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## Influence of Odonate Intermediate Host Ecology on the Infection Dynamics of *Halipegus* spp., *Haematoloechus longiplexus*, and *Haematoloechus complexus* (Trematoda: Digenea)

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**ABSTRACT:** The prevalences and relative densities of *Halipegus* spp., *Haematoloechus longiplexus*, and *Haematoloechus complexus* metacercarial infections in anisopteran (dragonfly) and zygopteran (damselfly) odonate intermediate hosts were examined. These measures of infection were compared in relation to the ecological habits of the host species. Also, the extent of second intermediate host specificity was compared between the 2 species of *Haematoloechus*. Eighteen species ( $N = 934$ ) of odonates were dissected and examined for metacercariae. *Halipegus* spp. generally had the highest prevalences and relative densities of infection when compared with *Haematoloechus* spp. in this system. Except for 1 host species, no significant differences in levels of infection were found between the 2 species of *Haematoloechus* in anisopterans. *Haematoloechus longiplexus* was a second intermediate host specialist, being found in anisopteran odonates only. In contrast, *Haematoloechus complexus* was a generalist and was found in both anisopteran and zygopteran hosts. Differences in infections among host species suggest that variations in odonate ecology are sufficient to influence the suitability of larval odonates to serve as intermediate hosts for these frog trematodes.

**KEY WORDS:** *Halipegus* spp., *Haematoloechus longiplexus*, *Haematoloechus complexus*, odonate, host specificity.

Bush et al. (1993) argued for the increased consideration of invertebrate intermediate hosts when investigating parasitic helminth community dynamics. Traditionally, vertebrate definitive hosts have received most of the attention in these analyses (Esch et al., 1990), although community studies of intramolluscan trematodes have received considerable attention recently (Kuris, 1990; Sousa, 1990, 1993; Fernandez and Esch, 1991a; Williams and Esch, 1991; Snyder and Esch, 1993; Esch and Fernandez, 1994; Laferty et al., 1994). Few studies have concentrated on parasites in invertebrate second or third intermediate hosts, i.e., those that have a closer ecological association with the definitive host, because the parasites rely on predator-prey pathways for transmission.

*Halipegus occidentalis* Stafford, 1905, is a hemiurid trematode that uses odonate (Insecta: Odonata; i.e., dragonfly and damselfly) naiads as third intermediate hosts. Naiads infected with metacercariae are ingested by the green frog, *Rana clamitans*, in which the parasites mature in the buccal cavity under the tongue (Goater et al., 1990). *Halipegus eccentricus* Thomas, 1939, is similar to *H. occidentalis*, except that adults mature in the eustachian tubes of the ranid definitive host. The life cycle of *H. eccentricus* has traditionally been thought to include only 3 hosts, with tadpoles ingesting infected microcrusta-

ceans (the second intermediate host) (Thomas, 1939). Thomas concluded that metacercariae would then reside in the host's stomach until the tadpole metamorphosed into an adult frog, at which time the worm would migrate up the esophagus to the eustachian tubes, where it would mature and live as an adult (Thomas, 1939). However, unpublished field data from our laboratory on the recruitment of this parasite into its definitive host (*R. clamitans*) suggest that *H. eccentricus* metacercariae also can be found in odonates, which presumably act as third intermediate hosts. This would make the life cycle of *H. eccentricus* similar to that of its congener, *H. occidentalis*. Thus, in habitats such as Charlie's Pond, where both congeners could be present within larval odonates, and because they are morphologically indistinguishable, they are referred to as *Halipegus* spp. for the purposes of this study.

*Haematoloechus complexus* Seely, 1906, and *H. longiplexus* Stafford, 1902, are frog lung flukes that use odonate naiads as second intermediate hosts. A number of previous studies have examined odonates for infections with *Halipegus* (Willey, 1930; Krull, 1935; Rankin, 1944; Macy et al., 1960; Kechemir, 1978; Goater, 1989; Fernandez, 1991) and *Haematoloechus* (Krull, 1930, 1931, 1932, 1933, 1934; Ingles, 1933; Grabda, 1960; Dronen, 1975, 1978; Bourgat and Kulo,

1979). However, these efforts predominantly have been surveys of odonate naiads as intermediate hosts in life history studies. Recently, Snyder and Janovy (1994) examined the second intermediate host specificity for 2 species of *Haematoloechus* and provided experimental evidence of important differences in host specificity between the species. However, field evidence for specificity of *Haematoloechus* spp. in odonates is lacking.

In this paper, we examine the prevalences and relative densities (as defined in Margolis et al., 1982) of *Halipegus* spp., *H. complexus*, and *H. longiplexus* metacercarial infections in several species of anisopteran (dragonflies) and zygopteran (damselflies) odonates in relation to the different ecological habits of these hosts. We also compare the prevalences and relative densities of 2 species of *Haematoloechus* to investigate whether strong second intermediate host specificity can be observed in samples of field-collected hosts.

#### Materials and Methods

Odonate naiads were sampled from several sites within Charlie's Pond, a 2-hectare pond in the Piedmont area of North Carolina, U.S.A., from June 1992 to June 1993. Because this study was associated with a more extensive investigation of trematode population dynamics in the definitive host, *Rana clamitans*, naiads were not sampled in the winter months (November–March) when frogs were inactive and not recruiting parasites. Odonates were collected using an aquatic sampling net and a 2-mm<sup>2</sup> mesh screen, placed in jars of pond water, and returned to the laboratory. Individuals were isolated at room temperature in 65-ml plastic jars filled with pond water. Naiads were examined under a dissecting microscope within 2 days of capture and identified to species according to Huggins and Brigham (1982). All parts of the odonate body were examined for metacercariae. *Halipegus* spp. were always found in the midgut of the naiads. *Haematoloechus complexus* was found in all parts of the body, occurring in a thin, hyaline cyst in the abdominal cavity, legs, and head. *Haematoloechus longiplexus* was unencysted and was always found associated with the branchial basket/respiratory structures of anisopteran odonates. Specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Halipegus* spp. (USNPC 85276), *Haematoloechus longiplexus* (USNPC 85277), and *Haematoloechus complexus* (USNPC 85278).

Data used to calculate prevalences were analyzed using chi-square (Zar, 1984). Relative density data were analyzed with an analysis of variance (ANOVA) using Systat (Wilkinson, 1989). Differences between groups were determined using Tukey's honestly significant difference (HSD) test. Probability values less than 5% ( $P < 0.05$ ) were considered statistically significant. In a few instances, relative density data were distributed nonnormally and were analyzed with a Kruskal-Wallis

nonparametric ANOVA. Due to the robustness of ANOVA, results from the nonparametric and parametric tests were consistent; thus, results are reported only from the parametric ANOVA for consistency.

#### Results

Eighteen species of odonates, representing 5 families, were examined ( $N = 934$ ). Those species with sample sizes adequate for statistical analysis are shown in Table 1. Other anisopterans that were examined, but not included in Table 1, were *Boyeria vinosa* ( $N = 6$ ; 4 infected with *Halipegus* spp., 1 infected with *H. complexus*) (Aeschnidae), *Neurocordulia alabamensis* ( $N = 1$ ) (Corduliidae), *Cerithemis monomelaena* ( $N = 1$ ), *Libellula deplanata* ( $N = 2$ ), and *Perithemis seminole* ( $N = 2$ ) (Libellulidae). Additional zygopterans included *Enallagma exsulans* ( $N = 15$ ; 4 infected with *Halipegus* spp., 2 infected with *H. complexus*), *En. signatum* ( $N = 1$ ), and *Ischnura verticalis* ( $N = 1$ ; 1 infected with *Halipegus* sp.) (Coenagrionidae).

*Halipegus* spp. generally were the most prevalent of the 3 trematode species (Table 1). Prevalences ranged from 55% in *Epitheca cynosura* to 3% in *Gomphus exilis*, which was significantly lower than in all other hosts ( $\chi^2 = 47.9$ ,  $df = 1$ ,  $P < 0.001$ ). There was no significant difference in *Halipegus* spp. infection among *Ep. cynosura*, *L. luctuosa*, *En. traviatum*, *En. basidens*, or *Erythemis simplicicollis* ( $\chi^2 = 2.28$ ,  $df = 4$ ,  $P > 0.05$ ); infection with *Halipegus* spp. in this group was significantly higher than in other hosts. Within individual host species, the prevalence of *Halipegus* spp. was significantly higher than the 2 species of *Haematoloechus* ( $P < 0.05$ ) except in *G. exilis* ( $\chi^2 = 1.24$ ,  $df = 2$ ,  $P > 0.05$ ) and *I. posita* ( $\chi^2 = 0.27$ ,  $df = 2$ ,  $P > 0.05$ ), for which there were no differences in prevalence of all 3 trematodes. Likewise, for *L. luctuosa* there was no difference between the prevalences of *Halipegus* spp. (54%) and *H. longiplexus* (41%) ( $\chi^2 = 1.43$ ,  $df = 2$ ,  $P > 0.05$ ).

The relative density of *Halipegus* spp. in *Er. simplicicollis* was significantly higher than in all other hosts except *En. traviatum* and *Ep. cynosura* (Table 1; Tukey HSD,  $P < 0.05$ ). *Halipegus* spp. generally had the highest relative densities of the 3 trematodes within individual host species as well. There were no significant differences among relative densities of any parasite for *A. fumipennis*, *G. exilis*, and *I. posita*. Only in *L. luctuosa* was the relative density of *Halipegus* spp. significantly lower than *H. longiplexus* ( $t =$

Table 1. Total prevalence, relative density, and range of infection of odonate naiads infected with metacercariae.

Odonate (habit*)	N	<i>Halipegus</i> spp.			<i>Haematoloechus longiplexus</i>			<i>Haematoloechus complexus</i>		
		No. infected (%)	Relative density ± SE (range)	No. infected (%)	Relative density ± SE (range)	No. infected (%)	Relative density ± SE (range)	No. infected (%)	Relative density ± SE (range)	
Suborder Anisoptera										
Gomphidae										
<i>Gomphus exilis</i> (B)	301	8 (3)	0.03 ± 0.01 (0-2)	13 (4)	0.05 ± 0.02 (0-2)	11 (4)	0.04 ± 0.01 (0-2)			
Cordulidae										
<i>Epicordulia princeps</i> (C, S)	68	21 (31)	0.9 ± 0.2 (0-9)	9 (13)	0.2 ± 0.08 (0-4)	5 (7)	0.7 ± 0.3 (0-1)			
<i>Epitheca cynosura</i> (C, S)	31	17 (55)	1.5 ± 0.4 (0-9)	5 (16)	0.4 ± 0.2 (0-5)	5 (16)	0.3 ± 0.1 (0-4)			
Libellulidae										
<i>Erythemis simplicicollis</i> (S)	90	38 (42)	2.7 ± 0.6 (0-40)	3 (3)	0.08 ± 0.05 (0-3)	9 (10)	0.3 ± 0.6 (0-7)			
<i>Libellula cyanea</i> (S)	200	76 (38)	0.6 ± 0.06 (0-4)	36 (18)	0.6 ± 0.2 (0-19)	32 (16)	0.3 ± 0.06 (0-7)			
<i>Libellula luctuosa</i> (S)	37	20 (54)	0.9 ± 0.2 (0-5)	15 (41)	1.0 ± 0.3 (0-9)	8 (22)	0.6 ± 0.3 (0-9)			
Suborder Zygoptera										
Coenagrionidae										
<i>Argia fumipennis</i> (C, S)	54	19 (35)	0.7 ± 0.2 (0-9)	0	0	7 (13)	0.5 ± 0.2 (0-7)			
<i>Enallagma basidens</i> (C)	33	15 (46)	1.0 ± 0.3 (0-5)	0	0	5 (15)	0.3 ± 0.1 (0-3)			
<i>Enallagma traviatum</i> (C)	41	20 (49)	1.7 ± 0.4 (0-10)	0	0	4 (10)	0.3 ± 0.2 (0-7)			
<i>Ischnura posita</i> (C)	50	10 (20)	0.3 ± 0.08 (0-3)	0	0	8 (16)	0.3 ± 0.1 (0-4)			

\* Ecological habits of odonates: B = burrower; C = climber; S = sprawler.

**Table 2.** Prevalence and relative density of infection of odonate naiads grouped by the ecological habit of the host. Only those species having a singular ecological habit designation are included.

Ecological habit	N	<i>Halipegus</i> spp.		<i>Haematoloechus longiplexus</i>		<i>Haematoloechus complexus</i>	
		Prevalence (%)	Relative density $\pm$ SE	Prevalence (%)	Relative density $\pm$ SE	Prevalence (%)	Relative density $\pm$ SE
Burrower*	301	2.7	0.03 $\pm$ 0.01	4.3	0.05 $\pm$ 0.02	3.7	0.04 $\pm$ 0.01
Climber†	124	36.3	1.0 $\pm$ 0.2	0	0	13.7	0.3 $\pm$ 0.9
Sprawler‡	327	41.0	1.2 $\pm$ 0.2	16.5	0.5 $\pm$ 0.1	15.0	0.3 $\pm$ 0.06

\* Burrower = *Gomphus exilis*.

† Climbers = *Enallagma basidens*, *Enallagma traviatum*, *Ischnura posita*.

‡ Sprawlers = *Erythemis simplicicollis*, *Libellula cyanea*, *Libellula luctuosa*.

2.25,  $df = 72$ ,  $P < 0.025$ ). There was no significant difference in densities of *Halipegus* spp. and *H. longiplexus* within *L. cyanea* ( $t = 0.1$ ,  $df = 398$ ,  $P > 0.05$ ).

*Haematoloechus longiplexus* infected anisopteran odonates only (Table 1). For anisopteran hosts, there were no significant differences in either the prevalence or the relative density of infection with *H. longiplexus* and *H. complexus* except in *L. luctuosa*. In the latter host species, the prevalence of *H. longiplexus* (41%) was significantly higher than that of *H. complexus* (22%;  $\chi^2 = 3.1$ ,  $df = 1$ ,  $P < 0.05$ ). Similarly, the relative density of *H. longiplexus* was significantly higher than for *H. complexus* ( $t = 6.11$ ,  $df = 72$ ,  $P < 0.001$ ).

In contrast to its congener, *H. complexus* infected both anisopteran and zygopteran odonates (Table 1). The prevalence of *H. complexus* in all species of zygopterans was lower than that of *Halipegus* spp. except in *I. posita* ( $\chi^2 = 0.27$ ,  $df = 1$ ,  $P > 0.05$ ). There were no significant differences in relative densities of infection with *H. complexus* and *Halipegus* spp. for *I. posita* ( $t = 0.51$ ,  $df = 98$ ,  $P > 0.05$ ) and *A. fumipennis* ( $t = 0.88$ ,  $df = 106$ ,  $P > 0.05$ ). However, there were significantly lower densities of *H. complexus* than *Halipegus* spp. in *En. basidens* and *En. traviatum* (Table 1; Tukey HSD,  $P < 0.05$ ).

There were no significant differences in the prevalence ( $\chi^2 = 0.74$ ,  $df = 1$ ,  $P > 0.75$ ) or relative density ( $t = 1.48$ ,  $df = 448$ ,  $P > 0.5$ ) of infection with *H. occidialis* between sprawling and climbing odonate species (Table 2). Likewise, there were no significant differences in the prevalence ( $\chi^2 = 0.09$ ,  $df = 1$ ,  $P > 0.9$ ) or relative density ( $t = 0.22$ ,  $df = 449$ ,  $P > 0.5$ ) of infection with *H. complexus* between these groups. When the sprawling and climbing habits were pooled and collectively compared with levels of infec-

tion of the burrowing habit (i.e., *G. exilis*), there were highly significant differences in both the prevalence and relative density (respectively) of infection for *H. occidialis* ( $\chi^2 = 133.0$ ,  $df = 1$ ,  $P < 0.001$ ;  $t = 8.4$ ,  $df = 750$ ,  $P < 0.001$ ), *H. longiplexus* ( $\chi^2 = 32.1$ ,  $df = 1$ ,  $P < 0.001$ ;  $t = 5.3$ ,  $df = 626$ ,  $P < 0.001$ ), and *H. complexus* ( $\chi^2 = 24.1$ ,  $df = 1$ ,  $P < 0.001$ ;  $t = 3.3$ ,  $df = 750$ ,  $P < 0.001$ ).

## Discussion

*Halipegus* spp. generally had the highest prevalences and relative densities of infection when compared with *Haematoloechus* spp. in this system. The prevalence of *Halipegus* spp. ranged from a high of 55% in *Ep. cynosura* to a low of 3% in *G. exilis*, a species that consistently had low prevalences and relative densities of all 3 parasites. Of all the species that were sampled, *Gomphus exilis* is the only burrower; i.e., the naiads burrow beneath the surface of the pond bottom mud, sand, or sediment (Huggins and Brigham, 1982). This life style or ecological "habit" would seem to restrict predation by this species on large numbers of infected ostracods (in the case of *Halipegus* spp.) or to reduce exposure of the host to the motile cercariae of *Haematoloechus* spp., relative to the other species of odonates. In contrast, all other odonate species had prevalences of *Halipegus* spp. that were at least 20%. These species represent 2 other ecological habits: climbers (*A. fumipennis*, *Enallagma* spp., *I. posita*, *Ep. cynosura*) and sprawlers (*Epi. princeps*, *Er. simplicicollis*, *Libellula* spp.) (Huggins and Brigham, 1982). Climbers are active predators that stalk their prey when foraging. Sprawlers are ambush predators, sitting and waiting for prey to move close to them (Huggins and Brigham, 1982). Species representing these latter 2 habits have relatively greater exposure

to the water column, including infected ostracods and motile cercariae.

There were no significant differences in patterns of infection between climbers and sprawlers. However, when collectively compared with the burrowing habit, the climber and sprawler habits had significantly greater prevalences and relative densities of infection of all 3 species of trematode. In light of this, we suggest that the ecological habit of the intermediate host may reflect real biological limits with respect to which species may play an important role in the dynamics of the parasite's life cycle. Given that all of these species were clearly susceptible to infection (notwithstanding the absence of *H. longiplexus* from zygopteran hosts), we feel that the differences in levels of infection among host species were primarily due to ecological/habitat determinants rather than patterns of host phylogenies (Bush et al., 1990). Thus, because of the sometimes large differences in levels of infection between hosts representing different ecological habits, care must be taken when generalizations are made with respect to a particular host "group." For example, Dronen (1978) examined several anisopteran and zygopteran odonate species, treating them as a single group because they reportedly served equally well as second intermediate hosts for *H. coloradensis* (= *H. complexus*; Kennedy, 1981). However, inspection of the present data suggests there is substantial variability in the suitability of a particular species as a host. Furthermore, much of this variability can be attributed to simple differences in the ecological habits of the hosts. When considering *H. longiplexus*, for example, treating both anisopteran and zygopteran odonates as a single group would have a serious impact on the assessment of prevalence and relative density of infection, given the strong specificity of this parasite for anisopteran naiads. Clearly, ecological habits of intermediate hosts must be considered when examining patterns of infection at the level of the component community.

Differences in host specificity were observed between *H. longiplexus* and *H. complexus*. *Haematoloechus longiplexus* infected only anisopteran odonates, whereas *H. complexus* infected both anisopterans and zygopterans. The apparent restriction of *H. longiplexus* to anisopterans in this system is in contrast to the work of Krull (1932), in which he described this species as occurring in the zygopteran *Lestes vigilax*. As we have no reason to doubt the experimental infec-

tions of Krull (1932), this discrepancy suggests that levels of host specificity may be more fine tuned than at the level of taxonomic suborders (i.e., anisopteran vs. zygopteran hosts). Because *Lestes* sp. does not occur in Charlie's Pond, potentially susceptible zygopteran hosts may not be present in this system. These differences also imply that regional differences in invertebrate intermediate host use may exist.

Except for 1 host species (*L. luctuosa*), there were no differences in either the prevalence or relative density of infection with *H. longiplexus* and *H. complexus* in anisopteran hosts. The similarities between the 2 congeners are interesting because the patterns do not reflect those seen at the level of the first intermediate hosts. In Charlie's Pond, *H. complexus* occurred in over 15% of *Physa gyrina*, its first intermediate host (Snyder and Esch, 1993). In contrast, *H. longiplexus* infected less than 1.5% of the snail *Helisoma anceps* for any given month (Fernandez and Esch, 1991b). We recognize that differences of this sort could be a result of different population sizes of the first intermediate hosts, but as population densities of the snail species have not been estimated, no conclusive comparisons are possible at this time. Instead, we suggest that despite the greater prevalence of *H. complexus* in its first intermediate host (*P. gyrina*), the similarity in the levels of infection with *H. longiplexus* and *H. complexus* in their second intermediate hosts (odonates) is a function of differing host specificities of these parasites.

Recently, Snyder and Janovy (1994) demonstrated that *H. complexus* is a second intermediate host generalist; this trematode was able to infect 9 arthropod species (representing 2 subphyla and 3 insect orders) exposed to cercariae. Whereas they did not test *H. longiplexus* in their study, a pattern similar to *H. longiplexus* was seen with *H. medioplexus*: it too infected only anisopteran naiads (Snyder and Janovy, 1994). They suggested that anuran definitive hosts might have a better chance of ingesting a food item infected by the generalist (*H. complexus*) because this parasite can infect a wider range of prey items. Using this logic, we suggest that, given a finite number of cercariae shed by an infected snail, a generalist parasite species would be expected to have a lower prevalence of infection in any particular second intermediate host species when compared with a parasite that was a specialist on that second intermediate host species. Assuming that *H. complexus* uses several other

aquatic arthropods in Charlie's Pond, we propose that the similarities in infection of anisopteran odonates with *H. longiplexus* and *H. complexus* in this system are created by the "dilution" of cercariae of *H. complexus* into other types of hosts (e.g., zygopterans). This would effectively counterbalance the initially large difference in prevalences of these parasites in their first intermediate hosts. Thus, the anisopteran-specialist *H. longiplexus* may be as prevalent in anisopteran hosts as the generalist *H. complexus*, despite the cercariae being shed from a much smaller proportion of its respective snail host.

Despite the variable levels of infection among odonate species, which, in this system, can be attributed primarily to different ecological habits, it does appear that a wide variety of the odonates could serve as suitable intermediate hosts. All of the trematodes in the present study are actively recruited by, and mature in, ranid frogs. Which odonate species act as the primary intermediate hosts in this system remains unknown. Presumably, species such as *Er. simplicicollis* and *L. cyanea* play an important role in the transmission dynamics of these parasites; they are abundant in the pond and have relatively high prevalences and relative densities of infection. For example, up to 40 metacercariae have been observed in 1 individual of *Er. simplicicollis*, which, if ingested by a frog, could represent an "instant" infestation in the definitive host. On the other hand, the burrowing *G. exilis*, although abundant in the pond (representing 32% of individuals sampled), consistently had the lowest levels of infection of any host sampled and thus would not be expected to contribute greatly to the transmission dynamics of these trematodes. Nevertheless, given the potentially large number of hosts that could be used, these parasites may be successfully "hedging their bets" against barriers to transmission and local extinction (Bush and Kennedy, 1994).

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## Pattern of Infection of *Gammarus aequicauda* (Amphipoda) with Metacercariae of *Levinseniella tridigitata* (Trematoda: Microphallidae)

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**ABSTRACT:** We investigated the pattern of infection of *Gammarus aequicauda* (Amphipoda) with metacercariae of *Levinseniella tridigitata* (Trematoda: Microphallidae) in a natural lagoon. Prevalence was significantly higher in females than in males, whereas there was no difference between sexes in parasitic intensity. Despite the very large size of *L. tridigitata* metacercariae, the mean abundance increased with host size and in both sexes, suggesting that the accumulation of this parasite has no significant effect on host survival. *Levinseniella tridigitata* is known not to alter its host's behavior. Our results contrast with those obtained with other helminths that alter host behavior in order to favor their transmission to the definitive host.

**KEY WORDS:** Trematoda, Amphipoda, *Levinseniella tridigitata*, *Gammarus aequicauda*, host survival.

Several advances in ecology have suggested that reduction in host survival caused by parasitism has immediate effects on the population dynamics and the community structure of both parasites and hosts (Crofton, 1971; Price, 1980; Anderson and Gordon, 1982; Freeland, 1983; Scott and Dobson, 1989; Minchella and Scott, 1991; Jaenike et al., 1995). However, we know little about parasite-induced host mortality in the field, mainly because it is often difficult to demonstrate. What we do know comes largely from laboratory observations or anecdotal evidence (Cox, 1989; Barker et al., 1991; Goater and Ward, 1992). Additionally, experimental investigations in the laboratory frequently involve the exposure of individuals to much higher levels of parasitism than those found in the field. Thus, before considering a parasite as an important biotic constraint on a host population, quantitative field measures are necessary.

For many species of parasites, particularly helminths (cestodes, nematodes, trematodes, and acanthocephalans), continuation of the life cycle requires predation on intermediate hosts by the definitive host. Several studies on crustaceans parasitized by helminth larvae reported a disappearance or an absence of heavily infected hosts in the oldest age classes (Seidenberg, 1973; Camp and Huizinga, 1980; Brown and Pascoe, 1989; Thomas et al., 1995a). Such studies concern helminths, the larvae of which may alter the behavior of their intermediate host and thereby enhance their transmission to the definitive host (i.e., "favorization", in the sense of Combes, 1991). For instance, in the crustacean *Gamma-*

*rus insensibilis*, infective larvae of *Microphallus papillorobustus* (Rankin, 1940) migrate into the brain, provoking aberrant behavior of the amphipod (Helluy, 1983a, b). The parasite transmission to aquatic birds (definitive host) feeding on gammarids is thus favored (Helluy, 1984). In a previous study (Thomas et al., 1995a), this trematode species was reported to severely affect the population dynamics of *G. insensibilis*. On the other hand, the same parasite on *Gammarus aequicauda* encysts in the abdomen, where it affects neither the behavior (Helluy, 1983a, b) nor the population dynamics of this alternative host (Thomas et al., 1995a). In *Gammarus aequicauda* infected with metacercariae of *Levinseniella tridigitata* (Creplin, 1837), such a "suicidal" behavior never occurs either, because cercariae do not migrate to the brain but also encyst in the abdomen where they a priori cannot provoke behavioral alterations (Helluy, 1981). However, metacercarial cysts of *L. tridigitata* are very large (i.e.,  $450 \times 650 \mu\text{m}$  versus  $270 \times 350 \mu\text{m}$  for *M. papillorobustus*; Rebecq, 1964). Thus, fundamental differences in virulence and in size do exist between these two parasite species. The aim of this study, based on data collected in the field, was to analyze the pattern of infection and evaluate the impact of *L. tridigitata* on the population of its intermediate host, *G. aequicauda*.

### Materials and Methods

A large sample of *G. aequicauda* ( $N = 929$ ) was collected in a lagoon from Palavas-les-Flots (southern France,  $43^{\circ}25'N$ ,  $3^{\circ}35'E$ ) during spring 1994. Gammarids were randomly sampled in the aquatic vege-

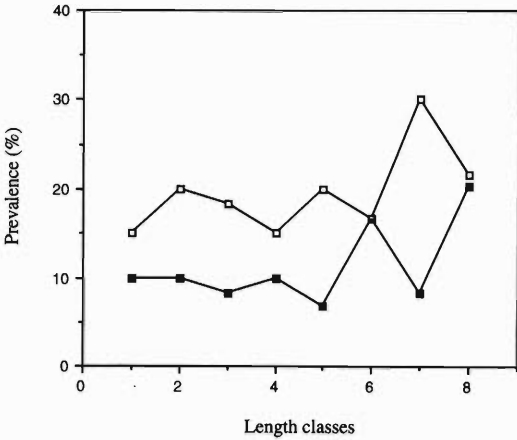


Figure 1. Changes in the prevalence of *Levinseniella tridigitata* with host size in *Gammarus aequicauda* (filled squares: males; open squares: females).

tation. The sampling site was no more than 40 cm in depth. All individuals were immediately preserved in 70% (v ethanol) and were later identified, sexed, measured in length, and dissected in order to count metacercariae of *L. tridigitata* (described in Rebecq, 1964). Following Margolis et al. (1982), we estimated (i) the prevalence (proportion of infected individuals), (ii) the mean parasitic intensity (mean parasite load of infected individuals), and (iii) the mean parasite abundance (total number of parasites divided by the total number of examined hosts). In gammarids, growth conforms to a logistic curve (Sutcliffe et al., 1981), but the relationships between size and age depends largely on temperature, food items, and sex (Sutcliffe et al., 1981). Here, males and females were analyzed separately and arranged in 8 length classes, assuming a positive correlation between age and size. In classes 2 to 7, steps are equal (1 mm for males and 0.5 mm for females). Class 1 includes all individuals that were too small to be in class 2 (i.e., smaller than 11 mm for males and 10 mm for females), and class 8 includes all individuals that were too large to be in class 7. We then analyzed changes in mean parasite abundance with host size.

Statistical tests are described in Siegel and Castellan (1988) and Sokal and Rohlf (1981). All tests are two-tailed. Results were considered significant at the 5% level.

**Results**

Changes in the prevalence of *L. tridigitata* with host size are presented in Figure 1. Prevalence was significantly higher in females than in males (19.6% for females and 11.3% for males, Fisher's exact test  $P = 0.0006$ ). For the mean intensity, there was no difference between sexes (males,  $I = 1.25$ ; females,  $I = 1.32$ ; Mann-Whitney  $U$ -test,  $U = 2271$   $P > 0.05$ ). The frequency distribution of *L. tridigitata* within its host conforms to a negative binomial distribution for males and for

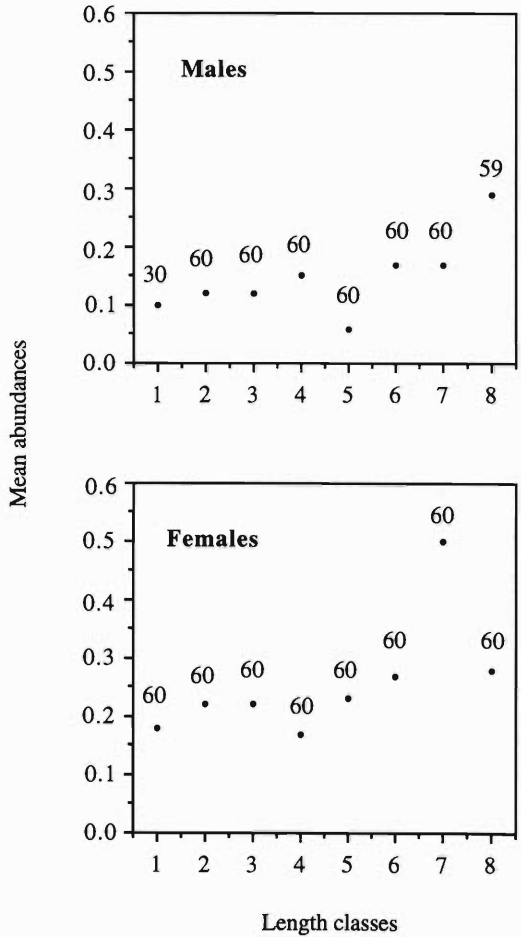


Figure 2. Changes in the mean abundance of *Levinseniella tridigitata* with host size in males and in females of *Gammarus aequicauda*. The number of hosts analyzed in each length class is indicated above each dot.

females (Kolmogorov-Smirnov test,  $P > 0.05$  in each case). The values of  $k$  (negative binomial parameter) were 0.36 and 0.64 and the means were 0.14 and 0.26 for males and females, respectively. Mean parasite abundance increased steadily with host size in both males ( $r_{\text{spearman}} = 0.75$ ,  $P < 0.05$ ) and females ( $r_{\text{spearman}} = 0.83$ ,  $P < 0.05$ ) (Fig. 2). The maximum mean abundances for males and for females reached 0.29 and 0.5, respectively.

**Discussion**

The fact that the prevalence was higher in females remains unexplained and contrasts with other studies concerning gammarids infected with

helminths. Indeed, prevalence previously has been reported significantly higher for males for *Gammarus pulex* infected with *Polymorphus minutus* (Ward, 1986) and *G. insensibilis* infected with *M. papillorobustus* (Thomas et al., in press a).

Following Anderson and Gordon (1982), when the rate of host mortality is positively correlated with parasite accumulation, curves of the host age as a function of the parasite abundance are convex, as a consequence of the death of the most heavily infected oldest hosts. However, for parasites of low virulence, continued acquisition through time acts to increase parasite abundance in older classes (Anderson and Gordon, 1982; Gordon and Rau, 1982). To our knowledge, infection with *L. tridigitata* provokes neither behavioral alterations nor color changes of the host (Helluy, 1981). Expectedly, the mean abundance of *L. tridigitata* increased with host size, indicating that, despite the very large size of *L. tridigitata* metacercariae, this parasite has no significant effect, through accumulation, on its host's survival. However, the absence of effect may come from the fact that this parasite does not accumulate enough to significantly affect its host's survival. Indeed, the maximum mean abundance is 4 times lower than what can be observed for *M. papillorobustus* on *G. insensibilis*, where host regulation indeed occurs (Thomas et al., 1995a). Nevertheless, a similar result has already been observed (Thomas et al., unpubl. data) for *G. aequicauda* infected with *Microphallus hoffmanni*, a trematode that also encysts in the abdomen, is unable to provoke behavioral alterations, and significantly accumulates in its host. Literature on parasites that enhance their host's susceptibility to predation by definitive hosts generally lacks quantitative field data. Consequently, the demographic impact of such parasitism on the host population remains mostly unknown. The contrasted pattern of mortality observed between *G. aequicauda* infected with *L. tridigitata*, *M. hoffmanni*, and *M. papillorobustus* and other crustaceans infected by debilitating parasites (Seidenberg, 1973; Camp and Huizinga, 1980; Brown and Pascoe, 1989; Thomas et al., 1995b) suggests that "favorization" has a real impact on the demography of host populations.

In conclusion, this study supports the idea that parasites that cause nonspecific symptoms, as does *L. tridigitata*, may well go unrecognized as

potential regulatory factors, being just "passengers" in their host with little impact on their host's population structure.

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## 1996 Meeting Schedule

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|------------------|--|
| 14 February 1996 | Nematology Laboratory, USDA, Beltsville, MD (Contact: David Chitwood, 301-504-5660)  |
| 20 March 1996    | Johns Hopkins Montgomery County Center, Rockville, MD (Contacts: Thomas Simpson, 410-366-8814 or Alan Scott, 410-955-3442) |
| 4 May 1996       | New Bolton Center, University of Pennsylvania, Kennett Square, PA (Contact: Gerhard Schad, 215-898-6680)                   |
| October 1996     | Site and date to be announced  |
| November 1996    | Site and date to be announced  |

## Larval Parasitic Nematodes Infecting Marine Crustaceans in Eastern Canada. 3. *Hysterothylacium aduncum*

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**ABSTRACT:** Third-stage larvae of *Hysterothylacium aduncum* were found infecting the hermit crab *Pagurus acadianus*, the gammaridean amphipod *Proboloides holmesi*, and the caprellid amphipod *Caprella linearis*. Five nematodes were found in 3 infected hermit crabs (100%) collected from the brackish Bras d'Or Lakes, Cape Breton Island, Nova Scotia. One infected hermit crab (0.3%) and 2 specimens of *P. holmesi* (7.7%) on the Scotian Shelf (the continental shelf off Nova Scotia) near Sable Island were each infected with single worms. One specimen of *C. linearis* (0.06%) in the St. Croix River, close to where it enters Passamaquoddy Bay, New Brunswick, was infected with a single nematode. No larva of *H. aduncum* was found in 18,210 amphipods belonging to 32 species other than *P. holmesi* and *C. linearis*, 1,254 mud shrimp (*Crangon septemspinosa*), 1,147 cumaceans, 417 isopods, or 4,819 polychaetes collected near Sable Island nor in 780 other hermit crabs (*P. arcuatus* and *P. pubescens*) collected from various areas on the Scotian Shelf and Georges Bank. Two fourth-stage larvae and one adult female *H. aduncum* were found in 2 amphipods (*Ceradocus torelli*) found among the gut contents of an Atlantic cod collected in the Cabot Strait. The intermediate host list of *Hysterothylacium* spp. compiled by Norris and Overstreet (1976) is updated.

**KEY WORDS:** *Hysterothylacium aduncum*, intermediate hosts, hermit crab, *Pagurus acadianus*, amphipod, *Proboloides holmesi*, *Caprella linearis*, *Ceradocus torelli*.

Nematode species of the genus *Hysterothylacium* (Ascaridoidea: Anisakidae) are extremely common intestinal parasites of teleosts, especially in the marine environment. In North Atlantic waters, *Hysterothylacium aduncum* (Rudolphi, 1802) Deardorff and Overstreet, 1981 (= *Thynnascaris adunca* = *Contracaecum aduncum*), generally is the only species recognized, but the taxonomy is unresolved (Køie, 1993). Off the Pacific and Atlantic coasts of North America, there are at least 76 species of teleost hosts of *H. aduncum* (see Margolis and Arthur, 1979; Love and Moser, 1983).

As with other anisakid nematodes, the life cycle involves an invertebrate intermediate host. Norris and Overstreet (1976) reviewed the known invertebrate hosts reported for *Hysterothylacium* spp. At that time, known hosts consisted of 10 copepods, 5 mysids, 1 isopod, 1 amphipod, 3 euphausiids, 17 decapods, 10 polychaetes, 5 gastropods, 2 cephalopods, 3 cnidarians, 1 ctenophore, 9 chaetognaths, and 1 starfish.

Since 1989, the Canadian Department of Fisheries and Oceans has conducted extensive surveys of invertebrates off Atlantic Canada. During the course of this survey, new intermediate hosts for *H. aduncum* were discovered and are reported herein, along with an update of Norris and Overstreet (1976).

### Materials and Methods

Marine invertebrates were collected from various areas off Nova Scotia and New Brunswick using a 0.5-m<sup>2</sup> Van Veen grab, epibenthic sled, scallop dredge, or coarse-meshed plankton net. Animals were collected in the Bras d'Or Lakes, Nova Scotia, from the CSS *Navicula* in August 1989; on the Scotian Shelf from the CSS *Alfred Needler* in February 1989 and 1990 and from the CSS *E. E. Prince* in May 1989 and 1990; on Georges Bank from a commercial scallop dragger in May 1991; and in the St. Croix River, New Brunswick, in June 1993. Collecting sites are shown in Figure 1. In addition, 2 infected amphipods (*Ceradocus torelli*) were removed from the stomach of an Atlantic cod (*Gadus morhua*) (collected from the CSS *Gadus Atlantica* with an otter trawl in the Cabot Strait between Cape Breton Island and Newfoundland, 47°05.5'N, 58°54.1'W) and frozen.

Invertebrates were fixed and preserved in 5% glycerol in 70% ethanol, sorted, identified, and dissected using a stereomicroscope. Nematodes were measured and identified using a compound microscope equipped with a calibrated ocular micrometer or with a calibrated digitizer and drawing tube. All measurements are given as means with the range in parentheses.

### Results

*Hysterothylacium aduncum* was found infecting Acadian hermit crabs (*Pagurus acadianus*), gammaridean amphipods (*Proboloides holmesi*), and caprellid amphipods (*Caprella linearis*) (Table 1). Each amphipod was infected with a single worm, and intensity ranged from 1 to 3 in hermit

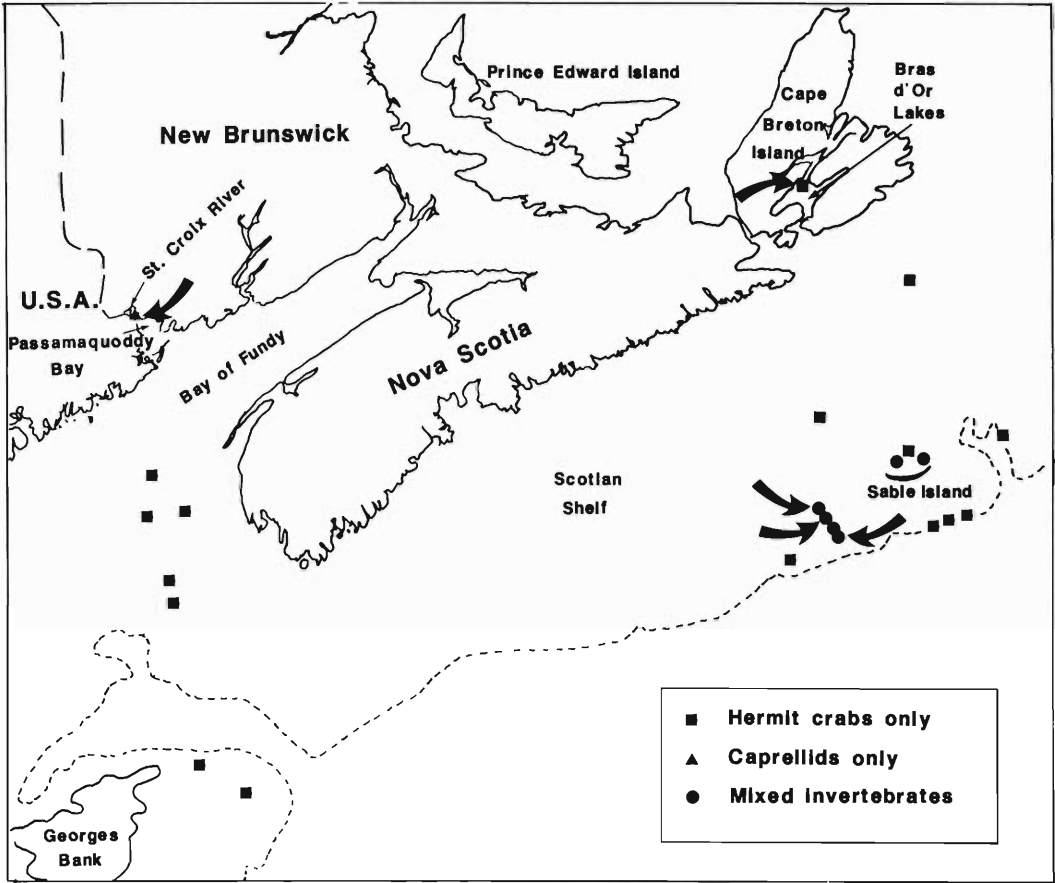


Figure 1. Map depicting invertebrate sampling locations in the Bras d'Or Lakes, on the Scotian Shelf and Georges Bank, Nova Scotia, and on the St. Croix River, New Brunswick. Large arrows indicate where crustaceans infected with *Hysterothylacium aduncum* were found.

crabs. Sites of collection of infected invertebrates are shown in Figure 1. No anisakid was found in 18,210 amphipods belonging to 32 other species, 1,254 mud shrimp (*Crangon septemspinosus*), 1,147 cumaceans, 417 isopods, or 4,819 polychaetes collected in the vicinity of Sable Island in 1989–1990 nor in 74 Acadian hermit

crabs from Georges Bank or 780 hairy hermit crabs (*P. arcuatus* and *P. pubescens*) collected from various areas on the Scotian Shelf and Georges Bank.

All specimens of *H. aduncum* possessed a boring tooth, an intestinal cecum, a short ventriculus, a ventricular appendix, and an excretory

Table 1. Prevalence (%) and mean intensity (M.I. = mean number/infected host) of *Hysterothylacium aduncum* in invertebrates collected in eastern Canada.

Species	N	P (%)	M.I.	Site
<i>Pagurus acadianus</i>	3	100	1.7	Bras d'Or Lakes
Benedict, 1901	331	0.3	1.0	Scotian Shelf
<i>Proboloides holmesi</i>	26	7.7	1.0	Scotian Shelf
Bousfield, 1973				
<i>Caprella linearis</i>	1,664	0.06	1.0	St. Croix River
(Linnaeus, 1767)				

Table 2. New invertebrate hosts for marine species of *Hysterothylacium* published since Norris and Overstreet (1976). Species previously recorded as natural or experimental hosts of any species of *Hysterothylacium* in Norris and Overstreet (1976) are omitted here.

Host	Parasite	Locality	Source
<i>Chnidaria</i>			
<i>Aglaitha digitale</i>	<i>Hysterothylacium</i> sp.	Oslofjord	Svendsen, 1990
<i>Ctenopora</i>			
<i>Mnemiopsis mccardyi</i>	<i>H. aduncum</i>	Black Sea	Gaevskaya and Mordvinova, 1993
<i>Arthropoda</i>			
<i>Copepoda</i>			
<i>Acartia biflora</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Acartia tonsa</i>	<i>H. aduncum</i>	Experimental	Køie, 1993
<i>Calanus sinicus</i>	<i>Thynnascaris</i> sp.	East China Sea	Shimazu, 1982
<i>Centropages hamatus</i>	<i>C. aduncum</i>	Experimental	Val'ter et al., 1979
	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Centropages typicus</i>	<i>Hysterothylacium</i> sp.	Oslofjord	Svendsen, 1990
<i>Cyclospina</i> sp.	<i>C. aduncum</i>	Experimental	Val'ter et al., 1979
<i>Eurytemora hirundoides</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Oncaea borealis</i>	<i>C. aduncum</i>	Experimental	Val'ter et al., 1979
<i>Pseudocalanus elongatus</i>	<i>H. aduncum</i>	Black Sea	Solonchenko and Kovaleva, 1985
<i>Mysidacea</i>			
<i>Mysis stenolepis</i>	<i>H. aduncum</i>	Bras d'Or Lakes	Jackson, 1995
<i>Neomysis integer</i>	<i>H. aduncum</i>	Y'than estuary	Gibson, 1972
	<i>H. aduncum</i>	Isefjord	Køie, 1993
<i>Neomysis intermedia</i>	<i>H. aduncum</i>	Lake Toro	Yoshinaga et al., 1987b
<i>Neomysis japonica</i>	<i>H. haze</i>	Experimental	Yoshinaga et al., 1989
<i>Isopoda</i>			
<i>Idothea</i> sp.	<i>H. aduncum</i>	Isefjord	Køie, 1993
<i>Jaera alibifrons</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
	<i>H. aduncum</i>	Experimental	Køie, 1993
	<i>H. aduncum</i>	Experimental	Køie, 1993
<i>Sphaeroma rugicauda</i>			
<i>Amphipoda</i>			
<i>Amphithoe valida</i>	<i>H. haze</i>	Experimental	Yoshinaga et al., 1989
<i>Anisogammarus kygi</i>	<i>H. aduncum</i>	Salmon hatchery, Japan	Moravec and Nagasawa, 1986
<i>Calliopius laeviscutus</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Caprella linearis</i>	<i>H. aduncum</i>	St. Croix River	This study



Table 2. Continued.

Host	Parasite	Locality	Source
<i>Corophium bonelli</i>	<i>H. aduncum</i>	Experimental	Køie, 1993
<i>Corophium uenoi</i>	<i>H. haze</i>	Experimental	Yoshinaga et al., 1989
<i>Gammarus finmarchicus</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Gammarus oceanicus</i>	<i>H. aduncum</i>	Baltic Sea	Fagerholm, 1982
<i>Gammarus salinus</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Gammarus zaddachi</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Grandidierella japonica</i>	<i>Hysterothylacium</i> sp.	Baltic Sea	Zander et al., 1994
<i>Microdeutopus gryllotalpa</i>	<i>H. aduncum</i>	Experimental	Køie, 1993
<i>Parathemisto abyssorum</i>	<i>H. haze</i>	Experimental	Yoshinaga et al., 1989
<i>Proboloides holmesi</i>	<i>Hysterothylacium</i> sp.	Oslofjord	Svensen, 1990
Euphausiacea	<i>H. aduncum</i>	Scotian Shelf	This study
<i>Thysanoëssa inermis</i>	<i>Hysterothylacium</i> sp.	Scotland	Smith, 1983
Decapoda			
<i>Callinectes sapidus</i>	<i>H. reliquens</i>	Gulf of Mexico	Deardorff and Overstreet, 1981
<i>Callinectes macandreae</i>	<i>H. aduncum</i>	Irish Sea	Calderon-Perez, 1986
<i>Carcinus mediterraneus</i>	<i>C. filiforme</i> (s.i.)	Black Sea	Naidenova and Mordvinova, 1985
<i>Crangon vulgaris</i>	<i>H. aduncum</i>	Ythan estuary	Gibson, 1972
<i>Munidia gregaria</i>	<i>T. adunca</i>	New Zealand	Hurst, 1984
<i>Pagurus acadianus</i>	<i>H. aduncum</i>	Bras d'Or Lakes; Scotian Shelf	This study
Chaetognatha			
<i>Eukrohnia hamata</i>	<i>Contracaecum</i> sp.	Argentine Sea	Mazzoni, 1986
<i>Sagitta crassa</i>	<i>Thynnascaris</i> sp.	Inland Sea of Japan	Shimazu, 1982
<i>Sagitta gazellae</i>	<i>T. adunca</i>	New Zealand	Hurst, 1984
	<i>Contracaecum</i> sp.	Argentine Sea	Mazzoni, 1986
<i>Sagitta minima</i>	<i>T. adunca</i>	New Zealand	Hurst, 1984
<i>Sagitta nagae</i>	<i>Thynnascaris</i> sp.	East China Sea	Shimazu, 1982
<i>Sagitta tasmanica</i>	<i>T. adunca</i>	New Zealand	Hurst, 1984
	<i>Contracaecum</i> sp.	Argentine Sea	Mazzoni, 1986
Echinodermata			
<i>Ophiopholis aculeatus</i>	<i>H. aduncum</i>	Øreslund	Køie, 1993
<i>Ophiura albida</i>	<i>H. aduncum</i>	Øreslund	Køie, 1993

pore situated near the nerve ring. Worms were divisible into 2 size groups: <3.5 mm and >10 mm. Small worms had a conical tail, and larger ones had a bulbous tail terminating in a spine. Similar developmental changes occurred in third-stage larvae of *H. aduncum* in K oie's (1993) laboratory study. The nematodes averaged 5.35 (1.35–11.72) mm in length and 0.094 (0.048–0.180) mm in width at the nerve ring. The mean length of the esophagus was 0.747 (0.207–1.473) mm, the intestinal cecum 0.303 (0.055–0.722) mm, the ventricular appendix 0.194 (0.073–0.850) mm, and the tail 0.091 (0.067–0.118) mm. The ratio of cecal to ventricular appendix lengths was 1:0.96–3.30. The nerve ring averaged 0.194 (0.096–0.299) mm from the anterior end. Voucher specimens (CMNPA 1995-0085–0089) are deposited in the Canadian Museum of Nature (P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4). In addition, 2 fourth-stage larvae, 33.3 and 35.5 mm in length, and 1 adult female *H. aduncum*, 42.4 mm in length, were found in 2 amphipods (*C. torelli*) removed from the stomach of a cod collected in the Cabot Strait. The 3 nematodes each had 3 large lips with short interlabia and a spinous tail. The adult measured 0.483 mm in width, its esophagus 1.952 mm, ventriculus 0.203 mm, intestinal cecum 1.537 mm, ventricular appendix 0.775 mm, and tail 0.343 mm in length, and its nerve ring was 0.508 mm from the anterior end. The other 2 nematodes were too badly decomposed to make measurements of internal structures. No specimens of *C. torelli* were collected in the invertebrate sampling program.

This is the first report of each of these crustaceans as intermediate hosts for *H. aduncum*. New invertebrate host records of marine species of *Hysterothylacium* published since the synopsis of Norris and Overstreet (1976) are listed in Table 2.

The only other parasitic nematode found infecting the invertebrates examined was a single unidentifiable spirurid in a specimen of the gammaridean amphipod *Lembos websteri* collected near Sable Island.

### Discussion

*Hysterothylacium* spp. are among the most widespread parasites in the marine environment, being found in numerous fish species as well as in over 100 invertebrate species from 7 phyla (see Norris and Overstreet, 1976; Table 2, this

study). Chaetognaths and decapods are very common hosts. K oie (1993) suggested that at least 1 intermediate host, a crustacean, is required for transmission of *H. aduncum* to fish. Noncrustacean hosts are successfully infected by consuming infected copepods (K oie, 1993).

Results herein and elsewhere suggest that *H. aduncum* is extremely common in the brackish Bras d'Or Lakes, with high abundances also being detected in mysids and chaetognaths (Jackson, 1995). The high water temperatures and confined nature of the lakes may create a focus of infection, allowing for greatly accelerated transmission. In contrast, infections in macroinvertebrates on the Scotian Shelf were extremely low. Other hermit crabs (*Clibanarius vittatus*) have also been reported elsewhere as hosts for *Hysterothylacium* sp. (Norris and Overstreet, 1976).

Reports of amphipods as hosts for *Hysterothylacium* spp. are becoming more common, and 14 different species have been documented (Table 2) since the synopsis of Norris and Overstreet (1976). Prevalence in amphipods in this study was much lower than in hermit crabs, with only 0.01% of 18,236 amphipods examined from Sable Island Bank being infected. It is curious that only *P. holmesi* was infected on Sable Island Bank. Little is known of the ecology of this species, other than it can be found in association with hydroids and ectoprocts (Bousfield, 1973), as are caprellids, which were infected in the St. Croix River. For some unknown reason, they may be exposed more often to infective stages of *H. aduncum*. Given the wide range of known invertebrate hosts of *H. aduncum*, it is unlikely that some sort of phylogenetic host specificity is involved.

It cannot be stated for certain that the *C. torelli* acts as an intermediate host because the fourth-stage and adult nematodes found may have been parasitic in the cod and migrated into the amphipods after ingestion by the fish. Other anisakid nematodes such as *Goezia minuta* Chandler, 1935, and *Iheringascaris inguies* (Linton, 1901) Deardorff and Overstreet, 1981, are known to occur in partially digested food items in the stomachs of their fish hosts (Deardorff and Overstreet, 1980b, 1981). However, large invertebrates previously have been reported infected with adult *Hysterothylacium* sp. (Margolis and Butler, 1954). On the Scotian Shelf, third- and fourth-stage larvae were found in the mysids *Er-*

*ythrops erythroptalma* and *Mysis mixta* and the shrimp *Crangon septemspinosa* recovered from flatfish stomachs (Martell and McClelland, 1995).

Infected crustaceans were found in both brackish (Bras d'Or Lakes, St. Croix River) and marine (Scotian Shelf) environments. Transmission of *H. aduncum* can also occur in freshwater habitats (Yoshinaga et al., 1987a, b). Similarly, *Hysterothylacium reliquens* (Norris and Overstreet, 1975) Deardorff and Overstreet, 1981, is found in habitats of varying salinity, although Deardorff and Overstreet (1980a) presented evidence suggesting that this species is transmitted only in highly saline environments. The euryhaline tolerance of *H. aduncum*, together with its lack of specificity for intermediate and definitive hosts, no doubt contributes to its broad geographic distribution, being found in both the Atlantic and Pacific oceans, in both the northern and southern hemispheres.

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## 1996 Beltsville Symposium

"Global Genetic Resources: Access, Ownership and Intellectual Property Rights" will be the topic of the 1996 Beltsville Symposium to be held 19-22 May at the Beltsville, Maryland, Agricultural Research Center. Scientists worldwide will explore issues related to ownership of and access to genetic resources and biological specimens around the world. Access to genetic resources affects the ability of scientists to do their job of providing knowledge to benefit the people of the world. While scientists desire free international distribution of germplasm and scientific information, current forces and trends are leading away from this position. A mutually beneficial compromise is needed. This meeting will explore these possibilities. The USDA's Agricultural Research Service and the Association of Systematics Collections will jointly sponsor the 1996 Beltsville Symposium and a 2-day pre-symposium workshop on public affairs advocacy. For more information about the pre-symposium workshop, call Elaine Hoagland (Phone: 202-347-2850; FAX 202-347-0072; e-mail: mnhas001@sivm.si.edu). For information about the symposium, contact Amy Y. Rossman (Phone: 301-504-5364; FAX 301-504-5810; e-mail amy@fungi.ars-grin.gov).

## *Dicelis keymeri* sp. n. (Nematoda: Drilonematidae) from the Earthworm *Octolasion pseudotranspadanum* Zicsi, 1971 (Oligochaeta: Lumbricidae)

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**ABSTRACT:** *Dicelis keymeri* sp. n. from the coelomic cavity of the lumbricid *Octolasion pseudotranspadanum* Zicsi, 1971, collected in Hungary is described. The new species is distinguished from the five other members of the genus by arrangement of male papillae, size of eggs, or shape of oral aperture.

**KEY WORDS:** Nematoda, Drilonematoidea, Drilonematidae, *Dicelis keymeri* sp. n., Oligochaeta.

The Drilonematoidea Chitwood, 1950, are parasitic in the body cavity, the genital tract, and even the excretory system of earthworms (Poinar, 1977, 1978; Spiridonov, 1992). Although the Asian fauna is well diversified, the Holarctic fauna is restricted to 1 genus, *Dicelis*. In this paper, we describe a new species of *Dicelis* recovered from the coelomic cavity of *Octolasion pseudotranspadanum* in Central Europe.

### Materials and Methods

A small collection of nematode parasites of terrestrial Oligochaeta has been deposited in the Natural History Museum of Geneva by Dr. A. Zicsi, Budapest. Nematodes were removed from the body cavity of preserved oligochaetes and were preserved in 70% ethanol before being cleared with lactophenol for study. Figures were made with the aid of a drawing tube. Measurements given are for the holotype male and the allotype female. Measurements in parentheses are the ranges of paratype males and females. All measurements are in micrometers.

### Description

#### *Dicelis keymeri* sp. n.

(Figs. 1–9)

**GENERAL:** Nematoda, Drilonematoidea, Drilonematidae, *Dicelis*. Transparent nematodes lacking lateral alae in both sexes. Cuticle thin.

Sexual dimorphism not prominent. Oral opening triangular with 3 lips. Four papillae on 4 elevations surrounding oral opening, inner papillae not visible and 2 small amphids (Fig. 8). Buccal cavity short (Figs. 1, 5). Esophagus with slightly enlarged valveless bulb (Figs. 4, 8). Nerve ring not visible. Excretory pore not visible.

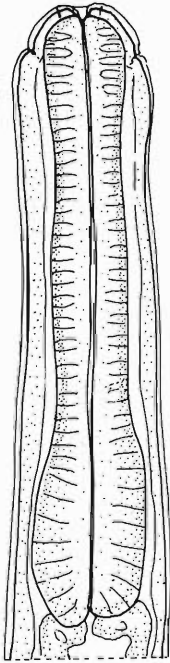
**MALE** (holotype and 2 paratypes) (Figs. 1–4):

Length 2,760 (2,200–2,750). Maximum width 45 (40–60). Esophagus 110 (110–125) long, 20 (15–19) wide. Nerve ring 60 (80–60), reflexed testis 360 (340–410) from anterior extremity. Tail 110 (98–102). Phasmids 90 (82–91) from posterior extremity. Spicules 49 (38–50) long. Gubernaculum 33 (30–36). Testis single and flexed. Tail without caudal alae. Six pairs of papillae: 4 pairs preanal, 1 pair adanal, and 1 postanal (Fig. 4). Spicules separate, gubernaculum present. Phasmid large between the adanal and the postanal pairs of papillae.

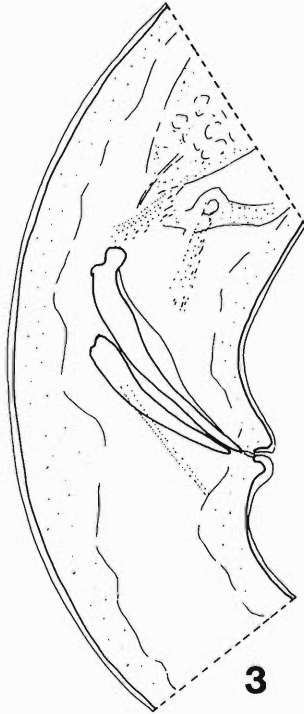
**FEMALE** (allotype and 4 paratypes): Length 3,400 (2,200–3,120). Maximum width 130 (110–110). Esophagus 120 (120–105) long, 20 (25–23) wide. Vulva 1,690 (1,760–1,700) from anterior extremity. Tail 210 (210–210). Phasmids 140 (155–160) from posterior extremity. Eggs 25–30 by 45–50. Tail elongate (Fig. 9). Phasmids large, posterior to anus (Fig. 9). Monodelphic, the ova-

Figures 1–4. *Dicelis keymeri* sp. n. male from *Octolasion pseudotranspadanum*. 1. Esophageal region, lateral view. 2. Caudal region, lateral view. 3. Caudal region, lateral view. 4. Entire worm. Scale bar: 1–3 = 50  $\mu$ m; 4 = 200  $\mu$ m.

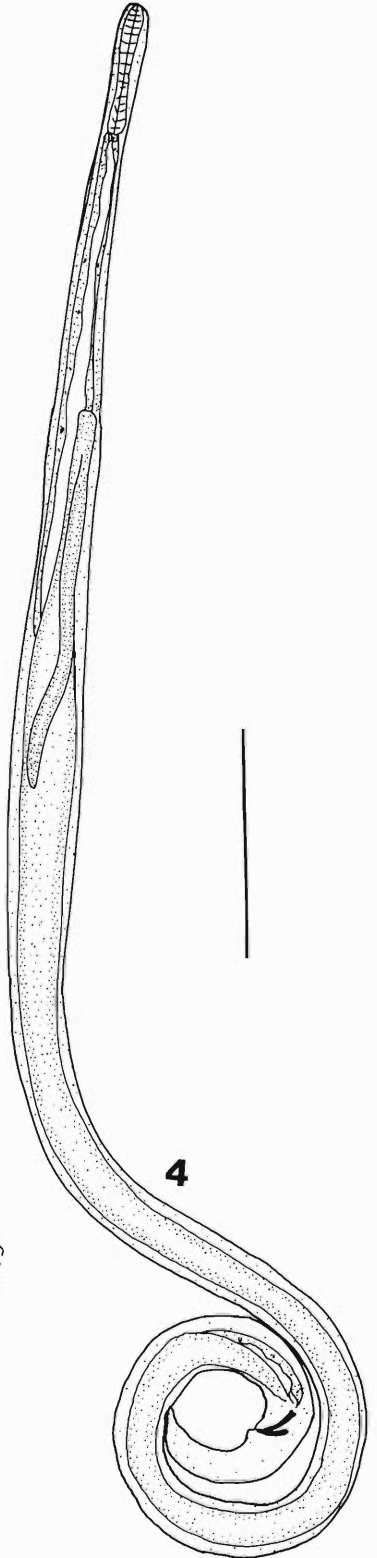
Figures 5–9. *Dicelis keymeri* sp. n. female from *Octolasion pseudotranspadanum*. 5. Cephalic extremity, lateral view. 6. Vulvar region, lateral view. 7. Entire worm. 8. Cephalic extremity in en face view. 9. Caudal extremity, lateral view. Scale bar: 5–6, 8–9 = 50  $\mu$ m; 7 = 400  $\mu$ m.



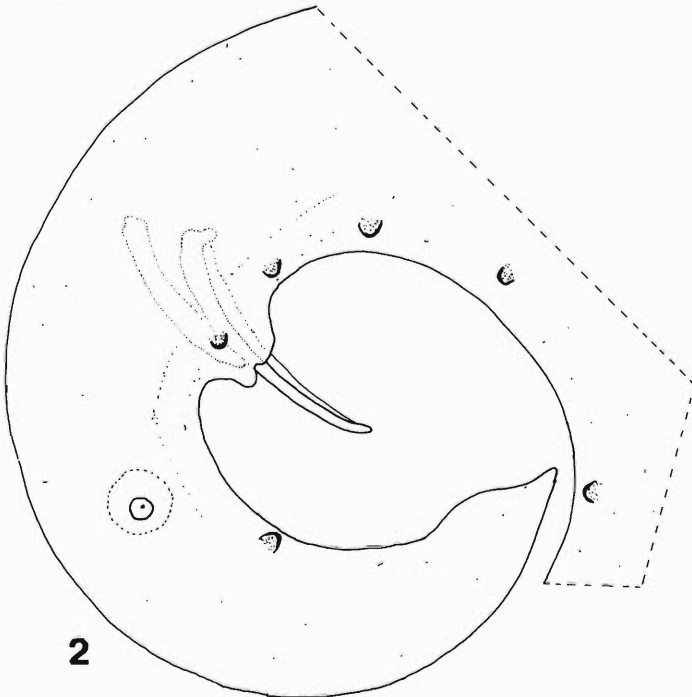
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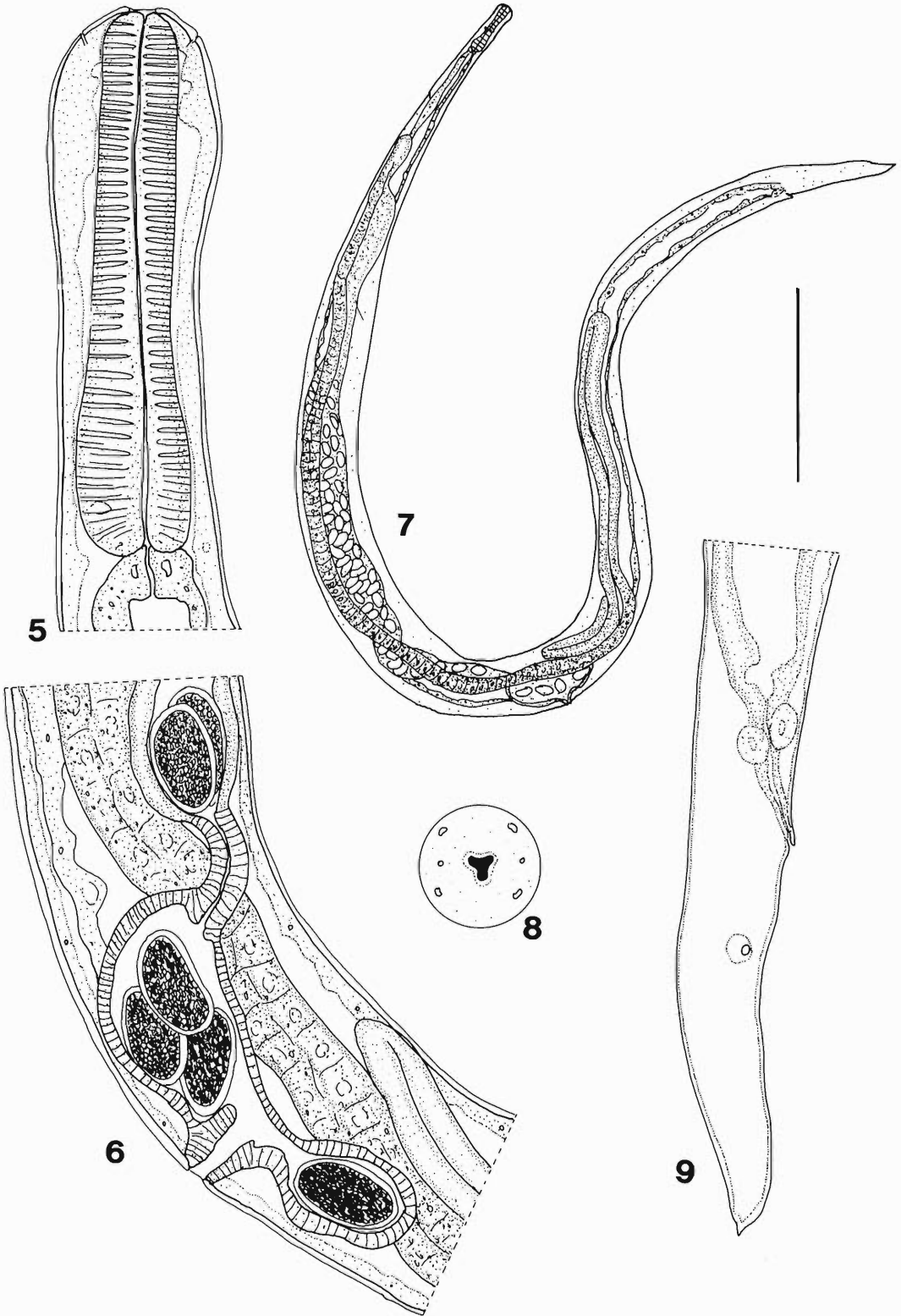
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4



2



ry extends posteriorly to midbody, flexes anteriorly, and then flexes posteriorly behind the esophagus before opening into the uterus. Vulva anterior to middle position leading to a vagina (Fig. 7). Eggs elliptical with smooth surface, numerous (more than 50), larvae absent.

HOST: *Octolasion pseudotranspadanum* Zicsi, 1971.

SITE IN HOST: Coelom.

LOCALITY AND DATE OF COLLECTION: Muratka, Hungary, 06-08-1978 by Dr. A. Zicsi.

SPECIMENS DEPOSITED: Museum d'Histoire Naturelle, CH-1211 Genève 6, Suisse. Holotype male No. 978.551, allotype female No. 978.533, and 4 paratypes, all from a single host specimen.

ETYMOLOGY: The species is named in honor of Dr. Ann Keymer.

### Discussion

Eleven species have been described in the genus *Dicelis*. The type species *Dicelis filaria* Dujardin, 1845, was described from "lombrics" (probably *Lumbricus terrestris*) in France (Dujardin, 1845). This species was redescribed by Wülker (1926) from *Lumbricus rubellus* in Germany but needs further study. Descriptions of Dujardin (1845) and Wülker (1926) do not mention the presence of papillae. However, the length of tail relative to that of the body is much greater in *Dicelis filaria* (9.7% in females, 6.7% in males) than in *Dicelis keymeri* sp. n. (6.2–6.7% in females; 3.7–4.5% in males).

*Dicelis nira* Chitwood and Lucker, 1934, from gonads of *Helodrilus caliginosus* (Oligochaeta) in Beltsville, Maryland (U.S.A.) differs from the present species in the round oral opening and the shape of spicules (Chitwood and Lucker, 1934).

Four species were described by Timm (1962, 1967): *Dicelis dendrobaenae* Timm, 1962, from the coelom of *Dendrobaena* sp. (Oligochaeta) but the locality is unknown; *Dicelis guatemalana* Timm, 1962, from the coelom of *Ramiellona balantina* (Oligochaeta) in Guatemala but the male is unknown; *Dicelis rossica* Timm, 1962, from *Eisenia kusenkoi* in Zailuskii and *Dicelis eiseniae* Timm, 1967, from *Eisenia carolinensis* in Louisiana, U.S.A. *Dicelis dendrobaenae* differs from the present species by the number of caudal papillae in males and in having larger eggs. The male of *Dicelis guatemalana* is unknown, but this species is distinguished by possessing very few large eggs in utero (9–10). The disposition of male

caudal papillae is different in *Dicelis rossica*. The egg shell of *Dicelis eiseniae* is covered with fine spines.

*Dicelis hyrcanus* Belostotskaya et al., 1987, was described from *Eisenia faetida* in South Arzərbayjan (Belostotskaya et al., 1987) and differs from the present species by the egg surface bearing tubercles.

Six species were described by Ivanova (1993, 1994) from oligochaetes collected in Eastern Europe or in Siberia: *Dicelis kimmeriensis* Ivanova, 1993, from *Dendrobaena veneta*; *Dicelis lovatiana* Ivanova, 1993, and *Dicelis lumbricola* Ivanova, 1993, from *Lumbricus rubellus*; *Dicelis pereliae* Ivanova, 1993, and *Dicelis rubidi* Ivanova, 1994, from *Eisenia nordenskioldi*; *Dicelis siberica* Ivanova, 1994, from *Dendrodriulus rubidus*. All these species differ from *Dicelis keymeri* sp. n. by the ornamented surface of their eggs.

The main characteristics that differentiate *Dicelis keymeri* sp. n. from all other species of the genus are a short esophagus and the smallest eggs.

### Acknowledgments

We thank Dr. A. Zicsi, Budapest, who collected the parasites when dissecting lumbricids, and Dr. S. Spiridonov, Institute of Parasitology, Moscow, for comments on the early draft of the manuscript. We also thank two referees for improving the text.

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## Obituary Notice

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Elected to Membership  
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Vice President, 1951

President, 1952

Anniversary Award, 1983

Life Membership, 1985

## Helminth Community Structure of Four Species of *Lepomis* (Osteichthyes: Centrarchidae) from an Oligohaline Estuary in Southeastern Louisiana

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**ABSTRACT:** A total of 141 centrarchids, *Lepomis macrochirus* ( $N = 65$ ), *L. punctatus* ( $N = 45$ ), *L. megalotis* ( $N = 17$ ), and *L. microlophus* ( $N = 14$ ) was collected from an oligohaline estuary in southeastern Louisiana and examined for adult helminths. Eight helminths (4 Trematoda, 2 Nematoda, 2 Acanthocephala) were recovered from the gastrointestinal and urogenital tracts of these hosts. The compound community of these estuarine hosts differed greatly from that of freshwater centrarchids. Two of the 3 most abundant helminths, the trematodes *Barbulostomum cupuloris* Ramsey, 1965, and *Genarchella* sp., have not been reported in freshwater centrarchids and seem to be restricted to an estuarine environment. The remaining helminths, *Crepidostomum cornutum* (Osborn, 1903) Stafford, 1904, *Phyllostomum pearsei* Holl, 1929, *Camallanus oxycephalus* Ward and Magath, 1916, *Spinitectus carolini* Holl, 1928, *Leptorhynchoides thecatus* (Linton, 1891) Kostylew, 1924, and *Neoechinorhynchus cylindricus* (Van Cleave, 1913) Van Cleave, 1919, are common parasites in freshwater centrarchids, but in general, with the exception of *L. thecatus*, their prevalence and abundance were low and their impact on the compound community minimal. *Lepomis punctatus* displayed the highest and *L. microlophus* the lowest levels of helminth diversity. Overall, there were differences in helminth species richness and diversity among the component community of these 4 hosts, and these differences were probably the result of host diet. Interspecific interactions among helminths did not play a significant role in structuring the infracommunity of these hosts.

**KEY WORDS:** parasite, helminth, Centrarchidae, *Lepomis*, estuary, Louisiana, community, site selection.

Holmes and Price (1986) recognized that helminth community structure varies greatly and that the make-up of each community may be regulated by a myriad of biotic and abiotic factors. However, they suggested that helminth communities may be generally characterized as being isolationist or interactive, with each community displaying unique attributes. Several factors that contribute to the development of isolationist communities were discussed by Kennedy et al. (1986). They concluded that, in general, fish hosts harbored a depauperate helminth fauna when compared with other taxonomic groups such as birds because of the simplicity of their enteric system, their ectothermic nature, low vagility, and diet.

Extensive surveys of the helminth fauna of freshwater centrarchids (Bangham, 1938; Bangham and Venard, 1942; Hare, 1943; McDaniel, 1963; McGraw and Allison, 1967; Spall, 1968; Becker and Houghton, 1969; Harley and Keefe, 1970; Meade and Bedinger, 1972; McDaniel and Bailey, 1974; Cloutman, 1975; Jilek and Crites, 1980) have shown that the helminth communities of these hosts display low species richness, and in most cases the helminth species are low in abundance and phylogenetically distant, sug-

gesting that the helminth communities of centrarchid hosts fit an isolationist community model. This conclusion is further supported by Goater et al. (1987), who showed that the composition of the community by phylogenetically unrelated helminth species may be a contributing factor to the isolationist nature of the helminth communities of salamanders.

Although the helminth fauna of freshwater centrarchids has been surveyed extensively, studies that utilize community measures (i.e., index of similarity, diversity index, and evenness) are lacking. Furthermore, no quantitative study has been done on the parasites of freshwater centrarchids when they occur in an estuarine habitat. We have utilized community measures to characterize the helminth community of 4 *Lepomis* species that occur in the oligohaline Lake Pontchartrain/Lake Maurepas estuary.

### Materials and Methods

A total of 65 bluegill, *Lepomis macrochirus* Rafinesque, 1819, 45 spotted sunfish, *L. punctatus* Valenciennes, 1831, 17 longear sunfish, *L. megalotis* Rafinesque, 1820, and 14 redear sunfish, *L. microlophus* Gunther, 1859, was collected from a 1.1-km section of the Interstate-55 canal located between the south bank of Pass Manchac and Ruddock, Louisiana, in St.

John Parish. This man-made canal is part of the Lake Pontchartrain/Lake Maurepas estuary, an oligohaline system located in southeastern Louisiana.

In order to minimize temporal variation within the helminth communities of these hosts, all 141 adult specimens were collected by angling within a 3-mo sampling period from 26 May to 26 August 1991. Most hosts were necropsied within 48 hr of capture; however, a few specimens were frozen and examined at a later date. The sex, weight, and standard length of each host was recorded, and the stomach, pyloric ceca, intestine, and urinary bladder were examined for adult helminths. To determine helminth site selection, and the possible presence of competition, the intestinal tract was partitioned into equal thirds before worm counts were made. All helminths were processed using standard parasitological procedures. When present, the gut contents of the hosts were collected, preserved in 70% ethanol, and identified later. Abbreviations used for all helminth species are found in Table 1.

Parasite data did not meet the parametric assumption of normality. Therefore, differences in helminth abundance between male and female hosts and correlations of helminth intensity with host length were tested using nonparametric tests. In addition, Friedman tests were utilized to determine site selection within the intestinal tract of the hosts.

The Shannon-Weiner diversity index (Shannon, 1948 as cited in Zar, 1984) was used to calculate the helminth diversity of the component community of these 4 hosts. We used *t*-tests to determine whether statistically significant differences existed among the helminth diversity indices of all host species (Zar, 1984). To compensate for the multiple comparisons necessary to test these differences, the *P*-value significance was adjusted to 0.008. The component communities were compared using Morisita's index of similarity (Krebs, 1989) and percent similarity. Prevalence, abundance, and mean intensity (Margolis et al., 1982) of all helminths and the mean number of helminth species per hosts were calculated for all host species. Voucher specimens have been deposited in the U.S. National Parasite Collection (Accession Nos. 84483–84490).

## Results

### Hosts

Among the host species *Lepomis punctatus* (standard length [SL] = 104.11 ± 10.15; range = 84.5–126.1 mm), *L. megalotis* (SL = 103.02 ± 10.42; range = 81.6–117.2 mm), and *L. microlophus* (SL = 125.81 ± 15.71; range = 104.0–155.2 mm), there were no significant differences in standard length between male and female hosts (*t*-test, *P* > 0.05). Although *Genarchella* sp. was more abundant in male than in female *L. punctatus* (Mann-Whitney *U*-test, *z* = -2.188, *P* < 0.05), males and females of these 3 host species were pooled for subsequent helminth community analysis. However, a significant difference in standard length was found between male (SL = 122.62 ± 14.59 mm) and female (SL = 113.53

± 13.92 mm) *L. macrochirus* (*t*-test, *P* < 0.05). In this host, *P. pearsei* was the only helminth that showed a statistically significant correlation with host length (Kendall's correlation, Tau = -0.043, *P* < 0.05). However, this helminth was so rare (Table 1) that the relationship was biologically unimportant. Therefore, male and female *L. macrochirus* also were pooled for subsequent helminth community analysis.

### Helminth community structure

Eight helminth species (4 Trematoda, 2 Nematoda, 2 Acanthocephala) were recovered from the alimentary and urogenital tracts of 4 centrarchid host species (Table 1). *Lepomis macrochirus* harbored all 8 helminth species, whereas only 3 species were found in *L. microlophus*. Five and 6 species of helminths were recovered from *L. punctatus* and *L. megalotis*, respectively (Table 2).

The most common trematodes were *Barbulostomum cupuloris* and *Genarchella* sp. Although they were the only 2 trematodes recovered from all 4 host species, their prevalence, abundance, and mean intensity were greater in *L. microlophus*. Only 3 specimens of *B. cupuloris* were recovered from a single specimen of *L. macrochirus*, and 4 specimens of *Genarchella* sp. were found in 1 *L. megalotis*. The remaining 2 trematode species were rare; 1 specimen of *Crepidostomum cornutum* and 2 specimens of *Phyllodistomum pearsei* were recovered from *L. macrochirus*. The most common nematode was *Camallanus oxycephalus*, which infected 3 of the 4 host species (Table 1). Overall, this helminth was uncommon within the compound community of these hosts, but it was the most prevalent (52%) and abundant (0.75) helminth in the component community of *L. macrochirus*. In addition, the intensity of this nematode displayed a statistically significant (Tau = -0.29, *P* < 0.05) negative correlation with the standard length of the host *L. megalotis*. *Lepomis macrochirus* and *L. megalotis* harbored *Spinitectus carolini*; however, this nematode displayed low prevalence, abundance, and mean intensity within the component community of both host species (Table 1). The acanthocephalan *Leptorhynchoides thecatus* was recovered from all 4 host species. Although rare within the component community of *L. macrochirus* and *L. punctatus*, this helminth was recovered from 100% of *L. megalotis* and *L. microlophus* and was the most abundant helminth in the component communities of these

Table 1. Prevalence, abundance, and mean intensity (range in parentheses) of all helminths recovered from 4 species of *Lepomis*.

<i>Lepomis</i> species	N	Helminth* (site†)									
		Bacu (SI)	Gesp (ST)	Crco (CE)	Phpe (UB)	Caox (CE, SI, LI)	Spca (SI)	Leth (SI)	Necy (SI)		
<i>L. macrochirus</i>	65										
Prevalence		1%	20%	1%	3%	52%	18%	3%			
Abundance		0.05	0.51	0.02	0.03	0.75	0.29	0.03			
Mean intensity		3-	2.5 (1-11)	1-	1 (1)	1.4 (1-4)	1.6 (1-3)	1 (1)			
<i>L. punctatus</i>	45										
Prevalence		36%	13%	0	0	36%	0	24%			
Abundance		1.09	1.42	0	0	0.71	0	0.58			
Mean intensity		3 (1-11)	10.6 (2-23)	0	0	2 (1-11)	0	2.4 (1-5)			
<i>L. megalotis</i>	17										
Prevalence		18%	6%	0	0	35%	12%	100%			
Abundance		0.29	0.24	0	0	0.53	0.18	9.94			
Mean intensity		1.6 (1-3)	4-	0	0	1.5 (1-4)	1.5 (1-2)	9.9 (4-31)			
<i>L. microlophus</i>	14										
Prevalence		86%	64%	0	0	0	0	100%			
Abundance		13.93	8.50	0	0	0	0	31.64			
Mean intensity		16.3 (1-33)	13.2 (1-54)	0	0	0	0	31.6 (2-88)			

\* Bacu, *Barbulostomum cupuloris*; Gesp, *Genarchella* sp.; Crco, *Crepidostomum cornutum*; Phpe, *Phyllodistomum pearsei*; Caox, *Camallanus oxycephalus*; Spca, *Spinitectus carolini*; Leth, *Leptorhynchoides thecatus*; Necy, *Neoechinorhynchus cylindricus*.

† CE, ceca; SI, small intestine; LI, large intestine; ST, stomach; UB, urinary bladder.

**Table 2.** Shannon-Weiner diversity index and evenness of the helminth component community of all host species, species richness, and the mean number of helminth species per host.

<i>Lepomis</i> host	<i>N</i>	Mean no. of helminth species per host	<i>H'</i>	<i>H'/H'</i> <sub>max</sub>	Species richness
<i>L. macrochirus</i>	65	1.0	0.609	0.674	8
<i>L. punctatus</i>	45	1.2	0.643	0.921	5
<i>L. megalotis</i>	17	1.8	0.244	0.314	6
<i>L. microlophus</i>	14	2.4	0.414	0.868	3

hosts (Table 1). A statistically significant correlation was found between this helminth and the standard length of *L. microlophus* ( $\text{Tau} = 0.538, P < 0.01$ ). *Neoechinorhynchus cylindratus* infected all host species except *L. microlophus* but showed its highest prevalence, abundance, and mean intensity in *L. punctatus*.

The Shannon-Weiner function showed that the helminth component community of *L. punctatus* had the highest species diversity ( $H' = 0.643$ ) and evenness ( $H'/H'_{\text{max}} = 0.921$ ; Table 2). In contrast, the lowest values of helminth species diversity and evenness were shown by *L. megalotis* ( $H' = 0.244, H'/H'_{\text{max}} = 0.314$ ). With the exception of the comparison between *L. macrochirus* and *L. punctatus* (Student's *t*-test,  $t = 0.8672, P > 0.05$ ), the helminth diversity among all other host species was significantly different (Student's *t*-test,  $t > 3.291, P < 0.001$ ; Table 3). Morisita's index of similarity, and percent similarity, showed that the helminth community of *L. microlophus* and *L. megalotis* displayed the highest similarity (Morisita's index = 0.869, percent similarity = 63%), whereas the component communities of *L. macrochirus* and *L. megalotis* were the least similar (Morisita's index = 0.086, percent similarity = 14.2%; Table 3).

Qualitative analysis of gut contents showed that *L. microlophus* ( $N = 4$ ) fed exclusively on amphipods, isopods, and bivalves. Insects were the primary prey of *L. macrochirus*. Insect remains were collected from 10 of 14 specimens with gut contents (71%), but this host also fed on crustaceans such as amphipods, isopods, and copepods, as well as small fish. Plant material, probably ingested while feeding on insects and other prey, was recovered from 7 specimens. Amphipods were the most common prey (92%) of 12 *L. punctatus* with gut contents; however, insects, sponges, and decapods were also ingested by this host. None of the 14 specimens of *L. megalotis* contained gut contents.

Friedman tests were used to analyze site selection in *B. cupuloris* and *L. thecatus*. These 2 helminths were the most abundant species found within the intestinal tract of their hosts and were chosen to examine whether competition played a significant role in structuring the helminth community. Results showed that these 2 helminths were site specific. The site preference of *B. cupuloris* in the absence of *L. thecatus* was examined in *Lepomis punctatus*. *Barbulostomum cupuloris* showed a preference for the anterior two-thirds of the intestinal tract. A total of 23 specimens was recovered from both the anterior and middle section of the intestine, but no worms were found occupying the posterior third ( $N = 13, \chi^2 = 9.90, P < 0.01$ ). In *Lepomis megalotis*, the site preference of *L. thecatus* was established in the absence of *B. cupuloris*. Totals of 142, 7, and 2 specimens of *L. thecatus* were recovered from the anterior, middle, and posterior sections of the intestinal tract, respectively ( $N = 14, \chi^2 = 22.26, P < 0.001$ ). *Barbulostomum cupuloris* and *L. thecatus* were most prevalent and abundant in *Lepomis microlophus* (Table 1); therefore, this host afforded us the opportunity to examine the site distribution of these helminths when concurrent infections were com-

**Table 3.** The *t*-values of Shannon-Weiner diversity index comparisons, percent similarity, and Morisita's index of similarity among the component community of 4 species of *Lepomis*.

Community comparison	<i>t</i> -value	% similarity	Morisita's index
<i>L. macrochirus</i> - <i>L. punctatus</i>	0.867	54.1	0.697
<i>L. macrochirus</i> - <i>L. microlophus</i>	5.228***	20.2	0.173
<i>L. macrochirus</i> - <i>L. megalotis</i>	7.084***	14.2	0.086
<i>L. punctatus</i> - <i>L. microlophus</i>	13.428***	55.7	0.611
<i>L. punctatus</i> - <i>L. megalotis</i>	10.134***	25.1	0.291
<i>L. megalotis</i> - <i>L. microlophus</i>	-4.545***	63.2	0.869

\*\*\*  $P < 0.001$ .

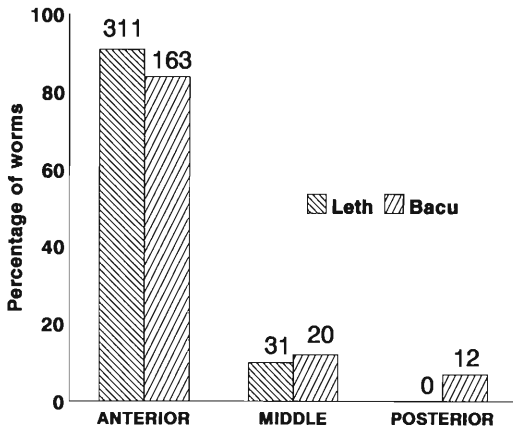


Figure 1. Percentage and total number of *Leptorhynchoides thecatus* and *Barbulostomum cupuloris* recovered from 3 intestinal sections of *Lepomis microlophus* (see Table 1 for abbreviations of scientific names).

mon and abundances were high. Both helminths overlapped in distribution and were recovered mostly from the anterior third of the intestinal tract. Totals of 163, 20, and 12 specimens of *B. cupuloris* ( $N = 12$ ,  $\chi^2 = 15.85$ ,  $P < 0.001$ ) and 311, 31, and 0 specimens of *L. thecatus* ( $N = 12$ ,  $\chi^2 = 22.37$ ,  $P < 0.001$ ) were recovered from the anterior, middle, and posterior segments respectively (Figure 1).

### Discussion

Lakes Pontchartrain and Maurepas are part of an oligohaline estuary that serves as a corridor to the Gulf of Mexico. The salinity of this large estuary ranges from 0‰ at the western shore of Lake Maurepas to 15‰ at the eastern shore of Lake Pontchartrain; however, at our study site it never exceeded 3‰. In this environment both freshwater and marine organisms are found. Such a unique habitat afforded us the opportunity to examine the role of ecological associations in structuring the helminth communities of freshwater hosts occupying a brackish water environment.

Unlike any published reports of centrarchid parasite communities, 2 of the 3 most abundant species in the compound community of these hosts were *Barbulostomum cupuloris* and *Genarchella* sp. Neither species has ever been found in centrarchid hosts inhabiting strictly freshwater habitats, and their distribution seems to be restricted to an estuarine environment. Ramsey (1965) described *B. cupuloris* from *L. punctatus*

and *L. microlophus* collected from the Lake Pontchartrain estuary. The absence of this trematode and the presence of the closely related *Homalometron armatum* in centrarchid hosts collected from freshwater ponds near the brackish estuary led Ramsey (1965) to conclude that *B. cupuloris* was dependent on an intermediate host restricted to a brackish water environment. This same explanation may account for the presence of *Genarchella* sp., an undescribed hemiurid sharing morphological characteristics with *G. isabellae* (Lamothe-Argumedo, 1977) and *G. overstreeti* (= *Paravitellotrema overstreeti* Brooks, Mayes, and Thorson, 1979) described from Mexican and Colombian freshwater fishes, respectively.

The remaining helminths in the compound community of these hosts have been reported previously from freshwater habitats (Bangham, 1938; Bangham and Venard, 1942; Hare, 1943; McDaniel, 1963; McGraw and Allison, 1967; Spall, 1968; Becker and Houghton, 1969; Harley and Keefe, 1970; Meade and Bedinger, 1972; McDaniel and Bailey, 1974; Cloutman, 1975; Jilek and Crites, 1980). However, with the exception of *L. thecatus*, these helminths displayed low prevalence and abundance. In addition, several adult helminths found in freshwater centrarchid hosts, such as *Rhipidocotyle septapapillata*, *Crepidostomum cooperi*, *Homalometron armatum*, *Pisciamphistoma reynoldsi*, *P. stunkardi*, *Phyllodistomum lohrenzi*, *Anallocreadium pearsei*, *Spinitectus gracilis*, *S. macrocanthus*, *Contraecaeum brachyurum*, *Rhabdochona decaturensis*, *Capillaria catenata*, *Proteocephalus* sp., and *Bothriocephalus claviceps* (see Bangham, 1938; Bangham and Venard, 1942; Spall, 1968; Meade and Bedinger, 1972; Jilek and Crites, 1980), were not recovered in our study.

The negative correlation displayed by the intensity of *C. oxycephalus* with the length of *L. megalotis* may be due to an ontogenetic shift in habitat or diet from smaller prey, such as copepods, which serve as intermediate hosts for this nematode, to larger prey as the host increases in size. Among the acanthocephalans, *Leptorhynchoides thecatus* showed a statistically significant correlation with host length in *Lepomis microlophus*. DeGiusti (1949) showed that the intermediate host of this acanthocephalan is the amphipod *Hyaella azteca*. The relationship between helminth intensity and host size may have been a result of an increase in the number of infected amphipods consumed by larger hosts,

or, as Ewald and Nickol (1989) concluded from their study of *L. cyanellus*, infrapopulations of this acanthocephalan may be regulated by the availability of cecal space. The trematode *P. pearsei* was rare, and the statistically significant correlation with host length in *L. macrochirus* was biologically insignificant. Overall, host sex did not play a major role in structuring the parasite community of these centrarchids. Similar results were obtained by Lawrence (1970), Cloutman (1975), and Aho et al. (1991). With the exception of *Genarchella* sp., which was more abundant in male than in female *L. punctatus*, host gender had no effect on helminth abundance.

Parasite community diversity differed among hosts. Bell and Burt (1991) concluded that helminth diversity was correlated with host body size and diet but that body size alone did not account for much of the variation in helminth diversity found among taxa. Diet analyses revealed that, in general, hosts that preyed primarily on benthic crustaceans and fish harbored a more diverse helminth fauna than did hosts that fed on detritus, vegetation, and insects (Bell and Burt, 1991). In general, in our study host size did not affect the abundance of helminth species. Because all of the helminths we recovered have indirect life cycles, we believe that differences among the helminth diversity of these four centrarchids may be attributed to diet. *Lepomis macrochirus*, a generalist feeder (Deselle et al., 1978; Levine, 1980), is exposed to a greater number of potential intermediate host species, thus resulting in higher helminth richness than *L. microlophus*, a specialized predator. In contrast to parasite species richness, differences in parasite prevalence or abundance among the component community of these centrarchids may represent a differential utilization of prey items by these hosts. However, because the mean number of helminth species per host does not seem to be related to diet diversity, we should note that some of the variation in species richness found among the component community of these hosts may be a result of sampling effort (Table 2).

*Barbulostomum cupuloris* and *Leptorhynchoides thecatus* did show site preference in *L. punctatus* and *L. megalotis*, respectively. Habitat shift within a host is a good indicator of interspecific interaction among parasites (Chappell, 1969; Holmes, 1973; Stock and Holmes, 1988). In *L. microlophus*, concurrent infections of *B.*

*cupuloris* and *L. thecatus* were common and both helminths were highly abundant. In this host, the spatial distribution of both helminths overlapped (Fig. 1) and neither helminth showed a shift from its preferred site, thus indicating a lack of interaction.

As Kennedy et al. (1986) found for freshwater fish in general, the helminth community of estuarine centrarchids in our study was depauperate and displayed attributes of an isolationist community as described by Holmes and Price (1986). Unlike prior surveys of freshwater centrarchids, 2 of the 3 helminths that seem to dominate the compound community of these estuarine hosts do not occur in freshwater habitats. Therefore, the presence of the trematodes *B. cupuloris* and *Genarchella* sp. seems to affirm the importance of ecological associations in structuring the helminth community of these estuarine hosts.

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## Redescription of *Dollfusentis heteracanthus* (Acanthocephala: Illiosentidae) from Bonefish, *Albula vulpes*, in the West Indies

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**ABSTRACT:** The original description of *Dollfusentis heteracanthus* (Cable and Linderoth, 1963) Golvan, 1969, was based on 6 immature adults and provided few measurements, which were incompatible with those of fully grown adults. The species is redescribed based on 27 fully developed mature adults collected from bonefish, *Albula vulpes* Linnaeus (Albulidae), in the West Indies. New information on sexual dimorphism, variability, and anatomical structures not included in the original description is added. The rarely observed copulatory interface is also described.

**KEY WORDS:** *Dollfusentis heteracanthus*, Acanthocephala, redescription, West Indies, *Albula vulpes*.

*Dollfusentis heteracanthus* (Cable and Linderoth, 1963) Golvan, 1969 (= *Illiosentis heteracanthus* Cable and Linderoth, 1963), was originally described from 6 immature adults (5 males, 1 female) from 4 marine fish species examined in Curacao, West Indies. Although the diagnostic features of the species are clearly definitive, reported measurements are inadequate and reflect only the very small size of the few immature structures measured. The only sex-linked measurements reported (in mm) are those of female trunk ( $3.68 \times 0.45$ ), distance between uterine bell and posterior end of trunk (0.5), male trunk  $3.0\text{--}4.43 \times 0.34\text{--}0.50$ , anterior testis (0.20–0.38 long), and posterior testis (0.18–0.30 long) (Cable and Linderoth, 1963). The few other measurements provided apply to both sexes: proboscis  $0.86\text{--}1.5 \times 0.07\text{--}0.15$ , crescent hooks 0.074–0.102 long, anterior hooks 0.032–0.051 long, middle hooks 0.032–0.041 long, posterior closely spaced hooks 0.010–0.020 long, trunk spines 0.020 long anteriorly and 0.030 posteriorly, proboscis receptacle 0.825–1.35 long.

### Materials and Methods

Twenty-seven sexually mature adult *D. heteracanthus* (males with sperm and gravid females) were collected from the intestines of 6 of 12 bonefish, *Albula vulpes* Linnaeus, netted in Bell Sound, 11.2 km north of South Caicos, Turks, and Caicos islands, British West Indies, in September 1992. Worms were fixed in alcohol–formalin–acetic acid, stained in Mayer's acid carmine, dehydrated in ascending concentrations of ethanol, cleared in graduated concentrations of terpineol in 100% ethanol, and whole mounted in Canada balsam. Measurements are of the longest and widest

dimensions of measured structures. Trunk measurements do not include neck or male bursa. Hooks were measured only in perfect profile and counted from at least 2 adjacent rows. All measurements are in micrometers unless otherwise specified (means are in parentheses). Figures are made to illustrate structures not included in or at variance with those presented by Cable and Linderoth (1963).

### Results

Some of our worms reached as long as 6 times those described by Cable and Linderoth (1963), and all other measured structures are correspondingly larger. Despite these size discrepancies, our worms were identified as *D. heteracanthus* based on having 26–29 proboscis hooks per row that are dorsoventrally dimorphic, dorso-lateral interruption of trunk spines, close proximity of the ventral crescent to other hooks, lateral proboscis papillae between ninth and eleventh hooks from posterior end of row, 6–8 reduced proboscis hooks at posterior end of each row, and shape of female posterior extremity.

The species is herein redescribed (based only on our material) to provide (1) a complete account of the typical size and usual variations in anatomical structures of fully developed, sexually mature adults, (2) a description of mature structures and measurements of other structures not previously reported or measured, e.g., reproductive system and lemnisci, (3) a description of variations, e.g., in trunk spines, not previously possible because of sample size, and (4) corrected and refined measurements of Cable and Linderoth (1963) material that are related to the im-

maturity of the type specimens, e.g., size of trunk spines; differential rate of growth may be involved.

Examination of the type material confirmed their immature state and the justification for re-description. One criterion of immaturity (in addition to the incomplete development of the reproductive system and size) is the relatively larger size of proboscis compared with trunk. In mature adults, the trunk continues to grow after the proboscis and its hooks have reached their full size (see Amin and Redlin, 1980; Amin, 1987). In Cable and Linderoth's (1963) specimens, the proboscis length : trunk length was ca. 1:3.0. In our specimens, this ratio was 1:5.5 in males and 1:8.0 in females. The small and unrepresentative size of the type specimens, the incompletely developed male reproductive system, the inapparent proboscis receptacle swelling and brain, and the limited variation in distribution and pattern of trunk spines were clearly visible.

The following redescription is based on 20 fully developed mature adults (10 males with sperm and 10 gravid females).

### Redescription

#### *Dollfusentis heteracanthus*

(Cable and Linderoth, 1963) Golvan, 1969

(Figs. 1-4)

**GENERAL:** With characters of the genus *Dollfusentis*. Shared structures larger in females than in males. Trunk cylindrical and elongate, widest just anterior to middle, with stout hypodermal spines that extend from narrow anterior end of trunk to level of half the length of proboscis receptacle to shortly past its posterior end ventrally and a shorter distance dorsally; anterior spines relatively longer than posterior. Field of trunk spines with a bare ovoid dorsolateral zone that is variably pronounced. Proboscis long, cylindrical, with 14 longitudinal rows of 26-29 rooted hooks each; posterior 6-8 (usually 7-8) hooks of each row much reduced in size, strongly recurved, closely spaced with posteriormost largest. Ventrolateral crescent of 6 large hooks just posterior to posteriormost hooks. Other proboscis hooks outside the crescent normal, anteriormost about as long as longest ventral hooks at middle of proboscis but less robust; middorsal hooks opposite midventral hooks markedly thinner and relatively shorter; a pair of lateral papillae present between ninth and eleventh hooks from posterior end of hook rows. Neck contin-

uous with proboscis and invariably bent ventrad. Proboscis receptacle slightly longer than proboscis and widest near its anterior end where the brain is located in a nearly rectangular enlargement. Lemnisci usually equal, about 3 times as long as proboscis receptacle, extending to posterior testis in males and to a corresponding distance in females, may be folded once or twice.

**MALES:** Trunk 5.200-11.800 (7.535) mm long by 0.760-1.200 (0.844) mm wide with trunk spines somewhat longer anteriorly, 25-45 (37), than posteriorly, 25-35 (32). Proboscis 1.120-1.820 (1.365) mm long by 112-210 (149) wide; anterior hooks 41-57 (50) long; midventral hooks 48-67 (56) long by 13-16 (15) wide at base; mid-dorsal hooks 35-45 (40) long by 3-9 (7) wide at base; largest posteriormost reduced hooks 23-35 (27); crescent hooks 80-118 (95) long. Proboscis receptacle 1.260-1.890 (1.621) mm long by 126-238 (176) wide at anterior enlargement (Fig. 1). Lemnisci 3.640-6.020 (4.459) mm long by 140-252 (180) wide. Male reproductive system (Fig. 2) robust and occupies posterior part of body cavity; testes contiguous, in tandem. Anterior testis 0.532-1.400 (0.832) mm long by 266-616 (367) wide. Shorter and wider posterior testis 0.420-1.260 (0.655) mm long by 280-560 (377) wide; 8 club-shaped tightly packed cement glands just behind posterior testis with anterior bulb 98-168 (145) in diameter; common sperm duct enlarged into a well-developed thick-walled bulboid seminal vesicle 252-658 (401) long by 168-518 (289) wide that opens into anterior end of cirrus; large Saeftigen's pouch 0.588-1.470 (0.920) mm long by 266-560 (360) wide; pyriform highly muscular cirrus 420-700 (512) long by 210-476 (291) wide; large well-developed bursa 0.602-1.204 (0.839) mm long by 0.448-1.050 (0.657) mm wide.

**FEMALES:** Trunk 7.000-19.520 (11.772) mm long by 0.800-1.600 (1.040) mm wide with trunk spines somewhat longer anteriorly, 25-64 (38), than posteriorly, 19-42 (30). Proboscis 1.050-1.820 (1.470) mm long by 112-280 (170) wide; anterior hooks 48-64 (53) long; midventral hooks 48-64 (54) long by 16-19 (16) wide at base; mid-dorsal hooks 45-48 (47) long by 7-9 (8) wide at base; largest posteriormost reduced hooks 29-45 (35) long; crescent hooks 96-112 (101) long. Proboscis receptacle 1.400-1.960 (1.590) mm long by 126-196 (156) wide at anterior enlargement. Lemnisci 3.500-5.180 (4.638) mm long by 112-168 (144) wide. Dorsoposterior end receded anteriorly above terminal gonopore (see Cable and

Linderoth, 1963); eggs (Fig. 3) elongate with polar prolongation of middle membrane, 48–64 (56) long by 9–16 (14) in diameter.

**DEFINITIVE HOSTS:** Yellowfin mojarra, *Gerres cinereus* (Walbaum) (Gerreidae); frillfin goby, *Bathygobius soporator* (Valenciennes) (Gobiidae); hairy blenny, *Labrisomus nuchipinnis* (Quoy and Gaimard) (Clinidae); flounder, *Platophrys ocellatus* (Agassiz) (Bothidae); bonefish, *Albula vulpes* Linnaeus (Albulidae).

**SITE OF INFECTION:** Intestine.

**LOCALITY:** West Indies at Curacao, South Caicos, Turks, and Caicos islands.

**DEPOSITED SPECIMENS:** U.S. National Parasite Collection No. 85059 (voucher males and females including those in figures).

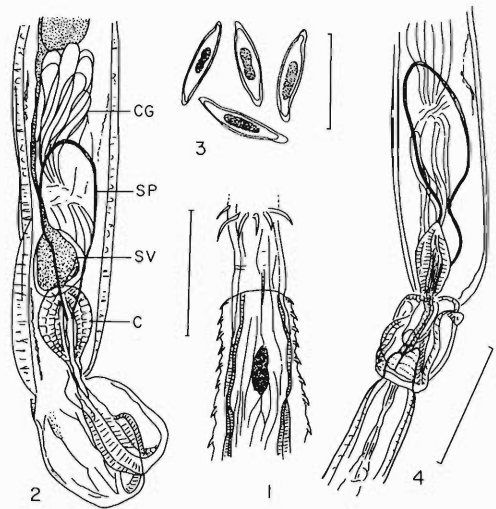
**EXAMINED SPECIMENS:** U.S. National Parasite Collection No. 60343 (holotype and paratypes).

**REMARKS:** This is the first description of mature adult *D. heteracanthus*. The specimens are from a new host, *Albula vulpes*, in the West Indies from where the type material was originally described. The distribution of this acanthocephalan, and perhaps other species of the genus, however, may not be limited to the West Indies and could well extend into the shallow marine waters of the southeastern United States (Amin, 1996).

Considering the state of maturity and development of our worms, bonefish are regarded as natural definitive hosts. Whether the other fish hosts of Cable and Linderoth's (1963) material may be accidental hosts where worms do not reach sexual maturity is unknown. The immature female and 2 males obtained from the flounder, *P. ocellatus*, were juveniles still enclosed within their cystic membranes (Cable and Linderoth, 1963).

Unlike the original description, our specimens show that anterior trunk spines of *D. heteracanthus* are somewhat larger than posterior ones. This feature, thus, becomes consistent in all species of *Dollfusentis*.

The limited number of specimens available to Cable and Linderoth (1963) did not allow the exploration of variability in the distribution of trunk spines. These spines extended ventrally to the level of half the length of proboscis receptacle (LPR) (in 6 males and 2 females), to 50–90% LPR (in 2 males and 4 females), to 100% LPR (in 1 male and 1 female; as in original description), and past the level of the posterior end of proboscis receptacle (in 1 male and 3 females). The bare dorsal area within the field of trunk



Figures 1–4. *Dollfusentis heteracanthus* from *Albula vulpes*. 1. Ventral view of anterior end of a male proboscis receptacle showing broad area housing the brain, the neck, and the posterior end of the proboscis with crescent hooks. 2. Ventral view of same male showing details of reproductive system. CG, cement gland; C, cirrus; SP, Saeftigen's pouch; SV, seminal vesicle. 3. Ripe eggs from the body cavity of a female. 4. Lateral view of posterior regions of a copulating male (above) with bursa enclosing the posterior end of the female. Scale bars = 1 mm (Figs. 1, 2, 4) and 50  $\mu$ m (Fig. 3).

spines was usually evident, but its extent varied considerably.

The number of reduced recurved hooks at the posterior end of proboscis hook rows varied between 6 and 8 (usually 7 or 8) but was more often 7 in males and more evenly distributed in females. The lateral sensory papillae were more often between proboscis hooks 10 and 11 than between 9 and 10 in females, from posterior end of hook rows, but more evenly distributed in males.

The position of the brain is the first to be precisely described in any species of the genus; it is anterior in the *D. heteracanthus* proboscis receptacle.

The lemnisci apparently continue to grow during later developmental stages, becoming about 3 times as long as the proboscis receptacle in our specimens; they were twice as long in immature adults (Cable and Linderoth, 1963).

Four of our worms had body wall anomalies (tegumental swelling) similar to those attributed to glycogen–phospholipid metabolic dysfunction and described in other acanthocephalan species by Amin (1984, 1989).

A male and a female collected and fixed in

copulatory position (Fig. 4) provided an opportunity to document copulatory interface as well as some fully developed adult reproductive structures not included in the original description. The copulating female was gravid with unripe eggs and some ovarian balls, suggesting that this might not have been its first copulation. Note the enclosing of female posterior end within the male bursa, contraction of the edge of the bursal musculature resulting in the constriction of the posterior end of the female trunk, and suction of her gonopore region into the male body. This observation clearly disputes Yamaguti's (1963) belief that evagination of the bursa is a post-mortem phenomenon.

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## Neotropical Monogenoidea. 27. Two New Species of *Telethecium* gen. n. from the Nasal Cavities of Central Amazonian Fishes and a Redescription of *Kritskyia moraveci* Kohn, 1990 (Dactylogyridae, Ancyrocephalinae)

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**ABSTRACT:** Two new species of Dactylogyridae (Ancyrocephalinae) are described from the nasal cavities of Central Amazonian fishes (Brazil): *Telethecium nasalis* sp. n. from *Osteoglossum bicirrhosum* Vandelli (Osteoglossidae), Furo do Catalão, near Manaus, Amazonas; and *T. paniculum* sp. n. from *Pellona flavipinnis* (Valenciennes) (Clupeidae), Rio Solimões, Ilha da Marchantaria, Manaus, Amazonas. *Telethecium* gen. n. is proposed for species having the terminal male genitalia located within a bag protruding from the midventral surface of the anterior trunk, a haptor armed with 14 hooks (12 marginal, 2 subcentral), overlapping gonads, a sinistrolateral vaginal aperture, a coiled male copulatory organ with counterclockwise rings, and a cephalic area lacking well-defined cephalic lobes; anchors, bars and 4A's are absent. *Kritskyia moraveci* Kohn, 1990, from the urinary tract of *Rhamdia quelen* (Quoy and Gaimard) (Pimelodidae) is redescribed. An emended diagnosis of *Kritskyia* Kohn, 1990, is provided.

**KEY WORDS:** Brazil, Monogenoidea, Dactylogyridae, *Telethecium* gen. n., *Telethecium nasalis* sp. n., *Telethecium paniculum* sp. n., *Kritskyia moraveci*, *Osteoglossum bicirrhosum*, *Pellona flavipinnis*, *Rhamdia quelen*.

Monogenoideans from sites other than the gills and skin of fishes have been infrequently studied with a few species recorded from nasal cavities, urinary and digestive systems, and lateral line pits of these hosts. In the Neotropics, only *Rhinoxenus* Kritsky, Boeger, and Thatcher, 1988 (with 4 species), and *Rhinonastes* Kritsky, Thatcher, and Boeger, 1988 (monotypic), have been proposed to accommodate species from the nasal cavities of freshwater fishes (Kritsky et al., 1988a, b; Boeger et al., 1995). Kohn (1990) proposed the monotypic *Kritskyia* for a species from the urinary bladder and ureters of a siluriform fish in Brazil. Although phylogenetic relationships are unknown, it appears that monogenoideans from sites other than the gills and skin form a unique fauna within the Neotropics. In the present paper, *Telethecium* gen. n. is proposed for 2 new species from the nasal cavities of distantly related hosts in the Brazilian Amazon. *Kritskyia moraveci* Kohn, 1990, is redescribed.

### Materials and Methods

Hosts, *Osteoglossum bicirrhosum* Vandelli (Osteoglossidae) and *Pellona flavipinnis* (Valenciennes) (Clupeidae), were collected with nets from the environs of

Manaus, Amazonas, Brazil, during 1984–1989. Methods of parasite collection and preparation of the helminths for study, measurement, and drawing are those of Kritsky et al. (1988a). Measurements (in micrometers) include the average followed by the range and number of structures measured in parentheses. Type specimens and vouchers are deposited in the collections of the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (IOC), the United States National Parasite Collection, Beltsville, Maryland (USNPC), and the University of Nebraska State Museum, Lincoln, Nebraska (HWML).

### Results

#### *Telethecium* gen. n.

**DIAGNOSIS:** Dactylogyridae: Ancyrocephalinae. Body fusiform, comprising cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Cephalic lobes undifferentiated, head organs present, cephalic glands indistinct. Eyes 4; granules elongate ovate. Mouth midventral; pharynx muscular, glandular; esophagus present; intestinal ceca (2) confluent in posterior trunk, lacking diverticula. Gonads overlapping, intercecal; testis dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle a dilation of vas deferens; 1 prostatic reservoir. Male cop-

ulatory organ consisting of sclerotized coiled tube with counterclockwise rings (Kritsky et al., 1985); accessory piece articulated to cirral base, membranous, bipartite, with distal portion serving as guide for male copulatory organ; copulatory complex (male copulatory organ + accessory piece) lying within bag that protrudes from anteroventral surface of trunk. Vaginal aperture sinistrolateral; seminal receptacle anterior to germarium; uterus indistinct; genital pore midventral. Vitellaria coextensive with intestinal ceca. Haptor posteroventrally concave, with 14 hooks (12 submarginal, 2 subcentral); 1 hook may be absent in some specimens. Anchors, bars, 4A's absent. Parasites of the nasal cavities of freshwater Neotropical fishes.

**TYPE SPECIES:** *Teletecium nasalis* sp. n. from *Osteoglossum bicirrhosum* Vandelli (Osteoglossidae).

**OTHER SPECIES:** *Teletecium paniculum* sp. n. from *Pellona flavipinnis* (Valenciennes) (Clupeidae).

**ETYMOLOGY:** The generic name is from Greek (*tele* = far, far off + *theco* = a case for something) and refers to the protruding bag containing the terminal male genitalia.

***Teletecium nasalis* sp. n.**  
(Figs. 1–5)

**HOST AND LOCALITY:** Nasal cavity of *Osteoglossum bicirrhosum* Vandelli (Osteoglossidae); Furo do Catalão, near Manaus, Amazonas, Brazil (10 January 1989).

**SPECIMENS STUDIED:** Holotype, IOC 33.639; 17 paratypes, USNPC 84842, HWML 38345.

**DESCRIPTION:** Body 306 (267–332;  $N = 10$ ) long; greatest width 95 (84–105;  $N = 5$ ) near midlength; cephalic region narrow. Eyes equidistant; members of posterior pair larger than those of anterior pair; eye granules small; accessory granules absent to numerous in cephalic region. Pharynx spherical, 21 (20–22;  $N = 5$ ) in diameter; esophagus short. Peduncle indistinct; haptor 32 (30–35;  $N = 5$ ) long, 51 (48–58;  $N = 5$ ) wide. Hooks 20 (19–21;  $N = 23$ ) long, similar; each with slightly protruding broad thumb, delicate point, shank comprised of 2 subunits; proximal subunit expanded; FH loop  $\frac{1}{2}$  shank length;

1 hook frequently absent with FH loop remaining (8 of 18 specimens with 13 hooks) (Fig. 1). Male copulatory organ with about 1.5 rings; base of male copulatory organ with variable sclerotized margin; male copulatory organ 82 (75–88;  $N = 4$ ) long, proximal ring diameter 19 (17–20;  $N = 10$ ). Accessory piece 26 (23–30;  $N = 10$ ) long, pincer-shaped. Gonads ovate; testis 44 (37–53;  $N = 5$ ) long, 26 (22–30;  $N = 3$ ) wide; seminal vesicle C-shaped, with delicate wall; prostatic reservoir saccate. Germarium 55 (50–67;  $N = 4$ ) long, 32 (30–33;  $N = 3$ ) wide; oviduct, ootype, uterus not observed. Vagina with sclerotized surface plate, distal sclerotized canal funnel-shaped, opening into large seminal receptacle.

**REMARKS:** *Teletecium nasalis* is the type species for the genus. It differs from its congener by having a larger accessory piece, a structurally complex vaginal aperture, and slightly protruding hook thumbs. In *T. paniculum*, the hook thumbs are depressed, the sclerotized vaginal aperture is simple, and the accessory piece lacks a pincer shape.

**ETYMOLOGY:** The specific name is from Latin (*nas/o* = the nose + *-alis* = pertaining to) and refers to the site of infestation on its host.

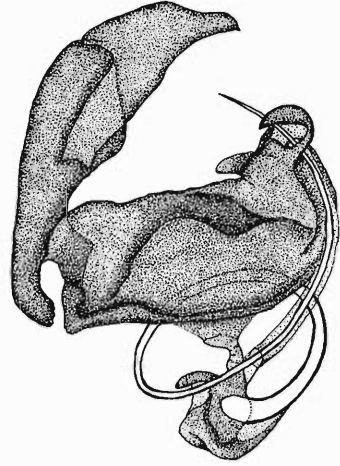
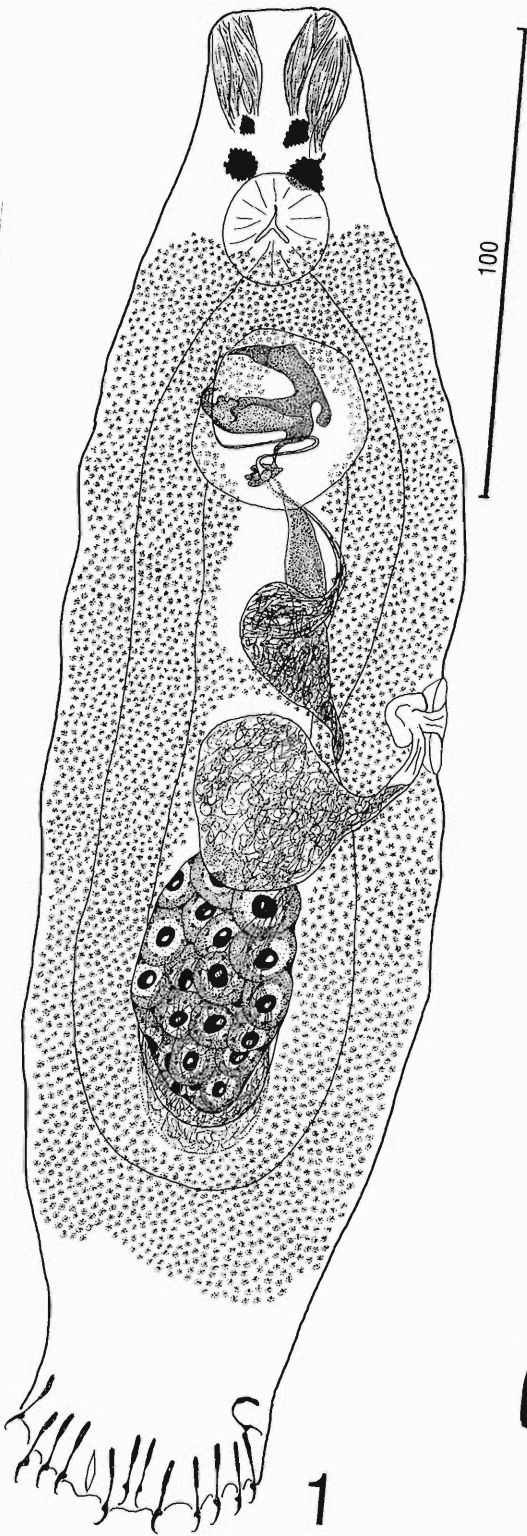
***Teletecium paniculum* sp. n.**  
(Figs. 6–9)

**HOST AND LOCALITY:** Nasal cavity of *Pellona flavipinnis* (Valenciennes) (Clupeidae); Rio Solimões, Ilha da Marchantaria, near Manaus, Amazonas, Brazil (14 September 1984).

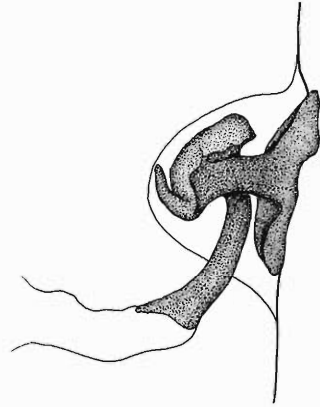
**SPECIMENS STUDIED:** Holotype, IOC 33.640; 4 paratypes, USNPC 84843, HWML 38344.

**DESCRIPTION:** Body 287 (276–297;  $N = 2$ ) long, conical; greatest width 87 (77–94;  $N = 3$ ) in posterior trunk. Cephalic margin narrow, tapered anteriorly. Eyes equidistant, compact; members of posterior pair larger than those of anterior pair; eye granules small; accessory granules uncommon in cephalic, anterior trunk regions. Pharynx subspherical, 23 (22–24;  $N = 3$ ) in diameter; esophagus short. Peduncle broad; haptor 42 (41–45;  $N = 3$ ) long, 71–72 ( $N = 2$ ) wide. Hooks 19 (18–20;  $N = 6$ ) long, similar; each with depressed thumb, delicate point, shank comprised of 2 subunits; proximal subunit ex-

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**Figures 1–5.** *Teletecium nasalis* gen. et sp. n. 1. Holotype (ventral view; note that 1 haptor hook in this specimen has been lost but the FH loop remains). 2. Copulatory complex. 3. Vagina. 4. Hook. 5. Lateral view of specimen showing relationship of body and copulatory bag (scale not provided). Figure 1 is drawn to the 100- $\mu$ m scale; Figures 2–4 are drawn to the 25- $\mu$ m scale.



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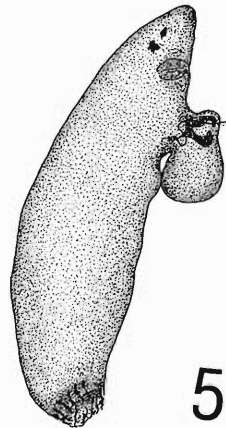


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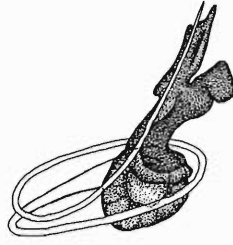
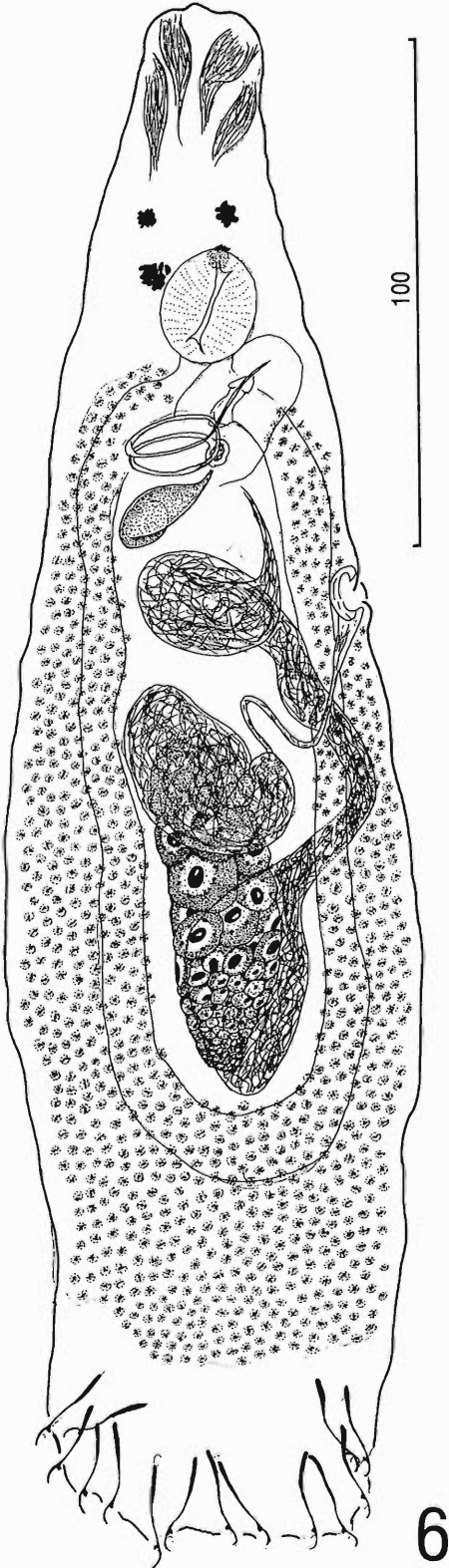
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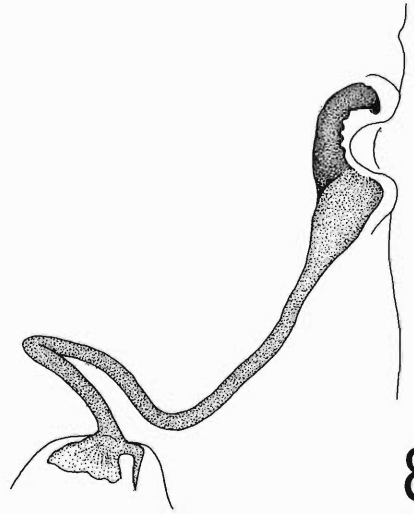
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panded; FH loop  $\frac{1}{2}$  shank length. Male copulatory organ with about 1.5 rings; base of male copulatory organ with wide sclerotized margin; male copulatory organ 90 (85–95;  $N = 2$ ) long, proximal ring diameter 17 (16–18;  $N = 4$ ). Accessory piece 17 (16–19;  $N = 3$ ) long, variable, distally bifurcate. Testis 39 (33–44;  $N = 2$ ) long, 26 (21–31;  $N = 2$ ) wide, ovate; seminal vesicle a loop of vas deferens; prostatic reservoir saccate, pyriform. Germarium bacilliform, 61 (53–68;  $N = 2$ ) long, 21 ( $N = 2$ ) wide; oviduct, ootype, uterus not observed. Vagina distally funnel-shaped with anteromedial lip; vaginal canal opening into large seminal receptacle.

REMARKS: *Telethecium paniculum* sp. n. is distinguished from *T. nasalis* by having the vaginal aperture anterior to the midlength of the body, a smaller accessory piece, and by the comparative morphology of the vaginae.

ETYMOLOGY: The specific name is from Latin (*panicula* = a small swelling) and refers to the midventral bag containing the terminal male genitalia.

#### *Kritskyia* Kohn, 1990

EMENDED DIAGNOSIS: Dactylogyridae: Ancyrocephalinae. Body elongate, fusiform, divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Cephalic lobes undifferentiated, head organs inconspicuous, cephalic glands indistinct. Eyes variable, comprising 4–6 accumulations of elongate-ovate granules. Mouth midventral at level of anterior margin of pharynx; pharynx muscular, glandular; esophagus present; intestinal ceca (2) confluent in posterior trunk, lacking diverticula. Gonads apparently tandem, intercecal; testis postgerminal. Vas deferens looping left intestinal cecum; seminal vesicle a dilation of vas deferens; 2 prostatic reservoirs. Male copulatory organ consisting of sclerotized coiled tube with counterclockwise rings (Kritsky et al., 1985); accessory piece non-articulated to base of male copulatory organ, membranous. Vaginal aperture sinistral in anterior trunk; seminal receptacle sinistral to anterior end of germarium; uterus indistinct; genital pore midventral. Vitellaria coextensive with intestinal ceca. Haptor cup-shaped, lacking anterior rim, armed with 14 marginal hooks. An-

chors, bars, 4A's absent. Parasites from urinary bladders and ureters of freshwater Neotropical fishes.

TYPE SPECIES: *Kritskyia moravecii* Kohn, 1990, from *Rhamdia quelen* (Quoy and Gaimard) (Pimelodidae).

#### *Kritskyia moravecii* Kohn, 1990 (Figs. 10–13)

HOST AND LOCALITY: Urinary bladder and ureters of *Rhamdia quelen* (Quoy and Gaimard) (Pimelodidae); Hydroelectric Power Station reservoir of "Passo Fundo," Rio Passo Fundo, São Valentim, Rio Grande do Sul, Brazil (May 1985).

SPECIMENS STUDIED: Three vouchers, USNPC 84844, HWML 38343.

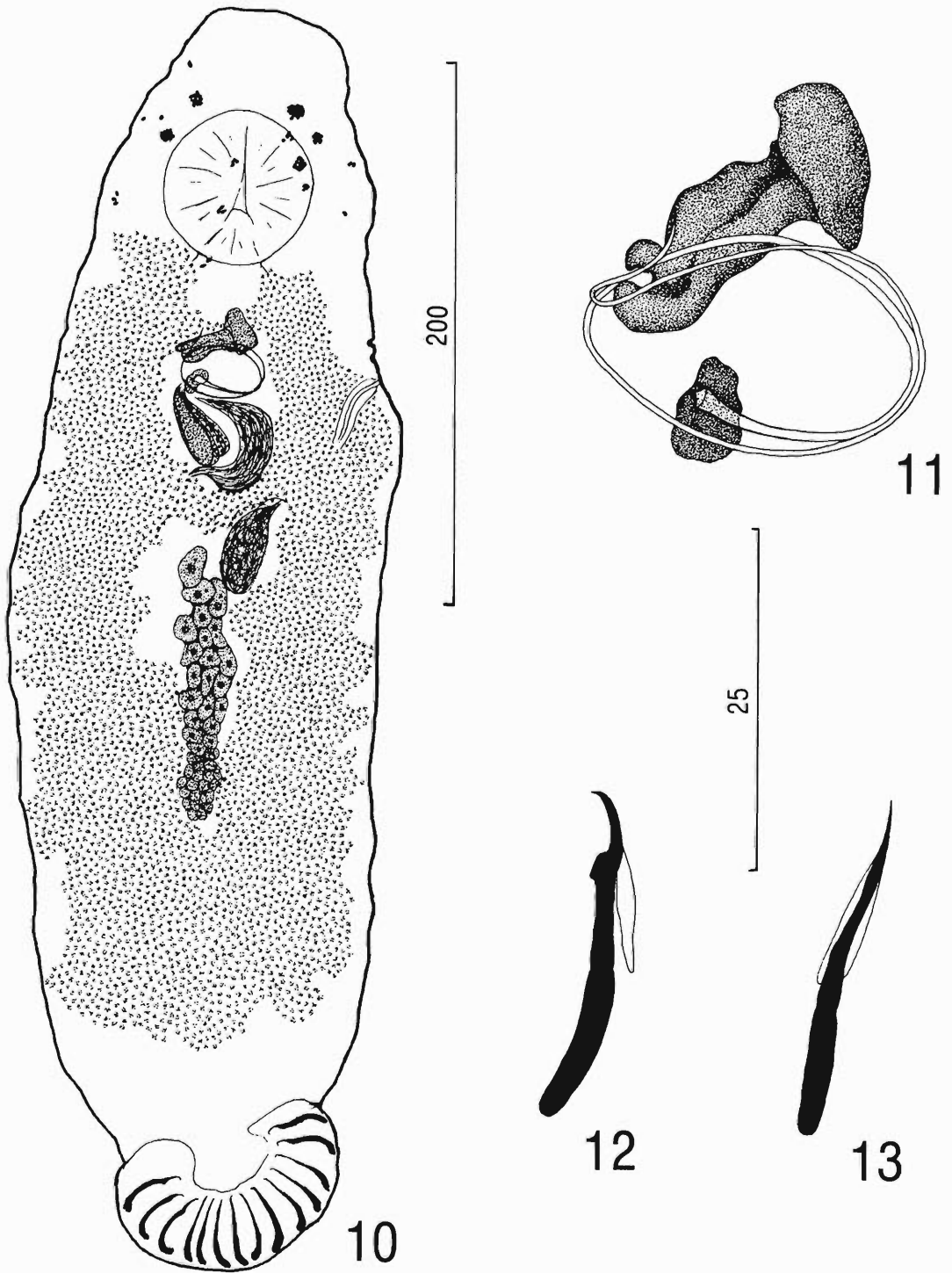
REDESCRIPTION: Body 438 (409–466;  $N = 2$ ) long; greatest width 150 (141–159;  $N = 2$ ) near midlength or in anterior trunk. Cephalic margin broad. Eyes subequal, lying dorsal to anterior margin of pharynx; accessory granules common in cephalic, anterior trunk regions. Pharynx spherical, 49 (48–50;  $N = 2$ ) in diameter; esophagus short. Peduncle broad; haptor 58 (56–59;  $N = 2$ ) long, 89 (84–94;  $N = 2$ ) wide. Hooks similar, 25–26 ( $N = 9$ ) long; each with flattened thumb, delicate point, expanded shank comprised of 2 subunits; FH loop  $\frac{1}{2}$  shank length. Male copulatory organ 143 (128–158;  $N = 2$ ) long, a loose coil of about 2 rings; base of male copulatory organ with sclerotized marginal flap; proximal ring diameter 28 (23–33;  $N = 2$ ). Accessory piece 28 (24–33;  $N = 3$ ) long, comprising variable grooved sheath. Testis not observed; seminal vesicle with delicate wall, C- or S-shaped; prostatic reservoirs saccate. Germarium with irregular margin, 73 (68–79;  $N = 2$ ) long, 30 (27–33;  $N = 2$ ) wide; oviduct, ootype, uterus not observed; vagina lightly sclerotized, opening into fusiform seminal receptacle.

REMARKS: The vouchers on which this redescription is based were collected from the same host specimens from which the type series for the species was obtained. We are grateful to Dr. A. Kohn for kindly providing them.

Kohn (1990) reported the accessory piece of the copulatory complex to be bipartite. In present specimens, the accessory piece is sheathlike with a longitudinal groove and a recurved (fold-

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Figures 6–9. *Telethecium paniculum* gen. et sp. n. 6. Holotype (ventral view). 7. Copulatory complex. 8. Vagina. 9. Hook. All figures are drawn to the 25- $\mu$ m scale except Figure 6 (100  $\mu$ m).



Figures 10–13. *Kritskyia moravecii* Kohn, 1990. 10. Whole mount (ventral view). 11. Copulatory complex. 12. Hook (lateral view). 13. Hook (ventral view). All figures are drawn to the 25- $\mu\text{m}$  scale except Figure 10 (200  $\mu\text{m}$ ).

ed) distal end. The distal fold is apparently the structure corresponding to the second portion of the accessory piece described by Kohn (1990).

Tandem gonads could not be confirmed in present specimens. A discrete cellular body occurred immediately posterior to the germarium. This body may be the testis, although no sperm cells could be seen and the origin of the vas deferens was not apparent.

### Discussion

Although species of *Telethecium* and *Kritskyia* inhabit different organs of their respective hosts, it appears that these genera may be closely related. Morphological characteristics supporting this relationship include the general organization of internal organ systems; presence of truncate cephalic margins; counterclockwise, loosely coiled male copulatory organs; sinistral vaginal apertures; and haptors lacking anchors, bars, and 4A's. These genera are differentiated by position of the gonads (overlapping in *Telethecium*; tandem in *Kritskyia*), presence of a midventral bag protruding from the anterior trunk and containing the terminal male genitalia in *Telethecium* (absent in *Kritskyia*), and morphology of the haptor (a simple posterior extension of the body armed with 14 hooks [12 marginal, 2 subcentral] in *Telethecium*; cup-shaped without an anterior rim and armed with 14 marginal hooks in *Kritskyia*).

Kohn (1990) included *Kritskyia* along with *Acolpenteron* Fischthal and Allison, 1940, and *Anonchohaptor* Mueller, 1938, in an unnamed group of primitive Monogenoidea based on presence of all haptor hooks being marginal and absence of haptor anchors and bars. This group is clearly polyphyletic, with its members representing 3 different familial taxa: *Kritskyia* (Ancyrocephalinae), *Acolpenteron* (Dactylogyridae), and *Anonchohaptor* (Pseudomurraytremitidae). In their analysis of character evolution within the Monogenoidea, Boeger and Kritsky (1993) considered presence of a bar a symplesiomorphy

for the Dactylogyridea, presence of anchors in at least 1 developmental (life cycle) stage a synapomorphy for the Monogenoidea, and 14 hooks marginal in the haptor a derived state resulting from loss of 1 hook pair from the plesiomorphic "16 marginal" state. If character evolution proceeded according to that suggested by Boeger and Kritsky (1993), both *Kritskyia* and *Telethecium* are clearly derived taxa that express secondary loss of anchors, 1 hook pair, and bars.

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## Relationships Between *Glyphelmins pennsylvaniensis* (Trematoda: Digenea) Infections and Host Size

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**ABSTRACT:** A total of 238 male spring peepers, collected from 3 different western West Virginia marshes during the spring breeding seasons of 1992, 1993, and 1994, were examined for *Glyphelmins pennsylvaniensis* infections. Prevalence was 66.4% with a mean intensity of 6.1. Hosts were divided into 5 sample populations, based upon year and site of collection, to examine relationships between 1) host weight and numbers of *G. pennsylvaniensis* individuals and 2) numbers of this digenean species versus their mean length. As infected host weight increased, the number of digeneans declined in all 5 host sample populations, but this inverse relationship was not significantly different from zero (i.e.,  $b = 0$ ) for 4 of those populations. Mean length of *G. pennsylvaniensis* individuals decreased as their numbers increased in a given host. This inverse relationship was significantly different from zero (i.e.,  $b \neq 0$ ) for individuals in all 5 host sample populations. Mean weights of infected hosts were significantly ( $P < 0.05$ ) lower than mean weights of uninfected hosts in 3 of the 5 sample populations.

**KEY WORDS:** *Glyphelmins*, *Pseudacris*, West Virginia, spring peeper.

In this paper we examine some relationships between the digenetic trematode, *Glyphelmins pennsylvaniensis* (Cheng, 1961), and its amphibian host, the northern spring peeper, *Pseudacris c. crucifer* (Weid-Neuweid). Because prevalence of *G. pennsylvaniensis* can exceed 50% in spring peeper populations, with a range of 1–20 (sometimes more) digeneans per host individual (Coggins and Sajdak, 1982; Muzzall and Peebles, 1991; Joy and Dowell, 1994), this parasite/host model offers the advantage of establishing databases of sufficient size for statistical analyses with a reasonable collection effort. There is the added opportunity to study effects of limited space on digenean parasites with this model because the small intestine of spring peepers is relatively small. Rankin (1937), observing trematodes in the genera *Brachycoelium*, *Plagitura*, and *Megalodiscus*, noted that, "Crowding of many individuals within a small area may account for small size, for when these flukes occur in small numbers, they are much larger." Willey (1941) and Fried and Nelson (1978), working with *Zygoctyle lunata*, and Nollen (1983), studying *Philophthalmus gralli*, also demonstrated stunting of adult digeneans in crowded conditions. Cheng (1961), Coggins and Sajdak (1982), Muzzall and Peebles (1991), and Joy and Dowell (1994) have provided prevalence and mean intensity data for *G. pennsylvaniensis* in spring peepers from a wide geographic range (Pennsylvania, Wisconsin,

Michigan, and West Virginia, respectively), but there is still much to learn about relationships between this digenean species and its amphibian host. In this paper we emphasize 1) differences between weights of infected versus uninfected hosts, 2) the relationship between host size (as weight) and number of *G. pennsylvaniensis* individuals present, and 3) the relationship between numbers of digeneans in a given host and their mean length in that host.

### Materials and Methods

A total of 238 male northern spring peepers, *Pseudacris c. crucifer* (Weid-Neuwied), were collected from 3 marsh areas in western West Virginia during the breeding seasons of 1992 through 1994. Two of the marshes, Beech Fork (BF) and Shoals (SM), are in Wayne County (USGS Topographic Map, Lavalette Quad). The third marsh, Green Bottom Wildlife Management Area (GB), is in northern Cabell County (USGS Topographic Map, Athalia Quad). Hosts from these 3 sites were segregated into 5 sample populations based on period of collection. Populations designated BF92, SM93, and SM94 were examined in different years, so their separation seemed appropriate. Host populations from Green Bottom were taken from the same site in 1994 and could have been grouped; however, we chose to consider the Green Bottom material as an early breeding season population (GBM94) and a late breeding season population (GBA94).

All hosts were captured between 2000 and 2200 hours, placed in 4-liter screw-cap jars (no more than 10 host individuals per jar) with moist paper towelling, returned to the laboratory, and placed in a refrigerator at 4°C. All hosts were necropsied within 12–24 hr after capture. Immediately prior to necropsy, each host individual was weighed to the nearest 0.1 g and measured for snout–vent length (SVL) with vernier calipers to

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**Table 1.** Combined probabilities for mean body weights of *Pseudacris c. crucifer* individuals infected ( $\bar{x}_i$ ) with *Glypthelmins pennsylvaniensis* compared with mean weights of uninfected ( $\bar{x}_u$ ) hosts in 5 different West Virginia host sample populations.

Host pop.	$N_i^*$	$\bar{x}_i (\pm 1 \text{ SD})$	$N_u^*$	$\bar{x}_u (\pm 1 \text{ SD})$	$t$	df	$P$	$\ln P^{\dagger}$
BF92	22	1.53 (0.184)	21	1.70 (0.227)	2.698	41	0.021	-3.863
SM93	25	1.70 (0.250)	25	1.85 (0.228)	2.212	48	0.033	-3.411
SM94	29	1.82 (0.257)	5	1.86 (0.291)	0.317	32	0.763	-0.270
GBM94	55	1.67 (0.249)	24	1.82 (0.318)	2.273	77	0.029	-3.540
GBA94	27	1.46 (0.199)	5	1.47 (0.237)	0.106	30	0.916	-0.088

\*  $N_i$  = number of infected hosts;  $N_u$  = number of uninfected hosts.  $\ln P$  = natural log of probability value.

† Reject  $H_0$ ;  $\bar{x}_i = \bar{x}_u$  because the observed value of  $-2 \sum \ln P$  (i.e., 22.344) >  $\chi^2_{.05(10)} = 18.307$ .

the nearest millimeter. Weight values, rather than SVL's, are used to describe host size throughout this paper because the former provided a wider range of values and thus a better estimation of host size.

After weighing, the host's spinal cord was severed at the base of the head with a surgical blade, and the small intestine was removed and examined.

*Glypthelmins pennsylvaniensis* individuals recovered from the small intestines of infected hosts at BF92 and SM93 were killed and fixed with 10% buffered formalin while under slight coverslip pressure. Length measurements of these *G. pennsylvaniensis* individuals were made with the aid of an ocular micrometer, and their mean length ( $\pm 1 \text{ SD}$ ) was calculated. Living trematodes recovered from infected hosts at SM94, GBM94, and GBA94 were segregated by host and placed directly into a series of 3.5-mm petri dishes containing 10 ml of tap water and a drop of stock mentholated alcohol solution (Abdel-Malek, 1951). Each appropriately labeled dish, containing this relaxing fluid and trematodes from a single host, was then refrigerated at 4°C for 1 hr (Fried, 1962). After 1 hr of refrigeration in the menthol solution, immobile trematodes were killed and fixed in 10% buffered formalin, then transferred to fresh formalin for storage. These trematodes were subsequently measured for total length with the aid of an ocular micrometer (without coverslip pressure) while in formalin mounts, and mean length ( $\pm 1 \text{ SD}$ ) was calculated.

Three null hypotheses were established for testing: 1) that mean weight of infected hosts was equal to that of uninfected hosts (i.e.,  $H_0: \bar{x}_i = \bar{x}_u$ ), 2) that there was no relationship between number of *G. pennsylvaniensis* individuals and size (as weight) of infected host (i.e.,  $b \neq 0$  for trematode numbers as a function of host weight), and 3) that there was no relationship between mean trematode length in a given infected host and the total number of trematode individuals in that host (i.e.,  $b = 0$  for mean trematode length as a function of total trematodes). The test statistic for the first hypothesis was a  $t$ -test, with a probability value for each host sample population given in Table 1. The test statistic for the latter two hypotheses was an  $F$ -test, with probability values given in the appropriate tables. Statistical tests were performed on an IBM compatible computer using Systat software (Systat, Inc., 1992).

Voucher specimens of *G. pennsylvaniensis* are deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705) under USNPC 83429.

## Results

A total of 238 male spring peepers, representing 5 West Virginia sample populations, were examined for *Glypthelmins pennsylvaniensis* infections. Mean infected host weight was significantly lower ( $P < 0.05$ ) than uninfected host weight in 3 of the 5 host populations (Figs. 1–5; Table 1). During the course of this investigation, 974 *G. pennsylvaniensis* individuals were recovered from 158 West Virginia spring peepers (Table 2). With the exception of the SM93 host population, there was no significant difference between mean trematode intensity levels (Table 2).

Larger infected hosts, in every host sample population, carried fewer *G. pennsylvaniensis* individuals than did smaller hosts. Still, this negative correlation between infected host weight and numbers of trematodes present was not significant (i.e.,  $b = 0$ ) for infected hosts in 4 of the 5 sample populations (Figs. 1–5; Table 3). Mean lengths of *G. pennsylvaniensis* individuals decreased as their numbers increased in a given host. This negative correlation was significant (i.e.,  $b \neq 0$ ) for all 5 host sample populations (Figs. 6–10; Table 4).

## Discussion

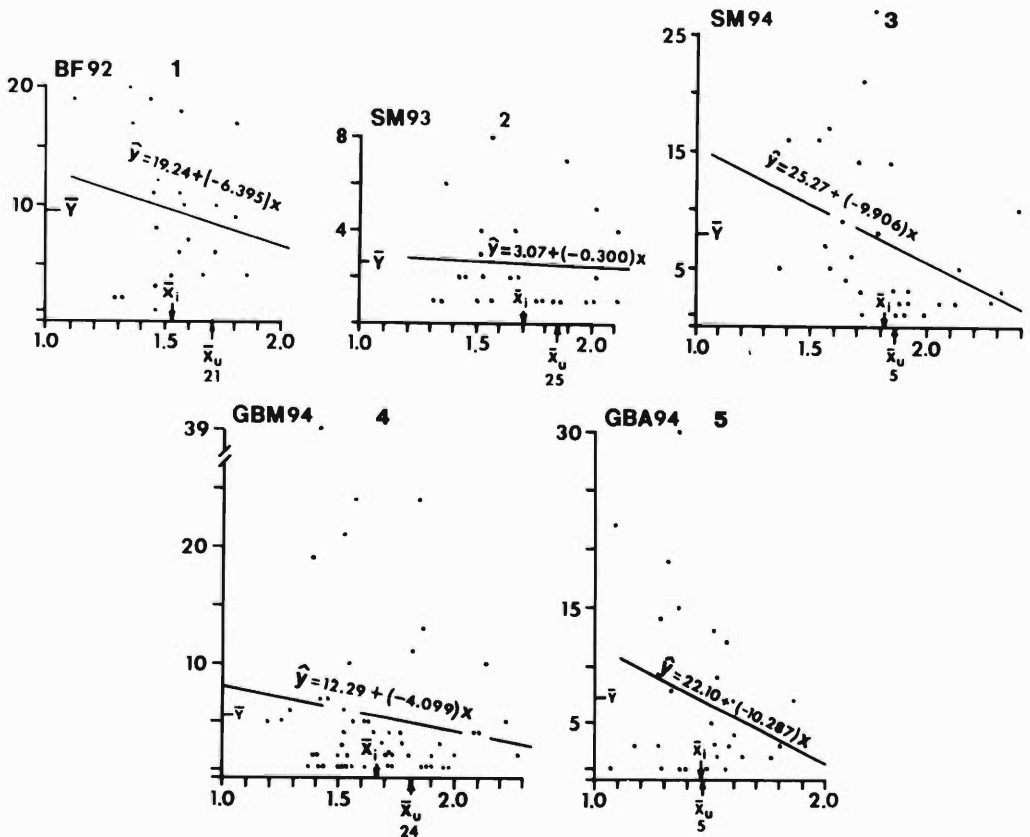
To evaluate the possible effect of *G. pennsylvaniensis* infections on spring peepers, we compared the weight of infected versus uninfected hosts. The test statistic for weight comparisons in each of 5 host populations was a  $t$ -test, with each test yielding a probability value. By combining probabilities and summing the natural log values for these 5 independent tests of significance, we were able to test the null hypothesis that mean infected host weight equaled mean uninfected host weight. It has been shown that  $-2 \sum \ln P$  is distributed as  $\chi^2$  with  $2k$  degrees of

**Table 2.** Mean intensities of *Flypthelmis pennsylvaniensis* in sample populations of *Pseudacris c. crucifer*. Means are significantly different ( $P < 0.05$ ) from each other if 95% confidence limits (LL = lower limit; UL = upper limit) do not overlap.

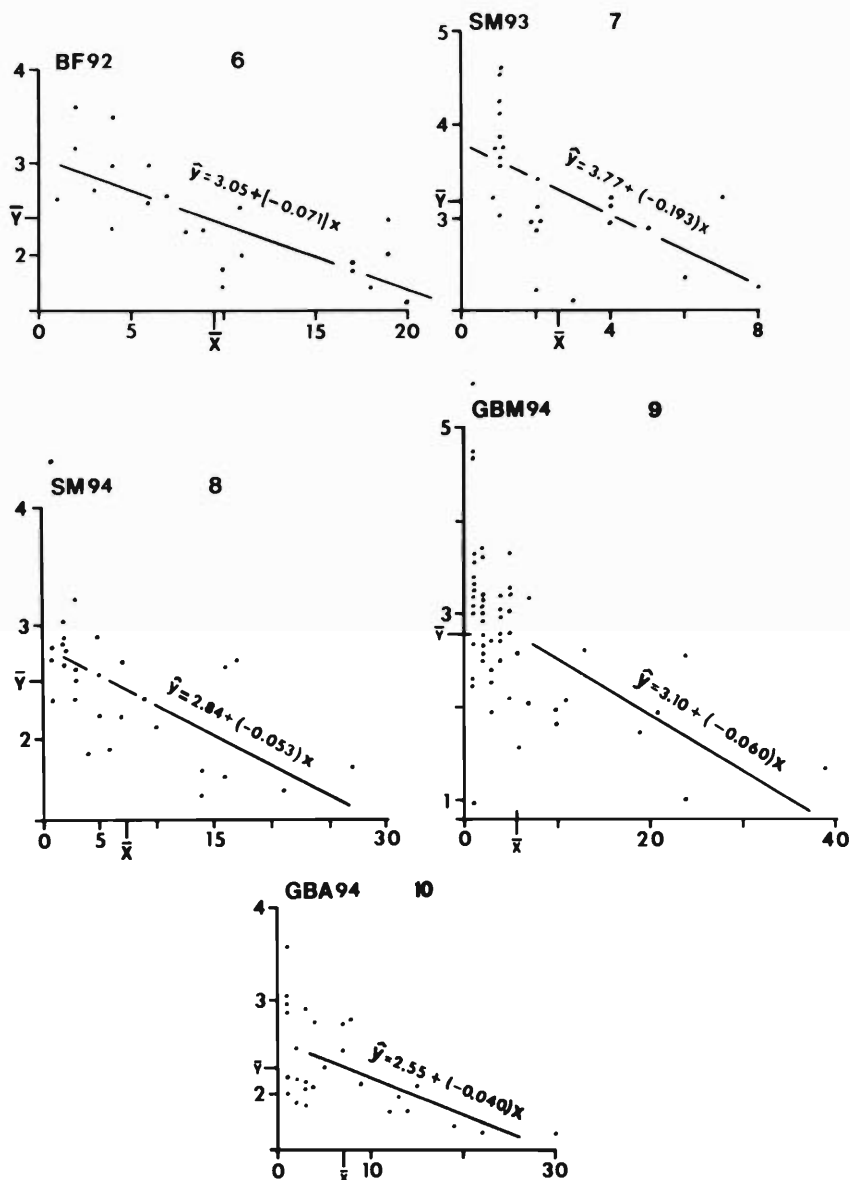
Host sample pop.	No. trematodes recovered	No. infected hosts	Intensity	95% confidence limits	
			$\bar{x} (\pm 1 \text{ SD})$	LL	UL
BF92	208	22	9.46 (6.29)	6.67	12.25
SM93	64	25	2.56 (2.06)	1.71	3.41
SM94	210	29	7.24 (6.88)	4.62	9.86
GBM94	300	55	5.46 (7.25)	3.50	7.42
GBA94	192	27	7.11 (7.50)	4.15	10.07

freedom, where  $k$  = the number of separate tests and probabilities (Sokal and Rohlf, 1981). Because our value of 22.344 for  $-2 \sum \ln P$  was greater than  $\chi^2_{0.05[10]} = 18.307$ , we rejected the null hypothesis and concluded that infected hosts were significantly smaller than their uninfected counterparts (Table 1).

There was an inverse relationship between host weight and numbers of *G. pennsylvaniensis* individuals in all 5 West Virginia host sample populations (Figs. 1-5). The slope of the regression line, however, was not significantly different from zero in 4 of those populations, allowing us to accept the null hypothesis (i.e.,  $H_0: b = 0$  for



**Figures 1-5.** Scatter diagrams showing numbers of *G. pennsylvaniensis* individuals (y-axis) as a function of host weight (g) (x-axis) for 5 West Virginia host collections. Each dot represents a single infected host.  $\bar{Y}$  = mean number of *G. pennsylvaniensis* individuals.  $\bar{x}_i$  = mean weight of infected hosts;  $\bar{x}_u$  = mean weight of uninfected hosts. Numbers below the  $\bar{x}_u$  notation represent the number of uninfected hosts. Differences between  $\bar{x}_i$  and  $\bar{x}_u$  are significant ( $P < 0.05$ ) for hosts at BF92, SM93, and GBM94.



Figures 6–10. Scatter diagrams showing mean length (mm) of *G. pennsylvaniensis* individuals (y-axis) as a function of total numbers of *G. pennsylvaniensis* individuals (x-axis) in infected hosts for 5 West Virginia host collections. Each dot represents the number of digeneans (and their mean length) from a single infected host.  $\bar{Y}$  = mean of mean length values;  $\bar{x}$  = mean number of digeneans in infected hosts (i.e.,  $\bar{x}$  values here correspond to the  $\bar{Y}$  values in Figs. 1–5).

trematode numbers as a function of host weight) in 4 of the 5 sample populations (Table 3). Similarly, Muzzall and Peebles (1991) found no relationship between helminth infections and length of *Rana sylvatica* and *P. c. crucifer* hosts.

There was also an inverse relationship between numbers of *G. pennsylvaniensis* individuals pres-

ent in a given infected host and the mean length of those trematodes in all 5 West Virginia spring peeper sample populations (Figs. 6–10). The slope of the regression line was significantly different from zero for all of those populations, allowing us to reject the null hypothesis (i.e.,  $H_0: b = 0$  for mean trematode length as a function of total

**Table 3. Significance of regression coefficients (i.e., b-values) for host weight (independent variable) versus the number of *G. pennsylvaniensis* individuals (dependent variable) (see Figs. 1–5).**

Sample pop.	b-value	F [1, N - 2]	P
BF92	-6.395*	0.72 [1, 20]	>0.05
SM93	-0.300*	0.03 [1, 23]	>0.05
SM94	-9.906†	4.28 [1, 27]	<0.05
GBM94	-4.099*	1.08 [1, 53]	>0.05
GBA94	-10.287*	2.02 [1, 25]	>0.05

\* Accept  $H_0$ : b = 0.

† Cannot accept  $H_0$ : b = 0.

trematodes) in all cases (Table 4). Such inverse relationships are not always evident. Nollen (1971) reported no relationship between numbers of *Philophthalmus megalurus* and their mean lengths. Later, however, Nollen (1983) found that *Philophthalmus gralli* in chickens were affected by crowded conditions, noting that adults "... from groups of over 40 per eye were significantly shorter than those in groups of up to 10 per eye." Fried and Nelson (1978) observed a similar effect with 2-wk-old *Zygocotyle lunata* individuals in chickens, where worms from single worm infections were more than twice as long as worms from initial infections of 100–500 cysts.

Information obtained during the course of this investigation corroborated some previously known aspects of *G. pennsylvaniensis* infections in spring peepers and revealed some new insights into this parasite/host relationship as well. Similar studies from other locations would be helpful in adding to our understanding of relationships between this digenean species and its amphibian host.

**Table 4. Significance of regression coefficients (i.e., b-values) for number of *G. pennsylvaniensis* individuals (independent variable) versus their mean length (dependent variable) (see Figs. 6–10).**

Sample pop.	b-value	F[1, N - 2]	P
BF92	-0.071*	26.71 [1, 20]	<0.001
SM93	-0.193*	12.15 [1, 23]	<0.005
SM94	-0.053*	15.37 [1, 27]	<0.001
GBM94	-0.060*	19.17 [1, 53]	<0.001
GBA94	-0.040*	13.83 [1, 25]	<0.005

\* Cannot accept  $H_0$ : b = 0.

### Acknowledgments

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## *Icosiella intani* sp. n. (Filarioidea: Onchocercidae), a Parasite of *Rana cancrivora* from South Kalimantan, Indonesia<sup>1</sup>

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**ABSTRACT:** *Icosiella intani* sp. n. (Filarioidea: Onchocercidae) is described from the muscles of the frog, *Rana cancrivora*, Gravenhorst, 1829, in South Kalimantan, Indonesia. *Icosiella intani* is distinguished by a microfilarial body length (121  $\mu\text{m}$ ) longer than that of the 7 known species in the genus and 4–7 tail nuclei in a single row. This species has a relatively short body length, a nonfiliform left spicule with its shaft about the same size as the “blade,” and a right spicule with a lanceolate terminus abruptly pointed. This is the first report of *Icosiella* from Indonesia and represents the eighth described species within this genus, with all hosts in the order Anura.

**KEY WORDS:** Filarioidea, Onchocercidae, *Icosiella intani* sp. n., *Rana cancrivora*, Kalimantan, Indonesia, morphology.

In February 1979, during a biomedical survey by the U.S. Naval Medical Research Unit No. 2 (Detachment) and the Indonesian Department of Health in Tanah Intan, South Kalimantan, Indonesia (3°20'S, 115°02'E) investigating zoonotic filariasis, filariid worms were found in the connective tissue of the hind leg muscle of a frog, *Rana cancrivora* Gravenhorst, 1829. Subsequent examination of these specimens revealed they were a new species within the genus *Icosiella* described herein.

### Materials and Methods

Adult worms were removed from the connective tissue of leg muscle, relaxed in 0.6% saline solution, fixed in hot 70% ethanol, and preserved in 70% ethanol/5% glycerin. All specimens were examined using a temporary lactophenol wet mount technique (Partono et al., 1977). Microfilariae were obtained from blood and thick blood smears processed and stained with Giemsa diluted 1:15 in pH 7.2 buffer for 15 min. Drawings (Figs. 1–9) were made with the aid of a camera lucida. All measurements are expressed as means followed by the range in parentheses and are given as length by width in micrometers ( $\mu\text{m}$ ) unless otherwise indicated.

### Results

#### *Icosiella intani* sp. n.

(Figs. 1–9)

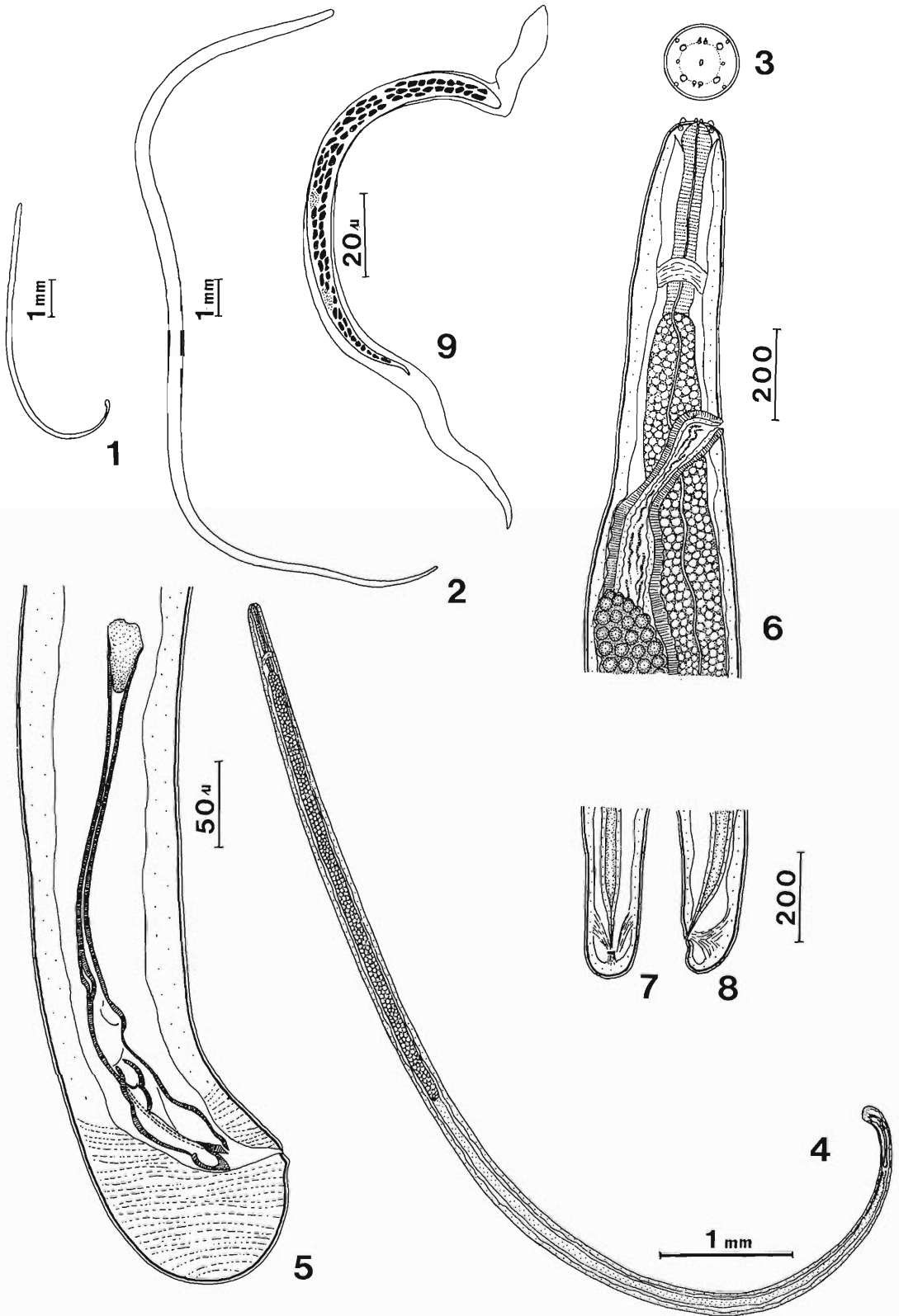
**DESCRIPTION:** Adult worms small and filiform, yellow to white when fixed. Anterior end

blunt. Posterior end conical and blunt for female, and protuberant in male (Figs. 1, 2). Anterior extremity with 4 pair submedian, 2 pair median labial cephalic papillae (spines) and 2 lateral amphids (Fig. 3). Buccal cavity absent. Esophagus divided into anterior muscular and posterior glandular portions, the latter being much longer and wider. Nerve ring at posterior half of muscular region of esophagus. Cuticle with finely transverse striations. Caudal alae and area rugosa absent. Anus subterminal.

**MALE** (based on 2 mature specimens): Body 9.9 (8.8–11.0) mm by 73  $\mu\text{m}$  (65–80) at level of head; width increasing posteriad, 138 (125–150) at level of nerve ring, 165 (155–175) at level of esophageal–intestinal junction, and 190 (180–200) at level of midbody, gradually decreasing posteriad and bulging at tail end, 115 (110–120) at level of cloaca. Esophagus (Fig. 4) 4,015 (4,010–4,020), distinctly divided into anterior muscular portion 525 (420–630) by 50 and posterior glandular portion 3,350 (3,100–3,600) by 90 (80–100). Muscular to glandular esophageal length ratio 1:4.9–8.5. Nerve ring 345 (310–380) from cephalic end. Caudal tail flexed ventrally 65 (55–75). The 2 spicules unequal in length and markedly dissimilar in appearance (Fig. 5). Larger (left) spicule 400 (380–420), composed of two sections: a slender tubular shaft 245 (240–250) with a proximal cup-shaped portion and a distal blade portion 155 (140–170) ending in a lanceolate-like tip. Smaller (right) spicule 103 (102–104); with a short proximal portion divided into 2 apparent segments with unevenly thickened edges and a longer portion wide distally, narrowing slightly, then widening and tapering abruptly to a thickened edged lanceolate point. The dorsal edge of the longer portion is markedly thicker.

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Spicule ratio 4.0 (3.7–4.1):1. Gubernaculum and caudal papillae absent.

**FEMALE** (based on 4 gravid specimens): Body 29.0 (24.6–38.2) mm by 98  $\mu$ m (90–100) at level of head; width increasing posteriad, 183 (170–200) at level of nerve ring, 280 (240–310) at level of vulval opening, 310 (280–330) at level of esophageal–intestinal junction, 330 (300–350) at midbody, gradually decreasing to 110 (80–150) at anal opening. Esophagus 4,500 (4,210–4,930), anterior muscular region 460 (380–590) by 54 (50–60) and posterior glandular region 4,040 (3,750–4,340) by 200 (190–210). Muscular to glandular esophageal length ratio 1:6.4–11.4. Nerve ring 300 (260–360) from cephalic end. Muscular vulva, opening as a transverse slit, just posterior to muscular–glandular esophageal junction, 1,070 (1,000–1,360) from cephalic end (Fig. 6). Vagina directed anteriorly, and flexing and looping posteriad, before receiving bifurcated uterus a varying distance below vulva. Uteri paired, loosely entwined, joining oviduct and extending anteriorly to within 900 (710–1,100) from cephalic end; posterior coil extending to within 510 (200–700) from tip of tail. Tail 50 (30–90) with blunt rounded end (Figs. 7, 8). Viviparous.

**MICROFILARIA** (based upon 25 specimens):

Body slender, ensheathed, and 121 (105–127) in length. Sheath much longer than body and unstained with Giemsa. Width at head level 3.7 (3–5), nerve ring 5.1 (4–6), excretory pore 5.0, anal pore 3.7 (3–4), and last tail nucleus 2.2 (2–3). Tail 4.5 (4–6) from last nucleus to posterior tip, tail nuclei 4–7 in single row (Fig. 9).

**HOST:** Mangrove frog, *Rana cancrivora* Gravenhorst, 1829.

**SITE IN HOST:** Connective tissue of hind leg muscle.

**LOCALITY:** Indonesia, South Kalimantan (Borneo), Tanah Intan rubber estate (3°20'S, 115°02'E).

**DATE OF COLLECTION:** February 1979.

**SPECIMENS DEPOSITED:** USNPC No. 81170. Holotype male, allotype female in 70% ethanol/5% glycerin, and 1 blood slide microfilariae (syn-types), Giemsa stained, are deposited in the U.S.

National Parasite Collection, Beltsville, Maryland 20705.

**ETYMOLOGY:** The specimens were obtained from a region of South Kalimantan purported to be rich in alluvial diamonds. *Intan*, translated from Indonesian, means diamond.

### Discussion

The monogeneric subfamily Icosiellinae Anderson, 1958, has been found in amphibia from Japan (Yamaguti, 1941; Hayashi, 1960), Malaysia (Yuen, 1962; Bain and Purnomo, 1984), Vietnam (Walton, 1935), and the Philippines (Schmidt and Kuntz, 1969). Johnston (1967) recorded *Icosiella* for the first time in Papua New Guinea. Of the 7 previously reported species of *Icosiella* Seurat, 1917 (Bain and Purnomo, 1984), *I. intani* represents the first report of this genus in an amphibian captured in Indonesia.

*Icosiella intani* adults can be differentiated easily from all previously described species by standard morphometric criteria. Specifically, adults are much smaller in length than adults of *Icosiella sasai* Hayashi, 1960. The spicule ratio is 4:1, whereas in *Icosiella neglecta* (Diesing, 1851) Seurat, 1917, *Icosiella kobayashii* Yamaguti, 1941, *Icosiella innominata* Yuen, 1962, and *Icosiella papuensis* Johnston, 1967, it is 3:1 or less. *Icosiella hoogstraali* Schmidt and Kuntz, 1969, has an enormous spicule ratio, 123:1. *Icosiella laurenti* Bain and Purnomo, 1984, has a total esophageal length only half that of *I. intani*. Most characteristic, *Icosiella intani* microfilariae differ from all other species by having a longer body length.

*Icosiella* has been described from 4 genera of frogs; *Rana*, *Discoglossus*, *Babina*, and *Cornufer*. Based on geographic proximity and host species (*Rana cancrivora*), *I. innominata* and *I. intani* might presumably share the most characters in common. However, as noted, the microfilaria of *I. intani* are significantly larger, more slender, and sheathed. The male spicule ratio differs enough to separate the 2. In particular, the larger (left) spicule is longer, falling out of the size range (300–370) given for *I. innominata* (Yuen, 1962). Additionally, the smaller (right) spicule is divid-

←

Figures 1–9. Adults and microfilaria of *Icosiella intani* sp. n. 1. Adult male, macroscopic. 2. Adult female, macroscopic. 3. En face view of female showing arrangement of 4 pair submedian, 2 pair median labial cephalic papillae and 2 lateral amphids. 4. Male, ventral view showing esophagus and spicules. 5. Caudal end, lateral view, of male showing left and right spicules and cloaca. 6. Anterior region, lateral view, of female. 7. Caudal end, ventral view, of female. 8. Caudal end, lateral view, of female. 9. Sheathed microfilaria from thick blood smear. Scale bar for Figures 1, 2, 4 in millimeters (mm). Scale bar for Figures 5–9 in micrometers ( $\mu$ m).

ed into 2 distinct segments proximally and tapers abruptly to a point, markedly different in appearance from *I. innominata*. However, because these apparent differences are based on only a few (7) mature male specimens by Yuen and our description (2 specimens), caution should be exercised in attributing strong significance to these measurements of spicule length and ratio because biological variability is difficult to assess. In fact, Yuen (1962) devised a taxonomic key to the 4 known *Icosiella* species at that time, using the length of the glandular esophagus (not spicules) as a diagnostic character. The length range for female *I. intani* (3.75–4.34 mm) exceeds that of *I. innominata* (2.9–3.6 mm) but is below that of *I. neglecta* (5.3 mm). Until more information on species distribution and additional material become available, some doubt will remain concerning the diagnostic significance of certain characters currently separating species of *Icosiella*.

The vector of *I. intani* is unknown. Desportes (1941) implicated a biting midge (Ceratopogonidae) and sand fly (Psychodidae), both nematoceran flies, as the probable biological vectors of *I. neglecta* based on successful larval development in the insect flight muscles. To our knowledge this is the only evidence of vector incrimination within this genus of filaria. Unfortunately, few investigations have explored the host-vector-parasite relationship between amphibians and hematophagous insects.

#### Acknowledgments

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## *Pharyngodon lepidodactylus* sp. n. (Nematoda: Pharyngodonidae) from the Mourning Gecko, *Lepidodactylus lugubris* (Lacertilia: Gekkonidae), from Hawaii

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**ABSTRACT:** Sixty-three *Pharyngodon lepidodactylus* sp. n. were recovered from the large intestines of 26 of 283 adult *Lepidodactylus lugubris* collected from Hawaii. Prevalence of infection was 9% (mean intensity 2.4, range 1–8). *Pharyngodon lepidodactylus* sp. n. represents the thirtieth species of the genus and can be distinguished from all other species of *Pharyngodon* by the presence of a unique “bottle-shaped” egg. This is the first report of the genus *Pharyngodon* from Hawaii.

**KEY WORDS:** *Pharyngodon lepidodactylus* sp. n., Pharyngodonidae, *Lepidodactylus lugubris*, Gekkonidae, Hawaii.

In a recent helminthological survey of lizards of Hawaii, 26 of 283 *Lepidodactylus lugubris* (Duméril and Bibron, 1836) were found to harbor 4 male and 59 female nematodes of a previously undescribed species of *Pharyngodon*. *Lepidodactylus lugubris*, the mourning gecko, has a wide distribution and is known from Oceania, India, Sri Lanka, Southeast Asia, Philippine Islands, Indonesia, Papua New Guinea, Australia, and the United States (Welch et al., 1990). It was probably introduced into Hawaii by early settlers approximately 1,000 years ago (Hunsaker and Breese, 1967). In Hawaii, *Lepidodactylus lugubris* is sympatric with the geckos *Gehyra mutilata*, *Hemidactylus frenatus*, and *Hemidactylus garnotii* and the skinks *Cryptoblepharus boutoni*, *Emoia cyanura*, *Lampropholis delicata*, and *Lipinia noctua* (McKeown, 1978).

The genus *Pharyngodon* was established by Diesing (1861) with *P. spinicauda* (Dujardin, 1845) from the intestine of a lizard, *Lacerta muralis*, taken at St. Malo, France, as type species. Skrjabin et al. (1960) revised the genus to retain only those species in which males have well-developed caudal alae forming a genital bursa enveloping all the anal pedunculate papillae and females have the vulva in the anterior half of the body. There are currently 29 species (an additional 4 species, *P. boulengerula* Ubelaker, 1965, *P. elongata* Markov and Bogdanov, 1961, *P. sphaerodactyli* Barus and Coy Otero, 1974, and *P. polypedatis* Yamaguti, 1941, are known only from female specimens and are designated as *species inquirenda*). Species of *Pharyngodon* occur primarily in lizards of the families Gekkon-

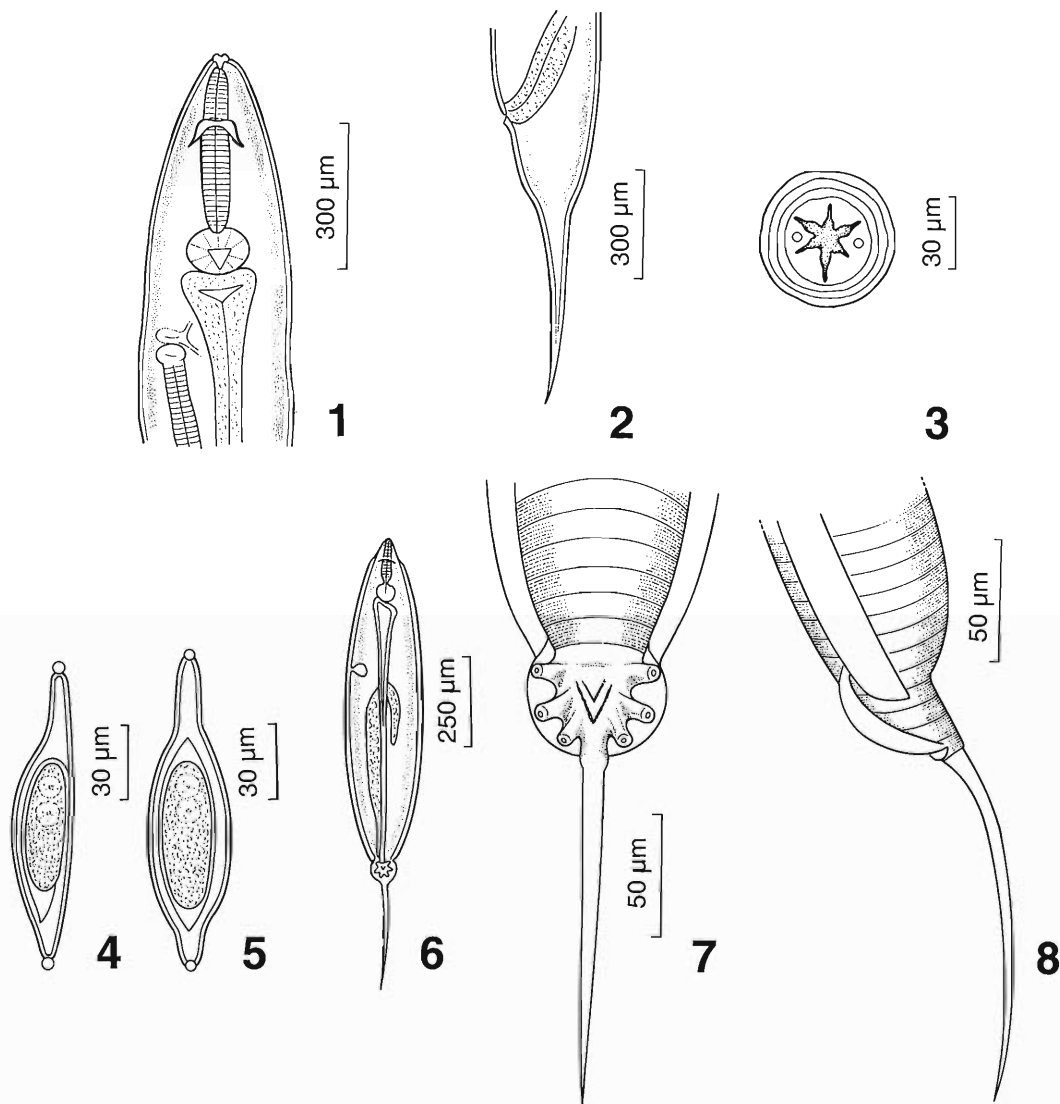
idae, Phrynosomatidae, Scincidae, and Teiidae; however, 2 species, *Pharyngodon bursatus* Rao, 1980, in *Euphlyctis cyanophlyctis* (= *Rana cyanophlyctis*) and *P. schistopapillatus* Rao, 1980, in *Bufo viridis*, are known only from amphibians (see Baker, 1987). Of the species infecting lizards, 9 are found in the Palearctic Zoogeographical Realm, 5 each in the Nearctic and Australian realms, 4 in the Neotropical Realm, 3 in the Oriental Realm, and 1 in the Ethiopian Realm.

### Material and Methods

Of the 283 *Lepidodactylus lugubris* captured by hand and fixed in neutral buffered 10% formalin, 35 were from Hawaii, Hawaii (1991, southeast corner of the island, 19°43'N, 155°05'W, from sea level to approximately 450 m elevation), and 248 were from Oahu, Hawaii (18 in 1991, eastern shore, 21°20'N, 157°52'W, from sea level to approximately 375 m elevation; 44 in 1992 at various sites along the northern, eastern, western, and southeastern shores from sea level to 100 m; 186 in 1993 at various sites on the northern, eastern, and western shores from 5 to 100 m). The body cavity was opened by a longitudinal incision from vent to throat and the gastrointestinal tract was removed and opened longitudinally. Nematodes were placed in glycerol, allowed to clear, and examined under a light microscope.

### Results

Four (11%) of the 35 *Lepidodactylus lugubris* collected from Hawaii, Hawaii, and 22 (9%) of the 248 from Oahu, Hawaii, were found to be infected. There was no significance difference in infection rates between the 2 islands ( $\chi^2 = 0.19$ ; 1 df,  $P > 0.05$ ); thus, the combined prevalence



Figures 1-8. *Pharyngodon lepidodactylus* sp. n. 1. Anterior end of female, lateral view. 2. Posterior end of female, lateral view. 3. Female, en face view. 4. Egg, lateral view. 5. Egg, dorsal view. 6. Male, entire. 7. Posterior end of male, ventral view. 8. Posterior end of male, lateral view. Scale bar values are given in micrometers.

of infection was 9% (mean intensity 2.4, range 1-8). A description of the new species follows.

*Pharyngodon lepidodactylus* sp. n.  
(Figs. 1-8)

**DESCRIPTION:** Males having caudal alae which envelop posterior postcloacal pair of pedunculate papillae; females having vulva in anterior half of body. Nematodes of small size with a cylindrical body tapering both anteriorly and

posteriorly. Cuticle with distinct transverse striations extending from behind lips to level of anus. Lateral alae present in males only. Mouth bounded by three lips; there is no buccal cavity. Esophagus ends in a valvulate, subspherical bulb which is separated from the esophageal body by a small constriction. In both sexes, there is an elongated tail.

**MALE** (based on 4 specimens; mean measurement and range in mm): Small, white, fusiform nematodes tapering both anteriorly and poste-

riorly; length 1.20 (1.10–1.50); maximum width 0.20 (0.15–0.25). Lateral alae 0.030 (0.029–0.034) wide extending posteriorly from the level of nerve ring to the middle of the genital bursa. Cuticle with fine cross striations at 1  $\mu$ m intervals, extending the entire length of the body. Mouth opening surrounded by 3 lips, V-shaped notch in each. One small, pedunculate amphid on each ventrolateral lip. Esophagus (including bulb) 0.186 (0.151–0.198); bulb length 0.055 (0.051–0.058); bulb width 0.059 (0.057–0.060). Nerve ring 0.036 (0.028–0.040), excretory pore 0.250 (0.228–0.274) from anterior end, respectively. Well-developed caudal alae present, 0.015 wide by 0.050 long. Three pairs of caudal papillae present; precloacal pair situated on slightly inflated anterior portion of caudal end, adcloacal pair posterolaterally directed and postcloacal pair enclosed by caudal alae, 0.030 behind adcloacal pair. Filiform tail extending 0.218 (0.211–0.234) beyond postcloacal papillae. Spicule absent; prominent genital cone with the posterior lip supported by a sclerotized V-shaped structure. Single vas deferens and testis; at level of excretory pore testis reflected posteriorly.

**FEMALE** (based on 10 gravid specimens): Small, white, cylindrical nematodes tapering anteriorly and posteriorly; posterior drawn out into subulate tail filament. Length (excluding tail filament) 3.40 (3.05–4.75); maximum width 0.285 (0.228–0.325). Lateral alae absent. Cuticle with fine cross striations at 1.2- $\mu$ m intervals. Esophagus (including bulb) 0.273 (0.245–0.297); bulb length 0.080 (0.074–0.086); bulb width 0.096 (0.086–0.103). Nerve ring 0.044 (0.034–0.063); excretory pore 0.330 (0.281–0.357) and vulva 0.345 (0.289–0.383) from anterior end. Vagina directed posteriorly, anterior thick, muscular and posterior glandular. Uterus didelphic, 1 uterine branch directed posteriorly, the other anteriorly. Ovaries with flattened oocytes arranged in single file. One ovary running anteriorly to join posteriorly directed oviduct and second ovary running posteriorly joining anteriorly directed oviduct. Ovarian and uterine coils postbulbar. Filamentous portion of tail 0.760 (0.650–0.845) and without spines. Thick-shelled, nonoperculated eggs flattened on 1 side, fusiform with 1 end extended, i.e., “bottle-shaped,” cuticular knob present at poles, 144  $\times$  36  $\mu$ m (131–151  $\times$  31–40  $\mu$ m). Pronucleus stage of development at deposition.

**TYPE SPECIMENS:** Holotype male, U.S. National Parasite Collection, Beltsville, Maryland,

Accession No. 84164. Allotype female, 84165. Paratypes: 3 males, 9 females, 84166.

**TYPE HOST:** *Lepidodactylus lugubris* (Duméril and Bibron, 1836) “mourning gecko.”

**TYPE LOCALITY:** Hawaii, Hawaii; prevalence 11% (4/35); mean intensity 1.5, range 1–2.

**OTHER LOCALITY:** Oahu, Hawaii; prevalence 9% (22/248) mean intensity 2.4, range 1–8).

**ETYMOLOGY:** The specific epithet is derived from the name of the host genus.

### Discussion

The general morphology of *Pharyngodon lepidodactylus* sp. n. allows its assignment to the superfamily Oxyuroidea Railliet, 1916, family Pharyngodonidae Travassos, 1919, which currently contains 21 genera (see Petter and Quentin, 1976). Of these, 3 genera characteristic of reptiles exhibit a vulvar opening in the anterior part of the body just behind the postbulbar excretory pore: *Pharyngodon* Diesing, 1861, *Spauligodon*, Skrjabin, Schikhobalova, and Lagodovskaja, 1960, and *Skrjabinodon*, Inglis, 1968. These genera are distinguished by the relationship of the caudal alae to the genital papillae: males of *Pharyngodon* have well-developed caudal alae that form a genital bursa enveloping all genital papillae; in males of *Spauligodon*, the posterior pair of papillae are excluded from the genital bursa; and males of *Skrjabinodon* lack caudal alae. The inclusion of the described specimens in the genus *Pharyngodon* is based on the position of the vulva and the configuration of the caudal alae.

Species of *Pharyngodon* are separated on the presence or absence of a spicule, the morphology of the caudal alae, the shape of the egg, the presence or absence of spines on the tail filament of adults, and geographical distribution (Table 1). Chabaud and Brygoo (1962) suggested that geographical distribution is the most important factor in the speciation of reptilian oxyurids. No other species of *Pharyngodon* has been reported to have “bottle-shaped” eggs; thus, *P. leptodactylus* sp. n. is easily distinguished from all other species of *Pharyngodon*. Geographically, the nearest species are found in Australia: *P. asterostoma* Adamson, 1984, *P. australis* Johnston and Mawson, 1942, *P. hindlei* Thapar, 1925, *P. kartana* Johnston and Mawson, 1941, and *P. tiliquae* Baylis, 1930; all have eggs with truncated ends. *Pharyngodon lepidodactylus* sp. n. is most like *P. inermicauda* Baylis, 1923, from the gecko *Tarentola annularis* of Egypt in that males of

Table 1. Geographic distribution and selected characters of species of *Pharyngodon* infecting lizards.

Biogeographic realm <i>Pharyngodon</i> sp.	Male characters		Female characters		Reference
	Spicule	Tail filament/bursa length	Tail	Egg morphology	
Australian					
<i>P. asterostoma</i>	Absent	Tail filament longer than bursa	Subulate, smooth	Truncated ends	Adamson, 1984
<i>P. australis</i>	Absent	Tail filament shorter than bursa	Subulate, smooth	Truncated ends	Johnston and Mawson, 1942
<i>P. hiradlei</i>	42 $\mu$ m	Tail filament equal to bursa	Subulate, smooth	Truncated ends	Thapar, 1925
<i>P. kartana</i>	60 $\mu$ m	Tail filament longer than bursa	Subulate, about 7 spines	Truncated ends	Johnston and Mawson, 1941
<i>P. tiltquae</i>	Absent	Tail filament longer than bursa	Subulate, smooth	Truncated ends	Baylis, 1930
Ethiopian					
<i>P. brevbursata</i>	Absent	Tail filament equal to bursa	Subulate, smooth	Truncated ends	Caballero Rodriguez, 1968
Oriental					
<i>P. frenatusi</i>	Absent	Tail filament longer than bursa	Subulate, smooth	Pointed ends, 1 with knob	Gupta, 1959
<i>P. gekko</i>	60 $\mu$ m	Tail filament longer than bursa	Subulate, spiny	Pointed ends	Chakravarty and Bhaduri, 1948
<i>P. kantzii</i>	Absent	Tail filament longer than bursa	Subulate, 17-22 spines	Pointed ends, each knobbed	Gupta, 1959
Neotropical					
<i>P. cesarpintoi</i>	Absent	Tail filament longer than bursa	Pointed, smooth	Truncated ends	Pereira, 1935
<i>P. micrurus</i>	Description not available				Baker, 1987
<i>P. travassosi</i>	Present	Tail filament equal to bursa	Pointed, smooth	Truncated ends	Pereira, 1935
<i>P. yucatanensis</i>	Absent	Tail filament longer than bursa	Subulate, 8-11 spines	Truncated ends	Chitwood, 1938
Nearctic					
<i>P. enemidophori</i>	Absent	Tail filament shorter than bursa	Pointed, smooth	Truncated ends	Read and Amrein, 1953
<i>P. kirbii</i>	Absent	Tail filament shorter than bursa	Pointed, smooth	Truncated ends	Specian and Ubelaker, 1974
<i>P. mudgi</i>	Absent	Tail filament shorter than bursa	Subulate, spiny	Oval, 1 end operculated	Specian and Ubelaker, 1974
<i>P. papillocauda</i>	Present	Tail filament shorter than bursa	Pointed, papillated	Not described	Hannum, 1941
<i>P. warneri</i>	Absent	Tail filament shorter than bursa	Pointed, smooth	Truncated ends, each plugged	Harwood, 1932
Palaearctic					
<i>P. gekkinis</i>	47-61 $\mu$ m	Tail filament longer than bursa	Subulate, smooth	Pointed ends	Liu and Wu, 1941
<i>P. hierrensis</i>	Absent	Tail filament equal to bursa	Subulate, smooth	Truncated ends	Solera-Puertas et al., 1988
<i>P. hispanicus</i>	Absent	Tail filament longer than bursa	Subulate, 3-8 spines	Truncated ends, each operculated	Astasio-Arbiza et al., 1987
<i>P. inermicauda</i>	Absent	Tail filament longer than bursa	Subulate, smooth	Pointed ends, each knobbed	Baylis, 1923
<i>P. mamillatus</i>	21-37 $\mu$ m	Tail filament longer than bursa	Subulate, smooth	Truncated ends, each plugged	Baylis, 1923
<i>P. neyrae</i>	Absent	Tail filament longer than bursa	Subulate, smooth	Pointed ends, 1/2 knobs	Calvente, 1948
<i>P. schikhobalovi</i>	Absent	Tail filament longer than bursa	Subulate, smooth	Oval, each end knobbed	Sharpilo, 1976
<i>P. spinicauda</i>	60 $\mu$ m	Tail filament longer than bursa	Subulate, spiny	Truncated ends, each operculated	Skrjabin et al., 1960
<i>P. termezensis</i>	Description not available				
Oceania					
<i>P. lepidodactylus</i> sp. n.	Absent	Tail filament longer than bursa	Subulate, smooth	Bottle-shaped, ends knobbed	This study



both species lack spicules and the tail filament is longer than the bursa, whereas females have smooth, subulate tails and the eggs are knobbed at each end; however, egg shape and geography separate the 2 species. This description of the thirtieth species of *Pharyngodon*, *P. lepidodactylus* sp. n., extends the range of the genus to Hawaii.

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***Parapharyngodon anomalus* sp. n. (Oxyurida, Pharyngodonidae)  
from the Australian Echidna *Tachyglossus aculeatus*,  
with Notes on the Thelandroinae**

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**ABSTRACT:** A new species of the genus *Parapharyngodon*, *Parapharyngodon anomalus*, is described from the Australian echidna, *Tachyglossus aculeatus*. This nematode is morphologically similar to 2 previously described species which occur in Australian reptiles. This is the first report of a member of this genus occurring in a mammal. The significance of this finding is discussed.

**KEY WORDS:** *Parapharyngodon anomalus* sp. n., Oxyurida, Pharyngodonidae, *Tachyglossus aculeatus*, Tachyglossidae, Australia.

Twenty-two specimens of a pinworm belonging to the genus *Parapharyngodon* Chatterji, 1933 were recovered from the colon of a road-killed echidna *Tachyglossus aculeatus* (Shaw, 1792) (Monotremata: Tachyglossidae). Adamson and Nasher (1984a) considered members of this genus to be close to ancestral pharyngodonids, and the presence of a member of this genus in a primitive mammal may be of evolutionary significance. Although *Parapharyngodon* has not been reported from mammals previously, 2 species of the genus are known from Australian reptiles. The specimens from the echidna differ morphologically from these, and are considered to belong to a new species described herein as *Parapharyngodon anomalus* sp. n. after its unusual host affinities. The significance of this new species is discussed.

**Materials and Methods**

A single specimen of *Tachyglossus aculeatus* was found dead, apparently killed by a motor vehicle, in the Perth hills suburb of Lesmurdie, Western Australia. The gastrointestinal tract was sent to the author for parasitological examination. Two male and 20 female worms recovered from the colon were washed in tap water and fixed in hot glycerine alcohol.

Specimens were cleared in lactophenol. Drawings and measurements were made with the aid of a drawing tube. All measurements are given in  $\mu\text{m}$  unless otherwise indicated. Measurements given in the descriptions are of the male holotype and female allotype specimens. Specimens have been deposited in the Western Australian Museum, Perth.

The anterior extremity of one female worm was removed, dehydrated to absolute ethanol, critical-point dried via amyl acetate and carbon dioxide (Hayes, 1973), and sputter-coated with gold for visualization by a Philips scanning electron microscope (Model XL20).

I also examined *Parapharyngodon fitzroyi* Jones, 1992 paratypes WAM 15-91 (Western Australian Museum) ex *Tiliqua multifasciata*; *P. kartana* (Johnston and Mawson, 1941) Mawson, 1971 (syn. *Thelandros kartana* Johnston & Mawson 1941) SAM V1248, V1244 (South Australian Museum) ex *Hemiergis peronii*; *Thelandros trachysauri* Johnston and Mawson (1947) SAM V1242, V1243 ex *Trachydosaurus rugosus*.

**Results**

***Parapharyngodon anomalus* sp. n.  
(Table 1, Figs. 1-11)**

**DESCRIPTION:** Oxyurida, Oxyuroidea, Pharyngodonidae, Thelandroinae. Robust worms with distinct transverse cuticular annulations. Mouth surrounded by 6 triangular lips. Cephalic extremity flattened, devoid of ornamentation, papillae, or external signs of amphids. Males with prominent lateral alae. Females without alae.

**MALE** (holotype WAM 5-95, paratype WAM 6-95): Figs. 1-3, Table 1. Length 5.28 mm, maximum width 490 at midbody. Lateral alae arise 1.1 mm from the anterior end and extend to about 100  $\mu\text{m}$  from the posterior end. Excretory pore 1.83 mm from anterior end. Annulations at level of excretory pore 28 apart. Nerve ring 188 from anterior end. Buccal cavity 7 deep and 22 wide. Esophagus 880 long, including valved bulb; narrow part 58 wide, with short isthmus before bulb which is 145 long and 185 wide. Intestine greatly expanded immediately posterior to esophageal bulb. Posterior end with prominent postanal cone, gradually tapering to a bluntly rounded tip, bearing a pair of minute papillae. Dorsally directed caudal appendage 60 long, bearing a pair of sessile papillae at the proximal third, and ending in a sharp point. Two pairs of mammiform papillae, 1 adanal and

**Table 1.** Measurements of *Parapharyngodon anomalus* sp. n. All measurements are in  $\mu\text{m}$  unless otherwise indicated.

	Females		Males	
	Allotype	Paratypes*	Holotype	Paratype
Length (mm)	10.52	8.05–10.68 (9.98)	5.28	5.58
Maximum width	980	830–980 (895)	490	570
Esophagus length	1,550	1,325–1,575 (1,458)	880	910
Esophagus width	73	63–80 (72)	58	53
Esophageal bulb length	195	195–230 (213)	145	145
Esophageal bulb width	265	245–280 (263)	185	180
Nerve ring	205	195–240 (209)	188	—
Excretory pore	2,600	2,025–2,625 (2,425)	1,825	1,800
Tail spike	145	100–160 (137)	60	58
Vulva (mm from anterior)	5.05	3.68–5.05 (4.67)	—	—
Vulva (% of body length)	48	42.7–49.8 (46.8)	—	—
Anus–tail tip	465	400–500 (456)	—	—
Egg length	90	83–95 (89)	—	—
Egg width	45	43–50 (46)	—	—
Spicule	—	—	63	—

\*  $N = 15$ ; measurements given are ranges, with means in parentheses.

slightly anterior to anus, the other posterolateral to anus. Anterolateral margins of anus with several (8 or 9) fingerlike projections, some of which are branched, approximately 10 long. Spicule 63 long, weakly sclerotized. Spicule pouch opens immediately posterior to the anal opening.

**FEMALE** (allotype WAM 7-95, paratypes WAM 8-95): Figs 4–11, Table 1. Length 10.52 mm, maximum width 980 immediately anterior to vulva. Excretory pore 2.60 mm from anterior end. Annulations 56 apart at level of excretory pore. Nerve ring 205 from anterior end. Esophagus 1,550 long, including bulb, narrow part 73 wide with bulb 195 long and 265 wide. Intestine greatly expanded immediately posterior to esophageal bulb. Four loops of ovary coiled around esophagus immediately anterior to bulb. Vulva 5.05 mm from anterior end. Anus a horizontal slit in a slight depression, 465 from posterior extremity. Tail ends in a prominent stout spike approximately 145 long. Phasmids open laterally just anterior to the base of the tail spike. Eggs asymmetrical, measuring  $90 \times 45$ , flattened on one side, with subpolar operculum.

**DIAGNOSIS:** *Parapharyngodon anomalus* most closely resembles the other two Australian representatives of the genus, but is considerably larger (Table 2). Males possess a smaller spicule than those of *P. fitzroyi*, and a smaller tail appendage than both *P. fitzroyi* and *P. kartana*. Eggs of *P. kartana* are shorter than those of both *P. anomalus* and *P. fitzroyi*. Males of *P. anomalus* have a smoothly tapering genital cone,

whereas those of *P. fitzroyi* have a small distal expansion on the genital cone.

## Discussion

The genus *Parapharyngodon* has been twice relegated to synonymy with *Thelandros* (see Jones, 1992). In a major revision of *Thelandros*, Adamson (1981) reinstated the genus *Parapharyngodon* and considered these genera to be readily distinguishable by the presence of a prominent genital cone in males of *Thelandros*, and by differences in the eggs. *Thelandros* eggs have a terminal operculum, whereas the operculum of *Parapharyngodon* eggs is subterminal. Eggs of *Thelandros* are larvated in utero, while those of *Parapharyngodon* are deposited at an earlier stage of cleavage. In addition, Adamson (1981) noted that *Thelandros* spp. are parasites of omnivorous and herbivorous reptiles, whereas *Parapharyngodon* is found in insectivorous reptiles and amphibians. Adamson and Nasher (1984a, b) expanded these distinguishing criteria, in that males of *Thelandros* have pedunculate preanal and adanal papillae and a spicule pouch markedly posterior to the anus, whereas these papillae on males of *Parapharyngodon* are mammiform and the spicule pouch opens directly into the anus.

Two Australian species were considered in Adamson's (1981) revision. *Thelandros trachysauri* Johnston and Mawson (1947) was to remain in the genus *Thelandros* according to the text of Adamson's paper. However, it appeared

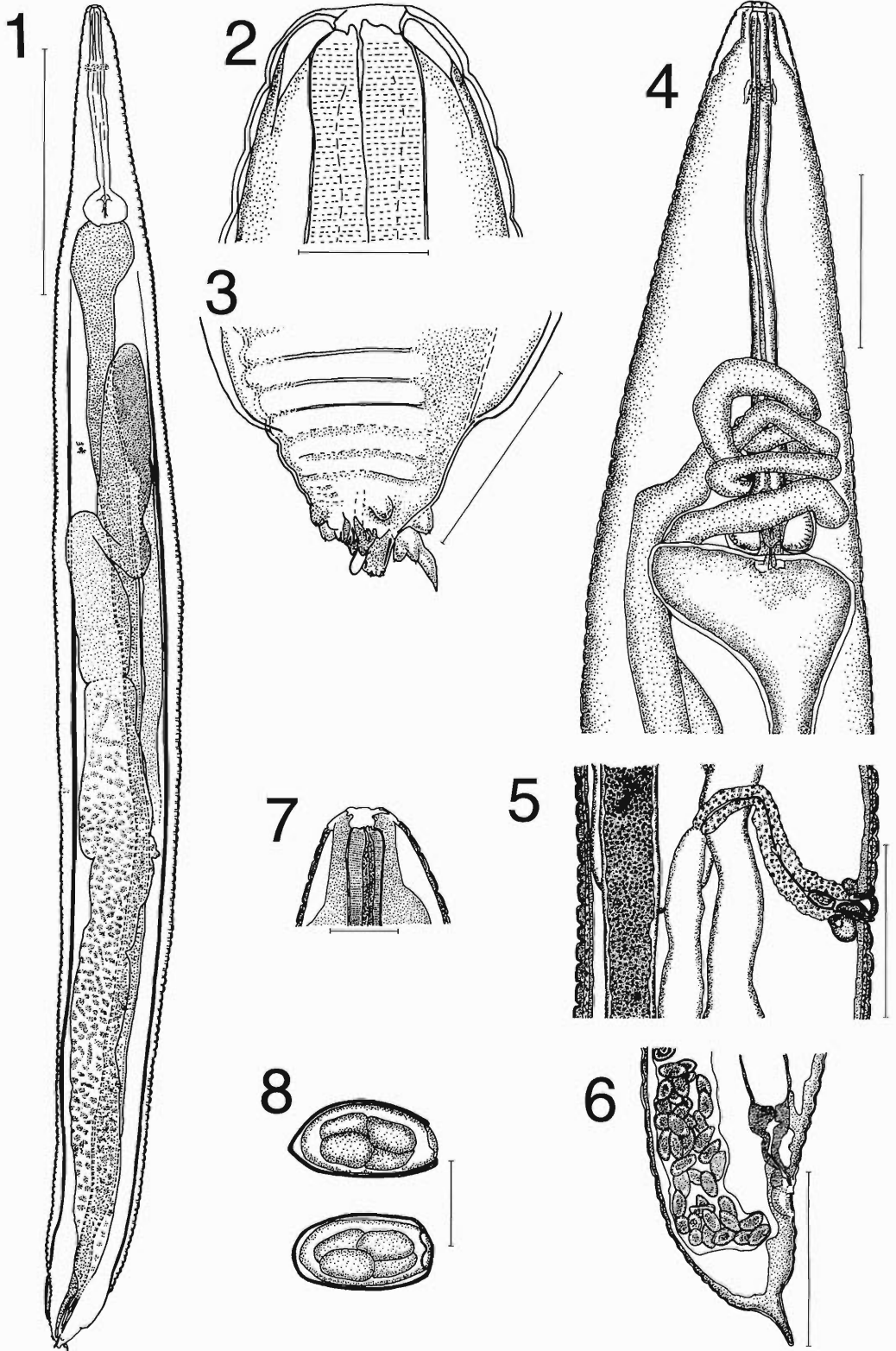


Table 2. Diagnostic comparison of the Australian species of the genus *Parapharyngodon*.

	<i>P. anomalus</i> sp. n.	<i>P. kartana</i>	<i>P. fitzroyi</i>
Body length (mm)			
Females	8.1–10.7	2.1–8.2	3.1–3.5
Males	5.3–5.6	1.6–4.2	2.0–2.4
Spicule length ( $\mu\text{m}$ )	63	55–65	80–92
Male tail appendage ( $\mu\text{m}$ )	58–60	50–90	60–80
Male genital cone	Smoothly tapering	—	Distal expansion
Egg length ( $\mu\text{m}$ )	83–95	75–90	88–96

in the abstract as being transferred to *Parapharyngodon*, and was not mentioned in a later compilation of *Thelandros* (Adamson and Nasher, 1984b). Type specimens viewed by the author, although not possessing pedunculate papillae, did display a prominent genital cone supported by a V-shaped sclerotized accessory piece, and eggs with terminal opercula, affirming their retention in *Thelandros*. Jones (1992) also considered this species to be a member of *Thelandros*.

Adamson (1981) placed the other Australian species, *T. kartana* Johnston and Mawson (1941) (= *T. khartana* of Adamson, 1981), in the genus *Parapharyngodon*. This species had already been transferred to *Parapharyngodon* by Mawson (1971), a fact that Adamson (1981) had overlooked. Although the male type specimen no longer has a posterior or anterior end, descriptions from the literature (Johnston and Mawson, 1941; Angel and Mawson, 1968; Mawson, 1971) indicate that the genital cone is not particularly prominent and is unlikely to be supported by a V-shaped sclerotized accessory piece. In the female type specimens, eggs have a subterminal operculum and the female has a rounded posterior with a stout spike tail. These characteristics all suggest *Parapharyngodon*. Note that material from *Hemiergis peronii*, listed as *Pharyngodon kartana* by Angel and Mawson (1968), is actually *Parapharyngodon kartana*; this error was transcribed in Adamson (1984).

In his description of *Parapharyngodon fitzroyi* from *Tiliqua multifasciata* in the northwest of Western Australia, Jones (1992) noted that this species could not be assigned easily to *Thelan-*

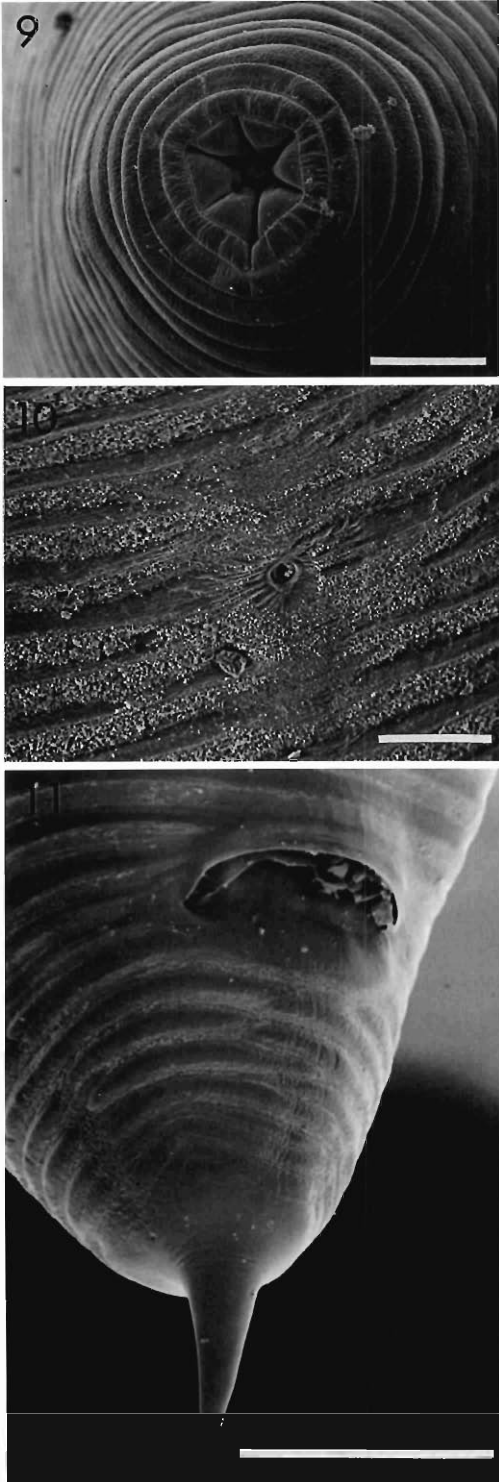
*dros* or *Parapharyngodon*. It conformed to *Parapharyngodon* in egg structure, in the shape of the female tail, and in the mammiform rather than pedunculate papillae in the males. However, the prominent postanal cone and the separate opening of the spicule pouch posterior to the anus aligned it to *Thelandros*. Thus, Jones (1992) concluded that the definitions of these 2 genera have yet to be satisfactorily resolved. *Parapharyngodon anomalus* sp. n. clearly fits *Parapharyngodon* for egg shape and shape of the female tail and male papillae, but it also possesses a separate opening to the spicule pouch, and the postanal cone is quite prominent. Therefore, all 3 Australian species of *Parapharyngodon* appear to share some characters usually diagnostic for *Thelandros*, which may suggest they are close to the ancestor of both genera.

The genus *Parapharyngodon* is considered to be a relatively ancient member of the Pharyngodonidae (Adamson and Nasher, 1984a), so the finding herein of a species of *Parapharyngodon* in a primitive mammal might be taken as evidence that mammals were the original vertebrate hosts of this family. Since the other 2 families of extant Oxyuroidea, Heteroxynematidae and Oxyuridae, are primarily parasites of mammals (Adamson, 1989), a single origin of parasitism in mammals could be indicated for the Oxyuroidea. However, Adamson (1989) argued persuasively that reptiles are the ancestral hosts of Pharyngodonidae, and that *Parapharyngodon* may have its origins in ancient insectivorous lizards (Adamson and Nasher, 1984a). The new *Parapharyngodon* is morphologically very sim-

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Figures 1–3. *Parapharyngodon anomalus* sp. n. male holotype. 1. Entire specimen anterolateral view. 2. Anterior end. 3. Posterior end. Scale lines: 1. 1 mm. 2. 50  $\mu\text{m}$ . 3. 100  $\mu\text{m}$ .

Figures 4–8. *Parapharyngodon anomalus* sp. n. female allotype. 4. Esophageal region. 5. Vulva. 6. Anus and tail tip. 7. Anterior end. 8. Eggs. Scale lines: 4. 500  $\mu\text{m}$ , 5. 500  $\mu\text{m}$ . 6. 500  $\mu\text{m}$ . 7. 100  $\mu\text{m}$ . 8. 50  $\mu\text{m}$ .



Figures 9–11. Scanning electron micrographs of *Parapharyngodon anomalus* sp. n. female. 9. En face.

ilar to other species of the genus found in modern Australian reptiles and it is likely that the line in reptiles would have diverged further than this had it originated in primitive mammals. Thus, *Parapharyngodon anomalus* sp. n., unlike other known parasites of echidnas (Inglis, 1968; Beveridge, 1980; Durette-Desset and Chabaud, 1981; McOrist and Smales, 1986), is most likely a relatively recent acquisition to the echidna parasite fauna, probably derived from host-switching with a recent reptilian species. This interpretation therefore does not refute the hypothesis of a reptilian ancestral host for the Pharyngodonidae.

A number of studies have reported helminths from echidnas, and the fauna is quite rich, with 2 endemic species of cestode, and 10 endemic trichostrongyloid nematodes (Spratt et al., 1990). However, these parasites appear to be relatively uncommon, and McOrist and Smales (1986) reported prevalences of only 12% both for the cestode *Linstowia echidnae* and for trichostrongyloid nematodes in a sample of 73 echidnas from Victoria in SE Australia. This combination of rich helminth fauna and low prevalence in echidnas, and the fact that Western Australia has been poorly sampled, supports the premise that this finding represents a new species and not just an incidental infection. The absence of this worm in the extensive survey of McOrist and Smales (1968) suggests that *P. anomalus* sp. n. may be restricted to Western Australia.

#### Acknowledgments

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10. Excretory pore. 11. Tail tip. Scale bars: 9. 50  $\mu$ m. 10. 50  $\mu$ m. 11. 200  $\mu$ m.

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## *Spauligodon goldbergi* sp. n. (Nematoda: Pharyngodonidae) and other Parasites of *Sonora semiannulata* (Serpentes: Colubridae) from New Mexico and Texas

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**ABSTRACT:** *Spauligodon goldbergi* sp. n. (Nematoda: Pharyngodonidae), a new oxyurid nematode, from the large intestine of *Sonora semiannulata* is described and illustrated. Seven of 48 (15%) adult specimens of *Sonora semiannulata* collected from Texas harbored a total of 181 specimens of *Spauligodon goldbergi* sp. n.; mean intensity was  $25.9 \pm 2.9$ , range was 15–34. *Spauligodon goldbergi* sp. n. is distinguished from all other Nearctic species in that the female possesses a pointed tail. This is the first report of a species of *Spauligodon* from a snake. The cestode *Oochoristica parvula* and cosmocercoid nematodes *Aplectana* sp. are also reported.

**KEY WORDS:** *Spauligodon goldbergi* sp. n., nematode, *Sonora semiannulata*, ground snake, Colubridae.

The ground snake, *Sonora semiannulata* Baird and Girard, 1953, is a small colubrid that ranges from SW Missouri and S Kansas to N Mexico and W to Nevada and California; disjunct populations occur in other western states (Conant and Collins, 1991). The ground snake is a secretive species having a preference for spiders, with centipedes, scorpions, and insects of the orders Lepidoptera, Coleoptera, Hymenoptera, and Orthoptera being eaten in smaller percentages (Kassing, 1961). Frost (1983) provided a summary of the biology of this snake in a species account. However, nothing, to our knowledge, is known about helminth parasites of *S. semiannulata*. The purpose of this note is to describe a new species of *Spauligodon* found in the large intestine of *Sonora semiannulata* from New Mexico and Texas and to report new host and geographic distribution records on other parasites of this snake.

### Materials and Methods

Between March 1986 and April 1991, 48 (22 males, 26 females) juvenile and adult (mean  $\pm$  SEM snout-vent length [SVL] =  $181.0 \pm 6.9$ , 90–256 mm) *S. semiannulata* were collected by hand from Hood ( $N = 20$ ), Johnson ( $N = 7$ ), Somervell ( $N = 18$ ), Upton ( $N = 1$ ), and Williamson ( $N = 1$ ) counties of Texas and San Miguel ( $N = 1$ ) County of New Mexico. Snakes were killed within 48 hr of capture with an overdose of sodium pentobarbital (Nembutal®) and examined for parasites. Blood samples were obtained from the exposed ventricle and stained with Giemsa for examination of hematozoa. Intestinal contents and feces were examined for coccidia. Tapeworms were stained with Semichon's acetocarmine and mounted in Damar.

Nematodes were placed in undiluted glycerol, allowed to clear, and examined under a light microscope.

Voucher specimens of *S. semiannulata* were deposited in the Arkansas State University Museum of Zoology as ASUMZ 5949–55, 5967–68, 7712–14, 8464–65, 8485–86, 8494, 8524–29, 8619, 8643–46, 8676–80, 11753–54, 16720, 16745, 16783, 17514, 17873–76. Specimens of parasites were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Oochoristica parvula* (USNPC 84397), *Aplectana* sp. (84398), *Spauligodon goldbergi* sp. n. (84518–20).

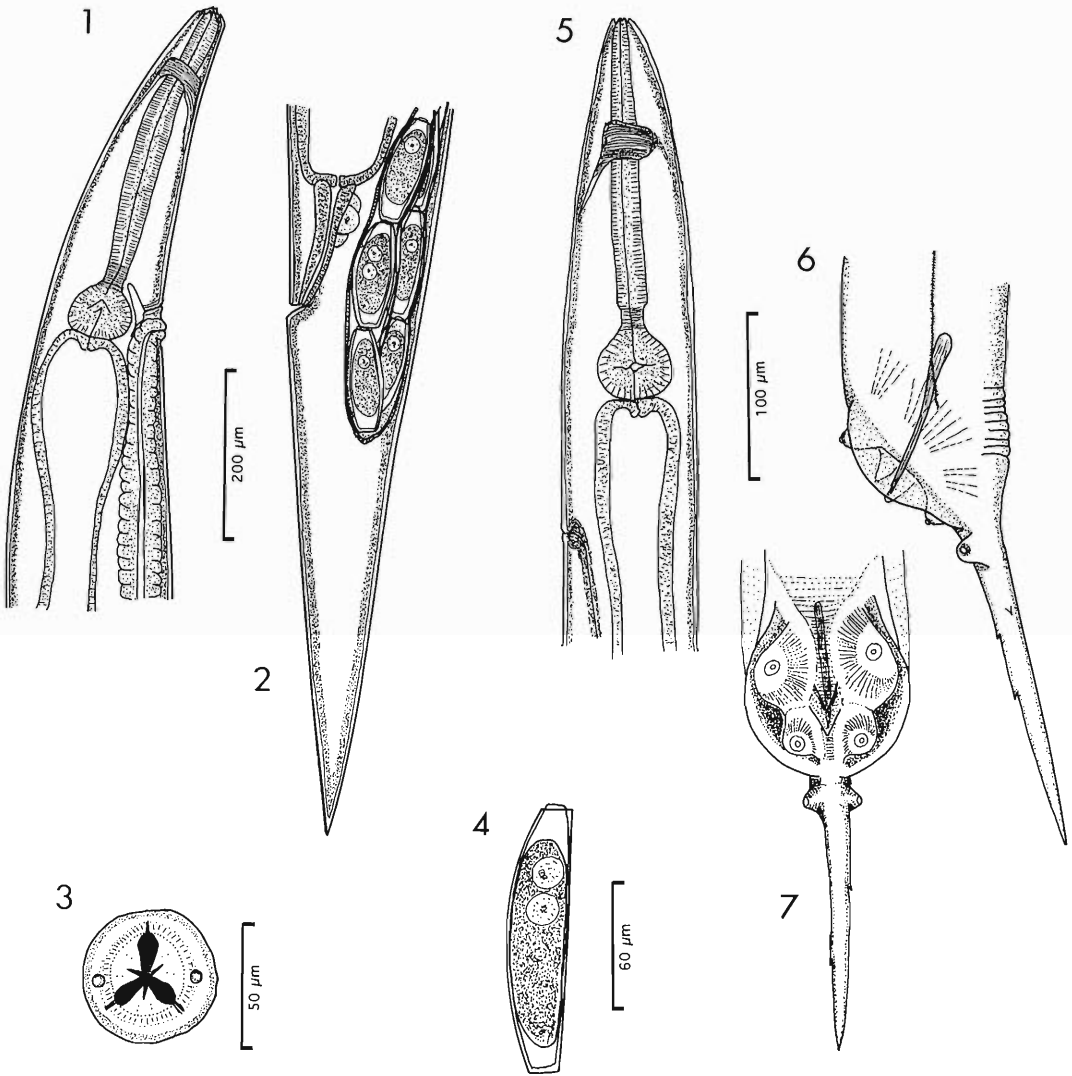
### Results

Twelve (25%) of the *S. semiannulata* (7 males, 5 females,  $201.0 \pm 7.4$ , 183–256 mm) were found to harbor parasites, including 1 (2%) with *Oochoristica parvula* (Stunkard, 1938) Stunkard, 1938, 5 (10%) with *Aplectana* sp., and 7 (15%) with an undescribed species of oxyurid nematode belonging to the genus *Spauligodon*. Blood smears were negative for hematozoa, and coccidia were not found in feces or intestinal contents. A description of the new species follows.

### *Spauligodon goldbergi* sp. n. (Figs. 1–7)

**DESCRIPTION:** Generic diagnosis after Skrjabin et al. (1960). Nematodes of small size with cylindrical body tapering both anteriorly and posteriorly. Cuticle transversely striated. Lateral alae present in males and females. Mouth opening triangular, bounded by 3 lips, each with shallow midline indentation. Esophagus ends in valvulate, subspherical bulb which is separated from





Figures 1–7. *Spauligodon goldbergi* sp. n. 1. Anterior end of female, lateral view. 2. Posterior end of female, lateral view. 3. Female, en face view. 4. Egg. 5. Anterior end of male, lateral view. 6. Posterior end of male, lateral view. 7. Posterior end of male, ventral view.

esophageal body by small constriction. Excretory pore behind esophageal bulb in males. Males having caudal alae which do not envelop posterior postcloacal pair of pedunculate papillae; females having vulva in anterior half of body.

**MALE** (based on 10 specimens; mean measurement and range in mm): Small, white, fusiform nematodes tapering both anteriorly and posteriorly; length, 2.14 (1.84–2.47); maximum width, 0.100 (0.091–0.114). Narrow lateral alae extend from halfway between nerve ring and lips to middle of caudal alae. Cuticle with striations

of approximately 1 μm in width. Mouth bounded by 3 lips each with midline indentation producing 2 pointed lobes. One small, pedunculate amphid on each ventrolateral lip. Esophagus (including bulb), 0.296 (0.285–0.333); bulb length, 0.057 (0.051–0.068); bulb width, 0.055 (0.048–0.068). Nerve ring, 0.126 (0.114–0.137); excretory pore, 0.404 (0.357–0.510) from anterior end. Narrow caudal alae present, 0.004 (0.004–0.005) wide by 0.079 (0.074–0.091) long. Three pairs of caudal papillae present; precloacal pair situated on slightly inflated ventral surface of caudal

end, first postcloacal pair posteriolaterally directed; second postcloacal pair not enclosed by caudal alae, 0.043 (0.037–0.048) behind first postcloacal pair. Prominent genital cone in mid-ventral line consisting of small, pointed anterior cloacal lip and larger, pointed posterior cloacal lip; spicule weakly sclerified, 0.085 (0.077–0.091). Cloacal opening 0.166 (0.157–0.177) from posterior extremity. Stiff tail spike extends 0.123 (0.112–0.131) beyond second postcloacal papillae; 5 (3–7) cuticular spines.

**FEMALE** (based on 10 gravid specimens): Small, white, nematodes tapering anteriorly and posteriorly; length, 5.26 (4.09–6.04); maximum width, 0.200 (0.160–0.222). Narrow lateral alae extending from between mouth and nerve ring to posterior lip of anus. Cuticle with striations of approximately 1–1.5  $\mu\text{m}$  width. Esophagus (including bulb), 0.430 (0.304–0.497); bulb length, 0.069 (0.051–0.081); bulb width, 0.070 (0.051–0.080). Nerve ring, 0.132 (0.125–0.154); excretory pore, 0.323 (0.239–0.382); vulva, 0.355 (0.291–0.439), from anterior end, respectively. Thick-walled muscular ovijector extends posteriorly 0.300 continuing as thin-walled vagina 0.300 joining 2 uteri, 1 directed anteriorly and the other posteriorly. Ovarian and uterine coils do not extend anteriorly as far as the esophageal bulb. Anus 1.21 (0.92–1.40) from posterior end of body. Postanal body region tapering to a point. Eggs barrel-shaped, ends truncated, operculate, 0.122 (0.111–0.131) by 0.032 (0.029–0.034), no polar adornment, no development, pronucleus stage at deposition.

**TYPE SPECIMENS:** Holotype. Male (U.S. National Parasite Collection, Beltsville, Maryland, accession no. 84518). Allotype: Female (84519). Paratypes (84520).

**TYPE HOST:** *Sonora semiannulata* Baird and Girard, 1853 "ground snake," CTM #860504–8, collected 4 May 1986, gravid adult female, 256 mm SVL, ASUMZ 5953.

**ADDITIONAL HOSTS:** *S. semiannulata*, 2 males, 4 females; 210.6  $\pm$  10.1, range 193–256 mm SVL, ASUMZ 8465–67, 8643, 8676, 8680.

**TYPE LOCALITY:** Somervell County, Texas ( $N = 3$ ), 1.6 km SW Nemo off FM 200 on county road 402.

**ADDITIONAL LOCALITIES:** Somervell County, Texas ( $N = 2$ ), 3.2 km SSW Nemo off FM 200 on county road 401; Hood County, Texas ( $N = 1$ ), off FM 2174 at Fort Spunky.

**SITE OF INFECTION:** Rectum.

**PREVALENCE:** Found in 7 of 48 (15%) *S. semiannulata* in Texas. Breakdown is as follows: 1/20 (5%) Hood County, 0/7 (0%) Johnson County, 6/18 (33%) Somervell County, 0/1 (0%) Upton County, and 0/1 (0%) Williamson County, Texas; 0/1 (0%) San Miguel County, New Mexico.

**ETYMOLOGY:** Named in honor of Dr. Stephen R. Goldberg, Whittier College, in recognition of his contributions to reptilian parasitology.

### Discussion

The general morphology of *Spauligodon goldbergi* sp. n. allows its assignment to the superfamily Oxyuroidea Railliet, 1916, family Pharyngodonidae Travassos, 1919, which currently contains 21 genera (see Petter and Quentin, 1976). Of these, 3 genera characteristic of reptiles exhibit a vulvar opening in the anterior part of the body just behind the excretory pore: *Pharyngodon* Diesing, 1861, *Spauligodon* Skrjabin, Schikhobalova, and Lagodovskaja, 1960, and *Skrjabinodon*, Inglis, 1968. These genera are separated by the relationship of the caudal alae to the genital papillae: males of the genus *Pharyngodon* have well-developed caudal alae which envelop all genital papillae; in males of the genus *Spauligodon*, the posterior pair of papillae are excluded from envelopment by the caudal alae; and males of the genus *Skrjabinodon* lack caudal alae. The inclusion of the described specimens in the genus *Spauligodon* is based upon the position of the vulva and the configuration of the caudal alae.

The genus *Spauligodon* contains species which are separated on the basis of the egg shape, tail shape, and geographical distribution (see Table 1 of Bursey and Goldberg, 1995). *Spauligodon goldbergi* sp. n. should be added to that table: Nearctic Realm; spicule length, 80–90  $\mu\text{m}$ ; male tail, 3–7 spines; female tail, smooth; eggs truncated. *Spauligodon goldbergi* sp. n. belongs to the subgroup in which the males have a spicule: *S. auziensis* (Seurat, 1917), *S. azerbaijanicus* Sharpilo, 1974, *S. carbonelli* Roca and Garcia-Adell, 1988, *S. extenuatus* (Rudolphi, 1819), *S. laevicauda* (Seurat, 1914), and *S. mearnsi* (Edgerly, 1952). However, in each of these species the female possesses a filiform tail; females of *S. goldbergi* sp. n. lack a filiform tail, the posterior extremity ends in a point.

Three previously described species are found in the Nearctic Realm: *S. californiensis* (Read

and Amrein, 1953) Skrjabin, Schikhobalova, and Lagodovskaja, 1960; *S. giganticus* (Read and Amrein, 1953) Skrjabin, Schikhobalova, and Lagodovskaja, 1960; and *S. mearnsi* (Edgerly, 1952) Skrjabin, Schikhobalova, and Lagodovskaja, 1960. The female of *S. goldbergi* sp. n. differs from these 3 species in that it possesses a pointed tail; females of the other Nearctic species have filiform tails. Males of *S. californienensis* and *S. giganticus* lack spicules; the male of *S. mearnsi* has a spicule and a smooth tail, whereas the male *S. goldbergi* sp. n. has a spicule and a spiny tail. These comparisons were based on published descriptions; no type specimens were examined.

In addition to the new species, other parasites found in *S. semiannulata* are reported for the first time. Three linstowiid tapeworms fitting the description of *Oochoristica parvula* were found in the small intestine of an adult male (SVL = 200 mm, ASUMZ 16783) *S. semiannulata* collected in August 1990 from San Miguel County, New Mexico. *Oochoristica parvula* was originally described from the gecko, *Coleonyx elegans* Gray, from Yucatan, Mexico (Stunkard, 1938). Measurements of *O. parvula* from *S. semiannulata* are as follows: 60–65 proglottids, 20–25 mm long by 0.68–0.77 mm wide, scolex 240–250  $\mu$ m, suckers 70–86  $\mu$ m, testes number 20–30, testes diameter 20–28  $\mu$ m. These measurements are within the ranges of those provided by Stunkard (1938).

A total of 11 male cosmocercoid nematodes, *Aplectana* sp. were found in the rectum of 2 adult (male and female, 188–208 mm SVL, ASUMZ 8465, 8559) *S. semiannulata* collected in Hood County, a single adult male (157 mm SVL, ASUMZ 11754) collected in Johnson County, and 2 adult males (190–202 mm SVL, 8644, 8677) from Somervell County, Texas. Mean intensity was  $2.2 \pm 0.73$  (range 1–5) worms/host. These nematodes possessed simple papillae in what appeared to be 2 rows of 8. The body, spicule, and gubernaculum length and shape appeared most similar to *Aplectana izocanensis* Bravo Hollis, 1943. However, without females, specific identification was not possible. *Aplectana* spp. have

been reported primarily from amphibians worldwide (Baker, 1987).

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## Helminth Parasites of Four Species of Aquatic Snakes from Two Habitats in Southeastern Louisiana

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**ABSTRACT:** A total of 202 specimens of water snakes (*Nerodia* spp.) and 17 cottonmouths (*Agkistrodon piscivorus*) were collected from 2 localities in southeastern Louisiana between March and December 1989, and examined for helminths. Six species of trematodes, 4 cestodes, 9 nematodes, and 2 acanthocephalans were recovered from these snakes. At the Head of Island locality, *Dasymetra villicaeca* and *Proteocephalus variabilis* had the highest prevalence and abundance in *Nerodia cyclopion*; *D. villicaeca*, *Styphlodora magna*, and *Terranova caballeroi* displayed the broadest host specificity, infecting all snakes from Head of Island. Species richness and mean number of individuals were highest in *N. cyclopion* and *A. piscivorus*, but diversity was highest in *N. fasciata*. *Proteocephalus perspicua* and *P. variabilis* were most prevalent and abundant in *N. cyclopion* at the Spanish Lake locality; *Ochetosoma aniarum* and *Kalicephalus rectiphilus* displayed broadest host specificity, infecting all snakes from Spanish Lake. Species richness and mean number of individuals were highest in *N. cyclopion*, while diversity was highest in *A. piscivorus*. In general, higher prevalence, abundance, species richness, and mean number of individuals occurred in snakes from Head of Island. The helminth fauna of *Nerodia* spp. were most similar, reflecting close phylogenetic affinities and broad overlap in diet. In contrast, the helminth fauna of the viperid, *A. piscivorus*, contained 5 helminths that were restricted to this snake. We propose that host diet, habitat differences, and phylogeny are the most important determinants of the helminth fauna of aquatic snakes.

**KEY WORDS:** Trematoda, Cestoda, Nematoda, Acanthocephala, helminths, survey, *Nerodia* spp., *Agkistrodon piscivorus*, snakes, Louisiana.

The helminth communities of fishes, birds, and mammals have been well studied and many theoretical predictions of parasite community organization have originated from these studies. However, the helminth communities of amphibians and reptiles have not been as well characterized as those of other vertebrates. Recently, Aho (1990) summarized what is known of the organization of helminth communities in these hosts. He concluded that their helminth communities are, in general, "highly variable, depauperate, and have traits characteristic of non-interactive community structure." Phylogenetic differences among hosts, as well as local environmental conditions, were shown to be important determinants of helminth community composition.

Several excellent studies of the helminth communities of frogs, salamanders, and turtles have been published (Esch et al., 1979a, b; Goater et al., 1987; Muzzall, 1991a, b), but little quantitative data exist for snake helminth communities. Most studies of the parasites of aquatic snakes have emphasized the taxonomy of certain groups

of parasites. However, several surveys have been concerned with the ecological relationships between helminth parasites and their snake hosts. Collins (1969) conducted a comparative study of the helminths of water snakes (*Nerodia* spp.) and the cottonmouth (*Agkistrodon piscivorus*) in North Carolina, and Detterline et al. (1984) compared the helminths of *A. piscivorus* and 3 species of *Nerodia* in Alabama. Studies of the overwintering of helminths in *Thamnophis sirtalis* (Rau and Gordon, 1978), host specificity of snake helminths (Rau and Gordon, 1980), and other general surveys have been published (Anderson, 1935; Gibson and Rabalais, 1973; Camp, 1980). The taxonomy and life histories of snake trematodes in Louisiana have been well studied (Byrd, 1935; Bennett, 1935, 1938; Rabalais, 1967, 1968, 1969a, b; Rabalais and Henson, 1968), but no complete surveys of the helminth fauna of Louisiana snakes have been published. In Louisiana, the cottonmouth and water snakes co-occur in many habitats and prey upon similar food items (Mushinsky, 1987). Because of these ecological similarities, this system presented an opportunity to compare the helminth faunas among these sympatric hosts. The objectives of this study were: 1) to determine the prevalence and abundance of helminths in aquatic snakes, 2) to compare

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the helminth faunas of *Nerodia* spp. and *A. piscivorus*, and 3) to compare the helminth community structures of aquatic snakes from 2 different localities.

### Materials and Methods

Snakes were collected from 2 freshwater localities in southeastern Louisiana. The Head of Island (HOI) study area was located near Head of Island, Louisiana, in Livingston Parish. The HOI locality consisted primarily of cypress-tupelo gum swamp, with some bottomland hardwood forest. The main waterway from which snakes were collected was the Petite Amite River, a natural meandering river with many fallen trees, stumps, and logs. At HOI, the swamp canopy closely follows the courses of the Petite Amite; few large open water areas are present.

The Spanish Lake (SL) study area was a swamp-bayou system located in Ascension Parish, Louisiana. This area has been described by Hebrard and Mushinsky (1978), but in general was characterized as a cypress-tupelo gum swamp with some areas of bottomland forest present. The major waterways are Spanish Lake, Alligator Bayou, Bayou Braud, and Bayou Paul. The presence of Spanish Lake, a large water body, differentiates the SL study area from HOI in that large open expanses of water are present. We observed a greater degree of fluctuation of water levels at SL than at HOI.

A total of 219 snakes including 108 green water snakes, *Nerodia cyclopion* (Dumeril, Bibron, and Dumeril, 1854), 59 broad-banded water snakes, *N. fasciata* (Linnaeus, 1766), 35 diamond-backed water snakes, *N. rhombifera* (Hallowell, 1852), and 17 cottonmouths, *Agkistrodon piscivorus* (Lacepede, 1789) were collected. Snakes were captured by hand, with Pillstrom tongs, or by shooting between March and December 1989. Most snakes were maintained in a refrigerator at 7°C to minimize parasite loss and were usually necropsied within 7 days of capture. Snakes were killed with MS-222 (ethyl m-aminobenzoate methane sulfonic acid) injected intracardially. Snakes that were shot were examined immediately or frozen at -18°C for later examination. Snakes that were frozen included 81 *N. cyclopion*; no other snake species were frozen before necropsy. Taxonomy of snake hosts follows Dundee and Rossman (1989).

The gastrointestinal tract, spleen, liver, gall bladder, heart, lungs, trachea, kidneys, reproductive tract, and oral cavity were examined for helminths. Larval parasites occurred commonly and in large numbers, but were not included in this study. Trematodes were fixed using Berland's solution (1 part formalin : 9 parts acetic acid) and placed in AFA. Cestodes were killed with hot water (90°C) and fixed in AFA. Nematodes were fixed with Berland's solution and transferred to a solution of 70% ethanol and 5% glycerin. Acanthocephalans were placed in 7°C distilled water for 24 hr, and then fixed in AFA. Trematodes, cestodes, and acanthocephalans were stained with Semichon's carmine. Whole mounts of nematodes were made using glycerin jelly. Voucher specimens of helminths were deposited in the United States National Parasite Collection, Beltsville, Maryland (accession nos. 84845-84861;

85272-85275). Ecological terminology conforms to the definitions of Margolis et al. (1982). Chi-square analysis was used to test for differences in prevalence between sexes. Mann-Whitney *U*-tests were used for 2-sample testing. Species richness is the mean number of helminth species per snake. Helminth species diversity was characterized with the Shannon-Weiner diversity index using common logarithms (Zar, 1984). Percent similarity and Jaccard's coefficients were also used to compare communities. These indices were calculated using Ecological Analysis-PC, Oakleaf Systems, P.O. Box 472, Decorah, Iowa 52101. Original data from all snakes are available from the senior author.

### Results

Body sizes (snout-vent length) of all snakes are presented in Table 1. Twenty-one helminth species (6 Trematoda, 4 Cestoda, 9 Nematoda, and 2 Acanthocephala) were recovered from the 4 snake species examined (Tables 2, 5). There was no significant difference in prevalence regardless of parasite species with respect to host sex for all snake species ( $P > 0.05$ ) from both localities. However, abundances of *Ochetosoma aniarum* and *Dasymetra villicaeca* were significantly higher in females of *N. cyclopion* at HOI ( $P < 0.05$ ). No significant difference in species richness ( $P > 0.05$ ) was found between sexes for all snake species from both localities. However, mean number of individual helminths per host was significantly higher in females of *N. cyclopion* at HOI ( $P < 0.01$ ).

Nineteen helminth species infected snakes from the HOI locality (Table 2). Within *Nerodia* spp., highest prevalence and abundance of *Dasymetra villicaeca* and *Pneumatophilus variabilis* occurred in *N. cyclopion*. Prevalence of *Ochetosoma aniarum* was highest in *N. cyclopion*, but abundance was greatest in *N. fasciata*. *Styphlodora magna* infected all species of HOI snakes, but prevalence and abundance was highest in *Agkistrodon piscivorus*. *Ochetosoma laterotrema* was abundant in *A. piscivorus*, but did not occur in other snakes. Similarly, *Proteocephalus agkistrodontis* and *P. marenzelleri* were prevalent (>70%), but their host distribution was limited to *A. piscivorus*. *Proteocephalus perspicua* infected all species of *Nerodia*, with highest prevalence and abundance in *N. cyclopion*. Abundance of *P. variabilis* was highest for all cestodes; however, this helminth only occurred in *N. cyclopion*. *Rhabdias fuscovenosa* was found in all *Nerodia* spp., but *R. eustreptos* was specific to *A. piscivorus*. Another nematode, *Terranova cabaleroi*, infected all species of snakes from HOI,

Table 1. Body sizes of aquatic snakes from Head of Island and Spanish Lake, Louisiana.

	<i>Nerodia cyclopion</i>		<i>Nerodia fasciata</i>		<i>Nerodia rhombifera</i>		<i>Agkistrodon piscivorus</i>	
	Head of Island	Spanish Lake	Head of Island	Spanish Lake	Head of Island	Spanish Lake	Head of Island	Spanish Lake
N	48	60	30	29	11	24	10	7
Snout-vent length (cm)	60.2 ± 1.2*	63.0 ± 1.5	55.1 ± 1.7	47.8 ± 2.0	81.7 ± 3.6	62.1 ± 4.6	60.5 ± 2.1	60.9 ± 6.8
Range (cm)	44.5-78.5	32.0-81.5	33.7-70.0	28.0-70.0	63.3-100.5	22.0-113.0	53.0-71.5	38.5-84.0

\* Standard error.

but was most abundant in *N. cyclopion* and *A. piscivorus*.

From a community perspective, species richness and mean number of individuals at HOI were highest in *N. cyclopion* and *A. piscivorus*, with a mean of >6 species of helminths and >300 individuals per snake (Table 3). Species richness and mean number of individuals were markedly lower in *N. fasciata* and *N. rhombifera*, but values were similar between these 2 snakes. The Shannon-Weiner species diversity index was highest for the helminth community of *N. fasciata*, while *A. piscivorus* harbored the least diverse helminth community. Jaccard's coefficient and percent similarity indicated that helminth community structure was most similar among *Nerodia* spp. The helminth community of *A. piscivorus* was distinct, reflecting the unique helminths restricted to this snake (Table 4).

Snakes from SL harbored a total of 17 helminth species (Table 5). *Ochetosoma aniarum*, *D. villicaeca*, and *P. variabilis* were most abundant in *N. rhombifera*. *Ochetosoma kansense*, *S. magna*, *Proteocephalus agkistrodontis* and *P. marenzelleri* were only found in *A. piscivorus*. *Proteocephalus perspicua* infected all species of snakes at SL, but highest prevalence and abundance occurred in *N. cyclopion*. *Proteocephalus variabilis* was the most prevalent and abundant helminth at SL, but was specific to *N. cyclopion*. *Kalicephalus rectiphilus* infected all snakes at SL, but was most prevalent and abundant in *A. piscivorus*. *Rhabdias fuscovenosa* infected all species of *Nerodia* at SL; however, *R. eustreptos* was specific to *A. piscivorus*.

Species richness was highest at SL in *N. cyclopion* and *A. piscivorus* (Table 3). Species richness was markedly lower for *N. fasciata* and *N. rhombifera*. Mean number of individuals was highest in *N. cyclopion* and *N. rhombifera*. Values of Shannon-Weiner species diversity were highest for the helminth communities of *A. piscivorus* and *N. fasciata*; *Nerodia cyclopion* had the least diverse helminth community. Analysis with Jaccard's coefficient revealed that helminth community structure of *Nerodia* spp. was most similar (Table 6). In general, the helminth community of *A. piscivorus* exhibited little overlap with *Nerodia* spp. However, analysis of percent similarity indicated that the helminths of *N. rhombifera* and *N. fasciata* were quantitatively most similar (Table 6).

When comparing snakes from HOI and SL, prevalence and abundance of most helminths

Table 2. Prevalence and abundance (mean ± SE) of helminths in 4 species of sympatric aquatic snakes from Head of Island, Louisiana.

Parasite species	<i>Nerodia cycloption</i> N = 48		<i>N. fasciata</i> N = 30		<i>N. rhombifera</i> N = 11		<i>Agkistrodon piscivorus</i> N = 10	
	Prevalence %	Abundance	Prevalence %	Abundance	Prevalence %	Abundance	Prevalence %	Abundance
<b>Trematoda:</b>								
<i>Ochetosoma aniarum</i> (Leidy, 1891)	88	19.1 ± 6.7	67	22.6 ± 8.6	73	9.6 ± 5.4	0	0
<i>O. laterotrema</i> (Byrd and Denton, 1938)	0	0	0	0	0	0	100	262.0 ± 37.9
<i>Dasymetra villicata</i> Byrd, 1935	90	97.0 ± 31.9	70	18.0 ± 6.9	73	19.0 ± 8.2	20	4.4 ± 3.7
<i>Pneumatophilus variabilis</i> (Leidy, 1856)	60	2.1 ± 0.5	17	0.5 ± 0.3	45	0.7 ± 0.3	0	0
<i>Styphlodora magna</i> Byrd and Denton, 1938	15	0.9 ± 0.4	30	1.4 ± 0.7	9	0.1 ± 0.1	80	5.2 ± 1.6
<b>Cestoda:</b>								
<i>Proteocephalus agkistrodonis</i> Harwood, 1933	0	0	0	0	0	0	80	4.0 ± 1.4
<i>P. marenzelleri</i> (Barrois, 1898)	0	0	0	0	0	0	70	2.2 ± 0.6
<i>P. perspicua</i> (LaRue, 1911)	96	16.1 ± 2.0	27	0.9 ± 0.4	55	4.4 ± 1.6	0	0
<i>P. variabilis</i> Brooks, 1978	100	156.0 ± 16.3	0	0	0	0	0	0
<b>Nematoda:</b>								
<i>Capillaria heterodontis</i> Harwood, 1932	0	0	3	0.1 ± 0.1	0	0	40	2.2 ± 1.3
<i>Cosmocercoides dukae</i> (Holl, 1928)	0	0	0	0	0	0	10	0.4 ± 0.4
<i>Falcaustra catesbetiana</i> Walton, 1929	10	0.2 ± 0.1	0	0	0	0	0	0
<i>Kalicephalus rectiphilus</i> Harwood, 1932	0	0	13	0.2 ± 0.1	0	0	30	0.5 ± 0.3
<i>Rhabdias eustreptos</i> (MacCallum, 1921)	0	0	0	0	0	0	70	2.4 ± 0.8
<i>R. fuscovenosa</i> (Railliet, 1899)	31	0.5 ± 0.1	47	2.7 ± 0.9	18	0.2 ± 0.1	0	0
<i>Strongyloides serpentis</i> Little, 1966	4	0.2 ± 0.1	0	0	0	0	0	0
<i>Terranova caballaeroi</i> Barus and Coy Otero, 1966	98	28.0 ± 2.8	97	14.3 ± 3.0	27	0.8 ± 0.5	100	22.7 ± 8.5
<b>Acanthocephala:</b>								
<i>Leptorhynchoides thecatus</i> (Linton, 1891)	27	1.3 ± 0.4	7	0.2 ± 0.2	0	0	0	0
<i>Neoechinorhynchus cylindricus</i> (Van Cleave, 1913)	15	0.3 ± 0.1	0	0	0	0	0	0

Table 3. Comparative diversity of helminth species in aquatic snakes.

	<i>Nerodia cycloption</i>		<i>Nerodia fasciata</i>		<i>Nerodia rhombifera</i>		<i>Agkistrodon piscivorus</i>	
	Head of Island (N = 48)	Spanish Lake (N = 60)	Head of Island (N = 30)	Spanish Lake (N = 29)	Head of Island (N = 11)	Spanish Lake (N = 24)	Head of Island (N = 10)	Spanish Lake (N = 7)
Species richness	6.4 ± 0.2*	3.0 ± 0.11	3.8 ± 0.3	1.3 ± 0.2	3.0 ± 0.5	1.5 ± 0.2	6.1 ± 0.4	3.0 ± 0.8
Mean no. of individuals	321.4 ± 44.0	79.4 ± 7.3	60.9 ± 12.6	4.2 ± 1.3	34.7 ± 13.2	43.9 ± 35.9	306.1 ± 41.9	7.1 ± 2.2
Shannon-Weiner diversity (H')	0.580	0.181	0.629	0.746	0.503	0.326	0.279	0.833
Hmax'	1.079	0.954	1.000	0.903	0.845	0.845	1.041	0.954
Evenness (H'/Hmax')	0.538	0.190	0.629	0.826	0.595	0.386	0.268	0.873

\* Standard error.

Table 4. Similarities between helminth communities of aquatic snakes from Head of Island, Louisiana.

Species	<i>Nerodia cycloption</i>	<i>N. fasciata</i>	<i>N. rhombifera</i>	<i>Agkistrodon piscivorus</i>
Jaccard's coefficient				
<i>Nerodia cycloption</i>	—	0.57	0.58	0.16
<i>N. fasciata</i>	0.47	—	0.70	0.31
<i>N. rhombifera</i>	0.45	0.62	—	0.20
<i>Agkistrodon piscivorus</i>	0.09	0.11	0.04	—
Percent similarity				

were much higher in HOI snakes (Tables 2, 5). This trend was especially true for trematodes. For example, *D. villicaeaca* infected 90% of *N. cycloption* at HOI with a mean abundance of 97.0. At SL, this trematode infected only 8.0% of *N. cycloption* and had an abundance value of 0.5. *Terranova caballeroi* was the most abundant nematode at HOI, but did not occur at SL. Despite these locality differences, a similar pattern of prevalence and abundance for *P. perspicua* and *P. variabilis* occurred in *N. cycloption*. Abundance of these cestodes was significantly higher for *N. cycloption* at HOI, but appeared to be only a difference in magnitude, as SL *N. cycloption* were also heavily infected.

From a community perspective, both species richness and mean number of individuals were higher in HOI snakes than in SL snakes (Table 3). Species richness was significantly higher in *N. cycloption* ( $P < 0.001$ ), *N. fasciata* ( $P < 0.001$ ), *N. rhombifera* ( $P < 0.01$ ), and *A. piscivorus* ( $P < 0.01$ ) from HOI than in conspecifics from SL. Mean number of individuals was significantly higher in *N. cycloption* ( $P < 0.001$ ), *N. fasciata* ( $P < 0.001$ ), and *A. piscivorus* ( $P < 0.001$ ) from HOI; however, this metric was significantly higher in *N. rhombifera* ( $P < 0.05$ ) from SL. Shannon-Weiner species diversity values were higher in *N. cycloption* and *N. rhombifera* from HOI when compared to conspecifics from SL (Table 3). Jaccard's coefficients for locality comparisons of the helminth communities of *N. cycloption*, *N. fasciata*, *N. rhombifera*, and *A. piscivorus* were 0.62, 0.50, 0.56, and 0.43, respectively. Percent similarity values for locality comparisons of the helminth communities of *N. cycloption*, *N. fasciata*, *N. rhombifera*, and *A. piscivorus* were 0.55, 0.51, 0.40, and 0.05, respectively. These community similarity indices indicated that the helminth community structure of *Nerodia* spp. was



Table 5. Prevalence and abundance (mean ± SE) of helminths in 4 species of sympatric aquatic snakes from Spanish Lake, Louisiana.

Parasite species	<i>Nerodia cyclopiion</i> N = 60		<i>N. fasciata</i> N = 29		<i>N. rhombifera</i> N = 24		<i>Agkistrodon piscivorus</i> N = 7	
	Prevalence %	Abundance	Prevalence %	Abundance	Prevalence %	Abundance	Prevalence %	Abundance
<b>Trematoda:</b>								
<i>Ochetosoma aniarum</i>	15	0.2 ± 0.1	34	0.7 ± 0.2	33	35.8 ± 33.4	14	0.1 ± 0.1
<i>O. kansense</i> (Crow, 1913)	0	0	0	0	0	0	14	1.3 ± 1.3
<i>Dasymetra villicaeca</i>	8	0.5 ± 0.3	3	1.2 ± 1.2	13	3.5 ± 2.4	0	0
<i>Pneumatophilus variabilis</i>	52	1.0 ± 0.1	17	0.2 ± 0.1	50	2.0 ± 0.7	0	0
<i>Styphlodora magna</i>	0	0	0	0	0	0	14	0.1 ± 0.1
<b>Cestoda:</b>								
<i>Proteocephalus agkistrodonis</i>	0	0	0	0	0	0	29	0.3 ± 0.2
<i>P. marenzelleri</i>	0	0	0	0	0	0	29	0.4 ± 0.3
<i>P. perspicua</i>	92	4.7 ± 0.5	24	0.4 ± 0.2	29	0.7 ± 0.3	43	0.9 ± 0.5
<i>P. variabilis</i>	95	72.1 ± 7.3	0	0	0	0	0	0
<b>Nematoda:</b>								
<i>Capillaria heterodontis</i>	0	0	0	0	8	0.9 ± 0.7	43	1.1 ± 0.6
<i>Cosmoecerooides dukae</i>	0	0	3	0.03 ± 0.04	0	0	0	0
<i>Kalicephalus rectiphilus</i>	3	0.03 ± 0.02	28	1.2 ± 0.5	13	1.0 ± 0.4	71	1.7 ± 0.6
<i>Oswaldocruzia pipiens</i> Walton, 1929	0	0	3	0.03 ± 0.04	0	0	0	0
<i>Rhabdias eustreptos</i>	0	0	0	0	0	0	14	0.4 ± 0.4
<i>R. fuscovenosa</i>	12	0.6 ± 0.4	21	0.5 ± 0.2	8	0.1 ± 0.1	0	0
<i>Strongyloides serpents</i>	2	0.03 ± 0.03	0	0	0	0	0	0
<b>Acanthocephala:</b>								
<i>Neoehinorhynchus cylindricus</i>	15	0.3 ± 0.1	0	0	0	0	0	0

**Table 6. Similarities between helminth communities of aquatic snakes from Spanish Lake, Louisiana.**

Species	<u>Nerodia cyclopion</u>	<u>N. fasciata</u>	<u>N. rhombifera</u>	<u>Agkistrodon piscivorus</u>
	Jaccard's Coefficient			
<u>Nerodia cyclopion</u>		0.55	0.60	0.20
<u>N. fasciata</u>	0.09		0.67	0.21
<u>N. rhombifera</u>	0.04	0.33		0.33
<u>Agkistrodon piscivorus</u>	0.06	0.39	0.08	
	Percent Similarity			

most similar between localities and that helminth community structure of *A. piscivorus* was least similar between localities.

#### Discussion

Based on patterns in the helminth communities of birds, fishes, and a mammal, Kennedy et al. (1986) predicted that host physiology, host vagility, breadth, and selectivity of diet, complexity of the alimentary canal, and host exposure to helminths with direct life cycles were important factors that contributed to helminth community structure and diversity. Results of the present study indicate that several of these factors also had great predictive value for comparison of aquatic snake helminth communities. Dietary differences, both among species of water snakes and between water snakes and cottonmouths, have been well documented (Mushinsky, 1987). Furthermore, those species of snakes that include a large proportion of tadpoles in their diet would be expected to harbor a rich trematode fauna, as tadpoles are commonly utilized as second intermediate hosts for most snake trematodes. At HOI, the high abundance of trematodes in *N. fasciata* may be a reflection of the diet of this snake, which includes many anurans (Mushinsky et al., 1982). Similarly, high helminth species diversity and evenness occurred in *N. fasciata* at HOI and SL, and this may be attributed to the euryphagic nature and generalized habitat preference of this snake (Mushinsky and Hebrard, 1977; Hebrard and Mushinsky, 1978). Conversely, prior to our study we expected that *N. cyclopion* would have a relatively depauperate helminth fauna since this snake has been reported to be piscivorous

throughout its life (Mushinsky et al., 1982). This pattern existed at SL, but conspecifics from HOI harbored helminths with much higher prevalence and abundance. One explanation for this difference is that *N. cyclopion* may have a broader diet than previously suggested and population differences in foraging ecology could be a factor contributing to the different helminth fauna between the 2 localities. While *Gambusia affinis* and centrarchid fish were common food items recovered from *N. cyclopion* at both HOI and SL, tadpoles (*Rana* sp.) were only found in snakes from HOI. Furthermore, Garton et al. (1970) reported salamanders (*Siren intermedia*) and centrarchids in stomachs of *N. cyclopion* from Illinois.

In our study, those host species with low sample sizes might have affected our overall conclusions concerning helminth occurrence. However, based on the high prevalence and abundance of many helminths, particularly in HOI snakes, our sampling would likely have included most of the helminths that parasitize aquatic snakes. Low helminth species diversity and evenness in *A. piscivorus* from HOI is likely due to a single species, *O. laterotrema*, which dominated the community of this snake. At SL, comparisons among snake species were more difficult due to poor colonization by helminths. For example, 3 species of trematodes were most abundant in *N. rhombifera*, while at HOI, these same species were more abundant in *N. cyclopion* and *N. fasciata*. Additionally, *S. magna* was only found in *A. piscivorus* at SL, but infected all species of snakes at HOI. Highest species richness and mean number of individuals in *N. cyclopion* and *A. piscivorus* is primarily due to the high prevalence and abundance of cestodes in these snakes.

Helminth community structure of HOI snakes was richer and more diverse than conspecifics from SL. One apparent locality difference that may be explained is the higher trematode abundance that occurred in HOI snakes as compared with that in SL snakes. A common factor shared by the trematodes reported in this study is the utilization of physid snails as first intermediate hosts. While snail abundance was not measured at our study sites, helminth survey data indicate that trematode transmission at SL was not as successful as at HOI, which might be a reflection of low snail densities. Qualitatively, habitat perturbation and environmental variability at SL was greater than at HOI. During this study, spoil bank construction and other anthropogenic disturbances were common at SL. However, the most influential factor was likely the extreme fluctuations of water level observed at SL. Furthermore, the SL locality differed from HOI in having greater expanses of open water, whereas the HOI locality consisted of a major waterway, the Petite Amite river, which was closely surrounded by swamp. For cestodes, which are not dependent on a snail intermediate host, abundance was more similar between localities. Nematodes of the genus *Rhabdias* were similar in abundance between localities and this possibly is due to their direct life cycle pattern. Further, *Kalicephalus rectiphilus* was actually more abundant in SL snakes than in HOI snakes. However, *Terranova caballeroi* was very abundant in HOI snakes, but absent from SL. Locality differences found in our study appear to be correlated with the life history strategy of particular helminth taxa and are very important in determination of helminth community organization of aquatic snakes. For example, if our study had only considered SL, then we would have concluded that aquatic snakes harbor a relatively depauperate helminth fauna.

Based on comparisons of our study with the data of Collins (1969) and Detterline et al. (1984), it appears that the trematode genera *Dasymetra*, *Ochetosoma*, *Pneumatophilus*, and *Styphlodora* characterize the helminth community of aquatic snakes. Taxonomic questions and uncertainties exist at the species level of these trematode genera, precluding a more specific analysis. Qualitatively, it appears that, regardless of locality, aquatic snakes host a similar suite of helminth parasites that are not shared with other reptiles or amphibians. However, it should be noted that quantitative differences in the helminth fauna (as

reported in the present study) may be locality-dependent. A total of 19 helminth species at HOI and 17 species at SL were recovered in the present study. Detterline et al. (1984) included snakes collected from discrete populations in Perry County, Alabama. They recovered only 10 species of helminths, including 2 species of pentastomids, from *Agkistrodon piscivorus*, *Nerodia erythrogaster*, *N. rhombifera*, and *N. sipedon*. Prevalence of *Ochetosoma aniarum* (90%) in *N. erythrogaster* and of *Proteocephalus perspicua* (58%) in *N. rhombifera* were reported by Detterline et al. (1984) and are comparable to our findings. Helminth community structure of snakes in the Alabama survey was similar to the present study in that the helminth fauna of *Nerodia* spp. was most similar, while *A. piscivorus* shared only 1 helminth species (*P. perspicua*) with water snakes. Collins (1969) reported 18 species of helminths, excluding larval forms, from *N. sipedon*, *N. erythrogaster*, and *N. taxispilota*, and 10 species of helminths from *A. piscivorus* from 8 counties in eastern North Carolina. Trematodes also were prevalent in his study with >80% prevalence of *O. aniarum* in *N. sipedon* and *N. erythrogaster*. Prevalence of 81% for the cestode *P. marenzelleri* in the North Carolina survey was similar to the prevalence (70%) recorded in *A. piscivorus* from HOI. In addition, Collins found that a nematode, *Terranova* sp., was very prevalent (>80%) in *N. erythrogaster*. In our study, prevalence of >97% was recorded for *Terranova caballeroi* in *N. cyclopion*, *N. fasciata*, and *A. piscivorus* at HOI. The occurrence of this nematode in piscivorous snakes suggests that a fish host may be involved in the life cycle since another related species, *T. crocodili*, is known to utilize fish (*Lates calcarifer*) as an intermediate host (Sprent, 1979). Abundance of *T. caballeroi* was lower in *N. rhombifera*, a snake that preys on different species of fish (catfish, shad), than in *N. cyclopion* and *N. fasciata*, which eat mainly mosquitofish and centrarchids (Mushinsky and Hebrard, 1977). Collins (1969) also reported that the helminth fauna of *A. piscivorus* was distinct when compared to *Nerodia* spp., with four helminths restricted to cottonmouths. In his study of snake trematodes, Rabalais (1967, 1969b) examined 11 genera and 14 species of snakes from various parishes in Louisiana. The host distribution of trematodes in the present study was in agreement with the data of Rabalais (1967, 1969b), and no new host records were found.

Broad overlap in diet and habitat preference

of *Nerodia* spp. and their close phylogenetic affinities are probably the proximate causes of the similarity of their helminth fauna. Aho (1990) suggested that host specificity was unimportant for the helminths of amphibians and reptiles. In our study, host generalists were found (e.g., *Styphlodora magna* and *T. caballeri*), but many host specialists existed in the helminth fauna of *A. piscivorus*. *Ochetosoma laterotrema*, *O. kansense*, *P. agkistrodontis*, *P. marenzelleri*, and *R. eustreptos* were all specific to *A. piscivorus*. Rabalais (1967, 1969b) also found that *O. kansense* and *O. laterotrema* occurred only in *A. piscivorus*. Our data, from both localities, indicate that *Nerodia* spp. harbor different species of *Ochetosoma*, *Proteocephalus*, and *Rhabdias* than cottonmouths. Support for this apparent phylogenetic component exists in that *A. piscivorus* is known to prey on many of the same food items as *Nerodia* spp. (Kofron, 1978). Furthermore, *O. laterotrema* has been experimentally demonstrated to be specific to cottonmouths (Sogandares-Bernal and Grenier, 1971). The specificity of *P. variabilis* for *N. cyclopion* is unknown. Brooks (1978) described *P. variabilis* and listed both *N. cyclopion* and *N. rhombifera* as hosts. Because *N. cyclopion* is piscivorous, it is interesting that *P. variabilis* is abundant in this host. Snake cestodes (*Proteocephalus*) have a life cycle that utilizes tadpoles as a second intermediate host (Thomas, 1941); therefore, infection of piscivorous snakes may indicate that fish also serve as intermediate hosts.

Because helminth prevalence and species richness of all snakes was not found to differ significantly with regard to host sex, it appears that males and females are equally exposed to helminth infections. However, mean number of individuals and abundance data for *O. aniarum* and *D. villicaeca* indicated that females of *N. cyclopion* were more heavily parasitized than males. We cannot explain this sexual difference, but dietary differences between males and females could account for this pattern.

Data concerning age of wild-caught snakes are not currently available. Therefore, our analysis of helminth infections in snakes is based on the assumption that body size is a reasonable indicator of age. For the purposes of our study, snakes that are less than 1 year old would probably not be exposed to as many helminths as would snakes that have completed their first full year of life. By comparing the body sizes of aquatic snakes recorded in the present study with neonate body

sizes of conspecifics (Dundee and Rossman, 1989), it was determined that most snakes examined during this study would likely be at least 1 year of age. One exception was a specimen of *N. rhombifera* from SL, which was of a size comparable to a young-of-the-year snake.

Several helminths that were recovered in our study appear to be accidental parasites of snakes. *Cosmocercoides dukae*, *Falcaustra catesbeiana*, and *Oswaldocruzia pipiens* usually infect amphibians and *Leptorhynchoides thecatus* and *Neoechinorhynchus cylindratus* normally infect fish hosts. The occurrence of these helminths may be explained by the inclusion of frogs and fish in the diet of aquatic snakes. Anderson (1935) also reported that *L. thecatus* was prevalent (21%) in *N. sipedon* from Ohio and considered the infection accidental, noting that the acanthocephalan was probably acquired by snakes eating infected fish.

Several factors that may play important roles in structuring the helminth community of aquatic snakes include the ecology of the host, phylogeny of the host, local environmental conditions, and other ecological factors such as seasonal changes in helminth populations. Our contribution is that we have addressed the first of these factors, host diet, host phylogeny, and effects of locality differences on snake helminth communities, providing a foundation on which other factors such as habitat differences and seasonal changes can be based.

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## Gastrointestinal Digenetic Trematodes of Olive Ridley's Turtle (*Lepidochelys olivacea*) from Oaxaca, México. Taxonomy and Infracommunity Structure

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**ABSTRACT:** Thirty-two Olive Ridley's turtles (*Lepidochelys olivacea* Eschscholtz) were collected from the coast of Oaxaca, México, and their digestive tracts were examined for helminths. Eight digenetic trematode species were found in the digestive tracts of 24 of 32 turtles: *Adenogaster serialis*, *Himasomum lobatus*, *Pyelosomum renicapite* (Pronocephalidae), *Pachypsolus irroratus* (Pachypsolidae), *Enodiotrema megachondrus* (Plagiorchiidae), *Orchidasma amphiorchis* (Telorchhiidae), *Prosorchis psenopsis* (Hemiuridae), and *Calycodes anthos* (Calycodidae). Oaxaca represents a new locality for all digenean species and *L. olivacea* represents a new host record for all except *O. amphiorchis*. *Adenogaster serialis* was the species with the highest prevalence of infection (53.1%), while *E. megachondrus* showed the highest abundance (28.2 digeneans/analyzed host). Digenean community structure at the infracommunity level is depauperate and isolationist according to the values of species richness, numbers of worms, and diversity. Vagility, and the possibility of a mixture of populations arriving at the nesting site, as well as broad feeding preferences, are suggested as the main factors that determine the digenean community structure in *L. olivacea*.

**KEY WORDS:** *Lepidochelys olivacea*, Digenetic trematodes, *Adenogaster serialis*, *Himasomum lobatus*, *Pyelosomum renicapite*, *Pachypsolus irroratus*, *Enodiotrema megachondrus*, *Orchidasma amphiorchis*, *Prosorchis psenopsis*, *Calycodes anthos*, infracommunity structure, México.

The Olive Ridley's turtle, *Lepidochelys olivacea* (Eschscholtz, 1829), is one of 7 extant species of marine turtles. All except the loggerhead, *Caretta caretta* (Linnaeus, 1756), are considered endangered (Waldichuk, 1987). The Mexican government has been concerned about the sea turtle stocks of both its Pacific and Atlantic coasts as 6 of the 7 species of sea turtles nest on Mexican beaches (Anonymous, 1988). The coast of Oaxaca State, México, is one of the main nesting areas for *L. olivacea*, but in the last several years nesting populations have decreased. The decrease is mainly because of commercial capture and illegal egg collecting. Development of resort areas near the nesting sites has also caused serious problems for the preservation of this species (Peñaflores and Nataren, 1988). In an attempt to solve these problems, the population biology and nesting behavior of this turtle has been studied intensively (Márquez et al., 1976; Casas, 1978; Frazier, 1983; Enciso and Barajas, 1993). On 31 May 1991, 13 months after our collections, the government initiated a prohibition on the capture of all sea turtles. These regulations also extend to all egg collecting.

Helminth parasites of marine turtles have been examined from a number of hosts and geographic localities (Ernst and Ernst, 1977; Blair and Limpus, 1982; Dyer et al., 1991). Most of the studies in México have investigated the parasites of *Chelonia mydas* (Linnaeus, 1758) (Caballero and Zerecero, 1950; Caballero-Rodríguez, 1960; Caballero 1962). Compared to other marine turtles, relatively little information is available on the parasites of Olive Ridley's. The only previous study on *L. olivacea* from México was by Parra (1983), who reported the occurrence of the digeneans *Plesiochorus cymbriformis* (Rudolphi, 1819) Looss, 1901 and *Pyelosomum cochlear* Looss, 1899. The objective of the present study was to identify and report the gastrointestinal digenetic trematodes of *L. olivacea* and to use patterns of infection to describe the digenean infracommunity structure.

### Materials and Methods

Turtles were collected in Mazunte, 5 km N of Puerto Angel, Oaxaca State, México in March 1990. The turtles were captured by commercial fishermen, taken to Mazunte beach, and killed by shooting. The digestive tracts were the only organs we could acquire from fishermen, because the rest of the body was sold.

The gastrointestinal tracts (esophagus, stomach, and intestine) from 32 Olive Ridley's turtles were refig-

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**Table 1.** Digeneans of Olive Ridley's turtles (*L. olivacea*) from Oaxaca, México.

Digenean	Habitat*	CHIBUNAM	USNPC
<b>Pronocephalidae:</b>			
<i>Adenogaster serialis</i> Looss, 1901	I	250-3	84868
<i>Himasomum lobatus</i> (Looss, 1901) Pérez and Brooks, 1995	I	250-11	84872
<i>Pyelosomum renicapite</i> Poche, 1926	I	250-12	84873
<b>Pachypsolidae:</b>			
<i>Pachypsolus irroratus</i> (Rudolphi, 1819) Looss, 1902	I, S	253-12	84874
<b>Plagiorchiidae:</b>			
<i>Enodiotrema megachondrus</i> (Looss, 1889) Looss, 1901	I	250-10	84871
<b>Telorchiiidae:</b>			
<i>Orchidasma amphiorchis</i> (Braun, 1899) Braun, 1901	I	253-14	84871
<b>Hemiuridae:</b>			
<i>Prosorchiopsis</i> Yamaguti, 1934	S	253-11	—
<b>Calycodidae:</b>			
<i>Calycodes anthos</i> (Braun, 1899) Looss, 1901	I	253-13	84869

\* I = intestine; S = stomach.

erated until examination. Digeneans were the only helminth group found. The worms were counted and their site of infection was recorded. They were then collected in petri dishes with 7.5% saline solution and killed with hot water. Most specimens were fixed with Bouin's fixative with light cover glass pressure for 18 hr and then stored in 70% ethanol. Remaining specimens were fixed and stored in 70% ethanol. Specimens were stained with Harris' haematoxylin, Mayer's pararcarmine, and Gomori's trichromic and mounted in Canada balsam. Representative specimens were deposited in the Colección Helmintológica del Instituto de Biología UNAM, México (CHIBUNAM) and in the U.S. National Parasite Collection, Beltsville (USNPC) (Table 1).

Prevalence and abundance of each digenean species follow definitions established by Margolis et al. (1982). For description of infracommunity structure, we used the number of helminth species per turtle (richness) and the number of worms per turtle, including uninfected hosts. Brillouin's index, appropriate for fully censused communities (Pielou, 1975), provided a measure of infracommunity diversity. Similarity at the infracommunity level was tested using Sorensen's coefficient (qualitative) and percent similarity (quantitative).

### Results and Discussion

Eight digenean species were recovered from 24 of 32 turtles and were identified as belonging to 6 different families, Pronocephalidae, Pachypsolidae, Plagiorchiidae, Telorchiiidae, Hemiuridae, and Calycodidae (Table 1). No other helminth group was collected. All species found represent new locality records and, except *O. amphiorchis*, new host records.

#### Taxonomic information

##### *Adenogaster serialis* Looss, 1901

One hundred sixty-eight digeneans were identified as *Adenogaster serialis* Looss, 1901. This

is the only valid species included within the genus, because *A. indica* Rao, 1975, reported from *Chelonia mydas* in India, was transferred to *Raogaster* by Groschaft and Tenora (1981).

*Adenogaster serialis* has a worldwide distribution. It has been reported from *Thalassochelys corticata* (= *Caretta caretta*) in Egypt (Looss, 1901), *Eretmochelys imbricata* (Linnaeus, 1766) in Cuba (Pérez-Vigueras, 1955), and *Chelonia mydas* in Panamá (Caballero et al., 1955) and México, from the states of Guerrero (Caballero-Rodríguez, 1960) and Colima (Parra, 1983).

##### *Himasomum lobatus* (Looss, 1901)

##### Pérez-Ponce de León and Brooks, 1995

Five specimens of a pronocephalid digenean were identified as *H. lobatus*. This species was originally described as *Glyphicephalus lobatus* by Looss (1901) but was transferred to *Pleurogonius* Looss, 1901 by Ruiz (1946) and later redescribed by Caballero et al. (1955). In the classification of Pronocephalidae proposed by Pérez-Ponce de León and Brooks (1995a), they mentioned that *Himasomum* Linton, 1910 and *Glyphicephalus lobatus* Looss, 1901 form a distinct group from *Pleurogonius* and *Glyphicephalus*. *Himasomum* was recognized as a valid genus, and Pérez-Ponce de León and Brooks (1995a) proposed that *Glyphicephalus lobatus* be reclassified as *Himasomum lobatus*.

This species has been found in *Chelonia mydas* from Egypt, Brazil, and Panamá (Looss, 1901; Ruiz, 1946; Caballero et al., 1955), *Eretmochelys squamosa* (Agassiz, 1857) from the Philippines (Palao Islands, Looss, 1902), and *E. imbricata*

from the United States (Florida), Jamaica (Yamaguti, 1971) and Puerto Rico (Fischthal and Acholonu, 1976; Dyer et al., 1991).

***Pyelosomum renicapite* Poche, 1926**

Thirty-one specimens, 15 mature and 16 immature, were identified as *P. renicapite*. Genus *Pyelosomum* contains 6 species originally placed by Yamaguti (1971) in 4 genera: *Pyelosomum* Looss, 1899; *Epibathra* Looss, 1902; *Astrorchis* Poche, 1925; and *Myosaccus* Gilbert, 1938. A comparative morphological study by Pérez-Ponce de León and Brooks (1995b) supports the placement of 6 species in the genus *Pyelosomum*, and this idea is in agreement with earlier suggestions by Ruiz (1946).

*P. renicapite* has been previously described from *Dermochelys coriacea* (Linnaeus, 1766) and *Caretta caretta* collected along the Atlantic coast of North America (Luhman, 1935; Yamaguti, 1971; Threlfall, 1978).

***Pachypsolus irroratus* (Rudolphi, 1819)  
Looss, 1902**

Two hundred twenty-five worms were identified as *Pachypsolus irroratus*. Until 1982, 7 species were included in the genus *Pachypsolus* Looss, 1901. Blair and Limpus (1982), studying the variability of the type species *P. irroratus*, subsequently proposed a reclassification in the specific composition of this genus. Their examination of the distribution of vitellaria and number of blind anterior caecal diverticula led them to declare all current species to be synonyms of *P. irroratus*, except *P. sclerops* (Travassos, 1922) Travassos, 1928. *Pachypsolus sclerops* was originally described from crocodilians in South America and the rest of the species had been described from marine turtles.

Following the proposal of Blair and Limpus (1982), the hosts for *P. irroratus* are *Caretta caretta*, *Chelonia mydas*, and *Eretmochelys imbricata*, with a worldwide distribution including the Mediterranean Sea; the Red Sea; the Atlantic coast of northwest Africa; Florida, U.S.A.; Puerto Rico; the Pacific coasts of Panama and México; New Guinea; and Australia (Yamaguti, 1971; Ernst and Ernst, 1977; Blair and Limpus, 1982).

***Enodiotrema megachondrus* (Looss, 1899)  
Looss, 1901**

Nine hundred five plagiorchids were identified as *Enodiotrema megachondrus* based on the de-

scription given by Looss (1901) and redescrptions made by Caballero-Rodriguez (1960), Euzet and Combes (1962), and Parra (1983). According to Blair and Limpus (1982), 7 species should be included in this genus: *E. megachondrus* (type species); *E. reductum* Looss, 1901; *E. instar* Looss, 1901 and *E. acariaeum* Looss, 1902 from the intestines of *Caretta caretta* and *Chelonia mydas* in Egypt (Looss, 1901); *E. microvitellatus* Chattopadhyaya, 1972 and *E. schikhobalovae* Gupta and Mehrotra, 1976 from the digestive tract of *Eretmochelys imbricata* in India; and *E. carettae* Blair and Limpus, 1982 from the liver and gall bladder of *Caretta caretta* in Australia. The known geographical distribution of *E. megachondrus* includes Egypt, France (Yamaguti, 1971), Acapulco (Caballero-Rodriguez, 1960), and Manzanillo (Parra, 1983) along the Pacific coast of México.

***Orchidasma amphiorchis* (Braun, 1899)  
Braun, 1901**

Seventy-one telorchids were identified as *O. amphiorchis*. This species was originally described as *Distomum amphiorchis* Braun, 1899 from the intestine of *Caretta caretta* in the Adriatic Sea and renamed *Orchidasma amphiorchis* (Braun, 1899) Braun, 1901. Later, 2 more species were described from sea turtles in India: *Orchidasma indica* Simha, Rao, and Chattopadhyaya, 1971 and *Orchidasma vitelloconfluens* Rao, 1972. Both are considered synonyms of *O. amphiorchis* by Blair and Limpus (1982). This telorchid represents a monotypic genus with a worldwide distribution and is found in 5 species of marine turtles (Blair and Limpus, 1982): *Caretta caretta*, *Eretmochelys imbricata*, *Chelonia mydas*, *Lepidochelys olivacea*, and *Podocnemys expansa* Schwergger, 1812. It was previously described in México by Caballero (1962) from Tamaulipas in the Gulf of México (in *Chelonia mydas*) and Salina Cruz, Oaxaca, on the Pacific coast (in *Eretmochelys imbricata*) by Caballero and Zerecero (1950).

***Prosorichis psenopsis* Yamaguti, 1934**

Three adult specimens were identified as *Prosorichis psenopsis*. The genus *Prosorichis* was created by Yamaguti (1934) to include specimens that apparently were hemiurids but unique in the preacetabular position of the testis. Interestingly, these digeneans are typically found in the gut and occasionally in the body cavity of marine teleosts. This is the first record of this worm from



**Table 2.** Prevalence and abundance of digeneans in 32 Olive Ridley's turtles (*L. olivacea*) from Oaxaca, México.

Digenean	Number infected (%)	Abundance mean $\pm$ SD
<i>Adenogaster serialis</i>	17 (53.1)	9.3 $\pm$ 4.1
<i>Himasomum lobatus</i>	1 (3.12)	0.2 $\pm$ 0.8
<i>Pyelosomum renicapite</i>	5 (15.6)	1.0 $\pm$ 3.4
<i>Pachypsolus irroratus</i>	4 (12.5)	7.0 $\pm$ 32.4
<i>Enodiotrema megachondrus</i>	14 (43.7)	28.2 $\pm$ 65.0
<i>Orchidasma amphiorchis</i>	5 (15.6)	7.7 $\pm$ 11.2
<i>Prosorthis psenopsis</i>	2 (6.2)	0.1 $\pm$ 0.4
<i>Calycodes anthos</i>	11 (34.4)	12.8 $\pm$ 14.8

the intestine of a marine turtle. *Prosorthis psenopsis* was described originally by Yamaguti (1934) from the esophagus of *Psenopsis anomala* (Temminck and Schlegel, 1844) in Japan. This phenomenon could represent an accidental infection; however, phylogenetic analysis by Pérez-Ponce de León and Brooks (1995a) suggests evidence for host-switching among members of the Pronocephalidae. These authors found 3 host-shifts from marine turtles to fish within the pronocephalid digeneans, so the reverse event should also be possible.

***Calycodes anthos* (Braun, 1899)  
Looss, 1901**

One hundred ninety-two specimens were identified as *Calycodes anthos*. Our specimens were identical to those redescribed by Parra (1983) from *Chelonia mydas* in Manzanillo, México. This species was originally described by Braun in a chelonian from Japan (host species and locality unknown) and later from *Thalassochelys mydas* (= *C. mydas*) from Egypt and Panama (Yamaguti, 1971). Fischthal and Acholonu (1976) described a second species in the genus, *C. caborojoensis*, but the description is based solely on the holotype. The establishment of a new species with only one specimen lacks taxonomic weight. Since we could not examine the original material, we consider *C. caborojoensis* as species inquirendae. Thus, *Calycodes* is still a monotypic genus.

**Digenean infracommunity structure**

Amphibians and reptiles represent excellent systems in which to study ecological and evolutionary relationships determining helminth species distribution and abundance (Aho, 1990). Helminths of marine turtles have been studied since the last century and, to date, many digenetic trematodes of most turtle species through

the world have been determined (Looss, 1901, 1902; Caballero, 1954; Caballero et al., 1955; Chattopadhyaya, 1972; Rao, 1975; Fischthal and Acholonu, 1976; Ernst and Ernst, 1977; Blair and Limpus, 1982; Dyer et al., 1991; this investigation). However, these studies have focused on taxonomic aspects and include little ecological or evolutionary information to be used as a comparative framework.

Twenty-four of the 32 turtles analyzed were parasitized by digenetic trematodes. No other helminths were found in our samples. Although Sey (1977) found *Porrocaecum sulcatum* (Rudolphi, 1819), *Kathlania leptura* (Rudolphi, 1819), and an unidentified larval trypanorhynch in *Caretta caretta*, our examination of *L. olivacea* found no helminths other than digeneans. Whether the 8 species we collected represent the entire community of gastrointestinal helminths of *L. olivacea* or only a subset of the assemblage can only be resolved by further studies. Resolving this question may be difficult because collection of marine turtles is now prohibited in México and in most places throughout the world.

The prevalence and abundance of infection of each digenean species is presented in Table 2. The digenean infracommunity was numerically dominated by *Enodiotrema megachondrus* with abundance of 28.2  $\pm$  65.2 individuals/analyzed host. Three species of flukes, *E. megachondrus*, *Adenogaster serialis*, and *Calycodes anthos*, were relatively common, with prevalences between 34.4% and 53.1%, and accounted for 79% of all individuals (Table 2). Individual turtles harbored between 1 and 5 species. Hosts with more than 2 species were rare (37.5%) with 62.5% of the infected turtles harboring 1 or 2 species of digeneans. The mean species richness was 1.8  $\pm$  1.4 for each analyzed host. The lowest number of worms per infracommunity was 1 and the highest was 365 (average 49.9  $\pm$  88.7). Twenty-

five percent of examined turtles were found free of digeneans. Brillouin's index ranged from 0.37 to 1.6, with a mean value of  $0.42 \pm 0.47$ . Qualitative as well as quantitative faunal similarity for all pairwise comparisons among hosts were quite low, with a mean value of  $39.5 \pm 30.8\%$  (Sorensen coefficient) and  $17.2 \pm 27.9\%$  (percent similarity) indicating high disparity in the number of individuals and the presence-absence of species across infracommunities.

In the literature, we found previous reports about helminth communities only in freshwater turtles. Some attempts have been made in this group of hosts to correlate the structure of helminth communities with geographic location or habitat conditions (Esch and Gibbons, 1967; Esch et al., 1979a, 1979b) but they do not provide data at the infracommunity level. As far as we know, the only documented study at this level besides the revision by Aho (1990) is Jacobson (1987, cited in Esch et al., 1990) in which the infra- and component community structure of intestinal helminths of the yellow-bellied slider, *Trachemys scripta* Schoepff, 1792 was examined. This author found a similar value for diversity index (0.46) to the value found in our study (0.41).

Our results are quite similar to those shown by Aho (1990) for the Testudines group for the mean species richness per individual host ( $1.8 \pm 1.14$  vs.  $1.52 \pm 0.20$ ) and the mean number of worms ( $49.9 \pm 88.7$  vs.  $66.6 \pm 21.9$ ). It is important to emphasize that the diversity obtained for Olive Ridley's turtle is greater than for most terrestrial and freshwater turtles and yet only digeneans were found in our analysis.

In this work we consider the digenean infracommunity of *L. olivacea* to be depauperate and isolationist in character, in accordance with the features established by Holmes and Price (1986).

The factors we consider to be the most important in determining the diversity, richness, and number of worms of the infracommunities are vagility of the host, feeding habits, and the mixture of turtle populations arriving at the nesting sites. The low similarity patterns between infracommunity pairs may also be the result of a combination of these factors. Additionally, the fact that these turtles remain in warm waters throughout much of their lives increases metabolic rate and input of food, exposing each host to frequent infections with different species of parasites.

*Lepidochelys olivacea* is the most numerous marine turtle today (Zwinnenberg, 1976) and is

widely distributed. It occurs in the east and west Pacific Ocean, in the Indian Ocean, and in both sides of the Atlantic Ocean (Bowen et al., 1991). The long distance movements of turtle populations between nesting and feeding sites increase the exposure to a variety of helminth species and contribute to the development of more complex helminth communities (see Kennedy et al., 1986). However, the high vagility and mixing of populations in breeding areas may limit the development of helminth infracommunities that are qualitatively and quantitatively predictable among individuals. These aspects need to be determined in further studies.

There is no available information on the life cycle patterns of the digeneans found, but the turtles may have become infected by the ingestion of different kinds of food. Olive Ridley's turtle is omnivorous and feeds on different marine invertebrates such as crabs, shrimp, molluscs, bryozoans, sipunculids, and ascidians, as well as fish eggs. It also feeds on fish and vegetation (Montenegro et al., 1986). The presence of pronoccephalids demonstrates that vegetation could be an important part of the diet of this host.

Additionally, information about the host-specificity of the digeneans mentioned herein shows that all of them can be considered as host generalists; although they all are parasites of marine turtles (with the exception of *P. psenopsis*), each has been recorded in 3 or 4 different host species.

The main goal of this paper was to provide information about the digenean infracommunity structure of *L. olivacea* as well as data to be used in the future to answer some of the questions addressed by Aho (1990, p. 162). We believe that our results force us to consider a different approach to analysis of the factors that determine the helminth community structure in reptiles. Many factors have been proposed as determinants of helminth community structure in different kinds of hosts, such as the habitat of the host (aquatic, semiaquatic, terrestrial), host feeding type (herbivorous, omnivorous, carnivorous), host vagility, etc. Most comparisons use nonphylogenetic classifications of vertebrates and, as a result, it has been proposed that the helminth richness and abundance increases according with the place of hosts within this classification (Freshwater Fish-Amphibians and Reptiles-Birds-Mammals) (see Bush et al., 1990). We propose to address questions concerning the

ecological determinants of helminth community structure between closely related groups of hosts, i.e., the chelonians should be compared with their sister group, the Sauria, and the paraphyletic reptiles should be compared with birds within the monophyletic amniota, and comparisons between reptiles and amphibians just because they are ectotherms, or between birds and mammals as endotherms, should be avoided.

There is still need for more information from many different reptile-parasite systems in order to be able to produce testable hypotheses to explain the causes of observed helminth community patterns. In summary, we must keep collecting more data about helminth communities of marine turtles and integrating this information, using a well-developed and robust method like that proposed by Brooks and McLennan (1991, 1993). We may then be able to decipher the complex evolutionary and biogeographical history of helminth-marine turtle associations and have clues to a better understanding of parasite community evolution.

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## Parasitic Helminths and Arthropods of Greater Shearwaters (*Puffinus gravis*) from Florida

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**ABSTRACT:** Fifteen greater shearwaters (*Puffinus gravis*) collected during June 1993 from the east coast of Florida were examined for parasites. Twenty-five species were identified and included 5 nematodes, 4 cestodes, 9 mites, 6 chewing lice, and 1 tick. All birds examined were infected with at least 4 species of helminths (mean 5.2, range 4–7 species). All are known from greater shearwaters except *Stegophorus diomedea*, *Stegophorus stellae-polaris*, and *Tetrabothrius minor*. The most common helminths were the cestodes *Tetrabothrius filiformis* and *Tetrabothrius laccocephalus* and the nematode *Stegophorus diomedea*, which occurred in prevalences of 100%, 93%, and 93%, respectively. All 15 birds were infested with chewing lice, but 4 birds were free of mites. Each bird was infested by at least 3 species of parasitic arthropods (mean 6.8, range 3–12 species). The most common arthropods were 2 chewing lice, *Austromenopon paululum* and *Halipeurus gravis*, both of which occurred on 100% of the birds. The bird tick, *Ixodes auritulus*, is reported for the first time from the east coast of the United States.

**KEY WORDS:** greater shearwater, *Puffinus gravis*, cestodes, nematodes, chewing lice, mites, ticks, survey, prevalences, Florida.

Greater shearwaters, *Puffinus gravis* (O'Reilly), are large, fairly common pelagic Atlantic seabirds in the order Procellariiformes. Following the breeding season in the south Atlantic, they migrate over the western Atlantic from May through June, after which they become widely distributed in the north Atlantic. Greater shearwaters irregularly frequent the east coast and, to a much lesser extent, the west coast of Florida in late spring and summer before they return to their breeding grounds in the fall. Highly gregarious the year round, greater shearwaters often occur in flocks of 50–100 or more and, when off the U.S. coast, remain well offshore (Stevenson and Anderson 1994). Parasitic helminths and arthropods have been reported from greater shearwaters in the north Atlantic by Bourgeois and Threlfall (1979) and Mobley and Miller (1981), and from their breeding grounds in the south Atlantic by Hoberg and Ryan (1989). Herein we report the parasitic helminths and arthropods collected from a sample of greater shearwaters from the Atlantic coast of Florida.

### Methods

Fifteen greater shearwaters (5 males, 10 females) were collected from the Atlantic Ocean adjacent to Brevard

(*N* = 6) and Martin (*N* = 9) counties on the east coast of Florida. These birds were part of a die-off that occurred in June 1993 and involved approximately 80 seabirds, most of which were greater shearwaters, but also included a few Cory's shearwaters (*Calonectris diomedea* (Scopoli)) and Leach's storm-petrels (*Oceanodroma leucorhoa* (Vieillot)). Most of the birds collected had washed up on shore following a period of high winds and heavy surf, and were alive, but died shortly after being transported to rehabilitation centers and before being treated. All birds examined were severely emaciated, and the cause of death seemed to be related to starvation (M. G. Spalding, pers. comm.). Three birds were placed in plastic bags, put on ice, and examined at necropsy within 24 hr. The other 12 birds were put in individual plastic bags and frozen until examination. Techniques for recovering, fixing, staining, and examining helminths followed Kinsella and Forrester (1972) and Forrester et al. (1974). Ectoparasites were collected by washing each bird in a bucket with soapy water, then rinsing it with a pressurized water spray. The soapy water and rinse water were poured through a 100-mesh screen. With the aid of a dissecting microscope, ectoparasites were removed and placed in vials containing 70% alcohol with glycerin. Representative voucher specimens have been deposited as follows: helminths in the U.S. National Parasite Collection (Beltsville, Maryland), mites in the Parasitology Collection of the USDA National Veterinary Services Laboratories (Ames, Iowa), chewing lice in the arthropod collections at the University of Minnesota and the K. C. Emerson Collection at Oklahoma State University, and the tick in the U.S. National Tick

**Table 1. Prevalence and intensity of helminths infecting 15 greater shearwaters from Florida.**

Helminth	USNPC accession no.	Number of birds		Intensity	
		Infected	%	Mean	Range
<b>Cestoda</b>					
<i>Tetrabothrius diomedea</i> Fuhrmann, 1900 (5)*	84034	8	53	1	1-2
<i>Tetrabothrius filiformis</i> Nybelin, 1916 (5)	84033	15	100	99	6-305
<i>Tetrabothrius laccocephalus</i> Spätlich, 1909 (5)	84032	14	93	7	1-22
<i>Tetrabothrius minor</i> (Lönnberg, 1893)† (5)	84186	1	7	3	—
<b>Nematoda</b>					
<i>Seuratia shipleyi</i> (Stossich, 1900) (1)	83289	9	60	4	1-8
<i>Stegophorus diomedae</i> (Johnson and Mawson, 1942)† (3, 4)	83290	14	93	4	1-12
<i>Stegophorus stellae-polaris</i> (Parona, 1901)† (4)	84035	3	20	1	1-2
<i>Contracaecum</i> sp. (larvae) (2)	—	12	80	9	1-67
Larval spirurids (1, 4)	—	3	20	5	1-13

\* Numbers in parentheses indicate locations in host: (1) stomach, (2) stomach wall, (3) gizzard, (4) koilon lining, (5) small intestine.

† New host record.

Collection (Georgia Southern University, Statesboro, Georgia).

## Results and Discussion

### Helminths

Nine species of helminths were collected from the 15 greater shearwaters, none of which was free of helminths. This included 4 species of cestodes and 5 species of nematodes. All are known from greater shearwaters except *Stegophorus diomedae*, *Stegophorus stellae-polaris*, and *Tetrabothrius minor*. Sites, prevalences, and intensities of each species are given in Table 1. All birds examined were infected with at least 4 species of helminths (mean 5.2, range 4-7 species). Multiple infections were as follows: 5 birds had 4 species of helminths, 3 had 5 species, 5 had 6 species, and 2 had 7 species. A total of 1,805 helminths was collected.

Three of the 4 species of *Tetrabothrius* have been collected also from *P. gravis* on its breeding grounds in the south Atlantic. Although prevalences of *Tetrabothrius* spp. in Florida were very similar to those reported at Gough Island by Hoberg and Ryan (1989), intensities were much lower. The mean intensities for cestodes were 108 in Florida and 1,157 at Gough Island, supporting the conclusion of Hoberg and Ryan that

*Tetrabothrius* spp. are acquired by shearwaters primarily on the breeding grounds and lost, without replacement, during migration and wintering. However, the lower intensities we report may also be due to the emaciated condition of the birds collected and the physiologic changes that take place during starvation. Bourgeois and Threlfall (1979) reported the range of *Tetrabothrius* spp. to be "1-several hundred," but did not give the mean intensity, so we cannot compare our results with theirs.

Nematodes of the genera *Seuratia* and *Stegophorus* have been reported from a variety of seabirds, including fulmars, petrels, and albatrosses (Wehr, 1934; Johnston and Mawson, 1942; Rodrigues and Mendonca, 1967), and seem to exhibit ecological rather than host specificity. *Seuratia shipleyi* now has been reported from *P. gravis* throughout its range in the Atlantic (Bourgeois and Threlfall, 1979; Hoberg and Ryan, 1989). *Stegophorus diomedae* and *S. stellae-polaris* have not been reported from greater shearwaters, although Bourgeois and Threlfall (1979) reported an unidentified species of *Stegophorus* from this host in the north Atlantic.

Larval *Contracaecum* have been reported also in greater shearwaters in all parts of their range, but appear to be incapable of developing to the

Table 2. Prevalence of parasitic arthropods infesting 15 greater shearwaters from Florida.

Species of arthropod	Number of birds		Total number collected
	Infected	%	
Feather mites			
<i>Zachvatkinia puffini</i> (Buchholtz, 1869)	11	73	105
<i>Brephosceles puffini</i> Peterson, 1971	11	73	85
<i>Ingrassia</i> sp.	8	53	104
<i>Microspalax</i> sp.	3	20	38
<i>Opetiopoda</i> sp.	1	7	2
<i>Alloptoides</i> sp.	1	7	1
Skin mites			
<i>Dermation</i> ( <i>Neodermation</i> ) sp.	3	20	4
<i>Harpyrhynchus</i> sp.	1	7	2
Nasal mites			
Mesostigmata: Rhinonyssidae	1	7	3
Chewing lice			
<i>Austromenopon paululum</i> (Kellogg and Chapman, 1899)	15	100	751
<i>Halipeurus gravis</i> Timmermann, 1961	15	100	355
<i>Trabeculus hexakon</i> (Waterston, 1914)	14	93	101
<i>Naubates harrisoni</i> Bedford, 1930	12	80	53
<i>Saemundssonina peusi</i> (Eichler, 1949)	3	20	4
<i>Ancistrona vagelli</i> (J. C. Fabricius, 1787)	2	13	6
Ticks			
<i>Ixodes auritulus</i> Neumann, 1904 (RML# 121491)*	1	7	1

\* U.S. National Tick Collection accession number.

adult stage in this host. Many of these larvae were encysted within the wall of the ventriculus and appeared to have been killed by the host's immune system.

Intensities of helminths were low in comparison with those reported by Hoberg and Ryan (1989), and appeared to have no causal relationship in the die-off of these birds on the Florida coast.

### Arthropods

Sixteen species of parasitic arthropods were collected, including 6 species of chewing lice, 6 feather mites, 2 skin mites, 1 nasal mite, and 1 tick. All are known from greater shearwaters except 3 of the feather mites, the skin mites, the nasal mite, and the tick. Prevalences and total numbers of each species collected are presented in Table 2. All 15 birds were infested with chewing lice, but 4 birds were free of mites. Each bird was infested by at least 3 species of parasitic arthropods (mean 6.8, range 3–12 species). Multiple infestations were as follows: 1 bird had 3 species of arthropods, 3 had 4 species, 1 had 6 species, 6 had 7 species, 3 had 9 species, and 1 had 12 species. A total of 1,615 arthropods was

collected and identified, but intensities could not be calculated because quantitative techniques were not used to obtain every parasitic arthropod from each host as they were for the parasitic helminths.

The 6 species of chewing lice taken from greater shearwaters represent all but 1 of the 7 known louse taxa recognized at this time from this host. *Austromenopon paululum*, by far the most common louse collected in this study, is distributed widely among the species of *Puffinus*, having been identified from 13 species of this host genus. Two additional species of *Austromenopon* do occur on other *Puffinus*, but in a very restricted fashion, being reported from only 1 and 3 species of *Puffinus*, respectively. Contrasted to this, *Halipeurus gravis*, the second most common louse we collected, is limited in its distribution to the greater shearwater. There apparently is a higher degree of host/louse-specificity within this louse genus, as 10 other species of *Halipeurus* are recognized on *Puffinus*, including each of 5 from a single host species, 4 from 2–3 host species, and 1 from 5 host species. Over 30 of the 50+ species of *Austromenopon* occur on the Charadriiformes, with a single species each on the Pelecaniformes

and Ciconiiformes; the almost 30 species of *Halipeurus* are found only on members of the Procellariiformes, with 25 of them on members of the family Procellariidae.

The 6 species of *Trabeculus* and the 8 species of *Naubates* are restricted in their distribution to hosts within the Procellariidae. *Trabeculus hexakon* is found not only on 5 species of *Puffinus*, in addition to *P. gravis*, but also on at least 13 host species in other genera. *Naubates harrisoni* occurs only on *Puffinus*, being known from 10 host species including *P. gravis*.

*Saemundssonina* is a very large genus, including over 85 species and a complexity of numerous named subspecies, many of the latter of dubious validity. The genus is distributed on 4 bird orders in addition to the Procellariiformes. There are 7 species of *Saemundssonina* on hosts within *Puffinus*, with *S. peusi* also known from Cory's shearwater (*Calonectris diomedea*). Only a single species of *Ancistrona*, *A. vagelli*, is known, which is found on more than 20 species of Procellariidae as well as on several species of Hydrobatidae. With more collecting, this species will probably prove to have a much broader distribution on hosts of these families.

In addition to the 6 genera and species of chewing lice discussed above from the greater shearwaters, Bourgeois and Threlfall (1979) reported *H. diversus* as an additional species *Halipeurus*, along with a *Docophoroides* sp. Both of these represent valid taxa from the Procellariidae. However, the first is most likely the result of an instance of natural stragglings, as it is not a regular normal inhabitant of the greater shearwater (R. L. Palma, pers. comm.). The *Docophoroides* sp., given as "1 F?" by Bourgeois and Threlfall (1979: Table 1), represents a misidentification that has been confirmed by the late K. C. Emerson; the slide actually carried only an immature homopteran and half of a pseudoscorpion together (R. L. Palma, pers. comm.).

Interestingly, Bourgeois and Threlfall (1979) found *T. hexakon* the most abundant louse (mean/host = 25) and *A. paululum* among the least common (mean/host = 2). While the former was third most common in our study, the latter was by far the most common. These differences are most likely a function of the collecting techniques utilized, although they might possibly indicate some meaningful difference in louse fauna composition.

Seven of the 9 mite taxa we collected from greater shearwaters (Table 2) are probably true

associates of this bird host. It seems likely that the other 2 mites were present on these birds as contaminants from the environment or other co-existing bird populations. In the latter group are a single female *Alloptoides* sp. (family Alloptidae) and 2 female *Harpyrhynchus* sp. (family Harpyrhynchidae) from 1 bird. The known species of *Alloptoides* are feather mites associated with ducks, but this genus is poorly understood, and species cannot be identified without male specimens. Harpyrhynchids are poorly known prostigmatid bird parasites usually associated with the host's skin. Most harpyrhynchids collected to date have come from evolutionarily advanced bird orders, with very few records from either less advanced or aquatic birds (Moss and Wojcik, 1977; Moss, 1979). Mites in this family are thought to be very host-specific. We know of no other collections from Procellariiformes.

The 3 most common feather mites collected were all present as males, females, and nymphs. The most common mite on *P. gravis* was *Zachvatkinia puffini* (family Avenzoariidae). This species is known from 4 genera in the family Procellariidae, including several *Puffinus* spp. besides *P. gravis* (Mironov, 1991). *Brephosceles puffini* (family Alloptidae) was present on as many birds as *Z. puffini*, but in slightly smaller numbers. Members of this genus are associated typically with a variety of aquatic birds (Peterson, 1971), and *Puffinus* spp. are favored. The greater shearwater is a known host of at least 2 species of *Brephosceles*, including *B. puffini*. *Ingrassia* sp. (family Xolalgidae) were as numerous as *Z. puffini*, but they occurred on fewer birds. The approximately 25 species in this genus are described from shorebirds, sea birds, grebes, and ducks (Gaud and Atyeo, 1981), but the descriptions and taxonomy of the few species from Procellariiformes are obscure and confusing. We find no previous records of *Ingrassia* from greater shearwaters, and the present specimens may represent a new, undescribed species.

*Microspalax* sp. (family Alloptidae) was the only other mite collected in large numbers, although only 1 male was taken among 37 females from 3 birds. *Microspalax* is the only genus in the alloptid subfamily Microspalacinae, and the 10 currently recognized species are all associated with various shearwaters and storm petrels (Aty eo and Gaud, 1991). Although specimens that are probably conspecific with our *Microspalax* mites were collected from greater shearwaters as early as 1883 (Dubinin, 1949), there is much uncer-



tainty about the taxonomic and host associations within the genus, and the mite from greater shearwaters remains unnamed and undescribed (Atyeo and Gaud, 1991).

We collected only 1 male and 1 female *Opetiopoda* sp. (family Xolalgidae) on 1 greater shearwater. At present, the only described species of the genus is *O. anadermura* Gaud & Atyeo from the wedge-tailed shearwater (*P. pacificus* (Gmelin)) (Gaud and Atyeo, 1981). Our specimens represent a second, undescribed species.

There were 4 female *Dermation* sp. (family Dermationidae) on 3 of our birds. All were similar and seem to belong in the subgenus *Neodermtion*, a group until now reported only on the skin of ducks (Fain, 1965). We are unaware of any previous records of *Dermation* from Procellariiformes, and the present specimens are probably a new species.

One male and 2 female mesostigmatid nasal mites in the family Rhinonyssidae were collected from 1 greater shearwater. There do not seem to be any previous records of rhinonyssids from any Procellariiformes (Maa and Kuo, 1965; Domrow, 1969; Pence, 1975; Spicer, 1987), and our mites probably represent an undescribed taxon.

The identification of a nymphal *Ixodes auritulus* is the first record of the occurrence of this bird tick from the east coast of the United States. *Ixodes auritulus* has a wide geographic distribution in the Southern Hemisphere, which is summarized by Arthur (1960). In the Northern Hemisphere, *I. auritulus* has been reported mainly from Central and North America. In North America this tick has been, for the most part, reported from the west coast of the United States and Canada. Its distribution in the United States includes Alaska, California, Colorado, Oregon, and Washington (Keirans and Clifford, 1978).

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## Acuarioid Nematodes in Whimbrels (*Numenius phaeopus hudsonicus*) Transient in Late Summer in Cape Breton, Nova Scotia, Canada

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**ABSTRACT:** Acuarioid nematodes collected from whimbrels (*Numenius phaeopus hudsonicus*) in Cape Breton, Nova Scotia, Canada, consisted of *Skrjabinocerca bennetti* sp. n.; *Ancyracanthopsis schikhobalovi* (Guschanskaya, 1950); *Skrjabinoclava snorrasoni* Anderson and Wong, 1992; and *Sciadiocara umbellifera* (Molin, 1860), and *Streptocara* sp. *Ancyracanthopsis schikhobalovi* and *S. snorrasoni* occurred in whimbrels in Iceland which also harbored 4 species of acuarioids not found in Cape Breton. *Skrjabinocerca bennetti* sp. n. is distinguished from the other 3 species in the genus by its unusually long right spicule (345  $\mu$ m versus 160  $\mu$ m or less).

**KEY WORDS:** Acuarioid nematodes, *Numenius phaeopus hudsonicus*, Nova Scotia, migratory birds.

There is increasing interest in information on the parasite fauna of migratory birds, because it can provide insights into the staging and wintering areas of hosts, as well as their foraging behavior. In this paper we report 5 species of acuarioid nematodes in whimbrels (*Numenius phaeopus hudsonicus* Latham, 1790) collected in Cape Breton, Nova Scotia, Canada while migrating to wintering grounds in the Gulf of Mexico and South America. These findings are then compared with the 6 acuarioid species found by Anderson and Wong (1992) and Wong and Anderson (1993) in whimbrels (*N. phaeopus phaeopus* (Linnaeus, 1758)) collected upon arrival in Iceland after migrating from wintering and staging areas in Europe and West Africa.

### Materials and Methods

Nine whimbrels (8 adults, 1 juvenile) were shot while feeding on crowberries (*Empetrum nigrum*) in upland areas at Capelin Cove (45°39'W, 60°25'N) in Cape Breton, Nova Scotia, Canada in late August 1993. Birds were placed in plastic bags and frozen prior to thawing and necropsy. The subspecies of whimbrel was identified as per Prater et al. (1977); the juvenile bird was identified by the presence of a bursa of Fabricius at necropsy. Nematodes found were fixed in hot 70% alcohol with 5% glycerine and cleared for study in pure glycerine. Measurements are presented in micrometers, unless otherwise specified. Specimens have been deposited in the U. S. National Parasite Collection in Beltsville, Maryland (Nos. 84957–84964).

### Results

Acuarioids were found only in the adult whimbrels; all were infected (Table 1). The following

lists the species found and provides relevant comments on identification.

#### *Skrjabinocerca bennetti* sp. n.

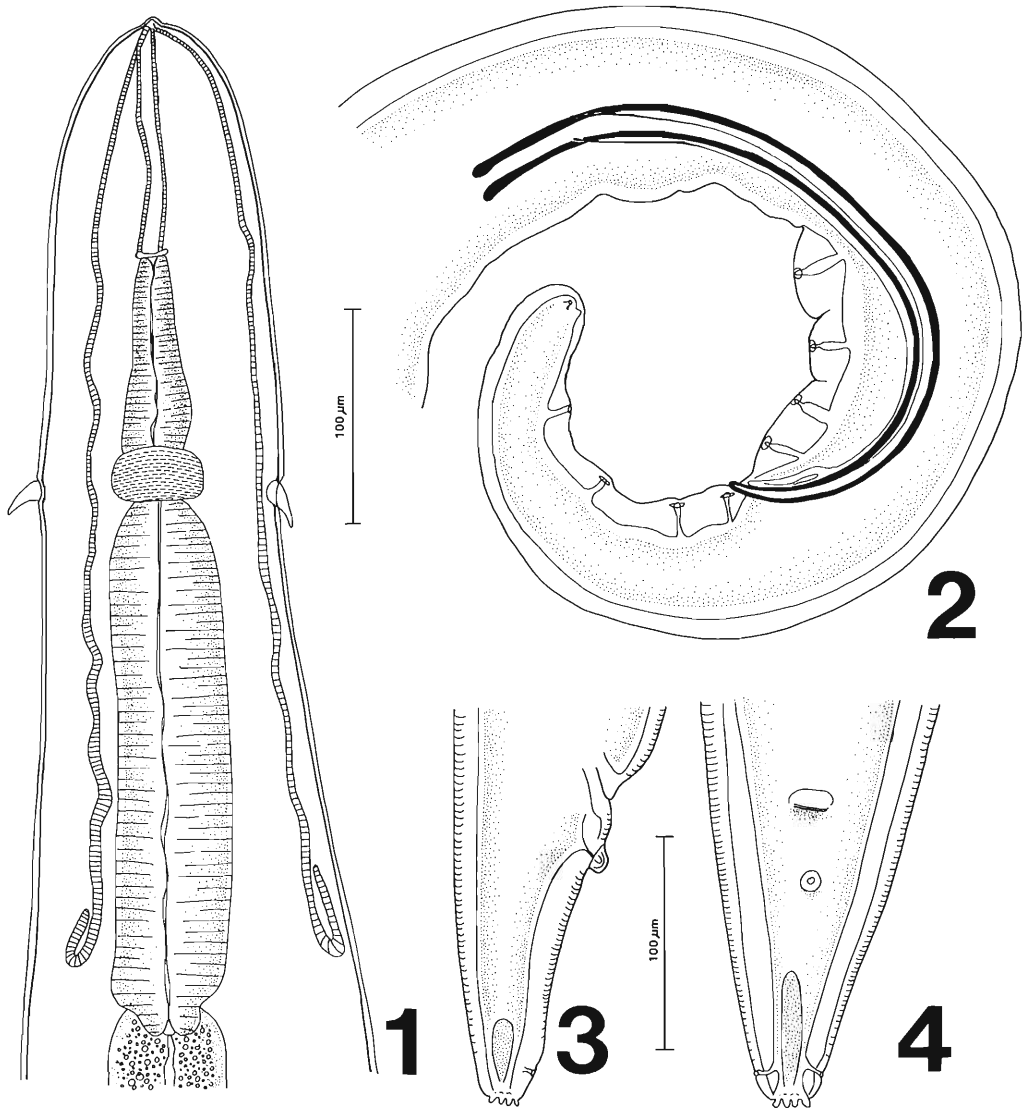
(Figs. 1–4)

**GENERAL:** Acuarioidea, Acuariidae, Acuariinae, *Skrjabinocerca* Schikhobalova, 1930. Small, delicate nematodes with prominent lateral alae with transverse striations. Cordons long, non-anastomosing, terminal ends often slightly recurrent. Deirids large, trifold. Muscular esophagus short; glandular esophagus long.

**MALE** (holotype): Length 5.1 mm. Maximum width 168. Buccal cavity 104 long. Nerve ring and deirids 230 and 233, respectively, from anterior extremity. Cordons 320 long. Muscular esophagus 458 and glandular esophagus 1.86 mm long. Spicules subequal, right 345 and left 365 long. Left spicule with tapered, blunt apex. Right spicule slightly flared at terminal end. Tail 172 long. Four pairs elongate, preanal and 5 pairs postanal papillae present.

**FEMALE** (first figure allotype): Length 5.3 (5.6, 5.9) mm. Maximum width 244 (220, 225). Buccal cavity 108 (100, 100) long. Deirids 290 (255, 210) from cephalic extremity. Nerve ring 225 (–, 230) from cephalic extremity. Cordons 490 (426, 420) long. Muscular esophagus 360 (440, 390) and glandular esophagus – (1.5, 2.0) mm long. Vulva 1.9 (1.3, 1.1) mm from posterior extremity. Tail 91 (150, 150) long, terminating in 6–8 blunt digitiform appendages. Eggs not larvated, 21–23 (22)  $\times$  46–48 (46) ( $N = 10$ ).

**TYPE HOST:** *Numenius phaeopus hudsonicus*, whimbrel (Scolopacidae).



Figures 1–4. *Skrjabinocerca bennetti* sp. n. 1. Anterior end female, ventral view 2. Caudal end male, lateral view showing spicules with left outlined more darkly than right 3. Caudal end female, lateral view 4. Caudal end female, ventral view.

TYPE LOCALITY: Capelin Cove, Cape Breton, Nova Scotia, Canada.

LOCATION IN HOST: Esophagus.

SPECIMENS: United States National Parasite Collection, Beltsville, Maryland, no. 84957 (holotype), no. 84958 (allotype), and no. 84959 (female paratypes).

ETYMOLOGY: The new species is named in honor of Mr. Thomas Bennett of Marion Bridge, Nova Scotia, who helped collect the whimbrels.

REMARKS: The new species is readily distinguished from the 3 other members of the genus

(*S. prima* Schikhobalova, 1930; *S. americana* Wong and Anderson, 1993; and *S. europaea* Wong and Anderson, 1993) by its subequal spicules. In other species the right spicule (120–160 µm) is much shorter than the left (285–445 µm).

*Ancyracanthopsis schikhobalovi*  
(Guschanskaya, 1950)

Specimens were found under the koilin lining of the gizzard. Dimensions and morphologic characters agree with the redescription based on specimens from whimbrels in Iceland (Wong and

**Table 1.** Numbers of adult acuarioid nematodes found in adult whimbrels (*Numenius phaeopus hudsonicus*) collected in August 1992, in Cape Breton, Nova Scotia, Canada.

Bird no.	<i>Ancyracanthopsis schikhobalovi</i>		<i>Skrjabinoclava snorrasoni</i>		<i>Skrjabinocerca bennetti</i> sp. n.		<i>Sciadiocara umbellifera</i>		<i>Streptocara</i> sp.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
500	4	3	—	2						
502	—	2								
509							6	7		
510										
511	5	8			1	3			—	1
512			1	1						
513			2	3						
526	1	2	1	—						

Anderson, 1993). *Ancyracanthopsis schikhobalovi* is 1 of 3 species in the genus in which ptilina are divided into sharply pointed posterior extensions (rather than rounded lobelike extensions). Also, this species has 4 groups of 4 extensions of the ptilinum although 1 in each group may be considerably smaller. The complex structure of the terminus of the left spicule, as well as the delicate fingerlike projection on the ventral side of the right spicule, distinguishes *A. schikhobalovi* from the 7 other members of the genus (Wong and Anderson, 1990).

***Skrjabinoclava snorrasoni*  
Anderson and Wong, 1992**

Specimens were found in the proventriculus. Morphology and dimensions agree with the original description based on specimens from whimbrels in Iceland. *Skrjabinoclava snorrasoni* is distinguished from all other members of the genus by the stout right spicule (65  $\mu\text{m}$  long) with a blunt terminus and the recurved distal end of the left spicule (370–410  $\mu\text{m}$  long) which is especially obvious in the extruded spicule but can also readily be observed in the fully retracted spicule.

***Sciadiocara umbellifera* (Molin, 1860)**

Specimens were found under the koilin lining of the gizzard. Dimensions and morphology agree with the redescription based on specimens from western willets (*Catoptrophorus semipalmatus*) and gray plovers (*Pluvialis squatarola*) (see Wong and Lankester, 1985). *Sciadiocara umbellifera* is markedly different from *S. legendrei* Petter, 1966 from a whimbrel in Europe in that the spicules are much larger and morphologically different. The spicules are also much larger in *S. umbellifera* than in *S. bihamata* (Mueller, 1897), a species found in whimbrels and other shorebirds in Iceland by Wong and Anderson (1993).

***Streptocara* sp.**

A single adult female was found under the koilin.

**Discussion**

Five species of acuarioid nematodes were found in the Whimbrels from Cape Breton. The 2 most common, *Ancyracanthopsis schikhobalovi* and *Skrjabinoclava snorrasoni*, were also found in whimbrels in Iceland (Wong and Anderson, 1993); *A. schikhobalovi* is also known from whimbrels in the Komi Republic, formerly part of the U.S.S.R. (Wong and Anderson, 1990). These 2 acuarioid species may, therefore, be widely distributed, especially since whimbrels from Europe stray to the Canadian Maritimes, including Nova Scotia (Tufts, 1986). The only other species of the genus *Skrjabinoclava* known to infect both New and Old World waders is *S. aculeata* (Creplin, 1825) of Dunlins (*Calidris alpina*) which, like whimbrels, have a number of distinct populations with specific migration routes (Anderson et al., 1994).

*Sciadiocara umbellifera* has previously been reported in whimbrels, specifically in Florida (Wong and Anderson, 1991), but not in Iceland. It also occurs in various other shorebirds in the New World, and early reports from the Old World may be in error (Wong and Anderson, 1991). Based on Wong and Anderson (1993), species of *Streptocara* appear to be sporadic parasites of waders; they are common in ducks, however. The single female found herein was not identifiable to species.

*Skrjabinocerca* was considered monotypic until Wong and Anderson (1993) recognized 2 new species. *Skrjabinocerca bennetti* sp. n. is distinctive because of its subequal spicules; it and *S. europaea* Wong and Anderson, 1993 both occur

in whimbrels, the latter species in Iceland. *Skrjabinocerca prima* Schikhobalova, 1930 and *Skrjabinocerca americana* Wong and Anderson, 1993 use amphipods as intermediate hosts and the latter is transmitted in freshwater habitats through *Hyalella azteca* (see Tsimbaliuk and Kulikov, 1966; Bartlett et al., 1989).

Four species found in Iceland were not found in Cape Breton, namely, *Voguracuaria lankesteri* Wong and Anderson, 1993; *Schistorophus coronatus* Sobolev, 1943; *S. cirripedesmi* Rhizhkov and Hhokhlova, 1964; and *Sciadiocara bihamata* (Mueller, 1897). These could be parasites confined to Old World waders since they have not been reported in the New World (Wong and Anderson, 1991).

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## ***Breinlia tinjili* sp. n. (Filarioidea: Onchocercidae), from the Malaysian Field Rat, *Rattus tiomanicus*, on Tinjil Island, West Java, Indonesia**

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**ABSTRACT:** *Breinlia tinjili* sp. n. (Filarioidea: Onchocercidae) is described from the thoracic and abdominal cavities of 4 rats, *Rattus tiomanicus* Miller, 1900, from Tinjil, an uninhabited island off the west coast of Java, Indonesia. *Breinlia tinjili* adult worms have 12 to 13 asymmetrical perianal papillae, a wide cup-shaped capitulum of both spicules, a 2.5:1 spicule ratio, a thick trilobed gubernaculum, and numerous cuticular bosses scattered along the dorsal and ventral surface of the worm. Microfilaria are unsheathed, measure between 273 and 300  $\mu\text{m}$  in length, and have 6–9 tail nuclei in a single row and a long lashlike tail. Descriptions are most similar to *B. spratti* and *B. booliati*, but differ in the following characteristics: the adults are smaller, the gubernaculum is trilobed, paired spicules are smaller, males have an extra pair of postanal papillae, and the microfilariae are much larger in *B. tinjili* than in *B. booliati*. *Breinlia spratti* has a reduced number and different arrangement of male perianal papillae, a smaller nongrooved gubernaculum, and smaller spicules compared to *B. tinjili*. This new species appears to share a number of close characters with other *Breinlia* species previously described from rodents. The diagnostic value of these characters is discussed.

**KEY WORDS:** Onchocercidae, *Breinlia tinjili* sp. n., *Rattus tiomanicus*, Indonesia, morphology.

During a biomedical expedition to Tinjil Island on 20–24 March 1989, U.S. Naval Medical Research Unit No. 2 staff, conducting routine capture and processing of native rodents, found adult filariid worms in the thoracic and abdominal cavities of 4 Malaysian field rats, *Rattus tiomanicus* Miller. Subsequent examinations of these specimens revealed that they represented a new species within the genus *Breinlia* which is described herein. Tinjil (6°58'S, 105°45'E) is an uninhabited coral island of approximately 600 hectares of undisturbed tropical forest located in the Indian Ocean 10 km off the south coast of West Java, Republic of Indonesia.

### **Materials and Methods**

Adult worms were removed from the thoracic and abdominal cavities of euthanized rats, fixed in hot 10% formalin and preserved in 70% ethanol/5% glycerin. All specimens were examined using a temporary lactophenol wet-mount technique (Partono et al., 1977). Microfilariae were obtained from a thick blood smear and stained for 15 min with Giemsa diluted 1:15 with pH 7.2 buffer. Drawings (Figs. 1–6) were made with the aid of a camera lucida. All measurements are expressed as means (range) and are given as length by width in micrometers ( $\mu\text{m}$ ) unless otherwise indicated.

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### **Results**

#### ***Breinlia tinjili* sp. n. (Figs. 1–6)**

**HOST:** Malaysian field rat, *Rattus tiomanicus* Miller, 1900.

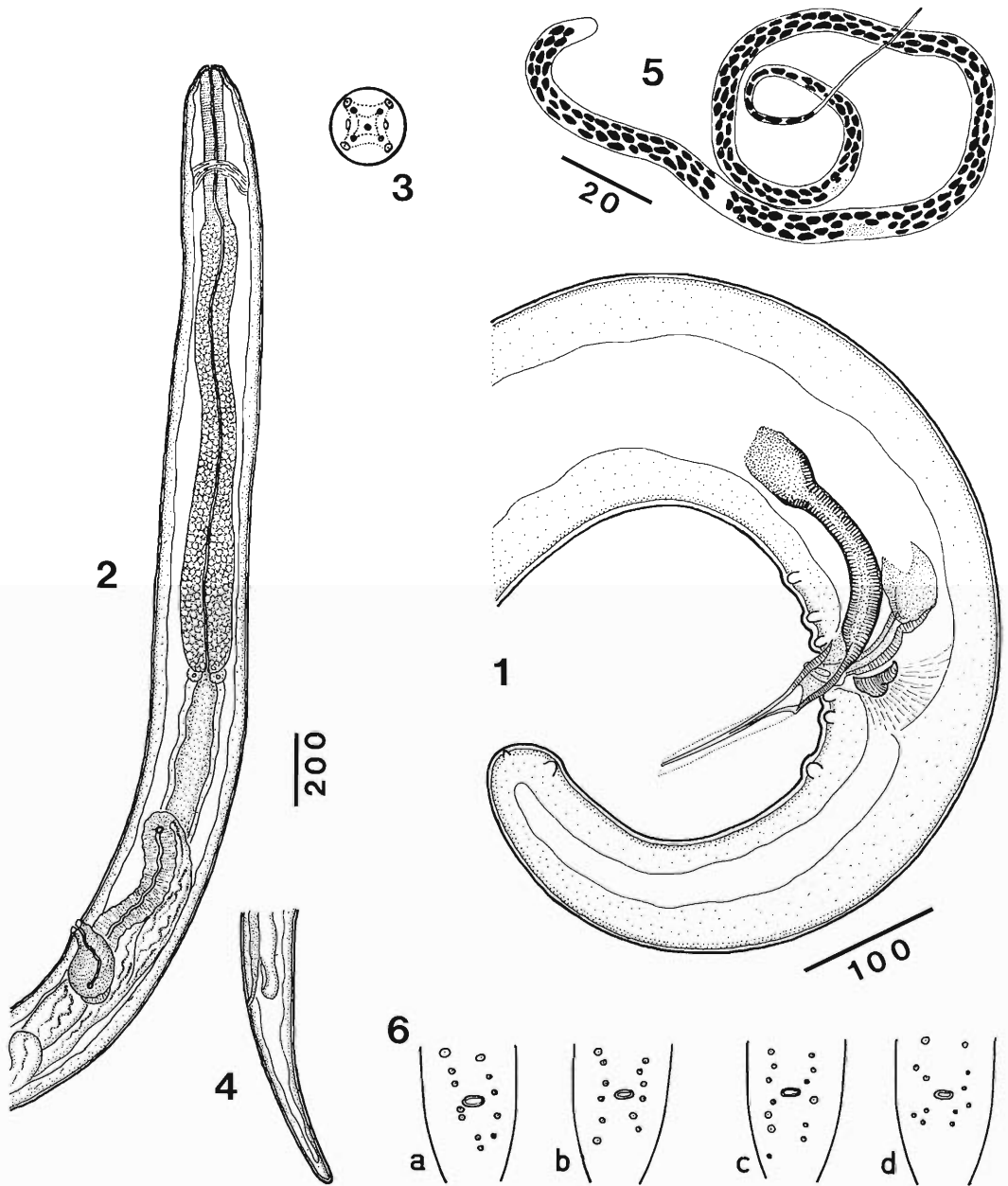
**LOCATION:** Thoracic and abdominal cavities.

**LOCALITY:** Tinjil Island, SW Java, Indonesia (6°58'S, 105°45'E).

**SPECIMENS DEPOSITED:** USNM Helm. Coll. nos. 80943 for both holotype male and allotype female, and 81169 for male and female paratypes, all in 70% ethanol/5% glycerin, and 4 slides of microfilariae (syntypes), Giemsa-stained, are deposited in the U.S. National Parasite Collection, Beltsville, Maryland, 20705 U.S.A.

**DESCRIPTION:** Adults filiform, yellow to white when fixed. Anterior and posterior ends blunt. Mouth simple, without lips. Buccal cavity indistinct with cuticular ring at base. Head with 4 pair submedian cephalic papillae arranged in 2 rings of 4 with 2 lateral amphids (Fig. 3). Cuticle finely striated transversely with small, slightly curved cuticular bosses covering worm at midbody to near cloacal aperture. Esophagus with unequal (ratios) anterior muscular and posterior glandular portions, the latter being slightly wider. Nerve ring at middle of muscular region of esophagus. Vulva postesophageal.

**MALE** (based on 5 mature specimens): Body 37.8 (31.1–42.2) mm by 150 (130–170) at level of nerve ring; width increasing posteriad, 157



Figures 1–6. *Breinlia tinjili* sp. n. adults and microfilaria from a rat from Tinjil Island, West Java. All scale bars in  $\mu\text{m}$ . 1. Caudal end of male, lateral view, showing left and right spicules, gubernaculum, and arrangement of pre- and postanal papillae. 2. Anterior region of female, lateral view, showing esophagus and oesjector. 3. En face view of female showing arrangement of 4 pairs submedian cephalic papillae and 2 lateral amphids. 4. Caudal end of female, lateral view, showing position of anus. 5. Unsheathed microfilaria. 6. a–d. Caudal end of male, ventral view, showing various arrangements of perianal papillae.

(140–180) at level of glandular region of esophagus, 170 (150–200) at esophageal-intestinal junction and 202 (185–215) at level of midworm, gradually decreasing to 105 (90–110) at level of

cloaca. Esophagus 1,651 (1,500–1,825), anterior muscular portion 510 (360–750) by 46 (35–55) (30% of entire length) and posterior glandular portion 1,141 (1,050–1,235) by 94 (90–100).



Nerve ring 264 (225–325) from cephalic end. Tail coiled (1–2 turns) into tight spiral 363 (310–430). Asymmetrical perianal papillae present: 6 preanal, 6 to 7 postanal, and 1 pair each at terminal and subterminal region of tail. Ratio of width at cloaca to length of tail 1:3.5 (2.8–4.1). Left and right spicules (Fig. 1) unequal in length and dissimilar in appearance. Left spicule 316 (295–340), composed of 4 sections; a thick-walled, tubular, striated, and granular proximal portion 159 (150–175); open, expanded, and cup-shaped capitulum 46 (40–50) wide; a thin-walled, semicircular, hyaline midsection 60 (50–70), and a narrow rodlike distal portion 97 (75–120) ending bluntly. Sheath of left spicule evident when extruded. Right spicule 130 (124–135) curved ventrad, divided into 3 distinct portions; a proximal section 56 (50–65), enlarged and rounded with fine striations on wall; a middle cylindrical thick-walled section 48 (35–53), and a distal cylindrical thick-walled section 27 (20–50) ending in a spatulate tip. Left to right spicule ratio 2.5 (2.2–2.6):1. Gubernaculum heavily sclerotized and trilobed in lateral view 36 (33–40) by 17 (15–20).

**FEMALE** (based on 11 gravid specimens): Body 77.6 (53.6–101.4) mm by 187 (160–210) at level of nerve ring; width increasing posteriad, 223 (170–260) at level of glandular region of esophagus, 246 (210–290) at esophagointestinal junction, 267 (230–300) at vulval opening, 338 (300–400) at midbody, gradually decreasing to 152 (100–230) at posterior uterine coils and 116 (100–140) at anal opening. Esophagus (Fig. 2) 1,819 (1,520–2,005), anterior muscular region 459 (400–675) by 58 (50–80) (25% of entire length) and a posterior glandular region 1,360 (1,120–1,570) by 132 (100–150). Nerve ring 261 (230–300) from cephalic end. Vulva opening as a transverse slit immediately posterior to esophageal-intestinal junction, 2,828 (1,930–3,680) from cephalic end. Ovejector pear-shaped, 148 (100–200) by 103 (75–125), with muscular wall. Vagina directed anteriorly, flexing and looping posteriad before receiving uteri at varying distances (500–1,000) below vulva. Uteri paired, loosely entwined, joining oviduct and extending anteriorly to within 2,287 (1,500–2,600) from cephalic end; posterior coil extending to within 990 (520–1,250) from tip of tail. Tail 638 (510–750) with blunt end, and 2 pairs of very small phasmids located subterminally. Viviparous.

**MICROFILARIA** (based on 30 specimens): Body slender, unsheathed, 285 (273–300) long. Width

at level of first nucleus 5.0 (5.0), nerve ring 5.1 (5.0–6.0), excretory pore 5.3 (5.0–6.0), and anal pore 4.1 (4.0–5.0). Cephalic space length 5.4 (3.0–5.0). Distance from anterior end to nerve ring 52 (50–55), excretory pore 78 (73–90), anal pore 203 (194–215), and last nucleus 251 (238–265). Tail 33 (30–37) from last nucleus to posterior tip, tail nuclei, 6–9 in single row (Fig. 5).

### Discussion

*Breinlia tinjili* shares a number of close morphological characters with other species in the genus described from rodents, specifically, *B. booliati* Singh and Ho, 1973 and *B. spratti* Bain et al., 1979. Differentiation can be made based on 1 or more of the following characteristics: the shape, length, and width of the gubernaculum and the length of microfilaria and adults. Additionally, *B. tinjili* differs from *B. booliati* and *B. spratti* in that adult males have an extra pair of postanal papillae with a complete papillar arrangement of 6 asymmetrical preanal, 6–7 asymmetrical postanal and 1 pair each of terminal and subterminal papillae (Fig. 6a–d). A single pair of adanal papillae as described for *B. booliati* was observed in 1 male specimen. Papillae arrangement appears variable and degrees of symmetry make it difficult to accurately judge positions of papillae respective to the anus. *Breinlia spratti* has the majority of papillae aligned along the midline with the anus.

The spicule ratio of *B. tinjili* is 2.5:1 and is similar to that of *Breinlia manningi* Bain et al., 1981, *B. spratti*, and *B. booliati*. However, the average lengths of both spicules were found to be intermediate between the 3 species. The capitula of both spicules are more expanded than illustrated for the other 3 species. The gubernaculum is wide, grooved and trilobed with an average length of 36  $\mu\text{m}$ . *Breinlia spratti* has a smaller structure (24  $\mu\text{m}$ ) that is smooth in appearance (lacks a ventral groove). *Breinlia booliati* is of similar length but is distinctly bilobate. Adult worms are similar in length to those of *B. spratti*, whereas adult *B. booliati* are considerably larger.

The cuticle has fine, transverse striations and small, elongate refractile bosses (longitudinal crests) beginning along the midregion of the body and terminating just anterior to the cloacal aperture. Cuticular bosses show irregular spacing and positions, being scattered on ventral and dorsal surfaces and reaching the lateral edges. This cuticular ornamentation is similar to that

**Table 1.** Selected character comparisons of 3 *Breinlia* species.

	<i>B. tinjili</i> <sup>1</sup>	<i>B. booliati</i> <sup>2</sup>	<i>B. spratti</i> <sup>3</sup>
Length (mm)			
Female	78 (54–101)*	197 (168–213)*	74
Male	38 (31–42)*	64 (46–77)*	31
Maximum width (μm)			
Female	338 (300–400)*	480 (370–521)*	330
Male	202 (185–215)*	245 (197–297)*	235
Length (μm)			
Left spicule	316 (295–340)*	371 (349–385)*	292
Right spicule	130 (124–135)*	144 (118–167)*	110
Gubernaculum (μm)			
Length	36 (33–40)*	35 (30–39)*	24
Width	17 (15–20)*	9 (6–12)*	—
Shape	3-lobed	2-lobed	smooth
Papillar arrangement			
Preanal	6	6	6
Adanal	—	2	—
Postanal	6–7	4	6
Tail tip	2 pair	2 pair	2 pair
Microfilariae (μm)			
Length	273–300	188–206	270–320
Width	5.3 (5–6)*	3.9 (3–5)	5.5

<sup>1</sup> Purnomo and Bangs sp. n. (1996).<sup>2</sup> Singh and Ho (1973).<sup>3</sup> Bain, Tibayrenc, and Mak (1979) (range measurements not given).

\* Mean (range).

described for *B. spratti* and *B. booliati* with the notable exception that bosses are far less dense on the caudal extremity (area rugosa) of both the male and the female. The long and slender microfilaria of *B. tinjili* (285 μm [273–300]) is longer than other known species, excluding *B. spratti* (270–320) and *B. dendrolagi* Solomon, 1933 (280–300). The combination of aforementioned morphologic characters allows for separation of *B. tinjili* from the other closely related *Breinlia* (Table 1).

Based on the study of marsupial filariid species from the Australasian region, Spratt and Varghese (1975) believed there was no morphological or biological justification for maintaining the genus *Breinlia*, Yorke and Maplestone, 1926 and subsequently placed the genus as a synonym of *Dipetalonema*. However, Bain et al. (1979) have retained the genus and its previous members as followed in this discussion. Of the 13 previously reported species of *Breinlia* (Bain et al., 1981), only *B. booliati* has been recovered from both *Rattus* and *Callosciurus* (Singh and Ho, 1973; Mak and Lim, 1974; Lim et al., 1975, 1978; Bain et al., 1979). Close morphological

similarities are seen between *B. tinjili*, *B. spratti*, and *B. manningi*, the last 2 parasites found in rodents in the family Sciuridae. The finding of *B. tinjili* in Muridae from Tinjil and nearby Deli Island represents the second report of a *Breinlia* filariid found in Indonesian mammals and the second species report of this genus from *Rattus* spp. Lim et al. (1978) reported finding *B. booliati* in *R. tiomanicus* from Ciloto, central-west Java, Indonesia. Although presumably true, only 1 complete male and 1 complete female were available for examination, and perianal papillae, cuticular ornamentation, and the microfilaria were not described.

Biological characteristics vary as well. The microfilarial periodicity of *B. tinjili* in the natural host *R. tiomanicus* is aperiodic. This is in contrast to the nocturnal periodicity described in *B. booliati* and its natural host, *Rattus sabanus* (Thos.) (Yap et al., 1975). Unlike *B. booliati*, which can develop in the albino rat, we were unsuccessful at establishing infection by subinoculation of *B. tinjili* in laboratory rats (Atmoseodjono and Purnomo, unpubl.). A potential natural vector for this filariid appears to be *Aedes*

(*Stegomyia*) *albolineatus* (Theobald) which is active in high density during daylight hours most of the year on both islands. Normal development of microfilariae to infective-stage larvae has been observed in the fat body cells of *Ae. albolineatus* and experimental laboratory mosquitoes, *Ae. (Finlaya) togoi*. Full development to the infective stage takes about 10 days, similar to descriptions by Ho et al. (1973).

The diagnosis of new species within the genus *Breinlia* can be a difficult task because many characters, such as papillar arrangement and spicular size and appearance, can be variable. Because of the close morphological similarity between *B. tinjili* and other *Breinlia*, especially *B. spratti*, found naturally in rodent hosts, a close ancestral relationship can be hypothesized. Based on the known geographic distribution of these filaria, we surmise a relatively recent allopatric speciation has taken place. It would be of interest to look at various molecular levels of expression in this group to help estimate genetic distances between species or investigate questions of possible conspecificity.

#### Acknowledgments

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## ***Diceronema versterae* gen. n., sp. n. (Atractidae: Cosmocercoidea) from the Black Rhinoceros, *Diceros bicornis bicornis*, in South Africa**

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**ABSTRACT:** *Diceronema versterae* gen. n., sp. n. (Atractidae: Cosmocercoidea) is described from the stomach of a black rhinoceros, *Diceros bicornis bicornis*, from the Umfolozi Game Reserve, KwaZulu-Natal Province, South Africa. The new genus and species differs from all other genera in the family Atractidae in the presence of a cup-shaped buccal capsule, the presence of symmetrical cervical papillae each with 4 prongs, the restriction of spines to the ventral surface of the female tail, the presence of caudal alae on the posterior end of the male, the ornamentation on the ventral surface of the male, and the structure and arrangement of the male caudal papillae.

**KEY WORDS:** *Diceronema versterae*, Atractidae, Cosmocercoidea, new genus, Nematoda, new species, taxonomy, black rhinoceros, *Diceros bicornis bicornis*, KwaZulu-Natal Province, Republic of South Africa.

Nematodes were collected from the stomach of a female black rhinoceros, *Diceros bicornis bicornis*, that died in the Umfolozi Game Reserve, Kwazulu-Natal Province, Republic of South Africa in June 1993. Approximately 2,100 specimens were present in the stomach contents. Examination of these nematodes revealed that they belonged to the family Atractidae (Railliet, 1917 subfamily) Travassos, 1919. Adamson and Baccam (1988) described the family as a morphologically diverse group whose only common characteristic is the capability for autoinfection, i.e., the viviparous females produce larvae which quickly develop into mature adults without passing out of their host into the external environment. The family has been clearly divided into 2 groups by Chabaud (1978) and Adamson and Baccam (1988) on the basis of the morphology of the esophagus and the presence of a monodelphic or didelphic female reproductive tract. The present specimens from the rhinoceros are monodelphic and have features not found in any other attractid. These nematodes are described and found to represent a new genus and species.

### **Materials and Methods**

The stomach ingesta was fixed in 10% formalin. Initially, one hundredth of the ingesta vol-

ume was examined microscopically. Subsequently, an additional nine hundredths of the ingesta volume was examined microscopically for recovery of further specimens for examination. Specimens were preserved in 70% alcohol with 2% glycerine added. They were cleared for examination in lactophenol and lactoglycerol and 1 specimen was mounted in Berlese's Fluid to examine the spicules. Some specimens were stained in Horen's trichrome. Drawings were made with the aid of an Olympus Drawing Attachment BH2-DA. Type specimens are deposited in the United States Department of Agriculture National Parasite Collection at Beltsville, Maryland, U.S.A. and the helminth collection of the International Institute of Parasitology, United Kingdom. Measurements are in millimeters unless otherwise stated.

### **Results**

#### **Family Atractidae (Railliet, 1917 subfamily) Travassos, 1919**

#### ***Diceronema* gen. n.**

**DIAGNOSIS:** Body elongate, attenuate, and transversally striated. Mouth with rudimentary dorsal and ventral lips; 2 pairs of submedian cephalic papillae lateral to mouth opening. Cup-shaped buccal capsule; anterior portion or cheilorhabdion divided dorsoventrally overlapping rim of mouth opening; posterior portion or pro-

\* An institute of CAB International.

habdion formed by dorsal and ventral curved semilunar plates. Cervical papillae present divided into 4 sharply pointed prongs articulate with plates in body wall. Esophagus divided, corpus distinct with distal bulb, posterior portion not distinguished into isthmus and bulb. The elongate nerve ring extends posteriorly from corpus of esophagus and excretory pore opens in region of nerve ring. Male tail coiled ventrally; 5 pairs of pedunculate caudal papillae asymmetrically arranged, 3 pairs of sessile papillae near the distal end with an additional pair of sensory organs, possibly the phasmids, adjacent to the median and distal pair; ventral cuticular flap with 2 small projections at distal tip of tail; spicules unequal, right spicule with a barb at distal tip; slender caudal alae present. Female vulva opens just anterior to anus; viviparous. Adults parasitic in stomach of rhinoceros.

*Diceronema versterae* sp. n.  
(Figs. 1–18)

**DESCRIPTION** (based on 15 male and 14 female specimens): Body elongate, attenuate, and transversally striated. Mouth with rudimentary dorsal and ventral lips; 2 pairs submedian cephalic papillae lateral to mouth opening. Cup-shaped buccal capsule (Figs. 3, 4, 9, 13), anterior portion or cheilorhabdion divided dorsoventrally overlapping rim of mouth opening; posterior portion or prorhabdion formed by dorsal and ventral curved semilunar plates. Cervical papillae symmetrical, projecting from body surface (Figs. 1, 2, 13), each with 4 sharply pointed prongs, joined at the base, which articulates with plates in body wall. Esophagus divided, corpus clearly defined with distal bulb, posterior portion not distinguished into isthmus and bulb (Fig. 13). Excretory pore posterior to distal bulb of anterior esophagus, small, difficult to see. Nerve ring elongate, cellular in appearance, extends poste-

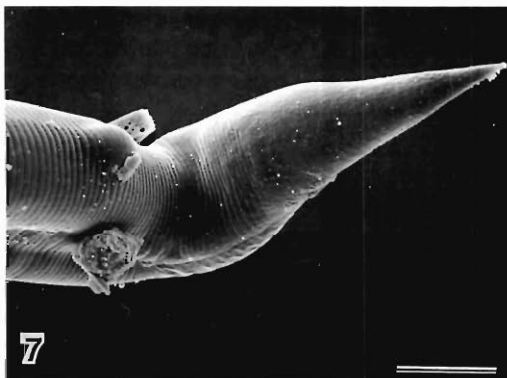
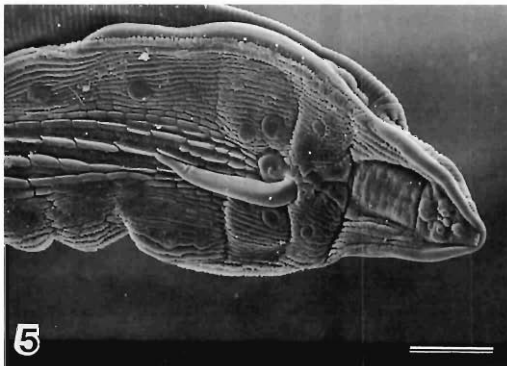
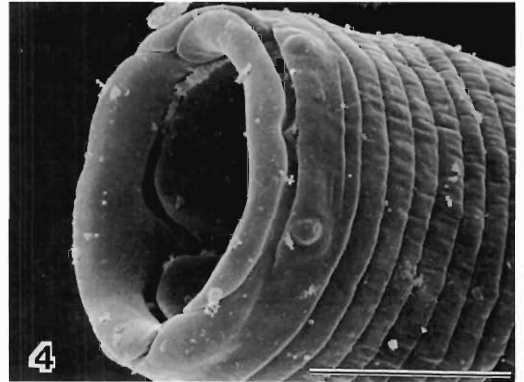
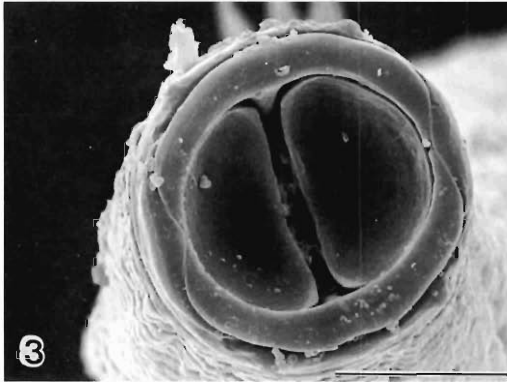
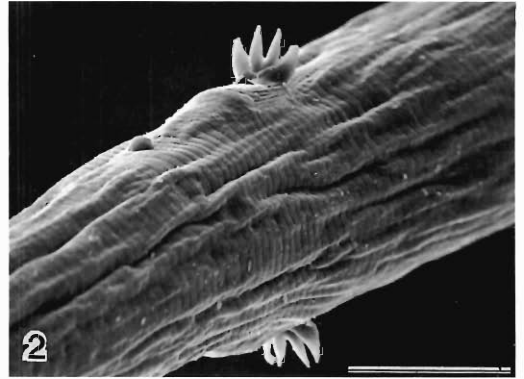
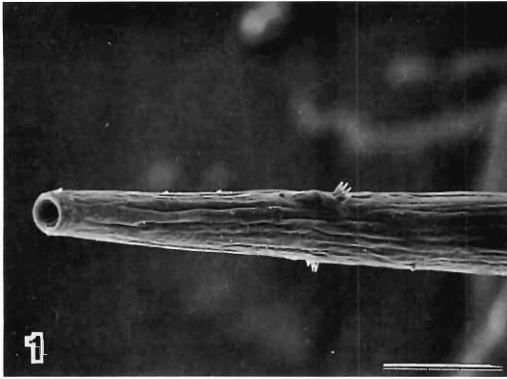
riorly from distal end of corpus of esophagus (Fig. 14).

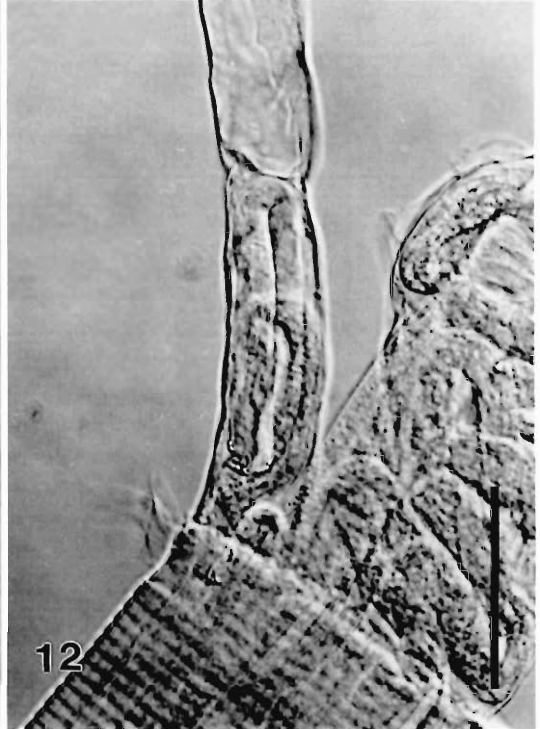
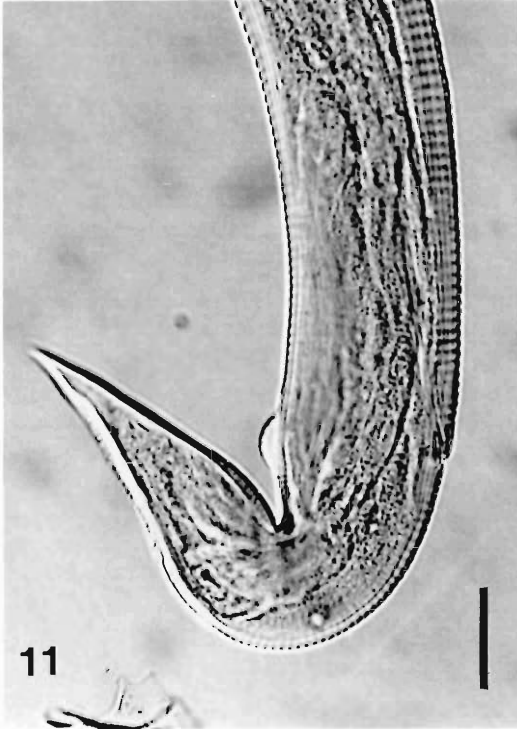
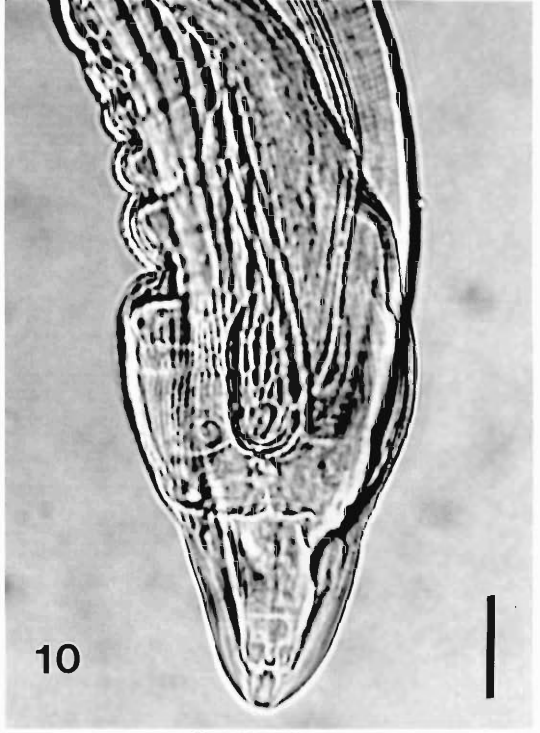
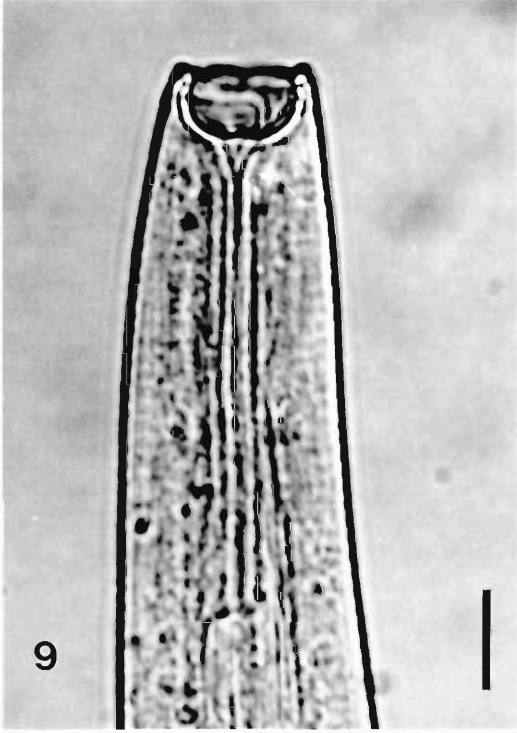
**MALE:** Body 2.2–2.9 long; maximum width 0.08–0.10, 0.040–0.068 wide just anterior to male caudal alae. Head 0.019–0.024 wide. Buccal capsule 0.014–0.020 wide, 0.010–0.012 deep. Esophagus 0.59–0.72 long, corpus 0.12–0.18 long, posterior esophagus 0.37–0.57 long. Cervical papillae, nerve ring, and excretory pore 0.11–0.16, 0.13–0.19, and 0.14–0.17, respectively, from anterior end. Nerve ring 0.03–0.05 long. Right and left spicules 0.09–0.11 and 0.16–0.22 long, respectively (Figs. 5, 6, 10, 17). Right spicule with distal barb (Fig. 18). Gubernaculum absent, dorsal wall of spicular pouch lightly sclerotized. Single sessile papilla just anterior to cloaca. Caudal papillae, 8 pairs (Fig. 16); 5 pairs pedunculate and asymmetrically arranged; third and fourth pair from anterior and open toward ventral median line, first, second and fifth open lateroventrally; 3 pairs sessile near distal tip, median pair separated by cuticular ridge; between median and distal pair of papillae a pair of small pores open which possibly represent the phasmids (Fig. 16). Two small refractive projections form distal margin of ventral cuticular flap on distal tip of tail. Slender caudal alae present. Discontinuous longitudinal cuticular ridges extend on ventral surface from commencement of caudal alae to just anterior to cloaca.

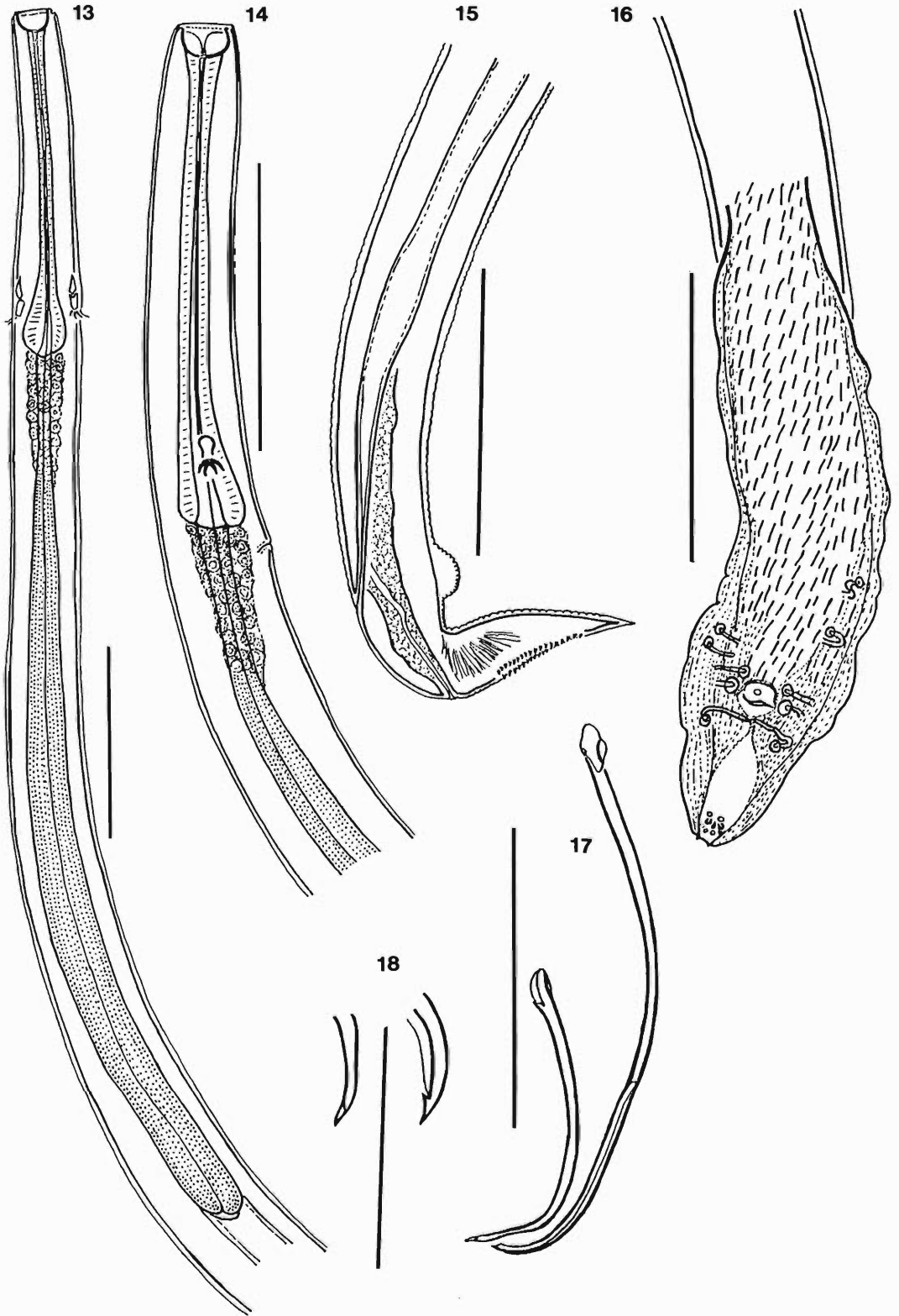
**FEMALE:** Body 2.70–3.24 long, width 0.09–0.12, 0.03–0.04 just anterior to vulva. Head 0.02–0.03 wide. Buccal capsule 0.01–0.02 wide, 0.01–0.14 deep. Esophagus 0.50–0.74 long, corpus 0.014–0.019 long, posterior esophagus 0.40–0.56 long. Cervical papillae, nerve ring, and excretory pore 0.13–0.17, 0.15–0.20, and 0.15–0.18, respectively, from anterior end. Nerve ring 0.04–0.06 long. Monodelphic, ovary 0.19–0.24 from distal end of esophagus. Vulva opens 0.10–0.14 from distal tip of tail and is near anus. Vesicular swelling of cuticle on dorsal surface directly op-

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**Figures 1–8.** Scanning electron micrographs of *Diceronema versterae* gen. n., sp. n. 1. Anterior end of female showing mouth and cervical papillae. Scale bar = 50  $\mu$ m. 2. Cervical papillae of female. Scale bar = 20  $\mu$ m. 3. *En face* view of female showing dorsal and ventral lips and semilunar plates of the buccal capsule. Scale bar = 10  $\mu$ m. 4. Anterior end of female showing cephalic papillae. Scale bar = 10  $\mu$ m. 5. Tail of male showing papillae, ventral view. Scale bar = 20  $\mu$ m. 6. Recurved tail of male showing postcloacal papillae and caudal alae. Scale bar = 10  $\mu$ m. 7. Tail of female showing vesicular swelling on dorsal surface of cuticle. Scale bar = 20  $\mu$ m. 8. Tail of female showing recurrent line of spines on ventral surface. Scale bar = 20  $\mu$ m.

**Figures 9–12.** Photomicrographs of *Diceronema versterae* gen. n., sp. n. 9. Anterior end of female showing cup-shaped buccal capsule. Scale bar = 10  $\mu$ m. 10. Posterior of male showing subequal spicules and caudal alae. Scale bar = 10  $\mu$ m. 11. Tail of female with vesicular swelling. Scale bar = 10  $\mu$ m. 12. Uterus with larvae. Scale bar = 10  $\mu$ m.









posite vulva (Figs. 7, 11, 15) 0.01–0.02 wide. Posterior margin of vesicle 0.09–0.10 from distal tip of tail. Viviparous, eggs and larvae present in uterus (Fig. 12). Tail 0.06–0.08 (Figs. 7, 8, 11, 15). Recurrent line of short anteriorly directed spines on ventral surface of tail, occasionally line double (Fig. 8). Pair of ventrolateral sensory organs 0.03–0.04 from distal tip of tail probably represent the phasmids.

**SPECIES:** *Diceronema versterae* sp. n.

**TYPE HOST:** *Diceros bicornis bicornis*.

**SITE:** Stomach.

**TYPE LOCALITY:** Umfolozi Game Reserve, KwaZulu–Natal, South Africa.

**ETYMOLOGY:** This genus is named after the host, *Diceros bicornis bicornis*, and the species is named after the late Professor Anna Verster, a prominent helminthologist.

**TYPE MATERIAL:** USDA National Parasite Collection; Holotype no. USNPC 84838, Paratype no. USNPC 84839. Helminth collection of the International Institute of Parasitology, United Kingdom; Paratype nos. B1059B, S1093B.

### Discussion

Although some of the features of the specimens described herein, particularly the morphology of the male tail, suggest that they should be placed in the order Spirurida, the esophagus is clearly divided into 2 parts, a precloacal sucker is absent on the male tail, the female vulva opens posteriorly, and the females are viviparous, all characters which suggest that they are close to the family Atractidae of the order Ascaridida. Skrjabin et al. (1964) reviewed the family Atractidae and pointed out that at first the genus *Atractis* was associated with the genus *Ascaris* but later was placed with *Oxyuris* in the superfamily Oxyuroidea. Chabaud and Petter (1960) referred the family Atractidae to the superfamily Cosmocercoida and this classification is now generally accepted. Chabaud (1965) confirmed the inclusion of the Atractidae within the Cosmocercoida but considered the group to be intermediary between the oxyurids and cosmocercids. Chabaud (1978) accepted 20 genera in the family

Atractidae. Adamson and Baccam (1988) accepted 14 genera in the family and characterized the Atractidae as having an esophagus distinctly divided at the junction of the corpus and the isthmus. They divided the family into 2 groups, the first didelphic with an esophagus with a long, narrow pharyngeal portion which includes *Fitsimmonsma* and *Probstmayria* and the second *Paratractis*, *Cyrtosomum*, *Rondonia*, *Monohysterides*, *Proatractis*, *Cobboldina*, *Orientaltractis*, *Labidurus*, *Grassinema*, *Leiperenia*, and *Crossocephalus*. Khalil and Gibbons (1988) and Gibbons et al. (1995) added 2 more genera to the second group, namely, *Buckleyatractis* and *Podocnematractis*, respectively. The specimens described herein show some similarities to the genus *Cobboldina* Leiper, 1911 from the hippopotamus in the shape and structure of the esophagus and the position and formation of the nerve ring. They differ from *Cobboldina* and all the other genera of the Atractidae in the presence of a cup-shaped buccal capsule, the structure of the cervical papillae, the restriction of spines to the ventral surface of the female tail, the presence of caudal alae on the posterior end of the male, the ornamentation on the ventral surface of the posterior end of the male, and the structure and arrangement of the male caudal papillae. For these reasons a new genus *Diceronema*, after the host *Diceros bicornis bicornis*, and a new species, *D. versterae*, after the late Professor Anna Verster, are erected for these specimens.

### Acknowledgments

The authors thank Mr. D. Boshoff and Mr. J. Lourens for technical assistance; Mr. H. Els for scanning electron microscopy; Mr. T. E. Krecek for assistance with drawings; Dr. L. F. Khalil for his helpful discussion on the various features of the specimens described and constructive criticism of the draft manuscript; Mrs. A. Lubbe for assistance with typing of the manuscript; and the Foundation for Research Development and University of Pretoria for financial support. This study forms part of the Wildlife Research Program at the Faculty of Veterinary Science, Uni-

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**Figures 13–18.** Drawing tube illustrations of *Diceronema versterae* gen. n., sp. n. 13. Anterior end of female, dorsal-ventral view. Scale bar = 0.05 mm. 14. Higher magnification of anterior end of female, lateral view. Scale bar = 0.10 mm. 15. Posterior end of female, lateral view. Scale bar = 0.10 mm. 16. Posterior end of male, composite ventral view from light and scanning electron microscopical studies. Scale bar = 0.10 mm. 17. Spicules dissected out of tissues. Scale bar = 0.10 mm. 18. Dorsal tip of right spicule. Scale bar = 0.05 mm.

versity of Pretoria. This is contribution no. J-3065 from the Montana State University Agricultural Experiment Station.

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## Research Note

# Development of *Onchocerca cervicalis* to the Third Larval Stage in *Simulium pictipes*

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**ABSTRACT:** The objective of this study was to assess the ability of a temperate black fly, *Simulium pictipes*, to support larval development by *Onchocerca cervicalis*, a filarial parasite of horses normally associated with ceratopogonid vectors. A proportion of both freshly isolated and cryopreserved microfilariae (MF) inoculated intrathoracically into *S. pictipes* completed development to the third larval stage (L3) in the thoracic and cephalic musculature. The first and second molts occurred as early as days 6 and 7 of infection, respectively. Relative to the course of *O. cervicalis* infection in a natural vector, *Culicoides nubeculosus*, the frequency of development in *S. pictipes* was low. Black flies inoculated with 50 freshly isolated MF ultimately yielded a mean of 3.3 L3 per fly, representing 6.5% of the original inoculum. In contrast, 2.1% of the cryopreserved MF were recovered as L3. Migration of L3 from the thoracic musculature to the heads and mouthparts of infected flies could not be ascertained. The mean length of L3 recovered from *S. pictipes* was 579  $\mu\text{m}$ , approximately 35% shorter than L3 derived from the natural vector. These results demonstrate that *S. pictipes* will support development of a proportion of inoculated *O. cervicalis* MF, making it a potential source of these parasite stages albeit an inefficient one. The relevance of this finding to the development of laboratory models for the study of onchocerciasis is discussed.

**KEY WORDS:** *Onchocerca cervicalis*, *Simulium pictipes*, black fly, microfilaria, third-stage larva, surrogate vector.

Efficient production of *Onchocerca volvulus* Leuckart, 1893 third stage larvae (L3) for laboratory research has constituted a major obstacle in the development of vaccines, macrofilaricides, and chemoprophylactics against human onchocerciasis. Onchocercids of livestock such as *O. lienalis* Stiles, 1892; *O. ochengi* Bwangamoi, 1969; and *O. gibsoni* Cleland and Johnston, 1910 in cattle, and *O. cervicalis* Railliet and Henry,

1910 in horses, have been proposed as models for basic research in this area (Copeman, 1979; Jones and Collins, 1979; Lok and Abraham, 1992; Trees, 1992). Temperate black flies such as *Simulium pictipes* Hagen, 1880 and *S. ornatum* Meigen, 1818 make highly efficient laboratory vectors for *O. lienalis* when inoculated intrathoracically with microfilariae (MF) (Lok et al., 1983; Bianco et al., 1989). It is noteworthy that these temperate black flies also support development of the allopatric, black-fly-associated filariae, *O. volvulus* (Ham and Bianco, 1983; Lok, et al., 1983) and *O. ochengi* (McCall and Trees, 1989), to the L3. *Culicoides* spp., the natural vectors of *O. cervicalis*, are highly susceptible to experimental infection with isolated MF of *O. cervicalis* via the oral route (Mellor, 1975; Collins and Jones, 1978), but to date no system of mass production of *O. cervicalis* L3 utilizing these flies has been demonstrated. The objective of this study was to determine whether the black fly *S. pictipes* could serve as an alternate source of *O. cervicalis* L3 for laboratory study.

Late larval instars of *S. pictipes* were collected from a bedrock cascade in Bushkill Creek near Resica Falls, Pennsylvania, U.S.A. These larvae were reared to adults in a closed-circulation rearing system as described by Brenner and Cupp (1980). Three initial infection trials were carried out with a batch of *O. cervicalis* MF collected from infected horses at slaughter, cryopreserved, and later thawed according to the technique of Ham et al. (1981). For a fourth infection trial, a fresh umbilical skin from an infected horse was shipped on wet ice via overnight courier from its collection site in South Carolina to the University of Pennsylvania, where MF were extracted and used immediately.

Freshly isolated or cryopreserved MF were suspended in Ham's Medium F12, containing

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**Table 1. Findings from dissection of *Simulium pictipes* experimentally infected with 50 *Onchocerca cervicalis* microfilariae and incubated at 27°C.**

Day of infection	Treatment of MF*	No. of flies dissected	% flies infected	No. of larvae recovered	Breakdown by stage			
					MF	L1	L2	L3
1	Cryopreserved	6	66.7	47	47	—	—	—
2	Cryopreserved	8	12.5	8	8	—	—	—
3	Cryopreserved	15	40.0	12	2	10	—	—
4	Cryopreserved	14	71.4	27	—	27	—	—
5	Cryopreserved	4	100	18	—	18	—	—
6	Cryopreserved	3	66.7	12	—	3	9	—
	Fresh	5	100	46	—	8	38	—
7	Cryopreserved	21	66.7	34	—	2	30	2
	Fresh	3	100	14	—	1	13	1
8	Cryopreserved	16	62.5	22	—	1	19	2
	Fresh	3	100	33	—	—	12	21
9	Cryopreserved	8	50.0	8	—	—	2	6
	Fresh	3	100	33	—	1	16	16
10	Cryopreserved	10	50.0	11	—	—	5	6
	Fresh	5	100	42	—	—	15	27
11	Cryopreserved	6	50.0	6	—	—	2	4
12	Fresh	3	100	9	—	—	4	5

\* Data on cryopreserved MF are pooled from 3 cohorts of flies infected with parasites from the same cryopreservation lot. Data on fresh MF are from a single infection cohort.

20% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B, and injected intrathoracically into female *S. pictipes* at a dose of 50 MF per fly using finely drawn glass micropipets as described by Lok et al. (1983) and Bianco et al. (1989). Flies were injected 24–48 hr after eclosion. Inoculated flies were incubated in humidified cages at 27°C and fed on a 30% sucrose solution via a filter paper wick.

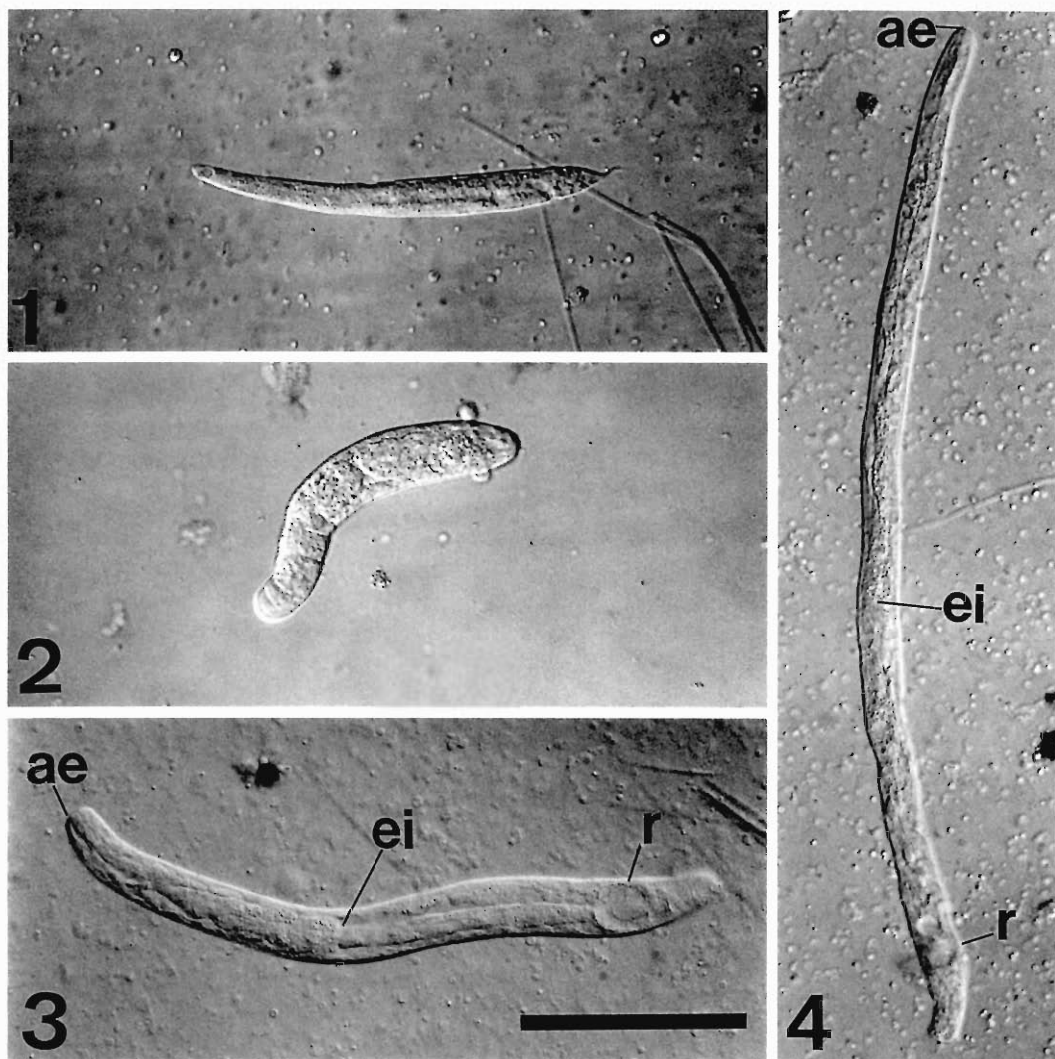
Each day, a sample of live flies was dissected and examined for developing larvae. In the trial involving freshly isolated MF, dissections were postponed until day 6 of infection in order to conserve inoculated flies and thus obtain a more accurate assessment of the system's potential to generate L3. Numbers of larvae in the head, thorax, and abdomen of each fly were recorded, and the parasites were measured and photographed under Nomarski differential interference contrast microscopy. They were assessed as to stage of development with the aid of morphological descriptions given by Mellor (1975) and Bain and Petit (1978). Dead flies were removed daily from the cohorts, counted, and discarded.

Quantitative findings from dissection of *S. pictipes* inoculated with *O. cervicalis* appear in Table 1. At 27°C the majority of larvae had matured to the "sausage form" first stage (L1; Figs. 1, 2) by day 3 of infection. In most cases, the molt to

the second stage (L2; Fig. 3) commenced on day 6 and the molt to the L3 (Fig. 4) commenced on day 7. Larval development was asynchronous, with L3 appearing as early as day 7 and L2 persisting as late as day 12. The total number of larvae, regardless of stage, recovered after inoculation of cryopreserved MF represents a developmental rate of 5.7%. The total number of L3 recovered represents 2.1% of the original inoculum. Much higher infection rates and parasite burdens were seen in flies infected with freshly isolated MF. The numbers of developing larvae and L3 recovered from flies inoculated with fresh MF (Table 1) represent 17.5% and 6.5% of the original inoculum, respectively.

There were frequent instances of abnormal development. Midbody constrictions and vacuolation of the posterior intestine were occasional findings, and examples of incomplete ecdysis were also seen.

The anatomical distribution of larval *O. cervicalis* recovered from *S. pictipes* is given in Table 2. At the time of injection, MF were dispersed throughout the heads, thoraces, and abdomens of the black flies, and the distribution of the MF among these segments remained random for approximately 48 hr. Although the majority of preinfective larvae were recovered from the thoraces of infected flies, low percentages of L1 and L2 as well as L3 were found in the heads. In



Figures 1–4. Developing larvae of *Onchocerca cervicalis* from the thoracic musculature of *Simulium pictipes* after intrathoracic inoculation with cryopreserved microfilariae. 1. Early L1, day 4 of infection. 2. Late L1, day 7 of infection. 3. L2, day 8 of infection. 4. L3, day 9 of infection. Bar = 100  $\mu$ m. Abbreviations: ei = esophageal/intestinal junction, ae = anterior extremity, r = rectum.

addition, a small number of L3 were found in the abdomens of flies on day 12 of infection with fresh MF.

High mortality occurred in the inoculated black flies between days 0 and 2 and days 6 and 8 (Fig. 5). Survival declined to 50% by day 5 of infection. By days 8 to 10 of infection, when significant numbers of L3 were seen, it was 10% or less.

These results demonstrate that MF of *O. cervicalis* can develop to the L3 when injected intrathoracically into *S. pictipes*. Infectivity of L3 derived in this manner remains to be ascertained.

The maximum efficiency of conversion of *O. cervicalis* MF to L3 in *S. pictipes* was 6.5%. This developmental rate was low considering that approximately 20% of *O. lienalis* MF develop to the L3 in this vector (Lok et al. 1983). On the other hand, developmental rates in the *O. cervicalis*/*S. pictipes* system are high compared with those seen in another surrogate vector, *Aedes aegypti*, in which only 1.6% of fresh, injected *O. cervicalis* MF complete development to the L3 (Lok et al. 1980). Comparable data on *O. cervicalis* in *Culicoides* sp. are not available. The

**Table 2. Anatomical distribution of *Onchocerca cervicalis* larval stages in experimentally infected *Simulium pictipes*.**

Larval stage	No. of parasites observed*	% of recovered parasites per body segment		
		Head	Thorax	Abdomen
MF	57	19.3	19.3	61.4
L1	73	4.1†	95.9	—
L2	174	9.2†	90.8	—
L3	92	6.5‡	90.2	3.3‡

\* Pooled data from all infection trials; fresh and cryopreserved MF included.

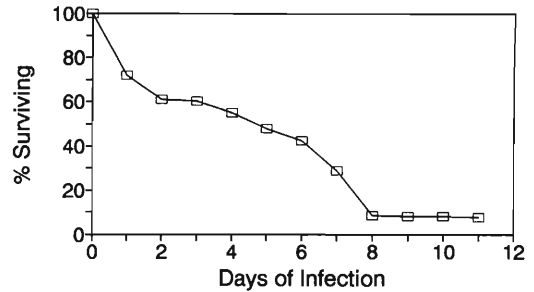
† Numbers of L1, L2, and L3 recovered from heads not significantly different ( $\chi^2 = 1.96$ ;  $P = 0.38$ ).

‡ L3 recovered day 12 of infection with fresh MF.

fact that extraction and purification of MF designated as "fresh" and "cryopreserved" were carried out in different laboratories does not permit a rigorous comparison of the infectivity of fresh and cryopreserved *O. cervicalis* MF in the context of this study. Given the wide applicability of the specified technique for cryopreservation (Ham et al., 1981) it seems likely that the observed differences in developmental success were due to batch variation in technique and not to the amenability of *O. cervicalis* MF to cryopreservation per se. Maintenance of the infected skin sample containing fresh MF on wet ice for a period of almost 24 hr during overnight shipment represents a departure from our standard technique for collection of *O. lienalis* MF, where skins are maintained at room temperature and are processed within 6 hours of collection. This technical factor may have resulted in diminished infectivity of fresh MF in the present study.

The mean length ( $\pm$ SD) of the MF used in the present study ( $223.7 \pm 27.6 \mu\text{m}$ ;  $N = 6$ ) was equivalent to published values for *O. cervicalis* (Mellor, 1975). However, at a mean length of  $579 \pm 80 \mu\text{m}$ , the L3 derived from *S. pictipes* were shorter than ceratopogonid-derived L3, which range in length from 680–870  $\mu\text{m}$  (Mellor, 1975; Bain and Petit, 1978). The lengths of L3 in the present study more closely approximate the mean length of  $558.8 \pm 32.2 \mu\text{m}$  reported for *O. cervicalis* L3 in *Aedes aegypti*. Thus, it appears that stunting of *O. cervicalis* L3 can occur as a result of development in an abnormal vector. Voucher specimens of *O. cervicalis* L3 recovered from *S. pictipes* have been deposited in the U.S. National Parasite Collection, USNPC No. 84909.

Aberrant and retarded development similar to



**Figure 5. Survival of *Simulium pictipes* inoculated intrathoracically with microfilariae of *Onchocerca cervicalis*.**

that observed in the present study have been reported for *O. cervicalis* in a natural vector, *Culicoides nubeculosus* (Mellor, 1975) and *Ae. aegypti* (Lok et al., 1980). Melanotic encapsulation, which figured prominently in abortive development by *O. cervicalis* in *Ae. aegypti*, was not observed in *S. pictipes*.

Another common feature of *O. cervicalis* infections in *S. pictipes* was the failure of fully formed, active L3 to migrate to the heads and mouthparts of the flies after 12 days' development at 27°C. The small number of L3 observed in the heads of these flies can be accounted for fully by the numbers of L1 and L2 apparently developing in this body segment. Development of larval filariae in the cephalic musculature has been observed in other vector-filaria systems. Laurence (1985) described the development of larval *Brugia pateri* in the pharyngeal musculature of an experimental mosquito vector, *Ae. togoi*. The fact that *C. variipennis* discharge a small number of *O. cervicalis* L2 during bloodfeeding on an artificial membrane (Collins and Jones, 1978) also suggests that preinfective larvae of this parasite may reside in the head of the vector. The finding of a few L3 in the abdominal hemocoels of flies 12 days after inoculation with fresh MF may indicate the beginning of migration by L3 at this time. However, high mortality among infected *S. pictipes* made it unfeasible to harvest significant numbers of parasites after periods of incubation longer than 12 days. Survivorship among black flies in the present study was much lower than in *S. pictipes* inoculated with *O. lienalis* previously (80% at day 12; Lok et al., 1983). This observation may indicate relatively high pathogenicity of *O. cervicalis* in *S. pictipes*. However, in the absence of the appropriate experimental controls, the hypothesis that low black

fly survival in the present study was due to substandard injection or rearing technique must be given equal weight.

The failure of L3 to migrate normally in *S. pictipes*, the stunting of the recovered L3, and the low rate of parasite development support a characterization of this black fly as an inefficient surrogate vector for *O. cervicalis*. Nonetheless, the present findings indicate that, until a practical system of mass production involving ceratopogonids is developed, *S. pictipes*, and perhaps other temperate black flies, may act as an alternative source of *O. cervicalis* L3. These findings also raise the prospect that black flies could serve as a source of L3 of another ceratopogonid-associated onchocercid, *O. gibsoni*.

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**Research Note**

**Taxonomy and Biology of *Mitotrema anthostomatum* Manter, 1963  
(Digenea: Cryptogonimidae) from Fishes of the Southern  
Great Barrier Reef, Australia**

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**ABSTRACT:** *Mitotrema anthostomatum* Manter, 1963 is redescribed from the serranid fishes *Epinephelus fuscoguttatus* and *Cromileptes altivelis* from the southern Great Barrier Reef, Australia. Distinctions of morphology and biology of these specimens in comparison with the original description are the presence of a uroproct and a distinctive lobe on the posterior margin of the ventral sucker. Metacercariae are recorded from the fish *Amphiprion akindynos*, *A. perideraion*, *Chaetodon aureofasciatus*, *C. pelewensis*, *Choerodon cyanodus*, *Chromis viridis*, *Dascyllus aruanus*, *Forcipiger flavissimus*, *Plectroglyphidodon dickii*, *Pomacentrus flavicauda*, *P. melanochir*, and *P. wardi*.

**KEY WORDS:** Digenea, Cryptogonimidae, *Mitotrema*, *Epinephelus*, *Cromileptes*, life cycle, Great Barrier Reef, Australia.

*Mitotrema anthostomatum* was described by Manter (1963) from a species of *Plectropomus* ("probably *maculatus*" according to Manter) from Fiji. Manter placed the genus in the subfamily Diplopharyngotrematinae, family Cryptogonimidae. Yamaguti (1971) transferred it to family Acanthostomidae, created the subfamily Mitotrematinae, and gave original figures of the terminal genitalia and proximal female system. The species was reported a second time by Gibson (in Lester and Sewell, 1989) from *Cromileptes altivelis* at Heron Island on the Great Barrier Reef. Here we report a new host record of adults and describe the metacercaria of this species.

All collections were made at the Heron Island and Wistari reefs on the southern Great Barrier Reef (23°27'S, 151°55'E). Trematodes were collected from freshly killed fish, fixed by pipetting them into nearly boiling 5% formalin, stained with Mayer's acid haemalum, cleared with methyl salicylate, and mounted in Canada balsam.

Drawings were made with the aid of a camera lucida. Measurements are quoted as range with mean in parentheses and are in micrometers. The following abbreviations are used: BM(NH), The Natural History Museum, London; QM, Queensland Museum, Brisbane; USNPC, United States National Parasite Collection, Beltsville, Maryland.

Over several years at Heron Island we have examined specimens of 15 species of Serranidae as follows: 18 *Cephalopholis boenak*, 5 *C. cyanostigma*, 19 *C. miniatus*, 1 *Cromileptes altivelis*, 6 *Diploprion bifasciatum*, 5 *Epinephelus cyanopodus*, 30 *E. fasciatus*, 2 *E. fuscoguttatus*, 1 *E. merra*, 13 *E. ongus*, 34 *E. quoyanus*, 1 *E. undulatostratus*, 34 *Plectropomus leopardus*, 1 *P. laevis*, and 1 *P. maculatus*. *Mitotrema anthostomatum* was found only in the 2 *E. fuscoguttatus* and the single *C. altivelis*.

***Mitotrema anthostomatum* Manter, 1963  
(Figs. 1–3, Table 1)**

**REDESCRIPTION** (based on 16 specimens; measurements in Table 1): Body very elongate, narrow. Forebody short, widest at level of ventral sucker. Hindbody narrows abruptly anteriorly and then widens gradually to level of gonads. Body surface covered with regular quincunxial array of closely packed minute spines, reach to posterior extremity. Oral sucker large, infundibuliform, with 6 muscular lobes (2 ventral, 2 lateral, 2 dorsal). Prepharynx long, narrow, with uniform breadth, wrinkled posteriorly. Pharynx subglobular. Esophagus short. Intestinal bifurcation immediately posterior to ventral sucker.



Table 1. Measurements of adult specimens of *Mitotrema anthostomatum* (in micrometers).

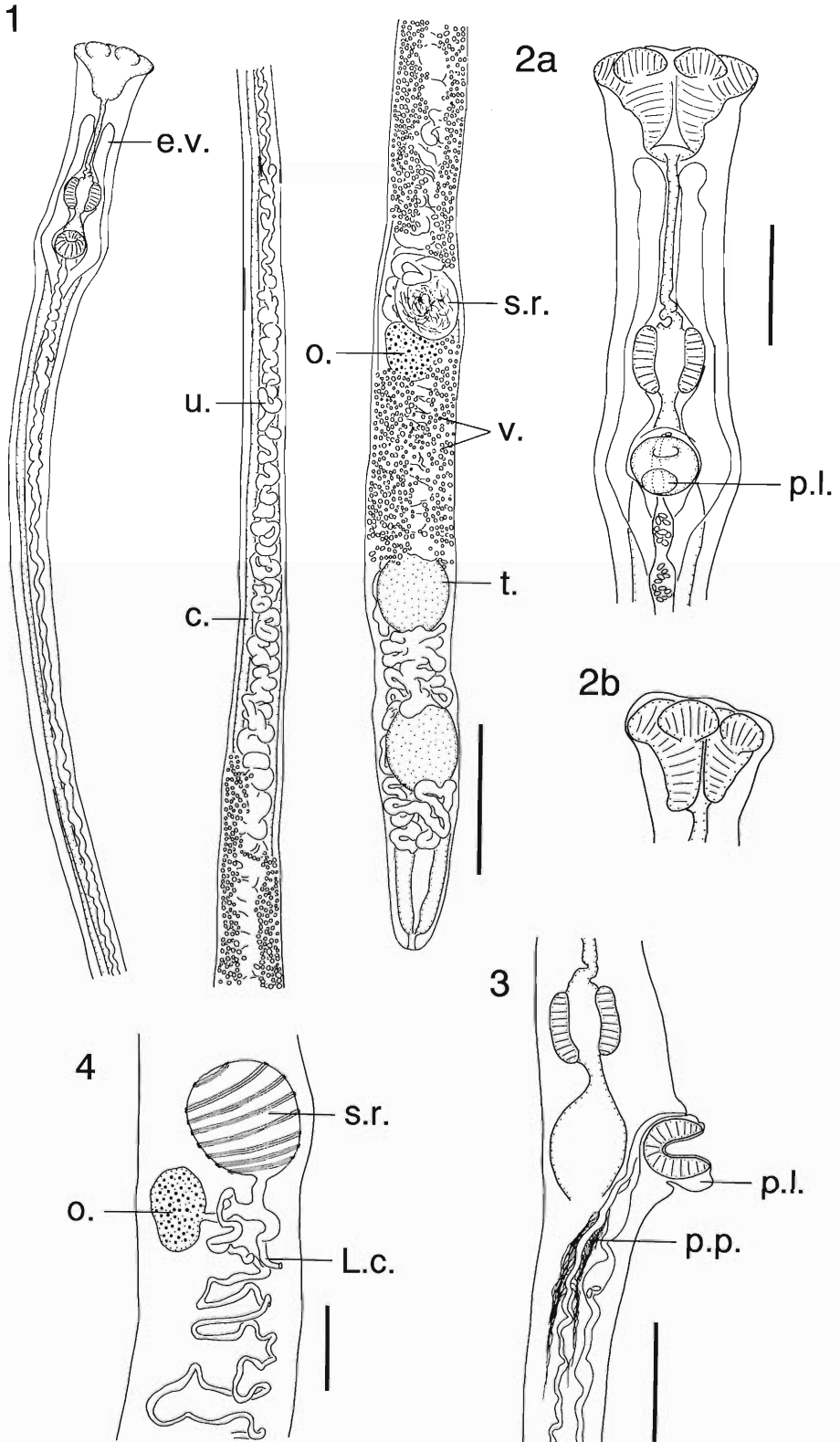
Host:	<i>Plectropomus</i> (prob) <i>maculata</i> (Manter, 1963) N = 5	<i>Cromileptes</i> <i>altivelis</i> (Queensland) N = 2	<i>Epinephelus</i> <i>fuscoguttatus</i> (Queensland) N = 5	Metacercaria (Queensland) N = 5
Length (L)	8,930–13,908	7,184–11,344	8,320–9,152	7,500–9,380
Width (W) VS	334–368	334–336	244–308	225–395
Width as % of L		3.23–4.65	2.67–3.09	2.63–4.21
Forebody (FB)	608–931	672–800	648–848	636–713
FB as % of L		7.05–9.35	7.08–8.56	6.94–9.08
Oral sucker L		154–173	193–225	148–218
W	241–368	199–205	212–302	180–218
Prepharynx L	268–335	212–244	244–308	231–308
Pharynx L	120–167	90–100	106–112	83–116
W	94–120	83–90	106–116	87–103
Esophagus L	30–40	26	19–58	
Ventral sucker L	114–154	83–96	100–116	90–96
W		96	106–116	96–103
Sucker width ratio	1:0.41–0.45	1:0.47–0.48	1:0.38–0.50	1:0.44–0.57
VS-ovary L (VS-Ov)		4,925–7,680	5,712–7,856	4,969–6,696
VS-Ov as % of L		68–69	66–70	66–73
Anterior extent of Vitellarium to Ov (Vit-Ov)		688–1,600	1,120–1,712	
Vit-Ov as % of VS-Ov		14–21	19–27	
Ovary L		167–244	183–250	87–116
W		148–225	148–199	58–148
Ov to anterior testis		352–912	464–768	443–571
Anterior testis L		212–353	263–385	176–212
W		212–321	225–295	119–177
Anterior testis to posterior testis		160–225	199–244	186–257
Posterior testis L		257–414	282–443	186–241
W		244–347	231–308	122–180
Posttesticular region L (PTR)	608–850	376–565	475–668	398–533
PTR as % of L		4.98–5.23	5.34–6.17	4.66–6.51
Eggs L × W	20–24 × 9–11	19 × 10	18–20 × 8–10	

Ceca may be distended in wide part of body at ventral sucker level, narrow in anterior hind-body, form uroproct at posterior extremity. Ventral sucker small, rounded, with distinctive lobe on its posterior margin (Figs 2a, 3).

Testes 2, subglobular to oval, tandem, separated by uterus, relatively close to posterior extremity. Seminal vesicle reaches from immediately anterior to seminal receptacle, initially relatively broad tubular, narrows abruptly passing through thicker-walled narrow duct into relatively broad saccular portion before narrowing to become very long, narrow, sinuous duct reaching to just posterior to ventral sucker; unites with metraterm at about level of posterior edge of ventral sucker. Pars prostatica at anterior end of seminal vesicle, indistinctly defined except for openings of prostate cells (Fig. 3); prostate cells extensive along at least 250  $\mu\text{m}$  of length of seminal vesicle. Genital sinus long, narrow, runs over

dorsal surface of ventral sucker. Genital pore median, immediately anterior to ventral sucker.

Ovary subglobular to oval, pretesticular, separated from testes by uterus, very distant from ventral sucker; oviduct leaves dorsal surface. Seminal receptacle subglobular, as large as or much larger than ovary, immediately antero-dorsal to ovary; spiral bands of thickened muscle in wall. Mehlis' gland prominent, dorsal to ovary. Laurer's canal short, opens dorsally to ovary, surrounded by gland cells, shares short common duct with seminal receptacle. Uterus passes posteriorly from ovary, filling space between ovary and testes, between testes and posttesticular region, then passes forward with subregular lateral slings in posterior half of ovary–ventral sucker region and narrow, undulating course distally; metraterm not differentiated. Eggs small, numerous; weakly tanned in region of uterus between ovary and posterior extremity; tanning in-



tensity (as seen by change of color from yellow to brown) increases from posterior extremity to about posterior testis; eggs in single file in distal narrow part of uterus. Vitelline follicles small, numerous; in two separated fields from seminal receptacle anteriorly for about one fifth of ovary-ventral sucker distance; second field from ovary to anterior testis; field continuous around dorsal surface of worm.

Excretory vesicle Y-shaped; bifurcation just posterior to posterior testis; arms passing ventral to intestinal ceca, extending to near oral sucker. Uroproct terminal.

**MATERIAL EXAMINED** (all Heron Island):

*Cromileptes altivelis* 2, Jan. 1991; *Epinephelus fuscoguttatus* 10, including one set of transverse sections, Jan. 1992; 4, including two sets of sagittal sections, Jan. 1993.

**SITE OF INFECTION:** Small intestine.

**SPECIMENS DEPOSITED:** Queensland Museum QM G 211566-211572; BM(NH) 1980.2.25.1-15; USNPC 84996.

#### **Metacercaria** (Figs. 4, 5)

Dissections of a wide range of small fish at Heron Island revealed metacercariae clearly attributable to *M. anthostomatum* in 12 species from 3 families. The metacercariae are tightly coiled in near-spherical cysts in the musculature.

**MATERIAL EXAMINED** (all from Heron Island):

Chaetodontidae—*Chaetodon aureofasciatus* 1, Jan. 1991; *C. pelewensis* 5, Jan. 1991; *Forcipiger flavissimus* 1, Jan. 1991. Pomacentridae—*Amphiprion akindynos* 6, Jan. 1991; *A. perideraion* 2, Jan. 1991; *Chromis viridis* 1, Jan. 1991; *Dascyllus aruanus* 1, Jan. 1991; *Plectroglyphidodon dickii* 1, Jan. 1991; *Pomacentrus flavicauda* 1, Mar. 1985; *P. melanochir* 3, Mar. 1985; *P. wardi* 1, 1985; 3, Mar. 1985. Labridae—*Choerodon cyanodus* 2, Nov. 1989.

**SPECIMENS DEPOSITED:** QM G 211573-211583.

**DESCRIPTION** (measurements in Table 1):

Body in adult form and near adult size. Di-

gestive system fully developed. Reproductive system fully developed except for absence of vitelline follicles. Egg-forming complex distinguishable except for vitelline reservoir. Spiral muscular thickening on seminal receptacle distinctive and far more prominent than in adult.

We here identify the present specimens as *Mitotrema anthostomatum*. In comparisons between our specimens and the description and figures given by Manter (1963) and from examination of a paratype specimen (USNM No. 59860) we found no significant differences except in a few details which Manter appears to have overlooked (presence of a uroproct and the distinctive lobe on the posterior margin of the ventral sucker). Nevertheless, this identification is made with some reservations. Notes of Professor H. W. Manter given to Professor J. C. Pearson indicate that he collected specimens of *Mitotrema* from *Cromileptes altivelis* when he visited Australia in 1963 and that he considered them distinct from *M. anthostomatum*. Although we can find no basis for this view ourselves, Manter obviously had access to the original specimens, so his opinion is worth noting.

We prefer to recognize *Mitotrema* within the Cryptogonimidae because we can find no strong basis for the recognition of the Acanthostomidae as a separate family.

Two specimens of *Cromileptes altivelis* (ours and that of Dr D. I. Gibson of The Natural History Museum, London) and 2 specimens of *Epinephelus fuscoguttatus* examined from Heron Island on the southern Great Barrier Reef were infected with *Mitotrema anthostomatum*. This is in contrast to the 168 specimens of 13 other species of Serranidae from the same location that were not infected with this species. All these serranids are carnivores and presumably most eat at least some of the wide range of pomacentrids and chaetodontids that we have identified as the intermediate host of this parasite. Therefore, this parasite appears to have high host-specificity for its definitive hosts even though *Cromileptes altivelis* and *Epinephelus fuscoguttatus* are not

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Figure 1. *Mitotrema anthostomatum*, adult, ventral. Scale bar = 0.5 mm. Abbreviations: c. = cecum, e.v. = excretory vesicle, o. = ovary, s.r. = seminal receptacle, v. = vitelline follicles, t. = testis, u. = uterus.

Figures 2-4. 2a. Oral sucker and terminal genitalia, ventral. 2b. Oral sucker, lateral. Scale = 0.2 mm. 3. Terminal genitalia, lateral. Scale = 0.2 mm. 4. Egg-forming complex of metacercaria. Scale bar = 0.1 mm: Abbreviations: L.c. = Laurer's canal, o. = ovary, p.l. = posterior lobe, p.p. = pars prostatica, s.r. = seminal receptacle.

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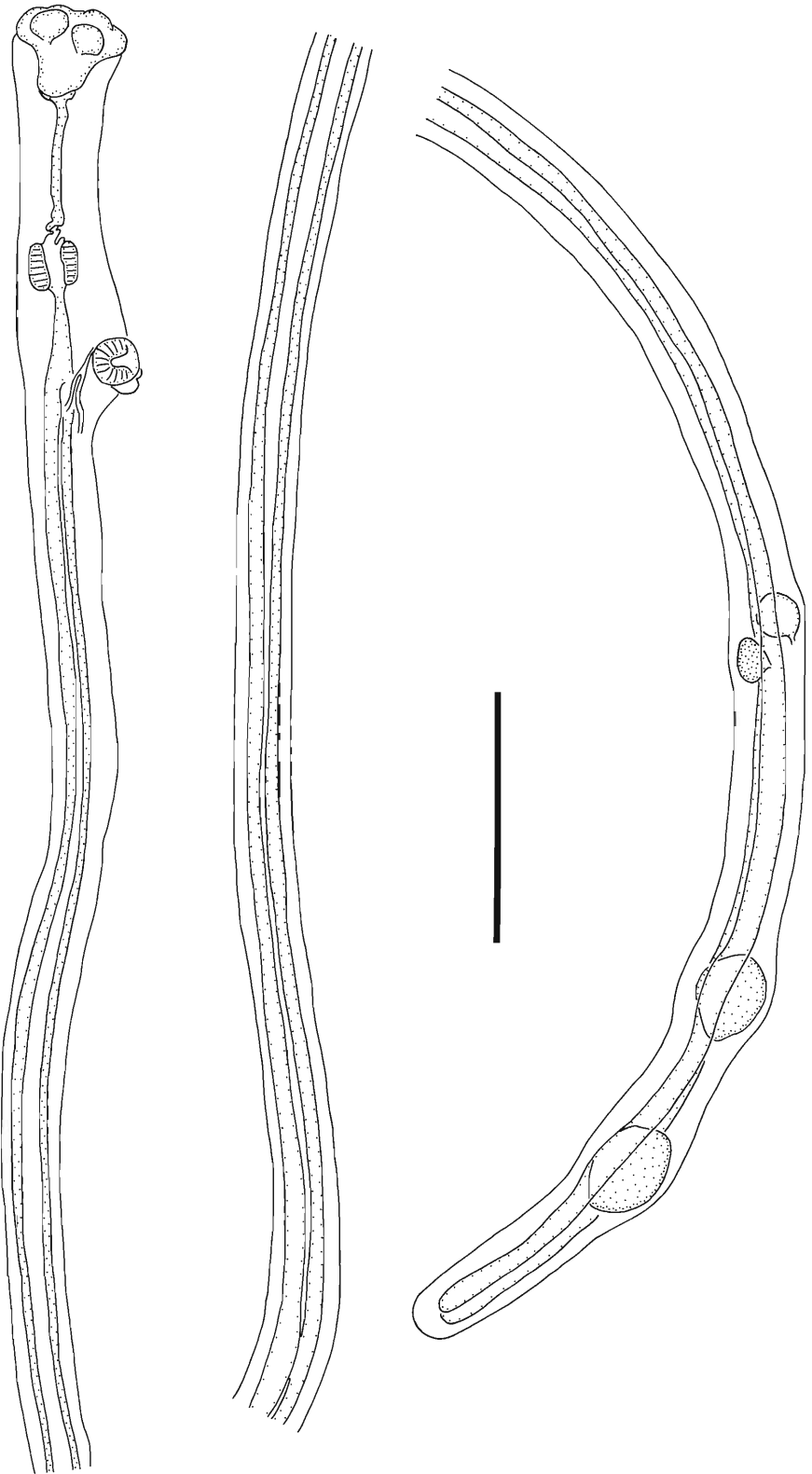


Figure 5. Metacercaria of *Mitotrema anthostomatum*. Scale bar = 0.5 mm.

closely related. This implies that the host-specificity is physiological rather than ecological. This in turn raises a problem because Manter recorded his Fijian specimens from a *Plectropomus* sp. ("prob *maculatus*") whereas our examinations of 36 specimens of 3 species of *Plectropomus* at Heron Island have not revealed any specimens of *Mitotrema* although they are undoubtedly exposed to infection. Three possible explanations present themselves: 1) the Fijian and Queensland specimens are in fact different species, 2) the same species has different host-specificity at the 2 locations, and 3) Manter recorded the identity of the host wrongly. We see no particular evidence to support any of these explanations and believe that the matter must remain unresolved for the present.

We thank Trudy Wright for assistance in the laboratory and Glenn Anderson for help in the field. This work was supported by grants from the Australian ARC and ABRS and from the Heron Island Research Station.

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#### Research Note

### Immature *Polyacanthorhynchus rhopalorhynchus* (Acanthocephala: Polyacanthorhynchidae) in Venton, *Hoplias malabaricus* (Pisces) from Moca Vie River, Bolivia, with Notes on its Apical Organ and Histopathology

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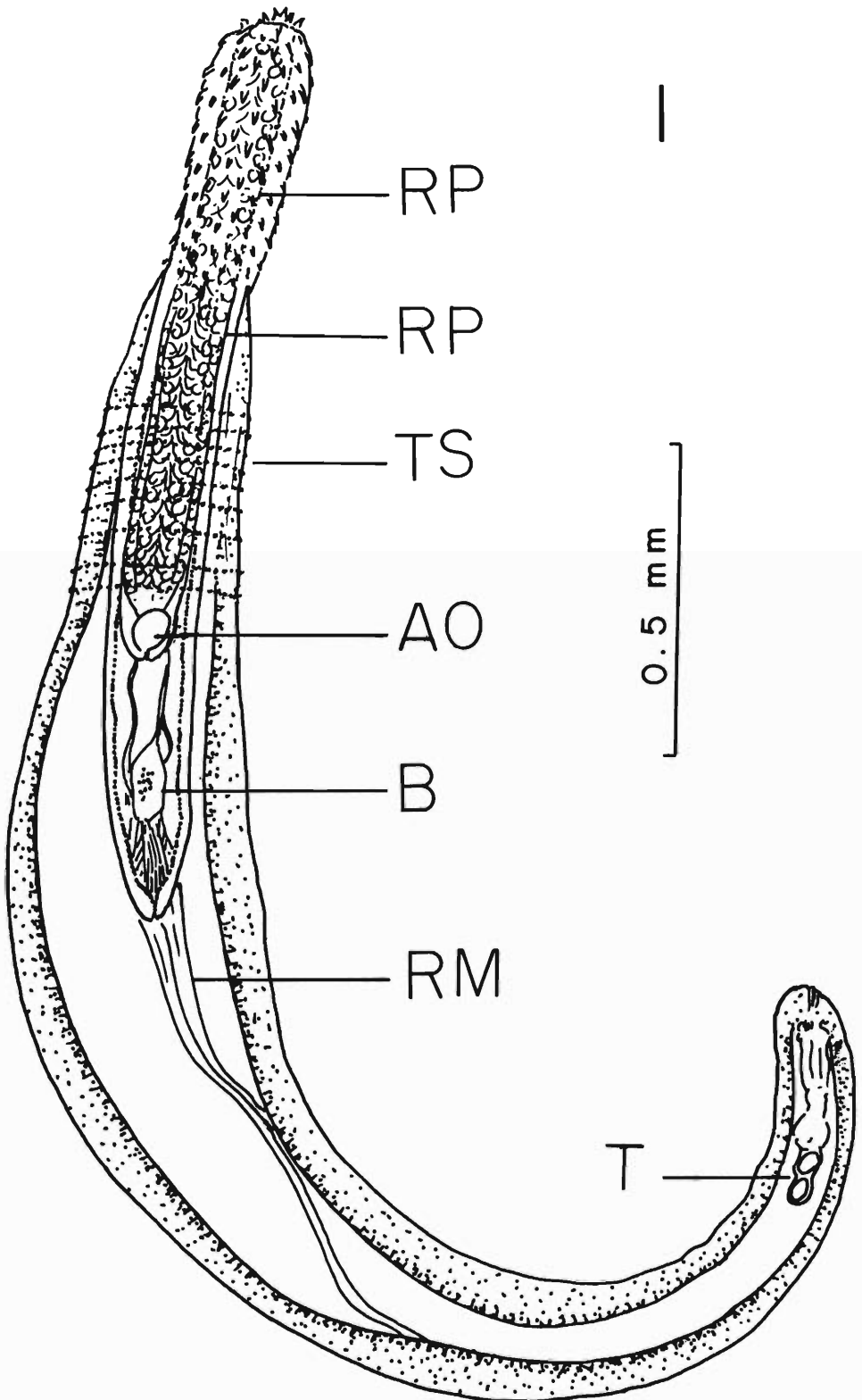
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**ABSTRACT:** Cystacanths of *Polyacanthorhynchus rhopalorhynchus* (Diesing, 1851) Travassos, 1920 were discovered in the viscera of venton, *Hoplias malabaricus* (Bloch, 1794) collected from the Moca Vie River at Las Palquitas, Bolivia. Apical proboscis organ and general morphological features were similar to but less developed than those of *Polyacanthorhynchus kenyensis* Schmidt and Canaris, 1967 cystacanths from Africa. Cystacanths of *P. rhopalorhynchus* were not deeply embedded in liver tissue. A collagenous connective tissue capsule surrounded and attached the cystacanths to the liver surface. Necrosis of hepatocytes and subsequent inflammatory response were observed near encapsulated acanthocephalans. Cystacanths attached to the intestine were encapsulated in the fibroserosa.

**KEY WORDS:** *Polyacanthorhynchus rhopalorhynchus*, Acanthocephala, cystacanths, *Hoplias malabaricus*, paratenic host, apical organ, histopathology, Bolivia.

Amin (1987) recently erected a new order, Polyacanthorhynchida, and a new class, Polyacanthocephala, for the monogeneric family Polyacanthorhynchidae. Adults of 3 of the 4 known species of the genus *Polyacanthorhynchus* Travassos, 1920 infect South American caimans (Alligatoridae). These species are *Polyacanthorhynchus macrorhynchus* (Diesing, 1856) Travassos,



1920 (genotype); *Polyacanthorhynchus caballe-roi* Diaz-Ungria and Rodrigo, 1960; and *Polyacanthorhynchus rhopalorhynchus* (Diesing, 1856) Travassos, 1920. Immatures are unknown. The fourth species, *P. kenyensis*, is known only from the cystacanth stage infecting freshwater fishes in Kenya and was originally reported by Baylis (1928). Schmidt and Canaris (1967) later described it as *Polyacanthorhynchus kenyensis*. That description was expanded and amended by Amin and Dezfuli (1995).

The original brief description of *P. rhopalorhynchus* was amended by Machado Filho (1947), and revised by Diaz-Ungria and Rodrigo (1960). Machado Filho (1947) and Yamaguti (1963) reported adults from various species of caimans of the genera *Arapaima* and *Caiman* in Brazil. This is the first report of immatures of any of the South American species of *Polyacanthorhynchus* in a fish paratenic host.

Twenty-seven venton, *Hoplias malabaricus* (Bloch, 1794) (17 males, 10 females) were captured with hand-held seines from the Moca Vie River at Las Palguitas near Trinidad-Bolivia jungles (latitude 14.8°S, longitude 64.8°W) on 1–8 June 1993. Fish weighed 85–361 gm (mean 148) and measured 168–262 mm (192) in standard length. Liver surface and intestinal peritoneum of 13 fishes (50%) were parasitized by 28 *P. rhopalorhynchus* cystacanths (1–4 per infected fish). Thirteen worms (10 males, 3 females) were processed for microscopical study and the remaining specimens were sectioned in situ for histopathological observations using the methods of Amin and Heckmann (1991). All measurements (range followed by mean in parentheses) are in  $\mu\text{m}$  unless otherwise specified. The proboscis length was calculated from the length of the everted and the inverted portions; all proboscides were partially invaginated.

The partial retraction of the club-shaped proboscis did not obscure the presence of an apical organ at its inverted anterior tip (Fig. 1). The retraction, however, made it impossible to count or measure proboscis hooks, and caused the posterior displacement of the well-developed brain. The number of proboscis hook rows was 16–18 posteriorly. Hooks gradually decreased in size

posteriorly; all had simple roots that are relatively shorter than blades and directed posteriorly. Anterior cuticular trunk spines are in 8–10 circles with about 34–40 minute spines each. Lemnisci not pronounced. Reproductive system poorly developed in both sexes and occupies narrow posterior end of trunk; genital pores terminal.

Males ( $N = 10$ ) (Fig. 1) 1.985–2.978 mm (2.552) long by 397–662 (480) wide; proboscis 1.430–1.690 mm (1.506) long by 169–208 (186) wide; proboscis receptacle 0.611–1.105 mm (0.848) long by 130–195 (184) wide; testes 26–39 (32) long by 26–40 (33) wide.

Females ( $N = 2$ ) 2.445–2.813 mm (2.629) long by 430–496 (463) wide; proboscis 1.196–1.430 mm (1.313) long by 169–208 (188) wide; proboscis receptacle 780–845 (813) long by 156–195 (175) wide.

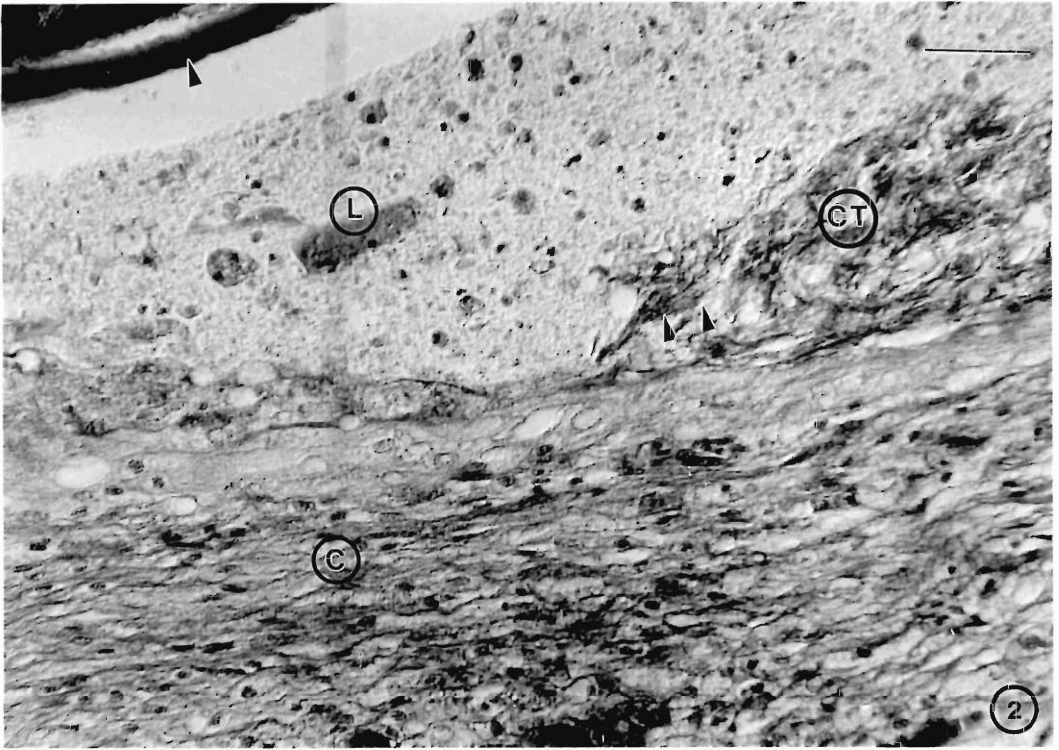
The general morphological features of the reported material were rather similar to but less developed than those of *P. kenyensis* cystacanths as reported by Amin and Dezfuli (1995). The size of the South American specimens is extremely small compared to the size of adult males and females (40–55 mm and 50–70 mm long, respectively, according to Machado Filho, 1947). Worms must undergo considerable growth once infected paratenic hosts are eaten by caimans.

Only cystacanths of *P. rhopalorhynchus* were found in the viscera of *H. malabaricus*. Host intestine was free from parasites. De Fabio (1982, 1983), however, reported nematode and acanthocephalan parasites, respectively, from the gut of the same host species near Campos, State of Rio de Janeiro, Brazil. The acanthocephalan parasites included *Quadrigyrus machadoi* De Fabio, 1983 and *Neoechinorhynchus macronucleatus* Machado Filho, 1954 from the intestine, and undetermined “echinorhynchis” cystacanths from the body cavity. The latter specimens do not belong to *Polyacanthorhynchus* because they are flask-shaped with short proboscides, long necks, and anterior trunk spines that are not organized in regular circles.

An apical organ was noted at the anterior end of the inverted proboscis (Fig. 1) of all worms. A similar structure was described in cystacanths

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Figure 1. Male *Polyacanthorhynchus rhopalorhynchus* cystacanth from *Hoplias malabaricus* body cavity. Abbreviations: AO = apical organ, B = brain, RM = retractor muscles, RP = retracted portion of proboscis, T = testes; TS = circles of trunk spines.



**Figure 2.** Cystacanth (arrow) invading host liver tissue (L). Necrosis of hepatocytes (double arrow) is evident with pycnotic nuclei invasion of connective tissue (CT). Capsule (C) of host liver is inflamed; many granulocytes and lymphocytes present. Measurement bar = 100 um.

**Figure 3.** Cystacanth (arrow) encapsulated on the surface of the fibroserosa of host intestine. Thin capsule (C) has formed around the immature acanthocephalan. Measurement bar = 100 um.



of *P. kenyensis* by Amin and Dezfuli (1995). However, no apical structures were observed in adults of any of the 3 South American species of the same genus. The original descriptions contained detailed illustrations of the proboscis. It is suggested that the apical structure in *P. rhopalorhynchus* cystacanths functions in secreting materials to aid in gut-wall penetration and invasion of body cavity tissues of the paratenic host. This is in agreement with the observations of Amin and Dezfuli (1995) on *P. kenyensis*.

The cystacanths appear to invade the surface of the liver, migrate under the connective tissue capsule, and cause necrosis of the surface hepatocytes (Fig. 2). There is an initial host tissue inflammatory response noted by granulocyte and lymphocyte aggregation at the site. Minimal hemorrhaging of damaged host tissue was observed. Pycnotic nuclei are observed with progression of cell necrosis (Fig. 2). Fibroblasts form a dense collagenous connective tissue capsule around the acanthocephalans. The capsule is infiltrated with lymphocytes and fat cells, and hypertrophy of connective tissue fibroblasts is also noted. The outer capsule surrounding the cystacanths represents an attempt by the host to isolate the organism from the organ (Fig. 2). The majority of the cystacanths were attached to the liver surface by a thin capsule. No acanthocephalans were "free" in the abdominal cavity of *H. malabaricus*. Those infecting the small intestine were attached to the surface of the fibroserosa with a thin layer of collagenous connective tissue surrounding the immature worms (Fig. 3)

**SPECIMENS:** Eight *P. rhopalorhynchus* cystacanths have been deposited in the University of Nebraska State Museum, Lincoln, Nebraska, Harold W. Manter Laboratory Coll. No. 38395.

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Research Note

Helminths of the Sonoran Green Toad, *Bufo retiformis* (Bufonidae),  
from Southern Arizona

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**ABSTRACT:** The gastrointestinal tracts, lungs, and urinary bladders from 49 *Bufo retiformis* were examined. Five species of nematodes, *Aplectana incerta*, *Aplectana itzocanensis*, *Oswaldocruzia pipiens*, *Physaloptera* sp. (larva), *Rhabdias americanus*, and one cestode, *Distoichometra bufonis*, were present. *Aplectana incerta* had the highest prevalence (61%) and greatest mean intensity (36.9). All represent new host records for *B. retiformis* but are previously known from other southwestern desert toads.

**KEY WORDS:** Cestoda, *Distoichometra bufonis*, Nematoda, *Aplectana incerta*, *Aplectana itzocanensis*, *Oswaldocruzia pipiens*, *Physaloptera* sp. (larva), *Rhabdias americanus*, Bufonidae, *Bufo retiformis*.

The Sonoran green toad, *Bufo retiformis* Sanders and Smith, 1951, occurs from south-central Arizona to west-central Sonora, Mexico, at elevations of 150 to 730 m (Stebbins, 1985). There are, apparently, no reports of helminths from *B. retiformis*. The purpose of this note is to report the prevalences and mean intensities of helminth parasites from a southern Arizona population of *B. retiformis* and to compare this helminth fauna with that of other North American southwestern desert toads.

Forty-nine *B. retiformis* (mean snout-vent length [SVL] = 49.4 mm ± 4.3 [SD]) (14 females, 35 males) were hand-collected in Pima County, Arizona at 516–969 m elevation during July–August. One toad was from 1992, 30 were from 1993, and 18 were from 1994. They came from several localities along Indian Routes 15 and 34 and Arizona Highways 85, 86, and 286, at approximately 32°14'–25'N, 112°1'–44'W. Toads were killed by immersion in 0.5% solution of 3-aminobenzoic acid ethyl ester (Sigma, St. Louis, Missouri) and were fixed in neutral-buffered 10% formalin.

The body cavity was opened by a longitudinal

incision from vent to throat and the gastrointestinal tract was removed by cutting across the anterior esophagus and rectum. The lungs, esophagus, stomach, small intestine, large intestine, and bladder of each toad were examined separately. Each helminth was identified utilizing the glycerol wet-mount procedure. Cestodes were stained with hematoxylin and mounted in balsam. Specimens were deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705: *Distoichometra bufonis* 84533, *Aplectana incerta* 84534, *Aplectana itzocanensis* 84535; *Oswaldocruzia pipiens* 84536, *Physaloptera* sp. (larva) 84537, and *Rhabdias americanus* 84538. All toads were deposited in the herpetology collection at Arizona State University, Tempe as ASU 30260–30266, 30268–30294, and 30296–30310.

Prevalence, location, and mean intensity for each parasite are given in Table 1. Terminology is in accordance with Margolis et al. (1982). There were no statistical differences in infection prevalences between female and male *B. retiformis*. *Distoichometra bufonis* was previously found in Arizona *Bufo cognatus*, *Scaphiopus couchii*, and *Bufo punctatus* (Goldberg and Bursey, 1991a, b) and New Mexico *B. cognatus*, *Bufo debilis*, and *Spea multiplicata* (Goldberg et al., 1995) and has also been reported in *Bufo terrestris*, *Bufo woodhousii*, and *Scaphiopus* sp. (Douglas, 1958; McAllister et al., 1989). *Distoichometra bufonis* was originally described from *Bufo terrestris* (= *lentiginosus*) by Dickey (1921).

*Aplectana incerta* and *A. itzocanensis* present an enumeration problem. While the adults are easily separated (*A. incerta* females have approximately 50 eggs, 99–123 × 54–62 μm; males have equal spicules, 135–143 μm. *Aplectana it-*

Table 1. Prevalence, mean intensity (range), and location of helminths from 49 *Bufo retiformis*.

Parasite	Prevalence (%)	Mean intensity (range)	Location*
Cestoda			
<i>Distiochometra bufonis</i>	27	1.7 (1-6)	b
Nematoda			
<i>Aplectana incerta</i>	61	36.9 (1-153)	b, c, e
<i>Aplectana itzocanensis</i>	57	23.1 (1-89)	b, c, e
<i>Oswaldocruzia pipiens</i>	2	18.0	a, b
<i>Physaloptera</i> sp. (larva)	2	1.0	a
<i>Rhabdias americanus</i>	35	3.3 (1-7)	d

\* a = stomach, b = small intestine, c = large intestine, d = lungs, e = bladder.

*itzocanensis* females have several hundred eggs, 70–82 × 42–51 μm; males have equal spicules, 172–203 μm, immature forms are not easily distinguished and here they have arbitrarily been separated based on the ratio of adults per host. *Aplectana incerta* was originally described by Caballero y C. (1949) from *Bufo marinus* from Chiapas, Mexico, and *A. itzocanensis* was originally described by Bravo Hollis (1943) from *S. multiplicata* from Puebla, Mexico. Both species were redescribed by Baker (1985). *Aplectana incerta* has been reported from Arizona *S. couchii* (Goldberg and Bursey, 1991a), and New Mexico *B. debilis* and *S. multiplicata* (Goldberg et al., 1995). *Aplectana itzocanensis* has been reported from Arizona *Bufo alvarius*, *B. cognatus*, and *B. punctatus* (Goldberg and Bursey, 1991a, b) and New Mexico *B. cognatus*, *B. debilis*, and *S. multiplicata* (Goldberg et al., 1995) and is also known from *B. marinus* and *B. woodhousii* (Brenes and Bravo Hollis, 1959; Caballero Deloya, 1974; Baker, 1985). In our current sample, only 1 *B. retiformis* was not infected with *Aplectana*. Simultaneous infections by *A. incerta* and *A. itzocanensis* were found on 10 occasions, infection by only *A. incerta* was found on 20 occasions, and infection by only *A. itzocanensis* was found on 18 occasions.

*Oswaldocruzia pipiens* was harbored by a single *B. retiformis*. It has been reported from Arizona *B. alvarius*, *B. cognatus*, *B. punctatus*, and *S. couchii* (Goldberg and Bursey, 1991a, b) and is also known from *Bufo americanus*, *Bufo houstonensis*, *B. woodhousii*, and *Scaphiopus holbrookii* (Brandt, 1936; Rankin, 1945; Campbell, 1968; Ashton and Rabalais, 1978; Baker, 1978a; Thomas et al., 1984).

Larval physalopterans have been reported from Arizona *B. alvarius* and *B. cognatus* (Goldberg

and Bursey, 1991a) and New Mexico *B. cognatus*, *B. debilis*, and *S. multiplicata* (Goldberg et al., 1995) and are also known from *B. americanus*, *Bufo microscaphus*, *Bufo speciosus* (= *compactilis*), and *B. woodhousii* (Kuntz, 1940; Parry and Grundmann, 1965; Ashton and Rabalais, 1978). Apparently, no cases of parasitism of toads by adult physalopterans have been reported.

Hosts for *R. americanus* include Arizona *B. alvarius* and *B. cognatus* (Goldberg and Bursey, 1991a), New Mexico *B. cognatus* and *B. debilis* (Goldberg et al., 1995), and also *B. americanus*, *B. woodhousii* (= *fowleri*), and *B. speciosus* (Kuntz, 1940; Reiber et al., 1940; Fantham and Porter, 1948; Campbell, 1968; Baker, 1978b; Williams and Taft, 1980).

North American southwestern desert toads appear to share a common helminth community: 1 monogenean species, *Pseudodiplorchis americanus* (see Tinsley, 1990); 2 cestode species, *D. bufonis* and *Nematotaenia dispar*; and 6 nematode species, *A. incerta*, *A. itzocanensis*, *O. pipiens*, *Physaloptera* sp. (larva), *Physocephalus* sp. (larva), and *R. americanus* (Goldberg and Bursey, 1991a, b; Goldberg et al., 1995). Subsequent examination of additional toad species will be required before this helminth community is completely known.

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Research Note

A New Host and Geographic Record for *Myxidium lesminteri*  
(Protozoa: Myxosporae) from *Tomopterna cryptotis*  
(Amphibia: Ranidae), in Namibia, South-West Africa

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**ABSTRACT:** A new host and geographic distribution record is reported for the myxosporae, *Myxidium lesminteri* Delvignier, Markus, and Passmore, 1992, from the stripe-burrowing frog, *Tomopterna cryptotis* (Ranidae) in Namibia, South-West Africa. One of 4 (25%) *T. cryptotis* was found to be harboring trophozoites and spores of *M. lesminteri* in the gall bladder. The species was originally described from a congeneric ranid, *Tomopterna krugerensis*, and also from *Bufo garrmani* (Bufonidae) and *Heleophryne natalensis* (Heleophrynidae) in South Africa.

**KEY WORDS:** Amphibia, Anura, *Myxidium lesminteri*, Myxosporae, Protozoa, Ranidae, spore, survey, trophozoite.

The gall bladder myxosporae, *Myxidium lesminteri* Delvignier, Markus, and Passmore, 1992, was recently described from 3 species of anurans from South Africa (Delvignier et al., 1992). During a survey of African amphibians for protozoan and helminth parasites, we found a myxosporae matching the description of *M. lesminteri* in a ranid frog from Namibia. Herein, we provide a new host and distributional record for the parasite.

During April 1992, 4 adult striped-burrowing frogs, *Tomopterna cryptotis* Boulenger, 1907, were collected by hand by P.S.F. from 2 sites in Namibia. One site was located in the Keetmanshoop District, 47.2 km S Keetmanshoop at the Löwen River (20°00'S, 18°00'E) and the other was in the Owambo District, 35 km W Oshakati (17°47'S, 16°05'E). Frogs were killed within 24 hr of capture by pithing and whole gall bladders were removed, preserved in 10% formalin, and temporarily stored in individual vials. Gall bladder contents were emptied onto microscopic slides for examination under low magnification. Detailed methods of fixation and staining follow those of McAllister et al. (1995). Specimens were identified based on the morphology of the trophozoite and spore. Cursor measurements of

trophozoites and spores were made with a calibrated ocular micrometer and are reported as means in micrometers ( $\mu\text{m}$ ) followed by the ranges in parentheses.

Voucher specimens of *T. cryptotis* were deposited in the Carnegie Museum of Natural History, Pittsburgh, Pennsylvania (CM). A voucher slide of *M. lesminteri* was deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705 as USNPC 84491.

One of 4 *T. cryptotis* (adult, 45 mm snout-vent length, CM 130226) from the Oshakati site was infected with a myxozoan matching the description of *M. lesminteri*. Trophozoites were ovoidal and floated freely in bile contents or adhered to gall bladder epithelium and measured 750 (500–1,000)  $\mu\text{m}$  in diameter ( $N = 10$ ). Ovoidal spores were visible in the medullary zone or endoplasm of trophozoites and measured (length  $\times$  width) 12.0 (10.0–14.0)  $\times$  6.5 (5.5–8.0)  $\mu\text{m}$  ( $N = 10$ ). Although there is size variation of trophozoites and spores among anuran hosts with *Myxidium* spp. (Kudo and Sprague, 1940; Kudo, 1943; Clark and Shoemaker, 1973; Clark, 1982; Delvignier, 1986; Delvignier et al., 1992; McAllister and Trauth, 1995; McAllister et al., 1995), our measurements accord well with those previously reported for *M. lesminteri* (see Delvignier et al., 1992). On closer examination, spores were without striations and contained 2 spheroidal polar capsules at each pole, features typical of *M. lesminteri* (Delvignier et al., 1992).

Delvignier et al. (1992) surveyed 409 anurans representing 50 species within 9 families from South Africa and Swaziland for gall bladder myxozoans. The authors found a very low overall prevalence of infection with *M. lesminteri*, as only 3 of 409 (0.7%) frogs harbored the parasite, including 1 of 7 (14%) sand frogs, *Tomopterna krugerensis* Passmore and Carruthers, 1 of 20

(5%) Garman's square-marked toads, *Bufo garmani* Meek, and 1 of 2 (50%) Natal ghost frogs, *Heleophryne natalensis* Hewitt, from the Transvaal region of South Africa. The new geographic locale noted herein for *M. lesminteri* is approximately 1,600 km NW of previous locales for *M. lesminteri* reported by Delvinqvier et al. (1992).

In summary, we add a fourth host species for this parasite, as *Myxidium lesminteri* is now reported from a bufonid, a heleophrynid, and 2 ranids from southern Africa. To date, 11 species of *Myxidium* have been reported from Africa. Fantham (1930) reported 3 species of *Myxidium* from South African saltwater fishes and Dubina and Isakov (1976) provided a description of *Myxidium gigantissimum* from a bathial fish collected off the South African coast. In other parts of Africa, Fomena and Bouix (1986) described 5 species of *Myxidium* from freshwater fishes in Cameroon and Okaeme et al. (1988) reported a *Myxidium* sp. in a Nigerian fish. However, to our knowledge, *M. lesminteri* is the first species of *Myxidium* known from Namibia in South-West Africa.

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Research Note

Gastrointestinal Helminths of the Anole *Anolis oculatus* (Polychridae) from Dominica, Lesser Antilles

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**ABSTRACT:** The gastrointestinal tracts of 20 *Anolis oculatus* from Dominica, West Indies were examined for helminths. Eight helminth species were present: *Ascarops* sp., *Oswaldocruzia lenteixeirai*, *Parapharyngodon cubensis*, *Physaloptera* sp., *Spauligodon cubensis*, *Spinicauda spinicauda*, *Mesocoelium monas*, and *Centrorhynchus* sp. Juvenile acanthocephalans (*Centrorhynchus* sp.) had the greatest prevalence (50%). *Spinicauda spinicauda* had the highest mean intensity (33.5). All are new host records for *A. oculatus*.

**KEY WORDS:** Polychridae, *Anolis oculatus*, *Mesocoelium monas*, *Ascarops* sp., *Oswaldocruzia lenteixeirai*, *Parapharyngodon cubensis*, *Physaloptera* sp., *Spauligodon caymanensis*, *Spinicauda spinicauda*, *Centrorhynchus* sp., prevalence, intensity.

*Anolis oculatus* Cope, 1879 is restricted to the island of Dominica, Lesser Antilles, where it occurs from sea level to about 914 m and is sympatric with 2 frogs, *Eleutherodactylus martinicensis* and *Leptodactylus fallax*; 1 turtle, *Geochelone carbonaria*; 4 lizards, *Gymnophthalmus pleei*, *Sphaerodactylus fantasticus*, *Sphaerodactylus vincenti*, and *Ameiva fuscata*; and 4 snakes, *Typhlops dominicana*, *Alsophis antillensis*, *Boa constrictor*, and *Liophis juliae* (Schwartz and Henderson, 1991). There are, apparently, no published accounts of helminths from *A. oculatus*. The purpose of this note is to report the helminths of *A. oculatus* from Dominica as part of an ongoing study of the biogeography of helminths in the Caribbean herpetofauna.

Twenty *A. oculatus* from Dominica (15°30'N, 16°20'W) were borrowed from the Division of Herpetology, Florida Museum of Natural History, University of Florida: (Saint Andrew Parish: UF 43568, 43569; Saint George Parish: UF 15811, 15819, 15822, 15824; Saint Patrick Parish: UF 15827–15830, 15833, 15835, 15837, 15839, 15845, 15847, 15849, 15850, 15852, 15854). Saint George Parish and Saint Patrick Parish specimens were collected in 1963; Saint Andrew Parish specimens are from 1977. The sample had a mean snout–vent length (SVL) of

65.8 mm ± 11.5 (SD) (range 47–84 mm) and consisted of 12 males (mean SVL = 73.4 mm ± 7.7 [SD]) and 8 females (mean SVL = 54.4 mm ± 3.9 [SD]). The mean SVLs of males and females are significantly different (Kruskal-Wallis statistic = 11.78, 1 df,  $P < 0.001$ ).

The body cavity was opened by a longitudinal incision from throat to vent and the gastrointestinal tract was removed by cutting across the anterior esophagus and rectum. The esophagus, stomach, small intestine, and large intestine were examined separately under a dissecting microscope. Helminths were removed and placed in a drop of undiluted glycerol on a glass slide. A coverslip was added and each helminth was allowed to clear before it was identified under a compound microscope. Terminology usage is in accordance with Margolis et al. (1982).

Six species of nematodes (*Ascarops* sp. as encysted larvae; *Oswaldocruzia lenteixeirai* Viguera, 1938; *Parapharyngodon cubensis* (Barus and Coy Otero, 1969) Barus, 1973; *Physaloptera* sp. as larvae; *Spauligodon caymanensis* Bursey and Goldberg, 1995; *Spinicauda spinicauda* (Olfers, 1819) Travassos, 1920), 1 trematode species (*Mesocoelium monas* (Rudolphi, 1819)), and 1 species of Acanthocephala (*Centrorhynchus* sp. [juveniles]) were found in *Anolis oculatus* (Table 1). All represent new host records. Specimens were placed in vials of alcohol and deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705: *Mesocoelium monas* (84469), *Ascarops* sp. (84471), *Oswaldocruzia lenteixeirai* (84470), *Parapharyngodon cubensis* (84468), *Physaloptera* sp. (84474), *Spauligodon caymanensis* (84473), *Spinicauda spinicauda* (84475), *Centrorhynchus* sp. (juvenile) (84472).

Larvae of *Ascarops* sp. have apparently not been previously reported from Caribbean amphibians and reptiles; however, larvae of *Agamospirura* sp. (a genus established for holding unidentified spirurids from herptiles) have been

**Table 1. Prevalence, mean intensity (range), and location of helminths from 20 *Anolis oculatus* from Dominica, Lesser Antilles.**

Parasite	Prevalence (%)	Mean intensity (range)	Location*
<i>Mesocoelium monas</i>	30	8.7 (1-39)	b
<i>Ascarops</i> sp.	40	25.8 (1-115)	a, d
<i>Oswaldocruzia lenteixeirai</i>	20	2.8 (1-4)	b
<i>Parapharyngodon cubensis</i>	30	2.0 (1-5)	a, c
<i>Physaloptera</i> sp.	25	4.4 (1-9)	a, d
<i>Spauligodon caymanensis</i>	5	12.0	c
<i>Spinicauda spinicauda</i>	10	33.5 (2-65)	c
<i>Centrorhynchus</i> sp.	50	4.3 (1-17)	a, b, c, d

\* a = stomach, b = small intestine, c = large intestine, d = body cavity.

reported from cysts on the stomachs of toads (*Peltaphryne empusa*, *P. gundlachi*, and *P. peltacephala*) and frogs (*Eleutherodactylus cuneatus*, *E. planirostris*, *E. sierramaestrae*, *E. varleyi*, and *E. zeus*) from Cuba (Barus, 1972; Coy Otero and Ventosa, 1984). Definitive hosts of *Ascarops* sp. and the closely related *Physocephalus* sp. are mammals of the orders Artiodactyla, Lagomorpha, and Rodentia; intermediate hosts include insects of the orders Coleoptera and Odonata, and paratenic hosts include amphibians, reptiles, birds, and mammals which have ingested beetles (Anderson, 1992).

*Oswaldocruzia lenteixeirai* has been reported from toads (*Peltaphryne empusa*, *P. gundlachi*, *P. longinasus*, *P. peltacephala*, and *P. taladaï*), frogs (*Eleutherodactylus atkinsi*, *E. cuneatus*, *E. dimidiatus*, *E. greyi*, *E. klinikowskii*, *E. pinaricensis*, *E. planirostris*, *E. sierramaestrae*, *E. zeus*, *E. zugi*, *Osteopilus septentrionalis*, and *Rana catesbeiana*), lizards (*Ameiva auberi*, *Anolis allisoni*, *A. allogus*, *A. baracoae*, *A. bartschi*, *A. bremeri*, *A. equestris*, *A. homolechis*, *A. loysianus*, *A. lucius*, *A. luteogularis*, *A. quadriocellifer*, *A. sagrei*, *Leiocephalus carinatus*, *L. cubensis*, *L. macropus*, *L. stictigaster*, *Chamaeleolis porcus*, and *Cyclura nubila*), and snakes (*Alsophis cantherigerus*, *Antillophis andreai*, and *Tropidophis pardalis*), from Cuba (Barus and Moravec, 1967; Barus, 1972, 1973; Barus and Coy Otero, 1968, 1978; Coy Otero and Barus, 1979; Coy Otero and Ventosa, 1984), *Eleutherodactylus portoricensis* from Puerto Rico (Schmidt and Whittaker, 1975), and *Anolis sagrei* from Bahamas (Goldberg et al., 1994).

*Parapharyngodon cubensis* has been reported from lizards (*Ameiva auberi*, *Anolis allisoni*, *A. allogus*, *A. bartschi*, *A. bremeri*, *A. homolechis*, *A. jubar*, *A. lucius*, *A. luteogularis*, *A. porcatus*,

*A. quadriocellifer*, *A. sagrei*, *A. vermiculatus*, *Gonatodes albogularis*, *Hemidactylus mabouia*, *Leiocephalus carinatus*, *L. cubensis*, *L. macropus*, *Sphaerodactylus cinereus*, and *S. torrei*), an amphisbaenid (*Amphisbaena cubana*), and snakes (*Alsophis cantherigerus*, *Trophidophis melanurus*, and *T. semicinctus*) from Cuba Barus and Coy Otero, 1969; Barus, 1973; Coy Otero and Barus, 1973, 1979); lizards (*Anolis lineatopus*, *A. grahami*, *A. sagrei*, and *A. valencienni*) from Jamaica (Bundy, et al., 1987; Vogel and Bundy, 1987); lizards (*Anolis bimaculatus*, *A. ferreus*, *A. gingivinus*, *A. lividus*, *A. pogus*, *A. sabanus*, *A. schwartzi*, and *A. watti*) from the Lesser Antilles (Dobson et al., 1992); and lizards (*Anolis bimaculatus leachi*, *A. grahami*) from Bermuda (Goldberg et al., 1995).

Only larval physalopterans were found in this study, but *Physaloptera retusa* has been found in the lizard *Cnemidophorus murinus* from Curaçao (Specian and Whittaker, 1980) and *Physaloptera squamatae* has been reported in the lizards *Ameiva ameiva*, *Anolis allogus*, *A. baracoae*, *A. bremeri*, *A. equestris*, *A. homolechis*, *A. lucius*, *A. sagrei*, *Leiocephalus carinatus*, *L. cubensis*, *L. macropus*, *L. raviceps*, and *L. stictigaster* from Cuba (Barus and Coy Otero, 1968; Coy Otero and Barus, 1979).

*Spauligodon caymanensis* was previously known only from *Anolis conspersus* from Grand Cayman Island, British West Indies (Bursey and Goldberg, 1995). Our finding it in *A. oculatus* from Dominica extends the range of this nematode approximately 1,450 km eastward to the Lesser Antilles. *Spinicauda spinicauda* has been reported from *Ameiva ameiva* from Trinidad (Everard, 1975). *Mesocoelium monas* (= *M. danforthi*, see Nasir and Diaz, 1971) has been reported from *Bufo marinus* from Jamaica (Met-



trick and Dunkley, 1968; Wong and Bundy, 1985) and *Bufo marinus*, *Ameiva exul*, *Anolis cristatellus*, *A. cuvieri*, *A. evermanni*, *A. gundlachi*, *A. krugi*, *A. poncensis*, *A. pulchellus*, *A. stratulus*, and *Diploglossus pleei* from Puerto Rico (Hoffman, 1935; Confresí-Sala and Rodríguez de Vega, 1963; Cofresí-Sala, 1964; Acholonu, 1976). An acanthocephalan, *Centrorhynchus* (?*spinosus*), has been reported from *Anolis garmani*, *A. grahami*, *A. lineatopus*, *A. sagrei*, and *A. valencienni* from Jamaica (Bundy et al., 1987, Vogel and Bundy, 1987) and from *Anolis bimaculatus*, *A. leachi*, *A. lividus*, *A. schwartzi*, and *A. watsi* from the Lesser Antilles (Dobson et al., 1992), where it is thought to be a parasite of the pearly-eyed thrasher (*Margarops fuscatus*) and the sparrowhawk (*Falco sparverius*).

Thus, all the helminths found in *Anolis oculatus* are shared with other Caribbean amphibians and reptiles. Determination of the extent of these shared helminths must await helminthological investigations of as yet unstudied Caribbean herptiles.

We thank David L. Auth, Herpetology Division, Florida Museum of Natural History, University of Florida, Gainesville for allowing us to examine *Anolis oculatus* for helminths.

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### Research Note

## *Sarcocystis* sp. (Apicomplexa) from the New Mexico Ridgenose Rattlesnake, *Crotalus willardi obscurus* (Serpentes: Viperidae) from Sonora, Mexico

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**ABSTRACT:** Two of 4 New Mexico ridgenose rattlesnakes, *Crotalus willardi obscurus* Harris, 1974, from Sonora, Mexico, were found to be passing oocysts and free sporocysts of a *Sarcocystis* sp. in their feces. Sporocysts measured  $11.9 \times 10.3$  ( $11.0-13.6 \times 9.6-11.2$ )  $\mu\text{m}$  ( $N = 20$ ) and had a shape index (length/width) of 1.15 (1.07-1.23). Attempts to transmit the *Sarcocystis* sp. experimentally to *Mus musculus*, *Peromyscus leucopus*, or *Microtus ochrogaster* were unsuccessful. This represents the first report of a parasite from this host.

**KEY WORDS:** Apicomplexa, *Sarcocystis* sp., Reptilia, Serpentes, Viperidae, ridgenose rattlesnake, oocysts, sporocysts, survey.

The New Mexico ridgenose rattlesnake, *Crotalus willardi obscurus* Harris, 1974, is a medium-sized viperid that ranges from the Animas and Peloncillo Mountains of extreme southwestern New Mexico south into the Sierra Madre Occidental to Zacatecas, Mexico (Stebbins, 1985; Campbell, et al., 1989). It is chiefly a mountain-dwelling snake occurring in the pine-oak and pine-

fir belts, but also inhabits foothill canyons of madrean habitat. Although Barker (1992) recently reported on various aspects of the biology of *C. willardi*, nothing, to our knowledge, has been published on parasites of this snake. Here, we provide the first report of a parasite from *C. willardi obscurus*.

As part of a long-term mark-recapture study, 4 *C. willardi obscurus* (1 male, 3 females; snout-vent length = 370-463 mm) were collected during March 1990 from an unnamed canyon north of Cañon El Diablo, Sierra San Luis, Sonora, Mexico (elev. 1,920 m). Feces were obtained and snakes were released unharmed at their original point of capture. Samples were placed in 2.5% (w/v) aqueous potassium dichromate and processed further for coccidia using previously described methods (Upton and McAllister, 1990). Measurements were made on 20 sporocysts using a calibrated ocular micrometer and are reported

as length  $\times$  width means  $\pm$  1 SE followed by the ranges in parentheses. Samples were 14 days old when measured and photographed.

Following the protocol of McAllister et al. (1995) for experimental transmission studies, 500 sporocysts were inoculated into each rodent (1 *Mus musculus*, 1 *Peromyscus leucopus*, and 1 *Microtus ochrogaster*). At 91 days postinoculation, rodents were killed with ether, and portions of tongue, diaphragm, heart, and skeletal muscle tissues were examined for sarcocysts.

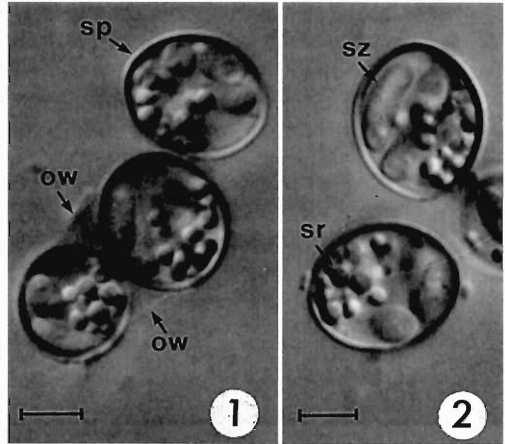
A voucher photo of *Sarcocystis* sp. is deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as USNPC 84752. A voucher photograph of *C. willardi obscurus* is deposited in the Arkansas State University Museum of Zoology as ASUMZ 20161.

Oocysts and free sporocysts of a *Sarcocystis* sp. (Figs. 1, 2) were found in feces of 2 of the snakes. Measurements on 20 sporocysts were  $11.9 \pm 0.2 \times 10.3 \pm 0.1$  ( $11.0\text{--}13.6 \times 9.6\text{--}11.2$ )  $\mu\text{m}$  and had a shape index (width/length) of  $1.15 \pm 0.01$  ( $1.07\text{--}1.23$ ). These length and width measurements were most similar to those reported for sporocysts of a *Sarcocystis* sp. (isolate SAR-26) from western diamondback rattlesnakes, *Crotalus atrox*, from Texas (McAllister et al., 1995).

All 3 species of rodents inoculated with sporocysts of *Sarcocystis* sp. were negative for detectable sarcocysts in tissues. Similarly, attempts to infect the same potential rodent intermediate hosts experimentally with several isolates of *Sarcocystis* spp. from *C. atrox* have been unsuccessful (McAllister et al., 1995), and strongly suggest that other species of rodents are involved in the life cycle.

Various species of *Sarcocystis* have been reported from snakes worldwide (Dubey et al., 1989). In North America, viperids previously reported to harbor *Sarcocystis* spp. include *Crotalus adamanteus*, *C. atrox*, *C. horridus*, *C. scutulatus*, *C. viridis*, *Agkistrodon contortrix*, *A. piscivorus leucostoma*, *Sistrurus catenatus*, and *S. miliarius streckeri* (see Upton and McAllister, 1990; Upton et al., 1992; McAllister et al., 1995). Although measurements of various isolates of *Sarcocystis* sp. suggest that multiple species exist in *C. atrox* and possibly in other viperids (McAllister et al., 1995), tissue stages recovered in the intermediate host are necessary for species identification.

In conclusion, we have provided the first report of an apicomplexan parasite from *C. willardi*



Figures 1, 2. Nomarski interference-contrast photomicrographs of oocysts and free sporocysts of *Sarcocystis* sp. from feces of *Crotalus willardi obscurus* from Sonora, Mexico. 1. View of oocyst containing 2 sporocysts and separate free sporocyst. 2. Free sporocysts. Abbreviations: ow = oocyst wall, sp = sporocyst, sr = sporocyst residuum, sz = sporozoite. Scale bars = 5.0  $\mu\text{m}$ .

*obscurus*. We suggest that additional snakes be surveyed to obtain sporocysts for further experimental transmission studies in alternate species of rodent intermediate hosts.

We thank our Mexican cooperator, David Lazcano, Museo de Historia Natural and Universidad Autonoma de Nuevo Leon, for providing samples, and Dr. B. Raphael, Bronx Zoo, and C. Garrett, Dallas Zoo, for technical assistance. The New Mexico Department of Game and Fish is gratefully acknowledged for financial support through Professional Services Contract no. 5-516.6-76-16.

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### Research Note

## Host-Induced and Geographical Variation in *Levinseniella cruzi* Travassos, 1920 (Digenea: Microphallidae)

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**ABSTRACT:** Morphological variation in *Levinseniella cruzi* (Digenea: Microphallidae) among the hosts from 3 localities, *Rollandia rolland chilensis* (Podicipedidae), *Himantopus melanurus* (Recurvirostridae), and *Vanellus chilensis lampronotus* (Charadriidae), was analyzed through an ANOVA test and with cluster analysis. A great variation in body shape and size of parasites is noted. Male pocket length and number, sucker diameter, pharynx and genital papillae length, and ratio of suckers appear to be the most constant features and, therefore, valuable for systematic purposes. The morphological variation is discussed in relation to host species and geographical distribution. A new host for *L. cruzi* is reported.

**KEY WORDS:** Digenea, Microphallidae, Aquatic birds, host-induced variations.

*Levinseniella cruzi* was previously reported by Martorelli (1988) from the ceca of 2 birds from Buenos Aires Province: the white tufted grebe, *Rollandia rolland chilensis* Lesson, 1828 (Podicipedidae) and the South American stilt, *Himantopus melanurus* Vieillot, 1817 (Recurvirostridae). We analyzed the morphological variation of *L. cruzi* among avian hosts from various geographic localities.

Definitive hosts were collected from 3 localities related with lentic freshwater environments in Buenos Aires Province (Argentina): Chascomús, a typical "pampa lagoon" which drains in Río Salado system (35°36'S, 58°00'W); Mar Chiquita, a large lagoon by the sea in contact with the Atlantic Ocean (37°46'S, 57°27'W) and Los

Talas, artificial and small lagoons related to the Río de La Plata system (34°52'S, 57°00'W).

Six specimens of each species of bird included in this study were examined: *R. r. chilensis* from Los Talas, *R. r. chilensis* from Chascomús, and *H. melanurus* and *Vanellus chilensis lampronotus* Wagler, 1827 (Charadriidae) from Mar Chiquita.

Voucher specimens of this parasite from different hosts and localities were deposited in the Museo de la Plata, La Plata, Buenos Aires, Argentina, Helminth Coll. no. 3303 a, b; 3304 a, b, c; 3305 a, b, and in USNPC 84905–84908.

All the digeneans measured were recovered alive from the bird's cecum, fixed in Bouin Hollande pressured with a cover glass, stained in Langeron alcoholic carmine, dehydrated in ethanol, cleared in creosote, and mounted in natural Canada balsam. All dimensions were given in millimeters. The morphological variation was studied taking into consideration the measurements shown in Table 1.

One-way analysis of variance (ANOVA) and Tukey's multiple range test were used to appraise differences in these morphological dimensions among 3 groups of specimens: 1) parasites from *R. r. chilensis*, 2) parasites from *H. melanurus*, and 3) parasites from *V. ch. lampronotus* (referred to as groups 1, 2, and 3 hereafter).

Moreover, in order to compare the specimens of *L. cruzi* from different hosts and their localities

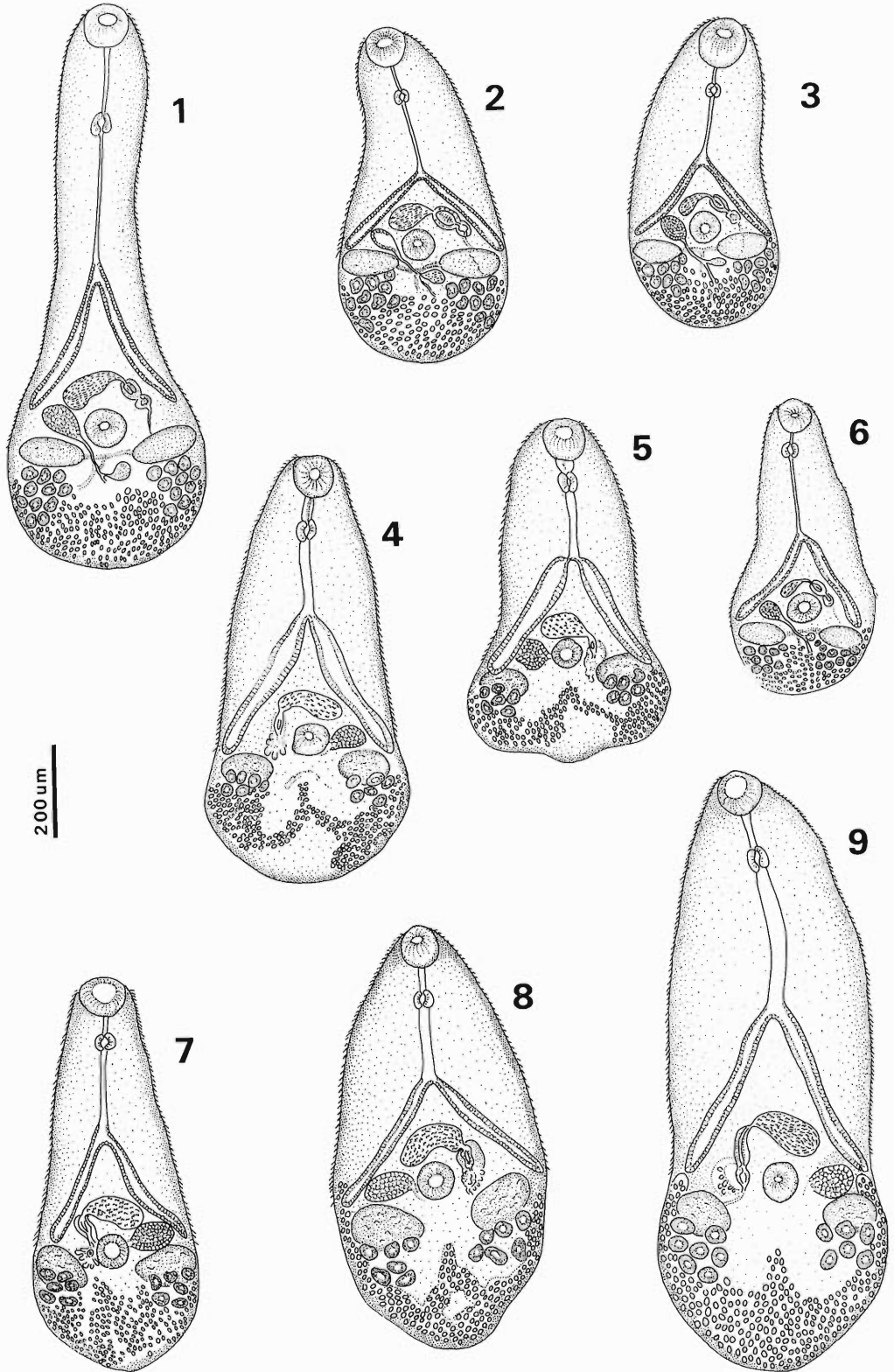
**Table 1.** Measurements of specimens of *Levinseniella cruzi* from different hosts. All measurements are given in mm.

	Body length	Body width	Body length		Oral sucker	Ventral sucker	Oral sucker		Pharynx length	Esophagus length	Body length		Pre-pharynx length	Intestinal ceca length
			Body width	Oral sucker			Ventral sucker	Forebody						
<i>R. r. chilensis</i>														
Mean	0.803	0.354	2.24	0.085	0.066	1.292	0.041	0.094	3.801	0.104	0.104	0.104	0.104	0.241
Maximum	1.319	0.459	2.95	0.102	0.079	1.593	0.048	0.199	5.841	0.179	0.179	0.179	0.179	0.329
Minimum	0.512	0.239	1.66	0.066	0.059	0.881	0.031	0.028	2.709	0.039	0.039	0.039	0.039	0.168
SD	0.245	0.069	0.36	0.011	0.006	0.208	0.003	0.044	1.017	0.034	0.034	0.034	0.034	0.053
N	20	20	20	19	20	19	19	17	17	16	16	16	16	14
<i>H. melanurus</i>														
Mean	0.702	0.285	2.52	0.078	0.061	1.321	0.038	0.139	2.719	0.041	0.041	0.041	0.041	0.25
Maximum	1.014	0.468	2.94	0.108	0.078	1.818	0.048	0.168	3.346	0.072	0.072	0.072	0.072	0.357
Minimum	0.592	0.208	2.05	0.059	0.044	1.108	0.024	0.084	2.141	0.028	0.028	0.028	0.028	0.179
SD	0.114	0.072	0.28	0.013	0.011	0.179	0.007	0.029	0.407	0.012	0.012	0.012	0.012	0.058
N	14	14	14	14	13	13	14	11	11	12	12	12	12	10
<i>V. ch. lampronotus</i>														
Mean	0.902	0.429	2.12	0.088	0.069	1.271	0.043	0.159	3.175	0.041	0.041	0.041	0.041	0.317
Maximum	1.329	0.539	2.81	0.109	0.088	1.492	0.052	0.286	4.527	0.072	0.072	0.072	0.072	0.403
Minimum	0.649	0.304	1.51	0.064	0.057	1.016	0.032	0.084	2.645	0.019	0.019	0.019	0.019	0.209
SD	0.159	0.063	0.32	0.012	0.008	0.125	0.006	0.044	0.461	0.013	0.013	0.013	0.013	0.059
N	25	25	25	24	24	24	22	22	22	20	20	20	20	20
	Male pocket number	Male pocket length	Egg length	Body length Egg length	Ovary length	Right testis	Left testis	Seminal vesicle length	Genital papillae length	Genital papillae width				
<i>R. r. chilensis</i>														
Mean	7.2	0.016	0.019	40.71	0.074	0.102	0.089	0.089	0.023	0.018				
Maximum	10	0.023	0.024	73.33	0.091	0.114	0.104	0.108	0.024	0.019				
Minimum	6	0.011	0.018	23.33	0.048	0.081	0.082	0.066	0.023	0.016				
SD	1.4	0.003	0.001	13.89	0.013	0.011	0.008	0.012	0.0005	0.002				
N	12	9	20	20	8	6	4	10	2	2				
<i>H. melanurus</i>														
Mean	6.8	0.016	0.019	35.51	0.077	0.061	0.054	0.086	0.023	0.018				
Maximum	8	0.016	0.021	49.78	0.108	0.069	0.054	0.088	0.035	0.019				
Minimum	6	0.016	0.018	30.39	0.052	0.053	0.054	0.083	0.012	0.016				
SD	0.8	0	0.001	6.005	0.017	0.008	0	0.002	0.009	0.002				
N	8	1	14	14	5	2	1	3	2	2				
<i>V. ch. lampronotus</i>														
Mean	7.8	0.018	0.019	47.22	0.081	0.107	0.102	0.141	0.031	0.021				
Maximum	10	0.035	0.024	78.24	0.093	0.121	0.109	0.152	0.039	0.024				
Minimum	6	0.01	0.017	31.57	0.075	0.099	0.087	0.131	0.026	0.018				
SD	1.3	0.007	0.002	11.22	0.007	0.009	0.007	0.006	0.004	0.002				
N	17	13	23	23	10	8	8	3	5	7				

SD = Standard deviation, N = number of parasites.

Figures 1–9. *Levinseniella cruzi*. 1, 2. Specimens of *L. cruzi* from *R. r. chilensis* in Chascomús (ventral view). 3. Specimens of *L. cruzi* from *R. r. chilensis* in Los Talas (ventral view). 4–6. Specimens of *L. cruzi* from *H. melanurus* in Mar Chiquita (Fig. 4: dorsal view, Figs. 5 and 6: ventral view). 7–9: Specimens of *L. cruzi* from *V. ch. lampronotus* in Mar Chiquita (Figs. 7 and 9: dorsal view, Fig. 8: ventral view).

Figure 10. Similarity dendrogram of specimens of *L. cruzi* from different hosts and localities. 1 = parasites from *R. r. chilensis* in Chascomús, 2 = parasites from *R. r. chilensis* in Los Talas, 3 = parasites from *H. melanurus* in Mar Chiquita, 4 = parasites from *V. ch. lampronotus* in Mar Chiquita.



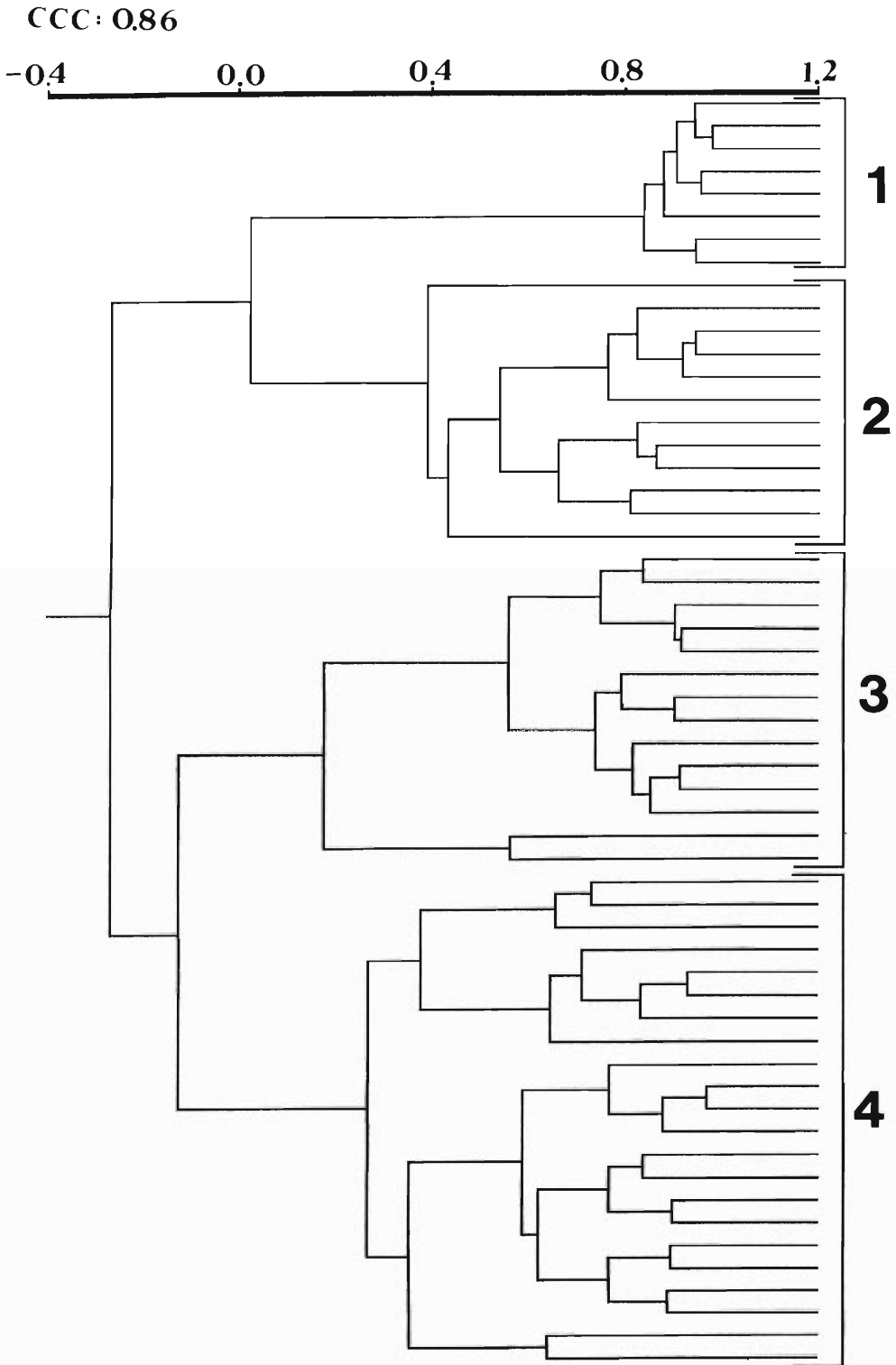


Table 2. Summary of one-way analysis of variance (ANOVA).

Source of variation	df	MS	F ratio	Significance level
Body length (Bl)	56	0.0359	5.149	0.008
Body width (Bw)	56	0.0048	19.924	0.000
Bl/Bw	56	0.113	6.311	0.003
Oral sucker (Os)	54	$1.4 \times 10^{-4}$	2.96	0.063
Ventral sucker (Vs)	54	$6.8 \times 10^{-5}$	4.765	0.012
Os/Vs	53	0.0305	0.350	0.706
Prepharynx length	45	0.0005	40.40	0.000
Pharynx length	52	$3.7 \times 10^{-5}$	2.615	0.082
Esophagus length	47	0.0018	11.08	0.000
Intestinal caeca length	41	0.0034	8.43	0.000
Right testis diameter	13	0.0082	3.999	0.044
Left testis diameter	11	$6.4 \times 10^{-5}$	29.22	0.000
Ovary diameter	20	$1.7 \times 10^{-4}$	0.487	0.621
Seminal vesicle length	13	$1.2 \times 10^{-4}$	25.63	0.000
Genital papillae length	6	$6.2 \times 10^{-5}$	1.112	0.388
Genital papillae width	6	$5.8 \times 10^{-6}$	3.797	0.099
Male pockets number	34	1.6351	1.677	0.202
Male pockets length	20	$4.1 \times 10^{-5}$	0.355	0.705
Bl/Forebody length	47	0.5153	8.094	0.001
Egg length (El)	54	$2.1 \times 10^{-6}$	1.120	0.333
Bl/El	54	134.39	4.654	0.013

df = degrees of freedom, MS = mean square.

taking into consideration all the measurements at the same time (Table 1), a cluster analysis was applied. In order to obtain a matrix with a low number of missing data, measurements such as testes and ovary diameter, male pockets, genital papillae, and seminal vesicle length were excluded from the analysis because such characters could not be measured in specimens where the eggs overlapped them. The product-moment corre-

lation coefficient ( $r$ ) was applied and the resulting dendrogram was constructed using the UPGMA method. The distortion caused by this method was measured by calculating the cophenetic correlation coefficient (CCC) (Rohlf, 1970).

We found *L. cruzi* in the cecum of the southern lapwing *V. ch. lampronotus* from Mar Chiquita as well as in *R. r. chilensis* and *H. melanurus* as previously reported by Martorelli (1988). In Figures 1-9, specimens of *L. cruzi* in different hosts are shown at the same magnification.

A summary of the ANOVA for the 3 groups is presented in Table 2. In Table 3, the contrasts among the groups of parasites for mean measurements are shown.

As noted in Figure 10, the results of the cluster analysis of *L. cruzi* specimens show the 2 largest clusters separated at a low value of correlation ( $r = -0.27$ ). One of them contains specimens from *H. melanurus* and *V. ch. lampronotus* from Mar Chiquita, and the other specimens from *R. r. chilensis* from Chascomús and Los Talas. This result might suggest the presence of geographical variation, as Kennedy (1980a) found for *He-matoloechus* sp.

The former cluster is divided into 2 groups ( $r = -0.12$ ): 1 contains specimens from *H. melanurus* and the other those from *V. ch. lampron-*

Table 3. Significant differences between groups (Tukey's multiple range test).

Source of variation	<i>R. r. chilensis-H. melanurus</i>	<i>R. r. chilensis-V. ch. lampronotus</i>	<i>H. melanurus-V. ch. lampronotus</i>
Body length (Bl)			*
Body width (Bw)	*	*	*
Bl/Bw			*
Ventral sucker			*
Prepharynx length	*	*	
Esophagus length	*	*	
Intestinal caeca length		*	*
Right testis diameter	*		*
Left testis diameter	*		*
Seminal vesicle length		*	*
Bl/Forebody length	*	*	
Bl/Egg length			*



*otus*, which indicates host-induced variation (Blankespoor, 1974; Kennedy, 1980b).

The latter cluster is formed on one hand by specimens from *R. r. chilensis* in Chascomús and on the other by specimens from this host in Los Talas ( $r = 0.02$ ).

The great variation in body size and shape of specimens in *L. cruzi* was one of the most notable features. Parasites from *R. r. chilensis* are usually pear-shaped with the forebody clearly prolonged in some specimens. Those from *H. melanurus* have a pear-shaped body but are smaller in size. Pear-shaped, oval, and tongue-shaped specimens could be seen parasitizing *V. ch. lampronotus* (Figs. 1–9).

The most important taxonomic features of *L. cruzi* include terminal genitalia and the position of vitelline glands (Deblock, 1971). We suggest that male pocket length and number, oral sucker and ovary diameter, pharynx and genital papillae length, and the oral sucker/ventral sucker ratio are less subject to variation and, therefore, valuable for systematic purposes.

Finally, the report of a new definitive host for *L. cruzi* confirms the low specificity for this group of parasites. As far as we know, *L. cruzi* has been reported from a mammalian host, *Scapteromys aquaticus* (Cricetidae), by Sutton and Lunaschi (1994), and avian hosts of 4 different families: *Annas bahamensis* (Anatidae) by Travassos (1920), *R. r. chilensis* (Podicipedidae) and *H. melanurus* (Recurvirostridae) by Martorelli (1988),

and *V. ch. lampronotus* (Charadriidae) in this study.

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## Research Note

# Parasitic Helminths of the Little Blue Heron, *Egretta caerulea*, in Southern Florida

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**ABSTRACT:** Twenty-four species of helminths, including 13 trematodes, 8 nematodes, 2 acanthocephalans, and 1 cestode, were collected from 33 of 35 (94%) little blue herons (*Egretta caerulea*) from southern Florida. A mean of 2.6 (range 1–10) species of helminths per host was recorded. Seventeen species are new host records, while 5 are reported from Florida for the first time.

**KEY WORDS:** little blue heron, *Egretta caerulea*, Florida, helminths, trematodes, nematodes, acanthocephalans, cestodes.

The little blue heron, *Egretta caerulea* (Linnaeus, 1758) is a small ciconiiform found in fresh- and saltwater habitats in North America, the Caribbean, and tropical South America (Walters, 1980). Little information is available on the parasites of this heron. Some endoparasites have been reported from North, Central, and South America (Vevers, 1923; Travassos, 1930; Polk, 1941; Viguera, 1944; Caballero and Hidalgo, 1955; Coil, 1955; Cable et al., 1960; Schmidt and Neiland, 1971, 1973). None of these, however, represent thorough surveys. Information on the parasites of little blue herons in Florida is restricted to the works by Stiles and Hassall (1894) and Leigh (1956), who reported cestodes of the genus *Taenia* and the trematode *Ascocotyle tenuicollis*, respectively. The purpose of the present study was to conduct the first systematic survey of helminths in little blue herons, and to determine the prevalence, intensity of infection, and abundance of each helminth species.

Thirty-five little blue herons were collected dead in Florida during November 1970 ( $N = 4$ ) and from April through August of 1987, 1988, 1989, 1990, and 1992 ( $N = 31$ ). Twelve of the birds were in good condition when examined at necropsy, and in the remaining 23 some degree of autolysis was found. Birds were collected from the following counties: Polk (7 birds), Okeecho-

bee (4), Palm Beach (1), Collier (1), Dade (6), and Monroe (12). The herons collected in 1970 came from the Everglades area; however, specific locality information was not available for these birds. The sample included a total of 11 nestling males, 7 nestling females, 12 nestlings of unknown sex, 1 juvenile female, 2 adult females, and 2 birds of unknown age. Birds were separated into age classes based on bill length and plumage characteristics. Techniques for the necropsy of birds and for the collection, fixing, and staining of helminths were similar to those described by Kinsella and Forrester (1972). The terms prevalence, intensity, and abundance used in this paper follow the definitions given by Margolis et al. (1982). Given that the tissues examined for parasites differed between birds, the prevalence for each species of helminth was determined by dividing the number of birds infected with a given helminth by the number of birds in which the tissue found to harbor that given species of helminth was examined (see Table 1). Representative specimens of each species of helminth have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNPC 84349–84372).

Twenty-four species of helminths (13 trematodes, 8 nematodes, 2 acanthocephalans, and 1 cestode) were collected from 33 of 35 (94%) birds examined. Infected birds harbored a mean of 2.6 helminths (range 1–10). The prevalence, intensity of infection, abundance, and location of each helminth are given in Table 1. Seventeen species have not been reported previously from little blue herons, while 5 are reported from Florida for the first time.

Thirty-four percent of the birds were infected with at least 1 species of trematode (mean 3, range 1–5). Three species of strigeids were recovered: *Posthodiplostomum macrocotyle*,

Table 1. Parasitic helminths of 35 little blue herons from southern Florida.

Helminth	USNPC no.	Sample size*	Pre-valence %	Intensity		Abundance
				Mean	Range	
<b>Trematoda</b>						
<i>Posthodiplostomum macrocotyle</i> Dubois, 1937 (2, 3, 4, 5)†‡§	84360	13	8	120	2-503	46
<i>Echinochasmus donaldsoni</i> Beaver, 1941 (3, 4, 5)†	84361	13	31	456	9-1,770	141
<i>Phagicola nana</i> (Ransom, 1920) (3, 4, 5)‡§	84351	13	31	49	22-90	15
<i>Clinostomum complanatum</i> (Rudolphi, 1814) (1)§	84355	19	26	2	1-2	<1
<i>Apharyngostrigea multiovata</i> (Perez Viguera, 1944) (2, 3)	84356	17	24	4	2-9	1
<i>Ascocotyle gemina</i> Font, Overstreet, and Heard, 1984 (2, 3, 4, 5)§	84349	13	23	7	1-18	2
<i>Phagicola diminuta</i> Stunkard and Haviland, 1924 (3)§	84352	17	18	38	5-100	7
<i>Microphallus turgidus</i> (Leigh, 1958) (3)	84358	17	12	15	10-19	2
<i>Pholeter anterouterus</i> Fischthal and Nasir, 1974 (3)§	84354	17	12	2	1-2	<1
<i>Ascocotyle</i> sp. (5)	84350	13	8	14	—	1
<i>Apharyngostrigea simplex</i> (Johnston, 1904) (3)‡§	84357	17	6	1	—	<1
<i>Prosthogonimus ovatus</i> (Rudolphi, 1803) (3)§	84353	17	6	1	—	<1
<i>Ribeiroia ondatrae</i> (Price, 1931) (2)	84359	35	3	3	—	<1
<b>Nematoda</b>						
<i>Contracaecum</i> spp. (2)‡§	84366-84367	35	69	25	1-236	17
<i>Tetrameres</i> sp. (1, 2)	84372	20	35	9	1-42	3
<i>Eustrongylides ignotus</i> Jaegerskiold, 1909 (2)	84370	35	20	4	1-10	<1
<i>Capillaria mergi</i> Madsen, 1945 (3)§	84365	17	6	3	—	<1
<i>Syncuaria</i> sp. (1)§	84371	19	5	1	—	<1
<i>Chandleronema longigutturata</i> (Chandler, 1942) (2)‡§	84369	35	3	30	—	<1
<i>Acuaria multispinosa</i> Perez Viguera, 1938 (2)‡§	84368	35	3	1	—	<1
<b>Cestoda</b>						
Unidentified proglottids (3)	84364	17	24	2	1-2	<1
<b>Acanthocephala</b>						
<i>Southwellina</i> sp. (3, 4)§	84362	17	6	2	—	<1
<i>Neoechinorhynchus</i> sp. (2)§	84363	35	6	1	—	<1

\* Number of organs examined differed between birds.

† Numbers in parentheses indicate location in host: (1) oral cavity/esophagus, (2) proventriculus/ventriculus, (3) small intestine, (4) large intestine, and (5) cloaca.

‡ New record for Florida.

§ New host record.

|| A complex of larvae and adults of 2 species: *C. multipapillatum* (Drasche, 1882) and *C. microcephalum* (Rudolphi, 1809).

*Apharyngostrigea multiovata*, and *Apharyngostrigea simplex*. *Posthodiplostomum macrocotyle* was the most prevalent trematode (38%), and represents the first record in little blue herons and in Florida. Specimens of *A. multiovata* were described originally from little blue herons in Cuba by Viguera (1944), and have been reported also in Florida from reddish egrets (*Egretta rufescens*) (Conti et al., 1986) and roseate spoonbills (*Ajaia ajaja*) (Sepúlveda et al., 1994). *Apharyngostrigea simplex* was collected from a single nestling from Dade County and is the first report of this parasite in the United States. It was reported previously from herons from Australia

and Argentina (Dubois, 1968; Ostrowoski de Nuñez, 1989). Ostrowoski de Nuñez (1989) reported the snail *Biomphalaria straminea* and fishes of the families Poeciliidae and Cichlidae as the first and second intermediate hosts, respectively, of *Apharyngostrigea simplex*.

Heterophyids were represented by the species *Ascocotyle gemina*, *Ascocotyle* sp., *Phagicola nana*, *Phagicola diminuta*, and *Pholeter anterouterus*. The flukes identified as *Ascocotyle* sp. may belong to the species *A. gemina*, since the distribution of the organs, vitellaria, and oral appendage was identical in both flukes. *Ascocotyle* sp., however, harbored large numbers of eggs

which made them much longer (1.6 mm vs. 0.64 mm) and wider (0.6 mm vs. 0.15 mm) than *A. gemina*. More taxonomic work is needed for a better understanding of these differences. Trematodes of the genus *Ascocotyle* and *Phagicola* frequently utilize fishes of the families Centrarchidae, Cyprinodontidae, Mugilidae, and Poeciliidae as second intermediate hosts (Lumsden, 1963). In Florida, metacercariae of *P. nana* develop in the centrarchiids *Micropterus salmoides*, *Lepomis microlophus*, *L. macrochirus*, and *L. humilis* (Font et al., 1984), while those of *Phagicola diminuta* have been reported from *Gambusia affinis* by Stein (1978).

Specimens of *Pholeter anterouterus* have been described in Florida from cystic cavities in the small intestine of brown and white pelicans *Pelecanus occidentalis*, and *P. erythrorhynchos*, respectively (Pearson and Courtney, 1977). In the present study, single specimens of *P. anterouterus* also were found buried inside nodules (2–3 mm wide) in the small intestine.

The families Microphallidae, Prosthogonimidae, Clinostomidae, Psilostomidae, and Echinostomatidae were represented by a single species each: *Microphallus turgidus*, *Prosthogonimus ovatus*, *Clinostomum complanatum*, *Ribeiroia ondatrae*, and *Echinochasmus donaldsoni*, respectively. *Microphallus turgidus*, *P. ovatus*, and *C. complanatum* have been reported in Florida from a number of avian hosts (Yamaguti, 1971; Heard and Overstreet, 1983).

Heard and Overstreet (1983) originally reported *Microphallus turgidus* from little blue herons collected in Mississippi. This microphallid utilizes snails of the genus *Littoridinops* as first intermediate hosts, and the crustaceans *Palaeomonetes paludosus*, *P. pugio*, and *P. vulgaris* as second intermediate hosts in South Florida (Heard and Overstreet, 1983). Dragonfly larvae and centrarchiid fishes have been reported as the intermediate hosts for *P. ovatus* and *C. complanatum*, respectively (Boddeke, 1960; Torres and Price, 1971).

Only one bird was infected with *Ribeiroia ondatrae*, a cosmopolitan parasite of fish-eating birds and mammals. This fluke was also reported from little blue herons in Puerto Rico (Cable et al., 1960), and from wood ducks, *Aix sponsa* (Thul et al., 1985), and raccoons (*Procyon lotor*) (Schaffer et al., 1981) in Florida.

*Echinochasmus donaldsoni* was the second most prevalent and the most abundant fluke encountered during this study. Most of the speci-

mens collected were immature, which suggests that little blue herons may represent secondary hosts for this helminth. Premvati (1968) reported this fluke from pied-billed grebes (*Podilymbus podiceps*) in Florida. Snails of the genus *Amnicola* and a variety of freshwater fishes can serve as intermediate hosts for this echinostome (Beaver, 1941).

Ninety-one percent of the little blue herons examined were infected with nematodes (mean 1.4, range 1–5). *Contraecaecum multipapillatum* and *Contraecaecum microcephalum* (family Heterocheilidae) were the most prevalent nematodes, infecting 69% of the birds. Nematodes of this genus have been reported in little blue herons from the British Guyana (*C. andersoni*; Vevers, 1923), and Nicaragua (*C. ardeae*; Schmidt and Neiland, 1973). In Florida, *C. multipapillatum* and *C. microcephalum* have been reported from ciconiiformes and pelecaniformes (Huizinga, 1971; Courtney and Forrester, 1974; Threlfall, 1982; Conti et al., 1986; Sepúlveda et al., 1994). In Florida, the nematode *C. multipapillatum* utilizes different families of freshwater fishes (Centrarchidae, Cyprinidae, and Poeciliidae) as second intermediate hosts (Huizinga, 1967).

The family Acuariidae was represented by 3 species: *Chandleronema longigutturata*, *Acuaria multispinosa*, and *Syncuaria* sp. Infections with *C. longigutturata* and *A. multispinosa* were detected in a single juvenile bird from Collier County. Little and Ali (1980) listed only raccoons and muskrats as hosts for *C. longigutturata*. The finding of this nematode in little blue herons constitutes the first record of this acuariid in an avian host and in Florida. A single female of the genus *Syncuaria* was recovered from an adult breeding female collected in Monroe County.

The families Trichuridae, Dioctophymidae, and Tetrameridae were represented by a single species each: *Capillaria mergi*, *Eustrongylides ignotus*, and *Tetrameres* sp., respectively. In Florida, the nematode *C. mergi* has been reported from roseate spoonbills (Sepúlveda et al., 1994) and specimens similar to *C. mergi* have been reported also from brown pelicans and white ibises (*Eudocimus albus*) (Courtney and Forrester, 1974; Bush and Forrester, 1976). The nematode *E. ignotus* was collected from the ventriculus of 20% of the birds. This parasite has been reported from several species of ciconiiformes in Florida, including little blue herons (Spalding et al., 1993). These authors also reported 10 species

of freshwater fishes (families Centrarchidae, Cyprinodontidae, Lepisosteidae, and Poeciliidae) as intermediate hosts for this nematode in southern Florida.

*Tetrameres* sp. was the second most prevalent nematode recovered during this study. Only males were collected from the esophagus and stomach of infected birds.

Infections with cestodes occurred in 24% of the herons. Only proglottids were recovered in each case, and thus identification to genus or species was not possible. There are 3 previous records of cestodes from little blue herons in America. The cestodes *Taenia* sp. (Stiles and Hassall, 1894), and *Dilepis hillis* (Polk, 1941), have been identified from little blue herons in North America (Florida, Oklahoma), while Schmidt and Neiland (1971) found *Parvitaenia aurita* in birds from Central America (Nicaragua).

Six percent of the birds were infected with the acanthocephalans *Southwellina* sp. (family Polymorphidae) and *Neoechinorhynchus* sp. (family Neoechinorhynchidae). In both cases, the parasites could not be identified to species because their proboscides were retracted. In Florida, members of the genus *Southwellina* have been identified from brown pelicans (Courtney and Forrester, 1974), white ibises (Bush and Forrester, 1976) and roseate spoonbills (Sepúlveda et al., 1994). Definitive hosts for *Neoechinorhynchus* spp., however, are fishes and turtles (Petrochenko, 1956), which suggests that the finding of this acanthocephalan in little blue herons may be accidental. These represent the first records of infections with acanthocephalans in little blue herons.

With the exception of infections with *P. macrocotyle*, *E. donaldsoni*, *Contracaecum* spp., and *E. ignotus*, the prevalence and mean intensity of parasites found in this study were relatively low and probably not associated with significant pathological changes. *Contracaecum* spp. have been implicated as pathogens in several species of aquatic birds (Huizinga, 1971), and infections with *E. ignotus* have been estimated to cause at least 80% mortality among ciconiiform nestlings in southern Florida (Spalding et al., 1993).

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## Research Note

# Hematozoa in Autumnal Migrant Raptors from the Hawk Ridge Nature Reserve, Duluth, Minnesota

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**ABSTRACT:** Blood from 159 raptors of 9 species from the Hawk Ridge Nature Reserve, Duluth, Minnesota was examined for hematozoa. *Accipiter striatus*, *A. cooperii*, *A. gentilis*, *Circus cyaneus*, *Buteo jamaicensis*, *B. platypterus*, *Falco peregrinus*, *F. columbarius*, and *Asio otus* harbored both *Leucocytozoon toddi* and *Haemoproteus*. *F. columbarius* and *Asio otus* had only *Leucocytozoon toddi*, and one *F. peregrinus* was not infected. The prevalence of hematozoans was similar between ages classes of the sharp-shinned hawk (*Accipiter striatus*) and goshawk (*Accipiter gentilis*), but in the Cooper's hawk (*Accipiter cooperii*) immatures were more frequently infected than adults. There were no significant intersexual differences in infection rates for accipiters.

**KEY WORDS:** Cooper's hawk, sharp-shinned hawk, northern harrier hawk, red-tailed hawk, broad-winged hawk, peregrine, merlin, goshawk, long-eared owl, *Leucocytozoon*, *Haemoproteus*, Lake Superior Flyway, fall migrants.

Only 2 studies, both from the eastern U.S.A., have been published on hematozoa of fall migrating raptors. Kirkpatrick and Lauer (1985) surveyed 11 species and found *Leucocytozoon toddi*, *Haemoproteus elani*, *H. tinnunculus*, *Plasmodium circumflexum*, and *Trypanosoma* sp. Powers et al. (1994) examined sharp-shinned hawks (*Accipiter striatus*) and compared packed cell volume, total solids, white blood cell counts, and prevalence of hemoparasites (*Haemoproteus* sp. and *Leucocytozoon toddi*). Here we provide baseline data from a central U.S. flyway regarding the prevalence of blood parasites in autumnal migrating raptors of known sex and age.

During 1992 and 1993, 159 fall migrating raptors of 9 species were trapped at the Hawk Ridge Nature Reserve, Duluth, Minnesota (49°N, 92°W) using mist nets and bow nets. For a description of the study area and trapping techniques, see Evans (1980) and Rosenfield and Evans (1980). Blood was taken with a syringe from the brachial vein. Thin smears were made on microscope slides, air-dried, fixed in methanol, and stained in Giemsa. Each slide was examined for at least 1 hr at 100, 200, 400, and 1,000 $\times$ . One positive

slide (37994–38003) from each species of bird was deposited in the University of Nebraska State Museum, Harold W. Manter Laboratory Collection, Lincoln, Nebraska 68588.

Statistical tests follow Zar (1984) and were conducted with StatXact-Turbo (Mehta and Patel, 1992). Significance was accepted at the 0.05 level.

Our data indicate that *Leucocytozoon toddi* and *Haemoproteus* sp. are relatively common in various species of fall migrating raptors at Duluth, Minnesota (Table 1). Our prevalence rates are generally similar to those reported for the same species by Kirkpatrick and Lauer (1985). However, they detected two parasites we did not: *Plasmodium* sp. and a microfilarial stage from red-tailed hawks.

Hematozoa were found in both sexes of immature and adult sharp-shinned and Cooper's hawks and both sexes of goshawks, but we did not detect *Haemoproteus* sp. in adult goshawks (Table 2). There was no statistical difference between the proportions of infected immatures and adults for sharp-shinned hawks or goshawks ( $P = 1.0$  and  $P = 0.22$ , respectively, Fisher's exact test). In the Cooper's hawk the proportion of infected immatures (100%) was significantly higher than the proportion of infected adults (69%) ( $P = 0.04$ , Fisher's exact test) (Table 3). There was no significant intersexual difference in infection rates for sharp-shinned hawks, Cooper's hawks, and goshawks ( $P = 1.0$ , 1.0, and 0.237, respectively, Fisher's exact test). In contrast to our findings for sharp-shinned hawks, and based on a smaller sample size than ours, Powers et al. (1994) found that immatures were more frequently infected than adults of that species among fall migrants in the eastern U.S.A. They did not detect *Haemoproteus* sp. in adult sharp-shinned hawks.

In an analysis of hematozoa from 30 avian families, Greiner et al. (1975) showed an inverse correlation between prevalence of parasites and

**Table 1. Prevalence of hematozoa in autumnal migrant raptors from the Hawk Ridge Nature Reserve in 1992 and 1993.**

Species	1992				1993			
	Ne*	Np (%)	NL (%)	Nh (%)	Ne	Np (%)	NL (%)	Nh (%)
Sharp-shinned hawk ( <i>Accipiter striatus</i> )	41	36 (88)	33 (80)	18 (44)	14	10 (71)	7 (50)	8 (57)
Cooper's hawk ( <i>Accipiter cooperii</i> )	20	14 (70)	15 (75)	7 (35)	7	7 (100)	6 (86)	5 (63)
Northern goshawk ( <i>Accipiter gentilis</i> )	38	23 (61)	21 (55)	2 (0.5)	10	8 (80)	3 (33)	2 (20)
Northern harrier ( <i>Circus cyaneus</i> )	7	6 (86)	6 (86)	0 (0)	2	2 (100)	1 (50)	2 (100)
Red-tailed hawk ( <i>Buteo jamaicensis</i> )	2	2 (100)	2 (100)	0 (0)	3	3 (100)	3 (100)	0 (0)
Broad-winged hawk ( <i>Buteo platypterus</i> )	7	6 (86)	6 (86)	0 (0)	3	2 (67)	2 (67)	2 (67)
Peregrine falcon ( <i>Falco peregrinus</i> )					1	0 (0)	0 (0)	0 (0)
Merlin ( <i>Falco columbarius</i> )	1	0	0	0	1	1 (100)	0 (0)	1 (100)
Long-eared owl ( <i>Asio otus</i> )	2	1 (50)	1 (50)	0 (0)				
Total	118	88 (75)	84 (71)	27 (23)	41	33 (80)	22 (54)	20 (49)

\* Ne = number examined, Np = number parasitized, NL = *Leucocytozoon*, Nh = *Haemoproteus*.

nest height on a local geographic basis—the higher the nest the fewer the parasites. That correlation did not appear to hold true for our study on nesting Cooper's hawks (Taft et al., 1994), nor does it appear to hold true for birds in this study, i.e., ground-nesting harriers *Circus cyaneus* have a high prevalence, but not significantly higher than other tree-nesting raptors.

Upon gross examination, none of the sampled birds in this study and that of Powers et al. (1994) exhibited signs of ill health when captured. It is thus difficult to speculate on the potential impact that these hematozoa may have on the sampled populations. According to Atkinson and Van Riper (1991), it is likely that hematozoa are pathogenic in their natural host, although little

**Table 2. Prevalence of hematozoa by sex and age for autumnal migrant accipiters at the Hawk Ridge Nature Reserve, 1992 and 1993.**

Species	Sex	Age	N	Only <i>L. toddi</i> (%)	Only <i>Haemoproteus</i> (%)	<i>L. toddi</i> and <i>Haemoproteus</i> (%)
Sharp-shinned hawk	Male	Immature	10	6/10 (60)	1/10 (10)	4/10 (40)
Sharp-shinned hawk	Male	> 1 year	8	2/8 (25)	2/8 (25)	1/8 (12.5)
Sharp-shinned hawk	Male	> 2 years	1	0/1	0/1	0/1
Sharp-shinned hawk	Female	Immature	21	6/21 (29)	0	10/21 (48)
Sharp-shinned hawk	Female	> 1 year	11	2/11 (18)	3/11 (27)	5/11 (45)
Sharp-shinned hawk	Female	> 2 years	4	0	1/4 (25)	0
Cooper's hawk	Male	Immature	7	4/7 (57)	0	3/7 (43)
Cooper's hawk	Male	> 1 year	3	0	0	1/3 (33)
Cooper's hawk	Male	> 2 years	1	0	0	1/1 (100)
Cooper's hawk	Female	Immature	7	4/7 (57)	0	2/7 (29)
Cooper's hawk	Female	> 1 year	4	0	0	2/4 (50)
Coopers' hawk	Female	> 2 years	5	4/5 (80)	1/5 (20)	0
Goshawk	Male	Immature	18	11/18 (61)	0	1/18 (0.55)
Goshawk	Male	> 1 year	2	2/2 (100)	0	0
Goshawk	Male	> 2 years	4	2/4 (50)	0	0
Goshawk	Female	Immature	6	4/6 (66)	0	2/6 (33)
Goshawk	Female	> 1 year	5	2/5 (40)	0	0
Goshawk	Female	2 years	13	5/13 (38)	0	0



**Table 3. The prevalence of hematozoans in age and sex classes of autumnal migrant accipiters at the Hawk Ridge Nature Reserve, 1992 and 1993.**

Species	Age	Infected (%)	Not infected (%)	Sex	Infected (%)	Not infected (%)
Sharp-shinned hawk	Immature	20 (65)	11 (35)	Male	15 (79)	4 (21)
	Adult	16 (67)	8 (33)	Female	27 (75)	9 (25)
Cooper's hawk	Immature	14 (100)	0	Male	9 (82)	2 (18)
	Adult	9 (69)	4 (31)	Female	14 (87.5)	2 (12.5)
Northern goshawk	Immature	19 (76)	6 (24)	Male	17 (71)	7 (29)
	Adult	13 (57)	10 (43)	Female	12 (50)	12 (50)

is known about the physiological, behavioral, and ecological costs of such infections in wild bird populations. However, recent research suggests that parasites ranging from protozoans to arthropods do affect host populations, as in the western fence lizard (*Scleropus occidentalis*) infected with malaria (Schall, 1983), red grouse harboring *Trichostrongylus tenuis* (Dobson and Hudson, 1992), and the coral reef fish (*Chromis nitida*) parasitized by *Anilocra pomacentri* (Adlard and Lester, 1994). The physiology of migrating raptors is poorly documented and understood (Kerlinger, 1989), and this makes it more difficult to determine the effect of hematozoa on wild populations. Powers et al. (1994) were the first to correlate differences in hematological parameters between parasitized and nonparasitized sharp-shinned hawks. They found no physiological differences when comparing hematocrits and hemoglobins of infected vs. noninfected sharp-shinned hawks. Unfortunately, no such comparison is possible in our samples, as we did not gather hematocrit and hemoglobin information. More sophisticated blood tests and a better understanding of raptor physiology will be needed to determine the effect of hematozoa on these migrating birds.

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## Research Note

# *Moniliformis clarki* (Acanthocephala: Moniliformidae) from the Pocket Gopher, *Geomys bursarius missouriensis*, in Missouri

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**ABSTRACT:** The North American moniliformid acanthocephalan, *Moniliformis clarki* (Ward, 1917) Chandler, 1921 (nec Van Cleave, 1924) is reported for the first time in Missouri from the pocket gopher, *Geomys bursarius missouriensis*. Intensity and mean intensity of *M. clarki* infection were 2–20 and 7.3 per individual, respectively. The diagnostic characteristics of proboscis armature and egg size conformed to those of *M. clarki*. Other anatomical structures were closer in size to those of the other cosmopolitan moniliformid acanthocephalan *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915.

**KEY WORDS:** *Moniliformis clarki*, pocket gopher, *Geomys bursarius missouriensis*, Missouri, new records.

Eleven species of the genus *Moniliformis* Travassos, 1915 have been described (Amin, 1985). Two of these species infect North American mammals. *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 (= *Moniliformis dubius* Meyer, 1932 designated for North American forms) is a cosmopolitan species that infects rats. It was first reported in the United States by Chandler (1921) from Houston, Texas. *Moniliformis clarki* (Ward, 1917) Chandler, 1921 (nec Van Cleave, 1924) is found only in North American squirrels, moles, chipmunks and deer mice, and less frequently in pocket gophers and skunks. It was first identified by Ward (1917), recognized as distinct by Chandler (1921), and described by Van Cleave (1924). The validity of the 2 North American species of *Moniliformis* has been established on morphological characteristics (Van Cleave, 1953; Buckner and Nickol, 1975a) and on experimental evidence (Buckner and Nickol, 1975b).

Fifty-one *Moniliformis clarki* (8 males, 43 females) were collected from 6 (3 males, 3 females) pocket gophers (*Geomys bursarius missouriensis* McLaughlin, 1958) trapped (using Victor gopher traps) 2.5 km NE of Chesterfield Airport, St. Louis County, Missouri (38°39'N, 90°39'W; elevation 154 m) on 26 March 1994. These are new host and geographical records. *M. clarki* was collected

from *Geomys bursarius illinoensis* in Illinois (Van Cleave, 1953). The intensity of *M. clarki* in each gopher ranged from 2–20 worms with a mean intensity of 7.3. These are considered light infections as compared to a record of 375 *M. clarki* specimens removed from the intestine of a single gray squirrel from Arkansas (Singleton et al., 1993).

Acanthocephalans were present throughout the whole intestine, where they were initially preserved in acetic acid-formalin-alcohol (AFA). Some were transferred to 75% ethanol, stained in acid carmine, dehydrated in ascending concentrations of ethanol, cleared in graded concentrations of terpineol in 100% ethanol, and mounted in Canada balsam. Measurements are in micrometers unless otherwise stated with means in parentheses following the range. Eggs were removed from the body cavities of 2 females before being measured.

Five males were 61–85 mm (72) long by 1.6–2.0 mm (1.8) maximum width and 25 females were 120–250 mm (156) long by 2.1–2.8 mm (2.4) wide. Specimens were identified as *M. clarki* based on their proboscis armature (12 or 13 longitudinal rows of 6 or 7 hooks each; largest hooks 23–28 (24) [ $N = 5$  males; not available in females], egg size 56–93 (81) long by 36–50 (43) wide) ( $N = 15$ ) and host. Other measurements from 5 males are: Proboscis 416–520 (463) long by 130–143 (137) wide. Proboscis receptacle 650–676 (658) long by 325–338 (330) wide. Lemnisci 3,462–4,793 (4,215) long by 132–182 (161) wide. Anterior testis 3,306–5,620 (4,417) long by 727–1,157 (903) wide. Posterior testis 3,957–4,297 (4,076) long by 723–1,171 (905) wide. All females were gravid with the internal structures obscured by eggs, retracted proboscides, and associated muscles.

The diagnostic characteristics of proboscis armature and sizes of hooks and eggs conformed to those of *M. clarki*. Other measurements were within the upper range of this species and the

“usual” range for *M. moniliformis*. These observations confirm the wide range of morphological variations reported for moniliformid acanthocephalans. These size variations could be a cause of some of the confusion on the taxonomic status of *M. clarki* and *M. moniliformis* in North America. Populations of these 2 acanthocephalan species exhibit considerable variability, depending on host and geographical distribution; see Chandler (1921, 1941), Van Cleave (1924, 1953), Petrochenko (1958), and Buckner and Nickol (1975a, b). This is the first morphological study of *M. clarki* from *G. bursarius missouriensis*.

**SPECIMENS:** Three male and 4 female *M. clarki* on 7 slides in the University of Nebraska State Museum, Harold W. Manter Laboratory Coll. 38227.

**HOSTS.** Skulls in the Museum of High Plains, Fort Hays, Kansas Coll. nos. 31075, 31077, 31108, 31122, 31126, 31135, 31141 (one gopher was not infected).

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#### Research Note

### The Raccoon as Intermediate Host of Three *Sarcocystis* Species in Europe

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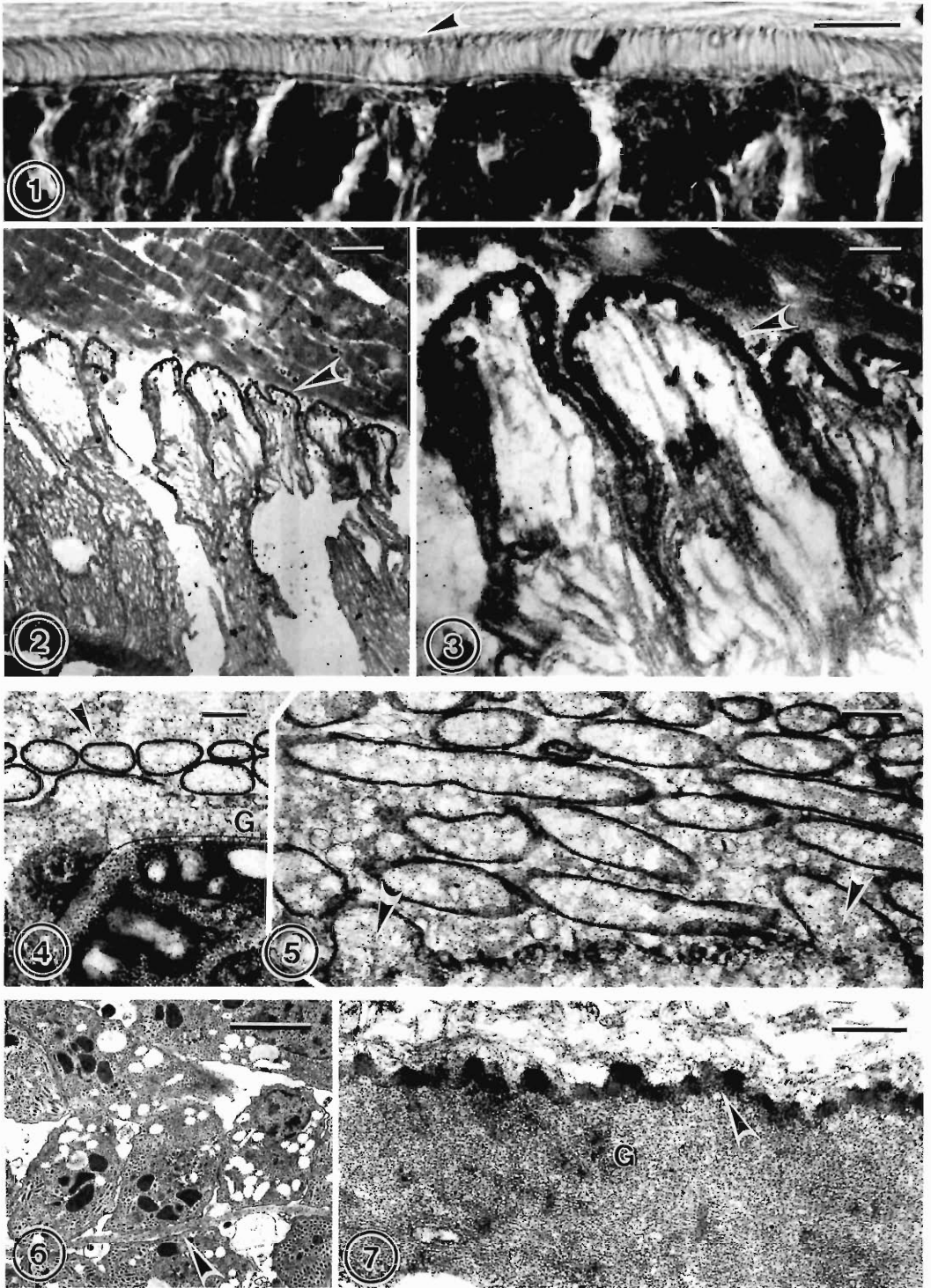
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**ABSTRACT:** One out of 12 raccoons from German Zoos was found to possess musculature infected by sarcocysts of 2 distinct *Sarcocystis* species (*S. sp. 1* and *S. sp. 2*). The cyst wall of *S. sp. 1* had fingerlike, and that of *S. sp. 2* hairlike, villar protrusions. Two out of 45 raccoons from a free-ranging population in Germany showed infection of the muscle by a third *Sarcocystis* species (*S. cf. sebeki*, without villar protrusions of the cyst wall). None of the 3 species is identical with *Sarcocystis kirkpatricki*, described from raccoons in North America.

**KEY WORDS:** raccoon, *Procyon lotor*, *Sarcocystis*.

Free-ranging raccoons, *Procyon lotor* L., in North America are known as intermediate hosts of *Sarcocystis kirkpatricki* Snyder et al., 1990. In Europe raccoons occur not only in zoos but also free in certain areas. Since we are unaware of reports of *Sarcocystis* spp. in raccoons in Europe, we decided to investigate whether the raccoons in North America and Europe are parasitized by the same or different *Sarcocystis* species.

Seven raccoons originated from the Leipzig



Figures 1-3. *Sarcocystis* sp. 1 from a zoo raccoon. Arrowheads point to the wall protrusions. 1. Light microscope photomicrograph of the sarcocyst in longitudinal section. Bar = 10  $\mu$ m. 2, 3. Transmission electron micrographs of the cyst wall and/or its villar protrusions. 2. Bar = 1.1  $\mu$ m. 3. Bar = 0.4  $\mu$ m.

Zoo, 5 from other East German zoos, and 45 animals were taken from a free-ranging population of the Buckow region (east of Berlin). For the history of this population see Grummt (1965), and for the integration into a parasitological research project see Lux and Priemer (1995). Muscle tissues were removed from esophagus and loin of the zoo animals and from tongue, esophagus, heart, chest, ribs, diaphragm, and thigh of the wild animals, and examined for the presence of sarcocysts by picking them to small pieces by means of fine needles and forceps under a dissecting microscope at 6.3-fold magnification. For histological examination, infected tissues were fixed in 4% formaldehyde, sectioned at 3 to 5  $\mu\text{m}$ , and stained with hematoxylin and eosin. The length of the bradyzoites was determined by measuring the more or less bent median line step by step from pole to pole, and the width was measured at the widest diameter. For transmission electron microscopy (TEM), portions of muscle from the single infected wild animal were fixed according to Pospischil and von Bomhard (1979). In the material from the zoo animal, a second *Sarcocystis* species was detected during the histological investigation. Therefore, this species could only be prepared for TEM investigation according to the re-embedding method described by Bergmann and Kinder (1987). For this purpose, the position of the sarcocyst within the musculature was marked on the back of the slide and the cover glass was removed. The section was first dipped into absolute ethanol, then into a mixture of ethanol and propylene oxide. The section was then covered with a mixture of glycid ether and propylene oxide, and a gelatin case filled with glycid ether was put on the marked place. After polymerization for 30 min at 105°C, the case was broken off and trimmed. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. All measurements are in micrometers unless stated otherwise. Abbreviations:  $\bar{x}$  = mean value, SD = standard deviation,  $N$  = number of elements measured.

Of the 12 raccoons from zoos, only one was

found to be infected with 2 *Sarcocystis* species, and only 2 of 45 free-ranging raccoons were infected by a third species.

#### *Sarcocystis* sp. 1 (Figs. 1–3)

ORIGIN: *Procyon lotor*, Leipzig Zoo, born in the zoo.

LOCALIZATION: Loin musculature.

DESCRIPTION: A single sarcocyst detected in histological sections, 99.5 wide by light microscopy; cyst wall thick, palisadelike, 4.6–6.9 wide ( $\bar{x}$  = 5.9, SD = 0.5,  $N$  = 50) by light microscopy; ground substance plus primary cyst wall 0.48–0.78 wide ( $\bar{x}$  = 0.62, SD = 0.09,  $N$  = 20) by electron microscopy, 0.46–1.20 ( $\bar{x}$  = 0.74, SD = 0.23,  $N$  = 50) with light microscopy. Fingerlike villar protrusions arise from cyst wall, closely packed, 5.8–7.4 long ( $\bar{x}$  = 6.1, SD = 0.4,  $N$  = 50) by light microscopy, with basal width of 1.12–1.38 ( $\bar{x}$  = 1.23, SD = 0.10,  $N$  = 5) in ultrathin sections; core of villar protrusions interwoven with microfilaments; surface of protrusions with small invaginations at short distances; invaginations 0.054–0.072 wide ( $\bar{x}$  = 0.059, SD = 0.005,  $N$  = 10) by electron microscopy; diameter of the compartments (created by septal invaginations) near cyst wall 9.2–19.9 ( $\bar{x}$  = 14.5, SD = 3.0,  $N$  = 20) at light microscopic level; depth in direction of center of sarcocyst 12.2–41.3 ( $\bar{x}$  = 27.2, SD = 6.9,  $N$  = 20).

#### *Sarcocystis* sp. 2 (Figs. 4, 5)

ORIGIN: *Procyon lotor*, Leipzig Zoo, born in the zoo.

LOCALIZATION: Wall of the esophagus, loin musculature.

DESCRIPTION: Two sarcocysts found, 1.8 and 1.9 mm long, 118–132 wide in the fresh state, extracted from muscle fibers; cyst wall thin with light microscopy; ground substance plus primary cyst wall 0.30–0.54 wide ( $\bar{x}$  = 0.38, SD = 0.06,  $N$  = 20) in ultrathin sections; numerous, predominantly diagonal and cross-cuts of hairlike

←  
 Figures 4, 5. *Sarcocystis* sp. 2 from a zoo raccoon. Transmission electron micrographs of the cyst wall. G = ground substance. Arrowheads point to the cuts of the hairlike villar protrusions. 4. Protrusions cut mainly crosswise. Bar = 0.4  $\mu\text{m}$ . 5. Protrusions cut mainly diagonally. Bar = 0.6  $\mu\text{m}$ .

Figures 6, 7. *Sarcocystis* cf. *sebeki* from a free-ranging raccoon. Transmission electron micrographs. 6. Section of the interior of a sarcocyst with bradyzoites and a septum (arrowhead). Bar = 1.7  $\mu\text{m}$ . 7. Section of the cyst wall consisting of the ground substance (G) and the membrane of the parasitophorous vacuole plus underlying osmiophilic layer with small invaginations (arrowhead). Bar = 0.4  $\mu\text{m}$ .

villar protrusions of cyst wall visible by electron microscopy; layer of these cut villar protrusions 0.50–1.26 wide ( $\bar{x}$  = 0.89, SD = 0.25,  $N$  = 20); villar protrusions 0.18–0.50 in diameter ( $\bar{x}$  = 0.35, SD = 0.08,  $N$  = 85), about 0.6 at bases; fine granulation visible in the interior of villar protrusions by electron microscopy; surface of cyst wall with small invaginations at short distances, 0.090–0.108 deep ( $\bar{x}$  = 0.101, SD = 0.011,  $N$  = 10) and 0.06–0.12 wide ( $\bar{x}$  = 0.088, SD = 0.019,  $N$  = 10) by electron microscopy; compartments 21.4–51.8 ( $\bar{x}$  = 29.9, SD = 9.5,  $N$  = 10) wide near cyst wall by light microscopy, depth in direction of center 19.5–48.8 ( $\bar{x}$  = 33.7, SD = 8.8,  $N$  = 10); bradyzoites 14.0–17.1 long ( $\bar{x}$  = 15, SD = 0.9,  $N$  = 30) and 3.4–3.9 wide ( $\bar{x}$  = 3.6, SD = 0.19,  $N$  = 30) by light microscopy, squashed out of extracted sarcocyst.

***Sarcocystis* cf. *sebeki***  
**(Tadros and Laarman, 1976)**  
**(Figs. 6, 7)**

**ORIGIN:** *Procyon lotor*, from the free-ranging population in the area of Buckow (east of Berlin).

**LOCALIZATION:** Muscle of loin, ribs, thorax, thighs, tongue, diaphragm, and wall of the esophagus.

**DESCRIPTION:** Sarcocysts 4–18 mm long and 27.5–112 (in histological sections 60.4–95.5) wide in the fresh state, extracted from muscle fibers; cyst wall thin and smooth at the light microscopic level, with no villar protrusions by electron microscopy, 1.06–1.71 wide ( $\bar{x}$  = 1.3, SD = 0.17,  $N$  = 30); surface of cyst wall with shortly spaced small invaginations, 0.072–0.126 deep ( $\bar{x}$  = 0.094, SD = 0.016,  $N$  = 20) and 0.060–0.126 wide ( $\bar{x}$  = 0.088, SD = 0.017,  $N$  = 20) by electron microscopy; compartments 6.9–16.2 wide near cyst wall ( $\bar{x}$  = 10.6, SD = 2.9,  $N$  = 10) and 8.5–14.6 ( $\bar{x}$  = 10.4, SD = 2.2,  $N$  = 10) deep in direction of center with light microscopy; bradyzoites 6.6–8.5 long ( $\bar{x}$  = 7.4, SD = 0.3,  $N$  = 50) and 1.3–1.8 wide ( $\bar{x}$  = 1.5, SD = 0.2,  $N$  = 50) by light microscopy, squashed out of extracted sarcocyst.

*Sarcocystis kirkpatricki* is the only known *Sarcocystis* species from raccoons in the U.S.A. (Seneviratna et al., 1975; Kirkpatrick et al., 1987). Its cyst wall is similar to type 11 (or lies between types 9 and 11) of the classification by Dubey et al. (1989) and differs distinctly in form and structure from all 3 species found by us in European raccoons.

The cyst wall of our *Sarcocystis* sp. 1 is similar

to type 10 of the classification by Dubey et al. (1989). Some features are recognizable (fingerlike outline of the villar protrusions and microfilaments in the core), although the TEM pictures are not optimal because of the re-embedding method applied. Thus it appears that the re-embedding method of Bergmann and Kinder (1987) allows a specific follow-up check of a paraffin section by means of TEM and allows ultrastructural characterization of the cyst wall at least in some cases. It is possible to get diagnostically usable ultrathin sections by means of this method that, however, cannot be compared with the sarcocysts embedded especially for TEM investigation in regard to the shrinkage and preservation state of the tissue (Figs. 2, 3). *Sarcocystis* sp. 1 is similar to *Sarcocystis* cf. *hofmanni*, a species occurring frequently in roe deer (*Capreolus capreolus*) in central Europe (see Sedlacek and Wesemeier, 1995), which is morphologically indistinguishable from *Sarcocystis hofmanni* Odening, Stolte, Walter, and Bockhardt, 1994, found in the European badger (*Meles meles*, Mustelidae) in Germany. Therefore, it is possible that the species usually occurring in roe deer sporadically infects badger and also raccoon.

*Sarcocystis* sp. 2 is morphologically indistinguishable from other species with hairlike villar protrusions of the cyst wall (type 7 of the classification by Dubey et al., 1989). From the zoo area and its environment, the following species would be in consideration: *Sarcocystis capreolicanis* (roe deer, see Sedlacek and Wesemeier, 1995), *S. cruzi* (cattle), *S. arieticanis* (sheep), and *S. hircicanis* (goats) (see Dubey et al., 1989). It is interesting that again a species from roe deer comes into question among them.

*Sarcocystis sebeki* was described from the musculature of *Apodemus sylvaticus* and *Mus musculus*. The definitive host is the tawny owl. Tadros and Laarman (1979) found similar sarcocysts in a European weasel and fed them to a tawny owl, in which a weak infection was obtained. Odening et al. (1994) described similar sarcocysts in European badgers (also mustelids), designating them as "*S. cf. sebeki*." Because the sarcocysts found in wild European raccoons are morphologically indistinguishable from those of weasel and badger, we use the same designation "*S. cf. sebeki*."

The occurrence of specific *Sarcocystis* species in the raccoon introduced into Europe is scarcely imaginable, because stable predator-prey relationships would not last with the raccoon as a

prey item in the absence of a predator regularly eating it. Thus it is possible that all 3 species found by us in European raccoons refer to "letters delivered to wrong address" which normally occur in other mammals. Voucher specimens are available from Institute of Zoo Biology and Wildlife Research, PF 1103, D-10252 Berlin, Germany: Collection of Protozoa, No. kT 68/60-W 1115/92 (histological sections of loin with *S. sp. 1*), and No. kT 66/57-W 24 (histological sections of tongue with *S. cf. sebeki*).

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### Research Note

## Helminths of Cetaceans on the Southeastern Coast of Brazil

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**ABSTRACT:** Seventy cetaceans accidentally captured in fishing nets in Rio de Janeiro State (Brazil) were dissected for parasites. *Sotalia fluviatilis* (Delphinidae) harbored *Braunina cordiformis*, *Halocercus brasiliensis*, and *Anisakis typica*. *Tursiops truncatus* (Delphinidae) was parasitized by *Nasitrema* sp. and *B. cordiformis*. *Steno bredanensis* (Delphinidae) had only *B. cordiformis*. *Sotalia fluviatilis* represents a new host record for *Braunina cordiformis* that is reported for the first time from Brazil. In an attempt to correlate these cetaceans' parasite infections with their food habits, a survey was made on fish of 20 species and *Loligo santapaulensis* (Cephalopoda) from the same area. Only *Bagre bagre*, *Macrodon ancylodon*, and *Nebris microps* contained *Anisakis* sp. larvae, a parasite species in-

fecting cetaceans. Lack of parasites in 42 *Pontoporia blainvillei* (Pontoporiidae) within our study area was probably related to the age of the hosts and differences in food habits between young and adults.

**KEY WORDS:** marine mammals, Cetacea, parasites, Brazil, *Braunina cordiformis*, *Anisakis typica*, *Halocercus brasiliensis*, *Nasitrema*.

Despite the long Brazilian coastline (about 8,700 km), the parasite fauna of marine mammals in this part of the Neotropical region is largely unknown. Previous studies in Brazil are restricted to only 2 reports: *Halocercus bras-*

*iliensis* Lins de Almeida, 1933 from the lungs of *Sotalia fluviatilis* (Gervais, 1853) (Delphinidae), from Guanabara Bay, Rio de Janeiro (Lins de Almeida, 1933), and *Anisakis alexandri* sp. nq. (Hsu and Hoeppli, 1933–1934) reported by Zam et al. (1970) in freshwater *Sotalia fluviatilis* imported from the upper Amazon river to Florida.

From September 1989 to June 1993, 70 cetaceans were accidentally captured throughout the year in fishing nets in Rio de Janeiro State, between Atafona (21°37'S, 41°02'W) and Macaé (22°23'S, 41°47'W): 42 *Pontoporia blainvillei* (Gervais and d'Orbigny, 1844) (Pontoporiidae), 23 *Sotalia fluviatilis* (Gervais, 1853) (Delphinidae), 4 *Tursiops truncatus* Montagu, 1821 (Delphinidae), and 1 *Steno bredanensis* Lesson, 1828 (Delphinidae).

Due to the fact that *Pontoporia blainvillei* and *Sotalia fluviatilis* are included in The Official List of Brazilian Fauna Threatened with Extinction, our sample of 70 cetaceans represents an important data set for the knowledge of the biology of these mammals and their helminths in the occidental South Atlantic Ocean.

Cetaceans were brought to the laboratory 24–48 h after death. In the necropsy, nasal cavity, mouth, and all internal organs were macroscopically examined in each animal. All parasites recovered were dead and some of them were damaged. For each host they were counted and fixed in 5% formalin.

To determine possible intermediate hosts, 117 fresh fish of 20 species and 28 squids from the same area were obtained from fishermen and examined for parasites. The trematodes were stained with alcoholic chlorhydric carmine (Langeron, 1949) and mounted in Canada balsam. Nematodes were studied after clearing in creosote, phenol, or acetic acid. The helminths were identified by one of us (C.P.S.) and are deposited in the Helminthological Collection of Oswaldo Cruz Institute, RJ with numbers 32.964 (*Nasitrema* sp.), 32.965 (*Braunina cordiformis*), 32.966 (*Anisakis typica*), and 32.967 (*Halocercus brasiliensis*).

In Rio de Janeiro State, of 23 *Sotalia fluviatilis* examined (12 males [107–193 cm long, mean = 165, SD = 27.3] and 11 females [158–193 cm long, mean = 177, SD = 10.6]), 19 were infected with the stomach trematode *Braunina cordiformis* Wolf, 1903 (mean intensity = 76, range 1–318, SD = 107.8, prevalence = 34%) and the nematodes *Anisakis typica* (Diesing, 1861) (stomach, mean intensity = 16, range 1–52, SD

= 19.9, prevalence = 48%) and *Halocercus brasiliensis* (lungs and trachea, mean intensity = 7, range 2–15, SD = 5.9, prevalence = 13%).

One of 4 specimens of *Tursiops truncatus* (3 males 184, 200, and 237 cm long, and one female 162 cm long), harbored 30 specimens of the trematode *Nasitrema* sp. in the nasal cavity and another 24 *Braunina cordiformis* in the stomach. Two were uninfected.

The single *Steno bredanensis* (female, 250 cm long) examined had 957 *Braunina cordiformis* in the stomach and the anterior part of the intestine.

Pearson's correlation test showed no significant correlation between sex and size of hosts (*S. fluviatilis*) with infections of *A. typica*, *H. brasiliensis*, and *B. cordiformis*, and with infections of all parasite species tested together, although all 4 uninfected *S. fluviatilis* were male and 2 of them were the smallest animals dissected (107 and 129 cm in length).

Forty-two *Pontoporia blainvillei* (18 males [14 measured: 95–118 cm long, mean = 105, SD = 6.9] and 24 females [21 measured: 74–141 cm long, mean = 116, SD = 17.4]) studied from the same area did not yield any parasites.

In order to find clues for these striking differences in infections of different host species, we looked for parasites in potential prey species we commonly find in the cetaceans' stomach in this region. Fishes examined by us include: Sciaenidae: 21 *Paralichthys brasiliensis* (Steindachner, 1875) (range 10–22.5 cm, mean = 16), 13 *Macrodon ancylodon* (Bloch and Schneider, 1801) (range 11–20 cm, mean = 16), 12 *Stellifer brasiliensis* (Schultz, 1945) (range 8–20 cm, mean = 14), 8 *Nebrius microps* Cuvier, 1830 (range 12–19 cm, mean = 15), 6 *Cynoscion leiarchus* (Cuvier, 1830) (range 9.5–16.5 cm, mean = 12), 3 *C. virescens* (Cuvier, 1830) (range 14–20 cm, mean = 17), 3 *Isopisthus parvipinnis* (Cuvier, 1830) (range 10.3–13.5 cm, mean = 11), and 1 *Menticirrus americanus* (Linnaeus, 1758) (15.2 cm); Stomateidae: 17 *Peprilus paru* (Linnaeus, 1758) (range 8.5–13 cm, mean = 10); Ariidae: 14 *Bagre bagre* (Linnaeus, 1766) (range 11–19.5 cm, mean = 16); Engraulidae: 6 *Anchoa spinifera* (Valenciennes, 1848) (range 12–17 cm, mean = 13); Tetraodontidae: 4 *Logocephalus laevigatus* (Linnaeus, 1766) (range 5–11 cm, mean = 7); Cynoglossidae: 3 *Symphurus tessellatus* (Quoy and Gaimard, 1824) (range 11–21 cm, mean = 16); Batrachoididae: 1 *Porichthys porosissimus* (Valenciennes, 1837) (20 cm); Haemulidae: 1 *Pomadasys corvinaeformis* (Steindachner, 1868) (10



cm); Ophichthidae: 1 *Ophichthys parilis* (Richardson, 1844) (45 cm); Pomadasyidae: 1 *Conodon nobilis* (Linnaeus, 1758) (10.5 cm); Soleidae: 1 *Trinectes paulistanus* (Ribeiro, 1915) (10 cm) and Trichiuridae: 1 *Trichiurus lepturus* Linnaeus, 1758 (37.5 cm).

Only one fish each of *Bagre bagre*, *Macrodon ancylodon*, and *Nebris microps* contained larval *Anisakis* sp., a species infecting cetaceans. Because loliginid squids also contribute to the diet of these cetaceans, 28 *Loligo sanpaulensis* Brakoniecki, 1984 (mantle length range 4–7 cm, mean = 5) were examined, but none of them were found infected.

Raga (1994) presented a list of parasites of *Tursiops truncatus* and *Steno bredanensis*. Previous studies of parasites of *Pontoporia blainvillei* revealed the following helminths: *Contraecium* sp. (Dailey and Brownell, 1972; Brownell, 1975, 1981; Praderi, 1984), *Polymorphus* (*P.*) *cetaceum* (Brownell, 1975, 1981; Praderi, 1985; Aznar et al., 1994); *Phyllobothrium delphini* (Testa and Dailey, 1976); *Anisakis typica* (Kagei et al., 1976; Praderi, 1984, 1985), *Procamallanus* sp. (Praderi, 1984, 1985), *Anisakis simplex* and Polymorphidae sp. (Aznar et al., 1994), *Hadwenius pontoporiae*, and *Pholeter gastrophilus* (Raga et al., 1994; Aznar et al., 1994). In *Sotalia fluviatilis* various authors reported *Amphimerus lancea*, *Halocercus brasiliensis* (Dailey and Brownell, 1972), *Anisakis typica*, and *A. alexandri* sp. inq. (Zam et al., 1970). For the first time, *Braunina cordiformis* is reported from the Brazilian coast, parasitizing 3 different hosts: *T. truncatus*, *S. bredanensis*, and *S. fluviatilis*. *Sotalia fluviatilis* constitutes a new host record for *Braunina cordiformis*.

The sample of *P. blainvillei* was not parasitized and this could be related to the age, size, and food habits of our specimens. Brownell (1984) reported that *Pontoporia* calves start taking solid food at about 3 months of age when animals are about 100 cm in length, and that males and females attain sexual maturity on average at lengths of about 131 and 140 cm, respectively, at a mean age of 2.7 years. The material we examined was composed mainly of younger individuals ranging from 74 to 141 cm long.

In the south of Brazil adults of *P. blainvillei* eat any prey as long as it is smaller than 50 mm and younger individuals generally eat more shrimps and squids than adults. The fish reported from their diet in southern Brazil (Pinedo, 1982) did not include any of those found to carry *An-*

*isakis* sp. in our area: *M. ancylodon* (total length 16.5 cm), *B. bagre* (total length 15 cm), and *N. microps* (total length 19 cm). On the other hand, *M. ancylodon* (larger than 1 cm) was found in the stomachs of *S. fluviatilis*, *S. bredanensis*, and *T. truncatus* studied by us. The lack of *A. typica* in *T. truncatus* and *S. bredanensis* is probably related to the small sample size, 4 and 1 hosts examined respectively.

In the Pontoporiidae studied, of which only 2 could be considered adults, age and preference for small prey (mainly squid instead of fish) could be a likely reason to explain the lack of infections of such hosts since the squid and small fish examined were not parasitized. The preference of *S. fluviatilis* and *T. truncatus* for prey larger than 100 mm reinforces this hypothesis. Absence of parasites in *Pontoporia blainvillei* in our study area could also be a local phenomenon related to the absence of suitable intermediate or transport hosts, or to the lack of infections of such hosts with cetacean parasites in our study area, since some helminth species were recovered from this host species by other researchers.

Raga et al. (1994) discussed the difference between the high prevalence of one trematode species, *Hadwenius pontoporiae*, found in *P. blainvillei* from Argentina, and the absence of this parasite in previous reports from Uruguayan waters just 500 km away. Aznar et al. (1994) also reported helminths in 46 *P. blainvillei*, with a mean age of 2.9 years, from Argentinean waters, suggesting that many characteristics of their helminth communities can at least partly be explained by ecological processes.

Studies on marine mammals generally do not report the age, size, and food habits of hosts, together with the occurrence of parasites. The lack of this information, especially in hosts with distinctive food habits, can make an understanding of the host–parasite relationship difficult. We suggest therefore that in future studies this information be included whenever possible.

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