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## Analyses of a Debilitating Parasite (*Microphallus papillorobustus*, Trematoda) and Its “Hitchhiker” Parasite (*Maritrema subdolum*, Trematoda) on Survival of Their Intermediate Host (*Gammarus insensibilis*, Amphipoda)

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**ABSTRACT:** Parasites that alter their intermediate host's behavior to favor its predation by the definitive host are known from a wide range of host–parasite associations. Recently, we found a new category of parasites, so-called “hitchhikers,” unable to modify the behavior of their intermediate host but exploiting the same host spectrum, that gain benefits in transmission success from the infection of these behaviorally manipulated hosts. Because the probability of successful transmission by “hitchhiking” depends on 1) the efficiency of the “favorization” process of the debilitating parasite and 2) the effects of infection by the hitchhiker parasite on the host survival, we investigated these two aspects. In the laboratory, we showed that, in the absence of a predator (definitive host), there is no significant difference between the mortality rates of *Gammarus insensibilis* (second intermediate host) uninfected and infected by the debilitating trematode *Microphallus papillorobustus*. In the field, we showed that the hitchhiker trematode *Maritrema subdolum* does not significantly reduce host survival. These results suggest that the higher mortality rate of manipulated hosts in the field could be explained by the predation by the definitive hosts and that *M. subdolum* does not alter, through survival reduction, the efficiency of the favorization process.

**KEY WORDS:** trematode, *Gammarus*, *Microphallus papillorobustus*, *Maritrema subdolum*, survival, hitchhiking strategy.

In a wide range of complex parasite life cycles, parasites have been shown to modify the behavior of their intermediate hosts to enhance their probability of predation by the definitive host (Bethel and Holmes, 1973; Camp and Hui-zinga, 1979; Giles, 1983; Hoogenboom and Dijkstra, 1987; Barnard and Behnke, 1990; Combes, 1991; Esch and Fernandez, 1993; Poulin, 1995). Even if they are difficult to quantify, these host manipulations are probably often costly to achieve (Poulin, 1995). Theory predicts that other parasites would benefit from such efficient mechanisms of “favorization,” through preferentially infecting hosts previously infected by a debilitating parasite (Combes, 1991; Poulin, 1994; Lafferty and Morris, 1996). These parasites could obtain a high probability of transmission, making no investment in manipulation themselves. Recently, Thomas et al. (1997) provided possible evidence for such a “hitchhik-

ing” strategy from a complex association involving two trematode parasites and one gammarid species as intermediate host: gammarids (*Gammarus insensibilis*) infected by *Microphallus papillorobustus* Rankin, 1940 (Trematoda, Microphallidae), are named “mad gammarids” because they display an aberrant behavior that renders them more vulnerable than uninfected ones to predation by aquatic birds, the definitive hosts (Helluy, 1983a, 1983b); *Maritrema subdolum* Jägerskiöld, 1909 (Trematoda Microphallidae), enhances its transmission to the definitive hosts by parasitizing amphipods previously infected by the debilitating trematode (i.e., *M. papillorobustus*).

Infection by parasites tends frequently to reduce the energy available for sustaining essential functions and, consequently, may increase the host mortality rate (Kinne, 1984; Combes, 1995). The probability of successful transmission by hitchhiking depends on 1) the efficiency of the “favorization” process of the debilitating parasite, and 2) the effects of infection by the

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hitchhiker parasite on the host survival. Although the demographic impact of *M. papillorobustus* is considerable in field populations of *G. insensibilis* (Thomas et al., 1995a), we do not know whether or not predation by definitive hosts (i.e., Charadriiform birds) is the major cause of "mad gammarids" mortality. In this paper, we first analyze whether mad gammarids, compared with uninfected ones, display a reduced vigor that could enhance their mortality rate even in the absence of predators. Second, we analyze the precise influence of *M. subdolum* on the mortality of its gammarid host in the field.

### Materials and Methods

#### Infection by *M. papillorobustus* and host mortality without predator

A large sample of unpaired *G. insensibilis* ( $n = 209$ ) was collected during May 1995 in the Thau's lagoon (southern France, 43°25'N, 3°35'E), following the method described in Thomas et al. (1995a). We attempted to obtain equal numbers of infected and uninfected individuals. In the field, infected individuals were identified through the aberrant behavior induced by the parasite (Helluy, 1983a, 1983b; Thomas et al., 1995a). In the laboratory, gammarids were maintained individually in small cups (diameter: 2 cm, height: 5 cm) filled with constantly aerated seawater (20°C, 38‰), without food, and at the natural photoperiod (14 h light:10 h dark). The cups were examined daily, and dead individuals were immediately sexed, measured by length (from head to tip of telson), and dissected to count cerebral metacercariae of *M. papillorobustus*. Metacercariae of this trematode are ovoid cysts (270 × 350 μm, Rebecq, 1964) located in the amphipod brain (Helluy, 1983a). We estimated prevalence (proportion of infected individuals, Margolis et al., 1982) and mean intensity of infection (mean parasite load of infected individuals) for both males and females. Since all individuals were entered simultaneously into the experiment and followed until death, we compared the survivorship between infected and uninfected males and females using a Kolmogorov-Smirnov test as recommended by Pyke and Thompson (1986).

#### Infection by *M. subdolum* and host mortality in the field

To determine whether metacercariae of *M. subdolum* induced host mortality, we collected a new sample ( $n = 700$ ) of *G. insensibilis* at Thau's lagoon during March 1996. Because *M. subdolum* is found mainly in "mad gammarids" (Thomas et al., 1997), we collected only gammarids with an aberrant behavior, following the same methodology as before. Later, gammarids were sexed, measured in length, and dissected to count the number of *M. subdolum* metacercariae present. Metacercariae of *M. subdolum* are small cysts (diameter: 250 μm, Rebecq, 1964) located in the abdomen (Helluy, 1981). Growth in gammarids conforms to a

logistic curve (Sutcliffe et al., 1981). Males and females were placed in 7 and 6 length classes, respectively (assuming there was a relation between age and size, Sutcliffe et al., 1981). In classes 2 to 6 for males, and 2 to 5 for females, steps were equal (i.e., 4 mm). Class 1 includes all individuals that were too small to be in class 2. Classes 7 for males, and 6 for females, include all individuals that were too large to be in classes 6 and 5, respectively. Following Anderson and Gordon (1982) and Rousset et al. (1996), we analyzed changes in mean parasite abundance of *M. subdolum* with host size.

Statistical tests were performed following Sokal and Rohlf (1981) and Siegel and Castellan (1988). Parametric statistics were used when appropriate; when conditions for their use were violated, equivalent non-parametric tests were used. All tests were two-tailed. Throughout the paper, values given are mean ± SD. The significance level chosen was 5%. All analyses were performed using Logitseq (V. Boy, Station biologique Tour du Valat).

### Results

#### Infection by *M. papillorobustus* and host mortality without predator

The lengths of infected (I) and uninfected (U) individuals were not significantly different for males (I: 12.97 mm ± 3.22, U: 12.85 mm ± 3.14; ANOVA,  $F_{1,111} = 0.03$ ,  $P > 0.05$ ), or for females (I: 12.02 mm ± 2.95, U: 11.73 mm ± 4.40; Mann-Whitney test,  $z = -1.7$ ,  $P > 0.05$ ). The mean mortality date was unrelated to the size of individuals in both infected (males:  $r = 0.23$ ,  $n = 55$ ; females:  $r = 0.08$ ,  $n = 44$ ;  $P > 0.05$  in both cases) and uninfected individuals (males:  $r = -0.05$ ,  $n = 58$ ; females:  $r = -0.13$ ,  $n = 52$ ;  $P > 0.05$  in both cases).

Death mortality distributions are shown in Figure 1. There was no significant difference between uninfected and infected individuals for males (Kolmogorov-Smirnov two-sample test,  $n_1 = 58$ ,  $n_2 = 55$ ,  $D = 0.13$ ,  $P > 0.05$ ) or females (Kolmogorov-Smirnov two-sample test,  $n_1 = 52$ ,  $n_2 = 44$ ,  $D = 0.10$ ,  $P > 0.05$ ).

Among infected individuals, the mean intensity between males (2.56 ± 2.33) and females (2.27 ± 2.33) was not significantly different (Mann-Whitney test,  $z = -0.2$ ,  $P > 0.05$ ). There was no significant relationship between the date of mortality and the parasite intensity (Spearman rank order correlation, males  $r_s = -0.18$ ; females  $r_s = -0.03$ ;  $P > 0.05$  in both cases).

#### Infection by *M. subdolum* and host mortality in the field

Mean sizes of males and females were 18.8 mm ± 6.6 and 14.9 mm ± 5.3, respectively. As

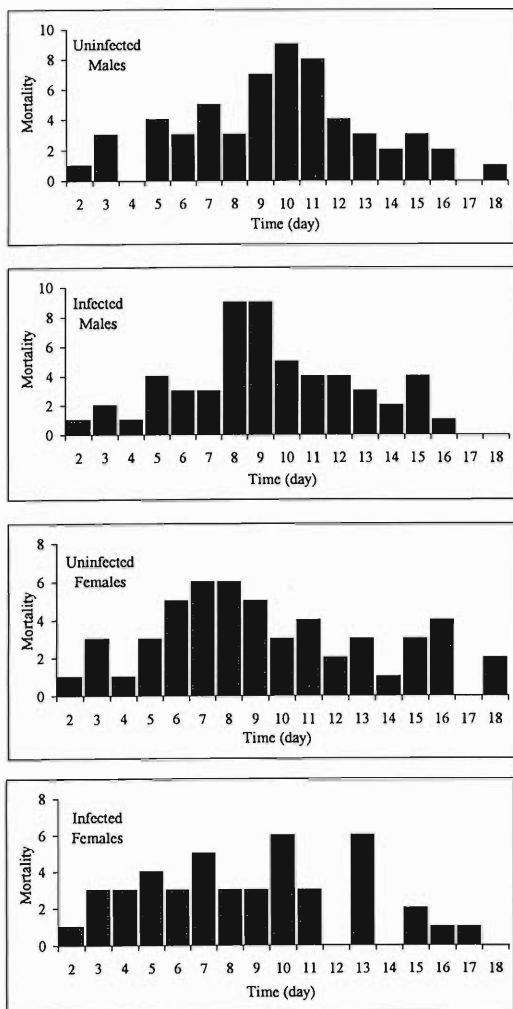


Figure 1. Mortality distributions (under starving condition) of *Gammarus insensibilis* uninfected and infected with metacercariae of *Microphallus papillorobustus*.

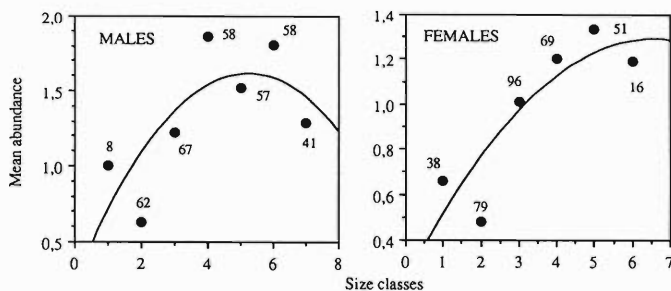


Figure 2. Changes in the mean abundance of *Maritrema subdolum* with host size in males and in females of *Gammarus insensibilis*. The number of hosts analyzed in each length class is indicated with each data point.

expected, all individuals were infected with cerebral metacercariae of *M. papillorobustus*. The mean intensities of *M. papillorobustus* for males were  $4.5 \pm 4.3$ , and for females,  $3.8 \pm 3.5$ , which is not significantly different (Mann-Whitney test,  $z = -0.07$ ,  $P > 0.05$ ). Prevalences of *M. subdolum* were 44% for males and 41% for females (Fisher' exact test,  $P = 0.68$ ). Mean abundance in *M. subdolum* for males were  $2.34 \pm 1.4$ , and  $1.7 \pm 1$  for females (Mann-Whitney test,  $z = -0.03$ ,  $P > 0.05$ ), and mean intensities were  $3.1 \pm 2.6$  and  $2.4 \pm 2$ , respectively (Mann-Whitney test,  $z = -1.9$ ,  $P > 0.05$ ). For both males and females, fits on mean abundance with size were better from a nonlinear regression than a simple linear regression (males,  $r^2 = 0.57$ ; females,  $r^2 = 0.77$ , Fig. 2). In males, a maximum mean abundance was not observed for larger hosts, but this effect was slight and was not supported in females, since the fitted polynomial curves did not show any decrease with host size. Relationships between variance to mean abundance ratio and size were not informative.

## Discussion

Studies on parasites that enhance their hosts' susceptibility to predation by definitive hosts have not provided much quantitative field data to date. Consequently, the demographic impact of such parasitism and the main causes of mortality of manipulated hosts in the field are unknown. Infected gammarids do not differ from uninfected ones in their capacity to resist starvation. Assuming there is a close relationship between resistance to starvation and physical condition, this suggests that the parasite load of *M. papillorobustus* does not reduce significantly the energy available for sustaining essential

functions. Thus, in the absence of predators (i.e., definitive hosts), *M. papillorobustus* seems to have no significant detrimental effect on its hosts' survival. This situation contrasts with the results obtained by Helluy (1984) from experimental tests using a definitive host (i.e., *Larus cachinans*). Indeed, experimentally, the differential predation of infected gammarids considerably reduced their survival compared with uninfected ones. The present study suggests that the strong demographic impact of *M. papillorobustus* on *G. insensibilis* populations (Thomas et al., 1995a) is unlikely to be explained by a reduced vigor of infected hosts compared with uninfected ones. Unless this impact is the result of the detrimental effect of *M. papillorobustus* on the amphipod growth (Thomas et al., 1996) or of another, unidentified cause of mortality, it could be explained by the predation by definitive hosts.

The results obtained on survival in starving conditions are also informative in the context of sexual selection. Indeed, in the mating system of many amphipod species, males guard females for several days until the fertilization of eggs is possible (i.e., precopulatory mate guarding, see Crespi, 1989, for a review). For males, mating success and ability to guard females are correlated positively (Ward, 1983, 1984; Sutcliffe, 1993). This period is costly for males in terms of reduced opportunity to feed, since their gnathopods are occupied in holding females (Robinson and Doyle, 1985; Sutcliffe, 1993). Our results suggest that infected males, compared with uninfected ones, are not limited by their ability to resist starvation when they hold a female.

Compared with the results obtained with *M. papillorobustus* (Thomas et al., 1995a), *M. subdolum* has much less influence on the host population. Indeed, when the rate of host mortality is correlated positively with parasite accumulation (Anderson and Gordon, 1982; Rousset et al., 1996) or parasite presence (Rousset et al., 1996), curves of the parasite abundance as a function of the host age are convex and peak at much smaller host sizes, as a consequence of the deaths of the oldest infected hosts. The absence of effect may come from the fact that *M. subdolum* does not accumulate enough to significantly affect its host's survival. Nevertheless, similar results have been obtained for two other species of Microphallidae (i.e., *Microphallus hoffmanni*, Thomas et al., 1995b, and *Levinseni-*

*iella tridigitata*, Thomas et al., 1995c) that, like *M. subdolum*, encyst in the abdomen of *G. insensibilis* and never alter the behavior. Thus, it appears that at least in the present case, *M. subdolum* seems not to be a costly passenger in its host.

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## *Chauhanotrema spiniacetabulum* sp. n. (Digenea: Waretrematidae) from *Hemiramphus marginatus* (Forsskal) (Hemiramphidae) from the Kuwaiti Coast of the Arabian Gulf

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**ABSTRACT:** *Chauhanotrema spiniacetabulum* sp. n. from the intestine of *Hemiramphus marginatus* is described. It differs from *C. indica* Zukov, 1972, the only other species in the genus, by having a shorter esophagus and consequently an intestinal bifurcation near the anterior level of the ventral sucker instead of the posterior level. *Chauhanotrema* (Waretrematidae: Chauhanotrematinae) is characterized by a spiny cuticle, a ventral sucker lined with spines, two caeca extending to testicular level, a single testis, large vitelline follicles, a pretesticular globular ovary, presence of a true seminal receptacle, and absence of a cirrus sac.

**KEY WORDS:** digenetic trematodes, Waretrematidae, Waretrematinae, *Chauhanotrema*, *Hemiramphus marginatus*, marine fishes, Arabian Gulf, Kuwait.

During the course of a survey of helminth parasites of Kuwaiti coast fishes carried out by the second author between October 1992 and December 1996, 3 blackedge white-banded half-beaks, *Hemiramphus marginatus*, were found harboring a large number of digeneans. These worms are believed to represent a new species.

### Materials and Methods

The fish were obtained from the local fish market. Following necropsy, the worms were washed in saline, fixed in cold AFA under slight coverglass pressure, stored in 70% ethanol, stained with alum- or acetocarmine, destained in diluted HCl, dehydrated in ascending concentrations of ethanol, cleared in methylsalicylate or clove oil, and mounted in Canada balsam. Specimens for scanning electron microscope (SEM) examination were dried, using the critical point technique, and coated with gold-palladium; the ventral sucker was observed and photographed using a JEOL, JSM-6300 SEM. Drawings of Figures 1–3 were made by microprojection and details filled in through microscopic examination; details of internal anatomy and male and female terminal reproductive structures are

based on 5 sectioned specimens stained with Delafield's hematoxylin and eosin. Measurements are in micrometers, given as range with mean in parentheses. Sucker ratio was calculated from the average of the length plus width (depth) and expressed with the oral sucker as 1. The holotype is deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland; and paratypes in the National Reference Collection (NRC), Department of Zoology, Kuwait University, Kuwait; the Natural History Museum BM(NH), London; and the Harold W. Manter Laboratory (HWML), Nebraska State Museum, Lincoln.

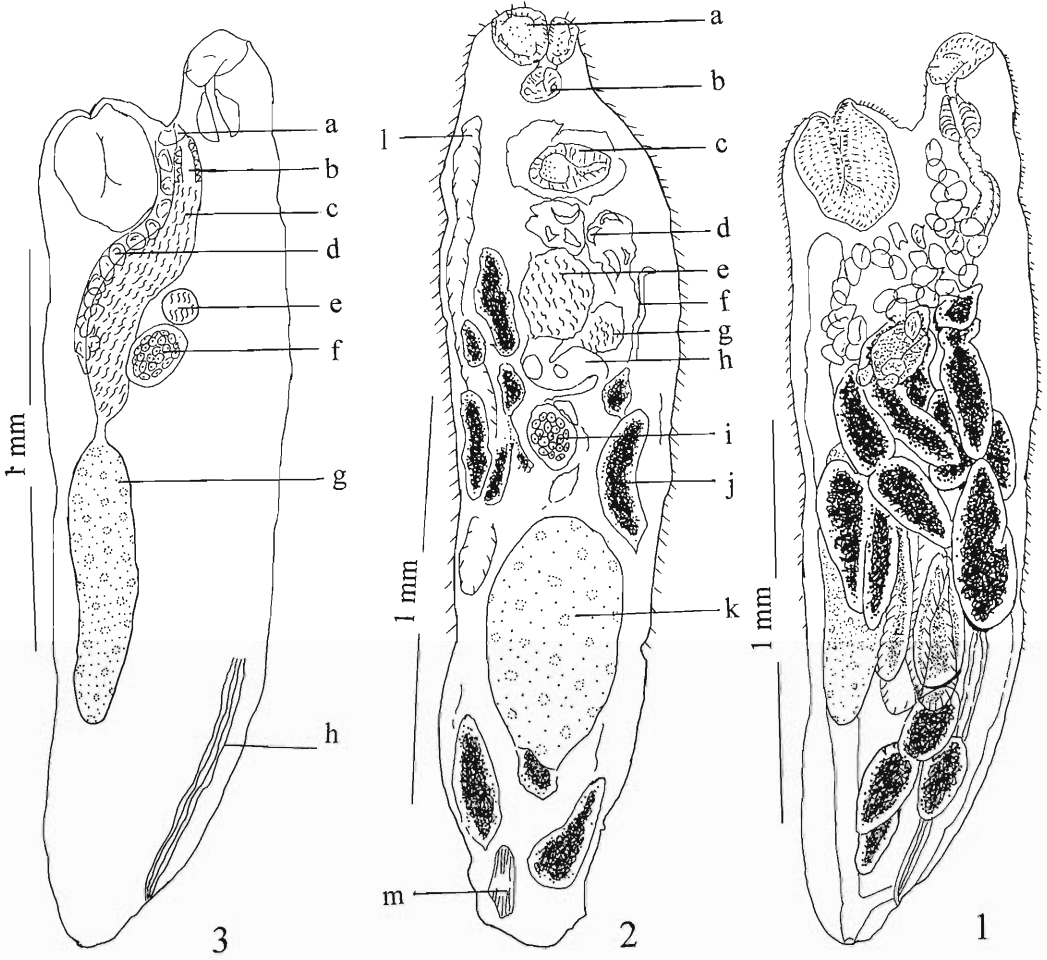
### *Chauhanotrema spiniacetabulum* sp. n. (Figs. 1–5)

**DESCRIPTION** (based on 26 mature worms, 5 sectioned specimens, and SEM micrographs): Body elongate to plump, 1180–2500 (1794) long, 325–625 (475) wide just posterior to ventral sucker. Tegument spinose, spines needle-like, 4–6 in length, extending to midlevel of testis. Eye spot pigments dispersed laterally in the pharyngeal area. Oral sucker terminal, 75–155 (98) long, 87–163 (130) wide; prepharynx short, about half pharyngeal length; pharynx 55–100 (75) long by 52–100 (76) wide; esophagus 50–125 (75), about same length as pharynx in well-extended specimens; intestinal bifurcation anterior to ventral sucker; caeca 2, narrow anteriorly, relatively wide posteriorly,

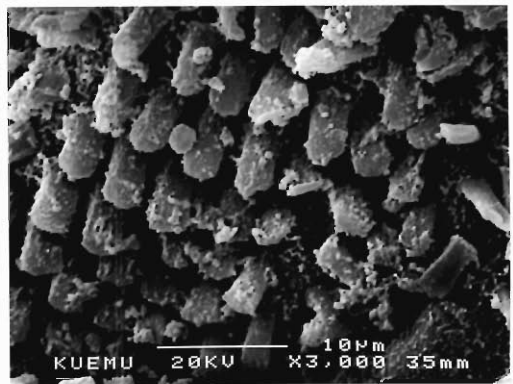
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Figures 1–5. *Chauhanotrema spiniacetabulum* sp. n., holotype from *Hemiramphus marginatus*, ventro-lateral view. 2. Tracing of an actual frontal section from a paratype: a. Oral sucker. b. Pharynx. c. Ventral sucker. d. Egg. e. Midsection of seminal vesicle. f. Laurer's canal. g. Seminal receptacle. h. Oviduct. i. Ovary. j. Vitelline follicle. k. Testis. l. Anterior segment of right caecum. m. Unidentified tubular structure. 3. Composite sketch from serial sections, male and female reproductive organs: a. Genital atrium. b. Pars prostatica. c. Seminal vesicle. d. Uterus. e. Seminal receptacle. f. Ovary. g. Testis. h. Unidentified tubular structure. 4. SEM photograph of ventral sucker showing acetabular spines, ×500 magnification. 5. Same as Figure 4, acetabular spines, ×3000 magnification.





4



5

extending to near posterior level of testis. Ventral sucker in anterior fifth of body, 140–270 (202) long by 180–300 (244) deep; its opening lined with 8–10 rows of spines (Fig. 4), each spine 6–8 long, somewhat flattened (Fig. 5). Sucker ratio 1:1.7–2.6 (1:2.2). Testis single, elongate, 400–750 (575) long by 200–300 (237) wide, approximately one third body length, mostly in upper posterior half of body; cirrus sac absent; vas deferens short; seminal vesicle long and wide, straight or sigmoid, approximately same length as testis, extending to anterior level of ventral sucker; pars prostatica cylindrical, short, surrounded by few cells, terminating by short muscular cirrus. Ovary globular, anterodorsal to testis, 138–200 (169) long, 163–180 (172) wide; Mehlis' gland and ootype lateral to ovary; seminal receptacle preovarian, adjacent to the base of the seminal vesicle; Laurer's canal present, near seminal receptacle, opening dorsally in anterior body third; uterus preovarian; metraterm weakly developed. Vitellaria 15–18 large, ovoid-to-elongate follicles of different sizes, 198–465 long by 100–150 wide, many larger than ovary, extending from near posterior level of ventral sucker to, but not reaching, posterior end of body. Eggs operculated, 62–70 by 42–50. Genital atrium small, thin-walled; pore median, between pharynx and ventral sucker. Excretory vesicle sac-like, small, excretory canals extending to near posterior level of ventral sucker.

#### Taxonomic summary

TYPE HOST: *Hemiramphus marginatus* (Forsskal, 1775) (Hemiramphidae).

SITE IN HOST: Intestine.

DATES OF COLLECTION: 15 October 1993 (6 in 1), 20 November 1996 (5 and 26 in 2).

PREVALENCE: 33.3% (3 of 9).

MEAN INTENSITY: 12.3 worms per host.

TYPE LOCALITY: Kuwaiti coast, Arabian Gulf.

HOLOTYPE: USNPC 87161.

PARATYPES: NRC 15, BM(NH) 1997.5.12.1–2, HWML 39355.

ETYMOLOGY: The species name reflects the worm's spiny ventral sucker.

REMARKS: *Chauhanotrema spiniacetabulum* is very similar to *C. indica* Zhukov, 1972, from *Hemiramphus far* (Forsskal) from India; this is evident in their shape, size, and measurements of most organs; it differs chiefly in having a shorter esophagus that extends to near anterior level of the ventral sucker and consequently more anterior location of the intestinal bifurcation. Zhukov (1972, p. 349) described the intestinal bifurcation "a little behind the lower edge of the ventral sucker" and the "gut-caeca, 0.49–0.58, as short, sac-like, lying in the middle of the body." In *C. spiniacetabulum*, the caeca are tubular. Zhukov's description of the ventral sucker is not very clear. He used the term "spines" to describe the tegument of *C. indica* but did not use the same term to describe the ventral sucker; his description is best translated as having a "fringe." Our description of the internal anatomy is based not only on observations made on whole mounts but also on 5 sets of serial sections, and our description of the ventral sucker, on SEM micrographs. The spines of the ventral sucker are about the same size as those of the

tegument except for a wider base. The SEM micrographs show several layers of spines (Figs. 4, 5), each having a flat rather than pointed tip. The presence or absence of a seminal receptacle in *Chauhanotrema* needs to be resolved; a seminal receptacle was not reported for *C. indica* and is not clearly evident in whole mounts of *C. spiniacetabulum*; some sperm are seen adjacent to the ovary, but whether the structure is a canalicular (true) or a uterine seminal receptacle could not be determined with certainty; several serial sections, however, suggest the presence of a distinct globular structure adjacent to the midsection of but independent of the seminal vesicle, with a tube (Fig. 2) leading toward the oviduct. If Zhukov's species lacks a canalicular seminal receptacle, then *C. spiniacetabulum* should be reassigned to a new genus and, perhaps, a new subfamily. Since Zhukov's description is based on whole mounts, and considering the difficulty we encountered in determining its presence in whole mounts, we prefer not to name a new genus.

Six specimens, slightly smaller than the others, collected 15 October 1993, are not included in measurements because of their poor condition; most of the tegumental spines and the acetabular spines were lost. In several specimens from the other hosts, the anterior part of the seminal vesicle was half as wide as shown in Figure 2. A thick-walled tubular structure (Figs. 1–3) terminating on the dorsal side near the posterior end of the body is seen in many whole mounts and serial sections, but its anterior origin could not be determined with certainty; it seems to originate behind the vitellaria, near the midlevel of the testis; it is not a Laurer's canal; its function is unknown. Such a structure was not described by Zhukov.

#### Discussion

The new species is placed in the genus *Chauhanotrema* on the basis of its similarity to *C. indica* Zhukov, 1972, from a related host, *Hemiramphus far* (Forsskal). The chief characteristics of *Chauhanotrema* are a spiny ventral sucker with rows of spines lining its opening, a single testis, and very large vitelline follicles, features that, in combination, do not fit into any known family described in Yamaguti (1971). *Chauhanotrema* bears a superficial resemblance to members of Haplospilachnidae Poche, 1926, especially in the presence of single testis (*Prohaplospilachnus diorchis* Tang and Lin, 1978, is the only exception) and the structure of the terminal reproductive organs. Traditionally, the ventral sucker has never been accorded more than a generic significance. However, other characteristics—the spiny ventral sucker, presence of 2 caeca, and a spiny tegument—rule it out, unless the family Haplospilachnidae is drastically revised. (For a review of Family Haplospilachnidae see Nahhas et al., 1997). Zhukov (1972)

placed the genus in Family Waretrematidae and subfamily Chauhanotrematinae.

### Acknowledgments

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### Erratum

An error was made in Table 1 for ages of owls in the article by S. J. Taft et al., 1997. Hematozoa of Spring- and Fall-Migrating Northern Saw-Whet Owls (*Aegolius acadicus*) in Wisconsin. *J. Helminthol. Soc. Washington* 64:296–298. The ages should read as follows: <1 Yr. 1 Yr. 2 Yr. ≥3 Yr.

## Taxonomic Status of *Halipegus* spp. (Digenea: Derogenidae) Parasitic in the Mouth and Eustachian Tubes of North American and Mexican Amphibians

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**ABSTRACT:** *Halipegus eccentricus* Thomas, 1939, and *H. amherstensis* Rankin, 1944, are shown to be junior synonyms, the latter only in part, of *H. occidualis* Stafford, 1905. The synonymy of *H. lermensis* Cabellero, 1941, with this latter species is also confirmed. Material redescribed as *H. occidualis* by Krull (1935) and *Cercaria sphaerula* Thomas, 1934, are shown to be junior synonyms of *Halipegus projecta* (Willey, 1930) n. comb. The type description for *H. amherstensis* Rankin, 1944, appears to be a composite based on specimens of *H. occidualis* and *H. projecta*. *Halipegus amherstensis* therefore becomes a junior synonym, in part, of *H. projecta* and, in part, of *H. occidualis*. We also review and correct reports of *Halipegus* spp. from North American and Mexican amphibians and indicate where continuing uncertainty exists.

**KEY WORDS:** amphibian, digenea, *Halipegus*, Mexico, North America, parasites.

Until recently, distinguishing the three North American species of *Halipegus* parasites in the mouths and eustachian tubes of frogs—*H. amherstensis* Rankin, 1944, *H. eccentricus* Thomas, 1939, and *H. occidualis* Stafford, 1905—has been considered impossible without reference to the morphology of the cercarial stage (Thomas, 1939; Rankin, 1944). However, as Goater (1989) has noted, since these digeneans mature in the mouth of the anuran definitive host and can be examined without killing the host, they offer unique opportunities for field studies that link host behavioral ecology with parasite population dynamics.

Using electrophoretic techniques, cercarial and adult morphology, and life history studies, Goater et al. (1990a, b) demonstrated that in *Rana clamitans*, worms they identified as *H. eccentricus* and *H. occidualis* showed a site fidelity that permits separation of these species—*H. eccentricus* being found in the eustachian tubes, *H. occidualis* being found under the tongue. They also confirmed subsequently that longer filaments project from the eggs of *H. occidualis* than from the eggs of *H. eccentricus*. These differences are in agreement with the description of *H. eccentricus* Thomas, 1939 (eustachian tube, egg filaments 56–58  $\mu\text{m}$ ), and the redescription of *H. occidualis* Stafford, 1905 (under tongue, egg filament 160–200  $\mu\text{m}$ ), provided by Krull (1935). Stafford (1900) first reported spec-

imens he later described as *H. occidualis* as the European frog ear-fluke, *H. ovicaudatum*, and noted he had collected the worms from the eustachian tubes of Ontario bullfrogs. Later (Stafford 1905) when he described the material as new he reported the egg filaments as “about 56  $\mu\text{m}$ ” but does not mention the site of infection in the host. It is clear that the original description of Stafford (1905) for *H. occidualis* is based on material which is in agreement with the description of worms designated *H. eccentricus* by Thomas (1939). Here we attempt to resolve certain taxonomic problems this presents, review and correct reports of *Halipegus* spp. from North American and Mexican amphibians, and indicate where there is continuing uncertainty over the identity of some *Halipegus* material. Previously, McCauley and Pratt (1961) dealt with *Halipegus aspina* Ingles, 1936, described from the stomach of *Rana boylei* in California. They proposed the genus *Deropegus* to accommodate this worm and identified it as normally a parasite of salmonid fishes.

### Materials and Methods

The following specimens were examined: *Halipegus amherstensis* Rankin, 1944: holotype plus two paratypes collected South Amherst, Massachusetts, from *Rana catesbeiana* (U.S. National Parasite Collection [USNPC] 36883); *Halipegus amherstensis* Rankin, 1944: 3 slides prepared by Cabellero (1947) in his redescription of this species, collected Xochimilco, Mexico, from *Rana montezumae* (Coleccion Helmintholo-

gica del Instituto, 22–13); *Halipegus eccentricus* Thomas, 1939: holotype and one immature collected Twin Lakes, Cheboygan County, Michigan, from *R. catesbeiana*, and one immature from *R. pipiens* tadpole infected experimentally from cyclops (USNPC 9203). Note: Thomas (1939) reports the type date as 2 July, 1933, but the type is labelled 1937; *Halipegus lermensis* Cabellero, 1941: single paratype collected in Lerma, Mexico, from *R. pipiens* or *R. montezumae* (USNPC 44988), plus an additional specimen identified by Cabellero (USNPC 87493); *Halipegus occidualis* Stafford, 1905: 20 vouchers collected by Brooks (1976) in Nebraska—18 from *R. catesbeiana* and 2 from *R. pipiens* (Harold W. Manter Laboratory, University of Nebraska State Museum, Lincoln [HWML] 20098–20102); and *Halipegus occidualis* Stafford, 1905: voucher reported as *Halipegus occidualis* sensu Krull (1935) by Russell and Wallace (1992) collected in northern Idaho from the eustachian tube of *R. pretiosa* (USNPC 81914).

### Results and Discussion

Apparently no type specimens now exist for *H. occidualis*. However, because material described by Thomas (1939) as *H. eccentricus* is identical to that described by Stafford (1905) as *H. occidualis*, *H. eccentricus* Thomas, 1939, must be considered a junior synonym of *H. occidualis* Stafford, 1905. The cystophorous *Cercaria californiensis* described by Cort and Nichols (1920) from Lake Temescal, near Oakland, California, possesses the cercarial streamers reported for *H. eccentricus* by Thomas (1939). Thomas (1939) noted that these forms were “similar in many respects,” and we can find nothing that distinguishes them from each other. However, as we also show here, there is uncertainty about the identity of several western North American *Halipegus* spp. or *Halipegus*-like worms. Although *C. californiensis* may indeed be a junior synonym of *H. occidualis*, we believe it is premature to synonymize these species until there is further study of the genus in western North America.

Material described as *H. lermensis* from Mexican *Rana montezumae* and *R. pipiens* by Cabellero (1941) was later (Rankin, 1944; Cabellero, 1947) regarded as a junior synonym of *H. occidualis*. The identity of the host is unclear. The *R. pipiens* complex includes more than 20 species (Hillis, 1988), but according to Flores-Villela (1993), *R. berlandieri* and *R. brownorum* are the only members of the complex in the area of the Yucatan Peninsula, near Lerma, where Cabellero (1941) collected his material. Cabellero (1941) reported that these worms were col-

**Table 1.** Comparison of morphometric features reported to separate *Cercaria projecta* Willey, 1930, and cercaria of *Halipegus occidualis* sensu Krull (1935). Means are in parentheses.

<i>H. projecta</i>	<i>H. occidualis</i>
Cercaria body 100–150 $\mu\text{m}$	Cercaria body 145–270 $\mu\text{m}$
Oral sucker; 20 $\mu\text{m}$	Oral sucker; 26–43 (33) $\mu\text{m}$
Ventral sucker; slightly larger than oral sucker	Ventral sucker; 22–53 (39) $\mu\text{m}$
Ceca extending almost to posterior end	Ceca extending to near posterior end
Cyst; 65–72 $\mu\text{m}$	Cyst; 72–83 (76) $\mu\text{m}$
Caudal appendage; 6 ridges	Caudal appendage; 4–6 (5) ridges
Delivery tube; 390–432 $\mu\text{m}$	Delivery tube; 420–470 (458) $\mu\text{m}$

lected from the eustachian tubes of frogs and that egg filaments were 74–78  $\mu\text{m}$ . It is difficult to get accurate measurements of filament lengths in *Halipegus* spp. when eggs are packed tightly in the uterus, as they are in the specimens we examined, but the two filaments we were able to measure were 58  $\mu\text{m}$  and 63  $\mu\text{m}$ . We are in agreement with Rankin (1944) and Cabellero (1947) that *Halipegus lermensis* is a junior synonym of *H. occidualis*.

It is clear that the specimens Krull (1935) used in his redescription of *H. occidualis* are different from those described by Stafford (1905). However, in describing his material, Krull noted similarities between it and *Cercaria projecta* of Willey (1930) collected from Henryville, Pennsylvania. On the basis of similarities between the rediae, Krull (1935) even went so far as to suggest that these might be identical species.

Krull (1935) distinguished his material from *C. projecta* on the basis of

the presence of cuticular projections at the anterior end of the body, the larger size of the cercaria body and suckers, longer ceca, the apparent difference in excretory systems, the apparent absence of a connection between the excretory system and the tail structures, larger cyst, number of ridges on the handle of the cyst, longer length of the delivery tube, absence of attachment of oral sucker to base of coiled delivery tube, and absence of uniform coiling of cercaria body in cyst.

Although Krull's material was somewhat larger than that described by Willey (1930), there is usually overlap in size (Table 1), and other dif-

**Table 2. Mollusk hosts recorded for North American *Halipegus* species.**

Parasite	Mollusk host	Source*
<i>Halipegus projecta</i>	<i>Heliosoma anceps</i>	Wiley (1930)
	<i>Planorbella trivolvis</i>	Thomas (1934)
	<i>H. anceps</i>	Krull (1935)
	<i>H. anceps</i>	Esch et al. (1997)
	<i>P. trivolvis</i>	Schmidt and Fried (1997)
<i>Halipegus occidualis</i>	<i>Planorbella occidentale</i>	Cort and Nichols (1920)
	<i>P. trivolvis</i> , <i>Physella gyrina</i> , <i>P. parkeri</i>	Thomas (1939)
	<i>P. gyrina</i> , <i>Physa</i> sp.	Ameel et al. (1949)
	<i>Physa</i> sp.	Guilford (1961)
	<i>P. trivolvis</i>	Brooks (1976)
	<i>P. gyrina</i>	Esch et al. (1997)
<i>Halipegus</i> sp.	<i>P. trivolvis</i>	Macy et al. (1960)

\* We have followed Burch (1989) for current nomenclature of mollusks, which is not necessarily the nomenclature that appears in these reports. The generic status of snails identified as *Physa* sp. in these reports is uncertain.

ferences are insignificant. The redescription of Krull (1935) is based largely on living material. He also provided some measurements of material fixed in 10% formalin, as well as stained and mounted specimens, noting that their smaller size is due to contraction. Wiley (1930) does not state whether his measurements are based on living or preserved specimens. Other differences may be due to the manner in which material was handled and maintained in the laboratory (Krull noted that cercariae kept in clean water lived up to 2 weeks, while Wiley kept his material alive more than 6 weeks under similar conditions) or the way in which observations were interpreted. The cuticular projections at the anterior end of the cercarial body reported by Krull (1935) are a good example. These projections, which are not present in the figures of Wiley (1930) are considered diagnostic by Krull (1935). However, examination of the photographs of *Halipegus* cercaria in Goater et al. (1990a) suggests that it would be easy to interpret these projections as present or absent in material examined by light microscope. We therefore consider *H. occidualis* of Krull, *née* Stafford, 1905, a new species whose larval stage is *Cercaria projecta*, and we rename it *Halipegus projecta* (Wiley, 1930) n. comb.

We also consider *Cercaria sphaerula* Thomas, 1934, collected from Mullet Lake, Michigan, a junior synonym of *C. projecta*. There appears to be nothing other than size distinguishing the two, a difference that could be due to development in different snail hosts. Thomas (1934) collected his material from the mollusk

*Heliosoma trivolvis* (= *Planorbella trivolvis*), whereas Wiley's specimens came from *H. antrosa* (= *H. anceps*). *Halipegus* spp. have been reported to use several species of pulmonate mollusks as intermediate hosts (Table 2).

Rankin (1944) described *H. amherstensis* on the basis of specimens collected from *R. catesbeiana* and *R. clamitans* from Massachusetts. *Halipegus amherstensis* was later reported from Mexico (Cabellero, 1947) but the material was reidentified subsequently as *H. lermensis* (= *H. occidualis*). The status of *H. amherstensis* reported from *R. esculenta* in Europe by Prudhoe and Bray (1982) is unknown. Russell and Wallace (1992) reported mistakenly that Bouchard (1951) collected *H. amherstensis* in Maine. Rankin (1944) reported that *H. amherstensis* used one of the intermediate snail hosts (*Physella gyrina*) reported by Thomas (1939) for *H. occidualis* and is found in the eustachian tubes of *R. clamitans* and *R. catesbeiana*, the same hosts of *H. occidualis*. Morphological differences between *H. amherstensis* and *H. occidualis* seem to be minor, except for egg filament lengths, which are reported as 120–170  $\mu\text{m}$ , close to the egg filament lengths of *H. projecta* as reported by Krull (1935). We have examined the type series (USNPC 36883) of *H. amherstensis* and find that the egg filament lengths of this material are much shorter. The filaments we were able to measure were 53  $\mu\text{m}$ , 55  $\mu\text{m}$ , and 58  $\mu\text{m}$  (paratype, slide 20), 52  $\mu\text{m}$  (paratype, slide 21), and about 50  $\mu\text{m}$  (holotype, slide 22). Filaments of the holotype are clearly of the short type, but they were either broken or packed so tightly that

we were unable to get precise measurements, even though some eggs were removed from the uterus. This suggests to us that these specimens are all *H. occidualis*. However, Rankin (1944, 1945) recovered *H. amherstensis* both from under the tongue and from the eustachian tube in natural and experimental infections. He also found some larval stages similar to *H. eccentricus* Thomas, 1939 (= *H. occidualis* Stafford, 1905), and others closer to *H. occidualis* Stafford, 1905, sensu Krull (1935) (= *H. projecta* (Willey, 1930)). However, we are unable to associate the cercarial stage illustrated by Rankin (1944) for *H. amherstensis* with either *H. eccentricus* Thomas, 1939, or *H. projecta*. Nonetheless, all other evidence indicates that the type description is a composite based on specimens of *H. occidualis* and *H. projecta*. *Halipegus amherstensis* Rankin, 1944, therefore becomes a junior synonym, in part, of *H. projecta* (Willey, 1930) and, in part, of *H. occidualis* Stafford, 1905.

Cabellero (1947) reported and redescribed *H. amherstensis* from *R. montezumae* from Xochimilco, Mexico. He does not indicate site of infection or provide measurements of egg filament length. We have examined the 3 slides in the series (Coleccion Helminologica del Instituto, 22–13). All are labelled “trompo de eustaquio,” and all eggs are of the short-filament type, indicating the material is *H. occidualis*. The identity of the host frog is, however, uncertain. Hillis et al. (1988) show frogs of both the Alpha and Beta species groups of the *R. pipiens* complex occupying the region of the Mexican plateau where collections were made.

Macy et al. (1960) described the adult of *H. occidualis* from the esophagus and upper stomach of the amphibians *R. aurora*, *Taricha granulosa*, and *Dicamptodon ensatus*, and its life cycle in *Helisoma subcrenatum* (= *Planorbella subcrenatum*) in northwestern Oregon. They report that cercariae are identical to those described by Krull (1935) (i.e., *H. projecta*), but the egg filament lengths in their material are noted as 61–100  $\mu\text{m}$ . The identity of one of these hosts, *Dicamptodon ensatus*, is now unclear. Populations in the area are now assigned to either *D. copei* or *D. tenebrosus* (see Good, 1989). We believe the specific identity of the *Halipegus* studied by Macy et al. (1960) is in need of confirmation.

Brooks (1976) reported *H. occidualis* from

the eustachian tube of *R. catesbeiana* and *R. pipiens* in Nebraska on the basis of the morphology of cercariae from *Helisoma trivolvis* (= *Planorbella trivolvis*). However, Brooks' (1976) vouchers (HWML 20098–20102) include some worms labelled as *H. eccentricus* and others as *H. occidualis*. We have examined 20 of these vouchers and find that 4 of those (2 from *R. catesbeiana*, 2 from *R. pipiens*) labelled as *H. occidualis* are of the long-egg filament type and therefore should be considered *H. projecta* (Willey, 1930), while the remaining 16, labelled *H. eccentricus* from *R. catesbeiana*, have a short egg filament and are therefore *H. occidualis* Stafford, 1905. Obviously, the report of Brooks (1976) includes both *Halipegus* species. We have also examined the Idaho specimens (USNPC 81914) from the eustachian tube of *R. pretiosa* reported as *H. occidualis* sensu Krull (1935) by Russell and Wallace (1992). Considering their location in the host and their egg filament lengths (53  $\mu\text{m}$ , 60  $\mu\text{m}$ , 65  $\mu\text{m}$ ), we have reidentified these specimens as *H. occidualis* sensu Stafford, 1905.

Given the work of Goater et al. (1990a, b) and the above discussion, it is evident that worms in the genus *Halipegus* found in the eustachian tubes of North American frogs, with egg filaments 53–65  $\mu\text{m}$  long, should be referred to *H. occidualis* Stafford, 1905, and that *H. eccentricus* Thomas, 1939, *H. lermensis* Cabellero, 1941, and *H. amherstensis* Rankin, 1944, are junior synonyms, the last only in part. *Halipegus* collected from under the tongues of North American frogs, with egg filaments of 160–200  $\mu\text{m}$ , should be referred to *H. projecta* (Willey, 1930), with *Cercaria sphaerula* Thomas, 1934, *H. occidualis* sensu Krull, 1935, and *H. amherstensis* Rankin, 1944, as junior synonyms—like-wise, the last only in part.

We propose that *Halipegus* species in North America inhabiting the eustachian tubes should be referred to *H. occidualis* Stafford, 1905. This includes reports from *R. catesbeiana* in Arkansas (Rosen and Manis, 1976), Idaho (Russell and Wallace, 1992), Michigan (Thomas, 1939; Muzzall, 1991), Nebraska (Brooks, 1976), and New Brunswick (McAlpine and Burt, 1998); *R. clamitans* in Massachusetts (Nickerson, 1898; Rankin, 1944), Michigan (Thomas, 1939; Ameel et al., 1947, 1949; and Muzzall, 1991), North Carolina (Goater et al., 1989), Wisconsin (Guilford, 1961; Williams and Taft, 1980), and New Bruns-

wick (McAlpine and Burt, 1998); and *R. pipiens* in Michigan (Thomas, 1939), Wisconsin (Williams and Taft, 1980), Nebraska (Brooks, 1976), and New Brunswick (McAlpine and Burt, 1998).

Species of *Halipegus* recovered from under the tongue of North American frogs should be referred to *Halipegus projecta* (Willey, 1930); their hosts are *R. catesbeiana* in Nebraska (Brooks, 1976); *R. clamitans* in Maryland (Krull, 1935), North Carolina (Goater, 1989; Goater et al., 1989; Goater et al., 1990a; Goater et al., 1990b), Massachusetts (Rankin, 1944), and Wisconsin (Gilford, 1961).

Esch et al. (1997), and numerous authors cited therein, studied *Halipegus* in the molluscan hosts *Helisoma anceps* and *Physa gyrina* (= *Physella gyrina*) and the frog *Rana clamitans* at the same study site in North Carolina as Goater (1989). These authors were able to confirm the identity of their material using cercarial morphology or a combination of site specificity and the length of the egg polar filament. Worms they reported as *H. occidualis* are here assigned to *H. projecta*. Worms they identified as *H. eccentricus* now become *H. occidualis*. Walton (1951) reported *H. occidualis* from *Rana sphenoccephala* in the United States with no supporting details, and it is therefore impossible to determine the specific identity of this material. Likewise, it is not possible to determine the specific identity of *Halipegus* sp. reported from *R. clamitans* in Michigan (Krull, 1935), *R. pretiosa* in Wyoming (Turner, 1958), or *R. pipiens* in Iowa (Ulmer, 1970). Bouchard (1951) reported *Halipegus* sp. from the eustachian tube and oral cavity of *R. clamitans*, *R. palustris*, and *R. septentrionalis* in Maine without indicating the site of infection in specific host species. Although *H. occidualis* would seem to be involved, it is not clear whether the eustachian tubes of all three hosts were infected, and it is likely that *H. projecta* was also present. On the basis of egg filament length, Jones (1956) assigned material to *H. occidualis* of Krull (1935, = *H. projecta*) but did not report the host or location of collection.

As noted above, the specific identity of *Halipegus* sp. from amphibians in some regions in western North America and Mexico remains unclear. We therefore recommend that investigators note as precisely as possible the site of infection in the amphibian host, report measurements of egg filament lengths, deposit vouchers of both

parasite and host in a permanent collection, and, wherever possible, examine cercariae.

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## ***Allopharynx macallisteri* sp. n. (Trematoda: Plagiorchiidae) from the Mourning Gecko, *Lepidodactylus lugubris*, from Guam, Mariana Islands, Micronesia, with a Key to the Species of the Genus *Allopharynx***

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**ABSTRACT:** *Allopharynx macallisteri* sp. n. (Trematoda: Plagiorchiidae), a new astriotrematid trematode from the small intestine of *Lepidodactylus lugubris*, is described and illustrated. Three of 21 (14%) adult specimens of *L. lugubris* collected from Guam harbored 7 specimens of *A. macallisteri* sp. n.; mean intensity was 2.3, range was 1–4. *Allopharynx macallisteri* sp. n. is distinguished from all other species in the genus by body size, location of cirrus and genital pore, distribution of vitellaria, and position of testes. This is the first report of a species of *Allopharynx* from a gecko host and the Pacific islands. A key to the species of *Allopharynx* is included.

**KEY WORDS:** digenea, Plagiorchiidae, *Allopharynx macallisteri*, gecko, *Lepidodactylus lugubris*, Guam, Micronesia.

Twenty-one female *Lepidodactylus lugubris* (Dumeril and Bibron, 1836) were collected by Richard D. Krizman from central Guam (13°27'N, 144°45'E) during 1976 and deposited in the herpetology collection of the Natural History Museum of Los Angeles County (LACM 141337–141366). All specimens were originally preserved in 10% formalin and later stored in 70% ethanol. Upon examination of the gastrointestinal tracts, we recovered 7 adult trematodes from 3 *L. lugubris*. Subsequent examination of these specimens revealed that they represented a new species within the genus *Allopharynx*, described herein.

### **Materials and Methods**

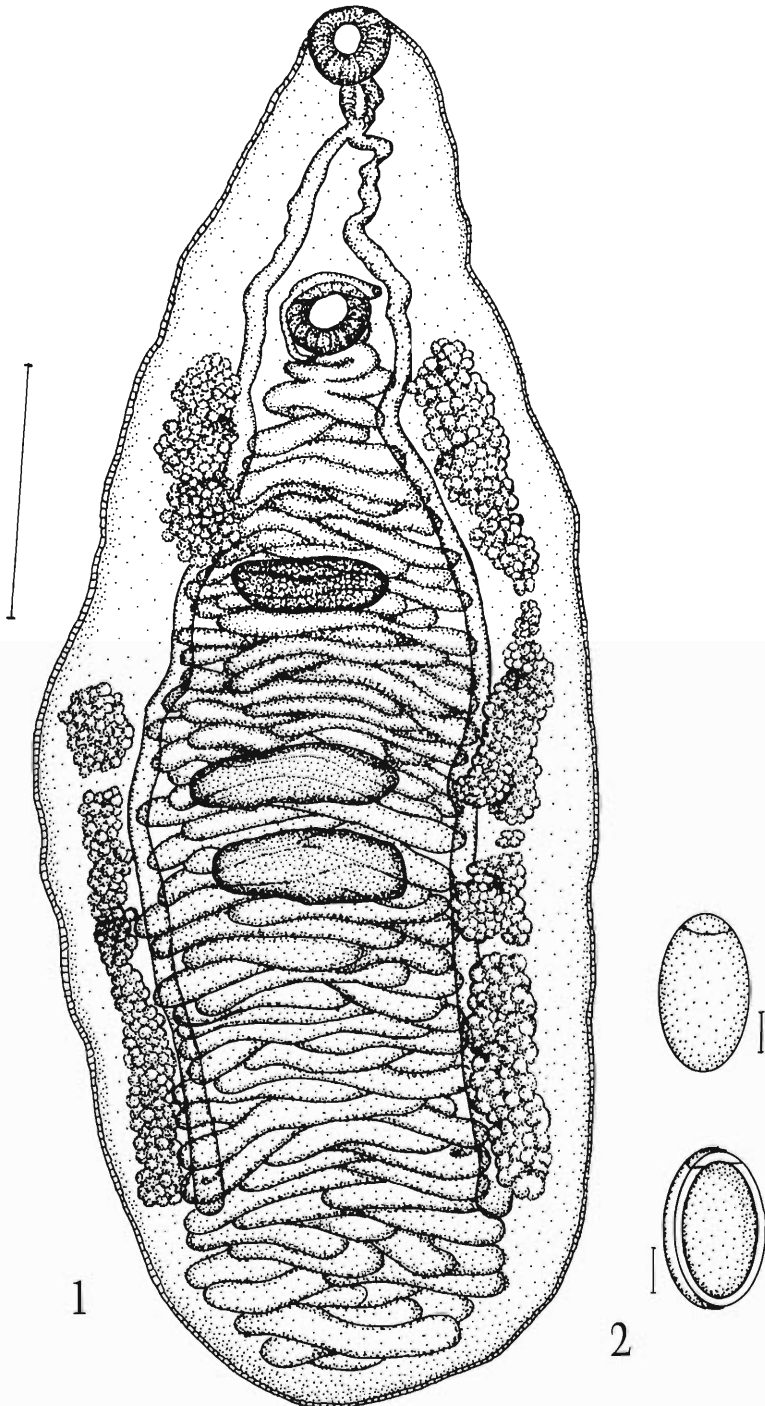
Adult worms were removed from the small intestines of 3 *L. lugubris*, rinsed in 70% ethanol, stained in Delafield's hematoxylin, dehydrated in ethanol, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube. Measurements are in micrometers unless otherwise indicated. The range is followed by the mean in parentheses. Type specimens were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland.

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### **Results**

#### ***Allopharynx macallisteri* sp. n. (Figs. 1 and 2)**

**DESCRIPTION** (based on 5 specimens): Plagiorchiidae (Lühe, 1901) Ward, 1917; Astriotrematinae Baer, 1924. Generic diagnosis based on Acholonu (1976). Body small, spatulate to pyriform, lacking spines, 2.2–3.6 mm (2.75) long, maximum width 0.60–0.95 mm (0.76) at midbody. Oral sucker terminal, ventrally directed, 124–171 (156) long by 124–175 (152) wide. Prepharynx very short to lacking. Pharynx 64–67 (66) long by 68–73 (71) wide. Esophagus very short. Ceca bifurcate just posterior to pharynx and extend to near posterior. Acetabulum approximates size of oral sucker, 126–156 (137) long by 144–192 (165) wide. Cirrus pouch elongate, 144–146 (145) long by 44–47 (45) wide, horizontal to anterior level of acetabulum with coiled seminal vesicle and short ejaculatory duct. Testes equatorial to postequatorial, transversely oval, anterior testis 127–130 (129) long by 443–447 (446) wide, posterior testis 150–154 (152) long by 406–410 (408) wide. Ovary preequatorial, transversely oval, 117–123 (120) long by 300–307 (305) wide, between acetabulum and testes. Mehlis' gland, seminal receptacle, and Laurer's canal



Figures 1, 2. *Alloharynx macallisteri* from *Lepidodactylus lugubris*. 1. Entire worm, ventral view. 2. Egg, longitudinal cut showing shell thickness and entire egg. Scale bars = 0.5 mm (Fig. 1) and 10  $\mu$ m (Fig. 2).

not observed. Vitellaria in solid fields of follicles, with gaps between fields, lateral to or slightly overlapping cecal arms, beginning just posterior to acetabulum and extending to near posterior extremity. Uterus extensive, extending to posterior end of body, overlapping cecal arms, highly convoluted, passing between testes, continuing between anterior testis and ovary before looping in preovarial region and passing dextral to acetabulum. Eggs, operculate, thick-shelled, 34–36 (35) long by 22–24 (23) wide. Genital pore, ventrosinistral, immediately anterior to acetabulum, approximately 120 posterior to cecal bifurcation.

**Taxonomic Summary**

TYPE HOST: *Lepidodactylus lugubris*.

TYPE LOCALITY: Guam, Mariana Islands, Micronesia.

SITE: Small intestine.

DEPOSITED SPECIMENS: Holotype USNPC 86935; paratype USNPC 86936.

ETYMOLOGY: The species is named after Chris T. McAllister for his contributions to the parasitology of amphibians and reptiles.

**Discussion**

The genus *Allopharynx* (Shtrom, 1928) Price, 1938, currently contains eleven species (Table 1). Gupta and Sharma (1973) have suggested suppressing the genera *Allopharynx* and *Microderma* Mehra, 1931, to subgeneric rank within the genus *Glossimetra* Mehra, 1937. However, *Microderma* and *Glossimetra* are known only from freshwater turtles and are morphologically distinct from *Allopharynx* in size and placement of the cirrus complex, which extends posteriorly to the level of the ovary in *Glossimetra* and nearly half that distance in *Microderma*, as well as in the shape and distribution of vitellaria. Thus, we believe the genus *Allopharynx* is valid and must be maintained.

*Allopharynx macallisteri* sp. n. most closely resembles *A. leiperi* Simha, 1965, *A. puertoricensis* Acholonu, 1976, and *A. parorchis* Wang, 1980, in small body size (all under 4 mm in length). It also resembles *A. tropidonoti* (MacCallum, 1918) Price, 1938, in shape of ovary and testes (transversely oval). However, *A. macallisteri* differs from *A. leiperi* and *A. tropidonoti* in lacking tegumental spines, and from these two species as well as *A. parorchis* in placement of cirrus and genital pore (lateral,

**Table 1. Species in the genus *Allopharynx*.**

Species	Length (mm)	Width (mm)	Egg (µm)	Locality	Host	Reference
<i>A. amudariensis</i> (Shtrom, 1928) Price, 1938	4.9	1.4	36 × 19.6	Kazakhstan	<i>Natrix tessellata</i>	Price, 1938
<i>A. japonica</i> Tamura, 1941	5.3	1.3	34 × 17	Japan	<i>Elaphe quadrivirgata</i>	Tamura, 1941
<i>A. leiperi</i> Simha, 1965	1.7–2.2	0.1–0.3	40 × 20	India	<i>Xenochrophis piscator</i>	Simha, 1965
<i>A. macallisteri</i> sp. n.	2.2–3.6	0.6–0.9	35 × 23	Guam	<i>Lepidodactylus lugubris</i>	(This paper)
<i>A. megorchis</i> Simha, 1961	4.4–5.5	2.1–3.2	11 × 1.7*	India	<i>Pygas mucosus</i>	Simha, 1961
<i>A. mehrai</i> (Gogate, 1935) Price, 1938	4.5	1.0–1.6	41 × 19	Burma	<i>Pygas korros</i> , <i>P. mucosus</i>	Gogate, 1935
<i>A. multispinosa</i> (Bennett, 1935) Price, 1938	5.4	1.5	27 × 15	U.S.A.	<i>Anolis carolinensis</i>	Bennett, 1935
<i>A. parorchis</i> Wang, 1980	3.3	1.4	36 × 21	China	<i>Dinodon rufozonatum</i>	Wang, 1980
<i>A. puertoricensis</i> Acholonu, 1976	3.5	1.5	28 × 17	Puerto Rico	<i>Anolis cristatellus</i>	Acholonu, 1976
<i>A. riopedrensis</i> Garcia-Diaz, 1966	4.9–6.7	1.6–2.1	20 × 14	Puerto Rico	<i>Anolis cristatellus</i>	Garcia-Diaz, 1966
<i>A. tropidonoti</i> (MacCallum, 1918) Price, 1938	5.0	1.7–2.0	37 × 19	Java	<i>Simonatrix trianguligera</i>	MacCallum, 1918

immediately above acetabulum in *A. macallisteri*; median, just posterior to cecal bifurcation in *A. leiperi*, *A. tropidonoti*, and *A. parorchis*. *Allopharynx macallisteri* differs from the other small-bodied species (*A. puertoricensis*) in lacking a long prepharynx as well as vitelline follicle arrangement and distribution (clustered in solid fields with gaps in *A. macallisteri*, not clustered and continuous in *A. puertoricensis*).

The type species for the genus *Allopharynx*, originally *Distomum tropidonoti*, was described from the gallbladder of the snake, *Sinonatrix trianguligera* (= *Tropidonotus trianguligerus*) from Indonesia. Price (1938) restudied the specimen and concluded that, in the original description, the anterior testis was mistaken for the ovary, a genito-intestinal canal that was described did not exist, and "the measurements were for the most part erroneous." Price (1938) also synonymized the genera *Ophiorchis* Mehra, 1937, *Ptyasiorchis* Mehra, 1937, and *Megacustis* Bennett, 1935, with *Allopharynx*, placing 4 species in the genus: *A. tropidonoti*; *A. amudariensis* (Shtrom, 1928); *A. mehrai* (Gogate, 1935) from species of snakes; and *A. multispinosa* (Bennett, 1935) from the lizard, *Anolis carolinensis*. The only other species of *Allopharynx* to be described from lizards are *A. riopedrensis* García-Díaz, 1966, and *A. puertoricensis* Acholonu, 1976, both from *Anolis cristatellus* in Puerto Rico. *Allopharynx macallisteri* is the first member of this genus to be reported from a gecko host.

Because distinguishing *A. macallisteri* from the other species of the genus is difficult given the overlap in body size measurements (Table 1), and because host species and locality are not necessarily good criteria (*A. puertoricensis* and *A. riopedrensis* are from the same host and locality), a key to the species of *Allopharynx* is included.

#### Key to the Species of *Allopharynx*

- |   |                       |
|---|-----------------------|
| 1a. Body length more than 4 mm .....                            | 2                     |
| 1b. Body length less than 4 mm .....                            | 8                     |
| 2a. Body tegument with spines .....                             | 3                     |
| 2b. Body tegument lacking spines .....                          | 7                     |
| 3a. Body length reaching 5 mm .....                             | 4                     |
| 3b. Body length less than 5 mm .....                            | 6                     |
| 4a. Cirrus pouch and metraterm separated by an acetabulum ..... | <i>A. tropidonoti</i> |

- |   |                          |
|---|--------------------------|
| 4b. Cirrus pouch and metraterm not as above ..... | 5                        |
| 5a. Egg length more than 30 $\mu$ m .....         | <i>A. japonica</i>       |
| 5b. Egg length less than 30 $\mu$ m ..            | <i>A. multispinosa</i>   |
| 6a. Body width more than 2 mm ...                 | <i>A. megorchis</i>      |
| 6b. Body width less than 2 mm .....               | <i>A. mehrai</i>         |
| 7a. Prepharynx present .....                      | <i>A. riopedrensis</i>   |
| 7b. Prepharynx absent .....                       | <i>A. amudariensis</i>   |
| 8a. Prepharynx present .....                      | <i>A. puertoricensis</i> |
| 8b. Prepharynx absent .....                       | 9                        |
| 9a. Genital pore lateral .....                    | <i>A. macallisteri</i>   |
| 9b. Genital pore median .....                     | 10                       |
| 10a. Body width less than 1 mm .....              | <i>A. leiperi</i>        |
| 10b. Body width more than 1 mm ....               | <i>A. parorchis</i>      |

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## ***Himasthla catoptrophori* sp. n. (Trematoda: Echinostomatidae) from Willets, *Catoptrophorus semipalmatus* (Charadriiformes: Scolopacidae), from the Galveston, Texas Area**

NORMAN O. DRONEN,<sup>1,3</sup> JANE E. BADLEY,<sup>1</sup> AND WILLIAM J. WARDLE<sup>2</sup>

<sup>1</sup> Laboratory of Parasitology, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843 and

<sup>2</sup> Department of Marine Biology, Texas A&M University—Galveston, Galveston, Texas 77553

**ABSTRACT:** During a study of digeneans of shore birds from the Galveston, Texas, area of the Gulf of Mexico, 40% (39 of 99) of willets, *Catoptrophorus semipalmatus* (Charadriiformes), were found to be infected with an undescribed species of Echinostomatidae (19 per infected host), *Himasthla catoptrophori* sp. n. The new species can be distinguished from others in the genus by the 40 collar spines, 2 more than the maximum reported previously. The new species most closely resembles *H. californiensis* and *H. rhigedana*, but in addition to having 2 more collar spines, *H. catoptrophori* sp. n. has a smaller ratio of the transverse diameter of the oral sucker to the acetabulum (1:2.0–2.1, as compared to 1:2.8 and 1:2.6–3.0, respectively). It is also smaller than *H. californiensis*, 18 mm in length as compared to 8 mm.

**KEY WORDS:** *Catoptrophorus semipalmatus*, Echinostomatidae, *Himasthla catoptrophori* sp. n., Gulf of Mexico, Galveston, Texas.

Of the 24 species of *Himasthla* Dietz, 1909, currently recognized, 13 have been reported from the western hemisphere: *H. alincia* Dietz, 1909; *H. californiensis* (sensu latu *H. rhigedana* Adams and Martin, 1963) Deblock, 1966; *H. compacta* Stunkard, 1960; *H. elongata* (Mehlis, 1831) Dietz, 1909; *H. incisa* Linton, 1928; *H. leptosoma* (Creplin, 1829) Dietz, 1909; *H. littorinae* Stunkard, 1966; *H. mcintoshi* Stunkard, 1960; *H. muehlensi* Vogel, 1933; *H. piscicola* Stunkard, 1960; *H. quissetensis* (Miller and Northrup, 1926) Stunkard, 1938; *H. rhigedana* Dietz, 1909; and *H. tensa* Linton, 1940. Stunkard (1960) considered *H. tensa* to be synonymous with *H. elongata*; however, unlike *H. tensa*, the vitellaria of *H. elongata* extend anteriorly to the level of the posterior margin of the cirrus sac, suggesting that *H. elongata* is distinct from *H. tensa*.

During a study of intestinal helminths of shorebirds from the Texas gulf coast, specimens of an undescribed species of *Himasthla* with 40 collar spines were found in willets, *Catoptrophorus semipalmatus* (Gremlin, 1789). *Himasthla rhigedana* (Russell, 1960) and *H. quissetensis* (Bush, 1990) have been reported previously from willets.

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### **Materials and Methods**

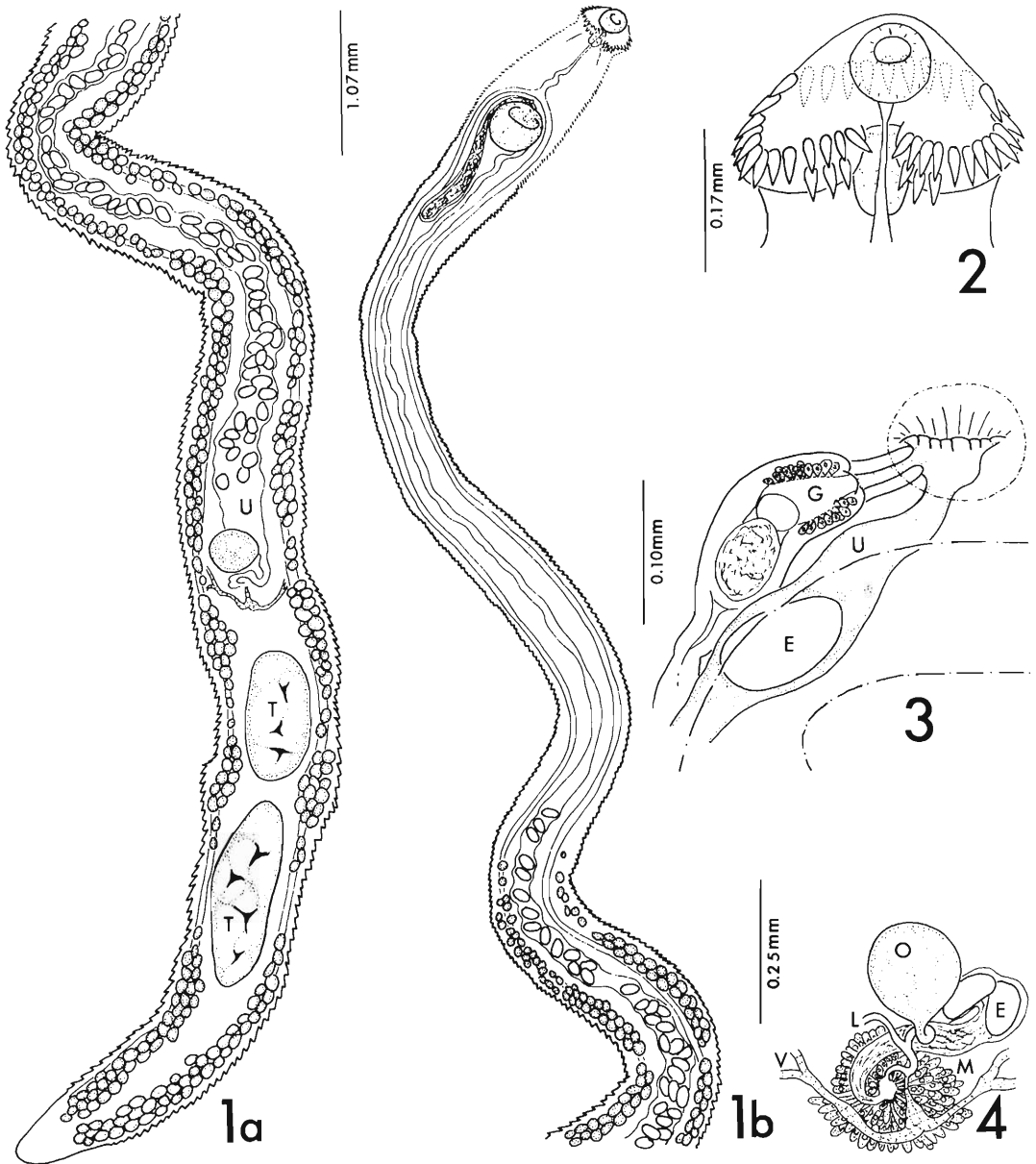
Ninety-nine willets were collected from the Galveston area of the Gulf of Mexico (89 between December 1977 and November 1978; 8 in August 1992; 2 in August 1994) and were examined for intestinal helminths. Trematodes were relaxed in saline, fixed in AFA, stained in Semichon's carmine or fast green, and mounted in Kleermount<sup>®</sup> or Canada balsam. Some specimens were sectioned by conventional paraffin technique. Drawings were done with the aid of a drawing tube. Measurements are in micrometers, with the mean followed by the range, in parentheses, unless otherwise stated.

### **Results**

Thirty-nine of 99 (40%) willets were infected with an undescribed species of *Himasthla*, with a mean intensity of 19 (3–21).

### ***Himasthla catoptrophori* sp. n. (Figs. 1–4)**

**DESCRIPTION** (based on 15 specimens): Echinostomatidae. Body with large spines 18 (9–32) mm long by 750 (373–980) wide, giving the tegument a serrated appearance. Forebody 805 (600–1,125) long, oral sucker 125 (100–155) long by 140 (110–165) wide with reniform collar bearing 40 spines 45 (40–50) long, arranged in a single continuous row of 34, with 3 additional corner spines in a separate row on each side. Acetabulum 284 (240–330) long by 280 (240–330) wide, located in upper 1/22 of body. Ratio of transverse diameter of oral sucker to



Figures 1–4. *Himasthla catoptrophori* sp. n. 1. Ventral view of body; a. posterior half showing testes (T) and uterus (U), b. anterior half. 2. Head collar and spine arrangement. 3. Genital pore area showing an egg (E), male terminal genitalia (G), and uterus (U). 4. Ovary and ootype region showing an egg (E), Laurer's canal (L), Mehlis' glands and ootype (M), ovary (O), and vitelline ducts (V).

acetabulum, 1:2.0–2.1. Mouth subterminal, pre-parynx short; pharynx 120 (110–135) long by 80 (70–95) wide; esophagus 525 (360–740) long, bifurcating immediately anterior to acetabulum; ceca long, terminating near posterior extremity. Testes smooth, tandem, in posterior ex-

trermity; anterior testis 1,020 (570–1,500) long by 295 (230–350) wide; posterior testis 1,095 (690–1,490) long by 280 (235–345) wide. Seminal vesicle bipartite, enclosed in cirrus sac, 790 (510–1,050) long, overreaching acetabulum by approximately 3 times its length into the hind-



body. Genital pore on ventral surface, immediately preacetabular on midline of body. Ovary smooth, spherical, 205 (110–290) long by 230 (130–325) wide, located approximately the length of one of the testes anterior to the anterior testis. Oviduct arises from ovary posteriorly, ootype situated immediately posterior to ovary, Laurer's canal present. Vitellaria in lateral fields extending from approximately midbody to near posterior extremity, often interrupted in testicular regions. Uterus essentially preovarian, intercecal, initial portion usually filled with spermatozoa. Eggs 99 (90–115) long by 75 (60–92) wide.

**TYPE HOST:** *Catoptrophorus semipalmatus* (Gremlin, 1789). Specimens deposited in the Texas Cooperative Wildlife Collection, Texas A&M University, College Station, Texas 77843. Museum no. 10458.

**TYPE LOCALITY:** Galveston County, Texas, west bay area.

**SITE OF INFECTION:** Small intestine.

**HOLOTYPE:** U.S. National Parasite Collection (USNPC) 75300.

**PARATYPES:** USNPC 75301 and the University of Nebraska State Museum, HWML (2 specimens) 39467.

**ETYMOLOGY:** The species name refers to the genus of its host, *Catoptrophorus*.

### Discussion

*Himasthla catoptrophori* sp. n. can be distinguished from other species in the genus by having 40 collar spines, 2 more than reported previously. Of the 13 species of *Himasthla* known to occur in the western hemisphere, only *H. alinca* (28–31 collar spines), *H. californiensis* (38), *H. leptosoma* (29), *H. mcintoshi* (35), *H. piscicola* (29), and *H. rhigedana* (34–38) are similar to *H. catoptrophori* n. sp. in having the

vitellaria commencing well behind the posterior margin of the cirrus sac. The new species resembles both *H. californiensis* described by Adams and Martin (1963) and *H. rhigedana* described by Dietz (1910), but in addition to having more collar spines, *H. catoptrophori* sp. n. differs from both of these species in having a smaller ratio of the transverse diameters of the oral sucker to acetabulum (1:2.0–2.1, as compared to 1:2.8 in *H. californiensis* and 1:2.6–3.0 in *H. rhigedana*) and an intermediate egg size (99 long, as compared to 112 in *H. californiensis* and 74–82 in *H. rhigedana*). It is also smaller than *H. californiensis* (18 mm long as compared to 8 mm).

### Acknowledgments

We thank the Texas Parks and Wildlife Department, whose cooperation made this study possible, and Dr. J. R. Lichtenfels, for access to specimens of *Himasthla* spp. We also thank Trudy Belz, Texas City, and Dr. Jackie Cole, Galveston, for help in collecting willets.

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***Choricotyle leonilavazquezae* sp. n. (Monogenea: Diclidophoridae)  
Parasitic on *Microlepidotus brevipinnis* (Osteichthyes: Haemulidae)  
from Chamela Bay, Jalisco, México**

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**ABSTRACT:** A new species of *Choricotyle* van Beneden and Hesse, 1863, is described from the gills of the sarangola *Microlepidotus brevipinnis* (Steindachner) Jordan and Fisher, (Haemulidae), from the Pacific Ocean in Chamela Bay, Jalisco State, México. Between May 1993 and May 1995, 53 specimens of *M. brevipinnis* were collected and examined for parasites. *Choricotyle leonilavazquezae* sp. n. is distinct from other species within the genus because of the presence of 2 well-developed horseshoe-shaped oral suckers, the shorter longitudinal axis of the haptor, the presence of 8 spines in the genital atrium, and the distribution of vitellaria that extend to the basal area of the peduncles.

**KEY WORDS:** monogenea, *Choricotyle leonilavazquezae* sp. n., *Microlepidotus brevipinnis*, gills, Chamela Bay, México.

Twenty species of the genus *Choricotyle* have been reported from marine teleosts belonging to different families in temperate and subtropical waters of the world (Crane, 1972; Luque et al., 1993). Other species of *Choricotyle* in teleosts along the Pacific coast of México include those recorded by Bravo (1953) (*C. caulolatlili* Meserve, 1938, parasitizing *Trachurops crumenophthalmus* Bloch), by Caballero and Bravo (1962) (*C. sonorensis* Caballero and Bravo, 1962, from *Microlepidotus inornatus* Gill), and by Bravo (1966) (*C. pacifica*, parasitizing *Umbina sinaloae* Scofield). *Choricotyle sonorensis* was considered as species inquirenda by Mamaev (1976) because its description was based on one specimen; however, Tantaleán et al. (1988) found that this species is a common parasite infecting the haemulid *Isacia conceptionis* from the central Peruvian coast. *Choricotyle pacifica* was transferred to *Hargicotyle* by Mamaev (1972) because of the presence of numerous spines in the genital atrium.

During a survey of the helminth parasites of fishes from Chamela Bay, Jalisco State, México, we collected numerous specimens of an undescribed species of *Choricotyle* parasitizing the sarangola, *Microlepidotus brevipinnis*. We describe that species herein.

#### Materials and Methods

Fifty-three specimens of *M. brevipinnis* were caught in Chamela Bay, using gill nets, between May 1993

and May 1995. Chamela Bay is located on the W coast of México (19°30', 19°32' latitude N and 105°06' longitude W). Dissection of hosts, collection, fixation, and staining of monogeneans follow procedures described by Leon-Regagnon et al. (1997). Specimens were deposited in the Colección Nacional de Helmintos (CNHE), Mexico City, and in the U.S. National Parasite Collection (USNPC), Beltsville, Maryland. Drawings were prepared with the aid of a camera lucida, and measurements, given in micrometers, are presented as the range followed by the mean  $\pm$  1SD, in parentheses. When the number of measurements differs from 10, sample size is mentioned. Terminology of the clamps follows that of Llewellyn (1958).

#### Results

##### *Choricotyle leonilavazquezae* sp. n. (Figs. 1–6)

**DESCRIPTION** (based on 31 flattened specimens, of which 10 were measured): Diclidophoridae, Choricotylineae. Body elongate, distinctly set off from haptor, flattened dorsoventrally; haptor relatively extended, palmate. Body length (including haptor) 910–3,370 (2,240  $\pm$  730), width 500–750 (350  $\pm$  160). Tegument smooth, 3–4 (3  $\pm$  2) thick. Prohaptor rounded, with 2 well-developed horseshoe-shaped suckers; prohaptor sucker 90–225 (140  $\pm$  50) long by 90–210 (120  $\pm$  50) wide, located immediately below a subterminal mouth surrounded by muscle; pharynx ovoid, posterior to suckers, 70–100 (80  $\pm$  20) long by 50–70 (60  $\pm$  20) wide; esophagus tubular, short. Intestinal bifurcation at level of genital atrium; intestinal caecae ramified, branches not extending beyond haptor.

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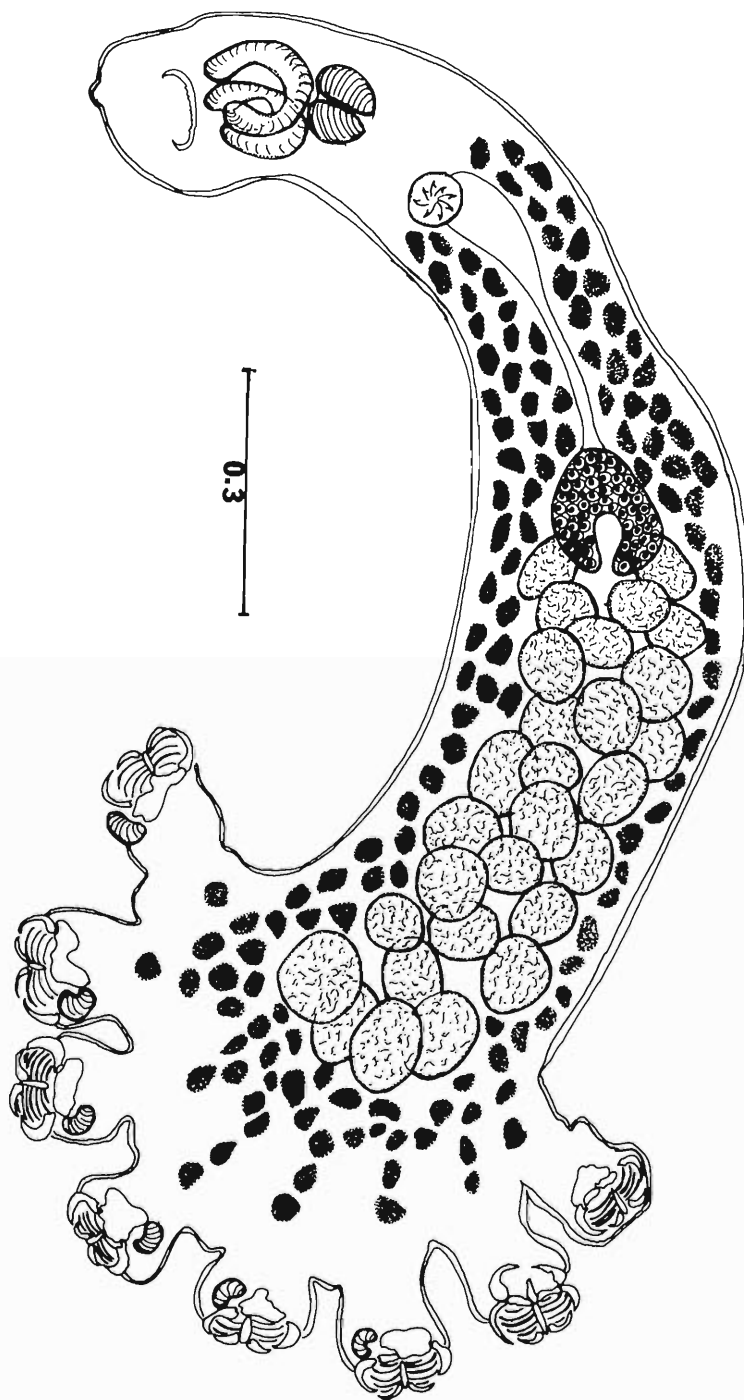
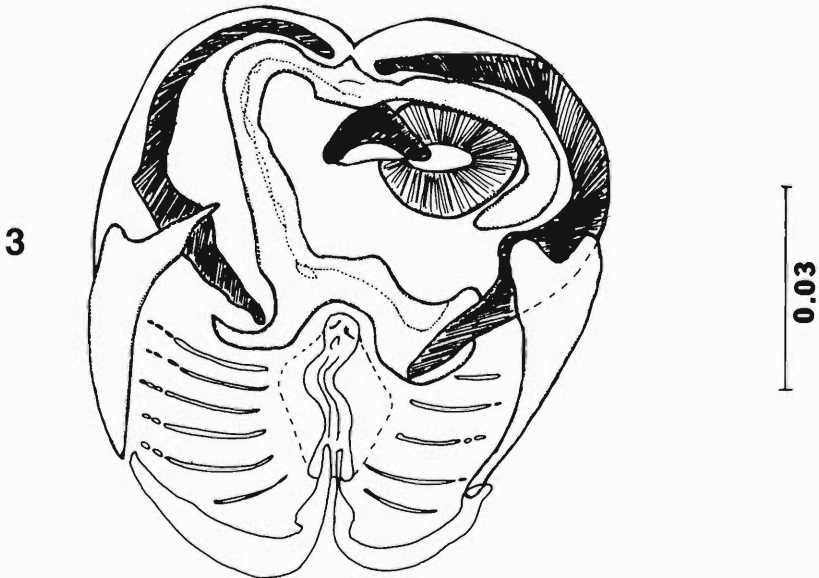
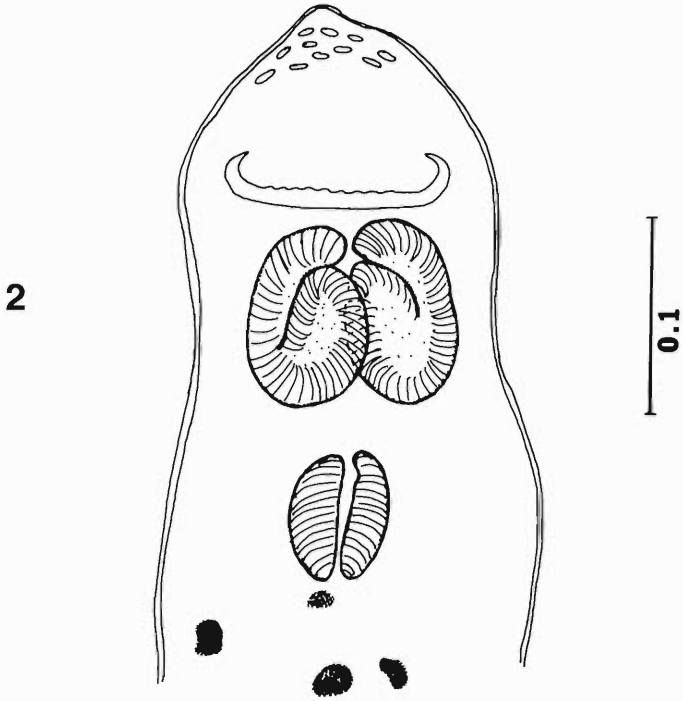
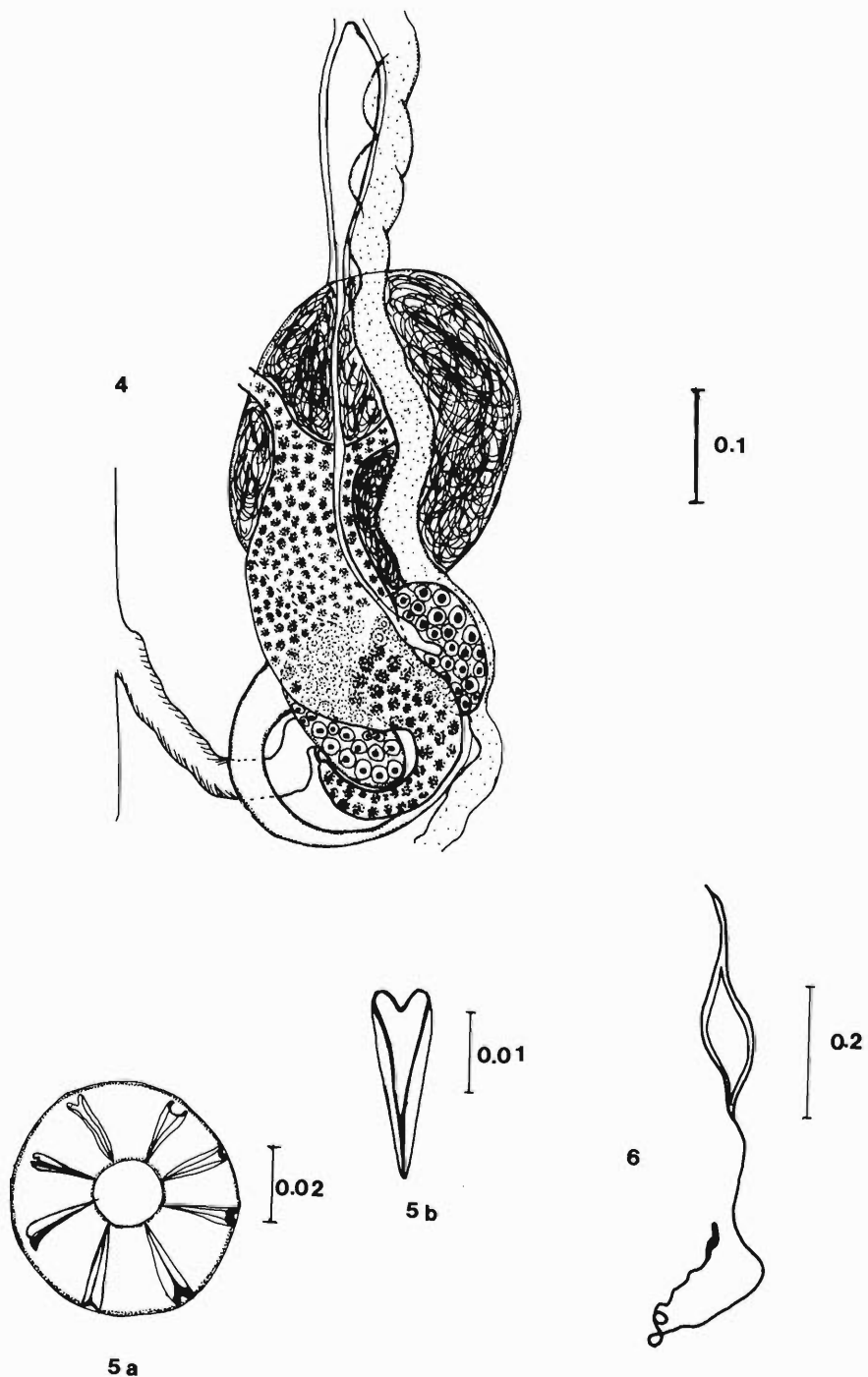


Figure 1. Whole mount illustration of *Choricotyle leonilavazquezae* sp. n., ventral view of the holotype.



Figures 2-3. 2. *Choricotyle leonilavazquezae* sp. n., anterior part of the body showing the horseshoe shape of oral suckers. 3. *Choricotyle leonilavazquezae* sp. n., structure of the clamp.



Figures 4–6. 4. *Choricotyle leonilavazquezae* sp. n., detail of the female reproductive system. 5. a. *Choricotyle leonilavazquezae* sp. n., genital atrium with 8 curved spines. b. Spine of the genital atrium. 6. *Choricotyle leonilavazquezae* sp. n., egg with polar filaments.

Haptor lacking terminal lappet, with 8 unequal clamps, clamp peduncles unequal, 50–100 ( $90 \pm 20$ ) long (excluding clamps). Two pairs of larval hooks occur between peduncles 4 and 5; first hook pair simple, 7.5 ( $n = 2$ ) long; second pair curved, 8.7 long. Clamps typical of *Choricotyle*; anterior midsclerite subtriangular, thick, curved anteriorly, possessing one muscular pad, associated with anterolateral and axial sclerites and small accessory sclerite. Anterior midsclerite articulated at base with the posterior midsclerite. Posterior midsclerite short, with midlongitudinal groove, slightly curved, base articulated with the first pair of posterolateral and axial sclerites (ventral); ventral axial sclerite curved, in contact with second pair of posterolateral and axial sclerites (dorsal). Posterior quadrants of clamp with 6–7 concentric arcs of skeletal rods.

Testes 33 to 39, intercecal, postovarian; each rounded to oval, shaped 40–120 ( $80 \pm 20$ ) long by 50–120 ( $90 \pm 20$ ) wide. Vas deferens sinuous, extending anteriorly in midline of body dorsal to ovary, opening in genital atrium. Genital atrium spherical, 30–60 ( $50 \pm 10$ ) in diameter, with 8 curved spines; spines 16–18 ( $17 \pm 0.4$ ) long by 6–7 ( $6.5 \pm 0.5$ ) wide.

Ovary bilobed (lobes directed backwards) 80–160 ( $130 \pm 30$ ); oviduct arising from right ovarian lobe, receiving ducts from seminal receptacle and vitellaria; transverse vitelline ducts join anteriorly to ovary to form saccular vitelline reservoir; oviduct surrounded by Mehlis gland. Ciliated genitointestinal canal originates from oviduct; seminal receptacle well developed, preovarian. Uterus extends anteriorly along midline of body to genital atrium. Vitelline follicles, coextensive and dorsal to intestinal ceca, from the level of genital pore into the haptor to base of peduncles. Eggs yellow, fusiform, with 2 polar filaments, 0.40–0.43 ( $0.41 \pm 0.01$ ) long by 0.07 wide (excluding filaments).

### Taxonomic Summary

TYPE HOST: *Microlepidotus brevipinnis* (Steindachner, 1869) Jordan and Fisher, 1895 (Haemulidae).

SITE: Gills.

TYPE LOCALITY: Bahía de Chamela, Estado de Jalisco, México (19°31'N, 105°04'W)

PREVALENCE AND MEAN INTENSITY: 22.6% (2.54 worms per infected host.)

SPECIMENS DEPOSITED: Holotype: Colección Nacional de Helminthos (CNHE), Mexico City,

No. 2836. Paratypes: CNHE Nos. 2837–2838, and United States National Parasite Collection, Beltsville, Maryland (USNPC) No. 87057.

ETYMOLOGY: The species is named after Dr. Leonila Vazquez García, eminent Mexican biologist, who dedicated 61 years of her life to study arthropods of México and who died in 1995.

### Discussion

*Choricotyle* van Beneden and Hesse, 1863, was established for *C. chrysophryi*, a parasite of *Chrysophrys aurata* in Belgium (Yamaguti, 1963). Composition of this genus is problematic, since there is no agreement between several authors about taxonomic validity of some species and transference of some of them to other genera (Mamaev, 1972; Oliva, 1987; Luque et al., 1993). In this work, we take the most conservative position and consider the genus to include 20 species (17 mentioned by Oliva (1987) and Luque et al. (1993); plus *C. exilis* Crane, 1972, parasitizing *Lyopsetta exilis* Jordan and Gilbert, from the coast of California; *C. brasiliensis* Luque, Amato, and Takemoto, 1993; and *C. orthopristis* Luque, Amato, and Takemoto, 1993, the latter 2 from *Orthopristis ruber* from Brazil).

*Choricotyle leonilavazquezae* is characterized by the presence of 2 well-developed suckers in the prohaptor, by the haptor's shorter longitudinal axis, by the presence of 8 spines in the genital atrium, and by the distribution of vitelline follicles extending into the haptor to the base of peduncles. With respect to the distribution of vitelline follicles along the body, the new species most closely resembles 9 of the 20 congeneric species: *C. sonorensis*; *C. caulolati*; *C. anisotremi* Oliva, 1987; *C. exilis*; *C. australiensis* Roubal, Armitage, and Rhode, 1983; *C. chrysophryi*; *C. polynemi* Mamaev, 1972; *C. hysteroncha* (Fujii, 1944) Sproston, 1946; and *C. brasiliensis*. Except for *C. exilis*, *C. chrysophryi*, *C. hysteroncha*, and *C. brasiliensis*, which possess 8–10, 8–9, 6–10, and 7–10 genital spines, respectively, *C. leonilavazquezae* differs from all of the other species by the number of spines of the genital atrium. The new species differs from *C. chrysophryi*, a very common parasite of sparid fishes in the world (Luque et al., 1993), by having larger oral suckers, by lacking terminal lappet, and because *C. chrysophryi* has cecal diverticules penetrating into peduncles. *Choricotyle leonilavazquezae* is similar to *C. hyster-*

*oncha*, a parasite of haemulid fishes in Florida, by having vitelline follicles extending into the base of peduncles, but *C. leonilavazquezae* differs from it by the lack of a terminal lappet in the haptor and by having a higher number of testes (33–39 vs. 13–28).

Apparently, *C. exilis* and *C. brasiliensis* are most similar to *C. leonilavazquezae* because of the vitelline follicles extending into the haptor and the complex structure of the clamps. However, *C. leonilavazquezae* differs from *C. exilis* by having shorter peduncles, by having vitelline follicles extending to base of peduncles, and by the size and shape of oral suckers (small and rounded in *C. exilis*, well-developed and horse-shoe-shaped in the new species). *Choricotyle exilis* was described by Crane (1972) as a parasite of *Lyopsetta exilis* Jordan and Gilbert from San Pedro, California, whereas the new species we describe herein was collected from *Microlepidotus brevipinnis* from Chamela Bay, in the Pacific of southern Mexico.

Finally, the new species differs from *C. brasiliensis* described from a haemulid (*Orthopristis ruber*) in Brazil by the number and size of testes (33–39 in the new species, measuring about 80 × 90, and 16–21 in *C. brasiliensis*, measuring 60 × 40), by the structure of clamps (anterior quadrant with 1 accessory sucker and posterior quadrant with 6–7 concentric arcs of skeletal rods in the new species, and anterior quadrant with 2 accessory suckers of unequal diameter and posterior quadrant with 4–6 skeletal rods in *C. brasiliensis*), and by the presence of 2 pairs of larval hooks with no terminal lappet in the new species (*C. brasiliensis* has a terminal lappet with 3 pairs of hooks).

The new species appears to be highly specific to the haemulid *Microlepidotus brevipinnis* in Chamela Bay, because it was the only host species in which it was found after analysis of 1,075 hosts representing 115 species. The genus *Choricotyle* is considered specific to perciform fishes worldwide (Mamaev, 1972), and at least 8 of the 20 species are found primarily or exclusively in 4 genera of the family Haemulidae (*Orthopristis*, *Microlepidotus*, *Haemulon*, and *Anisotremus*). The presence of some congeneric species in haemulid fishes could represent a particular clade within the phylogeny of these fishes. Possible patterns of coevolution and biogeography should be tested through a phylogenetic analysis of the genus, using methods described by Wiley (1981)

and Brooks and McLennan (1991, 1993). At this point, it seems that haemulids could represent the primitive host group for *Choricotyle* species and that several host-switching events to other members of the Perciformes took place during the evolutionary history of this taxon.

### Acknowledgments

We gratefully acknowledge Felipe Noguera, Chief of the Estación de Biología Chamela, for assistance in allowing us access to station facilities, and Elizabeth Castillo, Luis García, Mari-bel Garzón, Agustín Jimenez, Virginia León, Berenit Mendoza, Griselda Pulido, and Coral Rosas, who helped collect parasite specimens. This study was funded by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM No. IN201593) to R.L.A. and G.P.P.L.

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## Meeting Schedule

21 January, 1998	Johns Hopkins University, Montgomery County Center, Rockville, MD, 7:30 pm (Contact person: Thomas Simpson, 410-366-8814 or 757-787-7689)
11 March, 1998	Nematology Laboratory, BARC-West, Beltsville, MD, 7:30 pm (Contact person: David Chitwood, 301-504-8634)
9 May, 1998	University of Pennsylvania, New Bolton Center, Kennett Square, PA, 2:00 pm (Contact person: Phillip Scott, 215-898-1602)
October, 1998	Date and Place TBA
November, 1998	Date and Place TBA



## Neotropical Monogenoidea. 31. Ancyrocephalinae (Dactylogyridae) of Piranha and Their Relatives (Teleostei, Serrasalminae) from Brazil: Species of *Notothecium* Boeger and Kritsky, 1988, and *Enallothecium* gen. n.

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**ABSTRACT:** Eight species (7 new) of *Notothecium* and 4 species (3 new) of *Enallothecium* (Dactylogyridae, Ancyrocephalinae) are described or reported from the gills of 11 species of Serrasalminae from the Brazilian Amazon: *Notothecium circellum* sp. n., from *Serrasalmus gouldingi* and *Pristobrycon* sp.; *N. cyphophallum* sp. n., from *Pristobrycon eigenmanni*, *Pristobrycon* sp., *Serrasalmus compressus*, *S. elongatus*, *S. gouldingi*, *S. rhombeus*, *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ); *N. deleastoideum* sp. n., from *Serrasalmus* sp. ( $2n = 58$ ); *N. deleastum* sp. n., from *Serrasalmus elongatus*, *S. gouldingi*, *S. rhombeus*, and *Serrasalmus* sp. ( $2n = 58$ ); *N. mizellei* Boeger and Kritsky, 1988, from *Pygocentrus nattereri*; *N. modestum* sp. n., from *Serrasalmus spilopleura*; *N. phyleticum* sp. n., from *Serrasalmus rhombeus*; *N. reduvium* sp. n., from *Serrasalmus* sp. ( $2n = 58$ ) and *Serrasalmus* sp. (2 of Jégu); *Enallothecium aegidatum* (Boeger and Kritsky, 1988) comb. n. (syn. *Notothecium aegidatum* Boeger and Kritsky, 1988), from *Pristobrycon* sp., *Pygocentrus nattereri*, *Serrasalmus compressus*, *S. elongatus*, *S. gouldingi*, *S. rhombeus*, *S. spilopleura*, *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ); *E. cornutum* sp. n., from *Pristobrycon eigenmanni*, *Pristobrycon* sp., *Serrasalmus compressus*, *S. gouldingi*, *S. rhombeus*, *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ); *E. umbelliferum* sp. n., from *Serrasalmus compressus*, *S. rhombeus*, and *Serrasalmus* sp. (2 of Jégu); and *E. variabilum* sp. n., from *Pristobrycon striolatus*. *Enallothecium* gen. n. is proposed and is characterized by dactylogyrids with overlapping gonads, a C-shaped seminal vesicle, an oblique opening of the male copulatory organ, a flap-like thumb and an umbell of the accessory piece, and a vagina looping the left intestinal cecum and opening at the tip of a small papilla on the sinistrodorsal surface of the trunk. *Notothecium aegidatum* Boeger and Kritsky, 1988, is transferred to *Enallothecium* as the type species of the genus.

**KEY WORDS:** Monogenoidea, Dactylogyridae, Ancyrocephalinae, *Enallothecium* gen. n., *Notothecium*, *Enallothecium aegidatum* comb. n., *Enallothecium cornutum* sp. n., *Enallothecium umbelliferum* sp. n., *Enallothecium variabilum* sp. n., *Notothecium circellum* sp. n., *Notothecium cyphophallum* sp. n., *Notothecium deleastoideum* sp. n., *Notothecium deleastum* sp. n., *Notothecium mizellei*, *Notothecium modestum* sp. n., *Notothecium phyleticum* sp. n., *Notothecium reduvium* sp. n., Serrasalminae, *Pristobrycon eigenmanni*, *Pristobrycon striolatus*, *Pristobrycon* sp., *Pygocentrus nattereri*, *Serrasalmus compressus*, *Serrasalmus elongatus*, *Serrasalmus gouldingi*, *Serrasalmus rhombeus*, *Serrasalmus spilopleura*, *Serrasalmus* sp., Amazon Basin, Brazil.

This paper is the last of 4 contributions dealing with Ancyrocephalinae from the gills of Serrasalminae from the Brazilian Amazon (see Kritsky et al., 1996, 1997a, b) and includes the report or description of 8 species (7 new) of *Notothecium* Boeger and Kritsky, 1988, and 4 species (3 new) of *Enallothecium* gen. n. *Notothecium aegidatum* Boeger and Kritsky, 1988, is transferred to *Enallothecium* as its type species.

Methods of host (*Pristobrycon eigenmanni* (Norman); *P. striolatus* (Steindachner); *Pristo-*

*brycon* sp.; *Pygocentrus nattereri* (Kner); *Serrasalmus compressus* Jégu, Leão, and dos Santos; *S. elongatus* Kner; *S. gouldingi* Fink and Machado-Allison; *S. rhombeus* (Linnaeus); *S. spilopleura* Kner; *Serrasalmus* sp. ( $2n = 58$ ); and *Serrasalmus* sp. (2 of Jégu)) and parasite collection, and preparation of helminths for study, measurement, and illustration are those of Kritsky et al. (1986, 1996). Measurements, all in  $\mu\text{m}$ , represent straight-line distances between extreme points and are expressed as a mean followed by the range and number of specimens measured in parentheses; body length includes

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that of the haptor; length of the accessory piece is that of the distal rod. Measurements of internal organs (gonads and pharynx), the body, and haptoral bars were obtained from stained unflattened specimens; those of the anchors, hooks, and copulatory complex were from unstained specimens mounted in Gray and Wess' medium. Numbering (distribution) of hook pairs follows that recommended by Mizelle (1936; see Mizelle and Price, 1963). Type and voucher specimens are deposited in the helminth collections of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); the United States National Parasite Collection, Beltsville, Maryland (USNPC); and the University of Nebraska State Museum, Lincoln, Nebraska (HWML), as indicated in the respective descriptions or accounts. For comparative purposes, 2 paratypes (HWML 23363) and 3 vouchers (HWML 23397) of *Notothecium mizellei* were examined.

Presumed undescribed hosts have been provisionally identified by Jégu as *Pristobrycon* sp., *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ). Representative specimens, cataloged as *Pristobrycon* sp., *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ) from respective localities, are in the ichthyology collection of INPA.

#### Taxonomic Account

**Class Monogeneoidea Bychowsky, 1937**  
**Order Dactylogyridea Bychowsky, 1937**  
**Dactylogyridae Bychowsky, 1933**  
***Notothecium* Boeger and Kritsky, 1988**

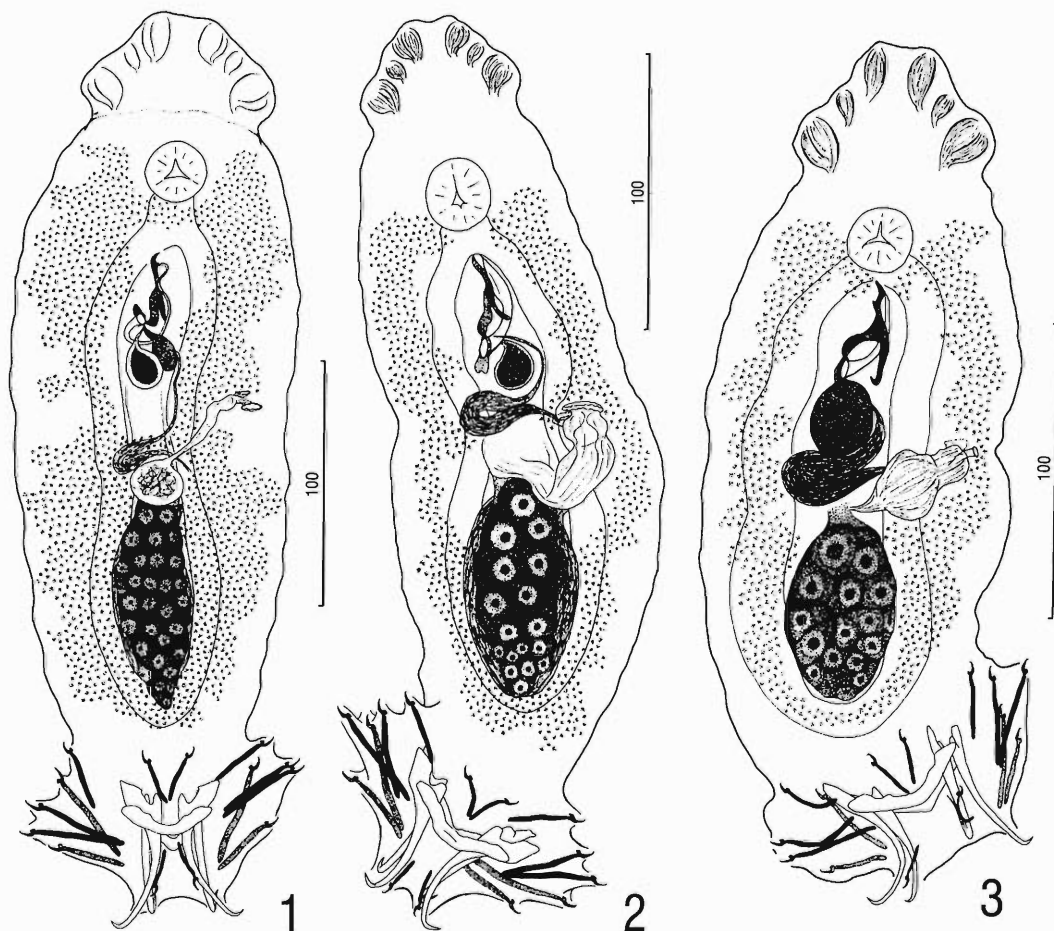
EMENDED DIAGNOSIS: Body somewhat flattened dorsoventrally, comprising cephalic region, trunk, peduncle, haptor. Tegument thin, smooth or with scaled annulations. Two terminal, 2 bilateral cephalic lobes; head organs present; cephalic glands unicellular, lateral, or posterolateral to pharynx. Eyes absent; accessory granules (when present) elongate ovate. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; intestinal ceca 2, confluent posterior to gonads, lacking diverticula. Gonads intercecal, overlapping; testis dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle a C-shaped dilated loop of vas deferens extending into right half of trunk; 1 or 2 prostatic reservoirs. Copulatory complex comprising articulated copulatory organ, accessory piece; copulatory organ usually

arcuate with 1 or 2 rami; accessory piece comprising distal rod with thumb, proximal articulation process. Seminal receptacle absent (functionally replaced by vaginal sperm storage) or present. Vagina looping left intestinal cecum, dilated, nonsclerotized, with subterminal ventral pouch; primary vaginal aperture simple, sinistrodorsal; ventral pouch may open as secondary vaginal aperture on sinistroversal body surface; vaginal vestibule absent. Genital pore midventral near level of intestinal bifurcation. Vitellaria coextensive with intestine. Haptor subhexagonal, with dorsal and ventral anchor/bar complexes, 7 pairs of hooks with ancyrocephaline distribution. Hooks similar; each with delicate point, truncate protruding thumb, expanded shank comprising two subunits; proximal subunit variable in length between hook pairs. FH loop extending to union of shank subunits. Ventral bar lacking anteromedial projection. Parasites of gills of serrasalmid fishes.

TYPE SPECIES: *Notothecium mizellei* Boeger and Kritsky, 1988, from *Pygocentrus nattereri*.

OTHER SPECIES: *Notothecium circellum* sp. n. from *Serrasalmus gouldingi* (type host) and *Pristobrycon* sp.; *N. cyphophallum* sp. n. from *P. eigenmanni*, *Pristobrycon* sp., *S. compressus*, *S. elongatus*, *S. gouldingi*, *S. rhombeus* (type host), *Serrasalmus* sp. ( $2n = 58$ ), and *Serrasalmus* sp. (2 of Jégu); *N. deleastoideum* sp. n. from *Serrasalmus* sp. ( $2n = 58$ ); *N. deleastum* sp. n. from *S. elongatus*, *S. gouldingi*, *S. rhombeus* (type host), and *Serrasalmus* sp. ( $2n = 58$ ); *N. modestum* sp. n. from *S. spilopleura*; *N. phyleticum* sp. n. from *S. rhombeus*; *N. reduvium* sp. n. from *Serrasalmus* sp. ( $2n = 58$ ) (type host), and *Serrasalmus* sp. (2 of Jégu).

REMARKS: Boeger and Kritsky (1988) proposed *Notothecium* for their new species, *N. mizellei* and *N. aegidatum*. The genus was characterized by species with a single vagina looping the left intestinal cecum, a sinistrodorsal vaginal aperture, overlapping gonads, and a C-shaped seminal vesicle. Our discovery of additional species of this group supports removal of *N. aegidatum* from *Notothecium* and proposal of *Enallothecium* gen. n. *Notothecium* is now characterized by an additional character, presence of a subterminal vaginal pouch that may open on the sinistroversal surface of the body (i.e., some species of *Notothecium* may have both sinistrodorsal and sinistroversal vaginal apertures) (see Figs. 1–3). It is separated from *Enallothecium* by lack-



Figures 1–3. Whole mount illustrations of *Notothecium* spp. (composite, ventral views). 1. *Notothecium circellum* sp. n. (from *Serrasalmus gouldingi*). 2. *Notothecium cyphophallum* sp. n. (from *Serrasalmus rhombus*). 3. *Notothecium deleastoideum* sp. n. All drawings are to respective 100- $\mu$ m scales.

ing an umbell in the accessory piece, a papillum at the vaginal aperture, a diagonal opening of the tip of the primary ramus of the copulatory organ, and an accessory flap extending over the thumb of the distal rod of the accessory piece (all apparent synapomorphic features of *Enallothecium*). *Notothecium* may be confused with *Calpidothecium*, but species in the latter genus lack the vaginal loop of the left intestinal cecum (vagina opening on the left lateral margin of the trunk in *Calpidothecium* species).

***Notothecium mizellei* Boeger and Kritsky, 1988**

(Figs. 4–11)

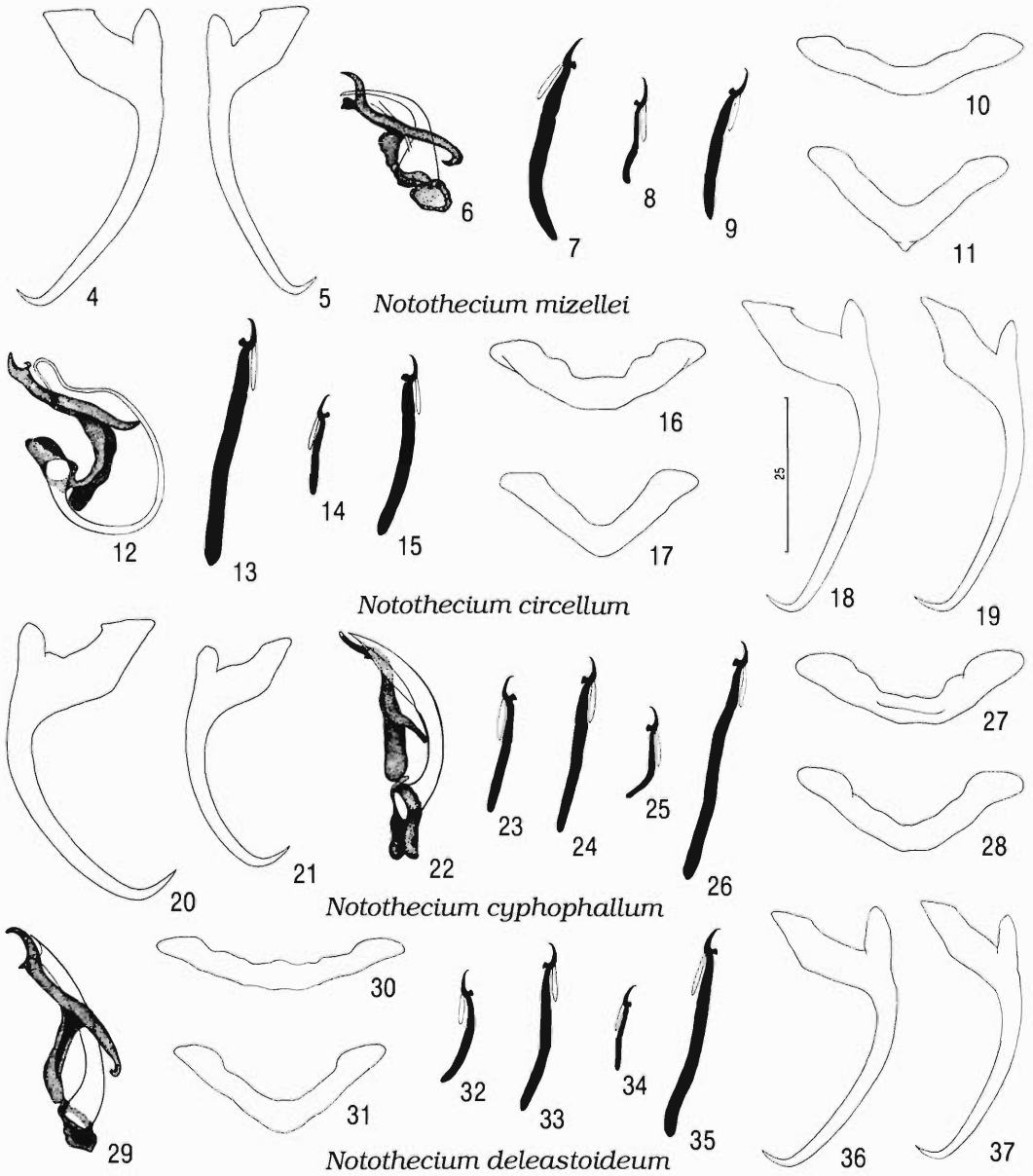
RECORDS: *Pygocentrus nattereri*: Rio Uatumã Lago Tapaná near Santana, Amazonas (3

November 1989); Furo do Catalão, Manaus, Amazonas (27 November 1984).

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

SPECIMENS STUDIED: Two paratypes, HWML 23363; 3 vouchers, HWML 23397; 13 vouchers (present collection), USNPC 86105, 86106, 86107.

MEASUREMENTS: Body length 270 ( $n = 1$ ), greatest width 104 ( $n = 1$ ); haptor length 66 ( $n = 1$ ), width 108 ( $n = 1$ ); pharyngeal diameter



Figures 4–37. Sclerotized structures of *Notothecium* spp. 4–11. *Notothecium mizellei* Boeger and Kritsky, 1988. 4. Ventral anchor. 5. Dorsal anchor. 6. Copulatory complex (ventral view). 7. Hook pair 7. 8. Hook pair 5. 9. Hook pair 1. 10. Ventral bar. 11. Dorsal bar. 12–19. *Notothecium circellum* sp. n. (from *Serrasalmus gouldingi*). 12. Copulatory complex (ventral view). 13. Hook pair 7. 14. Hook pair 5. 15. Hook pair 2. 16. Ventral bar. 17. Dorsal bar. 18. Ventral anchor. 19. Dorsal anchor. 20–28. *Notothecium cyphophallum* sp. n. (from *Serrasalmus rhombeus*). 20. Ventral anchor. 21. Dorsal anchor. 22. Copulatory complex (ventral view). 23. Hook pair 1. 24. Hook pair 2. 25. Hook pair 5. 26. Hook pair 7. 27. Ventral bar. 28. Dorsal bar. 29–37. *Notothecium deleastoideum* sp. n. 29. Copulatory complex (ventral view). 30. Ventral bar. 31. Dorsal bar. 32. Hook pair 1. 33. Hook pair 2. 34. Hook pair 5. 35. Hook pair 7. 36. Ventral anchor. 37. Dorsal anchor. All drawings are to the 25- $\mu$ m scale.

18 ( $n = 1$ ); ventral anchor length 50 (48–54;  $n = 10$ ), base width 21 (17–23;  $n = 10$ ); dorsal anchor length 47 (46–50;  $n = 7$ ), base width 18 (15–20;  $n = 6$ ); ventral bar 37 ( $n = 1$ ), dorsal bar 31 ( $n = 1$ ) long; hook pair 1—28 (27–31;  $n = 5$ ), pair 2—29–30 ( $n = 2$ ), pair 3—34 (32–37;  $n = 8$ ), pair 4—34 (31–36;  $n = 9$ ), pair 5—17 (16–18;  $n = 6$ ), pair 6—26 (25–28;  $n = 9$ ), pair 7—36 (33–38;  $n = 5$ ) long; copulatory organ 27 (25–31;  $n = 7$ ) long, accessory piece 27 (25–29;  $n = 10$ ) long; testis 56 ( $n = 1$ ) long, 32 ( $n = 1$ ) wide; germarium 57 ( $n = 1$ ) long, 32 ( $n = 1$ ) wide.

REMARKS: *Notothecium mizellei* was designated the type species for the genus by Boeger and Kritsky (1988). Our specimens did not differ from those used in the original description. In *N. mizellei*, the subterminal pouch of the vagina lacks a ventral aperture. *Notothecium mizellei* is the only member of the genus with 2 rami of the copulatory organ.

***Notothecium circellum* sp. n.**  
(Figs. 1, 12–19)

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

OTHER RECORD: *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988).

SPECIMENS STUDIED: Holotype, INPA PLH 308; 22 paratypes, INPA PLH 309, USNPC 86108, HWML 38754. 1 voucher from *Pristobrycon* sp., USNPC 86237.

COMPARATIVE MEASUREMENTS: Measurements of the specimen from *Pristobrycon* sp. follow those of the type series in brackets.

DESCRIPTION: Body 340 (285–380;  $n = 10$ ) long; cephalic region originating anteroventrally from trunk; greatest width 131 (110–159;  $n = 11$ ) usually in anterior trunk. Tegument smooth. Cephalic lobes moderately developed. Accessory eye granules usually absent, infrequently in cephalic, trunk regions. Pharynx spherical, 20 (18–22;  $n = 11$ ) in diameter. Peduncle broad; haptor 79 (71–87;  $n = 11$ ) long, 114 (103–129;  $n = 11$ ) wide. Anchors similar; each with elongate depressed superficial root, prominent deep root, elongate shaft, short point. Ventral anchor 52 (49–55;  $n = 12$ ) [48 ( $n = 1$ )] long, base 21 (19–23;  $n = 12$ ) [22 ( $n = 1$ )] wide; dorsal anchor 51 (49–53;  $n = 10$ ) [48 ( $n = 1$ )] long, base 17–18 ( $n = 4$ ) [17 ( $n = 1$ )] wide. Ventral bar 37 (36–40;  $n = 9$ ) long, U-shaped, with slightly

enlarged terminations, subterminal anterior expansions; dorsal bar 35 (33–38;  $n = 10$ ) long, V-shaped, with slightly enlarged ends. Hook pair 1—25 (23–27;  $n = 4$ ), pair 2—31 (29–33;  $n = 9$ ), pair 3—36 (34–39;  $n = 10$ ), pair 4—39 (37–41;  $n = 11$ ), pair 5—18 (16–23;  $n = 9$ ), pair 6—27 (25–29;  $n = 10$ ), pair 7—42 (39–45;  $n = 12$ ) long. Copulatory organ 77 (70–83;  $n = 10$ ) [60 ( $n = 1$ )] long, coiled (counterclockwise), with about 1 ring, distally sigmoid; base with sclerotized margin, small proximal flap; coil diameter 23 (21–27;  $n = 11$ ). Distal rod of accessory piece 25 (18–27;  $n = 12$ ) long, with terminal hook, subterminal thumb. Gonads ovate; testis 62 (54–74;  $n = 4$ ) long, 27 (22–33;  $n = 4$ ) wide; germarium 61 (56–66;  $n = 9$ ) long, 28 (20–34;  $n = 9$ ) wide. Seminal vesicle a slight dilation of vas deferens. Oviduct, ootype not observed; uterus delicate; vagina usually expanded into spherical chamber (seminal receptacle) proximally, slightly expanded along remaining length, bifurcating distally; sinistroventral branch open.

REMARKS: *Notothecium circellum* is the only species in the genus with a coiled copulatory organ. The specific name is from Latin (*circellus* ["a small ring"]) and refers to the copulatory organ.

***Notothecium cyphophallum* sp. n.**  
(Figs. 2, 20–28)

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

OTHER RECORDS: *Pristobrycon eigenmanni*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Negro near Manaus, Amazonas (28 December 1988). *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus compressus*: Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus elongatus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus rhombeus*: Rio Uatumã, Lago Tapaná near Santana, Amazonas (3 November 1989); Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus* sp. (2 of Jégu): Rio Uatumã, Lago Tapaná, Santana, Amazonas (3 November 1989). *Serrasalmus* sp. ( $2n = 58$ ):

Furo do Catalão, Manaus, Amazonas (30 January 1991).

**SPECIMENS STUDIED:** Holotype, INPA PLH 310; 20 paratypes, INPA PLH 311, USNPC 86109, 86110, 86111, HWML 38755 from *S. rhombeus*. Eleven vouchers from *P. eigenmanni*, USNPC 86116, 86117; 1 voucher from *Pristobrycon* sp., USNPC 86112; 3 vouchers from *S. compressus*, USNPC 86113; 10 vouchers from *S. elongatus*, USNPC 86114, 86115; 14 vouchers from *S. gouldingi*, USNPC 86118; 8 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 86119; 6 vouchers from *Serrasalmus* sp. ( $2n = 58$ ), USNPC 86120.

**COMPARATIVE MEASUREMENTS:** Table 1.

**DESCRIPTION:** Greatest body width in posterior trunk. Tegument usually smooth or infrequently with poorly developed scaled annulations on peduncle. Cephalic lobes moderately developed. Accessory eye granules usually in cephalic, anterior trunk regions, infrequently arranged in clusters. Pharynx spherical. Peduncle broad. Anchors similar; each with well-differentiated deep root, depressed superficial root, curved elongate shaft, short point. Ventral bar broadly U-shaped, with terminal enlargements; dorsal bar broadly U- or V-shaped. Copulatory organ tubular, arcuate, with elongate subterminal opening; base with prominent proximal flap. Distal rod of accessory piece sigmoid, with thumb reduced to inconspicuous keel. Gonads subovate; seminal vesicle prominent; single prostatic reservoir; oviduct short; ootype, uterus not observed; vagina distally bifurcate with sinistrodorsal and sinistroventral apertures, dilated, apparently serving as sperm reservoir; seminal receptacle absent; vitellaria limited in trunk, absent in regions of reproductive organs.

**REMARKS:** *Notothecium cyphophallum* is easily separated from congeners by the morphology of the copulatory organ and its elongate diagonal opening. The specific name is from Greek (*kyphos* ["humped, sloped, curved"] + *phallos* ["penis"]) and refers to the shape of the copulatory organ.

***Notothecium deleastoideum* sp. n.**  
(Figs. 3, 29–37)

**TYPE HOST AND LOCALITY:** *Serrasalmus* sp. ( $2n = 58$ ): Furo do Catalão, Manaus, Amazonas (5 January 1989; 30 January 1991).

**SPECIMENS STUDIED:** Holotype, INPA PLH

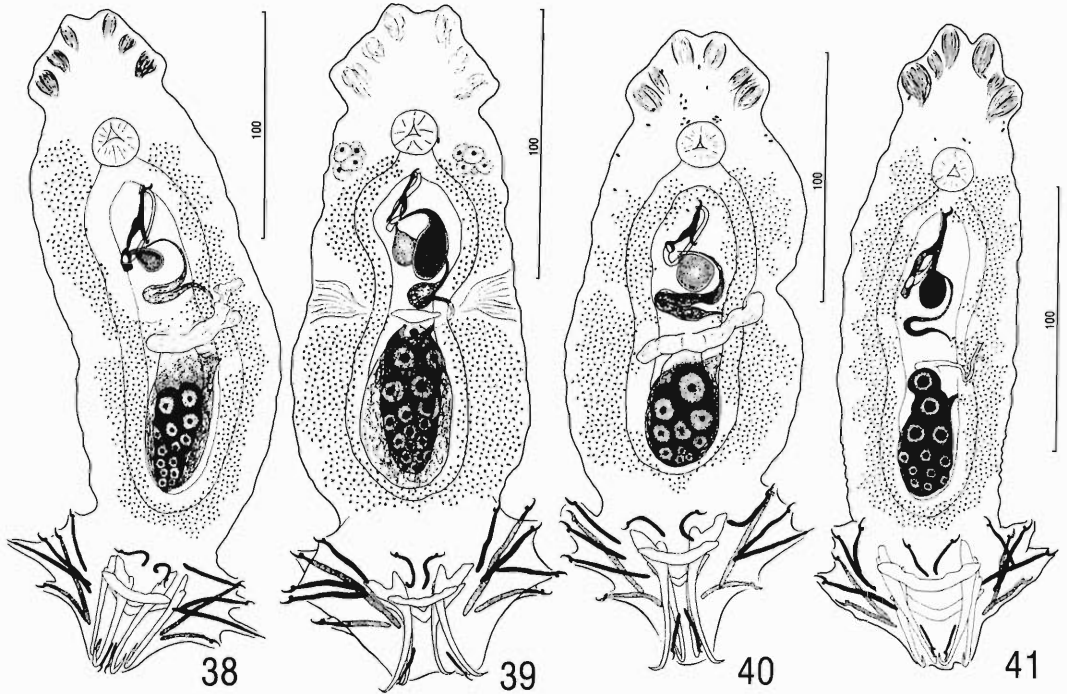
312; 7 paratypes, INPA PLH 313, USNPC 86121, 86122, HWML 38756.

**DESCRIPTION:** Body broad, 259 (224–294;  $n = 2$ ) long; greatest width 99 (85–113;  $n = 2$ ) near midlength. Tegument smooth or with scaled annulations over posterior half of trunk, peduncle. Cephalic lobes moderately developed. Accessory eye granules present in cephalic, trunk regions, infrequently absent. Pharynx spherical, 18 (16–20;  $n = 2$ ) in diameter. Peduncle broad to nonexistent; haptor 57 (56–58;  $n = 2$ ) long, 96 (78–114;  $n = 2$ ) wide. Anchors similar; each with elongate depressed superficial root, prominent deep root, elongate shaft, short point. Ventral anchor 45 (43–46;  $n = 6$ ) long, base 19 (17–22;  $n = 6$ ) wide; dorsal anchor 43 (41–44;  $n = 4$ ) long, base 16 (14–18;  $n = 4$ ) wide. Ventral bar 36–37 ( $n = 2$ ) long, gently curved, with slightly enlarged terminations; dorsal bar 34–35 ( $n = 2$ ) long, broadly V-shaped, with slightly enlarged ends. Hook pair 1–22 (21–23;  $n = 5$ ), pair 2–29 (26–30;  $n = 4$ ), pair 3–33 (32–35;  $n = 5$ ), pair 4–37 (35–38;  $n = 4$ ), pair 5–15 (14–16;  $n = 4$ ), pair 6–24 (23–26;  $n = 4$ ), pair 7–38 (37–41;  $n = 5$ ) long. Copulatory organ 37 (35–38;  $n = 6$ ) long, a broad arced tube terminating short of tip of accessory piece; base with sclerotized margin, small proximal flap. Distal rod of accessory piece 31 (27–33;  $n = 6$ ) long, with terminal hook, short subterminal thumb. Testis not observed; germarium 43 (36–51;  $n = 2$ ) long, 33 (28–38;  $n = 2$ ) wide, subovate. Seminal vesicle prominent. Single prostatic reservoir; oviduct, ootype, uterus not observed; vagina dilated, with sinistrodorsal, sinistroventral openings; seminal receptacle absent; vitellaria in trunk except absent in regions of reproductive organs.

**REMARKS:** *Notothecium deleastoideum* is similar to *N. reduvium* sp. n. and *N. deleastum* sp. n. in the general morphology of the male copulatory organ. It differs from *N. reduvium* by having less taper of the copulatory organ (copulatory organ tapering to a fine tube in *N. reduvium*) and by the comparative size of the anchors, bars, and hooks (smaller in *N. reduvium*). It differs from *N. deleastum* by having a short copulatory organ (copulatory organ flared distally and extending past the hook of the accessory piece in *N. deleastum*). In *N. deleastum*, the subterminal vaginal pouch is blind (open in *N. deleastoideum*). The specific name reflects the

Table 1. Comparative measurements (in micrometers) of *Notothecium cyphophallum* sp. n., from 8 serrasalmid hosts.

	<i>Pristobrycon</i> <i>eigenmanni</i>	<i>n</i>	<i>Pristobrycon</i> sp.	<i>n</i>	<i>Serrasalmus</i> <i>compressus</i>	<i>n</i>	<i>Serrasalmus</i> <i>elongatus</i>	<i>n</i>	<i>Serrasalmus</i> <i>gouldingi</i>	<i>n</i>	<i>Serrasalmus</i> <i>rhombeus</i>	<i>n</i>	<i>Serrasalmus</i> sp. (2n = 58)	<i>n</i>	<i>Serrasalmus</i> sp. (2 of Jégu)	<i>n</i>
Body																
Length	258 (229–288)	2	—	—	—	—	346 (310–376)	3	329 (305–359)	6	284 (189–329)	9	233	1	—	—
Width	66 (62–71)	2	—	—	—	—	86 (81–94)	3	121 (99–148)	6	98 (92–106)	9	107	1	—	—
Haptor																
Length	72 (65–80)	2	—	—	—	—	87 (83–92)	3	81 (78–85)	6	73 (61–80)	9	69	1	—	—
Width	88 (78–98)	2	—	—	—	—	103 (89–120)	3	128 (114–143)	6	111 (104–124)	9	103	1	—	—
Pharynx																
Diameter	16	2	—	—	—	—	17 (15–18)	3	20 (18–23)	6	19 (15–21)	9	19	1	—	—
Copulatory organ																
Length	42 (40–44)	9	—	—	43–44	3	42 (38–46)	6	44 (42–48)	8	43 (41–45)	10	43 (39–46)	5	42 (40–45)	7
Accessory piece																
Length	26 (25–28)	9	26	1	26	3	27 (26–30)	6	27 (25–29)	8	27 (25–30)	11	27 (26–28)	5	25 (24–27)	7
Dorsal anchor																
Length	43 (40–49)	9	43	1	49 (48–50)	2	52 (46–56)	6	49 (46–51)	7	47 (41–50)	8	44 (42–45)	2	47 (43–50)	6
Base width	17 (15–18)	8	15	1	16	1	18 (15–21)	3	18 (17–20)	3	17 (16–18)	6	17–18	2	18 (16–20)	5
Ventral anchor																
Length	49 (45–55)	9	46	1	55 (54–56)	2	57 (54–62)	6	53 (50–57)	7	51 (44–54)	11	49 (48–51)	2	50 (47–56)	7
Base width	22 (20–23)	8	21	1	20 (19–21)	2	21 (18–24)	6	23 (22–25)	7	22 (20–23)	9	22 (21–23)	2	21 (19–22)	7
Bar length																
Ventral	37 (36–38)	2	—	—	—	—	40	2	42 (38–48)	6	39 (31–43)	8	38	1	—	—
Dorsal	34–35	2	—	—	—	—	37–38	2	38 (36–40)	6	38 (36–41)	6	34	1	—	—
Hook lengths																
Pair 1	23 (22–24)	6	22	1	24	1	25 (24–26)	3	29	1	23 (21–24)	3	24–25	2	22	1
Pair 2	29 (27–30)	8	—	—	30	1	31 (26–34)	6	33 (31–37)	6	30 (28–34)	8	30–31	2	29 (27–31)	4
Pair 3	34 (32–36)	8	35	1	35	1	38 (35–40)	6	37 (36–39)	8	37 (32–43)	5	36	2	35 (34–37)	6
Pair 4	38 (36–41)	8	38	1	37	2	41 (37–43)	6	42 (40–43)	8	38 (34–41)	8	40 (38–41)	2	38 (33–41)	7
Pair 5	18	6	18	1	19	1	19 (18–20)	5	19 (18–20)	7	18 (17–19)	6	18	2	18 (17–19)	6
Pair 6	27 (25–29)	9	25	1	25	1	29 (28–30)	4	29	3	26 (25–27)	6	28	2	27 (26–28)	6
Pair 7	41 (40–42)	9	42	1	39 (38–40)	2	45 (41–48)	6	47 (45–49)	6	42 (39–44)	6	42	2	43 (38–47)	7
Germarium																
Length	43 (40–47)	2	—	—	—	—	69 (64–74)	3	63 (56–70)	5	58 (43–76)	7	42	1	—	—
Width	23 (20–26)	2	—	—	—	—	27 (22–32)	3	33 (30–36)	5	26 (19–32)	7	29	1	—	—
Testis																
Length	52 (49–54)	2	—	—	—	—	62 (61–63)	2	71 (54–95)	4	56 (46–68)	5	50	1	—	—
Width	30–31	2	—	—	—	—	26 (25–28)	2	44 (37–63)	4	38 (32–44)	4	38	1	—	—



Figures 38–41. Whole mount illustrations of *Notothecium* spp. (composite, ventral views). 38. *Notothecium deleastum* sp. n. (from *Serrasalmus rhombeus*). 39. *Notothecium modestum* sp. n. 40. *Notothecium phyleticum* sp. n. 41. *Notothecium redivium* sp. n. (from *Serrasalmus* sp. [ $2n = 58$ ]). All drawings are to respective 100- $\mu$ m scales.

presumed relationship of this species with *N. deleastum*.

***Notothecium deleastum* sp. n.**  
(Figs. 38, 42–50)

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

OTHER RECORDS: *Serrasalmus elongatus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus* sp. ( $2n = 58$ ): Furo do Catalão, Manaus, Amazonas (5 January 1989; 30 January 1991).

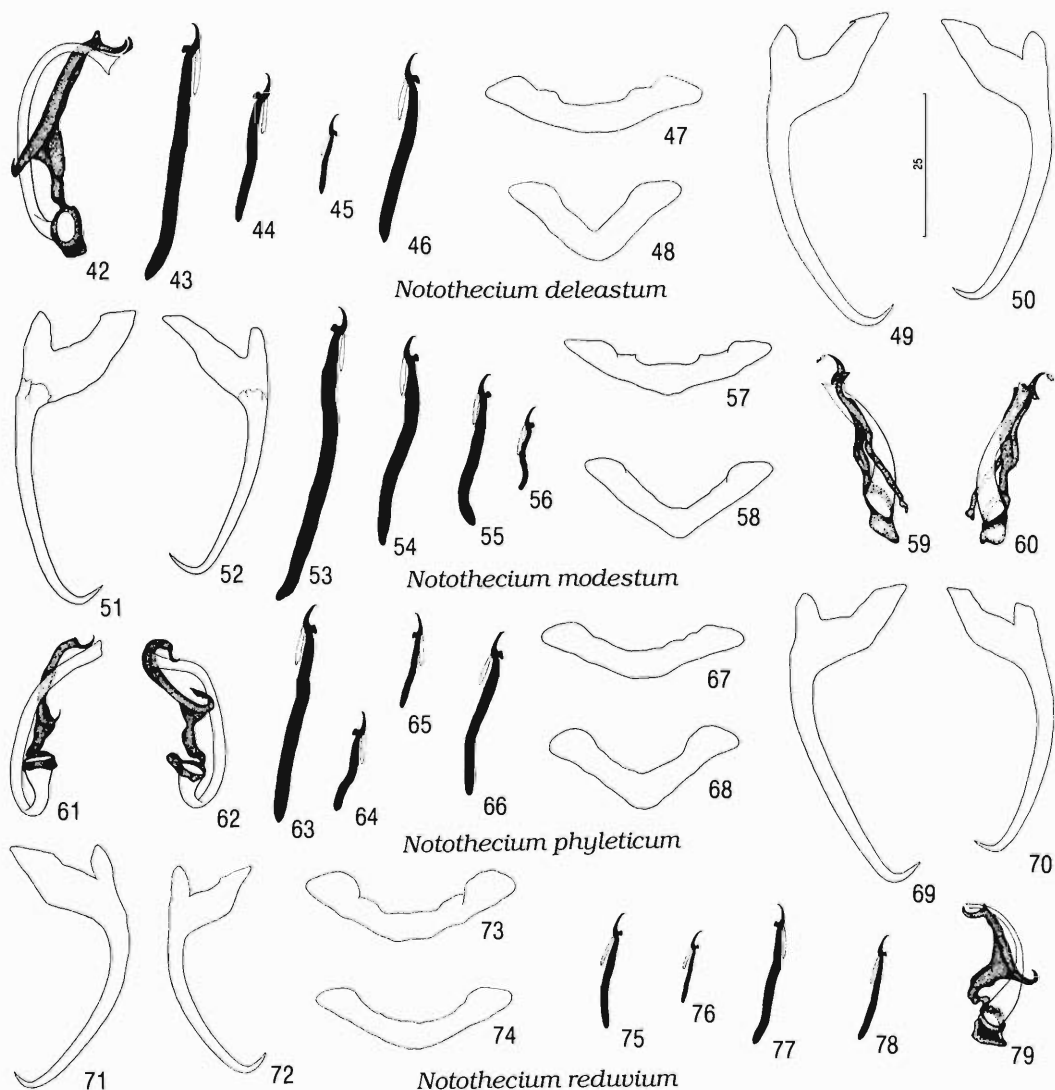
SPECIMENS STUDIED: Holotype, INPA PLH 314; 10 paratypes, INPA PLH 315, USNPC 86125, HWML 38757 from *S. rhombeus*. 10 vouchers from *S. elongatus*, USNPC 86123, 86124; 17 vouchers from *S. gouldingi*, USNPC 86128; 4 vouchers from *Serrasalmus* sp. ( $2n = 58$ ), USNPC 86126, 86127.

COMPARATIVE MEASUREMENTS: Table 2.

DESCRIPTION: Greatest width of body usually in posterior trunk. Tegument smooth. Cephalic lobes moderately developed. Accessory eye granules present in cephalic, trunk regions, infrequently absent. Pharynx spherical. Peduncle broad. Anchors similar; each with depressed superficial root, prominent deep root, elongate shaft, short point. Ventral bar gently curved, with slightly enlarged terminations, subterminal anterior expansions; dorsal bar V-shaped, with slightly enlarged ends. Copulatory organ a broadly U-shaped tube with slightly flared end; base with sclerotized margin, small proximal flap. Distal rod of accessory piece with terminal hook flexed dorsally, short subterminal thumb. Gonads subovate; seminal vesicle delicate. Single prostatic reservoir; oviduct, ootype, uterus not observed; vagina opening sinistrodorsally, expanded, with subterminal blind ventral pouch; seminal receptacle absent; vitellaria in trunk except absent in regions of reproductive organs.

REMARKS: This species resembles *Noto-*





Figures 42–79. Sclerotized structures of *Notothecium* spp. 42–50. *Notothecium deleastum* sp. n. (from *Serrasalmus rhombeus*). 42. Copulatory complex (ventral view). 43. Hook pair 7. 44. Hook pair 1. 45. Hook pair 5. 46. Hook pair 2. 47. Ventral bar. 48. Dorsal bar. 49. Ventral anchor. 50. Dorsal anchor. 51–60. *Notothecium modestum* sp. n. 51. Ventral anchor. 52. Dorsal anchor. 53. Hook pair 7. 54. Hook pair 3. 55. Hook pair 6. 56. Hook pair 5. 57. Ventral bar. 58. Dorsal bar. 59. Copulatory complex (ventral view). 60. Copulatory complex (dorsal view). 61–70. *Notothecium phyleticum* sp. n. 61. Copulatory complex (dorsal view). 62. Copulatory complex (ventral view). 63. Hook pair 7. 64. Hook pair 1. 65. Hook pair 5. 66. Hook pair 3. 67. Ventral bar. 68. Dorsal bar. 69. Ventral anchor. 70. Dorsal anchor. 71–79. *Notothecium reduvium* sp. n. (from *Serrasalmus* sp. [ $2n = 58$ ]). 71. Ventral anchor. 72. Dorsal anchor. 73. Ventral bar. 74. Dorsal bar. 75. Hook pair 2. 76. Hook pair 5. 77. Hook pair 7. 78. Hook pair 1. 79. Copulatory complex (ventral view). All drawings are to the 25- $\mu$ m scale.

*thecium deleastoideum* sp. n., from which it differs by having a longer copulatory organ and a blind subterminal vaginal pouch. The specific name is from Greek (*deleastikos* [“enticing”]).

***Notothecium modestum* sp. n.**

(Figs. 39, 51–60)

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

**Table 2. Comparative measurements (in micrometers) of *Notothecium deleastum* sp. n., from 4 serrasalmid hosts.**

	<i>Serrasalmus elongatus</i>	<i>n</i>	<i>Serrasalmus gouldingi</i>	<i>n</i>	<i>Serrasalmus rhombus</i>	<i>n</i>	<i>Serrasalmus</i> sp. ( $2n = 58$ )	<i>n</i>
Body								
Length	250	1	316 (291–331)	6	265 (217–294)	5	265	1
Width	85	1	118 (94–127)	6	91 (83–98)	5	106	1
Haptor								
Length	72	1	73 (67–82)	6	72 (68–80)	5	67	1
Width	101	1	125 (96–153)	6	105 (85–114)	5	96	1
Pharynx								
Diameter	15	1	21 (18–22)	6	19 (17–21)	5	20	1
Copulatory organ								
Length	40 (35–43)	8	38 (35–46)	11	42 (36–52)	6	43 (42–46)	3
Accessory piece								
Length	32 (30–34)	8	32 (29–38)	11	31 (24–35)	6	28 (26–30)	3
Dorsal anchor								
Length	54 (48–57)	6	49 (44–53)	9	49 (45–52)	4	46 (45–48)	3
Base width	15	1	17 (14–20)	7	18–19	2	12	1
Ventral anchor								
Length	56 (51–57)	7	51 (49–56)	11	55 (49–61)	4	50 (47–52)	3
Base width	20 (18–24)	6	21 (17–25)	10	20–21	4	20 (18–21)	3
Bar length								
Ventral	40	1	36 (34–37)	5	35 (33–39)	4	—	—
Dorsal	35	1	32 (30–34)	5	31 (29–33)	4	—	—
Hook lengths								
Pair 1	26	1	22	1	26	1	—	—
Pair 2	33 (31–36)	2	30 (28–33)	7	31 (28–34)	2	29	2
Pair 3	37 (36–39)	4	35 (33–40)	10	37 (35–40)	2	34 (33–35)	3
Pair 4	42 (39–44)	7	39 (35–42)	11	38 (35–41)	5	36	3
Pair 5	17 (16–18)	5	17 (16–19)	15	17	3	15 (14–17)	2
Pair 6	28 (27–29)	4	27 (26–31)	6	27 (26–28)	2	25	1
Pair 7	46 (45–49)	4	46 (42–52)	9	46 (43–49)	4	38 (34–41)	3
Germarium								
Length	39	1	52 (45–60)	5	44 (38–46)	5	—	—
Width	25	1	30 (23–39)	5	21 (19–22)	5	—	—
Testis								
Length	—	—	59 (52–69)	3	48 (44–50)	3	—	—
Width	—	—	31 (28–35)	3	27 (19–33)	3	—	—

OTHER RECORDS: *Serrasalmus spilopleura*: Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (14 September 1984); Furo do Catalão, Manaus, Amazonas (2 November 1993).

SPECIMENS STUDIED: Holotype, INPA PLH 316; 56 paratypes, INPA PLH 317, PLH 318, USNPC 86130, 86131, HWML 38758; 3 vouchers, USNPC 86129.

DESCRIPTION: Body 257 (222–294;  $n = 21$ ) long, with bilateral subtriangular zones lacking vitellaria near midlength of trunk; greatest width 102 (89–119;  $n = 23$ ) in posterior trunk. Tegument smooth. Cephalic margin broad; cephalic lobes moderately developed. Accessory eye granules uncommon in cephalic, anterior trunk

regions. Pharynx spherical, 17 (15–18;  $n = 24$ ) in diameter. Peduncle broad; haptor 70 (60–83;  $n = 23$ ) long, 102 (85–117;  $n = 21$ ) wide. Anchors similar; each with elongate deep root, depressed superficial root, delicate shaft, short point; ventral anchor 50 (47–53;  $n = 30$ ) long, base 19 (16–22;  $n = 30$ ) wide; dorsal anchor 47 (43–52;  $n = 28$ ) long, base 17 (14–19;  $n = 21$ ) wide. Ventral bar 36 (34–38;  $n = 20$ ) long, broadly V-shaped, with irregular anterior margin. Dorsal bar 30 (28–33;  $n = 15$ ) long, V-shaped, with slightly enlarged ends. Hook pairs 1, 6—26 (22–30;  $n = 26$ ); pair 2—32 (29–37;  $n = 10$ ); pairs 3, 4—37 (33–41;  $n = 57$ ); pair 5—15 (14–17;  $n = 23$ ); pair 7—52 (45–59;  $n =$

27) long. Copulatory organ 30 (29–32;  $n = 17$ ) long, a tapered sigmoid tube; base of copulatory organ with sclerotized margin, small proximal flap. Distal rod of accessory piece 31 (29–33;  $n = 23$ ) long, with terminal hook, subterminal thumb. Testis 47 (39–54;  $n = 7$ ) long, 29 (25–35;  $n = 7$ ) wide, elongate ovate; seminal vesicle fusiform, C-shaped, lying in left side of trunk; prostatic reservoirs pyriform. Germarium ovate, 44 (34–56;  $n = 19$ ) long, 27 (17–38;  $n = 19$ ) wide; oviduct, ootype, uterus, vagina, seminal receptacle not observed; vitellaria dense throughout trunk except absent in regions of reproductive organs.

REMARKS: The bilateral triangular zones lacking vitellaria near the midlength of the trunk may represent positions of vaginal apertures, but apertures and vaginal ducts, primary characters for generic assignment of ancyrocephalines parasitizing serrasalmids, could not be determined in available specimens. Thus, we assign this species to *Notothecium* based on the slight dorsoventral flattening of the body, the absence of eyes, and the general morphology of the copulatory complex and haptor structures. In *N. modestum*, the C-shaped seminal vesicle is limited to the left side of the trunk, while the seminal vesicle of all other congeneric species extends into the right side. The specific name is from Latin (*modestus* ["unassuming"]).

*Notothecium phyleticum* sp. n.  
(Figs. 40, 61–70)

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

OTHER RECORDS: *Serrasalmus rhombeus*: Rio Capucapú at its confluence with Rio Jatapú, Cachoeira das Garças, Amazonas (31 October 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 319; 7 paratypes, INPA PLH 320, USNPC 86132, 86133, 86134, HWML 38759.

DESCRIPTION: Body broad, 245 (195–282;  $n = 3$ ) long; greatest width 96 (84–103;  $n = 3$ ) usually in posterior trunk. Tegument smooth. Cephalic lobes moderately developed. Accessory eye granules usually present in cephalic, anterior trunk regions. Pharynx spherical, 18 (17–19;  $n = 3$ ) in diameter. Peduncle broad; haptor 73 (64–78;  $n = 3$ ) long, 92 (81–99;  $n = 3$ ) wide. Anchors similar; each with depressed superficial

root, prominent deep root, elongate shaft, short point; ventral anchor 51 (46–56;  $n = 5$ ) long, base 19 (17–20;  $n = 5$ ) wide; dorsal anchor 49 (47–51;  $n = 5$ ) long, base 14 (11–17;  $n = 4$ ) wide. Ventral bar 31 (28–34;  $n = 3$ ) long, bent near midlength, with slightly enlarged terminations; dorsal bar 31–32 ( $n = 3$ ) long, V-shaped, with enlarged ends. Hook pair 1–22 (21–24;  $n = 4$ ), pair 2–26 (20–31;  $n = 5$ ), pair 3–31 (29–33;  $n = 5$ ), pair 4–34 (32–36;  $n = 5$ ), pair 5–16–17 ( $n = 5$ ), pair 6–24 (23–25;  $n = 5$ ), pair 7–41 (39–43;  $n = 5$ ) long. Copulatory organ 35 (29–40;  $n = 5$ ) long, J-shaped; base with sclerotized margin, small proximal flap occasionally absent. Distal rod of accessory piece 20 (17–23;  $n = 5$ ) long, C-shaped, with terminal hook, small subterminal thumb. Gonads ovate to subspherical; testis 38 ( $n = 1$ ) long, 24 ( $n = 1$ ) wide; germarium 46 ( $n = 1$ ) long, 23 ( $n = 1$ ) wide. Seminal vesicle moderately developed. Single prostatic reservoir; oviduct, ootype, uterus not observed; vagina dilated, opening by indistinct slit on sinistrodorsal surface of trunk; ventral pouch poorly developed, blind; dextral vaginal branch present, blind; seminal receptacle absent; vitellaria limited in trunk, absent in regions of reproductive organs.

REMARKS: *Notothecium phyleticum* resembles several species of *Notothecium* including *N. reduvium* sp. n., *N. deleastum* sp. n., *N. deleastoideum* sp. n., *N. mizellei* Boeger and Kritsky, 1988, and *N. cyphophallum* sp. n. by the general morphology of the copulatory complex. It is distinguished from these species by having a J-shaped copulatory organ and a dextral vaginal branch. The specific name is from Greek (*phyletes* ["one of the same tribe"]).

*Notothecium reduvium* sp. n.  
(Figs. 41, 71–79)

TYPE HOST AND LOCALITY: *Serrasalmus* sp. ( $2n = 58$ ): Furo do Catalão, Manaus, Amazonas (5 January 1989; 30 January 1991).

OTHER RECORDS: *Serrasalmus* sp. (2 of Jégu): Rio Uatumã, Lago Tapaná near Santana, Amazonas (3 November 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 321; 12 paratypes, INPA PLH 322, USNPC 86137, 86138, HWML 38760. 7 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 86135, 86136.

COMPARATIVE MEASUREMENTS: Measurements

of specimens from *Serrasalmus* sp. (2 of Jégu) follow those of the type series in brackets.

DESCRIPTION: Body 245 (244–246;  $n = 2$ ) long; greatest width 85 (75–95;  $n = 2$ ) near midlength. Tegument smooth. Cephalic lobes moderately developed. Accessory eye granules usually present in cephalic, anterior trunk regions. Pharynx spherical, 16 (14–18;  $n = 2$ ) in diameter. Peduncle broad; haptor 68 (67–69;  $n = 2$ ) long, 90 (85–95;  $n = 2$ ) wide. Anchors similar; each with depressed superficial root, prominent deep root, elongate shaft, short point; ventral anchor 46 (43–50;  $n = 10$ ) [43 (41–45;  $n = 7$ )] long, base 17 (15–19;  $n = 11$ ) [17 (16–19;  $n = 7$ )] wide; dorsal anchor 39 (36–43;  $n = 6$ ) [40 (37–43;  $n = 6$ )] long, base 13 (11–15;  $n = 7$ ) [13–14 ( $n = 3$ )] wide. Ventral bar 35 ( $n = 2$ ) long, broadly U-shaped, with enlarged terminations; dorsal bar 32 ( $n = 1$ ) long, U- or V-shaped, with slightly enlarged ends. Hook pair 1–22 (21–24;  $n = 7$ ) [19 (18–21;  $n = 3$ )], pair 2–24 (21–25;  $n = 7$ ) [23 (22–25;  $n = 5$ )], pair 3–30 (27–31;  $n = 9$ ) [28 (26–30;  $n = 6$ )], pair 4–33 (30–36;  $n = 9$ ) [32 (31–33;  $n = 6$ )], pair 5–15–16 ( $n = 8$ ) [16–17 ( $n = 6$ )], pair 6–23 (22–24;  $n = 8$ ) [23 (21–24;  $n = 7$ )], pair 7–29 (26–32;  $n = 9$ ) [27 (23–30;  $n = 7$ )] long. Copulatory organ 28 (26–31;  $n = 7$ ) [29 (26–32;  $n = 6$ )] long, tapered; base with sclerotized margin, short proximal flap. Distal rod of accessory piece 21 (19–23;  $n = 11$ ) [20 (17–21;  $n = 5$ )] long, with terminal hook, short subterminal thumb. Gonads subovate to pyriform; testis 42 (40–44;  $n = 2$ ) long, 23–24 ( $n = 2$ ) wide; germarium 50 ( $n = 1$ ) long, 20 ( $n = 1$ ) wide. Seminal vesicle a slight dilation of vas deferens. Single prostatic reservoir; oviduct, ootype, uterus not observed; vagina slightly dilated, bifurcated distally, with sinistroventral, sinistrodorsal apertures; seminal receptacle absent; vitellaria in trunk except absent in regions of reproductive organs.

REMARKS: This species resembles *Notothecium deleastum* sp. n. and *N. deleastoideum* sp. n., from which it differs by having a more delicate tapered copulatory organ. The specific name is from Latin (*reduvia* ["a hangnail"]) and refers to the tip of the accessory piece.

#### *Enallothecium* gen. n.

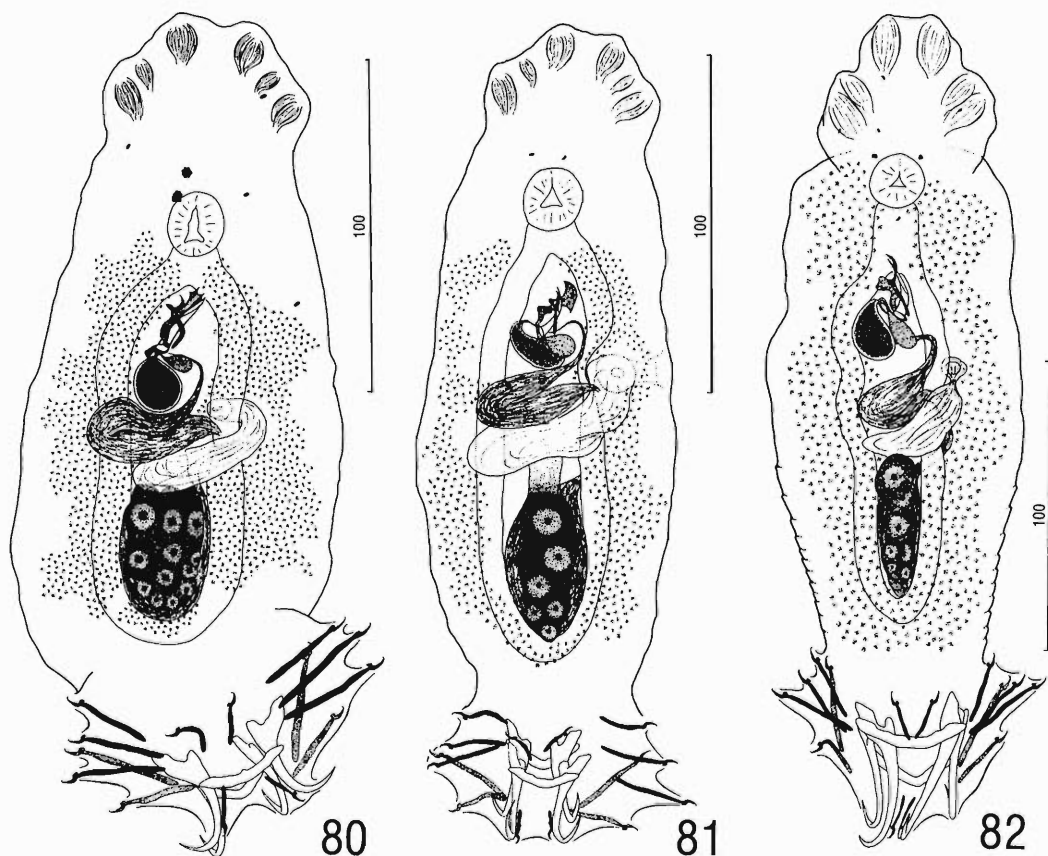
DIAGNOSIS: Body somewhat flattened dorsoventrally; comprising cephalic region, trunk, peduncle, haptor. Tegument thin, smooth, or

with scaled annulations. Two terminal, 2 bilateral cephalic lobes; head organs present; cephalic glands unicellular, lateral or posterolateral to pharynx. Eyes poorly developed or absent; granules elongate ovate. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; intestinal ceca 2, confluent posterior to gonads, lacking diverticula. Gonads intercecal, overlapping; testis dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle a C-shaped dilated loop of vas deferens extending into right half of trunk; 2 prostatic reservoirs. Copulatory complex comprising articulated copulatory organ, accessory piece; copulatory organ a short arced tube with diagonal opening. Accessory piece comprising distal rod, proximal articulation process; distal rod with terminal hook, thumb surrounded by poorly sclerotized mass, umbell originating from base of thumb. Seminal receptacle absent; vagina looping left intestinal cecum, dilated, nonsclerotized, opening on papilla lying on sinistrodorsal surface near body midlength. Genital pore midventral near level of intestinal bifurcation. Vitellaria coextensive with intestine. Haptor subhexagonal, with pairs of dorsal and ventral anchor/bar complexes, 7 pairs of hooks with ancyrocephaline distribution. Hooks similar; each with delicate point, truncate protruding thumb, expanded shank comprising two subunits; proximal subunit variable in length between hook pairs. FH loop extending to union of shank subunits. Ventral bar lacking anteromedial process. Parasites of gills of serrasalmid fishes.

TYPE SPECIES: *Enallothecium aegidatum* (Boeger and Kritsky, 1988) comb. n. from *Pristobrycon* sp., *Pygocentrus nattereri* (type host), *Serrasalmus compressus*, *S. elongatus*, *S. gouldingi*, *S. rhombeus*, *S. spilopleura*, *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ).

OTHER SPECIES: *Enallothecium cornutum* sp. n. from *Pristobrycon eigenmanni*, *Pristobrycon* sp., *Serrasalmus compressus*, *S. gouldingi*, *S. rhombeus* (type host), *Serrasalmus* sp. (2 of Jégu), and *Serrasalmus* sp. ( $2n = 58$ ); *E. umbelliferum* sp. n. from *S. compressus* and *S. rhombeus* (type host); and *E. variabilum* sp. n. from *P. striolatus*.

REMARKS: The type species of *Enallothecium* was originally placed in *Notothecium* by Boeger and Kritsky (1988). However, the 3 new species described below and *N. aegidatum* appear to form a monophyletic assemblage for



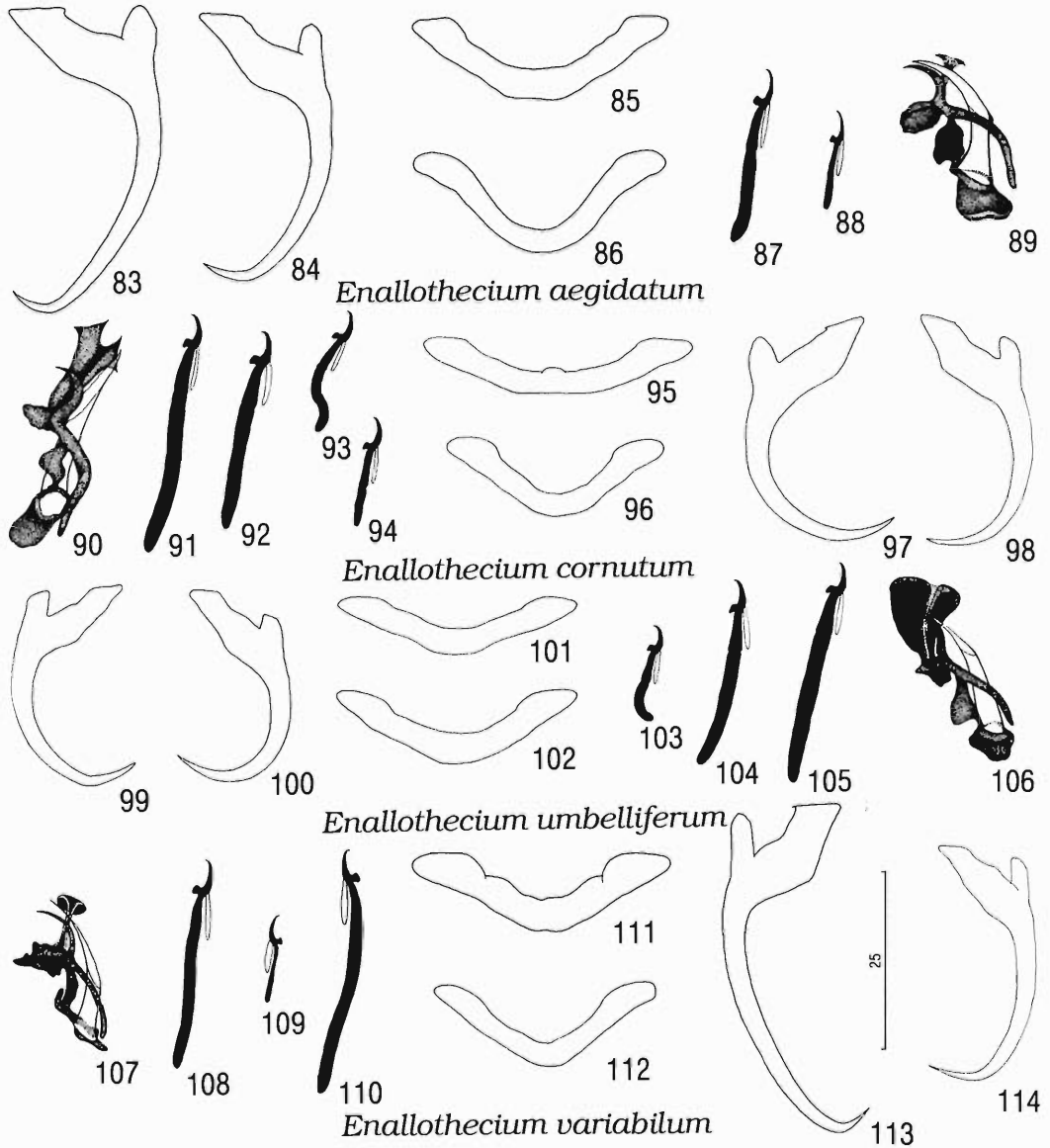
Figures 80–82. Whole mount illustrations of *Enallothecium* spp. (composite, ventral views). 80. *Enallothecium cornutum* sp. n. (from *Serrasalmus rhombeus*). 81. *Enallothecium umbelliferum* sp. n. (from *Serrasalmus rhombeus*). 82. *Enallothecium variabilum* sp. n. All drawings are to respective 100- $\mu$ m scales.

which *Enallothecium* is proposed. Apparent synapomorphic features of *Enallothecium* include a diagonal opening of the male copulatory organ, presence of an umbell and flap-like thumb of the accessory piece, and the vagina opening at the tip of a small papilla on the sinistrodorsal surface of the trunk.

*Enallothecium aegidatum*  
(Boeger and Kritsky, 1988) comb. n.  
(Figs. 83–89)

RECORDS: *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Pygocentrus nattereri*: Rio Uatumã, Lago Tapanã near Santana, Amazonas (3 November 1989); Furo do Catalão, Manaus, Amazonas (6 November 1984). *Serrasalmus compressus*: Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (28 October 1993). *Serrasalmus elongatus*: Furo do Catalão,

Manaus, Amazonas (27 November 1984); Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Serrasalmus rhombeus*: Rio Uatumã, Lago Tapanã near Santana, Amazonas (3 November 1989); Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (26 November 1984). *Serrasalmus spilopleura*: Rio Uatumã, Lago Tapanã near Santana, Amazonas (3 November 1989); Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (14 September 1984). *Serrasalmus* sp. (2 of Jégu): Rio Uatumã, Lago Tapanã near Santana, Amazonas (3 November 1989); Santa Luzia, Rio Uatumã, Amazonas (20 September 1985). *Serrasalmus* sp. ( $2n = 58$ ): Furo do Catalão, Manaus, Amazonas (5 January 1989, 30 January 1991).



Figures 83–114. Sclerotized structures of *Enallothecium* spp. 83–89. *Enallothecium aegidatum* (Boeger and Kritsky, 1988) (from *Serrasalmus gouldingi*). 83. Ventral anchor. 84. Dorsal anchor. 85. Ventral bar. 86. Dorsal bar. 87. Hook pair 3. 88. Hook pair 5. 89. Copulatory complex (ventral view). 90–98. *Enallothecium cornutum* sp. n. (from *Serrasalmus rhombeus*). 90. Copulatory complex (ventral view). 91. Hook pair 7. 92. Hook pair 3. 93. Hook pair 1. 94. Hook pair 5. 95. Ventral bar. 96. Dorsal bar. 97. Ventral anchor. 98. Dorsal anchor. 99–106. *Enallothecium umbelliferum* sp. n. (from *Serrasalmus rhombeus*). 99. Ventral anchor. 100. Dorsal anchor. 101. Ventral bar. 102. Dorsal bar. 103. Hook pair 1. 104. Hook pair 3. 105. Hook pair 7. 106. Copulatory complex (ventral view). 107–114. *Enallothecium variabilum* sp. n. 107. Copulatory complex (ventral view). 108. Hook pair 3. 109. Hook pair 5. 110. Hook pair 7. 111. Ventral bar. 112. Dorsal bar. 113. Ventral anchor. 114. Dorsal anchor. All drawings are to the 25- $\mu$ m scale.

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

SPECIMENS STUDIED: 1 voucher from *Pristobrycon* sp., USNPC 86141; 6 vouchers from *Pygocentrus nattereri*, USNPC 86142, 86143; 7 vouchers from *S. compressus*, USNPC 86150; 5 vouchers from *S. elongatus*, USNPC 86144, 86145; 14 vouchers from *S. gouldingi*, USNPC 86151; 11 vouchers from *S. rhombeus*, USNPC 86152, 86153, 86154; 5 vouchers from *S. spilopleura*, USNPC 86139, 86140; 3 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 86146, 86147; 10 vouchers from *Serrasalmus* sp. ( $2n = 58$ ), USNPC 86148, 86149.

COMPARATIVE MEASUREMENTS: Table 3.

REMARKS: *Enallothecium aegidatum* was originally described as *Notothecium aegidatum* from *Pygocentrus nattereri* by Boeger and Kritsky (1988). This species apparently has a wide host tolerance having herein been found on 9 species of *Pristobrycon*, *Pygocentrus*, and *Serrasalmus*. Although not reported in the original description, *E. aegidatum* does possess a small, weakly sclerotized umbell in the accessory piece. *Enallothecium aegidatum* differs from *E. cornutum* sp. n. and *E. umbelliferum* sp. n. by having anchors with elongate shafts and short points. It differs from *E. variabilum* sp. n. by having a more robust distal rod and a less developed umbell of the accessory piece and by the dorsal anchor being slightly smaller than the ventral anchor (anchors of noticeably different size in *E. variabilum*).

***Enallothecium cornutum* sp. n.**

(Figs. 80, 90–98)

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

OTHER RECORDS: *Pristobrycon eigenmanni*: Rio Negro near Manaus, Amazonas (28 December 1988); Nazaré, Rio Uatumã, Amazonas (17 September 1985). *Pristobrycon* sp.: Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus compressus*: Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (28 October 1993). *Serrasalmus gouldingi*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989). *Ser-*

*rasalmus rhombeus*: Rio Uatumã, Lago Tapaná near Santana, Amazonas (3 November 1989); Rio Negro near Manaus, Amazonas (28 December 1988). *Serrasalmus* sp. (2 of Jégu): Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Nazare, Rio Uatumã, Amazonas (17 September 1985). *Serrasalmus* sp. ( $2n = 58$ ): Furo do Catalão, Manaus, Amazonas (5 January 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 323; 3 paratypes, USNPC 86155, 86156, 86157. 4 vouchers from *P. eigenmanni*, USNPC 86158, 86159; 1 voucher from *Pristobrycon* sp., USNPC 86161; 3 vouchers from *S. compressus*, USNPC 86160; 11 vouchers from *S. gouldingi*, USNPC 86165; 2 vouchers from *Serrasalmus* sp. (2 of Jégu), USNPC 86163, 86164; 3 vouchers from *Serrasalmus* sp. ( $2n = 58$ ), USNPC 86162.

COMPARATIVE MEASUREMENTS: Table 4.

DESCRIPTION: Greatest body width in posterior trunk. Tegument smooth. Cephalic lobes moderately developed. Eyes usually absent; eye granules variable in size; accessory granules few or absent in cephalic, anterior trunk regions. Pharynx spherical. Peduncle broad. Anchors similar; each with well-developed roots, depressed superficial root, evenly curved shaft, elongate point. Ventral bar broadly U-shaped, with small terminal enlargements, short stubby anteromedial projection infrequently present; dorsal bar broadly U-shaped, with slightly enlarged ends. Copulatory organ slightly arched; base with small proximal flap. Distal rod of accessory piece sigmoid; cornate umbell with 2 terminal, 2 bilateral spines. Gonads subovate; seminal vesicle prominent. Oviduct short; ootype, uterus not observed; vitellaria limited in trunk, absent in regions of reproductive organs.

REMARKS: *Enallothecium cornutum* resembles *E. umbelliferum* sp. n. in the general morphology of the haptor armament. It differs from this species by having a spined umbell of the accessory piece (umbell large and unspined in *E. umbelliferum*). The specific name is from Latin (*cornutus* ["horned"]) and refers to the spines on the umbell of the accessory piece.

***Enallothecium umbelliferum* sp. n.**

(Figs. 81, 99–106)

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

Table 3. Comparative measurements (in micrometers) of *Enalothectium aegidatum* (Boeger and Kritsky, 1988) comb. n., from 9 serrasalmid hosts.

	<i>Pristibrycon</i> sp.	<i>Pygocentrus</i> <i>nattereri</i>	<i>Serrasalmus</i> <i>compressus</i>	<i>Serrasalmus</i> <i>elongatus</i>	<i>Serrasalmus</i> <i>gouldingi</i>	<i>Serrasalmus</i> <i>rhombus</i>	<i>Serrasalmus</i> <i>spitopleura</i>	<i>Serrasalmus</i> n sp. (2 of Jégu)	<i>Serrasalmus</i> sp. (2n = 58)
Body									
Length	—	—	—	—	310 (248–388)	7 299 (264–334)	2 270 (229–310)	2	— 222 (186–241)
Width	—	—	—	—	105 (91–122)	7 96	2 82	1	— 91 (85–94)
Haptor									
Length	—	—	—	—	70 (62–75)	7 72 (58–87)	2 69	1	— 59 (55–63)
Width	—	—	—	—	99 (92–108)	7 102 (98–107)	2 69	1	— 91 (85–94)
Pharynx									
Diameter	—	—	—	—	19 (16–21)	7 19–20	2 17	1	— 15 (14–16)
Copulatory organ									
Length	26	1 23 (21–28)	6 23 (22–24)	3 23 (21–25)	4 25 (23–26)	7 23 (21–24)	5 21–22	2	— 23 (22–25)
Length	22	1 22 (20–25)	6 21 (20–22)	2 21 (19–22)	4 22 (21–23)	6 20 (19–22)	5 20–21	2	27 1 21 (19–22)
Dorsal anchor									
Length	—	36 (32–41)	6 36 (35–37)	6 35 (31–39)	4 39 (38–41)	6 38 (36–40)	7 38 (37–39)	2 34 (31–36)	2 37
Base width	—	14 (13–16)	5 13–14	2 13 (11–16)	4 16 (14–18)	5 13–14	2 13	2 14	1 14
Ventral anchor									
Length	42	1 40 (36–46)	6 40 (37–41)	6 37 (32–42)	4 43 (42–44)	7 40 (37–43)	8 37–38	3 39 (37–41)	2 39 (36–42)
Base width	18	1 17 (14–18)	6 15 (14–16)	5 16 (15–17)	4 19 (18–21)	5 16–17	5 17 (15–18)	3 16 (14–18)	2 16 (15–17)
Bar length									
Ventral	—	42	1	—	—	36 (34–40)	6 37 (35–39)	2 35–36	2 38 (36–40)
Dorsal	—	37–38	2	—	—	33 (30–36)	6 33 (30–37)	2 32 (28–35)	2 32 (30–35)
Hook lengths									
Pair 1	—	22 (21–23)	2 21 (19–22)	4 24 (22–28)	3 22	1 21–22	2 20	1 21 (20–22)	3 21
Pair 2	26	1 26	1 24–25	2 24–25	2 27–28	2 27 (26–28)	5 25	1 25 (24–26)	2 24–25
Pair 3	34	1 30 (28–31)	5 29 (28–30)	4 29 (27–31)	3 32 (31–34)	5 31 (29–32)	7 31	2 32 (30–34)	2 32 (31–33)
Pair 4	31	1 30 (27–31)	5 29 (28–30)	4 29	3 32 (30–33)	5 30 (29–33)	9 29–30	2 33	1 32 (30–34)
Pair 5	14	1 16	4 15–16	4 15–16	2 16	4 15	3 15	1 14–15	2 15–16
Pair 6	24	1 20–21	2 22 (21–23)	2 20	1 22–23	2 23 (22–25)	5 22	1 21	1 22
Pair 7	41	1 33 (31–35)	4 34 (33–35)	4 35 (33–36)	4 39 (37–41)	3 39 (36–44)	5 34 (32–36)	2 39 (38–40)	2 36 (33–40)
Germinarium									
Length	—	—	—	—	—	52 (41–61)	6 49 (40–59)	2 43	1 33 (23–43)
Width	—	—	—	—	—	20 (19–23)	6 15 (10–20)	2 8	1 16–17
Testis									
Length	—	—	—	—	—	54 (52–57)	4 44	1	— 52
Width	—	—	—	—	—	28 (25–30)	4 21	1	— 20



Table 4. Comparative measurements (in micrometers) of *Enallothecium cornutum* sp. n., from 7 serrasalmid hosts.

	<i>Pristobrycon</i> <i>eigenmanni</i>	<i>n</i>	<i>Pristobrycon</i> sp.	<i>n</i>	<i>Serrasalmus</i> <i>compressus</i>	<i>n</i>	<i>Serrasalmus</i> <i>gouldingi</i>	<i>n</i>	<i>Serrasalmus</i> <i>rhombus</i>	<i>n</i>	<i>Serrasalmus</i> sp. (2 of Jégu)	<i>n</i>	<i>Serrasalmus</i> sp. (2 <i>n</i> = 58)	<i>n</i>
Body														
Length	201	1	—	—	—	—	314 (291–345)	6	250 (243–256)	2	—	—	265	1
Width	69 (67–71)	2	—	—	—	—	115 (94–135)	6	110 (104–115)	2	—	—	104	1
Haptor														
Length	55	1	—	—	—	—	65 (59–70)	6	57 (45–68)	2	—	—	59	1
Width	64	1	—	—	—	—	108 (96–124)	6	96 (89–102)	2	—	—	85	1
Pharynx														
Diameter	16–17	2	—	—	—	—	20 (19–21)	5	20 (18–21)	2	—	—	19	1
Copulatory organ														
Length	28 (27–29)	2	27	1	25 (24–27)	2	25 (22–29)	4	28–29	2	28	1	22	1
Accessory piece														
Length	24	2	25	1	23	2	23 (21–26)	4	26 (25–28)	2	25	1	—	—
Dorsal anchor														
Length	32 (30–33)	2	31	1	32 (29–33)	3	33 (31–35)	2	33	1	34	3	33	1
Base width	16	2	13	1	11	1	20	2	15	1	17	1	14	1
Ventral anchor														
Length	31–32	2	31	1	30 (29–31)	3	30 (27–33)	5	36 (34–38)	2	30 (28–33)	4	30–31	2
Base width	18–19	2	16	1	17 (16–18)	2	18 (15–21)	3	16–17	2	17 (15–19)	4	20	1
Bar length														
Ventral	29	1	—	—	—	—	37 (30–40)	5	38–39	2	—	—	37	1
Dorsal	26	1	—	—	—	—	33 (31–35)	5	33	2	—	—	33	1
Hook lengths														
Pair 1	18 (17–19)	2	—	—	—	—	22	1	20	1	18	1	18	1
Pair 2	24	1	27	1	24	1	25 (23–29)	4	24–25	2	27	2	24	1
Pair 3	28	1	29	1	28–29	2	27 (24–32)	5	30–31	2	30 (29–31)	2	29	2
Pair 4	28	1	29	1	28–29	2	30 (27–34)	7	30–31	2	31 (29–32)	3	30	1
Pair 5	17	1	16	1	15	1	16 (14–17)	2	16	1	16–17	2	—	—
Pair 6	20 (19–21)	2	24	1	22–23	1	24 (23–25)	3	23	1	23	2	22	2
Pair 7	28 (27–29)	2	33	1	31 (29–33)	2	34–35	3	34 (32–35)	2	32 (30–34)	2	32 (31–33)	2
Germaryum														
Length	39	1	—	—	—	—	48 (47–51)	5	43 (40–45)	2	—	—	35	1
Width	15	1	—	—	—	—	28 (20–37)	4	28	2	—	—	23	1
Testis														
Length	—	—	—	—	—	—	52 (46–57)	4	46	1	—	—	—	—
Width	—	—	—	—	—	—	33 (26–44)	4	29	1	—	—	—	—

OTHER RECORDS: *Serrasalmus compressus*: Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas (28 October 1993). *Serrasalmus* sp. (2 of Jégu): Rio Jatapú, Lago Maracana, Amazonas (2 November 1989).

SPECIMENS STUDIED: Holotype, INPA PLH 324; 9 paratypes, INPA PLH 325, USNPC 86166, HWML 38761. 4 vouchers from *Serrasalmus compressus*, USNPC 86168; 1 voucher from *Serrasalmus* sp. (2 of Jégu), USNPC 86167.

COMPARATIVE MEASUREMENTS: Measurements of specimens from *Serrasalmus compressus* follow those of the type series in brackets. The specimen from *Serrasalmus* sp. (2 of Jégu) was not measured.

DESCRIPTION: Body 266 (243–318;  $n = 6$ ) long; greatest width 94 (78–115;  $n = 6$ ) near midlength. Tegument smooth, infrequently with scaled annulations in posterior trunk, peduncle. Cephalic lobes moderately developed. Accessory eye granules usually present in cephalic, anterior trunk regions. Pharynx spherical, 18 (16–21;  $n = 6$ ) in diameter. Peduncle broad; haptor 57 (50–66;  $n = 6$ ) long, 89 (85–92;  $n = 6$ ) wide. Anchors similar; each with elongate, slightly depressed superficial root, prominent deep root, evenly curved shaft, long point; ventral anchor 28 (26–31;  $n = 5$ ) [30–31 ( $n = 3$ )] long, base 14 (13–15;  $n = 3$ ) [14 (13–16;  $n = 3$ )] wide; dorsal anchor 29–30 ( $n = 4$ ) [32 (31–33;  $n = 3$ )] long, base 15 (14–17;  $n = 2$ ) [13 (11–14;  $n = 2$ )] wide. Bars similar, broadly U-shaped, with slightly enlarged terminations; ventral bar 32 (29–36;  $n = 5$ ) long, dorsal bar 32 (29–35;  $n = 6$ ) long. Hook pair 1—[16 ( $n = 1$ )], pair 2—22 (21–24;  $n = 3$ ) [22–23 ( $n = 2$ )], pair 3—28 (26–29;  $n = 4$ ) [28 ( $n = 2$ )], pair 4—29 (27–31;  $n = 3$ ) [28 (27–29;  $n = 2$ )], pair 5—14–15 ( $n = 2$ ) [14 ( $n = 2$ )], pair 6—19 (18–20;  $n = 2$ ) [21 ( $n = 1$ )], pair 7—30 (27–32;  $n = 3$ ) [29–30 ( $n = 2$ )] long. Copulatory organ 23 (21–25;  $n = 4$ ) [26 (25–27;  $n = 3$ )] long, base with sclerotized margin, small proximal flap. Distal rod of accessory piece 27–28 ( $n = 4$ ) [24–25 ( $n = 3$ )] long, sigmoid, with terminal hook, subterminal thumb, large umbell. Gonads subovate; testis 46 (41–52;  $n = 3$ ) long, 24 (18–28;  $n = 3$ ) wide; germarium 41 (35–45;  $n = 6$ ) long, 17 (14–21;  $n = 6$ ) wide. Seminal vesicle prominent. Ootype, uterus not observed; vitellaria in trunk except absent in regions of reproductive organs.

REMARKS: *Enallothecium umbelliferum* dif-

fers from all other species in the genus by having a large umbell in the accessory piece. The specific name is from Latin (*umbella* [“a sun shade”] + *fero* [“to bear”]) and refers to the umbell of the accessory piece.

*Enallothecium variabilum* sp. n.

(Figs. 82, 107–114)

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

OTHER RECORDS: *Pristobrycon striolatus*: Rio Jatapú, Lago Maracana, Amazonas (2 November 1989); Santa Luzia, Rio Uatumã, Amazonas, (20 September 1985); Lago Samaumã, Rio Uatumã, Amazonas (25 September 1985); Rio Pitinga, Igarapé Agua Branca, Rio Uatumã, Amazonas (15 September 1985).

SPECIMENS STUDIED: Holotype, INPA PLH 326; 45 paratypes, INPA PLH 327, USNPC 86169, 86170, 86171, 86172, 86173, HWML 38762.

DESCRIPTION: Body 272 (219–337;  $n = 21$ ) long, cephalic region directed ventrally; greatest body width 87 (62–110;  $n = 21$ ) usually in anterior trunk. Tegument smooth or with scaled annulations over posterior trunk, peduncle. Cephalic lobes moderately developed. Eyes absent or small, comprised of few granules; posterior pair larger than anterior pair; accessory granules few or absent in cephalic, anterior trunk regions. Pharynx spherical, 14 (12–16;  $n = 22$ ) in diameter. Peduncle broad; haptor 66 (54–75;  $n = 23$ ) long, 92 (78–112;  $n = 22$ ) wide. Ventral anchor 45 (37–50;  $n = 20$ ) long, with well-developed roots, elongate curved shaft, short point; base 15 (13–17;  $n = 18$ ) wide. Dorsal anchor 36 (30–41;  $n = 19$ ) long, with well-developed roots, curved shaft, moderately long point; base 12–13 ( $n = 5$ ) wide. Ventral bar 34 (31–36;  $n = 21$ ) long, broadly U-shaped, with small terminal enlargements; dorsal bar 31 (28–34;  $n = 20$ ) long, broadly V-shaped, with slightly enlarged ends. Hook pair 1—22 (21–24;  $n = 3$ ), pair 2—26 (21–29;  $n = 12$ ), pairs 3, 4—31 (26–33;  $n = 39$ ), pair 5—14 (12–15;  $n = 18$ ), pair 6—20 (17–22;  $n = 9$ ), pair 7—36 (31–40;  $n = 15$ ) long. Copulatory organ 23 (22–24;  $n = 14$ ) long, arcuate; base with small proximal flap. Accessory piece 21 (20–22;  $n = 14$ ) long; distal rod delicate, with elongate terminal hook, sub-terminal flabellate thumb, small stalked umbell.

Testis 48 (34–67;  $n = 3$ ) long, 20 (19–21;  $n = 3$ ) wide, subovate; seminal vesicle prominent. Germarium 47 (26–68;  $n = 16$ ) long, 17 (12–21;  $n = 16$ ) wide, irregular; oviduct, ootype, uterus not observed; vitellaria throughout trunk except absent in regions of reproductive organs.

REMARKS: *Enallothecium variabilum* resembles *E. aegidatum* by general morphology of the anchors. It differs from *E. aegidatum* by possessing a more delicate distal rod of the accessory piece and a ventral anchor with a more elongate shaft. The specific name is from Latin (*variabilis* ["variable"]).

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## A New Species of *Pedibothrium* (Cestoidea: Tetraphyllidea) from the Short-tail Nurse Shark, *Pseudoginglymostoma brevicaudatum* (Elasmobranchii: Orectilobiformes), from Southwest Madagascar

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**ABSTRACT:** Examination of the contents of the spiral intestine of a preserved specimen of the short-tail nurse shark, *Pseudoginglymostoma brevicaudatum*, collected in the Mozambique Channel off Toliara, Madagascar, led to the discovery of *Pedibothrium toliarensis* sp. n. Among the 9 described species of *Pedibothrium*, the new species most closely resembles *Pedibothrium longispine* in its possession of bipronged hooks with axial and abaxial prongs that are conspicuously recurved. It is readily distinguished from the latter species in its possession of a greater number of testes, a larger scolex, and 2 (rather than 1) columns of testes in the postporal region of the segment. Species of *Pedibothrium* are now known from 3 of the 5 species of sharks belonging to the orectilobiform family Rhincodontidae. This record expands the known geographic range of the host species and parasite genus to include the southwestern coast of Madagascar. Several strobilar fragments consistent with the segment morphology of *Pedibothrium*, but differing from *P. toliarensis* in genital pore position and testes number, suggest that *P. brevicaudatum* may host additional species of *Pedibothrium*.

**KEY WORDS:** *Pedibothrium*, Tetraphyllidea, Onchobothriidae, *Pseudoginglymostoma*, Madagascar.

To date, the genus *Pedibothrium* includes 9 species of tapeworms. Verified host records for these 9 species are restricted to sharks belonging to the orectilobiform family Rhincodontidae. This family of sharks is interesting in that it currently consists solely of 5 monotypic genera. According to the most recent cladistic treatment of the group (see Dingerkus, 1986), these 5 shark species represent 3 lineages: (1) the Atlantic nurse shark (*Ginglymostoma cirratum* (Bonnatere, 1788)) plus the tawny nurse shark (*Nebrius ferrugineus* (Lesson, 1830)), (2) the whale shark (*Rhincodon typus* Smith, 1828) plus the zebra shark (*Stegostoma fasciatum* (Hermann, 1783)), and (3) a basal lineage consisting solely of the short-tail nurse shark (*Pseudoginglymostoma brevicaudatum* (Günther, 1866)). The 9 known species of *Pedibothrium* have been reported from 2 of the 5 shark species in the family representing each of the 2 nonbasal lineages of rhincodontids. According to Caira (1992), *G. cirratum* hosts: *Pedibothrium manteri* Caira, 1992; *Pedibothrium brevispine* Linton, 1909; *Pedibothrium longispine* Linton, 1909; *Pedibothrium globicephalum* Linton, 1909; *Pedibothrium maccallumi* Caira and Pritchard, 1986; and *Pedibothrium*

*servattorum* Caira, 1992. *Stegostoma fasciatum* hosts *Pedibothrium veravalensis* Shinde, Jadhav & Deshmukh, 1980; *Pedibothrium lintoni* Shinde, Jadhav & Deshmukh, 1980 (see Shinde et al., 1980; Butler, 1987; and Caira, 1992); and possibly also *Pedibothrium kerkhami* (see Caira, 1992). These records suggest that *Pedibothrium* is restricted to sharks of the family Rhincodontidae, but the extent of the association with sharks of this host family is unclear because, to date, no onchobothriid records exist for *N. ferrugineus*, *R. typus*, or *P. brevicaudatum*. In this study, we focused on the parasites of *P. brevicaudatum* in particular because of the basal position of this shark taxon on the rhincodontid tree. As we accept the proposal of Baer and Euzet (1962) that *Pedibothrium huttoni* Southwell, 1911 belongs in the genus *Pachybothrium* (see also Caira and Pritchard, 1986), we have omitted this species from consideration.

According to Compagno (1984), *P. brevicaudatum* is known only from the east coast of Tanzania and Kenya, and possibly the Mauritius and Seychelle islands. However, a collecting trip to Madagascar conducted in 1997 revealed not only that *P. brevicaudatum* is present in the waters of that country, but also that this shark species hosts at least 1 new species of *Pedibothrium* described below.

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## Materials and Methods

Interviews of fishermen working in the vicinity of Toliara, Madagascar, suggested that *P. brevicaudatum* (locally known as "Hia Hia") is a fairly common species in that region. We were unable to obtain freshly killed specimens of *P. brevicaudatum*. However, Dr. Edward Mare, the director of the museum at the Institut Halieutique et des Sciences Marines de l'Université de Toliara, kindly allowed us to necropsy a preserved museum specimen of *P. brevicaudatum*. This specimen had been caught off the coast of Toliara and had been stored in ethanol following fixation in formalin. The body cavity and spiral intestine had been opened with longitudinal incisions prior to fixation and thus fixative had penetrated into the internal regions of the spiral intestine when the shark was placed in formalin. To minimize disturbance of the host specimen, the spiral intestine was left attached within the body of the shark. Contents of the spiral intestine were removed through the existing longitudinal incision with a small curette and examined with a dissecting microscope. Worms collected from the contents of the spiral intestine were transferred to 70% ethanol for storage. Specimens prepared as whole mounts for light microscopy were hydrated in a graded ethanol series, stained in Gill's hematoxylin, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam on glass slides. The scolex of 1 specimen was prepared for scanning electron microscopy according to the procedure described by Freidenfelds et al. (1994). The dried specimen was attached to an aluminum stub with carbon tape, further grounded with carbon paint, sputter coated with approximately 200 Å of gold/palladium, and examined with a LEO/Zeiss DSM982 Gemini field emission scanning electron microscope. The stub was retained in the personal collection of the senior author.

All measurements are in micrometers unless otherwise stated and are given in the text as the range followed in parentheses by the mean, the standard deviation, the number of worms examined, and the number of observations made if more than 1 measurement for any character was taken per individual worm. Hook measurements were made following Caira (1992). Illustrations were prepared with the aid of a drawing tube. Abbreviations used for museums are as follows: DAB, Department of Animal Biology, % Dr. Daniel Rakotondravony, University of Antananarivo, Antananarivo, Madagascar; MNHN, Collection du Museum National d'Histoire Naturelle, Paris, France; USNPC, United States National Parasite Collection, Beltsville, Maryland, U.S.A.

## Results

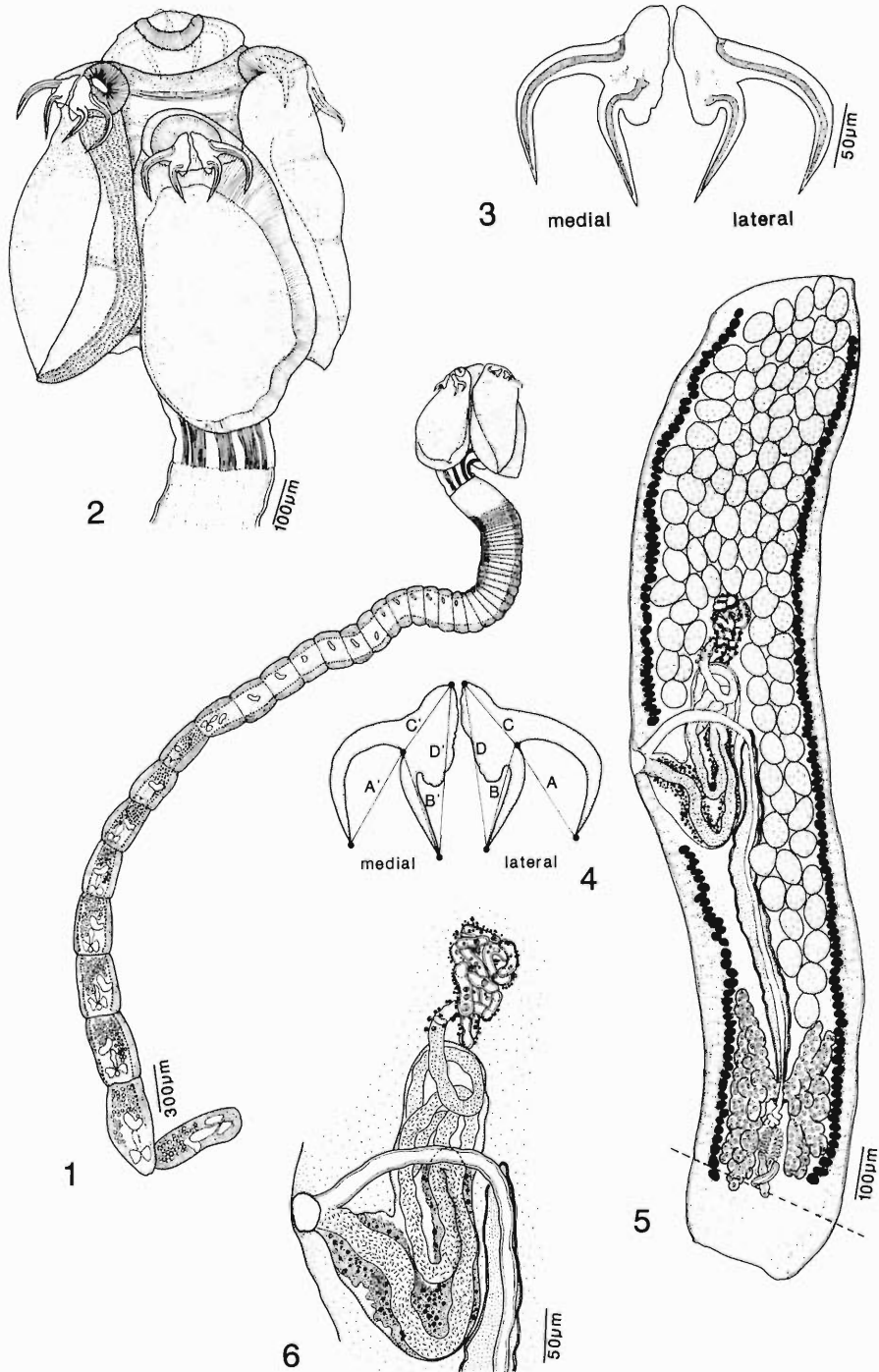
Six tapeworm specimens with scoleces, 6 strobilar fragments, and 3 free segments were found among the debris removed from the spiral intestine of the specimen of *P. brevicaudatum*. The 6 specimens with scoleces, 1 of the strobilar fragments, and 1 of the free segments were considered to represent the new species of *Pedibothrium* described below.

## *Pedibothrium toliarensis* sp. n.

(Figs. 1–6)

DESCRIPTION: (based on 1 complete specimen, 4 incomplete specimens with scoleces, 1 strobilar fragment, 1 free segment, and 1 scolex examined with SEM): Worms 1,950 ( $n = 1$ ) long; greatest width at level of scolex (Fig. 1); 23–51 ( $39 \pm 14.6$ ; 3) segments per worm; acraspedote, euapolytic; genital pores marginal, 42–44% ( $43 \pm 0.8$ ; 2; 2) of segment length from posterior end, irregularly alternating. Scolex with 2 dorsal and 2 ventral bothridia supported on conspicuous cephalic peduncle (Fig. 2). Each bothridium elongate oval in outline with rounded posterior margins, 698–833 ( $750 \pm 33.1$ ; 5; 18) long by 335–434 ( $390 \pm 31.1$ ; 5; 8) wide; consisting of 1 posthook loculus and anterior muscular pad with apical sucker (Fig. 2). Muscular pad 72 ( $n = 1$ ) long by 185 ( $n = 1$ ) wide; accessory sucker 39–48 ( $43 \pm 5.2$ ; 2; 3) long by 100–146 ( $116 \pm 20.6$ ; 2; 4) wide; loculus 536–704 ( $637 \pm 45.9$ ; 5; 17) long. Lateral margins of loculi well defined, not folded over one another along axis of bothridium. One pair of symmetrical bipronged hooks at boundary between muscular pad and loculus of each bothridium. Lateral and medial bothridial hooks similar in shape. Abaxial and axial prongs of each hook curved, with separate channels and pores (Fig. 3); abaxial prong channel opening on distal surface of hook, axial prong channel opening on proximal surface of hook. Lateral hook measurements (Fig. 4): A, 91–102 ( $97 \pm 3.9$ ; 3; 8); B, 89–100 ( $97 \pm 3.7$ ; 3; 8); C, 61–78 ( $70 \pm 6.0$ ; 3; 8); D, 139–155 ( $148 \pm 5.3$ ; 3; 8). Medial hook measurements (Fig. 4): A', 74–96 ( $86 \pm 7.6$ ; 3; 8); B', 91–100 ( $95 \pm 3$ ; 8); C', 55–72 ( $68 \pm 6.0$ ; 3; 8); D', 130–154 ( $146 \pm 7.4$ ; 3; 8). Bases of hooks embedded in musculature of scolex. Cephalic peduncle 309–600 ( $454 \pm 205.9$ ; 2) long. Scolex (including bothridia and cephalic peduncle) 1,017–1,380 (1,198; 2) long by 659–754 ( $725 \pm 38.4$ ; 5) wide. Microthrix pattern not observed.

Immature segments 49 in number, wider than long, becoming longer than wide with maturity; mature segments 2 in number, 1,519–1,866 ( $1,693 \pm 245.5$ ; 1; 2) long by 378–382 ( $380 \pm 3.0$ ; 1; 2) wide, length-to-width ratio 2.1–4.9:1 ( $3.3 \pm 1.4$ ; 1; 2); gravid segments not seen. Testes 90–111 ( $101 \pm 9.3$ ; 2; 4) in number, ap-



Figures 1–5. Line drawings of *Pedibothrium toliarensis* n. sp. 1. Holotype (DAB). 2. Scolex of paratype (USNPC No. 87580). 3. Hooks of paratype (USNPC No. 87580). 4. Illustration of hook measurements taken. 5. Mature segment of paratype (USNPC No. 87580). Note that segment is broken at level of dashed line. 6. Detail of terminal genitalia of segment illustrated in Figure 5.

proximately round, 30–59 ( $46 \pm 7.8$ ; 2; 12) long by 35–54 ( $48 \pm 5.1$ ; 2; 12) wide, filling anterior half of segment and extending to anterior margin of ovary on poral side of segment, absent from postporal region of segment, arranged in 1 plane, 5–6 irregular columns anterior to cirrus pouch (Fig. 5), 2 irregular columns posterior to cirrus pouch on aporal side of segment; vas deferens coiled, median, in middle of segment, entering base of cirrus sac from anterior. Cirrus sac elongated, J-shaped, 373–477 ( $425 \pm 73.6$ ; 2; 2) long by 113 ( $n = 1$ ) wide; containing coiled cirrus covered with spiniform microtriches; proximal half of cirrus sac submedian and more or less longitudinal, distal half posterior to proximal half, extending laterally towards genital pore. Ovary at posterior end of segment, 339–369 ( $354 \pm 21.5$ ; 1; 2) long by 204–217 ( $211 \pm 9.3$ ; 1; 2) wide, H-shaped in dorsoventral view, cross sections not prepared, weakly follicular, with thin ovarian isthmus; aporal lobe extending anteriorly approximately halfway to level of cirrus pouch; poral lobe extending slightly further anteriorly. Vagina slightly expanded at base, median, extending just anterior to middle of cirrus pouch, then laterally along anterior margin of cirrus pouch to genital pore. Uterus median, thick walled, extending anteriorly from ovarian isthmus to middle of cirrus pouch. Vitellaria follicular; follicles arranged in 2 lateral bands, extending from near anterior of segment to near posterior of segment, interrupted by cirrus sac, not interrupted by ovary; each band consisting of 1 dorsal and 1 ventral column of follicles. Excretory ducts not seen.

The scolex examined with scanning electron microscopy was found to be in poor condition, perhaps having suffered from preservation within the spiral intestine of its host. No useful microthrix data could be obtained from this specimen.

#### Taxonomic summary

TYPE HOST: *Pseudoginglymostoma brevicaudatum* (Günther, 1866), short-tail nurse shark.

TYPE LOCALITY: Mozambique Channel off Toliara, Madagascar.

SITE OF INFECTION: Spiral intestine.

SPECIMENS DEPOSITED: Holotype and 1 paratype (DAB); 1 paratype, MNHN No. 563HF, slide 63 CIX; 3 paratypes (1 specimen with scolex, 1 strobilar fragment, and 1 free segment, USNPC No. 87580).

ETYMOLOGY: This species is named for its type locality, Toliara, Madagascar.

#### Remarks

*Pedibothrium toliarensis* is a typical member of the genus *Pedibothrium* in that it possesses 4 uniloculated bothridia, bipronged hooks with separate channels in each of the 2 prongs and a J-shaped cirrus sac that is crossed by the vagina. In addition, like all described species in the genus except *P. veravalensis*, it lacks testes on the poral side of the segment between the cirrus sac and the ovary. *Pedibothrium toliarensis* most closely resembles *P. longispine* but can be distinguished from this species on the basis of its greater number of testes (90–111 vs. 54–70), its longer scolex (730–815 vs. 350), and its possession of a double column of testes rather than a single column of testes on the aporal side of the segment between the cirrus sac and the ovary. *Pedibothrium toliarensis* is easily distinguished from *P. servattorum*, *P. brevispine*, and *P. kerkhami*, in that the axial prongs of its medial and lateral hooks are curved rather than straight. It is further distinguished from *P. servattorum* and *P. brevispine* in that its testes extend posteriorly to the anterior margin of the ovary on the aporal side of the segment, rather than stopping just posterior to the cirrus pouch on the aporal side of the segment. It differs further from *P. kerkhami*, as emended by Caira (1992), in that it has a greater number of testes (90–111 vs. 75). *Pedibothrium toliarensis* differs from *P. globicephalum* and *P. manteri* in that the bases of its medial and lateral hooks touch one another and are shorter than the prongs, rather than being widely separated from one another and much longer than the prongs, and the lateral margins of the loculi do not, in fact perhaps cannot, fold over the distal surfaces of the hooks. Unlike *P. veravalensis*, *P. toliarensis* lacks testes on the poral side of the segment between the cirrus pouch and ovary; in addition, *P. toliarensis* has a greater number of testes than *P. veravalensis* (90–111 vs. 20–44). The genital pore of *P. toliarensis* is in the posterior half of the segment rather than the anterior half of the segment as in *P. maccallumi*, and *P. toliarensis* has fewer segments than *P. maccallumi* (23–51 vs. 86). Unlike the poorly known *P. lintoni*, the genital pores of *P. toliarensis* alternate irregularly, rather than being unilateral.

### Discussion

Toliara, Madagascar, is a new locality record for the shark *P. breviceaudatum*. The specimen from which the tapeworm species described here was collected remains intact at the Institut Halieutique et des Sciences Marines de l'Université de Toliara, in Toliara, Madagascar, as a voucher for this record. This report expands the known distribution of this shark species (see Compagno, 1984) to include the southwest coast of Madagascar. Given the limited collecting that has been conducted throughout the east coast of Africa, we believe this record suggests that this shark species has a broader geographic distribution than has currently been documented.

The discovery of *Pedibothrium toliarensis* brings the total number of species of *Pedibothrium* to 10. With the exception of *P. kerkhami*, verified records for each of these species suggest that each tapeworm species parasitizes only a single species of shark in the orectilobiform family Rhincodontidae. Unfortunately, host records for *P. kerkhami* remain in conflict. Southwell (1911; p. 222) originally reported this species from "a large specimen" of *Chiloscyllium indicum*, and then subsequently reported it (as *Pedibothrium longispine*) from *Galeocerdo arcticus* and *Rhina ancylostoma* (see Southwell, 1930). Baer and Euzet (1962) found specimens consistent with this species in Southwell's material reported to have been collected from *Galeocerdo tigrinum*, and Caira (1992) listed specimens consistent with Southwell's description of *P. kerkhami* from *Stegostoma fasciatum* collected near Balgal, Queensland, Australia. Caira's (1992; p. 303) indication of "*Nebrius ferrugineus* (Lesson, 1830) (= *Ginglymostoma concolor*)" as the type host for *P. kerkhami* is an error and should be disregarded. Given the host specificity of all of the other species of *Pedibothrium*, we believe it unlikely that this species actually parasitizes 5 host species representing 3 different orders of elasmobranchs. We suspect that either more than 1 species of tapeworm is involved, or there has been some confusion with the identity of the hosts from which the material was obtained. However, location and examination of Southwell's material and additional collections are required before the issue of host specificity in this species can be resolved. Specifically, collection of additional specimens of *P.*

*kerkhami* from *C. indicum* would at least serve to verify this shark species as the type host.

With respect to the issue of multiple species of *Pedibothrium* inhabiting the same host species, as seems to be the case for *G. cirratum* and *S. fasciatum* (see Caira, 1992), although we describe only a single species of *Pedibothrium* from *P. breviceaudatum* here, several of the strobilar fragments found along with *P. toliarensis* suggest that 1 and perhaps even 2 additional species of *Pedibothrium* may also parasitize this host species. This additional material included a segment lacking postporal testes on the oral side of the segment but with an extremely posterior genital pore, as well as several strobilar fragments with segments similar in general morphology to those of *P. toliarensis*, but with up to 142 testes. In general, the limited host material examined here gives us little confidence that we have discovered the entire compliment of *Pedibothrium* species hosted by the short-tail nurse shark. These ideas remain to be verified by the examination of additional specimens of *P. breviceaudatum*.

Three of the 5 species of sharks in the family Rhinodontidae are now known to host species of *Pedibothrium*. Given that these 3 species represent each of the 3 lineages of rhincodontids (see Dingerkus, 1986), it seems likely that the other 2 rhincodontid species will also be found to host this tapeworm genus. However, examination of the tapeworm fauna of the whale shark, *Rhincodon typus*, will be especially interesting given that, unlike the 4 other species in the family, the whale shark is a filter feeder rather than a bottom feeder.

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## A Redescription of *Cylicocycclus radiatus* (Nematoda: Cyathostominae), A Parasite of the Ass, *Equus asinus*, and Horse, *Equus caballus*

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**ABSTRACT:** *Cylicocycclus radiatus*, the type species of the genus, a rare, but cosmopolitan species of small strongyle from horses, is redescribed to provide the information required for its identification and differentiation from other species of the genus. Type specimens discovered in the British Museum of Natural History were included among the specimens from *Equus asinus* from Egypt and *Equus caballus* from Kazakhstan, Ukraine, Panama, Canada, and the United States of America that formed the basis for this redescription. *Cylicocycclus radiatus* is distinguished by its large buccal capsule with relatively thin, straight walls; the absence of a dorsal gutter; inconspicuous leaf crowns; and a small indistinct esophageal funnel. The most similar species is *C. triramosus* of zebras, which can be distinguished by the presence of a dorsal gutter and distinct dorsal and ventral notches in the mouth collar.

**KEY WORDS:** Nematode systematics, Cyathostominae, Strongylidae, horse, *Cylicocycclus*, *Equus caballus*.

The small strongyles (Subfamily Cyathostominae) can cause considerable morbidity and mortality in horses (Herd, 1990). Currently, research interest is high because of the recognition of a new disease syndrome caused by larval stages in the wall of the large intestine and caecum (Mair, 1994), the development of widespread drug resistance (Coles, 1994), efforts to develop alternative control methods (Bird and Herd, 1995), and research on alternative identification methods for larval stages. Progress in all areas of this research requires accurate species identification of adult stages of the nematodes.

Nematodes of the genus *Cylicocycclus* are among the most numerous among the Cyathostominae parasitic in horses, and the genus includes at least 12 species. Until recently, several species of this genus were among the most difficult to identify because of inaccurate or confusing descriptions in the literature. Recently, redescriptions (Kharchenko et al., 1997; Lichtenfels et al., 1997) of several species of *Cylicocycclus* have provided the information needed to identify some of the problem species including

*C. ashworthi* (Le Roux, 1924), *C. nassatus* (Looss, 1900), and *C. triramosus* (Yorke and Macfie, 1918). The objective of the present study is to provide an improved description of *C. radiatus* (Looss, 1900), the type species of the genus.

Although *C. radiatus* is not often present in large numbers in horses (Foster, 1936; Torbert et al. 1986), it is cosmopolitan in distribution and the species can be difficult to separate from the similar, more numerous species *C. ashworthi*, *C. nassatus*, and *C. leptostomus* (Kotlan, 1920).

### Materials and Methods

The discovery by Eileen Harris of type specimens of *C. radiatus* in the British Museum made this redescription possible. Other specimens studied are listed in Table 1. Many of the available specimens were in poor condition. Workers collecting this species in the future are encouraged to deposit specimens in museum collections to provide good specimens for future studies.

Nematodes were cleared for study in temporary wet mounts in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) and studied with the aid of interference contrast light microscopy. Measurements are in micrometers unless indicated otherwise (Table 2). Papillae of the genital cone are num-

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**Table 1. Geographic locality, host, number and sex of type, and voucher specimens of *Cylicocycclus radiatus* studied.**

Geographic locality	Host	Collection no.*	Number studied		Collector
			Males	Females	
Lethbridge, Alberta, Canada	<i>Equus caballus</i>	18804*	5	4	Voucher Hadwen, S.
Lethbridge, Alberta, Canada	<i>Equus caballus</i>	18934*	5	5	Voucher Hadwen, S.
Panama	<i>Equus caballus</i>	58487*	0	3	Voucher Foster, A. O.
Lexington, Kentucky	<i>Equus caballus</i>	78701*	2	2	Voucher Lyons, E. T.
Kazakhstan, Ural Region	<i>Equus caballus</i>	86813*	3	3	Voucher Kharchenko, V. A.
Ukraine	<i>Equus caballus</i>	86814*	1	0	Voucher Kharchenko, V. A.
Egypt	<i>Equus asinus</i>	1931:261–262†	1	1	Types Looss, A.
Egypt	<i>Equus asinus</i>	1968:249†	1	1	Voucher Looss, A.

\* U.S. National Parasite Collection Number, Beltsville, Maryland 20705.

† British Museum of Natural History, London.

bered following the system of Chabaud et al. (1970), in which the single ventral papilla is numbered "0" and the paired dorsal papillae are numbered "7's."

Photomicrographs were obtained with a 35-mm camera mounted on an Olympus Vanox research microscope, usually at a magnification of ×100–400 using Kodak Tmax 100 black and white negative film.

Drawings were prepared with the aid of a camera lucida.

**Redescription**

***Cylicocycclus radiatus* (Looss, 1900)  
Chaves, 1930  
(Figs. 1–18)**

- = *Cyathostomum radiatum* Looss, 1900
- = *Cylichnostomum radiatum* (Looss, 1900) Looss, 1902
- = *Cylicostomum radiatum* (Looss, 1900) Gedoelst, 1903
- = *Cylicostomum (Cylicocycclus) radiatus* (Looss, 1900) Ihle, 1922
- = *Trichonema radiatum* (Looss, 1900) Le Roux, 1924
- = *Cylicostomum prionodes* Kotlan, 1921; Skrjabin and Ershov, 1933

GENERAL: With characteristics of genus *Cylicocycclus* Ihle, 1922: mouth collar high with broad lateral papillae that extend through collar (Figs. 1–3, 11–13). Submedian papillae, with spindle-shaped tips, extend beyond lateral papillae (Figs. 2, 3, 14). External leaf crown (ELC) with about 26 long, narrow, inconspicuous elements that bend in the middle toward the mouth and do not extend beyond the mouth collar (Figs. 2, 3, 12). Internal leaf crown (ILC) with

about 50 small, thin rectangular plates (Figs. 2, 3, 11, 12). Buccal capsule slightly ellipsoidal, wider laterally (Figs. 2, 12) than dorsoventrally (Figs. 3, 11); walls thin anteriorly, thicken only slightly anterior to relatively large hooplike ring at base (Figs. 2, 3, 11, 12). Buccal cavity relatively large, twice as wide as deep in freshly mounted specimens (Figs. 2, 3, 11, 12) and 3 times as wide as deep in specimens partially flattened under the weight of a cover glass (Table 2). Duct of dorsal esophageal gland empties at base of buccal cavity (Fig. 15); dorsal gutter absent. Excretory pore (Fig. 1) and cervical papillae (Figs. 1, 10) at same level posterior to nerve ring at point where esophagus widens posteriorly.

MALES: (*N* = 18) (measurements in Table 2) Copulatory bursa of average size, but dorsal lobe slightly elongate and distinctly set off from lateral lobes (Figs. 5, 17). Distal branches of dorsal ray with accessory branches (Fig. 17). Ventral projection of genital cone surrounded by well-developed dermal collar (Figs. 7, 9, 18). Dorsal to vent genital cone bears paired, bilobed, bubblelike opaque genital appendages through each of which passes a single No. 7 papilla (Figs. 9, 18). The genital appendages also bear tiny nipplelike points (Fig. 18).

FEMALES: (*N* = 19) (measurements in Table 2) Posterior end of female relatively straight, but small lateral prominences present anterior to anus; tail tapers sharply to a thin cone for most of its length. Tail slightly shorter than vulva to anus distance (Figs. 6, 16). Vagina long, vestibule short, paired sphincters and infundibula elongate (Fig. 6), the latter slightly longer than the former (Table 2).

**Table 2. Morphometrics (in micrometers); range with mean in parentheses of males and females of *Cylicocyclus radiatus*.**

Character	Males	Females
Number of specimens	18	19
Body length (mm)	7.34–12.4 (9.39)	8.53–12.6 (10.6)
Diameter at E-I	300–465 (332)	281–401 (366)
Buccal capsule width	112–191 (149)	120–191 (151)
Buccal capsule depth	45–60 (56)	48–60 (56)
Nerve ring*	401–506 (451)	398–544 (470)
Cervical papillae*	454–675 (531)†	438–686 (552)‡
Excretory pore*	431–675 (518)	412–690 (558)§
Esophagus length*	806–1,050 (890)	907–1,068 (990)
Esophagus width at bulb	150–356 (218)	157–281 (222)
Egg (length × width)	—	80–100 (91) × 45–56 (51)
Vulva to anus distance	—	178–281 (220)
Vagina length	—	375–1,005 (701)
Vestibula length	—	75–135 (89)
Sphincter length	—	210–345 (288)
Infundibulum length	—	258–420 (298)
Spicule length (mm)	1.58–1.84 (1.69)	—
Gubernaculum length	206–300 (260)	—
Dorsal ray length	536–750 (650)	—
Tail length	—	131–210 (178)§

\* Measured from anterior end.

† *N* = 16.‡ *N* = 17.§ *N* = 18.|| *N* = 15.**Taxonomic summary**

TYPE HOST: *Equus* (Looss found it in both horse and ass; available types are from the latter).

LOCATION IN HOST: Colon and caecum.

TYPE LOCALITY: Egypt.

DISTRIBUTION: Cosmopolitan.

TYPE SPECIMENS: One male and 1 female in British Museum of Natural History, London.

COLLECTION NUMBER: 1931:261–262.

OTHER SPECIMENS: Table 1.

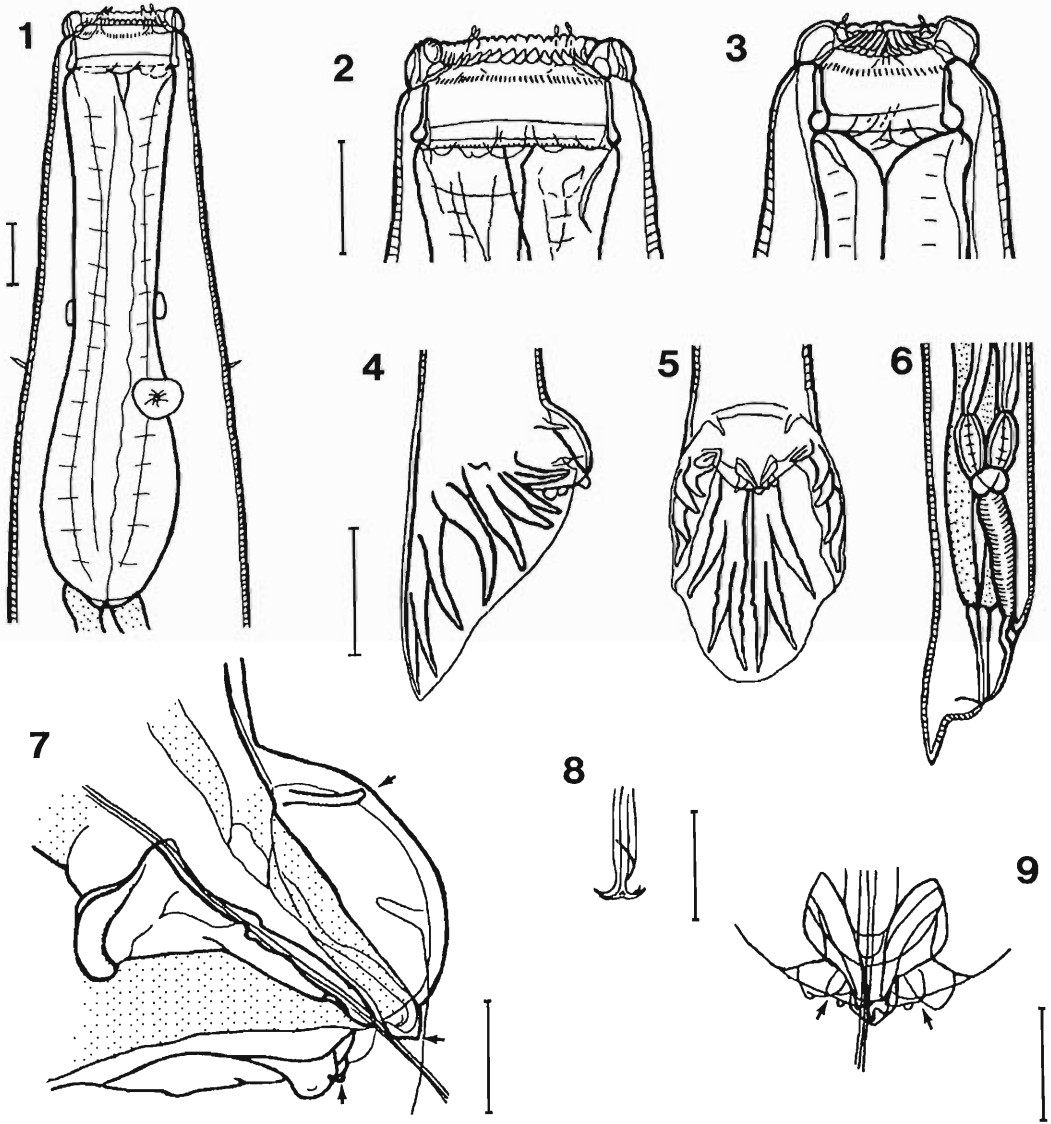
**Remarks**

Like all species of the Cyathostominae, *Cylicocyclus radiatus* can be distinguished in cleared whole specimens by characteristics of the buccal capsule and associated structures of the anterior end (Looss, 1900, 1902; Lichtenfels, 1975; Hartwich, 1986; Dvojnos and Kharchenko, 1994). The thin, relatively straight-walled, large buccal capsule and small esophageal funnel are sufficient to distinguish this species from its congeners (Lichtenfels, 1975). The smaller buccal capsule species of *Cylicocyclus* parasitic in *Equus caballus* or *Equus asinus* all have dorsal gutters, which *C. radiatus* lacks. Included in the

small buccal capsule group are *C. nassatus*, *C. ashworthi*, and *C. leptostomus*, all of which have dorsal gutters in addition to smaller buccal capsules. Two of these, *C. nassatus* and *C. ashworthi*, have been redescribed recently (Lichtenfels et al., 1997).

Two species of *Cylicocyclus* have been described with extremely shallow buccal capsules: *C. brevicapsulatus* (Ihle, 1920), which has a distinctive short, thin buccal capsule wall, and *C. prionodes* Kotlan, 1921, which has been synonymized with *C. radiatus* by Skrjabin and Ershov, 1933, who found this species to be composed of distorted specimens of *C. radiatus* in which the esophagus is pushed forward displacing the buccal capsule, flaring and spreading the elements of the leaf crowns. Lichtenfels (1975) confirmed this synonymy and illustrated such distorted specimens.

The larger buccal capsule species of *Cylicocyclus* include *C. auriculatus* (Looss, 1900), *C. ultrajectinus* (Ihle, 1920), *C. insigne* (Boulenger, 1917), and *C. elongatus* (Looss, 1900); all, like *C. radiatus*, lack a dorsal gutter, and can be distinguished from *C. radiatus* by their concave curve in the wall of the buccal capsule and the

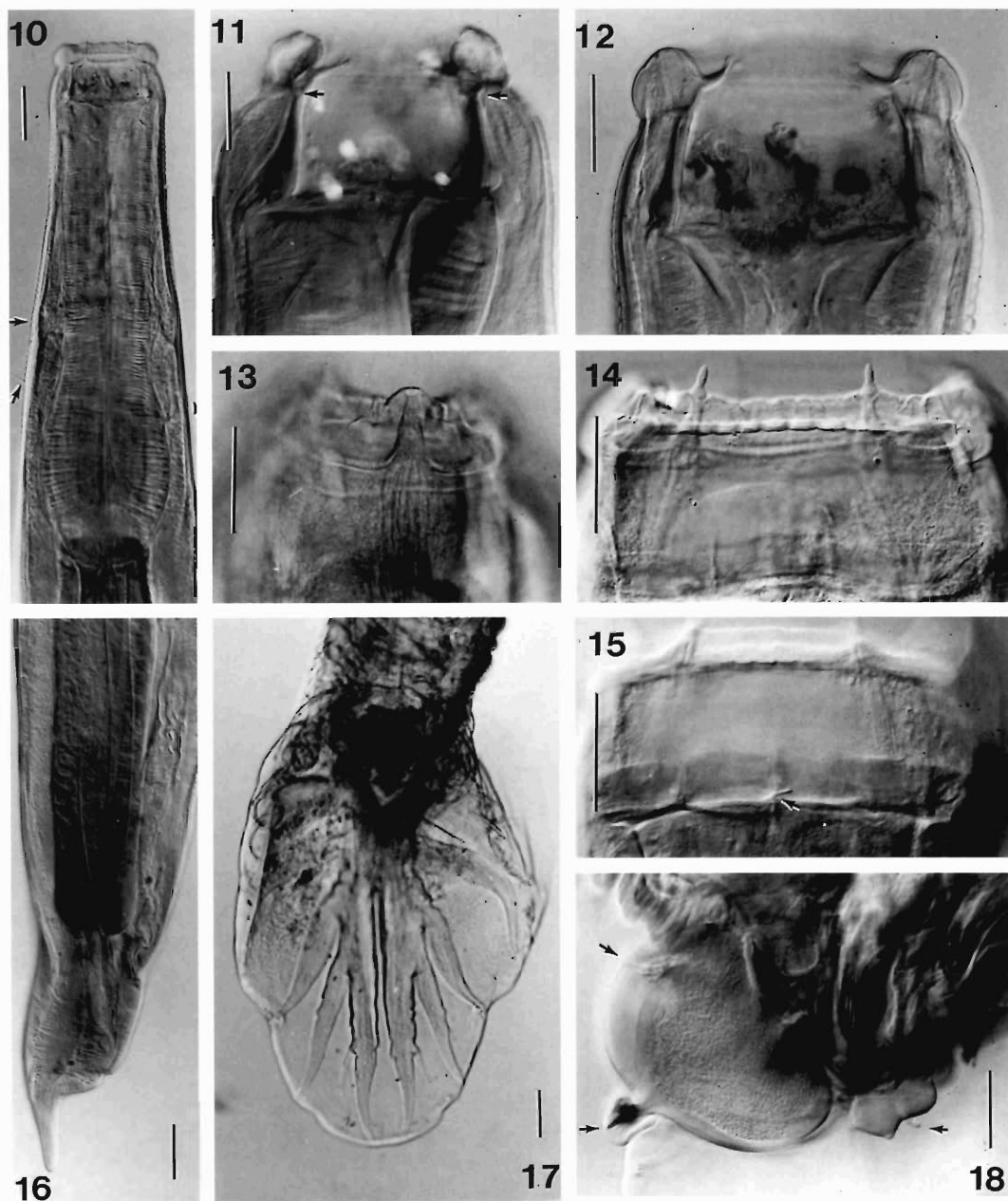


Figures 1–9. *Cylicocycclus radiatus*, drawings. Scale bars = 100  $\mu\text{m}$  (Figs. 1–3, 7, 9), 400  $\mu\text{m}$  (Figs. 4–6), and 50  $\mu\text{m}$  (Fig. 8). 1. Esophageal region, ventral view. 2. Buccal capsule, dorsoventral view. 3. Buccal capsule, lateral view. 4. Male tail, lateral view. 5. Male tail, ventral view. 6. Female tail, lateral view. 7. Genital cone of male, lateral view, showing positions of prebursal papillae (top arrow), papilla No. 0 (middle arrow), and papillae No. 7 (bottom arrow). 8. Fused spicule tips of male. 9. Appendages of genital cone, ventral view, showing paired dorsal papillae No. 7 (arrows).

distinctive natures of their prominent esophageal funnels (Lichtenfels, 1975). In addition, characteristics of lateral and submedian papillae and/or ELC and ILC (Lichtenfels, 1975) can be used to further differentiate these species from *C. radiatus*.

Two species of *Cylicocycclus* that occur only

in zebras, *C. triramosus* and an undescribed species of *Cylicocycclus*, are similar to *C. radiatus*. A recent redescription (Kharchenko et al., 1997) of *C. triramosus* clearly differentiated it from *C. radiatus*. Among all species of the genus, *C. triramosus* is most similar to *C. radiatus*, but differs in having a small dorsal gutter, distinctive



Figures 10–18. *Cylicocycclus radiatus*, photomicrographs. Scale bars = 50  $\mu\text{m}$  (Figs. 11–15, 18) and 100  $\mu\text{m}$  (Figs. 10, 16, 17). 10. Anterior end, dorsal view, showing shape of esophagus, position of nerve ring (anterior arrow), and cervical papillae (posterior arrows). 11. Buccal capsule, lateral view, showing inflated mouth collar, elements of ELC and ILC and extrachitinous supports (arrows). 12. Buccal capsule, dorsoventral view, showing lateral papillae extending through inflated mouth collar, and elements of ELC and ILC. 13. Mouth collar, lateral view, showing lateral papilla projecting through slightly collapsed collar. 14. Submedian papillae, dorsal view, showing spindle shape of papillae tips. 15. Anterior extremity of duct of dorsal esophageal gland (arrow) showing lack of dorsal gutter. 16. Female tail, lateral view, showing vulva and anus. 17. Male tail, ventral view, showing left externodorsal ray and 6 branches of dorsal ray. Small accessory branches are present on the distal or main branches of the dorsal ray. 18. Genital cone of male, lateral view, showing prominent ventral dermal collar with prebursal papillae (top left arrow),

dorsal and ventral notches in the mouth collar, and distinctive appendages of the genital cone. *Cylicocyclus gyalcephaloides* lacks a dorsal gutter and has a buccal capsule of similar shape to that of *C. radiatus*, but with thicker and slightly concave walls, a much larger and more prominent ILC, a prominent ELC that extends beyond the mouth collar, and a shorter, broader dorsal bursal ray.

#### Acknowledgments

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cuticular projection (lower left arrow), and one of paired bilobed genital appendages at dorsal edge of vent. The bilobed genital appendage bears tiny nipplelike points and surrounds one of a pair of dorsal papillae (No. 7's) (right arrow).

## Parasites of Florida Softshell Turtles (*Apalone ferox*) from Southeastern Florida

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**ABSTRACT:** A total of 15 species of helminths (4 trematodes, 1 monogenean, 1 cestode, 5 nematodes, 4 acanthocephalans) and 1 pentastomid was collected from 58 Florida softshell turtles (*Apalone ferox*) from southeastern Florida. *Spiroxys amydae* (80%), *Cephalogonimus vesicaudus* (80%), *Vasotrema robustum* (76%), and *Proteocephalus* sp. (63%) were the most prevalent helminths. Significant lesions were associated with the attachment sites of *Spiroxys amydae* in the stomach wall. *Contracaecum multipapillatum* and *Polymorphus brevis* are reported for the first time in reptiles. The pentastomid *Alofia* sp. is reported for the first time in North America and in turtles.

**KEY WORDS:** Softshell turtle, *Apalone ferox*, helminths, pentastomes, Florida.

The Florida softshell turtle (*Apalone ferox*) ranges from southern South Carolina, through southern Georgia to Mobile Bay, Alabama, and all of Florida except the Keys (Conant and Collins, 1991). Where it is sympatric with the Gulf Coast spiny softshell turtle (*Apalone spinifera aspera*) in the Florida panhandle, the Florida softshell is found more often in lacustrine habitats. In peninsular Florida, the Florida softshell can be found in both lacustrine and riverine habitats. Little is known about the parasites of this turtle. Previously, a small number of Florida softshells was examined and 8 species of helminths were reported (Lonnberg, 1894; Stunkard 1924, 1926, 1928; Cobb, 1929; Harwood, 1932; Loftin, 1960). None of these examinations were thorough surveys. Cobb (1929) described *Spiroxys amydae* from a *Apalone ferox* (= *Amyda ferox*) from the Mississippi River, and Harwood (1932) reported *Falcaustra chelvdrae* (Harwood, 1932) and *Serpinema trispinosus* (Leidy, 1852) (= *Camallanus trispinosus*) from an *Apalone ferox* from Houston, Texas. Both of these records lie well outside the range of *Apalone ferox* as currently recognized (Conant and Collins, 1991), and it is assumed that these turtles were either spiny softshells (*A. spinifera*) or smooth softshells (*A. mutica*), both of which occur in the Mississippi River and eastern Texas. In the present report, the parasites of 58 Florida

softshell turtles from southeastern Florida are discussed.

### Methods

A total of 58 Florida softshell turtles was examined. Fifty-seven were obtained from a commercial processor in Palm Beach County, Florida, between 1993 and 1995. Each of the 57 turtles was eviscerated during processing, and the organs and head were placed into a plastic bag and frozen until examined. The kidneys were not collected from these turtles. One turtle was collected from Collier County, Florida, in March 1995 and frozen whole until examined.

Seventeen of the turtles were examined at the Florida Game and Fresh Water Commission's Research Laboratory (GFCRL), Gainesville, Florida, and helminths were collected when seen, but parasite examinations were incomplete and not quantitative. The other 41 turtles were examined at the Department of Pathobiology, University of Florida (UF), Gainesville, where quantitative examinations for parasites followed the methods of Kinsella and Forrester (1972). Voucher specimens of helminths were deposited in the Harold W. Manter Collection (HWML), University of Nebraska State Museum, Lincoln.

### Results and Discussion

A total of 15 species of helminths (4 trematodes, 1 monogenean, 1 cestode, 5 nematodes, 4 acanthocephalans) and 1 pentastomid was collected. Prevalences and intensities of parasites for the 41 quantitative examinations are listed in Table 1. Multiple infections in the 41 turtles were as follows: 5 turtles had 2 species of parasites, 2 had 3 species, 7 had 4 species, 12 had

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**Table 1. Prevalences and intensities of parasites from 41 Florida softshell turtles in Florida.**

	HWML no.*	Location in host†	Prevalence		Intensity	
			No. inf.	%	Mean	Range
<b>Monogenea</b>						
<i>Neopolystoma orbiculare</i> (Stunkard, 1916)	39330	?	10	24	9	1–26
<b>Aspidogastrea</b>						
<i>Cotylaspis cokeri</i> Barker and Parsons, 1914	39329	SI	10	24	9	1–44
<b>Digenea</b>						
<i>Cephalogonimus vesicaudus</i> Nickerson, 1912	39327	SI	33	80	69	1–568
<i>Vasotrema robustum</i> Stunkard, 1928	39326	HT, LV, LN, SP	31	76	7	1–37
<i>Teloporia aspidonectes</i> (MacCallum, 1917)	39328	LI	3	7	2	1–2
<b>Cestoda</b>						
<i>Proteocephalus</i> sp.	39331	SI	26	63	13	1–51
<b>Nematoda</b>						
<i>Spiroxys amydae</i> Cobb, 1929	39340	ST, SI	33	80	10	1–41
<i>Contracaecum</i> sp. (larvac)	—	ST	12	29	13	1–66
<i>Falcaustra affine</i> (Lcidy, 1856)	—	LI	2	5	1	1
<i>Serpinema</i> sp. (larvac)	—	SI	1	2	32	—
<b>Acanthocephala</b>						
<i>Neoechinorhynchus chrysemydis</i> Cable and Hopp, 1954	39334	SI	5	12	2	1–4
<i>Polymorphus brevis</i> (Van Cleave, 1916)	39333	SI	3	7	1	1–2
<i>Acanthocephalus</i> sp.	39332	SI, LI	3	7	1	1–2
Acanthocephalan cystacanth	—	SI	1	2	1	—
<b>Pentastoma</b>						
<i>Alofia</i> sp.	—	LN, TR	33	80	6	1–40

\* Accession numbers of the Harold W. Manter Collection.

† Location in host: HT = heart, LI = large intestine, LN = lungs, LV = liver, SI = small intestine, ST = stomach, TR = trachea.

5 species, 7 had 6 species, 5 had 7 species, 2 had 8 species, and 1 had 9 species.

Eroding ulcerlike lesions, up to 8 mm in diameter, were associated with the attachment sites of *Spiroxys amydae* in the stomach wall. One to 4 ulcers containing 1 to 17 *S. amydae* were seen in the infected turtles. Cobb (1929) reported similar subspherical saccate stomach lesions, associated with *S. amydae* in a softshell from the Mississippi River. *Contracaecum* larvae were seen infrequently in the ulcers, but these were located mostly in the small intestine or encysted on the outside surface of the stomach and liver. Specimens of adult male *Contracaecum multipapillatum* (Drasch, 1882) (HWML# 39339)

were collected from the small intestine of 1 adult male turtle examined at the GFCRL. This is the first record of *C. multipapillatum* in reptiles; however, it is a common parasite in fish-eating birds of Florida (Huizinga, 1971; Deardorff and Overstreet, 1980; Sepúlveda et al., 1994; Kinsella et al., 1996). Moler and Berish (1995) reported that 146 (63%) of 233 softshell turtles they sampled had fish as a food item in the digestive tract. They indicated also that softshells are opportunistic feeders and probably eat fish on a regular basis, mainly through scavenging.

Lonnberg (1894) described *Tetrabothrium trionychinum*, now placed in the genus *Proteocephalus*, from *Apalone ferox* (= *Trionyx ferox*)

from Orange County, Florida, but Yamaguti (1959) considered his description too brief for adequate differentiation. Brooks (1978) thought that *T. trionychinum* might form a complex of closely related species with *Proteocephalus testudo* from *A. spinifera* and *P. australis* from a teleost fish in Texas, but recommended further material be collected from *A. ferox* in Florida. Identification to species was not possible due to the poor condition of our specimens, so this problem remains to be resolved.

Immature specimens of *Polymorphus brevis* were collected from 3 turtles. This acanthocephalan has been reported from several species of Florida birds, including the bald eagle (*Haliaeetus leucocephalus*) (Richardson and Cole, 1997) and the great blue heron (*Ardea herodias*) and yellow-crowned night-heron (*Nyctanassa violacea*) (Kinsella, unpubl.); however, this is the first report in reptiles.

Pentastomids of the genus *Alofia* were collected from the trachea, bronchi, and bronchia. Immature adults and nymphs were encapsulated also on the outside surface of the lungs. This is the first record of pentastomes in *Apalone ferox*, and of a species of *Alofia* in North America. Until now, pentastomid species in the genus *Alofia* were thought to be exclusively parasitic in crocodiles (Riley, 1994). The description of this new species is in progress.

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## Helminth Parasites of the Bald Eagle, *Haliaeetus leucocephalus*, in Florida

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**ABSTRACT:** Twenty species of helminths (9 trematodes, 9 nematodes, and 2 acanthocephalans), including 9 new host records, were collected from 40 bald eagles (*Haliaeetus leucocephalus*) from Florida. Intensities of infection were low and no lesions were attributed to the parasites. No species were considered specialists in bald eagles; 5 species were considered raptor generalists and the remainder, generalists in other orders of fish-eating birds. An undescribed species of *Hamatospiculum* was found in 3 birds. Most of the common helminths were acquired from eating fish intermediate hosts.

**KEY WORDS:** Helminths, bald eagle, parasites, *Haliaeetus leucocephalus*, *Hamatospiculum*.

The American bald eagle, *Haliaeetus leucocephalus* (L.), after a dramatic decline in the 1950's and 1960's due to pesticide contamination and illegal hunting, has made a remarkable recovery, and in 1995 was removed from the Endangered Species List and reclassified as a threatened species (Federal Register, 1995). Nesbitt (1996) estimated the population of bald eagles in Florida to be between 2,330 and 3,443 and pronounced it "healthy." Adults in southern Florida are permanent residents, but some adults from populations in north-central Florida and the panhandle migrate northward (Robertson and Woolfenden, 1992).

Because of its protection under the Bald Eagle Act of 1940, information on the helminth parasites of *H. leucocephalus* is limited. Kocan and Locke (1974) published a list of 10 species of helminths, and Tuggle and Schmeling (1982) gave data on 13 species found in bald eagles from the United States, including some from Florida, but included no data on prevalence or intensity. Richardson and Cole (1997) recorded 5 species of acanthocephala from bald eagles from the United States including Florida. In this report, we combine records of helminths collected from bald eagles from Florida at the Department of Pathobiology, University of Florida (UF), Gainesville, and the National Wildlife Health Center (NWHC), Madison, Wisconsin.

Twenty-one injured or dead bald eagles submitted to the Department of Pathobiology (UF)

between December 1992 and April 1995 were examined at necropsy according to the methods of Kinsella and Forrester (1972). Nineteen bald eagles submitted to the NWHC between January 1986 and December 1994 were examined for cause of death and helminths were collected when found, but parasite examinations were incomplete and not quantitative. Voucher specimens of helminths were deposited in the Harold W. Manter Laboratory, University of Nebraska State Museum, Lincoln.

Helminths representing 20 species (9 trematodes, 9 nematodes, and 2 acanthocephalans) were collected from the 40 bald eagles. Nine new host records were found. Prevalences and intensities of helminths from the 21 completely necropsied birds are listed in Table 1. Intensities of infection were low and no significant lesions were associated with any of the infections. Table 2 gives helminth prevalences for the 19 birds from the NWHC. Intensities are not listed since only samples of the helminths were collected for identification. Again, helminth infections were not implicated as the cause of significant lesions or death in these hosts.

Gravid worms were present for all species except for *Gnathostoma* sp. *Physaloptera* sp., and *Centrorhynchus kuntzi*, indicating that the bald eagle is a "competent" definitive host for most of the species found. Richardson and Cole (1997) recently reviewed all acanthocephalan records from bald eagles in North America, but reported no *C. kuntzi*. Two birds were infected in the present study with an adult male and an adult female, but the female contained no eggs.

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**Table 1. Prevalences and intensities of helminths of 21 Florida bald eagles examined at the University of Florida.**

	HWML acc. no.	Location in host*	Prevalence		Intensity	
			No. inf.	%	Mean	Range
<b>Trematoda</b>						
<i>Renicola thapari</i> Caballero, 1953†	39099	K	6	29	18	1-82
<i>Mesostephanus appendiculatoides</i> Price, 1934†	39098	SI	6	29	36	1-85
<i>Phagicola longa</i> Ransom, 1920	39094	SI	6	29	291	10-1,245
<i>Phagicola</i> sp.†	39097	SI	1	5	25	25
<i>Microparyphium facetum</i> Dietz, 1909†	39093	C	5	24	2	1-3
<i>Strigea falconis</i> Szidat, 1929	39100	SI	3	14	2	1-2
<i>Neodiplostomum attenuatum</i> (von Linstow, 1906)	39095	SI	3	14	5	3-9
<i>Posthodiplostomum minimum</i> (MacCallum, 1921)†	39096	SI	1	5	1	1
<b>Nematoda</b>						
<i>Contracaecum</i> spp.‡	—	E, V	9	43	10	1-76
<i>Capillaria falconis</i> (Goeze, 1782)	39110	SI	8	38	3	1-14
<i>Eucoleus contortus</i> (Creplin, 1839)	39109	E	8	38	6	1-18
<i>Desportesius invaginatus</i> (von Linstow, 1901)†	39113	E	2	10	12	11-13
<i>Chandleronema longigutturata</i> (Chandler, 1942)†	39112	V	1	5	5	5
<i>Gnathostoma</i> sp. (immature)	39108	SI	1	5	1	1
<i>Physaloptera</i> sp. (immature)	—	V	1	5	3	3
Immature spirurids	—	E, V	5	24	25	1-118
<b>Acanthocephala</b>						
<i>Centrorhynchus kuntzi</i> Schmidt and Neiland, 1966†	39338	SI	2	10	1	1
<i>Polymorphus brevis</i> (Van Cleave, 1916) (= <i>Arythmorhynchus brevis</i> )	39101	SI	5	24	4	1-10

\* Location in host: C = cloaca, E = esophagus, K = kidney, SI = small intestine, V = ventriculus.

† New host record for bald eagle.

‡ A complex of 2 species, *Contracaecum rudolphii* Hartwich, 1964 (HWML No. 39111), and *C. multipapillatum* (Von Drasche, 1882) (HWML No. 39342), combined because the immature stages could not be distinguished.

Kocan and Locke (1974) reported *Centrorhynchus* sp. from bald eagles in 4 states, but their specimens are not available for examination, so the status of the eagle as a competent host for *C. kuntzi* still remains to be determined.

A species of *Hamatospiculum* found in 3 birds appears to be the same undescribed species found recently in 2 Cooper's hawks, *Accipiter cooperi*, in Nebraska (Sterner and Kinsella, unpubl.). The specimens reported as *Serratospiculum amaculata* by Tuggle and Schmelting (1982) U.S. (National Parasite Collection No.

77004) were reexamined and determined to be the same *Hamatospiculum*. This casts into doubt the record of *S. amaculata* from the bald eagle by Kocan and Locke (1974), but those specimens were not available for examination. The description of the new species is in process.

The helminths listed in Tables 1 and 2 differ substantially from the list of Tuggle and Schmelting (1982), who examined 84 bald eagles from 18 states, including Florida. Tuggle and Schmelting collected parasites by sight and no fine-mesh screening was done, so only large specimens

**Table 2. Records of helminths from 19 Florida bald eagles examined at the National Wildlife Health Center, Madison, Wisconsin.**

	HWML acc. no.	Location in host*	Prevalence	
			No. inf.	%
<b>Trematoda</b>				
<i>Strigea</i> sp.	—	SI	3	16
<i>Neodiplostomum attenuatum</i> (von Linstow, 1906)	—	SI	1	5
<i>Phagicola nana</i> Ransom, 1920†	39337	SI	2	11
<i>Phagicola</i> sp.	—	SI	4	21
<i>Mesostephanus appendiculatoides</i> (Price, 1934)	—	SI	2	11
<i>Microparyphium facetum</i> (Dietz, 1909)	—	C	1	5
<i>Renicola</i> sp.	—	K	1	5
<b>Nematoda</b>				
<i>Contracaecum</i> spp.‡	—	E, V	8	42
<i>Eucoleus contortus</i> (Creplin, 1839)	—	E	1	5
<i>Hamatospiculum</i> sp.†	39341	A	3	16

\* Location in host: A = air sacs, C = cloaca, E = esophagus, K = kidney, SI = small intestine, V = ventriculus.

† New host record for bald eagle.

‡ A complex of 2 species, *Contracaecum rudolphii* Hartwich, 1964, and *C. multipapillatum* (von Drasche, 1882), combined because the immature stages could not be distinguished.

such as *Clinostomum* and *Contracaecum* were commonly collected. The present study is more representative of the overall helminth fauna of bald eagles.

In recent studies of helminths of hawks and falcons (Kinsella et al., 1995) and ospreys (Kinsella et al., 1996), 3 groups have been recognized; specialist, with the bulk of the population in a single host species; raptor generalist, found only in hawks and owls; and bird generalist, found in several orders of birds. None of the species found in this study can be considered specialists in bald eagles, and only 5 (*Strigea falconis*, *Neodiplostomum attenuatum*, *Capillaria falconis*, *C. kuntzi*, and possibly *Hamatospiculum* sp.) can be considered raptor generalists. The remaining species are found in a variety of fish-eating birds such as herons, spoonbills, and pelicans (e.g., *Phagicola* spp., *Mesostephanus appendiculatoides*, *Microparyphium facetum*, *Renicola thapari*, *Contracaecum* spp.). In contrast, the osprey, which commonly shares habitats with bald eagles, had 7 specialist species, none of which were found in the eagle. The osprey did share 1 raptor generalist (*C. falconis*) and several bird generalists (*Contracaecum* spp., *Phagicola* spp., *M. facetum*) with the eagle.

The diet of the bald eagle varies extensively

both with geographic location and time of the year (Gerrard and Bortolotti, 1988). In north-central Florida, 75% of the prey items found in or near eagle nests were fish, with coots and small mammals making up most of the remaining items (McEwan and Hirth, 1980). Nine of 20 species found in this study (e.g., *Contracaecum* spp., *Phagicola* spp., *P. brevis*) are known to have fish intermediate hosts (Huizinga, 1966; Deardorff and Overstreet, 1980; Font et al., 1984). In some cases, the helminths could act as biological tags, indicating whether the eagles had been feeding in freshwater or marine environments (e.g., *Phagicola longa* in mullets—*Mugil* spp., and *Phagicola nana* in centrarchids—*Lepomis* and *Micropterus* spp.).

No tapeworms were found here in eagles, although Kocan and Locke (1974) reported *Cladotaenia banghami* from bald eagles in 5 states, including Florida. The paucity of mammals in the diet of Florida eagles may explain the rarity of *Cladotaenia* spp., which use mammals as intermediate hosts.

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## In Vitro Growth of Swine Roundworm Larvae, *Ascaris suum*: Cultivation Techniques and Endocrine Regulation

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**ABSTRACT:** A stationary, multi-well bioassay for growth and development of *Ascaris suum* larvae has proven markedly sensitive for the study of endocrinological and pharmacological substances. Experiments were designed to assess physical factors of the cultivation procedure (larval density and type of flask) as well as the temporal sensitivity of the larvae to potential growth stimulants or inhibitors. Larvae at lower density (100/ml) did not grow as quickly as larvae at higher density (400/ml), coincident with their conversion to anaerobic metabolism. Large stationary flasks, irrespective of cap type, were similar in larval growth patterns. The present study demonstrated that the ecdysteroid agonists, RH-5849, but not RH-5992, had a biphasic effect on larval growth after 24-hr, premolt exposure; low concentrations (5 ng/ml) increased growth, and high concentrations ( $\geq 50$  ng/ml) decreased growth. Addition of 20-hydroxyecdysone (20-OH) after the third molt also decreased growth of the fourth-stage larvae; ecdysone did not share this effect over the same dose range. Decreased growth, when exposure after molting to 20-OH or an agonist, suggests that alternative pathways might be disrupted by the presence of these compounds.

**KEY WORDS:** Nematode, *Ascaris*, ecdysteroids, plumbagin, dibenzoyl hydrazines.

In nematodes, ecdysis is defined broadly as the deposition of a new cuticle under the old one, and then the removal of the old cuticle, or exsheathment (Willett, 1980). Regulation of these events in parasitic nematodes is understood incompletely but apparently involves neural (Davey, 1966), hormonal (Dennis, 1977), and enzymatic (Gamble et al., 1989) regulators. The model traditionally used for the study of molting in nematodes is the second molt of *Haemonchus contortus* (Rogers and Head, 1972), but this model involves only the final phase of the ecdysial process, i.e., exsheathment. Hence, synthesis of the new cuticle already is completed while the *Haemonchus* larvae are maintained in a dormant, infective stage, likely obviating any steroidal involvement in the final, short-term (30 min) process of exsheathment. In *Nematospo-roides dubius* (Dennis, 1977), *Ascaris suum* (Fleming, 1985a), and *Dirioflaria immitis* (Barker et al., 1990), very low concentrations of exogenous ecdysteroids enhanced ecdysis in vitro. Additionally, steroidal regulators also might serve as nonspecific growth modulators independent of molting in nematodes.

An in vitro growth bioassay system was developed (Fleming, 1985b) based on cultivation techniques of third- to fourth-stage *A. suum* larvae (Urban and Douvres, 1981), a developmental transition that encompasses both cuticle deposition as well as exsheathment. With this bio-

assay, the effects of steroids (Fleming, 1985a, b), biogenic amines (Fleming, 1988), and insect molting inhibitor (Fetterer and Fleming, 1991) have been examined in dose-response experimental designs, with exposure to the drugs limited to a brief (24 hr) premolt period. However, various aspects of the cultivation process have not been examined specifically relative to previously defined hormonal responses. These include aspects of larval culture density, optimal culture vessel, and postmolting or long-term exposure to potential regulators. These regulators comprise ecdysone, 20-hydroxyecdysone (20-OH), the ecdysteroid agonists (dibenzoyl hydrazines) RH-5849 (Wing, 1988) and RH-5992 (Smagge et al., 1995), and plumbagin (5-hydroxy, 2-methyl-1,4-naphthoquinone), an extract of plants of the *Plumbago* spp. that inhibits insect development (Kubo et al., 1983; Joshi and Sehna, 1989) and demonstrates antileishmanial activity (Croft et al., 1985). The experiments herein were designed to explore these variables on the in vitro growth of *A. suum* larvae in our previously defined bioassay (Fleming, 1985b).

### Materials and Methods

#### General larval cultivation procedures

Seven days after *per os* inoculation of 100,000 de-coated and embryonated eggs of *A. suum* (Urban et al., 1981), third-stage larvae were recovered from porcine lungs by Baermannization. These larvae were rinsed

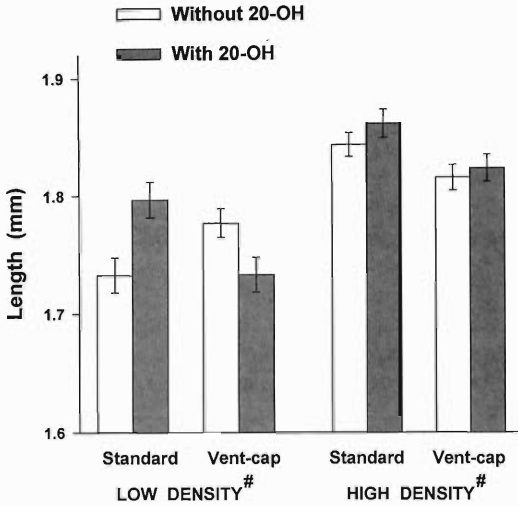


Figure 1. Mean lengths  $\pm$  SEM of fourth-stage *A. suum* larvae ( $N = 100$ /group) from 250-ml standard or vent-cap culture flasks at low or high density (100 or 400 larvae/ml) and in the presence (5 ng/ml) or absence of 20-hydroxyecdysone (20-OH). Treatments with the same symbol (#) are significantly different ( $P < 0.05$ ).

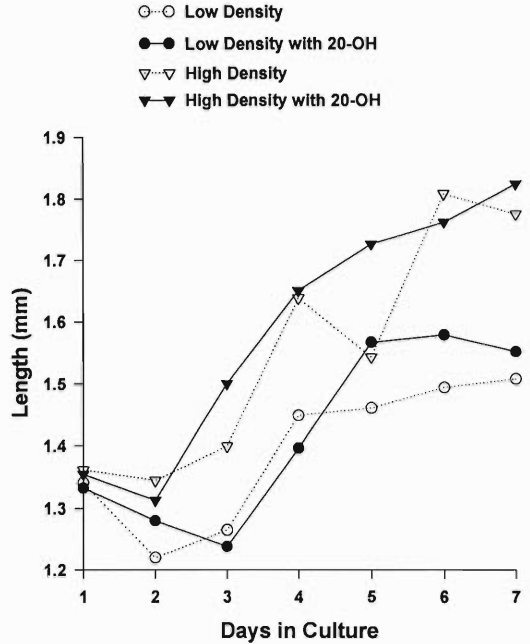


Figure 2. Growth curves of third- to fourth-stage *A. suum* larvae ( $N = 100$  day/group) multi-well plates at 2 different densities (100 or 400 larvae/ml) and in the presence (5 ng/ml) or absence of 20-hydroxyecdysone (20-OH) over 7 days of cultivation.

in Dulbecco's phosphate-buffered saline (DPBS) with penicillin/streptomycin/genomycin 5 times (1 hr each rinse) and rinsed 5 times with fresh DPBS (Urban and Douvres, 1981). After sedimentation, medium was aspirated, and larvae were resuspended in RPMI-1640 with 50  $\mu$ g/ml of cholesterol (Urban et al., 1983) and placed in either 24 multi-well culture plates or 250-ml stationary flasks. Cultures were incubated for 7 or 14 days at 37°C in 5% CO<sub>2</sub>:95% air, and then larvae were fixed in hot buffered formalin. Subsamples of larvae ( $N = 25$ /well or 100/flask) were magnified and projected onto the screen of a computer-linked digitizer (R & M Biometrics, Nashville, Tennessee), and fourth-stage larvae were measured. Data were analyzed by analysis of variance, and means were compared with Duncan's multiple range test (Freund and Little, 1981), with the exception of the time-course experiment. Because this experiment required sequential sampling of the same flasks, the analysis utilized a generalized linear model that recognized repeated measurements ( $n = 7$ ) from the same experimental unit, i.e., culture flask (Gill and Hafis, 1971). Means were considered significantly different at  $P < 0.05$ .

**Large flask cultivation**

Stationary 250-ml flasks, with or without vent caps (Corning Incorp., Corning, New York), were filled with 50 ml of medium and 100 or 400 third-stage larvae/ml. Half of each group of these flasks also were supplemented with 20-OH (5 ng/ml), a concentration that maximally stimulated growth in multi-well cultures (Fleming, 1985b). Cultures were terminated after 7 days of incubation.

**Time course**

Multi-well plates were incubated with 100 or 400 larvae/well (2 ml/well) with the addition or absence of 5 ng/ml of 20-OH. Eight wells from each treatment group were fixed each day during 7 days of incubation.

**Insect molting agonists**

Lung larvae (300/well) were incubated for 24 hr in multi-well plates that were coated with increasing concentrations of the insect molting agonists RH-5849 or RH-5992. Larvae were removed from culture wells and centrifuged, medium was aspirated, and larvae were returned to fresh medium and untreated culture plates for an additional 6 days of cultivation, then fixed and measured.

**Postmolting hormonal treatment**

Lung larvae were incubated in multi-well plates for 4 days, after which the majority had molted to fourth-stage larvae. Medium and larvae then were transferred to new plates that were coated with various concentrations of ecdysone, 20-OH, or ethanol vehicle and dried. After 3 days of further incubation, larvae were fixed and measured.

**Two-week incubation**

Third-stage larvae were incubated in multi-well plates for 1 wk and then transferred to new plates that



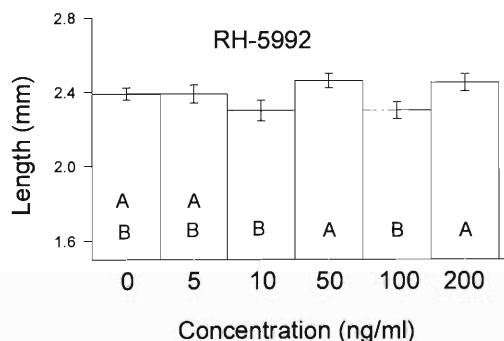
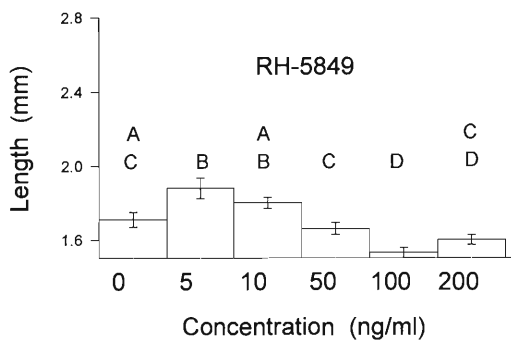


Figure 3. Mean lengths  $\pm$  SEM of fourth-stage *A. suum* larvae ( $N = 100/\text{dose}$ ) from 7-day multi-well cultures that were exposed to RH-5849 or RH-5992 for the initial 24 hr. Columns with different letters are significantly different ( $P < 0.05$ ).

were coated with various concentrations of 20-OH, plumbagin, or ethanol vehicles. After 7 days of further incubation, larvae were fixed.

### Results

The type of large cultivation flask or presence of 20-OH had no significant effect on the growth of third- to fourth-stage larvae. However, high cultivation density in large culture flasks resulted in significantly increased larval size (Fig. 1).

Stationary multi-well larval cultures at the higher density, irrespective of the presence of 20-OH, were significantly larger (15%) than cultures at low density with or without 20-OH, after 7 days of cultivation (Fig. 2). Divergence of growth rates occurred primarily after day 2.

Brief exposure to the ecdysteroid agonist RH-5849 generally decreased the subsequent size of larvae, although the sister compound, RH-5992, had no consistent effect on growth over the same dose range (Fig. 3).

Addition of ecdysone after molting (day 4)

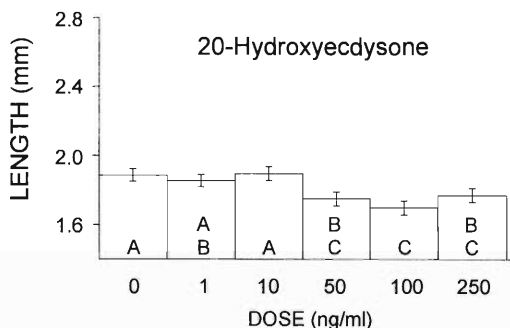
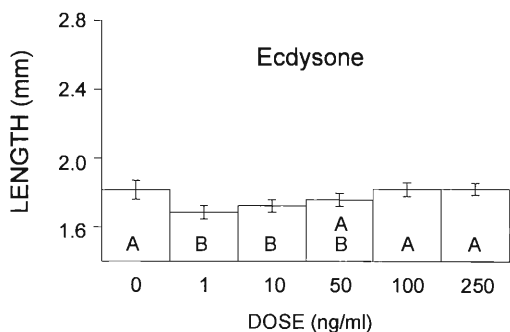


Figure 4. Mean lengths  $\pm$  SEM of fourth-stage *A. suum* larvae ( $N = 100/\text{dose}$ ) from multi-well plates after 7 days of incubation when ecdysone or 20-hydroxyecdysone (5 ng/ml) was added to culture medium on day 4 of incubation. Columns with different letters are significantly different ( $P < 0.05$ ).

significantly decreased growth at the lower concentration, but the higher concentrations were similar to control values (Fig. 4). In contrast, a similar temporal addition of 20-OH significantly decreased growth at the higher concentrations (Fig. 4). Growth of larvae from the multi-well plates was comparable to growth of larvae from the culture flasks (Fig. 1).

The exposure of fourth-stage larvae to 20-OH from days 7 through 14 of cultivation resulted in significant decreased growth at only the intermediate concentration of 100 ng/ml (Fig. 5). Plumbagin, with the same regimen of exposure and cultivation, resulted in fourth-stage larvae that were consistently 10% longer than control larvae at all drug concentrations.

### Discussion

The enhanced growth of the *A. suum* larvae at the 4-fold higher density suggests that certain metabolic processes function as positive stimuli

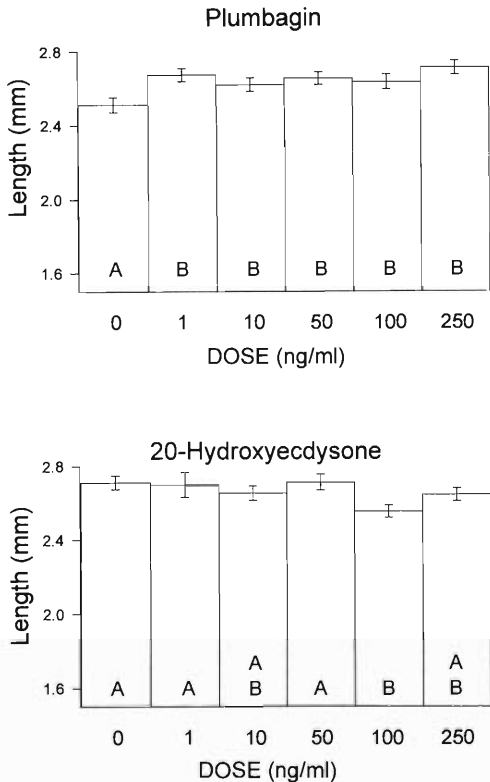


Figure 5. Mean lengths  $\pm$  SEM of fourth-stage *A. suum* larvae ( $N = 100$ ) from multi-well plates after 14 days of incubation when plumbagin or 20-hydroxyecdysone was added to medium on day 7. Columns with different letters are significantly different ( $P < 0.05$ ).

for growth. Significantly, this positive response in the time-course experiment is concurrent with the conversion of larval metabolism from aerobic to anaerobic (Komuniecki and Vanover, 1987; Komuniecki and Harris, 1995). Potentially, the higher concentration of larvae more rapidly converts the culture environment to an anaerobic condition, enhancing larval development into the fourth stage. Alternatively, the more rapid conditioning of the media with fermentation products, particularly those associated with the adult parasite (Komuniecki and Vanover, 1987; Vanover-Dettling and Komuniecki, 1989), might also contribute to a more favorable environment at higher larval density.

The results relative to the postmolting exposure to 20-OH support the hypothesis that the precise timing of this specific hormone is critical for its growth enhancement potential. When 20-

OH is applied earlier in the cultivation procedure (day 1) and for a short duration (24 hr), larvae molted earlier and grew longer (Fleming, 1985a). The later and longer exposure of 20-OH reported herein, however, did not elicit any growth enhancement. These time- and dose-specific effects argue for an early endogenous role of 20-OH in molting and development in nematodes.

The biphasic response of RH-5849 is similar to that observed with 20-OH in this bioassay (Fleming, 1985b), suggesting an ecdysonergic property in nematodes at low concentrations. The general lack of response with RH-5992 is similar to that reported as differential physiological responses of these 2 ecdysteroid mimics in a variety of insects, including *Plodia interpunctella* (Silhacek et al., 1990), *Spodoptera exigua* (Smaghe and Degheele, 1994), and *Leptinotarsa decemlineata* (Smaghe et al., 1995). Hence, RH-5992 might have distinct ecdysteroid activity that is not present in this stage of *Ascaris*, thus exhibiting a functional site distinguishing these insects from nematodes.

Hormonal, temporal, and cultivation factors have been further explored to define larval growth of *A. suum* and target windows in which specific responses are expressed. Prolonged or postmolting exposure to ecdysteroids inhibited growth in *A. suum* larvae in contrast to its effect with premolting incubation.

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## New Findings of Metacestodes and a Pentastomid from Rodents in Mongolia

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**ABSTRACT:** In this study, new findings of the immature-stage parasites of Mongolian rodents that require carnivorous mammals or birds of prey to complete their life cycle are reported. Six species of parasites, including metacestode stages of 5 cestodes (*Taenia mustelae*, *T. polyacantha*, *T. endotheracicus*, *Cladotaenia globifera*, and *Mesocestoides* sp.) and nymphs of 1 pentastomid (*Linguatula serrata*), were found. Of these, 2 species (*T. endotheracicus* and *L. serrata*) are reported for the first time in Mongolia. The new host records include *Microtus brandtii* for *T. polyacantha*, *T. mustelae*, *C. globifera*, and *Mesocestoides* sp.; *M. mongolicus* for *T. mustelae*; *Meriones meridianus* for *T. endotheracicus*; and *M. unguiculatus* for *T. endotheracicus* and *L. serrata*. The geographic distribution, prevalences, and morphology of these parasites are reported.

**KEY WORDS:** Cestoda, Metacestodes, Mongolia, Pentastomida, Rodentia.

The helminths of predatory hosts include many parasites of environmental health, medical, or veterinary importance. Many of these parasitize rodents as intermediate hosts. A review of the literature (Schumakovich, 1936; Galbadrah, 1972; Danzan, 1978; Daschzeveg et al., 1982; Suhbat and Ganzorig, 1988; Baatar and Handaa, 1989) indicates that 28 species of helminths have been reported in wild and domestic carnivores in Mongolia. Of those, 12 species, *Alaria alata* (Goeze, 1782); *Taenia polyacantha* Leuckart, 1856; *T. mustelae* Gmelin, 1790; *T. laeniaeformis* Batsch, 1786; *T. crassiceps* (Zeder, 1880); *T. pisiformis* (Bloch, 1780); *Mesocestoides lineatus* (Goeze, 1782); *Spirometra erinacei-europaei* (Rudolphi, 1819); *Macracanthorhynchus catulinus* Kostylew, 1927; *Trichinella spiralis* (Owen, 1835); *Toxascaris leonina* (Linstow, 1902); and *Toxocara cati* (Schrank, 1788), occur in the rodent intermediate host. But, only *Mesocestoides lineatus* tetrahyridia were reported from rodents in Mongolia (Dubinin and Dubinina, 1951; Danzan, 1978). During 1994–1996, we conducted a field survey to determine the biodiversity of the helminths in Mongolia. Special attention was given to parasites that cause significant economic and public health problems. In the present paper, the results of the survey of the parasites that have

environmental links to carnivorous mammals are reported.

### Materials and Methods

The hosts examined consisted of 1,524 rodents belonging to 34 species. Of those, immature stages of parasites were found in rodents of 10 species, including long-tailed souslik, *Spermophilus undulatus* Pallas, 1773 (Sciuridae); gray red-backed vole, *Clethrionomys rufocanus* Sundevall, 1846–1847; northern red-backed vole, *C. rutilus* Pallas, 1779; Mongolian vole, *Microtus mongolicus* Radde, 1861; Brandt's vole, *M. brandtii* Radde, 1861 (all Arvicolidae); gray hamster, *Cricetus migratorius* Pallas, 1773 (Cricetidae); Mongolian gerbil, *Meriones unguiculatus* Milne-Edwards, 1867; midday gerbil, *Meriones meridianus* (Pallas, 1773); great gerbil, *Rhombomys opimus* Lichtenstein, 1823 (all Gerbillinae); and Gobi jerboa, *Allactaga bullata* Allen, 1925 (Dipodidae). All were trapped or shot during field surveys in 1983–1992 and 1994–1996, in various places in Mongolia. The helminths from 9 *Microtus mongolicus* and 10 *Clethrionomys rutilus* were provided by Dr. H. Suhbat (National University of Mongolia) and Dr. B. I. Scheftel (Evolution, Morphology and Animal Ecology Institute named after A. N. Severtsov, Russian Academy of Sciences). The formalin-preserved carcasses of 160 *Microtus brandtii* were made available to us by Dr. A. A. Tarakanovskii (Institute of General and Experimental Biology, Academy of Sciences, Mongolia). The host-sampling procedure was carried out in spring, summer, and autumn, but mostly in summer. Captured mammals were dissected and studied immediately for helminths or after fixation with 10% formalin. The digestive organs, lungs, body cavity, and subcutaneous tissues were checked for helminths. The parasites were fixed in 10% formalin or in 70% ethanol. They were cleared in glycerin or lactic

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**Table 1. Prevalence and intensity of larval parasites of Mongolian rodents.**

Parasite species	Host (no. examined)	Prevalence	Intensity
<i>Taenia mustelae</i>	<i>Clethrionomys rufocanus</i> (31)	2 (6.45%)	7 and 15
	<i>C. rutilus</i> (58)	1 (1.7%)	30
	<i>Microtus mongolicus</i> (9)	1 (11.1%)	2
	<i>M. brandtii</i> (525)	1 (0.19%)	2
<i>T. polyacantha</i>	<i>Microtus brandtii</i> (525)	8 (1.52%)	1–19 (8.5)
	<i>Cricetulus migratorius</i> (6)	1	14
<i>T. endothoracicus</i>	<i>Meriones unguiculatus</i> (12)	1 (8.3%)	1
	<i>M. meridianus</i> (40)	1 (2.5%)	3
	<i>Rhombomys opimus</i> (25)	1 (4.0%)	1
<i>Cladotaenia globifera</i>	<i>Microtus brandtii</i> (525)	2 (0.38%)	3 and 11
<i>Mesocestoides</i> sp.	<i>Spermophilus undulatus</i> (194)	1 (0.5%)	1
	<i>Microtus brandtii</i> (525)	2 (0.38%)	1 and 46
	<i>Meriones unguiculatus</i> (12)	1 (8.3%)	8
	<i>Alactaga bullata</i> (2)	1	5
	<i>Clethrionomys rufocanus</i> (31)	1 (3.2%)	17
<i>Linguatula serrata</i>	<i>Meriones unguiculatus</i> (12)	1 (8.3%)	5

acid, after which a rostellum of each cestode was mounted in Hoyer's medium separately from the cyst. The nymphs of the pentastomid were fixed in 70% ethanol and cleared, using lactophenol. All measurements were made with a video micrometer. The measurements are in millimeters, with the range followed by the mean  $\pm$  1 standard deviation in parentheses. Drawings were made with the aid of a camera lucida. The specimens were deposited in the Department of Zoology of the National University of Mongolia (DZNUM) and in the Helminthological Collection of the Graduate School of Veterinary Medicine of the Hokkaido University (HKHU).

### Results

A total of 6 species of parasites, including 5 metacestodes (*Taenia mustelae*, *T. polyacantha*, *T. endothoracicus*, *Cladotaenia globifera*, and *Mesocestoides* sp.) and nymphs of 1 pentastomid, *Linguatula serrata*, were found. Of the helminths previously recorded from predatory hosts in Mongolia, we have registered larval stages of *T. polyacantha*, *T. mustelae*, and *C. globifera*. All other helminths represent new geographic records. Some of their definitive hosts remain unknown in Mongolia. The parasites' prevalence and intensity are shown in Table 1. Brief descriptions are given for all species.

#### Family Taeniidae Ludwig, 1886 *Taenia mustelae* Gmelin, 1790

SYNONYM: *Taenia tenuicollis* Rudolphi, 1819; *Cysticercus talpae* Rudolphi, 1819.

DESCRIPTION: The size of formalin-fixed cysts ( $n = 30$ ) recovered from livers of *C. rutilus* ranged from 2.46 to 5.74 ( $3.53 \pm 0.86$ ). Of

the 54 cysticerci observed, only 1 (1.85%) contained 2 scolices. Scolex diameter 0.330–0.476 ( $0.392 \pm 0.042$ ). Suckers 0.092–0.198 ( $0.147 \pm 0.024$ ) in diameter. Rostellum diameter 0.082–0.156 ( $0.102 \pm 0.017$ ), armed with 2 rows of hooks not differing significantly in size. Hook number 44–52, with a mean of 46 in 22 scolices examined. Hooks ( $n = 609$ ) 0.014–0.025 ( $0.018 \pm 0.0019$ ) in length. Base of hook 0.010–0.021 ( $0.015 \pm 0.002$ ) in length.

HOSTS: *Clethrionomys rufocanus*, *C. rutilus*, *Microtus mongolicus*, and *M. brandtii*.

SITE OF INFECTION: Liver.

LOCALITY: Hanh and Jargalant Counties in Hovsgol Province, railway station Davaani zorlog near Ulaanbaatar city (Fig. 1).

DEPOSITION OF VOUCHER SPECIMENS: DZNUM and HKHU (No. 742).

REMARKS: Adult worms were found in steppe polecat, *Mustela eversmanni* Lesson, 1827, at the same locality in Hovsgol Province (Suhbat and Ganzorig, 1988).

#### *Taenia polyacantha* Leuckart, 1856 (Fig. 2)

SYNONYM: *Tetratirotaenia polyacantha* (Leuckart, 1856) Abuladze, 1964; *Armatetrathyridium polyacantha* (Leuckart, 1856) Abuladze, 1964.

DESCRIPTION: Total length of armatetrathyridia ranged from 4 to 19.5. Scolex armed with 2 rows of characteristic hooks, 66 to 78 (71.8) in number in specimens from *M. brandtii*. Length of the large hook 0.169–0.209 ( $0.196 \pm 0.007$ ),



Figure 1. The sketch map of Mongolia with locations where parasites were found. Symbols: ■ *Taenia mustelae*; ● *T. polyacantha*; ▲ *T. endotheracicus*; ◊ *Cladotaenia globifera*; ▼ *Mesocestoides* sp.; □ *Linguatula serrata*.

blade and handle 0.081–0.115 ( $0.099 \pm 0.005$ ) and 0.096–0.136 ( $0.117 \pm 0.007$ ), respectively. Length of small hook 0.093–0.130 ( $0.117 \pm 0.005$ ), 0.061–0.106 ( $0.087 \pm 0.005$ ), and 0.049–0.070 ( $0.058 \pm 0.003$ ), respectively. Specimens from *C. migratorius* differed in number and length of the hook. There were 62 to 68 (65.4) hooks. Total length 0.180–0.228 ( $0.208 \pm 0.007$ ) and 0.114–0.139 ( $0.130 \pm 0.005$ ) for large and small hooks, respectively. Dimensions of the large hook blade and handle 0.087–0.127 ( $0.102 \pm 0.005$ ) and 0.091–0.135 ( $0.120 \pm 0.008$ ). Small hook 0.061–0.095 ( $0.081 \pm 0.008$ ) and 0.047–0.077 ( $0.065 \pm 0.005$ ).

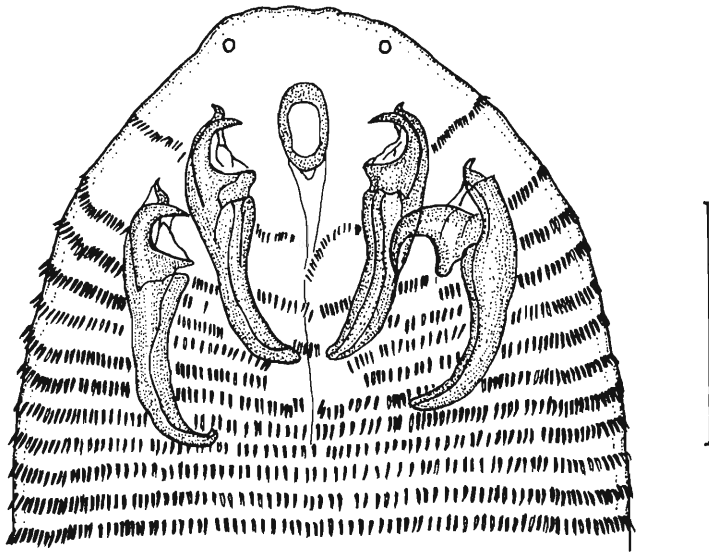
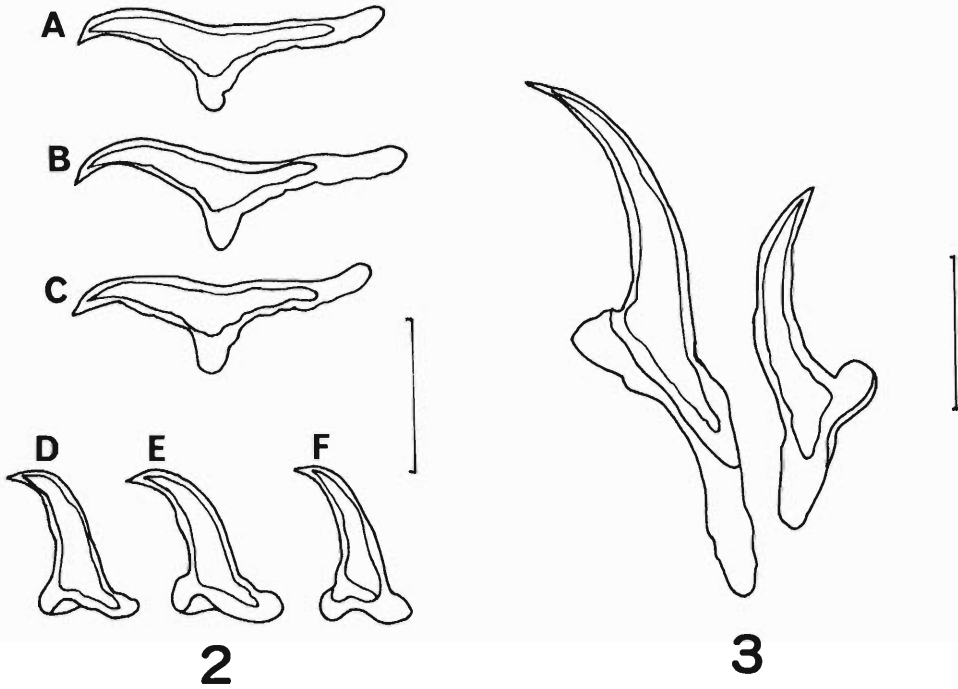
HOSTS: *Microtus brandti* and *Cricetulus migratorius*.

SITE OF INFECTION: Thoracic and peritoneal cavities.

LOCALITY: Bayan Ovoo County (Hentei Province), Zuil County and near waterfall Ulaan Tsutgalan (Ovorhangai Province), railway station Davaani zorlog near Ulaanbaatar city, Tsagaanvelger County (Dundgov Province), Mt. Eej Hairhan in Transaltai Gobi (Fig. 1).

DEPOSITION OF VOUCHER SPECIMENS: DZNUM and HKHU (No. 739–741, 2981).

REMARKS: The size and shape of the armatetrathyridia varied considerably. The variation in the size of the armatetrathyridia apparently represented age-related modifications (see Rausch and Fay, 1988a). Beside that, specimens from *C. migratorius* were characterized by lower numbers of rostellar hooks (65.4 vs. 71.8) and greater length of large (0.208 vs. 0.196) and small (0.130 vs. 0.117 mm) hooks than those from *M. brandti* (Fig. 2). These differences were statistically significant at the 0.1% level. There were no significant differences in size of rostellar hooks in specimens obtained from thoracic and abdominal cavities of *M. brandti*. The mean length of the large hook in specimens obtained from thoracic and abdominal cavities were 0.197 and 0.198 mm, respectively. Those for small hooks were identical, 0.117 mm in length. The prevalence was generally low (1.52% of 525 voles), but varied from 0 to 3.57% at Davaany zorlog, where 71 to 84 voles were trapped during each of 3 yr. Strobilar-stage cestodes were recorded from the corsac fox, *Vulpes corsac* L., 1768, in Mt. Hasagt Hairhan in Gov Altai Province and Erdeneburen County in Hovd Province (Danzan, 1978).



4

Figures 2-4. 2. Large and small hooks of *Taenia polyacantha* from different hosts: A, D from *Vulpes corsac*, B, E from *Cricetulus migratorius*, C, F from *Microtus brandti*. Scale bar = 0.1 mm. 3. Large and small hooks of *Taenia endothoracicus* from *Meriones unguiculatus*. Scale bar 0.1 = mm. 4. Anterior end of *Linguatula serrata*. Scale bar = 0.5 mm.

***Taenia endothoracicus* (Kirschenblatt, 1948)  
(Fig. 3)**

SYNONYM: *Multiceps endothoracicus* (Kirschenblatt, 1948); *Coenurus endothoracicus* Kirschenblatt, 1948.

DESCRIPTION: Only free coenuri were found. The number of scolices in 1 polycephalic metacestode varied from 6 to 10. Scolex 1.280–1.289 in diameter; rostellum 0.6 long, armed with a double row of 52 hooks. There were 4 suckers, 0.25 long by 0.47 wide. Length of large hooks 0.328–0.380 (0.354), blade and handle 0.144–0.198 (0.167) and 0.166–0.216 (0.193), respectively. Length of small hook 0.189–0.259 (0.221); blade length 0.106–0.140 (0.124); length of the handle 0.078–0.117 (0.103).

HOSTS: *Meriones unguiculatus*, *M. meridianus*, and *Rhombomys opimus*.

SITE OF INFECTION: Thoracic cavity.

LOCALITY: Borig Deliin Els in Uvs Province, Mt. Eej Hairhan, and oasis Shar Huls in Transaltai Gobi, Borzongiin Gobi in Omnogov Province (Fig. 1).

DEPOSITION OF VOUCHER SPECIMENS: DZNUM and HKHU (No. 743, 2982).

REMARKS: This is the first finding of *T. endothoracicus* in Mongolia. The definitive hosts in that country are unknown.

**Family Paruterinidae Fuhrmann, 1907  
*Cladotaenia globifera* Batsch, 1786**

SYNONYM: *Cladothyridium globifera* Batsch, 1876.

DESCRIPTION: Metacestodes 0.7–1.3 in length; scolex armed with 46–52 characteristically shaped hooks. Length of large hooks 0.032–0.040 and of small hooks, 0.023–0.029.

HOST: *Microtus brandti*.

SITE OF INFECTION: Liver.

LOCALITY: Tumentsogt County of Suhbaatar Province (Fig. 1).

DEPOSITION OF VOUCHER SPECIMENS: DZNUM (209/70).

REMARKS: Adult-stage cestodes were found in upland buzzard, *Buteo hemilasius* Temminck and Schlegel, 1844, and marsh harrier, *Circus aeruginosus* L., 1758, in Tov Province of Mongolia (Danzan, 1964). Danzan also reported the occurrence of *Cladotaenia fania* Meggitt, 1933 in tawny eagle, *Aquila rapax* Temminck, 1833, but *C. globifera* is distinguished by its greater number of hooks and their length.

**Family Mesocestoididae Fuhrmann, 1907  
*Mesocestoides* sp.**

SYNONYM: *Tetrathyridium* sp.

DESCRIPTION: The tetrathyridia found differed in size of body, and suckers as well. Tetrathyridia found in *A. bullata* measured 5.4 in length with maximal width 2.04. The suckers measured  $0.465 \times 0.207$ .

HOSTS: *Spermophilus undulatus*, *Clethrionomys rufocanus*, *Microtus brandti*, *Meriones unguiculatus*, and *Allactaga bullata*.

SITE OF INFECTION: Abdominal cavity, mesenteries, and liver.

LOCALITY: Hanh County in Hovsgol Province, Zuil County and near waterfall Ulaan Tsutgalan in Ovorangeai Province, Bayan Ovoo Country in Hentei Province, Delgerhangai County of Dundgov Province, railway station Davaani zorlog near Ulaanbaatar city (Fig. 1).

DEPOSITION OF VOUCHER SPECIMENS: DZNUM and HKHU (No. 745, 2983).

REMARKS: Previously, only *Mesocestoides lineatus* has been reported in Mongolia, the adult worms occurring in red fox, *V. vulpes* L., 1758; domestic cat, *Felis catus* L., 1758; northern three-toed jerboa, *Dipus sagitta* Pallas, 1773; and Mongolian hamster, *Cricetulus curtatus* Allen, 1925; and the tetrathyridia were in house mouse, *Mus musculus* L., 1758; Gobi jerboa, *A. bullata* (see Danzan, 1978); and Siberian marmot, *Marmota sibirica* Radde, 1862 (see Dubinin and Dubinina [1951]). Also, tetrathyridia were reported from multi-cellated racerunner, *Eremias multiocellata* Günther, 1872 (Reptilia: Lacertidae) (see Sharpilo, 1976). However, there is a clear-cut distinction between our material from *A. bullata* and *Mesocestoides lineatus* in the size of suckers,  $0.128-0.192 \times 0.115-0.166$  in the latter (see Tschertkova and Kosupko [1978]) and more than twice as large in the present larvae. Possibly, our specimens are closer to *Mesocestoides erschovi* Tschertkova and Kosupko, 1975, but it is difficult to identify the species based on juvenile characters only.

**Pentastomida**

**Family Linguatulidae Shipley, 1898  
*Linguatula serrata* Fröelich, 1789 (Fig. 4)**

DESCRIPTION: The nymphs possess an elongated body with 85 external annuli. Spines were present on the posterior border of each annulus.



Total length was 5.5–6.2; maximal width 1.175. The terminal segment 0.176 wide by 0.085 long. Mouth situated between the inner hooks, measuring  $0.114 \times 0.079$ . Two pairs of strong, double hooks arranged in an arc, measuring 0.481–0.500 in length.

HOST: *Meriones unguiculatus*.

SITE OF INFECTION: Thoracic and peritoneal cavity.

LOCALITY: Onjuul County of Tov Province (Fig. 1).

DEPOSITION OF VOUCHER SPECIMENS: DZNUM and HKHU (No. 744).

REMARKS: Excysted nymphs were removed from a large volume of bloody fluid that filled the body cavities. Our finding of the excysted nymphs may reflect postmortem migrations (see Riley, 1986). The present material was morphologically identical to the nymphs described by Rendtorff et al. (1962). Our finding constitutes a new host and distributional record.

### Discussion

The results of the present study showed a low prevalence of parasites, and the material was not sufficient for analysis, except that from *M. brandti* and *S. undulatus*. Mongolia is located at the junction of 2 large floral regions: the Siberian taiga holarctic subregion, covering the northern part of the country, and the Central Asian desert-steppe and desert subregion of the Ancient Mediterranean (see Information Mongolia [1990]). In that country, one may expect to find parasites of Holarctic and Palaearctic origins. Of the parasites found, 2, *T. mustelae* and *T. polyacantha*, are Holarctic; 1, *T. endothoracicus*, is Palaearctic; and 2, *C. globifera* and *L. serrata*, are cosmopolitan (see Abuladze [1964]; Self [1982]). Morphology and systematics of the holarctic cestodes has been studied predominantly on the basis of material from Europe and North America. Previously, it has been shown that *T. mustelae* of European and North American origins differ in hook length and also in extent of asexual reproduction in the juvenile stage (Wardle and McLeod, 1952; Abuladze, 1964; Verster, 1969; Murai, 1982). However, Todd et al. (1978) and Langham et al. (1990) found in North America that the hooks of the juvenile stage measured 0.020–0.021 mm in length, the same as those of European specimens. Morphologically, the cysticerci of *T. mustelae* from Mongolian rodents were similar to those of ro-

dentis in Europe (Tenora and Vanek, 1969; Murai, 1982) and Japanese specimens of *T. mustelae* (Iwaki et al., 1995). But, in contrast to European specimens, the material from Mongolia had a bicephalic cysticerci. Such cysticerci have been also reported in Japan (Iwaki et al., 1995). Compared with North American material, where all types of polycephalic and uniscolix cysts have been reported (Freeman, 1956), the Mongolian and Japanese materials consisted predominantly of the cysticercus-type metacestode and of polycephalic metacestodes that had only 2 scolices.

Another holarctic cestode, *T. polyacantha*, is widespread in Mongolia, appearing to consist of 2 morphologically different groups. Previously, 2 subspecies of *T. polyacantha* were recognized by Rausch and Fay (1988b). According to them, *Taenia p. polyacantha* Rausch and Fay, 1988 is distributed in Eurasia to the south of the zone of tundra, and *T. p. arctica* Rausch and Fay, 1988 is present throughout the holarctic tundra. Morphologically, our material from *M. brandti* is similar to the nominal subspecies, *T. p. polyacantha*. However, the armatetrazyridia that were obtained in *C. migratorius* in western Mongolia were different from those found in central and eastern provinces of Mongolia. On the basis of hook number and hook length, the material from *C. migratorius* resembled *T. polyacantha* from Kazakhstan (see Abuladze [1964]). The difference between isolates of *T. polyacantha* from various rodent hosts has not been reported in the literature. Thus, we suppose that the difference between western and eastern materials is based on geographic character, and probably there are 2 suprapopulations or races of *T. polyacantha* in Mongolia. Also, we note that the hook number (see Rausch and Fay [1988b]) of the *T. p. polyacantha* increases from 55–66 in western Eurasia (Hungary, Norway, Germany) to 66–78 in East Eurasia (central and eastern parts of Mongolia, and Inner Mongolia) and may present a clinal alteration in the number of hook and their length.

*Taenia endothoracicus* has been reported from Georgia, Turkmenistan, Kazakhstan, Russia, Iran, and Morocco (Abuladze, 1964; Ryjnikov et al., 1978). Others found were those of Khalil et al. (1979), which were registered as having Cheesman's gerbil, *Gerbillus cheesmani* Thomas, 1919, as intermediate host and red fox, *V. vulpes*, as definitive host in the State of Ku-

wait. Betterton (1977) reported that the taeniid metacestodes found in black rat, *Rattus rattus diardi* (Jentink, 1879), trapped in Malaysia closely resembled that of *T. endothoracicus* in number of hooks. However, that finding appears to represent metacestodes other than *T. endothoracicus*, since Kamiya et al. (1987) reported that bicephalic metacestodes found in the same host, and in the same country, are of a species different from *T. endothoracicus*. Thus, our finding significantly extends the eastern limit of the distribution of *T. endothoracicus*. The range of intermediate hosts was found to be only gerbils (Gerbillinae) (Abuladze, 1964; Ryjikov et al., 1978; Khalil et al., 1979), although Ryjikov et al. (1978) reported that steppe lemming, *Lagurus lagurus* Pallas, 1773, was also infected with the parasite. Thus, it may be suggested that *T. endothoracicus* is specific to gerbils, and the distribution of this parasite depends on the intermediate hosts.

*Linguatula serrata* is a cosmopolitan parasite of carnivorous mammals; its larval stage develops in herbivores (see Riley [1986]). The nymphs probably infect all members of the Lagomorpha and Artiodactyla (see Self [1982]). But the only record of *L. serrata* from rodents as intermediate hosts was that from the abdominal cavity of bandicoot rats (*Bandicota* sp.) in India (Raja, 1974). Thus, our case with *Meriones unguiculatus* is the second record in a rodent host. Of the parasites found, 2 species are medically important. Human cases with *M. lineatus* and *L. serrata* infections were reviewed by Riley (1986) and Tschertkova and Kosupko (1978).

During the field investigation of rodents, considerable attention was given to the study of *Echinococcus multilocularis* Leuckart, 1863, because this medically important parasite occurs in the regions of Russia and China that border Mongolia. Distribution of *E. multilocularis* in neighboring Russia (Martynenko et al., 1988) and China (Tang et al., 1988; Craig et al., 1991) includes Tuva, Altaiskii krai, Buryatia, Xinjiang, and Inner Mongolia, which border Mongolia on the north, northwest, southwest, and southeast. Moreover, almost every map on geographic distribution of *E. multilocularis* includes Mongolia (Matossian et al., 1977; McManus and Smyth, 1986; Schantz, 1986; Gemmel et al., 1987), but no official record of its occurrence in that country exists. We examined more than 1,500 rodents

from different geographic localities, but no *E. multilocularis* was found. So, the occurrence of *E. multilocularis* in Mongolia is still an open question and needs further investigation.

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## Evaluation of *Verticillium lecanii* Strains Applied in Root Drenches for Suppression of *Meloidogyne incognita* on Tomato

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**ABSTRACT:** Three-week-old tomato seedlings were transplanted from sand into 10-cm-diameter pots (540-ml volume). Each pot contained 600 g loamy sand to which either 1,000 or 5,000 *Meloidogyne incognita* eggs were added. Five strains of the fungus *Verticillium lecanii* were individually applied in root drenches to the tomato plants at the time of transplanting, at an application rate of about 0.08% (dry weight fungus/dry weight loamy sand). The strains were a wild type strain and four mutants induced from that strain. Control plants were treated with water only or with autoclaved (nonviable) fungus. The experiments ended 45 days after transplanting, when the number of eggs per pot, root infection ratings, root lengths, and shoot dry weights were determined. The numbers of eggs counted from fungus-treated plants did not differ significantly from the numbers on water-only control plants. Application of autoclaved wild type strain to pots treated with 5,000 eggs resulted in an infection rating significantly higher than infection ratings recorded from several other fungus treatments and from plants treated with water only, but not in increased egg numbers.

**KEY WORDS:** Biological control, fungus, *Lycopersicon esculentum*, root-knot nematode.

*Meloidogyne* (root-knot nematode) is one of the most destructive genera of plant-parasitic nematodes, affecting numerous crop plants worldwide. The identification and successful deployment of biological control organisms would greatly benefit existing management programs for this plant pest. Numerous studies have focused on fungi as microbial pest control agents for the species *Meloidogyne incognita* (Kofoid & White) Chitwood on tomato (e.g., Jansson et al., 1985; Mankau and Wu, 1985; Gaspard, 1986; Ibrahim et al., 1987; Cabanillas et al., 1989; Gaspard et al., 1990a, b; Leij et al., 1992a, b, c; Santos et al., 1992; Duponnois et al., 1995; Gautam et al., 1995). A commercial product containing *Arthrobotrys* was developed for management of root-knot nematode on tomato plants, but is not in widespread use (Stirling, 1991). *Verticillium lecanii* (A. Zimmermann) Viégas is another fungus investigated as a potential management agent for *M. incognita* (Meyer, 1994). A mutant strain (selected for increased benomyl tolerance) and a wild type strain, both found to have action against *Heterodera glycines* (soybean cyst nematode) on soybean (Meyer and Meyer, 1995, 1996; Meyer and Huettel, 1996), were tested in the greenhouse for antagonism to *M. incognita* on tomato plants (Meyer, 1994). An alginate granule formulation was selected for the tomato study because it was efficacious in soybean tests with *V. lecanii* (Meyer and Huettel, 1993, 1996; Meyer and

Meyer, 1995, 1996), and because alginate granules have been effectively employed in various tests of biocontrol fungi for plant-parasitic nematodes (e.g., Cabanillas et al., 1989; Schuster and Sikora, 1992a, b; Stirling and Mani, 1995). Application of the 2 *V. lecanii* strains in alginate granules decreased *M. incognita* populations on tomato in some experimental trials, but no application rate of either strain resulted in consistent, significant decreases in *M. incognita* populations (Meyer, 1994).

Because formulation is vital to success of biocontrol agents, the current study was initiated to determine whether a root drench would be efficacious for application of *V. lecanii* strains against root-knot nematode. The effects of root drenches applied at the time of tomato seedling transplant were investigated for the 2 previously tested *V. lecanii* strains and for the 3 other mutant strains that had been selected for benomyl tolerance (Meyer, 1992). All 5 strains were tested because biocontrol activity often varies with fungus strain.

### Materials and Methods

#### Preparation of fungi and nematodes

Mutant strains M1S1, M2S1, M9S1, and M10S1 (Agricultural Research Service Culture Collection, NRRL #'s 18725, 18726, 18727, and 18728, respectively) were induced from a wild type strain of *Verticillium lecanii* (American Type Culture Collection 58909) with ultraviolet radiation and selected for increased tolerance to the fungicide benomyl (Meyer, 1992). For the greenhouse tests, the fungi were grown

for 3 days in 1-L erlenmeyer flasks (each flask containing 250 ml potato dextrose broth) that were rotated on orbital shakers (240 rpm) at 25°C (Meyer, 1994). Mycelium was harvested by centrifugation of the broth cultures at 13,000 g (Meyer, 1994). Conidia produced in the broth cultures were collected with the mycelium, but our studies on shelf life of these *V. lecanii* strains indicated that the conidia are short lived; and therefore, they are not considered useful for nematode management applications (unpubl.). The collected mycelium was divided into 2 parts; half was autoclaved to be used as a nonviable control, while the other half was used live for addition to pots. Autoclaved treatments are given the suffix "A." All strains were refrigerated at 4°C overnight and used the day after harvest from the erlenmeyer flasks.

*Meloidogyne incognita* eggs for tomato plant infestation were collected from greenhouse cultures (Meyer, 1994).

### Greenhouse experiments

Tomato (*Lycopersicon esculentum* Mill.) cv. Marglobe seeds were sown in sand in styrofoam flats. Seedlings (ca. 3 wk old) were transplanted into 540-ml pots (10 cm diameter), each containing 600 g (air-dried weight) of loamy sand. The loamy sand was made from a compost/sand mixture (3 parts compost to 1 part sand) with a final composition of 79% sand, 11% silt, 7% clay, 3% organic matter, pH 6.9. *Meloidogyne incognita* eggs were mixed into the loamy sand just prior to transplanting of the tomato seedlings. The root-knot nematode eggs were added at 2 rates: 1,000 and 5,000 eggs per pot. For transplanting, a depression large enough for the plant roots was made in the loamy sand of each pot. Fungus (live or dead) in 60 ml of water was added to the depression in each pot. The fungus application rate was ca. 0.5 g dry weight fungus per pot, equivalent to ca. 0.08% dry weight fungus per dry weight loamy sand. Controls without live or dead fungus received 60 ml of water only. A tomato seedling was immediately transplanted into each depression. Ten pots were used for each treatment, and the experiment was later repeated for a total of  $N = 20$  pots per treatment (with a few exceptions where plants died during the course of the experiment). The pots were arranged in a randomized complete block design under daylight (greenhouse temperatures reaching up to ca. 40°C). Plants were fertilized with Sierra<sup>®</sup> Poinsettia Mix at recommended rates. The experiments were terminated 45 days after transplanting, at which time the tomato plants were cut off just above the soil line, and shoot weights were determined after drying at 63°C.

### Egg counts and infection class ratings

Root lengths were measured from the soil line to the tip of the main root. Nematode infection class numbers were assigned to roots (Daulton, 1959). Rating numbers indicate the following: 0 = free from galls; 1 = less than 5 galls; 2 = trace to 25 galls; 3 = 26 to 100 galls; 4 = moderate, numerous galls, mostly discrete; 5 = moderately heavy, numerous galls, many coalesced. Root-knot nematode eggs were collected as in Meyer (1994), except that egg masses from loamy

sand that was very dry at harvest had to be collected on the 60-mesh sieve and broken apart in a mortar and pestle or a manual tissue grinder. The eggs were then collected on a 500-mesh screen (pore size 25  $\mu$ m) and counted.

### Isolation of fungi from loamy sand

To test for the presence of the fungus at the end of the experiment, loamy sand from each pot was stirred in water (0.02% dry weight per volume water), plated onto semiselective media (0.05 ml of suspension per petri dish), and incubated at 25°C. Two media were used. One medium was PDA ABE 1000, similar to PDA ABE 100 (Meyer, 1994). Each liter of PDA ABE 1000 contained 39 g of PDA (potato dextrose agar), 970 ml of distilled water, 2 gr of benlate in 20 ml of distilled water (Benlate 50 Wetttable Powder or DF, E. I. Dupont de Nemours & Co., Wilmington, Delaware), and antibiotics (0.3 g of streptomycin sulfate plus 0.3 g of tetracycline in 10 ml of sterile water). Six milliliters of EtOH were used to rinse the flask in which the antibiotics were mixed and were then added to the medium. The second medium was modified Ausher's Medium Number 2 (Ausher et al., 1975), with PCNB replaced by benomyl. Loamy sand suspensions were plated out as follows: (1) water controls: 1 petri dish of Ausher's medium and 1 petri dish of PDA ABE 1000 per pot; (2) wild type strain treatments: 2 petri dishes of Ausher's medium per pot; and (3) mutant strain treatments: 2 petri dishes of PDA ABE 1000 per pot. Consequently, loamy sand from water control treatments was plated onto 78 petri dishes (1 pot was not sampled), and loamy sand from each fungus treatment was plated onto 80 petri dishes.

### Analysis of data

The experiments were combined and analyzed as an incomplete block design using the SAS procedure MIXED (SAS, 1992). The egg count values were wide ranging, differing in magnitude by as much as a factor of 10; the variances of the treatments were heterogeneous. To correct this, the data were  $\log_{10}$  transformed. The variables:  $\log_{10}$  (egg count), infection class, root length, and shoot dry weight were analyzed as mixed models. Experiment and the experiment by treatment interaction were considered random effects.

### Results and Discussion

When 1,000 eggs were initially applied to pots, the largest reductions in egg populations resulted from individual application of M1S1A, live wild type strain, and wild type A (Table 1). The latter 2 treatments resulted in a 32% reduction in egg numbers compared to water controls (based on egg count means transformed from  $\log_{10}$  egg counts), and M1S1A treatment resulted in a 28% decrease. However, the reductions were not significantly different from the egg numbers found in pots treated with water only. In the pots that had received 1,000 eggs, 2 of the 3 lowest infection ratings were recorded

**Table 1.** Effects of water, live *Verticillium lecanii*, and autoclaved *Verticillium lecanii* on tomato plants, number of galls, and mean numbers of *Meloidogyne incognita* eggs produced per pot. Pots initially received either 1,000 or 5,000 eggs.

Treatment	Number of eggs*		Infection rating		Shoot dry weight—grams†	
	1,000	5,000	1,000	5,000	1,000	5,000
Water	1,920	6,272 ab§	2.4	3.2 ab	38.7 a	40.0 bc
M1S1A‡	1,390	9,282 b	2.7	3.8 bc	41.4 c	39.5 abc
M1S1	2,124	4,982 ab	2.6	3.6 abc	40.0 abc	40.6 bc
M2S1A	1,745	3,011 a	2.4	3.0 ab	38.0 a	38.2 ab
M2S1	1,500	7,037 b	2.2	3.1 abc	40.1 bc	37.8 ab
M9S1A	2,408	4,226 ab	2.7	3.3 abc	38.7 ab	37.1 ab
M9S1	1,633	5,349 ab	2.6	3.1 abc	40.9 bc	40.3 bc
M10S1A	1,882	4,597 ab	2.0	2.9 ab	41.0 bc	39.4 ab
M10S1	2,600	5,083 ab	2.2	2.9 a	39.8 abc	41.5 c
Wild type A	1,309	5,780 ab	1.9	3.8 c	39.0 abc	37.7 ab
Wild type	1,308	7,254 ab	2.1	3.5 abc	38.7 ab	38.3 ab
F-value	6.39		5.59		2.52	
P-value	0.0001		0.0001		0.0108	

\* Values are the egg count means back transformed from  $\log_{10}$ .

† Values are least squares means.

‡ Treatments with letter A represent autoclaved fungal strains.

§ Numbers followed by the same letter are not significantly different at  $P = 0.05$ , based on analysis of  $\log_{10}$ -transformed data. Letters are comparable within columns but not between columns. Any apparent discrepancies in letter assignments (such as 2.9 a and 2.9 ab in the 5,000 infection rating column) are due to the fact that the standard error of the difference between the means is not the same for all pairs.

from the 2 wild type strain treatments (Table 1), but the infection ratings were similar for all treatments, generally falling in the 2–3 range (from more than 5 galls to less than 100). Root lengths among treatments were similar ( $P > 0.05$ ), ranging from 12.1 cm to 16.8 cm (least squares mean per treatment). The highest shoot dry weights were in plants treated with M1S1A (Table 1).

Trends recorded at 1 nematode population density did not appear at the other. When 5,000 eggs were initially added to each pot, the largest reduction in egg numbers (52% compared with water-treated controls) was recorded after treatment with M2S1A (Table 1). Despite the overall large reduction, there was high variability, and the 2 values were not significantly different. However, in the pots receiving 5,000 eggs, treatment with M2S1A did result in significantly fewer eggs than treatment with viable strain M2S1 or with M1S1A, and in one of the smallest root infection ratings. Applications of M1S1A or of the live wild type strain, which were associated with low egg counts when 1,000 nematodes were added per pot, were connected with high egg populations in the pots receiving 5,000 eggs. Additionally, M1S1A and wild type A treatments resulted in significantly higher in-

fection class ratings than some other treatments in the pots that received 5,000 eggs. Most of the other infection class ratings were similar to each other (ca. 3–4; from 26 galls to more than 100, galls mostly discrete), although plants in pots treated with strain M10S1 and 5,000 eggs had low infection ratings and also had the heaviest shoots (Table 1).

Delivery of a potential biocontrol fungus during transplant of tomato seedlings was previously tested with *Monacrosporium ellipsosporum* (Grove) Subr., which was applied on wheat grain substrate to tomato seedling transplant holes (Mankau and Wu, 1985). That study demonstrated a trend toward nematode suppression in fungus-treated plants, but there was not a significant difference from controls. Similarly, in the current study, none of the 5 strains applied to transplant holes significantly affected root-knot nematode populations on tomato plants. The lack of food source for the fungus in the root drench may have outweighed any advantage of direct root contact from the drench application, although *V. chlamydosporium* and *Monacrosporium ellipsosporum* were effective against *Meloidogyne* spp. on tomato when applied to soil without a food source (Leij and Kerry, 1991; Santos et al., 1992). Indeed, *V. chlamydospor-*

*ium* established in soil more readily without a food base, presumably because other microorganisms could feed upon the bran (Leij and Kerry, 1991). In the current experiment, there were only 4 isolations of *V. lecanii* from greenhouse pots: strain M2S1 was isolated from 1 pot (5,000-egg treatment) and strain M9S1 from 3 pots (2 pots treated with 5,000 eggs and 1 pot with 1,000 eggs). The 4 mutant strains of *V. lecanii* reduced *Heterodera glycines* populations even with poor fungus isolation rates from greenhouse pots (Meyer and Meyer, 1995, 1996; Meyer and Huettel, 1996). Consequently, it is not known whether *V. lecanii* was ineffective in the current experiment because of failure to survive in the soil, or because of a low level of activity against *M. incognita*. In either case, the strains were not efficacious management agents for this nematode.

Treatment with autoclaved alginate granules containing nonviable fungus did not affect *M. incognita* populations (Meyer, 1994), so no effects were anticipated following treatment with autoclaved fungus in root drenches. Interestingly, in the root drench application, treatment with 1 autoclaved fungus strain resulted in lower egg numbers than treatment with viable *V. lecanii* of the same strain. Application of M2S1A to pots treated with 5,000 eggs resulted in significantly fewer eggs (57% reduction in egg numbers) than were produced in pots treated with live M2S1. This may have been due to such factors as antagonistic breakdown products from decomposing mycelium or to increased populations of microorganisms feeding on the dead fungus, but these parameters were not measured in this study. In the pots receiving 5,000 eggs, M2S1A was also more effective at reducing egg populations than M1S1A (68% difference in final egg counts), and resulted in lower root infection ratings than application of wild type A. This suggests that there is some difference among the strains, although there are insufficient data at this time to determine what that difference might be. Leij et al. (1992c) found that as *M. incognita* densities increased, live *Verticillium chlamydosporium* became less effective against the nematode. In the current experiment, activity of M2S1A appeared to be enhanced by increasing the nematode population density, since none of the differences in egg numbers or infection ratings were found in pots treated with 1,000 eggs. If autoclaved strain M2S1 had demonstrated re-

markable suppression of *M. incognita*, it might be worthwhile to investigate impact on juveniles as well as on eggs, and to pursue application as an amendment or to study breakdown products from the dead mycelium. However, the effect compared with the water controls was not large enough to warrant study as a potential management agent.

A fungus that is effective against 1 plant-parasitic nematode is not necessarily active against another species. These strains of *Verticillium lecanii*, while efficacious against *H. glycines* under various greenhouse conditions (Meyer and Meyer, 1995, 1996; Meyer and Huettel, 1996), did not demonstrate similar activity against *M. incognita*.

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**Research Note**

**Parasites of Anadromous Arctic Char (*Salvelinus alpinus* L.) from Two Sites in Ungava Bay (Quebec, Canada)**

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**ABSTRACT:** Sixty-two anadromous Arctic char (*Salvelinus alpinus* L.) were collected at 2 sites in Ungava Bay (Quebec) in August 1994 and examined for parasites. Thirteen species were found: *Myxobolus arcticus*, *Tetraonchus alaskensis*, *Derogenes varicus*, *Hemiurus levinseni*, *Lecithaster gibbosus*, *Phyllodistomum umblae*, *Bothrimonus sturionis*, *Eubothrium salvelini*, *Contracecaecina* gen. sp. larva, *Philonema agubernaculum*, *Echinorhynchus gadi*, *Corynosoma strumosum*, and *Salmincola carpionis*. *Myxobolus arcticus* and *Hemiurus levinseni* are new Canadian records for Arctic char. No parasites of zoonotic significance were encountered.

**KEY WORDS:** myxozoans, helminths, parasites, Arctic char, *Salvelinus alpinus*, Quebec, Canada.

The Arctic char (*Salvelinus alpinus* L.) is a circumpolar species that has a broad distribution in the Canadian Arctic (Scott and Crossman, 1973). Populations of this species may be lake-resident, living in lakes and never entering the sea, or anadromous. In northern Canada, anadromous Arctic char migrate seaward during spring runoff in May–June (Gillis et al., 1982; Dempson and Green, 1985) and return to their natal rivers at the end of the summer, generally between mid-August and mid-September. This species supports commercial fisheries in Labrador (Dempson and Green, 1985) and Ungava Bay (Boivin et al., 1989) and is also an important traditional food resource for local indigenous people (Kinloch et al., 1992).

The parasite fauna reported from Arctic char in Canada is summarized by Margolis and Arthur (1979) and McDonald and Margolis (1995). Previous surveys on the parasites of this fish in

northern Canada are those of Mudry and McCart (1976), Beverley-Burton (1978), Curtis (1979), and Dick and Belosevic (1981) in the Northwest Territories; Hicks and Threlfall (1973) and Bouillon and Dempson (1989) in Labrador; and Hanek and Molnar (1974), Fraser and Power (1984), and Curtis et al. (1995) in Quebec. Of these, studies including the examination of anadromous Arctic char are those of Hicks and Threlfall (1973), Hanek and Molnar (1974), Mudry and McCart (1976), Dick and Belosevic (1981), and Bouillon and Dempson (1989). As part of a larger study investigating possible health risks posed by “country foods,” we investigated the parasites of anadromous Arctic char at 2 sites in Ungava Bay, northern Quebec.

A total of 62 anadromous Arctic char were sampled at 2 sites in Ungava Bay in August 1994. These included 37 fish from Sapukkait (lat. 59°28'N, long. 65°18'W) collected 23–24 August 1994, of length 17.0–69.3 cm (mean  $\pm$  SD = 42.8  $\pm$  12.7); weight 0.05–3.75 kg (1.18  $\pm$  0.87), and age 3–14 years (7.1  $\pm$  2.2); and 25 fish collected from the George River near Kangiquallujjaq (lat. 59°41'N, long. 65°57'W) on 25–26 August, of length 29.9–57.5 cm (46.5  $\pm$  7.4), weight 0.30–2.60 kg (1.46  $\pm$  0.65), and age 3–14 years (7.0  $\pm$  2.5). Salinities at the 2 sites at the time of collection were 0‰ and 14‰, respectively. Fish from Sapukkait were collected in the trap of a counting fence on a small freshwater stream located within 100 m of the sea from which they were arriving; those from the George River were gill-netted in the river estuary. Before being frozen for shipment, fish were measured, weighed, and sexed, and their saccular otoliths were removed for age determination.

Complete parasitological examinations were performed as follows: fish were thawed and the external surface rinsed. The rinse sediment was

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examined for ectoparasites using a stereomicroscope. Gills were removed and rinsed and the arches examined. The buccal cavity was rinsed, and the opercula, eyes, and rinse water were examined separately. The fins were removed and examined for encysted parasites. The internal organs (heart, liver, spleen, gallbladder, digestive tract, gonads, kidney, and urinary bladder) were inspected for parasites on their exterior surfaces. The stomach, pyloric caeca, and intestine were separated and opened longitudinally; their contents were rinsed into beakers, mixed with sodium bicarbonate, and settled to collect endoparasites. The walls of the stomach, pyloric caeca, and intestine, and the liver, spleen, kidney, and heart, were compressed between glass plates and examined for parasites. The body cavity was rinsed and the rinse examined. Squash preparations made from liver, spleen, kidney, gonads, intestine, muscle, and brain tissue, and scrapings from the urinary bladder and gallbladder, were examined for Protista and Myxozoa using a compound microscope at  $\times 400$  magnification. Preparations not found to harbor protistans or myxosporeans after 5 min examination were considered uninfected. The body musculature was removed from the vertebral column, and the fillets and flaps were sliced thinly and inspected for helminths and protistan and myxosporean cysts.

All parasites were sorted into major taxonomic groups, cleaned, counted for each organ (metazoans only), and fixed in 10% buffered formalin (myxozoans), 70% ethanol with 10% glycerine (nematodes, crustaceans), or alcohol-formalin-acetic acid (platyhelminths, acanthocephalans) for later identification to lowest possible taxon. Because of their abundance, stomach digeneans were counted in a subsample of 5 ml out of a 125 ml total volume, and total numbers were estimated. As necessary, digeneans, cestodes, and acanthocephalans were stained with Schneider's acetocarmine and mounted in Permount<sup>®</sup>. Nematodes and acanthocephalans were cleared by evaporation in glycerine in 70% ethanol and examined as temporary mounts in glycerine. Copepods were dissected and appendages examined as temporary mounts.

Voucher specimens are deposited in the collections of the Canadian Museum of Nature as collection nos. CMNPA 1997-0051 to CMNPA 1997-0062 and are also retained in the collection

of one of us (J.P.M.) at the Université du Québec à Rimouski.

Parasite data are presented as prevalence (percent infected) and intensity of infection (number of parasites per infected fish), expressed as mean  $\pm$  standard deviation, followed by the range, as recommended by Margolis et al. (1982).

Thirteen parasite species were identified: 1 Myxozoa, 4 Digenea, 1 Monogenea, 2 Cestoda, 2 Nematoda, 2 Acanthocephala, and 1 Copepoda (Table 1). Of these, 11 taxa were common to both localities, while two species (*Phyllodistomum umblae* (Fabricius, 1780) and *Tetraonchus alaskensis* Price, 1937) were encountered only at Sapukkait. Two parasite species have not been reported previously from Arctic char in Canada; *Myxobolus arcticus* Pugachev and Khokhin, 1979, and *Hemiurus levinseni* Odhner, 1905. In previous studies on Canadian Arctic char, *M. arcticus* may have been misidentified as *M. neurobius* Schuberg and Schröder, 1905 (see McDonald and Margolis, 1995).

There have been only a few studies on the parasite fauna of Arctic char from northern Quebec (Hanek and Molnar, 1974; Fraser and Power, 1984; Curtis et al., 1995), and of these, only Hanek and Molnar (1974), who examined 35 Arctic char from Matamek River and Matamek Lake, included examination of anadromous fish. Of the 16 parasite species identified in their study, only *Echinorhynchus gadi* and perhaps *Salmincola* sp. were also encountered during our investigation. The other three surveys conducted in the area near Ungava Bay (Labrador and Baffin Island) have reported finding a slightly higher number of parasite species than found during our study (22 by Hicks and Threlfall, 1973; 15 by Dick and Belosevic, 1981; and 18 by Bouillon and Dempson, 1989). In general, for species shared with our study (maximum of 8, for Dick and Belosevic [1981]), these studies report prevalences and intensities similar to our findings, except for the digeneans *Derogenes varicus* (O. F. Müller, 1784) and *Hemiurus levinseni*, whose abundances were much higher in our fish, and the cestode *Bothrimonus sturionis* Duvernoy, 1982, which was generally less abundant in our study.

None of the Arctic char examined carried parasites transmissible to humans, a finding potentially important to the health of local Inuit, who frequently consume raw fresh or frozen char (Lantis, 1981; Ross et al., 1989; Curtis and By-

**Table 1.** Summary of parasites of Arctic char (*Salvelinus alpinus*) from Sapukkait and the George River estuary (Ungava Bay, Quebec).

Parasite	Location*	Sapukkait (n = 37)			George River (n = 25)		
		P (%)†	Intensity‡	R§	P (%)	Intensity	R
<b>Myxozoa</b>							
<i>Myxobolus arcticus</i>	Brain	43.2	—	—	24.0	—	—
<b>Monogenea</b>							
<i>Tetraonchus alaskensis</i>	Gills	2.7	1.0 ± 0.0	1-1	—	—	—
<b>Digenea</b>							
<i>Derogenes varicus</i>	S	100.0	5,659.1 ± 4,119.6	8-13,775	100.0	5,750.4 ± 4,437.3	483-20,721
<i>Hemiurus levinseni</i>	S, I	100.0	3,450.8 ± 2,487.1	11-9,625	100.0	1,855.2 ± 1,714.5	288-8,575
<i>Lecithaster gibbosus</i>	S,   PC, I	86.5	27.4 ± 30.2	1-106	92.0	19.8 ± 26.2	1-99
<i>Phyllodistomum umblae</i>	U	21.6	13.0 ± 12.7	1-32	—	—	—
<b>Cestoda</b>							
<i>Bothriomus sturionis</i>	PC, I	35.1	4.3 ± 7.9	1-30	64.0	16.9 ± 31.2	1-128
<i>Eubothrium salvelini</i>	PC, I	29.7	2.9 ± 1.9	1-7	56.0	2.1 ± 1.2	1-5
<b>Nematoda</b>							
<i>Philonema agubernaculum</i>	BC	21.6	1.4 ± 0.7	1-3	56.0	3.0 ± 2.2	1-7
Contraeacinea gen. sp. (L)	I, S, Ms, BC	14.0	4.2 ± 6.6	1-16	60.0	1.9 ± 1.2	1-5
<b>Acanthocephala</b>							
<i>Corynosoma strimosum</i> (J)	I	10.8	2.0 ± 2.0	1-5	28.0	1.1 ± 0.4	1-2
<i>Echinorhynchus gadi</i>	I	8.1	2.0 ± 1.0	1-3	44.0	8.1 ± 12.1	1-42
<b>Copepoda</b>							
<i>Salmincola carponis</i>	Gills, Mo, O	43.2	4.1 ± 3.7	1-14	80.0	8.4 ± 12.2	1-45

\* Abbreviations: BC = body cavity, I = intestine, J = juvenile, L = larva, Ms = mesenteries, Mo = mouth, O = operculum, PC = pyloric caeca, S = stomach, U = ureter.

† P (%) = prevalence (percent infected).

‡ Intensity given as the mean ± SD.

§ R = range.

|| Findings in these locations are probably due to postmortem migration or to displacement of parasites during dissection.

lund, 1991). The only species of zoonotic importance known to occur in fishes from the study area is the pseudophyllidean cestode *Diphyllobothrium dendriticum* (Nitzsch, 1824) (Lantis, 1981; Curtis et al., 1988; Ross et al., 1989; Curtis and Bylund, 1991). Plerocercoids are found mainly in salmonids (Margolis and Arthur, 1979; McDonald and Margolis, 1995), with Arctic char being the main source of human infections in northern Canada (Laird and Meerovitch, 1961).

The extremely high numbers of hemiuroid trematodes (*Derogenes varicus* and *Hemiurus levinseni*) found in the stomachs of all fish examined are noteworthy. Other studies have not reported such high abundances of these parasites in Arctic char or in any other fishes in Canada.

Parasites have occasionally proved useful as biological tags for Arctic char, having been used to separate anadromous from resident fish (Dick and Belosevic, 1981; Bouillon and Dempson,

1989). In our study, the freshwater digenean *Phyllodistomum umblae* was found only at Sapukkait, which may make it potentially useful to fishery management as a indicator of fish originating from this area.

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### Research Note

## Trematodes from Fishes of the Río Hondo River and Freshwater Lakes of Quintana Roo, Mexico

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**ABSTRACT:** Twenty-seven species of Digenea (9 adults and 19 metacercariae) were found in 44 fish (11 species) from the Río Hondo River and two freshwater lakes ("lagunas") in Quintana Roo, Mexico. *Cichlasoma meeki* and *Astyanax fasciatus* had the highest number of species (12 and 9, respectively), and there were no significant differences in the composition of digeneans reported from previous surveys of cenote fishes from the same area. Fauna of trematodes found appeared to be most similar to that of the Neotropical region.

**KEY WORDS:** Digenea, freshwater fish, Yucatan Peninsula, zoogeography, survey.

The Yucatan Peninsula is situated in the transitional area between Nearctic and Neotropical regions. It has only two rivers, the Río Champoton situated on its western boundary in the State of Campeche, and the Río Hondo, State of Quintana Roo, which forms the boundary between Mexico and Belize.

Although surveys of parasites of freshwater fish from cenotes have been carried out (Moravec et al., 1995a,b; Scholz et al., 1995a,b, 1996), there is no information on the parasites of fish in rivers and lakes ("lagunas").

In January and February 1995, fish from four localities in the State of Quintana Roo, Mexico, were examined for the presence of helminths: 1) Río Hondo river at the village of La Unión (17°55'N, 88°51'W; 26 January 1995), *Astyanax fasciatus* (Cuvier)—8 specimens examined (family Characidae), 1 *Cichlasoma meeki* (Brind) (Cichlidae); 2) Río Hondo river at the village of Ramonal (18°16'N, 88°38'W; 26 January 1995), 1 *Dorosoma* sp. (Clupeidae), 1 *A.*

*fasciatus*, 2 *Arius felis* (L.) (Ariidae), 1 *Poecilia velifera* (Regan) (Poeciliidae), 4 *Cichlasoma aureum* (Günther); 7 *C. meeki* (Brind), 3 *C. octofasciatum* (Regan), 1 *C. synspilum* Hubbs; 3) Laguna Bacalar, a lake near the village of Bacalar (18°38'N; 88°25'W; 25 January 1995), 1 *A. felis*, 4 *Cichlasoma urophthalmus* (Günther), 1 *C. synspilum*; 4) Laguna Paiyegua, a lake near the village of Valle Hermoso (19°10'N; 88°30'W), 3 *Dorosoma* sp., 2 *A. fasciatus*, 1 *Rhamdia guatemalensis* (Günther) (Pimelodidae), 1 *C. meeki*, 1 *C. octofasciatum*, and 1 *C. urophthalmus*.

Voucher specimens were deposited in the helminthological collections of the Institute of Biology, National Autonomous University of Mexico (UNAM), Mexico City, Mexico (Nos. CNHE 2681, 2683, 2685-6, 2841, 2845-6), the Institute of Parasitology, České Budějovice, Czech Republic (Nos. D-311, 314-316, 321-327, 334, 335, 341, 345, and 349), and the U.S. National Parasite Collection, Beltsville, Maryland (Nos. 85969-85971 and 86399).

Table 1 summarizes prevalence, intensity, site of infection, and localities of trematodes found. The present study, even though based on a fairly limited number of examined fish, revealed the presence of as many as 27 species (9 adult trematodes and 19 species of metacercariae). Of these species, only one, identifiable at least to generic level (*Pelaezia* sp.), has not been reported from Mexico (see Pérez-Ponce de León et al., 1996; Salgado-Maldonado et al., 1997). It is probable that metacercariae unidentifiable to the species level ("Haplorchoides" type and "?"Heterophyidae) belong to species hitherto not reported from Mexico as well. Nevertheless, much more data, including obtaining adult worms, are necessary for their precise identification.

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**Table 1. Survey of trematodes found in freshwater fish from Quintana Roo, Mexico.**

Species	Site	Host	Locality, prevalence, and intensity of infection
Family Haploporidae Nicoll, 1914 <i>Saccocoelioides sogandaresi</i> Lumsden, 1963	Intestine	<i>P. velifera</i>	Ramonal (1 fish infected of 1 examined; 8 trematodes)
Family Angiodictyidae Looss, 1902 <i>Cichlasotrema ujati</i> Pineda and Andrade, 1989	Intestine	<i>C. synspilum</i>	Bacalar (1/1; 1)
Family Calodistomidae Poche, 1926 <i>Prosthenhystera obesa</i> (Diesing, 1850)	Gallbladder	<i>A. fasciatus</i>	La Unión (1/8; 1)
Family Homalometridae Cable and Hunninen, 1942 <i>Crassicuiis cichlasomae</i> Manter, 1936	Intestine	<i>C. aureum</i> <i>C. meeki</i> <i>C. synspilum</i> <i>C. urophthalmus</i>	Ramonal (1/4; 4) Ramonal (2/7; mean intensity 3 [range, 2–3]), Paiyegua (1/1; 7) Ramonal (1/1; 13), Bacalar (1/1; 29) Bacalar (4/4; 13 [1–22])
Family Macroderoididae McMullen, 1937 <i>Magnivitelinum simplex</i> Kloss, 1966	Intestine	<i>A. felis</i>	Ramonal (1/1; 1)
Family Acanthostomidae Poche, 1926 <i>Pelaezia</i> (?) sp.	Intestine	<i>A. felis</i>	Bacalar (1/1; 12)
Family Cryptogonimidae Ward, 1917 <i>Oligogonotylus manteri</i> Watson, 1976	Intestine	<i>C. octofasciatum</i> <i>C. synspilum</i> <i>C. urophthalmus</i>	Ramonal (1/3; 2) Bacalar (1/1; 6) Bacalar (3/4; 17 [8–31])
Family Derogenidae Lühe, 1910 <i>Genarchella astyanactis</i> (Watson, 1976) <i>Genarchella isabellae</i> (Lamothe-Argumedo, 1977)	Stomach Stomach	<i>A. fasciatus</i> <i>C. aureum</i> <i>C. meeki</i> <i>C. octofasciatum</i> <i>C. urophthalmus</i>	La Unión (6/8; 2 [1–2]) Ramonal (1/4; 4) Ramonal (3/7; 14 [1–32]) Ramonal (2/3; 4 [1–6]) Bacalar (3/4; 5 [2–7])
<b>METACERCARIAE</b>			
Family Echinostomatidae Poche, 1926 <i>Echinochasmus leopoldinae</i> Scholz, Ditrich, and Vargas-Vázquez, 1996 <i>Echinochasmus macrocaudatus</i> Ditrich, Scholz, and Vargas-Vázquez, 1996	Gills Gills	<i>C. synspilum</i> <i>A. fasciatus</i>	Ramonal (1/1; 70) La Unión (1/8; 22)
Family Acanthostomidae Poche, 1926 <i>Pelaezia loossi</i> (Pérez Viguera, 1955)	Muscles, including muscles of gill arches	<i>C. meeki</i> <i>C. octofasciatum</i> <i>C. synspilum</i> <i>C. urophthalmus</i>	Ramonal (1/7; 1) Ramonal (2/3; 1) Bacalar (1/1; 1); Ramonal (1/1; 3) Bacalar (1/4; 1) Ramonal (1/1; 265)
<i>Stunkardiella minima</i> (Stunkard, 1938) <i>Atrophecaecum</i> (?) <i>astorquii</i> (Watson, 1976)	Scales Fins and scales, rarely muscles and eyes	<i>P. velifera</i> <i>Dorosoma</i> sp.	Ramonal (1/1; 265) Paiyegua (3/3; 79 [56–105])

Table 1. Continued.

Species	Site	Host	Locality, prevalence, and intensity of infection
		<i>C. aureum</i>	Ramonal (1/4; 11)
		<i>C. meeki</i>	La Unión (1/1; 1); Ramonal (6/7; 52 [3–27]); Paiyegua (1/1; 316)
		<i>C. octofasciatum</i>	Ramonal (1/3; 73)
		<i>C. synspilum</i>	Ramonal (2/2; 278 [2–756])
Family Heterophyidae Odhner, 1914			
<i>Ascocotyle (Ascocotyle) tenuicollis</i> Price, 1935	Heart	<i>A. fasciatus</i>	La Unión (5/8; 8 [7–10]); Ramonal (1/1; 16); Paiyegua (1/2; 3)
<i>Ascocotyle (Ascocotyle) nunezae</i> Scholz, Vargas-Vázquez, Vidal-Martínez, and Aguirre-Macedo, 1997	Gills	<i>C. urophthalmus</i> <i>C. aureum</i>	Bacalar (1/4; 1) Ramonal (2/4; 34 [1–67])
<i>Ascocotyle (Phagicola) diminuta</i> (Stunkard and Haviland, 1924)	Gills	<i>C. meeki</i> <i>P. velifera</i>	La Unión (1/1; 3); Ramonal (4/7; 23 [2–70]); Paiyegua (1/1; 15) Ramonal (1/1; 482)
<i>Ascocotyle (Phagicola) nana</i> Ransom, 1920	Mesentery, intestinal wall, liver, spleen, muscles	<i>A. fasciatus</i> <i>P. velifera</i> <i>C. aureum</i> <i>C. meeki</i> <i>C. octofasciatum</i>	La Unión (4/8; 13 [1–20]) Ramonal (1/1; 242) Ramonal (1/4; 12) Ramonal (4/7; 4 [1–6]) Ramonal (1/3; 15)
Family Cryptogonimidae Ward, 1917			
<i>Oligogonotylus manteri</i> Watson, 1976	Intestinal wall, gills, fins	<i>C. meeki</i> <i>C. octofasciatum</i> <i>C. synspilum</i> <i>C. urophthalmus</i>	Ramonal (2/7; 13 [2–23]) Ramonal (1/3; 86) Bacalar (1/1; hundreds) Bacalar (4/4; thousands); Paiyegua (1/1; thousands)
Cryptogonimidae gen. sp.	Eye (humor body), fins, gills, muscles of fins	<i>A. fasciatus</i> <i>Dorosoma</i> sp. <i>C. meeki</i>	La Unión (6/8; 8 [1–18]) Ramonal (1/1; 72) Ramonal (1/7; 1)
Family Clinostomidae Lühe, 1901			
<i>Clinostomum complanatum</i> (Rudolphi, 1814)	Muscles	<i>P. velifera</i>	Ramonal (1/1; 1)
Family Diplostomidae Poirier, 1886			
<i>Diplostomum (Austrodiplostomum) compactum</i> (Lutz, 1928)	Eye (humor body)	<i>A. felis</i>	Ramonal (2/2; 12)
<i>Posthodiplostomum minimum</i> (MacCallum, 1921)	Muscles	<i>C. meeki</i> <i>C. meeki</i> <i>C. synspilum</i>	Ramonal (2/7; 11 [1–21]) Ramonal (2/7; 2) Ramonal (1/1; 5)
Family Proterodiplostomidae Dubois, 1936			
<i>Crocodilicola pseudostoma</i> (Wilemoes-Suhm, 1870)	Mesentery and muscles	<i>R. guatemalensis</i>	Paiyegua (1/1; 4)
Proterodiplostomidae gen. sp.	Muscles, rarely gills, swimbladder, or liver	<i>C. aureum</i> <i>A. fasciatus</i> <i>A. felis</i> <i>P. velifera</i> <i>C. aureum</i> <i>C. synspilum</i>	Ramonal (1/4; 1) Paiyegua (2/2; 6 [5–7]) Ramonal (1/2; 4) Ramonal (1/1; 4) Ramonal (1/4; 2) Ramonal (1/1; 5)

Table 1. Continued.

Species	Site	Host	Locality, prevalence, and intensity of infection
Family Strigeidae Railliet, 1919			
<i>Apharyngostrigea</i> sp.	Mesentery	<i>A. fasciatus</i> <i>C. meeki</i>	La Unión (1/8; 1) La Unión (1/1; 1); Ramonal (1/3; 1)
Trematoda gen. sp. 1 ("Haplorchoides" type)	Gills, fins, muscles	<i>C. aureum</i>	Ramonal (1/4; 5)
		<i>C. meeki</i>	Ramonal (4/7; 6 [1-19])
Trematoda gen. sp. 2 (? Heterophyidae)	Gills, fins, exceptionally eyes	<i>A. fasciatus</i>	La Unión (6/8; 47 [2-94])

The specimen identified as *Magnivittellinum simplex* found in *Arius felis* corresponded in its morphology to the species *M. simplex*, a trematode hitherto found only in the characid *Astyanax fasciatus* (Kloss, 1966; Jiménez-Guzmán, 1973; Scholz et al., 1995a). The finding of this trematode in the piscivorous catfish may be accidental because of predation of *A. fasciatus* by this fish host.

Acanthostomide trematodes found in *A. felis* were characterized by the presence of two intestinal caeca, opening posteriorly into the excretory system. On the basis of this feature, they fit well into the diagnosis of the genus *Pelaezia* (Lamothe-Argumedo and Ponciano-Rodríguez, 1986), which currently accommodates two species, *P. unami* (Pelaez and Cruz, 1953) and *P. loossi* (Pérez Viguera, 1955). Both species, however, differ from the present material by the number of circumoral spines (30 and 23, vs. 27).

Metacercariae of two proterodiplostomatid trematodes were found. They correspond to those designated as Proterodiplostomidae gen. sp. 1 and 2 by Scholz et al. (1995b). It is evident that the larvae found by these authors in *Rham-*

*dia guatemalensis* belong to the species *Crocodilicola pseudostoma*. The larvae can be differentiated one from another on the basis of body size and shape and size of the ventral sucker, which is more than 2 times smaller in Proterodiplostomidae gen. sp. (Proterodiplostomidae gen. sp. 2 in Scholz et al., 1995b) (diameter, 66-69 µm) than in *C. pseudostoma* (157-171 µm).

Fish harboring the highest number of trematode species were *Cichlasoma meeki* (12 species, including 10 metacercariae) and *Astyanax fasciatus* (2 adults and 7 metacercariae). In cenote fish, the highest number of trematode species (12) was also found in *C. meeki*; *C. octofasciatum*, *C. synspilum*, *C. urophthalmus*, and *A. fasciatus* harbored 10 trematodes (Scholz et al., 1995a, b).

Regarding the zoogeographical distribution, it is evident that most trematodes that could be identified to the species level belong to the Neotropical fauna. These include species limited to southeastern Mexico (*C. ujati*, *G. isabellae*, *E. leopoldinae*, *E. macrocaudatus*, *S. minima*, *A. nunezae*), to southeastern Mexico and Central America (*C. cichlasomae*, *O. manteri*, *G. astyanactis*, *A. ?astorquii*), southeastern Mexico and South America (*Magnivittellinum simplex*, *Pelaezia loossi*, *D. compactum*), southeastern Mexico and U.S.A. (*S. sogandaresi*), or Gulf of Mexico and South America (*A. tenuicollis*, *A. diminuta*, *A. nana*).

Comparison of the present data with results of a survey of helminth parasites of cenote fish (Scholz et al., 1995a, b) demonstrated that there are only minor differences in composition of trematode fauna; the number of species found is almost identical (Table 2). However, the number of fish examined from Río Hondo and internal lakes of Quintana Roo was rather limited, and this comparison should be considered prelimi-

Table 2. Number of trematode species found in fish from different freshwater bodies of the Yucatan Peninsula.

	Cenotes*	Rivers†	Lakes‡
Adult trematodes	10	8	5
Metacercariae	21	19	7
Total§	29	26	11

\* Scholz et al. (1995a, b).

† Río Hondo, two localities (La Unión, Ramonal); present study.

‡ Laguna Bacalar and Laguna Paiyegua; present study.

§ *Stunkardiella minima* and *Oligogonotylus manteri* were found both as adults and as metacercariae.





cidial exposure, 35 were alive at the end of the 6-wk observation period. The significantly greater survival rate of the control snails suggested that parasitism with larval *E. caproni* adversely affected snail survival. Exposure to *E. caproni* miracidia did not alter the growth of juvenile *B. glabrata* since there was no significant difference in shell diameter of exposed and control snails during the 6-wk observation period. The number of snail egg masses among the unexposed snails was 3.5 to 5.5 times greater than that of exposed snails, suggesting that infection with *E. caproni* adversely affected fecundity in *B. glabrata*.

KEY WORDS: *Echinostoma caproni*, Trematoda, *Biomphalaria glabrata*, Gastropoda, invertebrate-parasite relationships.

Effects of larval trematode infections on their first intermediate host snails have been studied in many host-parasite systems (see Thompson [1985, 1997] and Hurd [1990] for review). However, schistosomae-snail interactions have been studied more extensively because of the medical significance of schistosomes. The effects of larval schistosomes on the dynamics of snail populations, as defined by snail survival, growth, and fecundity, have been investigated. For example, Crews and Yoshino (1989) showed that *Schistosoma mansoni* infection reduced the fecundity of *Biomphalaria glabrata*; Mueleman (1972) demonstrated that infection of sexually mature *B. pfeifferi* with *S. mansoni* miracidia resulted in decreased fecundity and accelerated growth. In contrast, Raymond and Probert (1993) showed a reduction of growth of both immature and mature *Bulinus natalensis* infected with *S. margrebowiei*.

Effect of echinostome infection on survival, growth, and fecundity of snail hosts has received little attention. Christensen et al. (1980) noted that various species of *Biomphalaria* infected with miracidia of an Egyptian strain of *Echinostoma caproni* (referred to as *E. liei* in their study) showed an increased death rate compared with the uninfected control snails. Details of their experiments were not given. Kuris (1980) reported that infection with an Ethiopian strain of *E. caproni* (referred to as *E. liei* in his study) decreased the growth rate and increased the mortality of 1–2-mm juvenile *B. glabrata*, but had no effect on growth and survival of 4–6-mm juvenile snails.

The *E. caproni*-*B. glabrata* model has been relatively unexplored for studies on larval trematode-gastropod relationships. This model is

particularly useful because this echinostome can be cycled easily in the laboratory between *B. glabrata* snails and mice, chicks or hamsters (see Fried and Huffman [1996] for review). The purpose of the present study was to determine the effect of infection with an Egyptian strain of *E. caproni* on survival, growth, and fecundity of juvenile *B. glabrata* ( $4 \pm 0.2$  mm in shell diameter).

In each of 4 experiments, 10 *B. glabrata* snails ( $4 \pm 0.2$  mm in shell diameter) were exposed individually to 5 miracidia of *E. caproni* in 1.5 ml of artificial spring water (ASW; Ulmer, 1970) in a multiwell chamber for 24 hr, and an identical population of snails was treated the same way but not exposed to miracidia. Experimental and control snails were placed in separate 400-ml plastic beakers, 10 per 350 ml of ASW.

Snails were fed boiled leaf lettuce ad libitum supplemented with occasional feeding of TetraMin (Tetra Werka, Melle, Germany), maintained at  $23 \pm 1^\circ\text{C}$ , and the water was changed twice weekly. Snail shell diameters were measured weekly for 6 wk of postexposure (PE). The number of live snails, number of egg masses, and number of eggs per mass were recorded weekly. At 6-wk PE, the exposed snails were necropsied to determine the presence of rediae in the digestive gland-gonad complex (DGG).

Survival and growth data between exposed and control groups were compared using a two-tailed Kruskal-Wallis analysis of variance test (True Epistat™, Epistat Services, Richardson, Texas), with  $P < 0.05$  considered significant.

Of the 40 exposed and 40 control snails used, 24 and 35 snails, respectively, were alive at 6-wk PE. Of the 24 exposed snails examined at 6-wk PE, 16 were infected with *E. caproni* rediae. Mean weekly survival of exposed and control snails is shown in Figure 1. The survival of snails exposed to *E. caproni* miracidia decreased significantly during the 6-wk PE ( $P = 0.003$ ).

Increased snail mortality probably resulted from extensive damage to the DGG of infected snails (see Fig. 16 in Fried and Huffman [1996]). Such damage resulted in extensive disruption of the architecture of the DGG. The effects of redial infection with *E. caproni* on organs other than the DGG have not been studied.

Mean shell diameters of exposed and control snails are shown in Figure 2. There was no significant difference in the shell diameter of con-

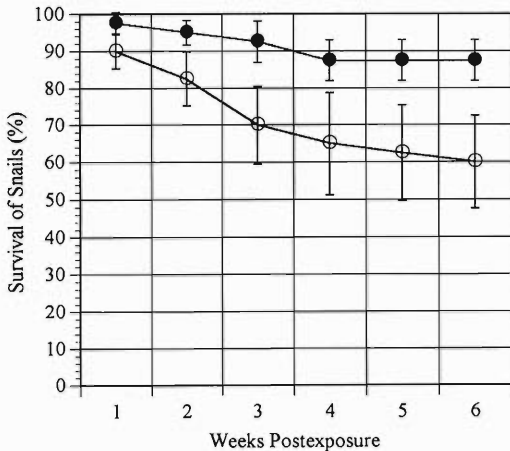


Figure 1. Percent survival of juvenile *Biomphalaria glabrata* with and without exposure to *Echinostoma caproni* miracidia (open circles = exposed snails; closed circles = unexposed snails).

control snails versus those exposed to *E. caproni* during the 6-wk PE ( $P = 0.851$ ).

Egg masses were first seen in 2 control groups and 1 exposed group at 5-wk PE. All control groups had egg masses at 6-wk PE, while only 2 of 4 of the experimental groups had egg masses at this time. Egg masses from infected snails were often misshapen and contained fewer embryos per egg mass than those from uninfected snails. The mean  $\pm$  SEM number of egg masses per snail in the control groups at weeks 5 and 6 PE was  $0.4 \pm 0.2$  and  $0.3 \pm 0.1$ , respectively. The mean  $\pm$  SEM number of egg masses per snail in the exposed groups at weeks 5 and 6 PE was  $0.1 \pm 0.1$ . The mean  $\pm$  SEM number of eggs per snail in the control groups at weeks 5 and 6 PE was  $3.3 \pm 1.9$  and  $3.2 \pm 1.0$ , respectively. The mean  $\pm$  SEM number of eggs per snail in the exposed groups at weeks 5 and 6 PE was  $0.6 \pm 0.6$  and  $0.9 \pm 0.5$ , respectively. The mean number of eggs in control groups was 5.5 times greater than in experimental groups at 5-wk PE and 3.5 times greater at 6-wk PE. The number of eggs in the experimental groups was significantly less ( $P < 0.05$ ) than that of the control groups at 5 and 6 weeks PE.

Survival of the exposed snails was significantly reduced compared to that of the unexposed snails ( $P = 0.003$ ), suggesting that larval parasitism by the Egyptian strain of *Echinostoma caproni* adversely affected the survival of juvenile *Biomphalaria glabrata*. Our finding

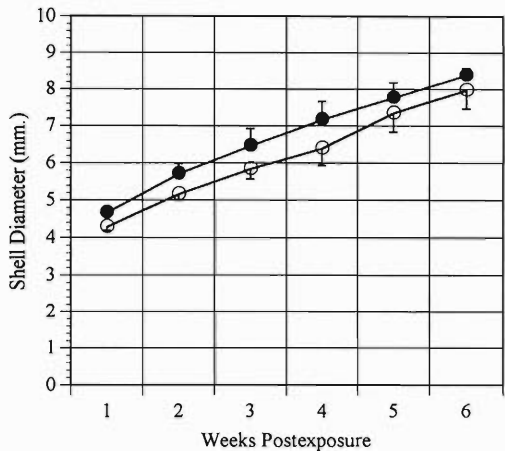


Figure 2. Mean shell diameter of juvenile *Biomphalaria glabrata* with and without exposure to *Echinostoma caproni* miracidia (open circles = exposed snails; closed circles = unexposed snails).

supports the previous observation by Christensen et al. (1980) on increased mortality of *Biomphalaria* snails infected with an Egyptian strain of *E. caproni*. However, our finding differs from that of Kuris (1980), in which exposure to an Ethiopian strain of *E. caproni* had no effect on the survival of 4–6-mm *B. glabrata*. Factors other than parasite strain differences may account in part for discrepancies seen in the 2 studies. Thus, whereas we infected individual snails each with 5 miracidia, Kuris exposed 25 snails en masse to 125 miracidia; temperature of snail maintenance was not given in that study, whereas we maintained our snails at  $23 \pm 1^\circ\text{C}$ .

Our results suggest that larval parasitism of *E. caproni* in *B. glabrata* did not alter snail growth, at least based on maximal shell diameters of the snails. Moreover, there is no evidence for gigantism in this model as reported for various snail–larval trematode systems (see Thompson [1985, 1997] and Hurd [1990] for review). Kuris (1980) noted a decline in growth of 1–2-mm *B. glabrata* infected with the Ethiopian strain of *E. caproni*, but no adverse effect of parasitism on the growth of snails exposed at 4–6 mm was recorded.

Fecundity of snails was not studied by Kuris (1980), but results of our studies show that the number of eggs laid by control snails was 3.5 to 5.5 times greater than that laid by the exposed snails at 5- to 6-wk PE. In accord with numerous studies on larval trematode infections in snails,

juvenile *B. glabrata* infected with *E. caproni* larvae showed reduced fecundity.

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### Research Note

## Helminth Parasites of the Spotted Salamander *Ambystoma maculatum* and Red-backed Salamander *Plethodon c. cinereus* from Northwestern Wisconsin

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**ABSTRACT:** Twenty spotted salamanders *Ambystoma maculatum* and 20 red-backed salamanders *Plethodon c. cinereus* were collected from NW Wisconsin in May 1996 and examined for helminth parasites. Two species of helminths infected the spotted salamanders, while 3 species infected the red-backed salamanders. The nematode *Batracholandro magnavulvaris* had the highest prevalence in spotted salamanders (45%), while the nematode *Rhabdias* sp. had the highest prevalence in red-backed salamanders (30%). The trematode *Brachycoelium salamandrae* had the highest mean intensity in both hosts, 3.3 in *A. maculatum* and 2.0 in *P. c. cinereus*. This is the first report of *B. magnavulvaris* from *Ambystoma maculatum* as well as the first report of it from Wisconsin.

**KEY WORDS:** *Ambystoma maculatum*, *Plethodon c. cinereus*, *Brachycoelium salamandrae*, *Batracholandro magnavulvaris*, *Rhabdias* sp., Wisconsin.

The spotted salamander *Ambystoma maculatum* Shaw, 1802, is a large, robust species of mole salamander reported from south-central Ontario to Nova Scotia, south to Georgia and eastern Texas (Vogt, 1981). The red-backed salamander *Plethodon c. cinereus* Green, 1818, is one of the smallest woodland species of lungless salamanders that occurs throughout the north-eastern United States and southeastern Canada, with populations in Ontario, Minnesota, Missouri, Arkansas, Oklahoma, Louisiana, and Georgia (Vogt, 1981). Both species inhabit mesic forests throughout northern Wisconsin. Although parasites of the spotted salamander, *Ambystoma maculatum*, and the red-backed salamander, *Plethodon c. cinereus*, have been studied by several

**Table 1.** Prevalence, abundance, and mean intensity of helminths of *Ambystoma maculatum* and *Plethodon c. cinereus*.

	<i>Ambystoma maculatum</i>			<i>Plethodon c. cinereus</i>			Location
	Prevalence*	Abundance ±1 SD	Mean intensity ±1 SD (range)	Prevalence*	Abundance ±1 SD	Mean intensity ±1 SD (range)	
<i>Brachycoelium salamandrae</i>	3 (15)	0.5 ± 1.3	3.3 ± 1.5 (2–5)	2 (10)	0.2 ± 0.6	2 (2)	Small and large intestine
<i>Batracholandros magnavulvaris</i>	9 (45)	0.8 ± 1.1	1.7 ± 1.1 (1–4)	1 (5)	0.05 ± 0.2	1 (1)	Small and large intestine
<i>Rhabdias</i> sp.	—	—	—	6 (30)	0.4 ± 0.8	1.3 ± 0.8 (1–3)	Body cavity

\* Number infected (percent infected).

authors (Chitwood, 1933; Rankin, 1937a, b, 1938, 1945; Rankin and Hughes, 1937; Fischthal, 1955a, b; Cheng, 1958, 1960; Cheng and Chase, 1960; Ernst, 1974; Rosen and Manis, 1976; Dunbar and Moore, 1979; Burse and Schibli, 1995), few studies are known from the Great Lakes area (Meserve, 1943; Coggins and Sajdak, 1982; Muzzall, 1990). Here we present new information on the parasites of Wisconsin salamanders.

Twenty adult red-backed salamanders (15 males and 5 females) were collected by overturning rocks and logs during the day, and 20 breeding spotted salamanders (15 males and 5 females) were collected by dip-net at an ephemeral pond in Bayfield County, Wisconsin, in May 1996. Animals were killed in MS 222 (ethyl m-aminobenzoate methane sulfonic acid) within 48 hours of capture. Snout-vent length and wet weight were recorded for each individual. The mean Snout-vent length ±1 SD (range) of spotted salamanders was 76 mm ± 8.8 (63–93). The mean Snout-vent length of red-backed salamanders was 44 mm ± 7 (30–52). At necropsy, the digestive tract, limb and body wall musculature, and internal organs were examined for helminths. Trematodes were preserved in 10% neutral buffered formalin, stained with aceto-carmine, dehydrated through ethanol, and mounted in Canada balsam. Nematodes were preserved in 10% neutral buffered formalin, dehydrated to 70% ethanol, cleared in glycerol, and identified as temporary mounts. Prevalence is the percentage of infected salamanders in a sample, mean intensity is the mean number of worms per infected salamander, and abundance is the mean number of individuals of a particular parasite species per host examined. Voucher specimens have been deposited in the Harold W. Manter

Laboratory, University of Nebraska State Museum, Lincoln (accession numbers HWML 39248—*Brachycoelium salamandrae* from red-backed salamander; 39249—*Brachycoelium salamandrae* from spotted salamander; 39250—*Rhabdias* sp.; 39251—*Batracholandros magnavulvaris*).

Eleven of 20 (55%) spotted salamanders were infected with helminths. Mean helminth abundance in spotted salamanders was  $1.25 \pm 1.60$ . Nine (45%) were infected with *Batracholandros magnavulvaris* Rankin, 1937, while 3 (15%) were infected with *Brachycoelium salamandrae* Frolich, 1789. Only one spotted salamander (5%) was concurrently infected with *B. magnavulvaris* and *B. salamandrae*. Eight of 20 (40%) red-backed salamanders were infected with 1 or more *B. magnavulvaris*, *B. salamandrae*, and *Rhabdias* sp.. Mean helminth abundance in red-backed salamanders was  $0.65 \pm 0.99$ . Only one red-backed salamander (5%) was concurrently infected with *B. magnavulvaris* and *B. salamandrae*. *Batracholandros magnavulvaris* had the highest prevalence in spotted salamanders, *Rhabdias* sp. had the highest prevalence in red-backed salamanders, and *B. salamandrae* had the highest mean intensity in both hosts (Table 1). There was no statistically significant correlation between helminth abundance and either weight or length for spotted salamanders ( $r = -0.228$ ,  $r = -0.111$ ) or red-backed salamanders ( $r = 0.027$ ,  $r = -0.096$ ). Because of the skewed sex ratio toward males, neither prevalence nor mean intensity was compared between the sexes in either host species.

*Batracholandros magnavulvaris* exhibits little host specificity, infecting plethodontids (Rankin, 1937b; Schad, 1960, 1963; Dyer et al., 1980; Goater et al., 1987; Muzzall, 1990; Joy et al.,

1993; McAllister et al., 1995; Bursey and Schibli, 1995) as well as salamandrids (Rankin, 1937b; Baker, 1987) and ambystomids (Muzzall and Schindlerle, 1992). In this study, 16 gravid female *B. magnavulvaris* were recovered from both salamander species, 15 from spotted salamanders and 1 from red-backed salamanders. All female nematode populations in salamanders has been reported by other investigators (Rankin, 1937b; Walton, 1940; Fischthal, 1955a; Joy et al., 1993). The spotted salamander is a new host record for *B. magnavulvaris*, and Wisconsin is a new locality record for this nematode in red-backed salamanders. Although both species of salamanders in this study are considered terrestrial, only the spotted salamander returns to water during its reproductive season. The red-backed salamander is totally terrestrial, with reproduction and development occurring on land (Vogt, 1981). Our prevalence data of *B. magnavulvaris* in spotted salamanders (45%) and red-backed salamanders (5%) agree with reports by Dunbar and Moore (1979) and Joy et al. (1993) that more aquatic salamanders are more likely to be infected with this nematode than are terrestrial species of salamanders. Although the differences observed in the present report may be due to chance, because of the small number of salamanders examined, Muzzall (1990) found 48 (28%) of 171 red-backed salamanders infected with this nematode, while Ernst (1974) found a 50% prevalence (6/12) in Virginia red-backed salamanders.

The 4 specimens of *Brachycoelium salamandrae* from red-backed salamanders and 10 specimens from spotted salamanders exhibited morphological variation. A statistically significant difference existed between the mean length, in millimeters  $\pm$  1 SD (range), of trematodes recovered from spotted salamanders, 2.82 mm  $\pm$  0.30 (2.28–3.30), and red-backed salamanders, 1.26 mm  $\pm$  0.47 (0.85–1.93) (one-tailed *t*-test,  $P < 0.05$ ), while no such difference existed in mean width of trematodes from spotted salamanders, 0.40 mm  $\pm$  0.07 (0.28–0.50), and red-backed salamanders, 0.34 mm  $\pm$  0.12 (0.25–0.52) (one-tailed *t*-test,  $P > 0.05$ ). Other apparent differences existed in body shape, position of testes, and distribution of vitellaria. *Brachycoelium* spp. are common parasites of salamanders (Dyer et al., 1980; Goater et al., 1987; Muzzall, 1990; McAllister et al., 1995; Bursey and Schibli, 1995), but controversy surrounds the as-

signment of species to this genus. Rankin (1938) reduced all known species to *B. salamandrae*. He concluded that heavy infections produce many small flukes, whereas light infections were usually made up of larger specimens. Other investigators (Parker, 1941; Cheng, 1958, 1960; Cheng and Chase, 1960; Couch, 1966) disagreed and described 13 species based on such morphological characteristics as body length and shape, length of esophagus, position of testes, and distribution of vitellaria. Although the trematodes we collected from spotted salamanders and red-backed salamanders exhibited morphological variation, we have adopted the conservative approach suggested by McAllister et al. (1995) to report *B. salamandrae* from North American salamanders.

*Rhabdias* sp. was the most frequently found parasite in red-backed salamanders. Six red-backed salamanders (30%) were found to be infected, with a total of 8 specimens recovered from the body cavity. *Rhabdias* spp. have been reported previously from a wide variety of salamander species (Chitwood, 1933; Walton, 1938, 1940; Lehmann, 1954; Landewe, 1963; Dyer and Peck, 1975; Price and St. John, 1980; Coggins and Sajdak, 1982; Muzzall and Schindlerle, 1992). However, the finding of this nematode in the red-backed salamander was unexpected, since it is a lungless salamander and should not be expected to harbor lung parasites. The study of Baker (1979) on *Rhabdias* revealed that subadult nematodes must invade the lungs if they are to mature and produce eggs, but numerous subadults can be found in the body cavity. All nematodes recovered in the present study were nongravid subadults from the body cavity and are probably accidental infections in this host.

Results of the current survey support previous work on salamander helminths, indicating that they are not strongly host-specific; rather, their distribution can be correlated with habitat preference and diet of the host (Fischthal, 1955a, b; Dunbar and Moore, 1979; Coggins and Sajdak, 1982; Goater et al., 1987). Recently, Kleeberger and Werner (1982, 1983) showed that in a northern hardwood forest, similar to the present study area, spotted salamanders spent 72% of their time underground, 21% under decaying logs, and 7% under wet leaf litter, while red-backed salamanders in the same area spent 61% of their time above the soil, primarily in the litter layers

and decaying logs. Thus, the differences in parasite prevalence and species found in the two hosts may be due to differences in host habitat utilization. Parasites may encounter only a limited number of potential hosts under natural conditions, giving the appearance of a much narrower host specificity (Kennedy, 1975). To our knowledge, the spotted salamander is a new host record for *B. magnavulvaris*, and Wisconsin is a new locality record for this nematode in either of the salamander species.

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### Research Note

## Nematodes of the Great Plains Narrow-mouthed Toad, *Gastrophryne olivacea* (Microhylidae), from Southern Arizona

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**ABSTRACT:** Thirty *Gastrophryne olivacea* from southern Arizona were examined for helminths. Two species of nematodes, *Aplectana incerta* and *Aplectana itzocanensis*, were found. Both represent new host records. *Aplectana itzocanensis* had the higher prevalence (70%) and greater mean intensity (13.0). In southern Arizona, *A. incerta* and *A. itzocanensis* also occur concurrently in other anurans.

**KEY WORDS:** *Gastrophryne olivacea*, Microhylidae, Nematodes, *Aplectana incerta*, *Aplectana itzocanensis*, Arizona.

The Great Plains narrowmouth toad, *Gastrophryne olivacea* (Hallowell, 1856), occurs from eastern Nebraska and western Missouri, through Oklahoma and Texas, west through northern México, into south central Arizona, from sea

level to 1250 m elevations (Stebbins, 1985). There are several reports of helminths in *G. olivacea*: Kansas (Freiburg, 1951), Oklahoma (Kuntz, 1941), and Texas (Harwood, 1932; McAllister and Upton, 1987). The purpose of this note is to report nematodes from a population of *G. olivacea* from southern Arizona.

Thirty *G. olivacea* (8 females, 22 males, mean snout-vent length = 27 mm ± 2.3 SD; range, 23–32 mm) were borrowed from the herpetology collection of the University of Arizona, Tucson (UAZ) 15824, 15826, 15827, 15830, 15833, 20561, 20563, 25858, 25861, 25863, 29026, 29027, 29032, 29035, 29036, 29039–29041, 29043, 29046–29049, 29051, 29052, 29055–29058, 32016. These specimens had been col-



Table 1. Known hosts of *Aplectana incerta* and *A. itzocanensis*.

Nematode host	Prevalence	Locality	Reference
<i>Aplectana incerta</i>			
<i>Bufo debilis</i>	69% (34/49)	New Mexico	Goldberg et al., 1995
<i>Bufo marinus</i>	Not given	Mexico	Caballero y C., 1949
<i>Bufo microscaphus</i>	1% (1/77)	Arizona	Goldberg et al., 1996a
<i>Bufo retiformis</i>	61% (30/49)	Arizona	Goldberg et al., 1996b
<i>Bufo woodhousii</i>	41% (25/61)	Arizona	Goldberg et al., 1996a
<i>Gastrophryne olivacea</i>	70% (21/30)	Arizona	(This paper)
<i>Scaphiopus couchii</i>	82% (62/76)	Arizona	Goldberg and Bursey, 1991a
<i>Spea multiplicata</i>	16% (5/31)	New Mexico	Goldberg et al., 1995
<i>Aplectana itzocanensis</i>			
<i>Bufo alvarius</i>	52% (49/95)	Arizona	Goldberg and Bursey, 1991a
<i>Bufo cognatus</i>	5% (1/21)	Arizona	Goldberg and Bursey, 1991a
	50% (18/36)	New Mexico	Goldberg et al., 1995
<i>Bufo debilis</i>	63% (31/49)	New Mexico	Goldberg et al., 1995
<i>Bufo marinus</i>	Not given	Costa Rica	Brenes and Bravo Hollis, 1959
	Not given	Mexico	Bravo Hollis, 1943
	Not given	Mexico	Caballero Deloya, 1974
<i>Bufo microscaphus</i>	19% (15/77)	Arizona	Goldberg et al., 1996a
<i>Bufo punctatus</i>	29% (6/21)	Arizona	Goldberg and Bursey, 1991b
<i>Bufo retiformis</i>	57% (28/49)	Arizona	Goldberg et al., 1996b
<i>Bufo woodhousii</i>	26% (16/61)	Arizona	Goldberg et al., 1996a
	Not given	California	Baker, 1985
<i>Gastrophryne olivacea</i>	40% (12/30)	Arizona	(This paper)
<i>Scaphiopus couchii</i>	<5%	Arizona	Tinsley, 1990
<i>Spea multiplicata</i>	Not given	Mexico	Bravo Hollis, 1943
	39% (12/31)	New Mexico	Goldberg et al., 1995

lected in oak-woodland habitat of the Pajarito Mountains (31°22'N, 111°04'W; elevation 914–1219 m), Santa Cruz County, Arizona, in 1961–1969, fixed in 10% formalin, and preserved in 70% isopropanol. The body cavity was opened, and the lungs, esophagus, stomach, small intestine, large intestine, bladder, and body cavity of each specimen examined.

The only helminths found were 2 species of nematodes: *Aplectana incerta* Caballero, 1949, and *Aplectana itzocanensis* Bravo Hollis, 1943. Each nematode was placed in a drop of glycerol on a glass slide; identifications were made from these temporary mounts. Representative samples were placed in vials of alcohol and deposited in the United States National Parasite Collection Beltsville, Maryland, accession numbers *Aplectana incerta* 87087 and *Aplectana itzocanensis* 87088.

Prevalence for *A. incerta* was 70%; mean intensity =  $13 \pm 12$  SD; range, 2–38; prevalence for *A. itzocanensis* was 40%; mean intensity =  $7 \pm 8$  SD; range, 1–28; infection sites were the small and large intestines. Eight *G. olivacea* had concurrent infections of both *A. incerta* and *A. itzocanensis*; 13 were infected with *A. incerta*

only, 4 were infected with *A. itzocanensis* only, 5 were not infected. There was no significant difference between male and female toads for either *A. incerta* or *A. itzocanensis* ( $\chi^2 = 0.5$ , 0.01, respectively, 1 df,  $P > 0.05$ ).

Both *A. incerta* and *A. itzocanensis* have been found in other anurans (Table 1), but occurrences are limited to toads. *Aplectana incerta* and *A. itzocanensis* closely resemble one another (*A. incerta* having shorter spicules and larger eggs than *A. itzocanensis*). Baker (1985) has suggested that the reports of *A. itzocanensis* in *Bufo marinus* of Costa Rica and México are referable to *A. incerta*, which was described from southern México. Baker (1985) has also suggested synonymy of *Aplectana hoffmanni*, a species originally reported in *Bufo marinus* collected in Puebla, México, by Bravo Hollis (1943) with *A. itzocanensis*. This synonymy is reflected in Table 1.

Both *A. incerta* and/or *A. itzocanensis* have been found in other desert-dwelling anurans from southern Arizona and New Mexico (Table 1). Thus, host specificity for both of these helminths is low. The current distribution of *A. incerta* and *A. itzocanensis* (Table 1) suggests that

these may be middle-American species that reach their northern limits in the deserts of the southwestern United States.

The helminth fauna of *Gastrophryne olivacea* east of the continental divide is completely different from that reported in this study. In Texas, McAllister and Upton (1987) found specimens of the cestode, *Cylindrotaenia americana*, and the nematode, *Cosmocercoides dukae*; Harwood (1932) had previously reported *C. dukae*. In Kansas, Freiburg (1951) found but did not identify nematodes. In Oklahoma, Kuntz (1941) reported 1 species of cestode and 2 species of nematodes in *G. olivacea* but did not identify them. More work will be required to determine whether the Continental Divide is the eastern boundary of the range of *A. incerta* and *A. itzocanensis*.

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## Research Note

# Helminths of the Prairie Rattlesnake, *Crotalus viridis viridis* (Serpentes: Viperidae), from Western South Dakota

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**ABSTRACT:** One hundred prairie rattlesnakes, *Crotalus viridis viridis*, from western South Dakota were examined for evidence of helminth parasites. Twenty-one percent were infected with 1 of 6 parasite species. The following species were recovered: *Manodistomum* sp., *Mesocestoides* sp., *Physaloptera* sp., 2 different species of oligacanthorhynchid acanthocephala, and an unidentified species of acanthocephalan. The occurrences of oligacanthorhynchid acanthocephala and *Manodistomum* sp. in *C. v. viridis* represent new host and locality records. A natural infection of *Mesocestoides* sp. tetrathyridia is documented in this host for the first time.

**KEY WORDS:** *Crotalus viridis viridis*, *Manodistomum* sp., Acanthocephala, *Mesocestoides* sp., *Physaloptera* sp., South Dakota, survey, Prairie rattlesnake.

Only a few helminth parasites have been reported from the prairie rattlesnake, *Crotalus viridis viridis* (Rafinesque, 1818) (Serpentes: Viperidae). Surveys from Colorado (Widmer, 1967; Olsen, 1980) and New Mexico (Pfaffenberger et al., 1989) indicate that this snake species harbors limited helminth fauna. In an effort to compare helminth populations from this host in western South Dakota with other areas, we examined 100 adult prairie rattlesnakes for helminths. In October 1995, 16 (8 male, 8 female) and 20 (4 male, 16 female) *C. v. viridis* were collected in Jones County (43°95'N, 100°68'W) and Stanley County (44°40'N, 100°74'W), South Dakota, respectively. In October 1994, 64 (32 male, 32 female) specimens were collected from various counties within western South Dakota. These were collected for their skins prior to consideration of this survey, so precise locality data were not documented. Snake carcasses and viscera were frozen at -20°C until examination of host tissues was performed. Visceral lengths ranged from 62 cm–91 cm (mean, 78.5 cm). The following tissues were examined for evidence of helminth infection: muscle fascia, coelomic cavity, mesentery, distal

esophagus, stomach, small and large intestines, distal trachea, reticular and membranous lungs, pericardial sac, heart, and gallbladder. The serosal surface of the gastrointestinal tract, liver, and kidneys were examined for evidence of encysted parasites.

Helminth parasites were collected between January and June 1996 and preserved initially in either 10% buffered formalin or AFA fixative. Trematode, cestode tetrathyridia, and acanthocephalan cystacanths were stained in borax-carmin, dehydrated in ethyl alcohol, cleared in xylene, and mounted in Canada balsam. Nematode specimens were either cleared and studied as temporary mounts in glycerin or stained with methyl green and mounted in Hoyer's mounting media, as described by Grundmann (1955).

Twenty one percent of the 100 prairie rattlesnakes examined from western South Dakota were infected with 1 of 6 helminth species. Infection rates of host specimens collected in 1995 are as follows: 0 of 8 (0%) males and 0 of 8 (0%) females from Jones County, South Dakota, and 1 of 4 (25%) males and 4 of 16 (25%) females from Stanley County, South Dakota, harbored helminth parasites. Of the 64 snakes collected from unspecified counties in 1994, 7 of 32 (22%) males and 9 of 32 (28%) females were infected.

The most prevalent helminth occurring in the prairie rattlesnake in this survey is *Physaloptera* sp. (Nematoda: Physalopteridae), with an intensity of 1 to 7 (1.7 mean intensity) specimens infecting a total of 11% of the hosts. Specimens were all nongravid females, found primarily between the rugal folds of the stomach mucosa, with one specimen being removed from the lumen of the distal esophagus, proximal small bowel, and distal large bowel of individual hosts. Species determination could not be made because of the absence of male specimens. Previous reports of *Physaloptera* sp. in *C. v. viridis*

have been recorded in New Mexico and Colorado by Pfaffenberger et al. (1989) and Widmer (1967), respectively.

*Mesocestoides* sp. tetrathyridia (Cestoidea: Cyclophyllidae) were harbored in 6% of the snakes examined, with an intensity of 1 to 56 (23.3 mean intensity) tetrathyridia per host. These were all encysted within the host mesentery and showed no evidence of asexual proliferation. *Crotalus viridis viridis* has been used numerous times as an experimental host for the metacestode stage of this parasite (Widmer et al., 1995; Engen and Widmer, 1993; Widmer and Specht, 1991; and Hanson and Widmer, 1985). This represents the first reported case of natural infection of *Mesocestoides* sp. tetrathyridia in *C. v. viridis*.

One snake possessed a single gravid *Manodistomum* sp. (Digenea: Plagiorchiidae) in the lumen of the distal esophagus, which represents a new host and locality record. Species determination was not possible because the distended ova-filled uterus obscured some taxonomic structures necessary for positive species assignment. However, morphological size, shape, and all discernible structures are consistent with *M. naticis* (Holl and Allison, 1935).

Two *C. v. viridis* harbored unidentified oligacanthorhynchid cystacanths (Acanthocephala: Oligacanthorhynchidae), each of which were hosts to different Acanthocephala.

One species consisted of 7 cystacanths whose inverted forms measured 3.7–4.1 mm (3.8 mm) long by 1.2–1.3 mm (1.25 mm) wide, and everted specimens 4.8–5.0 mm (4.9 mm) long by 1.2–1.3 mm (1.25 mm) wide. Proboscis armature was consistent with the Oligacanthorhynchidae. On the basis of proboscis shape, size, and juvenile trunk shape, these specimens appeared most similar to the genus *Oncicola*. Because they were collected from this northern geographical locality, they likely represent *O. canis* (Kaupp, 1909) Hall and Wigdor, 1918, which possess proboscis and hook measurements consistent with the current specimens. However, because of similarities between genera of juvenile forms of the Oligacanthorhynchidae, intraspecific morphological variation, and the inability to transfer specimens to an experimental host, these specimens are best documented as unidentified oligacanthorhynchid cystacanths.

The second species of encapsulated oligacanthorhynchid consisted of 2 specimens from the

mesentery of 1 host. Both specimens possess trunks in the inverted form, which are folded posteriorly at the midline within their cysts. Inverted form measured 5.4 mm long by 1.2 mm wide; with the everted specimen 5.9 mm long by 0.7 mm wide (trunk distorted during mounting process). The proboscis and hooks were larger and more robust than those possessed by the specimens discussed previously. These characteristics and trunk shape appeared most similar to some members of the genus *Oligacanthorhynchus*, but for the reasons given above, specimens must remain unclassified within the family Oligacanthorhynchidae.

A third single cystacanth was recovered from the mesentery of a separate host that remained inverted and, though probably an oligacanthorhynchid, was unidentifiable.

The only occurrences of oligacanthorhynchid cystacanths in the Serpentes suborder from the western United States are reported in a colubrid and viperid snakes from Texas and Arizona (Bolette, 1997a; 1997b). This report of oligacanthorhynchid cystacanths in *C. v. viridis* documents new host and locality records.

It appears from the observations of Meggitt (1934), Baker (1987), Pfaffenberger et al. (1989), and Widmer (1967) that the prevalence of helminth infections among wild populations of *C. v. viridis* is relatively high. However, the percent of this species harboring helminths in western South Dakota (21%) is substantially lower than *C. v. viridis* reported in New Mexico (66.6%) by Pfaffenberger et al. (1989) and in Colorado (72%) by Widmer (1967). Moreover, the helminth fauna recovered from *C. v. viridis* between these geographical areas appears different. Host populations studied previously by Pfaffenberger et al. (1989) and Widmer (1967) are infected with *Physaloptera* sp., as are hosts of the current study. However, these authors recovered adult specimens of *Oochoristica osheroffi* and *Kalicephalus inermis* from the intestines and a *Rhabdias* sp. from the lungs of this host species from New Mexico and Colorado. These parasite species were not recovered in this survey. Bowman (1984) reported studying a *Hexametra* sp. (probably *H. leidy*), (United States National Parasite Collection No. 28248) recovered previously from a captive *C. viridis* at the San Diego Zoo. However, this may represent an accidental infection of a captive host, since

this species of *Hexametra* is reported in *Crotalus horridus atricaudatus* from Louisiana.

Representative parasitic voucher specimens were deposited in the United States National Parasite Collection, Beltsville, Maryland: *Manodistomum* sp. (USNPC No. 86975), *Mesocestoides* sp. tetrathyridia (USNPC Nos. 86979, 86980), *Physaloptera* sp. (USNPC Nos. 86981-86983), Oligacanthorhynchid cystacanth (USNPC Nos. 86977, 86978), and unidentified cystacanth (USNPC No. 86976).

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**Research Note**

**Helminths of Four Lizards from Nayarit, México: *Anolis nebulosus* (Polychrotidae), *Ctenosaura pectinata* (Iguanidae), *Phyllodactylus lanei* (Gekkonidae), and *Sceloporus nelsoni* (Phrynosomatidae)**

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**ABSTRACT:** Four species of lizards, *Anolis nebulosus*, *Ctenosaura pectinata*, *Phyllodactylus lanei*, and *Sceloporus nelsoni*, were examined for gastrointestinal helminths. Twelve species of helminths were recovered. The most diverse helminth fauna was found in *Ctenosaura pectinata*, which had the largest body sizes and the most diverse diet. The greatest prevalence was for *Alaeuris mexicana* in *C. pectinata* (93%); the highest mean intensity was for *Atractis scelopori* in *C. pectinata* (2905).

**KEY WORDS:** *Anolis nebulosus* (Polychrotidae), *Ctenosaura pectinata* (Iguanidae), *Phyllodactylus lanei* (Gekkonidae), *Sceloporus nelsoni* (Phrynosomatidae), *Mesocestoides* sp., *Oochoristica* spp., *Centrorhynchus* sp., *Alaeuris mexicana*, *Atractis scelopori*, *Ozolaimus ctenosauri*, *Parapharyngodon alvarengai*, *Skrjabinodon scelopori*, *Strongyluris* sp., *Thubunaea ctenosauri*, prevalence, intensity.

México has one of the most diverse herpetofaunas in the world (Flores-Villela, 1993), yet there is little information on the helminths of these lizards (Bravo-Hollis, 1942; Caballero y Caballero, 1937; 1938a, b, c, 1939a, b; Goldberg et al., 1996). During August to November 1993 we were able to examine samples of 4 sympatric lizard species from the Pacific Coast of Nayarit, México, for helminths: the polychrotid, *Anolis nebulosus* (Wiegmann, 1834); the iguanid, *Ctenosaura pectinata* (Wiegmann, 1834); the gekkonid, *Phyllodactylus lanei* Smith, 1935; and the phrynosomatid, *Sceloporus nelsoni* Cochran, 1923, as part of an ongoing examination of reptilian helminth biogeography of México. All lizard species are endemic to México. *Anolis nebulosus* occurs from eastern Sonora southwards to northern Sinaloa, along the Pacific coast to southern Guerrero, and inland to Jalisco and Michoacán; *Ctenosaura pectinata* is a tropical species in México found in Durango, south to Chiapas, and along the Pacific coast; *Phyllodactylus lanei* occurs along the Pacific coast from Nayarit

to Guerrero (García and Ceballos, 1994). *Sceloporus nelsoni* occurs in Sonora, Chihuahua, and southward in Nayarit, Durango, Sinaloa, and Jalisco (Flores-Villela and Gerez, 1994). To our knowledge, there are no reports of the helminth fauna of reptiles from Nayarit, nor is there information on helminths from *Anolis nebulosus*, *Phyllodactylus lanei*, or *Sceloporus nelsoni*. However, there is a report of helminths in *C. pectinata* by Prado Vera (1971). The purpose of this note is to report on the helminths of 4 sympatric lizard species from the Mexican state of Nayarit. Description of new species and taxonomic details are presented elsewhere (Moravec et al. 1996, 1997).

A total of 139 lizards were examined: 44 *Anolis nebulosus*, 29 *Ctenosaura pectinata*, 56 *Phyllodactylus lanei* and 10 *Sceloporus nelsoni*, collected by hand from islands formed during the inundation of 13,000 ha of the Aguamilpa Dam Hydroelectrical Project near Tepic (21°5'32"N, 104°46'20"W), ca. 60 km E of the Pacific coast, Nayarit, México. The habitat consisted mainly of tropical deciduous or semideciduous forest, as well as xerophilous scrub. The *A. nebulosus* sample consisted of 5 females and 39 males and averaged 35.22 mm snout-vent length (SVL)  $\pm$  6.99 SD, range, 27–50; *C. pectinata*, 5 females and 24 males, mean SVL 281.58 mm  $\pm$  83.85 SD, range, 130–850; *P. lanei*, 38 females, 18 males, mean SVL 54.17 mm  $\pm$  12.21 SD, range, 31–70; *S. nelsoni*, 2 females, 8 males, mean SVL 108.22 mm  $\pm$  23.32 SD, range, 75–180. Lizards were deposited in the Herpetological Collection of Universidad Nacional Autónoma de México, Departamento de Zoología, Instituto de Biología (UNAM). All lizards were killed by freezing. The body cavity was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was excised by cutting across the anterior esophagus and the rectum.

**Table 1. Helminths of 4 species of reptiles of Aguamilpa, Nayarit, México.**

Host and helminth	Site	Prevalence	Mean intensity	Range	Abundance $\pm$ SD
<i>Phyllodactylus lanei</i> (n = 56)*					
<i>Oochoristica</i> sp. 1	Small intestine	10.71	1.66	1-3	0.17 $\pm$ 0.57
<i>Centrorhynchus</i> sp.†	Body cavity	1.78	1.0	1-1	0.017 $\pm$ 0.13
<i>Parapharyngodon alvarengai</i> (Freitas, 1957)	Large intestine	28.57	2.87	1-10	0.82 $\pm$ 1.77
<i>Skrjabinodon scelopori</i> (Caballero y Caballero, 1938)	Large intestine	30.35	9.64	1-48	2.92 $\pm$ 7.83
Spiruridae gen. sp.†	Body cavity	1.78	15	15-15	0.26 $\pm$ 2.0
<i>Anolis nebulosus</i> (n = 44)					
<i>Oochoristica</i> sp. 2	Small intestine	15.9	1.85	1-3	0.29 $\pm$ 0.76
<i>Mesocestoides</i> sp.†	Body cavity	2.27	45	45	1.02 $\pm$ 6.78
<i>Parapharyngodon alvarengai</i>	Large intestine	2.27	2.0	2-2	0.04 $\pm$ 0.30
<i>Ctenosaura pectinata</i> (n = 29)					
<i>Oochoristica</i> sp. 3	Small intestine	48.27	14.78	1-46	7.13 $\pm$ 12.13
<i>Mesocestoides</i> sp.†	Body cavity	6.89	24.5	1-48	1.68 $\pm$ 8.75
<i>Centrorhynchus</i> sp.†	Body cavity	3.44	1.0	1-1	0.03 $\pm$ 0.18
<i>Alaeuris mexicana</i> Moravec, Salgado-Maldonado, and Mayén-Peña, 1996	Stomach, large intestine	93.10	1,834.29	17-5,983	1,707.79 $\pm$ 1,651
<i>Atractis scelopori</i> Caballero y Caballero, 1938	Large intestine	51.72	2,905.8	8-12,721	1,503 $\pm$ 2,665
<i>Ozolaimus ctenosauri</i> Caballero y Caballero, 1938	Large intestine	58.62	18.0	3-59	10.55 $\pm$ 1.45
<i>Thubunaea ctenosauri</i> Moravec, Salgado- Maldonado, and Mayén-Peña	Large intestine	3.44	8	8-8	0.27 $\pm$ 15.70
<i>Sceloporus nelsoni</i> (n = 10)					
<i>Parapharyngodon alvarengai</i>	Large intestine	30	11	1-22	3.3 $\pm$ 6.9
<i>Strongyluris</i> sp.‡	Large intestine	10	6	6-6	0.6 $\pm$ 1.8

\* n = number of hosts examined.

† Larvae.

‡ Probably *Strongyluris similis* Caballero y Caballero, 1938.

Esophagus, stomach, and small and large intestines were examined separately. Each organ was slit longitudinally and examined under a stereomicroscope. Helminths were removed and counted. Cestodes were fixed under slight coverslip pressure using AFA fixative. Nematodes were fixed using 70% ethanol. Selected helminths were deposited in the Helminthological Collection, Institute of Biology, UNAM: *Oochoristica* sp. 2839, 2840; *Alaeuris mexicana*, 2648 to 2651; *Atractis scelopori*, 2646; *Ozolaimus ctenosauri*, 2645; *Parapharyngodon alvarengai*, 2647; *Skrjabinodon scelopori*, 2644; *Thubunaea ctenosauri*, 2643. Terminology is in accordance with Margolis et al. (1982).

The helminth fauna consisted of 4 species of cestodes, 3 unidentified species of *Oochoristica* and *Mesocestoides* sp. (tetrathyridia); 1 species of acanthocephalan, *Centrorhynchus* sp. (cysta-

canths); and 7 species of nematodes: *Parapharyngodon alvarengai* Freitas, 1957; *Skrjabinodon scelopori* (Caballero y Caballero, 1938); *Alaeuris mexicana* Moravec, Salgado-Maldonado and Mayén-Peña, 1996; *Atractis scelopori* (Geddoelst, 1919); *Ozolaimus ctenosauri* Caballero y Caballero, 1938; *Thubunaea ctenosauri* Moravec, Salgado-Maldonado and Mayén-Peña, 1997; and *Strongyluris* sp. (probably *similis*). Prevalence, location, mean intensity, and abundance for each host are given in Table 1. Differences of prevalence and mean intensity of helminth species among the sexes of lizard species were as follows: in *Phyllodactylus lanei*, *Oochoristica* sp. 1 was found in 16.66% of 38 female lizards and in 16.66% of 18 males; intensity in female hosts, 4 cestodes, range 1-2, mean 1.33; in male hosts, 6 cestodes, range 1-3, mean 2. All *Centrorhynchus* sp. cystacanths were recovered from fe-

male lizards. *Parapharyngodon alvarengai* was found in 28.94% of female lizards and in 22.22% of males; intensity in female hosts, 37 nematodes, range 1–10, mean 3.36; in male hosts, 5 nematodes, range 1–2, mean 1.25. *Skryabinodon scelopori* was found in 31.57% of female lizards and in 33.33% of males; intensity in female hosts, 118 nematodes, range 1–48, mean 9.83; intensity in male hosts, 50 nematodes, range 2–27, mean 8.33. All Spiruridae gen. sp. nematodes were recovered from male *Phyllodactylus lanei*. None of the 5 female *Anolis nebulosus* were parasitized. In *Ctenosaura pectinata*, *Oochoristica* sp. 3 was found in 50% of 6 female lizards and in 47.82% of 23 males, intensity in female hosts, 53 cestodes, range 1–46, mean 17.66; in male hosts, 154 cestodes, range 1–30, mean 14. *Mesocestoides* sp. and *Centrorhynchus* sp. were all recovered from male lizards. *Alaeuris mexicana* was found in 100% of female lizards and in 95.65% of males, intensity in female hosts, 9,237 nematodes, range 314–2,819, mean 204; in male hosts, 33,426 nematodes, range 17–5,983, mean 1,681.3. Three individuals of *Ozolaimus ctenosauri* were collected from a single female lizard (prevalence, 16.66%), 56.52% of male lizards were parasitized by this species, intensity in male hosts, 300 nematodes, range 4–59, mean 23.07. All *Thubunaea ctenosauri* nematodes were recovered from male *Ctenosaura pectinata*. All nematodes from *Sceloporus nelsoni* were recovered from male hosts.

A total of 94,014 individual helminths were recovered, 99.6% of which were nematodes. Most of these (99.6%) were recovered from *Ctenosaura pectinata*. *Phyllodactylus lanei* was the second most heavily parasitized species, from which 236 helminths were collected, 95% of these were nematodes. *Anolis nebulosus* contained 60 helminths, 95% of which were cestodes. *Sceloporus nelsoni* contained 39 nematodes. The highest prevalence (93%) was recorded for *Alaeuris mexicana* in *C. pectinata*; the greatest mean intensity (2,905) was recorded for *Atractis scelopori* in *C. pectinata*.

The helminth fauna consisted mainly of widely distributed species. Only *Alaeuris mexicana* and *Thubunaea ctenosauri*, which were described from specimens in the current study (Moravec et al., 1996, 1997), are not known from other species. All other helminths are shared with other reptilian hosts species (Baker,

1987; Goldberg and Bursey, 1990; Schmidt, 1986).

The 4 sympatric lizard species examined were found to have a unique and relatively diverse oxyurid fauna that is not shared among them. Only *Parapharyngodon alvarengai*, which infected 75% of the examined species, and *Mesocestoides* sp. and *Centrorhynchus* sp., which infected 50%, were found in more than 1 species. All 4 lizard species feed on insects, with *Ctenosaura pectinata* having a wider diet that also includes leaves, flowers, and fruits (García and Ceballos, 1994; Ramírez-Bautista, 1994), and all inhabit trees and bushes. *Ctenosaura pectinata* and *Phyllodactylus lanei* are also found on the ground. Arboreal and terrestrial lizards feed on different prey, which may help explain differences in helminth faunas. Also, arboreal lizards may be able to avoid fecal-contaminated soil better than ground dwellers.

*Ctenosaura pectinata*, which had the largest body sizes and the most varied diet, contained the richest helminth fauna, both in species diversity and in abundance in our study. Moreover, Prado-Vera (1971) previously found *Ozolaimus megatyphlon* and *Ozolaimus monhystera* in *Ctenosaura pectinata*, which brings the number of helminth species that infect this lizard to 9. Goldberg et al. (1993) similarly reported in their helminth study of xantusiid lizards that the large island night lizard, *Xantusia riversiana*, which ate a mixed diet of plant and animal material, had a more diverse helminth fauna than its smaller insectivorous mainland relatives, *Xantusia bolsonae*, and *X. henshawi*.

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### Research Note

## Paucity of Hematozoa in Peregrine Falcons (*Falco peregrinus*) in West Greenland and Coastal Texas

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**ABSTRACT:** Two adult gyrfalcons (*Falco rusticolus*) and 8 adult and 95 nestling peregrine falcons (*Falco peregrinus*) from Greenland were hema tozoa free and 2 of 60 adult peregrines from the east Texas coast harbored *Haemoproteus tinnunculi*.

**KEY WORDS:** *Falco peregrinus*, *Falco rusticolus*,

*Haemoproteus tinnuncule*, *Prosimulium ursinum*, *Aedes impiger*, West Greenland, Texas.

None of the published surveys of hematozoans in peregrine falcons (*Falco peregrinus*) is

based on large sample sizes. Crisp (1854) apparently observed adult filarial worms in cellular tissue "near the heart at the root of the great vessels" in 1 peregrine falcon from Great Britain. Greiner et al. (1975) examined hematozoan literature from North America and reported 6 peregrines as all being negative. Cheke et al. (1976) surveyed a diverse group of British birds for hematozoa and observed *Haemoproteus* spp. in 1 peregrine. Upon postmortem examination of 7 peregrines, Peirce (1980) found 1 with *Haemoproteus* spp. Peirce and Cooper (1977) observed leucocytozoons in 2 of 7 peregrines from Britain. Peirce et al. (1983) showed 1 of 3 peregrines from the United Arab Emirates harboring a microfilaria. Peirce and Marquiss (1983) reported that 25 *F. peregrinus* from Scotland were negative for hematozoa, and Stabler and Holt (1965) observed none in 5 peregrines from Colorado. We examined blood from 8 adult and 95 nestling (12–22 days old) peregrines in West Greenland during the summers of 1991–1993, as well as 60 adults ( $\geq 2$  years old) and immatures ( $\leq 1$  year old) during their spring and fall migration through Padre Island, Texas, in 1994. To our knowledge, this is the first study on hematozoans in *F. peregrinus* with large sample sizes and the first investigation of peregrines during both breeding and migration periods.

As part of wider ecological studies (see Hunt and Ward [1988]; Mattox and Seegar [1988]), adult and nestling peregrines were examined for hematozoa in West Greenland (66°45'N, 49°55'W) during the summers of 1991–1993. In addition, immature and adult peregrines were trapped during the spring and fall of 1994 and similarly surveyed on the Texas coast (27°10'N, 97°20'W). Two adult gyrfalcons were also examined in Greenland. Blood samples were taken, fixed in methanol, stained in Giemsa, and examined at  $\times 200$ , 400, 600, and 1,000 magnification for a minimum of 1 hr as reported by Taft et al. (1996). Adult *Aedes impiger* Walker, 1848 and *Prosimulium ursinum* Edwards, 1935 were collected in Greenland at or near nest sites or directly from nestlings using an aspirator. *Prosimulium ursinum* larvae were also collected from streams throughout the Greenland study area by hand. All specimens were placed in 70% ethanol, and over 100 *P. ursinum* adults and larvae were dehydrated and mounted in Balsam on microscope slides. One voucher specimen of *Haemoproteus tinnunculi* von Wasielewski and

Walker, 1918 (accession HWML 39029) from a Texas peregrine along with 2 adult *P. ursinum* (HWML 39245), 2 larval *P. ursinum* (HWML 39246), and 2 adult *A. impiger* (HWML 39247) were deposited in the University of Nebraska State Museum, Harold W. Manter Laboratory Collection, Lincoln, Nebraska.

Blood samples collected from 8 adult and 95 nestling peregrines during the summers of 1991–1993 in Greenland, as well as 2 adult gyrfalcons (*Falco rusticolus*), showed no detectable hematozoa.

Of 60 migrating peregrines at Padre Island, Texas, 2 (3%) harbored hematozoans most closely resembling *Haemoproteus tinnuncule* as described by Bennett and Peirce (1988) (an adult female captured on 22 April 1994 and an immature female on 27 September 1994). The former harbored 1 and the latter 2 organisms.

Along with blood samples, over 200 potential hematozoan vectors (*Prosimulium ursinum* and *A. impiger*) were collected in Greenland from eyries, nestlings, and surrounding habitats (streams and ponds), then mounted and identified. At the time, it was thought that if peregrines were infected we would later examine these diptera for hematozoan life cycle stages. According to Crosskey (1990), ornithophily is prevalent in various blackfly groups, but this feeding habit has been little documented in *Austrosimulium* and *Prosimulium* compared to other genera. In this study, we did aspirate feeding *P. ursinum* and *A. impiger* from nestling peregrines.

However, despite the presence of these potential vectors on Greenland nestlings, we were unable to detect hematozoa in peregrines there in the largest breeding season sample yet assembled. According to Wernsdorfer (1980), temperature may be the primary factor limiting the distribution of human malarial parasites to areas south of the 16°C summer isotherm. Temperatures ranged from 0–15°C in the study area (Mattox and Seegar, 1988) during July and August, and these temperatures may preclude the cycling of avian hemopsporidians as well. However, this does not explain the paucity of hematozoans in migratory peregrines. Only 3% of 60 spring and fall migrating peregrines in Texas were positive for hematozoa. Further, we have readily found hematozoa in other raptors in comparable sample sizes in both breeding (Taft et al., 1994) and migrational seasons (Taft et al., 1996).

These large samples on a half-hemisphere scale at diverse seasons, as well as other above-cited studies of other raptors, suggest that 1 or more ecological, behavioral, genetic, or physiological factors play a role in the general lack of hematozoans in the peregrine falcon.

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**Research Note**

**Gastrointestinal Helminths of Some Yellow-shafted Flickers, *Colaptes auratus luteus* (Aves: Picidae), from Allegheny County, Pennsylvania**

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**ABSTRACT:** Five yellow-shafted flickers, *Colaptes auratus luteus*, from Allegheny County, Pennsylvania, were examined for gastrointestinal helminths. The nematodes *Capillaria tridens* and *Dispharynx nasuta* are recorded in this host species for the first time, and new egg measurements are given for *C. tridens*. The acanthocephalan *Plagiorhynchus (Prosthorhynchus) cylindraceus* and an unidentifiable cestode were also found.

**KEY WORDS:** Yellow-shafted flicker, *Colaptes auratus luteus*, *Capillaria tridens*, *Dispharynx nasuta*, Nematoda, *Plagiorhynchus (Prosthorhynchus) cylindraceus*, Acanthocephala.

The northern flicker, *Colaptes auratus*, is an insectivorous bird that is somewhat abundant throughout its range, including Allegheny County, Pennsylvania. Yet, despite this abundance, there is a paucity of information concerning helminth infections in this species (Table 1), as well as in many other piciforms occurring in North America.

The opportunity to examine 5 yellow-shafted flickers, *Colaptes auratus luteus*, Bangs 1898 became available when they were presented to a local wildlife rehabilitator and subsequently died of unknown causes. Five adults (4 male, 1 female) were collected from July 1995 through November 1996 in Allegheny County (40.46895°N, 079.98119°W), Pennsylvania, and frozen at -20°C until examined for gastrointestinal helminths. Postmortem intervals were 1 wk for 3 specimens and 1 and 3 mo for the remaining birds. Voucher specimens of *C. a. luteus* are deposited in the Carnegie Museum of Natural History, Section of Birds (Pittsburgh, Pennsylvania); #'s T-20594 through T-20596.

Cestode and acanthocephalan specimens were initially preserved in 10% buffered formalin and AFA fixative, respectively; then, they were transferred to 70% ethyl alcohol, stained in borax-carmine or Mayer's hematoxylin, dehydrated, cleared in xylene, and mounted in Canada

balsam. Nematodes were preserved in 10% buffered formalin and studied as temporary glycerin wet mounts after clearing by the ethyl alcohol and glycerin evaporation technique.

All 5 birds harbored gastrointestinal helminths and were infected with one or more of the following species: *Capillaria tridens* (Dujardin, 1845) (Nematoda: Capillarinae); *Dispharynx nasuta* (Rudin, 1819) (Nematoda: Acuariidae); *Plagiorhynchus (Prosthorhynchus) cylindraceus* (Goeze, 1782) Schmidt and Kuntz, 1966 (Acanthocephala: Plagiorhynchidae); and 1 species of unidentifiable cestode (Cyclophyllidae: Davaineidae). Voucher helminth specimens are deposited in the United States National Parasite Collection, Biosystematic Parasitology Laboratory (U.S. Department of Agriculture, Beltsville, Maryland): *Capillaria tridens* (USNPC # 87117); *Dispharynx nasuta* (USNPC #'s 87118, 87119); *Plagiorhynchus (Prosthorhynchus) cylindraceus* (USNPC # 87120); unidentified cestode (USNPC # 87121).

*Capillaria tridens* occurred in the proximal half of the small intestine of 2 birds with an intensity of 24 and 73 specimens, and represents a new host and locality record. This report documents *C. tridens* in Pennsylvania for the first time and represents the sixth report of *C. tridens* in North America. This is only the third species of *Capillaria* shown to infect *C. auratus*. *Capillaria tridens* has previously been reported by Durbin (1952) as *Capillaria pirangae* in the scarlet tanager, *Piranga erythromelas*, from Maryland; in the eastern towhee, *Pipilo erythrophthalmus erythrophthalmus*, from Manitoba (Hodasi, 1963); in the brown-headed cowbird, *Molothrus ater ater*, from Ohio (Cooper et al., 1973); in the wild turkey, *Meleagris gallopavo*, from the southeast (Davidson et al., 1975); and Read (1949) reported males of the species in the red-winged blackbird, *Agelaius phoeniceus* from Prairie du Sac, Wisconsin. The capillarids in this

**Table 1. Helminths reported from *Colaptes auratus* in North America.**

Species	Host	Geographical locality	Reference
<b>Trematoda</b>			
<i>Posthodiplostomum minimum</i> (MacCallum, 1921) Dubois, 1936	<i>C. auratus</i>	Experimental	Palmieri, 1973
<b>Cestoda</b>			
<i>Liga punctata</i> (Weinland, 1856) Weinland, 1857; as synonyms <i>Liga brasiliensis</i> and <i>Fuhrmannia brasiliensis</i>	<i>C. auratus</i> <i>C. a. borealis</i>	Bowie, Maryland Manitoba, Canada	Ransom, 1909 Hodasi, 1963
<i>Raillietina (Paroniella) rhyngchota</i> (Ransom, 1909) Fuhrmann, 1920	<i>C. auratus</i>	Nebraska, Iowa, and Maryland	Ransom, 1909
<i>Raillietina (Raillietina) comitata</i> (Ransom, 1909) Fuhrmann, 1920	<i>C. auratus</i>	Nebraska, Iowa, and Maryland	Ransom, 1909
Unidentified cestode (Davaineidae)	<i>C. a. luteus</i>	Allegheny Co., Pennsylvania	This report
<b>Nematoda</b>			
<i>Capillaria longistriata</i> Walton, 1923	<i>C. a. luteus</i>	Monticello, Illinois	Walton, 1923
<i>Capillaria tridens</i> (Dujardin, 1845)	<i>C. a. luteus</i>	Allegheny Co., Pennsylvania	This report
<i>Capillaria venusta</i> (Freitas et Mendoca, 1958); as synonym <i>Thominx venusta</i>	<i>C. a. chrysocaulosus</i>	Baracoa, Soroa, and La Quira, Cuba	Barus, 1971
<i>Diplotrriaena americana</i> Walton, 1927	<i>C. auratus</i>	United States	Walton, 1927
<i>Diplotrriaena</i> sp. Railliet et Henry, 1909	<i>C. a. cafer</i>	Eugene, Oregon	Gullion, 1949
<i>Dispharynx nasuta</i> (Rudin, 1819)	<i>C. a. luteus</i>	Allegheny Co., Pennsylvania	This report
<i>Habronema colaptes</i> Walton, 1923	<i>C. a. luteus</i>	Monticello, Illinois	Walton, 1923
<b>Acanthocephala</b>			
<i>Mediorhynchus centurorum</i> Nickol, 1969	<i>C. auratus</i>	Experimental	Nickol, 1977
<i>Mediorhynchus robustus</i> Van Cleave, 1916	<i>C. a. luteus</i>	Illinois	Van Cleave, 1947
<i>Mediorhynchus</i> sp. Van Cleave, 1916	<i>C. a. cafer</i>	Eugene, Oregon	Gullion, 1949
<i>Plagiorrhynchus (Prosthorhynchus)</i> <i>cylindraceus</i> (Goeze, 1782) Schmidt and Kuntz, 1966; as synonym <i>Prosthorhynchus</i> <i>formosus</i>	<i>C. auratus</i> <i>C. a. cafer</i> <i>C. a. cafer</i> <i>C. a. luteus</i>	Bowie, Maryland Not specified Washington Allegheny Co., Pennsylvania	Van Cleave, 1918 Schmidt and Olsen, 1964 Schmidt and Neiland, 1966 This report

study concur with the description of *C. tridens* provided by Okulewicz (1991 (1992)) collected from the great tit, *Parus major*, in Poland. However, the eggs from the present specimens have a greater range in size than described for the species by Okulewicz (1991 (1992)). Measurements of 48 eggs from gravid *C. tridens* collected from *C. auratus* are 55–70  $\mu\text{m}$  (mean 64.1) long by 22.5–30  $\mu\text{m}$  (mean 28.1) wide.

The acuariid nematode *D. nasuta* was collected from the proventricular mucosa of 2 birds

that harbored 12 and 27 specimens and is reported in *C. auratus* for the first time. This species has been documented numerous times, primarily in a variety of colubriiform, galliform, and passeriform birds (Goble and Kutz, 1945; Martinez-Moreno et al., 1989 (1990); Silva et al., 1990), but it has also been reported in the piciforms (Barus, 1971).

*Plagiorrhynchus (P.) cylindraceus* was found in 2 of the birds examined and constitutes a new locality record for the species. Intensity of in-

fection was 1 and 7 specimens. This plagiorynchid has been formerly documented in *C. auratus* as its synonym *Plagiorynchus formosus* (Van Cleave, 1918; Schmidt and Olsen, 1964; Schmidt and Neiland, 1966). An unidentifiable cestode species (Davaineidae) occurred in 3 birds with an intensity of 2–21 (mean 8.7) specimens per host. A specific genus or species could not be determined because adequate staining could not be obtained. This may be attributed to the specimens being previously frozen within the host. However, they likely represent a species of *Raillietina*, based on minimal ascertainable features.

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*Research Note*

**Dispharynxiasis in a Least Flycatcher, *Empidonax minimus* (Passeriformes: Tyrannidae), and a Golden-breasted Starling, *Cosmopsarus regius* (Passeriformes: Sturnidae)**

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**ABSTRACT:** *Dispharynx* sp. collected from a least flycatcher (*Empidonax minimus*) is reported to occur for the first time in Allegheny County, Pennsylvania, and represents a new host record. Additionally, a golden-breasted starling (*Cosmopsarus regius*) is documented harboring *Dispharynx nasuta* within the National Aviary in Pittsburgh (Pittsburgh, Pennsylvania) and also represents a new host record. A severe proventriculitis associated with this helminth caused the death of this host.

**KEY WORDS:** *Dispharynx nasuta*, Nematoda, Acuariidae, *Empidonax minimus*, *Cosmopsarus regius*, new host, new locality.

The acuariid nematode *Dispharynx nasuta* (Rudolphi, 1819) commonly parasitizes primarily galliform, columbiform, and passeriform birds in which it firmly attaches to the host proventricular mucosa. Its distribution is cosmopolitan, it has been indicated as the causative agent of "grouse disease" (Allen, 1924), and it has been considered to have contributed to the death of various avian species (Allen, 1924; Cram, 1928; Goble and Kutz, 1945; Lindquist and Straffuss, 1980).

In August 1995, a wildlife rehabilitator submitted for parasitic evaluation 1 least flycatcher, *Empidonax minimus* (Baird and Baird, 1843) (Passeriformes: Tyrannidae), whose cause of death was unknown. The specimen was collected in Allegheny County (40.46°N, 079.98°W), Pennsylvania, frozen pending necropsy, and subsequently deposited in the Carnegie Museum of Natural History, Section of Birds, Pittsburgh, Pennsylvania, Collection No. A-6707. Two non-gravid female *Dispharynx* sp. were found attached to the proventricular mucosa on 3 June 1996. A specific species could not be determined, due to the paucity of specimens and the lack of adult males for morphological comparison. Both female specimens, however, are characteristic of *D. nasuta* (Nematoda: Acuariidae).

There did not appear to be any associated pathological change resulting from this low infection. All other organ structures appeared normal.

A 1.5-mo-old African golden-breasted starling, *Cosmopsarus regius* Reichenow, 1879 (Passeriformes: Sturnidae), died at the National Aviary in Pittsburgh (Pittsburgh, Pennsylvania) on 31 May 1995. It was refrigerated, and a necropsy was performed 5 hr postmortem. Tissues, which included a markedly enlarged, distended proventriculus, were formalin fixed and submitted for pathological evaluation.

Numerous specimens of *D. nasuta* were firmly attached deep within the mucosa of the proventriculus producing an acute, diffuse proventriculitis. Nineteen specimens (9 male, 10 female) were collected for identification. The remaining specimens were left attached to the proventricular mucosa and were embedded in paraffin for sectioning. Due to the lack of any significant pathological changes in all other organ structures, the host's death was attributed to the resulting debilitation and wasting associated with this infection.

Voucher specimens to both hosts are deposited in the United States National Parasite Collection, United States Department of Agriculture (Beltsville, Maryland), USNPC Nos. 86901 and 86902.

The presence of a *Dispharynx* sp. in *Empidonax minimus*, and *D. nasuta* in *Cosmopsarus regius*, are new host records. *Dispharynx* spp. (primarily *D. nasuta*) have been recorded in numerous passeriform birds in the same geographical area as Pennsylvania (Allen, 1924; Allen and Gross, 1926; Cram, 1932a, b; Venard, 1933; Goble and Cheatum, 1943; Webster, 1943; Stanley and Rabalais, 1971), but, to the author's knowledge, have never been reported to occur in this state.

Pathological change in the proventricular mu-

cosa associated with this parasite has been reported in galliformes and columbiformes (Allen, 1924; Cram, 1928; Goble and Kutz, 1945; Lindquist and Straffuss, 1980; Vassilev and Jooste, 1991). The author is also not aware of documented deaths associated with *D. nasuta* in passeriform birds. The only passeriform reported as being adversely affected is the gray catbird, *Dumetella carolinensis*, by Goble and Kutz (1945).

The golden-breasted starling, which was captive reared and recently fledged from wild caught parents within the National Aviary in Pittsburgh, obviously became infected from the consumption of infected intermediate hosts within the bird's enclosure. However, since *D. nasuta* infects other avian hosts with the same geographical distribution as the golden-breasted starling (Seurat, 1919; Vassilev and Jooste, 1991), it is likely that *C. regius* can become naturally infected as well.

#### Acknowledgments

Acknowledgment is due to Edwin C. Klein (University of Pittsburgh, Laboratory Animal Resources, Pittsburgh, Pennsylvania) for pathological assessment of infected tissues. Thanks are also due to Kenneth C. Parks (Carnegie Museum of Natural History, Section of Birds, Pittsburgh, Pennsylvania) for identification of the least flycatcher; the National Aviary in Pittsburgh (Pittsburgh, Pennsylvania) for providing helminth parasites and infected tissues of the *C. regius*; and Lois Sakolsky (Flying Mammal Wildlife Rehabilitation Center, Pittsburgh, Pennsylvania) for providing the *E. minimus* specimen.

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Research Note

Surface Ultrastructure of *Heterophyes heterophyes*  
(Trematoda: Heterophyidae) Collected from a Man

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ABSTRACT: Surface ultrastructure of *Heterophyes heterophyes* recovered from a man by treatment with praziquantel is reported. Whole surface of the body was covered with saw-toothed or alternatively brush-shaped tegumental spines with an average density of 42 per square micrometer. Sensory papillae were not identified. The gonotyl was posterosinistal to the ventral sucker and was protruded in 28% flukes. The rodlets were arranged radially along the gonotyl, occupying about 85% of the gonotyl circumference. Rodlets were seen to be composed of 3 to 6 spines in a row appearing as "cockscomb."

KEY WORDS: *Heterophyes heterophyes*, surface ultrastructure, scanning electron microscopy, rodlets.

Trematodes of the family Heterophyidae are minute flukes distributed in various regions of Middle and East Asia. The second intermediate hosts of these parasites are fresh and/or brackish water fishes. Adult flukes are found in fish-eating animals including humans (Beaver et al., 1984). *Heterophyes heterophyes* is distributed primarily in Egypt (particularly in the lower Nile Valley), Greece, and Israel. Human cases of *H. heterophyes* infection were obtained by eating brackish water fishes caught in the endemic areas (Kagei et al., 1980; Adams et al., 1986; Chai et al., 1986).

*Heterophyes heterophyes* and *H. nocens* have very similar morphological features. Taxonomically, there have been debates about the validity of *H. nocens* in contrast to the type species, *H. heterophyes*. Chai et al. (1986) suggested that they are 2 distinct species from the number of rodlets, 50 to 63 in *H. nocens* and 70 to 90 in *H. heterophyes*. Observations on the surface ultrastructure of the Heterophyidae were made in *Metagonimus* spp. (Saito, 1972; Fujino et al.,

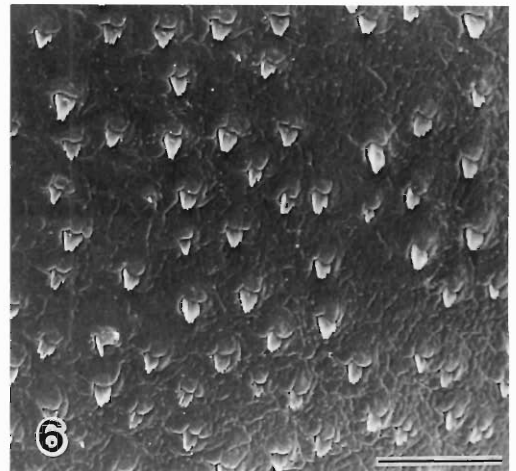
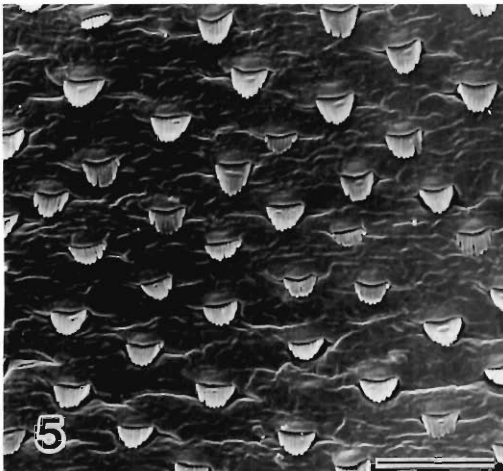
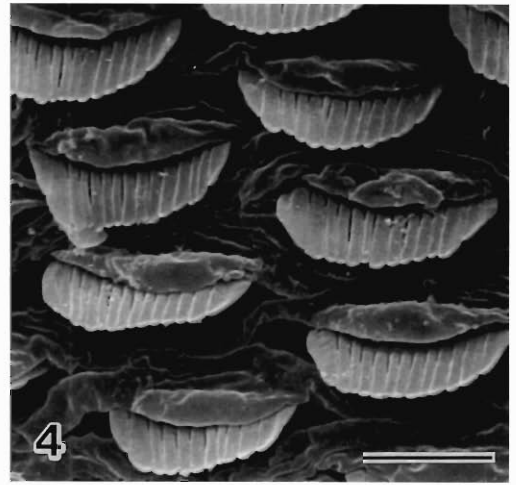
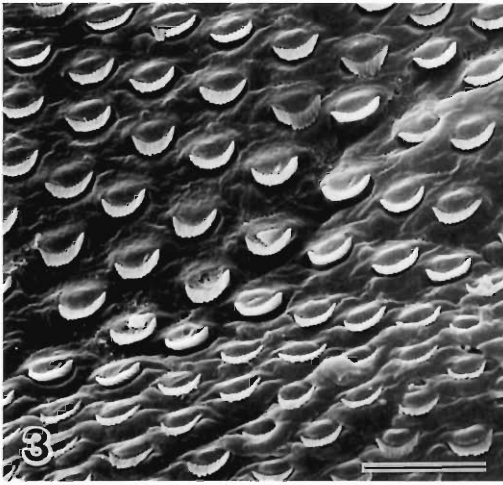
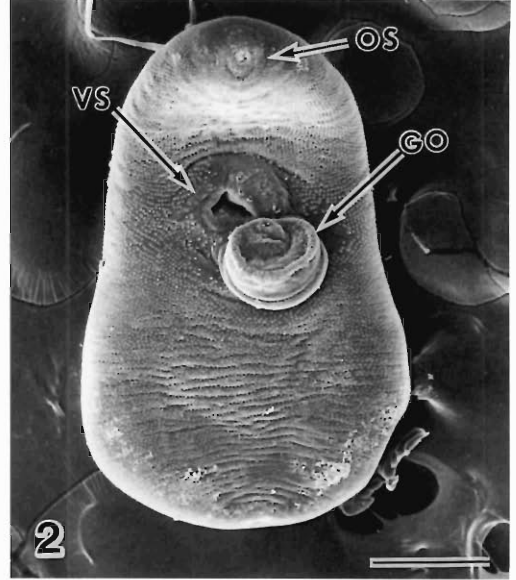
1989), *Haplorchis* (Fujino et al., 1989), and *Heterophyopsis continua* (Hong et al., 1991). However, the morphological details of the rodlets are not reported. The present study was undertaken to establish basic knowledge on the morphology of *H. heterophyes*, especially on the rodlets, by scanning electron microscopy.

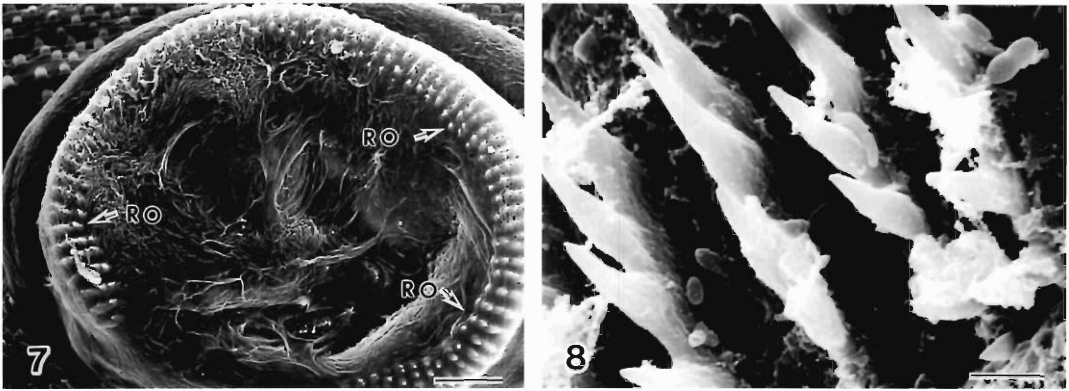
A total of 138 *H. heterophyes* were obtained from a 40-yr-old Japanese man after praziquantel administration. He returned to Japan in November 1994 after a 14-mo stay in Egypt as an engineer. Parasites were identified as *H. heterophyes* by light microscopy on the basis of their size, shape, and characteristic feature of both suckers. The details on the parasite collection will be reported elsewhere.

A total of 15 *H. heterophyes* were fixed in 10% formalin buffered with phosphate-buffered saline (pH 7.2), rinsed with PBS for 3 times at room temperature, and dehydrated with a graded series of ethyl alcohol. The specimens were transferred to absolute ethyl alcohol and to isoamyl acetate. The specimen was dried in a critical-point dryer (HCP-2, Hitachi, Tokyo, Japan), coated with gold (Ion Coater IB-3, Eiko, Tokyo, Japan), and observed under a scanning electron microscope (SEM) (JSM-T330A, Jeol, Tokyo, Japan). Several flukes were frozen in liquid nitrogen and fractured for observation of intrauterine eggs. Some of the remaining parasites in formalin have been deposited in the Meguro Parasite Museum, Tokyo, Japan (MPM Collection #19712).

Whole surface of the body was covered with tegumental spines (Figs. 1, 2). In the anterior part, tegumental spines were sawtoothed or alternatively brush shaped (mean length, 2  $\mu$ m), with a mean density of 42 per square micrometer (Fig. 3). The tegumental spines in the anterior part of the body were digitated into 14 to 17

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Figures 7, 8. Rodlets along the gonotyl tip of the genital sucker (7). Rodlets (RO). Higher magnification of rodlets (8). Bars = 10  $\mu\text{m}$  in 7 and 1  $\mu\text{m}$  in 8.

points (Fig. 4). The density and size of the tegumental spines decreased posteriorly (Figs. 5, 6). The number of points (or digits) of the tegumental spines in the posterior part was 7 to 10. The morphology and distribution of tegumental spines on the ventral surface were similar to those of the dorsal surface. Sensory papillae were not identified on either surface. The gonotyl was posterosinistal to the ventral sucker (Fig. 2). Of 108 flukes observed under a stereomicroscope, the gonotyl was protruded in 30 (28%) flukes, but not in 78 (72%). The rodlets were arranged radially along the gonotyl of the genital sucker (Fig. 7). Each rodlet was linear and consisted of 3 to 6 sharp spines (Fig. 8). The rodlets were 72–77 in number (mean 74) and occupied about 85% of the gonotyl circumference.

The uterus contained many oval-shaped eggs, with a mean length of 26.4  $\mu\text{m}$  (24.5–28.0  $\mu\text{m}$ ;  $n = 16$ ) and mean width of 15.2  $\mu\text{m}$  (15.0–15.6  $\mu\text{m}$ ;  $n = 16$ ). One end of the eggs was round and the other end had an operculum (5.2–6.4  $\mu\text{m}$ ). The basal edge of the operculum and the edge it fit into were both raised, forming narrow ridges. The entire egg surface was smooth.

The heterophyid trematodes comprise many species, and their morphologies have been studied extensively. Hong et al. (1991) observed sur-

face ultrastructures of *Heterophyopsis continua* and Chai et al. (1992) reported those of *Heterophyes nocens*, and our results agree with their findings in the following ways. First, tegumental spines are dense on anterior part of the body, whereas they are thin on the posterior part. Second, tegumental spines between the oral and ventral suckers have tips with 10 to 17 points in *H. continua*, but 12 to 17 points in *H. nocens* and 14 to 17 points in *H. heterophyes*. Sensory papillae were observed on the ventral surface of *Metagonimus* spp., *H. continua*, and *H. nocens* (Fujino et al. 1989; Hong et al. 1991; Chai et al. 1992), but we could not find any papillae in the *H. heterophyes* studied.

The number of rodlets on a gonotyl is important in the identification of species of the genus *Heterophyes*. *Heterophyes* with 50 to 63 rodlets are identified as *H. nocens* (*H. heterophyes nocens*), those with 52 to 57 as *H. katuradai*, and those with 70 to 90 as *H. heterophyes*. In the report by Chai et al. (1992), the gonotyl of *H. nocens* did not show the spines. They explain that “the tegument and spines were destroyed due to the effect of bithionol.” Kagei et al. (1980) reported light microscopic morphology of rodlets of *H. heterophyes* recovered from patients infected in Egypt. Miyazaki and Toh (1988) reported that rodlets are shaped like an

←

Figures 1–6. Dorsal (1) and ventral (2) surfaces of *H. heterophyes*. Oral sucker (OS); ventral sucker (VS); gonotyl (GO). Tegumental spines on anterior dorsal surface (3). Higher magnification of dorsoanterior tegumental spines (4). Tegumental spine on middle (5) and posterior dorsal surface (6). Bars = 200  $\mu\text{m}$  in 1 and 2; 5  $\mu\text{m}$  in 3, 5, and 6; and 1  $\mu\text{m}$  in 4.

antler, and an illustration was given, but the rodlets were not clear. In this study, rodlets could be photographed clearly. The rodlets were observed running around not 100% but about 85% of the gonotyl circumference radially, and this observation agrees with that reported by Chai et al. (1986). The present study revealed that the rodlets are not a single rod but are composed of 3 to 6 spines in a row appearing as "cockscomb."

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#### Research Note

### Applicability of Crude Extracts of Adult *Spirometra erinacei* for Serodiagnosis of Sparganosis

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**ABSTRACT:** Antigenicity of crude extracts of adult *Spirometra erinacei* was evaluated in comparison to those of the plerocercoid (sparganum) for serodiagnosis of human sparganosis. Patients' sera from 39 sparganosis, 77 other helminthic diseases, and 50 uninfected controls were tested by enzyme-linked immunosorbent assay (ELISA). When both extracts were used as antigen, specific antibody levels in sparganosis sera were highly correlated ( $r = 0.83$ ). The sensitivity and specificity of the adult worm extracts were 92.3 and 98.3%, while those of the sparganum were 94.3 and 96.6%, respectively. This result showed that crude

extracts of adult *S. erinacei* could be used as a diagnostic antigen of sparganosis.

**KEY WORDS:** *Spirometra erinacei*, sparganum, sparganosis, antigen, immunodiagnosis.

Human sparganosis is a parasitic disease caused by tissue-invading plerocercoids of *Spirometra* spp. (sparganum) such as *S. erinacei* Faust, Campbell, and Kellogg 1929 or *S. mansonioides* (Mueller 1935) Wardle, McLeod and

Stewart 1947. The parasites usually infect the subcutaneous and muscle layer but may invade the visceral organs and the central nervous system, sometimes eliciting severe neurological manifestations (Chang et al., 1992; Moon et al., 1993). The infection has been reported worldwide, but most cases were detected in China, Korea, Japan, and Southeast Asia (Holodniy et al., 1991).

In serological diagnosis of the disease, the crude extracts of the sparganum have been used as an antigen (Kim et al., 1984; Nishyama et al., 1994). Unlike in the past, however, it is getting difficult to obtain the worms from naturally infected hosts such as snakes or frogs due to the decrease of their population and degradation of the environment by industrialization. Unless the sparganum can be supplied in sufficient quantities from laboratory maintenance, we should search for another source of antigen for the serological diagnosis. In this respect, the adult worm may be a source if its antigenicity is shown to be useful. We evaluated here the applicability of crude extracts of adult *S. erinacei* as a diagnostic antigen.

Spargana, collected from snakes, *Rhabdophis tigrinus tigrinus* Boie, 1826, were washed with physiological saline and ground with a teflon-pestle tissue homogenizer followed by centrifugation at 20,000 g for 1 hr. The resulting supernatant was regarded as the crude sparganum extracts (Kong et al., 1994b). A dog was infected with 2 spargana *per os*. Two months later, adult strobila, which were confirmed under the dissecting microscope (wet weight: 8.2 g), were recovered from the dog's intestine. The strobila were washed and homogenized using a Waring blender 8 times at 20,000 rpm for a total of 15 min. Supernatant was obtained as described above. All procedures were carried out at 4°C. Protein content of the sparganum extracts was 6.4 mg/mL and that of adult extracts, 5.5 mg/mL, by the method of Lowry et al. (1951).

Sera from a total of 39 sparganosis, 24 cysticercosis, 5 each of *Taenia saginata* and *Diphyllobothrium latum* infections, 23 clonorchiasis, 20 paragonimiasis, and 50 uninfected controls without any possible infection were used. They were diagnosed either by positive antibody reaction (28 cases of sparganosis), or surgically (11 cases of sparganosis), or by egg detection (all other patients).

Using the crude extracts of spargana and adult

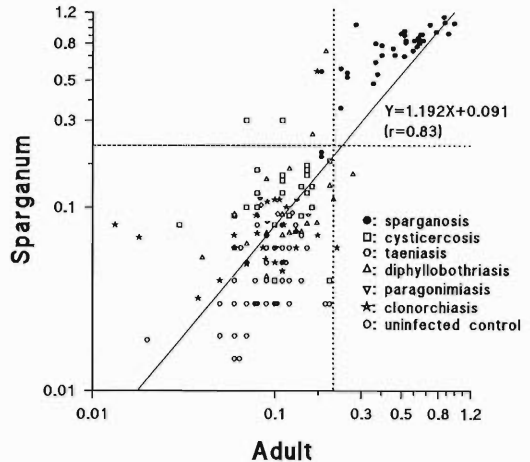


Figure 1. Correlation between antibody levels measured by ELISA absorbance using the adult and sparganum extracts as antigen. Sera from 39 sparganosis, 77 other helminthic diseases, and 50 normal controls were examined. Positive criteria at abs. 0.22 are indicated by dotted lines.

*S. erinacei* as antigens, antisparganum specific antibody (IgG) levels in the sera were measured. Antigen (200  $\mu$ L), diluted to 2.5  $\mu$ g/mL in carbonate buffer (0.05 M, pH 9.6), was coated to a microtiter plate (Costar, Cambridge, MA) overnight at 4°C. The sera (200  $\mu$ L) were diluted to 1:100 in phosphate-buffered saline containing 0.05% Tween 20 (PBS/T, pH 7.4) and reacted for 2 hr at 37°C. Peroxidase-conjugated antihuman IgG (heavy- and light-chain specific, Capel, Durham, NC) was diluted to 1:1,000 in PBS/T and further incubated for 2 hr at 37°C. The color reaction was developed using *o*-phenylenediamine and stopped by adding 25  $\mu$ L of 8 N H<sub>2</sub>SO<sub>4</sub>. Absorbance was read at 490 nm using an ELISA reader (Bio-Rad M 3550, Bio-Rad, Hercules, CA). Cut-off absorbance for a positive reaction was set at 0.22 for both antigens (Kim et al., 1984).

Figure 1 shows the correlation of antibody levels as expressed as absorbance (abs.) in the sera tested. Mean abs. in sera of 39 sparganosis patients was 0.51 ( $\pm$ 0.21) for the adult extracts, while that of the sparganum was 0.76 ( $\pm$ 0.22) (Table 1). Out of 39 sparganosis sera, 36 cases (92.3%) exhibited positive reactions to the adult antigen, whereas 37 sera (94.3%) were positive to the sparganum antigen. Sera of 2 paragonimiasis, 1 clonorchiasis, and 2 cysticercosis cases exhibited a positive reaction to the sparganum

**Table 1. Mean ELISA absorbance against each antigen preparation.**

Disease category	No. of sera examined	Mean absorbance $\pm$ standard deviation	
		Adult	Sparganum
Sparganosis	39	0.51 $\pm$ 0.21	0.76 $\pm$ 0.22
Cysticercosis	24	0.12 $\pm$ 0.05	0.12 $\pm$ 0.04
Taeniasis	5	0.11 $\pm$ 0.04	0.13 $\pm$ 0.04
Diphyllobothriasis	5	0.11 $\pm$ 0.06	0.10 $\pm$ 0.05
Paragonimiasis	20	0.13 $\pm$ 0.08	0.13 $\pm$ 0.14
Clonorchiasis	23	0.10 $\pm$ 0.05	0.09 $\pm$ 0.09
Uninfected control	50	0.09 $\pm$ 0.04	0.04 $\pm$ 0.02

extracts (specificity: 96.6%). Meanwhile, 2 paragonimiasis and 1 clonorchiasis serum showed a positive reaction to the *S. erinacei* extracts (specificity: 98.3%). The regression equation of the abs. was  $Y = 1.192X + 0.091$  ( $Y$  = absorbance for sparganum extracts,  $X$  = absorbance for *S. erinacei* extracts) ( $r = 0.83$ ,  $P < 0.01$ ). Antibody levels in other helminthic diseases and uninfected controls are summarized in Table 1.

Diagnosis of human sparganosis largely depends on the identification of the worms recovered from excisional biopsy. With progress in imaging diagnosis, however, preoperative presumption is now possible when the larva invades the central nervous system (Chang et al., 1992; Moon et al., 1993). Ultrasonographic findings of an elongated, folded bandlike hypoechoic structure in a heterogeneous hyperechoic mass are strongly suggestive of subcutaneous sparganosis (Chung et al., 1995). In addition, when unidentified worm sections were revealed in the pathological specimens, a specific antibody test is supplementary to confirm or disregard the presumption. The antibody test is also a useful seroepidemiological survey tool (Kong et al., 1994a).

The present study showed that the adult extracts of *S. erinacei* could be used in the serological diagnosis of sparganosis. Diagnostic sensitivity and specificity of both antigens were in 92–98%. The 2 sparganosis cases that did not react positively to either antigen were surgically confirmed chronic cerebral sparganosis in which highly degenerated worms were identified (Moon et al., 1993). These cases were included

in the antigen evaluation to cover the spectrum of the disease.

In conclusion, while the adult worm extracts exhibited lower activity than those of the sparganum in capturing the specific IgG antibody in the patients' sera, they revealed similar sensitivity and specificity with the sparganum extracts. In addition, adult worms have the benefit of providing large amounts of antigen with easy manipulation.

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## Research Note

# Free Radical Generation During Infection with *Nippostrongylus brasiliensis* (Nematoda) and/or *Eimeria nieschulzi* (Apicomplexa)

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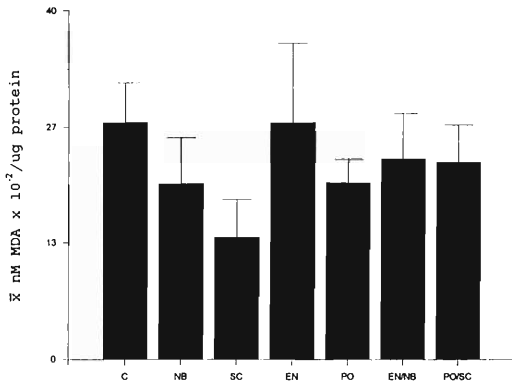
**ABSTRACT:** To determine if *Eimeria nieschulzi* suppresses *Nippostrongylus brasiliensis*-induced self-cure in Sprague-Dawley rats by inhibiting free radical production, jejunal free radical production was assessed using the Thiobarbituric Acid Assay in rats infected with  $1 \times 10^3$  third-stage larvae of *N. brasiliensis* and/or  $1 \times 10^5$  sporulated oocysts of *E. nieschulzi*. Sham infected rats administered saline served as controls. Rats infected with *N. brasiliensis* were killed on day 11 postinoculation (PI) and those infected with *E. nieschulzi* on day 8 PI. Rats infected with both parasites were killed when day 8 PI with *E. nieschulzi* coincided with day 11 PI *N. brasiliensis*. Free radical production was indirectly assessed by quantifying malondialdehyde (MDA), and data were expressed as  $\bar{x}$  nM MDA  $\times 10^{-2}/\mu\text{g}$  mucosal protein. No significant ( $P \geq 0.05$ ) differences in MDA production were observed among the groups. Results of this study show that *E. nieschulzi* does not suppress *N. brasiliensis* self-cure by inhibition of free radical production in Sprague-Dawley rats and, in fact, that this rat strain does not increase jejunal free radical production in response to infection with either parasite.

**KEY WORDS:** *Nippostrongylus brasiliensis*, *Eimeria nieschulzi*, free radical production, malondialdehyde, Thiobarbituric Acid Assay.

Smith and Bryant (1989) used a Thiobarbituric Acid (TBA) Assay to show that small intestinal free radical production was associated temporally with the expulsion of *Nippostrongylus brasiliensis* primary infections in female Wistar rats and suggested free radicals play an important role in the self-cure process. These authors focused their studies on the generation and effects of free radicals in rats infected only with *N. brasiliensis*. In nature, however, animals are usually infected with more than 1 species of parasite, and it has been shown that the host reaction to an infection with a single parasite species may be altered in the presence of another species. For example, Bristol et al. (1983) were able to show that Sprague-Dawley rats concurrently

infected with *N. brasiliensis* and *Eimeria nieschulzi* had significantly longer helminth patent periods when compared to rats that had only been infected with *N. brasiliensis*, suggesting that the host's immune and inflammatory response to *N. brasiliensis* was suppressed by *E. nieschulzi*. In separate reports, Broaddus et al. (1987) and Upton et al. (1987) demonstrated that *E. nieschulzi* suppressed *N. brasiliensis*-induced intestinal eosinophil lysophospholipase activity and relative peripheral eosinophilia, respectively. Although the mechanism by which *E. nieschulzi* suppresses self-cure of *N. brasiliensis* in Sprague-Dawley rats is unknown, we hypothesized it may be due, in part, to a suppression of free radical production since (1) it has been shown that *E. nieschulzi* suppresses intestinal eosinophilia (Broaddus et al., 1987), and (2) it is known that eosinophils are a major source of the free radicals generated in response to *N. brasiliensis* (Smith and Ovington, 1994). To test our hypothesis, specific pathogen-free mature male Sprague-Dawley *Rattus norvegicus*, each weighing 150–300 g, were used. Each rat was individually housed in an autoclaved cage that contained bedding (wood shavings) and was covered with a wire lid. Food and water were provided *ad libitum*. Rats were infected with  $1 \times 10^3$  third-stage larvae of *N. brasiliensis* and/or  $1 \times 10^5$  sporulated oocysts of *E. nieschulzi* and were killed on day 11 of the nematode infection ( $N = 5$ ), day 8 of the coccidian infection ( $N = 5$ ), or when day 8 of the *E. nieschulzi* infection coincided with day 11 of the *N. brasiliensis* infection ( $N = 5$ ). Uninfected sham-treated controls were administered 0.9% saline (NaCl) (w/v) and killed simultaneously along with uninfected-untreated controls. The jejunum was removed, slit open, washed to remove all debris and worms, and the number of worms present quantified. Intestinal mucosa (250 mg) was weighed and homogenized in a Virtis tissue homogenizer in phosphate-buffered saline (PBS),

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**Figure 1.** Malondialdehyde (MDA) production ( $\bar{x}$  nM MDA  $\times 10^{-2}/\mu\text{g}$  protein  $\pm$  SE) by small intestinal mucosa from rats infected with *Nippostrongylus brasiliensis* (NB) and/or *Eimeria nieschulzi* (EN), from sham-infected control rats that received saline subcutaneously (SC) and/or *per os* (PO), and from uninfected controls (C). N = 5 rats/group. NB = day 11 postinfection (PI); SC control. EN = day 8 PI; PO control. EN/NB = day 8 PI EN and day 11 PI NB; PO/SC control.

pH 7.4, with the final volume being 2.5 ml. Free radical levels were determined in the mucosa indirectly by using the TBA Assay (Asakawa and Matsushita, 1980) to measure malondialdehyde (MDA), an end product of the lipid peroxidation reaction generated by free radicals. A standard curve was prepared for use in determining MDA concentration in the mucosal homogenate samples by plotting the absorbance readings obtained from the following known concentrations of 1,1,3,3-tetramethoxypropane (malonaldehyde bis[dimethyl acetal]): 0.25, 0.5, 1, 2, and 4 nM. Absorbance was measured using a Beckman spectrophotometer at 532 nm. Protein concentrations in the homogenates were determined using the Bradford Assay, and data were expressed as  $\bar{x}$  nM MDA  $\times 10^{-2}/\mu\text{g}$  protein. Data were analyzed with a multivariate analysis of variance using a Wilks' Lambda ( $P$ -value = 0.6521).

The small intestinal mucosa from all experimental groups tested positive for the presence of low levels of MDA; however, no significant ( $P \geq 0.05$ ) differences in MDA production were found among the groups (Fig. 1).

Smith and Bryant (1989) suggested that free radicals play an important role in *N. brasiliensis* self-cure in Wistar rats. Results of the present study, however, suggest they are not important effectors of self-cure in Sprague-Dawley rats, since free radical levels during single or con-

current infection with *N. brasiliensis* and/or *E. nieschulzi* did not differ from those of uninfected control rats. These results are consistent with data obtained from mice infected with *N. brasiliensis* in our laboratory (Modric and Mayberry, 1994). The data also indicate *E. nieschulzi* does not inhibit self-cure of *N. brasiliensis* through suppression of free radical production.

Results of our TBA Assay show that low levels of MDA were produced during infection with *N. brasiliensis* or *E. nieschulzi* when compared to those levels observed by Smith and Bryant (1989) and Ovington and Smith (1992) in response to *N. brasiliensis* and *E. vermiformis*, respectively. It is possible that free radicals were produced in larger quantities in the Sprague-Dawley rats, but they were not able to participate in the lipid peroxidation reaction where MDA was formed due to the detoxification of free radicals by various host or parasite enzymes such as superoxide dismutase (SOD), catalase, and peroxidases (Ellis, 1990). In support of this argument, Batra et al. (1993) reported that *N. brasiliensis* contained SOD, glutathione peroxidase, and also catalase. It may be that the strains of *N. brasiliensis* used in our laboratory and Sprague-Dawley rats have higher levels of these enzymes than parasite or host strains used by other researchers such as Smith and Bryant (1989). An alternative explanation as to why our experiments with *N. brasiliensis* produced results that are contradictory to those reported by Smith and Bryant (1989) may be that they infected rats with  $6 \times 10^3$  third-stage larvae of *N. brasiliensis*, while we infected with  $1 \times 10^3$ . The increased number of larvae administered in the experiments by Smith and Bryant may have resulted in an intestinal worm burden large enough to induce a significantly higher level of free radical production and, thus, MDA when compared to the MDA levels of the current study. A third explanation may be that the production of parasite-induced free radicals may vary not only with the parasite burden and strain but with the strain of host as well. These are all important factors that must be considered when extrapolating results obtained in 1 laboratory or host system to results obtained in other laboratories with different strains of host and/or parasite.

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