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Can Autoinfection be Provoked in the *Strongyloides ratti*-infected Gerbil, *Meriones unguiculatus*?

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ABSTRACT: *Strongyloides ratti* infections in gerbils, *Meriones unguiculatus,* are chronic when compared to infections with this species in other rodent hosts. Furthermore, eggs are rarely found in freshly passed feces, instead, resembling *Strongyloides stercoralis* infection, rhabditiform, first-stage larvae are characteristically expelled. The longevity of *S. ratti* infection in gerbils is not, however, due to naturally occurring autoinfection, nor could autoinfection be induced by immunosuppression or prolongation of intestinal transit time.

KEY WORDS: nematode, *Strongyloides ratti,* gerbil, *Meriones unguiculatus,* autoinfection, prednisolone acetate, diphenoxylate.

Experimental murine strongyloidiasis, involving *Strongyloides ratti* and the rat, is a frequently used laboratory model for human strongyloidiasis. Unfortunately, however, this host–parasite association does not mimic the 2 most characteristic features of *Strongyloides stercoralis* infection in man, namely autoinfection and the unusual propensity to establish extremely chronic infections. Indeed, several attempts to provoke autoinfection in *S. ratti*-infected rodents have failed and adult worms are expelled from the usual rodent hosts (rats and mice) within the first 2 to 3 wk of infection (Dawkins, 1989). In this connection, it is interesting that an isolate of *Strongyloides* (probably *S. ratti*) from a muskrat survived in gerbils for a year (M. D. Little, pers. comm.).

Our own experience with a well-known strain of *S. ratti* indicates that infections in gerbils remain patent for at least 5 mo. This longevity of infection in gerbils, as well as our observation that in this host the eggs of *S. ratti* almost invariably hatch internally, prompted the 2 major questions addressed in this study: 1. Could the unusual longevity of a primary infection be due to naturally occurring autoinfection? and 2. Because the eggs hatch internally, could autoinfection be induced (or enhanced) by either increasing intestinal transit time and/or depressing the host's immune responses?

Materials and Methods

Parasite strain

Strongyloides ratti infective third-stage larvae (L_3) were obtained from fecal cultures of donor rats maintained in our laboratory. The strain (G60, a heterogonic strain) was obtained originally from Dr. M. E. Viney, University of Edinburgh, Edinburgh, Scotland.

Experimental animals

Purpose-bred female gerbils were purchased from Tumblebrook Farm, Inc. (West Brookfield, Massachusetts), housed 5 to a cage, and fed Purina Lab Chow and water ad libitum.

Experimental designs

To confirm that *S. ratti* infections in gerbils are in fact long-lasting, 2 4–6-week-old animals were injected subcutaneously (SQ) with 500 and 700 L_3, respectively. The number of L_1's shed in the feces was determined weekly using the McMaster technique, which has a lower detection limit of 100 larvae/g. A third gerbil received 1,000 L_3 SQ and 3 mg of prednisolone acetate on 4 alternate days beginning on the day of infection.

For an experiment designed to provoke (or enhance) autoinfection by immunosuppression, increasing the intestinal transit time, or by both treatments combined, 40 gerbils were injected with 1,000 L_3 *S. ratti* SQ. The infected animals were divided into 4 groups of 10 each: infected controls, prednisolone-treated, diphenoxylate-treated, and a group treated with both drugs.

Prednisolone acetate (3 mg/gerbil on alternate days for 4 treatments and then at 4-day intervals for the duration of the experiment) was selected as the immunosuppressive agent of choice, Kamiya and Sato (1990) having used it successfully to adapt the complete life cycle of *Echinococcus multilocularis* to the gerbil, a normally nonpermissive definitive host. To increase intestinal transit time, the motility-altering, antidiarrheal drug diphenoxylate was used at the dosage suggested by Megens et al., 1989 (8.15 mg/kg per os) on alternate days.

The animals in each group were divided into 2 lots of 5, 1 scheduled for euthanasia at about patency (days 6–8) and the other day 21, when infections normally have been expelled from rats and mice. These 2 necropsy times were based on unpublished observations and theoretical considerations (Schad, 1989) suggesting that autoinfection, it it were to occur, would maximize early in the infection (i.e., about the time of patency), and if it did extend the infection beyond its normal duration in rodents, would have to occur before the infection is normally expelled.

Table 1. Recovery of first-stage larvae of *Strongyloides ratti* from the intestines of gerbils at 2 times postinfection.

Group	Number of larvae recovered (geometric mean)*†	
	Day 6–8	Day 21
Infected controls	3,197.1	134.6[a]
Prednisolone-treated	4,187.1	332.6
Diphenoxylate-treated	9,319.8	103.6[b]
Prednisolone- and diphenoxylate-treated	458.4	854.1

* Results of dilution counts.

† Except as indicated by superscript letters, there were no statistically significant differences between groups. [a] = Significantly different ($P = 0.05$) from day 6–8 infected controls (Mann-Whitney *U*-test). [b] = Significantly different ($P = 0.04$) from day 6–8 diphenoxylate-treated group (Mann-Whitney *U*-test).

On the day of worm recovery, each gerbil was euthanatized and its intestines removed. The small intestine was slit longitudinally, rinsed of contents, and hung in a 50-ml centrifuge tube of phosphate-buffered saline (PBS). Its contents were suspended in a separate tube of PBS in which they were allowed to sediment for 30 minutes, at which time differential dilution counts were made of all life history stages represented. The cecum, large intestine, and rectum were also slit longitudinally and hung in a tube of PBS. Their contents were Baermannized at 37°C for 1 hr and any organisms recovered counted as above. The slit small and large intestines were incubated at 37°C for 3 hr when they were transferred to another tube of PBS for an additional 3 hr. After removal of the intestines, the suspended material in these tubes was allowed to sediment and parasites were counted as above.

Parasite fecundity was estimated in groups of hosts not given diphenoxylate by dividing the total number of larvae found in the intestines by the number of adult worms recovered.

Table 2. Recovery of adult *Strongyloides ratti* from the intestines of gerbils at 2 times postinfection.

Group	Number of adults recovered (geometric mean)*†	
	Day 6–8	Day 21
Controls	222.6	36.0
Prednisolone-treated	148.4	53.6
Diphenoxylate-treated	319.5	35.6[a]
Prednisolone- and diphenoxylate-treated	51.5	81.3

* Results of dilution counts.

† Except as indicated by the superscript letter, there were no statistically significant differences between groups. [a] = Significantly different ($P = 0.05$) from day 6–8 diphenoxylate-treated group (Mann-Whitney *U*-test).

Table 3. Fecundity of *Strongyloides ratti* adults in normal and immunodepressed gerbils.

Day post-infection	Group	Number of larvae recovered/ number of adults recovered*
6–8	Infected controls	14.3
	Prednisolone-treated	18.5
21	Infected controls	5.2[a]
	Prednisolone-treated	10.4

* Geometric mean (results of dilution counts). Except as indicated by the superscript letter, there were no statistically significant differences between groups. [a] = Significantly different ($P = 0.05$) from day 6–8 infected controls (Mann-Whitney *U*-test).

Statistical analysis

There was excessive mortality in the experimentally treated groups, so that 3 animals, rather than the statistically preferred 5 animals originally anticipated, were available for worm counts at 1 wk after infection. Only 4 and 3 animals, respectively, survived in the diphenoxylate and the combined treatment groups, at 3 wk after infection. Given the highly variable nature of worm count data and the small group sizes, geometric mean worm counts formed the basis for all statistical comparisons. All data (Tables 1–3) are presented as geometric means based on the ln(count + 1).

Results

The course of each of 3 *Strongyloides ratti* infections in gerbils is shown in Figure 1. The gerbil receiving the lowest larval dose remained positive by McMaster count for 22 wk and had no worms in its intestines when necropsied at 25 wk postinfection (PI). The second gerbil, last positive by McMaster at 24 wk PI, was still positive by coproculture, a more sensitive test, at 44 wk PI. The gerbil given 1,000 L_3 and initially treated with prednisolone maintained a higher level of larval output than either of the normal gerbils (Fig. 1) and at 29 weeks (the time at which this report was written) still had >100 L_1/g of feces. Other preliminary experiments (data not shown) indicated that doses exceeding 1,000 *S. ratti* L_3 were fatal in 4–5-week-old gerbils. *Strongyloides ratti* infective third-stage larvae obtained from coprocultures of the gerbil that received 1,000 L_3 were infective to mice and produced infections that were similar to those initiated by L_3's from rats. A gerbil that received 1,000 L_3's similarly derived has shown a pattern of larval shedding in the feces similar to the original 1,000 L_3 gerbil for 1 mo, the time interval to date (data not shown).

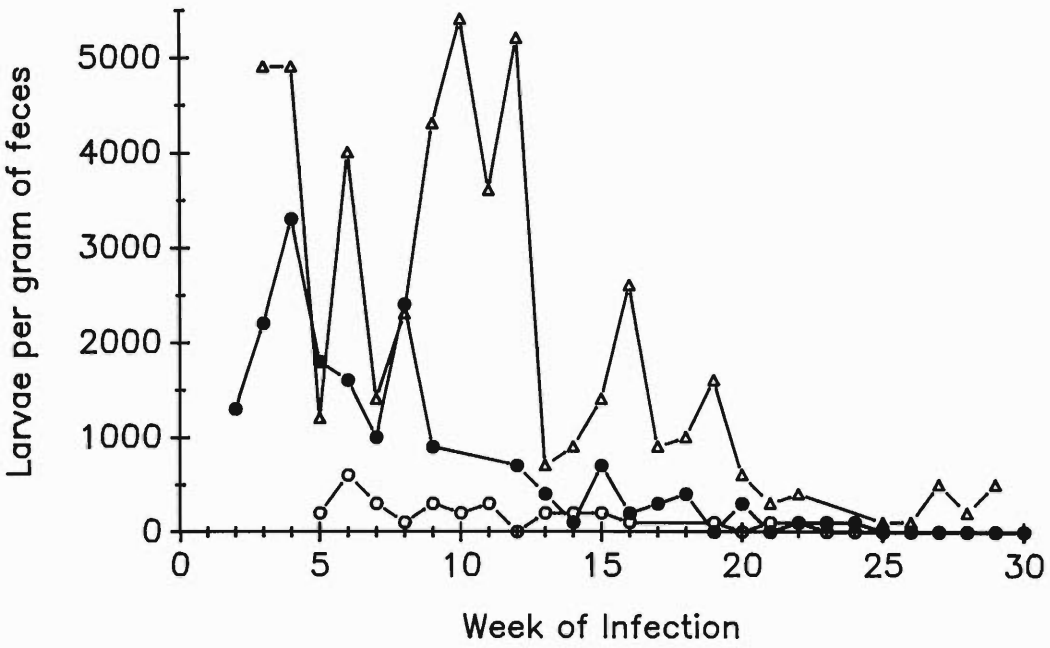

Figure 1. Course of infection of *Strongyloides ratti* in the gerbil as measured by the number of larvae in the feces. Gerbils were infected subcutaneously with 500 L_3's (third-stage larvae) (○——○), 700 L_3's (●——●), or 1,000 L_3's (△——△).

In groups of gerbils treated with prednisolone and/or diphenoxylate, *no* third-stage autoinfective larvae were found at either time period; only eggs , L_1's, and adult worms were recovered. Periodic weighing of the feces from the cages (5 gerbils/cage) of diphenoxylate-treated and untreated gerbils indicated that the treated gerbils produced less feces than untreated gerbils. The stage-specific parasite recoveries for the time of patency (1 wk) in the 3 experimentally treated groups were compared to those of the infected control group; none of the former differed significantly (Mann-Whitney *U*-test) from those of the latter (Tables 1, 2). Similarly, there were no statistically significant differences (Mann-Whitney *U*-test) between the worm populations of the experimentally treated groups necropsied after 3 wk and those of the control group (see Tables 1, 2). However, in most groups there appeared to be a marked decrease in the number of worms (both larvae and adults) between 1 and 3 wk postinfection, but given the small group sizes and the variability of the data, this decrease was significant in only 2 of 4 and 1 of 4 possible comparisons (see Tables 1 and 2, respectively).

There was excessive mortality of gerbils (7 of 29) in the diphenoxylate-treated groups, perhaps reflecting the relatively high parasite dose, approaching that fatal for gerbils, in interaction with a regimen of drug use designed for the rat.

Fecundity of *S. ratti* was compared in groups that were not treated with diphenoxylate. At 1 and 3 wk PI, there was a decrease in fecundity of border-line significance ($P = 0.05$) in the infected control group with only 36% of the week 1 larval output occurring at 3 wk. In contrast, there was no statistically significant decrease in the fecundity of worms between 1 and 3 wk PI in immunodepressed gerbils.

Discussion

Prompted by observations indicating that gerbils harbor unusually chronic *Strongyloides ratti* infections, we sought to determine whether this chronicity reflects an underlying autoinfection occurring in the *S. ratti*-infected gerbil. Our investigations were designed to determine if autoinfection occurs naturally in *S. ratti*-infected gerbils or if it could be induced (or enhanced) with immunosuppressive agents and/or intestinal motility-altering drugs. Treatment with immunosuppressive drugs, particularly corticosteroids such as prednisolone, is well known to increase autoinfection in *Strongyloides stercora-*

lis infections (Grove et al., 1983; Schad et al., 1984; Genta et al., 1986). Similarly, opium, a drug that decreases intestinal motility and, therefore, increases intestinal transit time increases autoinfection in experimental canine strongyloidiasis (Nishigori, 1928). However, no autoinfective larvae (L_3) were found in either the small or large intestines of the gerbils at either of 2 critical time points in any of the treatment groups, suggesting that autoinfection is not responsible for the prolongation of *S. ratti* infection in gerbils beyond the 3-wk interval of patency that normally occurs in other rodents. Under the conditions of this experiment, prednisolone acetate and diphenoxylate had no demonstrable effect on the number of L_1 or adult worms recovered. This may, however, be attributable to the marked variability in worm counts and the small sample size.

In contrast to the short infections in rats induced by infection with the usual large numbers of larvae, Graham (1940) was able to establish chronic infections of *S. ratti* in rats by infecting them with 1 larva. In these rats, the infection lasted for an average of 149 ± 8 days with a range of 8–477 days. Graham's results suggest that there may be a minimum antigenic load needed to cause the expulsion of the adult worms as there was no indication that autoinfection was maintaining the worm burden. A similar mechanism may be operating in the gerbil, allowing a small number of worms that survive the initial expulsion to remain without provoking a further immune response from the gerbil.

The exceedingly chronic *S. ratti* infections occurring in the gerbil more closely resemble human *S. stercoralis* infections than do *S. ratti* infections in rats and mice. Consequently, even though autoinfection does not occur in *S. ratti*-infected gerbils, this host–parasite association should prove useful for the experimental investigation of human strongyloidiasis. Furthermore, the chronicity of the infection in the gerbil makes it an excellent host for maintaining *S. ratti* in the laboratory. The postponement of the necessity to transfer the infection from 2 wk, as is the case in rats, to 5 mo (or even longer) saves animals and reduces the cost of strain maintenance substantially.

Acknowledgments

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Gonapodasmius williamsoni sp. n. (Digenea: Didymozoidae) from the Pink Snapper, *Pagrus auratus* (Teleostei: Sparidae) in Western Australia

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ABSTRACT: *Gonapodasmius williamsoni* sp. n. (Digenea: Didymozoidae: Gonapodasmiinae) is described from the muscle of the pink snapper, *Pagrus auratus* (Teleostei: Sparidae) from Shark Bay, Western Australia. The species is separable from the 15 described *Gonapodasmius* species most notably by the presence of a well-developed glandular "stomach" (Drüsenmagen), and by its site and host-specificity.

KEY WORDS: *Gonapodasmius williamsoni* sp. n., Digenea, Didymozoidae, Sparidae, *Pagrus auratus,* economic significance, Australia.

The developing export market of Western Australian pink snapper, *Pagrus auratus,* met some resistance in 1988 when complaints were received about bright yellow parasites in the flesh of the snapper. The parasite concerned was identified as a didymozoid trematode. The species does not agree with any described species and so is here described as new.

Materials and Methods

Parasites were dissected from either the flesh of the fish or in situ and fixed in hot or cold 5% or 10% formalin. Whole mounts were stained with Mayer's hematoxylin, cleared in methyl salicylate, and mounted in Canada balsam. Specimens for sectioning were embedded in paraffin wax, cut at 7–10 μm, and stained with Mayer's hematoxylin and eosin. Measurements are given in micrometers; figures in brackets are means. Drawings were made with the aid of a camera lucida.

Didymozoidae Poche, 1907

Gonapodasmiinae Ishii, 1935

Gonapodasmius williamsoni sp. n. (Figs. 1–9)

DESCRIPTION: Female observed from several more or less complete collections of fragments; measurements of suckers and pharynx from single individual. Body slender, slightly flattened dorsoventrally, 2 individuals (sums of fragments) 122 and 153 cm long, maximum width about 530. Tegument smooth. Mouth terminal (Fig. 2). Oral sucker 60 long and 55 wide. Prepharynx absent. Pharynx 33 long and 25 wide. Esophagus muscular, unstraightened length 347 long. Glandular "stomach" (=Drüsenmagen) prominent. Ceca simple, posterior extent not determined. Ventral sucker 57 wide and 37 long, 887 from anterior end of body. Ovary a single tube, extending anteriorly from egg-forming complex, distance of termination from anterior end not determined. Vitellarium a single narrow tube extending posteriorly to near posterior end of body (Fig. 3). Seminal receptacle, Mehlis' gland and Laurer's canal not observed. Uterus issues from egg-forming complex and passes posteriorly, without convolutions, to near posterior end of body then loops (Fig. 3) and passes anteriorly to ventral genital pore at level of posterior margin of pharynx. Testes and associated male reproductive system never observed, presumed absent. Eggs oval, tanned, operculate, 27–31 (30) by 10–13 (12) ($N = 10$). Excretory vesicle a single, simple tube opening subterminally at posterior end of body, divides near cecal bifurcation to form 2 irregular arms reaching midway between cecal bifurcation and oral sucker. Female system sometimes represented in males by single duct developed to greater or lesser extent; sometimes with malformed eggs.

Male observed only from incomplete fragments; measurements of suckers and pharynx from single individual. Body slender, approximately $\frac{1}{10}$ length of female, somewhat flattened dorsoventrally, 2 individuals (sums of fragments) 9 and 13 cm long, respectively, maximum width about 284. Tegument smooth. Mouth terminal (Fig. 4). Oral sucker 81 long and 47 wide. Prepharynx not distinguishable. Pharynx 37 long and 40 wide. Esophagus and "stomach" not observed. Ventral sucker 43 long and 53 wide. Testes paired, parallel, winding through most of body, posterior extent not determined.

TYPE HOST: *Pagrus auratus* (Bloch and Schneider) (Sparidae).

[3] Present address: CSIRO Division of Fisheries, Marmion, P.O. Box 20, North Beach 6020, Australia.

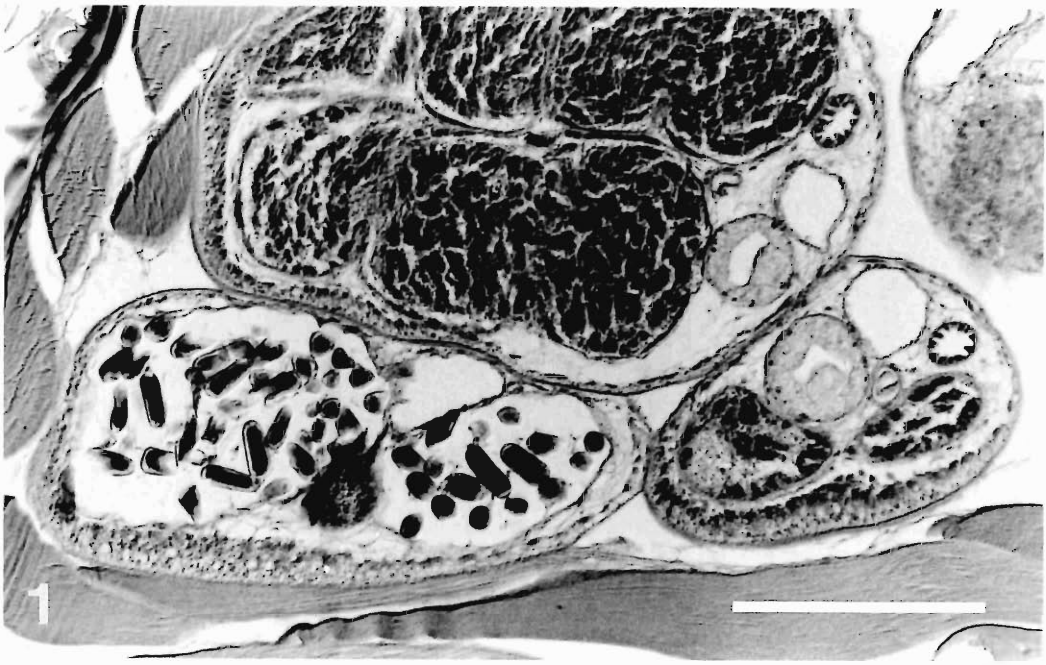


Figure 1. ***Gonapodasmius williamsoni*** **sp. n. in section in situ in fillet of** ***Pagrus auratus*****; 2 sections through male, 1 section through female. Scale bar = 200 μm.**

PREVALENCE OF INFECTION: 5.6% in 4,100 fish.

SITE IN HOST: Deep in lateral musculature, never observed in fins or head.

LOCALITIES: Vicinity Shark Bay, Western Australian coast, 23°–27°S.

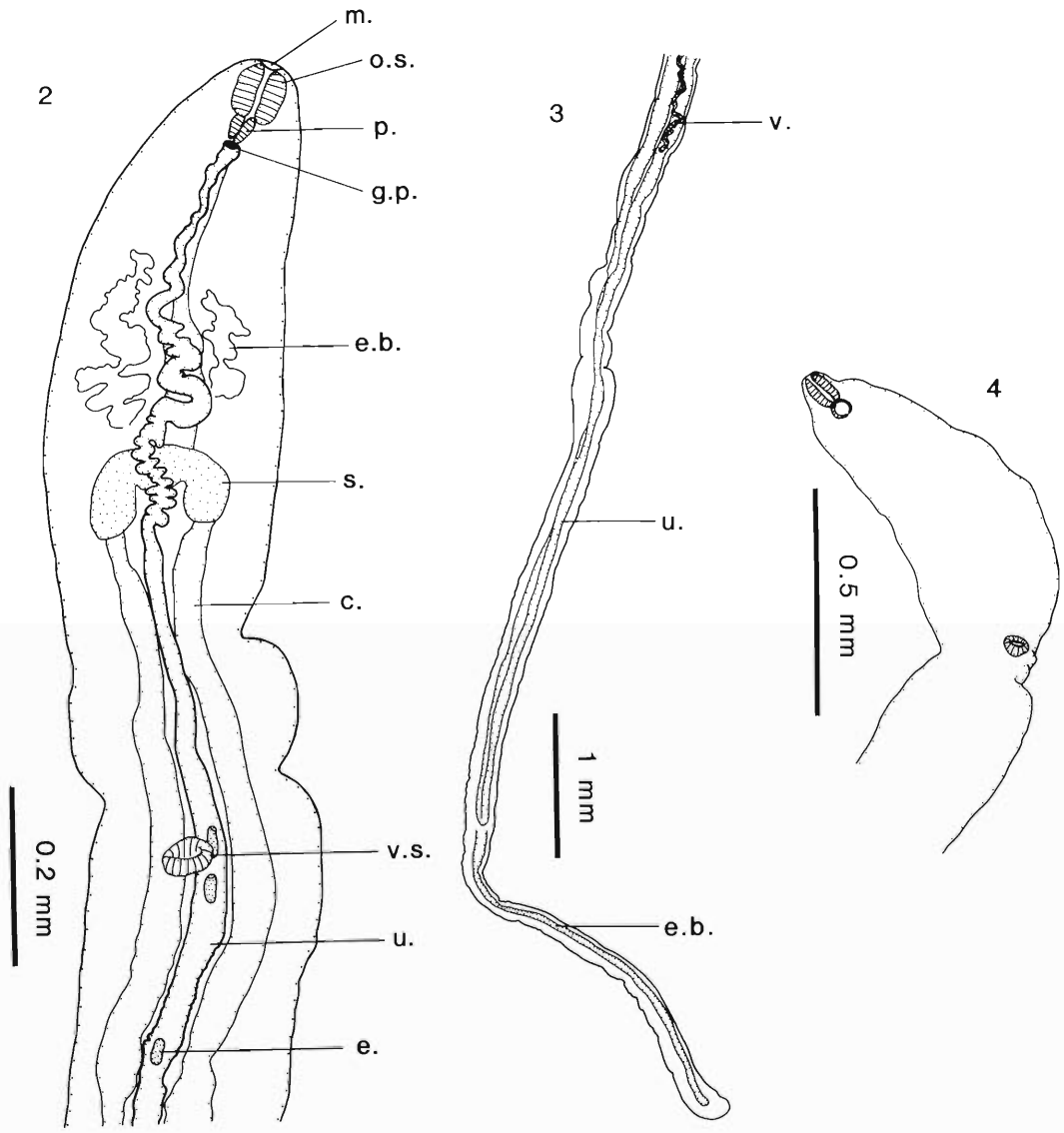
DEPOSITED MATERIAL: Holotype, coll. A. Williams, 6 Oct. 1988, Queensland Museum GL 1297. Four paratypes, coll. T. Cribb, Mar. 1989, QM GL 1298–1301. Sectioned nontype material: QM 12736–12745.

ETYMOLOGY: This species is named in honor of Messrs. Rodney and Mark Williamson upon whose boat this species was collected, and who facilitated this study.

Results and Discussion

This species presented considerable difficulties because the worms are long (over 1 m for females) and wind through the tissues of the host. Thus, specimens were difficult to recover. No specimens were recovered intact, and despite rapid fixation of specimens following collection, the preservation of the parasites was not ideal. The trematode was found in patches in the lateral musculature of the fish (Fig. 1), and though no further localization within the musculature was noted, the parasite was never found in any part of the head, the fins, or the viscera. As far as could be ascertained, the patches always consisted of 1 male and 1 female individual, i.e., no more than 2 anterior ends were ever recovered from a single patch. However, because the masses were so entangled in the flesh and the worms were never recovered intact, the possibility that more than 1 of each sex might be present on occasions cannot be discounted. The patches of worms were not encapsulated by a structure of parasite or host origin. Live trematodes tended to loop back and forth in the muscle of the fish, each loop separated from the next by host tissue. In what appeared to be older, perhaps moribund infections, the trematodes were less freely intertwined with host tissue. Dead parasites appeared as patches of dark, fragmented, granular material or formed discrete lumps.

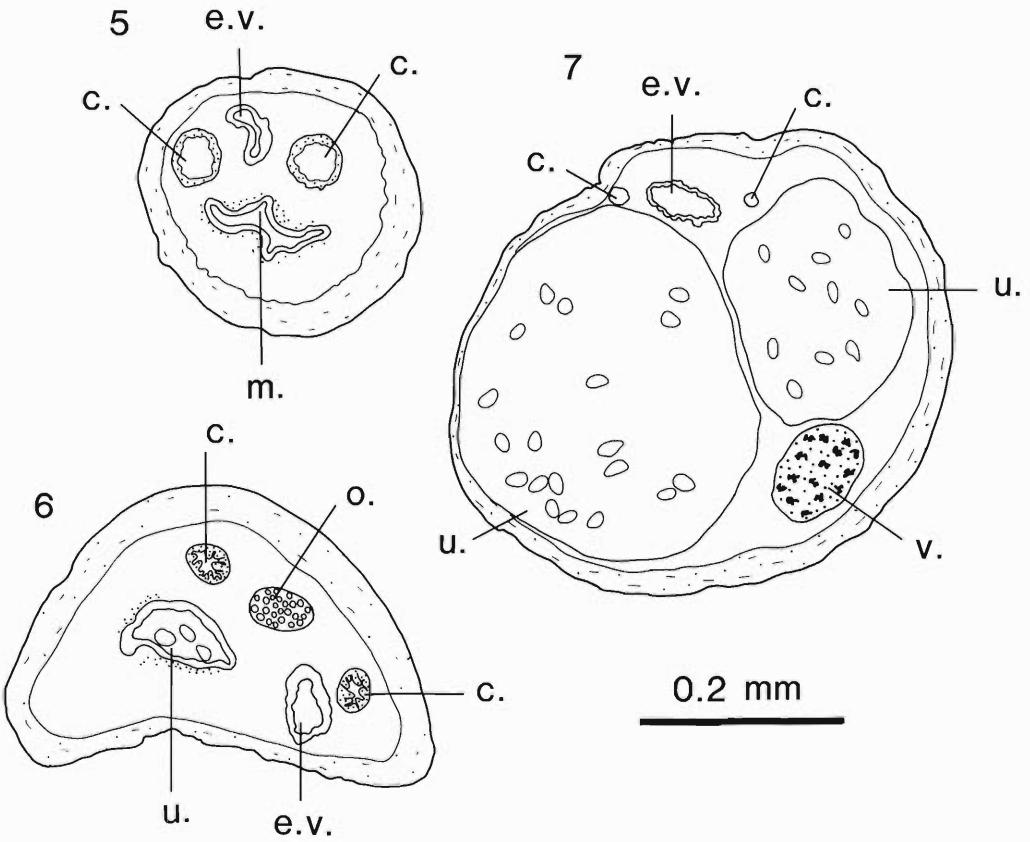
The skeletal musculature of 4 other commercial fish species taken by the snapper fishery in Shark Bay at the same time were also examined for *Gonapodasmius williamsoni.* It was not found in 185 *Lutjanus sebae* (Lutjanidae), 79 *Epinephelus multinotatus* (Serranidae), 429 *Lethrinus nebulosus,* or 184 *L. miniatus* (Lethrinidae), al-



Figures 2–4. ***Gonapodasmius williamsoni*** **sp. n. 2. Holotype, female, anterior end, ventral view. 3. Female, posterior end, orientation unknown. 4. Male, anterior end, ventral view (digestive and reproductive tracts not distinguished). c., cecum; e., egg; e.b., excretory bladder; g.p., genital pore; m., mouth; o.s., oral sucker; p., pharynx; s., stomach; u., uterus; v., vitellarium; v.s., ventral sucker.**

though *L. miniatus* was found to be host to another (undescribed) species of *Gonapodasmius*.

The classification of the Didymozoidae is based largely on that of Yamaguti (1971) who recognized 23 subfamilies of which 3, the Gonapodasmiinae, the Nematobothriinae, and the Glomeritrematinae, include threadlike forms such as the present species. Of these 3 subfamilies, only the Gonapodasmiinae includes forms with separate sexes, and it may be separated from the other 2 on that basis. Within the Gonapodasmiinae, 3 genera have been recognized—*Gonapodasmius* Ishii, 1935, *Paragonapodasmius* Yamaguti, 1938, and *Neogonapodasmius* Radhakrishnan and Nair, 1981. Yamaguti (1938, 1971) differentiated *Paragonapodasmius* from *Gonapodasmius* by the relative positioning of the



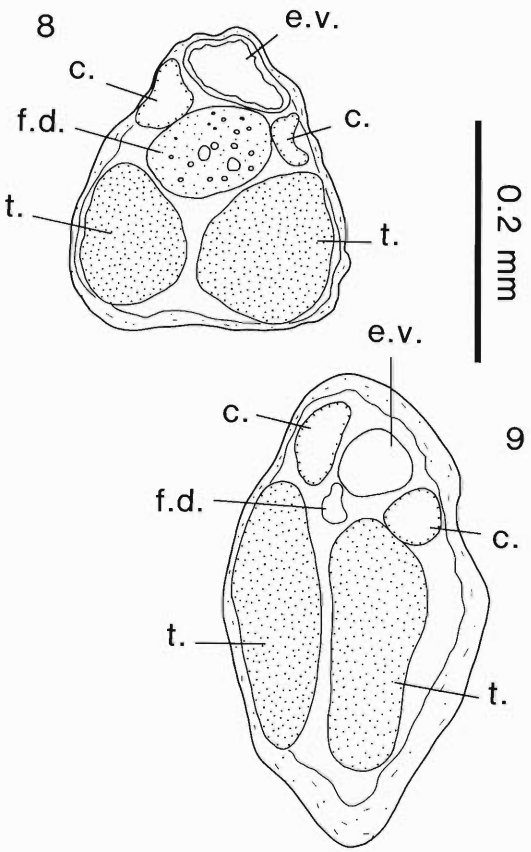
Figures 5–7. ***Gonapodasmius williamsoni*** **sp. n. in transverse section. 5. Female, level of metraterm. 6. Female, level of ovary and single loop of uterus. 7. Female, level of vitellarium and 2 loops of uterus. c., cecum; e.v., excretory vesicle; m., metraterm; o., ovary; u., uterus; v., vitellarium.**

testes, which are tandem in the former and parallel in the latter. Radhakrishnan and Nair (1981) distinguished *Neogonapodasmius* from the other 2 genera because the female has a distinct fore- and hindbody and the ovary and vitellaria are branched. Based on these criteria, the present species should therefore be placed in *Gonapodasmius*.

Fifteen *Gonapodasmius* species have been described and recorded (generally only in the original description): *G. branchialis* Yamaguti, 1970, from Hawaii, encysted in pairs or singly in "sea bass" and on or in the olfactory organ of *Epinephelus quernus* (Serranidae) (Shen, 1990) and in *Cephalopholis platycentron* (also Serranidae); *G. cypseluri* Yamaguti, 1940, from Japan, in the submucosa of the buccal cavity of *Cypselurus agoo* (Exocoetidae); *G. epinepheli* Abdul-Salam, Sreeltha, and Farah, 1990, from the Arabian Gulf, encysted on the gills of *Epinephelus tauvina* (Serranidae); *G. haemuli* (MacCallum and MacCallum, 1916) Ishii, 1935, from the New York Aquarium (presumed origin North Atlantic), in the pseudobranchs of *Haemulon flavolineatum* (Haemulidae); *G. hainanensis* Gu and Shen, 1983, from the China Sea, encysted on the gills of *Trisotropis dermopterus* (Serranidae) (see also Shen, 1990); *G. kovaljovae* Nikolaeva and Gaevskaya, 1985, from the Atlantic Ocean, encysted on the gills of *Cubiceps capensis* (Nomeidae); *G. menpachi* Yamaguti, 1970, from Hawaii, in the body cavity of *Myripristis berndti* (Holocentridae); *G. microovatus* Reimer, 1980, from the Gulf of Aden, in the body cavity of *Megalaspis cordyla* (Carangidae); *G. okushimai* Ishii, 1935, from Japan, in the muscle of *Pagrus auratus* (as *Pagrosomus major*) (Sparidae); *G. oxyporhamphii* Nikolaeva and Gichenock, 1981, from the Indian and Pacific oceans, from the fins of *Oxyporhamphus convexus* and *O. micropterus* (Hemirham-

phidae); *G. pacificus* Yamaguti, 1938, from Japan, in the gills of an "epinephelid" (presumably Serranidae); *G. pristipomatis* (Yamaguti, 1934) Yamaguti, 1938, from Japan, in the gills of *Pristipoma trilineatum* (Haemulidae) and the pharyngo-branchial region of *Epinephelus akaara* (Serranidae); *G. ryjikovi* Nikolaeva and Parukhin, 1971, from the Atlantic Ocean, in the pelvic and pectoral fins of *Cypselurus furcatus, Exocoetus volitans, E. monocirrhus* (all Exocoetidae), *Euleptorhamphus viridis,* and *Oxyporhamphus convexus* (both Hemirhamphidae); *G. spilonotopteri* Yamaguti, 1970, from Hawaii, free beneath the inner surface of the operculum and gill opening of *Cypselurus spilonotopterus* and *C. spilopterus,* and from the Bay of Bengal by Madhavi (1982) in connective tissue overlying the swim bladder of *C. comatus* (all Exocoetidae); *G. tomex* (Linton, 1907) Yamaguti, 1971, from Bermuda, in an unrecorded site in *Epinephelus striatus* (Serranidae).

We compared our specimens with the descriptions of these species and noted the following distinctions: *Gonapodasmius williamsoni* is the only species in the genus with a well-defined glandular stomach (=Drüsenmagen) at the cecal bifurcation. Although some of the other species have gland cells surrounding the beginning of the ceca, only *G. williamsoni* has a clearly demarcated "stomach." *Gonapodasmius pristipomatis* is distinct from *G. williamsoni* (and evidently all other species) in having 2 loops in the uterus. *Gonapodasmius haemuli* lacks a pharynx (it possesses only 1 muscular structure at the anterior end, which was interpreted as the pharynx by MacCallum and MacCallum [1916] but as the oral sucker by us). *Gonapodasmius okushimai* lacks a ventral sucker and has the genital pore much closer to the anterior end than does *G. williamsoni.* We found differences in the dimensions of the oral sucker, ventral sucker, and pharynx between *G. williamsoni* and some species, but because considerable ranges have been quoted for these features, and because they are often based on small sample sizes (in our description as well as in those of others), we find it difficult to draw useful conclusions from the differences noted. However, the ventral suckers of female *G. pacificus* and *G. menpachi* are recorded as being 125–150 and 120–180 μm in diameter, respectively—in each case more than twice the maximum diameter recorded for *G. williamsoni.* In *G. williamsoni* the ventral and oral suckers are of similar size, whereas in *G. microovatus* the



Figures 8, 9. ***Gonapodasmius williamsoni*** **sp. n. in transverse section. 8. Male, showing testes, gut, excretory vesicle, and "female duct" with malformed eggs. 9. Male, as previous with "female duct" weakly developed. c., cecum; e.v., excretory vesicle; f.d., female duct; t., testis.**

ventral sucker is much larger than the oral sucker, and in *G. ryjikovi* the oral sucker is much larger than the ventral sucker. The single described specimen of *G. tomex* is only 12 mm long as opposed to over 1 m for the specimens of *G. williamsoni. Gonapodasmius oxyporhamphii* and *G. kovaljovae* are far more robust than *G. williamsoni,* which is threadlike.

Gonapodasmius williamsoni appears to be distinct from other *Gonapodasmius* species, except *G. okushimai,* in the combination of the site of infection in the host and the identity of the host. These are the only 2 species recorded from muscle and also the only 2 known from sparids. This information can generally be used only with caution in the identification of helminths, and in the case of the Didymozoidae, the degree of site and host specificity has not been examined critically.

Indeed, it is salutary to note that only 4 of 15 *Gonapodasmius* species have been recorded in the literature more than once. Because of this, we use this information only as a posteriori support for further distinction of *G. williamsoni.* We believe that, in the present case, the evidence suggests that the site of infection and the host are valuable characters for use in association with the analysis of morphology. In support of this, we note that *G. williamsoni* always looked the same in the musculature (Figs. 1, 5–9) and that it was never seen other than in the lateral muscle. Furthermore, other fish species caught at the same sites as the infected snapper were not infected with this parasite.

In its host and site of infection *G. williamsoni* agrees entirely with *G. okushimai.* This special circumstance needs further consideration. *Gonapodasmius okushimai* was described from Japan from *Pagrosomus major,* a species now considered by Paulin (1990) to be a synonym of *Pagrus auratus,* the host of the present species. *Pagrus auratus* has a wide Indo-Pacific distribution from New Zealand and Australia to China and Japan, although northern and southern hemisphere populations of this species are "independent and reproductively isolated" (Paulin, 1990). This relationship between the 2 host populations sharpens the need for morphological separation of the 2 parasite species. We believe the differences we outlined earlier (presence/absence of Drüsenmagen and ventral sucker, position of genital pore) are strong characters. However, we recognize that should *G. okushimai* be found to possess a ventral sucker (which may have been overlooked in the original study), the case for recognition of 2 species would be weakened. Unfortunately, we were unable to examine specimens of *G. okushimai*; according to Dr. Shunya Kamegai of the Meguro Parasitological Museum, Japan, the location of the type specimens of this species is unknown. The infection of a single host species by more than 1 species of the same genus is a common occurrence within the Didymozoidae. For example, *Thunnus albacares* (yellowfin tuna) is reported to harbor 6 species of *Didymocystis* and 4 species of *Koellikeria.* Presence of 2 species within the 1 host therefore provides no special grounds for doubting the validity of the species.

Acknowledgments

Thanks are extended to the Williamson family of Carnarvon, Western Australia, who assisted in many ways with this research, including carrying us aboard their fishing vessel. Funding was provided by the Western Australian Fisheries Department (WAFD). We thank Dr. S. Kamegai of the Meguro Parasitological Museum for information on type specimens. Useful comments on the manuscript were given by Dr. Mike Moran (WAFD) and Dr. S. C. Barker and Miss S. C. Pichelin (Department of Parasitology, University of Queensland).

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Description of *Eocollis catostomi* sp. n. (Acanthocephala: Neoechinorhynchidae) from Two Species of Suckers (Catostomidae) in Alabama, with Comments on *Eocollis arcanus*

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ABSTRACT: *Eocollis catostomi* sp. n. is described from *Minytrema melanops* (Agassiz) and *Moxostoma poecilurum* (Jordan) in Alabama. *Eocollis catostomi* is distinguished from *E. arcanus* by having a more cylindrical trunk; longer proboscis hooks in anterior (71–81 [74] μm), middle (45–55 [49] μm), and posterior (24–28 [26] μm) circles; larger proboscis receptacle (273–395 [335] μm); lemnisci markedly unequal in length; an unelevated female genital pore; and wider eggs (24–26 [25] μm) that lack tubelike structures in the middle membrane. This is the first report of *Eocollis* from catostomid fishes in North America. *Eocollis arcanus* is reported from fishes collected in Alabama, Louisiana, and Mississippi. Also, the existence of a protuberance elevating the female genital pore and a middle egg membrane having tubelike structures is reported for *E. arcanus*.

KEY WORDS: *Eocollis catostomi* sp. n., *Eocollis arcanus*, Acanthocephala, Catostomidae, fish parasites, Alabama.

Two species of *Eocollis* Van Cleave, 1947, have been described: *E. arcanus* Van Cleave, 1947, from the United States and *E. harengulae* Wang, 1981, from China. Since its description from *Lepomis macrochirus* and *Pomoxis nigromaculatus* in the Ohio River and southern Illinois (Van Cleave, 1947), *E. arcanus* has been reported from *L. macrochirus* in Alabama (Williams and Rogers, 1982); *L. macrochirus* and *Pomoxis annularis* in Illinois (Lincicome, 1949); *L. macrochirus* in Louisiana (Arnold et al., 1968); and *Chaenobryttus coronarius, Lepomis cyanellus, L. macrochirus, P. nigromaculatus,* and *P. annularis* in Texas (Meade and Harvey, 1968; Meade and Bedinger, 1972).

During examination for acanthocephalans from fishes collected in the southeastern United States, I collected *E. arcanus* from several species of centrarchids in 3 states. Study of these specimens along with 10 paratypes (USNM Helm. Coll. No. 37666) provided information on the position of the female genital pore and the shape of the eggs not reported in the original description. I also collected females of an undescribed species of *Eocollis* from 2 species of catostomids collected in Alabama. This species is here described. This is the first report of *Eocollis* from catostomid hosts in North America.

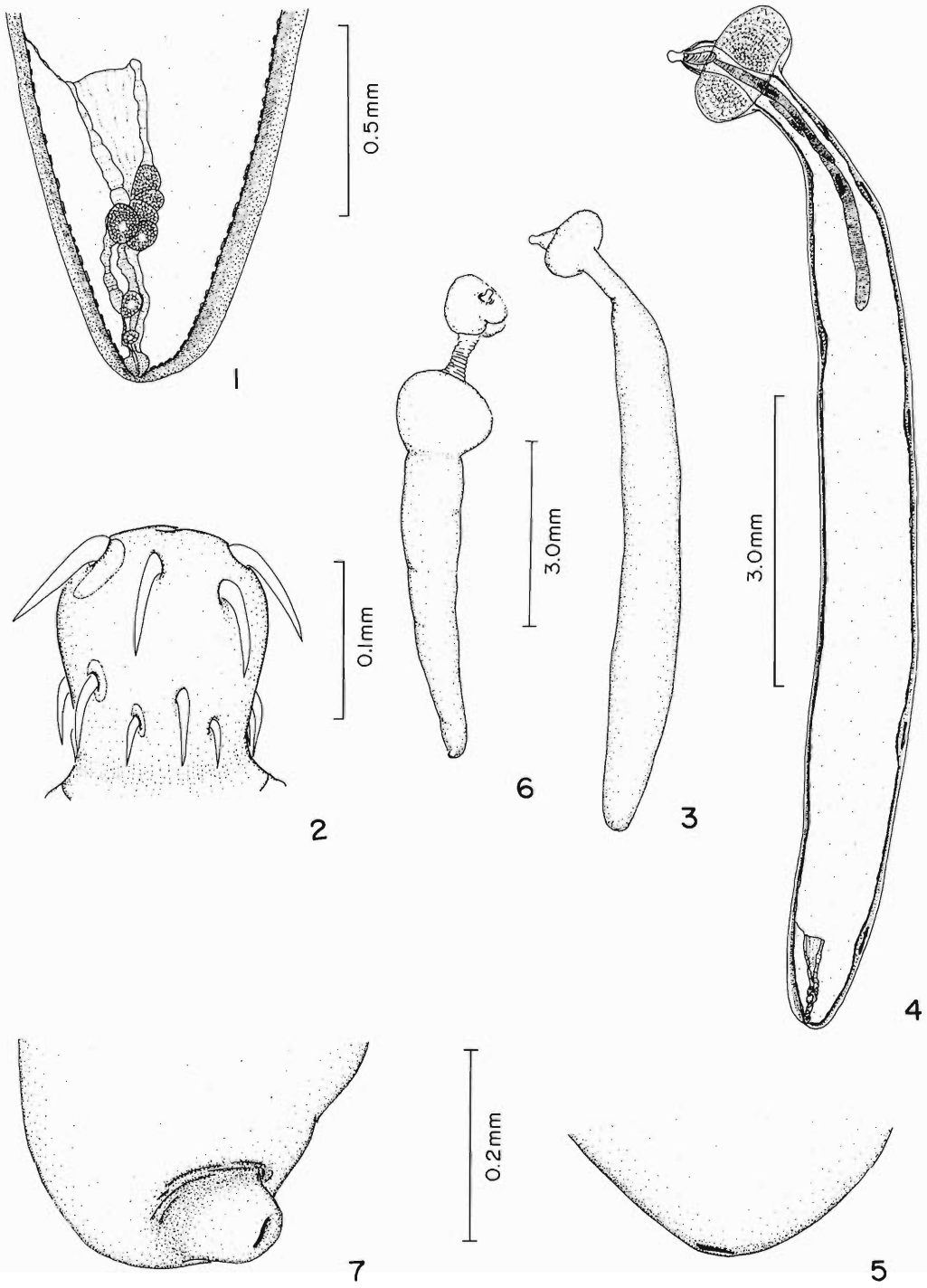
Materials and Methods

Fish were collected by trammel net or seine, kept in iced water, and examined within 24 hr of collection. Acanthocephalans were placed in distilled water to evaginate the proboscides and then fixed in alcohol–formaldehyde–acetic acid (AFA). Specimens were stained with Mayer's alum carmine, dehydrated, cleared in xylene, and mounted in Canada balsam. The description is of gravid females. Mature eggs are considered to be those with fully formed acanthors. Line drawings were prepared with the aid of a drawing tube. All measurements, unless otherwise noted, are in micrometers, with averages in parentheses.

Eocollis arcanus Van Cleave, 1947 (Figs. 6, 7, 11–13)

Eocollis arcanus was collected from *Pomoxis nigromaculatus* in Humphrey County, Mississippi; *Lepomis cyanellus* and *Lepomis marginatus,* in LeFlore County, Mississippi; *Lepomis macrochirus* in Washington Parish, Louisiana, and Choctaw and Dallas counties, Alabama; and from *Centrarchis macropterus* in Sumter County, Alabama. Of the 8 specimens collected, all were immature except for 1 female from *L. macrochirus* in Louisiana and 1 male from *L. macrochirus* in Alabama. The general body shape and sizes of proboscis hooks of these specimens agree with the original description of *E. arcanus*.

Van Cleave (1947) gave dimensions of "embryos within the body cavity" of *E. arcanus* as being 41 to 47 long by 10 wide but did not provide further description or an illustration of the eggs. Eggs were teased from the body cavity of the 1 mature female from the present collection. Most of these eggs were nearly mature and were within the reported size range. The middle membrane at the ends of the embryo in the nearly

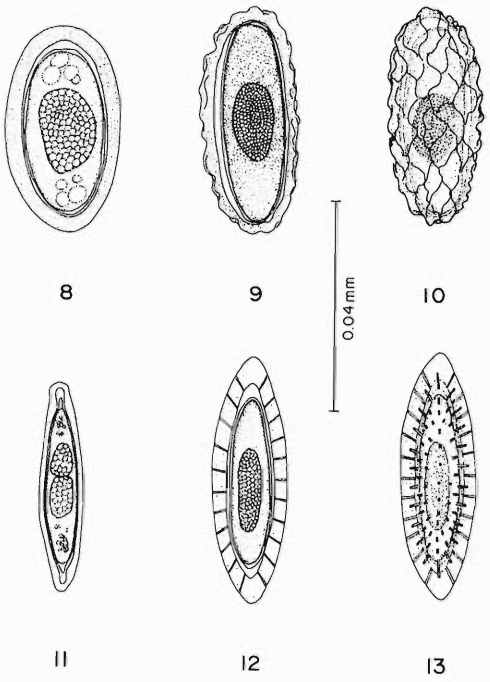


Figures 1–7. 1–5. *Eocollis catostomi* sp. n. holotype female. 1. Posterior end. 2. Proboscis. 3. Surface view. 4. Outline. 5. Surface view of posterior end. 6, 7. *Eocollis arcanus* paratypes. 6. Surface view of female. 7. Surface of posterior end of female. Scale between Figures 6 and 3 applies to both.

mature eggs (Fig. 11) was terminally constricted giving the appearance of polar protrusions. Mature eggs were 43–50 (47) long by 12–15 (13) wide (N = 12). These dimensions are slightly larger than the measurements originally reported and measured from examined paratypes. The polar protrusions were not seen in these mature eggs; the middle membrane was much thicker and contained tiny tubelike structures oriented perpendicularly to its surface (Figs. 12, 13). Eggs of paratypes were examined and measured. Most of these eggs appeared to have the polar protrusions. The egg membranes of many exhibited an opacity pattern suggestive of tubelike structures but might be an artifact of the age and shrinkage of specimens.

Female paratypes had a distinct genital protuberance not reported by Van Cleave (1947). This structure (Fig. 7), evident in all but the most contracted specimens, terminates with the genital pore. The immature females from the present collection did not show this structure and the posterior end of the 1 mature female was so contracted as to obscure its presence.

SPECIMEN DEPOSITION: The following voucher specimens of *E. arcanus* are deposited in the University of Nebraska State Museum's Harold W. Manter Laboratory Collection: 2 immature females from *L. marginatus* in Mississippi (HWML No. 34189), 1 mature female from *L. macrochirus* in Louisiana (HWML No. 34188), 1 immature female from *Centrarchis macropterus* (HWML No. 34190) in Alabama, and 1 mature male from *L. macrochirus* (HWML No. 34187) in Alabama.



Figures 8–13. 8–10. Eggs of *Eocollis catostomi* sp. n. 8. Immature egg outline. 9. Mature egg. 10. Surface of mature egg. 11–13. Eggs of *Eocollis arcanus*. 11. Nearly mature egg. 12. Mature egg. 13. Surface of mature egg.

Description

Eocollis catostomi sp. n. (Figs. 1–5, 8–10)

FEMALES (based on 32 gravid specimens): With characteristics of the genus *Eocollis*. Trunk (inclusive of trunk bulb and false neck) 5.7–15.0 (9.6) mm long. Trunk bulb 338–675 (518) long by 600–1,425 (1,083) wide. False neck 425–1,763 (938) long (measured from base of trunk bulb to point where body wall enlarges to form main trunk) by 213–450 (286) wide at narrowest point. Trunk proper nearly cylindrical, 525–1,650 (1,061) wide. Dorsal and ventral body wall about equal in thickness. Proboscis slightly longer than wide, 104–130 (119) long (N = 11) by 108–133 (109) wide (N = 17). Neck 31–40 (35) long by 92–124 (109) wide. Apical organ 97–111 (106) long. Proboscis hooks in anterior circle 71–81 (74) long by 12–17 (14) wide, in middle circle 45–55 (49) long, in posterior circle 24–28 (26) long. Proboscis receptacle 273–395 (335) long by 97–164 (118) wide. Lemnisci unequal in length; uninucleate lemniscus 55–97% (70%) of binucleate lemniscus length. Uninucleate lemniscus 956–1,963 (1,444) long, occupying 10–22% (15%) of trunk length; binucleate lemniscus 1,258–3,050 (2,160) long, occupying 11–33% (23%) of trunk length. Reproductive system 476–967 (744) long, occupying 7–11% (8%) of trunk length; uterus length variable depending on state of contraction, 62–484 (217); vagina 116–236 (173) long. Genital pore terminal, surrounding tegumental area unmodified. Mature eggs teased from the body cavity of mounted specimens ovoid with a wrinkled surface; 43–50 (48) long by 24–26 (25) wide.

MALES: Unknown.

Taxonomic summary

TYPE HOST: Spotted sucker, *Minytrema melanops* (Rafinesque) (Catostomidae).

OTHER HOST: Blacktail redhorse, *Moxostoma poecilurum* (Jordan) (Catostomidae).

SITE OF INFECTION: Intestine.

TYPE LOCALITY: Grinlin Lake, an oxbow lake off the Sucarnoochee River near its confluence with the Tombigbee River, east of Bellamy, Sumter County, Alabama, R1W T17N, Sec. 26, 10 April 1981.

SPECIMEN DEPOSITION: Holotype female, United States National Museum Helminthological Collection (USNM Helm. Coll.) No. 82068. Paratype females, USNM Helm. Coll. No. 82069 and HWML Coll. No. 34186. Other paratypes retained by the author.

ETYMOLOGY: The name *catostomi* refers to the family of piscine hosts.

Remarks

Eocollis catostomi is readily distinguished by having proboscis hooks much larger than those originally reported for *E. arcanus* (anterior circle 47–59 long and 6 thick, middle circle 23 long, and posterior circle 12 long). Also, *E. arcanus* has a smaller proboscis receptacle (80–210 long for 50–67 wide) and slightly shorter proboscis (76–117). The trunk proper of *E. catostomi* is nearly cylindrical, lacking the pronounced anterior expansion evident in *E. arcanus* (Figs. 3, 6). The lemnisci of *E. catostomi* are typically unequal in length, whereas those of *E. arcanus* are nearly equal in length. The eggs of *E. catostomi* are wider than those originally described for *E. arcanus* (41–47 by 10) and those observed by the author (43–50 by 12–15). The posterior end of female *E. catostomi* lacks the distinct genital protuberance observed in female paratypes of *E. arcanus* (Figs. 5, 7).

Eocollis catostomi also appears to differ from *E. arcanus* in host specificity. *Eocollis arcanus* in known only from centrarchid fish hosts, whereas *E. catostomi* was collected only from catostomid fishes, *Minytrema melanops* (5 specimens) and *Moxostoma poecilurum* (5), all fish infected. None of 11 other species of fish from 4 other families collected at the type locality of *E. catostomi* was found infected with this acanthocephalan. These piscine species (number examined in parentheses) include Centrarchidae: *Lepomis gulosus* (1), *L. macrochirus* (1), *L. megalotis* (3), *L. microlophus* (20), *Micropterus salmoides* (6), *Pomoxis annularis* (4), *P. nigromaculatus* (5); Ictaluridae: *Ictalurus natalis* (10), *I. nebulosus* (3), *I. punctatus* (1); and Scianidae: *Aplodinotus grunniens* (1).

Acknowledgment

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Branch Pattern for Anterior Proboscis Nerves in *Macracanthorhynchus hirudinaceus* (Acanthocephala)

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ABSTRACT: The anterior proboscis nerves of *Macracanthorhynchus hirudinaceus* are associated with the sensory support cell duct and sensory nerves. The entire complex of duct and nerves is largely surrounded by the proboscis retractor muscles. The pair of anterior proboscis nerves branch immediately posterior to the apical sensory organ. Transmission electron microscopy clearly shows this division as well as the morphology of adjacent sensory nerves and sensory support cell duct. The separation of the anterior proboscis nerves into 2 pairs at the base of the apical sensory organ is shown.

KEY WORDS: Acanthocephala, nerves, *Macracanthorhynchus hirudinaceus,* morphology.

Dunagan and Miller (1983) illustrated the relationship of sensory support cell to apical sense organ. Included in this illustration was a drawing showing the entry of the sensory nerves and sensory support cell duct into the cone-shaped apical sense organ. Dunagan and Miller (1983) reported that the anterior proboscis nerves branched at the posterior margin of this organ. Because those observations were made using light microscopy, it was not possible to capture the point where branching occurred. Indeed, the visual evidence presented by these authors requires a bit of faith to interpret. The present study describes observations with transmission electron microscopy (TEM). The greater magnification and thinner sections allow the branching of the anterior proboscis nerves to be shown clearly. In addition, the appearance and initial relationship of the posterior margin of the apical sense organ to the nerves and duct in this region are evident.

Materials and Methods

Macracanthorhynchus hirudinaceus were obtained from pigs through the courtesy of Reelfoot Meat Packaging in Union City, Tennessee. Worms were transported to the laboratory in intestinal contents placed in Dewar flasks. Four to 6 hr elapsed between collection and initial preparation for TEM. The protruded proboscis was removed immediately posterior to the neck and fixed for 1 hr at room temperature in a mixture of 2% glutaraldehyde and 2% freshly prepared formaldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 2.0 mM ethylene glycol-bis (β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA) and 1.0 mM $MgSO_4$. This preparation was placed in a refrigerator (5°C) for 3 days. Additional procedures were as described by Dunagan and Bozzola (1989). Sections were mounted on slot grids and examined in a Hitachi H500H transmission electron microscope. Position of dorsal and ventral surface is based on conclusions of Hyman (1951).

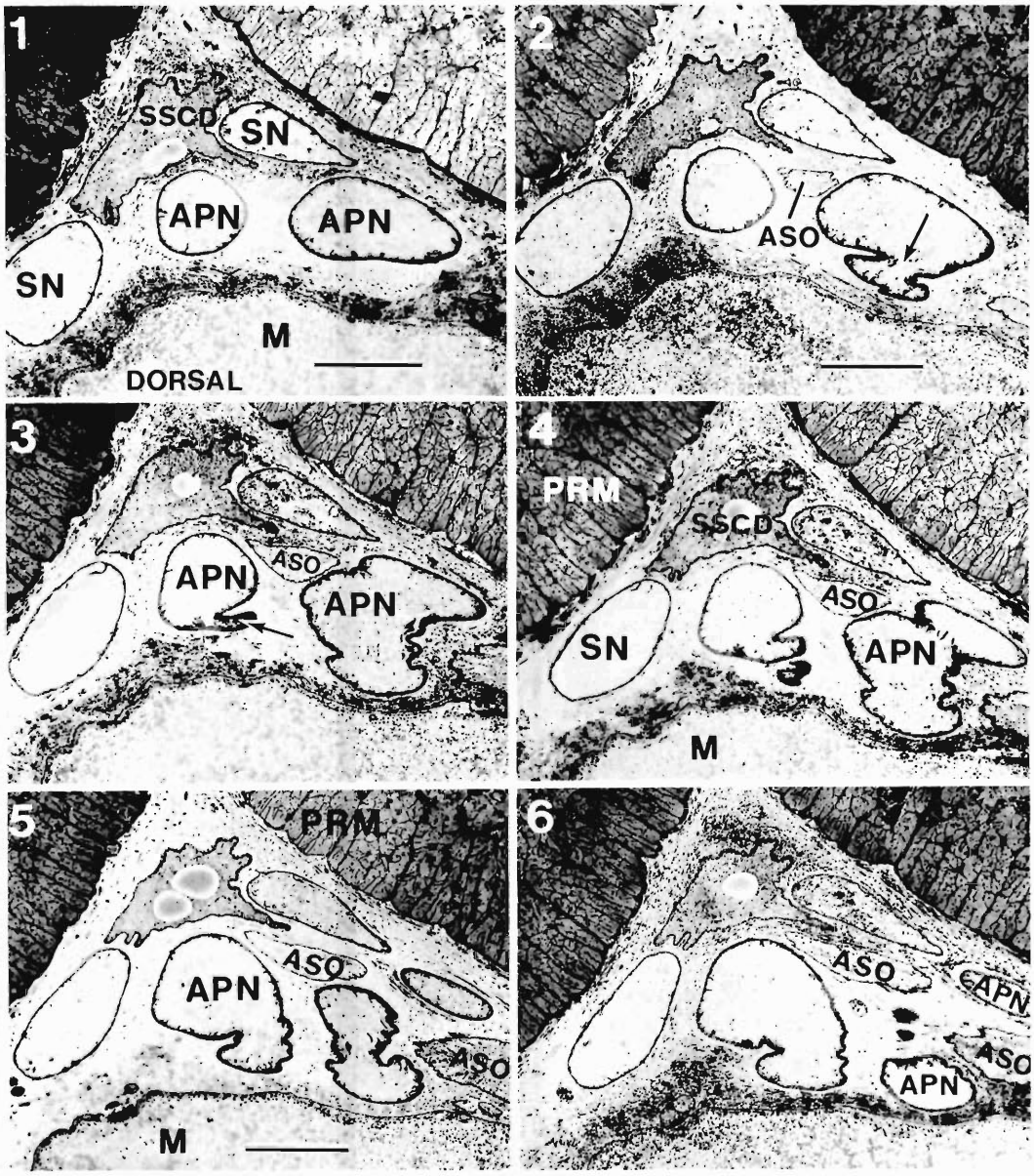
Results

The sensory support cell duct (SSCD), 2 sensory nerves (SN), and 2 anterior proboscis nerves (APN) occupy the same area immediately posterior to the base of the apical sense organ (Fig. 1). These structures are surrounded on 3 sides by proboscis retractor muscles (PRM) and on the fourth by a poorly defined structure called a "Markbeutel" (M) by 19th-century German investigators. At this level in the proboscis, the SSCD outline is very irregular but clearly separate from the SN. More anteriorly the SSCD and SN become so intertwined that they are difficult to separate.

A portion of the apical sense organ (ASO) appears in Figure 2 and gradually expands anteriorly (Figs. 3–10) until it separates the recently branched APN (Fig. 10). The ventral pair of APN will accompany the SSCD and SN until the latter enter the ASO after which both dorsal and ventral APN remain on the outer surface of the ASO.

The two APN do not divide simultaneously. The first division was initiated by the most peripheral APN (Fig. 2, arrow) and progresses (Figs. 2–5) until completed (Fig. 5). The remaining APN begins division more anteriorly (Fig. 3, arrow) and completes division (Fig. 7) after the more peripheral APN is completely separated by the base of the ASO. Following division of the original pair of APN, the newly formed branches and the SN have the same general appearance.

The SSCD remains pleomorphic throughout its remaining length, but structurally it becomes

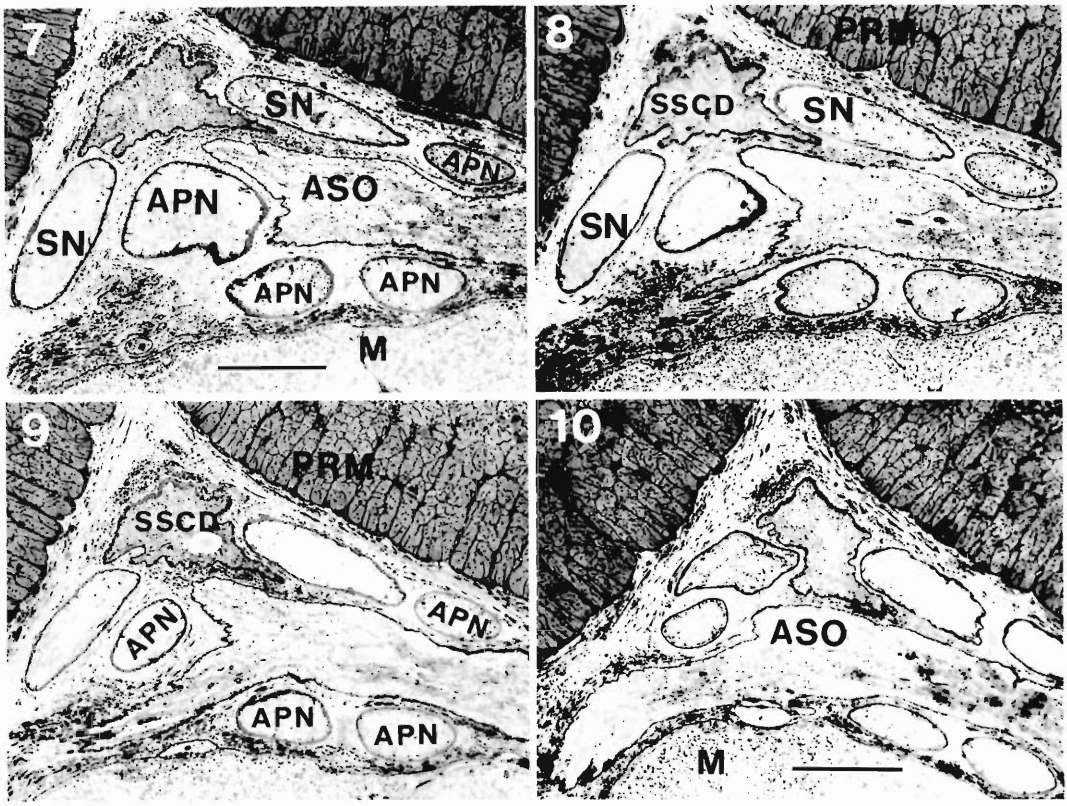


Figures 1–6. Electron photomicrographs of nerves and adjacent tissue in the proboscis of *Macracanthorhynchus hirudinaceus*. 1. Posterior to apical sensory organ. Sensory support cell duct highly vesiculated. 2. Posterior terminus of apical sense organ. One anterior proboscis nerve begins division. 3. Second APN initiates division. 4. Division of first APN nears completion. 5. Division of first APN completed. 6. Division of second APN nears completion. APN, anterior proboscis nerve; ASO, apical sensory organ; M, Markbeutel; PRM, proboscis retractor muscle; SN, sensory nerve; SSCD, sense support cell duct. Magnification ×9,000. Scale bar = 2.0 μm.

increasingly more complex as it approaches the anterior terminus of the ASO. The SN also become pleomorphic and eventually interact with the SSCD. In contrast, the APN are smooth surfaced before and after division. The target of the APN is thought to be retractor muscles.

Discussion

A literature review regarding the general construction of the ASO was made by Dunagan and Miller (1983). This review pointed out the lack of information regarding acanthocephalan recep-



Figures 7–10. Electron photomicrographs of posterior terminus of apical sense organ (ASO) and associated nerves and sensory support cell duct (SSCD) in proboscis of *Macracanthorhynchus hirudinaceus*. 7. Division of second anterior proboscis nerve (APN) completed. 8. Base of ASO separates both recently divided APN. 9. Base of ASO expanded but not yet isolating sensory nerve. 10. Base of ASO completely separates pair of branched APN from SN and SSCD. APN, anterior proboscis nerve; ASO, apical sense organ; M, Markbeutel; PRM, proboscis retractor muscle; SN, sensory nerve; SSCD, sensory support cell duct. Magnification ×9,000. Scale bar = 2.0 μm.

tor morphology as well as the absence of data on receptor function. These authors also reviewed the work of Kaiser (1893), Rauther (1930), and Kilian (1932), all of whom mentioned the presence of nerves at the "Tastpapille." Earlier work in this area was reviewed by Harada (1931). None of these studies was as well received as the study of Brandes (1899) who had his illustration appear in reviews by Hyman (1951) and Bullock and Horridge (1965). Brandes illustrated a single nerve to the ASO. This nerve was unbranched. Dunagan and Miller (1983) described a pair of sensory and anterior proboscis nerves to the ASO. The present investigation shows the point of division of the APN and the subsequent separation of these nerves by the ASO. Moreover, the relationship of this division to the ASO, SSCD, and SN is clearly illustrated in the figures presented herein.

Acknowledgments

We thank Mr. Steven Schmitt for assistance in electron microscopy. Financial support was provided by the Department of Physiology, Southern Illinois University, Carbondale, Illinois.

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J. Helminthol. Soc. Wash.
59(2), 1992, 167–169

Description of a New Species of *Eimeria* (Apicomplexa: Eimeriidae) from the Alligator Snapping Turtle, *Macroclemys temminckii* (Testudines: Chelydridae)

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ABSTRACT: Coccidian oocysts recovered from the feces of an alligator snapping turtle, *Macroclemys temminckii* (Harlan, 1835), in Arkansas, were found to represent a previously unreported eimerian. Oocysts of *Eimeria harlani* sp. n. are spherical to subspherical, 13.0 × 12.6 (10.4–14.4 × 10.4–13.8) μm, with a thin, single-layered wall; shape index (length/width) 1.03 (1.00–1.14). A micropyle is absent; oocyst residuum and polar granule present. Sporocysts are ovoidal, 8.9 × 5.2 (8.0–9.6 × 4.8–5.6) μm, with Stieda body; shape index 1.71 (1.57–1.85). A sporocyst residuum is present, consisting of 3–12 granules up to 1.0 μm in diameter. Sporozoites are elongate, 10.5 × 2.3 (8.0–12.0 × 2.0–2.6) μm in situ, arranged head-to-tail in sporocyst.

KEY WORDS: alligator snapping turtle, *Macroclemys temminckii*, Testudines, *Eimeria harlani* sp. n., Apicomplexa, coccidia.

The alligator snapping turtle, *Macroclemys temminckii* (Harlan, 1835), is a large aquatic reptile that ranges from southwestern Georgia and northern Florida west into eastern Texas and north into Kansas, Iowa, Illinois, and southwestern Kentucky (Conant and Collins, 1991). It inhabits rivers, lakes, oxbows, and sloughs where it feeds primarily on fish, other turtles, and carrion (Johnson, 1987). Unfortunately, this species is becoming rare over much of its range due to exploitation as a food resource (Pritchard, 1989).

Although information is available on the biology of the alligator snapping turtle (Pritchard, 1989), little is known about its endoparasites (Mackin, 1936; Cahn, 1937; Ernst and Ernst, 1977) and nothing has been published on coccidian parasites from alligator snapping turtles. Herein we report a new species of *Eimeria* from *M. temminckii* from Arkansas.

Materials and Methods

A single subadult *Macroclemys temminckii* (carapace length 32.5 cm) was collected accidentally in April 1991 by hoop net in the Black River, Jackson County, Arkansas. The turtle was returned to the laboratory, administered an overdose of sodium pentobarbital, and feces collected from the rectum. Feces were initially examined for coccidia using brightfield microscopy following flotation in a sucrose solution (sp. gr. 1.30). Fecal samples were then placed in a thin layer of 2.5% (w/v) aqueous $K_2Cr_2O_7$ solution in shallow Petri dishes, and unsporulated oocysts allowed to develop for 1 wk at room temperature (ca. 23°C). Oocysts were then concentrated by flotation and measured using an ocular micrometer; oocysts were photographed from wet mounts made using tap water. All measurements are presented in micrometers (μm), followed by the ranges in parentheses.

Results and Discussion

Numerous oocysts were found in the feces of the turtle, which proved to represent a previously undescribed species. Below we present a description of this new coccidian.

Eimeria harlani sp. n. (Figs. 1–3)

DESCRIPTION OF OOCYSTS: Oocysts spherical to subspherical, 13.0 × 12.6 (10.4–14.4 × 10.4–13.8) (N = 30); shape index (length/width) 1.03 (1.00–1.14). Wall of single thin layer, ca. 0.5 thick. Micropyle absent; oocyst residuum present, as delicate spherical or subspherical mass of granules usually surrounding vacuolelike structure; polar granule present. Sporocysts ovoidal, 8.9 × 5.2 (8.0–9.6 × 4.8–5.6) (N = 20), with thin wall ca. 0.4 thick; shape index 1.71 (1.57–1.83). Stieda body present, as thickening at 1 end of sporocyst; substieda body absent. Sporocyst residuum present, either as 3–12 scattered granules or as compact mass 3.1 × 2.9 (1.8–4.0 × 1.8–3.4) (N = 7). Sporozoites elongate, 10.5 × 2.3 (8.0–12.0 × 2.0–2.6) (N = 20) in situ, arranged head-to-tail within sporocyst. Posterior ends of sporozoites reflected back along 1 end of spo-

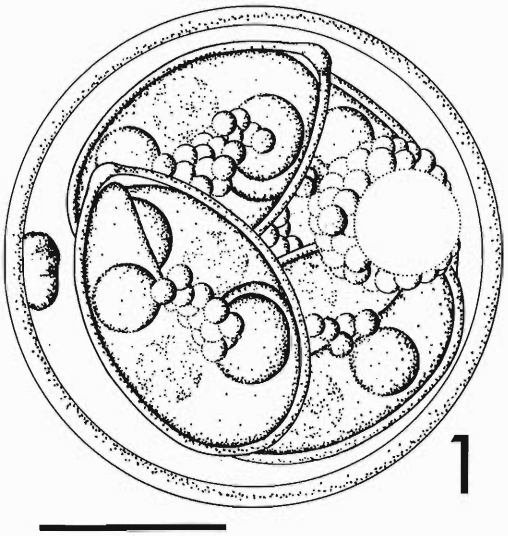
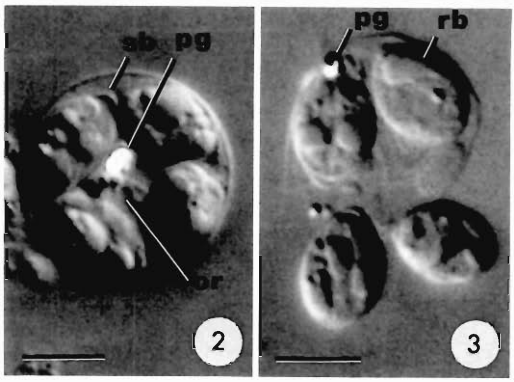


Figure 1. Composite line drawing of sporulated oocyst of *Eimeria harlani* sp. n. Scale bar = 5.0 μm.



Figures 2, 3. Nomarski interference-contrast photomicrographs of sporulated oocysts of *Eimeria harlani* sp. n. Abbreviations: or, oocyst residuum; pg, polar granule; rb, refractile body; sb, Stieda body. Scale bars = 5.0 μm.

rocyst. Each sporozoite contains a spherical anterior refractile body 1.8 (1.2–2.4) ($N = 20$) and a spherical posterior refractile body 2.2 (1.6–3.0) ($N = 20$). Nucleus located between refractile bodies.

TYPE HOST: *Macroclemys temminckii* (Harlan, 1835) "alligator snapping turtle" (Testudines: Chelydridae). Specimen collected 30 April 1991; voucher specimen deposited in the Arkansas State University Museum of Zoology as ASUMZ 17616.

TYPE SPECIMENS: Syntypes (oocysts in 10% formalin) have been deposited in the U.S. National Museum, Beltsville, Maryland 20705 as USNM No. 82005.

TYPE LOCALITY: Black River, Jackson County, Arkansas, 9.7 km west of Swifton.

SITE OF INFECTION: Unknown. Oocysts recovered from intestinal contents and feces.

SPORULATION: Exogenous. All oocysts were passed unsporulated and became fully sporulated within 1 wk at ca. 23°C.

ETYMOLOGY: The specific epithet is given in honor of Richard Harlan (1796–1843), American vertebrate paleontologist and comparative anatomist, who described the alligator snapping turtle in 1835 originally under the name *Chelonura temminckii* (see Bour, 1987).

REMARKS: No species of coccidian has been reported from alligator snapping turtles previously and oocysts of *Eimeria harlani* sp. n. are unlike any reported thus far from the family Chelydridae (McAllister and Upton, 1989; McAllister et al., 1990). Of the named *Eimeria* spp. reported thus far in the family (all from *Chelydra serpentina*), *Eimeria serpentina* McAllister, Upton, and Trauth, 1990 (probable synonym *E.* sp. of Wacha and Christiansen, 1980) has smaller oocysts and lacks an oocyst residuum (Wacha and Christiansen, 1980; McAllister and Upton, 1989; McAllister et al., 1990). *Eimeria chelydrae* Ernst, Stewart, Sampson, and Fincher, 1969, has larger oocysts and sporocysts and lacks a residuum. *Eimeria filamentifera* Wacha and Christiansen, 1979, is considerably larger overall (Ernst et al., 1969; Wacha and Christiansen, 1979). Although *E. mitraria* (Laveran and Mesnil, 1902) Doflein, 1909, has been reported from *Chelydra serpentina* by Wacha and Christiansen (1980), this coccidian may actually have been *Isospora chelydrae* McAllister, Upton, and Trauth, 1990, because the oocysts are similar morphologically (McAllister et al., 1990). Oocysts of these latter 2 species are irregular in shape (Laveran and Mesnil, 1902; McAllister et al., 1990) and cannot be confused with *E. harlani.*

Acknowledgments

We thank Mr. Anthony Holt for collecting the *M. temminckii.* The specimen was collected under the authorization of the Arkansas Game and Fish Commission under scientific collection permit #1048 issued to S.E.T.

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59(2), 1992, 170–176

Ultrastructure of *Frenkelia microti* in Prairie Voles Inoculated with Sporocysts from Red-tailed Hawks

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ABSTRACT: The ultrastructure of *Frenkelia microti* tissue cysts was examined in experimentally inoculated prairie voles (*Microtus ochrogaster*). Tissue cysts were lobate, thin-walled, and divided into compartments by septa. The host cell nucleus was often hypertrophic. Tissue cysts were enclosed by a primary cyst wall composed of the parasitophorous vacuole membrane that was highly ornamented with 0.2-μm, electron-dense, knoblike projections. The primary cyst wall was supported by granular ground substance. The entire tissue cyst wall was about 0.7 μm in thickness. Ground substance formed septa that extended into the cyst to produce compartments. Osmiophilic bodies about 60 nm in diameter and associated with microfilaments were observed in the ground substance and septa. Membrane fragments and membranous bodies were often observed within compartments. Metrocytes and mature bradyzoites were present in compartments both centrally and at the periphery of tissue cysts. Metrocytes divided by endodyogeny to produce bradyzoites. Rhoptries and micronemes were present in the anterior ¼ to ⅓ of each bradyzoite. The bradyzoite nucleus was often elongate and located in the posterior ½ of the parasite. Amylopectin granules were most abundant in the posterior portion of the bradyzoite.

KEY WORDS: ultrastructure, tissue cyst, prairie vole, *Microtus ochrogaster,* red-tailed hawk, *Buteo jamaicensis,* Apicomplexa, Sarcocystidae, *Frenkelia microti.*

Frenkelia microti (Findlay and Middleton, 1934) Biocca, 1968, is an obligatory, heteroxenous coccidium that produces tissue cysts in the brains of a variety of rodents (Dubey et al., 1989). Recently, Upton and McKown (1992) reported that sporocysts isolated from the feces of a red-tailed hawk (*Buteo jamaicensis*) collected in Kansas, U.S.A., induced infections in orally inoculated prairie voles (*Microtus ochrogaster*). The tissue cysts, found only in the brain, resembled *F. microti* when examined with light microscopy. They were unable to demonstrate infection in white-footed mice (*Peromyscus leucopus*) orally inoculated with sporocysts of the same isolate.

Previous studies of the ultrastructure of tissue cysts of *F. microti* have been done on naturally infected hosts, often using poorly fixed material (Tadros et al., 1972; Hayden et al., 1976; Kennedy and Frelier, 1986). The present study was conducted to examine the ultrastructure of the tissue cysts of *F. microti* in an experimentally infected prairie vole.

Materials and Methods

Tissue cysts were obtained from the brain of a prairie vole inoculated orally 114 days previously with *Frenkelia*-like sporocysts obtained from a red-tailed hawk (SAR-13 of Upton and McKown, 1992). Small portions (about 2–3 mm^3) of brain were fixed in 2.5% (v/v) glutaraldehyde in phosphate-buffered saline, post-fixed in 1% osmium tetroxide, dehydrated in ethanols, and embedded in Spurr's plastic (Polysciences, Inc., Warrington, Pennsylvania). Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 301 transmission electron microscope operating at 60 kV. One-micrometer thick sections were stained with toluidine blue and examined with light microscopy. Additional portions of the brain were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, sectioned at 8 μm and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) for light microscopic examination.

Results

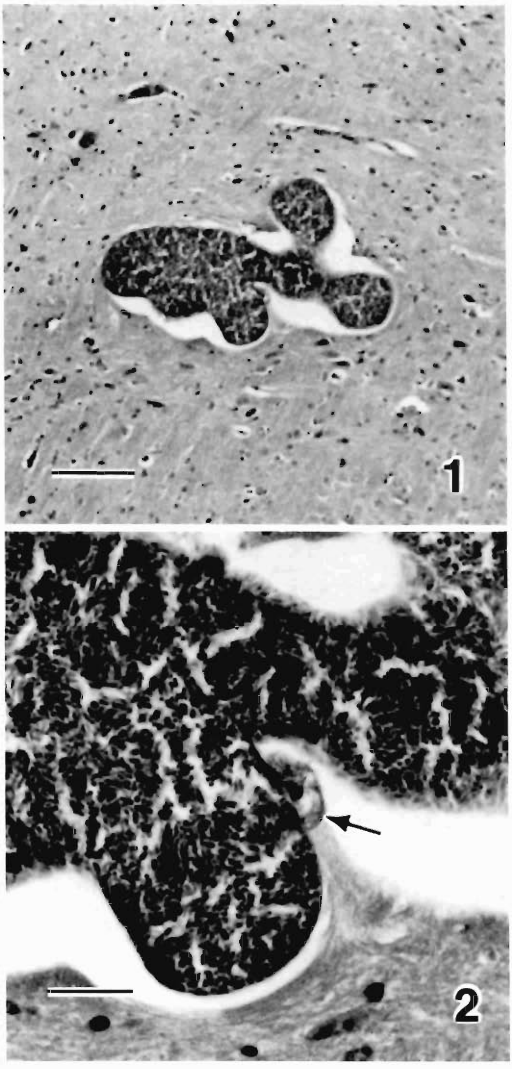
Seven tissue cysts were examined with light microscopy; 6 were lobate and irregular in shape (Fig. 1) and 1 was spherical. The tissue cyst walls were smooth and less than 0.5 μm thick. Septa were present but were difficult to visualize. Metrocytes contained few PAS-positive granules, whereas bradyzoites were intensely PAS-positive. Metrocytes were difficult to visualize in H&E stained sections but were readily seen in toluidine blue stained sections where they were faint blue in contrast to the dark blue bradyzoites. A hypertrophic nucleus of the infected host cell (Fig.

2) was seen adjacent to 2 tissue cysts. No other lesions were observed in sections of brain examined with light microscopy.

Three tissue cysts were examined with transmission electron microscopy (TEM). Their structure was similar (Figs. 3–5). Each tissue cyst was enclosed by a primary cyst wall composed of the parasitophorous vacuole (PV) membrane which was highly ornamented with electron dense knoblike projections. The knoblike projections were about 0.2 μm in length and were composed only of the PV membrane at the base. Electronlucent areas resembling holes were observed in portions of the primary cyst wall. They apparently resulted from sectioning through the bases of the knoblike projections (Fig. 5). The primary cyst wall was supported by granular ground substance, which composed the remainder of the tissue cyst wall. The entire tissue cyst wall was about 0.7 μm. The ground substance extended into the tissue cyst in the form of septa to produce a network of ill-defined compartments. Osmiophilic bodies about 60 nm and associated with microfilaments were often present in the ground substance and septa (Fig. 4). Metrocytes and mature bradyzoites were in compartments both centrally and at the periphery of tissue cysts (Fig. 3). However, some compartments at the periphery of tissue cysts appeared to contain only metrocytes. Membrane fragments and membranous bodies were often present within compartments (Fig. 4).

Metrocytes divided by endodyogeny to produce bradyzoites (Figs. 3, 4). Most major organelles disappeared prior to the beginning of bradyzoite production, but the conoid, micronemes, amylopectin granules, and mitochondria persisted well into bradyzoite formation. Bradyzoite anlagen developed in association with the parent nucleus, which became horseshoe-shaped and migrated into each of the developing bradyzoites. The conoids, rhoptries, micronemes, and subpellicular microtubules reappeared as the developing bradyzoites increased in size. The inner membrane complex of the metrocyte degenerated, and eventually the outer membrane of the developing bradyzoites fused with metrocyte outer pellicle membrane as mature bradyzoites were produced. Micropores were present in both metrocytes and bradyzoites.

Bradyzoites contained a conoid and at least 4 rhoptries. The ducts of rhoptries were often seen entering the conoid (Fig. 6). Numerous rodlike micronemes were present in the anterior ¼ to ⅓ of the bradyzoite (Figs. 6, 7). The bradyzoite nucleus was usually elongate and was located in the posterior ½ of the parasite (Fig. 7). Twenty-two subpellicular microtubules were present and they extended from the anterior end to about ½ the length of the bradyzoite. Amylopectin granules were present throughout the bradyzoites but were most abundant in the posterior portion of bradyzoites.



Figures 1, 2. Photomicrographs of *Frenkelia microti* tissue cysts in hematoxylin and eosin stained tissue sections. 1. Lobate tissue cyst. Note absence of host inflammatory response. Bar = 100 μm. 2. Higher magnification of tissue cyst in Figure 1. Note the hypertrophic host cell nucleus (arrow). Bar = 25 μm.

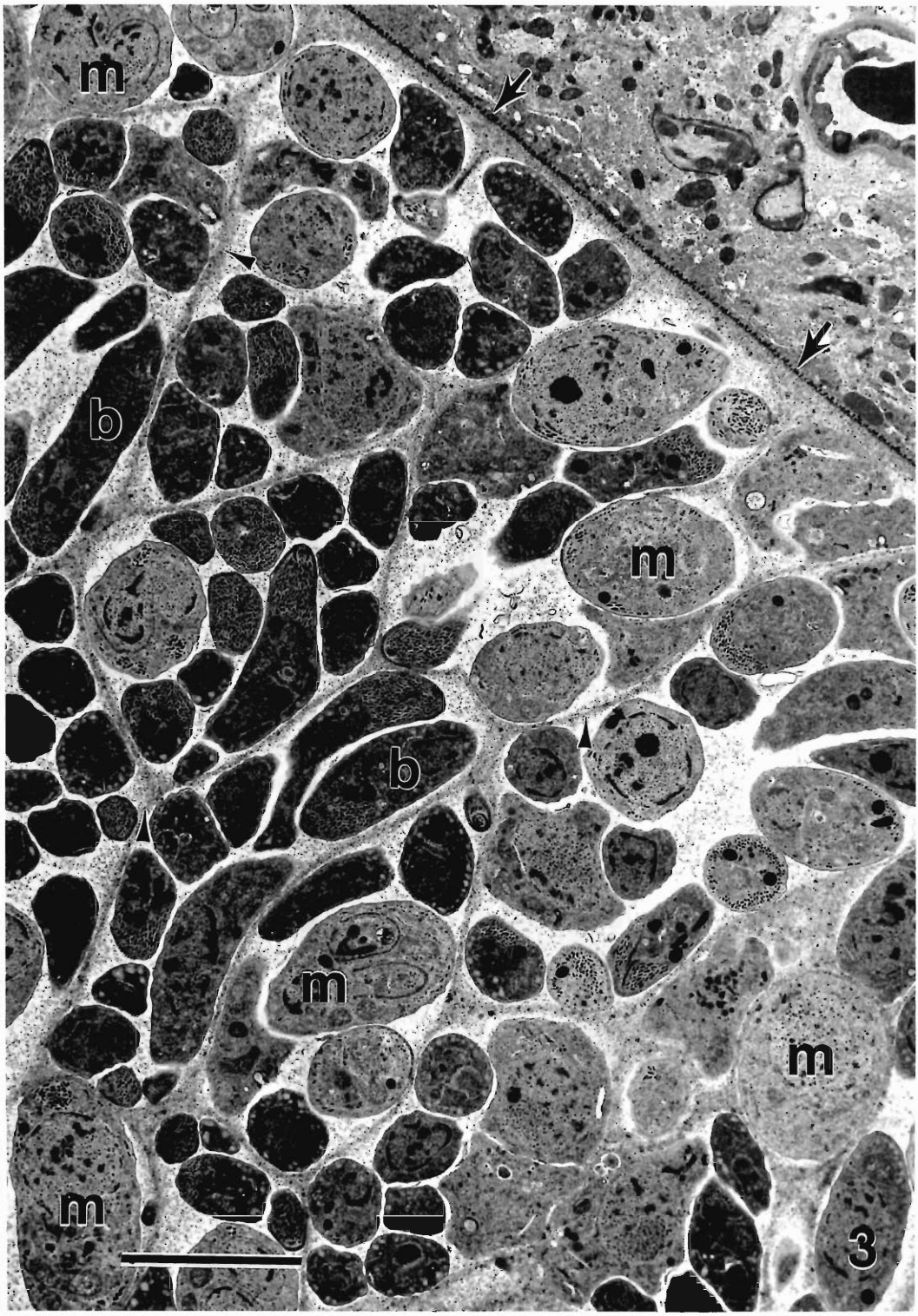


Figure 3. Electron micrograph of a portion of a *Frenkelia microti* tissue cyst. Numerous metrocytes (m) are located at the periphery and centrally within the tissue cyst. The compartments are separated by septa (arrowheads) and most contain both bradyzoites (b) and metrocytes. Arrows point to the tissue cyst wall. Bar = 5.0 μm.

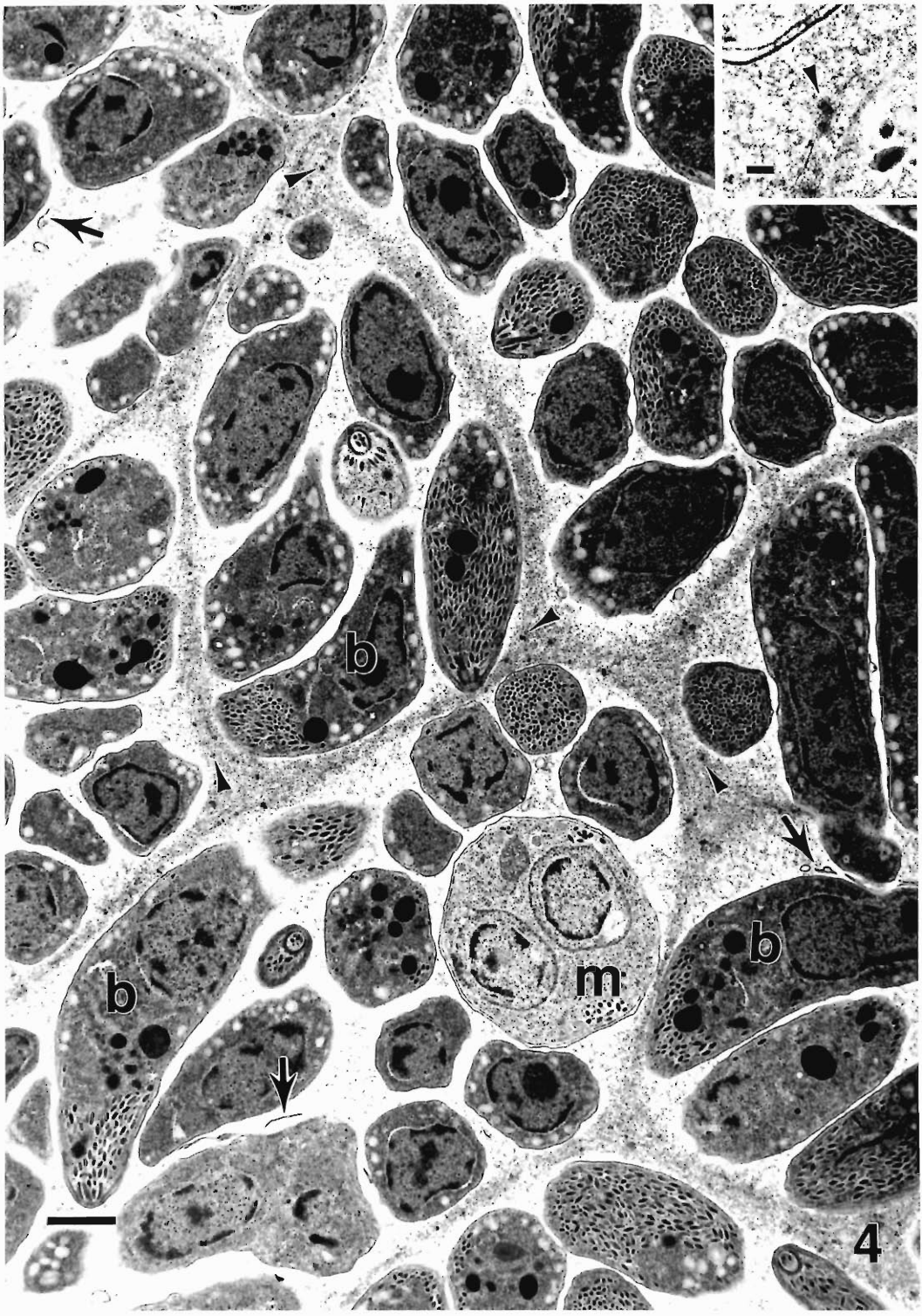


Figure 4. Electron micrograph of compartments within a *Frenkelia microti* tissue cyst. The compartments in this section contain mostly bradyzoites (b) and only an occasional metrocyte (m). Note that the labeled bradyzoites have micronemes that are confined to the anterior portion of the bradyzoite. Membrane fragments (arrows) are visible in several of the compartments, and osmiophilic bodies (arrowheads) are visible in septa. Bar = 1.0 μm. INSET. Osmiophilic body (arrowhead) demonstrating its association with microfilaments. Bar = 100 nm.

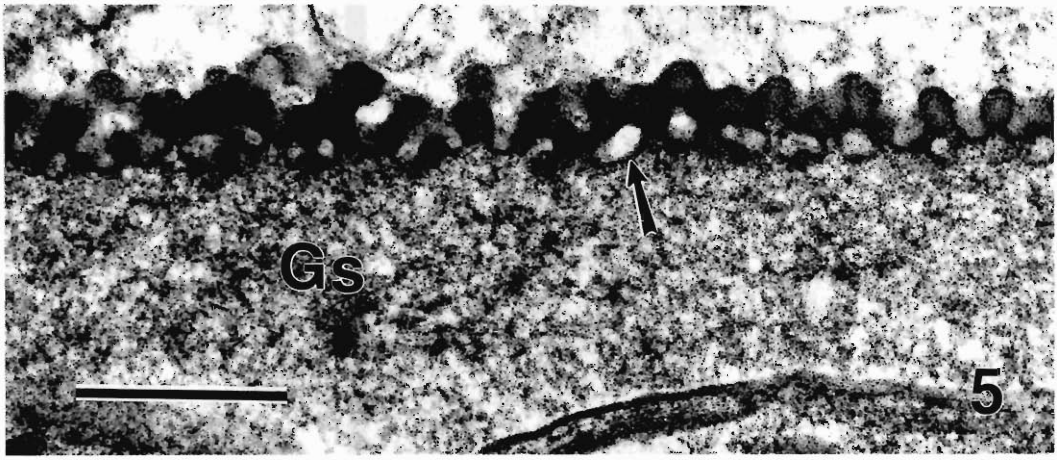


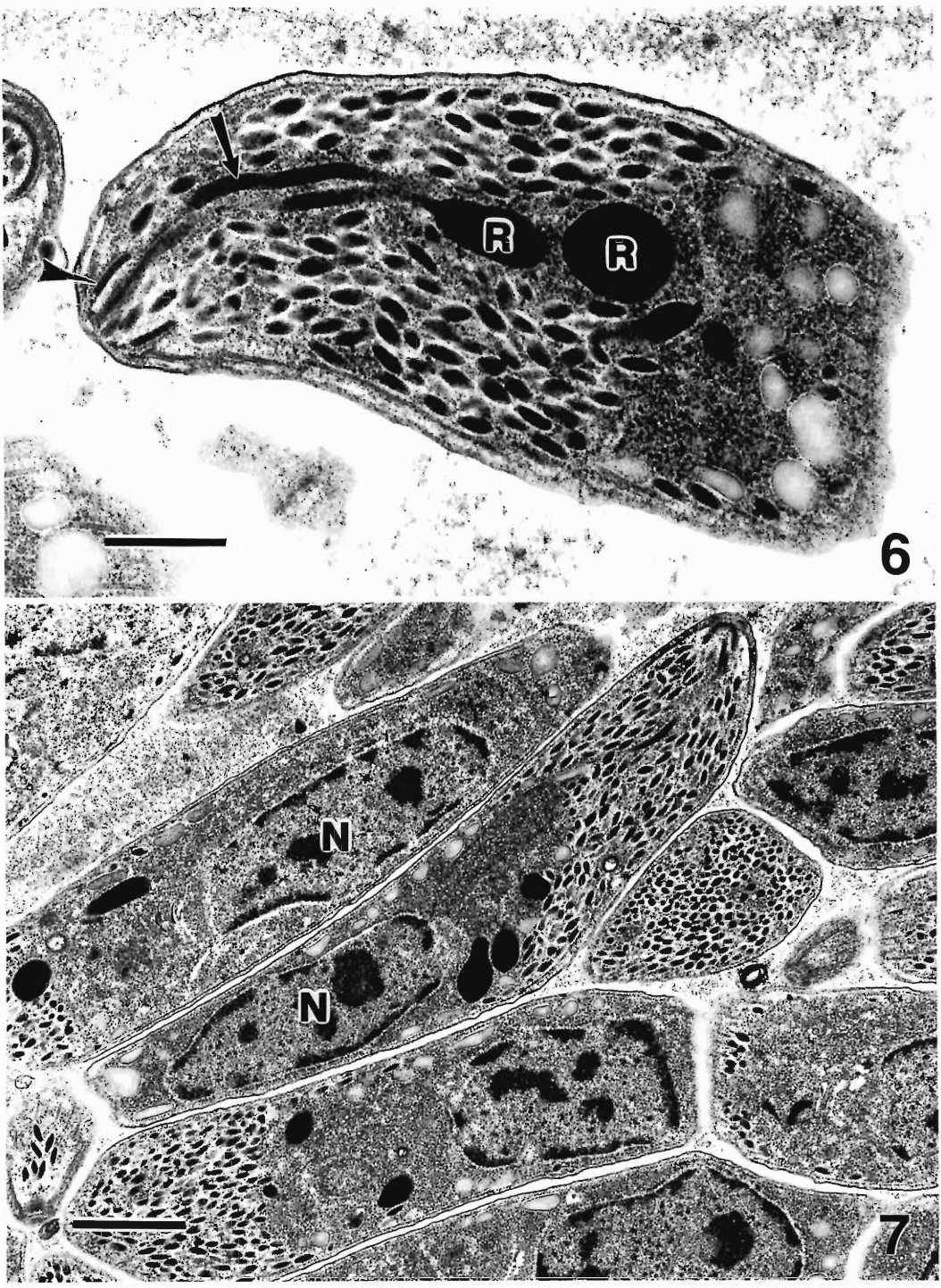
Figure 5. Tissue cyst wall of *Frenkelia microti*. Note the knoblike projections of the primary cyst wall and the apparent hole (arrow) that is a result of the plane of sectioning. The granular ground substance (Gs) is directly beneath the primary cyst wall. Bar = 0.5 μm.

Discussion

The light microscopic appearance of *Frenkelia microti* tissue cysts in brain sections of a prairie vole in this study was similar to tissue cyst structure reported by others in a variety of naturally infected mammals (Frenkel, 1956; Karstad, 1963; Hayden et al., 1976; Kennedy and Frelier, 1986). We did not observe lesions associated with tissue cysts, consistent with reports by others (Frenkel, 1956; Karstad, 1963). However, some reports indicate that microscopic lesions are associated with infection (Hayden et al., 1976; Kennedy and Frelier, 1986). The single spherical *F. microti* tissue cyst we observed may have been an artifact of tissue sectioning or an immature cyst. Geisel et al. (1979) reported that young *F. microti* tissue cysts were spherical.

Tadros et al. (1972) gave a brief description of the ultrastructure of tissue cysts of *F. microti* and *F. glareoli* in naturally infected voles (*Microtus agrestis* and *Clethrionomys glareolus,* respectively). Their report is difficult to interpret because tissues were poorly fixed and it is often unclear which species of *Frenkelia* they are describing. They did not provide an adequate description of the tissue cyst wall nor did they report the presence of osmiophilic bodies in septa or the membranous fragments and bodies seen within compartments. The origin of and significance of the osmiophilic bodies observed in the septa in the present study is unknown. The membranous fragments and bodies observed in compartments may represent byproducts of endodyogeny.

The structure of *F. microti* tissue cysts are similar to those of *Sarcocystis montanaensis* Dubey, 1983, found in heart, tongue, and various skeletal muscles of *Microtus* species (Dubey, 1983; Lindsay et al., 1991, 1992). Sarcocysts of some species of *Sarcocystis* can be found in the brain (Dubey et al., 1989), but sarcocysts of *S. montanaensis* were not reported in the brain tissue of the few voles that have been examined (Lindsay et al., 1992). The lobate structure of *F. microti* tissue cysts and their location in the brain can be used to differentiate it from *S. montanaensis* with light microscopy. Additionally, TEM can be used to demonstrate that bradyzoites of *F. microti* have micronemes which are restricted to the anterior region, whereas bradyzoites of *S. montanaensis* have micronemes that are located both anteriorly and posteriorly.



Figures 6, 7. Bradyzoites of *Frenkelia microti*. **1.** Anterior end of a bradyzoite demonstrating a conoid (arrowhead), rhoptries (R), and numerous rodlike micronemes. Note the duct of one rhoptry (arrow). Bar = 0.5 μm. **7.** A group of bradyzoites. Note the elongate nucleus (N) that is present and that the micronemes are located in the anterior portion of the bradyzoite. Bar = 1.0 μm.

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Zoological Nomenclature

The following Applications were published on 26 March 1992 in Vol. 49, Part 1 of the *Bulletin of Zoological Nomenclature.* Comment or advice on these Applications is invited for publication in the *Bulletin,* and should be sent to the Executive Secretary, I.C.Z.N., % The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.

Case 2251 *Bucephalus* Baer, 1827 and *B. polymorphus* Baer, 1827 (Trematoda): proposed conservation in their accepted usage

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Abstract. The purpose of this application is to conserve in their accepted usage the generic and specific names of an important trematode parasite of freshwater fishes—*Bucephalus polymorphus* Baer, 1827. The name *B. polymorphus* was based on cercariae, but it has been shown that these develop into the adult trematode first named as *Rhipidocotyle campanula* (Dujardin, 1844), a senior synonym of *R. illensis* (Ziegler, 1883). A neotype for *B. polymorphus* is proposed to avoid transfer of this long recognized name to *R. campanula,* with resulting confusion at both generic and specific levels.

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59(2), 1992, 177–181

Acanthatrium oregonense and *A. oligacanthum* (Digenea: Lecithodendriidae: Lecithodendriinae) from the Big Brown Bat in Oregon

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ABSTRACT: *Acanthatrium oregonense* Macy, 1939, is reported from the big brown bat, *Eptesicus fuscus bernardinus* Rhoads, a new host record. *Acanthatrium oligacanthum* Cheng, 1957, is reported from the same host species and for the first time from Oregon, where it is the only *Acanthatrium* species with short (4–5 μm) atrial spines. Measurements for the Oregon specimens overlap those of previously reported *A. oligacanthum* specimens but extend the upper limits of the ranges for most characters; the species is redescribed. The morphological variation may reflect geographic separation of populations of *A. oligacanthum*.

KEY WORDS: *Acanthatrium oligacanthum, Acanthatrium oregonense, Eptesicus fuscus,* infraspecific variation.

Within the subfamily Lecithodendriinae, only representatives of *Mesothatrium* Skarbilobich, 1948, and *Acanthatrium* Faust, 1919, possess a spined genital atrium. In *Mesothatrium* the vitellaria are posttesticular; in *Acanthatrium* they are pretesticular. Historically, the validity of various species of *Acanthatrium* has been debated. Dubois (1961) recognized 10 valid species and divided them into 2 subgenera, *Paracanthatrium* Dubois, 1961, and *Acanthatrium. Paracanthatrium,* distinguished by a preacetabular ovary, contained the sole species, *A. (P.) sphaerula* (Looss, 1896) Faust, 1919. *Acanthatrium,* characterized by a para- or postacetabular ovary, contained the remaining 9 species. Authors subsequent to Dubois (1961) seldom have mentioned the subgenera. Lotz and Font (1983) rejected Dubois' (1961) synonymy of *A. pipistrelli* Macy, 1940, with *A. eptesici* Alicata, 1932; otherwise, Dubois' (1961) revisions have been unchallenged and are the taxonomic foundation for this study.

Since 1961, 16 species of *Acanthatrium* (*Acanthatrium*) have been described. The 27 species of the genus have been reported at various localities worldwide, primarily from chiropterans, and they are distinguished by the size, distribution, and number of atrial spines. Within the Western Hemisphere only 3 species have been reported west of the Rocky Mountains: *A. eptesici, A. nycteridis* Faust, 1919, and *A. oregonense* Macy, 1939.

Materials and Methods

Sixty-six ovigerous specimens of *Acanthatrium* sp. were collected 5 January 1952 from the small intestine of a single big brown bat, *Eptesicus fuscus bernardinus* Rhoads, at Hubbard, Marion County, Oregon, by Ralph W. Macy. The specimens were fixed in Gilson's fluid, stained in hematoxylin, and counterstained with fast green, mounted whole in CMCP (General Biological Supply House), and donated in 1986 to the Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum, Lincoln (HWML Nos. 23413 and 33437). Observations were made using standard light microscopy and Nomarski differential interference contrast. Figures were drawn with the aid of a drawing tube. Measurements are stated in micrometers. Two specimens temporarily were dismounted for further flattening to enhance viewing of the atrial spines; 5 specimens were dismounted, sectioned sagittally at 5 μm, stained with Masson's trichrome stain, and mounted in Canada balsam.

The following specimens were studied: *A. beuschleini* Cheng, 1959 (paratype, USNM No. 38388), *A. eptesici* (paratype, USNM No. 30137), *A. jonesi* Sogandares-Bernal, 1956 (holotype, USNM No. 37255), *A. microcanthum* Macy, 1940 (paratype, HWML No. 20682), *A. oligacanthum* (paratype, USNM No. 38174), *A. oregonense* (paratypes HWML Nos. 23198, 23207, and additional specimens HWML No. 23300), and *A. sogandaresi* Coil and Kuntz, 1956 (paratype, USNM No. 38279).

Results

Two of the 66 specimens in the collection were identified as *Acanthatrium oregonense,* which is distinguished by the presence of more than 50 moderately sized (10–15 μm) atrial spines in a long, crescentic arrangement (Macy, 1939). The identification establishes *Eptesicus fuscus* as a new host for *A. oregonense.* The remaining specimens were identified as *A. oligacanthum,* which has a short row of approximately 10 atrial spines 4–5 μm in length.

Whereas the atrial spine length, number, and arrangement are virtually identical between the

Table 1. Summary of specimen data available for *Acanthatrium oligacanthum*.

	A. oligacanthum syn. *A. beuschleini* (from Dubois, 1961)*	*A. oligacanthum* (present study)	*A. oligacanthum* (combined)
Body			
Shape	oval to pyriform	oval	oval to pyriform
Size	180–490/160–320	290–972/210–544	180–972/160–544
Oral sucker	40–84/50–89	56–98/44–106	40–98/44–106
Acetabulum	50–67/50–60†	40–76/46–88	40–76/46–88
Pharynx (diam.)	14–40	23–65	14–65
Esophagus (length)	10–30	16–159	10–159
Prostate mass	20–71/40–75	44–320/76–240	20–320/40–240
Testes			
Position	slightly preacetabular	slightly preacetabular	slightly preacetabular
Size	32–108/31–98†	49–185/54–124	32–185/31–124
Ovary			
Position	submedian, postacetabular	posteromed. to rt. testis	posteromed. to rt. testis
Size	34–88/22–65†	43–148/51–144	32–148/22–144
Atrial spines			
Number	approx. 10	approx. 10	approx. 10
Length	4–5	4–5	4–5
Position	on anterior border of atrial diverticle	along inner ventral surface of atrium	along inner ventral surface of atrium
Arrangement	in a parallel manner, directed dorsad	arranged parallel in a short row, directed dorsad	in a parallel manner, directed dorsad
Vitellaria extent	from level of pharynx to anterior borders of testes	prececal, pretesticular, extending to level of pharynx	prececal, pretesticular, extending to level of pharynx
Eggs	29–33/18–20 (on the type)	22–36/12–18	22–36/12–20
Hosts	*Eptesicus fuscus fuscus*	*Eptesicus fuscus bernardinus*	*Eptesicus fuscus fuscus, Eptesicus fuscus bernardinus*
Geographic distribution	U.S.: Virginia, Wisconsin	U.S.: Oregon	U.S.: Oregon, Virginia, Wisconsin

* Dubois (1961) reviewed type specimens and made certain corrections in measurements provided by Cheng (1957, 1959).
† Measurements reported in Dubois (1961) have been corrected.

Oregon specimens and previously reported *A. oligacanthum* specimens, certain morphological traits vary substantially, necessitating an expansion of the earlier species description. The following redescription of *A. oligacanthum* is a composite of specimen data obtained from Cheng (1957, 1959), Dubois (1961), and the present study.

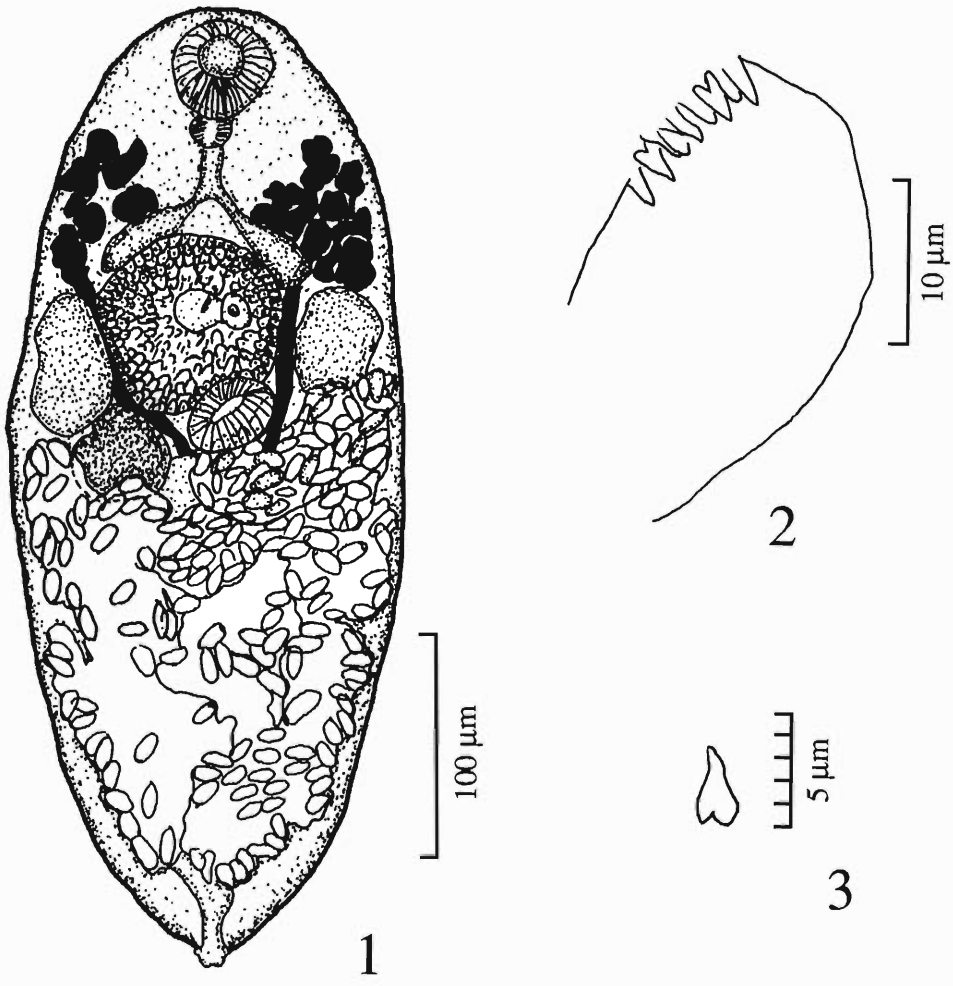
Acanthatrium oligacanthum Cheng, 1957 (Table 1, Figs. 1–3)

SYNONYM: *Acanthatrium beuschleini* Cheng, 1959.

HOSTS AND LOCALITIES: *Eptesicus fuscus fuscus* Beauvois in Albemarle and Russell counties, Virginia, and Eau Claire County, Wisconsin; *E. fuscus bernardinus* Rhoads in Marion County, Oregon.

SITE OF INFECTION: Small intestine.

REDESCRIPTION: With the characters of Lecithodendriidae, Lecithodendriinae. Body ovoid to pyriform, 180–972 long by 160–544 wide. Tegumental spines not visible. Oral sucker subterminal, 40–98 long by 44–106 wide. Acetabulum mesiad and equatorial or immediately preequatorial, 40–76 long by 46–88 wide. Sucker width ratio 1:0.69–1.28. Prepharynx absent. Pharynx 14–65 in diameter. Esophagus length variable, attaining maximum of 159 in relaxed specimens; bifurcation up to ⅕ of body length from anterior end; ceca short and widely divergent, confined to anterior third of the body. Testes 2, more or less ovoid, anterolateral to acetabulum, near margins of body; long axis usually parallel to midline; right testis 40–185 long by 40–122 wide; left testis 32–161 long by 31–124 wide. Two vasa efferentia arising from respective anterior margins of testes and uniting medially to form vas deferens. Vas deferens entering indistinct seminal vesicle leading into thicker walled ejaculatory duct. Prostate mass spheroid to ovoid, 20–320 long by 40–240 wide, equal to or greater than size of acetabulum and testes, intertesticular, overlapping acetabulum posteriorly and bor-



Figures 1–3. ***Acanthatrium oligacanthum*** **specimen from Oregon. 1. Adult, ventral view. 2. Genital atrium and atrial spines, lateral view. 3. Atrial spine, lateral view.**

dered anteriorly by ceca; genital atrium ovoid, median, armed with approximately 10 spines; genital pore muscular, submedian, and ventral to prostate mass. Atrial spines 4–5 long, cuneiform; arranged in short row along inner, ventral surface of atrium and directed dorsad. Ovary ovoid or somewhat irregular, posteromedial and dorsal to right testis, 32–148 long by 22–144 wide. Vitelline clusters paired, symmetrical, prececal, pretesticular, composed of several small to medium, nondendritic follicles, reaching level of pharynx; vitelline ducts extending from each cluster posteriad and mesiad, joining immediately posterior to acetabulum. Mehlis' gland irregularly ovoid, immediately postacetabular. Oötype not observed. Laurer's canal not visible. Uterine loops broad, filling most of hindbody and reaching posterior borders of testes; metraterm weakly muscular, with proximal sphincter, extending along ventral surface of prostate mass to atrium. Uncollapsed eggs operculate, amber, numerous, 22–36 long by 12–20 wide. Excretory pore terminal, stem of excretory vesicle short; extent of vesicle obscured by uterus.

The Oregon specimens provide the upper limits of the ranges for all but 1 morphometric trait, egg width, and differ from previously reported *A. oligacanthum* most prominently in the increased width of the prostate mass relative to the testes and acetabulum (Table 1). In individual Oregon specimens, the prostate mass width averages approximately twice the testes and ace-

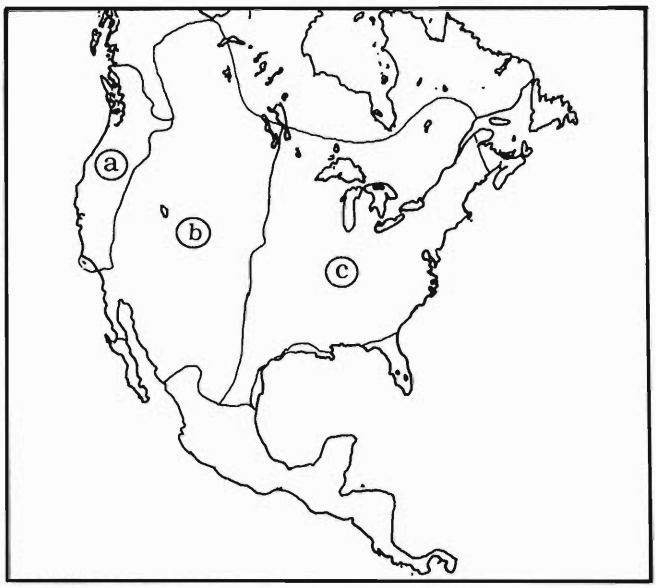


Figure 4. Geographical distribution of three *Eptesicus fuscus* subspecies (from Hall, 1981). a. *E. fuscus bernardinus*. b. *E. fuscus pallidus*. c. *E. fuscus fuscus*.

tabulum widths, whereas in previously reported specimens these same measurements are approximately equal.

Discussion

In addition to *Acanthatrium oligacanthum*, 2 other New World species possess very short (<10 μm) atrial spines: *A. microcanthum* and *A. molossidis* Martin, 1934. *Acanthatrium oligacanthum* differs from *A. microcanthum* in having only 10 spines; Dubois (1961) counted 70 in *A. microcanthum*. No specimen of *A. molossidis* could be obtained, but descriptions by Martin (1934) and Macy (1940) reveal several differences from *A. oligacanthum*. Although both species possess few, short atrial spines, the tegument of *A. molossidis*, unlike that of *A. oligacanthum*, is spined throughout its anterior third (Macy, 1940). Other differences include a more spheroid body shape, vitellaria extending more anteriorly, and the genital atrium placed near the acetabulum. In addition, *A. molossidis* was reported from a different host, *Molossus sinaloae* Allen, and locality, Honduras.

The Oregon *A. oligacanthum* specimens reveal infraspecific variation, most notably in regard to the increased size of the prostate mass as compared with earlier described specimens. Because the Oregon worms were recovered from a single bat, it is possible that similar morphological variation might be observed within this locality if worms from additional hosts were examined. Nonetheless, given (a) the mixed infection (*A. oligacanthum* and *A. oregonense*) and (b) the heavy infection of 64 specimens at various stages of maturity, it is doubtful that the bat obtained all metacercariae from a single insect.

Alternatively, the differences may reflect geographic separation of populations of *A. oligacanthum*. The Oregon specimens were recovered more than 1,500 miles from the nearest known *A. oligacanthum* locality. The definitive host species, *Eptesicus fuscus*, occurs throughout the United States but is divided into 11 subspecies that are nonmigratory (Barbour and Davis, 1969). The geographical distributions of 3 subspecies are shown in Figure 4. Comparison of the parasite and host species distributions indicates a correspondence of the Oregon and eastern *A. oligacanthum* populations to those of *E. fuscus bernardinus* and *E. fuscus fuscus*, respectively. The distribution of another subspecies, *E. fuscus pallidus*, corresponds roughly to the gap in the parasite distribution. Thus, the information to date suggests that the Oregon *A. oligacanthum* population is separated from other populations not only by space but also by host subspecies. Further work is necessary in 2 areas. First, collection of

additional *A. oligacanthum* within its known distribution is needed to evaluate the extent of morphological variation within populations in the eastern and western portions of the range. Second, sufficient *Eptesicus fuscus pallidus* individuals from the midwestern United States must be examined in order to determine if the *A. oligacanthum* distribution is indeed disjunct.

There are now 4 *Acanthatrium* species reported from the Pacific coast of North America: *A. eptesici, A. nycteridis, A. oligacanthum,* and *A. oregonense.* Of these species, only *A. oligacanthum* possesses very few and short atrial spines.

Acknowledgments

Thanks are due Ralph W. Macy for donation of the Oregon *Acanthatrium* specimens, J. Ralph Lichtenfels for loan of specimens from the USNM Helminthological Collection, and Mary H. Pritchard for direction of the study and use of the Harold W. Manter Laboratory facilities.

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Homalometron dowgialloi sp. n. (Homalometridae) from *Haemulon flavolineatum* and Additional Records of Digenetic Trematodes of Marine Fishes in the West Indies

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ABSTRACT: A total of 345 marine fishes representing 27 families, 31 genera, and 44 species from the coastal waters of southwestern Puerto Rico and Mona and Desecheo islands was examined for digeneans. Forty-five species of digeneans were identified including *Homalometron dowgialloi* sp. n. from the French grunt, *Haemulon flavolineatum* (Desmarest) (Haemulidae); 14 others were identified to genus. *Homalometron dowgialloi* differs from all known species of *Homalometron* in possessing an irregularly lobed ovary and vitellaria commencing at the equatorial level of the acetabulum. It is most similar to *H. caballeroi* and *H. armatum,* but differs from the former in having a prepharynx shorter than the pharynx, a proportionally shorter posttesticular space, noncontiguous testes, and smaller eggs and differs from the latter in being smaller, in having a larger sucker width ratio, and smaller eggs. Forty-one new host records and 2 new geographic locality records were reported. Most infections were of a single species and of low intensity.

KEY WORDS: *Homalometron dowgialloi,* Digenea, marine fish, Puerto Rico, Mona Island, Desecheo Island.

Initial reports on digeneans of marine fishes of Puerto Rico include the studies conducted by Cable (1954a, b, 1956a, b) and Le Zotte (1954). A more comprehensive survey was conducted later by Siddiqi and Cable (1960). More recently, Dyer et al. (1985) examined 1,019 marine fishes representing 76 families, 155 genera, and 252 species from the western and southwestern coasts of Puerto Rico. Nineteen families of digeneans representing 52 genera and 66 species were recorded including 4 new host records and 11 new geographic locality records.

The present study is a follow-up, especially in an attempt to examine additional fishes reported earlier as negative. Additional data were obtained from examining 345 marine fishes representing 27 families, 31 genera, and 44 species from coastal waters of southwestern Puerto Rico and Mona and Desecheo islands between November 1974 and August 1990.

Materials and Methods

Collection methods utilized throughout this study varied and included such techniques as variable mesh gill net, trawl net, seine, traps, spearfishing, and hook and line supplemented by quinaldine and rotenone to obtain species inhabiting reefs and shallow water. Fishes were placed in plastic bags containing seawater and transported to the laboratory where they were refrigerated and usually necropsied immediately.

Digeneans were washed in 0.7% saline, fixed with warm alcohol–formaldehyde–acetic acid (AFA), and stored in 70% ethanol. Specimens were stained with either Harris' hematoxylin or Grenacher's alcoholic borax carmine and prepared as whole mounts in Canada balsam. Voucher specimens of most species have been deposited in the National Parasite Collection, USDA, Beltsville, Maryland, as noted. The illustration was made with the aid of a camera lucida and a microprojector; measurements are in micrometers unless otherwise stated, with the range followed by the mean in parentheses.

Description

Homalometridae (Cable and Hunninen, 1942) Yamaguti, 1971

Homalometron dowgialloi sp. n. (Fig. 1)

DIAGNOSIS (based on 3 adult specimens): Elongate, spinulate distome with body tapering anteriorly, rounded posteriorly, 0.86–1.82 (1.24) mm long, 250–500 (336) wide at level of acetabulum. Oral sucker subterminal, 101–200 (141) long, 95–175 (125) wide. Acetabulum pre-equatorial, 183–286 (233) long, 184–350 (238) wide. Sucker length ratio 1:1.58–1.81 (1.66), width ratio 1:1.93–2.00 (1.98). Prepharynx 11–26 (17) long; pharynx 77–100 (81) long, 46–57 (52) wide; esophagus 33–56 (35) long, 23–44 (37) wide; ceca narrow, terminating subequally, 75–130 (100) from posterior extremity. Testes 2, smooth, tandem in posterior half of body; anterior testis round, 101–154 (125) long, 70–160 (114) wide; posterior testis longitudinally elongate, 101–200 (148) long, 79–148 (104) wide; posttesticular

space 260–340 (278) representing 17–30 (20) percent of total body length. Cirrus sac absent; seminal vesicle sacular, dorsosinestral to midline, 100–132 (125) long, 70–74 (72) wide, commencing at posterior margin of acetabulum, terminating at mid-level; prostatic complex inconspicious. Genital pore median, immediately preacetabular. Ovary pretesticular, irregularly lobed, dextromedian, slightly overlapping cecum, 88–117 (102) long, 48–99 (78) wide. Seminal receptacle preovarian, 75–114 (98) long, 30–48 (42) wide. Mehlis' gland median, preovarian; Laurer's canal not observed; vitellaria commencing at equatorial level of acetabulum, extending uninterrupted to posterior end of body, follicles overlapping ceca ventrally at testicular level, fields confluent posttesticularly; vitelline reservoir posterior to Mehlis' gland. Uterus overlapping ceca, coils between anterior testis and posterior third of acetabulum, overlapping ovary, seminal vesicle, and seminal receptacle; hermaphroditic duct short. Eggs in utero ($N = 20$), 44–52 (48) by 31–38 (33) wide. Excretory pore terminal, excretory vesicle tubular, extending from posterior end of body to terminus of posterior testis.

TYPE HOST: *Haemulon flavolineatum* (Desmarest) (Perciformes: Haemulidae); French grunt.

TYPE LOCALITY: La Parguera, Puerto Rico.

SITE: Small intestine.

HOLOTYPE: USNM Helm. Coll. No. 81658.

PARATYPE: USNM Helm. Coll. No. 81659.

ETYMOLOGY: Names for Michael Joseph Dowgiallo in recognition of his contributions to helminthology.

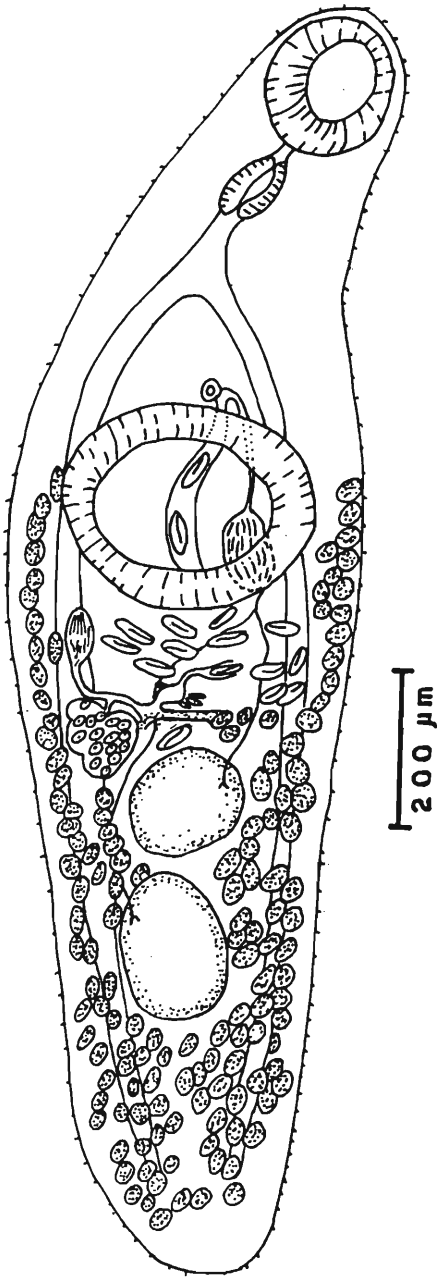

Figure 1. ***Homalometron dowgialloi*** **sp. n. Ventral view of holotype.**

Remarks

Species of *Homalometron* have been reported from marine, brackish, and freshwater fishes. Yamaguti (1971) listed 7 species: *H. pallidum* Stafford, 1904; *H. armatum* (MacCallum, 1895) Manter, 1947; *H. caballeroi* Lamothe Argumedo, 1965; *H. elongatum* Manter, 1947; *H. longulum* Travassos, Freitas, and Buhrnheim, 1965; *H. pearsei* (Hunter and Bangham, 1932) Manter, 1947; and *H. sophiae* (Stossich, 1886) Yamaguti, 1970. Miller (1959) redescribed *H. armatum* on the basis of 250 specimens collected from *Aplodinotus grunniens* (Rafinesque), *Lepomis humilis* (Girard), and *L. microlophus* (Günter) from Louisiana and designated *H. pearsei* as a synonym of *H. armatum* because of the large amount of variation occurring within the descriptions given for the 2 species.

Four additional species have been proposed as *Homalometron* subsequently: *H. foliatum* Siddiqi and Cable, 1960; *H. senegalense* Fischthal and Thomas, 1972; *H. carapevae* Amato, 1983; and *H. pseudopallidum* Martorelli, 1986. The presence of a prominent lymphatic system de-

Table 1. Digenetic flukes of marine fishes from coastal waters of Puerto Rico.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Bony fishes				
Acanthuridae (surgeonfishes)				
Acanthurus bahianus Castelnau (2/2)	*Hysterolecitha rosea* Linton, 1910	Sardinero, Mona Island, 22 April 1976	1/1/1	81569
	**Schikhobalotrema manteri* Siddiqi and Cable, 1960	La Parguera, 2 December 1977	1/1/1	81570
Acanthurus chirurgus (Bloch) (8/3)	*Dichadena acuta* Linton, 1910	La Parguera, 1 August 1990	7/2/8	81571
	Sterrhurus sp.	La Parguera, 10 February 1976	1/1/1	81572
Anomalopidae (flashlight fishes)				
Kryplophanaron alfredi Silvester and Fowler (1/1)	*Deretrema* sp.	Marganta reef, La Parguera, 11 April 1978	1/1/1	81573
Balistidae (leatherjackets)				
Balistes vetula Linnaeus (1/1)	**Lepidapedon truncatum* Sogandares-Bernal, 1959	Mona Island, 12 April 1975	1/1/15	81574
Bothidae (lefteye flounder)				
Bothus lunatus (Linnaeus) (4/2)	**Lecithochirium microcerus* (Manter, 1947)	Mona Island, 14 February 1975	1/1/10	81575
	**Leurodera decora* Linton, 1910	Mona Island, 14 February 1975	1/1/1	81576
	**Myzoxenus lachnolaimi* Manter, 1947	Mona Island, 12 April 1975	1/1/6	81577
Carangidae (jacks)				
Caranx hippos (Linnaeus) (2/2)	**Prosorhynchus stunkardi* Siddiqi and Cable, 1960	La Parguera, 17 April 1976	1/1/1	81578
	**Stephanostomum sentum* (Linton, 1910)	La Parguera, 17 March 1976	1/1/1	81579
Chloroscombrus chrysurus (Linnaeus) (2/1)	**Elytrophallus chloroscombri* (Siddiqi and Cable, 1960)	La Parguera, 17 April 1976	2/1/7	81580
Coryphaenidae (dolphins)				
Coryphaena hippurus Linnaeus (4/3)	*Dinurus* sp.	La Parguera, 14 February 1976	2/1/2	81581
		La Parguera, 20 March 1976	1/1/1	
	Dinurus tornatus Rudolphi, 1819	La Parguera, 2 February 1975	1/1/1	81582
Diodontidae (porcupinefishes)				
Diodon hystrix Linnaeus (2/2)	*Opistholebes diodontis* Cable, 1956	Enrique reef, La Parguera, 3 May 1975	1/1/8	81586
		Enrique reef, La Parguera, 3 March 1988	1/1/2	
Ephippidae (spadefishes)				
Chaetodipterus faber (Broussonet) (2/2)	*Apocreadium foliatum* (Siddiqi and Cable, 1960) Overstreet, 1969	Caracoles reef, La Parguera, 17 November 1974	1/1/1	81583
	Lecithaster sp.	Mario reef, La Parguera, 24 February 1976	1/1/6	81584
Exocoetidae (flyingfishes)				
Cypselurus sp. (1/1)	*Diplomonorcheides* sp.	Mona Island, 21 April 1976	1/1/5	81585
Gobiidae (gobies)				
Nes longus (Nichols) (2/2)	**Horatrema crassum* Manter, 1947	Laural reef, La Parguera, 11 August 1976	1/1/1	81587
	Lepocreadium sp.	Laural reef, La Parguera, 8 February 1977	1/1/1	81588

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Haemulidae (grunts)				
Haemulon carbonarium Poey (3/2)	**Lecithochirium microcercus*	San Cristobal reef, La Parguera, 24 February 1976	1/1/1	81589
	**Leurodera decora*	La Parguera, 5 September 1984	2/1/5	81590
	**Myzoxenus lachnolaimi*	Turrumotte reef, La Parguera, 5 September 1984	2/1/1	81591
Haemulon chrysargyreum Günther (1/1)	**Lecithochirium microcercus*	Media luna reef, La Parguera, 15 September 1978	1/1/1	81592
Haemulon flavolineatum Desmarest (234/221)	*Apocreadium foliatum*	La Parguera, 15 October 1976–15 October 1977	210/90/3	81593
		Mona Island, 15 October 1976–15 October 1977	5/4/4	
	Apopodocotyle oscitans (Linton, 1910)	La Parguera, 15 October 1976–15 October 1977	210/109/8	81594
		Mona Island, 15 October 1976–15 October 1977	5/3/5	
	Brachyphallus parvus (Manter, 1947)	La Parguera, 15 October 1976–15 October 1977	201/1/1	
	Derogenes crassus Manter, 1934	La Parguera, 16 November 1977	1/1/1	81595
	Diplangus paxillus Linton, 1910	La Parguera, 15 October 1976–15 October 1977	210/76/2	
	Homalometron dowgialloi sp. n.	La Parguera, 28 October 1976	11/11/4	81658 81659
	Infundibulostomum spinatum Siddiqi and Cable, 1959	La Parguera, 15 October 1976–15 October 1977	210/59/6	81596
		Mona Island, 15 October 1976–15 October 1977	5/2/1	
	Leurodera decora	La Parguera, 15 October 1976–15 October 1977	210/138/5	81597
		Mona Island, 15 October 1976–15 October 1977	5/5/5	
	Postmonorchis orthopristis Hopkins, 1941	La Parguera, 15 October 1976–15 October 1977	210/54/8	81598
	Pseudoplagioporus brevivitellus Siddiqi and Cable, 1960	La Parguera, 6 September 1976	6/6/4	81599
	Stephanostomum sentum (Linton, 1910)	La Parguera, 15 October 1976–15 October 1977	210/2/2	
Haemulon plumieri (Lacépède) (3/2)	*Diplangus ovalis* (Siddiqi and Cable, 1960)	Ensenada, 5 March 1977	2/1/6	81600
	Genolopa ampullacea Linton, 1910	Ensenada, 8 March 1977	1/1/1	81601
	**Leurodera decora*	Ensenada, 5 March 1977	2/1/1	81602
Haemulon sciurus (Shaw) (4/4)	**Apocreadium foliatum*	Mona Island, 15 April 1975	1/1/1	81603
	**Cainocreadium lintoni* (Siddiqi and Cable, 1960)	Mona Island, 15 April 1976	1/1/1	81604
	Lepocreadium sp.	Laural reef, La Parguera, 25 February 1975	1/1/3	81605
	**Leurodera decora*	Mona Island, 15 April 1975	3/3/2	81606
Holocentridae (squirrelfishes)				
Holocentrus rufus (Walbaum) (1/1)	**Leurodera decora*	La Parguera, 22 January 1975	1/1/3	81607

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Istiophoridae (billfishes)				
Makaira nigricans Lacépède (2/1)	*Hirudinella* sp.	La Parguera, 29 January 1975	1/1/1	81608
Kyphosidae (sea chubs)				
Kyphosus incisor (Cuvier) (3/1)	**Brachyphallus parvus* (Manter, 1947)	La Parguera, 29 October 1984	1/1/3	81609
Kyphosus sectatrix (Linnaeus) (4/2)	*Apocreadium foliatum*	Mona Island, 14 December 1975	1/1/6	81610
	**Enenterum aureum* Linton, 1910	Mona Island, 15 April 1975	1/1/6	81611
	**Hamacreadium mutabile* Linton, 1910	Mona Island, 15 April 1975	1/1/10	81612
	Opisthadena dimidia Linton, 1910	Mona Island, 15 April 1975	1/1/3	81613
	Stephanostomum sp.	Mona Island, 14 December 1975	1/1/10	81614
Labridae (wrasses)				
Lachnolaimus maximus (Wallbaum) (2/1)	*Myzoxenus lachnolaimi*	Enrique reef, La Parguera, 30 September 1977	1/1/1	81615
Lutjanidae (snappers)				
Lutjanus apodus (Walbaum) (4/3)	**Apocreadium foliatum*	Mona Island, 15 April 1975	1/1/1	81616
	Hamacreadium mutabile	Mona Island, 15 April 1975	1/1/3	81617
	**Lepidapedon holocentri* Siddiqi and Cable, 1960	La Parguera, 5 May 1977	1/1/1	81618
	**Lurodera decora*	Mona Island, 15 April 1977	1/1/1	81619
	Metadena adglobosa Manter, 1947	La Parguera, 19 February 1975	1/1/1	81620
Lutjanus griseus (Linnaeus) (5/4)	*Helicometra* (*Metahelicometra*) *torta* Linton, 1910	La Parguera, 26 July 1990	2/1/1	81621
	**Lepocreadium trulla* (Linton, 1907)	La Parguera, 4 December 1978	1/1/1	81622
	Metadena adglobosa	La Parguera, 9 March 1976	1/1/2	81623
Lutjanus jocu (Schneider) (1/1)	*Hamacreadium* sp.	Mona Island, 13 April 1975	1/1/10	81624
Ocyurus chrysurus (Bloch) (3/2)	**Hamacreadium mutabile*	La Parguera, 19 February 1975	1/1/7	81625
	**Siphoderina brotulae* Manter, 1934	Isla Guayacan, La Parguera, 5 March 1975	1/1/8	81626
Mullidae (goatfishes)				
Mullodicthys martinicus (Cuvier) (1/1)	*Opecoeloides vitellosus* (Linton, 1900)	La Parguera, 9 September 1984	1/1/4	81627
Pseudupeneus maculatus (Bloch) (2/2)	*Helicometra* sp.	La Parguera, 7 September 1977	1/1/1	81628
	Helicometra (*Metahelicometra*) *torta*	Enrique reef, La Parguera, 23 July 1990	1/1/2	81629
	Opecoeloides brachyteleus Manter, 1947	Enrique reef, La Parguera, 23 July 1990	1/1/2	81630
Muraenidae (morays)				
Gymnothorax funebris Ranzani (2/2)	*Dollfustrema* sp.	La Parguera, 19 February 1975	1/1/4	81631
	Sterrus sp.	Desecheo Island, 28 February 1975	1/1/50	81632
Gymnothorax moringa (Cuvier) (3/2)	**Dictysarca virens* Linton, 1910	La Parguera, 6 May 1977	1/1/2	81633
	**Dinurus tornatus*	La Parguera, 23 November 1974	1/1/1	81634
	Lecithochirium fusiforme Lühe, 1901	La Parguera, 6 May 1977	1/1/2	81635

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Gymnothorax vicinus (Castelnau) (1/1)	**Brachyphallus parvus*	La Parguera, 19 February 1975	1/1/5	81636
	**Lecithochirium fusiforme*	La Parguera, 19 February 1975	1/1/3	81637
Ostraciidae (boxfishes)				
Lactophrys bicaudalis (Linnaeus) (1/1)	*Pseudocreadium* sp.	Sardinero Playa, Mona Island, 15 April 1975	1/1/2	81638
Pomacanthidae (angelfishes)				
Pomacanthus arcuatus (Linnaeus) (5/5)	*Antorchis urna* (Linton, 1910)	La Parguera, 23 April 1976	2/2/4	81639
	Barisomum candidulum (Linton 1910)	Mona Island, 23 April 1976	3/3/2	81640
Priacanthidae (bigeyes)				
Priacanthus arenatus Cuvier (2/1)	**Tergestia laticollis* (Rudolphi, 1819)	La Parguera, 1 October 1984	1/1/3	81641
Sciaenidae (drums)				
Odontoscion dentex (Cuvier) (1/1)	*Manteriella crassa* (Manter, 1947)	Enrique reef, La Parguera, 7 February 1975	1/1/1	
Scombridae (mackerels)				
Scomberomorus regalis (Bloch) (1/1)	**Rhipidocotyle adbaculum* Manter, 1940	La Parguera, 22 October 1977	1/1/4	81642
Serranidae (sea basses)				
Ephinephelus adscensionis (Osbeck) (1/1)	**Myzoxenus lacknolaimi*	Mona Island, 13 April 1975	1/1/2	81643
Epinephelus cruentatus (Lacepède) (1/1)	**Cainocreadium lintoni*	Ensenada de Guanica, 14 October 1977	1/1/1	81644
Epinephelus fulvus (Linnaeus) (3/3)	**Hamacreadium mutabile*	Mona Island, 12 April 1975	2/2/2	81645
	Pseudoplagioporus brevivitellus	Mona Island, 14 April 1975	1/1/1	81646
Epinephelus striatus (Bloch) (6/5)	*Canocreadium lintoni*	Mona Island, 23 April 1976	1/1/2	81647
	**Hamacreadium mutabile*	Mona Island, 12 April 1975 Mona Island, 15 April 1975 Mona Island, 23 April 1976	3/2/3 1/1/6 1/1/2	81648
	Hamacreadium sp.	San Cristobal reef, La Parguera, 27 January 1976	1/1/1	81649
Mycteroperca tigris (Valenciennes) (1/1)	*Opechona* sp.	Mona Island, 14 April 1975	1/1/4	81650
	**Prosorhynchus atlanticus* Manter, 1940	Mona Island, 14 April 1975	1/1/4	81651
Mycteroperca venenosa (Linnaeus) (4/1)	**Helicometra mirzai* Siddiqi and Cable, 1960	Mona Island, 15 April 1975	1/1/9	81652
Sparidae (porgies)				
Calamus calamus (Valenciennes) (1/1)	**Pycnadena lata* (Linton, 1910)	La Parguera, 15 January 1978	1/1/1	81653
Sphyraenidae (barracudas)				
Sphyraena barracuda (Walbaum) (1/1)	*Sterrus* sp.	La Parguera, 4 March 1975	1/1/6	81654
Synodontidae (lizardfishes)				
Synodus intermedius (Agassiz) (7/4)	*Dichadena* sp.	La Parguera, 10 February 1976	1/1/4	81655
	Lecithochirium microcercus	La Parguera, 23 July 1990 La Parguera, 26 July 1990	1/1/12 4/1/2	81656
	**Myosaccium opisthonemae* (Siddiqi and Cable, 1960)	La Parguera, 8 February 1977	1/1/3	81657

tected in specimens of *H. foliatum* from *Haemulon aurolineatum* (Cuvier), *Haemulon carbonarium* (Poey), and *Haemulon parrai* (Desmarest) from Biscayne Bay, Florida, prompted Overstreet (1969) to transfer this species to the genus *Apocreadium.*

Homalometron dowgialloi may be readily distinguished from other members of the genus in that it possesses a lobed ovary and vitellaria commencing at the equatorial level of the acetabulum. Our form appears closest to *H. caballeroi* from *Balistes polylepis* (Steindachner) from Bahia Kino, Sonora, Gulf of California, and *H. armatum* from *Aplodinotus grunniens* from Lake Erie. *Homalometron dowgialloi* differs from *H. caballeroi* in having a prepharynx shorter than the pharynx rather than a prepharynx about the same length as the pharynx, a proportionally shorter rather than a proportionally longer posttesticular space, noncontiguous rather than contiguous testes, and shorter eggs (44–52 rather than 76–84 μm long). *Homalometron dowgialloi* differs from *H. armatum* in being smaller (0.86–1.82 rather than 1.74–3.44 mm long), in having a larger sucker width ratio (1:1.9–2.0 rather than 1:1.2–1.4), and shorter eggs (44–52 rather than 71–115 μm long).

Results

At least 1 trematode was detected in 303 (87.8%) of 345 fishes examined. Forty-five species were identified, including 1 new species; 14 others were identified to genus only. Of the 44 species of fishes that were infected, 20 (45.5%) harbored 1 species of digenean; 9 species (20%) 2; 6 species (13.6%) 3; 1 species (2.3%) 4; 1 species 5; and 1 species 11.

Thirty-one (68.9%) of the 45 species of digeneans occurred in 1 host species; 7 (15.6%) in 2; 2 (4.4%) in 3; 2 in 4; 2 in 5; and 1 (2.2%) in 7.

The intensity of a given species of digenean ranged from 1 to 15 specimens per host. Each of 31 fishes yielded 1 specimen of a given species; 96, 2; 99, 3; 26, 4; 148, 5; 64, 6; 2, 7; 167, 8; 1, 9; 2, 10; and 1, 15.

Because only a few specimens of most hosts were available for study, prevalence of their digeneans could not be ascertained. However, of 210 French grunts, *Haemulon flavolineatum* Desmarest, taken at La Parguera between 15 October 1976 and 15 October 1977, 65.7% were infected with *Leurodera decora* Linton, 1910, 51.9% with *Apopodocotyle oscitans* (Linton, 1910), 42.9% with *Apocreadium foliatum* (Siddiqi and Cable, 1960), 36.2% with *Diplangus paxillus* Linton, 1910, 28.1% with *Infundibulostomum spinatum* Siddiqi and Cable, 1959, 25.7% with *Postmonorchis orthopristis* Hopkins, 1941, 0.9% with *Stephanostomum sentum* (Linton, 1910), and 0.5% with *Brachyphallus parvus.*

Two of 43 digenean species are reported from Puerto Rico for the first time, namely, *Horatrema crassum* Manter, 1947, and *Derogenes crassus* Manter, 1934. Forty-one new host records are reported as indicated by an asterisk (*) preceding the parasite name (Table 1).

Discussion

Three studies of the digeneans of marine fishes from the western and southwestern coasts of Puerto Rico have now been completed. Siddiqi and Cable (1960) found 76 species of fishes harboring digeneans, 42 were infected with only 1 species (55.2%), 12 with 2 (15.8%), 9 with 3 (11.8%), 5 with 4 (6.6%), 3 with 5 (3.9%), 3 with 6, 1 with 7 (1.3%), and 1 with 9. Dyer et al. (1985) reported 70 species of fishes infected, 56 harbored 1 species (80%) of digenean, 8 species 2 (11.4%), and 2 species with 3 to a maximum of 5 (2.9%). These findings concur with those in the present study in that the majority of infections encountered constituted single infections and that the number of hosts with mixed infections decreased as the variety of digeneans increased.

Calculated from the data presented by Siddiqi and Cable (1960), 90 (73.2%) of the 123 species of digeneans found occurred in only 1 host species, 28 (22.8%) in 2 host species, 3 (3.4%) in 3, and 2 (1.6%) in 4. Dyer et al. (1985) reported that 52 (78.8%) of the 66 species of digeneans occurred in 1 host species, 10 (15.2%) in 2 host species, and the remaining 4 (6%) in 3, 5, 6, and 8 host species, respectively.

The intensity for each species of digenean could not be ascertained from the data presented by Siddiqi and Cable (1960). The intensity of a given species as reported by Dyer et al. (1985) ranged from 1 to 100 per host. Each of 88 fishes yielded 1–5 specimens; 18, 6–10; 4, 11–15; 3, 20; 5, 21–25; 3, 30–35; 3, 50; and 4 yielded 58, 76, 90, and 100 specimens, respectively. Comparison of these findings with those reported for the present study reveal that most of the fishes are infected with between 1 and 5 individuals of a given species of digenean.

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Meeting Schedule

HELMINTHOLOGICAL SOCIETY OF WASHINGTON 1992–1993

Oct 1992	Anniversary Dinner Meeting with the Trustees of the Brayton H. Ransom Memorial Trust Fund. Date to be announced later
(Wed) 4 Nov 92	"To Be Announced," Animal Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD
(Wed) 16 Dec 1992	"To Be Announced," Plant Protection Institute, U.S. Department of Agriculture, Beltsville, MD
(Wed) 13 Jan 1993	"To Be Announced," Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC
(Wed) 10 Feb 1993	"To Be Announced," U.S. Naval Medical Research Institute, Bethesda, MD
(Wed) 10 Mar 1993	"To Be Announced," Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD
Apr 1993	Date, place, and topic to be announced later
(Sat) 1 May 1993	"Vertical Transmission of Parasites," Annual Joint Meeting with the New Jersey Society for Parasitology, to be held at the New Bolton Center, University of Pennsylvania, Kennett Square, PA

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Eimeria rheemi sp. n. (Apicomplexa: Eimeriidae) from the Arabian Sand Gazelle, *Gazella subgutturosa marica* (Artiodactyla: Bovidae) in Saudi Arabia

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ABSTRACT: Oocysts of *Eimeria rheemi* sp. n. were detected in the feces of 35 out of 73 (48%) rheem or Arabian sand gazelles, *Gazella subgutturosa marica* Thomas, 1897, examined at King Khalid Wildlife Research Center, Thumamah, Riyadh Province, Saudi Arabia. Sporulated oocysts of *E. rheemi* sp. n. are spherical or ovoid, 25 × 21 (20–34 × 18–30) μm, with smooth, double-layered wall and micropyle, but no micropylar cap. The outer oocyst layer is yellow, almost twice as thick as the bluish-green inner one. Sporocysts oval, 10 × 8 (6–15 × 5–10) μm, each with a Stieda body and residuum. Sporozoites elongate 9 (7–11) μm, each with a single refractile globule 3 (2–4) μm in diameter at the wider end. Sporulation time 24 hr at 25 ± 2°C in aqueous $K_2Cr_2O_7$. *Eimeria rheemi* sp. n. is pathogenic to young rheem (2–4 mo old) causing mild to severe mucoid diarrhea, commensurate with the fecal oocyst count and has responded well to sulphonamide treatment.

KEY WORDS: antelope, coccidia, *Eimeria rheemi,* gazelle, mucoid diarrhea, oocyst, sporocyst, sporozoite, Stieda body.

There are 5 species of gazelles indigenous to the Arabian peninsula, 3 of which, *Gazella gazella* Pallas, 1766, *Gazella dorcas* Linnaeus, 1758, and *Gazella subgutturosa* Gueldenstaedt, 1780, are widespread (Groves and Lay, 1985; Groves, 1989). However, the subspecies *Gazella subgutturosa marica* Thomas, 1897, the Arabian sand gazelle or rheem of the Nafud desert of Saudi Arabia, is endangered (Honacki et al., 1982). Sizeable herds of the rheem and the other 2 endangered antelopes of Saudi Arabia, the idmi (*Gazella gazella*) and the Arabian oryx, *Oryx leucoryx* (Honacki et al., 1982), are kept together with the ifri (*Gazella dorcas*) at King Khalid Wildlife Research Center (KKWRC) of the National Commission for Wildlife Conservation and Development (NCWCD) in Thumamah, 80 km north of Riyadh, the capital of Saudi Arabia, for research, breeding, and later reintroduction into their now protected natural habitats in the country.

Recently, 2 new species of *Eimeria* have been described from these animals: *Eimeria saudiensis* from the Arabian oryx (Kasim and Al-Shawa, 1988) and *Eimeria idmii* from the idmi or Arabian mountain gazelle (Mohammed and Hussein, 1992). No *Eimeria* species was detected in the ifri (*G. dorcas*), but the rheem herd was infected with an unknown eimerian which is described in the present study.

Materials and Methods

The herds of gazelles and the Arabian oryx at KKWRC are born and bred in Thumamah and they are descendants of animals collected by the late King Khalid Ibn Abdul Aziz. A total of 73 (2–36 mo old) rheems, 55 adults (24–36 mo old) and 18 young (2–4 mo old), has been examined for parasitological assessment. The adults were sedated by darts and the young were sick gazelles that have been segregated in the treatment stalls of the veterinary clinic of the Center for diagnosis and treatment. Fresh fecal samples were collected directly from the rectum of each of the animals into wide-mouth, screw-cap, plastic containers. In the laboratory, the fecal samples were subjected to various parasitological examinations, including direct smear, sedimentation, and flotation over saturated sodium chloride solution, and the parasite prevalence and intensity for each animal was assessed by the modified McMaster technique (Anonymous, 1977). Fecal samples from the 18 young, sick gazelles were also sent to the microbiology laboratory of the Center for microbiological assessment.

Fecal samples with eimerian oocysts were sporulated at room temperature (25 ± 2°C) in thin layers of aqueous potassium dichromate as outlined by Mohammed and Hussein (1992). The 18 sick gazelles were drenched with an aqueous suspension of sulphadimidine at a dose of 30 mg per kg body weight for 10 consecutive days, and fecal samples were obtained from them for the assessment of the daily output of *Eimeria* oocysts. They were also clinically assessed throughout the treatment period, following which they were discharged to join the rest of the herd. Thereafter, sulphonamide treatments at low concentrations were periodically given in drinking water for all herds for the control of coccidiosis as outlined by Soulsby (1982).

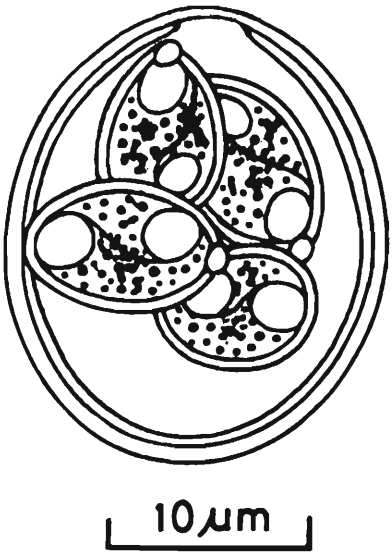


Figure 1. Camera lucida drawing of a sporulated oocyst of *Eimeria rheemi* sp. n.

Measurements were made by a calibrated ocular micrometer, photographs 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 in parentheses by the range.

Results

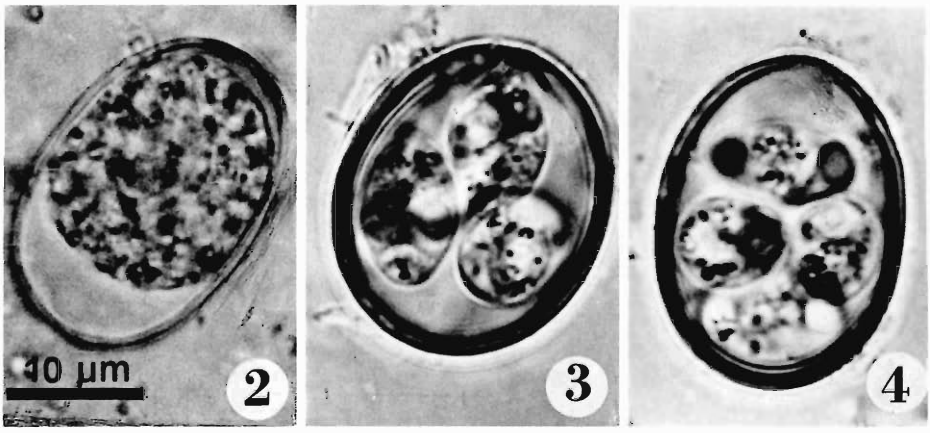
All of the 18 young, sick gazelles had mild to severe mucoid diarrhea and all were shedding large numbers (100,000–300,000 oocysts g^{-1} feces) of oocysts of an *Eimeria* sp. The severity of the diarrhea coincided with the oocyst count, but dramatically subsided with the initiation of the sulphonamide treatment. Daily counts progressively dropped from 100,000–300,000 oocysts g^{-1} feces on day 1 of treatment to 50–300 oocysts g^{-1} feces on day 10.

On the other hand, 15 of the 55 adult rheems were shedding oocysts of the same eimerian, but at considerably lower rates (100–10,000 oocysts g^{-1} feces) and all were healthy. Oocysts from young or adult rheems sporulated within 24 hr at room temperature (25 ± 2°C). Careful examination of the sporulated oocysts showed that they belong to an eimerian that is morphologically different from any described from gazelles or from any other antelope species of the family Bovidae. Hence, they represent a new *Eimeria* species that is described below.

Eimeria rheemi sp. n.
(Figs. 1–4)

DESCRIPTION: Oocysts spherical or ovoid. Oocyst wall 1.6 (1–2) thick, smooth, double-layered, outer layer yellow, almost twice as thick as bluish-green inner one. Micropyle present, 5.6 (5–6) wide, micropylar cap absent.

Sporulated oocysts (N = 500) 25 × 21 (20–34 × 18–30), length/width ratio 1.2 (1–1.6). Oocyst residuum, oocyst polar granule both absent. Sporocysts (N = 750) oval, 10 × 8 (6–15 × 5–10), length/width ratio 1.5 (1–2.4), with sporocyst re-



Figures 2–4. Photomicrographs of wet mounts of *Eimeria rheemi* sp. n. oocysts in aqueous $K_2Cr_2O_7$. 2. An unsporulated oocyst. 3. A spherical sporulated oocyst; the micropyle is clearly depicted in this figure. 4. An ovoid sporulated oocyst. The scale bar is for all figures.

siduum as dispersed fine granules. Stieda body present, substiedal body absent. Sporozoites (N = 750) ovoid to elongate, 9 (7–11) long, each with single spherical globule at the wider end, 3 (2–4) in diameter.

TYPE HOST: The rheem or the Arabian sand gazelle, *Gazella subgutturosa marica* Thomas.

TYPE LOCALITY: Thumamah, Riyadh Province, Saudi Arabia.

PREVALENCE: Found in 35 of 73 (48%) rheems.

SITE OF INFECTION: Unknown, oocysts recovered from feces.

SPORULATION: Exogenous. Oocysts sporulated in 24 hr at 25 ± 2°C in $K_2Cr_2O_7$.

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

PHOTOTYPES: Deposited in the National Parasite Collection, U.S. National Museum, USNM No. 82103.

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

Discussion

Six species of *Eimeria* have been described from the genus *Gazella* Blainville, 1816, of the family Bovidae and have recently been reviewed by Mohammed and Hussein (1992). These are *Eimeria abenovi* Svanbaev, 1979, *Eimeria elegans* Yakimoff, Gousseff, and Rastegaieff, 1932, and *Eimeria gazella* Musaev emend. Svanbaev, 1979, from the goittered gazelle, *G. subgutturosa*; *Eimeria chinkari* Pande, Bhatia, Chauhan, and Garg, 1970, and *Eimeria idmii* Mohammed and Hussein, 1992, from *G. gazella*; and *Eimeria dorcadis* Montovani, 1966, from *G. dorcas*. However, oocysts of *E. rheemi* sp. n. differ considerably from all of these species. Similar to other gazelle eimerians (Levine and Ivens, 1986; Mohammed and Hussein, 1992), oocysts of *E. rheemi* differ from those of *E. idmii* in being smaller and in lacking a micropylar cap. The outer layer of the oocyst wall of *Eimeria rheemi* is yellow and almost twice as thick as the bluish-green inner one. Both layers of the oocyst wall of *E. idmii* are similar in thickness, but the outer layer is green and the inner is yellow. Moreover, *E. rheemi* is pathogenic to young gazelles and sporulates within 24 hr at 25 ± 2°C in $K_2Cr_2O_7$, compared to the nonpathogenic *E. idmii* that sporulates in 7–8 days under the same conditions. Also, each sporozoite of *E. rheemi* has a single refractile globule at its wider end, whereas each of those of *E. idmii* has 2, 1 at either end (Mohammed and Hussein, 1992). Refractile globules are also present at either end of each sporozoite of *E. abenovi* and *E. chinkari* (Pande et al., 1970; Svanbaev, 1979; Levine and Ivens, 1986), but 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).

Eimeria rheemi is somewhat larger than either *E. chinkari* or *E. gazella,* both of which also lack a micropyle; *E. gazella* lacks a Stieda body (Pande et al., 1970; Svanbaev, 1979; Levine and Ivens, 1986). A micropyle is also found in both *E. abenovi* and *E. elegans,* but it is much larger than that of *E. rheemi,* and both lack Stieda bodies (Yakimoff et al., 1932; Svanbaev, 1979; Levine and Ivens, 1986). On the other hand, *E. dorcadis* is somewhat larger than *E. rheemi,* but it lacks a micropyle as well as Stieda bodies (Montovani, 1966; Levine and Ivens, 1986). With the exception of *E. abenovi,* all gazelle eimerians, including *E. rheemi,* have sporocyst residua, but all lack oocyst residua and polar granules (Svanbaev, 1979; Levine and Ivens, 1986; Mohammed and Hussein, 1992).

Moreover, *E. rheemi* can easily be differentiated from all of the 21 *Eimeria* species described from other antelopes and recently reviewed by Mohammed and Hussein (1992). It is smaller than *E. saudiensis, E. yakimovae, E. congolensis, E. kobi, E. macieli, E. talbotti, E. mirgai, E. impalae, E. neitzi, E. walleri, E. ismailovae, E. manafovae, E. saiga, E. tatarica,* and *E. tekenovae,* about the same size as *E. canna* and *E. chausinghi,* but is larger than *E. triffittae, E. gorgonis, E. connochaetei,* and *E. sajanica.* It also differs from *E. saudiensis, E. mirgai, E. tekenovi,* and some oocysts of *E. tatarica* in lacking a micropylar cap (refer to Table 1, Mohammed and Hussein, 1992). *Eimeria saudiensis, E. mirgai, E. canna, E. gorgonis, E. neitzi, E. manafovae,* and *E. saiga* are the only antelope eimerians that have oocyst polar granules; the rest, similar to *E. rheemi,* are devoid of these (Levine and Ivens, 1970, 1986; Pellérdy, 1974). Moreover, a micropyle is present in *E. rheemi* as well as in most antelope eimerians with the exception of *E. neitzi, E. connochaetei, E. gorgonis, E. talboti, E. chausinghi, E. triffittae, E. ismailovae, E. saiga,* and *E. sajanica* (Levine and Ivens, 1986; Mo-

hammed and Hussein, 1992). Stieda bodies are present in most antelope eimerians as well as in *E. rheemi,* but are absent from *E. triffittae, E. macieli, E. talboti, E. ismailovae, E. manafovae, E. saiga, E. sajanica, E. tatarica,* and *E. tekenovae* (Levine and Ivens, 1986; Mohammed and Hussein, 1992). Similar to *E. rheemi,* most antelope eimerians with the exception of *E. triffittae, E. congolensis, E. macieli, E. talboti,* and *E. impalae* have a sporocyst residuum (Levine and Ivens, 1986; Mohammed and Hussein, 1992).

Eimeria neitzi and *E. triffittae* are the only antelope eimerians whose oocysts are single-layered (Yakimoff, 1934; Levine and Ivens, 1970, 1986; McCully et al., 1970; Pellérdy, 1974). *Eimeria canna* and *E. walleri* are the only ones with triple-layered oocysts (Triffitt, 1924; Prasad, 1960; Levine and Ivens, 1970, 1986; Pellérdy, 1974), whereas all other antelope eimerians, as well as *E. rheemi,* have double-layered oocysts (Levine and Ivens, 1970, 1986; Pellérdy, 1974; Mohammed and Hussein, 1992). Similar to *E. idmii* (Mohammed and Hussein, 1992), both layers of the oocyst wall of *E. rheemi* are smooth, colored, and firmly attached to each other, whereas oocysts of both *E. congolensis* and *E. kobi* have a rough, granular, brown-colored outer layer that easily separates from its inner layer (Ricci-Bitti et al., 1973; Levine and Ivens, 1986). The oocyst walls of *E. sajanica* and *E. saiga* are colorless (Svanbaev, 1958; Levine and Ivens, 1970, 1986; Pellérdy, 1974) and that of *E. macieli* is radially striated (Yakimov and Matchuski, 1938; Levine and Ivens, 1970, 1986; Pellérdy, 1974). Hence, *E. rheemi* sp. n. appears to be a distinct and hitherto undescribed species.

The present results also demonstrate that *E. rheemi* sp. n. is pathogenic to young gazelles causing mucoid diarrhea that varies in severity with the fecal oocyst counts. Infections responded well to sulphonamide treatment. Animals at KKWRC are kept under excellent conditions with plenty of room to roam and are never allowed to crowd up in any single spot. Camels kept under similar conditions in Saudi Arabia never suffer from coccidiosis, but when they congregate in limited areas, either around water holes during the short rainy season, or in oases or in farms, young camels often develop clinical coccidiosis (Kawasmeh and El Bihari, 1983; Hussein et al., 1987). A similar situation was also reported in the impala in South Africa, which developed severe coccidiosis due to *E. impalae* infection only when they were brought together in small paddocks (Pinnaar et al., 1964; Bigalke, 1966). This indicates that *E. rheemi* might be more pathogenic than either *E. impalae* in South Africa (Pinnaar et al., 1964; Bigalke, 1966) or any of the camel eimerians reported in Saudi Arabia (Kawasmeh and El Bihari, 1983; Kasim et al., 1985; Hussein et al., 1987). In young rheems it causes mild to severe coccidiosis even under the excellent prevailing conditions at KKWRC. However, more studies are needed to determine the extent of *E. rheemi* pathogenicity.

Acknowledgments

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Schilbetrematoides pseudodactylogyrus gen. et sp. n. (Monogenoidea, Dactylogyridae, Ancyrocephalinae) from the Gills of *Schilbe intermedius* (Siluriformes, Schilbeidae) in Togo, Africa

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ABSTRACT: *Schilbetrematoides pseudodactylogyrus* sp. n. (Dactylogyridae, Ancyrocephalinae) is described from the gills of *Schilbe intermedius* Rüppell, 1832, Siluriformes, Schilbeidae, in Togo. *Schilbetrematoides* gen. n. is proposed for this species and is characterized, in part, by large, subspherical eye granules; intercecal, overlapping gonads (testis dorsal); a constriction of the vas deferens at its union with the seminal vesicle; dorsal anchors; a ventral pair of 4A's lying near the ends of a simple (vestigial?) ventral bar; a highly modified dorsal bar; 14 hooks in 7 pairs with slender shanks and protruding thumbs.

KEY WORDS: Togo, Africa, Monogenoidea, Dactylogyridae, Ancyrocephalinae, *Schilbetrematoides* gen. n., *Schilbetrematoides pseudodactylogyrus* sp. n., *Schilbe mystus, Schilbe intermedius.*

During the relatively short period when Dr. I. Paperna worked on the freshwater Dactylogyridae from Africa, he proposed 13 of the 23 genera currently accommodating Ethiopian species (see Euzet and Dossou, 1976, and Paperna, 1979). Together, these genera appear to represent a well-defined picture of the freshwater dactylogyrid fauna of Africa's freshwater fishes. Indeed, since 1979, only *Paraquadriacanthus* Ergens, 1988 (=*Quadriacanthoides* Kritsky and Kulo, 1988; see Kritsky, 1990), and *Insulacleidus* Rakotofiringa and Euzet, 1983, have been proposed to accommodate African species. Similarly, our collections from Togo have not resulted in the identification of many new generic taxa. In the present paper, however, a new genus is erected for an unusual worm from the gills of a siluriform, *Schilbe intermedius* Rüppel, 1832 (Schilbeidae), from Togo. Although apparently sister taxon to *Schilbetrema* Paperna and Thurston, 1968 (Ancyrocephalinae), the new genus is characterized by many features of the Dactylogyrinae.

Materials and Methods

Fish hosts, *Schilbe intermedius,* were collected from the Mono River near Kolokopé, Togo, during 1985–1989. Methods for collection, preservation, mounting, and illustration of helminths were as described by Kritsky et al. (1987). Measurements, all in micrometers, were made with a filar micrometer according to the procedures of Mizelle and Klucka (1953), except that cirral length was an approximation obtained by using a calibrated Minerva curvimeter on camera lucida drawings; average measurements are followed by ranges and the number (*N*) of specimens measured in parentheses. Type specimens were deposited in the helminthological collections of the U.S. National Museum (USNM), USDA, ARS, Agricultural Research Center-East, Beltsville, Maryland; the University of Nebraska State Museum (HWML), University of Nebraska, Lincoln, Nebraska; the Musée Royal de l'Afrique Centrale (MRAC), B-1980 Tervuren, Belgium; and the British Museum (Natural History) (BM[NH]), London, U.K., as indicated in the description. A specimen of the host, *Schilbe intermedius* Rüppel, was deposited in the American Museum of Natural History, New York, as AMNH 57312.

Schilbetrematoides gen. n.

DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Two bilateral, 2 terminal cephalic lobes; head organs present; cephalic glands unicellular, comprising 2 bilateral groups posterolateral to pharynx. Eyes present, generally compact; granules large, subspherical. Mouth subterminal, midventral; pharynx, esophagus present; intestinal ceca 2, confluent posterior to gonads. Gonads intercecal, overlapping; testis dorsal to ovary. Vas deferens looping left intestinal cecum, constricting at union with seminal vesicle; seminal vesicle fusiform, lying parallel to left intestinal cecum; distal vas deferens slightly dilated (secondary seminal vesicle?), delicate; single prostatic reservoir closely associated with cirral base. Copulatory complex comprising proximally articulated cirrus, accessory piece; cirrus a loose clockwise coil (see Kritsky et al., 1985); accessory piece comprising proximal articulating rod, distal complex with

grooved cirral guide. Oviduct short; uterus delicate; vagina dextral, opening near body midlength; seminal receptacle ventral to anterior end of ovary. Haptor armed with dorsal pair of anchors; ventral pair of 4A's lying near ends of simple ventral bar; dorsal, ventral bars; 14 (7 pairs) hooks with ancyrocephaline distribution (Mizelle, 1936; see Mizelle and Price, 1963); dorsal anchor filaments present. Hooks with slender shanks, protruding thumb.

TYPE SPECIES, HOST, AND LOCALITY: *Schilbetrematoides pseudodactylogyrus* gen. et sp. n., from *Schilbe intermedius* Rüppel, Mono River near Kolokopé, Togo.

ETYMOLOGY: The generic name indicates the apparent close relationship of this taxon to *Schilbetrema* Paperna and Thurston, 1968.

Schilbetrematoides pseudodactylogyrus gen. et sp. n. (Figs. 1–9)

DESCRIPTION: Body 388 (240 [contracted]–548; $N = 8$) long, fusiform; greatest width 79 (62–109 [contracted]; $N = 8$] in posterior trunk. Cephalic lobes, cephalic glands poorly differentiated. Eyes 4; posterior pair slightly larger, members of respective pairs equidistant; accessory granules uncommon in cephalic, anterior trunk regions. Pharynx spherical, 22 (16–25; $N = 8$) in diameter; esophagus short. Peduncle broad; haptor subhexagonal, 64 (56–78; $N = 5$) wide, 79 (57–89; $N = 7$) long. Dorsal anchor 62 (56–70; $N = 10$) long, with elongate superficial root having superficial pustule near midlength, short deep root, curved shaft, point; base width 26 (22–31; $N = 7$); anchor filament ancyrocephaline. Ventral 4A: 10–11 ($N = 3$) long, proximally expanded about ½ total length. Ventral bar 29 (25–34; $N = 8$) long, rod-shaped, with irregular anterior margin. Dorsal bar 17 (15–24; $N = 5$) long, platelike, with enlarged ends, large projection arising from anterior bar margin; projection complex, with 2 elbowlike bends, the last serving as guide for anchor points; length of most dorsal fold (anteroposterior measurement) 66 (60–70, $N = 7$). Hooks similar; each 16 (14–17; $N = 22$) long, with slightly depressed thumb, delicate point, shank; FH loop about 0.75 shank length. Cirrus a loose coil of less than 1 ring, frequently appearing J-shaped, cirral base with sclerotized margin; cirral length 48 (47–55; $N = 6$), ring diameter 12 (10–16, $N = 5$). Accessory piece 27 (24–31; $N = 4$) long, comprising proximal uniform connecting rod, distal sheath with several variable branches, 1 of which serving as cirral guide. Testis elongate ovate, 51 (42–61; $N = 3$) × 23 (22–24; $N = 3$); constriction of vas deferens with external spiral filament; seminal vesicle with distinct wall, fusiform; distal vas deferens pyriform; prostatic reservoir saccate. Ovary bacilliform, 43 (40–45; $N = 4$) × 20 (17–23; $N = 3$); vagina funnel-shaped, with distal loop opening into large central seminal receptacle; vitellaria dense throughout trunk, except absent in fields of reproductive organs.

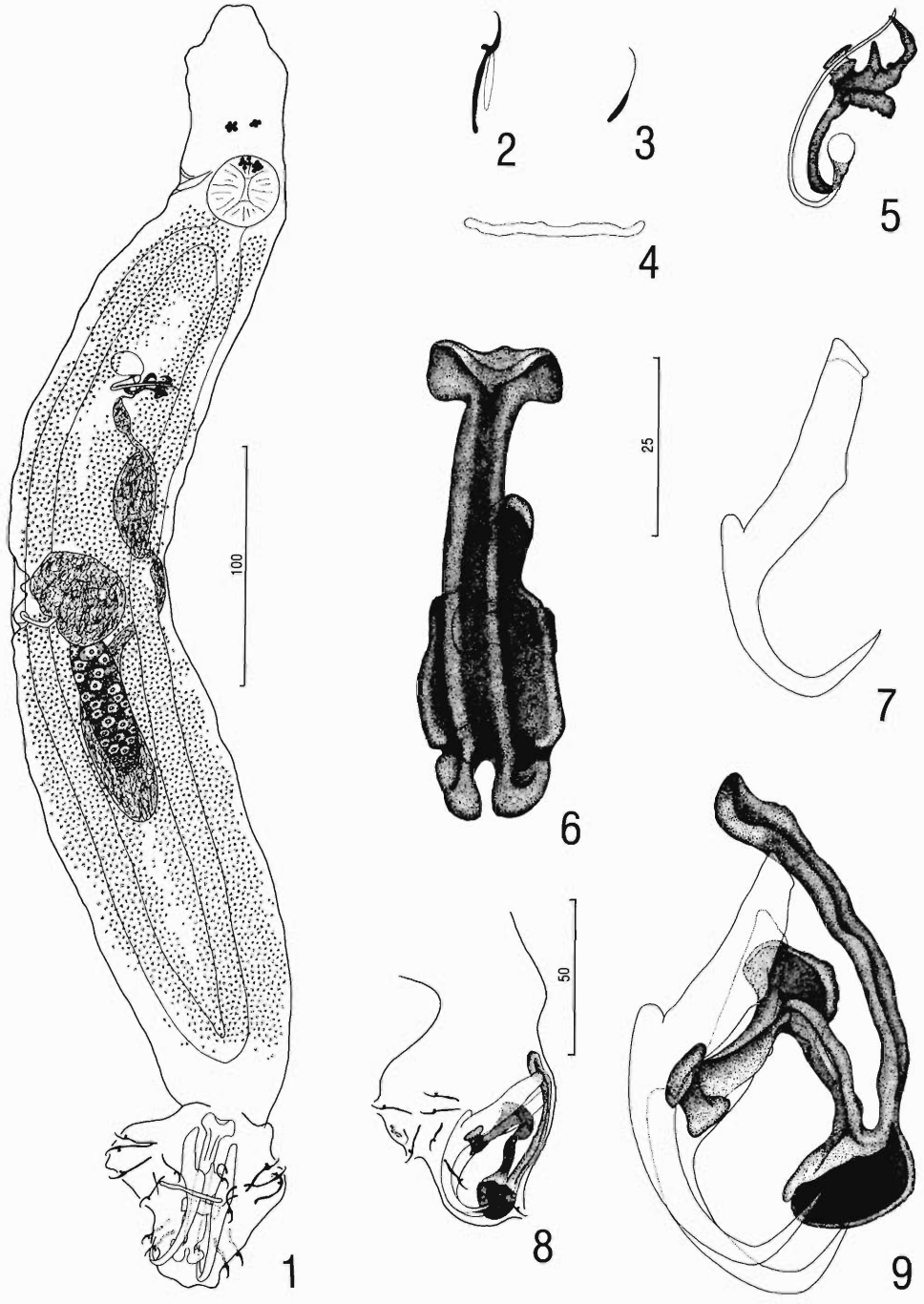
SPECIMENS STUDIED: Holotype, USNM 82093, 13 paratypes, USNM 82094, HWML 34290, MRAC 37.326, BM(NH) 1991.10.9.22.

ETYMOLOGY: The specific name refers to the superficial resemblance of the species to members of *Dactylogyrus* (Dactylogyridae, Dactylogyrinae).

Discussion

Our proposal of sister-group relationship of *Schilbetrematoides* and *Schilbetrema* Paperna and Thurston, 1968, is supported by several shared, apparently synapomorphic features. Among them, characteristics of the hooks, copulatory complex, eye granules, vagina, seminal vesicle, and distal vas deferens, and presence of a constriction of the vas deferens at its union with the seminal vesicle, appear to be the most significant (see Kritsky and Kulo, 1992). Basic features of the reproductive system of members of both genera are identical. However, these genera are presently differentiated primarily on haptoral structure. Although some components are highly modified, the distribution of haptoral sclerites in *Schilbetrema* spp. is typically ancyrocephaline, i.e., dorsal and ventral anchor/bar complexes present. In *Schilbetrematoides,* species possess a haptoral organization approaching that found in the Dactylogyrinae, i.e., dorsal anchor/bar complex present, ventral pair of 4A's present, ventral bar absent or vestigial.

Without totally discounting the possibility of dispersal of some dactylogyrine ancestor to a siluriform host and subsequent convergence of many morphologic features, occurrence of *Schilbetrematoides pseudodactylogyrus* on a schilbeid (Siluriformes) host further supports the sister-group relationship proposed herein. "Fahrenholz' Rule" states that the phylogeny of parasite groups usually directly corresponds to the natural relationships of their hosts (Eichler, 1948). Al-



Figures 1–9. ***Schilbetrematoides pseudodactylogyrus*** **gen. et sp. n. 1. Ventral view of holotype. 2. Hook. 3. 4A. 4. Ventral bar. 5. Copulatory complex. 6. Dorsal bar (dorsal view). 7. Dorsal anchor. 8. Lateral view of haptor (only sinistral members of hook pairs shown). 9. Dorsal anchor/bar complex (lateral view). All drawings are to the 25-μm scale, except Figures 1 and 8 (100-μm and 50-μm scale, respectively).**

though explanation of monogenoidean species on their hosts frequently requires instances of "host switching" (Boeger and Kritsky, 1989; Guégan and Agnèse, 1991; Wheeler and Beverley-Burton, 1989; among others), Fahrenholz' Rule is likely reflected in relationships of *S. pseudodactylogyrus* and *Schilbetrema* spp., all of which are exclusively parasites of African schilbeids.

Bychowsky and Nagibina (1978) split the Ancyrocephalinae from the Dactylogyridae and elevated it to family status based primarily on haptoral characteristics. These characters now appear to be insufficient to justify their action. Kritsky and Boeger (1989) provide evidence using cladistics that the Ancyrocephalidae (sensu Bychowsky and Nagibina, 1978) is paraphyletic and recommend that it be considered a junior synonym of the Dactylogyridae (sensu Yamaguti, 1963). Our finding of *Schilbetrematoides* and the characteristics it displays continue to cloud justification for the Ancyrocephalidae and, in addition, suggest probable polyphyly of the Dactylogyrinae (Dactylogyridae sensu stricto of Bychowsky and Nagibina, 1978) as well.

Kritsky and Boeger (1989), among others, consider the presence of a single pair of anchors (dorsal) in the Dactylogyrinae to represent a secondary loss of the ventral anchor pair. Taxa, based primarily on loss of structure(s), have a high risk of polyphyly because loss could have occurred many times with recognition of these independent occurrences difficult. In addition, structural loss in an evolutionary sense and particularly in parasites is potentially a more common occurrence than the development of new structures. As such, convergence, as shown by the absence of a structure, should be an expected outcome; proposal of new or the splitting of existing taxa based primarily on these characters, as engendered by Bychowsky and Nagibina, 1978, should be done conservatively.

Schilbetrematoides and perhaps some other genera (*Nanotrema* Paperna, 1969, *Trinidactylus* Hanek, Molnar, and Fernando, 1974, *Curvianchoratus* Hanek, Molnar, and Fernando, 1974, and *Acolpenteron* Fischthal and Allison, 1940) whose members occur on noncypriniform hosts and are considered by Gussev (1976, 1978) to comprise, in part, the Dactylogyridae sensu stricto, are potential groups in which loss of the ventral anchor pair may have occurred more than once throughout their evolutionary history. If independent loss of the ventral anchor pair happened in one or more of these genera, the family (s.s.) is likely polyphyletic. Thus, loss of an anchor pair has a very limited value in defining this familial taxon. Secondary loss of an anchor pair(s) has already been assumed to have occurred independently outside the Dactylogyridae (s.s.) in *Trinigyrus* Hanek, Molnar, and Fernando, 1974 (Ancyrocephalinae); *Pseudodactylogyrus* Gussev, 1965 (Pseudodactylogyrinae); *Anonchohaptor* Mueller, 1938, and *Icelanonchohaptor* Leiby, Kritsky, and Peterson, 1972 (Pseudomurraytrematidae); and *Anacanthorus* Mizelle and Price, 1965, and *Anacanthoroides* Kritsky and Thatcher, 1976 (Anacanthorinae). In species of the ancyrocephaline genera, *Eutrianchoratus* Paperna, 1969, *Heteronchocleidus* Bychowsky, 1957, and *Trianchoratus* Price and Berry, 1966, 1 member of the ventral anchor pair has been lost as a recognizable anchor but remains as a remnant resembling an early developing anchor.

Another feature that is used to place dactylogyrid species lacking 1 or both of the anchor pairs in the Dactylogyrinae is the presence of 4A's (Mizelle and Price, 1963) in the haptor (e.g., Gussev, 1978; Kritsky et al., 1978; Beverley-Burton, 1984). Circumstantial evidence, some of which is provided by *Schilbetrematoides pseudodactylogyrus,* continues to mount supporting the homology of the 4A with the respective lost anchor. This homology, implying each 4A to be an anchor vestige, was assumed by Kearn (1968) based on its relative position in the haptor and its temporal development in the larva and by Kritsky and Boeger (1989) based on parsimony. If *S. pseudodactylogyrus* is sister to *Schilbetrema* as proposed herein, the 4A's of the former are not homologues of those of the Dactylogyrinae, and presence or absence of this feature can no longer be used to separate dactylogyrids (sensu stricto) from so-called "ancyrocephalids." If 4A's are actually homologues of ventral anchors, they should be expected in any taxon within or without the Dactylogyrinae showing incomplete loss of the haptoral anchors.

We recognize that our proposal of the monotypic *Schilbetrematoides* gen. n. may result in paraphyly of *Schilbetrema,* because synapomorphic characters for the latter are not apparent. If *Schilbetrematoides pseudodactylogyrus* has a common ancestor (i.e., is sister species) with one or more of the *Schilbetrema* species infesting *Schilbe intermedius,* the new genus should be

considered a synonym of *Schilbetrema* with the new species a highly derived member. The spiral filament of the constriction of the vas deferens may be a character supporting this synonymy if its presence is the apomorphic state; a filamented constriction in *Schilbetrema* has only been observed in species infesting *Schilbe intermedius.* However, if the filament is synapomorphic for *Schilbetrema* + *Schilbetrematoides,* the character would not affect monophyly of the genera. Other characters, particularly associated with the highly modified haptoral sclerites in *Schilbetrema,* may provide synapomorphies for this genus. An objective analysis will be required to determine phylogenetic support for *Schilbetrematoides.*

In order to avoid potential confusion, we add the following concerning recent proposals for change in the nomenclature of the host. Based on the International Code of Zoological Nomenclature and on the type specimen of *Silurus mystus* Linnaeus, 1758, De Vos and Skelton (1990) indicate that the epithet "*Schilbe mystus*" must be applied to schilbeids currently assigned to *Eutropius niloticus* (Rüppel, 1829). As a result, these authors indicate that *Schilbe intermedius* Rüppel, 1832, is the oldest available name for fishes previously included in the taxon *S. mystus.* In the present paper, we accept De Vos and Skelton's (1990) nomenclatural changes for the host. It should be recognized, therefore, that the epithet "*Schilbe mystus*" used in previous parasitological literature refers to the species *S. intermedius,* and that "*Eutropius niloticus*" of the older parasitological literature is now referred to *S. mystus.*

Acknowledgments

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Report on the Brayton H. Ransom Memorial Trust Fund

The Brayton H. Ransom Memorial Trust Fund was established in 1936 to "encourage and promote the study and advance of the Science of Parasitology and related sciences." Income from the Trust currently provides token support of the *Journal of the Helminthological Society of Washington* and limited support for publication of meritorious manuscripts by authors lacking institutional or other backing. Contributions may be directed to the Secretary-Treasurer. Information about the Trust may be found in the following articles: *Proceedings of the Helminthological Society of Washington* (1936) 3:84–87 and (1983) 50:200–204.

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Helminths of the Salamanders *Ambystoma t. tigrinum* and *Ambystoma laterale* (Caudata: Ambystomatidae) from Southern Michigan

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ABSTRACT: Totals of 101 tiger salamanders, *Ambystoma t. tigrinum* (Green, 1825), and 302 blue-spotted salamanders, *Ambystoma laterale* Hallowell, 1856, were examined for helminths in 1989 and 1990 from 3 localities in southern Michigan. *Telorchis corti* Stunkard, 1915, *Diplostomum* sp., and *Proteocephalus* sp. infected larval and newly metamorphosed tiger salamanders. Overall prevalence of infection was 64%. Larval tiger salamanders harbored less diverse helminth communities than metamorphs. This was attributable to the changes in host habitat and feeding that precluded transmission of *T. corti* in metamorphosed salamanders. *Rhabdias ranae* Walton, 1919, *Spiroxys* sp., *Thelandros magnavulvaris* (Rankin, 1937), *Brachycoelium salamandrae* (Frölich, 1789), echinostome metacercariae, and an unidentified metacercaria infected blue-spotted salamanders. Our findings of *Rhabdias ranae, T. magnavulvaris,* and *B. salamandrae* in the blue-spotted salamander and *Diplostomum* sp. in the tiger salamander are new host records.

KEY WORDS: Trematoda, Nematoda, Cestoda, helminths, new host records, survey, ambystomid salamanders, Michigan.

The parasites of tiger salamanders, *Ambystoma t. tigrinum,* have been studied by Frandsen and Grundmann (1960), Waitz (1960), Watertor (1967), Ulmer (1970), Dyer and Brandon (1973), and Brooks (1976, 1978). Except for the Wisconsin study of Coggins and Sajdak (1982) on adult tiger and blue-spotted salamanders, *Ambystoma laterale,* little is known about the parasites of ambystomid salamanders in the Great Lakes area. The objectives of this study were: 1) to describe the prevalence and intensity of helminths in tiger and blue-spotted salamanders, 2) to describe the effects of metamorphosis on helminth community diversity in tiger salamanders, and 3) to compare prevalence and intensity of helminths of larval and adult blue-spotted salamanders from 2 different localities.

Materials and Methods

In May, June, and August 1989, 87 ($\bar{x}$ ± SD snout–vent length = 49 ± 23.7, range 15–82 mm) larval and 14 (79 ± 5.1, 72–88 mm) newly metamorphosed tiger salamanders were examined from Jackson Pond. Larvae were collected by dip net and newly metamorphosed individuals by hand from around the pond. This pond, located in south-central Michigan in Jackson County, is drained each fall.

Nine (55 ± 8.2, 42–68 mm) adult blue-spotted salamanders were collected by hand from the Otis Lake Area, Barry County, southwestern lower Michigan in March through May 1989. In May, June, and August 1989, 201 (23 ± 10.2, 7–44 mm) larvae were collected from 2 permanent ponds and 24 (40 ± 2.4, 36–46 mm) newly metamorphosed individuals were collected near the ponds where larvae were captured. Fifty-six (17 ± 3.6, 10–30 mm) larval and 12 (45 ± 11.5, 32–78 mm) adult blue-spotted salamanders were collected in March through June 1990 from a small permanent pond and swamp in the Rose Lake Wildlife Area, Clinton and Shiawassee counties, south-central Michigan. The ponds and swamp are surrounded by a mixed deciduous forest.

Salamanders were pithed or killed in MS222 (ethyl m-aminobenzoate methane sulfonic acid) and examined within 24 hr of collection. Helminths were processed using conventional techniques. Parasite species were not analyzed by salamander sex because of difficulties encountered in sexing. Prevalence is the percentage of salamanders infected in a sample; mean intensity is the mean number of worms per infected salamander, and values are expressed as a mean ± 1 SD. Brillouin's index, appropriate for fully censused communities and evenness (Pielou, 1975) were calculated for each infracommunity in tiger salamanders and included all helminths irrespective of their site of infection. Values were calculated using common logarithms. Species richness is the number of helminth species per salamander. Voucher specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Telorchis corti* (82122), *Diplostomum* sp. (82123), *Brachycoelium salamandrae* (82124), *Rhabdias ranae* (82125), *Thelandros magnavulvaris* (82126), and *Spiroxys* sp. (82127). Specimens of the other helminth species were not retained by the authors and therefore were not deposited.

Results

Three helminth species infected tiger salamanders with 59% of the larvae and 100% of newly metamorphosed individuals harboring at least 1 helminth. One small, immature *Proteocephalus* sp. occurred in the small intestine of a

Table 1. Monthly and overall prevalence and mean intensity of *Telorchis corti* and *Diplostomum* sp. in larval and newly metamorphosed *Ambystoma t. tigrinum* from Jackson Pond, Michigan in 1989.

Month	Number examined	*Telorchis corti* Prevalence*	*Telorchis corti* Mean intensity ± 1 SD (range)	*Diplostomum* sp. Prevalence*	*Diplostomum* sp. Mean intensity ± 1 SD (range)
May	41†	0 (0)	—	4 (10)	1.3 ± 1 (1–2)
June	30†	29 (97)	83.1 ± 58 (1–181)	29 (97)	7.4 ± 5 (1–21)
August	16†	14 (88)	247.9 ± 198 (3–720)	16 (100)	11.7 ± 4 (7–24)
Overall	87†	43 (49)	136.7 ± 143 (1–720)	49 (56)	8.0 ± 5 (1–24)
August	14‡	8 (57)	49.8 ± 98 (1–289)	14 (100)	10.4 ± 5 (2–21)

* Number infected (percent infected).
† Larvae.
‡ Newly metamorphosed individuals.

larval tiger salamander collected in May. Gravid *Telorchis corti* were found throughout the intestine, whereas *Diplostomum* sp. occurred unencysted in the lens of the eye. There was no significant difference (Student's *t*-test; $P > 0.05$) in the mean intensity of *Diplostomum* sp. between left (5.8 ± 2.8, $N = 30$) and right (5.3 ± 2.9, $N = 30$) lenses. The mean intensity of *T. corti* and the prevalence and mean intensity of *Diplostomum* sp. increased in larvae from May through August (Table 1). The prevalence and mean intensity of *T. corti* and mean intensity of *Diplostomum* sp., however, decreased in newly metamorphosed individuals in August when compared to infection values in larvae in the same month. Forty-two (48%) of 87 larval and 8 (57%) of 14 newly metamorphosed tiger salamanders were concurrently infected with *T. corti* and *Diplostomum* sp. *Diplostomum* sp. intensity was significantly correlated with larval snout–vent length ($r = 0.40$, $P < 0.01$). In newly metamorphosed individuals, there were no significant correlation coefficients between helminth intensities and snout–vent length.

Helminth species richness, helminth intensity, Brillouin's diversity index, and evenness for tiger salamanders are presented in Table 2. All mean values except helminth intensity were higher in newly metamorphosed individuals than in larvae. Correlation coefficients between snout–vent lengths of larval tiger salamanders and helminth community structure measures were significant. None of the correlation coefficients involving newly metamorphosed individuals was significant.

The lung nematode, *Rhabdias ranae,* had the highest prevalence and mean intensity of the 4 helminth species found in adult blue-spotted salamanders from the Otis Lake area (Table 3). Helminths were not found in the 201 larval and 24 newly metamorphosed blue-spotted salamanders. In the Rose Lake area, *Spiroxys* sp. infected adult blue-spotted salamanders and echinostome metacercariae infected larvae. The 30 larvae from the swamp were negative. Only 1 blue-spotted salamander from both localities was infected with 2 helminth species (*Spiroxys* sp. and echinostome metacercariae).

Discussion

The present study is the first survey on the helminths of ambystomid salamanders in Mich-

Table 2. Helminth species richness, helminth intensity, Brillouin's diversity index, and evenness for helminths in 87 larval and 14 newly metamorphosed *Ambystoma t. trigrinum* from Jackson Pond, Michigan, in 1989. Data are means ± 1 SD (range), and correlation coefficient.

Salamander stage	Helminth species richness	Helminth intensity	Brillouin's index	Evenness
Larvae	1.069 ± 0.95 (0–2), 0.92*	72.4 ± 125 (0–732), 0.61*	0.0556 ± 0.07 (0–0.25), 0.68*	0.1852 ± 0.24 (0–0.84), 0.67*
Newly metamorphosed	1.571 ± 0.51 (1–2), 0.17	38.8 ± 77 (2–303), 0.27	0.0903 ± 0.09 (0–0.23), 0.19	0.2998 ± 0.31 (0–0.76), 0.20

* $P < 0.01$.

Table 3. Site of infection, prevalence, and mean intensity of helminths in 9 adult *Ambystoma laterale* from the Otis Lake area and 12 adult and 26 larval *Ambystoma laterale* from the Rose Lake area, Michigan.

	Site*	Prevalence	Mean intensity ± 1 SD (range)
Otis Lake Area			
Adults (9)			
Rhabdias ranae†	L	67	14.2 ± 24 (1–56)
Thelandros magnavulvaris†	R	11	3
Brachycoelium salamandrae†	SI	11	1
Unidentified metacercaria	EL	11	1
Rose Lake Area			
Adults (12)			
Spiroxys sp.	SW	50	4.2 ± 4 (1–11)
Larval (26)			
Echinostome metacercariae	M	38	7.5 ± 5 (1–17)

* L, lungs; R, rectum; SI, small intestine; EL, encysted on outer surface of lungs; SW, encysted in stomach wall; M, encysted in mesenteries.

† Gravid individuals.

igan and the second in the Great Lakes area. The helminths are different from those reported in adult tiger and blue-spotted salamanders from Wisconsin by Coggins and Sajdak (1982), in Utah by Fransden and Grundmann (1960), and in Oklahoma by Dyer and Brandon (1973). Most salamanders examined in the present study were aquatic larvae; thus, helminths with terrestrial transmission were not expected. Other possible explanations for this compositional difference in these ambystomid salamander helminth assemblages may be the absence of the parasite in the study area, lack of an appropriate intermediate host, or both, as well as differences in times of salamander collections. Helminths with indirect life cycles infected tiger salamanders, whereas *Rhabdias ranae,* the most prevalent species in adult blue-spotted salamanders, has a direct life cycle.

Telorchis corti (conspecific with *T. bonnerensis*) has an extensive geographical range and has been found in tiger salamanders by Watertor (1967), Ulmer (1970), and the present study; in long-toed salamanders, *Ambystoma macrodactylum,* by Waitz (1960); in newts, *Notophthalmus viridescens,* by Muzzall (1991); and reported from other poikilotherms by MacDonald and Brooks (1989). Watertor (1967) demonstrated that amphibians and reptiles became infected with *T. corti* by eating infected *Physa gyrina. Physa* sp. was a very common food item of larval salamanders from Jackson Pond, thus explaining the high prevalence and mean intensity of *T. corti.* The significant correlation coefficient between *T. corti* intensity and larval salamander length was attributed to an increase in the number of *Physa* sp. eaten as salamanders increased in length. Dead and decomposing *T. corti* were found in the posterior intestine and rectum of newly metamorphosed individuals. The decrease in prevalence and mean intensity of *T. corti* in newly metamorphosed salamanders is similar to the results of Waitz (1960), who speculated that the loss of worms in adult long-toed salamanders was due to the change in diet that accompanies metamorphosis, resulting in a change in the microenvironment of the small intestine.

Kelley (1934) and Etges (1961) found *Diplostomum scheuringi* in the eye and brain of newts. Dunbar and Moore (1979) found *D. desmognathi* in the body cavity and musculature of desmognathine salamanders. Rankin (1937) reported *Diplostomulum ambystomae* in the body cavity of marbled salamanders, *Ambystoma opacum,* and spotted salamanders, *Ambystoma maculatum,* whereas Price and St. John (1980) found *Diplostomulum ambystomae* in the body cavity of small-mouthed salamanders, *Ambystoma texanum.* To our knowledge, our study is the first published report of *Diplostomum* sp. in the lens of an ambystomid salamander. Worms were active after they were removed from the lens, but it is not known if they were infective to the definitive host. Attempts to collect adult tiger salamanders returning to Jackson Pond in the spring of 1989 and 1990 to determine if they remained

infected with *Diplostomum* were unsuccessful. *Diplostomum* sp. had a higher mean intensity and prevalence in larval tiger salamanders in June than it did in walleye fingerlings, *Stizostedion vitreum* (see Muzzall et al., 1990). This may be due to salamanders staying in the pond for a longer period of time and being larger than walleyes.

The helminth infracommunities of tiger salamanders are in agreement with factors predicted by Kennedy et al. (1986) that lead to an isolationist community, such as ectothermy, having a simple alimentary canal and low vagility. The helminth infracommunities of newly metamorphosed salamanders were more diverse than those of larvae. This may have been the result of a higher helminth species richness and from a reduction in the number of *T. corti,* resulting in an increase in evenness that is related to a change in host habitat and physiology. These helminth community structure measures, except for helminth intensity, are similar to those values reported for desmognathine salamanders by Goater et al. (1987) and for newts by Muzzall (1991).

Of the 9 adult blue-spotted salamanders examined in the spring from the Otis Lake area, 8 were infected with 1 or more helminths. The high prevalence and mean intensity of *Rhabdias ranae* in blue-spotted salamanders were unexpected because *R. ranae* has never been reported from a salamander. Price and St. John (1980), however, reported that *Rhabdias* sp. was the most frequently found helminth in small-mouthed salamanders. *Ambystoma laterale* is also a new host for *Thelandros magnavulvaris* and *Brachycoelium salamandrae.* The occurrence of *T. magnavulvaris* and *B. salamandrae* in adults was not surprising because they commonly infected red-backed salamanders, *Plethodon c. cinereus,* in the same area (Muzzall, 1990). None of the larvae was infected even though their gastrointestinal tracts consistently contained cladocerans, copepods, ostracods, odonate larvae, mosquito pupae, and other invertebrates. No molluscs were seen in the Otis Lake area ponds when larval salamanders were collected, thus explaining the absence of trematodes. Newly metamorphosed salamanders contained little food in the gastrointestinal tract, indicating they were collected before the onset of heavy feeding. Only 6 adult and 10 larval blue-spotted salamanders from the Rose Lake area were infected, most with 1 helminth species. When the data for the blue-spotted salamanders from the 2 localities were combined, 4 helminth species infected adults, whereas 2 infected larvae. Four of the 6 helminth species found in blue-spotted salamanders have indirect life cycles.

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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 Eighth International Conference on Trichinellosis will be held in Orvieto, Italy, 7–11 September 1993. For information contact:

Dr. Edoardo Pozio
Laboratory of Parasitology
Istituto Superiore di Sanità
Viale Regina Elena
299-00161 Rome, ITALY

The Eighth International Congress of Parasitology will be held in Izmir, Turkey, 10–14 October 1994. For information contact:

Secretariat ICOPA VIII
P. K. 81 35042, Bornova
Izmir, TURKEY

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Bolbosoma turbinella (Acanthocephala) in a Blue Whale, *Balaenoptera musculus,* Stranded in the St. Lawrence Estuary, Quebec

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ABSTRACT: A stranded blue whale, *Balaenoptera musculus* (L.), on the north shore of the St. Lawrence River, Quebec, Canada, was examined for parasites. The acanthocephalan *Bolbosoma turbinella* (Diesing, 1851), found in all sections of the small intestine examined, was the only parasite observed. This is a new geographic and host record. The proboscis of adult *B. turbinella* has 18 to 21 longitudinal rows of hooks in males and 20–21 in females, with 6 to 7 hooks per row. Lemnisci were 9–18 mm long in males and 6–22 mm long in females.

KEY WORDS: *Bolbosoma turbinella,* Acanthocephala, blue whale, *Balaenoptera musculus,* St. Lawrence River, Canada.

The epizootiology of parasites of cetaceans is poorly understood. Early parasitological studies consisted of reports of parasites in whales, mostly mysticetes, taken during commercial whaling, particularly in Antarctica and the North Pacific Ocean. Parasite–host lists have been published by Margolis (1954), Delyamure (1955), Dailey and Brownell (1972), and Margolis and Dailey (1972). Dailey and Vogelbein (1991) examined the helminth fauna of 3 Antarctic whale species to determine its use as stock indicators.

Cowan (1967) examined helminths of pilot whales (*Globicephala melaena*) collected off Newfoundland. Over the past 30 yr at least 24 putative blue whales have stranded on the east coast of Canada (Béland et al., 1987; M. Kingsley, Dept. Fish. and Oceans, unpubl. data), but no parasitological data from these strandings have been published.

Materials and Methods

We examined a blue whale, *Balaenoptera musculus* (Linnaeus, 1758), which had stranded on 11 October 1990 on the north shore of the St. Lawrence River, 7 km west of Franquelin, Quebec, Canada (49°17′N, 67°54′W).

The whale was dissected on 12–13 October. The following organs were examined for parasites: liver, heart, left lung, proximal end of the esophagus, forestomach, main stomach, pyloric stomach, a randomly chosen 3-m section and 4 additional 1-m sections of the small intestine, and the colon. Parasites removed were placed in mammalian saline and later transferred to 70% ethanol.

Acanthocephalans were transferred to glycerin alcohol (9 parts 70% alcohol : 1 part glycerin) and cleared by evaporation. Some specimens were cleared in lactic acid or dissected. Others were prepared for scanning electron microscopy (SEM) by dehydration through a series of increasing concentrations of ethyl alcohol, postfixed in 1% osmium tetroxide, dried by critical point drying using carbon dioxide substitution, and coated with gold palladium. Acanthocephalans were studied using a JEOL JSM-T330 scanning electron microscope at 20 kV. Measurement and morphological study of acanthocephalans were made using a Leitz Diaplan microscope equipped with a drawing tube coupled with a digitizer tablet and computer. Measurements are in micrometers unless otherwise indicated.

Results

The whale was 19.65 m long from the tip of the snout to the notch between the tail flukes (measured in a straight line). Weight was estimated at 46.3 tonnes using the formula $W = aL^b$, given by Lockyer (1976). The whale was a young female estimated by length to be less than 2 yr old (Rudd et al., 1950). Time of death was estimated to have been no more than a few days previous. Most tissues were in good condition; however, decomposition was beginning (the epithelium of the skin was beginning to form blisters and slough off and there was some gas escaping from the body cavity as it was opened). No food was found in the portions of the gastrointestinal tract examined.

The only parasite found was *Bolbosoma turbinella* (Diesing, 1851) Porta, 1908, which was present in the small intestine. No definitive count of *B. turbinella* was made; however, hundreds were observed. As worms were removed, only a cylindrical cavity in the mucosa where the proboscis had been embedded was observed. A sample of 55 was collected for identification. Voucher specimens of *B. turbinella* (CMNP1991-0014)

Table 1. Values of mensural and meristic characters of *Bolbosoma turbinella* from the small intestine of *Balaenoptera musculus* (L.) from the St. Lawrence Estuary, Quebec, Canada.*

Character	Males (N = 10)	Females (N = 10)
Total length (mm)	19.3 ± 3.2 (15.2–24.9)	19.9 ± 2.7 (17.2–26.2)
Bulb width (mm)	2.3 ± 0.2 (2.1–2.7)	2.5 ± 0.3 (2.1–2.7)
Bulb length (mm)	1.0 ± 0.2 (0.8–1.3)	1.2 ± 0.1 (1.1–1.4)
Neck length	310 ± 95 (207–475)	325 ± 82 (212–399)
Neck width (maximum)	637 ± 64 (494–735)	725 ± 84 (628–903)
Proboscis length	758 ± 64 (648–833)	799 ± 66 (653–900)
Proboscis width (at basal row of hooks)	625 ± 65 (530–739)	680 ± 72 (580–766)
Proboscis receptacle length (mm)	1.5 ± 0.2 (1.2–1.9)	1.5 ± 0.1 (1.3–1.7)
Proboscis receptacle width (maximum)	629 ± 45 (559–689)	623 ± 97 (491–816)
No. longitudinal rows of hooks on proboscis	19 ± 1 (18–21)	20 ± 0.4 (20–21)
No. hooks per row on proboscis	(6–7)	(6–7)
Lemnisci length (mm)	13.3 ± 2.4 (9.3–18.4)	14.5 ± 4.0 (6.3–22.3)
Anterior testis† (mm)	14.0 ± 2.6 (10.0–18.1)	—
Diameter of anterior testis	796 ± 288 (471–1,434) × 732 ± 266 (357–1,211)	
Posterior testis† (mm)	13.0 ± 2.3 (9.0–16.9)	—
Diameter of posterior testis	800 ± 301 (435–1,450) × 779 ± 268 (428–1,197)	
Diameter of eggs‡	—	(41–61) × (14–22)

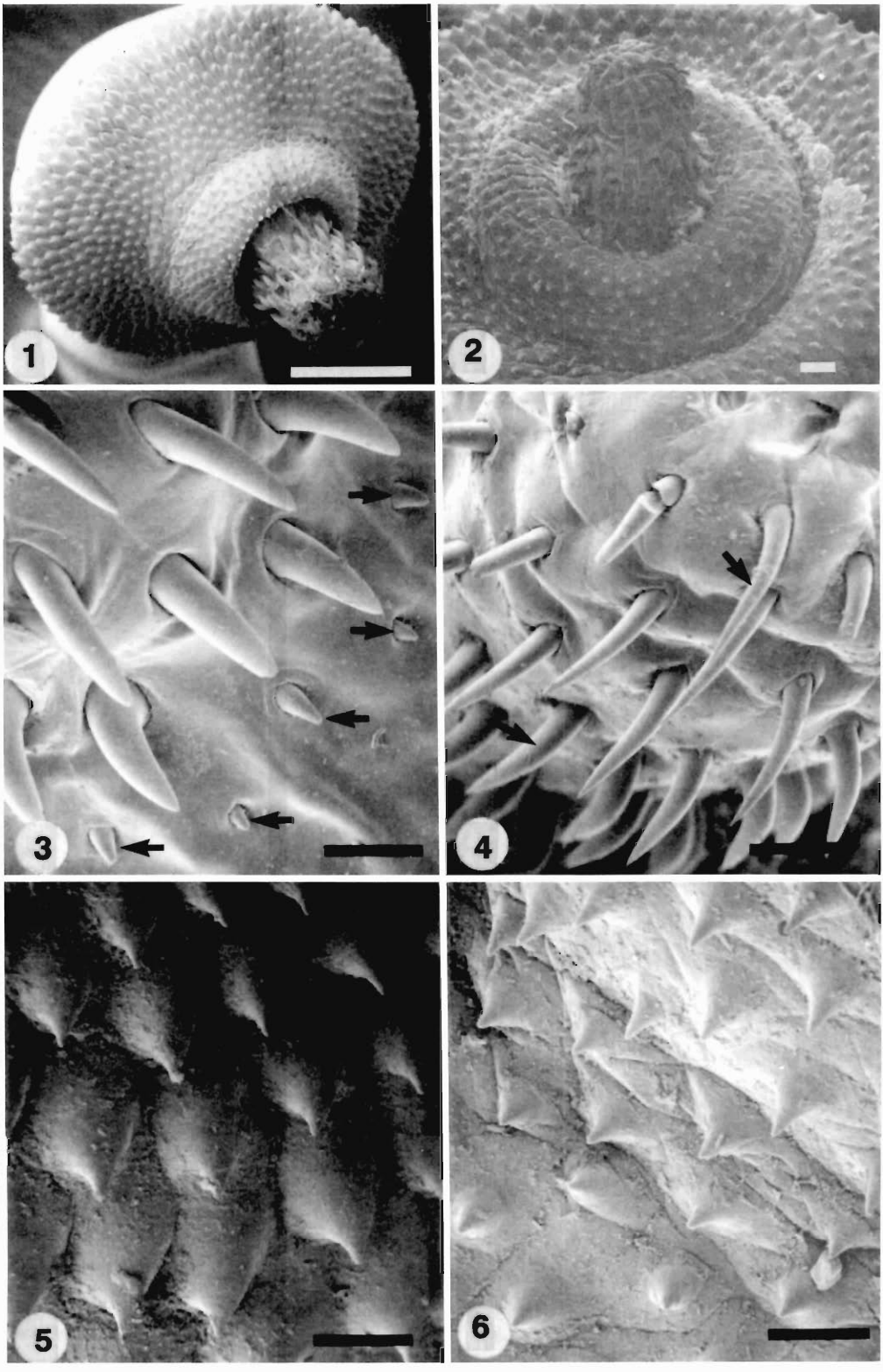
* Measurements (in micrometers unless otherwise noted) are mean ± standard deviation with range in parentheses.
† Distance from posterior extremity to anterior margin of testis.
‡ Eggs were not mature and were taken from the uterus and pseudocoel of 4 females. Other females had no eggs.

were deposited in the Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4.

Female *Bolbosoma turbinella* had immature eggs. Each egg contained an immature embryo but eggs lacked polar elongations. The proboscis was conical; males had 18–21 longitudinal rows of hooks and females had 20–21 (Table 1; Figs. 1, 7). There were 6–7 hooks per row on the proboscis of male and female specimens (Fig. 2). Apical hooks (N = 8) on the proboscis were slender (52–90 long in males and 88–114 long in females [Fig. 4]) with slender oblong roots (69–85 long in males and 80–98 long in females). Median hooks (N = 20) were robust (81–107 long in males and 82–127 long in females [Fig. 4]) with wide oblong roots (70–119 long in males and 96–125 long in females). Basal hooks (N = 20) were reduced (6–28 long in males and 10–90 long in females [Fig. 3]) with wide ovate roots (10–41 long in males and 10–93 long in females). The bulb (Fig. 1) had flat, scalelike spines located close to the outer perimeter of the bulb (Fig. 5) and erect spines located close to the base of the proboscis (Fig. 6). Spines (N = 10), were 17–119 long in males and 34–118 long in females. Spines were not observed elsewhere on the body. Lemnisci were long, often convoluted, and frequently terminated just posterior to the posterior testis. In a few specimens, lemnisci extended close to the posterior extremity of the body. Values of mensural and meristic characters are given in Table 1.

Discussion

This is the first report of *Bolbosoma turbinella* in blue whales from North American waters and the first from the eastern coast of North America. As the identification of *Bolbosoma* spp. is problematic, it may be useful to mention species of *Bolbosoma* reported in whales from North American waters. These include: *B. turbinella* in the sei whale (*Balaenoptera borealis*) from the Pacific Ocean off British Columbia (Margolis and Pike, 1955); *Bolbosoma capitatum* (Linstow, 1880) Porta, 1908, in pilot whales (*Globicephala melaena*) collected off Newfoundland (Cowan, 1967); *Bolbosoma* sp. from an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) stranded in Maine (Beverley-Burton, 1978); *Bolbosoma vasculorum* (Rudolphi, 1819) Porta, 1908, from a pigmy sperm whale (*Kogia breviceps*) stranded in Georgia (Pendergraph, 1971); *Bolbosoma balaenae* (Gmelin, 1790) Porta, 1908, from an unidentified whale examined at Seattle, Washington (Van Cleave, 1953); *Bolbosoma* sp. in the gray whale (*Eschrichtius robustus*) from the Pacific Ocean off California (Rice and Wolman,



1971); and *Bolbosoma* sp. in the sei whale, blue whale, and humpback whale (*Megaptera novaeangliae*) off California (Rice, 1963). Rice (1978) reported *B. nipponicum* Yamaguti, 1939, in blue whales collected and examined off California.

Amin (1985) lists 14 nominal species of *Bolbosoma*. Characters usually used to distinguish species are the number of longitudinal rows of hooks and the number of hooks per longitudinal row of hooks on the proboscis all of which, however, show considerable overlap. The number of longitudinal rows of hooks and the number of hooks per longitudinal row (in parentheses) on the proboscis of *B. turbinella* have been reported as 14–16 (7) (Porta, 1908), 20–22 (6–8) (Harada, 1931), 19–20 (6–7) (Meyer, 1933), 20–22 (7–8) (Margolis and Pike, 1955) and 6–7 hooks (?) (Diesing, 1851).

Bolbosoma turbinella is not restricted to blue whales. It also has been reported previously from several mysticetes (blue whale, sei whale, fin whale, humpback whale, and North Atlantic right whale [*Eubalaena glacialis*]) and 1 odontocete (the North Atlantic bottlenose whale [*Hyperoodon ampullatus*]), which is the type host according to Diesing (1851). Distribution of *B. turbinella* in whales includes the Atlantic and Pacific oceans in the northern and southern hemispheres.

Blue whales are stenophagous, i.e., they have a narrow range of food consumed. The diet of blue whales in the Gulf of St. Lawrence is unknown. However, Klumov (1963) lists food items in the stomach of blue whales examined elsewhere in the North Atlantic Ocean, including the euphausiids *Thysanoessa inermis, T. longicaudata* and *Meganyctiphanes norvegica*. In the lower estuary of the St. Lawrence, *T. inermis, M. norvegica,* and *T. raschi* occur in dense swarms of up to 700 km^2 at depths of 50–175 m during summer (Berkes, 1976; Simard et al., 1986; Runge and Simard, 1990). As blue whales usually feed below 50 m deep (Klumov, 1963), they are likely ingesting these euphausid species. Shimazu (1975) reported juvenile *Bolbosoma caenoforme* (Heitz, 1919) Meyer, 1935, from *T. longipes, T. raschi,* and unidentified species of euphausiids collected in the North Pacific Ocean. Female *B. turbinella* found in the present study contained immature eggs suggesting that blue whales are infected in the lower estuary of the St. Lawrence River or Gulf of St. Lawrence where they feed 9 mo of the year (Sears et al., 1990).

Two of the 4 other mysticetes reported infected with *Bolbosoma turbinella* are copepod specialists (sei whale, right whale), but they also feed on euphausiids (Mitchell, 1975). The fin whale and the humpback whale are generalists and euryphagous, feeding on swarming fish such as capelin, herring, cod, sardine, and mackerel (Nemoto, 1959; Klumov, 1963; Mitchell, 1975). Thus the latter 2 species of whales likely acquire *B. turbinella* from infected fish that probably serve

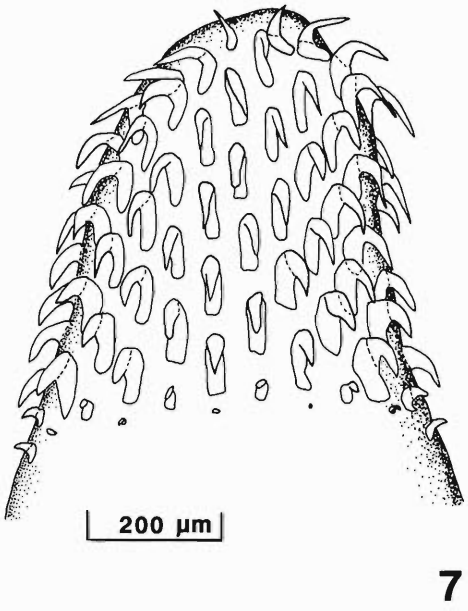


Figure 7. Proboscis of *Bolbosoma turbinella,* adult male. Note slender apical hooks, robust median hooks, and reduced basal hooks.

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Figures 1–6. Scanning electron micrographs of *Bolbosoma turbinella* from a blue whale, *Balaenoptera musculus* (L.). 1. Anterior extremity of adult female showing bulbous expansion of anterior part of trunk (=bulb) and proboscis. Scale bar = 500 µm. 2. Proboscis of adult female showing arrangement of longitudinal rows of hooks. Scale bar = 100 µm. 3. Proboscis showing reduced hooks (arrows) in basal row. Scale bar = 50 µm. 4. Subapical view of proboscis showing a slender apical hook (upper arrow) and a robust medial hook (lower arrow). Scale bar = 50 µm. 5. Flat scalelike spines on bulb located close to outer perimeter of bulb. Scale bar = 50 µm. 6. Erect spines on bulb, located close to base of proboscis. Scale bar = 50 µm.

as paratenic hosts. Reports of juvenile *Bolbosoma* sp. in fish involve members of Scombridae, Scorpaenidae, Carangidae, Trichiuridae, Gempylidae, Salmonidae, Berycidae, Lophotidae, Gadidae, and Belonidae (Harada, 1935; Kamegai, 1962a, b; Kato et al., 1963a, b; Mamaev and Baeva, 1963; Zhukov, 1963; Ichihara, 1964a–c; Mamaev, 1965; Sey, 1970; Bussieras and Baudin-Laurencin, 1973; Pennell et al., 1973; Butorina, 1976; Wang, 1980; Rego and Santos, 1983; Arthur, 1984; Arai, 1989). Most of these reports are from the North Pacific Ocean where *B. turbinella* is known to occur. However, Bakey and Zubchenko (1984) found *B. vasculorum* in the roundnose grenadier, *Coryphaenoides rupestris* (Macrouridae), collected in the North Atlantic Ocean.

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Angiostoma spiridonovi sp. n. (Nematoda: Angiostomatidae) from *Limax flavus* (Gastropoda: Limacidae)

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ABSTRACT: *Angiostoma spiridonovi* sp. n. (Rhabditida: Angiostomatidae) is described from limacid slugs in western Europe. The new species is distinguished from other members of the genus by the lack of lateral alae, shape of the buccal cavity, and number of caudal papillae.

KEY WORDS: *Angiostoma spiridonovi* sp. n., Nematoda, Angiostomatidae, taxonomy, slug, *Limax flavus*, Limacidae, Brittany, France.

The family Angiostomatidae contains the single genus *Angiostoma* which is composed of 6 species. One species has been described in North America (Chitwood, 1933), whereas the others have been found from various terrestrial molluscs in Europe and Central Asia (Spiridonov, 1985; Morand and Spiridonov, 1989). The present communication describes a new species of *Angiostoma* from limacid slugs in western Europe.

Materials and Methods

Slugs were collected in a garden from the city of Rennes, Brittany, France. Nematodes were removed from the anterior part of the intestine. The nematodes were fixed with hot 70% ethanol and cleared with lactophenol. Figures were made with the aid of a drawing tube. Measurements given are for the holotype male, the allotype female, and a larva from the uterus of a female. Measurements in parentheses are the ranges of paratype males and females. All measurements are in micrometers.

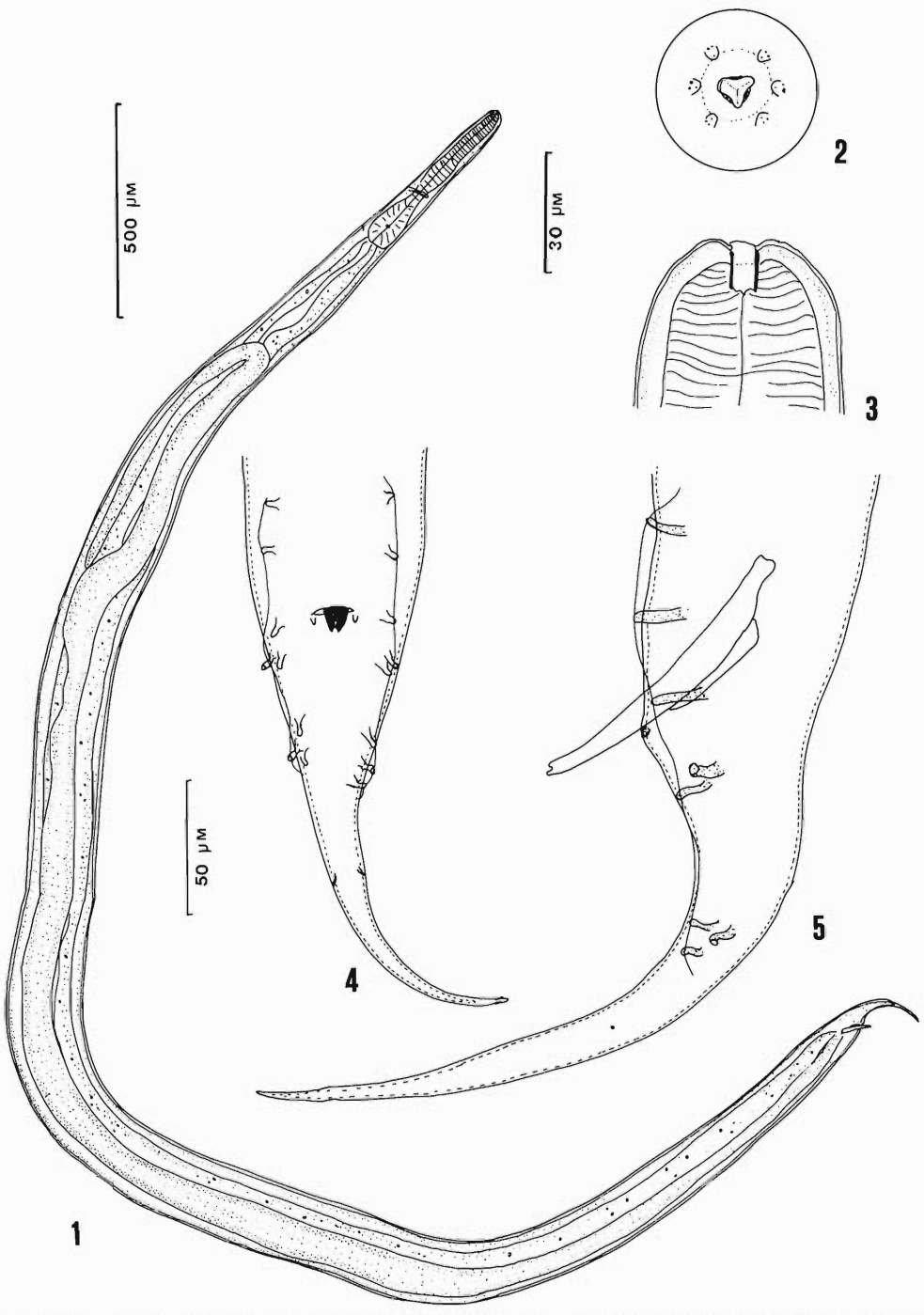
Description

Angiostoma spiridonovi sp. n. (Figs. 1–17)

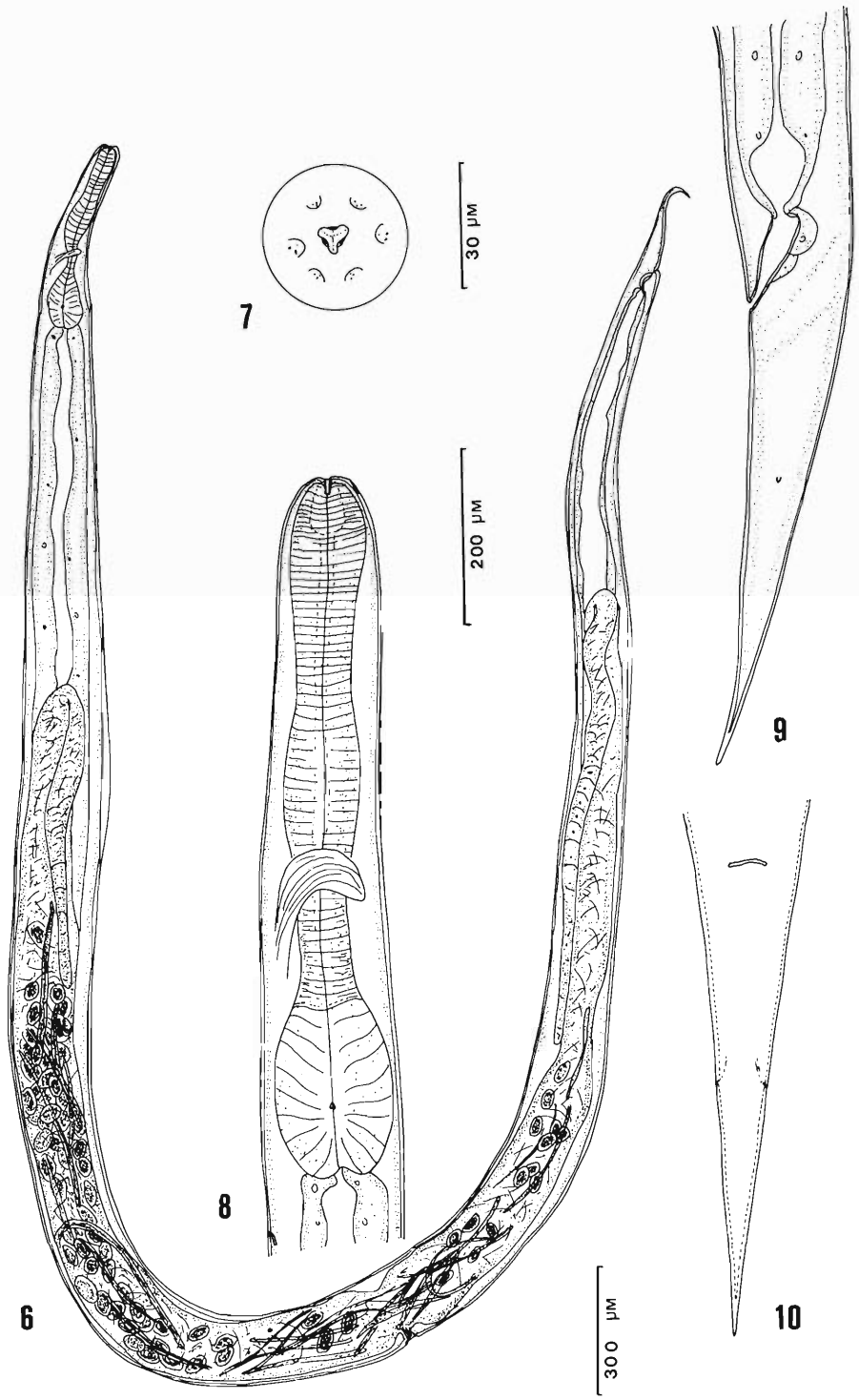
GENERAL: Nematoda, Rhabditida, Angiostomatidae, *Angiostoma*. Transparent nematodes lacking lateral alae in both sexes. Cuticle thin and nonstriated. Sexual dimorphism not prominent. Oral opening subtriangular with 3 lips. Ten papillae, 6 inner papillae, and 2 minute amphids on 6 elevations surrounding oral opening (Fig. 2). Cylindrical buccal cavity about 8 long and 5 wide (Fig. 3). Esophagus with corpus, isthmus, and bulb with minute valves (Fig. 8). Posterior extremity of corpus like a metacorpus without histological discontinuities (Fig. 8). Nerve ring at level of anterior part of isthmus. Excretory pore posterior to the esophago–intestinal junction.

MALE (holotype and 2 paratypes) (Figs. 1–5, 16): Length 2,630 (3,410–5,000). Maximum width 85 (80–100). Buccal cavity 16 (15–18) long and 8 (7–8) wide. Esophagus 320 (382–385) long: corpus 85 (90–95) long, isthmus 55 (88–70) long, and valved bulb 65 (75–105) long by 50 (55–60) wide. Nerve ring 250 (245–245), excretory pore 360 (430–440), and flexed portion of testis 660 (740–690) from anterior extremity. Caudal alae developed and supported by 8 pairs of pedunculate papillae: 2 pairs preanal, 1 pair adanal, and 5 postanal. Fifth and 8th pairs terminating on dorsal surface of bursa, whereas other pairs terminating on ventral surface (Figs. 4, 5). One pair of minute papillae immediately posterior to anus. Testis reflexed (Fig. 1). Spicules well chitinized, equal, expanded proximally, indented distally, poorly arcuate, 82 (81–91) long. Gubernaculum poorly chitinized, ellipsoidal in ventral view, 35 (36–41) long. Tail 160 (175–209) long. Phasmids 58 (70–90) posterior to anus.

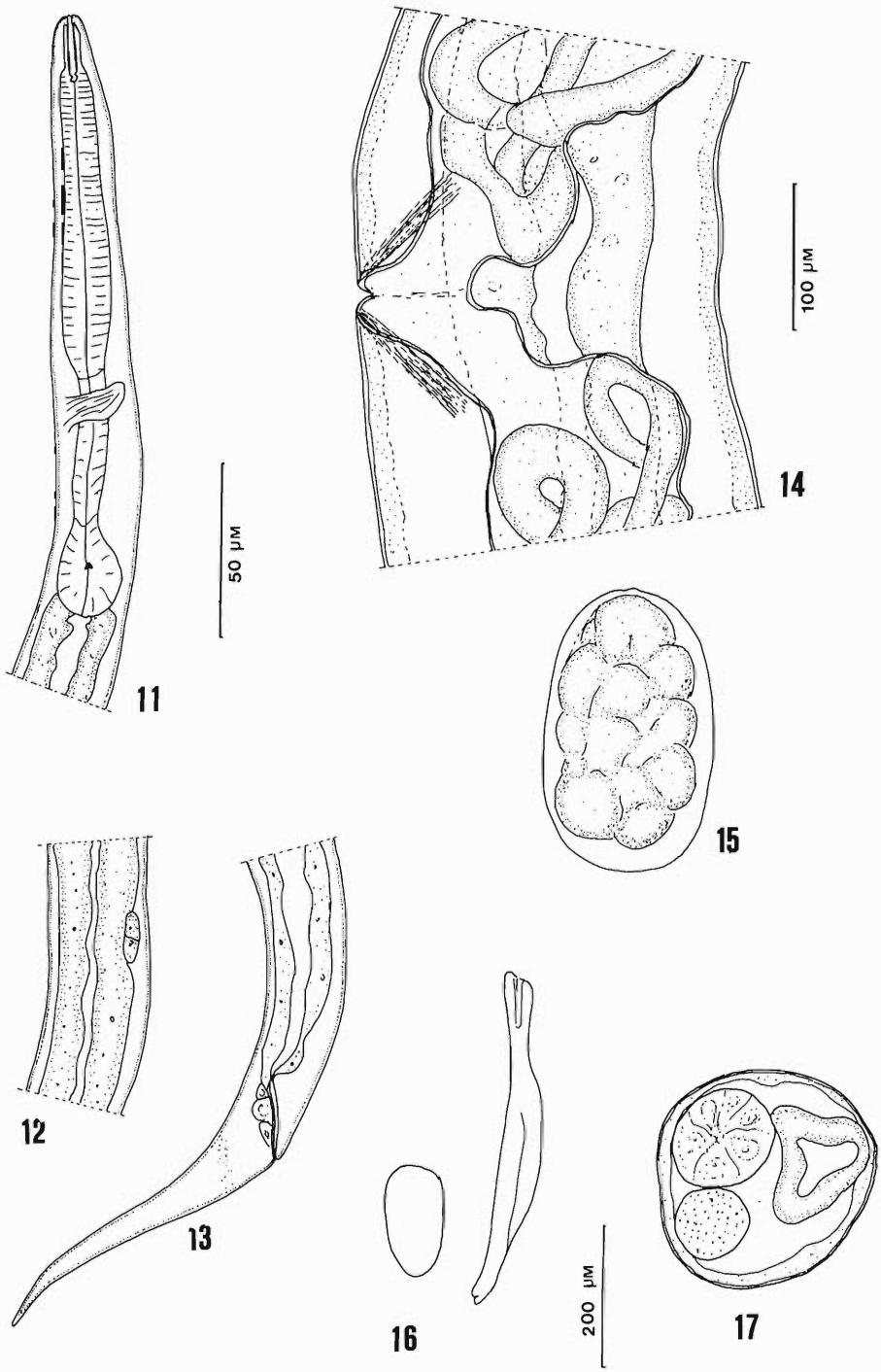
FEMALE (allotype and 2 paratypes): Length 4,080 (5,840–5,600). Maximum width 90 (110–90). Buccal cavity 10 (11–10) long and 7 (6–7) wide. Esophagus 365 (400–350) long: corpus 105 (110–100) long, isthmus 60 (65–60) long, and bulb 85 (105–72) long by 50 (72–65) wide. Nerve ring 225 (250–235), excretory pore 425 (465–410), and vulva 1,425 (2,840–2,750) from cephalic extremity. Tail elongated, 295 (260–235) long. Phasmids not conspicuous 160 (120–125) posterior to anus. Amphidelphic; uteri divergent, anterior uterus directing anteriorly, posterior uterus directing posteriorly; ovaries reflexed. Vulva in middle position leading to a short vagina (Fig. 14). Uteri contain numerous eggs 49–52 (50) by 79–93 (81) and larvae. Eggs elliptical,



Figures 1–5. ***Angiostoma spiridonovi*** **sp. n. male from** ***Limax flavus*** **in France. 1. Entire worm in lateral view. 2. Cephalic extremity in en face view. 3. Cephalic extremity in lateral view. 4. Caudal extremity in ventral view. 5. Caudal extremity in lateral view. Scale bars: 1 = 500 µm; 2 = 30 µm; 3–5 = 50 µm.**



Figures 6–10. ***Angiostoma spiridonovi*** **sp. n. female from** ***Limax flavus*** **in France. 6. Entire worm in lateral view. 7. Cephalic extremity in en face view. 8. Esophageal region, lateral view. 9. Caudal extremity in lateral view. 10. Caudal extremity in ventral view. Scale bars: 6 = 300 µm; 7 = 30 µm; 8–10 = 200 µm.**



Figures 11–17. ***Angiostoma spiridonovi*** **sp. n. from** ***Limax flavus*** **in France. 11–13. Larva removed from uterus of female. 11. Esophageal region in lateral view. 12. Middle region with genital primordium. 13. Caudal extremity in lateral view. 14. Vulvar region of the female. 15. Egg from uterus of female. 16. Spicule and gubernaculum of the male. 17. Transverse section of female through body. Scale bars: 11–13, 15, 16 = 50 µm; 14 = 100 µm; 17 = 200 µm.**

thin-shelled, containing embryos at all stages of development (Fig. 15).

LARVA (probably first-stage larva): Triangular oral opening. Cylindrical buccal cavity formed with a promesostoma, a metastoma without denticles, and a glottoid apparatus. Esophagus rhabditoid with swollen corpus, isthmus, and bulb without valves (Fig. 11). Phasmids and excretory pore not observed. Conical pointed tail (Fig. 13). Genital primordium consisting of few cells (Fig. 12). Length 637. Maximum width 28. Buccal cavity 18 long and 4 wide. Esophagus 180 long: corpus 110 long, isthmus 45 long, and bulb 25 long by 20 wide. Nerve ring 120 from cephalic extremity. Genital primordium 200 from cephalic extremity. Tail 97 long.

HOST: *Limax flavus* L., 1758.

SITE IN HOST: Pharynx.

LOCALITY: Rennes, Brittany, France.

DATE OF COLLECTION: December 1989.

SPECIMENS DEPOSITED: Laboratoire de Zoologie (Vers) Museum national d'Histoire naturelle, Paris. Holotype, allotype, and 4 paratypes. N° MNHN 118 HF.

ETYMOLOGY: The species name is dedicated to Dr. Sergueï Spiridonov.

Discussion

Six species of *Angiostoma* have been described previously: *Angiostoma limacis* Dujardin, 1845 (type species), from several species of Arionidae (*Arion ater, Arion circumscriptus, Arion silvaticus,* and *Arion subfuscus*) (Campana-Rouget and Théodoridès, 1956; Morand and Spiridonov, 1989); *Angiostoma stammeri* Mengert, 1953, from *Limax cinereoniger* and *Limax maximus*; *Angiostoma dentifera* Mengert, 1953, from *Limax cinereoniger*; and *Angiostoma aspersae* Morand, 1986, from *Helix aspersa* in Europe. *Angiostoma asamati* Spiridonov, 1985, was described from *Gigantomilax ferganus* in Central Asia and *Angiostoma plethodontis* Chitwood, 1935, from *Plethodon cinereus* in North America. The last species was collected from an amphibian, but it has been suggested (Adamson, 1986) that the salamander had acquired the infection by ingesting a parasitized mollusc.

Dujardin (1845) proposed *Angiostoma* for *A. limacis* and *A. entomelas*; the latter has been transferred to *Entomelas* Travassos, 1930. The diagnostic characters of the genus are: an enlarged buccal cavity, 6 elevations surrounding the oral opening, and a leptoderan rhabditoid bursa (Chitwood, 1933). The male bursa indicates that *A. spiridonovi* is an angiostomatid. Nevertheless, the species does not have the characteristic buccal cavity of the genus but most closely resembles free-living species of the genus *Rhabditis* (Andrassy, 1983).

The larva obtained from the uterus of a female worm has the same morphology as those from *A. aspersae* (Morand, 1989), *A. limacis,* and *A. dentifera* (Morand and Spiridonov, 1989).

The present species resembles *Angiostoma dentifera* by the triangular oral opening but differs by the absence of lateral alae in both sexes and by the number of caudal papillae in the male. *Angiostoma spiridonovi* sp. n. may be distinguished from *A. aspersae, A. plethodontis, A. asamati, A. stammeri,* and *A. limacis* in having a subtriangular oral opening, whereas these species exhibit a wide round oral opening. Also the number or the disposition of caudal papillae are different. The new species can also be differentiated from *A. plethodontis* and *A. limacis* by the presence of a metastomal apparatus.

The short and narrow buccal cavity of *A. spiridonovi,* which differs from those of all members of the genus, is an important character for the diagnosis of the species but not sufficient to erect a new genus. The genus *Angiostoma* is emended for *A. dentifera* and *A. spiridonovi*: oral opening round or subtriangular and buccal cavity large or narrow.

Acknowledgments

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SCHISTOSOMIASIS SYMPOSIUM

The Third SRP International Symposium on Schistosomiasis 14–18 February 1993, Cairo, Egypt

The Ministry of Health/USAID, Schistosomiasis Research Project (SRP) will hold its third international symposium on schistosomiasis, in Cairo, 14–18 February 1993.

Venue: Conference Hall, Nasr City, Cairo.

Registration: U.S. $150.00.

Deadline for receipt of abstracts: 31 August 1992.

Objectives of the conference:

To offer the large community of Egyptian scientists an update on the recent developments in all aspects of schistosomiasis research; and

To offer a forum at which the scientists funded by the SRP will present their results for discussion by international scientists in the field.

Program outline: The conference program will be based on the six SRP components:

—Vaccine development;
—Immuno-diagnosis;
—Chemotherapy;
—Epidemiology;
—Socio-economic aspects; and
—Operational research.

Participants: Speakers will include:

—SRP funded Egyptian scientists who will present the results of their research to date;
—U.S. collaborators with the SRP who will be invited to present results from their home laboratories;
—International scientists invited as keynote speakers in each of the six topics; and
—Free communications.

Posters: Poster facilities as an alternative to oral presentation will be available.

Social program: There will be a conference dinner, and post conference excursions to the Red Sea and Luxor/Aswan can be arranged on request.

For further details, please contact:

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Pattern of Apical Sensory Nerves in the Proboscis of *Macracanthorhynchus hirudinaceus* (Acanthocephala)

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ABSTRACT: The organization of the apical sensory organ in *Macracanthorhynchus hirudinaceus* includes a central core consisting of apical sensory nerves and a sensory support cell duct. Both nerves and duct terminate anteriorly in a pit or crateriform depression. About 30 μm posterior to the pit floor, the apical sensory nerves become quite pleomorphic. Simultaneously, they invade the sensory support cell duct. As the nerves move anteriorly, they branch repeatedly, whereas previously branched nerves are fusing. Some units remain on the outer surface of the sensory support cell duct while others remain enclosed by the duct. In either case, these combinations of nerves appear as thin sheets when viewed laterally. Those nerves on the outside periphery of the sensory support cell duct terminate near the apical pit and those enclosed by the duct become more nearly like thin cylinders that terminate in the walls of the pit.

KEY WORDS: Acanthocephala, nerves, proboscis, *Macracanthorhynchus hirudinaceus*.

The anterior terminus of the proboscis of *Macracanthorhynchus hirudinaceus* has a prominent cone-shaped elevation. This easily observed feature has generally been considered to serve in a sensory capacity. Dunagan and Miller (1983) reviewed the various terms that earlier authors had used in its description. Some of these terms such as "Tastpapille" suggest that this organ serves a chemosensory function. However, there is no experimental evidence to support this position. Nevertheless, circumstantial evidence, based on morphology, continues to suggest that this apical organ has a sensory role.

This study examines the relationship between apical sensory nerves and sensory support cell duct in the anterior terminus of the apical sensory organ of *M. hirudinaceus*.

Materials and Methods

Living worms were collected from pigs through the courtesy of Reelfoot Meat Packaging in Union City, Tennessee. Worms were transported to the laboratory in a Dewar flask containing intestinal contents. Detached specimens were rinsed in 30% seawater before fixing for 1 hr in a mixture of 2% glutaraldehyde and freshly prepared 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 2.0 mM EGTA and 1.0 mM $MgSO_4$. Specimens were then rinsed in 0.2 M cacodylate buffer at room temperature (RT) before being postfixed for 2 hr at RT in freshly prepared 1% OsO_4 and 1.5% $K_3Fe(CN)_6$. This procedure was followed by 3 20-min rinses in double-distilled water. Material was stained overnight in 1% aqueous uranyl acetate at 10°C. Specimens were dehydrated for 1–2 hr each in an ascending ethanol series (25, 50, 75, 95, 100%) followed by 3 20-min changes each in propylene oxide. The specimens were then infiltrated with increasing ratios (1:2, 1:1, 2:1) of propylene oxide : Spurr's epoxy resin over a 3-day period followed by 2 changes (1 day each) in pure Spurr's resin (1969). The specimens were then transferred into flat embedding molds, oriented as desired, and polymerized for 48 hr at 60°C. Serial sections were placed onto slot grids and examined in a Hitachi H500H transmission electron microscope operating at 50 kV.

Serial sections were cut at 80 nm thickness. Figures 5–42 depict every fifth section. Figure 4 is separated from Figure 5 by 560 nm and Figure 43 is separated from Figure 42 by 1.5 μm. The distance between Figure 4 and Figure 42 is 29.4 μm. The first section from each session of cutting was an orientation section of 1.4 μm in thickness.

Results

Posterior to the apical sensory core (Fig. 3), 2 anterior proboscis nerves, 2 sensory nerves, and a single sensory support cell duct are surrounded by muscles in the core of the proboscis. More anteriorly, this group encounters the apical sensory cone (ASC) where the anterior proboscis nerves divide and remain on the surface of the cone and the apical sensory nerves (SN) and sensory support cell duct (SSCD) enter the ASC (Fig. 2). One should note that while these last components are surrounded by the ASC they remain separated by 2 sets of membranes. In effect they are in a "hole" in the ASC. The SN and SSCD remain in the core of the ASC (Fig. 1) and eventually terminate in a pit on the apex of the proboscis. Approximately 30 μm (in the specimen shown) posterior to the pit, the well-defined SN and SSCD (Fig. 43) become progressively pleomorphic. The 2 nerves divide and redivide while becoming surrounded by the SSCD. This complex interaction is shown in Figures 4–43. (The

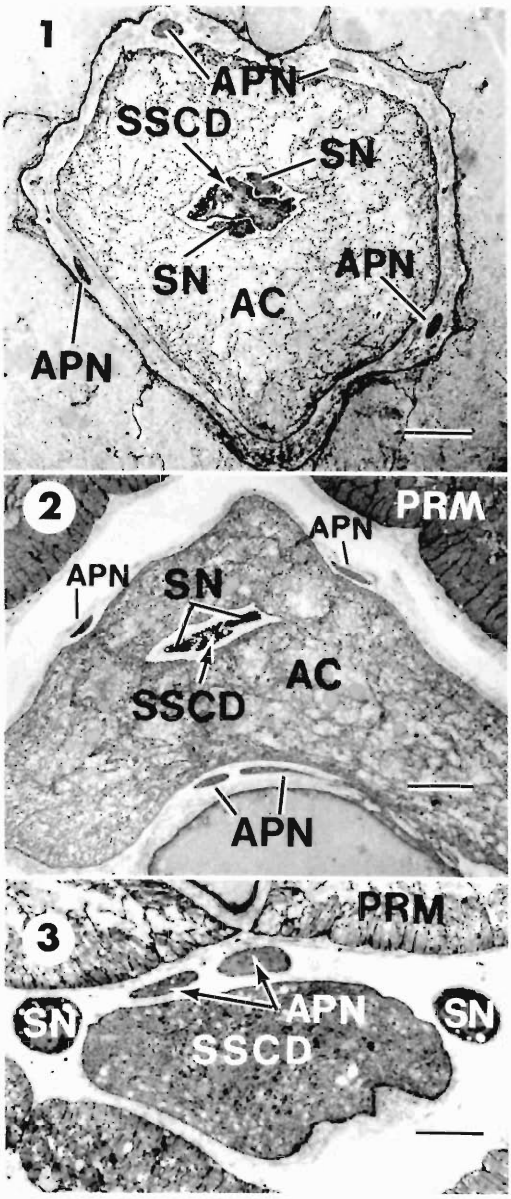
specimen is tilted slightly away from the viewer in the upper sections [Figs. 4–17] and results in an asymmetry in the pattern of nerves.) There is a constantly changing pattern in which the divided components unite only to divide once more. The individuality of the SN observed in Figure 43 is lost in this process. Eventually, they form discrete extensions (Figs. 5–13) going to the pit. Throughout this process there is close contact with the SSCD from the sensory support cell (stutzzelle) located adjacent to the cerebral ganglion.

Electron micrographs of different areas in the passage (Figs. 44–53) show the complexity of the events in this process. These pictures include the ASC through which the SN and SSCD are passing. Neither of the latter components reinvade the ASC in the process of passing through it. The ASC has not been included in the line drawings. Notice also that the SSCD has numerous fluid filled vesicles. These structures have not been included in the line drawings (Figs. 4–43).

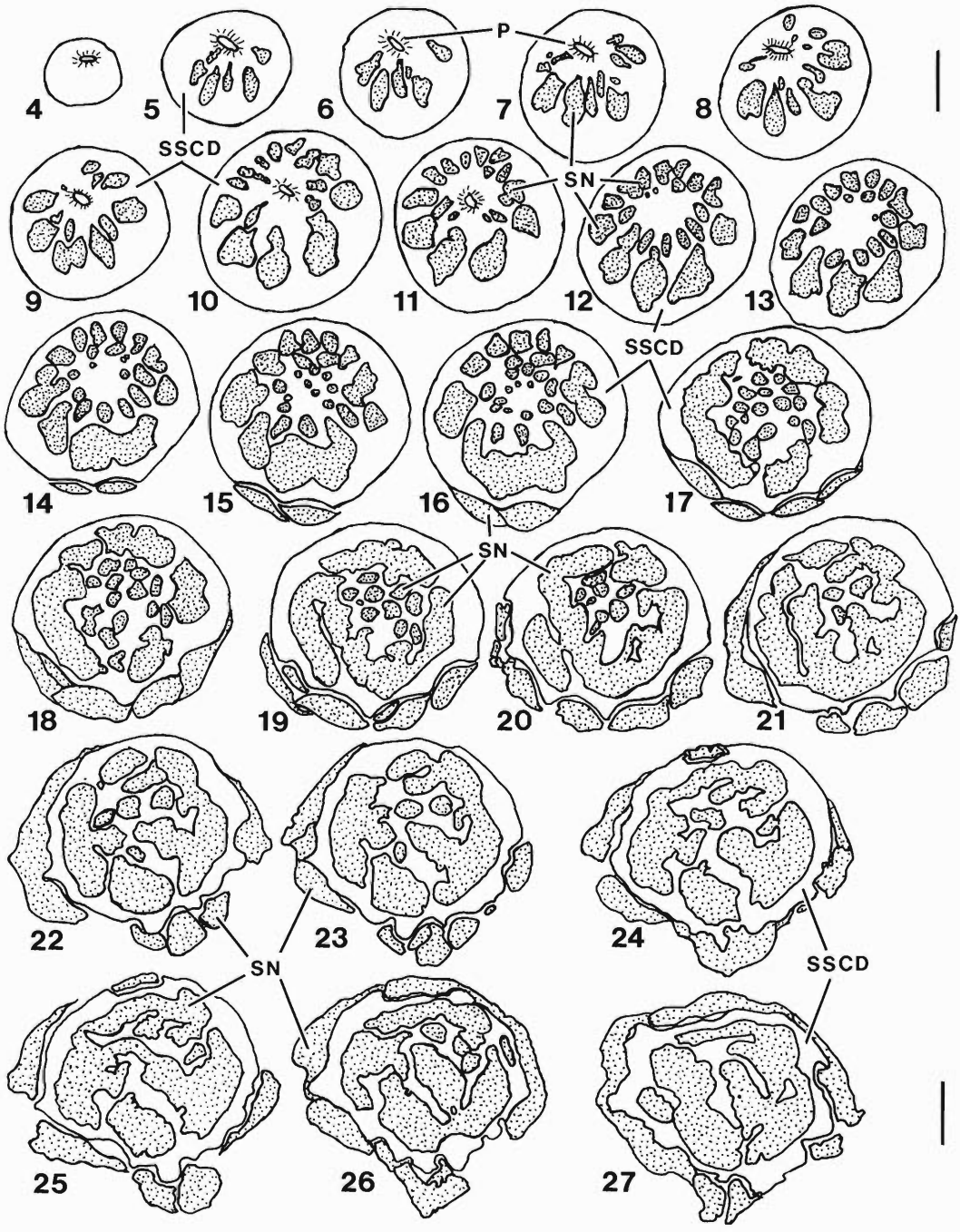
Discussion

Hyman (1951) indicated that the area beneath the pit had a coiled nerve with a fusiform ending. Rauther (1930, p. 458) stated that 2 fibers of the medial nerve wind convolutedly upward and enter the apical pore forming a sensory papilla. Kaiser (1893, part 2, p. 3) stated that a nerve (probably sensory) from the anterior part of the cerebral ganglion traveled between the retractor muscles to the proboscis tip where it ended. Kaiser (1893, part 2, pp. 8–9) expanded on this earlier statement by adding that in the apical sensory cone "two nerve fibers from the anterior medial nerve twist themselves into a thick ball . . . which represents a sensory papilla." It appears that Kaiser interpreted this papilla as a mechanoreceptor related to the eversion of proboscis hooks. In 1876, Leuckart, according to Harada (1931), observed that the anterior medial nerve connected with the tactile papillae on the proboscis tip. Dunagan and Miller (1983) outlined the apical sense organ including the point of entry of the sensory nerves and the branch pattern of the anterior proboscis nerves. However, these authors were unable to resolve the anterior terminal organization of these nerves.

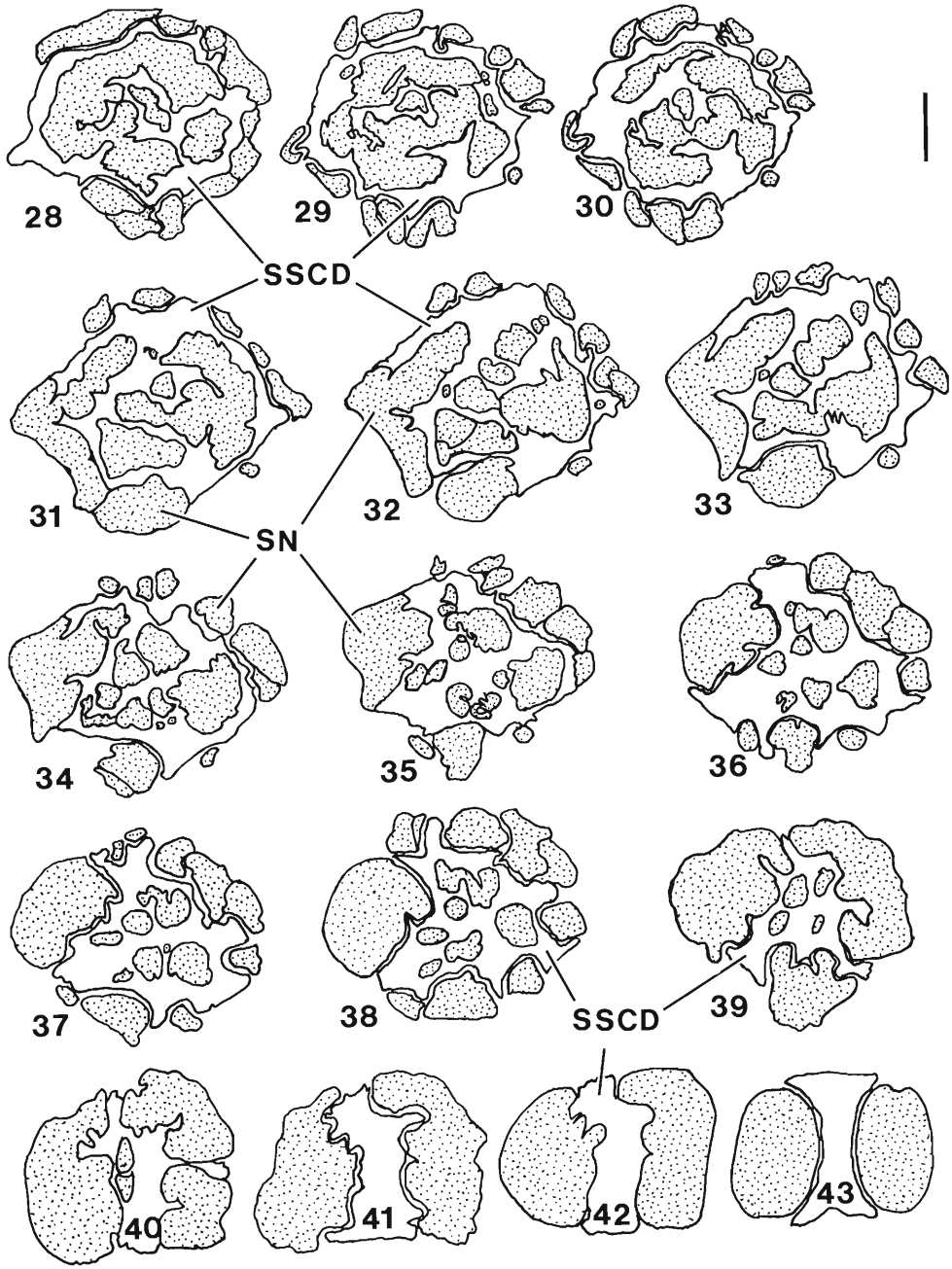
The figures presented herein depict an area of intense interaction of SN and SSCD in which the 2 original nerves branch many times throughout most of the anterior 30 μm of this system. Yet the branches do not remain separate but fuse in



Figures 1–3. Nerves and sensory support cell duct in proboscis of *Macracanthorhynchus hirudinaceus*. Abbreviations: apical sensory nerve (SN); anterior proboscis nerve (APN); apical sensory cone (AC); proboscis retractor muscles (PRM); sensory support cell duct (SSCD). 1. Pattern of nerves posterior to apical sensory cone. Scale bar = 8 μm. 2. Pattern of nerves immediately anterior to their entry into apical sensory cone. Scale bar = 10 μm. 3. Pattern of nerves at midlength in apical sensory cone. Scale bar = 2.3 μm.

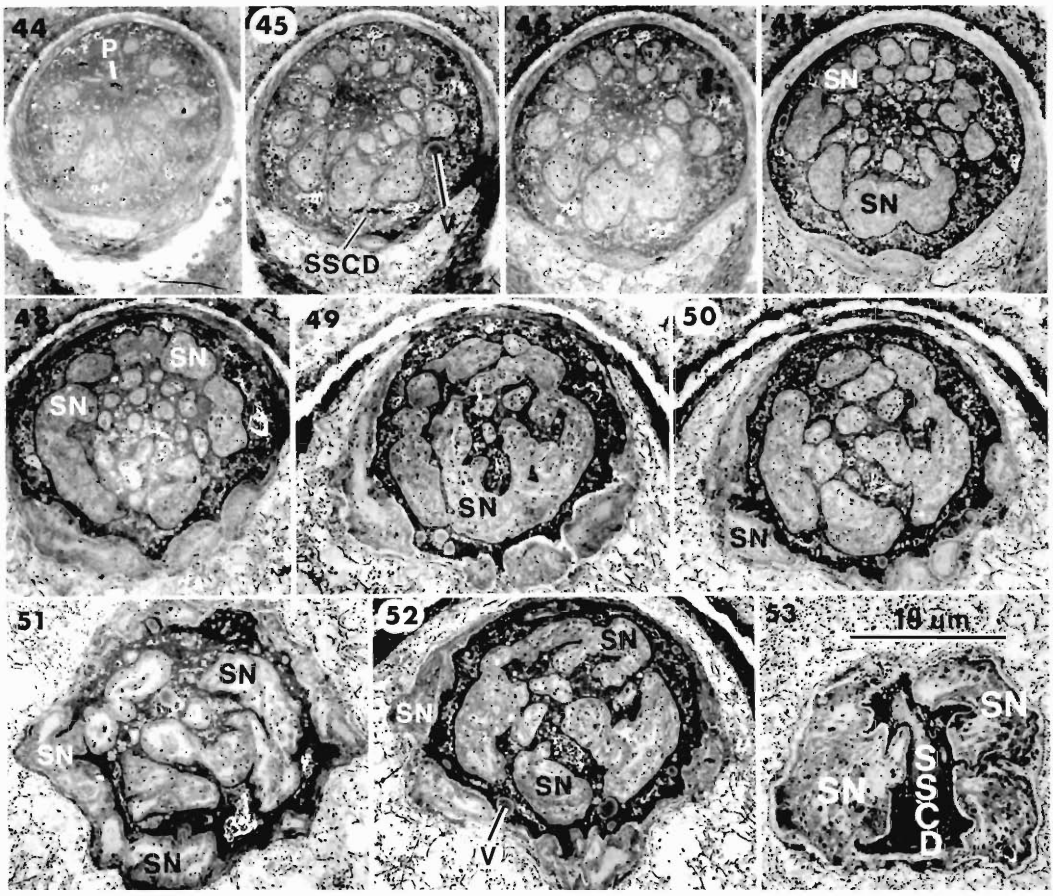


Figures 4–27. Illustrated cross sections of sensory (stippled area) and sensory support cell duct at anterior terminus of proboscis of *Macracanthorhynchus hirudinaceus*. Abbreviations: apical sensory nerve (SN); sensory support cell duct (SSCD); apical pitlike opening on anterior of proboscis (P). Note that the surrounding tissue of the apical sensory cone (see electron micrographs) has been omitted. Scale bar = 9.8 μm.



Figures 28–43. Illustrated cross sections of sensory nerves (stippled area) and sensory support cell duct at anterior terminus of proboscis of *Macracanthorhynchus hirudinaceus*. Abbreviations: apical sensory nerve (SN); sensory support cell duct (SSCD). Note that the surrounding tissue of the apical sensory cone (see electron micrographs) has been omitted. Scale bar = 9.8 μm.

unpredictable ways to form smaller numbers of larger units only to separate again into numerous branches near the pit of the anterior "papillae." We have completed 3 sets of serial sections on this area and the structural pattern (but not the exact points of branching) has remained the same. The failure of any of the SN components to interact with muscle suggests that these nerves have



Figures 44–53. Electron micrographs of core of apical sensory organ showing apical sensory cone enclosing apical sensory nerves and sensory support cell duct in proboscis of *Macracanthorhynchus hirudinaceus*. Abbreviations: apical sensory nerve (SN); apical pitlike opening on anterior of proboscis (P); sensory support cell duct (SSCD); vesicle (V). Scale bar = 19 µm.

nothing (directly) to do with hook movement or movement in other adjacent large muscle groups such as the proboscis retractors.

It is also clear that SN outside the SSCD terminate before the floor of the pit. The fact that some SN remain outside the SSCD and pit area suggests that the SN may be multifunctional.

Acknowledgments

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Acanthamoeba jacobsi sp. n. (Protozoa: Acanthamoebidae) from Sewage Contaminated Ocean Sediments

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ABSTRACT: A temperature-tolerant strain of *Acanthamoeba,* isolated from sewage contaminated ocean sediments, was characterized by isoenzyme analysis, mouse pathogenicity tests, and phase contrast microscopy and found to represent a new species. Intranasal inoculation of 10-g weanling mice killed 3/10 of them, and amebae were cultured from brain tissue. The new isolate, *Acanthamoeba jacobsi* sp. n., is described.

KEY WORDS: *Acanthamoeba,* Protozoa, sewage wastes.

Ocean sediments collected in 1974 from the discontinued New York 12-mile sewage disposal site yielded a temperature-tolerant strain of *Acanthamoeba* when cultured on freshwater agar medium (Sawyer et al., 1977). The new strain formed round cysts with an ectocyst wall that appeared to be smooth at low magnification, but rippled when viewed with an oil immersion objective, or with the electron microscope (Sawyer et al., 1987). At the time of original isolation, *Acanthamoeba culbertsoni* Singh and Das, 1970, was the only known species within the genus to form round cysts with a thin and delicate rippled ectocyst wall, to grow at mammalian body temperatures, and to kill experimentally infected laboratory animals (Culbertson et al., 1959; Singh and Das, 1970). Characteristics shared by the marine strain (Sawyer et al., 1977; Daggett et al., 1982) and *A. culbertsoni* led us to identify it tentatively as *A. culbertsoni.* Subsequent comparative studies using isoenzyme electrophoresis, morphology, and growth characteristics have shown sufficient differences between the Lilly A-1 type strain of *A. culbertsoni* (ATCC 30171) and the marine strain (ATCC 30732) to justify a new species designation for the latter. The new strain is designated *Acanthamoeba jacobsi* sp. n., in honor of Dr. Leon Jacobs (retired), former Director, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. Dr. Jacobs was one of the earliest investigators to recognize cysts of *Acanthamoeba* in cultures of monkey kidney cells (Jahnes et al., 1957).

Materials and Methods

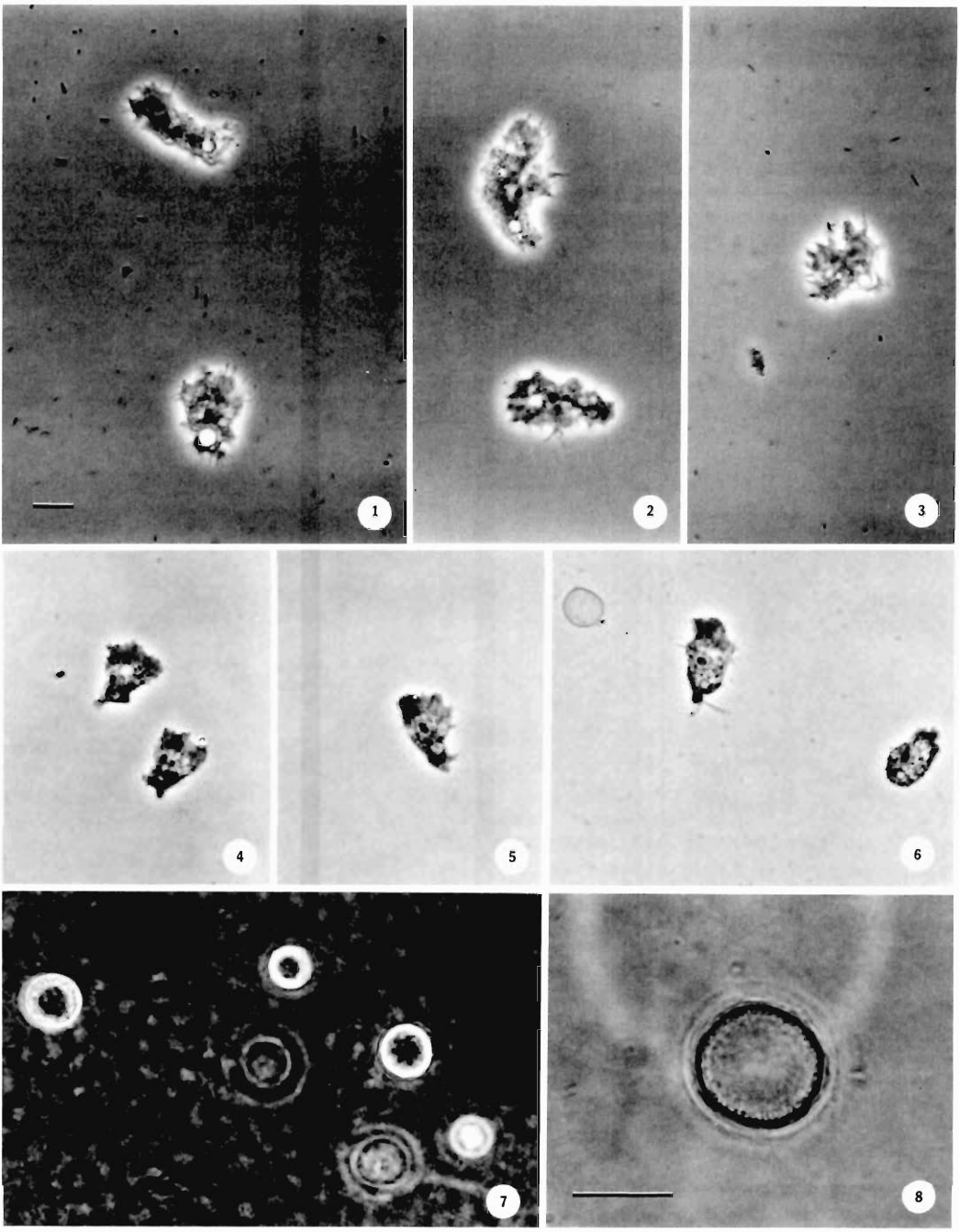
Bottom sediments were taken from the center of a near shore ocean sewage disposal site designated by the U.S. Environmental Protection Agency as the New York 12-mile Disposal Site. Sediments were taken with a Smith-McIntyre bottom grab and sampled by removing the top 1 cm with a sterile wooden tongue depressor as described earlier (Sawyer et al., 1987). Samples were taken in triplicate, stored in sterile plastic bags under refrigeration, and returned to the laboratory for culture studies. Six replicate cultures (2 each of 3 subsamples) were prepared by streaking one microbiological loopful of sediment on each of the 6 plates. Agar medium prepared by dissolving 0.1 g malt extract, 0.1 g yeast extract, and 15 g Difco agar in 1 liter distilled water, and sterilized by autoclaving, was used to discourage the growth of marine organisms otherwise requiring seawater medium. The surface of each agar plate was streaked with *Klebsiella pneumoniae* (ATCC 27889) as a food source for the amebae. Culture plates were inverted and stored in plastic boxes lined with moist paper towels; 3 cultures were incubated at room temperature and 3 at 38°–39°C.

One temperature-tolerant strain was isolated, cloned, and designated strain 31-B. Whole cell extracts of the new strain, and strain A-1 (ATCC 30171), were prepared and acid phosphatase (AP), leucine amino peptidase (LAP), and propionyl esterase (PE) isoenzymes were assayed using the method described by Nerad and Daggett (1979). Pathogenicity tests were carried out by instilling approximately 1×10^5 amebae suspended in 0.01 ml of Page's Saline (1988) intranasally into each of 25 20-g mice, 13 10-g mice, and 3 controls. Mice that died were necropsied and small fragments of brain tissue streaked on agar plates to test for the presence of amebae.

Living trophozoites and cysts were measured ($N = 25$) using phase contrast objectives. Trophozoites were also measured ($N = 25$) after staining with nuclear red (Kernechtrot) according to Page (1988). All measurements are given in micrometers.

Results

Cultures incubated at room temperature and at 38°–39°C were positive for cyst-forming amebae within 3 days of preparation. Encysted amebae were noted at all depths within the agar medium indicating that trophozoites had migrated



Figures 1–8. Trophozoites and cysts of *Acanthamoeba jacobsi* sp. n. Scale bar = 10 μm. For Figures 1–7, scale bar in Figure 1 applies. 1–3. Living trophozoites, phase contrast. Note phase halo effect and loss of nuclear detail due to contraction in response to light from microscope. 4–6. Trophozoites fixed in Nissembaum solution and stained with nuclear red, phase contrast. Note retraction of filose pseudopodia due to contraction during fixation, and uncollapsed empty cyst in Figure 6. 7. Living cysts on agar block, phase contrast. Note delicate rippled ectocyst and unfocused cysts at different depths in the agar medium. 8. Living cyst on agar, phase contrast. Photograph taken with 100× phase objective (oil), and phase ring in high dry position #2 to emphasize spherical contour of endocyst and delicate thin and rippled ectocyst.

throughout the agar matrix. Examination of cysts in wet mounts with 10×, 20×, and 40× phase contrast objectives indicated that there was little or no evidence of the irregular or rippled wall characteristic of amebae belonging to the genus *Acanthamoeba.* Examination with an oil immersion 100× objective, however, showed that a thin-rippled wall was present. Evidence of the wall was noted when small rectangular blocks of agar were removed from cultures and covered with a cover glass for study at 40×, probably due to slight pressure from the cover glass. Trophozoites had transitional acanthopodia characteristic of the genus when observed in wet mounts or hanging drops. Amebae contracted rapidly in the presence of light from the microscope and rarely could be photographed while undergoing typical locomotion (Figs. 1–3). Specimens measured under reduced light ranged 25.0–37.5 long × 10.0–17.5 wide (mean = 31.3 × 14.7). Stained specimens (Figs. 4–6) were smaller, probably due to fixation, ranging 15.0–27.0 long × 6.0–11.0 wide (mean = 21.0 × 8.6). Live cysts (Figs. 7, 8) had a spherical endocyst with a thin-rippled wall, and measured 12.5–17.5 in diameter. Cysts were present at all depths in the agar medium (Fig. 7).

The AP, LAP, and PE zymograms of *Acanthamoeba jacobsi* were distinct from the A-1 strain of *A. culbertsoni* (Fig. 9). With the possible exception of 1 PE isoenzyme band, no bands were shared between the strains.

None of the 20-g mice showed signs of infection during a 2-mo period of observation. Among the 10-g mice, however, 3/13 died within 5–24 days postinoculation. Control mice did not show evidence of infection. Cultures of brain tissue from 2/3 infected mice yielded *A. jacobsi.*

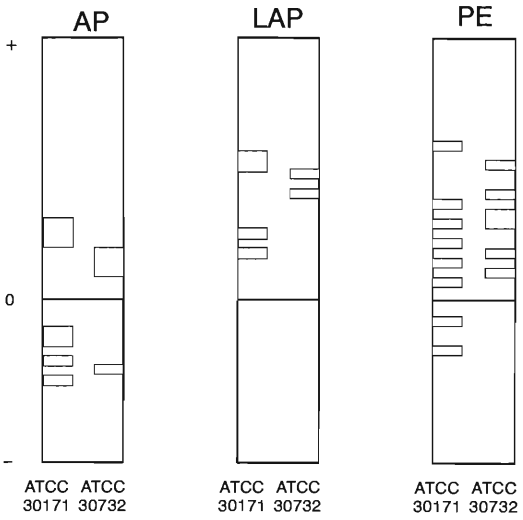


Figure 9. AP (alkaline phosphatase), LAP (leucine amino peptidase), and PE (propionyl esterase) zymograms of *A. culbertsoni* strain A-1 (ATCC 30171) and *A. jacobsi* sp. n. (ATCC 30732).

Description

Acanthamoeba jacobsi sp. n.

DIAGNOSIS: Trophozoites (Figs. 1–6) typical of the genus *Acanthamoeba,* 25.0–37.0 × 10.0–17.5 (mean = 31.3 × 14.7) in the living condition, and 15.0–27.0 × 6.0–11.0 (mean = 21.0 × 8.6) after fixation with Nissembaum's solution and staining with nuclear red. Amebae contract rapidly in the presence of light from the microscope lamp unless viewed with reduced or filtered light. Nucleus in stained specimens 4.0, nucleolus 2.5. Fine bristlelike pseudopodia transitional and arising randomly at all angles from the body surface. Pronounced posterior contractile vacuole. Cyst with a spherical endocyst closely appressed to the thin-rippled ectocyst wall. Cysts and trophozoites present at all depths within the agar medium. Excystment through an ostiole in the cyst wall; ostiole not evident by light microscopy. Growth at temperatures up to 38°–40°C. Amebae mildly pathogenic to weanling laboratory mice and culturable from brain tissue.

TYPE LOCALITY: Bottom sediments from an ocean sewage disposal site designated "New York 12-mile Site" in the New York Bight near Coney Island, approximately 40°27′ latitude and 73°45′ longitude.

TYPE SPECIMENS: Deposited at the American Type Culture Collection, Rockville, Maryland as strain ATCC 30732.

Discussion

Acanthamoeba jacobsi sp. n. was isolated in 1974 when *A. culbertsoni* and *A. palestinensis* were the only known members of the genus to form spherical cysts with a delicate sculptured or rippled ectocyst wall. The 2 species were readily distinguished by the more pronounced ectocyst wall and larger size of cysts of *A. palestinensis,* and its failure to grow at 37°C or higher. Strain 31-B seemingly had most of the features attributed to *A. culbertsoni* at the time, and was discussed and illustrated as such in 3 separate publications (Sawyer et al., 1977; Daggett et al.,

1982; Sawyer et al., 1987). Sawyer et al. (1987) designated strain 31-B simply as *Acanthamoeba* sp. 1 in 1 publication that illustrated the trophozoite and cyst as seen with the electron microscope. The cyst of *A. jacobsi* had a single ostiole, and the nucleus of the trophozoite was smooth-walled without evidence of pores in the nuclear membrane. Although *A. jacobsi* was pathogenic only to young 10-g mice, the recovery of amebae from brain tissue suggested that serial passage might lead to increased virulence.

Precise identification of strains of *Acanthamoeba,* especially those forming round cysts, has been complicated by the recent description of other new species, i.e., *A. lenticulata* Molet and Ermolieff-Braun, 1976, and *A. royreba* Willaert, Stevens, and Tyndall, 1978. Pussard and Pons (1977) proposed that all species of *Acanthamoeba* be placed within 1 of 3 groups on the basis of similar cyst morphology. Accordingly, *A. jacobsi* would be placed in group III, which includes those species with a round endocyst and a thinly rippled or wrinkled ectocyst wall. Careful study of all species included in group III has shown that cysts, except for those of *A. jacobsi,* may range from spherical to slightly angular or irregular, especially when densely crowded on agar culture plates. Isoenzyme studies on *A. jacobsi, A. culbertsoni, A. palestinensis, A. lenticulata,* and *A. royreba* have shown that they are distinct on the basis of zymograms and are valid species. Two other species of amebae, which also have round cysts, *A. glebae* and *A. invadens,* were previously included in the genus *Acanthamoeba.* Page (1988) stated that both species excyst by dissolution of the cyst wall rather than through an exit pore, and placed them within the genus *Protacanthamoeba* Page, 1981. The distinct pore, or ostiole, in the cyst wall of *A. jacobsi,* as seen with the electron microscope, clearly excludes this species from the genus *Protacanthamoeba.*

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Research Note

Sarcocystis felis sp. n. (Protozoa: Sarcocystidae) from the Bobcat (*Felis rufus*)

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ABSTRACT: *Sarcocystis felis* sp. n. was found in striated muscles of 4 of 6 bobcats (*Felis rufus*) from Arkansas. Sarcocysts were up to 2.1 mm long and up to 150 μm wide. The sarcocyst wall was about 1-μm thick and contained hobnaillike bumps and elongated villi. Bradyzoites were about 10 × 1.5 μm. *Sarcocystis felis* was structurally similar to sarcocysts from panthers, cougars, and domestic cats.

KEY WORDS: bobcat, *Felis rufus*, sarcocysts, *Sarcocystis felis*, panthers, cougars, cats.

Sarcocystis spp. undergo a 2-host life cycle involving prey and predator animals. Infection of muscles by *Sarcocystis* spp. is common in herbivores but rare in carnivores (Dubey et al., 1989). Sarcocysts have been reported in muscles of domestic cats (*Felis domesticus*) (Kirkpatrick et al., 1986; Everitt et al., 1987; Edwards et al., 1988; Fiori and Lowndes, 1988; Hill et al., 1988) from the U.S., in a leopard (*Panthera pardus*) and a lion (Bhatavedkar and Purohit, 1963; Somvanshi et al., 1987) from India, in Florida panthers (*Felis concolor coryi*) and cougars (*Felis concolor stanleyana*) from Florida and Texas (Greiner et al., 1989), and from Florida bobcats (*Felis rufus floridanus*) (Anderson et al., 1992), and a cougar from the National Zoo in Washington, D.C. (Kluge, 1967). In the present paper we describe sarcocysts in muscles of bobcats (*Felis rufus*).

Specimens of heart, esophagus, tongue, diaphragm, and masseter muscles of 6 (4 males and 2 females) bobcats from southwestern Arkansas were fixed in 10% neutral buffered formalin. Paraffin-embedded sections were cut at 5 μm thickness and examined after staining with hematoxylin and eosin. Formalin-fixed muscles from tongue were postfixed in osmium and processed for transmission electron microscopy. Sarcocysts were found in 4 bobcats (heart and tongue of 1, tongues alone of 2, masseter muscles of 1 and esophagus of 1). All measurements are in micrometers unless stated otherwise.

Sarcocystis felis sp. n. (Figs. 1–4)

DIAGNOSIS: Sarcocysts from tongue up to 2.1 mm long and up to 150 wide; cyst wall 1.0–1.5 thick with 0.4–1.2 long fingerlike villar projections (Figs. 1–3). Parasitophorous vacuole membrane (PVM) of the primary cyst wall folded into short hobnaillike bumps and villar projections 0.6–1.2 long × 0.3–0.4 wide at uneven distances, villi without microtubules, PVM including villi and hobnail bumps lined by 66-nm electron dense thick layer (Fig. 3). Ground substance 0.7–1.0 thick with amorphous material and few electron dense granules. Septa 0.1–0.2 thick, bradyzoites 7.0–10.0 × 1.5 long × 1.5–2.0 wide, micronemes in anterior (apical) third portion, nucleus terminal (Fig. 4).

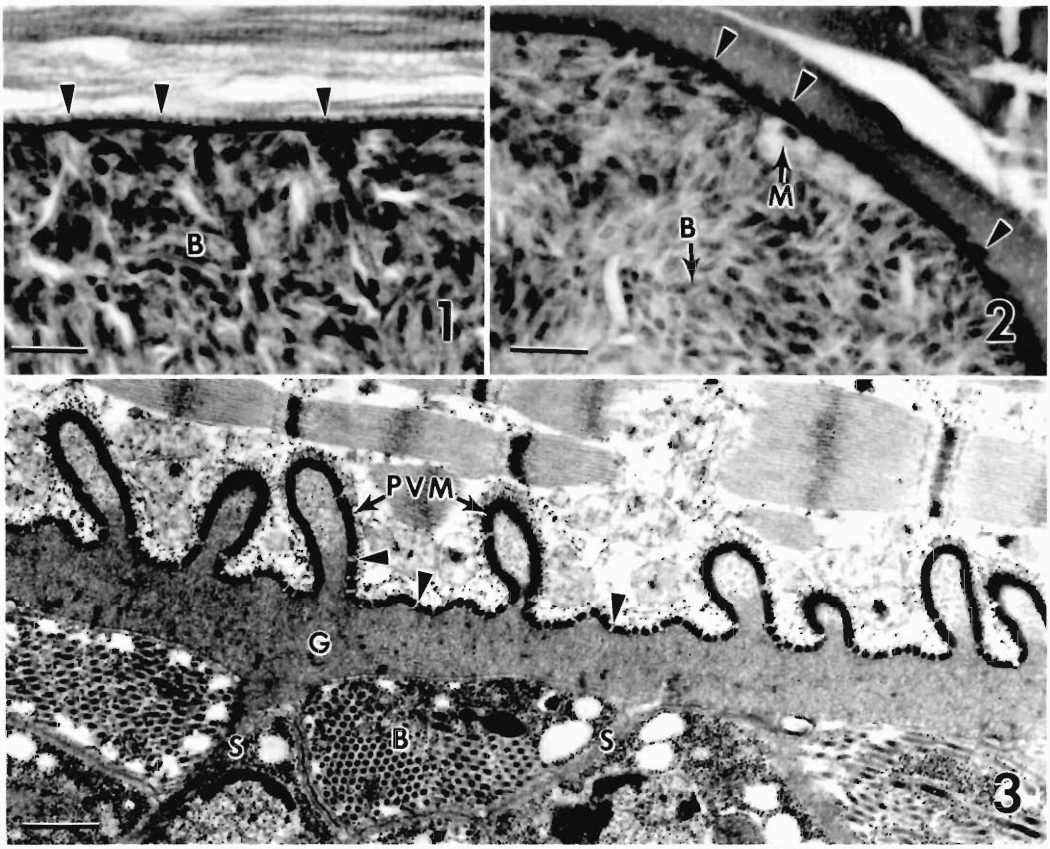
TYPE HOST: *Felis rufus* (bobcat). Other hosts: *Felis domesticus* (domestic cat), *Felis concolor coryi* (Florida panther), and *Felis concolor stanleyana* (cougar).

DISTRIBUTION: North America.

SYNTYPE: Section of tongue from the bobcat deposited in U.S. National Museum. USNM No. 82095.

Sarcocystis species are generally host specific. The structure of the sarcocyst wall is considered a reliable criterion for distinguishing *Sarcocystis* species within a given host (Dubey et al., 1989). Based on the structure of the sarcocyst wall, Du-

† Deceased.



Figures 1–3. ***Sarcocystis felis*** **sp. n. sarcocysts in tongue muscles of naturally infected bobcat. 1, 2. Light microscope photomicrographs of sarcocysts in longitudinal (Fig. 1) and oblique (Fig. 2) sections. Arrowheads point to villar projections on the cyst wall. Pale staining metrocytes (M) and banana-shaped bradyzoites are enclosed in the cyst. Hematoxylin and eosin stain. Bar = 10 μm. 3. Transmission electron micrograph of the cyst wall. Note differences in length of the villar projections and electron dense lining under the parasitophorous vacuole membrane (PVM). The electron dense lining is interrupted (arrowheads) both in the villar projections as well as in nonvillar portions. The ground substance (GS) lacks microtubules. Septa (S) arise from the GS. Bradyzoites (B) are juxtaposed under the GS. Bar = 0.66 μm.**

bey et al. (1989) grouped *Sarcocystis* species into 24 types. The ultrastructure of *S. felis* most closely resembles the type 9 sarcocysts occurring in rodents and voles (Dubey et al., 1989). However, in type 9 sarcocysts the villi have microtubules and villi taper at the free end.

The structure of sarcocysts from the bobcat resembles sarcocysts from domestic cats (Kirkpatrick et al., 1986; Everitt et al., 1987; Edwards et al., 1988; Fiori and Lowndes, 1988; Hill et al., 1988), panthers and cougars (Greiner et al., 1989), and Florida bobcats (Anderson et al., 1992). These *Sarcocystis* species from the Felidae have not been named because they were considered to be rare and their life cycles unknown. However, the present study and that of Greiner et al. (1989) and Anderson et al. (1992) indicate that sarcocysts in cats are not rare; collectively they have been found in 4 of 4 cougars, 11 of 14 panthers, and 4 of 6 bobcats (present study), and 30 of 60 Florida bobcats. Structurally, only 1 type of sarcocyst has been seen in bobcats, domestic cats, panthers, and cougars. Although the name *Sarcocystis felis* is proposed for the species in bobcats, the same species probably occurs in other Felidae. Nothing is known of the life cycle of these sarcocysts in Felidae; however, it most likely involves a predator or scavenger feeding on these cats (Greiner et al., 1989).

This study was in part supported by a grant

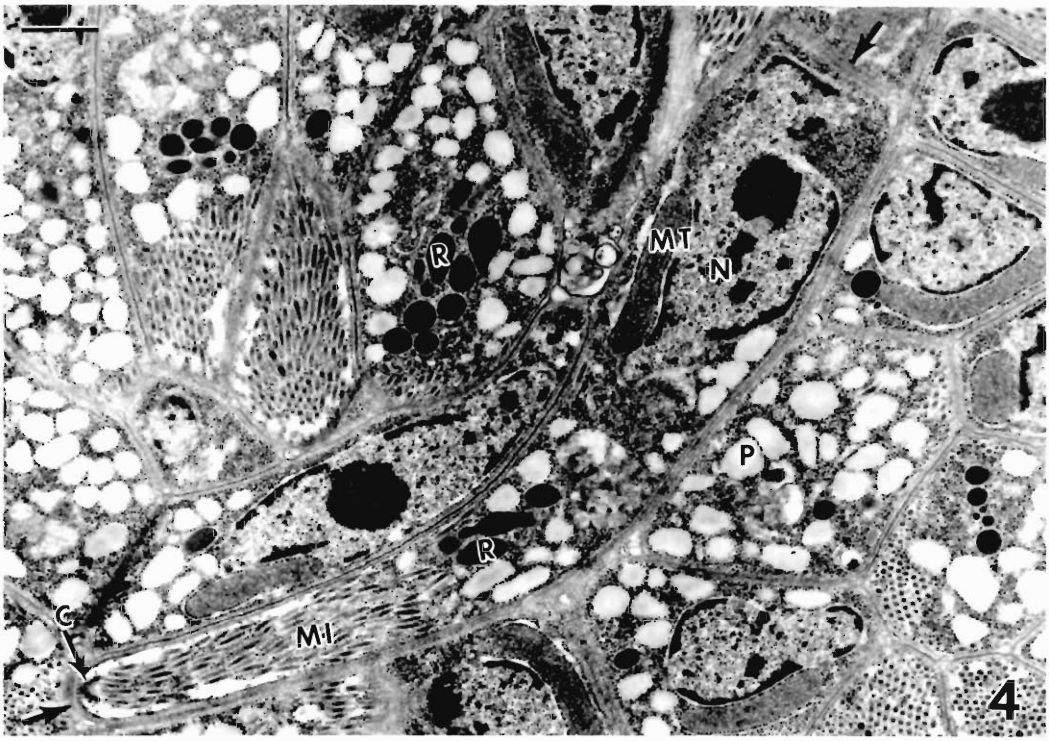


Figure 4. Transmission electron micrograph of bradyzoites of *Sarcocystis felis* sp. n. One bradyzoite (arrows) is cut longitudinally. Note an anteriorly located conoid (C), numerous micronemes (MI), few rhoptries (R), single mitochondrion (MT), and a posteriorly located nucleus (N). Bar = 0.76 μm.

from the commonwealth of Pennsylvania, Department of Agriculture.

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Research Note

Gastrointestinal Helminths of the Southwestern Earless Lizard, *Cophosaurus texanus scitulus,* and the Speckled Earless Lizard, *Holbrookia maculata approximans* (Phrynosomatidae)

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ABSTRACT: Fifty-three *Cophosaurus texanus scitulus* from Pima County, Arizona, and 15 *Holbrookia maculata approximans* from Cochise County, Arizona, were examined for helminths. One nematode species, *Thubunaea iguanae* (prevalence 8%), 1 cestode species, *Oochoristica* sp. (prevalence 2%), and a juvenile acanthocephalan, *Acanthocephalus* sp. (prevalence 2%) were recovered from *C. texanus scitulus* (helminth prevalence 11%). All are new host records. Two nematode species, *Atractis penneri* (prevalence 13%) and *Physaloptera* sp. (prevalence 7%), were recovered from *H. maculata approximans* (helminth prevalence 13%).

KEY WORDS: Cestoda, *Oochoristica* sp., Nematoda, *Thubunaea iguanae, Atractis penneri, Physaloptera* sp., Acanthocephala, *Acanthocephalus* sp., *Cophosaurus texanus scitulus, Holbrookia maculata approximans,* Phrynosomatidae, prevalence, intensity.

The greater earless lizard, *Cophosaurus texanus* Troschel, 1850, ranges from central Arizona through central and south Texas into Mexico; the lesser earless lizard, *Holbrookia maculata* Girard, 1851, ranges from southern South Dakota, south to Guanajuato, Mexico, and from western Arizona, east to the Great Plains (Stebbins, 1985). To our knowledge, there are 3 previous reports of helminths in *C. texanus* (Gambino, 1958; Specian and Ubelaker, 1974; McAllister, 1988) and 2 reports for *H. maculata* (Gambino, 1958; Gambino and Heyneman, 1960). In this note, we report the results of a helminth survey of 2 subspecies, the southwestern earless lizard, *Cophosaurus texanus scitulus* (Peters, 1951), and the speckled earless lizard, *Holbrookia maculata approximans* Baird, 1858.

We examined 53 *C. texanus scitulus* (mean snout–vent length, SVL ± SD = 60 mm ± 8.1, range 40–71 mm) from Sabino Canyon (elevation 883 m), Santa Catalina Mountains, west of Tucson, Pima County, Arizona (32°20′N, 110°49′W). Thirty-seven were collected in 1966, 10 in 1967, and 6 in 1969. Fifteen *H. maculata approximans* (mean SVL = 51 mm ± 6.6, range 39–60 mm) from the southern edge of Willcox, Cochise County, Arizona (32°14′N, 109°50′W, elevation 1,269 m) were also examined. Ten were collected in 1966 and 5 in 1967. Lizards were shot with 22 caliber dust shot and preserved in Bouin's fixative. They were later stored in ethyl alcohol. In 1991, each abdomen was opened and the esophagus, stomach, and small and large intestines were examined. Each organ was slit longitudinally and examined under a dissecting microscope. The liver and body cavity were also examined. Helminths were identified using glycerol wet mounts. The cestodes and the acanthocephalan were stained with Delafield's hematoxylin and mounted in Canada balsam. Voucher specimens were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): *Oochoristica* sp. (81959), *Thubunaea iguanae* (81958), *Acanthocephalus* sp. (81960), *Atractis penneri* (82096), and *Physaloptera* sp. (82097).

Six *C. texanus scitulus* were infected with helminths (prevalence 11%). One male lizard harbored 8 third-stage *Thubunaea iguanae* Telford, 1965 (prevalence 2%) in the esophagus, and 3 female lizards contained 4 adult female *T. iguanae* (prevalence 6%; mean intensity 1.3) in the stomach. One male lizard contained 3 *Oochoristica* sp. Lühe, 1898 (prevalence 2%) in the small intestines. One female lizard harbored 1 juvenile acanthocephalan *Acanthocephalus* sp. Koelreuther, 1771 (prevalence 2%) in the small intestines. All are new host records.

Two *H. maculata approximans* were infected with helminths (prevalence 13%); *Atractis penneri* (Gambino, 1957) (prevalence, 13%) and *Physaloptera* sp. Rudolphi, 1819 (prevalence, 7%). One female lizard harbored 31 *A. penneri* in the large intestine. One male contained 91 *A. penneri* in the large intestine as well as a concurrent stomach infection of 4 third-stage *Physaloptera* sp. The recovery of *Physaloptera* sp. represents a new host record.

Two species of nematodes have been previously reported from *C. texanus*. Gambino (1958)

reported 2 of 39 *C. texanus* to be infected with *Atractis penneri.* Specian and Ubelaker (1974) found *C. texanus* to be a host for *Parathelandros texanus* Specian and Ubelaker, 1974, but gave no additional details. *Thubunaea iguanae* is the third nematode species to be recovered from *C. texanus.*

One species of nematode has been previously reported from *H. maculata.* Gambino and Heyneman (1960) reported 23 of 48 *H. maculata* to be infected with *A. penneri. Physaloptera* sp. is the second nematode species to be recovered from *H. maculata.*

One species of cestode has been previously reported from *Cophosaurus texanus texanus.* McAllister (1988) reported 1 of 21 *C. texanus texanus* to be infected with *Mesocestoides* sp. Vaillant, 1863. *Oochoristica* sp. is the second cestode species to be recovered from *C. texanus.*

Although juvenile acanthocephalans have been reported occasionally from lizards collected in Arizona, this is apparently the first report of acanthocephalans from *C. texanus.* Benes (1985) reported 4 larval acanthocephalans from the coelom of 1 *Cnemidophorus tigris septentrionalis.* Goldberg and Bursey (1990a) found a juvenile acanthocephalan among the stomach contents of 1 *Cnemidophorus uniparens.* On another occasion, Goldberg and Bursey (1990b) recovered 3 unattached juveniles in the small intestines of 3 *Sceloporus jarrovii jarrovii.*

We thank Rana Tawil for assistance in removal of parasites.

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Research Note

Grenacher's Borax Carmine for Staining Nematodes Inside Insects

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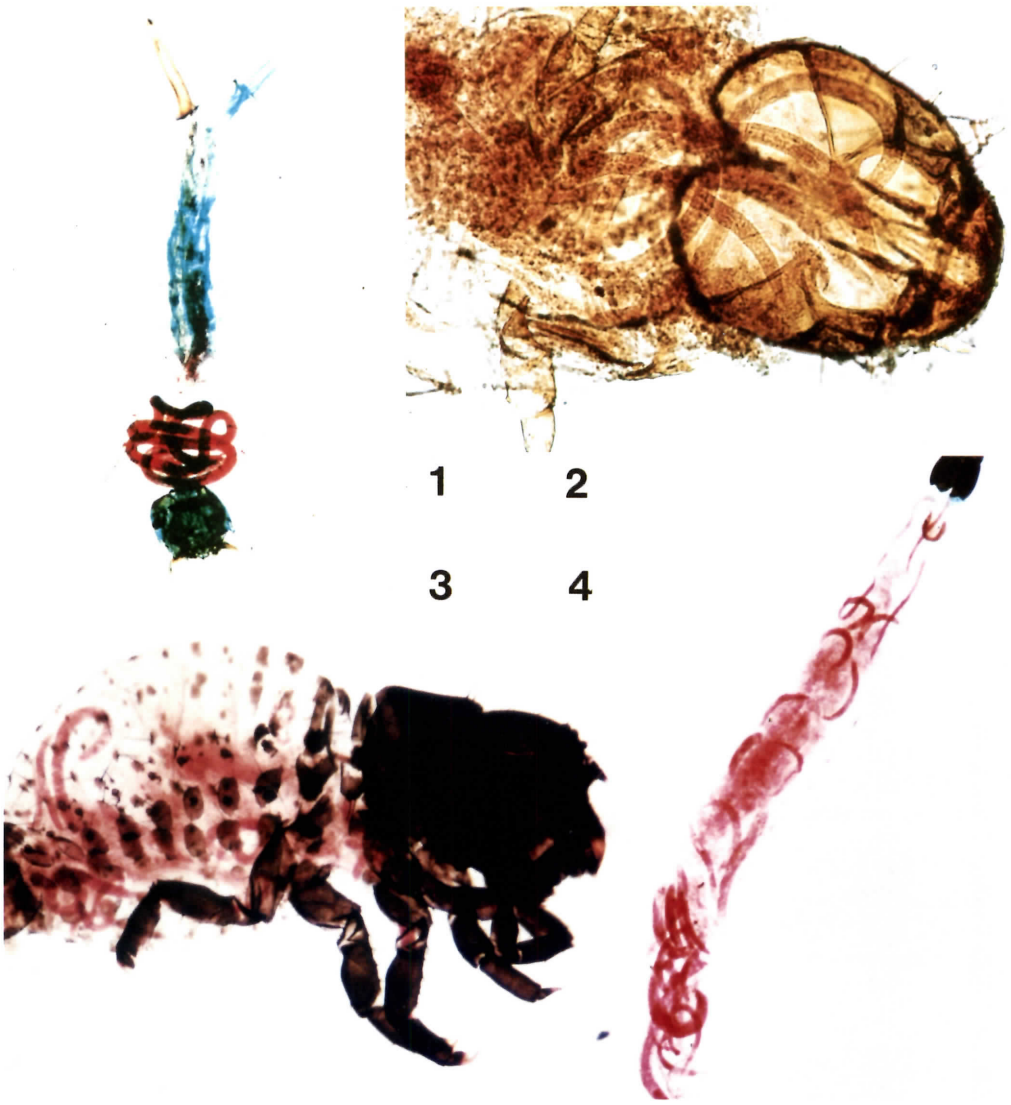
ABSTRACT: Grenacher's borax carmine stain was used to stain nematode parasites inside insects. This simple and useful procedure is described in detail. Examples of mermithid, *Romanomermis culicivorax,* and steinernematid nematodes, *Steinernema carpocapsae* and *S. feltiae,* stained well. The insects used were larval stages of both dipteran and coleopteran insect pests.

KEY WORDS: Nematoda, insect parasites, staining method, Grenacher's borax carmine, *Romanomermis culicivorax, Steinernema carpocapsae, Steinernema feltiae.*

During investigations on biological control of pest insects using nematodes, a need developed to show presence, size, and locations of nematodes inside bodies of insects. Usually insect lar-



Figures 1–4. Light micrographs of insect larvae parasitized by nematodes and stained with borax carmine. 1. The mosquito larva, *Culex pipiens quinquefasciatus,* parasitized by the mermithid nematode, *Romanomermis culicivorax.* 2. The western corn rootworm larva, *Diabrotica virgifera virgifera,* parasitized by the steinernematid nematode, *Steinernema carpocapsae.* 3. The Colorado potato beetle larva, *Leptinotarsa decemlineata,* parasitized by the steinernematid nematode, *Steinernema carpocapsae.* 4. The mushroom fly larva, *Lycoriella mali* parasitized by the steinernematid nematode, *Steinernema feltiae.*

vae are opaque because of high fat content. Therefore a technique was developed to clear the parasitized insect and to stain and destain nematode parasites.

The basic stain used in this procedure was the alcoholic borax carmine of Grenacher (1879) prepared using the recipe of Davenport (1960): 1.0 g carmine, 2.0 g borax, and 50.0 ml water.

Boil in a covered vessel for 30 min, or until the carmine dissolves; then add 50 ml of 70% ethyl alcohol (ETOH). Allow the solution to stand 1–2 days and filter through filter paper. The filtrate is then ready for use.

1. *Staining.* Begin procedure with parasitized insect larvae that have been fixed in 70% ETOH for at least 24 hr. Small holes made with a fine needle in bodies of corn rootworm and potato beetle let the stain go in efficiently. Transfer spec-

imens directly from 70% ETOH into Grenacher's borax carmine and leave overnight for at least 12 hr. Remove specimens from stain and rinse excess stain with several changes of 70% ETOH. A modified beem capsule (Day, 1974) was used during staining procedures and dehydration to avoid breaking the insect specimens during transfer. Both open ends of the tube were covered with nylon screen and then lids with holes cut in the center were placed on them.

2. *Destaining.* Destain in acid alcohol (70% ETOH + 2% HCl) until the nematode can be seen inside the insect. Destaining may be hastened by raising the HCl to 5%. Concentrations of HCl above 5% can destroy specimens. In the case of the mushroom fly, destaining did not take more than a few seconds. The potato beetle and corn rootworm took about 5–20 min depending upon the amount of fat in the insect. Transfer to plain 70% ETOH for overnight or weekend storage if destaining takes longer than a day. Stop destaining when the specimen becomes light pink and the darker nematode is visible inside. Destaining a little too much is preferred to not enough. Remove specimen from acid alcohol and transfer to plain 70% ETOH for 2 hr to remove acid from tissues.

3. *Dehydration.* Transfer specimen to 80% ETOH for 2 hr and then to 95% ETOH for 2 hr. Specimen may be stored overnight or over a weekend in any of these.

4. *Counterstaining.* Make a stock solution of 1% Fast green in 95% ETOH. Add 1 or 2 drops of this stock solution to a BPI or Syracuse watchglass of 95% ETOH. Dip specimen into diluted stain for 1–5 sec while watching under scope. Remove immediately to 95% ETOH and examine. The cuticle of the insect should have a barely perceptible green blush. Repeat if necessary. Fix color by transferring specimen into absolute ETOH. Let set 1–2 hr before clearing. The green will discolor and begin fading if left longer than 2 hr.

5. *Clearing.* Transfer specimen directly into methyl salicylate for approximately 5 min. As soon as the specimen has cleared, remove for mounting.

6. *Mounting.* Mount specimen directly from methyl salicylate into permount, damar balsam, or neutral Canada balsam. Do not use euparal, diatex, or harleco synthetic resin.

The staining procedure was used on dipterous insects, such as mosquito larvae, *Culex pipiens quinquefasciatus* (Say) (Fig. 1), and the larvae of the mushroom fly, *Lycoriella mali* (Fitch) (Fig. 4). Coleopteran larvae, such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Fig. 3), and the western corn rootworm, *Diabrotica virgifera virgifera* Le Conte (Fig. 2), were also processed using this staining technique. The mermithid nematode, *Romanomermis culicivorax* (Fig. 1), and the steinernematid nematodes, *Steinernema carpocapsae* (Figs. 2, 3) and *S. feltiae* (Fig. 4), were found to stain well using this technique.

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Research Note

A Simple Method for the Purification of Trichostrongyle Egg Shells

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ABSTRACT: Biochemical analysis of nematode egg shells requires the availability of pure material. Eggs of the ruminant nematode, *Haemonchus contortus,* were purified from sheep feces by flotation on sucrose and Percoll. Unembryonated eggs were disrupted by chopping and sonication, and pure shell preparations were recovered by differential centrifugation on Percoll gradients. For recovery of shells after hatching, eggs were allowed to embryonate, then shells and hatched larvae were separated on Percoll gradients.

KEY WORDS: Nematoda, *Haemonchus contortus,* egg shell, hatching.

The nematode egg shell serves as the primary barrier to adverse environmental conditions (Wharton, 1980). An in-depth knowledge of the composition of the egg shell would aid in understanding both how the shell functions in protecting the juvenile parasite and how it is degraded during the hatching process. However, studies on the nematode egg shell are limited by the lack of significant quantities of pure material. The inability to separate shell components from embryonic material makes biochemical analyses of shell components impractical. In order to study the composition of the egg shell of the ruminant trichostrongyle *Haemonchus contortus* (Rudolphi, 1803), it was necessary to develop methods for the recovery of large quantities of pure egg shells, both pre- and posthatching. Two methods are described here which should be applicable, with minor modifications, to egg shell recovery from other nematode species.

The Beltsville strain of *Haemonchus contortus* was maintained by serial passage in parasite-naive polled Dorset lambs (2–6 mo of age). For egg recovery, lambs were inoculated with 10,000–15,000 third-stage larvae obtained from 10-day fecal cultures. Egg production was monitored by flotation of fecal samples on 150% sucrose and eggs were enumerated by the modified McMaster technique (Whitlock, 1948). Fecal material was used for large scale egg recovery only when numbers of eggs per gram (EPG) of feces were $>10^3$. On average, 300 g of feces from 3 lambs shedding over 1,000 EPG was used to produce 0.5 ml of purified packed eggs and subsequently 50 μl of purified packed egg shells.

For egg recovery, feces from source lambs (300 g or 500 ml) were mixed with an equal volume of tap water, then homogenized briefly in a blender to create a slurry. Fecal material was then passed through a 300-μm mesh screen to remove large debris, and the screen rinsed with several volumes of tap water. The screened fecal suspension, containing eggs, was mixed with an equal volume of 150% sucrose, placed into 50-ml plastic tubes and centrifuged at a relative centrifugal force of 1,400 *g* in a Sorvall RC-3B centrifuge (Dupont Company, Wilmington, Delaware). Floated eggs were aspirated from the surface of the sucrose suspension, diluted in 100 volumes of tap water, and allowed to settle in a 2-liter beaker for 30 minutes. The supernatant was aspirated leaving settled eggs in 200 ml of water. This suspension was mixed with an equal volume of 150% sucrose and centrifuged as above. Following aspiration from the second sucrose flotation, eggs were pelleted, washed once in tap water by centrifugation (10 minutes at 1,400 *g*), and examined. Egg preparations from sucrose flotation contained varying amounts of fecal debris. For further purification, eggs were underlaid with Percoll (Sigma, St. Louis, Missouri) at concentrations of 30% and 45%, and centrifuged at 1,400 *g* for 15 min. Eggs recovered from the interface of the 30% and 45% Percoll layers were found to be free of fecal debris.

For the recovery of egg shells from unembryonated eggs, freshly recovered eggs were concentrated by centrifugation (1,400 *g*) to a small volume (1–2 ml) and chopped extensively with razor blades in a glass petri dish. Following chopping, shells were further disrupted by brief sonication (30% power for 1 min using a Microson Ultrasonic Cell Disrupter, Ultrasonic Heat Systems, Farmingdale, New York). This treatment was effective in releasing embryonic material without significant destruction of the shell. After sonication, 10–20 volumes of distilled water were

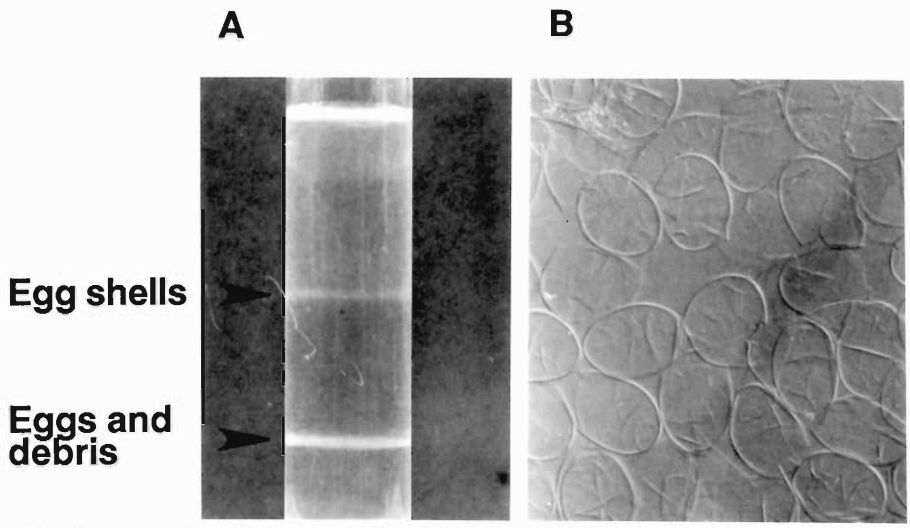

Figure 1. Recovery of egg shells from freshly collected, unembryonated eggs of *Haemonchus contortus*. A. Percoll gradient showing the separation of egg shells from undisrupted eggs and debris. B. Egg shells recovered from the Percoll gradient (×200).

added to dilute the preparation, then solubilized embryonic material was removed from shells and undisrupted eggs by centrifugation at 600 *g*. The supernatant, containing the majority of the embryonic debris, was aspirated and the pellet resuspended in 2–5 ml of distilled water. To recover shells from residual embryonic material and intact eggs, the chopped, sonicated preparations were underlaid with Percoll at various concentrations ranging from 10% to 50% and centrifuged at 600 *g* for 15 min. Based on recoveries from these gradients, 20% Percoll was found to be the most effective at separating shells from intact eggs. Using a 20% and 50% gradient, pure egg shells banded on top of the 20% Percoll, whereas debris, including intact eggs, was recovered from the interface of the 20% and 50% layers (Fig. 1). Egg shells did not appear to be substantially disrupted by this procedure and were free of embryonic debris when harvested from the gradients. Adequate separation of egg shells was achieved using a starting number of eggs ranging from 1.3 to 10.4 × 10^6 in a volume of 0.25–2.0 ml.

For recovery of egg shells following hatching, eggs were embryonated and hatched as follows. Eggs were purified as described above, then washed repeatedly in sterile tap water supplemented with 50 U/ml of penicillin, and 50 μg/ml of streptomycin. To assess sterility, a 50-μl sample of the final egg preparation was streaked onto nutrient agar plates prior to the initiation of hatching cultures. Preparations found to be contaminated with bacteria were discarded. Eggs, sterilized by antibiotic treatment, were incubated at 25°C for 42 hr in sterile tap water. After this time period, >90% of eggs had embryonated and larvae had hatched. For recovery of shells from hatched larvae and debris, cultures were concentrated to 2–5 ml by centrifugation (1,400 *g*), underlaid with an equal volume of various concentrations of Percoll ranging from 10% to 50% and centrifuged at 600 *g* for 15 min. Hatched egg shells were found to float on 10% Percoll, whereas debris floated on 35% Percoll and hatched larvae floated on 50% Percoll. Based on these results, a gradient was constructed consisting of 10%, 35%, and 50% Percoll. This gradient resulted in excellent separation of shells from debris, including unhatched eggs, and hatched larvae (Fig. 2). The egg shells recovered using this procedure were relatively intact. In addition, viable first-stage larvae could be recovered in large numbers. These larvae could be used further following active migration through 20-μm mesh nylon screens to recover viable larvae from dead or nonmotile worms.

Studies of nematode egg shells have been limited to morphological descriptions of the shell, histological staining, and, in a few cases, limited biochemical analyses (Clarke et al., 1967; Bird, 1971; Wharton, 1980, 1983). Further studies of

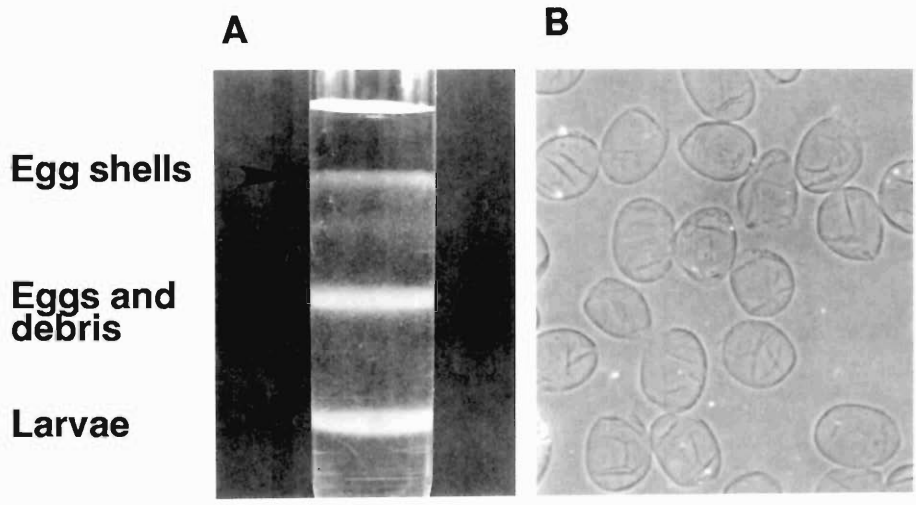

Figure 2. Recovery of egg shells from hatched *Haemonchus contortus* eggs. A. Percoll gradient showing the separation of egg shells, first-stage larvae, and unhatched eggs and debris. B. Egg shells recovered from the Percoll gradient (×200).

the nematode egg shell, its composition, formation, and degradation, have been limited by the availability of pure material. Using the procedures described here for obtaining pure egg shells, it should be possible to define, characterize, and separate the components of the egg shell of *Haemonchus contortus*. Additionally, purified egg shell components can be used to generate antibodies for localization of extracted components within the shell. Minor modifications of the described methods should make it feasible to recover significant amounts of pure egg shells from a variety of species of nematodes.

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Research Note

Induction of Increased Benomyl Tolerance in *Verticillium lecanii*, a Fungus Antagonistic to Plant-Parasitic Nematodes[1]

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ABSTRACT: Following exposure of *Verticillium lecanii* to ultraviolet radiation, 4 mutants were induced that exhibited greater tolerance to the fungicide benomyl than did the wild type. Colonies of the wild type strain increased in diameter at a rate of approximately 4–8 mm per week on 100 μg benomyl per ml potato dextrose agar; the mutants increased in diameter approximately 10–17 mm per week on the same agar.

KEY WORDS: *Verticillium, Heterodera,* soybean cyst nematode, nematode, biological control, genetic manipulation, mutation.

Verticillium lecanii (A. Zimmermann) Viégas has been studied as a control agent for insects and fungi (Hussey, 1984; Harper and Huang, 1986; Uma and Taylor, 1987; Heintz and Blaich, 1990), and strains have been commercially produced as biocontrol agents for aphids and whiteflies. Research on the fungus as a control agent for plant-parasitic nematodes has included isolation of *V. lecanii* from nematodes in the field, and laboratory experiments to determine whether the fungus affects nematode viability (Hänssler and Hermanns, 1981; Gintis et al., 1983; Rodríguez-Kábana and Morgan-Jones, 1988; Hänssler, 1990; Meyer et al., 1990). Four strains of *V. lecanii* were tested in petri dish cultures for antagonism to eggs of the soybean cyst nematode, *Heterodera glycines* Ichinohe (Meyer et al., 1990). Strain 58909 from the American Type Culture Collection caused a significant decrease in numbers of viable soybean cyst nematode (SCN) eggs.

The antagonistic strain 58909 was studied for tolerance to the fungicide benomyl (Meyer et al., 1991). Experiments were then conducted to determine whether benomyl tolerance could be increased by exposure to ultraviolet light. Previous mutagenesis studies on *V. lecanii* have produced changes in spore density and spore release, alterations in enzyme activity, strains with altered pigmentation on the undersides of colonies, and auxotrophic mutants (Jackson, 1984; Heale, 1987). There were several reasons for attempting to induce mutants with resistance or increased tolerance to benomyl. Benomyl can be deleterious to some strains of *V. lecanii* applied for biological control of insects in the greenhouse (Gardner et al., 1984; Hassan and Oomen, 1985). Benomyl is registered for use on soybean and other field crops, and may be applied as part of an integrated pest management (IPM) program. If *V. lecanii* was to be used as a biocontrol fungus in an IPM system, a strain with high benomyl tolerance might survive more readily than a fungus with no tolerance. In addition, benomyl could be incorporated into the fungus delivery system to discourage other organisms from growing on nutrients applied with the control fungus. A further potential benefit of increased benomyl tolerance is that it may serve as a marker to aid in identification of biocontrol strains. Another reason for employing benomyl is that some fungus mutants with increased benomyl tolerance have greater biocontrol capacity than the wild type strains, even when benomyl has not been applied to a crop (Papavizas, 1985).

Studies were conducted on nematode-antagonistic fungi to determine if benomyl tolerance or resistance would improve biocontrol ability (Gaspard and Mankau, 1985; Gaspard, 1986). Conidia of the fungi *Paecilomyces lilacinus* and *Verticillium chlamydosporium* were irradiated with ultraviolet light, and biotypes with resistance or tolerance to benomyl were induced. The isolates and the wild types were able to parasitize eggs of *Meloidogyne* spp., but neither the wild type nor the mutant strains of *P. lilacinus* significantly reduced root knot nematode egg numbers on tomato. Induction of benomyl resistant mutants from wild type strains of fungi that significantly reduce nematode populations may result in more successful biocontrol agents.

To induce mutants of *V. lecanii,* suspensions

[1] Mention of a trademark or proprietary product does not constitute a guarantee, warranty, or endorsement by the United States Department of Agriculture and does not imply its approval to the exclusion of other suitable products.

of conidia were made in sterile distilled water, and plated onto either potato dextrose agar (PDA) or PDA + benomyl. Benomyl (Benlate 50 Wettable Powder, E. I. du Pont de Nemours & Co., Wilmington, Delaware) amended plates contained 100 μg benomyl/ml PDA. This concentration of benomyl was selected because the wild type strain was not able to grow as quickly as on unamended PDA, but the benomyl level did not severely inhibit the growth of the fungus. The mutants could be selected by choosing colonies that grew the most quickly on the agar. Two hundred one petri dishes were each inoculated with approximately 200 conidia. Six petri dishes contained PDA; 195 petri dishes contained PDA + benomyl. Conidia in 3 petri dishes of PDA and 3 dishes of PDA + benomyl were not UV-irradiated. The rest of the conidia were exposed for 40 sec to ultraviolet radiation from a General Electric G30T8 30-watt germicidal bulb. If UV-irradiated spores of *Verticillium dahliae* and *Verticillium albo-atrum* are incubated in the light, fewer mutants are produced because photoreactivating enzymes repair DNA damage from ultraviolet light (Puhalla, 1973). Consequently, petri dishes containing irradiated conidia of *V. lecanii* were placed in boxes or were wrapped in foil to prevent exposure to light. Counts of viable colonies indicated that the survival rate after 40-sec irradiation was approximately 39%. After an incubation period of 8 days at 25°C, colony diameters were compared. Eight to 14 days after irradiation, 11 colonies on irradiated plates were greater in diameter than the other colonies growing on PDA + benomyl. To minimize genetic variability, 3–6 single spore isolates were made of each of the 11 colonies.

To determine whether the isolated colonies were mutants with increased tolerance to benomyl, plugs 9 mm in diameter were made from each single spore isolate and were inoculated onto PDA and PDA + 100 μg benomyl/ml medium. Colony growth was measured at 1 and 2 wk after inoculation. Seven of the strains that had originally appeared to grow more rapidly on benomyl-amended agar did not do so when tested in a quantifiable experiment. Those 7 colonies may have had large diameters on PDA + benomyl following irradiation because they grew from conidia that germinated quickly after inoculation, or because the large colonies formed when smaller colonies became confluent. Only 4 of the 11 strains had greater growth rates on the benomyl-amended agar than the wild type strain: strains 1, 2, 9, and 10. On PDA + benomyl, colonies of the wild type strain increased in diameter ca. 4 mm the first week and ca. 8 mm the second week. The mutants increased about 11–17 mm the first week and approximately 10–17 mm the second week. However, these strains did not grow as quickly as the wild type strain on the unamended agar. On PDA, colony diameters of the wild type strain increased approximately 18 mm the first week and 24 mm the second week, whereas mutants increased in diameter ca. 12–20 mm the first week and ca. 13–20 mm the second week.

These results show clearly that strains of *V. lecanii* with increased benomyl tolerance can be induced with exposure to ultraviolet light. In greenhouse experiments (Meyer, 1990; Meyer and Huettel, 1991), the wild type strain and a tested mutant both reduced nematode populations in the soil, but the mutant was more efficacious than the wild type at low levels of application. This result occurred in the absence of benomyl. A culture of each of the four strains has been deposited at the Agricultural Research Service Culture Collection (NRRL). The cultures have been assigned the strain numbers NRRL 18725, 18726, 18727, and 18728.

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Research Note

Occurrence of Larval *Contracaecum* sp. (Ascaridida: Anisakidae) in Rio Grande Lesser Sirens, *Siren intermedia texana* (Amphibia: Caudata), from South Texas

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ABSTRACT: Unencapsulated third-stage larval anisakid nematodes, *Contracaecum* sp. Railliet and Henry, 1912, were recovered from the coelomic cavity of all of 8 Rio Grande lesser sirens, *Siren intermedia texana* Goin, 1957, from southern Texas. Mean intensity was 2.1 (range 1–5) worms per host. This is the first report of larval *Contracaecum* sp. infecting a caudate amphibian.

KEY WORDS: Anisakidae, Ascaridida, Nematoda, *Contracaecum* sp., Caudata, *Siren intermedia texana,* Sirenidae.

The Rio Grande lesser siren, *Siren intermedia texana* Goin, 1957, is a large eellike salamander that ranges from the lower Rio Grande Valley of Texas to Tamaulipas, Mexico (Martof, 1973; Dixon, 1987). The species inhabits a wide variety of aquatic sites. In Texas, *S. i. texana* is considered an endangered taxon and is afforded protection by the Texas Parks and Wildlife Department.

Although a great deal of information is available on endoparasites of conspecific western lesser sirens, *S. i. nettingi* Goin, 1942 (Nickol, 1972; Dunagan and Miller, 1973; Dyer, 1973; Brooks and Buckner, 1976; Brooks, 1978; Buckner and Nickol, 1979), nothing has been published on parasites of *S. i. texana.* During a morphometric study of *S. i. texana* (McDaniel, 1969), several

sirens were observed to be infected with the anisakid nematode reported here.

During April 1968, the junior author collected 8 adult *S. i. texana* (mean ± SD snout–vent length [SVL] = 228.4 ± 30.8, range = 190–272 mm) by seining, hand, or with wire traps from freshwater ponds in the vicinity of Kingsville, Kleberg County, Texas (27°30′N, 97°51′W). Specimens were killed with a dilute solution of chloretone and a midventral incision was made to expose the viscera. Nematodes were collected from the coelomic cavity and fixed in 70% ethanol. Voucher specimens of hosts are deposited in the Texas A&I University Museum (AIM 574.21, 574.38, 574.43, 574.47, 575.77, 575.94, 575.109, and 575.111). Voucher specimens of *Contracaecum* sp. are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as USNM 82004.

All of 8 *S. i. texana* were infected with third-stage larvae of the anisakid nematode *Contracaecum* sp. Railliet and Henry, 1912. A total of 17 unencapsulated worms was recovered from the coelomic cavity of 8 sirens; a mean intensity of 2.1 (range 1–5) worms per host. The largest siren (SVL = 272 mm, AIM 574.38) was most heavily infected (5 worms).

Sirens have been reported to feed on a variety of prey items, including fish (Goin, 1957; Duellman and Schwartz, 1958; Altig, 1967; Hanlin, 1978) and crustaceans (Scroggin and Davis, 1956; Altig, 1967). Perhaps immature sirens become infected by ingesting invertebrates (copepods) harboring larval stages of *Contracaecum,* whereas the adults are infected from ingestion of encapsulated third-stage larvae found in fish (see Huizinga, 1967).

In summary, this is the first report of *Contracaecum* sp. from a member of the order Caudata and, to our knowledge, only the second time the parasite has been reported from amphibians. Coy Otero and Ventosa (1984) previously reported larval *Contracaecum* sp. from Cuban treefrogs, *Osteopilus septentrionalis* (Dumeril and Bibron, 1841), and bullfrogs, *Rana catesbeiana* Shaw, 1802, from Cuba.

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Research Note

Tetrathyridia of *Mesocestoides lineatus* (Cestoidea: Cyclophyllidea) in *Sceloporus undulatus hyacinthinus* (Sauria: Iguanidae) from Arkansas

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ABSTRACT: Twenty-nine northern fence lizards, *Sceloporus undulatus hyacinthinus* (Green, 1818), from 7 counties of Arkansas were examined for *Mesocestoides* sp. tetrathyridia. Only 1 (3%) of the lizards was found to be infected. Over 300 living tetrathyridia in 2 distinct size classes were found free in the coelomic cavity and encapsulated forms infected the liver, intestinal mesenteries, and musculature of this host. None of these tetrathyridia showed any evidence of asexual reproduction, even after up to 6 mo of maintenance in the body cavity of experimentally infected mice. When administered to hamsters by stomach tube, the tetrathyridia developed into gravid adults identified as *Mesocestoides lineatus* (Goeze, 1782). This constitutes a new host record for *M. lineatus* and represents only the second time a lizard from Arkansas has been reported to harbor tetrathyridia of *Mesocestoides*.

KEY WORDS: *Sceloporus undulatus hyacinthinus, Mesocestoides lineatus,* fence lizard, survey, tetrathyridia, Cestoidea, Cyclophyllidea, Arkansas.

Numerous North American lizards (Sauria) are known hosts of *Mesocestoides* sp. tetrathyridia (McAllister, 1988; Goldberg and Bursey, 1990a; McAllister, 1991; McAllister et al., 1991b). This metacestode has been reported frequently from lizards of the family Iguanidae, particularly *Sceloporus* species (Voge, 1953; Telford, 1970; Benes, 1985; McAllister, 1988; Goldberg and Bursey, 1990b).

As part of our continuing effort to collect information on infections of *Mesocestoides* sp. tetrathyridia in amphibians (McAllister, 1987; McAllister et al., 1989; McAllister and Conn, 1990) and reptiles (McAllister, 1988, 1991; Conn and McAllister, 1990; McAllister et al., 1991a, b, c), we examined several northern fence lizards, *Sceloporus undulatus hyacinthinus* (Green, 1818) from various localities in Arkansas for the parasite. Herein, we characterize an infection in 1 of these hosts.

Between April 1979 and 1980 and again between May 1989 and November 1990, 29 hatchling, juvenile, and adult *S. u. hyacinthinus* (snout–vent length [SVL] range = 22–68 mm) were collected by hand from (sample sizes in parentheses): Carroll (2), Izard (19), Marion (2), Perry (1), Polk (1), Pope (1), and Yell (3) counties of Arkansas and examined for *Mesocestoides* sp. tetrathyridia. Lizards were returned alive to the laboratory for examination and killed with an overdose of sodium pentobarbital (Nembutal®). Methods used for necropsy and processing hosts follow McAllister et al. (1991b). Formalin-fixed tissues were sectioned at 7 μm, stained with Mayer's hematoxylin and eosin, and mounted in gum damar. Live tetrathyridia were removed from the coelomic cavity of 1 host, rinsed in Dulbecco's phosphate-buffered saline (pH = 7.3), and mailed by air express to the junior author (D.B.C.) at St. Lawrence University. In an attempt to obtain adults, some of these living tetrathyridia were administered in groups of 3 by stomach tube to 9 young golden hamsters, *Mesocricetus auratus*. The remainder were either fixed for morphological study, or were inoculated intraperitoneally into 6 young laboratory-reared mice for maintenance; mice were necropsied up to a maximum of 6 mo postinoculation to check for asexual proliferation. Tetrathyridia and experimentally obtained adults were fixed in AFA (alcohol–formalin–acetic acid), stained in Semichon's acetocarmine, and mounted whole in gum damar.

Voucher specimens of lizards are deposited in the Arkansas State University Museum of Zoology (ASUMZ). Vouchers of *Mesocestoides lineatus* have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705, as USNM Helm. Coll. Nos. 81931 (tetrathyridia) and 81932 (adults).

Only 1 (3%) of the *S. u. hyacinthinus* was found to harbor *Mesocestoides* sp. tetrathyridia. The host was an adult female (68 mm SVL, ASUMZ 16998) collected on 23 November 1990 from

Yell County, Arkansas. Numerous encapsulated tetrathyridia were observed in the liver, intestinal mesenteries, and musculature. Each capsule contained from 1 to several tetrathyridia. In addition, over 300 individual live tetrathyridia were found free within the coelomic cavity. The tetrathyridia occurred as 2 distinct size classes, but otherwise were morphologically identical. None possessed multiple scolices, supernumerary suckers, buds, or any other morphological evidence of asexual proliferation; this was true of those taken directly from naturally infected lizards and those maintained for up to the maximum of 6 mo in experimentally infected mice. Furthermore, the number of tetrathyridia recovered from experimentally infected mice in each case was equal to or lower than the number inoculated.

Tetrathyridia administered to hamsters developed into gravid adults having morphological features fitting the description of *Mesocestoides lineatus* (Goeze, 1782). Tetrathyridia of both size classes developed into adults that were identical in both size and morphology.

Tetrathyridia of *Mesocestoides* have been reported from several North American *Sceloporus* lizards. Host reports include the western fence lizard, *S. occidentalis* Baird and Girard, 1852, from California (Voge, 1953; Specht and Voge, 1965), sagebrush lizards, *S. graciosus* Baird and Girard, 1852, from California (Telford, 1970), desert spiny lizards, *S. magister magister* Hallowell, 1854, from Arizona (Benes, 1985), Texas spiny lizards, *S. olivaceus* Smith, 1934, from Texas (McAllister, 1988), San Joaquin fence lizards, *S. occidentalis biseriatus* Hallowell, 1854, from California (Goldberg and Bursey, 1990a), Yarrow's spiny lizard, *S. jarrovii jarrovii* Cope, 1875, from Arizona (Goldberg and Bursey, 1990b) and bunch grass lizards, *S. scalaris slevini* Smith, 1937, from Arizona (Goldberg and Bursey, 1992). Among these, most were identified only as *Mesocestoides* sp. The only exception was that of Specht and Voge (1965) who identified their aberrant asexually proliferative tetrathyridia as *Mesocestoides corti* Hoeppli, 1925, an identity that was later questioned by Beaver (1989). However, Etges (1991) recently proposed the name *Mesocestoides vogae* for the species of asexually proliferative tetrathyridia originally described by Specht and Voge (1965).

The absence of any evidence of asexual proliferation in the present study further substantiates the rarity of asexually reproducing tetrathyridia in natural populations. Furthermore, the occurrence of more than 300 tetrathyridia in a single host, with more than 1 in many individual host capsules, is consistent with reports from other naturally infected hosts, thus further confirming that such large numbers do not result from asexual proliferation (Conn, 1990; McAllister and Conn, 1990). The 2 size classes of tetrathyridia reported here do not belong to different species, because both were of identical size and morphology as adults. The size difference may have resulted from 2 separate incidents of infection, with the smaller tetrathyridia having been acquired more recently by the host.

The size and morphological features of adult tapeworms obtained experimentally in the present study were identical to those reported as *M. lineatus* by Conn and Etges (1984) and Conn et al. (1984) from green anoles, *Anolis carolinensis* (Voigt, 1832), from Louisiana (USNM Helm. Coll. Nos. 78123 and 78124). In the latter cases, the worms were isolated as tetrathyridia from naturally infected lizards and developed into gravid adults in experimentally infected golden hamsters.

In conclusion, *S. u. hyacinthinus* constitutes a new host for *M. lineatus*. McAllister et al. (1991c) recently reported tetrathyridia of *Mesocestoides* from 2 of 51 (4%) six-lined racerunners, *Cnemidophorus sexlineatus* (Linnaeus, 1766), from Arkansas. Therefore, the present study represents only the second time tetrathyridia of *Mesocestoides* have been reported from an Arkansas lizard. In addition, Richardson et al. (1992) reported *Mesocestoides* spp. from 10 of 30 (33%) raccoons, *Procyon lotor* (Linnaeus, 1758), in north-central Arkansas. Perhaps raccoons are but 1 of many carnivores that serve as natural definitive hosts of species of *Mesocestoides* from lizards.

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MINUTES

Six Hundred Twenty-First Through Six Hundred Twenty-Eighth Meetings

621st Meeting: Uniformed Services University of the Health Sciences, Bethesda, MD, 9 October 1991. Nancy D. Pacheco presided over the business meeting. A slate of officers for 1992 was presented: David J. Chitwood, President; Ruth M. Kulstad, Vice-President; Joan E. Jackson, Secretary-Treasurer; and Yupin Charoenvit, Recording-Secretary. Bryce Reddington presided over the scientific program during which the following papers were presented: Prevalence of parasites in an Ethiopian study: its implications in immunodeficiency diseases, by Carolyn Masters; and AIDS in Zambia, by Subash Hira.

622nd Meeting: Animal Parasitology Unit, ARS, USDA, Beltsville, MD, 13 November 1991. Nancy D. Pacheco presided over the business meeting. The slate of officers was elected unanimously. Ronald Fayer presided over the scientific meeting and the following papers were presented: Diagnosis of cysticercosis, by Marcia Rhoads; Systematics of *Trichinella,* by J. Ralph Lichtenfels; Diagnosis and epidemiology of *Trichinella,* by Dante Zarlenga; Toxoplasmosis in pigs, by J. P. Dubey; and Treatment of cryptosporidiosis, by Ronald Fayer.

623rd Meeting: Smithsonian Institution, Washington, DC, 11 December 1991. Nancy D. Pacheco presided over the business meeting. Roy C. Anderson was elected to Honorary Membership and Milford E. Lunde, Everett L. Schiller, and Harley G. Sheffield to Life membership. Duane Hope presided over the scientific meeting. The following papers were presented: Life and times of N. A. Cobb, by Richard Sayre; Ultrastructure of cuticular exudations in parasitic juveniles of the sugar beet cyst nematode as related to cuticular structure, by Burton Endo; and Observations on the ultrastructure of members of the *Rhabdodomania* and their phylogenetic implications, by Duane Hope. The new officers were installed.

624th Meeting: Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD, 8 January 1992. David J. Chitwood presided over the business meeting. Allen W. Cheever presided over the scientific meeting and the following papers were presented: Oxidant stress, a potential target for rational drug design for malaria: cloning the *Plasmodium falciparum* glucose-6-phosphate gene, by Mohammed Sahabuddin; A new perspective on the phylogeny of *Entamoeba,* by Graham Clark; and IL-10 downregulates effector-function of macrophages against *Schistosoma mansoni,* by Isabel Oswald-Molenet.

625th Meeting: Naval Medical Research Institute, Bethesda, MD, 12 February 1992. David J. Chitwood presided over the business meeting and Stephen Hoffman presided over the scientific meeting. Papers presented were: Chloroquine resistant *Plasmodium vivax*: a serious emerging threat, by Stephen Hoffman; Malaria sporozoites and circumsporozoite proteins bind specifically to sulfated glycoconjugates, by Samuel J. Pancake; and Characterization of *Plasmodium yoelii* SSP2: a sporozoite, liver, and blood stage antigen, by Sylvie Mellouk.

626th Meeting: Walter Reed Army Institute of Research, Washington, DC, 4 March 1992. David J. Chitwood presided over the business meeting. The awarding of the 1991 Anniversary Award to Francis Tromba was announced. The Certificate of Life Membership was presented to Harley G. Sheffield. Treasurer Joan E. Jackson presented the financial report. The Society voted to increase dues for 1993: Membership: U.S. $20, Canada/Mexico $21, All Others $22; Subscribers: U.S. $37, Canada/Mexico $39, All Others $42. Willis Reed introduced the program on *Leishmania tropica* in U.S. Desert Shield/Storm Soldiers and the following papers were presented: Overview of entomological and potential transmission scenarios, by Phillip G. Lawyer; Diagnosis of patients and current developments, by Max Grogl; Clinical presentations and management of leishmaniasis patients, by Alan Magill; and Concluding perspectives on what the future holds, by Jonathan D. Berman.

627th Meeting: Johns Hopkins School of Hy-

giene and Public Health, Baltimore, MD, 8 April 1992; joint meeting with The Tropical Medicine Dinner Club of Baltimore. The meeting was called to order by Thomas Simpson. Vice-president Ruth Kulstad announced the election of Everett Schiller of the Johns Hopkins University to Life membership. Charles Wisseman introduced the speaker, Kenneth Linthicum, who spoke on "A satellite surveillance system to predict the potential for Rift Valley Fever activity in sub-Saharan Africa."

628th Meeting: University of Pennsylvania, New Bolton Center, Kennett Square, PA, 2 May 1992; joint meeting with the New Jersey Society for Parasitology. Gerhard Schad introduced Richard Seed, President of the American Society of Parasitologists, who gave a short address on the state of parasitology today and on the formation of a Federation of Societies for Parasitology. Herbert Haines, President of the New Jersey Society, presided over the symposium, "Strategies for Parasite Control into the 21st Century." Mervyn Turner spoke on future trends in parasitology; Gary Smith discussed future strategies for parasite control: the role of the universities; and Peter Perkins spoke on Leishmaniasis—a disease of civilian and military significance—future vector and parasite controls.

The Helminthological Society of Washington welcomed 25 new members to the Society during the meetings indicated: *621st:* Seham Kozman Abadir, Bolal Ahmed Soliman, Wayne Price, Kurt Pfister, Gerald S. Santo, Eileen M. Proctor, Harold J. Harlan, and Peter Seferian; *622nd:* Charlotte M. Christensen, Jean Mariaux, Sergei H. Movsessian, Lloyd L. Smrkovski, Aileen M. Marty, and Gary A. Conboy; *625th:* Victoria D. Fallarme, Helen H. Wang, and Serge Morand; *626th:* Arthur Abrams, Carl A. Lowenberger, and Linda S. Mansfield; *628th:* Cathy Leadabrand, Kenneth Nieves, Chrystal L. Mars, Dennis J. Richardson, and Nashaat AbdEl-Motaal Mahmoud.

Respectfully submitted,

Yupin Charoenvit
Recording Secretary

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KEY WORD AND SUBJECT INDEX FOR VOLUME 59

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Proceedings is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues are payable on notification of election. Send this completed form to:

The Recording Secretary
Helminthological Society of Washington
Post Office Box 368
Lawrence, Kansas 66044

Print name: _____ Date of birth: _____

Mailing address: _____
_____
_____

Degree and year received: _____

Present position: _____

Field of interest: _____
_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
(a member)

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	* Leo A. Jachowski, Jr.	1976
Mildred A. Doss	1961	* Horace W. Stunkard	1977
* Allen McIntosh	1962	Kenneth C. Kates	1978
* Jesse R. Christie	1964	* Everett E. Wehr	1979
Gilbert F. Otto	1965	* O. Wilford Olsen	1980
* George R. LaRue	1966	Frank D. Enzie	1981
* William W. Cort	1966	Lloyd E. Rozeboom	1982
* Gerard Dikmans	1967	Leon Jacobs	1983
* Benjamin Schwartz	1969	Harley G. Sheffield	1984
* Willard H. Wright	1969	A. Morgan Golden	1985
Aurel O. Foster	1970	Louis S. Diamond	1986
Carlton M. Herman	1971	Everett L. Schiller	1987
May Belle Chitwood	1972	Milford N. Lunde	1988
* Elvio H. Sadun	1973	J. Ralph Lichtenfels	1989
E. J. Lawson Soulsby	1974	A. James Haley	1990
David R. Lincicome	1975	Francis G. Tromba	1991
Margaret A. Stirewalt	1975		

HONORARY MEMBERS

* George R. LaRue	1959	John F. A. Sprent	1979
Vladimir S. Ershov	1962	Bernard Bezubik	1980
* Norman R. Stoll	1976	Hugh M. Gordon	1981
* Horace W. Stunkard	1977	E. J. Lawson Soulsby	1990
Justus F. Mueller	1978	Roy C. Anderson	1991

CHARTER MEMBERS 1910

* W. E. Chambers	* Philip E. Garrison	* Maurice C. Hall	* Charles A. Pfender
* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	* Benjamin Schwartz	1976
* Albert Hassall	1931	Mildred A. Doss	1977
* Charles W. Stiles	1931	* Everett E. Wehr	1977
* Paul Bartsch	1937	Marion M. Farr	1979
* Henry E. Ewing	1945	John T. Lucker, Jr.	1979
* William W. Cort	1952	George W. Luttermoser	1979
* Gerard Dikmans	1953	* John S. Andrews	1980
* Jesse R. Christie	1956	* Leo A. Jachowski, Jr.	1981
* Gotthold Steiner	1956	Kenneth C. Kates	1981
* Emmett W. Price	1956	Francis G. Tromba	1983
* Eloise B. Cram	1956	A. James Haley	1984
* Gerald Thorne	1961	Leon Jacobs	1985
* Allen McIntosh	1963	Paul C. Beaver	1986
* Edna M. Buhrer	1963	Raymond M. Cable	1986
* Benjamin G. Chitwood	1968	Harry Herlich	1987
Aurel O. Foster	1972	Glenn L. Hoffman	1988
Gilbert F. Otto	1972	Robert E. Kuntz	1988
* Theodor von Brand	1975	Raymond V. Rebois	1988
May Belle Chitwood	1975	Frank W. Douvres	1989
Carlton M. Herman	1975	Thomas K. Sawyer	1989
Lloyd E. Rozeboom	1975	* J. Allen Scott	1990
* Albert L. Taylor	1975	Judith H. Shaw	1990
David R. Lincicome	1976	Milford N. Lunde	1991
Margaret A. Stirewalt	1976	Everett L. Schiller	1991
* Willard H. Wright	1976	Harley G. Sheffield	1991

* Deceased.

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