

# Comparative Parasitology

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## Diplectanids (Monogonoidea: Dactylogyridea) from the Gills of Marine Fishes of the Persian Gulf off Kuwait

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**ABSTRACT:** Seventeen species of Diplectanidae were collected from the gills of 17 species of marine fishes from the Persian Gulf off Kuwait. *Lepidotrema kuwaitensis* sp. n. from *Terapon puta* (Teraponidae), *Lamellodiscus furcillatus* sp. n. from *Diplodus noct* (Sparidae), and *Protolamellodiscus senilobatus* sp. n. from *Argyrops spinifer* and *A. filamentosus* (Sparidae) are described. *Diplectanum cazauxi* from *Sphyaena jello* and *S. obtusata* (Sphyraenidae) (new host and geographic records), *D. sillagonum* from *Sillago sihama* (Sillaginidae) (new geographic record), *Pseudolamellodiscus sphyraenae* from *Sphyaena chrysotaenia* (Sphyraenidae) (new host and geographic records), and *Calydiscoides flexuosus* from *Nemipterus peronii* and *N. bipunctatus* (Nemipteridae) (new host and geographic records) are redescribed. An incidental geographic record for *C. flexuosus* on *N. japonicus* from the western coast of India is included. Ten diplectanid species from 8 hosts were unidentified for lack of sufficient specimens. *Diplectanum longipenis* (synonym: *Squamodiscus longipenis*) is transferred to *Lepidotrema*. *Squamodiscus* is removed from synonymy with *Diplectanum* and becomes a junior subjective synonym of *Lepidotrema*. *Calydiscoides indianus* (synonyms: *Lamellospina indiana* and *C. indicus*) is a junior subjective synonym of *C. flexuosus*.

**KEY WORDS:** Monogonoidea, monogenean, Diplectanidae, *Calydiscoides flexuosus*, *Diplectanum cazauxi*, *Diplectanum sillagonum*, *Diplectanum* sp., *Lamellodiscus furcillatus* sp. n., *Lamellodiscus* sp., *Lepidotrema kuwaitensis* sp. n., *Lepidotrema longipenis* comb. n., *Protolamellodiscus senilobatus* sp. n., *Pseudolamellodiscus sphyraenae*, *Pseudorhabdosynochus* sp., *Acanthopagrus berda*, *Acanthopagrus bifasciatus*, *Acanthopagrus latus*, *Argyrops filamentosus*, *Argyrops spinifer*, *Diplodus noct*, *Epinephelus areolatus*, *Epinephelus tauvina*, *Hemiramphus marginatus*, *Nemipterus bipunctatus*, *Nemipterus peronii*, *Otolithes argenteus*, *Sillago sihama*, *Sphyaena chrysotaenia*, *Sphyaena jello*, *Sphyaena obtusata*, *Terapon puta*, Persian Gulf, Kuwait.

A survey of the helminth parasites infesting marine fishes off the Kuwaiti coast by O.S. was conducted between October 1992 and December 1996. Species of Diplectanidae (Monogonoidea) were found on the gills of 17 marine fishes representing the Hemiramphidae, Nemipteridae, Sciaenidae, Serranidae, Sillaginidae, Sparidae, Sphyraenidae, and Teraponidae. The present paper includes descriptions and taxonomic considerations of 3 new and 5 previously described species.

### Materials and Methods

Hosts were obtained from the local fish market, Kuwait, and examined directly for helminth parasites. Diplectanids were removed from the gills of respective hosts, fixed, and stored as described by Sey and Nahas (1997); vials containing the helminths were then shipped to Idaho State University. Methods of staining, mounting, and illustration of diplectanids were those

described by Kritsky et al. (1986). Measurements, all in micrometers, were made with a filar micrometer according to procedures of Mizelle and Klucka (1953); average measurements are followed by ranges and number (*n*) of specimens measured in parentheses; unstained flattened specimens mounted in Gray and Wess' medium were used to obtain measurements of the haptoral sclerites and copulatory complex; other measurements were obtained from unflattened specimens stained in Gomori's trichrome and mounted in Canada balsam; the dimension of the pyriform ovary is the greatest width. Numbering of hook pairs follows the scheme proposed by Mizelle (1936; see Mizelle and Price, 1963). Type specimens of new species and voucher specimens of previously described species were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, and the helminth collection of the University of Nebraska State Museum (HWML), Lincoln, Nebraska, U.S.A., as indicated in the respective species accounts. For comparative purposes, the following specimens were examined: 3 voucher specimens of *Lepidotrema tenue* Johnston and Tiegs, 1922 (USNPC 63156); 4 voucher specimens of *Lepidotrema bidyana* Murray, 1931 (USNPC 63157); 5 voucher specimens of *Lepidotrema*

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*angusta* (Johnston and Tieg, 1922) (USNPC 63158); holotype, 22 paratypes of *Pseudolamellodiscus sphyraenae* Yamaguti, 1953 (Meguro Parasitological Museum, Tokyo, Japan [MPM] 22556); holotype, numerous paratypes of *Lamellodiscus convolutus* Yamaguti, 1953 (MPM 22558); holotype, 27 paratypes of *Lamellodiscus flexuosus* Yamaguti, 1953 (MPM 22557); holotype, 11 paratypes of *Squamodiscus longipenis* Yamaguti, 1934 (labeled as *S. longiphallus*) (MPM 22564); and 37 voucher specimens of *Calydiscoides flexuosus* Yamaguti, 1953 (USNPC 89024). Host names and synonyms follow those provided by the FAO Fish Base at <http://www.fao.org/waicent/faoinfo/fishery/fishbase/fishbase.htm>.

### Results

A total of 17 species of Diplectanidae was found on 17 species of marine fishes collected off the Kuwaiti coast. Specimens of only 7 of the 17 diplectanid species were sufficient for identification and description. Ten unidentified diplectanids and their hosts are listed in Table 1.

#### Class Monogonoidea Bychowsky, 1937 Order Dactylogyridea Bychowsky, 1937 Diplectanidae Monticelli, 1903

##### *Diplectanum cazauxi* Oliver and Paperna, 1984

##### (Figs. 1–8)

REDESCRIPTION (measurements of specimens from *Sphyraena obtusata* Cuvier, 1929, follow those from *Sphyraena jello* Cuvier, 1829 in brackets): Diplectaninae. Body 964 (729–1,080;  $n = 4$ ) [824 (608–1,070;  $n = 4$ )] long, fusiform; greatest width 170 (123–242;  $n = 4$ ) [156 (97–229;  $n = 4$ )] usually in posterior trunk at level of testis. Tegument smooth. Cephalic margin tapered; 2 terminal, 2 bilateral cephalic lobes poorly developed; head organs numerous; cephalic glands numerous in cephalic area, 2 bilateral groups posterolateral to pharynx. Eyes 4; members of posterior pair slightly larger, farther apart than anterior members; 1 anterior eye occasionally absent; granules small, ovate; accessory granules absent to numerous in cephalic region. Mouth subterminal, ventral to pharynx; pharynx 52 (39–68;  $n = 4$ ) [42 (32–48;  $n = 4$ )] wide, ovate to subrectangular in dorsoventral view; esophagus short or nonexistent; intestinal ceca blind. Peduncle short to elongate. Haptor 81–82 ( $n = 2$ ) [70 (69–72;  $n = 3$ )] long, 127 (113–140;  $n = 2$ ) [130 (120–137;  $n = 3$ )] wide, bilaterally lobed; squamodiscs similar, each 49 (36–60;  $n = 6$ ) [50 (46–61;  $n = 7$ )] long, 77

(61–88;  $n = 6$ ) [72 (64–86;  $n = 7$ )] wide, with 17–19 concentric rows of dumbbell-shaped rodlets, each with anterior lightly sclerotized blunt spinelet. Ventral anchor 30 (29–32;  $n = 11$ ) [31 (29–32;  $n = 6$ )] long, with elongate deep root, knob-like superficial root, straight shaft, moderately long point extending slightly past level of tip of superficial root; anchor base 9 (8–10;  $n = 3$ ) [7–8 ( $n = 1$ )] wide. Dorsal anchor 23 (22–24;  $n = 10$ ) [23 (22–25;  $n = 8$ )] long, with subtriangular base, slightly curved shaft, point extending past level of tip of superficial anchor root; anchor base 7–8 ( $n = 6$ ) wide. Ventral bar 72 (58–85;  $n = 10$ ) [66 (62–76;  $n = 6$ )] long, subrectangular, with tapered ends, ventral groove; paired dorsal bar 42 (36–46;  $n = 10$ ) [40 (37–44;  $n = 8$ )] long, spatulate medially. Hooks similar; each 10 (9–11;  $n = 19$ ) [10 (9–11;  $n = 11$ )] long, with protruding thumb with slightly depressed tip, delicate point, shank; hook pair 1 lying medial to anchors on short haptoral peduncles, pairs 2–4, 6 submarginal on lateral haptoral lobes, pair 5 associated with distal shaft of ventral anchor, pair 7 on dorsal surface of lateral haptoral lobe; filamentous hooklet (FH) loop shank length. Male copulatory organ 41 (39–44;  $n = 4$ ) [36 (31–40;  $n = 3$ )] long, weakly sclerotized, C shaped, with slightly enlarged base, nipple-like termination. Accessory piece absent. Testis 261 (185–300;  $n = 4$ ) [207 (129–292;  $n = 4$ )] long, 92 (70–130;  $n = 4$ ) [82 (65–105;  $n = 4$ )] wide, pyriform; course of vas deferens not observed; seminal vesicle a simple dilation of vas deferens, lying along body midline dorsal to vagina; 2 small prostatic reservoirs immediately anterior to male copulatory organ, saccate. Ovary 42 (31–56;  $n = 3$ ) [40–41 ( $n = 1$ )] wide, elongate pyriform, looping right intestinal cecum, lying transversely anterior to testis; oviduct elongate; ootype ventral, a small dilated portion of female duct; uterus delicate, extending along seminal vesicle; seminal receptacle not observed; vagina nonsclerotized, aperture sinistroventral near level of male copulatory organ; vitellaria throughout trunk, except absent in regions of major reproductive organs.

HOSTS AND LOCALITY: Pickhandle barracuda, *Sphyraena jello* Cuvier, 1829 (Sphyraenidae); Persian Gulf off Kuwait (15 October 1993). Obsolete barracuda, *Sphyraena obtusata* Cuvier, 1829 (Sphyraenidae); Persian Gulf off Kuwait (9 July 1993).

PREVIOUS RECORDS: Yellowtail barracuda,



**Table 1. Unidentified diplectanids infesting marine fishes off Kuwait.**

Host	Date of collection	Parasite	USNPC no.
<i>Acanthopagrus berda</i> (Forsskål, 1775) (Sparidae)	30 November 1996	<i>Lamellodiscus</i> sp. 1	89011
<i>Acanthopagrus bifasciatus</i> (Forsskål, 1775) (Sparidae)	10 May 1995	<i>Lamellodiscus</i> sp. 2	89012
	10 October 1995		89013
<i>Acanthopagrus latus</i> (Houttyn, 1782) (Sparidae)	28 March 1995	<i>Lamellodiscus</i> sp. 1	89014
		<i>Lamellodiscus</i> sp. 3	89015
<i>Diplodus noct</i> (Valenciennes, 1830) (Sparidae)	23 March 1996	<i>Lamellodiscus</i> sp. 4	89016
<i>Epinephelus areolatus</i> (Forsskål, 1775) (Serranidae)	15 October 1994	<i>Lamellodiscus</i> sp. 5	89017
<i>Epinephelus tauvina</i> (Forsskål, 1775) (Serranidae)	29 July 1993	<i>Pseudorhabdosynochus</i> sp. 1	89018
	16 June 1993	<i>Pseudorhabdosynochus</i> sp. 2	89019
	15 October 1994		89030
<i>Hemiramphus marginatus</i> (Forsskål, 1775) (Hemiramphidae)	15 June 1993	<i>Lamellodiscus</i> sp. 6	89029
<i>Otolithes argenteus</i> (Cuvier, 1830) (Sciaenidae)	10 March 1994	<i>Diplectanum</i> sp. 1	89031
	8 May 1995		89034
	18 October 1995		89033
	5 April 1996		89035
	15 October 1993	<i>Diplectanum</i> sp. 2	89032

*Sphyraena flavicauda* Rüppell, 1838 (Sphyraenidae): Gulf of Aqaba (Golfe D'Aquaba [sic]), Gulf of Suez (Egypt), Indian Ocean off Malindi (Kenya) (all Oliver and Paperna, 1984).

**SPECIMENS STUDIED:** 12 voucher specimens from *S. jello*, USNPC 89010, HXML 15023; 8 voucher specimens from *S. obtusata*, USNPC 89009.

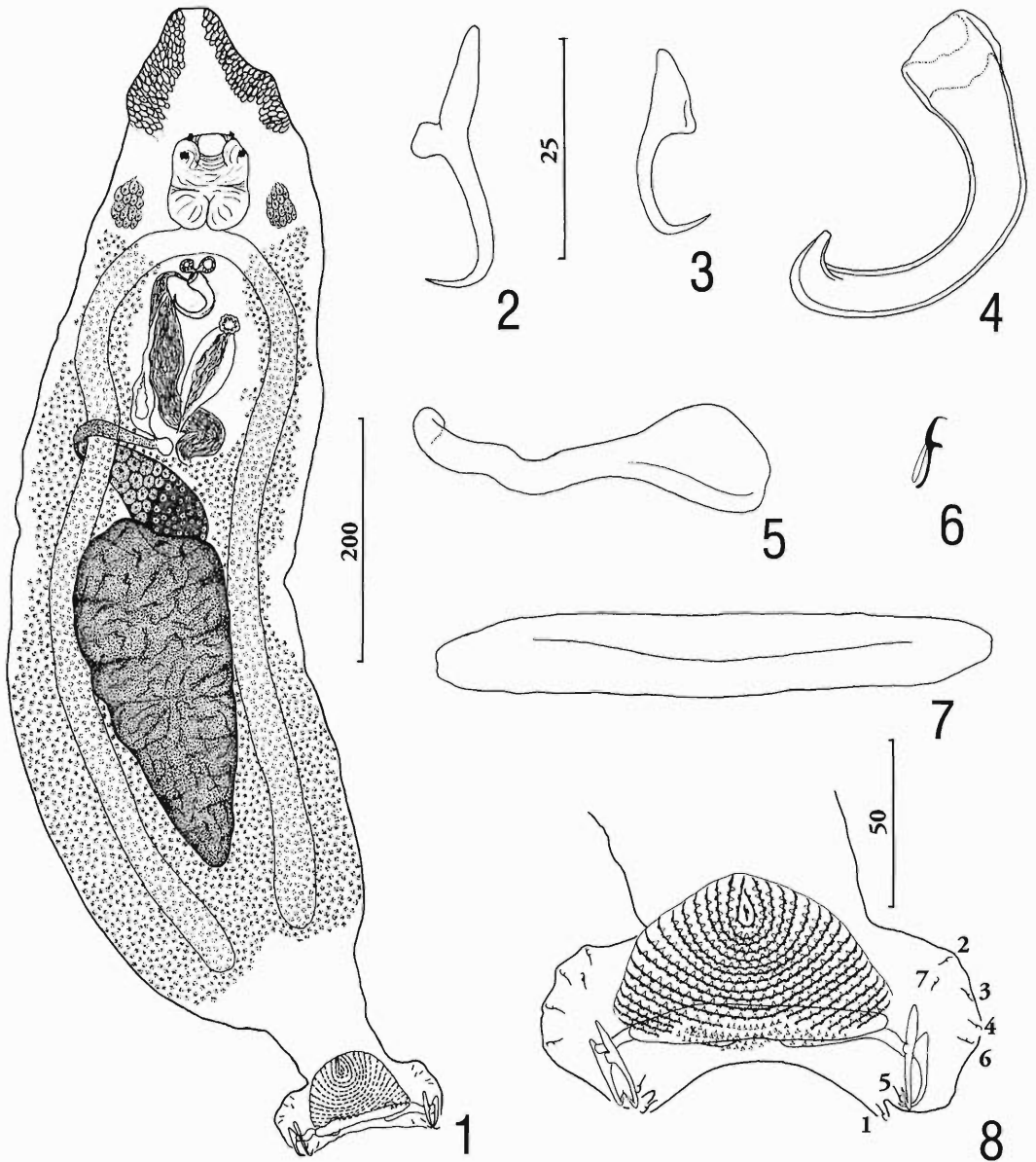
**REMARKS:** *Diplectanum cazauxi* is known only from species of barracuda (Sphyraenidae). Our report of this species on *S. jello* and *S. obtusata* from the Persian Gulf represents new host and geographic records. The known geographic distribution of *D. cazauxi* currently includes the western Indian Ocean and adjacent regions including the northern gulfs of the Red Sea and the Persian Gulf.

The original description of *D. cazauxi* is based on morphometrics of the squamodisc and sclerotized haptor and copulatory structures. Although Oliver and Paperna (1984) mentioned that the ovary loops the right intestinal cecum, a symplesiomorphic feature for all members of the Diplectanidae, other details of the internal anatomy were not considered. Our redescription

adds information on soft-tissue features of the reproductive, digestive, and nervous systems.

The morphometrics of the haptor and squamodisc in our specimens are in general agreement with those reported by Oliver and Paperna (1984) in the original description of *D. cazauxi*. Mounting media (Gray and Wess' medium, Malmberg's medium, and Hoyer's medium) commonly used to visualize the sclerites of monogenoideans apply pressure on the specimen. In *D. cazauxi*, this pressure results in significant distortion of the lightly sclerotized male copulatory organ. The copulatory organs of *D. cazauxi* shown in Figure 11 of Oliver and Paperna (1984) are clearly distorted, as were our specimens mounted in Gray and Wess' medium. Such artifacts are minimized when specimens are mounted in Canada balsam, which does not result in significant coverslip pressure on the specimen (compare Fig. 4 with Fig. 11 of Oliver and Paperna, 1984).

The copulatory complex, dorsal anchor, haptor bars, and squamodisc of *Diplectanum cazauxi* closely resemble those of *Laterocaecum pearsoni* Young, 1969, suggesting that these



Figures 1–8. *Diplectanum cazauxi* Oliver and Paperna, 1984. 1. Whole mount (composite, ventral; dorsal squamodisc not shown). 2. Ventral anchor. 3. Dorsal anchor. 4. Copulatory complex. 5. Dorsal bar. 6. Hook. 7. Ventral bar. 8. Ventral view of haptor showing ventral squamodisc and positions of hook pairs. All figures are drawn to the 25- $\mu$ m scale, except Figures 1 and 8 (200- $\mu$ m and 50- $\mu$ m scales, respectively).

species likely share a common evolutionary history. *Laterocaecum* was proposed by Young (1969) for a diplectanid collected from the obtuse barracuda, *S. obtusata*, from Moreton Bay, Queensland, Australia. Young (1969) differentiated the genus from other diplectanid genera

by species possessing lateral diverticula of the intestinal ceca (lateral diverticula absent in all other species of Diplectanidae) and 12 (6 pairs) hooks in the adult. If *D. cazauxi* actually shares a phylogenetic history with *L. pearsoni* as suggested by their similar morphology and host

preferences, separation of *Laterocaecum* from *Diplectanum* may not be justified, and the 2 unique characters presented by *L. pearsoni* may represent secondarily derived features within *Diplectanum*. We do not formally propose synonymy of the 2 genera at this time, however, because hypotheses on phylogenetic relationships within the Diplectanidae are lacking and *Diplectanum* may represent a paraphyletic group (see "Discussion"). *Diplectanum cazauxi* differs from *L. pearsoni* by having a knob-like superficial root on the ventral anchor (root elongate in *L. pearsoni*) and by possessing 7 pairs of hooks in the adult (6 pairs in *L. pearsoni*).

***Diplectanum sillagonum* Tripathi, 1957**

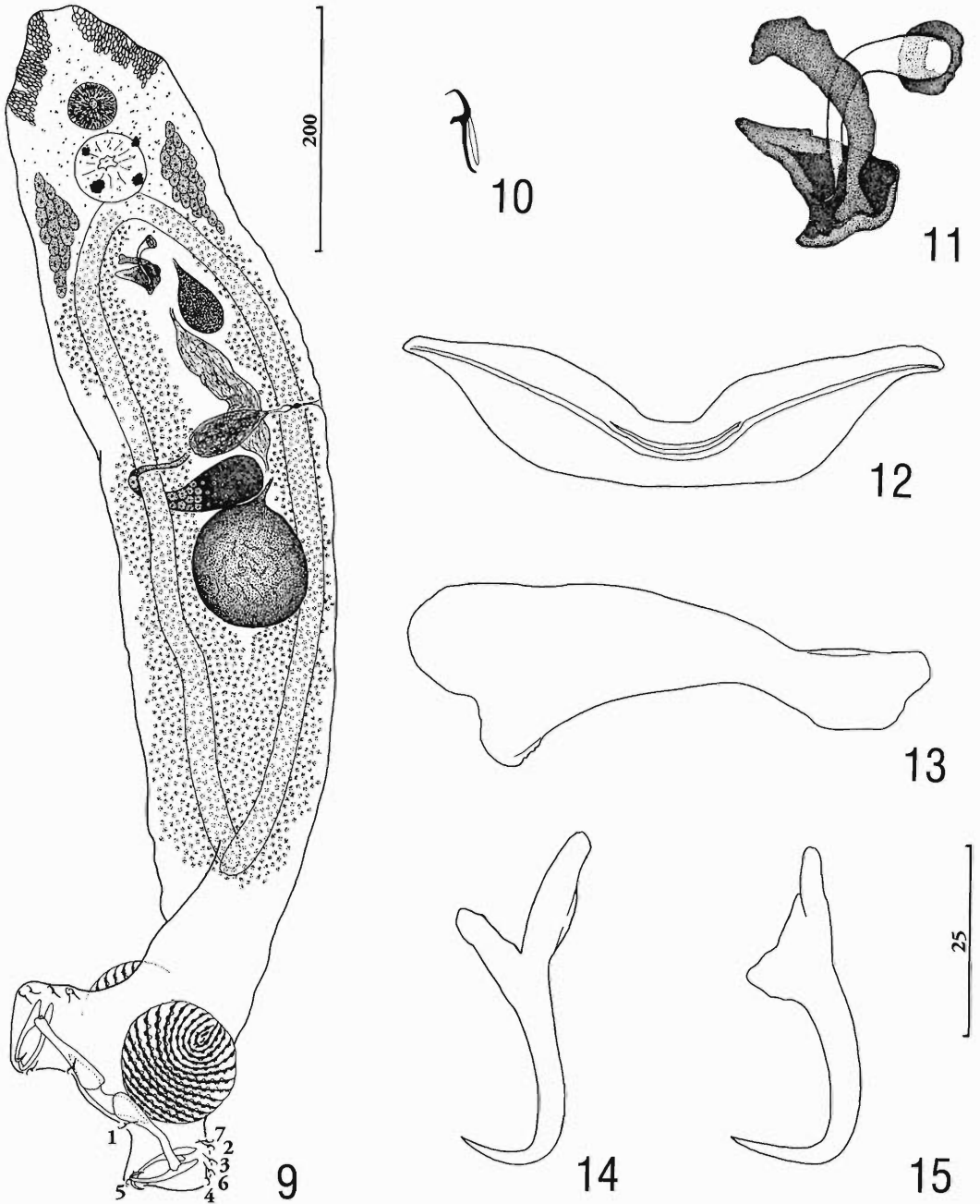
(Figs. 9–15)

REDESCRIPTION (Tripathi's [1957] original measurements and counts are in brackets following respective parameters of specimens from the Persian Gulf): Diplectaninae. Body 755 (694–815;  $n = 4$ ) [623–1,058] long, fusiform, somewhat flattened dorsoventrally; greatest width 131 (110–153;  $n = 4$ ) [114–144] usually in anterior trunk near level of copulatory organ. Tegument smooth. Cephalic margin tapered; 2 terminal, 2 bilateral cephalic lobes poorly developed; subspherical ventral pouch lying anterior to pharynx, opening to exterior via simple midventral pore. Head organs numerous; distributed in 3 poorly defined groups; anterior posterior groups associated with respective cephalic lobes. Cephalic glands lateral to pharynx, extending posteriorly past level of esophageal bifurcation. Eyes 4; members of posterior pair larger, closer together than anterior members; granules small, ovate; accessory granules numerous, distributed throughout cephalic, anterior trunk regions. Mouth subterminal, ventral to pharynx; pharynx 47 (40–53;  $n = 4$ ) [41–49] wide, subspherical; esophagus short or absent; intestinal ceca blind. Peduncle short, broad. Haptor 124 (113–137;  $n = 4$ ) [57] long, 159 (150–170;  $n = 4$ ) [133–152] wide, bilaterally lobed; squamodiscs similar, each 73 (62–83;  $n = 12$ ) [57–76] in diameter, subcircular, with 13–15 [11–15] concentric rows of dumbbell-shaped rodlets, each with anterior lightly sclerotized blunt spinelet. Ventral anchor 44 (38–50;  $n = 14$ ) [49–53] long, with elongate roots (deep root longest), straight shaft, recurved point extending slightly past level of tip of superficial anchor root; anchor base 14

(11–16;  $n = 8$ ) wide. Dorsal anchor 40 (38–44;  $n = 13$ ) [41–49] long, with subtriangular base, slightly curved shaft, recurved point extending past level of tip of superficial anchor root; anchor base 12 (10–14;  $n = 7$ ) wide. Ventral bar 74 (67–86;  $n = 10$ ) [60–72] long, with tapered ends, ventral groove; median anterior constriction. Paired dorsal bar 69 (63–75;  $n = 11$ ) [57–64] long, medial end expanded, bilobed. Hooks similar; each 12 (11–13;  $n = 29$ ) long, with protruding thumb with slightly depressed tip, delicate point, slender shank; hook pair 1 at level of tips of ventral bar, medial to anchors; pairs 2–4, 6, 7 submarginal in lateral haptor lobes; pair 5 associated with distal ventral anchor shaft; FH loop shank length. Male copulatory organ 34 (30–39;  $n = 6$ ) [41–45] long, a sigmoid tube originating from ring-like sclerotized base, with fine recurved tip. Accessory piece variable, comprising 2 articulated subunits, 1 subunit with bilobed proximal end articulating to other subunit. Testis 70 (69–71;  $n = 2$ ) [38–53 × 76–152] in diameter, subspherical; course of vas deferens not observed; seminal vesicle a simple elongate dilation of vas deferens, lying along body midline dorsal to seminal receptacle; prostatic reservoir saccate, posterior to male copulatory organ, frequently containing granules only at anterior end. Ovary 57 (42–71;  $n = 2$ ) [38 × 57] wide, elongate pyriform, looping right intestinal cecum, lying transversely anterior to testis; oviduct elongate; ootype, uterus not observed; seminal receptacle ovate, originating from short tubular vagina; vagina with small bead-like sclerotization having cupped proximal end; vaginal aperture sinistral; vitellaria throughout trunk, except absent in regions of major reproductive organs.

HOSTS AND LOCALITY: Silver sillago, *Sillago sihama* (Forsskål, 1775) (Sillaginidae): Persian Gulf off Kuwait (31 December 1993, 18 April 1996).

PREVIOUS RECORDS: *Sillago sihama*: Chandipore, Chilka Lake, Puri, all Bay of Bengal, India (Tripathi, 1957). *Sillago sihama*: Burdekin River, Duyfken Point, Point Samson, and Darwin, Australia; Phuket and Bang Saen, Thailand; Cendering and Kula Lumpur, Malaysia; Bali, Indonesia; Aberdeen market and Sai Kung, Hong Kong; Ring Ring, Kapa Kapa, and Sinapa, Papua New Guinea; and Madras, India (all Hayward, 1996). Slender sillago, *Sillago attenuata* McKay, 1985: Ras Lanura, Saudi Arabia (Hay-



Figures 9–15. *Diplectanum sillagonum* Tripathi, 1957. 9. Whole mount (composite, body ventral, haptor dorsal), showing position of hook pairs. 10. Hook. 11. Copulatory complex. 12. Ventral bar. 13. Dorsal bar. 14. Ventral anchor. 15. Dorsal anchor. All figures are drawn to the 25- $\mu$ m scale, except Figure 9 (200- $\mu$ m scale).

ward, 1996). Vincent's sillago, *Sillago vincenti* McKay, 1980: Kavanad, Kerala, India (Hayward, 1996).

SPECIMENS STUDIED: 14 voucher specimens, USNPC 89007, 89008, HWML 15022.

REMARKS: *Diplectanum sillagonum* was described by Tripathi (1957) from the gills of *S. sihama* from western coastal localities on the Bay of Bengal, India. His description of this species is of marginal value for species determination. Nonetheless, the original drawings of the copulatory complex, anchors, bars, and whole mount, while diagrammatic, strongly suggest conspecificity with our collection from the Persian Gulf. Persian Gulf specimens were obtained from the same host species as that of the type series, and respective measurements of specimens from the Persian Gulf and India are comparable. However, the types of *D. sillagonum* were not available for confirmation. General morphology of the sclerotized haptor structures and copulatory complex generally corresponds to figures of this species offered by Hayward (1996). However, Hayward (1996) did not mention the presence of the midventral pouch located anterior to the pharynx in his redescription of the species.

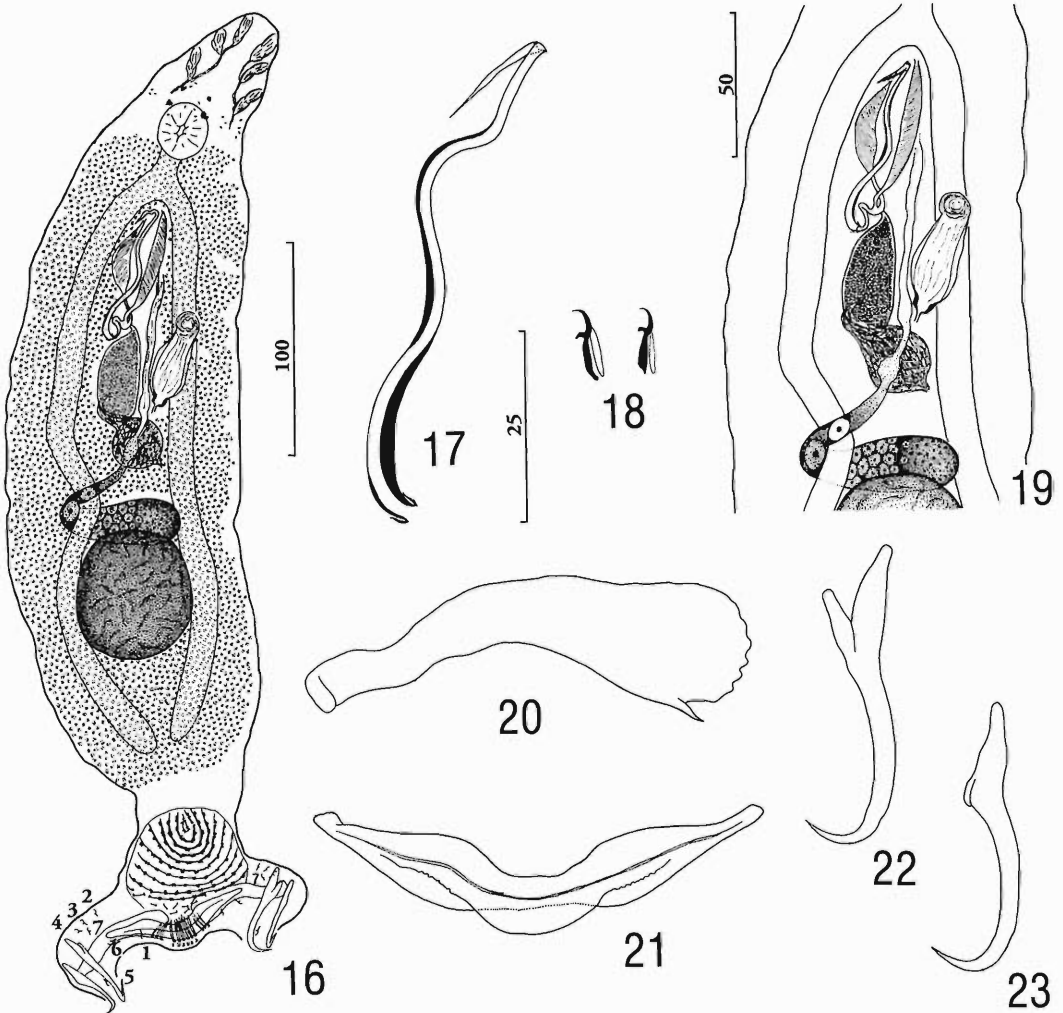
*Lepidotrema kuwaitensis* sp. n.

(Figs. 16–23)

DESCRIPTION: Diplectaninae. Body 504 (452–603;  $n = 8$ ) long, fusiform; greatest width 105 (90–121;  $n = 9$ ) near body midlength. Tegument smooth. Cephalic margin tapered; 2 terminal, 2 bilateral cephalic lobes poorly developed; 3 bilateral pairs of head organs with anterior, posterior pairs associated with respective cephalic lobes; cephalic glands not observed. Eyes 4, equidistant; members of posterior pair larger than anterior members; anterior eyes frequently absent, 1 or both posterior eyes occasionally dissociated; granules small, ovate, numerous accessory granules at eye level. Mouth subterminal, ventral to pharynx; pharynx 23 (19–26;  $n = 9$ ) wide, ovate to subspherical; esophagus short to absent; intestinal ceca blind. Peduncle short to elongate. Haptor 83 (65–100;  $n = 9$ ) long, 139 (124–151;  $n = 9$ ) wide, bilaterally lobed. Squamodiscs similar, each 37 (26–43;  $n = 4$ ) long, 40 (27–48;  $n = 6$ ) wide, subcircular, with 8–10 concentric rows of dumbbell-shaped rodlets becoming progressively more

delicate in posterior rows; 2–4 rows (layers) of elongate delicate spinelets wrap around posterior margin of both squamodiscs, spinelets frequently absent. Ventral anchor 42 (39–45;  $n = 25$ ) long, with elongate roots (deep root longest), evenly curved shaft with terminal indentation at articulation with recurved point; point extending slightly past level of tip of superficial anchor root; anchor base 9 (7–11;  $n = 13$ ) wide. Dorsal anchor 37 (32–40;  $n = 23$ ) long, with narrow base, long deep root, curved shaft, point extending past level of tip of superficial root of anchor base; anchor base 7 (6–9;  $n = 11$ ) wide. Ventral bar 59 (52–66;  $n = 21$ ) long, with tapered ends, ventral groove; paired dorsal bar 55 (47–60;  $n = 23$ ) long, spatulate, with posteromedial spine. Hook 10 (9–12;  $n = 37$ ) long, with protruding thumb with slightly depressed end, delicate point, shank dilated slightly in some specimens. Hook pair 1 lying medial to haptor lobes, posterior to bars; pairs 2–4, 7 in lateral haptor lobes; pair 5 associated with shaft of ventral anchor; pair 6 at level of or just anterior to dorsal bar. FH loop shank length. Male copulatory organ 68 (60–74;  $n = 11$ ) long, a sigmoid tube with wall of varying thickness along length, acute tip. Accessory piece absent. Testis subspherical, 54 (42–65;  $n = 9$ ) in diameter; course of vas deferens not observed; seminal vesicle a simple dilation of vas deferens, lying along body midline dorsal to ootype; prostatic reservoirs 3, saccate; anterior prostatic vesicles bilateral to male copulatory organ, with prostatic ducts fused prior to entering base of male copulatory organ via common duct; posterior reservoir caudal to male copulatory organ, apparently emptying independently into base of male copulatory organ. Ovary 23 (19–25;  $n = 3$ ) wide, pyriform, anterodorsal to testis, looping right intestinal cecum; oviduct elongate; ootype ventral, a small dilated portion of female duct; uterus delicate, extending anteriorly to left of prostatic reservoirs; seminal receptacle not observed; vaginal aperture sinistroventral, with circular muscular rim; vagina funnel-shaped, narrowing to short tube; vagina with proximally thickened walls; vitellaria throughout trunk, except absent in regions of major reproductive organs. Egg 83–84 ( $n = 1$ ) long, 56–57 ( $n = 1$ ) wide, ovate, with short proximal filament.

TYPE HOST: Small-scaled terapon, *Terapon puta* (Cuvier, 1829) (Terapontidae).



Figures 16–23. *Lepidotrema kuwaitensis* sp. n. 16. Whole mount (composite, ventral; dorsal squamodisc not shown), showing positions of hook pairs. 17. Copulatory complex. 18. Hooks. 19. Enlargement of worm at level of reproductive organs (composite, ventral). 20. Dorsal bar. 21. Ventral bar. 22. Ventral anchor. 23. Dorsal anchor. All figures are drawn to the 25- $\mu$ m scale, except Figures 16 and 19 (100- $\mu$ m and 50- $\mu$ m scales, respectively).

TYPE LOCALITY: Persian Gulf off Kuwait (9 July 1993, 15 October 1993, 26 March 1996).

INFECTION SITE: Gills.

DEPOSITED SPECIMENS: Holotype, USNPC 89020; 25 paratypes, USNPC 89021, 89022, 89023, HWML 15025.

ETYMOLOGY: This species is named for the country of Kuwait.

REMARKS: The primary distinguishing feature of *Lepidotrema* Johnston and Tiegs, 1922, is the presence of groups of elongate spinelets forming fan-like structures on the posterior portions of the

squamodiscs (Oliver, 1987). The genus currently includes 6 species, all from freshwater teraponids in Australia: *Lepidotrema therapon* Johnston and Tiegs, 1922; *L. angusta*; *L. bidyana*; *Lepidotrema fuliginosum* Johnston and Tiegs, 1922; *Lepidotrema simplex* Johnston and Tiegs, 1922; and *L. tenue*. Our finding of *L. kuwaitensis* on *T. puta* (Teraponidae) in the Persian Gulf is the first report of a member of *Lepidotrema* from a marine host. Existing descriptions of the 6 freshwater species are of marginal value for comparison with *L. kuwaitensis*, and most species require redescription



(see Johnston and Tiegs, 1922; Murray, 1931; Young, 1969).

In *L. kuwaitensis*, the posterior spinelets are delicate (or frequently absent, an apparent artifact resulting from deterioration of the specimen before fixation) and resemble those of *L. angusta* as depicted by Young (1969). These species are easily separated by the comparative morphology of the copulatory complexes (sigmoid in *L. kuwaitensis*; coiled with about 1 ring in *L. angusta*).

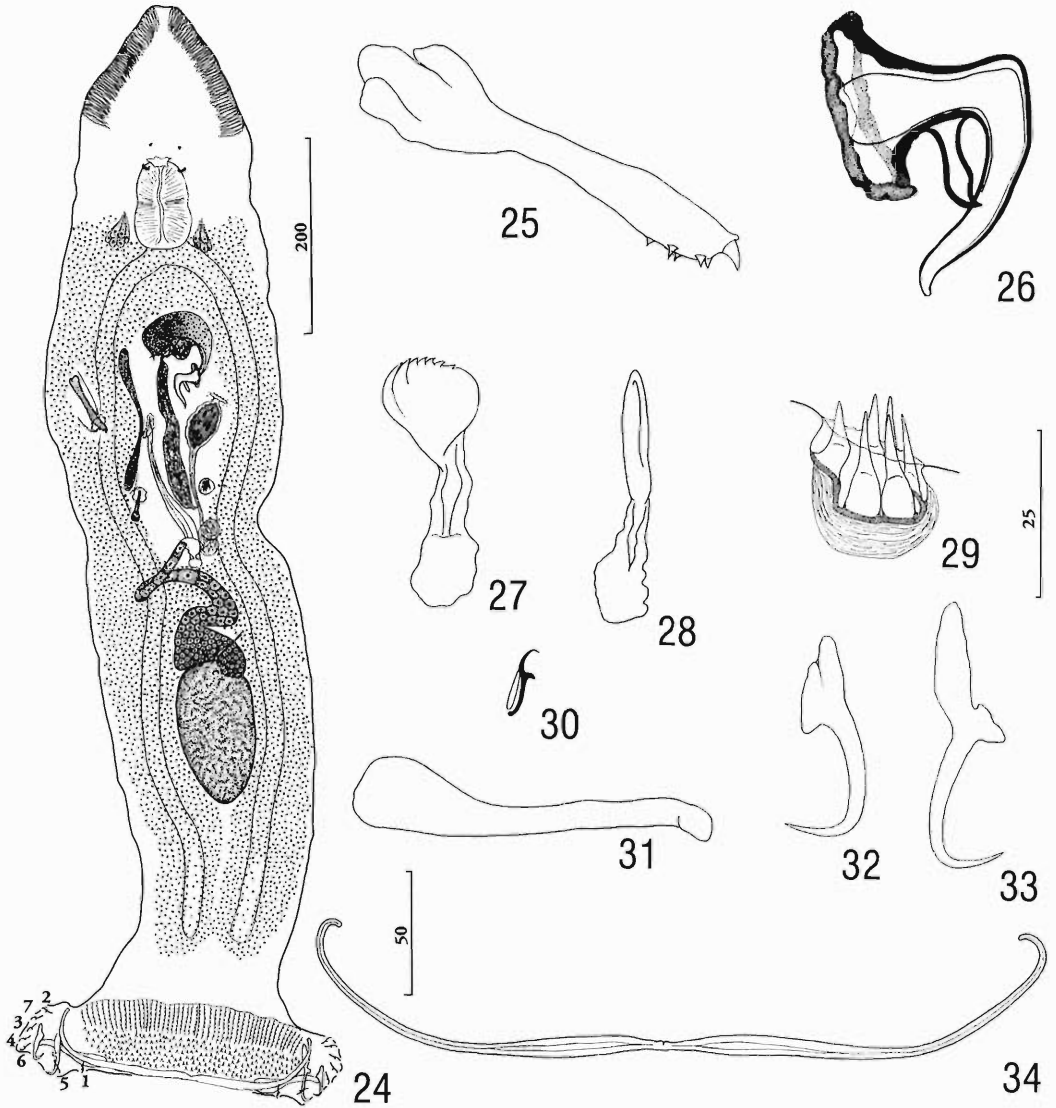
Examination of the types of *Diplectanum longipenis* (Yamaguti, 1934) Yamaguti, 1963 (= *Squamodiscus longipenis* Yamaguti, 1934), confirmed that *L. kuwaitensis* shares many features (general morphology and arrangement of the sclerotized haptor and copulatory sclerites and internal reproductive organs) with this species and may be more closely aligned to it than to those from fresh water. While staining procedures used by Yamaguti (1934) did not allow us to see spinelets near the posterior margin of the squamodisc in *D. longipenis*, similarities in the morphology of sclerotized structures and the general organization of the reproductive organs suggest that the 2 species are congeneric. Thus, we propose the transfer of *D. longipenis* to *Lepidotrema* as *L. longipenis* (Yamaguti, 1934) comb. n. *Squamodiscus* Yamaguti, 1934, is removed from synonymy with *Diplectanum* and becomes a junior subjective synonym of *Lepidotrema*. *Lepidotrema kuwaitensis* differs from *L. longipenis* by having delicate anchors (base of dorsal anchor in *L. kuwaitensis* narrow; broad in *D. longipenis*) and by the number of rodlet rows in the squamodisc (8–10 rows in *L. kuwaitensis*; 18–21 in *D. longipenis*).

***Pseudolamellodiscus sphyraenae* Yamaguti,  
1953**

(Figs. 24–34)

REDESCRIPTION: Diplectaninae. Body 1196 (1020–1354;  $n = 17$ ) long, flattened dorsoventrally; greatest width 244 (196–289;  $n = 16$ ) usually in anterior trunk. Trunk with anterior dextroventral sclerite, posterior dextroventral sclerite, sinistroventral spined pit. Anterior dextroventral sclerite 58 (48–72;  $n = 26$ ) long, with lobulate base, rod-shaped distal end protruding from small ventral pore, spined; number of spines variable. Posterior dextroventral sclerite 37 (32–45;  $n = 27$ ) long, spatulate, with incised distal margin; sinistroventral pit blind, with 4–6

spines, opening ventrally via small aperture through tegument; tips of spines usually protruding through pore. Tegument smooth. Cephalic margin tapered; cephalic lobes poorly developed; head organs numerous along anterolateral margins of cephalic area; cephalic glands posterolateral to pharynx. Eyes 4; members of posterior pair larger, slightly farther apart than anterior members; 1 member of each pair occasionally absent; granules small, irregular; accessory granules uncommon in cephalic region. Mouth subterminal, ventral to anterior portion of pharynx; pharynx 61 (53–70;  $n = 19$ ) wide, elongate, ovate; esophagus short to nonexistent; intestinal ceca blind. Peduncle broad. Haptor 337 (260–421;  $n = 18$ ) wide, 114 (93–148;  $n = 18$ ) long, bilaterally lobed; squamodiscs similar, each 61 (47–70;  $n = 17$ ) long, 249 (200–310;  $n = 17$ ) wide, with approximately 45 longitudinal parallel rows of dumbbell-shaped spines in anterior portion of squamodisc; posterior portion with numerous spine-like scales. Ventral anchor 41 (36–44;  $n = 25$ ) long, with elongate deep root, knob-like superficial root, slightly curved shaft, recurved point extending past level of tip of superficial anchor root; anchor base 11 (10–12;  $n = 3$ ) wide. Dorsal anchor 33 (31–36;  $n = 31$ ) long, with short deep root, triangular superficial root perpendicular to anchor base, curved shaft, point extending past level of tip of superficial root of anchor base; anchor base 9 (8–11;  $n = 6$ ) wide. Ventral bar 268 (218–328;  $n = 22$ ) long, narrowed medially, ends tapered, recurved anteriorly; ventral groove present. Paired dorsal bar 69 (59–87;  $n = 25$ ) long, club-shaped. Hooks similar; each 10–11 ( $n = 26$ ) long, with protruding depressed thumb, delicate point, shank. Hook pair 1 submarginal, lying posterior to bars near base of haptor lobes; pairs 2–7 located on lateral haptor lobes; FH loop shank length. Male copulatory organ 33 (31–35;  $n = 9$ ) long, with large base, bent shaft, acute bent tip, subbasal pointed projection. Accessory piece absent. Common genital pore absent; male genital pore lying ventrally to left of body midline slightly posterior to male copulatory organ; uterine pore ventral, slightly posterior to level of male genital pore, somewhat dextral to body midline. Testis 165 (144–184;  $n = 15$ ) long, 81 (59–98;  $n = 16$ ) wide, ovate; course of vas deferens not observed; 2 seminal vesicles simple dilations of vas deferens; proximal vesicle elongate, fusiform, lying along mid-



Figures 24–34. *Pseudolamellodiscus sphyraenae* Yamaguti, 1953. 24. Whole mount (composite, ventral; dorsal squamodisc not shown), showing positions of hook pairs. 25. Anterior dextroventral sclerite. 26. Copulatory complex. 27. Posterior dextroventral sclerite. 28. Posterodorsal accessory sclerite (lateral view). 29. Sinistroventral spinous cavity (lateral view). 30. Hook. 31. Dorsal bar. 32. Dorsal anchor. 33. Ventral anchor. 34. Ventral bar. All figures are drawn to the 25- $\mu$ m scale, except Figures 24 and 34 (200- $\mu$ m and 50- $\mu$ m scales, respectively).

line of body posterior to male copulatory organ; distal vesicle anterior to male copulatory organ, short, pyriform; prostatic reservoir saccate, anterior to male copulatory organ. Ovary 81 (62–107;  $n = 16$ ) wide, forming lobed cap on anterior margin of testis, with proximal sinistral loop before extending around right intestinal cecum; oviduct narrowing to small tube before joining

slightly expanded ootype; uterus delicate, extending to right of body midline; vaginal aperture sinistroventral; vagina tubular, frequently containing apparent spermatophore, joining small seminal receptacle lying to left of ootype; vitellaria throughout trunk, except absent in regions of reproductive organs.

HOST AND LOCALITY: Yellowstrip barracuda,

*Sphyraena chrysoaenia* Klunzinger, 1884 (Sphyraenidae): Persian Gulf off Kuwait (16 October 1996).

PREVIOUS RECORDS: *Sphyraena* sp.: Macassar, Celebes (Yamaguti, 1953). Great barracuda, *Sphyraena barracuda* (Walbaum, 1972): Nosy Bé, Madagascar (Rakotofiringa and Maillard, 1979).

SPECIMENS STUDIED: 34 voucher specimens, USNPC 89028, HWML 15020.

REMARKS: While Yamaguti (1953) did not adequately describe the haptor sclerites, male copulatory organ, and trunk sclerites of *Pseudolamellodiscus sphyraenae*, our examination of the holotype and paratypes of this species confirmed that our specimens were conspecific with *P. sphyraenea*. In the account of *P. sphyraenea* from Madagascar by Rakotofiringa and Maillard (1979), the morphology of the haptor and trunk sclerites were also not presented, but their figure of the trunk region, which includes a small drawing of the male copulatory organ, clearly supports their identification. However, both Yamaguti (1953) and Rakotofiringa and Maillard (1979) confused the vagina with the uterus. This error is supported by some of our specimens that contained a spermatophore in the tube that these authors described as the "uterus" and a developing egg in the tube they labeled "vagina."

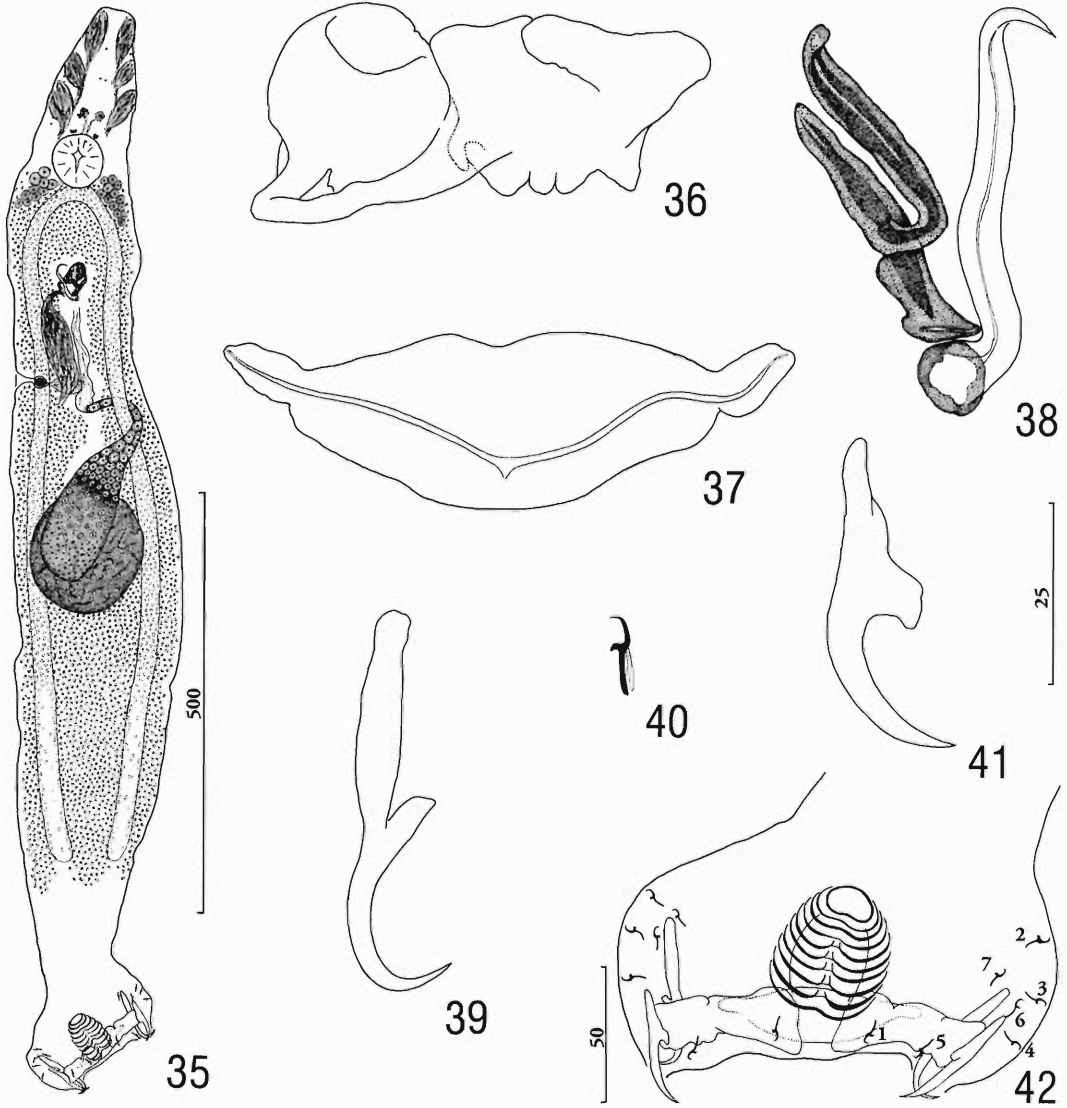
The slide containing the types of *P. sphyraenea* includes the holotype, 23 paratypes, and several fragments of specimens. Included in the 23 paratypes are 2 specimens clearly of an undescribed *Pseudolamellodiscus* species, characterized by having 1 large ventral trunk sclerite with a bifurcated, foliated proximal end.

#### *Lamellodiscus furcillatus* sp. n.

(Figs. 35–42)

DESCRIPTION: Lamellodiscinae. Body 1,092 (924–1,294;  $n = 4$ ), long, fusiform; greatest width 207 (183–226;  $n = 4$ ), usually in posterior trunk near level of testis. Tegument smooth. Cephalic margin tapered; 2 terminal, 2 bilateral cephalic lobes poorly developed; 3 bilateral pairs of head organs with anterior, posterior pairs associated with respective cephalic lobes; cephalic glands posterolateral to pharynx. Eyes 4; equidistant; members of posterior pair larger than anterior members; anterior eyes occasionally absent; granules ovate, variable in size; accessory

granules common in cephalic region. Mouth subterminal, ventral to pharynx; pharynx 54 (46–60,  $n = 4$ ) wide, ovate to subspherical; bilateral pair of prepharyngeal (buccal) glands anterior to pharynx; esophagus short to nonexistent; intestinal ceca blind. Peduncle broad. Haptor 163 (148–180;  $n = 4$ ) wide, 111 (104–117;  $n = 4$ ) long, bilaterally lobed; lobes short. Lamellogdiscs similar; each 58 (52–62;  $n = 4$ ) long, 42 (40–46;  $n = 4$ ) wide, ovate, with 10 lamellar rings; anterior (deep) lamella forming complete ring; intermediate lamellae superficially incomplete, medially indented; posterior (superficial) lamella indented, complete. Ventral anchor 58 (54–61;  $n = 8$ ) long, with elongate deep root, short depressed superficial root, evenly curved shaft, recurved point; point extending slightly past level of tip of superficial anchor root; anchor base 11–12 ( $n = 2$ ) wide. Dorsal anchor 48 (45–52;  $n = 8$ ) long, with elongate deep root, erect knob-like superficial root, evenly curved shaft, nonrecurved point; anchor base 14–15 ( $n = 2$ ) wide. Ventral bar 76 (70–82;  $n = 8$ ) long, plate-like, with ends constricted subterminally, ventral groove. Paired dorsal bar 62 (56–68;  $n = 8$ ) long, morphologically complex, broad. Hooks similar; each 12 (11–13;  $n = 7$ ) long, with protruding slightly depressed thumb, delicate point, shank; FH loop shank length. Hook pair 1 submedial at level of posterior margin of ventral bar; pairs 2–4 submarginal in lateral haptor lobes; pair 5 associated with ventral anchor shafts; pairs 6, 7 dorsal at level of tip of deep root of ventral anchor. Male copulatory organ 60 (58–65;  $n = 7$ ) long, a sigmoid tube with acute recurved tip. Accessory piece 53 (49–58;  $n = 4$ ) long, with subterminal elongate branch. Testis subspherical, 111 (110–113;  $n = 2$ ) in diameter, course of vas deferens not observed; seminal vesicle a simple dilation of vas deferens, lying to left of body midline dorsal to seminal receptacle; prostatic reservoir saccate, lying anterior to copulatory complex. Ovary 65 (64–67;  $n = 2$ ) wide, elongate pyriform, diagonal, looping right intestinal cecum, overlapping testis; oviduct elongate; ootype ventral, a small dilated portion of female duct; uterus delicate, seminal receptacle small. Vaginal aperture sinistral; vagina short, frequently containing apparent subspherical spermatophore. Vitellaria throughout trunk, except absent in regions of reproductive organs.



Figures 35–42. *Lamellodiscus furcillatus* sp. n. 35. Whole mount (composite, dorsal; ventral lamellocyte not shown). 36. Dorsal bar. 37. Ventral bar. 38. Copulatory complex. 39. Ventral anchor. 40. Hook. 41. Dorsal anchor. 42. Dorsal view of haptor showing dorsal lamellocyte and positions of hook pairs. All figures are drawn to the 25- $\mu$ m scale, except Figures 35 and 42 (500- $\mu$ m and 50- $\mu$ m scales, respectively).

TYPE HOST: Red Sea seabream, *Diplodus noct* (Valenciennes, 1830) (Sparidae).

TYPE LOCALITY: Persian Gulf off Kuwait (27 October 1995, 23 March 1996).

INFECTION SITE: Gills.

DEPOSITED SPECIMENS: Holotype, USNPC 89036; 7 paratypes, USNPC 89037, HWML 15024.

ETYMOLOGY: The specific name is from Lat-

in (*furcillatus* = a small fork) and refers to the accessory piece of the copulatory complex.

REMARKS: *Lamellodiscus furcillatus* sp. n. resembles *Lamellodiscus baeri* Oliver, 1974, in the morphology of the paired dorsal bars and general morphology of the copulatory complex. Oliver's (1974) description of *L. baeri* from the common seabream, *Pagrus pagrus* (Linnaeus, 1758), Sparidae, is brief and does not include

figures of the internal anatomy (whole mount), hooks, or lamellocdisc. However, *L. furcillatus* is easily differentiated from *L. baeri* by the presence of a nonrecurved point of the dorsal anchor (point of dorsal anchor recurved in *L. baeri*).

*Calydiscoides flexuosus* (Yamaguti, 1953)  
Young, 1969

(Figs. 43–52)

REDESCRIPTION (Table 2 for measurements): Lamellocdiscinae. Body long, fusiform; greatest width usually at level of testis. Tegument smooth. Cephalic margin tapered; 2 terminal, 2 bilateral cephalic lobes poorly developed; 3 bilateral groups of head organs with anterior, posterior groups associated with respective cephalic lobes; cephalic glands posterolateral to pharynx. Eyes 4; members of posterior pair larger, usually closer together than anterior members; 1 member of anterior pair occasionally absent; granules usually ovate, variable in size; accessory granules common in cephalic region. Mouth subterminal, ventral to pharynx; pharynx ovate to subspherical; esophagus short; intestinal ceca blind. Peduncle broad. Haptor bilaterally lobed; lamellocdiscs similar, each with 10 “telescoping” lamellae, posterior lamellae incomplete forming posterior superficial opening. Ventral anchor with elongate roots (superficial root longest) usually overlying one another (Fig. 51), evenly curved shaft, point recurved, not reaching level of tip of superficial anchor root. Dorsal anchor with short deep root, triangular superficial root, curved shaft, point extending past level of tip of superficial root. Ventral bar with tapered ends directed anterolaterally, ventral groove. Paired dorsal bar with bilobed medial end. Hooks similar; each with protruding thumb with slightly depressed end, delicate point, shank; hook pair 1 lying medial to ventral anchor at level of anterior margin of ventral bar, pairs 2 (anterior), 3 lateral to ventral lamellocdisc, pairs 4, 6 submarginal in haptor lobe, pair 5 associated with ventral anchor shaft, pair 7 lateral to dorsal lamellocdisc; FH loop nearly shank length. Male copulatory organ, accessory piece nonarticulated. Male copulatory organ C-shaped, variably sclerotized, with acute termination, 2 subterminal branches embedded in wall of genital atrium present or absent. Accessory piece variable, flattened. Testis ovate; course of vas deferens in relation to gut not observed; vas deferens tortuous

(Fig. 46), with anterior loop, expanded to form seminal vesicle; prostatic reservoir not observed. Ovary elongate pyriform, looping right intestinal cecum, lying transversely to diagonally anterodorsal to testis; oviduct elongate; ootype an expanded portion of female duct, surrounded by numerous glands; uterus with thick wall, ventral to proximal portion of vas deferens, extending dorsal to anterior loop of vas deferens. Vaginal aperture sinistroversal; vagina variable, funnel-shaped, narrowing to short tortuous tube; seminal receptacle absent, or represented by small expansion of vaginal duct prior to emptying into female duct; vaginal funnel with sclerotized clasp-like wall. Vitellaria coextensive with gut, absent in regions of reproductive organs.

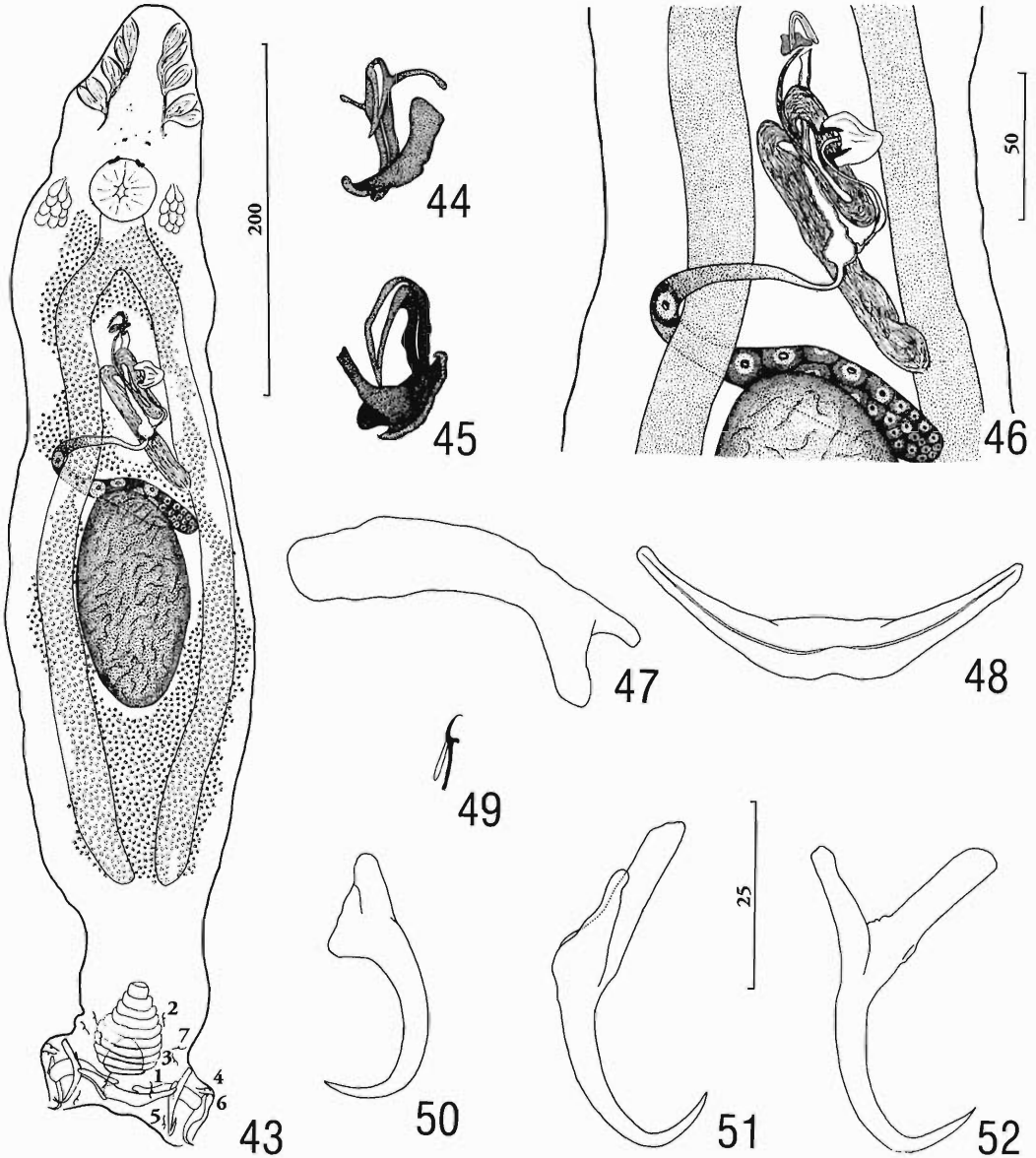
SYNONYMS: *Lamellocdiscus flexuosus* Yamaguti, 1953; *Lamellospina indiana* Karyakarte and Das, 1978; *Calydiscoides indicus* Venkatanarsaiah and Kulkarni, 1980.

HOST AND LOCALITIES: Notchedfin threadfin bream, *Nemipterus peronii* (Valenciennes, 1830) (originally identified as *N. tolu* [Valenciennes, 1830]) and Delagoa threadfin bream, *Nemipterus bipunctatus* (Valenciennes, 1830) (originally identified as *N. delagoae* Smith, 1941) (Nemipteridae): Persian Gulf off Kuwait (31 December 1993, 10 January 1994, respectively). Japanese threadfin bream, *Nemipterus japonicus* (Bloch, 1791) (Nemipteridae): Port of Okha, Gujarat, India.

PREVIOUS RECORDS: Ornate threadfin bream, *Nemipterus hexodon* (Quoy and Gaimard, 1824) (originally identified as *Synagris taeniopterus* (Valenciennes, 1830): Macassar, Celebes (Yamaguti, 1953). *Nemipterus japonicus*: Ratnagiri, west coast, Maharashtra, India (Karyakarte and Das, 1978); Kakinada, Bay of Bengal, India (Venkatanarsaiah and Kulkarni, 1980).

SPECIMENS STUDIED: 18 voucher specimens from *N. peronii*, USNPC 89025, HWML 15019; 14 voucher specimens from *N. bipunctatus*, USNPC 89026; 37 voucher specimens from *N. japonicus*, USNPC 89024.

REMARKS: Yamaguti (1953) described *Lamellospina flexuosus* from the gills of *Synagris taeniopterus* (= *Nemipterus hexodon*) collected at Macassar, Celebes. Young (1969) transferred this helminth to *Calydiscoides* Young, 1969, based on the presence of “telescoping lamellae” in the lamellocdisc, and Oliver (1987) recognized Young’s reassignment of *L. flexuosus* to *Calydiscoides*. In



Figures 43–52. *Calydiscoides flexuosus* (Yamaguti, 1953) Young, 1969. 43. Whole mount (composite, ventral; dorsal lamellogdisc not shown), showing positions of hook pairs. 44, 45. Copulatory complexes. 46. Enlargement of reproductive organs (composite, ventral). 47. Dorsal bar. 48. Ventral bar. 49. Hook. 50. Dorsal anchor. 51, 52. Ventral anchors. All figures are drawn to the 25- $\mu$ m scale, except Figures 43 and 46 (200- $\mu$ m and 50- $\mu$ m scales, respectively).

addition, Oliver (1987) transferred *Lamellospina indiana* Karyakarte and Das, 1978, to *Calydiscoides* as *C. indianus*, considered *Lamellospina* Karyakarte and Das, 1978, a junior synonym of *Calydiscoides*, and placed *C. indicus* Venkatanarsaiah and Kulkarni, 1980, in synonymy with *C.*

*indianus*. Present findings support Young's (1969) and Oliver's (1987) taxonomic proposals. Our examination of the holotype and paratypes of *L. flexuosus* and new voucher specimens of *C. flexuosus* collected from the Indian coast and Kuwait confirmed that *L. flexuosus* Yamaguti, 1953, and



**Table 2.** Comparative measurements (in micrometers) of *Calydiscoides flexuosus* (Yamaguti, 1953) Young, 1969, from 3 species of *Nemipterus* (Nemipteridae) from the Persian Gulf and Indian Ocean.

	<i>N. peronii</i> Kuwait	<i>N. bipunctatus</i> Kuwait	<i>N. japonicus</i> India	<i>N. hexodon</i> Celebes
<b>Body</b>				
Length	771 (675–843; <i>n</i> = 8)	776 (680–987; <i>n</i> = 9)	893 (781–1,044; <i>n</i> = 17)	528 (411–796; <i>n</i> = 16)
Width	142 (110–162; <i>n</i> = 11)	134 (107–162; <i>n</i> = 9)	136 (100–187; <i>n</i> = 19)	74 (62–91; <i>n</i> = 20)
<b>Haptor</b>				
Length	94 (78–103; <i>n</i> = 10)	102 (78–128; <i>n</i> = 9)	126 (113–142; <i>n</i> = 12)	88 (69–113; <i>n</i> = 17)
Width	107 (97–114; <i>n</i> = 10)	104 (88–116; <i>n</i> = 7)	121 (91–149; <i>n</i> = 8)	93 (76–119; <i>n</i> = 16)
<b>Lamellodisc</b>				
Length	49 (37–60; <i>n</i> = 10)	49 (43–58; <i>n</i> = 7)	68 (54–76; <i>n</i> = 13)	43 (31–56; <i>n</i> = 15)
Width	41 (34–46; <i>n</i> = 10)	37 (31–45; <i>n</i> = 6)	42 (36–53; <i>n</i> = 16)	31 (27–36; <i>n</i> = 20)
<b>Pharynx</b>				
Width	42 (35–48; <i>n</i> = 11)	38 (33–44; <i>n</i> = 8)	48 (23–56; <i>n</i> = 14)	23 (19–27; <i>n</i> = 18)
<b>Copulatory organ</b>				
Length	32 (29–37; <i>n</i> = 6)	36 (32–39; <i>n</i> = 7)	—	—
<b>Accessory piece</b>				
Length	21 (17–26; <i>n</i> = 6)	19 (15–24; <i>n</i> = 5)	—	—
<b>Dorsal anchor</b>				
Length	35 (32–40; <i>n</i> = 14)	35 (30–39; <i>n</i> = 13)	36 (29–48; <i>n</i> = 27)	27 (25–29; <i>n</i> = 19)
Base width	11 (10–12; <i>n</i> = 4)	10 (9–11; <i>n</i> = 2)	11 (9–14; <i>n</i> = 10)	9 (8–10; <i>n</i> = 2)
<b>Ventral anchor</b>				
Length	46 (43–48; <i>n</i> = 14)	46 (43–52; <i>n</i> = 14)	49 (42–56; <i>n</i> = 30)	39 (35–43; <i>n</i> = 21)
Base width	21 (16–26; <i>n</i> = 12)	22 (19–25; <i>n</i> = 10)	21 (18–25; <i>n</i> = 13)	17 (15–20; <i>n</i> = 3)
<b>Bar length</b>				
Dorsal	45 (39–54; <i>n</i> = 14)	43 (37–51; <i>n</i> = 14)	48 (38–66; <i>n</i> = 18)	37 (34–42; <i>n</i> = 25)
Ventral	54 (45–65; <i>n</i> = 11)	52 (47–58; <i>n</i> = 11)	56 (49–64; <i>n</i> = 10)	43 (39–48; <i>n</i> = 16)
<b>Hook</b>				
Length	12 (11–13; <i>n</i> = 9)	11 (10–13; <i>n</i> = 6)	12–13 ( <i>n</i> = 13)	11 (10–12; <i>n</i> = 9)
<b>Germinarium</b>				
Width	28 (24–34; <i>n</i> = 3)	29 (26–33; <i>n</i> = 3)	41 (37–45; <i>n</i> = 4)	—
<b>Testis</b>				
Length	149 (123–177; <i>n</i> = 9)	149 (121–178; <i>n</i> = 9)	183 (149–219; <i>n</i> = 9)	—
Width	63 (42–76; <i>n</i> = 9)	70 (51–89; <i>n</i> = 9)	79 (62–93; <i>n</i> = 9)	—

*Calydiscoides indianus* (Karyakarte and Das, 1978) Oliver, 1987, and its synonyms *Lamellospina indiana* Karyakarte and Das, 1978, and *Calydiscoides indicus* Venkatanarsaiah and Kulkarni, 1980, are synonyms of *C. flexuosus* (Yamaguti, 1953) Young, 1969.

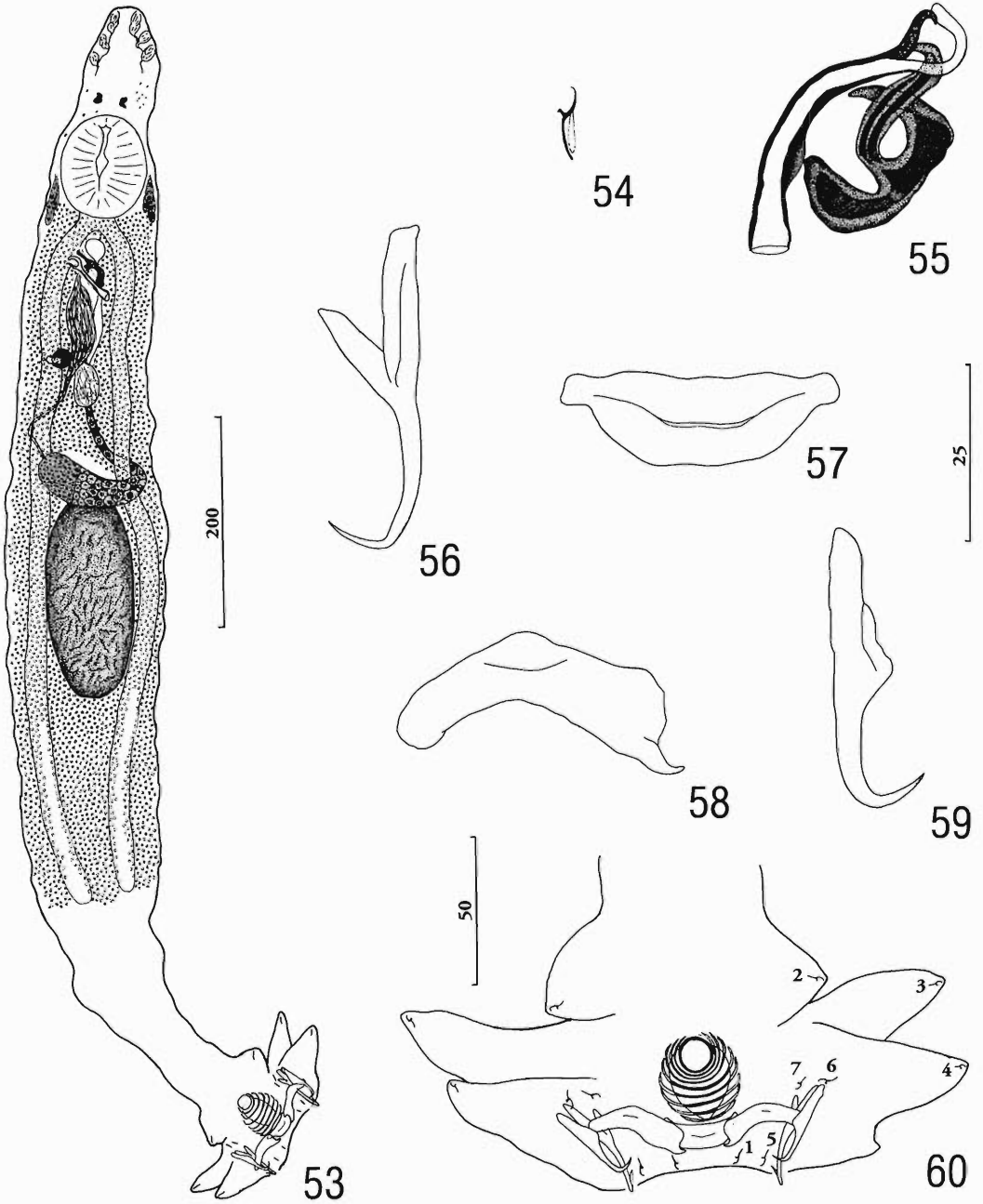
Specimens of *Calydiscoides flexuosus* from India, Kuwait, and the Celebes are morphologically indistinguishable. However, we did observe some differences in dimensions of the body and haptor sclerites (Table 2). Specimens from the Celebes were somewhat smaller than those from India, while those from Kuwait were intermediate in size. These differences are not considered sufficient to separate the collections

into distinct species, and could result from effects of different environmental and host factors on the parasite. All previous descriptions of this species lack detail and clarity of the morphological features necessary to identify the species; our redescription provides details of the morphology of the sclerotized parts of the haptor and copulatory complex.

***Protolamellodiscus senilobatus* sp. n.**

(Figs. 53–60)

DESCRIPTION (measurements of specimens from *A. filamentosus* follow those from the type host in brackets): Lamellodiscinae. Body 1,065 (720–1318; *n* = 8) [714 (673–755; *n* =



Figures 53–60. *Protolamellodiscus senilobatus* sp. n. 53. Whole mount (composite, dorsal; ventral lamellodisc not shown). 54. Hook. 55. Copulatory complex. 56. Ventral anchor. 57. Ventral bar. 58. Dorsal bar. 59. Dorsal anchor. 60. Dorsal view of haptor showing dorsal lamellodisc and positions of hook pairs (ventral lamellodisc not shown). All figures are drawn to the 25- $\mu$ m scale, except Figures 53 and 60 (100- $\mu$ m and 50- $\mu$ m scales, respectively).

2)] long, slender, fusiform; greatest width 185 (120–240;  $n = 9$ ) [164 (148–179;  $n = 2$ )] at level of testis. Tegument smooth. Cephalic margin narrow; 2 terminal, 2 bilateral cephalic lobes poorly developed; 3 bilateral pairs of head organs with anterior, posterior pairs associated with respective cephalic lobes; cephalic glands posterolateral to pharynx. Eyes 4; members of posterior pair slightly larger, closer together than anterior members; anterior pair frequently absent; granules irregular, variable in size; accessory granules common in cephalic region. Mouth subterminal, ventral to pharynx; pharynx 73 (61–89,  $n = 10$ ) [60 (51–70;  $n = 2$ )] wide, ovate or somewhat truncated posteriorly; esophagus short to nonexistent; intestinal ceca blind. Peduncle narrow, elongate. Haptor 206 (169–235;  $n = 8$ ) [155 (150–161;  $n = 2$ )] wide, 111 (104–117;  $n = 8$ ) [84 (79–89;  $n = 2$ )] long, with 3 bilateral pairs of lobes containing respective hook pairs 2, 3, 4 near apices; anterior lobes about half the length of more posterior lobes; lamellodiscs similar, each 44 (37–53;  $n = 10$ ) [37 (35–39;  $n = 2$ )] long, 32 (29–38;  $n = 10$ ) [30 (29–31;  $n = 2$ )] wide, with 1 complete, 8 incomplete lamellae lacking medial indentation; lamellae appear to telescope somewhat in dorsoventral view. Ventral anchor 45 (38–49;  $n = 11$ ) [42–43 ( $n = 1$ )] long, with elongate roots (deep root longest), evenly curved shaft, point acutely recurved not reaching level of tip of superficial root; base 14 (9–16;  $n = 8$ ) wide. Dorsal anchor 41 (37–44;  $n = 17$ ) [35–36 ( $n = 1$ )] long, with elongate deep root, short thickened superficial root, straight shaft, point reaching past level of tip of superficial root; base 9 (8–10;  $n = 13$ ) wide. Ventral bar 41 (34–47;  $n = 17$ ) [36 (34–38;  $n = 2$ )] long, plate-like, with short knob-like ends; dorsal bar 40 (35–46;  $n = 23$ ) [36 (34–38;  $n = 3$ )] long, with medial bend, spinous projection at proximal end. Hooks similar; each 10 (9–11;  $n = 28$ ) [9–10 ( $n = 3$ )] long, with protruding slightly depressed thumb, delicate point, shank; hook pair 1 lying near base of ventral anchor; pairs 2, 3, 4 at apices of respective haptor lobes; pair 5 posterior to ends of ventral bar; pair 6 near point of dorsal anchor; pair 7 near base of dorsal anchor. FH loop nearly shank length. Copulatory complex comprising articulated male copulatory organ, accessory piece. Male copulatory organ 45 (38–53;  $n = 22$ ) [42–43 ( $n = 1$ )] long, a curved heavily sclerotized tube with

subterminal recurved spine, distal loop terminating broadly; base of male copulatory organ lacking sclerotized margin. Accessory piece 28 (18–34;  $n = 16$ ) [32–33 ( $n = 1$ )] long, comprising flattened proximal portion, bifurcating near midlength to terminally acute elongately striated branch, spatulate branch frequently folded upon itself distally. Testis 107 (101–113;  $n = 2$ ) long, 52 (48–55;  $n = 2$ ) wide, ovate; vas deferens looping left intestinal cecum; seminal vesicle fusiform, simple dilation of vas deferens, lying slightly to left of body midline; prostatic reservoir saccate, lying anterior to copulatory complex. Ovary 47 (44–56;  $n = 5$ ) wide, pyriform, looping right intestinal cecum, lying transversely to diagonally anterior to testis; oviduct elongate; ootype, uterus not observed; vaginal aperture sinistrodorsal, submarginal; vagina short, nonsclerotized, with proximal chamber containing apparent spermaphore, opening into medial seminal receptacle; vitellaria dense throughout trunk, except absent in regions of reproductive organs. One egg (deformed during mounting) infrequently present in uterus, with short proximal filament.

TYPE HOST: King soldierbreem, *Argyrops spinifer* (Forsskål, 1775) (Sparidae).

TYPE LOCALITY: Persian Gulf off Kuwait (15 January 1994).

INFECTION SITE: Gills.

OTHER RECORD: Soldierbreem, *Argyrops filamentosus* (Valenciennes, 1830) (Sparidae): Persian Gulf off Kuwait (18 October 1995).

SPECIMENS STUDIED: Holotype, USNPC 89005; 28 paratypes from *A. spinifer*, USNPC 89006, HWML 15021; 3 voucher specimens from *A. filamentosus*, USNPC 89027.

ETYMOLOGY: The specific name is from Latin (*sen/i* = six + *lobat/o* = lobe) and refers to the 6 bilateral lobes of the haptor.

REMARKS: Oliver (1987) recognized 3 species of *Protolamellodiscus* from hosts of 3 marine teleost families: *Protolamellodiscus serranelli* (Euzet and Oliver, 1965) Oliver, 1969, from the comber, *Serranus cabrilla* (Linnaeus, 1758), the brown comber, *Serranus hepatus* (Linnaeus, 1758), and the painted comber, *Serranus scriba* (Linnaeus, 1758), Serranidae; *Protolamellodiscus convolutus* (Yamaguti, 1953) Oliver, 1987, from *N. hexodon*, Nemipteridae; and *Protolamellodiscus raibauti* Oliver and Radujkovic, 1987, from the annular seabream, *Diplodus annularis* (Linnaeus, 1758), Sparidae. The fourth

species, *P. senilobatus* sp. n., occurs on sparid hosts (*Argyrops* spp.). The new species most closely resembles *P. raibauti* in the comparative morphology of the copulatory complex but differs from this species by possessing a subterminal spine arising from the male copulatory organ, 3 bilateral pairs of haptor lobes (lobes lacking in *P. raibauti*), a flattened subrectangular ventral bar (bar rod-shaped in *P. raibauti*), and each dorsal bar with a proximal spine (see Oliver and Radujkovic, 1987). *Protolamellodiscus senilobatus* differs from *P. serranelli* in the comparative morphology of the copulatory complex. While Yamaguti's (1953) description of *P. convolutus* lacks details of the sclerotized structures of the haptor and copulatory complex, *P. senilobatus* is distinguished from this species by possessing 3 bilateral pairs of haptor lobes.

Oliver and Radujkovic (1987) described the vagina of *P. raibauti* as opening sublaterally on the left side of the body. In *P. senilobatus*, the vaginal aperture is submarginal on the sinistrodorsal body surface, midway between the ovary and copulatory complex. In *P. senilobatus*, the vas deferens loops the left intestinal cecum, while Euzet and Oliver (1965) reported the vas deferens to be intercecal in *P. serranelli*. Oliver and Radujkovic (1987) did not observe the course of the vas deferens relative to the intestine in *P. raibauti*. Confirmation of these 2 characters as potential diagnostic features of *Protolamellodiscus* is required.

Members of *Protolamellodiscus* Oliver, 1969, and *Calydiscoides* Young, 1969, are characterized, in part, by having a ventral and a dorsal lamellocdisc, each with several concentric unpaired lamellae, with the most anterior lamella forming a complete circle. *Calydiscoides* is, in part, diagnosed by the presence of telescoping lamellae. Depending on the orientation of the lamellocdisc when examined microscopically, specimens of *P. senilobatus* occasionally show that the deeper lamellae telescope, although not to the extent exhibited in described species of *Calydiscoides*. While outside the scope of the present study, it is possible that *Protolamellodiscus* and *Calydiscoides* are synonyms. Further study of all species in these genera combined with a phylogenetic analysis is necessary to clarify synonymy and/or validity of the genera.

### Discussion

In his revision of the Diplectanidae, Oliver (1987) divided the family into 4 subfamilies

based primarily on the morphology and presence/absence of the accessory adhesive organs of the haptor. He recognized the Diplectaninae Monticelli, 1903 ("squamosdiscs" composed of concentric rows of sclerotized rodlets): Lamellocdiscinae Oliver, 1969 ("lamellocdiscs" composed of concentric lamellae); Rhabdosynochinae Oliver, 1987 (lateral "placodiscs" unarmed); and Murraytrematoidinae Oliver, 1982 (accessory adhesive organs absent).

Oliver (1987) removed the then monotypic Rhamnocercinae Monaco, Wood, and Mizelle, 1954, from the Diplectanidae, elevated it to familial level, and placed it in the poorly supported superfamily Heterotesioidea Euzet and Dos-sou, 1979 (see Kritsky and Boeger, 1989), apparently because some previous descriptions of species of *Rhamnocercus* stated that the intestinal ceca are "apparently" united posterior to the gonads (Hargis, 1955, in *R. bairdiella* Hargis, 1955; subsequently by Luque and Iannacone [1991] in *R. oliveri* Luque and Iannacone, 1991). However, Monaco et al. (1954) and Seamster and Monaco (1956) did not mention the intestine in the respective descriptions of *R. rhamnocercus* Monaco, Wood, and Mizelle, 1954, and *R. stichospinus* Seamster and Monaco, 1956. Luque and Iannacone (1991) stated that the intestinal ceca end blindly in *Rhamnocercoides menticirrhoi* Luque and Iannacone, 1991, and *Rhamnocercus stelliferi* Luque and Iannacone, 1991. It appears that errors have been made concerning the morphology of the gut in some species of Rhamnocercinae, and the value of this character in determining familial relationships is limited. Along with Diplectaninae, Lamellocdiscinae, Rhabdosynochinae, and Murraytrematoidinae, we tentatively consider the Rhamnocercinae a member of the Diplectanidae, based on general haptor and internal morphology. However, these subfamilies all lack evolutionary support (phylogenetic analyses are lacking), and some or all may be unnatural.

With the exception of *Diplectanum* Diesing, 1858, and *Lamellocdiscus* Johnston and Tiegs, 1922, all diplectanid genera are defined by derived autapomorphic features, suggesting that *Diplectanum* and *Lamellocdiscus* are unnatural (paraphyletic) and currently serve as "catchall" groups for species lacking obvious derived characters. Kritsky and Boeger (1989) and Kritsky and Kulo (1992) discussed the probability of the

creation of paraphyletic taxa when new taxa are based primarily on autapomorphic features.

Oliver (1987) considered *Diplectanum* to include species having a squamodisc composed of concentric U-shaped rows of rodlets. Other diplectanine genera were diagnosed with characters thought to be lacking in *Diplectanum*, such as closed circular rows of rodlets (*Cycloplectanum* Oliver, 1968 [= *Pseudorhabdosynochus* Yamaguti, 1958, see Kritsky and Beverley-Burton 1986]), divergent rows of rodlets (*Heteroplectanum* Rakotofiringa, Oliver, and Lambert, 1987), lateral intestinal diverticula (*Latericaecum* Young, 1969), a row of elongate spines posterior to the squamodisc (*Lepidotrema* Johnston and Tiegs, 1922), 1 "squamodisc" (*Monoplectanum* Young, 1969), modified anchors (*Pseudodiplectanum* Tripathi, 1957), and parallel rodlets (*Pseudolamellodiscus* Yamaguti, 1953). Similarly, Oliver (1987) defined *Lamellodiscus* by species having paired (apparently incomplete) lamellae forming the lamellobiscs. The remaining genera in the Lamellobiscinae include forms with the following features absent in species of *Lamellodiscus*: *Calydiscooides* Young, 1969, with species having unpaired telescoping lamellae; *Furnestinia* Euzet and Audoin, 1959, with species lacking 1 "lamellobisc"; *Protolamellodiscus* Oliver, 1969, with species having closed or "O-shaped" lamella in the lamellobisc; and *Telegamatrix* Ramalingam, 1955, with a reproductive appendix containing the copulatory complex and vagina. Some of these genera might not be valid, as suggested by the apparent close relationship of *Diplectanum cazauxi* with *Laterocaecum pearsoni* and *Protolamellodiscus senilobatus* with species of *Calydiscooides* (see remarks under *D. cazauxi* and *P. senilobatus*).

That *Diplectanum* and *Lamellodiscus* are paraphyletic is supported by observations on specimens in the present study. Both genera include species with varying characters, which were not considered generic features by Oliver (1987), but that could be used to determine monophyletic groups in the 2 genera. Features such as presence/absence of an accessory piece, position of the vaginal aperture, and morphology of the copulatory complex, among others, may have value in determining monophyletic groupings in these genera. In *Lepidotrema*, which is characterized by species possessing a posterior shield of elongate spines as its autapomorphic character, at least 1 species, *L. longipenis*, apparently

lacks these structures. Thus, even some of the unique characters defining some of these genera (posterior spinous shield in *Lepidotrema*; gut diverticula in *Laterocaecum*) may not be valid for defining monophyletic groups within the Diplectanidae.

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## Obituary Notice

**MICHAEL J. PATRICK**

March 9, 1962–March 10, 2000

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## ***Langeronia burseyi* sp. n. (Trematoda: Lecithodendriidae) from the California Treefrog, *Hyla cadaverina* (Anura: Hylidae), with Revision of the Genus *Langeronia* Caballero and Bravo-Hollis, 1949**

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**ABSTRACT:** *Langeronia burseyi* sp. n. (Trematoda: Lecithodendriidae), a new trematode from the small intestine of *Hyla cadaverina* Cope, 1866, is described and illustrated. One (0.03%) of 36 adult specimens of *H. cadaverina* collected from Orange County, California, U.S.A., harbored 83 specimens of *L. burseyi* sp. n. *Langeronia burseyi* sp. n. is distinguished from all other species in the genus by body size, location of the cirrus, length of the ceca, placement of the vitellaria, and the shape of the excretory bladder. This is the first report of a species of *Langeronia* from a member of the Hylidae. An emended diagnosis and key to the genus *Langeronia* are presented.

**KEY WORDS:** Digenea, Lecithodendriidae, *Langeronia burseyi*, new species description, taxonomy, California treefrog, *Hyla cadaverina*, Orange County, California, U.S.A.

The taxonomic statuses of the genera *Langeronia* Caballero and Bravo-Hollis, 1949, and *Loxogenes* Stafford, 1904, have been the subject of much controversy. Caballero and Bravo-Hollis (1949) erected the genus *Langeronia* for a new species, *Langeronia macrocirra*, from the northern leopard frog, *Rana pipiens* Schreber, 1782, in Mexico. A second species, *Langeronia provitellaria*, was described by Sacks (1952) from the Florida leopard frog, *Rana sphenoccephala* Cope, 1886, in Florida, U.S.A. Yamaguti (1958) considered *Langeronia* synonymous with the genus *Loxogenes* Stafford, 1905. Brenes et al. (1959) examined specimens recovered from the cane toad, *Bufo marinus* (Linnaeus, 1758) in Costa Rica and disagreed with Yamaguti, concluding that *Langeronia* was a valid genus. Ubelaker (1965) collected trematodes from *B. marinus* in Nicaragua and published a redescription of *L. macrocirra*, concluding that *L. provitellaria* should be considered a synonym of that species. He also supported Yamaguti and his 1958 synonymy of the 2 genera. Christian (1970) studied specimens collected from the intestines of *R. pipiens* in Wisconsin, Ohio, and Vermont, U.S.A., which he identified as *L. provitellaria*, *Loxogenes* sp., and a new species, *Langeronia parva*, respectively. Christian (1970) disagreed with Yamaguti's (1958) opinion synonymizing

*Langeronia* and *Loxogenes* and supported Brenes et al. (1959) in validating the generic status of *Langeronia*. Christian (1970) did not mention the article by Ubelaker (1965) and the synonymy of *L. macrocirra* and *L. provitellaria*. However, Christian (1970) did state that in his opinion, according to the description and measurements given by Brenes et al. (1959) for "*L. macrocirra*," they were actually redescribing *L. provitellaria*. This would tend to explain why Ubelaker (1965) synonymized the 2 species, comparing the overlap of measurements from his specimens with the measurements given by Brenes et al. (1959). Yamaguti (1958) cited *Loxogenes* s. str. and *Langeronia* as subgenera of the genus *Loxogenes* s. lat. Babero and Golling (1974) reported 3 species of *L. provitellaria* from 2 bullfrogs (*Rana catesbiana* Shaw, 1802) collected in Nye County, Nevada, U.S.A.

### **Materials and Methods**

One of 36 California treefrogs, *Hyla cadaverina* Cope, 1866, examined (LACM No. 88937) from Orange County, California was infected with 83 trematodes in the large intestine. All *H. cadaverina* specimens had been collected between 1952 to 1967 and deposited in the herpetology collection of the Natural History Museum of Los Angeles County (LACM). They were originally preserved in 10% formalin and later stored in 70% ethanol.

Worms were removed from the large intestine, rinsed in 70% ethanol, stained in Delafield's hematoxylin, dehydrated in ethanol, and mounted in Canada

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balsam. Subsequent examination of the trematode specimens indicated that they represented an undescribed species of the genus *Langeronia*. Drawings were made with the aid of a drawing tube. Measurements are in micrometers unless otherwise indicated. The range is followed by the mean in parentheses. Type specimens were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A.

Some of the cotype specimens of *L. macrocirra* (USNPC No. 37127), the type specimens of *L. provitellaria* (USNPC No. 47569), and the type and paratype specimens of *L. parva* (USNPC No. 70557, 70558) were examined during this study.

## Results

### *Langeronia burseyi* sp. n.

(Fig. 1)

## Description

Based on 10 of 83 specimens: Lecithodendriidae (Lühe, 1901) Odhner, 1910; Pleurogenetinae Travassos, 1921. Body small, pyriform, 0.60–0.75 mm (0.66) long, maximum width 0.38–0.55 mm (0.49) at testicular level. Tegument thin, spinose. Oral sucker subterminal, 95–105 (102) long by 70–93 (81) wide. Prepharynx absent. Pharynx 60–68 (63) long by 38–45 (42) wide. Esophagus 23–28 (24) long by 12–15 (13) wide. Ceca bifurcate just anterior to midbody and extending posteriorly to anterior of testes. Acetabulum approximating size of oral sucker, 75–105 (92) long by 78–98 (90) wide. Cirrus pouch 225–287 (261) long by 53–70 (59) wide, arching transversely over acetabulum, then twisting medioventrally and opening into shallow thin-walled atrium with genital pore. Testes smooth, opposite, transversely oval, in posterior third of body. Right testis 92–155 (119) long by 98–163 (138) wide, left testis 110–125 (113) long by 125–188 (146) wide. Ovary round to oval, at acetabular level, anterior to right testis, 56–90 (68) long by 56–100 (77) wide. Seminal receptacle ovoid to spherical, 70 long by 45 wide. Mehlis' gland directly postacetabular, Laurer's canal not observed. Vitellaria dorsal, follicular, extending from just posterior to pharynx to anterior half of ceca on either side of esophagus. Uterus with irregular transverse loops filling post-testicular space. Eggs smooth, elliptical, 23–28 (24) long by 12–15 (13) wide. Excretory bladder V-shaped, excretory pore terminal.

## Taxonomic summary

TYPE HOST: California treefrog, *Hyla cadaverina* Cope, 1866, deposited in Natural History Museum of Los Angeles County as LACM 88937.

TYPE LOCALITY: Harding Canyon, Orange County, California, U.S.A. (33°42'N, 117°38'W).

COLLECTION DATE: 16 June 1965.

SITE OF INFECTION: Large intestine.

DEPOSITED SPECIMENS: Holotype and paratypes USNPC No. 89628

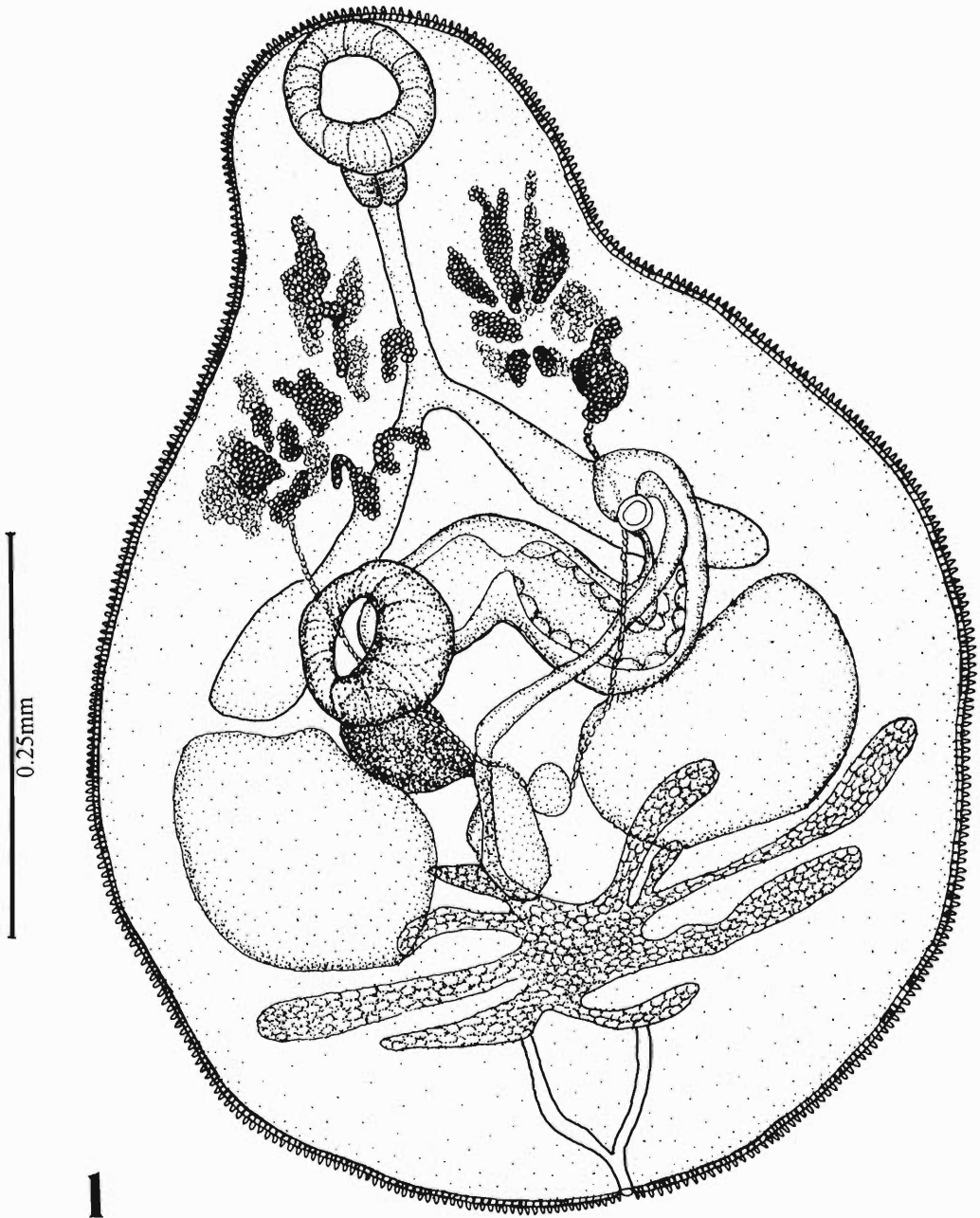
ETYMOLOGY: This species is named for Charles R. Bursey, Pennsylvania State University, Shenango, Pennsylvania, U.S.A., in recognition of his many contributions to the parasitology of amphibians and reptiles.

### *Langeronia* Caballero and Bravo-Hollis, 1949

EMENDED DIAGNOSIS: Lecithodendriidae, Pleurogenetinae. Body spatulate to pyriform, spined. Oral sucker well-developed, terminal or subterminal. Prepharynx present or absent; pharynx well-developed. Esophagus present; ceca wide, extending to midbody. Acetabulum equatorial. Testes symmetrical, postacetabular, and intercecal. Cirrus pouch elongate, twisted, extending transversely interceally in space between intestinal bifurcation and acetabulum. Genital pore preacetabular, ventral to or on internal border of left cecum. Ovary dextral to acetabulum, pretesticular. Uterine coils lateral, postequatorial; eggs smooth, operculate. Vitellaria follicular, in shoulder area, on either side of esophagus, not confluent. Excretory bladder Y- or V-shaped. Intestinal parasites of amphibians.

TYPE SPECIES: *Langeronia macrocirra* Caballero and Bravo-Hollis, 1949, from *R. pipiens* in Mexico.

OTHER SPECIES: In addition to *L. macrocirra*, the genus currently contains 2 other species, *Langeronia provitellaria* Sacks, 1952, and *L. parva* Christian, 1970. *Langeronia burseyi* sp. n. differs from all members of the genus in its small size (smallest in the genus), placement of cirrus at the acetabular level, cecal length, and V-shaped bladder. It most closely resembles *L. provitellaria* in the position of the vitellaria, with both beginning at the pharyngeal level. However, in *L. burseyi* the vitellaria end just



1 Figure 1. *Langeronia burseyi* sp. n. from *Hyla cadaverina*. Entire worm, ventral view.

posterior to the cecal bifurcation, while in *L. provitellaria*, they extend to the anterior of the cirrus. The new species resembles *L. macrocirra* and *L. parva* in that the ovary and testes are not deeply lobed as in *L. provitellaria*. The deeply lobed ovary and testes along with other features

(size, position of vitellaria and pharynx, length of ceca) distinguish *L. macrocirra* and *L. provitellaria* as separate species.

#### Key to the Species of *Langeronia*

- 1a. Body length more than 1.30 mm ..... 2



## Oxyuroids of Palearctic Testudinidae: New Definition of the Genus *Thaparia* Ortlepp, 1933 (Nematoda: Pharyngodonidae), Redescription of *Thaparia thapari thapari*, and Descriptions of Two New Species

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**ABSTRACT:** The generic diagnosis of *Thaparia* Ortlepp, 1933, is emended based on the study and redescription of *Thaparia thapari thapari* (Dubinina, 1949) from the cecum of *Testudo graeca* Linnaeus, 1758, collected in Settat, Morocco. In addition, 2 new species, *Thaparia carlosfeliui* sp. n. and *Thaparia bourgati* sp. n. from the cecum of *Testudo hermanni* Gmelin, 1789, collected in Catalonia, Spain, are described. Scanning electron microscopy studies revealed substantial differences in the structure of the mouth and the caudal end, which enabled us to differentiate the 2 new species from the others and from each other.

**KEY WORDS:** *Thaparia thapari thapari*, *Thaparia carlosfeliui* sp. n., *Thaparia bourgati* sp. n., Nematoda, Pharyngodonidae, *Testudo graeca*, spur-thighed tortoise, *Testudo hermanni*, Hermann's tortoise, Morocco, Spain.

The genus *Thaparia* was erected by Ortlepp (1933) for *Thaparia macrospiculum* Ortlepp, 1933, a parasite of the tent tortoise, *Psammobates tentorius* (Bell, 1828). Ortlepp (1933) gave the following diagnosis: Medium-sized worms possessing 3 lips and a relatively short esophagus consisting of an anterior muscular portion, a middle glandular portion, and a posterior bulb; excretory pore post-bulbar; lateral alae absent. Vulva approximated to anus; vagina very long; 2 uteri and 2 ovaries. Caudal extremity of male cut ventrally and continued backward to form a short truncated and alate tail. Four pairs of caudal papillae, 3 pairs circumcloacal and 1 pair toward tip of tail. Single spicule very long and stout, extending to or even anterior of the esophageal bulb. Type species *T. macrospiculum* from *P. tentorius*.

Walton (1942) described a second species, *Thaparia contortospicula*, a parasite of the Galapagos tortoise, *Geochelone nigra* (Quoy and Gaimard, 1824) from the Galapagos Islands. Fitzsimmons (1961) described *Thaparia capensis* from the South African bowsprit tortoise, *Chersina angulata* (Schweigger, 1812), in South Africa.

Petter (1966) described *Thaparia domerguei*, from the common spider tortoise, *Pyxis arachnoides* Bell, 1827, and the radiated tortoise, *Geochelone radiata* (Shaw, 1802), from Mada-

gascar. She also transferred the species *Tachygonetria thapari* Dubinina, 1949, described from the Central Asian tortoise, *Testudo horsfieldii* Gray, 1844, in Afghanistan and from other Palearctic tortoises, the spur-thighed tortoise *Testudo graeca* Linnaeus, 1758, and Hermann's tortoise, *Testudo hermanni* Gmelin, 1789, to the genus *Thaparia*. Petter (1966) also modified the diagnosis of the genus, which is now: Pharyngodoninae—mouth with 3 lips; short esophagus divided into 2 equal parts; 4 pairs of caudal papillae: 3 pairs at the level of the cloaca and 1 near tail extremity. Caudal alae present or absent in males. Type species: *T. macrospiculum* Ortlepp, 1933.

Petter and Douglass (1976) described 2 additional species, *T. macrocephala* and *T. microcephala*, from the Bolsón tortoise, *Gopherus flavomarginatus* Legler, 1959, in Mexico.

Baker (1987) cited the 7 species above plus 3 subspecies of *T. thapari*, following Petter (1966) (*Thaparia thapari thapari* (Dubinina, 1949), *Thaparia thapari australis* (Petter, 1966), and Johnson (1973a) (*Thaparia thapari rysavyi* Johnson, 1973, from *Testudo hermanni* from Albania). The 7 species of *Thaparia* are distributed in 5 biogeographical regions.

In this study, we describe 2 new species of *Thaparia* from a testudinid species and emend the diagnosis of the genus.

### Materials and Methods

A first collection of nematode parasites from 18 specimens of *Testudo graeca* from Settat, Morocco

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(deposited at the Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco), was made by one of us (S.B.). The second collection, from a single specimen of *T. hermanni* from Catalonia, Spain, was made by C. Feliu, Barcelona, Spain, and deposited at the Barcelona Zoo, Spain. Nematodes were preserved in 70% ethanol before being cleared with lactophenol for study. Figures were made with the aid of a drawing tube. Nematodes were dehydrated by passage through progressive ethanol concentrations to absolute ethanol and critical-point-dried (M scope 500, Hitachi, Japan). The scanning electron microscope used was a Hitachi S 520, Hitachi, Japan at 20 kV. Measurements given are for the holotype male and the allotype female. Measurements in parentheses are the ranges of paratype males and females. All measurements are in micrometers.

## Results

### *Thaparia thapari thapari* (Dubinina, 1949) (Figs. 1–10)

#### Redescription

**GENERAL:** The material examined consisted of 6 males and 15 females. Body medium-sized, stout. Mouth triangular, with 3 transparent lips. Buccal cavity with denticles. Cephalic sense organs consisting of inner circle of 6 nerve endings, papillae not pedunculate (Figs. 2, 3, 6, 7), the outer circle not observed, and amphids present. Esophagus divided into 2 portions: anterior muscular part, and comparatively longer posterior glandular part terminating in valvular bulb; 17 chitinoid pieces surrounding anterior end of esophagus (Figs. 1, 3). Excretory pore postesophageal.

**MALE:** Length 2,754–3,169; maximum thickness 191–229. In worm measuring 2,773, esophagus 388: corpus 180 and isthmus plus bulb 208. Nerve ring and excretory pore 161 and 889, respectively, from anterior end. Posterior extremity truncated. Tail 67 long. Spicule needle-shaped, 100 long. Gubernaculum V-shaped. Three pairs of caudal papillae: 2 circumcloacal (1 pair preanal and 1 pair postanal) and 1 pair at tail end. Preanal membrane, present with 6 lobes (Figs. 4, 5, 8, 9, 10), and caudal alae absent.

**FEMALE:** Length 4,282–4,716; maximum thickness 356–378. In a worm measuring 4,600, esophagus 615: corpus 240 and isthmus plus bulb 375. Nerve ring, excretory pore, and vulva at 180, 1,282, and 2,264, respectively, from anterior end. Tail 270 long.

#### Taxonomic summary

**HOST:** Spur-thighed tortoise, *Testudo graeca* Linnaeus, 1758.

**SITE IN HOST:** Cecum.

**TYPE LOCALITY/COLLECTION DATE:** Settat, Morocco, 32°30'45"N, 7°45'30"W, 22 July 1999, by S.B.

**SPECIMENS DEPOSITED:** Muséum National d'Histoire Naturelle, Paris, France. Number 825 HF.

#### Remarks

Thapar (1925) described the female of this species from *Testudo graeca*, as *Oxyuris* sp. Dubinina (1949) studied males, the structures of which made it possible to include the species in *Tachygonetria* Wedl, 1862. Petter (1966) redescribed and transferred this species to the genus *Thaparia* and divided it in 2 subspecies: *T. thapari thapari* and *T. thapari australis*.

The emended diagnosis characterizes *T. thapari thapari* with 17 chitinoid pieces, whereas Petter (1961, 1966) cited 6 chitinoid pieces. Cephalic sense organs consist of an inner circle of 6 nerve endings (papillae not pedunculate), 4 submedian and 2 lateral close to amphids; outer papillae were not observed.

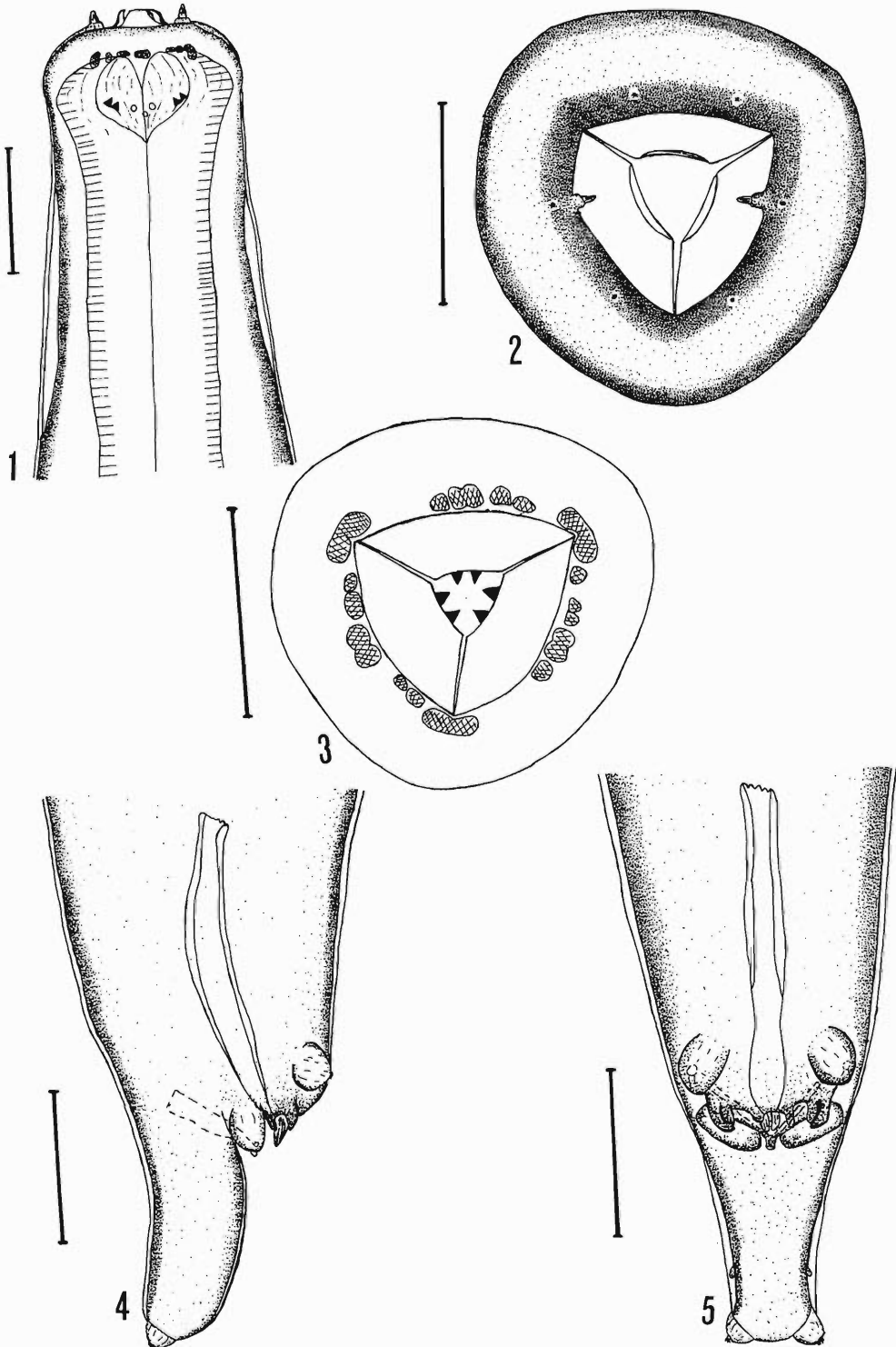
### *Thaparia carlosfeliui* sp. n. (Figs. 11–23)

#### Description

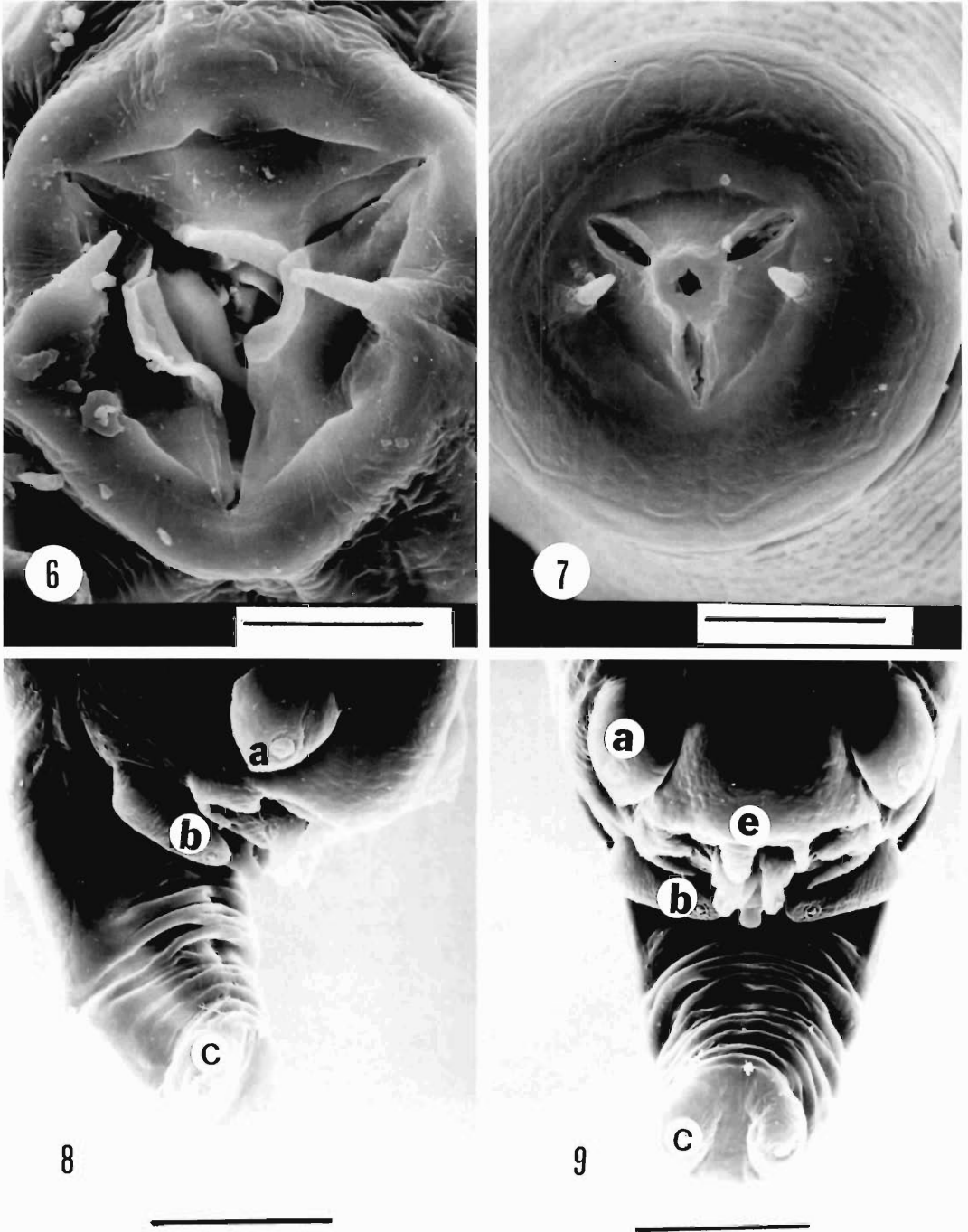
**GENERAL:** The material examined consisted of 20 males and 40 females. Nematoda, Oxyuroidea, Pharyngodonidae, *Thaparia*. Robust worms of small size. Mouth surrounded by 3 lips. Esophagus in 2 parts, with elongated isthmus. Amphids prominent. Differs from the diagnosis of the genus in the number of caudal papillae.

**MALE:** (holotype and 3 paratypes): Mouth surrounded by 3 V-shaped cut lips (Figs. 13, 21). Six oral papillae, arranged in 3 pairs (Fig. 13). Buccal cavity without denticles. Esophagus lobes visible. No chitinoid pieces visible. Tail without alae. Structure of caudal region complex (Figs. 15, 16, 22, 23). One pair of preanal papillae, 1 pair of large postanal elongated papillae. Posterior lip of anus with central nipple. Surrounding ventral membrane present lateral and anterior to anus, and second preanal membrane situated posterior to first membrane. Anterior lip of anus with 2 lobes, extremity of spic-

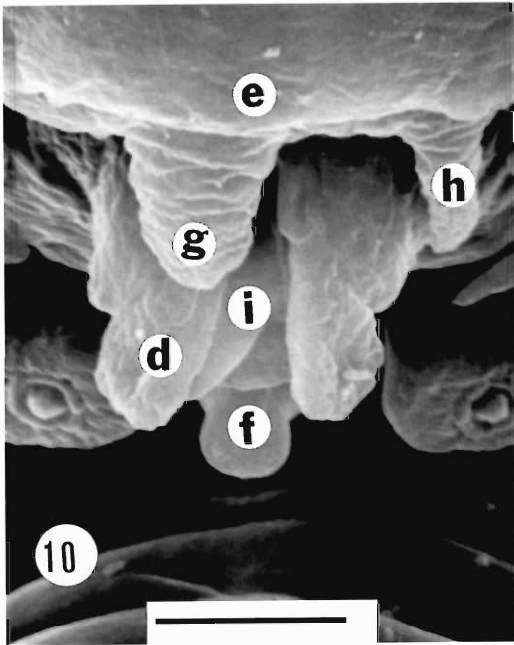




Figures 1–5. *Thaparia thapari thapari*, male. 1. Anterior end. 2. Cephalic end, en face view. 3. Cephalic end, deeper en face view. 4. Posterior end, lateral view. 5. Posterior end, ventral view. All scale lines = 50  $\mu\text{m}$ .



Figures 6-9. *Thaparia thapari thapari*, scanning electron micrographs: 6. Cephalic end of female. 7. Cephalic end of male. 8. Caudal end of male, ventral view (a = preanal papillae, b = postanal papillae, c = caudal papillae). 9. Caudal end of male, lateral view (a = preanal papillae, b = postanal papillae, c = caudal papillae, e = preanal membrane). Scale lines: Fig. 6 = 17.6  $\mu$ m; Fig. 7 = 20  $\mu$ m; Fig. 8 = 20  $\mu$ m; Fig. 9 = 20  $\mu$ m.



**Figure 10.** *Thaparia thaparia thaparia*, scanning electron micrograph: Cloacal view of male (d = anterior lip, e = preanal membrane, f = posterior lip, g = ventral lobe of preanal membrane, h = subventral lobe of preanal membrane, i = spicule). Scale line = 5  $\mu$ m.

ule visible between them. U-shaped gubernaculum. Third pair of papillae lateral at end of tail. Length 1,797 (1,324–1,943) with maximal width 180 (150–255). Nerve ring 112 (70–116) from anterior end. Excretory pore 586 (435–640) from anterior end. Esophagus 416 (351–421) long, corpus 165 (139–167) long, isthmus plus bulb 251 (212–254) long. Tail 45 (45–49) long. Spicule 123 (109–123) long.

**FEMALE** (allotype and 3 paratypes): Mouth surrounded by 3 V-shaped cut lips (Figs. 19, 20). Six oral papillae arranged in 3 pairs as in male (Fig. 19). Buccal cavity without denticles. No chitinoid pieces visible. Esophagus lobes visible. Length 3,471 (2,867–3,848), maximum width 426 (426–482). Nerve ring corpus 114 (114–188) from anterior end. Excretory pore 814 (575–814) from anterior end. Esophagus: 615 (482–634) long with corpus 244 (192–252) long and isthmus plus bulb 371 (290–382). Vulva 1,622 (1,528–2,056) from anterior end. Tail 244 (232–283) long. Eggs asymmetrical, measuring 89  $\times$  147 (69  $\times$  126–89  $\times$  147).

### Taxonomic summary

**TYPE HOST:** Hermann's tortoise, *Testudo hermanni* Gmelin, 1789.

**SITE IN HOST:** Cecum.

**TYPE LOCALITY/COLLECTION DATE:** South Catalonia, Spain, 41°23'14"N, 2°11'17"E, 17 December 1993 by Dr. Carlos Feliu.

**SPECIMENS DEPOSITED:** Museum National d'Histoire Naturelle, Paris, France. Number 826 HF.

**ETYMOLOGY:** The species is named in honor of Professor Carlos Feliu (University of Barcelona, Spain).

### Remarks

*Thaparia carlosfeliui* sp. n. differs from *T. macrospiculum* in the size of the spicule and from *T. domerguei* in the absence of caudal alae. *Thaparia capensis* differs from the new species in the presence of caudal alae and the length of the body.

*Thaparia contortospicula* resembles *T. carlosfeliui* in the size of the spicule but differs in the presence of caudal alae and the length of the body. *Thaparia macrocephala* and *T. microcephala* differ in the presence of caudal alae, the number of lips, and the length of the body.

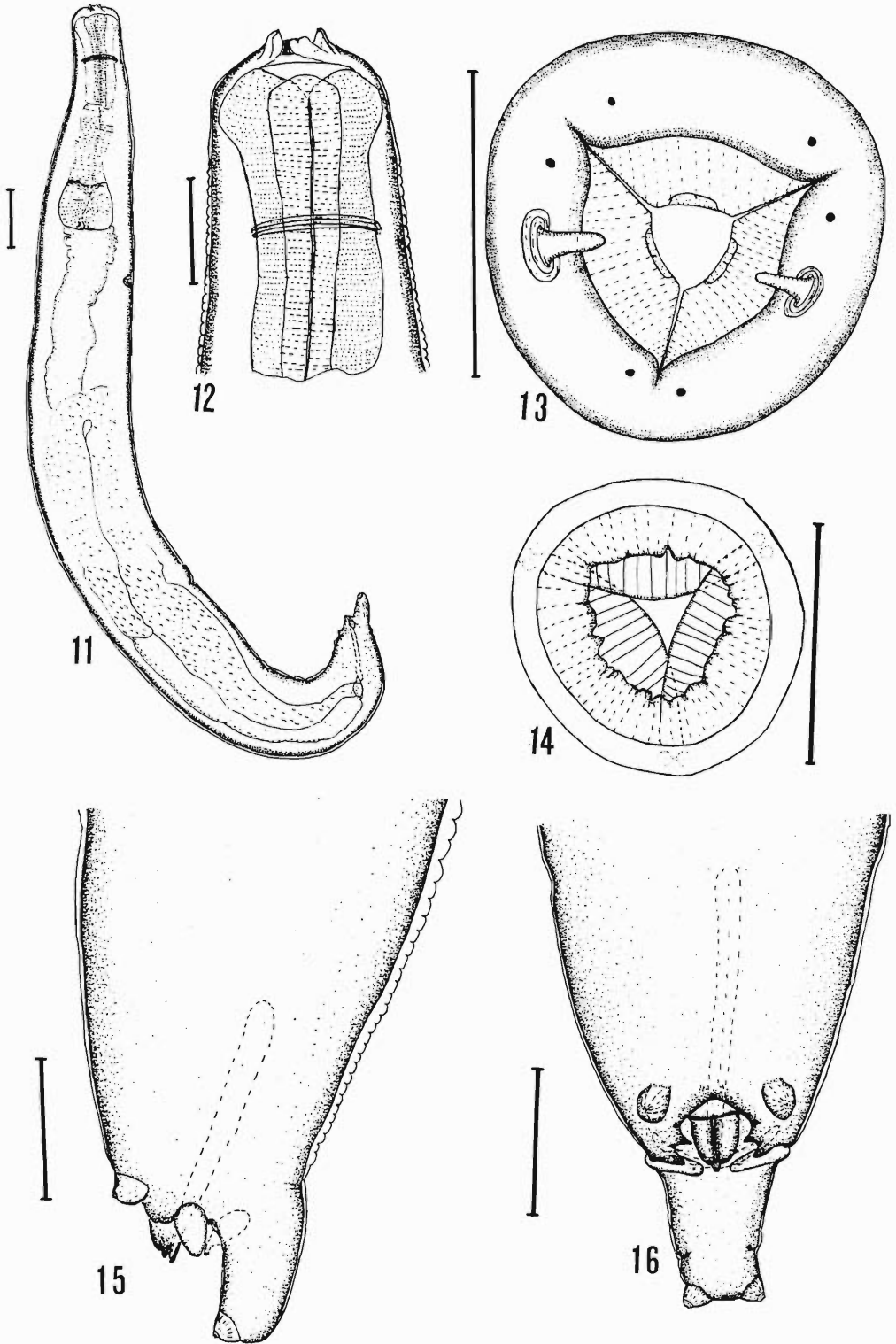
*Thaparia carlosfeliui* differs from *T. thaparia* (Dubinina, 1949) in the absence of teeth, the arrangement of labial papillae, and the absence of 6 chitinoid pieces around the mouth. *Thaparia carlosfeliui* differs from *T. thaparia australis* in the arrangement of labial papillae, the lack of a tip at the extremity of the male tail, and the absence of chitinoid pieces.

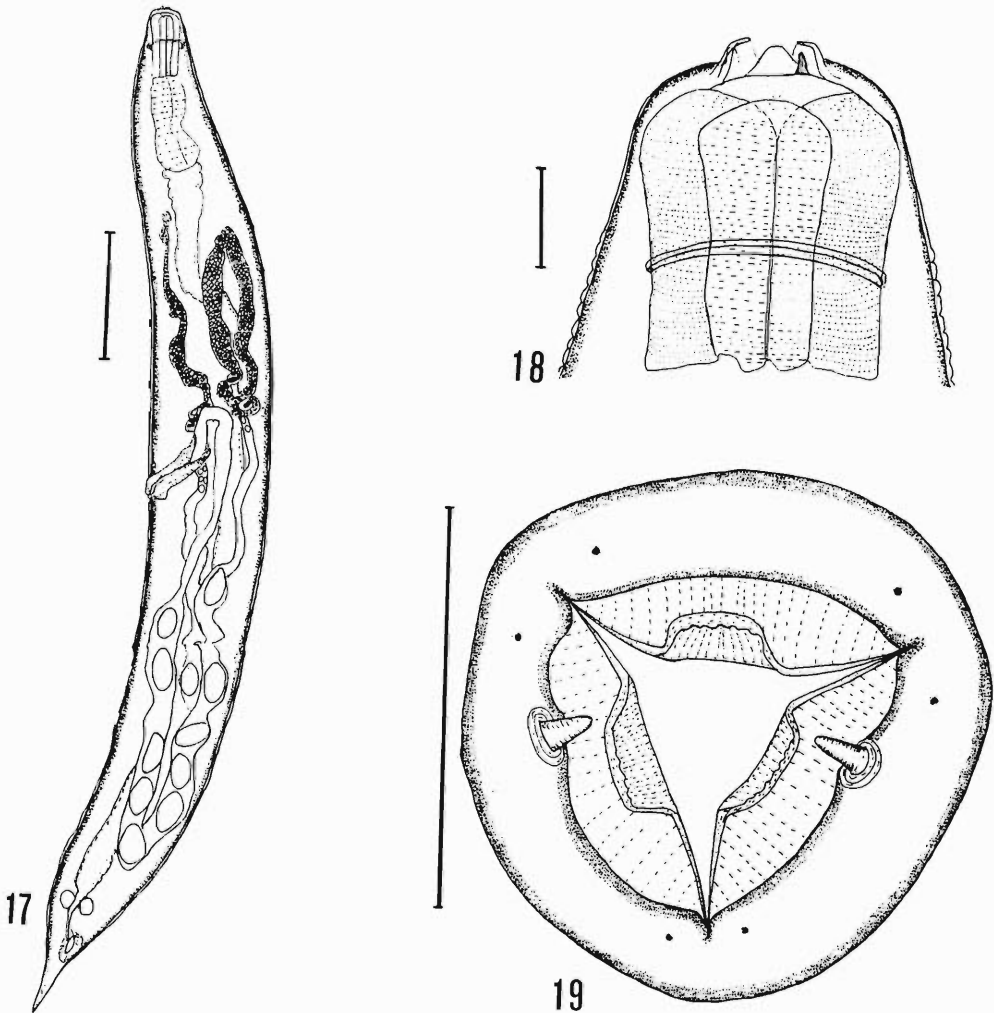
### *Thaparia bourgati* sp. n. (Figs. 24–31)

### Description

**GENERAL:** The material examined consisted of 10 males and 1 female. Nematoda, Oxyuroidea, Pharyngodonidae, *Thaparia*. Robust worms of small size. Mouth surrounded by 3 lips. Esophagus in 2 parts, with an elongated isthmus. Amphids prominent. Differs from the diagnosis of the genus in the number of caudal papillae.

**MALE** (holotype and 3 paratypes): Labial papillae conspicuous, arranged as in *T. carlosfeliui* sp. n. (Figs. 20, 28). Three projections visible inside mouth, forming diaphragm under lip. Buccal cavity without denticles. No chitinoid





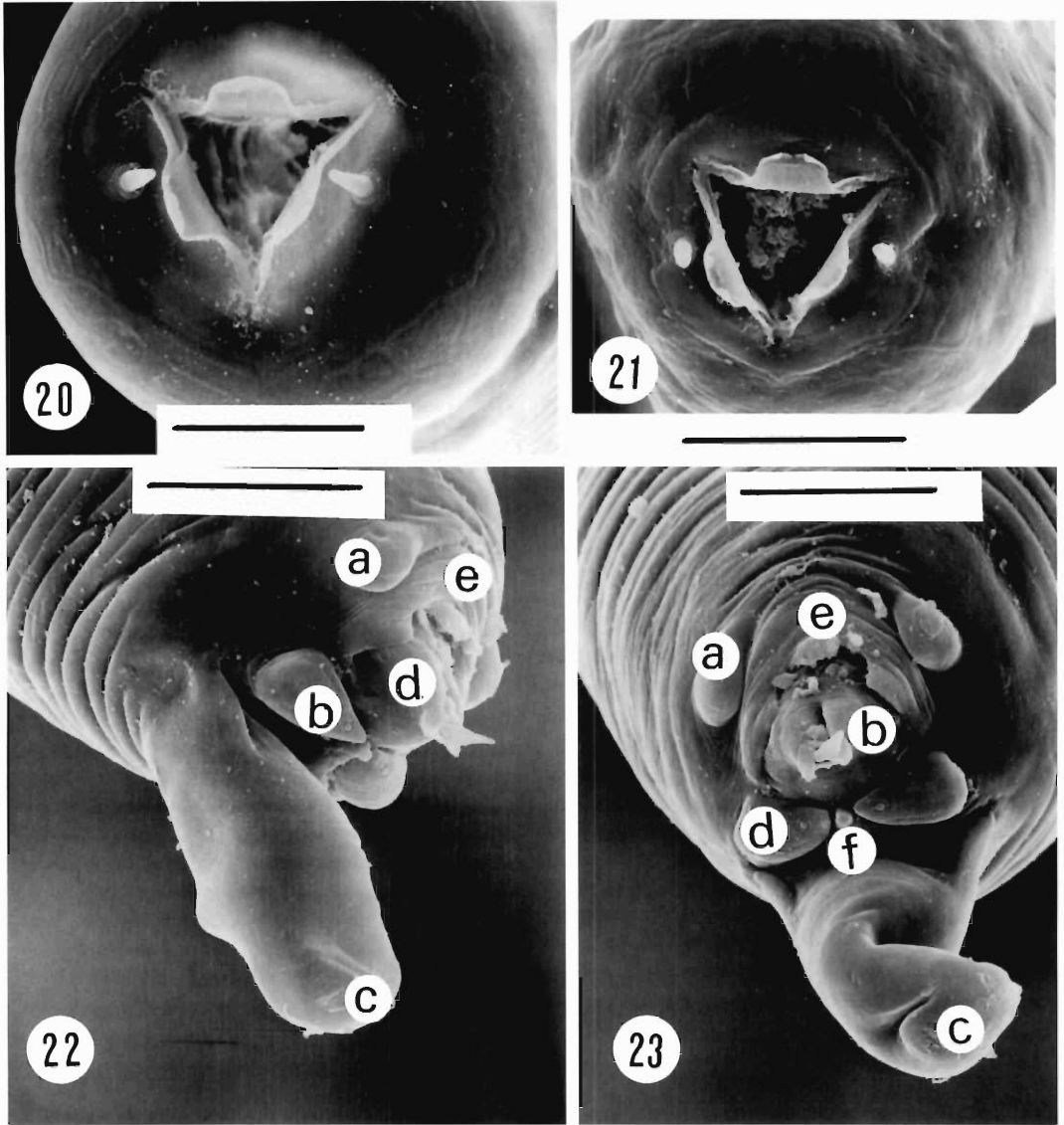
Figures 17–19. *Thaparia carlosfeliui* sp. n. female allotype. 17. Entire specimen, lateral view. 18. Anterior end. 19. Cephalic end, en face view. Scale lines: Fig. 17 = 400  $\mu\text{m}$ ; Figs. 18, 19 = 50  $\mu\text{m}$ .

pieces visible. Tail without alae. Three pairs of caudal papillae. Ventral membrane surrounding anus anteriorly, showing on each side 2 small submedian lobes and 2 large lateral lobes (Figs. 26, 27, 29–31). Anterior lip of anus composed of 2 submedian lobes congruent at distal extremity. Posterior lip of anus with central nipple. U-shaped gubernaculum. Length 3,122 (2,204–3,207), maximum width 221 (143–255). Nerve

ring 98 (68–104) from anterior end. Excretory pore 756 (705–851) from anterior end. Esophagus 439 (251–416) long, corpus 169 (97–169) long, isthmus plus bulb 270 (154–270) long. Tail 53 (45–53) long. Spicule 162 (112–225) long.

FEMALE (allotype): A single female has been found, which closely resembles females of the other species of the genus. Nerve ring not visible. Length 2,226, maximum width 166. Excre-

Figures 11–16. *Thaparia carlosfeliui* sp. n. male holotype. 11. Entire specimen, lateral view. 12. Anterior end. 13. Cephalic end, en face view. 14. Cephalic end, deeper en face view. 15. Posterior end, lateral view. 16. Posterior end, ventral view. Scale lines: Fig. 11 = 100  $\mu\text{m}$ ; Figs. 12–16 = 50  $\mu\text{m}$ .



Figures 20–23. *Thaparia carlosfeliui* sp. n., scanning electron micrographs. 20. Cephalic end of female. 21. Cephalic end of male. 22. Caudal end of male, lateral view (a = preanal papillae, b = postanal papillae, c = caudal papillae, d = anterior lip, e = preanal membrane). 23. Caudal end of male, ventral view (a = preanal papillae, b = postanal papillae, c = caudal papillae, d = anterior lip, e = preanal membrane, f = posterior lip). Scale lines: Fig. 20 = 30  $\mu$ m; Figs. 21–23 = 25  $\mu$ m.

tory pore 549. Esophagus 502, corpus 225 long, isthmus plus bulb 277 long. Vulva 804 from anterior end. Tail 131. Eggs asymmetrical, measuring 49  $\times$  90.

**Taxonomic summary**

TYPE HOST: Hermann’s tortoise, *Testudo hermanni* Gmelin, 1789.

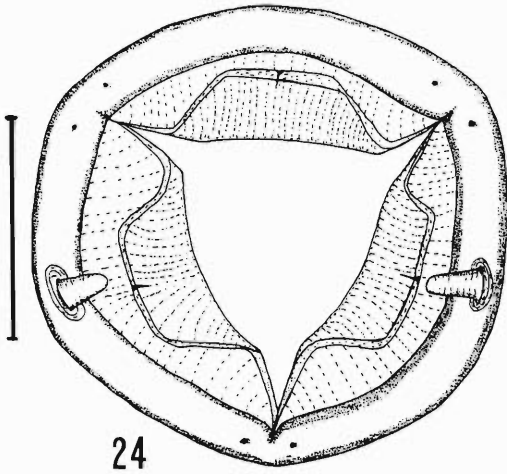
INFECTION SITE: Cecum.

TYPE LOCALITY/COLLECTION DATE: South Catalonia, Spain, 41°23’14”N, 2°11’17”E, 17 December 1993, by Dr. Carlos Feliu.

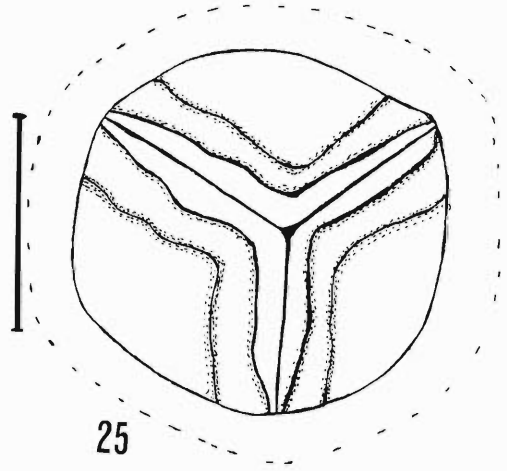
SPECIMENS DEPOSITED: Muséum National d’Histoire Naturelle, Paris, France. Number 827 HF.

ETYMOLOGY: The species is named in honor

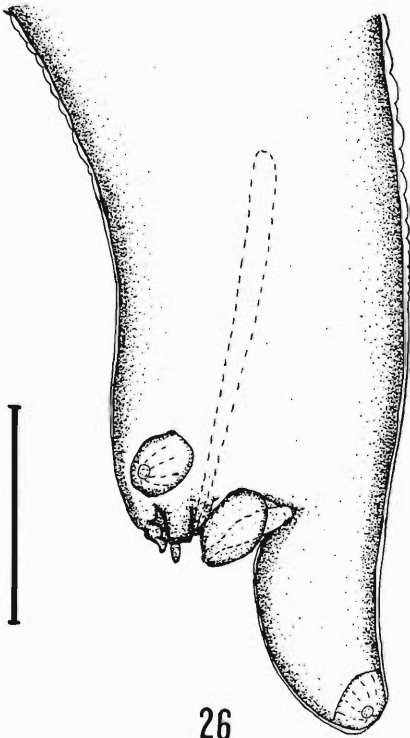




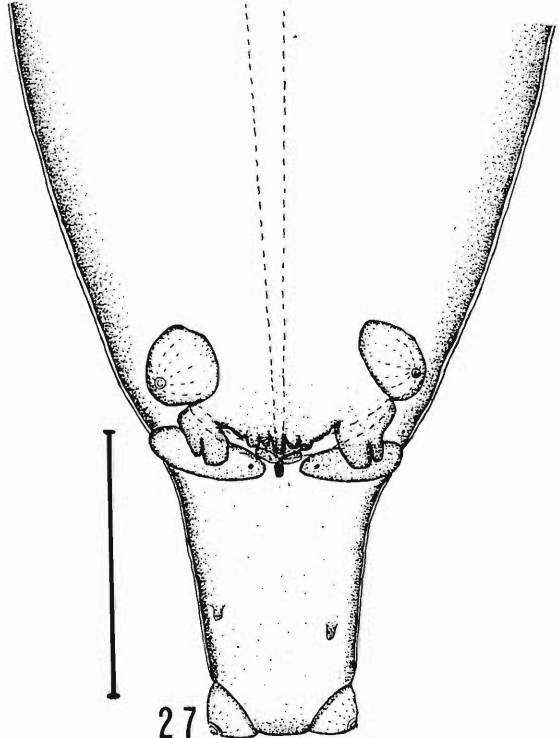
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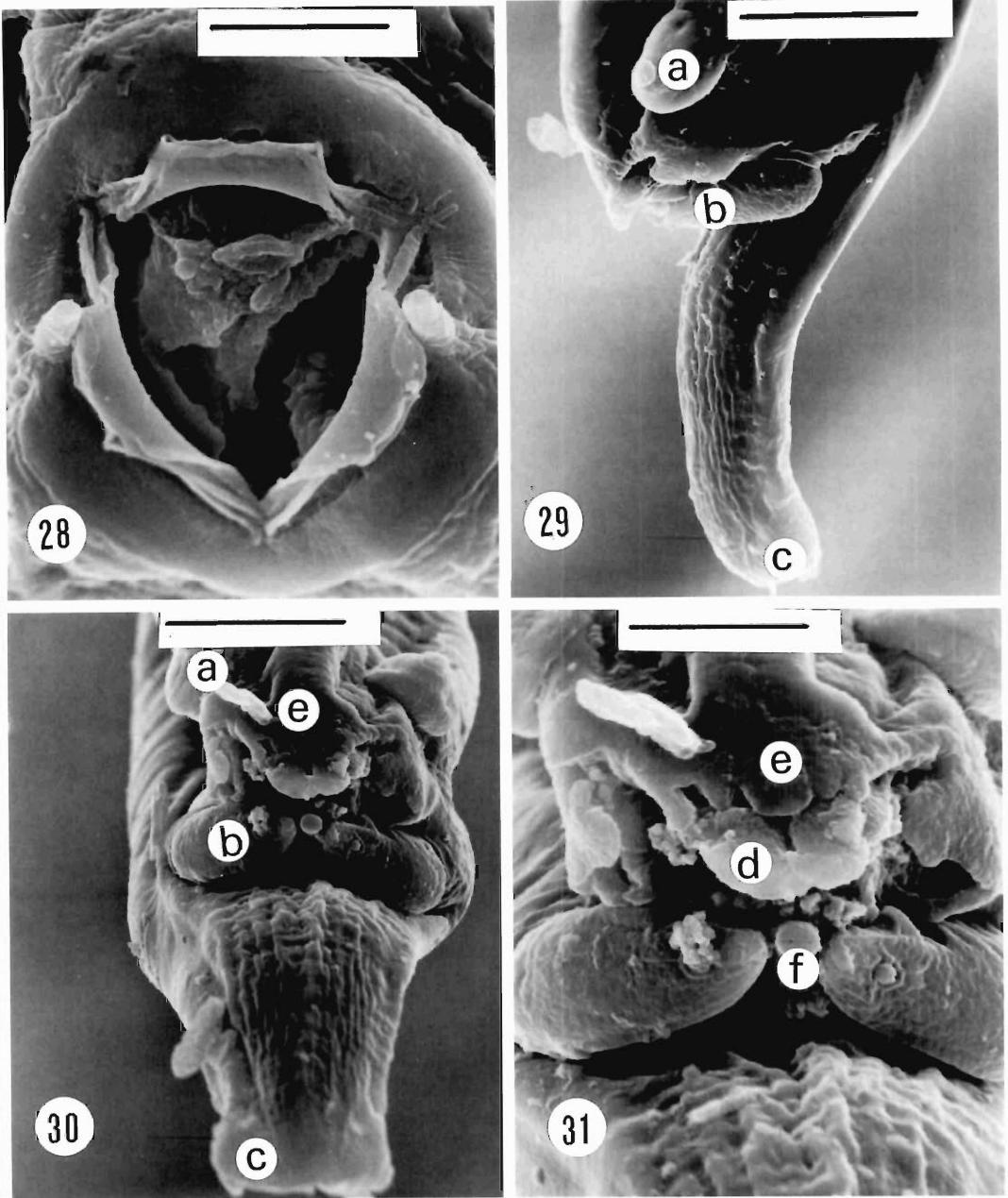


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27

Figures 24–27. *Thaparia bourgati* sp. n. male holotype. 24. Cephalic end, en face view. 25. Cephalic end, deeper en face view. 26. Caudal end, lateral view. 27. Caudal end, ventral view. All scale lines = 50  $\mu$ m.



Figures 28–31. *Thaparia bourgati* sp. n. male, scanning electron micrograph. 28. Cephalic end. 29. Caudal end, lateral view (a = preanal papillae, b = postanal papillae, c = caudal papillae). 30. Caudal end, ventral view (a = preanal papillae, b = postanal papillae, c = caudal papillae, d = anterior lip, e = preanal membrane). 31. Cloacal view (d = anterior lip, e = preanal membrane, f = posterior lip). Scale lines: Fig. 28 = 10  $\mu$ m; Fig. 29 = 17.6  $\mu$ m; Fig. 30 = 15  $\mu$ m; Fig. 31 = 7.5  $\mu$ m.

of Professor Robert Bourgat (University of Perpignan, France).

### Remarks

*Thaparia bourgati* sp. n. differs from all other species of the genus in the same characters as *T. carlosfeliui*. *Thaparia bourgati* differs from *T. carlosfeliui* in the shape of the preanal membrane in the male—9 lobes in *T. bourgati* and 4 lobes in *T. carlosfeliui*—and in the size of the eggs (smaller in *T. bourgati*).

### Discussion

*Thaparia bourgati* sp. n. and *T. carlosfeliui* sp. n. differ from all other species of the genus *Thaparia*, except *T. thapari*, in the lack of caudal alae. Both species differ from the subspecies *T. thapari australis* in the shape and the disposition of labial papillae, the lack of apical chitinous pieces, the lack of a tip at the end of the male tail, and the presence of a longer spicule compared with the length of the tail. They differ from the subspecies *T. thapari thapari* in the disposition of labial papillae, the lack of esophageal teeth and the lack of apical chitinous pieces, the shape of the preanal membrane, and the shape of the gubernaculum in the male, and from *T. thapari rysavyi* in the arrangement of labial papillae and in the shape of the adanal membrane in the male. Finally, *T. carlosfeliui* sp. n. resembles *T. bourgati* sp. n. in the arrangement of the labial papillae, but it is distinguished by the shape of the preanal membrane in the male and by the size of the eggs.

The genus *Thaparia* shows a wide geographical distribution, with 3 Palearctic species (*T. carlosfeliui* sp. n., *T. bourgati* sp. n., and *T. thapari*), 2 Nearctic species, 2 South African species, and 1 species from the Galapagos Islands. The question remains open concerning the presence of this genus in other members of the Palearctic tortoises (*T. horsfieldii*, *T. graeca*, *T. hermanni*, the Egyptian tortoise, *Testudo kleinmanni* Lortet, 1883, and the marginated tortoise, *Testudo marginata* Schoepff, 1792).

### Emended diagnosis of the genus *Thaparia*

The use of a scanning electron microscope allowed verification that the previously described adanal papillae are simple lobes. The lack of terminal nerves is confirmed by optical observation. The new diagnosis of the genus is:

Pharyngodonidae: Intestinal parasites of Tes-

tudinidae. Medium-sized, lateral alae present or absent. Mouth with 3 or 6 slightly bilobed lips. Esophagus rather short, divided into 2 parts of about equal length: an anterior muscular corpus and a posterior glandular isthmus terminating in a valvulated bulb. Excretory pore bulbar or postbulbar.

MALE: Tail truncated, spiked, or simple. Caudal alae present or absent. Spicule simple or contorted, may be very long. Gubernaculum U-, V-, or Y-shaped. Caudal papillae in 3 pairs: 2 circumcloacal, 1 pair at or near tail end.

FEMALE: Tail tapering to sharp point. Vulva postequatorial, sometimes very close to anus. Vagina long; ovjector present. Eggs thin-shelled, relatively few.

TYPE SPECIES: *Thaparia macrospiculum* Ortlepp, 1933, in *Psammobates tentorius*; South Africa.

### Key to the Species and Subspecies of the Genus *Thaparia*

This key follows Johnson (1973b) and includes *T. capensis* Fitzsimmons, 1961, *T. macrocephala* Petter and Douglass (1976), *T. microcephala* Petter and Douglass (1976), and *T. carlosfeliui* sp. n. and *T. bourgati* sp. n., both described herein.

- |  |  |
|--|--|
| 1. Caudal alae in male present .....                       | 2  |
| Caudal alae in male absent .....                           | 7  |
| 2. Tail in male spiked .....                               | 3  |
| Tail in male truncated .....                               | 5  |
| 3. Spicule contorted; vulva away from anus .....           |  |
| ..... <i>T. contortospicula</i> Walton, 1942               |  |
| Spicule simple; vulva far away from anus .....             | 4  |
| 4. Length of spicule 1/2 of body .....                     |  |
| ..... <i>T. macrocephala</i> Petter and Douglass (1976)    |  |
| Length of spicule 1/3 of body .....                        |  |
| ..... <i>T. microcephala</i> Petter and Douglass (1976)    |  |
| 5. Spicule simple; vulva far (more than 1,450 $\mu$ m)     |  |
| from anus .....  | <i>T. capensis</i> Fitzsimmons, 1961     |
| Spicule simple; vulva near (less than 200 $\mu$ m)         |  |
| anus .....   | 6  |
| 6. Spicule less than 1 mm in length .....                  |  |
| ..... <i>T. domerguei</i> Petter, 1966                     |  |
| Spicule more than 2.5 mm in length .....                   |  |
| ..... <i>T. macrospiculum</i> Ortlepp, 1933                |  |
| 7. Buccal cavity with 6 teeth .....                        |  |
| ..... <i>T. thapari thapari</i> Dubinina (1949)            |  |
| Buccal cavity without teeth .....                          | 8  |
| 8. Spicule less than 90 $\mu$ m; tail more than 90 $\mu$ m |  |
| in length .....  | <i>T. thapari australis</i> Petter, 1966 |
| Spicule more than 90 $\mu$ m; tail less than 90 $\mu$ m    |  |
| in length .....  | 9  |
| 9. Preanal membrane absent .....                           |  |
| ..... <i>T. thapari rysavyi</i> Johnson, 1973              |  |
| Preanal membrane present .....                             | 10                                       |

10. Preanal membrane with 4 lobes .....  
 ..... *T. carlosfeliui* sp. n.  
 Preanal membrane with 9 lobes .....  
 ..... *T. bourgati* sp. n.

**Acknowledgment**

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**Obituary Notice**

**MARION M. FARR**

1903–2000

Elected to Membership, 1938  
 Executive Committee Member at Large, 1943–1945  
 21<sup>st</sup> Recording Secretary, 1946  
 Vice President, 1946  
 34<sup>th</sup> President, 1951  
 Assistant Secretary-Treasurer, 1964  
 Society Representative to the Washington Academy of  
 Sciences, 1964–1965  
 Elected to Life Membership, 1979  
 Published in the Proceedings from 1939–1963 on  
*Eimeria* and *Histomonas*

## Parasites of Farm-Raised Trout in Michigan, U.S.A.

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**ABSTRACT:** A total of 635 trout (366 rainbow trout, *Oncorhynchus mykiss* Richardson, 1836; 166 brook trout, *Salvelinus fontinalis* Mitchill, 1814; 103 brown trout, *Salmo trutta* Linnaeus, 1758; Salmonidae) collected in March–July 1996, 1997, and 1998 from 12 trout farms in Michigan, U.S.A., was examined for parasites. Twelve parasite species (1 Acanthocephala, *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936; 1 Monogenea, *Gyrodactylus* sp.; 2 Cestoda, *Eubothrium salvelini* (Schrank, 1790), *Proteocephalus* sp.; 1 Nematoda, *Truttaedacnitis* sp.; 1 Copepoda, *Salmincola edwardsii* (Olsson, 1869); 1 Myxozoa, *Myxobolus cerebralis* (Hofer, 1903); 4 Ciliophora, *Capriniana* sp. [= *Trichophrya* sp.], *Chilodonella* sp., *Ichthyophthirus multifiliis* (Fouquet, 1876), *Trichodina* sp.; and 1 Mastigophora, *Ichthyobodo* sp. [= *Costia* sp.]) were found. Rainbow trout were infected with *A. dirus*, *Truttaedacnitis* sp., *E. salvelini*, *Proteocephalus* sp., *Gyrodactylus* sp., *M. cerebralis*, *Ichthyobodo* sp., *Capriniana* sp., *Chilodonella* sp., *I. multifiliis*, and *Trichodina* sp. Brook trout were infected with *A. dirus*, *S. edwardsii*, *E. salvelini*, *M. cerebralis*, and *Trichodina* sp. *Acanthocephalus dirus* was the only parasite infecting brown trout. *Eubothrium salvelini*, *A. dirus*, and *Trichodina* sp. infected trout from 9, 8, and 8 farms, respectively. *Acanthocephalus dirus* in all trout species and *S. edwardsii* on brook trout had the highest prevalences, mean intensities, and mean abundances.

**KEY WORDS:** trout, rainbow trout, *Oncorhynchus mykiss*, brook trout, *Salvelinus fontinalis*, brown trout, *Salmo trutta*, Salmonidae, helminths, protozoans, parasites, aquaculture, Michigan, U.S.A.

Newman and Kevern (1994) reported that more than half of the fish growers in the state of Michigan, U.S.A., raise rainbow, brook, and brown trout. These growers produce trout for sale: 1) to individuals or groups for stocking, 2) to retail stores or restaurants, and 3) through their own fee-fishing ponds. In 1996, predators and diseases were the leading causes of death for trout in culture conditions in Michigan, accounting for 53% and 13% of all fish lost, respectively (Anonymous, 1997). Except for a few reports in local newspapers of diseases of trout in the Michigan Department of Natural Resources hatcheries and the studies by Sawyer et al. (1974) and Yoder (1972), little has been published on the parasites of trout raised in culture in Michigan. The present study reports on the parasites infecting rainbow, brook, and brown trout from 12 privately owned farms in Michigan. The emphasis of this study was on the metazoan parasites of trout, but observations and comments are also made on protozoans. Furthermore, information is presented on the life cycles of some of the parasites, their pathogenicity, and factors influencing their occurrence.

### Materials and Methods

Trout were collected by dip net or seine from ponds or raceways (hereinafter referred to as ponds) in March–July 1996, 1997, and 1998 from 12 farms in

Michigan. These trout farms are in an area of the lower peninsula between 42.0° and 45.5°N and 84.0° and 86.5°W. Specific information on the locations of the trout farms, however, cannot be provided because of conditions of confidentiality imposed by the growers. Fish were either brought to the laboratory alive and necropsied within 48 hr of collection or were put on ice at the farm, brought to the laboratory, frozen, and examined later. Total length (mm) and sex were recorded during necropsy. The fins, external surface, buccal cavity, gills, brain, eyes, gonads, swim bladder, gastrointestinal tract, liver, spleen, and muscles (left or right side of each fish) were examined from all fish. In fish collected in 1997 and 1998, the skull bones and cartilage and 2 or more gill arches (without the filaments) were macerated separately into a slurry and examined with a compound microscope at 20×. During the entire study, rainbow trout were examined from 23 ponds, brook trout from 13 ponds, and brown trout from 7 ponds. These totals include some of the same ponds sampled on different dates and in different years. Parasite prevalence is defined as the percentage of fish infected, mean intensity as the mean number of metazoan parasites in infected fish, and mean abundance as the mean number of metazoan parasites in examined fish. Population numbers of each metazoan species at 1 facility were estimated as (prevalence) × (mean abundance) × (estimated number of trout) at the facility when the fish were sampled. It should be emphasized that the results of examination of frozen trout may not accurately reflect the occurrence and (or) numbers of protozoans and monogeneans. Therefore, mean numbers were not calculated for these parasite groups. Protozoan taxonomy follows that of Lom and Dykova (1992). Voucher specimens have been deposited in the United States National Parasite Collection

**Table 1. Numbers and mean lengths of *Oncorhynchus mykiss*, *Salvelinus fontinalis*, and *Salmo trutta* examined in 1996, 1997, and 1998.**

Species*	No. examined	Mean length $\pm$ SD (range, 95% confidence intervals)
OM, 1996	184	162 $\pm$ 71 (62–338, 152–172)
OM, 1997	50	177 $\pm$ 59 (93–279, 161–195)
OM, 1998	132	215 $\pm$ 64 (94–409, 204–226)
SF, 1996	112	154 $\pm$ 72 (56–378, 141–168)
SF, 1997	27	222 $\pm$ 45 (153–328, 204–239)
SF, 1998	27	200 $\pm$ 26 (160–260, 190–210)
ST, 1996	88	146 $\pm$ 69 (43–283, 131–161)
ST, 1997	15	208 $\pm$ 25 (147–244, 194–222)

\* OM = *Oncorhynchus mykiss*; SF = *Salvelinus fontinalis*; ST = *Salmo trutta*.

(USNPC), Beltsville, Maryland, U.S.A., with the following accession numbers: *Eubothrium salvelini* (89483), *Acanthocephalus dirus* (89484), *Salmincola edwardsii* (89485).

## Results

Totals of 366 rainbow trout, 166 brook trout, and 103 brown trout were examined for parasites from 12 Michigan farms in March–July 1996, 1997, and 1998. All farms did not raise all species; thus, unequal numbers of each species were examined. The mean lengths of the trout species examined each year are in Table 1. Rainbow trout in 1998 were significantly larger than those in 1996 and 1997 (analysis of variance,  $F = 24.4$ ,  $P < 0.0001$ ). Brook trout in 1996 were significantly smaller than those examined in 1997 and 1998 (analysis of variance,  $F = 15.6$ ,  $P < 0.0001$ ). Brown trout in 1997 were significantly larger than those examined in 1996 (Student's  $t$ -test,  $t = -6.34$ ,  $P < 0.0001$ ).

Twelve parasite species were found in trout in this study (Table 2). Eleven parasite species infected rainbow trout, 5 species infected brook trout, and only 1 species infected brown trout. The prevalences, mean intensities, and mean abundances of the parasites found in trout in ponds varied dramatically. Of the metazoan parasite species found, *Eubothrium salvelini* (Schrank, 1790), *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936, and *Salmincola edwardsii* (Olsson, 1869) were gravid. The sites (in parentheses) where the parasites were found in trout were: *E. salvelini* (pyloric ceca, small intestine); *Proteocephalus* sp. (intestine); *Gyrodactylus* sp. (gills), *Truttaedac-*

*nitis* sp. (small intestine); *A. dirus* (intestine); *S. edwardsii* (primarily gills, inner operculum, base of fins); *Myxobolus cerebralis* Hofer, 1903 (head bones and cartilage, gill arches); *Ichthyobodo* sp., *Capriniana* sp., *Chilodonella* sp., *Ichthyophthirus multifiliis* (Fouquet, 1876), and *Trichodina* sp. (gills and [or] head area).

*Acanthocephalus dirus* was the most common parasite species occurring in the gastrointestinal tract of each trout species. When *S. edwardsii* occurred, it commonly infested brook trout. Although several small immature *S. edwardsii* were seen on many brook trout, mean intensities and mean abundances of *S. edwardsii* reflect only gravid females counted. *Trichodina* sp. was the most common external protozoan found on rainbow and brook trout. *Myxobolus cerebralis* infected 13 of 22 rainbow trout at 1 farm, 2 of 15 rainbow trout at another farm, and 1 of 20 brook trout at a third facility. *Capriniana* sp., *Chilodonella* sp., *I. multifiliis*, and *Ichthyobodo* sp. each infected fish in only 1 pond.

All farms had trout that were infected with at least 1 parasite species. *Eubothrium salvelini*, *A. dirus*, and *Trichodina* sp. infected trout from 9 (75%), 8 (67%), and 8 (67%) farms, respectively, of the 12 farms from which trout were examined. *Acanthocephalus dirus* and *Trichodina* sp. each infected rainbow trout from 52% of the 23 ponds examined (Table 3). *Eubothrium salvelini*, *A. dirus*, and *S. edwardsii* each infected brook trout from 31% of the 13 ponds. *Acanthocephalus dirus* infected brown trout from 2 of 7 ponds.

Trout were examined for parasites from 1 farm in March and July 1997. Prevalences, mean abundances, and estimated numbers of helminths varied dramatically between these months (Table 4). In March, 4 parasite species infected trout, and *A. dirus* and *S. edwardsii* were common. *Acanthocephalus dirus* had the highest mean abundances and estimated number of helminths. In July, only *A. dirus* infrequently infected trout.

There were no significant differences in the prevalence (chi-square analysis,  $P > 0.05$ ) and intensity (Mann–Whitney test,  $P > 0.05$ ) of *E. salvelini*, *A. dirus*, and *S. edwardsii* between female and male trout of each species. At the farms where these 3 parasite species were common, examined trout did not vary enough in length to determine if these parasite infections had a significant relationship with length.



## Discussion

Twelve parasite species (2 Cestoda, 1 Monogenea, 1 Nematoda, 1 Acanthocephala, 1 Copepoda, 1 Myxozoa, 1 Mastigophora, 4 Ciliophora) were found in 635 trout examined from 12 farms in the present study. Parasites of these trout and their infection values varied between ponds and years, a variability characteristic of wild trout populations as well. Most if not all of these parasite species have been found in wild trout from Michigan environments (Muzzall, 1984, 1986; Hernandez and Muzzall, 1998). Digenic trematodes, however, found in wild trout by Muzzall (1984, 1986), did not infect trout from culture ponds.

Of the parasitic species found in the present study, *E. salvelini*, *A. dirus*, *S. edwardsii*, and *M. cerebralis* had prevalences of 50% or more in at least 1 pond and deserve further discussion. *Eubothrium salvelini* was a common parasite of rainbow and brook trout. It utilizes copepods as intermediate hosts and commonly infects wild salmonids in inland waters (Hernandez and Muzzall, 1998) as well as in the Great Lakes (Muzzall, 1993, 1995a, b). Muzzall (1984) found immature *Eubothrium* sp. in brook trout from a Michigan creek. Boyce (1969) reported that *E. salvelini* reduced the growth, swimming performance, and survival of salmon. Smith and Margolis (1970) suggested that this cestode caused indirect damage to young salmonids. Hendee in 1980 believed it reduced the growth of brook trout in the state of New Hampshire, U.S.A. (in Hoffman, 1999).

*Acanthocephalus dirus* had the highest prevalences, mean intensities, and mean abundances of all parasites found. It is widespread in Michigan trout farms and is common in some natural environments of Michigan (Muzzall, 1984). Muzzall (1984) also reported that the isopod, *Caecidotea intermedius* Forbes, 1876, was the intermediate host for this parasite in the Rogue River. In the present study, some individuals of all 3 trout species infected with 100 worms or more from 3 farms appeared emaciated, and the head appeared large for the size of the fish. Bullock (1963) demonstrated that the most pronounced effects of *A. dirus* (= *A. jacksoni*) in rainbow and brook trout in a New Hampshire hatchery were damage to the intestinal epithelium and proliferation of connective tissue, leading to malnutrition and emaciation. Furthermore,

he stated (p. 33) that "this worm seriously impairs the health of the fish." Allison (1954) discussed the advancements in prevention and treatment of parasitic diseases of fish and listed 13 parasitic genera that warranted discussion. However, *A. dirus* was not listed, and acanthocephalans in general receive little attention in hatchery manuals about fish diseases. It is not known if *A. dirus* was common when Allison wrote his article or if it has become increasingly common in Michigan.

The presence of *S. edwardsii* on brook trout and its absence from rainbow and brown trout were not unexpected, because it parasitizes only the former species (Kabata, 1969). Some brook trout infested with *S. edwardsii* had 1 or both opercula folded underneath itself, and the distal portions of many gill filaments showed hyperplasia and clubbing. These characteristics also occurred on uninfected trout, suggesting previous infestation by this parasite. This copepod has a direct life cycle and is a common parasite of brook trout in Michigan (Allison and Latta, 1969; Muzzall, 1984, 1986). The mean intensities of *S. edwardsii* are higher than those found on trout in Michigan lotic environments but are comparable to the high mean intensities found on brook trout in Michigan lakes by Allison and Latta (1969). Many studies on *Salmincola* spp. suggest that they debilitate their hosts but may not be direct causes of trout mortality. Allison and Latta (1969) found no relationship between *S. edwardsii* and brook trout mortality in Michigan lakes.

Owners of 2 Michigan farms told me that they had seen a parasitic copepod on the gills of rainbow trout in their ponds. However, in this study, none was found infesting rainbow trout. A copepod that infests rainbow trout is *Salmincola californiensis* (Dana, 1853), which is native to streams in the Pacific Northwest, U.S.A., and Canada (Kabata, 1969). Hoffman (1984) reported on its eastward movement in North America, being transferred on live fish and with shipments of trout eggs. Sutherland and Wittrock (1985) believed that *S. californiensis* entered central Iowa through the importation of infested rainbow trout from a Missouri farm. They found a mean intensity of 4.6 adults and suggested that this copepod may be responsible for host mortality if fish are sufficiently stressed. Perhaps this species has made its way to Michigan but is infrequent on rainbow trout in this state.

**Table 2. Prevalences, mean intensities, mean abundances of parasites found in *Oncorhynchus mykiss*, *Salvelinus fontinalis*, and *Salmo trutta* from farms in 1996, 1997, and 1998.**

Parasite	Farm number, year	Trout species*	No. examined	Prevalence No. infected (%)	Mean intensity $\pm$ SD (max)	Mean abundance $\pm$ SD	
<b>Cestoda</b>							
<i>Eubothrium salvelini</i>	4, 96	OM	25	1 (4)	1.0	0.04 $\pm$ 0.20	
	4, 96	OM	13	1 (8)	1.0	0.08 $\pm$ 0.27	
	5, 96	OM	15	3 (20)	1.7 $\pm$ 1.2 (3)	0.33 $\pm$ 0.82	
	5, 97	OM	15	4 (27)	2.0 $\pm$ 2.0 (5)	0.53 $\pm$ 1.30	
	6, 98	OM	16	1 (6)	1.0	0.06 $\pm$ 0.25	
	8, 98	OM	20	14 (70)	2.4 $\pm$ 1.5 (5)	1.65 $\pm$ 1.66	
	9, 98	OM	18	13 (72)	20.7 $\pm$ 44.9 (163)	14.94 $\pm$ 6.63	
	11, 98	OM	22	5 (23)	2.6 $\pm$ 1.8 (5)	0.59 $\pm$ 1.37	
	3, 96	SF	14	1 (7)	2.0	0.14 $\pm$ 0.54	
	12, 96	SF	4	1 (25)	1.0	0.25 $\pm$ 0.50	
	1, 98	SF	19	1 (5)	1.0	0.05 $\pm$ 0.23	
	12, 98	SF	2	1 (50)	1.0	0.50 $\pm$ 0.71	
	<i>Proteocephalus</i> sp.	4, 96	OM	25	2 (8)	1.0	0.08 $\pm$ 0.28
		1, 97	OM	5	2 (40)	1.0	0.40 $\pm$ 0.55
		11, 98	OM	22	4 (18)	1.5 $\pm$ 0.6 (2)	0.27 $\pm$ 0.63
<b>Monogenea</b>							
<i>Gyrodactylus</i> sp.	11, 98	OM	22	1 (5)	—	—	
<b>Nematoda</b>							
<i>Truttaedacnitis</i> sp.	5, 96	OM	15	3 (20)	1.0	0.20 $\pm$ 0.41	
<b>Acanthocephala</b>							
<i>Acanthocephalus dirus</i>	2, 96	OM	12	4 (33)	93.5 $\pm$ 107.5 (209)	31.20 $\pm$ 72.6	
	4, 96	OM	13	1 (8)	1.0	0.08 $\pm$ 0.28	
	4, 96	OM	13	1 (8)	2.0	0.15 $\pm$ 0.56	
	4, 96	OM	25	10 (40)	2.3 $\pm$ 1.6 (5)	0.92 $\pm$ 1.49	
	5, 96	OM	15	15 (100)	44.7 $\pm$ 43.0 (127)	44.70 $\pm$ 43.00	
	5, 97	OM	15	15 (100)	56.5 $\pm$ 110.5 (432)	56.50 $\pm$ 110.50	
	5, 98	OM	5	1 (20)	22.0	4.40 $\pm$ 9.80	
	5, 98	OM	16	9 (56)	3.2 $\pm$ 3.3 (11)	1.81 $\pm$ 2.93	
	7, 98	OM	20	7 (35)	17.9 $\pm$ 39.4 (107)	6.25 $\pm$ 23.87	
	8, 98	OM	20	16 (80)	98.1 $\pm$ 137.8 (490)	78.40 $\pm$ 128.90	
	10, 98	OM	20	1 (5)	1.0	0.05 $\pm$ 0.22	
	11, 98	OM	22	5 (23)	5.6 $\pm$ 9.2 (22)	1.27 $\pm$ 4.67	
	5, 96	SF	20	20 (100)	42.7 $\pm$ 48.6 (172)	42.70 $\pm$ 48.60	
	5, 96	SF	16	3 (19)	2.0 $\pm$ 1.4 (4)	0.38 $\pm$ 1.03	
	5, 97	SF	15	15 (100)	46.3 $\pm$ 43.8 (116)	46.3 $\pm$ 43.8	
	8, 98	SF	6	6 (100)	11.7 $\pm$ 6.9 (20)	11.7 $\pm$ 6.9	
	5, 96	ST	20	20 (100)	36.0 $\pm$ 24.3 (87)	36.0 $\pm$ 24.3	
5, 96	ST	15	3 (20)	3.3 $\pm$ 4.0 (8)	0.67 $\pm$ 2.05		
5, 97	ST	15	15 (100)	75.7 $\pm$ 80.6 (298)	75.7 $\pm$ 80.6		
<b>Copepoda</b>							
<i>Salmincola edwardsii</i>	1, 96	SF	10	10 (100)	39.5 $\pm$ 20.9 (71)	39.5 $\pm$ 20.9	
	5, 96	SF	20	20 (100)	3.6 $\pm$ 3.1 (11)	3.6 $\pm$ 3.1	
	1, 97	SF	12	7 (58)	6.6 $\pm$ 6.4 (20)	3.8 $\pm$ 5.8	
	5, 97	SF	15	14 (93)	45.4 $\pm$ 31.2 (88)	42.4 $\pm$ 32.2	
<b>Myxozoa</b>							
<i>Myxobolus cerebralis</i>	5, 97	OM	15	2 (13)	—	—	
	11, 98	OM	22	13 (59)	—	—	
	5, 97	SF	20	1 (5)	—	—	
<b>Ciliophora</b>							
<i>Capriniana</i> sp.	8, 98	OM	20	8 (40)	—	—	
<i>Chilodonella</i> sp.	2, 96	OM	12	3 (25)	—	—	
<i>Ichthyophthirus multifiliis</i>	3, 96	OM	20	8 (40)	—	—	

Table 2. Continued.

Parasite	Farm number, year	Trout species*	No. examined	Prevalence No. infected (%)	Mean intensity $\pm$ SD (max)	Mean abundance $\pm$ SD
<i>Trichodina</i> sp.	1, 96	OM	10	10 (100)	—	—
	1, 96	OM	24	14 (58)	—	—
	2, 96	OM	12	3 (25)	—	—
	4, 96	OM	13	2 (15)	—	—
	4, 96	OM	25	7 (28)	—	—
	1, 97	OM	10	3 (30)	—	—
	1, 97	OM	20	2 (10)	—	—
	1, 97	OM	5	1 (20)	—	—
	7, 98	OM	20	4 (20)	—	—
	9, 98	OM	18	10 (56)	—	—
	11, 98	OM	22	4 (18)	—	—
	12, 98	OM	11	2 (18)	—	—
	1, 96	SF	20	5 (25)	—	—
	1, 96	SF	10	10 (100)	—	—
	1, 97	SF	12	5 (42)	—	—
	1, 98	SF	19	5 (26)	—	—
	8, 98	SF	6	2 (33)	—	—
	12, 98	SF	2	1 (50)	—	—
	Mastigophora					
<i>Ichthyobodo</i> sp.	3, 96	OM	20	2 (10)	—	—

\* OM = *Oncorhynchus mykiss*; SF = *Salvelinus fontinalis*; ST = *Salmo trutta*.

*Myxobolus cerebralis* has been present in Michigan waters since at least 1968, when it was discovered in 3 commercial trout hatcheries. Yoder (1972) discussed the spread of *M. cerebralis* into native trout populations in the Tobacco River, Michigan, from 1 of these hatcheries. The protozoan spread down the first 6 mi of water, and factors involved in this spread were the high incidence of disease at the hatchery, abundance of susceptible trout, and trout movement. In 1998 and 1999, *M. cerebralis* was reported from at least 6 privately owned trout farms in Michigan. It has been suggested that it was endemic in 1 or more facilities and transferred to other facilities with infected fish or by piscivorous birds that ate infected fish. In the present study, *M. cerebralis*-infected trout were detected in 3 farms. Infected trout, however, did not have clinical symptoms. Furthermore, Sutherland (1999) reported that *M. cerebralis* has been found in fish from the Au Sable and Manistee rivers in lower Michigan.

In the present study, parasites and their numbers infecting trout in a pond may dramatically change the next time the fish are sampled and examined. One reason for this is that trout are moved into and out of facilities during the year. An example of this was evident at 1 farm, when,

in March 1997, 4 parasite species infected trout and 2 were common (Table 4). In July, only 1 species infrequently infected trout after the infected trout were moved out and uninfected ones were moved in. Also, informing the owner of the facility on the parasites found can affect infection levels from 1 sampling date to the next. After an owner was informed that brook trout were infested with *S. edwardsii*, he told me that "a treatment had been done to the pond." Approximately 2 months later, the prevalence and mean intensity of *S. edwardsii* on trout from the same pond were dramatically reduced. Another suggestion for these differences is that parasite species may exhibit a seasonal cycle in their occurrence.

Hare and Frantsi (1974) found 12 parasite species, 10 parasite species, and 1 parasite species infecting, respectively, Atlantic salmon, *Salmo salar* Linnaeus, 1758; brook trout; and rainbow trout, in 13 Canadian hatcheries in the Maritime provinces. *Hexamita salmonis* (Moore, 1923) Wenyon, 1926; *Trichophyra piscium* Buetschli, 1889; *Diplostomum spathaceum* (Rudolphi, 1819) Braun, 1893; *Acanthocephalus lateralis* (Leidy, 1851); and *S. edwardsii* were considered to be serious fish pathogens, based on the work of other authors. Buchmann and

**Table 3. Parasites of *Oncorhynchus mykiss* examined from 23 ponds, *Salvelinus fontinalis* from 13 ponds, and *Salmo trutta* from 6 ponds in 1996, 1997, and 1998.**

Parasite	Trout species*	No. (%) of ponds where parasites occurred
<b>Cestoda</b>		
<i>Eubothrium salvelini</i>	OM	9 (39)
	SF	4 (31)
<i>Proteocephalus</i> sp.	OM	3 (13)
<b>Monogenea</b>		
<i>Gyrodactylus</i> sp.	OM	1 (4)
<b>Nematoda</b>		
<i>Truttaedacnitis</i> sp.	OM	1 (4)
<b>Acanthocephala</b>		
<i>Acanthocephalus dirus</i>	OM	12 (52)
	SF	4 (31)
	ST	2 (33)
<b>Copepoda</b>		
<i>Salmincola edwardsii</i>	SF	4 (31)
<b>Myxozoa</b>		
<i>Myxobolus cerebralis</i>	OM†	2 (17)
	OM†	1 (8)
	SF†	1 (25)
<b>Ciliophora</b>		
<i>Capriniana</i> sp.	OM	1 (4)
<i>Chilodonella</i> sp.	OM	1 (4)
<i>Ichthyophthirus multifiliis</i>	OM	1 (4)
<i>Trichodina</i> sp.	OM	12 (52)
	SF	6 (46)
<b>Mastigophora</b>		
<i>Ichthyobodo</i> sp.	OM	1 (4)

\* OM = *Oncorhynchus mykiss*; SF = *Salvelinus fontinalis*; ST = *Salmo trutta*.

† OM examined from 12 ponds and SF from 4 ponds in 1997 and 1998.

Bresciani (1997) listed investigations performed on the parasites of farmed salmonids, and reported 22 parasite species (12 protozoans and 10 metazoans) infecting 805 pond-reared rainbow trout from 5 freshwater farms in Denmark. Based on these and the present studies, there is a relationship between the number of trout examined and number of parasite species found. As the number of fish examined increases, so does the number of parasite species found. Hnath (1993) suggested that a sample size of 60 individuals should be examined from a population of 2,000 fish or more in a pond in order to detect a pathogen. The numbers of parasite species found in rainbow and brook trout in the present study are low compared with the numbers found by Hare and Frantsi (1974) and by Buchman and Bresciani (1997). More rainbow and brook trout were examined in those studies than in the present one.

The total numbers of parasite species found in rainbow, brook, and brown trout in this study are low compared with the numbers for each species listed by Hoffman (1999) in North America. This may be explained by the artificial conditions in trout farms, which harbor very few potential intermediate invertebrate hosts. In most ponds, snails, which serve as intermediate hosts for digenetic trematodes, were never collected. In contrast, protozoans with direct life cycles are easily introduced and spread between fish. In conversations with several farmers, it was apparent that they used several "antiparasitic" drugs to treat against ectoparasitic infections when they were aware that their fish exhibited signs of infection. This treatment regime also explains the paucity of parasites. The current and increasing use of well water and spring wa-

**Table 4. Prevalence (P), mean abundance (MA), and estimated number (EN) of parasites from 1 farm in March and July 1997.**

Parasite	March				July			
	Trout species (n)*	P	MA ± SD (Max.)	EN	Trout species (n)*	P	MA ± SD (Max.)	EN
<i>Acanthocephalus dirus</i>	SF (20)	100	42.7 ± 49 (172)	106,750	SF (16)	19	0.38 ± 1.03 (4)	14
	ST (20)	100	36.0 ± 24 (87)	90,000	ST (15)	20	0.67 ± 2.05 (8)	27
	OM (15)	100	44.7 ± 43 (127)	312,900	OM (15)	0	—	—
<i>Eubothrium salvelini</i>	OM (15)	20	0.33 ± 0.82 (3)	462	OM (15)	0	—	—
<i>Truttaedacnitis</i> sp.	OM (15)	7	0.20 ± 0.41	98	OM (15)	0	—	—
<i>Salmincola edwardsii</i>	SF (20)	100	3.60 ± 3 (11)	9,000	SF (16)	0	—	—

\* SF = *Salvelinus fontinalis*; ST = *Salmo trutta*; OM = *Oncorhynchus mykiss*. (no. examined)

ter and fiberglass or concrete ponds and tanks will also reduce the incidence of parasites.

The effects of natural bodies of water and the fish in them serving as a source of parasites in culture should be addressed. This involves facilities with "flow-through" systems (those that receive water from lentic or lotic environments). Eggs, other infective stages, and hosts can be carried with water that flows into and through facilities. Obviously this facilitates infection of fish. In general, trout in the present study being raised in flow-through systems had more parasite species and more individuals of the species present in comparison with the other systems. Similarly, Valtonen and Koskivaara (1994), studying the relationships between parasites of wild and cultured fishes in 2 lakes and a fish farm in Finland, reported that the source of parasites in the fish farm was the water-supplying lake.

Cone and Cusack (1988) reported on the occurrence of 2 monogeneans, *Gyrodactylus colemanensis* Mizelle and Kritsky, 1967, and *Gyrodactylus salmonis* Yin and Sproston, 1948, on brook and rainbow trout, and Atlantic salmon, *Salmo salar* Linnaeus, 1758, in a farm in Nova Scotia, Canada, and discussed the origins of infection and their dispersal in the farm. Sources of infection with *G. salmonis* were stocks of infected rainbow trout brought into the facility from another farm, as well as wild infected Atlantic salmon and brook trout gaining access to the hatchery. Parasite dispersal in the farm involved infected fish jumping and wriggling from one pond to the next and the workers using transfer nets and buckets that contained live parasites. Also, brood stocks were infected and constituted internal reservoirs of infection.

The effects of fish culture on natural waters receiving water from the farms has been a subject of increasing debate. If surveillance of parasites in the water above and below the fish facility is not continuous, little will be known about where the parasite really originated or how long it has been present. Regarding *M. cerebralis*, fish known to have whirling disease were imported into a commercial trout farm in Michigan in 1968. The receiving stream (a brook and brown trout stream) of this facility yielded *M. cerebralis*-infected rainbow trout escapees directly below the positive facility effluent. Valtonen and Koskivaara (1994) suggested that the farm itself was unlikely to affect the fish

parasite fauna of the water-recipient lake, although some ectoparasites could originate from the farm.

Muzzall (1995c), studying the parasites of pond-reared yellow perch, *Perca flavescens* (Mitchill, 1814) in Michigan, suggested that conditions of a pond associated with producing a good crop of fish also support a good crop of helminths that infect fish. Later, Muzzall (1996) referred to this as "the good fish crop—good helminth crop" relationship. Based on the results of the parasites infecting trout in the present study, this relationship does not occur. Of the parasite species found by Muzzall (1995c) infecting perch, 8 were represented as only larval stages, 6 of which were digenetic trematodes. Only 2 genera (generalist protozoans, *Trichodina* sp., *Capriniana* sp.) infesting perch were also found infesting trout. The dramatic differences in parasites found in yellow perch and trout from culture conditions can be explained by many factors. Probably the most important are the types of ponds used to culture the particular species, water temperatures, water sources, whether ponds are periodically drained, the surroundings of the ponds, and animals associated with the ponds.

The state of control and prevention of parasites and diseases of fishes in culture in Michigan is difficult to assess. I refer to it as "crisis fisheries health," which can be defined as follows: "Some state and university officials, extension specialists, aquaculture centers, and trout farmers are not apparently concerned with fish health in aquaculture and in nature unless there is a crisis health problem, then action takes place." This approach is understandable with so many interested parties having different motives and the low priority of funding for parasite and disease work. I suggest that more studies on fish parasites and diseases in Michigan be encouraged and supported by the interested groups. Surveillance and surveys are needed to determine what parasites are infecting trout in culture conditions and in the surrounding waters.

As mentioned earlier, growers in Michigan are involved in 3 activities in producing and selling trout. In regard to the first, the sale of infected trout for stocking could transfer some parasites to other fish directly or contaminate the watershed with other parasites. However, most if not all parasites reported in this study have been found infecting trout in the wild. Second, no par-

asites were found that could infect humans if poorly cooked infected meat was eaten. Third, the sale of infected fish in fee-fishing ponds should not play a role in transmitting parasites, unless these fish are placed in other environments or the ponds have effluents to public waters. Obviously it should be emphasized that if trout are not routinely examined, light infections will not be noticed; when the infections do become evident, it may be too late to help the diseased fish.

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J. Ralph Lichtenfels and Janet W. Reid

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## Six New Host Records and an Updated List of Wild Hosts for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae)

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**ABSTRACT:** Six new host records and an updated list of wild hosts for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae) are provided. We report specimens of *N. melleni* from the skin of a whitefin shark-sucker (*Echeneis neucratoides* Zuiew [Echeneidae]) caught off Mayagüez, Puerto Rico; from the skin of a mosquitofish (*Gambusia xanthosoma* Greenfield [Poeciliidae]) caught in Little Salt Creek, Grand Cayman Island, British West Indies; from a freshwater immersion bath of red grouper (*Epinephelus morio* (Valenciennes) [Serranidae]) caught in the Gulf of Mexico off Sarasota, Florida, U.S.A.; from the skin of a garden eel (*Heteroconger hassi* (Klausewitz and Eibl-Eibesfeldt) [Congridae]) in the Toledo Zoo, Toledo, Ohio, U.S.A.; from the skin of a raccoon butterflyfish (*Chaetodon lunula* (Cuvier) [Chaetodontidae]) in the Fort Wayne Children's Zoo, Fort Wayne, Indiana, U.S.A.; and from the gill cavity of a red snapper (*Lutjanus campechanus* (Poey) [Lutjanidae]) in holding facilities at the Gulf Coast Research Laboratory, Ocean Springs, Mississippi, U.S.A. *Neobenedenia melleni* had not been reported previously from a suspected wild host in the Gulf of Mexico (i.e., *E. morio*) or from a member of Echeneidae, Atheriniformes, or Anguilliformes. Published host records indicate that *N. melleni* exhibits a relatively low degree of host specificity among captive and wild hosts; in nature, *N. melleni* infests predominantly shallow-water or reef teleosts.

**KEY WORDS:** *Neobenedenia melleni*, *Echeneis neucratoides*, *Gambusia xanthosoma*, *Epinephelus morio*, *Heteroconger hassi*, *Chaetodon lunula*, *Lutjanus campechanus*, Monogenea, Capsalidae, host specificity, zoogeography, public aquaria, aquaculture, U.S.A., Puerto Rico, British West Indies, Florida, Mississippi, Gulf of Mexico.

The capsalid *Neobenedenia melleni* (MacCallum, 1927) is relatively unusual among members of Monogenea in that it has been reported from a wide range of hosts. This capsalid infests the eyes, fins, gill cavity, nasal cavity, and skin of over 100 species of marine teleosts (Whittington and Horton, 1996). Most of these records are from fishes in aquaria and aquaculture systems where the parasite is identified as a lethal pathogen (e.g., MacCallum, 1927; Jahn and Kuhn, 1932; Nigrelli and Breder, 1934; Mueller et al., 1994). However, there is no report of disease associated with infestations of *N. melleni* among wild fishes. *Neobenedenia melleni* had been reported previously from wild hosts in the Caribbean Sea, Gulf of California, and eastern Pacific Ocean off the coasts of Chile, Mexico, and the United States (Table 1). Published

accounts of *N. melleni* infesting wild hosts (see references in Table 1) are relatively scarce, and little is known about the breadth of host specificity exhibited by this parasite in nature. Therefore, reports of *N. melleni* from wild hosts are significant because they offer insight into the natural geographic distribution and host range of this parasite. We report 6 new host records for *N. melleni*: 3 from wild fishes and 3 from captive fishes.

### Materials and Methods

Worms were fixed in 10% neutral buffered formalin, 70% ethanol, or Bouin's fixative. Eight worms were stained in Van Cleave's hematoxylin containing several additional drops of Ehrlich's hematoxylin and were then dehydrated to 70% ethanol. Several drops of aqueous saturated lithium carbonate were then added, followed by several drops of 6% butylamine solution. Stained worms were dehydrated in an ethanol series, cleared in clove oil, and mounted permanently on glass slides using neutral Canada balsam. Five

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**Table 1. Wild hosts for *Neobenedenia melleni* (MacCallum, 1927).**

Host	Site	Locality	Reference
<b>ATHERINIFORMES</b>			
Poeciliidae			
<i>Gambusia xanthosoma</i> Greenfield, 1983	Skin	Little Salt Creek, Grand Cayman Island, British West Indies	Present study (USNPC No. 89159)
<b>SCORPAENIFORMES</b>			
Scorpaenidae			
<i>Sebastes capensis</i> (Gmelin, 1789)	Skin	Southeast Pacific Ocean off northern Chile	Gonzalez and Acuna, 1998
<i>Sebastes melanops</i> Girard, 1856 (as <i>Sebastodes melanops</i> )	Gills	Northeast Pacific Ocean off San Juan County, Washington, U.S.A.	Whittington and Horton, 1996
<i>Sebastes serranoides</i> (Eigenmann and Eigenmann, 1890)	Mouth and skin	Northeast Pacific Ocean off Diablo Cove, California, U.S.A.	Love et al., 1984 (as <i>Neobenedenia girellae</i> )
Hexagrammidae			
<i>Hexagrammos decagrammus</i> (Pallas, 1810)	Gills	Northeast Pacific Ocean off San Juan County, Washington, U.S.A.	Whittington and Horton, 1996
Cottidae			
<i>Leptocottus armatus</i> Girard, 1854	Not indicated	Northeast Pacific Ocean off La Jolla, California, U.S.A.	Gaida and Frost, 1991 (as <i>Neobenedenia girellae</i> )*
<b>PERCIFORMES</b>			
Serranidae			
<i>Epinephelus guttatus</i> (Linnaeus, 1758)	Gills	Caribbean Sea off La Pargüera, Puerto Rico	Dyer et al., 1992 (as <i>Neobenedenia pargueraensis</i> )
<i>Epinephelus morio</i> (Valenciennes, 1828)	Not indicated	Gulf of Mexico off Sarasota, Florida, U.S.A.	Present study (HWML Coll. No. 15064; USNPC No. 89162)
<i>Epinephelus striatus</i> (Bloch, 1792)	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia melleni</i> )
<i>Mycteroperca rosacea</i> (Gilbert, 1892) (as <i>Mycteroperca pardalis</i> )	Gills	Gulf of California off La Paz, Mexico	Bravo-Hollis, 1957 (as <i>Benedenia girellae</i> )
Echeneidae			
<i>Echeneis neucratoides</i> Zuieww, 1789	Skin	Caribbean Sea off Mayagüez, Puerto Rico	Present study (HWML Coll. No. 15063; USNPC No. 89161)
Lutjanidae			
<i>Lutjanus apodus</i> (Walbaum, 1892) (as <i>Lutianus apodus</i> )	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia melleni</i> )

Table 1. Continued.

Host	Site	Locality	Reference
Sparidae			
<i>Archosargus probatocephalus</i> (Walbaum 1792)†	Eyes and skin	Northeast Pacific Ocean off La Jolla, California, U.S.A.	Whittington and Horton, 1996
Chaetodontidae			
<i>Chaetodon capistratus</i> Linnaeus, 1758	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
<i>Chaetodon ocellatus</i> Bloch, 1787	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
<i>Chaetodon striatus</i> Linnaeus, 1758	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
Pomacanthidae			
<i>Holocanthus ciliaris</i> (Linnaeus, 1758)	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
<i>Holocanthus tricolor</i> (Bloch, 1795)	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
<i>Pomacanthus arcuatus</i> (Linnaeus, 1758)	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
<i>Pomacanthus paru</i> (Bloch, 1787)	Not indicated	Caribbean Sea off Bimini, British West Indies	Nigrelli, 1947 (as <i>Benedenia meleni</i> )
Kyphosidae			
<i>Girella nigricans</i> (Ayres, 1860)	Skin	Northeast Pacific Ocean off La Jolla, California, U.S.A.	Hargis, 1955 (as <i>Benedenia girellae</i> )
	Fins and skin	Northeast Pacific Ocean off Santa Catalina Island, California, U.S.A.	Goldberg et al., 1991 (as <i>Neobenedenia girellae</i> )
<i>Medialuna californiensis</i> (Steindachner, 1876)	Skin	Northeast Pacific Ocean off Santa Catalina Island, California, U.S.A.	Gaida and Frost, 1991 (as <i>Neobenedenia girellae</i> )
Embiotocidae			
<i>Embiotoca jacksoni</i> Agassiz, 1853	Exterior	Northeast Pacific Ocean, Naples Reef off Santa Barbara, California, U.S.A.	Whittington and Horton, 1996
<i>Embiotoca lateralis</i> Agassiz, 1854	Exterior of head	Northeast Pacific Ocean, San Francisco Bay, California, U.S.A.	Whittington and Horton, 1996
	Exterior	Northeast Pacific Ocean near San Francisco, California, U.S.A.	Moser and Haldorson, 1982 (as <i>Neobenedenia girellae</i> )
<i>Rhacochilus vacca</i> (Girard, 1855) (as <i>Damalichthys vacca</i> )	Exterior	Northeast Pacific Ocean near San Francisco, California, U.S.A.	Moser and Haldorson, 1982 (as <i>Neobenedenia girellae</i> )

Table 1. Continued.

Host	Site	Locality	Reference
Labridae <i>Semicossyphus pulcher</i> (Ayers, 1854) (as <i>Pimelometopon pulchrum</i> )	Skin	Northeast Pacific Ocean off La Jolla, California, U.S.A.	Brooks and Mayes, 1975 (as <i>Neobenedenia girellae</i> )
Scaridae <i>Scarus perrico</i> Jordan and Gilbert, 1882	Gills	Northeast Pacific Ocean, Bahía Banderas off Nayarit, Mexico	Bravo-Hollis and Deloya, 1973 (as <i>Benedenia girellae</i> )
Acanthuridae <i>Acanthurus chirurgus</i> (Bloch, 1787)	Gills	Caribbean Sea, Parguera Bay, Puerto Rico	Whittington and Horton, 1996

\* Record needs verification.

† The sheephead, *Archosargus probatocephalus*, does not occur in the Pacific Ocean, and it is likely that this record is in error and should be the sheephead, *Semicossyphus pulcher* (see Robins et al., 1986).

worms intended for study using Nomarski illumination were dehydrated, cleared in clove oil, and mounted unstained in neutral Canada balsam. Worms were identified using the original description of *N. melleni* (as *Epibdella melleni* MacCallum, 1927), the redescription of *N. melleni* contained in a recent revision of *Neobenedenia* Yamaguti, 1963, and the key to the species of *Neobenedenia* (see Whittington and Horton, 1996). We primarily used 1) anterior attachment organs circular and not bipartite; 2) anterior hamuli recurved, nonserrated (i.e., smooth), and robust (i.e., width usually greater than that of both accessory sclerites and posterior hamuli and with root of consistent width along total length [i.e., root not tapered, constricted, or pinched]); 3) glands of Goto not evident, and 4) other specific features indicated by Whittington and Horton (1996). Nomenclature used herein for members of *Neobenedenia* follows that of Whittington and Horton (1996). Specimens of *N. melleni* from *Gambusia xanthosoma* (Poeciliidae) and *Lutjanus campechanus* (Poey, 1860) (Lutjanidae) were deposited in the United States National Parasite Collection (USNPC) at Beltsville, Maryland, U.S.A. (USNPC Nos. 089159 and 089160), and specimens from *Echeneis neucratoides* (Echeneidae), *Epinephelus morio* (Serranidae), *Heteroconger hassi* (Klausewitz and Eibl-Eibesfeldt, 1959) (Congridae), and *Chaetodon lunula* (Cuvier, 1831) (Chaetodontidae) were deposited there (USNPC Nos. 089161, 089162, 089163, and 089164) and in the helminth collections of the H. W. Manter Laboratory (HWML) of the University of Nebraska State Museum at Lincoln, Nebraska, U.S.A. (HWML Nos. 15063, 15064, 15065, and 15066).

### Results and Discussion

Regarding our new host records, 2 specimens of *N. melleni* were collected from the skin of a whitefin sharksucker (*E. neucratoides*), a remora that was attached to a West Indian manatee (*Trichechus manatus* Linnaeus, 1758 [Trichechidae]) off Mayagüez, Puerto Rico. This is the first report of *N. melleni* from a remora and may help to explain in part the wide geographic distribution of *N. melleni*. Although carriers of infested remoras may not travel between oceans, infested remoras may transfer infestations of *N. melleni* among fish, mammalian, and turtle species and individuals with which they associate. In addition, remoras can attached to or mingle with their carriers for prolonged periods of time. This habit may provide *N. melleni* opportunity to infest the remora's carrier host or other fishes in close proximity to the infested remora. Various cleaner fishes (e.g., bluehead wrasse, *Thalassoma bifasciatum* (Bloch, 1791) [Labridae]; neon goby, *Gobiosoma oceanops* (Jordan, 1904) [Gobiidae]; and cleaning goby, *Gobiosoma genie* Böhlke and Robins, 1968) were effective in controlling infestations of *N. melleni* among aquar-

ium-kept fish (see Cowell et al., 1993). Some species of remora feed on ectoparasites (Cressey and Lachner, 1970), and, because of this, aquaculturists eventually may use remoras to control infestations of *N. melleni* on large hosts. However, as previously suggested, remoras may transport worms to adjacent groups of fishes.

A specimen of *N. melleni* was collected from the skin of a mosquitofish (*G. xanthosoma*) from Little Salt Creek (western shore of North Sound, Grand Cayman, British West Indies). *Neobenedenia melleni* had not been reported previously from a member of Atheriniformes or from the western Caribbean Sea. The specimen of *N. melleni* was conspicuous, 3 mm in total length, and attached to the dorsal surface of the head at the level of the eyes of a mosquitofish that was 33 mm in total length. *Gambusia xanthosoma* is apparently endemic to the high salinity mangrove habitats throughout North Sound (Abney and Heard, personal communication); therefore, it is of ecological interest to report on the occurrence of nonendemic parasites, such as *N. melleni*, that infest a wide range of hosts and that are identified as lethal pathogens among confined fishes. Nigrelli (1947) reported several wild hosts for *N. melleni* in the Caribbean Sea off Bimini (see Table 1). Robinson et al. (1992) and Hall (1992) reported heavy infestations of *N. melleni* among cultured, seawater-acclimated red hybrid tilapia in floating cages off southern Jamaica. Cowell et al. (1993) reported infestations of *N. melleni* on Florida red tilapia (descendants of an original cross between *Oreochromis urolepis hornorum* (Norman, 1922) [Cichlidae] and *Oreochromis mossambicus* (Peters, 1852)) in aquaria at the Caribbean Marine Research Center (CMRC), Lee Stocking Island, Exuma Cays, Bahamas. However, because *N. melleni* has a broad host range and wide geographic distribution and heavily infests some hosts in aquaculture, we cannot determine if, when, or how it was introduced to the endemic population of *G. xanthosoma*.

At least 3 specimens of *N. melleni* infested the red grouper (*E. morio*); they were caught off Sarasota, Florida, U.S.A., in January 1993. Material of *N. melleni* was collected from a freshwater immersion bath at the Mote Marine Laboratory (MML), Sarasota, Florida, when the fish were initially treated after being captured from the Gulf of Mexico. Nevertheless, *N. melleni* later became established in culture facilities at the

MML. *Neobenedenia melleni* was previously reported from *E. morio* and *Mycteroperca microlepis* (Goode and Bean, 1879) (Serranidae) in recirculating-seawater tanks in northwestern Florida (Florida State University Marine Laboratory, Turkey Point, Florida, U.S.A.) by Mueller et al. (1994) and from other members of the sea bass family in the Caribbean Sea and the Gulf of California (see Table 1); however, this is the first report of *N. melleni* from a suspected wild host in the Gulf of Mexico.

We also report numerous adult and juvenile specimens of *N. melleni* from the skin of a garden eel (*H. hassi*) from the Toledo Zoo, Toledo, Ohio, U.S.A. This is the first report of *N. melleni* from any member of Anguilliformes, and to the best of our knowledge, it is also the first report of *N. melleni* from a host that lacks scales. Whereas the exact geographic origin of the eel was not known, we suspect that it became infested while confined in a compartmentalized quarantine system at the Toledo Zoo. One of us (J.H.) observed a cream angelfish (*Apolectichthys xanthurus* (Bennett, 1832) [Pomacanthidae]) in this same water system that harbored numerous specimens of a platyhelminth on its skin that were probably *N. melleni*. Nigrelli and Breder (1934) reported that some angelfishes were foci for epidemics of *N. melleni* in the New York Aquarium. Specimens of *N. melleni* have yet to be reported from *A. xanthurus*. However, because the aforementioned worms from this host were not available for identification, we did not report this fish as a host for *N. melleni*.

Numerous specimens of *N. melleni* were also collected from a raccoon butterflyfish (*C. lunula*) that died while in quarantine at the Fort Wayne Children's Zoo, Fort Wayne, Indiana, U.S.A. We are not certain of the exact geographic origin of that wild-caught fish or whether it was infested in the wild. However, *C. lunula* is a reef species that ranges from East Africa to Polynesia (Randall et al., 1990), and that raccoon butterflyfish most likely came from there (i.e., Indo-Pacific Region). *Neobenedenia melleni* has been reported from 3 members of *Chaetodon* in the Caribbean Sea (Nigrelli, 1947; Table 1).

A single specimen of *N. melleni* was collected from the gill cavity of a red snapper (*L. campechanus*) caught in the northern Gulf of Mexico and maintained in an aquaculture tank with other red snapper at the Gulf Coast Research



Laboratory (GCRL), Ocean Springs, Mississippi, U.S.A. The tank and filtration system that supported this host had been sanitized before adding any fish, and no other fishes shared the water of this system. There was no history of infestation by this monogenean in culture facilities at GCRL. Therefore, it is likely that this red snapper was infested with *N. melleni* in the wild. In addition, 3 juvenile red snapper (each approximately 120 mm in total length) that were spawned and reared at the GCRL aquaculture facility and then transferred to the GCRL Marine Education Center (MEC), Biloxi, Mississippi, U.S.A., became heavily infested with *N. melleni*. These red snapper were maintained in a 7,700-liter aquarium with a spadefish (*Chaetodipterus faber* (Broussonet, 1782) [Ephippidae]) that was also heavily infested with the monogenean. Nigrelli (1947) reported *Lutjanus apodus* (as *Lutianus apodus*) as a wild host for *N. melleni* (as *Benedenia melleni*) in the West Indies. We suspect that *L. campechanus* also may be a wild host of *N. melleni* in the northern Gulf of Mexico. However, it does not seem to be a common host, because we have yet to observe a specimen of *N. melleni* on a red snapper directly from the wild, in spite of examinations of at least 276 such fish.

The most recent list of captive and wild hosts for *N. melleni* was presented by Lawler (1981). Whittington and Horton (1996) subsequently provided a list of hosts for *N. melleni*; however, that list did not distinguish between captive and wild hosts. Because a list identifying wild hosts for *N. melleni* has not been presented in 18 years, we consider Table 1 a useful update.

Rarely does a monogenean species, let alone a capsalid, occur in more than 1 ocean and infest more than 1 host species, and if so, those hosts are usually closely related species (e.g., Byrnes and Rohde, 1992; Whittington, 1998). *Neobenedenia melleni* has now been reported from 27 species comprising 18 genera, 14 families, and 3 orders of wild hosts (Table 1). These records suggest that *N. melleni* is a parasite of predominantly shallow-water or reef-dwelling marine teleosts. *Neobenedenia melleni* exhibits a relatively low degree of host specificity among both captive and wild hosts. Nigrelli and Breder (1934) studied the host-parasite relationship between *N. melleni* and several fishes held at the New York Aquarium. However, the factors that allowed it to infest a broad array of hosts in

captivity and in the wild were not clearly understood. In some cases, horizontal transfer and levels of infestation may be limited initially only by the physical distance between parasite and potential host. This could, in part, explain the apparent abundance of *N. melleni* among reef fishes that live in close proximity to one another in the wild and among those and other fishes held in public aquaria and aquaculture systems. Further study of this unique monogenean utilizing molecular techniques could possibly reveal population differences.

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## *Hymenolepis nana* in Pet Store Rodents

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**ABSTRACT:** The rodent tapeworm, *Hymenolepis nana*, is a zoonotic pathogen transmissible through the ingestion of eggs in feces or cysticercoids in arthropods. Since data addressing the potential for acquiring human infections of *H. nana* from pet rodents are lacking, a survey of pet stores in southern Connecticut, U.S.A., was conducted. Fecal flotation analysis revealed 9.1% overall prevalence in 110 samples collected weekly from cages holding group-housed small animals, from 3 stores for 4 weeks. Of 11 species, only cages holding rats (3 of 22 samples), mice (6 of 30 samples), and prairie dogs (1 of 2 samples) were positive. Necropsies of 38 rats, 72 domestic mice, and 39 golden hamsters purchased from 9 stores showed prevalences of 31.6%, 22.2%, and 10.3%, respectively. Mean intensity was 66 worms per rat, 14 worms per mouse, and 15 worms per hamster. Overall, 75% of surveyed pet stores were selling infected rats, mice, or hamsters, indicating that pet store rodents pose a potential threat to public health.

**KEY WORDS:** *Hymenolepis nana*, pets, rodents, survey, zoonosis.

*Hymenolepis nana* Siebold, 1852, infects 75 million people worldwide (Crompton, 1999), of whom the majority are children (Little, 1985; Markell et al., 1999). *Hymenolepis nana* has a cosmopolitan distribution, with human, Old World monkey, and rodent definitive hosts becoming infected through ingestion of infective cysticercoids within beetle or flea intermediate hosts or through ingestion of eggs in feces. In the latter route, cysticercoids develop in intestinal villi, with worms later reemerging and attaching to the mucosa (Roberts and Janovy, 2000). Direct transmission through the ingestion of eggs may be the most common route of infection in humans (Turner, 1975).

It has been suggested that 2 morphologically identical subspecies of *H. nana* exist, yet this tapeworm is typically classified as a zoonotic and is capable of horizontal transmission between human and nonhuman animals (Fox et al., 1984; Jacoby and Fox, 1984; Chomel, 1992). Human infections “produce either no symptoms or vague abdominal disturbances. In fairly heavy infections, children may show lack of appetite, abdominal pain with or without diarrhea, anorexia, vomiting, and dizziness” (Neva and Brown, 1994). Such nondescript symptoms may account for the low number of reported clinical cases, and many subclinical infections may go undiagnosed. Available prevalence data regarding human populations were obtained in most cases from fecal surveys conducted in develop-

ing nations. For example, *H. nana* was found in 20.5% of Australian aborigines (Meloni et al., 1993), 8–10% of oncology patients in Mexico (Guarner et al., 1997), 16% of Egyptian school children (Khalil et al., 1991), and 0.6% of Thai laborers (Wilairatana et al., 1996). In developed regions such as Western Europe and North America, human infections are seldom identified or acknowledged, and survey data are often patchy and scarce (Croll and Gyorkos, 1979; Jacobs, 1979; Seaton, 1979; Cooper et al., 1981). Still, *H. nana* is estimated to be an important cause of cestodiasis in the southeastern United States, with infections found in approximately 1% of school children (Roberts and Janovy, 2000) and 4% of pediatric clinic patients (Flores et al., 1983). In 1987, 34 state diagnostic laboratories identified *H. nana* in collected stool samples (0.4%), with Connecticut reporting a prevalence of 0.8%, Massachusetts 0.4%, and Rhode Island 1.6% (Kappus et al., 1991).

Most studies focus on human infection, but fail to adequately address potential zoonotic sources of the infection. In Turkey, 5.6% of surveyed wild mice and rats harbored *H. nana* (Sahin, 1979), and in Saudi Arabia, *H. nana* was reported from baboons living in close proximity to humans (Ghandour et al., 1995). In the United States, Stone and Manwell (1966) reported infection in 21% of mice and 9% of hamsters from Syracuse University, Syracuse, New York, U.S.A., animal rooms and various commercial vendors. In the same study, pet mice and ham-

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**Table 1. Results of cage sampling for *Hymenolepis nana* using fecal flotation.**

Host species	No. of cage samples	Samples (+) for <i>H. nana</i>	Cage prevalence (%) <sup>*</sup>
Domestic spiny mouse (Heteromyidae)	3	0	0
Long-tailed chinchilla ( <i>Chinchilla lanigera</i> Molina, 1782)	6	0	0
Black-tailed prairie dog ( <i>Cynomys ludovicianus</i> Ord, 1815)	2	1	50 <sup>†</sup>
Guinea pig ( <i>Cavia porcellus</i> Linnaeus, 1758)	19	0	0
Golden hamster ( <i>Mesocricetus auratus</i> Waterhouse, 1839)	18	0	0
Domestic mouse ( <i>Mus musculus</i> Linnaeus, 1758)	30	6	20
Ferret ( <i>Mustela putorius furo</i> Linnaeus, 1758)	3	0	0
Mongolian gerbil ( <i>Meriones unguiculatus</i> Milne-Edwards, 1867)	7	0	0
European rabbit ( <i>Oryctolagus cuniculus</i> Linnaeus, 1758)	11	0	0
Siberian hamster ( <i>Phodopus sungorus</i> Pallus, 1773)	1	0	0
Norway rat ( <i>Rattus norvegicus</i> Berkenhout, 1769)	22 <sup>‡</sup>	3	14

\* Overall prevalence of infected cages was 9.1%.

† A total of 5 individual prairie dogs was surveyed from the 2 cage samples.

‡ *Hymenolepis diminuta* eggs were also detected in the cage sample.

sters showed prevalences of 66% and 44% respectively.

Pet stores are traditionally implicated as potential sources of human parasite infections, but emphasis is centered upon feline, canine, or avian species rather than rodents. However, *H. nana* is a common zoonosis of pet rodents (Chomel, 1992), and in 1969 infection was detected in Mongolian gerbils purchased as pets from a department store (Lussier and Loew, 1970). Given that children have less than optimal hygiene habits, and immune-compromised individuals, such as those with the acquired immunodeficiency syndrome (AIDS) or undergoing cancer treatment, are at greater risk for disease (Gerba et al., 1996), pet rodent infections raise obvious public health concerns. Additionally, there is a lack of survey data addressing the assumption that golden hamsters are more often parasitized with *H. nana* than are other rodents (Chomel, 1992; Teclaw et al., 1992). The purpose of this study was to assess health risks associated with human and rodent interaction as they pertain to *H. nana*, through a survey of small animals sold by pet stores in southern Connecticut.

#### Materials and Methods

Once a week for 4 weeks beginning in July 1999, a fecal survey was conducted on all small animal cages from 3 pet stores. Samples of 5–10 fecal pellets were collected from the bedding of cages housing grouped animals and analyzed by fecal flotation (Hendrix, 1998). A total of 110 cage samples was obtained from representatives of 11 domesticated small animal species (Table 1).

Based on the findings from fecal analysis of small

animal cages, individual rodents were purchased from 9 different pet stores not included in the fecal survey, and a postmortem examination of the intestinal tract of each rodent was performed. Necropsy was conducted on a total of 38 rats, 39 golden hamsters, and 72 domestic mice. Animals were killed by CO<sub>2</sub> narcosis, and the small intestine, from the pyloric sphincter to the ileocecal juncture, was removed, placed in a Petri dish of tap water, and opened longitudinally. Worms were removed and counted. Representative specimens were stained, mounted, and deposited in the United States National Museum Parasite Collection in Beltsville, Maryland, U.S.A. (USNPC No. 089330.00).

#### Results

Fecal analysis showed that 9.1% of cages housed infected animals, with animals from 1 pet store testing positive for 3 of 4 weeks. Domestic mice and Norway rats exhibited prevalences of 30.0% and 13.6%, respectively. One of 2 black-tailed prairie dog cage samples revealed *H. nana*. All other species, including golden hamsters, were negative by fecal flotation analysis (Table 1).

Necropsy results of purchased animals revealed that 7 of 9 pet stores were selling infected rats, domestic mice, and/or golden hamsters. Prevalence was highest in rats (31.6%), with mean intensity (MI) of 66 worms per host. Mouse prevalence was lower at 22.2% (MI = 15), and only 4 golden hamsters (10.3%) were infected (MI = 15). One rat was infected with *Hymenolepis diminuta* Rudolphi, 1819 (Table 2).

#### Discussion

Rodents typically remained in pet stores approximately 7–10 days. The prepatent period for

**Table 2. Presence of *Hymenolepis nana* in necropsied rats, mice, and hamsters.**

Host species	No. of individuals necropsied	No. (%) of individuals infected	Mean intensity
Golden hamster	39	4 (10.3)	15
Domestic mouse	72	16 (22.2)	14
Norway rat	38	12 (31.6)*	66

\* One rat was infected with *Hymenolepis diminuta*.

*H. nana* is approximately 25 days (Hunninen, 1935; Jacoby and Fox, 1984), leading to the conclusion that animals arrived infected from commercial vendors or private breeders, rather than acquiring infection through exposure at pet stores. Because of the high demand for and quick turnover rate of rats, mice, and hamsters, the majority of pet stores surveyed purchased their rodents from various vendors rather than relying on in-house breeding programs. In this study, 7 of the 12 pet stores purchased animals from 5 different vendors, while the other 5 stores relied on in-house breeding programs or various suppliers, either private or commercial

sources. Rodents in those 7 vendor-supplied stores tested positive for *H. nana*, while only 3 of the other 5 stores revealed positive rodents (Table 3).

Evidence for direct transmission of *H. nana* as the common route of infection in rodents can be derived from the concomitant presence of *H. diminuta* and *H. nana* within the same rat cage. Transmission of *H. diminuta* requires an arthropod intermediate host (Roberts and Janovy, 2000). Since the same arthropods may serve as intermediate hosts for both tapeworms, lower prevalence of *H. diminuta* and higher prevalence of *H. nana* indicate transmission through direct rather than indirect routes. If *H. nana* were using an intermediate host, one would assume the prevalences of the 2 tapeworms to be nearly equivalent.

Traditionally, *H. nana* is considered a tapeworm of mice (Markell et al., 1999). However, the public health significance of the higher prevalence in rats becomes apparent when the type of pet most often purchased for children is considered. According to pet store owners, mice are usually sold as feeder animals, but hamsters and

**Table 3. Summary of survey data on *Hymenolepis nana* in rodents from pet stores in southern Connecticut, U.S.A.**

Pet store location	Sample size	Store prevalence (%) <sup>*</sup>	Species prevalence, No. sampled % infected			Source of animals <sup>†</sup>		
			Mouse	Rat	Hamster	Mouse	Rat	Hamster
<b>Necropsy animals<sup>‡</sup></b>								
Hamden	20	5.0	10/0	5/20	5/0	I	I	I
Wallingford	17	29.4	7/14.3	5/80	5/0	II	II	II
Meriden	10	0	0/—	5/0	5/0	—	Varied	Varied
East Haven	17	5.8	10/10	2/0	5/0	II	II	In-house
North Branford	15	33.3	10/40	5/20#	0/—	III	III	—
Fairfield	21	42.9	10/70	5/40	6/0	Varied	Varied	Varied
Orange	13	46.2	7/42.9	1/0	5/60	II	II	II
Stratford	18	27.8	8/0	5/80	5/20	IV	IV	IV
Naugatuck (a)	18	0	10/0	5/0	3/0	Varied	Varied	Private
<b>Fecal sample<sup>  </sup></b>								
Naugatuck (b)	37	16.2	10/50	15/6.7	5/0	I	II	V
Seymore (a)	23	17.4¶	4/25	4/50#	14/0	Private	Private	Private
Seymore (b)	9	0	6/0	3/0	0/—	NA	NA	—

\* Overall, 75% of pet stores were selling infected animals.

† Different vendors are indicated by roman numeral (I, II, ...) rather than by name; NA = not available; In-house = animals bred at pet store; Private = animals from small local breeders or customers.

‡ Store prevalence calculation includes rats, mice, and hamsters.

|| Store prevalence calculation includes only rats, mice, hamsters, prairie dogs, and gerbils sampled during the 4-week sampling period.

¶ Store selling the infected prairie dog.

# *Hymenolepis diminuta* present.



rats are more often purchased as pets. Surprisingly, our results indicate that pet store rats constitute a more important reservoir for *H. nana* than do mice or hamsters. Also, this is the first report of *H. nana* from a pet prairie dog, a non-traditional or exotic animal that is becoming common in pet stores (Storer and Watson, 1997). Improper hygiene following handling of all rodents, including feeders, may lead to transmission. A pet ownership profile (Teclaw et al., 1992) showed that approximately 50% of households, primarily those with children between the ages of 6–17 years, owned some type of pet. Further, 2% of Florida AIDS patients interviewed owned pet rodents, but most health care providers failed to advise them of possible zoonoses from their companion animals (Conti et al., 1995).

Overall, 75% of surveyed pet stores were selling animals infected with *H. nana*. Despite the fact that *H. nana* infection in rodents is easily treatable with praziquantel (Harkness and Wagner, 1995), none of the pet stores reported practicing antihelminthic treatment and control measures. The combination of high prevalence and absence of control measures demonstrates that pet rodents pose a zoonotic threat to pet store personnel, animal care workers, and customers. Surveys of human populations, together with further epidemiological information, are needed to assess the extent to which this potential health threat is actually being realized.

#### Acknowledgments

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## NEW BOOK AVAILABLE

**Echinostomes as Experimental Models for Biological Research.** Edited by Bernard Fried and Thaddeus K. Graczyk. 2000. Kluwer Academic Publications, Dordrecht, The Netherlands. 284 pp. ISBN 0-7923-6156-3. Hardcover. US\$ 150.00/NLG 250.00/GBP 88.00. **Abstract:** “. . . Considerable but scattered literature has been published on the subject of echinostomes and a synthesis of this wide range of topics has now been achieved with the publication of this book, which represents a wide range of topics in experimental biology related to the use of echinostomes as laboratory models. It will have a special appeal to advanced undergraduates and graduate students in parasitology and should also appeal to professional parasitologists, physicians, veterinarians, wildlife disease biologists, and any biomedical scientists interested in new model systems for studies in experimental biology.”

## Seasonal Occurrence and Community Structure of Helminth Parasites from the Eastern American Toad, *Bufo americanus americanus*, from Southeastern Wisconsin, U.S.A.

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**ABSTRACT:** From April to September 1996, 47 American toads, *Bufo americanus americanus* Holbrook, were collected from Waukesha County, Wisconsin, U.S.A., and examined for helminth parasites. Forty-six (98%) of 47 toads were infected with 1 or more helminth species. The component community consisted of 6 species, 3 direct-life-cycle nematodes, and 3 indirect-life-cycle helminths (2 trematodes and 1 metacestode). Totals of 2,423 individual nematodes (92%), 45 trematodes (2%), and 155 cestodes (6%) were found, with infracommunities being dominated by skin-penetrating nematodes. A significant correlation existed between wet weight and overall helminth abundance, excluding larval platyhelminths. Helminth populations and communities were seasonally variable but did not show significant differences during the year. However, a number of species showed seasonal variations in location in the host, and these variations were related to recruitment period.

**KEY WORDS:** *Bufo americanus*, American toad, *Cosmocercoides variabilis*, *Rhabdias americanus*, *Oswaldocruzia pipiens*, *Mesocestoides* sp., *Gorgoderina* sp., Trematoda, Nematoda, Cestoda, echinostome metacercariae, seasonal study, Wisconsin, U.S.A.

American toads, *Bufo americanus americanus* Holbrook, 1836, are large, thick-bodied terrestrial anurans found in North America near marshes, oak savannas, semiopen coniferous and deciduous forests, and agricultural areas. They range from Labrador and Hudson Bay to eastern Manitoba, south to eastern Oklahoma and the coastal plains, and are distributed throughout Wisconsin (Vogt, 1981). Toads are active foragers, differing from most anurans that are sit-and-wait predators (Seale, 1987). Although a number of surveys and natural history studies on the helminths and ecology of toads exists (Bouchard, 1951; Odlaug, 1954; Ulmer, 1970; Ulmer and James, 1976; Williams and Taft, 1980; Coggins and Sajdak, 1982; Joy and Buntzen, 1997), no studies have used measures of helminth communities. Here we report on the helminth infracommunity and component community structure in American toads from southeastern Wisconsin.

### Materials and Methods

American toads were collected from April to November of 1996 by driving 4.0-km sections of highways N and 67 (42°54'N, 88°29'W) in Eagle, Wau-

kesha County, Wisconsin, U.S.A., during the night and collecting individuals as they crossed roads. Animals were placed in plastic containers, transported to the laboratory, stored at 4°C, and killed 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. Toads were individually toe-clipped and frozen. At necropsy, the digestive tracts, limbs, body wall musculature, and internal organs were examined for helminth parasites. Each organ was individually placed in a Petri dish and examined under a stereomicroscope. The body cavity was 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. Echinostome metacercariae were badly damaged during necropsy, and these were identified but not retained. Prevalence, mean intensity, and abundance are according to Bush et al. (1997). Mean helminth species richness is the sum of helminth species per individual amphibian, including noninfected individuals, divided by the total sample size. All values are reported as the mean  $\pm$  1 SD. Undigested stomach contents were identified to class or order following Borror et al. (1989). Stomach contents are reported as a percent = the number of arthropods in a given class or order, divided by the total number of arthropods recovered  $\times$  100. Voucher specimens have been deposited in the helminth collection of the H. W. Manter Laboratory (HWML), University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (accession numbers HWML

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**Table 1.** Prevalence, mean intensity, mean abundance, and total numbers of helminths found in 47 specimens of *Bufo americanus americanus*.

Species	Prevalence, No. (%)	Mean intensity $\pm$ 1 SD (range)	Mean abundance $\pm$ 1 SD	No. of worms recovered	Location
<b>Trematoda</b>					
<i>Echinostome metacercariae</i> *	3 (6.3)	13 $\pm$ 19 (1–35)	0.8 $\pm$ 5.1	39	Kidneys, body cavity
<i>Gorgoderina</i> sp.	1 (2.1)	6 $\pm$ 0 (6)	0.1 $\pm$ 0.9	6	Bladder
<b>Cestoda</b>					
<i>Mesocostoides</i> sp.*	6 (12.7)	25.8 $\pm$ 22 (12–70)	3.3 $\pm$ 11.3	155	Leg muscles
<b>Nematoda</b>					
<i>Oswaldocruzia pipiens</i>	41 (87)	8.5 $\pm$ 7 (1–31)	7.4 $\pm$ 7.1	349	Small intestine
<i>Cosmocercoides variabilis</i>	43 (91)	32.3 $\pm$ 31.5 (1–135)	29.6 $\pm$ 31.5	1,392	Lungs, body cavity, large and small intestine
<i>Rhabdias americanus</i>	43 (91)	15.8 $\pm$ 17.9 (1–75)	14.5 $\pm$ 17.7	682	Lungs, body cavity

\* Underestimate.

15051, male *Oswaldocruzia pipiens*; 15052, male *Cosmocercoides variabilis*; 15053, *Rhabdias americanus*; 15054, *Gorgoderina* sp.; 15055, *Mesocostoides* sp.).

The chi-square test for independence was calculated to compare differences in prevalence among host sex, seasonal differences in prevalence, and seasonal differences in location of nematodes in the host. Yates' adjustment for continuity was used when sample sizes were low, and a single-factor, independent-measures analysis of variance was used to compare among seasonal differences in mean intensity and mean helminth species richness (Sokal and Rohlf, 1981). 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 amphibian. Because WW gave a stronger correlation than SVL in each case, it is the only parameter reported. Because of low sample sizes during certain collection periods, data were pooled on a bimonthly basis to form samples of 15 to 16 toads per season. Larval platyhelminths were not included in the seasonal analysis, because they can accumulate throughout an amphibian's life.

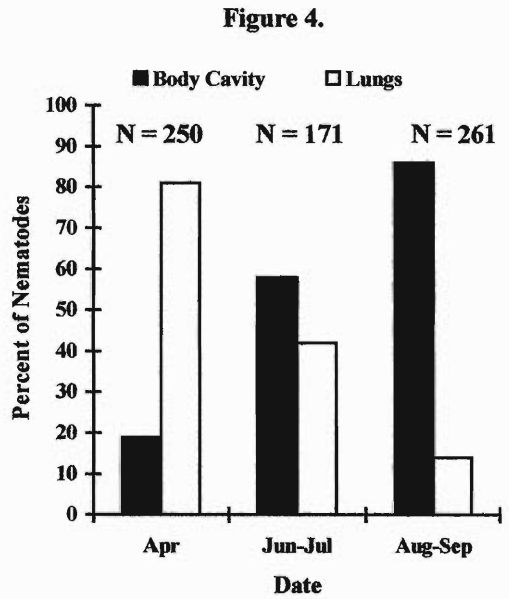
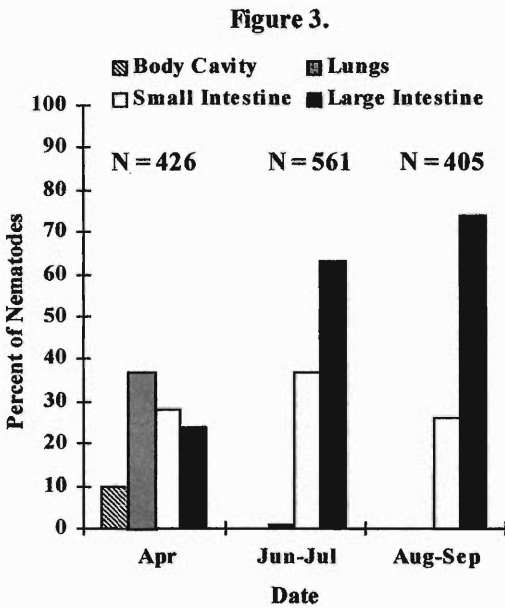
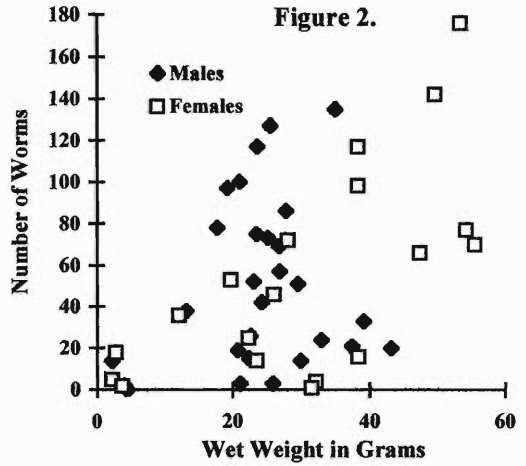
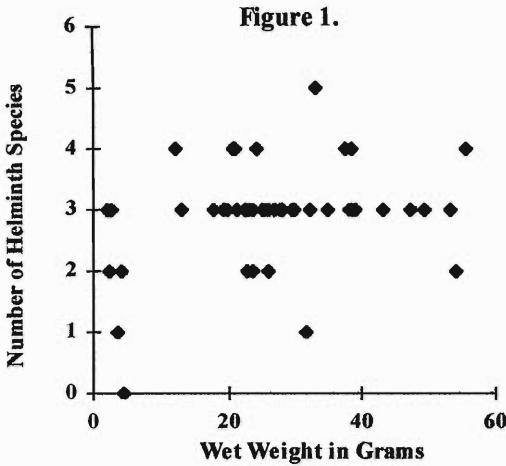
## Results

A total of 47 American toads, 28 males and 19 females, was collected. The overall mean SVL and WW of toads was 56.6  $\pm$  12.5 mm (range = 26.2–72.6 mm) and 26.6  $\pm$  13.5 g (range = 2.19–55.5 g), respectively. No significant difference existed in numbers of male toads and female toads collected throughout the

year ( $\chi^2 = 1.72$ ,  $P > 0.05$ ). Although female toads were larger (58.2  $\pm$  15.4 mm) and heavier (30.5  $\pm$  17.3 g) than males (55.5  $\pm$  10.2 mm, 23.9  $\pm$  9.7 g), these differences were not significant ( $t = 0.73$ ,  $P > 0.05$ ,  $t'_s = 1.50$ ,  $P > 0.05$ ). Stomach contents analyses revealed that the toads fed mostly on ants (98%), with beetles and other terrestrial arthropods representing a small portion of the diet (2%).

Forty-six (98%) of 47 toads were infected with 1 or more species of helminths. The component community consisted of 6 species, 3 direct-life-cycle nematodes, and 3 indirect-life-cycle helminths (2 trematodes and 1 metacestode). Overall mean helminth abundance, excluding larval platyhelminths, was 55.7  $\pm$  45.3 worms per toad infracommunity (range = 0–180). Prevalence was highest for nematodes, ranging from 91% for *Cosmocercoides variabilis* Harwood, 1930, and *Rhabdias americanus* Baker, 1978, to 87% for *Oswaldocruzia pipiens* Walton, 1929. Prevalence for indirect life cycle parasites was generally low, being highest for the cestode *Mesocostoides* sp. (12.7%) and lowest for *Gorgoderina* sp. (2.1%) (Table 1). No significant differences existed in prevalence between male and female toads for any of the 6 helminth species recovered. Mean intensity differed significantly only in *Mesocostoides* sp., being higher in male (32.7  $\pm$  32) than in female toads (19  $\pm$  4.6,  $t'_s = 4.70$ ,  $P < 0.05$ ).

Mean helminth species richness was 2.9  $\pm$  0.9



Figures 1-4. 1. Wet weight versus number of helminth species per individual of the American toad, *Bufo americanus americanus*,  $r = 0.31$ ,  $P < 0.05$ . 2. Wet weight versus total helminth abundance, excluding larval platyhelminths, in American toads, *B. a. americanus*, overall  $r = 0.47$ ,  $P < 0.01$ ; female toads  $r = 0.57$ ,  $P < 0.01$ ; and male toads  $r = 0.20$ ,  $P > 0.05$ . 3. Seasonal distribution of the relative proportions of *Cosmocercoides variabilis* recovered in the body cavity, lungs, small intestine, and large intestine of *B. a. americanus*.  $N$  equals number of nematodes recovered in each sampling period. 4. Seasonal distribution of the relative proportions of *Rhabdias americanus* recovered in the body cavity and lungs of *B. a. americanus*.  $N$  equals number of nematodes recovered in each sampling period.

species per toad. Infections with multiple species were common, with 0, 1, 2, 3, 4, and 5 species occurring in 1, 2, 6, 30, 7, and 1 host, respectively. No statistically significant differences in mean helminth species richness were found between male ( $3.0 \pm 0.86$ ) and female toads ( $2.8$

$\pm 0.85$ ,  $t = 0.83$ ,  $P > 0.05$ ). There was a significant positive correlation between WW and helminth species richness per toad ( $r = 0.31$ ,  $P < 0.05$ , Fig. 1). However, this relationship became insignificant when a single uninfected toad was removed ( $r = 0.20$ ,  $P > 0.05$ ). A significant

**Table 2.** Seasonal prevalence and mean intensity of 3 species of nematodes in *Bufo americanus americanus*.

Species		Apr	Jun–Jul	Aug–Sep	Statistic	P
<i>Cosmocercoides variabilis</i>	Prevalence	93% (14/15)	88% (14/16)	94% (15/16)	$\chi^2 = 0.5$	$P > 0.05$
	Mean intensity $\pm 1$ SD	30.4 $\pm$ 28.5	40 $\pm$ 31.7	27 $\pm$ 34.6	F = 0.65	$P > 0.05$
<i>Oswaldocruzia pipiens</i>	Prevalence	93% (14/15)	88% (14/16)	81% (13/16)	$\chi^2 = 1.1$	$P > 0.05$
	Mean intensity $\pm 1$ SD	7 $\pm$ 6.7	10 $\pm$ 8.1	8.3 $\pm$ 6.1	F = 0.63	$P > 0.05$
<i>Rhabdias americanus</i>	Prevalence	93% (14/15)	100% (16/16)	81% (13/16)	$\chi^2 = 3.7$	$P > 0.05$
	Mean intensity $\pm 1$ SD	17.8 $\pm$ 21.6	10.7 $\pm$ 11.2	20 $\pm$ 19.8	F = 1.12	$P > 0.05$

positive correlation existed between WW and overall helminth abundance, excluding larval platyhelminths ( $r = 0.47$ ,  $P < 0.01$ , Fig. 2), although this correlation was only significant for female toads ( $r = 0.57$ ,  $P < 0.01$ ) and not for males ( $r = 0.20$ ,  $P > 0.05$ ). Similar results were obtained for *C. variabilis* ( $r = 0.41$ ,  $P < 0.01$ ) and *O. pipiens* ( $r = 0.43$ ,  $P < 0.01$ ), whereas no significant correlation was observed for *R. americanus* ( $r = 0.27$ ,  $P > 0.05$ ). When these analyses were performed separately for male and female hosts, significant positive correlations occurred only in female toads (*C. variabilis*,  $r = 0.52$ ,  $P < 0.05$ ; *O. pipiens*,  $r = 0.58$ ,  $P < 0.01$ ; *R. americanus*,  $r = 0.54$ ,  $P < 0.05$ ).

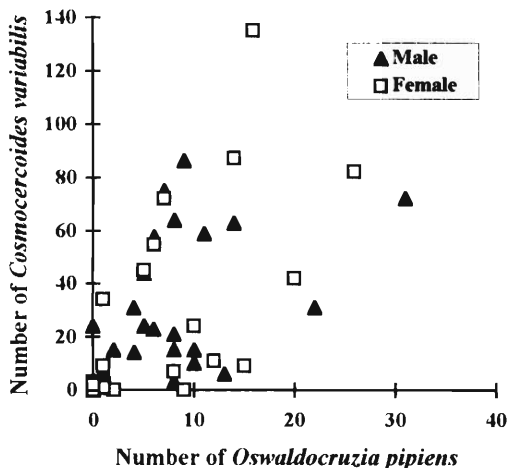
Only 1 male toad was infected with 6 *Gorogoderina* sp. during the early spring (April) collection. There was no significant seasonal difference in prevalence or mean intensity for any

of the nematodes recovered (Table 2). Prevalence was highest during early spring for *O. pipiens* (93%), early summer (June–July) for *R. americanus* (100%), and late summer–early fall (August–September) for *C. variabilis* (94%). Mean intensities were higher during early summer for *C. variabilis* and *O. pipiens* and in late summer–early fall for *R. americanus*. Seasonally, there was a significant difference in location of *C. variabilis* ( $\chi^2 = 556$ ,  $P < 0.01$ ), and *R. americanus* ( $\chi^2 = 232$ ,  $P < 0.01$ ). *Cosmocercoides variabilis* occurred in greater numbers in the body cavity and lungs during the early spring and in the small and large intestines during early summer and late summer–early fall collections (Fig. 3). Individuals of *Rhabdias americanus* were found primarily in the lungs during early spring and progressively increased in numbers in the body cavity during early summer and late summer–early fall collections (Fig. 4). The nematode *O. pipiens* showed no seasonal variation in location and was found in the small intestine throughout the year. *Oswaldocruzia pipiens* was significantly correlated with *C. variabilis* ( $r = 0.54$ ,  $P < 0.01$ ) in both male ( $r = 0.51$ ,  $P < 0.01$ ) and female toads ( $r = 0.58$ ,  $P < 0.01$ ) (Fig. 5) but was not significantly correlated with *R. americanus* ( $r = 0.23$ ,  $P > 0.05$ ) in either male ( $r = 0.23$ ,  $P > 0.05$ ) or female toads ( $r = 0.24$ ,  $P > 0.05$ ).

Mean species richness varied throughout the year, being highest ( $3.26 \pm 0.79$ ) in early spring, intermediate ( $2.88 \pm 0.71$ ) in early summer, and lowest during the late summer–early fall collection ( $2.62 \pm 0.95$ ), although these differences were not statistically significant ( $F = 2.3$ ,  $P > 0.05$ ). Wet weight of toads showed similar seasonal variability, but these differences were also not significant ( $F = 2.99$ ,  $P > 0.05$ ).

### Discussion

The component community of Wisconsin toads was similar to those of other published re-



**Figure 5.** Number of *Oswaldocruzia pipiens* versus *Cosmocercoides variabilis* in *Bufo americanus americanus*, overall  $r = 0.54$ ,  $P < 0.01$ ; female toads  $r = 0.58$ ,  $P < 0.01$ ; and male toads  $r = 0.51$ ,  $P < 0.01$ .

ports (Bouchard, 1951; Odlaug, 1954; Ulmer, 1970; Ulmer and James, 1976; Williams and Taft, 1980; Coggins and Sajdak, 1982; Joy and Bunten, 1997; Yoder, 1998). Toad helminth infracommunities were dominated by 3 species of skin-penetrating nematodes with high overall prevalence and mean intensities and with few toads infected by indirect-life-cycle parasites.

Bladder flukes of the genus *Gorgoderina* are common parasites of amphibians, but few life-cycle studies exist (Prudhoe and Bray, 1982). The life cycles of *Gorgoderina attenuata* Stafford, 1902, and *Gorgoderina vitelliloba* Olsson, 1876, have been determined. For these species, amphibians acquire infection by feeding on semiaquatic insect larvae or tadpoles, and the worms excyst in the stomach and migrate to the kidneys and bladder (Rankin, 1939; Smyth and Smyth, 1980). The low prevalence (2.1%) and intensity (6) of *Gorgoderina* sp. observed in our study were not surprising, since analyses of stomach contents revealed few kinds of arthropods. This is characteristic of actively foraging species such as toads. Ants made up the largest portion of the diet, with beetles and other terrestrial arthropods being found less frequently. Kirkland (1904) also found that ants and beetles made up the greatest portion of the diet of 149 toads from New England, U.S.A. These results are similar to other investigations on diet of species of *Bufo* (Toft, 1981; Collins, 1993; Indraneil and Martin, 1998), where ants and beetles appeared to be an important food item in the diet of toads. Toft (1981) reported that ants made up 64% to 91% of the arthropods consumed by 3 South American toad species, while Collins (1993) mentioned that ants and beetles were important items in the diet of 5 species of *Bufo* from Kansas, U.S.A. Because most trematodes of amphibians use aquatic or semiaquatic arthropods as intermediate hosts (Prudhoe and Bray, 1982), these observations may indicate why toads usually have low species richness and prevalence of adult trematodes (Williams and Taft, 1980; Coggins and Sajdak, 1982; McAllister et al., 1989; Goldberg and Bursey, 1991a, 1991b, 1996; Goldberg et al., 1995; Bursey and Goldberg, 1998).

The most commonly occurring nematode was *C. variabilis*, with a total of 1,392 worms recovered. Vanderburgh and Anderson (1987) studied the seasonal transmission of this species in American toads from Ontario, Canada. They

observed J<sub>4</sub> larvae in the lungs during the breeding season and adult worms in the rectum of toads throughout the year. They suggested that toads may acquire *C. variabilis* soon after emerging in the spring and that transmission may decline during summer and fall. However, they stated that this may have been an artifact of sampling, because all toads collected after the breeding season were from another location and may have had a lower prevalence and mean intensity of *C. variabilis*. Vanderburgh and Anderson (1987) also observed larvae in the lungs of 5 toads collected in October of the following year and concluded that transmission probably occurs throughout the year.

Data from the present study suggest that the breeding period may be important in transmission of *C. variabilis* in adult toads. All our toads were collected from the same general location and had high prevalence and mean intensities throughout the year. Although the differences were not significant, mean intensity increased after the breeding season and decreased during the late summer-early fall collection. Ten percent of the worms recovered during April were located in the body cavity, 37% were located in the lungs, and 28% and 24% were located in the small and large intestine, respectively. Subsequent sampling revealed that only 1 toad collected during June had 6 larvae in the lungs, with all other worms being recovered from the small and large intestine. Baker's (1978a) study on the life cycle of *Cosmocercoides dukae* Holl, 1928 (= *C. variabilis*) in toads revealed that 8–10 days are required for larvae to reach the lungs, and more than 30 days at 14–18°C to migrate to the rectum and develop to a gravid stage. Our observations suggest that toads became infected during the breeding season, and there appeared to be a decline during summer and early fall. Unfortunately, no toads were collected during October, and therefore it is not known if infection may occur during the fall (but see below). All adult female worms recovered throughout the year were gravid, indicating that eggs were being produced from April through September.

The second most frequently recovered nematode was *R. americanus*, primarily a parasite of toads (Baker, 1979a, 1987). Few studies exist on the seasonal occurrence of species of *Rhabdias* in amphibians (Lees, 1962; Plasota, 1969; Baker, 1979b). Lees (1962) studied the seasonal occur-



rence of *Rhabdias bufonis* Schrank, 1788, in its host, *Rana temporaria* Linnaeus, 1758, in England, and Baker (1979b) studied the seasonal occurrence of *Rhabdias ranae* Walton, 1929, in the wood frog, *Rana sylvatica* Le Conte, 1825, in Canada. Prevalence and intensities in these species were lowest during summer and highest in spring and early fall. Baker (1979b) observed many subadult worms in the body cavity of wood frogs during late summer and early fall, with no worms being found in the body cavity during early spring and few worms in the fall. Worms occurred in the lungs during early spring and fall, while few were found in the lungs during late summer and early fall. Baker (1979b) concluded that transmission of *R. ranae* occurs throughout the summer and early fall, with worms maturing in the lungs during the fall and overwintering in their hosts.

During this study, no significant differences in prevalence or mean intensity were observed throughout the collection period, although the location (lungs or body cavity) of worms varied during the year. Most *R. americanus* recovered in April occurred in the lungs, with numbers of worms in the lungs decreasing during early summer (June–July) and late summer–early fall (August–September). In contrast, the number of worms in the body cavity increased during the June–July and August–September collections. Therefore, transmission of this species occurred during the summer and early fall, with worms overwintering in their host. These results are consistent with earlier studies of seasonal distribution of other species of *Rhabdias* (Lees, 1962; Baker, 1979b).

*Oswaldocruzia pipiens* also had high prevalence but lower mean intensities than the other 2 species of nematodes recovered. Because of its fast migration, reaching the stomach and small intestine within 1 to 3 days of infection (Baker, 1978b), differences in location of these worms within the host were not observed. Prevalence and mean intensity were variable but not significant over the course of this study. Baker (1978b), in a seasonal study of *O. pipiens* in wood frogs, observed peak prevalence and intensity during spring (May–June) and early fall (September–October) in Ontario, Canada. He stated that worms overwintered in the host and that transmission occurred in early spring, with an initial decline during early summer and continued transmission during summer and early

fall. A significant positive correlation existed for this species and *C. variabilis* but not for *R. americanus*, which suggested that toads became infected with *O. pipiens* and *C. variabilis* during the same time and in the same places, while infection with *R. americanus* occurred at a later time during the summer. Hosts that contain different species of adult parasites that co-occur in an infracommunity may contaminate an area when they release eggs in their hosts' feces. Therefore, acquisition of a certain parasite species by a host may often be coupled with the acquisition of other species into the infracommunity. The spring breeding period may also be important in the recruitment of *O. pipiens* in toads at our study site. Because toads were not collected during October, it is not known if recruitment occurs during this time. If infection occurs during the fall, as Baker's (1978b) data for wood frogs imply, *C. variabilis* may also be acquired in the fall.

Significant, positive relationships between WW and species richness and abundance were observed in toad helminth communities. However, significant relationships between WW and abundance were observed only in female toads. Interestingly, the largest toads collected were females, and these had the highest intensities of helminths; therefore, they may provide a greater surface area for colonization by skin-penetrating nematodes. Similar observations were reported by McAlpine (1997) for female leopard frogs, which were significantly larger than males. Although species richness also showed a significant positive relationship with WW, once the single noninfected individual was excluded from the calculation, the relation became nonsignificant. Therefore, no conclusions can be drawn from this relationship. Seasonal variance in species richness was not significant in *B. a. americanus*, with toad infracommunities being dominated by 3 skin-penetrating nematodes throughout the year. The toad's terrestrial habitat and diet of ants and beetles may be important in excluding transmission of adult and larval trematodes, unlike other anurans such as semiaquatic species of *Rana*, which spend more time in an aquatic environment and have a broader diet of semiaquatic invertebrates (Muzzall, 1991; McAlpine, 1997; Bolek, 1998).

#### Acknowledgments

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## Meeting Notices

The **8<sup>th</sup> European Multicolloquium of Parasitology (EMOP-8)** will be held on 10–14 September, 2000 in Poznań, Poland. “Parasitology at the Turn of the Millenium” is the theme of the EMOP-8, and the program will consist of: **6 symposia** (Malaria in European Travelers; Congenital Toxoplasmosis; *Toxocara* and Toxocariasis; Tapeworm Zoonoses; Human and Animal Trematode Infections; and the Status of Food-Born Parasites at the Dawn of the Millenium), **8 scientific sessions** (Biology and Taxonomy; Molecular Biology; Immunology, Including Vaccines; Epidemiology and Control; Parasitic Infections and Diseases; Antiparasitic Drugs and Drug Resistance; The Parasites of Fishes and Other Hosts from the Aquatic Environment; and General Parasitology, including SNOPAD), and **5 technical workshops** (Microscopy Updated; New Methods in Serological Diagnosis of Parasitic Infections; Molecular Methods in Diagnosis of Parasitic Infections; Education in Parasitology on CD-ROM; and Parasitology on the Internet). **Contact information:** Organizing Committee (Prof. Z. S. Pawlowski and Prof. K. Boczoń), Department of Biology and Medical Parasitology, Karl Marcinkowski University of Medical Sciences, Fredry Street 10, 61-701 Poznań, Poland. Telephone/Fax (48-61) 852-71-92, e-mail: emop8@eucalyptus.usoms.poznan.pl. Internet: <http://www.emop8.am.poznan.pl>.

The **4<sup>th</sup> International Symposium on Monogenea** will be held on 9–13 July 2001 at the Women’s College of the University of Queensland, Brisbane, Queensland, Australia. A local committee has been established to organize details for the various scientific sessions, social events, excursions and accompanying persons program. Subject to sufficient demand, there will be a post-symposium workshop on Heron Island on the Great Barrier Reef. Calls for expressions of interest to attend the symposium, and details regarding registration, submission of abstracts, and a preliminary scientific program will be available later in 2000. **Contact information:** Dr. Ian D. Whittington and Dr. Leslie A. Chisolm, Department of Microbiology and Parasitology, The University of Queensland, Brisbane, Queensland 4072, Australia. Fax +61 7 3365 4620, e-mail: i.whittington@mailbox.uq.edu.au or l.chisolm@mailbox.uq.edu.au. For further information and notices see the Internet web page at: <http://www.biosci.uq.edu.au/micro/academic/ianw/ism4.htm>.

## Ecological Aspects of Endohelminths Parasitizing *Cichla monoculus* Spix, 1831 (Perciformes: Cichlidae) in the Paraná River near Porto Rico, State of Paraná, Brazil

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**ABSTRACT:** We examined 136 specimens of *Cichla monoculus* Spix, 1831, collected in the Paraná River near Porto Rico, State of Paraná, Brazil, from July 1996 through October 1997. Of the total number of fish, 133 (97.8%) were infected with at least 1 species of helminth. A total of 8 helminth species was recorded: 3 Digenea, *Clinostomum* sp., *Diplostomum* (*Austrodiplostomum*) *compactum* (Lutz, 1928), and *Diplostomum* sp.; 3 Cestoda, *Proteocephalus microscopicus* Woodland, 1935, *Proteocephalus macrophallus* (Diesing, 1850), and *Sciadocephalus megalodiscus* Diesing, 1850; 1 Nematoda, *Contracaecum* sp.; and 1 Acanthocephala, *Quadrigyrus machadoi* Fábio, 1983. *Proteocephalus microscopicus* and *P. macrophallus* showed the highest values of prevalence and intensity of infection, followed by *Contracaecum* sp. In the endoparasite community of *C. monoculus*, the cestodes are both dominant and codominant species. The typical pattern of overdispersion or aggregation was observed for *P. microscopicus*, *P. macrophallus*, *S. megalodiscus*, *Q. machadoi*, and *Contracaecum* sp. Prevalence and total host length were positively correlated in fish parasitized by *P. microscopicus*, *P. macrophallus*, and *S. megalodiscus*. Infection intensity and host length were positively correlated only for *P. microscopicus*. There were significant differences in the prevalence of *P. macrophallus* and *Q. machadoi* in males and females of *C. monoculus*. *Clinostomum* sp., *Diplostomum* sp., *D. (A.) compactum*, and *Q. machadoi* were found for the first time in *C. monoculus*.

**KEY WORDS:** ecology, endohelminths, Digenea, Cestoda, Nematoda, Acanthocephala, freshwater fish, tucunaré, *Cichla monoculus*, Cichlidae, Teleostei, Paraná River, Brazil.

Of the main factors influencing the composition of endoparasite communities, the feeding habits of the hosts are most important, since diverse animals that serve as intermediate hosts for the hosts' parasites are found in their diets (Dogiel, 1970). Changes in the diet and feeding habits of fishes also influence the composition of their parasite fauna (Dogiel, 1970) and account for the differences in the parasite faunas of young and adults (Burn, 1980; Scott, 1982; Moser and Hsieh, 1992). It is also well understood that the fluctuations in water level characteristic of floodplains may modify the feeding habits of fish because of changes in the quantity and quality of available food (Junk, 1980; Lowe-McConnell, 1987; Brasil-Sato and Pavanelli, 1999). The influence of the sex of the hosts is another important factor responsible for the variation in the composition of their parasitofauna and may be related to behavioral, biological, and physiological differences between male and fe-

male fish (Paling, 1965; Muzzall, 1980; Fernández, 1985; Moser and Hsieh, 1992; Takemoto et al., 1996; Machado et al., 1994). The number of studies in Brazil on the ecology of helminth parasites of fish, especially in floodplain environments, is still small.

The tucunaré, *Cichla monoculus* Spix, 1831, the object of this investigation, is an important commercial and sport fish in the Upper Paraná River. It is a native of the Amazon Basin and was first recorded in the Paraná River in 1986 (Agostinho et al., 1994). It is a predator (Lowe-McConnell, 1969), considered piscivorous because of the predominance of fish in its diet (FUEM/CIAMB/PADCT, 1995) and carnivorous because it eats shrimp (Fontenele and Peixoto, 1979; Géry, 1984; Bonetto and Castello, 1985) and benthopelagic fish (Ortega and Vari, 1986).

This study was intended to extend our existing knowledge of the helminth fauna parasitizing the fishes of the Paraná River floodplain, to show the structure and diversity of the endoparasitic infrapopulations of the tucunaré, and to

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**Table 1. Prevalence (P%), mean intensity of infection (MII), mean abundance (MA), range of variation (Rx), and sites of infection of the endohelminths of 136 specimens of *Cichla monoculus* collected in Pau Véo Bayou near Porto Rico, state of Paraná, Brazil, from July 1996 through October 1997.\***

Parasite	Ni	Np	P (%)	MI I ± SD	MA ± SD	Rx	Site of infection
<b>Digenea</b>							
<i>Clinostomum</i> sp. (M)	2	18	1.5	9.0 ± 9.9	0.13 ± 1.4	2–16	Branchial cavity and stomach
<i>D. (A.) compactum</i> (M)	7	19	5.2	2.7 ± 1.7	0.1 ± 0.7	1–5	Vitreous humor (eye)
<i>Diplostomum</i> sp. (M)	12	16	8.8	1.3 ± 0.5	0.1 ± 0.4	1–2	Vitreous humor (eye)
<b>Cestoda</b>							
<i>Proteocephalus microscopicus</i> (A)	128	36,863	94.1	288.0 ± 793.0	271.1 ± 772.1	1–8,594	Stomach and intestine
<i>Proteocephalus macrophallus</i> (A)	61	1,121	44.9	18.4 ± 74.6	8.2 ± 50.6	1–573	Stomach and intestine
<i>Sciadocephalus megalodiscus</i> (A)	18	154	13.2	8.6 ± 11.0	1.1 ± 4.9	1–42	Stomach and intestine
<b>Nematoda</b>							
<i>Contracaecum</i> sp. (L)	96	1,034	70.6	10.8 ± 32.1	7.6 ± 27.3	1–309	Mesentery
<b>Acanthocephala</b>							
<i>Quadrigyrus machadoi</i> (L)	30	76	22.1	2.5 ± 2.0	0.6 ± 1.4	1–7	Mesentery (encysted L); stomach and intestine (free L)

\* Ni = number of infected fish; Np = number of parasites; M = metacercaria; A = adults; L = larvae.

analyze the possible influences of sex and length of the hosts on these infrapopulations.

### Materials and Methods

The fish were collected monthly in Pau Véo Bayou on Mutum Island in the floodplain of the Upper Paraná River, state of Paraná, Brazil (22°45'00"S, 53°16'50"W) from July 1996 through October 1997. After capture and identification, the fish were measured, weighed, and sexed. They were eviscerated, and the visceral cavity, eyes, digestive tract and associated organs, kidney, urinary and reproductive tracts, and gonads were removed. The organs were separated and placed in Petri dishes containing 0.65% physiological solution and examined individually with a stereomicroscope. The digenetic trematodes were compressed between slides and/or coverglasses and fixed in cold AFA. The cestodes and nematodes were fixed in warm formol. The acanthocephalans were killed in distilled water in Petri dishes under refrigeration and fixed unpressed in AFA. All of the worms were preserved in 70% alcohol. The digenetic trematodes, cestodes, and acanthocephalans were stained with acetic carmine or Delafield's hematoxylin. All of the worms were dehydrated in a graded ethanol series, cleared with beechwood creosote, and mounted in Canada balsam. For identification of the parasites, the following works were used: Diesing (1850), LaRue (1914), Woodland (1933, 1935), Yamaguti (1963), Freze (1965), Travassos et al. (1969), Schmidt and Huggins (1973), Moravec (1994), Rego (1994), Takemoto and Pavanelli (1996), Sholtz et al. (1996), and Silva-Souza (1998).

Helminths were deposited in the Helminthological

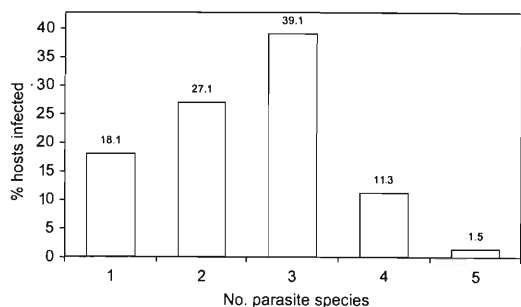
Collection of the Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, state of Rio de Janeiro, Brazil, under the following accession numbers: *Clinostomum* sp. 34235, *Diplostomum (Austrodiplostomum) compactum* 34233, *Diplostomum* sp. 34232, *Proteocephalus microscopicus* 34234, *Proteocephalus macrophallus* 34230, *S. megalodiscus* 33951, 33952, and 33953 a-c, *Contracaecum* sp. 34231, and *Quadrigyrus machadoi* 34236.

Parasite diversity was evaluated by the Shannon diversity index ( $H'$ ). The possible variation in parasite diversity was analyzed in relation to sex of the hosts by Student's  $t$ -test, and in relation to the total length of the hosts by the Spearman rank correlation coefficient ( $r_s$ ) (Ludwig and Reynolds, 1988). The importance value ( $I$ ) proposed by Bush, according to Thul et al. (1985), was used to classify the parasite community components. Species in the larval stage were not considered in this classification. The dispersion index was used to determine the distribution of the infrapopulation in the sample. The degree of overdispersion or aggregation was calculated using Green's index (Ludwig and Reynolds, 1988). These tests were applied only to the endohelminth species present at prevalences higher than 10%. The correlation between total host length and the intensity of infection of the parasite species was evaluated by Spearman rank correlation coefficient ( $r_s$ ) (Zar, 1996). The existence of a correlation between total host length and prevalence of infection was tested using Pearson's correlation coefficient ( $r$ ) (9 length classes between 13.1 and 49 cm were established) after angular transformation of the prevalence data ( $\arcsin\sqrt{x}$ ) (Zar, 1996). Student's  $t$ -test was used to compare the total lengths of male and

**Table 2. Monthly values of prevalence (P%) and mean intensity of infection (MII) of the endohelminths of 136 specimens of *Cichla monoculus* collected in Pau Véio Bayou near Porto Rico, state of Paraná, Brazil, from July 1996 through October 1997 (N = number of fish examined).**

Month	N	<i>Clinostomum</i> sp.		<i>Diplostomum</i> (A.) <i>compactum</i>		<i>Diplostomum</i> sp.		<i>Proteocephalus</i> <i>microscopicus</i>		<i>Proteocephalus</i> <i>macrophallus</i>		<i>Sciadocephalus</i> <i>megalodiscus</i>		<i>Contracaecum</i> sp.		<i>Quadrigyus</i> <i>machadoi</i>	
		P(%)	MII	P(%)	MII	P(%)	MII	P(%)	MII	P(%)	MII	P(%)	MII	P(%)	MII	P(%)	MII
1996																	
Jul	8	12.5	16.0	—	—	25.0	1.5	87.5	195.6	50.0	4.8	62.5	17.0	50.0	5.5	25.0	3
Aug	2	—	—	—	—	50.0	2.0	50.0	37.0	50.0	2.0	—	—	100.0	7	50.0	1
Sep	2	—	—	—	—	—	—	100.0	42.0	100.0	1.0	—	—	50.0	16	—	—
Oct	7	—	—	—	—	42.9	1.3	100.0	448.9	71.4	12.8	57.1	7.5	100.0	4	14.3	1
Nov	5	—	—	—	—	20.0	1.0	80.0	2,562.5	80.0	179.5	—	—	60.0	1.7	—	—
Dec	3	—	—	—	—	—	—	100.0	240.0	33.3	53.0	33.3	1.0	33.3	10	—	—
1997																	
Jan	2	—	—	100.0	3.5	—	—	100.0	1,074.0	100.0	4.0	—	—	50.0	3	100.0	3
Feb	32	—	—	3.1	1.0	—	—	84.4	1.0	12.5	1.0	6.3	2.0	59.4	3.9	25.0	0.3
Mar	24	4.2	2.0	8.3	2.5	8.3	1.5	100.0	293.8	66.7	9.8	8.3	1.0	66.7	3.6	20.8	1
Apr	13	—	—	7.7	5.0	15.4	1.0	100.0	335.7	46.2	6.0	7.7	1.0	100.0	6.9	30.8	3.5
May	10	—	—	10.0	1.0	—	—	100.0	180.2	40.0	1.8	—	—	70.0	10.3	10.0	5
Jun	8	—	—	—	—	12.5	1.0	100.0	98.4	37.5	3.3	—	—	100.0	49.1	12.5	2
Jul	7	—	—	—	—	—	—	100.0	131.3	42.9	1.0	—	—	85.7	25.2	14.3	1
Aug	9	—	—	—	—	—	—	100.0	112.4	22.2	2.5	—	—	44.4	10.5	33.3	2
Sep	1	—	—	—	—	—	—	100.0	134.0	100.0	1.0	—	—	100.0	18	—	—
Oct	3	—	—	—	—	—	—	100.0	531.7	100.0	12.0	—	—	100.0	13	33.3	1





**Figure 1.** Parasite richness in 136 specimens of *Cichla monoculus* collected in Pau Véo Bayou near Porto Rico, state of Paraná, Brazil from July 1996 through October 1997.

female hosts. The effect of sex of the host on the prevalence of each parasite species was evaluated by the log-likelihood G test using a  $2 \times 2$  contingency table (Zar, 1996), and the intensities of infection of each species of parasite in the male and female hosts were compared using the Mann-Whitney *U*-test (Siegel, 1975). In the data analysis, only values with significance levels of  $P \leq 0.05$  were considered significant. Ecological terms are based on Bush et al. (1997).

### Results

Of the 136 hosts examined, 133 (97.8%) were parasitized by 1 or more species of endohelminth, of which 39,301 specimens were collected. The endohelminths included 3 species of digeneans (*Clinostomum* sp., *Diplostomum* (*Austrodiplostomum*) *compactum* (Lutz, 1928), and *Diplostomum* sp.); 3 species of cestodes (*Proteocephalus microscopicus* Woodland, 1935, *Proteocephalus macrophallus* (Diesing, 1850), and *Sciaodocephalus megalodiscus* Diesing, 1850); 1 species of nematode (*Contracaecum* sp.); and 1 species of acanthocephalan (*Quadrigyrus machadoi* Fabio, 1983) that were found free in the intestine or encysted in the mesentery in the same stage of development (Tables 1 and 2). Parasite richness varied from 1 to 5 species, and 52 fish were infected by 3 species of parasites (Fig. 1).

The cestodes were the most frequently encountered parasites, corresponding to 97% of the helminths collected, and were present in 130 hosts. *Proteocephalus microscopicus* showed the highest percentages of parasitism, followed by *Contracaecum* sp. (Table 1). *Proteocephalus microscopicus* also was the species that presented the highest monthly values of prevalence and mean intensity (Table 2). The acanthocephalan *Q. machadoi* made up 0.2% of the parasite spec-

**Table 3.** Classification and Bush's Importance values (I) of the species of endohelminth parasites of 136 specimens of *Cichla monoculus* collected in Pau Véo Bayou near Porto Rico, state of Paraná, Brazil from July 1996 through October 1997.

Parasite	I
Dominant species	
<i>Proteocephalus microscopicus</i>	98.50
<i>Proteocephalus macrophallus</i>	1.40
Co-dominant species	
<i>Sciaodocephalus megalodiscus</i>	0.06

imens collected, while the digenetic trematodes represented 0.14%.

The mean parasite diversity according to the Shannon index ( $H'$ ) was 0.1329 (SD = 0.1879), and the maximum diversity was 1.3716. Parasite diversity did not differ significantly between male and female hosts ( $t = 0.6004$ ,  $P = 0.5492$ ), and was not correlated with total length of the hosts ( $r_s = 0.03124$ ,  $P = 0.7180$ ). Total host length varied from 13.5 to 45.7 cm (mean 25.1 cm).

Using the importance value (I) proposed by Bush, 2 species were classified as dominants and 1 as co-dominant (Table 3). The parasites of *C. monoculus* showed the typical pattern of overdispersion or aggregation of the parasite populations. *Proteocephalus macrophallus* and *S. megalodiscus* showed the highest values of Green's index of aggregation (Table 4). There was no significant difference in length between the 61 male and 75 female tucunarés examined ( $t = 0.6130$ ,  $P = 0.5409$ ).

There was a positive correlation between total length of the hosts and prevalence for fish parasitized by *P. microscopicus*, *P. macrophallus*, and *S. megalodiscus*. A positive correlation be-

**Table 4.** Dispersion Index (DI) and Green's Index of Aggregation (GI) for the endohelminth parasite species of 136 specimens of *Cichla monoculus* collected in Pau Véo Bayou near Porto Rico, state of Paraná, Brazil from July 1996 through October 1997.

Parasite	DI	GI
<i>Proteocephalus microscopicus</i>	2,198.96	0.059
<i>Proteocephalus macrophallus</i>	312.23	0.278
<i>Sciaodocephalus megalodiscus</i>	21.83	0.136
<i>Quadrigyrus machadoi</i>	3.27	0.030
<i>Contracaecum</i> sp.	98.06	0.094

**Table 5.** Values of Spearman's rank correlation coefficients (rs) and Pearson's correlation coefficients (r), to evaluate the relationship between intensity and prevalence of infection, respectively, of the endo-helminth fauna with the total length of 136 specimens of *Cichla monoculus* collected in Pau Véio Bayou near Porto Rico, state of Paraná, Brazil from July 1996 through October 1997.

Parasite	rs	P*	r	P
<i>Proteocephalus microscopicus</i>	0.3596	$P < 0.0001$	0.7501	$P = 0.0199$
<i>Proteocephalus macrophallus</i>	0.2175	$P = 0.0922$	0.9048	$P = 0.0008$
<i>Sciadocephalus megalodiscus</i>	0.1943	$P = 0.4397$	0.7603	$P = 0.0174$
<i>Contracaecum</i> sp.	-0.0755	$P = 0.4648$	0.2509	$P = 0.5149$
<i>Quadrigrurus machadoi</i>	-0.2954	$P = 0.1130$	0.5697	$P = 0.1093$

\* Level of significance.

tween the total length of the hosts and the intensity of parasite infection was seen only for *P. microscopicus* (Table 5).

In the cestode *P. macrophallus* and the acanthocephalan *Q. machadoi*, prevalence and intensity of infection were influenced by sex of the host (Table 6). For *P. macrophallus* these indices were higher in the males, and for *Q. machadoi* in the females (Table 6). In addition to these species, *S. megalodiscus* showed a significant difference in intensity of infection according to sex of the host, with higher intensity in males (Tables 6 and 7).

### Discussion

In the tucunaré, the proteocephalid cestodes *P. microscopicus* and *P. macrophallus* showed the highest values of mean intensity of infection. This is explainable by the feeding habits of the tucunaré, which includes in its diet fish species that act as intermediate or paratenic hosts of these parasites. The cestodes found in the present work appear to be exclusive to the tucunaré, never having been recorded in other fish (Rego, 1994).

For the acanthocephalans, the main factor regulating the prevalence and intensity of the par-

asitoses is also predation by the fish on the intermediate or paratenic hosts (Amin and Burrows, 1977). In the specimens of *C. monoculus* studied, only larvae of *Q. machadoi* in the same stage of development were found free in the intestine or encysted in the mesentery, which may indicate that these fish are intermediate or paratenic hosts of this parasite. The natural predators of tucunaré in the study region are carnivorous fish or piscivorous birds. The small number of fish infected by *Clinostomum* sp., together with the fact that the worms were found free in the stomach, may indicate that they were ingested accidentally with prey.

Only 2 individuals of *C. monoculus* showed the maximum parasite richness found, i.e., 5 species of endoparasites. Most of the population was parasitized by 3 species of helminths, of which *P. microscopicus* was always present. Holmes (1990) pointed out that parasite richness is higher in fishes of intermediate trophic levels, since they harbor both adult and larval stages of parasites.

The relationship between body length or age of the host and parasite diversity is based on the process of temporal accumulation and on the increase in the dimensions of the sites of infection

**Table 6.** Results of the log-likelihood test (G) to compare prevalence between males and females and of the Mann-Whitney U-test to compare intensity of infection between males and females of 136 specimens of *Cichla monoculus* collected in Pau Véio Bayou near Porto Rico, state of Paraná, Brazil from July 1996 through October 1997.

Parasite	G	P	Z	P
<i>Proteocephalus microscopicus</i>	1.067	$0.5 > P > 0.25$	0.16	$P > 0.25$
<i>Proteocephalus macrophallus</i>	5.321	$0.025 > P > 0.01$	7.32	$P < 0.0005$
<i>Sciadocephalus megalodiscus</i>	2.210	$0.25 > P > 0.10$	3.18	$0.01 > P > 0.005$
<i>Contracaecum</i> sp.	0.111	$0.75 > P > 0.50$	1.05	$0.25 > P > 0.10$
<i>Quadrigrurus machadoi</i>	5.410	$0.025 > P > 0.01$	5.71	$P < 0.005$

P = level of significance; Z = value of normal approximation of U-test.

**Table 7. Prevalence and mean intensity of infection by helminth parasites of *Cichla monoculus* collected in Pau Véio Bayou near Porto Rico, state of Paraná, Brazil from July 1996 through October 1997.\***

Parasite	Nm	Nf	Nmi	Nfi	Pm (%)	Pf (%)	Mim	Mif
<b>Digenea</b>								
<i>Clinostomum</i> sp.	61	75	0	2	0.0	2.7	—	9.0 ± 9.9
<i>Diplostomum</i> (A.) <i>compactum</i>	61	75	2	5	3.3	6.7	4.0 ± 0.0	2.2 ± 1.8
<i>Diplostomum</i> sp.	61	75	6	6	9.8	8.0	1.2 ± 0.4	1.5 ± 0.6
<b>Cestoda</b>								
<i>Proteocephalus microscopicus</i>	61	75	56	72	91.8	96.0	434.7 ± 1,160.2	173.9 ± 227.6
<i>Proteocephalus macrophallus</i>	61	75	34	27	55.7	36.0	26.4 ± 99.0	8.4 ± 15.3
<i>Sciadocephalus megalodiscus</i>	61	75	11	7	18.0	9.3	9.3 ± 12.0	7.4 ± 10.0
<b>Nematoda</b>								
<i>Contracaecum</i> sp.	61	75	44	52	70.5	70.7	14.5 ± 46.4	7.7 ± 8.8
<b>Acanthocephala</b>								
<i>Quadrigrurus machadoi</i>	61	75	8	22	13.1	29.3	2.0 ± 1.1	2.7 ± 2.2

\* Nm = number of males examined; Nf = number of females examined; Nmi = number of males infected; Nfi = number of females infected; Pm and Pf = prevalence of males and females, respectively; MIm and MIf = mean intensity of infection of males and females, respectively.

as a function of growth (Luque et al., 1996). Such a relationship has been shown not to exist in other species of freshwater fishes (Adams, 1986; Janovy and Hardin, 1988; Machado et al., 1996). The lack of this relationship in the tucunaré may indicate a homogeneity in their feeding habits during ontogenetic development.

The independence of diversity in relation to the sex of *C. monoculus* may constitute evidence that the occupation of the habitat and the diet are similar in males and females. Adams (1986), Janovy and Hardin (1988), and Machado et al. (1996) obtained similar results for other species of freshwater fishes.

The cestodes *P. microscopicus*, *P. macrophallus*, and *S. megalodiscus* must be considered as basic components of the parasite community of *C. monoculus*. The first 2 species were classified as dominants, and the third as codominant (Thul et al., 1985). The dominant and codominant species of endohelminths showed a pattern of spatial aggregation, in agreement with the typical pattern of endoparasitism demonstrated by other investigators (Skorping, 1981; Janovy and Hardin, 1987; Oliva et al., 1990; Takemoto, 1993; Machado et al., 1996).

The positive correlation observed between the total length of the hosts and the prevalence of *P. microscopicus*, *P. macrophallus*, and *S. megalodiscus* indicates the occurrence of a cumulative process. A positive correlation between the standard length of hosts and the prevalence

and/or intensity of infection was observed also by Conneely and McCarthy (1986), Machado et al. (1994), and Takemoto and Pavanelli (1994); the latter 2 investigations were also carried out in the Paraná River.

Esch et al. (1988) pointed out that the sex of the hosts may also be one of the factors that influence levels of parasitism. The influence of physiological factors (hormones, mucosity) was demonstrated by Paling (1965) and Moser and Hsieh (1992), who hypothesized that certain species of parasites possess a greater facility to infect male or female hosts. Muzzall (1980) and Takemoto and Pavanelli (1994) found no influence of the sex of the host on the parasite fauna, showing that the ecological relationships (behavior, habitat, and diet) of males and females are similar. In *C. monoculus*, the sex influences the prevalence and intensity of infection of *P. macrophallus* and *Q. machadoi* and only the intensity of *S. megalodiscus*. This can be explained by the fact that some species of fish become more susceptible in the breeding season because of physiological and behavioral changes.

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## Prevalence of Hookworms (*Uncinaria lucasi* Stiles) in Northern Fur Seal (*Callorhinus ursinus* Linnaeus) Pups on St. Paul Island, Alaska, U.S.A.: 1986–1999

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**ABSTRACT:** Prevalence of hookworms (*Uncinaria lucasi*) was studied in northern fur seal (*Callorhinus ursinus*) pups at necropsy on St. Paul Island, Alaska, U.S.A. Gross examination of 2,121 pups during the period 1986–1998 revealed that only 13 had a light hookworm infection and 2 other pups had hookworm disease (moderate hookworm infection, enteritis, and anemia). Specific parasitologic inspections of 230 pups in 1988 and 1996–1999 included examinations of feces for eggs or intestines for adult worms. Hookworm eggs were present in fecal samples of 1 (2%) of 48 pups in 1988 and none of 19 pups in 1996. Specimens of *U. lucasi*, found mostly during qualitative examinations, were in the intestines of 4 (5%) of 77 pups in 1997, 4 (9%) of 47 in 1998, and 1 (3%) of 39 in 1999. The 9 infected pups harbored 1–8 hookworms each. Observations in this study indicate a dramatic decline in hookworm prevalence in *C. ursinus* pups on St. Paul Island compared with that of several years previously.

**KEY WORDS:** *Uncinaria lucasi*, adult hookworms, prevalence, northern fur seal pups, *Callorhinus ursinus*, St. Paul Island, Alaska, U.S.A.

*Uncinaria lucasi* Stiles, 1901, and *Uncinaria hamiltoni* Baylis, 1933, are the only 2 species of hookworms described from pinnipeds (Baylis, 1933, 1947; Stiles, 1901). However, there are types with measurements intermediate between *U. lucasi* and *U. hamiltoni* (Baylis, 1933; Dailey and Hill, 1970). Specimens of *Uncinaria* are common in otariids (eared seals) and rare in phocids (earless or true seals) (George-Nascimento et al., 1992). Classification of hookworms in pinnipeds remains uncertain. George-Nascimento et al. (1992) considered all hookworms in otariids to be the same species, *U. lucasi*. In the present paper, the hookworms are designated as *U. lucasi* because they were first described and named from northern fur seals (*Callorhinus ursinus* Linnaeus, 1758). This research was done to compare the current prevalence of adult *U. lucasi* in northern fur seal pups on St. Paul Island, Alaska, U.S.A., with prevalences found in earlier studies.

### Materials and Methods

Dead fur seal pups were collected from 2 rookeries, Northeast Point and Reef, on St. Paul Island, Alaska (57°09'N, 170°13'W), for pathologic studies, from

1986 to 1999. Some of these pups were selected for specific research on hookworms. The pups were gathered daily from early July through the first 2 weeks of August of each year. Pups selected had not been dead for more than about 24 hr. Collection was by a person working on a catwalk over a rookery and using a 5-m-long pole equipped with a noose. Pup carcasses recovered from rookeries were taken directly to the research laboratory on St. Paul Island for necropsy.

Gross examination of 2,121 dead pups, for the overall period from 1986 to 1998, included observations for hookworms in the opened ileocecal area. About 95% of the pups were from Northeast Point and 5% from Reef rookeries. The infections were separated into 2 categories: 1) light hookworm infections, when only a few parasites were noted, and 2) hookworm disease, defined as moderate hookworm infection, when a number of parasites, enteritis, and anemia were evident.

Special parasitologic examinations of 230 pups were conducted. Fecal samples were obtained from the colons of 48 pups in 1988 and 19 pups in 1996, placed in glass vials or plastic bags containing 5% formalin, and examined for hookworm eggs (Lyons et al., 1976). Determinations (Lyons, 1963; Olsen and Lyons, 1962, 1965) for presence of adult hookworms in the intestines were made for 77 pups in 1997, for 47 in 1998, and for 39 in 1999. Parts of the intestines examined were 1) approximately 60–100 mm of the ileum, the entire cecum, and about 45–60 mm of the proximal end of the colon for all 77 pups in 1997; 2) up to about 300 mm of the ileum, the entire cecum, and approximately 150 mm of the colon, including the proximal end, for 36 pups, and the entire intestinal tract for 11

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**Table 1.** Summary of results of gross examination of the ileocecal area of the intestines of northern fur seal pups ( $n = 2,121$ ) for hookworm (*Uncinaria lucasi*) infection and hookworm disease at necropsy from 1986 through 1998 on St. Paul Island, Alaska, U.S.A.

Year	No. of pups		
	Examined	With light hookworm infection	With hookworm disease*
1986	39	0	0
1987	90	5	0
1988	91	1	0
1989	113	2	1
1990	364	0	0
1991	248	1	0
1992	227	2	0
1993	141	0	0
1994	251	0	0
1995	130	0	0
1996	172	0	1
1997	165	1	0
1998	90	1	0
Total	2,121	13	2

\* Moderate hookworm infection, enteritis, and anemia.

pups in 1998; and 3) the entire intestinal tract for 39 pups in 1999.

### Results

Results of gross examination of the entire sample of 2,121 pups at necropsy from 1986 to 1998 are summarized in Table 1. Only 13 pups had light hookworm infections, and 2 other pups were considered to have hookworm disease.

Examinations of fecal samples revealed hookworm eggs in only 1 (2%) of 48 pups in 1988 and none of 19 pups in 1996. Prevalence of *U. lucasi*, based on recovery of adult specimens in the intestines of pups at necropsy, was <10% (Table 2). Only 4 (5%) of 77 pups in 1997, 4 (9%) of 47 in 1998, and 1 (3%) of 39 in 1999 were positive for *U. lucasi*. The overall prevalence of hookworms for these 163 pups was 6%. There were 1–8 hookworms per infected pup. Examination of the complete intestinal tracts of 50 of the 163 pups indicated that 2 (4%) were infected with 1 hookworm each. Representative voucher specimens of 1 male and 1 female of the *U. lucasi* recovered were deposited in the University of California, Davis Nematode Collection (UCDNC) as UCDNC accession number 3665.

**Table 2.** Results of examination of intestines\* of northern fur seal pups at necropsy on St. Paul Island, Alaska, U.S.A., for the hookworm, *Uncinaria lucasi*, in 1997, 1998, and 1999.

Year	No. of pups		No. (mean) of hookworms/infected pups		
	Examined	Infected (%)	Male	Female	Total
1997	77	4 (5)	0.50	0.75	1.25
1998	47	4 (9)	0.75	2.00	2.75
1999	39	1 (3)	1.00	0.00	1.00
Total	163	9 (6)	0.67	1.38	1.89

\* Ileocecal areas for 113 pups and complete intestinal tracts for 50 pups.

### Discussion

In this study, prevalence of *U. lucasi* in northern fur seal pups on St. Paul Island was low. This was determined mostly from gross examination of the ileocecal area of the pups' intestines. The observed prevalence might have been higher if the entire small and large intestine had been scrutinized for every pup. However, Olsen (1958) demonstrated that *U. lucasi* concentrate in the ileum, cecum, and proximal colon; these areas were examined in all fur seal pups in the present study.

Current rates of infection of adult *U. lucasi* in northern fur seal pups appear to be much lower than those found in several previous surveys on St. Paul Island 40–100 years ago (1897, 1952, 1953, 1955, 1960) (Table 3). Data from the 1897 study (Lucas, 1899) apparently were based on deaths of pups attributed to *U. lucasi* and are not comparable to the later surveys for presence or absence of these parasites (Olsen, 1954, 1956, 1958; Lyons and Olsen, 1960). However, the study by Lucas is an excellent indicator of prevalence in the late 19th century. In the interim period (1950s and 1960s), overall prevalence for 873 pups was 69%; variation was 18–92% for the rookeries investigated. More recently, the best indication of the infection rate of *U. lucasi* on St. Paul Island was in 1977, when hookworm eggs were found in feces of 27 (90%) of 30 live pups on Northeast Point (Lyons et al., 1978).

In the early observations by Lucas (1899) and later by Olsen (1958), hookworm prevalence was higher in pups on rookeries with sandy versus rocky terrain. Sandy areas are considered more conducive for development, survival, and transmission of hookworm eggs/free-living third-stage larvae ( $L_3$ ) than more solid rocky ter-

**Table 3. Prevalence of *Uncinaria lucasi* in intestines of northern fur seal pups at necropsy in some previous surveys on St. Paul Island, Alaska, U.S.A.**

Reference	Year of study	Rookery	No. of pups	
			Examined	Infected (%)
Lucas (1899)*	1897	Gorbach	33	15 (45)
		Kitovi	17	7 (41)
		Lagoon	4	0 (0)
		Lukanin	12	7 (58)
		Northeast Point	10	7 (70)
		Polovina	10	6 (60)
		Reef	57	12 (21)
		Tolstoi	109	52 (48)
		Zapadni	<u>93</u>	<u>38 (41)</u>
		Total	345	144 (42)
Olsen (1954)	1952	Unknown	42	38 (90)
Olsen (1954)	1953	Polovina	26	24 (92)
Olsen (1956, 1958)	1955	Kitovi	28	5 (18)
		Polovina	164	120 (73)
		Reef	4	13 (27)
		Tolstoi	100	73 (73)
		Vostochni†	112	89 (79)
		Zapadni	<u>100</u>	<u>67 (67)</u>
	Total	553	367 (66)	
Lyons and Olsen (1960)	1960	Little Polovina	30	20 (67)
		Polovina	112	86 (77)
		Reef	63	35 (56)
		Vostochni†	30	22 (73)
		Zapadni	<u>17</u>	<u>11 (65)</u>
		Total	252	174 (69)

\* These data apparently are for causes of death of pups due to *U. lucasi* rather than actual prevalence.

† A rookery on Northeast Point.

rain. The ground where fur seals now breed on the 2 rookeries (Northeast Point and Reef) in the present study is generally rocky. Previously, when populations of fur seals were much higher, breeding animals were more widely dispersed to include sandy surfaces on these rookeries (E.T.L., personal observation). Possibly the decline in numbers of animals breeding on sandy areas has contributed to the dramatic decrease in prevalence of *U. lucasi*.

Table 4 summarizes literature on prevalences of adult *U. lucasi* in northern fur seal pups in some localities other than St. Paul Island. On the Commander Islands (Bering Island and Medny Island), Russia, and the Channel Islands (San Miguel Island), California, U.S.A., the hookworm prevalence is currently high, based on examinations of relatively small numbers of dead pups.

No definite cause has been determined for the spectacular decline of hookworm infections in northern fur seal pups on St. Paul Island. Per-

haps it is related to one or more unknown factors in combination with a corresponding decline in the herd. Numbers of fur seals in the 20th century peaked in the 1950s and 1960s and began to decline in the 1970s (Trites, 1992). Estimated size of the fur seal population on the Pribilof Islands (St. Paul Island and St. George Island) was about 1.5 million in the 1960s (Baker, 1957; Riley, 1961). This population is now estimated at about 973,000 (York et al., 2000). The overall population decreased about 40% in the last 40 years, but the number of pups born declined much more; i.e., about 60% or from about 500,000 to 200,000 (York et al., 2000).

Although it is known that parasitic third-stage larvae ( $L_3$ ) of *U. lucasi* can live for many years in the tissues of northern fur seals, there is no definite information on the time period or number of lactations for clearance of these larvae through the mammary system. In experimental infections of the nematode *Strongyloides ransomi* Schwartz and Alicata, 1930, in pigs, colos-

**Table 4.** Prevalence of *Uncinaria lucasi* in intestines of northern fur seal pups at necropsy in some studies in Russia and in California, U.S.A.

Location	Reference	Year of study	No. of pups	
			Examined	Infected (%)
Russia	Kolevatova et al. (1978)	1997		
Commander Islands				
Bering Island				
Northern rookery			30	6 (20)
Northwestern rookery			199	147 (74)
Medny Island				
Southeastern rookery			11	0 (0)
Urillie rookery			11	7 (64)
Bering Island	Mizuno (1997)	1995		
Unknown rookery			26	22 (85)
California, U.S.A.	Lyons et al. (1997)	1996		
Channel Islands				
San Miguel Island				
West Cove rookery			1	0 (0)
Adams Cove rookery			24	24 (100)

trum samples were collected for 4 consecutive lactations from 6 individual sows (Stewart et al., 1976). There was an exponential decline of parasitic  $L_3$  of *S. ransomi* with each lactation. The mean number of larvae/ml of colostrum was 1.1 for the first lactation and 0.06 for the fourth lactation.

Several aspects of the life cycle of *U. lucasi*, as studied in northern fur seals on St. Paul Island (Lyons, 1994), may have a role in the decline in prevalence: 1) the only source of adult hookworms in pups is from parasitic  $L_3$  passed in the mother's milk for <24 hr; 2) female fur seals are, in effect, intermediate hosts; 3) only pups have adult hookworms, and therefore the only source of free-living  $L_3$  on rookeries is from eggs passed in pups' feces; 4) pups lose intestinal adult hookworm infections by a few (<3) months of age; 5) free-living  $L_3$  on the rookery enter fur seals (percutaneously and orally) and do not mature, even in pups, but locate as parasitic  $L_3$  in tissues, especially the ventral abdominal area (predominantly blubber); 6) free-living  $L_3$  definitely enter pups, but it is unknown if they enter older fur seals; 7) parasitic  $L_3$  can live for many years (>6) in tissues of fur seals; 8) parasitic  $L_3$  are confined to tissues except in periparturient females, from which some of them exit in the "first milk"; 9) fur seal cows may live for >20 yr and therefore parasitic  $L_3$  can potentially be passed in milk for years; and 10)

all parasitic  $L_3$  in tissues are not passed in milk in a single lactation. Population dynamics of the fur seal hosts also have a bearing on the infections with *U. lucasi*. Some of these include 1) a decrease of the breeding fur seal population means that eventually there will be a corresponding decrease in hookworms; 2) if female fur seals have low numbers of parasitic  $L_3$  in tissues as a result of depletion or minimal acquisition of free-living  $L_3$ , there is an increasingly smaller chance of transmission of parasitic  $L_3$  to their pups; 3) the successful life cycle of *U. lucasi* is dependent on the presence of sufficiently high numbers of parasitic  $L_3$  in tissues of female fur seals and adult hookworms in pups to supply enough free-living  $L_3$  on the rookery to maintain the cycle; and 4) even if the breeding population of fur seals is decreased, reproductive groups are concentrated, which should favor hookworm transmission; however, pups (and older individuals) roam around on the rookery and when populations of fur seals and hookworms were higher, there was a much greater chance of acquisition of free-living  $L_3$  from the rookery.

In a large population of mammals, exclusive transmammmary transmission of parasites seems to be an ideal manner of infection. In such a situation, offspring will almost certainly be infected with larval stages when they nurse. Also, perpetuation of the parasites is assured. How-

ever, at the present time on St. Paul Island, hookworm infections and numbers of infected fur seals are so low that these parasites appear to be at a minimal or subminimal level for continued existence. If older fur seals had infections of adult hookworms contributing eggs to the environment, continued success of the hookworms presumably would be more likely. Possibly the highly evolved, exclusive manner of transmammalian transmission of *U. lucasi* has become a detriment for the species under the present conditions on St. Paul Island.

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## Life History of *Spiroxys hanzaki* Hasegawa, Miyata, et Doi, 1998 (Nematoda: Gnathostomatidae)

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**ABSTRACT:** The life history of *Spiroxys hanzaki* Hasegawa, Miyata, et Doi, 1998 (Nematoda: Gnathostomatidae), a stomach parasite of the Japanese giant salamander, *Andrias japonicus* (Temminck, 1836) (Caudata: Cryptobranchidae), was studied. The eggs developed in water to liberate sheathed second-stage larvae with a cephalic hook. They were ingested by the cyclopoid copepods, *Mesocyclops dissimilis* Defaye et Kawabata, 1993, and *Macrocyclus albidus* (Jurine, 1820) and developed to infective third-stage larvae in the hemocoel. Natural infections with third-stage larvae were also found in the cobitid loaches, *Misgurnus anguillicaudatus* (Cantor, 1842) and *Cobitis biwae* (Jordan et Snyder, 1901). The largest third-stage larva from *A. japonicus* had almost the same body size as the smallest immature adult.

**KEY WORDS:** *Spiroxys hanzaki*, Nematoda, Gnathostomatidae, life history, *Andrias japonicus*, Japanese giant salamander, Caudata, Cryptobranchidae, Copepoda, *Mesocyclops*, *Macrocyclus*, Japan.

The Japanese giant salamander, *Andrias japonicus* (Temminck, 1836) (Cryptobranchidae), is an endangered amphibian distributed only in West Japan and protected by Japanese national law. From this salamander, a new nematode, *Spiroxys hanzaki* Hasegawa, Miyata, et Doi, 1998 (Gnathostomatidae), was described recently (Hasegawa et al., 1998). Although it was suggested that the salamander acquired the infection by ingesting freshwater fish harboring the infective stage of *S. hanzaki* (Hasegawa et al., 1998), there is insufficient evidence for this. Recently, viable eggs of *S. hanzaki* were unexpectedly available, allowing attempts to experimentally infect copepods as intermediate hosts. In addition, freshwater fish captured in the rivers where the giant salamanders live were examined for larvae of *S. hanzaki*. The larval stages were also compared with those observed in the definitive host. We present herein the results of these observations, with a discussion on the developmental stages of gnathostomatoid nematodes.

### Materials and Methods

#### Experiments on embryonic and larval development

On 4 July 1998, 1 *A. japonicus* reared in the Suma Aqualife Park, Kobe, Hyogo Prefecture, Japan, vomited a half-digested loach, *Misgurnus anguillicaudatus* Cantor, 1842, that had been given on the previous day as food. Many individuals of *S. hanzaki* at various de-

velopmental stages were found invading the skin, muscles, and viscera of the loach. The loach was kept at 4°C and transported to the Department of Biology, Oita Medical University, for further examination. On arrival (6 July 1998), all the worms were still alive. Eggs were obtained by tearing the uteri of 2 gravid females. Meanwhile, the remaining worms were fixed with 70% ethanol at 70°C for routine morphological examination or were stored at -25°C for future biochemical analysis.

The eggs were incubated in distilled water in a Petri dish (9 cm in diameter) at 15°C for 11 days, and then the temperature was raised to 20°C to facilitate hatching. When larvae hatched, 1 or 2 were transferred by a capillary pipette to each of several small Petri dishes (3 or 4 cm in diameter) containing about 5 ml of pond water. Copepods were collected in a nearby pond or paddy with a plankton net and were introduced to the dishes containing *S. hanzaki* larvae. Each copepod was observed daily thereafter under a stereomicroscope to examine the development of *S. hanzaki* larvae inside the body. Identification of copepods was based on Ueda et al. (1996, 1997).

Some newly hatched larvae were fixed by slight heating to observe their morphology. Infected copepods were dissected in physiological saline at various days of infection, and recovered larvae were killed by slight heating or by placing them in 70% ethanol at 70°C. Heat-killed larvae were examined immediately, whereas those fixed in 70% ethanol were cleared in glycerol-alcohol solution by evaporating the alcohol, mounted on a glass slide with 50% glycerol aqueous solution, and observed under a Nikon Optiphot microscope equipped with a Nomarski differential interference apparatus. Measurements are in micrometers unless otherwise stated.

#### Larvae parasitic in naturally infected fish

Between May and November 1998, the following fish were netted in the Hatsuka River and the Okuyama

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River, Kobe, Hyogo Prefecture, Japan, and were examined for larvae of *Spiroxys*: from the Hatsuka River: 47 *Zacco temmincki* (Temminck et Schlegel, 1846) (Cyprinidae) (body length 37–137 mm); 2 *Moroko juyi* (Jordan et Snyder, 1901) (Cyprinidae) (54–58 mm); 2 *Pungtungia herzi* Herzenstein, 1892 (Cyprinidae) (90–95 mm); 4 *Misgurnus anguillicaudatus* (Cantor, 1842) (Cobitidae) (46–80 mm); 13 *Cobitis biwae* Jordan et Snyder, 1901 (Cobitidae) (38–95 mm); 4 *Rhinogobius flumineus* (Mizuno, 1960) (Gobiidae) (33–45 mm); and 1 *Odontobutis obscura* (Temminck et Schlegel, 1845–1846) (Gobiidae) (96 mm); from the Okuyama River: 10 *Z. temmincki* (41–75 mm). Their viscera were pressed between 2 thick glass plates and observed under a stereomicroscope with transillumination to detect *Spiroxys* larvae. The remaining portions of the fish were minced and digested with artificial gastric fluid for 3 hr at 37°C. The residues were transferred to a Petri dish and examined for nematode larvae under a stereomicroscope. Larvae detected were processed as described above for morphological observation. Scientific names of the fishes follow those adopted by Masuda et al. (1984).

The third-stage larvae of *Spiroxys japonica* Morishita, 1926, from the Asian pond loach, *M. anguillicaudatus*, and frogs, *Rana nigromaculata* Hallowell, 1860, and *Rana rugosa* Schlegel, 1838, captured in Niigata and Akita Prefectures, northeastern Japan, where *A. japonicus* does not occur, were examined for comparison.

Voucher nematode specimens were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A., Nos. 89629–89638.

## Results

### Embryonic development

When the culture started, the nematode eggs contained 1- to 4-cell-stage embryos. After 2 days of culture, they developed to 16-cell to morula stage. On days 7 and 8 of culture, tadpole-stage embryos were seen. On day 10, first-stage larvae showed movement within the eggshell, and some larvae began to molt to become second stage. On day 11, molted larvae were observed. On day 18, eggs began to hatch (Fig. 1), and hatched second-stage larvae were still enclosed in a sheath, adhered by the tips of their tails to the bottom of the culture dish. They seldom swam in the water.

**MORPHOLOGY OF HATCHED SECOND-STAGE LARVAE ( $n = 4$ ):** Stumpy worm with tapered posterior portion (Fig. 1). Enclosed within double-layered sheath: outer layer lacking striations, and inner layer with reticular markings (Figs. 2, 3). Length 330–435, maximum width 25–32. Anterior end with dorsal sclerotized hooklet with elongated base (Fig. 2). Esophagus 118–173 long, widened posteriorly and narrowed at

level of nerve ring. Nerve ring 65–85 from anterior extremity. Intestinal wall with brown granules. Excretory pore, genital primordium, and anus indiscernible.

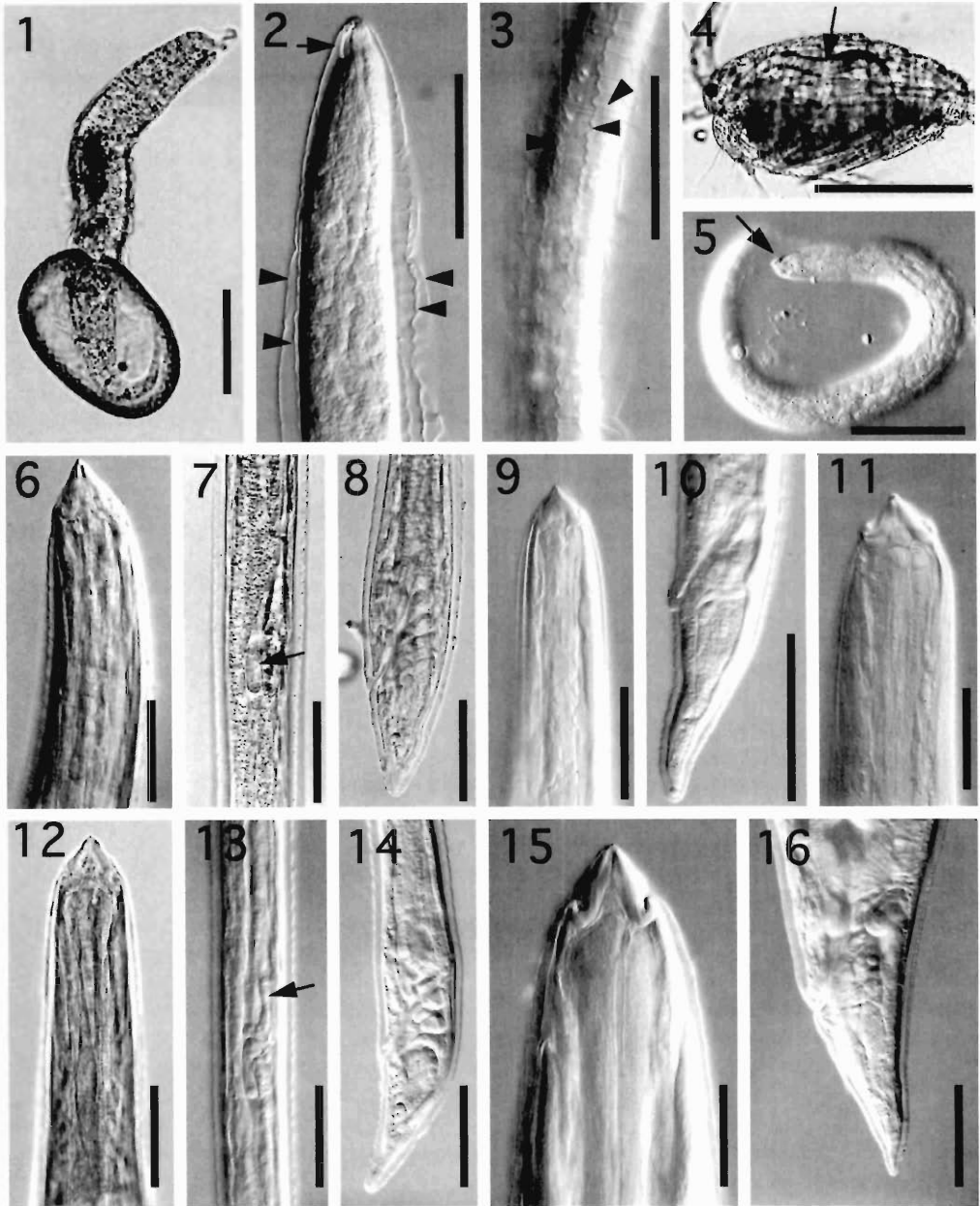
### Development in intermediate host

Several species of copepods were used for experimental infection. Preliminary trials revealed that *Mesocyclops dissimilis* Defaye et Kawabata, 1993, *Macrocyclus albidus* (Jurine, 1820), and 3 species of unidentified cyclopoids readily ingested the hatched larvae, but infection was established only in the former 2 species. The other species could not tolerate the infection and soon died. Hence, the following results were based on the experiments using *M. dissimilis* and *M. albidus* as intermediate hosts.

After being ingested by the copepods, the larvae soon migrated to the hemocoel of the host (Fig. 4). The sheath was not observed in the larvae that had migrated to the hemocoel. Among 31 *M. dissimilis* challenged, 15 were found to ingest the larvae, whereas worm uptake was not confirmed in the remaining individuals. The larvae disappeared from the hemocoel of 3 *M. dissimilis* by day 7 after infection. The copepods harboring *S. hanzaki* larvae became emaciated, 5 of them died by day 10, and 6 more died by day 20. The larvae recovered by dissecting these dead copepods showed little development, still possessing the cephalic hooklet (Fig. 5). In 1 *M. dissimilis*, disseminated fatal infection with unidentified flagellates was caused after migration of *S. hanzaki* larvae. Ultimately, only 1 *M. dissimilis* survived for more than 25 days. When dissected on the 35th day of infection, this copepod harbored 1 living third-stage larva and 1 dead second-stage larva.

Among 10 *M. albidus* challenged, only 2 were found to harbor the larvae in the hemocoel on day 2 after infection, but 1 of them died by day 10. The remaining individual died on day 24, but 1 third-stage larva was recovered from it by dissection. The control copepods, 36 *M. dissimilis* and 10 *M. albidus*, were not observed to be infected with any nematode throughout the experiment.

**MORPHOLOGY OF THE SECOND-STAGE LARVAE COLLECTED FROM THE INFECTED COPEPODS:** Identical with that of the hatched larvae but lacking sheaths; size gradually increased as the duration of infection lengthened. On day 8 after infection, length 313–333, maximum width



Figures 1–3. Second-stage larva of *Spiroxys hanzaki*. 1. Hatching larva (scale bar = 50  $\mu$ m). 2. Anterior portion, lateral view, showing cephalic hooklet (arrow) and double-layered cuticle (darts) (scale bar = 25  $\mu$ m). 3. Inner layer of cuticle showing reticulated nature (darts) (scale bar = 25  $\mu$ m).

Figure 4. *Spiroxys hanzaki* larva (arrow) in the hemocoel of *Mesocyclops dissimilis* on day 1 after exposure (scale bar = 200  $\mu$ m).

Figure 5. Second-stage larva collected from the hemocoel of *M. dissimilis* at 15 days after infection. Arrow indicates cephalic hooklet (scale bar = 50  $\mu$ m).

Figures 6–8. Third-stage larvae collected from the infected copepod, lateral view (scale bars = 50  $\mu$ m). 6. Anterior portion. 7. Genital primordium (arrow). 8. Posterior portion.

Figures 9–10. Third-stage larva of *S. hanzaki* in naturally infected sand loach, *Cobitis biwae*, lateral view (scale bars = 50  $\mu$ m). 9. Anterior portion. 10. Posterior portion.

**Table 1.** Measurements of third-stage larvae of *Spiroxys hanzaki* collected from experimentally-infected copepods, naturally infected fish, and salamanders (measurements in micrometers unless stated otherwise).

	<i>Mesocyclops dissimilis</i> and <i>Macrocyclops albidus</i>	<i>Misgurnus anguillicaudatus</i> and <i>Cobitis biwae</i>	<i>Andrias japonicus</i>	<i>Andrias japonicus</i>
No. measured	2	3	2	5*
Body length, mm	1.39–1.80	1.33–2.01	1.76–2.00	6.70–9.00
Maximum width	61–80	46–56	56–90	208–286
Nerve ring†	140–198	118–144	176–143	384–455
Excretory pore†	175–219	149–205	214–190	462–539
Deirids†	220–296	226–304	293–296	666–813
Esophagus length, mm	0.50–0.70	0.41–0.58	0.57–0.65	1.83–2.16
Esophagus width	32–34	28–38	28–32	78–102
Genital primordium, mm‡	0.46–0.47	0.43–0.77	0.54–0.65	2.36–3.02
Tail length	45–56	56–69	54–64	150–183

\* Advanced third-stage larvae.

† Distance from cephalic extremity.

‡ Distance from caudal extremity.

18–19 at posterior esophagus level, nerve ring 69–72 from anterior extremity and esophagus 115–143 long ( $n = 2$ ). On day 15, length 345, maximum width 18, nerve ring 75 from anterior extremity and esophagus 148 long ( $n = 1$ ).

**MORPHOLOGY OF THE THIRD-STAGE LARVAE COLLECTED FROM THE INFECTED COPEPODS:** Body slender. Cuticle with fine transverse striations. Lateral alae absent. Anterior extremity with lateral pseudolabia with trilobed internal sclerotized structure of which dorsal and ventral lobes much smaller than median lobe, directed anteriorly (Figs. 6, 17). Large submedian papillae and amphidial pore present (Fig. 17). Esophagus club-shaped. Intestinal wall densely packed with brown granules. Genital primordium with elongated 2 branches extending anteriorly and posteriorly (Fig. 7). Tail conical, with prominent phasmidial pores and blunt extremity (Fig. 8). Measurements are presented in Table 1.

#### Natural infection of fish with *Spiroxys* larva

A total of 83 individuals of 7 fish species belonging to 3 families was examined during the

period from May to November 1998. Only 1 *M. anguillicaudatus* and 2 sand loach, *Cobitis biwae* (Jordan et Snyder, 1901), were found to be infected each with 1 larva of *Spiroxys*. Two of the larvae were found encysted on the stomach wall and liver surface, whereas the remaining larva was recovered by artificial digestion. The morphology was identical with that of the larvae recovered from the experimentally infected copepods (Figs. 9, 10, 18). Measurements are also comparable with those of the third-stage larvae from the experimentally infected copepods as shown in Table 1.

The third-stage larva of *S. hanzaki* is readily distinguished from that of *S. japonica*, because the latter has inwardly curved dorsal and ventral lobes of the internal sclerotized structure in the pseudolabium (Figs. 11, 19).

#### Morphology of *S. hanzaki* larvae and immature adults vomited from *A. japonicus*

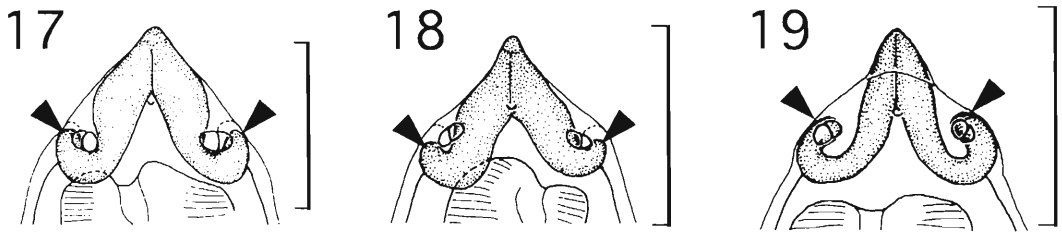
**THIRD-STAGE LARVAE** (Figs. 12–14): Morphology comparable with those from the exper-

←

**Figure 11.** Third-stage larva of *Spiroxys japonica* collected from pond loach, *Misgurnus anguillicaudatus* from Hachiro-gata, Akita Prefecture, Japan, lateral view, showing inwardly bent dorsal and ventral lobes of the sclerotized structure in pseudolabium (scale bar = 50  $\mu$ m).

**Figures 12–14.** Smallest third-stage larva of *S. hanzaki* collected from *Andrias japonicus*, lateral view (scale bars = 50  $\mu$ m). 12. Anterior portion. 13. Genital primordium (arrow). 14. Posterior portion.

**Figures 15, 16.** Advanced third-stage larva vomited by *A. japonicus*, lateral view (scale bars = 50  $\mu$ m). 15. Anterior extremity. 16. Posterior extremity.



Figures 17–19. Cephalic extremities of third-stage larvae of *Spiroxys* spp., lateral view (scale bars = 25  $\mu$ m). 17, 18. *Spiroxys hanzaki* collected from experimentally infected copepod, *Mesocyclops dissimilis* (17), and naturally infected sand loach, *Cobitis biwae* (18). 19. *Spiroxys japonica* collected from naturally infected *Rana rugosa* (tadpole) in Akita, Akita Prefecture, Japan.

imentally infected copepods or naturally infected fish. Measurements are stated in Table 1.

ADVANCED THIRD-STAGE LARVAE (Figs. 15, 16): Morphology identical with that of the third-stage larvae described above but with much larger body (Table 1).

IMMATURE ADULTS: Morphology identical with that of mature adults described in Hasegawa et al. (1998), but much smaller in size: males 10.2–13.3 mm long ( $n = 5$ ) and females 10.2–15.5 mm long ( $n = 5$ ).

### Discussion

Although only a few third-stage larvae were recovered from the experimentally infected copepods, it is apparent that *S. hanzaki* utilizes copepods as its intermediate host, like most gnathostomatoids for which life histories have been elucidated (cf. Anderson, 1992). Compared with *Spiroxys contortus* (Rudolphi, 1819) and *S. japonica* (cf. Hedrick, 1935; Hasegawa and Otsuru, 1978), *S. hanzaki* shows some different features in the life history. Hatched larvae are much larger (175–294 long in *S. contortus*, and 148–207 long in *S. japonica*). The hatched larva attaches at the bottom, like that of *S. contortus*, whereas the larva of *S. japonica* often swims in the water. Moreover, the period to attain the third stage in the copepods is much longer (10 to 14 days and 6 to 8 days in *S. contortus* and *S. japonica*, respectively).

The large size of the hatched larva and the slow development may be responsible for the high mortality rate of the infected copepods. Penetration of such a large larva through the alimentary canal wall of the copepod may result in perforation, through which pathogenic organisms could easily invade the hemocoel, as shown by the disseminated infection with flagellates in

the present experiment. Meanwhile, it is also probable that the larva in the hemocoel would stimulate some defense mechanism of the copepods to eliminate the invader, because the larvae often died without further development.

The worm size and morphology of the third-stage larvae from the copepods are similar to those of the smallest third-stage larva found in the salamander. This suggests that the third-stage larva developed in copepods could be infective to the final host. However, most of the gnathostomatoids require the second intermediate or paratenic hosts, often fish, in which the third-stage larva becomes the so-called advanced third stage, that shows significant gain in body size but without essential morphological change (cf. Anderson, 1992). A similar pattern was also postulated for *S. hanzaki* (Hasegawa et al., 1998). Because the low tolerance of the copepods to the infection in our experiments prevented experimental infection of fish with raised larva, it remains unknown whether the larvae grow to the advanced third stage in fish.

In most parasitic nematodes, the fourth stage exists between the infective third stage and fifth (adult) stage. However, the presence of the fourth-stage larva in *Spiroxys* is doubtful. Hedrick (1935) stated that the third and fourth molts of *S. contortus* were observed in the definitive host turtles but did not present the morphology of the fourth stage. In the life history study of *S. japonica*, Hasegawa and Otsuru (1978) could not find any larva that was distinguishable morphologically from the third-stage larva in the experimentally infected definitive host, frogs. Berry (1985) described the larvae of *Spiroxys chelodinae* Berry, 1985, collected from the stomach ulcers of Australian chelonians, as fourth stage, but the morphology resembles that of third-stage

larvae of *S. contortus* or *S. japonica*. In the present study, the largest third-stage larva from the salamander had nearly the same body size as that of the smallest immature adult. These facts suggest that *Spiroxys* lacks a fourth larval stage.

The presence of the fourth larval stage is also unclear for other gnathostomatoids. In *Gnathostoma* spp., there has been no description of the fourth-stage larva. In the life history study of *Gnathostoma procyonis* Chandler, 1942, Ash (1962) termed the larva of which a cross-section was presented as the fourth stage in the figure caption. However, he did not use this term in the text or describe a stage morphologically different from both the third stage and the adult stage. Moreover, we recently observed that *Gnathostoma doloresi* Tubangui, 1925, larvae in molting to the adult stage had the cuticle with typical arrangement of cephalic hooklets of the third stage (specimens courtesy of Dr. J. Imai). Further careful study is required to determine whether gnathostomatoids molt only once in the definitive host.

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## Inducible Nitric Oxide Synthase in the Muscles of *Trichinella* sp.– Infected Mice Treated with Glucocorticoid Methylprednisolone

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**ABSTRACT:** The dynamics of inducible nitric oxide synthase (iNOS) activity in mice infected with *Trichinella spiralis* larvae were followed between the first and tenth week postinfection (p.i.). During infection with *T. spiralis*, a bimodal stimulation of iNOS activity to 371% of the control value by day 21 p.i. and to 285% by day 70 p.i. was observed. The first increase in iNOS activity was abolished by glucocorticoid treatment. In *T. pseudospiralis* infection, the dynamics of iNOS stimulation differed from that in mice infected with *T. spiralis*: a constant but much weaker stimulation of iNOS starting on day 21 p.i. lasted until the end of the study. The results suggest that nitric oxide synthase activity is induced in muscle of the mouse during trichinellosis and that nitric oxide may participate in the host's biochemical defense mechanism.

**KEY WORDS:** iNOS, inducible nitric oxide, *Trichinella spiralis*, *Trichinella pseudospiralis*, muscle, mouse, glucocorticoid treatment, methylprednisolone.

The past decade has witnessed an increase in the number of papers devoted to the role of nitric oxide (NO) synthase in the pathogenesis of many diseases. Part of this surge in interest is related to the discovery of a role in both signal transduction and cell toxicity for NO. Induction of inducible nitric oxide synthase (iNOS) has been observed in the course of many human diseases. The parasitic infections investigated until now include malaria (Tsuji et al., 1995); leishmaniasis (Stenger et al., 1996); and toxoplasmosis (Holscher et al., 1998). The role of NO in killing protozoans of the genus *Leishmania* was studied in greater detail as early as 1993 (Callahan et al., 1993), when it was established that the course of the disease is dependent to a considerable extent on the type of lymphokines generated by T lymphocytes. During infection with such protozoans as *Trypanosoma cruzi* Chagas, 1909 (Rottenberg et al., 1996), or *Toxoplasma gondii* Nicolle et Manceaux, 1908 (Hayashi et al., 1996), NO has both antiparasitic and immunosuppressive effects. Recent publications have also reported modulation of the expression of messenger RNA responsible for tumor necrosis factor- and prostaglandin E<sub>2</sub>-independent synthesis of iNOS and production of NO in *Entamoeba histolytica* Schaudinn, 1903, infection (Wang et al., 1994). The type of free radicals contributing to pathogenesis in specific parasitic invasion depends on the developmental stage of

the parasite, and the protective function of NO seems to be tissue-specific (Scharton-Kersten et al., 1997).

Nitric oxide generated by nitrogen free radicals (RNI), specifically one generated in inflammatory conditions by the inducible form of NOS (iNOS), is associated with macrophages and plays a fundamental role in killing or suppressing various pathogens (Gross and Wolin, 1995). The mechanism whereby NO influences the cell includes, among others, an effect on both respiration and oxygen potential in mitochondria and Fe-S proteins engaged in the Krebs cycle and in electron transport (Kroncke et al., 1995). In the case of NO overproduction, the concentration of oxygen in the environment plays an important role in regulating the functions of mitochondria. The balance between RNI and oxygen free radicals (ROS) is of special importance.

Nitric oxide also participates in modulating enteritis during the intestinal phase of infection with *Trichinella spiralis* Owen, 1935, since inflammatory changes in the intestine of animals infected with *T. spiralis* were eliminated with a specific iNOS inhibitor. This suggests that iNOS may participate in the disease process associated with intestinal invasion by adult forms of *T. spiralis* (Hogaboam et al., 1996) and may through its influence on enteritis play an important role in rejection of adult worms.

Our laboratory proposed a hypothesis that RNI may also play a role in protective mechanisms during the muscular phase of trichinellosis.

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is. Using histochemical methods our group demonstrated NOS in basophilically transformed muscle fibers in *T. spiralis*-infected mice (Hadaś et al., 1999). In a separate paper we reported on the participation of ROS in the biochemical protective mechanisms in host muscle infected with *T. spiralis* larvae (Wandurska-Nowak et al., 1998). In the same paper we demonstrated that administration of the glucocorticoid methylprednisolone had a profound effect on the activity of antioxidant enzymes that were examined (superoxide dismutase [SOD] and peroxidase). According to Connors and Moncada (1991), glucocorticoid also inhibits iNOS.

The initiation of research on the participation of iNOS in biochemical defense mechanisms of the host in *T. spiralis* infection was also important from the point of view of its possible participation in the mechanism of uncoupling of oxidative phosphorylation, which can be observed in the mitochondria of tissue infected with helminths (Michejda and Boczoń, 1972; Van den Bosche et al., 1980; Boczoń and Bier, 1986; Ruhle et al., 1989). It was shown that the expected temporal correlation between the increase in the activity of SOD and peroxidase and the peaks in trichinellosis phosphorylation uncoupling did not occur (Wandurska-Nowak et al., 1998).

The objectives of the present investigation were to determine 1) quantitative changes in the activity of iNOS in muscles from hosts infected with *T. spiralis* or *Trichinella pseudospiralis* Garkavi, 1976, and 2) if glucocorticoid prevents changes in the quantity of NO generated in infected tissues.

### Materials and Methods

Experimental tissue consisted of muscles removed from uninfected mice (2-mo-old female mice, strain BALB/C) and from mice infected per os with 700–800 infective larvae of either *T. spiralis* (strain MSUS/PO/60/ISS3) or *T. pseudospiralis* (strain MPRO/US/72/ISS13). The infective larvae obtained after pepsin–HCl digestion after about 2 hr for *T. spiralis* larvae and about 1–1.5 hr for *T. pseudospiralis* were administered per os to mice anesthetized with ether. The mice were killed by decapitation. The amount of larvae per 1 g of muscle tissue obtained after pepsin–HCl digestion at 6–8 wk post-infection (p.i.) were 10,000–12,000 and 5,000 for *T. spiralis* and *T. pseudospiralis*, respectively.

Mice were bred and housed in the animal laboratory, which ensured approximately constant temperature, humidity, and ad libitum access to LMS Labofeed B

(Feed and Concentrates Production Plant) granulated food and water.

Only 1 group of animals infected with *T. spiralis* larvae was treated with methylprednisolone (Depomedrol [Jelfa, Poland], a drug with prolonged action) administered on day 7 p.i. by subcutaneous injection at a dose of 20 mg/kg of body weight. Quadriceps muscles from hind legs were removed and homogenized for 15 to 30 sec in a sucrose medium of the following content (in final concentration): 0.25 M sucrose, 0.002 M EGTA, 0.01 M Tris HCl buffer (pH 7.3), and 20  $\mu$ l heparin with a concentration of 500 units/g per 10 ml medium. The homogenate was centrifuged for 10 min at 4,500 rpm, and the resulting supernatant was centrifuged for 12 min at 15,000 rpm.

The activity of iNOS was measured in the latter supernatant spectrophotometrically by Green's method as modified by Lepoivre (Lepoivre et al., 1989), using the following solutions: A) Griess' reagent containing 0.5% sulphanilamide dissolved in 1 N HCl and 0.15% *N*-(1-naphthyl) ethylenediamine mixed in a ratio of 1:1 and B) consisting of (in final concentrations) 40 mM Tris HCl buffer (pH 8.0), 2 mM NADPH, and 7 mM arginine. Enzyme activity was measured in 140  $\mu$ l of supernatant after 30 min of incubation (to induce the enzyme activity) at 1-wk intervals at a wavelength of  $\lambda = 540$  nm in a cuvette containing 1,200  $\mu$ l of solution A and 100  $\mu$ l of solution B. In some pilot experiments 1.5 mM CaCl<sub>2</sub> was added. Absorption readings were taken after a 30-min incubation period at a temperature of 24 °C, and NO concentration was determined using a NaNO<sub>2</sub> standard curve. Protein was measured applying Lowry's method (Lowry et al., 1951).

The measurements were carried out in 4 groups of animals: for *T. spiralis*-infected mice (I+NaCl), *T. spiralis*-infected mice under treatment (I+D), and also for 2 control groups (C+NaCl and C+D). Both infected and untreated mice (I+NaCl) and those from the respective control group (C+NaCl) were given intramuscular injections of 0.9% NaCl. Activity measured in the respective control groups was taken as 100% for the calculation of percentage changes in such activity during *T. spiralis* infection and treatment.

Analysis of variance or the Mann–Whitney test was used for statistical comparison between groups;  $P < 0.01$  (very significant) or  $< 0.05$  (significant).

### Results

The lack of influence of Ca<sup>++</sup> ions on NOS activity indicated that activity measured in our experiments was that of the inducible NOS form and not the constitutive form that is Ca<sup>++</sup> dependent.

The iNOS activity within the C+NaCl group amounted to  $0.14 \pm 0.01$  nmoles/mg of protein/min, and it was similar in C+D group ( $0.11 \pm 0.003$  nmoles/mg of protein/min). The results of iNOS activity within 2 groups of animals, *T. spiralis* infected and untreated and *T. spiralis*

**Table 1. Activity of iNOS (in nmoles/mg of protein/min) in *T. spiralis*-infected (I+NaCl) and infected + methylprednisolone-treated mouse muscles (I+D).**

Days postinfection (d.p.i.)	I+NaCl*		I+D†	
	Activity ( $\pm$ SEM)	% of control	Activity ( $\pm$ SEM)	% of control
Controls	0.14 $\pm$ 0.01 (8)	—	0.11 $\pm$ 0.003 (3)	—
7	0.08 $\pm$ 0.007 (2) ( $P < 0.05$ )	57	0.20 $\pm$ 0.026 (4)	182
14	0.15 $\pm$ 0.007 (4)	107	0.11 $\pm$ 0.053 (5)	100
21	0.52 $\pm$ 0.1 (5) ( $P < 0.01$ )	371	0.16 $\pm$ 0.003 (3) ( $P < 0.05$ )	145
34	0.11 $\pm$ 0.003 (6)	79	0.14 $\pm$ 0.056 (11)	127
42	0.07 $\pm$ 0.003 (5) ( $P < 0.05$ )	50	0.06 $\pm$ 0.003 (5)	56
49	0.07 $\pm$ 0.003 (6) ( $P < 0.05$ )	50	0.07 $\pm$ 0.003 (6) ( $P < 0.05$ )	64
70	0.40 $\pm$ 0.063 (6) ( $P < 0.01$ )	285	0.37 $\pm$ 0.254 (6)	336
90	0.04 $\pm$ 0.003 (3) ( $P < 0.05$ )	29	0.04 $\pm$ 0.001 (3)	37

\* The statistically significant differences in column I+NaCl when the values of activity in infected mice were compared with normal mice. The number in parentheses is the number of measurements.

† The comparison of the results from infected and infected and treated by glucocorticoid methylprednisolone mice was carried out using a Mann-Whitney test. The number in parentheses is the number of measurements.

infected and treated, between 7 and 70 days p.i., are presented in Table 1.

The investigations of iNOS activity in muscles of mice infected with *T. spiralis* larvae showed a 2-stage increase in enzyme activity during the course of trichinellosis. An initial peak of activity was seen at 21 days p.i., and a second rise in activity occurred at 70 days pi (285% of the activity in the control group). Statistically the changes in activity during the stages of trichinellosis mentioned above varied significantly from controls ( $P < 0.01$  or  $P < 0.05$ ).

The investigation of muscle enzyme activity was carried out on mice infected with *T. spiralis* and treated simultaneously with glucocorticoid methylprednisolone (I+D) at the same intervals as those for the group of infected and untreated animals. The I+D group mice had higher enzyme activity than those of the I+NaCl group on day 7 p.i. (182% of the control values) and at 70 days p.i. (up to 336% of the control value) with a statistically significant result compared with the control group ( $P < 0.01$ ). At 90 days p.i. enzyme activity fell in a manner similar to that seen in *T. spiralis*-infected and untreated animals. Therefore, it may be assumed that methylprednisolone exerted a normalizing influence on iNOS activity only in the initial stage of the muscular phase, i.e., at day 21 p.i.

The comparison of changes in iNOS activity in 2 infections, 1 caused by nonencysting *T. pseudospiralis* larvae and the other by encysting *T. spiralis* larvae, as presented in Figure 1, revealed a totally different dynamic for iNOS changes in muscles of infected mice. A statistically significant difference existed between the activity of the enzyme examined in mice infected with the *T. spiralis* and *T. pseudospiralis* invasion during all phases of trichinellosis ( $P < 0.01$ ). In general, unlike *T. spiralis*, infection with *T. pseudospiralis* was characterized by an absence of iNOS stimulation during the first weeks, while considerable stimulation (up to approximately 280% of the control) lasted throughout and continued up to the end of the muscle phase (from 42 to 70 days p.i.).

### Discussion

The biochemical defense mechanisms for killing *T. spiralis* larvae by eosinophils are mediated by peroxidase (POX), with inclusion of the process in which both neutrophils and eosinophils produce hypochlorous acid, which is toxic to the larvae of the parasite (Buys et al., 1981).

The quantitative results of research on the activity of the inducible NOS isoform (iNOS) presented in this paper clearly indicate that this enzyme, supplying NO lethal to many parasites, is

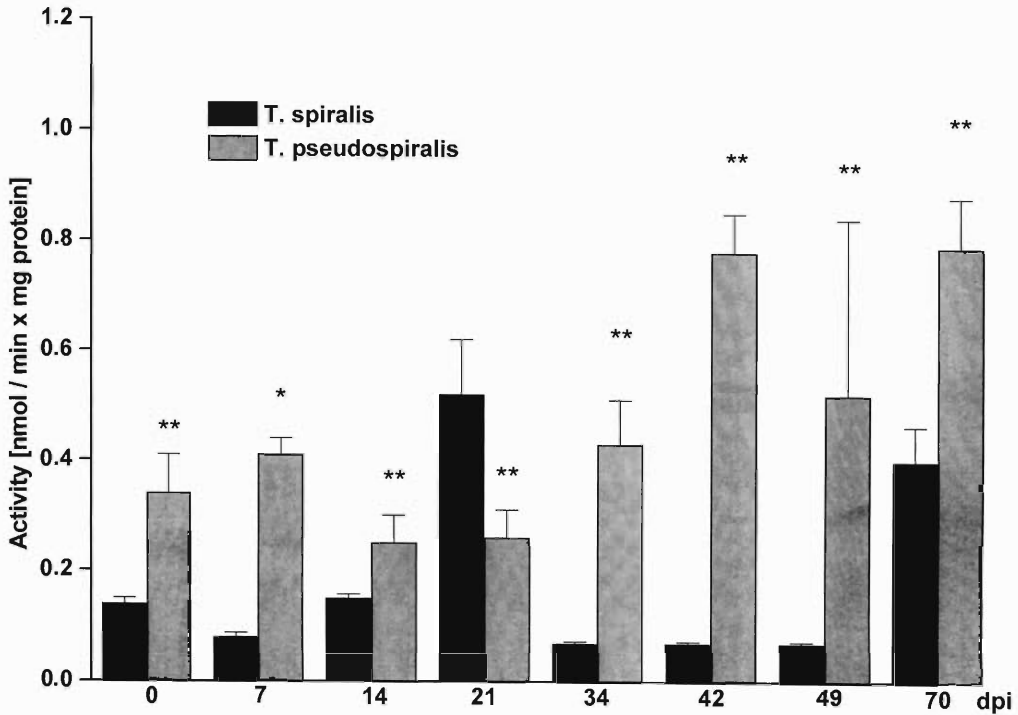


Figure 1. Comparison of the changes of the iNOS activity (mean  $\pm$  SEM) during *T. spiralis* and *T. pseudospiralis* infection between 0–70 days postinfection. Values with \* or \*\* ( $P < 0.05$  and  $P < 0.01$ , respectively) in *T. pseudospiralis* are significantly different from the respective values in *T. spiralis* infection.

strongly activated during certain phases of trichinellosis in mice. It underwent a 4-fold stimulation 3 weeks after infection, a period during infection when histochemical evidence shows that NO is induced in numerous cells located near the larval sac (eosinophils and lymphocytes) and in macrophages (Hadaś et al., 1999).

The increase in NO within host cells may be associated with suppression of cytochrome oxidase (OX), leading to a decrease in production of adenosine triphosphate within the cell. Recently, it has been stressed that uncoupling of oxidative phosphorylation in mice muscle mitochondria in trichinellosis is accompanied by reduced oxygen consumption (in state III), continuing through all phases of trichinellosis (Wandurska-Nowak et al., 1998). Two of the strongest increases in uncoupling were expressed as a decrease in the respiratory control index (RCI) to approximately 40% of the control value on days 20 and 45 p.i., respectively. The correlation between the almost 4-fold iNOS stimulation in the host's muscles on day 23 p.i. with the first decrease in the RCI values is striking. These data

may suggest the participation of an iNOS producing nitric oxide in this phase of the phosphorylation uncoupling process in the host's muscles, which suppresses OX (Brown, 1995). At this point during infection, the larva has created the nurse cell–infective L<sub>1</sub> larva complex (Despommier, 1990), and a rapid increase in the number of eosinophils in host muscles takes place (Butterworth, 1980). Eosinophils, which may destroy *Trichinella* larvae through the production of ROS (Boczoń et al., 1996), may also be responsible for the generation of RNI. In tissue invasions, RNI generation is frequently more intense than ROS generation (Callahan et al., 1993). It has also been observed in trichinellosis that both peroxidase and superoxide dismutase (Derda, 1998) are considerably weaker and delayed in relation to the first maximum of iNOS induction.

The mechanism of long-term uncoupling of phosphorylation that follows in the later muscular phase (34–70 days p.i.) appears complex. Generally, glucocorticoids have an anti-inflammatory effect, which includes destruction of T

lymphocytes and suppression of NO production. In the present study, the immunosuppressive drug had no effect on iNOS induction in *T. spiralis*-infected mice muscles. On the other hand, during the muscular phase of trichinellosis, methylprednisolone exerts a suppressive influence on the subpopulation of CD3 lymphocytes (Boczoń et al., unpublished data) with the degree of change dependent on the doses of larvae used to infect the host.

Similar investigations on *T. pseudospiralis* were not performed because human cases of *T. pseudospiralis* have not been reported. Nevertheless, in severe cases of human trichinellosis caused by *T. spiralis*, glucocorticoid chemotherapy is popular, although still disputable.

It is well known that larvae of *T. pseudospiralis* are less immunogenic than those of *T. spiralis* (Flockhart, 1986). Irrespective of the fact that they share 60% of antigens with *T. spiralis* larvae, they still possess 8 different proteins. In the intestinal phase, the adult form of *T. pseudospiralis* causes a much weaker inflammatory reaction than that of *T. spiralis* (Flockhart, 1986). In the late muscular phase (70 days p.i.), the presence of *T. pseudospiralis* larvae resulted in a level of iNOS stimulation similar to that caused by *T. spiralis* larvae (approximately 240%).

Taking into account that the intensity of the *T. pseudospiralis* infection was 2 times lower than that of *T. spiralis*, we can assume that despite the lower immunogenicity of the former, it induced much stronger RNI generation. For example, on day 7 p.i. the iNOS activity recalculated per 1,000 muscle larvae of *T. pseudospiralis*/g of tissue was about 0.08 nmoles/mg of protein/min, and during the muscular phase measured on day 42 p.i. was 0.16 nmoles/mg of protein/min. In *T. spiralis* infection, the respective values for iNOS activity were 0.007 and 0.006 nmoles/mg of protein/min, being about 10 and 26 times lower, respectively, than in *T. pseudospiralis* infection.

Thus, the continuous migration of *T. pseudospiralis* larvae causes a much greater degree of damage, but instead of an immunological response, a set of biochemical defense reactions, including RNI generation, take place.

The agents present in excretory-secretory products of larvae induced iNOS for almost 3 months. Still, in order to establish the possible duration of this effect, it would be necessary to

carry out a long-term study for at least 6 to 8 mo p.i.

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## The Expulsion of *Echinostoma trivolvis*: Worm Kinetics and Intestinal Cytopathology in Jirds, *Meriones unguiculatus*

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**ABSTRACT:** Worm kinetics and cytopathology of jirds, *Meriones unguiculatus* Milne-Edwards, 1967, infected with *Echinostoma trivolvis* (Cort, 1914) Kanev, 1985, were reported and compared with previous studies on echinostome infections in murine hosts. Seven jirds were each infected with 40 metacercarial cysts, and the worms were recovered at days 5, 8, 10, 12, 15, and 17 postinfection (p.i.). Worm recoveries were 35.4, 10.7, and 0.4 at days 5, 10, and 15 p.i., respectively. Worm expulsion occurred on about day 10 p.i., corresponding to the peak increase in the number of goblet cells at  $24.3 \pm 0.6$ /villus-crypt unit (VCU) at day 10 p.i. These data showed that worm expulsion of *E. trivolvis* in jirds occurred earlier than that in C3H and BALB/c mouse hosts. The difference in expulsion times and rates reflects differences in the peak number of goblet cells in the host intestines of jirds versus mice. The number of mucosal mast cells increased slightly and peaked at  $1.1 \pm 0.32/10$  VCU at day 10 in jirds. An increase in mucosal mast cells occurred earlier and was smaller in jirds than in BALB/c mice. Scanning electron microscope observations showed an irregular arrangement of microvilli in the small intestine of infected jirds. Transmission electron microscope observations also showed damage in the distal parts of villi in infected jird intestines and the appearance of numerous vesicles in the infected epithelium.

**KEY WORDS:** *Echinostoma trivolvis*, worm expulsion, worm kinetics, intestine, cytopathology, jird, *Meriones unguiculatus*, SEM, TEM.

*Echinostoma trivolvis* (Cort, 1914) Kanev, 1985, is expelled within several weeks of infection from the intestines of various strains of mice (*Mus musculus* Linnaeus, 1758): ICR (Hosier and Fried, 1986; Weinstein and Fried, 1991), BALB/c (Fujino et al., 1993), C3H (Fujino and Fried, 1993a; Fujino et al., 1996), and Swiss Webster (Hosier and Fried, 1986) mice, whereas this echinostome species is retained for more than 15 weeks in the intestines of golden hamsters (*Mesocricetus auratus* Waterhouse, 1839) (Huffman et al., 1986; Franco et al., 1986). Fujino et al. (1993, 1996) noted that worm expulsion is mainly caused by an increased secretion of mucins by hyperplastic goblet cells. Fujino and Fried (1993b; 1996) examined glycoconjugates in intestinal mucins in C3H mice versus golden hamsters infected with *E. trivolvis* and reported that goblet cells in mice

contained sulfomucins, whereas those in hamsters contained sialomucins. Probably differences in mucin characteristics account in part for differences in infectivity of *E. trivolvis* in these hosts. Thus, differences in infectivity depend in part on genetic differences in murine strains used as hosts of echinostomes.

Studies on *E. trivolvis* in jirds have not been done. However, Christensen et al. (1990) described survival and fecundity of an allopatric echinostome species, *Echinostoma caproni* Richard, 1964, in hamsters and jirds (*Meriones unguiculatus* Milne-Edwards, 1867). Mahler et al. (1995) noted considerable differences in the reproductive capacity of *E. caproni* in hamsters versus jirds. The hamster was more susceptible to *E. caproni* infection than was the jird.

The purpose of the present study was to report information on the infection, growth, and distribution of *E. trivolvis* in jirds and to compare our data with previous studies on this species in mouse strains and in the golden hamster. Path-

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**Table 1. Infectivity and distribution of *Echinostoma trivolvis* in jirds.**

Group	Day postinfection	No. of exposed (infected) jirds	Mean ( $\pm$ SE) No. (%) of worms recovered	No. of worms located in the:		
				Small intestine		Colon + rectum
				Total (I II III) <sup>1</sup>	Cecum	
A	5	7 (7)	14.1 $\pm$ 2.4 (35.4)	97 ( 4 49 44)	1	1
B	8	7 (7)	13.6 $\pm$ 2.2 (33.9) <sup>†</sup>	90 (30 21 39)	4	0
C	10	7 (7)	4.3 $\pm$ 0.9 (10.7)	30 (13 8 9)	0	0
D	12	7 (5)	1.7 $\pm$ 1.8 (4.3)	10 ( 3 4 3)	1	1
E	15	7 (1)	0.1 $\pm$ 0.4 (0.4)	1 ( 1 0 0)	0	0
F	17	7 (0)	0	0	0	0

\* I = anterior; II = middle; III = posterior.

<sup>†</sup> Not significant.

ological, histochemical, and electron microscopical studies of the intestines of jirds infected with *E. trivolvis* were also carried out.

### Materials and Methods

Metacercarial cysts of *Echinostoma trivolvis* were obtained from the kidney and pericardial sac of laboratory-infected *Biomphalaria glabrata* (Say, 1816) snails. The worm strain was previously described by Fujino and Fried (1993a). Forty cysts were fed via a stomach tube to each jird, and 7 jirds were lightly anesthetized with ether and killed by cervical dislocation at days 5, 8, 10, 12, 15, and 17 postinfection (p.i.). Six groups of untreated control jirds, 7 per group, were also killed on the same days as the infected hosts. The jirds were starved for about 12 hr prior to necropsy to avoid food residue in the intestine. The intestine was removed and opened longitudinally to determine worm location. The worms were counted, and their distribution was recorded in the small intestine, which was divided equally into anterior, middle, and posterior regions, and in the cecum and colon plus rectum. Where applicable, Student's *t*-test was used to analyze differences between means, and  $P < 0.05$  was considered statistically significant.

For histological samples, pieces of intestine (2 cm long) located 10 cm anterior to the cecum, corresponding to the middle jejunum to ileum, were excised and fixed for 3 hr in Carnoy's fixative. The samples were dehydrated with an ethanol series and embedded in paraffin. Histological sections 5  $\mu$ m thick were stained with periodic-acid Schiff for goblet cell mucins. Mucosal mast cells were stained with alcian blue (pH 0.3) and safranin O. All counts were expressed as the number of cells per villus-crypt unit (VCU) (Miller and Jarrett, 1971) for goblet cells and cells per 10 VCU for mast cells. Thirty to 50 VCUs were analyzed per host. Logarithmic transformation of data: geometric means (antilog of mean log of data) was performed. For comparison of the cell counts, goblet cell numbers were multiplied 10 times as for the mast cells. This transformation tends to stabilize variance and to normalize such data.

For scanning electron microscopy (SEM), the intestinal tissue from jirds infected with *E. trivolvis* at 8

and 10 days p.i. and control tissues were excised from the upper ileum, opened longitudinally with fine needles, and pinned on small rubber boards in physiological saline. The intestinal debris was removed by gentle flow of saline forced over the surface with a pipette. After a brief rinse in 0.1M sodium cacodylate buffer (pH 7.4), the specimens were fixed for 3 hr with 3% glutaraldehyde, postfixed for 3 hr in 0.1 M osmium tetroxide (pH 7.4), and then dehydrated in an ethanol series. The material was dried in a carbon dioxide critical-point drying apparatus (Hitachi HCP-2, Tokyo, Japan), coated with palladium in a Hitachi E 1030, Tokyo, Japan ion sputter, and examined in a Hitachi S-450 SEM, Tokyo, Japan at 10 kV. For transmission electron microscopy (TEM), the material was prepared as described for SEM procedures in Fujino and Fried (1993a). Ultrathin sections stained with uranyl acetate and lead acetate were viewed in a JEOL JEM 1210 electron microscope operating at 80 kV.

### Results and Discussion

Infectivity and worm recovery data are presented in Table 1. All jirds were infected with *E. trivolvis* at days 5, 8, and 10 p.i., and this was confirmed by fecal examination under the microscope. By day 12 p.i., 5 of 7 jirds were infected, but only 1 was infected by day 15 p.i. Worm recoveries were 35.4% and 33.9% at days 5 and 8 p.i., respectively, and this difference was not statistically significant. The recovery data dropped to 10.7% at day 10 p.i., fell markedly to 4.3% by day 12 p.i., and finally to 0.4% by day 15 p.i. Most worms were expelled between days 10 and 15 p.i., and all were expelled by day 17 p.i. Most worms were found in the middle to posterior part of the small intestine at day 5 p.i. The worms moved mainly anterior to the middle of the small intestine by day 10 p.i. Such an anterior worm shift was reported previously in ICR mice infected with *E. trivolvis* at day 21 p.i. (Weinstein and Fried, 1991). In the present

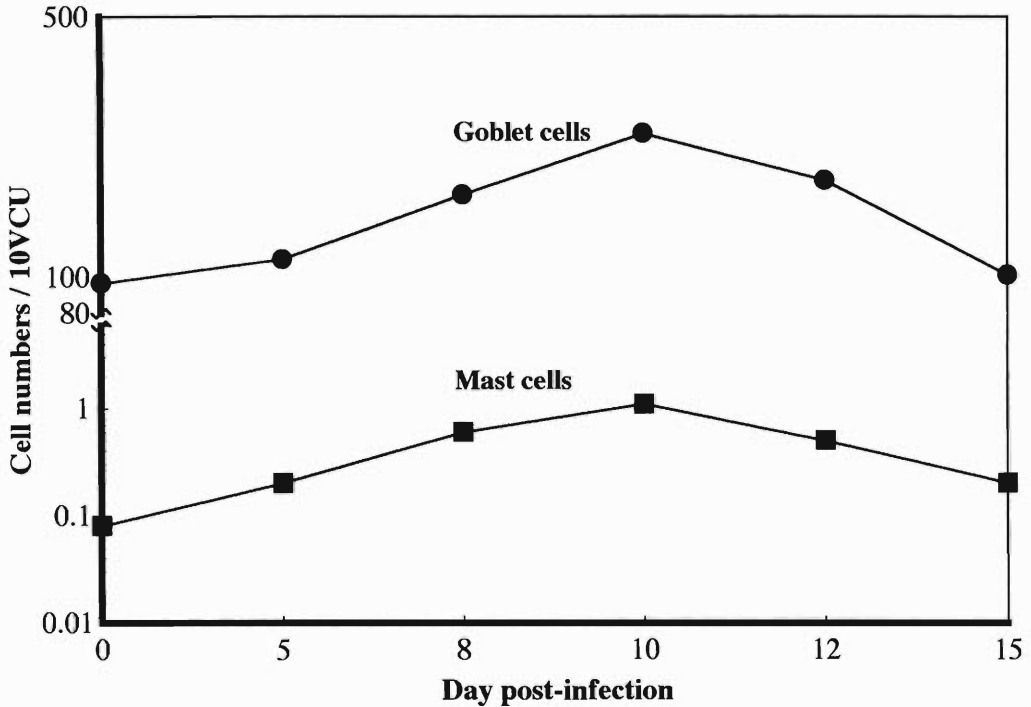
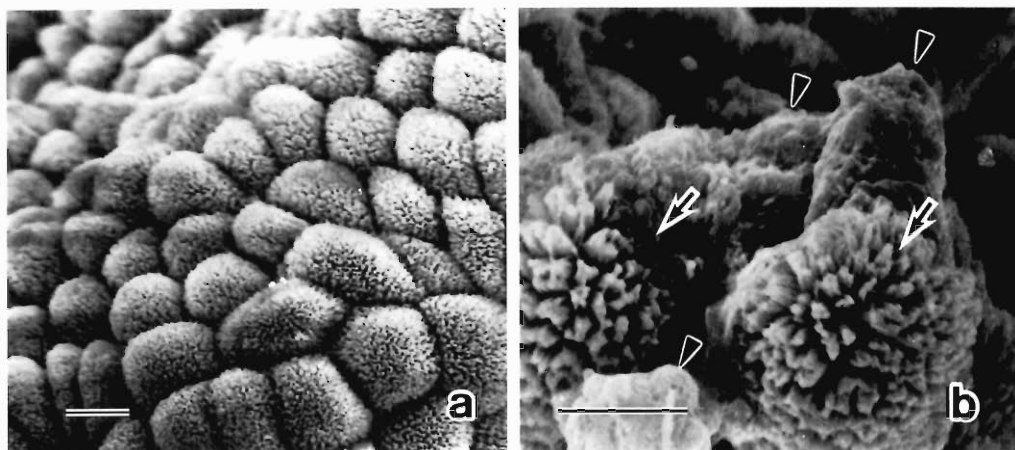


Figure 1. Goblet cell (mean  $\pm$  SE)/VCU (●) and mast cell (■) (mean  $\pm$  SE)/VCU numbers of the anterior section of the ileum of jirds, each of which was infected with *Echinostoma trivolvis* metacercarial cysts. A logarithmic transformation was performed on the number of the cells for normalizing the data. For comparison of the cell counts, goblet cell numbers were multiplied 10 times as for the mast cells.

study, the worms moved posteriad to the cecum and colon plus rectum by day 12 p.i. In BALB/c mice infected with *E. trivolvis*, the recovery rate of the worms was over 44% for days 6–10 p.i. and worm expulsion occurred from day 10 to 12 p.i., corresponding to the peak increase in goblet cells (Fujino et al., 1996). Those worm recovery rates were much higher than what is seen in the present study on jirds, i.e., 35.4% and 33.9% at days 5 and 8 p.i., respectively. Therefore, worm expulsion occurred from days 8 to 12 p.i. These data showed that worm expulsion in jirds occurred earlier than in murine hosts, probably reflecting a difference in the peak number of goblet cells in jirds and mice. Christensen et al. (1990) examined the establishment, survival, and fecundity in *E. caproni* and the allopatric species of *E. trivolvis* in hamsters and jirds. They noted that the jird exhibited an overall low susceptibility to *E. caproni* infection. The jird's low susceptibility to *E. caproni* is different from that of *E. trivolvis*. According to Ellerman and Morrison-Scott (1951), the jird

(*M. unguiculatus*) belongs to the subfamily Gerbillinae of the family Muridae and differs both taxonomically and genetically from the golden hamster (*M. auratus*) of the subfamily Cricetinae and also from various mouse strains of *Mus musculus* of the subfamily Murinae. It is known that Gerbillinae is genetically closer to Murinae than Cricetinae (Ellerman and Morrison-Scott, 1951). The present infection data on *E. trivolvis* in jirds generally correspond to the above-noted taxonomic and genetic differences in murine hosts. In conclusion, the recoveries of *E. trivolvis* from jirds were lower than those from mice and much lower than those from golden hamsters. It is possible that these differences in recoveries reflect the genetic differences among these 3 hosts, jirds, mice, and hamsters.

Kinetic changes in the number of goblet cells/VCU at the anterior sections ( $n = 50$ ) of the ileum with or without the parasites present are shown in Figure 1. The number of goblet cells in infected jirds increased markedly, peaked at  $24.3 \pm 0.6/\text{VCU}$  at day 10 p.i. and then de-



**Figure 2.** SEM of the control and infected intestinal surface of jirds. **a.** Control (normal) intestine, with round villi having regularly arranged microvilli. Scale bar = 5.0  $\mu\text{m}$ . **b.** Intestine infected for 10 days with *Echinostoma trivolvis*. The intestinal microvilli appear irregularly arranged (arrows) and partly peeled off (arrowheads). Scale bar = 5.0  $\mu\text{m}$ .

clined. The number of goblet cells in the control was  $9.6 \pm 0.4/\text{VCU}$ . The numbers of mucosal mast cells were so small that their kinetic changes were examined in 10 VCU, although the number of mast cells in infected jirds increased gradually from  $0.2 \pm 0.42/10$  VCU at day 5 p.i. to reach a peak of  $1.1 \pm 0.32/10$  VCU at day 10 p.i. and then declined gradually. For comparison of the cell counts, goblet cell numbers were multiplied 10 times as for the mast cells. The numbers of cells were log-transformed to normalize the data in Figure 1. Fujino et al. (1993) examined the worm kinetics and intestinal cytopathology in conventional and congenitally athymic BALB/c mice and noted that worm rejection was caused by goblet cell hyperplasia and not by mast cells.

The SEM observations of the surface of the intestinal villi infected with *E. trivolvis* and the control showed a rough and irregular arrangement of microvilli in the infected intestine compared with the regular microvilli arrangement in the control intestine (Fig. 2). The intestine infected with worms was partly damaged by day 10 p.i., and its epithelial surface was eroded. The TEM observations showed that the intestinal epithelium appeared more electron-dense than that in the control (not shown). The distal ends of the villi were partly broken and the microvilli were partially eroded. Numerous vesicles of various sizes appeared in the intestinal epithelium. The appearance of these vesicles was also re-

ported in BALB/c and C3H mice infected with *E. trivolvis* by Fujino et al. (1993) and Fujino and Fried (1993a), respectively. Matrices of many small rounded mitochondria were granular and irregularly condensed. Elongate nuclei with an irregular peripheral margin had heterochromatin arranged in small patches and randomly distributed. Fujino and Fried (1996) noted histopathological differences in mouse versus hamster small intestine infected with *E. trivolvis* and showed no marked histopathological and histochemical changes in the hamster intestines. They suggested that the response of the hamster to *E. trivolvis* infection was relatively weak and that this host showed only a limited capacity to expel *E. trivolvis*.

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## 2000–2001 MEETING SCHEDULE OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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| 11 October 2000  | George Washington University, Washington, DC (Contact Person: Ralph Eckerlin, 703-323-3234).   |
| 15 November 2000 | Anniversary Dinner, Location to be announced.  |
| 17 January 2001  | Nematology Laboratory, Beltsville Agricultural Research Service, USDA, Beltsville, MD (Contact Person: Lynn Carta, 301-504-8787).  |
| 14 March 2001    | Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD (Walter Reed Forest Glen Annex Bldg. 503) (Contact Person: Eileen Franke-Villasante, 301-319-7667).    |
| 5 May 2001       | Joint Meeting with the New Jersey Society for Parasitology at the New Bolton Center, University of Pennsylvania, Kennett Square, PA (Contact Person: Jay Ferrell, 215-898-8561). |

## Effects of a High-Carbohydrate Diet on Growth of *Echinostoma caproni* in ICR Mice

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**ABSTRACT:** The effects of a high-carbohydrate diet (HCD) on the host-parasite relationship of *Echinostoma caproni* Richard, 1964, in ICR mice were studied. The experimental diet was a customized HCD containing 63% carbohydrates, 14% protein, 4% fat, and 19% cellulose. The control diet, a standard laboratory diet, contained 31% carbohydrate, 20% protein, 7% fat, and 42% cellulose. Thirty-six mice were each infected with 35 metacercarial cysts; 18 mice were fed the HCD and the remaining mice received the control diet. Equal numbers of experimental and control mice were necropsied at 2, 3, and 4 weeks postinfection (p.i.). Comparisons of worm body area in uniformly fixed and stained worms were made at 2, 3, and 4 weeks p.i. There was no significant difference in body area in worms from each group at 2 and 3 weeks p.i. At 4 weeks p.i. the body area of worms from hosts on the HCD was significantly greater than that of worms from hosts on the control diet. The findings suggest that the HCD contributes to growth enhancement of *E. caproni* in ICR mice.

**KEY WORDS:** trematodes, high-carbohydrate diet, *Echinostoma caproni*, ICR mice, growth.

Previous studies in our laboratory have examined the effects of various experimental diets of hosts on growth and development of *Echinostoma caproni* Richard, 1964, in Institute for Cancer Research (ICR) mice. Sudati et al. (1996, 1997) used this model to study the effects of high-lipid and high-protein diets, respectively, in ICR mice. Rosario and Fried (1999) examined the effects of a protein-free host diet on growth and development of *E. caproni* in ICR mice.

Although studies are available on the effects of a high-carbohydrate host diet on gastrointestinal trematodes, this topic has been studied extensively in rats infected with hymenolipid cestodes (e.g., Read; 1959; Read and Simmons, 1963). It is clear from the literature that hymenolipids thrive best in rodent hosts maintained on high-carbohydrate diets (see Von Brand, 1973, for review). Because of the lack of information on gastrointestinal trematodes maintained in rodent hosts fed a high-carbohydrate diet (HCD), we initiated this study to examine the effects of such a diet on worm recovery, growth, and distribution of *E. caproni* in ICR mice. *Echinostoma caproni* now is a well-established model for conducting such studies of intestinal trematode infections in nutritionally altered hosts.

Graczyk and Fried (1998) examined the recent literature on human echinostomiasis and noted that it is a common but forgotten food-borne disease. Because echinostomiasis may oc-

cur in people from socioeconomic groups that have relatively high-carbohydrate, low-protein diets, studies on the effects of HCD on the model echinostome, *E. caproni*, seemed appropriate.

### Materials and Methods

Metacercarial cysts of *Echinostoma caproni* were removed from the kidney/pericardial region of experimentally infected *Biomphalaria glabrata* (Say, 1818) snails and fed by stomach tube (35 cysts per mouse) to 36, 6 to 8-week-old, female ICR mice (Manger and Fried, 1993). The experimental mice were fed a customized HCD in pellet form containing 63% cornstarch as a source of carbohydrate, 14% protein, 4% fat, and 19% cellulose (Dyets Inc., Bethlehem, Pennsylvania, U.S.A.). The control mice were fed a standardized rat-mouse-hamster (RMH) 3000 diet in pellet form containing 31% carbohydrate, 20% protein, 7% fat, and 42% cellulose (US Biochemicals Co., Cleveland, Ohio, U.S.A.). Both diets contained essential vitamins and minerals as described previously. The HCD was about 1.3 times more calorific than the normal diet (Rosario and Fried, 1999).

A total of 36 mice was used in the experiment; 18 mice were maintained on the HCD, and the remainder on the RMH diet. On the day of infection, the mice were weighed and maintained 6 per cage on either the HCD or the RMH diet. Food and water were provided ad libitum. Six mice on the HCD and 6 mice on the RMH diet were each necropsied at 2, 3, and 4 weeks post infection (p.i.). Mice were weighed on the day they were fed cysts and at necropsy. At that time, the small intestine was removed from the pyloric sphincter to the ileocecal valve and divided into 5 equal sections numbered 1–5, beginning with the pylorus. Worms were removed from the small intestine, and their location and number in each section were recorded. Worms were rinsed in Locke's solution and fixed in hot (85°C) alcohol-formalin-acetic acid. Twenty

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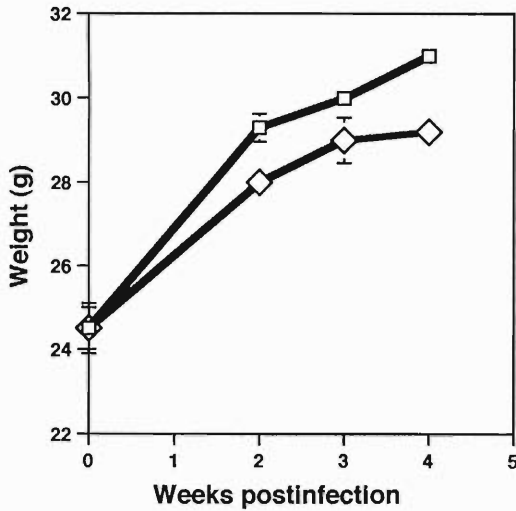


Figure 1. Mean ( $\pm$  SE) weights of mice on high carbohydrate (diamonds) versus control (squares) diet at 0–4 weeks postinfection.

worms at each data point were selected at random from mice on the HCD and RMH diets and stained in Gower's carmine, dehydrated in ethanol, cleared in xylene, and mounted in Permount™ (Kaufman and Fried, 1994). Length and maximum width measurements of worms were made with the aid of a calibrated ocular micrometer to give body area in  $\text{mm}^2$  for control and experimental worms at 2, 3, and 4 weeks p.i. Length and width measurements were also made on the gonads and suckers to determine if there were significant differences in organ sizes between worms on HCD versus RMH diet (Sudati et al., 1997). Whenever possible, differences in means between groups were determined using Student's *t*-test, with  $P < 0.05$  being considered significant.

### Results

Mean weights of mice on both the HCD and RMH diet are shown in Figure 1. Mouse weight in both groups increased rapidly until 2 weeks p.i. and then less rapidly until 4 weeks p.i. Although the weights of mice on the RMH diet were slightly higher than those of mice on the HCD diet, there was no significant difference in mouse weight between groups at any week p.i. There was no apparent difference in food consumption in mice on either diet.

From 2 to 4 weeks p.i., the small intestines of hosts on the HCD were yellow compared to the tan-colored intestines of hosts on the RMH diet; the intestines of mice on the HCD were thinner, more translucent, and more brittle than those of hosts on the RMH diet. All worms from hosts on both diets were ovigerous at 2 to 4 weeks p.i.

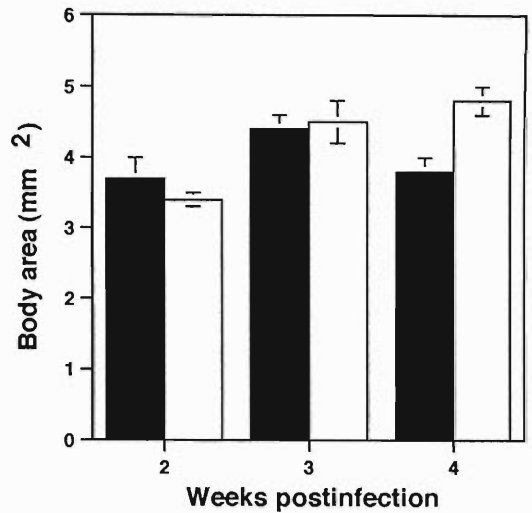


Figure 2. Effects of diet on mean ( $\pm$  SE) *E. caproni* worm body area; control diet (closed bar) and high-carbohydrate diet (open bar).

Eggs taken at random from some worms maintained on the HCD, when incubated in artificial spring water, produced miracidia that were capable of infecting *B. glabrata*.

The mean body areas of worms from the hosts on the RHM diet and on the HCD are shown in Figure 2. At 2 and 3 weeks p.i., there were no significant differences in the body areas of worms from either group. However, a significant increase in body areas was seen in worms from the experimental hosts at 4 weeks p.i. compared with that of worms from the control hosts. There was a significant difference at 4 weeks p.i. in the length of the anterior and posterior testes and in the diameter of the acetabulum and oral sucker of worms from the HCD group compared with those on the RMH diet.

The percent worm recovery is shown in Figure 3 and was similar in control and experimental mice at all 3 sampling points with about 50% recovery in both groups at all data points. More worms from hosts on the RMH diet were located in segments 3 and 4 than those from hosts on the HCD at sampling points. Considerably more worms on the HCD were located in segment 5, compared with worms on the RMH diet at all sampling points. Worms from the HCD group were more widely dispersed in their hosts than those from hosts on the RMH diet; worms from HCD hosts were also located more posteriorly than those from hosts on the RMH diet.



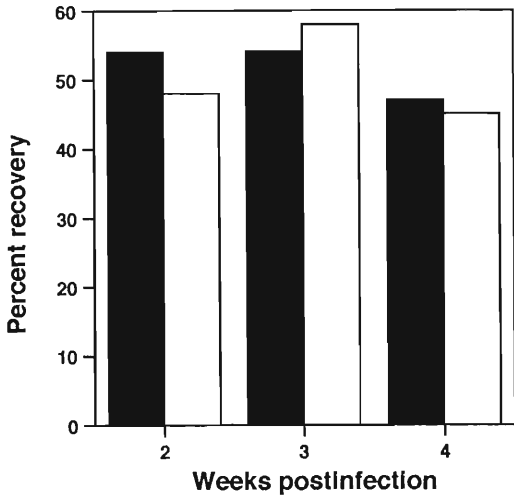


Figure 3. Effects of diet on *E. caproni* worm recovery in mice exposed to 35 cysts/hosts; control diet (closed bar) and high-carbohydrate diet (open bar).

### Discussion

Worms from hosts on the HCD, when compared with those from mice on the RMH diet, showed a marked increase in body area at 4 weeks p.i. This is the first report that documents enhanced growth of a digenean maintained in an experimental vertebrate host fed an HCD. Echinostomes on the HCD showed greater body area by 4 weeks p.i. Reasons for the increase in worm body area are not readily apparent from the findings in this study. We have no way of knowing if the HCD had a direct effect on worm growth (i.e., if the worms consumed more carbohydrates from the HCD than the RMH diet) or had an indirect effect by altering gut constituents. Our results suggest that the intestines of hosts on the HCD showed a loss of normal integrity. The HCD at 4 weeks p.i. could have contributed to the altered gut that allowed for a large number of mucosal epithelial cells to be sloughed off, thereby increasing the food supply available to the echinostomes in hosts on the HCD. Perhaps such an increased food supply

was a factor in the enhanced worm growth. Since there were no control uninfected mice on the HCD, there is also no way of knowing if some of the changes in host guts may not have been caused by interactions between diet and worms.

Distribution data are interesting in that, in hosts on the HCD, worms were more spread out and also located more posteriorly than worms from hosts on the RMH diet. The disparate arrangement of the worms in the HCD hosts is similar to previous observations on mice infected with *E. caproni* and maintained on diets with altered amounts of fats and proteins (Sudati et al., 1996, 1997; Rosario and Fried, 1999).

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## Surface Ultrastructure of Larval *Gnathostoma* cf. *binucleatum* from Mexico

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**ABSTRACT:** We examined the morphology of gnathostome larvae obtained in Temazcal and Sinaloa, Mexico, mainly using scanning electron microscopy. The mean body length was 4.67 mm. The head had 4 transverse rows of hooklets, and the mean number of each row was 40, 44, 47, and 50. The bodies were wholly covered with minute cuticular spines along their transverse striations. The mean number of striations varied from 227 to 275. The cervical papillae were situated between the 13th and 17th transverse striations, and most specimens had them between the 14th and 15th transverse striations. An excretory pore was also located between the 24th and 28th transverse striations. We identified this Mexican gnathostome as *Gnathostoma* cf. *binucleatum* Almeyda-Artigas, 1991.

**KEY WORDS:** *Gnathostoma* cf. *binucleatum*, scanning electron microscopy, morphology, Mexico.

Gnathostomiasis is an important parasitic zoonosis, mainly endemic in such countries as Japan, Thailand, and Vietnam, where people often eat raw freshwater fish. For this reason, this food-borne disease was thought to be limited to Southeast Asian countries. In 1970, however, a case of human gnathostomiasis was reported in Mexico (Peláez and Pérez-Reyes, 1970). The patient was neither a traveler nor an immigrant from Southeast Asia. After this initial discovery, the number of gnathostomiasis patients increased drastically; more than 1,000 cases have been diagnosed in Mexico. The endemic area in Mexico includes 6 states, which are roughly divided into 3 regions, including the Pacific coast (Culiacán), Atlantic coast areas (Tampico), and regions (Veracruz) adjacent to Central American countries (Ogata et al., 1998). Lamothe-Argumedo et al. (1989) and Almeyda-Artigas (1991) examined the morphology of gnathostome larvae from fish in Oaxaca-Veracruz. Later Aka-hane et al. (1994) examined by light microscopy the morphology of the larvae collected from pelicans in the same area.

We herein report the morphology of specimens of *Gnathostoma* cf. *binucleatum* Almeyda-

Artigas, 1991, from Mexico, which were examined using scanning electron microscopy (SEM). The results were compared with our previous SEM study of larvae of *Gnathostoma spinigerum* Owen, 1836, *Gnathostoma doloresi* Tubangui, 1925, and *Gnathostoma hispidum* Fedtschenko, 1872, obtained in Japan, China, and Thailand (Koga et al., 1987, 1988, 1994).

### Materials and Methods

Three American white pelicans (*Pelecanus erythrorhynchos* Gmelin, 1789) were collected in the Presidente Miguel Alemán Reservoir in Temazcal, Oaxaca, Mexico, and their muscles were examined for gnathostome larvae. The muscles were removed, chopped into small pieces, and then cut into thin slices. The slices were then placed between 2 glass plates (10 × 10 cm, 2 mm thick), pressed by hand, and examined under a dissecting microscope. The muscle remnants were then digested in artificial gastric juice (0.2 g pepsin in 0.7 ml HCl/100 ml distilled water) for 3 hours at 37°C to collect any larvae that might have been overlooked. The muscles of another ichthyophagous bird, a great egret (*Egretta alba* Linnaeus, 1758), captured at a dike of the San Lorenzo River in Culiacán, were also examined. These larvae were processed for morphological examination by both light microscopy and SEM. Paraffin sections of specimens were prepared by conventional methods and stained with Mayer's hematoxylin and eosin.

For the SEM specimen preparations, 10 viable lar-

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vae from Temazcal and 3 from Culiacán were washed in distilled water and stored in a refrigerator until the worms relaxed completely. They were then fixed in 10% formalin for 7 days. The larvae then were washed overnight in running tap water to remove the fixative and were transferred to distilled water. The specimens were rinsed twice in Millonig's phosphate buffer and postfixed overnight in 0.5% OsO<sub>4</sub> in the same buffer. All specimens were then carefully and gradually dehydrated in an ascending series of ethanol, since such specimens often shrink or have surface wrinkles because of rapid dehydration. They were transferred into amyl acetate and CO<sub>2</sub> critical-point dried with a Hitachi HCP-2 dryer (Tokyo, Japan). The specimens were sputter-coated with gold and examined with a JEOL JSM-U3 SEM (Tokyo, Japan) operated at 15 kV.

### Results

As many as 570 larvae were obtained from the 3 pelicans in Temazcal. Only 3 larvae were found in 5 egrets in Culiacán. The mean body length (10 larvae) was 4.67 mm, measured in a relaxed state after natural death in cold distilled water. The heads had 4 transverse rows of hooklets (Fig. 1), and the mean number in each row was 40, 44, 47, and 50 hooklets. The typical hooks on the head bulb had sharp tapering points composed of hard keratin that emerged from an oblong chitinous base (Fig. 2). The bodies were wholly covered with minute cuticular spines along their transverse striations. The mean number of striations varied from 227 to 275. A pair of cervical papillae was laterally situated between the 13th and 17th transverse striations (Fig. 3). In most specimens, the papillae were located between the 14th and 15th striations. A ventral excretory pore was located between the 24th and 28th transverse striations (Fig. 4). A wide terminal anal opening was visible on the ventral surface, and the transverse striations on the body were limited to the extent of this opening (Fig. 5). Both ends of the larva had a pair of lateral phasmidial pores (Fig. 6).

The intestinal cells had multiple nuclei in the larvae from Temazcal (Fig. 7). The larvae from Sinaloa had 2 to 7 nuclei in each intestinal cell (Fig. 8).

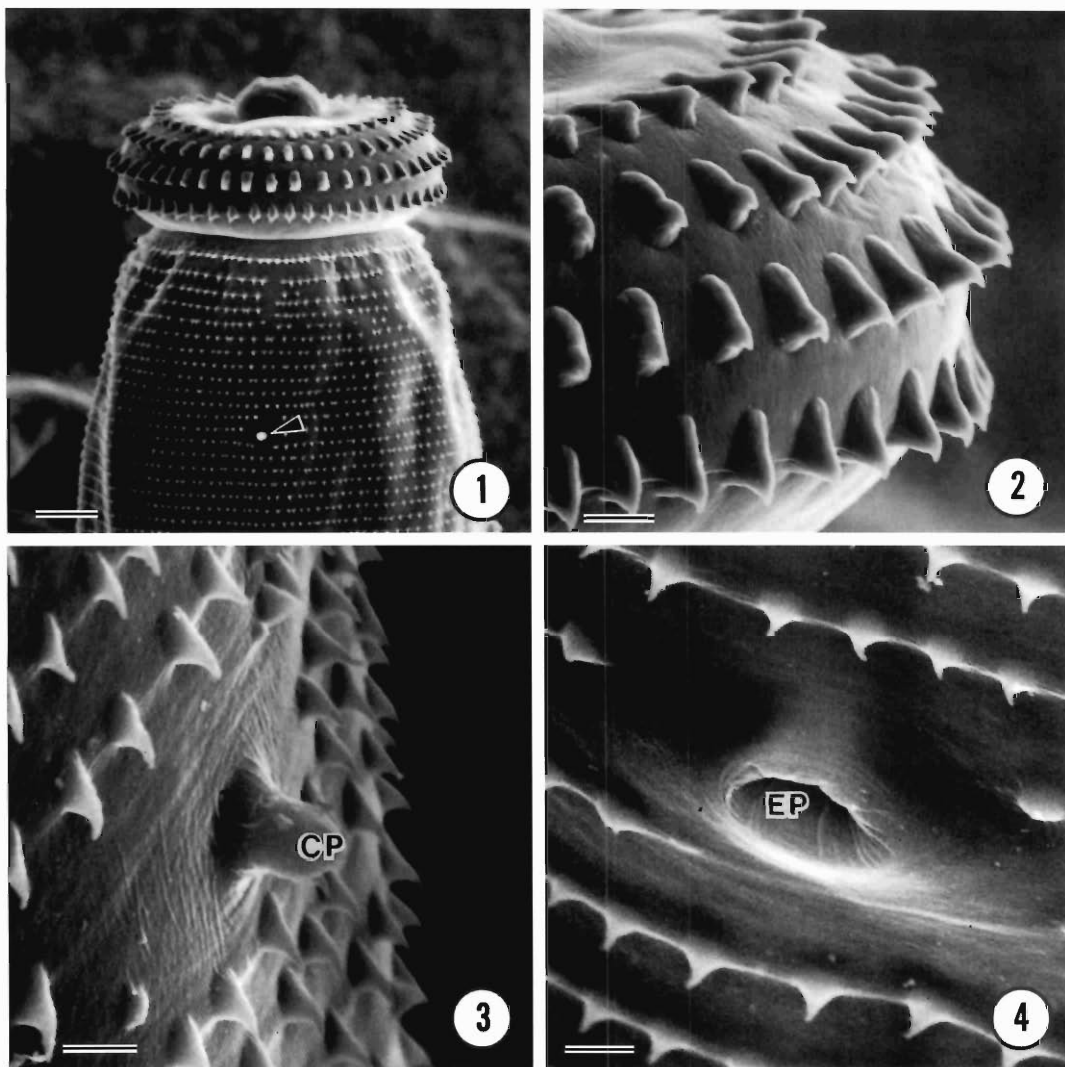
### Discussion

Lamothe-Argumedo et al. (1989) determined their larval gnathostome specimens obtained from Temazcal to be *Gnathostoma* sp. However, based on our observations, their specimens seemed to be the same as those reported by Almeyda-Artigas (1991); both specimens of larvae were from both fish and waterfowl in the same

endemic area of human gnathostomiasis, and the descriptions of the larval morphology were quite similar. We attributed this specimen as *G. binucleatum*. Lamothe-Argumedo et al. (1989) had previously observed larvae in Oaxaca, Temazcal, Mexico. We think that their SEM observations were insufficient, especially regarding the location of excretory pores and numbers of the transverse striations on the larval bodies. We reexamined the Temazcal specimens using SEM and made some new observations. We also examined the surface structures of the specimens from Sinaloa, Culiacán. Previously, 5 specimens from Sinaloa were examined by Camacho et al. (1998) using SEM. They mentioned the numbers of hooklets of 4 rows on the head bulb as 39, 42, 44, and 49. Furthermore, they recognized 1 pair of cervical papillae located between the 13th and 15th striations of the cuticular spines on a single larva. The number of transverse striations on the body was more than 200. There were no descriptions regarding the location of the excretory pore. The locations of the cervical papillae, the excretory pore, and the number of transverse striations are very important for the identification of species of gnathostome larvae. As shown in Table 1, the number of transverse striations is more than 200 in *G. spinigerum*. However, the number is less than 200 in most specimens of *G. doloresi*. On the other hand, the cervical papillae and excretory pores of *G. hispidum* were situated more anteriorly than those of the other 2 species.

In the present study, we compared the larvae from 2 districts in Mexico, Temazcal and Culiacán, and found no differences between them in the larval morphology (Table 1). In particular, the surface ultramorphologies were very similar. However, when our findings were compared with those of *G. spinigerum* in Thailand (Table 1), they were the same, including the shape of the larval hooks, which had oblong chitinous bases and are known to be one of the characteristic structures of *G. spinigerum* (Miyazaki, 1960). Akahane et al. (1994) also compared the number of hooklets in each row on the head bulb of the Temazcal larvae and the larvae of *G. spinigerum* in Thailand by light microscopy and concluded that the numbers of hooklets in Temazcal larvae were slightly less than those of *G. spinigerum*.

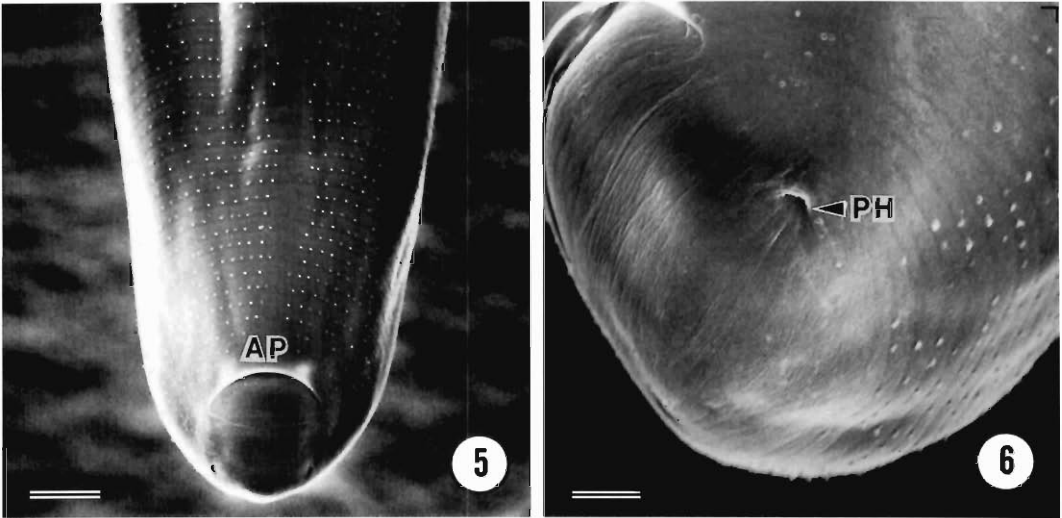
The intestinal epithelium of Temazcal specimens consisted of a single layer of intestinal cells, and each columnar cell had 2 to 5 nuclei



Figures 1-4. Scanning electron micrographs of *Gnathostoma* cf. *binucleatum*. 1. Lateral view of the head bulb of Temazcal specimen. The arrow indicates the cervical papilla. Scale = 50  $\mu\text{m}$ . 2. An enlarged view of the hooklets. The base of each hooklet has an oblong shape. Sharp keratin hooks armed posteriorly. Scale = 10  $\mu\text{m}$ . 3. A mammary form of the cervical papilla (CP) protruding from the tegument. Scale = 3  $\mu\text{m}$ . 4. The oval-shaped opening of the excretory pore (EP), which opens ventrally. Scale = 3  $\mu\text{m}$ .

(Akahane et al., 1994). This feature closely resembles that of the Sinaloa specimen. Once again, no differences were observed in intestinal cells between the larvae from Temazcal and Sinaloa, and we conclude that both should be included in the same species (*G. binucleatum*). Further, we could not differentiate *G. binucleatum* from *G. spinigerum* based on the number of nuclei in the intestinal cells. Most specimens of *G. spinigerum* from Thailand also had 2 to 4

nuclei in the intestinal cells. On the other hand, the number of nuclei in the intestinal cells of other Asian species, e.g., *G. hispidum* and *G. doloresi*, have only 1 nucleus per cell (Akahane et al., 1994). Almeyda-Artigas' light microscopic observations of the larvae were limited regarding the number of hooklets in each row and the number of nuclei in the intestinal cells. Recently Koga et al. (1999) experimentally obtained the adults of this Mexican gnathostome

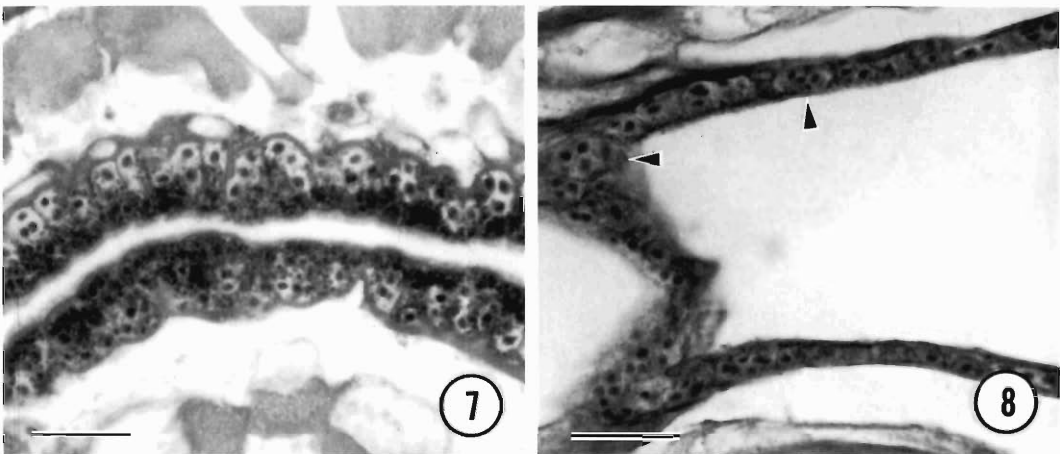


Figures 5, 6. Scanning electron micrographs of *Gnathostoma cf. binucleatum*. 5. The terminal end of a larva where the anal opening (AP) is clearly visible on the ventral surface of the larva, which has a crescent shape. Scale = 3.5  $\mu\text{m}$ . 6. The terminal extremity of a larva, showing a lateral phasmidial pore (PH). Scale = 12  $\mu\text{m}$ .

and found that the eggs have no surface pits. Furthermore, Kuramochi et al. (unpublished data, 1999) found arrangement differences in the mitochondrial DNA of adult Thai specimens of *G. spinigerum* and the adult Mexican gnathostome. Although our SEM observations did not show typical differences in larval stages between these 2 species, we think that this Mexican gnathostome may be a separate species.

Such designation must, however, await a more detailed analysis.

*Gnathostoma spinigerum* was reported in Ecuador in 1981 (Ollague et al., 1981), yet their description remains unclear. The adult of this species should be re-examined more precisely. We assume that this human-infecting Latin American gnathostome may be the same as that of *G. binucleatum*.



Figures 7, 8. Cross-sections of the larval intestines of *Gnathostoma cf. binucleatum*. 7. A cross section of the Temazcal larva. Multiple nuclei are evident in 1 cell. Scale = 20  $\mu\text{m}$ . 8. A cross-section of the Sinaloa larva. Arrows indicate the cells with 5 nuclei each. Scale = 20  $\mu\text{m}$ .

**Table 1. Morphological dimensions of the advanced third-stage larvae of species of *Gnathostoma* (data obtained by SEM).\***

<i>Gnathostoma</i> species: Locality	No. of hooklets on head bulb				Location between transverse striations of		No. of transverse striations (No. of larvae examined)	References
	I	II	III	IV	Cervical papillae	Excretory pore		
<i>G. binucleatum</i> :								
Temazcal	40	43	46	49	12th–13th	about 30th	260 (12)	Lamothe-Argumedo et al. (1989)
Present specimens:								
Temazcal	39	44	46	50	12th–15th	24th–28th	227–225 (10)	This report
Sinaloa	40	44	45	49	12th–15th	23rd–24th	228–256 (3)	This report
<i>G. spinigerum</i> :								
Thailand	40	43	46	50	11th–16th	22nd–28th	225–256 (8)	Koga et al. (1994)
<i>G. doloresi</i> :								
Japan	39	39	36	38	15th–19th	25th–28th	176–211 (10)	Koga and Ishii (1987)
<i>G. hispidum</i> :								
China	40	41	47	48	10th–13th	19th–20th	202–216 (10)	Koga et al. (1988)
<i>G. procyonis</i> :								
U.S.A.	33	37	41	45	ND*	ND	ND (15)	Ash (1962)

\* ND = not described.

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## Report on the Brayton H. Ransom Memorial Trust Fund

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Contributions from Members of the Helminthological Society of Washington:	
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1999 (in memory of Dr. Francis G. Tromba) .....	\$50.00
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## Research Note

# Helminth Parasites in Six Species of Shorebirds (Charadrii) from Bristol Bay, Alaska, U.S.A.

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**ABSTRACT:** Nineteen species of gastrointestinal helminth parasites were recovered from 6 species of charadriid shorebirds (Aves: Charadriiformes) from Bristol Bay, Alaska: the surfbird *Aphriza virgata*, the western sandpiper *Calidris mauri*, the rock sandpiper *Calidris ptilocnemis*, the whimbrel *Numenius phaeopus*, the northern phalarope *Phalaropus lobatus*, and the black-bellied plover *Pluvialis squatarola*. Cestode species were dominant ( $N = 14$ ), followed by trematode species ( $N = 4$ ) and an acanthocephalan ( $N = 1$ ). No nematodes were observed. Only the cestode *Aploparaksis daviesi* infected more than 1 species of host, the surfbird *Aphriza virgata* and the northern phalarope *Phalaropus lobatus*. All species of helminths have been reported from birds on other continents, particularly Eurasia.

**KEY WORDS:** Helminth parasites, Aves, Charadrii, surfbird, *Aphriza virgata*, western sandpiper, *Calidris mauri*, rock sandpiper, *Calidris ptilocnemis*, whimbrel, *Numenius phaeopus*, northern phalarope, *Phalaropus lobatus*, black-bellied plover, *Pluvialis squatarola*, littoral zone, Bristol Bay, Alaska, U.S.A.

Bristol Bay, Alaska, and adjacent tundra provide significant habitat to nesting and postbreeding shorebirds (Suborder Charadrii) (Gill and Handel, 1981). The bay is shallow and shorebirds are easily observed foraging on extensive sand–mud habitats exposed at low tide. Observations and counts (A.G.C.) near the mouth of the Egegik River, Bristol Bay, over the summers of 1991, 1993, 1996, and 1997 indicated arrival and utilization of the littoral zone by large numbers of postbreeding shorebirds. There is no published information on helminth parasites of shorebirds from this important area and very little from the other localities on Bristol Bay. Schmidt and Neiland (1968) recorded 19 species of cestodes and trematodes from 5 species of shorebirds collected on Kvichak Bay, Bristol

Bay. Deblock and Rausch (1968) reported *Aploparaksis leonovi* Spasskii, 1962, and *Aploparaksis stricta* Spasskii, 1962, from the western sandpiper *Calidris mauri* Cabanis, 1857, from the watersheds of Bristol Bay. Van Cleave and Rausch (1950), in the only previous report of helminths from the surfbird *Aphriza virgata* Gmelin, 1789, recovered 2 immature specimens of the acanthocephalan *Arythmorhynchus comptus* Van Cleave and Rausch, 1950, near Juneau, Alaska. We examined these 2 host species and 4 additional species from Bristol Bay and herein report our findings.

Birds were obtained from a 6.0-km-long section of littoral zone just north of the mouth of the Egegik River, Bristol Bay, Alaska, between Bishop Creek (58°14'31"N, 157°29'43"W) and Big Creek (58°17'01"N, 157°32'25"W). All species of hosts were common except the surfbird and rock sandpiper *Calidris ptilocnemis* Coues, 1873, which were rare. Five surfbirds *A. virgata*, 5 western sandpipers *C. mauri*, 4 whimbrels *Numenius phaeopus* Linnaeus, 1758, and 1 *C. ptilocnemis*, were collected in 1996, and 5 *A. virgata*, 10 black-bellied plovers *Pluvialis squatarola* Linnaeus, 1758, 10 northern phalaropes *Phalaropus lobatus* Linnaeus, 1758, in 1997. Birds were collected with a shotgun between July 2 and July 23 and examined within 6 hr. All internal organs were examined. The koilon of the ventriculus was removed, and both the ventriculus and proventriculus tissues were teased apart. Skin and blood were not examined for parasites.

Acanthocephalans, cestodes, and trematodes were fixed and preserved in alcohol–formalin–acetic acid, stained in Ehrlich's hematoxylin, cleared in methyl salicylate, and mounted in Canada balsam. Voucher specimens were deposited in the United States National Helminthol-

<sup>3</sup> Corresponding author.

**Table 1. Helminth parasites of 6 species of shorebirds (Charadrii) from Bristol Bay, Alaska, U.S.A.**

Host and parasite	Number infected	Mean intensity	Range	Other localities
Black-bellied plover, <i>Pluvialis squatarola</i> Linnaeus, 1758 ( <i>N</i> = 10)				
<i>Anomotaenia ericetorum</i> (Krabbe, 1869)	7	14.9	1–72	Europe
<i>Aploparaksis diagonalis</i> Spasskii and Bobova, 1961	1	0.1	—	Russia
<i>Liga brevis</i> (Linstow, 1884)	5	11.7	6–55	Eurasia
<i>Proterogynotaenia variabilis</i> Belopol'skya, 1954	5	129.5	1–1,155	Russia
<i>Schistocephalus solidus</i> (Mueller, 1776)	1	0.1	—	Eurasia, North America
<i>Wardium squatarolae</i> Kornyushin, 1970	4	4.6	1–34	Eurasia
<i>Echinoparyphium recurvatum</i> (Linstow, 1873)	6	62.0	1–468	Cosmopolitan
<i>Polymorphus magnus</i> (Southwell, 1927)	1	0.2	—	Russia
Northern phalarope <i>Phalaropus lobatus</i> Linnaeus, 1758 ( <i>N</i> = 10)				
<i>Aploparaksis daviesi</i> Deblock and Rausch, 1968	1	0.2	—	Alaska, U.S.A.
<i>Echinocotyle tenuis</i> Clerc, 1906	2	0.4	1–3	Russia
<i>Trichocephaloides megalcephala</i> (Krabbe, 1869)	1	0.1	—	Eurasia
<i>Plagiorchis morosovi</i> Sobolev, 1946	3	0.7	1–3	Russia
Surfbird <i>Aphriza virgata</i> Gmelin, 1789 ( <i>N</i> = 10)				
<i>Aploparaksis diagonalis</i> Spasskii and Bobova, 1961	6	10.5	1–56	Africa, Eurasia
<i>Dictyometra nymphaea</i> (Schränk, 1790)	8	22.4	2–66	Eurasia
<i>Lacunovermes</i> sp. Ching, 1965	1	89.3	—	British Columbia, Canada
Western sandpiper <i>Calidris mauri</i> Cabanis, 1857 ( <i>N</i> = 5)				
<i>Aploparaksis leonovi</i> Spasskii, 1961	2	7.4	1–36	Russia
<i>Kowalewskiella cingulifera</i> (Krabbe, 1869)	2	2.6	1–12	Eurasia, Guadeloupe
Whimbrel <i>Numenius phaeopus</i> Linnaeus, 1758 ( <i>N</i> = 4)				
<i>Brachylaima fuscatum</i> (Rudolphi, 1819)	1	0.25	—	Australia, Europe, North America, Russia
Rock sandpiper <i>Calidris ptilocnemis</i> Coues, 1873 ( <i>N</i> = 1)				
<i>Wardium amphitricha</i> (Rudolphi, 1819)	1	2.0	—	Europe, North America, Russia

ological Collection, Beltsville, Maryland, U.S.A., accession numbers 89038–89055.

Nineteen species of helminths were recovered from the 6 species of hosts. Cestode species were dominant (*N* = 14), followed by trematode species (*N* = 4) and an acanthocephalan (*N* = 1). No nematodes were observed. Each of the 6 species of host was parasitized by at least 1 helminth species. Only the cestode *Aploparaksis daviesi* Deblock and Rausch, 1968, infected more than 1 species of host—the surfbird *A. virgata* and northern phalarope *P. lobatus* (Table 1). All are new host records for Alaska. All species of helminths were previously reported from birds on other continents, particularly from Eurasia (Table 1).

Generally, trematode species are dominant in marine habitats, and cestodes are dominant in freshwater environments (Bush, 1990; Canaris and Kinsella, 1998). In both our study and that

by Schmidt and Neiland (1968), cestode species were dominant (72% and 79%, respectively). This may reflect the hosts' recent association with the terrestrial (freshwater) nesting area, an absence of proper intermediate molluscan hosts for trematodes in Bristol Bay, or both. Also, it may reflect early summer season examination of hosts in both studies. In this study, the bulk of the trematodes was obtained later in July. Trematodes obtained earlier in July were often immature or recently mature, as indicated by the presence of small numbers of eggs and lack of pigmentation of the eggshell. Small numbers or absence of species of acanthocephalans and nematodes in Bristol Bay have also been found in studies done in Canada on 3 species of shorebirds: the long-billed curlew *Numenius americanus* Bechstein, 1812 (Goater and Bush, 1988); the American avocet *Recurvirostra americana* Gmelin, 1789 (Edwards and Bush, 1989); and

the whimbrel *Catoptrophorus semipalmatus* Gmelin, 1789 (Bush, 1990). The absence of nematodes from the upper digestive tract in this study is also somewhat puzzling. Anderson et al. (1996) reviewed records for these nematodes in shorebirds from North and South America. As in the present study, they found no species of the genus *Skrjabinoclava* Sobolev, 1943, or ventricular nematodes in 15 surfbirds (*A. virgata*) or 44 northern phalaropes (*P. lobatus*). However, species of *Skrjabinoclava* were common in 83 black-bellied plovers (*P. squatarola*), 93 western sandpipers (*C. mauri*), and 8 whimbrels (*N. phaeopus*). It is possible that the intermediate hosts of these nematodes are absent in Bristol Bay or that they were not detected in our relatively small sample sizes. Part of the explanation, at least in our study, was that skin and blood were not examined for nematodes.

Most helminths reported herein are not host specific (Baer, 1962; Deblock and Rausch, 1968; Schmidt and Neiland, 1968). Low overlap in helminth species may be attributed to small sample size, but it may also be influenced by specialized feeding habits of shorebirds (Storer, 1971) prior to arrival from the nesting grounds and in Bristol Bay. Natural sorting out of shorebird species into preferred feeding habitats as the tide recedes, as reported by Ehrlich et al. (1988), is easily observed (A.G.C.) on the sand-mud habitats of Bristol Bay.

All species of shorebirds nesting at Bristol Bay migrate to more distant southern wintering localities, many to other continents. We expect that further studies will reveal more relationships of helminth species of shorebirds on Bristol Bay to those from distant localities.

The littoral zone of Bristol Bay is an important postbreeding locality for many species of shorebirds. Studies of helminth communities need to be extended, in both time and location, to understand the dynamics of the helminth communities and their interactions among the many species of shorebirds during the long days but relatively short summer.

We wish to thank Hilda Ching for her opinion on *Lacunovermis* sp. and Jerry Solie and Jerry

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### Research Note

## *Colobomatus embiotocae* (Copepoda: Philichthyidae) from Shiner Perch, *Cymatogaster aggregata* (Osteichthyes: Embiotocidae) in Canadian Waters

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**ABSTRACT:** During an examination of the parasitic crustacean fauna of shiner perch, *Cymatogaster aggregata* (Embiotocidae) from eastern Vancouver Island in Nanaimo, British Columbia, Canada, the copepod, *Colobomatus embiotocae* Noble, Collard, and Wilkes, 1969 (Philichthyidae), was noted in the sensory ducts of the preopercular cephalic canals. Prevalence and mean intensity of *C. embiotocae* were 59.2% and  $1.36 \pm 0.57$ , respectively. This parasite was also recovered from 68.4% (mean intensity =  $1.62 \pm 0.65$ ) of shiner perch sampled near Bamfield Marine Station on the western coast of Vancouver Island. The high prevalence of *C. embiotocae* probably reflects increased transmission resulting from the aggregation behavior of the fish host. These results establish a range extension for *C. embiotocae* in *C. aggregata* to include Canadian Pacific waters.

**KEY WORDS:** *Colobomatus embiotocae*, Copepoda, shiner perch, *Cymatogaster aggregata*, British Columbia, Canada.

Members of the poecilostome family Philichthyidae are endoparasitic copepods that occupy the subcutaneous spaces associated with the sensory canals of the skull bones and lateral line of marine fishes (Kabata, 1979). They are highly specialized parasitic copepods, with pronounced sexual dimorphism and females exhibiting reduced organs of attachment, reduced appendages, and bizarre morphological processes projecting from their bodies.

The richest genus of this family, *Colobomatus*, is recorded from a diversity of marine teleosts and elasmobranchs (Kabata, 1979; West, 1992). *Colobomatus embiotocae* Noble, Collard, and Wilkes, 1969, was first described from shiner perch, *Cymatogaster aggregata* Gibbons, 1854, and was found infecting several other spe-

cies of embiotocid fishes in California and Oregon in the United States and in Mexico (Noble et al., 1969). Samples were not collected from Canadian waters, though the range of *C. aggregata*, among the most widely distributed embiotocid fish species, extends from Port Wrangel, Alaska, U.S.A., to Quintin Bay, Baja California, Mexico (Odenweller, 1975). Arai et al. (1988) did not find *C. embiotocae* during their study of metazoan parasites of *C. aggregata* from British Columbia. To date, the only species of *Colobomatus* recorded from Canadian waters is *Colobomatus kyphosus* Sekerak, 1970, from *Sebastes alutus* Gilbert, 1890, and several species of *Sebastes* (Sekerak, 1970; Sekerak and Arai, 1977; Kabata, 1988).

Females and males of *C. embiotocae* have 11 body segments; in the female the fourth and fifth are fused. The average length for females and males is approximately 3.7 mm and 1.2 mm, respectively (Noble et al., 1969). Diagnostic morphological features distinguishing the female parasite from other species of *Colobomatus* include the caudal furcae with a spine on their inside lateral surfaces, the egg-laying apparatus with a bulbous structure equipped with a flagellate seta, and 3 eyes arranged in a compact cluster. Males of *C. embiotocae* are distinguished on the basis of their 6-segmented first antennae and 1-segmented mandibles (Noble et al., 1969).

During an investigation of the parasitic crustacean fauna of *C. aggregata* from Piper's Lagoon, Nanaimo, British Columbia, males and females of *C. embiotocae* were noticed infecting the sensory canals of the skull. A total of 76 *C. aggregata* was seined from the littoral region during March 1996, returned to the laboratory, and killed in concentrated anesthetic (MS-222), and their cephalic sensory canals and lateral

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lines were carefully examined. Live males and females of *C. embiotocae* were teased out of the canals with fine needles. The prevalence and mean  $\pm$  SD intensity were 59.2% and  $1.36 \pm 0.57$ , respectively. There was no association between host size and copepod intensity ( $n = 45$ ,  $r = 0.09$ ,  $P = 0.557$ ). Females were less prevalent (22.4% females vs. 48.7% males) and abundant (mean intensity of  $1.0 \pm 0$  females vs.  $1.19 \pm 0.46$  males) than males. There was no significant difference in the intensity of males compared with the intensity of females in infected hosts ( $F_{1,54} = 2.99$ ,  $P = 0.09$ ).

*Colobomatus embiotocae* were also present in *C. aggregata* caught in a trawl (March 1996) in Trevor Channel on the western coast of Vancouver Island near the Bamfield Marine Station, British Columbia. Nineteen fish were necropsied for the presence of *C. embiotocae* in the cephalic canals. The prevalence was 68.4% and the mean intensity was  $1.62 \pm 0.65$ . The aggregating behavior characteristic of this fish species may be one factor explaining the high prevalence of this parasite, because host aggregation likely increases contact with *C. embiotocae* larvae.

Only 1 of the 95 fish examined from both localities had 2 female *C. embiotocae* sharing the same canal. The presence of a gravid female within the cephalic sensory canals may prevent or inhibit other females from establishing themselves within such a space-constrained microhabitat. The 2 females were found aligned head to furca in the left preopercular canal. All females were recovered from either the left or right preopercular canals. Males were found in all of the skull's sensory canals. Only 1 male was recovered from the lateral line, and several males were observed exiting the fish via the pores associated with the sensory canals.

These observations establish the first definitive record of *C. embiotocae* in Canadian eastern Pacific waters and add another species to the diverse list of shiner perch parasites in Canada (Margolis and Arthur, 1979; McDonald and Margolis, 1995). Very little is known about the population biology of philichthyid copepods, probably because they are endoparasites inhabiting a unique and seldom studied microhabitat (Kabata, 1988) and are mostly found in fish species that are of limited commercial importance (West, 1992). We urge other investigators to include the sensory canals and lateral line system

of marine fish as sites to routinely examine for these copepods. The site and host specificity of these unusual parasites might inspire experimental and field-based studies examining how seasonality and aspects of the fish host's behavior and ecology interact to influence the parasite's transmission dynamics.

Voucher specimens have been deposited in the United States National Parasite Collection, Bethesda, Maryland, U.S.A. (USNPC accession No. 87634). We thank Jason Lewis for assisting with fish collections and Cameron Weighill for necropsy assistance. The manuscript benefited from constructive comments by Bob Kabata and Cam Goater.

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**Research Note**

**Parasites of the Green Treefrog, *Hyla cinerea*, from Orange Lake, Alachua County, Florida, U.S.A.**

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**ABSTRACT:** Four species of parasites (1 trematode, 2 nematodes, and 1 protozoan) were identified from 60 green treefrogs, *Hyla cinerea* (Schneider), collected in north-central Florida, U.S.A. The most prevalent parasites were the nematode *Cosmocercella haberi* (Steiner) Baker and Adamson (23%) and the protozoan *Opalina* sp. Purkinje and Valentin (47%). The trematode, *Clinostomum attenuatum* Cort, had a prevalence of 2%, and the other nematode, *Rhabdias* sp. Stiles and Hassall, had a prevalence of 5%. Seven females and seven males were infected with *C. haberi*. The prevalence and intensity of *C. haberi* were correlated positively with host size (wet weight and snout-vent length). There was no statistically significant difference between gender and intensity of *C. haberi* infection. Fourteen females and 14 males were infected with *Opalina* sp. The prevalence of *Opalina* sp. was correlated negatively with host size. Both *C. haberi* and *Opalina* sp. have been reported previously from *H. cinerea*. The green treefrog represents a new host record for *C. attenuatum* and *Rhabdias* sp.

**KEY WORDS:** *Hyla cinerea*, Hylidae, green treefrog, helminths, Trematoda, *Clinostomum attenuatum*, Nematoda, *Cosmocercella haberi*, *Rhabdias* sp., Protozoa, *Opalina* sp., prevalence, intensity, Florida, U.S.A.

The green treefrog, *Hyla cinerea* (Schneider, 1799), is a small, bright green, yellow, or greenish-gray treefrog with a sharply defined light stripe along the upper jaw and side of the body. Its North American range extends from Delaware south to the Florida Keys, west to Texas, and north to Illinois. *Hyla cinerea* is found predominantly near permanent water. In the southern parts of its range it breeds from March to October (Behler and King, 1997) and is probably the most abundant species of treefrog in the Gainesville region of north-central Florida (Kilby, 1945).

Many parasites have been reported from hylid frogs in the United States and Canada. Walton (1946) listed primarily nematodes, trematodes, and protozoans as being parasitic in *H. cinerea*. Esch and Fernandez (1993) suggested factors that may influence parasite populations. Two of these included gender and host age or, as may be inferred for some animals, host size. To our knowledge there is no standard aging technique for treefrogs; however, Koller and Gaudin (1977) stated that “larger (hence older) frogs” usually have a greater species diversity and greater intensity of infections than “smaller, younger individuals.” It was assumed for this study that host size is a rough indicator of host age.

We are not aware of any comprehensive studies on the parasites of green treefrogs in Florida. The purpose of this study was to examine the parasites of *H. cinerea* in north-central Florida, to determine the prevalence and intensity of parasitic infections, and to determine whether relationships exist between gender, wet weight, snout-vent length, and parasitic infections.

Sixty green treefrogs were collected from Orange Lake (29°27'20"N 082°10'20"W), about 32 km southeast of Gainesville, Florida, U.S.A. Treefrogs were collected from a small stand of oak trees at the edge of the lake using the PVC pipe technique described by Boughton (1997). The PVC pipes were checked twice a week, during the day. Thirty treefrogs were collected from September to October 1998, and 30 treefrogs were collected from January to February 1999. All laboratory work was conducted at the Wildlife Disease Research Laboratory of the University of Florida's College of Veterinary Medicine. Treefrogs were killed with tricaine methane sulfonate (MS-222) following the methods of Gold-

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**Table 1. Prevalence, intensity, abundance, and location of parasites in 60 green treefrogs collected from Orange Lake, Alachua County, Florida, U.S.A., 1998–1999.**

Parasite species	Prevalence (%)	Intensity		Abundance	Location*
		Mean	Range		
Trematoda					
<i>Clinostomum attenuatum</i> †	2	1	—	0.02	SK
Nematoda					
<i>Cosmocercella haberi</i>	23	94	1-236	21.6	CL,LI,SI,ST
<i>Rhabdias</i> sp.†	5	3	2-5	0.15	LU
Protozoa					
<i>Opalina</i> sp.	47	—	—	—	CL,LI,SI

\* Location in host: CL = cloaca; LI = large intestine; LU = lungs; SI = small intestine; SK = skin; ST = stomach.

† New host record.

berg et al. (1996) and dissected within 24 hours of capture. Gender, wet weight, and snout-vent length were recorded for each individual. The skin, liver, heart, lungs, esophagus, stomach, small intestine, large intestine, cloaca, bladder, and kidneys were evaluated for parasites in separate Petri dishes under a dissecting microscope. Protozoans were fixed in Zn-PVA and stained with Giemsa. The trematode was fixed in Roudabush's AFA, stained with acetocarmine, and mounted in neutral Canada balsam. Nematodes were fixed in 70% ethanol containing 10% glycerine and mounted in lactophenol for identification. Voucher specimens have been deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A. The prevalence and intensity of parasites were correlated with wet weights and snout-vent lengths of *H. cinerea* using Pearson product moment correlations. A *t*-test was used to determine whether gender was related to intensity of *Cosmocercella haberi* (Steiner, 1924) Baker and Adamson, 1977, infections (Minitab, 1998). We did not conduct statistical tests on *Clinostomum attenuatum* Cort, 1913, and *Rhabdias* sp. Stiles and Hassall, 1905, because of their low prevalences. Terminology used follows Bush et al. (1997).

Thirty-one female and 29 male green treefrogs were collected from Orange Lake (mean wet weight  $\pm$  SD = 3.5 g  $\pm$  1.5 g; mean snout-vent length  $\pm$  SD = 4.2 cm  $\pm$  0.6 cm). The prevalences, intensities, abundances, and locations of parasites are listed in Table 1. Twenty-two treefrogs had no parasites, 22 had only *Opalina* sp., 8 had only *C. haberi*, 4 had both

*C. haberi* and *Opalina* sp., 2 had both *C. haberi* and *Rhabdias* sp., 1 had both *Rhabdias* sp. and *Opalina* sp., and 1 had both *C. attenuatum* and *Opalina* sp. No lesions were associated with the parasites.

One green treefrog was infected with *C. attenuatum* (USNPC No. 88956) encysted under the skin on the back. We used 2 features to identify the trematode as *C. attenuatum* rather than *C. complanatum*, which also occurs in amphibians (McAllister, 1990): the body is uniform in width (rather than wider in the hindbody as in *C. complanatum*), and the testes and ovary are postequatorial (rather than medial as in *C. complanatum*) (Cort, 1913; Baer, 1933; Ukoli, 1966). Yamaguti (1971) indicated that *C. attenuatum* is found in frogs, primarily species of the genera *Bufo* Laurenti, 1768, and *Rana* Linnaeus, 1758. The definitive hosts include the great blue heron (*Ardea herodias* Linnaeus, 1758), American bittern (*Botaurus lentiginosus* Rackett, 1813), green-backed heron (*Butorides striatus* Linnaeus, 1758), and double-crested cormorant (*Phalacrocorax auritus* Lesson, 1831). *Hyla cinerea* is a new host record for *C. attenuatum*.

Fourteen green treefrogs were infected with *C. haberi* (USNPC Nos. 88959 and 88960). *Cosmocercella haberi* has been reported previously in *H. cinerea* by Steiner (1924) and Walton (1946). A voucher specimen of *C. haberi* from *H. cinerea* was collected in Arkansas and deposited in the USNPC by C. T. McAllister in 1994 (USNPC No. 84259). This nematode is a fairly common parasite of hylids and has been identified in other species such as *Hyla versicolor* LeConte, 1825; *Hyla arenicolor* Cope,

1866; and *Hyla wrightorum* (Taylor, 1939) (Campbell, 1968; Goldberg et al., 1996). *Cosmocercella haberi* was found in the stomach, small intestine, large intestine, and cloaca of *H. cinerea*. Seven females and 7 males were infected with the parasite. The prevalence of *C. haberi* was correlated positively with the wet weights ( $r = 0.283$ ,  $P = 0.029$ ) and snout-vent lengths ( $r = 0.268$ ,  $P = 0.039$ ) of *H. cinerea*. There was no statistically significant difference between gender and intensity of *C. haberi* infection. The intensity of *C. haberi* infection was correlated positively with the wet weights ( $r = 0.678$ ,  $P = 0.008$ ) and snout-vent lengths ( $r = 0.760$ ,  $P = 0.002$ ) of the 14 infected green treefrogs.

Three green treefrogs were infected with *Rhabdias* sp. (USNPC No. 88958). This nematode was found at low intensities in the lungs. *Rhabdias* spp. are considered "cosmopolitan" in reptiles and amphibians (Baker, 1978). Other hyliids, such as *Hyla regilla* Baird and Girard, 1852, and *Pseudacris crucifer* (Wied-Neuwied, 1838) have been reported having these parasites (Koller and Gaudin, 1977; Muzzall and Peebles, 1991; Yoder and Coggins, 1996). There is no record of *Rhabdias* sp. from *H. cinerea*.

Twenty-eight green treefrogs were infected with *Opalina* sp. Purkinje and Valentin, 1835 (USNPC No. 88957). *Opalina obtrigonoidea orbiculata* has been reported previously in the green treefrog by Walton (1946). A voucher specimen of *Opalina* sp. from *H. cinerea* was collected in Arkansas and deposited in the USNPC by C. T. McAllister in 1994 (USNPC No. 84277). *Opalina* sp. is a common parasite in treefrogs (McAllister, 1991). It has also been reported in *Hyla avivoca* Viosca, 1928; *Pseudacris clarkii* Baird, 1854; and *Hyla chrysoscelis* Cope, 1880 (McAllister, 1991; McAllister et al., 1993; Bolek and Coggins, 1998). *Opalina* sp. was found at high intensities in the small intestine, large intestine, and cloaca of *H. cinerea*. Fourteen females and 14 males were infected with this protozoan. The prevalence of *Opalina* sp. was correlated negatively with the wet weights ( $r = -0.357$ ,  $P = 0.005$ ) and snout-vent lengths ( $r = -0.387$ ,  $P = 0.002$ ) of *H. cinerea*. Schorr et al. (1990) studied the population changes of *Opalina* spp. and found population declines and even loss of the parasite at metamorphosis of some anurans. No change, however, was observed in others. They attribut-

ed the decline or loss of *Opalina* spp. in some hosts to morphological and physiological changes in the host at metamorphosis. The negative correlation of host wet weights and snout-vent lengths with prevalence of *Opalina* sp. may therefore be due to the loss of parasites as the treefrogs increase in size or age. The intensity of *Opalina* sp. infection was not determined, because of the large numbers of *Opalina* sp. per treefrog.

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### Research Note

## Atypical Specimens of Helminth Parasites (*Anoplocephala perfoliata* and *Thelazia lacrymalis*) of Horses in Kentucky, U.S.A.

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**ABSTRACT:** During a survey of internal parasites in horses at necropsy at a diagnostic laboratory in Kentucky, U.S.A., in 1998, atypical specimens of 2 species were found. Two specimens of the cecal tapeworm,

*Anoplocephala perfoliata*, were fused at the midportion of each individual. One eyeworm, *Thelazia lacrymalis*, had 3 rather than the normal 2 uteri.

**KEY WORDS:** atypical morphology, Cestoda, cecal tapeworm, *Anoplocephala perfoliata*, Nematoda, eyeworm, *Thelazia lacrymalis*, horses, Kentucky, U.S.A.

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Several horses, all with unknown antiparasitic treatment, were examined at necropsy in Kentucky, U.S.A., in 1998 in a prevalence survey for various species of internal parasites. Specimens of 2 species were atypical. One was the cecal tapeworm, *Anoplocephala perfoliata* (Goeze, 1782) Blanchard, 1848. The other was the eyeworm, *Thelazia lacrymalis* (Gurlt, 1831) Railliet and Henry, 1910.

The usual habitat of *A. perfoliata* in the horse is the large intestine, mainly the cecum. In past surveys of dead horses in Kentucky, prevalence of *A. perfoliata* was about 50–60% and no differences in infection with age of the horse were found (Benton and Lyons, 1994). Detrimental effects of *A. perfoliata* are not always evident. Some of the problems, mainly at the attachment sites of the tapeworms, are ulceration, inflammation, edema, and a resulting diphtheritic membrane (Proudman and Trees, 1999). Possible life-threatening effects attributed to *A. perfoliata* are intussusception, perforation, and hypertrophied small intestine (Proudman and Trees, 1999).

Among 265 *A. perfoliata* found in a 29-year-old Thoroughbred gelding in the present study were 2 atypical specimens joined together at the midportion (Fig. 1). Possibly there had been incomplete separation of 2 eggs during embryogenesis. Alternatively, in early development, there may have been injury to 1 specimen, and the other somehow partially invaded the afflicted individual. The authors were unable to find any reference in the literature to this type of anomaly in *A. perfoliata*. However, several other types of abnormalities, including 1–4 extra suckers on the scolex and tri- and tetra- radiate strobila, have been reported for *A. perfoliata* (Lyons et al., 1997).

*Thelazia lacrymalis* uses muscid flies, e.g., *Musca autumnalis* (deGeer, 1776), as intermediate hosts. Negative effects caused by *T. lacrymalis* are usually limited to conjunctivitis and excessive lacrimation (Patton and McCracken, 1981). In past surveys for *T. lacrymalis*, about 40–50% of horses under 5–6 years of age were infected; older horses had much lower prevalences (Lyons et al., 1986). This eyeworm species is associated with several parts of the eyes, including the lacrimal glands, lacrimal ducts, conjunctival sac, and nictitating membrane gland plus ducts. Typically, females in most groups of nematodes have a double or bifurcate

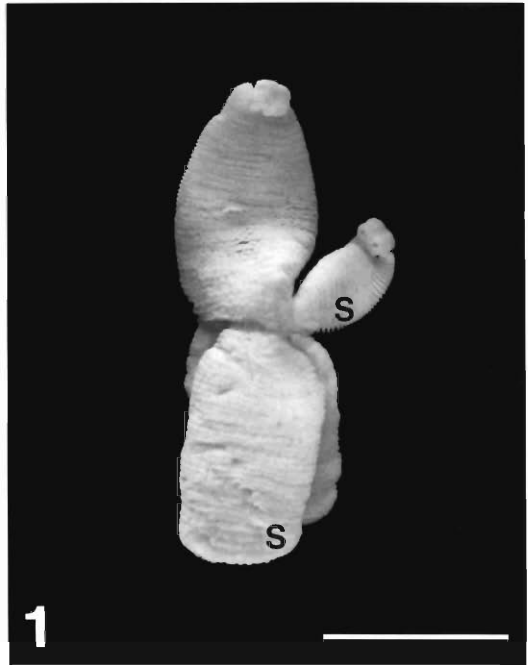


Figure 1. Anomalous *Anoplocephala perfoliata*: 2 specimens joined in midportion, 1 smaller (S) than the other. Scale bar = 10.0 mm.

reproductive system consisting of a vulva, vagina, and 2 uteri (Fig. 2A) and 2 ovaries. In the present study, 1 of 3 female *T. lacrymalis* recovered from the eyes of a yearling male Thoroughbred had 3 uteri (Fig. 2B). This aberration was observed by chance, because all female *T. lacrymalis* in the survey were examined for the presence of embryos with the aid of a compound microscope. The specimen with the 3 uteri accidentally ruptured at the location shown in the accompanying photomicrograph (Fig. 2B), which was taken to record the embryos. Later, it was realized that the presence of 3 uteri was not normal. No references could be found regarding such an anomaly in *T. lacrymalis*. Hyman (1951) mentioned that polydelphic female nematodes may have more than 2, and as many as 10 or 11, ovaries and uteri. This situation occurs particularly in the Physalopteridae, which are spirurids (Hyman, 1951). *Thelazia* spp., while also spirurids, are in a different family. Chandler (1924) found 3 instead of the usual 2 ovaries and uteri in the ascarid, *Ascaris lumbricoides* (Linnaeus, 1758), and considered this highly unusual.

Causes of anomalies of internal parasites are

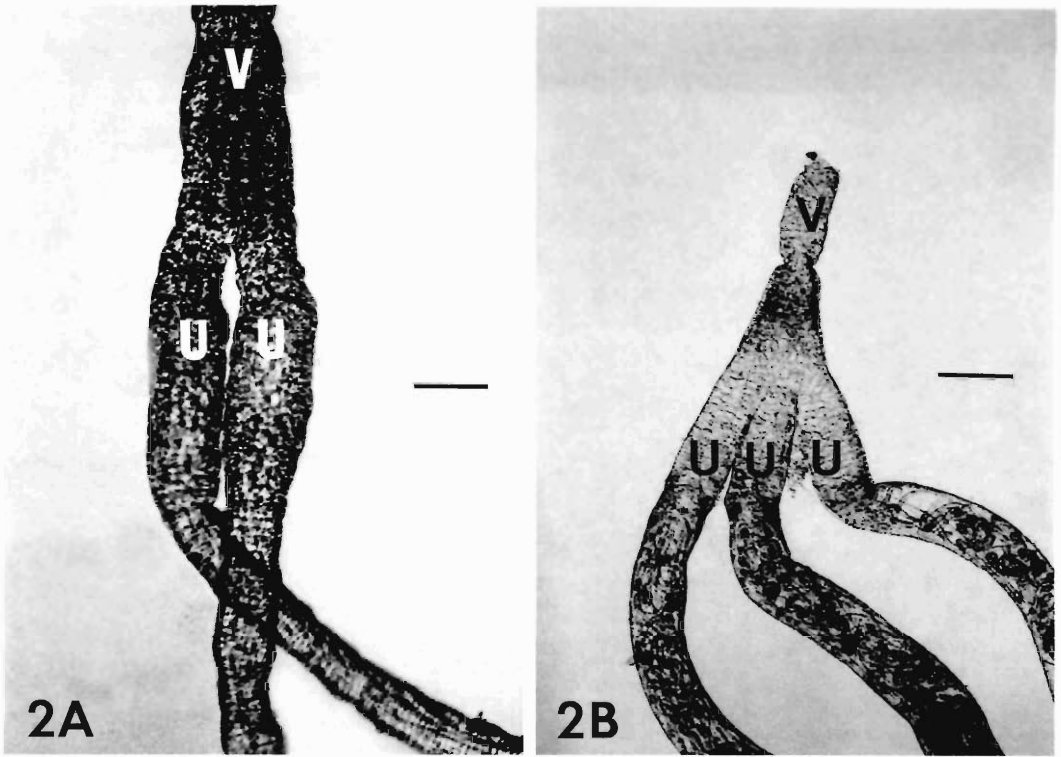


Figure 2. Photomicrographs of *Thelazia lacrymalis*. Part of vagina (V) and uterus (U). A. Normal individual with 2 uteri. B. Abnormal individual with 3 uteri and embryos visible in each branch. Scale bar = 50  $\mu$ m.

difficult to document. It is of interest that Becklund (1960) recorded an association of phenothiazine given to sheep and morphological anomalies of male *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898.

This investigation was done in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the director as paper No. 99-14-120.

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## Anniversary Award

### The Helminthological Society of Washington

FRANK W. DOUVRES



**J. Ralph Lichtenfels, right, presents the 1999 Anniversary Award to Frank W. Douvres**

Mr. President, Members and Guests, Ladies and Gentlemen, as Chair of the Awards Committee, I am honored to be able to present, on behalf of the Helminthological Society of Washington, the 1999 Anniversary Award to an outstanding scientist, the world authority on the *in vitro* cultivation of nematode parasites of livestock and a friend and mentor to many in our society, Dr. Frank W. Douvres.

Frank was born to immigrant parents in the Borough of Harlem in New York City, April 16, 1927. He grew up there, speaking Greek at home, and received an outstanding education at Benjamin Franklin High School, where he graduated in 1943 at the age of 16, ranking third in his class, just behind his classmate Daniel Patrick Moynihan. Frank's classmates correctly predicted that Moynihan would go into politics, but they were off the mark when they predicted that Frank Douvres would become a Russian Commissar. This prediction was based on Frank's outspoken support for the Russian war effort against Germany in World War II.

Frank completed 2 years of premed at Fordham University in December 1944. He transferred to the University of Maryland in January 1945, again in premed, but April 12, 1945, just before his eighteenth birthday, he enlisted in the navy as a hospital corpsman. On learning that Frank had enlisted, Germany immediately surrendered. Later, when Frank completed basic training, Japan surrendered!

Frank was discharged from the Navy in 1947 and returned to the University of Maryland, where he switched his major to microbiology and received his B.S. degree in 1948, before reaching the age of 20 yr. At the University of Maryland Frank began to meet some really interesting people who called themselves parasitologists, so he decided to stay and work on a graduate degree. He was interested in ichthyology and completed his Master of Science degree at Maryland in 1951 after completing a study program that included a survey of the parasites of fish.

The parasitologist at Maryland was William O. Negherbon, who counted among his students Frank Tromba, T. Bonner Stewart, Conrad Yunker, Will Smith, and Les Costello. Professor Negherbon was studying rabies and he hired Frank Tromba to collect little brown bats, for which he paid \$2 each. Douvres helped Tromba collect bats and along the way discovered a new stomach

worm, *Rictularia lucifugus* (Douvres, 1956) in the bats. Frank published the description of the new species in the *Proceedings of the Helminthological Society of Washington*. He went on to complete a Ph.D. at the University of Maryland on the microanatomy of *Rictularia lucifugus* under Professor Josh Brown in 1958.

In 1953 Frank married Angelica "Kiki" Vlangas, whom he met in the Greek community of Baltimore. Typical of Frank, he told Kiki on their first date that he was going to marry her. After working briefly as a cook in a New York diner (and seriously considering staying in the restaurant business), Frank followed the example of his fellow graduate students Frank Tromba and Bonner Stewart and obtained a job with the U.S. Department of Agriculture. Frank was hired by Benjamin Schwartz, Chief of the Zoological Division of the Bureau of Animal Industry, who had obtained some new money for work on parasites of cattle.

His first assignment was at Tifton, Georgia. Frank worked at Tifton with Harry Herlich, Bonner Stewart, and Dale Porter from 1953 until 1955 on parasites of cattle. It was at Tifton where Frank did his landmark work on "The Morphogenesis of the Parasitic Stages of *Ostertagia ostertagi*," the "Morphogenesis of the Parasitic Stages of *Trichostrongylus axei* and *T. colubriformis*," and "Keys to the Identification and Differentiation of the Immature Parasitic Stages of Gastrointestinal Nematodes of Cattle." These papers are standard references, still in use today.

After transferring to Beltsville in 1955, Frank teamed up again with his old pal from graduate school, Frank Tromba, and with John Lucker on numerous studies on the morphogenesis and development of nematode parasites of cattle, sheep, and pigs.

During the time Frank was a student at Maryland and later at Beltsville, he, like many of us, was fortunate to have available the advice and expertise of MayBelle Chitwood. Frank called her "coach."

In 1959, Lou Diamond, who was then working at Beltsville, invited Frank to try some of his nematodes in Diamond's media developed for the in vitro culture of protozoa. The success that they had with these experiments changed the direction of Frank's research. For the next 25 years, Frank Douvres made breakthrough after breakthrough in successfully culturing important nematode parasites of large food animals in clear, cell-free media.

In addition to Lou Diamond, Frank credits Paul Weinstein with mentoring his early in vitro cultivation work. Clearly, however, Frank Douvres became the recognized world authority on the in vitro cultivation of nematode parasites of animals. He collaborated with Frank Tromba on the cultivation and the description of developmental stages of parasites of swine, including *Stephanurus dentatus* and *Ascaris suum*, and, with John Lucker, Halsey Vegors, Don Thompson, and later Harry Herlich, Rob Rew and Lou Gasbarre, on parasites of cattle.

From the late 1960s until Frank retired in 1985, he was assisted by George Malakatis, a world-class technician with an international reputation of excellence, earned first in the navy with Bob Kuntz and Harry Hoogstraal and later at Beltsville with Frank.

In the early 1980s Frank began a short but extremely productive collaboration with Joe Urban that included numerous papers, perhaps the most significant of which were (1) Douvres and Urban. 1983. Factors contributing to the in vitro development of *Ascaris suum* from second-stage larvae to mature adults. *Journal of Parasitology* 69:549–558 and (2) Douvres and Urban. 1986. Development of *Ascaris suum* from in vivo-derived third-stage larvae to egg-laying adults in vitro. *Proceedings of the Helminthological Society of Washington* 53:256–262. During this period a distinguished visiting scientist from China worked with Frank and Joe and was a coauthor on several of their papers. Dr. Xu Shoutai, Chief, Shanghai Laboratory of Animal Schistosomiasis, spent a productive 6-month sabbatical with Frank learning his in vitro methods.

Frank credits Dr. A. O. Foster with strong support and encouragement for the in vitro work. Others who worked with Frank and benefited from his expertise included Lou Gasbarre, Ray Fetterer, Rob Rew, and Bob Romanowski. Frank asked me to be sure to mention some of the support staff who made significant contributions to his research, including Ray Rew, Ken Goodson, and Don Thompson.

Frank retired from the U.S. Department of Agriculture in December of 1985 and became an international consultant, traveling to Townsville, Australia, where he instructed Bruce Copeman's

laboratory on the *in vitro* cultivation of nematodes for several months prior to the 1986 International Congress of Parasitology in Brisbane.

After the Australian trip, Frank settled into retirement and took up the role of grandfather, which he now plays for grandsons Christopher and Tim and daughter Nicky. Like everything else in his professional life, Frank plays the grandfather role with enthusiasm, a strong personal style, and a sense of duty and devotion.

It was these same values that made Frank Douvres not just an outstanding scientist, but one of the most unforgettable personages for his colleagues and friends. At meetings, Frank could be counted on for a direct, to-the-point question meant to be provocative. Not everyone understood and appreciated this approach, but things were never dull when Frank was around.

In retirement, Frank has continued to be active in his church and the National Association of Retired Federal Employees and was the local NARFE chapter president in 1995, prior to a serious illness from which a long recovery is now almost complete. Frank and Kiki have also generously supported HelmSoc through the activities of the Brayton H. Ransom Memorial Trust Fund.

The Anniversary Award of the Helminthological Society of Washington is given either for scientific achievement or for service to the Society. Dr. Frank Douvres qualifies in both respects, having served in most of the offices of the society and on the editorial board. On behalf of the society, it is a great pleasure for me to present the 1999 Anniversary Award to Dr. Frank W. Douvres. Congratulations, Frank.

J. Ralph Lichtenfels  
November 17, 1999

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## MINUTES

### Six Hundred Sixty-Sixth Through Six Hundred Seventieth Meeting

*666th Meeting:* Beltsville Agricultural Research Center, United States Department of Agriculture, Beltsville, Maryland, 13 October 1999. President Eric Hoberg presided over the business meeting and the scientific session, which consisted of 3 presentations: Dr. Benjamin Rosenthal provided an overview of the phylogeography of deer ticks in eastern North America, Dr. John Carroll spoke on black-legged ticks and Lyme disease in Maryland, and Dr. Eric Hoberg provided a summary of nematode parasites of ruminants in the Mackenzie Mountains. New members included Santiago Mas-Coma (Spain), Eun-Taek Han (Korea), Marie-Claude Durette-Desset (France), Pan Cangsang (People's Republic of China), Richard Botzler (U.S.A.), Austin MacInnis (U.S.A.), and Scott Monks (Mexico).

*667th Meeting:* Sabang Restaurant, Wheaton, Maryland, 17 November 1999. The anniversary

dinner meeting and program were presided over by President Eric Hoberg. The slate of officers for 2000 was elected and installed by the membership in attendance: Dennis J. Richardson, president; Lynn K. Carta, vice president; Pat Carney, recording secretary; and Nancy Pacheco, corresponding secretary-treasurer. Willis A. Reid, Jr., and Janet W. Reid continued in office as editors. Dr. Ralph Lichtenfels introduced the recipient of the Anniversary Award, Dr. Frank Douvres. Dr. Douvres reviewed his research career, particularly his pioneering work with the *in vitro* culture of nematodes. Dr. Hoberg's final action as president was to turn the meeting over to the new president, Dr. Dennis Richardson. Dr. Richardson's first action was to adjourn the 667th meeting of the society and advise the membership that the next meeting would be held at the National Museum of Natural History, Smithsonian Institution, Washington, DC, on Wednesday, 19 January 2000, at 1900 h, with William Moser serving as the host.

**668th Meeting:** National Museum of Natural History, Smithsonian Institution, Washington, DC, 19 January 2000. President Dennis Richardson presided over the business meeting, which he summarized for the membership, and reminded the membership that the 669th meeting of the society would be held at the Johns Hopkins Montgomery County Center in Maryland, with Dr. Thomas Simpson in charge of making the local arrangements. He then introduced William Moser, who chaired the scientific session, which consisted of 4 papers: the first paper, authored by Mr. Dan Holiday and Dr. Dennis Richardson and presented by Mr. Holiday, dealt with archaeoparasitology on the Chiribaya Culture of southern Peru; the second, by Dr. Jeff Bates, provided an overview of the molecular phylogeny of the Adenophorea; and the third, by Dr. Jon Norenburg, reviewed his phylogenetic studies of the phylum Nemertea. The final speaker was Dr. Duane Hope, who provided an overview of the phylogenetic relationship between the marine nematode genera *Rhabdodemia* and *Pandolaimus*. New members included Benjamin Rosenthal (U.S.A.) and Alan Fedynich (U.S.A.).

**669th Meeting:** Johns Hopkins Montgomery County Center, 22 March 2000. The business meeting was opened by the vice president, Lynn Carter, and presided over by President Dennis Richardson. President Richardson welcomed members and guests to the meeting, and a moment of silence was observed in memory of recently deceased society members James H. Turner, Bryce C. Walton, Richard M. Sayer, Francis G. Tromba, Everett L. Schiller, and Marion M. Farr. President Richardson then introduced Dr. Alan L. Scott, who chaired the scientific program, which consisted of 2 presentations. Dr. David Sullivan summarized his work on the formation and inhibition of heme poly-

mers in parasites. His presentation was followed by Dr. Christopher V. Plowe's discussion of a molecular marker for chloroquine-resistant falciparum malaria. Following the presentations and questions from the members and guests, President Richardson thanked the speakers for their informative and digestible summaries of malaria and schistosomiasis at the molecular level, and he also thanked Dr. Tom Simpson, who arranged the meeting. Finally, he reminded the membership that the last meeting of the season would be held at the New Bolton Center, University of Pennsylvania, Kennett Square, together with the New Jersey Society of Parasitologists, on 6 May 2000. New members included Al Canaris (U.S.A.), Peter Hotez (U.S.A.), Nicole Havas (U.S.A.), John Janovy, Jr. (U.S.A.), and Alan Scott (U.S.A.).

**670th Meeting:** New Bolton Center, University of Pennsylvania, Kennett Square, with the New Jersey Society of Parasitologists, 6 May 2000. The business meeting was presided over by President Richardson. Dr. Jay Farrell presided over the scientific meeting, which consisted of 3 presentations. Dr. Thomas Klei discussed immunity to equine strongyle infections. His paper was followed by Dr. David Sibley's discussion of motility and invasion of *Toxoplasma*. The final presentation was provided by Dr. James B. Lok on the Dauer pathway in *Caenorhabditis elegans* as a model for regulation of infective larval development in parasitic nematodes. New members included Ian Whittington (Australia), M. Rocio Ruiz de Ybañez (Spain), Francisco Jimenez-Ruiz (U.S.A.), Glen Dappen (U.S.A.), Alan Kocan (U.S.A.), Aaron McCormick (U.S.A.), Robin LePardo (U.S.A.), Megan Collins (U.S.A.), Mike Barger (U.S.A.), Megan Ryan (U.S.A.), Tamara Cook (U.S.A.), Kashinath Ghosh (U.S.A.), and Richard Clopton (U.S.A.).

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# THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

## MISSION AND VISION STATEMENTS

May 7, 1999

### THE MISSION

The **Helminthological Society of Washington**, the prototype scientific organization for parasitological research in North America, was founded in 1910 by a devoted group of parasitologists in Washington, D.C. Forging a niche in national and international parasitology over the past century, the **Society** focuses on comparative research, emphasizing taxonomy, systematics, ecology, biogeography, and faunal survey and inventory within a morphological and molecular foundation. Interdisciplinary and crosscutting, comparative parasitology links contemporary biodiversity studies with historical approaches to biogeography, ecology, and coevolution within a cohesive framework.

Through its 5 meetings in the Washington area annually, and via the peer-reviewed *Comparative Parasitology* (continuing the *Journal of the Helminthological Society of Washington* in its 67th Volume), the **Society** actively supports and builds recognition for modern parasitological research. Taxonomic diversity represented in the pages of the **Society's journal** treats the rich helminth faunas in terrestrial and aquatic plants, invertebrates, and vertebrates, as well as parasitic protozoans and arthropods. Parasitology, among the most integrative of the biological sciences, provides data critical to elucidation of general patterns of global biodiversity.

### THE VISION

The **Helminthological Society of Washington** celebrates a century of tradition and excellence in global parasitology, solving challenges and responding to opportunities for the future of society and the environment.

Members of the **Helminthological Society of Washington** contribute to understanding and protecting human health, agriculture, and the biosphere through comparative research emphasizing taxonomy, systematics, ecology, biogeography, and biodiversity assessment of all parasites. The **Society** projects the exceptional relevance of its programs to broader research and education in global biodiversity and conservation biology through the activities of its members and its journal, *Comparative Parasitology*.

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*Edna M. Buhner	1960	*O. Wilford Olsen	1980
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