex. 35. Hook. 36. 4A. 37–40. *Anacanthorus dipelecinus*. 37, 38. Two views of the copulatory complex. 39. 4A. 40. Hook. All figures are drawn to the 25-μm scale.

$N = 8$) wide. Cirrus, accessory piece articulated by copulatory ligament 9 (8–10; $N = 9$) long. Cirrus 61 (53–71; $N = 14$) long, sigmoid, with terminal flare, large base lacking flange. Accessory piece 41 (37–44; $N = 13$) long, 4 branches; 2 branches forming claw.

REMARKS: *Anacanthorus chelophorus* resembles *A. glyptophallus* and *A. acuminatus* spp. n. based on the comparative morphology of the accessory piece and hooks. It differs from *A. glyptophallus* by lacking a second accessory piece and from *A. acuminatus* by possessing a large claw-shaped portion of the accessory piece. The specific name is from Greek (*chele* = claw + *phoros* = bearing).

Anacanthorus cornutus sp. n. (Figs. 34–36)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

TYPE SPECIMENS: Holotype, INPA PA357; paratypes, USNM 81683, HWML 33350.

DESCRIPTION (based on 10 specimens): Body 392 (324–438; $N = 6$) long, robust, noticeably flattened, foliform; trunk subovate in dorsoventral view; peduncle elongate; greatest width 153 (140–178; $N = 6$) at trunk midlength. Cephalic lobes large, well developed; anterior lobes fused along midline; additional small lobe on cephalic margin between bilateral lobes and cephalic tip. Four eyes equidistant; members of posterior pair larger than those of anterior pair, anterior pair absent or poorly developed; granules moderately large, elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx ovate to subspherical, 25 (24–26; $N = 6$) wide. Haptor 53 (41–59; $N = 6$) long, 69 (53–85; $N = 6$) wide. Hooks similar; each with slightly depressed truncate thumb; shank conspicuously inflated, gently tapering toward both ends; hook lengths variable, 31 (26–35; $N = 32$); FH loop about 0.3 shank length. 4A's similar; each 9–10 ($N = 5$) long, proximally expanded about 0.5 length. Gonads overlapping significantly; testis 39 (28–53; $N = 5$) long, 32 (22–39; $N = 5$) wide; ovary 58 (47–69; $N = 5$) long, 35 (21–46; $N = 6$) wide. Cirrus, accessory piece articulated by copulatory ligament 5–6 ($N = 10$) long. Cirrus 55 (50–58; $N = 10$) long, sigmoid, with subterminal spine or flare, large base lacking flange. Accessory piece 34 (33–36; $N = 9$) long, with 4 branches, 2 bladelike, 2 with blunt ends.

REMARKS: *Anacanthorus cornutus* resembles *A. alatus, A. carinatus, A. lygophallus,* and *A. strongylophallus* spp. n. by possessing hooks with shanks inflated near midlength and tapered toward both ends. It is most similar to *A. alatus,* from which it differs by being larger and by possessing 2 bladelike branches of the accessory piece (1 in *A. alatus*). The specific name is from Latin (*cornutus* = horned) and refers to the accessory piece and its branches.

Anacanthorus dipelecinus sp. n. (Figs. 37–40)

TYPE HOST AND LOCALITY: *Roeboides myersii* Gill; Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (1 November 1984).

OTHER RECORD: *Roeboides myersii* Gill, Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

TYPE SPECIMENS: Holotype, INPA PA358; paratypes, USNM 81684, 81685, HWML 33351.

DESCRIPTION (based on 21 specimens): Body 440 (403–484; $N = 4$) long, robust, fusiform, gently tapered toward each end; greatest width 101 (85–108; $N = 9$) at midlength. Cephalic lobes moderately developed; anterior lobes fused along midline. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules variable in size, subovate; accessory granules few to numerous in cephalic region, or absent. Pharynx subspherical, 27 (23–31; $N = 8$) in diameter. Haptor 50 (43–66; $N = 5$) long, 71 (59–77; $N = 5$) wide. Hooks similar, each with truncate depressed thumb, small proximal enlargement of shank; hook pairs 25 (23–26; $N = 20$) long; FH loop about 0.5 shank length. 4A's similar; each 11 (10–12; $N = 7$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 41 (31–48; $N = 4$) long, 32 (28–36; $N = 4$) wide; ovary 41 (35–49; $N = 8$) long, 42 (35–50; $N = 9$) wide. Cirrus, accessory piece articulated by copulatory ligament 6 (5–8; $N = 13$) long. Cirrus 86 (75–101; $N = 15$) long, sigmoid, expanded, with terminal flare, large base lacking flange. Accessory piece 69 (62–75; $N = 13$) long, with 2 branches; each branch with submedial short flat branch, terminal spines.

REMARKS: *Anacanthorus dipelecinus* possesses characteristics of the haptor and copulatory complex similar to those of *Anacanthorus* species infesting *Triportheus angulatus, T. albus,* and *T. elongatus.* However, it is most similar to *A. cuticulovaginus* Kritsky and Thatcher, 1974, a parasite of *Salminus affinis.* It differs from this species by possessing a hatchetlike projection on

each arm of the accessory piece (*A. cuticulovaginus* possesses undifferentiated irregular margins of the accessory piece). The specific name is from Greek (*pelecy* = hatchet) and refers to the submedial branches of the accessory piece.

Anacanthorus euryphallus sp. n. (Figs. 41–43)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORDS: *Triportheus angulatus* (Spix), Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988); *T. elongatus* (Guenther), Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989); *T. albus* Cope, Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA359; paratypes, USNM 81686, 81687, 81688, 81689, HWML 33352.

DESCRIPTION (based on 16 specimens): Body 309 (264–357; $N = 5$) long, fusiform or gently tapered anteriorly from level of testis; greatest width 67 (53–80; $N = 8$) near midlength or at testicular level. Cephalic lobes large, well developed. Four eyes equidistant; members of posterior pair larger than those of anterior pair, 1 or both members of anterior pair poorly developed or absent; granules small, elongate ovate; accessory granules rare in cephalic region. Pharynx subspherical 15 (11–17; $N = 9$) in diameter. Haptor 39 (30–52; $N = 5$) long, 57 (54–60; $N = 6$) wide. Hooks similar; each with truncate thumb, robust shank, small proximal enlargement of shank; hook pairs 20 (19–23; $N = 49$) long; FH loop about 0.5 shank length. 4A's similar; each 7 ($N = 5$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 39 ($N = 2$) long, 23 (22–24; $N = 2$) wide; ovary 41 (40–42; $N = 4$) long, 25 (15–34; $N = 5$) wide. Cirrus, accessory piece articulated by copulatory ligament 2–3 ($N = 9$) long. Cirrus 31 (28–35; $N = 14$) long, broadly tubular, sigmoid, with slight terminal flare, base lacking flange. Accessory piece 26 (23–28; $N = 15$) long, with 2 branches; longer branch terminally bifid.

REMARKS: This species resembles *Anacanthorus carinatus* sp. n. in comparative morphology of the accessory piece. They differ most significantly by *A. euryphallus* lacking expanded hook shanks and a secondary accessory piece developed from the cirral base. The specific name is from Greek (*eurys* = wide, broad + *phallos* = penis).

Anacanthorus formosus sp. n. (Figs. 44–46)

TYPE HOST AND LOCALITY: *Triportheus elongatus* (Guenther); Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORD: *Triportheus* sp., Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (1 November 1984).

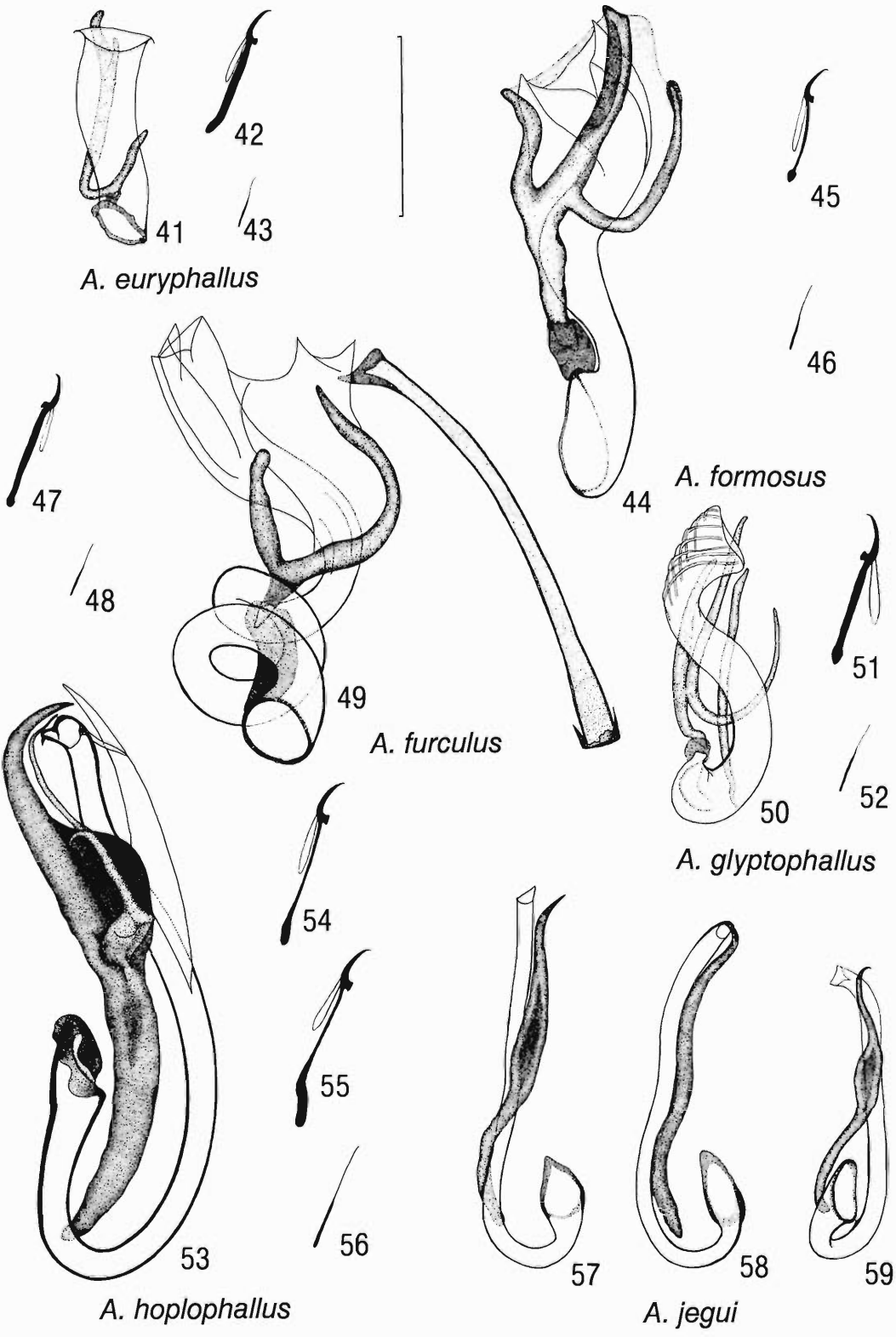
TYPE SPECIMENS: Holotype, INPA PA360; paratypes, USNM 81690, 81691, HWML 33353.

DESCRIPTION (based on 8 specimens): Body 295 (263–346; $N = 6$) long, fusiform, gently tapered toward both ends from midlength; greatest width 81 (62–100; $N = 7$). Cephalic lobes large, well developed; anterior lobes fused along midline. Four eyes equidistant; members of anterior pair poorly developed or absent, smaller than those of posterior pair; granules small, subovate; accessory granules absent or few in cephalic, anterior trunk regions. Pharynx subspherical, 16 (14–17; $N = 7$) in diameter. Haptor 45 (39–49; $N = 6$) long, 58 (48–71; $N = 5$) wide. Hooks similar; each with truncate thumb, slender shank with small proximal enlargement; hook pairs 18 (17–19; $N = 18$) long; FH loop about 0.6 shank length. 4A's similar; each 9–10 ($N = 4$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 36 (33–43; $N = 4$) long, 23 (18–26; $N = 4$) wide; ovary 39 (35–46; $N = 6$) long, 28 (20–38; $N = 6$) wide. Cirrus, accessory piece articulated by copulatory ligament 9 (8–10; $N = 7$) long. Cirrus 67 (62–72; $N = 8$) long, sigmoid, with terminal flare, large base lacking flange. Accessory piece 45 (41–47; $N = 8$) long, with long basal root, 3 blunt branches; central branch flattened.

REMARKS: Based on comparative morphology of the accessory piece, *Anacanthorus formosus* most closely resembles *A. cornutus* and *A. quinqueramus* spp. n. It differs from *A. cornutus* by lacking an inflated hook shank and from *A. quinqueramus* by lacking 2 small subterminal branches on the main accessory piece branches. The specific name is from Latin (*formosus* = beautiful).

Anacanthorus furculus sp. n. (Figs. 47–49)

TYPE HOST AND LOCALITY: *Triportheus elongatus* (Guenther); Rio Solimões near Manaus,



Amazonas, Brazil (22 November 1983, 6 January 1989).

OTHER RECORD: *Triportheus elongatus* (Guenther), Manaus Fish Market, Manaus, Amazonas, Brazil (10 March 1979).

TYPE SPECIMENS: Holotype, INPA PA361; paratypes, USNM 81692, 81693, HWML 33354.

DESCRIPTION (based on 38 specimens): Body 454 (290–584; $N = 22$) long, fusiform, with slight posterior taper from level of copulatory complex; greatest width 89 (52–140; $N = 26$). Cephalic lobes large, well developed, terminal lobes fused along midline. Two eyes, anterior pair absent; granules small, variable in shape, frequently ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 25 (17–29; $N = 24$) in diameter. Haptor 56 (40–71; $N = 21$) long, 70 (47–92; $N = 22$) wide. Hooks similar; each with truncate depressed thumb, shank slightly expanded with small proximal enlargement; hook pairs 19 (17–21; $N = 78$) long; FH loop about 0.5 shank length. 4A's similar; each 6 (5–7; $N = 3$) long, proximally expanded about 0.5 length. Gonads tandem or slightly overlapping; testis 84 (60–107; $N = 12$) long, 35 (30–40; $N = 12$) wide; ovary 36 (25–47; $N = 4$) long, 28 (18–32; $N = 4$) wide. Cirrus, primary accessory piece articulated by copulatory ligament 12 (10–14; $N = 25$) long. Cirrus 58 (46–76; $N = 29$) long, a clockwise coil of about 2.5 rings, with terminal membranous flare, base lacking flange; proximal ring diameter 21 (18–23; $N = 38$). Two accessory pieces; primary accessory piece 34 (28–45; $N = 17$) long, distally C shaped, comprised of 2 unequal branches. Secondary accessory piece 59 (49–69; $N = 28$), nonarticulated to cirrus, appearing as straight tube spined distally with proximal circular base.

REMARKS: Only *Anacanthorus glyptophallus, A. carinatus,* and *A. furculus* spp. n. are known to possess 2 accessory pieces. Unlike that of *A. furculus,* however, the secondary accessory piece of *A. carinatus* is a development of the cirral base. *Anacanthorus furculus* differs from *A. glyptophallus* by having a coiled cirrus. A coiled cirrus is also a feature of *A. spiralocirrus* Kritsky, Thatcher, and Kayton, 1979, *A. strongylophallus, A. calophallus,* and *A. mastigophallus. Anacanthorus furculus* differs from these species in comparative morphology of the primary accessory piece and haptoral hooks. The specific name is from Latin (*furcula* = small fork) and refers to the primary accessory piece.

Anacanthorus glyptophallus sp. n. (Figs. 50–52)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

TYPE SPECIMENS: Holotype, INPA PA362; paratypes, USNM 81694, HWML 33355.

DESCRIPTION (based on 36 specimens): Body 270 (228–320; $N = 22$) long, robust, fusiform; greatest width 82 (64–100; $N = 29$) near level of ovary or in posterior half. Cephalic lobes large, well developed; anterior lobes fused along midline. Four eyes; members of posterior pair larger, slightly closer together than members of anterior pair; granules small, ovate to elongate ovate; accessory granules uncommon in cephalic, anterior trunk regions. Pharynx subspherical, 16 (14–19; $N = 21$) in diameter. Haptor 41 (35–53; $N = 16$) long, 60 (50–70; $N = 20$) wide. Hooks similar; each with truncate thumb, heavy shank with small proximal enlargement; hook pairs 20 (19–21; $N = 44$) long; FH loop about 0.5 shank length. 4A's similar; each 8 ($N = 4$) long, proximally expanded about 0.6 length. Gonads slightly overlapping; testis 36 (26–46; $N = 10$) long, 25 (18–33; $N = 10$) wide; ovary 35 (26–49; $N = 15$) long, 26 (19–34; $N = 14$) wide. Seminal vesicle fusiform, with distal cork-screw configuration continuous with distal vas deferens. Cirrus, accessory piece articulated by copulatory ligament 4–5 ($N = 23$) long. Cirrus 41 (37–45; $N = 22$) long, sigmoid, with subterminal ridges, base lacking flange. Two accessory pieces; primary accessory piece 28 (24–31; $N = 21$) long, with 2 submedian branches articulating with primary branch at same point forming U-shaped structure; secondary ac-

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Figures 41–59. Sclerotized parts of *Anacanthorus* spp., continued. 41–43. *Anacanthorus euryphallus.* 41. Copulatory complex. 42. Hook. 43. 4A. 44–46. *Anacanthorus formosus.* 44. Copulatory complex. 45. Hook. 46. 4A. 47–49. *Anacanthorus furculus.* 47. Hook. 48. 4A. 49. Copulatory complex. 50–52. *Anacanthorus glyptophallus.* 50. Copulatory complex. 51. Hook. 52. 4A. 53–56. *Anacanthorus hoplophallus.* 53. Copulatory complex. 54. Hook pairs 1, 2, 5–7. 55. Hook pairs 3, 4. 56. 4A. 57–59. *Anacanthorus jegui.* 57, 58. Copulatory complexes of worms collected from *Serrasalmus spilopleura.* 59. Copulatory complex of worm collected from *Serrasalmus* sp. (2n = 58). All figures are drawn to the 25-μm scale.

cessory piece 33 (30–34; $N = 6$) long, rod shaped, with pointed ends, not articulating to cirrus or primary accessory piece.

REMARKS: *Anacanthorus glyptophallus* and *A. furculus* spp. n. are the only species of the genus with 2 accessory pieces, 1 of which is nonarticulated to the cirrus. These species are differentiated by the comparative morphology of the cirrus (sigmoid in *A. glyptophallus,* coiled in *A. furculus*). Based on the morphology of the primary accessory piece, *A. glyptophallus* resembles *A. acuminatus.* It differs from *A. acuminatus* by possessing ridges on the distal cirrus. In general, corresponding strucures of the copulatory complex of *A. acuminatus* are more delicate than those of *A. glyptophallus.* The specific name is from Greek (*glyptos* = carved + *phallos* = penis) and refers to the cirral ridges.

Anacanthorus hoplophallus sp. n. (Figs. 53–56)

TYPE HOST AND LOCALITY: *Myleus rubripinnus* (Mueller and Troschel); Nazare, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (10 September 1985).

TYPE SPECIMENS: Holotype, INPA PA363; paratypes, USNM 81695.

DESCRIPTION (based on 3 specimens): Body 379 (348–431; $N = 3$) long, fusiform, gently tapered toward both ends; greatest width 120 (73–150; $N = 3$) at midlength. Bilateral cephalic lobes reduced or absent; terminal lobes well developed, fused along midline. Four eyes equidistant; members of posterior pair much larger than those of anterior pair; granules small, subovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 19 ($N = 1$) in diameter. Haptor 49 (46–50; $N = 3$) long, 94 (79–108; $N = 3$) wide. Hooks similar; each with truncate thumb, shank with proximal enlargement about 0.2–0.3 shank length; hook pairs 1, 2, 5–7: 24 ($N = 2$), pairs 3, 4: 26–27 ($N = 4$) long; FH loop about 0.3–0.5 shank length. 4A's similar; each 11–12 ($N = 2$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 36 ($N = 1$) long, 35 ($N = 1$) wide; ovary 39 ($N = 1$) long, 28 ($N = 1$) wide. Cirrus, accessory piece nonarticulated. Cirrus 81 (74–87; $N = 3$) long, J-shaped, with delicate pointed flap arising from distal half of cirral tube, small base lacking flange. Accessory piece 78 (74–81; $N = 3$) long, with recurved acute tip, submedial keel, submedial branch with hairlike anterior extension.

REMARKS: The copulatory complex of *Anacanthorus hoplophallus* possesses features of some species infesting piranha (Van Every and Kritsky, 1992). The delicate flap arising from the midlength of the cirral shaft, the recurved lip of the cirrus, and the submedial branch of the pointed accessory piece suggest close relationship of this species to those on piranha. The specific name is from Greek (*hoplon* = tool, weapon + *phallos* = penis).

Anacanthorus jegui Van Every and Kritsky, 1992 (Figs. 57–59)

HOSTS AND LOCALITIES: *Serrasalmus spilopleura* Kner, from Rio Solimões, Ilha da Marchantaria near Manaus, Amazonas, Brazil (14 September 1984, 26 November 1984) (USNM 81696, HWML 33356); *Serrasalmus* sp. (2 of Jégu), from Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985) (USNM 81697, HWML 33357), Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985) (USNM 81698, HWML 33358), and Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985) (USNM 81699); *Serrasalmus* sp. (2n = 58), from Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989) (USNM 81700, HWML 33359); *Pristobrycon eigenmanni* (Norman), from Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985) (USNM 81701, HWML 33360), Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985) (USNM 81702, HWML 33361); *Pristobrycon* sp., from C. Miriti, Rio Uatumã, Amazonas, Brazil (26 September 1985) (USNM 81703).

REMARKS: *Anacanthorus jegui* was described from *Serrasalmus rhombeus* (Linnaeus) and characterized by double marginal expansions near the midlength of the accessory piece (Van Every and Kritsky, 1992). Morphologic variants, which we tentatively assign to *A. jegui,* have been found on other serrasalmid hosts. These variants differ primarily in the extent of the marginal expansions of the accessory piece (compare our Figs. 57–59 with figs. 24–26 in Van Every and Kritsky, 1992). Based on a comparatively high host specificity of the Dactylogyridea, it is possible that these populations comprise a species complex of closely related forms, each of which is associated with a particular serrasalmid host species.

Anacanthorus lepyrophallus sp. n. (Figs. 60–62)

TYPE HOST AND LOCALITY: *Serrasalmus elongatus* Kner; Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

OTHER RECORDS: *Serrasalmus* sp. (1 of Jégu), Rio Solimões near Ilha da Marchantaria near Manaus, Amazonas, Brazil (26 November 1984); *Serrasalmus* sp. (2n = 58), Lago do Rei, Paraná, Ilha do Careiro, Amazonas, Brazil (28 February 1986), and Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

SPECIMENS: Holotype, INPA PA364; paratypes, USNM 81704, HWML 33362; vouchers, USNM 81705, 81706, 81707.

DESCRIPTION (based on 39 specimens from *S. elongatus*): Body 412 (277–533; $N = 21$), fusiform, gently tapered toward both ends; greatest width 85 (70–101; $N = 21$) near midlength. Cephalic lobes well developed; anterior lobes fused medially. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules variable in size, ovate to elongate ovate; accessory granules scattered in cephalic, trunk regions. Pharynx subovate, 26 (22–35; $N = 25$) wide. Haptor 41 (33–49; $N = 15$) long, 72 (60–83; $N = 13$) wide. Hooks similar; each with short point, truncate depressed thumb, proximal enlargement of shank about 0.5 shank length; hook pairs 27 (24–31; $N = 44$) long; FH loop about 0.5 shank length. 4A's similar; each 14–15 ($N = 4$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 105 (41–167; $N = 10$) long, 33 (26–41; $N = 11$) wide; ovary 55 (36–70; $N = 18$) long, 25 (16–38; $N = 18$) wide. Cirrus, accessory piece nonarticulated. Cirrus 59 (52–67; $N = 28$) long, J-shaped, with subterminal aperture, submedial cirral "feather," base with small flange. Accessory piece 47 (43–55; $N = 24$) long, with sickle-shaped tip, small subterminal flap, indistinct submedial (muscle) articulation point.

REMARKS: The phylogenetic relationships of this species is provided by Van Every and Kritsky (1992). The above description is based only on specimens collected from *Serrasalmus elongatus*. However, specimens of *A. lepyrophallus* from other *Serrasalmus* species do not differ significantly in morphology; their measurements fall within ranges for corresponding values of *A. lepyrophallus* from the type host. The specific name is from Greek (*lepyron* = a husk + *phallos* = penis) and refers to the featherlike appendage on the cirrus.

Anacanthorus lygophallus sp. n. (Figs. 63–65)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA365; paratypes, USNM 81708.

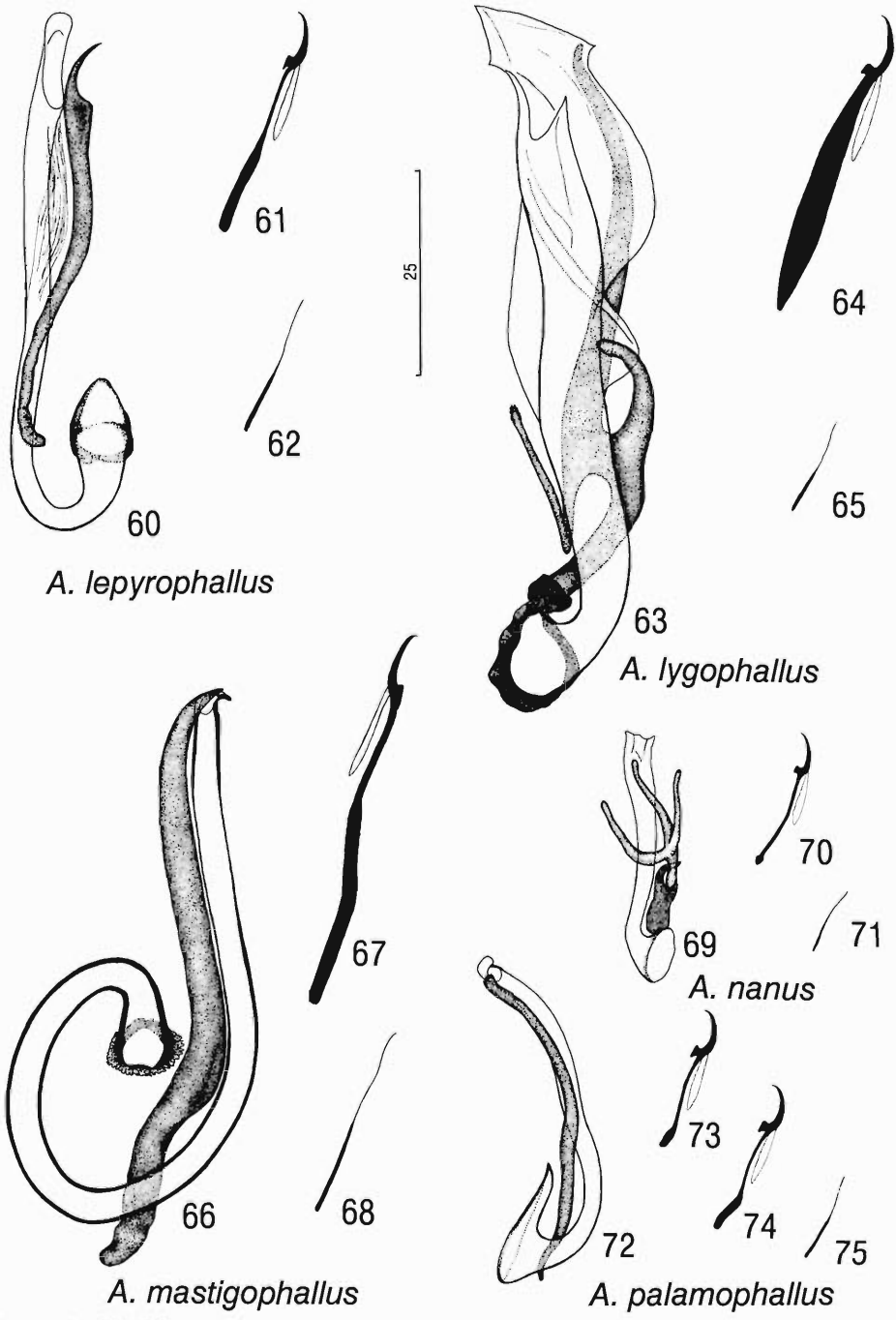
DESCRIPTION (based on 3 specimens): Body 531 ($N = 1$) long, robust; greatest width 132 ($N = 1$) in anterior trunk; peduncle moderately elongate. Cephalic lobes well developed. Two eyes, anterior pair absent; granules small, elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 24 ($N = 1$) in diameter. Haptor 62 ($N = 1$) long, 88 ($N = 1$) wide. Hooks similar; each with truncate thumb; shank conspicuously inflated, gently tapering toward both ends; hook pair 7 more robust, slightly larger than other pairs; hook pairs 35 (31–39; $N = 12$) long; FH loop about 0.3 shank length. 4A's similar; each 9 ($N = 1$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 33 ($N = 1$) long, 42 ($N = 1$) wide; ovary 58 ($N = 1$) long, 54 ($N = 1$) wide. Cirrus, accessory piece articulated by copulatory ligament 3–4 ($N = 3$) long. Cirrus 84 (79–87; $N = 3$) long, sigmoid, with subterminal flaps, terminal flare, large base lacking flange. Accessory piece 68 (64–72; $N = 3$) long, with long base, 3 branches; primary branch, 1 secondary branch separated from base by short lateral extension.

REMARKS: *Anacanthorus lygophallus* is likely the sister species of *A. cornutus* sp. n. based on morphology of the hooks and accessory piece. They differ by *A. lygophallus* having 3 branches of the accessory piece (4 in *A. cornutus*). The specific name is from Greek (*lygos* = twig + *phallos* = penis).

Anacanthorus mastigophallus sp. n. (Figs. 66–68)

TYPE HOST AND LOCALITY: *Pristobrycon eigenmanni* (Norman); Nazare, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (17 September 1985).

OTHER RECORD: *Pristobrycon eigenmanni* (Norman), Santa Luzia, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (20 September 1985).



Figures 60–75. Sclerotized parts of *Anacanthorus* spp., continued. 60–62. *Anacanthorus lepyrophallus*. 60. Copulatory complex. 61. Hook. 62. 4A. 63–65. *Anacanthorus lygophallus*. 63. Copulatory complex. 64. Hook. 65. 4A. 66–68. *Anacanthorus mastigophallus*. 66. Copulatory complex. 67. Hook. 68. 4A. 69–71. *Anacanthorus nanus*. 69. Copulatory complex. 70. Hook. 71. 4A. 72–75. *Anacanthorus palamophallus*. 72. Copulatory complex. 73. Hook pairs 1, 2, 5–7. 74. Hook pairs 3, 4. 75. 4A. All figures are drawn to the 25-μm scale.

TYPE SPECIMENS: Holotype, INPA PA366; paratypes, USNM 81709, 81710, HWML 33363.

DESCRIPTION (based on 8 specimens): Body 584 (543–635; $N = 4$) long, robust, fusiform, gently tapered toward both ends; greatest width 170 (150–191; $N = 3$) near midlength. Cephalic lobes well developed. Four eyes; members of posterior pair larger, slightly farther apart than those of anterior pair; granules moderate in size, elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 40 (38–44; $N = 3$) in a diameter. Haptor 76 (70–88; $N = 3$) long, 95 (78–108; $N = 3$) wide. Hooks similar; each with flattened thumb, long proximal enlargement of shank; hook pairs 48 (46–53; $N = 13$) long; FH loop about 0.3 shank length. 4A's similar; each 18 (16–19; $N = 6$) long, proximally expanded about 0.5 length. Gonads slightly overlapping or tandem; testis 119 (95–144; $N = 2$) long, 66 (57–74; $N = 2$) wide; ovary 51 (46–56; $N = 2$) long, 49 (48–50; $N = 2$) wide. Cirrus, accessory piece nonarticulated. Cirrus 69 (66–72; $N = 6$) long, a counterclockwise coil of about 1 ring; base lacking flange, aperture directed posteriorly; ring diameter 30 (27–32; $N = 7$). Accessory piece 72 (69–75; $N = 5$) long, rod-shaped, with acute curved tip, small submedial flap; submedial (muscle) articulation point indistinct.

REMARKS: The phylogenetic relationships of this species is provided by Van Every and Kritsky (1992). The specific name is from Greek (*mastigos* = whip + *phallos* = penis) and refers to the shape of the cirrus.

Anacanthorus nanus sp. n. (Figs. 69–71)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

TYPE SPECIMENS: Holotype, INPA PA367; paratypes, USNM 81711, HWML 33364.

DESCRIPTION (based on 14 specimens): Body 315 (263–390; $N = 9$) long, robust, fusiform, gently tapered toward both ends; peduncle poorly developed; greatest width 80 (66–93; $N = 11$) near midlength. Cephalic lobes well developed. Four eyes equidistant; members of anterior pair absent or poorly developed, smaller than those of posterior pair; granules small to moderate, elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 17 (15–18; $N = 9$) in diameter. Haptor 45 (36–52; $N = 9$) long, 53 (43–63; $N = 10$) wide. Hooks similar; each with truncate thumb, slender shank with small proximal enlargement; hook pairs 17–18 ($N = 15$) long; FH loop about 0.5 shank length. 4A's similar; each 7–8 ($N = 2$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 41 (33–60; $N = 8$) long, 27 (21–36; $N = 8$) wide; ovary 55 (47–64; $N = 7$) long, 25 (18–30; $N = 7$) wide. Cirrus, accessory piece articulated by copulatory ligament 7–8 ($N = 12$) long. Cirrus 31 (29–34; $N = 8$) long, sigmoid, with small terminal flare, large base lacking flange. Accessory piece 18 (16–20; $N = 12$) long, with 3 subequal blunt branches; 2 lateral branches forming U-shape.

REMARKS: This species resembles *Anacanthorus formosus* sp. n. in morphology of the haptoral hooks, accessory piece, and cirrus. The copulatory complex is significantly smaller and the 2 lateral branches of the accessory piece arise from the same level in *A. nanus,* whereas the lateral arms of the accessory piece in *A. formosus* originate at different levels along the main branch. The specific name is from Greek (*nannos* = dwarf) and refers to the small size of this worm.

Anacanthorus palamophallus sp. n. (Figs. 72–75)

TYPE HOST AND LOCALITY: *Pristobrycon eigenmanni* (Norman); Nazare, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (17 September 1985).

OTHER RECORD: *Pristobrycon eigenmanni* (Norman), Santa Luzia, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (20 September 1985).

TYPE SPECIMENS: Holotype, INPA PA368; paratypes, USNM 81712, 81713, HWML 33365.

DESCRIPTION (based on 26 specimens): Body 313 (196–428; $N = 10$) long, fusiform, gently tapered from midlength toward both ends; greatest width 68 (45–94; $N = 14$). Cephalic lobes well developed. Four eyes; members of posterior pair larger, slightly closer together than those of anterior pair; granules small, elongate ovate; accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 17 (14–22; $N = 11$) in diameter. Haptor 41 (28–64; $N = 8$) long, 62 (48–72; $N = 7$) wide. Hooks similar; each with truncate slightly depressed thumb, shank proximally enlarged about 0.2–0.3 shank length; hook pairs 1, 2, 5–7: 18 (17–20; $N = 23$), pairs 3, 4: 19–20 ($N = 11$) long; FH loop about

0.5 shank length. 4A's similar; each 12 (11–13; $N = 8$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 45 (26–77; $N = 9$) long, 22 (16–26; $N = 9$) wide; ovary 35 (24–53; $N = 9$) long, 20 (16–26; $N = 9$) wide. Cirrus, accessory piece nonarticulated. Cirrus 46 (40–53; $N = 15$) long, C-shaped, with subterminal aperture, terminal flap, anteriorly acute base. Accessory piece 42 (36–48; $N = 11$) long, rod-shaped, with blunt tip, indistinct submedial (muscle) articulation point.

REMARKS: This species is sister to *Anacanthorus stachophallus* sp. n. and *Anacanthorus thatcheri* Boeger and Kritsky, 1988 (see Van Every and Kritsky, 1992). It differs from these species by lacking a knob on the cirral base and by possessing an unflared blunt tip of the accessory piece. The specific name is from Greek (*palame* = palm + *phallos* = penis).

Anacanthorus paraspathulatus sp. n. (Figs. 76–78)

TYPE HOST AND LOCALITY: *Mylossoma duriventris* (Cuvier); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (6 January 1989).

TYPE SPECIMENS: Holotype, INPA PA369; paratypes, USNM 81714, HWML 33366.

DESCRIPTION (based on 7 specimens): Body 379 (328–446; $N = 6$) long, fusiform, gently tapered toward both ends; greatest width 69 (55–85; $N = 6$) at various levels along trunk. Cephalic lobes well developed. Four eyes; members of posterior pair larger, closer together than those of anterior pair; granules small, ovate; accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 22 (20–25; $N = 4$) in diameter. Haptor 49 (39–57; $N = 6$) long, 77 (70–82; $N = 6$) wide. Hooks similar; each with truncate depressed thumb, shank proximally enlarged about 0.3 length; hook pairs 26 (24–28; $N = 16$) long; FH loop about 0.5 shank length. 4A's similar; each 13–14 ($N = 2$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 57 ($N = 1$) long, 16 ($N = 1$) wide; ovary 53 ($N = 1$) long, 13 ($N = 1$) wide. Cirrus, accessory piece articulated by copulatory ligament 4 ($N = 6$) long. Cirrus 37 (35–42; $N = 5$) long, sigmoid or C-shaped, with terminal flare, large base lacking flange. Accessory piece 22 (20–23; $N = 5$) long, with hooked tip, submedial branch or shelf.

REMARKS: *Anacanthorus paraspathulatus* is similar to *A. spathulatus* Kritsky, Thatcher, and Kayton, 1979. Both species possess an accessory piece with a submedial shelf and hooks with depressed thumbs. The new species differs from *A. spathulatus* by having an acute termination of the accessory piece (spathulate in *A. spathulatus*). The specific name (*paraspathulatus*) reflects similarity of this species with *A. spathulatus*.

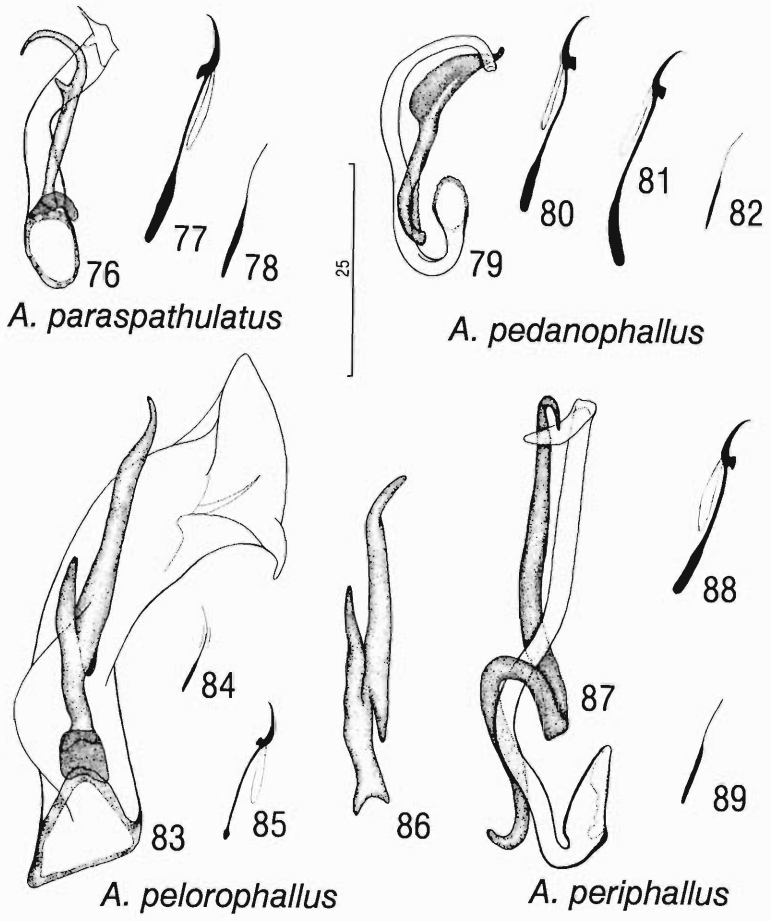
Anacanthorus pedanophallus sp. n. (Figs. 79–82)

TYPE HOST AND LOCALITY: *Myleus rubripinnus* (Mueller and Troschel); Nazare, Rio Uatumã, Amazonas, Brazil (10 September 1985).

TYPE SPECIMENS: Holotype, INPA PA370; paratypes, USNM 81715, HWML 33367.

DESCRIPTION (based on 19 specimens): Body 475 (369–577; $N = 8$) long, fusiform, gently tapered toward both ends; greatest width 90 (70–114; $N = 8$) at various levels along trunk. Bilateral cephalic lobes usually absent; terminal lobes well developed. Four eyes equidistant; members of posterior pair much larger than those of anterior pair; granules variable in size, ovate to elongate ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical to subovate, 23 (16–31; $N = 8$) wide. Haptor 53 (41–67; $N = 9$) long, 73 (66–83; $N = 9$) wide. Hooks similar; each with truncate slightly depressed thumb, proximally enlarged shank; hook pairs 1, 2, 5–7: 24 (22–26; $N = 21$), pairs 3, 4: 27 (25–28; $N = 14$) long; FH loop about 0.5 shank length. 4A's similar; each 13 (12–14; $N = 5$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 66 (41–81; $N = 7$) long, 26 (21–39; $N = 6$) wide; ovary 55 (42–60; $N = 8$) long, 24 (18–31; $N = 7$) wide. Cirrus, accessory piece nonarticulated. Cirrus 33 (25–49; $N = 13$) long, J-shaped with recurved termination, base lacking flange. Accessory piece 27 (21–32; $N = 12$) long, with subterminal tapered flap beginning near midlength.

REMARKS: *Anacanthorus pedanophallus* undoubtedly is related to the group of anacanthorine parasites infesting *Serrasalmus, Pristobrycon,* and *Pygocentrus* hosts (Boeger and Kritsky, 1988; Van Every and Kritsky, 1992; nobis) as shown by the common features of 1) a J-shaped cirrus, 2) nonarticulated cirrus and accessory piece, and 3) general morphology of the haptoral hooks. It could most easily be confused with the nontype form of *A. reginae* Boeger and Kritsky, 1988, in that both possess recurved cirral tips and a sub-



Figures 76–89. Sclerotized parts of *Anacanthorus* spp., continued. 76–78. *Anacanthorus paraspathulatus*. 76. Copulatory complex. 77. Hook. 78. 4A. 79–82. *Anacanthorus pedanophallus*. 79. Copulatory complex. 80. Hook pairs 1, 2, 5–7. 81. Hook pairs 3, 4. 82. 4A. 83–86. *Anacanthorus pelorophallus*. 83. Copulatory complex. 84. 4A. 85. Hook. 86. Accessory piece. 87–89. *Anacanthorus periphallus*. 87. Copulatory complex. 88. Hook. 89. 4A. All figures are drawn to the 25-μm scale.

terminal flap on the accessory piece. The specific name is from Greek (*pedanos* = short + *phallos* = penis).

Anacanthorus pelorophallus sp. n. (Figs. 83–86)

TYPE HOST AND LOCALITY: *Triportheus elongatus* (Guenther); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (22 November 1983; 6 January 1989).

OTHER RECORD: *Triportheus elongatus* (Guenther), Manaus Fish Market, Manaus, Amazonas, Brazil (10 March 1979).

TYPE SPECIMENS: Holotype, INPA PA371; paratypes, USNM 81716, 81717, HWML 33368.

DESCRIPTION (based on 13 specimens): Body 407 (273–509; $N = 11$) long, fusiform, gently tapered toward both ends from midlength; greatest width 81 (69–104; $N = 11$). Cephalic lobes well developed. Four eyes equidistant; members of anterior pair absent or poorly developed, smaller than those of posterior pair; granules small, ovate; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 21 (14–26; $N = 12$) in diameter. Haptor 48 (36–59; $N = 8$) long, 58 (48–71; $N = 9$) wide. Hooks similar; each with truncate thumb, slender shank with small proximal enlargement; hook pairs 18 (17–19; $N = 26$) long; FH loop about 0.6 shank length. 4A's similar; each 9–10

(N = 2) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 66 (55–83; N = 8) long, 37 (29–44; N = 8) wide; ovary 44 (35–59; N = 4) long, 26 (22–32; N = 4) wide. Cirrus, accessory piece articulated by copulatory ligament 5–6 (N = 11) long. Cirrus 64 (57–69; N = 13) long, sigmoid, expanded, with submedian hump, large terminal flare, large base lacking flange. Accessory piece 38 (34–42; N = 13) long, comprising 2 rods joined laterally at respective proximal, distal ends.

REMARKS: Based on morphology of the haptoral hooks, *Anacanthorus pelorophallus* resembles *A. quinqueramus, A. calophallus, A. formosus, A. nanus,* and *A. chaunophallus* spp. n. It is distinguished from all of these by having a cirrus with a submedian "hump" and a relatively simple accessory piece. The specific name is from Greek (*peloros* = monstrous + *phallos* = penis).

Anacanthorus periphallus sp. n. (Figs. 87–89)

TYPE HOST AND LOCALITY: *Serrasalmus* sp. (2n = 58); Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORD: *Serrasalmus* sp. (1 of Jégu), Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984).

TYPE SPECIMENS: Holotype, INPA PA372; paratypes, USNM 81718, 81719, HWML 33369.

DESCRIPTION (based on 8 specimens): Body 318 (238–377; N = 7) long, robust, fusiform, gently tapered toward both ends; greatest width 106 (87–134; N = 6) near midlength. Cephalic lobes well developed. Four eyes; members of posterior pair larger, usually closer together than those of anterior pair; granules variable in size, irregular to elongate ovate; accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 24 (20–26; N = 6) in diameter. Haptor 47 (38–62, N = 6) long, 87 (65–107; N = 6) wide. Hooks similar; each with truncate slightly depressed thumb, shank proximally enlarged about 0.3 length; hook pairs 25 (22–28; N = 21) long; FH loop about 0.5 shank length. 4A's similar; each 12 (10–14; N = 5) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 50 (N = 1) long, 28 (N = 1) wide; ovary 41 (N = 1) long, 27 (N = 1) wide. Cirrus, accessory piece nonarticulated. Cirrus 53 (47–57; N = 8) long, modified J-shape, with submedial bend, large base lacking flange, subterminal opening. Accessory piece 52 (45–55; N = 7) long, rod-shaped, wrapped around cirral shaft, terminally acute; submedial (muscle) articulation point indistinct.

REMARKS: Although *Anacanthorus periphallus* differs significantly from other *Anacanthorus* spp. infesting serrasalmid hosts by having the accessory piece wrapped around the cirral shaft, it clearly belongs to the large and complicated subgroup having J-shaped cirri wtih nonarticulating accessory pieces. Its phylogenetic relationships are provided by Van Every and Kritsky (1992). The specific name from Greek reflects the looping of the accessory piece around the cirrus (*peri* = around + *phallos* = penis).

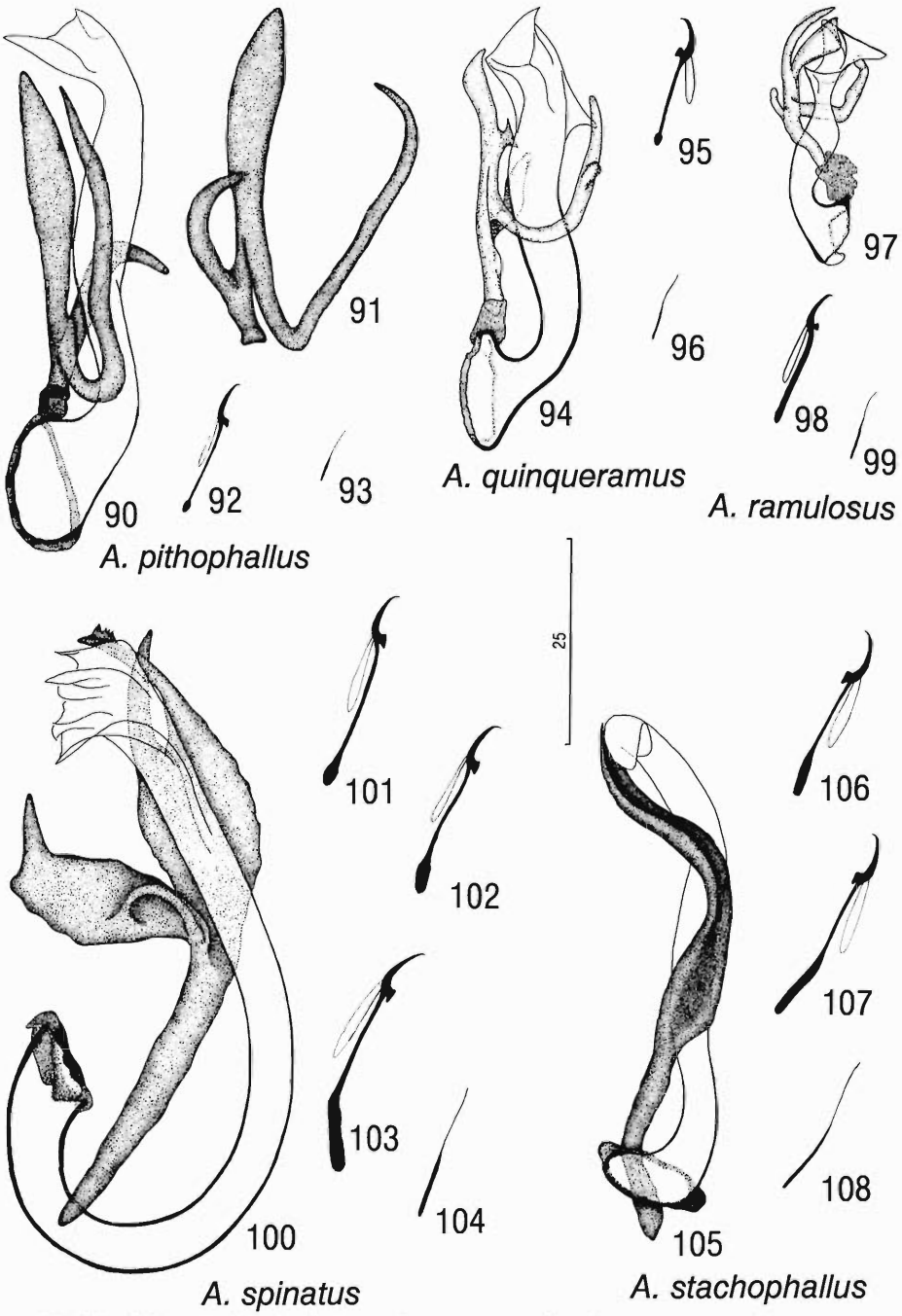
Anacanthorus pithophallus sp. n. (Figs. 90–93)

TYPE HOST AND LOCALITY: *Triportheus angulatus* (Spix); Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988).

TYPE SPECIMENS: Holotype, INPA PA373; paratypes, USNM 81720, HWML 33370.

DESCRIPTION (based on 5 specimens): Body 267 (222–290; N = 4) long, robust, fusiform, gently tapered toward both ends; greatest width 85 (75–94; N = 5) at midlength. Cephalic lobes well developed. Four eyes equidistant; members of anterior pair slightly smaller than those of posterior pair; granules small, variable in shape; accessory granules apparently absent. Pharynx subspherical, 19 (17–21; N = 5) in diameter. Haptor 39 (33–43; N = 5) long, 58 (53–64; N = 4) wide. Hooks similar, each with truncate thumb, small proximal enlargement of slender shank; hook pairs 18 (17–19; N = 12) long; FH loop about 0.5 shank length. 4A's similar; each 6 (N = 2) long, proximally expanded about 0.5 length. Gonads slightly overlapping or tandem; testis 32 (24–39; N = 4) long, 26 (22–31; N = 4) wide; ovary 37 (28–42; N = 5) long, 31 (25–40; N = 4) wide. Cirrus, accessory piece articulated by copulatory ligament 3–4 (N = 5) long. Cirrus 63 (59–69; N = 5) long, sigmoid, with terminal flare, large base lacking flange. Accessory piece 39 (37–40; N = 5) long, with 3 branches, primary branch club-shaped.

REMARKS: This species resembles *Anacanthorus formosus* sp. n. in the general morphology of the accessory piece and cirrus. They differ most significantly by *A. pithophallus* having a comparatively shorter accessory piece base with a club-shaped primary branch. In addition, the lat-



Figures 90–108. Sclerotized parts of *Anacanthorus* spp., continued. 90–93. *Anacanthorus pithophallus*. 90. Copulatory complex. 91. Accessory piece. 92. Hook. 93. 4A. 94–96. *Anacanthorus quinqueramus*. 94. Copulatory complex. 95. Hook. 96. 4A. 97–99. *Anacanthorus ramulosus*. 97. Copulatory complex. 98. Hook. 99. 4A. 100–104. *Anacanthorus spinatus*. 100. Copulatory complex. 101. Hook pairs 1, 5–7. 102. Hook pair 2. 103. Hook pairs 3, 4. 104. 4A. 105–108. *Anacanthorus stachophallus*. 105. Copulatory complex. 106. Hook pairs 1, 2, 5–7. 107. Hook pairs 3, 4. 108. 4A. All figures are drawn to the 25-μm scale.

eral branch of the accessory piece is noticeably longer in *A. pithophallus*. The specific name is from Greek (*pithos* = a wide-mouth jar + *phallos* = penis).

Anacanthorus quinqueramus sp. n. (Figs. 94–96)

TYPE HOST AND LOCALITY: *Triportheus albus* Cope; Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORDS: *Triportheus elongatus* (Guenther), Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989); *Triportheus* sp., Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (1 November 1984).

TYPE SPECIMENS: Holotype, INPA PA374; paratypes, USNM 81721, 81722, 81723, HWML 33371.

DESCRIPTION (based on 30 specimens): Body 288 (217–344; $N = 16$) long, robust, gently tapered posteriorly from level of copulatory complex; greatest width 92 (57–116; $N = 23$). Cephalic lobes well developed; anterior lobes fused along midline. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules small, variable in shape; few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 21 (16–26; $N = 23$) in diameter. Haptor 37 (27–47; $N = 13$) long, 59 (50–74; $N = 13$) wide. Hooks similar; each with truncate thumb, small proximal enlargement of slender shank; hook pairs 16 (14–17; $N = 22$) long; FH loop about 0.5 shank length. 4A's similar; each 10 ($N = 3$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 37 (30–41; $N = 4$) long, 27 (25–29; $N = 3$) wide; ovary 28 (19–32; $N = 6$) long, 27 (23–33; $N = 6$) wide. Cirrus, accessory piece articulated by copulatory ligament 6 (5–8; $N = 28$) long. Cirrus 53 (48–57; $N = 19$) long, sigmoid, with subterminal spine, large conical base lacking flange. Accessory piece 33 (30–35; $N = 16$) long, with 5 branches.

REMARKS: *Anacanthorus quinqueramus* resembles *A. glyptophallus* and *A. chelophorus* spp. n. by having a U-shaped medial branch of the accessory piece. It differs from these species by having a total of 5 branches on the accessory piece and by having hooks with delicate shanks. The specific name is from Latin (*quinque* = five + *ramus* = branch) and refers to the accessory piece.

Anacanthorus ramulosus sp. n. (Figs. 97–99)

TYPE HOST AND LOCALITY: *Triportheus albus* Cope; Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

OTHER RECORDS: *Triportheus elongatus* (Guenther), Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA375; paratypes, USNM 81724, 81725, HWML 33372.

DESCRIPTION (based on 24 specimens): Body 210 (173–261; $N = 11$) long, robust, gently tapered anteriorly from level of testis; greatest width 64 (51–77; $N = 10$). Cephalic lobes well developed. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules small, elongate ovate; few to many accessory granules scattered throughout cephalic, trunk regions. Pharynx subspherical, 17 (15–19; $N = 9$) in diameter. Haptor 31 (29–35; $N = 10$) long, 56 (47–73; $N = 7$) wide. Hooks similar; each with truncate thumb, slightly inflated shank with small proximal enlargment; hook pairs 17–18 ($N = 36$) long; FH loop about 0.6 shank length. 4A's similar; each 7–8 ($N = 4$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 32 (29–33; $N = 4$) long, 22 (18–23; $N = 5$) wide; ovary 35 (25–45; $N = 7$) long, 23 (17–30; $N = 7$) wide. Cirrus, accessory piece articulated by copulatory ligament 8 (7–9; $N = 22$) long. Cirrus 33 (29–38; $N = 18$) long, sigmoid, with terminal flare, large base lacking flange. Accessory piece 22 (19–26; $N = 14$) long, with 4 branches; 1 medial branch "strong-arm"-shaped.

REMARKS: Based on the comparative morphology of the accessory piece and cirral base, this species resembles *Anacanthorus quinqueramus* and *A. formosus* spp. n. *Anacanthorus ramulosus* differs from *A. quinqueramus* by lacking a subterminal branch of the ventral medial arm of the accessory piece and by possessing slightly inflated hook shanks. It differs from *A. formosus* by the primary branch of the accessory piece being bifurcated into bluntly pointed and spathulate arms. The specific epithet is from Latin (*ramulosus* = full of small branches) and refers to the accessory piece.

Anacanthorus spinatus sp. n. (Figs. 100–104)

TYPE HOST AND LOCALITY: *Myleus rubripinnus* (Mueller and Troschel); Nazare, Rio Uatu-

mã, a tributary of Rio Amazonas, Amazonas, Brazil (10 September 1985).

TYPE SPECIMENS: Holotype, INPA PA376; paratypes, USNM 81726, HWML 33373.

DESCRIPTION (based on 12 specimens): Body 388 (274–668; N = 10) long, robust, fusiform, usually strongly contracted; greatest width 168 (108–229; N = 10) near midlength. Cephalic margin usually rounded, lobes poorly developed. Four eyes equidistinct; members of posterior pair larger than those of anterior pair; granules small, irregular to elongate ovate; few accessory granules scattered in cephalic region. Pharynx subspherical, 27 (22–32; N = 7) in diameter. Haptor 59 (45–78; N = 8) long, 117 (92–157; N = 9) wide. Hooks similar; each with truncate slightly depressed thumb, shank with proximal enlargement varying in length with hook pair; hook pairs 1, 5–7: 25 (23–27; N = 16), pairs 2, 3, 4: 30 (29–32; N = 13) long; FH loop about 0.6 shank length. 4A's similar; each 15 (14–16; N = 8) long, proximally expanded about 0.5 length. Gonads slightly overlapping (all available specimens unstained). Cirrus, accessory piece nonarticulated. Cirrus 80 (77–87; N = 9) long, J-shaped, with terminal flare, base lacking flange. Accessory piece 70 (62–77; N = 8) long, with submedial bifurcation, branches terminally spined.

REMARKS: All available specimens of *Anacanthorus spinatus* were unstained and strongly contracted, both of which precluded reliable measurements of the reproductive organs. However, this species is distinct by having an accessory piece with 2 "antler-like" branches distally spined. It is apparently related to the group of species infesting Serrasalmidae and characterized by possessing an accessory piece nonarticulated to the J-shaped cirrus. The specific name is from Latin (*spinatus* = spined) and refers to the accessory piece.

Anacanthorus stachophallus sp. n. (Figs. 105–108)

SYNONYM: *Anacanthorus* sp. (of Boeger and Kritsky, 1988).

TYPE HOST AND LOCALITY: *Pygocentrus nattereri* Kner; Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (15 August 1984; 6, 25, 27 November 1984).

OTHER RECORD: *Pygocentrus nattereri* Kner, Furo do Catalão near Manaus, Amazonas, Brazil (27 November 1984).

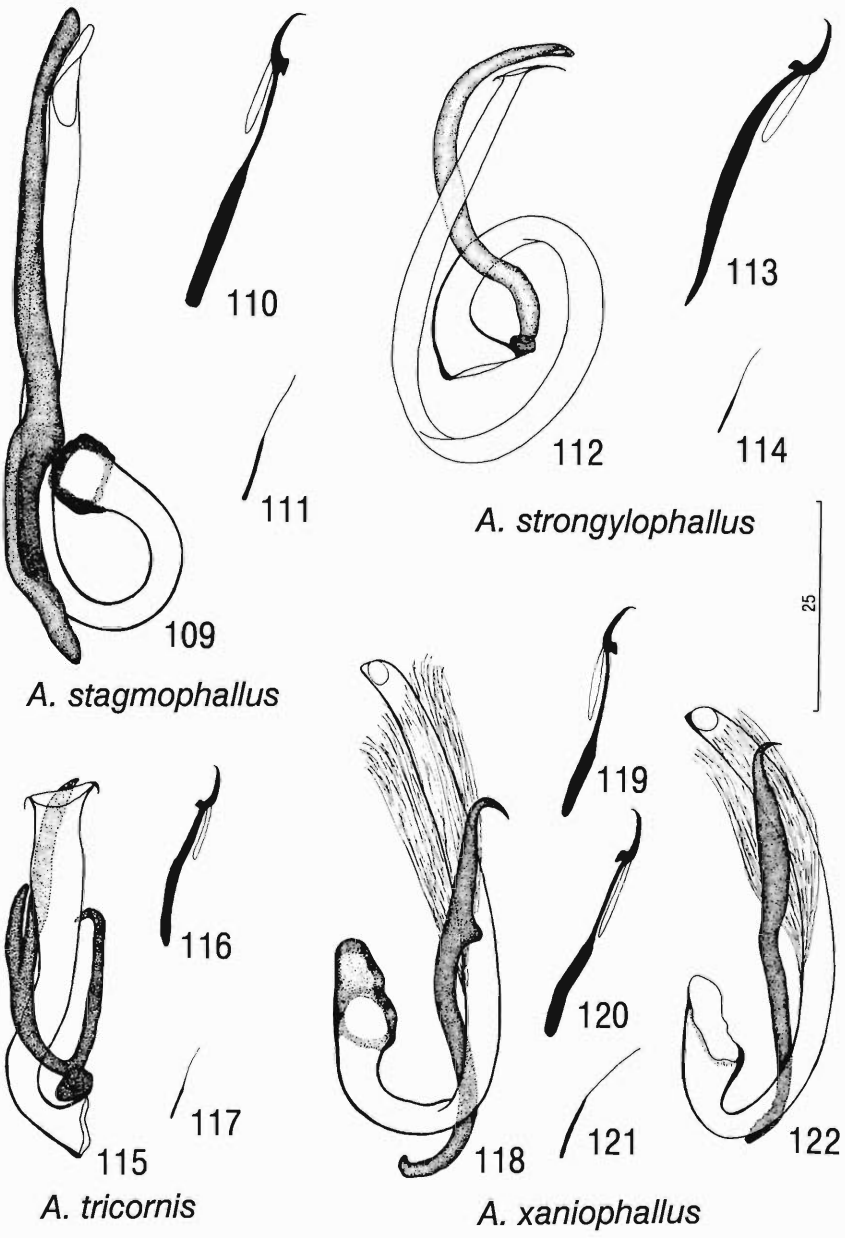
TYPE SPECIMENS: Holotype, INPA PA377; paratypes, USNM 79197, 81727, HWML 23371.

DESCRIPTION (based on 7 specimens): Body 564 (466–655; N = 7) long, fusiform, gently tapered toward both ends from midlength; greatest width 163 (118–195; N = 6). Cephalic lobes well developed. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules variable in size, elongate ovate; accessory granules absent to many in cephalic, anterior trunk regions. Pharynx subspherical, 36 (N = 1) in diameter. Haptor 73 (52–91; N = 4) long, 111 (84–136; N = 4) wide. Hooks similar; each with truncate slightly depressed thumb, shank enlarged proximally 0.3–0.5 shank length; hook pairs 1, 2, 5–7: 22 (21–23; N = 6), pairs 3, 4: 25 (24–26; N = 5) long; FH loop about 0.5 shank length. 4A's similar; each 13–14 (N = 3) long, proximally expanded about 0.5 length. Gonads slightly overlapping, indistinct. Cirrus, accessory piece nonarticulated. Cirrus 58 (54–65; N = 7) long, C-shaped, with subterminal aperture, large base having small proximal knob. Accessory piece 59 (50–65; N = 7) long, rod-shaped, with flared distal end, submedial (muscle) articulation point indistinct.

REMARKS: All available specimens of *Anacanthorus stachophallus* were unstained and mounted in Gray and Wess' medium which tend to flatten the specimens. As a result, measurements of soft body parts, including the body length and width, may not be totally comparable with measurements of other species described herein. However, *A. stachophallus*, recognized by Boeger and Kritsky (1988) as an undescribed species, is easily distinguished by the morphology of the copulatory complex. It is sister species to *A. thatcheri* Boeger and Kritsky, 1988, and *A. palamophallus* sp. n., from which it differs in the comparative morphology of the accessory piece. *Anacanthorus stachophallus* exhibits a basal to diagonal opening at the proximal end of the cirrus, whereas that exhibited by the other 2 species is lateral (Van Every and Kritsky, 1992). The specific epithet is from Greek (*stachys* = a spike + *phallos* = penis).

Anacanthorus stagmophallus sp. n. (Figs. 109–111)

TYPE HOST AND LOCALITY: *Myleus rubripinnus* (Mueller and Troschel); Nazare, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (10 September 1985).



Figures 109–122. Sclerotized parts of *Anacanthorus* spp., continued. 109–111. *Anacanthorus stagmophallus*. 109. Copulatory complex. 110. Hook. 111. 4A. 112–114. *Anacanthorus strongylophallus*. 112. Copulatory complex. 113. Hook. 114. 4A. 115–117. *Anacanthorus tricornis*. 115. Copulatory complex. 116. Hook. 117. 4A. 118–122. *Anacanthorus xaniophallus*. 118. Copulatory complex of worm collected from *Pristobrycon* sp. from the Rio Uatumã. 119. Hook pairs 1, 2, 5–7. 120. Hook pairs 3, 4. 121. 4A. 122. Copulatory complex (typical) of worm collected from *Pristobrycon eigenmanni*. All figures are drawn to the 25-μm scale.

TYPE SPECIMENS: Holotype, INPA PA378; paratypes, USNM 81728, HWML 33375.

DESCRIPTION (based on 12 specimens): Body 496 (311–657; $N = 6$) long, fusiform, gently tapered from midlength toward both ends; greatest width 92 (78–123; $N = 6$). Cephalic lobes well developed; anterior lobes fused along midline. Four eyes equidistant; members of posterior pair much larger than those of anterior pair; granules small, subovate to elongate ovate; few accessory

granules scattered in cephalic, anterior trunk regions. Pharynx ovate, 25 (21–27; $N = 6$) wide. Haptor 65 (50–76; $N = 6$) long, 72 (61–88; $N = 4$) wide. Hooks similar; each with truncate slightly depressed thumb, shank with proximal enlargement about 0.5 total length; hook pairs 41 (35–48; $N = 43$) long; FH loop about 0.3 shank length. 4A's similar; each 14–15 ($N = 7$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 84 (63–96; $N = 4$) long, 21 (17–27; $N = 4$) wide; ovary 69 (55–89; $N = 5$) long, 26 (23–30; $N = 5$) wide. Cirrus, accessory piece nonarticulated. Cirrus 70 (64–76; $N = 7$) long, J-shaped, with subterminal aperture, small base lacking flange. Accessory piece 74 (62–80; $N = 6$) long, rodshaped with blunt distal end.

REMARKS: This species resembles *Anacanthorus palamophallus* sp. n. by possessing a simple rod-shaped accessory piece with blunt termination. They differ in the cirrus of *A. stagmophallus* being in the shape of a "J", whereas that of *A. palamophallus* has a lateral basal opening. The specific name is from Greek (*stagmatos* = a drop + *phallos* = penis).

Anacanthorus strongylophallus sp. n. (Figs. 112–114)

TYPE HOST AND LOCALITY: *Triportheus elongatus* (Guenther); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (22 November 1983; 6 January 1989).

OTHER RECORD: *Triportheus elongatus* (Guenther), Manaus Fish Market, Manaus, Amazonas, Brazil (10 March 1979).

TYPE SPECIMENS: Holotype, INPA PA379; paratypes, USNM 81729, 81730, HWML 33376.

DESCRIPTION (based on 11 specimens): Body 326 (243–407; $N = 6$) long, fusiform; greatest width 81 (75–94; $N = 6$) near midlength. Cephalic lobes well developed. Two eyes; granules small, subovate or irregular; accessory granules absent or rare in older specimens, scattered throughout trunk, cephalic regions in younger specimens. Pharynx subspherical, 21 (16–25; $N = 7$) in diameter. Haptor 45 (35–56; $N = 7$) long, 58 (51–64; $N = 7$) wide. Hooks similar; each with truncate thumb, conspicuously inflated shank gently tapered anteriorly, posteriorly from midlength, proximally pointed; hook pairs 34 (31–39; $N = 26$) long; FH loop about 0.3 shank length. 4A's similar; each 8 ($N = 3$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 57 (55–61; $N = 4$) long, 33 (29–36; $N = 4$) wide; ovary 46 (41–53; $N = 4$) long, 26 (20–33; $N = 4$) wide. Cirrus, accessory piece articulated by copulatory ligament 4–5 ($N = 10$) long. Cirrus 115 (98–143; $N = 10$) long, a clockwise coil of 1.5 rings, with small base lacking flange; ring diameter 32 (28–36; $N = 11$). Accessory piece 38 (33–42; $N = 11$) long, curved, rod-shaped, with blunt tip.

REMARKS: Based on hook morphology, *Anacanthorus strongylophallus* resembles the following species infesting *Triportheus*: *A. alatus, A. carinatus, A. cornutus,* and *A. lygophallus* spp. n. It differs from all of these species by possessing a single rod-shaped accessory piece and a coiled cirrus. It superficially resembles *A. mastigophallus* from *Pristobrycon eigenmanni* (Serrasalmidae), which also possesses a coiled cirrus and rod-shaped accessory piece. However, the coiled states of these 2 species apparently represent homoplasies; that of *A. strongylophallus* being derived from the sigmoid state found in most other *Anacanthorus* species from *Triportheus,* and that of *A. mastigophallus* being derived from the J-shaped cirrus of *Anacanthorus* species found on serrasalmid hosts in the region (Van Every and Kritsky, 1992). Similarly, the "rod-shaped" states of the accessory pieces of these species likely are homoplasies with different origins. The specific name is from Greek (*strongylos* = rounded + *phallos* = penis).

Anacanthorus tricornis sp. n. (Figs. 115–117)

TYPE HOST AND LOCALITY: *Triportheus elongatus* (Guenther); Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (22 November 1983; 6 January 1989).

OTHER RECORDS: *Triportheus elongatus* (Guenther), Manaus Fish Market, Manaus, Amazonas, Brazil (10 March 1979); *T. angulatus* (Spix), Bairro de São Jorge, Manaus, Amazonas, Brazil (31 December 1988) and Furo do Catalão near Manaus, Amazonas, Brazil (5 January 1989).

TYPE SPECIMENS: Holotype, INPA PA380; paratypes, USNM 81731, 81732, 81733, 81734, HWML 33377.

DESCRIPTION (based on 9 specimens): Body 314 (240–355; $N = 6$) long, fusiform, gently tapered from midlength toward each end; greatest width 83 (59–115; $N = 5$). Cephalic lobes well developed. Four eyes equidistant; members of anterior pair frequently absent or poorly developed, farther apart than those of posterior pair; granules moderately large, subovate; few acces-

sory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical, 19 (17–21; N = 4) in diameter. Haptor 41 (31–59; N = 4) long, 62 (48–82; N = 5) wide. Hooks similar; each with truncate thumb, slightly inflated shank with indistinct proximal enlargement; hook pairs 21 (19–24; N = 28) long; FH loop about 0.3 shank length. 4A's similar; each 8–9 (N = 5) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 36 (33–38; N = 2) long, 23 (20–27; N = 2) wide; ovary 44 (26–69; N = 3) long, 29 (14–54; N = 3) wide. Cirrus, accessory piece articulated by copulatory ligament 4–5 (N = 8) long. Cirrus 48 (44–54; N = 9) long, sigmoid, with terminal flare, base lacking flange. Accessory piece 38 (34–46; N = 8) long, with 3 blunt branches.

REMARKS: Based on the comparative morphology of the accessory piece and haptoral hooks, *Anacanthorus tricornis* resembles *A. bellus* and *A. acuminatus* spp. n. It differs from *A. bellus* by having a comparatively short nonbifurcated branch of the accessory piece and from *A. acuminatus* by the blunt terminations of all accessory piece branches. The specific name is from Latin (*tri* = three + *cornu* = horn) and refers to the 3 branches of the accessory piece.

Anacanthorus xaniophallus sp. n. (Figs. 118–122)

TYPE HOST AND LOCALITY: *Pristobrycon eigenmanni* (Norman); Nazare, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (17 September 1985).

OTHER RECORDS: *Pristobrycon eigenmanni* (Norman), Santa Luzia, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (20 September 1985); *Pristobrycon* sp. n., C. Miriti, Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (26 September 1985), Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

SPECIMENS: Holotype, INPA PA381; paratypes, USNM 81737, 81738, HWML 33378; vouchers, USNM 81735, 81736.

DESCRIPTION (based on 92 specimens from *P. eigenmanni*; 70 measured): Body 481 (341–696; N = 19) long, fusiform, gently tapered from midlength toward both ends; greatest width 81 (57–106; N = 25). Cephalic lobes well developed. Four eyes equidistant; members of posterior pair larger than those of anterior pair; granules variable in size, ovate to elongate ovate; usually few accessory granules scattered in cephalic, anterior trunk regions. Pharynx subspherical to subovate, 22 (15–27; N = 29) wide. Haptor 62 (46–82; N = 14) long, 79 (55–100; N = 15) wide. Hooks similar; each with truncate slightly depressed thumb, shank proximally enlarged about 0.5 length; hook pairs 1, 2, 5–7: 26 (22–31; N = 63), pairs 3, 4: 29 (26–34; N = 39) long; FH loop about 0.5 shank length. 4A's similar; each 15 (13–16; N = 18) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 69 (44–105; N = 13) long, 25 (16–39; N = 13) wide; ovary 63 (48–109; N = 16) long, 23 (16–34; N = 16) wide. Cirrus, accessory piece nonarticulated. Cirrus 53 (49–60; N = 43) long, J-shaped, with featherlike sheath arising from midlength of cirral shaft, base with lateral flange. Accessory piece 44 (38–48; N = 44) long, rod-shaped with terminal recurved point, variably developed submedial (muscle) articulation point.

REMARKS: The phylogenetic relationships of *Anacanthorus xaniophallus* are provided by Van Every and Kritsky (1992). The specific name is from Greek (*xanion* = a comb + *phallos* = penis) and refers to the featherlike sheath on the cirrus.

In addition to the type host, *A. xaniophallus* was collected from an undescribed species of *Pristobrycon* from the Rio Negro and Rio Uatumã. Specimens of *A. xaniophallus* from this host in the Rio Uatumã possess a distinct submedial (muscle) articulation point on the accessory piece (Fig. 118), while specimens from *P. eigenmanni* from the Rio Uatumã and *Pristobrycon* sp. from the Rio Negro have a small to indistinct point (Fig. 122). Measurements of the 3 populations do not differ significantly from one another.

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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

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ABSTRACT: Thirteen new species of *Anacanthorus* are described from the gills of 9 piranha species from the central Amazon: *Anacanthorus amazonicus* sp. n. from *Serrasalmus rhombeus* (Linnaeus), *Serrasalmus* sp. (2 of Jégu), and *Pristobrycon striolatus* (Steindachner); *A. cinctus* sp. n., *A. crytocaulus* sp. n., and *A. lasiophallus* sp. n. from *P. striolatus*; *A. cladophallus* sp. n. and *A. scapanus* sp. n. from *S. spilopleura* Kner; *A. gravihamulatus* sp. n. from *S. rhombeus, Serrasalmus* sp. (2 of Jégu), and *P. eigenmanni* (Norman); *A. jegui* sp. n. from *S. rhombeus*; *A. mesocondylus* sp. n. from *S. elongatus* Kner, *S. rhombeus, S. spilopleura, Serrasalmus* sp. (1 of Jégu), *Serrasalmus* sp. (2 of Jégu), *P. eigenmanni,* and *Pristobrycon* sp.; *A. prodigiosus* sp. n. from *S. elongatus, S. rhombeus, Serrasalmus* sp. (1 of Jégu), and *Serrasalmus* sp. (2 of Jégu); *A. ramosissimus* sp. n. from *S. elongatus*; *A. sciponophallus* sp. n. from *S. elongatus, S. rhombeus, S. spilopleura, Serrasalmus* sp. (2n = 58), *Serrasalmus* sp. (1 of Jégu), and *Serrasalmus* sp. (2 of Jégu); and *A. serrasalmi* sp. n. from *S. elongatus, S. rhombeus, Serrasalmus* sp. (2n = 58), *Serrasalmus* sp. (1 of Jégu), and *Serrasalmus* sp. (2 of Jégu). Utilizing 12 homologous series comprising 28 character states, a hypothesis for the phylogeny of 22 central Amazonian species of *Anacanthorus* from piranha is proposed. Monophyly of the ingroup is supported by 2 synapomorphies: a cirrus with a tendency to curl into J-shaped configurations and an accessory piece nonarticulated to the cirral base. Based on parasite data, a preliminary hypothesis for the phylogeny of 10 species of piranha (Serrasalmidae) in *Pygocentrus, Pristobrycon,* and *Serrasalmus* is proposed. The host cladogram indicates that *Serrasalmus* and *Pristobrycon* are paraphyletic. A parasite–host list of *Anacanthorus* species is provided.

KEY WORDS: Brazil, taxonomy, Monogenoidea, Dactylogyridae, Anacanthorinae, cladistics, coevolution, piranha, *Anacanthorus amazonicus* sp. n., *Anacanthorus cinctus* sp. n., *Anacanthorus cladophallus* sp. n., *Anacanthorus crytocaulus* sp. n., *Anacanthorus gravihamulatus* sp. n., *Anacanthorus jegui* sp. n., *Anacanthorus lasiophallus* sp. n., *Anacanthorus mesocondylus* sp. n., *Anacanthorus prodigiosus* sp. n., *Anacanthorus ramosissimus* sp. n., *Anacanthorus scapanus* sp. n., *Anacanthorus sciponophallus* sp. n., *Anacanthorus serrasalmi* sp. n., *Anacanthorus stachophallus, Anacanthorus thatcheri, Anacanthorus palamophallus, Anacanthorus periphallus, Anacanthorus mastigophallus, Anacanthorus reginae, Anacanthorus beleophallus, Anacanthorus xaniophallus, Anacanthorus lepyrophallus, Serrasalmus rhombeus, Serrasalmus elongatus, Serrasalmus spilopleura, Serrasalmus* sp., *Pristobrycon striolatus, Pristobrycon eigenmanni, Pristobrycon* sp., *Pygocentrus nattereri,* Serrasalmidae.

The Serrasalmidae consists of 13 genera of characoid fishes endemic to South America. Included in this family are *Pygopristis, Pygocentrus, Pristobrycon,* and *Serrasalmus,* which make up the popular and often publicized group referred to as "piranha." Species from 2 of these, *Pygocentrus* and *Serrasalmus,* are notorious for their occasional threat to man. The creative legendry concerning the habits of these fishes far exceeds our knowledge of their ecology, behavior, and systematics. Myers (1972), Fink and Fink (1979), and Goulding (1980) indicate that the classification of piranhas is confusing, with species identification often difficult. This is evidenced by several undescribed piranha in our collections that have been provisionally identified as *Serrasalmus* sp. 1, *Serrasalmus* sp. 2, *Serrasalmus* sp. (karyotypic form 2n = 58), and *Pristobrycon* sp. by Michel Jégu, ORSTOM, INPA, Manaus, Amazonas, Brazil. Although a phylogenetic hypothesis for the serrasalmid genera has been proposed by Machado-Allison (1983), virtually nothing is known concerning evolutionary relationships of these hosts at the species level. Further, only *Pygocentrus nattereri* Kner has had its monogenoidean fauna documented (Mizelle and Price, 1965; Boeger and Kritsky, 1988; Kritsky et al., 1988). Twenty-four species comprising 7 genera of Dactylogyridae have been described from this host.

Boeger and Kritsky (1988) suggest that the Monogenoidea of *Pygocentrus nattereri* comprise monophyletic groups that may provide useful models for testing hypotheses on coevolution and biogeography. Additionally, Brooks (1981) and O'Grady and Deets (1987) have shown that

in the absence of a host phylogeny, host relationships can be derived by using their parasites as characters. The latter is accomplished by first determining phylogenetic relationships of the parasites, and then converting the parasite cladogram into a multistate character tree that reflects the genealogical relationships of the hosts. The objectives of this study were to (1) describe new species of *Anacanthorus* infesting 6 species of *Serrasalmus* and 3 species of *Pristobrycon*; (2) propose a phylogeny for the *Anacanthorus* species parasitizing these hosts and *Pygocentrus nattereri* in the central Amazon; and (3) develop a phylogenetic hypothesis for these serrasalmid hosts using the anacanthorine parasites as indicators of host evolution.

Materials and Methods

Fish hosts were collected from central Amazonia during 1984–1989. Methods of parasite collection, preparation of helminths for study, measurement and illustration are those of Kritsky et al. (1986, 1992). Measurements, all in micrometers, represent straight-line distances between extreme points and are expressed as the average followed by the range and number of specimens measured in parentheses; body length includes that of the haptor. Hook numbering is according to Mizelle (1936) (see Mizelle and Price, 1963). Generic characters are those given in an emended diagnosis by Kritsky et al. (1992). Type specimens collected from the type host were used exclusively for development of species descriptions. Type specimens and vouchers (specimens collected from other hosts) were deposited in the collections of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); the U.S. National Museum, Beltsville, Maryland (USNM); the University of Nebraska State Museum, Lincoln, Nebraska (HWML); the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (IOC); and the Zoological Institute, U.S.S.R. Academy of Sciences, Leningrad (ZIAC), as indicated in the respective descriptions. Museum numbers have not been received from IOC and ZIAC for publication. For comparative purposes, the following type and voucher specimens were also examined: Holotype, *Anacanthorus anacanthorus* Mizelle and Price, 1965 (USNM 60459); holotype, 4 paratypes, *A. beleophallus* Kritsky et al., 1992 (INPA PA350, USNM 81669, HWML 33343); holotype, *A. brazilensis* Mizelle and Price, 1965 (USNM 60460); holotype, 31 paratypes, *A. catoprioni* Kritsky et al., 1992 (INPA PA354, USNM 81677, 81678, HWML 33347); holotype, 2 paratypes, *A. hoplophallus* Kritsky et al., 1992 (INPA PA363, USNM 81695); holotype, 38 paratypes, *A. lepyrophallus* Kritsky et al., 1992 (INPA PA364, USNM 81704, HWML 33362); holotype, 7 paratypes, *A. mastigophallus* Kritsky et al., 1992 (INPA PA366, USNM 81709, 81710, HWML 33363); holotype, *A. neotropicalis* Mizelle and Price, 1965 (USNM 60461); holotype, 25 paratypes, *A. palamophallus* Kritsky et al., 1992 (INPA PA368, USNM 81712, 81713, HWML 33365); holotype, 6 paratypes, *A. paraspathulatus* Kritsky et al., 1992 (INPA PA369, USNM 81714, HWML 33366); holotype, 18 paratypes, *A. pedanophallus* Kritsky et al., 1992 (INPA PA370, USNM 81715, HWML 33367); holotype, 7 paratypes, *A. periphallus* Kritsky et al., 1992 (INPA PA372, USNM 81718, 81719, HWML 33369); 67 vouchers, *A. reginae* Boeger and Kritsky, 1988 (USNM 81799, 81800); 17 vouchers, *A. spathulatus* Kritsky et al., 1979 (USNM 81798); holotype, 11 paratypes, *A. spinatus* Kritsky et al., 1992 (INPA PA376, USNM 81726, HWML 33373); holotype, 6 paratypes, *A. stachophallus* Kritsky et al., 1992 (INPA PA377, USNM 79197, 81727, HWML 23371); holotype, 11 paratypes, *A. stagmophallus* Kritsky et al., 1992 (INPA PA378, USNM 81728, HWML 33375); 139 vouchers, *A. thatcheri* Boeger and Kritsky, 1988 (USNM 81797); holotype, 91 paratypes, *A. xaniophallus* Kritsky et al., 1992 (INPA PA381, USNM 81737, 81738, HWML 33378).

An initial hypothesis on the evolutionary relationships of the species of *Anacanthorus* parasitizing 10 serrasalmid fishes was constructed using Hennigian argumentation (Hennig, 1966; Wiley, 1981) and tested with Phylogenetic Analysis Using Parsimony (PAUP) (D. L. Swofford, Illinois Natural History Survey, Champaign). Twenty-eight character states comprising 12 homologous series were utilized in the analysis. Polarization was initially determined by outgroup comparison and optimized according to procedures described by Watrous and Wheeler (1981) and Maddison et al. (1984); functional outgroups as defined by Watrous and Wheeler (1981) were used when character states were totally within the ingroup. Outgroups included *A. catoprioni, A. spathulatus, A. paraspathulatus,* and the *Anacanthorus*-species group from *Triportheus* spp. described by Kritsky et al. (1992).

The host phylogeny was generated utilizing the methods of Brooks (1981, 1990), Cressey et al. (1983), and O'Grady and Deets (1987). An additive binary-coded matrix of the parasite cladogram was constructed by mapping each host onto their respective parasite taxa. Since all hosts utilized in the analysis harbored more than 1 parasite species (Fig. 1) and no prior host phylogeny at the species level was available, this matrix was compressed via inclusive ORing (O'Grady and Deets, 1987, and Brooks, 1990) and subjected to PAUP analysis for development of the hypothesis. Parasite species representing less than 2% of the total specimens collected from a host were considered potential accidental occurrences and were not used in the analysis (Fig. 1).

Results

Dactylogyridae Bychowsky, 1933

Anacanthorinae Price, 1967

Anacanthorus Mizelle and Price, 1965 (emended, Kritsky et al., 1992)

Anacanthorus cladophallus sp. n. (Figs. 2, 10–12)

TYPE HOST AND LOCALITY: *Serrasalmus spilopleura* Kner, Rio Solimões near Ilha da Mar-

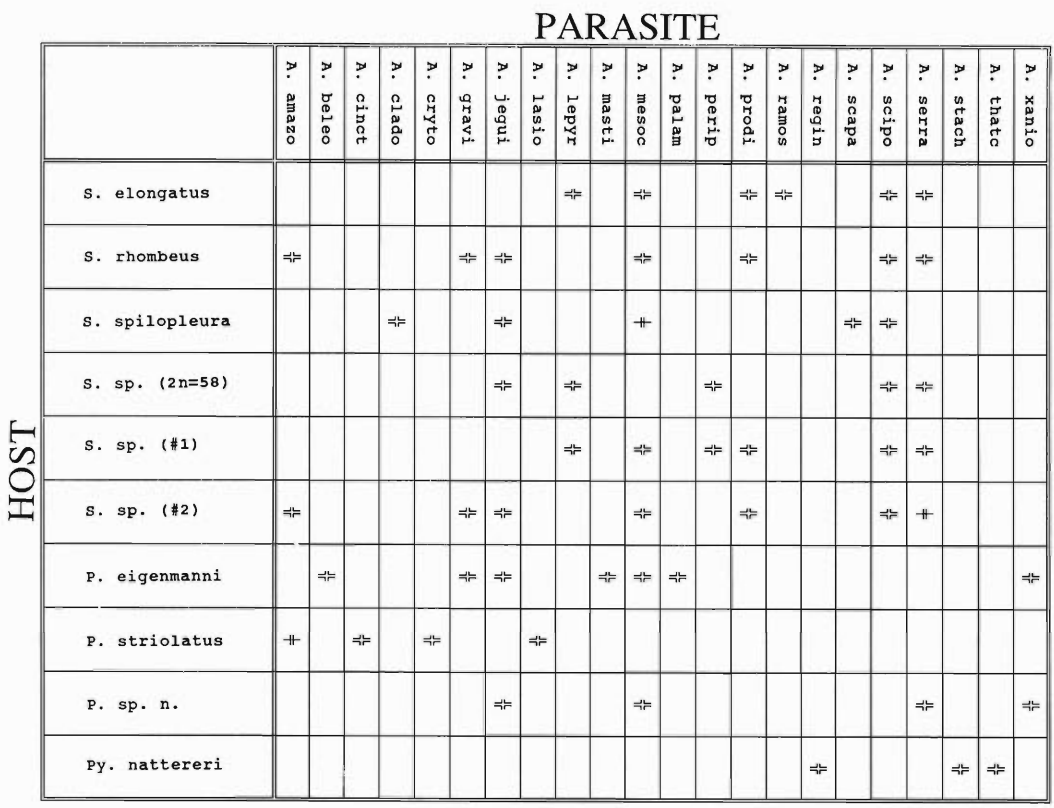


Figure 1. The occurrence of 22 species of *Anacanthorus* on their piranha hosts. ⋕ = parasites that were considered potentially accidental occurrences representing less than 2% of the total assemblage from each host and were not used in host phylogeny reconstruction. *S.* = *Serrasalmus, P.* = *Pristobrycon, Py.* = *Pygocentrus.*

chantaria, Manaus, Amazonas, Brazil (14 September 1984, 26 November 1984).

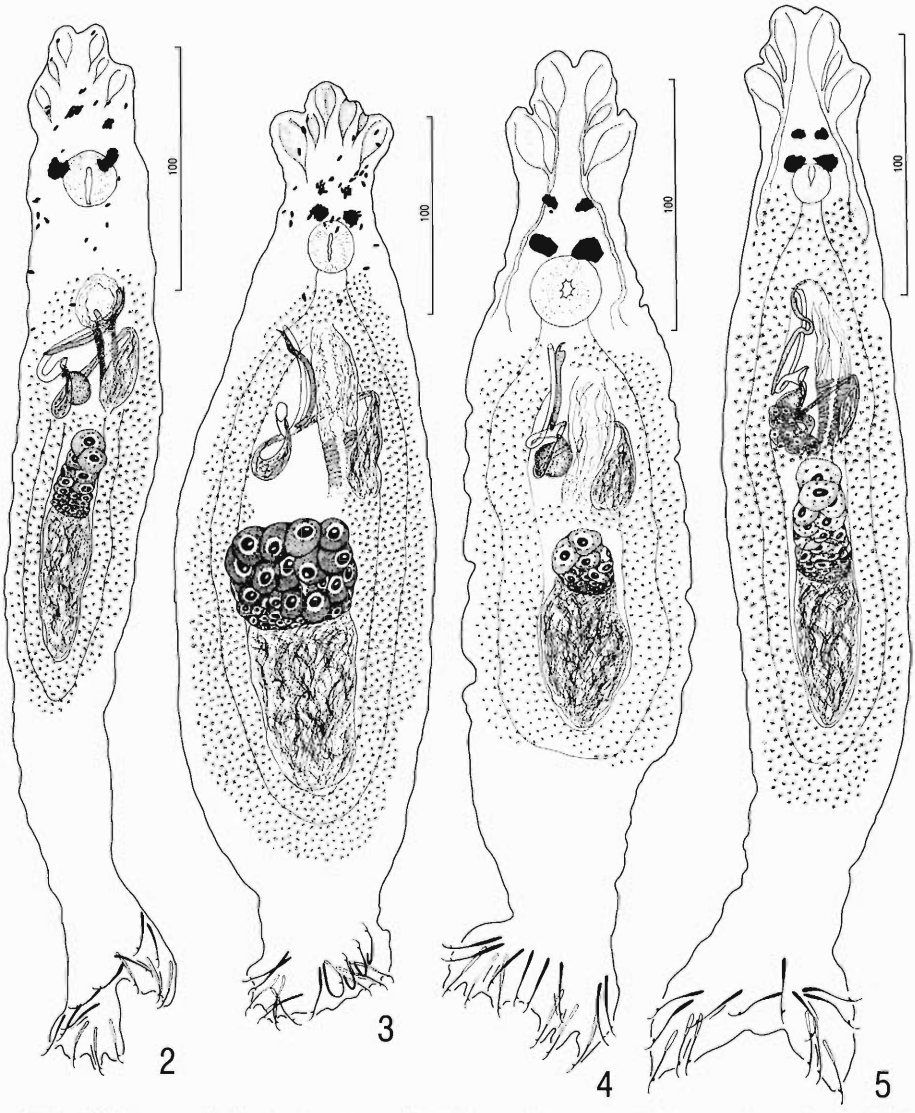
TYPE SPECIMENS: Holotype, INPA PA334; paratypes, USNM 81739, HWML 33379.

DESCRIPTION (based on 49 specimens): Body fusiform, 359 (238–542; $N = 21$) long; greatest width 74 (59–90; $N = 21$) near midlength or in anterior half. Cephalic lobes moderately developed. Four eyes, equidistant; anterior eyes frequently dissociated, smaller than posterior eyes; granules elongate ovate, large; accessory granules scattered in anterior trunk, cephalic area. Pharynx spherical to subspherical, 24 (21–28; $N = 22$) in diameter. Haptor 39 (31–49; $N = 12$) long, 63 (56–81; $N = 13$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 3, 4: 27 (25–28; $N = 19$) long, proximal expansion about 0.5 shank length; pairs 1, 2, 5–7: 22 (20–24; $N = 41$) long, proximal expansion about 0.4 shank length; filamentous hooklet (FH) loop approximately 0.5 shank length. 4A's similar, each 13 (12–14; $N = 11$), proximally expanded about 0.4 length. Gonads slightly overlapping; testis 55 (35–70; $N = 12$) × 29 (21–39; $N = 12$); ovary 33 (22–41; $N = 12$) × 22 (17–32; $N = 12$). Cirrus, accessory piece nonarticulated. Cirrus 54 (46–60; $N = 29$) long, J-shaped, with small basal flap, slight terminal thickening of wall of shaft. Accessory piece 48 (42–56; $N = 31$) long, rod-shaped, with acute distal termination, submedial (muscle) articulation point expanded forming a broad branch.

REMARKS: *Anacanthorus cladophallus* is sister species to *A. ramosissimus* sp. n. (Fig. 70). It differs from this species by having a shorter proximal expansion of the shank on hook pairs 3 and 4. The specific name is from Greek (*klados* = a branch + *phallos* = penis).

Anacanthorus scapanus sp. n. (Figs. 3, 13–15)

TYPE HOST AND LOCALITY: *Serrasalmus spilopleura* Kner, Rio Solimões near Ilha da Mar-

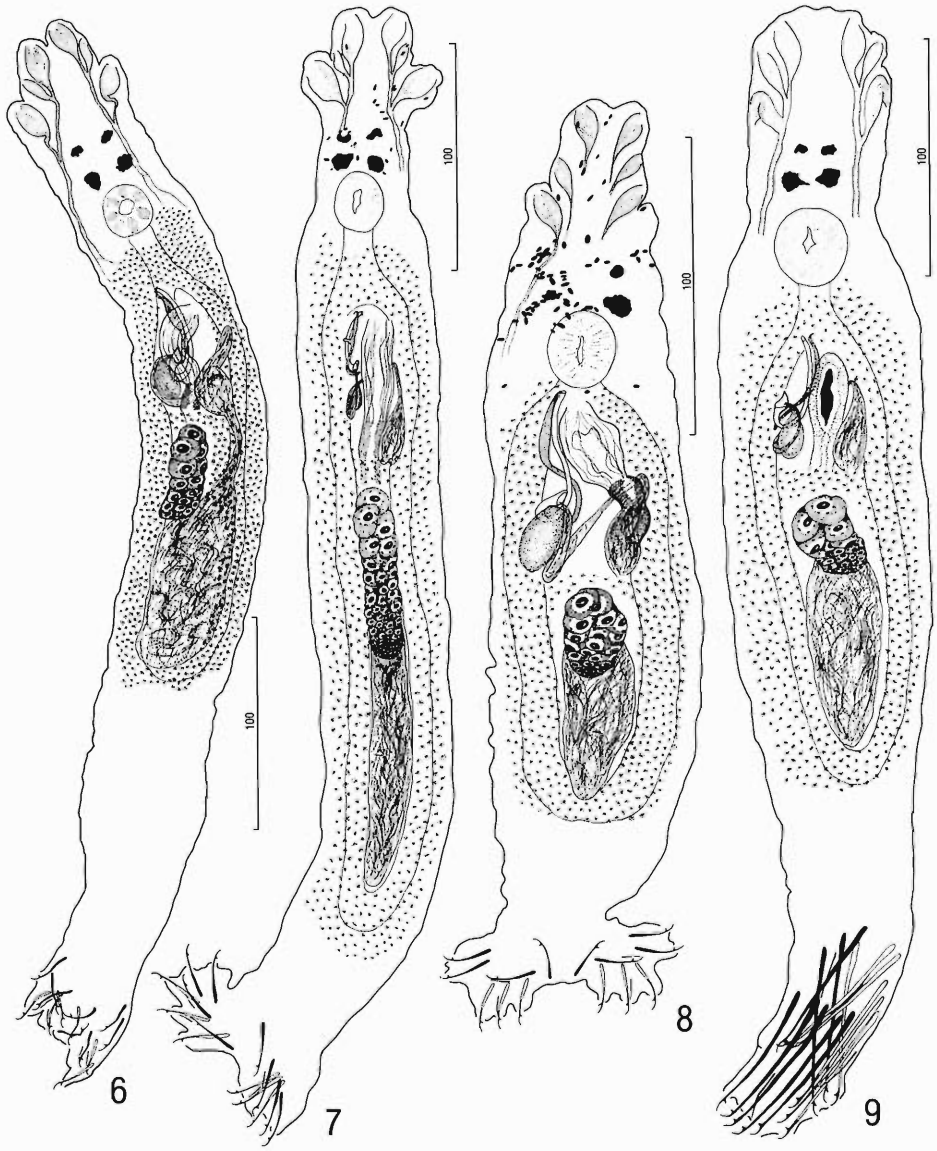


Figures 2–5. Whole mount illustrations (ventral) of *Anacanthorus* spp. 2. *Anacanthorus cladophallus* (holotype). 3. *Anacanthorus scapanus* (holotype). 4. *Anacanthorus amazonicus* (holotype). 5. *Anacanthorus crytocaulus* (holotype).

chantaria, Manaus, Amazonas, Brazil (14 September 1984).

TYPE SPECIMENS: Holotype, INPA PA335; paratypes, USNM 81740, HWML 33380.

DESCRIPTION (based on 6 specimens): Body fusiform, 487 (470–503; $N = 2$) long; greatest width 133 (130–136; $N = 2$) near midlength. Cephalic lobes well developed. Four eyes; anterior eyes occasionally dissociated; members of posterior pair larger, farther apart than those of anterior pair; granules large, elongate ovate; accessory granules peripharyngeal, scattered in cephalic area. Pharynx subspherical, 26–27 ($N = 2$) in diameter. Haptor 39 (38–40; $N = 2$) long, 77 (76–78; $N = 2$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 2–4, 6, 7: 32 (31–34; $N = 9$) long, proximal expansion 0.6 shank length; pairs 1, 5: 30 ($N = 1$) long, proximal expansion 0.5 shank length; FH loop approximately 0.4 shank length.



Figures 6–9. Whole mount illustrations (ventral) of *Anacanthorus* spp. 6. *Anacanthorus jegui* (holotype). 7. *Anacanthorus ramosissimus* (holotype). 8. *Anacanthorus serrasalmi* (holotype). 9. *Anacanthorus gravihamulatus* (holotype).

4A's similar; each 15–16 ($N = 2$) long, proximal expansion about 0.5 length. Gonads slightly overlapping; testis 90 ($N = 1$) × 51 ($N = 1$); ovary 58 (57–59; $N = 2$) × 62 (60–63; $N = 2$). Cirrus, accessory piece nonarticulating. Cirrus 53 (49–57; $N = 6$) long, broadly J-shaped, basal flap with smooth margins, slight terminal thickening of shaft wall. Accessory piece 45 (44–47; $N = 4$) long, slender, rod-shaped, with small subterminal expansion, acute distal termination; submedial (muscle) articulation point indistinct.

REMARKS: Based on the comparative morphology of the copulatory complex, *Anacanthorus scapanus* is similar to *A. serrasalmi* sp. n. It differs from this species by having hooks with longer proximal bases and by lacking a median

Table 1. Comparative measurements (in micrometers; mean with range in parentheses) of *Anacanthorus amazonicus* from 3 piranha hosts.

	*Serrasalmus rhombeus**	*N*	*Serrasalmus* sp. 2	*N*	*Pristobrycon striolatus*	*N*
Body length	469 (313–668)	20	367 (294–405)	3		
Greatest width	86 (53–103)	23	88 (79–101)	3		
Pharynx	28 (22–32)	31	25 (23–27)	5		
Haptor length	45 (36–59)	14	38 (36–40)	3		
Haptor width	75 (61–90)	14	77 (71–80)	3		
Hooks 1–7	30 (25–34)	111	29 (25–33)	76	30 (28–31)	5
4A	16 (14–17)	10	15 (14–16)	8	16–17	2
Testis length	115 (55–206)	19	66 (55–77)	2		
Testis width	34 (18–49)	20	34 (25–44)	2		
Ovary length	53 (29–77)	24	40 (34–47)	2		
Ovary width	24 (15–36)	25	26 (19–32)	2		
Cirrus length	60 (51–68)	46	62 (58–72)	16	64 (62–66)	2
Accessory piece	47 (43–52)	40	49 (46–55)	22	52 (48–55)	2

* Type series.

bend of the accessory piece. The specific name is from Greek (skapanē = spade) and refers to the morphology of the cirral base.

Anacanthorus amazonicus sp. n. (Figs. 4, 16–19)

TYPE HOST AND LOCALITIES: *Serrasalmus rhombeus* (Linnaeus), Rio Pitinga, Igarape Agua Branca, Rio Uatumã a tributary of Rio Amazonas, Amazonas, Brazil (15 September 1985) (type); Rio Uatumã, Amazonas, Brazil (collection date unknown); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

OTHER RECORDS: *Serrasalmus* sp. (2 of Jégu), from Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985); Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985); Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985). *Pristobrycon striolatus* (Steindachner), from Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985); Lago Samaumã, Rio Uatumã, Amazonas, Brazil (25 September 1985).

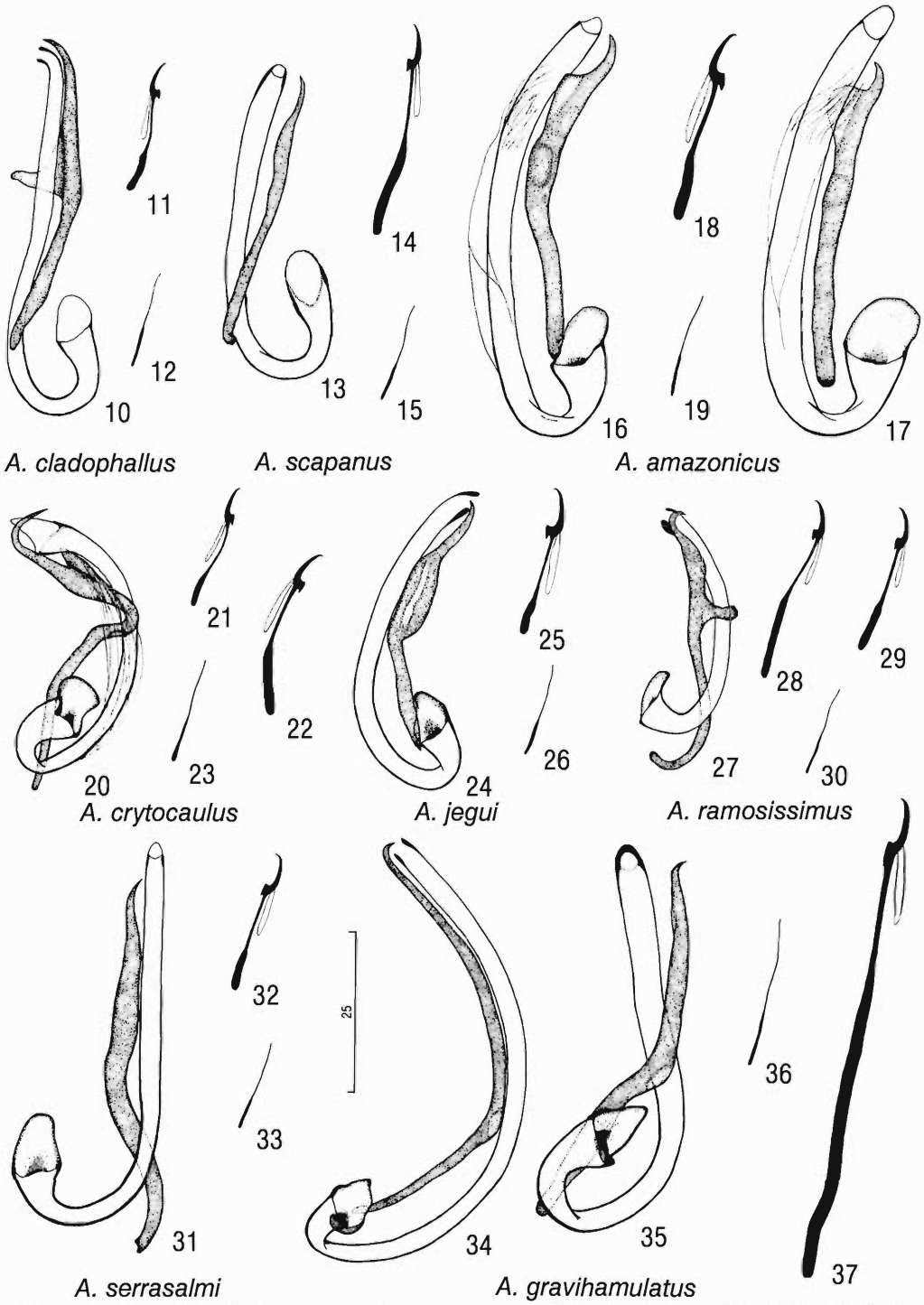
SPECIMENS: Holotype, INPA PA336; paratypes, USNM 81741, 81742, 81743, HWML 33381; vouchers, USNM 81744, 81745, 81746, 81747, 81748.

DESCRIPTION (based on 73 specimens): Body fusiform, greatest width near midlength. Cephalic lobes well developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; granules elongate ovate, small; accessory granules occasionally in cephalic region. Pharynx subspherical. Hooks similar; each with slightly depressed thumb, proximal expansion of shank; proximal expansion about 0.5 shank length; FH loop approximately 0.5 shank length. 4A's similar; each expanded about 0.5 length. Gonads slightly overlapping. Cirrus, accessory piece nonarticulating. Cirrus J-shaped, with short basal flap, submedial "feather," slight thickening of terminal shaft wall. Accessory piece rod-shaped, with hooked acute tip, submedial (muscle) articulation point slightly elevated, variable subterminal flap. Comparative measurements provided in Table 1.

REMARKS: *Anacanthorus amazonicus* resembles *A. lepyrophallus* Kritsky, Boeger, and Van Every, 1992, and *A. xaniophallus* Kritsky, Boeger, and Van Every, 1992, in the comparative morphology of the cirrus and hooks. It is easily distinguished from *A. xaniophallus* by having a more robust cirrus and a flap on the distal portion of the accessory piece. *Anacanthorus amazonicus* differs from *A. lepyrophallus* by lacking a subterminal opening of the cirral tip. The specific name refers to the Amazon Basin where the specimens were collected.

Anacanthorus crytocaulus sp. n. (Figs. 5, 20–23)

TYPE HOST AND LOCALITIES: *Pristobrycon striolatus* (Steindachner), Lago Samaumã, Rio Uatumã a tributary of Rio Amazonas, Amazonas, Brazil (25 September 1985) (type); Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985); Rio Pitinga, Igarape Agua Branca,



Figures 10–37. Sclerotized structures of *Anacanthorus* spp. 10–12. ***Anacanthorus cladophallus.*** 10. Copulatory complex. 11. Hook. 12. 4A. 13–15. ***Anacanthorus scapanus.*** 13. Copulatory complex. 14. Hook. 15. 4A. 16–19. ***Anacanthorus amazonicus.*** 16, 17. Copulatory complexes. 18. Hook. 19. 4A. 20–23. ***Anacanthorus crytocaulus.*** 20. Copulatory complex. 21. Hook pairs 1, 2, 5–7. 22. Hook pairs 3, 4. 23. 4A. 24–26. ***Anacanthorus jegui.*** 24. Copulatory complex. 25. Hook. 26. 4A. 27–30. ***Anacanthorus ramosissimus.*** 27. Copulatory complex. 28. Hook

Rio Uatumã, Amazonas, Brazil (15 September 1985).

TYPE SPECIMENS: Holotype, INPA PA337; paratypes, USNM 81749, 81750, HWML 33382.

DESCRIPTION (based on 110 specimens): Body fusiform, 404 (259–570; $N = 41$) long; greatest width 71 (52–101; $N = 51$) near midlength. Cephalic lobes well developed. Four eyes; members of posterior pair closer together, larger than members of anterior pair; granules elongate ovate, small; accessory granules absent. Pharynx subspherical, 17 (12–23; $N = 49$) in diameter. Haptor 42 (33–53; $N = 43$) long, 79 (59–119; $N = 43$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 3, 4: 29 (26–31; $N = 33$) long, proximal expansion about 0.4 shank length; pairs 1, 2, 5–7: 25 (21–28; $N = 44$) long, proximal expansion about 0.3 shank length; FH loop approximately 0.5 shank length. 4A's similar; each 14 (12–16; $N = 9$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 71 (42–97; $N = 38$) × 23 (13–38; $N = 36$); ovary 46 (29–67; $N = 39$) × 22 (13–28; $N = 40$). Cirrus, accessory piece nonarticulating. Cirrus 42 (34–51; $N = 52$) long, J-shaped, with curved shaft, small basal flap, submedial "feather," flared tip. Accessory piece 47 (39–56; $N = 41$) long, rod-shaped, with moderately raised (muscle) articulation point proximal to submedial loop, subterminal thumb, acute terminal hook.

REMARKS: *Anacanthorus crytocaulus* is sister species to *A. cinctus* sp. n. (Fig. 70). It is easily differentiated from *A. cinctus* by possessing a cirral "feather" and by the absence of a corrugated peduncle. *Anacanthorus crytocaulus* also resembles *A. lasiophallus* sp. n. but is easily differentiated from this species by having an unique median loop of the accessory piece and in the comparative morphology of the proximal expansion of the hook shank. The specific name is from Greek (*cryto* = curved, + *caulis* = stem) and refers to the curvature of the accessory piece.

Anacanthorus jegui sp. n. (Figs. 6, 24–26)

TYPE HOST AND LOCALITIES: *Serrasalmus rhombeus* (Linnaeus), Rio Uatumã, a tributary of Rio Amazonas, Amazonas, Brazil (collection date unknown) (type); Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985).

TYPE SPECIMENS: Holotype, INPA PA338; paratypes, USNM 81751, HWML 33383.

DESCRIPTION (based on 10 specimens): Body fusiform, 478 (458–503; $N = 4$) long; greatest width 77 (69–91; $N = 5$) along trunk. Cephalic lobes well developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; granules small, elongate ovate; accessory granules absent. Pharynx subspherical, 27 (24–30; $N = 5$) in diameter. Haptor 35 (34–36; $N = 2$) long, 69 (52–87; $N = 2$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 3, 4: 25 (24–26; $N = 3$) long, proximal expansion about 0.4 shank length; pairs 1, 2, 5–7: 23 (22–23; $N = 12$) long, proximal expansion about 0.3 shank length; FH loop approximately 0.6 shank length. 4A's similar; each 15 (15–16; $N = 4$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 96 (87–107; $N = 3$) × 31 (29–35; $N = 3$); ovary 48 (36–58; $N = 3$) × 24 (19–33; $N = 3$). Cirrus, accessory piece nonarticulated. Cirrus 48 (45–51; $N = 10$) long, J-shaped, with short basal flap, curved shaft, terminal thickening of shaft wall. Accessory piece 39 (35–42; $N = 9$) long, rod-shaped, with acute termination, submedial (muscle) articulation point indistinct, subterminal expansion originating from both margins of shaft.

REMARKS: *Anacanthorus jegui* resembles *A. serrasalmi* sp. n. in the comparative morphology of the copulatory complex and hooks. *Anacanthorus jegui* differs from this species by having a submedian expansion of the accessory piece that originates from both margins. Morphotypes differing primarily in the extent of the marginal expansions of the accessory piece have been found on several other serrasalmid hosts (compare Fig. 24 to figs. 57–59 in Kritsky et al. 1992). Based on the generally high host specificity exhibited by the Monogenoidea, these forms may comprise a closely related group of species. This species is named for Michel Jégu who collected and identified the majority of hosts for this study.

←

pairs 3, 4. 29. Hook pairs 1, 2, 5–7. 30. 4A. 31–33. *Anacanthorus serrasalmi*. 31. Copulatory complex. 32. Hook. 33. 4A. 34–37. *Anacanthorus gravihamulatus*. 34, 35. Copulatory complexes. 36. 4A. 37. Hook. All figures are drawn to the same 25-μm scale.

Anacanthorus ramosissimus sp. n. (Figs. 7, 27–30)

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

TYPE SPECIMENS: Holotype, INPA PA339; paratypes, USNM 81752, HWML 33384.

DESCRIPTION (based on 41 specimens): Body fusiform, 439 (360–545; $N = 16$) long; greatest width 82 (54–104 $N = 23$) along trunk. Cephalic lobes well developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; granules elongate ovate, small; accessory granules occasionally scattered in cephalic area. Pharynx spherical, 22 (17–29; $N = 22$) in diameter. Haptor 42 (35–48; $N = 11$) long, 76 (61–84; $N = 11$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 3, 4: 29 (26–32; $N = 18$) long, proximal expansion about 0.6 shank length; pairs 1, 2, 5–7: 26 (23–30; $N = 33$) long, proximal expansion about 0.5 shank length; FH loop approximately 0.4 shank length. 4A's similar; each 13–14 ($N = 5$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 87 (63–114; $N = 15$) × 29 (20–41; $N = 15$); ovary 60 (48–78; $N = 17$) × 29 (20–39; $N = 18$). Cirrus, accessory piece nonarticulated. Cirrus 33 (32–36; $N = 10$) long, approaching J-shaped, with small basal flap, curved shaft, terminal thickening of shaft wall. Accessory piece 36 (33–40; $N = 12$) long, sigmoid, with shaft slightly expanded subterminally, heavy acute terminal hook; submedial (muscle) articulation point modified into a protruding submedian branch.

REMARKS: *Anacanthorus ramosissimus* is sister species to *A. cladophallus* sp. n. (Fig. 70). *Anacanthorus ramosissimus* is easily differentiated from this species by having a sigmoid accessory piece which extends past the proximal portion of the cirrus and by lacking a distinctly J-shaped cirrus. The specific name is from Latin (*ramus* = a branch + *-issimus* = most) and refers to the submedial (muscle) articulation point (branched) on the accessory piece.

Anacanthorus serrasalmi sp. n. (Figs. 8, 31–33)

TYPE HOST AND LOCALITIES: *Serrasalmus rhombeus* (Linnaeus), Rio Pitinga, Igarape Agua Branca, Rio Uatumã a tributary of Rio Amazonas, Amazonas, Brazil (15 September 1985) (type); Rio Uatumã, Amazonas, Brazil (collection date unknown); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

OTHER RECORDS: *Serrasalmus elongatus* Kner, from Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988). *Serrasalmus* sp. (2 of Jégu), from Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985). *Serrasalmus* sp. (2n = 58), from Rio Negro near Manaus, Amazonas, Brazil (5 January 1989). *Pristobrycon* sp., from C. Miriti, Rio Uatumã a tributary of Rio Amazonas, Amazonas, Brazil (26 September 1985).

SPECIMENS: Holotype, INPA PA340; paratypes, USNM 81753, 81754, HWML 33385; vouchers, USNM 81755, 81756, 81757, 81758, 81759.

DESCRIPTION (based on 24 specimens): Body fusiform, greatest width near midlength or in posterior trunk. Cephalic lobes well developed. Four eyes poorly developed, frequently dissociated; members of anterior pair smaller, closer together than those of posterior pair; granules elongate ovate, variable in size; accessory granules in anterior trunk, cephalic region. Pharynx subspherical. Hooks similar; each with slightly depressed thumb, proximal expansion of shank; hook pairs 3, 4 proximal expansion about 0.5 shank length; pairs 1, 2, 5–7 proximal expansion about 0.4 shank length; FH loop approximately 0.5 shank length. 4A's similar; each proximally expanded about 0.5 length. Gonads slightly overlapping. Cirrus, accessory piece nonarticulated. Cirrus J-shaped, with short basal flap, slight terminal thickening of shaft wall. Accessory piece rod-shaped, with moderate angular bend near midlength, indistinct submedial (muscle) articulation point, acute termination. Comparative measurements provided in Table 2.

REMARKS: *Anacanthorus serrasalmi* is similar to *A. scapanus* and *A. gravihamulatus* spp. n. in the comparative morphology of the copulatory complex. *Anacanthorus serrasalmi* is differentiated from *A. scapanus* by possessing a more robust accessory piece with an angular bend near midlength. It is differentiated from *A. gravihamulatus* in the comparative morphology of the hooks. The specific name is derived from the generic name of the host.

Table 2. Comparative measurements (in micrometers; mean with range in parentheses) of *Anacanthorus serrasalmi* from 6 piranha hosts.

	*Serrasalmus rhombeus**	*N*	*Serrasalmus elongatus*	*N*	*Serrasalmus* sp. 2	*N*	*Serrasalmus* sp. 1	*N*	*Serrasalmus* sp. (2n = 58)	*N*	*Pristobrycon* sp.	*N*
Body length	367 (271–445)	11	332	1	319	1	528	1				
Greatest width	70 (54–90)	13	71	1	57	1	105	1				
Pharynx	24 (20–34)	14	21	1	20	1	33	1				
Haptor length	41 (36–48)	8			28	1						
Haptor width	69 (62–85)	7			59	1						
Hooks 3, 4	27 (25–28)	11	27	2	26	1	26–27	3			26–27	3
Hooks 1, 2, 5–7	24 (22–25)	21	24	2			23–24	5			23 (22–24)	3
4A	14 (13–14)	5	13	1			14	1			14	1
Testis length	69 (39–98)	11	58	1	54	1	74	1				
Testis width	25 (16–33)	11	28	1	14	1	40	1				
Ovary length	40 (25–49)	12	32	1	37	1	61	1				
Ovary width	23 (17–31)	12	26	1	16	1	34	1				
Cirrus length	56 (49–64)	22	59–60	2	50	1	62 (59–64)	4	57 (51–63)	2	56–57	2
Accessory piece	52 (48–57)	15	53–54	2	48	1	57 (56–58)	4	47 (42–51)	2	55–56	2

* Type series.

Anacanthorus gravihamulatus sp. n. (Figs. 9, 34–37)

TYPE HOST AND LOCALITIES: *Serrasalmus rhombeus* (Linnaeus), Rio Pitinga, Igarape Agua Branca, Rio Uatumã a tributary of Rio Amazonas, Amazonas, Brazil (15 September 1985) (type); Rio Uatumã, Amazonas, Brazil (collection date unknown).

OTHER RECORDS: *Pristobrycon eigenmanni* (Norman), from Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985). *Serrasalmus* sp. (2 of Jégu), from Nazare, Rio Uatumã a tributary of Rio Amazonas, Amazonas, Brazil (17 September 1985); Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985).

SPECIMENS: Holotype, INPA PA341; paratypes, USNM 81760, HWML 33386; vouchers, USNM 81761, 81762, 81763, 81764.

DESCRIPTION (based on 34 specimens): Body fusiform, greatest width near midlength or in anterior half of trunk. Cephalic lobes broad, well developed. Four eyes; members of posterior pair larger, closer together than those of anterior pair; granules elongate ovate, small; accessory granules absent. Pharynx subspherical. Hooks similar; each with flattened thumb, shank expanded proximally; proximal expansion about 0.8 shank length; FH loop approximately 0.2 shank length. 4A's similar; each proximally expanded about 0.4 length. Gonads slightly overlapping. Cirrus, accessory piece nonarticulated. Cirrus J-shaped, with variable spathulate basal flap, terminal wall of shaft thickened. Accessory piece rod-shaped, with moderate angular bend near midlength, acute termination; submedial (muscle) articulation point indistinct. Comparative measurements provided in Table 3.

REMARKS: *Anacanthorus gravihamulatus* is sister species to *A. mastigophallus* Kritsky, Boeger, and Van Every, 1992 (Fig. 70). *Anacanthorus gravihamulatus* is differentiated from this species by (1) possessing a simple J-shaped cirrus with the basal opening directed anteriorly (*A. mastigophallus* has a secondarily derived coiled cirrus with the aperture directed posteriorly) and (2) having significantly longer hooks. *Anacanthorus gravihamulatus* is the only known species of this genus with hooks exceeding 55 μm in length. The specific name is from Latin (*gravis* = burdened with + *hamulatus* = a small hook) and refers to the haptoral hooks.

Anacanthorus sciponophallus sp. n. (Figs. 38, 43–54)

TYPE HOST AND LOCALITIES: *Serrasalmus elongatus* Kner, Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984) (type); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

OTHER RECORDS: *Serrasalmus rhombeus* (Linnaeus), from Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 Sep-

Table 3. Comparative measurements (in micrometers; mean with range in parentheses) of *Anacanthorus gravihamulatus* from 3 piranha hosts.

	*Serrasalmus rhombeus**	*N*	Serrasalmus sp. 2	*N*	*Pristobrycon eigenmanni*	*N*
Body length	549 (342–716)	13	538	1	521 (466–578)	2
Greatest width	95 (77–125)	18	112	1	103	1
Pharynx	36 (29–42)	16	32	1	37 (36–38)	2
Haptor length	90 (69–105)	12	87	1	63	1
Haptor width	61 (52–70)	12	73	1	69	1
Hooks 1–7	70 (56–78)	41	63 (61–65)	14	63 (58–70)	6
4A	22 (21–23)	5	21	1	23	1
Testis length	107 (65–211)	9	110	1	55	1
Testis width	33 (19–44)	9	45	1	12	1
Ovary length	57 (36–87)	14	50	1	84 (79–89)	2
Ovary width	28 (23–39)	14	30	1	32–33	2
Cirrus length	63 (56–71)	9	59 (53–66)	4	43 (42–44)	2
Accessory piece	59 (55–65)	7	58 (52–61)	3	40	1

* Type series.

tember 1985); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988). *Serrasalmus spilopleura* Kner, from Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (14 September 1984, 26 November 1984). *Serrasalmus* sp. (1 of Jégu), from Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984). *Serrasalmus* sp. (2 of Jégu), from Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985). *Serrasalmus* sp. (2n = 58), from Lago do Rei, Paraná, Ilha do Careiro, Amazonas, Brazil (28 February 1986); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

SPECIMENS: Holotype, INPA PA342; paratypes, USNM 81765, HWML 33387; vouchers, USNM 81766, 81767, 81768, 81769, 81770, 81771, 81796.

DESCRIPTION (based on 27 specimens): Body fusiform, greatest width near midlength. Cephalic lobes well developed. Four eyes; members of posterior pair larger, farther apart than those of anterior pair; granules elongate ovate, variable in size; accessory granules absent. Hooks similar; each with slightly depressed thumb, shank expanded proximally; proximal expansion of hook pairs 3, 4 about 0.5 shank length; pairs 1, 2, 5–7 proximal expansion about 0.4 shank length; FH loop approximately 0.5 shank length. 4A's similar; each proximally expanded about 0.5 length. Gonads slightly overlapping. Cirrus, accessory piece nonarticulated. Cirrus J-shaped, basal flap small ovate, shaft wall terminally thickened. Accessory piece rod-shaped, with acute slightly recurved tip; submedial (muscle) articulation point indistinct to slightly elevated. Comparative measurements provided in Table 4.

REMARKS: *Anacanthorus sciponophallus* resembles *A. serrasalmi* sp. n. in the comparative morphology of the copulatory complex and the hooks. *Anacanthorus sciponophallus* is most easily differentiated from this species by having a smaller, more ovate cirral base and a long straight accessory piece with a slightly recurved tip. Several morphotypes (Figs. 43, 47, 51) of this species were found which differ in the morphology of the cirral base and accessory piece. These forms, apparently restricted to specific hosts, may comprise a closely related complex of species. The specific name is from Greek (*skipōnos* = a staff + *phallos* = penis) and refers to the morphology of the copulatory complex.

Anacanthorus mesocondylus sp. n. (Figs. 39, 55–58)

TYPE HOST AND LOCALITIES: *Serrasalmus elongatus* Kner, Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984) (type); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

OTHER RECORDS: *Pristobrycon eigenmanni* (Norman), from Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988). *Pristobrycon* sp., from C. Miriti, Rio Uatumã, Amazonas, Brazil (26 September 1985). *Serrasalmus rhombeus* (Linnaeus), Rio Uatumã, Amazonas, Brazil (collection date unknown); Rio

Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988). *Serrasalmus spilopleura* Kner, Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984). *Serrasalmus* sp. (1 of Jégu), Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984). *Serrasalmus* sp. (2 of Jégu), from Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985); Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985); Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985).

SPECIMENS: Holotype, INPA PA343; paratypes, USNM 81772, HWML 33388; vouchers, USNM 81773, 81774, 81775, 81776, 81777, 81778, 81779, 81780, 81781, 81782, 81783, 81784, 81785.

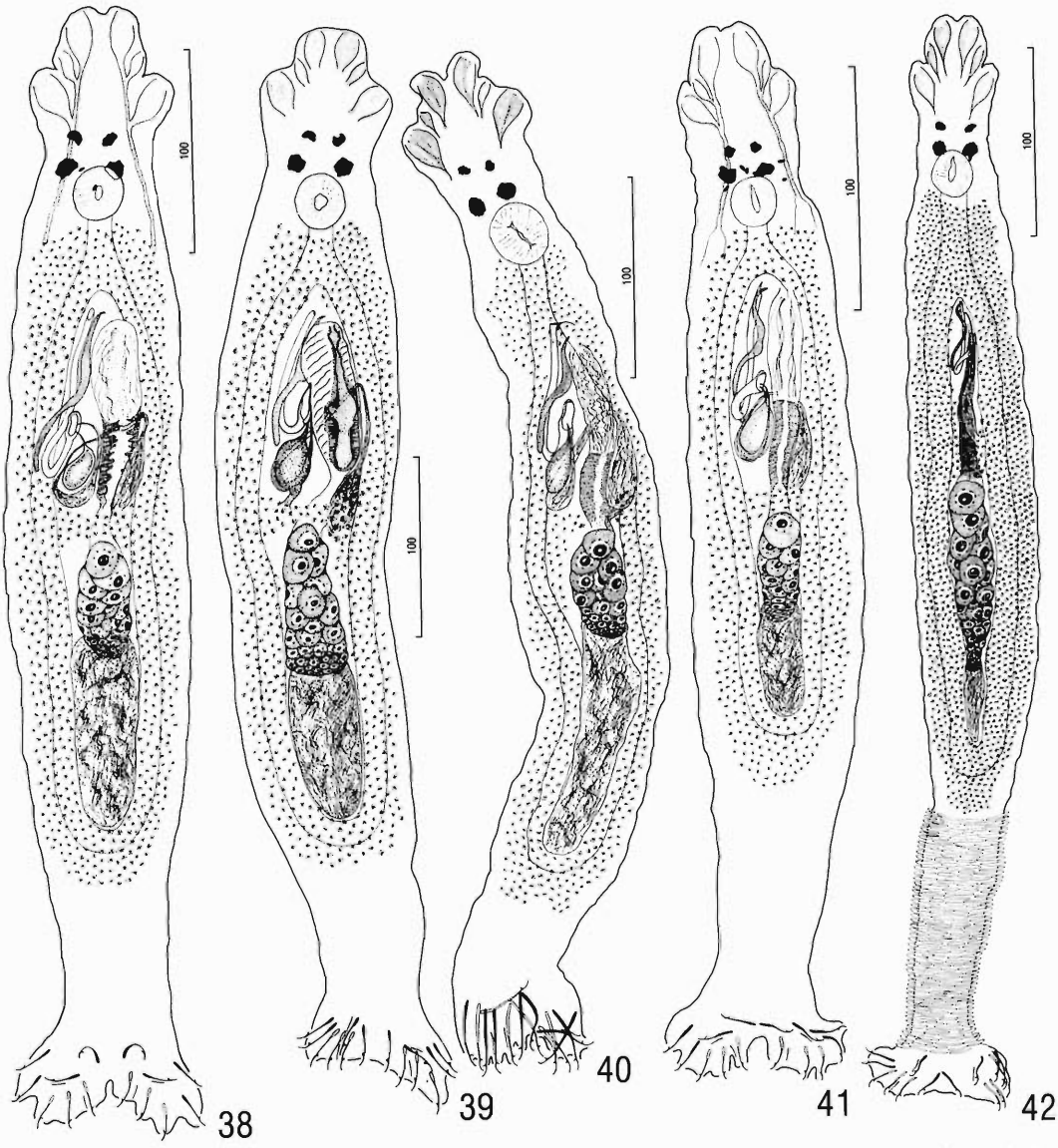
DESCRIPTION (based on 23 specimens): Body fusiform, greatest width near midlength or in anterior half. Cephalic lobes well developed. Four eyes; members of posterior pair larger, farther apart than those of anterior pair; granules elongate ovate, small; accessory granules absent. Pharynx subspherical. Hooks similar; each with depressed thumb, proximal expansion of shank; hook pairs 3, 4 proximal expansion about 0.5 shank length; pairs 1, 2, 5–7 proximal expansion about 0.4 shank length; FH loop approximately 0.5 shank length. 4A's similar; each proximally expanded about 0.5 length. Gonads slightly overlapping. Cirrus, accessory piece nonarticulated. Cirrus broadly J-shaped, with elongate basal flap extending to proximal apex, slight terminal thickening of shaft wall. Accessory piece rod-shaped, with acute slightly recurved termination; submedial (muscle) articulation point a raised, rounded knob. Comparative measurements provided in Table 5.

REMARKS: *Anacanthorus mesocondylus* is sister species to *A. cladophallus* sp. n., *A. ramosissimus* sp. n., and *A. reginae* Boeger and Kritsky, 1988 (Fig. 70). *Anacanthorus mesocondylus* is easily differentiated from these species by having an elongate, pointed flap on the cirral base, and a conspicuous rounded medial knob on the accessory piece. This species was found on numerous hosts; no significant morphological or size differences were observed among the populations. The specific name is from Greek (*mesos* = middle + *kondylos* = knob) and refers to the submedial (muscle) articulation point of the accessory piece.

Table 4. Comparative measurements (in micrometers; mean with range in parentheses) of *Anacanthorus sciponophallus* from 6 piranha hosts.

	*Serrasalmus elongatus**	*N*	*Serrasalmus spilopleura*	*N*	*Serrasalmus rhombeus*	*N*	*Serrasalmus* sp. 1	*N*	*Serrasalmus* sp. 2	*N*	*Serrasalmus* sp. (2n = 58)	*N*
Body length	431 (287–556)	17	426 (356–481)	15	376 (374–379)	2	384 (332–486)	7	260	1		
Greatest width	78 (59–94)	17	106 (80–133)	15	73 (60–84)	5	84 (62–94)	8	86	1		
Pharynx	25 (21–29)	18	34 (29–36)	14	29 (22–33)	3	25 (21–27)	7	24	1	25	1
Haptor length	38 (26–55)	11	41 (32–49)	14	33	1	36 (31–42)	5				
Haptor width	82 (63–117)	11	86 (70–109)	13	66	1	72 (66–79)	5				
Hooks 3, 4	22 (20–24)	6	23 (22–25)	6	25	4	23 (22–24)	9	21 (19–23)	2	24 (23–26)	9
Hooks 1, 2, 5–7	19 (17–20)	10	20 (19–22)	6	22 (21–23)	6	20 (19–22)	16			20 (19–21)	11
4A	12–13	3	12	1	12–13	2	13	6			12–13	3
Testis length	65 (50–87)	10	73 (52–83)	11	52	1	20	1				
Testis width	27 (23–31)	10	38 (28–59)	11	28 (23–33)	2						
Ovary length	50 (38–62)	12	49 (42–55)	12	39	1						
Ovary width	28 (19–38)	11	36 (27–50)	12	16	1	27 (25–29)	2				
Cirrus length	79 (69–91)	26	76 (68–87)	18	82 (74–95)	7	79 (72–87)	17	79 (76–82)	3	81 (72–92)	16
Accessory piece	76 (65–83)	24	74 (65–78)	18	79 (73–85)	7	76 (68–83)	17	76 (74–77)	2	76 (69–84)	15

* Type series.



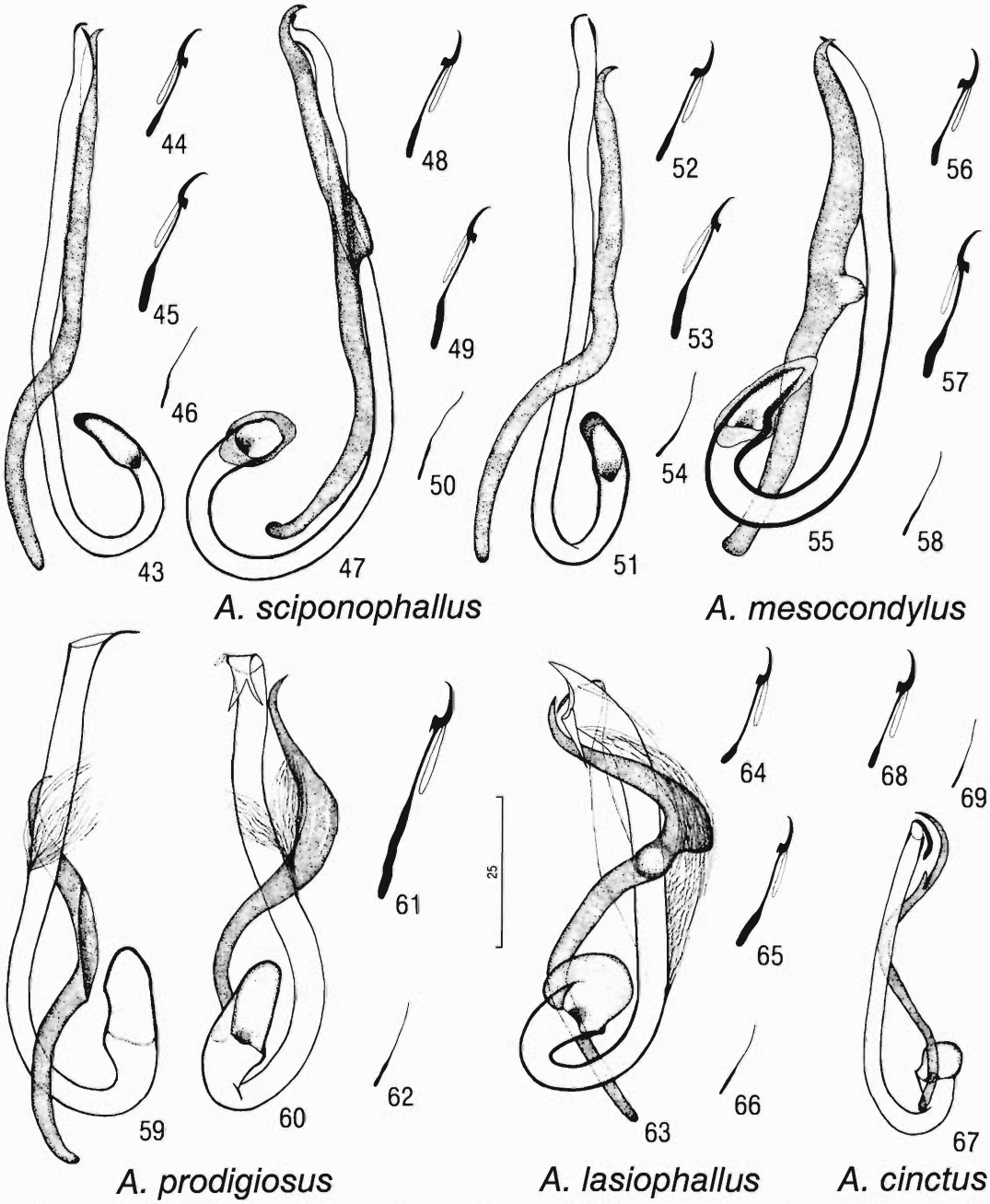
Figures 38–42. Whole mount illustrations (ventral) of *Anacanthorus* spp. 38. *Anacanthorus sciponophallus* (holotype). 39. *Anacanthorus mesocondylus* (holotype). 40. *Anacanthorus prodigiosus* (holotype). 41. *Anacanthorus lasiophallus* (holotype). 42. *Anacanthorus cinctus* (holotype).

Anacanthorus prodigiosus sp. n. (Figs. 40, 59–62)

TYPE HOST AND LOCALITY: *Serrasalmus elongatus* Kner, Rio Negro near Manaus, Amazonas, Brazil (28 December 1988).

OTHER RECORDS: *Serrasalmus rhombeus* (Linnaeus), from Rio Uatumã, Amazonas, Brazil (collection date unknown); Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985); Rio Negro near Manaus, Amazonas, Brazil (28 December 1988). *Serrasalmus* sp. (1 of Jégu), Rio Solimões near Ilha da Marchantaria, Manaus, Amazonas, Brazil (26 November 1984). *Serrasalmus* sp. (2 of Jégu), Nazare, Rio Uatumã, Amazonas, Brazil (17 September 1985); Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985).

SPECIMENS: Holotype, INPA PA344; paratypes, USNM 81786, HWML 33389; vouchers



Figures 43–69. Sclerotized structures of *Anacanthorus* spp. 43–54. *Anacanthorus sciponophallus* from 3 different hosts. 43–46. *A. sciponophallus* from *Serrasalmus elongatus*. 43. Copulatory complex. 44. Hook pairs 1, 2, 5–7. 45. Hook pairs 3, 4. 46. 4A. 47–50. *A. sciponophallus* from *S. rhombeus*. 47. Copulatory complex. 48. Hook pairs 1, 2, 5–7. 49. Hook pairs 3, 4. 50. 4A. 51–54. *A. sciponophallus* from *S. spilopleura*. 51. Copulatory complex. 52. Hook pairs 1, 2, 5–7. 53. Hook pairs 3, 4. 54. 4A. 55–58. *Anacanthorus mesocondylus*. 55. Copulatory complex. 56. Hook pairs 1, 2, 5–7. 57. Hook pairs 3, 4. 58. 4A. 59–62. *Anacanthorus prodigiosus*. 59, 60. Copulatory complexes. 61. Hook. 62. 4A. 63–66. *Anacanthorus lasiophallus*. 63. Copulatory complex. 64. Hook pairs 1, 2, 5–7. 65. Hook pairs 3, 4. 66. 4A. 67–69. *Anacanthorus cinctus*. 67. Copulatory complex. 68. Hook. 69. 4A. All figures are drawn to the same 25-μm scale.

Table 5. Comparative measurements (in micrometers; mean with range in parentheses) of *Anacanthorus mesocondylus* from 7 piranha hosts.

	*Serrasalmus elongatus**	*N*	*Serrasalmus rhombeus*	*N*	*Serrasalmus spilopleura*	*N*	*Serrasalmus* sp. 1	*N*	*Serrasalmus* sp. 2	*N*	*Pristobrycon* sp.	*N*	*Pristobrycon eigenmanni*	*N*
Body length	457 (316–601)	9	410 (336–535)	8			418 (363–491)	4	359 (320–398)	2	386 (321–418)	4	358 (302–414)	2
Greatest width	84 (66–107)	10	81 (64–97)	8			100 (95–112)	4	74 (58–89)	2	79 (59–94)	6	75 (73–78)	2
Pharynx	26 (22–31)	12	27 (22–29)	6			28 (26–31)	4	25 (23–28)	2	26 (22–30)	5	25 (23–27)	2
Haptor length	39 (29–47)	9	41 (34–48)	5			41 (38–43)	4	39 (38–40)	2	35 (34–36)	4	38 (36–41)	2
Haptor width	77 (59–94)	9	75 (65–80)	5			79 (71–92)	4	69 (62–76)	2	69 (62–80)	4	84 (80–88)	2
Hooks 3, 4	23 (21–24)	8	23–24	6	24–25	2	23	2	23 (20–25)	7	23 (22–25)	13	23 (21–26)	12
Hooks 1, 2, 5–7	20 (18–21)	17	19 (18–21)	17			19 (18–20)	4	20 (19–25)	14	19 (18–21)	29	19 (18–22)	32
4A	11 (11–12)	3	13 (12–14)	4			12–13	2	11–12	3	13 (12–14)	6	13 (12–14)	7
Testis length	171 (95–208)	9	73 (47–114)	3			72	1	48 (33–62)	3	75 (64–86)	2	44	1
Testis width	71 (50–104)	9	24 (19–31)	3			39	1	22 (19–27)	3	33 (29–38)	2	32	1
Ovary length	58 (31–78)	9	55 (46–65)	2			50 (49–51)	2	43 (36–57)	3	57	1	31	1
Ovary width	29 (20–38)	9	19 (18–20)	2			30 (28–31)	2	22 (13–26)	3	26	1	25	1
Cirrus length	72 (67–76)	22	74 (67–80)	15	85	1	74 (70–82)	6	78 (69–86)	13	77 (72–85)	16	77 (68–84)	11
Accessory piece	74 (61–81)	20	77 (72–80)	15	81	1	76 (72–82)	6	80 (72–86)	13	78 (72–83)	16	79 (69–84)	11

* Type series.

USNM 81787, 81788, 81789, 81790, 81791, 81792.

DESCRIPTION (based on 27 specimens): Body fusiform, greatest width near midlength. Cephalic lobes well developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; granules elongate ovate, small; accessory granules absent. Pharynx spherical. Hooks similar; each with slightly depressed thumb, shank expanded proximally; proximal expansion about 0.6 shank length; FH loop approximately 0.5 shank length. 4A's similar; each proximally expanded about 0.5 length. Gonads slightly overlapping. Cirrus, accessory piece nonarticulated. Cirrus J-shaped, robust, with elongate rounded basal flap, cirral "feather" originating near midlength, terminal bipartite flap at tip of shaft. Accessory piece rod-shaped, with acute tip, broad flaplike expansion along distal half; submedial (muscle) articulation point indistinct. Comparative measurements provided in Table 6.

REMARKS: *Anacanthorus prodigiosus* is similar to *A. lepyrophallus* Kritsky, Boeger, and Van Every, 1992, and *A. lasiophallus* sp. n. in the comparative morphology of the copulatory complex. It is easily differentiated from *A. lepyrophallus* by having a broad subterminal expansion of the accessory piece and an extended bipartite flap on the distal end of the cirrus. *Anacanthorus prodigiosus* differs from *A. lasiophallus* by lacking a distinct modification of the submedial (muscle) articulation point. Further the hooks of *A. prodigiosus* have a proximal expansion nearly ⅔ of the shank length (hooks of *A. lepyrophallus* and *A. lasiophallus* have a basal expansion of ½ or less). The specific name is from Latin (*prodigiosus* = full of wonder).

Anacanthorus lasiophallus sp. n. (Figs. 41, 63–66)

TYPE HOST AND LOCALITIES: *Pristobrycon striolatus* (Steindachner), Rio Pitinga, Igarape Agua Branca, Rio Uatumã, Amazonas, Brazil (15 September 1985) (type); Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985); Lago Samaumã, Rio Uatumã, Amazonas, Brazil (25 September 1985).

TYPE SPECIMENS: Holotype, INPA PA345; paratypes, USNM 81793, 81794, HWML 33390.

DESCRIPTION (based on 71 specimens): Body fusiform, 379 (212–551; $N = 25$) long; greatest width 77 (51–102; $N = 28$) near midlength. Ce-

Table 6. Comparative measurements (in micrometers; mean with range in parentheses) of *Anacanthorus prodigiosus* from 4 piranha hosts.

	*Serrasalmus elongatus**	*N*	*Serrasalmus rhombeus*	*N*	*Serrasalmus* sp. 1	*N*	*Serrasalmus* sp. 2	*N*
Body length	438 (377–510)	14	481 (420–626)	5	470 (441-526)	3		
Greatest width	78 (66–91)	16	84 (72–94)	4	106 (90–127)	3		
Pharynx	31 (27–34)	16	29 (26–31)	4	34–35	3		
Haptor length	42 (35–49)	9	56	1	48 (45–50)	2		
Haptor width	71 (59–82)	8	96	1	87 (80–94)	2		
Hooks 1–7	36 (31–40)	40	39 (34–42)	19	36 (33–38)	5	40–41	3
4A	15 (14–16)	7	15–16	3	14	1	16	1
Testis length	82 (67–103)	8	99	1	94	1		
Testis width	24 (14–31)	8	28	1	35	1		
Ovary length	47 (33–59)	12	50	1	61	1		
Ovary width	29 (23–36)	12	26 (24–28)	3	39	1		
Cirrus length	74 (67–78)	24	71 (68–76)	10	68 (67–70)	5	65 (60–69)	2
Accessory piece	60 (55–67)	25	61 (56–65)	9	57 (55–60)	5	53 (50–57)	2

* Type series.

phalic lobes well developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; granules elongate ovate, variable in size; accessory granules occasionally scattered in pharyngeal area. Pharynx subspherical, 17 (13–20; $N = 34$) in diameter. Haptor 37 (27–49; $N = 23$) long, 78 (62–92; $N = 23$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 3, 4: 22 (20–23; $N = 43$) long, proximal expansion 0.4 shank length; pairs 1, 2, 5–7: 18 (16–20; $N = 62$) long, proximal expansion about 0.25 shank length; FH loop approximately 0.5 shank length. 4A's similar; each 11 (10–12; $N = 11$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 50 (28–86; $N = 22$) × 25 (18–41; $N = 21$); ovary 42 (18–59; $N = 23$) × 23 (14–35; $N = 22$). Cirrus, accessory piece nonarticulating. Cirrus 65 (54–73; $N = 48$) long, J-shaped, with rounded basal flap, submedial "feather," curved shaft terminally flared, subterminal opening. Accessory piece 64 (53–73; $N = 48$) long, rod-shaped, with acute terminal hook, broad flap extending from subterminal bend; submedial (muscle) articulation point rounded, protruding.

REMARKS: *Anacanthorus lasiophallus* is sister species to *A. crytocaulus* and *A. cinctus* spp. n. (Fig. 70). All 3 species exclusively parasitize *Pristobrycon striolatus* (Fig. 1). *Anacanthorus lasiophallus* is differentiated from *A. crytocaulus* by having a more robust accessory piece with a broad flap on the subterminal bend. It is differentiated from *A. cinctus* by having a cirral "feather" and a distinct modification of the submedial (muscle) articulation point of the accessory piece. The species name is from Greek (*lasios* = hairy + *phallos* = penis) and refers to the cirral "feather."

Anacanthorus cinctus sp. n. (Figs. 42, 67–69)

TYPE HOST AND LOCALITIES: *Pristobrycon striolatus* (Steindachner), Lago Samaumã, Rio Uatumã, Amazonas, Brazil (25 September 1985) (type); Santa Luzia, Rio Uatumã, Amazonas, Brazil (20 September 1985).

TYPE SPECIMENS: Holotype, INPA PA346; paratypes, USNM 81795, HWML 33391.

DESCRIPTION (based on 24 specimens): Body fusiform, 455 (308–579; $N = 11$) long; greatest width 82 (73–90; $N = 7$) near midlength. Cephalic lobes well developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; granules elongate ovate, variable in size; accessory granules absent. Pharynx spherical, 21 (18–25; $N = 9$) in diameter. Peduncle elongate, surface corrugated. Haptor 31 (27–36; $N = 7$) long, 72 (64–84; $N = 7$) wide. Hooks similar; each with slightly depressed thumb, shank expanded proximally; hook pairs 3, 4: 21 (20–23; $N = 11$) long, proximal expansion about 0.4 shank length; pairs 1, 2, 5–7: 19 (17–20; $N = 20$) long, proximal expansion about 0.25 shank length; FH loop approximately 0.6 shank length. 4A's similar; each 10–11 ($N = 3$) long, proximally expanded about 0.5 length. Gonads slightly overlapping; testis 32 (30–34; $N = 4$) × 11 (9–12; $N = 3$); ovary 68 (42–99; $N = 8$) × 25 (19–30; $N = 7$). Cirrus, accessory piece

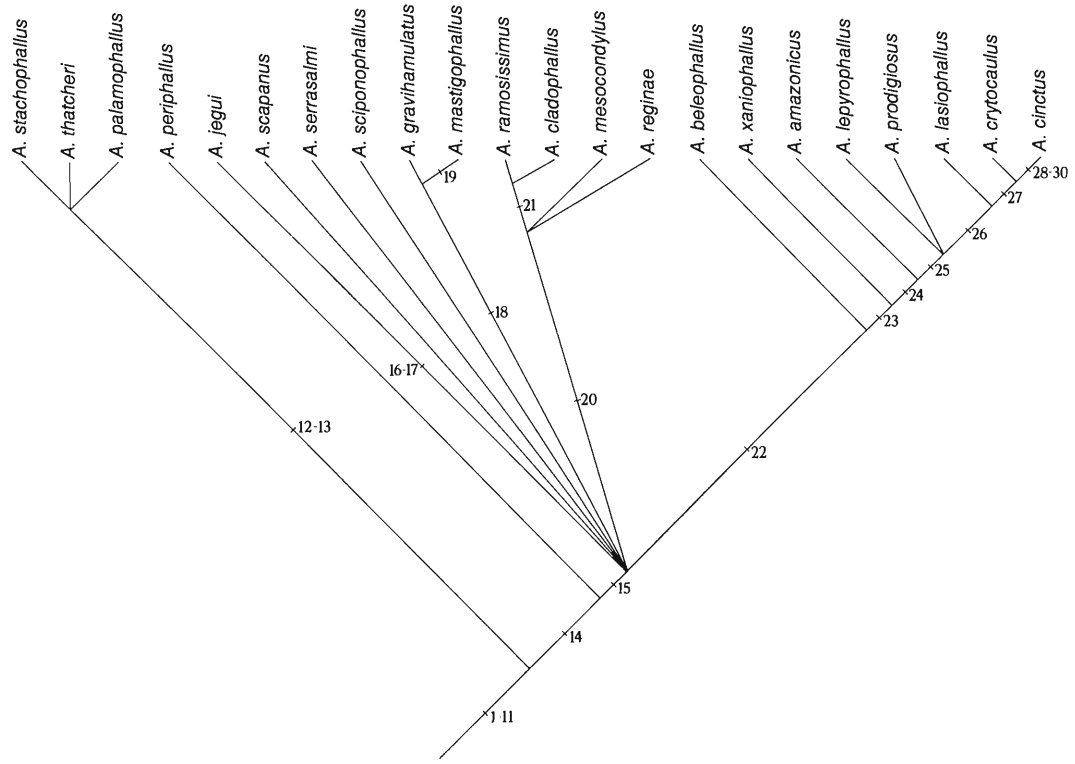


Figure 70. Cladogram depicting evolutionary relationships of *Anacanthorus* species parasitizing 10 species of piranha (Serrasalmidae) from the central Amazon. Numbered slashes refer to postulated evolutionary changes in character states as indicated in the character analysis.

nonarticulated. Cirrus 48 (45–52; $N = 12$) long, J-shaped, with small basal flap, slightly curved shaft, terminally thickened wall, subterminal opening. Accessory piece 45 (40–52; $N = 10$) long, rod-shaped, with moderate curvature near midlength, small subterminal thumb, acute terminal hook; submedial (muscle) articulation point indistinct.

REMARKS: *Anacanthorus cinctus* is sister species to *A. crytocaulus* sp. n. (Fig. 70). *Anacanthorus cinctus* differs from this species by lacking a distinct subterminal loop of the accessory piece and by the absence of a cirral "feather." The cladogram (Fig. 70) suggests that *Anacanthorus cinctus* secondarily lost the cirral "feather" but has retained the derived morphology of the cirral tip and the subterminal thumb of the accessory piece. The corrugated peduncle is an autapomorphy for *A. cinctus*. Further, *A. cinctus* differs from other species of *Anacanthorus* in having an ovarian length nearly twice that of the testis. The specific name is from Latin (*cinctus* = girdled) referring to the tegumental surface of the peduncle.

Phylogenetic Analysis

CHARACTER ANALYSIS: Homologous series utilized in the analysis of parasite phylogeny are listed below. Bold numbers in parentheses indicate the location of postulated character-state changes on the cladogram (Fig. 70); the character-state matrix for the species of *Anacanthorus* is presented in Table 7. Polarity is determined by out-group comparison unless stated otherwise.

1. *Articulation of accessory piece to cirral base.* Plesiomorphy: present. Apomorphy: absent (**1**). The apomorphic state is a synapomorphy for the ingroup.

2. *Shape of cirrus.* Plesiomorphy: straight or with tendency for twisting. Apomorphy: tendency for curling (**2**). Both plesiomorphic and apomorphic states may incorporate forms with coiled cirri. However, differentiation of each state depends on means by which the coil is derived. A coil developed from twisting of the cirrus does not require change in the relative position of the cirral base and/or terminal aperture, while 1

Table 7. Matrix of characters for central Amazonian species of *Anacanthorus* parasitizing 10 species of piranha. Sequence of characters corresponds to listing in character analysis. Homologous series 3 and 9 were unordered in the analysis.

A. amazonicus	1	1	2	1	1	0	0	1	0	0	0	0
A. beleophallus	1	1	1	1	1	0	0	0	9	0	0	0
A. cinctus	1	1	0	1	0	0	0	1	1	0	1	0
A. cladophallus	1	1	0	1	1	0	2	0	9	0	0	0
A. crytocaulus	1	1	2	1	0	0	1	1	1	0	0	0
A. gravihamulatus	1	1	0	1	1	0	0	0	9	0	0	1
A. jegui	1	1	0	1	1	0	0	1	2	0	0	0
A. lasiophallus	1	1	2	1	0	0	1	1	0	0	0	0
A. lepyrophallus	1	1	2	1	0	0	0	1	0	0	0	0
A. mastigophallus	1	1	0	2	1	0	0	0	9	0	0	1
A. mesocondylus	1	1	0	1	1	0	1	0	9	0	0	0
A. palamophallus	1	1	0	0	0	1	0	0	9	1	0	0
A. periphallus	1	1	0	1	0	0	0	0	9	0	0	0
A. prodigiosus	1	1	2	1	0	0	0	1	0	0	0	0
A. ramosissimus	1	1	0	1	1	0	2	0	9	0	0	0
A. reginae	1	1	0	1	1	0	1	0	9	0	0	0
A. scapanus	1	1	0	1	1	0	0	0	9	0	0	0
A. sciponophallus	1	1	0	1	1	0	0	0	9	0	0	0
A. serrasalmi	1	1	0	1	1	0	0	0	9	0	0	0
A. stachophallus	1	1	0	0	0	1	0	0	9	1	0	0
A. thatcheri	1	1	0	0	0	1	0	0	9	1	0	0
A. xaniophallus	1	1	2	1	1	0	0	0	9	0	0	0
Ancestor	0	0	0	0	0	0	0	0	0	0	0	0

formed by curling implies a changed position of either end of the cirrus in relation to the cirral shaft. Cirral shafts developed through twisting are demonstrated in cirri of *Anacanthorus* species infesting *Triportheus* (see Kritsky et al., 1992) and *Salminus* species (see Kritsky and Thatcher, 1974); cirral shafts developed through curling apparently represent a synapomorphy for the *Anacanthorus* ingroup infesting *Pygocentrus, Pristobrycon,* and *Serrasalmus.* In the latter, the coiled cirrus of *A. mastigophallus* Kritsky, Boeger, and Van Every, 1992, appears to be secondarily derived from the J-shaped cirrus of its ancestor (Fig. 70), which in turn has developed from a relatively straight tube (see cirrus of *A. stachophallus* Kritsky, Boeger, and Van Every, 1992 [fig. 105 in Kritsky et al., 1992]).

3. *Cirral "feather."* Plesiomorphy: absent (**3, 28**). Apomorphies: moderately developed, terminal filaments absent (fig. 10 in Kritsky et al., 1992) (**22**); well developed, terminal filaments present (Fig. 63) (**23**).

4. *Basal aperture of cirrus.* Plesiomorphy: aperture opening posterolaterally or laterally (figs. 72, 105 in Kritsky et al., 1992) (**4**). Apomorphies: aperture opening anteriorly or anterolaterally, cirrus J-shaped (Fig. 10) (**14**); aperture opening posteriorly, cirrus curled to form 1 or more rings (fig. 66 in Kritsky et al., 1992) (**19**).

5. *Distal aperture of cirrus.* Plesiomorphy: subterminal (Figs. 20, 60, 63) (**5, 25**). Apomorphy: terminal or diagonal (Figs. 24, 35) (**15**).

6. *Cirral base.* Plesiomorphy: heellike projection absent (Figs. 10, 13) (**6**). Apomorphy: heellike projection present (fig. 105 in Kritsky et al., 1992; figs. 6–8, 23 in Boeger and Kritsky, 1988) (**12**).

7. *Submedial (muscle) articulation point of accessory piece.* Plesiomorphy: flat or minimally elevated (Figs. 17, 31) (**7, 29**). Apomorphies: small protuberance (Figs. 55, 63) (**20, 26**); protruding branch (Figs. 10, 27) (**21**). The submedial (muscle) articulation appears as a roughened area near the midlength of the accessory piece. In contrast, the subterminal expansion (character 8) has a smooth margin.

8. *Subterminal expansion of accessory piece.* Plesiomorphy: absent (Fig. 31) (**8**). Apomorphy: present (Figs. 17, 20, 24) (**16, 24**).

9. *Shape of subterminal expansion of accessory piece* (when present). Plesiomorphy: flaplike, originating from single margin (Figs. 16, 63). Apomorphies: thumblike, from single margin (Figs. 20, 67) (**27**); expanded from both margins (Fig. 24) (**17**). Species lacking a subterminal expansion (see character 8) receive a "9" in the matrix code. Polarity determined by functional outgroup comparison.

10. *Distal tip of accessory piece.* Plesiomorphy: acute (Figs. 20, 31) (**9**). Apomorphy: blunt (figs. 72, 105 in Kritsky et al., 1992) (**13**).

11. *Peduncle surface.* Plesiomorphy: smooth (**10**). Apomorphy: corrugated (Fig. 42) (**30**).

12. *Morphology of thumb on haptoral hooks.*

Plesiomorphy: slightly depressed (Figs. 11, 14, 18) (**11**). Apomorphy: flattened (Fig. 37) (**18**).

Our hypothesis on the phylogenetic relationships of the parasites is based primarily on characters of the copulatory complex. The internal anatomy of *Anacanthorus* species is redundant and, therefore, provides no evolutionary information for the present analysis. Similarly, with the exception of the morphology of the hook thumb, features of the haptor were of limited value for phylogenetic reconstruction.

Since length of the proximal expansion of the hook shank varies both inter- and intraspecifically, ratios between length of the basal expansion and the total shank length were compared across the same hook pair among species (i.e., 1-1, 2-2, etc. [7 possible homologous series]) and also pairwise within a species (i.e., 1-2, 1-3, 2-3 etc. [21 possible series]). This analysis resulted in numerous instances of homoplasy in evolution of the haptor that tended to outweigh effects of all other characters in the analysis. In effect, use of these hook characters masked pertinent information of the other 12 homologous series biasing the cladogram toward hook characteristics. Because the amount of required homoplasy was high when hook ratios were incorporated, we excluded these series from the analysis.

Further, hook pairs 3 and 4 have obviously longer proximal expansions of the shank than those of the remaining hook pairs in the outgroups and all but 4 ingroup species (*Anacanthorus amazonicus* sp. n., *A. prodigiosus* sp. n., *A. mastigophallus* Kritsky, Boeger, and Van Every, 1992, *A. gravihamulatus* sp. n.). Although the original descriptions of these 4 species do not indicate differences in the proximal expansion of the respective hook pairs, reexamination of the haptors confirms that hook pairs 3 and 4 are subtly larger than the remaining pairs. We consider this character to be symplesiomorphic for the ingroup and do not include it in the phylogenetic analysis.

PARASITE PHYLOGENY: The cladogram depicting phylogenetic relationships of the *Anacanthorus* species parasitizing 10 serrasalmid species from the central Amazon is presented in Figure 70. The consistency index (CI = 76.2%) was the highest obtained for hypotheses produced through the PAUP anaylsis utilizing the 12 homologous series. Monophyly of the ingroup is supported by 2 synapomorphies (character-state changes 1, 2): a cirrus with a tendency to curl into J-shaped to coiled configurations and an accessory piece nonarticulated to the cirral base. Character-state changes 3–11 represent symplesiomorphies for the ingroup (see character analysis).

Development of the cirral "feather" is a synapomorphy for the clade containing *Anacanthorus beleophallus* Kritsky, Boeger, and Van Every, 1992, and its 7-species sister group. The cladogram indicates that the ancestral state (character-state change 22) exhibited by *A. beleophallus* is an intermediate step in the full development of the "feather" as found in all but 1 of the remaining species in the clade. Even though a secondary loss of the "feather" has occurred in *A. cinctus* sp. n., the sister relationship of *A. cinctus* and *A. crytocaulus* sp. n. is strengthened by the derived morphology of the subterminal expansion of the accessory piece (Figs. 20, 67, 70).

HOST PHYLOGENY: One tree (Fig. 71) depicting phylogenetic relationships of the 10 serrasalmid hosts with a consistency index (CI = 71.4%) was produced through PAUP analysis using the matrix from parasite data. The positions of *Serrasalmus spilopleura* and species of *Pristobrycon* in the cladogram indicate that these genera are paraphyletic.

Discussion

In order to minimize effects of geographic and environmental variation on parasite communities (see Boeger and Kritsky, 1988), the species of *Anacanthorus* used in this study were purposely limited to those occurring in the central Amazon near Manaus. As a result, 5 previously described species from *Pygocentrus nattereri* were not included: *Anacanthorus maltai* and *A. rondonensis* reported and described by Boeger and Kritsky (1988) from the Brazilian state of Rondônia; and *A. anacanthorus, A. brazilensis,* and *A. neotropicalis* collected from aquarium hosts by Mizelle and Price (1965) in the United States. Boeger and Kritsky (1988) suggest that host specimens of the latter 3 species may have been misidentified, which limits the value of their parasites for coevolutionary studies.

The species of *Anacanthorus* infesting piranha in the central Amazon appear to form a monophyletic group defined by 2 synapomorphies: a cirrus with a tendency to curl and a nonarticulated accessory piece and cirrus. *Anacanthorus hoplophallus, A. pedanophallus, A. spinatus,* and *A. stagmophallus,* all described by Kritsky et al.

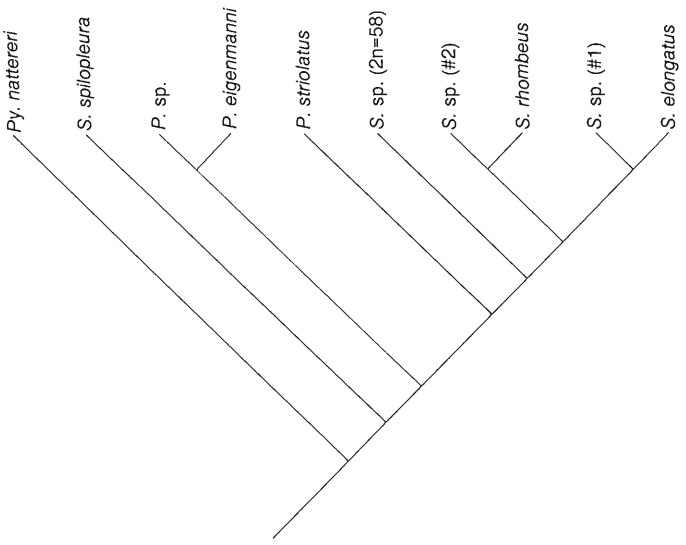


Figure 71. Cladogram depicting evolutionary relationships of 10 piranha species from the central Amazon based on their parasite fauna. *S.* = *Serrasalmus, P.* = *Pristobrycon, Py.* = *Pygocentrus.*

(1992) from *Myleus rubripinnus,* and an undescribed species of *Anacanthorus* from *Metynnis* sp. (Kritsky, unpubl.) also may have their origins within our ingroup. Since 1 objective of this study was to examine phylogeny and coevolution of the Anacanthorinae infesting *Pygocentrus, Pristobrycon,* and *Serrasalmus* (the 3 terminal serrasalmid genera of Machado-Allison, 1983), these 5 species were not included in the analysis. Further, our collections did not include any species from the potential host genus *Pygopristis,* which Machado-Allison (1983) places as sister to the 3 genera examined herein. The species of *Anacanthorus* from these and other serrasalmid genera (*Utiaritichthys, Acnodon,* and *Mylesinus,* and possibly *Piaractus*) also will likely fall within our ingroup and collectively provide an important model for studies on coevolution and biogeography of the entire Serrasalmidae.

Our hypothesis on phylogenetic relationships of *Anacanthorus* reveals several polychotomies (Fig. 70). These points of nonresolution may be attributable to a lack of informative homologous series for the ingroup species. However, it is possible that these unresolved nodes reflect historical geologic and climatic events that fragmented an ancestral distribution producing isolated populations in each of which speciation of the parasites could have occurred.

Considerable data exist to support periodic habitat changes in South America as a consequence of Quaternary climatic cycles (Cracraft and Prum, 1989). Although Weitzman and Weitzman (1982) question whether the evolution of higher taxa of Amazonian freshwater fishes corresponds to Pliocene and Pleistocene climatic events (Refugia), they suspect that these events may have been important in evolution at the minor generic and species levels. If the periodic drying and wetting of the Amazon Basin with consequent development of refugia played a role in fish speciation in the Neotropics (Weitzman and Weitzman 1982), the anacanthorine fauna could also have coevolved and speciated along with their hosts. Since refugia theory (Haffer, 1982 and others) assumes fragmentation of ancestral distributions, it could in part explain the unresolved topology of the parasite cladogram.

An alternative to the refugia theory that could also explain the unresolved nodes in our cladogram has recently been put forth by Frailey et al. (1988). The existence of a recent freshwater lake, Lago Amazonas, over the whole of the Amazon Basin may have provided a significant speciation mechanism not typically provided in a riverine system. Species diversity among Amazonian fishes and their parasites may be a result of la-

custrine resource partitioning, similar to that proposed to explain cichlid diversity in some African lakes (Lowe-McConnell, 1987) and Pliocene diversity in Lake Idaho (Smith, 1975). This single event of a "Lago Amazonas" could account for the evolution of several fish species from a single ancestor with the coinciding parasite fauna paralleling these speciation events.

Brooks (1979) suggests that coevolving hosts and parasites have experienced a common set of historical isolating events that may be reflected by occurrences and distributions of extant parasites on their hosts. He suggests 3 possible scenarios from these events. In the first, the parasites speciate while their hosts do not, which is reflected by extant sister species of parasites occurring on the same host. In his second scenario, the hosts speciate and parasites do not, resulting in the same extant species of parasite occurring on closely related hosts. In the third scenario, both hosts and parasites speciate, with the scenario being expressed by extant sister species of parasites occurring on sister host species. Our cladogram of *Anacanthorus* suggests that at least 2 of these 3 scenarios were involved during the evolutionary history of the ingroup. The sister relationships of *A. lasiophallus, A. crytocaulus,* and *A. cinctus* spp. n. and that of *A. thatcheri* Boeger and Kritsky, 1988, and *A. stachophallus* Kritsky, Boeger, and Van Every, 1992, are best explained by Brook's (1979) first scenario. Brook's (1979) second hypothesis is evident by several examples of closely related hosts harboring the same *Anacanthorus* species (see Figs. 1, 71). The third scenario may have also been involved in the evolution of the ingroup, but direct evidence from our data may be masked by the large, unresolved node in our cladogram. In any case, interpretation of coevolutionary relationships from our parasite cladogram requires explanation involving numerous instances of dispersal and/or extinction. However, the lack of an independently derived host phylogeny at the species level clearly limits the extent to which we can determine coevolutionary extinction and dispersal events.

While the generic relationships evident in our host cladogram (Fig. 71) generally support those offered by Machado-Allison (1983) for the genera *Pygocentrus, Pristobrycon,* and *Serrasalmus,* our host hypothesis suggests that the latter 2 are paraphyletic. Paraphyly of *Pristobrycon* was also suggested by Machado-Allison et al. (1989, Fig. 18B), who considered *P. striolatus* to lack a sister group relationship with any other members of the genus. It is clear that future tests of these emerging and possibly competing hypotheses on host evolution are necessary before the evolutionary history of the Serrasalmidae becomes clear.

Anacanthorus sciponophallus sp. n., *A. jegui* sp. n., and *A. xaniophallus* Kritsky, Boeger, and Van Every, 1992, occur on more than 1 host species as different morphotypes (see Figs. 43, 47, 51 this study; figs. 57–59, 118, 122 in Kritsky et al., 1992). Similarly, slight morphologic differences exist between specimens of *A. serrasalmi* sp. n. collected from different hosts. These terminal taxa with distinct morphologic forms may comprise collapsed clades composed of very closely related species. If this is the case, each clade could provide individual and independent tests of host relationships.

Although preliminary, this study demonstrates that the Anacanthorinae, and the Monogenoidea in general, provide useful models for the study of biogeography and coevolution in the Neotropics. However, caution should be used when interpreting coevolutionary relationships proposed from our parasite data. A number of hosts in our collections harbor distantly related *Anacanthorus* species, and O'Grady and Deets (1987) state that the use of inclusive ORing may give rise to phylogenetic inconsistencies when this occurs. In addition, host material may have been insufficient to ensure that all parasite species capable of infesting a host were found. Ideally, future studies to test hypotheses proposed herein would involve examination of parasites from a greater number of host specimens and species from a larger geographic area, utilization of other monogenoidean taxa, and incorporation of homologous series of host features into the matrix derived from the parasite cladogram.

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Addendum

Parasite–Host List of *Anacanthorus* Species*

SERRASALMIDAE
SERRASALMINAE

1. *Anacanthorus amazonicus* Van Every and Kritsky, 1992–*Serrasalmus rhombeus* (Linnaeus) (type); *Serrasalmus* sp. (2 of Jégu); *Pristobrycon striolatus* (Steindachner).
2. *Anacanthorus anacanthorus* Mizelle and Price, 1965–*Pygocentrus nattereri* Kner.
3. *Anacanthorus beleophallus* Kritsky, Boeger, and Van Every, 1992–*Pristobrycon eigenmanni* (Norman).
4. *Anacanthorus brazilensis* Mizelle and Price, 1965–*Pygocentrus nattereri* Kner.
5. *Anacanthorus cinctus* Van Every and Kritsky, 1992–*Pristobrycon striolatus* (Steindachner).
6. *Anacanthorus cladophallus* Van Every and Kritsky, 1992–*Serrasalmus spilopleura* Kner.
7. *Anacanthorus crytocaulus* Van Every and Kritsky, 1992–*Pristobrycon striolatus* (Steindachner).
8. *Anacanthorus gravihamulatus* Van Every and Kritsky, 1992–*Serrasalmus rhombeus* (Linnaeus) (type); *Serrasalmus* sp. (2 of Jégu); *Pristobrycon eigenmanni* (Norman).
9. *Anacanthorus jegui* Van Every and Kritsky, 1992–*Serrasalmus rhombeus* (Linnaeus) (type); *S. spilopleura* Kner; *Serrasalmus* sp. (2 of Jégu); *Serrasalmus* sp. (2n = 58); *Pristobrycon eigenmanni* (Norman); *Pristobrycon* sp.
10. *Anacanthorus lasiophallus* Van Every and Kritsky, 1992–*Pristobrycon striolatus* (Steindachner).
11. *Anacanthorus lepyrophallus* Kritsky, Boeger, and Van Every, 1992–*Serrasalmus elongatus* Kner (type); *Serrasalmus* sp. (1 of Jégu); *Serrasalmus* sp. (2n = 58).
12. *Anacanthorus maltai* Boeger and Kritsky, 1988–*Pygocentrus nattereri* Kner.
13. *Anacanthorus mastigophallus* Kritsky, Boeger, and Van Every, 1992–*Pristobrycon eigenmanni* (Norman).
14. *Anacanthorus mesocondylus* Van Every and Kritsky, 1992–*Serrasalmus elongatus* Kner (type); *S. rhombeus* (Linnaeus); *S. spilopleura* Kner; *Serrasalmus* sp. (1 of Jégu); *Serrasalmus* sp. (2 of Jégu); *Pristobrycon eigenmanni* (Norman); *Pristobrycon* sp.
15. *Anacanthorus neotropicalis* Mizelle and Price, 1965–*Pygocentrus nattereri* Kner.
16. *Anacanthorus palamophallus* Kritsky, Boeger, and Van Every, 1992–*Pristobrycon eigenmanni* (Norman).
17. *Anacanthorus periphallus* Kritsky, Boeger, and Van Every, 1992–*Serrasalmus* sp. (2n = 58) (type); *Serrasalmus* sp. (1 of Jégu).
18. *Anacanthorus prodigiosus* Van Every and Kritsky, 1992–*Serrasalmus elongatus* Kner (type); *S. rhombeus* (Linnaeus); *Serrasalmus* sp. (1 of Jégu); *Serrasalmus* sp. (2 of Jégu).
19. *Anacanthorus ramosissimus* Van Every and Kritsky, 1992–*Serrasalmus elongatus* Kner.
20. *Anacanthorus reginae* Boeger and Kritsky, 1988–*Pygocentrus nattereri* Kner.
21. *Anacanthorus rondonensis* Boeger and Kritsky, 1988–*Pygocentrus nattereri* Kner.
22. *Anacanthorus scapanus* Van Every and Kritsky, 1992–*Serrasalmus spilopleura* Kner.
23. *Anacanthorus sciponophallus* Van Every and Kritsky, 1992–*Serrasalmus elongatus* Kner (type); *S. rhombeus* (Linnaeus); *S. spilopleura* Kner; *Serrasalmus* sp. (1 of Jégu); *Serrasalmus* sp. (2 of Jégu); *Serrasalmus* sp. (2n = 58).
24. *Anacanthorus serrasalmi* Van Every and Kritsky, 1992–*Serrasalmus rhombeus* (Linnaeus) (type); *S. elongatus* Kner; *Serrasalmus* sp. (2 of Jégu); *Serrasalmus* sp. (2n = 58); *Pristobrycon* sp.
25. *Anacanthorus stachophallus* Kritsky, Boeger, and Van Every, 1992–*Pygocentrus nattereri* Kner.
26. *Anacanthorus thatcheri* Boeger and Kritsky, 1988–*Pygocentrus nattereri* Kner.
27. *Anacanthorus xaniophallus* Kritsky, Boeger, and Van Every, 1992–*Pristobrycon eigenmanni* (Norman) (type); *Pristobrycon* sp.

MYLEINAE

28. *Anacanthorus hoplophallus* Kritsky, Boeger, and Van Every, 1992–*Myleus rubripinnus* (Mueller and Troschel).
29. *Anacanthorus paraspathulatus* Kritsky, Boeger, and Van Every, 1992–*Mylossoma duriventris* (Cuvier).
30. *Anacanthorus pedanophallus* Kritsky, Boeger, and Van Every, 1992–*Myleus rubripinnus* (Mueller and Troschel).
31. *Anacanthorus spathulatus* Kritsky, Thatcher, and Kayton, 1979 (syn. *A. spatulatus* Kritsky et al., 1979, a misspelling)–*Colossoma bidens* (Spix) (type); *C. macropomum* (Cuvier).
32. *Anacanthorus spinatus* Kritsky, Boeger, and Van Every, 1992–*Myleus rubripinnus* (Mueller and Troschel).
33. *Anacanthorus stagmophallus* Kritsky, Boeger, and Van Every, 1992–*Myleus rubripinnus* (Mueller and Troschel).

CATOPRIONINAE

34. *Anacanthorus catoprioni* Kritsky, Boeger, and Van Every, 1992–*Catoprion mento* (Cuvier).

CHARACIDAE
CHARACINAE

35. *Anacanthorus dipelecinus* Kritsky, Boeger, and Van Every, 1992–*Roeboides myersii* Gill.

BRYCONINAE

36. *Anacanthorus acuminatus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix) (type); *T. elongatus* (Guenther); *T. albus* Cope.
37. *Anacanthorus alatus* Kritsky, Boeger, and Van Every, 1992–*Triportheus albus* Cope (type); *T. elongatus* (Guenther).

* Host classification is based on Géry (1977; *Characoids of the World.* T. F. H. Publications, Inc., Neptune City, New Jersey, 672 pp.). *Anacanthorus* species are listed alphabetically according to families and subfamilies of their hosts.

38. *Anacanthorus andersoni* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
39. *Anacanthorus bellus* Kritsky, Boeger, and Van Every, 1992–*Triportheus albus* Cope (type); *T. elongatus* (Guenther); *Triportheus* sp.
40. *Anacanthorus brevis* Mizelle and Kritsky, 1969–*Brycon melanopterus* (Cope).
41. *Anacanthorus calophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus elongatus* (Guenther).
42. *Anacanthorus carinatus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
43. *Anacanthorus chaunophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
44. *Anacanthorus chelophorus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix) (type); *Triportheus* sp.
45. *Anacanthorus colombianus* Kritsky and Thatcher, 1974–*Salminus affinis* Steindachner (type) (also *Tilapia mossambica* (Peters), Cichlidae [accidental]).
46. *Anacanthorus cornutus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
47. *Anacanthorus cuticulovaginus* Kritsky and Thatcher, 1974–*Salminus affinis* Steindachner.
48. *Anacanthorus elegans* Kritsky, Thatcher, and Kayton, 1979–*Brycon melanopterus* (Cope).
49. *Anacanthorus euryphallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix) (type); *T. elongatus* (Guenther); *T. albus* Cope.
50. *Anacanthorus formosus* Kritsky, Boeger, and Van Every, 1992–*Triportheus elongatus* (Guenther) (type); *Triportheus* sp.
51. *Anacanthorus furculus* Kritsky, Boeger, and Van Every, 1992–*Triportheus elongatus* (Guenther).
52. *Anacanthorus glyptophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
53. *Anacanthorus kruidenieri* Kritsky, Thatcher, and Kayton, 1979–*Brycon melanopterus* (Cope).
54. *Anacanthorus lygophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
55. *Anacanthorus nanus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
56. *Anacanthorus pelorophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus elongatus* (Guenther).
57. *Anacanthorus pithophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus angulatus* (Spix).
58. *Anacanthorus quinqueramus* Kritsky, Boeger, and Van Every, 1992–*Triportheus albus* Cope (type); *T. elongatus* (Guenther); *Triportheus* sp.
59. *Anacanthorus ramulosus* Kritsky, Boeger, and Van Every, 1992–*Triportheus albus* Cope (type); *T. elongatus* (Guenther).
60. *Anacanthorus spiralocirrus* Kritsky, Thatcher, and Kayton, 1979–*Brycon melanopterus* (Cope).
61. *Anacanthorus strongylophallus* Kritsky, Boeger, and Van Every, 1992–*Triportheus elongatus* (Guenther).
62. *Anacanthorus tricornis* Kritsky, Boeger, and Van Every, 1992–*Triportheus elongatus* (Guenther) (type); *T. angulatus* (Spix).

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Cestoda from Lake Fishes in Wisconsin: The Ecology and Interspecific Relationships of Bothriocephalid Cestodes in Walleye, *Stizostedion vitreum*

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ABSTRACT: A total of 1,812 fishes of 32 species from Silver Lake (Kenosha County) and Tichigan Lake (Racine County), southeast Wisconsin, and 1,543 fishes of 27 species from connected waters, were examined for parasites. Only walleye, *Stizostedion vitreum,* from Silver Lake were infected with both *Bothriocephalus formosus* (a new host record) and *B. cuspidatus.* Green sunfish, *Lepomis cyanellus,* from Tichigan Lake canal were also infected with *B. formosus.* The description of *B. formosus* is addended from autumn and spring collections. Available museum specimens were examined critically and some were reidentified. The relationship between growth and development of *B. formosus* was related to season (temperaturee) and host species. *Bothriocephalus formosus* was more dominant than *B. cuspidatus.* It was present in walleye all year, but its major recruitment and reproductive seasons were autumn and summer, respectively. *Bothriocephalus cuspidatus,* which competed with *B. formosus* for pyloric ceca, was absent from walleye during summer when *B. formosus* reached its peak intensity of infection and reproductive activity. Peak reproductive season of *B. cuspidatus* was in the autumn. This is considered to be a case of temporal segregation of reproductive niches. Prevalence and intensity of infection of both *Bothriocephalus* species did not appear to be related to host size or sex. *Pomoxis annularis, P. nigromaculatus, S. vitreum, Micropterus salmoides,* and *Ambloplitis rupestris* appear to be paratenic hosts of *Bothriocephalus* in Silver Lake; the first to be reported.

KEY WORDS: cestodes, Wisconsin, *Bothriocephalus, Stizostedion vitreum,* morphology, ecology, interspecific relationships, seasonality, host size and sex, site selection.

This report addresses various ecological relationships between 2 species, *Bothriocephalus formosus* Mueller and Van Cleave, 1932, and *Bothriocephalus cuspidatus* Cooper, 1917, infecting walleye, *Stizostedion vitreum,* from 2 eutrophic lakes in southeast Wisconsin. Early studies of fish parasites in various Wisconsin waters included parsite–host lists, which were occasionally annotated. Both species of cestodes were previously reported in western Wisconsin (Fischthal, 1947; Kuntz and Font, 1984), but *B. cuspidatus* was more widespread elsewhere in northern and eastern Wisconsin and in other Great Lakes states (see Hoffman, 1967). The fact that both species of cestodes utilized the same fish species as the definitive host in this study provided a unique opportunity to study their interspecific associations, habitat occupancy, host relationships, and variations in their temporal pattern of reproduction. Morphological comparisons and ecological information contained herein are reported in the North American literature for the first time.

Materials and Methods

Walleye were examined from Silver Lake (Kenosha County), a 188-ha eutrophic land-locked lake, and Tichigan Lake (Racine County), a 458-ha lake in an advanced state of eutrophication on the Fox River (tributary of the Mississippi River). Seasonal biweekly collections were made from both lakes during spring (April), summer (June, July, and early August), and autumn (late October and November) between 1977 and 1979 and also from Silver Lake during the summer of 1976.

Fish were electro-shocked and dissected shortly after capture. The stomach (region A), the first and second loops of the small intestine (B_1 and B_2) and the first and second halves of the large intestine (C_1 and C_2) were systematically examined for parasites. Cestodes were fixed, stained, and mounted as in Amin (1986a) and categorized as juveniles (strobila with no or only immature proglottids), mature adults (posterior proglottids sexually mature), and gravid (at least some proglottids with eggs). Mean refers to the number of worms recovered/number of fish examined, and prevalence is the number of fish infected/number examined × 100. Representative specimens were deposited in the U.S. National Museum Helminthological Collection (USNM Helm. Coll.) and in the University of Nebraska State Museum Harold W. Manter Laboratory Collection (HWML Coll.).

Results

Only walley from Silver Lake were found infected with either species of cestodes.

Variability

The morphometrics of *B. formosus* from walleye in Wisconsin were similar in the autumn and

Table 1. Comparison between major anatomical features of *Bothriocephalus formosus* from Wisconsin and the type material.*

Character	Wisconsin material		Type material	
	Autumn/spring $N = 8$	Summer $N = 11$	Mueller and Van Cleave (1932) description	USNM Helm. Coll. No. 8690 cotypes $N = 6$
Strobilar length (mm)	102.50 (74.00–130.00)†	29.50 (18.00–38.00)	Rarely exceeding 30	27.15 (21.20–34.00)
Max. width (mm)	2.06 (1.60–2.44)	1.11 (0.84–1.37)	1.3	1.05 (1.00–1.12)
Scolex length (mm)	1.16 (1.12–1.26)	0.83 (0.70–0.98)	0.32–0.475	0.46 (0.42–0.49)
Max. width (mm)	0.45 (0.36–0.52)	0.37 (0.27–0.42)	0.13–0.23	0.20 (0.14–0.25)
Neck width (mm)	0.27 (0.22–0.31)	0.18 (0.15–0.31)	Similar to scolex width	0.16 (0.14–0.17)
Mature seg. length (mm)	0.60 (0.36–0.76)	0.40 (0.28–0.49)	0.4–0.6	0.54 (0.40–0.60)
Width (mm)	2.00 (1.37–2.44)	0.88 (0.71–1.09)	0.8–1.2	0.97 (0.88–1.12)
Gravid seg. length (mm)	0.97 (0.72–1.20)	0.53 (0.42–0.63)	—	0.69 (0.60–0.84)
Width (mm)	1.95 (1.60–2.44)	1.11 (0.84–1.37)	1.3	1.03 (0.92–1.12)
No. of segments	380.2 (277–443)	119.9 (81–158)	—	105.7 (92–120)
% gravid	10.7 (8.0–16.0)	17.7 (8.0–30.0)	—	14.5 (6.0–27.0)
Testis dimensions (μm)	120 (106–134) × 110 (80–128)	89 (76–109) × 76 (61–99)	—	81 (70–112) × 68 (58–90)
No.	50.7 (45–58)	44.1 (34–60)	30–45	41.5 (32–55)‡
Cirrus sac dimensions (μm)	285 (238–350) × 251 (196–336)	204 (154–238) × 186 (140–238)	—	151 (115–196) × 132 (96–154)§
Ovary width (μm)	630 (490–728)	399 (294–560)	—	274 (238–322)
Egg length (μm)	55 (48–64)	53 (48–64)	53–59	56 (51–61)
Width (μm)	39 (32–45)	38 (32–48)	33–35	41 (38–48)
Vitellaria dimensions (μm)	69 (48–83) × 54 (42–64)	54 (38–64) × 44 (35–51)	—	42 (32–51) × 33 (26–42)

* All the Wisconsin specimens and the cotypes were gravid.

† Mean (range). Two testes, cirrus sacs, ovaries, eggs, and vitelline glands were measured and/or counted in each cestode.

‡ Only developing testes could be counted until obscured by forming vitellaria. Numbers shown may be underestimates.

§ The size of the cirrus sac increases considerably in posteriormost proglottids of complete worms. Indicated size was probably affected by a few missing segments from 3 worms.

Table 2. Prevalence and mean intensity of *Bothriocephalus formosus* and *B. cuspidatus* infections in *Stizostedion vitreum* from Silver Lake, 1976–1979.

	Autumn (late Oct., Nov.)	Spring (April)	Summer (June–early Aug.)	Total
B. formosus				
Fish inf./exam. (%)	15/23 (65)	20/21 (95)	6/10 (60)	41/54 (76)
Worms (mean/exam. fish) max.	313 (13.6) 112	529 (25.2) 93	368 (36.8) 285	1,210 (22.4) 285
B. cuspidatus				
Fish inf./exam. (%)	5/23 (22)	12/21 (57)	0/10	17.54 (31)
Worms (mean/exam. fish) max.	62 (2.7) 47	415 (19.8) 120	0	477 (8.8) 120

spring collections; data were combined (Table 1). Summer worms were considerably shorter and measured structures were smaller. Meristic and morphometric features in 19 worms with complete strobilae were compared with the incomplete description of Mueller and Van Cleave (1932), as well as with measured samples of their cotypes (Table 1). Available museum specimens of *B. formosus* and *B. claviceps* were also examined for verification of identity.

Seasonality

Prevalence and intensity of infection were considerably higher for *B. formosus* (76%, 22.4) than for *B. cuspidatus* (31%, 8.8) (Table 2). Walleye were most frequently and heavily infected with *B. formosus* during summer and with *B. cuspidatus* during spring (Table 2). Summer infections with *B. formosus* were influenced by 1 heavily infected fish that had 285 worms.

Table 3. Seasonal development of *Bothriocephalus formosus* and *B. cuspidatus* in *Stizostedion vitreum* from Silver Lake, 1976–1979.

Cestode developmental stages	No. of worms	Number and prevalence (%) of worms: Autumn (late Oct., Nov.)	Spring (April)	Summer (June–early Aug.)
B. formosus				
All stages	1,210	313	529	368
Juvenile (%)*	973	293 (94)	432 (82)	248 (67)
Mature (%)	184	8 (2)	88 (17)	88 (24)
Gravid (%)	53	12 (4)	9 (2)	32 (9)
B. cuspidatus				
All stages	477	62	415	0
Juvenile (%)	335	0 (0)	355 (86)	0 (0)
Mature (%)	93	41 (66)	52 (13)	0 (0)
Gravid (%)	29	21 (34)	8 (2)	0 (0)

* The percent prevalence compares data in vertical columns.

Walleye harbored juvenile and gravid *B. formosus* during all seasons; most worms recovered during autumn were juveniles (94%), and the highest proportion of mature and gravid adults (33%) was obtained during the summer (Table 3). No *B. cuspidatus* were found in walleye during summer; most spring worms were juveniles (86%), and all autumn worms were mature (66%) or gravid (34%) adults (Table 3).

Host relations

Parameters of *B. formosus* and *B. cuspidatus* infections were not affected by host sex. Prevalence and mean intensity of *B. formosus* infections were 79% and 19.0 in 28 male and 73% and 26.0 in 26 female walleye. *B. cuspidatus* infections were 36% and 9.1 and 30% and 8.5 in male and female walleye, respectively. No particular relationship between rates of infection and fish size was apparent; males and females were not significantly different in size.

Site of infection

Pyloric ceca of walleye appear to be the preferred sites of infection with both species of *Bothriocephalus,* particularly *B. cuspidatus,* during all seasons (Table 4). However, gravid worms occurred with similar frequency in both cecal and intestinal sites.

Infections in other hosts

The only adult *B. formosus* not collected from *S. vitreum* were 13 gravid worms from the pyloric ceca of 3 green sunfish, *Lepomis cyanellus,* captured in Tichigan Lake Canal during the summer of 1979. In addition, 13 larval *B. formosus* (Bf) and 9 larval *B. cuspidatus* (Bc) were found during the spring (20) and summer (2) of 1978 on the liver of 6 fishes, 1 *Pomoxis annularis* (1 Bc), 2 *S. vitreum* (3 Bf), 1 *Pomoxis nigromaculatus* (1 Bc), 1 *Micropterus salmoides* (11 Bf, 5

Table 4. Seasonal site selection of *Bothriocephalus formosus* and *B. cuspidatus* in *Stizostedion vitreum* from Silver Lake, 1976-1979.

Season	No. of worms	Percent of worms in intestinal regions (% of juveniles, adults, gravid worms)*				
		A	Ceca	B	C_1	C_2
B. formosus						
Autumn	313	5 (100, 0, 0)	59 (90, 5, 5)	32 (97, 0, 3)	4 (100, 0, 0)	0
spring	529	1 (83, 17, 0)	68 (76, 22, 2)	30 (95, 4, 1)	0.4 (50, 50, 0)	0.4 (100, 0, 0)
Summer	368	12 (66, 29, 5)†	50 (68, 25, 7)	37 (67, 20, 13)	1 (50, 0, 50)	0
B. cuspidatus						
Autumn	62	0	97 (0, 65, 35)	1.5 (0, 100, 0)	1.5 (0, 100, 0)	0
Spring	415	0	85 (86, 12, 2)	15 (84, 14, 2)	0	0

* Percent of worms in the stomach (A), ceca, small intestine (B), and first and second halves of large intestine (C_1 and C_2) (percent of juveniles with only immature segments if segmented, adults with sexually mature segments, adults with segments gravid with eggs).

† It is possible that regurgitation was a variable affecting this anterior localization of worms.

Bc) and 1 *Ambloplites rupestris* (1 Bc) from Silver Lake. Cestodes from walleye were 3–22 mm long and with 6–70 segments each. Those from other hosts were 2–9 mm long and with 2–35 segments each. The scolex was well defined in all juveniles.

Discussion

Morphometric and meristic features of *B. cuspidatus* were similar in shape and size to those originally described by Cooper (1917) and subsequent observers (Van Cleave and Mueller, 1934; Hugghins, 1972). Thus, morphometric analysis was not warranted. The smaller summer *B. formosus* from Wisconsin were similar to those in the original description (Table 1). Mueller and Van Cleave's (1932) specimens were collected during the summer. Therefore, their description of *B. formosus* does not express the full range of variation exhibited by *B. formosus* in this study (Table 1). The scolex of *B. formosus* from Wisconsin agrees with the text description of Mueller and Van Cleave (1932). However, the scolex of Oneida Lake cestodes (Mueller and Van Cleave, 1932, fig. 20) was markedly smaller (Table 1) and its anterior tip was more dome-shaped. The dome-shaped scolex tip of the 6 cotypes (USNM Helm. Coll. No. 8690) (Table 1) was barely to moderately pronounced. Observed scolex differences are not considered sufficient to warrant considering Wisconsin material as a new species. Proglottids of the Wisconsin material were similar to those in the original description known only from summer material.

Reference specimens made available for study included *B. formosus* cotypes collected by J. F. Mueller in 1932 from *Percopsis omiscomaycus* in Oneida Lake (USNM Helm. Coll No. 8690) and *B. formosus* from *Etheostoma nigrum* in Chippewa County, Wisconsin (USNM Helm. Coll. No. 78221), which were identical to my material. Misidentifications included "*B. formosus*" from *E. nigrum* in Ingram County, Michigan (USNM Helm. Coll. No. 76699) and from *Pimephalus notatus* in Posey County, Indiana (HWML Coll. No. 21487), as well as "*B. claviceps*" (actually *B. formosus*) from *Lepomis cyanellus* in Keith County, Nebraska (HWML Coll. Nos. 19827, 19893).

Summer *B. formosus* and their reproductive structures (except egg size) were considerably smaller compared to autumn–spring worms (Table 1). Number of proglottids/strobila was also considerably fewer, but percent of gravid segments was greater in summer (Table 1). Clearly these data represent a seasonal (temperature-related) growth-maturity phenomenon. Growth appears to proceed quickly during the autumn (early in the infection cycle) and large size is retained in the overwintering worms. In the summer, rapid maturation, perhaps of new recruits, leads to small size adults. Musselius (1962 *in* Davydov, 1978) reported that cestodes in fish intestines at water temperatures of 22–25°C mature and produce eggs almost twice as fast as at 16–19°C. It took *Bothriocephalus acheilognathi* Yamaguti, 1934, 12–14 and 22–25 days to attain sexual maturity at 22–25° and 15–18°C, respectively (Davydov, 1978). Granath and Esch (1983) noted that development of *B. acheilognathi* from North Carolina was stimulated by water temperature. Growth and fecundity of *B. acheilognathi* were also found by Davydov (1978) to be

dependent on worm size and affected by temperature. Moravec (1985) reported that gravid *B. claviceps* from Czechoslovakia were larger in May to July than in August. Robert et al. (1988) observed many large segments of *Bothriocephalus gregarius* from France during July compared to the fewer and smaller segments observed later in the season. Bona (1983) shed more light on this phenomenon with his careful biometrical study of the dilepidid cestode *Dendrouterina pilherodiae* Mahon, 1956. He analyzed differential rate of growth versus development and proposed a model showing how adult proglottids of different sizes can form. He concluded that growth assumes an S-shaped curve with an increasing, then a decreasing, rate. Wisconsin material also indicates that this growth-development phenomenon is additionally affected by host species. The 13 gravid *B. formosus* collected from *L. cyanellus* in Tichigan Lake Canal during the summer were similar in size to the larger autumn–spring worms obtained from walleye in Silver Lake (Table 1). This may represent an ecological phenomenon related to *L. cyanellus* becoming active and feeding on intermediate hosts earlier in the spring, or to its gut size. Riggs et al. (1987) also noted the relationship between size and fecundity of *B. acheilognathi* and cyprinid and poecilid fish host species and gut size in North Carolina. The absence of other collections from green sunfish from Wisconsin during other seasons does not allow further elaboration on that point.

Bothriocephalus cuspidatus is a common parasite of walleye (Van Cleave and Mueller, 1934; Hugghins, 1972; Deutsch, 1977; Sutherland and Holloway, 1979; Robinson and Jahn, 1980; and others). In land-locked Silver Lake, *B. cuspidatus* was far less common than *B. formosus* in walleye (Table 2). Neither species was found in 55 walleye examined from Tichigan Lake and its canal. Appropriate intermediate hosts are probably present in Tichigan Lake system as indicated by infection of *L. cyanellus* from the Tichigan Lake Canal. However, the vagile green sunfish might have picked up the infection elsewhere, e.g., the Fox River. Other helminth species also found to be more dominant in Silver Lake fishes compared to Tichigan Lake include *Proteocephalus ambloplitis* (Leidy, 1887) Benedict, 1900 (see Amin, 1990a; Amin and Cowen, 1990), caryophyllaeid cestodes (Amin, 1986a), and *Neoechinorhynchus* spp. (Amin, 1986b). The opposite trend was observed in *Pomphorhynchus bulbocolli* Linkins *in* Van Cleave, 1919 (see Amin, 1987) and similar infection parameters were noted for *Proteocephalus pinguis* La Rue, 1911, in both lakes (Amin, 1990b). Environmental pecularities and ecological variables, relating to differences in the life cycles of each of the parasitic groups, particularly the intermediate host, may account for the expression of the above patterns. Parasites using cyclopoid copepods are less represented in Tichigan than in Silver Lake.

The similar prevalence and intensity of both *B. formosus* and *B. cuspidatus* infections in male and female walleye of all sizes over 15 cm appear to reflect a relatively uniform level of infection in fish paratenic hosts since walleye become piscivorous at about 6–8 cm (Niemuth et al., 1972). No second intermediate host is necessary in the cycle of both species of cestodes. This pattern of infection with *Bothriocephalus* was previously noted by Essex (1928), Mitchell and Hoffman (1980), and others. In the present study, the stomachs of some larger walleye contained walleye and bluegill, *Lepomis macrochirus.* These prey fish may represent a link in the chain of walleye infection in Silver Lake. The increased prevalence of *Bothriocephalus* sp. in female yellow perch, *Perca flavescens,* in Ontario was apparently related to increased predation by older females which, unlike males, "showed an increase in food size . . . (crayfish and fish) . . . with increasing size . . ." (Cannon, 1973). Similar relationships between intensity of infection and host size were also reported for *B. claviceps* from perch, *Perca fluviatilis,* in Czechoslovakia (Scholz, 1986) and for *B. gregarius* from turbot, *Psetta maxima,* in France (Robert et al., 1988).

Of the 54 walleye examined from Silver Lake, 41 (76%) were infected with *B. formosus* and 17 (31%) with *B. cuspidatus* (Table 2). All 17 fish infected with *B. cuspidatus* were also concurrently infected with *B. formosus.* Both species occupied pyloric cecal sites (Table 4). Site selection, interspecific competition, and/or seasonality of intermediate hosts may have influenced the reproductive strategy of both cestode species in walleye. At the infrapopulation level, individual concurrently infected walleye with high intensity of *B. formosus* infections usually had light infections with *B. cuspidatus*; the opposite was true. *Bothriocephalus formosus* appears to be the dominant cestode species in Silver Lake walleye. Recruitment, maturation, and reproduction of *B. formosus* occur during all seasons (Tables 2, 3).

The major recruitment season appears to be autumn when 94% of cestodes were juveniles; partial generation overlap is indicated. Cestode build-up and maturation increased reaching a maximum intensity (36.8) (Table 2) and proportion of mature and gravid worms (33%) (Table 3) during summer when the highest proportion in nonpyloric cecal sites (37%) was noted (Table 4). No collections were made during winter. The seasonality of *B. formosus* has not been reported in any other location in North America, but comparable patterns were reported in other bothriocephalid cestodes, e.g., *B. claviceps* from eel (*Anguilla anguilla*) in Czechoslovakia (Moravec, 1985), *B. claviceps* from perch (*Perca fluviatilis*) in Czechoslovakia (Scholz, 1986), and *B. acheilognathi* from *Gambusia affinis* in North Carolina (Marcogliese and Esch, 1989).

The reproductive cycle of *B. cuspidatus* in Silver Lake may be related to its decreased competitive success compared to *B. formosus*. In summer, when *B. formosus* reached its peak abundance and reproductive activity, *B. cuspidatus* was absent. This absence might have been affected by sample size of walleye but not its sex or size, which were shown above to be unrelated to prevalence or intensity of infection. Peak reproductive activity of *B. cuspidatus* (100% mature and gravid worms) was evident in the autumn (Table 3). This is in contrast to evidence from other studies of *B. cuspidatus* in single infections, which suggests that the usual breeding season of *B. cuspidatus* is summer in its fish definitive hosts (Van Cleave and Mueller, 1934; Amin, 1975; and others). The seasonal displacement of *B. cuspidatus* and change in its reproductive cycle allowed temporal niche sharing in the pyloric ceca of the same host species. The possible involvement of the seasonal distribution of intermediate hosts, or other ecological variables, in this phenomenon is not known.

The recovery of juveniles of both species of *Bothriocephalus* from the liver of *P. annularis, P. nigromaculatus, S. vitreum, M. salmoides,* and *A. rupestris* contrasts with Essex's (1928) original contention about the role of small fish as a source of infection of the definitive fish host. Essex (1928) originally thought that *B. cuspidatus* could be transmitted from fish to fish if the prey fish was recently ingested and had an infected copepod in its stomach. The fact that walleye of the sizes examined in this study (15–45 cm) are piscivorous suggests that the above species of fish serve as paratenic hosts in Silver Lake. This is the first report of fish paratenic hosts for either species of *Bothriocephalus*. Other sites for paratenic infection have, however, been noted, e.g., *B. gregarius* metacestodes infected the intestine of goby (Robert et al., 1988).

Deposited Specimens

Bothriocephalus formosus: HWML Coll. Nos. 31701–31707 and USNM Helm. Coll. Nos. 81264–81268. *Bothriocephalus cuspidatus:* HWML Coll. Nos. 31708, 31709 and USNM Helm. Coll. Nos. 81269, 81270.

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Surface Topography of Adults and Eggs of *Gnathostoma doloresi* (Nematoda: Spirurida) from Wild Boars (*Sus scrofa leucomystax*)

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ABSTRACT: As few works were found on surface morphology of *Gnathostoma doloresi* Tubangui, 1925, adult specimens and the eggs of this species were examined by scanning electron microscopy. Worms had a subglobular head-bulb with 1 pair of anterior lateral lips. The head-bulb was armed with 7–9 rows of cephalic hooks. Multidigitate cuticular spines were spaced unevenly on transverse cuticular striations on the anterior half of the body. The lengths of the spines were variable with tridentate spines longer than bidentate spines. The posterior half of the body was covered densely with long unidentate spines. Labial papillae and amphids, cervical papillae, caudal papillae, small papillae, and phasmids were also present on the bodies. Eggs recovered from the uteri of female worms were covered with circular pits of comparatively equal size and depth.

KEY WORDS: *Gnathostoma doloresi,* ultrastructure, scanning electron microscopy, adult worms, eggs.

Gnathostoma doloresi Tubangui, 1925, was first reported from a pig in the Philippines. Since that discovery, the endemic area of this parasite has been expanded to include most of southeast Asia and Oceania. Adult worms are normally found in shallow nodules on the stomach wall of pigs and wild boars, the main reservoir hosts for this parasite. Human infections with the advanced third-stage larvae of *G. doloresi* have recently been identified in Japan. This species of gnathostome is currently recognized as an important cause of clinical disease (Ogata et al., 1988; Nawa et al., 1989).

The morphology of adult specimens and larval stages of *G. doloresi* has been described in detail by light microscopy (Tubangui, 1925; Miyazaki, 1950, 1954; Ishii, 1956; Chiu, 1959; Dissamarn et al., 1966; Tada, 1968; Lin and Chen, 1988). More detailed studies of the second-stage, and early and advanced third-stage larvae of *G. doloresi* from cyclops and snakes, respectively, have been done by scanning electron microscopy (SEM) (Koga and Ishii, 1987; Imai et al., 1988; Koga et al., 1989). Observations of adult worms and eggs by SEM have been reported, but the entire outer surface of the worms was not examined (Ishii and Tokunaga, 1970; Sakaguchi et al., 1985; Imai et al., 1989). The present study was designed to examine the surface of adult specimens and eggs of *G. doloresi* in greater detail.

Materials and Methods

Adult specimens of *Gnathostoma doloresi* were collected from the stomachs of naturally infected wild boars (*Sus scrofa leucomystax*) from Kyushu, Japan. Six worms, including males and females, were washed in a glass vial with several changes of tap water and finally rinsed with physiological saline. The worms were fixed with 10% formalin for 7 days, soaked in running tap water overnight to remove the fixative, rinsed with distilled water, and then postfixed in 1% osmium tetroxide for 4 hr. During the postfixation, the specimens were cut transversely into 6 pieces to facilitate observations by SEM. The tissue was then dehydrated with an ethanol series and critical point dried with a Hitachi HCP-2 critical point dryer. After coating with gold in an ion sputter coater (JEOL FC-1100), the specimens were examined with a JEOL JSM-U3 scanning electron microscope operated at 15 kV. All measurements are given in micrometers.

Results

Adult specimens of *Gnathostoma doloresi* are elongate cylindrical nematodes that are covered with evenly spaced rows of transverse striations (Fig. 1). Adult male and female worms examined in this study had a hemispherical head-bulb armed with 7–9 transverse rows of cephalic hooks (Figs. 1, 2). The slender hooks measured about 16 in length and had tapering points (Fig. 3). The mouth was located in the center of the head-bulb and had 1 pair of ellipsoidal lateral lips (pseudolabia). Labial papillae occurred in pairs on each lip and measured about 13 in diameter. Each labial papilla was comprised of fused double papillae (the cephalic and outerlabial papillae). An amphid was present between each pair of papillae (Fig. 4). The body was covered entirely with cuticular spines that originated from the transverse striations. One pair of balloonlike cervical papillae measuring about 20 × 15 in size was located laterally near the 20th striation (Fig. 5). Four pairs of mammiform caudal papillae were located on the ventral surface of the tail of male

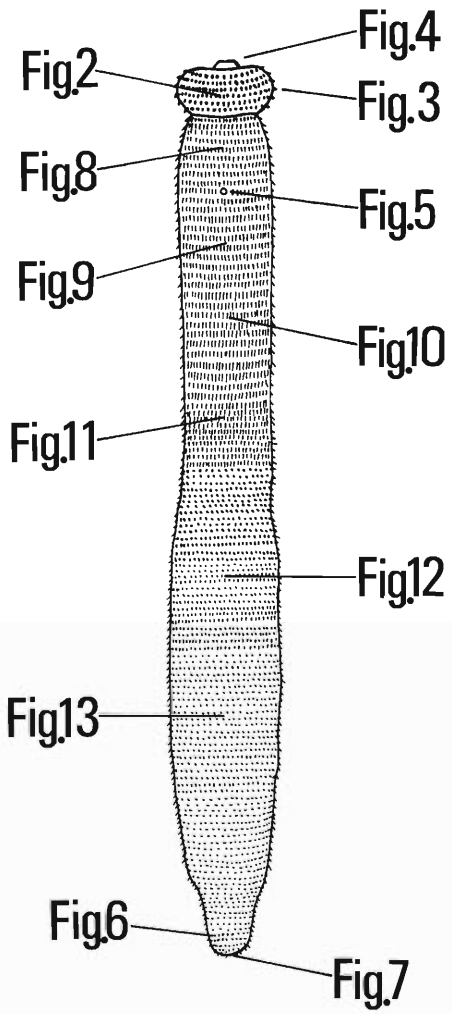


Figure 1. A schematic diagram of an adult *Gnathostoma doloresi*. Lines identify areas of the worm that are illustrated by specific figures.

specimens. An additional 4 pairs of small, dome-shaped papillae, including a pair of cloacal papillae, were located between the larger papillae. The cloacal papillae marked the bilateral edges of the anal opening and were difficult to distinguish at low magnifications (Fig. 6). Slightly elevated bilateral circular phasmids measuring 10 × 12 in size were located at the posterior end of female worms (Fig. 7). These phasmids were not covered with spines.

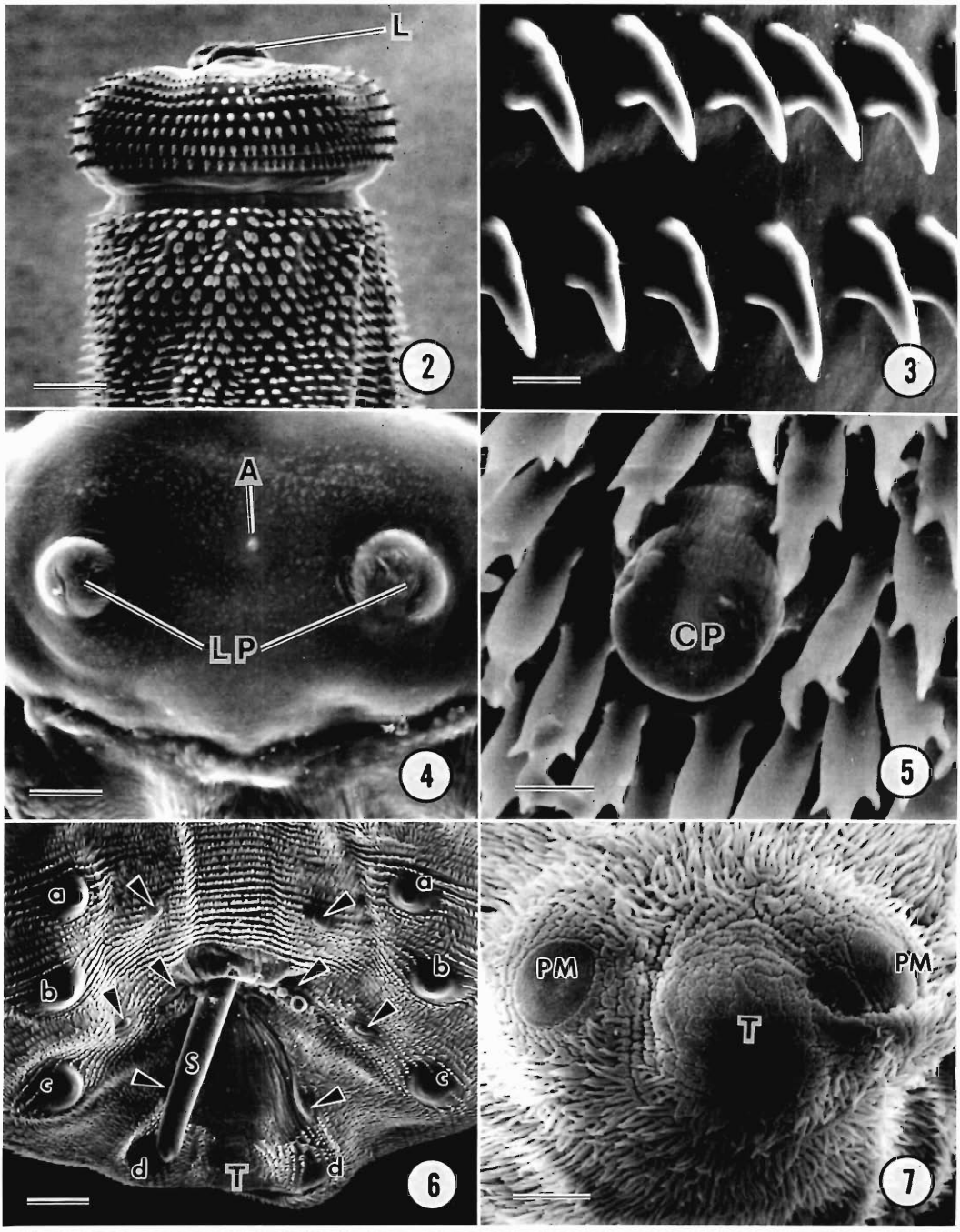
Spines were more variable in shape on the anterior half of worms. Those located immediately behind the head-bulb were broad and stumpy, measured about 17 × 15 in size, and had 5–6 teeth (Fig. 8). Spines became longer and changed in shape as distance from the anterior end of the worm increased, eventually becoming tridentate and reaching sizes of 50 × 6 on the anterior quarter of the body (Fig. 9). These tridentate spines merged gradually into bidentate spines measuring about 40 × 5 in size (Fig. 10) and eventually into unidentate spines (about 40 × 3.5) slightly anterior to the mid-body (Fig. 11). The unidentate spines decreased in length to 15–30 and became more slender and hairlike towards the posterior end of the worms (Figs. 12, 13). The tail of male worms was covered densely with small spines, but the anal opening was surrounded by a spineless area (Fig. 6). Except for the terminal projection and the area of phasmids, the posterior end of female worms was covered densely with hairlike spines (Fig. 7).

Fertilized uterine eggs of *G. doloresi* were ellipsoidal and measured about 40 × 20 in size. The eggs had a pair of opercula and were covered with round pits of uniform depth that were approximately 6 in diameter (Figs. 14, 15).

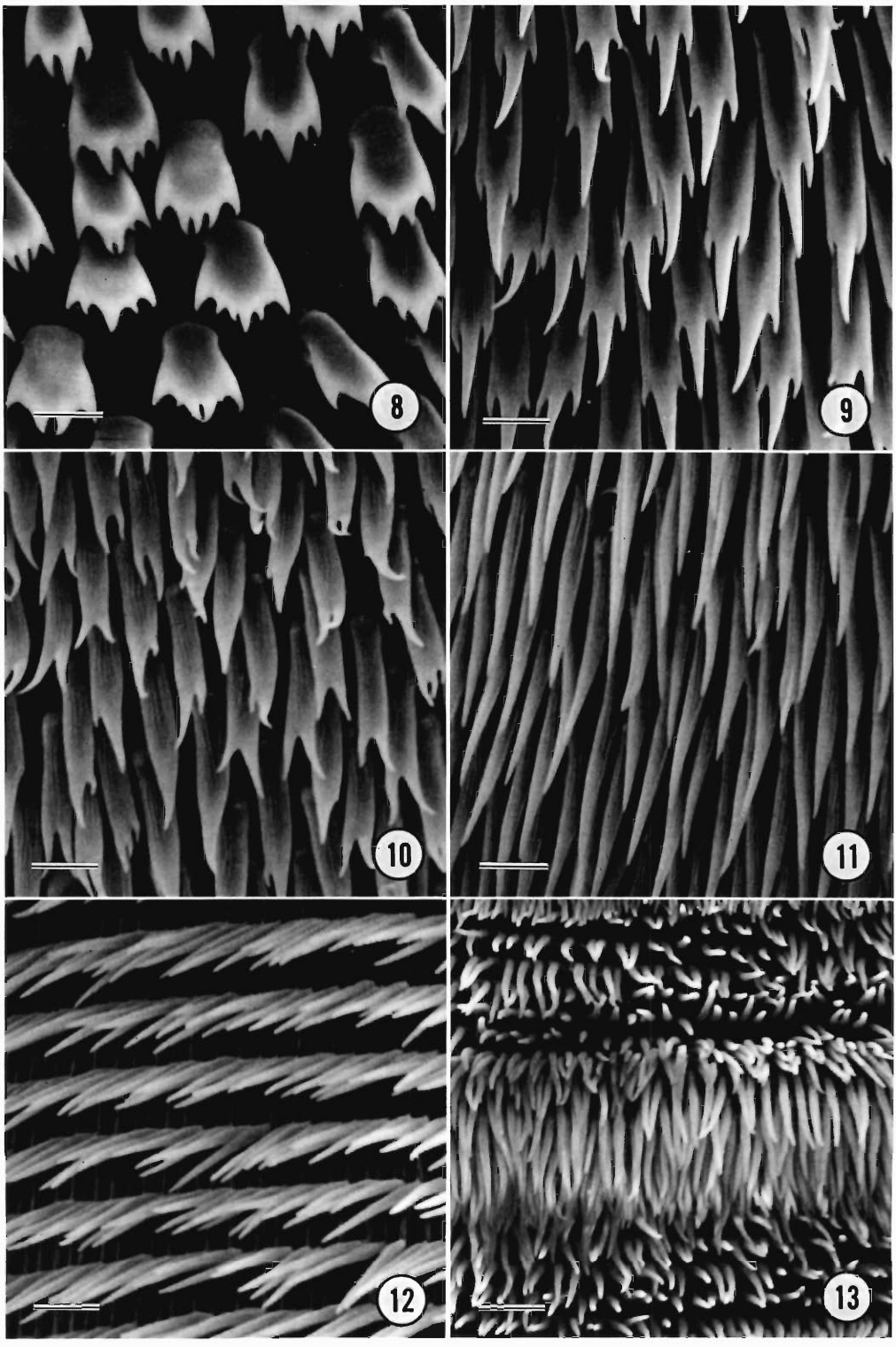
Discussion

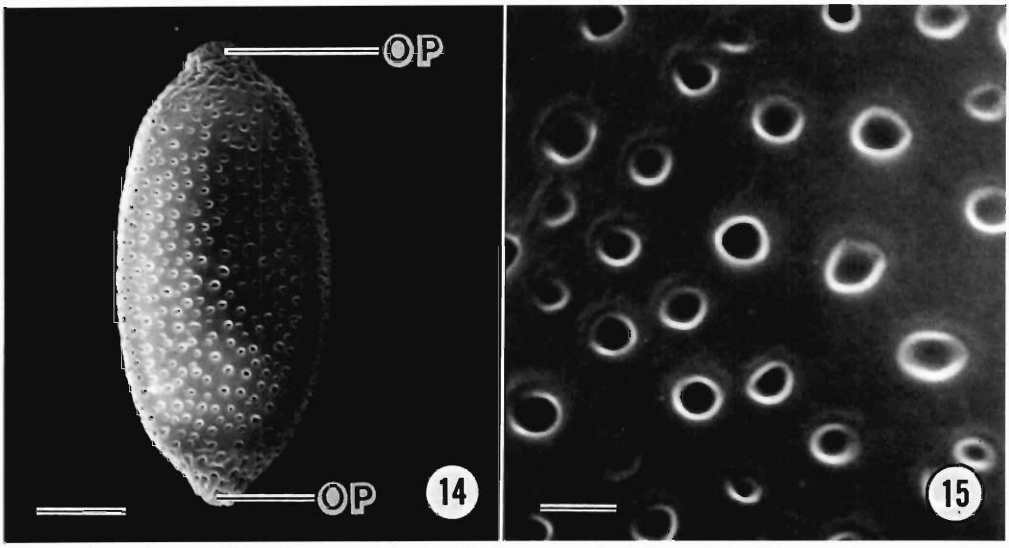
Previous studies of adult specimens of *Gnathostoma doloresi* by SEM provided few details about the location and morphology of papillae. Sakaguchi et al. (1985) did not describe these structures and Imai et al. (1989) only illustrated a pair of cervical papillae. We were able to demonstrate the presence of labial papillae, an amphid, cervical papillae, caudal papillae, small papillae, and phasmids on the adult specimens. The locations of these structures were almost identical to those in other species of gnathostomes (Koga and Ishii, 1981; Koga et al., 1984; Huang et al., 1986; Scholz and Ditrich, 1990). We were unable to demonstrate the excretory pore and posterior body papillae in our specimens of *G. doloresi,* but suspect that they were covered by the abundant, hairlike body spines of this nematode. The mammiform caudal papillae and dome-shaped small papillae on the ventral surface of the tail end of male worms, and the circular, spineless phasmids that occur on the tail ends of female worms, are similar to those that have been described in specimens of *Gnathostoma hispidum* Fedtschenko, 1872 (Koga et al., 1984; Huang et al., 1986).

Miyazaki (1960) conducted a detailed study of the spines of *G. doloresi* by light microscopy and showed that the body is covered entirely by long, slender spines. Sakaguchi et al. (1985) and Imai et al. (1989) demonstrated by SEM that the num-



Figures 2–7. Scanning electron micrographs of adult *Gnathostoma doloresi*. 2. Lateral view of the head-bulb. The bulb has 8 transverse rows of hooks. L, Lip. Scale bar = 80 μm. 3. Hooks located on the head-bulb are unidentate and have sharp tips. Scale bar = 7 μm. 4. Frontal view of the head-bulb. A pair of dome-type labial papillae (LP) and an amphid (A) are visible on 1 of the 2 semicircular pseudolabia. Scale bar = 10 μm. 5. Balloon-shaped cervical papilla (CP) are located on the anterior surface of the body. Scale bar = 7 μm. 6. Four pairs of dome-type caudal papillae (a–d) are present on the ventral side of the male tail. Another 4 pairs of smaller dome-type papillae (arrowheads) are more medially located. S, Spicule; T, Terminal projection. Scale bar = 30 μm. 7. The extreme end of the female tail has 2 bare phasmids (PM) which are elevated slightly above the tegument. The terminal projection (T) has no spines. Scale bar = 10 μm.





Figures 14, 15. Eggs of *Gnathostoma doloresi*. 14. Two opercula (OP) are located at opposite ends of the egg. Scale bar = 7 μm. 15. The surface of the eggshell has many round pits of relatively equal size and depth. Scale bar = 1 μm.

ber of teeth in these spines decreased as distance from the anterior end increased. Structures associated with the tail end of male and female worms were either unrecognizable or not described. Observations from our study on the spacing of spines were most similar to those of Miyazaki (1960), Sakaguchi et al. (1985), and Imai et al. (1989). As seen by SEM, spacing of spines of *G. hispidum* is very similar to that of *G. doloresi*. Spines of *G. hispidum* change size and shape between the anterior quarter and one-third of the body. Around the anterior quarter regions are found the stumpy spines (about 47 × 26 in size) having 5–10 teeth, and they progressively increased in size to about 105 × 12 bearing 3 teeth with the middle 1 markedly elongated at the anterior one-third region. Then the spines abruptly changed shapes to those with 2 denticles. Spines with 1 denticle followed the 2-denticle ones posterior to the anterior one-third body region. These 1-toothed spines entirely covered the rest of the body. Spines at about the mid-body measured 35–65 × 2 and at the posterior extremity about 25–35 × 2 (Koga et al., 1984). Spines of *G. hispidum* seem to be much longer than those of *G. doloresi* in comparable regions.

Early study of the eggs of *G. doloresi* by SEM found ellipsoidal pits in the eggshell (Ishii and Tokunaga, 1970). These structures were more rounded in our specimens, although some pits were oval in shape. The shape of these pits appears to be characteristic for the eggs of *G. doloresi*. The pits resemble those that are found in eggs of *G. hispidum*, but are different from those of *Gnathostoma spinigerum* Owen, 1836, and *Gnathostoma nipponicum* Yamaguti, 1941 (Zaman, 1987; Koga and Ishii, 1981) whose pits have irregular shapes and depths.

To obtain clear, crisp, and concise observations of the location and morphology of spines, papillae, amphids, and phasmids on male and female specimens of *G. doloresi* is very difficult using only light microscopy. There are no descriptions of the cervical papillae and eggshell pits by light microscopy. Our present results may aid in the identification of the adults and eggs of this species of nematode.

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Figures 8–13. Scanning electron micrographs of cuticular spines of *Gnathostoma doloresi*. Depending on location, spines on the surface of adult worms differ in size and shape. Scale bars = 10 μm. 8. Spines immediately behind the head-bulb have 5 or 6 teeth and are broad and stumpy. 9. Slender tridentate spines reach the greatest lengths. 10. Bidentate spines located on the anterior one-third of the body. 11. Unidentate spines are located posterior to bidentate spines and extend to the posterior extremity. 12, 13. Fine, eyelashlike spines cover the posterior half of the body.

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Description of *Trypanosoma* (*Megatrypanum*) *stefanskii* sp. n. from Roe Deer (*Capreolus capreolus*) in Poland[1]

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ABSTRACT: *Trypanosoma* (*Megatrypanum*) *stefanskii* sp. n. is described from 12 of 18 (66.6%) roe deer examined between 1984 and 1988 from Puszcza Niepolomicka in south-central Poland. Trypomastigotes were compared with unnamed trypanosomes from roe deer in Germany, with *T. cervi* Kingston and Morton, 1975, from North American cervids, with *T. theileri* Laveran, 1902, from North American cattle, and with *T. melophagium* Flu, 1908, from sheep in Germany. Qualitatively, trypanosomes from roe deer in Poland differed from all the above in that 90 (56%) of 162 specimens examined lacked a free flagellum extending beyond the body. Trypanosomes from roe deer in Poland differed also in most mensural values from trypanosomes from roe deer in Germany and from trypanosomes from North American cervids and cattle. Trypanosomes from roe deer in Poland most closely resemble *T. melophagium* from sheep in Germany, but conspecificity is not considered possible inasmuch as the latter species is held to be markedly host specific. A discussion of *Trypanosoma* (*Megatrypanum*) spp. from various hosts in Europe and North America is provided.

KEY WORDS: *Trypanosoma stefanskii* sp. n., (*Megatrypanum*), roe deer, *Capreolus capreolus,* Poland.

Ruminant stercorarian trypanosomes, subgenus *Megatrypanum,* were known, until recently, from only a limited number of host species worldwide. They included *Trypanosoma theileri* Laveran, 1902, from bovids (principally cattle, *Bos taurus* L., 1766); *T. melophagium* Flu, 1908, from sheep, *Ovis aries* L.; and *T. theodori* Hoare, 1931, from goats, *Capra hircus* L. Wrublewski (1909, 1912) reported *T. theileri* from European bison, *Bison bonasus* L., from Puszcza Bialowieska in eastern Poland although some controversy arose regarding this designation (Wladimiroff and Yakimoff, 1909; Yakimoff, 1915).

Reports of trypanosomes from cervids were also rare. Knuth (1909) reported on a species of "*Herpetomonas*" (surely an erroneous identification) observed in blood films from the heart of a roe deer, *Capreolus capreolus* Gray, 1821, from Westerwald district in Germany. *Trypanosoma evansi* Balbiani, 1888, a salivarian trypanosome, was reported from *Cervus unicolor* (Kerr, 1792) in Mauritius (Adams and Lionnet, 1933); in muntjak, *Muntiacus muntjak* Zimmermann, 1780, the axis deer, *Axis axis* Erxleben, 1777, and *Cervus timorensis* (Blainville, 1822) in Indonesia (Kraneveld and Mansjoer, 1952); and from roe deer in the Soviet Union (Kazakhstan) (Galuzo and Novinskaia, 1958). In the Western Hemisphere, stercorarian, subgenus *Megatrypanum,* trypanosomes, *T. mazamarum* Mazza, Romana, and Fiora, 1932, and *T. theileri*-like forms were recovered from *Mazama* spp. Rafinesque, 1817, and *Odocoileus virginianus* (Zimmermann, 1780) in Argentina and Brazil (Mazza et al., 1932; Deane, 1961) and Colombia (Ayala et al., 1973). In North America the first report of a *Trypanosoma* sp. in cervids (white-tailed deer, *Odocoileus virginianus*) was from blood cultured from deer from the southeastern United States (Kistner and Hanson, 1969). Subsequently, *Trypanosoma* sp. was reported from mule deer (*Odocoileus hemionus* (Rafinesque, 1817)) in Colorado and New Mexico (Clark, 1972) and Wyoming (Kingston et al., 1975); elk (*Cervus canadensis* (Erxleben, 1779)) in Wyoming (Kingston and Morton, 1973) and Colorado and New Mexico (Davies and Clark, 1974); reindeer (*Rangifer tarandus* L.) in Alaska (Kingston et al., 1982) and Finland (Kingston and Nikander, 1985); and moose (*Alces alces* L.) in Alaska and Wyoming (Kingston et al., 1981).

More recently, *Trypanosoma* (*Megatrypanum*) spp. were reported from red deer (*Cervus elaphus* L.), fallow deer (*Cervus dama* L.), and roe deer from Germany (Hoffman et al., 1984),

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Table 1. Morphological comparison of trypanosomes from roe deer, *Capreolus capreolus*, from Poland with trypanosomes from cervid species and cattle from North America and with trypanosomes from roe deer and sheep from Germany.

Host species	PK	KN	PN	NA
Roe deer (84) *Trypanosoma stefanskii* Poland $N = 50$	15.9 ± 6.39* 0–28†	5.8 ± 2.07 0–10	23.3 ± 5.59 13–33	32.0 ± 5.1 20–40
Roe deer (88) *Trypanosoma stefanskii* Poland $N = 40$	14.2 ± 5.66 5–27	5.8 ± 1.32 3–9	19.88 ± 6.3 9–33	28.2 ± 6.07 14–39
Roe deer (88) *Trypanosoma stefanskii* Poland $N = 72$	13.5 ± 5.32 2–24	6.3 ± 1.96 3–15	19.57 ± 4.94 11–30	27.8 ± 6.71 11–42
Composite roe deer *Trypanosoma stefanskii* Poland $N = 162$	14.39 ± 5.81 0–28	6.0 ± 1.86 0–15	20.8 ± 5.73 9–33	29.2 ± 6.36 11–42
Roe deer‡§ *Trypanosoma* sp. Germany $N = 86$	9.8 ± 5.7	5.8 ± 1.4	15.5 ± 5.7	18.1 ± 5.0
All deer‖ *Trypanosoma cervi* North America $N = 174$	11.5 ± 5.60 3–27	7.0 ± 2.10 2–14	18.5 ± 6.34 8–36	23.3 ± 7.30 10–43
Cattle¶ *Trypanosoma theileri* North America $N = 304$	7.4 ± 3.3 0–17	8.9 ± 2.6 2–20	16.2 ± 5.1 5–33	20.2 ± 6.3 7–36
Sheep**§ *Trypanosoma melophagium* Germany $N = 111$	14.7 ± 2.9	5.1 ± 1.1	19.8 ± 3.5	19.5 ± 1.9

* SD.
† Range.
‡ Hoffman et al., 1984 (roe deer).
§ Range not given.
‖ Includes elk, Kingston and Morton, 1975; mule deer, Matthews et al., 1977; white-tailed deer, Kingston and Crum, 1977; reindeer, Kingston et al., 1982; moose, Kingston et al., 1985.

and in roe deer (Kingston and Bobek, 1985), red deer, and elk in Poland (Kingston et al., 1985a). Hinaidy (1987) reported on the recovery of *Trypanosoma* (*Megatrypanum*) sp. from 2 of 105 roe deer in Austria. None of the European reports identified trypanosomes from deer as other than *Trypanosoma* (*Megatrypanum*) spp. Also, *Trypanosoma* (*Megatrypanum*) sp. was rediscovered in 4 of 38 wisent, i.e., European bison, *Bison bonasus*, in Poland from Puszcza Bialowieska (Kingston et al., 1987).

Kingston and Morton (1975) compared hemoflagellates from cattle and elk in North America and concluded that the form from elk was a new species, which they designated *Trypanosoma* (*Megatrypanum*) *cervi* Kingston and Morton, 1975. Subsequently, trypanosomes from mule deer (Matthews et al., 1977), white-tailed deer (Kingston and Crum, 1977), reindeer (Kingston et al., 1982), and moose (Kingston et al., 1985b) were considered as conspecific with *T. cervi* from North American elk. Cross-transmission experiments of *T. cervi* from elk to cattle (6 trials—intact and splenectomized recipients) failed to produce infections in the experimental, putative recipient hosts (Kingston and Morton, 1973). Blood containing *T. cervi* from an infected mule deer was transferred to an uninfected elk in which

Table 1. Continued.

BL	FF	L	W	FF:BL	KI	NI
55.1 ± 9.23 37–71	0	55.1 ± 9.23 34–71	5.66 ± 1.65 3–10	00	1:3.66 ± 1.44 0–8	0.73 ± 0.15 0.43–1.1
48 ± 11.5 26–70	0	48 ± 11.5 26–70	5.8 ± 1.94 2–11	00	1:3.46 ± 0.89 1.7–5.4	0.71 ± 0.15 0.41–0.93
47.3 ± 10.3 26–68	7.7 ± 2.73 4–17	55 ± 10.34 37–75	6.53 ± 2.5 2–13	1:6.9 ± 2.72 1:2–13.4	1:3.34 ± 1.09 1–6	0.73 ± 0.25 0.41–2.27
49.9 ± 10.81 26–71	7.7 ± 2.73 0–17	55.02 ± 10.34 26–75	6.1 ± 2.16 2–13	1:6.9 ± 2.72 1:2–13	1:3.47 ± 1.17 0–8	0.73 ± 0.20 0.41–2.27
33.6 ± 9.5	10.4 ± 2.5	44.0 ± 8.5	not given	1:2.93 ± 7.72	1:2.9 ± 0.98	0.87 ± 0.16
42.0 ± 12.44 21–74	8.2 ± 3.24 0–21	50.1 ± 13.64 26–83	5.5 ± 2.48 1–13	1:6.1 ± 3.24 1:0–27	1:2.7 ± 0.96 1:1.23–7	0.8 ± 0.22 0.42–2.67
36.4 ± 10.5 13–59	14.2 ± 4.5 1–37	50.5 ± 12.7 16–90	3.3 ± 2.02 1–13	1:2.8 ± 2.29 1:0.89–39	1:1.86 ± 0.42 1–4	0.88 ± 0.22 0.43–1.67
39.3‡‡	6.0 ± 1.6	45.3 ± 4.1	3.1‡‡	1:6.55‡‡	1:3.8‡‡	1.1‡‡

¶ Matthews et al., 1979; McKenzie, unpublished M.S. thesis, 1980.
** Buscher and Friedhoff, 1984.
‡‡ SD not given.
Underlined data calculated.

a cryptic infection (detected by culture only) resulted, persisting for approximately 30 days (Matthews et al., 1977). In a third experiment, *T. cervi* from an infected reindeer from Alaska were inoculated into 2 uninfected elk calves and an uninfected bovine calf but failed to result in infection (Kingston et al., 1982). Living, culture derived, cryopreserved trypanosomes from cattle (*T. theileri*) and North American bison (*Trypanosoma* sp.) were inoculated into homologous and heterologous hosts. A transient infection developed in 1 bison inoculated with *T. theileri*; no infections developed in the other recipients. The trypanosomes recovered from the experimentally infected bison were compared with *T. theileri* from Wyoming cattle (Matthews et al., 1979) and the forms from these hosts were considered conspecific (Kingston et al., 1986). Recent transmission studies in Germany using naturally infected species of tabanid flies harboring species of cattle and deer trypanosomes have also demonstrated that trypanosomatid species from cattle and deer are distinct (Bose et al., 1987).

Although trypanosomes from a relatively large number of cervid (and other) hosts from North and South America, Germany, Austria, and Poland have been described and some named, many

remain nameless pending further analysis. The description, comparison, and analysis of 162 trypanosomes of the new species from 12 of 18 roe deer collected in Puszcza Niepolomicka in south-central Poland in 1984 and 1988 form the basis for this paper. The species is named in honor of the late Prof. dr hab. Witold Stefanski, founder of the Institute of Parasitology-PAN, which bears his name.

Materials and Methods

Blood samples were collected in heparinized or plain tubes from the heart, pleural, or body cavities of hunter-killed male roe deer (*Capreolus capreolus*) during 1–10 August 1984 (8 deer) and 22–31 July 1988 (10 deer) in Puszcza Niepolomicka (south-central Poland). Direct examinations (DE) of samples using phase, or brightfield, microscopy were conducted within 2 hr following collection using the microhematocrit concentration technique of Bennett (1962). When trypanosomes were detected microscopically (10× objective) in tubes swimming in plasma, or serum, above the buffy coat, tubes were scored, broken, and trypanosomes, plasma or serum, white blood cells, and a few red blood cells, to serve as a marker, were expressed onto microscope slides using a stylet to push against the sealant (Crito seal®). Conventional thin blood films were prepared, air-dried, fixed in absolute methanol, and stained in Giemsa's stain, and later examined microscopically. Trypanosomes were photographed using a photo-equipped Reichert Zetopan compound microscope at 1,000× on color slide film (Orwochrome or Kodachrome 25). A stage micrometer was also photographed at the same magnification to allow for calibration of the measuring device (Curvometer, Alvin 1112). The processed slides were projected from a standard distance and the trypanosome images sketched onto tracing paper. The sketches were measured (Hoare, 1972; Kingston and Morton, 1975) for various morphological parameters: PK = posterior end to kinetoplast, KN = kinetoplast to nucleus, PN = posterior end to nucleus, NA = nucleus to anterior end, BL = body length, FF = free flagellum, L = length, W = width; and various indices calculated, FF:BL = free flagellum to body length ratio, NI = PN/NA, KI = PN/KN. Results were recorded in μm after calculating values from the projected image of the stage micrometer and were processed through a VAX8800 computer, using SPSS-X Data Analysis System 3.0 (Statistical Package for the Social Sciences, Version 8) for a one-way analysis of variance utilizing Duncan's multiple range test to compare the differences (confidence interval, $P \leq 0.05$) between group means of trypanosomes from North American deer and cattle, and roe deer from Poland.

Results

Mensural values of *T. stefanskii* from roe deer from Poland were compared to similar values of *T. cervi* from North American deer and *T. theileri* from cattle (Table 1).

Seven of 8 roe deer from Puszcza Niepolomicka sampled in early August 1984 were infected with trypanosomes (Kingston and Bobek, 1985). Trypanosomes from 4 of the deer were studied ($N = 50$) and all apparently lacked a free flagellum. Most specimens were trypomastigotes, but some broad, predivision forms with 2 kinetoplasts and sometimes with a short flagellum were seen. Further sampling in late July 1988 in the same locality resulted in recovery of trypanosomes ($N = 426$ trypanosomes on 71 slides) from 5 of 10 bucks examined. Seventy-two of 112 trypanosomes studied possessed a free flagellum 7.7 (4–17) μm in length (Fig. 1); the remaining 40 lacked a free flagellum (Fig. 2). Some specimens on a given slide lacked a free flagellum, whereas other specimens on the same slide possessed one. In both types of trypanosome, the undulating membrane was usually well developed. With the exception of this flagellar difference, a one-way analysis of variance (ANOVA) of the means of other mensural values revealed no significant differences ($P \leq 0.05$). Some differences ($P \geq 0.05$) were noted in PN, NA, and BL of trypanosomes from deer sampled in 1984 and those examined in 1988. It was not possible to compare FF:BL ratios where FF was lacking.

All 112 trypanosomes examined in 1988 were from 3 of the 5 infected deer. No differences were noted in PK, KN, FF (where present), W, NI, and KI ($P \leq 0.05$). Some variation ($P \geq 0.05$) was seen in specimens from 2 of the deer in PN, AN, BL, L, and the FF:BL ratio. These variations are essentially reflections of differences in body length.

Discussion

Trypanosoma cervi from North American deer, and *T. stefanskii* from roe deer in Poland, superficially appear to be similar. Nearly all mensural values (PK, KN, PN, NA, BL, W, KI, and FF:BL), however, were found to be significantly different. Total length values for *T. theileri* and *T. cervi* are similar ($P \leq 0.05$); however, this similarity is a reflection of the shorter FF of *T. cervi* (8 μm) as compared with *T. theileri* (14 μm). Free flagellar lengths of *T. cervi,* and of *T. stefanskii* (which possessed this organelle), did not differ significantly ($P \leq 0.05$), 8.2 and 7.7 μm, respectively. Nuclear index (NI) values for *T. theileri* and *T. cervi* did not differ significantly ($P \leq 0.05$), whereas this value for *T. stefanskii* from roe deer differed from both ($P \geq 0.05$) (Table 1).

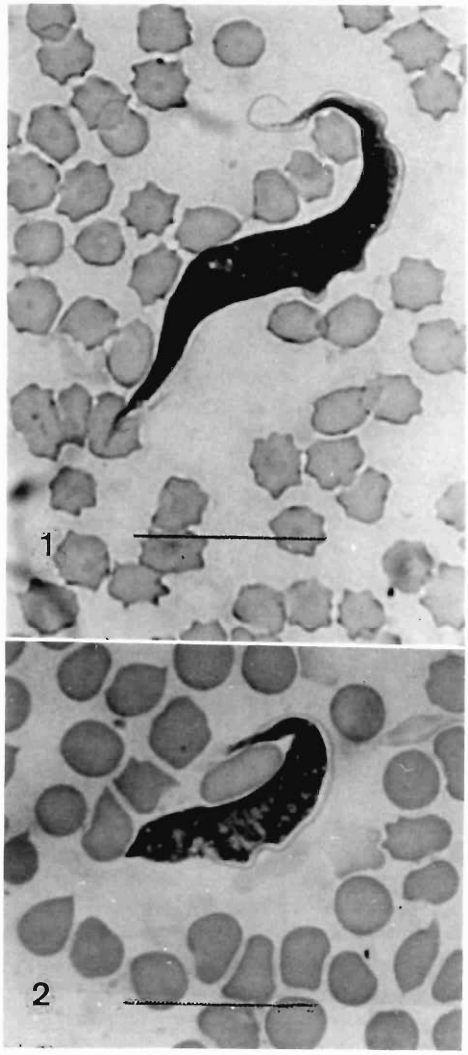
Except in mean NA, BL, and W values, *T.*

stefanskii from roe deer in Poland closely resembles *Trypanosoma melophagium* from sheep in Germany (Table 1) (Buscher and Friedhoff, 1984). This resemblance must be considered superficial if one adheres to the widely held principle of strict host specificity of most stercorarian trypanosomes (Hoare, 1972).

Trypanosomes from roe deer in Poland collected in 1984 and 1988 differed from trypanosomes reported from roe deer in Germany collected in 1983 (Hoffman et al., 1984) (Table 1). Qualitatively, 90 of 162 (55.6%) of the trypanosomes from roe deer in Poland lacked a free flagellum, whereas all 86 of the trypanosomes from Germany possessed this organelle (Hoffman et al., 1984). The basis for this difference is unknown. These aflagellate forms from Polish roe deer may represent the vector-infective, aflagellate, so-called "stumpy" forms seen in other species of trypanosomes (Hoare, 1972), but never noted by us in North American *Megatrypanum* species. Also, the mean FF (when present in the Polish material) values between these 2 groups of trypanosomes differed, those from Germany being approximately 25% longer (10.4 vs. 7.7 μm, respectively). Although unclear, this anomalous condition may have resulted from genetic drift producing forms with and without a free flagellum in the same parasite species. Other quantitative differences are obvious in PK, KN, NA, BL, and possibly in L, FF:BL, and KI values, all being smaller in the German material. The only numerical correspondence appears to occur between trypanosomes from roe deer from Poland which lack a free flagellum and those from trypanosomes from roe deer in Germany (all of which had a free flagellum) in KN values.

Based on morphologic data, there appear to be 2 or more distinct populations of *Megatrypanum* trypanosomes in Europe. In the West, trypanosomes appear homogeneous in the possession of a free flagellum and otherwise quantitatively different from forms found more easterly which appear homogeneous in all values except for the presence or absence of a free flagellum. Hoffman et al. (1984) implied that the trypanosome from roe deer was, perhaps, a separate species but felt cross-transmission experiments were needed to satisfy this question.

The trypanosomal material from roe deer in Poland is clearly morphologically distinct from trypanosomes from North American deer spp. (except for FF values), perhaps owing to host differences or their long-term temporal and wide geographic separation. In addition, these trypanosomes differ from trypanosomes in Germany. Thus, the trypanosomes from roe deer in Poland warrant specific recognition and are designated *Trypanosoma stefanskii* sp. n.



Figures 1, 2. ***Trypanosoma (Megatrypanum) stefanskii* sp. n. 1. Bloodstream trypomastigote with free flagellum, roe deer. 2. Bloodstream trypomastigote lacking free flagellum, roe deer. Scale bar = 20 μm.**

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1991 Honor Award

Helminthological Society of Washington member Isi A. Siddiqui, Assistant Director of Food and Agriculture, received the 1991 Honor Award from NASDA. His accomplishments, for which the award was given, were in pest prevention in California, particularly Mexican and Mediterranean fruit fly eradication programs.

Dr. Isi A. Siddiqui (right) receiving the 1991 Honor Award from the National Association of State Departments of Agriculture.

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The Genus *Bitylenchus* Filip'ev, 1934 (Nematoda: Tylenchida) with Descriptions of Two New Species from Spain

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ABSTRACT: The taxonomic position of the genus *Bitylenchus* Filip'ev, 1934, is discussed and its diagnosis emended. Two new species, *B. serranus* sp. n. and *B. pratensis* sp. n., from southeastern Spain are described and illustrated using light and scanning electron microscopy (SEM). *Bitylenchus serranus* sp. n. is characterized by a hemispherical lip region with 6–8 annuli, stylet 21 μm long, lateral field with regularly areolated outer bands, a short postanal intestinal sac extending up to one-fourth of the tail length, and a broadly conoid tail usually ventrally curved (about 90% of specimens studied). *Bitylenchus pratensis* sp. n. is characterized by a subcylindrical female tail regularly tapering to a rounded and striated terminus and a basal esophageal bulb as long as or longer than the isthmus. Several populations of *B. maximus* (Allen, 1955) Siddiqi, 1986, from Scotland, Spain, Trinidad, and U.S.A. are described and compared. The Spanish population is characterized by the presence of males and inseminated females; SEM observations are provided. A population of *B. huesingi* (Paetzold, 1958) Siddiqi, 1986, is described. A table with the morphometric data of each species is included.

KEY WORDS: *Bitylenchus huesingi, Bitylenchus maximus, Bitylenchus pratensis* sp. n., *Bitylenchus serranus* sp. n., plant-parasitic nematodes, SEM observations, Spain, taxonomy.

During a nematode survey on several plant communities in southeastern Spain, 2 new and 2 known species of the genus *Bitylenchus* Filip'ev, 1934 (Siddiqi, 1986), were collected. Females and males of the 4 species are described herein as *B. serranus* sp. n., *B. pratensis* sp. n., *B. maximus* (Allen, 1955) Siddiqi, 1986, and *B. huesingi* (Paetzold, 1958) Siddiqi, 1986. Morphology of 3 female populations of *B. maximus* from Trinidad, U.S.A., and Scotland is reported. Morphometry and morphology using SEM observations are also given.

Materials and Methods

Nematodes were killed by gentle heat and fixed in a 4% solution of formaldehyde, then dehydrated and processed to glycerine according to Seinhorst's method. Body length and curved structures were measured with the aid of a precision curvimeter; straight structures, such as maximum body width, stylet, anal body width, etc., were measured using a micrometer-scale in the eyepiece of a high-power (×1,250) microscope. All measurements are in micrometers unless otherwise stated.

For examination under the scanning electron microscope (SEM), specimens were processed with Spurr's low viscosity epoxy resin, coated with gold (De Grisse, 1973), and examined with a Zeiss DSM 950 scanning electron microscope at accelerating voltages of 10 and 15 kV.

Description

Bitylenchus serranus sp. n. (Table 1, Figs. 1–14)

HOLOTYPE (female in glycerine): L = 883 μm; a = 32.7; b = 6.1; b_1 = 10.3; V = 53; c = 17.3; c′ = 2.4; stylet = 22 μm; m = 53; S = 1.5; O = 9; tail annuli = 40.

PARATYPE FEMALES (N = 36): Morphometrics are given in Table 1. Body moderately thin, slightly curved ventrally upon relaxation. Body annuli opposite mid-esophagus 1–2 and opposite mid-body 1 wide. Lateral field with 4 lines, (8–11) wide at mid-body, and outer bands areolated. Lip region hemispherical, anteriorly flattened, set off by a slight constriction from the body; 4 high and 7–9 wide, bearing 6–8 annuli. Labial plate fused with labial sectors (Figs. 10, 11). Longitudinal indentations on lip annuli behind amphids present (Figs. 10, 11). Cephalic framework lightly sclerotized. Stylet 2.5–2.7 times longer than lip region width, conus 11–12 long. Basal knobs rounded, anterior surface posteriorly sloping, flattened, or slightly indented, 4–5 across. Dorsal esophageal gland orifice 2 from stylet base. Procorpus slender, 1.1–1.6 times longer than isthmus. Median esophageal bulb round-oval, strongly muscular, 13–17 long × 10–14 wide,

Table 1. Morphometric data for *Bitylenchus serranus* sp. n. female and male paratypes (measurements in μm).

	Females (N = 36)		Males (N = 18)	
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range
L	824.0 ± 47.3	734.0–922.0	756.0 ± 61.4	650.0–906.0
a	30.0 ± 1.5	26.0–33.0	32.0 ± 2.1	28.0–36.0
b	6.0 ± 0.5	5.0–7.0	5.0 ± 0.4	5.0–6.0
b_1	10.0 ± 0.8	8.0–12.0	10.0 ± 0.5	8.0–10.0
V%	52.0 ± 1.5	49.0–54.0	—	—
T%	—	—	48.0 ± 5.7	39.0–58.0
G_1	25.0 ± 3.4	18.0–32.0	—	—
G_2	27.0 ± 3.5	19.0–34.0	—	—
c	17.0 ± 1.5	14.0–20.0	15.0 ± 1.4	13.0–19.0
c′	2.6 ± 0.2	2.0–3.0	2.7 ± 0.2	2.5–3.0
Stylet length	21.0 ± 0.6	19.0–22.0	20.0 ± 0.6	19.0–21.0
m	52.0 ± 0.9	50.0–55.0	52.0 ± 1.2	50.0–53.0
o	10.0 ± 1.1	9.0–13.0	11.0 ± 1.1	10.0–13.0
S	1.4 ± 0.3	1.0–2.0	1.5 ± 4.2	1.4–1.5
MB	51.0 ± 1.6	49.0–55.0	52.0 ± 1.7	49.0–55.0
Nerve ring	96.0 ± 7.5	79.0–113.0	91.0 ± 7.2	79.0–104.0
Excretory pore	121.0 ± 9.2	97.0–137.0	113.0 ± 8.0	101.0–128.0
Esophagus length	145.0 ± 10.9	116.0–161.0	137.0 ± 9.8	124.0–156.0
Maximum body width	28.0 ± 1.4	25.0–31.0	24.0 ± 1.9	19.0–27.0
Anal body width	19.0 ± 1.5	16.0–22.0	19.0 ± 1.0	17.0–20.0
Tail length	50.0 ± 5.4	40.0–62.0	52.0 ± 4.1	45.0–57.0
Tail annuli	38.0 ± 4.3	31.0–46.0	—	—
Spicule length	—	—	28.2 ± 1.4	26.0–31.0
Gubernaculum length	—	—	14.2 ± 0.7	13.0–16.0

with refractive cuticular valve 4 long. Basal bulb pyriform, 21–29 long, 12–15 wide. Excretory pore located opposite anterior half of basal bulb level. Hemizonid occupying 3–4 annuli, located 2–4 annuli anterior to excretory pore. Esophago–intestinal junction with 2 cardial cells. Fasciculi (lateral canal, serpentine canal) present, occasionally obscure.

Vulva transverse, flush with body surface or slightly raised; body cuticle just anterior to vulva slightly thickened; body just behind vulva slightly constricted. Vagina straight, 10–12 long. Spermatheca round, 12–18 wide, filled with round sperm, 2–3 wide. Egg in uterus oval, 18–19 × 60–61. Ovaries outstretched, posterior usually longer than anterior, with a single row of oocytes. Tail subcylindroid, with rounded terminus, and usually ventrally curved (about 90% of specimens studied), rarely straight. Terminus coarsely annulated, terminal cuticle 5–9 thick. Short postanal intestinal sac 10–19 long, extending up to one-fourth of tail length. Phasmids porelike, located anterior to middle of tail, and at 7–17 annuli posterior to anus level, seldom opposite.

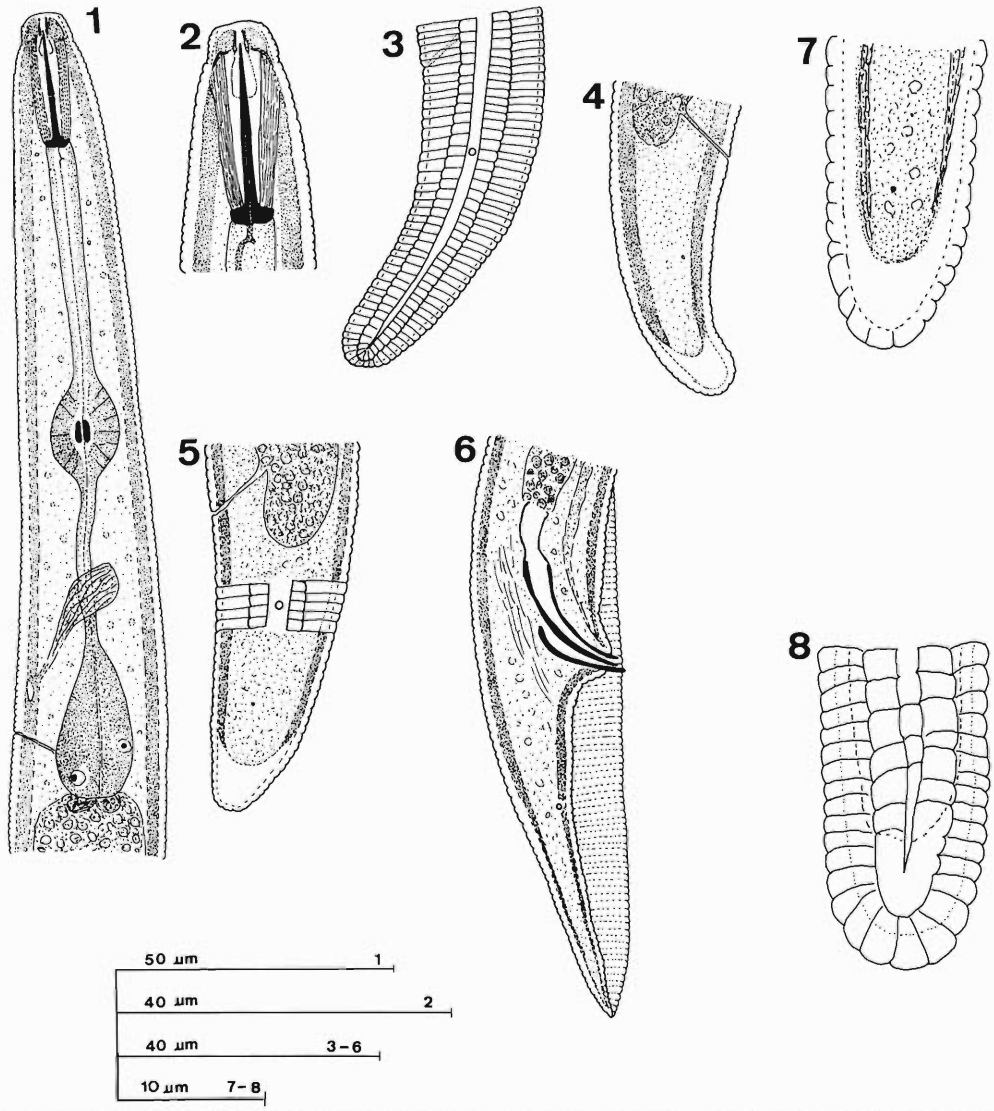
ALLOTYPE (male in glycerine): L = 805 μm; a = 31.0; b = 5.8; T = 52; c = 14.6; c′ = 2.7; stylet = 19 μm; m = 52; S = 1.5; O = 10; spicules = 30 μm; gubernaculum = 14 μm.

PARATYPE MALES (N = 18): Morphometrics are given in Table 1. Similar to female in measurements and morphology, except for the sexual dimorphism of tail region. Testis well developed, with 2 rows of spermagonia, 301–499 long. Spicules ventrally curved, with pointed tip. Gubernaculum bent, not protruding. Tail conoid, enveloped by a crenate bursa 80–103 long. Phasmid located 16–26 from anus.

TYPE HABITAT AND LOCALITY: Specimens collected around the roots of pinus (*Pinus nigra salzmanni*) at Nava del Espino, Sierra de Cazorla, Jaén, Spain.

TYPE SPECIMENS: Holotype female, allotype male, 29 female and 15 male paratypes (slides BTC 1–BTC 28) at nematode collection of Centro de Investigación y Desarrollo Agrario, Granada, Spain; 6 female and 2 male paratypes (slides BTC 24–BTC 28) at CAB International Institute of Parasitology (CIP), St. Albans, Herts., England.

DIAGNOSIS AND RELATIONSHIPS: *Bitylenchus serranus* sp. n. is characterized by a hemispherical lip region with 6–8 annuli, stylet 19–23 long,



Figures 1–8. ***Bitylenchus serranus*** **sp. n. 1. Female anterior region. 2. Lip region. 3–5. Female tails. 6. Male tail. 7, 8. Tail termini of females.**

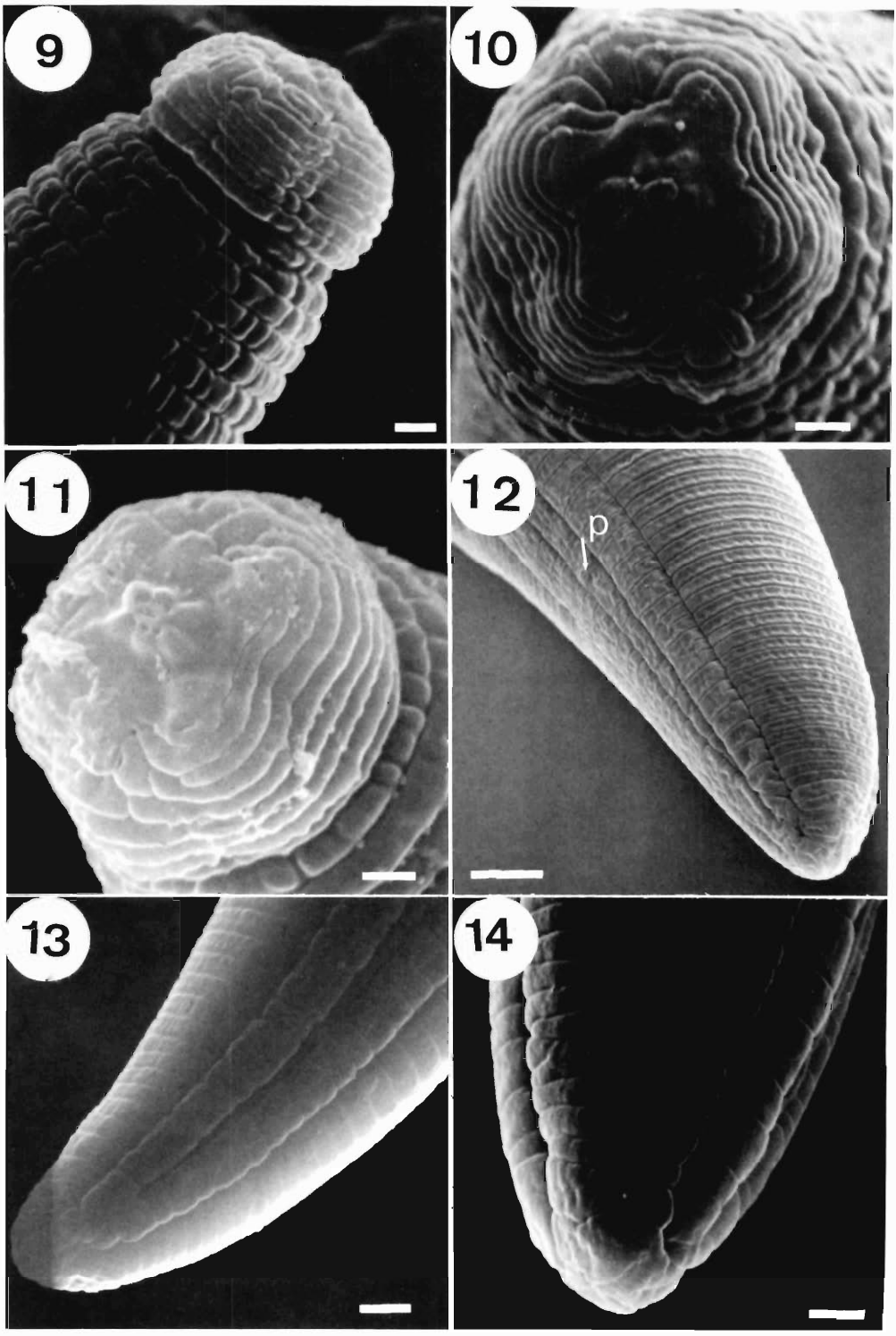
lateral field with regularly areolated outer bands, a short postanal intestinal sac extending up to one-fourth of tail length, and tail subcylindroid, usually ventrally curved, rarely straight.

Bitylenchus serranus is most similar to *B. canalis* and *B. wilskii*. It differs from *B. canalis* in having the excretory pore located opposite the anterior half of the basal bulb vs. opposite the esophageal base; a short postanal intestinal sac extending up to one-fourth of the tail length vs. long and completely filling tail cavity, and female tail with fewer than 50 annuli and terminus being coarsely annulated. It differs from *B. wilskii* in finer body annuli: 1 vs. 2–3 wide, in stylet length: 19–23 vs. 24–27, larger stylet knobs, and female tail terminus being coarsely annulated.

The SEM face view is quite similar to that of *B. tobari* as it is shown by Sauer (1985).

Bitylenchus pratensis sp. n. (Table 2, Figs. 15–28)

HOLOTYPE (female in glycerine): L = 1,070 µm; a = 34.5; b = 6.4; b_1 = 10.2; V = 53; c =



Figures 9–14. SEM micrographs of *Bitylenchus serranus* sp. n. 9. Lip region. 10, 11. Face views. 12, 13. Tail region. 14. Tail terminus. Scale bars: 9–11 = 1 μm; 12, 13 = 5 μm; 14 = 2 μm. p, phasmid.

Table 2. Morphometric data for *Bitylenchus pratensis* sp. n. female and male paratypes (measurements in μm).

	Females (N = 18)		Males (N = 6)	
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range
L	1,106.0 ± 127.8	828.0–1,296.0	1,033.0 ± 35.3	979.0–1,078.0
a	36.9 ± 3.9	29.9–46.3	41.8 ± 3.1	37.3–45.2
b	7.5 ± 0.8	5.5–9.0	6.9 ± 0.4	6.5–7.4
b_1	11.7 ± 1.0	10.0–13.8	11.5 ± 0.5	11.1–12.1
V%	52.0 ± 1.5	50.0–53.0	—	—
T%	—	—	43.0 ± 17	24.0–64.0
G_1	28.0 ± 2.9	22.0–31.0	—	—
G_2	26.0 ± 4.0	18.0–30.0	—	—
c	18.3 ± 1.9	14.8–22.7	19.0 ± 1.3	17.5–21.2
c′	2.8 ± 0.2	2.6–3.4	3.0 ± 0.1	2.9–3.1
Stylet length	20.5 ± 0.6	20.0–22.0	20.3 ± 0.4	20.0–21.0
m	55.0 ± 2.2	53.0–60.0	54.0 ± 1.7	53.0–56.0
o	10.7 ± 1.4	9.5–12.2	12.2 ± 4.6	9.5–17.5
S	1.5 ± 0.1	1.4–1.5	1.5 ± 0.1	1.4–1.6
MB	55.0 ± 2.1	52.0–59.0	54.0 ± 2.3	51.0–55.0
Nerve ringe	101.0 ± 9.8	83.0–115.0	102.0 ± 7.8	92.0–107.0
Excretory pore	129.0 ± 12.7	108.0–149.0	127.0 ± 4.0	123.0–131.0
Esophagus length	148.0 ± 12.4	124.0–168.0	150.0 ± 9.9	137.0–162.0
Maximum body width	30.0 ± 4.3	24.0–37.0	25.0 ± 1.7	23.0–27.0
Anal body width	21.6 ± 2.7	17.0–26.0	18.2 ± 1.0	17.0–19.0
Tail length	61.0 ± 6.1	48.0–70.0	54.0 ± 3.1	49.0–57.0
Tail annuli	28.0 ± 2.7	24.0–33.0	—	—
Spicule length	—	—	34.0 ± 0.8	33.0–35.0
Gubernaculum length	—	—	15.3 ± 1.0	14.0–16.0

16.7; c′ = 2.7; stylet = 21 μm; m = 53; S = 1.4; O = 9.5; tail annuli = 28.

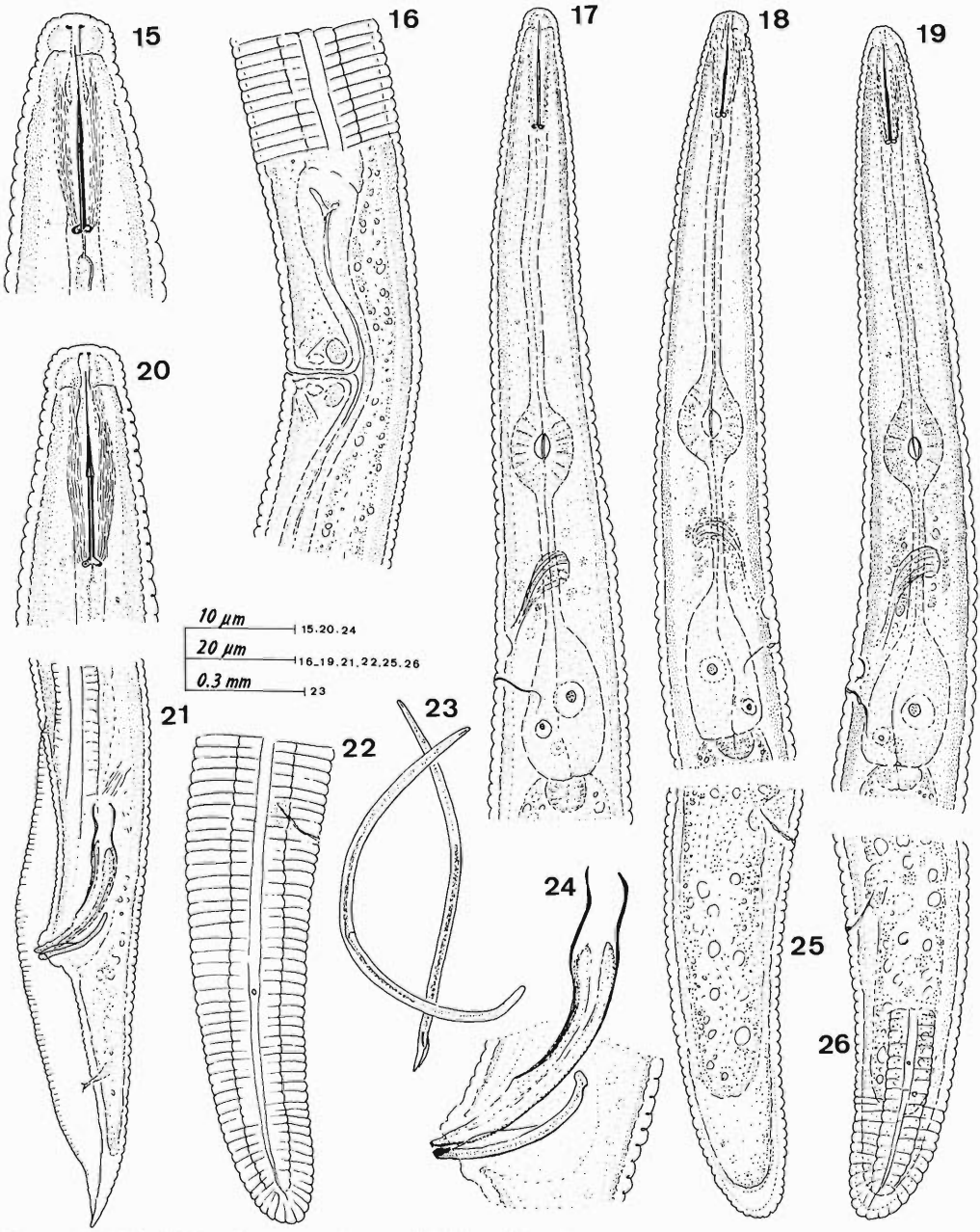
PARATYPE FEMALES (N = 18): Morphometrics are given in Table 2. Body ventrally arcuate to an open C-shaped; annuli prominent, 1.5–2.0 wide near mid-body. Lateral field 9–12 wide at mid-body; with 4 incisures, outer ones crenate; outer bands regularly areolated. Lip region conoid-rounded, clearly set off, 7–8 wide × 4–5 high, with 5–6 distinct annuli and an inconspicuous labial disc. SEM face view shows a squarish labial disc and that the anteriormost cephalic annulus is divided into 6 sectors (Fig. 27), and 6 longitudinal grooves on other lip annuli. Cephalic framework lightly sclerotized. Stylet 1.4–1.5 times as long as lip region width, with small rounded posteriorly sloping knobs 3–3.5 across. Dorsal esophageal gland orifice 2–3 from stylet base. Median esophageal bulb oval 14–19 long, with refractive valve 5–6 long. Basal bulb saccate to pyriform, 19–31 in length and as long as or longer than isthmus. Esophago-intestinal valve well developed, rounded, 5–7 long, positioned slightly into base of esophagus. Excretory pore just behind hemizonid or 1–3 annuli posterior to it, usually opposite anterior half of basal bulb.

Vulva transverse; vagina straight, 13–17 long. Ovaries outstretched, anterior usually longer than posterior, with a single row of oocytes, except 2 in multiplication zone. Spermatheca spherical, 16–18 wide, filled with rounded sperm 2.5–3 wide. Egg in uterus oval, 31 wide × 93 long. Intestine with fasciculi and refractive globules. Postanal extension of intestine extending ⅔ to ¾ of tail length. Tail subcylindrical, regularly tapering to a small rounded, striated terminus. Terminal hyaline portion 5–8 long. Phasmids large, 9–13 annuli posterior to level of anus.

ALLOTYPE (male in glycerine): L = 1,039 μm; a = 40.0; b = 7.2; T = 37; c = 21.2; c′ = 2.9; stylet = 20 μm; m = 53; S = 1.4; O = 17.5; spicules = 34 μm; gubernaculum = 16 μm.

PARATYPE MALES (N = 6): Morphometrics are given in Table 2. Similar to female in most details. Body usually straight to *slightly ventrally* curved. Testis outstretched. Bursa crenate, enveloping tail, 89–108 long. Spicules arcuate, pointed; gubernaculum arcuate and not protrusible, with a distinct velum. Tail conoid, enveloped by a large bursa. Phasmid located 24–27 posterior to anus.

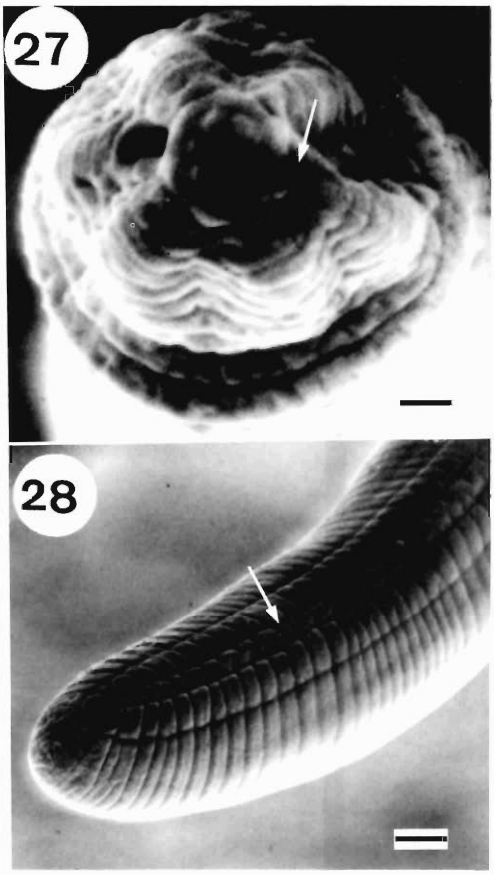
TYPE HABITAT AND LOCALITY: Specimens collected from soil in a grassy field at Sierra Morena, Andujar, Jaén, Spain.



Figures 15–26. ***Bitylenchus pratensis*** **sp. n. 15. Female head region. 16. Vulva region and lateral field. 17–19. Female esophageal regions. 20. Male head region. 21. Male tail terminus. 22, 25, 26. Female tails. 23. Female and male in death posture. 24. Spicule and gubernaculum.**

TYPE SPECIMENS: Holotype female, allotype male, 7 female and 2 male paratypes (slides BTA 10–BTA 14) at nematode collection of Centro de Investigación y Desarrollo Agrario, Granada, Spain; 10 female and 4 male paratypes (slides BTA 16–BTA 21) at CAB International Institute of Parasitology (CIP), St. Albans, Herts., England.

DIAGNOSIS AND RELATIONSHIPS: *Bitylenchus pratensis* sp. n. is characterized by a conoid



Figures 27, 28. SEM micrographs of *Bitylenchus pratensis* sp. n. 27. Face view. 28. Tail region. Scale bars: 27 = 1 μm; 28 = 5 μm.

rounded lip region, with 5–6 distinct annuli; stylet 20–21 long; lateral field with regularly areolated outer bands; and a subcylindrical tail, regularly tapering to a small, rounded, striated terminus.

Bitylenchus pratensis sp. n. most closely resembles *B. maximus* and *B. serranus*. It differs from *B. maximus* in body posture (ventrally arcuate to an open C-shaped vs. C-shaped to loose spiral); female tail shape (subcylindrical, regularly tapering to a small rounded terminus vs. cylindrical with broadly rounded terminus with fewer annuli in terminal hyaline region); basal esophageal bulb (as long as or longer than isthmus vs. clearly shorter than isthmus, see Figs. 29–35). From *B. serranus* it differs in lip region shape (conoid-rounded vs. hemispherical); smaller stylet knobs; tail shape (subcylindrical, straight vs. broadly conoid, usually ventrally curved); labial disc in SEM observations (squarish labial disc and anterior cephalic annulus divided into 6 sectors vs. labial disc fused with labial sectors; Figs. 15 and 10, 11, respectively), and spicule length (26–31 vs. 33–35).

Bitylenchus maximus (Allen, 1955) Siddiqi, 1986 (Table 3, Figs. 29–41)

FEMALE: Body C-shaped to loose spiral. Lateral field 9–13 wide at mid-body, with 4 lines, outer bands regularly areolated along body and inner band irregularly areolated at tail region (Fig. 41). Lip region hemispherical with flattened anterior end; 7–8 wide × 4–5 high. There are some differences in the lip region between Spanish and other populations. Spanish specimens (bisexual population) lips set off from body and have 7–9 annuli between anterior end of body to end of outer extension of cephalic framework; whereas U.S.A. and Scotland specimens are slightly set off and have 10–11 annuli. Labial disc and anterior cephalic annulus divided into 6 sectors (Figs. 37, 38). Longitudinal grooves on lip annuli behind amphids (Fig. 37). Stylet attenuated, with posteriorly inclined basal knobs. Procorpus cylindrical, 43–65 long. Median esophageal bulb oval, 16–19 long, strongly muscular with refractive valve 5.5–6 in length. Excretory pore located opposite the begining of basal bulb. Hemizonid 2–3 annuli long, located 2–3 annuli anterior to excretory pore.

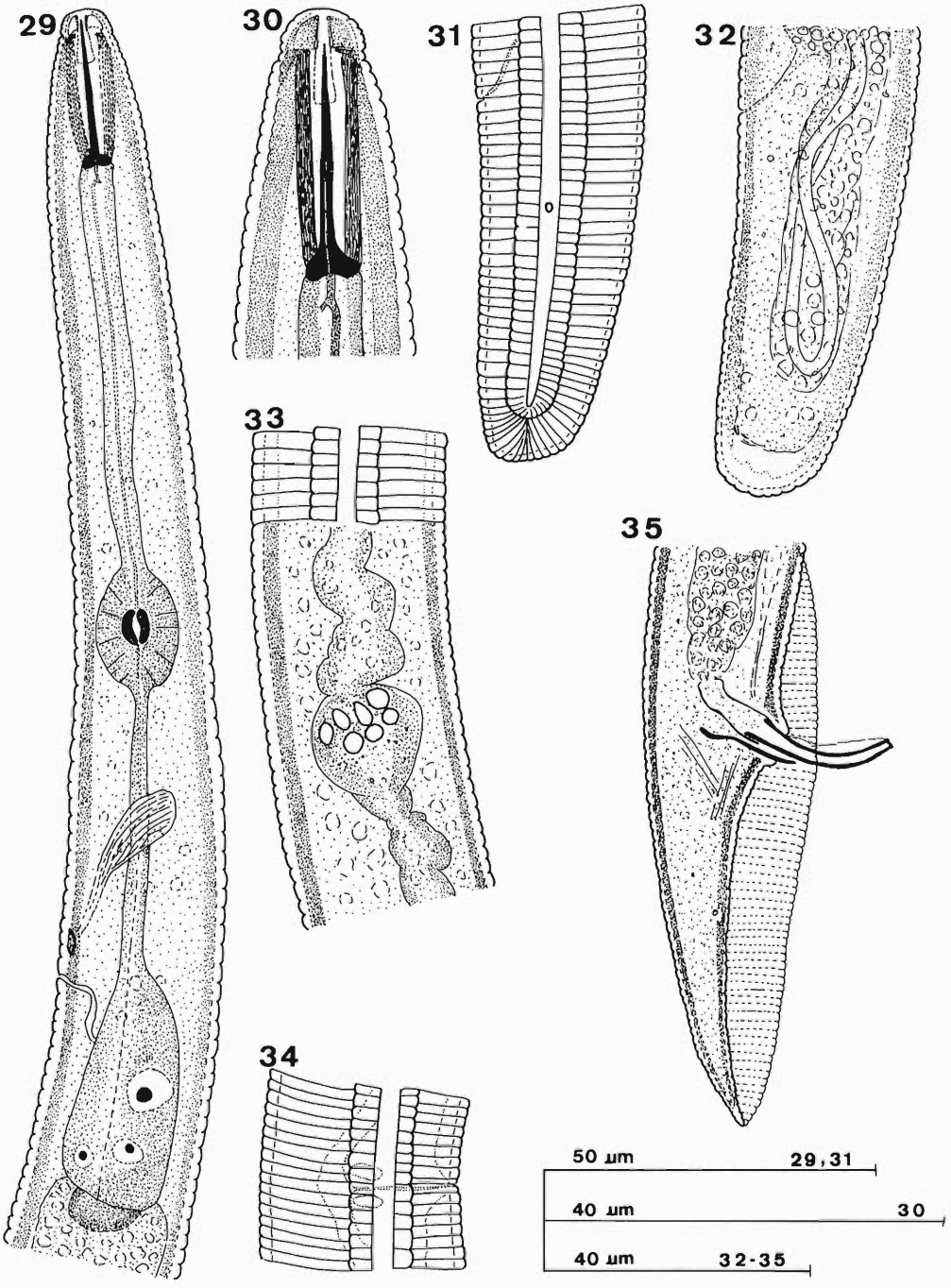
Ovaries outstretched, equally developed. Spermatheca round 13–18 wide, filled with rounded sperm (2–2.5 wide) in Spanish population only. Fasciculi well developed. Postanal intestinal sac long. Phasmid located 11 (9–14) annuli posterior to anus. Tail cylindrical with length different between Spanish and the other populations (see c′ values in Table 3). Anus a rounded pore (Fig. 39). Terminal hyaline region 6–8 long.

MALE: Relatively common in Spanish population but absent in the populations from U.S.A., Trinidad, and Scotland. Similar to female in general morphology and measurements except in a slight difference in size of the lip region (lower in males), 6.5–7 wide × 3.5–4 high. Gubernaculum with a slight proximal curvature. Bursa crenate, surrounds tail completely, 86–108 long. Phasmid 23–30 from anus.

HABITAT AND LOCALITIES: For Spanish population same as for *B. serranus* sp. n. Other localities are from trees by river bank in Minne-

Table 3. Morphometric data for different populations of *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986.

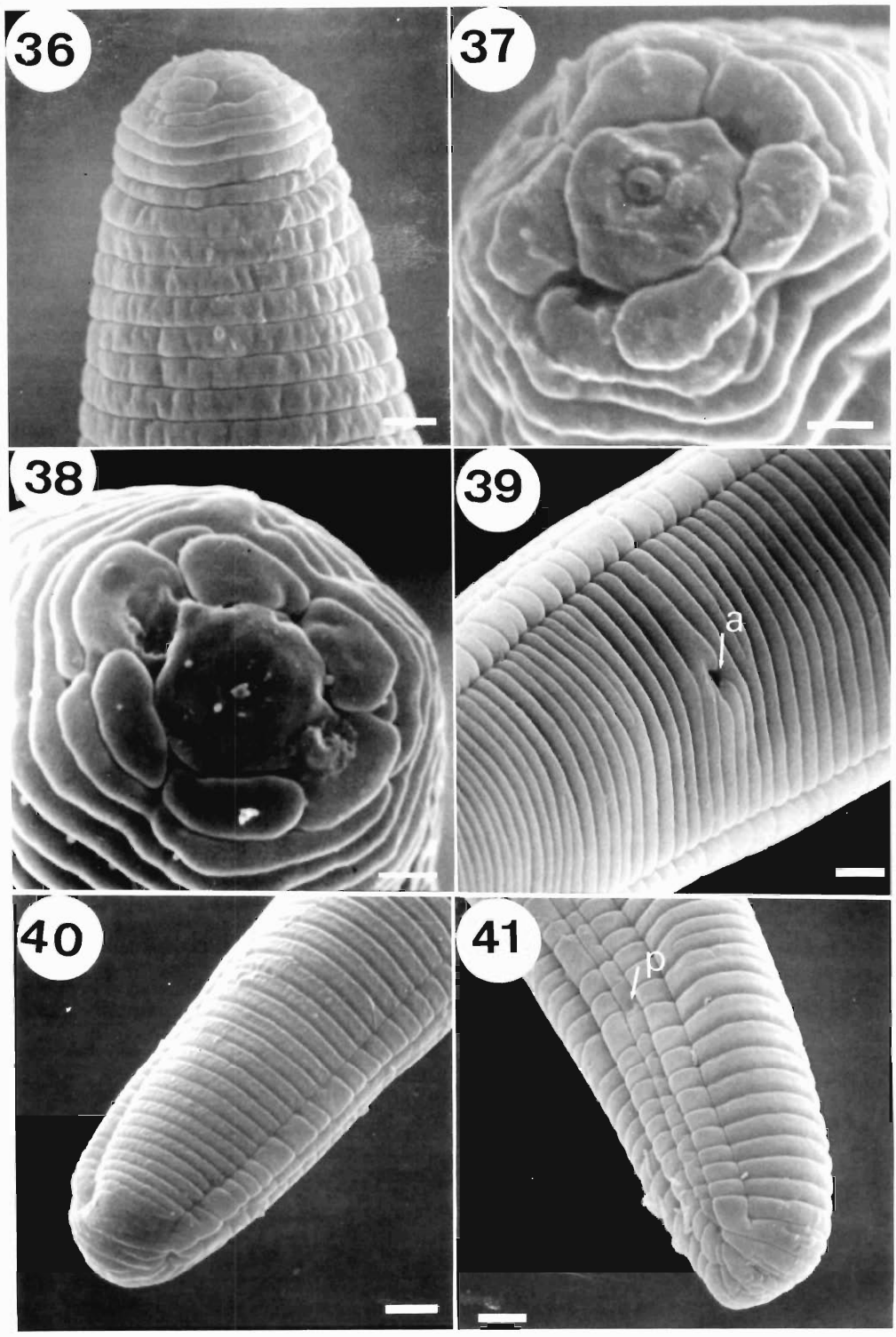
	Spain				U.S.A		Scotland		Trinidad
	Females (N = 31)		Males (N = 12)		Females (N = 4)		Females (N = 5)		Females (N = 2)
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	Range
L	1,278.0 ± 162.0	1,031.0–1,617.0	935.0 ± 67.8	797.0–1,023.0	1,125.0 ± 83.0	1,019.0–1,212.0	1,236.0 ± 48.0	1,191.0–1,294.0	1,172.0–1,328.0
a	42.6 ± 4.3	33.9–51.2	36.2 ± 2.5	31.9–40.0	45.5 ± 3.4	43.1–50.5	42.5 ± 2.2	40.1–44.9	42.8–43.4
b	7.6 ± 0.7	6.4–9.2	5.9 ± 0.6	4.9–7.0	6.9 ± 0.3	6.6–7.3	7.5 ± 0.3	7.1–8.0	6.5–7.5
b_1	12.6 ± 1.3	10.0–15.5	9.9 ± 1.0	8.6–11.7	11.8 ± 0.8	11.2–12.9	12.7 ± 0.4	12.2–13.3	10.2–11.8
V%	52.0 ± 1.6	50.0–56.0	–	–	51.5 ± 2.4	50.0–55.0	48.4 ± 2.5	44.0–50.0	50.0
T%	–	–	50.0 ± 6.5	38.0–61.0	–	–	–	–	–
G_1	22.0 ± 3.9	14.0–27.0	–	–	19.0 ± 3.0	16.0–23.0	20.0 ± 1.4	19.0–21.0	–
G_2	21.0 ± 3.7	12.0–26.0	–	–	19.0 ± 3.9	15.0–24.0	19.0	–	–
c	22.3 ± 2.1	18.5–27.3	17.2 ± 2.0	13.7–19.6	18.0 ± 0.9	16.7–18.8	18.0 ± 1.3	16.2–19.4	17.5–18.0
c′	2.5 ± 0.3	2.0–3.1	2.7 ± 0.3	2.5–3.3	3.6 ± 0.4	3.3–4.1	3.3 ± 0.3	3.1–3.8	3.0–3.4
Stylet length	22.0 ± 1.0	20.0–23.0	21.3 ± 1.0	20.0–23.0	21.0	–	23.0 ± 1.5	21.0–24.5	21.0–22.5
m	53.0 ± 2.0	50.0–57.0	55.0 ± 1.7	52.0–57.0	56.7 ± 1.5	56.0–59.0	57.0 ± 1.7	56.0–59.0	56.0
o	9.0 ± 1.3	8.0–13.0	11.0 ± 1.6	9.0–13.0	9.5	–	10.0	–	2.0–2.5
S	1.4 ± 0.1	1.1–1.6	1.5 ± 0.05	1.4–1.6	1.4 ± 0.1	1.3–1.6	1.4	–	1.4–1.5
MB	54.0 ± 2.0	51.0–58.0	54.0 ± 1.9	51.0–57.0	52.3 ± 0.5	52.0–53.0	54.0 ± 1.7	51.0–55.0	57.0–58.0
Nerve ring	116.0 ± 9.8	98.0–136.0	107.0 ± 9.3	89.0–120.0	106.0 ± 3.8	101.0–109.0	107.0 ± 6.7	97.0–115.0	120.0–127.0
Excretory pore	146.0 ± 14.1	116.0–172.0	125.0 ± 9.0	105.0–137.0	128.0 ± 5.1	123.0–134.0	131.0 ± 9.7	119.0–140.0	142.0–146.0
Esophagus length	169.0 ± 14.4	141.0–192.0	159.0 ± 13.6	141.0–176.0	164.0 ± 6.4	155.0–169.0	165.0 ± 12.5	150.0–181.0	177.0–180.0
Maximum body width	30.0 ± 3.0	24.0–35.0	26.0 ± 1.5	23.0–29.0	25.0 ± 1.7	23.0–27.0	29.0 ± 0.5	28.5–30.0	27.0–31.0
Anal body width	23.0 ± 3.1	18.0–28.0	20.0 ± 1.1	18.0–22.0	17.0 ± 1.2	16.0–18.5	21.0 ± 1.1	19.0–22.0	19.0–25.0
Tail length	58.0 ± 6.0	48.0–73.0	54.0 ± 3.4	47.0–60.0	63.0 ± 4.3	57.0–66.0	69.0 ± 6.6	62.0–80.0	65.0–76.0
Tail annuli	33.0 ± 2.4	28.0–37.0	–	–	36.0 ± 2.1	34.0–39.0	40.5 ± 0.7	40.0–41.0	36.0–37.0
Spicules	–	–	32.0 ± 1.3	30.0–34.0	–	–	–	–	–
Gubernaculum	–	–	16.0 ± 1.2	14.0–18.0	–	–	–	–	–



Figures 29–35. ***Bitylenchus maximus*** **(Allen, 1955) Siddiqi, 1986, from Spain. 29. Female anterior region. 30. Lip region. 31, 32. Female tails. 33. Spermatheca. 34. Mid-body region. 35. Male tail.**

apolis (U.S.A.), coconut soil from Trinidad, and grass from Nottinghamshire, England.

REMARKS: This interesting species has a worldwide distribution being recorded from U.S.A. (Allen, 1955; Thorne and Malek, 1968), Canada (Anderson, 1977), Pakistan (Maqbool and Shahina, 1987), Spain (S'Jacob et al., 1959; Zancada and Bello, 1981), Germany (Sturhan,



Figures 36–41. SEM micrographs of *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986, from Spain. 36. Lip region. 37, 38. Face view. 39. Ventral view of anus. 40, 41. Tail termini. Scale bars: 36 = 2 μm; 37, 38 = 1 μm; 39 = 2.5 μm; 40, 41 = 5 μm. a, anus; p, phasmid.

Table 4. Morphometric data for *Bitylenchus huesingi* (Paetzold, 1958) Siddiqi, 1986 (measurements in μm).

	Females (N = 25)		Males (N = 3)	
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range
L	674.0 ± 62.4	581.0–847.0	700.0 ± 81.6	609.0–766.0
a	28.9 ± 2.3	25.2–35.3	32.0 ± 5.1	26.4–36.3
b	5.7 ± 0.4	5.0–6.5	5.5 ± 0.8	4.6–6.2
b_1	10.2 ± 0.7	9.0–11.0	9.7 ± 1.7	8.0–11.3
V%	54.0 ± 1.5	51.0–57.0	–	–
T%	–	–	59.0 ± 4.0	55.0–63.0
G_1	29.0 ± 5.1	20.0–35.0	–	–
G_2	28.0 ± 3.5	22.0–34.0	–	–
c	13.7 ± 1.2	12.0–16.1	18.0 ± 1.4	16.9–19.6
c′	2.7 ± 0.3	2.4–3.3	2.2 ± 0.2	2.0–2.4
Stylet length	18.0 ± 1.0	16.0–20.0	17.7 ± 0.6	17.0–18.0
m	54.0 ± 2.7	50.0–59.0	56.0	–
o	13.0 ± 2.8	10.0–20.0	12.0 ± 0.7	11.0–12.0
S	1.3 ± 0.1	1.1–1.5	1.5	–
MB	51.0 ± 1.2	49.0–53.0	52.0 ± 1.7	50.0–53.0
Nerve ring	77.0 ± 7.5	67.0–90.0	85.0 ± 3.6	82.0–89.0
Excretory pore	94.0 ± 10.5	76.0–114.0	104.0 ± 6.7	96.0–108.0
Esophagus length	118.0 ± 10.4	99.0–135.0	127.0 ± 3.5	124.0–131.0
Maximum body width	23.0 ± 2.1	21.0–27.0	22.0 ± 1.7	20.0–23.0
Anal body width	18.0 ± 1.5	16.0–21.0	17.0 ± 1.5	15.0–18.0
Tail length	50.0 ± 5.1	39.0–59.0	39.0 ± 4.0	35.0–43.0
Tail annuli	47.0 ± 4.1	40.0–54.0	–	–
Spicules	–	–	26.7 ± 0.6	26.0–27.0
Gubernaculum	–	–	12.3 ± 0.6	12.0–13.0

1966), Netherlands (Dao, 1970), Poland (Brzeski, 1977), and France (Scotto La Massese and Du Merle, 1978). However, only 2 populations with females and males are known: from Canada (Anderson, 1977) and Spain (this paper). But there is an important difference between them because in Canadian females no sperm were found in the spermatheca of females whereas Spanish females were clearly inseminated with sperm. General morphology and measurements fit well with those given by Allen (1955) and others such as Thorne and Malek (1968), Anderson (1977), and Maqbool and Shahina (1987).

Bitylenchus huesingi (Paetzold, 1958) Siddiqi, 1986 (Table 4)

FEMALE: Body ventrally curved upon relaxation. Lateral field 6–8 wide at mid-body, with 4 lines, outer bands regularly areolated. Lip region continuous with body, bearing 5–7 annuli and measuring 3–4 high × 6–7 wide. Stylet knobs rounded, slightly directed posteriad, 3–4 in diameter. Esophagus typical of the genus, with a slender isthmus 23–28 long. Excretory pore located opposite first third of basal bulb. Hemizonid 2–3 annuli long, located 3–5 annuli anterior to excretory pore.

Ovaries outstretched, equally developed. Tail straight, cylindrical, with annulated terminus. Terminal hyaline region 4–5 long. Postanal intestinal sac fills the tail cavity. Phasmids located 12–21 annuli posterior to anus level.

MALES: Uncommon. Similar to female in general morphology as well as measurements. Tail finely annulated, conoid. Spicules and gubernaculum ventrally curved. Bursa crenate, surrounds the tail completely, 64–70 long.

HABITAT AND LOCALITY: Pasture soil from Arroyo Frio, Sierra de Cazorla, Jaén, Spain.

REMARKS: Morphology as well as general measurements of this population are in close conformity with those given for the species by Paetzold (1958). It is well distributed in Europe where it has been recorded from Germany (Sturhan, 1966), France (Scotto La Massese and Du Merle, 1978), and Spain (Romero et al., 1970; Arias and Romero, 1971).

Discussion

Remarks on the genus *Bitylenchus* Filip'ev, 1934

Bitylenchus was proposed as a subgenus of the genus *Tylenchus* by Filip'ev (1934). It was synonymized with *Tylenchorhynchus* Cobb, 1913,

by Filip'ev (1936). Jairajpuri (1982) resurrected *Bitylenchus* as a subgenus of *Tylenchorhynchus* Cobb, 1913, and gave a key to its species. Siddiqi (1986) recognized it as a valid genus, gave a diagnosis to it, and differentiated it from *Tylenchorhynchus* "in having outer bands of lateral fields areolated, a large postanal intestinal sac containing intestinal granules and fasciculi, relatively more thickened cuticle at female tail tip, and a non-protrusible gubernaculum."

Fortuner and Luc (1987) rejected the genus *Bitylenchus,* arguing that it "was defined using very secondary characteristics that are not known for many taxa, and that, when known, do not clearly differentiate this genus from *Tylenchorhynchus.*" They, however, assigned *Sauertylenchus* Sher, 1974, a close relative of *Bitylenchus,* to the subfamily Belonolaiminae because, according to them, it "may be seen as a relict of ancestral forms from which evolved the present day belonolaimids," and because it "shares some derived characters with members of this subfamily (strong valve, elongate stylet)." We reject this apparently misleading "phylogeny" of *Sauertylenchus* on grounds discussed below. The argument put forward by Siddiqi (1986) that this genus is similar to *Bitylenchus* except for the differences in the structure of the lip region is supported by our study of SEM face views of *Bitylenchus pratensis* sp. n. and *B. maximus* (Figs. 27, 37).

Sher (1974) stated that the type species *Sauertylenchus labiodiscus* Sher, 1974, appeared similar to *Geocenamus* Thorne and Malek, 1968, in having a set-off lip region with the labial disc, a weakly developed cephalic framework, and a long thin stylet, but because it had 4 incisures (as against 6 in the latter), spicules with distal velum, a gubernaculum that protrudes from the cloacal aperture, and the absence of hypoptygma, it could be placed in the genus *Tylenchorhynchus.* He placed *Sauertylenchus* in the subfamily Tylenchorhynchinae, even though it had a lip region and stylet similar to the species of *Geocenamus* of the subfamily Merliinae and stated, "These two subfamilies are considered to be well defined (Siddiqi, 1971) after examining most of the species and many undescribed species in these taxa." Fortuner and Luc (1987) did not accept this statement and synonymized Merliniinae with Telotylenchinae because, according to them: 1) morphology of lateral field is not a primary differentiating character, 2) shapes of spicule and gubernaculum are accepted as generic characters only, and 3) the exact appearance of corpus, vulva, and spermatheca is not well defined in most species in the taxa concerned.

We concur with Sher (1974) and Siddiqi (1971) in regarding Merliniinae as a well defined group, separate from Telotylenchinae (which includes tylenchorhynchs). Those who have studied a large number of species in these groups can easily see that Merliniinae is distinct on the basis of the following characteristics: male and female genital structures (phylogenetically strong characters), such as spicules, gubernaculum, hypoptygma, and shapes of vulva and spermatheca; SEM face views and the occurrence of 6 incisures in the lateral field; and presence of deirids (absence of deirids in *Scutylenchus* is a derived character state in the group Merliniinae). In addition, and contrary to Fortuner and Luc's (1987) argument, *Merlinius* Siddiqi, 1970, in our opinion resembles *Amplimerlinius* Siddiqi, 1976, and both genera belong in Merliniinae, whereas *Tylenchorhynchus* resembles *Paratrophurus* Arias, 1970, and both belong in Telotylenchinae.

Fortuner and Luc (1987) assigned *Sauertylenchus* and *Geocenamus* to Belonolaiminae, differentiating the latter from Telotylenchinae by the well marked round labial disc and "a tendency towards an elongation of the stylet to reach inside the roots." As discussed here, we regard *Sauertylenchus* as a member of Telotylenchinae and *Geocenamus* as a member of Merliniinae. We believe that the development of a round labial disc is associated with the elongation of the stylet, the latter having occurred independently in various groups of Tylenchina. An SEM face view is required to see a round labial disc in some species, e.g., *Bitylenchus maximus, B. pratensis, B. velatus* (Sauer and Annells, 1981) comb. n. However, *Bitylenchus maximus,* with stylet 20.0–24.5, has a round labial disc, but *B. serranus* with stylet 19.0–22.5 as well as other *Bitylenchus* spp. with stylets under 19.0 such as *B. goffarti* (Sturhan, 1966) Siddiqi, 1986, and *B. tobari* (Sauer and Annells, 1981) Siddiqi, 1986, have a squarish labial disc, and annuli following it are broken by lateral indentations (Figs. 10, 63 and fig. 5B in Sher and Bell [1975]). In contrast, *Tylenchorhynchus* (see SEM face view of *T. cylindricus* Cobb, 1913, in fig. 5A in Sher and Bell [1975]) as well as *Paratrophurus* and *Histotylenchus* Siddiqi, 1971, have complete labial annuli following a squarish labial disc.

We believe that Fortuner and Luc (1987) are unjustified in using the characteristics of the elongation of the stylet in the classification of these groups and would like to quote from Sid-

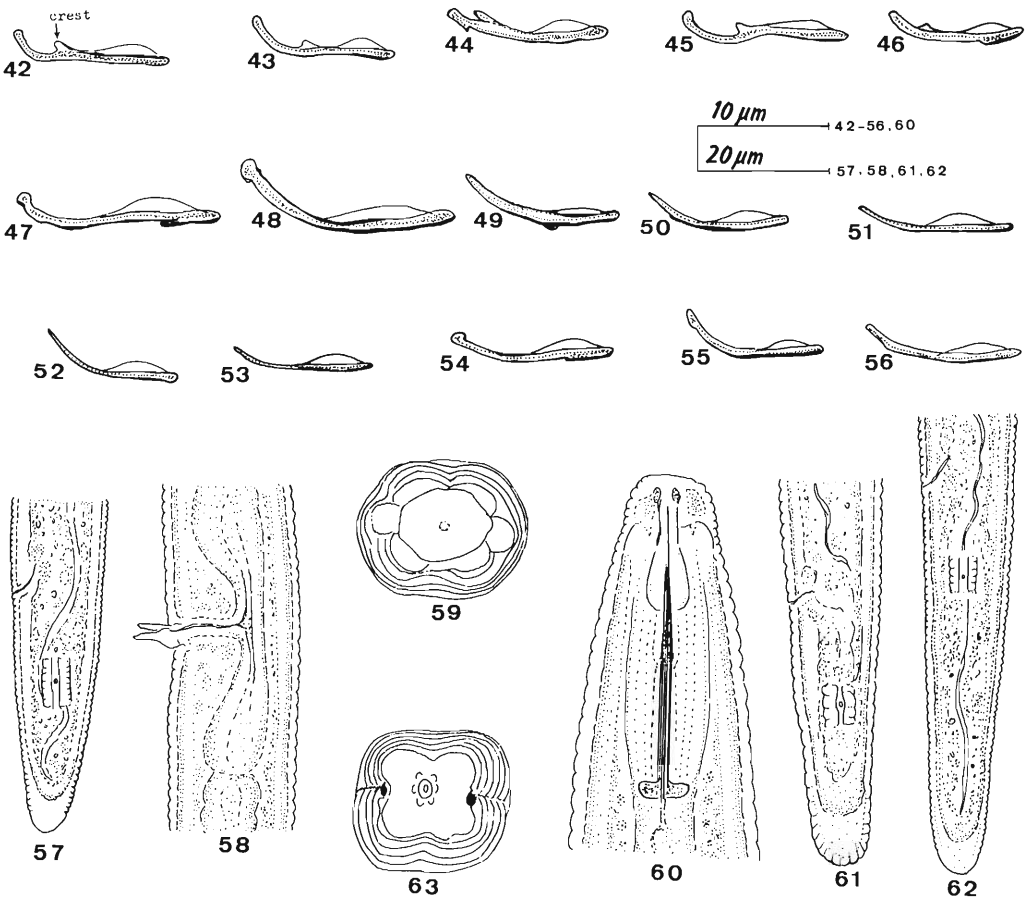
diqi (1986, p. 174) the following, to which we fully subscribe:

> "The elongation of the stylet in Dolichodoridae occurred independently in Dolichodorinae, Macrotrophurinae, Tylenchorhynchinae (*Sauertylenchus*) and Merliniinae (*Hexadorus*). Stylets with a solid-appearing tip (e.g. in *Tylenchorhynchus, Merlinius, Trophurus*), seldom reach a length over 30 μm; those with a tubular conus have a greater chance of exceeding this length. Stylet length can be used as a generic character with care, but it can not be the basis for grouping genera, e.g. *Dolichodorus, Belonolaimus* and *Macrotrophurus,* into one subfamily solely because they have excessively long stylets."

For rejecting *Bitylenchus,* Fortuner and Luc (1987) used some of the characters of *Bitylenchus* spp. that were, as it would appear from their discussion, based on published data and not actual examination of specimens. For example, they stated that a postanal intestinal sac was absent from *B. tobari* and *B. ventrosignatus* (Tobar Jiménez, 1969) Siddiqi, 1986. Upon examination of paratypes of these species, we found it to be present. In fact the sac extends into the entire tail cavity and contains intestinal granules and fasciculi (Figs. 57, 62). We also examined paratypes of *B. aerolatus* (Tobar Jiménez, 1970) Siddiqi, 1986, and found the species to belong to the genus *Tylenchorhynchus* to which it is here returned. In this species, there is no intestinal sac extending into the female tail and the gubernaculum has a winglike expansion in its proximal half (Fig. 44) different from the gubernaculum of all known species of *Bitylenchus.* We found this winglike expansion of the gubernaculum (crest) in several species of *Tylenchorhynchus* including the type species *T. cylindricus* Cobb, 1913 (Figs. 42–46). Because the crest is absent in *Bitylenchus,* it serves as a useful character in differentiating between the 2 genera. We have also found that the gubernaculum in several species of *Bitylenchus* is protrusible through the cloacal aperture when the spicules are protruded. A pair of pedunculated papillae are seen on either side of the cloacal aperture when spicules are protruded. A round labial disc and 6 sectors of the first cephalic annulus are seen in SEM face views of *B. pratensis* and *B. maximus* (Figs. 27, 28 and 36–41). A similar face view is seen in *T. velatus.* We examined paratypes of *T. velatus* and found that the species shows various characteristics of *Bitylenchus,* including a postanal intestinal sac, typical gubernaculum, and modified vulva lips; hence, it is transferred to *Bitylenchus* as *Bitylenchus velatus* (Sauer and Annells, 1981) comb. n. In this and various other morphological characters (stylet and esophageal structures, genital structures, female tail having a large intestinal sac, etc.) these 3 species are similar to *Sauertylenchus labiodiscus* and should belong to 1 genus. However, at this stage we do not propose to synonymize *Sauertylenchus* with *Bitylenchus* but we regard it as a separate genus characterized by an elongated body (1.4–2.0 mm long), an offset labial disc, an elongated stylet measuring 33–40 μm long in type species, and a characteristic gubernaculum that is distally recurved and has titillae. Because the characters of the labial disc and first head annulus being divided into 6 sectors are visible only by using SEM, and because these conditions are supposedly derived by the relative elongation of the stylet, we propose to retain *B. maximus, B. velatus,* and *B. pratensis* in the genus *Bitylenchus* and to emend the diagnosis of *Bitylenchus* in the light of the above discussion.

The genus *Bitylenchus* Filep'ev, 1934

Diagnosis (after Siddiqi, 1986; emended): Telotylenchinae: Small to medium size (0.4–1.5 mm long). Cuticle with fine but distinct annuli. Lateral field with 4 incisures, outer bands areolated. Deirids absent. Cephalic region with or without fine annuli, usually offset by a depression or constriction, and with lateral longitudinal indentations on annuli behind amphidial apertures. Labial disc with 6 labial sensilla usually roughly squarish in outline, but in some species round, and anteriormost lip annulus 6-sectored. Stylet attenuated, small, of medium strength, 10–24 μm long; conus anteriorly attenuated and solid-appearing; knobs small, rounded, usually posteriorly sloping. Median esophageal bulb well developed, oval. Basal esophageal bulb offset from intestine, containing esophageal glands; cardia well developed. Vulva a small transverse slit, postmedian lips generally modified. Ovaries paired. Spermatheca round, axial. Postanal intestinal sac large, filling one-quarter or more of tail cavity, with intestinal granules and fasciculi. Female tail more than 2 anal body widths long, subcylindrical, cylindrical, or subclavate, with a rounded, usually striated terminus; terminal cuticle thickened but not excessively so. Male tail completely enveloped by a large, finely crenate



Figures 42–63. 42–46. Gubernacula in *Tylenchorhynchus*. 47–56. Gubernacula in *Bitylenchus*. 42. *Tylenchorhynchus cylindricus* Cobb, from U.S.A. 43. *T. brassicae* Siddiqi, paratype. 44. *T. aerolatus* Tobar Jiménez, paratype. 45. *T. mashhoodi* Siddiqi and Basir, paratype. 46. *T. clarus* Allen, England. 47. *Bitylenchus maximus* (Allen), England. 48. *B. pratensis* sp. n., paratype. 49. *B. velatus* (Sauer and Annells, 1981) comb. n., paratype. 50. *B. goffarti* (Sturhan), Cyprus. 51. *B. indicus* (Siddiqi), paratype. 52. *B. teeni* (Hashim), paratype. 53. *B. colombianus* (Siddiqi) comb. n., paratype. 54. *B. botrys* (Siddiqi) comb. n., paratype. 55. *B. dubius* (Bütschli), England. 56. *B. parvus* (Allen), England. 57. *B. ventrosignatus* (Tobar Jiménez), paratype female, tail end. 58–61. *B. velatus* (Sauer and Annells). 58. Vulva region. 59. En face view taken from SEM by Sauer and Annells (1981). 60. Head end. 61. Tail end. 62. *B. tobari* (Sauer and Annells), paratype, tail end. 63. *B. tobari,* en face view taken from SEM by Sauer and Annells (1981).

bursa. Spicules distally flanged, 19–39 μm long; tip narrow, minutely rounded or indented. Hypoptygma absent; a pair of pedunculated papillae are seen on either side of cloacal aperture when spicules are protruded. Gubernaculum large, 10–18 μm long, usually protrusible through cloacal aperture when spicules are protruded, distally boat-shaped in lateral view, and lacking crest (an expansion in distal half), telamon, or titillae.

Type species: Bitylenchus dubius (Bütschli, 1873) Siddiqi, 1986, syn. *Tylenchus dubius* Bütschli, 1873, *Tylenchorhynchus dubius* (Bütschli, 1873) Filip'ev, 1936.

Other species (for synonymies see Siddiqi, 1986): *B. botrys* (Siddiqi, 1985) comb. n., syn. *Tylenchorhynchus* (*Bitylenchus*) *botrys* Siddiqi, 1985; *B. colombianus* (Siddiqi, 1985) comb. n., syn. *Tylenchorhynchus* (*Bitylenchus*) *colombianus* Siddiqi, 1985; *B. pratensis* sp. n.; *B. serranus* sp. n.; *B. velatus* (Sauer and Annells, 1981) comb. n., syn. *Tylenchorhynchus velatus* Sauer and Annells, 1981.

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Some Digenea from Freshwater Fishes of Alabama and Florida including *Allocreadium* (*Neoallocreadium*) *lucyae* sp. n. (Digenea: Allocreadiidae)

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ABSTRACT: *Allocreadium* (*Neoallocreadium*) *lucyae* sp. n. is described from the bandfin shiner, *Notropis zonistius*, of east central Alabama. It differs from its most similar species, *Allocreadium* (*Neoallocreadium*) *elongatum*, in having contiguous testes, cecal bifurcation at the level of the acetabulum, and vitellaria commencing at the acetabular level. Comparison is made to all species of *Allocreadium* from New World fishes. Twenty-eight new host records for *Azygia longa*, *Bucephaloides pusillus*, *Crepidostomum cooperi*, *Pisciamphistoma stunkardi*, and *Posthodiplostomum minimum* in freshwater fishes are noted. Slight variations from the description in our specimens of *Alloglossidium corti* are discussed.

KEY WORDS: Digenea, *Allocreadium* (*Neoallocreadium*) *lucyae*, *Crepidostomum cooperi*, *Alloglossidium corti*, freshwater fishes, new host record, new locality records, Alabama, Florida, *Azygia longa*, *Bucephaloides pusillus*, *Pisciamphistoma stunkardi*, *Posthodiplostomum minimum*.

The present report is concerned with the description of a new species of *Allocreadium* from the bandfin shiner of Alabama which represents the fourth species of this large genus recorded from New World fishes. In addition, 28 new host records and numerous new locality records in Alabama and Florida are given.

Materials and Methods

Fishes were collected with monofilament gill nets, backpack shocker, and 4.6-m seine. Live specimens of digeneans were removed from the hosts within a few hours of capture. Metacercariae were removed from cysts. Worms were fixed in hot 10% formalin; whole mounts were stained in Semichon's carmine. Some worms were embedded in paraffin, sectioned at 10 μm, and stained with Harris' hematoxylin and eosin. All specimens are deposited in the U.S. Helminthological Collection (USNM). Measurements are in micrometers unless otherwise indicated. Drawings were made with the aid of a microprojector.

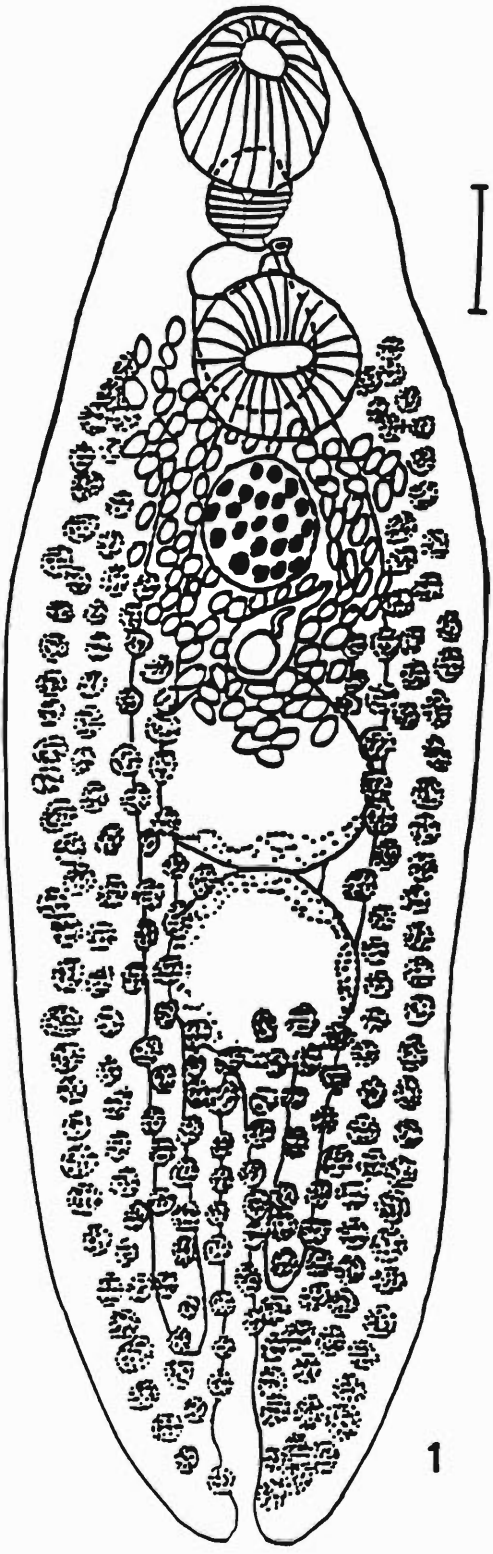
Allocreadiidae Stossich, 1903

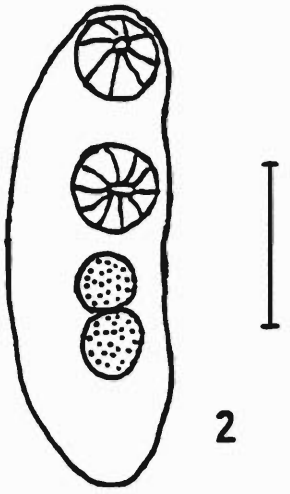
Allocreadiinae Looss, 1902

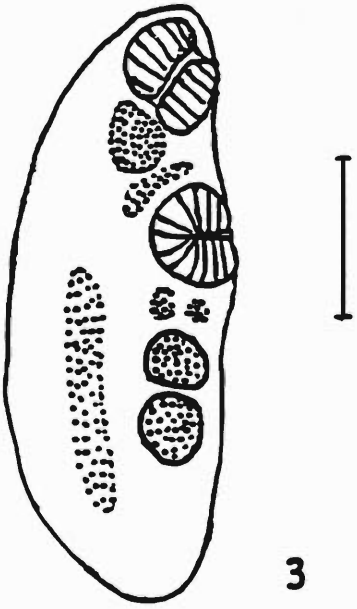
Allocreadium (*Neoallocreadium*) *lucyae* sp. n. (Figs. 1–4)

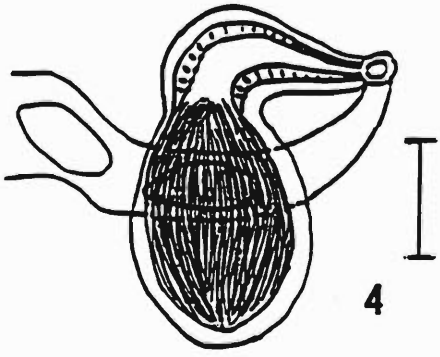
DESCRIPTION: Measurements based on 2 whole mounts and 1 sectioned specimen ($N = 3$ unless noted). With characters of genus and subgenus. Body oval, flattened, tapering at both ends, 2.05–2.58 (2.36) mm long, 692–823 (775) wide. Oral sucker subterminal, 239–289 (264) long, 249–324 (282) wide. Acetabulum in anterior one-third of body, slightly larger than oral sucker, 264–349 (297) long, 289–374 (325) wide. Oral sucker connects directly with pharynx without prepharynx. Pharynx well developed, 134–167 (148) long, 129–154 (141) wide. Esophagus long, 181 ($N = 1$). Cecal bifurcation dorsal of acetabulum; ceca terminate blindly near posterior end of body, subequal. Testes contiguous, subequal in size, in middle one-third of body, oval, smooth, tandem, median; anterior testis 264–274 (269) long, 249–344 (294) wide; posterior testis 304–349 (324) long, 314–324 (321) wide. Post-testicular space 561–790 (683). Seminal receptacle pear-shaped between ovary and anterior testis, in dorsoventral plane of ceca, 154–174 (168) long, 97–105 (102) wide. Cirrus pouch well developed, positioned ventral of cecal bifurcation and dorsal of acetabulum, curling ventrally anterior of acetabulum, 244–309 (276) long, 139–174 (152) wide. Genital pore median, preacetabular. Ovary smooth, subspherical, just posterior to acetabulum, largely in dorsoventral plane of ceca, 194–224 (210) long, 204–214 (209) wide. Uterus extends from anterior of testis to acetabulum. Eggs oval, measured in utero ($N = 20$), 71–79 long, 47–51 wide. Vitellaria commencing at acetabulum and extending uninterrupted to posterior end of body, median and lateral posteriorly, lateral anteriorly with few follicles extending into median field. Excretory pore terminal, excretory vesicle I-shaped, extending from terminal end of body to posterior of posterior testis.

TYPE SPECIMENS: Holotype (USNM 81414), 2 paratypes (1 sectioned) (USNM 81415), 3 immatures (USNM 81416).









TYPE HOST: Bandfin shiner, *Notropis zonistius* (Jordan) (Cypriniformes: Cyprinidae).

TYPE LOCALITY: Mill Creek north of Valley, Alabama (18 February 1971); Lat. 32°51′N, Long. 85°12′W.

ADDITIONAL HOST AND LOCALITY: Rough shiner, *Notropis baileyi* Suttkus and Raney, Choclafala Creek, northeast of Tuskegee, Macon County, Alabama (4 April 1972).

OCCURRENCE: Intestine of host. Adults and immature worms 1 to 2 per host. Of 29 type hosts at the type locality, 6 were infected with adult worms. Seven specimens of the type host collected from the same creek on the same day east of Valley were negative for this worm. Thirty-two specimens of the type host collected from the type locality 10 March 1972 were also negative.

REMARKS: The authors agree with Yamaguti (1971) in recognizing 3 subgenera of the genus *Allocreadium,* namely, *Allocreadium* Looss, 1900, *Allocreadioides* Koval, 1949, and *Neoallocreadium* Akhmerov, 1960, based on the location of the cirrus pouch and uterus and the distribution of the vitellaria. Our specimens conform to the diagnosis for *Neoallocreadium* in that the uterus extends ventral to the anterior testis, the vitellaria extend well into the forebody, and the large cirrus pouch may reach to the posterior end of the acetabulum. The new species is designated *Allocreadium* (*Neoallocreadium*) *lucyae* and most closely resembles *A.* (*N.*) *elongatum* Akhmerov, 1960 (syn. *Neoallocreadium pseudaspii* Akhmerov, 1960), described from *Erythroculter mongolicus* of the Amur River, but differs in having contiguous rather than noncontiguous testes, cecal bifurcation at the level of the acetabulum rather than at the level of the anterior margin of the ovary, and vitellaria commencing at the acetabular level rather than anterior to the acetabulum or almost at the level of the pharynx.

Three species of *Allocreadium* are recognized from New World fishes, namely, *Allocreadium* (*Allocreadium*) *lobatum* Wallin, 1909 (North America), *Allocreadium* (*Neoallocreadium*) *mexicanum* Osorio-Sarabia, Pérez-Ponce de León, and Salgado-Maldonado, 1986 (Michoacán, Mexico), and *Allocreadium* (*Neoallocreadium*) *centropomi* Fischthal and Nasir, 1974 (Venezuela) (Yamaguti, 1971). Of New World species, *A.* (*N.*) *lucyae* most closely resembles *A.* (*A.*) *lobatum,* but differs in having spherical to oval instead of lobate testes, vitellaria commencing at the level of the acetabulum instead of the ovary, and an excretory bladder that does not reach the posterior testis. The new species differs from *A.* (*N.*) *centropomi* by having a long rather than a short esophagus, smooth rather than lobate testes, an excretory bladder that does not reach the posterior testis, and vitellaria commencing at the acetabulum rather than posterior to the acetabulum. It differs from *A.* (*N.*) *mexicanum* in that the testes are located in the middle third of the body rather than the posterior third, ceca terminating near the posterior end of the body rather than at the anterior edge of the posterior testis, and an acetabulum slightly longer than the oral sucker and located in the anterior third of the body rather than twice as long and located in the middle third.

This digenean was not found in more than 2,500 specimens of 141 species of freshwater fishes examined in the southeastern U.S.A. (Amin and Williams, 1983). It did not occur in 175 specimens of 13 other species of *Notropis* examined from 24 other collecting sites.

ETYMOLOGY: This specimen is named for Dr. Lucy Bunkley-Williams.

Crepidostomum cooperi Hopkins

NEW HOST AND LOCALITY (date): Spotted sunfish, *Lepomis punctatus* (Valenciennes) (Perciformes: Centrarchidae), unnamed tributary of Chattahoochee River near Huguley, Alabama, at Interstate 85 intersection (24 February 1972) (USNM 81368).

HOSTS AND NEW LOCALITIES (dates): Redbreast sunfish, *Lepomis auritus* (Linnaeus), same location and date as above (USNM 81367); longear sunfish, *Lepomis megalotis* (Rafinesque), Calebee Creek, south of Tuskegee, Alabama (11 February 1969) (USNM 81369).

REMARKS: Cooper (1915) briefly described specimens of *Crepidostomum* from *Perca flaves-*

←

Figures 1–4. ***Allocreadium* (*Neoallocreadium*) *lucyae* sp. n. 1. Ventral view largely of the holotype, some details from sectioned paratype. Scale = 0.2 mm. 2, 3. Immature specimens showing oral sucker, acetabulum, and testes with developing stages of ovary, pharynx, and ceca. Scale = 0.2 mm. 4. Terminal genitalia of paratype. Scale = 0.1 mm.**

Table 1. Digenea from some Alabama and Florida freshwater fishes.

Digenea Host	N/H*	Site	I/E†	Sizes (cm)	Locality	Date	USNM no.
Alloglossidium corti (Lamont, 1921)							
Lepomis gulosus	1	int.	1/1	14.0	Euphapy Creek, SW of Auburn, Alabama	8 Jul 1970	
Apophallus venustus (Ransom, 1920)							
Lepisosteus osseus	13	mouth‡	1/1	91.4	Devil's Channel, Mobile Bay, Alabama	9 Apr 1970	
Azygia longa (Leidy, 1851)							
Esox americanus§	1–2	stom.	2/3	20.3	Euphapy Creek, SW of Auburn, Alabama	8 Jul 1970	81492
Bucephaloides pusillus (Stafford, 1937)							
Esox americanus§	50	int.	1/1	32.9	Euphapy Creek, SW of Auburn, Alabama	28 Mar 1970	81494
Crepidostomum cooperi Hopkins, 1931							
Esox niger	1	int.	1/1	9.3	Chattahoochee River, Huguley, Alabama	15 Nov 1982	
Homalometron armatum (MacCallum, 1895)							
Aplodinotus grunniens	2	int.	1/2	41.0	Cahaba River, NW of Selma, Alabama	12 Jan 1973	
Neochasmus ictaluri Sogandares-Bernal, 1953							
Ictalurus furcatus	3	int.	1/2	30.5	Tombigbee River, S of Demopolis, Alabama	20 Apr 1970	
Pisciamphistoma stunkardi (Holl, 1929)							
Esox americanus§	7	int.	2/3	20.3	Euphapy Creek, SW of Auburn, Alabama	8 Jul 1970	81493
Plagiocirrus primus Van Cleave and Mueller, 1932							
Notemigonus crysoleucas	4–12	int.	5/5	13.0–25.0	Santa Fe River, N of Gainesville, Florida	12 Dec 1972	
Polylekithum ictaluri (Pearse, 1924)							
Ictalurus furcatus	3	int.	1/2	30.5	Tombigbee River, S of Demopolis, Alabama	20 Apr 1970	

Posthodiplostomum minimum (MacCallum, 1921)

Carpiodes velifer§	1	mes.	1/1	27.9	Euphapy Creek, SW of Auburn, Alabama	27 May 1971	81495
Centrarchus macropterus§	1	mes.	1/1	7.6	Euphapy Creek, SW of Auburn, Alabama	1 Apr 1970	81496
Cottus pygmaeus§	2	mes.	1/5	2.9–3.2	Cold Spring, W of Oxford, Alabama	24 Sep 1971	81497
Elassoma zonatum§	2	mes.	1/5	2.5	Euphapy Creek, SW of Auburn, Alabama	28 Mar 1970	81498
Erimyzon oblongus§	2	mes.	1/45	22.9	Beaver Swamp Creek, S of Lanett, Alabama	6 Feb 1971	81499
Erimyzon tenuis§	1	mes.	1/11	34.1	Fish River, SE of Fairhope, Alabama	24 Feb 1973	81500
Esox americanus§	2	mes.	1/3	12.7	Euphapy Creek, SW of Auburn, Alabama	26 Mar 1970	81501
Hypentelium etowanum§	13	mes.	1/2	22.9	Loblockee Creek, Auburn, Alabama	14 Jan 1971	81502
Ictalurus furcatus§	2	mes.	1/2	30.5	Tombigbee River, S of Demopolis, Alabama	20 Apr 1970	81503
Ictalurus serracanthus§	1	mes.	1/1	26.7	Santa Fe River, N of High Springs, Florida	14 Feb 1972	81504
Micropterus coosae§	10	mes.	1/2	20.5	Loblockee Creek, Auburn, Alabama	14 Jan 1971	81505
Minytrema melanops§	4	mes.	1/1	15.2	Euphapy Creek, SW of Auburn, Alabama	1 Apr 1970	81506
Moxostoma carinatum§	1	mes.	1/3	53.8	Sandy Creek, N of Bosworth, Alabama	24 Apr 1973	81507
Nocomis leptocephalus§	2	mes.	1/2	12.8	Mill Creek, N of Valley, Alabama	18 Feb 1971	81508
Notropis baileyi§	1–34	mes.	4/4	5.1	Euphapy Creek, SW of Auburn, Alabama	16 Mar 1970	81509
Notropis bellus§	1	mes.	1/1	7.6	Euphapy Creek, SW of Auburn, Alabama	16 Mar 1970	81510
Notropis caeruleus§	2	mes.	1/1	6.0	Cahaba River, NW of Selma, Alabama	12 Jan 1973	81511
Notropis callistius§	3	mes.	1/5	11.5	Salt Creek, SE of Munford, Alabama	22 Jan 1974	81512
Notropis chrysocephalus§	3–7	mes.	2/2	5.0–13.0	Euphapy Creek, SW of Auburn, Alabama	16 Mar 1970	81513
Notropis texanus§	2	mes.	1/2	7.8	Lake Martin, W of Dadeville, Alabama	13 Aug 1973	81514
Notropis venustus§	6	skin	1/1	7.6	Euphapy Creek, SW of Auburn, Alabama	16 Mar 1970	81515
Notropis zonistius§	5	mes.	1/7	15.5	Mill Creek, E of Valley, Alabama	18 Feb 1971	81516
Noturus leptacanthus§	1	mes.	1/1	4.5	Santa Fe River, N of Gainesville, Florida	14 Feb 1972	81517
Percina carpiodes§	3	mes.	1/1	10.2	Euphapy Creek, SW of Auburn, Alabama	16 Mar 1970	81518

* Number of worms per host.
† Number of hosts infected/number of hosts examined.
‡ Immature in cysts in mouth and tongue.
§ New host record.

cens, Lepomis gibbosus, Etheostoma nigrum, and *E. exile* taken at Go-Home Bay, Ontario, under the name *Crepidostomum laureatum* (Zeder). Hopkins (1931) re-examined Cooper's specimens and ascertained that the specimens from *Perca flavescens* and those from *Etheostoma nigrum* represented 2 distinct new species that were designated as *Crepidostomum cooperi* for the former and *Crepidostomum canadense* for the latter. In her revision of North American species of papillose allocreadiids, Caira (1989) pointed out that although Hopkins (1931, 1934) listed several criteria with which to distinguish *Crepidostomum cooperi* from *Crepidostomum cornutum* (Osborn, 1903) Stafford, 1904, the criteria are not always consistent and variation in these characters overlaps between the 2 species. Likewise, the characters utilized by Amin (1982) in his key to the North American species of *Crepidostomum* do not adequately distinguish between these 2 species. Our specimens concur with the description of *C. cooperi* as given by Caira (1989) who was able to distinguish adults of these 2 species based on differences in the size of the seminal vesicle and subsequently the position of the pars prostatica within the cirrus sac.

Alloglossidium corti (Lamont)

HOSTS AND NEW LOCALITY (date): Yellow bullhead, *Ictalurus natalis* (Lesueur) (Siluriformes: Ictaluridae), Euphapy Creek at Interstate 85 intersection, southwest of Auburn, Alabama (26 March 1970) (USNM 81408); unnamed tributary of Chattahoochee River near Huguley, Alabama, at Interstate 85 intersection (24 February 1972) (USNM 81409); brown bullhead, *Ictalurus nebulosus* (Lesueur), Euphapy Creek at Interstate 85 intersection, southwest of Auburn, Alabama (16 March 1970) (USNM 81407).

REMARKS: Our specimens varied slightly from the description of this species. Vitellaria usually began anteriorly just anterior of acetabulum, but occasionally at posterior margin of pharynx. Vitellaria usually end posteriorly between testes, but occasionally extend beyond posterior end of last testis. Some follicles extended into the median field. Acetabulum usually smaller than oral sucker, but occasionally equal or larger. This species has been reported from the channel catfish, *Ictalurus punctatus* (Rafinesque) (Ictaluridae), in Alabama (Allison, 1957) and from this host, in general, from the southeastern U.S.A. (Plumb, 1985).

Collection records for 10 species of digeneans from freshwater fishes from Alabama and Florida are given in Table 1. New host and locality records are noted. Records, which do not represent new hosts, are included because few records of Digenea in freshwater fishes from Alabama have been reported.

Acknowledgments

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Amazilolepis trinidadensis gen. n., sp. n. (Cestoidea: Hymenolepididae) from the Copper-rumped Hummingbird, *Amazilia tobaci,* in Trinidad, West Indies

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ABSTRACT: *Amazilolepis trinidadensis* gen. n., sp. n. (Cestoidea: Hymenolepididae) is described from the small intestine of the copper-rumped hummingbird, *Amazilia tobaci,* from Trinidad, West Indies. The new genus differs from all unarmed cestode members of genera in Hymenolepididae found in mammals by having the vagina anterior to the cirrus sac. Of the 3 genera of Hymenolepididae whose members lack a rostellum and are found in birds, it is closest in morphology to *Woodlandia* Yamaguti, 1959. It differs from *Woodlandia* in arrangement and location of testes, ovary shape and placement, as well as host. *Woodlandia* is found in Pelicaniformes.

KEY WORDS: Cestoidea, *Amazilolepis trinidadensis,* Hymenolepididae, copper-rumped hummingbird, *Amazilia tobaci,* Trinidad, West Indies.

For several years the Caribbean Institute of Epidemiology, Trinidad, W.I., collected hundreds of birds to sample blood for viruses. After bleeding, the carcasses were frozen. Among them were 2 copper-rumped hummingbirds, *Amazilia tobaci* (Gmelin, 1788), from Port-of-Spain. Both were infected with tapeworms representing a new genus and species that are described here.

Materials and Methods

Birds were collected in a mist-net, bled, and frozen. After thawing and dissection, the worms were fixed in AFA, stained in Semichon's carmine, and mounted in Canada balsam. The following description is based on 3 nearly complete specimens and several fragments. All measurements are in micrometers unless otherwise indicated.

Amazilolepis gen. n.

GENERIC DIAGNOSIS: Hymenolepidinae Perrier, 1897. Rostellum on apical organ absent, suckers weak, unarmed. Neck present. Proglottids numerous, wider than long. Genital pores unilateral. Protandry marked; testes disappear before ovary appears. Testes 3, in transverse row, internal to osmoregulatory canals. Cirrus pouch nearly reaches median field, containing small seminal vesicle. External seminal vesicle large, filling at about same time ovary appears. Cirrus unarmed. Vagina opens to genital atrium anterior to cirrus sac. Ovary oval, antiporal. Vitellarium round to oval, slightly poral to ovary. Uterus a simple transverse sac, filling most of gravid proglottid. Parasites of hummingbirds.

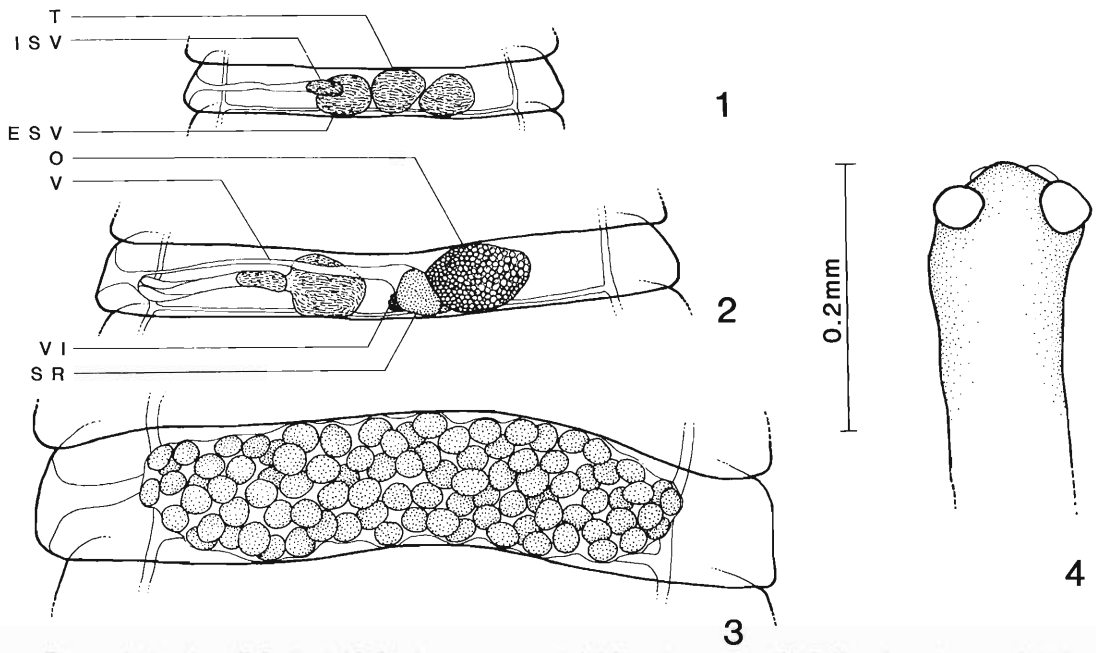
Amazilolepis trinidadensis sp. n. (Figs. 1–4)

SPECIFIC DESCRIPTION: Strobila 20–40 mm long, 560–600 in greatest width when gravid. Proglottids craspedote (Figs. 1–3): Mature proglottids (male) 40–45 long, 280–300 wide, (female) 45–50 long, 300–320 wide. Gravid proglottids 106–120 long, 545–600 wide. Scolex (Fig. 4) 200 long, 135 in greatest width. Neck about 2.5 mm long. Suckers rounded, 54 × 40. Genital pores unilateral, about equatorial in mature proglottids. Genital ducts pass dorsal to osmoregulatory canals. Genital atrium 13–18 deep, 7–10 wide. Male reproduction system matures before female system.

Male genitalia: Testes, 3, in transverse row, 3–35 long by 45–50 wide in mature segments. Cirrus sac elongate, oval, 90–100 long, 10–15 in greatest width. Cirrus small, unarmed. Internal seminal vesicle present, 40–55 long, 10–15 wide, vas deferens expanded into external seminal vesicle, 80–90 long, 55–60 wide.

Female genitalia: Ovary ovoid, aporal, 57–65 wide, 47–65 long. Vitellarium round, slightly poral to ovary, 26–35 wide, 28–41 long. Vagina anterior to cirrus sac. Seminal receptacle elongate, 70 long by 40 wide. Uterus forms a transverse, irregular sac, overlapping osmoregulatory canals. Eggs thin shelled, 24–26 wide. Onchosphere hooks 12–14 long.

[3] Deceased 17 October 1990.



Figures 1–4. ***Amazilolepis trinidadensis*** **gen. n., sp. n. 1. Male mature proglottid. 2. Female mature proglottid. 3. Gravid proglottid. 4. Scolex. All figures drawn to same scale. Abbreviations: ESV, external seminal vesicle; ISV, internal seminal vesicle; O, ovary; SR, seminal receptacle; T, testes; V, vagina; VI, vitellarium.**

Taxonomic summary

TYPE AND ONLY SPECIES: *A. trinidadensis* sp. n.

REMARKS: *Amazilolepis* differs from all the unarmed genera found in mammals, *Gvosdilepis* Spasskii, 1953, *Mathevolepis* Spasskii, 1948, *Soricina* Spasskii et Spasskaja, 1954, *Mytolepis* Spasskii, 1954, *Cryptocotylepis* Skrjabin et Mathevossian, 1948, *Insectivorolepis* Zarnowski, 1956, and *Hymenolepis* Weinland, 1858, in having the vagina anterior to the cirrus sac. It differs from the 3 genera of Hymenolepidinae that lack a rostellum which are found in birds by the following: *Amphipetrovia* Spasskii et Spasskaja, 1954, has the testes antiporal to the ovary; *Arhynchotaeniella* Saakova, 1958, where the vagina has a distal sclerotized clamp; and *Woodlandia* Yamaguti, 1959, in which the testes are arranged in a triangular pattern external to the excretory canals. *Amazilolepis trinidadensis* is closest in morphology to *Woodlandia* Yamaguti, 1959, found in shags, *Phalacrocorax carbo,* in Asia, but clearly differs, as stated above, in having testes arranged in a transverse row between the osmoregulatory canals instead of in a triangular pattern, 1 poral and 2 antiporal, located lateral to the osmoregulatory canals; in having a compact, antiporal ovary instead of median; and in host, *Woodlandia* being found in Pelicaniformes where *Amazilolepis* is the first species of Hymenolepididae to be reported from hummingbirds.

HOST: Copper-rumped hummingbird, *Amazilia tobaci.*

LOCALITY: Port-of-Spain, Trinidad, West Indies.

HABITAT: Small intestine.

HOLOTYPE: USNM Helm. Coll. No. 81858.

PARATYPES: USNM Helm. Coll. No. 81859.

ETYMOLOGY: Named for genus of host and its country of collection.

Discussion

Three species of cestodes have been reported from the Trochilidae, birds that occur only in the New World. All 3 are in the Dilepididae: *Anonchotaenia trochili* Fuhrmann, 1908; *Anomaloporus hesperiphonae* Voge et Davis, 1953; and *Arostellina reticulata* Neiland, 1955 (Schmidt, 1986). It is interesting to note that cestodes of these 3 genera previously found in hummingbirds, as well as *Amazilolepis,* lack rostella, but belong to families in which an armed rostellum is usual. Of the many of the nearly 100 hummingbirds representing many genera and species

from South, Central, and North America dissected by 1 of us (G.D.S.), this is the first time tapeworms have been found. The stomach of nearly every bird examined contained tiny spiders, fruit flies, wasps, and other insects. These arthropods probably were attracted to the flowers or each other, and mainly have mouth parts incapable of ingesting bird feces. The limited possibility of these insects to ingest tapeworm onchospheres probably accounts for the paucity of cestodes in this large family of about 320 species of birds.

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Eimeria idmii sp. n. (Apicomplexa: Eimeriidae) from the Arabian Mountain Gazelle, *Gazella gazella*, in Saudi Arabia

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ABSTRACT: Fecal examination of 47 idmi, the Arabian or dark-colored mountain gazelle, *Gazella gazella* Pallas, 1766, at King Khalid Wildlife Research Center in Thumamah, Riyadh Province, Saudi Arabia, yielded oocysts of an undescribed coccidian, *Eimeria idmii* sp. n. Sixteen of the 47 idmi (34%) were infected. Sporulated oocysts of *E. idmii* sp. n. are ellipsoidal, flattened at micropylar end, 42 × 30 (36–48 × 27–37) μm, length/width ratio 1.4 (1.2–1.6), with smooth, double-layered oocyst wall, the inner yellow, the outer green, with micropyle covered with dome-shaped micropylar cap, 9 (2–15) μm wide, 2 (1–4) μm high. Sporocysts elongate, ovoidal, 18 × 10 (10–23 × 8–13) μm with Stieda body and sporocyst residuum. Sporozoites elongate, 15 × 6 (10–18 × 4–9) μm, each with a large and a small refractile body, 5 (3–8) μm and 3 (1–5) μm in diameter, respectively. Sporulation time 7–8 days at 25 ± 2°C.

KEY WORDS: antelope, Coccidia, *Eimeria idmii* sp. n., gazelle, oocyst, sporocyst, sporozoite, Stieda body.

Eimeria infections are common in both domestic and wild animals in Saudi Arabia. Several species were reported from camels (Kawasmeh and El Bihari, 1983; Kasim et al., 1985; Hussein et al., 1987), sheep (Kasim and Al-Shawa, 1985a), cattle (Kasim and Al-Shawa, 1985b), rabbits (Kasim and Al-Shawa, 1987), the Arabian oryx (Kasim and Al-Shawa, 1988a), the rock agama, *Agama sinaita* (Kasim and Al-Shawa, 1988b), the Arabian quail, *Coturnix delegorguei* (Amoudi, 1987), the Indian peacock, *Pavo cristatus* (Amoudi, 1988), and the grey monitor, *Varanus griseus* (Amoudi, 1989). However, none is yet reported from Saudi Arabian gazelles.

Herds of antelopes indigenous to the Kingdom of Saudi Arabia including the 3 main species of gazelles, *Gazella gazella, Gazella subgutturosa,* and *Gazella dorcas* (Groves and Lay, 1985; Groves, 1989), and the Arabian oryx, *Oryx leucoryx,* are kept at King Khalid Wildlife Research Center (KKWRC) of the National Commission for Wildlife Conservation and Development (NCWCD) in Thumamah, some 80 km N of Riyadh, the capital of Saudi Arabia, for research, breeding, and later reintroduction to their now protected natural habitats in the Kingdom. During the course of parasitological assessment of these animals, oocysts of various *Eimeria* species were detected in the feces of these animals. Those recovered from the feces of the idmi, the Arabian or dark-colored mountain gazelle, *G. gazella,* proved to be a new species that is described in the present study.

Materials and Methods

Fresh fecal samples were collected into wide-mouth, screw-cap, plastic containers directly from the rectum of each of 47 idmi (2–36 mo old) sedated by darts at KKWRC. These animals were born in Thumamah and are the descendants of a herd collected by the late King Khalid Ibn Abdul Aziz. In the laboratory, the fecal samples were subjected to various parasitological examinations, including direct smear, sedimentation, and floatation over saturated sodium chloride solution (Anonymous, 1977). The parasitic burden carried by each animal was assessed by the modified McMaster technique (Anonymous, 1977).

Samples with eimerian oocysts were ground up in a mortar, thoroughly mixed with 2.5% (w/v) aqueous solution of potassium dichromate ($K_2Cr_2O_7$), strained with a fine-mesh wire strainer, and suspended in shallow layers of the solution in petri dishes at room temperature (25 ± 2°C) for sporulation. These were examined daily and the sporulation time was recorded. Measurements were made by a calibrated ocular micrometer, photomicrographs were taken by a Nikon camera (Nikon Company, Japan) attached to a Zeiss compound microscope (Karl Zeiss, Jena, Germany), and drawings were made using an attached Zeiss camera lucida. All measurements are in micrometers (μm): means followed by the range in parentheses.

Results

Sixteen of the 47 idmi (34%) were infected with a single species of *Eimeria.* The numbers of oocysts per gram of feces shed by these animals were 50–9,000. The oocysts (Figs. 1–4) are dif-

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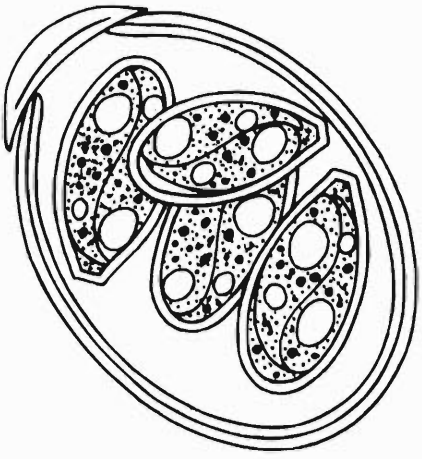


Figure 1. Camera lucida drawing of a sporulated oocyst of *Eimeria idmii* sp. n.

ferent from any of those described from gazelles or from any other antelope species. Hence, they represent a new species of *Eimeria* that is described below.

Eimeria idmii sp. n. (Figs. 1–4, Table 1)

DESCRIPTION: Oocysts ellipsoidal, flattened at micropylar end. Oocyst wall 1.9 (1–2) thick, smooth, double-layered, each of about the same thickness, outer layer green, inner layer yellow, with wide micropyle covered by dome-shaped micropylar cap 9 (2–15) wide, 2 high.

Sporulated oocysts ($N = 105$) 42 × 30 (36–48 × 27–37), length/width ratio 1.4 (1.2–1.6). Oocyst residuum, oocyst polar granule both absent. Sporocysts elongate, ovoidal ($N = 500$) 18 × 10 (10–23 × 8–13); length/width ratio 1.8 (1–2). Stieda body present, substieda body absent. Sporocyst residuum present, consists of diffuse, coarse, refractile granules. Sporozoites elongate ($N = 500$), 15 (10–18), each with a large refractile body at wide end, 5 (3–8) in diameter, and a smaller one at narrow end, 3 (1–5) in diameter.

TYPE HOST: The idmi, Arabian or dark-colored mountain gazelle, *Gazella gazella* Pallas.

TYPE LOCALITY: Thumamah, north of Riyadh, Saudi Arabia.

PREVALENCE: Found in 16 of 47 idmi (34%).

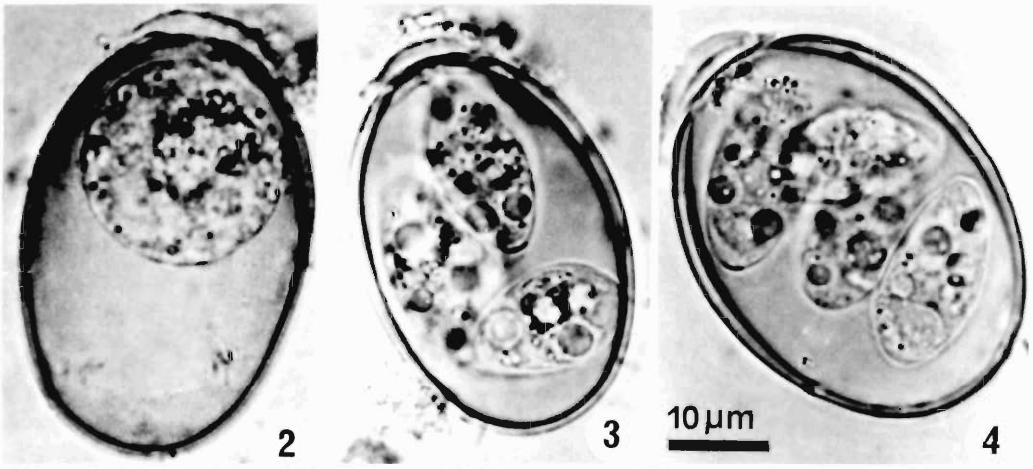
SITE OF INFECTION: Unknown, oocysts recovered from feces.

SPORULATION: Exogenous, within 7–8 days at 25 ± 2°C in 2% $K_2Cr_2O_7$.

TYPES: Phototypes and preserved materials in authors' collection at KKWRC and at the Zoology Department, College of Science, King Saud University.

PHOTOTYPES: Deposited in National Parasite Collection, U.S. National Museum, USNM No. 82006.

ETYMOLOGY: The specific name is derived from the common Arabic name of the type host.



Figures 2–4. Photomicrographs of *Eimeria idmii* sp. n. oocysts. 2. An unsporulated oocyst. 3. A narrower, ellipsoidal sporulated oocyst. 4. A wider, ellipsoidal sporulated oocyst. Scale bar is for all figures.

Table 1. Morphometric comparison between *Eimeria idmii* sp. n. and *Eimeria* species described from gazelles and other antelopes.

Eimeria species	Oocyst: Mean size (range) (μm)	Oocyst: Micropyle	Oocyst: Micropylar cap	Oocyst: Polar granule	Sporocyst: Stieda body	Sporocyst: Residuum	Type host
E. idmii sp. n.	42 × 30 (36–48 × 27–37)	+	+	−	+	+	The idmi, *Gazella gazella*
E. abenovi Svanbaev, 1979	32 × 23 (24–27 × 19–26)	+	−	−	−	−	Goitered gazelle, *Gazella subguttrosa*
E. chinkari Pande, Bhatia, Chauhan, and Garg, 1970	25 × 22 (24–27 × 19–26)	−	−	−	+	+	The chinkara, *Gazella gazella*
E. dorcadis Montovani, 1966	29 × 18 (26–31 × 15–26)	−	−	−	−	+	Dorcas gazelle, *Gazella dorcas*
E. elegans Yakimoff, Gousseff, and Rastegaieff, 1932	(23–45 × 16–25)	+	−	±	−	+	Goitered gazelle, *Gazella subguttrosa*
E. gazella Musaev, 1970, emend. Svanbaev, 1970	24 × 20 (20–28 × 17–25)	−	−	−	−	+	Goitered gazelle, *Gazella subguttrosa*
E. saudiensis Kasim and Al-Shawa, 1988	31 × 25 (24–37 × 20–28)	+	+	+	+	+	Arabian oryx, *Oryx leucoryx*
E. canna Triffitt, 1924	(23–24 × 16–20)	+	−	+	+	+	The eland, *Taurotragus oryx*
E. triffittae Yakimoff, 1934, emend. Levine and Ivens, 1970	21 × 18 (21–24 × 15–19)	−	−	−	−	−	The eland, *Taurotragus oryx*
E. yakimovi Rastegaieff, 1929	(27–41 × 20–29)	+	−	−	±	±	The nilgai, *Boselaphus tragocamelus*
E. chausinghi Pande, Bhatia, Chauhan, and Garg, 1970	25 × 18 (20–27 × 15–21)	−	−	−	+	+	The 4-horned antelope, *Tetracerus quadricornis*
E. congolensis Ricci-Bitti, Pampiglione, and Kabala, 1973	30 × 22 (27–33 × 19–24)	+	−	−	+	−	The waterbuck, *Kobus defassa*
E. kobi Ricci-Bitti, Pampiglione, and Kabala, 1973	38 × 28 (34–41 × 26–30)	+	−	−	+	+	The waterbuck, *Kobus defassa*
E. macieli Yakimoff and Matchulski, 1938	30 × 21 (24–34 × 20–24)	+	−	−	−	−	The waterbuck, *Kobus defassa*
E. talboti Prasad and Narayan, 1963	36 × 25 (35–38 × 22–28)	−	−	−	−	−	The hartbeeste, *Alcalaphus cokei*
E. gorgonis Prasad, 1960	23 × 17 (20–26 × 15–18)	−	−	+	+	+	The wildebeest, *Connochaetus taurinus*
E. connochaetei Levine and Ivens, 1970	22 × 14 (20–27 × 13–15)	−	−	−	+	+	The wildebeest, *Connochaetus taurinus*
E. mirgai Chauhan, Bhatia, and Arora, 1972	49 × 30 (39–55 × 26–32)	+	+	+	+	+	The blackbuck, *Antelope cervicapra*
E. impalae Prasad and Narayan, 1972	33 × 22 (30–36 × 20–24)	+	−	−	±	−	The impala, *Aepyceros melampus*
E. neitzi McCully, Basson, DeVos, and DeVos, 1970	32 × 30 (29–34 × 28–33)	−	−	+	+	+	The impala, *Aepyceros melampus*
E. walleri Prasad, 1960	29 × 24 (27–30 × 22–25)	+	−	−	+	+	The gerenuk, *Litocranus walleri*
E. ismailovi Musaev, 1970	29 × 22 (23–33 × 18–24)	−	−	−	−	+	The saiga, *Saiga tatarica*
E. manafovae Musaev, 1970	37 × 20 (29–47 × 17–26)	+	−	+	−	+	The saiga, *Saiga tatarica*
E. saiga Svanbaev, 1958	31 × 30 (28–34 × 27–32)	−	−	+	−	+	The saiga, *Saiga tatarica*
E. sajanica Machulskii, 1974	21 × 18 (18–23 × 16–20)	−	−	−	−	+	The saiga, *Saiga tatarica*
E. tatarica Musaev, 1970	30 × 24 (25–35 × 19–30)	+	±	−	−	+	The saiga, *Saiga tatarica*
E. tekenovi Svanbaev, 1979	29 × 22 (23–33 × 18–24)	+	+	−	−	+	The saiga, *Saiga tatarica*

+ = present; ± = present or absent; − = absent.

Discussion

Levine and Ivens (1986) have listed only 5 species of *Eimeria* from members of the antelope genus *Gazella* Blainville, 1816, of the family Bovidae, and *E. idmii* sp. n. is very much different from all of these parasites. It has the largest and the only oocyst with a micropylar cap among gazelle eimerians (Table 1). It can also be differentiated from *E. chinkari, E. dorcadis,* and *E. gazella* by having a micropyle and from both *E. dorcadis* and *E. gazella* by having a micropyle and Stieda bodies (Table 1). Similar to other gazelle eimerians, *E. idmii* lacks oocyst residuum and polar granules, though some oocysts of *E. elegans* have polar granules (Yakimoff et al., 1932; Levine and Ivens, 1970, 1986; Pellérdy, 1974). Moreover, with the exception of *E. abenovi,* all gazelle eimerians, including *E. idmii,* have sporocyst residuum (Svanbaev, 1979; Levine and Ivens, 1986). Similar to *E. abenovi* and *E. chinkari* (Pande et al., 1970; Svanbaev, 1979; Levine and Ivens, 1986), sporozoites of *E. idmii* have refractile bodies that are absent from those of *E. elegans* and *E. gazella* (Yakimoff et al., 1932; Montovani, 1966; Levine and Ivens, 1970, 1986; Pellérdy, 1974; Svanbaev, 1979).

Of the other antelopes kept at KKWRC in Thumamah, both the rheem (*G. subgutturosa*) and the Arabian oryx were each infected with a different species of *Eimeria,* whereas no eimerian oocysts were recovered from the Dorcas gazelle. The rheem was infected with an *Eimeria* species that has a small oocyst devoid of a micropylar cap and the Arabian oryx with the recently described *E. saudiensis.* There are indications that the species of *Eimeria* found in the rheem could well be yet another undescribed species that is currently under investigation. On the other hand, oocysts of both *E. saudiensis* and *E. idmii* have a dome-shaped micropylar cap, but that of the former is very much smaller than that of the latter. Moreover, each *E. saudiensis* oocyst has an average of 5 (1–8) polar bodies that were considered by Kasim and Al-Shawa (1988b) to represent an oocyst residuum. The sporulation times of the 2 species are also different: *E. saudiensis* is 5 days at 25 ± 2°C, whereas *E. idmii* is 7–8 days at the same temperature. Oocysts of *E. idmii* have smooth, double-layered walls, whereas the outer layer of the *E. saudiensis* oocyst is finely pitted (Kasim and Al-Shawa, 1988b).

Eimeria saudiensis is the only species of *Eimeria* described from the antelope genus *Oryx* Blainville, 1816 (Kasim and Al-Shawa, 1988b). However, about 20 other species of *Eimeria* have been described from other antelope genera (Levine and Ivens, 1970, 1986; Pellérdy, 1974). A morphometric comparison that sets *E. idmii* apart from all of these species is shown in Table 1. As far as size is concerned, oocysts of *E. idmii* are second only to those of *E. mirgai*; both, together with *E. saudiensis, E. tekenovi,* and rarely *E. tatarica,* are the only antelope eimerians that have micropylar caps. Moreover, *E. mirgai* also differs from *E. idmii* in having oocyst residuum and polar granules; its oocyst wall is thicker and its micropylar cap is transparent and helmet-shaped. *Eimeria idmii* can also be differentiated from *E. tekenovi, E. tatarica,* and *E. macieli,* as well as from all *Eimeria* species described from the saiga and from most of the sporocysts of both *E. impalae* and *E. yakimovi,* by having Stieda bodies. It can also be differentiated from *E. triffittae* and *E. talboti* by having a micropyle, Stieda body, and sporocyst residuum. The micropyle is also absent from *E. chausinghi, E. gorgonis, E. connochaeti, E. neitzi, E. ismailovi, E. saiga,* and *E. sajanica,* and the sporocyst residuum from *E. congolensis, E. impalae,* and *E. yakimovi.* Oocysts of *E. idmii* lack polar granules that are present in those of *E. canna, E. gorgonis, E. manafovae, E. mirgai, E. neitzi,* and *E. saiga* (Table 1).

Two of the antelope eimerians, *E. neitzi* and *E. triffittae,* have single-layered oocyst walls (Yakimoff, 1934; McCully et al., 1970; Levine and Ivens, 1970, 1986; Pellérdy, 1974), those of *E. canna* and *E. walleri* are triple-layered (Triffitt, 1924; Prasad, 1960; Levine and Ivens, 1970, 1986; Pellérdy, 1974), and the rest, including *E. idmii,* are double-layered. Both outer and inner layers of *E. idmii* oocysts are smooth, firmly clinging to each other, the outer is green and the inner yellow, and both are penetrated by the micropyle. Oocysts with double-layered walls also occur in *E. saiga* and *E. sajanica*; however, both of their walls are colorless and are thinner than those of *E. idmii.* Oocysts of both species are smaller than those of *E. idmii,* both lack a micropyle as well as a micropylar cap and Stieda bodies. Polar granules and oocyst residuum occur in *E. saiga* oocysts, but both are absent from the other 2 species (Svanbaev, 1958; Levine and Ivens, 1970, 1986; Pellérdy, 1974). *Eimeria congolensis* and *E. kobi* oocysts have rough, granular, brown-colored outer oocyst walls that can easily peel off their inner counterparts and their micropyles do not penetrate their inner walls (Ricci-Bitti et al., 1973; Levine and Ivens, 1986).

Oocysts of *E. macieli* are also double-layered, but both of their walls are radially striated (Yakimov and Matchuski, 1938; Levine and Ivens, 1970, 1986; Pellérdy, 1974).

From the many structural differences discussed, it is clear that *E. idmii* is a distinct and hitherto undescribed species.

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Research Note

Gastrointestinal Helminths of the Lizard, *Sceloporus malachiticus* (Sauria: Iguanidae) from Costa Rica

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ABSTRACT: Three species of nematodes were recovered from the gastrointestinal tracts of 9 of 41 (22%) *Sceloporus malachiticus* from Costa Rica: *Spauligodon oxkutzcabiensis* (prevalence 7%, mean intensity 25); third-stage *Ascarops* sp. (prevalence 20%, mean intensity 7); and third-stage *Physaloptera* sp. (prevalence 2%, mean intensity 13). All are new host records.

KEY WORDS: Nematoda, *Spauligodon oxkutzcabiensis, Ascarops* sp., *Physaloptera* sp., Iguanidae, *Sceloporus malachiticus,* prevalence, intensity, Costa Rica.

Sceloporus malachiticus Sumichrast, 1882, is a medium-sized iguanid lizard that occurs from Guatemala to Panama in relatively mountainous regions from about 600 to 3,600 m (Villa et al., 1988). To our knowledge, there are no helminthological surveys of this species. The purpose of this paper is to present data from a survey of helminths from a population of *S. malachiticus* from Costa Rica.

The specimens utilized for this study are from a collection first used by Marion and Sexton (1971) for a study of the reproductive cycle of *Sceloporus malachiticus* and were collected January–December 1968 within a 50-km radius of San José, Costa Rica (9°50′N, 84°05′W) in the provinces of San José, Cartago, Alajuela, and Heredia at elevations 800–3,200 m. The 2 major collecting regions were San Ramon de Tres Ríos and Volcán Irazú. Tres Ríos is a pre-montane wet forest at about 1,100 m elevation, whereas Volcán Irazú is a montane wet forest at 2,400–3,000 m elevation. San José is at about 1,160 m elevation. Forty-one of these specimens (mean snout–vent length 74.2 ± 1.0 mm SE, range 59–85 mm) were examined for helminths. The body cavity was opened by a longitudinal incision from vent to throat and the gastrointestinal tract was excised by cutting across the anterior esophagus and the rectum. The esophagus, stomach, small intestine and large intestine were examined separately. Each helminth was identified utilizing a glycerol wet mount. Selected intact specimens were placed in vials of alcohol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *Spauligodon oxkutzcabiensis* (81860), *Ascarops* sp. (81861), and *Physaloptera* sp. (81862).

Nematodes (Table 1) were recovered from 9 of 41 *Sceloporus malachiticus* examined (prevalence 22%). *Spauligodon oxkutzcabiensis* (Chitwood, 1938) was recovered from the fecal material within the large intestine (prevalence 7%, mean intensity 25), third-stage larvae of *Physaloptera* Rudolphi, 1819, from the stomach lumen (prevalence 2%, intensity 13), and larvae of *Ascarops* Beneden, 1873, from cysts within stomach musculature (prevalence 20%, mean intensity 7). Each of the 3 lizards infected with *Spauligodon oxkutzcabiensis* had concurrent infection of *Ascarops* sp. All represent new host records.

Seventy-five (2 male, 73 female) nematodes matching the description of and within the range of measurements for *S. oxkutzcabiensis* as reported by Chitwood (1938) were recovered from the large intestine of 3 (1 female, 2 male) *S. malachiticus* collected at San José. The 2 male specimens were 1.8 and 2.0 mm long and 160 and 165 μm wide, respectively. The cloacal orifice was prominent; the caudal alae did not enclose the postanal papillae. The esophagus was 270 μm long; the excretory pore was opposite the esophageal–intestinal junction. Gravid female specimens were 3.0–5.3 mm long by 200–350 μm wide. The esophagus was 410–430 μm in length. The excretory pore was located 25–50 μm posterior to the esophageal–intestinal junction. The vulva was 25–50 μm posterior to the excretory pore. The tail had 11 to 15 spines. The elongated, ellipsoidal eggs measured 36 × 120 μm and had small terminal knobs at each end. *Spauligodon* (=*Pharyngodon*) *oxkutzcabiensis* was first described in the gecko, *Thecadactylus rapicaudus,* from Yucatan, Mexico by Chitwood (1938) and has not since been reported until now. Of the 23 species of *Spauligodon* so far described, 8 are

Table 1. Prevalence of nematodes in *Sceloporus malachiticus* by date of capture and location.

Nematode	Apr Volcán Irazú	Oct–Nov Tres Ríos	Dec Volcán Irazú	Dec San José	Total
Spauligodon oxkutzcabiensis	0% (0/10)	0% (0/12)	0% (0/12)	43% (3/7)	7% (3/41)
Spirurid larvae (*Ascarops* sp.)	0% (0/10)	8% (1/12)	8% (1/12)	86% (6/7)	20% (8/41)
Third-stage *Physaloptera* sp.	10% (1/10)	0% (0/12)	0% (0/12)	0% (0/7)	2% (1/41)

from the western hemisphere, and with 1 exception (Baylis, 1923), the species have been recovered only from lizards. In the western hemisphere they have been found as parasites of the large intestine of gekkonid, iguanid, and teiid lizards although they may occasionally spill over into the small intestine (Goldberg and Bursey, 1990). Pearce and Tanner (1973) considered the effects of *Spauligodon giganticus* on its host to be negligible and to be more a commensal than a parasite. Although we report a prevalence of 7% for *S. oxkutzcabiensis* in *S. malachiticus,* had we examined only the population from San José, prevalence would have been much different as it was absent from the Tres Ríos and Volcán Irazú samples (Table 1).

Fifty-six third-stage spirurid larvae, which we identify as *Ascarops* sp., were recovered from cysts within stomach musculature of 8 (4 male, 4 female) *S. malachiticus.* The distinguishing differential features of the third-stage larvae of *Ascarops* sp. and seen in our specimens are (1) the right and left anterolateral body wall is prolonged into liplike projections, and (2) the tip of the tail possesses a smooth knoblike process. Goldberg and Bursey (1989) listed paratenic hosts of *Ascarops* sp. within the United States.

Thirteen third-stage physalopterid larvae, which we identify as *Physaloptera* sp., were recovered from the stomach of 1 female *S. malachiticus.* There are 4 genera of the family Physalopteridae reported from the western hemisphere: *Physaloptera, Abbreviata, Skrjabinoptera,* and *Thubunaea.* To identify larval forms is always difficult, but we have based identification of our specimens on the presence of a collarette, symmetrical lips, triangular teeth, and finely striated cuticle. We consider *Abbreviata* to have asymmetrical lips, *Skrjabinoptera* to lack a finely striated cuticle, and *Thubunaea* to lack a collarette. Baker (1987) listed the records of occurrence for species of *Physaloptera, Abbreviata, Skrjabinoptera,* and *Thubunaea.* Considering their occurrences in western hemisphere lizards, of the 57 species of *Abbreviata* currently recognized, only *A. baracoa* is known from the western hemisphere where it occurs in snakes and toads but no lizards. Two of the 8 species of *Skrjabinoptera,* 4 of 16 *Thubunaea* species, and 4 of 15 *Physaloptera* species have been reported from lizards in the western hemisphere.

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Research Note

Sarcocystis sehi sp. n. (Protozoa: Sarcocystidae) from the Porcupine (*Erethizon dorsatum*)

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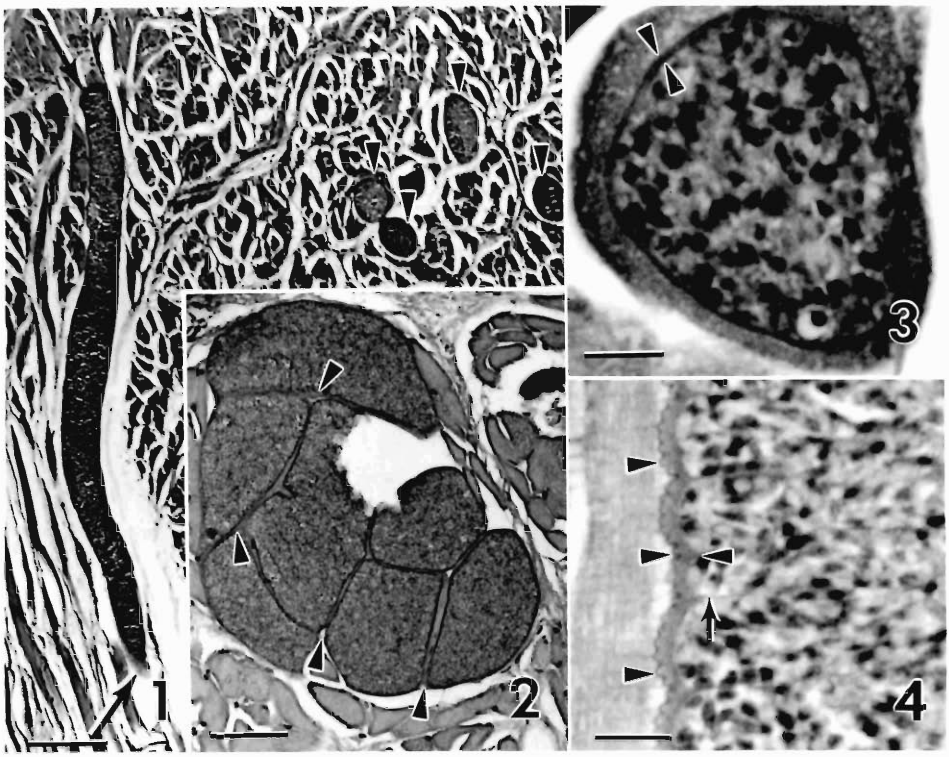
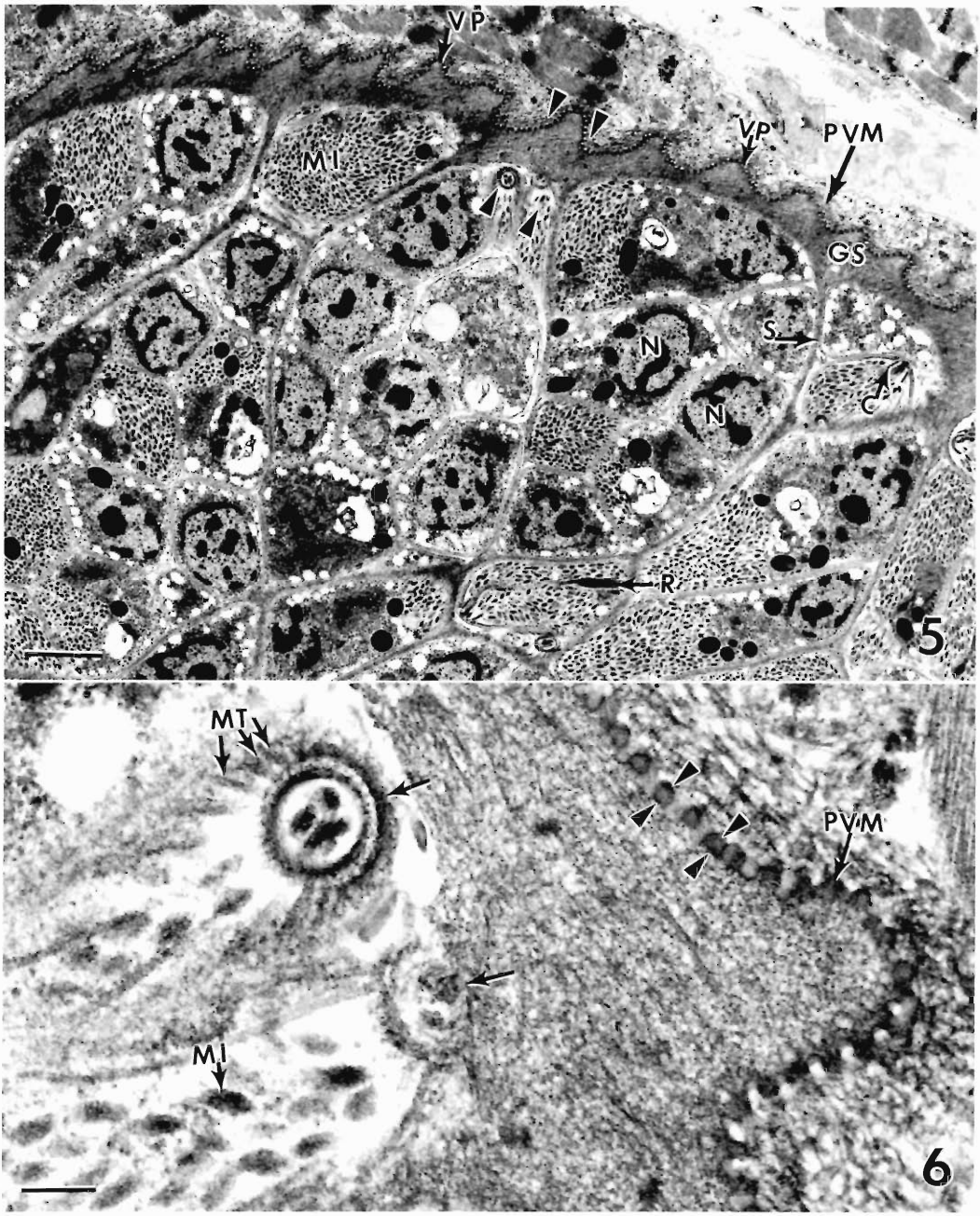
ABSTRACT: Sarcocysts were found in muscles of tongue, heart, esophagus, diaphragm, and masseter in 10 porcupines (*Erethizon dorsatum*) from Sullivan County, Pennsylvania. The sarcocysts were up to 2.3 mm long and 135 μm wide. The sarcocyst wall was 0.3–1.2 μm thick, including the projections on the wall. The parasitophorous vacuole membrane of the sarcocyst wall had conical protuberances at irregular distances and short (60 nm) electron-dense blebs. The bradyzoites were approximately 7 × 1.5 μm and the micronemes were restricted to the anterior half of the bradyzoites. Only 1 morphologic type of sarcocyst was found in all porcupines and it is named *Sarcocystis sehi.*

KEY WORDS: Protozoa, Apicomplexa, Coccidia, *Sarcocystis sehi,* sarcocysts, porcupine, *Erethizon dorsatum.*



Figures 1–4. Sarcocysts in tongues of naturally infected porcupines. Hematoxylin and eosin stain. 1. One longitudinally cut (arrows) sarcocyst and 4 sarcocysts in cross section. Scale bar = 160 μm. 2. A group of sarcocysts within 1 myocyte or in adjacent myocytes. Arrowheads point to sarcocyst boundaries. Scale bar = 75 μm. 3. Sarcocyst in cross section. The cyst wall (arrowheads) is thin and septa are not visible. Scale bar = 6.6 μm. 4. Sarcocyst in longitudinal section. The cyst wall has serrations (arrowheads) on its outer wall layer. Septa originate from the inner wall layer. Opposing arrowheads demarcate the cyst wall thickness. Compare the thickness of sarcocyst wall in Figure 3 at the same magnification. Scale bar = 6.6 μm.



Figures 5, 6. Transmission electron micrographs of the sarcocyst wall from the tongue of a naturally infected porcupine. 5. The parasitophorous vacuole membrane (PVM) has conical villar projections. The sarcocyst wall is cut tangentially toward the left side. Scale bar = 1.1 μm. 6. Higher magnification of the sarcocyst wall marked by opposing arrowheads in Figure 5. Note electron-dense, short, stubby thickenings of PVM (opposing arrowheads). The conoidal ends of 2 bradyzoites (arrow) lie beneath the sarcocyst wall. Microtubules (MT) originate from the conoidal ring. MI = micronemes; GS = ground substance; N = nucleus of bradyzoite; R = rhoptry; S = septum; VP = villar projections; C = conoid. Scale bar = 0.18 μm.

Numerous species of *Sarcocystis* are found in muscles of mammals, reptiles, and birds, but none has been reported from the porcupine (Dubey et al., 1989). In this paper, a new species of *Sarcocystis, S. sehi,* is reported from muscles of the porcupine, *Erethizon dorsatum* Linnaeus, 1758.

Specimens of esophagus, diaphragm, masseter muscles, tongue, and heart of 17 (5 males and 12 females) porcupines were fixed in 10% buffered neutral formalin. The porcupines were live-trapped during 1989 and 1990 in State Gamelands-13, Sullivan County, Pennsylvania (76°N, 41°20'W), euthanatized, and necropsied as part of baseline data collection at site selections for a potential mainland field trial with an oral recombinant rabies vaccine for raccoons (Rupprecht et al., 1986). Paraffin-embedded sections were cut at 5–6 μm, stained with hematoxylin and eosin, and examined microscopically. Formalin-fixed muscles from tongue of 1 porcupine were processed for transmission electron microscopy.

Mature sarcocysts (Figs. 1–4) were found in 10 (7 females and 3 males) porcupines, in masseter muscles of 9 of 16, tongues of 8 of 17, diaphragms of 7 of 15, esophagi of 3 of 17, and in heart of 1 of 17. Sarcocysts were longer in masseter muscles than in other muscles. Some sarcocysts were located either in a single myocyte or in adjacent myocytes giving the impression of thick compartments within a single sarcocyst (Fig. 2). Under the light microscope the sarcocyst wall was less than 1 μm thick and had minute serrations (Fig. 4).

Sarcocystis sehi sp. n. (Figs. 1–6)

DIAGNOSIS: Sarcocysts in myocytes, up to 2.3 mm long and 125 μm wide, cyst wall with spiny villar projections. Ultrastructurally, sarcocyst wall 0.6–1.3 μm thick including the villar projections, parasitophorous vacuole membrane wavy with conical villar projections without microtubules at uneven distances and short (60 nm) electron-dense circular to tombstone-like blebs. Bradyzoites approximately 7 × 1.5 μm with typical apicomplexan organelles, including numerous micronemes restricted to anterior half of bradyzoites (Figs. 5, 6).

HOST: *Erethizon dorsatum.*

DISTRIBUTION: United States.

SYNTYPE SPECIMENS: Section of tongue and masseter muscles from a naturally infected porcupine deposited in U.S. National Museum. USNM Nos. 81942 and 81943.

ETYMOLOGY: The species name is derived from the Hindi name sehi for the porcupine.

Sarcocystis species generally are considered host specific. The structure of the sarcocyst wall is a reliable criterion for distinguishing *Sarcocystis* species in a given host but not between different hosts (Dubey et al., 1989). Based on the structure of the sarcocyst walls, Dubey et al. (1989) grouped *Sarcocystis* species into 24 types. The structure of the sarcocyst wall of *S. sehi* is distinct from sarcocysts of any other species. The blebs on the sarcocyst walls in porcupines are structurally similar to those on the sarcocyst walls of type 1 species (e.g., *Sarcocystis muris*), but in type 1 species, the sarcocyst wall lacks the conical villar projections.

Sarcocystis species have a prey–predator 2-host life cycle. The predator becomes infected by eating sarcocysts from the tissues of the prey. Natural predators of porcupines include bobcats, coyotes, fishers, wolves, and cougars (Dodge, 1982).

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Research Note

Helminths of the Bunch Grass Lizard, *Sceloporus scalaris slevini* (Iguanidae)

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ABSTRACT: Thirty-eight *Sceloporus scalaris slevini* Smith, 1937, from Cochise County, Arizona were examined for helminths. Tetrathyridia of the cestode, *Mesocestoides* sp. Vaillant, 1863, were found in the coelom of 3 lizards. Third-stage larvae of the nematode, *Physaloptera* sp. Rudolphi, 1819, were recovered from the stomach of 1. Cestode prevalence was 8%, mean intensity 39.3; nematode prevalence was 3%, intensity 6. The finding of these helminths represents new host records.

KEY WORDS: Cestoda, *Mesocestoides* sp., Nematoda, *Physaloptera* sp., prevalence, intensity, *Sceloporus scalaris*, Iguanidae.

The bunch grass lizard, *Sceloporus scalaris*, occurs in the Huachuca, Dragoon, Santa Rita, and Chiricahua Mountains of Arizona, the Animas Mountains of New Mexico, and in the Sierra Madre Occidental and Sierra del Nido of Mexico, mostly above 1,830 m (a few isolated valley populations as low as 1,220 m) (Stebbins, 1985). To our knowledge, there have been no reports of helminth parasitism in this species. The purpose of this note is to report the results of a helminth survey of *Sceloporus scalaris slevini* Smith, 1937.

We examined 38 *Sceloporus scalaris* (mean snout–vent length 49 ± 5 mm SD, range 34–58 mm) from the Angelo State Natural History Collections, Angelo State University, San Angelo, Texas (Appendix 1). Lizards had been preserved in 10% formalin and were later stored in ethyl alcohol. Eight had been collected 13 km W of Portal, Chiricahua Mountains, 31°55′N, 109°16′W, Cochise County, Arizona (2,438 m elevation) in September 1972; 30 were from the vicinity of Rustler's Park, Chiricahua Mountains, 31°54′N, 109°16′W, Cochise County (2,560 m elevation) May–June 1973. The abdomen was opened and the esophagus, stomach, and small and large intestines were removed. Each organ was slit longitudinally and examined under a dissecting microscope. The liver and body cavity were also examined. Helminths were identified after preparation of glycerol wet mounts.

Only 3 of the 38 *S. scalaris*, all from the 1973 collection, were infected with helminths (prevalence 8%). These had 58, 52, and 8 tetrathyridia of *Mesocestoides* sp., respectively (mean intensity 39.3), within their body cavities. One lizard also had 6 third-stage *Physaloptera* sp. in its stomach (prevalence 3%). Representative helminths were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): *Mesocestoides* sp. (81343) and *Physaloptera* sp. (81344).

Mesocestoides is a cosmopolitan genus of cyclophyllidean cestodes for which the complete life cycle is still unknown. Its unique larval form, the tetrathyridium, is commonly found in mammalian, avian, and reptilian intermediate hosts and is readily infective to predatory definitive hosts (Schmidt, 1986). Goldberg and Bursey (1990) have reviewed the prevalences of *Mesocestoides* sp. in North American lizards. Our recovery of this parasite from *S. scalaris* represents a new host record and is the thirty-first lizard species from which it has been reported. The prevalence of 8% for tetrathyridia of *Mesocestoides* sp. is within the range of prevalences (0–27%) reported in other North American lizards.

Physaloptera is a cosmopolitan genus of spirurid nematodes occurring in reptiles, birds, and mammals; the life cycle is indirect, requiring an intermediate host (Olsen, 1974). Eggs contain first-stage larvae when laid and are passed in the host's feces. They are immediately infective to arthropod intermediate hosts. Arthropods harboring third-stage larvae are the source of infection in lizards (Olsen, 1974). Our recovery of third-stage *Physaloptera* sp. from *S. scalaris* represents a new host record.

Our finding of only immature helminths suggests a limited parasite fauna for *S. scalaris*. We have no explanation for the lack of adult helminths. Baker (1987) listed an average of 2.7 (range 1–8) adult nematodes for 14 species of

Sceloporus. This difference between *S. scalaris* and other sceloporine lizards may lie in ecological attributes. *Sceloporus scalaris* inhabits grass clumps whereas most other sceloporine lizards are geophilic. This habitat difference may be enough to eliminate the majority of insect intermediate hosts from the diet as well as remove the lizard from areas contaminated by feces. Further investigation of the helminths of this species would be appropriate.

We thank Raymond Stone, Jr., Department of Biology, Angelo State University, San Angelo, Texas, for allowing us to examine *S. scalaris* from the Angelo State Natural History Collections.

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Appendix 1

Sceloporus scalaris examined from Angelo State Natural History Collection: 10728–10735, 11019, 11348–11351, 11358, 11360–11369, 11382, 11383, 11387, 11390, 11392–11394, 11492, 11499, 11609, 11612–11615.

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Research Note

Helminths of the Marine Toad, *Bufo marinus* (Anura: Bufonidae), from American Samoa

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ABSTRACT: The gastrointestinal tracts, lungs, livers, and urinary bladders of 97 *Bufo marinus* were examined for helminths. The nematode, *Parapharyngodon kartana,* was recovered from 1 toad (prevalence 1%). This occurrence represents a new host record. The trematode, *Mesocoelium monas,* was also recovered; prevalence 100% (mean intensity 101) from *B. marinus* collected on Tutuila Island and prevalence 80% (mean intensity 19) for toads from Aunu'u Island. This finding extends the range of *M. monas* to the Pacific Islands.

KEY WORDS: Trematoda, *Mesocoelium monas,* Nematoda, *Parapharyngodon kartana,* Bufonidae, *Bufo marinus,* prevalence, intensity, American Samoa.

The marine toad, *Bufo marinus* (Linnaeus, 1758), originally ranged from southern Texas to central Brazil (Zug and Zug, 1979), but was introduced to many areas including the Caribbean Islands, Pacific Islands, and Australia (Easteal, 1981). It was introduced into Tutuila Island, American Samoa, from Hawaii in 1953 to control insects and later to Aunu'u Island. The population on Tutuila Island was estimated to be 2,296,000 in 1976 by Amerson et al. (1982). To our knowledge, the helminth fauna of *B. marinus* from American Samoa has not been investigated. The purpose of this report is to present findings of an examination of *B. marinus* from American Samoa for helminths.

Ninety-seven *B. marinus* were examined. Eighteen (mean snout–vent length [SVL] 89.5 mm, range 53–127 mm) were collected April–May 1989 from Tutuila Island, American Samoa (14°17′S, 170°41′W); 23 (mean SVL 91.7 mm, range 75–122 mm) were collected January 1990 also from Tutuila Island. Fifty-six (mean

SVL 77.6 mm, range 20–111 mm) were collected January 1990 from Aunu'u Island (14°17'S, 170°33'W). The 1989 sample was examined for cestodes and nematodes; the 1990 sample was examined for cestodes, nematodes, and trematodes. Specimens of the toads were deposited in the herpetology collection of the Los Angeles County Museum of Natural History (LACM): Tutuila Island, 138688–138728; Aunu'u Island, 138729–138784. The body cavity was opened and the esophagus, stomach, and small and large intestines were removed to a dissecting pan, slit longitudinally, and examined under a dissecting microscope. The surfaces of the liver, body cavity, lungs, and urinary bladder were also examined for helminths. Each helminth was identified utilizing a glycerol wet mount. Representative helminths were deposited in the U.S. National Parasite Collection, USDA, ARS, Beltsville, Maryland 20705: *Parapharyngodon kartana* (81917) and *Mesocoelium monas* (81918).

Mesocoelium monas (Rudolphi, 1819) was found in both Tutuila and Aunu'u Island populations of *B. marinus*. In American Samoa, *B. marinus* is restricted to Tutuila and Aunu'u Islands (Amerson et al., 1982). Prevalence of *M. monas* was 100% (23/23), mean intensity 101, range 2–696 in the Tutuila Island population, and 80% (45/56), mean intensity 19, range 1–148 in the Aunu'u Island population. There were significant differences in prevalence and mean intensity of *M. monas* between island populations ($\chi^2 = 5.24$, 1 df, $P < 0.05$ and 56.03, 1 df, $P < 0.001$, respectively). This difference may relate to the relative abundance of sigmurethrid snails (see Fischthal and Kuntz, 1967) on the 2 islands, but to our knowledge a study of relative abundance of Samoan land snails has not been published.

Freitas (1963) has synonymized 19 species of the genus *Mesocoelium* from a wide variety of amphibians and reptiles with *M. monas*. The list of synonymized species was expanded to 23 by Nasir and Diaz (1971) who in the process recognized only 4 species. Nasir and Diaz (1971) believed only 2 characters, the sucker ratio and egg size, are necessary for species determination. We have identified our specimens as *M. monas* because they have a sucker ratio of 1:1 and eggs that average 35 μm in diameter. *Mesocoelium monas* has been recovered from *B. marinus* from widely separated geographical regions such as Brazil, Colombia, Costa Rica, Hawaii, Paraguay, and Puerto Rico (Nasir and Diaz, 1971). Our findings extend the range of this parasite to the Pacific Islands.

Five female nematodes, which we identified as *Parapharyngodon kartana* (Johnston and Mawson, 1941) (prevalence 6%, 1/18), were found in 1 of the toads collected in 1989. No nematodes were found in the 1990 sample; thus, by combining the samples, prevalence is reduced to 1% (1/97). Although no male nematodes were recovered, we base our identification on a sample of *P. kartana* taken from the skinks, *Emoia nigra* and *Emoia samoense,* also collected on Tutuila Island in 1990 (Goldberg and Bursey, 1991). The measurements of the specimens from *B. marinus* were within the range of those reported by Angel and Mawson (1968) and are identical to the nematodes we recovered from *E. nigra* (Goldberg and Bursey, 1991). Currently 33 species of *Parapharyngodon* are recognized and various species have been recovered from hosts in 11 families of lizards, 2 of snakes, and 2 of frogs (Baker, 1987). Given the cosmopolitan distribution of the genus and its absence in bufonid amphibians, we believe its occurrence in 1 toad to be a case of pseudoparasitism. Because *B. marinus* is sympatric with *E. nigra* and *E. samoense,* it is possible that skinks may occasionally be taken as prey.

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Research Note

Cryopreservation of Infective Third-stage Larvae of *Strongyloides ratti*

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ABSTRACT: Infective third-stage larvae of *Strongyloides ratti* were successfully cryopreserved using a modification of the procedure developed for *Strongyloides stercoralis*. The larvae were frozen in a mixture of DMSO and dextran (10% of each in water) in the vapor phase of liquid nitrogen. Cryopreserved larvae were thawed into RPMI-1640 cell culture medium, and incubated overnight in an invertebrate saline to allow time for injured worms to die. The surviving larvae accounted for only 1% to 10% of those frozen, but when they were injected into rats a patent infection was produced.

KEY WORDS: *Strongyloides ratti*, cryopreservation.

Strongyloides ratti, a parasite of rats, is a frequently used laboratory model for species of *Strongyloides* that infect domestic animals and man. Because the rat eliminates an *S. ratti* infection in 3 to 4 wk, it is expensive to maintain this parasite, especially if more than 1 strain is being used. In fact, when we decided recently to re-establish this parasite in our laboratory, we were unable to find a laboratory in the United States that was maintaining it. Although both *Strongyloides stercoralis* (Nolan et al., 1988) and *Strongyloides papillosus* (Van Wyk et al., 1977) have been cryopreserved, the latter was not infective for its host (sheep) upon thawing. Therefore, this investigation was undertaken to determine whether our method for freezing *S. stercoralis* would not only cryopreserve *S. ratti*, but also maintain its infectivity.

The strain of *S. ratti* used in this investigation was G-60 (given to us by Dr. M. E. Viney, University of Edinburgh, Edinburgh, Scotland). This is a heterogonic strain originally isolated from a wild rat by Dr. G. Graham at the University of Pennsylvania. In our laboratory this strain was maintained in both Wistar rats and multimammate rats (*Mastomys natalensis*). Third-stage infective larvae (L_3) were obtained from 7-day-old coprocultures by baermannization, and washed twice in distilled water. These larvae were held in the freezing medium described for *S. stercoralis* (10% DMSO and 10% dextran; Nolan et al., 1988) for 15 to 90 min, depending on the experiment. They were then frozen in the vapor phase of liquid nitrogen and stored there for 7 to 330 days. The length of time spent frozen had no effect on the survival of the larvae, as was also described for other parasitic nematodes (Nolan et al., 1988; Van Wyk et al., 1990).

The larvae were thawed as described (Nolan et al., 1988) for *S. stercoralis* and were then resuspended in BU, a buffered saline designed for invertebrates (Hawdon and Schad, 1991). It is important to wash the larvae several times after thawing in order to remove all of the freezing medium since it is slightly toxic to the L_3's. Counts made 30 min after thawing showed no significant difference in survival (as judged by movement) between larvae frozen after incubation at room temperature for 15 to 90 min (Fig. 1). However, when the same larvae were counted approximately 20 hr after being thawed, survival had decreased significantly in all groups, but significantly more survived in the group given a 60-min incubation than in either the 15- or 30-min

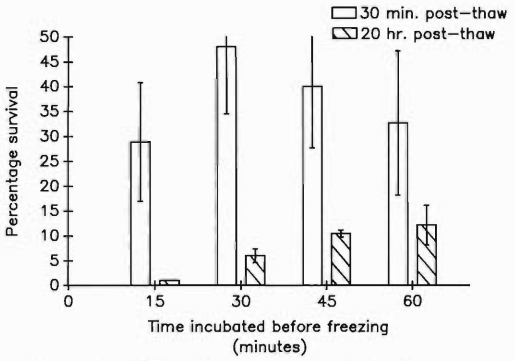

Figure 1. Effect of pre-freezing incubation in cryoprotectant on the survival of cryopreserved *Strongyloides ratti* third-stage larvae (L_3). Combined results of 8 experiments. Error bars = 1 standard deviation.

groups ($P < 0.05$, Mann-Whitney U-test). Using the same freezing method, the survival of *S. ratti* L_3's (32.7 ± 14.5%), as measured 30 min after thawing (60-min incubation group), was lower than that seen for *S. stercoralis* (55.0 ± 5.5%; Nolan et al., 1988).

When either multimammate or Wistar rats were injected subcutaneously with cryopreserved larvae within 1 hr of thawing, the infection became patent in very few animals (Table 1). However, when the larvae were incubated overnight in BU at 25°C, all rats that were injected developed patent infections (Table 1). After this overnight incubation, only about 1% of the thawed larvae were highly active, although 10.2% were still alive (a decrease from 32.7% at 30 min after thawing). Live larvae were separated from dead with a modified baermann apparatus. Tissue paper was placed in a sieve that was partially submerged in BU, the thawed larvae were placed on the tissue paper and incubated for 1 to 2 hr before they were collected from the BU by centrifugation. Only small numbers of larvae were finally recovered, suggesting that only the highly active larvae were penetrating the tissue paper.

Coprocultures made from Wistar rats injected with larvae 24 hr after thawing produced enough L_3's to infect another rat that developed a patent infection. From this rat, normal numbers of L_3's were obtained from charcoal-fecal cultures. Although rats receiving between 1,000 and 2,500 L_3's 24 hr after thawing developed patent infections, only a few adult worms were found in their intestines 10 to 14 days postinjection (Table 1). To increase the number of worms becoming adult, rats were immunosuppressed with methylprednisolone acetate (Depo-Medrol, Upjohn, Kalamazoo, Michigan). Subcutaneous injections of methylprednisolone acetate (2.5 mg/kg) were given on day −1, day 0, and days 5, 8, and 12. The 2 rats thus immunosuppressed were found to have more adult worms than the untreated rats (Table 1), but still far fewer than rats given third-stage larvae that had never been cryopreserved. When 1,000 normal L_3's were given to 2 rats, an average of 316 adult worms was recovered from the small intestine 10 days postinjection.

Although the system developed for freezing *S. stercoralis* (Nolan et al., 1988) is less effective for *S. ratti,* it can be used to cryopreserve the latter provided that the following modifications are

Table 1. Infections of rats with cryopreserved *Strongyloides ratti* third-stage larvae (L_3).

Dose of L_3's	Host	Time of injection of L_3's (after thawing)	Immuno-suppressed?	Result of charcoal-fecal culture	Prepatent period (days)	No. of worms recovered*
3,000	*Mastomys*	<1 hr	no	−	NA	ND
3,000	*Mastomys*	<1 hr	no	−	NA	ND
10,000	*Mastomys*	<1 hr	no	+	7	ND
12,000	*Mastomys*	<1 hr	no	−	NA	ND
20,000	*Mastomys*	<1 hr	no	−	NA	ND
1,000	*Rattus*	<1 hr	no	−	NA	0 (10)
1,000	*Rattus*	>20 hr	no	+	6	2 (10)
2,000	*Rattus*	>20 hr	no	+	7	0 (11)
2,500	*Rattus*	>20 hr	no	+	7	1 (14)
2,500	*Rattus*	>20 hr	yes	+	8	10 (14)
9,000	*Rattus*	>20 hr	yes	+	6	13 (27)

* (Day postinjection on which worms were recovered).
NA = not applicable; ND = not done.

used: 1. Because the recovery after thawing is less than 1%, large numbers of L_3's must be frozen (>100,000). 2. Upon thawing, the larvae should be incubated in BU at room temperature for 20 to 24 hr, allowing the injured L_3's to die. Thereafter, the living L_3's are collected. 3. Although it is not absolutely necessary, immunosuppression of the rat, before and after the subcutaneous injection of the L_3's, can be used to increase the number of adults maturing in the small intestine. 4. Because few adults develop from cryopreserved L_3's, it is necessary to amplify the infection by passage through another rat to obtain sufficient *S. ratti* for most experimental purposes.

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Research Note

Histochemical Observations on *Cyathostoma lari* (Strongyloidea: Syngamidae)

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ABSTRACT: The location and relative abundance of chemical components of adult female *Cyathostoma lari* were determined using a variety of histochemical methods. Carbohydrates, acid mucopolysaccharides, lipids, and proteins were widely distributed throughout the nematode. Host blood and hemoglobin were detected in the gut lumen, with hemoglobin also being demonstrated in the pseudocoel. Ribonucleic acid, mitochondria, succinic dehydrogenase, and acid and alkaline phosphatases were located in the digestive and reproductive systems. The data suggest that the anterior intestine plays the most important role in digestion. The role and importance of the various substances are discussed.

KEY WORDS: histochemistry, Nematoda, *Cyathostoma lari*.

Much work has been performed on the physiology and biochemistry of metazoan parasites, particularly digeneans and cestodes (von Brand, 1979; Chappell, 1980; Barrett, 1981; Smyth and McManus, 1989). One of the least examined groups has been the nematodes, with species of medical and veterinary importance receiving the most attention. Within this group larvae and eggs have been the objects of greatest attention. In examining the chemical constituents of nematodes, studies have to a large extent been performed using homogenates of whole worms (Singh and Sharma, 1981; Chopra, 1986; Rao and Rajlingam, 1989). Few studies have been concerned with determining the precise location of various substances within nematodes (Sood and Kalra, 1977; Sood and Sehajpal, 1978; Maki and Yanagisawa, 1980; Sharma and Singh, 1985).

The distribution of the chemical components of a parasite is a reflection of where biochemical processes are occurring, with intensity and type of reaction perhaps changing at different times during the host's life cycle. Host physiology and location of the parasite within the host will also affect the physiological status of the parasite. Phylogenetic differences among hosts and among parasite species might also be reflected in the parasite's physiology. As noted by Threlfall et al.

(1990), data to support such a contention are rare. The present histochemical study was undertaken to determine the distribution and relative concentrations of various substances throughout the body of adult female *Cyathostoma lari* Blanchard, 1849, recovered from herring gull (*Larus argentatus* Pontoppidan, 1763) chicks taken in both Wales and Newfoundland, and to compare the results with previous works. *Cyathostoma lari* is an obligate, blood-sucking nematode that lives in the nasal cavities of a variety of birds including gulls (*Larus* spp.) (Barus et al., 1978). A plug of host mucosa is drawn into the buccal cavity of the worm and lacerated with cutting plates so that blood may flow freely through the worm (Threlfall, 1966; Colam, 1971a). This study also expands on the work of Colam (1971a) who studied the gut ultrastructure and digestive physiology of this species, as well as *Rhabdias bufonis, R. sphaerocephala,* and *Cosmocerca ornata* (Colam, 1971b, c).

Worms were fixed in situ by flooding the sinuses of freshly killed gull chicks with a variety of fixatives, including Altmann's, cold acetone (4°C), 90% ethyl alcohol, Susa's, and Zenker's depending on the tests to be performed. The worms were then removed from the sinuses, blocked at 54°C in paraffin wax, and sectioned at 5, 10, and 15 μm. A small number of worms were recovered alive, quick-frozen, and sectioned at 10 and 15 μm using a freezing microtome. Carbohydrates were detected in Zenker's-fixed worms, using Best's carmine, and the PAS reaction, with and without amylase treatment (Gurr, 1956, 1958; Pearse, 1968). The presence of acid mucopolysaccharide was revealed in Susa's-fixed specimens using the thionin, toluidine blue, and Hale's dialysed iron methods outlined by Gurr (1958). Lipids were stained using Sudan Black B in specimens fixed in Altmann's and Zenker's or in frozen specimens, or copper phthalocyanin using methanol fast blue stain (Pearse, 1968). Controls were with Sudan Black B plus pyridine extraction. Mercuric bromphenol blue with and without deaminase was used to detect proteins in Zenker's-fixed worms (Mazia et al., 1953; Pearse, 1968). The digestive tract of the nematode contained host blood, and the pseudocoel was filled with a red pigment-containing fluid. To determine the distribution of hemoglobin within the worm, Zenker's-fixed sections were stained with Giemsa stain and benzidine (Gurr, 1958). Ribonucleic acid was detected using the pyronine/methyl green method with and without ribonuclease (Gurr, 1958). Mitochondria were demonstrated in frozen sections by the presence of succinic dehydrogenase using the NBT technique (Pearse, 1972; Bancroft, 1975) and in Altmann's-fixed sections with Metzner's stain (Metzner and Krause, 1928). Acid phosphatase was detected using the Gomori lead nitrate, Burnstone's, and the modified Gomori methods (Pearse, 1968), whereas alkaline phosphatase was demonstrated using the calcium cobalt (Gomori, 1952) and modified Gomori methods (Pearse, 1968). Tests for phosphatases were performed on material that had been fixed in cold acetone (4°C), except in the case of the modified Gomori test for alkaline phosphatase where specimens were fixed in 90% ethanol. Controls were performed as follows: acid phosphatase, incubated as for the test but 0.01 M sodium fluoride was included in the reaction medium; alkaline phosphatase, 3% sodium-b-glycerophosphate in the medium was replaced by distilled water (Chayen et al., 1973).

The distribution and amounts or activity of various chemical components in the digestive and reproductive tracts of *C. lari* are shown in Table 1. Glycogen comprises the principal carbohydrate reserve in nematodes, with the amount varying from species to species (Fairbairn, 1958, 1960; Barrett, 1981). A general orthochromasia was noted throughout the worm with the highest concentrations of carbohydrates being seen in the anterior intestine (Table 1). Sood and Sehajpal (1978) noted similar results in *Haemonchus contortus.* The ovaries of *C. lari* showed a similarly intense reaction. The cuticle of this helminth is extremely thin, particularly when compared to that of many intestinal inhabitants, e.g., *Ascaris lumbricoides,* and was negative in tests for carbohydrates, whereas the hypodermis and cytoplasmic portion of the muscle cells stained lightly. A similar situation was noted by Sood and Kalra (1977) in *Haemonchus contortus* and *Xiphinema insigne.* Acid mucopolysaccharide, an ubiquitous substance in nematodes (Barrett, 1981), was present in all the body tissues except the cuticle and the eggshell/membranes. Sood and Kalra (1977) noted the presence of this substance in the inner cortical layer of the cuticle of *H. contortus* and *X. insigne.* Sood and Sehajpal (1978) were unable to demonstrate acid mucopolysaccharide or glycogen in the brush border of the gastrodermis of *H. contortus.* In the present study, considerable amounts of both glycogen and acid mucopolysaccharide were present in the

Table 1. Histochemistry of the digestive and reproductive systems of adult female *Cyathostoma lari*.

	Body region								
	Buccal region	Esoph-agus	Intestine			Rectum	Ovary	Ovum (in shell)	Egg-shell
			Ant.	Mid.	Post.				
Carbohydrates	+	+	++	+	+	+	++	+	−
Acid mucopolysaccharide	+	+	+	+	+	+	+	+	−
Lipids	−	−	+++	++	++	−	++	+	++
Proteins	+	+	++	++	+	+	+++	++	+
Ribonucleic acid	−	−	+++	++	++	+	+++	+	−
Mitochondria	−	−	+++	++	++	+	++	+	−
Succinic dehydrogenase	−	−	+++	++	++	+	++	+	−
Acid phosphatase	−	−	+++	++	+	+	++	+++	−
Alkaline phosphatase	−	−	+++	++	+	+	++	+++	−

+++ = strongly positive; ++ = moderately positive; + = weakly positive; − = negative.

gastrodermis. Monné (1959) showed the presence of acid mucopolysaccharide in the eggshell and membranes of 3 species of lungworms, a finding at odds with the present work. Helminths such as *C. lari* that have access to a rich supply of oxygen via the host blood or live in aerobic conditions tend to have little glycogen in their tissues. This is unlike the situation seen in anaerobic or anoxybiotic organisms such as *Ascaris lumbricoides* and *Porrocaecum decipiens* (Barrett, 1981).

Lipids were detected in the cuticle, hypodermis, and muscle cell cytoplasm. This distribution was similar to that noted by Sood and Kalra (1977). The intestine was particularly rich in lipid globules as noted previously by Colam (1971a). Sood and Sehajpal (1978) observed lipids in the esophagus and intestine of *H. contortus* and noted that lipids may well form a major storage product in nematodes. No lipids were detected in the esophagus of *C. lari,* whereas the intestine stained intensely for these substances. The concentration of lipid globules in the intestinal wall of *C. lari* may represent the end-product of digestion in the gut lumen and the syncytium of the gastrodermis. The greater concentration in the anterior region of the intestine suggests that this is where the greatest biochemical activity is occurring. The ovaries were well supplied with lipids, with lesser amounts being detected in the ovum. The eggshell was rich in lipids, a phenomenon previously reported in other nematode species (Rogers, 1962; Seesee, 1977). Lipids were confined to the outer and inner layers of the 3-layered eggshell.

Proteins form the major structural component of *C. lari* as reflected by the general orthochromasia with mercuric bromphenol blue. The cuticle, hypodermis, lateral cords, and muscles, particularly the myofilaments, stained lightly. The intestine and reproductive system were rich in proteins. Many enzymes are proteinaceous in nature. The present results suggest that the anterior intestine is most heavily involved in the digestive process. This observation supports the work of Colam (1971a), but refines our knowledge by showing that enzymatic activity may vary along the length of the intestine. The ovary is a region of intense protein, DNA, and mitochondrial activity resulting in the formation of ova. The eggshells stained quite intensely with mercuric bromphenol blue, probably as a result of their being composed of lipoproteins (Seesee, 1977).

RNA was detected in the intestine; the most intense reaction was in the anterior portion, with less intense reactions in the mid and posterior portions and rectum. The ovaries and ova were also rich in this substance. The hypodermis and hypodermal cords did not stain for RNA, which differs from the results of Sood and Kalra (1977), who noted a reaction in the hypodermis, hypodermal cords, and inner cortical layer of the cuticle of *H. contortus*. Barrett (1981) noted the types of RNA found in nematodes and discussed their importance in protein building. The gut contents of the nematode, host blood containing nucleated erythrocytes and leucocytes, stained intensely for RNA.

Cyathostoma lari is an obligate hematophage, with its "normal" red coloration being due to the presence of host blood in its gut and a red pigment in its pseudocoel. Tests revealed the presence of hemoglobin (Hb) in the intestinal lumen, and in the protein-containing pseudo-

coelomic fluid. No attempt was made to characterize the Hb in the pseudocoel. Colam (1971a) noted Hb in the gut lumen of *C. lari* and as small granules below the brush layer of the gastrodermis after hemolysis. Sood and Sehajpal (1978) noted Hb in the same location in the intestine of *H. contortus,* whereas Sood and Kalra (1977) identified Hb in the cuticle of *H. contortus.* Rose and Kaplan (1972), working on the closely related species *Syngamus trachea,* noted that the Hb from the worm was composed of only 1 component and had a different molecular weight than that extracted from the host blood. This result differs from that of van Grembergen (1954) who showed that the Hb of *Heterakis gallinae* had characteristic alpha and beta absorption bands that were similar to those shown by the Hb from host blood. Hemoglobins in nematodes seem to have a very high affinity for oxygen, even at very low partial pressures. Oxyhemoglobin will, therefore, only dissociate at very low tissue concentrations of oxygen, and it seems improbable that the Hb in an aerobic species, such as *C. lari,* will be used for oxygen transport. The differences noted above in the types of Hb found in nematodes, as well as the function of Hb, are deserving of further investigation.

Mitochondria, indicated by the presence of succinic dehydrogenase, were detected in greatest amounts in the anterior part of the intestine. Lesser amounts were present in the remainder of the digestive tract (except for the wall of the buccal cavity and esophagus where they were absent). The reproductive tract was also positive. These locations all correspond to sites of great biochemical activity. Colam (1971a) showed that digestion and absorption of blood cells occurred in the gastrodermis of *C. lari,* and that numerous multicristate mitochondria were present in specific regions of the tissue. Monné (1959) discussed the mitochondria of developing lungworm ova, and noted that they are numerous both at this stage and in adult worms.

Acid and alkaline phosphatases were widely distributed in the digestive and reproductive tracts, regions where intense biochemical activity might be expected. Again the anterior intestine appeared to be the region where most activity occurred, with a decline of activity in the posterior regions. Both these substances are associated with digestion (Colam, 1971a; Riley, 1973). The presence of acid phosphatase has been used as an indicator of lysosomal activity (Duvé, 1963; Novikoff, 1963) and would be expected to occur in areas where intense biosynthesis is occurring. The role, and distribution, of phosphatases in cestodes is somewhat better understood than in nematodes (Threlfall et al., 1990). Arme and Read (1970) and Mayberry and Tibbitts (1972) suggested that alkaline phosphatase is involved in active transport and/or digestion. Other workers (Sood and Kalra, 1977; Sood and Sehajpal, 1978; Maki and Yanagisawa, 1980) have shown a more general distribution of phosphatases in nematodes than was seen in *C. lari.* In the present work the cuticle, hypodermis, hypodermal cords, and muscles appeared to be free of phosphatases.

Cyathostoma lari is typical of many strongyloids in possessing a limited number of cells (Chitwood and Chitwood, 1974) and a syncytial intestinal wall (Colam, 1971a). The presence of a syncytial intestine, which has a well-developed bacillary (microvillar) layer, may be an adaptation to hematophagy. Differences noted in the amounts of various substances along the length of the intestine suggest differential enzymatic action along its length and are worthy of further study.

It became obvious that marked differences do occur in the presence and distribution of substances in different nematode species. Our knowledge of such differences is at present rudimentary. A more complete understanding of the physiological and biochemical processes occurring in nematodes will be aided by further histochemical studies, including a comparison of male and female worms. A preliminary study of male *C. lari,* which are much rarer and smaller than the females (Burt and Eadie, 1958), revealed chemical substances and distributions similar to those noted above. It is possible that similarities or differences among nematode species might be partially explained by differing habitats of the parasites, stage of development, host phylogeny, and/or host physiological differences.

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Research Note

Experimental Fascioliasis in Llamas

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ABSTRACT: Three llamas and 2 domestic sheep were inoculated orally with metacercariae of liver flukes, *Fasciola hepatica.* The prepatent period in llamas and sheep was 8–12 wk. Sizes of fluke eggs passed in feces were similar between llamas and sheep. At necropsy, the percentages of original inoculum recovered from the llamas and sheep were 24% and 22%, respectively. Sizes of flukes recovered from livers were similar between llamas and sheep. The gross appearance of the livers from the llamas varied from slight discoloration with some bile duct thickening to marked fibrosis and scarring. Llama livers were similar histologically. Bile duct hyperplasia, portal fibrosis, and granulomas, often containing degenerated trematode eggs and necrotic debris, were hallmarks of infection. These changes resembled chronic fascioliasis in sheep. The data indicate that llamas, like domestic sheep, have low resistance to liver fluke infection.

KEY WORDS: *Fasciola hepatica,* liver flukes, experimental infection, llama, *Lama glama,* sheep, *Ovis aries.*

Fasciola hepatica is a prevalent and economically important trematode parasite of cattle and sheep in the United States. In endemic areas goats, rabbits, swine, horses, and man may also become infected (Leathers et al., 1982; Soulsby, 1982; Malone, 1986; Wescott and Foreyt, 1986). In the United States, natural infections of *F. hepatica* have been reported in 1 llama in Texas and 1 llama in Oregon (Cornick, 1988; Rickard and Bishop, 1991). The purpose of this study was to determine the prepatent period of *F. hepatica* in llamas, describe the lesions associated with mature infections, and compare them with those in domestic sheep.

Three healthy adult female llamas (*Lama glama*), 5–7 years old, were donated for research purposes because of reproductive or conformational problems. All were maintained on pasture and were supplemented with hay when needed. All pastures used were known fluke-free pastures and none of the animals had any history of liver fluke infections prior to experimental infection. All llamas were clinically normal. Two of the llamas (nos. 2 and 3) were also given larvae of meningeal worm, *Parelaphostrongylus tenuis,* on day 18 of this experiment. Because *P. tenuis* in llamas is confined to the neurologic system (Baumgartner et al., 1985; Krogdahl et al., 1987), it was considered that it would not directly affect the liver fluke infection. Two healthy domestic sheep (*Ovis aries*), 1.5-year-old wethers, were purchased as lambs from a known *F. hepatica*-free area, and were housed on pasture until the start of the experiment when they were moved indoors and housed on concrete.

On day 0, 250 (llama 1) or 500 (all other animals) metacercariae of *F. hepatica* (Baldwin Enterprises, Monmouth, Oregon) were administered to each animal orally either by stomach tube (llamas) or gelatin capsule (sheep). Rectal fecal samples were collected and animals were weighed at approximately 2-wk intervals throughout the trial. Animals were observed daily for signs of clinical parasitism.

Five grams of feces was examined at each sampling period for eggs of *F. hepatica* with a sedimentation technique. For llama 1, the samples were scored as negative or positive, and for the other animals, actual numbers of fluke eggs per gram of feces were determined. A minimum of 20 eggs from each positive sample were measured using a microscope equipped with an ocular micrometer.

On day 157 postinfection, llama 1 was euthanized for reasons unrelated to parasitism. On

postinfection day 69, llama 2 was euthanized because of incoordination caused by *P. tenuis* infection (Foreyt et al., 1991). The following day sheep 2 was euthanized for comparative purposes. On day 83 postinfection, llama 3 died from causes related to *P. tenuis* infection (Foreyt et al., 1991). Sheep 1 was euthanized the next day for comparative purposes. At necropsy, the liver and duodenum were removed intact. Recovery and enumeration of flukes were as previously described (Rickard and Bishop, 1991) except live, intact flukes from all animals except llama 1 were measured to the nearest mm after relaxation in water. Representative pieces of liver were fixed in 10% neutral buffered formalin and were later processed by routine histologic techniques and stained in hematoxylin and eosin.

In the first llama, the prepatent period (PPP) of *F. hepatica* was 84 days, whereas in the other 2 llamas it was only 56 days (Table 1). For the sheep, the PPP was 63 days (Table 1), 1 wk longer than that for the llamas infected at the same time. The total number of liver flukes recovered from each animal, with percentage of original inoculum in parentheses was: llama 1, 82 (32.8%); llama 2, 68 (13.6%); llama 3, 154 (30.8%); sheep 1, 129 (25.8%); and sheep 2, 87 (17.4%). The mean lengths of flukes recovered from llamas 2 and 3 (13.0 ± 3.9 mm and 16.8 ± 3.0 mm, respectively) were slightly less than those from flukes of the same age recovered from sheep 2 and 1 (15.9 ± 3.7 mm and 17.7 ± 3.6 mm) (Table 2). However, the differences in size were minimal with almost complete overlap in range. Mean sizes of *F. hepatica* eggs were 123.2 × 68.6 mm in llamas ($N = 240$) and 125.4 × 67.8 mm in sheep ($N = 200$).

Weights of all llamas fluctuated during the experiment with some weight loss, but all were in good body condition at necropsy with adequate amounts of mesenteric and subcutaneous fat present. Both sheep were in excellent condition at the time of necropsy.

The gross appearance of the livers of llamas 1 and 2 was similar. Peripheral margins were slightly discolored being lighter than normal. Multifocal, hardened white nodules 1–2 mm in diameter were present throughout the livers. The ventral surfaces were slightly irregular with the main bile duct thickened. On cut surface, the bile ducts were irregularly thickened and contained flukes throughout the liver. The appearance of the liver of llama 3 was much different. Three cysts, about 1 cm in diameter, were present in the lateralmost section of the lateral lobe of the liver. White streaks on the capsular surface were evident, and the ventral surface was markedly irregular with numerous, raised firm nodules 2–10 mm in diameter. Similar, but less severe lesions, were present on the dorsal surface of the liver. On cut surface, the nodules and streaks were seen to be fibrotic bile ducts. Many ducts contained caseous, tan-green material as did the 3 cysts. The main bile duct was markedly and irregularly thickened and sacculated. The saccules contained bile and numerous flukes. Flukes were also found in bile ducts throughout much of the liver.

All 3 llama livers were similar on histologic examination; however, changes were more severe in llama 3. Regional differences in histologic changes were apparent. Bile duct hyperplasia (Fig. 1) and portal fibrosis were present in most areas. These changes were striking near the hilus where lobulation was accentuated by biliary hyperplasia that bridged adjacent portal areas. Bile ductules often contained basophilic granular material. Scattered granulomas containing necrotic debris and degenerated fluke eggs were present primarily in portal areas (Fig. 2). Eggs were also present in dilated bile ductules. Eosinophils, lymphocytes, plasma cells, and neutrophils were

Table 1. Numbers of *Fasciola hepatica* eggs per gram of feces from llamas and sheep.

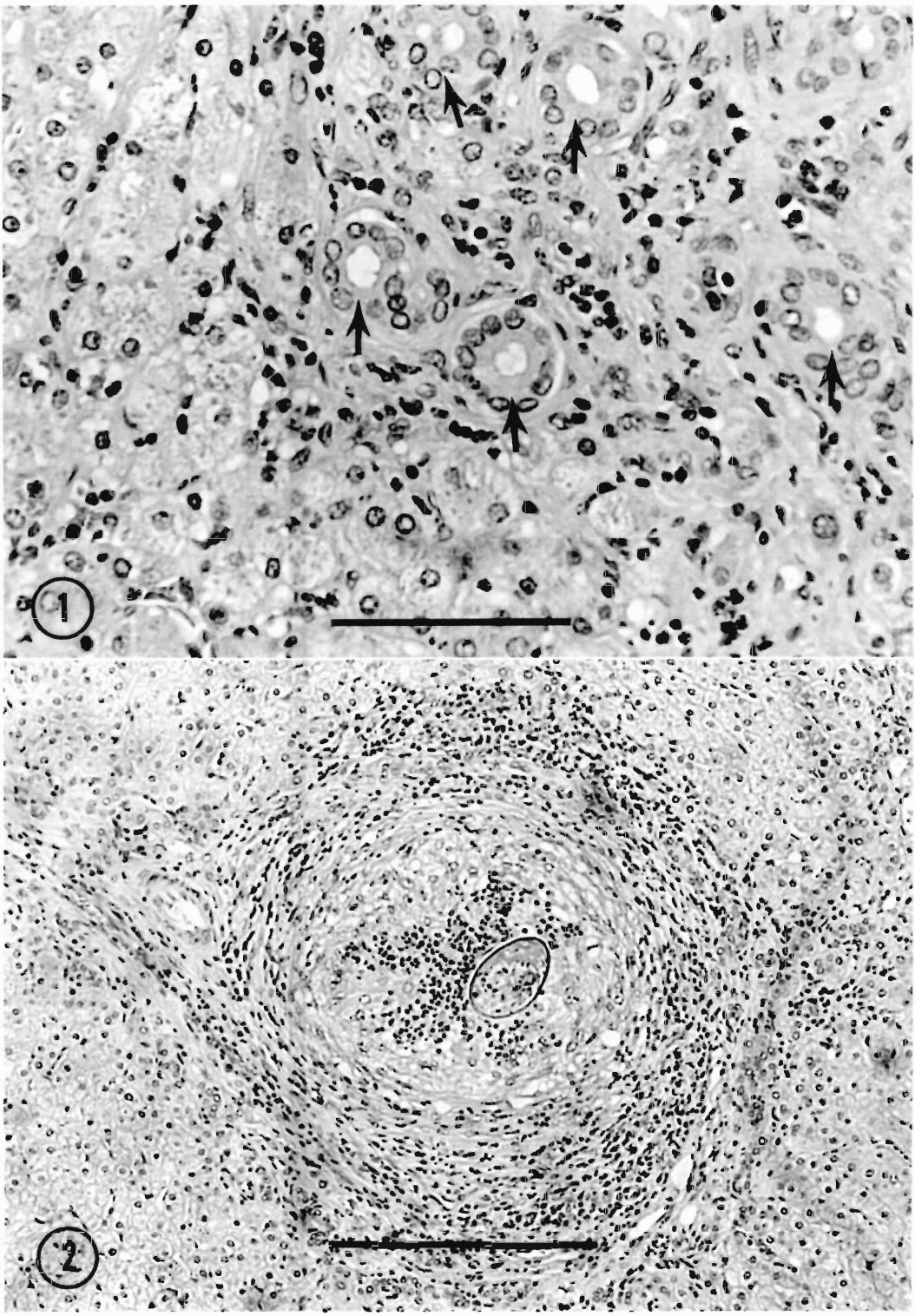
Animal no.	Days postinfection					
	0–49	56	63	69	77	83
Llama 2	0	0.6	3.2	4.4	NS	NS
Llama 3	0	6.6	27.8	23.8	253.2	69.4
Sheep 2	0	0	0.4	10.0	NS	NS
Sheep 1	0	0	2.4	9.8	46.2	32.6

NS = no sample as animal euthanized prior to the sampling date.

Table 2. Lengths (mm) of *Fasciola hepatica* recovered from llamas and sheep.

Animal no.	Days PI*	N	$\bar{x} \pm SD$	Range
Llama 2	69	33	13.0 ± 3.9	4–19
Sheep 2	70	51	15.9 ± 3.7	7–25
Llama 3	83	108	16.8 ± 3.0	8–27
Sheep 1	84	93	17.7 ± 3.6	7–25

* Days postinfection.



Figures 1, 2. 1. Biliary hyperplasia (arrows) in liver from llama 1. Scale bar = 100 μm. 2. Egg granuloma in liver from llama 1. Scale bar = 250 μm.

present surrounding the granulomas and throughout the portal areas. A calcified nodule was present in llama 1, corresponding to the white nodules seen on gross examination, and probably represented a fluke migration tract.

The appearance of the livers of both sheep was similar and did not differ substantially from previous descriptions (Dow et al., 1968; Rushton and Murray, 1977). Flukes were found in bile ducts throughout the livers of both animals. The majority of flukes were mature with eggs in their uteri.

The PPP of *F. hepatica* in sheep and cattle is variable, but is usually between 8 and 12 wk (Ross et al., 1966; Rushton and Murray, 1977; de Leon et al., 1981; Soulsby, 1982). Although the PPP in 2 llamas was 1 wk shorter than the sheep infected at the same time, the 8–12 wk PPP for all llamas and the 9 wk for the sheep are within the range in sheep and cattle.

Because the llamas were euthanized for reasons unrelated to the liver fluke infection, the patent period cannot be determined from these data. However, shedding of eggs was uninterrupted once it began and, in llama 1, continued for 9 wk.

Little difference existed in the numbers or sizes of flukes recovered from llamas and sheep at necropsy. The overall percentages of flukes recovered (llamas, 24%; sheep, 22%) were also similar. Yet, the severity of the gross pathologic changes present was somewhat dissimilar. Llamas 1 and 2 (82 and 68 flukes, respectively) had less severe lesions than sheep 2 (84 flukes). However, in llama 3 (154 flukes) gross lesions approximated those of the sheep. Histologic appearance of the llama livers was similar to that described for chronic fascioliasis in sheep and cattle (Ross et al., 1966; Dow et al., 1967, 1968; Rushton and Murray, 1977), including the presence of egg granulomas. A primary difference, however, between mature infections in cattle and sheep is the mineralization of bile ducts. This occurs in cattle beginning by week 16 of infection (Ross et al., 1966), but does not occur in sheep (Boray, 1969; Rushton and Murray, 1977). In the present study, no bile duct calcification was noted in llama 1 at 22 wk postinfection, indicating this may not be a feature of fascioliasis in llamas.

Various hosts differ in susceptibility to infection with *F. hepatica* and the degree of resistance has been cited as the underlying factor in the production of acute or chronic fascioliasis. Boray (1969) divided the more common hosts of *F. hepatica* into 3 groups based on an early, delayed, or low level of resistance. The early resistance hosts (group I; domestic pigs) possess tissues that are not suitable for the parasite resulting in a high degree of natural resistance. The infection is self-limiting without harming the host. The delayed resistance hosts (group II; cattle and horses) have a resistance which is acquired during the first weeks of a primary infection or during challenge infection. A delayed host reaction controls flukes during tissue migration, and chronic reactions including bile duct calcification lead to eventual elimination of infection. Mortality is not common. Group III hosts (sheep and goats) have low resistance resulting in severe tissue reactions that do not immobilize or eliminate the parasites. In the chronic condition, there is no calcification of the bile ducts and flukes often survive the life of the host. Mortality in both the acute and chronic phases is common. Neither acute nor chronic fascioliasis has been described in llamas in North America; however, both conditions are reported to occur in alpacas in South America (Hernandez and Condorena, 1967; Guerrero and Leguia, 1987). This, together with the histologic evidence, indicates that llamas may have low resistance to *F. hepatica.*

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Research Note

Trichinella pseudospiralis Infections in Free-living Tasmanian Birds

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ABSTRACT: Muscle tissues from 91 birds comprising 13 species were examined for the presence of *Trichinella pseudospiralis* larvae. *Trichinella* infection was detected in 2 masked owls, *Tyto novaehollandiae,* and 1 marsh harrier, *Circus aeruginosus.* These findings confirm that carnivorous or carrion-feeding birds are naturally infected with this nematode. Intestinal infection was also achieved in a 6-day-old marsh harrier after oral dosing. The source of infections and the significance of avian hosts in the epizootiology of *T. pseudospiralis* are discussed.

KEY WORDS: *Trichinella pseudospiralis,* avian infections, Australia.

Following the detection of *Trichinella pseudospiralis* Garkavi, 1972, in Tasmania, investigations were commenced to determine which free-living vertebrate hosts are responsible for the transmission and maintenance of this parasitic infection (Obendorf et al., 1990). Studies to date have suggested that *T. pseudospiralis* in Tasmania is predominantly maintained by dasyurid marsupials, in particular Tasmanian devils, *Sarcophilus harrisii,* eastern quolls, *Dasyurus viverrinus,* and spotted-tailed quolls, *D. maculatus.*

In the northern hemisphere, there are several records of free-living carnivorous birds, particularly carrion feeders, being infected with *Trichinella* sp. presumed to be *T. pseudospiralis* (Boev et al., 1979). In the Tien Shan mountain region of U.S.S.R., *T. pseudospiralis* has also been recorded in 2 crows, *Corvus frugilegus* (Shaikenov, 1980), out of a total of 744 birds. It is also quite likely that *T. pseudospiralis* was recovered from a common buzzard, *Buteo buteo,* in Spain (Calero et al., 1978). Records of *Trichinella* sp. in North American birds include the great horned owl, *Bubo virginianus* (Zimmermann and Hubbard, 1969), the pomarine jaegar, *Stercorarius pomarinus* (Rausch et al., 1956), and Cooper's hawk, *Accipiter cooperi* (Wheeldon et al., 1983).

In this study, 13 avian species with carnivorous habits were examined for the presence of *Trichinella* infection in muscles. Samples of muscle were obtained from birds killed as a result of road accidents, malicious shooting, or poisoning and trapping. Some forest ravens were obtained by authorized trapping. In addition, a 6-day-old raptor was experimentally infected with

muscle tissue containing *T. pseudospiralis* derived from a naturally infected Tasmanian devil.

Skeletal muscle removed from the chest and legs was chopped and macerated. Ten-gram samples of these muscles were digested for 12–16 hr in a solution of 1% pepsin and 0.5% concentrated hydrochloric acid. Digest fluid was passed through a 53-μm sieve and the collected material examined by light microscopy at ×40 magnification. In 2 of the 3 birds infected with *Trichinella*, counts of larvae per gram in muscle were conducted. Muscle tissues were fixed in 10% formol-saline and processed for routine histology using hematoxylin and eosin for the staining of 5-μm-thick tissue sections. A 6-day-old marsh harrier, *Circus aeruginosus*, was fed for 3 days on minced muscle from a Tasmanian devil. The bird consumed 50 g of muscle containing 34 larvae/g. The bird died 4 days after commencing the experimental feeding; the cause of death was not attributable to *Trichinella* infection. Portions of proximal, middle, and distal small intestine were fixed in 10% formol-saline for histological processing. The mucosa of each portion of intestine was scraped off the muscle wall and subsequently digested for 2 hr in pepsin/hydrochloric acid solution. The digests were carefully examined for the presence of *Trichinella*.

A list of avian species tested for the presence of *Trichinella* larvae is presented in Table 1. Larvae were detected in 2 masked owls, *Tyto novaehollandiae*, from the Deloraine district and 1 marsh harrier, *C. aeruginosus*, from the central highlands region. One masked owl had 2,130 larvae/g, whereas the marsh harrier had 650 larvae/g. These counts are considerably higher than those reported from the 3 dasyurid species; the highest muscle larva count in a marsupial was 508 larvae/g recorded from an eastern quoll (Obendorf et al., 1990). Histologically, the larvae were found within hypertrophied muscle cells and were structurally indistinguishable from *Trichinella* larvae found in the dasyurid marsupials. In 1 owl, the nematodes were associated with some host inflammatory response and myodegeneration of parasitized muscle fibers. The larvae in all 3 birds appeared viable.

Several immature male and female *Trichinella* were recovered from the intestine of the experimentally infected marsh harrier. A total of 15 females and 6 males were collected with the majority of the nematodes (13 of 21) present in the mid-small intestine. In histological section, several *Trichinella* were seen within intra-epithelial sites along the intestinal villi.

Table 1. List of birds examined for *Trichinella pseudospiralis* larvae by muscle digestion.

Avian species	No. positive/ no. examined
Order Accipitiformes (diurnal raptors)	
Grey goshawk	
Accipiter novaehollandiae	0/7
Brown goshawk	
A. fasciatus	0/5
Collared sparrowhawk	
A. cirrhocephalus	0/5
Marsh harrier	
Circus aeruginosus	1/11
Brown falcon	
Falco berigora	0/8
Wedge-tailed eagle	
Aquila audux	0/6
Order Strigiformes (owls)	
Masked owl	
Tyto novaehollandiae	2/12
Barn owl	
T. alba	0/1
Southern boobook	
Ninox novaeseelandiae	0/3
Order Caprimulgiformes (frogmouths and nightjars)	
Tawny frogmouth	
Podargus strigoides	0/4
Order Passeriformes (perching birds)	
Black currawong	
Strepera fuliginosa	0/2
Australian magpie	
Gymnorhina tibicen	0/1
Forest raven	
Corvus tasmaniensis	0/26
Total	3/91

It has been suggested that meat-eating birds may be important in the maintenance of *T. pseudospiralis* in nature (Boev et al., 1979). This study was conducted to determine if free-living Tasmanian birds are naturally infected. The sample size was small (91) and no attempt was made to sample avian species from areas of the state where the prevalence of infection in dasyurid marsupials was especially high.

The recovery of *Trichinella* infection in the marsh harrier was not unexpected, as this raptor regularly feeds on carrion (Baker-Gabb, 1982), including road-killed Tasmanian devils (Mooney, 1991). Marsh harriers migrate across Bass Strait, arriving in Tasmania in August and departing in February (Green, 1977). This repre-

sents a potential dispersal mechanism for the parasite into southeastern Australia. The ability of *T. pseudospiralis* to be maintained in mainland Australia may, however, be affected by the absence of the large dasyurid fauna, which exists in Tasmania.

The recovery of numerous *Trichinella* larvae in the muscles of 2 free-living masked owls confirms that this bird also consumes *Trichinella*-infected meats. The masked owl in Tasmania is recognized as a distinct subspecies, *T. n. castanops,* which has been geographically isolated from mainland Australia for at least 10,000 years. Although most of the masked owl's diet consists of small- to medium-sized mammals, it also includes the dasyurid marsupials, *Dasyurus maculatus* and *D. viverrinus* (Mooney, 1990). No naturally acquired infections of *T. pseudospiralis* have been detected in native or introduced rodents (Obendorf et al., 1990), although only a small number were examined. Current evidence suggests that dasyurid marsupials are predominantly responsible for the transmission and maintenance of *T. pseudospiralis* (Obendorf et al., 1990). The most likely source of *Trichinella* infection for these 2 avian species is thought to be dasyurids.

This study confirms that birds are naturally infected with the Tasmanian *T. pseudospiralis.* Domestic chickens (*Gallus* sp.) have been experimentally infected with the Tasmanian *T. pseudospiralis* isolate (Obendorf et al., 1990) while isolates from North America and U.S.S.R. have also been successfully used to infect a very wide range of avian species (Tomasovicova, 1975; Tomasovicova and Hovorka, 1982; Bober and Dick, 1983). The presence or absence of *T. pseudospiralis* infection in individual birds appears to be related to their dietary preference and availability of *Trichinella*-infected muscle tissues for ingestion. The high muscle larval recovery in the 2 birds for which counts were conducted suggests that they are suitable hosts for *T. pseudospiralis.*

Carrion feeding of infected Tasmanian devil muscle to a 6-day-old marsh harrier resulted in a successful intestinal infection. Due to the unexpected death of this bird only 4 days after commencement of feeding, the nematodes in the bowel were immature. The viability of the *Trichinella* larvae in the muscle used for dosing was low as the muscle had been kept refrigerated for several weeks prior to dosing. This may explain the small number of intestinal nematodes recovered. The same *Trichinella*-infected muscle was also used successfully to infect domestic cats, *Felis domesticus.* These findings suggest that the Tasmanian *T. pseudospiralis* is capable of infecting a wide range of animals including placental and marsupial mammals and birds.

Several other Tasmanian birds are recorded as feeding on Tasmanian devil carcasses; these include wedge-tailed eagle, white-breasted sea-eagle (*Haliaetus leucogaster*), grey goshawk, brown goshawk, brown falcon, forest raven, grey currawong (*Strepera versicolor*), grey butcherbird (*Craticus torquatus*), and grey shrike-thrush (*Colluricincla harmonica*) (Mooney, pers. comm.). All these species are potentially capable of being infected with *T. pseudospiralis.*

It is unclear whether birds are important in the epizootiology of *T. pseudospiralis* on the island of Tasmania. The presence of infection in marsh harriers, a species that migrates to and from Tasmania, is noteworthy. Nevertheless, based on the high prevalence of infection in the dasyurid marsupials, it appears that infections are principally transmitted and maintained through these carrion-feeding and cannibalistic marsupials, with certain carnivorous birds occasionally becoming infected.

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Research Note

Prevalence of *Acetodextra amiuri* (Trematoda: Cryptogonimidae) in Channel Catfish, *Ictalurus punctatus,* from Kentucky Lake, Kentucky–Tennessee

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ABSTRACT: One hundred seventy-three channel catfish, *Ictalurus punctatus,* were collected in Kentucky Lake by monthly gill netting from April through October 1988, and examined for the presence of the digenean *Acetodextra amiuri.* Only the ovaries of mature females were infected. Mature females constituted 46.8% (81/173) of the sample and 18.5% (15/81) were infected. The highest prevalences were observed during June and July (70% and 50%, respectively).

KEY WORDS: channel catfish, *Acetodextra amiuri, Ictalurus punctatus,* prevalence, Kentucky Lake, Digenea.

The prevalence of *Acetodextra amiuri* (Stafford) in channel catfish, *Ictalurus punctatus* (Rafinesque), was studied in Kentucky Lake, an impoundment of the Tennessee River. One hundred seventy-three channel catfish (>300 mm) were collected with gill nets from April through October 1988. Based upon its large size (64,800 hectares), the lake was divided into 3 sampling areas extending from Kentucky Dam in Kentucky (Tennessee River Mile 22) south to Pickwick Dam (TRM 207) in Tennessee. Area 1 (TRM 22–66) was characterized as lacustrine; area 2 (TRM 66–116) was transitional between areas 1 and 3; area 3 (TRM 116–207) was narrow and riverine, included the Pickwick Dam tailwaters, and was nearly as long as areas 1 and 2 combined. Voucher specimens were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as *Acetodextra amiuri* (No. 81240).

Acetodextra amiuri was observed only in the ovaries of adult channel catfish. *Acetodextra amiuri* was not observed in air bladders of either males or females as observed by Perkins (1956). Eighty-one (46.8%) of the fish collected were mature females and 15 of these (18.5%) were infected. Females infected with *A. amiuri* had a mean total length of 498 mm (range 386–601 mm), a mean weight of 1,512 g (range 687–2,471 g), and a mean ovary weight of 79 g (range 2–206 g). Females without *A. amiuri* had a mean total length of 460 mm (range 300–611 mm), a mean weight of 1,163 g (range 341–2,821 g), and a mean ovary weight of 23 g (2–237 g). There were no significant differences between fish with and without *A. amiuri* for lengths, weights, or ovary weights ($P < 0.05$). *Acetodextra amiuri* was found in ovaries before and after spawning. Channel catfish spawn in June and July (Marzolf,

1957). The highest prevalences in hosts were 70% in June (area 3) and 50% in July (area 2). Lower prevalences, ranging from 0% to 33%, were observed in individual areas in other collection months. The prevalences of *A. amiuri* and sample sizes of females in each area from April through October were: 31.0% for area 3 (33 fish), 21.1% for area 2 (19 fish), and 3.4% for area 1 (29 fish). The number of channel catfish infected with *A. amiuri* was lower in the lacustrine than the riverine area of Kentucky Lake. Monthly prevalences for the 3 areas combined were: 0 for April and May (15 fish), 41.1% for June (17 fish), 37.5% for July (8 fish), 8.7% for August (23 fish), 11.1% for September (9 fish), and 11.1% for October (9 fish).

Several hundred *A. amiuri* were observed per ovary. Although intensity was not measured for all fish, over 500 adult *A. amiuri* were observed in 1 ovary that was preserved. This observation is consistent with the findings of Warner and Hubert (1975) and Perkins (1956) who sometimes found more than 1,000 adult *A. amiuri* in a single ovary. Edwards et al. (1977) reported a low prevalence (1.0%) in channel catfish from the Kentucky River with few *A. amiuri* per host. They did not separate mature females from the total number of females and males.

Acetodextra amiuri parasitizes many ictalurids (Perkins, 1956; Hoffman, 1967; Aliff, 1977) including: channel catfish; yellow bullhead, *Ictalurus natalus*; black bullhead, *Ictalurus melas*; brown bullhead, *Ictalurus nebulosus*; stone cat, *Noturus flavus*; and tadpole madtom, *Noturus gyrinus*. *Acetodextra amiuri* has not been reported from blue catfish, *Ictalurus furcatus* (Hoffman, 1967). During the present study, 190 mature female blue catfish were also examined. *Acetodextra amiuri* was not observed in any blue catfish examined. Why *A. amiuri* does not parasitize blue catfish is enigmatic.

The pathogenicity of *A. amiuri* on channel catfish is unknown, but Perkins (1956) and Hoffman (1979) indicated it has the potential for reducing channel catfish reproduction by damaging ovarian tissue and eggs.

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