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Development and Specificity of *Oochoristica javaensis* (Eucestoda: Cyclophyllidea: Anoplocephalidae: Linstowiinae)

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ABSTRACT: Because assumptions of strict host specificity and geographic isolation apparently have been used as criteria in determining species of *Oochoristica*, studies were conducted to address the effects that these assumptions could have on resolving the taxonomy of *Oochoristica*. Experimental infections of native fence lizards, *Sceloporus undulatus undulatus*, and Indo-Pacific geckos, *Hemidactylus garnotii*, demonstrated that the exotic tapeworm *Oochoristica javaensis* lacked host specificity. In addition, tapeworms with gravid proglottids, a stage of development that has not been previously reported for any species of *Oochoristica*, were obtained from both hosts. Evidence against the assumption of geographic isolation stems from the fact that lizard species known to harbor *Oochoristica* spp. have been introduced beyond their native ranges, and in some cases, these introductions predate the species descriptions. Lack of support for either assumption indicates a need for more rigorous analyses and experimentation to determine species of *Oochoristica*.

KEY WORDS: *Oochoristica javaensis*, Cestoda, *Hemidactylus turcicus*, *Hemidactylus garnotii*, *Sceloporus undulatus undulatus*, Mediterranean geckos, Indo-Pacific geckos, fence lizards, development, host specificity, biogeography, taxonomy.

Approximately 80 species have been described in the cosmopolitan genus *Oochoristica* Lühe, 1898 (Bursey and Goldberg, 1996a, b; Bursey et al., 1996, 1997; Brooks et al., 1999). These anoplocephalid cestodes predominantly parasitize lizards, but also snakes, turtles, and a few marsupials (Schmidt, 1986; Beveridge, 1994). Development has been examined for *Oochoristica vacuolata* Hickman, 1954, *Oochoristica osheroffi* Meggitt, 1934, and *Oochoristica anolis* Hardwood, 1932 (Hickman, 1963; Widmer and Olsen, 1967; Conn, 1985). Although larval and adult development has been examined for 3 species of *Oochoristica*, only Conn (1985) tried to determine host specificity experimentally. In his experiments, however, he was unable to infect wall lizards, *Podarcis muralis* (Laurenti, 1768) and mice, *Mus musculus* Linnaeus, 1758. Curiously, no other attempts have been made to determine the specificity of a species of *Oochoristica*. This is unfortunate in that, as Brooks et al. (1999) pointed out, one of the major criteria used in resolving the taxonomy of *Oochoristica* has been the assumption of a high degree of host specificity exhibited by species in this genus. They also mentioned restriction to particular geographic regions as a criterion that has ostensibly been used in the past to identify species of *Oochoristica*.

In a survey of helminths of the Mediterranean gecko, *Hemidactylus turcicus* (Linnaeus, 1758), from Louisiana, U.S.A., a species of *Oochoristica* was recovered (C. D. Criscione, unpublished data); however, there were difficulties in identifying this species. These problems were associated in part with the assumptions of strict host specificity and geographic isolation. It became apparent that in addition to the lack of specificity experiments, introduced and native host distributions were often ignored when identifying species of *Oochoristica*. Neither assumption has been properly addressed, and in order to validate the identification of any species of *Oochoristica*, these assumptions should be tested. In light of these problems with the taxonomy of *Oochoristica*, the primary objective of this study was to examine the development of *Oochoristica javaensis* Kennedy, Killick, and Beverley-Burton, 1982, and to test the assumption of specificity via experimental infections. In addition, we comment on the assumption of geographic isolation.

Materials and Methods

To minimize variation among hosts, gravid proglottids of *O. javaensis* were obtained from 15 worms recovered from the small intestine of a single female

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Table 1. Measurements of *Oochoristica javaensis* from experimentally infected *Hemidactylus turcicus* (HETU), *H. garnotii* (HEGA), and *Sceloporus u. undulatus* (SCUN) for days 1–30 postexposure; measurements in μm unless noted otherwise.

	HETU	HEGA		SCUN	
	Day 1 n = 4*	Day 7 n = 1	Day 28 n = 4	Day 10 n = 2	Day 30 n = 2
Total L† (mm)	0.26–0.37 (0.3 ± 0.03)‡	0.52	5.97–10.5 (8.32 ± 0.95)	0.36–0.37 (0.37 ± 0.01)	5.16–5.2 (5.18 ± 0.02)
Proglottid No.	0	0	30–47 (38.5 ± 3.6)	0	25–34 (29.5 ± 4.5)
Scolex W	109–129 (120 ± 4.4)	125	98–137 (117 ± 8.6)	117–133 (125 ± 8)	113–164 (139 ± 25.5)
L	44–101 (81.2 ± 13.1)	78	66–105 (84.8 ± 8.0)	82–86 (84 ± 2)	70–113 (91.5 ± 21.5)
Sucker W	43–51 (46 ± 1.9)	51	39–51 (45 ± 2.6)	51 (51 ± 0.0)	39–66 (52.5 ± 13.5)
L	51–74 (58.7 ± 5.2)	59	47–59 (53 ± 2.6)	55–62 (59 ± 3.5)	51–78 (64.5 ± 13.5)
Neck W	N/A§	N/A	125–179 (146 ± 13.1)	N/A	48–176 (162 ± 14)
L (mm)	N/A	N/A	0.65–1.32 (0.91 ± 0.14)	N/A	0.89–1.12 (1.01 ± 0.12)

* Number of worms used for measurements, which in this table, also refers to the sample size of each character measured.
† L = length, W = width.
‡ Range followed by mean ± 1 SE in parentheses.
§ N/A = not applicable.

Mediterranean gecko that was collected from Louisiana State University (LSU) in Baton Rouge, Louisiana, U.S.A. (30°24.92′N; 91°10.81′W). Laboratory-raised flour beetles, *Tribolium castaneum* Herbst, 1797, were used as potential intermediate hosts in the experiments. Groups of 10 flour beetles were placed in 100- × 15-mm plastic petri dishes lined with filter paper; the beetles were starved for 48 hr. Eight gravid proglottids were removed from each of the 15 worms, lightly dusted with flour beetle medium from Carolina Biological Supply Company, and placed in a single dish. Each petri dish contained gravid proglottids from a different parental worm. After 24 hr of exposure, the filter paper was replaced and beetles were fed ad libitum with flour beetle medium and slices of potatoes. Flour beetles were maintained at 25 ± 1°C until necropsy. Metacestodes recovered from *T. castaneum* on day 60 postexposure (PE) were used to infect experimental definitive hosts that included *H. turcicus* (n = 16), *Anolis carolinensis* Voigt, 1832 (green anole, n = 10), *Hemidactylus garnotii* Duméril and Bibron, 1836 (Indo-Pacific gecko, n = 10), *Sceloporus undulatus undulatus* (Bosc and Daudin, 1801) (southern fence lizard, n = 5), and *Rana sphenocephala* Cope, 1886 (southern leopard frog, n = 5). Indo-Pacific geckos were ordered from Glades Herp Inc. in Florida, U.S.A.; the fence lizards and tadpoles of the leopard frogs that had undergone metamorphosis in the laboratory were obtained from Glenn's Pond in Harrison County, Mississippi, U.S.A. Green anoles were caught on the campus of Southeastern Louisiana University in Hammond, Louisiana (30°30.67′N; 90°27.98′W), and Mediterranean geckos were collected in Fairview Riverside State Park, Madisonville, Louisiana (30°24.55′N; 90°08.41′W). To determine if the experimental hosts were naturally infected with tapeworms, feces were examined for proglottids 2 wk prior to infection. Only specimens not shedding proglottids were used in experimental infections.

Each potential definitive host was inoculated via stomach tube with 10 metacestodes obtained from the day 60 PE flour beetles. Experimental definitive hosts were maintained at 25 ± 4°C in 11.36-liter containers (40.64 × 27.94 × 15.24 cm) and provided refuge. Hosts were fed ad libitum with laboratory-reared crickets and mealworms and given a constant supply of water. Experimental vertebrate hosts were killed using an overdose of ether, and the body cavity, musculature, and all internal organs were examined for helminth parasites under a dissecting microscope. Tapeworms were killed with hot water (90°C), fixed and stored in alcohol–formalin–acetic acid (AFA), stained in Semichon's acetocarmine, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. All measurements are in μm unless specified otherwise. We deposited voucher specimens of *O. javaensis* from *H. turcicus* in the United States National Parasite Collection (USNPC) (nos. 90344–90348). Specimens from experimental infections are available upon request from the senior author.

Results

Tribolium castaneum was readily infected with *O. javaensis* and represents a new inter-

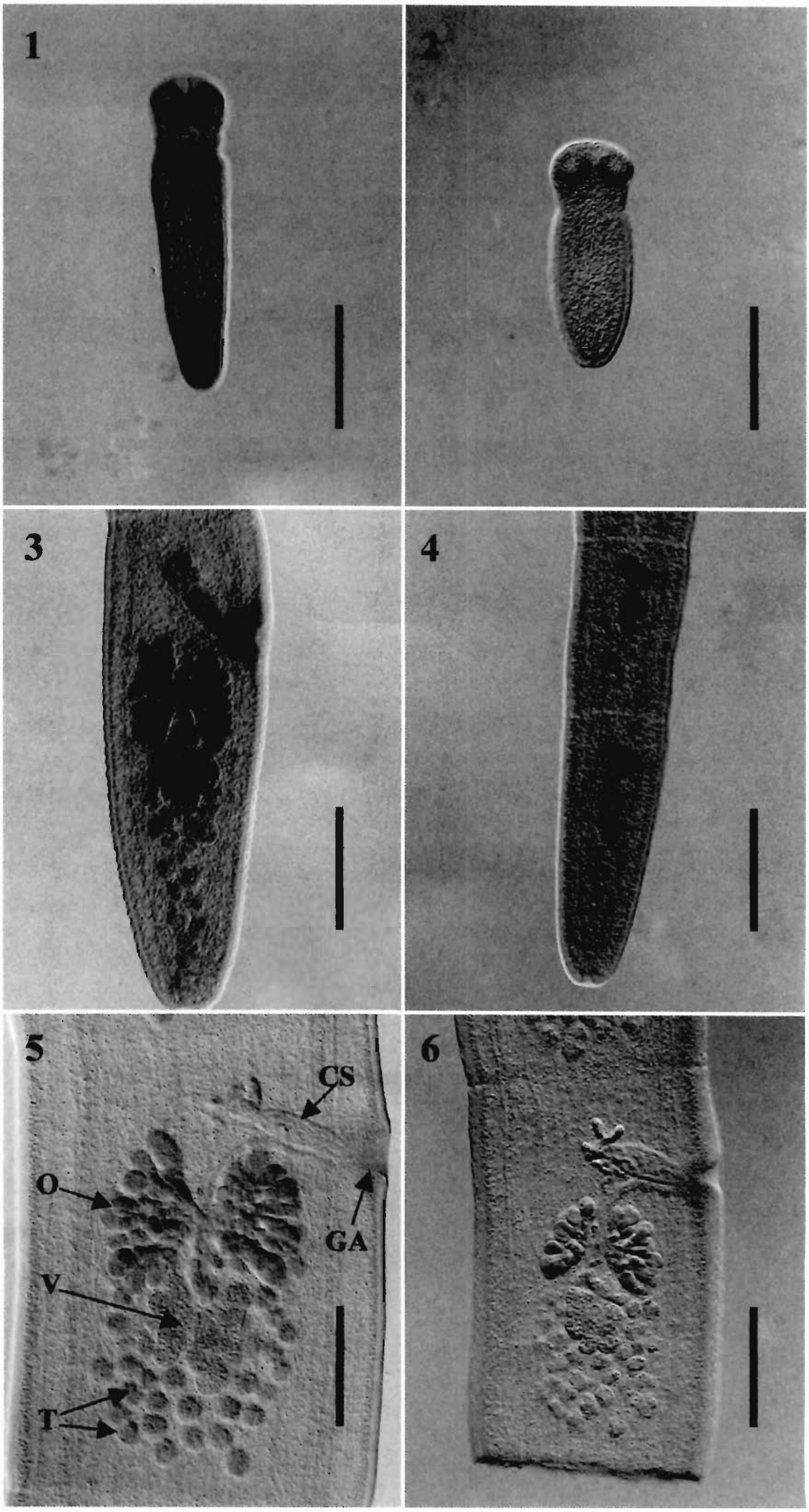
Table 2. Measurements of *Oochoristica javaensis* obtained from experimentally infected *Hemidactylus garnotii* (HEGA), and *Sceloporus u. undulatus* (SCUN) for day 105 postexposure; measurements in µm unless noted otherwise.

Variable		HEGA Sample size*	HEGA n = 5†	SCUN Sample size	SCUN n = 1
Total	L‡ (mm)	5	61.6–86.9 (67.7 ± 48.3)§	1	54.5
Proglottid number		5	109–144 (128 ± 5.7)	1	140
Neck	W	5	166–229 (191 ± 10.7)	1	221
	L (mm)	5	1.16–1.79 (1.52 ± 0.12)	1	1.12
Scolex	W	5	90–191 (152 ± 17.7)	1	218
	L	5	78–277 (140 ± 36.3)	1	164
Sucker	W	5	35–90 (61.8 ± 9.5)	1	82
	L	5	47–82 (66.4 ± 6.4)	1	105
Immature proglottid	W	15	482–561 (520 ± 6.6)	3	379–403 (390 ± 7.1)
	L	15	300–387 (348 ± 7)	3	427–466 (443 ± 11.9)
Genital pore position‖		15	0.24–0.28 (0.27 ± 0.004)	3	0.24–0.28 (0.26 ± 0.01)
Mature proglottid	W	15	593–695 (643 ± 9.1)	3	403–411 (406 ± 2.7)
	L	15	616–790 (712 ± 12.4)	3	648–695 (679 ± 15.7)
Cirrus sac	W	15	43–51 (46.7 ± 0.47)	3	47–51 (49.7 ± 1.3)
	L	15	137–164 (146 ± 1.99)	3	109–121 (117 ± 4.0)
Ovary	W	15	265–351 (314 ± 6.0)	3	168–211 (91 ± 12.6)
	L	15	195–265 (230 ± 5.1)	3	133–187 (159 ± 15.7)
Vitellaria	W	15	137–191 (169 ± 4.5)	3	86–105 (96.3 ± 5.6)
	L	15	82–144 (118 ± 4.3)	3	82–101 (89.7 ± 5.8)
Testis	W	15	39–47 (43.5 ± 0.77)	3	35–39 (36.3 ± 1.3)
	L	15	35–55 (46.2 ± 1.3)	3	39–43 (41.7 ± 1.3)
Testes number		15	23–35 (30.8 ± 0.78)	3	21–30 (26.3 ± 2.7)
Gravid proglottid	W	15	571–749 (629 ± 12.6)	3	433–473 (453 ± 11.6)
	L (mm)	15	1.50–2.28 (1.92 ± 0.05)	3	1.48–1.52 (1.49 ± 0.01)
Oncosphere	W	15	20–34 (25.9 ± 1.04)	3	22 (22 ± 0.0)
	L	15	16–28 (21.5 ± 0.79)	3	18–20 (19.3 ± 0.67)
Hook	L	15	10–12 (11.6 ± 0.21)	3	12 (12 ± 0.0)

* Sample size refers to the total number of each character that was measured.
† Number of tapeworms measured.
‡ L = length, W = width.
§ Range followed by mean ± 1 SE in parentheses.
‖ Genital pore position was calculated as a ratio of the position along the length of the mature proglottid from the anterior end (length to the center of the genital pore ÷ length of proglottid).

mediate host for the genus *Oochoristica*. *Oochoristica javaensis* became established in 7 individuals of the experimental definitive hosts. Successful infection occurred in only 1 of 16 *H. turcicus*; this Mediterranean gecko was examined on day 1 PE and had an intensity of 4. *Oochoristica javaensis* was recovered from 3 of 10 *H. garnotii* on days 7, 28, and 105 PE and had intensities of 1, 7, and 6, respectively. Three of 5 *S. u. undulatus* were infected with 2, 3, and 1 tapeworms on days 10, 30, and 105 PE, respectively. The 10 *A. carolinensis* and 5 *R. sphenocephala* were negative for infections. Measurements for specimens from experimental infections are in Tables 1 and 2.

Proglottid formation did not occur prior to day 28 PE (Figs. 1 and 2). Terminal proglottids of tapeworms recovered on day 28 PE from *H. garnotii* had developing ovaries, testes, vitellaria, and cirrus sacs (Fig. 3). A median terminal excretory pore was present in each terminal proglottid, and in 1 worm there was a developed genital atrium. Terminal proglottid length ranged from 514 to 822 (mean = 659, SE = 38.7, n = 7) and width from 174 to 277 (mean = 216, SE = 12.4, n = 7). Two of the worms from *S. u. undulatus* examined on day 30 PE also had developing reproductive organs (Fig. 4). One measured 442 × 158 (L × W); the other had been torn. The third tapeworm recovered from day 30 PE had been damaged in the mounting process; however, prior examination had shown that no more than 15 proglottids had formed, sexual primoridia had just begun to develop, and total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length did not exceed 1.2 mm. By day 105 PE, development of *O. javaensis* progressed to strobilas with gravid proglottids in both *H. garnotii* (Fig. 5) and *S. u. undulatus* (Fig. 6). Although it was possible that tapeworms from day 105 PE were natural infections because experimental definitive hosts were not laboratory-raised, prior fecal examinations were negative for all hosts used in experiments.

Discussion

Development of *Oochoristica javaensis*

Prior to the present study, the most developed stage experimentally obtained for a species of *Oochoristica* was a terminal mature proglottid (Conn, 1985). Our experimental infections with *O. javaensis* were successful in obtaining gravid specimens. Susceptible definitive hosts for *O. javaensis* included *H. turcicus, H. garnotii,* and *S. u. undulatus*; however, the fact that only 1 of 16 control hosts, *H. turcicus,* became infected suggests that exposure techniques may have been flawed. Possible problems may have been the temperature at which experimental hosts were housed or the inoculation method. When dealing with small hosts, the stomach tube may not be the best method, and another, such as placing metacestodes in gel capsules, may prove to be more efficient. Despite the scarcity of infections, however, the developing worms obtained from *H. garnotii* and *S. u. undulatus* indicated a lack of specificity for *O. javaensis.*

On day 105 PE, Conn (1985) recovered a single specimen of *O. anolis* from *A. carolinensis* that had mature proglottids with fully formed male and female reproductive systems. The terminal proglottid, however, still had a median excretory pore, suggesting that this specimen had not yet shed any proglottids. Specimens of *O. javaensis* from *H. garnotii* on day 28 PE also had median excretory pores in the terminal proglottids. Although most of the specimens that we recovered from day 28 PE did not have fully developed reproductive organs, their stage of development had greatly surpassed the developmental stage of *O. anolis* from day 28 PE (Conn, 1985). *Oochoristica anolis* from green anoles on day 28 PE had just begun to form proglottids with genital anlages and had a maximum total length of 3.25 mm (Conn, 1985). Widmer and Olsen (1967) reported immature *O. osheroffi* with a maximum total length of 4.14 mm on day 28 PE in the prairie rattlesnake, *Crotalus viridis* Rafinesque, 1818, but the mean total worm length from our study for day 28 PE was 8.32 mm (Table 1). These comparisons suggest a more rapid development in the definitive host for *O. javaensis* than for *O. anolis* or *O. osheroffi.* However, small sample sizes in our study and infection techniques differing from previous life cycle studies of *Oochoristica* spp. prevented definitive comparison of developmental patterns among species. Likewise, the low number of infections precluded examination of host-induced variation for *O. javaensis.* Although development in *S. u. undulatus* appeared slightly slower than in *H. garnotii* up to day 30 PE (Table 1), measurements of gravid specimens from *H. garnotii* and *S. u. undulatus* on day 105 PE (Table 2) may suggest plasticity for some characters.

Host specificity and geographic isolation

Results from our study experimentally demonstrated for the first time that a single species of *Oochoristica* can infect more than 1 species of host. *Oochoristica javaensis* infected hosts representing 2 unrelated lizard families, Phrynosomatidae for *S. u. undulatus* and Gekkonidae for *H. garnotii* (Estes et al., 1988; Pough et al., 1998). It is not known if the ecology of either *S. u. undulatus* or *H. garnotii* would predispose natural populations of these hosts to the establishment of *O. javaensis.* Development in different hosts demonstrated via our laboratory experiments, however, raises questions with regard to the use of host specificity as a taxonomic criterion for species of *Oochoristica.* Specificity in the laboratory and in the field should be examined for other species of *Oochoristica* in light of these results for 2 reasons. First, lack of speci-

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Figures 1–6. Development of *Oochoristica javaensis* from *Hemidactylus garnotii* (HEGA) and *Sceloporus u. undulatus* (SCUN) at different days postexposure. Photomicrographs were taken with differential interference contrast. Bars = 200 μm. 1. Day 7 in HEGA. 2. Day 10 in SCUN. 3, 4. Terminal proglottids from day 28 in HEGA and day 30 in SCUN, respectively. 5, 6. Mature proglottids from day 105 in HEGA and SCUN, respectively. CS = cirrus sac, GA = genital atrium, O = ovary, T = testis, and V = vitellaria.

ficity means that tapeworms of the same species will be exposed to different environments in different species of hosts, thus presenting opportunities for host-induced variation. If variation is induced, then this may affect the current morphometrically based taxonomy of species of *Oochoristica.* Second, a better understanding of the degree to which species of *Oochoristica* can switch hosts is imperative in light of the conservation implications associated with introduced organisms and their parasites (see Barton, 1997).

Introduced lizards will have consequences not only for conservation, but also for parasite taxonomy. If in the past, species of *Oochoristica* have been transmitted with their exotic lizard hosts, then current assumptions of biogeographic isolation may be incorrect. This is not to say that there was never a biogeographic pattern that paralleled *Oochoristica* speciation, but especially because of anthropogenic effects, species of *Oochoristica* may have colonized new areas before many of them were ever described (see Bursey et al. [1996] for a list of authority dates). This possibility exists because records of some introduced geckos, i.e., *H. turcicus* in Florida (Stejneger, 1922) and *Hemidactylus mabouia* (Moreau de Jonnès, 1818) in South America and the Caribbean (Kluge, 1969), predate many *Oochoristica* species descriptions.

It is interesting to note that several authors (Bursey and Goldberg, 1996b; Bursey et al., 1996; Brooks et al., 1999) listed *O. vanzolinii* as a Neotropical species of *Oochoristica* from Brazil without regard to the fact that it was described from the introduced house gecko, *H. mabouia* (Rêgo and Oliveira Rodrigues, 1965). It has been hypothesized that *H. mabouia* naturally colonized the New World via rafting or was transported during the slave trades over 400 yr ago (Kluge, 1969). In either case, *H. mabouia* colonized the New World from Africa, thus presenting the opportunity for parasite transport. It is also interesting to note that in describing *O. javaensis,* Kennedy et al. (1982) listed *O. vanzolinii* as the species that most resembled their specimens.

Brooks et al. (1999) stated that both assumptions, specificity and geographic isolation, were not evidence for new species, and suggested that in describing new species many morphological characters should be provided. We strongly support their contention; however, the full extent to which certain features are plastic is still unknown for this genus. As indicated by Criscione and Font (2001), proglottid morphology of *O. javaensis* exhibited a high degree of plasticity that may have resulted from a crowding effect (Read, 1951). Providing more characters may alleviate some problems, but it will not solve the underlying difficulties associated with the taxonomy of *Oochoristica.* The morphologically based taxonomy of *Oochoristica* will only be validated upon experimentation establishing the variation of characters within the genus and/or the use of molecular data.

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Artifactual and Natural Variation of *Oochoristica javaensis*: Statistical Evaluation of In Situ Fixation

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ABSTRACT: Lack of knowledge of the extent of natural morphological variation can undermine proper taxonomic decisions. Confounding this problem is artifactual variation that arises from improper fixation techniques. For the morphologically based taxonomy of the cestode genus *Oochoristica,* little information exists on the plasticity of important taxonomic characters. In addition, paratypes of several species of *Oochoristica* are highly contracted and contorted. These paratypes were recovered from preserved hosts; thus, the tapeworms were killed and fixed inside the host (in situ fixation). Experiments demonstrated that in situ fixation of *Oochoristica javaensis* results in highly contracted specimens, and statistical comparisons between relaxed and in situ-fixed tapeworms revealed significant differences for proglottid measurements. Natural variation for the paratypes recovered from preserved hosts is likely misrepresented by the artificial variation induced by in situ fixation. Lastly, data from natural infections suggested that proglottid characters of *O. javaensis* are plastic and may be subject to crowding effects.

KEY WORDS: *Oochoristica javaensis,* Cestoda, *Hemidactylus turcicus,* Mediterranean gecko, fixation techniques, crowding effects, morphological variation, taxonomy.

The taxonomy of the cestode genus *Oochoristica* Lühe, 1898, has been based solely on morphology without knowledge of the extent of natural intraspecific morphological variation. Parasite morphological variation may be the result of genetic determinants, host-induced effects, parasite intensity effects, or external habitat influences. Stunkard (1957) and Haley (1962) discussed the importance of environmental and host-induced variation for the systematics of helminth parasites, citing such factors as different host species, host age, host diet, or infection intensity as causes of variation. They also emphasized the need to assess experimentally the stability of taxonomic characters when identifying a species.

In addition to the lack of knowledge on intraspecific variation, natural variation of some species of *Oochoristica* may be masked by artificial morphological variation induced by fixation techniques. Several paratype specimens of *Oochoristica* examined from the U.S. National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A. were highly contracted and contorted. Examination of the respective species descriptions revealed that these paratypes (listed below) were obtained from formalin-fixed hosts. That is, they were removed from host specimens deposited in museum collections without regard to the effects of host fixation on internal parasites. Bakke (1988) and others have qualitatively illustrated the distorting effects of improper fixation techniques on the morphology of soft-bodied helminths, but comparisons of fixation techniques have not been tested statistically to examine for quantitative differences in the measurements of important taxonomic characters.

The purpose of our report was to provide a quantitative assessment of the artifactual morphological variation induced by killing and fixing tapeworms within a host, i.e., in situ fixation. In order to address the effects that improper fixation methods may have on the morphologically based taxonomy of *Oochoristica,* statistical comparisons of in situ-fixed tapeworms to ones that were collected alive and killed in a relaxed state were conducted with specimens of *Oochoristica javaensis* Kennedy, Killick, and Beverley-Burton, 1982. In addition, we provide data regarding the effects of intensity on *O. javaensis* morphology.

Materials and Methods

Fixation experiments

To mimic lizard fixation techniques used for museum collections, 15 Mediterranean geckos, *Hemidactylus turcicus* (Linnaeus, 1758), were collected from the campus of Louisiana State University (LSU), Baton Rouge, Louisiana, U.S.A. (30°24.92′N; 91°10.81′W), where they were known to have a high prevalence of

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infection with *O. javaensis* (C. D. Criscione, unpublished data). Geckos were killed using an overdose of ether and immediately fixed via subcutaneous, oral cavity, and body cavity injections of unheated 10% formalin. Oral cavity injections insured that the tapeworms were killed immediately. After 6 days in 10% formalin, geckos were soaked in water for 24 hr to remove the formalin. Geckos were then transferred to 70% ethanol for storage until dissection 4 days later. In situ-fixed tapeworms recovered upon necropsy were stored in 70% ethanol, stained in Semichon's acetocarmine, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. Comparisons were made with relaxed tapeworms that were killed with hot water (90°C) and fixed and stored in alcohol–formalin–acetic acid solution (AFA). Relaxed worms were obtained in a helminth survey of *H. turcicus* from LSU in the summer of 1998 (C. D. Criscione, unpublished data); staining and mounting techniques were the same as for the in situ-fixed specimens.

Quantitative analyses included measurements of mature proglottids from 5 relaxed and 5 in situ-fixed specimens of *O. javaensis.* Three mature proglottids, located just anterior to the first proglottid displaying evidence of egg production, were selected from each individual. Length and width were measured for each mature proglottid and for the ovary, vitellaria, and 1 testis within each proglottid. One testis was randomly selected from each proglottid, ovary length was measured for the ovary lobe opposite the genital atrium, and ovary width was measured across both lobes. Although multiple testes are present within a single proglottid, only 1 was chosen in order to facilitate the use of appropriate statistical tests. In order to test for in situ-fixation effects, a nested ANOVA design was used to control the pseudoreplication of measuring 3 proglottids from 1 tapeworm. Tapeworms nested within type of fixation constituted the experimental units, i.e., true replicates, with the error term being the proglottids, i.e., pseudoreplicates, nested within individual tapeworms. Principal components analysis (PCA) with Varimax rotation was used as a data reduction technique and to examine latent relationships among the variables. A variable was considered to load on a factor if its correlation to the factor was $>|0.5|$ (Hair et al., 1999). The resulting factors with their standardized factor scores were then tested for differences between relaxed and in situ-fixed tapeworms in the nested design. Statistical significance was determined at $P < 0.05$.

Analysis of natural morphological variation

The analysis of intensity effects on the morphology of *O. javaensis* included 5 tapeworms from each of 3 Mediterranean geckos that were naturally infected with 15, 28, and 64 tapeworms. Criteria and morphological characters used for measurements were the same as those used in the fixation experiments. Experimental design using factor scores from a PCA with Varimax rotation was also the same, in that a nested ANOVA was used to test for intensity effects. A priori contrasts of 15 versus 28 and 28 versus 64 were computed. In order to conduct this analysis, the 5 tapeworms from each intensity level were treated as true replicates, when in fact they were pseudoreplicates.

In addition, 10 relaxed tapeworms that were killed with hot water (90°C) were selected to provide measurements representative of *O. javaensis* recovered in a helminth survey of *H. turcicus* (C. D. Criscione, unpublished data). This representative data set included specimens from geckos with intensities ranging from 1 to 64, and had at least 1 tapeworm from each of 5 collection locations in southeastern Louisiana, U.S.A. [Bayou Segnette State Park in Westwego (29°53.18′N; 90°09.80′W); Fairview-Riverside State Park in Madisonville (30°24.55′N; 90°08.41′W); a residential neighborhood in Metairie (30°00.76′N; 90°08.90′W); Southeastern Louisiana University in Hammond (30°30.67′N; 90°27.98′W); and LSU]. PCA with Varimax rotation was applied to this data set to examine for latent relationships among the same variables used in the fixation and intensity analyses.

Specimens examined

Museum specimens examined from the USNPC and the Canadian Museum of Nature (CMNPA), Ottawa, Ontario, Canada included the following: *O. javaensis,* 2 paratypes (CMNPA nos. 1982-0693, 1982-0695); *Oochoristica anolis* Hardwood, 1932, 1 voucher (USNPC no. 75748) and the holotype (USNPC no. 30898); *Oochoristica bezyi* Bursey and Goldberg, 1992, 2 paratypes (USNPC no. 81874); *Oochoristica bresslaui* Fuhrmann, 1927, 1 voucher (USNPC no. 89087); *Oochoristica chinensis* Jensen, Schmidt, and Kuntz, 1983, 2 paratypes (USNPC no. 077168); *Oochoristica islandensis* Bursey and Goldberg, 1992, 1 paratype (USNPC no. 82225); *Oochoristica macallisteri* Bursey and Goldberg, 1996, 1 voucher (USNPC no. 89267) and 2 paratypes (USNPC no. 86196); *Oochoristica mccoyi* Bursey and Goldberg, 1996, 2 vouchers (USNPC nos. 85403, 85408) and 1 paratype (USNPC no. 86343); *Oochoristica novaezealandae* Schmidt and Allison, 1985, 5 paratypes (USNPC no. 78407); *Oochoristica osheroffi* Meggitt, 1934, 1 voucher (USNPC no. 80433); *Oochoristica parvula* (Stunkard, 1938), 3 vouchers (USNPC no. 84397); *Oochoristica piankai* Bursey, Goldberg, and Woolery, 1996, 1 voucher (USNPC no. 88189) and 1 paratype (USNPC no. 84589); *Oochoristica scelopori* Voge and Fox, 1950, 2 vouchers (USNPC nos. 84234, 87529). We deposited voucher specimens of *O. javaensis* from *H. turcicus* in the USNPC (nos. 90344–90348).

Results

For the fixation analysis, PCA revealed 3 latent variables from the 8 measured (Table 1). Examination of the variable factor loadings revealed that factor 1 consisted of all the vertical measurements, while factors 2 and 3 were characterized by horizontal measures. Factors 1, 2, and 3 were renamed vertical, horizontal-1, and horizontal-2, respectively. All 3 factors showed significant worm-to-worm variation within each type of fixation (F_{vertical} = 9.20, $F_{\text{horizontal-1}}$ =

Table 1. *Oochoristica javaensis*: variable factor loadings, factor eigenvalues, and percent total variance accounted for by each factor from the Varimax rotated correlation matrix of the fixation data set.

	Varimax rotated loading matrix		
	Factor 1 (vertical)	Factor 2 (horizontal-1)	Factor 3 (horizontal-2)
Testis length	**0.917***	−0.112	0.209
Ovary length	**0.875**	−0.176	0.209
Vitellaria length	**0.863**	−0.237	0.262
Proglottid length	**0.805**	−0.497	0.023
Ovary width	−0.203	**0.912**	−0.082
Proglottid width	−0.236	**0.869**	−0.096
Testis width	−0.092	0.469	**−0.835**
Vitellaria width	0.463	0.177	**0.793**
Eigenvalues	3.320	2.184	1.498
Percent of total variance explained by the factor	41.495	27.296	18.723

* Bold print shows loadings where variable loaded onto factor.

42.20, $F_{horizontal\text{-}2} = 5.31$, df = 8, 20, $P < 0.001$); thus, the fixation main effect was tested with the mean-square values of the subgroups, tapeworms nested within fixation method. The vertical factor showed a significant effect between in situ-fixed and relaxed tapeworms (Fig. 1) ($F_{1,8} = 11.927$, $P = 0.009$); however, neither horizontal factor was significant. Table 2 provides the raw measurements of the variables used in the analysis.

PCA revealed that the 8 variables used in the intensity data set constituted only 1 factor (Table 3), thus showing that all 8 characters from relaxed specimens varied together. Even though there was significant worm-to-worm variation for this factor ($F_{12,30} = 12.62$, $P < 0.001$), there was still a significant intensity effect ($F_{2,12} = 13.42$, $P < 0.001$) (Fig. 2). The a priori contrast between 15 and 28 was significant ($F_{1,12} = 10.58$, $P = 0.007$), but 28 versus 64 was not. Figure 3A–C displays mature proglottid variation for tapeworms recovered from intensities of 15, 28, and 64, and Table 4 gives the raw measurements of the variables. Measurements of 10 *O. javaensis* tapeworms from Louisiana are given in Table 5. Based on results from the fixation experiments, only tapeworms exhibiting little to no wrinkling (i.e., contraction) were used to provide the representative measurements of *O. javaensis* collected in our survey. The fact that the 8 variables in the representative data emerged as only 1 factor from the PCA (Table 3) again demonstrated that all 8 characters from relaxed specimens varied together.

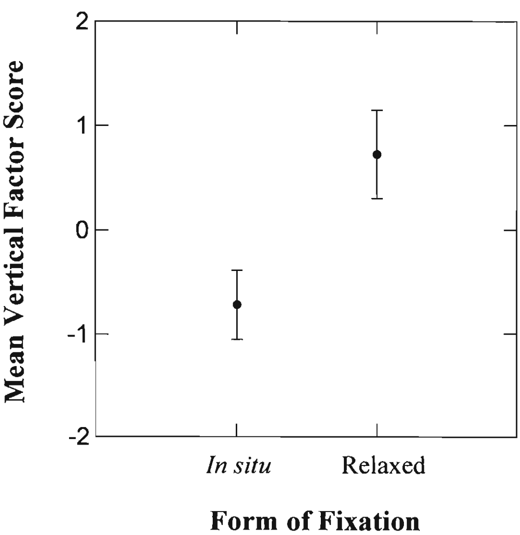

Figure 1. Plot of the mean factor scores and 95% confidence intervals for the vertical factor of relaxed and in situ-fixed specimens of *Oochoristica javaensis*.

Discussion

Variation resulting from different fixation techniques or conditions only confounds taxonomic problems in which the range of natural morphological variation is not known. Experimental data revealed 2 quantitative problems with using in situ-fixed tapeworms. The first was that a significant reduction in the vertical factor without a significant change in horizontal factors produced an accordion effect. High vertical factor scores were determined by large length measurements of the proglottid and its ovary, vitel-

Table 2. Measurements of mature proglottids from specimens of *Oochoristica javaensis* fixed in a relaxed state and specimens fixed in situ; all measurements are in μm.

	Sample size*	Relaxed $n = 5$†	In situ-fixed $n = 5$
Proglottid width	15	482–648 (589 ± 15.8)‡	403–845 (635 ± 34.1)
Proglottid length	15	356–640 (493 ± 25.8)	213–490 (312 ± 23.0)
Ovary width	15	246–351 (288 ± 8.06)	261–355 (310 ± 7.01)
Ovary length	15	152–238 (201 ± 6.63)	109–183 (144 ± 5.69)
Vitellaria width	15	125–195 (161 ± 5.11)	113–148 (130 ± 2.86)
Vitellaria length	15	90–137 (104 ± 3.83)	43–94 (70.5 ± 4.11)
Testis width	15	31–43 (38.7 ± 0.83)	35–47 (43.5 ± 0.95)
Testis length	15	27–47 (39.5 ± 1.23)	20–35 (27.1 ± 1.07)

* Sample size refers to the total number of each character that was measured.
† Number of tapeworms used in measurements.
‡ Range followed by mean ± 1 SE in parentheses.

laria, and testes (Table 2); thus, relaxed tapeworms had a higher mean factor score (Fig. 1). Contraction from in situ fixation resulted in mature proglottids wider than long (Fig. 3D–F), but completely relaxed tapeworms from hot-fixed specimens yielded mature proglottids longer than wide (Fig. 3A–C).

The second quantitative effect pertained to the correlative relationships among the mature proglottid characters and was revealed by the PCA itself. If only relaxed specimens are incorporated into the PCA, i.e., the intensity and representative data sets, all 8 characters vary together as 1 factor (Table 3); but when in situ-fixed tapeworms are incorporated into the PCA, i.e., the fixation data set, vertical measurements become independent of horizontal measurements (Table 1). Contraction of the in situ-fixed tapeworms altered the correlative nature of the 8 variables and divided 1 factor into 3 factors.

Contraction of helminth parasites resulting from improper fixation has been documented many times in the parasite literature (Bakke, 1988); however, our study may be the first to quantify the effects of different forms of fixation and to analyze the data statistically. The empirical evidence provided in the current study not only supported the conclusions of Bakke (1988)

Table 3. *Oochoristica javaensis*: variable factor loadings, factor eigenvalues, and percent total variance accounted for by each factor from the correlation matrix of the intensity data set and representative data set.

	Loading matrices for the	
	Intensity data set Factor 1	Representative data set Factor 1
Ovary width	**0.924***	**0.909**
Vitellaria width	**0.921**	**0.854**
Ovary length	**0.916**	**0.814**
Proglottid width	**0.893**	**0.851**
Vitellaria length	**0.866**	**0.784**
Testis width	**0.841**	**0.825**
Testis length	**0.753**	**0.749**
Proglottid length	**0.751**	0.443
Eigenvalues	5.929	4.996
Percentage of total variance explained by the factor	74.116	62.445

* Bold print shows loadings where variable loaded onto factor.

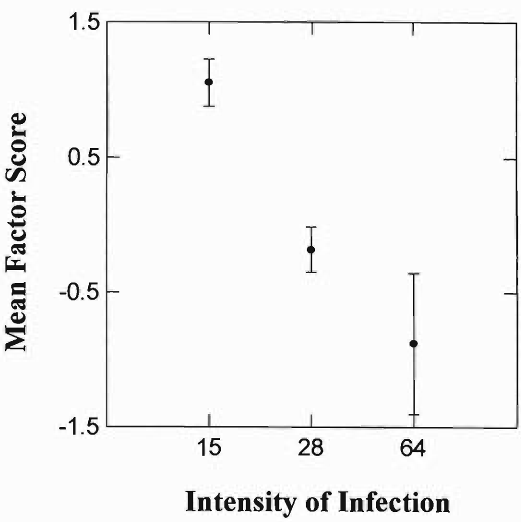

Figure 2. Plot of the mean factor scores and 95% confidence intervals for tapeworms collected at intensities of 15, 28, and 64.

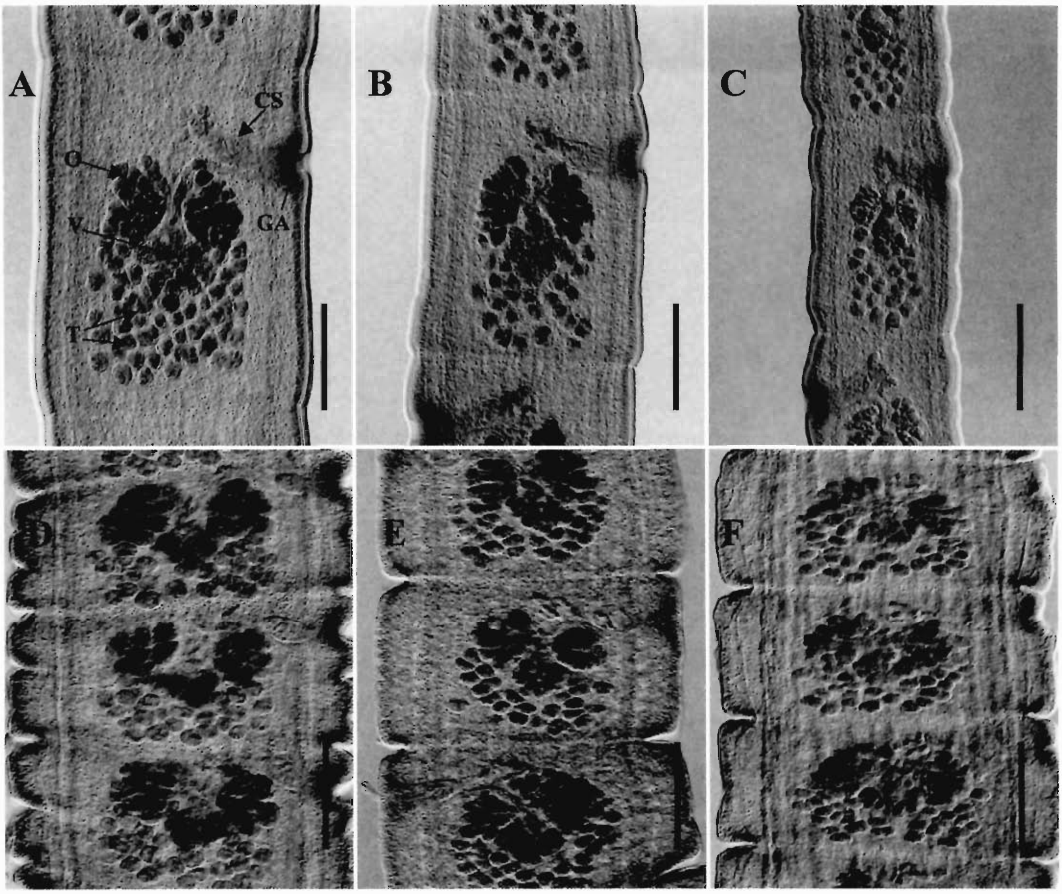

Figure 3. Mature proglottid variation of *Oochoristica javaensis* from *Hemidactylus turcicus*. Photomicrographs were taken with differential interference contrast. A–C. Natural variation of specimens from intensities of 15, 28, and 64, respectively; all were fixed in a relaxed state. D–F. Artificial variation showing contraction that resulted from in situ fixation. Bars = 200 μm. CS = cirrus sac, GA = genital atrium, O = ovary, T = testis, and V = vitellaria.

Table 4. Mature proglottid measurements of *Oochoristica javaensis* from *Hemidactylus turcicus* with intensities of 15, 28, and 64; all measurements are in μm.

Level of intensity	Sample size*	15 $n = 5$†	28 $n = 5$	64 $n = 5$
Proglottid width	15	506–616 (560 ± 7.06)‡	387–450 (409 ± 5)	269–545 (387 ± 31.7)
Proglottid length	15	545–751 (624 ± 14.5)	395–553 (470 ± 14.7)	419–545 (463 ± 8.7)
Ovary width	15	238–293 (268 ± 3.89)	195–254 (225 ± 4.85)	121–281 (198 ± 14.2)
Ovary length	15	156–226 (189 ± 4.48)	125–179 (160 ± 3.83)	78–183 (126 ± 7.9)
Vitellaria width	15	129–203 (161 ± 5.78)	86–133 (117 ± 3.37)	59–117 (90.4 ± 5.19)
Vitellaria length	15	74–137 (105 ± 4.95)	70–113 (90.3 ± 3.3)	43–101 (73.5 ± 4.91)
Testis width	15	39–47 (42.7 ± 0.61)	35–43 (39 ± 0.78)	27–43 (35 ± 1.29)
Testis length	15	39–51 (43 ± 0.87)	31–47 (39 ± 1.03)	27–47 (36.6 ± 1.4)

* Sample size refers to the total number of each character that was measured.
† Number of tapeworms used in measurements.
‡ Range followed by mean ± 1 SE in parentheses.

Table 5. Measurements of *Oochoristica javaensis* from naturally infected *Hemidactylus turcicus* in southeastern Louisiana; measurements in μm unless noted otherwise.

Variable		Sample size*	n = 10†
Total	L‡ (mm)	10	22.2–105 (53.4 ± 7.4)§,‖
Proglottid number		10	86–164 (131 ± 7.8)‖
Neck	W	10	158–237 (205 ± 8.1)‖
	L (mm)	10	1.12–1.58 (1.38 ± 0.05)‖
Scolex	W	10	148–246 (195 ± 9.1)‖
	L	10	98–183 (140 ± 8.9)
Sucker	W	10	51–90 (74.1 ± 3.9)‖
	L	10	62–117 (89 ± 5.3)‖
Immature proglottid	W	30	261–506 (408 ± 14.5)
	L	30	237–371 (297 ± 7.6)
Genital pore position#		30	0.24–0.33 (0.28 ± 0.004)‖
Mature proglottid	W	30	277–648 (490 ± 19.5)‖
	L	30	395–751 (509 ± 17.8)‖
Cirrus sac	W	30	43–55 (47.7 ± 0.58)
	L	30	86–144 (116 ± 2.8)‖
Ovary	W	30	133–390 (268 ± 11.8)‖
	L	30	78–316 (184 ± 0.6)‖
Vitellaria	W	30	62–269 (144 ± 8.9)‖
	L	30	43–221 (106 ± 6.6)‖
Testis	W	30	27–51 (40.2 ± 0.98)‖
	L	30	31–47 (40.9 ± 0.9)
Testes number		30	17–46 (26.6 ± 1.4)‖
Gravid proglottid	W	30	158–650 (492 ± 29.4)‖
	L (mm)	30	0.85–1.99 (1.26 ± 0.05)‖
Oncosphere	W	30	20–34 (25.3 ± 0.69)‖
	L	30	18–28 (23.2 ± 0.52)‖
Hook	L	30	8–12 (11.5 ± 0.18)‖

* Sample size refers to the total number of each character that was measured.
† Number of tapeworms measured.
‡ L = length, W = width.
§ Range followed by mean ± 1 SE in parentheses.
‖ Indicates that the range of the character extends values reported in the original description (Kennedy et al., 1982).
\# Genital pore position was calculated as a ratio of the position along the length of the mature proglottid from the anterior end (length to the center of the genital pore ÷ length of proglottid).

but also quantitatively demonstrated the misleading representation of morphological characters used in the taxonomy of *Oochoristica* resulting from in situ fixation. We believe that our more rigorous analysis is important because, despite the elegant studies of Bakke (1988) and previous workers, the practice of describing improperly fixed specimens is still widespread among parasite taxonomists.

In addition to the quantitative changes resulting from in situ fixation, 2 qualitative effects further demonstrated the inappropriateness of using in situ-fixed specimens in species descriptions. Distortion of the scolex (Fig. 4A, B) and proglottids (Fig. 4C, D) prevented accurate measurements of these characters. This is not to say that every scolex and mature proglottid fixed in situ will be rendered useless for species identification, but the number of appropriate characters available for analysis will be greatly reduced. As seen in Figure 4A–D, one would have difficulty in finding a true scolex width, and contortion in the strobila would limit the number of proglottids suitable for examination.

Species descriptions based on contracted or disfigured specimens will misrepresent the true natural variation by decreasing the means of vertical characters and artificially inflating the dispersion of measurements if used in conjunction with relaxed specimens. Such may be the case with several paratype (*O. bezyi, O. islandensis, O. macallisteri, O. mccoyi, O. piankai*) and voucher (*O. mccoyi, O. parvula, O. piankai, O. scelopori*) specimens examined *in our study*. These specimens may represent true species, but their reported natural variation is more than like-

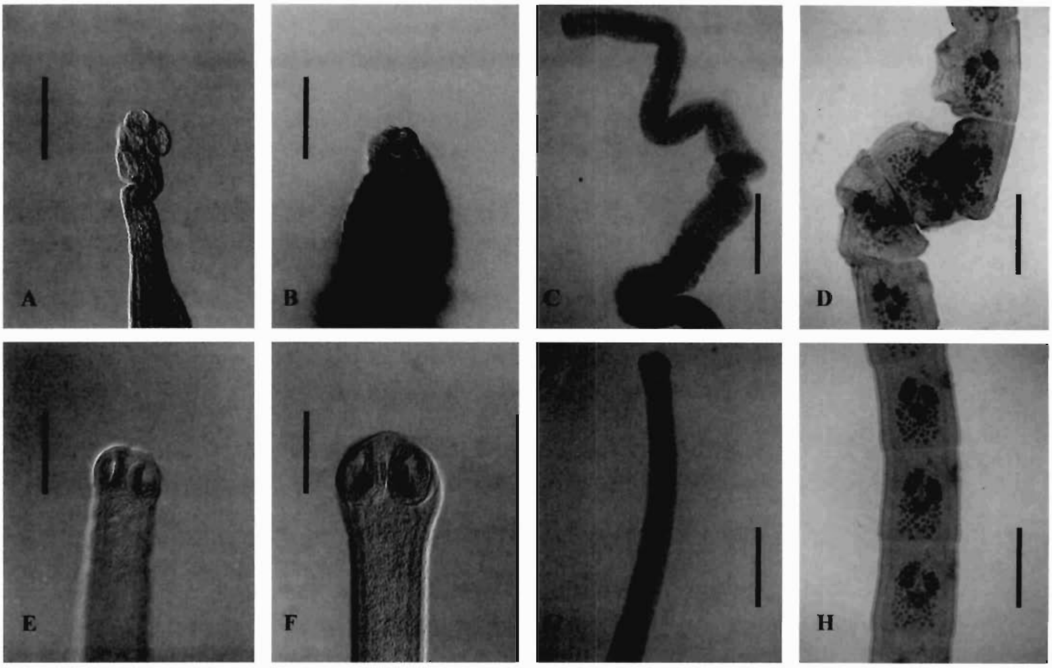

Figure 4. Comparisons of relaxed and in situ-fixed specimens of *Oochoristica javaensis*. A–D. Distorting effects of in situ fixation on scolices and proglottids. E–H. Relaxed specimens. Bars = 200 μm for A, B, E, and F (differential interference contrast). Bars = 500 μm for C, D, G, and H (brightfield).

ly masked within the artificial variation induced by in situ fixation. Characters that do not reflect their natural variation should not be used to describe species. *Oochoristica* spp. recovered from fixed museum hosts may provide historical abundance data, but identification of these specimens should be made with extreme caution, and ideally, in conjunction with specimens fixed appropriately.

Intensity effects were examined because a crowding effect has been documented as a cause of variation in the size of tapeworms (Read, 1951), and because of the occurrence of different intensities in naturally infected *H. turcicus*. PCA for the intensity data set produced 1 factor in which all 8 variables loaded high (Table 3); therefore, individual proglottids with large measurement values received high factor scores. Although not quantified, there was no apparent crowding effect observed for natural infections with intensities between 1 and 15. Tapeworms from an intensity of 15 had significantly greater factor scores than specimens from 28 and 64 (Fig. 2), thus indicating that crowding reduces the size of the respective morphological characters (Table 4) in *O. javaensis*. Brooks and Mayes (1976) reported similar crowding effects for *O. bivitellobata* recovered from the prairie racerunner, *Cnemidophorus sexlineatus* Lowe, 1966. Their results and ours, however, should be considered only preliminary for 2 reasons. First, data were obtained from natural infections; thus other factors that induce variation were not controlled. Second, the intensity levels were not replicated. Ideally, one would wish to sample tapeworms from multiple hosts harboring all possible intensity levels. Both reports, however, suggest that morphological characters for species of *Oochoristica* can be variable and may be subject to intensity levels.

Measurements of the 10 *O. javaensis* specimens given in Table 5 extend the ranges of several characters provided in the original description of *O. javaensis* (Kennedy et al., 1982). These measurements were based on specimens with little or no contraction and are provided to give a representation of *O. javaensis* collected from *H. turcicus* in southeastern Louisiana. Means of several characters (Table 5) do not match those provided by Kennedy et al. (1982); however, based on the lack of host specificity displayed in laboratory experiments (Criscione and Font, 2001), the indication of plasticity in morphological characters (Table 4), and the examination of *O. javaensis* paratypes, it was determined that the specimens from *H. turcicus* in southeastern Louisiana were *O. javaensis.*

In summary, statistical analyses demonstrated that measurements of in situ-fixed tapeworms, i.e., specimens recovered from preserved hosts, distorted the true natural variation of *O. javaensis.* The intraspecific variation of several species of *Oochoristica* may be misrepresented because they were described from highly contracted, in situ-fixed specimens. Additionally, morphological characters used in the taxonomy of *Oochoristica* have not been examined for their stability when exposed to different environmental or host-induced conditions. Our analyses indicated that proglottid morphology was highly variable and that this plasticity may have resulted from crowding.

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Notice of Dues Increase

At the January 2001 meeting of the Helminthological Society of Washington, the Society voted to increase the membership dues to US$30.00 for individual U.S. members and to US$33.00 for individual foreign members. Institution subscription rates will increase as follows: US$55.00 (U.S.A.), US$57.00 (Canada and Mexico), US$60.00 (all other countries). This is the first dues increase in six years and is necessitated by increased management costs to the Society. The increases will take effect in January, 2002.

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Seasonal Occurrence and Community Structure of Helminth Parasites in Green Frogs, *Rana clamitans melanota,* from Southeastern Wisconsin, U.S.A.

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ABSTRACT: From April to October 1996, 75 green frogs, *Rana clamitans melanota,* were collected from Waukesha County, Wisconsin, U.S.A. and examined for helminth parasites. Seventy-one (94%) of 75 green frogs were infected with 1 or more helminth species. The component community consisted of 12 species: 5 trematodes, 2 cestodes, and 5 nematodes. Approximately 2,790 (72%) trematodes, 447 (11.5%) cestodes, and 636 (16.5%) nematodes were found. A significant correlation existed between wet weight and helminth species richness. Helminth populations and communities were seasonally variable and/or did not show significant differences during the year. *Haematoloechus varioplexus* showed seasonal variation in size during the year that was related to recruitment period. The helminth fauna of green frogs was depauperate and dominated by indirect-life-cycle parasites. Host diet and aquatic habitat were important in the transmission dynamics of these species. Host size, sex, and time of collection were also important factors in structuring helminth communities of green frogs and may mask any simple explanations.

KEY WORDS: *Rana clamitans, Haematoloechus varioplexus, Halipegus eccentricus, Glypthelmins quieta, Cosmocercoides* sp., *Oswaldocruzia pipiens, Waltonella* sp., *Mesocestoides* sp., metacercariae, Trematoda, Nematoda, Cestoda, Amphibia, seasonal study, Wisconsin, U.S.A.

Studies by Kennedy et al. (1986) on freshwater fish, birds, and a mammal have developed predictions in determining helminth community structure, particularly that ectotherm and endotherm helminth communities are fundamentally different: the former are species poor and noninteractive, while the latter are diverse and interactive. A review by Aho (1990) indicated that helminth communities of amphibians are highly variable, depauperate, and noninteractive in structure, but there is a need to examine and reexamine more species from different locations. To date there are few studies that utilized helminth community measures in amphibian hosts (Goater et al., 1987; Aho, 1990; Muzzall, 1991a, b; Goldberg et al., 1995; Barton, 1996; Yoder and Coggins, 1996; McAlpine, 1997; Bolek and Coggins, 2000). A number of helminth surveys of green frogs, *Rana clamitans melanota* Rafinesque, 1820, have been published (Campbell, 1968; Williams and Taft, 1980; Coggins and Sajdak, 1982; McAlpine and Burt, 1998), but there are few studies on the helminth infracommunity and component community structure of this species (Muzzall, 1991a; McAlpine, 1997), and none that incorporated a seasonal component.

Green frogs are large, semiaquatic frogs inhabiting freshwater ponds, lakes, swamps, and slow-moving streams in North America. They spend most of their time around the water's edge. They occur from Newfoundland to western Ontario, and south to eastern Oklahoma, southern Illinois, northern Georgia, and eastern North Carolina. In Wisconsin, these frogs overwinter buried in the mud and are active from early April through October (Vogt, 1981). Green frogs are largely sit-and-wait gape-limited predators, feeding on any accessible prey of appropriate size, including aerial, aquatic, and terrestrial invertebrates, primarily insects (Seale, 1987; Werner et al., 1995). Here we report on the seasonal helminth community structure of green frogs from southeastern Wisconsin. Specifically, we were interested in how host habitat, age and/or size, diet, sex, and seasonality were important in determining helminth populations and communities of green frogs.

Materials and Methods

A total of 75 green frogs, *R. clamitans melanota,* was collected from April to October 1996 at a small spring-fed permanent pond located at the Carroll College field station in Waukesha County, Wisconsin,

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U.S.A. (42°59′N; 88°21′W). Ten to 15 frogs were collected monthly around the periphery of the pond by a dip-net. Animals were placed in plastic containers, transported to the laboratory, stored at 4°C, and euthanized in MS 222 (ethyl m-aminobenzoate methane sulfonic acid) within 72 hr of capture. Snout–vent length (SVL) and wet weight (WW) were recorded for each individual. Frogs were individually toe-clipped and frozen. At necropsy, the digestive tracts, limbs and body wall musculature, and internal organs were examined for helminth parasites. Each organ was placed individually in a petri dish and examined under a stereomicroscope. The body cavities were rinsed with distilled water into a petri dish and the contents examined. All individuals were sexed by gonad inspection during necropsy. Worms were removed and fixed in alcohol–formaldehyde–acetic acid or formalin. Trematodes and cestodes were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Nematodes were dehydrated to 70% ethanol, cleared in glycerol, and identified as temporary mounts. Prevalence, mean intensity, and abundance are according to Bush et al. (1997). Mean intensity was not calculated for the unidentified kidney metacercariae because they could not be counted accurately, and overall abundance was reported as an estimate of encysted metacercariae counted on the surface of the kidneys. Mean helminth species richness is the sum of helminth species per individual frog, including noninfected individuals, divided by the total sample size. All values are reported as the mean ± 1 standard deviation. Undigested stomach contents were identified to class or order following Borror et al. (1989). Stomach contents are reported as a percent = the number of prey items in a given class or order, divided by the total number of prey items recovered × 100. Voucher specimens have been deposited in the H. W. Manter Helminth Collection, University of Nebraska, Lincoln, Nebraska, U.S.A. (accession numbers HWML 15354, *Haematoloechus varioplexus* Stafford, 1902; 15355, *Glypthelmins quieta* Stafford, 1900; 15356, kidney metacercariae; 15357, *Mesocestoides* sp.; 15358, diplostomid metacercariae; 15359, *Halipegus eccentricus* Thomas, 1939; 15360, unidentified adult tapeworm; 15361, *Cosmocercoides* sp.; 15362, unidentified larval nematode; 15363, unidentified species of *Waltonella* Schacher, 1974; 15364, *Oswaldocruzia pipiens* Walton, 1929).

The chi-square test for independence was calculated to compare differences in prevalence among host sex. Yates' adjustment for continuity was used when sample sizes were low. A single-factor, independent-measures ANOVA and Scheffe's posthoc test were used to compare among seasonal differences in mean intensity and mean helminth species richness. When variances were heteroscedastic, the Kruskal–Wallis test and the Kolmogorov–Smirnov two-sample test were used. Student's *t*-test was used to compare differences in mean intensity and mean helminth species richness between sex of hosts. Approximate *t*-tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1981). Pearson's correlation was used to determine relationships among host SVL and WW and abundance of helminth parasites, excluding larval platyhelminths. Pearson's correlation was calculated for host SVL and WW and helminth species richness per individual frog. Because WW gave a stronger correlation than SVL in each case, it is the only parameter reported. Because of low sample size during certain collection periods, data were pooled on a bimonthly basis to form samples of 15–20 frogs per season. Larval helminths were not included in the seasonal analysis, because they can also accumulate throughout the amphibian's life and thus mask monthly recruitment dynamics in adult frogs.

Results

A total of 75 adult green frogs, 43 males and 32 females, was collected during April through October 1996. No significant difference existed in the number of male and female frogs collected throughout the year ($\chi^2 = 7.01$, $P > 0.05$). The overall means of SVL and WW of green frogs were 68.8 ± 10.8 mm (range 39.8–89.4 mm) and 35.8 ± 15.5 g (5.4–75.6 g), respectively. There was no significant difference in mean SVL ($t = 0.10$, $P > 0.05$) or mean WW ($t = 0.24$, $P > 0.05$) in male and female frogs. Stomach content analyses of green frogs revealed a broad range of aerial, terrestrial, and aquatic invertebrates. Sixteen different groups of invertebrates were recovered from stomach contents of green frogs, with coleopterans, gastropods, and diplopodans making up the largest percentage.

Seventy-one (94%) of 75 *R. clamitans melanota* were infected with helminth parasites. The component community consisted of 12 species (5 trematodes, 2 cestodes, and 5 nematodes). Of these, 8 have indirect life cycles, 1 has a direct life cycle, and the life cycles of 3 are unknown. Overall mean helminth abundance, excluding larval platyhelminths, was 16.5 ± 38 with most frog infracommunities having 10 or fewer worms. In terms of abundance, digeneans dominated adult helminth communities (61.5% of total adult helminths). Prevalence ranged from 80% for kidney metacercariae to 1.3% for an unidentified adult cestode, a filarid nematode of the genus *Waltonella,* and an unidentified encysted nematode. Values for overall prevalence, mean intensity, mean abundance, and total number of helminths recovered are summarized in Table 1.

Statistically significant differences in prevalence or mean intensity existed between male and female frogs for *H. varioplexus, H. eccentricus,* kidney metacercariae, and unidentified species of *Mesocestoides* Valunt, 1863 and *Cos-*

Table 1. Prevalence, mean intensity (MI), mean abundance (MA), and total helminths found in 75 specimens of *Rana clamitans melanota* in Wisconsin.

Species	Prevalence: number (%)	MI ± 1 SD (range)	MA ± 1 SD	Number of worms recovered	Location
Trematoda					
Haematoloechus varioplexus	33 (44)	5.3 ± 7 (1–30)	2.3 ± 5.4	176	Lungs
Halipegus eccentricus	17 (22.6)	1.9 ± 1.7 (1–8)	0.4 ± 1.1	33	Eustachian tubes
Glypthelmins quieta	11 (14.6)	35.5 ± 34.7 (1–110)	5.2 ± 18	391	Small intestine
Unidentified metacercariae*	60 (80)	NC‡	NC	>1,770	Kidneys, body cavity
Diplostomid metacercariae*	12 (16.0)	35 ± 41 (1–100)	5.7 ± 20.7	420	Leg muscles
Cestoda					
Unidentified adult cestode	1 (1.3)	1	0.01 ± 0.1	1	Small intestine
Mesocestoides sp.*	14 (18.6)	31.9 ± 22 (12–86)	5.9 ± 15.3	446	Leg muscles, lungs
Nematoda					
Oswaldocruzia pipiens	21 (28)	14.7 ± 23 (1–90)	4.1 ± 13.7	310	Small intestine, stomach
Cosmocercoides sp.	19 (25)	3.3 ± 4.3 (1–19)	0.8 ± 2.6	62	Large intestine, small intestine
Waltonella sp.†	1 (1.3)	4	0.05 ± 0.5	4	Body cavity
Larval nematode	9 (12)	25.9 ± 61 (1–200)	3.5 ± 23	259	Large intestine
Encysted nematode	1 (1.3)	1	0.01 ± 0.1	1	Small intestine

* Underestimate.
† New host record.
‡ Not counted.

mocercoides Harwood, 1930 (Table 2). Both *H. varioplexus,* a lung trematode, and *H. eccentricus,* a trematode of the eustachian tubes, had significantly higher mean intensities in male frogs, while the kidney metacercariae occurred at a higher prevalence in males. Female frogs had significantly higher mean intensities of *Mesocestoides* sp. and *Cosmocercoides* sp.

Mean helminth species richness was 2.68 ± 1.29 species per frog. Infections with multiple species were common, with 0, 1, 2, 3, 4, and 5 species occurring in 4, 8, 23, 20, 13, and 7 frogs, respectively. No statistically significant differences in mean helminth species richness were found between male (2.76 ± 1.01) and female frogs (2.56 ± 1.52, $t = 0.66$, $P > 0.05$). A nonsignificant positive correlation was found between overall helminth abundance, excluding larval platyhelminths, and WW ($r = 0.04$, $P > 0.05$). Nonsignificant relationships were also observed for most helminth species: unidentified adult cestode ($r = -0.01$, $P > 0.05$), *H. varioplexus* ($r = 0.10$, $P > 0.05$), *H. eccentricus* ($r = 0.19$, $P > 0.05$), *G. quieta* ($r = -0.02$, $P > 0.05$), *O. pipiens* ($r = -0.12$, $P > 0.05$), unidentified larval nematode ($r = -0.04$, $P > 0.05$), *Waltonella* sp. ($r = 0.20$, $P > 0.05$), and encysted nematodes ($r = -0.19$, $P > 0.05$). The nematode *Cosmocercoides* sp. had a significant positive correlation with WW ($r = 0.31$, $P < 0.01$). A significant positive Pearson's correlation also existed between species richness and WW ($r = 0.31$, $P < 0.01$). However, correlations between frog WW and species richness were not significant in May–June ($r = 0.31$, $P > 0.05$), July–August ($r = 0.01$, $P > 0.05$), and September–October ($r = 0.29$, $P > 0.05$) but were significant for the April collection ($r = 0.60$, $P < 0.02$).

The trematodes *H. varioplexus* and *H. eccentricus* occurred throughout the year, with highest prevalences observed during the fall (September–October) collection, 65% and 30%, respectively. The intestinal trematode, *G. quieta,* was

Table 2. Prevalence (Pr) and mean intensity (MI) of helminth parasites in male and female green frogs, *Rana clamitans melanota*.

Species	Measure of parasitism	Males $N = 43$	Females $N = 32$	Statistic	P
Trematoda					
Haematoloechus varioplexus	Pr	46.5 (20/43)	40.6 (13/32)	$\chi^2 = 0.24$	>0.05
	MI ± 1 SD	7 ± 8.6	2.8 ± 2.2	$t'_s = 3.07$	<0.05
Halipegus eccentricus	Pr	23.3 (10/43)	21.9 (7/32)	$\chi^2 = 0.02$	>0.05
	MI ± 1 SD	2.3 ± 2.1	1.4 ± 0.5	$t'_s = 2.71$	<0.05
Glypthelmins quieta	Pr	11.6 (5/43)	18.8 (6/32)	$\chi^2 = 0.46$	>0.05
	MI ± 1 SD	21.8 ± 27.9	47 ± 38.1	$t = 1.35$	>0.05
Unidentified metacercariae*	Pr	90.7 (39/43)	65.6 (21/32)	$\chi^2 = 5.72$	<0.05
	MI ± 1 SD	NC‡	NC		
Diplostomid metacercariae*	Pr	18.6 (8/43)	12.5 (4/32)	$\chi^2_{adj} = 0.16$	>0.05
	MI ± 1 SD	45.3 ± 46.1	14.5 ± 23.7	$t = 1.23$	>0.05
Cestoda					
Unidentified adult cestode	Pr	2.3 (1/43)	0 (0/32)	$\chi^2_{adj} = 0.02$	>0.05
	MI ± 1 SD	1	0		
Mesocestoides sp.*	Pr	23.3 (10/43)	12.5 (4/32)	$\chi^2_{adj} = 0.78$	>0.05
	MI ± 1 SD	24.9 ± 16.2	49.3 ± 24.8	$t = 6.69$	<0.05
Nematoda					
Oswaldocruzia pipiens	Pr	27.9 (12/43)	28.1 (9/32)	$\chi^2 = 0.00$	>0.05
	MI ± 1 SD	16.6 ± 25.8	12.2 ± 19.6	$t = 0.44$	>0.05
Cosmocercoides sp.	Pr	20.9 (9/43)	31.3 (10/32)	$\chi^2 = 1.00$	>0.05
	MI ± 1 SD	2 ± 1.7	4.4 ± 5.6	$t'_s = 3.81$	<0.05
Waltonella sp.	Pr	0 (0/43)	3.1 (1/32)	$\chi^2_{adj} = 0.02$	>0.05
	MI ± 1 SD	0	4		
Larval nematode	Pr	9.3 (4/43)	18.8 (6/32)	$\chi^2_{adj} = 1.67$	>0.05
	MI ± 1 SD	11.5 ± 11.2	35.5 ± 80.6	$t'_s = 1.67$	>0.05
Encysted nematode	Pr	0 (0/43)	3.1 (1/32)	$\chi^2_{adj} = 0.02$	>0.05
	MI ± 1 SD	0	1		

* Underestimate.
‡ Not counted.

first observed during midspring (May–June) with a prevalence of 5%. Prevalence for this species reached its maximum (30%) during summer (July–August) and decreased during the fall collection (20%). Seasonal mean intensity of adult platyhelminths followed similar patterns as prevalence, but no significant differences existed for any of the adult platyhelminths recovered, *H. eccentricus* (adjusted $H = 2.02$, $P > 0.05$), *H. varioplexus* ($F = 0.34$, $P > 0.05$), or *G. quieta* ($t = 0.43$, $P > 0.05$).

Although prevalence and intensity of *H. varioplexus* did not vary significantly throughout the collection period, mean length of worms did (Fig. 1). Greatest mean length of worms (4.1 mm) was recorded in early spring (April), when all individuals were gravid adults, and reached a minimum during midspring (1.84 mm), when immature individuals were common. Statistically significant differences in mean length were observed for April and May–June collections, April and July–August collections, May–June and September–October collections, and July–August and September–October collections ($F = 11.8$, $P < 0.05$, single-factor, independent-measure ANOVA; $P < 0.05$ for all pair-wise comparisons, Scheffe's test).

The nematodes *Waltonella* sp. and an unidentified encysted larva were recovered infrequently as single infections during midspring and fall collections, respectively. Prevalence and intensity values for *O. pipiens*, *Cosmocercoides* sp., and unidentified larval nematodes were low and/or erratic over the 7-mo period. The nematodes *O. pipiens*, *Cosmocercoides* sp., and unidentified larval nematodes were first observed during midspring and persisted until fall, with prevalence being highest in summer for *O. pipiens* (62%) and midspring and summer for *Cosmocercoides* sp. (40%) and larval nematodes (20%). However, only *O. pipiens* exhibited statistically significant differences (adjusted H =

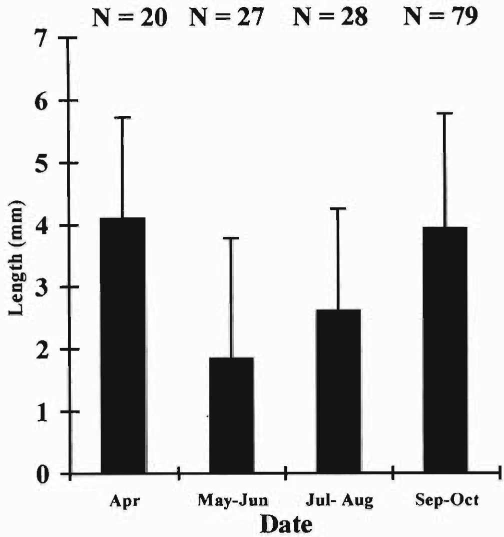

Figure 1. Mean lengths of *Haematoloechus varioplexus* from *Rana clamitans melanota*. *N* = number of worms measured from all frogs recovered in each sampling period.

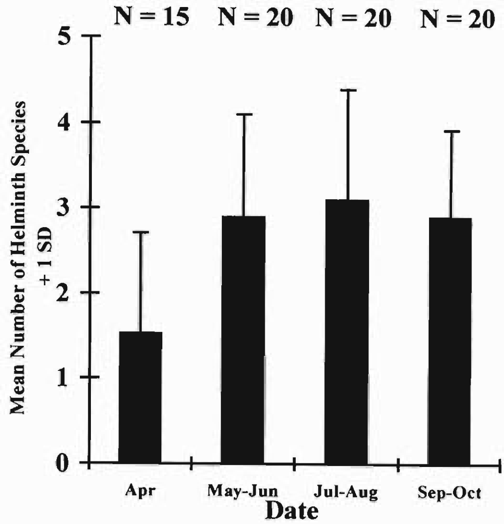

Figure 2. Mean helminth species richness of *Rana clamitans melanota* during April, May–June, July–August, and September–October 1996. *N* = number of frogs collected on each date.

9.45, $P < 0.05$) in mean intensity. The two-sample Kolmogorov–Smirnov test revealed significant differences in mean intensities during the May–June (27 ± 30) and July–August (9.8 ± 17) collections.

Mean helminth species richness fluctuated seasonally (Fig. 2) and was lowest (1.53) during early spring and highest (3.1) during the summer collections. Statistically significant differences in mean helminth species richness were observed for April and May–June collections, April and July–August collections, and April and September–October collections ($F = 6.01$, $P < 0.05$, single-factor, independent-measure ANOVA; $P < 0.05$ for all pairwise comparisons, Scheffe's test). No statistically significant differences in frog WW were observed during the year ($F = 0.37$, $P > 0.05$).

Discussion

Wisconsin green frogs had high overall helminth prevalence, with parasite infracommunities being dominated by indirect life-cycle parasites. Of the identified parasites, only 1 direct life-cycle nematode, *O. pipiens*, was present, with most helminth species displaying a prevalence below 50% and/or low mean intensities below 30.

Most green frogs contained identifiable stomach contents containing predominantly beetles, gastropods, and diplopods. In total, 16 different groups of terrestrial, aerial, and aquatic invertebrates comprised their stomach contents. These results appear similar to those of other investigators (Hamilton, 1948; Stewart and Sandison, 1972; McAlpine and Dilworth, 1989; Werner et al., 1995). Hamilton (1948) found that the principal foods of green frogs collected in New York, U.S.A. consisted of beetles, flies, and grasshoppers, with a total of 15 different items recovered from adult frogs and 20 different prey items recovered from various sized individuals.

The most common helminth recovered was an unidentified kidney metacercaria. This larval trematode had an overall prevalence of 80% and mean intensity of over 30 worms per frog. Four other digeneans were recovered from green frogs: a diplostomid metacercaria encysted in the musculature, and 3 adult trematodes: *H. varioplexus*, *H. eccentricus*, and *G. quieta*. Frogs become infected with *H. varioplexus*, a lung trematode, and *H. eccentricus*, a eustachian tube trematode, by eating infected odonates (Krull, 1931; Dronen, 1975, 1978; Wetzel and Esch, 1996). *Glypthelmins quieta*, a trematode of the small intestine, is acquired when frogs ingest prey such as tadpoles, frogs, and/or shed skin infected with metacercariae (Prudhoe and Bray, 1982). Therefore, diet was important in the transmission dynamics of these 3 trematode species in this study.

Haematoloechus varioplexus and *H. eccentricus* were recovered from frogs throughout the year, increasing, although not significantly, in both prevalence and mean intensity during the fall collection. Recently, Wetzel and Esch (1997) have shown that the life span of *H. eccentricus* may be variable, with trematodes capable of maturing in as little as 1 wk and being lost the following week. Because of the small number of these flukes recovered in our study, little can be said about their recruitment throughout the year. Krull (1931) estimated that the life-span of *Haematoloechus medioplexus* Stafford, 1902, averaged 1 yr, while studies by Kennedy (1980) on species of *Haematoloechus* have shown that trematodes can reach full length in only 60 days. The size differences observed for *H. varioplexus* during the year (Fig. 1) may be significant in understanding recruitment of this species. The seasonal variation in length of *H. varioplexus* suggests that adult worms are lost during early spring and that new infections begin during midspring and continue throughout the year. These results are similar to those of Ward (1909), who observed lung flukes of *Rana pipiens* Schreber, 1782, being lost during breeding, and recruitment occurring throughout the year.

Two cestode species were recovered from green frogs during this study, the larval tetrathyridium of *Mesocestoides* sp. and a single specimen of an adult cestode that could not be identified because the scolex was lost. The complete life cycles of *Mesocestoides* spp. are currently unknown; however, a number of mammals, amphibians, and reptiles are known to serve as second intermediate hosts, while carnivorous mammals serve as definitive hosts. The tetrathyridian stage has been reported from a variety of mammals and reptiles but is rare in amphibians (McAllister and Conn, 1990). The life cycles of these 2 species of cestode are unknown, although frog diet may be important in their transmission dynamics.

Nematodes in the genus *Waltonella* typically are found in the body cavity of species of *Rana*. Adult worms release microfilaria into the bloodstream, and mosquitoes serve as vectors, infecting frogs while feeding (Witenberg and Gerichter, 1944). The only report of filarial worms in *R. clamitans* is of microfilaria recovered from 1 frog in Ontario, Canada (Barta and Desser, 1984). Therefore, *R. clamitans melanota* is apparently a new host record for *Waltonella* sp. (Esslinger, 1986; Baker, 1987). *Waltonella americana* was previously reported and described in Wisconsin leopard frogs by Walton (1929).

The nematode *Cosmocercoides* sp. was recovered from the large intestine of green frogs. Confusion exists in the literature on the identification of species of *Cosmocercoides* in amphibians and reptiles (Baker, 1987; Vanderburgh and Anderson, 1987). The major difference in species identification is the number of rosette papillae per subventral row in males, with male *Cosmocercoides dukae* Holl, 1928 (gastropod parasite) having 9–21 rosette papillae, averaging 13–14, and *C. variabilis* (amphibian parasite) having 15–25, averaging 20 or 21. Because of this overlap and the presence of only 5 damaged males out of 62 nematodes recovered, species identification was not possible. Interestingly, no worms were found in the lungs or body cavity of any green frogs, and *Cosmocercoides* sp. occurred in frogs in months when gastropods were commonly found in the stomach contents. We suspect that specimens of *Cosmocercoides* sp. recovered are *C. dukae,* although this cannot be confirmed.

Differential infection between host sex and prevalence or mean intensity was observed for a number of helminth species. Male frogs had a significantly higher prevalence of kidney metacercariae and significantly higher mean intensities of *H. varioplexus* and *H. eccentricus* than female frogs. Male green frogs are territorial during the breeding season and defend their aquatic breeding site from potential competitors (Martof, 1953; Oldham, 1967). Thus, unlike the females, they remain in the water for longer periods of time and may be exposed to cercariae of the kidney trematode for longer periods. Because males remain in a relatively small area of the pond during the breeding season, they may occur in a microhabitat conducive to becoming infected with digeneans. Recently, Wetzel and Esch (1997), in a seasonal study of *Halipegus occidualis* Stafford, 1905 and *H. eccentricus* in green frogs, suggested that certain areas of a pond may be "hot spots" for infection with digenetic trematodes. Therefore, male frogs in these "hot spots" may feed more often on emerging odonates containing metacercariae of species of *Haematoloechus* and *Halipegus,* explaining the higher mean intensities of these trematodes observed in male frogs (Wetzel and Esch, 1996).

Female frogs had significantly greater mean intensities of *Cosmocercoides* sp. and *Mesocestoides* sp. than males. Although *Cosmocercoides* sp. could not be identified to species, both *C. variabilis* and *C. dukae* occur in terrestrial habitats. Female frogs spend more time on the ground and have a higher probability of encountering these nematodes in a terrestrial habitat, either by skin-penetrating *C. variabilis* or by feeding on terrestrial mollusks, hosts for *C. dukae*. Unfortunately, nothing can be stated about the transmission dynamics of *Mesocestoides* sp., and no conclusions can be drawn from this difference. The observed differences in host sex are probably due to ecological differences in their habitat preference throughout the year.

Significant positive relationships between WW and species richness were observed in green frogs. In this study, frogs in the later collections had greater species richness than in early collections (Fig. 2); therefore, time of exposure was more important in developing richer helminth communities than was frog weight during the May–June, July–August, and September–October collections. This is supported by the results showing significant differences in species richness over time and nonsignificant correlations between WW and species richness. Observations linking higher species richness with larger host size have been reported in green frogs and other species of *Rana* by Muzzall (1991a) and McAlpine (1997). These investigators suggested that older individuals may have a longer exposure time and possess more surface area for colonization by skin-penetrating nematodes and digenean metacercariae. Also, larger frogs possess a greater gape size and may feed on larger, and a wider number, of intermediate hosts than smaller individuals. As in their studies, our data also support the island size hypothesis, which predicts that larger host individuals should support higher species richness than smaller individuals (Holmes and Price, 1986). McAlpine (1997) also stated that aspects of host ecology, such as diet and habitat, and parasite transmission may confound any simple relationship between the diversity of helminth communities and size of hosts. Data from the present study suggest that time of transmission may also have a similar confounding effect.

The depauperate helminth community structure of Carroll College green frogs was similar to the community structure of green frogs examined from Michigan, U.S.A. and New Brunswick, Canada by Muzzall (1991a) and McAlpine (1997), respectively. Of the 120 frogs examined from Michigan, 108 (90%) were infected with a total of 13 species of helminths (8 trematodes, 1 cestode, and 4 nematodes) while 164 of 234 (70.1%) green frogs examined from Canada were infected with 18 species of helminths (10 trematodes, 3 cestodes, and 5 nematodes). As in our study, in terms of abundance, digeneans dominated the adult helminth communities of frogs in Michigan (96.5%) and New Brunswick (60.8%), indicating that diet and a semiaquatic habitat were also important in structuring helminth communities dominated by indirect-life-cycle parasites at those locations. Similarly, in both of those studies, adult frogs had higher species richness than young-of-the-year individuals, indicating that size and/or age were also important in acquiring new species of helminths into the infracommunity of these hosts. Data from our study and the Michigan and New Brunswick frogs suggest that although helminth species composition, richness, and prevalence may be variable depending on collection site and the ecological factors influencing variation in life history traits in local populations at those locations, green frog helminth communities are dominated by digenetic trematodes acquired in a semiaquatic habitat and/ or through the frogs' diet.

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Announcement of the FOURTH INTERNATIONAL CONGRESS OF NEMATOLOGY at the Tenbel Resort, Tenerife, Canary Islands, Spain June 8–13, 2002

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Blood Parasites of the Ring-Necked Duck (*Aythya collaris*) on Its Wintering Range in Florida, U.S.A.

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ABSTRACT: Five species of parasites were found in blood smears from 283 ring-necked ducks, *Aythya collaris* (Donovan, 1809) overwintering in Florida. These included the following (with overall prevalences in parentheses): *Haemoproteus nettionis* (Johnson and Cleland, 1909) (5.3%), *Leucocytozoon simondi* Mathis and Leger, 1910 (9.2%), *Splendidofilaria fallisensis* (Anderson, 1954) (39.2%), and 2 unidentified species of filaroids, Species I (6.0%), and Species II (13.8%). Ninety-seven ducks were infected with 1 species only, 43 with 2 species, and 8 with 3 species. The most common combined infection was *L. simondi* and *S. fallisensis*, which occurred 15 times. Prevalences of *H. nettionis* were significantly higher in ducks from the state's panhandle (far northwest) and north-central regions, whereas prevalences of *L. simondi* were higher in the panhandle. Microfilariae of unidentified Species II were more prevalent in the north-central and southern regions. Microfilariae of *S. fallisensis* and the 2 unidentified species were more prevalent in female ducks than in males. *Leucocytozoon simondi* was more prevalent in juvenile ducks than in adults, whereas the prevalences of the microfilariae of both unidentified species were higher in adult ducks.

KEY WORDS: Ring-necked duck, *Aythya collaris, Haemoproteus nettionis, Leucocytozoon simondi, Splendidofilaria fallisensis,* microfilariae, blood parasites, Hematozoa, Florida, U.S.A.

The ring-necked duck, *Aythya collaris* (Donovan, 1809) is a fairly common and sometimes abundant transient and winter visitor throughout the state of Florida, U.S.A. (Robertson and Woolfenden, 1992). This duck is the most heavily hunted species of waterfowl in Florida; the mean annual total harvest by hunters for the 10-yr period of 1981–1990 was 64,165 (Martin, 1996). Although there have been a number of published reports of the occurrence and prevalence of blood parasites of this host elsewhere in North America (see Bennett et al., 1982; Bishop and Bennett, 1992), there is no such information on this species in Florida.

The objectives of our study were to identify the species of blood parasites present in ring-necked ducks overwintering in Florida and to determine the relationships of location within the state, gender, and age of the hosts to the prevalences of these hematozoans.

Materials and Methods

The sample consisted of 283 ring-necked ducks that were collected by shooting during November to March of 1979–1982 from 3 regions. Region 1 consisted of 2 counties in the Florida panhandle (Jefferson and Leon) (30°30′N; 84°00′W); Region 2, 3 counties in north-central Florida (Alachua, Hamilton, and Putnam) (29°50′N; 82°30′W); and Region 3, 1 county in southern Florida (Broward) (26°10′N; 80°20′W). Ages were determined by analyses of wing plumage and bursal development. The gender of each bird was determined by plumage and confirmed by cloacal and gonad examination at necropsy.

Thin blood films were made with blood obtained by cardiac puncture and stained for 1 hr with Giemsa at pH 7 after fixation in 100% methanol. Smears were scanned microscopically at low power (160×) in order to detect larger hematozoans, and a total in excess of 10,000 red blood cells was examined at higher powers (400× and 1,000×). Hematozoans found at lower powers were examined further at high power (1,000×) to confirm specific identification.

The length of each microfilaria and the measurement of a stage micrometer were traced onto a sheet of paper using a camera lucida. A curvimeter was calibrated with the stage micrometer tracing, and then the length of each microfilaria tracing was measured and recorded. Widths were measured directly with an ocular micrometer. Where possible, at least 10 microfilariae of each type were traced and measured for each blood film; in 19 instances, it was not possible to find 10 microfilariae to measure because of low intensities of infection. Measurements of microfilariae included the cephalic space and 3 fixed points (i.e., excretory cell, inner body, and anal pore, measured from the anterior end of the microfilaria to the beginning of the fixed point area) expressed as percentages of body length. All measurements are given in micrometers; means are followed by ranges in parentheses.

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Voucher specimens have been deposited in the International Reference Centre for Avian Haematozoa at the Queensland Museum, Brisbane, Australia (accession nos. G462509–G462640) and the U.S. National Parasite Collection in Beltsville, Maryland, U.S.A. (accession nos. 88248–88254). Prevalence data for each parasite were evaluated by using PROC FREQ for 2-tailed Fisher's exact tests with regard to locality, gender, and age (SAS Institute, 1989). Significance was taken at $P < 0.05$. Terminology used was according to Bush et al. (1997).

Results

Five species of blood parasites were identified, 2 protozoans (*Haemoproteus nettionis* (Johnson and Cleland, 1909) and *Leucocytozoon simondi* Mathis and Leger, 1910) and microfilariae of 3 nematodes (*Splendidofilaria fallisensis* (Anderson, 1954) and 2 unidentified species). The microfilariae of *S. fallisensis* (n = 1,096) were 75.1 (41–130) in length and 4.9 (4.1–6.0) in width, with a rounded anterior, and a tapering tail that ended bluntly. Sheaths were lacking.

Microfilariae of 2 unidentified filaroid species were observed in 36 ring-necked ducks. Microfilariae of Species I were long and slender with a bluntly rounded anterior and an overall uniform body width that tapered slightly to a rounded posterior. These microfilariae (n = 94) were 150 (110–190) in length and 5.8 (5.0–6.0) in width. They had a well-defined sheath that could be seen at 1 or both ends of the body. The cephalic space was 4.8 (3–7, n = 10). Fixed points (n = 10), expressed as percentages of body length, were as follows: excretory cell 36.4 (35–38), inner body 64.9 (62–67), anal pore 86.6 (83–90). Microfilariae of Species II (n = 287) were similar in appearance to Species I, except they were 150 (120–210) in length and 5.8 (5.0–6.0) in width and lacked a sheath. The cephalic space was 5.2 (3–6, n = 10). Fixed points (n = 10) were as follows: excretory cell 36.5 (35–39), inner body 64.7 (63–67), anal pore 86.6 (85–90).

The overall prevalences were *H. nettionis* (5.3%), *L. simondi* (9.2%), *S. fallisensis* (39.2%), filaroid Species I (6.0%), and filaroid Species II (13.8%). Of the sample, 97 ducks were infected with 1 species of parasite, 43 with 2, and 8 with 3. The most common multiple infection was a combination of *L. simondi* and *S. fallisensis* (n = 15), followed by combinations of the 2 unidentified filaroids (n = 10).

There were no significant differences in prevalences of the 5 species of parasites among the collection periods, and therefore data for all years were combined. Prevalences of *H. nettionis* in Regions 1 (7%) and 2 (7%) were significantly higher (P = 0.03) than in Region 3 (0%). The prevalence of *L. simondi* was significantly higher (P = 0.0005) in Region 1 (19%) than in Regions 2 (8%) and 3 (1%). Microfilariae of Species II were more prevalent (P = 0.016) in Regions 2 (14%) and 3 (22%) than in Region 1 (6%). There were no regional differences in the prevalences of the microfilariae of *S. fallisensis* and Species I.

Microfilariae of *S. fallisensis,* Species I, and Species II were more prevalent in female ducks (46%, 9%, and 23%) than in males (33%, 3%, and 5%) (P = 0.028, 0.042, and 0.00002). The prevalences of *H. nettionis* and *L. simondi* were similar.

Leucocytozoon simondi was more prevalent (P = 0.021) in juveniles (16%) than in adults (6%). On the other hand, filaroid Species I and II were both more prevalent (P = 0.029 and 0.001) in adults (8% and 18%) than in juveniles (1% and 4%). There were no age differences in the prevalences of *H. nettionis* and *S. fallisensis.*

Discussion

Although this is the first report of *H. nettionis* and *L. simondi* from ring-necked ducks in Florida, both have been identified in ring-necked ducks overwintering in Texas, U.S.A. (Loven et al., 1980). Prevalences in the 20 ducks examined in Texas were similar to those of our Florida birds, i.e., 5% for *H. nettionis* and 10% for *L. simondi.* No microfilariae were reported in the Texas study. Unidentified microfilariae were seen in 1 of 6 ring-necked ducks examined in Maine, U.S.A.; the authors stated that their microfilariae were 45–65 long and 4–6 wide with a short, narrow, and slightly twisted tail (Nelson and Gashwiler, 1941). In a study of the blood parasites of 178 ring-necked ducks in the Maritime Provinces of Canada (New Brunswick, Nova Scotia, and Prince Edward Island), the prevalence of *H. nettionis* was higher (10%), and of *L. simondi* was lower (5%), than those in our birds from Florida (Bennett et al., 1975). No microfilariae were reported in the Maritime study. In another study, Bennett and Inder (1972) found microfilariae in 1 of 10 ring-necked ducks from Newfoundland, Canada, but provided no descriptions or measurements.

Anderson's (1956) description of the microfilariae of *S. fallisensis* was similar to our specimens, with the exception of ranges of the lengths and the lack of sheaths; however, he noted that the sheaths of *S. fallisensis* were extremely delicate, and he was unable to see them in most specimens stained with Giemsa. Anderson (1956) also described a microfilaria from a European teal (*Anas crecca* Linnaeus, 1758) that he called Type D. His Type D microfilariae were similar to those of *S. fallisensis* except for the length (range = 110–138) and the lack of a sheath. The microfilariae in our ring-necked ducks that we are calling *S. fallisensis* could actually represent 2 species. However, it is possible that because we measured twice as many microfilariae as did Anderson (1956), that we have determined that the range of lengths for the microfilariae of this species is more extensive than previously recognized. Therefore, we are calling those microfilariae that fell in the range of 41–130 in length, but otherwise conformed to Anderson's (1956) description, *S. fallisensis*. The high number of combined infections of *S. fallisensis* and *L. simondi* in the same bird (n = 15) was probably due to the fact that both parasites utilize the same species of simuliid blackflies as vectors (Fallis et al., 1951; Anderson, 1968).

Of the 36 ducks that had unidentified microfilariae, 2 had Species I only, while 17 had Species II only. Seventeen of 36 ducks had both Species I and II microfilariae. The fact that Species I usually occurred with Species II and only twice by itself and that the range of lengths and several fixed points were almost equal may support the idea that these are variations of a single species. In many ways (morphologic and metric) our microfilariae resemble those of *Chandlerella bushi*, described by Bartlett and Anderson (1987) from American coots (*Fulica americana* Gmelin, 1789) in Manitoba, Canada. They were not able to see the sheaths on microfilariae of *C. bushi* in blood films made from fresh heart blood and stained with Giemsa. However, they were able to see the sheaths on Giemsa-stained blood films of heart blood taken from thawed carcasses or specimens teased from lung tissue. The lack of visible sheaths of microfilariae in our ring-necked duck blood films might be because they were made from fresh heart blood. The identification of the microfilariae from ring-necked ducks will have to await the discovery of adult worms and further study and comparison of their intrauterine microfilariae with those from the blood.

The regional differences in the prevalences of *H. nettionis* and *L. simondi* may have been a reflection of the location of the breeding grounds and flyways used by different subpopulations of ring-necked ducks. Because transmission of the blood parasites of waterfowl does not occur in Florida (Thul et al., 1980; Thul and O'Brien, 1990; Forrester et al., 1994), the ducks must become infected either on the breeding grounds or during migration. The types and numbers of arthropod vectors found on various breeding grounds might differ and thereby influence the acquisition of these blood parasites in various segments of the North American population. Ring-necked ducks that overwinter in Florida are known to breed during the summer months in various prairie provinces of Canada across to Ontario and the eastern U.S.A. (Bellrose, 1976). Some ducks that breed in the more western regions of Canada migrate eastward and then move southward. Others migrate southward and pass through Wisconsin, Indiana, Tennessee, and Georgia. Most of the ring-necked ducks in Florida originate from Ontario, Manitoba, and the District of Mackenzie (Bellrose, 1976). The lower prevalence of infections of *L. simondi* in adults may be due to age-related immunity. Reasons for the gender differences in prevalences are unknown.

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Parelaphostrongylus tenuis (Nematoda: Protostrongylidae) and Other Parasites of White-Tailed Deer (*Odocoileus virginianus*) in Costa Rica

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ABSTRACT: Parasites were collected from 2 female white-tailed deer (*Odocoileus virginianus*) in the Area de Conservación Guanacaste, Costa Rica, in early June 1999. Both deer were parasitized by the ticks *Amblyomma parvum* and *Haemaphysalis juxtakochi* as well as the hippoboscid fly, *Lipoptena mazamae*. One deer also hosted the ticks *Boophilus microplus, Ixodes affinis,* and *Anocentor nitens*. Both deer were infected by larvae of the nasopharyngeal botfly *Cephenemyia jellisoni,* and the helminths *Eucyathostomum webbi, Gongylonema pulchrum, Parelaphostrongylus tenuis,* and *Paramphistomum liorchis,* whereas *Setaria yehi,* an undescribed species of *Ashworthius,* and *Onchocerca cervipedis* occurred in single hosts. A cysticercus of *Taenia omissa* was found encapsulated in the lung parenchyma of 1 host. This is the first report of these endoparasites from Central America.

KEY WORDS: *Ashworthius* sp., biodiversity, ticks, *Boophilus microplus, Gongylonema pulchrum, Haemaphysalis juxtakochi, Ixodes affinis, Odocoileus virginianus,* white-tailed deer, helminth parasites, *Parelaphostrongylus tenuis,* cysticercus, Costa Rica.

The white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780) has a widespread Nearctic and Neotropical range, extending from southern Canada and the United States through Mexico and Central America to Bolivia, the Guianas, and northern Brazil (Reid, 1997). The subspecies described from Costa Rica, *Odocoileus virginianus truei* Merriam, 1898, ranges from the southeastern edge of Mexico to northeastern Panama (Whitehead, 1972; Mendez, 1984). The parasite fauna of *O. virginianus* and other cervids is well documented in North America (Walker and Becklund, 1970; Davidson et al., 1981). However, very little information is available on parasites of cervids in the southern parts of their range, including Central America. This is significant because white-tailed deer are hosts to several serious pathogens and parasites of cervids and other animals, including the tick *Ixodes scapularis* Say, 1821, which is the main North American vector of the agent of Lyme borreliosis. Additionally, one of the most important parasites in *O. virginianus* is *Parelaphostrongylus tenuis* (Dougherty, 1945), the meningeal worm. This species is not pathogenic in *O. virginianus,* but when snails infected with its larvae are ingested by other ruminants such as moose (*Alces alces* (Linnaeus, 1758)), fallow deer (*Dama dama* (Linnaeus, 1758)), reindeer (*Rangifer tarandus* (Linnaeus, 1758)), and llamas (*Lama* spp.), severe neurologic disease can result from adult worms in the brain and central nervous system (Anderson, 1964, 1970; Nettles et al., 1977; Krogdahl et al., 1987; Rickard et al., 1994).

The following report is part of a biodiversity inventory of eukaryotic parasites of vertebrates in the Area de Conservación Guanacaste (ACG) in northwestern Costa Rica.

Materials and Methods

We collected 2 adult female *O. virginianus* within the ACG, Guanacaste, Costa Rica (10°57′N; 85°48′W) in early June 1999. Ectoparasites were collected within 1 hr postmortem. Internal organs were then removed, following procedures suggested by Nettles (1981), and examined for endoparasites. In addition to onsite ex-

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amination, several aliquots of abomasal and intestinal contents were fixed and later searched for parasites. Contents from the abomasum and small intestine were suspended in 2 liter of water. Duplicate aliquots of 200 ml were removed, fixed in 5% formalin, and subsequently examined using a stereomicroscope. Digeneans were fixed in hot 10% formalin and stored in 70% ethanol. Ectoparasites and nematodes were stored in 70% ethanol without fixation in formalin. Prior to examination, nematodes were cleared in either glycerine or phenol–alcohol. Tissues examined for sarcocysts were embedded in paraffin, sectioned, and stained with both hematoxylin and eosin and periodic acid-Schiff stains for diagnosis. Amphistome digeneans were prepared using the same methods and identified using descriptions by Kennedy et al. (1985) and Sey (1991). Voucher tick specimens are deposited in the U.S. National Tick Collection (USNTC), Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, Georgia, U.S.A. Voucher specimens of other parasites are deposited in the U.S. National Parasite Collection (USNPC), United States Department of Agriculture, Beltsville, Maryland, U.S.A. Accession numbers are listed in Table 1.

Results

A total of 18 parasite species was found in the 2 deer (Table 1). Both deer hosted the hippoboscid louse fly *Lipoptena mazamae* and the ixodid ticks *Amblyomma parvum* and *Haemaphysalis juxtakochi.* We also found second- and third-stage larvae of *Cephenemyia jellisoni* (identified using keys in Bennett and Sabrosky [1962]) in the nasal sinuses of both deer. One deer also hosted the ticks *Anocentor nitens, Boophilus microplus,* and *Ixodes affinis.*

Both deer hosted the digenean trematode *Paramphistomum liorchis* in their rumens. They also were infected by 3 species of nematodes: *Gongylonema pulchrum* in the submucosa of the esophagus, *Eucyathostomum webbi* in the large intestine, and *Parelaphostrongylus tenuis* in the inner surface of the dura and from cranial sinuses and nerves. Meristic data for the latter species (Table 2) did not differ from those reported for *P. tenuis* in *O. virginianus* from North America (Anderson, 1963; Carreno and Lankester, 1993), although the esophageal lengths of the 2 males were greater than those reported in North American specimens.

A cysticercus of *Taenia omissa* was found encapsulated in fibrous connective tissue in the lung parenchyma. Identification of the cysticercus was based on structure and measurements of rostellar hooks and the occurrence in deer; morphology of the intact cysticercus was consistent with observations by Rausch (1981).

Two specimens of *Setaria yehi* were collected from 1 deer, 1 in the rectum, and the other in the posterior region of the body cavity. From the abomasal intestinal aliquots, few nematodes in the abomasa and none in the small intestines were found. A female specimen of *Mazamastrongylus* sp. occurred in the abomasum of 1 deer. It could not be identified to species based on the diagnostic features within the genus. Hoberg (1996) demonstrated polymorphism in vulvar anatomy, and no males were found. In the abomasum of the second deer, specimens of an undescribed species of *Ashworthius* were found.

Protozoan infections were not obvious in these animals (blood smears and fecal examinations were negative), except for the presence of unidentified sarcocysts in the hind leg muscles of 1 host.

Discussion

Parasites in white-tailed deer

This report constitutes the first data on parasites of *O. virginianus* in Costa Rica. The ranges of *C. jellisoni, P. liorchis, E. webbi, O. cervipedis,* and, most importantly, *P. tenuis* are now extended south of North America. These range extensions suggest that the parasites may also be present in Mexico, other parts of Central America, and perhaps South America, coinciding with the distribution of this cervid.

There is little information on the parasites of cervids south of the United States. Captive *O. virginianus* from the Yucatan Peninsula have been reported as hosts of nematodes (*Haemonchus* sp., *Cooperia* spp., *Isospora* spp., *Eimeria* spp., *Trichuris* spp., *Strongyloides* spp.) and cestodes (*Moniezia* spp.), based on examination of fecal samples and fecal culture of nematode L3 larvae (Montes-Pérez et al., 1998). Several parasites have been listed from *O. virginianus* from Mexico and Central America, including arthropods, *Cephenemyia* sp., *L. mazamae, A. nitens, H. juxtakochi, I. affinis,* and nematodes, *Haemonchus contortus* (Rudolphi, 1802) Cobb, 1898, and *Gongylonema pulchrum,* as well as several other species not found in the present study (Mendez, 1984). In South America, *O. virginianus* has been reported as a host for the nematodes *H. contortus, Setaria* sp., *Oesophagostomum asperum* Railliet and Henry, 1913, and *Mecistocirrus* sp., as well as the arthropods

Table 1. Parasites recovered from *Odocoileus virginianus* in Guanacaste, Costa Rica.

Parasite	Deer 1	Deer 2	Accession number
Protozoa			
Sarcocystis sp.	–	+	USNPC 90056
Arthropoda: Acari			
Amblyomma parvum Aragão, 1908	+	+	RML 122837, RML 122838
Anocentor nitens (Neumann, 1897)	+	–	RML 122838
Boophilus microplus (Canestrini, 1887)	+	–	RML 122838
Haemaphysalis juxtakochi (Cooley, 1946)	+	+	RML 122837, RML 122838
Ixodes affinis Neumann, 1899	+	–	RML 122838
Amblyomma sp. (immature stages)	+	+	RML 122837, RML 122838
Arthropoda: Diptera			
Lipoptena mazamae Rondani, 1878	+	+	USNPC 90062, USNPC 90063
Cephenemyia jellisoni Townsend, 1941	7	10	USNPC 90053, USNPC 90054
Trematoda			
Paramphistomum liorchis Fischoeder, 1901	+ (>1,000)	+ (<100)	USNPC 90055, USNPC 90067
Cestoda			
Taenia omissa Lühe, 1910	–	+	USNPC 90052
Nematoda			
Setaria yehi (Desset, 1966)	1 male 1 female	–	USNPC 90059
Ashworthius sp.	2 males 3 females	–	USNPC 90048, USNPC 90049, USNPC 90050
Gongylonema pulchrum Molin, 1857	17 males 29 females	4 males 7 females	USNPC 90057, USNPC 90058
Eucyathostomum webbi Pursglove, 1976	5 males 19 females	1 male 1 female	USNPC 90064, USNPC 90065
Parelaphostrongylus tenuis (Dougherty, 1945)	1 male 5 females	3 males 4 females	USNPC 90060, USNPC 90061
Mazamastrongylus sp.	1 female	–	USNPC 90051
Onchocerca cervipedis Wehr and Dikmans, 1935	–	6 males 22 females	USNPC 90066

Amblyomma spp., *Boophilus* spp., *Lipoptena* sp., *Dermatobia* sp., *Calliphora* sp., and *Demodex* sp. (Brokx, 1984). No voucher specimens are available from these published studies, making confirmation of species identifications impossible. In addition, parasite identifications based on eggs or larvae recovered from feces are often unreliable.

Arthropods

The louse fly *L. mazamae* is a widespread ectoparasite of deer in North, Central, and South America (Maa, 1963). Although this ectoparasite can be abundant on white-tailed deer, it is not known to transmit any pathogens to them (Strickland et al., 1981). The nasopharyngeal bot fly *C. jellisoni* has been recorded from much of North America (Bennett and Sabrosky, 1962; Capelle, 1971), but it has not previously been reported from Central America. The current records from Costa Rica suggest that *C. jellisoni* may also occur in other Central American countries.

The tick fauna of Costa Rica is sparsely documented (Tonn et al., 1963), but the USNTC contains unpublished records and specimens for this country representing 44 different species of ticks. The collection of at least 5 species of ixodid ticks (immature stages of *Amblyomma* spp. [Table 1] could not be identified to species and could therefore represent additional species) from just 2 deer at 1 site is suggestive of a diverse tick fauna in the Guanacaste region. Although 18 species of ticks have been recorded from *O. virginianus* in the United States (Strickland et al., 1981), only a few of these species are commonly collected from this host. Further, most of these tick species are typically segre-

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Table 2. Morphometric measurements for *P. tenuis* recovered from *O. virginianus* in Guanacaste, Costa Rica; measurements are given in microns (μm) unless otherwise noted.

	N	Mean	Range
Males			
Length (mm)	1	47.00	—
Width (posterior to esophagus)	2	170.00	140–200
Esophagus length	2	807.50	795–820
Nerve ring from anterior	2	120.00	107–133
Excretory pore from anterior	1	110.00	—
Gubernaculum length	3	117.00	110–131
Crura length	3	36.17	25.7–44.0
Spicules	6	225.00	211–236
Spicule branch	2	71.50	70–73
Females			
Length (mm)	1	105.50	—
Width (posterior to esophagus)	2	210.00	190–230
Nerve ring from anterior	2	105.50	102–109
Excretory pore from anterior	2	108.50	106–111
Vulva from posterior	8	185.50	165–214
Anus from posterior	8	56.96	45.5–79.0

gated geographically and seasonally. For example, in Alabama, Durden et al. (1991) recovered 4 species of ticks from 537 *O. virginianus* examined during winter (November–January), but only 2 of these species (*I. scapularis* and *Dermacentor albipictus* (Packard, 1869)) were common. In Alberta, Samuel et al. (1980) recorded just 1 species of tick (*D. albipictus*) from 148 *O. virginianus* examined in all months, whereas Smith (1977) recorded 7 tick species from this host from 12 southeastern states, again with just 2 species (*I. scapularis* and *Amblyomma americanum* (Linnaeus, 1758)) being common. In southern Texas, Samuel and Trainer (1970) recovered 6 species of ticks from 404 *O. virginianus* examined, with 3 of these species (*A. americanum, Amblyomma inornatum* Banks, 1909, and *Amblyomma maculatum* Koch, 1844) being prevalent.

The known geographical range of *A. parvum* extends from Mexico to Argentina. Throughout this range it parasitizes a wide variety of mammals (Fairchild et al., 1966; Jones et al., 1972). In Panama, Fairchild et al. (1966) recorded *A. parvum* from white-tailed deer, cattle, domestic cats, sloth, human, anteater (*Tamandua* sp.), and cotton rats (*Sigmodon* spp.). There is an unpublished USNTC record of this tick from a horse in Costa Rica.

The tropical horse tick, *A. nitens,* is a pest of equines in the neotropics and is the main vector of *Babesia equi* (Laveran, 1901), the protozoan that causes equine piroplasmosis (Strickland et al., 1976). In adjoining Panama, Fairchild et al. (1966) reported this tick from horses, cattle, and deer. The USNTC contains 8 collections of *A. nitens* from Costa Rica: 6 from horses, 1 from a domestic cat, and 1 from a rabbit (*Sylvilagus* sp.).

The southern cattle tick, *B. microplus,* was formerly widespread throughout the New World as a major pest of domestic cattle, where it caused tremendous economic damage through its role as a vector of *Babesia bigemina* (Smith and Kilborne, 1893), an agent of bovine piroplasmosis (=Texas cattle fever) (Strickland et al., 1976; Bram and George, 2000). This tick also is a vector of *Anaplasma marginale* Theiler, 1910 and *Babesia bovis* (Babes, 1888) Starcovici, 1893. In Costa Rica, Hermans et al. (1994) reported high infection rates for these 3 hemoparasites in *B. microplus* and high seroprevalences to them in cattle. In Panama, Fairchild et al. (1966) reported this tick from cattle, horses, pigs, and dogs, with single collections from a goat and a deer. The USNTC contains 23 collections of *B. microplus* from Costa Rica: 15 from cattle, 2 from horses, 2 from vegetation, and 1 each from a human, a tapir (*Tapirus* sp.), a gray fox (*Urocyon cinereoargenteus* (Schreber, 1775)), and a fringe-lipped bat (*Trachops cirrhosus* (Spix, 1823)).

Deer are the preferred hosts of *H. juxtakochi,* which is widely distributed from Mexico to Argentina (Jones et al., 1972). However, this tick has occasionally also been collected from rodents, humans, tapirs, coatimundis (*Nasua* sp.), peccaries (*Tayassu* spp.), porcupines (*Coendou* spp.), and lagomorphs (Fairchild et al., 1966; Jones et al., 1972). The USNTC contains 1 other Costa Rican collection of *H. juxtakochi,* also from a white-tailed deer.

Ixodes affinis has a disjunct geographical distribution, with 1 focus in the southeastern U.S.A. (coastal Florida, Georgia, and South Carolina), and the other focus extending from Mexico to Brazil (Fairchild et al., 1966; Durden and Keirans, 1996). Because it is a member of the *Ixodes ricinus* complex, several members of which are vectors of the Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde et al., 1984, this tick could be an enzootic vector of this zoonotic pathogen. It parasitizes a range of host species, but most collections, especially of adults, are from deer and larger carnivores (Fairchild et al., 1966; Durden and Keirans, 1996). The USNTC includes 5 additional Costa Rican collections of *I. affinis*: 2 from ocelots (*Leopardus pardalis* (Linnaeus, 1758)), and 1 each from a human, a horse, and a long-tailed weasel (*Mustela frenata* Lichtenstein, 1831).

Digeneans

Brokx (1984) and Mendez (1984) reported amphistome digeneans, *Cotylophoron* sp. and *Paramphistomum cervi* (Zeder, 1790), respectively, in the stomach of *O. virginianus*. Unfortunately no voucher specimens exist from those accounts, so we cannot confirm their identifications. The only amphistomes we found were *P. liorchis*, the species most commonly reported from *O. virginianus* in North America (Kennedy et al., 1985).

Cestodes

Taenia omissa has a broad geographic distribution in the Western Hemisphere, coinciding with the range of the cougar, *Puma concolor* (Linnaeus, 1771), and deer intermediate hosts including *Odocoileus* and *Mazama* in North and South America (Rausch, 1981; Rausch et al., 1983). Consistent with the current study, cysticerci generally are found in the thoracic cavity, including the lungs and pericardium (Forrester and Rausch, 1990). Although cysticerci have been reported in brocket deer (*Mazama* cf. *gouazoubira* (Fischer, 1814)) from eastern Colombia (Rausch, 1981), there are apparently no prior records from Central America. Prevalence and intensity of infection in deer may be influenced by differences in population density of cougars across the range of this parasite–host assemblage (Forrester and Rausch, 1990).

Nematodes

This is the first record of *P. tenuis* south of the United States. The presence of elaphostrongyline nematodes in cervids is a major concern in the translocation of these animals in wildlife projects and the game ranching industry (Lankester and Fong, 1989; Samuel et al., 1992; Miller and Thorne, 1993; Davidson et al., 1996). *Parelaphostrongylus tenuis* is of great concern in future wildlife management and conservation practices in Central America. An overall decline of *O. virginianus* populations in Mexico and Central America due to overhunting and habitat loss (Mendez, 1984) raises the possibility of reintroducing deer to areas where they have been extirpated. The effects of *P. tenuis* on the only other Central American cervid, the brocket deer (*Mazama americana* (Erxleben, 1777)), are unknown. As *P. tenuis* is highly pathogenic in most cervids other than *O. virginianus*, it may also be pathogenic in *Mazama* spp. Until the pathogenic significance (if any) of *P. tenuis* to *M. americana* has been determined, the translocation of both it and Central American *O. virginianus* may be problematic in areas inhabited by other cervids that may be susceptible to parelaphostrongylosis.

The translocation of infected *O. virginianus* to areas in which *P. tenuis* is absent may result in the establishment of the parasite in other areas. The importation of deer from Pennsylvania to an island off the Georgia coast may have resulted in the establishment of *P. tenuis* in an area outside its normal range (Davidson et al., 1996). Similarly, the translocation of reindeer (*Rangifer tarandus*) from Norway to Newfoundland has led to the establishment of *Elaphostrongylus rangiferi* Mitskevitch, 1960, another pathogenic species, in this region of North America (Lankester and Fong, 1989). Other cervids such as red deer (*Cervus elaphus* Linnaeus, 1758) and moose (*Alces alces* (Linnaeus, 1758)) may also harbor *Elaphostrongylus* species, and North American elk have been shown to have potential for surviving infection with and passing larvae of *P. tenuis* (Samuel et al., 1992). These studies indicate a need for reliable diagnosis of *P. tenuis* in ungulates and the need to determine the full distribution of this parasite. This information can help to prevent the spread of the parasite to uninfected host populations. Cervids of western North America are of particular concern, as *P. tenuis* has not been recorded in western states and provinces (Miller and Thorne, 1993).

The presence of *P. tenuis* in Central American deer is also of evolutionary significance. As yet, we do not know if *M. americana* or any of the South American cervids such as *Pudu* spp. and *Ozotoceros* spp. are hosts for protostrongylids. Phylogenetic analysis of the family Protostrongylidae and comparison with host distribution, however, indicates that cervids are the basal hosts of these parasites (Carreno and Hoberg, 1999). Discovery of new or already described protostrongyles in these hosts will contribute further to an understanding of the evolution of the Protostrongylidae.

The presence of an undescribed species of *Ashworthius* is also of interest. Species of this genus of the Haemonchiinae have never been reported from hosts in the Western Hemisphere, although both *Haemonchus* and *Mecistocirrus* are known in Central and South America. In Africa and Eurasia, species of *Ashworthius* have been reported from cervids and members of 2 subfamilies of the Bovidae, but not species of *Bos* (Pike, 1969; Drozdz et al., 1998). The presence of *Ashworthius* sp. in a wild cervid in the Western Hemisphere may have been the result of introduction and colonization from bovine hosts following European settlement since the 1500s (Hoberg, 1997). The host distribution for this genus and its apparent absence in domestic bovids, however, suggest otherwise. Alternatively, the distribution of *Ashworthius* spp. in Central America may be relatively archaic, reflecting an extended history with endemic cervids. Historical reconstruction of the biogeography of *Ashworthius* in the New World is currently hampered by the paucity of survey data regarding parasitism in wild ruminants in Central and South America. Additionally, it is important to note that superficially some species of *Ashworthius* may resemble and be confused with *Haemonchus* spp.

Conclusions

The purpose of parasite inventories is 2-fold. First, the information obtained from parasite faunas can contribute valuable integrative information on our knowledge of the biosphere that serves as an indicator of biodiversity (Brooks and Hoberg, 2000). Secondly, in the case of the white-tailed deer, it is important to develop an understanding of the distribution of potential pathogens of wildlife. Many wildlife pathogens pose a serious threat to global biodiversity and also include several zoonoses (Daszak et al., 2000), and it is thus necessary to assess carefully the global distribution of potentially pathogenic parasites (Hoberg, 1997). The results of this study are an important contribution to biodiversity initiatives in Costa Rica. They have important implications in conservation projects in the region, as some parasites of white-tailed deer, such as *P. tenuis,* may be pathogenic in other, less common endemic hosts such as *M. americana.*

The uncertainty of species identifications in other reports, such as the amphistome digeneans (Brokx, 1984; Mendez, 1984), demonstrates the need for deposition of suitable voucher specimens in documenting parasite fauna. Are we dealing with a widespread and relatively uniform parasite fauna of white-tailed deer throughout their range, or one that is highly localized depending on habitat? A lack of voucher specimens to confirm identifications, and a lack of sufficient expert taxonomists available to provide those identifications (the taxonomic impediment: Brooks and Hoberg, 2000), prevents us from making such assessments. And such assessments, in turn, are critical for management policy, including game farming, sport hunting, and the interface between wildlands and agroscape.

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Cepedietta michiganensis (Protozoa) and *Batracholandros magnavulvaris* (Nematoda) from Plethodontid Salamanders in West Virginia, U.S.A.

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ABSTRACT: The gastrointestinal tracts of 38 plethodontid salamanders (25 *Plethodon punctatus* and 13 *Plethodon wehrlei*), collected at high elevation sites in Pendleton and Randolph counties, West Virginia, U.S.A., were examined for parasites in 1996. Sixty percent of *P. punctatus* and 23% of *P. wehrlei* were infected by the ciliate *Cepedietta michiganensis*, while prevalences of the nematode *Batracholandros magnavulvaris* were 52% and 30% for *P. punctatus* and *P. wehrlei*, respectively. Mean intensities were 3.4 nematodes per infected host for *P. punctatus* and 4.0 for *P. wehrlei*. Only 2 of 61 *B. magnavulvaris* collected were males. This is the first report of parasites from these plethodontid species and the first record of *C. michiganensis* from West Virginia.

KEY WORDS: *Batracholandros magnavulvaris, Cepedietta michiganensis, Plethodon punctatus, Plethodon wehrlei*, Ciliata, Nematoda, West Virginia, U.S.A.

The Cow Knob salamander, *Plethodon punctatus* Highton, 1972, is a large plethodontid salamander known only from higher elevations (>730 m) of the Shenandoah and Great North mountains in Augusta, Rockingham, and Shenandoah counties of Virginia (Buhlmann et al., 1988; Conant and Collins, 1991). The range of this rare species in West Virginia is restricted to higher elevations (>730 m) of Hardy and Pendleton counties in the eastern panhandle. All 25 *P. punctatus* examined for this study were collected on Shenandoah Mountain in Pendleton County, West Virginia, from June through August 1996, under a permit granted by the West Virginia Division of Natural Resources (WVDNR) and written permission from the U.S. Fish and Wildlife Service.

Wehrle's salamander, *Plethodon wehrlei* Fowler and Dunn, 1917, is considered a near sibling of *P. punctatus,* and has been recorded from a wide range of elevations in 28 of West Virginia's 55 counties (Green and Pauley, 1987). The geographic range of *P. wehrlei* extends from southwestern New York to northwestern North Carolina (Conant and Collins, 1991). All 13 *P. wehrlei* individuals used in this study were collected from Shaver's Mountain in Randolph County, West Virginia, from May through August 1996, under a permit from the WVDNR.

The original purpose of collecting these plethodontid species was to obtain reproductive and ecological data for use in forest and wildlife management plans. Because there are no published reports of parasites from either species, these collections also offered the opportunity to examine them for parasites.

Materials and Methods

All salamanders were anesthetized in Chloretone® within 48 hr of collection. Snout-to-vent lengths (SVL) were measured with vernier calipers to the nearest 0.1 mm. Salamanders were killed by decapitation, sexed, and the small and large intestines were removed for examination. The SVL for *P. punctatus* (*n*/mean in mm ± 1 SD) was 18/63.8 ± 6.9 for males and 7/65.3 ± 7.9 for females. Because the difference in mean SVL for males versus females was not significant ($t_{0.05,23}$ = 0.469), individuals of both host sexes were combined to calculate prevalence of ciliate infection and prevalence and mean intensity of nematode infection. The SVL for *P. wehrlei* (*n*/mean in mm ± 1 SD) was 6/59.8 ± 9.3 for males and 7/59.9 ± 11.7 for females. Again the difference in mean SVL for males versus females was not significant ($t_{0.05,11}$ = 0.017), and the data for both host sexes were combined for calculations of prevalence and mean intensity.

During necropsy it was evident that both salamander species harbored astomatous ciliates and nematodes. Whole mounts of these ciliates and nematodes were prepared by staining in Semichon's acetic carmine, dehydrating in an ethanol series, clearing in xylene, and mounting in Permount®. Voucher specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, U.S.A., under accession numbers USNPC 89838 (*Cepedietta michiganensis*) and USNPC 89839 (*Batracholandros magnavulvaris*).

Results

The astomatous ciliate, *C. michiganensis* (Woodhead, 1928) Corliss, de Puytorac, and

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Table 1. Published reports of amphibian hosts harboring *Cepedietta michiganensis*, with prevalences (P) by locality; host scientific names, taxonomic authorities, and dates after Frost (1985).

Host common name and species	P (%)	Locality	Reference
Ambystoma jeffersonianum (Green, 1827) (Jefferson salamander)	1/NI*	Michigan	Woodhead, 1928
Ambystoma opacum (Gravenhorst, 1807) (marbled salamander)	7	North Carolina	Rankin, 1937a
Desmognathus fuscus (Green, 1818) (northern dusky salamander)	0/12†	North Carolina	Rankin, 1937a
Desmognathus phoca (Matthes, 1855) (seal salamander)	6	North Carolina	Rankin, 1937a
Eurycea bislineata (Green, 1818) (northern two-lined salamander)	9	North Carolina	Rankin, 1937a
	6	New Hampshire	Muzzall et al., 1997
Eurycea cirrigera (Green, 1830) (southern two-lined salamander)	18	North Carolina	Mann, 1932
Eurycea guttolineata (three-lined salamander)‡	16	North Carolina	Mann, 1932
	0/21†	North Carolina	Rankin, 1937a
Hemidactylium scutatum (Temminck and Schlegel, 1838) (four-toed salamander)	70	Michigan	Woodhead, 1928
Plethodon albagula Grobman, 1944 (western slimy salamander)	5	Arkansas	McAllister et al., 1993
Plethodon cinereus (Green, 1818) (red-backed salamander)	1/NI*	Ohio	Hazard, 1937
	9	New Hampshire	Muzzall et al., 1997
	18	Michigan	Muzzall, 1990
Plethodon fourchensis Duncan and Highton, 1979 (Fourche Mountain salamander)	33	Arkansas	Winter et al., 1986
Plethodon glutinosus (Green, 1818) (northern slimy salamander)	5	North Carolina	Mann, 1932
	11/7†	North Carolina	Rankin, 1937a
	41–72§	Tennessee	Powders, 1970
	20	Arkansas	Winter et al., 1986
Plethodon jordani Blatchley, 1901 (Jordan's salamander)	14	Great Smoky Mts. of Tennessee–North Carolina	Powders, 1967
	0–49	Tennessee	Powders, 1970
Plethodon ouachitae Dunn and Heinze, 1933 (Rich Mountain salamander)	48	Arkansas	Winter et al., 1986
Plethodon punctatus Highton, 1972 (Cow Knob salamander)	60	West Virginia	Present study
Plethodon wehrlei Fowler and Dunn, 1917 (Wehrle's salamander)	23	West Virginia	Present study
Pseudotriton montanus Baird, 1849 (midland mud salamander)	33	North Carolina	Rankin, 1937a
Rana clamitans Latreille, 1801 (southern green frog)	—	Ohio	Odlaug, 1954
Rana sylvatica LeConte, 1825 (wood frog)	20	Ohio	Hazard, 1937

* Single individual infected/sample size not included.

† Prevalence in Durham, North Carolina, U.S.A. area of the central Piedmont/prevalence in the mountains.

‡ *Eurycea guttolineata*, the three-lined salamander, is considered a subspecies of the long-tailed salamander, *E. longicauda* (Green, 1818).

§ Prevalences inversely related to altitude and higher in fall months.

Lom, 1965, was found in 60% (15/25) and 23% (3/13) of *P. punctatus* and *P. wehrlei*, respectively (Table 1). This ciliate species was primarily aggregated in the duodenum of both host species, either free in the lumen or attached to the intestinal epithelium. *Cepedietta michiganensis* individuals were often so numerous that they appeared to occlude the duodenum; however, because there was no gross distention of this organ it was unlikely that any blockage of food materials actually occurred. No damage to cells of the host's intestinal epithelium was evident. There were a few instances where small numbers of ciliates were found in the middle and posterior small intestine, large intestine, or gall bladder of *P. punctatus*, as well.

The oxyurid nematode, *Batracholandros magnavulvaris* (Schad, 1960) Petter and Quentin, 1976, was found in the large intestine of 52% (13/25) *P. punctatus* and 31% (4/13) *P.*

Table 2. Published reports of hosts harboring *Batracholandros magnavulvaris*, with prevalences (P) and mean intensities ($\bar{x}$) by locality; host scientific names, taxonomic authorities, and dates after Frost (1985).

Host common name and species	P (%)	$\bar{x}$	Locality	Reference
Aneides flavipunctatus (Strauch, 1870) (black salamander)	50	—	California	Lehmann, 1954
Aneides aeneus Cope and Packard, 1881 (green salamander)	24	3	West Virginia	Schad, 1963
Desmognathus brimleyorum Stejneger, 1895 (Ouachita dusky salamander)	77	5	Arkansas	Winter et al., 1986
	30	3	Arkansas	McAllister et al., 1995
Desmognathus fuscus (Green, 1818) (northern dusky salamander)	48	<1*	North Carolina	Rankin, 1937a, b†
	—	—	Tennessee	Walton, 1940
	10	2	New York	Fischthal, 1955
	6	—	Tennessee	Dunbar and Moore, 1979
	27	—	Illinois	Dyer et al., 1980
	—	—	Illinois	Dyer, 1991
Desmognathus monticola Dunn, 1916 (seal salamander)	48	—	Tennessee	Dunbar and Moore, 1979
	60	1*	North Carolina	Goater et al., 1987
	50	2	West Virginia	Joy et al., 1993
Desmognathus ochrophaeus Cope, 1959 (Allegheny Mountain dusky salamander)	23	<1*	North Carolina	Rankin, 1937a, b†
	14	—	Tennessee	Dunbar and Moore, 1979
	40	<1*	North Carolina	Goater et al., 1987
	14	1	West Virginia	Joy et al., 1993
Desmognathus phoca (Matthes, 1855) (seal salamander)	25	<1*	North Carolina	Rankin, 1937a, b†
Desmognathus quadrimaculatus Holbrook, 1840 (black-bellied salamander)	15	<1*	North Carolina	Rankin, 1937a, b†
	7	—	Tennessee	Dunbar and Moore, 1979
	31	<1*	North Carolina	Goater et al., 1987
Eurycea bislineata (Green, 1818) (northern two-lined salamander)	27	<1*	North Carolina	Rankin, 1937a, b†
	11	—	Tennessee	Dunbar and Moore, 1979
	2	1	New Hampshire	Muzzall et al., 1997
Eurycea guttolineata (three-lined salamander)	0/64‡	3*	North Carolina	Rankin, 1937a, b†
Eurycea lucifuga Rafinesque, 1822 (cave salamander)	100	1	Tennessee	Dyer and Peck, 1975
Leurognathus marmoratus Moore, 1899 (shovel-nosed salamander)	6	<1*	North Carolina	Goater et al., 1987
Notophthalmus viridescens (Rafinesque, 1820) (red-spotted newt, red eft)	100	3*	North Carolina	Rankin, 1937a, b†
Notophthalmus viridescens (red-spotted newt, adult)	8	<1*	North Carolina	Rankin, 1937a, b†
Plethodon caddoensis Pope and Pope, 1951 (Caddo Mountain salamander)	12	1	Arkansas	Winter et al., 1986
Plethodon cinereus (Green, 1818) (red-backed salamander)	0/2‡	<1*	North Carolina	Rankin, 1937a, b†
	50	2	Virginia	Ernst, 1974
	28	2	Michigan	Muzzall, 1990
	—	—	Illinois	Dyer, 1991
	9	2	Pennsylvania	Bursey and Schibli, 1995
Plethodon fourchensis Duncan and Highton, 1979 (Fourche Mountain salamander)	33	1	Arkansas	Winter et al., 1986
Plethodon glutinosus (Green, 1818) (northern slimy salamander)	0/3‡	<1*	North Carolina	Rankin, 1937a, b†
Plethodon ouachitae Dunn and Heinze, 1933 (Rich Mountain salamander)	14	1	Arkansas	Winter et al., 1986
Plethodon punctatus Highton, 1972 (Cow Knob salamander)	52	3	West Virginia	Present study
Plethodon serratus Grobman, 1944 (southern red-backed salamander)	22	1	Arkansas	Winter et al., 1986
Plethodon wehrlei Fowler and Dunn, 1917 (Wehrle's salamander)	31	4	West Virginia	Present study
Plethodon yonahlossee Dunn, 1917 (Yonahlossee salamander)	33	<1*	North Carolina	Rankin, 1937a, b†

* These values appear to be mean abundance rather than mean intensity.

† Same host species listed in both references, but prevalence and mean abundance (rather than mean intensity) given only in Rankin (1937a). Original species description for *Oxyuris magnavulvaris* provided in Rankin (1937b).

‡ Prevalence in Durham, North Carolina, U.S.A. area of the central Piedmont/prevalence in mountains.

wehrlei individuals (Table 2). Mean intensities (±1 SD) were 3.4 (±1.9) and 4.0 (±4.0) for *P. punctatus* and *P. wehrlei,* respectively. Of the 61 *B. magnavulvaris* individuals collected, only 2 (both in *P. wehrlei*) were males.

Discussion

Prevalences of 60% and 23% for *C. michiganensis* in *P. punctatus* and *P. wehrlei* from West Virginia fall within ranges cited by previous investigators for this species (Table 1). Finding *C. michiganensis* species throughout the intestinal tract, with heavy aggregations in the duodenum, is consistent with the observations of Winter et al. (1986), who noted that *C. michiganensis* could be found throughout the intestine of *Plethodon ouachitae* Dunn and Heinze, 1933, but was concentrated in the anterior third of this organ. There was a single case of *C. michiganensis* infection in the gallbladder of *P. punctatus,* but ciliates were not attached to the epithelium of the gall bladder. Winter et al. (1986) also reported *C. michiganensis* from the gall bladder of *P. ouachitae,* adding that the ciliates were not attached to the epithelium. Three other reports mentioned occurrence of this ciliate in the host's gall bladder: Muzzall (1990) for *Plethodon cinereus* (Green, 1818); McAllister et al. (1993) for *Plethodon albagula* Grobman, 1944; and Muzzall et al. (1997) for *Eurycea bislineata* (Green, 1818) and *P. cinereus.* Previous reports of amphibian hosts harboring species of *C. michiganensis* are summarized in Table 1.

Prevalences of 52% and 31% recorded in the present study for *B. magnavulvaris* in *P. punctatus* and *P. wehrlei,* respectively, are not unusual. This nematode species exhibits little host specificity and is found in widely varying prevalences (McAllister et al., 1995), an observation supported by the findings of other investigators summarized in Table 2. McAllister et al. (1995) also reported that prevalence for *B. magnavulvaris* in *Desmognathus brimleyorum* Stejneger, 1895 varies seasonally, being highest (34%) in mid-March versus only 17% for late May. While we had relatively few salamanders in our sample, all *B. magnavulvaris* individuals were collected from 13 of 21 *P. punctatus* in June and July. Similarly, 4 of the 9 *P. wehrlei* examined in May through July were infected. None of the 8 plethodontids (4 *P. punctatus* and 4 *P. wehrlei*) sampled in August were infected, suggesting that the variations in prevalence by season noted by McAllister et al. (1995) may be a normal pattern. Mean intensities of infection at 3.4 and 4.0 for *P. punctatus* and *P. wehrlei,* respectively, were relatively high compared with previous reports (Table 2). Only 2 *B. magnavulvaris* individuals collected in the present study were males. This heavily female-biased sex ratio for *B. magnavulvaris* is similar to the observations of previous investigators (Dyer et al., 1980; Muzzall, 1990; Joy et al., 1993; McAllister et al., 1995).

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Some Adult Endohelminths Parasitizing Freshwater Fishes from the Atlantic Drainages of Nicaragua

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ABSTRACT: Adults of 12 endoparasitic helminths were recorded from 8 freshwater fish species from the South Atlantic Autonomous Region, Nicaragua: 8 digeneans *Crassicutis cichlasomae, Magnivitellinum simplex, Oligogonotylus manteri, Prosthenhystera obesa, Saccocoelioides sogandaresi, Saccocoelioides* sp. 1, *Saccocoelioides* sp. 2, and Allocreadiidae gen. sp. ("*Crepidostomum*" sp.); 3 nematodes *Procamallanus* (*Spirocamallanus*) *rebecae, Procamallanus* (*Spirocamallanus*) *neocaballeroi,* and *Rhabdochona kidderi kidderi*; and 1 acanthocephalan *Neoechinorhynchus golvani.* Comparative measurements among *S. sogandaresi* from *Poecilia velifera, Saccocoelioides* sp. 1 from *Cichlasoma maculicauda,* and *Saccocoelioides* sp. 2 from *Astyanax fasciatus,* as well as drawings of the 2 latter species are given for future reference. All but *C. cichlasomae* and *O. manteri* are reported from Nicaragua for the first time, and most taxa also represent new geographical records for Central America. The majority of species have previously been found in freshwater fishes from southeastern Mexico, which indicates a close similarity of the helminth faunas of both regions, in accordance with previous data on the larval stages of endohelminths and gill monogeneans.

KEY WORDS: Helminths, parasites, Digenea, Nematoda, Acanthocephala, freshwater fishes, Nicaragua.

During a short visit by 4 of the authors (M.L.A.M., T.S., V.M.V.M., and G.A.T.) to Nicaragua in March 1999, brackish and freshwater fishes from the Autonomous Region of the South Atlantic were examined for helminth parasites. Because information on helminths parasitizing freshwater fishes in Nicaragua is limited to the report by Watson (1976), in which several species of trematodes from Lake Nicaragua were reported, a list of adult endohelminths is provided, with morphological data on some taxa. The results of a survey of larval stages of endohelminths found in the same fish hosts and ancyrocephaline monogeneans from the gills of cichlids, have already been published (Aguirre-Macedo et al., 2001; Vidal-Martínez, Scholz, and Aguirre-Macedo, 2001).

Materials and Methods

A total of 56 fish of the following 8 species was examined: tetra *Astyanax fasciatus* (Cuvier, 1819) (8 specimens examined) (family Characidae); pastel cichlid *Amphilophus alfari* (Meek, 1907) (3); convict cichlid *Archocentrus nigrofasciatus* (Günther, 1869) (3); blackbelt cichlid *Cichlasoma maculicauda* (Regan, 1805) (12); jaguar cichlid *Cichlasoma managuense* (Günther, 1867) (13); butterfly cichlid *Herotilapia multispinosa* (Günther, 1867) (8) (Cichlidae); molly *Poecilia velifera* (Regan, 1814) (5) (Poeciliidae); and long-whiskered catfish *Rhamdia nicaraguensis* (Günther, 1864) (4) (Pimelodidae). Fish were collected by hook and line and throw nets from 7 localities of the Atlantic drainages of Nicaragua in the Autonomous Region of the South Atlantic (Región Autónoma del Atlántico Sur—RAAS): Torsuani River (11°47′06″N; 83°52′38″W); Mahogany River (12°03′22″N; 83°59′07″W); Caño Negro Stream (12°00′55″N; 84°01′10″W), in Bluefields City; Walpatara Bridge (12°00′14″N; 83°45′58″W); Loonku Creek (11°59′05″N; 83°46′48″W); Caño Marañón Stream (12°00′10″N; 83°46′39″W); and Puente Chino (12°00′30″N; 83°46′13″W). The number of fish sampled in individual localities and map locations are given in Aguirre-Macedo et al. (2001).

Fish were transported alive to the laboratory of the Bluefields Indian and Caribbean University (BICU), where they were examined by routine helminthological procedures outlined by Vidal-Martínez, Aguirre-Macedo et al. (2001). All helminths were studied in fresh preparations and counted in situ. Adult helminths were considered those with fully developed reproductive organs regardless of the presence of eggs. Eventually, some digeneans were fixed with a glycerin-ammonium picrate (GAP) mixture following the methodology out-

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Table 1. Some endoparasitic helminths collected from Nicaraguan freshwater fishes.

Helminth species	Hosts (no. infected/examined)	Intensity range	Locality	Deposit accession nos. CNHE/CHCM
Digenea				
Crassicutis cichlasomae Manter, 1936	*Cichlasoma maculicauda* (5/7)	1–8	Caño Marañón	4193/
Magnivitellinum simplex Kloss, 1966	*Astyanax fasciatus* (1/4)	1	Torsuani River	4195/
	(1/2)	6	Loonku Creek	
Oligogonotylus manteri Watson, 1976	*C. maculicauda* (2/7)	3–6	Torsuani River	4196/
	Cichlasoma managuense (1/2)	1	Puente Chino	
?*Prosthenhystera obesa* (Diesing, 1850)	*A. fasciatus* (1/2)	1	Loonku Creek	4194/
Saccocoelioides sogandaresi Lumsden, 1964	*Poecilia velifera* (1/1)	1	Caño Marañón	/383
Saccocoelioides sp. 1	*C. maculicauda* (1/3)	17	Torsuani River	/384
	(6/7)	4–42	Caño Marañón	
Saccocoelioides sp. 2	*A. fasciatus* (2–4)	1–3	Torsuani River	/380
Allocreadiidae gen. sp.	*A. fasciatus* (1/4)	1	Torsuani River	
	(1/2)	4	Loonku Creek	
Nematoda				
Procamallanus (*Spirocamallanus*) *rebecae* Andrade-Salas, Pineda-López, and Osorio-Sarabia, 1994	*Amphilophus alfari* (1/3)	29	Torsuani River	4142/
	C. maculicauda (2/11)	1–2	Loonku Creek	/393, 393-1
	Herotilapia multispinosa (3/8)	1–3		
Procamallanus (*Spirocamallanus*) *neocaballeroi* (Caballero-Deloya, 1977)	*A. fasciatus* (1/9)	3	Mahogany River	4143/
	C. maculicauda (1/11)	1	Torsuani River	/396
Rhabdochona kidderi kidderi Pearse, 1936	*C. maculicauda* (1/11)	1	Torsuani River	
Acanthocephala				
Neoechinorhynchus golvani Salgado-Maldonado, 1978	*A. alfari* (1/1)	1	Loonku Creek	4197/
	C. managuense (1/2)	1	Puente Chino	
	C. managuense (2/4)	1–8	Caño Negro	
	H. multispinosa (1/4)	3	Loonku Creek	
	H. multispinosa (1/3)	2	Puente Chino	

lined by Ergens (1969). Measurements are given in micrometers. Drawings were made with the aid of a drawing tube. Reference specimens were deposited in the Colección Nacional de Helmintos (CNHE), Mexico City, Mexico, and the Laboratory of Parasitology, CINVESTAV-IPN (CHCM), Mérida, Mexico.

Results

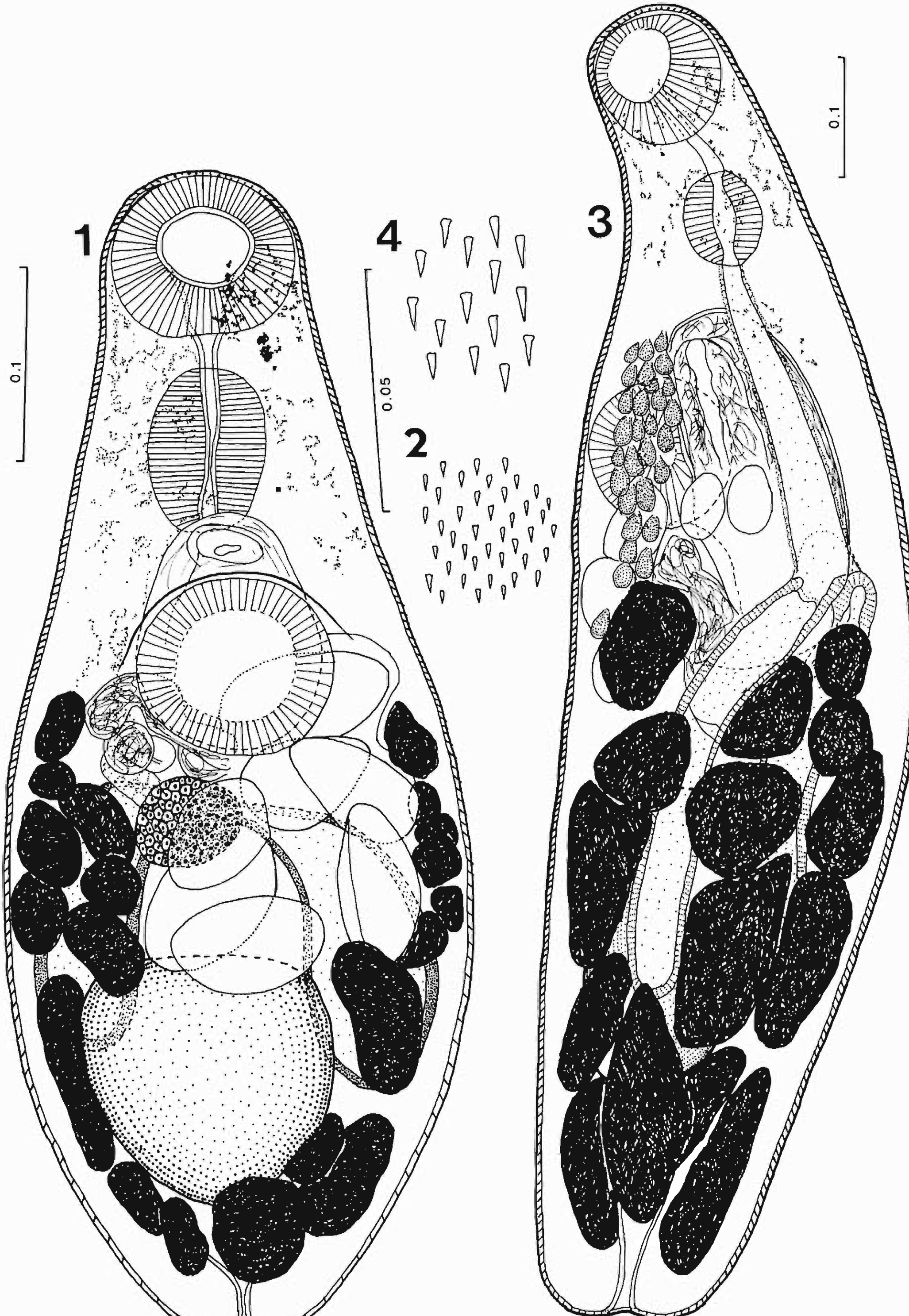
A total of 12 helminth species was found. Data on the hosts, localities, and infection range are provided in Table 1. All but 1 species (?*Prosthenhystera obesa*) were located in the intestine; ?*P. obesa* inhabited the gall blader.

Among the species found, 3 trematodes were not identified to species level: 2 species of *Saccocoelioides* and a trematode of the subfamily Allocreadiinae (Allocreadiidae). Measurements of the first 2 species (Figs. 1–4), together with those of a congeneric species (*Saccocoelioides sogandaresi*) from *Poecilia velifera* are presented in Table 2.

Discussion

The number of species of adult endohelminths recorded was lower than that of larval stages, in particular metacercariae of digeneans, found in the same fish hosts (Aguirre-Macedo et al., 2001). Adult trematodes represented the dominant group (8 species) in this study, whereas nematodes were fewer (only 3 species). Only 1 acanthocephalan occurred in fishes examined.

No adult cestodes were found, even in the pimelodid catfish *Rhamdia nicaraguensis,* but only 4 fish of this species were examined. Thus, it is probable that more fish and localities need to be sampled to find adult cestodes, especially considering the low prevalence (<10% in 229 fish examined from several localities) of species such as *Bothriocephalus* sp. (=*Bothriocephalus pearsei* Scholz, Várgas-Vázquez, and Moravec, 1996) and *Nomimoscolex* sp. (=*Proteocephalus brooksi* García-Prieto, Rodríguez, and Pérez-



1
0.1
4
0.05
2
3
0.1

Table 2. Measurements of species of *Saccocoelioides* from Nicaraguan freshwater fishes (*n* = number of specimens measured).

Host	*Saccocoelioides sogandaresi* (*n* = 1) *Poecilia velifera**	*Saccocoelioides* sp. 1 (*n* = 3) *Astyanax fasciatus*	*Saccocoelioides* sp. 2 (*n* = 15) *Cichlasoma maculicauda*
Body shape	Widely oval	Elongate, with tapering ends	Oval to elongate
Body length	995	1,070–1,210	470–680
Maximum width	310	290–320	150–335
Oral sucker	100 × 122	90–112 × 100–120	67–105 × 75–125
Ventral sucker	123 × 130	105–115 × 113–125	56–120 × 50–125
Sucker ratio	1.14:1	0.83–1.01:1	0.63–1.30:1
Position of acetabulum	48% of body length	33–35%	31–39%
Prepharynx	38	45–50	36–72
Pharynx	90 × 98	70–78 × 63–69	50–82 × 47–75
Oral sucker/pharynx ratio	1.18:1	1.35–1.61:1	1.01–1.46:1
Extent of ceca	Anterior line of testis	About midline of testis	About midline to ⅔ of testis
Testis	102 × 82	274–290 × 140–173	72–175 × 58–145
Hermaphroditic sac	150 × 76	245–280 × 143–160	77–162 × 62–135
Ovary	—	98–105 × 80–104	37–87 × 35–87
Extent of vitellarium	—	Far postacetabular	About midline of acetabulum
Eggs	—	73–75 × 46–50	67–81 × 36–47

* Specimens fixed with GAP under pressure.

Ponce de León, 1996) from fish of the genus *Rhamdia* in the Yucatán Peninsula (see Scholz et al., 1996).

In South America, cestodes appear to be the dominant component of the fauna of endohelminths in freshwater fishes, in terms of the number of species and genera (Thatcher, 1991; Rego et al., 1999). These cestodes belong almost exclusively to the order Proteocephalidea, and they occur most frequently in siluriform fishes, including pimelodids (de Chambrier and Vaucher, 1999; Rego, 2000).

The endohelminth fauna of fishes from the Atlantic coastal drainages of Nicaragua closely resembles that of southeastern Mexico. Similar to the larval stages of endohelminths (Aguirre-Macedo et al., 2001), a majority of species found occur in congeneric fish hosts from the Yucatán Peninsula (Moravec et al., 1995; Scholz et al., 1995, 1996; Salgado-Maldonado et al., 1997; Scholz and Vargas-Vázquez, 1998; Vidal-Martínez, Aguirre-Macedo et al., 2001). This similarity indicates close relationships between the helminth faunas of freshwater fishes in Central America and southeastern Mexico, in accordance with the analysis of Vidal-Martínez and Kennedy (2000) and the general biogeography of the neotropics (Briggs, 1984). Vidal-Martínez, Scholz and Aguirre-Macedo (2001) also found a marked resemblance between gill monogeneans of cichlids from Nicaragua and those from Yucatán.

Three species of trematodes, *Magnivitellinum simplex*, *?P. obesa*, and *S. sogandaresi*, all nematodes, and the acanthocephalan *Neoechinorhynchus golvani*, previously found in North and South America (Travassos et al., 1969; Thatcher, 1991; Pérez-Ponce de León et al., 1996; Salgado-Maldonado et al., 1997; Moravec, 1998), are reported from Central America for the first time. With the exception of the trematodes *Oligogonotylus manteri* and *Crassicutis cichlasomae* reported from Lake Nicaragua by Watson (1976), all species also represent new geographical records from Nicaragua. This reflects the shortage

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Figures 1–4. 1, 2. *Saccocoelioides* sp. 1 from *Cichlasoma maculicauda*. 1. Total view from the ventral aspect. 2. Tegumental spines at pharyngeal level. 3, 4. *Saccocoelioides* sp. 2 from *Astyanax fasciatus*. 3. Total view ventral from the ventral aspect. 4. Tegumental spines at pharyngeal level. Scale bars in millimeters.

of data on helminths of freshwater fishes in this country and in Central America in general.

Three species of the genus *Saccocoelioides* Szidat, 1954, were found in this study, differing from each other in the size and shape of the body, their spination, relative size of the pharynx and hermaphroditic sac, extent of the vitellaria, egg size, etc. However, 2 of them (Figs. 1–4) remain unidentified to species level because they differ from all hitherto described taxa (see Szidat, 1954; Lunaschi, 1984). They are not described as new species because of the unsatisfactorily resolved taxonomy of the genus, with numerous taxa having been inadequately described. To provide data for subsequent species identification, measurements of all 3 species are provided in Table 2.

The allocreadiid trematode found in *Astyanax fasciatus* most resembles in its morphology the species *Crepidostomum platense* Szidat, 1954, and *Crepidostomum stenopteri* Mañé-Garzón and Gascón, 1973, described from the intestine of several pimelodid catfishes from Argentina and from the characid fish "dentudo transparente" *Charax* (=*Asiphonichthys*) *stenopterus* (Cope, 1894) from Uruguay, respectively (see Szidat, 1954; Mañé-Garzón and Gascón, 1973). However, there are marked differences among the present specimens and both species of *Crepidostomum* in the extent of the vitelline follicles, position of the ventral sucker, and in the shape and relative positions of the testes. It is, therefore, probable that the trematode from Nicaragua represents at least a new species. Nevertheless, it is not described formally in this paper because it differs, as do both species from South America, in its morphology from members of Holarctic species of the genus *Crepidostomum* Braun, 1900, and may even represent a different genus.

The present report is the second on adults of helminth parasites from Nicaragua, following that of Watson (1976). It is evident that much more data on the fish parasites from larger samples of fish hosts must be obtained for better understanding of their species composition and relationships to those from other areas of the Neotropical region.

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Relocation of the Onderstepoort Helminthological Collection

We have been notified that the Onderstepoort Helminthological Collection has moved and been renamed, and is now under the curatorship of Professor J. Boomker, Department of Veterinary Tropical Diseases, and Dr. E. van den Berg, Plant Protection Unit, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. The collection is now known as the National Collection of Animal Helminths and is fully accessible. Prospective lenders, or those seeking further information, can notify Professor Boomker at the above address, phone: +27-12-529-8166, fax: +27-12-529-8312, or e-mail: jboomker@op.up.ac.za.

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Helminth Parasites of Freshwater Fishes of the Balsas River Drainage Basin of Southwestern Mexico

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ABSTRACT: This study presents the results of the first survey of the helminth parasites in fishes in the Balsas River drainage, southwestern Mexico. A total of 25 species of helminth parasites in 13 freshwater fish species (n = 1,045) was collected between December 1995 and September 1998. The most prevalent and widespread helminth species was the Asian tapeworm *Bothriocephalus acheilognathi.* Two features characterize the helminth fauna of the Balsas River basin fishes: (1) a predominance of nematode and trematode species coupled with a scarcity of monogeneans and acanthocephalans; and (2) all helminths found had previously been reported from other regions of Mexico; therefore the composition of the helminth fauna of the fishes of the Balsas River drainage is not very distinct from that of fishes from other previously studied freshwater basins in Mexico.

KEY WORDS: Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala, freshwater fish, Balsas River, southwestern Mexico, survey.

The Balsas River basin is the largest river drainage in southwestern Mexico. The Balsas River has its source at about 3,660 m altitude in the Sierra Madre del Sur. It flows generally east–west through the states of Tlaxcala, Puebla, Guerrero, and Michoacán and receives several major inflows from the states of Oaxaca, México, Morelos, and Jalisco before emptying into the Pacific Ocean. This river has a fish fauna composed of 37 species of 26 genera in 10 families. In addition to native fishes, exotic species such as Asian cyprinids (carps) and African cichlids (tilapias) have been introduced into many areas.

Little information exists about the occurrence of helminth parasites of fishes from the Balsas River (Osorio-Sarabia, 1982, 1984; Salgado-Maldonado et al., 1998; Caspeta-Mandujano and Moravec, 2000; Caspeta-Mandujano et al., 2000; Moravec, 2000; Moravec et al., 2000), and the present report is the first survey of the helminth parasites of fishes of this drainage system. The aim of this work is to report the survey results, and the distribution and intensity data for these helminth parasites.

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Materials and Methods

A total of 1,045 fish was collected from 28 sites, mostly rivers, in the Balsas River basin between December 1995 and September 1998 (Table 1, Fig. 1).

Fish at each site were captured by electrofishing or by gill nets. Live fish were brought to the laboratory and examined within 48 hr after capture using standard procedures. The following fishes were examined (* indicates species endemic to the Balsas River basin): Cyprinidae—**Hybopis boucardi* (Günther, 1868) (Balsas shiner, n = 111); Characidae—*Astyanax fasciatus* (Cuvier, 1819) (Mexican tetra, n = 166); Ictaluridae—**Ictalurus balsanus* (Jordan and Snyder, 1899) (Balsas catfish, n = 1); *Ictalurus punctatus* (Rafinesque, 1818) (channel catfish, n = 2); Goodeidae—*Goodea atripinnis* Jordan, 1880 (blackfin goodea, n = 6); **Ilyodon whitei* (Meek, 1904) (Balsas splitfin, n = 59); Poeciliidae—*Heterandria bimaculatus* (Heckel, 1848) (spottail killifish, n = 88), *Poecilia reticulata* Peters, 1860 (guppy, n = 20), *Poecilia sphenops* Valenciennes *in* Cuvier and Valenciennes, 1846 (Mexican molly, n = 261), *Poeciliopsis gracilis* (Heckel, 1848) (porthole livebearer, n = 156), *Poeciliopsis infans* (Woolman, 1894) (Lerma livebearer, n = 20); Cichlidae—**Cichlasoma istlanum* (Jordan and Snyder, 1899) (redside cichlid, n = 32), *Cichlasoma nigrofasciatum* (Günther, 1867) (convict cichlid, n = 123). Fish sample sizes per site are given in Table 1.

All helminths recovered from each fish were counted. Digeneans (adults and metacercariae), cestodes, and nematodes were fixed in hot 10% neutral formalin. Acanthocephalans were placed in distilled water and refrigerated overnight (6–12 hr) to evert the proboscis,

Table 1. Helminths found in freshwater fishes from the Balsas River basin, Mexico.

Species	Site	Hosts	*Locality (no. of hosts infected/no. of hosts examined; total no. of helminths; abundance ± SD [range of intensity])
Monogenea			
Gyrodactylus sp.	Gills	*P. gracilis*	PL (2/18; 7; 0.4 ± 1.1 [3–4])
		P. infans	VJ (1/20; 1)
Urocleidoides cf. *costaricensis* (Price and Bussing, 1967) Kritsky and Leiby, 1972	Gills	*A. fasciatus*	PL (3/13; 12; 0.9 ± 1.8 [3–5]), AM (5/26; 21; 0.8 ± 2.9 [1–15]), CU (2/11; 3; 0.3 ± 0.6 [1–2]), PT (5/10; 30; 3.0 ± 3.9 [1–11])
Trematoda			
Saccocoelioides sogandaresi Lumsden, 1961	Intestine	*I. whitei*	CH (1/22; 3)
		P. sphenops	AR (1/7; 1), CH (2/43; 2; 0.05 ± 0.2 [1–1])
		P. gracilis	AR (9/11; 17; 1.5 ± 1.1 [1–4])
Magnivitellinum simplex Kloss, 1966	Intestine	*A. fasciatus*	CU (1/11; 1)
Diplostomum cf. *compactum* (Lutz, 1928)	Eyes, brain, body cavity	*P. reticulata*	AC (3/20; 3; 0.2 ± 0.4 [1–1])
		P. sphenops	HA (2/15; 16; 1.1 ± 3.9 [1–15])
Posthodiplostomum minimum (MacCallum, 1921)	Muscle, liver, eyes, mesentery, body cavity	*H. boucardi*	JU (1/8; 1), IX (5/10; 18; 1.8 ± 3.2 [1–10]), PL (4/15; 2.5 ± 6.5 [3–25]); AM (1/2; 1)
		G. atripinnis	JU (6/6; 801; 134.0 ± 37.5 [83–182])
		H. bimaculata	HJ (1/25; 1)
		P. sphenops	HA (5/15; 39; 2.6 ± 6.0 [1–22]), AC (1/22; 1), XO (2/16; 10; 0.6 ± 2.0 [2–8]), HJ (1/40; 1), AM (1/16; 1); OT (1/2; 1)
		P. infans	VJ (19/20; 307; 15.4 ± 14.3 [3–53])
		C. istlanum	AM (1/4; 2)
		C. nigrofasciatum	CO (5/44; 5; 0.1 ± 0.3 [1–1]), HJ (14/21; 53; 2.5 ± 2.6 [1–9]) AM (8/20; 25; 1.2 ± 2.3 [1–9])
Uvulifer sp.	Skin, fins	*H. boucardi*	CU (5/14; 10; 0.7 ± 1.3 [1–4]), IX (2/10; 4; 0.4 ± 1.0 [1–3])
		A. fasciatus	PL (1/13; 1), HJ (1/5; 1)
		P. sphenops	CO (1/10; 1), HJ (10/40; 14; 0.3 ± 0.7 [1–2])
		P. gracilis	PL (1/18; 2), HJ (2/3; 3; 1.0 ± 1.0 [1–2])
		C. istlanum	AM (3/4; 157; 39.2 ± 53.3 [16–118]), TE (1/11, 1)
		C. nigrofasciatum	CH (17/22; 125; 5.7 ± 5.7 [1–22]), CO (42/44; 342; 7.7 ± 7.7 [1–37]), HJ (21/21; 348; 16.6 ± 13.1 [1–55]), AM (18/20; 207; 10.3 ± 14.1 [1–58])
Clinostomum complanatum (Rudolphi, 1814)	Fins, opercula, body cavity	*A. fasciatus*	AM (4/26; 5; 0.2 ± 0.5 [1–2])
Centrocestus formosanus (Nishigori, 1924)	Gills	*A. fasciatus*	AM (2/26; 5; 0.2 ± 0.7 [2–3])
		I. whitei	CH (18/22; 1926; 87.5 ± 97.4 [1–426]), AM (2/4; 224; 56 ± 111.3 [1–223])
		P. sphenops	CH (6/43; 28; 0.6 ± 2.3 [1–13]), PL (1/13; 1)
		P. gracilis	CH (3/15; 4; 0.3 ± 0.6 [1–2]), PL (1/18; 8), AM (1/18; 11)
		C. nigrofasciatum	CH (2/22; 2; 0.1 ± 0.3 [1–1]), HJ (1/21; 1), AM (2/20; 13; 0.6 ± 2.0 [6–7])

Table 1. Continued.

Species	Site	Hosts	*Locality (no. of hosts infected/no. of hosts examined; total no. of helminths; abundance ± SD [range of intensity])
Cestoda			
Bothriocephalus archeilognathi Yamaguti, 1934	Intestine	*H. boucardi*	CU (15/19; 103; 5.4 ± 10.8 [1–46]), HA (3/4; 7; 1.7 ± 1.5 [1–3]), RP (6/7; 20; 2.9 ± 1.9 [1–6]), IX (1/10; 1), PT (7/7; 53; 7.6 ± 6.4 [1–20]), MI (6/10; 15; 1.5 ± 1.6 [1–4]), PL (5/15; 8; 0.5 ± 1.0 [1–4]), AM (1/2; 1)
		A. fasciatus	CU (1/11; 1)
		H. bimaculata	CO ((1/13; 1), HJ (5/25; 7; 0.3 ± 0.7 [1–3])
		P. reticulata	AC (1/20; 1)
		P. sphenops	PT (1/5; 1), MI (1/15; 2), XO (3/16; 16; 1.0 ± 3.5 [1–14]), CH (1/43; 1), CO (2/10; 4; 0.4 ± 0.9 [1–3]), PL (2/13; 2; 0.1 ± 0.4 [1–1]), HJ (4/40; 10; 0.2 ± 1.1 [1–7]), PT (1/5; 1)
		P. gracilis	CO (2/19; 2; 0.1 ± 0.3 [1–1]), TE (10/16; 26; 1.6 ± 2.6 [1–10])
		C. istlanum	TE (1/11; 2)
		C. nigrofasciatum	HJ (3/21; 5; 0.2 ± 0.7 [1–3])
Glossocercus auritus (Rudolphi, 1819) Bona, 1994	Body cavity, mesentery, liver	*P. sphenops*	HA ((2/15; 2; 0.1 ± 0.4 [1–1]), TE (1/6; 2) XO (3/16; 6; 0.4 ± 0.8 [2–2])
		P. gracilis	TE (9/16; 15; 0.9 ± 1.1 [1–3])
		A. fasciatus	CU (1/11; 1)
Parvitaenia cochlearii Coil, 1955	Liver	*P. gracilis*	TE (1/16; 1)
Parvitaenia macropeos (Wedl, 1855) Baer and Bona, 1960	Liver	*C. istlanum*	TE (3/11; 11; 1.0 ± 2.6 [1–9])
Valipora minuta (Coil, 1950) Baer and Bona, 1960	Liver, gall bladder	*P. sphenops*	TE (2/6; 18; 3.0 ± 6.0 [3–15])
		P. gracilis	TE (4/16; 19; 1.2 ± 2.4 [2–8])
Nematoda			
Capillaria cyprinodonticola Huffman and Bullock, 1973	Intestine, liver	*A. fasciatus*	PL (1/13; 5)
		P. sphenops	CH (7/43; 42; 1.0 ± 2.7 [1–10]), CO (2/10, not counted), AM (1/16; 1), PL (7/13; 43; 3.3 ± 4.5 [1–13]), HJ (5/40; 116; 2.9 ± 11.1 [5–65]), YE (4/20, not counted).
		C. nigrofasciatum	CH (1/22; 1)
Rhabdochona canadensis Moravec and Arai, 1971	Intestine	*H. boucardi*	JU (5/8; 15; 1.9 ± 2.1 ([1–6]), CU (12/14; 205; 14.6 ± 12.3 [1–45]). HA (3/4; 9; 2.2 ± 1.7 [2–4]), RP (6/7; 80; 11.4 ± 9.2 [3–25]), IX (3/10; 5; 0.5 ± 0.9 [1–3]), PT (2/7; 2; 0.3 ± 0.5 [1–1]), MI (7/10; 34; 3.4 ± 4.4 [1–14]), PL (4/15; 5; 0.3 ± 0.6 [1–2])
Rhabdochona kidderi Pearse, 1936	Intestine	*C. istlanum*	CO (1/1; 4), AM (4/4; 143; 35.8 ± 16.8 [1–56])
		C. nigrofasciatum	AR (8/16; 35; 2.2 ± 3.6 [1–14]), CH (11/22; 21; 0.9 ± 1.2 [1–4]), CO (14/44; 25; 0.6 ± 1.0 [1–6]), HJ (17/21; 99; 4.7 ± 5.6 [1–22]), AM (14/20; 56; 2.8 ± 3.0 [1–10])
Rhabdochona lichtenfelsi Sánchez-Álvarez et al., 1998	Intestine	*G. atripinnis*	JU (5/6; 33; 5.5 ± 4.9 [1–13])
Rhabdochona mexicana Caspeta-Mandujano et al., 2000	Intestine	*A. fasciatus*	CO (2/13; 2; 0.1 ± 0.4 [0–1]), PL (2/13; 2; 0.1 ± 0.4 [1–1]), AM (1/26; 1), CU (2/11; 2; 0.2 ± 0.4 [1–1]), PT (1/10; 1), AT (8/15; 12; 0.8 ± 0.9 [1–3]), RP (1/2; 1)

Table 1. Continued.

Species	Site	Hosts	*Locality (no. of hosts infected/no. of hosts examined; total no. of helminths; abundance ± SD [range of intensity])
Eustrongylides sp.	Muscle	*P. sphenops*	YE (2/20; 2; 0.1 ± 0.3 [1–1]), HA (5/15; 5; 0.3 ± 0.5 [1–1]), XO (8/16; 11; 0.7 ± 0.8 [1–2]), CH (2/43; 2; 0.05 ± 0.2 [1–1])
		P. gracilis	HA (1/10; 1)
		C. nigrofasciatum	CH (1/22; 1)
Contracaecum sp.	Mesentery, liver, muscle	*A. fasciatus*	OT (3/30; 3; 0.1 ± 0.3 [1–1])
		P. sphenops	XO (2/16; 2; 0.1 ± 0.3 [1–1])
		C. nigrofasciatum	HJ (1/21; 1)
Spiroxys sp.	Intestine	*A. fasciatus*	YE (1/2; 1), OT (1/30; 4)
Hysterothylacium sp.	Intestine	*C. nigrofasciatum*	AM (1/20; 1)
Acuariidae gen. sp.	Intestine	*C. nigrofasciatum*	HJ (1/21; 1)
Acanthocephala			
Neoechinorhynchus golvani Salgado-Maldonado, 1978	Intestine	*C. istlanum*	TE (1/11; 6)

* State of Puebla: La Huerta (HU) (18°15′11″N; 98°00′53″W) (date of collection, mo/yr; 2/98); Oaxaca: San Pedro Alpoyeca (PE) (18°04′01″N; 97°41′45″W) (9/98), Cuyotepeji (CU) (17°57′35″N; 97°41′06″W) (2/98, 9/98), Petlalcingo (PT) (18°04′35″N; 97°55′29″W) (2/98, 9/98), Ahuehuetitlán (AH) (17°54′00″N; 97°47′25″W) (6/98), Santa María Chilapa (CI) (17°50′03″N; 97°43′19″W) (9/98), Huajuapan de León (HA) (17°45′25″N; 97°48′03″W) (2/98), San Agustín Atenango (AT) (17°39′03″N; 97°57′00″W) (2/98), Michapa (MI) (17°30′11″N; 98°04′35″W) (9/98), San Francisco Paxtlahuaca (PA) (17°41′16″N; 97°57′00″W) (9/98); Morelos: Las Planchas (PL) (18°49′03″N; 99°30′14″W) (3/96), Huajintlán (HJ) (18°38′47″N; 99°27′02″W) (5/96), Amacuzac (AM) (18°38′47″N; 99°27′02″W) (5/96), Contlalco (CO) (18°38′58″N; 99°27′38″W) (4/96), El Chisco (CH) (18°33′00″N; 99°13′00″W) (12/95); Guerrero: Río Petatlán (RP) (17°35′31″N; 99°00′27″W) (2/98), Xalitla (XA) (17°59′55″N; 99°32′30″W) (6/98), Acatlán (AC) (17°39′17″N; 99°09′09″W) (6/98), Tlacuitlapa (TL) (18°39′41″N; 99°41′20″W) (6/98), Atenango del Río (AR) (18°06′02″N; 99°06′28″W) (6/98), Presa Tepecoacuilco dam (TE) (18°18′01″N; 99°28′16″W) (6/98), Xochihuehuetlán (XO) (17°54′28″N; 98°29′31″W) (9/98); Jalisco: Presa Valle de Juárez dam (VJ) (19°56′05″N; 102°57′31″W) (12/97); Michoacán: Presa San Juanico dam (JU) (19°50′36″N; 102°40′41″W) (12/97), Puente Corondiro (CR) (18°59′28″N; 102°07′08″W) (12/97), Puente Las Yeguas (YE) (19°00′46″N; 102°16′21″W) (12/97), Río Los Otates (OT) (19°07′45″N; 102°50′50″W) (12/97); Estado de México: Río San Jerónimo, Ixtapan de la Sal (IX) (18°51′40″N; 99°15′00″W) (6/98).

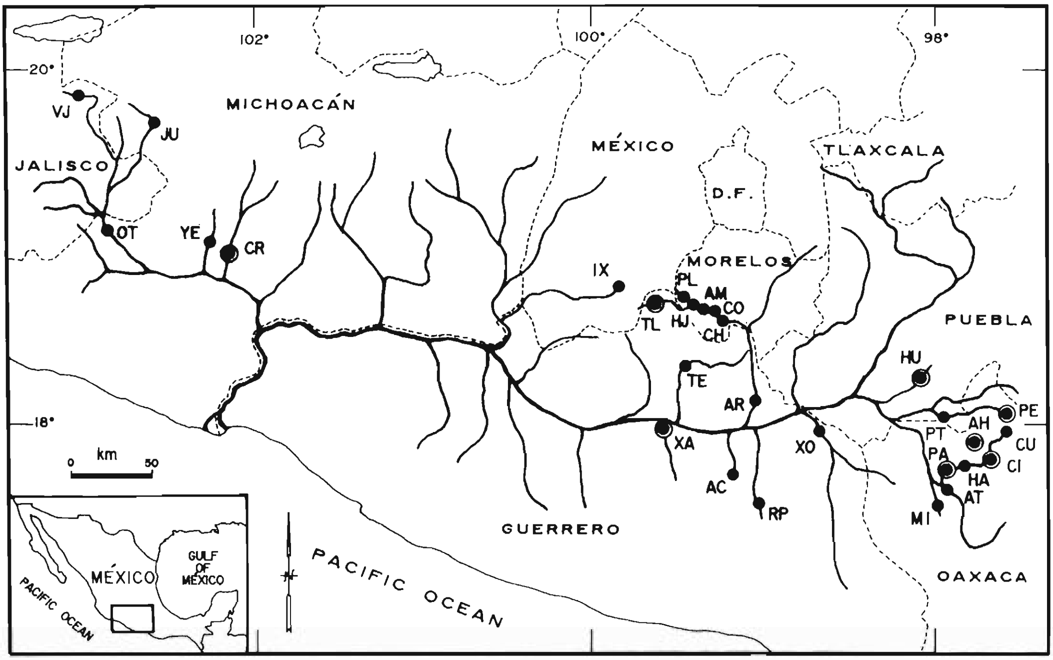

Figure 1. The Balsas River drainage basin of southwestern Mexico, showing the fish collection sites. Double circles indicate sites where no infected fish were collected.

then fixed in hot 10% formalin. Digeneans, cestodes, and acanthocephalans were stained with Mayer's paracarmine or Ehrlich's hematoxylin, dehydrated through a graded alcohol series, cleared in methyl salicylate, and whole-mounted. Nematodes were cleared with glycerine for light microscopy and stored in 70% ethanol. Voucher specimens of all taxa have been deposited in the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México. Infection parameters utilized are those proposed by Margolis et al. (1982), that is, prevalence (% infected) and abundance of infection (number of parasites per examined fish), expressed as mean ± standard deviation, followed by the range of intensity.

Results

The parasites encountered, their hosts, collection locations, infection sites, and prevalence, abundance, and range of intensity of helminth species are summarized in Table 1.

Only 18 of the 1,045 host fish examined harbored monogeneans. Eight *Gyrodactylus* sp. were collected from 2 *P. gracilis* and 1 *P. infans*. Fifteen *A. fasciatus* were found to harbor 66 *Urocleidoides* cf. *costaricensis*.

Only 36 (3.4%) of the necropsied fish (3 species) were parasite-free. These fish included both ictalurid species, and 33 *I. whitei* from CR. Fish at 8 of the 28 collection sites sampled were not infected at all: HU, PE, CI, PA, XA, TL, AH, and CR (Fig. 1).

The most prevalent and widespread helminth parasite was the cestode *Bothriocephalus acheilognathi* that was recorded in 8 of the 13 Balsas River fish species, at infection intensities from 1 to 46.

Discussion

Data from this survey provide further evidence to support Moravec's (1998) contention that nematodes represent a significant component of helminth faunas in tropical freshwater fishes. They also corroborate the statement of Salgado-Maldonado and Kennedy (1997) that richness in digenean species is a characteristic of these helminth communities. In contrast, acanthocephalans were found to be very rare in the Balsas River survey, supporting the claim of Salgado-Maldonado et al. (1992) that adult acanthocephalans are generally very rare parasites in Mexican freshwater fishes. Adult cestodes are not common parasites in Mexican freshwater fishes; however, this survey found 4 metacestode species. Previous surveys from most other geographical areas in Mexico (Pineda-López et al., 1985; León, 1992; Jiménez-García, 1994; Salgado-Maldonado et al., 1997) did not reveal a rich fauna of cestodes (but see Scholz et al., 1996). Monogeneans have only exceptionally been reported from freshwater fishes in Mexico (Lamothe-Argumedo, 1981), but a number of species have been found recently, in particular in southeastern Mexico (Kritsky et al., 1994, 2000; Mendoza-Franco et al., 1997, 1999).

Most of the parasites recorded in this survey are shared with freshwater fishes inhabiting other Mexican drainage basins (see Pineda-López et al., 1985; Jiménez-García, 1994; Moravec, Vivas-Rodríguez, Scholz, Vargas-Vázquez, Mendoza-Franco, and González-Solís, 1995; Moravec, Vivas-Rodríguez, Scholz, Vargas-Vázquez, Mendoza-Franco, Schmitter-Soto, and González-Solís, 1995; Scholz et al., 1995, 1996; Salgado-Maldonado et al., 1997; Moravec, 1998; Moravec et al., 2000; Scholz and Vargas-Vázquez, 1998; Scholz and Salgado-Maldonado, 2000). Six adult species are of neotropical origin: *Urocleidoides* cf. *costaricensis, M. simplex, R. kidderi, R. lichtenfelsi, R. mexicana,* and *N. golvani. Saccocoelioides sogandaresi, Rhabdochona canadensis,* and *C. cyprinodonticola* have been recorded in various freshwater fishes in Canada and southern North America (Lumsden, 1963; Moravec and Arai, 1971; Moravec, 1998).

Twelve of 25 helminth species recorded during this survey were larval forms that utilized small freshwater fishes as intermediate hosts. All these allogenic species are widespread taxa, with wide distributions within Mexico and broad host specificity. Thus, they can be regarded as an ecological component of the fish parasite communities in the Balsas River basin. The metacercariae of *C. complanatum, P. minimum,* and *Diplostomum* cf. *compactum,* as well as the larvae of nematodes *Eustrongylides* sp., *Contracaecum* sp., and Acuariidae gen. sp. have been commonly recorded in cichlids, poeciliids, characiids, pimelodids, and other fish families from southern Mexico (Pineda-López, 1985; Pineda-López et al., 1985; Osorio-Sarabia et al., 1987; Jiménez-García, 1994; Moravec, Vivas-Rodríguez, Scholz, Vargas-Vázquez, Mendoza-Franco, Schmitter-Soto, and González-Solís, 1995; Scholz et al., 1995; Salgado-Maldonado et al., 1997). They have also been reported in atherinids, goodeids, and other fish families from the Lerma Santiago River basin in the highland plateau of central Mexico (Osorio-Sarabia et al., 1986; Salgado-Maldonado and Osorio-Sarabia, 1987; León, 1992; Peresbarbosa et al., 1994). All these helminth species are widely distributed in North America, and some are worldwide (Hoffman, 1967; Yamaguti, 1971; Gibson, 1996).

Some of the helminths found have been introduced to Mexico with exotic fish or other animals. The Asian fish tapeworm (*B. acheilognathi*) has been disseminated globally in association with Asian cyprinids (grass and common carp) introduced to several countries for use in aquaculture (Salgado-Maldonado et al., 1986). This tapeworm has broad host specificity and now occurs in more than 15 freshwater fish species in Mexico (García and Osorio-Sarabia, 1991). We found *B. acheilognathi* widely distributed within the Balsas River basin, parasitizing 8 fish species, mainly poeciliids.

Another example is the heterophyid trematode *C. formosanus* that was introduced into Mexico most probably with the imported thiarid snail *Melanoides tuberculata* (Müller, 1774) serving as the first intermediate host. This trematode has rapidly spread to an extensive area, including central Mexico and both the Atlantic and Pacific coasts, apparently aided by the previous expansion of *M. tuberculata* within Mexico. The metacercariae of *C. formosanus* are encysted in the gills of a wide spectrum of native fishes including members of Atherinidae, Cichlidae, Cyprinidae, Eleotridae, Goodeidae, Ictaluridae, and Poeciliidae (see Scholz and Salgado-Maldonado, 2000). The adults are parasites in piscivorous birds and mammals. An increasing number of recent records of *C. formosanus* in numerous new hosts and regions, including the Balsas River drainage, suggests that this helminth is continuing to expand its distribution (Scholz and Salgado-Maldonado, 2000).

Too few studies have been undertaken to draw conclusions about the zoogeographic characteristics of the helminth communities in the freshwater fish species of the Balsas River basin. However, two general statements can be made about these faunas. The first is that nematode and trematode species predominate, with only a few monogeneans and acanthocephalans being present. Second, all helminths found had previously been reported from other regions of Mexico; therefore the taxonomic composition of the helminth fauna of the fishes of the Balsas River drainage is not very distinct from that seen in other previously studied freshwater basins in Mexico.

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A Checklist of Helminth Parasites of Freshwater Fishes from the Lerma-Santiago River Basin, Mexico

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ABSTRACT: A checklist based on previously published records and original data is presented for the helminth parasites reported from 33 freshwater fish species from the Lerma-Santiago river basin, west-central Mexico. The checklist contains 43 helminth species, 6 (14%) of which are endemic to the basin. Fourteen of the 43 are allogenic species, mostly Nearctic in origin. Three species are anthropogenically introduced colonizers, of which the Asian fish tapeworm *Bothriocephalus acheilognathi* is the most widely distributed species in the basin. The checklist includes 75 new host records, and records of 12 localities where no previous surveys had been conducted.

KEY WORDS: Digenea, Monogenea, Cestoda, Nematoda, Acanthocephala, freshwater fishes, Lerma-Santiago river basin, west-central Mexico, survey.

At least 375 freshwater fish species, of which approximately 60% are endemic, occur in Mexico, and over 500 species occur if those living in estuaries and coastal lagoons are included (Miller, 1982; Espinosa-Pérez, 1993). The Lerma-Santiago river basin in west-central Mexico has the highest percentage of endemism of any major river basin in Mexico, with 30 of its 42 (72%) fish species found nowhere else (Espinosa-Pérez, 1993; Soto-Galera et al., 1998).

This river basin (Fig. 1) drains much of west-central Mexico and consists of 2 major rivers, the Lerma and the Santiago. The Lerma River basin is the most important hydrologic system of the Mexican Central Highland Plateau. It originates in the State of México at an elevation of 3,000 m and flows for 700 km through the states of Querétaro, Guanajuato, Michoacán, and Jalisco before emptying into Chapala Lake at 1,500 m elevation. The Santiago River drains from Chapala Lake, flowing through the state of Jalisco to the Pacific Ocean.

The fish fauna of the Lerma-Santiago river basin has long been studied by ichthyologists (Díaz-Pardo et al., 1993; Soto-Galera et al., 1998). No regional survey of the parasite fauna of these fish has been published, and the literature is scattered in taxonomic papers (Flores-Barroeta, 1953; Lamothe-Argumedo, 1970, 1981, 1988; Lamothe-Argumedo and Cruz-Reyes, 1972; Osorio-Sarabia et al., 1986; Salgado-Maldonado et al., 1986; Salgado-Maldonado and Osorio-Sarabia, 1987; Alarcón, 1988; Alarcón and Castro-Aguirre, 1988; García-Prieto et al., 1988; García-Prieto and Osorio-Sarabia, 1991; León-Règagnon, 1992; Peresbarbosa-Rojas et al., 1994; Pérez-Ponce de León et al., 1994; Espinosa-Huerta et al., 1996; Mendoza-Garfías et al., 1996; Astudillo-Ramos and Soto-Galera, 1997; Pineda-López and González, 1997; Sánchez-Álvarez et al., 1998; Guzmán-Cornejo and García-Prieto, 1999; Caspeta-Mandujano et al., 1999; Moravec et al., 2000, 2001; Scholz and Salgado-Maldonado, 2000, 2001). This paper compiles the extant information on the helminth parasites of freshwater fishes in the Lerma-Santiago river basin and includes original data derived from our own research. The species referred to in theses and scientific meetings do not constitute formal publications and are consequently not considered herein. This checklist should facilitate future research on the ecology, zoogeography, and biodiversity of this important river basin.

Materials and Methods

As part of an ongoing parasitological investigation into the helminth fauna of the freshwater fishes of Mexico, a

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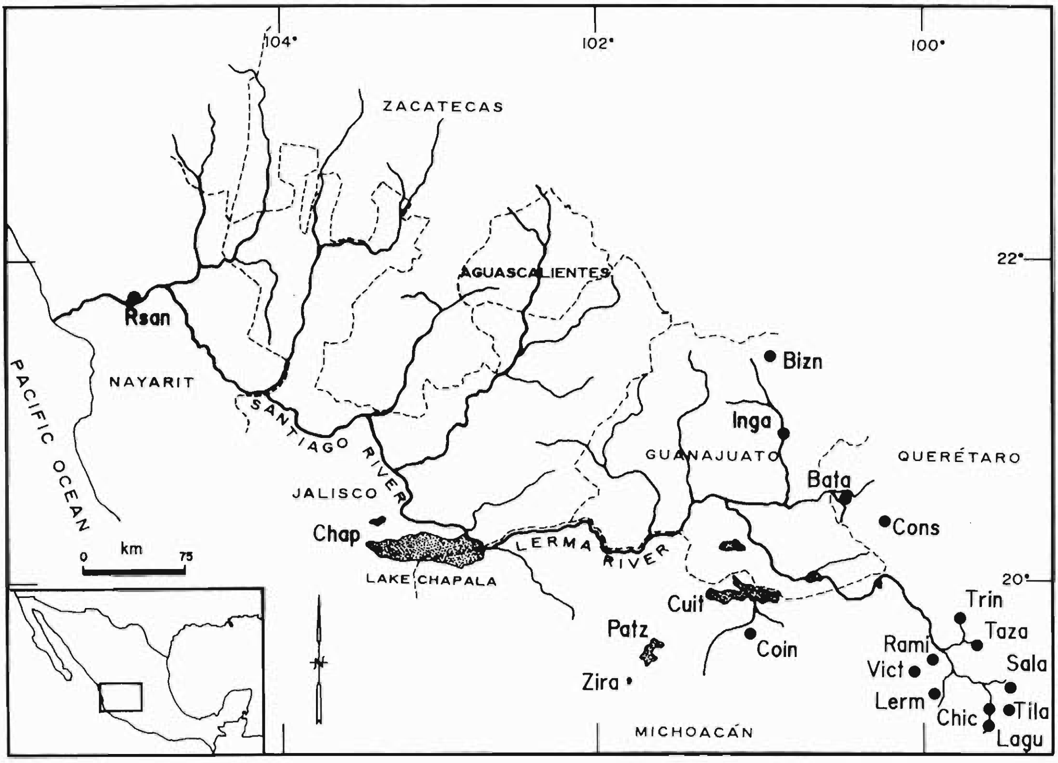

Figure 1. The Lerma-Santiago River drainage basin of west-central Mexico, showing the fish collection sites. Locality codes as in Table 1.

review of the literature dealing with freshwater fish helminth parasites in the entire Lerma-Santiago river basin was made. In addition, a total of 1,177 fish of 18 species (Table 1), from 11 localities in the Lerma-Santiago river basin (Table 2, Fig. 1) was examined for the presence of helminths from January to October 1997 and from January to March 1998.

At each site, fish were captured using electrofishing or gill nets. The numbers of fish examined at each locality and collection data are given in the parasite–host list (Table 3). After capture, the fish were taken live to the laboratory and examined within 48 hr using standard procedures. Briefly, all the external surfaces, viscera, and musculature of each fish host were examined under a stereomicroscope, and all the helminths encountered in each fish were counted. Digeneans (adults and larvae), cestodes, and nematodes were fixed in hot 4% neutral formalin. Acanthocephalans were placed in distilled water, refrigerated overnight (6–12 hr) to evert the proboscis, and then fixed in hot 10% formalin. Digeneans, cestodes, and acanthocephalans were stained with Mayer's paracarmine or Ehrlich's hematoxylin, dehydrated using a graded alcohol series, cleared in methyl salicylate, and whole mounted. Nematodes were cleared with glycerine for light microscopy and stored in 70% ethanol. Voucher specimens of all taxa have been deposited in the National Helminth Collection (Colección Nacional de Helmintos [CNHE]), Institute of Biology, National Autonomous University of Mexico (UNAM), Mexico City. Infection parameters utilized are those proposed by Margolis et al. (1982), that is, prevalence (% infected) and mean intensity of infection (number of parasites per examined fish).

Voucher specimens of the following species, found in fish from the Lerma-Santiago river basin and deposited in the CNHE, were examined: *Posthodiplostomum minimum* (MacCallum, 1921) (nos. 001253, 001476, 001748); *Bothriocephalus acheilognathi* Yamaguti, 1934 (no. 000434); *Proteocephalus pusillus* Ward, 1910 (nos. 000383–000386); *Proteocephalus* sp. (no. 000425); *Ligula intestinalis* (Linnaeus, 1758) [nos. 448(F) and 449(F)], and *Contracaecum* sp. [nos. 002508(F) and 002253(F)].

Results

A host–parasite checklist is presented herein as Table 3. In this study, 43 helminth species are reported from 33 species of freshwater fishes of the Lerma-Santiago river basin, west-central Mexico. Six (14%) of the 43 species are endemic to the basin: *A. mexicanum, M. bravoae, O. mexicanum, R. lichtenfelsi, Spinitectus* sp., and *B. nayaritensis*. Fourteen are allogenic species that mature in, and are transported by, birds: *C.*

Table 1. Fish species from the Lerma-Santiago river basin of west-central Mexico that were examined for helminths in 1997 and 1998.

Fish species	Common name	Sample size (n)
Cyprinidae		
**Algansea tincella* (Valenciennes *in* Cuvier and Valenciennes, 1844)	Spottail chub	17
†*Cyprinus carpio* Linnaeus, 1758	Common carp	45
**Notropis sallei* (Günther, 1868)	Azteca chub	37
**Yuriria alta* (Jordan, 1880)	Lerma chub	49
Goodeidae		
**Girardinichthys multiradiatus* (Meek, 1904)	Darkedged splitfin	503
**Goodea atripinnis* Jordan, 1880	Blackfin goodea	143
**Xenotoca variatus* (Bean, 1887)	Jeweled splitfin	56
Poeciliidae		
Poecilia sphenops Valenciennes *in* Cuvier and Valenciennes, 1846	Mexican molly	23
**Poeciliopsis infans* (Woolman, 1894)	Lerma livebearer	16
Poeciliopsis sp.		13
Atherinidae		
**Atherinella crystallina* (Jordan and Culver *in* Jordan, 1895)	Blackfin silverside	48
**Chirostoma humboldtianum* (Valenciennes *in* Cuvier and Valenciennes, 1835)	Shortfin silverside	46
**Chirostoma jordani* Woolman, 1894	Mesa silverside	64
**Chirostoma labarcae* Meek, 1902	Sharpnose silverside	3
**Chirostoma riojai* Solorzano and López, 1966	Toluca silverside	78
Cichlidae		
Cichlasoma beani (Jordan, 1889)	Sinaloan cichlid	32
Centrarchidae		
† *Lepomis macrochirus* Rafinesque, 1819	Bluegill	2
Gobiidae		
Awaous tajasica (Lichtenstein, 1822)	River goby	2

* Species endemic to the Lerma-Santiago river basin.
† Species introduced to the Lerma-Santiago river basin.

complanatum, *Diplostomum* sp., *P. minimum*, *C. formosanus*, *L. intestinalis*, *C.* cf. *ralli*, *P. caballeroi*, *P.* cf. *urseus*, *P. cochlearii*, *V. campylancristrota*, *V. mutabilis*, *Eustrongylides* sp., *Contracaecum* sp., and *P. brevis*. Three species are recent, anthropogenically introduced colonizers: *C. formosanus*, *P. tomentosa*, and *B. acheilognathi*, which is the most widely distributed species in the basin. Twelve of the 33 fish species examined have not previously been surveyed for parasites, and present data expand the spectrum of fish hosts, to the effect that the list provides 75 new records for hosts and locations.

Discussion

Only 8 fish species have been examined in sufficient numbers to enable evaluation of helminth community composition and structure: *Algansea lacustris*, *Chirostoma estor*, *C. attenuatum*, *Goodea atripinnis*, *Alloophorus robustus*, *Allotoca diazi*, *Micropterus salmoides*, and *Cyprinus carpio*. Pátzcuaro Lake has been systematically sampled, while other localities have only been sampled occasionally and with few fish examined. From large areas of the basin no data on fish parasites exist at all. There is also limited information on the parasites of fish in rivers and other water bodies. Parasitological knowledge for the Lerma-Santiago river basin is fragmentary, as many studies did not record all the helminth species because they were prepared for taxonomic ends, such as the description of a single species. As a result, most of the research in the basin merely indicates where data are most needed.

A notable aspect of the present data is a highly characteristic endemic helminth component in the Lerma-Santiago river basin. Of the 43 re-

Table 2. Codes and features of the localities sampled or reported in the literature from which hosts were collected.

Code	Locality name	Habitat type	State (coordinates)
Bata	Presa El Batán	AR*	Querétaro (20°13′13″N; 100°24′39″W)
Bizn	Presa La Biznaga	AR	Guanajuato (21°25′30″N; 100°52′52.7″W)
Chap	Lago de Chapala	NL	Jalisco (20°08′–20°22′N; 102°42′–103°25′W)
Chic	Lago de Chicnahuapan ("Almoloya del Río")	NL	Estado de México (19°11′N; 99°30′W)
Coin	Presa Cointzio	AR	Michoacán (19°36′46″N; 101°17′58″W)
Cons	Presa Constitución de 1917	AR	Querétaro (20°25′00″N; 100°05′00″W)
Cuit	Lago de Cuitzeo	NL	Guanajuato–Michoacán (20°04′34″–19°53′25″N; 101°19′34″–100°50′20″W)
Igna	Presa Ignacio Allende	AR	Guanajuato (20°55′N; 100°50′W)
Lagu	La Lagunilla	WL	Estado de México (19°08′30″N; 99°30′12″W)
Lerm	Ciénega de Lerma	WL	Estado de México (19°22′41″N; 99°59′39″W)
Patz	Lago de Pátzcuaro	NL	Michoacán (19°41′–19°32′N; 101°27′–101°53′W)
Rami	Presa Ignacio Ramírez	AR	Estado de México (19°26′54″N; 99°59′32″W)
Rsan	Río Santiago (Aguamilpa)	RI	Nayarit (21°46′42″N; 104°55′36″W)
Sala	Lago de Salazar	NL	Estado de México (19°21′5″N; 99°21′55″W)
Taza	Las Tazas	AR	Estado de México (not located)
Tila	Santiago Tilapa, Laguna de Guadalupe Victoria	NL	Estado de México (19°11′15″N; 99°23′56″W)
Trin	Trinidad Fabela	AR	Estado de México (19°48′N; 99°46′W)
Vict	Villa Victoria	AR	Estado de México (19°26′28″N; 100°4′33″W)
Zira	Lago de Zirahuén	NL	Michoacán (19°21′14″–19°29′32″N; 101°30′33″–101°46′15″W)

* AR = Artificial reservoir; NL = natural lake; WL = wetland; RI = river.

corded helminth species, 6 (14%) are endemic to the basin: the digeneans *A. mexicanum* and *M. bravoae,* from atherinids and the goodeid *G. multiradiatus,* respectively; the monogenean *O. mexicanum,* a parasite of the cyprinid *A. lacustris*; and the nematodes *R. lichtenfelsi,* from the goodeids *A. robustus, A. diazi,* and *G. atripinnis,* and a species of *Spinitectus,* previously referred to as *S. carolini,* from the atherinids *C. attenuatum* and *C. estor.* Additionally, the nematode species *B. nayaritensis,* a parasite of *C. beani* in the Santiago River, may be endemic to this basin, because there is no other record of this species in Mexico (Moravec, 1998), and cichlids are the best studied fish family from a parasitological point of view (Salgado-Maldonado et al., 1997; Vidal-Martínez and Kennedy, 2000).

It is thought that the present hydrological configuration of the Lerma-Santiago river basin was created during the Pliocene Age by orogenic activity that isolated it from the ocean (Barbour, 1973; Echelle and Echelle, 1984). The fish fauna of the basin consists of the descendants of marine ancestors that invaded the freshwater bodies, as well as Nearctic components such as cyprinids. It is assumed that by at least 5 million yr ago the fish species in the basin had established themselves, evolving and diversifying from their original marine ancestors. The parasite fauna must also have evolved and diversified during this period of isolation, the current assemblage of endemic helminth species being the product of these evolutionary processes. To the extent to which the fish species adapted to these environments and speciated within them, so did their helminth communities, with some being lost and others developing in the new hosts. In other words, both the fish of the Lerma-Santiago river basin, and their parasites developed in isolation.

The fish parasite fauna of this basin is also enriched through colonization by allogenic species transported by birds. As a result, the fish helminth communities in the basin have an abundant (14 of the total 43 species) component of allogenic species that mature in, and are transported by, birds: *C. complanatum, Diplostomum* sp., *P. minimum, C. formosanus, L. intestinalis, C.* cf. *ralli, P. caballeroi, P.* cf. *urseus, P. cochlearii, V. campylancristrota, V. mutabilis, Eustrongylides* sp., *Contracaecum* sp., and *P. brevis,* most of which occur throughout the American continent or are cosmopolitan. Many factors may have favored this colonization. They include the small size of the fish in this basin, their gregarious habits, their shallow water habitat, their status in the food web, and

Table 3. Parasite–host list of helminths collected from fish of the Lerma-Santiago river basin of west-central Mexico.

Parasite	Host/infection site(s)*	Locality	Number of hosts examined	Prevalence/ mean intensity	Reference(s)
Adult Trematoda					
Family Allocreadiidae Stossich, 1903					
Allocreadium mexicanum Osorio-Sarabia, Pérez and Salgado-Maldonado, 1986	*C. estor*/I	Pátz	216	6%/5.3	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. attenuatum/I	Pátz	195	4%/2.9	Pérez-Ponce de León et al., 1994
	A. crystallina/I	Rsan	48	23%/3	Present work
	C. riojai/I	Tila	8	13%/1	Present work
Crepidostomum cooperi Hopkins, 1931	*M. salmoides*/Pc, I	Pátz	209	24%/13	Salgado-Maldonado and Osorio-Sarabia, 1987
Margotrema bravoae Lamothe-Argumedo, 1970	*G. multiradiatus*/I	Lagu	64	"Low"/?	Lamothe-Argumedo, 1970
	/I	Vict	5	40%/22.5	Present work
Family Gorgoderidae (Looss, 1901)					
Phyllodistomum lacustris (Loewen, 1929)	*I. dugesi*/I	Chap	?	?/?	Lamothe-Argumedo, 1988
Larval Trematoda					
Family Cryptogonimidae Ciurea, 1933					
Cryptogonimidae gen. sp.	*A. tincella*/I	Igna	17	6%/1	Present work
Family Proterodiplostomidae Dubois, 1936					
Proterodiplostomum sp.	*A. tincella*/Bc	Igna	17	6%/1	Present work
	N. sallei/Bc	Rami	15	67%/24.6	Present work
	G. multiradiatus/Bc	Chic	94	45%/6.4	Present work
	/Bc	Lagu	50	20%/2.6	Present work
	/Bc	Rami	75	15%/3	Present work
	/Bc	Sala	3	33%/1	Present work
	/Bc	Trin	31	3%/1	Present work
	/Bc	Vict	5	20%/1	Present work
	G. atripinnis/Bc	Bizn	18	61%/2.4	Present work
	/Bc	Igna	20	30%/9.7	Present work
	/Bc	Trin	29	3%/1	Present work
	C. humboldtianum/L	Vict	46	28%/13.6	Present work
	C. riojai/Bc	Rami	14	36%/4.8	Present work
Family Clinostomidae Lühe, 1901					
Clinostomum complanatum (Rudolphi, 1814)	*A. robustus*/?	Cuit	30	90%/32.8	Guzmán-Cornejo and Garcia-Prieto, 1999
	/L, M	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/L, M	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts examined	Prevalence/ mean intensity	Reference(s)
	G. atripinnis/?	Cuit	30	13%/4.7	Guzmán-Cornejo and Garcia-Prieto, 1999
	/L	Pátz	178	0.6%/4	Salgado-Maldonado and Osorio-Sarabia, 1987
	/L	Igna	22	5%/9	Present work
	X. variatus/?	Cuit	41	27%/4.5	Guzmán-Cornejo and Garcia-Prieto, 1999
Family Diplostomidae Poirier, 1886					
Diplostomum sp.	*C. estor*/B	Pátz	216	10%/6	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. jordani/M	Bizn	38	79%/3.2	Present work
	P. sphenops/Bc	Rsan	22	9%/4	Present work
	Y. alta/M	Igna	9	11%/1	Present work
Diplostomum (*Tylodelphys*) sp.	*G. atripinnis*/?	Cuit	30	7%/1	Guzmán-Cornejo and García-Prieto, 1999
	C. attenuatum/?	Zira	42	14%/1.6	Espinosa-Huerta et al., 1996
	C. jordani/?	Cuit	30	30%/5	Guzmán-Cornejo and García-Prieto, 1999
Posthodiplostomum minimum (MacCallum, 1921) Dubois, 1936	*A. lacustris*/M	Pátz	390	5%/2.5	Mendoza-Garfías et al., 1996
	N. sallei/?	Lerm	6	?/?	León-Règagnon, 1992
	A. robustus/?	Cuit	30	93%/57.1	Guzmán-Cornejo and García-Prieto, 1999
	/L, M, Mu, E	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/L, M, Mu, E	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
	G. multiradiatus/?	Lerm	9	?/?	León-Règagnon, 1992
	G. atripinnis/?	Cuit	30	87%/26.5	Guzmán-Cornejo and García-Prieto, 1999
	/L, Mu	Pátz	178	62%/13.3	Salgado-Maldonado and Osorio-Sarabia, 1987
	/L, M, Mu, E	Pátz	35	?/?	Guzmán-Cornejo and García-Prieto, 1999
	X. variatus/?	Cuit	41	80%/26.1	Guzmán-Cornejo and García-Prieto, 1999
	C. attenuatum/L, M, Mu	Pátz	30	100%/1433	Espinosa-Huerta et al., 1996
	/L, M, Mu, E, B	Pátz	195	98%/111.3	Pérez-Ponce de León et al., 1994
	/L, M, Mu	Zira	42	81%/31.9	Espinosa-Huerta et al., 1996
	C. estor/L, Mu, E, B	Pátz	216	95%/66.1	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. jordani/?	Cuit	30	67%/10.3	Guzmán-Cornejo and García-Prieto, 1999
	O. aureus/?	Cuit	30	7%/1	Guzmán-Cornejo and García-Prieto, 1999
	A. tincella/M	Igna	17	82%/18.4	Present work
	N. sallei/M	Rami	15	7%/1	Present work
	/M	Lagu	1	100%/1	Present work
	/M	Chic	8	22%/4	Present work
	Y. alta/I, L, M	Igna	10	80%/54.8	Present work
	G. multiradiatus/M	Chic	118	22%/1	Present work
	/M	Rami	13	8%/3	Present work

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts exam-ined	Prevalence/ mean intensity	Reference(s)
	G. atripinnis/M	Bizn	25	60%/10.8	Present work
	/L, M	Igna	22	55%/5.7	Present work
	/M	Trin	4	25%/7	Present work
	X. variatus/L, M	Igna	35	57%/13.8	Present work
	P. sphenops/M	Rsan	22	5%/1	Present work
	P. infans/L, M	Igna	9	100%/10.6	Present work
	/M	Rami	2	100%/4	Present work
	C. humboldtianum/L	Vict	46	48%/13.9	Present work
	C. jordani/L, M	Igna	23	52%/3.6	Present work
	C. labarcae/L, M	Igna	2	100%/1.5	Present work
	C. riojai/L, M	Rami	23	26%/0.8	Present work
	/L, M	Tila	13	8%/1	Present work
Family Plagiorchiidae Lühe, 1901					
Ochetosoma sp.	*A. diazi*/I	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
Family Heterophyidae Odhner, 1914					
Centrocestus formosanus (Nishigori, 1924)	*A. tincella*/G	Igna	17	18%/58	Scholz and Salgado-Maldonado, 2000
	Y. alta/G	Igna	14	50%/142	Scholz and Salgado-Maldonado, 2000
	G. atripinnis/G	Igna	11	27%/5	Scholz and Salgado-Maldonado, 2000
	P. sphenops/G	Rsan	1	100%/1	Scholz and Salgado-Maldonado, 2000
	P. infans/G	Igna	5	20%/1	Present work
	Poeciliopsis sp./G	Rsan	13	62%/74.1	Present work
	A. crystallina/G	Rsan	48	38%/96.6	Present work
	L. macrochirus/G	Rsan	2	50%/105	Present work
Monogenea					
Family Dactylogyridae Bychowsky, 1933					
Sciadicleithrum sp.	*C. beani*/G	Rsan	25	12%/1.6	Present work
Family Gyrodactylidae Cobbold, 1864					
Gyrodactylus elegans Nordmann, 1832	*G. multiradiatus*/G	Chic	46	28%/1.8	Present work
Gyrodactylus sp.	*P. sphenops*/G	Rsan	22	23%/1.2	Present work
Family Discocotylidae Price, 1936					
Octomacrum mexicanum Lamothe-Argumedo, 1981	*A. lacustris*/G	Pátz	390	62%/5.1	Mendoza-Garfías et al., 1996

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts examined	Prevalence/ mean intensity	Reference(s)
Adult Cestoda					
Order Caryophyllidea van Beneden *in* Carus, 1863					
Caryophyllidea gen. sp.	*A. lacustris*/I	Pátz	390	0.3%/1	Mendoza-Garfías et al., 1996
Family Bothriocephalidae Blanchard, 1849					
Bothriocephalus acheilognathi Yamaguti, 1934	*A. rubescens*/I	Chap	?	?/?	García-Prieto and Osorio-Sarabia, 1991
	A. lacustris/I	Pátz	390	5%/12.8	Mendoza-Garfías et al., 1996
	N. sallei/I	Lerm	6	?/?	León-Règagnon, 1992
	C. carpio/I	Pátz	178	13%/6.4	Salgado-Maldonado and Osorio-Sarabia, 1987
	/I	Lerm	5	?/?	León-Règagnon, 1992
	A. robustus/I	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/I	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
	G. multiradiatus/I	Lerm	9	?/?	León-Règagnon, 1992
	G. atripinnis/I	Bata	41	12%/4	Pineda-López and González, 1997
	/I	Chap	?	?/?	García-Prieto and Osorio-Sarabia, 1991
	X. variatus/I	Cons	36	8%/3.2	Pineda-López and González, 1997
	C. attenuatum/I	Pátz	30	13%/3.2	Espinosa-Huerta et al., 1996
	/I	Pátz	195	7%/3.5	Pérez-Ponce de León et al., 1994
	/I	Zira	42	24%/7.6	Espinosa-Huerta et al., 1996
	C. estor/I	Pátz	216	2%/7	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. humboldtianum/I	Coin	234	2%/66	
	C. ocotlane/I	Chap	?	?/?	García-Prieto and Osorio-Sarabia, 1991
	C. grandocule/I	Pátz	?	?/?	García-Prieto and Osorio-Sarabia, 1991
	Chirostoma sp./I	Bata	25	24%/1.3	Pineda-López and González, 1997
	/I	Cons	43	40%/4.6	Pineda-López and González, 1997
	M. salmoides/I	Pátz	209	1%/3	Salgado-Maldonado and Osorio-Sarabia, 1987
	O. niloticus/I	Cons	40	8%/5	Pineda-López and González, 1997
	A. tincella/I	Igna	17	6%/1	Present work
	N. sallei/I	Rami	15	13%/1	Present work
	Y. alta/I	Igna	17	24%/3.2	Present work
	/I	Rami	3	33%/57	Present work
	C. carpio/I	Rami	43	2%/1	Present work
	/I	Trin	2	100%/5	Present work
	G. multiradiatus/I	Chic	63	3%/1	Present work
	/I	Lagu	50	26%/2.5	Present work
	/I	Rami	75	3%/1	Present work

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts examined	Prevalence/ mean intensity	Reference(s)
	X. variatus/I	Igna	21	10%/1	Present work
	A. crystallina/I	Rsan	48	6%/1	Present work
	C. jordani/I	Bizn	38	68%/4.1	Present work
	/I	Igna	23	35%/2.4	Present work
	C. labarcae/I	Igna	1	100%/2	Present work
	C. riojai/I	Tila	13	15%/1.5	Present work
Family Proteocephalidae La Rue, 1911					
Proteocephalus pusillus Ward, 1910	*G. atripinnis*/I	Pátz	178	34%/2.2	Salgado-Maldonado and Osorio-Sarabia, 1987
Metacestodes					
Family Diphyllobothriidae Lühe, 1910					
Ligula intestinalis (Linnaeus, 1758)	*G. multiradiatus*/Bc	Lerm	?	?/?	Lamothe-Argumedo and Cruz-Reyes, 1972
	/Bc	Trin	563	16%/1.6	Astudillo-Ramos and Soto-Galera, 1997
	G. atripinnis/Bc	Pátz	?	?/?	García-Prieto et al., 1988
	C. bartoni/Bc	Chap	?	?/?	Flores-Barroeta, 1953
	C. consocium/Bc	Chap	?	?/?	García-Prieto et al., 1988
	C. estor/Bc	Pátz	?	?/?	García-Prieto et al., 1988
	N. sallei/Bc	Lagu	1	100%/1	Present work
	/Bc	Rami	15	27%/1.3	Present work
	G. multiradiatus/Bc	Lagu	50	2%/3	Present work
	/Bc	Rami	5	80%/1.7	Present work
Family Proteocephalidae La Rue, 1911					
Proteocephalidea gen. sp.	*A. lacustris*/M	Pátz	390	0.3%/1	Mendoza-Garfías et al., 1996
	A. robustus/L, I, M	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/L, I, M	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
	G. atripinnis/L, I, M	Pátz	35	?/?	Peresbarbosa-Rojas et al., 1994
	M. salmoides/I	Pátz	209	0.5%/11	Salgado-Maldonado and Osorio-Sarabia, 1987
	N. sallei/M	Rami	15	13%/1.5	Present work
	P. infans/M	Igna	9	22%/1	Present work
Family Dilepididae Railliet and Henry, 1909					
Cyclustera cf. *ralli* (Underwood and Dronen, 1986)	*C. carpio*/M	Rami	42	2%/1	Scholz and Salgado-Maldonado, 2001
	N. sallei/M	Rami	15	13%/1.5	Scholz and Salgado-Maldonado, 2001
	A. robustus/M	Pátz	25	8%/2.5	Scholz and Salgado-Maldonado, 2001
	G. multiradiatus/M	Chic	211	10%/0.1	Scholz and Salgado-Maldonado, 2001

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts examined	Prevalence/ mean intensity	Reference(s)
	X. variatus/M	Igan	24	8%/1	Scholz and Salgado-Maldonado, 2001
Paradilepis caballeroi Rysavy and Macko, 1973	*C. jordani*/M, L	Bizn	38	3%/2.0	Scholz and Salgado-Maldonado, 2001
Paradilepis cf. *urceus* (Wedl, 1855)	*C. jordani*/L	Igna	23	22%/8.2	Scholz and Salgado-Maldonado, 2001
Paradilepis sp.	*C. jordani*/L	Bizn	38	3%/1	Scholz and Salgado-Maldonado, 2001
Parvitaenia cochlearii Coil, 1955	*A. crystallina*/L	Rsan	48	2%/1	Scholz and Salgado-Maldonado, 2001
Valipora campylancristrota (Wedl, 1855)	*C. humboldtianum*/Gb	Vict	46	2%/2	Scholz and Salgado-Maldonado, 2001
	C. jordani/Gb	Rami	3	33%/12	Scholz and Salgado-Maldonado, 2001
	C. riojai/Gb	Rami	20	10%/1	Scholz and Salgado-Maldonado, 2001
	G. multiradiatus/Gb	Lagu	50	4%/1	Scholz and Salgado-Maldonado, 2001
		Rami	75	9%/1.8	Scholz and Salgado-Maldonado, 2001
		Trin	31	3%/1	Scholz and Salgado-Maldonado, 2001
Valipora mutabilis Linton, 1927	*C. beani*/L, Gb	Rsan	25	4%/1	Scholz and Salgado-Maldonado, 2001
Order Cyclophyllidea					
Cyclophyllidea gen. sp.	*C. attenuatum*/I	Pátz	195	0.5%/8	Pérez-Ponce de León et al., 1994
Adult Nematoda					
Family Capillariidae Neveau-Lemaire, 1936					
Pseudocapillaria tomentosa (Dujardin, 1843)	*A. robustus*/I	Pátz	20	5%/3	Present work
	G. atripinnis/I	Pátz	178	10%/2.7	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. attenuatum/I	Pátz	195	0.5%/1	Pérez-Ponce de León et al., 1994
	C. estor/I	Pátz	216	2%/5.2	Salgado-Maldonado and Osorio-Sarabia, 1987
	/I	Pátz	110	1%/1	Moravec et al., 2000
		Pátz	43	7%/1.7	Moravec et al., 2001
		Pátz	75	8%/4.2	Present work
	C. carpio/I	Pátz	184	5%/5.3	Salgado-Maldonado and Osorio-Sarabia, 1987
	N. sallei/I	Rami	3	33%/1	Present work
Remarks: This species was originally described as *Capillaria patzcuarensis* by Osorio-Sarabia et al. (1986) but Moravec et al. (2001) demonstrated that it is in fact *P. tomentosa* introduced from Europe with cyprinids.					
Capillariidae gen. sp.	*G. atripinnis*/I	Igna	20	5%/2	Present work
	/I	Bizn	25	4%/1	Present work
Family Cucullanidae Cobbold, 1864					
Dichelyne mexicanus Caspeta-Mandujano, Moravec and Salgado-Maldonado, 1999	*C. beani*/I	Rsan	7	14%/1	Caspeta-Mandujano et al., 1999

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts examined	Prevalence/ mean intensity	Reference(s)
Family Philometridae Baylis and Daubney, 1926					
Philometridae gen. sp.	*A. lacustris*/Bc	Pátz	390	0.5%/1	Mendoza-Garfías et al., 1996
Family Rhabdochonidae Travassos, Artigas, and Pereira, 1928					
Rhabdochona lichtenfelsi Sánchez-Álvarez, García, and Pérez, 1998	*A. robustus*/I	Cuit	360	?/?	Sánchez-Álvarez et al., 1998
	/I	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/I	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
	G. atripinnis/I	Cuit	20	40%/18	Sánchez-Álvarez et al., 1998
	/I	Pátz	178	8%/7.8	Salgado-Maldonado and Osorio-Sarabia, 1987
	/I	Pátz	35	?/?	Peresbarbosa-Rojas et al., 1994
Beaninema nayaritense Caspeta-Mandujano, Moravec, and Salgado-Maldonado, 2000	*C. beani*/I	Rsan	25	44%/7.3	Caspeta-Mandujano et al., 2001
Family Cystidicolidae Skrjabin, 1846					
Spinitectus sp.	*C. attenuatum*/I	Pátz	30	10%/1.3	Espinosa-Huerta et al., 1996
	/I	Pátz	195	14%/2.9	Pérez-Ponce de León et al., 1994
	/I	Zira	42	43%/10.3	Espinosa-Huerta et al., 1996
	C. estor/I	Pátz	216	12%/5.2	Salgado-Maldonado and Osorio-Sarabia, 1987
Remarks: The nematodes were originally reported as *Spinitectus carolini* Holl, 1928, but they in fact belong to a separate species that is to be described (F. Moravec, Academy of Sciences of the Czech Republic, personal communication).					
Larval Nematodes					
Family Dioctophymatidae Railliet, 1915					
Eustrongylides sp.	*A. robustus*/M, Bc	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	G. atripinnis/Mu	Pátz	178	2%/1.3	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. attenuatum/M	Pátz	195	2%/1.3	Pérez-Ponce de León et al., 1994
	/?	Pátz	30	13%/1.5	Espinosa-Huerta et al., 1996
	M. salmoides/Mu	Pátz	209	1%/1	Salgado-Maldonado and Osorio-Sarabia, 1987
	G. atripinnis/Mu	Bizn	10	10%/1	Present work
Family Anisakidae Railliet and Henry, 1912					
Contracaecum sp.	*A. lacustris*/I	Pátz	390	0.3%/1	Mendoza-Garfías et al., 1996
	A. tincella/M	Igna	17	6%/1	Present work
	N. sallei/M	Rami	15	13%/1	Present work
	Y. alta /M	Igna	9	11%/3	Present work
	G. atripinnis/M, Bc	Igna	22	5%/1	Present work
	X. variatus/M	Igna	35	31%/1.4	Present work

Table 3. Continued.

Parasite	Host/infection site(s)*	Locality	Number of hosts exam-ined	Prevalence/ mean intensity	Reference(s)
	P. infans/M	Igna	9	11%/1	Present work
	C. jordani/M	Bizn	38	3%/1	Present work
	/M	Igna	23	17%/1.7	Present work
	C. beani/L, M	Rsan	25	12%/1	Present work
	A. tajasica/M	Rsan	2	50%/3	Present work
Family Gnathostomatidae Railliet, 1895					
Gnathostoma sp.	*A. robustus*/L	Pátz	20	5%/1	Present work
Spiroxys sp.	*A. lacustris*/I	Pátz	390	0.8%/1	Mendoza-Garfías et al., 1996
	C. carpio/I	Pátz	184	2%/4.5	Salgado-Maldonado and Osorio-Sarabia, 1987
	A. robustus/M, I	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/M, I	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
	G. atripinnis/M, I	Pátz	35	?/?	Peresbarbosa-Rojas et al., 1994
	/I	Pátz	178	1%/1	Salgado-Maldonado and Osorio-Sarabia, 1987
	M. salmoides/I	Pátz	209	1%/3.5	Salgado-Maldonado and Osorio-Sarabia, 1987
	N. sallei/I	Rami	3	33%/1	Present work
	G. multiradiatus/I	Rami	13	15%/1	Present work
	G. atripinnis/I	Bizn	18	6%/1	Present work
	/I	Trin	29	3%/1	Present work
	X. variatus/I	Igna	21	5%/1	Present work
Acanthocephala Adult					
Family Neoechinorhynchidae Ward, 1953					
Neoechinorhynchus golvani Salgado-Maldonado, 1978	*C. beani*/I	Rsan	25	20%/3.4	Present work
Acanthocephala Larvae					
Family Polymorphidae Meyer, 1931					
Polymorphus brevis Van Cleave, 1916	*A. lacustris*/M	Pátz	390	0.3%/1	Mendoza-Garfías et al., 1996
	C. carpio/L, M	Pátz	184	2%/2	Salgado-Maldonado and Osorio-Sarabia, 1987
	A. robustus/M, Mu	Pátz	41	?/?	Peresbarbosa-Rojas et al., 1994
	A. diazi/M, Mu	Pátz	31	?/?	Peresbarbosa-Rojas et al., 1994
	G. atripinnis/L, M	Pátz	178	3%/1	Salgado-Maldonado and Osorio-Sarabia, 1987
	C. attenuatum/L, M	Pátz	195	4%/2.8	Pérez-Ponce de León et al., 1994
	C. estor/L, M	Pátz	216	8%/1.2	Salgado-Maldonado and Osorio-Sarabia, 1987
	M. salmoides/L, M	Pátz	209	3%/2.7	Salgado-Maldonado and Osorio-Sarabia, 1987
	X. variatus/L	Igna	35	6%/1	Present work

* B = brain; Bc = body cavity; E = eyes; G = gills; Gb = gall bladder; I = intestine; L = liver; M = mesentery; Mu = muscle; Pc = pyloric cecum.

their situation along the annual migratory routes of Nearctic birds. Additionally, the low number of helminth species in the basin may have readily allowed invasion of these communities by allogenic species.

Three helminth species on the list are recent, anthropogenically introduced colonizers. The first is the cestode *B. acheilognathi,* which is the most widely distributed species in the basin and is found in 22 host species. The second is the heterophyid trematode *C. formosanus.* The actual distribution of this helminth within the basin has not been evaluated, because the intermediate host, the thiarid snail *Melanoides tuberculata* Müller, 1774, has established along riverbanks and in riverbeds, where few fish have been sampled. Both these species were introduced recently into Mexico; the cestode together with Asian carp (Salgado-Maldonado et al., 1986), and the trematode most probably with the intermediate snail host (Scholz and Salgado-Maldonado, 2000). The third species is the capillariid nematode *P. tomentosa,* reported from atherinids and goodeids, as well as from cultured carp, *C. carpio,* from Mexico, where it was probably introduced along with its fish host from Europe (Moravec, 1998; Moravec et al., 2001).

The proportions among the helminth groups that constitute the communities in the fish of the Lerma-Santiago river basin are also distinctive. The dominance in species numbers of nematodes and trematodes (principally metacercariae) is a pattern characteristic of the fish helminth communities of southeastern Mexico (Scholz et al., 1995; Salgado-Maldonado and Kennedy, 1997; Scholz and Vargas-Vázquez, 1998) and the Balsas River basin in central Mexico (Salgado-Maldonado et al., 2001). However, data in the present study show that cestodes, both adults and metacestodes, are almost as important in the Lerma-Santiago river basin in terms of numbers as the nematodes and trematodes. Most cestodes found, such as *V. campylancristrota,* occur throughout the American continent or are cosmopolitan (see Scholz and Salgado-Maldonado, 2001). The presence of 5 monogenean species in the basin is also notable, as it is a higher number than recorded in other drainages in central Mexico. However, the monogenean fauna of freshwater fishes in southeastern Mexico is even richer (Kritsky et al., 1994, 2000; Mendoza-Franco et al., 1997, 1999, 2000).

It is still not possible to form conclusions about the zoogeographic characteristics of the fish helminth parasite communities in the Lerma-Santiago river basin, as very few studies have been done. However, the data that do exist suggest that the proportion of endemic parasites is high, and thus very distinctive, as compared for example to the lack of endemic species among the helminth parasites of fishes from the Balsas River drainage (Salgado-Maldonado et al., 2001). The helminth communities were probably initially poor, and have been invaded by allogenic, Nearctic species transported by birds that have enriched these multispecific assemblages.

Research into fish helminth parasites in the Lerma-Santiago river basin has been restricted to descriptions of some species, and more detailed studies have been carried out only in Pátzcuaro Lake. Obviously, more complete inventories of the fish parasites in this basin are urgently required. Almost 7% of the fish species that originally inhabited the basin are extinct, and an additional 23% are classified as endangered or vulnerable because of population decline associated with continuous habitat degradation and introduction of competing and predatory species that are added to the natural predation pressures in these ecosystems (Soto-Galera et al., 1998).

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Singhiatrema vietnamensis sp. n. (Digenea: Ommatobrephidae) and *Szidatia taiwanensis* (Fischthal and Kuntz, 1975) comb. n. (Digenea: Cyathocotylidae) from Colubrid Snakes in Vietnam

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ABSTRACT: A new digenean is described and a second species is redescribed from the colubrid rear-fanged water snakes *Enhydris chinensis* (Gray) and *Enhydris plumbea* (Boie) captured from several regions in Vietnam during 1996–1998. *Singhiatrema vietnamensis* sp. n. (Ommatobrephidae) from the small intestine of both snakes is characterized by the extent of the ceca, the position of the vitellaria, the size of the eggs, and the host. *Szidatia taiwanensis* (Fischthal and Kuntz, 1975) comb. n. (Cyathocotylidae) is redescribed from the holotype and specimens from the gallbladder of both snakes. The species is transferred from the genus *Mesostephanoides* primarily because it does not have a large cirrus that is spined; it is characterized by the shape of the seminal vesicle, length of the ceca, body size relative to the forebody, number of testes in hindbody, egg size, size of tribocytic organ, and the infection site in the host. Concerning the classification of *Singhiatrema* Simha within Echinostomatiformes, we consider Singhiatrematinae Simha a junior synonym of Ommatobrephinae Poche. We discuss the classification of *Gogatea* Lutz and *Szidatia* Dubois and consider Gogatinae Mehra a junior synonym of Szidatiinae Dubois. The use of different fixation methods can produce artifacts characteristic at the generic level.
KEY WORDS: *Singhiatrema vietnamensis* sp. n., Ommatobrephidae, *Szidatia taiwanensis* comb. n., Cyathocotylidae, fixation artifacts, *Enhydris chinensis*, *Enhydris plumbea*, Colubridae, snakes, Vietnam.

Two unrelated digeneans, a new intestinal ommatobrephid and a gallbladder cyathocotylid, each infecting 2 species of rear-fanged colubrid water snakes (*Enhydris chinensis* (Gray, 1842) and *Enhydris plumbea* (Boie, 1827)) from Vietnam, are herein described. The life history of neither parasite is known. The new ommatobrephid is most similar to species of *Singhiatrema* Simha, 1954, which are usually found in the intestines of water snakes. Exceptions include *Singhiatrema najai* Chattopadhyaya, 1967, from the intestine of the Indian cobra *Naja naja* (Linnaeus, 1758), and *Singhiatrema lali* Chakrabarti, 1967, from the intestine of a freshwater emydid turtle (*Hardella thurgii* (Gray, 1831)) (Chattopadhyaya, 1967). All prior known species are reported from the Indian subcontinent only.

The gallbladder cyathocotylid that we found in Vietnam also occurs in one of the same hosts in Taiwan. It exhibits a relationship with *Szidatia joyeuxi* (Hughes, 1929), which infects the intestine of the colubrid water snake *Natrix maura* (Linnaeus, 1758) (as *Tropidonotus viperinus* (Sonnini and Latreille, 1802)) in an oasis in Tunis, Morocco, and has a cercaria that is shed from the freshwater snail *Melanopsis* sp. and develops in the leg muscles of the frog *Rana rudibunda* Pallas, 1771 (as *Rana esculenta rudibunda*; see Langeron, 1924; Hughes, 1929; Dubois, 1938).

Materials and Methods

The second and third authors captured a total of 43 specimens of *E. chinensis* and 51 specimens of *E. plumbea* in Ha Noi, Nam Ha, Thai Binh, Na Nam, Nam Dinh, Hai Duong, and Hai Phong provinces (Red River Delta) in Vietnam. They collected digeneans live from the intestines and gallbladders of both snakes from all the provinces. For the first collections, they relaxed the specimens in distilled water, then placed them in physiological saline, and finally fixed them in a cold 5% formalin solution. However, specimens collected later were used for the descriptions. These were placed directly into saline, then fixed with near boiling water without coverslip pressure, and finally pipetted into 5% formalin. Additional unmeasured specimens were fixed under pressure for examination of specific features. Whole mounts of worms were prepared by staining specimens with either carmine or Van Cleave's hematoxylin with additional Ehrlich's hematoxylin. These were dehydrated, cleared with clove oil, and mounted on slides with Canada balsam. Measurements are given for the holotype, followed in parentheses by the range of measurements of each feature derived from specimens heat-fixed without pressure. Measurements are given in micrometers unless otherwise stated. Specimens were deposited in the United States National Parasite Collection (USNPC), Belts-

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ville, Maryland, U.S.A., and the Harold W. Manter Laboratory (HWML) of the University of Nebraska State Museum, Lincoln, Nebraska, U.S.A.

Results

Ommatobrephidae Poche, 1926
Singhiatrema vietnamensis sp. n.
(Figs. 1 and 2)

Description

Based on 5 specimens: Ommatobrephinae Poche, 1926. Body pyriform, elongate, 2.56 mm (2.41–2.61 mm) long, 0.76 mm (0.76–0.91 mm) wide at maximum width near posterior end of body, lacking tegumental spines but possessing minute interrupted longitudinal striae. Oral sucker with subterminal mouth, 170 (170–207) long, 189 (189–217) wide. Head crown having single row of 22–23 spines 31–56 long, arranged 11–12 per side; row interrupted dorsally and ventrally. Prepharynx short, less than ½ length of pharynx; pharynx oval, 95 (95–106) long, 78 (78–106) wide. Esophagus 640 (547–640) long, 89 (80–105) wide. Ceca extending beyond posterior extent of testes. Acetabulum 290 (285–329) long, 379 (363–407) wide, lying between anterior ⅓ and anterior ½ of body. Ratio of widths of oral sucker to acetabulum 1:1.8–2.0.

Testes 2, weakly (incompletely) lobed, lying opposite, located near terminal end of body, with anterior margins diverging from each other; left testis 292 (285–340) long, 217 (200–234) wide; right testis 279 (251–335) long, 234 (195–234) wide. Cirrus sac oval, 179 (179–227) long, 102 (102–110) wide, medial, lying between cecal bifurcation and acetabulum, containing large bipartite seminal vesicle, short pars prostatica, and short muscular ejaculatory duct; pars prostatica a short duct surrounded by prostatic cells at anterior of sac; external seminal vesicle lacking.

Ovary pretesticular, spherical, 73 (73–95) in diameter, slightly dextral in 4 of 5 specimens, slightly sinistral in 1 specimen. Oviduct communicating with Laurer's canal, then forming ootype; Laurer's canal straight, directed dorsally from region of ootype, opening on dorsal surface at level of ovary; ootype surrounded by Mehlis' gland; Mehlis' gland compact, usually larger than ovary, consisting of relatively small cells; ootype receiving relatively long common vitelline duct from vitelline reservoir, communicating with uterus; uterus intercecal, postacetabular, with proximal portion a uterine seminal receptacle and with distal portion a metraterm; metraterm thick walled, approximately length of cirrus sac, sinistral to cirrus sac, entering genital atrium anteriorly; genital atrium relatively small, ventral to anterior portion of cirrus sac in heat-killed specimens (directed anteriorly in cold-killed specimens when fixed under pressure with cirrus sac displaced anteriorly); genital pore opening medially or submedially near anterior region of cirrus sac in heat-killed specimens (near anterior acetabular margin in contracted specimens). Vitellarium consisting of 2 lateral bands of irregularly shaped follicles; bands lying ventral to ceca between posterior level of acetabulum and middle of testes, communicating to vitelline reservoir by left and right transverse main collecting channel; left channel about 195 long, right channel about 223–280 long; vitelline reservoir approximately 45–56 long, 111–195 wide. Eggs 95–117 long, 61–75 wide, with thickened knob at posterior end, with eyespots visible in miracidia of some specimens; eyespots 2, lightly pigmented in developing miracidia, darkly pigmented and fused in developed miracidia.

Excretory vesicle Y-shaped, with triangular posterior bladder; main stem slender, medial, concealed by overlapping lobes of testes, extending anteriorly from bladder and bifurcating at anterior margins of testes; arms reaching anteriorly to approximate level just posterior to pharynx; excretory pore subterminal, opening on dorsal surface.

Taxonomic summary

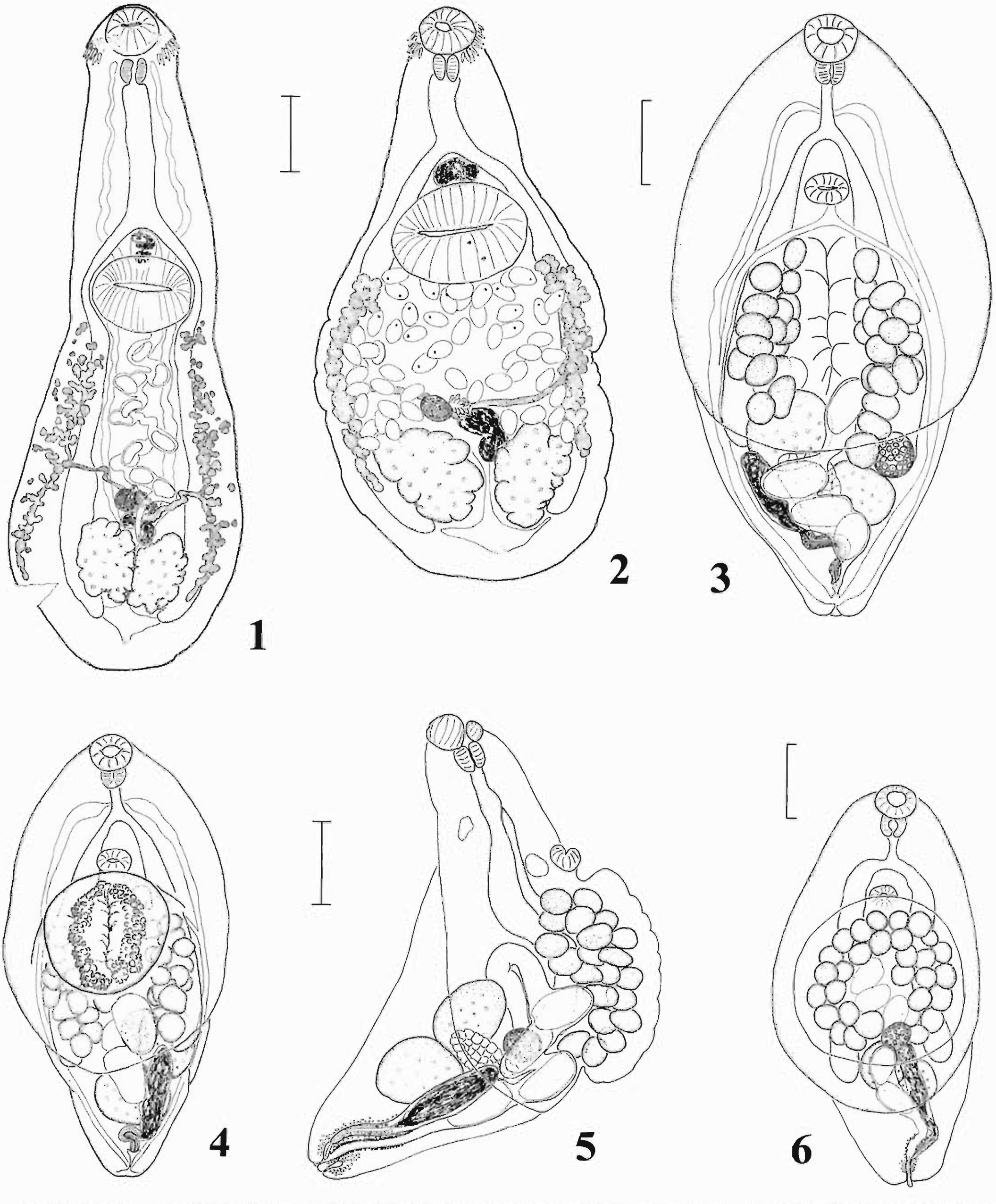
TYPE HOST: *Enhydris chinensis* (Gray, 1842), rear-fanged water snake or Chinese water snake (Colubridae). Other host: *Enhydris plumbea* (Boie, 1827), rear-fanged water snake, rice paddy snake, or plumbeous water snake (Colubridae).

TYPE LOCALITY: Hà Nôi Province, Vietnam. Other localities: throughout Red River Delta, Vietnam, in Nam Hạ, Thái Bình, Nam Hạ, Nam Định, Hải Dủỏng, and Hải Phòng provinces.

INFECTION SITE: Small intestine.

PREVALENCE AND INTENSITY OF INFECTION: Ten of 43 specimens of *E. chinensis* (23%) each hosted 1–5 individual worms; 9 of 51 specimens of *E. plumbea* (18%) each hosted 1–3 worms.

SPECIMENS DEPOSITED: Holotype USNPC No. 90037; paratypes USNPC No. 90038, HWML Nos. 15388 (*E. chinensis*), 15389 (*E. plumbea*).



Figures 1–6. **1. Ventral view of holotype of *Singhiatrema vietnamensis* sp. n.; scale bar = 300 μm. 2. Ventral view of specimen of *S. vietnamensis* exposed to fresh water and slight pressure prior to and during cold fixation; scale bar = 300 μm. 3. Ventral view of *Szidatia taiwanensis*; scale bar = 200 μm. 4. Ventral view of *S. taiwanensis* showing detail of tribocytic organ; scale bar = 200 μm. 5. Lateral view of *S. taiwanensis*; scale bar = 200 μm. 6. Ventral view of *S. taiwanensis* exposed to fresh water prior to cold fixation; scale bar = 275 μm.**

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

Remarks

Singhiatrema vietnamensis is consistent with members of Ommatobrephidae because it has a preacetabular cirrus sac with an internal seminal vesicle; large embryonated eggs, with some containing an oculate miracidium; and opposite incompletely lobed testes located in the posterior region of the body, often with axes diverging anteriorly. The species belongs in Ommatobrephinae rather than Parorchiinae Lal, 1936, because the collar of spines is interrupted dorsally and ventrally as opposed to being arranged in a continuous row. In addition, the tegument is smooth rather than spinous, an external seminal vesicle is absent, spines do not occur on the intromittent organ, and the specimens are parasites in reptiles rather than birds. The 2 genera within Ommatobrephinae, *Singhiatrema* and *Ommatobrephus* Nicoll, 1914, are distinguished by the presence of a dorsally interrupted row of collar spines in the former and the absence of the collar spines in the latter. The presence of a dorsally and ventrally interrupted row of 22–23 collar spines in *S. vietnamensis* enables us to assign the worms to the genus *Singhiatrema. Singhiatrema* was originally unveiled to science in an oral presentation and abstract at the Forty-first Session of the Indian Science Congress (Simha, 1954). The genus was described in more detail by Simha (1958) and placed in Echinostomatidae Poche, 1926, by the author without a subfamily designation. *Singhiatrema singhia* Simha, 1954, from a colubrid ratsnake (*Ptyas mucosus* (Linnaeus, 1758)) from Hyderabad in southern India, was designated the type species (Simha, 1954). Two other species, *Singhiatrema longifurca* Simha, 1958, and *Singhiatrema hyderabadensis* Simha, 1958, parasitize another colubrid water snake, the checkered keelback *Xenochrophis piscator* (Schneider, 1799) (as *Tropidonotus piscator*), from the same locality (Simha, 1958). Three other Indian species have since been added to the genus. *Singhiatrema najai* parasitizes the Indian cobra (*N. naja*) in Hyderabad, *S. lali* parasitizes the turtle *H. thurgii* in Lucknow, and *Singhiatrema piscatora* Dwivedi, 1968, parasitizes the water snake *X. piscator* in Chhindwara (Chakrabarti, 1967; Chattopadhyaya, 1967; Dwivedi, 1968).

Singhiatrema vietnamensis differs from its congeners, with the exception of *S. lali*, by having ceca that extend to the posterior region of the body and vitelline follicles that lie ventral and lateral to the ceca in bands stretching from the posterior margin of the acetabulum to the midlevel of the testes. *Singhiatrema vietnamensis* differs from *S. lali* by having larger eggs (103–119 μm long by 45–56 μm wide vs. 65–87 μm long by 34–39 μm wide), ceca that reach beyond the posterior level of the testes rather than to their midlevel, longer collar spines (31–56 μm rather than 7–9 μm), and a snake rather than a turtle definitive host. Yamaguti (1971) reported 24 collar spines for *S. lali*; however, Chakrabarti (1967) did not report the number of collar spines in the description of *S. lali*, and the illustration of the species did not permit the collar spines to be counted. Specimens of *S. lali* were apparently never deposited in a lending museum. We report 22–23 collar spines in our specimens of *S. vietnamensis*. Three of 5 of our specimens had 22 collar spines and the remaining 2 had 23 collar spines. It is possible that some specimens could lose spines in life or handling, but biological variation probably exists.

Initially, we obtained 4 specimens of *S. vietnamensis* that had been placed in fresh water prior to their fixation with slight pressure in unheated formalin (see Fig. 2). The overall shape of these specimens, as well as their measurements, varied dramatically from those specimens fixed with heat and used in the above description. Specimens subjected to fresh water and pressure had an oval rather than pyriform body shape, and their width was greater (1.0–1.6 mm vs. 0.75–0.91 mm). The pharynx was swollen (131–158 μm long by 127–140 μm wide vs. 95–106 μm long by 78–106 μm wide), and the esophagus was contracted in length but swollen in width (285–415 μm long by 77–203 μm wide vs. 547–640 μm long by 80–105 μm wide). In addition, the ceca were contracted slightly, reaching to the posterior level of the testes rather than beyond the posterior level of the testes, and the eggs were swollen or collapsed and therefore larger in whole mounts (109–117 μm long by 81–95 μm wide vs. 103–119 μm long by 45–56 μm wide). The long side of the oval cirrus sac was oriented laterally rather than vertically, with the pore on the sinistral end rather than at the anterior end, and the testes were larger (411–560 μm long by 258–339 μm wide compared with 250–340 μm long by 195–234 μm wide). If the methods of fixation had not been known, these differences would be great enough to suspect different species.

Cyathocotylidae Poche, 1926

Szidatia taiwanensis (Fischthal and Kuntz, 1975) comb. n. (Figs. 3–6)

Redescription

Based on holotype and 9 specimens from Vietnam: Szidatiinae Dubois, 1938. Body bipartite; forebody pyriform, 0.85 mm (0.81–1.15 mm) long, not accounting for slight curl; hindbody conical; entire body 1.20 mm (1.03–1.57 mm) long, 0.57 mm (0.47–0.93 mm) wide at maximum width just posterior to midforebody. Ratio of forebody to total body length 1:1.39 (1:1.27–1.36). Tegument with spines over entire forebody, with few or no spines on hindbody, with numerous minute papillae near posterior end. Oral sucker with subterminal mouth, (90–119) long, 119 (99–144) wide. Prepharynx very short. Pharynx oval, (60–75) long, (60–80) wide. Esophagus 94 (67–149) long, 31 (23–40) wide. Ceca bifurcating at about anterior ⅓ of forebody, extending to level of posterior margin of ovary. Acetabulum 82 (62–95) long, 98 (85–112) wide, situated between anterior ⅓ and anterior ½ of forebody, always smaller than oral sucker. Tribocytic organ oval, protruding slightly from ventral surface, 327 (283–447) long, ~250 (246–423) wide, with medial-longitudinal slit with basal layer of dark-staining cells.

Testes 2, oblique; anterior testis spanning both forebody and hindbody, ~80 (149–213) long, 124 (114–169) wide; posterior testis in hindbody 91 (144–199) long, 142 (129–229) wide. Cirrus sac in posterior end of body, club-shaped, 410 (332–362) long, 65 (60–73) wide, extending anteriorly to middle of anterior testis, containing internal seminal vesicle, pars prostatica with small prostatic cells, and short unspined ejaculatory duct (not true cirrus); internal seminal vesicle 241 (206–339) long, 45 (57–110) wide at thickest portion; pars prostatica 111 (43–198) long, ~30 (23–25) wide, sinuous in some specimens, surrounded externally by free gland cells; ejaculatory duct 45 (24–74) long, 20 (8–14) wide, thin walled, eversible, surrounded by gland cells.

Ovary nearly spherical, 85 (65–129) long, 75 (70–99) wide. Vitellarium comprised of 2 pairs of lateral fields of follicles underlying tribocytic organ, with the 2 on each side overlying each other, ventral to ovary and testes; fields not confluent anteriorly; right field totaling ~12–14 (16–20) follicles; left field totaling ~14 (16–23) follicles; follicles spherical to ovate, ranging 37–43 (50–99) long, >50 (55–124) wide. Vitelline reservoir lying posterior to ovary and between testes, 145 (119–149) long, 80 (78–144) wide, (159–167) thick (thickness measured from 2 laterally mounted specimens). Uterus reaching anteriorly to near level of anterior extent of vitellarium; distal portion an indistinct metraterm; metraterm slightly longer than cirrus sac (coiled in holotype and some other specimens), demarcated by transverse muscular band, surrounded by lining of free gland cells, emptying into genital atrium separate from ejaculatory duct; genital atrium an open funnel in distal portion of body, surrounded by gland cells. Eggs 5 (4–12) in number, 150 (140–169) long, 77 (80–99) wide, with indistinct operculum.

Excretory vesicle V-shaped; arms united both anteriorly and medially; anterior junction at level of and dorsal to midesophagus; medial junction at level of anterior extent of tribocytic organ, ventral to ceca, leading to small bladder associated with base of acetabulum; excretory pore subterminal, opening ventral to and separate from constricted genital atrium.

Taxonomic summary

HOSTS: *Enhydris chinensis* (Gray, 1842), rear-fanged water snake or Chinese water snake (Colubridae), and *Enhydris plumbea* (Boie, 1827), rear-fanged water snake, rice paddy snake, or plumbeous water snake (Colubridae).

LOCALITIES: Hà Nôi, Nam Hạ, Thái Bình, Hạ Nam, Nam Định, Hải Dủỏng, and Hải Phòng provinces in Vietnam.

INFECTION SITE: Gallbladder (holotype USNPC No. 73148 from Taiwan in *E. chinensis* from intestine).

PREVALENCE AND INTENSITY OF INFECTION: Sixteen of 43 specimens from intestine of *E. chinensis* (37%) each hosted 1–9 individual worms; 24 of 51 specimens of *E. plumbea* (47%) each hosted 1–9 worms.

SPECIMENS DEPOSITED: Voucher specimens USNPC Nos. 90039 and 90040 (*E. chinensis*), HWML No. 15390 (2 slides, *E. chinensis*).

Remarks

Fischthal and Kuntz (1975) described *Mesostephanoides taiwanensis* from the small intestine of *E. chinensis* from Taipei Prefecture in Taiwan on the basis of a single specimen. We examined that specimen, USNPC No. 73148, and, even though it was not from the gallbladder, we considered our specimens from Vietnam conspecific with it. Data for the holotype fit those for our specimens. We, however, interpret differently the terminal genitalia described and illustrated by Fischthal and Kuntz (1975) in their Figure 15. Perhaps they misinterpreted the non-illustrated muscular looping of the metraterm as spines. In any event, the cirrus sac of the holotype contained a club-shaped seminal vesicle measuring about 260 μm long, a straight pars prostatica 113 μm long, and a short ejaculatory duct 45 μm long. The metraterm coiled once prior to descending to the genital atrium. We observed spines in none of these features. Because of the lack of a spined cirrus and lack of anterior confluence of the bands of vitelline follicles, we consider that the species belongs to *Szidatia* Dubois, 1938, as *Szidatia taiwanensis* (Fischthal and Kuntz, 1975) comb. n.

Dubois (1951) differentiated the monotypic genus *Mesostephanoides* Dubois, 1951, from other cyathocotylid genera on the basis of *Mesostephanoides burmanicus* (Chatterji, 1940), having a forebody to total body length ratio that we estimate as 1:1.14–1.18, a spined cirrus 300 μm long, and vitelline follicles confluent anteriorly. We consider the first 2 features of questionable generic significance, and an examination of *M. burmanicus* and related species may show that *Mesostephanoides* is a synonym of *Gogatea* Lutz, 1935, a genus with species having anteriorly confluent vitelline follicles.

Szidatia taiwanensis resembles its 2 congeners in that the vitelline follicles are in separate lateral fields, as opposed to being in a single confluent field underlying the tribocytic organ as reported for species of *Gogatea,* the only other genus in Szidatiinae. *Szidatia taiwanensis* most closely resembles *Szidatia nemethi* Dollfus, 1953, from the water snake *N. maura* (as *N. viperina*) from Charrat, Morocco, but differs from this species by having a much smaller tribocytic organ (283–447 μm long by 246–423 μm wide vs. 885 μm long by 765 μm wide), larger eggs (140–169 μm long by 80–99 μm wide vs. 105 μm long by 55 μm wide), and a club-shaped rather than a coiled seminal vesicle. *Szidatia taiwanensis* differs from *S. joyeuxi,* the type and remaining species in the genus, by having a longer esophagus (67–149 μm compared with 40 μm), ceca that reach only to the level of the anterior testis rather than beyond the posterior testis, an acetabulum that lies between the middle and anterior ⅓ of the forebody rather than exactly in the middle, and oblique rather than tandem testes; the anterior testis is almost entirely within the forebody rather than in the hindbody, the seminal vesicle is club-shaped rather than coiled, and the eggs are larger (140–169 μm long by 80–99 μm wide vs. 100 μm long by 70 μm wide). The minute papillae on the tegument at the posterior end, too small to illustrate to scale, probably serve a sensory function during mating or egg deposition. The intestine, the infection site of the specimen reported by Fischthal and Kuntz (1975), may have resulted from postmortem migration from the gallbladder. A few specimens of other species normally found in the gallbladder occasionally occur in the intestine normally. We do not think the difference in sites based on 1 specimen is significant.

As with the echinostomate specimens of *S. vietnamensis,* the initial specimens of *S. taiwanensis* had been placed in fresh water prior to fixation in unheated formalin (see Fig. 6). Unlike those of *S. vietnamensis,* these specimens were not fixed under any pressure; nevertheless, their measurements and features varied dramatically from those obtained from specimens fixed with heat and used for the above description. Hindbody length was generally shorter (146–506 μm vs. 326–762 μm), and both the anterior and posterior testes occurred entirely in the forebody of specimens exposed to water, whereas the posterior testis was always in the hindbody of heat-killed specimens. The cirrus sac was generally straighter in osmotically stressed specimens and more bent or curled in the region of the pars prostatica in heat-killed specimens. In addition, the tribocytic organ was greatly enlarged (492–650 μm long by 520–685 μm wide vs. 283–447 μm long by 245–423 μm wide) in the stressed specimens. The most important difference due to fixation techniques pertained to the configuration of the vitellarium. In the stressed specimens, vitelline follicles appeared confluent anteriorly (horseshoe-shaped distribution) but were in independent lateral bands in heat-killed specimens. Because the presence or absence of an anterior vitelline confluence differentiates *Gotatea* from *Szidatia,* and the appearance of confluence can be influenced in cold-killed or freshwater-soaked specimens, the methods of fixation clearly have a bearing on taxonomic interpretations.

Discussion

Classification of *Singhiatrema*

The position of *Singhiatrema* within Echinostomatiformes La Rue, 1957, continues to be debated by taxonomists. Ommatobrephinae Poche, 1926, was created for *Ommatobrephus* and *Singhiatrema,* and Parorchiinae Lal, 1936, was included in Ommatobrephidae on the basis of the generic level character of the collar spines (see Simha and Chattopadhyaya, 1967). Singhiatrematinae Simha, 1962, was later created to house *Singhiatrema,* presumably because the author considered the presence of collar spines to be of taxonomic importance at the subfamiliar rather than generic level. These changes resulted in Ommatobrephidae containing Ommatobrephinae, Parorchiinae, and Singhiatrematinae, each containing a single genus. Members of Ommatobrephinae and Singhiatrematinae differ only in the presence or absence of a single row of collar spines. We consider the presence or absence of collar spines to represent a generic feature only and, therefore, consider Singhiatrematinae a junior subjective synonym of Ommatobrephinae. Yamaguti (1971) considered Parorchiinae a subfamily in Philophthalmidae Travassos, 1918. We agree with Yamaguti in returning Parorchiinae to Philophthalmidae because members of that subfamily do not possess characters consistent with Ommatobrephidae. Most notably, parorchiines have tegumental spines, a conspicuous prepharynx, an external seminal vesicle, a spined cirrus, opposite testes that do not diverge anteriorly, and life cycles that involve birds and estuarine molluscs. Although no knowledge of the larval stages of species in *Singhiatrema* or *Ommatobrephus* is available, all known adults of species in these genera infect freshwater or terrestrial reptilian hosts, indicating a freshwater rather than an estuarine life cycle.

Classification of *Szidatia*

Contrary opinions regarding the status of key generic features of *Gogatea* and *Szidatia* have formed the basis for debates over whether the 2 cyathocotylid subfamilies Szidatiinae and Gogatinae Mehra, 1947, should be synonymized (see Dubois, 1938, 1951, 1953; Mehra, 1947; Sudarikov, 1962; Yamaguti, 1971). Both subfamilies contain only a single genus, but the above discussion of *Mesostephanoides* should be noted.

Lutz (1935) erected *Gogatea* and created the combination *Gogatea serpentum* (Gogate, 1932) for *Prohemistomum serpentum* Gogate, 1932, a parasite in the intestine of the colubrid snake *X. piscator* (as *Natrix piscator*) from the Union of Myanmar (as Burma). Lutz (1935) included *Gogatea* in his new subfamily Prohemistominae Lutz, 1935. Szidat (1936) added the new combination *Gogatea joyeuxi* (Hughes, 1929) for the cyathocotylid previously considered *Prohemistomum joyeuxi* (Hughes, 1929) from the colubrid water snake *Natrix natrix scutata* Pallas, 1771 (as *Tropidontus natrix persa*), by Joyeux and Baer (1934). That species developed in the snake when fed diplostomula consistent with diplostomula described by Hughes (1929) from Tunis, Morocco (Joyeux and Baer, 1934). *Gogatea indicum* Mehra, 1947, was subsequently described from *X. piscator* in India. Dubois (1938) observed that the vitellarium of *G. joyeuxi* consisted of 2 distinct rows of vitelline follicles, 1 on either side of the tribocytic organ. The vitellarium of other species in *Gogatea* consists of a confluent arch of follicles, lying dorsal to the tribocytic organ. Dubois (1938) erected *Szidatia* Dubois, 1938, on the basis of the differing configuration of the vitellaria and created the new combination *S. joyeuxi* for *G. joyeuxi.* Dubois (1938) also created the subfamily Szidatinae, emended as Szidatiinae by Yamaguti (1958), to house both *Szidatia* and *Gogatea.* Mehra (1947) did not consider that the difference in vitelline configuration warranted generic distinction and rejected *Szidatia* and therefore Szidatiinae. Instead, he created Gogatinae to contain all species of *Gogatea,* with *G. serpentum* as the type species. Dollfus (1953), Sudarikov (1962), and Yamaguti (1971) all accepted the generic distinctions established by Dubois (1938) and considered Gogatinae a junior synonym for Szidatiinae, retaining *G. serpentum* and *S. joyeuxi* as the type species for their respective genera. We concur with these authors and think that the different configuration of the vitellarium can be used as an informative generic level taxonomic distinction.

Fixation techniques

A wide variety of techniques has been used by parasitologists to fix specimens. Most of those techniques yield satisfactory and consistent results; however, those that produce results inconsistent with other procedures should be avoided. We advocate collecting digeneans by initially placing them live in physiological saline solution (7.7–8.5 g NaCl per liter of distilled water). They should never be exposed to distilled or tap water while alive. Unless specimens are to be used for ultrastructural or other specific purposes, they should be killed rapidly with heat. They can be killed by a flame under a slide with worms in a small amount of saline or by pouring a relatively large volume of hot or boiling saline or tap water over specimens immersed in little or no saline. The specimens then should be transferred, without touching them with forceps, into 5–10% buffered formalin solution soon after being killed. Killing with hot formalin solution or other fixatives is acceptable but produces harsh fumes. Killing with heat produces consistently fixed specimens, ideal for making comparative measurements. Fixation with pressure may be useful for examining certain organ systems such as the female reproductive complex or the terminal genitalia, but specimens fixed under pressure should be used with care for taxonomic purposes because the entire specimen or specific structures may be distorted.

Comparison of heat-killed specimens with specimens bathed in fresh water and then cold-killed revealed differences. Specimens of *S. vietnamensis* that were osmotically stressed prior to fixation were a different shape, they were wider, the pharynx and esophagus were distorted, the ceca were slightly shorter, the testes were larger, and the cirrus sac was oriented differently. Any one of these conditions might be used to misidentify a species, incorrectly describe specimens, or provide misleading information. Stressed specimens of *S. taiwanensis* exhibited some distortions at the specific level, but more importantly, the confluent vitelline follicles represent a generic distinction for *Gogatea*. Lack of knowledge about the methods used on specimens for descriptions of some species of *Gogatea* and *Szidatia* shows the need to reevaluate those species with heat-killed specimens. Until such material is available, we prefer to treat the genera separately.

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Symposium Announcement

PARASITOLOGY IN SCIENCE AND SOCIETY

Sponsored by The Helminthological Society of Washington
Saturday, October 27, 2001, 1:00–5:00 pm
S. Dillon Ripley Center of the South Quadrangle, Room 3111
National Museum of Natural History of the Smithsonian Institution
Washington, D.C.

The 676th meeting of the Helminthological Society of Washington, to be held on October 27, 2001 at the Smithsonian Institution, will be a special event. The Helminthological Society of Washington, in cooperation with The American Society of Parasitologists, will conduct a symposium entitled *Parasitology in Science and Society*. The purpose of the symposium is to assess the state of parasitology as a discipline, clearly define the role of parasitology in science, and explore ways that various parasitological and related societies and governmental agencies can work together to strengthen our discipline. Dr. Richard O'Grady, Executive Director of the American Institute of Biological Sciences, will speak on the power and importance of collaborative efforts in science. Dr. Eric P. Hoberg of the United States Department of Agriculture's Beltsville Agricultural Research Center, will discuss the seminal importance of phylogenetic studies and taxonomic inventories and collections to contemporary parasitology. Additionally, leaders of various parasitological and related societies and governmental agencies are being asked to clearly articulate the missions and visions of their organizations as they relate to parasitology. Because each society and agency is a unique entity, we all have special strengths to offer to our broader discipline. Further, participants are being asked to identify contemporary societal issues which the discipline of parasitology can address, and indicate specific ways that their organization is, and can aid in, addressing these issues. We invite you to make every effort to attend this important meeting of the Helminthological Society of Washington.

INVITED SOCIETIES, AGENCIES, AND ORGANIZATIONS

American Association of Veterinary Parasitologists
American Heartworm Society
American Institute of Biological Sciences
American Society of Parasitologists
American Society of Tropical Medicine and Hygiene
Canadian Zoological Society
Centers for Disease Control and Prevention
Entomological Society of America
Helminthological Society of Washington
National Institutes of Health
National Science Foundation
Society of Nematologists
Society of Protozoologists
United States Department of Agriculture
United States Department of the Interior
Wildlife Disease Association

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Rhabdias ambystomae sp. n. (Nematoda: Rhabdiasidae) from the North American Spotted Salamander *Ambystoma maculatum* (Amphibia: Ambystomatidae)

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ABSTRACT: *Rhabdias ambystomae* sp. n. is described on the basis of specimens found in the lungs and body cavity of the spotted salamander (*Ambystoma maculatum*) from northwestern Wisconsin, U.S.A. The new species differs from *Rhabdias bermani* in tail shape, arrangement of circumoral lips, and position of vulva, from *Rhabdias tokyoensis* in the morphology and size of the buccal capsule and the shape of the esophagus, and from *Rhabdias americanus* in the absence of pseudolabia at the cephalic extremity and the shape of the tail. *Rhabdias ambystomae* sp. n. is the first species of the genus described from salamanders in North America.

KEY WORDS: Nematoda, Rhabdiasidae, *Rhabdias ambystomae* sp. n., salamanders, *Ambystoma maculatum*, Wisconsin, U.S.A.

Nematodes of the genus *Rhabdias* Stiles and Hassall, 1905, are globally distributed lung parasites of amphibians and reptiles. Among amphibian hosts, the vast majority of *Rhabdias* species have been reported from anurans (frogs and toads), whereas only 2 species of the genus have been described from caudatans (salamanders): *Rhabdias bermani* Rausch, Rausch, and Atrashkevich, 1984, from the Siberian newt *Salamandrella keyserlingii* Dybowski, 1870, in the eastern Palearctic (Rausch et al., 1984) and *Rhabdias tokyoensis* Wilkie, 1930, from *Cynops* spp. in Japan (Wilkie, 1930). In North America, *Rhabdias* spp. previously have been found in the lungs and body cavities of several species of salamanders (Lehmann, 1954; Dyer and Peck, 1975; Price and St. John, 1980; Coggins and Sajdak, 1982; Muzzall and Schinderle, 1992; Bolek and Coggins, 1998; Goldberg et al., 1998). These nematodes were identified as either *Rhabdias* sp., *Rhabdias ranae* Walton, 1929, or *Rhabdias joaquinensis* Ingles, 1935, the latter 2 species normally restricted to anuran amphibians.

In the course of investigations of the helminth fauna of Wisconsin amphibians, infections by a species of *Rhabdias* were detected in the lungs and body cavities of 2 specimens of the spotted salamander *Ambystoma maculatum* (Shaw, 1802). Morphological examination revealed these worms to represent a new species of the genus *Rhabdias*. This species is described herein as *Rhabdias ambystomae* sp. n.

Materials and Methods

Amphibians were collected from a roadside wetland near Pigeon Lake, Bayfield County, Wisconsin, U.S.A. A total of 26 gravid and 110 subadult nematodes were found in 2 of 4 *A. maculatum*. Nematodes were fixed in hot formalin and postfixed in 70% ethanol. Prior to light microscopic examination, worms were cleared in glycerol by gradual evaporation from a 5% solution of glycerol in 70% ethanol. Nematodes to be examined with scanning electron microscopy (SEM) were postfixed in ethanol, dehydrated in a graded series of ethanol and acetone, and critical point dried in a Desk II Critical Point Dryer® (Denton Vacuum, Inc., Moorestown, New Jersey, U.S.A.) with CO_2 as the transition fluid. The specimens were mounted on stubs, coated with gold, and examined with a Hitachi 2460N® scanning electron microscope (Hitachi USA, Mountain View, California, U.S.A.) at an accelerating voltage of 10–15 kV.

Five specimens of *R. bermani* from *S. keyserlingii* collected in Magadanskaya Region, Russia, 10 specimens of *R. tokyoensis* from the brown newt *Cynops ensicauda* (Hallowell, 1860) collected on Okinawa Island, Japan, 20 specimens of *R. ranae* from the northern leopard frog *Rana pipiens* (Schreber, 1782) collected in Wisconsin, U.S.A., and 18 specimens of *Rhabdias americanus* Baker, 1978, from the American toad *Bufo americanus* Holbrook, 1836, collected in Wisconsin, U.S.A. were examined by light microscopy and measured after being cleared as above. All measurements are given in micrometers unless otherwise stated. Measurements are given for the holotype followed by minimum and maximum measurements of paratypes in parentheses.

[4] Corresponding author.

Table 1. Measurements taken from gravid *Rhabdias ambystomae* sp. n. (type series; *n* = 18) (measurements in micrometers unless otherwise noted).

Character	Holotype	Paratypes (mean [min.–max.])
Body length (mm)	12.6	10.5 (6.8–13.0)
Body width	350	342 (210–430)
Buccal capsule depth	15	14 (12–15)
Buccal capsule width	17	17 (15–17)
Width of esophagus, anterior end	45	43 (40–45)
Width of esophagus, muscular region	50	54 (45–67)
Minimum width of esophagus, glandular region	57	59 (42–67)
Esophageal bulb width	90	85 (65–100)
Distance, anterior end of esophagus to nerve ring	160	159 (130–180)
Distance, anterior end of esophagus to nerve ring (as % of esophagus length)	28.6	28.9 (22.8–34.6)
Esophagus length	560	546 (450–590)
Esophagus length (as % of body length)	4.5	5.4 (4.1–7.4)
Distance from anterior end to vulva (mm)	7	5.8 (3.8–7.4)
Distance from anterior end to vulva (as % of body length)	56	55 (47.4–58.2)
Tail length	210	236 (190–300)
Tail length (as % of body length)	1.7	2.3 (1.6–3.1)

Results

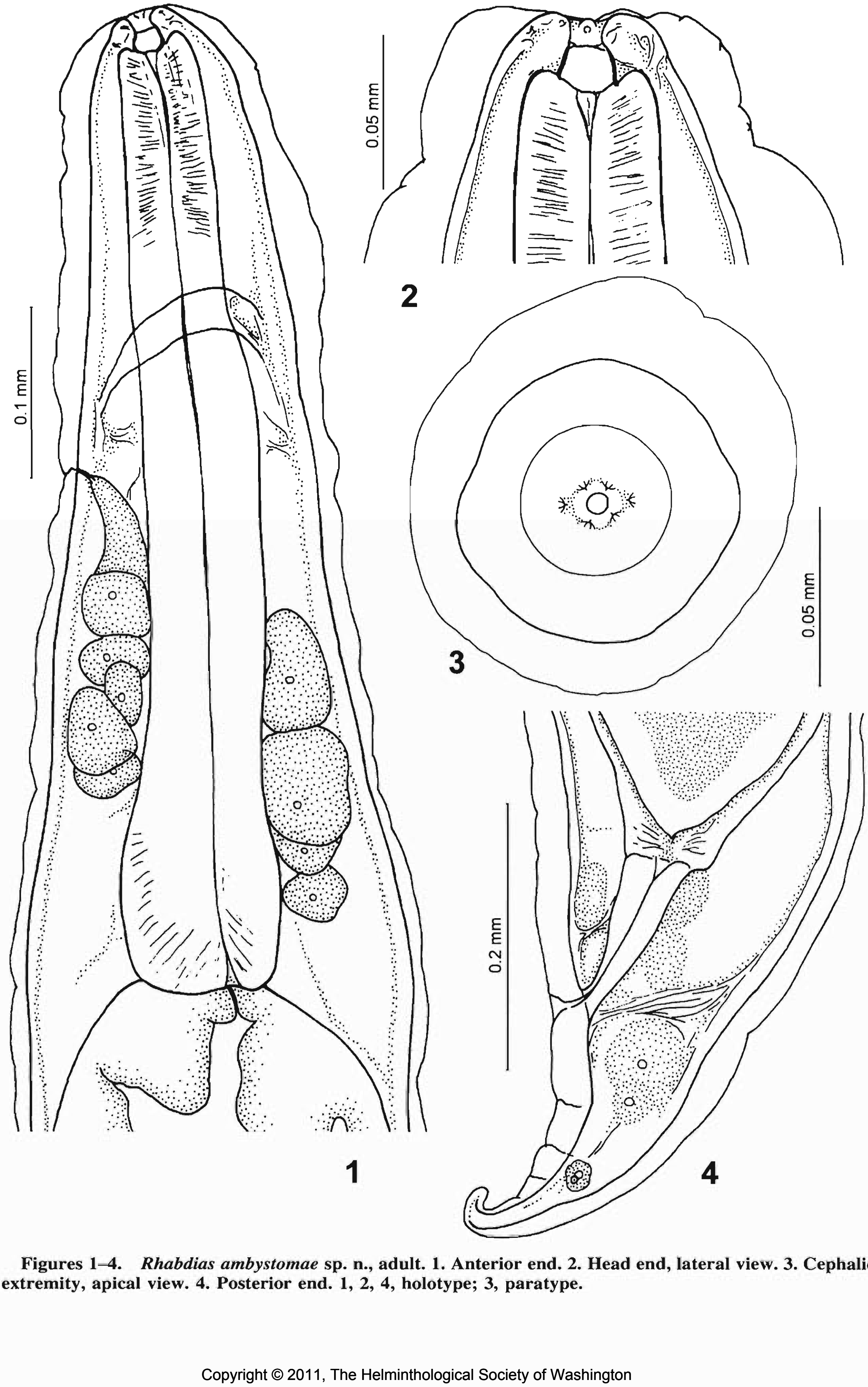
Rhabdias ambystomae sp. n. (Figs. 1–15)

Description

Because both gravid and subadult nematodes were found in the same host specimens, each of these stages is described separately.

GRAVID SPECIMENS (Table 1): Body elongated 12.6 (6.8–13.0) mm long, 350 (210–430) wide. Anterior end rounded, posterior end tapered. Body cuticle swollen, especially on anterior and posterior thirds of body. Irregularly arranged transverse folds formed by cuticular surface. Round oral opening surrounded by 6 small lips, each bearing 1 elongated conical inner papilla and 2 minute outer papillae. Inner papillae directed toward oral opening. Flat cuticular ring separating each lip from edge of oral opening. Buccal capsule cup-like in lateral view, round in apical view. Buccal capsule depth 15 (12–15), width 17 (15–17). Esophagus club-shaped, 560 (450–590) in length, with short anterior muscular portion and long posterior glandular portion. Nerve ring at level of border between muscular and glandular regions of esophagus, 160 (130–180) from esophagus anterior end. Large optically dense hypodermal cells prominent along glandular region of esophagus. Excretory glands indistinct, excretory duct short. Two large anterior coelomocytes situated between posterior end of esophagus and loop of anterior genital limb. Intestine thick, filled with brown or black contents. Intestinal walls thinner in posterior than in anterior region of body. Muscular sphincter between intestine and rectum present. Rectum lined with thick cuticle. Tail wide, conical, 210 (190–300) in length. Vulva usually postequatorial with indistinct lips. Genital system amphidelphic. Ovaries straight or slightly twisted, lying along intestine. Proximal regions of both ovaries overlap level of vulva. Both limbs of genital system bend backward at level of oviducts. Anterior oviduct occasionally forms 2 loops as it bends. Seminal receptacles short, thick walled. Uteri wide, thin walled, filled with numerous eggs. Egg size 112–130 × 55–65.

SUBADULT SPECIMENS (Table 2): Body length 4.15 (3.3–4.8) mm, width 111 (100–120). Anterior end rounded, posterior end tapered. Body cuticle thin and smooth, slightly swollen at anterior and posterior ends. Head structures similar to those in gravid worms. Lateral lips situated farther from oral opening than submedian lips. Buccal capsule and esophagus shapes similar to those in adults. Buccal capsule 12 (10–12) deep, 17 (15–17) wide. Esophagus 432 (400–460) long. Two elongated narrow excretory glands stretch from posterior edge of nerve ring to anterior end of intestine. Pair of coelomocytes situated subventrally, close to anterior limb of genital system. Intestine thick, reddish. Rectum sclerotized. Tail elongated, 171 (150–190) in length. Bulbous projection of body wall slightly posteriad to anal opening. Vulva postequatorial. Vulva lips indistinct. Genital system



Figures 1–4. ***Rhabdias ambystomae*** **sp. n., adult. 1. Anterior end. 2. Head end, lateral view. 3. Cephalic extremity, apical view. 4. Posterior end. 1, 2, 4, holotype; 3, paratype.**

Table 2. Measurements of *Rhabdias ambystomae* sp. n. subadult specimens (n = 15) (measurement in micrometers unless otherwise noted).

Character	Mean	Minimum	Maximum
Body length (mm)	4.2	3.3	4.9
Body width	111	100	120
Buccal capsule depth	12	10	12
Buccal capsule width	17	15	17
Width of esophagus, anterior end	37	35	40
Width of esophagus, muscular region	39	35	42
Minimum width of esophagus, glandular region	42	37	47
Esophageal bulb width	57	50	60
Distance, anterior end of esophagus to nerve ring	149	110	170
Distance, anterior end of esophagus to nerve ring (as % of esophagus length)	34.4	26.8	37.8
Esophagus length	432	400	460
Esophagus length (as % of body length)	10.5	8.8	12.0
Distance from anterior end to vulva (mm)	2.4	1.9	2.8
Distance from anterior end to vulva (as % of body length)	57.4	54.4	61.8
Tail length	171	150	190
Tail length (as % of body length)	4.1	3.5	5.0

completely developed, but eggs absent. Proximal regions of gonads overlap level of vulva. Each gonad forms a single loop as it bends. Uteri narrow, lacking eggs.

Taxonomic summary

TYPE HOST: Spotted salamander *Ambystoma maculatum* (Shaw, 1802).

TYPE LOCALITY: Roadside wetland near Pigeon Lake, Bayfield County, Wisconsin, U.S.A.; 46°20′84″N, 91°20′58″W.

SITES OF INFECTION: Lungs, body cavity.

TYPE SPECIMENS: The type series consists of the gravid specimens only. Holotype: U.S. National Parasite Collection, Beltsville, Maryland, U.S.A., USNPC 90869. Paratypes: USNPC 90870 (9 specimens); Department of Parasitology, Institute of Zoology, Kiev, Ukraine, Vial N 847 (8 specimens).

ETYMOLOGY: The new species is named in reference to the generic name of its type host.

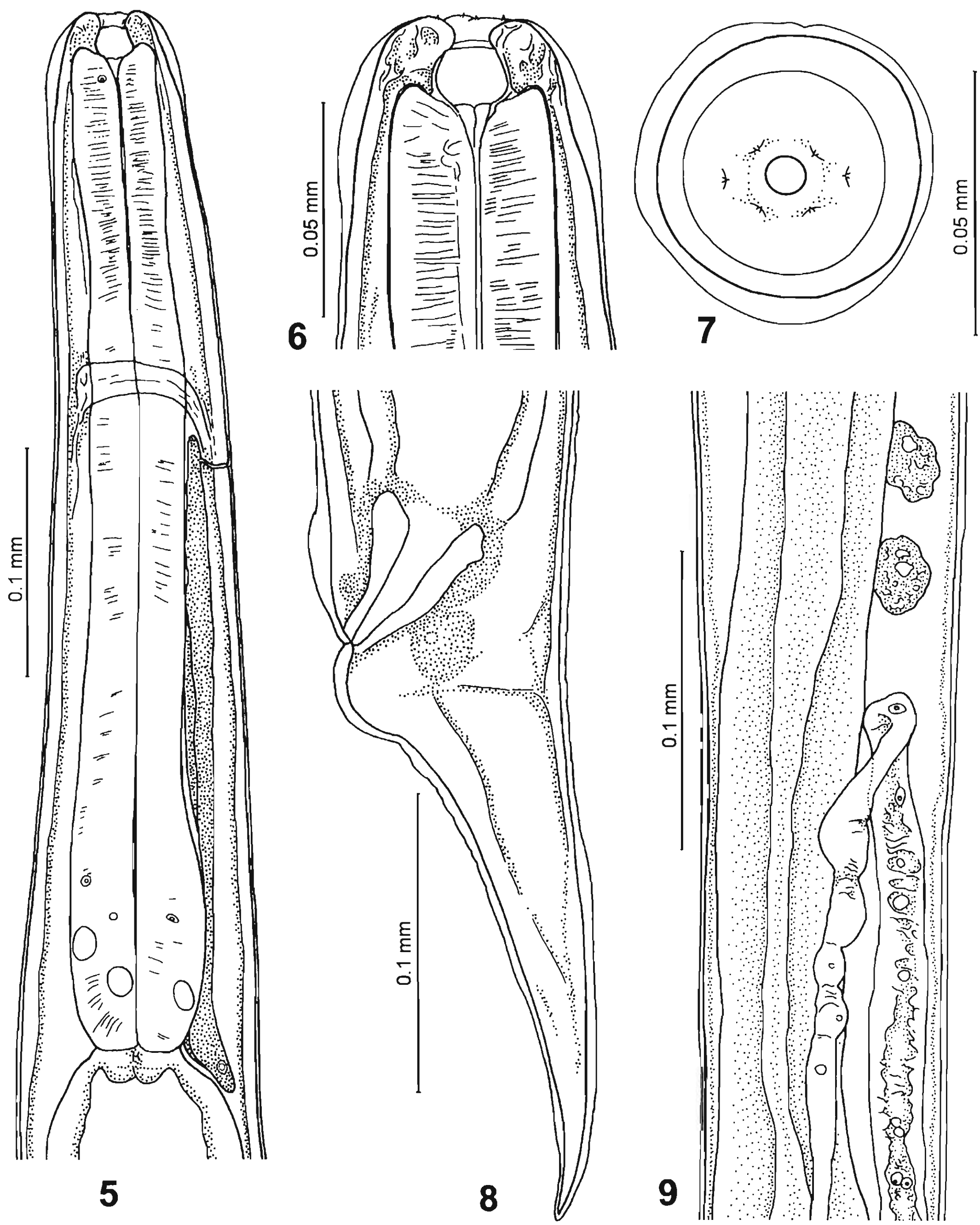
PREVALENCE AND INTENSITY: Two of 4 specimens of spotted salamander; 20–116 specimens of *R. ambystomae* (5–21 adult nematodes in lungs and 15–95 subadults in body cavity).

Remarks and Discussion

Rhabdias ambystomae sp. n. is most similar morphologically to *R. bermani* and *R. tokyoensis,* the only other species of *Rhabdias* described from salamanders. The 3 species are similar in body size and shape and in egg size (Table 3). *Rhabdias ambystomae* sp. n. differs from *R. bermani* in the absence of a lancet-like cuticular swelling of the posterior extremity characteristic of the latter species. In *R. bermani,* the circumoral lips are arranged in 2 lateral groups; in each group, the lateral lip is situated closer to the oral opening than are the submedian lips (Rausch et al., 1984). In contrast, the lateral lips of *R. ambystomae* sp. n. are situated farther from the oral opening than are the submedian lips, a character more prominent in subadult worms than in adults (Figs. 3, 7). Additionally, the vulva of *R. ambystomae* sp. n. is usually postequatorial, whereas it is equatorial in *R. bermani* (Rausch et al., 1984). The most apparent differences between *R. ambystomae* sp. n. and *R. tokyoensis* are the markedly smaller buccal capsule and narrower esophagus of *R. ambystomae* (Table 3).

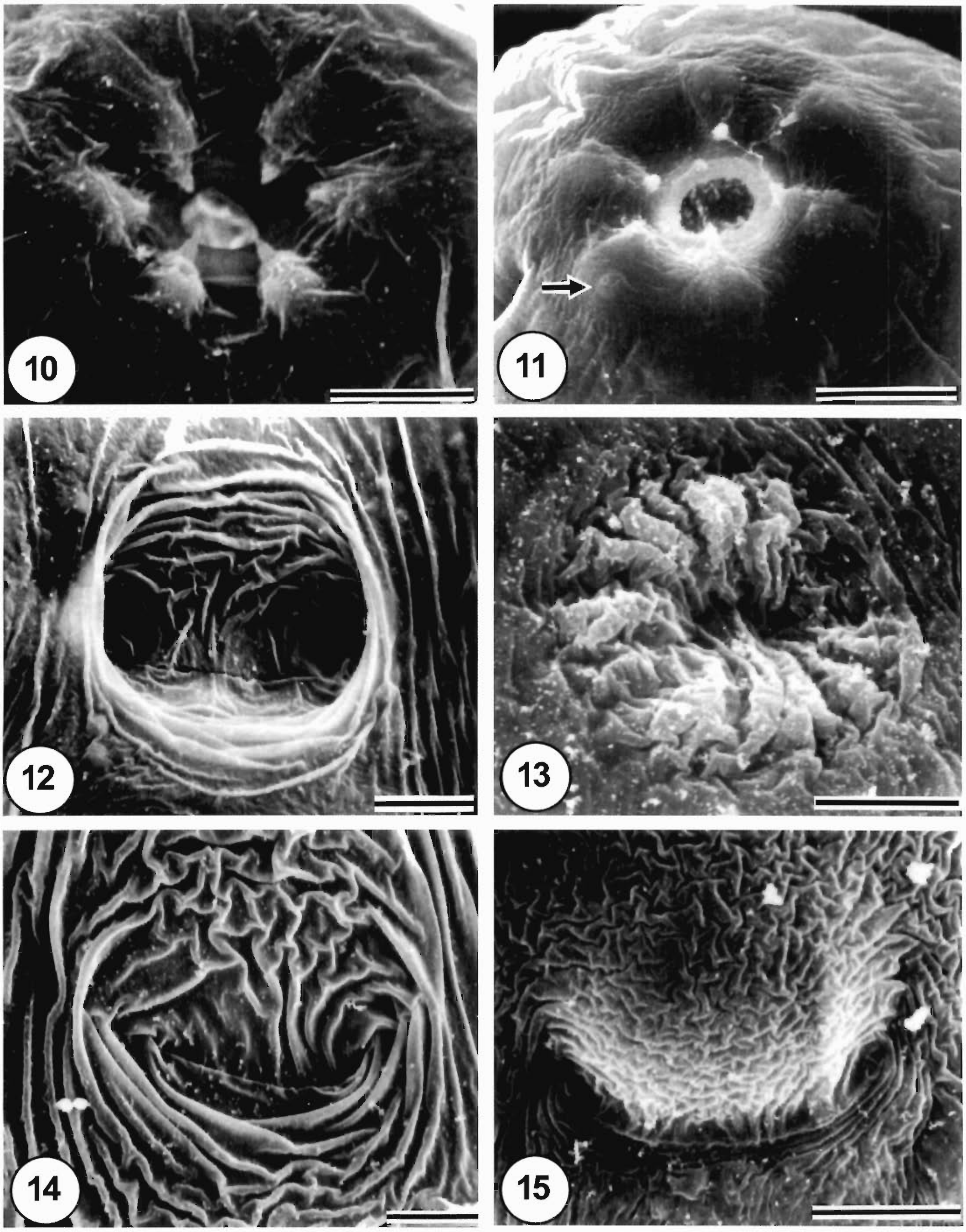
Rhabdias ambystomae sp. n. can be distinguished readily from 2 *Rhabdias* species common in North American amphibians, *R. americanus* and *R. ranae,* by the absence of lateral pseudolabia on the cephalic end. The presence and shape of pseudolabia in *R. americanus* and *R. ranae* were well documented by Baker (1978) and confirmed by examination of specimens collected as part of the present study. In addition, *R. americanus* has a more elongated tail (cf. Baker, 1978, fig. 3) compared with that of *R. ambystomae* sp. n.

Parasitic nematodes of the genus *Rhabdias* have been reported in several species of salamanders from the midwestern U.S.A. Price and St. John (1980) reported finding an undeter-



Figures 5–9. ***Rhabdias ambystomae*** **sp. n., subadult. 5. Anterior end. 6. Head end, lateral view. 7. Cephalic extremity, apical view. 8. Posterior end. 9. Anterior loop of genital system and coelomocytes.**

mined species of *Rhabdias* in the smallmouth salamander *Ambystoma texanum* (Matthes, 1855) from Illinois. Adult specimens were found in the lungs of the host, whereas "larval" (=subadult) nematodes were found in the body cavity. Bolek and Coggins (1998) found undetermined subadult *Rhabdias* in the body cavity of the lungless red-backed salamander *Pletho-*



Figures 10–15. External morphology of *Rhabdias ambystomae* sp. n. 10. Cephalic extremity of adult. 11. Cephalic extremity of subadult; note amphid marked with an arrow. 12. Vulva of adult. 13. Vulva of subadult; note obliteration of the female genital opening at this stage. 14. Anus of adult. 15. Anus of subadult. Scale bars = 10μm.

Table 3. Comparison of characters in *Rhabdias ambystomae* sp. n., *Rhabdias bermani* Rausch, Rausch, and Atrashkevich, 1984, and *Rhabdias tokyoensis* Wilkie, 1930 (measurements in micrometers unless otherwise noted).

Character	*R. ambystomae*	*R. bermani*		*R. tokyoensis*		
	This study	After Rausch et al. (1984)	Original data, 5 specimens	After Wilkie (1930)	After Yamaguti (1935)	Original data, 10 specimens
Body length (mm)	6.8–13.0	6.1–10.7	8.4–10.0	12	8.4–11.7	12.5–17.2
Maximum body width	210–430	294–458	440–548	520	370–500	506–664
Distance to vulva (as % of body length)	47.4–58.2	50.4*	49.7–50.7	53.3	50.0–55	50.8–55.8
Buccal capsule depth	12–15	—	10–12	28	16–27	16–20
Buccal capsule width	15–17	—	16	25	21–27	30–34
Width of esophagus, anterior end	40–45	—	36–40	—	—	54–64
Width of esophagus, muscular region	45–67	—	—	—	—	68–74
Minimum width of esophagus, glandular region	42–67	—	—	—	—	66–80
Esophageal bulb width	65–100	60–107	86–100	—	—	96–132
Esophagus length (as % of body length)	4.1–7.4	—	4.8–5.5	5.6	—	3.2–5.3
Tail length (as % of body length)	1.6–3.1	—	1.9–2.9	2.3	—	1.0–1.6
Egg size	112–130 × 55–65	99–130 × 43–60	—	11 × 50	99–126 × 48–62	—

* Mean.

don cinereus (Green, 1818) from collecting sites within a few kilometers of where *R. ambystomae* sp. n. was collected as part of the present study (J. R. Coggins, University of Wisconsin, Milwaukee, personal communication). Oddly enough, no *Rhabdias* were found in *A. maculatum* collected by Bolek and Coggins (1998) from the same area. This information suggests that *R. ambystomae* sp. n. may also parasitize *P. cinereus* in northern Wisconsin but cannot reach maturity in this lungless amphibian. Further examinations will be necessary for confirmation.

Other authors identified lung nematodes collected from salamanders in Wisconsin and Michigan as *R. ranae* (Coggins and Sajdak, 1982; Muzzall and Schinderle, 1992). This species has been recorded in a number of North American anurans, most of which belong to the family Ranidae (Baker, 1978; Yoder and Coggins, 1996; Bursey and DeWolf, 1998). In our opinion, the determination of the material from salamanders as *R. ranae* is questionable because of the high level of host specificity demonstrated by most representatives of *Rhabdias*; members of this genus have never been found to parasitize hosts from more than a single order (Rausch et al., 1984). Similarly, the report by Goldberg et al. (1998) of a frog parasite, *Rhabdias joaquinensis* Ingles, 1935, from salamanders in California may also represent a misidentification and is in need of confirmation.

Rhabdias ambystomae sp. n. is the first species of this genus described from North American salamanders. The rich salamander fauna of North America and the strict specificity of rhabdiasids to their hosts indicate that further studies of material from the field or museum collections may reveal the presence of more species of *Rhabdias* unique to the salamanders of the New World.

Acknowledgments

We thank Dr. Gennadiy Atrashkevich, who kindly provided several specimens of *S. keyserlingii* for our investigation, and Dr. Hideo Hasegawa for the loan of *R. tokyoensis*. We are grateful to Dr. Robert Wise for assistance with SEM. Collection of amphibians in Wisconsin was conducted under a permit provided by the Wisconsin Department of Natural Resources. This research was supported by a grant from the Vander Putten International Fund of the University of Wisconsin Oshkosh.

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Supplemental Diagnosis of *Myxobolus gibbosus* (Myxozoa), with a Taxonomic Review of Myxobolids from *Lepomis gibbosus* (Centrarchidae) in North America

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ABSTRACT: *Myxobolus gibbosus* Herrick, 1941 (Myxosporea) is reported from the connective tissue of gills of *Lepomis gibbosus* (Centrarchidae) in Algonquin Park, Ontario, Canada. The new material (formalin-preserved) is used to supplement the original taxonomic diagnosis of nearly 60 yr ago. Spores are round to oval in valvular view, 11–14 μm long and 10–11 μm wide, with a distinctly blunt capsular region. The polar capsules are relatively large for the size of the spore, measuring 6–7 μm long and 3.5–4.0 μm wide, and aligned almost parallel to each other. There are 8–12 loose filament coils lying up to 45° to the long axis of the capsule. The taxonomy of species of *Myxobolus* described or reported from *L. gibbosus* in North America is examined, and the following are considered to be valid taxa: *Myxobolus dechtiari* Cone and Anderson, 1977; *M. gibbosus*; *Myxobolus magnaspherus* Cone and Anderson, 1977; *Myxobolus osburni* Herrick, 1936; *Myxobolus paralintoni* Li and Desser, 1985; and *Myxobolus uvuliferus* Cone and Anderson, 1977. Comparative photographs of spores accompany differential diagnoses of the 6 species. *Myxobolus gibbosus* Li and Desser, 1985, and *Myxobolus lii* Desser, 1993, are junior synonyms of *M. uvuliferus*. *Myxobolus lepomicus* Li and Desser, 1985, is considered a *species inquirendae,* and the reports of *Myxobolus cyprinicola* Reuss, 1906, and *Myxobolus poecilichthidis* Fantham, Porter, and Richardson, 1939, from *L. gibbosus* are considered misidentifications.
KEY WORDS: *Myxobolus gibbosus,* Myxosporea, redescription, differential diagnoses, pumpkinseed sunfish, *Lepomis gibbosus,* Centrarchidae, Algonquin Park, Canada.

Myxobolus gibbosus Herrick, 1941 (Myxozoa) was described from connective tissue of the gill arch of pumpkinseed sunfish (*Lepomis gibbosus* (Linnaeus, 1758)) from the island region of western Lake Erie (Herrick, 1941). In subsequent surveys of myxosporean parasites of pumpkinseed (Cone and Anderson, 1977a, b; Li and Desser, 1985; Hayden and Rogers, 1997), the parasite was not encountered. However, during a new survey of myxosporeans of fish in Algonquin Park, a single pseudocyst of *M. gibbosus* was discovered. This rare find enabled the author to assess information provided in the original species description and to critically compare the parasite with other species of the genus reported from pumpkinseed. The present study describes the new material and reviews the taxonomy of myxobolids from pumpkinseed in North America.

Materials and Methods

Nine pumpkinseed (6–8.9 cm in total length) were collected in baited trapnets set 20 June 1994 and 21 June 1995 in the shallows of Lake Sasajewan (45°35′N; 78°30′W), Algonquin Park, Ontario, Canada. The fish were pithed and necropsied. All body organs and tissues were examined microscopically for myxosporean pseudocysts, and, when found, they were fixed in 10% buffered formalin. Fixed pseudocysts were punctured and the spore contents stabilized in temporary mounts prepared with 1% agar (Lom, 1969). Spores were photographed with interference contrast optics. Enlarged photographic prints of individual spores were used to determine spore dimensions. Descriptive terminology follows Lom and Dyková (1992). Measurements are presented in micrometers. The sample of *M. gibbosus* was compared with other species of *Myxobolus* in the author's collection, namely *Myxobolus dechtiari* Cone and Anderson, 1977; *Myxobolus magnaspherus* Cone and Anderson, 1977; *Myxobolus osburni* Herrick, 1936; *Myxobolus paralintoni* Li and Desser, 1985; and *Myxobolus uvuliferus* Cone and Anderson, 1977. Syntype slides of *M. gibbosus* (NMCICP 1984-0359), *Myxobolus lepomicus* (NMCICP 1984-0362), and *M. paralintoni* (NMCICP 1984-0364) housed in the parasite collection of the Canadian Museum of Nature were also examined. A photo-voucher (negative film) is deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A.

Results

Myxobolus gibbosus Herrick, 1941 (Figs. 1 and 2)

Supplementary diagnosis

Pseudocyst egg-shaped, gray-white and minute (250 long), embedded in connective tissue surrounding base of gill arch. Spores round to

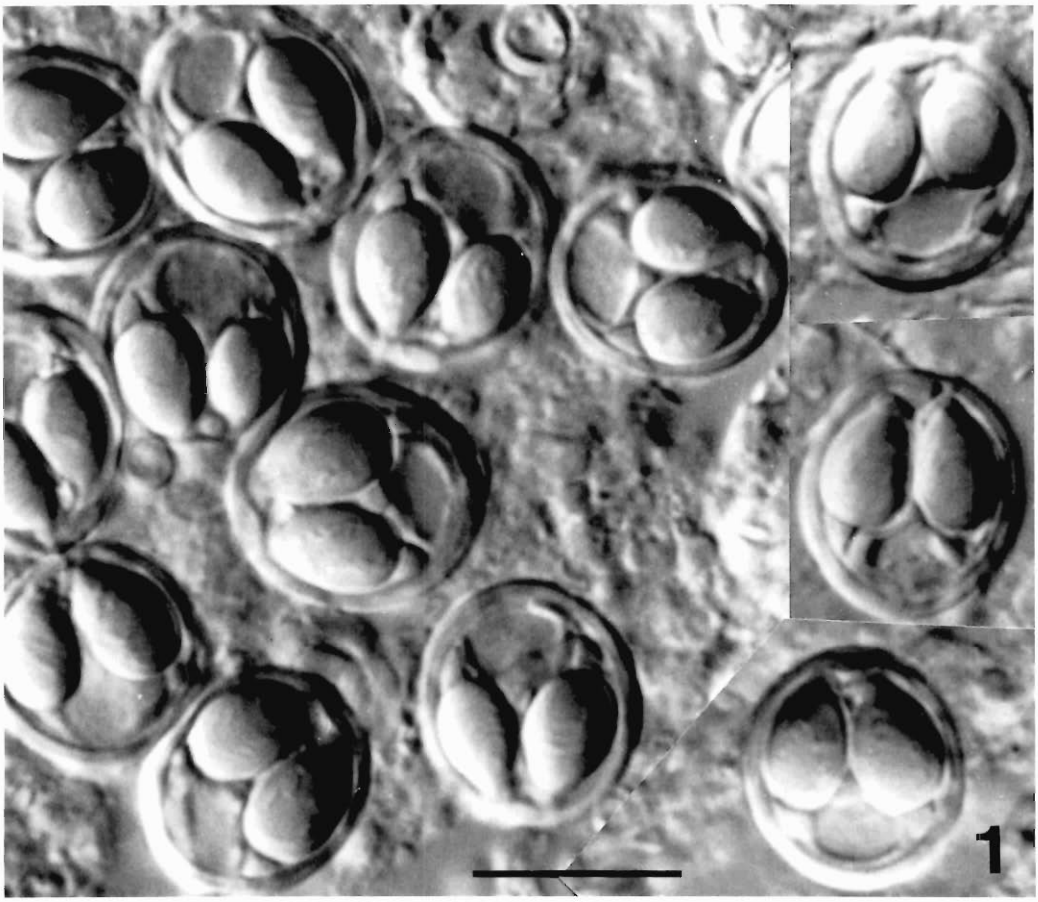


Figure 1. Spores of *Myxobolus gibbosus*. Scale bar = 10 μm.

oval in valvular view, with blunt capsular edge. Spores 11.8 ± 0.9 (11–14, n = 10) long and 10.6 ± 0.5 (10–11) wide. Width-to-length ratio 1:1.09 ± 0.08 (1.05–1.3). Polar capsules oval, 6.8 ± 0.3 (6–7) long and 4.0 ± 0.3 (3.5–4) wide, aligned almost parallel to each other. Polar filaments in 8–12 loose coils, lying up to 45° to long axis of capsule. Capsulogenic nuclei prominent, triangular. Shallow intercapsular appendix evident in some spores. Sutural ridge thin and smooth.

Taxonomic summary

HOST: Pumpkinseed sunfish (*Lepomis gibbosus*) (Centrarchidae); total length 6.2 cm, 1+ yr old.

LOCALITY/COLLECTION DATE: Lake Sasajewan, Algonquin Park, Ontario, Canada (45°35′N; 78°30′W), 20 June 1994.

SITE OF INFECTION: Connective tissue of gill arch.

PREVALENCE AND INTENSITY OF INFECTION: One of 9 fish infected with 1 pseudocyst.

SPECIMENS DEPOSITED: Photo-voucher USNPC No. 091157.00.

Remarks

Myxobolus gibbosus has not been reported in surveys of myxozoans in *L. gibbosus* from Algonquin Park (Cone and Anderson 1977a, b; Li and Desser, 1985). It was probably not overlooked, for the parasite has several distinct diagnostic features. It forms small but obvious pseudocysts in the connective tissue of the gill arch and produces round spores with a blunt capsular end. The polar capsules are relatively large, the length being about half the length of the spore, and they are arranged almost parallel

Table 1. Comparison of pertinent taxonomic information about *Myxobolus gibbosus* reported in the original species description and that observed in the present study.

	Herrick (1941)*	Present study†
Host	*Lepomis gibbosus*	*Lepomis gibbosus*
Locality	Lake Erie	Lake Sasajewan
Tissue site	Connective tissue of gill	Connective tissue of gill
Pseudocyst	Round, 0.75 mm	Round, 0.25 mm
Spore length	10.6–12.3	11–14
Spore width	9.8–12.3	10–11
Spore thickness	6.5–8.2	—
Polar capsule length	5.7–7.4	6–7
Polar capsule width	3.3–4.1	3.5–4
Polar filament coils	8–12	8–11

* Based on fresh material in a hanging drop preparation.
† Based on formalin-fixed material in agar wet mounts.

to each other. It appears then that *M. gibbosus* is simply rare in this region. The dimensions of the preserved spores found in the present study are similar to those described by Herrick (1941) from fresh material (Table 1). It should be noted that dimensions of fixed spores are often smaller than those of fresh spores because shrinkage can take place during fixation. This means that fresh spores of *M. gibbosus* in Algonquin Park may be slightly larger than those described originally by Herrick (1941).

Spores of other species of *Myxobolus* (*M. dechtiari, M. magnaspherus, M. osburni, M. paralintoni,* and *M. uvuliferus*) from *L. gibbosus* are presented for comparative purposes (Figs. 3–7). Each species has a distinct spore shape and specific tissue site in which it develops and is readily identified by these indicators. *Myxobolus paralintoni* (Fig. 4) has oval spores in frontal view and develops in the bulbus arteriosus of the heart (Hayden and Rogers, 1997; Cone and Overstreet, 1998). *Myxobolus dechtiari* (Fig. 5) has spores that are broadly pyriform in frontal view and develops in gill tissue (Cone and Anderson, 1977a). *Myxobolus uvuliferus* has slightly compressed spores in frontal view usually with the width greater than length, often has polar capsules dissimilar in the length, and develops in the connective tissue capsule surrounding the metacercaria of *Uvulifer ambloplites* (Hughes, 1927) Dubois, 1938 (Cone and Anderson, 1977a). *Myxobolus osburni* has round spores in frontal view and develops in the exocrine tissue of the pancreas (Cone and Anderson, 1977a). *Myxobolus magnaspherus* has round spores in frontal view that are huge, often 20 μm in diameter, and develops in connective tissue of the body, including the peritoneum (Cone and Anderson, 1977a).

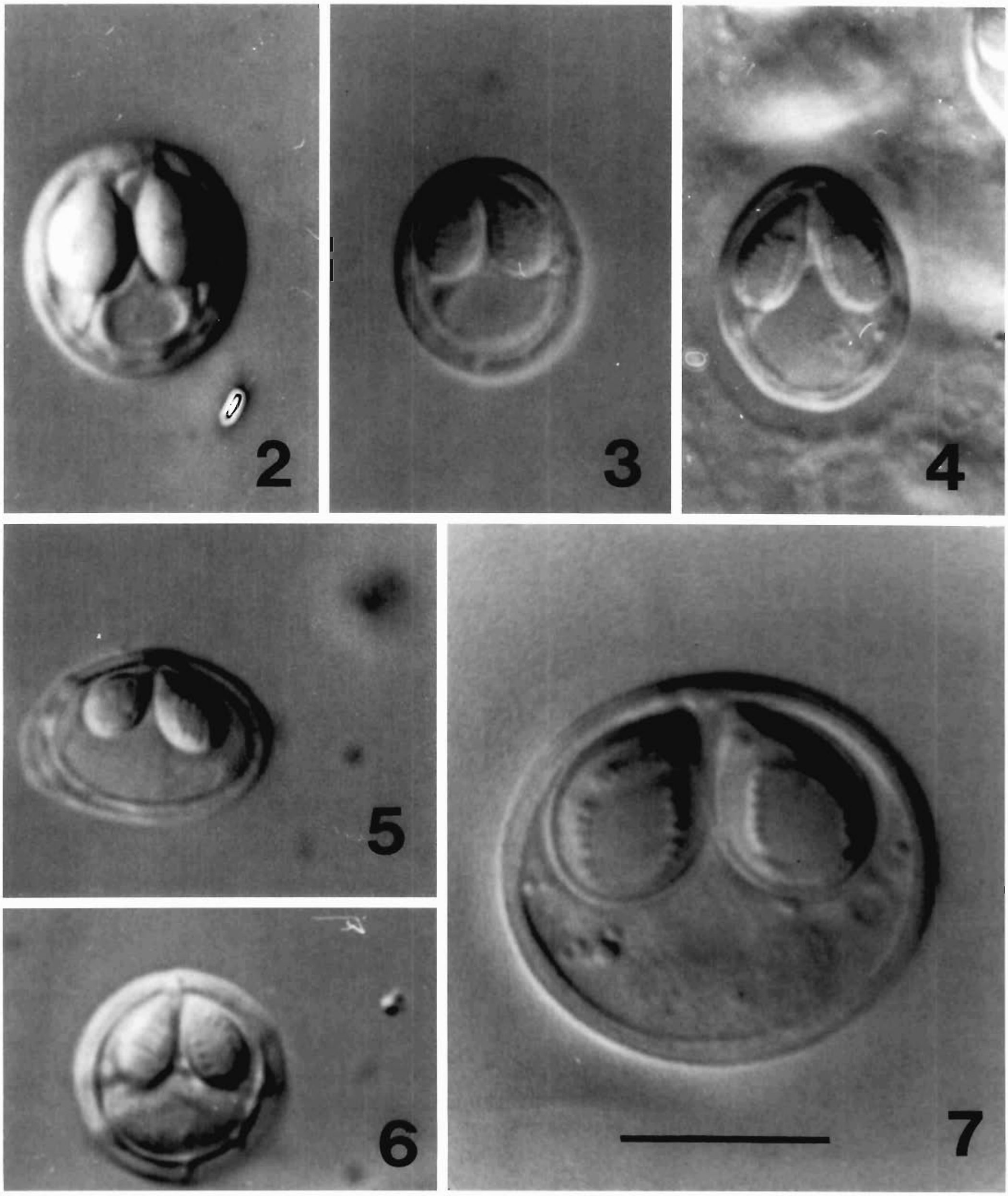
Taxonomic Key to the Species of *Myxobolus* Infecting Pumpkinseed Sunfish

1a. Spore length more than 16 μm .... *M. magnaspherus* (Fig. 8)
1b. Spore length less than 16 μm 2
2a. Polar capsules aligned more or less parallel .... *M. gibbosus* (Fig. 9)
2b. Polar capsules converged anteriorly 3
3a. Spore circular in frontal view .... *M. osburni* (Fig. 10)
3b. Spore not circular in frontal view 4
4a. Spore width greater than spore length .... *M. uvuliferus* (Fig. 11)
4b. Spore width less than spore length 5
5a. Spore oval in frontal view .... *M. paralintoni* (Fig. 12)
5b. Spore broadly pyriform in frontal view .... *M. dechtiari* (Fig. 13)

Discussion

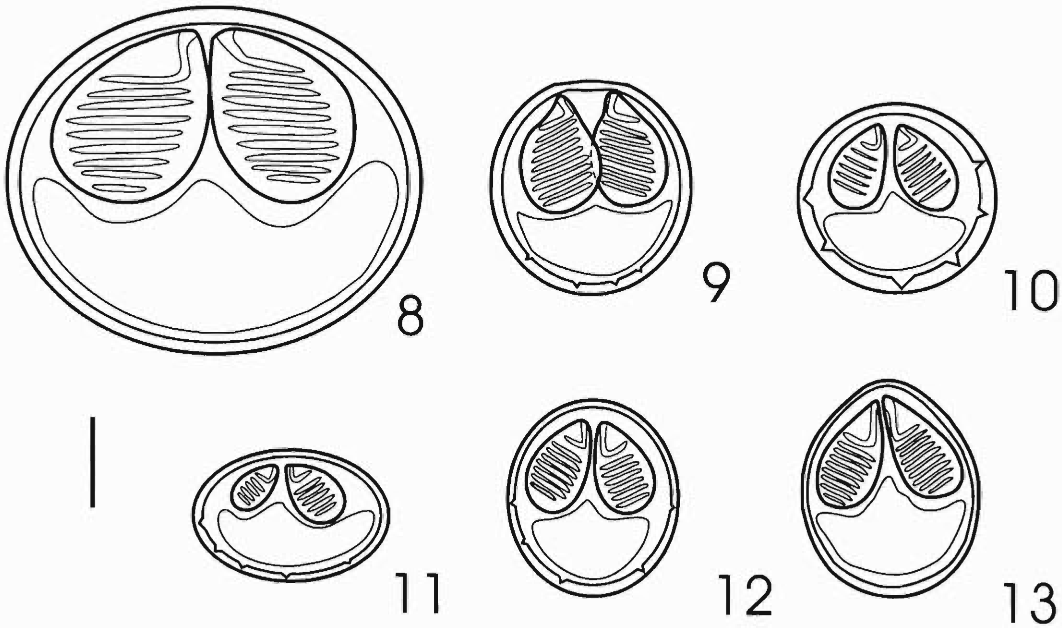
Ten species of *Myxobolus* Bütschli, 1882 (Myxosporea) have been reported from *L. gibbosus* in North America (Herrick, 1936, 1941; Cone and Anderson, 1977a, b; Ingram and Mitchell, 1982; Li and Desser, 1985; Desser, 1993; Cone and Overstreet, 1998). The author has necropsied *L. gibbosus* from Algonquin Park and from Lake Erie and has to date encountered 6 of the 10 species, namely *M. dechtiari, M. gibbosus, M. magnaspherus, M. osburni, M. paralintoni,* and *M. uvuliferus.*

The reports of *Myxobolus cyprinicola* Reuss, 1906, and *Myxobolus poecilichthidis* Fantham, Porter, and Richardson, 1939, from the brain and heart and from the gills, respectively, of *L. gib-*



Figures 2–7. Photographs of spores in frontal view of species of *Myxobolus* known to parasitize *Lepomis gibbosus* in North America. 2. *Myxobolus gibbosus* (formalin-preserved). 3. *Myxobolus paralintoni* (formalin-preserved). 4. *Myxobolus dechtiari* (formalin-preserved). 5. *Myxobolus uvuliferus* (formalin-preserved). 6. *Myxobolus osburni* (formalin-preserved). 7. *Myxobolus magnaspherus* (fresh spore). Scale bar = 10 μm and applies to all figures.

bosus in Algonquin Park (Li and Desser, 1985) are considered misidentifications. Both of these species have similarities in tissue site and spore morphology to *M. dechtiari* and *M. paralintoni* and could have easily been confused with them. Li and Desser (1985) were apparently unaware of the study by Cone and Anderson (1977a) in nearby Ryan Lake.



Figures 8–13. Drawings of spores in frontal view of species of *Myxobolus* known to parasitize *Lepomis gibbosus* in North America. 8. *Myxobolus gibbosus*. 9. *Myxobolus paralintoni*. 10. *Myxobolus dechtiari*. 11. *Myxobolus uvuliferus*. 12. *Myxobolus osburni*. 13. *Myxobolus magnaspherus*. Scale bar = 5 μm and applies to all figures.

The type material of *M. lepomicus* Li and Desser, 1985, described from a variety of organs of *L. gibbosus*, has deteriorated, and spores are not to be found on the slide. The species description includes a schematic drawing of the spore. Until additional samples are obtained the species is considered a species inquirendae.

Myxobolus gibbosus Li and Desser, 1985, is a homonym of *M. gibbosus* Herrick, 1941. Desser (1993) proposed *Myxobolus lii* as a nomen novum to replace *M. gibbosus* Li and Desser, 1985. However, Landsberg and Lom (1991) considered *M. gibbosus* Li and Desser, 1985, to be a junior synonym of *M. uvuliferus*, and thus both *M. gibbosus* and *M. lii* become junior synonyms of *M. uvuliferus*. The report by Hoffman (1998) that *M. gibbosus* Li and Desser, 1985, is a junior synonym of *M. osburni* cannot be supported on the basis of spore shape.

The 6 confirmed species of *Myxobolus* mentioned above are known to parasitize *L. gibbosus* or related centrarchid fishes in North America. *Myxobolus magnaspherus* and *M. paralintoni* have been found in redear sunfish (*Lepomis microlophus* (Günther, 1859)) in Mississippi, U.S.A. (D. K. Cone, Saint Mary's University, and R. M. Overstreet, Gulf Coast Research Laboratory, unpublished data) and redbreast sunfish (*Lepomis auritus* (Linnaeus, 1758)) in Maryland, U.S.A. (Hayden and Rogers, 1997), respectively. *Myxobolus osburni* has been reported (Herrick, 1936; Otto and Jahn, 1943) from bluegill sunfish (*Lepomis macrochirus* Rafinesque, 1819), smallmouth bass (*Micropterus dolomieu* Lacépède, 1802), and black crappie (*Pomoxis nigromaculatus* (Lesueur, 1829)). The genus clearly has undergone a diverse radiation in these hosts, and it is of ecological interest that all 6 species are found in *L. gibbosus* in Algonquin Park and that all occupy distinct and very specific tissue sites in this host species.

Acknowledgments

The research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Research Grant awarded to the author. Thanks are extended to the staff of the Harkness Research Laboratory for their help and hospitality and to Sherwin Desser, University of Toronto, for providing constructive comments on a draft of the paper.

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2001–2002 MEETING SCHEDULE OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

27 October 2001	Symposium—Parasitology in Science. 1:00 PM. Smithsonian Institution, Quad Building, (Room 3111), Washington, DC (Contact Persons: Bill Moser, 202-357-2473 or Dennis Richardson, 203-582-8607).
28 October 2001	Presidential Summit of Parasitology Societies. 8:30 AM. Smithsonian Institution, Quad Building, (Room 3112), Washington, DC (Contact Persons: Bill Moser, 202-357-2473 or Dennis Richardson, 203-582-8607).
28 November 2001	Anniversary Dinner. 6:30 PM. 94th Aero Squadron, College Park, MD (Contact Person: Bill Moser, 202-357-2473).
January 2002	Date, time, and place to be announced.
March 2002	Date, time, and place to be announced.
May 2002	Date, time, and place to be announced.

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Cuticular Changes in Fergusobiid Nematodes Associated with Parasitism of Fergusoninid Flies

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ABSTRACT: In the stylet-bearing nematode *Fergusobia* sp. (Tylenchida: Neotylenchidae), we hypothesize an additional separation (apolysis) and loss (ecdysis) of the adult cuticle, without the formation of a new cuticle, during the transition from the preparasitic to parasitic female. This pattern is in direct contrast to the characteristic 4-molt pattern accepted for most nematodes. Transmission electron microscope comparisons of the cuticle of an adult parthenogenetic female, male, and preparasitic female from the plant-parasitic phase of the fergusobiid life cycle revealed a relatively simple cuticle with an epicuticle, amorphous cortical/median zone, and a striated basal zone that is underlain by a relatively thin epidermis and striated somatic muscles. In contrast, the parasitic female from the adult fly was without its stylet and cuticle, the epidermis was enlarged, the outer edges of the epidermis were modified into microvilli, and the somatic muscles and esophagus were degenerate. The apparent hypertrophy and development of epidermal microvilli greatly expand the surface area of the parasitic female and presumably increase the nematode's ability to absorb nutrients directly through the epidermis from the host's hemolymph without cuticular interference.

KEY WORDS: *Fergusobia,* parasitism, *Fergusonina,* cuticle, epidermis, TEM, molting, nematode, fly, Myrtaceae, Australia.

In the only known mutualistic association between nematodes and insects (Maggenti, 1982), nematodes of the genus *Fergusobia* Currie, 1937, together with flies of the genus *Fergusonina* Currie, 1937, induce galls in young meristematic tissues of myrtaceous hosts in Australasia (Giblin-Davis et al., 2001). The nematode is apparently responsible for gall induction (Currie, 1937; R. M. Giblin-Davis, unpublished data), and the fly for dispersal and sustenance of the nematode. The female fly deposits its eggs and juvenile nematode parasites in plant tissue (Currie, 1937). As these nematodes feed, a gall begins to form, and the nematodes develop into parthenogenetic females that lay eggs giving rise to amphimictic male and female nematodes. Inseminated preparasitic females are infective and invade mature female third-instar fly larvae. Inside the fly, the nematodes develop into parasitic females that deposit eggs in the fly's hemolymph. Juvenile nematodes that hatch from these eggs move to the oviducts of the adult fly and, together with the fly's eggs, are deposited into appropriate plant tissue to begin the next generation.

During dissections of mature third-instar fly larvae from a variety of myrtaceous hosts (swamp bloodwood *Corymbia ptychocarpa* (F. Mueller, 1859), South Australian blue gum *Eucalyptus leucoxylon* F. Mueller, 1855, and broad-leaved paperbark *Melaleuca quinquenervia* (Cavanilles, 1797) S. T. Blake, 1958), we observed apparent separation (apolysis) and loss (ecdysis) of the adult cuticle during transition from the preparasitic to parasitic female without the formation of a new cuticle (K. A. Davies, unpublished data). This assumes that the first molt occurs in the egg in *Fergusobia,* as with other Tylenchida, and that 3 molts occur after emergence from the egg through to the preparasitic female. This pattern is surprising because nematodes characteristically undergo 4 molts in their development from the juvenile to the adult stage (Bird and Bird, 1991). We report on the ultra-

[5] Corresponding author.

structure of the cuticle of adults at different phases of the life cycle of *Fergusobia*.

Materials and Methods

Multilocular flower bud galls of undescribed species of *Fergusobia* and *Fergusonina* were collected on 9 August 1999 from *C. ptychocarpa* at the Sherwood Arboretum in Sherwood, Queensland, Australia (27°32.06′S; 152°58.39′E). Galls were dissected. Adult parthenogenetic female and amphimictic male and preparasitic infective female nematodes present in the plant tissue were placed separately into Trump's fixative for transmission electron microscopy or in formalin-aceto-alcohol fixative (Southey, 1970). Mature fly larvae (third-instar) and adults were dissected from the galls in phosphate-buffered saline (pH 7.2). Parasitic female nematodes were removed from the hemocoel and placed into Trump's fixative. Specimens were postfixed in 2% formaldehyde (prepared from paraformaldehyde), 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for 18 hr at 4°C. After repeated rinsing in buffer, specimens were postfixed in 2% OsO_4 in 0.1 M cacodylate buffer at pH 7.2 for 3 days at 4°C. Nematodes were rinsed in water, fixed with 1% aqueous uranyl acetate, dehydrated through 100% ethanol into 100% acetone, and infiltrated with Spurr's epoxy resin. Blocks were sectioned on an RMC® ultramicrotome. Sections were poststained with 5% aqueous uranyl acetate and lead citrate before viewing on a Zeiss EM10® transmission electron microscope at 80 kV.

Results

Examination of the cuticle of an adult parthenogenetic female and a male nematode revealed a relatively simple cuticle with a striated basal zone, an amorphous cortical/median zone, and a distinct epicuticle (Figs. 1, 2). It is underlain by relatively thin epidermis that covers the striated somatic muscles.

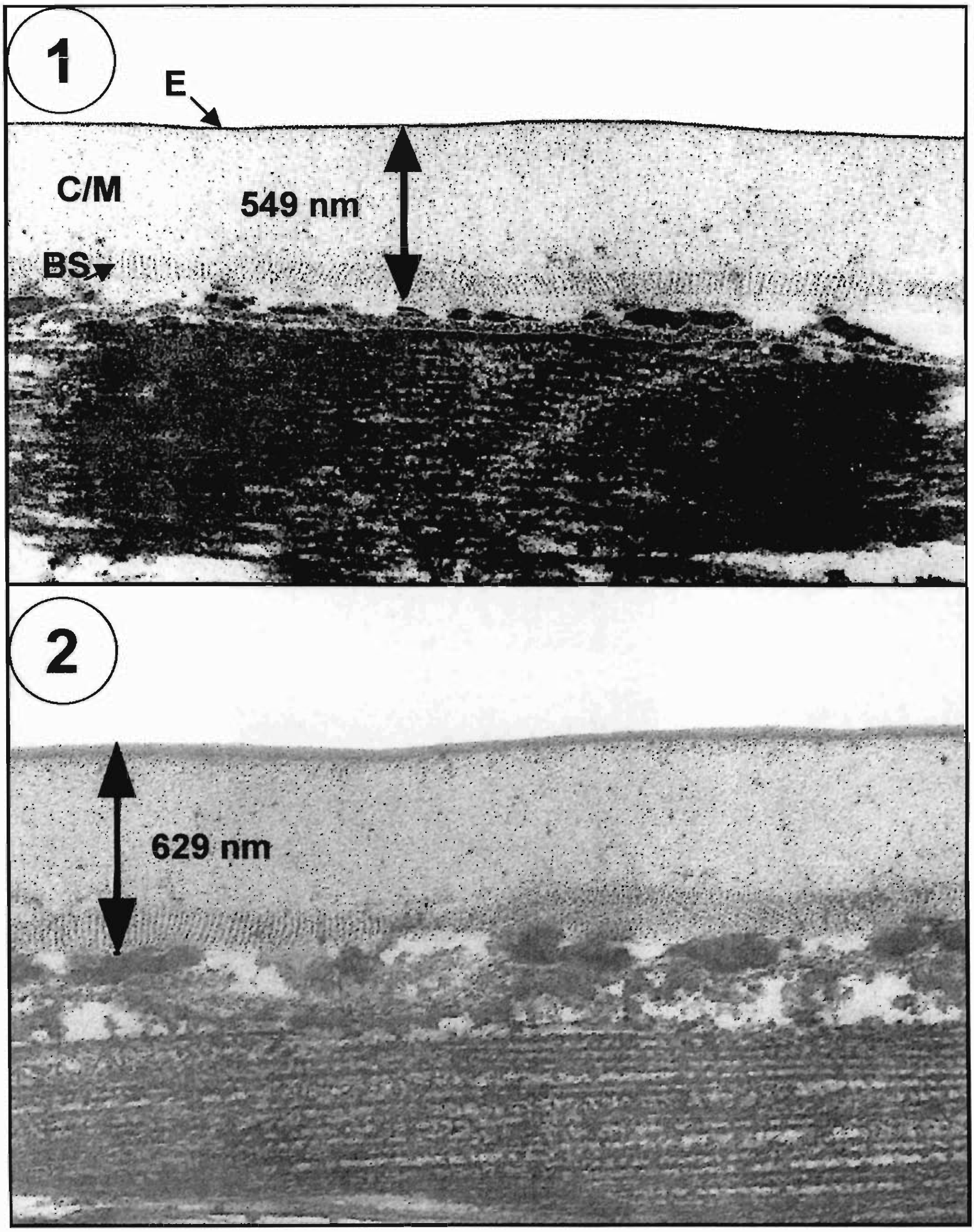
Comparisons of the preparasitic female nematode from the plant gall and the parasitic female nematode from the adult fly show dramatic differences (Figs. 3–7). The preparasitic female has cuticle, epidermis, and muscles similar to those described for the male and parthenogenetic female from the plant host (Figs. 3, 4). However, the cuticle appears thinner (200–250 nm vs. 450–550 nm for the parthenogenetic female and 630–680 nm for the male). The parasitic form of the nematode from the adult fly has no cuticle. The epidermis is greatly enlarged, and the outer edge of the epidermis appears to be modified into microvilli (Figs. 5–7). The somatic muscles appear degenerated (Fig. 6).

During the transition from the preparasitic to the parasitic female nematode in the larval fly, the stylet is lost and the esophagus and intestine appear to degenerate. In a parasitic female from a fly larva, the remnant of the adult epicuticle was present (Fig. 5), but it was not present in the parasitic female from an adult fly (Figs. 6, 7). The apparent hypertrophy and development of epidermal microvilli greatly expand the surface area of the parasitic female and presumably increase the nematode's ability to absorb nutrients directly through its epidermis from the host's hemolymph without cuticular interference. Interestingly, the cuticle represents a form of protection against insect host defense mechanisms. However, these mechanisms may be modified or lacking in the female larva, pupa, and adult fly in this mutualistic association. Whether there is a strong defense system in male flies to prevent parasitism by *Fergusobia* or the nematodes fail to penetrate the male fly larvae is not known.

Discussion

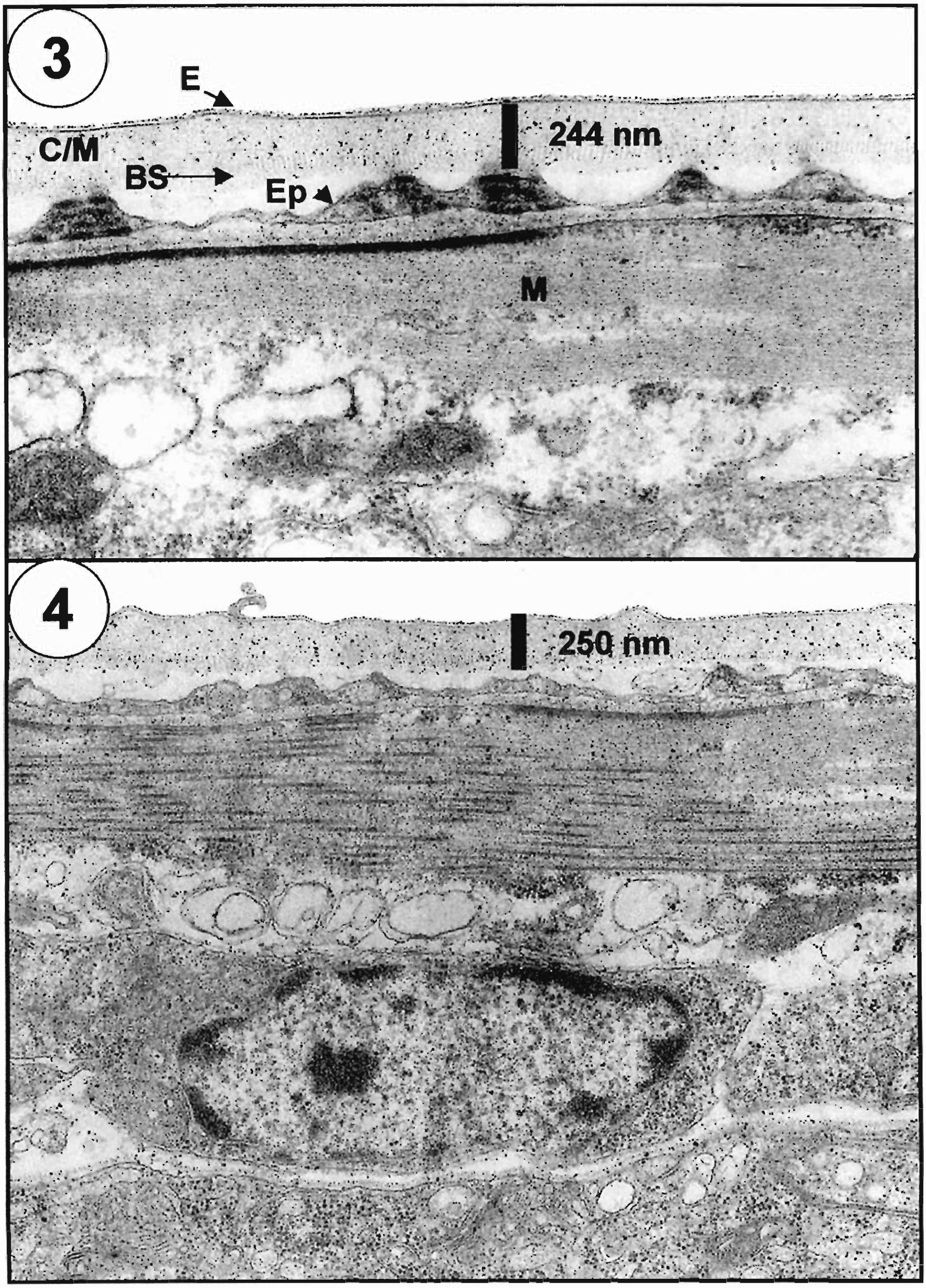
Riding (1970) reported that microvilli were present on the outside of the parasitic female stage of *Howardula husseyi* Richardson, Hesling, and Riding, 1977 (=*Bradynema* sp.) (Allantonematidae), a tylenchid parasite of the phorid fly, *Megaselia halterata* Wood, 1910. A cuticle was not observed in this stage of the nematode, suggesting that the microvilli were of epidermal origin and that there could have been an additional apolysis and ecdysis without cuticular replacement, as appears to occur in *Fergusobia*. The epidermis in this nematode was hypertrophied. In addition, the stylet and esophagus are not present in this form of *H. husseyi* (Poinar, 1979). Subbotin et al. (1994) reported that entomoparasitic females of *Wachekitylenchus bovieni* (Wachek, 1955) Slobodyanyuk, 1986 (Parasitylenchidae), and *Bradynema rigidum* (von Siebold, 1836) zur Strassen, 1892 (Allantonematidae), had similar body wall morphology to *H. husseyi*. Entomoparasitic females of the tylenchid *Skarbilovinema laumondi* Chizhov and Zakharenkova, 1991 (Iotonchiidae), exhibited a body wall composed of a "spongy" layer of the epidermis formed by interwoven and fused microvilli without a cuticle (Subbotin et al., 1993).

In contrast, the epicuticle is apparently retained by the entomoparasitic amphimictic female of *Paraiotonchium nicholasi* Slobodyanyuk, 1975 (=*Heterotylenchus* sp.) (Iotonchiidae) (Nicholas, 1972). Ultrastructural differences

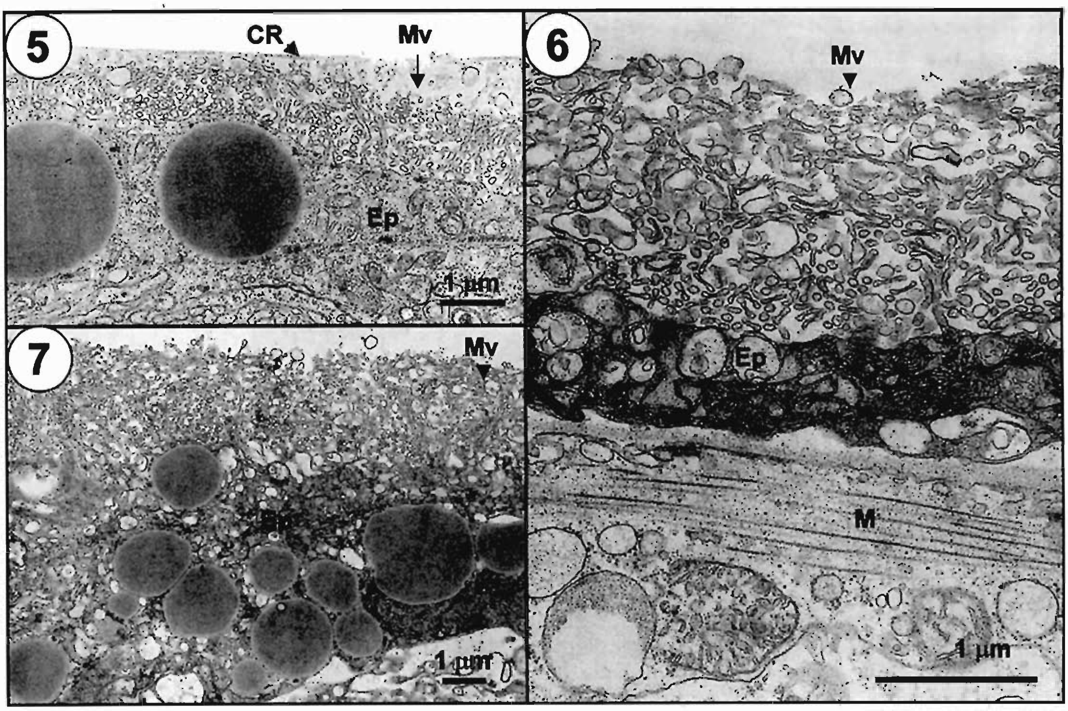


Figures 1, 2. Longitudinal sections of the cuticle of *Fergusobia* sp. ex *Corymbia ptychocarpa* from galled flower buds. 1. Adult parthenogenetic female. 2. Adult male. BS = basal striations; C/M = cortical/median zone; E = epicuticle.

were observed between the amphimictic female, parthenogenetic female, and amphimictic-phase juvenile of *P. nicholasi* from the body cavity of the Australian bush fly *Musca vetustissima* Walker, 1857 (Nicholas, 1972). Fourth-stage juvenile (J4) nematodes are deposited into cow dung where they mate, "metamorphose" into the infective form, penetrate the fly larva, and



Figures 3, 4. Longitudinal sections of the cuticle of a preparasitic adult female of *Fergusobia* sp. ex *Corymbia ptychocarpa* from galled flower buds. 3. Close-up showing epidermal folds. 4. Different section. BS = basal striations; C/M = cortical/median zone; E = epicuticle; Ep = epidermis; M = muscle.



Figures 5–7. Longitudinal sections of the cuticle of parasitic females of *Fergusobia* sp. ex *Corymbia ptychocarpa* from different stages of *Fergusonina* fly hosts. 5. Adult female from third-instar fly larva. 6. High magnification of adult female from adult female fly. 7. Lower magnification of adult female from adult female fly. CR = cuticle remnant; Ep = epidermis; M = degenerated muscle; Mv = microvilli.

then mature. The cuticle of the J4 *P. nicholasi* is typical of other free-living tylenchids with epicuticle, cortical/median, and basal layers. Unfortunately, the cuticle of the preparasitic female from cow dung was not observed before entry into the fly. Presumably, it is similar to the cuticle of the J4. Cuticle was observed from the mature parasitic amphimictic female of *P. nicholasi* from the fly hemocoel, where the esophagus is degenerate but the stylet is retained. Because the stylet and part of the cuticle are present in this form, there may be no additional molt in the transition from preparasitic female to mature female. The cortical and median layers of the cuticle appear to be absent, and the epicuticle is underlain with numerous irregularly shaped "canals" (or microvilli?) that apparently open to the surface. These canals likely function in the assimilation of nutrients from the host. The epicuticle in these females may not constitute much of a barrier to nutrient assimilation, while still providing some protection against insect host defenses. The "maturation" that Nicholas (1972) referred to during the adult female stage of *P. nicholasi* (preparasitic to parasitic stage) involves the epidermis becoming hypertrophied and microvillar in appearance under an epicuticle remnant layer from a partially resorbed cuticle or, alternatively, the epicuticle of a fifth cuticle after shedding of the adult cuticle. This pattern indicates an additional apolysis in the adult stage without ecdysis and formation of a new cuticle or, alternatively, the molting of the adult cuticle and the retention of a very meager cuticle and stylet. Another possibility is that the J4 female is mated and retains sperm prior to the molt to the adult stage, which occurs inside the insect. The mature parasitic female of another insect-parasitic tylenchid, *Deladenus siricidicola* Bedding, 1968 (Neotylenchidae Thorne, 1941), from the hemocoel of its siricid woodwasp host, may have scattered clusters of epidermal microvilli under its cuticle (Riding, 1970), suggesting a less complete apolysis and a greater reliance on transcuticular uptake than in *P. nicholasi*.

The body walls of entomoparasitic females of the tylenchids *Wachekitylenchus bembidi* Zakharenkova and Chizhov, 1991, and *Allantonema mirabile* Leuckart, 1884 (Allantonematidae), were similar to those of *P. nicholasi,* being composed of a hypertrophied epidermis with microvilli that was covered by a cuticle-like layer (Subbotin et al., 1994).

Insect hemolymph characteristically has high levels of amino acids, trehalose, other nonamino organic acids, and salts (Chapman, 1972), making it a nutrient-rich environment for parasites that can overcome innate host defense mechanisms. Insect-parasitic tylenchid nematodes have adapted to the challenges of obtaining nutrition from a living insect host in a variety of ways, including acquisition per os (through the mouth), through a modified or absent cuticle, or through prolapsis and modification of the uterus as in the Sphaerulariinae (Sphaerulariidae). Tylenchids from the Neotylenchidae, Allantonematidae, Iotonchiidae, and Parasitotylenchidae have insect-parasitic forms that are obese and have degenerate esophagi, intestines that are degenerate or modifed as storage organs, and the stylet often sunken into the body or even lacking (Siddiqi, 2000), suggesting that they employ some form of transcuticular or transepidermal uptake.

Deladenus (Neotylenchidae), *Paraiotonchium* (Iotonchiidae), *Howardula* (Allantonematidae), *Skarbilovinema* (Iotonchiidae), and *Fergusobia* (Neotylenchidae) may represent contemporary examples of an evolutionary trend from per os to transepidermal nutrient acquisition in insect-parasitic Tylenchida. Of course, this is a highly speculative exercise until more information about the transition between preparasitic and parasitic females is known and some independent phylogenetic data are available. The evolutionary trend is hypothesized to be: 1) Per os acquisition via a stylet, esophagus, and gut. This strategy takes advantage of the existing stylet for feeding on fungi, plants, or other invertebrates. It is a less energy- and time-efficient method of nutrient acquisition for a hemocoelic parasite because obtaining food through the stylet requires expending energy to maintain and operate its esophagus and intestine. 2) Per os acquisition with thinning and partial apolysis of the cuticle and coincident epidermal folding to increase surface area for supplemental transcuticular uptake of nutrients (possibly *Deladenus* spp.). 3) Early per os acquisition followed by apolysis, partial absorption of the cuticle without the creation of a new cuticle, and folding of the epidermis such that uptake is transcuticular and somatic muscles, esophagus, and gut degenerate (e.g., *Paraiotonchium*). 4) Early per os acquisition followed by full apolysis and ecdysis without the creation of a new cuticle. There is hypertrophy and folding of the epidermis, and nutrient uptake is transepidermal, somatic muscles and esophagus degenerate, and the gut degenerates or is transformed into a storage organ (e.g., *Howardula, Skarbilovinema,* and *Fergusobia*). The epidermal hypertrophy and folding are superficially similar to the formation of plicae (epidermal folds) during the development of a new cuticle (Bird and Bird, 1991) but are more extensive and apparently are not accompanied by the formation of a new cuticle.

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Report on the Brayton H. Ransom Memorial Trust Fund

The Brayton H. Ransom Memorial Trust Fund was established in 1936 to "Encourage and promote the study and advance of the Science of Parasitology and related sciences." Income from the Trust currently provides token support of *Comparative Parasitology* and limited support for publication of meritorious manuscripts by authors lacking institutional or other backing. Donations or memorial contributions may be directed to the Secretary-Treasurer. Information about the Trust can be found in the following articles: *Proceedings of the Helminthological Society of Washington* (1936) 3:84–87; (1983) 50:200–204 and *Journal of the Helminthological Society of Washington* (1993) 60:144–150.

Financial Report for 2000

Balance on hand, January 1, 2000	$24,459.06
Receipts:	$1,402.64
Contributions from Members of the Helminthological Society of Washington will be credited in 2001	
Interest received in 2000	$1,402.64
Disbursements	($6.00)
Grant to the Helminthological Society of Washington for 2000—$50.00 (to be debited in 2001)	
Membership in the American Association for Zoological Nomenclature for 2000—$50.00 (to be debited in 2001)	
Bank charges	($6.00)
On hand, December 31, 2000	$25,855.70

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Tegumentary Ultrastructure (SEM) of Preadult and Adult *Lobatostoma jungwirthi* Kritscher, 1974 (Trematoda: Aspidogastrea)

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ABSTRACT: Larval *Lobatostoma jungwirthi* Kritscher, 1974 (Trematoda: Aspidogastrea) parasitize the digestive gland of *Heleobia parchappii* (d'Orbigny, 1835) (Mollusca: Hydrobiidae) and, as adults, the posterior intestine of the chameleon cichlid *Cichlasoma facetum* (Jenyns, 1842) (Pisces, Cichlidae). Currently, *L. jungwirthi* is the only aspidogastrid reported from freshwater fishes in Argentina. Tegumentary structures of preadults and adults of *L. jungwirthi* were observed under scanning electron microscopy. In the preadult, 2 types of sensory receptors were observed: monociliate papillae of intermediate length on the walls and crests of the ventral adhesive disc as well as on the disc periphery and oral lobules, and nonciliate dome-shaped papillae on the crests of the ventral adhesive disc, neck, and oral lobules. In adults, other types of sensory receptors could be observed: in the posterior dorsal region, monociliate papillae with longer cilia than those found in the preadult, and a multiciliate structure in the dorsal region at the posterior third of the body. This is the first record of a surface multiciliate receptor in aspidogastreans. The pores of marginal glands were found only between the anterior alveoli.

KEY WORDS: Aspidogastrea, *Lobatostoma jungwirthi,* tegument, sensory papillae, SEM, Argentina.

Lobatostoma jungwirthi Kritscher, 1974 (Trematoda: Aspidogastrea), is the only species of the genus that parasitizes freshwater fishes. It was first found in 1974, in the stripefin earth-eater *Gymnogeophagus rhabdotus* (Hensel, 1870), in the Sinus River, Brazil (Kritscher, 1974). Lunaschi (1984) found it in the posterior intestine of the chameleon cichlid *Cichlasoma facetum* (Jenyns, 1842) (Pisces: Cichlidae) at 2 localities in Buenos Aires Province. Later, Zylber and Ostrowski de Núñez (1999) described the larval stages of *L. jungwirthi* from the gonad of *Heleobia castellanosae* (Gaillard, 1974) (Gastropoda: Hydrobiidae) collected in an artificial pond in Buenos Aires City.

To date, the morphology of the larval (Zylber and Ostrowski de Núñez, 1999) and adult (Kritscher, 1974; Lunaschi, 1984) stages of this species is known only at the light microscopy level. Several investigators have described the tegumentary ultrastructure of adult aspidogastrids, such as *Aspidogaster conchicola* Baer, 1826 (Halton and Lyness, 1971), and *Cotylogaster occidentalis* Nickerson, 1902 (Ip and Desser, 1984). The variability of tegumentary sensory structures of the cotylocidia of *C. occidentalis* (Fredericksen, 1978), the development and growth of the ventral adhesive disc of *C. occidentalis* and *A. conchicola* (Fredericksen, 1980), and the sensory receptors of the larval stage of *Lobatostoma manteri* Rohde, 1973 (Rohde and Watson, 1989a, b, 1992), and *Multicotyle purvisi* Dawes, 1941 (Rohde and Watson, 1990b, c, d), were also studied. The aim of the present paper is to describe the tegumentary ultrastructure of juvenile and adult specimens of *L. jungwirthi* under scanning electron microscopy (SEM).

Materials and Methods

Parasites removed from the posterior intestine of *C. facetum* were identified as *L. jungwirthi* on the basis of the descriptions of Kritscher (1974) and Lunaschi (1984). Juvenile stages were found in the digestive gland of *Heleobia parchappii* (d'Orbigny, 1835) (Mollusca: Hydrobiidae), and adult specimens were obtained from the posterior intestine of *C. facetum.* Both host species were naturally parasitized by this aspidogastrid in Saladita Pond, Avellaneda District, Buenos Aires.

The specimens were fixed in 10% formalin and washed in distilled water. They were dehydrated by 2 changes in 35, 50, 70, and 90% acetone for 15 min each and 3 changes in 100% acetone. The material was critical point dried, then mounted on stubs and coated for SEM observation (JEOL 100).

The immature stage was named the postacetabular juvenile, following the nomenclature used by Fredericksen (1980). Two stages could be distinguished according to the development of the ventral adhesive disc: a recently formed postacetabular juvenile, with little differentiation of alveoli and the buccal opening

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without oral lobules; and a preadult characterized by the presence of oral lobules and a distinct differentiation between alveoli, with the external morphology similar to that of the adult.

Results

Postacetabular juvenile

In the recently formed postacetabular juvenile (Fig. 1), the oral disc characteristic of the adult phase is not observed. The mouth is a simple opening, without lobules (Fig. 2).

The preadult shows a rough dorsal cone. Central and marginal alveoli, completely differentiated and varying in number, are observed on the ventral adhesive disc (Fig. 3). The posterior alveoli are less developed. Monociliate sensory papillae are found in the internal wall of the marginal alveoli (Fig. 4). The pores of the marginal bodies can be observed on the external border between the marginal alveoli. They are more developed in the anterior region of the ventral disc (Figs. 4, 5). The oral disc has 3 ventral and 2 dorsal lobules as in the adult, though they are not completely developed (Fig. 6). Monociliate sensory papillae and dome-shaped papillae are observed on the posterior surface of the oral disc. In this region, there is no regular distribution pattern of sensory structures (Fig. 7).

In the dorsal region of the neck, immediately behind the oral disc, there are pores and dome-shaped papillae (Fig. 8). The monociliate papillae each have a cilium emerging from a bulbous surface.

Adult

The ventral adhesive disc has 16 marginal pairs and 32 central alveoli. The limit between both groups of central alveoli cannot be clearly observed (Fig. 9). The anterior region of the ventral adhesive disc shows a neat differentiation among the alveoli, with many sensory structures (Fig. 10). Completely differentiated pores of the marginal bodies are found between the marginal alveoli (Fig. 11). Monociliate papillae are located on the internal wall of each alveolus, arranged in 2 concentric circles. Two rows of dome-shaped papillae occur on the external edge of the marginal alveoli (Figs. 11–13). Monociliate and dome-shaped papillae (Fig. 14) are present on both sides of the transverse dividing line between the central alveoli. The excretory pore can be seen in the dorsal cone (Fig. 15). Two kinds of monociliate receptors (Fig. 16) and a single multiciliate structure (Fig. 17) were observed in the posterior dorsal region.

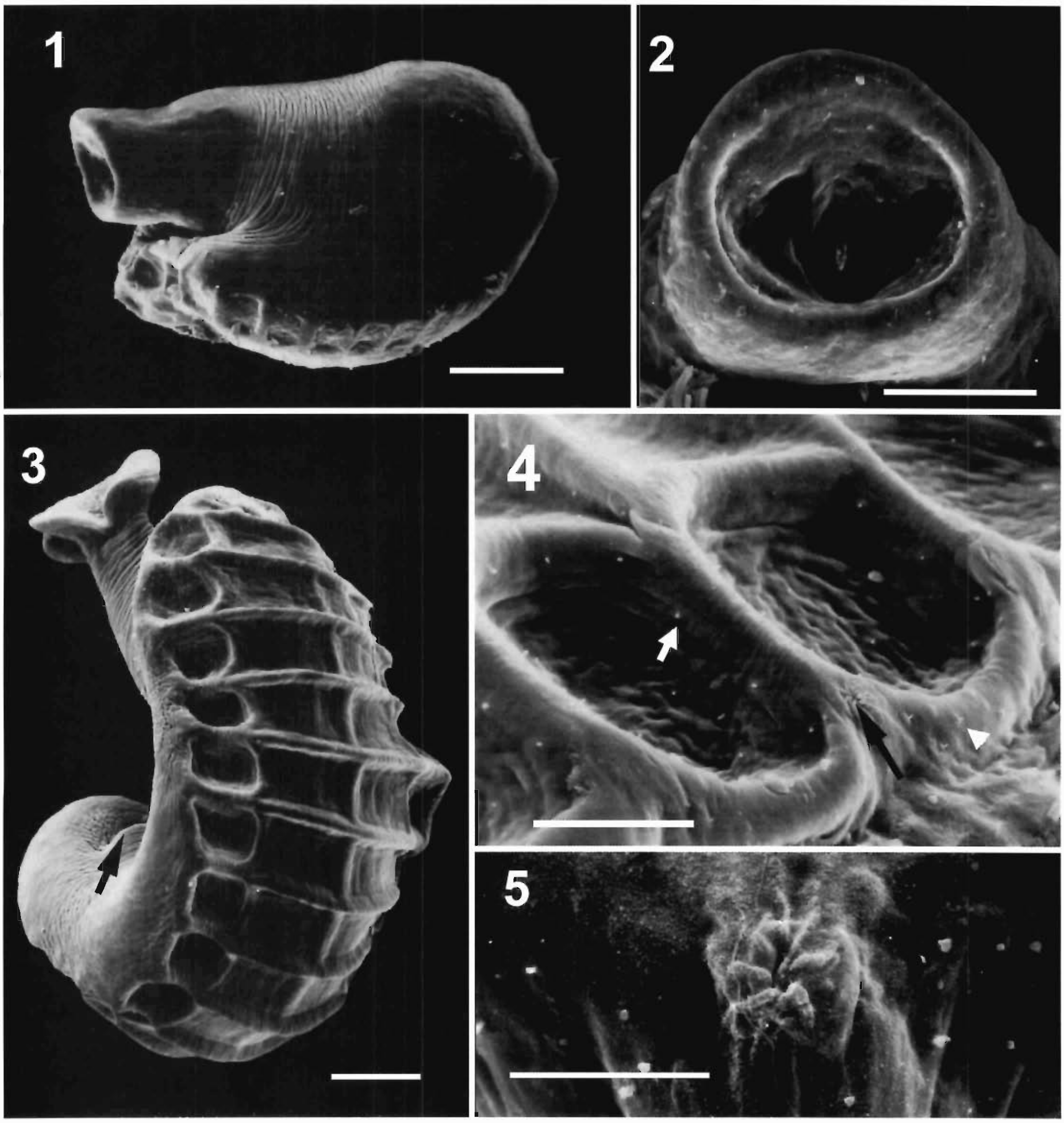
Discussion

The external morphology of the preadult of *L. jungwirthi* is very similar to that of the adult from *C. facetum.* Both juveniles and adults show a single excretory pore that ends at the channel formed by the union of the lateral ducts, as described by Lunaschi (1984). In agreement with the observations of Kritscher (1974) and Lunaschi (1984), the adult stage does not show a pore of Laurer's canal.

Four types of sensory receptors were observed by SEM:

MONOCILIATE PAPILLAE WITH A SHORT CILIUM: This type of receptor was irregularly distributed on the dorsal tegument, in the posterior surface of the oral lobules, and in the neck of juvenile *L. jungwirthi.* A defined pattern of distribution was observed only on the edges of the alveoli of the ventral adhesive disc. Rohde and Watson (1992) described this structure as a receptor formed by a cilium of intermediate length, being the most common type on the surface of *L. manteri.* Halton and Lyness (1971) described this type of papilla as the most frequent receptor on the body surface of *A. conchicola.* This receptor is more abundant in the oral lobules and in the central, marginal, and peripheral regions of the ventral adhesive disc of *L. jungwirthi.* This distribution agrees with that observed by Halton and Lyness (1971) in *A. conchicola.* The type of monociliate papilla found in *L. jungwirthi* may also correspond to that described by Fredericksen in the juvenile acetabulum of *C. occidentalis* and the simple uniciliate sensory structures observed in the cotylocidium larva of the same species (Fredericksen, 1978). Likewise, they are similar to type I sensilla found in adult *C. occidentalis* (Ip and Desser, 1984). Monociliate receptors were observed in *Lobatostoma* sp. (Rohde, 1972), and the type I receptor has been observed in the tegument of posterior suckerlets of larval *Multicotyle purvisi* (Rohde and Watson, 1990c). Monociliate tegumental receptors were also found in the buccal complex of *Polylabroides australis* (Murray, 1931) (Monogenea, Microcotylidae) (Rohde and Watson, 1995b) and in *Udonella* sp. (Platyhelminthes) (Rohde et al., 1989).

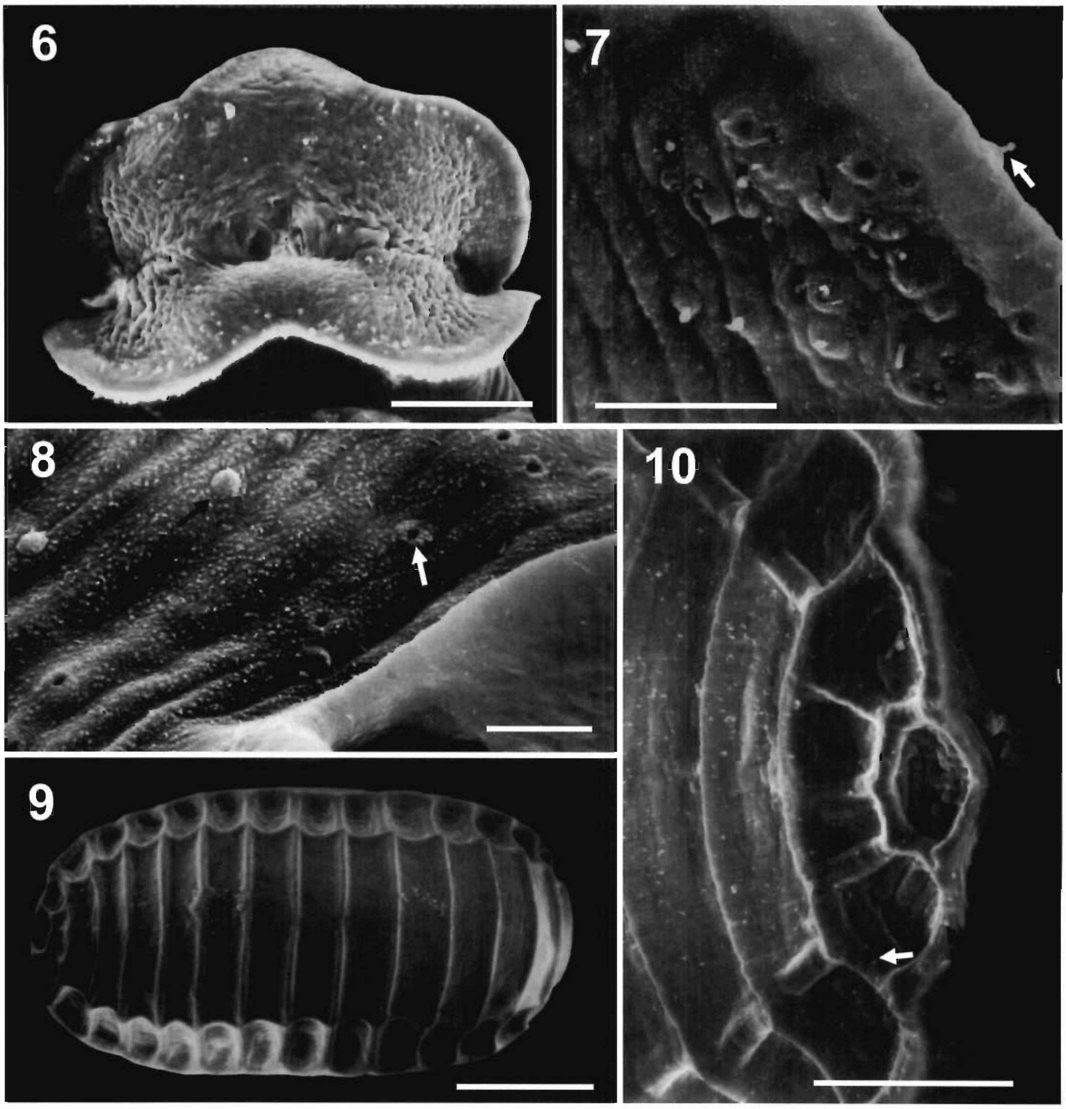
MONOCILIATE PAPILLAE WITH A LONG CILIUM:



Figures 1–5. Immature stages of *Lobatostoma jungwirthi*. 1. Postacetabular juvenile showing a simple buccal cavity (without lobules) and the ventral adhesive disc with poorly differentiated alveoli. 2. Frontal view of the buccal cavity of the postacetabular juvenile. 3. Alveoli of the ventral adhesive disc, oral lobules, and a rough posterior cone (dorsal) (black arrow) in latero-ventral view of the preadult. 4. Marginal alveoli of the ventral adhesive disc of the preadult, with sensory receptors of the monociliate type (white arrow), dome-shaped receptor (arrowhead), and the pores of the marginal glands not yet formed between the posterior alveoli (black arrow). 5. Pore of a marginal gland (=marginal body) of the preadult. Scales: 1, 2 = 10 μm; 3 = 50 μm; 4 = 25 μm; 5 = 10 μm.

These were found only on the surface of the dorsal cone of the adult. This type of receptor may correspond to the receptor with a long cilium in the larva of *L. manteri* (Rohde and Watson, 1992). It is also similar to the receptor type A described by Ip et al. (1982) in adult *C. occidentalis*.

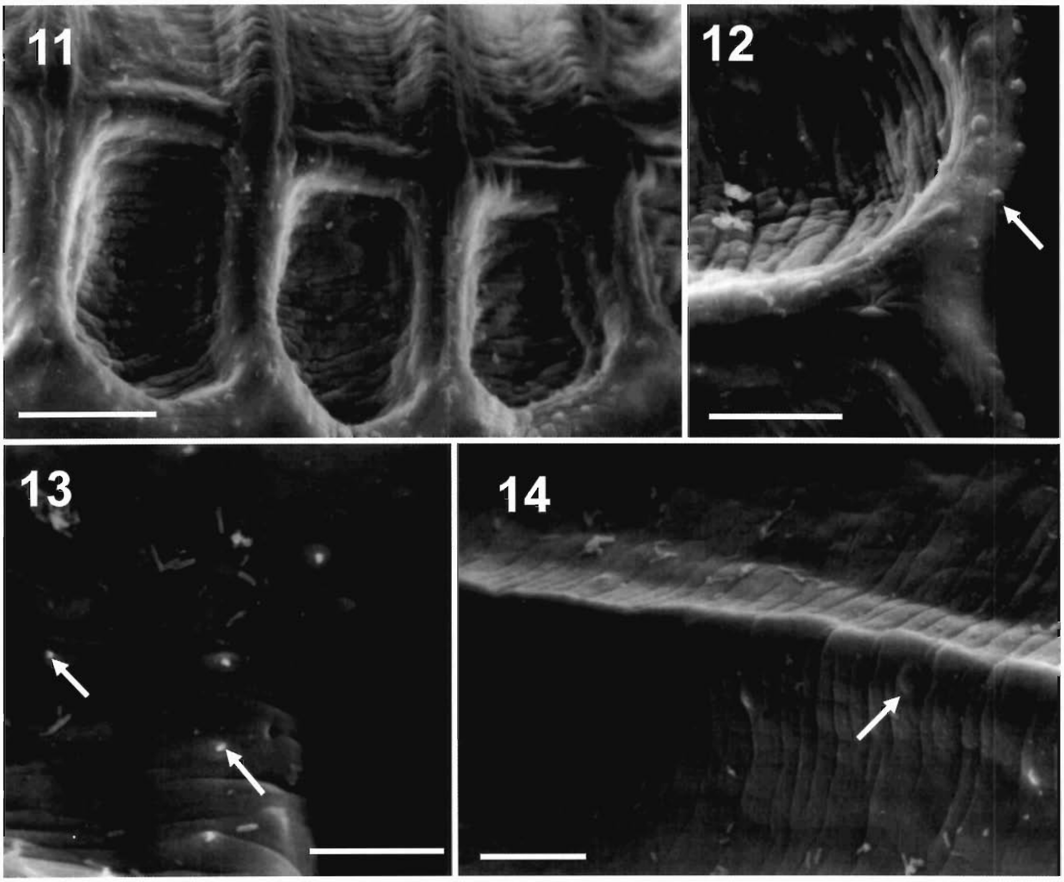
NONCILIATE DOME-SHAPED RECEPTORS: These were found on the posterior surface of the oral lobules and in the neck of the juvenile. They



Figures 6–10. Preadult and adult of *Lobatostoma jungwirthi*. 6. Frontal view of the preadult showing the 5 oral lobules. 7. Posterior surface of the oral lobules showing monociliate papillae (arrow) and dome-shaped papillae arranged without defined pattern. 8. Neck region (ventral) with pores (white arrow) (in some cases with secretion) and dome-shaped papillae. 9. General ventral view of the adult. A clear differentiation of the longitudinal septum of the ventral adhesive disc cannot be observed. 10. Anterior end of the ventral adhesive disc showing dome-shaped and monociliate papillae (arrow) on the edge of the walls. Scales: 6 = 50 μm; 7, 8 = 10 μm; 9 = 25 μm; 10 = 100 μm.

also were distributed on the borders between the alveoli of the adhesive disc of both juvenile and adult worms. Nonciliate sensory receptors were found by Rohde and Watson (1990b) in the external ventral tegument of the ventral suckerlets in *M. purvisi.* This structure may correspond to the nonciliate disc-shaped receptors or to the nonciliate type with a long root described by Rohde and Watson (1992) in the larva of *L. manteri.* Nonciliate tegumental receptors were also found in the juvenile of *Astramphilina elongata* Johnston, 1931 (Monogenea) (Rohde and Watson, 1990a).

A SINGLE MULTICILIATE RECEPTOR: This was

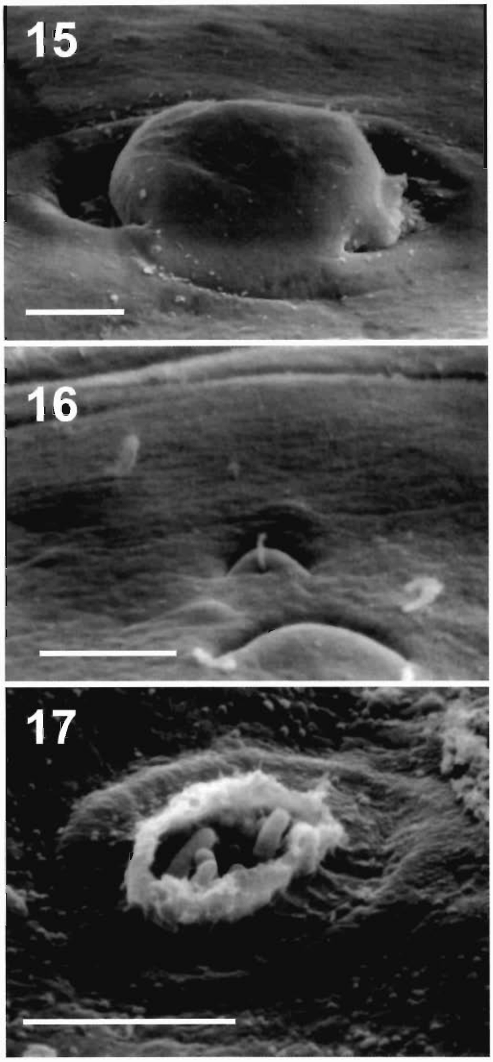


Figures 11–14. Ventral adhesive disc of adult *Lobatostoma jungwirthi*. 11. General view of the marginal alveoli on the marginal region at midbody level of the ventral adhesive disc. 12. Walls of the marginal alveoli showing 2 circles of dome-shaped papillae (arrow) and marginal gland pore. 13. Detail of the internal wall with almost 1 complete circle of monociliate papillae (arrows). 14. Ventral view: transverse septum with monociliate (black arrow) and nonciliate papillae (white arrow). Scales: 11 = 50 μm; 12 = 25 μm; 13, 14 = 10 μm.

observed in the posterior third of the dorsal region of the adult of *L. jungwirthi*. Paired multiciliate receptor complexes with 10 short cilia were described by Rohde and Watson (1990d) to be located dorsally to the mouth cavity of larval *M. purvisi,* but our record is the first of a surface multiciliate receptor in aspidogastreans. However, multiciliate receptors are found in other groups of parasitic Platyhelminthes, i.e., in the taste organ of the buccal complex of *Pricea multae* Chauhan, 1945 (Monogenea, Gastrocotylidae). Rohde and Watson (1996) found these receptors concentrated in small pits. Additionally, multiciliate pit-receptors were observed in the buccal complex of *Polylabroides australis* (Rohde and Watson, 1995b) and in specimens of an undescribed species of *Proseriata* (Monocelididae: Minonidae) (Rohde and Watson, 1995a).

Pores of the marginal bodies were observed on the external border between the marginal alveoli of juveniles and adults of *L. jungwirthi*. Kritscher (1974) found similar structures in the adult of the species. These pores were described as part of a glandular system in the adhesive disc of aspidogastrids (Rohde, 1994).

Unlike the preadult, the youngest juvenile does not have oral lobules. A few less-developed alveoli were observed on the ventral adhesive disc. Two sensory receptors were found in the preadult of *L. jungwirthi*: a ciliate receptor with a cilium of intermediate length, and a nonciliate dome-shaped receptor. These correspond to 2 of



Figures 15–17. Tegument of posterior dorsal region of *Lobatostoma jungwirthi*. 15. Excretory pore on the dorsal cone. 16. Type of monociliate papilla in the posterior cone region. 17. Multiciliate sensory receptor in the posterior dorsal region. Scales: 15, 17 = 5 μm; 16 = 10 μm.

the 4 types of receptors described by Rohde and Watson (1992) in the larva of *L. manteri*. In addition, a third kind of receptor was observed that corresponds to the marginal body complex in the adult. However, the complex formed by the marginal bodies was observed as described for the adult, though the pores of these glands are not yet developed in the posterior region of the ventral adhesive disc in the preadult.

The most common receptors in the preadult and in the adult were the intermediate length monociliate and the nonciliate dome-shaped papillae. The monociliate structures may correspond to some of the types I to V of monociliate dome-shaped papillae described for adult *L. manteri* (Rohde and Watson, 1989a). The nonciliate dome-shaped papillae may be homologous to those of type VI A and B found in the adult of *L. manteri*. Both types of receptors were abundant over the entire body, arranged in circles on the ventral adhesive disc. Longer monociliate papillae and multiciliate receptors were found exclusively in the adult. Probably more than one type of nonciliate and monociliate papillae may exist. It would be interesting to study these papillae further under transmission electron microscopy.

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New Books Available

The Flagellates: Unity, Diversity and Evolution. Barry S. C. Leadbeater and J. C. Green, Editors. 2000. The Systematics Association Special Volume Series 59, Taylor and Francis Limited, 29 West 35th Street, New York, NY 10001-2299. i–xi, 401 pp. ISBN 0-7484-0914-9. 7″ × 9¾″, hard cover. Cost: US$130.00 or Canadian $195.00, per copy plus shipping and handling. **Abstract:** 35 Authors have contributed to the 17 chapters of this examination of the blood parasite group. "[The] book sets out to examine flagellates from a multidisciplinary standpoint. Of primary concern are the unifying structures, mechanisms and processes involved in flagellate biology. [It begins] with a review of the complex history of flagellate studies from the first use of microscopes . . . to the present. [This is] followed by a series of chapters on common aspects of flagellates, . . . [including] a discussion of the problems inherent in being a flagellate, and reviews of the structure and function of the flagellum itself, the cytoskeleton, surface structures and sensory mechanisms. The diversity of flagellates is recognized in the next series of chapters, which include reviews of trophic strategies of both free-living and parasitic groups, and contributions on ecology, biogeography and population genetics. The final chapters . . . are concerned with the occurrence and loss of organelles, and other aspects of flagellate evolution and phylogeny."

Interrelationships of the Platyhelminths. D. T. J. Littlewood and R. A. Bray, Editors. 2001. The Systematics Association Special Volume Series 60, Taylor and Francis Limited, 29 West 35th Street, New York, NY 10001-2299. i–xii, 356 pp. ISBN 0-7484-0903-3. 8½″ × 11″, hard cover. Cost: US$125.00 or Canadian $188.00, per copy plus shipping and handling. **Abstract:** This book's 27 chapters have been prepared by no fewer than 50 different expert contributors. It "has been split into four sections, rather dissimilar in length, that highlight the underlying goals [of bringing together workers from divergent areas to produce a work unified by modern approaches to phylogenetic analysis]. The first section takes a broader perspective on the status of the Platyhelminthes, its monophyly, placement in relation to other Metazoa and the nature of the basal taxa. The second section deals with the interrelationships of major free-living taxa, and the third on symbiotic and parasitic taxa. The final section encompasses contributions that view phylogeny and phylogenetic inference from the point of view of particular characters or techniques."

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Research Note

Infectivity and Comparative Pathology of *Echinostoma caproni*, *Echinostoma revolutum*, and *Echinostoma trivolvis* (Trematoda) in the Domestic Chick

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ABSTRACT: We examined the clinical and pathological effects of 3 species of 37-collar-spined *Echinostoma* in domestic chicks. Three groups of 6 chicks each were infected with 50 metacercariae of either *Echinostoma caproni, Echinostoma revolutum,* or *Echinostoma trivolvis.* A group of 6 chicks was not infected and served as the uninfected controls. The chicks were necropsied on day 14 postinfection (PI). Infectivity and worm recovery rates for *E. caproni* were 100% and 24%, respectively; for *E. revolutum,* they were 67% and 9%, respectively; and for *E. trivolvis,* they were 83% and 15%, respectively. *Echinostoma caproni* was located in the middle third of the small intestine, whereas *E. revolutum* and *E. trivolvis* were located in the lower third, showing that niche selection of the different echinostomes varied. The echinostomes became ovigerous on days 10, 12, and 14 PI for *E. caproni, E. trivolvis,* and *E. revolutum,* respectively. Goblet cell proliferation in the host intestinal mucosa occurred in all infections.

KEY WORDS: *Echinostoma caproni, Echinostoma trivolvis, Echinostoma revolutum,* Trematoda, domestic chicks, echinostomiasis, pathology, clinical effects, goblet cell, infectivity.

Because echinostomiasis has produced significant mortality in ducks raised for commercial production in Europe and Asia (Kishore and Sinha, 1982), studies on experimental avian models to define the clinical and pathological features of the echinostomes are needed. Except for the experimental studies by Kim and Fried (1989) on gross and histopathological effects of *Echinostoma caproni* Richard, 1964, in an experimental avian model, such studies are lacking.

In North America, avian hosts in the wild are often infected with *Echinostoma trivolvis* (Cort, 1914) and *Echinostoma revolutum* (Froelich, 1802) and species of *Echinoparyphium* (43- and 45-collar-spined echinostomes). Interestingly, the habitat of species of *Echinoparyphium* in the gut of birds is more anteriad than that of either *E. trivolvis* or *E. revolutum. Echinostoma caproni* also tends to localize more anteriad in the avian gut than either *E. trivolvis* or *E. revolutum,* and may serve as a useful model for *Echinoparyphium* infections. Therefore, information obtained from single infections of the 3 echinostome species examined in this study may be useful to wildlife studies of birds naturally infected with 3 or more species of echinostomes.

The objectives of this study were to determine the following parameters in *E. caproni-, E. revolutum-,* and *E. trivolvis*-infected birds: packed cell volume, hemoglobin concentration, and the relative splenic and hepatic weights of infected and noninfected domestic chicks. Parasite recovery and location were recorded from infected animals. We also examined tissues grossly and microscopically for evidence of pathological changes. Metacercarial cysts of *E. caproni* and *E. trivolvis* were obtained from the kidneys and pericardial sacs of laboratory-infected *Biomphalaria glabrata* (Say, 1816) snails (Huffman and Fried, 1990). Metacercarial cysts of *E. revolutum* were obtained from experimentally infected *Lymnaea elodes* (Say, 1821) snails (Sorenson et al., 1997). Twenty-four-d-old unfed domestic chicks were obtained from Reich Poultry Farm (Marietta, Pennsylvania, U.S.A.). All chicks were infected on day 1 prior to feeding. All animals were provided food (Country Egg Producer®, Agway Inc., Syracuse, New York, U.S.A.) and water ad libitum throughout the study. Group A ($N = 6$) was not infected and served as controls for the study. Chicks in Groups B–D each received 50 metacercarial cysts per os of either *E. caproni* (Group B, $N = 6$), *E. trivolvis* (Group C, $N = 6$), or *E. revolu-*

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Table 1. Mean worm recovery, mean percentage of recovery, percentage infected, and range of parasites recovered from chicks infected with *Echinostoma caproni* (Group B, $N = 6$), *Echinostoma trivolvis* (Group C, $N = 6$), and *Echinostoma revolutum* (Group D, $N = 6$). Uninfected controls are represented by Group A ($N = 6$).

Group	No. cysts administered	Mean no. worms recovered (range)	Mean percentage of worms recovered	Day of first appearance of eggs in feces	Percentage of chicks infected
A	0	0 (0)	0	0	0
B	50	12 (8–20)	24	10	100
C	50	8 (0–21)	15	12	83
D	50	5 (0–16)	9	14	67

tum (Group D, $N = 6$). Fecal samples were collected from each infected animal and checked for echinostome eggs daily, starting on day 8 postinfection (PI). Approximately 1 g of feces was emulsified in distilled water and examined by light microscopy. Animals were weighed every 2 d to monitor weight gain in the chicks.

Blood samples were collected from the jugular veins of all chicks on day 14 PI into tubes containing 0.13 M sodium citrate, refrigerated, and processed within 24 hr. Packed cell volume and hemoglobin concentration were measured and recorded.

All chicks (Groups A–D) were necropsied on day 14 PI. The small intestines, ceca, and cloacas were opened, and the location and number of echinostomes in the infected chicks were recorded. Hepatic, splenic, and intestinal tissue samples from all groups were fixed in 10% neutral buffered formalin for 48 hr and then dehydrated in a series of graded alcohols, cleared in xylene, embedded in paraffin, and sectioned at 6 μm. At necropsy, the relative spleen and liver weights were determined.

Infected and control tissues were stained in hematoxylin and eosin to evaluate histopathological effects. The occurrence of immunological cells from the infected and control tissues was also evaluated.

Echinostome eggs were first seen in the fecal samples of all chicks from Group B on day 10, of 5 chicks from Group C on day 12, and of 4 chicks from Group D on day 14 PI. All chicks exposed to *E. caproni* became infected. The number of worms recovered from their intestines ranged from 8 to 20, with a 24% recovery. The number of parasites recovered from Group C ranged from 0 to 21, a 15% recovery. Four of 6 chicks (67%) exposed to *E. revolutum* cysts were infected, and the numbers of worms recovered ranged from 0 to 16, averaging 9%. These data are summarized in Table 1.

The locations of the recovered worms from the infected chicks varied according to species. *Echinostoma caproni* was located in the mid-third of the intestine, between the pylorus and the cloaca. Both *E. trivolvis* and *E. revolutum* were found toward the end of the intestine near the cloaca, but typically, *E. revolutum* was found more posteriad than *E. trivolvis*. No differences were noted between the number of parasites recovered per chick and the location of the worms. *Echinostoma caproni* tended to be in groups, but single worms were also found. *Echinostoma revolutum* and *E. trivolvis* were found singly and in groups.

There was no significant weight loss ($P > 0.05$) in chicks infected with any species of echinostome versus the uninfected (control) group. No differences were seen in liver or spleen weights of the infected chicks versus the control group, nor were there differences in liver or spleen weights between the infected groups. There were no notable differences in either the measured packed cell volume or hemoglobin concentration of the infected chicks compared with the uninfected chicks.

Histologically, the liver and spleen tissues of Groups B–D showed no sign of immunological response or damage from the parasites. Damage to the intestinal villi of chicks infected with *E. caproni* was observed at the site of parasite attachment, villi were atrophic, and the circular musculature was hypertrophied with collagen-like fibers present. There was a proliferation of goblet cells. Hemorrhage occurred at the site of attachment. In chicks infected with *E. trivolvis*, damage to villi also occurred, with lymphocytic infiltration and goblet cell proliferation but with no hemorrhage noted. The response to *E. revo-*

lutum was less severe than with the other 2 parasites, but damage to the intestinal villi was observed.

The presence of eggs in the host's feces is used for diagnosis of echinostomiasis. The time of deposition of eggs in the feces will vary among species (Huffman and Fried, 1990). In this study, *E. caproni* eggs were first noted in the chick's feces on day 10 PI, followed by *E. trivolvis* eggs on day 12 PI, and eggs of *E. revolutum* were found on day 14 PI.

Infectivity in the chicks varied between the different *Echinostoma* species in this study. Factors such as age, size of cyst inoculum, pretreatment of metacercarial cysts, and host-gut emptying time influence the infectivity of *E. trivolvis* in experimentally infected chicks (Fried et al., 1997). Huffman and Fried (1990) reported *E. trivolvis* to have infectivity varying between 50 and 69%. Fried (1984) reported 100% infectivity when preselected cysts were used. In this study, the *E. trivolvis* metacercariae administered to the chicks averaged 83% infectivity. Experimental infection with *E. caproni* cysts in this study resulted in 100% infectivity, agreeing with a previous study on this species conducted by Fried et al. (1988), which reported 97% infectivity. *Echinostoma revolutum* infectivity in this study (67%) is congruent with the report of Humphries et al. (1997) on the species in experimentally infected chicks (64%).

Huffman and Fried (1990) summarized findings of average worm recoveries for *E. trivolvis* in experimentally infected chicks. These results varied between 6 and 21%. In this study, a mean of 15% of the flukes were recovered from chicks infected with *E. trivolvis*. *Echinostoma caproni* infections resulted in 24% worm recovery, concurring with the report by Fried et al. (1988) of 28% worm recovery. An average of 9% of the administered cysts were recovered as adult worms in the *E. revolutum* infections. This differed from the 32% worm recovery of *E. revolutum* reported by Humphries et al. (1997) and the 21% worm recovery reported for the same species of *Echinostoma* in domestic chicks (Fried et al., 1997).

Echinostoma trivolvis distribution along the intestine of the domestic chick varied in numerous past studies (Huffman and Fried, 1990). On day 14 PI of our study, *E. trivolvis* was found mainly in the lower intestine near the cloaca. *Echinostoma revolutum* also was seen in the posterior aspect of the intestine, congruent with results by Humphries et al. (1997) and Fried et al. (1997). *Echinostoma caproni* was found more anteriad than the other echinostomes in this study, mainly clustered in the midthird of the intestine.

Weight gain, spleen and liver weights, packed cell volume, and hemoglobin concentrations of the infected chicks compared with the controls were not affected by the presence of any of the echinostomes. As noted in previous studies, liver or spleen tissue damage did not occur. Huffman, Iglesias, and Fried (1986) noted increased pathology in golden hamsters infected with echinostomes and increased pathology when greater numbers of parasites infected the host. Infectivity in the present study was less compared with other studies. In the study by Fried and Wilson (1981), high worm burdens caused a decrease in chick weight. Changes in blood parameters and tissue damage (Huffman, Michos, and Fried, 1986) have been noted in rodent hosts infected with echinostomes.

In conclusion, there are differences in the host–parasite relationships for each of the echinostomes used in this study. An understanding of these differences will contribute to better understanding the biosystematics of the 37-collar-spined echinostome group. Some of these differences may help elucidate species distinctions when echinostomes are recovered from naturally infected hosts in both single and multiple infections.

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Research Note

Excystation and Distribution of Metacercariae of *Echinostoma caproni* in ICR Mice

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ABSTRACT: In vivo excystation and distribution of newly excysted metacercariae of *Echinostoma caproni* Richard, 1964, were studied in 16 ICR mice, each fed 400 metacercarial cysts and necropsied at various intervals from 1 to 24 hr postinfection (p.i.). Excysted metacercariae were recovered from the stomach and intestine (duodenum and jejunum) at 1 hr p.i. In vivo excystation in this echinostome occurred in the stomach and the anterior part of the small intestine. Encysted metacercariae were recovered from the stomach, small intestine, and cecum–large intestine at 1 and 2 hr p.i. Recovery of encysted metacercariae was rare at 3 hr and nil at 4 and 24 hr. At 3, 4, and 24 hr, the encysted metacercariae had either excysted or were voided. Excysted metacercariae were widely scattered throughout the small intestine at all times, with about 75% located in segments 1, 2, and 3 (duodenal–jejunum zone) of the small intestine at 3, 4, and 24 hr p.i.

KEY WORDS: trematodes, in vivo excystation, *Echinostoma caproni*, metacercariae, ICR mice.

Although information is available (Fried and Emili, 1988; Fried, 1994; Ursone and Fried, 1995) on chemical excystation of metacercarial cysts of *Echinostoma caproni* Richard, 1964, there are no studies on in vivo excystation of this echinostome in mice. Metacercariae of most intestinal digeneans excyst in the vertebrate small intestine, but details on in vivo excystation and the microhabitat where excystation occurs are poorly understood in the Digenea. Simonsen et al. (1989) stated that *E. caproni* metacercarial cysts excysted in the duodenum of the mouse and the newly excysted metacercariae migrated to the posterior third of the small intestine. However, Simonsen et al. did not do experimental studies on in vivo excystation of the metacercarial cyst.

The purpose of this research was to examine in vivo excystation of *E. caproni* metacercariae at various times up to 24 hr postinfection (p.i.) and to determine the distribution of newly excysted metacercariae in ICR mice. The ICR mouse is widely used as a laboratory host for this echinostome (see review in Fried and Huffman, 1996). The only previous study that has reported distribution of preovigerous worms of *E. caproni* is that of Manger and Fried (1993), who showed that, by 2 days p.i., more than 90% of the juvenile worms were located in segment 3 posterior to the pylorus (equivalent to the jejunum) in the ICR mouse.

Metacercarial cysts of *E. caproni* were removed from the pericardial cavity and kidney of experimentally infected *Biomphalaria glabrata* Say, 1818, snails and fed (400 cysts/mouse) via stomach tube to 16 6–8-wk old, outbred, female

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Table 1. Percentage of encysted (EN) and excysted (EX) metacercariae (M) of *Echinostoma caproni* from 16 mice, each fed 400 cysts.

Group*	Time postinfection (hr)	M	Stomach	Segments of the small intestine 1	2	3	4	5	Cecum–large intestine	Total
A	1	EN	0.9	1.5	1.3	0.7	0.4	0.4	0.2	5.4
		EX	1.0	1.9	1.6	2.5	0.5	0	0	7.5
B	2	EN	0.3	0.5	0.5	0.4	0.5	0.2	0.1	2.5
		EX	0.8	2.4	1.8	1.3	1.7	0.6	0	8.6
C	3	EN	0	0	0.1	0	0.1	0.1	0.1	0.4
		EX	0.5	2.6	2.9	3.1	2.0	0.3	0	11.4
D	4	EN	0	0	0	0	0	0	0	0
		EX	0.2	2.3	2.6	2.9	1.7	0.4	0	10.1
E	24	EN	0	0	0	0	0	0	0	0
		EX	0	1.6	2.7	3.3	2.2	0.5	0	10.3

* Groups A and B each with 2 mice; Groups C, D, and E each with 4 mice.

ICR mice (Hosier and Fried, 1991). Preliminary studies on 2 mice each fed 100 metacercarial cysts and necropsied at 1 and 2 hr p.i. showed metacercarial recoveries (combined data of excysted and encysted metacercariae) of about 10% at necropsy. The preliminary work showed the inherent difficulties in recovering these small organisms (excysted metacercariae measuring about 250 μm in length and encysted metacercariae about 150 μm in diameter) from the intestinal tract. Moreover, the color, size, and motility of the villi made it difficult to distinguish them from excysted metacercariae. Empty cysts were also seen at necropsy but were not counted. On the basis of our experiences with the preliminary study, we increased the cyst inoculum to 400 per host in the study reported herein.

Groups of 2 mice each were necropsied at 1 and 2 hr p.i. (Groups A and B, respectively; Table 1), and groups of 4 mice each were necropsied at 3, 4, and 24 hr p.i. (Groups C, D, and E, respectively; Table 1). The numbers of encysted and excysted metacercariae in the stomach, in 5 intestinal segments of equal length (approximately 10 cm each), beginning at the pylorus and ending at the ileocecal valve, and in the combined cecum–large intestine were counted. Empty cysts were seen but not counted in hosts necropsied at 1 and 2 hr p.i. The numbers were converted to percentages and the information is presented in Table 1. In Group A, the greatest percentage of encysted metacercariae was in segment 1 of the small intestine, and excysted metacercariae were recovered as far posteriad as segment 4 of the small intestine. Most of the excysted metacercariae recovered at 1 hr p.i. were located in segment 3. Some excysted metacercariae were in the stomach at 1 hr and were alive and active. These organisms either had excysted in the stomach or, possibly, could have excysted in the small intestine and migrated anteriad to the stomach. In Group A, the finding of most excysted metacercariae in segments 1, 2, and 3 of the small intestine (duodenum–jejunum region) provides support for claims that in vivo excystation takes place in the anterior part of the small intestine. This finding supports the statement of Simonsen et al. (1989) referenced above.

With time, the ratio of encysted to excysted metacercariae declined (see last column in Table 1), and by 4 hr p.i., encysted metacercariae were not found. These findings suggest that by 4 hr p.i. most of the encysted metacercariae had excysted or were voided. The idea of metacercariae being voided by 4 hr p.i. is consistent with the fact that the usual transit time for ingested food in the mouse digestive tract is 4 hr (Barrachina et al., 1997). Fecal examinations to determine the possible presence of excysted or encysted metacercariae in the stool were not made.

About 75% of the excysted metacercariae in Groups C, D, and E were located in segments 1, 2, and 3. Hence, newly excysted juveniles, up to at least 24 hr p.i., are more dispersed in the gut than are older worms. Manger and Fried (1993) showed that by day 2 p.i. more than 90% juvenile *E. caproni* were localized in segment 3 (the jejunum), and by day 4 and beyond, worms tended to migrate even more posteriad, with most being found in segment 4 (jejunum–ileum zone).

In conclusion, this study provides information on in vivo excystation of *E. caproni* metacercariae during the first day after infection in ICR mice. The in vivo studies show that most metacercariae excyst in the duodenum and migrate to the jejunum to become juveniles. The distribution of excysted metacercariae is quite variable during the first 24 hr of infection. The low recovery rates of organisms (only about 10% when mice received 100 cysts, and from 10.1% to 12.9% when mice received 400 cysts) attest to the fact that these organisms are difficult to detect in the first 24 hr after excystation. Perhaps some of these missing larvae are in sites other than the intestinal lumen, e.g., ducts or crypts associated with the intestine or other unknown locations. Manger and Fried (1993) did not report recovery percentages of juvenile worms of *E. caproni* from ICR mice at 2 d p.i. They did report a wide range of worm recoveries (18 to 95%) from 2 to 8 d p.i. The fact that their recoveries were higher than the 10–13% in this study would suggest that some of the newly excysted metacercariae had been overlooked or had reemerged from extra-luminal sites.

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Research Note

Helminths Collected from *Rattus* spp. in Bac Ninh Province, Vietnam

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ABSTRACT: Helminthological examination was made on 35 rats (12 *Rattus tanezumi*, 14 *Rattus argentiventer*, and 9 *Rattus losea*) captured in 3 different habitats, i.e., residential, paddy field, and hilly areas, all in Bac Ninh Province, northern Vietnam. One trematode (*Notocotylus* sp.), 2 cestodes (*Raillietina celebensis, Hymenolepis diminuta*), 6 nematodes (*Strongyloides ratti, Strongyloides venezuelensis, Nippostrongylus brasiliensis, Orientostrongylus* cf. *tenorai, Syphacia muris, Gongylonema neoplasticum*), and 1 acanthocephalan (*Moniliformis moniliformis*) were collected. The species composition and prevalence of these helminths differed among the habitats, apparently because of biological characters of the parasites and environmental conditions of the localities.

KEY WORDS: helminths, rat, *Rattus tanezumi, Rattus* *argentiventer, Rattus losea,* Trematoda, *Notocotylus* sp., Cestoda, *Raillietina celebensis, Hymenolepis diminuta,* Nematoda, *Strongyloides ratti, Strongyloides venezuelensis, Nippostrongylus brasiliensis, Orientostrongylus* cf. *tenorai, Syphacia muris, Gongylonema neoplasticum,* Acanthocephala, *Moniliformis moniliformis,* prevalence, ecology, Vietnam.

There have been only limited reports on the parasites of rats from Vietnam (see Segal et al., 1968). Most previous surveys were carried out before 1970, and no data are available to assess the parasitological condition of rats at the present time. In 1999, we had an opportunity to examine helminths collected from rats trapped near Hanoi, northern Vietnam. Ten helminth species, including some of taxonomic and ecological interest, were found as recorded herein.

Rats were collected with live traps in 3 different habitats, i.e., residential areas, paddy fields, and low hilly areas, all in Bac Ninh Province, Vietnam, in December 1999. They were anesthetized with ether and killed. Their viscera were fixed in 10% formalin solution and transported to the Oita Medical University, Oita, Japan. Their heads were also fixed in 10% formalin for species identification on the basis of skull morphology. On examination, the lung, heart, and liver were minced in water with fine forceps under a stereomicroscope to detect helminths parasitic in these organs. Then, the alimentary canal was cut open and washed on a stainless steel sieve with aperture size of 0.1 mm. The residues left on the sieve were transferred to a Petri dish and observed under a stereomicroscope to recover helminths. The stomach wall was observed under a stereomicroscope with transillumination to find nematodes dwelling in the wall.

Helminths collected were cleared in a glycerol–alcohol solution by evaporating alcohol and mounted on glass slides with a 50% glycerol solution. Some trematodes were stained with alum carmine or Heidenhain's iron hematoxylin, dehydrated in an alcohol series with ascending concentration, cleared in xylene and creosote, and mounted with Canada balsam. Voucher parasite specimens and host skulls are deposited in the National Science Museum, Tokyo (NSMT), Japan, with the accession numbers NSMT-Pl 5073–5076, NSMT-As 2944–2953, and NSMT-M 31601–31606.

The 35 rats examined included 12 black rats *Rattus tanezumi* Temminck, 1844 (=so-called Asian-type *Rattus rattus* Linnaeus, 1758; cf. Musser and Carleton (1993)), 14 ricefield rats *Rattus argentiventer* (Robinson and Kloss, 1916), and 9 lesser ricefield rats *Rattus losea* (Swinhoe, 1871). Helminths were not detected from the lung and liver, although *Angiostrongylus cantonensis* (Chen, 1935) and *Calodium hepaticum* (Bancroft, 1893) (syn. *Capillaria hepatica* (Bancroft, 1893)) have been previously recorded from those organs of rats in Vietnam (cf. Segal et al., 1968). Meanwhile, 10 helminth species, comprising 1 trematode, 2 cestodes, 6 nematodes, and 1 acanthocephalan, were collected from the alimentary canal (Table 1). Most of these helminths are common rat parasites, being widely distributed in the surrounding countries (Myers and Kuntz, 1964, 1969; Ow-Yang, 1971; Singh and Cheong, 1971; Wiroreno, 1978; Sinniah, 1979; Ow-Yang and Durette-Desset, 1983; Hasegawa, 1990; Hasegawa et al., 1992, 1994; Hasegawa and Syafruddin, 1995). *Orientostrongylus* sp. was found only in 2 rats from the paddy fields. Because no males were found, species identification is withheld, although it is strongly suggested to be *Orientostrongylus tenorai* Durette-Desset, 1970, a common rat parasite widely distributed from Afghanistan to Taiwan (Durette-Desset, 1970; Ow-Yang and Durette-Desset, 1983; Ohbayashi and Kamiya, 1980; Hasegawa, 1990; Hasegawa et al., 1994; Hasegawa and Syafruddin, 1995).

Among the parasites recovered, 2 cestodes, *Hymenolepis diminuta* (Rudolphi, 1819) and *Raillietina celebensis* (Janicki, 1902); 1 nematode, *Gongylonema neoplasticum* (Fibiger et Ditlevsen, 1914); and 1 acanthocephalan, *Moniliformis moniliformis* (Bremser, 1811) have been recorded previously from Vietnamese rats (Segal et al., 1968). *Notocotylus* sp. is of special interest because trematodes of this genus have been reported only rarely from rats of the subfamily Murinae, although some members have often been recorded from voles of the subfamily Arvicolinae (cf. Yamaguti,

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Table 1. Helminthic infections among rats collected from 3 different habitats in Bac Ninh Province, Vietnam.

Habitat:	Residential area	Paddy field			Low hilly area	
Rattus species:	*R. tanezumi*	*R. argentiventer*	*R. losea*	*R. tanezumi*	*R. argentiventer*	*R. losea*
No. rats examined:	10	7	7	2	7	2
Head and trunk length (cm) range:	14–19	12–20	10–18	17–17.5	16–21	13–14
(Mean):	(16.7)	(15.4)	(14.2)	(17.3)	(17.5)	(13.5)
Trematoda						
Notocotylus sp.	—	1 (14%)*	2 (29)	—	3 (43)	1 (50)
Cestoda						
Raillietina celebensis	2 (20)	—	—	—	—	—
Hymenolepis diminuta	—	1 (14)	—	—	—	—
Nematoda						
Strongyloides spp.†	—	2 (29)	3 (43)	—	1 (14)	—
Nippostrongylus brasiliensis	1 (10)	6 (86)	3 (43)	2 (100)	7 (100)	2 (100)
Orientostrongylus cf. *tenorai*	—	1 (14)	1 (14)	—	—	—
Syphacia muris	6 (60)	4 (57)	3 (43)	2 (100)	2 (29)	2 (100)
Gongylonema neoplasticum	4 (40)	—	—	—	—	—
Acanthocephala						
Moniliformis moniliformis	1 (10)	—	—	—	—	—

* No. rats infected (prevalence in parentheses).
† *Strongyloides ratti* and/or *S. venezuelensis*.

1971). The morphological characteristics of *Notocotylus* sp. are summarized below.

Description: *Notocotylus* sp. Trematoda: Notocotylidae. Body foliate, attenuated anteriorly, 1.39–2.64 mm long by 0.57–0.91 mm wide; 3 longitudinal rows of prominent ventral glands present on ventral surface, each row composed of 12 to 14 glands; oral sucker terminal; esophagus short; ceca diverticulated, terminating just posterior to ovary; testes lobed, located lateral to terminal portion of ceca; genital pore immediately posterior to oral sucker; ovary intertesticular, lobed; vitellaria in lateral fields, extending from middle of body to anterior margins of testes; metraterm and cirrus sac almost equal in length; egg ellipsoidal, 22–24 by 11–12 μm, with polar filaments, 2 to 3 times longer than egg length.

Hosts: *Rattus argentiventer* (Robinson et Kloss, 1916) and *Rattus losea* (Swinhoe, 1871).

Site in hosts: Intestine.

Locality: Paddy field and low hilly area in Bac Ninh Province (21°7′N; 105°59′E), Vietnam.

Specimens deposited: National Science Museum, Tokyo, NSMT-Pl 5073, 5074.

Remarks: In the neighboring areas of Vietnam, only 2 *Notocotylus* species have been recorded from mammals: *Notocotylus mamii* Hsu, 1954, of which adults were experimentally raised in rabbits, and *Notocotylus ratti* Yie, Qiu, Weng, Li, and Li, 1956, the only representative exclusively known from *Rattus*, both from southern China (Hsu, 1954; Yie et al., 1956). *Notocotylus mamii* resembles the present form in the position of the genital pore but is readily distinguished in that the ceca are simple and the eggs have extremely elongated filaments, often more than 10 times longer than the egg length (Hsu, 1954, 1957). Although *N. ratti* resembles the present species in having diverticulated ceca, it is clearly distinguished by possessing a genital pore located posterior to the cecal bifurcation and only 5 to 6 ventral glands in the median row (Yie et al., 1956). Presumably, the present worms represent a new species. However, proposal of a new taxon is withheld because the present worms were contracted by unsuitable fixation, obscuring some of the key structures.

The species composition and prevalence of the rat helminths differed greatly among the habitats surveyed. Only *Nippostrongylus brasiliensis* (Travassos, 1914) and *Syphacia muris* (Yamaguti, 1935) were common to all 3 habitats. *Raillietina celebensis, G. neoplasticum,* and *M. moniliformis* were collected only from the rats captured around houses. Strongyloidid and trematode infections were not found in the rats captured around houses. *Notocotylus* sp.

was recovered from the rats collected in the paddy and hilly areas but not from those in the residential area.

The differences in species composition and prevalence among the habitats could be explained by the biological characters of each helminth and by the environmental conditions. Because members of *Notocotylus* require a freshwater snail as the intermediate host (cf. Yamaguti, 1975), their presence in the paddy fields is reasonable. The hilly area surveyed in the present study contained many small ponds that might provide habitats for the snails. Also not unexpected is that *G. neoplasticum* and *M. moniliformis* were found only in the rats captured around the houses, because their intermediate hosts, usually cockroaches, are abundant in the residential areas. *Nippostrongylus brasiliensis* and *Strongyloides* spp. require moist soil for their embryonic and larval development and transmission. Their low prevalence or absence in the residential areas seems to be due to the dried soil around the houses in that season. *Syphacia muris*, the only helminth with relatively stable prevalence among the habitats, has a quite simple life cycle passed nearly entirely within the body of the host, and hence, its prevalence is less affected by the external environment.

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Research Note

Nematodes of the Tribe Cyathostominea (Strongylidae) Collected from Horses in Scotland

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ABSTRACT: Nematodes of the tribe Cyathostominea are important parasites of horses. They live in large numbers in the large intestine and include over 50 species worldwide. This report describes an enumeration study of species found in a small population of horses in western Scotland. As found previously in a wide range of geographic regions, the 7 most abundant species of Cyathostominea, of the 18 recorded in this study, accounted for over 94% of the total population. One major exception to the results of previous studies was the presence of the most common species in this population, *Cylicocyclus ashworthi.* This species has not been recorded in the U.K. since its original description in 1924 and is morphologically very similar to another member of the same genus, *Cylicocyclus nassatus,* from which it has not been distinguished in previous studies in this geographical region. A rare species, *Tridentoinfundibulum gobi,* was found in low numbers in 3 of 4 horses.

KEY WORDS: Nematoda, Cyathostominea, species survey, prevalence, intensity, horses, morphological identification, Scotland.

Nematodes of the tribe Cyathostominea are the most common helminth parasites of the horse and are ubiquitous in all breeds. Members of the tribe Cyathostominea (Strongylidae) have been commonly referred to as small strongyles, cyathostomins (for the tribe), or cyathostomes (for the genus *Cyathostomum* Molin, 1861) (Hartwich, 1986). However, in order to avoid possible confusion with members of the nematode genus *Cyathostoma* Blanchard, 1849 (Syngamidae), which are sometimes referred to as cyathostomes, we will use the common name cyathostomins to refer to the 51 species included in the tribe Cyathostominea as listed by Lichtenfels et al. (1998). Infections with these nematodes are complex: 51 species of cyathostomins have been recorded in horses, donkeys, and zebras worldwide (Lichtenfels et al., 1998), but 10 of these species have been reported only from zebras or donkeys, and a few other species are known to have very limited distributions. However, most horses carry a burden of 5 to 10 common species, including many thousands (sometimes more than 100,000) of lumen-dwelling adult nematodes and as many larval stages in the walls of the large intestine (Reinemeyer et al., 1984; Bucknell et al., 1995). Clinically, cyathostomins are associated with various syndromes, the most dramatic of which is larval cyathostominosis, a fatal enteritis that occurs secondary to synchronized reactivation of arrested larvae from the intestinal mucosa (Giles et al., 1985; van Loon et al., 1995). The major obstacles to understanding, and therefore controlling, these parasites are their complexity, our inability to identify eggs in the feces, and the difficulty in identifying larvae on pasture. Until recently, the parasitic stages of cyathostomins could be identified only by adult worm morphology. However, recent studies have examined the molecular relationship of these species with a view to developing molecular probes for use in identifica-

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tion of both preparasitic and parasitic stages. For such studies, it is critical that the cyathostomins be identified and classified as consistently as possible. Modern identification manuals exist (Lichtenfels, 1975; Hartwich, 1986; Dvojnos and Kharchenko, 1994), but problems in identifying several species persist (Lichtenfels et al., 1997). In 1997, workers convened an international workshop to clarify the systematics of the Cyathostominea (Sun City, Republic of South Africa), and an agreement was reached on a consensus recommendation for the taxonomy of 51 species as detailed in Lichtenfels et al. (1998).

Despite the importance of these parasites, information is still lacking on species prevalence in Britain, especially since the development of widespread anthelmintic resistance. The last detailed study of species prevalence and infection intensity in the U.K. was in 1976 (Ogbourne, 1976). The present report describes the species of cyathostomins present in the large intestine of a population of ponies from western Scotland. The nematodes were collected to provide DNA sequence information for the development of diagnostic tools and for phylogenetic analysis of the nematodes.

Adult parasites were collected from intestinal contents of 4 Welsh–Shetland cross ponies aged from 9 to 15 mo originating from a local horse population. The history of anthelmintic treatment of the ponies is unknown. These animals were euthanized at the University of Glasgow Veterinary School for reasons other than parasite infestation. Intestinal contents were coarse-filtered with household plastic sieves. After sieving, the contents were passed through a Baermann apparatus with milk filters and then through 38-μm wire-mesh sieves. Individual adult parasites were washed in sterile phosphate-buffered saline (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.47 mM KH_2PO_4, pH 7.2). Where possible, a total of 200 parasites were collected from the ventral and dorsal colon; however, the cecum often contained fewer than 50 adult parasites. With the aid of a dissection microscope, the heads were excised with a scalpel because the bodies were subsequently used for DNA extraction. The heads were stored in 200 μl of 5% buffered formalin, then mounted on glass slides in a few drops of phenol–alcohol (80% melted phenol crystals and 20% absolute ethanol) to which glycerine had been added at about 5% of the volume, and studied with an Olympus BX50® differential interference contrast microscope. The parasites were identified according to the key of Lichtenfels (1975), supplemented by more recent descriptions of certain species (Lichtenfels and Klei, 1988; Kharchenko et al., 1997; Lichtenfels et al., 1997, 1999). The taxonomy used in this report follows the checklist of genera and species recommended by the 1997 international workshop (Lichtenfels et al., 1998). Representative heads of 14 species of cyathostomins and *Craterostomum acuticaudatum* and photomicrographs of 2 species of cyathostomins have been deposited in the U.S. National Parasite Collection, U.S. Department of Agriculture, Beltsville, Maryland 20705-2350 as accession numbers 90698–90714. Two species of cyathostomins, *Cylicocyclus elongatus* and *Cylicocyclus radiatus,* and *Gyalocephalus capitatus* could not be documented by either method.

Eighteen cyathostomin species, representing 5 genera, were identified. Table 1 shows the total numbers of parasite species per animal identified morphologically and the relative numbers of each species collected from each animal. Eight species occurred in all 4 ponies. One rare species, *Tridentoinfundibulum gobi,* was found in Scotland for the first time. It had been reported previously only in Asia and North America (Lichtenfels et al., 1998). In addition, individuals of the genera *Craterostomum* and *Gyalocephalus* were isolated but in smaller numbers than most of the cyathostomin species. The 7 most abundant cyathostomin species were, in descending order, *Cylicocyclus ashworthi, Cyathostomum catinatum, Cylicostephanus longibursatus, Cyclostephanus minutus, Cylicocyclus nassatus, Cylicocyclus insigne,* and *Cylicostephanus goldi.* These species comprised over 94% of the total cyathostomin burden. These results are similar to recent enumeration studies performed in several geographically distinct regions, for example in the U.S.A., Europe, and Australia (Reinemeyer et al., 1984; Mfitilodze and Hutchinson, 1985; Bucknell et al., 1995; Gawor, 1995). In addition, in terms of local studies performed previously in the U.K., the species identified here were very similar to those reported by Mathieson in Scotland (1964); Ogbourne in southwest England (1976), and Love and Duncan in Scotland (1992). Ogbourne (1976) performed the most extensive study and identified 21 species in 86 horses of various ages

Table 1. Numbers of specimens of nematodes, by species, collected from 4 ponies from western Scotland.

Parasite species	Pony 1	Pony 2	Pony 3	Pony 4
Cylicocyclus ashworthi (Le Roux, 1924) McIntosh, 1933	94	77	81	83
Cylicocyclus nassatus (Looss, 1900) Chaves, 1930	10	51	47	15
Cylicocyclus insigne (Boulenger, 1917) Chaves, 1930	24	6	43	14
Cylicocyclus ultrajectinus (Ihle, 1920) Ershov, 1939	5	1	1	0
Cylicocyclus leptostomum (Kotlan, 1920) Chaves, 1930	0	1	3	0
Cylicocyclus radiatus (Looss, 1900) Chaves, 1930	0	0	1	0
Cylicocyclus elongatus (Looss, 1900) Chaves 1930	0	0	1	0
Cyathostomum catinatum Looss, 1900	20	51	80	73
Cyathostomum pateratum (Yorke and Macfie, 1919) K'ung, 1964	9	0	6	0
Coronocyclus coronatus (Looss, 1900) Hartwich, 1986	1	2	0	0
Coronocyclus labiatus (Looss, 1900) Hartwich, 1986	0	0	0	1
Cylicostephanus calicatus (Looss, 1900) Ihle, 1922	1	2	3	0
Cylicostephanus longibursatus (Yorke and Macfie, 1918) Cram, 1924	24	11	85	23
Cylicostephanus minutus (Yorke and Macfie, 1918) Cram, 1924	32	55	6	49
Cylicostephanus goldi (Boulenger, 1917) Lichtenfels, 1975	26	13	19	4
Cylicostephanus bidentatus (Ihle, 1925) Lichtenfels, 1975	2	4	9	1
Cylidodontophorus bicoronatus (Looss, 1900) Ihle, 1922	0	2	0	0
Tridentoinfundibulum gobi Tshoijo, in Popova, 1958	1	1	3	0
Craterostomum acuticaudatum (Kotlan, 1919) Ihle, 1920	4	3	0	1
Gyalocephalus capitatus Looss, 1900	2	0	0	0

and breeds. In the latter study, 80% of these horses had *C. longibursatus, C. goldi, C. calicatus, C. catinatum, C. coronatus,* and *C. nassatus*. The most notable exception between the current study and all of these previous studies is the presence of *C. ashworthi*, the most prevalent species identified in our population. *Cylicocyclus ashworthi* was last reported in the U.K. as a new species (Le Roux, 1924) and has not been reported there since. Of note is that, in a comparable study performed several years earlier on worm populations derived from the same pastures as those used here, Love and Duncan (1992) identified 6 species, and *C. nassatus* was one of the most numerous. *Cylicocyclus ashworthi* and *C. nassatus* are morphologically very similar, and it is highly likely that these and other workers misidentified *C. ashworthi* as *C. nassatus* prior to the recent redescriptions of these species (Lichtenfels et al., 1997). *Cylicocyclus nassatus* is characterized by a cuticular shelf on the inner surface of the buccal capsule, a dorsal gutter that is as long as 50% of the buccal capsule depth, and 20 elements in the external leaf crown. *Cylicocyclus ashworthi* can be distinguished from *C. nassatus* by the absence of the shelf from the inner surface of the buccal capsule, by its much shorter dorsal gutter, and by 25–29 external leaf crown elements that differ in shape from those of *C. nassatus* (Lichtenfels et al., 1997). The ability to clearly observe the cuticular shelf in the buccal capsule is dependent on the clearing agent used, and this may have contributed to the difficulty in identifying this unique feature in previous studies.

In addition to the historical difficulty in separating *C. nassatus* and *C. ashworthi, C. ashworthi* has also been misidentified as *C. triramosus,* which has also been confused with *C. nassatus* prior to its recent redescription (Kharchenko et al., 1997). We now know that *C. triramosus* is exclusively a parasite of zebras. It is imperative that *C. nassatus* and *C. ashworthi* be correctly differentiated because they are 2 of the most common nematodes found in the ventral colon of horses, and if DNA probes are to be developed on the basis of morphological delineation, then consistent identification is a prerequisite. Interestingly, Hung et al. (1997) performed sequencing of the first (ITS-1) and second (ITS-2) internal transcribed spacers of 5.8S ribosomal DNA of these species and found that *C. nassatus* and *C. ashworthi,* differentiated by head morphology, were sufficiently different at the DNA level to assign them to separate species. These results are similar to work performed on the intergenic spacer region of the nuclear DNA, where over 50% DNA sequence difference was found between these 2 species (Kaye et al., 1998), with low intraspecific variation

(0.3% for *C. ashworthi* and 1.9% for *C. nassatus*). Subsequently, oligoprobes designed from these IGS sequences have been used successfully to distinguish DNA of individual *C. nassatus* and *C. ashworthi* (Hodgkinson et al., 2001). Furthermore, pairwise evolutionary distances, calculated under maximum likelihood with a GTR model estimated from data from the mitochondrial large ribosomal RNA subunit and ITS-2 DNA, showed a 9.5% difference between the 2 species (McDonnell et al., 2000).

A consistent feature of all of the species incidence studies, including the present work, is that a small number of species, usually 5 to 10, constitute more than 80% of the infective load. Furthermore, the proportions of species have remained remarkably stable over many years, despite the widespread use of anthelmintics and the development of resistance. The rarer species are found at less consistent levels, but this is expected because the small populations may have gone undetected in cases where small subsamples of the worm populations have been examined for practical reasons. Consequently, much of the data on the least commonly discovered species are certain to underestimate true prevalence. Chapman et al. (1999) reported that 9 to 15 species were found in a single animal when 200 specimens were identified, but the number increased to 20 to 29 when all nematodes in an entire 5% aliquot were identified.

In the current study, *Strongylus* species were not found. This is probably indicative of the efficacy of anthelmintics and their strategic use in parasite control programs. In older studies, 100% prevalence of *Strongylus* species was reported (Le Roux, 1924; Foster and Ortiz, 1937). More recently, Bucknell et al. (1995) reported a prevalence of 38% *Strongylus* species in a study in which the deworming history of the horses was not known. Here, it was observed that, whereas relatively few species occurred exclusively in one or other parts of the intestines, most followed distinct site distributions (not shown), strongly biased in favor of a particular region, and (with the exception of *C. ashworthi*) these distributions were as described by Ogbourne (1976). Here, as in Ogbourne's study, the majority of *C. pateratum, C. insigne,* and *C. longibursatus* individuals were found in the dorsal colon, whereas most of the *C. nassatus, C. ultrajectinus, C. catinatum,* and *C. goldi* adults were found in the ventral colon. Some caution must be taken in the interpretation of these data because some of the species were found only in very low numbers. Also consistent with the findings of Ogbourne (1976) was that the cecum was the most sparsely populated region of the large intestine.

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Research Note

Helminth Parasites of the Green Frog (*Rana clamitans*) from Southeastern Wisconsin, U.S.A.

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ABSTRACT: Between 13 August and 3 September 1999, 26 green frogs *Rana clamitans* Rafinesque, 1820, were collected from 2 ponds at the University of Wisconsin–Milwaukee Field Station in Ozaukee County, Wisconsin, U.S.A. Hosts were euthanized and organs were examined for helminth parasites. All host individuals were infected with 1 or more helminth parasites. A total of 11 helminth species infected *R. clamitans* at this location: 9 platyhelminths (7 trematodes, 2 cestodes) and 2 nematodes. Mean abundance of infection was 65.5 ± 79.7 worms per host (range = 1–330). This is the first report of *Clinostomum* sp. from green frogs in Wisconsin.

KEY WORDS: Amphibia, aquatic, Cestoda, *Clinostomum,* ephemeral pond, *Haematoloechus varioplexus,* green frog, helminth, Nematoda, parasites, *Rana clamitans,* survey, temporary pond, Trematoda, Wisconsin, U.S.A.

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The green frog *Rana clamitans* Rafinesque, 1820, occurs from Newfoundland, where the population was introduced (Conant and Collins, 1991), to western Ontario, Canada, in the northern extent of its range and from North Carolina to eastern Oklahoma, U.S.A. in the south (Vogt, 1981). Although reports of green frog parasites are numerous, only 3 studies have been conducted in Wisconsin, U.S.A. (Williams and Taft, 1980; Coggins and Sajdak, 1982; Bolek, 1998).

A total of 26 green frogs were collected by hand between 13 August and 3 September 1999 from 2 temporary ponds at the University of Wisconsin–Milwaukee Field Station, Ozaukee County, Wisconsin (43°23′N; 88°2′W). Frogs were transported to the laboratory and euthanized in MS-222 (ethyl m-aminobenzoate sulfonic acid). Body surface, mouth, eustachian tubes, celom, lungs, stomach, small intestine, colon, urinary bladder, liver, kidneys, and leg musculature in individual containers were examined with a dissecting microscope for the presence of helminth parasites. Nematodes were preserved in 70% ethanol and mounted in glycerin for identification. Larval and adult platyhelminths were fixed in alcohol–formalin–acetic acid, stained with acetic carmine, and mounted in Canada balsam. Voucher specimens were deposited at the H. W. Manter Helminth Collection, University of Nebraska, Lincoln, Nebraska (Table 1). Use of ecological terms follows the suggestions of Bush et al. (1997).

All host individuals were infected with 1 or more helminths (prevalence = 100%). The component community of green frogs consisted of 11 helminth species: 7 trematodes, 2 cestodes, and 2 nematodes (Table 1). Overall mean abundance of helminths was 65.5 ± 79.7 worms per frog (range = 1–330). *Haematoloechus varioplexus* occurred with highest mean abundance, mean intensity, and prevalence of infection (Table 1). Nematodes occurred in low numbers and in few hosts (Table 1).

Adult green frogs breed in a variety of permanent bodies of water (May–July in Wisconsin) and inhabit the periphery of these aquatic habitats throughout the summer (Vogt, 1981). During this time, adult frogs feed upon a variety of animals, including several species of insects with aquatic life histories (Jenssen and Klimstra, 1966). Whereas green frogs are known to migrate prior to hibernation, they are thought to seek out aquatic habitats that are well oxygenated and do not freeze entirely in winter (Lamoureux and Madison, 1999). The ponds sampled in the present study are ephemeral. Even in years when some water remains over winter, these ponds freeze solid. The green frogs that we collected seem to have moved into these ponds as a place to feed prior to hibernating in other areas.

The species composition and numbers of helminths in green frog infracommunities at this location were similar to those reported previously (Rankin, 1945; Bouchard, 1951; Najarian, 1955; Campbell, 1968; Williams and Taft, 1980; Coggins and Sajdak, 1982; Muzzall, 1991; McAlpine, 1997; Bolek, 1998; McAlpine and Burt, 1998). The aquatic habitat and diet of green frogs correspond with helminth communities consisting mostly of platyhelminths with indirect life cycles and relatively few direct life cycle nematodes. In the present study, *H. varioplexus* occurred with the highest values of prevalence, mean intensity, and mean abundance. These values are also high compared with those reported in previous studies. Muzzall (1991) reported 57% of 120 green frogs infected with *H. parviplexus,* synonymous with *H. varioplexus* (Kennedy, 1981), with a mean intensity of 29. Najarian (1955) reported 48% of 40 green frogs infected with *H. parviplexus* and 42% prevalence for *H. breviplexus* but did not provide values for intensity or abundance of infection. Bolek (1998) reported a prevalence of 44% for *H. varioplexus* from 75 green frogs with a mean intensity of 5.3. Others have reported prevalence values of 25% or less for *Haematoloechus* spp. from *R. clamitans* (Rankin, 1945; Bouchard, 1951; Campbell, 1968; Williams and Taft, 1980; McAlpine and Burt, 1998). *Haematoloechus varioplexus* has been reported previously from wood frogs (*Rana sylvatica* Le Conte, 1825) and spring peepers (*Pseudacris crucifer* Wied, 1839) from the same ponds sampled in the current study (Yoder and Coggins, 1996). It is therefore likely that infected intermediate hosts are present in these ponds. Additionally, large numbers of immature *H. varioplexus* were recovered from green frogs, indicating that hosts are being infected while feeding at these locations. Odonates serve as second intermediate hosts for species of *Haematoloechus*. Muzzall (1991) reported that the absence of fish predators may have increased the number of adult odonates emerging from Turkey Marsh, Michigan, U.S.A., resulting in richer helminth communities than those occurring in habitats where both frogs and fish occur. The absence of fish from these ephemeral ponds may have had a similar result in terms of high values of parasitism by *H. vario-*

Table 1. Number, prevalence, mean intensity, range, and mean abundance of helminth parasites from *Rana clamitans*.

Helminth species (accession no.)	No. helminths	Site*	Prevalence (%)	Mean intensity ± SD (range)	Mean abundance ± SD
Trematoda					
m9*Haematoloechus varioplexus* Stafford, 1902 (HWML 15377)					
Mature	915	L	80.7	43.6 ± 45.5 (1–84)	35.2 ± 44.3
Immature	717	L	69.2	39.8 ± 51.6 (1–176)	27.6 ± 46.5
Total	1,632	L	84.6	74.2 ± 81.2 (1–330)	62.8 ± 79.3 (0–330)
Halipegus eccentricus Thomas, 1939 (HWML 15378)	4	ET	7.7	2.0 ± 0 (1–2)	0.2 ± 0.5
Glypthelmins quieta Stafford, 1900 (HWML 15379)	51	SI	26.9	7.3 ± 7.5 (1–19)	2.0 ± 5
Gorgoderina bilobata Rankin, 1937 (HWML 15380)	1	UB	3.9	1.0	0.04 ± 0.2
Megalodiscus temperatus Stafford, 1905 (HWML 15381)	3	C	7.7	1.5 ± 0.7 (1–2)	0.1 ± 0.4
Clinostomum sp. Leidy, 1856 (HWML 15382)†	NC‡	BC	3.9	NC	NC
Metacercaria†	NC	M	15.4	NC	NC
Cestoda					
Proteocephalus sp. Weinland, 1858 (HWML 15383)†	2	BC	7.7	1.0 ± 0	0.1 ± 0.3
Mesocestoides sp. Vaillant, 1863 (HWML 15384)†	NC	M	15.4	NC	NC
Nematoda					
Oswaldocruzia pipiens Walton, 1929 (HWML 15386)	5	SI	11.5	1.7 ± 1.2 (1–3)	0.2 ± 0.6
Cosmocercoides sp. Wilkie, 1930 (HWML 15387)	2	C	7.7	1.0 ± 0.0	0.1 ± 0.3

* BC = body cavity; C = colon; ET = eustachian tube; L = lung; M = mesentery; SI = small intestine; UB = urinary bladder.

† Larval stage.

‡ NC = not counted.

plexus. This is the first report of *Clinostomum* sp. from Wisconsin green frogs.

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Research Note

Gastrointestinal Helminths of Spinner Dolphins *Stenella longirostris* (Gray, 1828) (Cetacea: Delphinidae) Stranded in La Paz Bay, Baja California Sur, Mexico

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ABSTRACT: Thirty-one spinner dolphins *Stenella longirostris* stranded in La Paz Bay, Baja California Sur, Mexico, were examined for endoparasitic helminths. The following species were identified: *Zalophotrema pacificum* and *Hadwenius tursionis* (Digenea); *Strobilocephalus triangularis, Trigonocotyle* sp., and Tetraphyllidea gen. sp. larva (Cestoda); immature *Bolbosoma hamiltoni* (Acanthocephala); and *Anisakis typica*

(Nematoda). Except for *H. tursionis,* all the identified helminths are reported for the first time in Mexico. *Stenella longirostris* represents a new host for *H. tursionis* and *A. typica.*

KEY WORDS: Cetacea, spinner dolphin, *Stenella longirostris,* parasites, Digenea, Cestoda, Nematoda, Acanthocephala, Gulf of California, Mexico.

Although cetaceans, including dolphins, are common in marine waters of Mexico, their parasite fauna is poorly known. To date, only 2 reports on the helminth parasites of cetaceans in Mexico have been published. Lamothe-Argumedo (1987) identified the trematode *Hadwenius tursionis* (Marchi, 1873) in the intestine of the vaquita *Phocoena sinus* Norris and McFarland, 1958 (Phocoenidae), from the northern Gulf of California, and Morales-Vela and Olivera-Gómez (1993) reported the trematode *Nasitrema globicephala* Neiland, Rice, and Holden, 1970, and the nematodes *Stenurus globicephalae* Baylis and Daubney, 1925, *Stenurus minor* (Kuhn, 1829), and *Crassicauda* sp. in the pilot whale *Globicephala macrorhynchus* Gray, 1846 (Delphinidae), from Cozumel Island, Quintana Roo (Caribbean Sea). The present report provides data on helminth occurrence in spinner dolphins *Stenella longirostris* (Gray, 1828) from the state of Baja California Sur, Mexico.

In August 1993, 31 spinner dolphins were stranded in La Paz Bay (24°07′–24°21′N; 110°17′–110°40′W), 20 km SW of the city of La Paz, Baja California Sur. The stranded dolphins consisted of 17 males (total length 130–188 cm, weight 19–57 kg, ages 1–18 yr) and 14 females (161–186 cm, 33–45 kg, 5.5–15 yr). The animals died of unknown causes during the strand, and they were kept deep frozen (−22°C) until examination. During necropsy, the digestive tract of each animal was separated from its other viscera and examined for parasites. Trematodes and cestodes were fixed with Bouin's fluid and preserved in 70% ethanol, and acanthocephalans and nematodes were fixed and preserved in 70% ethanol. All helminths identified during the examination have been deposited in the Colección Nacional de Helmintos (National Helminth Collection) (CNHE) of the Universidad Nacional Autónoma de México.

Seven helminth species were recovered from the 31 dolphins. These include 2 trematodes: *Zalophotrema pacificum* Dailey and Perrin, 1973 (bile ducts, prevalence 19%, mean intensity 6 worms per parasitized host, range 1–16, CNHE No. 4018) and *Hadwenius tursionis* (Marchi, 1873) (intestine, 6%, 1, 1–1, CNHE No. 4017); 3 cestodes: *Strobilocephalus triangularis* (Diesing, 1850) (rectum, 6%, 2, 2–2, CNHE No. 4019), *Trigonocotyle* sp. (intestine, 90%, 5, 1–27, CNHE No. 4021; the poor condition of specimens preclude identification of species), and larval stages of Tetraphyllidea (intestine, 16%, 31, 5–69, CNHE No. 4020); the nematode *Anisakis typica* (Diesing, 1860) (stomach, 77%, 18, 1–98, CNHE No. 4023); and the immature acanthocephalan *Bolbosoma hamiltoni* Baylis, 1929 (posterior intestine, 51%, 4, 1–9, CNHE No. 4022).

The helminth parasites of *S. longirostris* have been reported by Delyamure (1955), Dailey and Brownell (1972), and Dailey and Perrin (1973). The previously recorded helminth fauna for this dolphin species includes the following: the trematodes *Oschmarinella laevicaecum* (Yamaguti, 1942), *Campula rochebruni* (Poirier, 1886), *Delphinicola tenuis* Yamaguti, 1933, *Lecithodesmus nipponicus* Yamaguti, 1942, and *Z. pacificum*; the cestodes *Diphyllobothrium fuhrmanni* Hsü, 1935, *S. triangularis, Tetrabothrium forsteri* (Krefft, 1871), *Phyllobothrium delphini* (Bosc, 1802), *Phyllobothrium* sp., *Monorygma grymaldii* (Moniez, 1881), and *Monorygma* sp.; the nematodes *Anisakis simplex* (Rudolphi, 1809), *Halocercus delphini* Baylis and Daubney, 1925, and *Mastigonema stenellae* Dailey and Perrin, 1973; and the acanthocephalans *Bolbosoma vasculosum* (Rudolphi, 1819), *Bolbosoma balaenae* (Gmelin, 1790), and *Corynosoma* sp. In the present study, the previously recorded *Z. pacificum* and *S. triangularis* are identified, and new host records are reported for *H. tursionis, Trigonocotyle* sp., tetraphyllidean *B. hamiltoni,* and *A. typica.* All but 1 of the identified species (*H. tursionis*) are recorded for the first time in Mexico.

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Research Note

The Lung Nematodes (Metastrongyloidea) of the Virginia Opossum *Didelphis virginiana* in Southern California, U.S.A.

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ABSTRACT: The lungworm *Heterostrongylus heterostrongylus* (Nematoda: Metastrongyloidea) is reported for the first time from the Virginia opossum *Didelphis virginiana* in North America. Seventeen of 31 opossums (55%) examined from San Diego County, California, U.S.A., were infected with *H. heterostrongylus,* with intensities ranging from 8 to 128 worms per host (mean 41). Another species of metastrongyloid nematode, *Didelphostrongylus hayesi,* was found in 74% of the lungs examined, with intensity ranging from 2 to 1,328 worms per host (mean 312).

KEY WORDS: lungworm, *Heterostrongylus heterostrongylus,* Nematoda, opossum, *Didelphis virginiana, Didelphostrongylus hayesi,* California, U.S.A.

The Virginia opossum *Didelphis virginiana* Kerr, 1792, is the only marsupial inhabiting North America, occurring in tropical, subtropical, and temperate habitats from southern Canada to Costa Rica (Gardner, 1973). California, U.S.A., was outside the original range of *D. virginiana* until its accidental introduction into Los Angeles County and the San Jose area from various eastern states between 1890 and 1910. By 1958, *D. virginiana* was distributed widely in all the areas of California below 1,500 m altitude (Hunsaker, 1977).

Until recently, the metastrongyloid lungworms of *D. virginiana* have been studied only in the midwestern and eastern U.S.A. Alden (1995) reviewed helminth records from the Virginia opossum and listed records of *Didelphostrongylus hayesi* Prestwood, 1976, from North Carolina, Georgia, Louisiana, and Tennessee, U.S.A. Subsequently, Baker et al. (1995) record-

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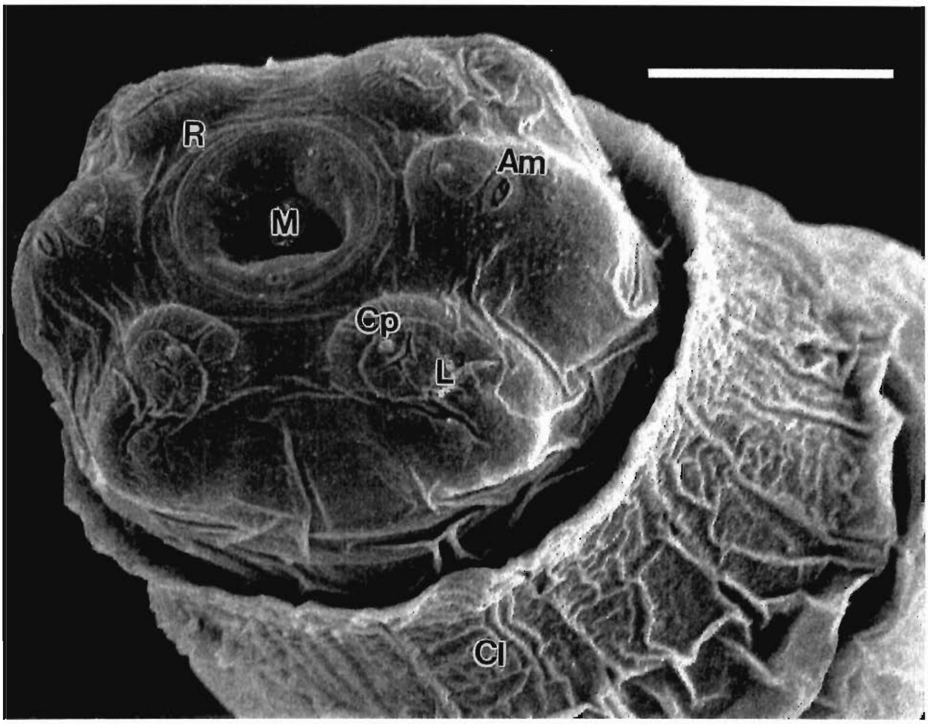

Figure 1. **Cephalic end of the lung nematode *Heterostrongylus heterostrongylus* from the Virginia opossum *Didelphis virginiana*. Male, frontal view. SEM. Am = amphid; Cl = collarette; Cp = cephalic papilla; L = lip; M = mouth opening; R = ring surrounding mouth. Scale bar = 20 μm.**

ed *D. hayesi* from the Virginia opossum from Sacramento County in northern California. The objective of our study was to determine the identity and prevalence of lung parasites in feral Virginia opossums from southern California.

Thirty-one Virginia opossums from San Diego County, California, killed by cars or euthanized after trauma, were examined for lung parasites from March 1999 to June 2000. All samples were obtained from a local nonprofit organization, Project Wildlife, Opossum Team, members of which also carried out the necropsy of the animals. All specimens were categorized, on the basis of weight, into juveniles (0.14–0.90 kg) or adults (1.2–3.4 kg). The lungs with attached trachea of 7 juveniles and 24 adults were examined grossly and under the dissecting microscope. The trachea, bronchi, and bronchioles were split, and the lung parenchyma was teased apart gently. Worms recovered were fixed in 5% formalin or alcohol-formalin-acetic acid (AFA). For light microscopy, worms were examined as temporary whole mounts in glycerine after clearing in glycerine–alcohol with a Diastar® microscope equipped with a Photostar® camera system and were measured in micrometers. For scanning electron microscopy (SEM), the specimens were postfixed in 1% osmium tetroxide, followed by dehydration in an ethanol series, critical point dried with liquid CO_2, sputter coated with gold–palladium, and examined with a Hitachi S-2700® scanning electron microscope. Voucher specimens of nematodes were deposited in the H. W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (accession numbers 15617–15619).

In all, 7,381 lungworms were found in adult and juvenile animals examined. The parasites were identified as metastrongyloid nematodes. Of these, 91.1% were identified as *D. hayesi* and 8.9% were identified as *Heterostrongylus heterostrongylus* Travassos, 1925. This is the first record of *H. heterostrongylus* from *D. virginiana* and the first record of this nematode in North America. Previous records of *H. heterostrongylus* were from another species of opossum, *Didelphis marsupialis* Linnaeus, 1758, from Colombia and Brazil, South America (Travassos, 1925; Vaz and Pereira, 1934; Anderson et al., 1980).

Morphologic and morphometric features of *H. heterostrongylus* in the opossums from southern California resembled those of specimens from South America described by Anderson et al. (1980). In *D. virginiana,* the male worms were slightly smaller, and the female worms were larger than in *D. marsupialis.* The mean length and width of *H. heterostrongylus* males from *D. virginiana* were correspondingly 6.5 mm (5.0–7.2) and 280 μm (245–320). For females, the mean length was 9.7 mm (8.6–13.4) and the mean width was 380 μm (350–475). SEM study showed some features of the cephalic structures that were not noted by Anderson et al. (1980). The 6 lips are completely fused, and each of them, in addition to 2 cephalic papillae, bears an amphid opening on the surface by an amphidial canal (Fig. 1). The shape of the mouth opening varies from triangular to circular (Fig. 1). A delicate ring surrounding the mouth opening and a collarette formed by a dilated cuticle are considered as permanent structures (Fig. 1). A new character of the female caudal extremity found in our study is a pair of small caudal papillae near the tip of the short blunt tail. The morphology of the male bursa, with its large lobe formed by the dorsal ray and short (93–100 μm), complex and slightly arcuate spicules, is the same as previously reported.

In the infected opossums, *H. heterostrongylus* were found lying freely in the bronchi. In 1 case, worms were recovered from the trachea.

Seventeen of 31 opossums (55%) were infected with *H. heterostrongylus,* and intensities of infection ranged from 8 to 128 (mean 41). Infections were found in 58% of adult animals, with 12 to 128 worms per host (mean 41), and 43% of juveniles, with intensities of 8 to 80 worms per host (mean 44). The other species of lung nematodes, *D. hayesi,* was found under the pleura and was piercing lung tissue in 23 of 31 opossums (74%). Intensities of infection ranged from 2 to 1,328 worms per host (mean 312). All animals infected by *H. heterostrongylus* were also infected by *D. hayesi.*

Baker et al. (1995) reported on the prevalence and treatment of *D. hayesi* infections in opossums collected in Yolo, Solano, and Sacramento counties in northern California. Infections were found in 23 of 33 opossums (70%). Because most of their infections were diagnosed by the presence of metastrongylid larvae in the feces and only 2 infections were confirmed by necropsies, it is possible that mixed infections of *H. heterostrongylus* and *D. hayesi* were also present in that area of California. Examination of additional host samples is desirable both in California and the eastern U.S.A. to determine the local range of *H. heterostrongylus.*

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Research Note

New Host and Distribution Records of *Cosmocephalus obvelatus* (Creplin, 1825) (Nematoda: Acuariidae), with Morphometric Comparisons

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ABSTRACT: This is the first record of *Cosmocephalus obvelatus* (Creplin, 1825) Seurat, 1919 (Nematoda: Acuariidae), from Argentina (Valdés Peninsula, province of Chubut) and from the Magellanic penguin *Spheniscus magellanicus* (Aves: Spheniscidae). The prevalence of this parasite was 31.3% and the mean intensity was 5.4. Despite the wide geographic distribution and the great variety of hosts parasitized by *C. obvelatus* (14 families belonging to 8 orders), there were no significant differences in morphological characteristics and measurements from previous records. Both the wide distribution and the morphometrical stability of *C. obvelatus* may be explained by its ecology and mode of transmission.

KEY WORDS: *Cosmocephalus obvelatus*, Acuariidae, Nematoda, *Spheniscus magellanicus*, Spheniscidae, marine birds, Argentina.

Cosmocephalus obvelatus (Creplin, 1825) Seurat, 1919, an acuariid nematode with a wide distribution, has been previously reported in Europe, Asia, Africa, New Zealand, and North America (Wong and Anderson, 1982). In South America, there is only 1 record of *C. obvelatus*, described as *Cosmocephalus tanakai* by Rodrigues de Olivera and Vicente (1963) from the black-backed gull *Larus dominicanus* Lichtenstein, 1823, in Brazil. Later, *C. tanakai* was synonymized with *C. obvelatus* by Anderson and Wong (1981). This parasite has a wide range of hosts, having been previously recorded in members of Lariidae, Pelecanidae, Rynchopidae, Sternidae, Anatidae, Podicipedidae, Phalacrocoracidae, Gaviidae, Ardeidae, Stercoraridae, Haematopodidae, Treschiornitidae, and Accipitridae (Baruš and Majudmar, 1975; Borgsteede and Jansen, 1980; Anderson and Wong, 1981; Tuggle and Schmeling, 1982). Among members of the Spheniscidae, *C. obvelatus* has been cited only from the rockhopper penguin *Eudyptes crestatus* (Miller, 1784) caught in Chile and transferred to the Japanese Zoological Garden (Azuma et al., 1988).

This note reports the first record of *C. obvelatus* in the Magellanic penguin *Spheniscus magellanicus* (Forster, 1781) (Aves: Spheniscidae). It is also the first time that *C. obvelatus* has been found in Argentina. Measurements of the specimens in this study are compared with those given by previous authors. Morphological details seen in the scanning electron microscope (SEM) and dates of prevalence and mean intensity are provided.

At irregular intervals from 1996 to 2000, 16 specimens of *S. magellanicus*, all of which had recently died of unknown but presumably natural causes, were collected along the coasts of the Valdés Peninsula (42°04′–42°53′S, 63°38′–64°30′W), province of Chubut, Argentina. After dissection, the digestive tract was fixed in 10% formalin. Acuariid nematodes were removed from the esophagus and stored in 70% ethanol. The specimens were cleared in lactophenol and studied under the light microscope. Some specimens were dried by the critical point method, examined by SEM (Jeol/SET 100®), and photographed. Voucher specimens were deposited in the Helminthological Collection of the Museo de La Plata (CHMLP), La Plata, Argentina (Accession no. 4811).

The measurements of our specimens and those given by previous authors are listed in Table 1. Morphological details are shown in Figures 1–6. The prevalence was 31.25% and the mean intensity was 5.4. The esophagus was the only site of infection. We observed several de-

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Table 1. Comparative measurements of *Cosmocephalus obvelatus* from different hosts and localities.

	Cram (1927)	Khalil (1931)	Rao (1951)	Rodrigues de Olivera and Vicente (1963)	Anderson and Wong (1981)	Bowie (1981)	Azuma et al. (1988)	This report
Host*	Several	*Pelecanus* sp.	*Larus* sp.	*Larus* sp.	*Larus delawarensis*	*Larus dominicanus*	*Eudypes crestatus*	*Spheniscus magellanicus*
Locality	Europe	Egypt	Canada	Brazil	Canada	New Zealand	Chile	Argentina
Female								
n	—	1 immature	—	1	10	4	19	10
Total length (mm)	9.7–20	5.7	37122	12.5	19.4 (15.8–22.3)	10.85 (7.61–17.5)	11.7–22.8	16 (13.5–22.12)
Maximum width (μm)	300–380	—	200–400	277	393 (320–500)	270 (130–440)	280–480	425 (296–627)
Buccal capsule (μm)	—	—	—	363	615 (570–730)	—	480–760	566 (525–637)
Nerve ring (μm)	—	—	—	—	684 (640–770)	398 (378–421)	440–840	646 (585–780)
Deirids (μm)	490	—	—	—	685 (610–790)	458 (368–647)	450–900	687 (611–793)
Excretory pore (μm)	—	—	—	—	813 (705–940)	—	37118	777 (650–962)
Muscular esophagus (mm)	—	—	—	0.77	1.3 (1.2–1.5)	—	0.80–1.56	0.98 (0.72–1.2)
Glandular esophagus (mm)	—	—	—	3.43	4.7 (4.1–5.1)	—	2.32–5.24	4.04 (3.16–4.98)
Total esophagus (μm)	—	680	—	4.2	6.0 (5.2–6.6)	3.37 (2.83–3.5)	3.12–6.80	5.07 (3.95–6.19)
Postdeirids	—	—	—	—	End of lateral alae	End of lateral alae	—	8.4
Vulva (from anterior end) (mm)	5.5	Midbody	Midbody	6.2	8.4 (7.4–10.4)	44.5% of body length	4.3–13.6	7.6 (6.27–9.23)
Vagina vera (μm)	—	—	—	—	Long	—	—	85 (45–150)
Vagina uterina (μm)	—	—	—	—	Short	—	—	173 (120–240)
Egg length (μm)	36	—	35–37	36	43 (40–45)	39 (36–42)	34–37	36 (33–40)
Egg width (μm)	20	—	17–18	19	25	21 (19–23)	18–22	20 (18–21)
Tail (μm)	230	180	—	175	301 (220–380)	—	200–300	248 (182–373)
Male								
n	—	2	—	2	10	10	8	9
Total length (mm)	5.7–12.2	7.6	37085	9.5–12	12.4 (9.9–14.3)	10.89 (8.97–12)	9.6–13	9.47 (8.08–10.4)
Maximum width (μm)	240–255	—	150–270	250–280	279 (200–350)	275 (230–390)	240–300	272 (195–390)
Buccal capsule (μm)	—	—	—	369	418 (380–510)	—	440–500	415 (360–481)
Nerve ring (μm)	—	—	—	462	474 (420–530)	—	460–580	460 (390–552)
Deirids (μm)	430	—	—	399	450 (350–540)	469 (442–493)	440–600	491 (369–671)
Excretory pore (μm)	—	—	—	532–630	583 (500–680)	550 (531–578)	520–720	589 (474–820)
Muscular esophagus (mm)	—	—	—	0.98–1.32	1.1 (1.0–1.3)	—	0.8–1.08	0.82 (0.68–1.06)
Glandular esophagus (mm)	—	—	—	2.85–4.61	4.0 (3.6–4.3)	—	2.76–4.08	3.24 (2.52–3.99)
Total esophagus (μm)	—	930	—	3.83–5.93	5.1 (4.6–5.4)	5.29 (4.91–5.79)	3.56–5.16	4.05 (3.30–5.05)
Postdeirids	—	—	—	—	End of lateral alae	60.1% of body length	—	6.1

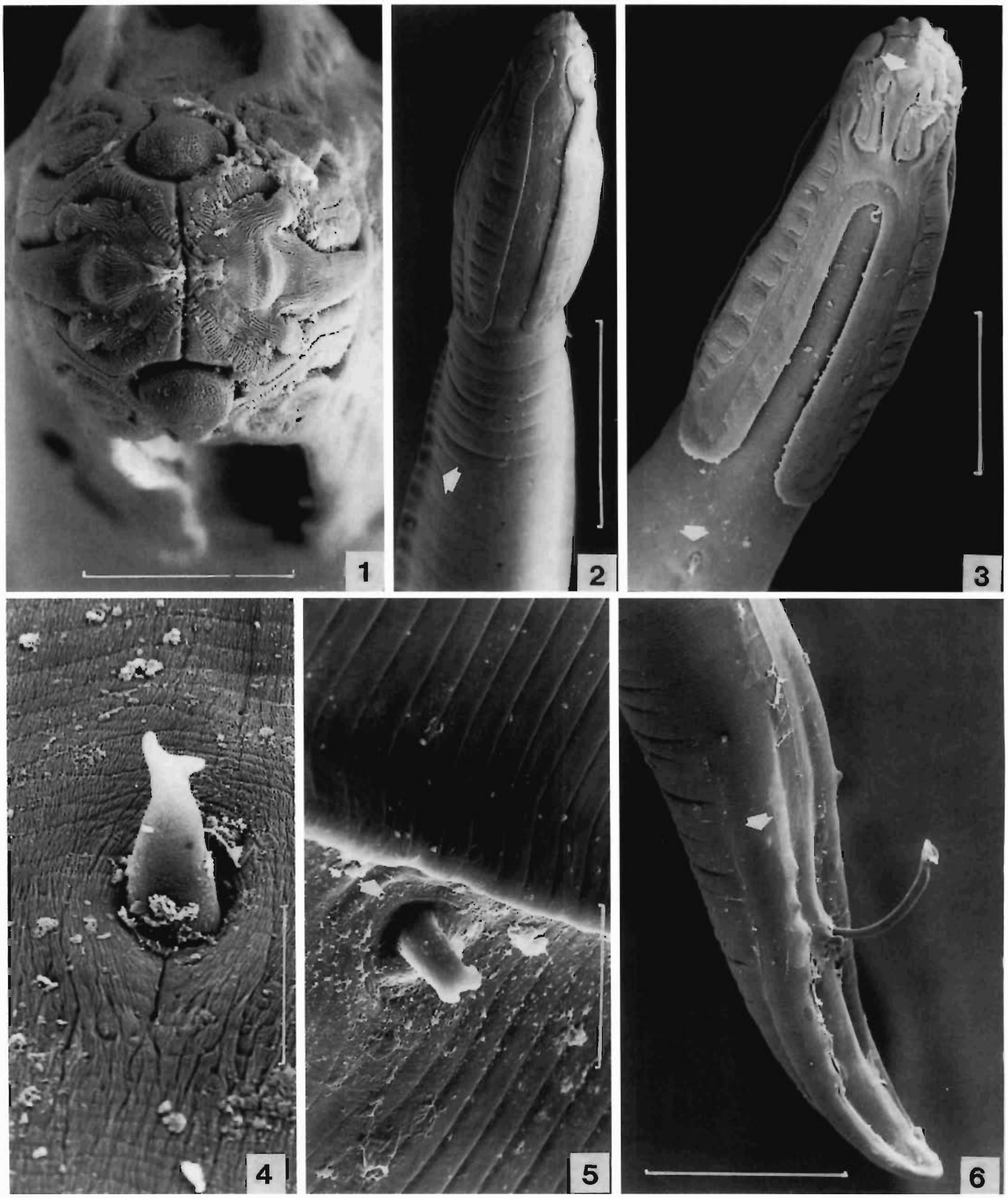
Table 1. Continued.

	Cram (1927)	Khalil (1931)	Rao (1951)	Rodrigues de Olivera and Vicente (1963)	Anderson and Wong (1981)	Bowie (1981)	Azuma et al. (1988)	This report
Right spicule (μm)	130–155	160	140–150	—	195 (180–220)	145 (129–161)	160–180	162 (127–212)
Left spicule (μm)	420–540	540	480–540	—	633 (590–700)	542 (537–568)	560–640	526 (474–575)
Tail (μm)	420	270	—	415	450 (400–500)	—	380–440	316 (285–373)
Precloacal papillae (no.)	4	4	4	4	4	4	4	4
Postcloacal papillae (no.)	5–6	5	5	5	5	5	5	5

* Hosts: *Actitis* spp., *Alca* spp. *Cotorrhactes pachyrhynchus* Viellot, 1816, *Larus* spp., *Mergus serrator* Linnaeus, 1758, *Puffinus* sp., *Sterna* spp., and *Totanus* spp.

tails under the SEM. Each cordon is long, recurrent, laterally anastomosing, and runs along the margin of membranous plates that extend posteriorly. The cordons have 2 inflexions at the level of the anterior end (Figs. 1–3). The descending and ascending arms are scalloped on the inner edges. The membranous plate shows both transverse and longitudinal markings (Figs. 1–3). The deirids are bicuspid (Fig. 4). Postdeirids are located asymmetrically near the end of the lateral alae; they have 2 tips (Fig. 5). At the posterior end of the male, the proximal pair of precloacal papillae lies outside the line of distribution of the other precloacal papillae (Fig. 6). In addition, we observed under the optical microscope that the vagina vera is shorter than the vagina uterina (85 μm and 173 μm, respectively).

In spite of the numerous records of *C. obvelatus* worldwide, few authors have given complete measurements, usually doing so only while describing the nematode as a new species. However, it is useful to give measurements when reporting a new host and/or locality, if only for comparative purposes. General morphology and measurements of our specimens agree with those presented by Cram (1927), Khalil (1931), Rao (1951), Rodrigues de Olivera and Vicente (1963), Anderson and Wong (1981), Bowie (1981), and Azuma et al. (1988) (Table 1) with the exception of the esophagus as reported by Khalil (1931), who considered esophagus length to be only that of the muscular portion. Other discrepancies arise with the vagina vera and vagina uterina lengths. Anderson and Wong (1981) observed a long vagina vera and a short vagina uterina, without giving measurements, and Lafuente et al. (1999) agreed with them. We also observed a terminal papilla on the female tail, as previously mentioned by Rodrigues de Olivera and Vicente (1963), Bowie (1981), Azuma et al. (1988), and Anderson and Wong (1981). Postdeirids were mentioned only by Anderson and Wong (1981), and Bowie (1981). Possibly because they are difficult structures to see, we assume that the postdeirids were present in the other cases. Despite the wide geographical distribution and the great variety of hosts parasitized by *C. obvelatus* (14 families belonging to 8 orders), there are no significant variations among populations in morphological characteristics and measurements. In contrast, in another acuariid species, *Synhimantus* (*Synhimantus*) *la-*



Figures 1–6. ***Cosmocephalus obvelatus*** **from** ***Spheniscus magellanicus*****. 1. Apical view. 2. Anterior extremity showing lateral alae (arrow), dorsal view. 3. Anterior extremity showing cephalic papillae (arrow), inflexions of chordons and deirid (arrow), lateral view. 4. Detail of deirid. 5. Detail of postdeirid located near end of lateral alae (arrow). 6. Posterior extremity of male with left spicule protruded and showing papillae arrangement with proximal pair of precloacal papillae lying out of line of distribution (arrow), latero-ventral view. Scale bars: 1 = 50 μm, 2 = 500 μm, 3 = 100 μm, 4 = 10 μm, 5 = 20 μm, and 6 = 200 μm.**

ticeps (Rudolphi, 1819), which also is cosmopolitan but has a narrower range of hosts, Etchegoin et al. (2000) found differences in measurements among specimens from different localities.

The shape of the cordons, the morphology and size of the cervical papillae, and the location in the definitive host (habitats) seem to be of fundamental importance when establishing relationships in the acuariid group (Baruš and Majudmar, 1975). The cordons of *Cosmocephalus* have a complex structure and are relatively wide. *Cosmocephalus obvelatus* is always located in the esophagus of the host. The genera *Synhimantus* and *Cosmocephalus* are closely related; they have similar cordons and cervical papillae, but members of the former genus live under the cuticle of the gizzard. Etchegoin et al. (2000) reported morphometric differences that they considered as intraspecific variations in specimens from different hosts and localities. However, we observed that *C. obvelatus* varies little, even in different hosts and localities. This morphometrical stability may indicate that *Cosmocephalus* is better adapted to different hosts and diverse localities because all hosts have similar environmental and feeding habits (eating fish). The intermediate hosts of *C. obvelatus* are amphipods, and it uses fish as paratenic hosts (Anderson, 1992). These characteristics may play an important role in the cosmopolitan distribution of *C. obvelatus*. Moreover, the distributions of many species of fish-eating birds overlap in their breeding and/or wintering grounds.

The intensity of infection recorded here is similar to the intensities found by Keppner (1973) from the California gull *Larus californicus* Lawrence, 1854 (Lariidae) (prevalence [P] = 23.5% and mean intensity [I] = 4.25), by Courtney and Forrester (1974) from the brown pelican *Pelecanus occidentalis* Linnaeus, 1776 (Pelecanidae), in North America (P = 40% and I = 4), and by Lafuente et al. (1999) from Audouin's gull *Larus audouinii* Payraudeau, 1826 (Lariidae), in the Mediterranean Sea (P = 82.76% and I = 5.08). This intensity is higher than that given by Threlfall (1968) from the black-backed gull *Larus marinus* Linnaeus, 1758 (Lariidae), in Newfoundland (P = 9.39% and I = 1).

Boero and Led (1970) described a new species, *Cosmocephalus argentinensis,* from 1 female specimen found in a Magellanic penguin in the Zoological Garden in La Plata, Argentina. We consider this acuariid as a species inquirendae because the description is very poor and no type materials (which were never deposited in a museum collection) are available for our examination.

We gratefully acknowledge the staff of the Servicio de Microscopía Electrónica de Barrido, Museo de La Plata, for their technical assistance and Lucy Shirlaw for revision of the English. This study was funded by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and by the Comision de Investigaciones Científicas de la Provincia de Buenos Aires (CIC).

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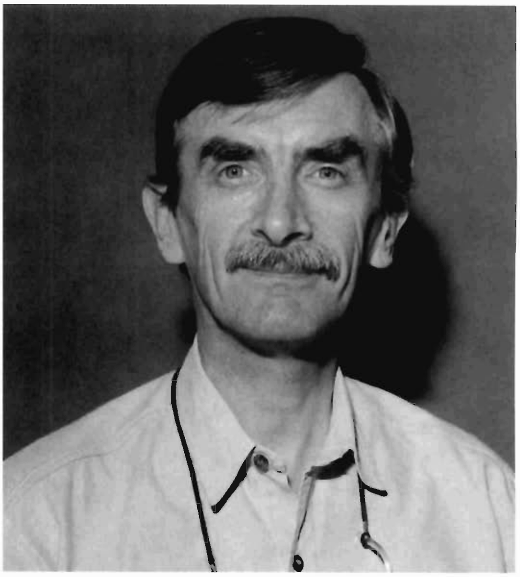
JOHN S. MACKIEWICZ
Elected to Life Membership in the Helminthological Society of Washington November 15, 2000



GRAHAM C. KEARN
Elected to Life Membership in the Helminthological Society of Washington November 15, 2000

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Anniversary Award

The Helminthological Society of Washington

NANCY D. PACHECO

Harley G. Sheffield, left, presents the 2000 Anniversary Award to Nancy D. Pacheco

As Chairman of the Anniversary Award Committee of the Helminthological Society of Washington, it is my duty to present the 2000 Anniversary Award to Nancy Pacheco. Not only is it a duty, it is an honor and a pleasure to be able to present this award to such an outstanding member of the Society.

The Award is authorized by the Society's Constitution and is to be given to a member for one or more achievements of the following nature: an outstanding contribution to the science of parasitology or related sciences that brings honor and credit to the Society, an exceptional paper read at a meeting of the Society or published in the Society's journal, outstanding service to the Society, or another achievement or contribution of distinction that warrants the highest recognition by the Society. The Awards Committee determined that Nancy qualifies in all of the above categories.

Nancy was born and educated in Kansas. During her junior year of high school, she was fortunate to be an exchange student under the American Field Service Program and lived 6 months in New Zealand. She subsequently received the Bachelor of Science Degree from Washburn University in Topeka. Following graduation, she came to the National Institutes of Health and received a position as a biologist in the National Heart Institute. Working with physicians and postdocs, Nancy was engaged in studies on cardiac muscle physiology. Maybe there was a lot of twitching in that job because for some reason, she saw the light and soon turned to parasitology. In 1968, and for the next 8 years, she worked as a biologist in the Animal Parasitology Institute (API) of the U.S. Department of Agriculture doing research on poultry and bovine parasites. Shortly after her arrival at API, I received a call from her supervisor, Dr. Vetterling, saying that he had hired a new person for his electron microscopy lab, and I should come over to meet her. That began our long scientific and social association. In 1976, Nancy moved to the Naval Medical Research Institute as a research microbiologist. There, she studied parasite immunology in relation to the development of malaria vaccines and later worked on cytokine regulation in wound repair. In 1994, she somehow slipped out of parasitology, without seeking advice of the Helminthological Society of Washington, and worked in the Wound Repair Program at NMRI until her retirement in 1997.

Nancy's résumé lists numerous publications. They illustrate her research contributions in the ultrastructure of intracellular parasites, techniques for isolation of large numbers of malaria parasites, which is a prerequisite to vaccine development, development of an oral vaccine against *Campylobacter* infection, development of a method to study local inflammatory action, and the study of the effects of cytokines in preventing translocation of bacteria in hemorrhagic shock.

A predominant factor in the Committee's decision was Nancy's excellent service to the Society, of which, undoubtably, most of you are aware. She has a record that is hard to beat. To the best of my knowledge, and with a little help from her curriculum vitae, Nancy has served in every office and has been on every committee of the Society, with the exception of Editor and the Editorial Committee. After holding the position of vice president in 1980, she moved up to president the next year. She must have done something right because she was re-elected president in 1991, a feat that, with one exception, has been unmatched in recent Society history. In 1999, she was elected to the position of corresponding secretary-treasurer, which she currently holds.

In spite of the considerable time that she has donated to the Society, Nancy has offered her expertise in various roles in other societies such as the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene. Outside of the scientific area, she has been active in many church-related events with her husband Jim. There is one other activity that might be noted. As mentioned before, Nancy spent a number of years working with poultry coccidia. You all know what people in that field do—they search through chicken droppings and count the coccidial oocysts that they find. Well, Nancy must have developed a high degree of excellence in counting because she has steadily moved upward through the ranks in the H&R Block organization and is now a senior tax preparer.

Nancy, I am very pleased to present to you, on the behalf of the Anniversary Awards Committee, the Helminthological Society of Washington's Anniversary Award for 2000.

Harley G. Sheffield, Ph.D.
November 15, 2000

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MINUTES

Six Hundred Seventy-First through the Six Hundred Seventy-Fifth Meeting of the Helminthological Society of Washington

671st Meeting: George Washington University, Washington, DC, 12 October 2000. President Dennis Richardson conducted the business meeting. Ralph Eckerlin welcomed members and their guests and introduced the President, who briefly summarized the Executive Council meeting. Dr. Eckerlin introduced the speakers. Robert Gwadz gave an overview of the National Institutes of Health (NIH)-funded malaria research in Mali. This NIH program funds electives for students with interests in either basic sciences or clinical aspects of malaria. Albert

Nieto reviewed recent advances in the use of synthetic peptides for cystic hydatid disease serology. John Hawdon provided an account of his research on potential hookworm vaccine candidates. Finally, Dr. Eckerlin reviewed his studies of fleas from flying squirrels in Virginia. New and renewal members included Russell C. Van Horn (U.S.A.), Eric Panitz (U.S.A.), and Riccardo Fiorillo (U.S.A.).

672nd Meeting: 94th Aero Squadron, College Park, Maryland, 15 November 2000. The anniversary dinner meeting and program were presided over by President Dennis Richardson. The slate of officers for 2001 were elected and installed by the membership in attendance: Dennis J. Richardson, president; William E. Moser, vice president; W. Patrick Carney and Nancy D. Pacheco continue as reporting secretary and corresponding secretary-treasurer, respectively. Nancy Pacheco was presented the Anniversary Award by Harley Sheffield. John S. Mackiewicz and Graham C. Kearn were elected to Life Membership (accepted for him by Gene Hayunga) and Honorary Membership (accepted for him by Sherman Hendrix), respectively.

673rd Meeting: Nematology Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland, 17 January 2001. President Dennis Richardson presided over the business meeting, and Vice President Lynn Carta presided over the scientific session. Peter Maser summarized his paper "Comparative Biochemistry of Parasitic and Free-Living Nematodes." William Wergin gave an overview of "Low Temperature Scanning Microscopy and its Application in Agriculture." Dr. Carta finished the session with "Integrating Nematode Morphology and Molecules in Plant-Parasitic and Free-Living Nematodes." Two new members were announced: Wellington A. Oyibo (Nigeria) and Analía Cristina Paola (Argentina).

674th Meeting: Walter Reed Army Institute of Research/Naval Medical Research Center, Silver Spring, Maryland, 12 March 2001. President Dennis Richardson presided over the business meeting and Eileen Franke-Villasante presided over the scientific session. Ed Rowton reviewed the "Status of the Recent Outbreak of Canine Leishmaniasis in the United States." His paper was followed by David Fryauff's report on "A New Twist on an Old Drug: Recent DOD Studies of Primaquine for Malaria Prophylaxis." Kent Kester summarized "Advances in Pre-Erythrocytic Malaria Vaccine Development," and Dr. Ling presented the final paper on "The Labor Involved in Malaria Field Studies in Indonesia."

675th Meeting: Biology Department, Gettysburg College, Gettysburg, Pennsylvania, 5 May 2001. President Richardson presided over the business meeting. Robin Overstreet, vice president of the American Society of Parasitologists (ASP), was welcomed and introduced to the members and guests by the president. Dr. Overstreet advised members that ASP has resources to provide travel grants for students who present papers at ASP meetings and to support speakers who are invited to present papers at meetings of affiliated societies. Sherman Hendrix presided over the scientific session. The first paper, presented by Dr. Overstreet, covered "Shrimp Parasites and Diseases," followed by Eric Hoberg's paper on the "Ancient Mariner—A History of Seabirds and Tapeworms on the Deep Blue Sea." Ann Barse summarized "New Host and Geographic Records of Monogenea of Billfishes." Sherman Hendrix presented the final paper on "Some Aspects of Biology of *Bothitrema bothi* (Platyhelminthes: Monogenea)."

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KEYWORD AND SUBJECT INDEX FOR VOLUME 68

APPLICATION FOR MEMBERSHIP
in the
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Present Position and Name of Institution (If you are a student, write "Student" and indicate the degree you are working for and the university and department): ____________________

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May the Society publish your mailing address, phone and fax numbers, and e-mail addresses in its membership directory listing on the web site? If not, what information should be withheld? ______

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Mail the completed application along with a check (on a U.S. bank) or money order (in U.S. currency) for the first year's dues [US$25 (2001) or US$30 (2002) for domestic active members and US$28 (2001) or US$33 (2002) for foreign active members] to the Corresponding Secretary-Treasurer, Nancy D. Pacheco, 9708 DePaul Drive, Bethesda, MD 20817, U.S.A.

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Helminthological Society of Washington Home Page: http://www.gettysburg.edu/shendrix/helmsoc.html

ANNIVERSARY AWARD RECIPIENTS

*Edna M. Buhrer	1960	*O. Wilford Olsen	1980
*Mildred A. Doss	1961	*Frank D. Enzie	1981
*Allen McIntosh	1962	Lloyd E. Rozeboom	1982
*Jesse R. Christie	1964	*Leon Jacobs	1983
*Gilbert F. Otto	1965	Harley G. Sheffield	1984
*George R. LaRue	1966	A. Morgan Golden	1985
*William W. Cort	1966	Louis S. Diamond	1986
*Gerard Dikmans	1967	*Everett L. Schiller	1987
*Benjamin Schwartz	1969	Milford N. Lunde	1988
*Willard H. Wright	1969	J. Ralph Lichtenfels	1989
*Aurel O. Foster	1970	A. James Haley	1990
*Carlton M. Herman	1971	*Francis G. Tromba	1991
*May Belle Chitwood	1972	Thomas K. Sawyer	1992
*Elvio H. Sadun	1973	Ralph P. Eckerlin	1993
E. J. Lawson Soulsby	1974	Willis A. Reid, Jr.	1994
David R. Lincicome	1975	Gerhard A. Schad	1995
Margaret A. Stirewalt	1975	Franklin A. Neva	1996
*Leo A. Jachowski, Jr.	1976	Burton Y. Endo	1997
*Horace W. Stunkard	1977	Sherman S. Hendrix	1998
*Kenneth C. Kates	1978	Frank W. Douvres	1999
*Everett E. Wehr	1979	Nancy D. Pacheco	2000

HONORARY MEMBERS

*George R. LaRue	1959	Roy C. Anderson	1991
*Vladimir S. Ershov	1962	Louis Euzet	1992
*Norman R. Stoll	1976	John C. Holmes	1993
*Horace W. Stunkard	1977	Purnomo	1994
*Justus F. Mueller	1978	Naftale Katz	1995
John F. A. Sprent	1979	*Robert Traub	1996
Bernard Bezubik	1980	*Alan F. Bird	1997
Hugh M. Gordon	1981	Graham C. Kearn	2000
E. J. Lawson Soulsby	1990		

CHARTER MEMBERS 1910

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

LIFE MEMBERS

*Maurice C. Hall	1931	*Everett E. Wehr	1977
*Albert Hassall	1931	*Marion M. Farr	1979
*Charles W. Stiles	1931	*John T. Lucker, Jr.	1979
*Paul Bartsch	1937	George W. Luttermoser	1979
*Henry E. Ewing	1945	*John S. Andrews	1980
*William W. Cort	1952	*Leo A. Jachowski, Jr.	1981
*Gerard Dikmans	1953	*Kenneth C. Kates	1981
*Jesse R. Christie	1956	*Francis G. Tromba	1983
*Gotthold Steiner	1956	A. James Haley	1984
*Emmett W. Price	1956	*Leon Jacobs	1985
*Eloise B. Cram	1956	*Paul C. Beaver	1986
*Gerald Thorne	1961	*Raymond M. Cable	1986
*Allen McIntosh	1963	Harry Herlich	1987
*Edna M. Buhrer	1963	Glenn L. Hoffman	1988
*Benjamin G. Chitwood	1968	Robert E. Kuntz	1988
*Aurel O. Foster	1972	Raymond V. Rebois	1988
*Gilbert F. Otto	1972	Frank W. Douvres	1989
*Theodor von Brand	1975	A. Morgan Golden	1989
*May Belle Chitwood	1975	Thomas K. Sawyer	1989
*Carlton M. Herman	1975	*J. Allen Scott	1990
Lloyd E. Rozeboom	1975	Judith H. Shaw	1990
*Albert L. Taylor	1975	Milford N. Lunde	1991
David R. Lincicome	1976	*Everett L. Schiller	1991
Margaret A. Stirewalt	1976	Harley G. Sheffield	1991
*Willard H. Wright	1976	Louis S. Diamond	1994
*Benjamin Schwartz	1976	Mary Hanson Pritchard	1994
*Mildred A. Doss	1977	John S. Mackiewicz	2000

*Deceased.

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