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A New Monogenean and Cestode from the Deep-Sea Fish, *Macrourus berglax* Lacépède, 1802, from the Flemish Cap off Newfoundland

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ABSTRACT: A new trematode, *Macruricotyle newfoundlandiae* sp. n. (Monogenea: Diclidophoridae), and a new cestode, *Parabothriocephalus macruri* sp. n. (Cestoda: Parabothriocephalidae), are described from 46 specimens of the macrourid fish, *Macrourus berglax* Lacépède, 1802, taken in the Newfoundland region of the North Atlantic Ocean (45°31′–46°43′N, 44°08′–48°23′W). *Macruricotyle newfoundlandiae* is the second species named in the genus, and is distinguished from *M. clavipes* Mamaev and Lyadov, 1975 by its much larger pharynx, smaller first pair of clamps, "callous" on the second and third pairs of clamps, and the shape of the distal median sclerite in the posterior clamp jaw. *Parabothriocephalus macruri* sp. n. is differentiated from the three other species in the genus by its narrow scolex and segment shape, 77–112 testes per segment, and lack of a vaginal armature and sphincter.

During May–June 1981, 218 fishes (21 spp.) were examined off the eastern slope of the Grand Bank and in the Flemish Cap area off Newfoundland. In this paper we describe a new species of *Macruricotyle* (Monogenea) and a new species of *Parabothriocephalus* (Cestoda) from that collection. Noble et al. (1972) and Zubchenko (1979, 1981) have reported pseudophyllidean cestodes from *Macrourus berglax* and other macrourids in the northwest Atlantic, but did not identify them. Specimens of *Macruricotyle* and *Parabothriocephalus* are unknown from *M. berglax* or other fishes in this region. Discussion of the entire metazoan parasite fauna of *M. berglax* and other dominant deep-benthic fishes of this region will be presented in another paper.

Materials and Methods

Forty-six specimens of *M. berglax*, taken in 12.5-m Gulf of Mexico shrimp trawls and standard survey-type bottom trawls fished at 367–811 m, were examined at sea. A few fish were moribund when obtained, but most had recently died. Examinations for parasites began immediately. Fish from large samples that could not be examined immediately were refrigerated and examined the following day. Organs and tissues were observed with the aid of a dissecting microscope. The monogenetic trematodes were fixed in cold alcohol-formalin-acetic acid (AFA) without cover-glass pressure. Cestodes were relaxed in tap water for 10–20 min prior to fixation by immersion in AFA. All parasites were later transferred to 70% ethanol prior to staining. Trematodes were stained with Mayer's paracarmine and cestodes were stained in Harris' hematoxylin. Whole mounts were dehydrated in ethanol, cleared in methyl salicylate, and mounted in Canada balsam. Serial transverse sections, cut at 6 μm, of mature segments of *Parabothriocephalus* were stained in Harris' hematoxylin and counterstained with eosin.

Descriptive measurements are expressed as length by width, and are in micrometers unless otherwise indicated. The mean is given following the range for some characters.

Macruricotyle newfoundlandiae sp. n.
(Figs. 1–7)

DESCRIPTION (based on 14 specimens; 10 mature specimens measured): Body proper elongate, rather slender, tapering anteriorly, 3.6–8 mm (5.18 mm). Maximum width at ovarian level, 360–1,000 (789). Total length 4.2–9.1 mm (6.8 mm) including opisthaptor. Opisthaptor 824–1,800 (1.15 mm) by 2–4.2 mm (3.16 mm), consisting of 2 symmetrical, palmate lobes, each bearing 4 pedunculated clamps; lobes directed at right angles to body. Origin of lobes marked by inverted V-shaped depression on ventral body surface (Fig. 1). Terminal lappet about 120 by 160, bearing 1 or 2 pairs of minute hooks. Clamps about equal, wider than long, 160–272 by 192–264; anterior pair of clamps often slightly smaller. Clamp cavity surrounded by 8 sclerites; opening exhibiting well-developed muscular rim (Fig. 5). Interior of posterior valve bearing riblike thickenings and smaller, distal, median sclerite with flared lateral wings (Figs. 5, 6). Posterior (dorsal) surfaces of second and third clamp valves (Fig. 1) bearing well-developed pad or "callous" (sic, Mamaev and Lyadov, 1975). Callous slightly bilobed, about same dimensions as its associated clamp. Muscle bundles traversing opisthaptor to clamps well developed, especially large for the third clamp pair.

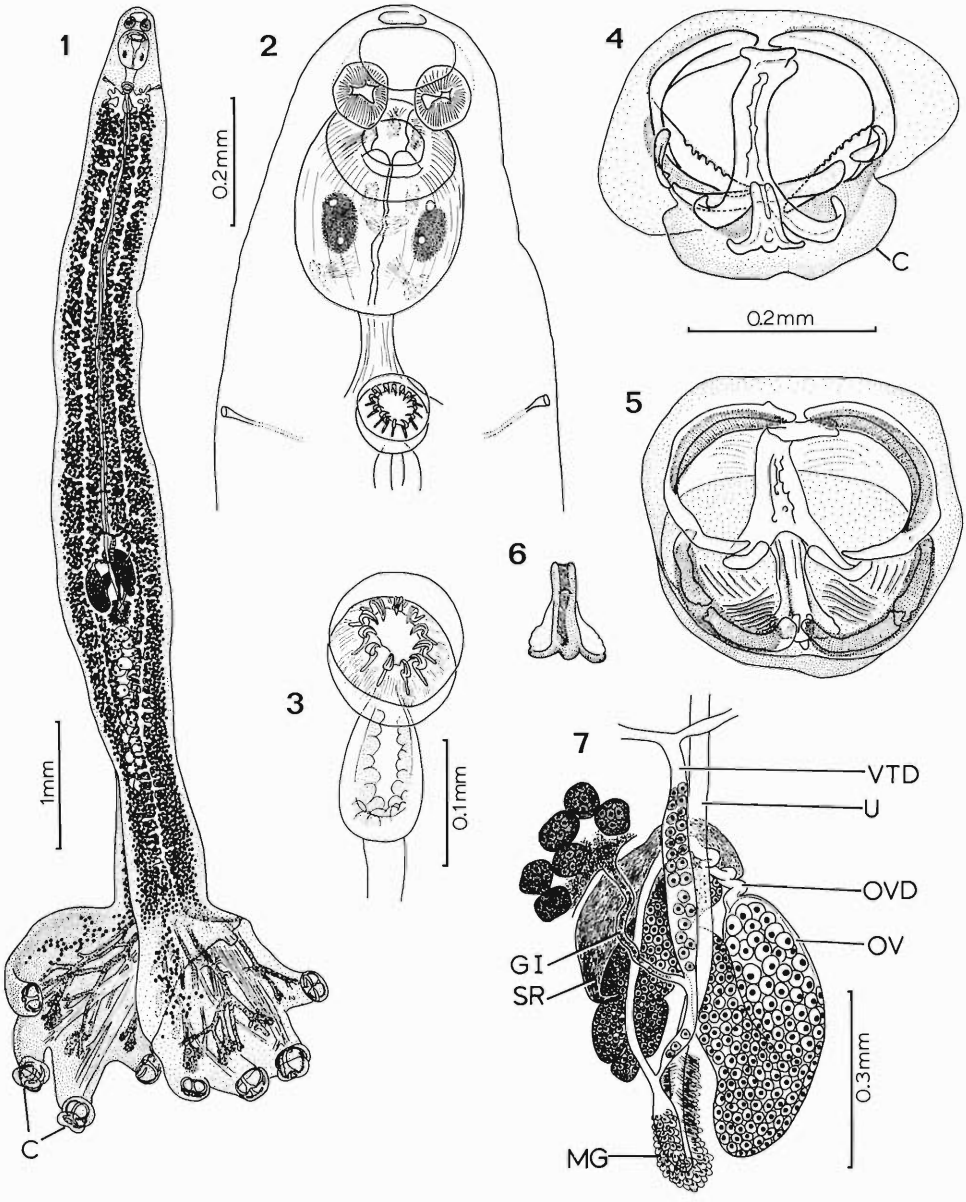
Mouth ventral, subterminal. Intrabuccal suckers 72–128 (104) in diameter. Pharynx conspicuous, 256–360 (309) by 224–288 (261), containing 2 indistinct masses of gland cells. Esophagus short. Intestinal bifurcation at level of genital pore; ceca forming numerous lateral diverticula, terminating in large branches within opisthaptor.

Testes subspherical, about 50–70 in number, in postovarian intercecal space. Cirrus 80–136 (105) in diameter, armed with a corona of 10–14 sickle-shaped hooks. Prostatic vesicle droplet-shaped, immediately posterior to cirrus; prostate cells large but indistinct.

Ovary reverse J-shaped when viewed ventrally, situated at midbody, maximum dimensions 320–600 by 248–400, mature end sinistral. Oviduct passing anteriorly and across to right side of ovary, then descending beneath immature end of ovary, entering ootype in postovarian space (Fig. 7). Seminal receptacle large, unlobed, overlapping right anterior ½ of ovary. Genito-intestinal canal ventral, recurving anterolaterally from main vitelline duct, emptying into right cecum. Mehlis' gland small, indistinct. Vaginal openings paired, on lateral body margins opposite genital pore. Uterus ascending directly in midline to genital atrium. Vitelline follicles subspherical to irregular, 42–68 by 38–46, numerous, distributed around ceca from level of prostatic vesicle to end of body proper; linear series of follicles scattered through anterior portion of opisthaptor lobes in direction of first pair of clamps. Eggs not observed.

HOST: *Macrourus berglax* Lacépède, 1802 (Macrouridae).

COLLECTION LOCALITIES AND DEPTHS: 45°38′N, 48°16′W at 715–720 m; 46°31′N, 45°52′W at 425–464 m; 46°35′N, 45°51′W at 367–390 m; 46°43′N, 44°08′W at 514–521 m.



Figures 1–7. ***Macruricotyle newfoundlandiae*** **sp. n. 1. Holotype, dorsal view. Note callous (C) on second and third clamps. 2. Anterior end, showing pharynx. 3. Detail, male terminalia. Note cirrus hooks. 4. Posterior (dorsal) view, second clamp on right side, showing callous (C) of a paratype. Figures 4–6 to same scale. 5. Anterior (ventral) view of second clamp on left side (holotype). 6. Detail of distal median sclerite in posterior clamp jaw. 7. Detail, female reproductive system. Abbreviations: CP, cirrus pouch; GI, genito-intestinal canal; GP, genital pore; MG, Mehlis' gland; OV, ovary; OVD, oviduct; SR, seminal receptacle; T, testis; U, uterus; UD, uterine duct; UP, uterine pore; V, vagina; VD, vas deferens; VTD, vitelline duct.**

LOCATION: Gills.

PREVALENCE: On 10 of 46 fish examined.

INTENSITY: One to 2 worms per host.

TYPE SPECIMENS: USNM Helm. Coll. Nos. 77096 (holotype) and 77097 (paratype); additional paratypes in Harold W. Manter Laboratory, University of Nebraska State Museum No. 21399, and in the British Museum (Natural History).

ETYMOLOGY: The species is named after Newfoundland, in the geographical region of collection.

Remarks

The genus *Macruricotyle* was created by Mamaev and Lyadov (1975) for *M. clavipes* found on the gills of *Macrourus* sp. taken in the southern Indian Ocean. Diagnostic characteristics of the genus are a prostatic vesicle, an armed cirrus, paired vaginae, and clamps with an adhesive (?) callous. Because of its diagnostic characters, Mamaev and Lyadov (1975) considered *Macruricotyle* to occupy an intermediate position among the subfamilies of the Diclidophoridae, with an advanced structural organization above the most primitive subfamily, Diclidophoropsinae.

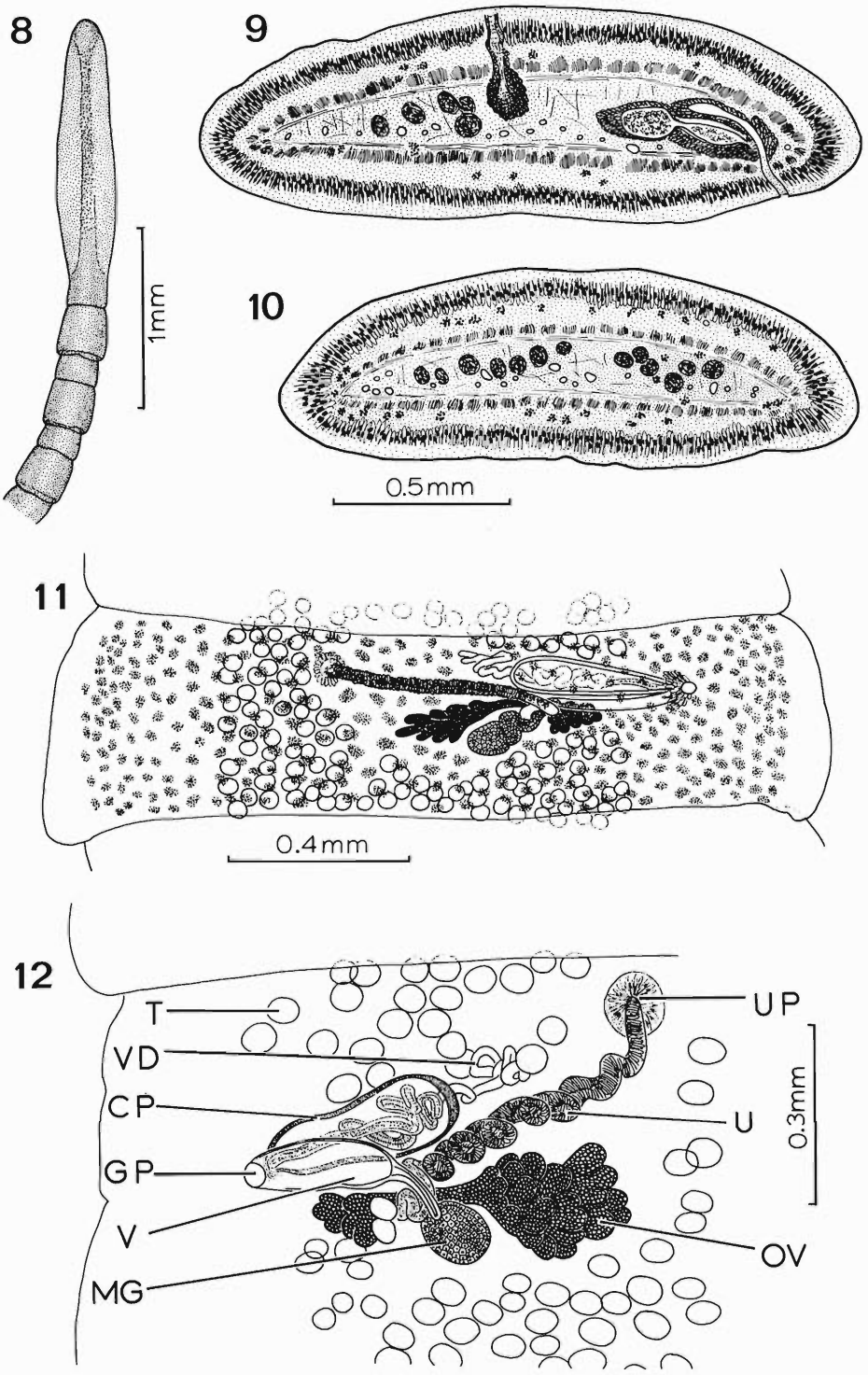
Macruricotyle newfoundlandiae may be differentiated from *M. clavipes*, the only other species described in the genus, by the following combination of characters: (1) a much larger pharynx (256–360 by 224–288 versus 140–160 by 120–150 for *M. clavipes*); (2) the first pair of clamps smallest (fourth pair of clamps smallest in *M. clavipes*); (3) a callous present on both the second and third clamp pairs (on only the third clamp pair of *M. clavipes*); and (4) shape of the distal median sclerite of the posterior clamp jaw (compare Fig. 6 with figures of Mamaev and Lyadov, 1975).

Parabothriocephalus macruri sp. n. (Figs. 8–12)

DESCRIPTION (based upon 3 complete specimens with mature segments): Strobila filiform, delicate, tapering to maximum width of 2.34 mm. Total length 14.5–26.9 cm; 840 segments in largest specimen. Scolex elongate, pointed, 1,460–2,240 by 340–380. Bothria shallow. Neck absent. Segments craspedote; first segment merging with scolex, succeeding segments elongate, about 400 by 280, rapidly becoming wider than long, about 200 by 600. Genital primordia first apparent in segments measuring 280 by 2,000; testes apparent in segments measuring 380 by 2,100, segments elongating to 860 by 2,340 with further maturation. Secondary division of segments apparent throughout strobila; segments up to 540 by 1,840 still dividing. Fully mature primary segments 460–560 by 1,960–2,340, segments resulting from secondary division narrower, up to 240 by 2,080. Genital

→

Figures 8–12. ***Parabothriocephalus macruri*** **sp. n. 8. Scolex (holotype). 9. Transverse section through genital pore, vagina, and uterine pore of mature segment. Figures 9–10 to same scale. 10. Transverse section of mature segment. Note disposition of testes, vitelline follicles, and osmoregulatory ducts. 11. Mature segment. 12. Detail of reproductive system (vitellaria not shown). Abbreviations as in Figures 1–7.**



pores dorsosubmarginal, above nerve cords, irregularly alternating. Testes spherical to subspherical, 56–88 in diameter, 77–112 (90) per segment, encircling reproductive organs within medullary zone, continuous from segment to segment. Cirrus sac pyriform, thick-walled, 352–440 by 96–184, oriented transversely or slightly anteromedially. Cirrus armed with small spines. Vas deferens tightly coiled at proximal end of cirrus sac.

Ovary bilobed, maximum dimensions 80–208 by 520–624, submedian, immediately posterior to cirrus sac. Ovarian lobes asymmetrical, subdivided into lobules; antiporal lobe 240–336 by 104–208; poral lobe 88–236 by 80–184. Ootype and Mehlis' gland form a globular complex, 106–208 by 112–152, posterior to ovarian isthmus. Vagina divided into 2 parts of about equal length; proximal portion tubular, not coiled, surrounded by gland cells; distal portion thin-walled, saccate, 320–360 by 48–120, reaching maximum dimensions in oldest segments. No vaginal armature, vaginal sphincter, or seminal receptacle. Uterus extending anterolaterally to uterine pore, tightly coiled, surrounded by gland cells. Uterine pore ventral, near anterior margin of segment, submedian to antiporal side by 6–8% of segment width. Vitelline follicles spherical to subspherical, 56–80 in diameter, filling entire circumcortical zone; some follicles in medullary zone. Eggs not observed. Osmoregulatory ducts in a longitudinal series across dorsal medulla (Fig. 9).

HOST: *Macrourus berglax* Lacépède, 1802 (Macrouridae).

COLLECTION LOCALITIES AND DEPTHS: 46°31′N, 46°52′W at 425–464 m; 46°35′N, 45°51′W at 367–390 m.

LOCATION: Scolex in pyloric cecum, strobila in intestinal lumen.

PREVALENCE: In 3 of 46 fish examined.

INTENSITY: One worm per host.

TYPE SPECIMENS: USNM Helm. Coll. Nos. 77098 (holotype) and 77099 (paratype).

Remarks

Yamaguti (1934) created *Parabothriocephalus* for a cestode commonly found in a shallow-water teleost, *Psenopsis anomala* (Temminck and Schlegel, 1850), from the Inland Sea of Japan. The type species, *P. gracilis*, is unusual in having a vaginal armature. Since the creation of the genus, that feature has not been found in the two species added to the genus. A vaginal armature is also absent in *P. macruri* sp. n. Other differences distinguishing *P. macruri* from *P. gracilis* Yamaguti, 1934 are its larger size (14–26 cm versus 6 cm) and its consistently wider-than-long mature segments (longer than broad in *P. gracilis*).

The two remaining species in the genus are *P. sagitticeps* (Sleggs, 1927) and *P. johnstoni* Prudhoe, 1969. Jensen (1976) correctly reassigned *Dibothrium sagitticeps* Sleggs, 1927 to *Parabothriocephalus*. *Parabothriocephalus sagitticeps*, from the California rockfish, *Sebastes paucispinis* Ayres, 1854, is the largest species in the genus to date (up to 42 cm by 8 mm). Other characteristics separating *P. sagitticeps* from *P. macruri* are its testes number (135–384 versus 77–112 in *P. macruri*), a large scolex with projecting bothria, a centered uterine pore, a sharply recurved distal portion of the uterus, and smooth ovarian lobes. The last three characters were determined by examining Jensen's specimens (USNM Helm. Coll. Nos. 73463–73467).

Parabothriocephalus johnstoni described by Prudhoe (1969), was discovered in a deep-sea fish, *Coryphaenoides whitsoni* Regan, 1913. The specimens were collected northeast of Cape Ann, Antarctica, during the B.A.N.Z. Antarctic Expedition of 1929–1931. After examining the type specimens of *P. johnstoni* (whole mounts and serial sections), we found that it possesses a very large vaginal sphincter, about 50 testes per segment, a rather powerful longitudinal musculature, and has the Mehlis' gland situated dorsal to the ovarian isthmus. Those features clearly differ from our species, *P. macruri* sp. n., which lacks a vaginal sphincter, possesses 77–112 (90) testes per segment, has smaller longitudinal muscle bundles, and has the Mehlis' gland situated posterior to the ovarian isthmus.

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Thanks are due to Dr. John Anderson, Scientist-in-Charge, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland, for providing research space aboard *F-V Gadus Atlantica* for R. Campbell; and to Dr. Richard L. Haedrich, Memorial University of Newfoundland, for supplying equipment and fish for examination. This work was supported, in part, by National Sciences and Engineering Research Council of Canada Grant A-7230 to R. L. Haedrich.

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Monogenea (Capsaloidea) from Philippine Marine Fishes

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ABSTRACT: Herein presented are monogenetic trematodes of the family Capsalidae from the gills of Philippine marine teleosts: *Caballerocotyla philippina* sp. n., *Allometabenedeniella plataxi* gen. et sp. n., and *Neobenedenia manilae*, all from *Platax orbicularis* (Forskäl); *Benedenia malaboni* sp. n. from *Epinephelus undulosus* (Quoy & Gaimard); and a new record for *Tristomella pricei* (Hidalgo, 1959) Price, 1960 from *Sphyraena* sp.

The only record of Capsaloidea from Philippine marine fishes is that of *Encotyllabe caballeroi* Velasquez, 1977 from *Lethrinus nebulosus* (Forskäl) (Velasquez, 1977). The present paper on the family Capsalidae is based on specimens from some Philippine marine teleosts. Descriptions are presented of *Allometabenedeniella plataxi* gen. et sp. n., *Neobenedenia manilae* sp. n., and *Benedenia malaboni* sp. n., all belonging to the subfamily Benedeniiae, and of *Caballerocotyla philippina* sp. n. and a new record for *Tristomella pricei* (Hidalgo, 1959) Price, 1960, both belonging to the subfamily Capsalinae.

Collection and staining methods were the same as those used previously (Velasquez, 1977). Measurements are in micrometers unless otherwise stated; means are in parentheses. Drawings were made with the aid of a camera lucida.

Family Capsalidae Baird, 1853
Subfamily Capsalinae Johnston, 1929
Genus *Caballerocotyla* Price, 1960

Caballerocotyla philippina sp. n.
(Fig. 1a–h)

DIAGNOSIS (based on 10 specimens out of 20 examined): Body elongate, 1,020–1,890 long (1,420) by 390–850 wide (570). Tegument thin, smooth; marginal papillae absent. Prohaptors weak, ventrolateral, 150–240 (193) by 110–180 (139). Opisthaptor open, circular, 230–540 (359) by 220–550 (361), with 3 pairs of anchors and 14 marginal hooks, surrounded by delicate pleated membrane, 20–70 (40.2) wide. Anterior pair of anchors (Fig. 1g) stout, pointed anteriorly, bifid posteriorly, 96–160 (126) long; middle pair (Fig. 1c) variable, posterior end bent anteriorly, 60–100 (65) by 20–50 (27); posterior pair (Fig. 1b–d) 60–70 (67) by 10 (10). Two pairs of eyes. Mouth mid-ventral, 160–250 (217) from anterior tip of body; pharynx spherical, 80–160 (115) in diameter, internally constricted, delimited by a thin covering; esophagus short; gut bifurcated, with lateral and medial diverticulae, crura confluent posteriorly. Testes follicular, numbering 22–35, 40–104 (60) by 29–80 (52). Ducts from testicular follicles uniting to form vas deferens, which passes forward on left side of ovary, bending over a short distance from ovarian zone, entering flask-shaped cirrus sac (Fig. 1h). Cirrus with minute papillae, prominent when retracted. Cirrus sac and uterine duct uniting to form genital atrium opening via common genital pore on left side of body at posterior margin

of prohaptor. Ovary smooth, 50–130 (88) by 50–150 (103), median, immediately pretesticular. Oviduct short, extending to left and entering ootype immediately anterior to vitelline reservoir; vagina short, opening to left margin of body at level of intestinal crura. Vitellaria occupying greater part of body, extending to cephalic lobe. Egg elongate, broadly filamented at both ends, 20 by 12.

HOST: *Platax orbicularis* (Forskäl).

LOCATION: Gills.

LOCALITY: Malabon, Rizal, Luzon Island, Philippines.

HOLOTYPE: USNM Helm. Coll. No. 76331.

PARATYPES: C. C. Velasquez Coll. No. 538 (2)b.

Remarks

Price (1960) proposed the genus *Caballerocotyla*, characterized by the open central haptoral area and testes confined to the inter-intestinal field. Although Yamaguti (1963) relegated it to subgeneric rank, I agree with Stunkard (1962) in accepting Price's (1960) proposal, with the type species *Caballerocotyla biparasitica* (Goto, 1894). Stunkard (1962) presented a new key to the species and recognized *C. caballeroi* (Winter, 1955) on the gills of *Sarda orientalis*; *C. magronum* (Ishii, 1936) on the gills of *Thynnus orientalis* (synonym of *Thunnus thynnus*); *C. manteri* (Price, 1951) on the gills of *Euthynnus alleteratus*; *C. gouri* (Chauhan, 1952) on the operculum of *Thynnus thunnina* (synonym of *Euthynnus yaito*); *C. pelamydis* on the gills of *Sarda sarda*; *C. katsuwoni* (Ishii, 1936) on the gills of *Katsuwonus vagans*; *C. foliacea* (Goto, 1894) on the gills of an undetermined Japanese fish; and *C. klawei* Stunkard, 1962 from the nasal capsule of *Neothunnus macropterus*.

This is the first representative of *Caballerocotyla* to be described from a marine teleost in the Philippines. It differs from all known species by the shape and size of prohaptor, opisthaptor, and opisthaptoral hooks, the size and number of testes, character and size of the egg, and host.

Subfamily Benedeniinae Johnston, 1931

Allometabenedeniella plataxi gen. et sp. n. (Fig. 2a–c)

DIAGNOSIS (based on 10 out of 27 examined): Body subtriangular, 640–1,350 (1,200) long including opisthaptor, maximum width 370–1,200 (990) at posterior ⅔ of body proper. Prohaptor a pair of oval ventrolateral suckers, 64–210 (147) by 88–220 (178); anteroventrally separated by a distance of 40–200 (114) and ventrally by 40–80 (43). Opisthaptor muscular, 220–680 (390) by 270–680 (480), with narrow frilled membrane without marginal hooklets; 3 pairs of anchors, anterior pair (Fig. 2a) wedge-shaped, bifid, 40–150 (59) by 4–32 (16), pointed at free anterior end; middle anchors (Fig. 2c) slender, needlelike, 40–90 (65) by 4–16 (9), overlapping posterior, very slender, pointed anchors, 24–80 (40) by 4–8 (4) (Fig. 2b). Two pairs of eye spots, anterior smaller than posterior; mouth oval, 20–80 (63) wide, opening immediately between posterior pair of eye spots; pharynx 48–140 (94) by 72–210 (156), notched on each side anterodorsally. Crura numerous, branched laterally and medially, branches dendritic, not confluent posteriorly. Testes oval, immediately postequatorial, juxtaposed, right one 80–144

(113) by 80–176 (129), left one 80–144 (91) by 48–168 (124). Vas deferens sinuous, ascending along sinistrodorsal side of ovary, turning to right, and joining muscular ejaculatory duct, on its course to cirrus. Cirrus pouch thin, muscular, conspicuously globular, occupying a space 96–232 (191) by 80–208 (108) enclosing a circularly winding sclerotized eversible cirrus; distal end of cirrus with tiny openings. Prostatic reservoir filled with fine granules between ovary and right crus. Ovary ovoid, 40–144 (98) by 24–180 (122), in the middle half of body proper. Germiduct arising from dorsal side of ovary, joining vitelline duct from vitelline reservoir, turning forward anterodextral to ovary, receiving short duct from spherical seminal receptacle; ootype and winding uterine duct surrounded by shell-gland cells containing oculate developing embryos. Uterus proper expanded anteriorly, 80–168 (135) long by 9.6–64 (34) wide, sclerotized, running obliquely forward across left crus and opening dorsally on left margin of body at level of pharynx. In each of 2 specimens, in the expanded uterus proper was found a single dark yellow polyhedral egg 104–112 (108) by 40 at anterior end and 88–96 (92) at posterior end with short twirled filament. Uterus proper opening posterolateral to pharynx. Vagina tubular on left margin of body; vaginal duct short, narrow, winding. Vitellaria coextensive with intestine and its branches; vitelline reservoir anterolateral to ovary, receiving vitelline ducts from lateral sides; vitelline ducts joining germiduct from dorsal side of vitelline reservoir. Parasite of marine teleosts.

HOST: *Platax orbicularis* (Forskäl).

LOCATION: Gills.

LOCALITY: Manila Bay, Luzon Island.

HOLOTYPE: USNM Helm. Coll. No. 76332.

PARATYPES: C. C. Velasquez Coll. No. 538 (2)b2.

Remarks

Allometabenedeniella plataxi sp. n. is the type and only species of the new genus *Allometabenedeniella*. It differs from the most closely related *Metabenedeniella hoplognathi* (Yamaguti, 1942) essentially in the following respects: (1) shape and size of body; (2) anchor sizes and shapes; (3) unusual shape of the cirrus pouch; (4) circularly winding, long, eversible, sclerotized cirrus; and (5) uterus enclosed in a thin-walled sac, characteristically expanded anteriorly.

***Allometabenedeniella* gen. n.**

GENERIC DIAGNOSIS—CAPSALIDAE, BENEDENIINAE: Oculate. Prohaptor suckerlike. Opisthaptor with frilled marginal membrane, marginal hooks missing. Three pairs of anchors; anterior pair wedge-shaped, bifid at posterior end, others needlelike. Pharynx notched. Crura with numerous side branches not united posteriorly.

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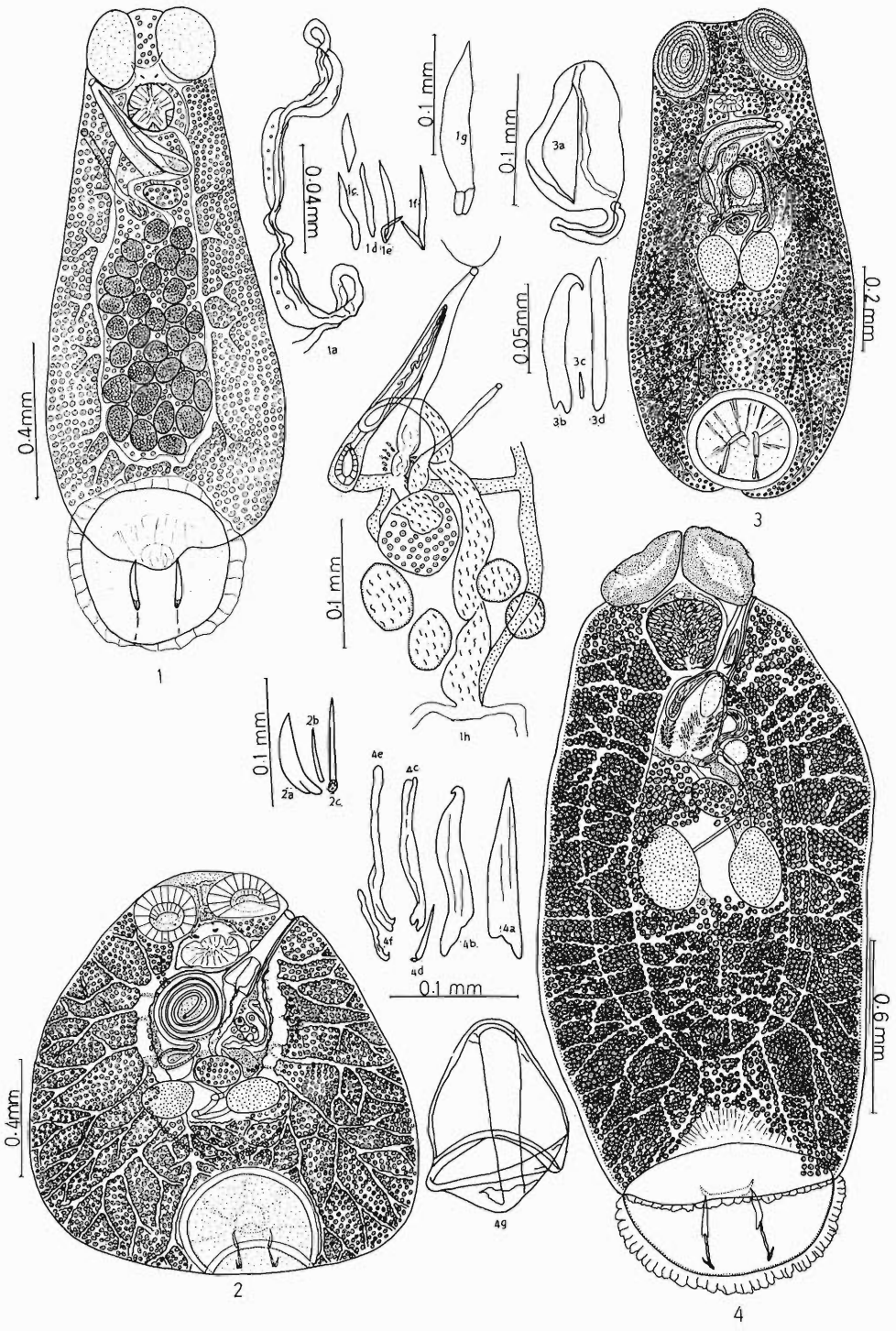
Figures 1–4. Whole-mount drawings are ventral view, except Figure 1, which is dorsal view. 1. *Caballerocotyla philippina* sp. n. a, eggs; b and g, anterior anchors; c and d, middle anchors; e and f, posterior anchors; h, cirrus sac. 2. *Allometabenedeniella plataxi* gen. et sp. n. a, anterior anchor; b, posterior anchor; c, middle anchor. 3. *Neobenedenia manilae* sp. n. a, egg; b, anterior anchor; c, posterior anchor; d, middle anchor. 4. *Benedenia malaboni* sp. n. a and b, anterior anchors; c and e, middle anchors; d and f, posterior anchors.



Testes juxtaposed, postequatorial. Vas deferens winding anteriorly, then posteriorly, entering into muscular ejaculatory duct, thence to a prominent circularly winding, long, eversible, sclerotized cirrus. Prostatic reservoir between ovary and right crus. Prostatic bulb elongate, thick-walled, muscular, with its distal end entering into globular cirrus pouch, enclosing the circularly winding, sclerotized cirrus. Ovary subspherical, equatorial, just anterior to left testis. Uterus enclosed in a wide, thin-walled sac, surrounded by shell-gland cells; anteriorly expanded, sclerotized, opening dorsolateral to genital pore. Eggs polyhedral, with twirled filament at posterior end. Vagina tubular, elongate, opening adjacent to uterine pore. Vitellaria coextensive with intestine and its branches; vitelline reservoir well developed immediately anterior to ovary. Parasitic on gills of marine teleosts.

GENOTYPE: *Allometabenedeniella plataxi* sp. n.

Genus *Neobenedenia* Yamaguti, 1963

Neobenedenia manilae sp. n. (Fig. 3a–d)

DIAGNOSIS (based on 6 out of 10 examined): With characters of the genus. Oculate. Body elongate, 1,050–1,940 by 500–1,060 (688) maximum width. Prohaptor elliptical, weak, 180–270 (210) by 130–170 (150), concentric surface markings distinct. Opisthaptor circular, not indented, 200–320 (280) by 250–380 (310), marginal membrane relatively wide, 20–40 (35). Marginal hooklets present. Anterior pair of haptoral hooks 70–90 (76) by 20, pointed at anterior end, 10 long, curved, posterior end truncated, slightly bifid; middle pair of hooks fairly stout, 70–85 (75) by 10; posterior pair of hooks very small, pointed at anterior end, $^{1}/_{7}$–$^{1}/_{10}$ of middle pair. Mouth midventral, posterior to prohaptor; muscular pharynx 50–120 (80) by 100–220 (140). Esophagus short, intestinal crura dendritic, extending to posterior extremity of body. Testes equatorial, smooth, oval, right one 100–240 (110) by 80–200 (120); left one 130–250 (180) by 100–200 (130). Vas deferens coiling anteriorly from testes, intercecal, curving right to cirrus pouch, 150–380 (260) by 40–50 (45), enclosing muscular prostatic reservoir, 70–150 (120) by 50–70 (60); seminal vesicle continuing as ejaculatory duct; prostatic glands linearly aligned into 2 lateral symmetrical groups. Genital atrium submedian. Glands of Goto minute, posttesticular. Ovary median, pretesticular, 50–130 (80) by 60–110 (90); seminal receptacle 50–100 (80) by 50–100 (80) in 1 specimen. Vitellaria follicular, extending from oral sucker to posterior end of body. Vitelline ducts uniting medially to form a transverse reservoir just anterior to ovary. Vagina absent. Single egg collapsed, 107 by 33 (Fig. 3a), with short, curved, stout appendages at one end and slender at opposite end. In 1 dorsoventrally flattened immature egg, wider end with 1 long filament and 2 lateral, short, blunt, marginal processes.

HOST: *Platax orbicularis* (Forskäl).

LOCATION: Gills.

LOCALITY: Manila Bay, Luzon Island.

HOLOTYPE: USNM Helm. Coll. No. 76333.

PARATYPES: C. C. Velasquez Coll. No. 560 (1)b.

Remarks

Neobenedenia manilae sp. n. differs from the most closely related species, *N. muelleri* (Meserve, 1938) Yamaguti, 1963 and *N. longiprostata* Bravo-Hollis, 1971, in (1) size of body, (2) anchor sizes and shapes, (3) character of the cirrus complex, (4) distribution of the prostatic glands, and (5) host.

Genus *Benedenia* Diesing, 1858

Benedenia malaboni sp. n.

DIAGNOSIS (based on 3): With characters of the genus. Oculate. Body elliptical, 990–1,470 (1,270) long by 465–675 (560) wide. Tegument fairly thick, smooth. Prohaptors suckerlike, 135–180 long (150), 120–195 (160) wide. Opisthaptor almost globular, 300–450 (350) long, 255–360 (300) wide, aseptate, surrounded by a thin, pleated, marginal membrane, 30 wide, with 3 pairs of anchors in linear series and 14 marginal hooklets. Anterior pair stout, 60–90 (75) long, 15 maximum width in holotype; right anchor pointed anteriorly (Fig. 4a), left one sinuous, recurved, slender anterior end (Fig. 4b); in the paratypes, shape of anchors as in left one; middle pair 45–77 (65) by 3, posterior end recurved, slightly bifid (Fig. 4c, e); posterior pair ⅓ length of middle pair, sinuous, irregular, with sharp, recurved posterior pointed end (Fig. 4d, f). Mouth midventral at level of posterior margin of prohaptors, continuing to pharynx, 120–150 (135) by 90–180 (135). Gut bifurcated, branching medially and laterally, not confluent posteriorly. Two testes side by side, equatorial, smooth, oval, 135–180 (165) by 90–135 (114); vasa efferentia anastomosing to form sinuous vas deferens, paralleling left intestinal crus on its course to cirrus. Cirrus complex consisting of cirrus, prostatic reservoir, seminal vesicle in cirrus pouch. Cirrus 75 long, 30 wide; prostatic reservoir 45–60 (54), 38–45 (42) wide. Prostatic gland cells prominent, posterior to prostatic reservoir. Glands of Goto not distinct. Common genital pore submarginal on left near anterior level of pharynx, immediately at posterior margin of prohaptor. Ovary protesticular, oval, 60–105 (81) long, 60–90 (75) in diameter; oviduct receiving duct from vitelline reservoir proceeding anteriorly to ootype. Ootype wide, surrounded by Mehlis' gland cells; uterus fairly short, opening into genital atrium. Vitellaria follicular, occupying almost entire available space of body proper. Vitelline reservoir anterosinistral to ovary. Egg filamented, 150 long, 120 maximum width. Unknown organ of Yamaguti (1934), 45–60 (50) by 38–45 (43). Vagina narrow, opening at left margin near genital atrium.

HOST: *Epinephelus undulosus* (Quoy and Gaimard).

LOCATION: Gills.

LOCALITY: Dagatdagatan, Malabon, Rizal, Luzon Island.

HOLOTYPE: USNM Helm. Coll. No. 76334.

PARATYPES: C. C. Velasquez Coll. No. 438d3.

Remarks

Benedenia malaboni sp. n. is apparently closely related to either *B. sebastodis* (Yamaguti, 1934) Meserve, 1938 or *B. epinepheli* (Yamaguti, 1937) Meserve, 1938; however, it differs from both in the (1) sizes and shapes of the prohaptors and

opisthaptor, (2) sizes and shapes of the haptoral hooks, (3) character of the cirrus prostatic reservoir complex, and (4) size of egg.

Capsalinae Johnston, 1929

Tristomella pricei (Hidalgo, 1959) Price, 1960 (Fig. 5a–h)

REDESCRIPTION (based on 1 specimen): Body almost circular, 7,560 long by 6,370 wide; dorsal surface slightly convex with 2 rows of spines at lateral margins, an inner row of 14–18 spines on each side, 60 by 30, with 3–4 cuspids, and an outer row of about 63–100 spines on each side, 42 by 18, with 1–4 cuspids (Fig. 4d–h). Prohaptors circular, disklike, 1,320 by 1,320. Opisthaptor saucer-shaped, 3,500 by 3,200, surrounded by a delicate pleated membrane; central heptagonal area open, with 7 rays radiating from it; ventral surface papillate. Opisthaptoral anchors lancet-shaped, almost straight, left partly cut, 434 by 79, right 579 by 105. Marginal hooklets present. Oral aperture median, 158 by 395. Pharynx constricted; digestive tract as in type. General aperture at level of constriction of pharynx and at distal margin of left prohaptor. Cirrus pouch pestle-shaped, 1,850 long, 263 maximum width. Cirrus with numerous pulvinate elevations. Testes numerous, interintestinal, partly extraintestinal within extralateral limits of longitudinal nerves. Ovary lobate, 1,000 by 1,250. Vitelline follicles extensive as in type. Vagina narrow, opening posterior to and adjacent to genital aperture. Ootype posterior to cirrus pouch. No egg.

HOST: *Sphyraena* sp.

LOCATION: Gills.

LOCALITY: Visayan Islands, Philippines.

SPECIMEN: USNM Helm. Coll. No. 76336.

Remarks

This single specimen from *Sphyraena* sp. was given to the author by Mrs. Lolita Santos Basio of the Manila Medical Center. It appears smaller than those described by Hidalgo (1959) from *Makaira mitzukurii* (Jordan and Snyder) and Price (1960) from *Makaira marlina* Jordan and Evermann. The host may have been misidentified.

Acknowledgments

This work was supported in part by grant AI 02575-05 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, with the cooperation of the Smithsonian Institution, Washington, D.C., % Dr. I. E. Wallen and a grant-in-aid from the National Research Council of the Philippines, I.E. Project 53. Thanks are due to Dr. J. Ralph Lichtenfels, Animal Parasitology Institute, Agricultural Research Service, U.S.D.A., for space facilities and loan of type specimens, and to Mr. D. E. Zwerner for furnishing a microfiche copy of

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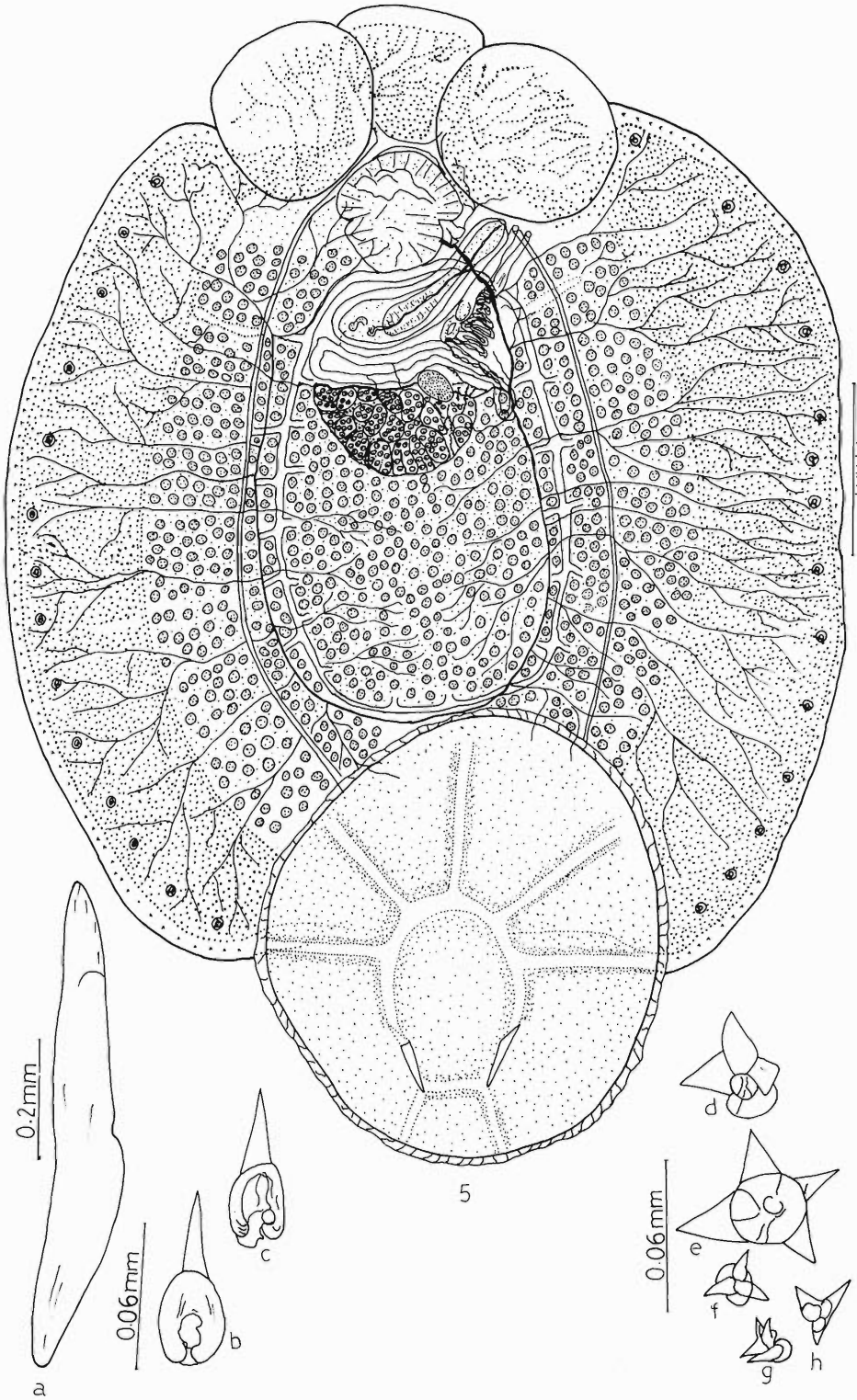
Figure 5. ***Tristomella pricei*** **(Hidalgo, 1959) Price, 1960. a, anchor; b–h, body spines on dorsal surface.**



the world bibliography on monogenetic trematodes. The U.P. Natural Science Research Center, Diliman, Quezon City, provided space facilities.

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Proc. Helminthol. Soc. Wash.
49(2), 1982, pp. 185–188

Paracreptotrematina limi gen. et sp. nov. (Digenea: Allocreadiidae) from the Mudminnow, *Umbra limi*

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ABSTRACT: *Paracreptotrematina limi* gen. et sp. nov. is described from the intestine of the mudminnow, *Umbra limi*, in Wisconsin, Ohio, and Michigan. The new genus is distinguished from *Creptotrematina* Yamaguti, 1954, primarily by having ceca extending to the posterior extremity instead of terminating at about midbody.

This paper reports a new papillose allocreadiid genus and species from the intestine of mudminnows, *Umbra limi*, collected in Wisconsin, Ohio, and Michigan. The genus is diagnosed and the type species is described.

Wisconsin specimens, obtained between 1977 and 1981, were refrigerated in distilled water overnight, then fixed in AFA for whole mounts and stained in Semichon's carmine; those from Ohio and Michigan, collected between 1953 and 1957, were fixed in hot 10% formalin and either stained with Harris' haematoxylin or Semichon's carmine for whole mounts or sectioned at 10 or 20 micrometers.

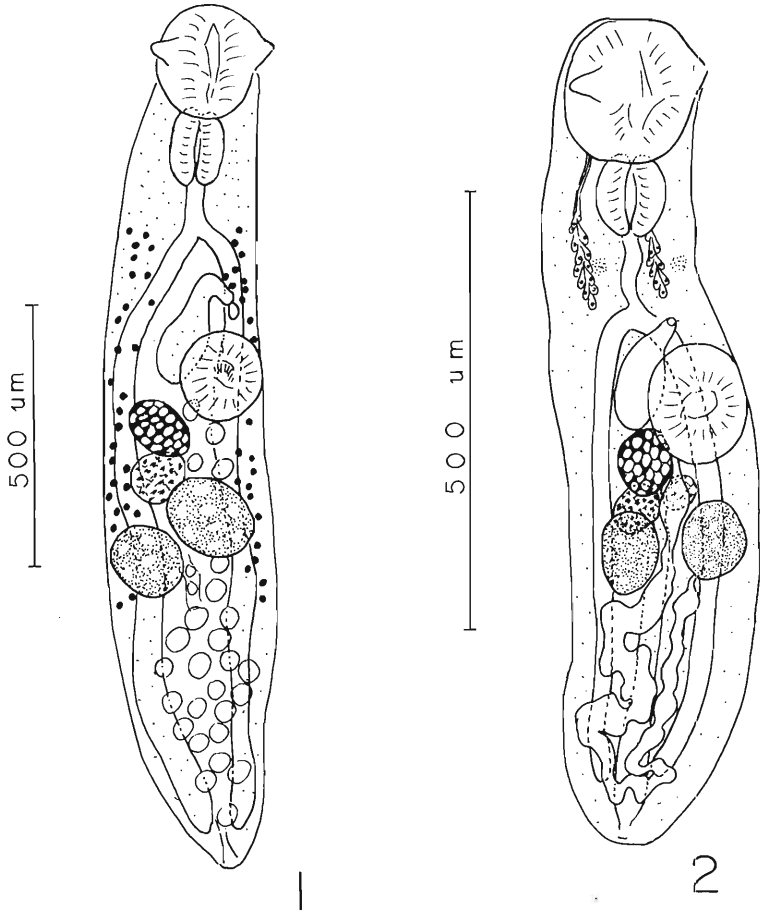
Figures were drawn with the aid of a microprojector. All measurements are in micrometers, with means in parentheses. Eggs were measured in the uterus of whole mounts.

Paracreptotrematina gen. nov.

GENERIC DIAGNOSIS: Allocreadiidae, Megalogoniinae. Body elongated-fusiform, oval in cross section, smooth; eyespot pigment in forebody. Oral sucker with ventrolateral pair of triangular lobes. No prepharynx. Pharynx variable, esophagus somewhat short, ceca dorsal, extend to near posterior extremity. Suckers equal or subequal, acetabulum pre-equatorial. Testes distinctly to barely diagonal, well removed from posterior end of body. Cirrus sac to right of acetabulum, enclosing tubular seminal vesicle and eversible cirrus. Genital pore median or nearly so, slightly anterior to acetabulum. Ovary submedian, posterolateral to acetabulum. Seminal receptacle present. Vitelline follicles variable, in lateral fields from near cecal bifurcation to past testes. Uterus extends to posterior extremity, filling most post-testicular intercecal space; metraterm present. Eggs numerous, containing oculate embryos. Excretory vesicle dorsal, tubular, reaching ovary. Intestinal parasites of freshwater fishes. Type species:

Paracreptotrematina limi sp. nov. (Figs. 1, 2)

DESCRIPTION (based on 10 gravid specimens: 3 from Wisconsin and 7 from Ohio): With characters of the genus, 879–1,652 (1,106) by 168–308 (260). Oral sucker subterminal, 126–196 (169) by 112–196 (160). Pharynx large, 84–140 (105) by 63–114 (91). Acetabulum subspherical, smaller than oral sucker, ⅓–½ body length from anterior end, 111–168 (133) by 98–168 (138). Eyespot pigment dorsal,



Figures 1, 2. ***Paracreptotrematina limi*****, ventral views. 1. Holotype from Wisconsin. 2. Paratype from Ohio, showing uterine and excretory vesicle outlines, eyespots, and the paired gland clusters.**

lateral to esophagus, especially prominent in live worms. One pair of gland clusters, 15 cells each, dorsolateral to esophagus and pharynx, with ducts opening near oral sucker. Testes smooth, oval to spheroid, somewhat postequatorial; anterior testis on left, 67–154 (101) by 40–140 (80); posterior testis 78–154 (107) by 50–126 (74). Cirrus sac 128–267 (168) by 47–84 (64), club-shaped, overlapping acetabulum; contains saclike seminal vesicle, glandular pars prostatica, tubular ejaculatory duct with pouch at distal end, and weakly muscular cirrus. Ovary smooth, rounded, pretesticular, 61–154 (94) by 55–112 (83). Seminal receptacle overlapping ovary dorsoposteriorly, 98–126 (108) by 70–98 (88). Mehlis' gland and Laurer's canal not observed. Vitelline reservoir just anterior to anterior testis; vitelline follicles small, few, extending to just past testes, 16–26 (22) by 13–22 (16). Metraterm ventral to cirrus sac. Up to 50 eggs in uterus; eggs thin-shelled, 42–86 (66) by 35–74 (55).

HOLOTYPE: USNM Helm. Coll. No. 76643 from Tichigan Lake, Wisconsin.

PARATYPES: USNM Helm. Coll. No. 76644, 2 gravid specimens from Tichigan Lake, Wisconsin; USNM Helm. Coll. No. 59689, 1 whole mount and sections of 1 specimen from Coffee Creek, Ohio; HWML (University of Nebraska State Museum, H. W. Manter Laboratory) Coll. Nos. 21335, 21337, whole mounts of 7 immature specimens from Coffee Creek, Ohio and 1 from Saline, Michigan; and Nos. 21336, 21338, sections of 7 gravid specimens from Coffee Creek and 2 from Saline.

TYPE HOST: Mudminnow, *Umbra limi* (Umbridae).

TYPE LOCALITY: Tichigan Lake Canal, Racine County, Wisconsin.

OTHER LOCALITIES: Coffee Creek and Mosquito Creek, Trumbull County; Oak Openings Swanton Township, Lucas County; Styx River, Medina County (Ohio); Saline, Washtenaw County (Michigan).

SITE OF INFECTION: Intestine.

ETYMOLOGY: The generic name indicates a close relationship to the South American genus *Creptotrematina* Yamaguti, 1954; the specific name refers to the type host.

Remarks

Only three gravid specimens were recovered from the intestine of one mudminnow collected in the spring from the Tichigan Lake Canal, Wisconsin. Nine, 66, and 10 more mudminnows collected from the same location in the spring, summer, and autumn, respectively, were negative for *P. limi* infections, as were an additional 2,430 fishes representing 39 species in 12 families collected from the same lake.

In Ohio, a single *Umbra limi,* taken in Lucas County in April 1953, yielded 8 *P. limi,* and 17 of 76 mudminnows collected in Medina and Trumbull counties between February and June 1957 harbored 31 worms, with a maximum of nine in one host. Eighty-six other mudminnows from collections made in Trumbull, Portage, and Ottawa counties, Ohio, on other dates were not infected. Detailed data on the 1955 Michigan collection are not available. Most of the Michigan and Ohio specimens (except those collected in April) were immature.

The Ohio specimens were slightly smaller than the more mature Wisconsin ones, which had relatively larger reproductive organs. In the Ohio material, eyespot pigment and the paired gland clusters were more conspicuous, whereas the vitelline follicles were less distinct (Fig. 2). Also, the esophagus was relatively longer and the testes less distinctly diagonal than in the Wisconsin material.

The new genus is easily separated from *Megalogonia,* which has four testicular lobes, and from *Creptotrema,* in which the uterus is confined to the pretesticular area. It most closely resembles the South American genus *Creptotrematina* Yamaguti, 1954, which, however, has ceca reaching only just past midlength of the body. Yamaguti (1954) erected *Creptotrematina* for *Creptotrema dissimilis* Freitas, 1941, with which he synonymized *Creptotrema dispar* Freitas, 1941. The new species further differs from *C. dissimilis* in having a narrower body, less widely spaced testes, oral sucker larger than acetabulum, oral lobes less anteriorly located on the oral sucker, a considerably larger pharynx, a less extensive uterus, and smaller and fewer vitelline follicles. The latter in *C. dissimilis* showed considerable variation in size (Freitas, 1941a), being largest in bigger worms. In his smallest worm (Fig. 6), which was of about the same size as our holotype (Fig.

1), the size of vitellaria appeared comparable. Vitelline follicles in *C. dispar* were considerably larger (Freitas, 1941b).

Creptotrematina aguirrepequenoi Jimenez-Guzman, 1973, from *Astyanax fasciatus mexicanus* (Characidae) in Mexico, is herein transferred to genus *Paracreptotrematina*, primarily because of its long ceca. It differs from *P. limi* in having a larger, yellow body (121–2,800 by 392–770), equal suckers with acetabulum in anterior one-tenth to one-fifth of body, smaller pharynx (35–70 by 46–91), large vitelline follicles (21–105 by 21–70) extending to the posterior extremity, markedly diagonal testes with the anterior testis on the right side of body, and more numerous and elongate eggs (Jimenez-Guzman, 1973).

Acknowledgments

We are grateful to Professor J. H. Fischthal, State University of New York, Binghamton, for taxonomic comments based on his examination of the Wisconsin material.

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Proc. Helminthol. Soc. Wash.
49(2), 1982, pp. 189–195

Redescription of *Phyllodistomum americanum* Osborn, 1903 with a Discussion of the Taxonomic Status of *Phyllodistomum coatneyi* Meserve, 1943 and *Phyllodistomum bufonis* Frandsen, 1957 (Trematoda: Gorgoderidae)

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ABSTRACT: A redescription of *Phyllodistomum americanum* Osborn, 1903 is based on new collections from Wisconsin *Ambystoma tigrinum* and examination of type material of this species, type material of *P. coatneyi*, and specimens of *P. bufonis* previously collected in Colorado. *Phyllodistomum americanum*, *P. coatneyi*, and *P. bufonis* are considered valid species. Errors associated with the original description of *P. americanum* by Osborn are rectified. Discrepancies in characters used to differentiate *P. coatneyi* and *P. americanum* are corrected, and presumed host specificity based on present and previous collections of *P. americanum* and *P. bufonis* is discussed.

This report deals with three species of *Phyllodistomum* having amphibian hosts in the north-central and western United States. Osborn (1903) described *P. americanum* from Minnesota urodeles, identified as *Ambystoma punctatum* in the title but as *A. tigrinum* in the text of his paper. Later, Crawford (1940) reported *P. americanum* from *A. tigrinum* and *Bufo boreas* in Colorado. Meserve (1943) described *P. coatneyi* in Wisconsin, and Frandsen (1957) described *P. bufonis* from *Bufo boreas* in Utah.

During 1979–1980, the authors recovered *P. americanum* from the urinary bladder of *A. tigrinum* in southeastern Wisconsin. Quantities recovered warrant a redescription of *P. americanum* and a reappraisal of characters used to differentiate this species from *P. coatneyi* and *P. bufonis*. For this purpose the authors' material was supplemented with type specimens and other collections of the three species.

After specimens of *P. americanum* were examined alive, they were fixed with AFA and stored in 70% ethanol until prepared as whole mounts or as serial sections cut at 6 micrometers and stained with hematoxylin and eosin. Whole mounts were stained with borax carmine or Mayer's hematoxylin and mounted in Canada balsam. Measurements are in millimeters unless otherwise stated; mean values are in parentheses. Figures were drawn with the aid of a camera lucida. Additional collection data may be obtained from Coggins and Sajdak (1982).

Phyllodistomum americanum Osborn, 1903 (Figs. 1–3)

REDESCRIPTION (based on 19 specimens): Body elongate in young to fusiform or spatulate in older individuals, flattened dorsoventrally, 1.22–4.82 (2.43) long

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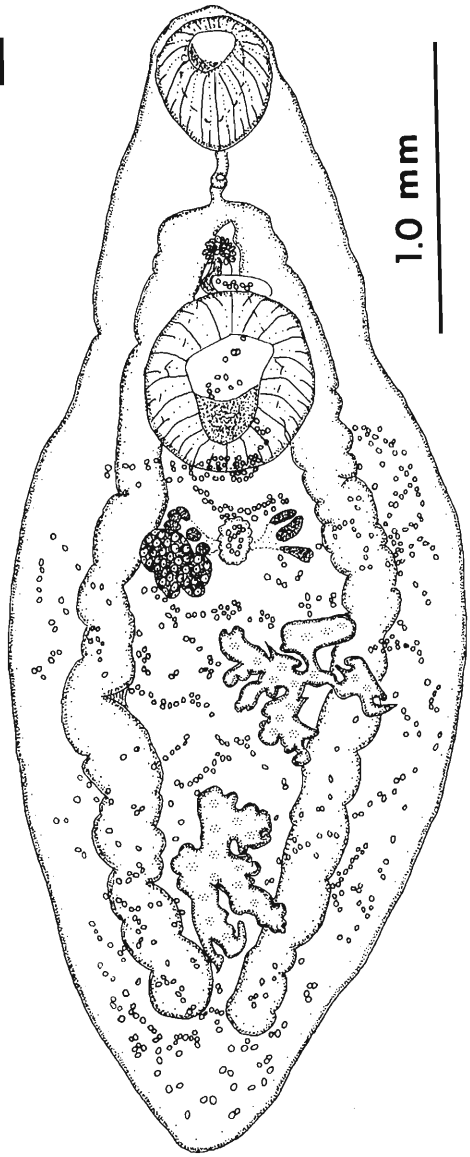
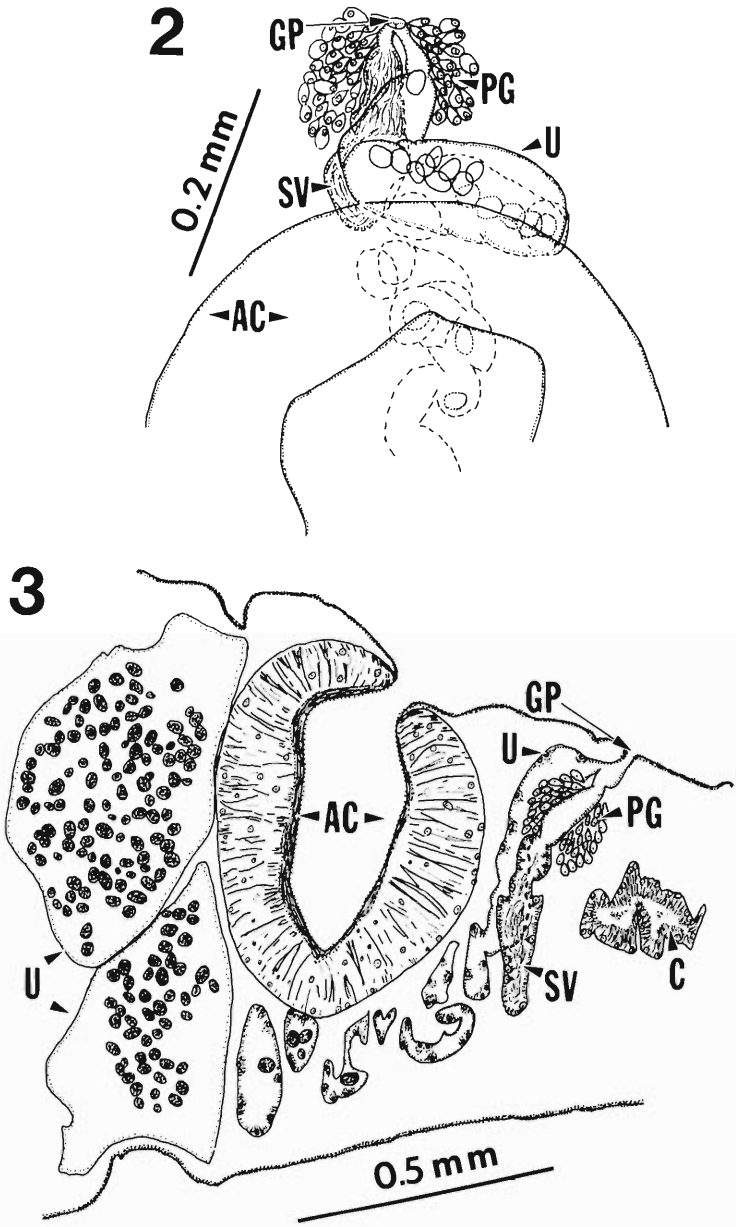


Figure 1. ***Phyllodistomum americanum*** **Osborn, 1903. Ventral view of hypotype collected from** ***Ambystoma tigrinum*** **in Wisconsin. Abbreviations: AC, acetabulum; C, caecum; GP, genital pore; PG, prostate gland; SV, seminal vesicle; U, uterus.**

by 0.25–2.05 (0.68) wide. Oral sucker subterminal, 0.16–0.56 by 0.14–0.52 (0.29 × 0.25). Acetabulum in anterior half of body, 0.20–0.84 by 0.18–0.83 (0.38–0.36). Ratio of sucker length 1:1.08–1.50 (1:1.27); ratio of sucker width 1:1.27–1.75 (1:1.47). Testes tandem to slightly oblique, deeply lobed. Anterior testis 0.084–0.74 by 0.091–0.80 (0.29 × 0.23); posterior testis 0.10–0.91 by 0.07–1.12 (0.35 × 0.22). Ovary irregularly to deeply lobed, pretesticular, postacetabular; 0.049–0.41 by 0.049–0.23 (0.18 × 0.14). Vitellaria less than ¼ the size of the



Figures 2, 3. ***Phyllodistomum americanum*** **Osborn, 1903 (hypotype). Terminal genitalia. 3.** ***Phyllodistomum americanum*** **Osborn, 1903 (hypotype). Parasagittal section of terminal genitalia, acetabulum, and uterus posterior to acetabulum. Abbreviations as for Figure 1.**

ovary, follicular, lying close together near center of the body. Mehlis' gland dorsomedial to level of ovary, 0.05–0.16 by 0.05–0.21 (0.086 × 0.081). Seminal vesicle 0.035–0.24 by 0.021–0.14 (0.11 × 0.07). Caeca extending to or somewhat beyond posterior testis. Eggs non-operculate, 0.021–0.028 by 0.016–0.021 (0.025 × 0.020).

HOST: *Ambystoma tigrinum tigrinum* (Green).

SITE IN HOST: Urinary bladder.

LOCALITIES: Waukesha County and Sheboygan County, Wisconsin, new localities.

HYPOTYPE SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 76917 and 76918 and UNSM Manter Helm. Coll. Nos. 21356–21359. Other hypotypes have been retained in the authors' collections.

SYNTYPE SPECIMENS: After conducting a search for the original specimens of *P. americanum* and *P. coatneyi*, syntypes were discovered at Hamline University and the University of Nebraska State Museum, respectively. Three syntypes of *P. americanum*, a whole mount, a specimen in cross section, and a specimen sectioned parasagittally, have been designated as the lectotype and paralectotypes, respectively. The syntype of *P. coatney* has been designated the lectotype. The 19 specimens of *P. americanum* in Wisconsin are designated hypotypes. This terminology is in accordance with that proposed by Frizzell (1933).

LECTOTYPE OF *P. AMERICANUM* (whole mount): USNM Helm. Coll. No. 76548.

PARALECTOTYPES OF *P. AMERICANUM* (cross and parasagittal sections): USNM Helm. Coll. Nos. 76549 G 35-16 and 76549 G 35-17.

LECTOTYPE OF *P. coatneyi* (whole mount): USNM Helm. Coll. No. 76578.

Type materials to describe *P. americanum* Osborn, 1903 and *P. coatneyi* Meserve, 1943 were studied, in addition to 19 specimens of *P. americanum* collected in Wisconsin. All specimens of *P. americanum* agree with the original description except that mature eggs range from 0.021 × 0.016 to 0.028 × 0.021 (0.025 × 0.020), rather than 0.052 × 0.050.

Discussion

Meserve (1943) differentiated *P. coatneyi* from *P. americanum* by the following characters: testes larger, more lobular, and more anterior; uterine loops less numerous and different in extent; seminal vesicle larger; vitellaria about one-half the size of the ovary; and eggs much smaller. Based on a reexamination of Osborn's specimens, it is evident that he erred in measuring egg size. It is apparent that egg size of these two species does not differ significantly. Certain other diagnostic differences between *P. americanum* and *P. coatneyi* listed by Meserve are also in error (Table 1): the vitellaria of *P. americanum* are not approximately the same size as the ovary, but are slightly less than one-fourth the size of the ovary, and testes of *P. americanum* are slightly smaller, but are more lobed and more anterior in extent than in *P. coatneyi*. Although the uterine loops are more numerous in *P. americanum*, they extend laterally to the body margins.

Crawford (1939, 1940) collected digeneans, reported as adult *P. americanum*, from naturally infected *B. boreas* and *A. t. tigrinum* in Colorado. He described the life cycle and was able to infect *B. boreas* experimentally, but not *A. tigrinum*. From 60 *B. boreas* from the same locality, Tonn (1961) recovered 927 worms that he identified as *P. bufonis*. Careful comparison revealed that Crawford's specimens were *P. bufonis*, not *P. americanum*. Specimens of *P. bufonis* were reported to differ from *P. americanum* in sucker ratio, size of eggs, and absence of a posterior notch in the body (Frandsen, 1957). However, collections of *P. bufonis* from *B. boreas* in Colorado (Tiekotter, 1977; USNM Helm. Coll. No. 76450–76455; UNSM Helm. Coll. No. 21309–21315) and the authors' specimens

Table 1. Comparative measurements of *P. americanum*, *P. bufonis*, and *P. coatneyi*.

	P. americanum (present study)	*P. americanum* (Osborn, 1903)	*P. americanum* (lectotype; Osborn, 1903)	*P. bufonis* (Frandsen, 1957)	*P. bufonis* (Tiekotter, 1977)	*P. bufonis* (Tonn, 1961)	*P. bufonis* (Crawford, 1940 by Tonn, 1961)	*P. coatneyi* (Meserve, 1943)
Total length	1.22–4.82 *(2.39)	4.2	3.5	7.5–9.0 (8.0)	2.20–9.80	3.60–6.40	3.00–4.10	3.3–7.0
Total width	0.25–2.05 (0.67)	0.88	0.79	2.0–2.75 (2.07)	0.67–2.30	0.80–2.60	0.70–1.20	0.7–1.7
Oral sucker	0.16 × 0.14 0.56 × 0.52 (0.29 × 0.25)	N.A.†	0.41 × 0.35	0.425–0.55 (0.475)	0.27–0.59 × 0.26–0.57	0.32–0.62	0.32–0.38	0.30–0.47
Acetabulum	0.20 × 0.18 0.84 × 0.83 (0.38 × 0.36)	N.A.	0.53 × 0.48	0.875–1.0 (0.922)	0.35–1.01 × 0.35–1.0	0.45–1.07	0.53–0.64	0.45–0.63
Sucker size ratio	1:1.08–1:1.50 (1:1.27)	N.A.	1:1.29	1:1.73–1:2.0	1:1.34–1:2.10 (1:1.67)	1:1.09–1:1.84	1:1.40–1:1.87	1:1.43 (lectotype)
Length and width of ovary	0.05 × 0.049 0.41 × 0.23 (0.18 × 0.14)	N.A.	0.27 × 0.25	0.63 × 0.43	0.75–1.0 × 0.18–0.30	0.19–0.36 × 0.19–0.40	0.36–0.51 × 0.11–0.19	0.33–0.55 × 0.25–0.43
Length and width of anterior testis	0.08 × 0.09 0.74 × 0.80 (0.29 × 0.23)	N.A.	0.51 × 0.25	1.00–1.37 × 0.40–0.82	0.72–1.65 × 0.31–0.40	0.55–0.94 × 0.26–0.79	0.30–0.44 × 0.17–0.28‡	0.49–1.23 × 0.36–0.86
Length and width of posterior testis	0.10 × 0.070 0.91 × 1.12 (0.11 × 0.07)	N.A.	0.58 × 0.27	1.15–1.75 × 0.40–0.80	0.91–1.97 × 0.32–0.41	0.58–1.25 × 0.30–0.75	0.42–0.63‡ × 0.15–0.28‡	0.94 × 0.64 (lectotype)
Seminal vesicle	0.04 × 0.02 0.24 × 0.14 (0.11 × 0.07)	N.A.	N.A.	N.A.	0.17 × 0.30 0.80 × 0.64 (0.31 × 0.39)	N.A.	N.A.	0.09–0.26 × 0.13–0.41
Mehlis' gland	0.05 × 0.05 0.16 × 0.21 (0.09 × 0.08)	N.A.	N.A.	N.A.	0.10 × 0.10 0.21 × 0.19 (0.14 × 0.13)	N.A.	N.A.	N.A.
Egg size	0.021 × 0.016 0.028 × 0.021 (0.025 × 0.020)	0.052 × 0.50	0.022–0.032 × 0.017–0.028	0.028–0.034 × 0.022–0.026 (0.024)	0.018 × 0.010 0.032 × 0.020 (0.024 × 0.017)	N.A.	N.A.	0.021–0.029 × 0.015–0.020

*.Numbers in parentheses are means.
† N.A. = not available.
‡ Badly distorted.

of *P. americanum* from *A. tigrinum* in Wisconsin show that sucker ratio ranges of the two species definitely overlap, although the average ratio is greater in *P. bufonis*. Their eggs are not significantly different in size (Table 1), and individuals of both species exhibit varying degrees of posterior indentation.

Ubelaker and Kimbrough (1970) reported *P. bufonis* from *B. boreas* and *A. tigrinum* in Colorado. Although Ubelaker and Olsen (1972) experimentally infected *B. boreas* with *P. bufonis*, they were unable to infect *A. tigrinum*. Thus, experimental infections of *P. bufonis* in *A. tigrinum* have not been established, and the occurrence of *P. bufonis* in *A. tigrinum* must be examined more critically.

In the present study, 29 *A. tigrinum*, 20 *A. maculatum*, and 10 *Bufo americanus* were collected from the same localities. *Phyllodistomum americanum* was recovered from 12 *A. tigrinum* (41%); *A. maculatum* harbored no bladder flukes. Another bladder fluke, *Gorgoderina bilobata*, was recovered from *B. americanus*. *Phyllodistomum americanum* was found only in *A. tigrinum* in the present study and those collections previously reported from *B. boreas* are apparently in error. Consequently, there appears to be definite host specificity of *P. americanum* in *A. tigrinum*. This further supports the hypothesis that the host referred to by Osborn (1903) was *A. tigrinum* rather than *A. maculatum*.

Conclusions

Based on collections of *P. bufonis* from *B. boreas* (Frandsen, 1957; Tonn, 1961; Tiekotter, 1977) and the examination of *A. tigrinum*, *A. maculatum*, and *B. americanus* in the present study, the authors believe that both *P. bufonis* and *P. americanum* exhibit host specificity and are restricted to *B. boreas* and *A. tigrinum*, respectively. Although *P. coatneyi* has not been reported since Meserve (1943) recovered this trematode from *A. maculatum*, it appears to be a valid species specific to *A. maculatum*.

The most valid taxonomic character for identification of *P. americanum*, *P. bufonis*, and *P. coatneyi* appears to be their presumed host specificity. Other taxonomic characters include the size of the vitellaria in relation to the ovary, testicular location and lobation, size of the seminal vesicle, and the sucker ratio. These characters are valid only when large numbers of individuals can be obtained and compared. Each of these three species exhibit prominent intraspecific variations. Thus, characteristics such as total length, width, and, to a lesser degree, sucker ratio should only be considered when a large number of individuals have been secured and compared, as in Table 1.

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Adult Trematodes (Digenea) from Lake Fishes of Southeastern Wisconsin, with a Key to Species of the Genus *Crepidostomum* Braun, 1900 in North America

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ABSTRACT: Eleven species in six families of digenetic trematodes are reported from eleven species of fish collected from two inland lakes in southeastern Wisconsin between 1976 and 1981. New host records include *Allocredium lobatum* Wallin, 1909 from *Lepomis macrochirus* and *Macroderoides spiniferus* Pearse, 1924 from *Amia calva*. *Lissorchis mutabile* (Cort, 1919) is reported for the first time in Wisconsin. All records, except that of *L. attenuatum* Mueller and Van Cleave, 1932, are new to southeast Wisconsin. A key to species of *Crepidostomum* Braun, 1900 in North America is included. Morphological variations for each trematode species are documented and host and seasonal distribution are also noted.

This is the second in a series of three reports on the trematode parasites of fishes from two southeastern Wisconsin lakes in Racine and Kenosha counties. In the other reports, a new species of Allocreadiidae is described (Amin and Myer, 1982) and the larval forms (metacercariae) are treated (Amin, 1982). Most of the recovered species were reported previously from Wisconsin (Fischthal, 1953), but morphological variability of many remained to be documented. This is done in the present report, and comparisons with previous accounts are made when available. A review of the species of *Crepidostomum* Braun, 1900 in North America and of keys constructed by Hopkins (1931, 1933) indicated the need for a new key, which is included in this report. Ecological observations are also included.

Materials and Methods

The fishes examined were from Silver Lake, a 200-ha eutrophic land-locked lake in Kenosha County, and from Tichigan Lake (Racine County), a 108-ha lake in an advanced state of eutrophication on the Fox River (a tributary of the Mississippi River). Seasonal collections were made from both lakes during the spring (April), summer (June, July, and early August), and autumn (late October and November) between 1977 and 1981, and from Silver Lake during the summer of 1976. A total of 1,812 fishes representing 32 species and 10 families (Amiidae, 1 species; Catostomidae, 7; Centrarchidae, 9; Cyprinidae, 2; Esocidae, 2; Ictaluridae, 4; Lepisosteidae, 1; Percidae, 2; Salmonidae, 2; Serranidae, 2) were captured by electroshocking from both lakes. An additional 1,543 fishes representing 27 species and 11 families (Amiidae, 1; Catostomidae, 3; Centrarchidae, 6; Cyprinidae, 5; Cyprinodontidae, 2; Esocidae, 2; Gasterosteidae, 1; Ictaluridae, 4; Percidae, 3; Serranidae, 1; Umbridae, 1) were primarily seined or minnow trapped in a channel draining the swampy western area of Tichigan Lake during 1978, 1979, and 1981.

Fish were systematically dissected shortly after capture. Specimens were processed as in Amin and Myer (1982). Line drawings were made with the aid of a microprojector. All measurements (length by width with averages in parentheses)

Table 1. Distribution of adult trematodes in infected fish species from Silver and Tichigan lakes, 1976–1981.

Trematode species	Host species	Silver Lake 1976–1979	Tichigan Lake 1976–1978	Tichigan Canal 1978, 1979, 1981
Allocreadiidae				
Allocreadium lobatum	*Lepomis macrochirus*	0, 0/301*	0, 0/212	2, 1/121
Bunoderina eucalia	*Culaea inconstans*	0, 0/0	0, 0/0	139, 36/182
Bunoderina succulata	*Perca flavescens*	5, 4/67	0, 0/77	0, 0/2
Crepidostomum cornutum	*Lepomis gibbosus*	4, 1/16	0, 0/60	0, 0/14
	Lepomis macrochirus	7, 6/301	0, 0/212	0, 0/121
Paracreptotrematina limi	*Umbra limi*	0, 0/0	0, 0/0	3, 1/86
Azygiidae				
Azygia angusticauda	*Amia calva*	0, 0/18	2, 1/23	0, 0/37†
	Lepomis macrochirus	1, 1/301	0, 0/212	0, 0/121
Azygia longa	*Amia calva*	19, 5/18	2, 2/23	0, 0/37†
	Esox lucius	2, 1/20	0, 0/22	0, 0/2
	Stizostedion vitreum	3, 1/54	0, 0/52	0, 0/3
Cryptogonimidae				
Caecincola purvulus	*Micropterus salmoides*	1, 1/72	0, 0/44	0, 0/3
Lissorchiidae				
Lissorchis attenuatum	*Catostomus commersoni*	1, 1/10	0, 0/54	7, 1/11
Lissorchis mutabile	*Erimyzon sucetta*	33, 6/94	0, 0/0	0, 0/1
Macroderoidae				
Macroderoides spiniferus	*Amia calva*	94, 2/18	0, 0/23	0, 0/37†

* No. parasites recovered, no. hosts infected/no. fish examined.
† Most were fingerlings.

are in micrometers, unless otherwise noted, and include a few measurements of eggs and vitelline follicles from each specimen. Fish size is expressed in total length (cm). Representative specimens were deposited in the U.S. National Museum Helminthological Collection (USNM Helm. Coll.), where all specimens drawn in Figures 1–12 are included, and in the Milwaukee Public Museum Collection (MPM Coll.).

Results and Discussion

The following 11 species of digenetic trematodes representing six families were recovered from eleven species of fish in Silver and Tichigan lakes as summarized in Table 1.

Family Allocreadiidae (Looss, 1902) Stossich, 1903

Allocreadium lobatum Wallin, 1909 (Fig. 1)

Identification of this species was based on the systematic revision of the genus by Peters (1957). Only two gravid specimens were recovered from the intestine of one 6.5-cm-long *Lepomis macrochirus* in the Tichigan canal in May 1978. Body length of *L. macrochirus* from Tichigan Lake ranged between 6 and 29 cm. This is a new host record. Only Arnold et al. (1968) reported *Allocreadium* sp. from *L. macrochirus* in Louisiana.

DIMENSIONS (N = 2): Body 2.16 (2.16) by 0.72 mm (0.72), oral sucker 280–294 (287) by 294 (294), acetabulum 308–350 (329) by 294–308 (301), pharynx 168–182 (175) by 154 (154), anterior testis 322–392 (357) by 252–322 (287), posterior testis 322–476 (399) by 280 (280), cirrus sac 238–308 (273) by 140–182 (161), ovary 280–336 (308) by 280 (280), vitelline follicles 64–102 (89) by 48–80 (56), eggs 48–67 (56) by 38–51 (43).

Body size was smaller than that reported by Wallin (1909; 3–6.7 by 0.72–1.5 mm) but comparable to that reported by Mueller (1934; 2–4 mm long). All organs in Wallin's material were relatively larger and the esophagus was longer than in my specimens; eggs were only slightly larger, 67–85 long by 46–57 wide. The cecal bifurcation was distinctly anterior to the acetabulum as described by Wallin, and not dorsal to it as reported by Mueller. My specimens otherwise fit the descriptions given by them.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 76647.

Bunoderina eucaliae Miller, 1936
(Fig. 2)

This species was represented by 139 worms from 39 of 182 *Culaea inconstans* from the Tichigan canal (Table 1). Summer infections were heavier (121 worms in 35 of 135 fish examined) than spring infections (15 worms in 4 of 45 fish examined).

DIMENSIONS (N = 28): Body 0.31–1.68 (1.06) by 0.11–0.53 mm (0.28), oral sucker 49–182 (144) by 56–182 (134), acetabulum 77–252 (175) by 77–238 (171), pharynx 35–98 (70) by 35–91 (64), anterior testis 56–140 (101) by 42–98 (78), posterior testis 63–182 (116) by 42–154 (98), ovary 56–140 (116) by 56–126 (98), vitelline follicles 32–102 (59) by 22–86 (44), eggs 54–96 (69) by 32–54 (39).

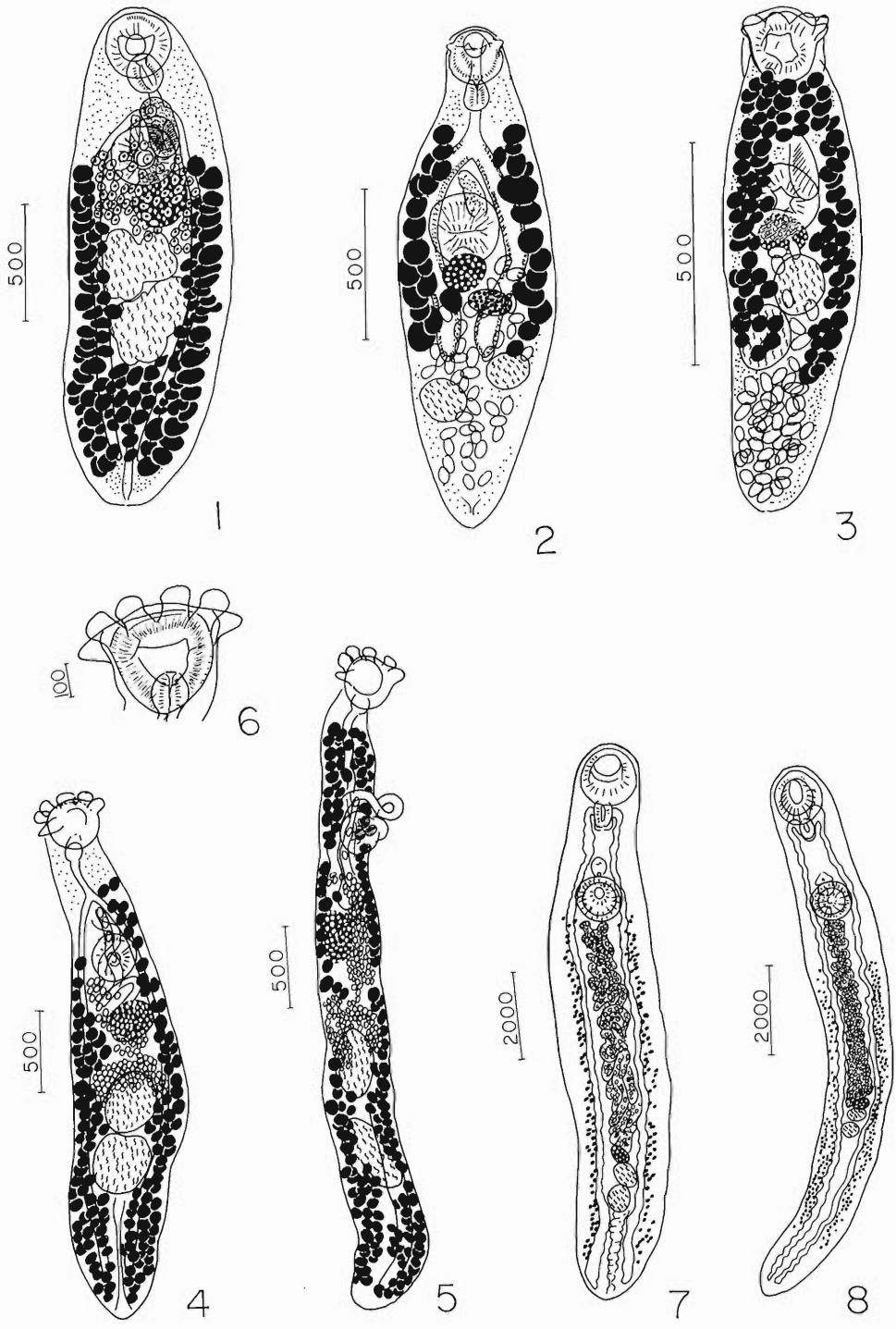
My specimens were larger than those described by Miller (1936) from the same host in Canada and had a considerably wider size range. Miller measured only the body (0.83–1.06 by 0.31–0.35 mm) and eggs (70–83 by 44–47) (N = ?). The Wisconsin material (Fig. 2) otherwise resembled Miller's except that the pharynx appeared smaller, the esophagus straight and somewhat shorter, and the cecal bifurcation markedly more anterior in my specimens. Furthermore, in many of my smaller specimens (gravid or nongravid), the testes were relatively more posteriorly located. Size of eggs and worms essentially did not correlate; very large eggs were observed in some of the smallest specimens, which had even smaller testes than eggs.

DEPOSITED SPECIMENS: 20 in USNM Helm. Coll., Nos. 76658–76660; 16 in MPM Coll., No. IZ365a.

Bunoderina sacculata (VanCleave and Mueller, 1932) Yamaguti, 1958
(Fig. 3)

Five gravid specimens were recovered from the anterior intestine of four 10-cm-long *Perca flavescens* only in July 1979, when 38 perches were examined from Silver Lake. VanCleave and Mueller (1932) did not find the species in Oneida Lake perches before August 8, whereas Cannon (1972, 1973) reported spring and autumn peaks in *B. sacculata* populations, particularly in larger *P. flavescens* from Ontario.

DIMENSIONS (N = 1): Body 1.12 by 0.31 mm, oral sucker 168 by 154, acetab-



Figures 1–8. 1. *Allocreadium lobatum*; seminal receptacle not shown. 2. *Bunoderina eucaliae*. 3. *Bunoderina sacculata*. 4, 5. A normal and a more extended specimen of *Crepidostomum cornutum* drawn to same scale. 6. Detail of oral sucker of *C. cornutum*. 7. *Azygia angusticauda*. 8. *Azygia longa*.

ulum 168 by 168, anterior testis 126 by 98, posterior testis 154 by 126, ovary 98 by 98, vitelline follicles 42–64 (57) by 35–61 (45), eggs 64–80 (74) by 42–58 (47).

Worms were similar to those reported by VanCleave and Mueller (1932) in size and anatomical detail (particularly their fig. 3, pl. 7), except that in my specimens the posterior testis was considerably closer to the posterior extremity (Fig. 3). Uterine expansion in the gravid specimens must, at least, partially account for this observation (see fig. 1, pl. 7, in VanCleave and Mueller). The above authors provided measurements of only the body (0.5 to >2.0 mm long) and eggs (66–85 by 40–49) (N = ?). My specimens were also comparable to those described by Hopkins (1934), who measured only body length (0.5–2.00 mm) and eggs (65–85 by 38–58) (N = ?).

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 7657.

Crepidostomum cornutum (Osborn, 1903) Stafford, 1904 (Figs. 4–6)

Eleven gravid worms were recovered from the stomach and ceca of one *L. gibbosus* and six *L. macrochirus* in Silver Lake (Table 1) only in the spring, when six and 62 fishes were examined, respectively. Infected hosts were 17–21 cm long and of both sexes, whereas the length of examined fish ranged between 6 and 22 cm; most were below 17. A similar relationship between *C. cooperi* infections and size of *P. flavescens* was observed by Cannon (1973).

DIMENSIONS (N = 9): Body 2.80–4.40 (3.60) by 0.36–0.80 mm (0.60), oral sucker 266–378 (318) by 252–336 (300), knobbed dorsal papillae on oral sucker 98–112 (105) by 70–98 (83) and conical lateral papillae 140–196 (168) by 98–112 (101), acetabulum 224–350 (276) by 224–294 (279), pharynx 98–126 (114) by 98–112 (107), anterior testis 350–490 (438) by 252–378 (308), posterior testis 378–644 (480) by 252–364 (315), ovary 238–294 (255) by 196–238 (218), vitelline follicles 54–154 (99) by 40–115 (75), eggs 67–86 (78) by 42–64 (52).

My specimens were similar to the ones studied by Hopkins (1934) from various sources in the United States and Canada, and to those of VanCleave and Mueller (1934) from Oneida Lake. They were, however, markedly larger than Hopkins' (1.0–3.7 mm long, width ¼–⅓ length) and VanCleave and Mueller's (1.0–3.0 mm long) specimens, but egg sizes were comparable, 65–90 (81) by 35–68 (56) and 60–90 by ≤⅔ length, respectively. The only other measurements provided by any of the above authors is the diameter of the oral sucker, 210–450 by VanCleave and Mueller. The ratios of various organ dimensions to others reported by Hopkins were not sufficiently species specific, and varied considerably with the state of relaxation of specimens (Figs. 4, 5).

The four dorsal papillae on the oral sucker in local material were not conical as previously described (Hopkins, 1934), but distinctly knobbed distally and broader at the base (Fig. 6). Further, my specimens were not widest at the oral sucker level and were less robust (average width/length = 1/6; Hopkins: 1/4 to 1/3), had the posterior extent of the uterus to the middle of the anterior testis, and showed the anterior extent of the vitellaria between the pharynx and cecal bifurcation (Figs. 4, 5). Extreme variations were reported by Simer (1929) and Hopkins (1934), who noted extension of the uterus to the posterior edge of posterior testis, and by Lyster (1939), who observed oral suckers three to four times the size of the acetabulum.

The keys to species of *Crepidostomum* provided by Hopkins (1931, 1933) are considered inadequate and outdated. The inclusion of certain European forms not represented in North America and of a number of synonyms is confusing. Usage of the widely variable and overlapping ratios of various organ dimensions to others renders the keys even more ineffective. The generic diagnosis of Yamaguti (1958, p. 130) is strictly applied, and only seven North American species with two testes and six oral papillae each are recognized. *Crepidostomum ictaluri* VanCleave and Mueller (1934) is synonymous with *Megalogonia ictaluri* (Surber, 1928) Hopkins, 1934, and is not included in the following key. For this and other synonymies see Yamaguti (1971). A simple key to North American species of *Crepidostomum* is provided below.

Key to North American Species of *Crepidostomum*

1. Genital pore anterior to intestinal bifurcation 2
   Genital pore ventral or posterior to intestinal bifurcation 3
2. Acetabulum distinctly larger than oral sucker ..... *C. farionis*
   Suckers equal or oral sucker slightly larger than acetabulum ..... *C. isostomum*
3. Vitellaria postacetabular only ..... *C. brevivitellum*
   Vitellaria pre- and postacetabular 4
4. Cirrus sac stout, rarely reaching beyond acetabulum ..... *C. lintoni*
   Cirrus sac slender, extending well beyond acetabulum 5
5. Median dorsal pair of oral papillae notched at tip ..... *C. illinoiense*
   No notched papillae 6
6. Oral sucker larger than acetabulum; vitellaria not confluent posterior to testes ..... *C. cornutum*
   Suckers subequal; posttesticular vitellaria confluent posterior to testes ..... *C. cooperi*

DEPOSITED SPECIMENS: 5 in USNM Helm. Coll., Nos. 76645 and 76646; 3 in MPM Coll., No. IZ365b.

Paracreptotrematina limi Amin and Myer, 1982

Three specimens infected one *Umbra limi* from the Tichigan Lake canal in May 1978. Other specimens were collected from the same host species in Ohio and Michigan. For additional information see Amin and Myer (1982).

Azygiidae (Lühe, 1909) Odhner, 1911

Azygia angusticauda (Stafford, 1904) Manter, 1926 (Fig. 7)

Azygia longa (Leidy, 1851) (Fig. 8)

Manter (1926) reduced eight nominal North American species of *Azygia* to three that VanCleave and Mueller (1934) further reduced to two, *A. angusticauda* and *A. longa*. Both were recovered in the present study. Their highly muscular bodies and great contractability cause taxonomic difficulties related to processing meth-

ods. However, the two species are distinguished by three relatively stable differences. The testes are more posterior, the anterior extent of the vitellaria is closer to the acetabulum, and the sucker is farther from the anterior end in *A. angusticauda* (Fig. 7) than in *A. longa* (Fig. 8).

Both species of *Azygia* infected fish from both lakes, living in the stomach (87%) and upper intestine end of the host (13%). *Azygia angusticauda* infected *Amia calva* in the spring and *L. macrochirus* in the summer, whereas *A. longa* infected *Stizostedion vitreum* in the spring, *Esox lucius* in the autumn, and *A. calva* in all three seasons (24% of 21 specimens in spring, 67% in summer, and 9% in autumn). Two juveniles of *A. longa* from *A. calva* and one of *A. angusticauda* from *L. macrochirus* were also recovered during the spring (not included in following measurements).

DIMENSIONS OF *A. ANGUSTICAUDA* (N = 2): Body 12.16–13.92 (13.04) by 2.36–2.40 mm (2.38), oral sucker 1,240–1,280 (1,260) by 1,160–1,240 (1,200), acetabulum 960–1,000 (980) by 1,040 (1,040), pharynx 440–480 (460) by 400 (400), anterior testis 600–640 (620) by 480–520 (500), posterior testis 640 (640) by 600–640 (620), ovary 680–720 (700) by 400 (400), vitelline follicles 112–210 (143) by 84–140 (106), eggs 38–58 (48) by 22–32 (25).

DEPOSITED SPECIMENS: 1 in USNM Helm. Coll., No. 76648; 1 in MPM Coll., No. IZ365c.

DIMENSIONS OF *A. LONGA* (N = 14): Body 4.28–13.48 (10.24) by 0.80–1.80 mm (1.22), oral sucker 600–1,080 (852) by 520–1,000 (804), acetabulum 440–840 (632) by 440–840 (636), pharynx 240–480 (352) by 220–360 (292), anterior testis 400–600 (476) by 200–400 (320), posterior testis 320–600 (456) by 280–440 (364), ovary 360–600 (480) by 200–720 (328), vitelline follicles 70–196 (118) by 42–154 (76), eggs 35–61 (48) by 22–32 (29).

DEPOSITED SPECIMENS: 14 in USNM Helm. Coll., Nos. 76649–76651; 6 in MPM Coll., No. IZ365d.

Cryptogonimidae (Ward, 1917) Cirurea, 1933

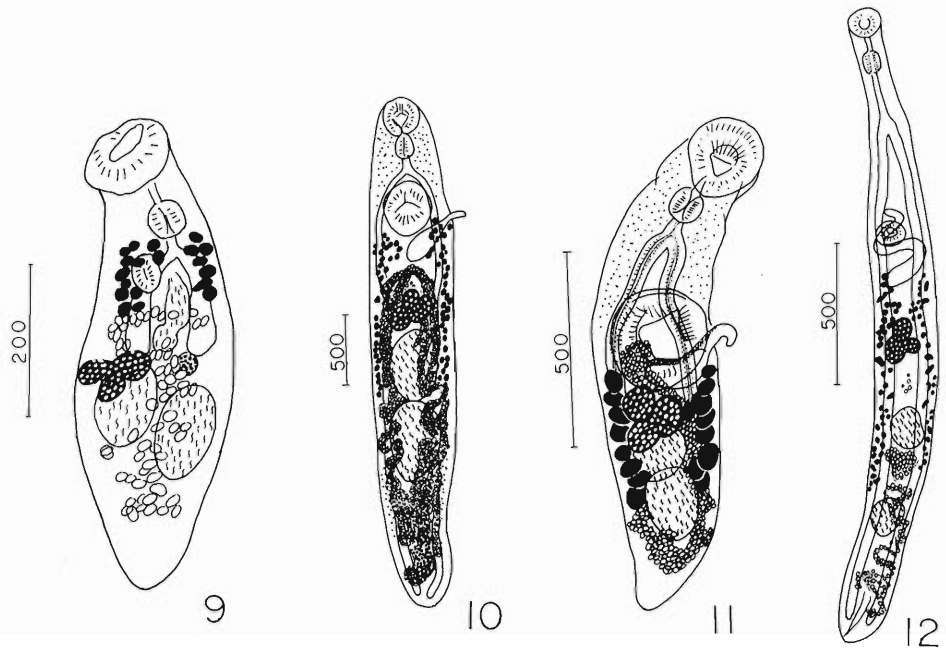
Caecincola parvulus Marshall and Gilbert, 1905 (Fig. 9)

One gravid specimen was recovered from one *Micropterus salmoides* in Silver Lake in April 1978. VanCleave and Mueller (1934) found thousands of *C. parvulus* from that host species during late August. "*Caecincola parvulus*" reported by Pearse (1924b) from *Ambloplites rupestris* in Lake Michigan, Wisconsin (USNM Helm. Coll. No. 7617), was later identified as *Cryptogonimus* by Yamaguti (1971).

DIMENSIONS (N = 1): Body 0.62 by 0.21 mm, oral sucker 122 by 125, acetabulum 35 by 32, pharynx 38 by 38, anterior testis 112 by 82, posterior testis 122 by 73, ovary 93 by 80, vitelline follicles 19–26 (23) by 16–19 (17), eggs 19–26 (22) by 13–16 (14).

My specimen agrees with the description given by Marshall and Gilbert (1905) as supplemented by VanCleave and Mueller (1934) and Lundahl (1941). Yamaguti's (1971) figure 547 of the species, however, unaccountably shows a small bilobed ovary, many small vitelline follicles, and an acetabulum much larger than the pharynx.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 76653.



Figures 9–12. 9. *Caecincola parvulus*. 10. *Lissorchis attenuatum*. 11. *Lissorchis mutabile*. 12. *Macroderoides spiniferus*.

Lissorchiidae (Poche, 1926) McMullen, 1937

Lissorchis attenuatum Mueller and VanCleave (1922) (Fig. 10)

Eight gravid worms were found in the stomach and throughout the length of the intestine of one *Catostomus commersoni* from each of Silver Lake and Tichigan Lake canal, only during the summer, when two and one suckers were examined, respectively, from those waters.

DIMENSIONS (N = 7): Body 3.0–4.12 (3.44) by 0.52–0.60 mm (0.57), oral sucker 210–294 (263) by 196–280 (252), acetabulum 294–378 (346) by 294–378 (336), pharynx 140–154 (151) by 126–147 (137), anterior testis 490 (490) by 252–364 (318), posterior testis 490–616 (550) by 238–336 (304), ovary 252–350 (304) by 196–308 (242), vitelline follicles 29–67 (45) by 22–45 (30), eggs 11–16 (15) by 10–16 (11).

Mueller and VanCleave (1932) and Mueller (1934) measured only the type specimen, 3.5 by 0.75 mm, and the eggs, 20 by 12 (N = ?); no mention of variability was made. My specimens differed from theirs by having more elongate pharynx, shorter eggs, a more distinctly lobed ovary farther from the acetabulum, testes less irregular in outline, vitellaria usually extending to intertesticular level, and more fleshy suckers. The latter might have been due to the partially macerated condition of some specimens upon recovery. My specimens were also comparable to the smaller ones reported by Amin (1974) from the same host in Pike and Root rivers, southeastern Wisconsin.

DEPOSITED SPECIMENS: 3 in USNM Helm. Coll., Nos. 76654 and 76655; 31 from the Pike River in MPM Coll., No. IZ365e.

Lissorchis mutabile (Cort, 1919)
(Fig. 11)

Thirty-three gravid worms were found throughout the length of the intestine of six 14–26-cm-long *Erimyzon sucetta* of both sexes from Silver Lake in autumn. During that season, 42 chubsuckers were examined; the remainder were almost equally divided between spring and summer.

DIMENSIONS (N = 10): Body 1.05–1.92 (1.41) by 0.24–0.35 mm (0.29), oral sucker 140–238 (193) by 154–210 (188), acetabulum 182–280 (238) by 196–266 (227), pharynx 56–112 (85) by 56–112 (83), anterior testis 140–196 (164) by 98–126 (110), posterior testis 140–224 (178) by 98–126 (116), cirrus sac 168–280 (235) by 70–98 (80), ovary 140–182 (161) by 126–168 (147), vitelline follicles 42–84 (69) by 28–56 (45), eggs 13–26 (19) by 10–16 (12).

Except for the larger size of body and posterior extent of the vitellaria to the midlevel of the posterior testis, my specimens agree closely with *L. mutabile* as described from the same host species in Michigan by Wallace (1941). The ovary in all of my specimens was deeply trilobed, and not just "moderately" so as Fischthal's (1942) key indicated.

DEPOSITED SPECIMENS: 8 in USNM Helm. Coll., No. 76656; 9 in MPM Coll., No. IZ365i.

Macroderoididae McMullen, 1937

Macroderoides spiniferus Pearse, 1924
(Fig. 12)

This study reports *M. spiniferus* for the first time from *A. calva,* the host of three other species of *Macroderoides*: *M. flavus, M. typicus,* and *M. parvus.* Ninety-four mostly gravid worms infected the posterior 75% of the intestine of two of eight *A. calva* examined during the summer from Silver Lake.

DIMENSIONS (N = 20): Body 2.07–2.94 (2.32) by 0.15–0.27 mm (0.20), oral sucker 56–98 (74) by 56–98 (77), acetabulum 70–112 (95) by 91–126 (104), pharynx 42–70 (63) by 42–84 (60), anterior testis 112–168 (147) by 84–140 (115), posterior testis 126–196 (155) by 91–140 (116), ovary 98–154 (129) by 98–140 (120), vitelline follicles 19–54 (33) by 16–35 (24), eggs 22–54 (34) by 12–32 (20).

The shape and size of the body and various organs in my specimens are comparable to those given by Pearse (1924a) as emended by Simer (1929) and supported by Leigh (1958). They reported the species from various fishes, particularly gar, in Wisconsin and Florida. Measurements of the pharynx given by Pearse (12 long by 88 wide) are obviously in error. The testes may be spherical as well as ellipsoidal, and are about equal in size, as stated by Pearse. Distance of the acetabulum from the anterior end in my specimens is 27–37% (32%) of the body length as compared to 25–50% reported by Simer and 1/7 by Pearse. The ovary was distinctly and invariably three-lobed, as emended by Simer. In his key to genera of Macroderoidinae, Yamaguti (1971) relied on the original description of *M. spiniferus,* and erred by placing *Macroderoides* with genera having unlobed ovary. Yamaguti's (1971) new figure (no. 315) of the ovarian complex of a paratype from Pearse's material further shows a smooth but not entirely ovoid ovary.

DEPOSITED SPECIMENS: 29 in USNM Helm. Coll., No. 76652; 17 in MPM Coll., No. IZ365f.

Conclusion

Of 3,355 fishes representing 40 species in 12 families, only 71 fishes from 11 species and seven families (Amiidae, Catostomidae, Centrarchidae, Esocidae, Gasterosteidae, Percidae, Umbridae) were infected with adult digenetic trematodes. Infections were generally light; the heaviest was 89 *M. spiniferus* in a female *A. calva* 29 cm long. Light infections with adult trematodes appear to be common elsewhere in Wisconsin (Fischthal, 1952). Local digenetic trematodes apparently do not maintain dense populations and their spread, as a group, across the familial boundaries of their hosts is apparently influenced by restricted host specificity, which was marked for all species except those of *Azygia*. Each of the remainder parasitized only one fish species, except *C. cornutum*, which occurred in two. Three species were common to both lakes: *A. angusticauda*, *A. longa* and *L. attenuatum*. Two others (*B. eucalia*, *P. limi*) were restricted to Tichigan Lake canal and one (*L. mutabile*) to Silver Lake, possibly because of the virtual absence of their hosts in the other habitat (Table 1). Of the species recovered from hosts common to both lakes, one (*A. lobatum*) is reported from Tichigan Lake and four (*B. sacculata*, *C. parvulus*, *C. cornutum*, *M. spiniferus*) from Silver Lake. Whether this is at all related to the fact that Silver Lake is land-locked or that the more eutrophic and polluted Tichigan Lake is associated with the Mississippi River drainage system is yet to be clarified.

For most of the trematode species reported herein, information concerning intraspecific variability has been amplified by studying and measuring them in greater detail than has been done before.

Acknowledgments

I am grateful to Dr. J. H. Fischthal, State University of New York at Binghampton, for comments on *C. parvulus* and *L. attenuatum* and to Dr. M. H. Pritchard, The H. W. Manter Laboratory, University of Nebraska, Lincoln, for comments on *M. spiniferus*.

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Two Larval Trematodes (Strigeoidea) of Fishes in Southeastern Wisconsin

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ABSTRACT: Metacercariae of *Posthodiplostomum minimum* (MacCallum, 1921) and metacercariae morphologically identical to *Ornithodiplostomum ptychocheilus* (Faust, 1917) were recovered from 12 and 11 fish species (mostly centrarchids) from Tichigan and Silver lakes, respectively, in southeastern Wisconsin. It is suggested that the *O. ptychocheilus* larvae from centrarchids may represent a new subspecies. The prevalence of infection with both trematode larvae in bluegills, *Lepomis macrochirus*, increased with host size. No difference between host sex and prevalence was observed in bluegills from Tichigan Lake, but a higher prevalence was noted in females from Silver Lake. The lowest prevalence of *P. minimum* infection in bluegills occurred in the summer in both lakes and of *O. ptychocheilus* in this host from Tichigan Lake.

This is the third report on the trematode parasites of fishes from two lakes in southeastern Wisconsin; the first two reports were concerned with adult trematodes (Amin, 1982; Amin and Myer, 1982). The morphology of *Posthodiplostomum minimum* metacercariae was similar to that described by other workers. Based on morphology, the other strigeoid metacercaria was identified as *Ornithodiplostomum ptychocheilus*. Although this larva has been previously reported primarily from cyprinids (Hoffman, 1958a, 1967), it was found in this study in cyprinid, ictalurid, percid, and centrarchid hosts, with the latter predominating.

Materials and Methods

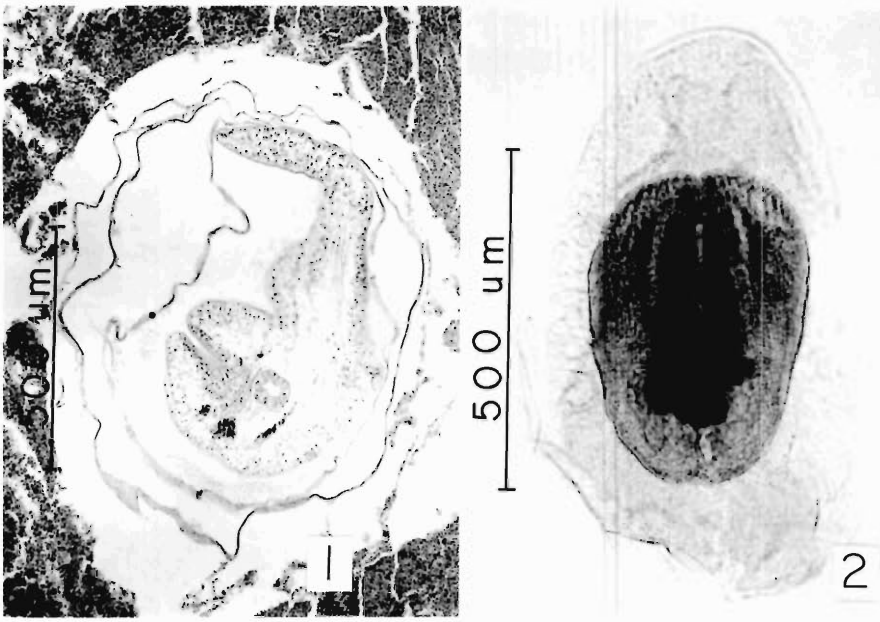
Fishes were examined for parasites during the spring (April), summer (late June, July, August), and autumn (late October, November) of 1976–1979 from Silver Lake (200-ha eutrophic lake in Kenosha County) and in these same periods of 1977–1978 from Tichigan Lake (108-ha lake in a late eutrophic stage on the Fox River, a tributary of the Mississippi River, in Racine County). Fishes from a canal draining the western swamps of Tichigan Lake were also examined from 1977–1981. Metacercariae were processed as described by Amin and Myer (1982). Larvae were recovered from the liver, spleen, heart, gonads, stomach, intestine, peritoneum, and mesenteries; cranial sites were not examined. No attempt was made to recover all larvae, especially from heavily infected fish. Sections were stained with hematoxylin and eosin, and whole mounts with Semichon's carmine. Line drawings were made with the aid of a microprojector. Measurements are in micrometers; means are followed by ranges in parentheses.

Results and Discussion

Posthodiplostomum minimum (MacCallum, 1921) Dubois, 1936
Ornithodiplostomum ptychocheilus (Faust, 1917) Dubois, 1936

Morphology

The morphology of *P. minimum* metacercariae examined in this study was similar to that given by Hughes (1928) in the original description. Variations in body form and oral sucker outline as reported by Ferguson (1937) and Palmieri



Figures 1, 2. 1. Section of *Posthodiplostomum minimum* in *Lepomis macrochirus* liver showing "three" cyst walls. 2. Metacercaria and cyst of *Ornithodiplostomum ptychocheilus* from liver of *Pomoxis nigromaculatus*.

(1975) were observed. Eighty-five cysts from bluegills, *Lepomis macrochirus*, from Tichigan Lake measured 890 (630–1,148) long by 536 (280–840) wide, and 85 metacercariae 878 (546–1,484) by 350 (210–560). No attempt was made to distinguish between *P. m. minimum* and *P. m. centrarchi* Hoffman, 1958. The double-walled cyst of host and parasite origins reported by many authors and immunologically confirmed by Crider and Meade (1975) was seen in situ in sections of *P. minimum*. However, a third wall was observed in a few sections (Fig. 1).

Metacercariae of *O. ptychocheilus* examined here were morphologically comparable to the description given by Hughes and Piszczek (1928). These larvae typically filled only a small portion of the cyst (Figs. 2–6). Identification of *O. ptychocheilus* from bluegills was independently confirmed by Hendrickson (1981, pers. comm.) and Hoffman (1981, pers. comm.). Eighty-five cysts from bluegills from Tichigan Lake measured 843 (672–1,092) long by 468 (350–658) wide, and 85 metacercariae 489 (350–616) by 273 (154–378).

Hosts

Metacercariae of *O. ptychocheilus* and *P. minimum* were found in 11 and 12 host species belonging to four and five families of fishes, respectively (Tables 1, 2). The only hosts not shared by these two larvae were the catostomid *Erimyzon sucetta* and the centrarchids *Micropterus dolomieui* and *Pomoxis annularis*. The bluegill was the most commonly collected host.

The following fishes from Silver Lake, Tichigan Lake, and Tichigan Lake Canal were not infected with either larval species (numbers examined are enclosed in

Table 1. Prevalence of *Posthodiplostomum minimum* metacercariae in fishes from Silver and Tichigan lakes.

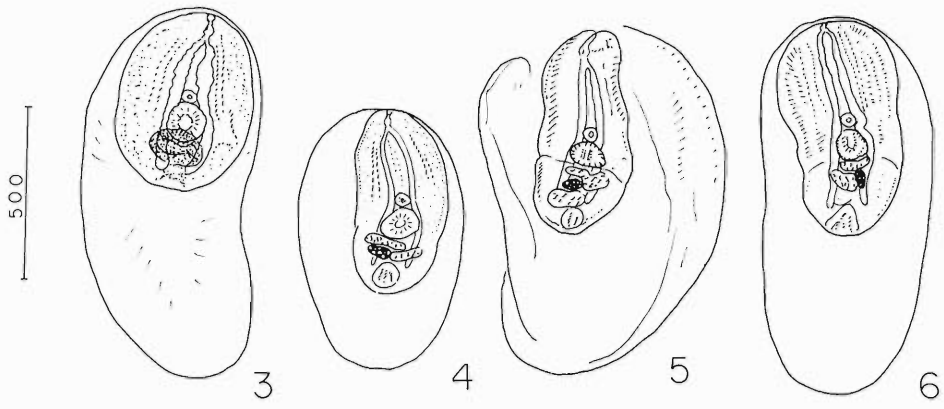
Fish species	Silver Lake (1976–1979)				Tichigan Lake (1977–1978)			
	Spring	Summer	Autumn	Total	Spring	Summer	Autumn	Total
Cyprinidae*								
Cyprinus carpio	0/16†	0/36	0/0	0/52	0/14	0/22	1/30 (3)	1/66 (1)
Catostomidae								
Erimyzon sucetta	1/27 (4)	0/25	0/42	1/94 (1)	0/0	0/0	0/0	0/0
Ictaluridae								
Ictalurus punctatus	0/0	0/0	0/0	0/0	0/17	0/12	1/6 (17)	1/35 (3)
Centrarchidae								
Ambloplites rupestris	0/4	1/8 (12)	2/13 (15)	3/25 (12)	0/2	0/0	0/0	0/2
Lepomis cyanellus	1/3 (30)	5/14 (36)	0/0	6/17 (35)	2/7 (29)	1/5 (17)	1/6 (17)	4/18 (21)
L. gibbosus	6/6 (100)‡	0/9	0/48	6/63 (10)	3/15 (20)	12/32 (39)	7/13 (54)	22/60 (37)
L. macrochirus	42/62 (68)§	26/98 (27)	83/141 (59)	151/301 (50)	21/51 (41)	23/74 (31)	64/87 (74)	108/212 (51)
Micropterus dolomieui	0/2	0/2	0/0	0/4	2/6 (33)	0/10	0/2	2/18 (11)
M. salmoides	4/28 (14)	3/38 (8)	0/6	7/72 (10)	1/2 (50)	3/19 (16)	3/23 (12)	7/44 (16)
Pomoxis nigromaculatus	1/25 (4)	0/4	0/18	1/47 (2)	0/70	1/33 (3)	0/59	1/162 (1)
Percidae								
Stizostedion vitreum	1/21 (5)	0/10	0/23	1/54 (2)	0/4	0/20	0/28	0/52
Total	56/194 (29)	35/244 (14)	85/291 (29)	176/729 (24)	29/188 (15)	40/227 (18)	77/254 (30)	146/669 (22)

* Of the cyprinid hosts examined from the Tichigan Lake Canal, only *Pimephales promelas* (765 examined) was infected.
† Number of infected fish/number examined (% infected).
‡ One infected pumpkinseed recovered from the stomach of a largemouth bass.
§ Two infected bluegills recovered from the stomachs of two bowfins.

Table 2. Prevalence of *Ornithodiplostomum ptychocheilus* metacercariae in fishes from Silver and Tichigan lakes.

Fish species	Silver Lake (1976–1979)				Tichigan Lake (1977–1978)			
	Spring	Summer	Autumn	Total	Spring	Summer	Autumn	Total
Cyprinidae*								
Cyprinus carpio	1/16 (6)†	0/36	0/0	1/52 (2)	0/14	0/22	0/30	0/66
Ictaluridae								
Ictalurus punctatus	0/0	0/0	0/0	0/0	0/17	0/12	1/6 (17)	1/35 (3)
Centrarchidae								
Ambloplites rupestris	1/4 (25)	2/8 (25)	0/13	3/25 (12)	0/2	0/0	0/0	0/2
Lepomis cyanellus	1/3 (33)	1/14 (7)	0/0	2/17 (12)	1/7 (14)	0/5	1/6 (17)	2/18 (11)
L. gibbosus	3/6 (50)‡	0/9	0/48	3/63 (5)	0/15	4/32 (13)	6/13 (46)	10/60 (17)
L. macrochirus	38/62 (61)§	27/98 (28)	14/141 (10)	79/301 (26)	5/51 (10)	2/74 (3)	36/87 (41)	43/212 (20)
Micropterus salmoides	0/28	1/38 (3)	0/6	1/72 (1)	0/2	0/19	0/23	0/44
Pomoxis annularis	1/1 (100)	0/0	0/0	1/1 (100)	0/7	0/5	0/3	0/15
P. nigromaculatus	0/25	0/4	0/18	0/47	0/70	0/33	3/59 (5)	3/162 (2)
Percidae								
Stizostedion vitreum	1/21	0/10	0/23	1/54	0/4	0/20	0/28	0/52
Total	46/166 (28)	31/217 (14)	14/249 (6)	91/632 (14)	6/189 (3)	6/222 (3)	47/255 (18)	59/666 (9)

* *Pimephales promelas* from Tichigan Lake Canal was less heavily infected with *O. ptychocheilus* than with *P. minimum*.
† Number of infected fish/number examined (% infected).
‡ One infected pumpkinseed recovered from the stomach of a largemouth bass.
§ One infected bluegill recovered from the stomach of a bowfin.



Figures 3–6. Metacercariae of *Ornithodiplostomum ptychocheilus* from *Lepomis macrochirus* body cavity organs, particularly liver and mesenteries.

parentheses in that order): *Amia calva* (18, 23, 37) (Amiidae); *Carpiodes carpio* (0, 3, 0), *C. cyprinus* (0, 19, 0), *Catostomus commersoni* (10, 54, 11), *Moxostoma anisurum* (0, 4, 0), *M. erythrurum* (0, 8, 10), *M. carinatum* (0, 3, 0) (Catostomidae); *Chaenobryttus gulosus* (1, 0, 0) (Centrarchidae); *Notropis cornutus* (0, 0, 107), *N. umbratilis* (16, 17, 0) (Cyprinidae); *Fundulus notatus* (0, 0, 19), *F. notti* (0, 0, 6) (Cyprinodontidae); *Esox americanus* (3, 0, 2), *E. lucius* (20, 22, 2) (Esocidae); *Culaea inconstans* (0, 0, 182) (Gasterosteidae); *Ictalurus melas* (1, 8, 0), *I. natalis* (4, 8, 5), *I. nebulosus* (3, 10, 3), *Schilbeodes gyrinus* (0, 0, 2) (Ictaluridae); *Lepisosteus osseus* (14, 9, 0) (Lepisosteidae); *Etheostoma nigrum* (0, 0,

Table 3. Infection of *Lepomis macrochirus* with *Posthodiplostomum minimum* and *Ornithodiplostomum ptychocheilus* from Silver and Tichigan lakes.

	Silver Lake (1976–1979)		Tichigan Lake (1977–1978)	
	P. minimum	*O. ptychocheilus*	*P. minimum*	*O. ptychocheilus*
No. infected hosts	151	79	108	43
No. (%) concurrent infections	64 (42)	64 (81)	37 (34)	37 (86)
No. (%) single infections	87 (58)	15 (19)	71 (66)	6 (14)
Total infect./total exam. (%)	166/301 (55)		114/212 (54)	
Relationship to host length (cm):				
No. infect./no. exam. (%): (6–10)	0/19 (0)		0/9 (0)	
(11–13)	23/61 (38)		0/10 (0)	
(14–16)	76/134 (57)		15/29 (52)	
(17–19)	52/71 (73)		50/89 (56)	
(20–29)	15/16 (94)		49/75 (65)	
Relationship to host sex:				
No. infect./no. exam. males (%)	73/151 (48)		48/85 (56)	
No. infect./no. exam. females (%)	93/150 (62)		66/127 (52)	
Mean fish length in cm (range):				
Males	14.3 (7–21)		17.9 (6–29)	
Females	15.4 (9–22)		18.0 (7–23)	

123), *Perca flavescens* (67, 77, 2) (Percidae); *Salmo gairdneri* (0, 1, 0), *S. trutta* (0, 1, 0) (Salmonidae); *Roccus chrysops* (0, 21, 2), *R. mississippiensis* (0, 1, 0) (Serranidae); and *Umbra limi* (0, 0, 86) (Umbridae).

Ecology

In both lakes the prevalence of infection in the different hosts was in general highest with *P. minimum* (Tables 1, 2). The prevalences of each trematode species were similar in the two lakes: 24% and 22% with *P. minimum* and 14% and 9% with *O. ptychocheilus* in Silver Lake and Tichigan Lake, respectively. Prevalence was in general highest in the centrarchid hosts.

The prevalence of infection of bluegills in both lakes was highest for *P. minimum* (Tables 1, 2). The lowest prevalence of *P. minimum* infections in this host occurred in the summer, and this was also true for *O. ptychocheilus* in bluegills from Tichigan Lake.

The prevalence of infection with both larval species in five bluegill size classes increased with host size (Table 3). No difference between host sex and prevalence of infection was apparent in bluegills from Tichigan Lake, but a higher prevalence was evident in females from Silver Lake (Table 3). It was not determined if this difference was statistically significant.

Conclusions

Some workers have noted developmental stages of *P. minimum* that are similar to *O. ptychocheilus* metacercariae (Ferguson, 1937; Hendrickson, 1981, pers. comm.). Because the two trematode larvae in this study were from essentially the same hosts (Tables 1, 2), there is the possibility of misidentification. However, much of the *O. ptychocheilus* material included fully developed metacercariae, and a number of fish were infected only with this larva (Table 3).

No morphological differences were seen in *O. ptychocheilus* metacercariae from the different host species in this study. Previously this species has been reported primarily from cyprinids, whereas here it was collected from seven centrarchids, two cyprinids, one percid, and one ictalurid. It is suggested that the *O. ptychocheilus* larvae from centrarchids may represent a new subspecies or race. A situation comparable to that known to occur with *P. minimum minimum* and *P. minimum centrarchi* (Hoffman, 1958b) may be present; experimental studies would be needed to substantiate this view. The possibility of a cranial versus a visceral strain of *O. ptychocheilus* has been suggested by Radabaugh (1980).

A direct correlation between prevalence of infection and host size in bluegills was found with both trematode species in this study. Spall and Summerfelt (1970) and McDaniel and Bailey (1974) reported similar results with *P. minimum,* in that larger centrarchids were more frequently infected. Additionally, Colley and Olson (1963) and Avault and Allison (1965) observed larger fish to be more heavily infected. Chubb (1979) suggested continual seasonal infection by cercariae, metacercarial longevity, and fish age as factors that might account for this correlation.

DEPOSITED SPECIMENS: *Posthodiplostomum minimum:* 14 slides in USNM Helm. Coll., Nos. 76661–76669, and 11 slides in MPM (Milwaukee Public Museum) Coll., No. IZ365g. *Ornithodiplostomum ptychocheilus:* 14 slides in USNM Helm.

Coll., Nos. 76670–76675, and 13 slides in MPM Coll., No. IZ365h. All from *Lepomis macrochirus*.

Acknowledgments

I am very grateful to Drs. Glenn L. Hoffman, Fish Farming Experiment Station, Stuttgart, Arkansas, and Gary L. Hendrickson, Humboldt State University, Arcata, California, for their examination of and critical comments on the *O. ptychocheilus* material.

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A Simple Technique for Counting *Fasciola* and *Paramphistomum* Eggs in Feces of Cattle and Sheep

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ABSTRACT: A rapid method for assessing the number of eggs of *Fasciola* spp. and *Paramphistomum* spp. in the feces of cattle and sheep is described. Unskilled persons who used the method for estimating the number of *F. hepatica* eggs per gram of cattle feces obtained a mean count that did not differ statistically from the mean resulting from employment by one of the authors of a more complicated technique. The laboratory utensils required for the new method are obtainable from suppliers.

The egg-counting technique (Method I) described by one of us (Dorsman, 1956) is rather complicated and requires equipment that is not commercially available. The following technique (Method II), which is a simplified variation of the former, is performed more easily and in less time, with laboratory utensils obtainable from suppliers.

Materials and Methods

Procedure of Method II

1. Two 20-cm-diameter sieves, with metal gauze and pore apertures of about 0.180 mm or more (sieve A) and of no more than 0.050 mm (sieve B), are soaked with water. Sieve A is then fitted on top of sieve B, and the pair is deposited in a sink in such a way that free flow of water underneath sieve B is possible.

2. A sample, equivalent to 3 g feces, is drawn from a thoroughly mixed fecal suspension. The sample is transferred with water to the screen of sieve A, and the material is washed with plenty of water from a perforated nozzle attached to the tap by a length of rubber tubing. In this procedure, overflow of sieve B must be prevented.

3. The sieves are separated and the material retained in sieve B (containing the eggs) is washed to a small area at one side of the screen, using a fine jet of water at the last stage. A few drops of a 3.55% aqueous solution of methylene blue are then distributed over the accumulated material.

4. After cleaning sieve A, the excess of stain is washed away from sieve B and the stained debris is washed to one side of the screen once more.

5. The debris is quantitatively washed with water from the sieve into a 400-ml beaker. More water is added to make the total volume in the beaker 300 ml.

6. The contents of the beaker are mixed, by means of a magnetic stirrer, as vigorously as possible without introducing air into the water. At least two aliquots of 10 ml are withdrawn from the stirring fluid with a measuring pipette. These volumes are transferred to transparent counting dishes.

7. All fluke eggs settled on the bottom of each dish are counted under a low-power microscope, preferably a dissecting microscope, at about 40×. The mean of the counts, multiplied by 10, represents the assessment of the number of eggs per gram of feces.

Technical details

The fecal suspension is obtained by thoroughly mixing a weighed quantity of feces with a measured amount of water. For cattle feces the proportion 1:1 is convenient; sheep feces have to be diluted more (1:2 to 1:4), and often soaking is necessary before a homogeneous suspension can be made. Mixing is adequately performed by use of a powerful stirrer with propeller-shaped rotor. Representative samples, containing the equivalent of 3 g feces, can be drawn by gradually filling a measuring spoon, taking small amounts from the revolving suspension.

If the method is to be applied for counting fluke eggs in sheep feces, it is advisable to have sieve B provided with gauze with a pore aperture of 0.045–0.050 mm, because the feces of sheep contain a greater amount of very fine fibers and particles than those of cattle.

The tip aperture of the 10-ml measuring pipette used in step 6 must be no less than 1.2 mm in diameter to avoid blocking. Even partial blocking may have an undesired sieving effect. As soon as sucking fluid into the pipette is stopped, sedimentation of the eggs inside begins. One should therefore not try to measure the 10-ml amount very accurately. A delay will result in loss of eggs from the pipette.

We advise using counting dishes with a bottom surface of at least 10 cm^2, especially if the method is to be applied to sheep feces.

The counting dishes must have sloping inner side walls, because the meniscus at perpendicular walls interferes with microscopic observation at the periphery of the bottom. We found the lid of Cooper tissue-culture dishes, 60 × 15-mm style (Falcon, Div. Becton, Dickinson & Co., U.S.A.), with a bottom 4 cm in diameter, satisfactory, especially if parallel lines, about 1.5 mm apart, were scratched on the bottom surface to facilitate systematic searching under the microscope.

Results

Method II was applied by 15 students who had no experience in laboratory work. Each student collected a 6-ml sample from a well-mixed suspension of *Fasciola hepatica* eggs containing cattle feces and water (1:1). They all performed the whole procedure, pipetted 10 ml from the beaker twice, and thus obtained two counts. The resulting egg counts are given in Table 1. The 15 determinations ranged from 23.5 to 36 eggs, with a mean of 29.1 and standard deviation of 3.3. Thus the estimation by these 15 persons gave $291 \pm 33/\sqrt{15} = 291 \pm 8.5$ eggs per gram feces.

One of us (A.C.B.) used the same fecal suspension for estimating the number of *F. hepatica* eggs by means of Method I. The whole procedure, beginning with drawing a 6-ml sample of fecal suspension, was carried out six times and the eggs were counted in the two compartments of the counting slide every time. The counts are presented in Table 2. The six determinations ranged from 24.5 to 32.5, with a mean of 29.2 and standard deviation of 2.9. Thus these determinations resulted in an estimate of $292 \pm 29/\sqrt{6} = 292 \pm 11.8$ eggs per gram feces.

Discussion

The overall results obtained with the two methods are strikingly similar. Application of Student's *t* test for comparison of the means of two normal distri-

Table 1. ***Fasciola hepatica*** **egg counts obtained with Method II.**

Student	First count	Second count	Mean
Lo	32	32	32.0
Ni	28	28	28.0
Jo	28	29	28.5
Fr	33	14	23.5
Ma	33	20	26.5
Ri	27	31	29.0
Re	35	25	30.0
On	35	37	36.0
Ar	25	28	26.5
No	32	29	30.5
Er	34	28	31.0
An	27	36	31.5
Ge	30	18	24.0
To	36	28	32.0
Mr	28	26	27.0

Mean count with standard error: 29.1 ± 0.85.
Standard deviation of means: 3.3.

butions (Kreyszig, 1970) shows that there is no significant difference at the 5% level between the means for the two methods. It should be noted that the difference is so small that the test is hardly asked for in this case. Some difference between the results of the two methods would have been quite natural, taking into account the random variation present in the data. The results here obtained with the new method are promising, but more extensive experience is necessary before a final choice between the two methods is to be made. On the basis of the results hitherto obtained, it may be assumed that the mean recovery of eggs will be about the same in both methods. For Method I this recovery was earlier shown to be close to 100%. The application of Method II by students has shown that this technique is satisfactorily performed by unskilled persons. We have applied Method I for many years for counting eggs of *F. hepatica* and those of *Paramphistomum* spp. in feces of cattle and sheep. Method II will also be suitable for these purposes and for counting eggs of *F. gigantica*.

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We are grateful to Mr. M. Keuls, Department of Mathematics, Agricultural University, Wageningen, for statistical analysis and interpretation.

Table 2. ***Fasciola hepatica*** **egg counts obtained with Method I.**

Sample	Number of eggs in slide		Mean
1	31	26	28.5
2	32	30	31.0
3	28	37	32.5
4	31	31	31.0
5	28	27	27.5
6	26	23	24.5

Mean count with standard error: 29.2 ± 1.18.
Standard deviation of means: 2.9.

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HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1982–83 MEETING SCHEDULE

15 October	Uniformed Services University of the Health Sciences Bethesda, Maryland
12 November	Animal Parasitology Institute, Beltsville Agricultural Research Center-East, USDA, Beltsville, Maryland
3 December	Plant Protection Institute, Beltsville Agricultural Research Center-West, USDA, Beltsville, Maryland
14 January	National Institutes of Health Bethesda, Maryland
11 February	Naval Medical Research Institute Bethesda, Maryland
11 March	Walter Reed Army Institute of Research Washington, D.C.
15 April	School of Hygiene and Public Health, Johns Hopkins University Baltimore, Maryland
14 May	New Bolton Center, University of Pennsylvania Kennett Square, Pennsylvania

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Pistana eurypharyngis gen. et sp. n. (Cestoda: Pseudophyllidea) from the Bathypelagic Gulper Eel, *Eurypharynx pelecanoides* Vaillant, 1882, with Comments on Host and Parasite Ecology

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ABSTRACT: *Pistana eurypharyngis* gen. et sp. n. is described as an intestinal parasite of the deep-sea teleost *Eurypharynx pelecanoides* (Lyomeri: Eurypharyngidae). Fish were taken by trawls in Block, Norfolk, and Wilmington submarine canyons in the northwest Atlantic and south of Flemish Cap in Newfoundland waters. Additional fish, representing both eastern and western North Atlantic localities, were examined from museum collections. Bottom depths in the vicinity of capture ranged from about 750 to 4,000 m. The new cestode is a triaenophorid pseudophyllidean most similar to *Eubothrioides* Yamaguti, 1952, but can be distinguished from this and other triaenophorids by the following combination of characters: unarmed, hastate scolex with deep bothria; no apical disk on scolex or surficial grooves on strobila; rectangular segments longer than broad; numerous testes in two longitudinal bands medial to nerve trunks; cirrus sac extending well into medulla; submedian, dendritic ovary; vitelline follicles occupy entire circumcortical zone; operculate, embryonated eggs; and nerve cord ventral to vagina and cirrus sac on poral side of segment. In contrast, *Eubothrioides* differs from *Pistana* in having a fleshy strobila with longitudinal surficial grooves, trapezoidal segments that are wider than long, vitellaria confined to the posterior region of segment, cirrus sac confined to the cortical zone, compact ovary, a nerve cord dorsal to the vas deferens and vagina on the poral side, and eggs that are elongated and bowed. The presence of operculate, embryonated eggs in *Anoncocephalus chilensis* Lühe, 1902, another triaenophorid, was confirmed by examination of fresh material taken from the type host in Chilean coastal waters. Host ecology is also discussed.

Few cestode parasites from true deep-sea fishes have been described in detail. This is especially true for bathypelagic fishes, which inhabit the largest potential living space on earth. Their parasite fauna in general is only poorly known, and the evidence gleaned thus far has led to contradictory predictions, some as yet unpublished, concerning its true ecological nature (Gusev, 1957; Collard, 1970; Noble and Orias, 1975; Orias et al., 1978; Gartner, 1981).

The new species of cestode described herein is part of a collection of helminths obtained by J. Gartner, Jr. during parasitological examinations of 18 species, totalling 668 specimens, of mesopelagic and bathypelagic fishes. The fishes were taken by otter trawls during ecological studies made by the Virginia Institute of Marine Science (VIMS) in the western North Atlantic in the region of Block, Norfolk, and Wilmington submarine canyons. Adult tapeworms were recovered from two specimens of the bathypelagic "gulper" eel, *Eurypharynx pelecanoides* Vaillant, 1882, of which a total of 16 specimens were initially examined. An additional nine hosts were among 38 fish obtained from museum collections: VIMS (7 specimens); Rosenstiel School of Atmospheric and Marine Science,

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Table 1. Host collection data for *Pistana eurypharyngis*. (Numbers of the host, *Eurypharynx pelecanoides*, examined/infected were: 23/3 (VIMS), 10/6 (UMML), 15/2 (ISH). The symbol + following fish length indicates a portion of the tail was missing.)

Collection	Lat., long.	Depth	Host TL (mm)	Sex	No. worms
VIMS	39°46′N, 71°28′W	1,020 m	384+	?	1
	38°27′N, 73°03′W	2,068 m	413+	?	2
	37°09′N, 74°19′W	1,753 m	419	♀	1
UMML	24°39′N, 76°27′W	1,591 m	255	?	1
	25°08′N, 77°43′W	2,341 m	295	?	1
	24°54′N, 77°42′W	2,242 m	413	?	1
	23°48′N, 77°03′W	1,360 m	483	♀	1
	23°48′N, 77°03′W	1,360 m	342+	?	1
	23°56′N, 75°58′W	1,758 m	500	♀	3
ISH	30°27′N, 66°08′W	1,800 m	513+	♂	2
	30°27′N, 66°08′W	1,800 m	406+	♀	1

Miami (UMML; 10 specimens); Institut für Seefischerei, Hamburg (ISH; 15 specimens); and the United States National Museum (USNM; 6 specimens). During cruises from New England north to Flemish Cap, R. Campbell examined 168 meso- and bathypelagic fishes (17 spp.), including 6 specimens of *Eurypharynx*, but found few metazoan parasites and no adult cestodes. The size range of the *Eurypharynx* examined was 187–550 mm total length (TL). The 11 hosts measured 255–513 mm TL. Host collection data, summarized in Table 1, revealed the parasite to be distributed in the western North Atlantic though fish were examined from the eastern Atlantic southwest of the Azores, 35°20′N, 30°16′W. Hosts were found in Exuma Sound to southwest of Bermuda and north to Block Submarine Canyon, 25°08′–39°46′N, 66°08′–74°19′W. Following measurements the worms were divided into segments for sectioning and whole mounts. Serial transverse sections of segments were stained with Harris' hematoxylin and eosin. Whole mounts were stained with hematoxylin or Mayer's paracarmine. All preparations were dehydrated, cleared, and mounted in Canada balsam according to standard procedures. Descriptive measurements are expressed as length by width and include the number of measurements (*N*), the mean, and the range in parentheses. All measurements are in micrometers unless otherwise indicated.

Pistana gen. n.

DIAGNOSIS: Scolex unarmed, hastate, with 2 freely projecting fleshy bothria. Apical disk lacking. External and internal segmentation present. Segments longer than wide. Poral longitudinal nerve cord ventral to vas deferens and vagina. Genital atrium marginal, irregularly alternating. Cirrus pouch extends into medulla. Cirrus unarmed. Testes in 2 lateral bands, medial to nerve trunks. Vagina passes anterior to cirrus sac. Ovary posterior, submedian, comprised of 2 multilobed masses. Seminal receptacle absent. Vitellaria occupy entire circumcortical zone. Uterus median, uterine sac and pore midventral. Eggs operculate, embryonated. Parasites of marine teleosts.

TYPE AND ONLY SPECIES: *Pistana eurypharyngis* sp. n.

ETYMOLOGY: *Pistana* (=arrowhead) from the Latin refers to the scolex shape and is feminine in gender.

Pistana eurypharyngis sp. n.
(Figs. 1–9)

DESCRIPTION (based on 15 specimens): *Pistana*. Scolex distinctly hastate, maximum dimensions up to 1,320 by 2,160; 2 deep dorsoventral bothria with fleshy walls, 1,060–1,560 by 480–760, posterior margins free and flared. Neck absent. Strobila filiform, longitudinal furrows lacking, 0.6–24.6 cm long by 2,020; segments craspedote, rectangular, anapolytic, total number about 650. First segments 220–336 by 240–512; mature segments 1,220–1,500 by 820–1,440, maturation rapid; gravid segments 1,220–2,500 by 700–1,540. Secondary segmentation apparent. Genital pores marginal, irregularly alternating, in posterior ⅓ of segment. Wall of genital atrium may be everted as a bulbous protrusion. Cirrus sac (N = 30) 224–296 (272) by 64–120 (85.3), curving anteriorly into medulla, terminating near midline. Invaginated cirrus lined with hairlike processes. Vas deferens tightly coiled, extending from cirrus sac to uterus, thereupon turning posteriad for a short distance parallel to uterus. Testes spherical or slightly subspherical, diameter (N = 20) 19–49 (34), forming 2 continuous longitudinal bands in lateral medulla; testes number 68–97 per segment (N = 20), 11–23 postovarian. Vitelline follicles subspherical, circumcortical, continuous from segment to segment, interspersed among longitudinal muscle bundles. Vagina initially expanded, 42–52 by 110–114, nonmuscular, parallels cirrus pouch along posterior border then narrows to 19–23 in diameter, and turns posteriad to join ootype. Vaginal sphincter and seminal receptacle absent. Ovary (N = 30) 360–620 wide in mature segments, divided into 2 masses of radiating filamentous acini joined by a short isthmus; branches just reach lateral margins of testicular bands and overreach osmoregulatory ducts on poral side. Mehlis' gland posteroventral to ovary. Uterus originates on poral side of midline, proceeds anterolaterally across vaginal duct, then curves toward midline and ascends uncontorted to terminate near anterior margin of segment. Uterine sac pyriform, about 288 by 264, pointing anteriorly. Uterine pore ventromedian in anterior 30–40% of segment length. Eggs (N = 50) 45.6–57 (52.5 ± 4.8) by 30.4–38 (34.2 ± 2), operculate, thin-shelled, oval, embryonated. Longitudinal nerve cords lateral to osmoregulatory ducts; poral nerve cord ventral to vagina and vas deferens; antiporal nerve cord at mid-depth opposite genital pore. Ventral osmoregulatory ducts largest, ventromedial to dorsal pair.

HOST: *Eurypharynx pelecanoides* Vaillant, 1882; "gulper or swallower" eel (Eurypharyngidae).

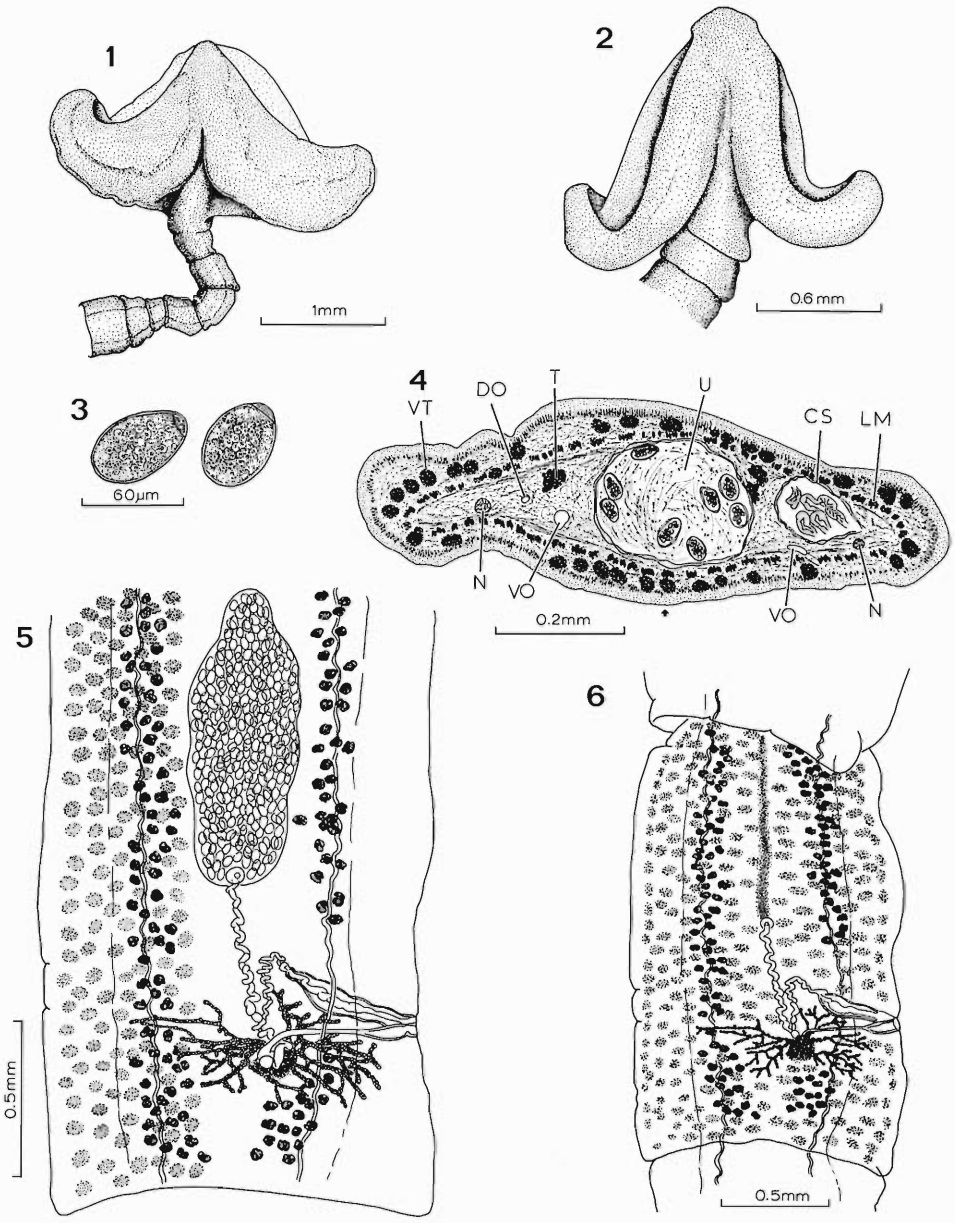
TYPE LOCALITY: Block Submarine Canyon (39°46′N, 71°28′W); other localities listed in Table 1.

HOLOTYPE AND PARATYPE: USNM Helm. Coll. Nos. 76928 and 76929.

ETYMOLOGY: The species name, *eurypharyngis*, refers to the fish host.

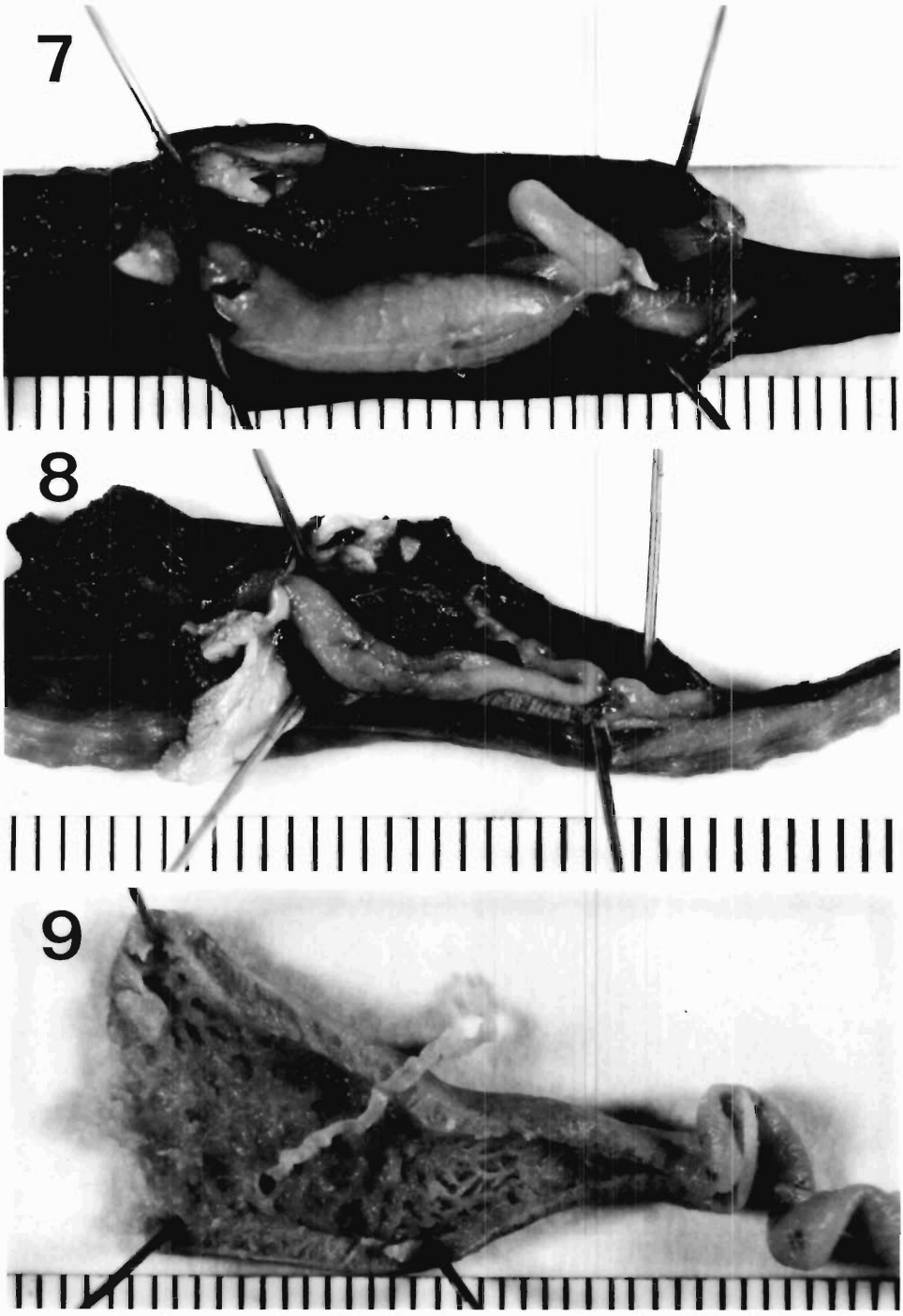
Remarks

A total of 15 *Pistana* were recovered from 11 hosts (Table 1). In all but three cases the worms were firmly attached to the intestinal mucosa—11 scoleces were attached just posteriad to the pylorus and the others were situated about midintestine. In several instances, the scolex was deeply embedded in the intestinal wall, creating a hole ca. 1 mm in diameter around the strobila (Fig. 9). Of the 11 hosts, eight harbored a single cestode, two had two tapeworms each, and one fish contained three worms. Because pseudophyllidean cestodes tend to be large



Figures 1–6. New cestode from *Eurypharynx*. 1. Scolex, holotype. 2. Scolex, paratype. 3. Intrauterine eggs. 4. Cross section of early gravid segment. Arrow indicates ventral surface. 5. Detail of reproductive system in ventral view (some vitelline follicles not shown). 6. Mature segment, dorsal aspect. Abbreviations: CS, cirrus sac; DO, dorsal osmoregulatory duct; LM, longitudinal muscle; N, nerve; T, testis; U, uterus; VO, ventral osmoregulatory duct; VT, vitelline follicle.

(anapolytic), their numbers may be reduced in infrapopulations due to intraspecific competition. The specimens of *P. eurypharyngis* recovered ranged from 0.6 to 24.6 cm long (mean 11.3 cm) in fish hosts of 25.5–51 cm total length. Only four of the 15 *Pistana* found were shorter in length than the host's intestine, the others



Figures 7–9. Gross morphology of intestine of *Eurypharynx pelecanoides*. 7. Distended intestine containing *Pistana eurypharyngis*. Host length 295 mm TL. Vertical lines indicate millimeters. 8. Intestine of uninfected specimen, 280 mm TL. 9. Opened intestine showing cestode embedded in intestinal wall.

were considerably longer, resulting in a tight coiling of the strobila. Externally, the host's intestine appeared markedly dilated when compared with those of uninfected fish (Figs. 7, 8). Internally, this appeared to cause mechanical blockage of the intestine. There was little evidence of pathology except where the bothria contacted the mucosal epithelium, causing metaplasia from columnar to pavement-type cells with a noticeable reduction of mucus-secreting cells. Dilation of the intestine apparently is due only in part to the physical presence of large worms. Two hosts that harbored small worms (3.7 and 4.5 cm) had swollen intestines in the region of scolex attachment despite the small size of the strobila. However, no evidence of hyperaemia was found.

Systematics

Pistana resembles the triaenophorid genera *Anoncocephalus* Lühe, 1902, *Eubothrioides* Yamaguti, 1952, and *Fistulicola* Lühe, 1899 because of its unarmed bothriate scolex, marginal genital pore, ventral uterine pore, and operculate eggs. However, it may be differentiated from these and other triaenophorids by the following: a fleshy, hastate scolex lacking an apical disk; rectangular segments; circumcortical vitellaria; cirrus sac extending into the medullary zone; and dendritic ovary. The arrowhead-shaped scolex and fleshy bothria of *Pistana* are especially reminiscent of *Anoncocephalus chilensis* (Riggenbach, 1896). Internally, however, the vitellaria of *Anoncocephalus* are medullary and form a layer ventral to the testes, whereas those of *Pistana* are circumcortical and continuous, thereby forming a sleeve around the testes.

Pistana shares many characteristics with *Eubothrioides,* its closest known relative, but major differences of *Eubothrioides* include: median longitudinal furrows on strobila, trapezoidal segments (wider than long), a compact ovary, vitellaria confined to the posterior half of the segment (see Yamaguti, 1952, p. 14 and fig. 15), cirrus sac in the cortical zone, and nerve cord dorsal to vas deferens and vagina on poral side of segment. Variations in these characters may prove insufficient to separate them as new species are found.

Fistulicola, unlike *Pistana,* has an apical disk, and the bothria give the scolex a less defined arrowhead shape because the bothrial margins are not as fleshy and projecting as in *Pistana.* A pseudoscolex may replace the primary scolex of *Fistulicola,* but we do not know if this occurs in *Pistana.* Other major differences that separate *Fistulicola* from *Pistana* are the distribution of testes in a single continuous medullary field traversing the lateral nerve trunks, and widely separated nerve trunks that lie dorsal to the cirrus sac and vagina. In *Pistana* the testes form two narrow bands in the lateral medulla and the nerve cord on the poral side lies ventral to the cirrus sac and vagina, but is found at mid-depth on the opposite side.

A feature of these triaenophorids that is apparent, though of questionable generic importance at present, is the contrasting segment shape, which thus far has proved rather short and narrow in other members of the family. Segments of these worms are often fleshy and some have marginal expansions (described as "leaf-like" in *Fistulicola*; Yamaguti, 1959). Those of *Eubothrioides* are distinctly trapezoidal. However, the segments of *Pistana* are distinctly longer than broad and have parallel sides. Even the youngest segments are almost square, and their

length increases rapidly with growth until their length is one and a half times their width at maturity and about three times their width when gravid.

Pistana shares characteristics intermediate to the three genera compared above. In scolex shape it is most like *Anoncocephalus,* with its flared bothria and in lacking an apical disk, a feature seen in *Fistulicola.* A neck is absent in all. The testes and vitellaria are distributed more like those of *Eubothrioides,* but *Pistana* has an elongated cirrus sac like *Anoncocephalus.* An accessory excretory stem lies medial to the nerve cord in young segments of *Pistana,* but is characteristic throughout the strobila of *Eubothrioides.* Other common features shared by these triaenophorids are marginal, irregularly alternating genital pores; vaginal opening posterior to the cirrus; a median, elongate uterus with uterine sac; a midventral and slightly preequatorial uterine pore; submedian ovary displaced to the poral side; and operculate eggs. (The presence of operculate, embryonated eggs in *Anoncocephalus chilensis* was confirmed for us by Dr. Juan Carvajal G., Catholic University of Santiago, Chile, who obtained fresh specimens from the type host, *Genypterus chilensis.*) The few known members of these genera are parasitic in marine teleosts, mostly from widely separated geographic areas and over a wide bathymetric range.

Host ecology

The host, *Eurypharynx pelecanoides,* is a bathypelagic eel possessing the black coloration, small eyes, watery body, and reduced muscle and bone structure typical of bathypelagic fishes (Idyll, 1964; Marshall, 1971; Childress and Nygaard, 1973). Its most distinctive feature is a delicate but enormous mouth that comprises about 25% of the total length of the fish. According to Grey (1956), *Eurypharynx* is a circumglobal inhabitant, mainly of temperate and tropical seas. In the Atlantic Ocean, it has been caught at depths of 700 m to deeper than 5,000 m, with centers of distribution between 1,400 and 2,800 m in the western Atlantic, 3,400–4,200 m in the eastern Atlantic. Host specimens examined in the present study were captured by bottom trawls fished between 1,020 and 2,250 m. Virtually nothing certain is known about the ecology and population biology of *Eurypharynx.* Current theory suggests that *Eurypharynx* probably engulfs prey by slowly swimming over them with its enormous mouth open (Böhlke, 1966). The diet consists of a wide variety of fishes and invertebrates; identifiable remains include *Sergestes atlanticus, Acanthephyra purpurea,* calanoid copepods, and conchoecid ostracods (Bertin, 1934; Gartner, unpubl. data). There are several reports of benthic prey items in the stomachs of *Eurypharynx* (Vaillant, 1888, cited in Bertin, 1934; Gartner, unpubl. data), which suggests that *E. pelecanoides* may occasionally occur close to the bottom. Because of the delicate and poorly developed jaw skeleton and musculature, the possibility of direct benthic feeding by *Eurypharynx* is remote (C. R. Robins, pers. comm.); net feeding in the otter trawl was equally unlikely (Gartner, unpubl. data). Sedberry and Musick (1978) have suggested that bottom currents could suspend materials in the water overlying the bottom, and it may be that *E. pelecanoides* near the bottom are able to occasionally ingest suspended benthic organisms. An increasing body of evidence has been found indicating that "midwater" fishes, such as *Eurypharynx,* regularly occur near the bottom, especially in regions where the midwater habitats intersect submerged topographic features (e.g., continental slopes). Observers in submersibles have often noted bathypelagic fishes in close proximity (<2 m) to the

bottom (J. E. Craddock, pers. comm. on myctophids; J. A. Musick, pers. comm. on *Gonostoma bathyphilum*). Midwater fishes also have been found in the stomachs of demersal and benthic predators who range only a few meters off the bottom (Sedberry and Musick, 1978; Gartner, pers. obs.). Markle and Wenner (1979) have provided evidence that several species of midwater fishes approach the bottom in large numbers at certain times of the year for spawning purposes.

Acknowledgments

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In Vitro Culture of *Rhodobothrium mesodesmatum* (Cestoda: Tetraphyllidea), Parasite of a Chilean Clam

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ABSTRACT: The plerocercus larva of *Rhodobothrium mesodesmatum*, a parasite of the foot of the clam *Mesodesma donacium*, was cultured in vitro using a saline medium containing urea. In vitro development of the parasite was evaluated by the hatching from the swollen capsule of the plerocercus through the apical pore, by the strobilization of the larva as it leaves the capsule, and by the appearance of genital primordia (testes and vagina) in the most distal proglottids. In nature *R. mesodesmatum* matures in the spiral intestine of the bat stingray *Myliobatis chilensis*.

Information on larval tetraphyllidean parasites (plerocerci) of lamellibranchs is scarce. The presence of these larvae as parasites in many species of bivalves of the North Atlantic has been reported (Gallien, 1949; Dollfus, 1964, 1974; Cake, 1976). In the Southern Hemisphere Bahamonde and López (1962) reported the presence of plerocerci in the clam *Mesodesma donacium* from Chile. Campbell and Carvajal (1979) reviewed the systematics of this worm. There is no experimental work available that could help us understand the biology of these parasites. Hamilton and Byram (1974) cultured tetraphyllidean plerocercoids in vitro in a medium rich in urea, and obtained differentiation of the scolex that allowed the identification of the larvae as members of the family Onchobothridae, genus *Acanthobothrium*.

In the present work we have attempted to culture a tetraphyllidean plerocercus of the family Phyllobothriidae, obtaining excystment of the parasite, strobilization, and appearance of genital primordia in the distalmost proglottids. In tetraphyllidean cestodes none of these stages had previously been obtained experimentally.

Materials and Methods

Rhodobothrium mesodesmatum larvae were taken from the foot region of the clam *Mesodesma donacium* obtained in the local seafood market. The culture medium consisted of (g/liter) NaCl 14.645, KCl 0.328, $CaCl_2 \cdot 2H_2O$ 0.216, $MgCl_2 \cdot 6H_2O$ 0.651, Na-pyruvate 0.110, glucose 1.00, urea 18.018, and Tris buffer 1.210. The culture medium was sterilized by filtering through a Millipore filter with a pore size of 0.2 μm and was stored at −40°C in sterile culture bottles. Immediately before use, 100 IU of penicillin, 10 μg of streptomycin, and 0.1 ml of 56°C-heated human blood serum were added to the culture medium. The final pH of the medium was 7.4 and the osmolarity was 784 mosmol per ml.

The larvae were cultured individually in 10-ml screw-cap vials and maintained at 10°C, which is the temperature of the seawater in which the final host (*Myliobatis chilensis*) lives. The medium was changed twice daily after recording the changes undergone by the larvae.

One hundred eighty-nine larvae were used and were divided into three groups as follows.

Group I: 42 larvae were treated for 1 hr in a 0.5% pepsin (NBC 3× crystalline) solution prepared in culture medium, washed in fresh medium, treated for 1 additional hour in 0.25% trypsin (Calbiochem 2,970 NF units/mg) solution prepared in culture medium, washed, and then transferred to the culture vials.

Group II: 137 larvae were transferred immediately to the culture vials.

Group III: 10 larvae were fixed in 10% formalin immediately after removal from the clam, dissected out, and their length was measured.

At the end of culture the worms from Groups I and II were fixed in 10% formalin and their length was measured. The distal segments of the strobila were stained with Ehrlich hematoxylin, dehydrated, cleared, and mounted in Permount in order to evaluate the development of testes and vagina.

Results

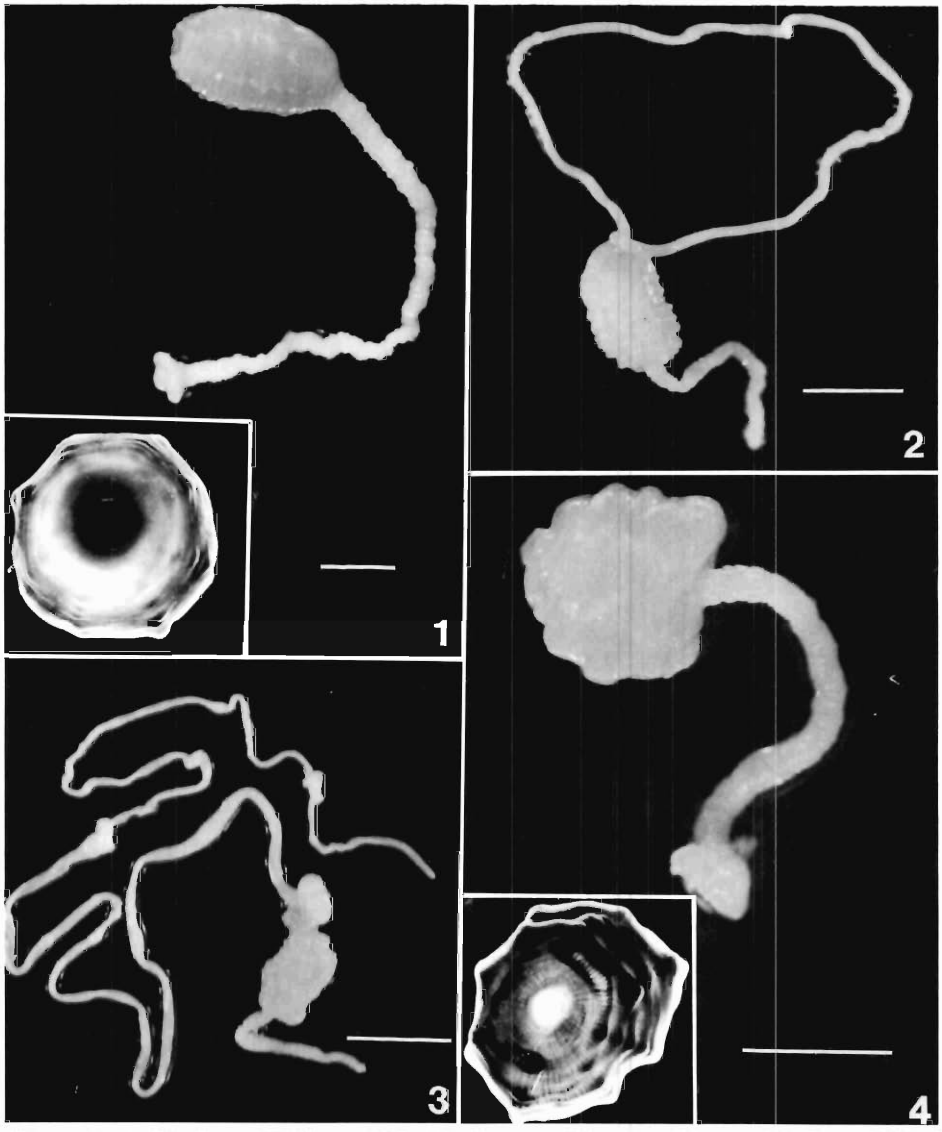
The morphological characteristics of these larvae concur with those given by Bahamonde and López (1962) and Campbell and Carvajal (1979); i.e., they have a swollen capsule 10.5 mm by 7.5 mm, with eight longitudinal rows of nine to 15 tubercles over their exterior; and the cylindrical peduncle is about 3.5 cm in length, in contracted state and ends in a distinct sucker (Fig. 1).

Live *R. mesodesmatum* larvae removed from the molluscan host are characterized by their pink color and contraction and extension movements. At the center of the anterior region of the swollen capsule there is a circular area covered by a thin membrane. The parasite hatches through this thin membrane; the process starts with the exit of the invagination tube by means of contraction and relaxation movements that allow the evagination of the parasite (Fig. 2). The scolex can be seen only at the end of the evagination process, and it remains attached to the swollen capsule by loose connective tissue. At this time, the scolex, strobila, and claviform capsule (Fig. 3) can still respond to mechanical stimuli, which seems to enable the scolex and chain of proglottids to become separated from the claviform capsule. The empty capsule shows the evagination pore open (Fig. 4) and can still respond to mechanical stimuli for a period of 15 hr. The post-scolex portion that appears first from the apical pore of the capsule exhibits strobilization (Fig. 5), and at this stage it is possible to see the excretion channels at the lateral margins of the strobila.

The process of evagination (Table 1) was completed within the first 24 hr in Group I, whereas in Group II the evagination process was spread through a long period of time lasting up to 15 days. Moreover, the percentage of evagination was higher (66%) in the groups treated with enzymes than in the group with no treatment (45%).

The earliest time at which testes development was observed was 5 days after evagination. The percentages of larvae developing testes (60%) were similar in Groups I and II. The semispherical testes appeared distributed in the medullar field of the proglottids (Fig. 6).

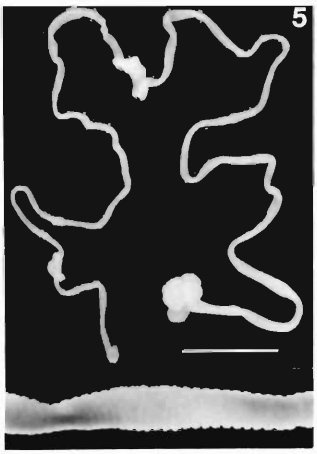
It was possible to observe vagina development only after the termination of culture, due to the fact that specimens must be stained. We were not able to see



Figures 1–4. Developmental stages of *Rhodobotrium mesodesmatum*. Bar equals 0.5 cm. 1. Fresh plerocercus just obtained from the clam. In the insert the closed apical pore can be seen. 2. Formalin-fixed specimen hatching from the swollen capsule after 4 hr of culture. 3. Formalin-fixed specimen evaginated and loosely attached to the plerocercus after 20 hr of culture. 4. Empty capsule as observed after hatching. In the insert the opened apical pore can be seen.

Table 1. Time of evagination of plerocercus larva under different conditions.

Group	Treatment	Total no. of larvae in culture	Total larvae evaginated (%)	No. of larvae evaginated at							
				4 hr		16 hr		24 hr		48 hr	
				No.	%	No.	%	No.	%	No.	%
I	Enzyme	42	28 (66.7)	17	40.5	4	9.5	7	16.7	—	—
II	None	137	61 (44.5)	3	2.2	1	0.7	20	14.6	37	27



Figures 5–7. ***Rhodobothrium mesodesmatum.*** **Bar equals 0.5 cm in Figure 5 and 100 μm in Figures 6 and 7. 5. Whole worm after separation from the capsule. Note strobilization (insert). 6. Hematoxylin-stained proglottids of a worm after 10 days of culture. Note the distribution of testes in the medullar field. 7. Posterior region of a larva mechanically removed from the plerocercus and stained with hematoxylin. Note the absence of genital primordia.**

ripe proglottids even though we were able to maintain the worms for up to 51 days in culture. On the other hand, the study of the posterior region of larvae obtained from the plerocerci immediately after removal from the clam showed only the tegumental striation and the highly convoluted excretory channels (Fig. 7), without the presence of testes and vagina. The lengths of worms cultured in vitro were compared with those of larvae obtained from dissection of plerocerci immediately after recovery from the clam (Group III). The length of the plerocercus larvae was 4.71 cm ± 0.45 (SE) (N = 10) and the length of the worms after culture was 12.46 ± 0.52 (SE) (N = 10).

Discussion

Information, other than on morphology of the site at which tetraphyllideans complete their life cycle, is scarce (Williams, 1968; Carvajal and Dailey, 1975). Hamilton and Byram (1974) cultured in vitro a tetraphyllidean plerocercoid larva belonging to the family Onchobothriidae and obtained from the marine snail *Fasciolaria tulipa*. Their results showed development of the scolex with a pair of bifid hooks per bothridia. In the present work, we have cultured in vitro a tetraphyllidean larva of the plerocercus type (family Phyllobothridae), and have obtained excystment of the larva from the swollen capsule, strobilization, and appearance of testes and vagina.

In nature the plerocercus of *R. mesodesmatum* develops to adulthood in the spiral intestine of the bat stingray *Myliobatis chilensis*, where it reachs a length of 33 cm (Campbell and Carvajal, 1979). In the present report, differentiation has been induced using a compound (urea) present in high concentrations in elasmobranchs (Read et al., 1959), but because of the marked host specificity exhibited by these cestodes, there may be other factors, such as P_{CO_2}, P_{O_2}, temperature, and osmotic pressure, that may have some bearing on the maintenance and full development of the strobila to give ripe proglottids.

Therefore, the possibility of obtaining viable and fertile adults of *R. mesodesmatum* in vitro may have to await elucidation of the biochemical and physiological characteristics in the spiral valve of *Myliobatis chilensis*.

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Neoechinorhynchus limi sp. n. (Acanthocephala: Neoechinorhynchidae) from the Central Mudminnow, *Umbra limi*

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ABSTRACT: *Neoechinorhynchus limi* sp. n. is described from specimens recovered from the central mudminnow, *Umbra limi*, collected in south-central Michigan. The diminutive trunk lengths of *N. limi* (males 1.06–2.63 mm, females 1.67–3.80 mm) place it closest to *N. golvani* Salgado-Maldonado, 1978 and *N. pungitius* Dechtiar, 1971. It is separated from these species by having smaller proboscis hooks; lengths of apical, middle, and basal hooks are 29–37 (33), 17–24 (20), and 13–19 (16) for males and 30–39 (35), 19–27 (22), and 16–20 (18) for females. *Neoechinorhynchus limi* possesses lemnisci of approximately equal lengths. It is differentiated from other species of *Neoechinorhynchus* having lemnisci of equal lengths, and parasitizing North American fishes, by its smaller trunk, proboscis, and proboscis hooks.

During the course of a study of the parasites of the central mudminnow, *Umbra limi* (Kirtland), in the Looking Glass River, a member of the genus *Neoechinorhynchus* Hamann, 1892 was recovered from the intestines of 48 of the 286 fish examined. Based on comparisons of measurements to other species of this genus, the specimens are considered to be a new species of *Neoechinorhynchus*, which is described herein.

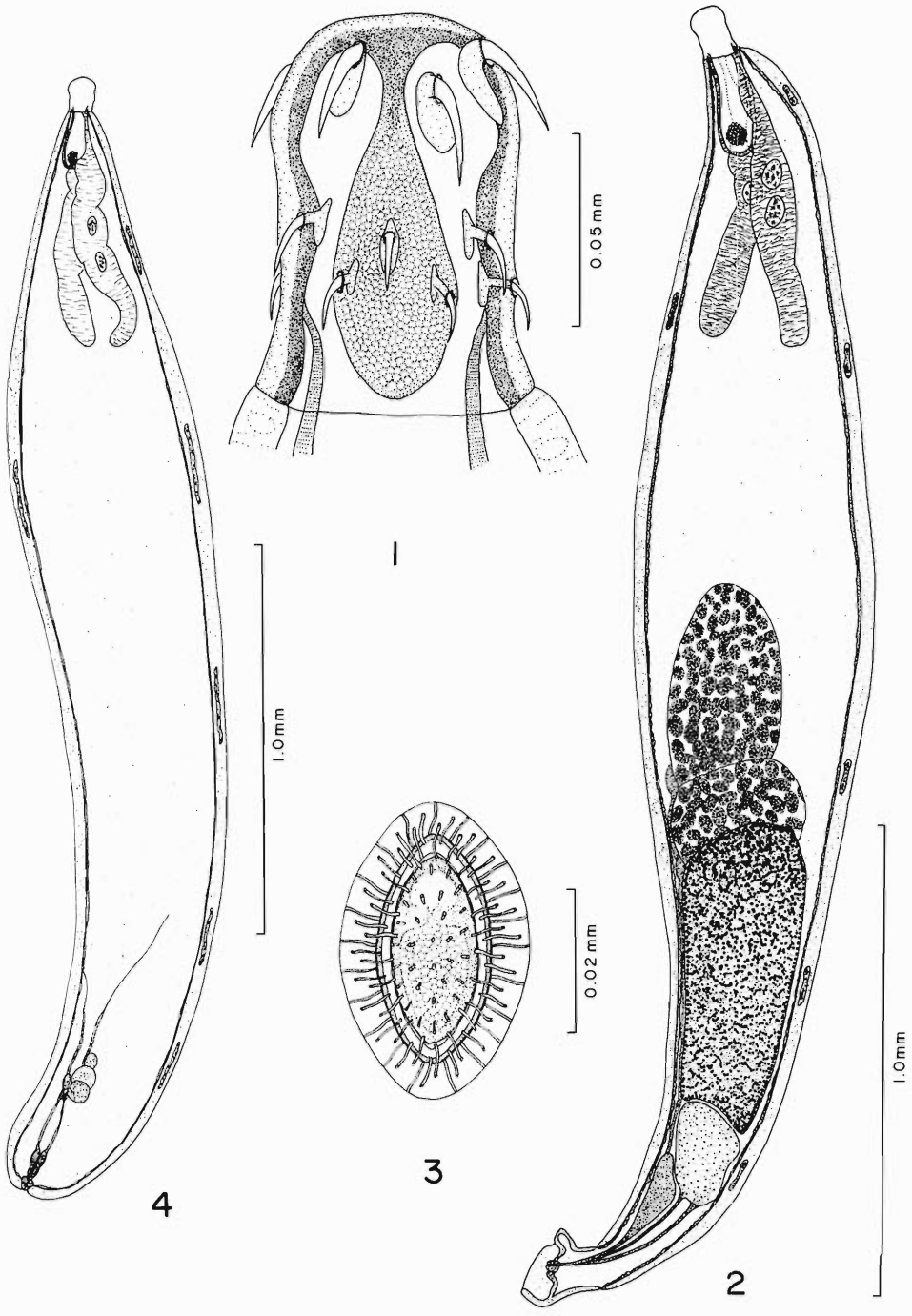
Materials and Methods

Mudminnows were collected with a seine from the Looking Glass River, Woodbury Avenue, Shiawassee County, Michigan, in October and November 1979, May through November 1981, and March through July 1981. Fish were brought to the laboratory alive and examined within 24 hr of collection. After removal from the hosts, acanthocephalans were placed in distilled water to evaginate the proboscis and then were fixed in AFA. Specimens were stained with Mayer's alum carmine or with Lynch's precipitated borax carmine and mounted in Canada balsam.

Measurements were recorded from fully extended specimens having an everted proboscis. Description is based on mature specimens: males with spermatozoa in their vasa deferentia and females with eggs. Width measurements refer to maximum width. Mature eggs that were measured in glycerine came from unfixed and fixed gravid females. All measurements are in micrometers, with means in parentheses, unless otherwise stated.

Neoechinorhynchus limi sp. n. (Figs. 1–4)

GENERAL DESCRIPTION: With characteristics of the genus *Neoechinorhynchus*. Trunk small and oblong to spindle-shaped, tapering toward anterior and posterior ends in males; posterior end of female rounded. Females longer than males.



Figures 1–4. Camera lucida drawings of *Neoechinorhynchus limi* sp. n. 1. Proboscis of holotype male. 2. Holotype male, lateral view. 3. Egg, from pseudocoel of preserved female. 4. Allotype female, lateral view.

Proboscis globular, armed with 3 circles of 6 hooks; anterior hooks notably larger, with prominent root. The dorsal body wall does not show conspicuous thickening. Two lemnisci, similar in length and width; one having 2 nuclei, the other having 1 nucleus. Both sexes with a single, ventral, giant hypodermal nucleus and usually 5, but sometimes 4, dorsal giant hypodermal nuclei; giant hypodermal nuclei not easily observed in several mounted specimens. Eggs with a delicate continuous outer shell membrane, with numerous tubelike structures; outer membrane difficult to observe in mounted specimens.

MALES (based on 21 specimens): Trunk 1.06–2.63 mm (1.95) long by 216–649 (456) wide. Proboscis 54–79 (69) long by 41–99 (67) wide. Apical organ 56–113 (87) long by 14–35 (27) wide. Apical hooks of proboscis 29–37 (33) long, middle hooks 17–24 (20) long, basal hooks 13–19 (16) long. Neck 26–36 (30) long by 43–86 (71) wide. Proboscis receptacle 163–294 (230) long by 48–100 (72) wide. Uninucleate lemniscus 409–757 (567) long by 62–108 (86) wide; binucleate lemniscus 416–726 (575) long by 72–129 (90) wide. Anterior testis 136–515 (332) long by 114–294 (212) wide. Posterior testis 186–407 (278) long by 133–309 (236) wide. Syncytial cement gland in contact with and partially overlapping posterior testis; larger than testes, 216–690 (437) long by 152–340 (244) wide. Cement reservoir 98–227 (151) long by 76–200 (129) wide. Total length of genitalia from end of the anterior testis to the posterior end of the trunk 808–1,158 (1,129), occupying 44–76% (58%) of trunk lengths. Everted bursa 138–304 (222) long.

FEMALES (based on 18 specimens): Trunk 1.67–3.80 mm (2.76) long by 196–624 (438) wide. Proboscis 60–81 (68) long by 57–76 (67) wide. Apical organ 68–80 (75) long by 17–35 (27) wide. Apical hooks of proboscis 30–39 (35) long, middle hooks 19–27 (22) long, basal hooks 16–20 (18) long. Neck 26–38 (33) long by 52–72 (64) wide. Proboscis receptacle 143–214 (167) long by 48–86 (62) wide. Uninucleate lemniscus 390–814 (622) long by 41–113 (79) wide; binucleate lemniscus 474–901 (661) long by 57–103 (83) wide. Reproductive system from anterior margin of the uterine bell to terminal genital pore 300–552 (453) long, occupying 14–18% (16%) of trunk length. Eggs (40 measured) ovoid with a delicate continuous outer shell membrane with numerous tubelike structures; measurements including outer shell membrane 31–48 (43) long by 20–30 (26) wide; without outer shell membrane 29–38 (34) long by 14–18 (16) wide.

TYPE HOST: Central mudminnow, *Umbra limi* (Kirtland) (Umbridae).

OTHER HOSTS: Immature specimens were recovered from brook sticklebacks, *Culaea inconstans* (Gasterosteidae).

SITE OF INFECTION: Anterior and middle intestine.

TYPE LOCALITY: Looking Glass River, Woodbury Avenue, Shiawassee County, Michigan.

TYPE SPECIMENS: Holotype male, USNM Helm. Coll. No. 76839. Allotype female, USNM Helm. Coll. No. 76840. Paratype, USNM Helm. Coll. No. 76841; several paratypes have been retained in the author's collection.

ETYMOLOGY: Named after its host.

Discussion

Neoechinorhynchus limi belongs to the group of species of *Neoechinorhynchus* possessing uninucleate and binucleate lemnisci of approximately equal length.

With the exception of *N. pungitius* Dechtiar, 1971 and *N. golvani* Salgado-Maldonado, 1978, *N. limi* differs from the other species of *Neoechinorhynchus* in the group occurring in North American fishes by having a shorter trunk, proboscis, and proboscis hooks.

Neoechinorhynchus golvani was described by Salgado-Maldonado (1978) from a cichlid, *Cichlasoma aureum,* of Mexico. Its trunk lengths (males 0.837–1.561 mm, females 0.217–3.059 mm) and proboscis lengths (56–75) are similar to those of *N. limi.* The presence of larger apical hooks and the unusual disparity in apical-hook size between males and females (males 52–78, females 45–48), plus the uniformity of middle and basal hook lengths (18), clearly separate this species from *N. limi.*

Neoechinorhynchus pungitius, described from *Pungitius pungitius* of Lake Huron by Dechtiar (1971), also has trunk lengths (males 0.811–2.300 mm, females 1.302–2.830 mm) and proboscis lengths (63–90) similar to *N. limi.* The smaller proboscis hooks of *N. limi* distinguish it from *N. pungitius.* Lengths of apical, middle, and basal proboscis hooks reported for *N. pungitius* are 36–45, 25–29, and 18–23 for males and 44–56, 23–33, and 18–27 for females. *Neoechinorhynchus limi* is also more slender than the distinctly robust *N. pungitius.* The trunk widths given for *N. pungitius* are 400–860 for males and 443–1,380 for females. Several specimens of *N. pungitius* were made available for study through the courtesy of Dr. W. L. Bullock (University of New Hampshire). Comparison of these with our specimens confirmed *N. limi* as a distinct species.

Two species of *Neoechinorhynchus* are known from the mudminnow, *N. rutili* reported by Bangham (1955) and now *N. limi. Neoechinorhynchus rutili* occurs in a variety of hosts (Van Cleave and Lynch, 1950), whereas *N. limi* exhibits a high degree of specificity for the mudminnow. Of the 13 species of fish examined for parasites, mature specimens of *N. limi* occurred only in the mudminnow. A total of three immature specimens were recovered from three of 18 brook sticklebacks, *Culaea inconstans.* The following piscine species collected from the Looking Glass River (number examined in parentheses) were not infected: Catostomidae—*Catostomus commersoni* (11), *Minytrema melanops* (2); Centrarchidae—*Lepomis gibbosus* (9), *L. macrochirus* (8), *Pomoxis nigromaculatus* (9); Cyprinidae—*Notropis cornutus* (17), *Pimephales promelas* (10); Esocidae—*Esox americanus* (4); Ictaluridae—*Ictalurus melas* (13); Percidae—*Perca flavescens* (6), *Percina maculata* (15).

Acknowledgment

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Description of Larval *Acanthocephalus parksidei* Amin, 1975 (Acanthocephala: Echinorhynchidae) from Its Isopod Intermediate Host

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ABSTRACT: Stages of larval *Acanthocephalus parksidei* Amin, 1975 were identified and sequentially traced from natural infections in the isopod intermediate host, *Caecidotea militaris* (Hay, 1878), in the Pike River, southeastern Wisconsin. Acanthor structures and their fate were delineated. Young acanthellae completed their development with the formation of proboscis receptacle, proboscis primordia, and early elements of genital primordia that were not yet assignable to either sex. Further organogenesis in subsequent stages of male and female acanthellae was not, however, correlated with time of giant subcuticular nuclei fragmentation. Acanthellae and/or cystacanths of either sex appear precocious and have actively eversible proboscis and bursa. This is the first account of larval development for any species of genus *Acanthocephalus* from North America.

The early monographs by European workers (e.g., Linstow, 1872; Leuckart, 1876; Hamann, 1891; Kaiser, 1893) contained detailed descriptions of developmental stages of various acanthocephalans including *Acanthocephalus anguillae*, *A. clavula*, *A. lucii*, and *A. ranae*. More recent accounts were presented by Rojanapaibul (1976, on *A. clavula*), Hine (1977, on *A. galaxii*), Kurbanov (1978, on *A. ranae*), Andryuk (1979a, b on *A. anguillae* and *A. lucii*), and Brattey (1980, on *A. lucii*). This is the first description of larval stages of any member of the genus *Acanthocephalus* in North America.

Materials and Methods

The material described in this report includes about 240 specimens obtained from natural infections of the isopod *Caecidotea militaris* (Hay, 1878) from the Pike River, southeastern Wisconsin, between May 1976 and May 1977 (as reported by Amin et al., 1980), as well as about 140 other specimens obtained during summer 1978. Isopods were refrigerated in stream water until dissected shortly after collection. Obtained *A. parksidei* larvae were refrigerated overnight in distilled water, then fixed in chilled AFA, stained in Mayer's acid carmine, cleared in terpineol, and whole-mounted in Canada balsam.

Four major developmental stages were recognized and are herein defined: (1) acanthor: with no primordia; (2) young acanthella: with primordia of organ systems except reproductive; (3) male and female acanthellae: with genital primordia of each sex recognizable, proboscis apparatus not fully developed, body wall soft and delicate; (4) cystacanth: with all adult structures present, proboscis apparatus functional, body wall tough and leathery.

All line drawings were made with the aid of a microprojector. All measurements are in micrometers. Structures labelled proboscis retractor occasionally also include uncinogenous bands that were less distinct and thus not drawn. Some of the structures common to both sexes were more elaborately demonstrated in males or females to avoid duplication. The following abbreviations are used in Figures 1–34:

A, acanthor; AN, apical nucleus; AS, acanthor spine; Br, brain; BrP, brain

primordium; Bu, bursa; BuP, bursa primordium; BWMP, body-wall musculature primordia; C, cortex; CG, cement gland; CGD, cement-gland duct; CGP, cement-gland primordium; E, entoblast; EN, entoblast nuclei; FM, fertilization membrane; FNLR, fragmented nuclei of lemnisci ring; FSN, fragmented subcuticular nucleus; GG, genital ganglion; GP, genital primordium; GPo, genital pore; GSN, giant subcuticular nucleus; HP, hook primordium; IM, inner membrane; IPr, invaginated proboscis; L, lemniscus; LSa, ligament sac; LSt, ligament strand; NLR, nuclei of lemnisci ring; NNR, nuclei of neck retractor; NPrRt, nucleus of proboscis retractor; NR, neck retractor; NRPr, nuclear ring of proboscis; NRcW, nuclei of receptacle wall; NRWM, nuclei of receptacle wall musculature; OB, ovarian ball; OM, outer membrane; Pe, penis; PBC, pockets of bursal cap; Pr, proboscis; PrN, proboscis nuclei; PrP, proboscis primordium; PrRc, proboscis receptacle; PrRt, proboscis retractor; PUC, pouch of urogenital canal; R, retinaculum; SN, subcuticular nuclei; SP, Saefftigen's pouch; SV, seminal vesicle; T, testis; TP, testicular primordium; U, uterus; UB, uterine bell; UBP, uterine bell primordium; UP, uterus primordium; V, vagina; VD, vas deferens; VE, vas efferens; VP, vagina primordium.

Results and Discussion

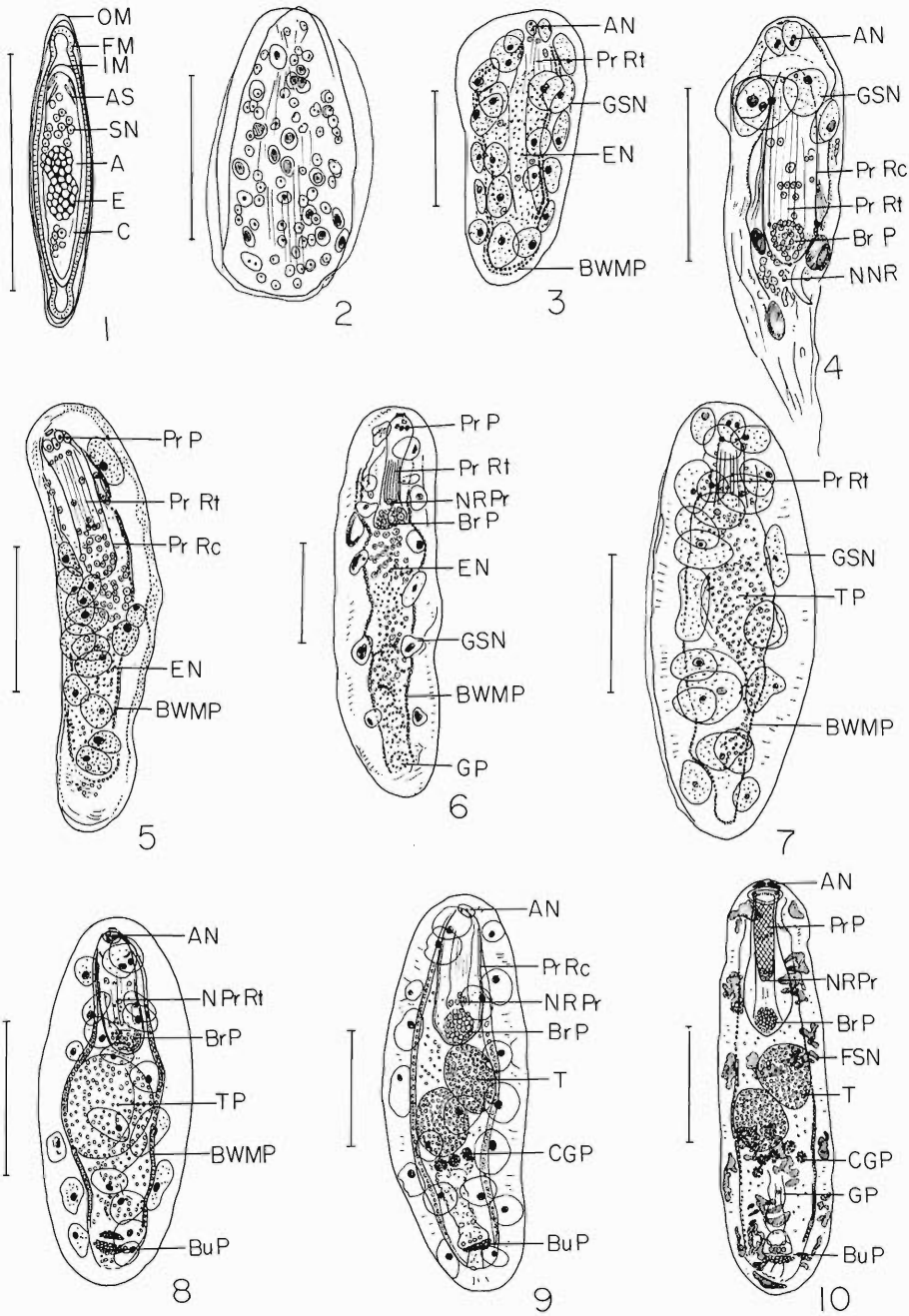
The acanthor

The spindle-shaped, blunt-ended, shelled acanthors (may also be referred to as eggs) were 64–70 long (N = 7). Each was surrounded by four membranes and included the acanthor spines, the entoblast, and a number of subcuticular nuclei that appeared to be more numerous anteriorly than posteriorly (Fig. 1). The latter eventually become the giant subcuticular nuclei, giving rise to adult cortical structures. The number and origin of the cortical nuclei appear to be species specific. The pattern described for *Polymorphus minutus* (14 anterior and 10 posterior cortical nuclei originating from nuclei that always remain outside the entoblast) by Nicholas and Hynes (1963a) appears to be comparable to that of *A. parksidei*. The entoblast nuclei, destined to give rise to most adult organs and the body-wall musclature, were compacted in two distinct masses.

The young acanthella

Early acanthellae associated with the host gut were not observed. The least developed acanthella obtained (Fig. 2) was a 350 long teardrop-shaped specimen. It included a number of developing giant subcuticular nuclei, entoblast nuclei, and protoplasmic strands and was surrounded, like all subsequent stages, with a thin capsule membrane. The origin of the latter could not be determined. Wanson and Nickol (1973) reviewed the literature on the envelope surrounding larval acanthocephalans.

The subsequent stage is formed by the growth and elongation of the entoblast nuclei nearly filling the cortical area and pushing the now-enlarged giant subcuticular nuclei to the periphery. The outermost layer of the entoblast nuclei has now formed two layers of myoblast nuclei, which make up the primordia of body-wall musculature (Fig. 3). The anterior part of the entoblast has begun to differentiate, with the giant subcuticular nuclei assuming near-cortical position and the apical nuclei more clearly recognizable.



Figures 1–10. Shelled acanthor, young, and male acanthellae of *Acanthocephalus parksidei*. 1. Acanthor, from within isopod gut (68 μm long). 2–6. Young acanthellae showing progressive organogenesis (350, 476, 392, 588, and 784, respectively). 7–10. Male acanthellae showing successive stages of development of reproductive system and of proboscis apparatus (630, 490, 700, and 728). Value of scale: 50 (Fig. 1), 200 (Figs. 2–10).

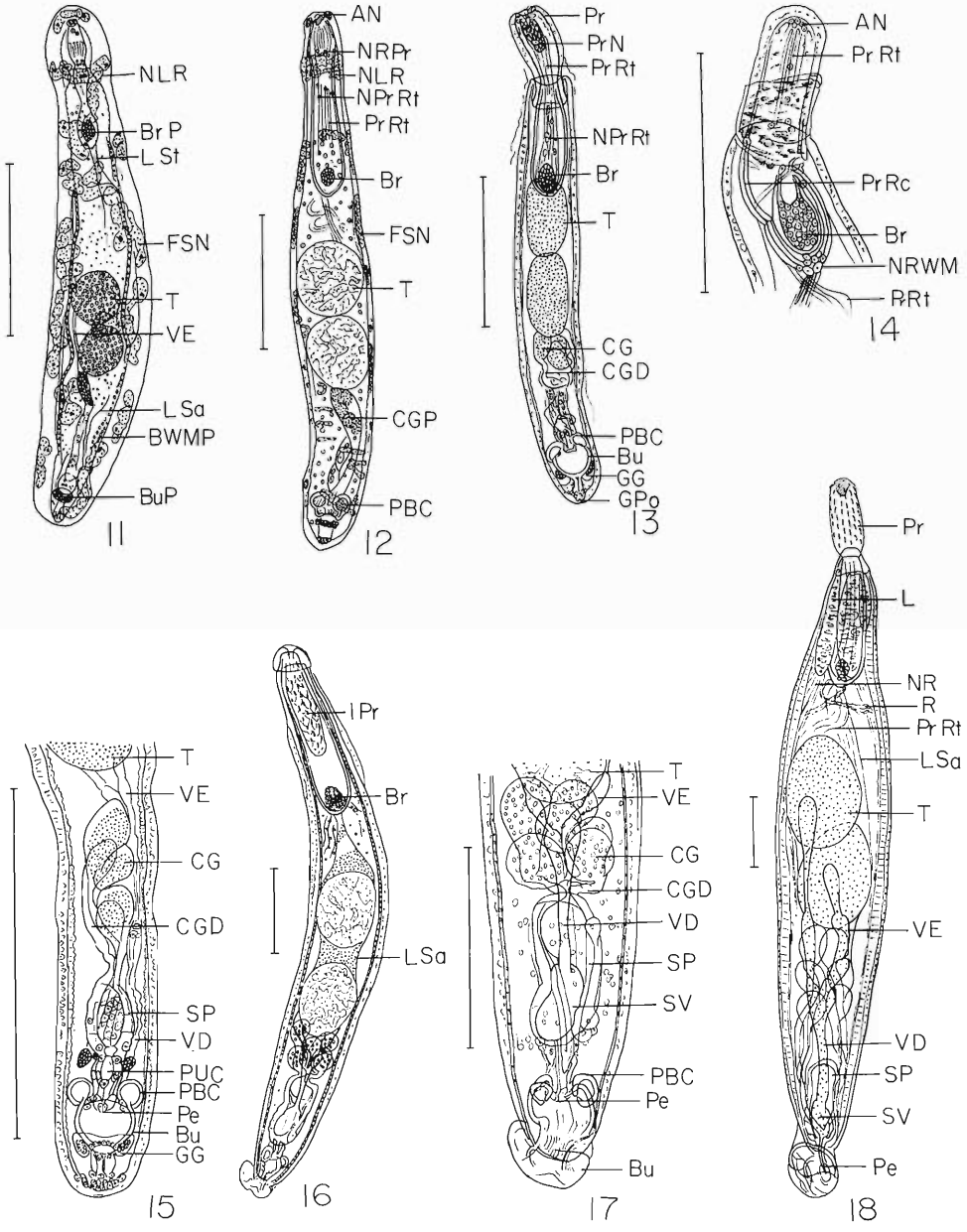
As the giant subcuticular nuclei continue to grow, the first differentiated entoblastic structure, the proboscis receptacle, takes shape (Fig. 4), with the enclosed brain primordium and proboscis retractor becoming evident. With the continued growth and elongation of entoblastic material, the giant subcuticular nuclei become less rounded and the proboscis primordium becomes more evident. The latter takes the form of anterior proboscis nuclei, posterior nuclear ring connected by uncinogenous bands overlapping the proboscis retractor (Figs. 5, 6). In the largest young acanthella observed (784 long, Fig. 6), the giant subcuticular nuclei assumed near-symmetrical bilateral organization.

The male acanthella

The smallest male acanthella observed was 490 long (Fig. 8). The posterior group of entoblast cells proliferates into two testicular fields (primordia), the first structure of the male reproductive system to become apparent (Fig. 7). Up to 22 giant subcuticular nuclei were observed (Fig. 7). Hine (1977) observed only 12 giant nuclei, in pairs, in *A. galaxii*. In *A. anguillae*, *A. ranae*, and *A. lucii*, at least 30, 31, and 18 were evident (Leuckart, 1876, fig. 387b; Kurbanov, 1978, fig. 2; Andryuk, 1979b, fig. 2a, respectively). *Leptorhynchoides thecatus*, *Echinorhynchus lageniformis*, and *Polymorphus minutus* had 14, 18, and 24 (DeGiusti, 1949; Olson and Pratt, 1971; Nicholas and Hynes, 1963b, respectively). As the testicular fields become more distinct and their early side-by-side arrangement evident, the bursa primordium followed by elements of cement-gland primordia starts appearing (Fig. 8). Subsequently, elements of the reproductive system become more independently discernible, e.g., cement-gland primordia, as well as components of the proboscis and the brain (Fig. 9).

The subsequent stage (Fig. 10), observed in acanthellae of about the same size as in the previous stage (Fig. 9), is characterized by fragmenting giant subcuticular nuclei, more oblong body form, and more developed reproductive system and proboscis apparatus (Fig. 10). The proboscis develops in an inverted position, as is typical in Palaeacanthocephala, and later everts, then reinverts in the cystacanth stage. In *Echinorhynchus truttae*, however, the proboscis appears to develop in the everted position (Awachie, 1966). Further elongation of body, differentiation of reproductive system, and fragmentation of the giant subcuticular nuclei correspond with the formation of an anterior constriction girdled by the nuclei of the lemnisci ring at a level dividing the body into presoma and soma (Fig. 11). The giant subcuticular nuclei that eventually become the nuclei of the lemnisci ring mark that same location in earlier developmental stages, though somewhat less conspicuously. All the structures evident in the previous stage (Fig. 11) are differentiated further in these longer acanthellae (Fig. 12), particularly the cement-gland primordia and the pockets of the bursal cap.

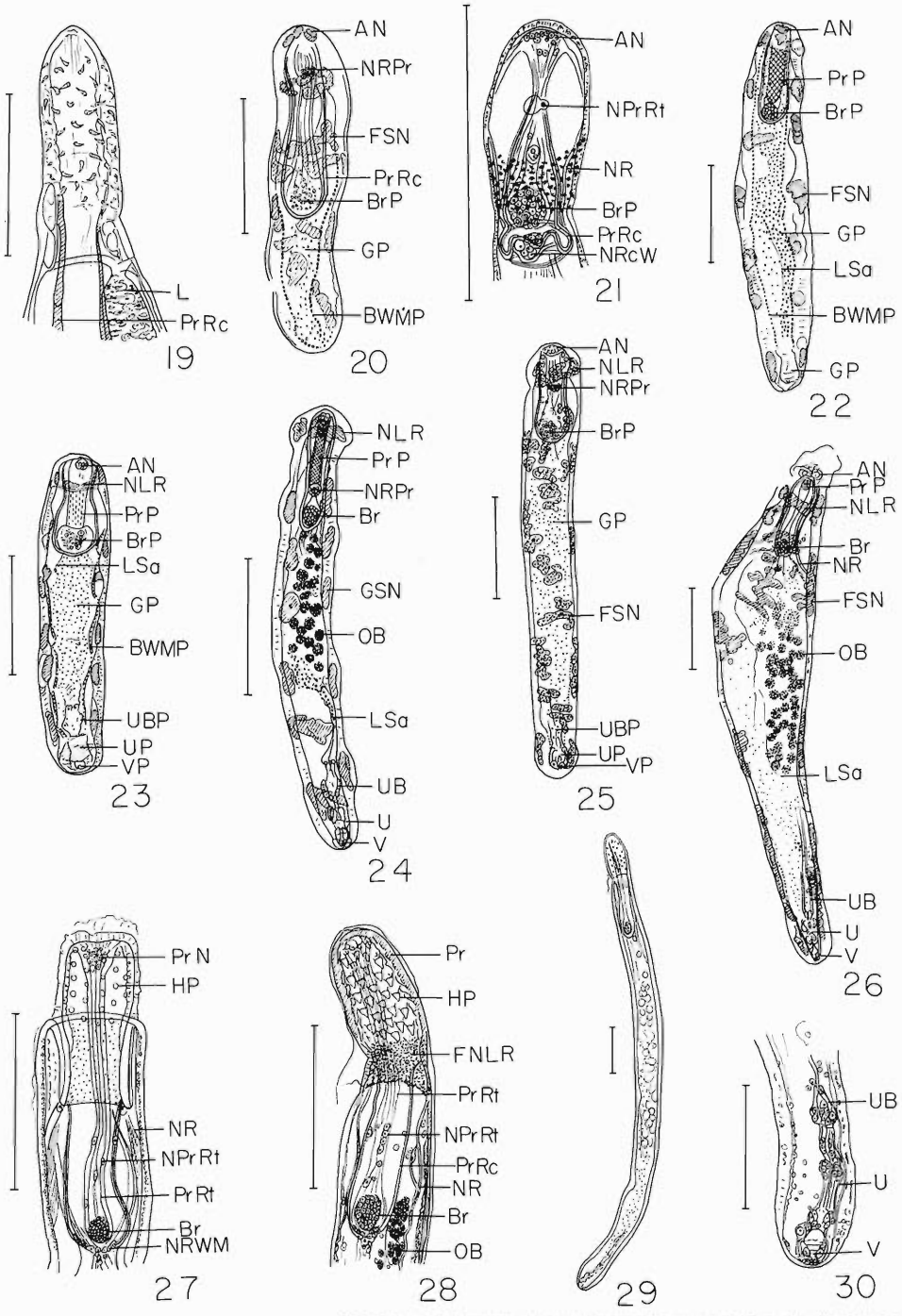
Significant transformations marking the next stage (Fig. 13) include the almost complete fragmentation of the giant subcuticular nuclei, eversion of the proboscis, enlargement and near-complete formation of the bursa and associated structures, formation of the genital ganglion, and the delineation of cement-gland ducts. A closer look at the anterior end of an acanthella at the same stage of development (Fig. 14) shows that hook bulges (primordia) have not yet formed on the uncinogenous bands. Detail of the reproductive system of one of the largest acanthellae recovered (1,820; Fig. 15) shows that all the reproductive structures of adult males are now present, complete with vas efferens and vas deferens.



Figures 11–18. Male acanthellae and cystacanths of *Acanthocephalus parksidei*. 11–13. Acanthellae in subsequent developmental stages (1,218, 1,610, and 1,358 μm long). 14. Anterior part of an acanthella (1,550) at a developmental stage comparable to that in Figure 13. 15. Posterior end of a male acanthella (1,820). 16. Early cystacanth (2,660). 17. Posterior part of cystacanth in Figure 16. 18. Fully developed cystacanth (4,004). Value of scale: 400 (Figs. 11–18).

The female acanthella

The sequence of development of proboscis receptacle and proboscis was comparable to that of male acanthellae. The smallest female acanthella recovered (714 long) is illustrated in Figure 20. This figure shows that fragmentation of the giant



Figures 19–30. Male cystacanth and female acanthellae of *Acanthocephalus parksidei*. 19. Proboscis of a male cystacanth (3,430 μm long) with abnormal hooks. 20. Early female acanthella (714). 21. Anterior part of a female acanthella (896) at a developmental stage comparable to that in Figure 20. 22–26. Female acanthellae in subsequent developmental stages (1,134, 812, 980, 1,246, and 1,932). 27, 28. Anterior parts of two female acanthellae (3,150 and 2,240), showing hook development. 29. Female acanthella (3,094). 30. Reproductive system of acanthella in Figure 29. Value of scale: 300 (Figs. 19–30).

subcuticular nuclei might proceed quite early, even before the genital primordia begin differentiating. Detail of the anterior end of a female acanthella (896 long) at a comparable stage of development is shown in Figure 21; note neck retractor and nucleus of proboscis retractor and detail of proboscis receptacle.

The next three stages (Figs. 22–24) are of three progressively more mature acanthellae, as judged by the differentiating proboscis and genital primorida, respectively. In these three acanthellae, the giant subcuticular nuclei have barely begun to fragment, if at all. It thus appears that much of the organ-system primordia, e.g., reproductive and proboscis apparatus, may begin and proceed with differentiation (all the way to the breaking of the ovary into ovarian balls, Fig. 24) before the giant subcuticular nuclei begin to fragment. The opposite is also true (Fig. 20). It is thus suggested that the rate of differentiation and/or growth of the above structures is not parallel and is not essentially correlated with events affecting giant-nuclei fragmentation. The same was observed in developing male acanthellae, though less dramatically.

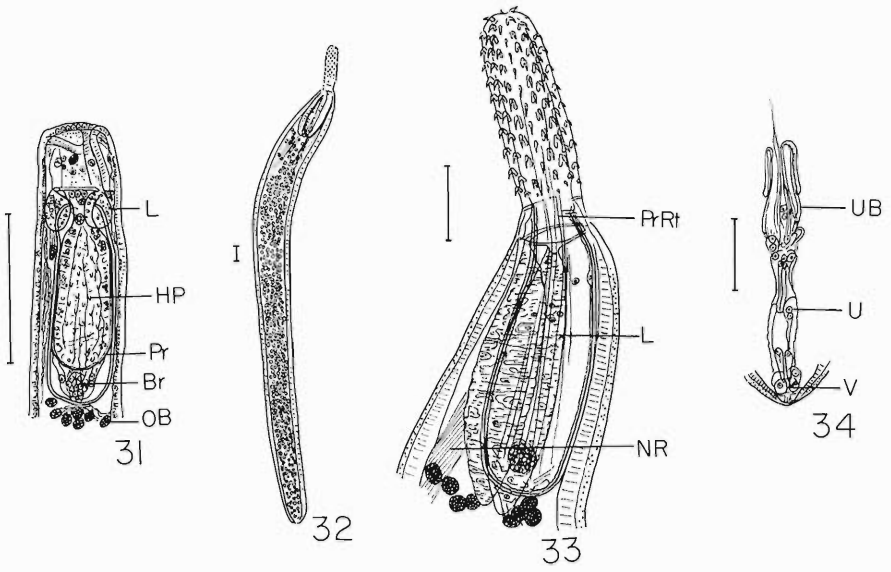
A developmental stage with a conspicuous ovary, which would have preceded the stage in Figure 24, was not observed. The specimen in Figure 25, although significantly larger than that in Figure 24, demonstrates either delayed formation of ovarian balls and development of proboscis primordium or somatic acceleration. The acanthella in Figure 26 (1,932 long) is twice as large as that in Figure 24 (980 long), but its proboscis primordium is less well developed.

In many acanthocephalans, the ovary does not develop sufficiently to break into ovarian balls while still in the intermediate host (Schmidt and Olsen, 1964). In *A. parksidei,* this advanced stage of development is attained as early as in an intermediate acanthella stage. This precocious state was previously observed in other acanthocephalans, e.g., *Prosthorhynchus formosus* and *Echinorhynchus truttae,* by Schmidt and Olsen (1964) and Awachie (1966), respectively. Such conditions, particularly when associated with related developments, e.g., in bursa and proboscis (see following section on cystacanths), might be conducive to rapid growth and early fertilization once in the final host.

The anterior parts of two 3,150 and 2,240 long acanthellae (Figs. 27, 28, respectively) show two stages of hook-primordia development in the everting proboscis that occur after the giant subcuticular nuclei are fragmented. Note the neck retractor and the fragmented nuclei of lemnisci ring at the presoma–soma level (Fig. 28). The subsequent stage is characterized by marked elongation of body (Fig. 29) and complete differentiation of the reproductive system (Fig. 30). The last structures to form in the female acanthella are the lemnisci. These start out as two small inpocketings of the body wall directly behind the region of the lemnisci ring (Fig. 31).

The male cystacanth

In early male cystacanths (Fig. 16), the development of the proboscis apparatus, brain, and reproductive system (Fig. 17) is completed. Reproductive structures, however, remain tightly confined within the ligament sac. The proboscis has now reinverted, the bursa is eversible, and the body wall becomes more tough and opaque. The fully grown cystacanth (Fig. 18) is indistinguishable from adult males; the proboscis is eversible and the reproductive system appears to be functional. This precocious state appears to be analogous to the early development



Figures 31–34. Female acanthella and cystacanth of *Acanthocephalus parksidei*. 31. Anterior part of acanthella (2,800 μm long). 32. Fully developed cystacanth (9,058). 33, 34. Anterior part and reproductive system of cystacanth in Figure 32. Value of scale: 300 (Figs. 31–34).

of ovarian balls in females. Acanthocephalans typically complete their sexual development in the final host, and larval proboscis and bursa do not usually evert in the intermediate host. These events might be correlated. Cystacanths and acanthellae with actively eversible proboscis and bursa appear to be found mostly in precocious species, e.g., *A. parksidei* and *E. truttae*, as described by Amin (this paper) and Awachie (1966), respectively.

Of 47 male cystacanths studied (like other specimens, all were refrigerated in distilled water overnight before fixing), 13% and 38% had fully and partially evaginated proboscises, respectively. Seventy percent had six cement glands each and 30% had four, five, seven, or eight. Lobed, branched, or multiple lemnisci (three or four) were noted in 22% and subequal lemnisci in 8% of the specimens. Only one spindle-shaped, monorchid male was observed. Another specimen was short and robust with the testes filling practically all the body-cavity space. Another slender cystacanth had two large body-wall bulges extending around its "enormous testes." The contorted and abnormal proboscis hooks observed in the monorchid cystacanth (Fig. 19) might have resulted from improper inversion of hooks as the proboscis reinverted. A similar observation was made in *P. formosus* by Schmidt and Olsen (1964). The body wall of three specimens had blisterlike swellings, as described in adult *A. parksidei* by Amin (1975) and *Echinorhynchus salmonis* by Amin and Redlin (1980), resulting from glycogen-phospholipid metabolic dysfunction (Lester and Wright, 1978).

The female cystacanth

Like the male cystacanth, the fully grown female cystacanth (Fig. 32) is indistinguishable from young adults. It has a functional proboscis apparatus, fully

differentiated tegument (Fig. 33), and a fully developed reproductive system (Fig. 34) that lacks many of the nucleated elements characterizing the earlier stages.

Of 78 female cystacanths studied, 19% and 49% had fully and partially evaginated proboscises, respectively. Twenty percent had lobed, branched, or multiple (3, 4, or 5) lemnisci. Subequal lemnisci were less common, and no specimens with body-wall blisters were observed.

Specimens

Materials deposited at the United States National Museum Helminthological Collection (USNM Helm. Coll.) include: USNM Helm. Coll. No. 76326, acanthors on one slide; USNM Helm. Coll. No. 76327, many acanthellae on 10 slides; USNM Helm. Coll. No. 76378, five male and five female cystacanths on nine slides.

Conclusions

The larval development of *A. parksidei* in *Caecidotea militaris* is rather similar to that reported for other species of the genus *Acanthocephalus*, with minor variations. The cortex is considerably thicker, particularly at the terminal regions in the earlier stages of *A. anguillae* (Leuckart, 1876, fig. 387a, b) compared to those of *A. parksidei*. The very brief description (with no illustrations) of the developmental stages of *A. clavula* in *Asellus meridianus* observed weekly by Rojanapaibul (1976) varies in some minor details from that for *A. parksidei* (e.g., the lemnisci differentiated earlier in *A. clavula*). Most notably, however, ovarian-ball formation and bursa eversion in *A. clavula* occurred only at the end of the first week postinfection of the definitive fish host. Only a few disjunct stages in the development of *A. galaxii* larvae in *Paracalliope fluviatilis* have been very briefly described; no early development or proboscis/bursa eversions were noted, and the number of giant nuclei was 12. The development of *A. anguillae* and *A. lucii* in *A. aquaticus* is more similar to that of *A. parksidei*. However, precocious development was not evident in the two European species, and early eversion of proboscis and bursa was not demonstrated (Andryuk, 1979a, b). Sexual maturity of *A. anguillae* and *A. lucii* was attained in fish intestine 15 and 21 days after infection, respectively (Andryuk, 1979a, b). Brattey's (1980) abstract, however, refers to sexually fully developed *A. lucii* in *A. aquaticus*, with copulation occurring less than 24 hr at 20°C in the definitive host.

The described pattern of development in *A. parksidei* is also comparable to patterns described for other palaeacanthocephalans, e.g., *L. thecatus* by DeGuisti (1949) and Uznanski and Nickol (1980), *E. lageniformis* by Olson and Pratt (1971), and *P. minutus* by Nicholas and Hynes (1963b); specific differences are noted in previous sections.

Two significant features appear to distinguish the developmental pattern of *A. parksidei*. First, the differentiations of the proboscis apparatus and the reproductive system do not appear to proceed in a parallel fashion and are not correlated with the pattern of giant subcuticular nuclei fragmentation, which may begin before the genital primordia start to differentiate or much later in development (after ovarian balls break). It is thus concluded that factors affecting differentiation and growth of various structures might act independently from each other as well as from those regulating fragmentation of the giant subcuticular

nuclei. This observation has not been previously reported. Second, the precocious condition observed in the development of *A. parksidei* appears to be correlated with the early eversibility of the proboscis of both sexes and male bursa while still in the intermediate host. Under these conditions, rapid growth and early fertilization in the definitive host might be expected.

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Hysterothylacium pelagicum sp. n. and *H. cornutum* (Stossich, 1904) (Nematoda: Anisakidae) from Marine Fishes

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ABSTRACT: *Hysterothylacium pelagicum* sp. n. from the digestive tract of the dolphin, *Coryphaena hippurus*, is described from the Atlantic and Pacific oceans. It is most similar to *H. cornutum*, but differs from it by lacking ventral crests on the males and having the vulva located within the anterior 30–40% rather than 18–25% of the female body. Specimens of *H. cornutum* are reported from *Thunnus albacares*, a previously unrecorded host, off Hawaii.

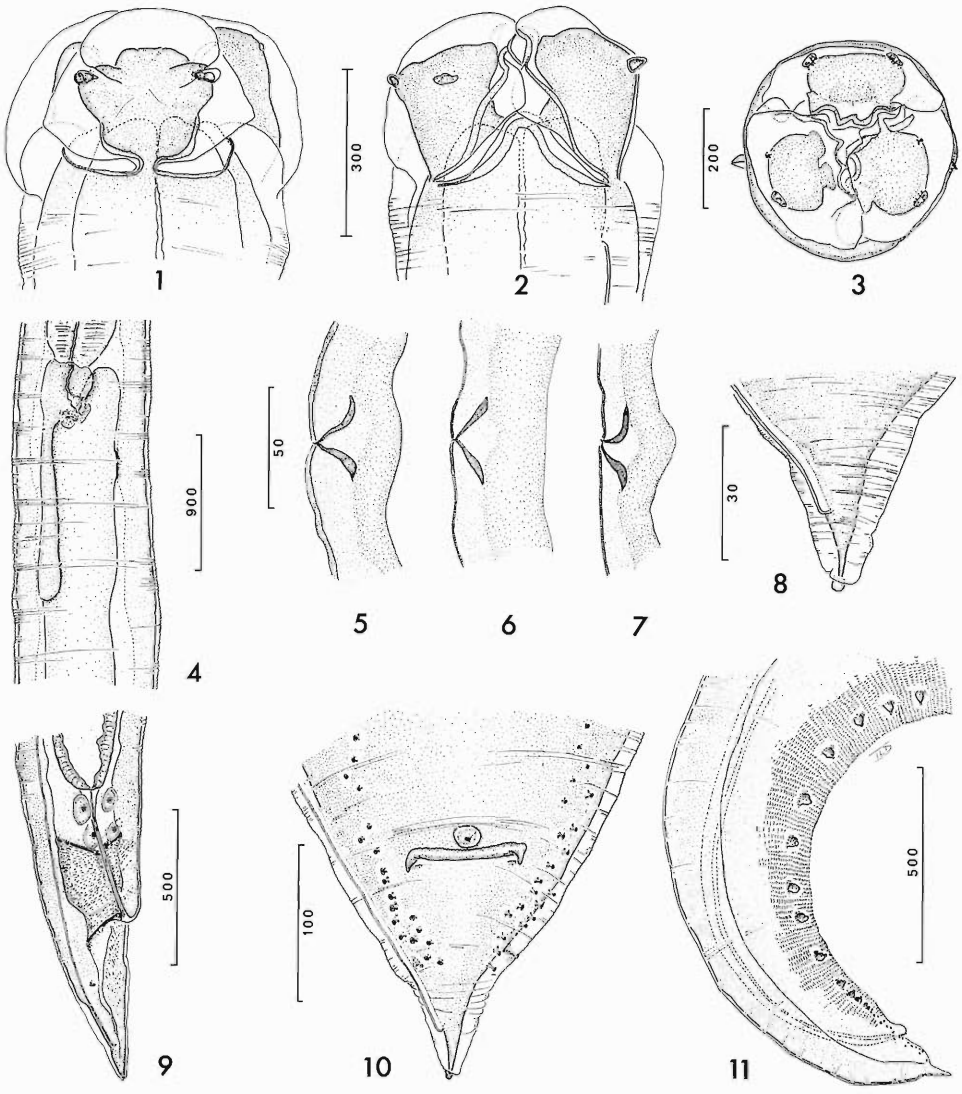
Because features of ascaridoid specimens collected from the dolphin, *Coryphaena hippurus* Linnaeus, from the Pacific and Atlantic oceans, including the Gulf of Mexico, resemble descriptions of *Hysterothylacium cornutum* (Stossich, 1904), specimens of the latter were critically examined. We determined, based on specimens from the same lot as the type material and from others collected near Hawaii, that the specimens from the dolphin differed, and therefore we describe them below.

Living nematodes were fixed in glacial acetic acid or 10% formalin, stored in a solution of 5 parts glycerin and 95 parts 70% ethanol, examined either in glycerin after evaporation of the alcohol or in lactic acid when rapid clearing was required. All measurements are in micrometers unless otherwise stated, and illustrations were drawn with the aid of a drawing tube. Serial sections of portions of a worm were stained with hematoxylin and eosin.

Hysterothylacium pelagicum sp. n. (Figs. 1–10)

GENERAL: Body reaching greatest width near midbody. Cuticle with fine annulations; lateral alae distinct along each side, becoming increasingly apparent at levels posterior to rectum and anterior to nerve ring; alal supports with slight V-shape in cross section. Lips approximately equal in size, wider than long; flanges constricted at anterior ¼ of lip; pulp conspicuously narrow near base. Interlabia with height equal to or slightly greater than width at base; interlabial grooves deep; adjacent grooves nearly merging at base of each lip. Esophagus 8–12% of body length. Ventriculus proper usually longer than wide, narrower than widest level of esophagus; ventricular appendage projecting posteriad from posterior portion of ventriculus. Nerve ring located at anterior 9–17% of esophagus. Excretory pore immediately posterior to level of nerve ring. Excretory duct extending posteriad to left lateral cord and joining excretory canal; canal extending posteriorly to at least midbody. Tail gradually tapering, with rounded nipplelike structure at posterior extremity; structure lacking spinules.

MALE (based on 10 mature specimens): Body 28–67 mm long by 0.4–1.0 mm at greatest width; ratio of greatest width to length 1:60–67. Lips 144–284 long by 192–339 wide. Nerve ring (center) 556–898 from anterior extremity, 43–61 in breadth. Esophagus 3.5–6.1 mm long by 154–321 wide. Ventriculus 144–197 long



Figures 1–11. ***Hysterothylacium pelagicum*** **(1–10) and** ***H. cornutum*** **(11). 1. Dorsal view of lip. 2. Ventral view of lips showing interlabium. 3. En face. 4. Body at level of intestinal-ventricular junction, female. 5–7. Cross section of lateral ala at level of nerve ring (5), middle of body (6), and immediately anterior to anus (7) of female. 8. Posterior extremity of male tail, lateral view. 9. Female tail, lateral view. 10. Posterior end of male showing postanal papillae, ventral view. 11. Posterior end of** ***H. cornutum*** **showing both caudal papillae and annules on ventral surface, lateral view of male.**

by 156–278 wide; ventricular appendage 0.6–1.2 mm long by 79–135 wide; ratio of ventricular appendage to esophageal lengths 1:4.0–6.3. Intestinal cecum 98–271 long by 154–228 wide; ratio of cecal to ventricular appendage lengths 1:4.4–6.3; ratio of cecal to esophageal lengths 1:18–36. Spicules 3–5% of body length, nearly equal; left spicule 1.3–2.4 mm long by 19–30 wide; right spicule 1.4–2.4 mm long by 19–30 wide; spicule ratio 1:0.9–1.0. Caudal papillae 44–47 pairs; preanal pairs 32–35; postanal pairs 10; para-anal pairs 2, not clearly distinguished from others.

Medioventral preanal organ papillated, simple, at middle of preanal fold, single, larger than other papillae. Tail flexed ventrad, 166–247 long, with digitiform process at posterior extremity; process 37–55 including rounded nipplelike structure.

FEMALE (based on 10 mature specimens): Body 17–82 mm long by 0.2–1.0 mm wide; ratio of greatest width to length 1:59–80. Lips 72–321 long by 123–398 wide. Nerve ring (center) 376–992 from anterior extremity, 36–61 in breadth. Esophagus 1.8–7.5 mm long by 91–426 wide. Ventriculus 91–321 long by 84–346 wide; ventricular appendage 1.0–1.7 mm long by 21–72 wide; ratio of ventricular appendage to esophageal lengths 1:1.0–8.5. Intestinal cecum 125–390 long by 55–204 wide; ratio of cecal to ventricular appendage lengths 1:3.5–13.8; ratio of cecal to esophageal lengths 1:14–16. Vulva without salient lips, opening 30–40% of body length from anterior extremity. Uterus didelphic, opisthodelphic. Ovaries rarely extending beyond anterior level of vulva. Eggs 49–61 in diameter. Tail gradually tapering, 149–977 long.

TYPE HOST: *Coryphaena hippurus* Linnaeus, dolphin (Coryphaenidae).

SITES OF INFECTION: Lumens of stomach, pyloric ceca, and intestine.

LOCALITIES: Offshore from Hawaii (type locality); Gulf of Panama (6°16′N, 78°11′W); offshore from Orange Beach, Alabama; offshore from South Carolina; and Papua New Guinea (specimens from New Guinea not used for description).

PREVALENCE: In 19 of 33 (57.6%) fish from Hawaii.

INTENSITY: Range, 1–35; mean, 10.

SPECIMENS DEPOSITED: Holotype, male, USNM Helm. Coll. No. 76615; allotype, female, USNM Helm. Coll. No. 76616; paratypes, USNM Helm. Coll. No. 76617.

ETYMOLOGY: The Latinized Greek *pelagicum* means "of the sea" and refers to the habitat of the host.

Remarks

We examined nematodes from *Coryphaena hippurus* that were deposited in the U.S. National Museum and British Museum (Natural History). Specimens identified by A. O. Foster as *Contracaecum* sp. from the Gulf of Panama (USNM No. 58323, personal communication) and others identified as *C. cornutum* from Papua New Guinea (BMNH 1973.282–285) correspond with our material. Other specimens deposited by Foster as *Porrocaecum* sp. (USNM No. 58565) from the Gulf of Panama also appear to be *H. pelagicum*, but specific identification could not be confirmed because of the opaque condition of the material. Not all ascaridoids from *C. hippurus* deposited at the U.S. National Museum are *H. pelagicum*. Linton's specimens (USNM No. 6366), which he identified (Linton, 1901) as *A. increscens* Molin, 1858, lack crests, but differ from *H. pelagicum* by having a multispinous process on the posterior extremity of a male; unidentified worms from Panama (USNM No. 58571) deposited by Foster are the fourth-stage larva of *Anisakis* sp. According to Punt (1941), *H. increscens*, a parasite of lophiid fishes, may be a synonym of *H. rigidum* (Rudolphi, 1809).

By possessing 10 postanal and 2 para-anal papillae, *H. pelagicum* most closely resembles *H. cornutum*, but differs conspicuously by lacking modified ventral annules (ventral crests) on the males (Figs. 10–11) and by having a vulva in the anterior 30–40% of the female rather than the anterior 18–25%. *Hysterothylacium pelagicum* constitutes the seventh species in the genus from the Gulf of Mexico,

not counting *H. eurycheilum* (Olsen, 1952) from Tortugas, Florida (Deardorff and Overstreet, 1981b). It brings to at least 50 the number of species in the genus. Some are questionably differentiated from others, whereas some not placed in the genus are based on incompletely described adults or larvae and surely belong there (Deardorff and Overstreet, 1981a).

Hysterothylacium cornutum (Stossich, 1904) Deardorff and Overstreet, 1981 (Fig. 11)

Ascaris cornuta Stossich, 1904.
Contracaecum cornutum: Baylis, 1923.
Thynnascaris legendrei Dollfus, 1933.
Contracaecum legendrei: Dollfus, 1935.
Thynnascaris cornuta: Hartwich, 1975.

Remarks

We obtained *H. cornutum* in 4 of 17 stomachs of the yellowfin tuna, *Thunnus albacares* (Bonnaterre), offshore from Hawaii. In contrast, we did not see the species in any of 19 individuals of that fish host examined from the northern Gulf of Mexico, and apparently neither did Ward (1962) in 45 specimens from the same area.

Stossich (1904) briefly described *Ascaris cornuta* from the bluefin tuna, *T. thynnus* (Linnaeus) (as *T. vulgaris*) from Trieste, Italy. Baylis (1923), apparently using material from the same lot as the type material, provided a more detailed description. Although the species has been adequately described for most features, most recently by Berland (1961) and Hartwich (1975) from *T. thynnus* and by Petter (1969) from the albacore, *T. alalunga* (Bonnaterre) (as *T. germo* Lacépède), we reexamined those specimens described by Baylis (BMNH Reg. No. 1931.10.20.80–89). In addition to that described, the specimens possess a pair of lateral alae extending from the base of the lips to just anterior of the posterior extremity, and have modified ventral annules on the males extending from just anterior to the anus to well beyond the anteriormost precloacal papillae. Berland (1961) mentioned "ridges, probably reduced alae, running laterally from near head to tail" and "longitudinal striation very fine, visible on entire body, except just behind head, and most prominent near tail."

By possessing ventral crests, *H. cornutum* shows similarity to *H. corrugatum* Deardorff and Overstreet, 1981 from *Xiphias gladius* (Linnaeus); *H. incurvum* (Rudolphi, 1819) from *X. gladius, Tetrapturus albidus* Poey, and *T. brevirostris* (Playfair); and possibly *H. histiophori* (Yamaguti, 1935) from *Istiophorus platypterus* (Shaw and Nodder). All these pelagic hosts are related in the Scombroidei. *Hysterothylacium cornutum,* however, differs significantly from these species in the number and arrangement of the postanal papillae.

In addition to the new record of *H. cornutum* in *T. albacares* from Hawaii and its well-established presence as an adult in the type host, *T. thynnus,* from Trieste (Mediterranean Sea) (Baylis, 1923), the North and Baltic seas (Hartwich, 1975), and the southwestern Norwegian coast (Berland, 1961) and from *T. alalunga* in the Bay of Biscay (Dollfus, 1933, 1935; Petter, 1969), the species has been reported (as *Contracaecum legendrei*) with inadequate or no descriptive information from *T. alalunga* [as *T. alalonga* (Gmelin)] in the "Pacific" by Yamaguti

(1941); from *T. maccoyii* (Castelnau) (as *T. maccoyi*), *Promicrops lanceolatus* (Bloch), and *Argyrosomus hololepidotus* (Lacépède) (as *Sciaena antarctica* Castelnau) off Queensland, New South Wales, and Victoria by Johnston and Mawson (1943, 1951); and tentatively from *Polyprion oxygeneios* (Bloch and Schneider) in Dusky Sound, Fiordland, New Zealand, by Brunsdon (1956). Johnston and Mawson (e.g., 1943, 1945, 1951) also listed various fishes from Australia as hosts of larval and immature specimens. Identification of specimens from the last three definitive hosts should be confirmed or more individuals of those fishes examined for ascaridoids, because other than those reports, the species is known from tunas only.

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On Two New Nematode Parasites (Trichostrongyloidea: Molineidae) from Amphibians and Reptiles

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ABSTRACT: *Kentropyxia sauria* gen. et sp. n. from the lizard *Kentropyx calcaratus* (Teiidae) of Brazil most closely resembles the genus *Oswaldocruzia* in the morphology of the caudal bursa, presence of a synlophe, and lack of a gubernaculum. It may be distinguished from *Oswaldocruzia* by the presence of a corona radiata on the cephalic extremity and by bursal rays 8 terminating beside rays 6. *Oswaldocruzia polycercus* sp. n. from the amphibian *Bufo polycercus* of Cameroon, Africa, is closely related to the group of *Oswaldocruzia* species in Central Africa that have spicules divided into five distal points. It may be distinguished by the morphology of the spicule points.

Two new species of trichostrongyles (Molineidae) are described from material in the Museum National d'Histoire Naturelle, Paris: *Kentropyxia sauria* gen. et sp. n. from a Brazilian lizard, and *Oswaldocruzia polycercus* n. sp. from an African toad.

Results

Kentropyxia gen. n.

Trichostrongyloidea, Molineidae, Molineinae. Corona radiata present, formed by 9 unequal lappets. Bursal rays 2–3 and 4–6 forming 2 separate groups each with common base; rays 2–3 parallel, ray 4 diverging from 5–6, which are parallel. Terminal papilla of ray 8 close to ray 6.

TYPE SPECIES: *Kentropyxia sauria* sp. n.

Kentropyxia sauria sp. n.

TYPE MATERIAL: Male holotype, female allotype, 2 male and 4 female paratypes, Museum National d'Histoire Naturelle (MNHN) No. 207RL.

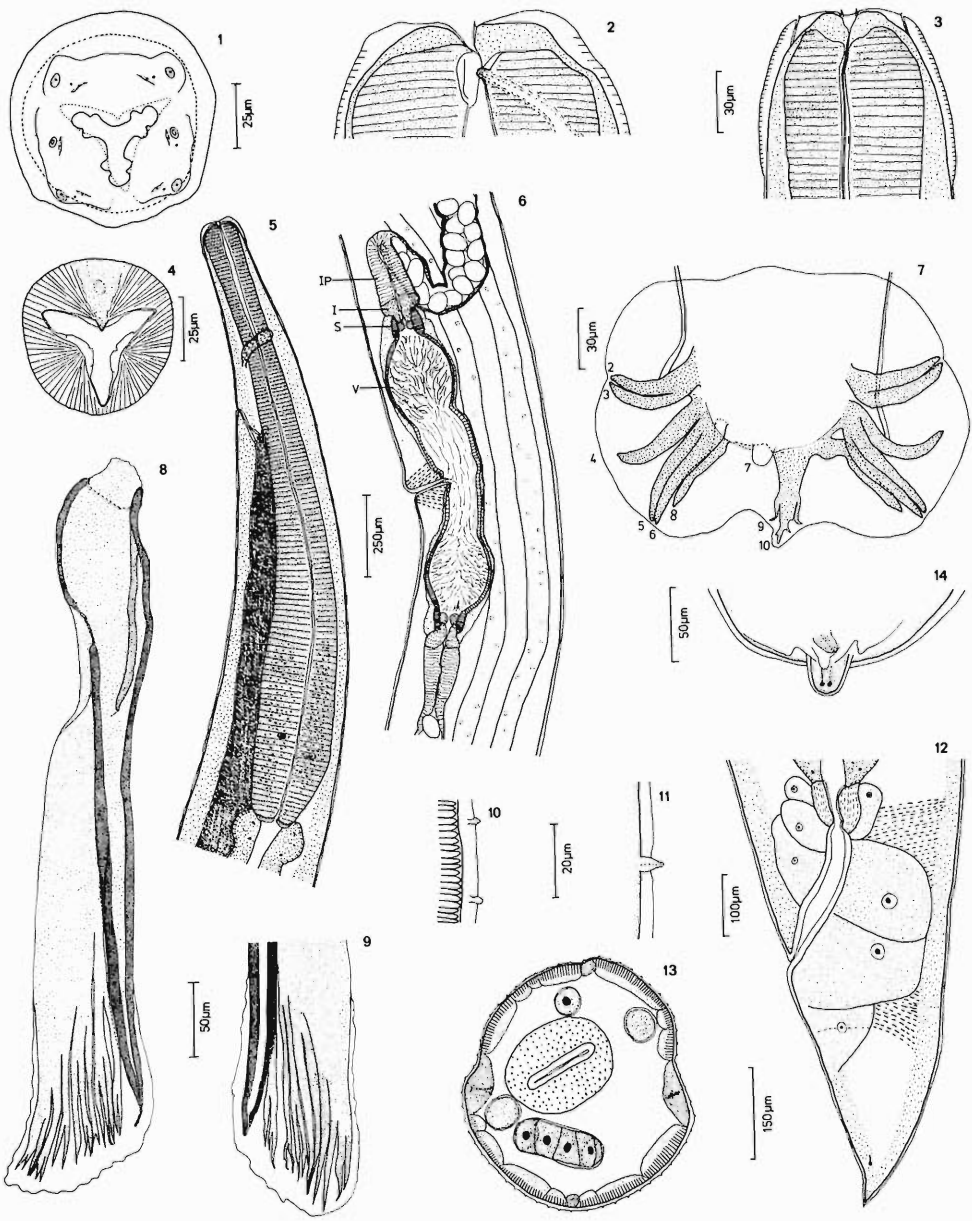
HOST: *Kentropyx calcaratus* Spix (Teiidae).

LOCALITY: Belem, Brazil.

LOCATION: Intestine.

DESCRIPTION (Figs. 1–14): Oral opening triangular, surrounded by corona radiata in form of 9 unequal lappets (3 dorsal, 3 on each subventral side). Mouth opening into short funnel-shaped cavity. Cephalic lips not present, but cephalic extremity with dorsal and 2 subventral elevations bearing papillae and amphids. Six small inner papillae, 6 small outer papillae, and 4 cephalic papillae present. Small dorsal esophageal tooth present, projecting into thick cuticular pad on opposite side of esophagus. Cephalic inflation conspicuous. Cervical alae absent. Excretory pore at anterior ⅓ of esophagus. Cervical deirids small, digitiform, located near excretory pore. Synlophe with numerous small ridges perpendicular to body and extending from behind cephalic inflation to posterior portion of tail in females and to bursa in males. Ridges of synlope with small supports. Number of ridges variable, slightly less numerous at anterior and posterior ends. In midbody males with 50 crests, females with 58.

MALE (holotype): Total length 12.7 mm, maximum width 293 μm. Cephalic



Figures 1–14. *Kentropyxia sauria* **gen. et sp. n. 1. Anterior extremity, apical view. 2. Anterior extremity, lateral view. 3. Anterior extremity, dorsoventral optical section at level of amphids. 4. Anterior end of esophagus, section at level of dorsal tooth. 5. Anterior end, lateral view. 6. Ovejector, lateral view. 7. Caudal bursa, ventral view. The numbering of the rays is indicated. 8. Left spicule, ventral view. 9. Distal end of left spicule, dorsal view. 10. Detail of crests of synlophe, transverse section. 11. Deirid, lateral view. 12. Tail of female, lateral view. 13. Synlophe of female near midbody. 14. Genital cone of male, ventral view. Abbreviations: I, infundibulum; IP, intermediary portion; S, sphincter; V, vestibule.**

inflation 84 μm long, 103 μm wide. Nerve ring 406 μm, excretory pore 588 μm from anterior extremity. Esophagus 1.22 mm long. Bursa with thick dorsal ray extending slightly posterior to lateral lobe. Prebursal rays 1 not observed. Rays 2–3 with common origin, parallel, terminating at edge of bursa. Rays 4–6 with common origin: 4 separating from 5–6 and curved anterolaterally; 5–6 parallel and directed posterolaterally. Rays 8 originating from base of dorsal ray, parallel to rays 5–6. Genital cone conspicuous, with 2 papillae. Ventral edge of anus with single digitiform papilla and papilla-like cuticular projection. Prebursal papillae not conspicuous. Spicules equal, 513 μm long, shaft divided in distal ⅓ into robust outer process and thin, wide, dorsal inner and ventral inner processes. Outer process forming single point. Dorsal and ventral inner processes divided distally into 14 and 18 fine points, respectively. Gubernaculum absent.

FEMALE (allotype): Total length 18.4 mm, maximum width 390 μm. Cephalic inflation 100 μm long, 122 μm wide. Nerve ring 390 μm, excretory pore 620 μm, and vulva 12.0 mm from anterior extremity. Esophagus 1.71 mm long. Anterior portion of ovejector with vestibula 212 μm, sphincter 252 μm, infundibulum 75 μm, intermediary portion (thickened terminal portion of uterus) 281 μm long; posterior portion divided into parts 151 μm, 235 μm, 70 μm, and 312 μm long, respectively. Sphincters with markedly thick walls. Anterior uterus with 211 eggs, posterior with 243. Eggs 91–100 μm long and 53–56 μm wide, at morula stage. Tail thick, 206 μm long, with sharp terminal point.

PARATYPES: Dimensions of 2 males and 4 females are as follows: *Males*—length 12.1–13.9 mm; nerve ring 350–405 μm, excretory pore 556–725 μm from anterior extremity; esophagus 1.17–1.36 mm and spicules 500–531 μm long. *Females*—length 16.8–19.1 mm; nerve ring 367–405 μm, excretory pore 544–582 μm, vulva 11.2–12.6 mm from anterior extremity; esophagus 1.48–1.86 mm, tail 231–234 μm long.

Remarks

In bursal morphology, presence of a simple synlophe, and lack of gubernaculum, *Kentropyxia* gen. n. most closely resembles *Oswaldocruzia* (Molineinae). However, the presence of a vestigial corona radiata easily distinguishes it from all *Oswaldocruzia* for which the cephalic end has been studied in apical view. Because the presence of a corona radiata in the Trichostrongyloidea is considered to be a primitive character indicating a phyletic relationship to the Strongyloidea, a new genus has been proposed. *Kentropyxia* may also be distinguished from *Oswaldocruzia* by bursal rays 8, which are parallel to, and terminate beside, rays 6. In all *Oswaldocruzia* species rays 8 are curved away from rays 6 in their distal portions.

***Oswaldocruzia polycercus* sp. n.**

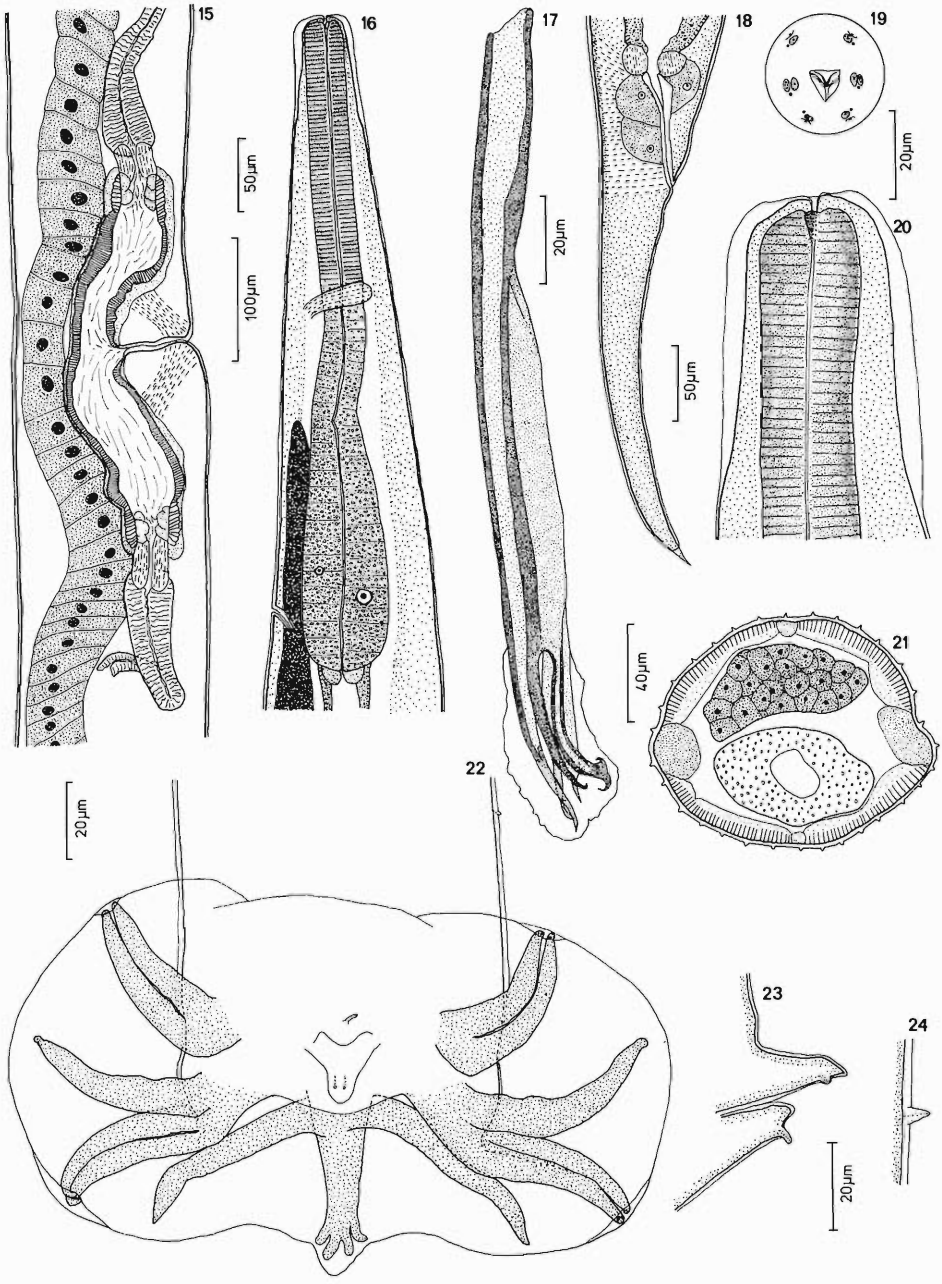
TYPE MATERIAL: Male holotype, female allotype, 2 male and 3 female paratypes, MNHN 95KB.

HOST: *Bufo polycercus* Wern. (Bufonidae).

LOCALITY: Kribi, Cameroon.

LOCATION: Intestine just posterior to stomach.

DESCRIPTION (Figs. 15–24): Trichostrongyloidea, Molineidae, Molineinae. Oral opening triangular, opening into short funnel-shaped cavity lined with thin cuticle. Conspicuous dorsal esophageal tooth present. Six conspicuous inner papillae, 6



Figures 15–24. *Oswaldocruzia polycercus* **sp. n. 15. Ovejector, lateral view. 16. Anterior end, lateral view. 17. Spicule, ventral view. 18. Tail of female, lateral view. 19. Anterior extremity, apical view. 20. Anterior extremity, lateral view. 21. Synlophe of female near midbody. 22. Caudal bursa, ventral view. 23. Genital cone of male, lateral view. 24. Deirid, dorsoventral view.**

small outer papillae, and 4 small cephalic papillae present. Cephalic inflation conspicuous. Cervical alae absent. Excretory pore near posterior end of esophagus. Cervical deirids small, digitiform, located near excretory pore. Synlophe with numerous small ridges perpendicular to body and extending from just behind

the cephalic inflation to midregion of tail in females and to bursa in males. Number of ridges variable, less numerous at anterior and posterior ends. In midbody males with about 31 ridges, females with 54.

MALE (holotype): Total length 5.2 mm, maximum width 110 μm. Cephalic inflation 65 μm long, 47 μm wide. Nerve ring 187 μm, excretory pore 368 μm from anterior extremity. Esophagus 418 μm long. Bursa with thick dorsal ray, dorsal lobe not extending posterior to lateral lobe. Rays 2–3 with common origin, parallel, terminating at edge of bursa. Rays 4–5–6 with common origin: 4 separating from 5–6 and curved anterolaterally; 5–6 parallel and directed posterolaterally. Rays 4 terminating 6 μm from edge of bursa, rays 5–6 terminating at edge of bursa. Rays 8 originating from base of dorsal ray, terminating 3 μm from edge of bursa. Genital cone elongate, with 2 small papillae. Ventral edge of anus with single digitiform papilla. Prebursal papillae inconspicuous. Spicules equal, 172 μm long, shaft with large ala-like expansion on inner side extending from anterior ⅓ to distal end. Shaft divided in distal ¼ into 2 sharply pointed processes, ala-like expansion divided distally into 2 sharply pointed processes and 1 robust process forming anvil-like terminal end. Gubernaculum absent.

FEMALE (allotype): Total length 10.5 mm, maximum width 140 μm. Cephalic inflation 62 μm long, 47 μm wide. Nerve ring 193 μm, excretory pore 334 μm, and vulva 7.1 mm from anterior extremity. Esophagus 434 μm long. Anterior portion of ovejector with vestibula 70 μm, sphincter 48 μm, infundibulum 44 μm, intermediary portion 152 μm long; posterior portion divided into parts 92 μm, 35 μm, 52 μm, and 180 μm long, respectively. Anterior uterus with 29 eggs, posterior with 23. Eggs 94–100 μm long and 62–69 μm wide, at morula stage. Tail slender, 258 μm long, with conspicuous terminal spike about 15 μm long. Phasmids on posterior ½ of tail.

PARATYPES: Dimensions of 2 males and 3 females are as follows: *Males*—length 5.8–6.4 mm; nerve ring 163–209 μm, excretory pore 306–368 μm from anterior extremity; esophagus 353–359 μm and spicules 203–218 μm long. *Females*—length 11.8–13.2 mm; nerve ring 193–200 μm, excretory pore 350–403 μm, vulva 7.9–9.6 mm from anterior extremity; esophagus 443–497 μm, tail 322–353 μm long.

Remarks

In common with six *Oswaldocruzia* species from amphibians and reptiles of Africa (see Hörchner, 1963; Durette-Desset and Vaucher, 1979), *O. polycercus* sp. n. has spicules divided in the posterior quarter into five points. No other species outside of Africa have spicules of this type. Among this group, *O. polycercus* is easily differentiated from *O. chamaeleonis* Hörchner from *Chamaeleo* sp. of Africa or Madagascar (locality not given precisely) and *O. cricogaster* Durette-Desset and Vaucher from *Phrynobatrachus cricogaster* of Cameroon by the shape of the dorsal lobe of the bursa. It may be easily differentiated also from *O. johnstoni* Durette-Desset and Vaucher from *Pedropedetes johnstoni* of Cameroon in that it lacks cervical alae, and from *O. gracilipes* Durette-Desset and Vaucher from *Bufo gracilipes* of Cameroon in that rays 5–6 are relatively shorter. Finally the spicules of *O. polycercus* have four slender, sharply pointed, distal processes and one robust process with an anvil-like end, whereas *O. gassmanae*

Durette-Desset and Vaucher from *Chamaeleo wiedersheimi* of Cameroon has three slender and two robust processes, and *O. perreti* Durette-Desset and Vaucher from *Bufo latifrons* of Cameroon has three straight processes, one process that is curved proximally, and one robust process.

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Factors Influencing Egg Hatching of the Soybean Cyst Nematode *Heterodera glycines* Race 3

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ABSTRACT: Individual cysts of the soybean nematode *Heterodera glycines* contained an average of 323 eggs. Hatching increased linearly as the incubation temperature was raised during a 2-wk test. Insignificant differences in egg hatching were found over 25°C or after pre-exposure to −4°C temperatures for 3 wk prior to 25°C incubation. Optimal hatching rates were obtained with 25% soil moisture and pH 6.0. Increases or decreases in these values depressed egg hatching of *H. glycines*. Soybean root leachate at 4.86 root-g-hr/ml significantly elevated hatching when compared to the control, whereas Hoagland's solution, a plant nutrient, showed a tendency to reduce hatching. Incubation in ionic solutions revealed various effects. Sodium, calcium, potassium, and chloride revealed no effect and, indirectly, osmotic influences may be negligible. Incubation in nitrate and ferric concentrations showed insignificant increases in hatching. In contrast, exposure of *H. glycines* eggs to zinc chloride gave a pronounced increase of egg hatching.

Races of the soybean cyst nematode *Heterodera glycines* are economically significant pathogens of soybeans. Since the initial report of this nematode from Japan, *H. glycines* has been found throughout the southeastern United States. The production losses in Illinois alone due to the nematode are estimated in millions of dollars annually.

A number of studies have concerned various developmental aspects of *H. glycines*, and both free and, mainly, encysted eggs have been used. Endo (1962) found that eggs hatched for up to 4 days at 0–8.5% relative humidity, whereas 100% relative humidity facilitated hatching for 16 days. Okada (1971a) reported that the pre-soaking of cysts in water increased hatching at 25 or 30°C.

Tsutsumi and Sakurai (1966) and Okada (1971b) found that host-root diffusates increased egg hatching, but non-hosts had no influence. Okada (1972a) also described a hatching stimulant in *H. glycines* eggs. This stimulant was organic, water soluble, and tolerant to 120°C for 30 min. Additionally, an inhibitory factor for egg hatching, which was dosage dependent in its effect, was present in *H. glycines* cysts (Okada, 1972b).

In contrast, Slack and Hamblen (1961) found that root leachate and diffusate failed to stimulate larval emergence from cysts. However, their study showed that larval emergence from cysts was optimal at 24°C but failed below 16°C or above 36°C. Light had no effect, whereas mild desiccation and the absence of aeration reduced larval emergence. Environmental temperatures have been related to seasonal emergence of larvae from cysts (Ross, 1963).

Lehman et al. (1971) reported that egg masses were insensitive to pH, but larval emergence from the cyst was greatest at pH 3.5 and reduced at pH 5.5. Larval emergence from cysts was inhibited by NH_4^+ or NO_3^-, but stimulated by $CaSO_4$. Clarke and Shepherd (1966) examined the hatching of encysted eggs of nine *Heterodera* species during exposure to a wide variety of inorganic ions. Twelve ions stimulated the hatching of *H. glycines*; Fe^{+2} and Zn^{+2} were most pronounced in their effect. Hatching was inhibited by Cd^{+2}. Water and root diffusates gave an average 4% hatch.

The present study examined a number of chemical and physical factors that potentially influence hatching of free eggs in the population of *H. glycines* Race 3 in Illinois. Clarification of the geographic differences that are reported in the literature and the biological differences of free versus encysted eggs is needed for an understanding of the reproductive capability of the soybean cyst nematode.

Materials and Methods

Cysts of *Heterodera glycines* Race 3 were obtained from laboratory- or greenhouse-reared soybean plants (Union variety). Cysts were used as a source of eggs for experimentation or for maintenance of the life cycle (Skotland, 1957). Cyst age throughout the study ranged from 6 wk to 3 mo, but cysts for any individual trial were used within a 3-wk period of age.

Cysts were opened manually and the freed eggs were collected in distilled water. Eggs were placed in test tubes and the indicated test compounds were added to give selected final concentrations in a 2-ml volume. Alternatively, the eggs were maintained in 2 ml of distilled water for treatment by physical factors. Each dosage or treatment was conducted in triplicate and the results were averaged. The number of larvae that hatched was determined microscopically by enumeration with a calibrated counting chamber. At least five counts were done during a 2-wk period to determine rate of egg hatching and the mean percentage of hatched eggs. Eggs were maintained at a temperature of 25°C unless stated otherwise. At least 300 eggs were used per test.

The effects of temperature on hatching were studied by constant incubation of eggs at 16, 20, 25, 30, and 36°C for the 2-wk period. Test tubes were covered to maintain a constant volume. After establishment of this background, the influence of initial exposure to variable temperatures was examined. Eggs were held at −5, 4, 9, 16, and 25°C for a 3-wk period prior to incubation at a constant temperature of 25°C for 2 wk. Hatching was determined with a calibrated counting chamber.

Moisture effects were studied by addition of known numbers of eggs of *H. glycines* into petri dishes that contained a distilled water–soil mixture. Moisture levels were established on a weight basis at 15, 21, 25, 30, and 33% in sterile soil. Dishes were weighed daily for replacement of any evaporated water and were examined for 2 wk to determine hatching. Soil was obtained from an infested soybean field in Elkville, Illinois.

The influence of pH on the hatching of free eggs was tested also. Test tubes with eggs in distilled water were adjusted to pH 5.4, 6.0, 6.7, 7.2, and 8.0 with dibasic sodium phosphate–monobasic potassium phosphate (0.067 M) buffer. Enumeration was as described previously.

Any alteration of egg hatching by soybean root leachate was investigated by similar methods. The root mass of living plants was maintained in 36 ml of distilled water for 48 hr. After preparation, the root mass was removed and weighed to quantify the host leachate as root-g-hr/ml. The resultant solution contained 4.86 root-g-hr/ml. This leachate was tested at 100, 75, 50, and 25% concentrations after dilution with distilled water for any influence on *H. glycines* eggs. Distilled water was used as a control.

Additionally, Hoagland's solution was evaluated for influences on egg hatching, due to its usage as a nutrient for host plants. The cultivation medium was prepared

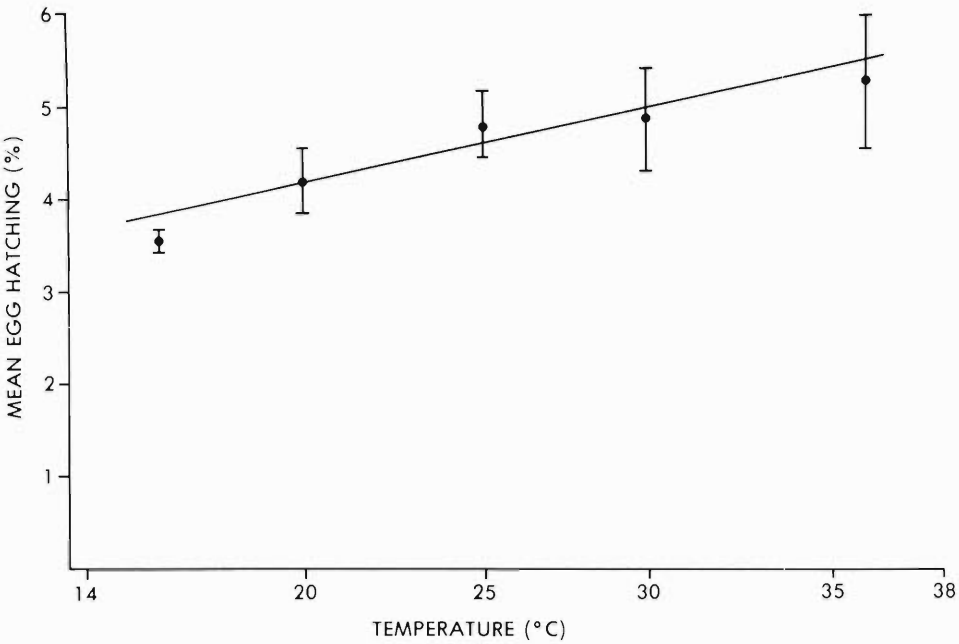


Figure 1. Mean percentage of egg hatching of *Heterodera glycines* during 2 wk at various incubation temperatures (MSE = 0.44).

according to Hoagland and Arnon (1938). Eggs of *H. glycines* were exposed for 2 wk to 100, 75, 50, and 25% concentrations at 25°C. Distilled water served as a control.

Selected inorganic ions were used to study their effect on hatching of *H. glycines*. Eggs were incubated in 85, 170, 340, 510, and 850 mM sodium chloride or 33, 66, 100, 133, and 266 mM calcium chloride to determine any effect of sodium or calcium. A second experiment employed a 171–134 mM solution with sodium chloride:potassium chloride ratios of 1:0, 3:1, 1:1, 1:3, and 0:1, respectively.

Influences of nitrate were tested also. Eggs were incubated for 2 wk in 60, 120, 180, 360, and 480 mM sodium nitrate at 25°C. Zinc chloride and ferric chloride were tested similarly at 1, 2, 3, and 5 mM concentrations. Alternatively, monobasic potassium phosphate and dibasic sodium phosphate were used at a 7:1 ratio (33–400 mM) at a constant pH of 6. Distilled water was the control in the above trials.

Data were evaluated by linear regression or analysis of variance. The 0.05 probability level was considered significant.

Results

Initially, the number of eggs per cyst of *H. glycines* was determined as baseline data for these and future studies of reproductive potential. Individual enumeration of 147 cysts gave a mean content of 323.4 eggs/cyst (MSE = 8.61). This value was within the range reported by other investigators. Based on this information, egg concentrations could be approximated at appropriate levels for subsequent study.

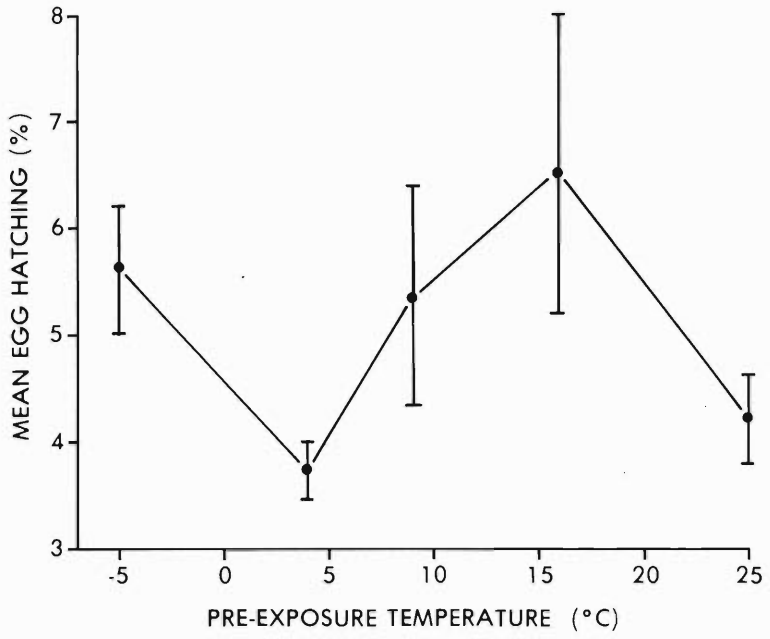
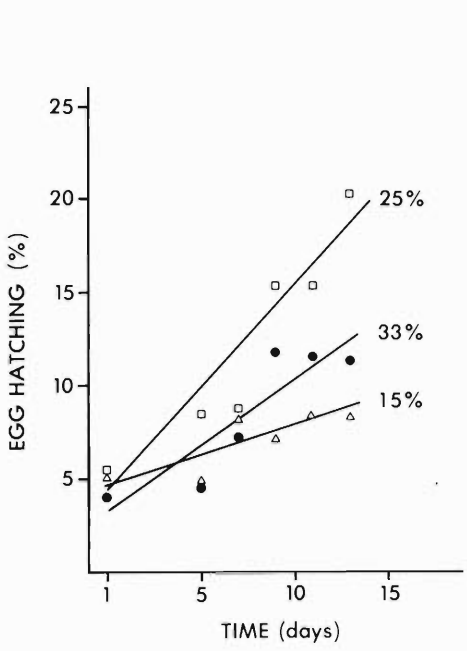


Figure 2. Mean percentage of egg hatching of *Heterodera glycines* during 2 wk after a 3-wk exposure to the indicated temperatures prior to incubation at 25°C (MSE = 0.72).

Incubation temperature affected the rate of egg hatching in *H. glycines* (Fig. 1). The mean percentage of hatching for the 2-wk period increased linearly ($r = 0.92$) as the incubation temperature was increased. No significant difference was found among temperatures between 20 and 36°C, although slightly more eggs hatched at higher temperatures. However, the hatching response at 16°C was lower than at other tested temperatures. The mean percentage of eggs that hatched in the 2-wk period increased 46% with a temperature elevation from 16°C to 36°C. The rate of hatching was elevated also as incubation temperature was raised.

In contrast to the above results, exposure of cysts to temperature extremes for 3 wk prior to test incubation at 25°C revealed no apparent effect on egg hatching (Fig. 2). The mean percentage of eggs that hatched from pre-exposure temperatures of −4 or 5°C was similar to that for the 25°C control and other moderate temperature regimes ($r = 0.08$). Considerable variation was found among treatments in this study, but no obvious trend was evident. Exposure to the tested temperatures apparently has no influence on subsequent hatching within the examined periods.

The effect of soil moisture on the hatching of *H. glycines* eggs is shown in Figure 3 for selected data. The mean rate of hatching increased by 75% as the moisture level was raised from 15% to 25%. Further increases in the water content to 33% caused a decline in hatching so that the rate was similar to dry soil and also to the water-only control. The 20% and 30% moisture conditions gave hatching rates that were intermediate to those of Figure 3. Based on these results, the eggs of *H. glycines* hatched maximally at a moisture level of 25%. However, the soil composition cannot be eliminated as a source of possible interaction.



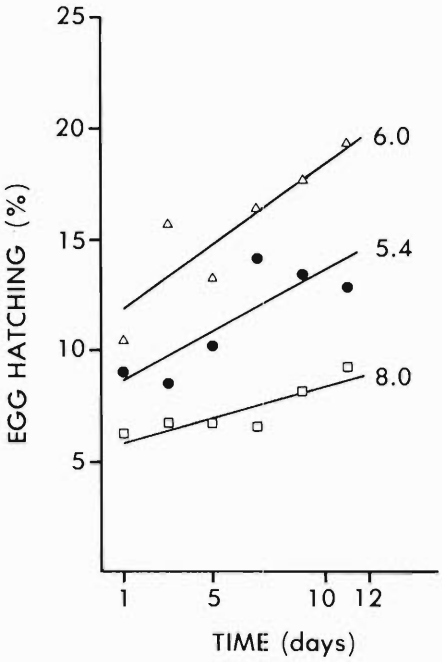


Figure 3. Rate of egg hatching of *Heterodera glycines* in sterile soil at 15, 25, and 33% moisture levels (r = 0.82, 0.95, and 0.90, respectively).

Figure 4. Rate of egg hatching of *Heterodera glycines* at the pH values of 5.4, 6, and 8 (r = 0.84, 0.88, and 0.85, respectively).

The pH of the incubation solution also influenced the rate of egg hatching in the soybean cyst nematode (Fig. 4). When the pH was elevated from 5.4 to 6.0, the mean rate of hatching increased 50%. However, further elevation of the pH over 6.0 eliminated the increase and caused a 100% reduction in hatching at pH 8.0. Based on the tested pH values, optimal hatching responses are elicited by slightly acidic conditions around pH 6.0. The hatching rates at pH 6.7 and 7.2 were less than at pH 6.0, but greater than the pH 8.0 results.

The effect of soybean root leachate on hatching of *H. glycines* eggs is shown in Figure 5. The mean rate of hatching increased linearly as the concentration of root leachate became greater (r = 0.89). The 100% leachate solution resulted in a hatching rate of 12.09%, or a 62% increase over the 7.48% rate that was found in the distilled-water control. The hatching response to the 100% leachate was different from that caused by water or 25% leachate, but not from other dilutions. Stronger concentrations of root leachate may cause even greater rates of hatching, but were not tested in this preliminary study.

Hoagland's solution showed no significant effect on egg hatching at lower concentrations when compared to the control (Fig. 6). However, hatching of *H. glycines* eggs in 100% Hoagland's solution was less than in the water control and all other tested concentrations. At that concentration, the hatching response of *H. glycines* was only 39% of the mean of all other solutions. A more marked reduction may result with a more concentrated solution. However, those levels are not reached in plant cultivation due to dilution by watering.

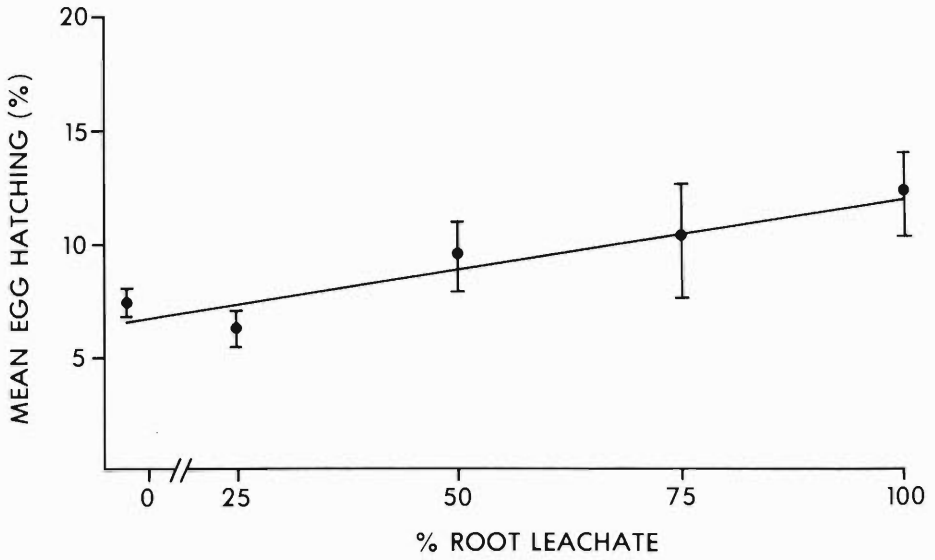


Figure 5. Mean percentage of egg hatching of *Heterodera glycines* during 2 wk in various concentrations of soybean root leachate (MSE = 1.41).

The influences of selected ions on egg hatching were varied. Exposure of *H. glycines* eggs to 0.85 M sodium chloride for 2 wk revealed no effect on the mean egg hatch or the rate of hatching. Likewise, a maximal concentration of calcium chloride exhibited no clear trend, and alteration of the sodium:potassium ratio revealed no clear dosage dependency. Based on the above results, sodium, calcium, potassium, and chloride ions do not alter egg hatching within the tested ranges. Any osmotic pressures within the above ranges also appear negligible, as

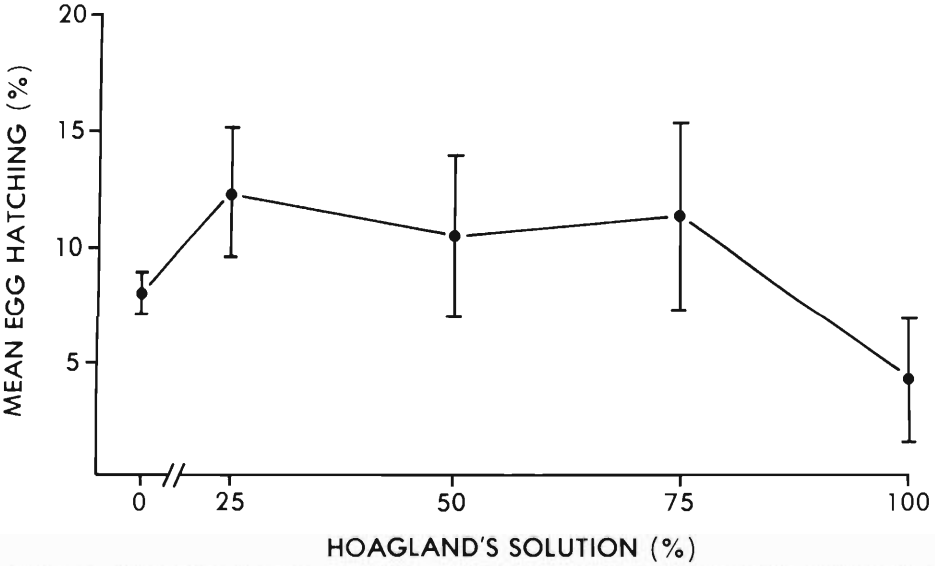


Figure 6. Mean percentage of egg hatching of *Heterodera glycines* in the indicated concentrations of Hoagland's solution (MSE = 2.99).

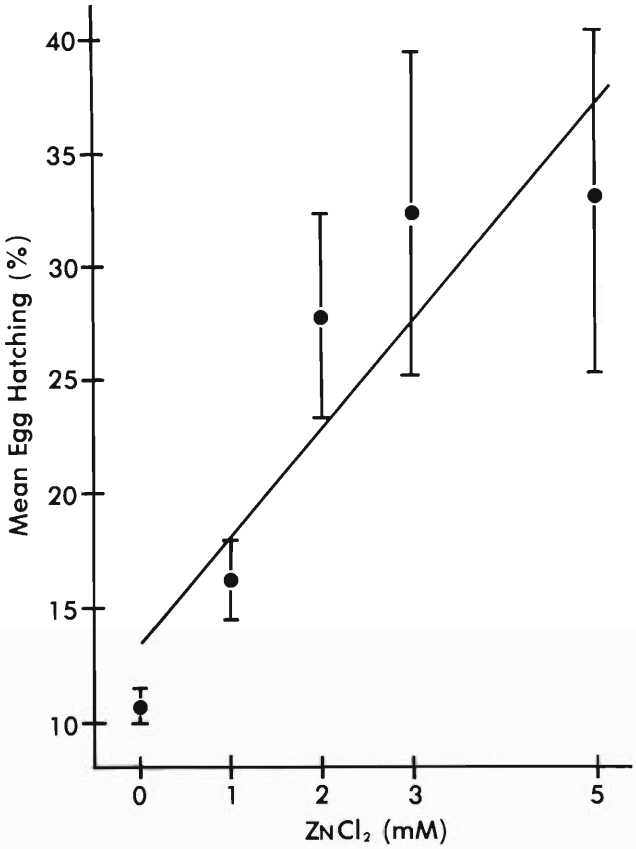


Figure 7. Mean percentage of egg hatching during 2 wk in various concentrations of zinc chloride (MSE = 4.37).

evidenced by no hatching effects with sodium chloride. These ionic studies also represented a positive control for the pH results.

The nitrate and ferric ion concentrations that were used had no significant effect on the mean percentage or rate of egg hatching, although a trend of increased hatching was evident. Phosphate at the greatest concentration caused an insignificant decrease in the mean hatching. In contrast, zinc had a pronounced influence on egg hatching (Fig. 7). The mean percentage of egg hatching increased linearly with metal concentrations. The hatching in 5 mM zinc chloride was threefold greater than in the water control.

Discussion

The hatching of *H. glycines* eggs is influenced by a number of factors with both stimulatory and inhibitory results. This study confirms the optimal hatching temperature of 24°C for free eggs, as found by Slack and Hamblen (1961) for encysted eggs. Pre-exposure of eggs to freezing temperatures failed to induce any refractoriness to the optimal temperature for hatching. Different results might be obtained after more prolonged periods of cold.

In addition to an optimal temperature, egg hatching of *H. glycines* appears maximal at pH 6 and at certain moisture levels. Based on indirect results, osmotic pressure apparently has only minor influences within natural ranges.

Heterodera glycines eggs increase their hatching in root exudates as do other *Heterodera* (Clarke and Shepherd, 1966). We suggest that the literature differences probably result from nonstandardization of concentrations and absence of dosage analysis.

Some 5–10% of the eggs used in this study hatched in a 2-wk period. This range is within that reported by a number of investigators. Combination of selected environmental parameters and plant exudates might provide greatly enhanced hatching rates and laboratory cultivation of *H. glycines*.

Rogers and Brooks (1976) suggested that exsheathment of the zooparasitic nematode *Haemonchus contortus* involved leucine aminopeptidase, a probable zinc metallo-enzyme. The enhanced hatching of eggs in micromolar zinc concentrations indicates that a similar enzyme may be present in *H. glycines*. Environmental levels of zinc may be partially responsible for the degree of soybean loss to cyst nematode pathology. Continued study of hatching conditions may enable prediction of population levels and potential economic loss so that control practices may be rationally applied.

Acknowledgments

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Biology of the Marine Piscicolid Leech *Johanssonia arctica* (Johansson) from Newfoundland

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ABSTRACT: In Newfoundland, the piscicolid leech *Johanssonia arctica* feeds on the blood of several species of marine fishes and uses the crab *Chionoecetes opilio* as a substrate for cocoon deposition. It lives at −1–2°C, takes eight to nine blood meals, deposits about 62 cocoons in its lifetime, which is about 2½ yr, and achieves a maximum length of 4 cm. Amount of blood ingested varies from two to four times its body weight. Time for digestion of a blood meal (47–105 days) is dependent on the size of the leech and the quantity of blood ingested. Some individuals can fast for at least 1 yr after repletion. Cocoons hatch in 176–253 days, but emergence of the young is dependent on temperature. Although newly emerged leeches occur at all times of the year, maximum abundance coincides with the onshore migration of fish in late spring. Winter might represent a period of starvation. Although the leech feeds on a broad spectrum of hosts, certain fish species such as Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*), are preferred to others. Following the fourth and subsequent blood meals, leeches attach to recently molted crabs strictly for deposition of cocoons. Location of fish hosts is substantially increased by the foraging activity of the crab. Several behavioral and physiological factors are responsible for the success of this sanguivorous leech.

Johanssonia arctica is a marine piscicolid leech that has been commonly encountered in Arctic seas (Johansson, 1899; Selensky, 1915; Moore and Meyer, 1951; Epshtein, 1961, 1962) and more recently from the northwestern Atlantic coast of North America (Meyer and Khan, 1979). It occurs commonly on the spider crab, *Chionoecetes opilio* (O. Fabricius), rarely on the toad crab, *Hyas coarctatus* Leach, and occasionally on marine fishes on which it is known to feed (Khan, 1976, 1977). Consequently, a study was initiated to determine its distribution and life history.

Materials and Methods

Field observations

Specimens of *J. arctica* were obtained from spider crabs caught in Japanese conical traps and gill nets set at depths of 150–320 m and 5–70 km offshore in the Newfoundland area. Bottom temperatures varied from −1 to 3°C. Occasionally, crabs were trapped in Placentia, Fortune, St. Mary's, Conception, Bonavista, and White bays, but monthly samples were obtained from areas off the Avalon Peninsula. Observations from 1972 through 1981 were recorded either on board longliners or after the crabs were returned to the laboratory. Observations included the condition and width of the carapace, as well as numbers, length, and distribution of leeches and viable cocoons. Also recorded were the presence of young leeches (6–8 mm) and the feeding state of all leeches.

Laboratory observations

Leeches were relaxed in either MS222 or 5–15% cold ethanol in seawater before being measured (mm). Leeches of equal length were routinely weighed in groups of 10 prior to and after engorgement (within 6–12 hr after the blood meal). They were dried on absorbent paper prior to weight determination.

Rates of cocoon deposition and periods of incubation, growth, and digestion of blood meals were determined mostly at 0–1°C and occasionally at −1–0 and −1–4°C. Cocoon deposition was recorded at about 5-day intervals. Mean cocoon deposition per leech was based on dead and surviving leeches held individually or in groups in 500-ml glass jars filled with the seawater that was changed about twice weekly. Mortality among these leeches was variable (5–50%).

Leeches that fed on fish used in host-preference studies were collected 24 hr after exposure to various piscine hosts held in tanks 1.3 m in diameter × 0.9 m or 0.7 × 0.7 × 0.2 m. To identify the origin of their blood meal, specific antisera were prepared against the various fish species by inoculating 0.5 ml of whole blood intravenously into rabbits at weekly intervals for 4 wk. Rabbits were then bled (10 ml at each bleeding) for antisera. The highest dilution of antigen to give a positive reaction was used. The immunodiffusion method used for detection of specific antisera was performed on agar-covered glass slides. A central trough 65 × 3 mm was made with 11 wells (3-mm diameter, 5 mm apart on either side). Antiserum was introduced into the trough, specific antigen (whole blood) into uneven-numbered wells and the blood meals, taken from leeches that fed on the test fish, in even-numbered wells. Slides were incubated in a humid chamber at 5°C for 24–48 hr, washed 24 hr in saline (0.14 M NaCl), and dried overnight under a layer of filter paper. The slides were then immersed in 5% acetic acid, the filter paper was removed, the slides were stained with amido black for 30 min, and the excess stain was removed by immersion in acetic acid (5%). Precipitation reactions were then read as arcs of identity, partial identity, and distinctiveness. No antisera prepared against the various fish species formed arcs of identity. This procedure has been used previously to identify food of ectoparasites (Boreham and Gill, 1973) and predators (Davies et al., 1978).

R. J. Miller (pers. comm.) classified spider crabs on the basis of shell hardness and color. Stage one crabs have soft red shells; in stage two animals the shell is hard, with iridescence on the tips of the claws; stage three crabs have hard shells with no barnacles or iridescence on the claws; and in stage four the shell appears to be degenerating and is invariably encrusted with barnacles. Although this scheme appears somewhat arbitrary, expecially between stages two and three, after some practice an observer finds little difficulty in distinguishing the various stages.

Results

Field observations

Johanssonia arctica was encountered on the male spider crab (*C. opilio*) and occasionally on the toad crab (*H. coarctatus*), taken more often in baited Japanese crab traps than in gill nets, from depths of 160–310 m (water temperature −1–0°C) about 5–70 km off the Avalon Peninsula, Newfoundland (Miller, 1975). Leeches were attached mainly to recently molted crabs (soft-shelled), especially on the ventral femoral surface of the first and second pairs of walking legs (Table 1; Fig. 1). Leeches also occurred in smaller numbers on the third and fourth pairs of walking legs, and occasionally on the claws, but less often on the dorsal and ventral surfaces of the body. Fewer leeches were observed on crabs with darkened and hardened shells, especially when encrusting barnacles were large and abundant. The total number of leeches collected from a single crab varied con-

Table 1. Distribution (mean ± standard deviation) of the leech *Johanssonia arctica on the claws (CL) and legs (1 to 4) of the spider crab, *Chionoecetes opilio*, at different stages of carapace hardness.†**

Stage	No. examined	CL	1	2	3	4
Right appendages						
2	120	1.0 ± 0.5	3.0 ± 2.2	2.6 ± 2.3	1.3 ± 0.8	0
3	125	1.2 ± 0.5	4.6 ± 3.3	4.4 ± 2.2	1.2 ± 0.1	0.1
4	120	1.0 ± 0.7	1.5 ± 0.4	2.5 ± 2.1	2.0 ± 1.9	0
Left appendages						
2	120	1.2 ± 0.5	2.9 ± 2.1	1.8 ± 1.2	1.4 ± 1.1	0
3	125	0.6 ± 0.4	4.5 ± 4.1	4.6 ± 2.2	1.2 ± 0.5	0.1
4	120	0	2.0 ± 1.0	2.3 ± 1.5	2.0 ± 1.8	0

* Samples taken monthly over 1 year (1974–1975) about 5 km off St. John's, Newfoundland.
† See text for stage description.

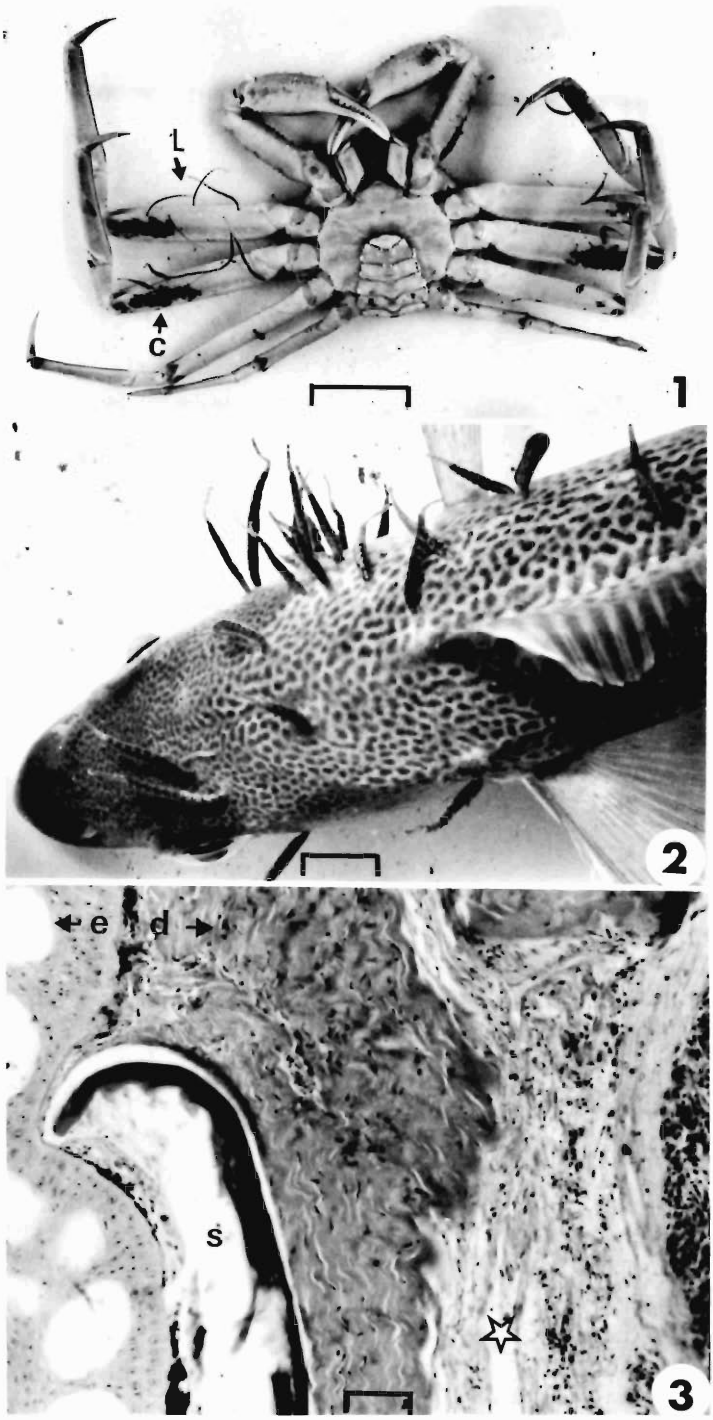
siderably, from zero to over 100. As many as 137 leeches were collected from a crab taken off St. John's in 1976. Many more leeches were collected during the months of May–December than at other times of the year. For example, the mean number of leeches collected from 50 crabs (all stages inclusive) in November ($\bar{x}$ = 8) was greater than that for February ($\bar{x}$ = 5). The leeches deposited their cocoons primarily on the ventral femoral surfaces of the first and second pairs of legs (Table 2). Cocoons occurred less often on the other joints, and were rare on the claws, carapace, and ventral surface of the body. Viable cocoons, golden-brown in color, were more abundant on recently molted crabs than on hard-shelled crabs encrusted with barnacles. Moreover, the total number of cocoons varied considerably, from none to as many as 1,246 on a crab 12 cm in carapace width. Cocoons tend to darken with age, and after emergence of the young remained on the crab up to 1 yr before dropping off. Crabs with encrusted shells and with varying numbers of empty cocoons were collected at all times of the year.

The number of cocoons deposited by a leech after each blood meal was variable. Thirteen wild-caught leeches (25–40 mm), when held in the laboratory in-

Table 2. Distribution (mean ± standard deviation) of cocoons of *Johanssonia arctica on the claws (CL) and legs (1 to 4) of the spider crab, *Chionoecetes opilio*, at different stages of carapace hardness.†**

Stage	No. examined	CL	1	2	3	4
Right appendages						
2	151	0.2 ± 0.1	8.9 ± 3.1	17.7 ± 9.6	3.2 ± 2.1	0.1 ± 0.1
3	180	0.8 ± 0.4	23.8 ± 14.2	35.3 ± 17.1	9.4 ± 4.7	0.3 ± 0.1
4‡	255	0.3 ± 0.2	15.9 ± 9.6	31.9 ± 11.8	8.3 ± 6.2	0.4 ± 0.3
Left appendages						
2	151	0.4 ± 0.2	8.1 ± 4.7	15.2 ± 10.1	1.7 ± 1.2	0.1 ± 0.1
3	180	0.7 ± 0.5	25.4 ± 17.1	34.5 ± 16.8	8.9 ± 3.9	0.3 ± 0.2
4‡	255	0.4 ± 0.2	14.4 ± 12.3	31.3 ± 12.3	7.7 ± 6.4	0.4 ± 0.2

* Samples taken monthly over 1 year (1974–1975) about 5 km off St. John's Newfoundland.
† See text for stage description.
‡ More than 70% were empty.



Figures 1–3. 1. Ventral aspect of spider crab with leeches (l) and cocoons (c). Note latter on distal femoral surfaces. Scale bar = 5 cm. 2. Atlantic cod with engorged leeches. Scale bar = 2 cm. 3. Subcutaneous hemorrhage (*) in the skin of Atlantic cod. d, dermis; e, epidermis; s, scale. Scale bar = 10 μm.

dividually, deposited an average of 13.5 (range 7–36) cocoons after a blood meal. The digestive tracts of these leeches were bright red in color, suggestive of recent engorgement. Because cocoon deposition rarely occurred prior to darkening of the blood meal, possibly the above-mentioned leeches did not produce cocoons prior to their retention. Seven of these 13 lived over a year at 0–1°C without additional blood meals.

Young leeches (6–8 mm) were observed throughout the year, but were most abundant from May through October on crabs with carapaces recently hardened after molting. Because the incubation period of cocoons appeared to be about 253 days (to be discussed later) and the molting period about 3 mo, possibly the small leeches emerged on older crabs and subsequently migrated to recently molted animals. It was observed, moreover, that recently emerged leeches wandered from the site of emergence on one leg and relocated on other joints, but less often on the carapace and ventral surfaces of the body.

Laboratory observations

HOST PREFERENCES: Leeches were collected naturally from an Atlantic cod (*Gadus morhua*), a polka-dot seasnail (*Liparis cyclostigma*), and three American plaice (*Hippoglossoides platessoides*), caught in gill nets 5–12 km off St. John's, Newfoundland. The leeches were all bright red in color, and examination of the stomach contents in saline revealed erythrocytes of fish. This observation led to the belief that *J. arctica* might feed on piscine hosts. Consequently, a number of fish were exposed to groups of assorted sizes (8–30 mm) of fasted leeches (Fig. 2). Immunodiffusion studies indicated in the first trial that the leeches fed more often on American plaice (51% [44%] of 82) and Atlantic cod (35%) than on striped wolffish, *Anarhichas lupus* (9%), Laval's eelpout, *Lycodes lavalaei* (2%), or thorny skate, *Raja radiata* (1%). In the second trial, Atlantic cod (74% [60] of 81) was preferred to striped wolffish (13%), longhorn sculpin, *Myoxocephalus octodecemspinosus* (5%), winter flounder, *Pseudopleuronectes americanus* (4%), or oceanpout, *Macrozoarces americanus* (4%). In the third trial, 80% (52) of the leeches fed on Atlantic cod in preference to longhorn sculpin (11%), winter flounder (6%), and thorn skate (3%). It appeared, then, that the leeches fed more often on American plaice and Atlantic cod than on other species of fish. When American plaice and Atlantic cod were placed in aquaria 0.9 m in depth, the leeches (8–30 mm) fed preferentially on plaice (67% of 372). Cod tend to swim close to the surface and were probably unavailable to leeches at the bottom of the tank. However, when the fish were placed in a shallow aquarium (0.2-m depth), more leeches fed preferentially on Atlantic cod (59% of 369) than on American plaice (41%).

These preliminary observations suggested that some hosts were preferred to others. Evidence in support of this view was obtained by exposing various species of fish individually to fasted leeches. The results of these studies indicated that *J. arctica* fed readily on fish such as Atlantic cod, American plaice, etc. (Table 3). A number of fish species were utilized only after prolonged exposure, lasting up to 8 days, and the leeches did not feed on some other fish species.

Fasted leeches displayed behavioral characteristics typical of other Hirudinea. When placed in aquarium with fish, they floated at the surface of the water or swam actively by undulating motions and attached to their hosts. When the water

Table 3. Host* preferences of the marine leech *Johanssonia arctica* based on exposure of individual fish to fasted leeches (in groups of 5–10). At least 6 exposures of each individual species were made.

Hosts fed on readily	Hosts† fed on less readily	Hosts not fed on
Gadus morhua	*Anarhichas lupus*	*Agonus decagonus*
Hippoglossoides platessoides	*Pseudopleuronectes americanus*	*Stichaeus punctatus*
Lycodes lavalaei	*Macrozaorces americanus*	*Triglops murrayi*
Lycodes vahlii	*Limanda ferruginea*	*Cyclopterus lumpus*
Salmo salar	*Raja radiata*	*Gasterosteus aculeatus*
Salmo trutta	*Anguilla rostrata*	*Tautogolabrus adspersus*
Microgadus tomcod	*Hemitripterus americanus*	*Lumpenus lumpretaeformis*
Reinhardtius hippoglossoides	*Myoxocephalus octodecemspinosus*	*Raja ocellata*
Glyptocephalus cynoglossus	*Myoxocephalus scorpius*	
Melanogrammus aeglefinus	*Anarhichas minor*	
	Anarhichas denticulatus	

* Names from Leim and Scott (1966).
† Exposure periods lasted up to 8 days to achieve 20–100% feeding.

current was swift, they generally sought out their hosts by looping over the substratum. They attached to fish with their anterior suckers and fed subsequently with both suckers attached. They appeared to select feeding sites on the host at random, but in some species (such as cod) the head region was preferred, whereas in flatfishes (such as American plaice) feeding occurred on areas adjacent to the fins. Fifty-three percent (310) of 590 leeches (15–30 mm) attached and fed on the heads of 10 cod, in contrast to 17% on the trunk and 30% on the caudal region. Fewer leeches fed on the ventral, lighter surfaces. Additionally, 60% (132) of the leeches (15–30 mm) fed on the dorsal and ventral fins of four American plaice, whereas 26% (58) and 14% (34) fed on the head and tail, respectively. Preferences of the newly emerged leeches (8 mm) appeared similar. Among juvenile winter flounder (12–22 cm), 80% of the leeches (44) fed on the head, in contrast to 20% on the rest of the body. Leeches fed more rapidly in the dark. Ninety percent (104) of 130 leeches fed in the dark to repletion within 6 hr after attachment, in contrast to 58% (65) of 112 in the presence of light after the same time.

Following engorgement of leeches, subcutaneous hematomas were observed in host fish. Histological examination of skin from the feeding site revealed damage to the epidermal cells, including the malpighian layer, and subsequent infiltration with erythrocytes (Fig. 3). The dermis, which normally contains fibrous tissue, showed evidence of edema, hemorrhages, and ruptured blood vessels. Vasodilation of intact blood vessels was also apparent. However, hematomas disappeared after 8–10 days, and by this time both the epidermis and dermis returned to their normal architecture.

QUANTITY OF BLOOD INGESTED: Leeches fed within 3–6 hr after attachment to their hosts. The amount of blood ingested by the leech was dependent on its size; small individuals generally ingested less blood than larger ones (Fig. 4). Leeches of all sizes ingested more than twice their body weight, but among juveniles (7 mm) it was about three times. Large (>30 mm) engorged leeches, taken from the field, were observed with bodies constricted into two or more regions. The bulges were stomach ceca into which abnormal quantities of blood

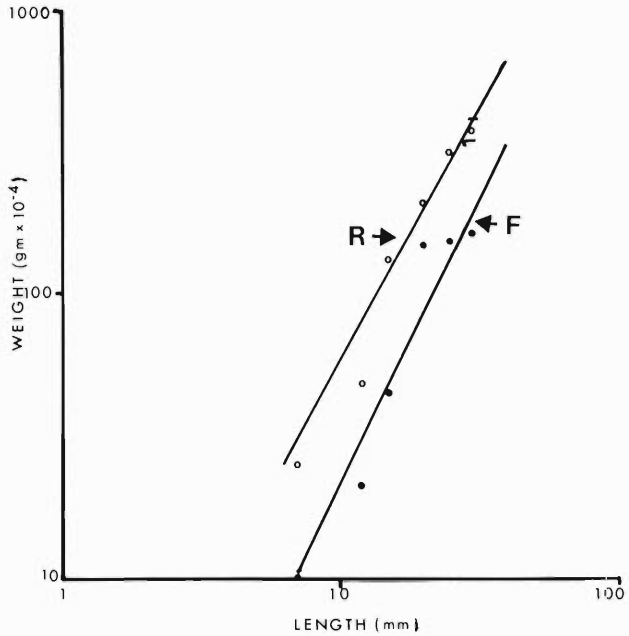


Figure 4. Relationship (log-log) between leech length (mm), mean weight (g), and weight of blood ingested. F, fasted; R, replete.

were ingested. Blood in these leeches was estimated to be about three to four times their body weight.

TIME FOR BLOOD DIGESTION: The time required for digestion of a blood meal was dependent on the size of the leech (or quantity of blood ingested) and on the hemoglobin level of the host. Digestion was completed in a shorter time (39–47 days) in small (6–8 mm) leeches than in leeches of intermediate sizes (12–15 mm; 54–69 days) or in larger ones (20–40 mm), in which digestion was accomplished in about twice the time (>85 days). The rate of digestion varied among leeches (25–40 mm) that fed on cod and other hosts (Table 4). Digestion time was shorter in animals that fed on Laval's eelpouts than in those that fed on Atlantic cod (one instance), longhorn sculpin, or winter flounder. Leeches that fed on anemic Arctic eelpouts, moreover, digested their blood meal in a shorter time (65–75 days) than those that fed on normal animals (80 or more days).

COCOON DEPOSITION AND HATCHING: Following engorgement, leeches detached from their piscine hosts and reattached by looping shortly thereafter to the crab *C. opilio* in preference to inanimate objects (walls of the aquarium, stones, mussel or scallop shells). Twenty-seven of 44 engorged leeches attached within a minute to a crab (carapace width 8 cm) after it was placed in an aquarium (48 × 30 × 25 cm). The remaining leeches followed within an hour. Fasted leeches behaved similarly, as all of 46 attached to the crab within 1 hr. The stimulus responsible for this attraction is unknown, but better aeration, a site for cocoon deposition, and subsequent dispersal of the young leeches might be the underlying advantages.

Engorged leeches first attached in a random manner on the crab, but within 48

Table 4. Percent digestion of a blood meal at 0–1°C in *Johanssonia arctica following engorgement on different hosts.**

Host	Total fed	Days after ingestion (% digested)						
		41	57	65	75	85	92	105
Laval's eelpout	130	3	51	75	94	100	—	—
Laval's eelpout†	70	—	94	100	—	—	—	—
Laval's eelpout	60	—	27	47	91	100	—	—
Laval's eelpout†	56	—	77	82	100	—	—	—
Atlantic cod	62	—	21	57	84	100	—	—
Atlantic cod	41	—	18	24	40	66	83	100
Atlantic cod	47	—	32	67	95	—	—	—
Winter flounder	72	—	16	28	40	50	55	80
Longhorn sculpin	90	—	38	61	78	90	97	100
Shorthorn sculpin	116	—	—	—	—	90	100	—

* All leeches were 25–40 mm in length.
† Animal anemic.

hr they relocated on the ventral femoral surfaces of the legs, especially the first and second pairs, where they remained and deposited cocoons. Eighty percent (166) of 208 leeches that were exposed to two crabs attached to first and second pairs of legs. There appeared to be no difference between the right and left sides. When the first or second pair of legs was absent, the third pair was used as a substitute. Leeches, moreover, attached more readily to the legs of crabs that had recently molted than to those with encrusted shells. Of 210 engorged leeches placed in an aquarium with crabs, 57% (124) attached to a crab with a recently hardened shell (stage three), 46% (96) to a crab with a soft shell (stage two), and 5% (14) to a crab with a dark and encrusted integument (stage four). A repetition with 178 different leeches resulted in 67% (120) attaching to the stage three crab, 26% (46) to the stage two crab, and 7% (12) to the stage four crab. It would appear then that the recently hardened integument, following a molt, was selected for cocoon deposition rather than the "soft" shell or crabs with dark ventral surfaces and encrusted shells. Mating occurred on the crab, and involved an exchange of spermatophores.

Cocoons, deposited by laboratory-reared leeches 8–15 days after the blood meal, occurred primarily on the femurs of the first and second pairs of walking legs. Ninety-nine percent (108) of 109 cocoons deposited by 23 leeches 29 days after engorgement were observed on the first and second pairs of legs of a spider crab. At 62 days, 94% (213) of 227 cocoons were present on the first and second pairs of legs, and the remainder were on the third and fourth pairs and on the carapace. None was observed on the claws or on the ventral aspect of the body, despite the large surface area. The incubation period was temperature dependent. When cocoons, deposited on the sides of 500-ml jars, were held at the ambient temperature, −1–12°C, young emerged 143 days after deposition. However, when cocoons were placed at 0–1°C, emergence of the young varied from 176 to 209 days. If held at −1–0°C, a temperature at which both crabs and leeches live, the incubation period increased to 253 days. Each cocoon contained one egg.

GROWTH OF THE LEECH: Young leeches fed on piscine hosts within a week after emergence, but some survived for 4 or more weeks without feeding. Al-

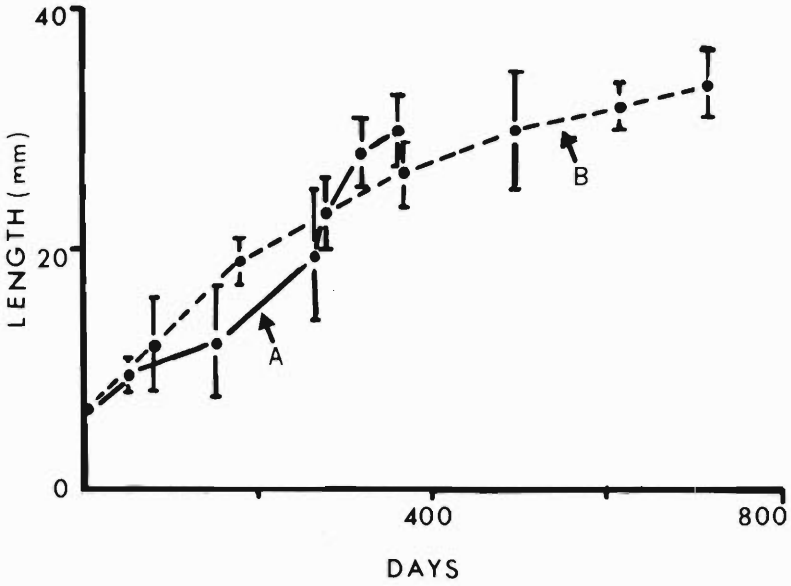


Figure 5. Rate of growth of *Johanssonia arctica* held at 0–5°C (A) and 0–1°C (B).

though they exhibited host preferences, the young fed occasionally on species, such as thorny skates, that were rejected by larger leeches. In a laboratory population that emerged in 1974, the leeches were placed in an aquarium together with a crab and three winter flounder, on which they fed periodically. The water temperature was maintained at 0–1°C for 175 days and subsequently at ambient, which varied from −1 to 4°C. Some were alive for almost 1 yr (Fig. 5A), but all died by June as the water temperature (4.5°C) rose.

Because it was not ascertained that mortality of the leeches was attributed to age or water temperature, a second laboratory population was started in 1975 (Fig. 5B), but the leeches were maintained at 0–1°C continuously. Growth was slightly slower, but the leeches lived 2 or more yr. Cocoon deposition occurred after the third blood meal, but only 11 of 48 leeches deposited cocoons. After the fourth blood meal, however, all leeches deposited cocoons. Fourteen to 18 cocoons were produced by each leech. Following the fifth to the eighth blood meals, the number of cocoons deposited varied from 2 to 26. At this time, the leeches were at least 2 yr old and deposited an average of 15.6 cocoons per clutch. A mean of 62 (49–66) cocoons was produced during the animal's reproductive period.

The effect of temperature and salinity

To ascertain the effect of temperature and salinity on survival of *J. arctica*, groups of 20 fasted leeches were held in 500-ml jars at 0, 5, 10, and 15°C at seawater concentrations of 24.3 to 32.4‰ salinity. All leeches held at 5–15°C died within 14 days, whereas those held at 0°C were alive after this time. Additionally, 75% of 612, both fasted and replete leeches, died within 16 days following removal from 0 to 5°C (32.0‰ salinity). Fasted leeches rarely fed at temperatures above

5°C, and were generally less active. In the laboratory, leeches were routinely reared in seawater of variable salinity (30.8–32.7‰) at 0°C. These observations suggest that the animals could survive varying concentrations of salinity provided the temperature is not changed drastically.

Discussion

The occurrence of *J. arctica* is associated with that of the spider crab, *C. opilio*, which is widely distributed on the continental shelf off Newfoundland (Miller, 1975). Temperature has a considerable influence on its distribution. Other factors include food, depth, and type of substratum. Most of this northern shelf area, where the crab is abundant, is under the influence of the Labrador Current (Sutcliffe et al., 1977), which is characterized by low temperature (<0°C) and high salinity (>32‰), especially in the benthic zone. High osmolarity ($\bar{x}$ 900 ± 100 mosmol/liter) in the body fluids of both crab (blood) and leech is associated with low temperature (Khan, unpubl. data). Large adult males prefer muddy bottoms, whereas small males and females are also found in rock outcrops. The crab has variable food habits, but prefers seastars, clams, and other invertebrates. It molts once each year from June to September, and the shell hardens within 3 mo. Males mature later than females. They remain in mud during the molt, possibly to avoid predators. Their attraction to gill nets, in which large numbers are caught, might be the result of a stimulus initiated by blood oozing from captured fish. Unpublished data of the Newfoundland Biological Station indicate that concentrations of male crabs occur off the coast of Labrador especially in the vicinity of the Hawke Channel and on the northeast coast of Newfoundland. Lower concentrations occur on the south and west coasts of Newfoundland and northern Gulf of St. Lawrence. Offshore distribution, with the exception of the Labrador region, appears sparse, and few specimens have been taken in the northern Grand Bank and none have been reported from the southern Grand or St. Pierre banks. The distribution of *J. arctica* parallels that of *C. opilio*. Few leeches occur on crabs taken from Placentia Bay and the northern Gulf of St. Lawrence, but they are numerous on crabs from Hawke Channel, Labrador, and along the north and east coasts off Newfoundland (Khan, unpubl. data). Leeches are also more abundant on crabs taken at 8 km than at 70 km offshore from St. John's.

The life cycle of *J. arctica* is reconstructed on the basis of field and laboratory observations. Adult, juvenile, and embryonic stages of *J. arctica* occur throughout the year on the spider crab, *C. opilio*. This observation suggests an asynchronous life cycle, which is not surprising, because the benthic environment of the leech apparently is a stable one. However, a greater number of small leeches (6–8 mm) occur on crabs from May through August than during other months of the year. During spring, some groundfish species, especially American plaice, move closer inshore, where the crabs occur and where young and fasted leeches are able to obtain blood meals. Blood is obtained probably by palpating the anterior sucker and extending a protrusible proboscis that secretes proteolytic enzymes. Large numbers of crabs with engorged leeches are caught in gill nets from spring to early summer. Moreover, bright red blood in these leeches is indicative of recent engorgement. In late spring–early summer, the capelin migrate inshore to spawn and are pursued by Atlantic cod and other species of fish.

Thus, the leeches have a broader selection of hosts on which to feed. Coincidentally, the crabs commence their molt 5–70 km along the northeast and east coasts of Newfoundland, where 50–70% of a 3,000-kg catch is recently molted crabs. The majority of crabs molt from July through September, and it takes about 2–3 mo for the integument to harden. Leeches attach preferentially to recently molted crabs. They mate by exchanging spermatophores while attached to the crab. Cocoons are deposited from September onward on the first and second pairs of walking legs. Because the incubation period is about 8 mo, the peak period for emergence of young occurs from May through August. Following digestion of each blood meal, leeches feed two to three times during the season when several fish species are inshore. After offshore migration of the fish and during the months of December to March, most of the leeches will have digested their blood meal. They then live, some without feeding, until the following spring, when they encounter fish. The leeches will feed once more and deposit cocoons, usually after their fourth blood meal, on crabs. About 15.2 cocoons are deposited after each blood meal, for a total of about 62 per leech. Many of them possibly die at the end of their second year (i.e., after about eight to nine blood meals). *Johanssonia arctica* is therefore unique in that it is the only marine leech currently known to live more than 1 yr (Llewellyn, 1965; Gibson and Tong, 1969; Daniels and Sawyer, 1975; Sawyer et al., 1975; Khan and Meyer, 1976). Other species are annuals that die after breeding in spring and produce fewer offspring (Khan and Meyer, 1976, 1978).

Associations between decapod crustaceans and leeches have been reported previously and are summarized by Meyer and Barden (1955). *Carcinobdella kanibir* occurs on the spider crab (*C. opilio*) off Japan (Oka, 1927). *Notostomobdella cyclostoma* was collected from the king crab (*Paralithodes camtschatica*) on which it deposited its cocoons (Johansson, 1899), and Moore and Meyer (1951) reported specimens from skates (*Raja* sp.) taken in Alaskan waters. Specimens of *Platybdella olriki,* originally described infesting another crab (*Hyas araneus*) and subsequently *Sclerocrangon boreas* (=*Crangon boreas*), is now suspected to feed on marine fish (Wesenberg-Lund, 1926; Meyer and Khan, 1979). *Myzobdella lugubris* was initially described from specimens taken from the blue crab (*Callinectes sapidus*) and later from oysters (*Crassostrea* sp.) and a prawn (*Palaemonetes vulgaris*) in United States waters (Meyer and Barden, 1955). Recently, Daniels and Sawyer (1975) demonstrated that association between *M. lugubris* and the crab was a phoretic one. The leech feeds on the catfish (*Ictalurus catus*) and uses its crustacean host primarily for cocoon deposition. *Notostomobdella laeve,* originally described as a parasite of fish of the Greenland seas, was subsequently reported from crabs in the same area (Wesenberg-Lund, 1926). It would appear, therefore, from the examples cited, that the association between leeches and arthropod invertebrates is primarily as a substratum for cocoon deposition and secondarily for transport. The nutritional relationship, reported previously, is therefore somewhat dubious, although Burreson and Allen (1978) provide evidence that *Mysidobdella borealis* feeds on the mysid *Neomysis americana.*

Studies in the Newfoundland area provide evidence that *J. arctica* is probably the main vector of blood protozoa, viz., a trypanosome, *Trypanosoma murmanensis* (Khan, 1976, 1977; Khan et al., 1980); a hemogregarine, *Haemogregarina uncinata* (Khan, 1978); a piroplasm, *Haemohormidium beckeri* (Khan, 1980); and

an undescribed trypanoplasm (Khan, unpubl. data). Its life span of 2 or more yr permits several blood meals. During spring through summer of each year many species of migratory fish move into coastal areas for feeding and reproductive purposes. The leeches then feed on a variety of fish depending on their availability. During this period, protozoan infections are acquired and/or transmitted. As a vector of hematozoa, therefore, *J. arctica* is more efficient than the leech species that remain permanently attached to their hosts (Khan and Meyer, 1976; Meyer and Khan, 1979). Consequently, some infections, e.g., trypanosomes, are not encountered in leeches with specific host preferences (Khan et al., 1980). In this respect, *J. arctica* is comparable to some hematophagous arthropods that feed intermittently on several mammalian hosts, such as tsetses (*Glossina* spp.) that transmit the *T. brucei* complex (Hoare, 1972).

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Parasites of Chinook Salmon (*Oncorhynchus tshawytscha*) and Coho Salmon (*O. kisutch*) from the Mad River and Vicinity, Humboldt County, California

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ABSTRACT: Of 84 chinook and 96 coho salmon necropsied from northern California, 97.8% harbored at least one of the 27 species of parasites recorded. *Bucephalopsis gracilescens, Plagioporus shawi, Derogenes varicus, Lampritrema miescheri, Rhabdochona* sp., *Rhadinorhynchus trachuri,* and *Lironeca vulgaris* are reported from new hosts, and *B. gracilescens, P. shawi, L. miescheri,* and *R. trachuri* are reported for the first time from California. The relationship between migrations of the host fish and their parasite faunas and potential use of parasites as biological tags is also discussed.

With the exception of Haderlie (1953) and a few short papers, little is known about parasite faunas of northern California chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*). Numerous publications, summarized by Margolis (1970a) and Margolis and Arthur (1979), have dealt with parasites of salmon in Canadian waters. Olson (1978) examined parasites of chinook and coho salmon taken off the Oregon coast. This study was conducted to provide information about parasite faunas of northern California salmon, to explore the possibility that parasites might be used as biological tags to indicate geographical areas of salmonid origin, and to obtain data on prevalence of any parasites that might be of potential public-health importance.

Materials and Methods

Three age categories of chinook and coho salmon were examined for parasites: Juvenile (young fish still inhabiting fresh water), immature (ocean-dwelling fish), and adult (sexually mature fish that have returned to fresh water). Juvenile fish were obtained by angling, netting, and electrofishing the tributary streams (North Fork of the Mad River, Lindsay Creek, and Maple Creek) and lower portions of the Mad River during the spring and fall of 1979 and 1980 (Fig. 1). Netting procedures were those of Taniguchi (1970). Immature fish were caught in the Pacific Ocean off of the coast of Humboldt County, California, during May 1979, from a rented charter boat. During the spring of 1980, gills, eyes, and viscera of immature fish were obtained from commercial salmon fishermen. Adult fish were obtained from the Mad River Hatchery, California Department of Fish and Game, Blue Lake, during the spawning season (late fall or early winter depending on rainfall).

Fish were placed on ice for transport to the laboratory and were usually necropsied within 24 hr. Specimens that could not be examined within 48 hr were frozen for later examination. The external surface, mouth, nasal cavities, gills, eyes, musculature, viscera, and mesenteries were examined for parasites. Standard helminthological techniques were used in preparing parasites for identifi-

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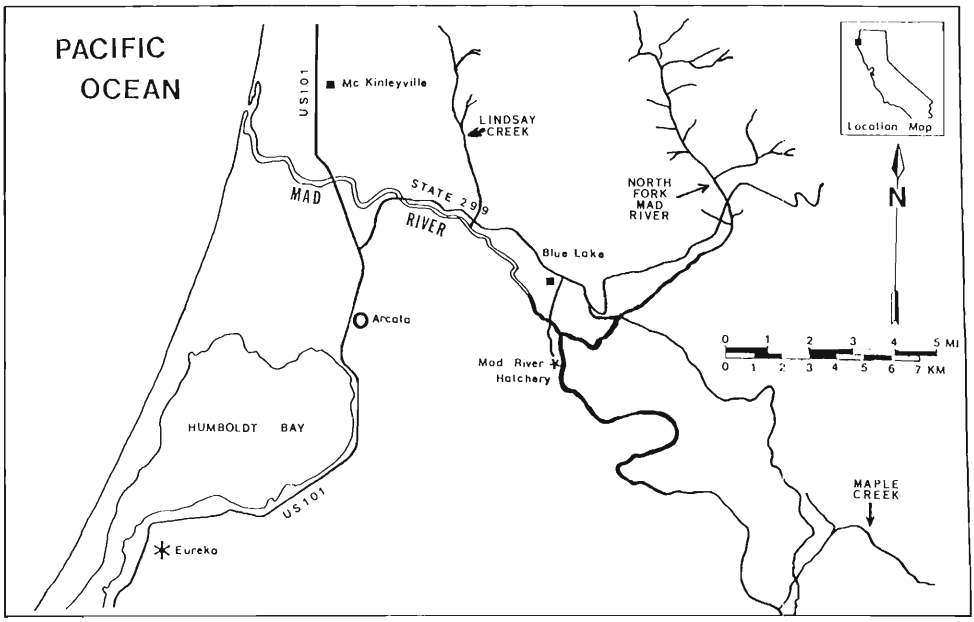

Figure 1. Map of the study area, showing the location of the Mad River and other sampling sites.

cation. Ecotypes of most specimens have been deposited in the United States National Museum Helminthological Collection. Accession numbers are listed in Tables 1 and 2. Remaining specimens have been retained in the collections of the authors.

Results

The 84 chinook and 96 coho salmon examined harbored 27 species of parasites (Tables 1, 2). A total of 97.8% of these fish were infected with at least one kind of parasite. Plerocercoids of the cestode *Phyllobothrium caudatum* and *Anisakis simplex* juveniles were the most common parasites encountered. Several juvenile helminths could not be specifically identified. The single larval Acarina from the intestine of a juvenile chinook salmon is included in this listing, even though it was probably a food item. However, it was alive when first examined. Eight parasites are reported from new hosts and four parasites are reported for the first time from California.

Discussion

Previous studies on parasites of anadromous salmonids have revealed several changes in the parasite fauna of the host fish during its lifetime (Dogiel, 1966; Dogiel et al., 1970). Each change is directly related to the migration of salmon from fresh water to the marine environment and vice versa. Margolis (1963, 1965), Boyce (1966, 1969), Pennell et al. (1973), and Olson (1978) have extensively documented this phenomenon in Pacific salmonids. The results of this study are in agreement with these authors.

Juvenile fish acquired a parasite fauna strictly of freshwater origin (*Plagioporus*

Table 1. Parasites, anatomic locations, incidence, and intensities in all groups of chinook salmon (4 juveniles, 19 immature, and 61 adults) collected from the Mad River and vicinity.

Parasite	USNM no.	No. of fish infected (%)				Mean no. per infected fish				Range in nos. per fish infection	Location of parasites
		Juv.	Imm.	Ad.	Total	Juv.	Imm.	Ad.	Total		
Protozoa											
Ichthyophthirius multifilis	—	—	—	6 (9.8)	6 (7.1)	—	—	*	*	*	Skin, gills
Trematoda											
Diplostomum spathaceum sensu lato (metacercaria)	76959	—	1 (5.3)	24 (39.3)	25 (29.8)	—	5.0	47.0	45.3		Eye lens, vitreous chamber
Bucephalopsis gracilescens†‡										2–167	
(adult)	76961	—	—	21 (34.4)	21 (26.2)	—	—	*	*	1–1,000's	Intestine
(metacercaria)	—	—	—	3 (4.9)	3 (3.6)	—	—	*	*	1–1,000's	Internal organs (cysts)
Nanophyetus salmincola (metacercaria)	—	—	—	1 (1.6)	1 (1.2)	—	—	20.0	20.0	20	Kidney (cysts)
Plagioporus shawi†‡	76964	4 (100.0)	—	—	4 (4.8)	8.8	—	—	8.8	4–15	Intestine
Derogenes varicus†	—	—	—	1 (1.6)	1 (1.6)	—	—	1.0	1.0	1	Stomach
Deropegus aspina	76967	2 (50.0)	—	1 (1.6)	3 (3.6)	1.0	—	2.0	1.3	1–2	Stomach
Hemiuris levinseni	76968	—	—	6 (9.8)	6 (7.1)	—	—	4.5	4.5	1–10	Stomach
Lecithaster gibbosus	76969	—	—	16 (26.2)	16 (19.0)	—	—	11.7	11.7	1–80	Intestine
Tubulovesicula lindbergi	76971	—	—	1 (1.6)	1 (1.2)	—	—	1.0	1.0	1	Stomach
Lampritrema miescheri (immature)†‡	76972	—	1 (5.3)	1 (1.6)	2 (2.4)	—	1.0	1.0	1.0	1	Stomach
Unidentified Digenea	—	—	—	1 (1.6)	1 (1.2)	—	—	1.0	1.0	1	Stomach
Cestoda											
Eubothrium sp. (immature adult)	76973	—	5 (26.3)	22 (36.1)	27 (32.1)	—	2.0	3.1	2.9	1–10	Intestine, stomach, pyloric caecae
Phyllobothrium caudatum (plerocercoid)	76975	—	13 (68.4)	59 (96.7)	72 (85.7)	—	*	*	*	1–1,000's	Intestine, pyloric caecae
Nematoda											
Capillaria sp. (juvenile)	—	—	—	1 (1.6)	1 (1.2)	—	—	1.0	1.0	1	Intestine
Thynnascaris sp. (juvenile)	76988	—	4 (21.1)	8 (13.1)	12 (14.3)	—	1.8	2.1	2.0	1–4	Intestine, stomach, pyloric caecae
Anisakis simplex (juvenile)	76986	—	19 (100.0)	49 (80.3)	68 (81.0)	—	5.1	5.2	5.1	1–41	Intestine, internal organs, musculature
Rhabdochona sp. (juvenile and adult)	—	1 (25.0)	5 (26.3)	7 (11.5)	13 (15.5)	1.0	2.4	1.4	1.8	1–8	Intestine, pyloric caecae
Unidentified Nematoda (juvenile)	—	—	1 (5.3)	—	1 (1.2)	—	2.0	—	2.0	2	Intestine (cysts)
Acanthocephala											
Rhadinorhynchus trachuri†‡	76978	—	6 (31.6)	6 (9.8)	12 (14.3)	—	1.3	1.5	1.4	1–3	Intestine
Annelida											
Piscicola salmositica	76980	—	—	14 (23.0)	14 (16.7)	—	—	58.7	58.7	1–325	Skin, gills
Copepoda											
Salmincola californiensis	76982	—	—	3 (4.9)	3 (3.6)	—	—	2.0	2.0	1–3	Gills
Lepeophtheirus salmonis	76983	—	2 (10.5)	—	2 (2.4)	—	2.0	—	2.0	2	Skin
Isopoda											
Lironeca vulgaris†	76990	—	1 (5.3)	—	1 (1.2)	—	2.0	—	2.0	2	Gills
Arachnida											
Unidentified Acarina (larval)	76985	1 (25.0)	—	—	1 (1.2)	1.0	—	—	1.0	1	Intestine

* Value not calculated.
† New host record.
‡ New state record.

Table 2. Parasites, anatomic locations, incidence, and intensities in all groups of coho salmon (18 juveniles and 78 adults) collected from the Mad River and vicinity.

Parasite	USNM no.	No. of fish infected (%)			Mean no. per infected fish			Range in nos. per fish infection	Location of parasites
		Juv.	Ad.	Total	Juv.	Ad.	Total		
Protozoa									
Ichthyophthirius multifilis	—	—	4 (5.1)	4 (4.2)	—	*	*	*	Skin, gills
Trematoda									
Diplostomum spathaceum sensu lato (metacercaria)	76960	—	25 (32.1)	25 (26.0)	—	18.7	18.7	1–200	Eye lens
Bucephalopsis gracilescens†‡ (adult)	76962	—	4 (5.1)	4 (4.2)	—	4.3	4.3	1–13	Intestine
(metacercaria)	76963	—	3 (3.8)	3 (3.1)	—	7.0	7.0	1–18	Kidney (cysts), heart (cysts)
Plagioporus shawi‡ (adult)	76965	—	1 (1.3)	1 (1.0)	—	1.0	1.0	1	Intestine
(immature)	76966	12 (66.7)	—	12 (12.5)	3.3	—	3.3	1–10	Intestine
Lecithaster gibbosus	76970	—	5 (6.4)	5 (5.2)	—	2.4	2.4	1–6	Intestine
Unidentified strigeid metacercaria	—	1 (5.6)	2 (2.6)	3 (3.1)	15.0	*	*	*	Skin
Cestoda									
Eubothrium sp. (immature adult)	76974	—	9 (11.5)	9 (9.4)	—	1.2	1.2	1–2	Intestine, pyloric caecae
Phyllobothrium caudatum (plerocercoid)	76976	—	72 (92.3)	72 (75.0)	—	*	*	1–1,000's	Intestine, pyloric caecae, gall bladder
Unidentified cystidean plerocercoid†	76977	—	1 (1.3)	1 (1.0)	—	1.0	1.0	1	Heart (cyst)
Nematoda									
Capillaria sp. (juvenile)	—	—	2 (2.6)	2 (2.1)	—	1.5	1.5	1–2	Intestine
Thynnascaris sp. (juvenile)	76989	—	3 (3.8)	3 (3.1)	—	1.3	1.3	1–2	Intestine, stomach
Anisakis simplex (juvenile)	76987	—	68 (87.2)	68 (70.8)	—	6.9	6.9	1–65	Internal organs, musculature
Rhabdochona sp. (juvenile and adult)	—	3 (16.7)	23 (29.5)	26 (27.1)	4.3	1.7	2.0	1–11	Intestine
Unidentified Nematoda (juvenile)	—	—	1 (1.3)	1 (1.0)	—	1.0	1.0	1	Intestine
Acanthocephala									
Rhadinorhynchus trachuri‡	76979	—	13 (16.7)	13 (13.5)	—	2.0	2.0	1–8	Intestine
Annelida									
Piscicola salmositica	76981	—	17 (21.8)	17 (17.7)	—	5.1	5.1	1–21	Skin, gills
Copepoda									
Lepeophtheirus salmonis	76984	—	1 (1.3)	1 (1.0)	—	2.0	2.0	2	Skin

* Value not calculated.
† New host record.
‡ New state record.

shawi, Deropegus aspina, Rhabdochona sp., and strigeoid metacercariae). Because juvenile coho salmon generally spend 1 yr in California streams before migrating to the ocean (Fry, 1973; Moyle, 1976), they are likely to acquire a more definitive freshwater parasite fauna than juvenile chinooks, which migrate soon after emerging from the streambed gravel (Taniguchi, 1970; Fry, 1973; Moyle, 1976). Juvenile chinooks, however, were observed with a definitive freshwater parasite fauna when forced to remain in their natal streams over the summer.

Upon migration to sea, both species of immature salmon obtain approximately 70% of their parasite faunas (Tables 1, 2). According to Boyce (1969) and Pennell et al. (1973), most marine hemiurid trematodes and cestodes are acquired by smolts soon after they begin to feed in brackish water. As salmon migrate into the open ocean, they begin to feed on different intermediate hosts and their endoparasitic fauna begins to change. Short-lived trematodes, such as *Lecithaster gibbosus,* are no longer observed in salmon that have been in the open ocean for a year or more (Margolis and Boyce, 1969), and nematodes, such as *Anisakis simplex,* begin to be encountered in large numbers in fish at this time. This nematode is long-lived and remains viable throughout the life of the fish (Margolis, 1970b).

As salmon migrate back into coastal areas, they once again acquire short-lived trematodes, a fact brought out by the 26.2 and 6.4% infection rates of *Lecithaster gibbosus* in adult chinook and coho salmon. Once in fresh water, the marine ectoparasite fauna (*Lepeophtheirus salmonis, Lironeca vulgaris*) drops off and is entirely replaced by a freshwater ectoparasite fauna (*Ichthyophthirius multifilis, Piscicola salmositica,* and *Salmincola californiensis*) in a few days. Because the Mad River hatchery is only 13 km from the ocean, many dead marine ectoparasites were still observed attached to salmon taken at the hatchery. *Ichthyophthirius multifilis* disappeared after a large decrease in water temperature (to 7°C) at the hatchery. This effect of lowered water temperature on *I. multifilis* in salmonid hatcheries was previously noted by Carl (1939).

The finding of *Nanophyetus salmincola* metacercariae, *Deropegus aspina, Plagioporus shawi, Rhabdochona* sp., and *Diplostomum spathaceum* metacercariae in adult fish supports the evidence that some parasites can survive throughout the life cycle of the fish (Dogiel, 1966). For *N. salmincola* this has already been shown experimentally (Farrell et al., 1964). *Deropegus aspina* and *Plagioporus shawi* were both found in precocious male salmon (''jacks''), fish that have been to sea for 1 yr or less. The adult (jack) coho that contained the specimen of *P. shawi* was marked, and thus was known to have been at sea for only 6 mo, a time well within the life span of the trematode (Olson, 1978).

Metacercariae of *Diplostomum spathaceum* appear to be the only parasites to have any potential for use as biological tags. Specimens were obtained from immature fish taken in the ocean, and adult fish are apparently reinfected with more metacercariae when they ascend streams to spawn. Such adult fish have two layers of metacercariae on the eye lens: one layer of metacercariae of a smaller size superimposed on a thicker layer of larger, more active metacercariae. More studies are needed to fully understand the life history of *D. spathaceum* from this area.

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Gastrointestinal Helminths of the Central Newt, *Notophthalmus viridescens louisianensis* Wolterstorff, from Southern Illinois

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ABSTRACT: A total of 76 adult and 19 immature central newts, *Notophthalmus viridescens louisianensis*, from two ponds located in Jackson County, Illinois, was examined for gastrointestinal parasites. Prevalence of infection in adults was 43%. No significant ($P = 0.69$) difference in parasite prevalence by sex was observed, although female newts had a significantly ($P < 0.01$) higher number of parasites per infected animal. No parasites were recovered from immatures. *Plagitura salamandra* and *Cosmocercoides dukae* are reported for the first time from *N. v. louisianensis*. A brief comparison of prevalence and diversity of parasites in the central newt and red-spotted newt (*N. v. viridescens*) is made.

The only helminth parasites reported from the central newt, *Notophthalmus viridescens louisianensis*, are *Bothriocephalus rarus* Thomas, 1937 from Michigan and South Carolina (Thomas, 1937), *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932 from Florida (Parker, 1941), *Manodistomum parvum* Stafford, 1905 from Illinois (Landewe, 1963), and *Brachycoelium salamandrae* (Froelich, 1789) Dujardin, 1845 from Louisiana (Rabalais, 1970). Several extensive surveys have reported numerous helminth parasites from the red-spotted newt, *Notophthalmus viridescens viridescens* (Fig. 1). With the exception of Berry's (1953) study, all have been conducted in eastern states. Geographic distribution of both salamander subspecies is indicated in Figure 1.

The present study in southern Illinois was undertaken to facilitate a comparison of newt parasites in the eastern and western parts of the hosts' ranges and to examine variations in the parasitic fauna of *N. v. louisianensis* and *N. v. viridescens*.

Materials and Methods

Newts were collected by seines and dip nets from McGuire's Pond, approximately 10 km south of Carbondale, Jackson County, Illinois, on 21 March and 13 August 1979, and from Etherton Pond, approximately 11 km south of Murphysboro, Jackson County, Illinois, on 7 March 1980. Both ponds had extensive vegetation and accumulated bottom detritus.

Newts were anesthetized and sacrificed within 24 hr of capture. Gastrointestinal tracts were removed and examined in 0.75% saline. Trematodes were fixed in 10% buffered formalin, stained in Harris' hematoxylin, cleared in Beechwood creosote, and mounted in Canada balsam. Nematodes were killed in 70% ethanol at 70°C, cleared in glycerine, and studied in temporary mounts.

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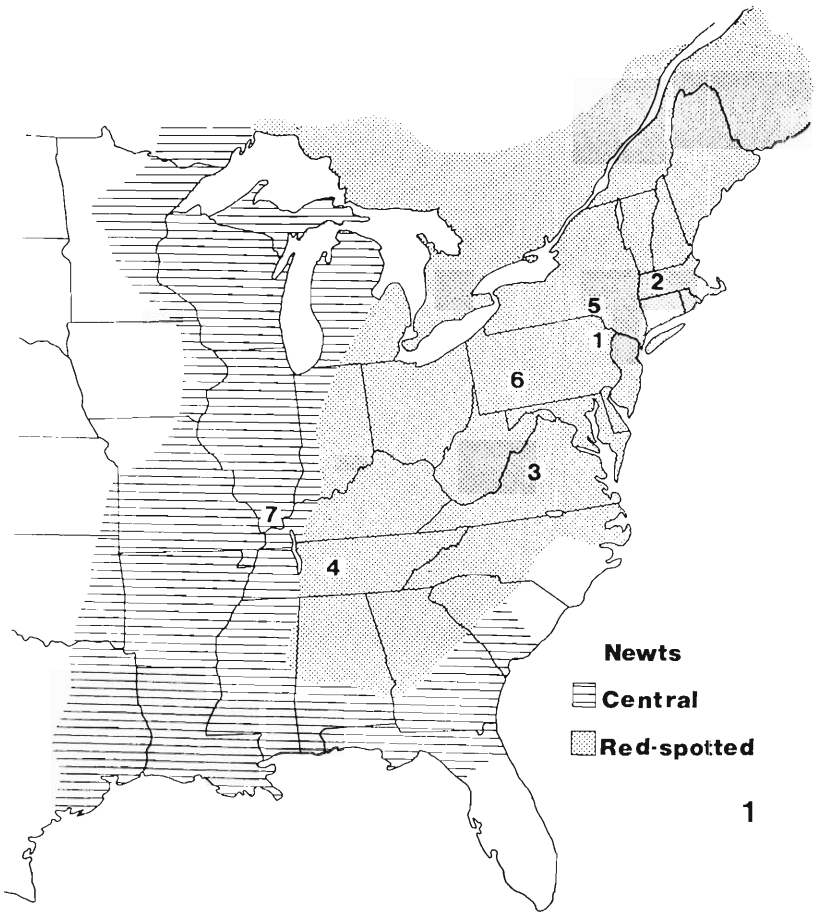

Figure 1. Geographic distribution of red-spotted and central newts, modified from Conant (1975). Numbers on map refer to location of major parasite surveys: 1, Kelly (1934); 1, Kelley (1934), is incorrect and should be in southwestern Pennsylvania just north of the Maryland and West Virginia borders; 2, Rankin (1945); 3, Russell (1951); 4, Berry (1953); 5, Fischthal (1955); 6, Jackson and Beaudoin (1967); and 7, present study.

Statistical studies were performed on an IBM Model 370/158 computer using SAS (Statistical Analysis System).

Results and Discussion

A total of 95 newts was examined from the two ponds. All newts collected in March (38 from McGuire's Pond and 38 from Etherton Pond) were abranchiate adults. All 19 specimens collected from McGuire's Pond in August were immature and harbored no parasites. Parasites found and average number per host are reported in Table 1.

Byrd (1937) and Cheng (1958) indicated the genus *Brachycoelium* may be divided into two major groups on the basis of vitellaria joining on the dorsomedial plane. In some of our specimens of *Brachycoelium* sp., vitellaria joined on the dorsomedial plane and in others they did not. Byrd (1937), after examination of

Table 1. Helminth parasites found in gastrointestinal tracts of adult *Notophthalmus viridescens louisianensis* from two southern Illinois ponds.

Pond	Total no. infected	*Plagitura**† *salamandra*	*Brachycoelium* sp.	*Megalodiscus rankini*	*Cosmocercoides*† *dukae*	Nematode cysts
McGuire's (N‡ = 38)	11	4/6	6/6	0/0	3/1	0/0
Etherton (N = 38)	22	20/4	4/3	2/4	3/2	2/37

* Numbers listed below each helminth species correspond to number of newts infected and mean number per host, respectively.
† *Plagitura salamandra* and *Cosmocercoides dukae* are reported for the first time from *Notophthalmus viridescens louisianensis*.
‡ N = total number of newts collected per pond.

B. meridionalis Harwood, 1932 paratypes (U.S. National Museum Helminthological Collection No. 30875), reported a break in the vitellaria and suggested that, if the holotype showed a break, *B. meridionalis* should be suppressed in favor of *B. trituri* Holl, 1928. After examination of the *B. meridionalis* holotype (No. 30874), we have decided there is not a break. Examination of holotype and paratype specimens of *B. trituri* (Nos. 7994 and 7995) was also inconclusive, because the holotype shows few vitelline glands with no overlap and the paratype shows many glands with extensive overlap on the dorsomedial plane. Therefore, we feel it is impossible at this time to assign our specimens to either *B. trituri* or *B. meridionalis*. Representative specimens of *Brachycoelium* sp. and other parasites found have been deposited in the U.S. National Museum Helminthological Collection (*Brachycoelium* sp., No. 76347; *Plagitura salamandra* Holl, 1928, No. 76348; *Megalodiscus rankini* Bravo, 1941, No. 76349; *Cosmocercoides dukae* (Holl, 1928) Wilkie, 1930, No. 76350).

Prevalence of parasitism in adult newts collected from Etherton Pond is significantly ($P < 0.01$) greater than in those from McGuire's Pond. However, the intensity in adults is not significantly ($P = 0.69$) different. Although the number of infected females does not differ significantly ($P = 0.12$) from that of males, females have a significantly ($P < 0.01$) greater intensity. The absence of parasites in larval stages is probably explained by Brophy's (1980) observation that small newts in McGuire's Pond positively select for ostracods and large newts for *Physa*. A significant ($P < 0.01$) inverse relationship between abundance of trematodes and abundance of nematodes in parasitized newts was observed.

An extensive review of the literature established diversity of parasites to be much greater in the red-spotted newt, for which nearly 30 identified species have been reported. Only six species, including *Plagitura salamandra* and *Cosmocercoides dukae* reported for the first time here, have been reported from the central newt. This observation is probably biased, due to the numerous studies that have been completed on the red-spotted newt. Most extensive surveys of the red-spotted newt indicate greater than 70% infection. Central newts from Etherton Pond were 58% infected, but only 29% of adults in McGuire's Pond were infected.

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Parasites of *Gammarus pseudolimnaeus* and *Hyalella azteca* (Crustacea: Amphipoda) in Three South-central Michigan Localities

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ABSTRACT: The parasite species found in *Gammarus pseudolimnaeus* collected from the Little Maple River (Parks Road), Clinton County, Michigan, were *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 (Acanthocephala: Pomphorhynchidae), *Polymorphus marilis* Van Cleave, 1939 (Acanthocephala: Polymorphidae), and *Allocreadium lobatum* Wallin, 1909 (Trematoda: Allocreadiidae). *Pomphorhynchus bulbocolli* infected 227 of the 3,125 *G. pseudolimnaeus* examined and had a mean number of worms for the total sampling period of 1.1 per host. Amphipods in all 13 length classes were infected with *P. bulbocolli*. Although the mean number of worms was similar in every month sampled during the period August 1980 through October 1981, prevalence was high in August through November 1980 and September and October 1981. *Gammarus pseudolimnaeus* is a new intermediate host for *P. bulbocolli*. Only two of the 172 *Hyalella azteca* collected from this locality were infected with *P. bulbocolli*. *Allocreadium lobatum* occurred in seven *G. pseudolimnaeus* collected from the Little Maple River. *Polymorphus marilis* infected *G. pseudolimnaeus* from the Little Maple River and also occurred in *G. pseudolimnaeus* and *H. azteca* from Augusta Creek (C. Avenue), Kalamazoo County, Michigan. The prevalence and mean number of *P. marilis* in the amphipod species from both localities were low. The state of Michigan is a new locality record for *P. marilis*, and *G. pseudolimnaeus* and *H. azteca* represent new intermediate hosts for this parasite species.

There are no published studies on the parasites of amphipods in Michigan besides the investigations of DeGiusti (1962) and Hazen and Esch (1977). Because of this paucity of information, a study was conducted to survey the parasites that utilize *Gammarus pseudolimnaeus* Bousfield and *Hyalella azteca* (Saussure) as hosts in three localities in south-central Michigan.

Materials and Methods

Gammarus pseudolimnaeus and *Hyalella azteca* were collected by dip net (1-mm mesh) from the Little Maple River (Parks Rd.), Clinton County, during August 1980 through October 1981 and from Augusta Creek (C. Avenue), Kalamazoo County, during July and September 1980. *Hyalella azteca* was also sampled during December 1979, June and August 1980, and May through August 1981 from the Looking Glass River (Woodbury Avenue), Shiawassee County. The collecting site at the Little Maple River has an average width of 3 m, and its bottom is composed of alternating areas of sand-mud and stones. The Augusta Creek site has a bottom consisting of fine gravel and stones up to 2 inches in diameter; some areas are covered with sand and silt. The width of the stream at this site is approximately 6 m. The Looking Glass River site has an average width of 5 m, and the bottom consists of silt and mud. Water depth at all three localities during time of collection was 2 m or less.

Amphipods collected were brought to the laboratory alive and examined within 48 hr; they were measured to the nearest millimeter, from the base of the antennae to the base of the telson. Routine procedures for fixing and staining the parasites were employed. Prevalence is the percentage of infected hosts in a given sample. Specimens of *Pomphorhynchus bulbocolli*, *Polymorphus marilis*, and *Allocread-*

Table 1. Prevalence and mean number of *Pomphorhynchus bulbocolli* and *Polymorphus marilis* in *Gammarus pseudolimnaeus* and *Hyalella azteca*.

Locality	No. examined	No. infected (%)	No. parasites found	Mean no. of worms ± SE*	Mean length (mm) ± SE of infected amphipod
Little Maple River					
P. bulbocolli	3,125†	227 (7.3)	254	1.1 ± 0.04	8.9 ± 0.18
	172‡	2 (1.2)	2	1.0	3.9 ± 0.20
P. marilis	3,125†	19 (0.6)	20	1.1 ± 0.05	8.0 ± 0.54
Augusta Creek					
P. marilis	380†	3 (0.8)	3	1.0	6.3 ± 0.59
	662‡	4 (0.6)	4	1.0	6.0 ± 0.81

* Standard error.
† *G. pseudolimnaeus*.
‡ *H. azteca*.

ium lobatum from *G. pseudolimnaeus* have been deposited at the U.S. National Museum (USNM) (USNM Helm. Coll. Nos. 76456, 76457, and 76458, respectively).

Results and Discussion

Pomphorhynchus bulbocolli Linkins in Van Cleave, 1919 (Acanthocephala: Pomphorhynchidae)

A total of 227 (7.3%) *Gammarus pseudolimnaeus* out of 3,125 examined from the Little Maple River was infected with larval stages of *Pomphorhynchus bulbocolli* (Table 1). Pigmentation differences between infected and noninfected amphipods were not observed. Of the infected amphipods, 204 (90%) had one acanthocephalan, 19 (8%) had two acanthocephalans, and four (2%) had three acanthocephalans. The total number of *P. bulbocolli* recovered from the infected amphipods was 254. The mean number of worms per host for the total sampling period was 1.1 ± 0.04. Of the worms that were sexed, 103 were males and 112 were females. There was a significant difference between the number of cystacanths with their presomas directed posteriorly (134) and anteriorly (73) ($\chi^2 = 17.97$, $P < 0.005$).

Cystacanths were found in the amphipod hemocoel dorsal or ventral to the digestive tract, and could be seen through the amphipod cuticle under a dissecting microscope. Female and male worms were similar in size and could only be differentiated with the aid of a compound microscope. The mean length of cystacanths for the entire sampling period was 5.2 mm ± 0.11. On several occasions the proboscis of a cystacanth appeared to be sticky when evaginated, because it would actually stick to the side and/or bottom of the stender dish; this "sticky" proboscis may aid *P. bulbocolli* in attaching to the intestine of the fish definitive host. The testes, seminal vesicles, and cement glands of male cystacanths stained darkly, indicating the possible presence of sperm. Five (4.9%) of the males examined were monorchids. Many female cystacanths had one large ovarian mass, or the ovarian mass was fragmented, forming a maximum number of eight smaller masses of ovarian balls.

Table 2. Prevalence and mean number of *P. bulbocolli* recovered from 3,125 *G. pseudolimnaeus* of various length classes examined during August 1980 through October 1981.

Length class (mm)	No. amphipods examined	No. infected (prevalence)	No. worms found	Mean no. of worms ± SE*
4	84	4 (4.8)	5	1.3 ± 0.50
5	412	23 (5.6)	25	1.1 ± 0.48
6	442	23 (5.2)	27	1.1 ± 0.45
7	377	28 (7.4)	30	1.1 ± 0.38
8	345	46 (13.3)	55	1.2 ± 0.41
9	181	25 (13.5)	26	1.1 ± 0.28
10	161	22 (13.7)	24	1.1 ± 0.43
11	122	11 (9.0)	13	1.2 ± 0.40
12	206	10 (4.9)	11	1.1 ± 0.32
13	276	17 (6.2)	19	1.1 ± 0.33
14	275	12 (4.4)	12	1.0
15	134	4 (2.9)	4	1.0
16	110	2 (2.0)	3	1.5 ± 0.71

* Standard error.

Amphipods in all length classes were infected with *P. bulbocolli* (Table 2). The mean length of infected amphipods was 8.9 mm ± 0.18. Prevalence increased from 4.8% in amphipods 4 mm in length to its maximum (13.7%) in amphipods 10 mm in length, and then decreased as amphipod length increased. Although amphipods 8 mm in length were infected with the largest number of worms, the mean number of worms did not vary significantly among host length classes (analysis of variance, $F = 0.52$, $P > 0.80$).

Pomphorhynchus bulbocolli occurred in amphipods in every month sampled (Table 3). Prevalence was high in August through November 1980, low in December 1980 through August 1981, and high in September and October 1981.

Table 3. Prevalence, mean number, and mean size of *Pomphorhynchus bulbocolli* in *Gammarus pseudolimnaeus*.

Month	No. amphipods examined	No. infected (prevalence)	No. worms found	Mean no. of worms ± SE*	Mean size of worms ± SE	Mean size of infected amphipods ± SE
Aug. (1980)	403	41 (10.2)	48	1.2 ± 0.01	4.1 ± 0.18	5.6 ± 0.23
Sept.	383	48 (12.5)	56	1.2 ± 0.06	4.7 ± 0.20	7.5 ± 0.18
Oct.	126	15 (11.9)	18	1.2 ± 0.08	6.0 ± 0.39	9.6 ± 0.42
Nov.	106	15 (14.2)	17	1.1 ± 0.13	5.8 ± 0.27	11.5 ± 0.42
Dec.	142	6 (4.2)	7	1.2 ± 0.18	5.6 ± 0.20	12.2 ± 0.70
Jan. (1981)	117	3 (2.6)	3	1.0	5.7 ± 0.62	11.0 ± 0.58
Feb.	146	8 (5.5)	9	1.1 ± 0.13	6.5 ± 0.31	13.1 ± 0.35
Mar.	386	20 (5.2)	22	1.1 ± 0.07	6.8 ± 0.16	13.1 ± 0.73
Apr.	84	4 (4.8)	4	1.0	6.8 ± 0.36	13.3 ± 0.48
May	153	4 (2.6)	4	1.0	6.6 ± 0.44	14.0 ± 0.71
June	183	1 (0.5)	1	1.0	4.0	5.0
July	238	1 (0.4)	1	1.0	4.3	6.0
Aug.	207	3 (1.4)	3	1.0	5.0 ± 0.72	6.7 ± 0.19
Sept.	231	30 (13.0)	31	1.0 ± 0.01	4.5 ± 0.42	7.6 ± 0.20
Oct.	220	28 (13.0)	30	1.1 ± 0.26	5.9 ± 0.24	8.9 ± 0.20

* Standard error.

There was no significant difference in the mean number of *P. bulbocolli* between monthly samples (analysis of variance, $F = 0.39$, $P > 0.80$). Both the mean size of cystacanths and infected amphipods increased from August 1980 through May 1981. It appears that a new generation of amphipods entered the environment in June 1981 or earlier, because 72% of the amphipods collected in this month were 6 mm or less as compared to April and May, when all amphipods collected were 9 mm or longer. In June through October 1981, the mean lengths of both cystacanths and amphipods increased. Significant correlation coefficients for August 1980 through May 1981 ($r = 0.90$, $P < 0.01$) and June through October 1981 ($r = 0.89$, $P < 0.01$) suggest that cystacanths of *P. bulbocolli* grow with the amphipods they infect during these time periods. These results, plus the occurrence of acanthellae in amphipods during August through October 1980 and September and October 1981, indicate that, although no significant monthly changes were found in the mean number of *P. bulbocolli* in amphipods, the length range of the worms underwent seasonal change. During September 1980 and March 1981, the only months in which fish were sampled, gravid *P. bulbocolli* were found in golden shiners (*Notemigonus crysoleucas*), white suckers (*Catostomus commersoni*), and rock bass (*Ambloplites rupestris*).

In February and March 1981, 257 ovigerous female amphipods were examined; 12 (4.6%) of these were infected with *P. bulbocolli,* thus indicating female amphipods infected with *P. bulbocolli* can carry eggs. Similarly, Van Maren (1979) found that several female *G. fossarum* infected with *P. laevis* carried eggs or juveniles.

Awachie (1967) reported that both *Echinorhynchus truttae* and *Polymorphus minutus* utilize the amphipod *G. pulex* as an intermediate host, but simultaneous infections in nature were rare. However, in laboratory studies he demonstrated that co-invasion of both species in one amphipod is possible. In the present study, single *G. pseudolimnaeus* were not found concurrently infected with *P. bulbocolli, Polymorphus marilis,* and *Allocreadium lobatum,* even though amphipods were always collected in the same area of the Little Maple River. Circumstances that play a role in this lack of natural simultaneous infections are not known.

Two of 172 *Hyalella azteca* collected from the Little Maple River during October 1980 were infected with cystacanths of *P. bulbocolli* (Table 1). A total of 880 *H. azteca* was examined for parasites from the Looking Glass River; all were negative.

In the North American continent and surrounding waters, there are only three published reports of species of *Gammarus* serving as intermediate hosts for acanthocephalans that utilize fish as definitive hosts. These are Johnson and Harkema (1971), Bullock and Samuel (1975), and Johnson (1975). Jensen (1952) experimentally infected *Gammarus* sp. with *P. bulbocolli* and reported that its development in *Gammarus* sp. was limited. The present study, which is the fourth published report, demonstrates that *G. pseudolimnaeus* is a new intermediate host for *P. bulbocolli* and that *P. bulbocolli* can utilize both *G. pseudolimnaeus* and *H. azteca* as intermediate hosts.

Polymorphus marilis Van Cleave, 1939 (Acanthocephala: Polymorphidae)

Cystacanths of *Polymorphus marilis* were easily seen with the unaided eye, appearing as orange spheres within the hemocoel dorsal to the digestive tract of

both *G. pseudolimnaeus* and *H. azteca*. Upon dissection, each orange sphere was enclosed by a sheath that was light orange in color. At both localities, the prevalence and mean number of *P. marilis* in the amphipods were low (Table 1). Maximum number of *P. marilis* was two, which occurred in only one *G. pseudolimnaeus*; this is low when compared to the field studies of Spencer (1974), who found four cystacanths of *P. minutus* infecting *G. lacustris*, and Hynes and Nicholas (1957), who found as many as seven fully developed cystacanths of *P. minutus* infecting *G. pulex*. None of the *G. pseudolimnaeus* infected with *P. marilis* were ovigerous females. Infected amphipods ranged in length from 4 to 12 mm; none of the 795 *G. pseudolimnaeus* 13 mm or over from the Little Maple River were infected with *P. marilis*.

Since its original description, *P. marilis* has been found in several species of ducks (Van Cleave and Starrett, 1940; Denny, 1968). Denny (1968) found that *G. lacustris* is the intermediate host in the Edmonton, Alberta, Canada, area. The present report demonstrates that *G. pseudolimnaeus* and *H. azteca* represent new intermediate hosts for this parasite species, and the state of Michigan is a new locality record for *P. marilis*.

Allocreadium lobatum Wallin, 1909 (Trematoda: Allocreadiidae)

Seven specimens (five nongravid, two gravid) of *A. lobatum* were found in six *G. pseudolimnaeus* collected from the Little Maple River in December 1980 and January and October 1981. The mean size and range of the infected amphipods were 10.3 mm ± 0.88 (8–14). This is the third report of progenetic individuals of *A. lobatum* occurring in aquatic crustaceans; the other two are DeGiusti (1962) and Muzzall (1981).

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Development of the Ox–Cat Cycle of *Sarcocystis hirsuta*

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ABSTRACT: The ox–cat cycle of *Sarcocystis hirsuta* was studied by killing 18 calves between 7 and 110 days after inoculation (DAI) with sporocysts from cats. At 7 DAI, sporozoites were found free and within leukocytes in the lumen of arteries associated with mesenteric lymph nodes and in endothelial cells of mesenteric arteries. First-generation meronts were found 7–23 DAI in arteries associated with mesenteric lymph nodes and intestines. Meronts matured between 7 and 10 DAI. First-generation meronts were 37.2 × 22.3 μm and contained more than 100 merozoites, which measured 5.1 × 1.2 μm. Merozoites measuring 5.4 × 1.5 μm were found free in the peripheral blood of a calf 11 DAI. Second-generation meronts were found in capillaries of striated muscles and heart 15–23 DAI; meronts matured between 15 and 16 DAI. Second-generation meronts were 13.9 × 6.5 μm and contained three to 35 merozoites that measured 4 × 1.5 μm. Second-generation individual merozoites were seen within myofibers and in tissue macrophages in muscles 16–23 DAI. Sarcocysts formed between 25 and 75 DAI in striated muscles, but not in heart; esophagus was the most heavily infected organ. At 30 DAI, a sarcocyst contained two metrocytes. At 62 DAI, sarcocysts were up to 550 μm long and 35 μm wide and contained only metrocytes; the wall was up to 3 μm thick and cross-striated. Bradyzoites developed between 62 and 75 DAI. Mature sarcocysts were up to 800 μm long and the wall was up to 6 μm thick. Sarcocysts became infective for cats at 75 DAI. Cats shed sporocysts and oocysts 8–10 days after ingesting infected muscles.

Of the three species of *Sarcocystis* that occur in the ox (*S. cruzi* [*S. bovicanis*], *S. hirsuta* [*S. bovifelis*], and *S. hominis* [*S. bovihominis*]), developmental stages of only *S. cruzi* are known (for review see Levine and Ivens, 1981). In this report the development of *S. hirsuta* is described. Clinical signs and lesions will be reported separately.

Materials and Methods

Inoculation of calves

Sporocysts of *S. hirsuta* were obtained originally by feeding tongue and esophagus of a naturally infected cow from the Montana State University Agricultural Experiment Station to a specific-pathogen-free (SPF) cat. Sporocysts were collected from intestinal scrapings and stored in a balanced salt–antibiotic mixture (Dubey, 1980, 1981). Two experiments were performed.

In experiment 1, seven 8–12-week-old calves were killed 75, 88, 89, 104, 110, 170, and 220 days after oral inoculation (DAI) with 1 million, 1 million, 250,000, 1.4 million, 5,000, 5,000, and 5,000 sporocysts, respectively, and their muscles were fed to 22 SPF cats.

In experiment 2, 11 1–2-week-old calves were necropsied 7, 10, 15, 16, 20, 23, 30, 35, 42, 62, and 82 DAI with 15, 25, 1, 15, 25, 15, 15, 1, 1, 3, and 1 million sporocysts, respectively. Two 7-day-old calves served as uninoculated controls, and were killed 30 and 82 days after the initiation of the experiment.

Inoculated calves were housed separately from uninoculated calves for 7 DAI, and their excreta were incinerated to kill sporocysts that might have passed unexcysted in their feces. They were fed milk until 6–8 weeks of age and then were fed grain and hay. Calves in experiment 1 were housed outdoors and had access

to hay, grain, and pasture. Calves in experiment 2 were housed individually indoors.

Examination for parasitemia

In experiment 2, blood (7 ml) was drawn from each inoculated calf into vacuum tubes containing ethylenediaminetetracetic acid twice weekly and on the day of necropsy. Smears of buffy coat were air dried, fixed with methanol, stained with Giemsa's stain, and examined as described (Dubey, 1982a). Smears were considered negative when parasites were not seen in about 1 ml of blood.

Necropsy of calves

Calves were killed by electrocution, exsanguinated, and necropsied immediately. Portions of bone marrow, brain, spinal cord, eyes, pituitary, salivary and adrenal glands, thymus, lungs, heart, diaphragm, spleen, kidneys, liver, gallbladder, urinary bladder, omentum, rumen, reticulum, omasum, abomasum, small and large intestines, esophagus, skeletal muscle, lymph nodes (superficial cervical, mandibular, retropharyngeal, mediastinal, hepatic, gastric, mesenteric, subiliac), cerebrum, cerebellum, pons, medulla, and tongue were fixed in 10% Millonig's buffered formalin (MBF). In two calves necropsied 7 and 10 DAI, sections of every ⅓ m of intestines were examined. Selected tissues were also fixed in Bouin's fluid (BF), in Helly's fixative (HF), or in 1% glutaraldehyde and 4% formaldehyde (GF) mixture.

Paraffin-embedded sections were cut at 5 μm. Selected tissues were embedded in glycol methacrylate and sectioned at 3 μm. Sections were stained with hematoxylin and eosin (HE), Heidenhain's iron hematoxylin (IH), or periodic acid–Schiff's hematoxylin (PASH).

Inoculation of cats

Cats used in this study were obtained from the SPF cat colony maintained in the Veterinary Research Laboratory, Montana State University, Bozeman, Montana. They were never fed raw meat until used in experiments. Cats were fed ground muscles of experimentally inoculated calves over a period of 1–7 days, and their feces were examined for sporocysts after sugar flotation (Dubey, 1976).

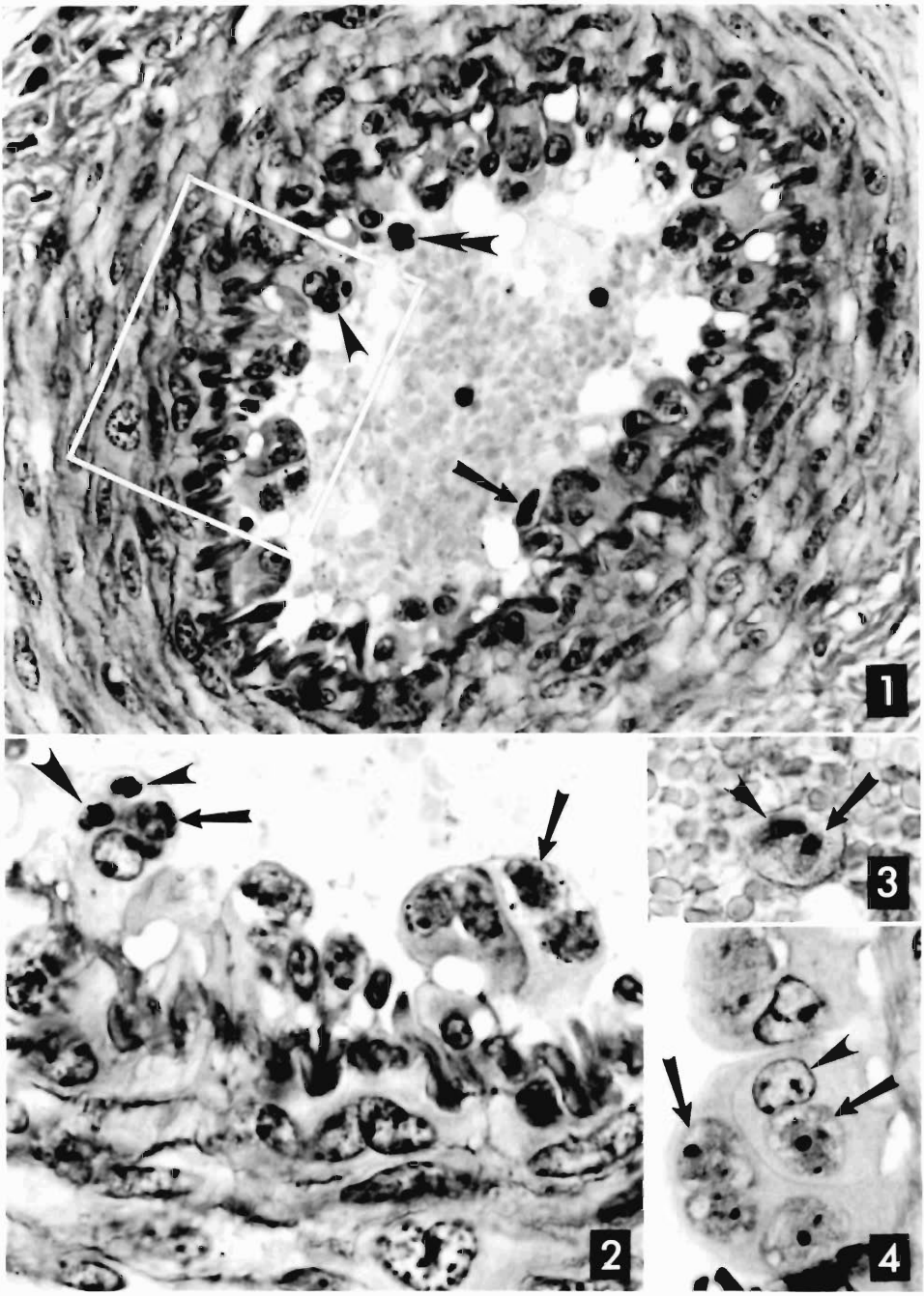
Results

First-generation meronts

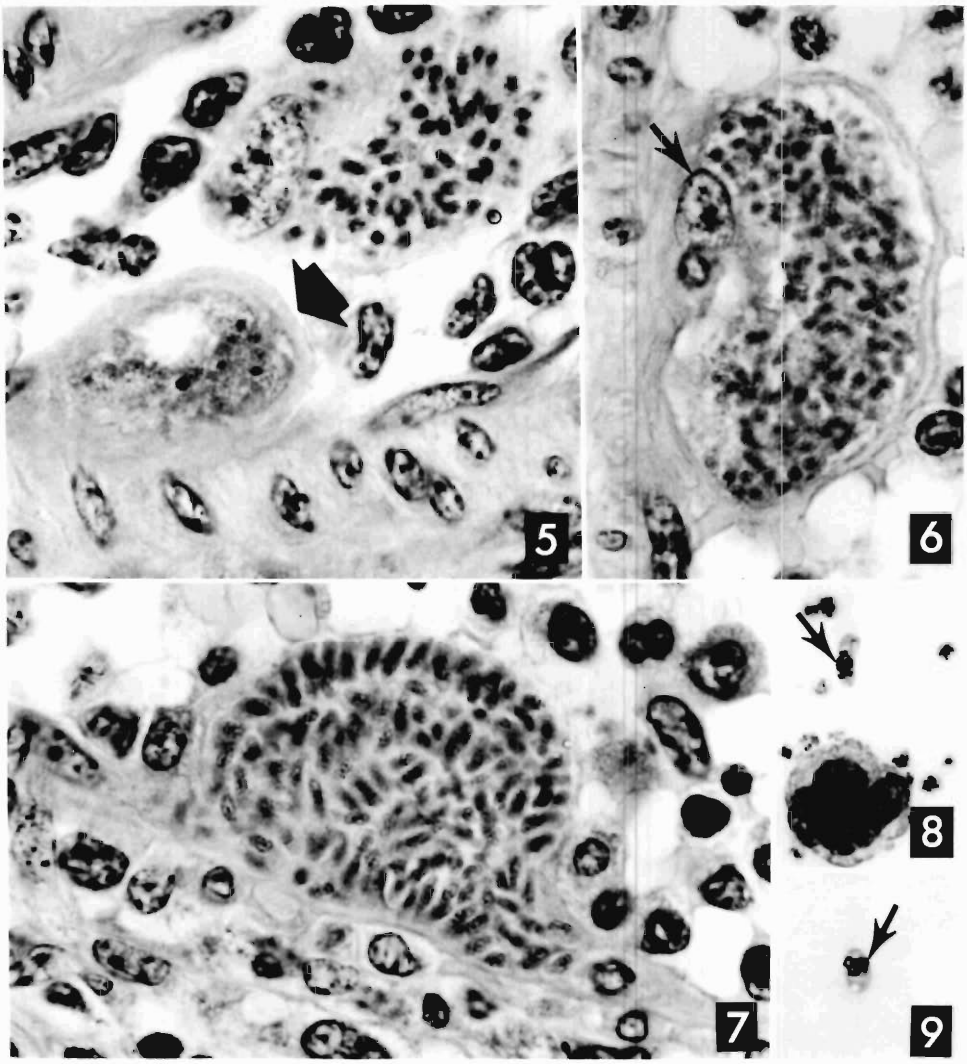
At 7 DAI, sporozoites were found free and within leukocytes in the lumen of arteries at the periphery of mesenteric lymph nodes (Figs. 1–3). In HE-stained sections, sporozoites had a central nucleus with a very small nucleolus, a pink-staining area anterior to the nucleus, and a pale area posterior to the nucleus

→

Figures 1–4. 1. Cross section of an artery in mesentery of a calf, 7 DAI with 15 million *Sarcocystis hirsuta* sporocysts. Note sporozoites in a leukocyte close to the endothelial lining (arrowhead), in close approximation to endothelial lining (arrow), and within endothelial lining (arrowhead). Host cells containing meronts and sporozoites are enlarged and protruding into the arterial lumen. BF, PASH; 5 μm ×400. 2. Enlargement of the area marked in Figure 1. Note two sporozoites (arrowheads) and two

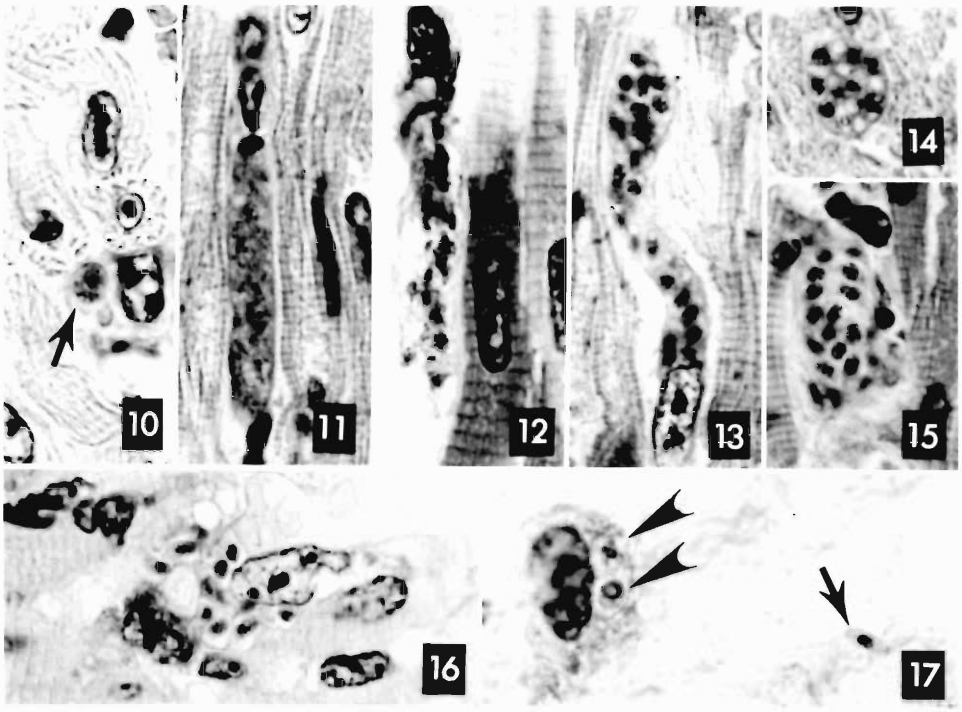


meronts (arrow, one is out of focus) in a single host cell in the left corner and several young meronts in the right corner. PAS-positive granules are larger in sporozoites than in meronts. ×1,000. 3. A sporozoite (arrow) in a leukocyte in mid-lumen of a mesenteric artery of calf in Figure 1. The leukocyte nucleus (arrowhead) is out of focus. Note subterminal nucleus of the sporozoite. BF, HE. ×1,000. 4. A 3-μm section of mesenteric artery of the calf in Figure 1. Note that the host cell nucleus (arrowhead) and a young meront (arrow) are enclosed within a membrane. In the adjoining cell there are three meronts. Note different-sized nucleoli. HF, IH. ×1,000.



Figures 5–9. 5–7. First-generation meronts in 3-μm sections of arteries in mesenteric lymph nodes of a calf, 10 DAI with 25 million sporocysts. ×1,000. 5. Immature meront (arrow) protruding into arterial lumen. Note thick covering, several prominent nucleoli, and dispersed chromatin. Another meront with differentiated nuclei is lying free in the arterial lumen. HF, IH. 6. Meront with differentiated nuclei and enlarged host-cell nucleus (arrow) covered by a thick capsule. BF, HE. 7. Mature meront with merozoites arranged randomly and in a row at the periphery. BF, HE. 8, 9. Merozoites (arrows) in smear of blood from the jugular vein of a calf, 11 DAI with 15 million sporocysts. Methanol, Giemsa's stain. ×1,000. Note three nuclei in Figure 9.

(Fig. 3). In PASH-stained sections, a large PAS-positive area and several small granules were found in sporozoites (Fig. 3). Sporozoites appeared to be within endothelial cells, and some lay across the length of the host cell. Six longitudinally cut sporozoites were 5.6 × 2.5 μm (5–7 × 2–3). The next stage was a round to ovoid, uninucleate meront with a central nucleus (Figs. 2, 4). The nucleolus in young meronts was larger than that in the sporozoite, and PAS-positive granules



Figures 10–17. Second-generation meronts and merozoites in 3-μm sections of heart of a calf, 16 DAI with 15 million sporocysts. All meronts are located in capillaries. ×1,000. 10, 16, 17, HF, HE; 11–15, BF, IH. 10. Uninucleate meront (arrow). Note prominent nucleolus. 11. Immature meront with undifferentiated nucleus or nuclei. 12. Immature meront with differentiating nuclei. 13. Multinucleated meront. 14. Cross section of a mature meront. Both ends of merozoites have unstained areas, thus the vacuolated appearance of the meront. 15. Mature meront with longitudinally sectioned merozoites. 16. Ruptured meront with spilled merozoites. The capillary lumen has not yet collapsed. 17. One extracellular merozoite (arrow) and two merozoites (arrowheads) within a tissue macrophage.

decreased with the development of meronts (Fig. 2). Meronts were 9.5 × 5.8 μm (4–14 × 3.5–7; N = 16) and contained up to five nucleoli. It was difficult to determine whether these were separate nucleoli or part of a single nucleus. Infected host cells were hypertrophied and meronts were seen both below and above the host-cell nucleus. One host cell contained two meronts and two sporozoites. Some arteries were heavily parasitized, whereas most of them were not infected.

At 10 DAI, meronts were in arteries within mesenteric lymph nodes, mesentery, and intestines. In the small intestine, the middle part was the most heavily infected; meronts were not seen in the first and the last meter segments. More meronts were in intestinal arteries than in arteries in the mesentery. The host covering (capsule) was thicker in immature meronts than in mature meronts (Figs. 5–7). Chromatin in the undifferentiated nucleus of meronts was dispersed and granular, and it condensed as nuclei became differentiated. Nuclei in meronts were sometimes arranged in groups. The number of nuclei in meronts was difficult to count, but appeared to be more than 100. Meronts with undifferentiated nuclei were 22.7 × 12.1 μm (10–35 × 3–18; N = 10) and with differentiated nuclei were 37.2 × 22.3 μm (28–56 × 17–40; N = 30). Merozoites were arranged peripherally

as well as randomly (Fig. 7). Several immature and mature meronts, and merozoites, were free in the arterial lumen. Merozoites in sections were 5.1 × 1.2 μm (5–6.5 × 1–1.5; $N = 9$) and in smears were 6.3 × 1.4 μm (5.5–7 × 1–1.5; $N = 12$). Mature meronts were PAS-negative, and there was no residual body.

At 15, 16, 20, and 23 DAI, very few meronts were seen, and these were in arteries associated with mesenteric lymph nodes.

Parasitemia

Merozoites were found in the peripheral blood of a calf at 11 DAI with 15 million sporocysts. All 15 merozoites seen were free in plasma. Merozoites were 5.4 × 1.5 μm (5–6 × 1–2; $N = 13$) and contained a subterminal nucleus (Fig. 8). One merozoite had three nuclear lobes or three separate nuclei (Fig. 9).

Second-generation meronts

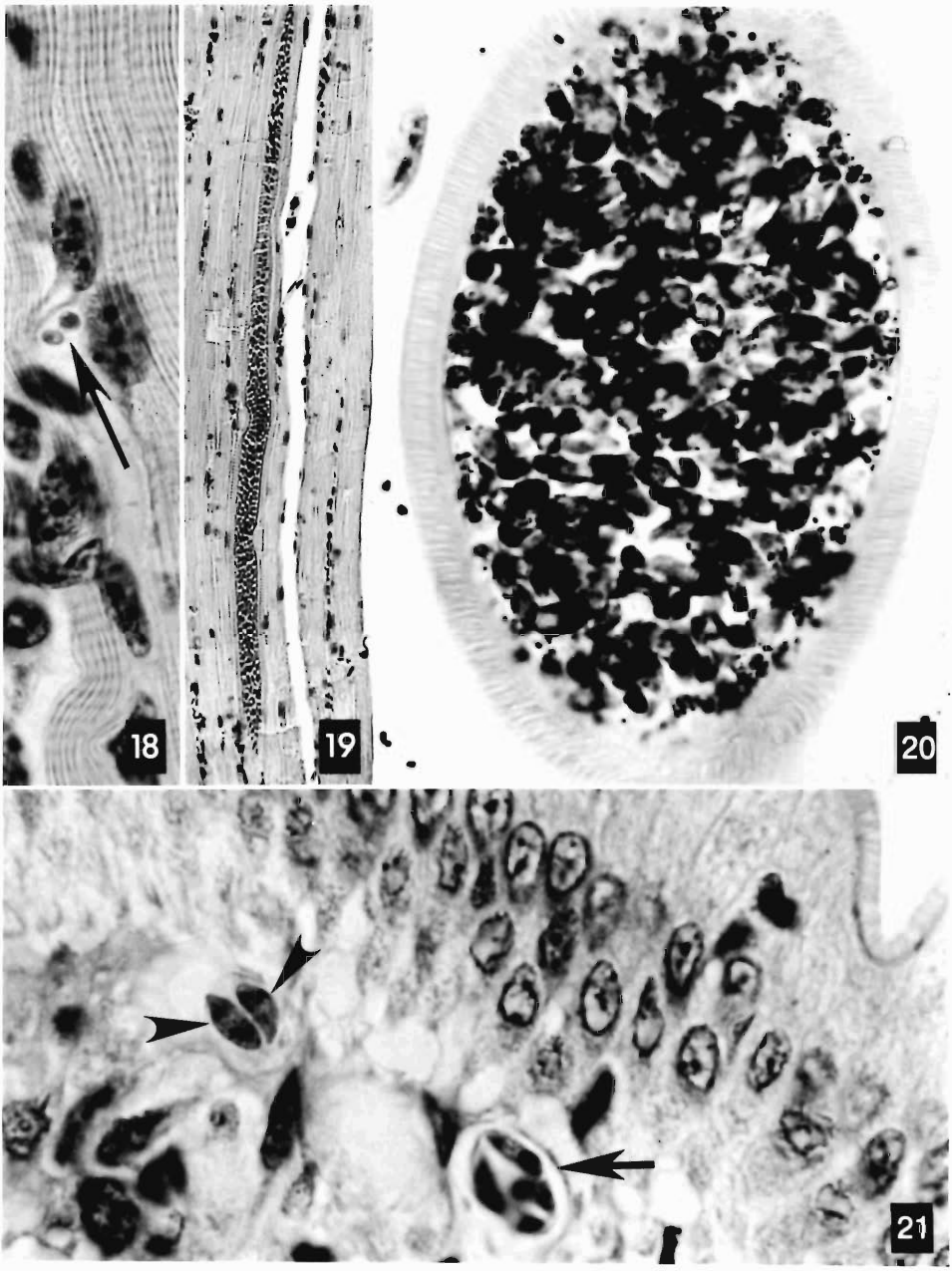
Second-generation meronts were found in capillaries of heart, thigh, diaphragm, tongue, and eye 15–23 DAI; most of them were in the heart. The youngest uninucleate meront was 5 × 3.5 μm. In immature meronts, the chromatin was dispersed and there were one to four nucleoli either in separate nuclei or in lobes of a single nucleus. Meronts with differentiated nuclei were 13.9 × 6.5 μm (7–45 × 3–11; $N = 55$) and the number of nuclei was 12.4 (3–35; $N = 55$). Merozoites in meronts were loosely arranged, with spaces between them. Meronts were PAS-negative. Ten longitudinally cut merozoites averaged 4 × 1.5 μm; most of them were 3 × 1.5 μm. Merozoites had a vesicular central nucleus and two clear areas at both ends in sections stained with hematoxylin; thus, meronts appeared vacuolated (Fig. 14).

Individual merozoites in tissues

Individual merozoites outside of meronts were seen 16–23 DAI in muscles where second-generation meronts developed. Some merozoites appeared to be within myofibers. One to three merozoites were seen in tissue macrophages, especially around blood vessels (Fig. 17). Intraleukocytic merozoites had a vesicular nucleus and were structurally similar to second-generation meronts. One merozoite appeared to have two nuclei. Nuclei in extracellular merozoites were often pyknotic.

Sarcocysts

The esophagus was the most heavily infected organ, followed by tongue, eye muscles, and muscles of legs. Sarcocysts were not found in the heart. At 30 and 35 DAI, single sarcocysts (7 × 5 μm) containing two metrocytes were found in sections of skeletal muscle (Fig. 18). At 42 DAI, one sarcocyst measuring 90 × 14 μm was found in a section of tongue; its wall was thin and contained only metrocytes. At 62 DAI, sarcocysts were up to 550 μm long and 35 μm wide (Fig. 19); the wall was 1.5–3.0 μm thick and cross-striated. In sarcocysts at 62 DAI, only metrocytes were seen, which were about 5 × 3.5 μm in sections. At 82 and 89 DAI, the sarcocyst wall was 3–6 μm thick and both bradyzoites and metrocytes were seen. At 105 and 110 DAI, sarcocysts were up to 800 μm long and 60 μm wide; the sarcocyst wall was up to 6 μm thick and contained mostly bradyzoites



Figures 18–21. 18. Sarcocyst in 3-μm section of thigh muscle of calf 595, 30 DAI with 15 million sporocysts. BF, HE. ×1,000. 19. Immature sarcocyst in 5-μm section of tongue of a calf, 62 DAI with 3 million sporocysts. HF, HE. ×100. 20. Sarcocyst in 3-μm section of esophagus of a calf 110 DAI with 5,000 sporocysts. Note cross-striated PAS-negative wall and hundreds of PAS-positive bradyzoites. BF. ×1,000. 21. A 3-μm section of small intestine of a cat, 12 days after it was fed meat infected with *Sarcocystis hirsuta*. Note two sporocysts in the lamina propria. Arrowheads point to sporozoites. BF, HE. ×1,000.

(Fig. 20). Bradyzoites in sections were about 13 × 3 μm and contained several large PAS-positive granules; the sarcocyst wall was PAS-negative (Fig. 20).

Both thin-walled sarcocysts (*S. cruzi*) and thick-walled (*S. hirsuta*) sarcocysts were found in calves of the first experiment. Only thick-walled sarcocysts were found in the calves of the second experiment.

Gametogony

Sarcocysts were infectious to cats at 75 DAI. Cats shed sporocysts 8–10 days after ingesting infected meat. The prepatent period was 7 days in two cats, 8 days in eight cats, and 9 days in 10 cats. The two cats fed heart of calves killed 75 and 88 DAI did not shed sporocysts, whereas those fed other muscles from the same calf did. Oocysts were located in the lamina propria and in the villar epithelial tips of small intestine of cats (Fig. 21). In intestinal scrapings of cats, sporulated oocysts were 17.1 × 12.7 μm (16–18 × 11–14; N = 10). They contained sporocysts measuring 12.8 × 8.4 μm (11–14 × 7–9; N = 20), each with four elongate sporozoites. A Stieda body was absent. The sporocystic residuum varied from being compact to being a few scattered granules. Living sporozoites were 8.2 × 1.9 μm (7.5–9 × 1.5–2; N = 10), with anterior pointed end. In sections of sporozoites, the nucleus was subterminal and there were several PAS-positive granules (Fig. 21).

Control calves

No parasites were found in the calf killed 30 DAI. A few immature sarcocysts were found in the other calf killed 82 DAI, probably acquired from the *S. hirsuta*-infected calf housed next to it.

Discussion

This is the first study of the intravascular development of *S. hirsuta*. After the sporocysts excyst in the gut of an ox, the sporozoites reach the mesenteric arteries by an unknown route. The mechanism of transport and penetration of endothelial lining of mesenteric arteries is unknown. It is probable that sporozoites are carried in the leukocytes to the site of their development within the endothelial lining. The PAS reaction proved useful in tracing the development of sporozoites into meronts, because PAS-positive granules found in sporozoites disappeared during the development of first-generation meronts. The type of host cell parasitized was not determined. It is suspected that merogony occurs in host cells between endothelium and tunica intima.

Parasitemia in *S. hirsuta* infection was transient and of low degree. The size of merozoites and the timing indicate that parasitemia was due to first-generation merozoites.

The occurrence of second-generation meronts only in muscles is unusual when compared to occurrences of the other species of *Sarcocystis* in domestic animals (for review see Levine and Ivens, 1981). The occurrence of merozoites in macrophages in muscles suggests that the second-generation merozoites are transported locally from capillaries to myofibers; this might explain why second-generation merozoites were not found in peripheral blood.

Sarcocysts formed between 25 and 30 DAI. At 62 DAI, the sarcocyst wall was thick and cross-striated. Photographs published by Boch et al. (1978) indicate

Table 1. Comparison of developmental stages of *S. cruzi* and *S. hirsuta*.

	S. cruzi (ox–coyote)	*S. hirsuta* (ox–cat)
First-generation meronts		
Location	Several organs	Mesenteric and intestinal arteries
Duration (DAI)	7–26	7–23
Peak development (DAI)	15	10
Size of meronts (μm)	41.0 × 17.5	37.2 × 22.3
No. of merozoites	>100	>100
Size of merozoites (μm)	6.3 × 1.5	5.1 × 1.2
Second-generation meronts		
Location	Several organs	Striated muscles, heart
Duration (DAI)	19–46	15–23
Peak development (DAI)	24–28	16
Size of meronts (μm)	19.6 × 11.0	13.9 × 6.5
No. of merozoites	4–37	3–35
Size of merozoites (μm)	7.9 × 1.5	4 × 1.5
Parasitemia		
Duration (DAI)	24–46	11
Intraleukocytic multiplication	Yes	No
Sarcocysts		
Wall (μm)	Thin (<1.0)	Thick (3–6)
Location		
CNS	Yes	No
Heart	Yes	No
Striated muscles	Yes	Yes
Smooth muscles	No	No

that the wall becomes cross-striated between 50 and 61 DAI; no other details were given. Gestrich et al. (1975) described the ultrastructure of *S. hirsuta* sarcocysts in two calves killed 98 and 160 DAI. At 98 DAI, sarcocysts contained both metrocytes and bradyzoites, but only bradyzoites were present at 160 DAI.

Cats shed sporocysts 8–10 days after ingesting meat infected with *S. hirsuta*. The number of sporocysts shed was much smaller than that shed by canines ingesting *S. cruzi* (Fayer, 1977; Dubey, 1980), suggesting that domestic cats are not a very good host of *S. hirsuta*. These observations in experimental infections are similar to those in cats fed beef naturally infected with *S. hirsuta* (Dubey and Streitel, 1976). Gametogony of *S. hirsuta* in the present study was similar to that reported by Heydorn and Rommel (1972). The prepatent period and the morphology of sporocysts and sarcocysts indicate that the parasite studied in Germany by Heydorn et al. (1975) was the same as in the present study.

Newborn calves were used in experiment 2 of this study in order to minimize natural infections. Weaned calves in experiment 1 housed outdoors became infected with *S. cruzi*. Natural infections with *S. cruzi* were not found in control calves used in the present study and in 10 other newborn calves killed in other projects on sarcocystosis and toxoplasmosis in our laboratory.

The development of *S. hirsuta* in newborn calves is compared in Table 1 with the development of the ox–coyote cycle of *S. cruzi* studied under identical con-

ditions in this laboratory (Dubey, 1982b). Differences and similarities are apparent. One striking difference is the absence of second-generation meronts of *S. hirsuta* in kidneys, whereas those of *S. cruzi* occur predominantly in renal cortex. Also, sarcocysts of *S. hirsuta* were not found in heart, whereas those of *S. cruzi* occur predominantly in heart.

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Further Studies on Toxic Extracts from *Eimeria tenella*-infected Chicken Ceca

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ABSTRACT: The active toxic component of extracts prepared from *Eimeria tenella*-infected chicken ceca (ICE) was localized in the "microsomal" fraction of the crude ICE. This component can clot human plasma and persists after repeated washings in buffer. Slow intravenous perfusion of ICE into healthy chickens resulted in increased prothrombin times. The ICE toxic component proved lethal regardless of the site of injection. Toxic activity occurred in extracts of both the mucosal and muscular layers of the infected ceca. The toxic component is stable and can be extracted from ceca frozen for 1 mo. We suggest the increased prothrombin times observed in *E. tenella* infections may in part be the result of this toxic activity. The release of the toxic activity may deplete clotting factors and lead to the hypocoagulative state observed.

Bradford et al. (1947) noted a toxic activity in extracts from the cecal contents of *E. tenella*-infected birds. They suggested that, if the "activity" were leaked from the cecal lumen, through the tissue, and into the bloodstream during an infection, it may lead to a hypocoagulative state in the live chicken. The hypocoagulative state would arise from the slow depletion of clotting factors resulting in an increased prothrombin time.

Witlock (1980) demonstrated that a component was also present in tissue extracts from *Eimeria tenella*-infected chicken ceca that was toxic when injected into otherwise healthy chickens. This toxic component produced a "thromboplastin-like" activity and was present in the cecal tissue as early as 4 days postinoculation. The component was heat stable and caused intravascular coagulation.

The following experiments were undertaken to expand the work of Witlock (1980), to further localize and identify the origin and nature of the toxic activity in cecal tissue, and to provide additional information on the physiological characteristics and effects of the activity and its impact on the hemostatic system.

Materials and Methods

Preparation of extracts

Noninfected cecal extract (NCE) and *E. tenella*-infected cecal extract (ICE) were prepared from 3-wk-old White Leghorn cockerels as outlined by Witlock (1980). Briefly, ceca were collected from the infected cockerels at 5 days postinoculation. This roughly corresponds to the time when second-generation schizonts are present. The parasite used was the Beltsville (NAPL) strain of *E. tenella* cultured at the Animal Parasitology Institute (Laboratory Strain #49). Usually

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10^5 sporulated oocysts were administered per os, and in all cases a severe infection (+4 lesion score; Johnson and Reid, 1970) followed.

Preparation of cellular subfractions

Ceca removed from infected cockerels or their noninfected brood mates were slit lengthwise, rinsed with buffer (0.05 M imidazole, pH 7.0, containing 0.025 M $CaCl_2$) to remove the contents, and weighed. The organs were pooled within treatments to give equal weight of tissue and were homogenized in imidazole buffer (1.5:1, v/w) on a Sorvall Omnimixer at maximum speed for 10 min. Further disruption of the tissue was accomplished by use of a Polytron (Brinkman Instruments) for 1 min.

The homogenates were separated into crude subcellular constituent fractions by a modification of the method of Unkeless et al. (1974). Briefly, the homogenates were spun on a Sorvall refrigerated centrifuge with an SS-34 head at 800 *g* for 10 min to remove cell debris and nuclei. Mitochondria were sedimented from the post-nuclear supernatant at 6,000 *g* for 10 min. Post-mitochondrial supernatant was centrifuged at 15,000 *g* for 20 min to sediment a crude lysosomal fraction. Post-lysosomal supernatant, spun at 100,000 *g* for 1 hr on a Beckman ultracentrifuge equipped with a type 30 head, yielded a microsomal pellet and a soluble fraction. Prior to each centrifugation, a sample was removed from each supernatant fraction for an in vivo assay. All pellets were resuspended in imidazole buffer with the Polytron and brought up to the volume of the supernatant fraction from which they had been prepared. No further characterization of the cellular subfractions was attempted.

For isolation of the microsomes alone, homogenates were prepared as described above, followed by Sorvall centrifugation at 20,000 *g* for 20 min. Ultracentrifugation of the resulting supernatant fraction at 100,000 *g* for 1 hr provided the preparations used for comparative in vivo and in vitro assays of cecal tissue activity. Fractions were assayed for protein with a modified Lowry method (Layne, 1957).

Assays of extract activity

IN VIVO: The maximum nonlethal dose of NCE or microsomes from uninfected ceca (NCM) that could be injected into the left wing vein of noninfected White Leghorns was determined. An equal volume of ICE or microsomes from infected ceca (ICM), equalized on the basis of protein content, was then injected into another bird. The assay time covered the interval between conclusion of the injection and the death of the chicken. Each test was run in duplicate, and the chickens used in the assay were of the same age and approximate weight.

IN VITRO: This assay, as outlined by Witlock (1980), was essentially a modified prothrombin-time assay. However, ICE, NCE, or cellular fractions were substituted for chick-brain thromboplastin. Plasma from control White Leghorns was prepared from citrated blood obtained by cardiac puncture. Prothrombin time, used as a positive control, was conducted according to the method of Doerr et al. (1975), using acetone-dehydrated chick-brain thromboplastin. All assays were replicated four times.

In one series of assays, human blood was collected in citrated tubes and the

plasma separated and used in the in vitro procedure. In all other cases, chicken plasma was used.

Experimental Procedures

Experiment I

To test the effect of cold storage on the activity of ICE, both infected and uninfected ceca were removed, washed with buffer, and stored at −15°C for 1 mo. After the storage period the ceca were thawed and both ICE and NCE were prepared and compared with freshly prepared ICE and NCE. The comparison was conducted via the in vivo assay.

Experiment II

This experiment was designed to assess the role of passage through the liver on the lethal activity in the ICE. Chickens were injected in either the right or left wing vein, femoral artery, or the heart. For the femoral injections, chickens were lightly anesthetized with ether and the right or left femoral artery was exposed from the surrounding musculature. The extract was then injected directly into the artery and the in vivo assay timed as described previously. Cardiac and wing vein injections of the extracts were done on unanesthetized birds.

Experiment III

Ether-anesthetized White Leghorn cockerels were slowly perfused with ICE to simulate the slow release of ICE that might be expected during an *E. tenella* infection. NCE or buffer perfusion was used for comparison. A Spectroderm slow-speed pump (Spectroderm International, Fairfax, Va.) fitted with a 7013 head was calibrated to deliver the extracts at the rate of 0.12 ml/min into the left wing vein. This rate was determined by constraints of the pump and is probably faster than the actual release of the activity in vivo.

ICE and NCE were perfused in volumes two to four times the amount of the predetermined single injected lethal ICE dose. At the end of a perfusion, the cockerel was bled via cardiac puncture into a citrated tube (0.18 M sodium citrate, 1:9) and the blood was stored on ice. Plasma was collected by centrifugation. Prothrombin times were assayed (Doerr et al., 1975) using the plasma of the perfused cockerels and appropriate controls.

Experiment IV

ICE and NCE and their corresponding microsomes (ICM and NCM) were prepared according to the scheme described above. These fractions were then tested for activity with the in vitro assay and, in the case of the microsomes, with the in vivo lethality assay.

ICM and NCM fractions were also prepared using a mucosal scraping or the muscular layer of infected and noninfected ceca.

Results

Experiment I

ICE prepared from infected ceca that had been frozen for 1 mo was as lethal as extracts prepared from fresh ceca (Table 1). NCE from either fresh or frozen ceca was not lethal at the level injected.

Table 1. Effect of freezing *Eimeria tenella*-infected ceca or noninfected ceca on the activity of *Eimeria tenella*-infected cecal extract (ICE) or noninfected cecal extract (NCE).

Extract	Storage	mg protein injected	Results of in vivo assay*
NCE	fresh	4.5	no death
ICE	fresh	4.5	death (34.9 sec)
ICE	frozen	4.5	death (29.7 sec)
NCE	frozen	4.5	no death

* In vivo assay measures both the time from extract injection to death of chicken and lethality of extracts.

Experiment II

A single ICE lethal dose injected into various locations did not alter the time of death (Table 2), except perhaps for the intracardiac route. Injection of ICE directly into the heart caused death more quickly than injection at any other site. Equal doses of NCE administered at all sites proved nonlethal.

Experiment III

A single rapid injection of 0.2 ml of ICE into nonperfused cockerels usually caused death in 32.4 sec. Slow perfusion of ICE into the wing vein in quantities up to four times the single lethal dose did not produce mortality, but increased prothrombin times (Table 3). Similarly, perfusion with NCE also increased prothrombin time. Buffer, however, had no effect. Perfusion of four times the lethal dose did not appreciably increase the prothrombin time over that seen with a doubling of the lethal dose.

Experiment IV

In general, both the supernatants and the pellets from all ICE fractions had more activity than did those from NCE (Table 4). Although both ICM and NCM were more active than any other subfraction on a microgram-of-protein basis, ICM was the more active microsomal preparation of the two. The post-microsomal supernatants from both ICE and NCE had the least activity of the tissue subfractions tested. However, post-microsomal supernatant from ICE was the more active of the two.

When ICM, derived from ICE, were washed two additional times with buffer, the activity in the in vitro assay remained unchanged. ICM also reduced prothrombin times when human plasma was substituted for chicken plasma in the in

Table 2. Effects of injection site on in vivo assay time of *Eimeria tenella*-infected cecal extract (ICE).*

Injection site	*N*	In vivo assay time† (sec)
L. wing vein	4	23 ± 1
R. wing vein	4	23 ± 1
L. femoral artery	3	23 ± 1
R. femoral artery	2	24 ± 1
Cardiac puncture	2	16 ± 5

* Injection of noninfected cecal extract (NCE) was not lethal. 1.6 mg of extract protein was injected in all sites for both extracts.
† $\bar{x}$ ± SEM. In vivo assay measures the time from extract injection to death of chicken.

Table 3. Effect of extract perfusion via wing vein on prothrombin times.

Source*	Amount perfused (ml)	Prothrombin time $\bar{x}$ ± SEM (sec)	Death
ICE	0.4†	17.2 ± 0.04	0
ICE	0.8	18.2 ± 0.1	0
NCE	0.4	18.4 ± 0.2	0
NCE	0.8	19.3 ± 0.3	0
Buffer	0.2	12.4 ± 0.2	0
Buffer	0.8	13.6 ± 0.2	0
Anesthetized control	—	12.6 ± 0.5	0
Control	—	13.0 ± 0.5	0

* ICE = *E. tenella*-infected cecal extract; NCE = noninfected cecal extract.
† Lethal injection of ICE was 0.2 ml (1.58 mg protein) of an equal protein, single injection of NCE was not lethal.

vitro assay (Table 5). The human plasma did not react as rapidly as that from the chicken, however, and its use increased the interval between the assay times for NCM and ICM in dose-response curves (data not shown).

An attempt to localize the lethal activity within the microsomes of either the mucosal (parasitized cells) or muscular (nonparasitized cells) layers of the infected ceca was not entirely successful (Table 6). The activity was present in both the infected mucosal scrapings and in the muscular layer. Activity was not present in the control tissue at the protein levels injected.

To compare the relative activity of the crude extracts (ICE and NCE) and their respective microsomes (ICM and NCM), both were subjected to the in vivo and in vitro assays (Tables 7, 8). In both trials the NCM and ICM were more active

Table 4. In vitro assay of fractions from cecal extracts.

Source*	Fraction level	Type	Protein (mg/ml)	In vitro assay time† (sec)
NCE	—	—	800	12.2 ± 0.2
ICE	—	—	800	9.5 ± 0.6
NCE	mitochondial	pellet	200	12.5 ± 0.3
ICE	mitochondial	pellet	200	9.0 ± 0.1
NCE	mitochondial	supernatant	700	10.2 ± 0.2
ICE	mitochondial	supernatant	700	7.5 ± 0.2
NCE	lysosomal	pellet	200	10.1 ± 0.2
ICE	lysosomal	pellet	200	7.7 ± 0.2
NCE	lysosomal	supernatant	500	11.3 ± 0.4
ICE	lysosomal	supernatant	500	8.1 ± 0.2
NCE	microsomal	pellet (NCM)	100	8.6 ± 0.1
ICE	microsomal	pellet (ICM)	100	6.9 ± 0.1
NCE	microsomal	supernatant	600	25.0 ± 0.2
ICE	microsomal	supernatant	600	15.1 ± 0.5
ICE	microsomes	unwashed (ICM)	100	6.5 ± 0.5
ICE‡	microsomes	washed (ICM)	100	7.2 ± 0.4

* NCE = noninfected cecal extract; ICE = *E. tenella*-infected cecal extract; ICM = infected cecal microsomes; NCM = noninfected cecal microsomes.
† All values are $\bar{x}$ ± SEM, $N = 4$. In vitro assay is a modified prothrombin time using extracts or fractions as a thromboplastin source.
‡ These microsomes were washed twice in buffer.

Table 5. Effect of plasma type on in vitro assay.

Fraction*	Plasma type	In vitro assay time† (sec)
NCM	chicken	14.8 ± 1.0
ICM	chicken	11.2 ± 0.1
NCM	human	62.0 ± 1.3
ICM	human	39.3 ± 1.5

* NCM = noninfected cecal microsomes; ICM = *E. tenella*-infected cecal microsomes.
† All values are $\bar{x}$ ± SEM. In vitro assay is a modified prothrombin time using extracts or fractions as a thromboplastin source.

than the corresponding crude extract. The ICM, when injected at 1.5 mg/kg, was about five times more active than the ICE at 8 mg/kg (Table 7).

Discussion

The ability of the chicken to withstand a perfusion of up to four times the lethal dose of a single injection with ICE (Table 3), the nondependence on the site of injection (Table 2), and the increase in the post-infusion prothrombin time (Table 3) all support the existence of the "hypocoagulative" state proposed by Bradford et al. (1947) and expanded on by Witlock (1980). The slow perfusion (0.12 ml/min) of the ICE attempts to simulate the release of the "thromboplastin-like" activity from the *E. tenella*-infected cecum. This slow release of activity places the chicken in a negative clotting phase, as indicated by the increased prothrombin times in this study (Table 3), and suggests an explanation for the elevated prothrombin times observed during *Eimeria* sp. infections reported in previous studies (Ruff et al., 1978). NCE, although not lethal when injected rapidly, will increase the prothrombin time when slowly perfused into chickens (Table 3). This then suggests that the altered prothrombin times observed during *E. tenella* infections may be related only to the tissue destruction and not to the parasite proper. The absence of any effect related to injection site in the in vivo assay further suggests that the toxic component of ICE is not rapidly removed from the bloodstream and, if produced in ceca during natural infections, could exert its hemostatic effects systemically.

Further localization of the activity was obtained by ultracentrifugation (Table 4). This yielded "microsomes" (ICM and NCM) that have the same or slightly lower in vitro assay times (increased activity) as their respective crude extracts (ICE and NCE) while containing only one-eighth the protein. These data suggest

Table 6. Localization of toxic activity in cecal tissue microsome using the in vivo assay.

Source of microsomes	ml injected	Results of in vivo assay* (sec)	Protein injected (mg)
Control mucosal scraping	0.1	no death	0.23
Infected mucosal scraping	0.1	death (31.6)	0.23
Control muscle	0.25	no death	0.36
Infected muscle	0.25	death (22.9)	0.36
	0.1	death (70.3)	0.14

* In vivo assay measures both the time from extract injection to death of chicken and lethality of the extracts.

Table 7. In vivo assay comparison of the crude extracts of *E. tenella*-infected (ICE) and noninfected ceca (NCE) and the microsomes prepared from those extracts (ICM/NCM).

Source*	Injected protein (mg/kg)	Results of in vivo assay† (sec)
NCE	19.1	death (30.2)
NCE	12.6	no death (>134)
NCM	2.0	death (32.1)
NCM	1.5	no death (>190)
ICE	19.1	death (21.2)
ICE	12.6	death (25.3)
ICE	8.0	death (35.6)
ICM	2.0	death (26.9)
ICM	1.5	death (34.2)
ICM	1.0	death (82.3)

* NCE = noninfected cecal extract; NCM = noninfected cecal microsomes; ICE = *E. tenella*-infected cecal extracts; ICM = *E. tenella*-infected cecal microsomes.
† In vivo assay measures both the time from extract injection to death of chicken and lethality of the extract.

that the toxic component resides in the microsomal fraction. Furthermore, in each of the prior subcellular fractions, the activity appears to be roughly equal in both supernatant and pellet of either crude extract. However, when the microsomes are removed, there is an abrupt loss of activity in the supernatant. The toxic component in the pelleted ICM is maintained even after repeated resuspension and washing (Table 4). These data suggest that the activity is associated with membranes in general, and with the membranes of the endoplasmic reticulum specifically, rather than with soluble components of the cell. When the crude extracts (ICE and NCE) are compared with their respective microsomal fractions (ICM and NCM) in the in vivo assay, the microsomal fractions are clearly more active (Table 7). Both the NCE and NCM at high enough levels will cause mortality. Conversely, ICE maintains activity at much lower protein levels (Table 7). This is in agreement with past studies (Witlock, 1980). The ICM has greater activity than ICE, NCM, and NCE. Similarly, in the in vitro assay (Table 8) the activity of the NCM and ICM, when compared to their respective crude extracts, is increased. Both the ICM and ICE in general have more activity than either NCM or NCE.

In further characterization of the activity, the ability of the ICM, and to some extent the NCM, to react with and clot human plasma (Table 5) suggests that the

Table 8. In vitro assay comparison of the crude extracts of *E. tenella*-infected (ICE)/noninfected (NCE) ceca and the microsomes derived from those extracts (ICM/NCM).

Source*	Protein (mg)	In vitro assay time† (sec)
NCE	2.6	12.0 ± 1
NCM	0.4	17.0 ± 1
ICE	2.6	10.6 ± 0.4
ICM	0.4	14.0 ± 0.4

* NCE = noninfected cecal extract; NCM = noninfected cecal microsomes; ICE = *E. tenella*-infected cecal extract; ICM = *E. tenella*-infected cecal microsomes.
† All values are $\bar{x}$ ± SEM. In vitro assay is modified prothrombin time using extracts or fractions as a thromboplastin source.

activity is nonspecific. Even with heterologous plasma, however, the ICM is clearly more active than the NCM. The activity is stable after freezing (Table 1), has been shown to be heat stable (Witlock, 1980), and is not localized within a specific area of the *E. tenella*-infected cecum (Table 6). This last point is supported by data showing that ICM from both mucosal scrapings and the muscular layer of *E. tenella*-infected ceca have increased activity when compared to NCM of both areas (Table 6).

The presence of toxic components in the microsomes of the muscular layer, where the parasite is not usually found, suggests that the activity may not be directly associated with the parasite itself. This suggestion is further supported by the presence of reduced clotting activity, but not its absence, in NCE.

McDougald (1981) observed that ICE made from the ceca of birds receiving drugs known to inhibit the activity of prostaglandins had no lethal activity. Similarly, *E. tenella*-infected chickens receiving antiprostaglandins showed decreased lesion scores and lessened cecal inflammation when compared to nontreated *E. tenella*-infected chickens.

Prostaglandins have been implicated in disseminated intravascular coagulation and other diseases (Bell et al., 1980). They are formed from the release of arachidonate from ruptured phospholipid membranes (Bell et al., 1980). The toxic activity elicited from both ICE and NCE may be related to prostaglandins. Washed ICE microsomes still maintain their lethal activity when compared to unwashed ICE microsomes (Table 4). The pellets of the ultracentrifuge fractions, as well as the supernatants, retain their lethal activity, and the cell cytoplasm loses activity when microsomes are removed (Table 4).

Similarly, the findings of Witlock et al. (1981) on the physiological stresses present in moribund *E. tenella*-infected chickens indicated that the requisite changes in O_2 tension and pH for prostaglandin formation (Bell et al., 1980) are present. Because prostaglandin formation is linked to membrane disruption and liberation of arachidonic acid, this may account for the lethal activity associated with the subcellular membrane pellets.

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Research Note

Parasites of Bocaccio, *Sebastes paucispinis*, from Southern and Central California

From January 1976 to September 1981, 29 species of parasites were collected from 145 bocaccio, *Sebastes paucispinis*, taken off the coast of southern and central California. Bocaccio is the most important rockfish in California, comprising approximately 35–40% of the annual commercial rockfish catch (Fitch, 1974, Offshore Fishes of California. Calif. Dep. Fish and Game). Many of the parasites are quite visible to commercial fishermen, sportsmen, and fish-market customers. Tapeworms emerging from the anus, coiled nematodes in the viscera, and puslike material exuding from ruptured protozoan cysts in fillets are unappetizing and aesthetically displeasing. Heavily infected fish are sometimes discarded altogether, and are considered unsafe to eat. The present study was undertaken primarily to ascertain which species of parasites were most obvious to laymen, but efforts were made to identify all parasites encountered. To date there have been no comprehensive surveys on the parasites of bocaccio from California, although Sekerak and Arai (1977, Syesis 10:139–144) reported the helminths and copepods of this fish from Canadian waters.

The parasites collected consisted of seven species of protozoa, 18 species of helminths, and four species of crustaceans (Table 1). The parasites most conspicuous to commercial and sport fishermen were *Kudoa clupeidae*, *Paraboth-riocephalus sagitticeps*, *Anisakis simplex*, *Phocanema decipiens*, and *Lafystius* sp.

Cysts of *K. clupeidae* were located in the skeletal muscle and were easily ruptured when fish were filleted. The contents have the appearance of thick pus, and the presence of numerous cysts makes the fillets difficult to sell. Customers of seafood outlets often refuse to purchase infected fish, and the marketing practice of calling cysts "flavor buds" or "fat bodies" is sometimes used to promote the sale of the product.

The tapeworm *P. sagitticeps* is commonly encountered, as it is not unusual for worms to be forced out of the torn or severed intestine while the fish is being filleted. Two- to 3-yr-old fish ordinarily harbored one or two worms, whereas bocaccio five years or older were generally infected with several dozen worms. The sagittate scolex is extremely mobile and remains active for several days in uncleaned, refrigerated fish.

From a public-health standpoint, larvae of *A. simplex* and *P. decipiens* were the most important parasites observed in this study. Human infections could result from consumption of viable larvae of either species. *Anisakis simplex* was present only in the viscera, but burdens were oftentimes heavy. Larvae of *P. decipiens* were located mainly in the skeletal muscle; in fact, it is not uncommon to find live brown worms of *Phocanema* within cellophane packages of rockfish fillets in many local grocery stores.

Amphipods of *Lafystius* sp., referred to as lice by sportsmen, were more common in fish taken over sandy or muddy bottoms than in fish taken over rocky areas.

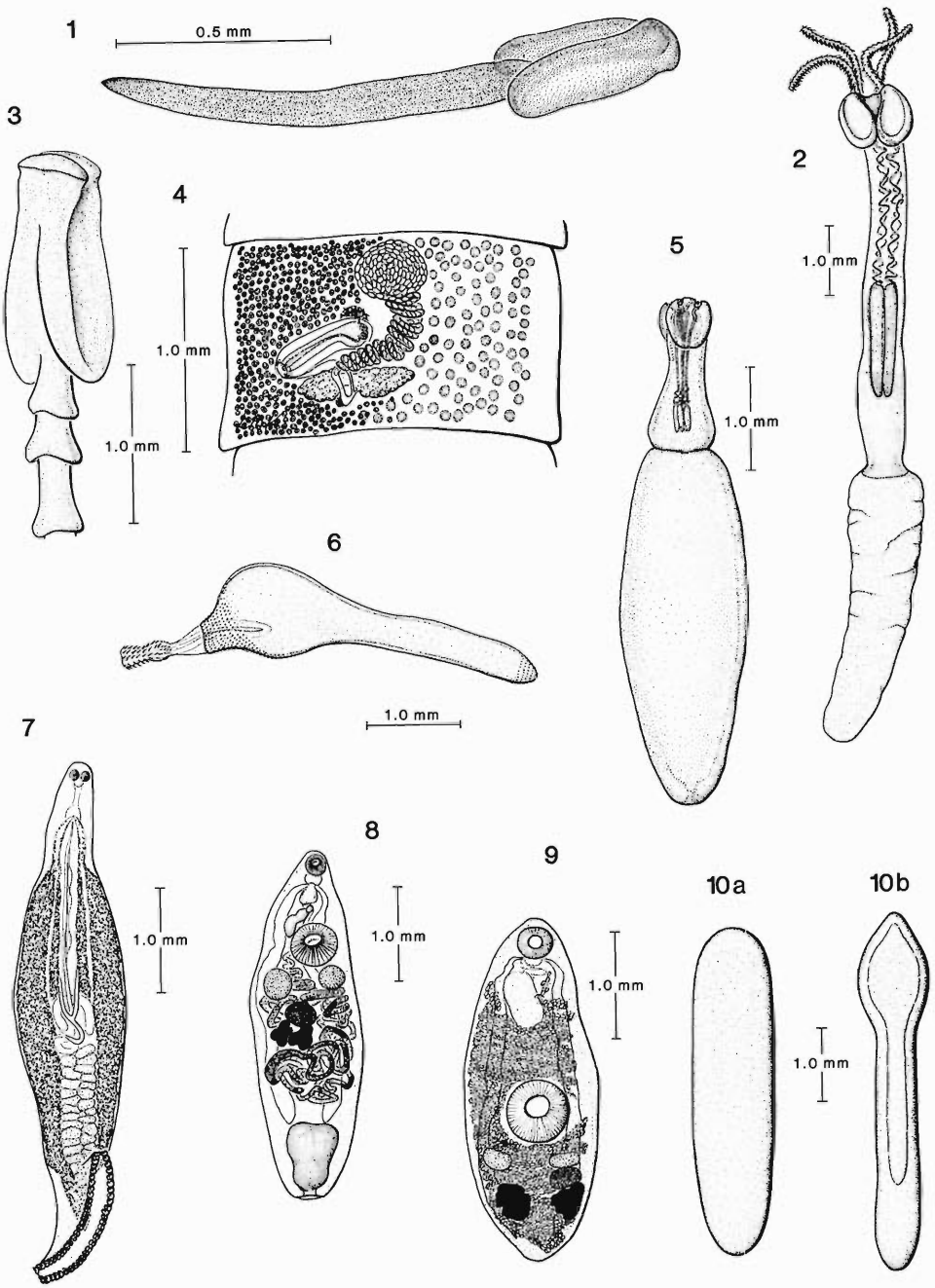
Table 1. Parasites of *Sebastes paucispinis* from southern and central California.

Parasite	No. fish infected/no. fish examined	Mean no. per fish	Site of infection*	USNM Helm. Coll. No.
Myxosporida				
Ceratomyxa elegans	5/100	†	GB	76792
Ceratomyxa hopkinsi	23/100	†	GB	76792
Ceratomyxa sebasta	37/100	†	GB	76792
Leptotheca informis	41/100	†	GB	76792
Myxidium calcariferi	9/100	†	GB	76792
Henneguya sebasta	31/112	†	BT	72793
Kudoa clupeidae	47/145	†	SM	76794
Monogenea				
Microcotyle sebastis (Fig. 7)	106/115	15.1	G	76795
Digenea				
Derogenes varicus (Fig. 9)	3/114	1	S	76796
Sterrhurus exodicus (Fig. 8)	13/114	1.2	S	76797
Psettarium sebastodorum	35/112	2.1	H	76798
Deretrema cholaeum	14/100	1.4	BD	76799
Stephanostomum sp. (metacercaria)	3/100	1 cyst	PF	76800
Cestoidea				
Parabothriocephalus sagitticeps (Figs. 1, 3, 4)	92/112	5.3	I	76801
Grillotia smarisgora (metacestode)	81/115	9.3	GBW, M, SW	76803
Lacistorhynchus tenuis (metacestode) (Fig. 10a, b)	94/115	7.9	IW, L, M, PC, SM, SW, Sp	76804
Callitetrarhynchus(?) sp. (metacestode) (Fig. 2)	14/115	1.4	M	76802
Trypanorhyncha gen. sp. (metacestode) (Fig. 5)	3/115	10	M, SW	76805
Scolex pleuronectis, uniloculate form	4/112	†	I	76806
Acanthocephala				
Echinorhynchus sp.	1/112	1	I	76807
Corynosoma strumosum (cystacanth) (Fig. 6)	3/115	1	M	76808
Nematoda				
Hysterothylacium aduncum	19/112	3.4	I, S	76809
Cucullanus sp.	2/112	1	I	76810
Anisakis simplex (larva)	101/115	15	M, L, SW	76811
Phocanema decipiens (larva)	10/145	1.1	M, SM, SW	76812
Crustacea				
Naobranchia occidentalis	22/115	3.2	G	76813
Lepeophtheirus sp.	1/145	1	ES	76814
Gnathia sp.	1/145	1	ES	76815
Lafystius sp.	5/145	10.4	DF, ES, PF	76816

* Abbreviations: BD, bile duct; BT, bulbus and truncus arteriosus; DF, dorsal fin; ES, exterior surface; G, gills; GB, gall bladder; GBW, gall bladder wall; H, heart; I, intestine; IW, intestinal wall; M, mesentery; PC, pyloric ceca; PF, pectoral fin; S, stomach; SM, skeletal muscle; SW, stomach wall; Sp, spleen.

† The mean number of parasites in some fish was not calculated due to difficulty in determining accurate counts.

Parasites observed in this study that heretofore have not been reported from *S. paucispinis* include *Callitetrarhynchus*(?) sp., *Gnathia* sp., and *Lafystius* sp. The metacestode *Callitetrarhynchus*(?) sp. and the adult acanthocephalan *Echinorhynchus* sp. are probably undescribed species.



Figures 1–10. Parasites of *Sebastes paucispinis*. **1.** Preadult of *Parabothriocephalus sagitticeps*, dorsoventral view. **2.** Metacestode of *Callitetrarhynchus* sp. **3.** Scolex of *P. sagitticeps*, lateral view. **4.** Gravid proglottid of *P. sagitticeps*, dorsal view. **5.** Unidentified trypanorhynchan metacestode. **6.** Cysticanth of *Corynosoma strumosum*. **7.** *Microcotyle sebastis*. **8.** *Sterrhurus exodicus*. **9.** *Derogenes varicus*. **10a.** Encapsulated metacestode of *Lacistorhynchus tenuis*. **10b.** Encapsulated metacestode of *L. tenuis*; note shape of the blastocyst.

We thank Linda and Wayne Caywood, Michelle and Mark Ridgway, and Jon Christensen for providing fish for this study. L. Margaret Kowalczyk illustrated the parasites; David G. Huffman provided the meristogram analysis for *Echinorhynchus* sp.; D. I. Gibson, Robin M. Overstreet, and Tom E. Mattis assisted in the identification of some of the trypanorhynchs; Richard C. Brusca confirmed the identity of the isopod; and J. Laurens Barnard identified the amphipod.

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Research Note

Internal Parasites of *Odocoileus hemionus* of Central Utah

Sixty female and 16 male mule deer, *Odocoileus hemionus*, were examined for internal parasites from June to November 1981 in central Utah. The study was undertaken primarily to determine if mule deer are a significant reservoir for parasites that infect man and domestic animals. This is the first comprehensive survey of the internal parasites of *O. hemionus* in Utah, although several accounts of specific parasites are noted in the literature. Wehr and Dickmans (1935, Zool. Anz. 110:202–208) reported *Elaeophora schneideri*; Doman and Rasmussen (1944, J. Wildl. Manage. 8:317–338) recovered *Dictyocaulus viviparus* and also cysticerci of *Taenia hydatigena*; Grundmann (1958, J. Parasitol. 44:425–429) found *Thysanosoma actinioides*; Yuill et al. (1961, Wildl. Dis. No. 10) reported *Onchocerca cervipedis*; and Walker and Becklund (1970, Spec. Publ. No. 1, Index-Catalogue of Medical and Veterinary Zoology) identified *Trichuris* sp. and *Protostrongylus macrotis*.

The animals examined in the present study were killed by personnel of the Utah State Division of Wildlife Resources as part of a control program to prevent crop damage. The abomasum, small and large intestines, pancreas, liver, bile ducts, kidney, spleen, diaphragm, lungs, and heart were examined macroscopically in the laboratory. Because the deer were to be auctioned to the general public and could not be mutilated, the skeletal muscle, subcutaneous tissue, and

Table 1. Internal parasites recovered from *Odocoileus hemionus* of central Utah.

Parasite	No. deer infected/ no deer examined	Site of infection*	USNM no.
Protozoa			
Eimeria mccordocki†	9/76	F	76734
Cestoidea			
Thysanosoma actinioides	3/76	SI	76728
Moniezia benedeni†	15/76	SI	76729, 76740
Moniezia expansa†	1/76	SI	76730
Taenia hydatigena (cysticercus)	25/76	H, M, L, Lu	76731
Taenia ovis krabbei (cysticercus)†	1 animal infected‡	D	76732
Taenia omissa (cysticercus)†	4/76	D, M	76930
Nematoda			
Ostertagia circumcincta†	38/76	A	76735
Trichuris sp.	1/76	LI	76736
Dictyocaulus viviparus	2/76	B	76737
Protostrongylus macrotis	1/76	B	76738
Elaeophora schneideri	1 animal infected‡	Ar	76739
Onchocerca cervipedis	2 animals infected‡	ST	76785
Filarioid larvae†	4/26	Bl	76733

* Abbreviations: A, abomasum; Ar, artery; B, bronchi; Bl, blood; D, diaphragm; F, feces; H, heart; L, liver; LI, large intestine; Lu, lung; M, mesentery; SI, small intestine; SM, skeletal muscle; ST, subcutaneous tissue.

† Not previously reported from mule deer in Utah.

‡ Prevalence not calculated due to incomplete examination of skeletal muscle, subcutaneous tissue, and the arterial system.

the arteries in the neck were not thoroughly dissected; therefore, the prevalences for parasites found in these locations were not calculated. Fecal samples from all animals were checked microscopically for protozoa and helminth eggs, and blood samples from 26 deer were examined for microfilariae.

One protozoan species and 13 helminth species were recovered (Table 1). Parasites not previously reported from *O. hemionus* in Utah included *Eimeria mccordocki, Moniezia benedeni, M. expansa, Taenia ovis krabbei, T. omissa, Ostertagia circumcincta,* and undetermined microfilariae. The most prevalent parasite detected was *O. circumcincta,* and deer may serve as a reservoir of this parasite for domestic ruminants. Other helminths identified that have veterinary importance were *M. benedeni, T. hydatigena, D. viviparus,* and *E. schneideri.* The microfilariae were not identified to species, and did not resemble those described from deer by Hibler and Adcock (1971, Parasitic Diseases of Wild Mammals. Iowa State Univ. Press). Our filarioid larvae ranged in size from 67 to 95 by 7 μm, and were characterized by a sheath and a blunt tail.

Two deer each possessed a single hepatic cyst, irregularly shaped, approximately 5 mm in diameter, uniloculate, and containing only a translucent substance. The geographic area surveyed is endemic for *Echinococcus granulosus* (Loveless and Andersen, 1978, Am. J. Vet. Res. 38:499–502), and hydatid cysts have been reported in mule deer from both California (Brunetti and Rosen, 1970, J. Parasitol. 56:1138–1140) and Oregon (Hall, 1925, J. Parasitol. 12:105). Because the cysts previously found in mule deer were in the lungs, the hepatic cysts found in our study cannot be identified specifically as *E. granulosus.*

We are indebted to Harold Blackburn, Rodney T. John, and Jordan C. Pederson

of the Utah State Division of Wildlife Resources for their cooperation in this study. Thanks are due to David E. Worley for assisting in the identification of *P. macrotis* and Charles P. Hibler for confirming that the filarioid larvae were not *E. schneideri* or *O. cervipedis*. This study was supported in part by NIH Grant AI-10588-10.

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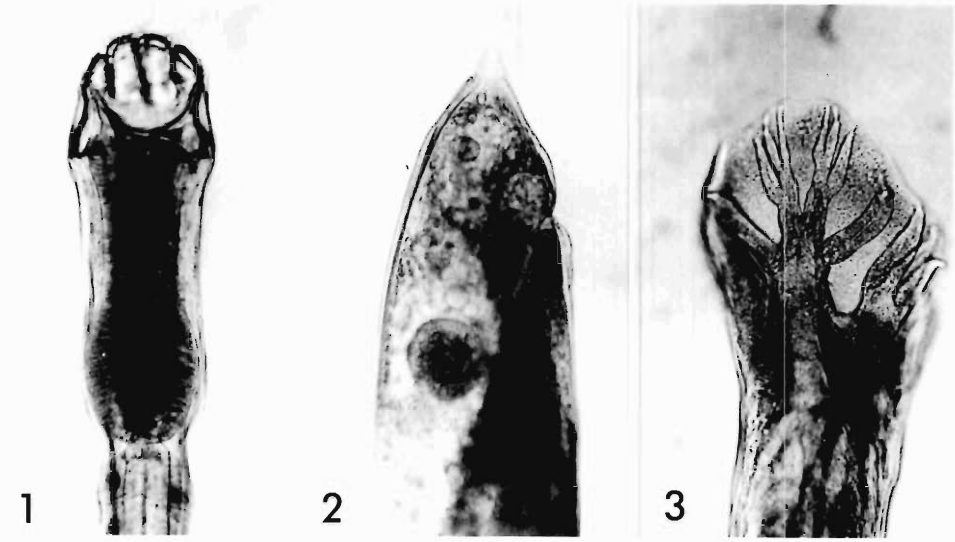
Research Note

Helminths of *Cryophis hallbergi* (Serpentes: Colubridae), a Cloud Forest-dwelling Snake from Oaxaca, Mexico

Cryophis hallbergi Bogert and Duellman, 1963 is known only from cloud forest habitat in the Atlantic drainage of the Sierra Madre del Sur, Oaxaca, Mexico. To our knowledge, the only report of this species was the original description based on two specimens (Bogert and Duellman, 1963, Am. Mus. Novit. 2162:1–15). In expeditions into southern Mexico during 1978 and 1980 we collected five additional specimens. The snakes were taken near Valle Nacional, Oaxaca, where cloud forest habitat is steep and rugged. The elevation was approximately 1,100 m above sea level, and the flora was characterized by *Liquidamber, Acer,* and *Quercus* as the dominant trees, with tree ferns, bromeliads, and orchids abundant. The restricted range of *C. hallbergi,* coupled with increasing disturbance of cloud forest habitat by logging operations, makes it doubtful that this host will again be available for parasitological examination. Therefore, the helminths we recovered and the ecological observations we made on the five specimens of *C. hallbergi* form the basis of this brief report.

Hosts were taken in the vicinity of small waterfalls, but not necessarily restricted to them. One snake was found crawling up the vertical, heavily vegetated, rock face of a waterfall, and a second specimen was collected at the margin of a pool at the base of another waterfall. Two additional snakes were crossing a nearby road. These four snakes were all collected while they were foraging at night; the fifth snake was taken during the day under a log.

Three of the *C. hallbergi* were infected in the esophagus and small intestine with five, four, and four *Kalicephalus* (*Inermiformis*) *macrovulvus* Caballero, 1954, respectively (Figs. 1–3). These diaphanocephalid nematodes are hookworm-like parasites of reptiles with direct life cycles of free-living juveniles and parasitic adults. In Schad's (1962, Can. J. Zool. 40:1035–1165) revision of *Kalicephalus,* he regarded *K. inermis* Molin, 1861 (from Brazil), *K. macrovulvus* Caballero, 1954 (from Guatemala), and *K. coronellae* Ortlepp, 1923 (from the United States and Mexico) as subspecies of the oldest-named *K. inermis*.



Figures 1–3. ***Kalicephalus (Inermiformis) macrovulvus*** **from** ***Cryophis hallbergi*****. 1. Anterior end with rounded face (×80). 2. Female tail (×80). 3. Male copulatory bursa (×80).**

Our specimens were collected from a locality that lies between the southern edge of the range of *K. (I.) coronellae* (Guerrero and Veracruz, Mexico) and that of *K. (I.) macrovulvus* (Guatemala). The rounded face, bulbed esophagus, and palmate branches of the copulatory bursa of the male conform to Schad's concept of *K. (I.) macrovulvus*. Notable, however, are the short, conical tails of the females in our collection, which are more similar to that described by Schad for *K. (I.) inermis* from Brazil. Measurements for our specimens are presented in Table 1.

A single, encysted acanthocephalan was found in the mesenteries of one *C. hallbergi* that was also infected with four of the *Kalicephalus*. We were unable to identify this immature worm.

Table 1. Measurements of *K. (I.) macrovulvus* from Oaxaca, Mexico (in mm).

	Females (N = 8)	Males (N = 5)
Length	9.3–11.3	6.6–8.7
Maximum width	0.26–0.55	0.30–0.32
Length of buccal capsule	0.18–0.20	0.15–0.18
Length of esophagus	0.35–0.40	0.33–0.35
Width of esophagus	0.19–0.20	0.15–0.16
Vulvar ratio*	1.65–2.05	—
Distance from anus to posterior end	0.16–0.20	—
Spicule length	—	0.60–0.67

* Length of prevulvar body divided by postvulvar body.

Examination of the stomach contents of four of the hosts revealed no clue as to their diet. We suspected that these snakes may feed on frogs and lizards, since these are the prey items of the snake genus *Leptodeira,* which Bogert and Duellman (1963, loc. cit.) postulated as one of the closest relatives phylogenetically of *Cryophis*. A search was initiated for frogs that may have been a potential food source. This yielded several leptodactylid frogs that were far too large to be consumed by *C. hallbergi,* but this does not preclude the tadpole or juvenile from being preyed upon. The only lizards found at this elevation were *Xenosaurus grandis* (Gray) and *Sceloporus* sp. No fish were present in any of the pools examined.

Specimens of *K. (I.) macrovulvus* and the encysted acanthocephalan were deposited as USNM Helm. Coll. Nos. 76608 and 76609, respectively. Four of the *C. hallbergi* were deposited in the Louisiana State University Museum of Zoology as 39051, 36979, 39532, and 39568; the fifth was returned to Mexico in compliance with permit regulations.

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Research Note

Infection of *Microtus pinetorum* with the Nematode *Capillaria gastrica* (Baylis, 1926) Baylis, 1931

From May 1978 to March 1979 a total of 298 pine voles, *Microtus pinetorum,* were snap-trapped from apple orchard habitats located in Botetourt County in southwestern Virginia. All voles were examined at necropsy on the day of capture. Of the voles examined, two lactating females collected during the month of May 1978 were found to have stomachs that were grossly enlarged, thickened, and externally rugose in appearance. The opened stomach showed a capacity much reduced in volume as a result of extreme folding and thickening of the gastric wall. Stomach tissues were preserved in 10% formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin.

Histologic examination of the stomach sections revealed severe hyperplasia of the mucosa, in which were embedded myriads of nematodes and double-oper-

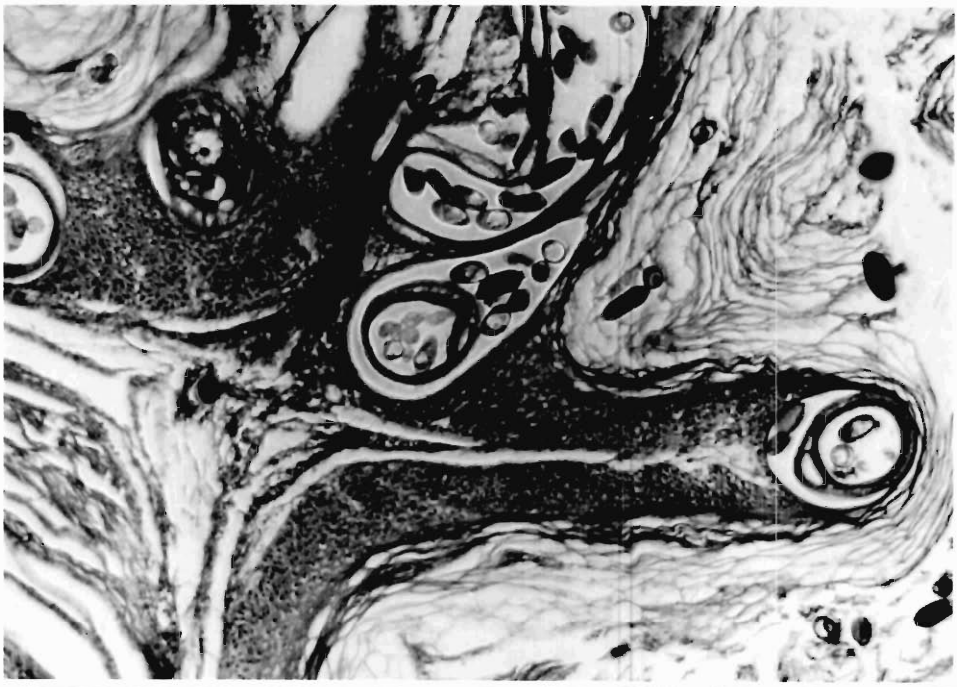

Figure 1. Photomicrograph showing the severe hyperplasia and folding of the gastric mucosa with embedded *Capillaria gastrica* and eggs in the vole *Microtus pinetorum*, ×10.

culated eggs of the species *Capillaria gastrica* (Baylis, 1926, J. Trop. Med. Hyg. 29:226–227) (Fig. 1). The eggs (N = 30) measured, on average, 60 by 26 μm. All nematodes were embedded in the gastric mucosa, and none was observed free in the gastric lumen.

Levine (1980, Nematode Parasites of Domestic Animals and of Man, 2nd ed. Burgess Publ. Co., Minneapolis. 447 pp.) listed *C. gastrica* as occurring in *Rattus norvegicus, Rattus rattus, Apodemus sylvaticus,* and the voles *Microtus socialis* and *Microtus schidlovskii.* Obendorf (1979, Aust. J. Zool. 27:867–879) reported the occurrence of *C. gastrica* in *Rattus fuscipes* and *Rattus lutreolus.* This is the first report of *C. gastrica* in the pine vole.

Dunaway et al. (1968, Bull. Wildl. Dis. Assoc. 4:18–20) found infections of an unidentified species of *Capillaria* in the gastric mucosa of the vole *Microtus ochrogaster,* with gastric tissue reactions similar to those described in this paper. Cosgrove and O'Farrell (1965, J. Mammal. 46:510–513) described stomachs collected from pine voles, which showed markedly thickened epithelia with rough, papillary hyperplasia of the mucosal surface. They could not identify the cause of the observed papillary lesions. They did observe a protozoan parasite in the gastric muscle fibers of one pine vole; however, no other gastric parasites were seen. Their descriptions indicated that the observed voles were possibly recovering from a previous infection with *C. gastrica.* No other report of a possible or actual *Capillaria* infection in the species *M. pinetorum* could be found. The prevalence of infection in our sample of 298 pine voles was low (<1%). However,

this was only an estimate of prevalence of the severe infections described above. Voles harboring low to moderate burdens of this parasite may not have shown such visible hyperplasia of the stomach wall, and thus were not detected.

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Research Note

Leeches of Some Marine Fishes from Puerto Rico and Adjacent Regions

Sawyer et al. (1975, J. Nat. Hist. 9:633–667), Wallace and Sawyer (1976, Bull. Mar. Sci. 27:347–348), and Sawyer and Kinard (1980, Caribb. J. Sci. 15(3–4):83–85) reported three leeches from three species of marine fishes in Puerto Rico (Table 1). The following report notes the leeches collected from over 2,000 fishes, representing over 500 species, examined principally from Puerto Rico and Mona Island; in addition some fishes were examined from the U.S. and British Virgin Islands, central and southern Bahamas, the south coast of the Dominican Republic, Jamaica, Barbados, Trinidad, Tobago, Curaçao, Bonaire, Bermuda, Colombia, and Panama. Fishes were collected with seines, monofilament gill and trammel nets, hook and line, gig, dip net, rotenone, trawl, spear gun, and fish trap. They were either examined immediately or held individually in plastic bags and examined within 1 hr of capture. Examination included the surface, nostrils, eye sockets, mouth, gills, pseudobranchs, and gill chambers of all fishes. Specimens are deposited in the United States National Museum. Isopods from these hosts were reported by Williams and Williams (1977, Proc. Assoc. Isl. Mar. Lab. Caribb. 13:14). One leech not previously noted to occur in the West Indies and 13 new host records are reported.

Table 1. Leeches collected from some marine fishes in Puerto Rico and adjacent areas.

Leech (no. per host)	Host	No. infested/ no. examined	Location on host
*Branchellion ravenelii**			
(1)	*Aetobatius narinari* (Euphrasen), spotted eagle ray	1/5	Left nasal basket
Malmiana philotherma			
(3)	*Hemiramphus brasiliensis* (Linnaeus), ballyhoo†‡	3/123	Posterior to base of pectoral fin
Stibarobdella macrothela			
(1–10)	*Ginglymostoma cirratum* (Bonnaterre), nurse shark	14/14	Roof of mouth or tongue
(1)	*Galeocerdo cuvieri* (Peron and Leseur), tiger shark§	3/17	Roof of mouth
(3)	*Negaprion brevirostris* (Poey), lemon shark§	1/5	Roof of mouth
(1–2)	*Sphyrna mokarran* (Ruppell), great hammerhead§	2/4	Mouth
Trachelobdella lubrica			
(1)	*Acanthurus bahianus* (Castelnau) ocean surgeon§	1/54	Gill filaments
(1)	*Archosargus rhomboidalis* (Linnaeus), sea bream§	1/8	Gill filaments
(1)	*Cantherhines macrocerus* (Hollard), whitespotted filefish§	1/4	Gill filaments
(1)	*Epinephelus guttatus* (Linnaeus), red hind§	1/34	Gill filaments
(1)	*Epinephelus striatus* (Bloch), Nassau grouper§	2/29	Under opercular cover
(1–2)	*Haemulon album* Cuvier, margate§	3/9	Gills
(1)	*Haemulon flavolineatum* (Desmarest), French grunt§	2/258	Under opercular cover
(1–3)	*Haemulon sciurus* (Shaw), bluestriped grunt§	2/25	Under opercular cover
(1–10)	*Lachnolaimus maximus* (Walbaum), hogfish§	15/29	Gills chamber
(—)	*Lutjanus cyanopterus* (Cuvier), cubera snapper‡‖	1/3	Gills chamber
(1)	*Scorpaena plumieri* Bloch, spotted scorpionfish§	1/4	Ventral surface

* New record for the West Indies.
† Wallace and Sawyer (1976).
‡ Sawyer and Kinard (1980).
§ New host record.
‖ Sawyer et al. (1975).

BRANCHELLION RAVENELII (GIRARD, 1850): Although this leech has been reported from the ventral surface of various rays and skates from the Atlantic and Gulf coast of the United States (Sawyer et al., 1975, loc. cit.) a specimen occurred in the right nasal capsule of a spotted eagle ray in Puerto Rico. Seven Monogenea (Trematoda) occurred in the left nasal capsule, but none occurred in the right capsule with the leech.

STIBAROBDELLA MACROTHELA (SCHMARDA, 1861): This is a very common parasite of the nurse shark. Every nurse shark examined possessed at least one of these leeches and one shark was infested with 10. Normally the leeches occurred on the roof of the mouth; occasionally on the tongue, gill filaments, arches, or chamber; and rarely on the fins or body.

TRACHELOBDELLA LUBRICA (GRUBE, 1840): This leech seems to occur rather erratically on a variety of hosts in southwestern Puerto Rico. The 11 hosts represent eight different families of fishes. It occurs very commonly on the gills of adult hogfish in Puerto Rico, and was collected on this host in Tobago.

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Edgardo Ortiz, and Jorge Rivera López for help in collecting and examining hosts. Special thanks to Dr. Eugene M. Burreson, Virginia Institute of Marine Sciences, for review of the manuscript and confirmation of leech identifications. Support was provided in part by Grant No. 8852 from the American Philosophical Society.

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Research Note

Abnormal Morphology in *Contracaecum* sp. (Anisakidae: Anisakinae)

An unusual abnormality occurred in a species of *Contracaecum* Railliet and Henry, 1912 removed from the stomach of a Laysan or Hawaiian monk seal, *Monachus schauinslandi* Matschie. One of 13 specimens of *Contracaecum* sp. was found to possess a posteriorly directed intestinal cecum (Fig. 1).

The Hawaiian monk seal, an endangered species, was found dead on French Frigate Shoals in the northwestern Hawaiian Islands in 1977 and necropsied by Whittow et al. (1978, Elepaio 38:83–84). The method of fixation for these worms is unknown; however, they were stored in a solution of 5 parts glycerin and 95 parts 70% ethyl alcohol. All worms were in good condition. I stripped the cuticle from the anterior portion of the abnormal worm and verified that the distal portion of the intestinal cecum was attached by short fibers to the adjacent basement membrane of the worm (Fig. 2). The worm measured 23 mm long, with a ratio of cecal to esophageal lengths of 1:1.8, a ratio of cecal to ventricular appendage lengths of 1:0.4, and a ratio of ventricular appendage to esophageal lengths of 1:4.0, as compared with 1:1.4–1.8, 1:0.4–0.6, and 1:3.0–6.0 for the other specimens, respectively.

Because the presence, length, and presumably other features of the cecum determine generic significance, the recognition of an abnormal one is important. The intestinal cecum of members of the genus *Contracaecum*, and all other ascaridoid nematodes that possess one, is normally directed anteriorly and attached by fibers to the inner wall. This report is the first account of a posteriorly directed cecum in members of the genus *Contracaecum*; however, it is the second report for this anomaly in ascaridoid nematodes. Punt (1941, Mem. Mus. R. Hist. Nat. Belg. 98:1–110) illustrated a recurved intestinal cecum that occurred in *Hysterothylacium aduncum* (Rudolphi, 1802) (as *Contracaecum a.*) but did not indicate if it was attached at its distal aspect.

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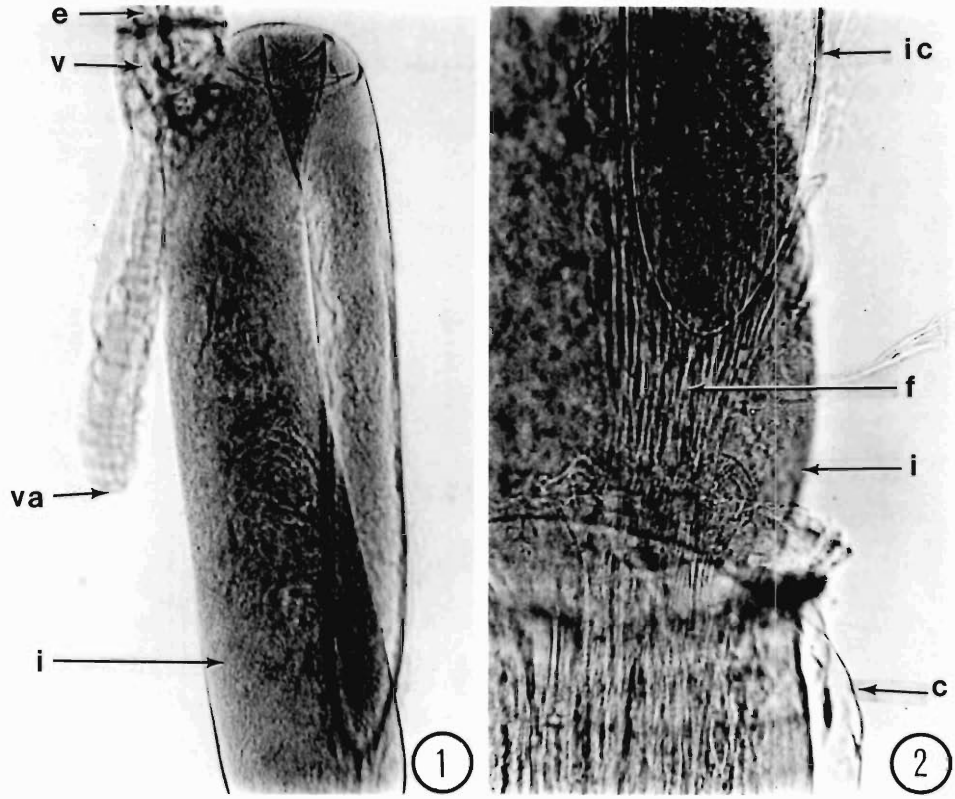

Figures 1, 2. Abnormal *Contracaecum* sp. 1. Anterior extremity of female with stripped cuticle showing the intestinal cecum–ventricular appendage junction, lateral view, ×6.3. 2. Distal portion of intestinal cecum showing attachment, ×16. c, cuticle; e, esophagus; i, intestine; ic, intestinal cecum; f, attachment fibers; v, ventriculus; va, ventricular appendage.

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Research Note

The Chick as an Experimental Host of *Cyclocoelum mutabile* (Zeder, 1800) (Digenea)

Adult *Cyclocoelum mutabile* (Zeder, 1800) parasitize the air sacs of coots (*Fulica* spp.) and other aquatic birds (McDonald, 1969, Bur. Sport Fish. and Wildl. Spec. Sci. Rep. Wildl. 126. 692 pp.). After ingestion, young flukes spend the first 12–15 days in the liver, before migrating to the air sacs where they mature (McLaughlin, 1977, Can. J. Zool. 55:274–279). Unfortunately, detailed studies on the host–parasite relationship during fluke migration are difficult because young coots are only available seasonally and are difficult to rear in sufficient numbers for experimental purposes. As the domestic chick is a suitable experimental host for several digeneans of waterfowl (e.g., Macy et al., 1968, J. Parasitol. 54:28–38; Fried and Nelson, 1978, Parasitology 77:49–55), we undertook the following experiments to assess the suitability of the chick as a laboratory host for *C. mutabile*.

General techniques for rearing and infecting coots, *Fulica americana* (Gm.), and snails, *Promenetus exacuous* (Say), and necropsy procedures have been described previously (McLaughlin, 1976, Can. J. Zool. 54:48–54), as have techniques for the excystment of metacercariae and parenteral infection of coots (McLaughlin, 1980, Can. J. Zool. 58:71–74).

Coots used in this study were 5–7 wk old when exposed; white rock chicks, purchased from a local hatchery, were 5–9 days old when exposed. The birds were maintained on a commercial diet of unmedicated duck and goose crumbles ad lib.

Six coots and six chicks each received 20 *C. mutabile* metacercariae per os and necropsied at 6 days postinfection (PI). The results are presented in Table 1. Four of the coots harbored flukes in the liver; none of the chicks was infected. As the metacercariae had all been drawn from the same pool, it was concluded that the failure of chicks to become infected was not due to the metacercariae but rather to conditions in the host.

Bile is known to affect excystment and to play a role in the host specificity of a number of helminths (see Lackie, 1975, Biol. Rev. 50:285–323 for summary). To test whether bile might play a role in the failure of *C. mutabile* to establish in chicks, two groups of 20 metacercariae were drawn from a pool of 100 metacercariae. One group was incubated in a medium containing 0.5% coot bile + 0.5% trypsin (Anachemia 1:100) in Tyrode's solution at 41°C; 0.5% chick bile was substituted for coot bile in the other group. Both groups were examined at 15-min intervals for 1 hr. The results are presented in Table 2. Excysted flukes were removed at each interval, placed in the same type of medium as that in which they excysted, and their activity was observed continuously for the next 15 min. The total number of metacercariae excysting in either medium did not differ significantly; however, significantly more metacercariae had excysted in coot bile by 30 min than in chick bile (chi-square; $P < 0.05$). *Cyclocoelum mutabile* will excyst in 0.5% trypsin in Tyrode's at 41°C, but do so more slowly than when coot bile is present (unpubl. data). Coot bile therefore enhances excystment, but

Table 1. Results of oral (o) and intraperitoneal (i.p.) exposure of coots and chicks to *C. mutabile* metacercariae.

Host	*N*	Method	Days infected	No. infected	Total flukes	% alive
Chick	6	o	6	0	0	0
Coot	6	o	6	4	8	100
Chick	11	i.p.	6	10	32	80
Chick	10	i.p.	12	5	11	45.5
Chick	10	i.p.	15	1	1	0
Coot	5	i.p.	18	5	25	100

is not an absolute requirement. It is thus questionable whether the chick bile had an effect on excystment or whether this was due to trypsin alone. Excysted metacercariae placed in a medium containing chick bile were virtually inactive after 10 min. Those placed in the medium containing coot bile were still contracting vigorously 15 min after transfer, when the observations were terminated. These results suggest that the conditions in the chick gut are an effective barrier to infection by *C. mutabile,* as few metacercariae seem to excyst quickly enough or remain active long enough to penetrate the intestine.

As the conditions in the chick intestine apparently provide an effective barrier to infection by orally administered metacercariae, attempts were made to establish infections parenterally, thus bypassing the intestinal phase.

Five coots and 31 chicks each received 20 excysted metacercariae intraperitoneally. Eleven, 10, and 10 chicks were examined at 6, 12, and 15 days PI; the coots were examined on day 18. The results are summarized in Table 1. No significant differences in the number of infected coots, chicks at day 6, or chicks at day 12 were found. Significantly more coots and chicks at day 6 were infected than chicks at day 15 (Fisher's exact test; $P = 0.002$ and $P = 0.003$, respectively).

All flukes recovered from coots were alive, but survivorship in chicks declined steadily with time (Table 1). No living flukes and only a single recognizable dead fluke were found in the livers of chicks on day 15. Several amorphous masses were found in the chick livers on day 12 and a few on day 15. Subsequent examination revealed that these contained dead flukes. As only representative specimens of these masses were saved, these data were not included in Table 1. Survivorship at day 12, based on recognizable flukes (Table 1), is thus overestimated. No flukes were found in either the body cavity or air sacs of chicks.

Comparison of flukes from chicks and from coots (coot data from this study and McLaughlin, 1977, loc. cit.) revealed that growth and development were

Table 2. Comparison of excystment of *C. mutabile* metacercariae in media containing 0.5% coot bile and media containing 0.5% chick bile.

		Number excysting between (min)				
Medium with	*N*	0 and 15	16 and 30	31 and 45	46 and 60	Unexcysted
Coot bile	20	3	11	1	1	4
Chick bile	20	0	2	3	4	11

Table 3. Comparison of the mean lengths of *C. mutabile* from coots and chicks at 3, 6, and 12 days postinfection.

Days PI	Mean length ± standard deviation	
	Coot	Chick
3	0.64 ± 0.07	—
6	1.59 ± 0.14	0.54 ± 0.11
12	4.99 ± 0.43	1.49 ± 0.29

retarded in the chick. Six- and 12-day-old flukes from chicks were significantly shorter than flukes of the same age from coots (Table 3). Six-day-old flukes from chicks closely resembled 3-day-old flukes from coots in both size and degree of development, whereas 12-day-old flukes from chicks were similar to 6-day-old flukes from coots.

Previous studies have shown that 1-day-old chicks can be infected successfully with waterfowl digeneans by oral intubation of metacercariae pretreated in either acid-pepsin and trypsin or in sodium bicarbonate (Macy et al., 1968, loc. cit.; Fried and Nelson, 1978, loc. cit., respectively). We do not know whether pretreatment of *C. mutabile* metacercariae and/or the use of younger hosts would have resulted in infections in orally exposed chicks; however, the apparent toxic effects of chick bile suggests that few, if any, flukes would establish themselves. Further, there is no reason to assume that growth or survivorship would differ from that observed for parenterally administered flukes.

In view of the foregoing, the domestic chick is not a suitable laboratory host for *C. mutabile*.

We thank Mr. Peter Ward and Dr. Bruce Batt for use of facilities at the Delta Waterfowl Research Station, Delta, Manitoba. The Canadian Wildlife Service (Winnipeg office) kindly provided the necessary permits to collect and rear coots. The work was supported by a Natural Science and Engineering Research Council of Canada Grant A6979.

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Research Note

Preliminary Observations on Survival and Pairing of *Schistosoma mansoni* on the Chick Chorioallantois

Although numerous studies are available on the survival and development of hermaphroditic digenetic trematodes on the chick chorioallantois (Fried, 1969, Proc. Pa. Acad. Sci. 43:232–234), there are no similar studies on mammalian or avian schistosomes. Survival and egg-laying of turtle blood flukes (spirorchids) on the chick chorioallantois were reported (Fried and Tornwall, 1969, Proc. Helminthol. Soc. Wash. 36:86–88). This note reports preliminary pairing of *Schistosoma mansoni* adults on the chick chorioallantois.

Chick embryos were prepared according to Zwilling (1959, Transplant. Bull. 6:115–116) as modified by Fried (1973, J. Parasitol. 59:591–592) and were used on day 10 postembryonation. Six- to 8-wk-old adult *S. mansoni* worms were perfused from mice (Radke et al., 1961, J. Parasitol. 47:366–368; Imperia et al., 1980, J. Parasitol. 66:682–684), rinsed briefly in a sterile Locke's antibiotic solution (Fried, 1973, loc. cit.), and transferred to eggs within 0.5 hr after perfusion. Most worm pairs were cooled in an ice bath, separated by gentle prodding, then placed individually on the chick chorioallantois. In two eggs (eggs 1 and 9, Table 1), unseparated worm couples were placed on the chorioallantois. Eggs were maintained at 37.5 ± 0.5°C and relative humidity of 50–60%, then examined on the first and second days following inoculation.

As shown in Table 1, on day 1 all worms were alive. These worms contained black pigment within their intestinal ceca. This pigment, presumably egested by the flukes, was also present on the chorioallantois. Both sexes were very active, and showed typical peristaltic, writhing movements, as described by Fetterer et al. (1977, Exp. Parasitol. 43:286–294). By day 2 most of the worms were dead or moribund, although live worms were recovered from eggs 8 and 9. Some worms were encapsulated on the surface of the chorioallantois, as described for spirorchids (Fried and Tornwall, 1969, loc. cit.). Worms were usually localized near the center of the chorioallantois, and were not found in sites other than the chorioallantois. Contrary to the previous study on spirorchids, worm eggs were not found on the chorioallantois. Survival of *S. mansoni* adults on the chorioallantois appears limited to 2 days, whereas other trematodes survive in this site for 7–14 days (Fried, 1962, J. Parasitol. 48:545–550; 1973, loc. cit.; Fried and Butler, 1978, J. Parasitol. 64:175–177).

Worm-pairing is defined as a proximity of 5 mm or less between worms (Fried and Roberts, 1972, J. Parasitol. 58:88–91; Fried et al., 1980, J. Parasitol. 66:1014–1018), and is summarized in the last column of Table 1. Examination of chorioallantoic membranes within 1–2 min postinoculation indicates that flukes are usually within 1–6 cm of each other. Our pairing observations on days 1 and 2 indicate that schistosomes moved toward each other on the chorioallantois. A previous in vitro study showed that single females of *S. mansoni* were significantly attracted to individual males (Imperia et al., 1980, loc. cit.).

Egg 2, which received an uncoupled male and female from the same pair contained a pair in copula on day 1. Egg 9, inoculated with 3 worm pairs, contained

Table 1. Survival and pairing of adult *Schistosoma mansoni* on the chick chorioallantois.

Egg no.	No. of worms transferred		No. of days on chorio-allantois	No. of live worms recovered		No. of dead worms recovered		Pairing data*
	Males	Females		Males	Females	Males	Females	
1†	2	2	1	2	1	—	0	M–M
2	1	1	1	1	1	—	—	M–F
3	3	3	1	2	3	0	—	M–F
4	2	2	2	0	1	0	1	—
5	1	1	2	0	0	1	1	M–F
6	5	—	2	0	—	2	—	M–M
7	—	4	2	—	0	—	3	F–F
8	4	4	2	2	0	0	4	F–F
9†	3	3	2	2	2	0	0	M–F
Total	23	22	1–2	9	8	3	9	

* M = male; F = female.
† Worms transferred as couples.

2 worm pairs in contact on day 2. Results of our study indicate that *S. mansoni* adults have a tendency to both heterosexual and homosexual pairing on the chick chorioallantois.

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MINUTES

Five Hundred Forty-First Through Five Hundred Forty-Eighth Meetings

541st Meeting: Uniformed Services University of Health Sciences (USUHS), Bethesda, Maryland, 16 October 1981. The following slate of officers was presented: Louis S. Diamond (President), Milford N. Lunde (Vice President), Sherman S. Hendrix (Corresponding Secretary-Treasurer), Raymond V. Rebois (Recording Secretary). Life membership was presented to Dr. Leo A. Jackowski by President N. D. Pacheco. Dr. Kenneth C. Kates was announced as a Life Membership recipient. Honorary Membership was given to Dr. Hugh McLeod Gordon, University of Sydney, Australia. The following papers were presented, LTC Bryce C. Redington presiding: "Welcome to USUHS", J. W. Bullard; "Immunoelectron Microscopy of *Plasmodium falciparum*", S. G. Langreth; "Isolation and Characterization of Surface Glycoproteins from *Schistosoma mansoni* using Lectin Affinity Chromatograph", E. G. Hayunga; "Specific Malarial and Schistosomal Circulating Antibodies and Antigens in Nigerian Children", M. Stek.

542nd Meeting: Animal Parasitology Institute, USDA, Beltsville, Maryland, 13 November 1981. The Anniversary Award was presented to Dr. Frank D. Enzie by Dr. K. G. Powers. In Dr. Enzie's absence the award was accepted by Dr. H. Herlich. The slate of officers presented at the 541st meeting was unanimously elected. The Incorporation date of the Society was announced as 4 November 1981. Dr. Harry Herlich presided over the following papers: "Development of Hybridoma Antibodies Directed Against Avian Coccidia", H. C. Danforth; "Immunological Studies on Swine Ascariasis", J. F. Urban and F. G. Tromba; "Demonstration of Anti-horse Red Blood Cell Antibodies in a *Sarcocystis* sp infected Horse", L. C. Gasbarra and R. Fayer.

543rd Meeting: Plant Protection Institute, USDA, Beltsville, Maryland, cosponsors, U.S. Food and Drug Administration, Division of Veterinary Research, Bureau Veterinary Medicine and the Division of Microbiology, Bureau of Foods. 4 December 1981. President L. S. Diamond announced the following executive appointments: Judith H. Shaw and Larry D. Hendricks (Executive Committee Member-at-Large), Ralph Eckerlin (Assistant Corresponding Secretary), Leon Jacobs (Business Advisory Committee), Ralph Beudoin (Awards Committee), Gerhard A. Schad (Custodian of Back Issues), David R. Lincicome (Archivist), and Patricia A. Pilitt (Librarian). The following papers were presented, Drs. R. V. Rebois and K. G. Powers presiding: "Ultrastructure of the Stomatal Region of the Soybean Cyst Nematode", B. Y. Endo; "The Columbia Root-knot Nematode, A Serious Parasite on Potatoes in Five Western States", A. M. Golden; "Occult and Microfilaremic Dirofilariasis in Dog", K. G. Powers and J. M. Stiller; "Diphyllobothriasis Associated with Salmon in the United States, Results of 1981 Salmon Survey", J. W. Bier.

544th Meeting: National Institutes of Health, Laboratory of Parasitic Diseases, Bethesda, Maryland, 15 January 1982. Dr. A. W. Cheever presided over the

following papers: "Iodination of Surface Membrane Proteins of *Leishmania tropica*", Peter Gardiner; "Are All Parasites Created Equal" or "The Response of Inbred Mice to Infection with *Trypanosoma cruzi* Clones Isolated from a Single Strain", Marium Postan; "Development of a Solid Phase RIA for Quantitation of IgE in Schistosomiasis: Correlation with Skin Tests and Histamine Release", Rabia Hussain; "Can Molecular Biology Solve the Identity Crisis of Schistosomes?", Andrew Simpson.

545th Meeting: Naval Medical Research Institute, Bethesda, Maryland (Cosponsored by Oxford Laboratory, NOAA), 12 February 1982. Vice President M. N. Lunde announced the Audit Committee would give an interim report to the Executive Committee pending time needed to resolve some discrepancies in the bookkeeping. N. D. Pacheco summarized a letter from Hugh Gordon, written in appreciation of his having received an Honorary Membership to the Society in 1981. Drs. Wilton Vannier and Aaron Rosenfield presided over the following papers: "Induction of Resistance to Infection with *Schistosoma mansoni*", Beverly L. Mangold and David A. Dean; "Suppression of Parasite-antigen Specific Lymphoid Blastogenesis in African Trypanosomiasis", Yupin Charoenvit, Gary H. Cambell and Sei Tokudo; "Occurrence of a New Rhynocodid Ciliate in the American Oyster *Crassostrea virginica*", Steve Cooper; "Developmental Aspects of *Glugea stephani*", Ann Cali.

546th Meeting: Walter Reed Army Institute of Research, Washington, D.C., 12 March 1982. M. D. Ruff presented the "Report of the Audit Committee for 1981" which was approved and accepted by the Executive Committee. S. S. Hendrix handed out the "Statement of Cash Receipts and Disbursements for the Year Ending December 31, 1981" to the Society members present. The following papers were presented, COL. D. Davidson presiding: "Experimental Chemotherapy of Cutaneous Leishmaniasis in a Laboratory Model", Patrick McGreevy; Analysis of *Leishmannia* Species and Strain Keinetoplast DNA", Peter R. Jackson, John A. Wohlhieter and Wayne Hockmeyer; "Antimalarial activity of 2-acetylpyridine thiosemicarbazones *in vitro*", Chris Lambros.

547th Meeting: The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, 16 April 1982. G. F. Otto announced the Society is expected to run a deficit of about $2,300 in 1982 unless corrective action such as increasing page charges is instituted. President L. Diamond announced that A. James Haley requested to be retired from his position as Editor in 1983. G. F. Otto was appointed Chairman of the Committee to select a new editor. Dr. T. W. Simpson presided over the following papers: "Modulation of Immune Response in Chronic Chagas Disease", Renato Gusmao; "Chemoprotective Effects of an Antischistosomal Compound", Sherry S. Ansher; "The Detection of Distinct and Homologous Antigens of *Onchocerca volvulus* and *Dirofilaria immitis* by Enzyme-linked Immuno-inhibition Assay (ELIA)", William M. Boto; "Onchocerciasis Transmission: Parasite Prevalence in the Host and Vector", Victor Barbiero.

548th Meeting: The University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, 8 May 1982. This was a joint meeting with the New

Jersey Society of Parasitologists. President L. Diamond summarized a report by Dr. G. F. Otto, Chairman of Business Advisory Committee which projected a potential financial deficit status for the Society for 1982. It was announced that the Executive Committee had approved increasing page charges to twenty dollars per page. The title for the Symposium was "The Vertebrate Intestine as a Habitat for Parasites". Dr. Gerhard S. Schad presided. The following papers were presented: "Microbial Ecology; The Influence of Other Organisms", Sherwood L. Gorback; "Physiological Aspects", David F. Mettrick; "Effect of Host Diet on the Intestinal Habitat", Michael Ruff; "Immunological Aspects", Joseph Urban. The following 30 new members were elected at the meetings indicated: *541st:* Bernice M. Fernandes, Scott L. Gardner, Larry N. Gleason, Naim Akhtar Khan, Anna Kohn, Fan-Yao Kung, Becky A. Lasee, Vincent Z. Lopez, Susan V. Marcquenski, Michael G. Pappas, Roberto M. Pinto, and Howard T. Underwood. *543rd:* H. Ray Gamble, Zafar Ahmed Handoo, and Douglas P. Jasmar. *544th:* John F. Finerty, Henry L. Francis, Takashi Schimazu, and Joseph E. Toole. *545th:* Richard J. Cawthorn, Philip E. Coyne, Jr., David A. Levy, David J. De Mont, and Dennis A. Thoney. *546th:* M. A. Idris and Jeffrey D. Shields. *547th:* Yupin Charoenvit. *548th:* Raymond H. Fetterer, Herbert W. Haines and Gene E. Miller.

RAYMOND V. REBOIS
Recording Secretary

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