Number 2

PROCEEDINGS

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Studies on Monogenea of Pakistan. III. Status of the Calceostomatidae (Parona and Perugia, 1890) with a Redescription of Neocalceostoma elongatum Tripathi, 1957

and the Proposal of Neocalceostomoides gen. n.

D. C. KRITSKY,¹ J. D. MIZELLE,² AND F. M. BILQEES³

ABSTRACT: Neocalceostoma elongatum Tripathi, 1957 (Dactylogyridae: Calceostomatinae) is redescribed from the gills of Arius serratus (Day), Ariidae, from the Karachi Coast, Pakistan. The diagnosis of Neocalceostoma Tripathi, 1957, is emended, and Neocalceostomoides gen. n. is proposed for N. arii (Unnithan, 1964) comb. n. A new dactylogyrid subfamily, the Pseudomurraytrematinae, is erected for Pseudomurraytrema Bychowsky, 1957, Anonchohaptor Mueller, 1938, Myzotrema Rogers, 1967, and Icelanonchohaptor Leiby, Kritsky, and Peterson, 1972. The Calceostomatidae (Parona and Perugia, 1890), containing Calceostoma van Beneden, 1858, Calceostomella Palombi, 1943, Neocalceostoma Tripathi, 1957, Paracalceostoma Caballero and Bravo-Hollis, 1959, Pseudocalceostoma Yamaguti, 1963, Bychowskya Nagibina, 1968, Dicrumenia Mamaev, 1969, and Neocalceostomoides gen. n., is reduced to a subfamily of the Dactylogyridae Bychowsky, 1933.

Yamaguti (1963) included the following in the Dactylogyroidea: Bothitrematidae, Calceostomatidae, Dactylogyridae, Diplectanidae, and Protogyrodactylidae. Based on the study of *Protogyrodactylus* spp., Price and Pike (1969) suppressed the Protogyrodactylidae and placed the included genera in the Ancyrocephalinae (Dactylogyridae). Recently, one of us (F.M.B.) collected numerous Neocalceostoma elongatum Tripathi, 1957 (Calceostomatidae) from the gills of Arius serratus (Day) (Ariidae) from the Karachi Coast, Pakistan. Examination of these specimens and members of other genera of Calceostomatidae has revealed that separation of this family from the Dactylogyridae is not warranted.

Methods used in the collection, preservation, and preparation of Monogenea for study are those described by Kritsky et al. (1972), except that Mayer's acid carmalum, Semichon's carmalum, and Delafield's hematoxylin were used individually to differentiate internal anatomy; serial transverse sections of two N. elongatum were also used. Measurements, in microns, were made according to the recommendations of Mizelle and Klucka (1953). A camera lucida and microprojector were used in the preparation of the plate. Specimens of N. elongatum were deposited in the helminthological collections of the U.S. National Museum (No. 73106) and the University of Nebraska State Museum (No. 20689).

Neocalceostoma Tripathi, 1957

EMENDED DIAGNOSIS: Dactylogyridae; Calceostomatinae. Body divisible into cephalic region, trunk, peduncle and haptor. Tegument thin, smooth. Head organs large; cephalic lobes well developed. Cephalic glands numerous. Pharynx muscular, glandular; esophagus moderate; intestinal crura (2) lacking diverticula, nonconfluent posteriorly. Gonads tandem, intercecal; ovary pretesticular. Seminal

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vesicle a dilation of vas deferens; vas deferens looping left intestinal crus; cirrus, accessory piece present. Oviduct elongate; vagina dextral; vitellaria well developed. Haptor disc shaped, with 14 (7 pairs) ventral hooks and one pair ventral anchors. Haptoral bar present. Parasitic on gills of marine and brackish water fishes.

Type AND ONLY SPECIES: Neocalceostoma elongatum Tripathi, 1957.

Neocalceostoma elongatum Tripathi, 1957 (Figs. 1–6)

Redescription (based on 40 whole mounts, 20 measured; and serial transverse sections of 2 specimens): Body fusiform; length 2,052 (1,360-3,056), greatest width 324 (178-454) slightly posterior to testis. Three bilateral pairs of cephalic lobes, median pair inconspicuous. Cephalic glands numerous, present as 2 bilateral groups immediately posterior to pharynx; 1 head organ in each cephalic lobe, each composed of groups of cephalic-gland ducts. Eyes approximately equidistant; members of posterior pair larger, each with anterior lens; accessory eye granules absent. Mouth subterminal; pharynx 119 (81–165) wide, consisting of 2 subspherical parts. Peduncle moderate to narrow; haptor concave ventrally, with delicate marginal membrane (Fig. 6); haptor 301 (216-346) long, 353 (216-583) wide. Anchors 22 (21–23) long, stout, with well-developed roots; base 12-13 wide; anchor filament absent. Bar 13-14 long, delicate, with medial undulation. Hooks similar, 5 pairs marginal, 1 pair near anchor-bar complex, 1 pair slightly posterolateral to anchors; each 18 (17-19) long, with straight perpendicular thumb, delicate point, slightly curved shank; filamentous hooklet loop 0.7 shank length. Gonads in anterior half of body. Testis subovate, 159 (97-227) wide, 179 (145-356) long; vas deferens enlarged anteriorly; seminal vesicle near midline; prostates indistinct. Cirrus 67 (60–76) long, rod shaped, with spiral flange becoming a keellike ridge on distal C-shaped termination; accessory piece 88 (81-95) long, lightly sclerotized, distally flabellate, nonarticulated with cirrus base. Ovary pyriform, contiguous with anterior margin of testis, 111 (75–146) wide, 150 (108–189) long; oviduct looping posteroventrally over anterior portion of ovary; Mehlis' gland butterfly shaped, comprising several elongate unicellular glands; uterus indistinct, near midline; vagina dextral opposite ovary; seminal receptacle small; vitelline ducts lying anterior to ovary; vitellaria coextensive with gut; egg 32 in diameter, subspherical, with single elongate filament.

Remarks

Tripathi (1957) described N. elongatum from the gills of Arius arius and Osteogeneosus *militaris* in India. Based on the morphology of the copulatory complex and anchors, the position of the gonads, the general shape of the body, and the host, there is little doubt that our specimens are N. elongatum, even though we observed significant differences from the original description. Present specimens differ from Tripathi's description principally by (1) a larger size; (2) the presence of a large vas deferens which loops the left intestinal crus (not mentioned by original author); (3) a dextral vagina (Tripathi states that the vagina is submedian opening toward the right side); (4) a small seminal receptacle; (5) a ventral bar supporting the single pair of anchors (not observed by original author); (6) seven pairs of ventral hooks, five marginal, two subcentral (Tripathi did not observe the subcentral pairs; (7) a moderately long instead of short esophagus; and (8) small follicular vitellaria (vitelline follicles are large in the original drawing).

Neocalceostoma arii Unnithan, 1964, was described from the gills of Arius sp. (Aris, sic) in India. In the original description, Unnithan (1964) reports that the vas deferens extends along the midline of the body after originating from the anterior right corner of the testis, the haptoral anchors are widely separated and lack a supporting bar, the haptor is armed with 10-12 (?) marginal hooks, and the vagina

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Figures 1-6. Neocalceostoma elongatum Tripathi, 1957. 1. Whole mount (ventral view). 2. Enlargement of reproductive organs (ventral). 3. Anchor-bar complex. 4. Hook. 5. Copulatory complex. 6. Ventral view of haptor. Figures 3, 4, 5 are drawn to the same scale (20 μ).



opens on the dorsolateral surface of the body above the right intestinal crus. In view of present information on *Neocalceostoma* (see emended diagnosis), Unnithan's species cannot be placed in this genus. Thus, *Neocalceostomoides* gen. n., with *N. arii* (Unnithan, 1964) comb. n. as the type species, is proposed (see diagnosis below).

Discussion

The Dactylogyridae Bychowsky, 1933, and Calceostomatidae (Parona and Perugia, 1890) Poche, 1926, comprise genera with many common features. They have been differentiated largely on (1) the presence in some species of Calceostomatidae of large cephalic lobes or lappets which are absent in the Dactylogyridae; (2) the presence of a weakly muscular, cupshaped haptor armed with paired anchors, hooks, and/or two variously developed suckers in the Calceostomatidae, whereas in Dactylogyridae the haptor is muscular and lacks suckers; (3) the existence in some calceostomatids of intestinal diverticula (absent in Dactylogyridae); (4) a compact or follicular testis in Calceostomatidae (usually compact in Dactylogyridae); (5) a copulatory complex which may lack an accessory piece in Calceostomatidae (almost always present in Dactylogyridae); and (6) a compact, lobed or tubular ovary in the Calceostomatidae (compact in Dactylogyridae). Yamaguti (1963) included the following genera in the Calceostomatidae: *Calceostoma* van Beneden, 1858, Acolpenteron Fischthal and Allison, 1940, Anonchohaptor Mueller, 1938, Calceostomella Palombi, 1943, Fridericianella Brandes, 1894, Neocalceostoma Tripathi, 1957, Paracalceostoma Caballero and Bravo-Hollis, 1959, and Pseudocalceostoma Yamaguti, 1963; and placed *Pseudacolpenteron* Bychowsky and Gussev, 1955, in synonymy with Acolpenteron. Since Yamaguti's (1963) revision, Dicrumenia Mamaev, 1969, and Bychowskya Nagibina, 1968, have also been assigned to this family.

Based on the obvious similarity of the reproductive system of Anonchohaptor with those of Pseudomurraytrema, Myzotrema, and Icelanonchohaptor, Leiby et al. (1972) transferred Anonchohaptor to the Dactylogyridae and emended the diagnosis of this family to include forms with cephalic lappets. We concur with this action and propose that these four genera be assigned to a new dactylogyrid subfamily, the Pseudomurraytrematinae. Members of this subfamily are characterized by having the ovary looping the right intestinal crus and a welldeveloped ejaculatory ampulla surrounding the seminal vesicle. Also, the cirrus, shaped as a U with a subterminal flange and guided by a lightly sclerotized multiramus accessory piece, and the dextral vagina, composed of a distal funnel and a proximal loose coil, are unique for these genera. The diagnosis of Pseudomurraytrematinae subfam. n. is given below.

Bychowsky (1957) included Adcolpenteron and *Pseudacolpenteron* in the Dactylogyrinae (Dactylogyridae), but considered the placement of the former genus in this subfamily as conditional since one species (A. ureteroecetes Fischthal and Allison, 1940) occurred in a centrarchid fish (an atypical host for the Dactylogyrinae). Without subfamily designation, Rogers (1968) also placed these genera in the Dactylogyridae. Recently, we collected A. catostomi Fischthal and Allison, 1942, from the ureters of Catostomus ardens Jordan and Gilbert (Snake River, American Falls, Idaho) and P. pavlovskii Bychowsky and Gussev, 1955, from the gills and skin of Cyprinus carpio L. (confluence of Grand River and Oahe Reservoir, South Dakota). After comparing these specimens, we concur with Bychowsky that they belong to the Dactylogyrinae. This is supported by the presence in both of a haptor armed with seven pairs of normal hooks (5 pairs marginal, 2 pairs central) and one pair of reduced (4A) hooks situated near the center of the haptor. Head organs are poorly developed in both species, and the vas deferens loops the left intestinal crus in A. catostomi (not seen in P. pavlovskii).

Bychowsky and Gussev (1955) used the presence of a thin cuticle, two pairs of distinct eyes, and a well-developed haptor armed with 14 hooks to differentiate *Pseudacolpenteron* from *Acolpenteron*. Rogers (1968) also considered these genera distinct based on differences in the eyes, head organs, and site of attachment to the host. However, critical examination of our material has shown that many of these differences do not exist and others are certainly not of sufficient magnitude to warrant separation of these genera. Although eye granules are usually dissociated and scattered in the cephalic region and anterior trunk of A. catostomi, several of our specimens possessed well-developed compact eyes. Head organs and the tegument in both species are similar morphologically. Moreover, the position of the hooks in the haptor were the same in both, all being directed toward the ventral surface with five pairs situated near the lateral margin and two pairs near the center of the haptor. Therefore, we conclude that *Pseudacolpenteron* should be considered a junior synonym of *Acolpenteron* as originally proposed by Yamaguti (1963).

Bychowsky (1957) did not combine the Calceostomatidae with the Dactylogyridae principally because of the presence of 12 or fewer normal hooks in the haptor of calceostomatid genera as compared to 14 for the Dactylogyridae. Subsequently, however, Euzet and Ktari (1973) have demonstrated 14 hooks in the adult haptors of *Calceostoma*, *Calceo*stomella, and Dicrumenia. Also, 14 hooks occur in Bychowskya (see Nagibina, 1968) and Neocalceostoma (nobis). Thus, it appears that further study of the remaining genera will verify that the normal number of hooks in all Calceostomatidae is 14.

Excluding Fridericianella, the remaining genera which Yamaguti (1963) included in the Calceostomatidae (i.e. Calceostoma, Calceostomella, Pseudocalceostoma, Paracalceostoma, and Neocalceostoma) along with Dicrumenia, Bychowskya, and Neocalceostomoides appear to form a natural taxonomic grouping. However, in view of the above transfers of *Anonchohaptor* and Acolpenteron from the Calceostomatidae to Dactylogyridae, the characteristics formerly used to separate these two families can no longer be considered diagnostically sufficient. For example, cephalic lappets and a weakly muscular, cup-shaped haptor, present in Anonchohaptor, are now included with the Dactylogyridae. Also, intestinal diverticula, a follicular testis, a copulatory complex without an accessory piece, and a lobed or tubular ovary are not common characteristics of all the remaining genera. Therefore, we propose that the Calceostomatidae be reduced to a subfamily of the Dactylogyridae and include the above eight genera. Thus, this subfamily is generally characterized by dactylogyrids with welldeveloped cephalic regions, and a cup-shaped haptor armed with one or two pairs of ventral anchors, hooks (probably always 14), and/or two variously developed suckers or loculi (absent in *Neocalceostoma*). In all genera except *Neocalceostoma*, the vas deferens is reported to be intercecal. The latter now has been verified by us in *Calceostoma* sp., which we (Bilqees) collected from *Pseudosciaena diacanthus* (author?) from the Karachi Coast, Pakistan. The diagnosis of Calceostomatinae is given below.

The monotypic genus, *Fridericianella*, was proposed by Brandes (1894) for a helminth collected from the eggs of *Arius commersonii* Lacepede in Brazil. We consider this taxon *incertae sedis*, since it has not been sufficiently studied to be assigned at the subfamily level.

Diagnoses of Referred Taxa Pseudomurraytrematinae subfam. n.

DIAGNOSIS: Dactylogyridae. Cephalic region developed into cephalic lobes or lappets; head organs present or absent; cephalic glands in 2 bilateral groups in anterolateral trunk. Four eyes. Ovary pretesticular, looping right intestinal crus; vagina dextral or dextroventral, comprising a distal lightly sclerotized funnel and proximal coiled portion. Testis intercecal; vas deferens looping left intestinal crus; seminal vesicle enclosed in ejaculatory ampulla; cirrus U shaped, with subterminal flange; accessory piece multiramus, lightly sclerotized, articulating with cirrus base. Haptoral hooks 14; 2 anchor pairs, when present, supported by 2 or 3 bars.

INCLUDED GENERA: Pseudomurraytrema Bychowsky, 1957; Anonchohaptor Mueller, 1938; Myzotrema Rogers, 1967; Icelanonchohaptor Leiby, Kritsky, and Peterson, 1972.

Calceostomatinae (Parona and Perugia, 1890)

DIAGNOSIS: Dactylogyridae. Cephalic region developed into cephalic lobes or lappets. Head organs present or absent; cephalic glands present. Eyes 4. Ovary pretesticular, intercecal; vagina dextral. Testis compact or follicular, intercecal; vas deferens may or may not loop left intestinal crus; copulatory complex frequently lacking accessory piece. Haptor cup shaped, armed with 1 or 2 ventral pairs of anchors, ventral hooks (probably always 14), and/or 2 variously developed suckers or loculi. INCLUDED GENERA: Calceostoma van Beneden, 1858; Calceostomella Palombi, 1943; Neocalceostoma Tripathi, 1957; Paracalceostoma Caballero and Bravo-Hollis, 1959; Pseudocalceostoma Yamaguti, 1963; Bychowskya Nagibina, 1968; Dicrumenia Mamaev, 1969; Neocalceostomoides gen. n.

Neocalceostomoides gen. n.

DIAGNOSIS: Dactylogyridae; Calceostomatinae. Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Head organs, cephalic lobes well developed. Pharynx muscular, glandular; esophagus short; intestinal crura (2) lacking diverticula, nonconfluent posteriorly. Gonads tandem, intercecal; ovary pretesticular, compact; vagina dextrodorsal; vitellaria well developed. Testis single; seminal vesicle a dilation of vas deferens; vas deferens intercecal; cirrus, accessory piece present. Haptor cup shaped, with 10–12 marginal hooks (?), pair of widely separated ventral anchors, 2 incipient loculi on inner margin; haptoral bar absent. Parasitic on gills of brackish water fishes.

TYPE AND ONLY SPECIES: Neocalceostomoides arii (Unnithan, 1964) comb. n., from Arius sp., Trivandrum, India.

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Lepocreadium manteri sp. n. (Trematoda: Lepocreadiidae) from the California Grunion, Leuresthes tenuis, and its Hyperparasitic Microsporidan

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ABSTRACT: Lepocreadium manteri sp. n. is described from the California grunion, Leuresthes tenuis, from beaches of southern California and adjacent Baja California Norte, Mexico. It differs from the similar L. bravoae chiefly by having a subterminal oral sucker, the genital pore anterior to the acetabulum, fewer and less extensive vitellaria in the posterior region, and larger eggs; and from L. sogandaresi by having larger eggs and vitellaria extending to the level of the pharynx. Eggs hatch after 13 to 15 days in seawater. A hyperparasitic microsporidan is reported.

Examinations of the beach-spawning California grunion, *Leuresthes tenuis* (Ayres) (Osteichthyes: Atherinidae), from beaches of southern California and adjacent Baja California Norte, Mexico, have yielded specimens unassignable to any of the species in the trematode genus *Lepocreadium* Stossich, 1903. They are described herein as a new species. A hyperparasitic microsporidan also is reported.

The trematodes were recovered from the expanded anterior intestine ("stomach") and studied alive or were fixed with or without flattening usually in hot AFA solution. Living worms were examined in physiological saline under coverslip pressure. Whole mounts were stained with Mayer's carmalum or Harris' hematoxylin and mounted in Permount. Serial sections were stained with hematoxylin and eosin. The whole mount drawing was made with the aid of a microprojector. All measurements are in micrometers.

Lepocreadium manteri sp. n. (Figs. 1-3)

DESCRIPTION (based on 20 slightly flattened gravid specimens): Body elongate oval, narrower anteriorly, extremities rounded, 486– 1,404 long, 162–540 wide at acetabulovarian level; body spines scalelike anterior to level of ovary, becoming pointed and less dense posteriorly. Eyespot pigment dispersed. Mouth subterminal. Oral sucker 57–111 long and 49–119 wide. Acetabulum 52–114 long, 49–116 wide. Sucker width ratio 1: 0.83–1.24 (mean 1: 0.99). Forebody 33–51% body length, decreasing with increasing body length. Prepharynx longer or shorter than pharynx depending upon state of contraction. Pharynx 36-77 long by 32-75 Esophagus longer or shorter than wide. pharynx. Intestinal bifurcation about midway between acetabulum and pharynx. Ceca terminating near or to 1/3 distance into posttesticular space. Genital pore median or slightly sinistral, about midway between intestinal bifurcation and acetabulum. Testes ovoid, in posterior ½ of body, intercecal, usually tandem, occasionally slightly oblique, contiguous; anterior testis 59–184 long by 70–188 wide; posterior testis 67-216 long by 70-205 wide. Posttesticular space 7-15% body length. Vasa efferentia from anterior margins of testes, converging at external seminal vesicle. External seminal vesicle elongate, approaching size of cirrus sac. Vas deferens short. External prostate cells dorsal, between acetabulum and posterior edge of ovary, with long ducts entering cirrus sac at proximal foramen. Cirrus sac elongated claviform about 1/5 body length, thick walled, overlapping right side of acetabulum, sometimes reaching ovarian zone, and containing subspherical internal seminal vesicle, prostatic vesicle, prostate cells and cirrus. Prostatic vesicle filled with large cells, receiving ducts from external prostate cells. Cirrus sinuous when inverted, often everted. Ovary anterodextral to testes, globular, 41-188 in diameter. Ovicapt dorsal to ovary, proximal end ciliated. Oviduct receiving common duct of seminal receptacle and Laurer's canal. Seminal receptacle sinistral to ovary, transversely oval, 52-Laurer's canal opening dorsally 101 long. above anterior left quarter of anterior testis,



Figures 1-3. Lepocreadium manteri sp. n. from California grunion, Leuresthes tenuis, San Diego Bay, San Diego Co., California. 1. Holotype, ventral view. 2. Terminal genitalia, ventral view. 3. Female complex of a considerably flattened specimen, ventral view.

proximal ¹/₃ ciliated. Oviduct ciliated from Laurer's canal to ootype sphincter, receiving common vitelline duct. Mehlis' gland poorly defined. Uterus chiefly anterior to testes, sometimes overlapping anterior testis or looping further posteriorad, extending to genital pore as a muscular metraterm about ¾ length of cirrus sac. Up to 55 eggs, undeveloped, oval, operculate with small anopercular knob, shell thin and light yellow, viable eggs 65–77 by 35–47, eggs in permanent mounts 68–77 by 40–50. Vitelline follicles 20–85 by 14–46 in two lateral rows dorsal, ventral and lateral to ceca; extending from level of posterior border of pharynx to beyond tips of ceca, sometimes confluent at anterior end, usually not confluent at posterior end; lateral collecting ducts ventral to ceca, uniting posteriorly; transverse ducts immediately pretesticular; reservoir somewhat triangular, median. Excretory pore terminal. Excretory bladder I shaped, extending dextrally to between level of anterior margin of ovary and midacetabulum; primary trunks ciliated distally, opening into bladder at level of anterior testis.

Host: Leuresthes tenuis (Ayres), California grunion.

SITE: Anterior intestine.

LOCALITIES AND PREVALENCE: Estero Beach, 10 km south of Ensenada, Baja California Norte, Mexico (9 in 4 of 20 hosts; 1–4 per host); Mission Beach (39 in 8 of 20 hosts; 1–10 per host); San Diego Bay (635 in 50 of 55 hosts; 1–43 per host, San Diego Co.; San Clemente Beach (7 in 2 of 20; 1–6 per host), Orange Co., California. Holotype from San Diego Bay.

HOLOTYPE: USNM Helm. Coll. No. 73873.

PARATYPES: USNM Helm. Coll. Nos. 73874 (whole mount) and 73875 (serial sections); HWML Nos. 20880 (serial sections) and 20881–2 (whole mounts); and in collection of author.

ETYMOLOGY: The species is named to honor the late Harold W. Manter, who contributed significantly to the knowledge of trematodes, including this genus.

Discussion

Lepocreadium manteri most resembles L. bravoae Lamothe, 1965 and L. sogandaresi Nahhas and Powell, 1971 in that the three species have vitellaria which extend to the intestinal bifurcation, testes which are tandem and entire, an ovary which is entire, and eggs longer than 54. All three agree with the characters of L. bravoae in Edwards and Nahhas' (1968) key to the species in the genus. Lepocreadium manteri differs from L. bravoae by being more attenuate anteriorly and by having a subterminal rather than terminal oral sucker, a genital pore anterior rather than lateral to the acetabulum, less extensive vitellaria in the posterior region, larger and fewer vitelline follicles, and somewhat larger egg size (size range 65–77 by 35–50 as compared with 64–72 by 32–40). It differs from *L. sogandaresi* by having vitellaria which extend beyond the intestinal bifurcation and eggs larger than 60 by 36. In addition, it differs from both species by host and locality.

Eggs usually masked the reproductive organs located between the acetabulum and testes. Those organs and their interrelationships were best observed in stained sections and considerably flattened unfixed specimens. As shown in Figure 3, that flattening resulted in displacement of structures such as the ovicapt which is not in its normal position dorsal to the ovary.

Eggs hatch after 13 to 15 days in seawater at room temperature $(20-25^{\circ}C)$.

Microsporidans were found in 14 of 159 (8.8%) *L. manteri* from San Diego Bay grunion collected 20 June 1977. The spores measure 3 by 1 and occupied the vitelline follicles, ducts and reservoir, and the infected flukes contained no eggs. Four of 12 flukes in one fish contained microsporidans and no eggs while the uninfected flukes each contained eight to 14 eggs. The identification of the microsporidan to genus and species must await further investigation. It does represent a host and a distribution not listed by Canning (1975) in her account of microsporidan parasites of platy-helminths.

Acknowledgments

I would like to acknowledge Raphael Lamothe-Argumedo, the late Harold W. Manter, Fuad Nahhas and the late Satyu Yamaguti, all of whom examined specimens from the California grunion and suggested they represented an undescribed species.

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The Chromosomes of *Cotylogaster occidentalis* and Cotylaspis insignis (Trematoda: Aspidogastrea) with Evolutionary Considerations¹

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ABSTRACT: The chromosome number and morphology for two species of Aspidogastrea were studied. The chromosome number of Cotylogaster occidentalis is 2n = 12 and for Cotylaspis insignis is 2n = 22. Cotylogaster occidentalis has two pairs of metacentric chromosomes, two pairs that are submetacentric and two pairs that are acrocentric. The largest pair of chromosomes are $6 \mu m$ long, while the shortest are $2 \mu m$. Only meiotic chromosomes were observed for *Cotylaspis insignis*. Phylogenetic implications of chromosome numbers in the Aspidogastrea are discussed.

Among the Trematoda, the Monogenea and Digenea have been subjected to cytological analysis, while in the Aspidogastrea incidental observations have been made in two cases and a definite chromosome number report in one instance (Table 1).

Osborn (1905) depicted an anaphase cell and several cells at the pachytene stage of meiosis (pl. 14, figs. 42-45, p. 241; pl. 15, fig. 52, p. 252, respectively) of Cotylaspis insignis, but referred to them as "chromatine" masses in the cytoplasm. Brinkman (1957) described "with safety 6 pair of daughter chromosomes" from Macraspis elegans but concluded the chromosome number to be at least "6 probably 8 diploid." Rohde (1976), the first to report an actual chromosome number, observed 2n = 14 for Lobatostoma manteri.

This report is a study on the chromosome numbers and morphology of two aspidogastrid species.

Materials and Methods

Cotylogaster occidentalis was obtained from the pericardial cavity of Legumia recta collected near Douglas Lake, Michigan; Cotylaspis insignis from the gills of Anodonta corpulentum from Lake Pepin, Minnesota.

The parasites were fixed in a modified Carnoy's fluid (3 parts glacial acetic acid; 1

part 95% ethanol) for approximately 4 hr, after which they were stained with either aceticorcein (LaCour, 1941) or Gomori's chrom alum hematoxylin (Short and Menzel, 1960). The ovaries and testes usually with surrounding tissue were removed by dissection and handled separately from the remaining tissue which was cut into small pieces. Pieces of tissue (ovaries, testes, etc.) were placed on a clean slide, rinsed with 45% acetic acid to remove excess stain, and squashed under a coverslip. Pressure from the thumb was sufficient to adequately spread the chromosomes. The slides were sealed temporarily with paraffin and later were made permanent by the method of Conger and Fairchild (1953).

Observations

In Cotylogaster occidentalis eight cells in mitosis showed metaphase chromosomes suitable for detailed observations. Each cell had 12 chromosomes (2n = 12) (Figs. 1-3). There were two pairs of acrocentrics, two pairs of submetacentrics, and two pairs of metacentrics. The longest chromosome measured ca. 6 μ m in length while the shortest was ca. $2 \ \mu m$ long. No meiotic chromosomes were observed for Cotylogaster occidentalis. However, in Cotylaspis insignis 20 different meiotic metaphase cells obtained from testes had the haploid number 11 (2n = 22) (Fig. 4). Mitotic metaphase chromosomes observed in Cotylaspis insignis were judged as not reliable for chromosome number determination or characterization.

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Table 1. Chromosome number determinations for the Trematoda.*

Mongenea	Digenea	Aspidogastrea†		
2 families	26 families	1 family		
3 genera	60 genera	3 genera		
3 species	85 species	3 species		

* See Walton, 1959; Short and Menzel, 1960; Pickel and Jones, 1967; Rohde, 1973; Filippone and Fried, 1974; LoVerde, 1974; Fried, 1975. † Includes this report.

Discussion

The generally accepted phylogenetic hypothesis is that a rhabdocoele stock of turbellarians (the Dalyellioidae) gave rise to the Trematoda (see Walton, 1959; Llewellyn, 1965; Stunkard, 1967; Rohde, 1972, 1973). It is also generally assumed that the Monogenea and Digenea were derived independently most likely from different rhabdocoele stocks.



Figures 1–3. Mitotic metaphase of Cotylogaster occidentalis, 2n = 12. Figure 4. Meiotic metaphase of Cotylaspis insignis, n = 11.

 Table 2. Chromosome numbers for three families of trematodes.

Family	Species	Haploid number*
Heronimidae	Heronimus chelydrae	10
Microscaphildae Paramphistomidae	Megalodiscus temperatus Gigantocotyle bathocotyle Gastrothylax crumenifer Zygocotyle lunata Cotylophoron elongatum	8, 9 6 7 7 8

* See Walton, 1959 for exact references.

Evidence presented by Stunkard (1963) and more recently by Rohde (1971, 1972, 1973) indicates that the Aspidogastrea are more closely allied to the Digenea than to the Monogenea, and that the Aspidogastrea exhibit archaic features; Rohde (1972, p. 143) suggests that they "stand close to the root of the Digenea, i.e., to the hypothetical Prodigenea.' Both authors agree that the Aspidogastrea and Digenea descended from a common ancestor. Cable (1974), on the other hand, has suggested that the Aspidogastrea could as well have been derived from "heteroxenous ancestors." All agree that it is unlikely the Aspidogastrea and Digenea evolved independently from a common turbellarian stock.

The chromosomal data presented here have a bearing on the phylogeny of these groups. In the dalyellioid rhabdocoeles the haploid chromosome number ranges from 2-4 (Ruebush, 1937, 1938). For the three species of Monogenea whose chromosome numbers are known. haploid numbers of 4 and 6 have been reported (Pickel and Jones, 1967). In the Digenea, the haploid chromosome number ranges from 6 to 14, with 11 being the most common (Britt, 1947; Walton, 1959). The three species of Aspidogastrea exhibit 6, 7, and 11 as haploid numbers. In a comparative study of the Turbellaria, Ruebush (1938) found that the acoelids and alloecoelids, considered to be the most primitive families on noncytological grounds, consistently possessed the highest number of chromosomes. Conversely, in the Trematoda, where chromosomal evolution seems to be proceeding by aneuploidy also, it appears to be correlated with phylogenetic advancement. The aspidogastrids studied so far exhibit the haploid chromosome numbers 6, 7, and 11 which are within the upper range of the Dalyellioidea and the lower range of the

Digenea. According to the scheme of Cable (1974), the Aspidogastrea would be derived from an evolutionary line leading to the Heronimidae, Microscaphiidae, and Paramphistomidae. The chromosome numbers that have been reported for these families are in Table 2. Cable argued (p. 188) that "whether the heronimid cycle is primitive or not, it is just one "step" from the cycle of the aspidobothrians as far as generations are concerned and equally close in its host-parasite relationships." The chromosome data indicate that it is plausible that the Aspidogastrea were derived from paramphistome stock as suggested by Wootton (1966).

Nevertheless, the chromosome data do support the notion that members of the Aspidogastrea stand close to the root of the Digenea.

It is hoped that further studies of chromosome number and morphology in the Aspidogastrea will aid in establishing the relationships between them and the dalyellioid rhabdocoele group of turbellarians and digenea on one hand, and their interspecific relationships on the other.

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ANNOUNCEMENT

The Division of Systematic Zoology of the American Society of Zoologists will sponsor a symposium entitled, "Contemporary Methods in Systematic Parasitology" which will be presented during the joint meetings of the American Society of Zoologists and American Microscopical Society December 27–30, 1978 in Richmond, Virginia. Numerical and nonnumerical approaches will be presented and their applications discussed. For further information contact either Daniel R. Brooks, Department of Biology, University of Mississippi, University, Mississippi 38677 or W. Wayne Moss, The Academy of Natural Sciences of Philadelphia, Department of Entomology, Nineteenth and the Parkway, Philadelphia, Pennsylvania 19103.

Growth and Development of the Tetracotyle of *Cotylurus strigeoides* (Trematoda) in the Chick, on the Chorioallantois and in Vitro

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ABSTRACT: Isolated tetracotyles of *Cotylurus strigeoides* from *Physa heterostropha* snails produced minimal infection in domestic chicks, whereas chicks fed *Physa* containing tetracotyles were readily infected. Total length and hindbody length of worms grown in chicks increased most rapidly between days 1 and 5, and worms became ovigerous by day 4. Excysted tetracotyles implanted on the chick chorioallantois survived there for 8 days but only showed minimal postmetacercarial development. Excysted tetracotyles cultivated in vitro in the defined medium NCTC-135 supplemented with 50% chicken serum developed vitellaria, whereas tetracotyles cultured in NCTC-135 with 40% chicken serum and 20% upper chicken intestine mucosal extract became ovigerous within 6 days. In vitro worms contained eggs with abnormal shells.

Fried and Butler (1977) studied neutral lipids in *Cotylurus* sp. which possessed abundant vitelline follicles in the forebody as described by Basch (1969) for *Cotylurus lutzi*. Based on information in Dubois (1958, 1968) the species has been identified as *Cotylurus strigeoides* Dubois, 1958.

Although many species of *Cotylurus* have been grown experimentally in birds other than the natural host (Acholonu, 1965; Basch, 1969; Campbell, 1973a; Nasir, 1960; Van Haitsma, 1931; Williams, 1966), only limited success has been obtained using the domestic chick as the host (Acholonu, 1965; Campbell, 1973a; Nasir, 1960; Ulmer, 1957; Van Haitsma, 1931). Fried and co-workers (see review by Fried, 1969) have used the chick chorioallantois to observe trematode development, but species of *Cotylurus* have not been studied on this site. Interestingly, Voge and Jeong (1971) and Basch et al. (1973) have cultivated tetracotyles of *Cotylurus lutzi* in vitro to ovigerous adults.

The present investigation reports on the growth and development of the tetracotyle of C. strigeoides in the domestic chick, on the chick chorioallantois and in vitro.

Materials and Methods

Tetracotyle cysts were dissected from naturally infected *Physa heterostropha* (Say) snails obtained from Lopatcong tributaries in Warren County, New Jersey (Fried and Butler, 1977). To obtain infections in chicks, either isolated cysts or whole snails were fed to white leghorn chicks (Table 1). Following exposure, chicks were either maintained without food, or fed on a commercial mash diet (Pacemaker Starter, Agway, Inc., Syracuse, New York), or fed on a commercial frozen codfish diet of 15 g of fish per day per chick (Table 1).

Excysted tetracotyles were needed for chorioallantoic transplant and in vitro cultivation studies. Attempts to excyst encysted tetracotyles of *C. strigeoides* in Locke's solution or Earle's BSS maintained at 39 to 42 C were unsuccessful. However, the use of a trypsin-bile salt procedure (Fried and Roth, 1974) provided for successful chemical excystation of these tetracotyles.

A total of 250 tetracotyles excysted in trypsin-bile salts was transferred through five changes of sterile Locke's and then implanted 10 to 20 per egg on the chorioallantoic membranes of 20 8- to 12-day-old white leghorn chick embryos which were maintained at 39 to 40 C. Worms were recovered 1 to 8 days postinoculation.

In vitro culture media consisted of the defined medium NCTC-135 (Grand Island Biological Co., Grand Island, New York) supplemented with 50% inactivated chicken serum (Voge and Jeong, 1971) or NCTC-135 supplemented with 40% chicken serum and 20% upper chicken intestine mucosal extract (Basch et al., 1973). Both media contained 200 units/ml penicillin and 200 μ g/ml streptomycin. Chemically excysted tetracotyles were trans-

Exp.		-	Exposure data					
	Age of chicks at exposure (days)	No. of chicks exposed	No. of cysts fed/chick	No. of snails fed/chick	Diet*	Age of worms at necropsy (days)	No. of infected chicks	Range of worm recovery and (avg.)
A	1-2	4	50	_	mash	2-3	1	6 (6)
В	14†	2	100	_	mash	4	0	0
С	1 - 2	20		10	mash	1 - 6	11	1-65(14)
D	1 - 2	23		10	fish	1 - 6	19	1-70 (13)
Е	1	4	—	10	no food	4	4	2-12 (8)

Table 1. Infectivity of Cotylurus strigeoides in the domestic chick.

* See text for further explanation of diet. † Maintained on mash diet until one day prior to exposure; all other chicks not fed prior to exposure.

ferred through four successive 30 min washes of sterile Locke's containing 200 units/ml penicillin and 200 μ g/ml streptomycin. Worms were as eptically transferred 10/tube (12×75 mm sterile plastic culture tubes) to 3 ml of medium. Tubes were sealed with plastic caps and incubated in an upright position at 41 C. The gas phase was air, and half the medium in each tube was replaced with fresh medium every other day from day 2 until cultures were terminated, usually by day 7.

Excysted tetracotyles, chick worms, chorioallantoic worms, and cultured worms were pipetted into hot AFA (Fried, 1962) stained in Gower's (1939) carmine, dehydrated in ethanol, cleared in xylene, mounted in Permount and used for growth measurements and development studies. Some chorioallantoic worms were prepared in situ as cryostat sections fixed in neutral buffered formalin and then stained with Harris' hematoxylin and eosin.

Results

In dissections of 150 P. heterostropha, infected snails contained from 1 to 130 encysted tetracotyles (avg. 8). The encysted tetracotyles were uniform in size and appearance (see Fig. 7 in Fried and Butler, 1977) and were the only larval trematodes from Physa. Preliminary studies indicated that all tetracotyles grown in chicks belonged to C. strigeoides.

The results of infectivity studies in the domestic chick are presented in Table 1. Worms were recovered singly, in pairs, or in clusters tenaciously attached to the villi of the upper ileum.

Body length measurements were expressed as the mean and range of five to 10 excysted tetracotyles of chick worms. Growth was most pronounced between days 1 and 5. Day-old and 5-day-old worms averaged 0.33 mm and 1.1 mm in length, respectively, and during this time hindbody growth more than quadrupled. Worms grown in chicks were ovigerous by day 4, and by day 6 they usually contained eight to 12 eggs (Fig. 4).

A total of 75 live worms was recovered from chorioallantoic membranes from 1 to 8 days postinoculation. These worms were found singly, in pairs, or in clusters with up to 15 worms/cluster on the chorionic surface, and were usually surrounded by cellular debris (Fig. 3). Chorioallantoic worms usually contained hematinlike material in their ceca, an enlarged hindbody, and genital anlage (Fig. 2). Worm growth and development on the membrane never progressed beyond that observed in 2-day-old chick worms.

In vitro cultivated worms survived for 7 days at which time cultures were terminated. These worms varied markedly in growth and development and by day 7 some looked no different than excysted tetracotyles, whereas others resembled worms grown in chicks for about 4 days. Of approximately 50 worms cultivated in vitro for 7 days more than 20 showed obvious postmetacercarial growth and development. The average length of five optimal specimens grown in the Voge and Jeong (1971) medium for 6 days was 0.68 mm. These worms did not develop beyond the vitellogenesis stage of Smyth (1966). By day 6 some worms grown in the Basch et al. (1973) medium were ovigerous (Fig. 5), and five optimal specimens averaged 0.75 mm. Eggs from cultured worms contained thin shells and appeared not to be embryonated. It was not determined if eggs were ever released into culture vessels.



Figures 1-5. Photomicrographs of *Cotylurus strigeoides* from the chick, the chorioallantois and in vitro. Unless otherwise stated specimens were fixed in hot AFA and stained in Gower's carmine. 1. Excysted tetracotyle. 2. Chorioallantoic worm, 2 days old. 3. Cryostat section of 2-day-old worm on the chorioallantois; fixed in NBF and stained with H&E. 4. Chick worm, 6 days old. 5. Excysted tetracotyle cultured in vitro for 6 days in NCTC-135 with 40% chicken serum and 20% upper chicken intestine mucosal extract. Abbreviations: A = allantoic endoderm; C = chorionic ectoderm; D = cellular debris; E = egg; G = genital anlage; M = mesenchyme; V = vittellaria. Scale bars equal approximately 100 μ m.

Discussion

From comparison with information in Dubois' (1968) key, our specimens are either Cotylurus gallinulae vitellosus or Cotylurus strigeoides. The pharynx of the former species is smaller than that of the latter. Pharyngeal measurements of our worms are in essential accord with similar measurements given for C. strigeoides by Dubois (1958). Dr. Ronald Campbell examined some of our specimens from chicks and concurred with our specific identification.

Campbell (1973a) had limited success infecting chickens with isolated tetracotyles of *C. flabelliformis.* Ulmer (1957) was unable to infect a 12-week-old chicken with either isolated tetracotyles or snails containing *C. flabelli*- formis tetracotyles. Nasir (1960) failed to infect chicks fed numerous C. brevis cysts. However, Acholonu (1965), Campbell (1973a) and Van Haitsma (1931) infected chicks with either whole snails removed from their shells, or crushed snails containing C. flabelliformis tetracotyles. In the present study, chicks fed P. heterostropha snails containing C. strigeoides tetracotyles became readily infected, whereas isolated tetracotyles fed to chicks produced only limited infection.

Some chicks maintained on either mash or fish diets or without food became infected. Because it was not possible to determine the number of cysts administered per chick, infectivity data from these experiments are inconclusive. Our in vivo growth and development studies on *C. strigeoides* are in agreement with other studies on *Cotylurus* spp. (Campbell, 1973a; Williams, 1966) which also show rapid hindbody growth and sexual maturation usually within 4 days. Campbell (1973a, b) reported that *C. flabelliformis* became ovigerous within 48 hr in ducks and suggested that such rapid development may be an adaptation to the migratory habits of the definitive hosts.

Cotylurus strigeoides showed some postmetacercarial growth and development on the chorioallantois, but did not become ovigerous in this site. Fried (1970) cultured the strigeid metacercaria of Posthodiplostomum minimum minimum to the ovigerous adult on the chorioallantois. Madsen and Johnson (1974) were unsuccessful in their attempts to develop mesocercariae of Alaria spp. on the chick chorioallantois and Kannangara and Smyth (1974) achieved only minimal development of Diplostomum spp. metacercariae in this site.

Voge and Jeong (1971) activated the tetracotyle of C. *lutzi* at 41 C in the absence of digestive enzymes and bile salts. Basch et al. (1973) placed tetracotyles of C. *lutzi* directly into their culture medium without specific activation procedures. In our studies on C. *strigeoides*, activation of tetracotyles in a trypsin-bile salt solution at an elevated temperature was an absolute prerequisite for in vitro cultivation or chorioallantoic transplantation studies.

Voge and Jeong (1971) cultivated tetracotyles of *C. lutzi* in NCTC-135 supplemented with 50% chicken serum and obtained adults that produced nonviable eggs. Basch et al. (1973) added upper chicken intestine mucosal extract to the Voge and Jeong (1971) medium and obtained adult *C. lutzi* which produced eggs capable of embryonation and hatching. Miracidia from the eggs were capable of infecting *Biomphalaria glabrata* snails. *Cotylurus strigeoides* is more fastidious than *C. lutzi* and was incapable of producing eggs in the Voge and Jeong (1971) medium and produced eggs with abnormal shells in the Basch et al. (1973) medium.

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Philophthalmus pulchrus sp. n. (Digenea: Philophthalmidae) from the Intestinal Ceca of a Malaysian Moorhen¹

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ABSTRACT: Philophthalmus pulchrus closely resembles P. indicus but differs by possessing uterine loops not extending as far posteriorly, a relatively smaller acetabulum and pharynx, and by lacking tegumental spines. Philophthalmus pulchrus further inhabits the intestinal ceca of the moorhen Gallinula chloropus orientalis in Malaysia, whereas P. indicus occurs in the eye of an Indian vulture.

Most philophthalmid digeneans inhabit the orbital areas of their avian hosts, although some reported species are intestinal forms. The specimens upon which the following description is based occurred in the intestinal ceca of a Malaysian moorhen, *Gallinula chloropus orientalis*. Worms were removed from the host ceca, flattened with minimal coverslip pressure, fixed with AFA, and stored in 70% ethanol. They were stained with Mayer's hematoxylin and mounted in Histoclad for study as whole mounts. Figures were drawn with the aid of a drawing tube; measurements are in micrometers unless otherwise stated.

Philophthalmus pulchrus sp. n. (Figs. 1-3)

DESCRIPTION (based on 8 specimens): Body elongate, 3.2–4.4 mm long by 1.0–1.2 mm wide in midhindbody. Tegument aspinose. Oral sucker subterminal, 204–324 long by 264–360 wide. Acetabulum 336–456 long by

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Figures 1-3. Philophthalmus pulchrus. 1. Ventral view of holotype. 2. Ventral view of terminal genitalia (acetabulum omitted), paratype. 3. Dorsal view of female complex, paratype.

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360–468 wide. Ratio of oral sucker width to acetabular width 1:1.23–1.45. Forebody 21–24% of total body length. Prepharynx lacking. Pharynx 252–348 long by 228–324 wide. Ratio of oral sucker width to pharyngeal width 1:0.83–0.95. Esophagus 12–120 long. Cecal bifurcation 13–17% of total body length from anterior end; ceca extending near posterior end.

Testes intercecal near cecal tips, tandem to obliquely tandem, shallowly and irregularly lobate. Anterior testis 276–432 long by 252– 336 wide; posterior testis 276–432 long by 264– 360 wide. Posttesticular space 5–7% of total body length. Cirrus sac dorsal to acetabulum, occasionally extending slightly postacetabular, 672–900 long by 78–108 wide; containing saccate internal seminal vesicle and unspined eversible cirrus.

Ovary presticular, spherical to subspherical, 144–180 long by 144–180 wide. Mehlis' gland immediately postovarian; Laurer's canal posterodorsal to ovary. Uterus occupying available space between anterior testis and acetabulum with extracecal loops; uterus terminating with prominent muscular metraterm 600–900 long; space occupied by uterus equal to 45-57% of total body length. Short uterine seminal receptacle present. Vitellaria tubular with irregularly spaced thickenings, in paired extracecal longitudinal series uniting immediately postovarian forming a U-shaped structure. Vitellaria extending from immediately postacetabular to immediately postovarian levels, or from 32-39% of total body length from anterior end to within 16-21% of total body length from posterior end. Thickenings of vitellaria 42–90 long by 18-48 wide. Genital atrium shallow, ventral to juncture of esophagus and cecal bifurcation, 14-17% of total body length from anterior end. Eggs 73-84 long by 29-35 wide.

Excretory vesicle Y shaped with arms reaching dorsal to pharynx; bifurcation posttesticular; pore terminal.

HOST: Gallinula chloropus orientalis.

SITE: Intestinal ceca.

LOCALITY: Vic. Kuala Lumpur, Malaysia.

HOLOTYPE: USNM Helm. Coll. No. 73056.

PARATYPES: USNM Helm. Coll. No. 73057; Univ. Nebraska State Museum, Manter Laboratory No. 20863; and in collections of authors.

Philophthalmus pulchrus appears extremely similar to P. indicus Jaiswal and Singh, 1954 from the orbit of an Indian vulture. Both species possess tubular vitellaria with irregular thickenings, median genital pores at the juncture of the esophagus and cecal bifurcation, prominent metraterms, vitellaria extending anteriorly to the posterior margin of the acetabulum, cirrus sacs extending posteriorly to near the posterior margin of the acetabulum, and similarsized eggs. *Philophthalmus pulchrus* inhabits the intestinal ceca rather than the orbital areas. It further differs from *P. indicus* by possessing irregular testes with lobate rather than smooth margins, uterine loops which extend posteriorly to the anterior margin of the anterior testis rather than to the lateral margins of the posterior testis, and by having an oral sucker width to acetabular width ratio of 1 : 1.23–1.45 rather than 1:1.69 and an oral sucker width to pharyngeal width ratio of 1:0.83-0.95 rather than 1: 1.22. Jaiswal and Singh (1954) stated that P. indicus possessed a spinose tegument, whereas the tegument of *P. pulchrus* lacks spines.

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Editor's Note

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.

Redescription of Nagmia floridensis Markell, 1953 with Discussion of the Composition of the Anaporrhutinae Looss, 1901 (Digenea: Gorgoderidae)

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ABSTRACT: Nagmia floridensis, infecting Dasyatis sabina and the new host D. americana from the Gulf of Mexico in the new localities of near Tallahassee, Florida and Ocean Springs, Mississippi, is redescribed, because the original description was based on a single specimen. Also the morphology of N. floridensis provides additional information clarifying the position and status of the Anaporrhutinae. All specimens collected lacked Laurer's canals, a trait which appears to be constant among gorgoderid digeneans infecting elasmobranchs. Further, N. floridensis exhibits vitellaria which extend from entirely intercecal to almost entirely extracecal. Because of the variation in vitelline extent shown by N. floridensis, we do not accept the erection of the Probilotrematinae for species which purportedly possess vitellaria that are entirely extracecal rather than those which possess vitellaria that are entirely intercecal, and declare the Probilotrematinae a junior synonym of the Anaporrhutinae. Further, we declare Nagmioides a junior synonym of Nagmia because the only distinction between the genera is the vitelline configuration.

Markell (1953) described Nagmia floridensis based on a single specimen from Dasyatis sabina (LeSueur) (listed as Amphotistius sabinus) in Lemon Bay, Florida. Further reports of the species are lacking, although Yamaguti (1971) listed Dasyatis americana Hildebrand and Schroeder as an additional host. While studying specimens identified as N. floridensis, we discovered that the holotype had been lost. We therefore present the following redescription based on our specimens and on Markell's (1953) description.

Worms were removed from hosts, examined alive, flattened with minimal coverslip pressure, and fixed with AFA, then stored in 70% ethanol. They were stained with Mayer's hematoxylin and mounted in Histoclad for study as whole mounts. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube.

Nagmia floridensis Markell, 1953 (Figs. 1, 2)

REDESCRIPTION (based on 15 specimens): Body 3.9–13.4 mm long by 3.2–12.6 mm wide immediately postacetabular. Oral sucker terminal with subterminal mouth, 342–1,140 long by 475–1,596 wide. Prepharynx lacking. Pharynx 190–665 long by 247–817 wide. Ratio of oral sucker width to pharyngeal width 1: 0.38–0.61 (1: 0.50). Acetabulum 741– 2,090 long, by 779–2,185 wide. Forebody 22– 30% (28%) of total body length. Ratio of oral sucker width to acetabular width 1: 1.27–1.67 (1: 1.53). Esophagus 190–665 long. Cecal bifurcation 10–20% (15%) of total body length from anterior end. Ceca terminating within 8–15% (11%) of total body length from posterior end of body.

Testes composed of paired, symmetrical, extracecal groups of follicles in midhindbody. Posttesticular space 13-34% (20%) of total body length. Left testis comprising 22-26 (24) follicles, right testis comprising 13-30 (24) follicles; total number of follicles 39-52 (48); follicles 152-665 in diameter. Vasa efferentia joining elongate seminal vesicle immediately anterodorsal to acetabulum. Seminal vesicle 1,140-2,736 long by 190–513 wide, terminating in short ejaculatory duct surrounded by gland cells. Genital pore 13-25% (18%) of total body length from anterior end.

Ovary postacetabular, submedian, spherical to subspherical, 114–275 long by 114–437 wide. Seminal receptacle preovarian, 266–1,425 long by 285–1,520 wide. Digitiform paired vitellaria lateral to ovary, extending ventrally from slightly intercecally to slightly extracecally, 570–855 long by 275–437 wide. Uterus looping in postacetabular intercecal space, occasionally extending to posterior end of body, terminat-



Figures 1, 2. Nagmia floridensis. 1. Female ducts. 2. Terminal genitalia. Abbreviations: M = Mehlis' gland; O = oviduct; S = seminal receptacle; V = vitelline reservoir.

ing anteriorly in muscular metraterm. Eggs hatching in utero, 45–60 long by 36–43 wide.

Excretory vesicle I shaped, extending anteriorly to midtesticular level; pore dorsal, subterminal.

Hosts: Dasyatis sabina (LeSueur), D. americana Hildebrand and Schroeder, new host.

LOCALITIES: Gulf of Mexico, Alligator Harbor, Franklin County, Florida, vic. Ocean Springs, Mississippi, *new localities*.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74852 and No. 74853.

In the original description, Markell (1953) failed to illustrate either the female ducts or the terminal genitalia; we present them here (Figs. 1, 2). We also confirmed the absence of a Laurer's canal in this anaporrhutine species.

Five gorgoderid genera, considered by many to constitute the subfamily Anaporrhutinae Looss, 1901, are comprised of species exhibiting foliate hindbodies, lacking Laurer's canals, and possessing pharynges, intercecal uterine loops, and seminal receptacles; additionally, all inhabit elasmobranch body cavities. Two of those genera, *Petalodistomum* Johnston, 1913 and Nagmia Nagaty, 1930, are characterized further by the presence of I-shaped excretory vesicles and of cecal diverticula. Nagmia species have two follicular testes, whereas Petalodistomum species possess multiple lobulate testes. Two other genera exhibit unbranched ceca and H-shaped excretory vesicles: Anaporrhutum Ofenheim, 1900, with testicular follicles extending extracecally and intercecally and Staphylorchis Travassos, 1922, with entirely extracecal follicles. A Y-shaped excretory vesicle and nondiverticulate ceca characterize the fifth genus, Probilotrema Looss, 1902. Probilotrema species reportedly differ from those of the other four genera by possessing extra-rather than intercecal vitellaria, a trait which Yamaguti (1971) considered so important that he erected a subfamily separate from the Anaporrhutinae for *Probilotrema*. Similarly, Dollfus (1971) proposed Nagmioides for Nagmioides trygonis because it resembled species of Nagmia

but exhibited vitelline follicles ventral to the ceca. However, Nagmia floridensis exhibits vitellaria which extend from inter- to extracecal. so we therefore consider the lateral extent of the vitellaria an unreliable character for discerning supraspecific relationships and place all five above-mentioned genera in a single subfamily, the Anaporrhutinae. We declare Nagmioides Dollfus, 1971, a junior synonym of Nagmia; further, we declare the Probilotrematinae Yamaguti, 1971 a junior synonym of the Anaporrhutinae.

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Morphology of Euclinostomum multicaecum Tubangui and Masilungan, 1935 (Trematoda: Clinostomatidae) from Ardea purpurea¹

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ABSTRACT: Adult specimens of Euclinostomum multicaecum Tubangui and Masilungan, 1935, previously only known from its metacercarial stage, were collected from the esophagus of Ardea purpurea (purple herons) in Central Sulawesi, Indonesia. Structures of the adult stage are described, figured and compared with those of E. heterostomum, the only other recognized species of Euclinostomum. The oral field of E. multicaecum has an adhesive function.

Euclinostomum multicaecum was first described by Tubangui and Masilungan (1935) from metacercariae encysted in the muscles of fish, Channa (Ophicephalus) striatus, from the Philippines. Subsequently, Velasquez (1959) reported finding unencysted metacercariae beneath the visceral peritoneum of the same host species.

In July 1973, 20 adult specimens of E. multicaecum were recovered from the esophagus of three herons, Ardea purpurea, collected near Paku and Muara in the Lindu Valley of Central Sulawesi, Indonesia. All worms were fixed and preserved in 10% formalin. Whole mounts were stained with Gower's carmine; histological sections were stained with H&E. Drawing was prepared with the aid of a Bausch & Lomb microprojector. Measurements are in millimeters.

Results

DIAGNOSIS: Measurements were derived from 7 ovigerous specimens. Diagnosis otherwise based on study of 20 ovigerous specimens (Fig. 1).

Body fusiform slightly truncate at anterior end, broad at middle and rounded at posterior end, with maximum width occurring at region of anterior testis. Body wall at anterior end invaginated to form a collar around a protrusible, broad and dome-shaped oral field. Integument spineless. Body length 16.3 (9.0-22.2), width 6.24 (5.1-7.5). Acetabulum

¹ The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large. Reprint requests to Publications Office, NAMRU-2, Box 14, APO San Francisco 96263, USA or 7-1 Kung Yuan Road, Taipei, Taiwan, Republic of China. ² U.S. Naval Medical Research Unit No. 2 (NAMRU-2) Detachment, National Institute of Health Research and Development, P.O. Box 226, Jakarta, Indonesia. ³ U.S. Naval Medical Research Unit No. 2 (NAMRU-2), Taipei, Taiwan, Box 14, APO San Francisco, Calif. 96263. ⁴Zoonotic Disease Division, Armed Forces Institute of Pathology, Washington, D.C. 20306.



Figure 1. Euclinostomum multicaecum from the esophagus of a purple heron (Ardea purpurea), Sulawesi, Indonesia (ventral view).

large, 1.70 $(1.5-2.0) \times 1.71 (1.6-1.9)$, strong, in anterior fifth of body. Y-shaped excretory bladder opens into excretory pore on middorsal surface near posterior end.

Oral sucker subterminal, in center of oral field, length 0.71 (0.66-0.78), width 0.36 (0.30-0.45); ratio of length of oral to ventral sucker, 1: 2.0–2.9. Prepharynx exits from the posteroventral margin of oral sucker and enters the pharynx at its ventroanterior end. Pharynx leads dorsally to bulb-shaped esophagus (not illustrated in Fig. 1 since it is dorsal to pharynx), 0.30×0.15 in dimension, from which cecal bifurcations originate. Each cecum forks into 2 primary lateral canals. Primary intestinal ceca extend posteriad, converge slightly mediad behind posterior testis and end blindly near posterior end. Primary intestinal ceca give rise to several secondary blind diverticula of unequal length, most of which extend to posterior end. Number of secondary diverticula vary, 7–10 on left, 8–10 on right (ventral view).

Reproductive organs intercecal in middle third of body. Ovary oval (0.35 [0.20-0.50] \times 0.34 [0.20-0.48]) in intertesticular parenchyma slightly to right of median line. Oviduct arises from middorsal aspect of ovary. Laurer's canal branches from oviduct and terminates on middorsal surface. Vitelline follicles small, 0.07-0.06 in diameter, dispersed between primary and secondary ceca. Vitelline duct joins oviduct at junction of oviduct with ootype. Uterus originates as terminal coil of ootype, proceeds anteriad ventral to ootype but dorsal to left margin of anterior testis. Uterus turns abruptly approximately 2 mm in front of anterior testis and proceeds posteriad as a broad uterine sac, length 2.14 (1.15-3.20). A bulbous metraterm (0.30×0.24) arises from the ventroposterior limit of uterine sac and empties into a ventromedian genital-atrium. Testes tandem in posterior half of body. Anterior testis U shaped, length 1.05 (0.7-1.6), width 1.55 (0.9–2.0); posterior testis heart shaped, length 0.83 (0.5-1.25), width 1.31 (0.8-1.79). Host: Ardea purpurea (purple heron).

HABITAT: Esophagus.

LOCALITY: Paku and Muara, Lindu Valley of Central Sulawesi, Indonesia (1°19'S, 120° 04'E, 950 m).

PLESIOTYPES: Two specimens in USNM Helm. Coll. No. 74355. Remaining specimens in Helm. Coll., U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, Indonesia. Nos. Djllv. 0029, 0035, 0036.

ATTACHMENT: Euclinostomum multicaecum was attached to host esophageal tissue by



Figures 2, 3. Attachment of *E. multicaecum* to esophageal tissues. 2. Sagittal section through oral region. 3. Oral field–esophagus interface. BM, blood meal; E, esophagus; I, interface of oral field and host esophageal tissue; HE, host esophageal tissue; OF, oral field; OS, oral sucker; P, pharynx; PP, prepharynx.

means of its acetabulum and oral field. The oral attachment, surprisingly, did not involve the oral sucker (Fig. 2). Instead, the tegument of the oral field adhered to the submucosa of the esophagus (Fig. 3).

The mucosa was eroded at the attachment site and the oral sucker interfaced directly with submucosal connective tissue. Adjacent to and surrounding the attachment site there was an extensive granulomatous reaction composed predominately of numerous foamy histiocytes. In addition, there were scattered mononuclear cells, both lymphoid and plasmacytic types, and occasional polymorphonuclear leucocytes. The attachment site was also characterized by necrosis and nuclear debris. Marked edema occurred in the surrounding tissues.

Discussion

Ukoli (1966) and Dennis and Sharp (1973) recognized only two species in the genus Euclinostomum Travassos (1928), namely E. heterostomum, and E. multicaecum. The adult morphology of E. heterostomum was carefully redescribed by Dennis and Sharp (1973). Euclinostomum multicaecum, however, was described from encysted and unencysted metacercarial stages (Tubangui and Masilungan, 1935; Velasquez, 1959). The most distinguishing feature of the metacercarial stage of E. multicaecum, by which it was distinguished from E. heterostomum, is that in the former all cecal diverticula reach the posterior end of the body. Our study of adult specimens corroborated the distribution of cecal diverticula as a

distinguishing feature for ovigerous specimens of *E. multicaecum*. Ovigerous *E. multicaecum* can also be separated from *E. heterostomum* by the following: (1) The two main intestinal ceca do not form an H-shaped configuration; (2) the number of secondary cecal diverticula (ventral view) in *E. multicaecum* (8–10 right and 7–10 left) is less than in *E. heterostomum* (12–15 right and 9–12 left); and (3) adult specimens of *E. multicaecum* are larger (16.3 in length \times 6.24 in width) than *E. hetero*stomum (8.92 in length \times 2.92 in width) as measured by Dennis and Sharp (1973).

The pharynx of *E. multicaecum* metacercariae was considered rudimentary (Tubangui and Masilungan, 1935; Velasquez, 1959). However, in adult specimens, as one might suspect, we observed a well-developed prepharynx, pharynx and a bulb-shaped esophagus similar to those described for *E. heterostomum* (Dennis and Sharp, 1973).

An adhesive function for the oral field of clinostomid trematodes has not been reported to our knowledge. In strigeoids an adhesive area, or organ, is a distinguishing feature and excorporeal digestion and absorptive functions are attributed to this organ (Erasmus, 1972). In our material, even though the epithelium of the host's esophagus had been eroded, the function of this area appeared to be one of attachment. Perhaps this phenomenon is related to the precarious habitat *E. multicaecum* occupies. Without such a strong attachment these flukes might be scraped off the oral or esophageal epithelium when fish or other bulky foods were swallowed.

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Editorial Acknowledgment

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Digenetic Trematodes of the Marine Fish, Girella nigricans (Ayres), from Southern California with the Description of Two New Species

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ABSTRACT: The following digenetic trematodes have been found in the digestive tract of the marine fish *Girella nigricans* (Ayres) collected in southern California: *Opisthadena cheni* sp. n.; *Vitellibaculum girellicola* sp. n.; *Opechona orientalis* (Layman, 1930) Ward and Fillingham, 1934; *Opecoelus adsphericus* Manter and Van Cleave, 1951; and *Schikhobalotrema girellae* (Manter and Van Cleave, 1951) Skrjabin and Guschanskaja, 1955. Major differences between the new species and others in their genera are different number of oral papillae and absence of diverticula from the anterior excretory crura in *Opisthadena cheni*, and the presence of four columns of vitellaria in *Vitellibaculum girellicola*.

Species of *Girella* occur on both sides of the Pacific Ocean. Barnhart (1936) stated that *G. nigricans* is found from Cape San Lucas to San Francisco. The presently known distribution of *Girella* in the western Pacific is the seas adjacent to Japan, Taiwan and the Philippines (Lindberg and Krasyukova, 1969). Digenetic trematodes have been reported from these fish by Manter and Van Cleave (1951), Montgomery (1957), Winter (1960) and Yamaguti (1940).

In the present study, five *Girella nigricans* were examined, four collected at White Point, San Pedro, California and one collected in the San Pedro area; the latter was held for more than one year in an aquarium at the Cabrillo Marine Museum. All were parasitized by digenetic trematodes. These worms were fixed in hot 5% formalin without pressure, stained with Mayer's paracarmine, dehydrated in ethyl alcohol, cleared in methyl benzoate and mounted in Canada balsam. Measurements are in micrometers and averages in parentheses. Drawings were made with the aid of a camera lucida.

Family Hemiuridae Lühe, 1901 Subfamily Opisthadeniinae Yamaguti, 1970

Opisthadena cheni sp. n. (Figs. 1–3)

The terminology recommended by Manter (1970) is followed, where appropriate, in de-

scribing this species. Description based on 5 mature worms from 1 fish and 1 immature specimen from another. Body elongate, nearly uniform in width and lacking ecsoma. Tegument smooth except for scattered papillae and fine transverse striae posterior to acetabulum. Large (33 by 26, with nuclei about 3), oval unicellular glands scattered through body. Body length 2,710–3,875 (3,254); body width 279-341 (310). Oral sucker 78-144 (112) long, 90–113 (98) wide, partially surrounded by a variable lobe (possibly comparable to Manter's "preoral lobe") with thin tegument. Lobe bears at least 6 papillae and varies in size depending on the degree of retraction of the oral sucker. Six papillae surround mouth. Acetabulum 255–333 (294) long, 200–300 (249) wide; ratio of average oral sucker and acetabular widths 1: 2.5. Acetabulum circled by band of muscle fibers and about 8 papillae. Wedge-shaped ridges at postlateral margins of acetabulum (Figs. 1, 2) transversely joined and provided with muscle fibers. Preacetabular pit lacking. Prepharynx extremely short or lacking. Pharynx 89–122 (110) long, 90–114 (101) wide. Esophagus and portions of adjacent, expanded ceca lined with continuation of body tegument. Ceca pass laterad to terminate blindly near posterior end of body.

Genital pore ventral, median, from immediately posterior to pharynx to near midway between pharynx and acetabulum; 2 papillae on each side of genital pore. Testes 2, nearly spherical to triangular, tandem, separated by



uterine coils, in posterior half of body. Testes slightly overlap in immature specimen. Anterior testis 100–133 (118) long, 111–178 (146) wide; posterior testis 122-166 (140) long, 122-178 (146) wide. Seminal sac 80-133 (97), nearly as wide, enclosing coiled seminal vesicle, postacetabular. Prostate postacetabular, cylindrical, about 499 long, 99 wide. A narrow tube leads from prostate to sinus sac. Sinus sac 133–166 (149) long, 72–129 (102) wide containing gland cells and terminal portions of male and female ducts. Hermaphroditic duct bipartite opening at tip of sinus organ (Fig. 3). Genital atrial wall surrounding proximal portion of sinus organ, with muscular rings. Ovary transversely elongate, posttesticular, in posterior third of body. Ovary length 80–111 (95), width 111–178 (149). Seminal receptacle pyriform, immediately anterior to or sometimes overlapping ovary. Laurer's canal not seen, probably lacking. Two transverse vitelline bodies immediately posterior to ovary. Uterus extends posteriad beyond vitellaria, occasionally to anterior border of excretory bladder stem, then proceeds anteriorly in short transverse loops to hermaphroditic duct. Gland cells surround uterus near sinus sac. Eggs with smooth shells, 28-44 (36) long, 13–18 (15) wide.

Excretory pore terminal. Excretory bladder with tubular stem and crura extending laterally and anteriorly to unite dorsal to posterior portion of oral sucker. Anterior crura without lateral diverticula.

HOST: *Girella nigricans* (Ayres), opaleye. LOCATION: Stomach.

LOCALITY: White Point, San Pedro, California.

HOLOTYPE: Opisthadena cheni sp. n. de-

posited as No. 771, Hancock Parasite Collection, Univ. So. California.

The specific name is in honor of the late Dr. T. T. Chen.

The genus *Opisthadena* was erected by Linton (1910) with O. dimidia as type from the stomach of *Kyphosus sectatrix* collected in the Dry Tortugas, Florida. Manter (1947) redescribed this species from the same locality and added a new host Kyphosus incisor. Srivastava (1941) described a new species of trematode from *Clupea illisha* collected in Pakistan. He placed it in the genus Sterrhurus but Chauhan (1954) erected the genus Ahemiuris for it. Yamaguti (1958) declared Ahemiuris a synonym of Opisthadena. Johnson and Copsey (1953) described O. bodegensis from Cebidichthys violaceus collected at Dillon Beach, California. Bravo-Hollis (1965, published 1966) described O. cortesi from Kyphosus elegans collected in Baja California, Mexico. Overstreet (1969) found greater variation in O. dimidia than had been reported previously which forced him to declare O. cortesi a synonym of O. dimidia. Yamaguti (1970) described O. kyphosi from Kyphosus cinerascens collected in Hawaii. Opisthadena cheni differs from the above in number of papillae on the preoral lobe and around the oral sucker, in size of major organs and in lacking lateral diverticula from the anterior portions of the excretory crura (where they have been described in other species).

Kyphosid fish probably are the disseminators of opisthadenids around the world. Girellids evidently are closely related to kyphosids because they have been shifted between the Families Kyphosidae and Girellidae (stated to me by Dr. Basil Nafpaktitis, ichthyologist, U.S.C.).

←

Figures 1-3. Opisthadena cheni. 1. Lateral view. 2. Ridge at postlateral margin of acetabulum. 3. Terminal genitalia.

Abbreviations: A = acetabulum; C = cirrus; CS = cirrus sac; E = excretory bladder; EG = egg; ES = eyespot pigment; EV = excretory vessel; G = genital pore; GA = genital atrium; H = hermaphroditic sac; HD = hermaphroditic duct; L = lymph sac; M = muscular body (sucker?); MB = muscle bands; O = ovary; OS = oral sucker; P = pharynx; PD = prostate ducts; PR = prostate; S = internal seminal vesicle; SA = sinus sac; SE = external seminal vesicle; SO = sinus organ; SR = seminal receptacle; SS = seminal sac; T = testis; U = uterus; V = vitellaria.



Figures 4, 5. Opechona orientalis. 4. Ventral view. 5. Terminal genitalia.

Whether the two populations of Pacific *Girella* were parasitized by opisthadenids before or after separation remains unsolved.

Family Lepocreadiidae Nicoll, 1934 Subfamily Lepocreadiinae Odhner, 1905 Opechona orientalis (Layman, 1930) Ward and Fillingham, 1934 (Figs. 4, 5)

Thirteen ovigerous specimens were taken from the intestines of 2 fish. Description as figured. To date this is the only species of *Opechona* with 3 longitudinal main excretory tubes. Only the median tube contains excretory concretions. In most respects these specimens agree with Manter's (1940) description. The few discrepancies probably are due to differences in pressure during fixation.

This species has been reported from both sides of the Pacific. It has been found in *Girella nigricans* by Manter and Van Cleave (1951) and Montgomery (1957) collected at La Jolla, California.



Figures 6, 7. Vitellibaculum girellicola. 6. Dorsal view. 7. Terminal genitalia. Figure 8. Schikhobalotrema girellae, protruded cirrus.

Family Opecoelidae Ozaki, 1925 Subfamily Opecoeliinae Stunkard, 1931 Opecoelus adsphericus Manter and Van Cleave, 1951

One specimen of this species was found and its measurements fall within the ranges given by Manter and Van Cleave (1951). They found it in *Girella nigricans* and *Clinocottus analis australis* collected at La Jolla, California.

Family Haploporidae Nicoll, 1914 Vitellibaculum girellicola sp. n. (Figs. 6, 7)

Description based on 10 specimens including 3 nonovigerous. The latter are 1,210–1,550 (1,395) long, 186–264 (202) wide.

Body 1,440–2,080 (1,700) long, 186–264 (225) wide; tegument spined except on dorsal posterior half of body; large circular glands between pharynx level and anterior end of body; evespot pigment heavy; oral sucker 67-111 (81) long, 80–111 (101) wide; acetabulum 111–171 (142) long, 100–158 (126) wide; prepharynx 166-280 (214) long; pharynx 100-133 (121) long, 89-111 (100) wide; esophagus 144-311 (226) long; gut bifurcation at acetabular level, ceca end short distance from posterior end of body. Gonads tandem in posterior half of body; posterior testis 92–256 (196) long, 108–162 (152) wide; anterior testis 110-215 (163) long, 92-164 (142) wide; ovary immediately pretesticular, nearly spherical, 50-123 (114) long, 49-118 (87) wide; external seminal vesicle long, tubular, convoluted; uterus between ovary and hermaphroditic sac; seminal receptacle uterinum present; vitellaria in 4 lateral and median columns from just posterior to acetabulum to near posterior end of body, confluent posteriorly; eggs 49-62 (57) long, 31-39 (34) wide. Hermaphroditic sac oval, preacetabular, containing internal seminal vesicle, prostate cells, terminal portions of genital ducts, a muscular body which probably can be everted as a sucker and a hermaphroditic duct. Genital pore between acetabulum and pharynx. Four lymph sacs at posterior end of body. Excretory bladder tubular with sphincter and cellular mass around exit.

HOST: Girella nigricans.

LOCATION: Intestine.

LOCALITY: White Point, San Pedro, California.

HOLOTYPE: Vitellibaculum girellicola sp. n. deposited as No. 772, Hancock Parasite Collection.

The genus Vitellibaculum was erected by Montgomery (1957) with V. girellae as type from the intestine of G. nigricans at La Jolla, California.

Montgomery described only lateral vitellaria, but I found a few dorsal and median follicles in his type specimen. He reported a seminal receptacle in this species but Durio and Manter (1968) found it to be a seminal receptacle uterinum. The seminal vesicle extends farther posteriad than Montgomery indicated. In the type specimen it terminates nearer the ovary than the acetabulum. Siddiqi and Cable (1960) erected a new genus Allomegasolena for two species, A. spinosa and A. attenuata, from Puerto Rican fish. Durio and Manter (1968) declared Allomegasolena a synonym of Vitellibaculum. Vitellibaculum girellicola differs from the other species in the genus in the possession of four longitudinal columns of vitellaria.

Family Haplosplanchnidae Poche, 1925 Schikhobalotrema girellae (Manter and Van Cleave, 1951) Skrjabin and Guschanskaja, 1955

This species was found in all the Girella examined, sometimes in large numbers (103, 202). The fish kept in an aquarium for over one year had only one specimen that probably was acquired before captivity. Manter and Van Cleave (1951) described this species from Girella nigricans collected at La Jolla, California and placed it in the genus Haplosplanchnus. Skrjabin and Guschanskaja (1955) transferred most of the species of Haplosplanchnus to their genus Schikhobalotrema mainly on the basis of more extensive vitellaria in the latter. Manter and Van Cleave described a papilla on the midventral wall of the oral sucker but did not mention the approximately 24 gland cells between the oral sucker and the acetabulum whose ducts converge to this papilla. They mentioned an ovoid to elongate prostatic vesicle but not the approximately 40 prostate cells alongside the acetabulum whose ducts empty into the male duct (Fig. 8). In all my specimens the cirrus was extruded.

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Staphylocystis (Staphylocystis) suncusensis sp. n. (Cestoda: Hymenolepididae) from the Musk Shrew, Suncus murinus (Soricidae), from Taiwan, with a Key to the Known Species of Staphylocystis Villot, 1877

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ABSTRACT: Compared to the four species previously described from the musk shrew (Suncus murinus) in India, Staphylocystis (Staphylocystis) suncusensis sp. n. from this host on Taiwan differs markedly. Staphylocystis (S.) suncusensis has 11-14 rostellar hooks 16-18 μ long and a strobila 6.4-18.2 mm long. Staphylocystis (S.) sanchorensis Nama and Khichi, 1975 has 30 hooks 15-17 μ long and a strobila 3.11-10.77 mm long. Staphylocystis (S.) sindensis Nama, 1976 has 20 hooks 22-23 μ long and a strobila 7-8.5 mm long. Staphylocystis (S.) minutissima (Meggitt, 1927) has 12 hooks 16-18 μ long and a strobila 2 mm long. Staphylocystis (S.) solitaria (Meggitt, 1927) has 16 hooks 16-17 μ long and a strobila 2 mm long. The last two species were reported from Crocidura murina which is probably Suncus murinus. Rostellar hooks of each species are distinct in shape and characteristic of it. Staphylocystis (S.) acuta (Rud., 1819) and S. (S.) bacillaris (Goeze, 1782) are transferred to the genus Vampirolepis Spassky, 1950 because of the large number of characteristically shaped rostellar hooks and very long strobilae. The generic description is revised to exclude them. Hymenolepis asketus Brooks and Mayes, 1977 is transferred to S. (Staphylocystoides) on the basis of two poral testes in relation to the vitelline gland and small strobila. Hymenolepis pauciproglottis Neiland, 1953, as used by Olsen (1969), is recognized as belonging the S. (Staphylocystoides). A key to the known species of Staphylocystis together with illustrations of rostellar hooks of the species are included.

Hymenolepidid cestodes collected from the musk shrew *Suncus murinus* Linnaeus, in Wu Shi, Nantous Hsien, Taiwan, are considered an undescribed species of *Staphylocystis* Villot, 1877. The collection consists of five strobilae that appear complete, one gravid strobila without a scolex, 10 additional scolices, and a number of fragments which formed the basis for the description.

Materials and Methods

The fixing fluid used is unknown. Specimens were stained in Semicohn's acid carmine and mounted in Permount. Illustrations were made with the aid of a camera lucida. Measurements in the text are given in micrometers except when stated otherwise.

Staphylocystis (Staphylocystis) suncusensis sp. n.

DESCRIPTION: Characters of the genus. Strobila 6.4–18.2 mm long, with five worms having 101, 131, 216, 257 and 259 recognizable proglottids. Mature proglottids up to 477 wide by 180 long; gravid ones up to 700 by 180 long; neck smooth, up to 234 long; mature and gravid proglottids serrate. Scolex 197-239 wide by 128-213 long; suckers more or less circular, 67-82 in diameter. Rostellum 40-45 in diameter. Rostellum 40-45 in diameter by 85-146 long; 11-14 rostellar hooks (11 on 5 scolices, 12 on 3, 13 on 6, 14 on 1) 16.2–18.4 (mean 17.5) long; blade long, slender and pointed, guard shorter and thick, and handle very short and slightly upturned terminally. Genital pores unilateral, slightly posterior to middle of margin of proglottid. Cirrus not extruded and details of its structure not ascertained. Excretory canals not seen. Cirrus sac slightly S shaped, extending externally from near anterior margin of proglottid to genital pore, 80-154 long by 27-80 wide; testes 1 poral and 2 aporal to ovary and vitelline gland and tandem, oval, 53-160 long by 32-64 wide. Ovary small, 18-27 by 18-29, located at anterior margin of 2 posterior testes; vitelline gland posterior to
ovary, 32–69 long by 24–35 wide. Gravid uterus saccate, fills proglottid. Eggs 29–40 long by 20–32 wide.

HOST: Suncus murinus Linnaeus, 1776.

SITE: Small intestine.

LOCALITY: Wu Shi, Nantous Hsien, Taiwan. PARATYPES: USNM Helm. Coll. No. 74543. HOLOTYPE: USNM Helm. Coll. No. 74657.

Discussion

The genus Staphylocustis was proposed by Villot (1877a) for grapelike clusters of cysticercoids found in the millipede Glomeris limbata Latreille. The cysticercoids are formed by successive branching and external proliferation of secondary ones. There is no external membrane enclosing the cluster (Ransom, 1904; Wardle and McLeod, 1952) as distinct from *Coenurus* and *Echinococcus* with internal budding and external enclosing membrane. From these clusters of cysticercoids, Villot (1877a) described S. bilarius from the Malpighian tubules, and later (1877b) S. micracanthus from the fat bodies. In a subsequent paper (1877c), he recognized S. bilarius as the cysticercoids of *Taenia scutigera* Dujardin, 1845 and T. scalaris Dujardin, 1845, and S. micracanthus as Taenia pistillum Dujardin, 1845.

Joyeux and Baer (1935) demonstrated experimentally that *S. micracanthus* Villot, 1877a develops into *Hymenolepis pistillum* (Dujardin, 1845).

Hughes (1940) pointed out that the genus *Hymenolepis* contained 320 recognized species. He (1941) prepared a key, acknowledged as not entirely reliable, together with figures from the literature of the rostellar hooks of the species.

Skrjabin and Matevosyan (1948) reproduced from the original literature specific descriptions and figures of the species of Hymenolepididae reported from mammals.

Spassky (1950) pointed out that inasmuch as *Staphylocystis micracanthus* Villot, 1877 is a synonym of *Taenia pistillum* Dujardin, 1845, it shall be regarded as the genotype of *Staphylocystis*, i.e., *S. pistillum* (Dujardin, 1845).

In his classification of the hymenolepidid cestodes of mammals, Spassky (1954) provided a description of the genus *Staphylocystis*. In it he pointed out that the species are small tapeworms but gave no indication of

the number of proglottids that might be considered in the category of small, as he used it.

Yamaguti (1959) divided the genus Staphylocystis Villot, 1877 into two subgenera. The subgenus Staphylocystis Villot, 1877, with Hymenolepis pistillum (Dujardin, 1845) as type, contains 16 species, 9 of the same ones included by Spassky (1945). The members of this subgenus include those species of the genus with one testis poral to the vitelline gland and two aporal. The species listed by Yamaguti range from 0.6 mm long with as few as 10-12proglottids [S. (S.) pistillum (Duj. 1845)] to 150 mm long and probably with hundreds of proglottids [S. (S.) bacillaris (Goeze, 1782)].

The subgenus Staphylocystoides Yamaguti, 1959, with Hymenolepis sphenomorphus Locker and Rausch, 1952, as type, contain six species. The subgenus is characterized by two testes being poral to the vitelline gland and one aporal. They range in size from 0.475 mm long with 7–10 proglottids [S. (S.) parvissima (Voge, 1953)] to 20 mm long with 158 recognizable proglottids [S. (S.) sengeri (Neiland, 1953)].

Yamaguti's (1959) description of the genus varies from that of Spassky (1954) in such a manner as to be inconsistent with the species that he (Yamaguti) included. He stated that the species are "small worms," as did Spassky, but added "proglottides not numerous . . . , which statement Spassky's description does not include. These two statements by Yamaguti are contradictory and meaningless when S. (S.) pistillum and S. (S.) bacillaris as extremes, with intermediaries of short and long lengths, are included. Yamaguti's description of the gravid uterus is "horseshoe-shaped . . . or a simple sac." Since both authors included some species with a horseshoe-shaped uterus and others with a sae-shaped uterus, this change by Yamaguti appears logical.

Because of the discrepancy between Yamaguti's (1959) description of the genus and the number of proglottids in the species, it is emended as follows to rectify the inconsistency.

Staphylocystis Villot, 1877 emended

HYMENOLEPIDINAE: Small to medium-sized worms with very few to many proglottids. Rostellum well developed, with rostellar sheath and a crown of 8–10 or more hooks; guard and blade of hook well developed. Proglottids

















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elongated transversely, trapezoidal or almost quadrate in mature and gravid ones. Excretory stems of 2 pairs; inner longitudinal muscle bundles of four pairs. Testes 3, in a triangle or transverse row; 1 poral, 2 antiporal, to vitelline gland, or vice versa. Cirrus pouch may or may not reach median line. External and internal seminal vesicle present. Accessory sac and stylet absent. Ovary compact or slightly lobed, median or submedian; vitelline gland median or submedian. Uterus horseshoe shaped, with apex directed forwards, or a simple sac. Parasites of Insectivora or Chiroptera.

GENOTYPE: Staphylocystis pistillum (Dujardin, 1845) Spassky, 1954.

Not conforming to the generic description of *Staphylocystis* in the large size of the strobilae and numerous (up to 50) Y-shaped rostellar hooks with the handle comparatively long and the guard broad and equal to or slightly more or less as long as the blade, *S.* (*S.*)*bacillaris* (Goeze, 1782) and *S.* (*S.*)*acuta* (Rud., 1819) are transferred to the genus Vampirolepis (Spassky, 1954).

Prokopic (1967) in his studies on S. (Staphylocystis) murissylvatici (Rud., 1819) from Apodemis considered it a synonym of Varilepis crenata (Goeze, 1782).

Staphylocystis (Staphylocystis) suncusensis sp. n. in the musk shrew, Suncus murinus from Taiwan with 11–14 rostellar hooks 16–18 μ long and of characteristic shape (Figs. 2, 20) and longer strobila differs markedly from S. (S.) sanchorensis Nama and Khichi, 1975, with 30 hooks 15–17 μ long (Fig. 19) and S. (S.) sindensis Nama, 1976, with 20 hooks 22–23 μ long (Fig. 17) from Suncus murinus sindensis in India.

Of those species with fewer than 20 rostellar hooks in the range of $16-18 \mu \log_3$, it is closest to S. (S.) minutissima in Crocidura murina¹ from Rangoon described by Meggitt (1927) as having 12 hooks $16-18 \mu \log_3$, whose guard and blade are much larger than the short pointed handle (Fig. 15) and S. (S.) solitaria Meggitt (1927), also from *Crocidura murina* from Rangoon with 16 hooks 16–17 μ long, whose guard is massive in comparison to the blade or handle (Fig. 21). Further differentiation from other species may be made with the aid of the key that follows.

On the basis of the small strobila and the arrangement of the testes with two being poral to the vitelline gland and one aporal, *Hymenolepis asketus* Brooks and Mayes, 1977, from the short-tailed shrew, *Blarina brevicauda*, is transferred to S. (*Staphylocystoides*).

Key to the Species of the Genus Staphylocystis Villot, 1877

- One testis poral to vitelline gland, two aporal; subgenus *Staphylocystis* Villot, 1877 ______ 2 Two testes poral to vitelline gland, one aporal. Subgenus *Staphylocystoides* Yamaguti, 1959 ______ 17
- Testes more or less in straight line across proglottid ______ 3 Testes definitely in triangular arrangement with aporal ones more or less tandem ______ 6
- Thirty-eight to forty hooks 17.6 μ long; testes and ovary in straight line and on same level; strobila 3-5.5 mm long; eggs 27-34 μ. Fig. 9. In Crocidura, Sorex

..... S. (Staphylocystis) tiara (Duj., 1845) Eighteen or fewer rostellar hooks 4

4. Ten rostellar hooks 33-40 μ long, blade and handle nearly equal in length, guard short; strobila 4-6 mm long; uterus crescent shape with concavity anterior. Fig. 10. In Sorex S. (Staphylocystis) toxometra (Baer, 1932) Twelve to thirteen rostellar hooks 5

¹ Probably Crocidura murina is a synonym of Suncus murinus, according to Allen (1906), Allen (1938), and Tate (1947).

4

Figures 1–8. Staphylocystis (Staphylocystis) suncusensis sp. n. 1. Scolex. 2. Rostellar hook. 3. Egg with oncosphere. 4. Proglottid where both male and female reproductive organs first appear simultaneously in strobila. 5. Mature proglottid. 6. Proglottid with uterus beginning to form. 7, 8. Gravid proglottids of different shape. All scales of reproduction 100 μ except where otherwise given. Abbreviations: c, cirrus pouch; e, external seminal vesicle; i, internal seminal vesicle; o, ovary; s, seminal receptacle; t, testis; u, uterus; v, vitelline gland.



 Ovary anterior to testes; 12 rostellar hooks 12–14 μ long, blade extremely large, guard longer than handle; strobila 1.15–1.8 mm long, with up to 8 proglottids. Embryo 20–24 μ. Fig. 12. In Sorex

(Neiland, 1953) Testes anterior to ovary; 12–13 rostellar hooks 26–33 μ long, handle longest, blade longer than short guard; strobila 15 mm long, proglottids numerous; eggs 64 \times 70 μ . Fig. 13. In *Crocidura* S. (Staphylocystis) scalaris (Duj., 1845)

- hooks ______ 8
 Rostellar hooks 10 μ long, 14–22 in number, wrench shaped; strobila 0.62–2 mm long; uterus horseshoe shaped; eggs 51 × 46 μ. Fig. 15. In Crocidura, Sorex ______ S. (Staphylocystis) pistillum (Duj., 1845)

Rostellar hooks 20 μ or more in length 9

 Rostellar hooks 20 μ long, 20–23 in number; strobila 15 mm long; eggs 43 × 32 μ. No Fig. of hooks. In *Pipistrellus* S. (Staphylocystis) sydarensis

(Skarbilovitsch, 1946)

- Twenty rostellar hooks 22–23 μ long; scolex 110–190 μ in diameter. Fig. 16. In Suncus ______ S. (Staphylocystis) sindensis Nama, 1976 Twenty-two to twenty-eight rostellar hooks 26–28 μ long; scolex 210 μ in diameter. Fig. 17. In Crocidura, Neomys, Sorex ______ S. (Staphylocystis) furcata (Stieda, 1862)
 Rostellar hooks 10μ long, 14–22 in
- number, wrench shaped; strobila 0.65–
 2 mm long; scolex 120–125 μ in diameter; uterus horseshoe shaped; eggs 57
 × 46 μ. Fig. 15. In Crocidura, Sorex
 S. (Staphylocystis) pistillum (Duj., 1845)
 Rostellar hooks more than 10 μ long ... 12
- 13. Rostellar hooks 32–34 μ long, 12 in number; scolex 360–380 μ in diameter; strobila 15 mm long. Eggs 67–72 μ. Fig. 18. In Crocidura __________S. (Staphylocystis) dodecacantha (Baer, 1925)

Rostellar hooks 25–29 µ long 14

 2 Hilmy (1936) reported rostellar hooks of S. (S.) loossi and of S. (S.) fuelleborni to be similar in shape.

←

Figures 9-22. Rostellar hooks of known species of Staphylocystis (Staphylocystis) presented without relation to respective sizes. 9. S. (S.) tiara (Duj., 1845), after Hughes (1941). 10. S. (S.) toxometra (Baer, 1932), after Hughes (1941). 11. S. (S.) chrysochloridis (Janicki, 1904), after Skrjabin and Matevosyan (1948). 12. S. (S.) pauciproglottis (Nciland, 1953), after Neiland (1953). 13. S. (S.) scalaris (Duj., 1845), after Hughes (1941). 14. S. (S.) sanchorensis Nama and Khichi, 1975, after Nama and Khichi (1950). 15. S. (S.) pistillum (Duj., 1845), after Skrjabin and Matevosyan (1948). 16. S. (S.) sindensis Nama, 1976, after Nama (1976). 17. S. (S.) furcata (Stieda, 1862), after Hughes (1941). 18. S. (S.) dodecacantha (Baer, 1925), after Hughes (1941). 19. S. (S.) loossi (Hilmy, 1936) and S. (S.) fuelleborni (Hilmy, 1936), after Skrjabin and Matevosyan (1948). 20. S. (S.) suncusensis sp. n. 21. S. (S.) minutissima (Meggitt, 1927), after Meggitt (1927). 22. S. (S.) solitaria (Meggitt, 1927), after Meggitt (1927).

Figures 23-28. Rostellar hooks of known species of *Staphylocystis* (*Staphylocystoides*) presented without relation to respective sizes. 23. S. (S.) sengeri (Neiland, 1953), after Neiland (1953). 24. S. (S.) longi (Oswald, 1951), after Oswald (1951). 25. S. (S.) serrula (Oswald, 1951), after Oswald (1951). 26. S. (S.) parvissima (Voge, 1953), after Voge (1953). 27. S. (S.) asketus (Brooks and Mayes, 1977), after Brooks and Mayes (1977). 28. S. (S.) sphenomorphus (Locker and Rausch, 1952), after Locker and Rausch (1952).

strobila 8 mm long; ovary deeply lobed. Fig. 19. In *Crocidura*, *Sorex* S. (*Staphylocystis*) fuelleborni

(Hilmy, 1936)²

or handle. Fig. 22. In *Crocidura*³ S. (*Staphylocystis*) solitaria (Meggitt, 1927

- 18. Cirrus pouch 140 μ long × 20 μ in diameter and reaching almost across width of proglottid which is 192 μ wide; strobila incomplete and without scolex. No Fig. of hooks. In Orycto-lagus⁴ S. (Staphylocystoides) evansi (Skrjabin and Matevosyan, 1943) Cirrus pouch 80–120 μ long × 20 μ in diameter; strobila up to 20 mm long × 370 μ wide, with 151 recognizable proglottids; 10 hooks 51–56 μ long. Fig. 23. In Sorex S. (Staphylocystoides) sengeri
 - (Neiland, 1953)
- 19. Eight rostellar hooks20Ten rostellar hooks21
- 20. Hooks 20.6–24 μ long, with guard markedly larger than handle or blade; scolex 170–237 μ in diameter, 6–8 proglottids; testes 38–55 μ in diameter. Fig. 24. In Sorex

S. (Staphylocystoides) longi (Oswald, 1951)

Hooks 18–21 μ long, with guard and

(Oswald, 1951)

21. Ovary in anterior half of proglottid; rostellar hooks $20-24 \ \mu$ long, blade, guard, handle about equal in length; cirrus pouch $60-86 \ \mu$ long. Fig. 26. In Sorex

...... S. (Staphylocystoides) parvissima (Voge, 1953)

- Ovary in posterior half of proglottid 22 Hooks 12, 15 m long, grand and blade
- Hooks 13–15 μ long, guard and blade about equal in size, very large and longer than straight, slender handle; strobila 1–2 mm long with 6–8 proglottids; scolex 136–142 μ in diameter; ovary dumbbell shaped; eggs 23–35 μ. Fig. 27. In *Blarina*

 - Hooks 16–20 μ long, guard and blade not large, handle longest; strobila up to 0.8 mm long with 15 or more proglottids; scolex 90 μ in diameter; ovary round or oval. Fig. 28. In Sorex S. (Staphylocystoides) sphenomorphus

(Locker and Rausch, 1952)

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Passerilepis schmidti sp. n. (Cestoidea: Hymenolepididae) from the Blue Jay, Cyanocitta cristata L. in Nebraska

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ABSTRACT: Passerilepis schmidti sp. n. is described from a blue jay, Cyanocitta cristata, from Nebraska. It may be distinguished from the other members of the genus by possessing 10 rostellar hooks 21–26 μ m long, a cirrus sac 173–231 μ m long, eggs 45–68 μ m in diameter, 2 to 5 lobed vitellaria, and proglottids 2 times wider than long. Passerilepis nebraskensis (Rolan and Leidahl, 1969) comb. n. is transferred from the genus Mayhewia.

A cestode from the small intestine of a blue jay, *Cyanocitta cristata* L., collected in Nebraska represents a new species of *Passerilepis* Spassky and Spasskaya, 1954. Previous reports of cestodes infecting *C. cristata* include two reportings of *Oligorchis* sp. (Boyd et al., 1956; Loftin, 1960) and one reporting of *Culcitella* sp. (Boyd et al., 1956). Specimens were relaxed in cold pond-water, fixed in AFA, stained in Mayer's hematoxylin, cleared in methyl benzoate, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube, and all measurements are in micrometers unless stated otherwise.

Passerilepis schmidti sp. n. (Figs. 1–7)

DESCRIPTION (based on 6 mature specimens; n equals number of measurements utilized): Scolex 242–270 long by 265–332 wide (n = 6), set off from neck by conspicuous constriction. Suckers 107–130 in diameter (n = 12), unarmed. Rostellum retracted in all specimens, 76–94 long by 55–71 wide (n = 5); rostellar sac 213–216 long by 87–124 wide (n = 5); extending posterior to sucker; rostellar hooks numbering 10 (n = 6), 21–26 long (n = 5). Neck 243–355 long by 118–282 wide (n = 6).

Strobila 4.5–5.5 cm long by 1.9–1.95 cm wide (n = 5) near posterior end. Proglottids craspedote; mature proglottid 255–480 long by 0.90–1.54 cm wide (n = 10), ratio of width to length 1 : 0.33–0.45 (n = 12); gravid proglottid 510–780 long by 1.26–1.54 cm wide (n = 10), ratio of width to length 1 : 0.27–0.50 (n = 10). Ventral osmoregulatory canals lateral, 10–21 in diameter (n = 10), with transverse canal near posterior extremity of each

proglottid; dorsal canals lateral, generally overlapping ventral canals, 4–5 in diameter (n = 10). Reproductive system protandrous; genital pores unilateral; pore within anterior $\frac{1}{3}$ of proglottid; terminal genital duct dorsal to osmoregulatory canals; genital atrium 10–34 deep by 10–21 wide (n = 13).

Testes dorsal, 1 poral in posterior region of proglottid between osmoregulatory canals and vitellaria, 2 aporal, 1 anteriad and 1 posteriad, both between osmoregulatory canals and vitellaria, irregularly shaped with smooth margins, 113–186 long by 124–225 wide (n = 30). Vas deferens in anterior, medial field. Cirrus pouch with basal portion projecting slightly anteriad, 173–231 long by 42–89 wide (n = 19), proximal ¹/₄ extending internal to osmoregulatory canals. External seminal vesicle pyriform, 18–94 wide (n = 10).

Ovary medial, multilobed, 113–270 long by 169–440 wide (n = 20). Vitellaria with 2 to 5 lobes, dorsal to posterior portion of ovary, ventral to testes, 73–141 long by 68–141 wide (n = 16). Vagina posteroventral to cirrus pouch. Seminal receptacle in anterior field, expanding to anterior margin of proglottid when full, 110–166 long by 200–236 wide (n = 15), persisting conspicuously in gravid proglottids. Eggs spherical, 45–68 in diameter (n = 40), numbering 124–180 per proglottid (n = 10), each containing single oncosphere; oncospheres sperical, 21–50 in diameter, with thin membrane; embryonic hooks 16–24 long (n = 10).

Host: *Cyanocitta cristata* (Linnaeus), blue jay, (Corvidae).

SITE: Small intestine.

LOCALITY: 2.5 km north, 6.5 km west of Guide Rock, Webster County, Nebraska.



Figures 1-6. Passerilepis schmidti sp. n. 1. Scolex. 2. Mature proglottid. 3. Rostellar hooks. 4. Normal egg. 5. Gravid proglottid. 6. Atypical egg, 3 of 40 eggs possessing 7 embryonic hooks.

HOLOTYPE: USNM Helm. Coll. No. 74502; paratype No. 74503.

ETYMOLOGY: This species is named in honor of Dr. Gerald D. Schmidt, professor at the University of Northern Colorado, in recognition of his numerous contributions to cestode taxonomy.

Discussion

By possessing 10 rostellar hooks 21–26 long, P. schmidti most closely resembles P. passeris (Gmelin, 1790) Spassky and Spasskaya, 1954, P. crenata (Goeze, 1782) Sultanov and Spasskaya, 1958, *P. intermedius* (Clerc, 1906) Spassky and Spasskaya, 1954, and *P. spasskii* (Sudarikov, 1950) Spassky and Spasskaya, 1954 from Eurasia. *Passerilepis schmidti* has a cirrus sac 173–231 long, whereas that of *P. intermedius* is 360 long and those of *P. passeris*, *P. crenata*, and *P. spasskii* measure between 140 and 180 long. Eggs of *P. schmidti* range from 45–68 in diameter, whereas those of *P. passeris*, *P. crenata*, *P. intermedius*, and *P. spasskii* have been reported as 95 long by 67 wide, 85–98 in diameter, 100 in diameter, and 61–64 long by 36–39 wide, respectively. Both P. intermedius and P. spasskii contain elongated rather than subspherical testes, and proglottids of P. spasskii average more than 4 times wider than long rather than 2 times wider in P. schmidti. The shape of rostellar hooks in P. crenata differs from that of those in P. schmidti.

Spassky and Spasskaya (1964) revised the Hymenolepididae of birds and synonymized *Mayhewia* Yamaguti 1956 with *Passerilepis*, an action with which we agree. Rolan and Leidahl (1969) described *M. nebraskensis* from the rock dove, *Columba livia* (Gmelin), in Nebraska. That species possesses a single circle of wrench-shaped rostellar hooks, three triangularly arranged testes, and unilaterally located genital pores which are characteristic of species of *Passerilepis*. Although Rolan and Leidahl (1969) failed to find gravid proglottids and thereby establish the worm's generic status, we consider *M. nebraskensis* as *P. nebraskensis* (Rolan and Leidahl, 1969) comb. n.

In addition to *P. nebraskensis*, five other species of *Passerilepis* occur in North America. *Passerilepis chiapensis* (Coil, 1952) Spassky, 1963 from the acorn woodpecker; *Melanerpes* formicivorus (Swainsen), in Mexico has 18 rostellar hooks each 51–59 long and a smaller cirrus sac than that of *P. schmidti*; *P. neb*raskensis possess 8 rostellar hooks each 13.3 long; *P. corvi* (Mayhew, 1925) Spassky and Spasskaya, 1964, from the crow, *Corvus* brachyrhynchus (Brehm), in Illinois contains 8–10 rostellar hooks each 33–36 long, a sucker diameter of 80, and smaller testes than *P*. schmidti. Spassky and Spasskaya (1964) also included Hymenolepis hopkinsi Schiller, 1951, from the black duck, Anas rubripes (Brewster), from Wisconsin in the genus Passerilepis since it possessed 10 rostellar hooks each 26 long, but we do not consider H. hopkinsi in Passerilepis because it contains a U-shaped uterus. Spassky (1959) transferred Oligorchis cyanocitti Coil, 1955, infecting the stellar jay, Cyanocitta stellari (Gmelin), in Mexico to Passerilepis, but since proglottids of O. cyanocitti contain four rather than three testes, it should be placed back into its original genus, Oligorchis.

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Acanthotaenia overstreeti sp. n. (Cestoda: Proteocephalidae) from a Puerto Rican Lizard, the First Acanthotaeniine in the New World

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ABSTRACT: Acanthotaenia overstreeti from the iguanid lizard Cyclura stejnegeri in Puerto Rico most closely resembles A. nilotica in having a very prominent apical organ, A. shipleyi and A. daileyi in relative size of cirrus sac, and A. nilotica, A. shipleyi, A. daileyi, and A. beddardi in testes number. It differs from all species by having a much smaller scolex, 108–116 μ m wide rather than more than 225 μ m, from A. woodlandi by having fewer testes, and from all but A. shipleyi by having a cirrus sac less than 250 μ m long.

The proteocephalidean subfamily Acanthotaeniinae Freze, 1963 contains cestode species parasitizing monitor lizards of the genus *Varanus* occurring throughout the Indo-Pacific. Specimens collected by Dr. E. W. Price from an iguanid lizard Cyclura stejnegeri Barbour and Noble on Mona Island, Puerto Rico, in 1929 and deposited in the National Helminthological Collection, Beltsville, Maryland represent a new species of Acanthotaenia Linstow, 1903, constituting the first record of the subfamily in the New World. We obtained the specimens as whole mounts stained with acetocarmine and mounted in Canada balsam and as alcoholic fragments. Measurements are in μ m unless otherwise stated; figures were drawn with the aid of a drawing tube.

Acanthotaenia overstreeti sp. n. (Figs. 1-3)

DESCRIPTION (measurements of holdfast structures based on 5 measurements, those of proglottids and their structures based on 25 measurements): Scolex, neck, and strobila Scolex 154-189 long by 108-116 spinose. wide; apical organ 91–113 wide at base; apical sucker 51-61 in diameter; scolex suckers 47-61 long by 41-58 wide. Neck immediately posterior to scolex 72-75 wide. Neck up to 40 mm long. Immature proglottids quadrate to longer than wide. Mature proglottids 1.13-1.34 mm long by 0.40-0.50 mm wide; ratio of width to length 1: 2.66-3.11 (1: 2.85). Testes 48-89 (64.2 \pm 12.5, n = 38) in number, 10-30 (17.8) preporally, 9-17 (12.6) postporally, 14-50 (33.8) antiporally; preporal and antiporal fields often confluent in anterior half of proglottid; testes 37–73 in diameter. Vas deferens coiled in median portion of anterior $\frac{1}{2}$ of proglottid. Cirrus sac 145–197 (160) long by 61-95 (77) wide; ratio of cirrus sac length to proglottid width 1:2.20-2.96 (1:2.61). Cirrus armed with spines 6-9 long. Genital pores alternating in anterior 42-57% (49%) of proglottid; genital papillae may be prominent. Vagina anterior or posterior to cirrus sac, surrounded by gland cells porally; vaginal sphincter present; aporal end of vagina dilated to form seminal receptacle. Ovary bialate with lobate wings, 180–265 wide at isthmus: wings 180-265 long; Mehlis' gland prominent. Uterus not preformed in mature proglottids. Vitellaria follicular, medullary, in paired lateral, roughly single file rows extending longitudinally from anterior end of proglottid to near posterior end. Vitelline reservoir dorsomedian to ovarian isthmus. Ovarian wings reaching nearly to vitelline fields. Gravid proglottids 1.32–1.82 mm long by 0.51-0.60 mm wide; ratio of width to length 1: 2.22-3.57. Genital pores alternating in anterior 40-47% of proglottid. Cirrus sac 203-219 (215) long by 81-97 wide; ratio of cirrus sac length to proglottid width 1: 2.52-2.96. Ovary 227-309 long by 325-406 wide at isthmus. Uterus a narrow tube with irregular shallow branches. Eggs not contained in capsules, collapsed in our specimens; oncospheres 10-15 in diameter.

Host: *Cyclura stejnegeri* Barbour and Noble (Sauria: Iguanidae).

SITE OF INFECTION: Intestine.



Figures 1-3. Acanthotaenia overstreeti. 1. Scolex. 2. Mature proglottid. 3. Terminal genitalia.

LOCALITY: Mona Island, Puerto Rico.

HOLOTYPE: USNM Helm. Coll. No. 73060. PARATYPES: USNM Helm. Coll. No. 73061. Unmounted fragments in original vial, USNM Helm. No. 26907.

ETYMOLOGY: The species is named for Dr. Robin M. Overstreet, Gulf Coast Research Laboratory, in recognition of his contributions to helminth taxonomy and ecology.

We agree with Schmidt and Kuntz (1974) that Acanthotaenia contains nine nominal species, including those Freze (1965) placed in his genus Rostellotaenia. Morphological data adequate for differentiation and comparison are lacking for four of those nine species (see Freze, 1965, for species inquirendae). By possessing 48 to 89 testes per proglottid, A. overstreeti resembles A. shipleyi Linstow, 1903 (40–65), A. nilotica Beddard, 1913 (45–80), A. beddardi (Woodland, 1925) Schmidt and Kuntz, 1974 (60-80), and A. daileyi Schmidt and Kuntz, 1974 (46-82), but differs from A. woodlandi (Moghe, 1925) Schmidt and Kuntz, 1974 (90–130). The scolex of the new species is 108 to 116 µm wide, and only A. shipleyi with a scolex 150 to 230 μ m wide possesses a scolex less than 225 μ m in width. The relatively prominent apical organ of the Puerto Rican species is most similar to that of A. nilotica from Africa. The new species further resembles A. shipleyi by having a cirrus sac which does not exceed 250 μ m in length, and A. shipleyi and A. daileyi by exhibiting a cirrus sac extending medially more than 1/3 of the total proglottid width.

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Intermediate Hosts for Tegorhynchus furcatus and Dollfusentis chandleri (Acanthocephala)¹

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ABSTRACT: Cystacanths of Tegorhynchus furcatus are described from the haustoriids Lepidactylus sp. and Haustorius sp., and those of Dollfusentis chandleri are described from Lepidactylus sp., the aorid Grandidierella bonnieroides, and the corophid Corophium lacustre. These infected amphipods, inhabiting marine and brackish environs in Mississippi and adjacent regions, represent the first intermediate hosts known for illiosentid or closely related acanthocephalans. In addition to infecting a variety of teleost definitive hosts, both acanthocephalan species also infected dasyatid rays, but were not observed to develop shelled acanthors in the elasmobranchs.

An intermediate host for a member of the Illiosentidae has not been reported. In fact, no intermediate hosts have been reported for "rhadinorhynchids" in the broad sense unless we consider Leptorhynchoides thecatus (Linton, 1891) Kostylew, 1924 in that group (see DeGiusti, 1949). Our study reports amphipod hosts as well as some fishes for two illiosentids, describes the cystacanths, and discusses the infections.

Materials and Methods

Amphipods were collected with a finemeshed dipnet, and the acanthocephalans removed from them, placed in tap or distilled water to promote eversion of the proboscides

¹ This study was conducted in cooperation with the U.S. Department of Commerce, NOAA, National Marine Fisheries Service, under PL 88-309, Project No. 2-262-R. ² Division of Natural Sciences and Mathematics, Liv-ingston University, Livingston, Alabama 35470. ³ Gulf Coast Research Laboratory, Ocean Springs, Mis-sissippi 39564. ⁴ Dauphin Island Sea Lab, Dauphin Island, Alabama 36528.

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before fixing them in AFA, staining in Mayer's carmalum or Van Cleave's hematoxylin, and mounting in Histoclad or Canada balsam. Descriptions of cystacanths include only those features, excepting length and width of trunk, not modified by growth that substantiate our identifications. Sectioning of amphipods allowed examination of the host-parasite relationship. All measurements are in micrometers, unless otherwise specified, with averages in parentheses; figures were drawn with the aid of a camera lucida. An asterisk in the host lists signifies that the host harbored some gravid females. Specimens were deposited in the U.S. National Museum (USNM) and The Manter Laboratory, University of Nebraska State Museum (UNSM).

Tegorhynchus furcatus (Van Cleave and Lincicome, 1939) Bullock and Mateo, 1970

The species was described from Menticirrhus americanus from Louisiana as Illiosentis furcatus. Bullock and Mateo (1970) placed Illiosentis Van Cleave and Lincicome, 1939 as a junior synonym of Tegorhynchus Van Cleave, 1921, but were not specific about the status of all species of Illiosentis, a matter still requiring attention. In any event, I. africanus Golvan, 1955 (=I. furcatus africanus Golvan, 1955) from Senegal, Africa, I. edmondsi Golvan, 1960 (=I. furcatus sensu Edmonds, 1957) from Western Australia, and I. cetratus Van Cleave, 1945 from California are not identical with T. furcatus.

Cystacanth (Figs. 1-2, 5-8)

AMPHIPOD HOSTS AND LOCALITY: Lepidactylus sp., Haustorius sp. (both are undescribed species of Haustoriidae, see text); from Horn Island, Mississippi.

SITE: Hemocoel.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74600 (pair); UNSM No. 20855 (pair).

DESCRIPTION (based on 20 male and 27 female cystacanths): Trunk elongate, cylindrical, with sheathed cuticular spines encircling anterior portion of trunk and extending posteriorly from junction with neck to slightly past midlevel of proboscis receptacle. Proboscis elongate, clavate, with armature of 3 distinct types: 6 to 8 greatly enlarged hooks in ventral portion of basal circle; 4 rows of thick, strongly recurved hooks in middorsal region, extending posteriorly from level of sensory papillae, becoming less recurved and spinelike towards proboscis base; remaining hooks longer and widely separated in anterior portion of proboscis, decreasing in length and becoming less recurved and more crowded towards proboscis base, with dorsal hooks longer, thinner, and less recurved than ventral hooks. Sensory papillae a lateral pair at level of 11th to 14th (usually 12th or 13th) hook from base of proboscis.

Male: 3.2–6.7 (5.5) mm long by 0.24–0.66 (0.45) mm maximal width. Cuticular spines 38-53 (47) long anteriorly, gradually decreasing to 18–25 (20) long posteriorly. Genital spines lacking. Proboscis 1,025-2,200 (1,875) long by 200-250 (225) wide at anterior and 153-180 (171) wide at base, armed with 13-14 (usually 14) longitudinal rows of 28-34 (usually 32) hooks each; enlarged hooks in basal circle 70–113 (93) long; hooks in modified middorsal rows numbering 10-13 per row, 28-35 (33) long anteriorly and 28-40 (35) long posteriorly; remaining unmodified hooks reaching maximal length anteriorly with dorsal hooks 75–88 (84) long and ventral hooks 73–93 (80) long, at level of sensory papillae dorsal hooks 43–58 (50) long and ventral hooks 53–63 (59) long; unmodified ventral hooks in basal circle 33-48 (40) long.

Female: 4.1–9.1 (6.8) mm long by 0.26– 0.63 (0.45) mm maximal width. Cuticular spines 43–55 (49) long anteriorly, gradually decreasing to 18–28 (21) long posteriorly. Posterior end of trunk modified into dorsal and ventral protuberances, with vaginal opening on dorsal of posterior protuberance; muscular fan-shaped structure underlying area between protuberances. Genital spines present on both protuberances. Proboscis 1,700–2,490 (2,161)

Figures 1-4. Female tegorhynchid cystacanths. 1. Proboscis of *Tegorhynchus furcatus*. 2. Posterior end of *T. furcatus*. 3. Proboscis of *Dollfusentis chandleri*. 4. Posterior end of *D. chandleri*.



long by 270–310 (282) wide at anterior and 150–210 (183) wide at base, armed with 14–15 (usually 14) longitudinal rows of 29–35 (usually 33) hooks each; enlarged hooks in basal circle 95–120 (109) long; hooks in modified middorsal rows numbering 11–15 per row, 30– 40 (35) long anteriorly and 30–58 (41) long posteriorly; remaining unmodified hooks reaching maximal length anteriorly with dorsal hooks 80–95 (89) long and ventral hooks 80–95 (86) long, at level of sensory papillae dorsal hooks 45–65 (53) long and ventral hooks 33–63 (42) long.

Adult

PISCINE HOSTS AND LOCALITIES: *Menticirrhus americanus (Linnaeus) southern kingfish (Sciaenidae); Fundulus similis (Baird and Girard) longnose killifish (Cyprinodontidae); Dasyatis sabina (Lesueur) Atlantic stingray; D. sayi (Lesueur) bluntnose stingray; D. americana Hildebrand and Schroeder southern stingray (Dasyatidae); from Horn and Ship Islands, Mississippi.

SITES: Rectum and occasionally intestine (spiral valve in rays).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74601–2 (pair from both *M. americana* and *D. sayi*); USNM No. 20856–7 (pair from same hosts).

DESCRIPTION: Van Cleave and Lincicome (1939) provided a description of the adults, and Bullock (1957) reported some differences.

Remarks

A few specimens not described above from both *Lepidactylus* sp. and *Menticirrhus americanus* had 16 rows of longitudinal hooks not exhibiting the dorsoventral dissymmetry.

We observed various stages of ovarian fragmentation in the described cystacanths. In one female 4.5 mm long, a single elliptical ovarian mass 275 by 63 μ occurred in the posterior portion of the worm, and in four females 4.4 to 6.3 mm long, there occurred double masses 43 to 112 (77) μ by 33 to 100 (58) μ situated end to end.

Because we observed most infections of *T*. *furcatus* in *Lepidactylus* sp., that relationship was investigated in some detail. One to three individuals developed within and occupied most of a host's hemocoel, displacing many of that amphipod's internal organs (Figs. 5–7). They were folded near their middle with portions either juxtaposed or twisted around each other. A delicate envelope surrounded the cystacanth and associated fluid (Fig. 8). Wanson and Nickol (1973) support and list references suggesting a parasitic rather than host origin for a similar envelope in other acanthocephalans.

The acanthocephalan, typically longer than its host amphipod, appears to affect that host. When comparing the average lengths of 25 infected with 24 uninfected amphipods from the same sample, averages were 4.27 ± 0.07 and 3.90 ± 0.07 , respectively, a significant difference according to the Mann-Whitney rank test. However, individual amphipods were not sexed, but neither were infections restricted to a single sex. Bousfield (1973: 107) stated that male haustoriines are smaller than females, and by using a different lot of 61 specimens roughly 2 mm long, we determined that males averaged 0.3 mm smaller. Nevertheless, parasitism probably increased host size. Additionally, an increase in number of worms per amphipod probably resulted in a decrease in worm length. To exemplify this, males and females in single infections from one collection averaged 5.8 and 6.9 mm long, whereas those in triple infections measured 5.4 and 5.6 mm long. Only males, 5.4 mm long, occurred in the double infections. Confirmation of field observations for both worm affecting host growth, and worm density affecting worm length necessitates experimental infections.

Lepidactylus sp. and Haustorius sp., a close relative of H. canadensis Bousfield, are both being described as new species by Bousfield (pers. comm.). Lepidactylus sp. tolerates water of both low and high salt content, but infections, sometimes reaching high prevalance, occurred solely in high salinity areas at Horn Island (Table 1). Infected hosts may exhibit special behavioral traits because a high percentage of amphipods taken from the stomachs of small Dasyatis sabina and D. sayi had infections. Other acanthocephalan species modify the behavioral patterns of their amphipod hosts (e.g. Holmes and Bethel, 1972). Young of both rays actively feed on amphipods, isopods, cumaceans, and other small crustaceans in Mississippi and Georgia (Heard, unpublished).

Location	Habitat	Date	No. examined	No. infected/%	Remarks
Horn Island, SW end	shallow mud-sand lagoon		213	31/14.6	4 double, 3 triple, 4 Dollfusentis chandleri
Marsh Point, West end	relatively low salinity, "high" energy, sand beach	23 Sept 74	250	0/0.0	1 D. chandleri
East of OS-Biloxi bridge	sandy beach	17 Oct 74	220	0/0.0	
Horn Island, NW end	shallow sandy beach	20 Jun 75	125	8/6.4	1 double
Horn Island, NW end	shallow sandy beach	2 Jul 75	100	3/3.0	
Horn Island, NW end	>1.5 m sandy area	2 Jul 75	150	7/4.7	1 D. chandleri
Horn Island, NW end	>1.5 m sandy area	7 Jul 75	175	10/5.7	
Horn Island, NW end	shallow	8 Aug 75	700	40/5.7	1 double
Horn Island, NW end	shallow	4 Dec 75	500	11/2.0	15 amphipods gravid

Table 1. Cystacanths of Tegorhynchus furcatus in Lepidactylus sp. from Mississippi.

Elasmobranchs do not typically host acanthocephalans; but specimens attached to the spiral valve of the rays, as well as to the gut of *Fundulus similis*, had not developed to sexual maturity. On the other hand, most southern kingfish near the barrier islands off Mississippi harbor mature worms, and some individuals possessed more than 50.

Dollfusentis chandleri Golvan, 1969

Classification of the prevalent acanthocephalan in the rectum of Micropogonias undulatus from the northern Gulf of Mexico is in a state of confusion. Specimens from this host have been referred to by Chandler (1934) and Bullock (1957) as Telosentis tenuicornis (Linton, 1891) Van Cleave, 1947 [=Rhadinorhynchus tenuicornis (Linton, 1891) Van Cleave, 1918] and by Cable and Linderoth (1963) as Illiosentis ctenorhynchus Cable and Linderoth, 1963. Golvan (1969) applied the new name Dollfusentis chandleri Golvan, 1969 specifically to specimens described by Chandler. Bullock and Mateo (1970) considered D. chandleri a synonym of D. longispinus (Cable and Linderoth, 1963) Golvan, 1969; however, Bullock (pers. comm.) presently is uncertain about the status of D. longispinus and considers all specimens from the northern Gulf of Mexico to be D. chandleri.

Cystacanth (Figs. 3–4)

AMPHIPOD HOSTS AND LOCALITIES: Lepidactylus sp. (same species hosting T. furcatus, Haustoriidae) from high-salinity water at Horn Island; Grandidierella bonnieroides Stephensen (Aoridae) from low-salinity ponds and upper reaches of bayous of Mississippi; *Corophium lacustre* Vanhoffen (Corophidae) from low-salinity region of Atchafalaya Bay, Louisiana.

SITE: Hemocoel.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74603 (pair).

DESCRIPTION (based on 1 male and 3 female cystacanths): Trunk elongate, cylindrical with large sheathed cuticular spines investing anterior portion of trunk. Genital spines absent in both sexes. Proboscis elongate and slender, with 14 longitudinal rows of hooks; dorsal and ventral basal hooks similar in size and shape: posterior 6 or 7 hooks of each longitudinal row shorter, thicker, more strongly recurved, and closer together than anterior ones, appearing as vertical rows; anterior hooks more separated, arranged in quincunx pattern; dorsal hooks longer, thinner, and less recurved than ventral ones; anterior ventral hooks strongly recurved, gradually becoming longer and less recurved posteriorly; ventral crescent of 8 enlarged hooks up to 68 long, located about 36 μ posterior to basal proboscis hooks. Sensory papillae: a lateral pair at level of 6th or 7th hook from base of proboscis.

Male: 3.4 mm long by 0.31 mm maximal width. Cuticular spines up to 75 long anteriorly and to 23 long posteriorly. Proboscis 1,125 long with 24–25 hooks per row; hooks posterior to sensory papillae 15–18 long; dorsal hooks near anterior of proboscis 40 long, increasing in length posteriorly to maximal length of 50 at 13th hook from anterior; ventral hooks 30 long near anterior increasing to maximum of 50 at 14th hook.

Female: 5.3-6.2 (5.6) mm long by 0.39-0.51 (0.44) maximal width. Cuticular spines



Figures 5–8. 5. Cross section of anterior end of *Tegorhynchus furcatus* in *Lepidactylus* sp., Harris' hematoxylin and eosin, \times 78. 6. Cross section of uninfected *Lepidactylus* sp. to compare with Fig. 5 for displacement of host's organs, Harris' hematoxylin and eosin, \times 79. 7. Cross section of folded posterior end of the same specimen shown in Fig. 5, Harris' hematoxylin and eosin, \times 103. 8. Close-up of anterior end of same specimen showing relationship between cystacanth and host, Heidenhain's iron hematoxylin, \times 354.

75–80 (77) long anteriorly, decreasing to 18-25 (20) posteriorly. Posterior end of trunk ummodified. Proboscis about 1,250 long with 26–27 hooks per row; hooks posterior to sensory papillae 18-25 (21) long; dorsal hooks near anterior of proboscis 48 long increasing to 50–60 (54) at 9th hook; anterior ventral hooks 33 long increasing to 50 at 17th hook.

Adult

PISCINE HOSTS AND LOCALITIES: *Micropogonias undulatus (Linnaeus) Atlantic croaker; Leiostomus xanthurus Lacépède spot; *Bairdiella chrysura (Lacépède) silver perch (Sciaenidae), *Orthopristis chrysoptera (Linnaeus) pigfish (Pomadasyidae), *Archosargus probatocephalus (Walbaum) sheepshead (Sparidae); Dasyatis sabina (Lesueur) (Dasyatidae); from Mississippi Sound and adjacent waters. SITES: Rectum and occasionally intestine (spiral valve in ray).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74604 (pair from *M. undulatus*), No. 74605 (female from *D. sabina*).

DESCRIPTION: Golvan (1969) presented an adequate description with a partial synonymy including references to other descriptions.

Remarks

Several specimens from amphipods were not described because proboscides showed incomplete evagination. Living cystacanths appeared as those of T. furcatus when removed from amphipods, except that the anterior portion of the trunks bearing cuticular spines was inverted.

Corophium lacustre and Grandidierella bonnieroides, even though both capable of inhabiting relatively high-salinity habitats, occur primarily in low-salinity areas in Mississippi (Farrell, 1970; Myers, 1970; Heard, unpublished). Based on extensive collections, considerably more Atlantic croaker, the principal definitive host in Mississippi, revealed infections in low- than in high-salinity samples. In high-salinity (about 32‰) regions at the barrier islands, *Lepidactylus* sp. was infected, and most infected fish in that region harbored immature specimens. *Orthopristis chrysoptera* and occasionally other fishes, however, did have a few mature specimens and these may act as reservoirs for the typically estuarine acanthocephalan.

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Associations Between Nematodes (Nematoda) and Oligochaetes (Annelida)

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ABSTRACT: The associations between oligochaetes and nematodes are discussed. Oligochaetes can serve as phoretic, paratenic, intermediate or sole hosts for representatives of 10 families and one superfamily of nematodes.

A list citing all types of natural relationships between oligochaetes and nematodes (with over 150 nematode citations) is included and a brief summary is given of the groups of nematodes found in oligochaetes.

Representatives of the thelastomatids (Oxyurida) probably represent the most ancient group of nematode parasites associated with oligochaetes. Through intermediate forms like *Mesidionema*, the thelastomatids gave rise to the Drilonematoidea, unuusual parasites of the coelomic cavity of earthworms. Associations involving oligochaetes as intermediate or paratenic hosts of nematodes were probably secondarily acquired to enhance the chances of locating definitive hosts.

The Associations between nematodes and oligochaetes are more extensive than one might suspect. The present work discusses these relationships and presents a host list of oligochaetes known to be associated with specific nematodes.

Perhaps the simplest type of association between these two groups of animals is phoresis, where the nematode is simply carried around in a nonfeeding state (usually as a dauer) somewhere in the oligochaete. In contrast to the phoretic nematodes on insects, which occur mostly on insects, all cases of nematode phoresis in oligochaetes is internal, usually with the nematodes occurring in the excretory system of the host. The nematodes are microbivorous (free-living) forms which return to the environment for development and reproduction or feed on the worm after it dies. Benefits derived by the nematodes are protection during adverse periods and dissemination. There is no obvious damage to the oligochaete, even when the dauer stages sometimes initiate development in the excretory system of the earthworm.

Aside from serving as phoretic hosts, oligochaetes may also serve as paratenic hosts to nematodes. In this case, a juvenile nematode enters the internal tissues of the oligochaete but does not develop until the earthworm is ingested by another (developmental) host. The annelids do not appear to be affected by these nematodes. When the nematode also develops in the initial earthworm host, the oligochaete is known as the intermediate host and the final (second) host the definitive host. Again, there is no evidence of damage to the earthworm.

Finally, some groups of nematodes utilize oligochaetes as the sole host and remain inside the annelid during their entire developmental period. Representatives of this category can cause mortality of the annelid.

Below is a summary of each group of nematodes known to have associations with oligochaetes. The nematode family (or superfamily) is followed by its abbreviation used in the host list, while the nematode order is included in parenthesis.

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Figures 1-4. 1. A juvenile of *Rhabditis pellio* Schneider in the excretory system of *Aporrectodea* trapezoides (Duges). 2. Infective stage of an unknown mermithid nematode utilizing the oligochaete Limnodrilus silvani Eisen as a paratenic host. 3. Adult female drilonematoidid, Mesidionema praecomasculatis Poinar, removed from the coelom of Eudrilus eugeniae Kinberg. 4. Adult female thelastomatid, Thelastoma endoscolicum Poinar, removed from the gut lumen of Eudrilus eugeniae Kinberg.



Ascarididae (A) (Ascaridida). Some members of the genus *Porrocaecum* deposit eggs that hatch when ingested by earthworms. These newly hatched juveniles then penetrate the gut wall and enter the blood vessels of the worms where they develop and molt to the infective stage. The earthworm serves as a true intermediate host with birds serving as the final hosts.

Dioctophymatidae (Dio) (Enoplida). Both Dioctophyme renale and Hystrichis tricolor, parasites of mammals and birds, respectively, utilize earthworms as intermediate hosts. The eggs hatch in the gut of the oligochaete and develop to the infective stage in the blood vessels. They continue their development when ingested by a vertebrate host.

Diplogasteridae (Di) (Rhabditida). All members of this family have phoretic associations with earthworms and apparently occur (mostly as dauer forms) in the excretory system or coelom of their host. The adult stages are free-living.

Drilonematoidea (D) (Rhabditida). All members of this superfamily live in the coelom (some may occasionally enter the lumen of the intestine) of earthworms where they are considered as true parasites, utilizing the oligochaete as the sole host. They may occur free in the coelom, in sperm sacs or embedded in various tissues (especially muscle) of the earthworm (Fig. 3).

Heterakidae (H) (Ascaridida). There is some question whether members of the genus *Heterakis* actually develop in the earthworm before reaching the vertebrate (bird) host. Thus, the oligochaete may simply serve as a paratenic host.

Mermithidae (M) (Enoplida). Members of this family can use earthworms as paratenic hosts (where they lie coiled up in various tissues) (Fig. 2) or as sole developmental hosts. In case of the latter, the earthworms are probably killed after the nematodes make their exit.

Metastrongylidae (Me) (Strongylida). Eggs of some species of both *Metastrongylus* and *Choerostrongylus* hatch when ingested by earthworms. The juveniles penetrate the gut wall and develop to a stage infective to pigs. While in the oligochaetes, the nematodes may occur in the calciferous glands, esophageal wall, heart, crop, intestine, dorsal blood vessel, or gizzard. In these cases, the earthworm serves as an intermediate host.

Rhabditidae (R) (Rhabditida). Nematodes of this family utilize earthworms as phoretic hosts. Usually the dauer, but also developing juveniles and even the adult, may occur in the excretory system or tissues of oligochaetes (Fig. 1). The adults are free-living or may develop after the host dies and consume the cadaver.

Syngamidae (Sy) (Strongylida). Representatives of both Syngamus and Stephanurus utilize earthworms to reach the final vertebrate host. With Syngamus it is not clear if the earthworm serves as a paratenic or intermediate host. The infective stages of S. trachea occur in the oligochaete muscles. With Stephanurus dentatus, there is apparently some development and the infective-stage juveniles can be found throughout the earthworm's digestive tract or in the brown bodies.

Thelastomatidae (Th) (Oxyurida). Recently, a representative of this family was recovered from the gut of an African earthworm. This is the first case of a thelastomatid (generally found in arthropods) from an annelid. The nematodes develop and mate in the intestinal lumen of the earthworm and it is assumed that the egg is the infective unit after it passes out of the host into the soil. There is only a single host in the life cycle of these nematodes (Fig. 4).

Trichuridae (T) (Enoplida). Certain representatives of the genus *Capillaria* utilize earthworms as paratenic or intermediate hosts. The eggs hatch in the oligochaete gut and the nematode juveniles enter the intestine and encyst in muscles of the body wall. With *C. plica*, only the first-stage juvenile occurs in the worm and it is doubtful whether any development occurs. However some growth supposedly occurs in other species of *Capillaria* utilizing earthworms as initial hosts.

The purpose of the following host list is to cite all types of relationships between oligochaetes and nematodes. At least the worm and nematode genus (with one exception) had to be known for inclusion in the host list. Attempts were made to include all natural occurrences. Experimental results were also cited when it was probable that the association occurred in nature. Only the earliest most complete reference to specific nematode-oligo-

Oligochaete	Nematode	Reference
Acanthodrilidae		
Diplocardia sp. Parachilota sp. Plutellus sp. Plutellus sp.	M-unknown D-Ungella sucofera Timm, 1962 D-Plutellonema clitellatum T. & M., 1966 D-Siconema ovimammillatum Timm, 1966	Timm, 1969 Timm, 1962a Timm & Maggenti, 1966 Timm, 1966b
Criodrilidae		
Criodrilus lacuum Hoffmeister	Dio-Hystrichis tricolor Duj., 1845	Karmanova, 1959
Eudrilidae		
Eudrilus eugeniae Kinberg Eudrilus eugeniae Kinberg	D-Mesidionema praecomasculatis Poinar, 1978 Th-Thelastoma endoscolicum Poinar, 1978	Poinar, 1978a Poinar, 1978b
Glossoscolecidac		
Aptodrilus festae Cognetti Thamnodriloides yunkeri Gates	D-Opistonema minutum Pier., 1916 D-Diceloides mirabilis Timm, 1967	Pierantoni, 11916 Timm, 1967c
Lumbricidae		
Allolobophora chlorotica Savigny Allolobophora dubiosa Savigny Allolobophora dubiosa	Di-Anchidiplogaster eurycephalus (Völk, 1950) R-Caenorhabditis dolichura (Schneider, 1866) Di-Diplogasteritus stoeckherti (Völk, 1950) R-Diploscapter lycostoma Völk, 1950) R-Pelodera cylindrica (Cobb, 1898) R-Pelodera strongyloidas (Schneider, 1860) R-Pelodera teres Schneider, 1866 R-Rhabditis aspera Bütschli, 1873 R-Rhabditis elongata (Schneider, 1866) R-Rhabditis oxpera Bütschli, 1873 R-Rhabditis oxpera Bütschli, 1875 R-Rhabditis pellio (Schneider, 1866) R-Rhabditis verneti Maupas, 1900 Di-Rhabditolainus stigmatus Steiner, 1930 Dio-Hystrichis tricolor Duj., 1845	Völk, 1950 Völk, 1950 Karmanova, 1959
Allobophora tenuis Eisen Aporrectodea giardi Ribar. (=Allolobophora terrestris Savigny)	Me-Metastrongylus elongatus (Duj., 1845) R-Rhabditis pellio (Schneider, 1866)	Alicata, 1936 Cuénot, 1898
Aporrectodea longa (Ude) Aporrectodea longa (Ude) Aporrectodea longa (Ude)	T-Capillaria annulata (Molin, 1859) T-Capillaria caudinflata Molin, 1858 Me-Choerostrongylus pudendotectus	Allen, 1950 Savvateeva, 1966 Shope, 1941
Aporrectodea longa (Ude) Aporrectodea longa (Ude) Aporrectodea rosea (Savigny) Aporrectodea rosea (Savigny) Aporrectodea rosea (Savigny) Aporrectodea trapezoides (Duges)	R-Rhabditis maupasi Seurat, 1919 Sy-Syngamus trachea (Montagu, 1811) R-Rhabditis craspedocerca Völk, 1950 R-Rhabditis paupasi Seurat, 1919 R-Rhabditis pellio (Schneider, 1866) Me-Choerostrongglus pudendotectus	Johnson, 1913 Taylor, 1935 Völk, 1950 Völk, 1950 Völk, 1950 Schwartz & Alicata, 1935
Aporrectodea trapezoides (Duges) Aporrectodea trapezoides (Duges) Aporrectodea trapezoides (Duges) Bimastos parvus (Eisen) Dendrobaena kurashvilii K. & K., 1974 Dendrobaena octaedra Savigny Dendrodrilus rubidus (Savigny) Dendrodrilus rubidus (Savigny) Dendrodrilus rubidus (Savigny) Dendrodrilus rubidus (Savigny) Dendrodrilus rubidus (Savigny) Eisenia foetida Savigny Eisenia foetida Savigny	(Wost., 1905) Me-Metastrongylus elongatus Duj., 1845 Me-Metastrongylus salmi Gedoelst, 1923 R-Rhabditis pellio (Schneider, 1866) H-Heterakis perspicillus Rud., 1803 D-Dicelis kurashvili K., 1974 R-Rhabditis pellio (Schneider, 1866) T-Capillaria caudinflata Molin, 1858 T-Capillaria caudinflata Molin, 1858 D-Dicelis dendrobaena Timm, 1967 R-Rhabditis maupasi Seurat, 1919 T-Capillaria annulata (Molin, 1859) Me-Choerostrongylus pudendotectus (Wost, 1905)	Schwartz & Alicata, 1935 Alicata, 1936 Poinar & Thomas, 1975 Scott, 1913 Kakulia & Kvavadze, 1974 Völk, 1950 Savvateeva, 1966 Enigk, 1950 Timm, 1967c Johnson, 1913 Wehr, 1936 Schwartz & Alicata, 1935
Eisenia foetida Savigny Eisenia tetradar (Savigny) Eisenoides carolinensis (Michaelsen) Eisenoides lonnbergi (Michaelsen)	D-Dicelis' filaria' Duj., 1845 H-Heterakis gallinarum (Schrank, 1788) Me-Metastrongylus elongatus (Duj., 1845) A-Porrocaecum ensicaudatum(Zeder, 1800) R-Rhabditis maupasi Seurat, 1919 Sy-Stephanurus dentatus Diesing, 1839 Sy-Syngamus trachea (Montagu, 1811) D-Dicelis rossica Timm, 1962 R-Rhabditis pellio (Schneider, 1866) D-Dicelis ciseniae Timm, 1967 Me-Choerostrongylus pudendotectus (Wost, 1905)	Keilin, 1915 Lund et al., 1966 Schwartz & Alicata, 1935 Jogis, 1967 Johnson, 1913 Tromba, 1955 Clapham, 1934 Clapham, 1934 Timm, 1962b Völk, 1950 Timm, 1967 Dayton, 1957
Eisenoides lonnbergi (Michaelsen) Helodrilus caliginosus Savigny	Me-Metastrongylus elongatus (Duj., 1845) T-Capillaria aerophila (Creplin, 1839)	Dayton, 1957 Borovkova, 1941 (In Rysavy 1969)
Helodrilus caliginosus Savigny	T-Capillaria annulata (Molin, 1859)	Wehr, 1936

Oligochaete Hosts of Nematodes

Oligochaete Hosts of Nematodes (Continued)

Oligochaete	Nematode	Reference	
Lumbricidae (Continued)			
Helodrilus caliginosus Savigny Helodrilus caliginosus Savigny Helodrilus caliginosus Savigny Helodrilus caliginosus Savigny Helodrilus caliginosus Savigny Helodrilus caliginosus Savigny Helodrilus caliginosus Savigny Lelodrilus rhodensis Cogn. Lumbricus rubellus Hoff.	T-Capillaria caudinflata (Molin, 1858) D-Dicclis nira Chit. & Luck., 1934 H-Heterakis gallinarum (Schrank, 1788) A-Porrocaccum ensicaudatum (Zeder, 1800) Me-Metastrongylus apri Gmelin, 1790 Sy-Syngamus trachea (Montagu, 1811) D-Mesonema rhodense Pier., 1916 T-Capillaria aerophila (Crelpin, 1839)	Morehouse, 1944 Chitwood & Lucker, 1934 Lund et al., 1966 Atlavinyte, 1963 Dunn, 1955 Taylor, 1935 Pierantoni, 1916 Borovkova, 1941 (In Rysavy 1969)	
Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus rubellus Hoff.	T-Capillaria caudinflata (Molin, 1858) T-Capillaria mucronata Molin, 1858 T-Capillaria plica (Rud., 1819) Me-Choerostrongylus pudendotectus (Wost., 1905)	Savvateeva, 1966 Skarbilovic, 1950 Petrov & Borovkova, 1942 Alicata, 1936	
Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus rubellus Hoff. Lumbricus terrestris L.	D-Dicelis filaria Duj., 1845 Me-Metastrongylus elongatus (Duj., 1845) A-Porrocaecum ensicaudatum (Zeder, 1800) R-Rhabditis anomala Hertwig, 1922 R-Rhabditis maupasi Seurat, 1919 R-Rhabditis pellio (Schneider, 1866) T-Capillaria aerophila (Creplin, 1839)	Wulker, 1926 Alicata, 1936 Joges, 1967 Goodchild & Irwin, 1971 Johnson, 1913 Goodchild & Irwin, 1971 Borovkova, 1941 (In Rysavy 1969)	
Lumbricus terrestris L. Lumbricus terrestris L.	T-Capillaria plica (Rud., 1819) Me-Choerostrongylus pudendotectus (Wost., 1905)	Enigk, 1950 Alicata, 1936	
Lumbricus terrestris L. Lumbricus terrestris L. Cotolasion cyaneum (Savigny) Octolasion tyrtaeum (Savigny) Octolasion tyrtaeum (Savigny)	D-Dicelis filaria Duj., 1845 H-Heterakis gallinarum (Schrank, 1788) Me-Metastronglus clongatus (Duj., 1845) Me-Metastronglus salmi Gedoelst, 1923 R-Pelodera strongyloides (Schneider, 1860) R-Pelodera teres Schneider, 1866 A-Porrocaccum ensicaudatum (Zeder, 1800) R-Rhabditis ananala Hertwig, 1922 R-Rhabditis maupasi Seurat, 1919 R-Rhabditis verneti Maupas, 1900 Sy-Stephanurus dentatus Diesing, 1839 Sy-Syngamus trachea (Montagu, 1811) Sy-Syngamus trachea (Montagu, 1811) R-Rhabditis maupasi Seurat, 1919 D-Mesonema acuminatum Pier., 1916 D-Opistonema acuminatum Pier., 1916 A-Porrocaecum ensicaudatum (Zeder, 1800) R-Rhabditis maupasi Seurat, 1919	Dujardin, 1845 Lund et al., 1966 Alicata, 1936 Völk, 1950 Völk, 1950 Cori, 1898 Goodchild & Irwin, 1971 Johnson, 1913 Goodchild & Irwin, 1971 Välk, 1950 Tromba, 1955 Clapham, 1934 Walker, 1886 Johnson, 1913 Pierantoni, 1916 Pierantoni, 1916 Levin, 1957 Völk, 1950	
Lumbriculidae			
Lumbriculus variegatus Müller Lumbriculus variegatus Müller	Dio-Diotophyme renale (Goeze, 1782) R-Rhabditis lumbriculi v. Linst., 1895	Karmanova, 1960 von Linstow, 1895	
Megascolecidac			
Perionyx macintoshi Beddard Perionyx sp. Perionyx sp. Pheretima alexandri (Beddard) Pheretima alexandri (Beddard) Pheretima alexandri (Beddard) Pheretima alexandri (Beddard) Pheretima a. analecta Gates Pheretima andersoni Michaelsen Pheretima asiatica Michaelsen Pheretima benguetensis Beddard Pheretima benguetensis Beddard Pheretima bulmeri Gates Pheretima bulmeri Gates Pheretima carinensis (Rosa) Pheretima carinensis (Rosa) Pheretima defecta Gates Pheretima defecta Gates Pheretima defecta Gates Pheretima doliaria Gates Pheretima fucosa Gates Pheretima fucosa Gates Pheretima longicauliculata Gates Pheretima longicauliculata Gates Pheretima longicauliculata Gates	D-Scolecophilus gatesi Baylis, 1943 D-Scolecophilus lumbricicola B. & D., 1922 D-Scolecophilus lumbricicola B. & D., 1922 D-Scolecophilos lumbricicola B. & D., 1943 D-Synoccnema perionychis Baylis, 1943 D-Homungella siamense Timm, 1966 D-Adieronema mirabile Timm, 1967 D-Adieronema mirabile Timm, 1967 D-Filiponema ovicoronatum Timm, 1966 D-Filiponema ophilippinense T. & M., 1966 D-Filiponema philippinense T. & M., 1967 D-Filiponema builtopensis Timm, 1967 D-Gatesnema bilobatum Timm, 1967 D-Jonema pheretimae Timm, 1971 D-Adgungella gatesi Timm, 1967 D-Adungella major Timm, 1967 D-Siconema succaturum Timm, 1966 D-Siconema succaturum Timm, 1966 D-Siconema sicarmatus Timm, 1966 D-Siconema and Timm, 1966 D-Siconema ovicostatum Timm, 1966 D-Succamphida robustum Timm, 1966 D-Siconema ovicostatum Timm, 1966 D-Jionyx guineensis Pier, 1916 D-Homungella monodontium Timm, 1966 D-Hosonema hurmense Timm, 1966	Timm, 1967a Baylis & Daubney, 1922 Baylis, 1943 Jaylis, 1943 Timm, 1966a Timm, 1966b Timm, 1967c Timm, 1967c Timm, 1967b Timm, 1967b Timm, 1967b Timm, 1971 Timm, 1971 Timm, 1967b Timm, 1967b Timm, 1967b Timm, 1967b Timm, 1966b Timm, 1966c Timm, 1966c Timm, 1966a Timm, 1966a Timm, 1967a	
Pheretima angleauticulata Gates	D-Adungella manicatae Timm, 1966	Timm, 1966b Timm, 1967b	

Oligochaete	Nematode	Reference
Megascolecidae (Continued)		
Pheretima mekongianus (Cognetti) Pheretima mira Gates Pheretima montana (Kinberg) Pheretima montana (Kinberg) Pheretima omtrekensis Cogn. Pheretima posthuma (L. Vaillant) Pheretima posthuma (L. Vaillant) Pheretima posthuma (L. Vaillant) Pheretima posthuma (L. Vaillant) Pheretima sp. Pheretima sp. Pheretima sp. Pheretima schuanensis Chen. Pheretima terrigena Gates Pheretima vigio (Beddard) Pheretima vitiata (Goto & Hatai) Pheretima vitiata (Goto & Hatai) Pheretima wendessiana Cogn. Pheretima terrigensus Gates Tonoscolex depressus Gates Tonoscolex depressus Gates Tonoscolex sp.	 D-Pharyngonema mekongianus Pier., 1923 D-Miranema mirae Timm, 1967 D-Synoecnema drawidae Baylis, 1943 D-Diouyx acutifrons Pier., 1916 D-Synoecnema gatesi Timm, 1962 D-Perodira pheretimae Timm, 1960 D-Pharyngonema pheretimae Timm, 1959 D-Synoecnema anseriforme Timm, 1959 D-Synoecnema neseriforme Timm, 1959 D-Synoecnema hirsutum Timm, 1959 D-Synoecnema hirsutum Timm, 1959 D-Synoecnema hirsutum Timm, 1959 D-Synoecnema hirsutum Timm, 1967 D-Diotonema subtile Pier., 1916 D-Buronanema singulare Timm, 1967 D-Siconema luor Timm, 1966 D-Adaramphida sinense Timm, 1966 D-Cephalonema macrocephalum Pier., 1916 D-Cephalonema microcephalum Pier., 1916 D-Dicelis pleurochaetae Beddard, 1883 D-Tonoscolecinema baroum Timm, 1967 D-Tonoscolecinema gatos Timm, 1967 	Pierantoni, 1923 Timm, 1967b Timm, 1967b Pierantoni, 1916 Timm, 1962a Timm, 1962a Timm, 1959b Timm, 1959a Timm, 1959a Baylis, 1943 Pierantoni, 1916 Timm, 1967c Timm, 1967b Timm, 1967b Timm, 1966b Isoda & Kato, 1956 Timm, 1966b Pierantoni, 1916 Pierantoni, 1916 Pierantoni, 1916 Pierantoni, 1916 Pierantoni, 1916 Pierantoni, 1916 Pierantoni, 1916 Pierantoni, 1916 Timm, 1967c Timm, 1967c Timm, 1967c Timm, 1967c
Moniligastridae		
Drawida ampullacea Gates Drawida dolosa Gates Moniligaster gravelyi Stephenson	D-Perodira alata Baylis, 1943 D-Synoecnema drawidae Baylis, 1943 D-Creagrocercus barbatus Baylis, 1943	Baylis, 1943 Baylis, 1943 Baylis, 1943
Octochaetidae		
Dichogaster duwonica Cognetti Dichogaster gestri Cognetti Dichogaster italiensis (Mich.) Eutyphoeus bullatus Gates Eutyphoeus rarus Gates Eutyphoeus rarus Gates Hoplochaetella anomala Steph. Hoplochaetella anomala Steph. Ramiellona balantina Gates	D-Dionyx minuta Pier., 1916 D-Dionyx cognettii Pier., 1916 unknown D-Iponema minor T. & M., 1966 D-Adieronema eutyphoei Timm, 1967 D-Ungella secta Cobb, 1928 D-Perodira alata Baylis, 1943 D-Synoecnema hoplochaetellae Baylis, 1943 D-Dicclis guatemalana Timm, 1962	Pierantoni, 1916 Pierantoni, 1916 Cognetti de Martiis, 1909 Timm & Maggenti, 1966 Timm , 1967 Cobb, 1928 Baylis, 1943 Baylis, 1943 Timm, 1962b
Tubificidae		
Limnodrilus silvani Eisen	M-unknown	Poinar, 1976

Oligochaete Hosts of Nematodes (Continued)

chaete associations was cited. The oligochaete families, genera and species are listed in alphabetical order. When more than one nematode is recorded under the same oligochaete, then the genera and species of nematodes are also arranged in alphabetical order. Abbreviations before the scientific name of the nematode represent the family or superfamily of that particular nematode. These abbreviations and their corresponding families (or superfamilies) and orders are discussed above, along with a brief summary of each family in regards to its association with oligochaetes. The last column of the host list gives the references to the most complete report of the association.

Discussion

It is clear from the above host list that oligochaetes can harbor many types of nematodes in a variety of associations.

From the standpoint of human welfare, perhaps the most important associations are the nematode parasites of domestic animals that utilize earthworms as paratenic or intermediate hosts. This topic has been discussed further by Rysavy (1969). From the standpoint of basic science, nematodes of the superfamily Drilonematoidea represent one of the more interesting groups of oligochaete parasites since they have so many unique morphological characters and show no direct relationship to any of the free-living nematodes. For those attempting to culture earthworms, mermithid nematodes would be the only group showing potential danger since they usually kill their host. However their occurrence in oligochaetes is rare, insects being the preferred host to most mermithids. It is possible that those representatives of the Rhabditidae and Diplogasteridae which enter the excretory system of earthworms, could rupture the nephridia and cause a general septicemia of the oligochaete.

Since annelids are considered the most primitive coelomate animals known (Raymond, 1950), it is interesting to consider in evolutionary terms when the various groups of nematodes became associated with earthworms.

The Oxyurida are probably the most ancient group of nematodes and Inglis (1965) considers the Thelastomatidae as the most primitive family in this order. Representatives of this family, whose members probably arose from terrestrial Rhabditidae, thus represent one of the earliest known groups of animal parasites, and thus *Thelastoma endoscolicum* might have become associated with earthworms in the Lower Cambrian.

Representatives of the thelastomatids probably gave rise to the Drilonematoidea, another very ancient group of nematode parasites of the coelomic cavity of earthworms (Timm, 1964). An intermediate form sharing characters of the gut-inhabiting thelastomatids and coelom-inhabiting drilonematoidids is *Mesidionema praecomasculatis*.

Aside from rare cases of earthworm parasitism by mermithid nematodes, the two abovementioned groups are the only nematodes that utilize earthworms as sole hosts.

Most of the other nematodes cited in the host list utilize earthworms as intermediate or paratenic hosts. Such associations were probably secondarily acquired and enhanced the possibility of the nematodes finding the definitive hosts.

Those nematode groups which contain representatives that utilize earthworms as phoretic hosts and then lead a free-living existence in the environment may also have a long-standing association with their hosts.

Acknowledgments

The author would like to thank Dr. G. E. Gates for examining the host list and making the

necessary nomenclatural changes for the oligo-chaetes.

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genera and seven new species of the family Ungellidae. Pak. J. Biol. Agric. Sci. 10: 13-21.

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Report on the Brayton H. Ransom Memorial Trust Fund

Balance on hand, 1 January 1977	\$4,217.25
Receipts: Interest received in 1977	288.49
•	\$4,505.74
Disbursements: Grant to Helminthological Society of Washington On hand, 31 December 1977	\$ 10.00 \$4,495.74
A. Morga	N GOLDEN
Secretary-'	Treasurer

Helminth Acquisition by Wild Turkeys (Meleagris gallopavo osceola) in Florida¹

LARRY T. HON,² DONALD J. FORRESTER,³ AND LOVETT E. WILLIAMS, JR.⁴

ABSTRACT: During 1970 and 1971, 113 wild turkeys (Meleagris gallopavo osceola) ranging from 1 day to 9 months in age were collected on the Fisheating Creek Wildlife Management Area in southern Florida. In the summer of 1971 poults acquired helminths during their first week. The nematode, Dispharynx nasuta, was the first helminth to appear, with birds as young as 3 days infected. One-week-old poults collected in 1970 were free of helminths, indicating variation between years in the early acquisition of parasites. Rainfall and creek levels, which affected the feeding range of turkey broods and inhabitable range of intermediate hosts, appeared to influence Dispharynx burdens. The nine most common helminths had five patterns of seasonal occurrence: peaks in summer (Dispharynx nasuta and Cyrnea colini), peaks in both summer and winter (Ascaridia dissimilis), peaks in winter (Zygocotyle lunata and Trichostrongylus tenuis), persistently high intensity (Metroliasthes lucida, Strongyloides sp., and Capillaria sp.), and irregular fluctuations (Echinoparyphium recurvatum). It is suggested that these patterns were influenced by factors such as host age, food habits, climatic conditions, life cycles of the helminths in relation to cycles utilized, and longevity of parasite forms.

In 1969, a study was initiated to gather information on the prevalence and distribution of parasites and diseases of wild turkeys in Florida. Several reports have been published on viruses (Busch and Williams, 1970; Colwell et al., 1973; Grant et al., 1975), blood protozoans (Forrester et al., 1974; Telford and Forrester, 1975; Young et al., 1977), and helminths (Hon et al., 1975; Dubois and Hon, 1973; Davidson et al., 1977). The present report is concerned with results of a helminth acquisition study undertaken as a part of that program.

Materials and Methods

Birds were collected from the Lykes Fisheating Creek Wildlife Management Area and Refuge near Palmdale (Glades County) in southern Florida. From June 1970 through February 1971 monthly samples of young turkeys of various ages were collected. During April, May, June and July 1971, additional young poults were collected from broods in the

area (Table 1). Methods of collecting and aging turkeys have been presented (Forrester et al., 1974; Hon et al., 1975). Techniques for recovering, killing, fixing, preserving and staining helminths followed those described by Kinsella and Forrester (1972). Representative specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland) as USNM Helm. Coll. Nos. 74516-74537.

Results of long-term studies (Williams et al., 1969, 1973) of wild turkey population biology on the Fisheating Creek area provided background information for the present study. A detailed description of the area has been published (Williams et al., 1973). Rainfall avcraged approximately 50 inches annually.

Table 1. Summary of young wild turkeys examined for helminth parasites.

Date	Age	Number examined
June 1970 July 1970 August 1970 September 1970 October 1970 December 1970 December 1970 January 1971 February 1971 April 1971 May 1971 June 1971 May 1971 May 1971 May 1971	5-6 days 1 month 2 months 3 months 4 months 5 months 6 months 6 months 7 months 1 day $3-4 days$ $8-14 days$ $21-37 days$ $50-87 days$	$9 \\ 10 \\ 9 \\ 11 \\ 11 \\ 5 \\ 11 \\ 7 \\ 9 \\ 2 \\ 6 \\ 9 \\ 11 \\ 3 $
Total		113

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Figure 1. Monthly precipitation and average monthly temperatures for 1970 and 1971 at the weather station in Labelle, Florida (U.S. Dept. of Commerce, 1970–71).

Monthly precipitation and average monthly temperatures for 1970 and 1971 at a nearby weather station in Labelle, Florida are presented in Figure 1. Water levels in Fisheating Creek are shown in Figure 2. Periodic flooding of the creek resulted in inundation of portions of the habitat for several weeks at a time. One half of the 30 square mile study area was open to hunting of hens and gobblers in the past and more recently to gobblers only. The estimated prenesting turkey population in 1970 and 1971 was one hen per 75 acres with an approximately equal sex ratio (Williams et al., 1973).

Results and Discussion

Acquisition of helminths during the first five weeks of life

Poults acquired helminths during their first week in 1971 (Table 2). The number of species in the poult population reached a maximum of five in 21- to 25-day-old birds (Fig. 3). Prevalence of infections increased from 67% in 3- to 4-day-old birds to 100% in the 8- to 14-day-old group and remained at this level. The mean helminth burden increased steadily to reach a peak in 21- to 25-day-old poults, but fell slightly in the month-old birds (Fig. 4).

Dispharynx nasuta was the first helminth to be acquired; infected 3- and 4-day-old birds harbored single immature worms. Burdens and prevalences of *D. nasuta* increased rapidly in the next age-group of poults, while the trematode, Stomylotrema vicarium, and the cestode, Metroliasthes lucida, also appeared. Thus, by the end of the second week, poults harbored representatives of the three major helminth taxa found in turkeys. Two other nematode species, Ascaridia dissimilis and Strongyloides sp., ap-



Figure 2. Average monthly creek levels of Fisheating Creek for 1970 and 1971. Time of peak hatch of poults is indicated by asterisk (*). (U.S. Dept. of Interior, Geological Survey, Water Resources Division, unpublished records for Palmdale, 02256400, for 1970–71).

peared first in 21- and 24-day-old birds, respectively.

Poults acquired helminths at an earlier age in 1971 than in 1970. Nine poults 5 and 6 days old collected in 1970 were free of helminths, while *D. nasuta* infections were encountered in 3- and 4-day-old birds in 1971. This yearly difference in burdens of *D. nasuta* for poults 35 days old or less was significant at the $P \leq 0.01$ level of confidence (Student's *t*-test). Only one specimen of S. *vicarium* (from a 51-day-old bird) was collected in 1970, but this trematode reached a prevalence of 67% in 29- to 37-day-old birds in 1971.

Development of *D. nasuta* to the infective stage occurs in an isopod intermediate host

Table 2.	Helminths ac	quired during	the first 5 week	s by wild turke	y poults in 1971.
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Helminth	Age (days): 3-4 No. examined: 6	<u>8–14</u> 9	21-25	29-37
Stomylotrema vicarium		$\frac{22(3.5)}{1-6}$ *	60(5.0) 1-9	67(3.0) 1-6
Metroliasthes lucida		$\frac{11}{2}$		-
Ascaridia dissimilis	—	_	40(4.5)	$\frac{17}{3}(3.0)$
Dispharynx nasuta	$\frac{67}{1}$	$ \begin{array}{r} 100 (5.6) \\ 1-18 \end{array} $	100_{1-16}	$\frac{67}{3-10}$
Strongyloides sp.	_	_	20 (2.0)	

% prevalence (mean intensity)

Intensity range



Figure 3. The acquisition of species of helminths by poults in 1971. Legend: — mean number of species per bird; ——— cumulative number of species.

Chaetophiloscia Sowbugs, (Cram, 1931). floridana (Crustacea: Isopoda), were found to be intermediate hosts for *D. nasuta* on the Fisheating Creek Area. Water levels of Fisheating Creek probably influenced the host-parasite relationship of D. nasuta. According to Williams et al. (1973), turkey broods on the area move directly from their nests in the saw palmetto (Sereona repens) to the cypress woods, where they remain for several weeks. The cypress woods and the closely associated bayheads were the two plant communities found to be inhabited by sowbugs. In these areas they occurred on and around the bases of cypress trees (Taxodium distichum), under cow feces and other debris, and crawling across the ground, in easy reach of turkey poults. During periods when the swamp floor was flooded, sowbugs were found to migrate vertically on the cypress trunks and survive under loose bark one to several feet above the water line, their



Figure 4. The acquisition of populations of helminths by poults in 1971. Legend: _____ mean helminth burden; ____ prevalence (%).



Figure 5. Intensity of *Dispharynx nasuta* infections in poults in 1970 and 1971.

vulnerability to poults undoubtedly being reduced. From Figure 2 it can be seen that creek levels were lower in the early summer of 1971 than in the corresponding hatching and brood season of 1970. The lower creek levels were reflected in higher prevalence and larger worm burdens in young turkeys hatched in 1971 (Fig. 5). Yearly differences in *D. nasuta* burdens therefore appear to be a function of environmental factors, one of which is the variability in creek levels.

Helminth acquisition and seasonal variation

Young turkeys were hosts of 28 species of helminths during their first 9 months of life. These included those listed in Table 1 of Hon et al. (1975), except Ascotyle sp., Brachylaima virginianum, Zonorchis sp., Hymenolepis carioca, Splendidofilaria sp., and an unidentified species of subfamily Splendidofilarinae. Of these, 19 species appeared infrequently and in low numbers in the population, or in one or a few birds as accidental infections.

The remaining nine species were viewed as major components of the helminth fauna of young turkeys on the Fisheating Creek area. Five patterns of occurrence were discernible when monthly prevalances of the major helminths were graphed (Figs. 6–14). Two species demonstrated summer peaks in prevalence, one showed a summer-winter peak, two had winter peaks, three had high, fluctu-



Figures 6-14. Seasonal variation in prevalence of: 6. Dispharynx nasuta, 7. Cyrnea colini, 8. Ascaridia dissimilis, 9. Zygocotyle lunata, 10. Trichostrongylus tenuis, 11. Metroliasthes lucida, 12. Strongyloides sp., 13. Capillaria sp., and 14. Echinoparyphium recurvatum.

ating prevalences, and one showed irregular fluctuations in occurrence.

Peak prevalences of *Dispharynx nasuta* and Cyrnea colini (Figs. 6, 7) occurred in the summer and early fall. Dispharynx reached a peak prevalence of 89% in August then dropped, disappearing almost entirely by November. One factor believed to contribute to this pattern is seasonal change in food habits of the host. Analysis of crop and gizzard contents of poults collected during this study (Barwick et al., 1974) revealed a change in food habits in November. The diet shifted from a mixture of insects, arachnids, and other invertebrates along with an assortment of plant material (grasses, seeds, and leaves) to one composed almost exclusively of plant material with only a trace of animal matter. Seasonal change in climate with lower temperatures probably accounted for the disappearance of invertebrates in the diet. At the same time acorns (Quercus spp.) and cabbage palm (Sabal palmetto) seeds became available and were heavily used. The direct result of these changes was a removal of the source of Dispharynx infection, the sowbug intermediate host, from the host's diet.

Prevalence of *Dispharynx* had begun to decline several months before the change in food habits. This suggests that some other factor(s) may also be involved, such as a physiological change with age or an acquired immunity to the parasite. Bendell (1955) found that *D. nasuta* infections in blue grouse (*Dendragapus obscurus fuligionsus*) were restricted also to young chicks.

Since turkeys lost *Dispharynx* infections during the winter months the question arises as to how the nematode is maintained in the environment to infect poults of the succeeding summer. Experimental evidence from sowbug colonies maintained in the laboratory (Hon, unpublished data) indicated that *Dispharynx* larvae can survive at least 6 months in the intermediate host. In addition, naturally infected sowbugs were encountered on the study area in the spring of 1971. Given this longevity, *D. nasuta* larvae could overwinter in sowbugs infected in the summer and fall, when most young turkeys were passing *Dispharynx* eggs, and infect the following year's poults.

Another possible mechanism of maintaining *Dispharynx* on the area is a reservoir host

system in which one or more other common birds harbor the nematode through the winter and spring. High prevalences of *D. nasuta* were found in common crows (*Corvus brachyrhynchos*) and blue jays (*Cyanocitta cristata*) collected on the study area in the spring (Hon et al., 1975). Since these two species live in the same habitat as turkeys it appears likely that they contribute to the "seeding" of the environment with *Dispharynx* eggs throughout the year.

The summer peak of *Cyrnea colini* also appeared to be related to food habits of the turkeys. This species has been shown experimentally to use a cockroach (*Blatella germanica*) as an intermediate host (Cram, 1931). The seasonal prevalence of the nematode may be influenced by the availability of roaches, which are common in turkey habitat in Florida.

Ascaridia dissimilis was the only helminth showing a distinct summer-winter peak (Fig. 8). This nematode has a direct life cycle and so should be, and is, prevalent in the winter when the diet of turkeys is mainly vegetarian. At this time ova in the soil are more likely to be ingested as turkeys feed on seeds and other plant material on the ground. The rapid peak in young birds during early summer apparently reflects the presence of viable eggs still in the soil from winter and spring infected birds. As environmental conditions and food habits of turkeys discourage infections, prevalence of the nematode drops through the summer and fall. A major environmental factor influencing infections is believed to be creek levels. From Figure 2 it can be seen that creek levels rose in June 1970 and remained high into November, then fell. Prevalence of Ascaridia increased in November and reached a peak in January 1971, while creek levels remained low. During this period eggs could have embryonated in soil not inundated by the creek.

The third type, with a rise in prevalence during winter, is represented by two helminths, Zygocotyle lunata and Trichostrongylus tenuis (Figs. 9, 10). From the standpoint of food habits of the host, both are direct life cycle forms. Infective metacercariae of Z. lunata encyst on debris or vegetation near water (Byrd, 1972). Low water during winter months allowed turkeys to utilize otherwise flooded marsh areas and ponds that were also frequented by Florida ducks, Anas platyrhynchos fulvigula, of which this is a characteristic parasite (Kinsella and Forrester, 1972). The foraging activities of turkeys there probably accounted for the winter peak of this trematode.

Trichostrongylus tenuis infections began rising in January 1971 and were highest in birds collected during February. Food habits of turkeys and soil conditions favorable for survival of larvae are believed responsible for the increase at this time. Levine (1968) gives optimum conditions for pasture transmission of *Trichostrongylus* in sheep and goats as 2 inches or more total monthly precipitation and 43 to 68°F mean monthly temperature. If these optimum conditions are similar to those of *Trichostrongylus* in turkeys, they are present from November through March on the Fisheating Creek Area (Fig. 1).

Three helminths had persistently high prevalences. Two of these species, *Metroliasthes lucida* and *Strongyloides* sp., were the two most commonly encountered species during the study. *Capillaria* sp. (*Capillaria* sp. 1 of Hon et al., 1975) also showed this pattern, with no seasonal trend in infections discernible. Graphs of monthly prevalences for the three helminths are shown in Figures 11, 12, and 13.

Prevalence of *Metroliasthes* rose sharply in poults during the summer. According to Reid (1962) several species of grasshoppers are utilized as intermediate hosts by this tapeworm. These insects were plentiful on the area and were common in the summer and fall diet of turkeys. Adults and subadult birds from the previous year probably maintained *M. lucida* infections and infected the range when grasshoppers become plentiful in the spring prior to the brood season.

The persistently high prevalence of Strongyloides sp. is believed to be due to at least two factors. First, the relatively high prevalence of the nematode in the adult birds at all times during the year (Hon et al., 1975) insures infection of poults at an early age. Prevalence jumped to 90% in 1-month-old birds and reached 100% in poults collected during October. Second, the unique life cycle of the parasite, with its free-living and parasitic generations, maintains a plentiful supply of infective larvae in the environment. Cram (1929) observed that eggs of the parasitic generation hatched in 18 hr at $50-57^{\circ}F$, hence the life cycle could be completed at most times during the year at Fisheating Creek (Fig. 1). Cram also found that parasitic females began passing eggs within 5 days after infection. This short generation time undoubtedly enhanced the prevalence of *Strongyloides* in the turkey population.

Capillaria sp. also showed a pattern of relatively high prevalence with no definite seasonal trend. Prevalence of the nematode reached 60% in 1-month-old birds and remained fairly high with intermittent fluctuations. The life cycle of the species is unknown and reasons for this type of pattern are not obvious. Life cycles of most capillarids from birds are unknown; those which are known are either direct, or indirect, utilizing an earthworm intermediate host (Levine, 1968). A decline in prevalence of Capillaria sp. occurred in December, January, and February. Without further collections it is not possible to ascertain if there is a definite loss of infections during winter. However, it is possible that the helminth demonstrates a broad summer-fall peak in prevalence rather than a trendless pattern. From the standpoint of turkey food habits this would indicate an indirect life cycle utilizing an annelid intermediate host.

The irregular type with low prevalences and no apparent seasonal trend was represented by Echinoparyphium recurvatum (Fig. 14). Prevalence of the trematode reached a peak of 40% in 2-month-old poults collected in August, then declined through late summer to disappear in October. Fluctuations occurred during the winter months, but prevalence remained low. Kinsella and Forrester (1972) found the prevalence of *E. recurvatum* in Florida ducks on the Fisheating Creek area to increase through the summer and reach a peak in November, then fall to a low in February and March. The high prevalence of the trematode in ducks during summer could account for the peak in turkeys at that time. A factor contributing to higher prevalences in both species during summer and fall is probably the availability of the snail or tadpole second intermediate host. Turkeys appeared to be accidentcal hosts of the parasite with only sporadic infections occurring in areas inhabited by Florida ducks.

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Acknowledgments

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Microbial Flora of Cuticular Lesions on Strongylus edentatus

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ABSTRACT: Four types of cuticular lesions on specimens of Strongylus edentatus recovered from horses were characterized as (1) filamentous, (2) flat, (3) cratered, and (4) proliferate. The filamentous and flat lesions contained Enterobacter aerogenes, Escherichia coli, Micrococcus sp., Streptococcus sp., and Streptococcus faecalis. In addition, flat lesions contained Proteus vulgaris. Cratered lesions contained only S. faecalis and E. aerogenes. Proliferate lesions contained microbial flora similar to those of the other types of lesions but did not contain P. vulgaris or E. coli. Both E. coli and E. aerogenes were recovered from worm surfaces having no lesions, but healthy cuticle specimens generally did not contain S. faecalis; the only S. faecalis associated with normal strongyle cuticle was in the debris that accumulates on female cuticle around the genital pore during copulation. This debris also contained coliform microorganisms and other cocci but did not contain E. coli.

Although dermatoses of the ascarid cuticle have been most frequently studied, such lesions are not confined to these large roundworms. The earliest described cuticular lesions of helminth parasites of vertebrate hosts were on specimens of *Parascaris equorum* (Weinberg and Keilin, 1912). Lubinsky (1931) found similar lesions on five nematode genera, including *Strongylus*.

Cuticular lesions of helminths have been found as both single or multiple lesions (Dollfus, 1946; Stewart and Godwin, 1963; McKinnon and Lubinsky, 1966; Anderson et al., 1971), and several microbial types have been found associated with diseased cuticle (Manter, 1929; Stewart and Godwin, 1963; Anderson et al., 1971). Candida sp., Escherichia coli, and Pseudomonas sp. were recovered from swine ascarid cuticular lesions by Stewart and Godwin (1963), but only the pseudomonad was capable of causing lesions on healthy worms. A differing microbial flora in each of two grossly distinguishable types of cuticular lesions on swine ascarids was reported by Anderson et al. (1971). In addition to either or both the coliform organisms and enteric streptococci found in the small, often multiple, lesions, the larger, singly occurring, lesions contained a Candida sp.

While studying the microbial flora of *Stephanurus dentatus*, Anderson et al. (1973) found a cuticular lesion on one of the worms differing grossly from cuticular lesions found on ascarids but resembling lesions they had observed on a parasite of horses, *Strongylus*

vulgaris. Since cuticular lesions are infrequently found on *S. dentatus*, possibly because of their brief sojourn in the swine intestine, an opportunity to examine *Strongylus edentatus* having cuticle lesions provided the possibility of studying dermopathies that might resemble that found on *S. dentatus*.

The microbial associations in both diseased and healthy cuticles of *S. edentatus* were examined microscopically and with bacteriologic techniques. Correlations between lesion flora and lesion morphology, as well as relationships between the microbial flora of normal and diseased worms, were investigated.

Materials and Methods

From 200 to 400 living S. *edentatus* were obtained from horses having natural infections. After gross examination for cuticular lesions, worm specimens were prepared either for histologic examination by light microscopy (LM) or scanning electron microscopy (SEM) as described by Anderson et al. (1971). Representative types of cuticular lesions and pieces of healthy cuticle were excised and immediately inoculated into various bacteriologic media using methods previously described by Anderson et al. (1971). These methods were used also to test the microbial flora of the body fluids of lesion-free and diseased worms.

A substance grossly resembling cuticular lesions and found on the cuticles of only female strongyles was subjected to microscopic and bacteriologic examination as a possibly unique type of cuticular pathology.



Figures 1–4. Scanning electron micrographs of *Strongylus edentatus* cuticular lesions. 1. Filamentous type. \times 850. 2. Flat type. \times 525. 3. Cratered type. \times 500. 4. Proliferate type. \times 150.

Methods described in Gibbs and Skinner (1966), Gibbs and Shapton (1968), and Oetjen and Harris (1973) were used to identify microbial isolates from the various specimens.

So that stained histologic sections of strongyles previously examined by LM could be studied with SEM, slides were immersed in xylene to remove cover glasses were etched

Figures 5–8. Scanning electron micrographs of *S. edentatus* cuticle lesions. Associated microbial flora. 5. Filamentous. × 9,000. 6. Flat. × 2,100. 7. Cratered. × 3,350. 8. Proliferate. × 2,900.

Figures 9–11. Scanning electron micrographs of *S. edentatus* genital pore and copulatory debris. 9. Genital pore with debris removed. \times 260. 10. Debris covering genital pore. \times 720. 11. Microbial flora associated with copulatory debris. \times 2,850.



Table	1.	Microb	ial i	solates	from	cuti	cle lesions,
healthy	cı	iticles,	and	body	fluids	of	Strongylus
edentat	us.						

		Mi	crobia	l isol	ates	
	Enterobacter aerogenes	Escherichia coli	Micrococcus sp.	Proteus vulgaris	Streptococcus sp.	Streptococcus faecalis
Healthy cuticle	(+)	(+)	(+)	(–)	(+)	(–)
Cuticular lesions: Filamentous Flat Cratered Proliferate	(+) (+) (+) (+)	(+) (+) (-) (-)	(+) (+) (-) (+)	(-) (+) (-) (-)	(+) (+) (-) (+)	(+) (+) (+) (+)
Lesion-free Worm body fluid*	(+)	(–)	(+)	(–)	(+)	(+)
Debris associated Worm genital pore	(+)	(–)	(+)	(-)	(+)	(+)

* Similar patterns of microbial isolates were found in body fluids of worms with cuticle lesions.

with a diamond-point scriber, broken into pieces suitable for placement on stubs, coated, and put into the SEM specimen chamber. Otherwise, such samples were handled in the same manner as samples obtained and prepared specifically for SEM.

Results

Four types of cuticular lesions were distinguishable. These types could be characterized as (1) filamentious—small, with intertwining mat of bacteria and cuticular tissue projecting above, but not penetrating deeply into the cuticular surface; (2) flat—large, closely adhering to, but not penetrating deeply into, the cuticular surface; (3) cratered—large, markedly pitting and penetrating the cuticular surface; and (4) proliferate—small, numerous, distributed over the entire cuticular surface, slightly raised, but not penetrating deeply into the cuticular surface. The distinguishing morphologic features of these four types of cuticular lesions and their associated microbial flora can be seen in Figures 1–4 and 5–8, respectively. Lesions of the first three types were found at a frequency of one per worm, but those of the fourth type were found in great numbers over the entire worm (Fig. 4). The ratio of worms with multiple lesions to worms with single lesions was approximately 4 to 1. Worm selection was not sufficiently random to ascertain validly a ratio of worms with lesions to worms with healthy cuticles.

Microorganisms recovered from the lesions are listed in Table 1. Only two of the microbial strains recovered from cuticular lesions, Strepto*coccus faecalis* and *Proteus vulgaris*, were not present in the microbial flora of healthy cuticle; moreover, only flat lesions contained P. vulgaris. Of the lesions tested, cratered lesions had the least extensive microbial flora-only two recoverable microbial strains, S. faecalis and Enterobacter aerogenes. Although associated with healthy cuticle and with two types of cuticular lesions, Escherichia coli was absent from the microbial flora of cratered and proliferate lesions, the lesions that had the most severe localized or disseminated impact on the worm cuticle. The various types of lesions differed by as few as one and as many as four constituent bacterial genera in their microbial flora (Table 1).

When body fluids of lesion-free worms were cultured, S. *faecalis*, other cocci, and E. *aerogenes* were recovered; however, no *Escherichia coli* were isolated (Table 1). Results were similar when the body fluids of worms with cuticular lesions were cultured.

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Figures 12–17. Scanning electron and light micrographs of *S. edentatus* cuticular lesions. 12. Scanning electron micrograph of unstained tissue section (oblique) showing extent of cuticular penetration by proliferate lesions (arrows). \times 125. 13. Light micrograph of Gram-stained tissue sections. Bracketed area between lesions indicates microbial aggregations. \times 500. Phase contrast. 14. Light micrograph of Gram-stained tissue section. Microbial aggregations (arrows) adjacent to cuticular lesion (CL). \times 1,250. Phase contrast. 15. Gram-stained tissue section (oblique) (4 μ m thick). Scanning electron micrograph showing microbial aggregations (arrows) between lesions. 16. As in Fig. 15. Microbial aggregations (arrows) adjacent to lesion edge (CL). \times 1,500. 17. As in Figs. 15, 16. Intracuticular microbial aggregation (center arrow of Fig. 16). \times 15,000.



The substance suspected of being cuticular lesions, and observed on the cuticles of only female strongyles, proved to be the by-product of a very natural and nonpathologic process; that is, a debris that accumulates around the genital pore while the male strongyle is attached for copulation (Figs. 9–11). The debris was easily removed and contained enteric cocci and rods but no *E. coli* (Table 1). The microbial flora of the debris was the only exception to the observation that *S. faecalis* was not found associated with healthy cuticle.

Results derived from gross, LM, and SEM observations of the lesions generally agreed. But LM of stained histologic sections of proliferate lesions, as well as SEM of unstained sections, revealed deeper pentration of the cuticle than was apparent by superficial examination (Figs. 12, 13). Plugs of deteriorated cuticle retained within these small lesions gave them the gross appearance of being shallow.

During LM examination of histologic sections of cuticles having proliferate lesions, apparent microbial aggregations were noted within the cuticles between and to either side of the lesions (Figs. 13, 14). Such aggregations suggested a mode of dissemination for proliferate lesions. Because LM observation could not confirm the microbial morphology of the constituents of these aggregations, SEM was used to examine the stained cuticle sections. Figures 15–17 reveal that constituents of these aggregations did have a microbial morphology even though, with an 4- μ -thick sections, they were subsurface to the SEM beam.

Discussion

As with ascarid cuticle lesions (Anderson et al., 1971), lesions of strongyle cuticle varied not only in size, number, and degree of tissue destruction, but also in the microbial genera found associated with them. Microorganisms associated with lesion-free worm cuticle and those cultured from worm body fluids constituted a part of the microbial milieu to which these strongyles were exposed. All bacteria recovered from the worms, with the possible exception of *P. culgaris*, are considered regular inhabitants of the horse intestine; however, *Proteus* sp. are occasional inhabitants of the mammalian gut. It may not be a coincidence that the flattened, spreading lesions included

in their microbial flora P. vulgaris, a bacterial species noted for its spreading colonial growth on solid media. Although S. faecalis was not found associated with healthy cuticle as a constituent of the regular microflora, it was very definitely a constituent of the strongyle gut microbial flora. Although E. coli was recovered from healthy and some diseased cuticles it did not seem to be a gut inhabitant of the worms; this is borne out by the absence of E. coli from the microbial flora of the copulatory debris. It is interesting to speculate whether the absence of *E*. *coli* from the microbial flora of proliferate and cratered lesions could possibly account for, or be a result of, the severity of these lesions.

Microorganisms found in the cuticle lesions could have originated from either the horse intestine or worm gut, with the exception of *E. coli* and *P. vulgaris*. The vertebrate host intestine would seem a more likely source of these two microbial isolates of cuticular lesions.

Unfortunately, none of the cuticle lesions on S. edentatus resembled the hypertrophic lesions on cuticles of Stephanurus dentatus and S. vulgaris (Anderson et al., 1973). All lesions had a more or less eroded appearance similar to that seen by Anderson et al. (1971) in ascarid cuticular lesions. Observations of cuticle lesions on strongyles and ascarids indicate a relationship between the cuticle lesion histopathology and its constituent microbial flora. Thus, a differing microflora might account for the differing superficial appearance of flat and proliferate lesions. Conversely, cratered lesions may represent a progressively severe stage of filamentous lesions in which the deteriorating cuticular tissue is inimical to all but two of the bacterial genera found in filamentous lesions. The foregoing suggests that the microbial flora of hypertrophied-type lesions would differ from that found in erosive-type lesions. At present, the exact nature of that difference remains unknown.

McKinnon and Lubinsky (1966) found that cuticle lesions on swine ascarids they examined occurred more frequently on female than on male worms. They also found these lesions occurred mainly on the anterior part of the worm body but did not associate this finding with proximity to the genital pore. Their observations, in light of our findings, suggest a possible etiology of some helminth cuticle lesions. Although the location of the nematode genital pore varies and accessory male organs such as bursae may be present, copulatory mechanisms in most nematodes are probably essentially similar. Because the male strongylc deposits its sperm through a cloaca, the origin of the debris and its microbial flora around the genital pore is not difficult to understand. Because S. *faecalis* was associated with all types of strongyle cuticle lesions, as well as with copulatory debris microflora, one might speculate that at least some lesions of helminth cuticles may be a "venereal disease." Obviously, this would not account for all lesions of worm cuticles and the cuticle would presumably have to become in some way predisposed to microbial infection.

Data obtained from examining stained sections of proliferate lesions with SEM suggested that some cuticular lesions of nematodes are not discrete dermatoses but may have an intracuticular dissemination under appropriate as yet undetermined conditions.

Acknowledgments

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Microbial Flora Associated with Migrating Stephanurus dentatus Larvae and with Tissue of Parasitized Swine

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ABSTRACT: Grossly normal tissues from swine parasitized with Stephanurus dentatus yielded: Streptococcus sp. (lung, ureter); Enterobacter sp., Escherichia coli (diaphragm, gastrohepatic lymph (GHL) nodes, liver, perirenal (PR) fat, ureter); Proteus mirabilis (GHL nodes); Actinobacillus sp., Bacillus sp., and Micrococcus sp. (ureter). Petechiae and pus pockets from livers of parasitized swine contained Enterobacter sp., E. coli, P. mirabilis, and Streptococcus sp.; whereas, ureteral cysts and cyst fluids from these swine contained Actinobacillus sp., Bacillus sp., Enterobacter sp., E. coli, Micrococcus sp., and Streptococcus sp. Microbial associates of larval S. dentatus recovered from swine tissues included Enterobacter sp., E. coli (diaphragm, liver, portal vein (PV), PR fat); Pseudomonas aeruginosa (diaphragm); P. mirabilis (liver); Streptococcus sp. (liver and PR fat). No larvae were recovered from lung or GHL node tissues. Only E. coli and Enterobacter sp. were found associated with adult S. dentatus recovered from ureteral cysts. Bacteriuria was demonstrable in parasitized swine and consisted of Enterobacter sp., E. coli, and Streptococcus sp. Microbial associates of tissues from helminth-free swine included P. aeruginosa (diaphragm and GHL nodes) and Streptococcus sp. (liver and PV). No bacteria were found in other helminth-free swine tissues examined and no bacteriuria was demonstrated in these swine. Ancillary data from examining the ulcerated colon of a helminth-free swine indicated that the ulcer had a microbial flora similar to that found in pathologic liver tissue from parasitized swine. This similarity in flora suggested the possible intestinal origins of the microbial strains associated with liver lesions in parasitized swine.

Stephanurus dentatus larvae in their wideranging tissue migrations may lodge and cause lesions in various swine abdominal and thoracic organs and tissues (Schwartz and Price, 1931). Unlike lesions caused by many other tissueinvading nematodes, those caused by S. dentatus were acute and inflammatory and were reminiscent of those produced by bacterial infection (Schwartz and Price, 1932). Ashizawa et al. (1972a) attributed at least one type of pathologic change noted in livers from swine infected with S. dentatus to bacterial contamination of the worm body.

A systematic survey of microorganisms associated with adult *S. dentatus* revealed two or three bacterial genera distributed in various worm organs and tissues (Anderson et al., 1973). On the basis of that information, it seemed useful to determine what microorganisms might be associated with migrating *S. dentatus* larvae and with the swine tissues from which the larvae were recovered.

Tissues from both parasitized and helminthfree swine and pathologic materials from swine livers and ureters were examined. The microbial flora of larval and adult worms recovered from affected tissues was compared with the microbial flora of those tissues. In addition, the urine from these swine was examined for bacteria and compared with previous results (Anderson et al., 1973) from bacteriologic examination of swine urine cultures.

An ulcerated colon from a helminth-free pig was also examined. A comparison of the microbial flora of this intestinal lesion with the flora found associated with extraintestinal tissues of parasitized swine seemed pertinent because the intestinal tract flora of swine is thought to be carried by migrating *S. dentatus* larvae to other tissue loci and to contribute to the lesions seen in stephanuriasis.

Materials and Methods

All swine tissues examined were from animals maintained at the Animal Parasitology Institute (Beltsville, Md.) for swine kidney worm research. Five parasitized swine had been experimentally infected *per os* approximately 8 months before the time their tissues were examined. Four helminth-free swine had never been experimentally infected and showed no evidence of natural *S. dentatus* infection. Both swine tissue and *S. dentatus* samples were obtained from freshly killed animals and were examined and cultured immediately thereafter. Specimens for histological examination by light microscope (LM) were prepared as previously described (Anderson et al., 1973).

The following tissues from three helminthfree swine were examined for their microbial flora: lung; diaphragm; gastrohepatic lymph (GHL) nodes; liver; portal vein (PV); perirenal (PR) fat; ureter; and ulcerated colon. Tissues from parasitized swine that were examined bacteriologically included lung, diaphragm, GHL nodes, grossly normal and pathologic liver, PV, PR fat, ureter, and ureteral cysts. These tissues, as well as ureteral cyst fluid, adult worms, and tissue larvae, were cultured with bacteriologic techniques as described in Anderson et al. (1973). After it was established that the helminth-free pig with an ulcerated colon showed no evidence of natural S. dentatus infection, microbiologic examination of this animal was confined to study of the colon lesion and this animal was excluded from data pertaining to helminth-free swine.

In addition, urine from swine under study was collected and cultured according to methods set forth previously (Anderson et al., 1973). Methods described by Gibbs and Skinner (1966), Gibbs and Shapton (1968), and Oetjen and Harris (1973) were used to identify microbial isolates. Examination for, and recognition of, gross and microscopic tissue lesions in swine parasitized with *S. dentatus* was aided by consulting the papers of Ashizawa et al. (1972a, b, c).

Results

Microbial flora of helminth-free swine tissues

Pseudomonas aeruginosa was recovered from diaphragm and GHL node specimens obtained from three helminth-free swine. Liver and PV specimens from these swine contained Streptococcus sp. No signs of gross or microscopic lesions were noted in tissues from helminthfree swine from which bacteria were recovered. No bacteria were found in lung, PR fat, or ureter tissue specimens from helminth-free swine. Moreover, no bacteriuria was demonstrated in these swine.

Microbial flora of swine tissues parasitized with S. dentatus

Grossly normal tissues from three parasitized swine contained *Streptococcus* sp. (lung, ureter); *Enterobacter* sp., *Escherichia coli* (diaphragm, GHL nodes, liver, PR fat, ureter); *Proteus mirabilis* (GHL nodes); *Actinobacillus* sp., *Bacillus* sp., *Micrococcus* sp. (ureter). Light microscopy of stained sections of the grossly normal tissues from parasitized swine revealed no abnormalities and no larvae were noted in the sections.

Liver lesions (petechiae and pus pockets) from two parasitized swine contained *Enterobacter* sp., *E. coli*, *P. mirabilis*, and *Streptococcus* sp. These petechiae, pus pockets, and ureteral cysts were the only gross lesions observed in any of the tissues from these animals. No sections for LM were prepared from these lesions. Bacteriura was demonstrable in these two parasitized swine, and *E. coli*, *Enterobacter* sp., and *Streptococcus* sp. were isolated.

Microorganisms associated with S. dentatus

Larval S. dentatus recovered from swine tissues yielded the following microbial associates: E. coli; Enterobacter sp. (diaphragm, liver, PV, PR fat); P. aeruginosa (diaphragm); P. mirabilis (liver); and Streptococcus sp. (liver and PR fat). No larvae were recovered from lung or GHL node tissues. Only E. coli and Enterobacter sp. were found associated with adult S. dentatus recovered from ureteral cysts. The results of the bacteriological examination of the above specimens are summarized in Table 1.

Several comments on the microbial isolates are appropriate. Identification of certain microbial isolates to the species level was accomplished with relative ease, whereas those identified to only the generic level proved difficult to speciate unequivocally after several attempts to do so. Streptococci isolated included both enteric and nonenteric strains. The enteric strains were cultured from larvae recovered from liver and PR fat, as well as from liver

				Mic	robial iso	lates			
Source	Actinobacillus sp.	Bacillus sp.	Enterobacter sp.	Escherichia coli	Micrococcus sp.	Proteus mirabilis	Pseudomonas aeruginosa	Streptococcus sp.	None
Helminth-free swine (3)* Tissues† Lung Diaphergen									+
Gastrobepatic lymph (GHL) nodes Liver Portal vein (PV) Perirenal (PR) fat Ureter Urine							÷	+ +	+ + +
Larval Worms From‡ Diaphragm Liver PV PR fat			-+- +- +- -+	+++++++++++++++++++++++++++++++++++++++		+	+	+++++++++++++++++++++++++++++++++++++++	
Parasitized swine (5) Grossly normal tissues (3) Lung Diaphragm GHL nodes Liver Pyre c			+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++		+		+	+
rk fat Ureter	+	+	++	+++++++++++++++++++++++++++++++++++++++	+			+	
Pathologic liver (2)			-1-	+		+		+	
Ureter Cyst and Fluid (2)	+	+	+	-+-	+			+	
Adult Worms (2)			+	+					
Urine (2)			+	+				+	

Table	1	Deculte /	of	bacteriologic	examination	of	helminth.fr	ee swine	b and	swine	norositized	hv	S	dentatus
I able	1.	Results	01	Dacteriologic	examination	- 01	merminin-n	ee swine	: anu	swine	Darasitizeu	υv	э.	aentatus.

* Figures in parentheses indicate the number of swine from which tabulated microbial isolations made. + Ulcerated colon tissue, mentioned in text and excluded from Table, had microbial flora similar to that in pathologic liver from parasitized swine. + No larvae were recovered from lung or GIIL node tissues.

lesions, ureter tissue, ureteral cyst fluid, and urine from parasitized swine. Nonenteric strains were recovered from helminth-free swine tissues and grossly normal lung tissue from parasitized swine. The Actinobacillus sp. was initially isolated from ureteral cyst fluid, and its unexpected appearance led to a reexamination of the materials cultured. The only other material yielding actinobacilli was grossly normal ureter tissue from parasitized swine.

Examination of bacterial isolates from lesions of the ulcerated colon of a helminth-free pig revealed a microbial flora similar to that found associated with liver lesions from parasitized swine (Table 1). No attempt was made to determine the precise microbiologic etiology of the colon lesion.

Discussion

Microorganisms found associated with tissue of swine parasitized with adult S. dentatus and with the adult worms were in general accord with previous findings that were derived from renal tissues and adult S. dentatus recovered from naturally infected swine (Anderson et al., 1973). At variance with previous data was the bacteriuria demonstrated in parasitized swine and the Actinobacillus sp. isolated from ureter tissue and ureteral cyst fluid. The finding of bacteriuria in these parasitized swine may have been due to the stage of the kidney worm infection at which the urine was sampled. Ross et al. (1972) indicated that although actinobacilli are only occasionally isolated from swine, they had recovered several strains related to *Actinobacillus suis* from vaginal exudate of postparturient sows. Thus, the actinobacilli isolated could very well have originated from an ascending progression of these bacteria. The observation that actinobacilli were limited to ureteral tissue seemed to support this possibility.

Only nonenteric streptococci were found in tissues from both helminth-free and parasitized swine. Although the ubiquity of these bacteria could account for their presence in tissues from helminth-free swine, the isolates of *P. aeruginosa* recovered from these tissues are not as casily explained. Interestingly, these pseudomonads were isolated also from larvae recovered from diaphragm tissue but could not be found in parasitized swine tissues. Thus, the impact of these larva-associated pseudomonads on swine tissue is uncertain.

Interesting though these observations may be, they are not as important as the consistent association of enteric microorganisms with tissues from parasitized swine infected *per os*, as well as with *S. dentatus* larvae recovered from many of those tissues, that was demonstrated. Such associations indicate that migrating *S. dentatus* larvae do indeed carry swine intestinal flora components to other tissues and suggest that these bacteria may contribute significantly to histopathology of stephanuriasis. A remaining question concerns what the microbiologic manifestations might be in swine infected percutaneously with *S. dentatus* because then the intestine is bypassed.

Of the tissues from parasitized swine examined, only those from liver and ureter were demonstrably affected by intrusion of the helminth-bacteria association. Because the ureteral cyst pore offers free access to the ureter lumen, bacteria associated with encysted *S. dentatus* can and did contribute to the swine urine flora. The microbial flora found associated with the colon ulcer in a helminth-free pig provided data indicating the intestinal source of bacteria strains associated with tissues of parasitized swine. Although the precise microbiologie etiology of the colon ulcer was not determined, the ancillary data regarding the microbial flora of the ulcer suggested that these bacteria can cause lesions in the apparent absence of helminth parasitization.

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The Effect of UV Radiation on Survival of the Free-Living Stages of *Haemonchus contortus*

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ABSTRACT: The effect of UV radiation on the free-living stages of *Haemonchus contortus* was examined, and only third-stage larvae were found to be resistant to UV radiation. Third-stage larvae were then subjected to desiccation followed by rehydration and UV exposure, desiccation and UV exposure concurrently, or UV exposure followed by desiccation. Ultraviolet radiation and desiccation were found to have an additive deleterious effect on the parasite. Thus, UV radiation should be considered in any future experimentation involving parasitic-nematode survival in relation to bioclimatic factors.

Many researchers have suggested that sunlight is harmful to both the egg and larval stages of parasitic nematodes (Mönnig, 1930; Spindler, 1934, 1940; Kauzal, 1936; Roberts, 1937; Dinaburg, 1944; Dinnik and Dinnik, 1958, 1961; Marquardt et al., 1959; Becklund, 1964: Kates, 1965). Wertejuk (1959), however, felt that infective larvae of gastrointestinal nematodes of sheep were characteristically resistant to sun rays with the exception of larvae of Strongyloides papillosus. Temperature and desiccation, which are in part a function of sunlight, have been examined in great detail; however, to my knowledge no work has been conducted to determine what effect the UVradiation component of sunlight has on the freeliving stages of parasitic nematodes. These experiments were an attempt to examine the effect of UV radiation on the survival of freeliving stages of Haemonchus contortus.

Materials and Methods

Unembryonated eggs collected in fecal pellets from sheep infected with *H. contortus* were processed by the techniques of Todd et al. (1970) in order to obtain the five principle freeliving stages (the unembryonated and embryonated egg and first-, second-, and third-stage larvae) of the parasite. Aliquots containing

200-300 or more of each stage were placed in separate 35×10 mm plastic petri dishes, and the volume of water in these dishes was brought to 2 ml. Duplicate samples were also prepared for use as controls. All samples (Treatment A) were placed under a General Electric UV source fitted with a medium pressure quartz-mercury lamp; however, the control samples were protected from UV radiation by a cardboard covering. The UV source produced radiation in the range of 254-365 nm with a combined energy of ~1,626 erg \cdot sec⁻¹ \cdot cm⁻². This range includes radiation that is normally filtered from sunlight by the earth's ozone layer and which is strongly absorbed by nucleic acids and proteins (Giese, 1968). All samples were periodically observed for development and/or activity of the various stages. The temperature at the exposed site ranged from 26.8-29.5 C and that at the covered site was about 0.5 C cooler.

Only third-stage larvae were used to examine the combined effect of radiation and desiccation. These larvae were prepared as previously described, except that they were washed twice in distilled water following baermannization. This step was included because Todd et al. (1970) showed that distilled water gave better survival on desiccation for *H. contortus* than did tap water. The third-stage larvae were then

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Tabl	e 1.	Effec	ts of desice	atio	n followed b	oy rehyd	ra-
tion	and	UV	exposure	on	third-stage	larvae	of
Haer	nonc	hus c	ontortus (Trea	atment B).		

Treat	tment	
Desiccation (hr)	UV exposure (hr)	% active (days active)
12	24	0
12	0	9 (30+)
24	24	0
24	0	5 (7)
48	24	0
48	0	0
0	24	10 (8)
0	0	95 (30+)

Table 2. Effects of desiccation and UV exposure concurrently on third-stage larvae of *Haemonchus* contortus (Treatment C).

Trea	tment				
Desiccation (hr)	UV exposure (hr)	% active (days active)			
12	12	0			
12	0	22(30+)			
24	24	0			
24	0	3 (30+)			
48	48	0			
48	0	0			

subjected to one of three types of treatment: (1) desiccation followed by rehydration and UV exposure (Treatment B), (2) desiccation and UV exposure concurrently (Treatment C), and (3) UV exposure followed by desiccation (Treatment D).

Results and Discussion

Ultraviolet radiation at the wavelengths and energies used in Treatment A was extremely deleterious to all free-living stages of H. con*tortus* except the third-stage larvae. Development and/or activity ceased within 30 hr or less for each of the first four free-living stages; however, third-stage larvae remained active for almost 2 weeks under continuous UV These results agree well with exposure. Wertejuk's (1959) general conclusion that infective larvae of gastrointestinal nematodes of sheep are characteristically resistant to sunrays. Toward the end of the two-week period a majority of third-stage larvae shed the cuticle of the old second-stage larva (exsheathment). This cuticle apparently protects the third-stage larva against UV damage. Prior to exsheathment, the cuticle may provide sufficient insulation from UV radiation simply because this type of radiation is not highly penetrating. When the cuticle was lost, however, third-stage larvae became susceptible to UV damage and soon died. Under normal conditions the thirdstage larva is the only stage of the parasite which is exposed to UV radiation, since the other stages are usually found within the sheep pellet where UV radiation does not penetrate.

Since only third-stage larvae demonstrated

resistance to UV radiation, that stage alone was used to examine the combined effects of UV radiation and desiccation on survival. Desiccation and UV exposure separately were harmful but tolerable for moderate periods of time, while Treatment B was lethal at even the lowest levels examined (12 hr of desiccation followed by rehydration and 24 hr of UV exposure; Table 1). Control samples which received neither treatment showed a high rate of survival (Table 1). Larvae subjected to Treatment C were readily killed, while control samples subjected only to desiccation were less severely affected (Table 2). When larvae were exposed to Treatment D (Table 3), they survived longer and in greater numbers than either of the previous two treatment schemes. Again control larvae subjected only to desiccation

Table 3. Effects of UV exposure followed by desiccation on third-stage larvae of *Haemonchus* contortus (Treatment D).

Treat	ment	
UV exposure (hr)	Desiccation (hr)	% active (days active)
12	24	92(30+)
0	24	93(30+)
24	24	74(30+)
0	24	85 (30+)
48	24	24(30+)
0	24	52(30+)
72	24	16(30+)
0	24	87 (30+)
96	24	5(30+)
0	24	79(30+)
120	24	4 (11)
0	24	93(30+)
144	24	0
0	24	94 (30+)

were less severely affected than those treated with UV radiation followed by desiccation (Table 3).

It can be concluded that for *H. contortus* only third-stage larvae exhibit significant UV resistance, and that UV radiation and desiccation have an additive deleterious effect on the parasite. Therefore, UV radiation should be considered in any future experimentation involving parasitic-nematode survival in relation to bioclimatic factors.

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Swarming Disease of Nematodes: Host Range and Evidence for a Cytoplasmic Polyhedral Virus in *Tylenchorhynchus martini*

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ABSTRACT: Host and geographical ranges based on swarming symptoms and reports of swarming nematodes from the United States, Puerto Rico, Europe, and Africa indicate that swarming in plantparasitic and free-living nematodes is a phenomenon of common occurrence and worldwide distribution. Electron microscopy of swarming *Tylenchorhynchus martini* Fielding, 1956 sections showed high numbers of symmetrical viruslike inclusion bodies (VLIB) in nematode tissues; no viruslike particles were observed in tissues of healthy, nonswarming *T. martini*. Cell-free extracts of swarming nematode tissues yielded large numbers of honeycomblike fragments resembling the symmetrical VLIB and small numbers of crystalline bodies with polyhedra also resembling the symmetrical VLIB. Current evidence suggests the swarming phenomenon is a disease of nematodes associated with inclusion bodies similar to the cytoplasmic polyhedral viruses causing cytoplasmic polyhedroses in insects.

The swarming phenomenon in plant-parasitic and frec-living nematodes was discovered independently by Meyl (1955) and Hollis (1958). Meyl observed swarming in *Hemicycliophora typica* de Man 1921 and gave it the name "Nesterbildung" and ascribed to it a sexual function.

Evidence on swarming has come from experiments with the plant-parasitic nematode Tylenchorhynchus martini Fielding, 1956.Swarming results from a stickiness of cuticle, which has not been modifiable by chemicals, except trypsin which masks the stickiness and inhibits swarming (Hollis, 1962). The effect is reversible; the specimens swarm again after the removal of the trypsin by washing. Electron micrographs of T. martini (Ibrahim, 1967) showed the presence of morphological modifications in the cuticle of swarming nematodes. Swarming specimens exhibited swelling and disruption of the external cuticle and separation of external and internal cortical layers. Poinar (1973) described a cuticular infection caused by bacterialike microorganisms (not further identified) in adults of *Thelastoma pterygoton*. He suggested that these microorganisms were capable of dissolving parts of the nematode's cuticle and establishing colonies on the cuticle surface.

Additional studies on the fine structure of the

cuticle of swarming and nonswarming T. martini were conducted by Ibrahim and Hollis (1973) utilizing transmission and stereoscan electron microscopes. The basic cuticular ultrastructure (sequence of layers) was the same in both swarming and nonswarming T. martini. Nonswarming specimens of T. martini exhibited normal intact cuticle without any morphological changes, identical to that previously described by Ibrahim (1967). On the other hand, swarming specimens showed morphological changes in the cuticle cortex which appeared first as an increase in electron density of the outer and inner sublayer. Later changes appeared as dissolution of some parts of the cortical layer and the occurrence of randomly scattered cuticular projections. Cuticular projections which were outgrowths of both the cortical and matrix layers appeared mainly on the lateral and sublateral ridges. Partial dissolution of the cortex and matrix probably resulted in the formation of sticky materials on the surface of the cuticle. Rupture and breakdown of the cuticular layers were characterized generally by the appearance of irregular cracks on the sublateral areas of the body. The authors concluded that the irregular and drastic character of the morphological changes in swarming suggested a diseased condition and subsequently it was reported that swarming in T. martini is caused by a virus (Ibrahim et al., 1973). The purpose of this paper is to list the nematode species involved in swarming disease and the

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		Observer			
Nematode species	Geographical location	Name	Year of record		
Hemicycliophora typica de Man, 1921 Tylenchorhynchus martini Fielding, 1956 Tylenchorhynchus martini Fielding, 1956 Tylenchorhynchus sp. Rotylenchulus reniformis Lindford and Oliviera, 1940 Hemicycliophora sp. Dorylaimus pusillus Cobb, 1893 Oncholaimus sp. Hemicycliophora sp. Rotylenchulus sp. Scatellonema sp. Tylenchorhynchus ewingi Hopper, 1959 Hemicycliophora sp. Scatellonema sp. Tylenchorhynchus claytoni Steiner, 1937 Helicotylenchus nannus Steiner, 1945 Helicotylenchus nannus Steiner, 1945 Tylenchorhynchus claytoni Steiner, 1937 Criconemoides xenoplax Raski, 1952 Tylenchorhynchus claytoni Steiner, 1937 Tylenchorhynchus claytoni Steiner, 1937 Martine Steiner, 1937 Griconemoides xenoplax Raski, 1952 Tylenchorhynchus dubius	Germany Louisiana, USA Kentucky, USA Louisiana, USA Louisiana, USA Louisiana, USA Louisiana, USA Puerto Rico Kenya Kenya Louisiana, USA Kenya Louisiana, USA Florida, USA Florida, USA Florida, USA Georgia, USA Georgia, USA England	A. H. Meyl J. P. Hollis R. A. Chapman J. P. Hollis J. P. Hollis J. P. Hollis J. P. Hollis G. Steiner A. G. Whitehead A. G. Whitehead A. G. Whitehead J. P. Hollis J. P. Hollis J. P. Hollis J. P. Hollis J. P. Hollis J. P. Hollis J. M. McBride R. P. Esser R. P. Esser R. P. Esser R. P. Esser R. P. Esser E. J. Wehunt E. J. Wehunt E. J. Wehunt D. C. Hooper J. K. A. Drebing	1955 1957 1958 1960 1960 1960 1961 1961 1961 1961 1961		

Table 1. Occurrence of the swarming disease in nematodes.

morphology of the viruslike inclusion bodies in swarming T. martini and its cell-free preparations.

Materials and Methods

Methods of nematode culture in clay pots containing soil or sand planted with rice or sugarcane in the greenhouse and induction of the swarming phenomenon in T. martini were described by Hollis (1958, 1960, 1962), Hollis and McBride (1962), and McBride (1963).

Techniques for making thin sections of swarming and nonswarming specimens of T. *martini* were described by Ibrahim and Hollis (1973), except that specimens were embedded in epoxy resin. Nematode sections were stained with phosphotungstic acid to obtain negative staining of the viruslike inclusion bodies (Dawes, 1971).

A method of obtaining cell-free extracts was developed for isolation of viruslike inclusions. Two extracts were prepared; one from swarming and the other from nonswarming populations of T. martini. Approximately 40,000 nematodes were concentrated in a small volume of water by centrifugation, frozen in liquid nitrogen and ground in mortar. The frozen suspension was remoistened, diluted with water, filtered through cheesecloth and centrifuged for 5 min at 3,000 rpm; this procedure was repeated once. Five volumes of 1/1 mixture of 0.05 M NaCl and 0.05 M Na₂CO₃ were added to one volume of the filtrate. The suspension was centrifuged at 3,000 rpm for 15 min. The clarified supernatant was centrifuged at 40,000 rpm for 40 min. The resulting pellet at the bottom of the centrifuge tube was dissolved in a small volume of sterile distilled water. A drop of this suspension was placed on a carboncoated grid and prepared for examination in the electron microscope by standard shadowing technique using platinum-palladium alloy, and also by staining in uranyl acetate and lead hydroxide solutions (Dawes, 1971).

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Figure 1. Viruslike inclusion bodies (VLIB) in *Tylenchorhynchus martini* Fielding, 1956 swarmers. A, B. Cross sections of *T. martini* showing cuticle (C), muscle layer (M) and symmetrical VLIB (V). \times 48,000 and \times 50,000, respectively. C. Cross section through the ovary of *T. martini* showing negatively stained symmetrical VLIB. \times 38,250. D. Symmetrical VLIB on the cuticle surface. \times 21,000. E. Symmetrical VLIB in cell-free extract stained with uranyl acetate and lead hydroxide solutions. \times 56,000. F. Symmetrical VLIB in cell-free extract shadowed with platinum-palladium. \times 67,000.





Results

Occurrence of the swarming disease of nematodes is shown in Table 1. There is a minimum number of 17 species of plant-parasitic and free-living nematodes in three taxonomic orders which have manifested swarming symptoms. Five of these species are in the genus *Tylenchorhynchus*.

Electron microscopy of thin sections and cellfree extracts of swarming specimens of T. martini revealed the presence of cytoplasmic viruslike inclusion bodies (VLIB). Symmetrical VLIB of variable size were observed in nematode sections and in cell-free extracts (Fig. 1). They had an average diameter of 100 nm. Weak alkali treatment of cell-free extracts resulted in the apparent fragmentation and aggregation of symmetrical VLIB which now appeared in the extracts as honeycomblike fragments ranging in diameter from 50 to 120 mm. Many of these fragments in both size and peripheral morphology resembled the symmetrical VLIB in the sections, but in addition they manifested polyhedral sockets internally in which viruslike subunits could have been occluded. The cores appeared 5- or 6-sided with a range of 3.8 to 5.7 nm and an average 4.8 nm diameter. Diameter dimensions from the middle of one wall to another across the subunit polyhedra averaged 7.4 nm (Fig. 2A, B).

Another type of inclusion body (crystalline bodies 250-500 nm diameter) appeared in unfiltered *T. martini* swarming homogenates (Fig. 2C). These crystalline bodies contained numerous polyhedral units which were of the same size range and superficial pattern as the symmetrical VLIB. Furthermore, these polyhedral units each in turn contained subunits in a crystalline-structured, geometrical honeycomb pattern matching closely the size (7.3–7.5 nm), shape, and arrangement of the individual polyhedral sockets in the honeycomblike fragments (Fig. 2B).

Figure 2. Viruslike inclusion bodies (VLIB) in cell-free extracts of *Tylenchorhynchus martini* Fielding, 1956 swarmers. A, B. Honeycomblike fragments containing sockets of polyhedral subunits designated by arrows. \times 137,000 and \times 394,000, respectively. C. Cytoplasmic crystalline body containing viruslike polyhedral units. \times 103,000.

Thin sections and cell-free extracts of nonswarming T. martini examined with the electron microscope showed no VLIB and the nematode tissues were intact.

Discussion

Interpretation of the inclusion bodies in T. martini swarmers as manifestations of a cytoplasmic polyhedral virus (CPV) accounts for all of the observed information and is in agreement with all published evidence on cytoplasmic polyhedroses of insects (Smith, 1967a, b).

The inclusion bodies in T. martini swarmers are of two types: (1) symmetrical VLIB in sections equals honeycomblike fragments in cell-free extracts, (2) crystalline body. The VLIB, the honeycomblike fragments and the individual polyhedra of the crystalline body are comparable in size, shape, and internal dimen-Based strictly upon morphological sions. criteria, and in the absence of biochemical and serological data, it is evident that each of these three structures may represent the virus; in this discussion the word virus will be substituted for them, individually or collectively, with the understanding that portions of their structure may be missing as a result of chemical or physical action during preparation.

Size variation of the virus, described above, is similar to the size variation found in insect CPV (Aruga et al., 1963). This variation has constituted a stumbling block in recognition of the viral nature of nematode swarming disease because particles of other types of viruses known to virologists are of uniform sizes.

Japanese workers have published extensively on the cytoplasmic polyhedrosis and the CPV of the silkworm, *Bombyx mori* Linn. This disease and its virus in silkworms are well known and furnish guidelines for fundamental studies on other insects and nematodes (Smith, 1967a). Fine structures of the CPV in silkworm have been delineated by Hosaka and Aizawa (1964). Smith (1967b), noting morphological similarities between CPV of insects, has emphasized the question of how far the many CPV are one and the same.

Insect CPV are confined to the gut cells and may exert benign effects on the host. By contrast, *T. martini* is severely affected by CPV distribution in cells of the ovary, muscle layers and hypodermis, and by disruption of the cuticle. The function of swarming as a phenomenon now is clear: The CPV swarming disease immobilizes, localizes, separates or quarantines the diseased individuals from the healthy population which moves freely in search of living space and food. It is probable that swarming populations will die of starvation, if not of disease, although swarming T. *martini* specimens have been observed to feed on rice roots and lay eggs in agar cultures.

Points of correspondence between the *T.* martini CPV and insect CPV include their partial dissolution in weak alkali; their near spherical but possible polyhedral shape (Fig. 1F); their difficulty of isolation, purification, transmission and use in biological control studies (Smith, 1967a). The peripheral "burst pattern" of the *T. martini* CPV, best seen in the negatively stained particles (Fig. 1C), is a characteristic differing from CPV of insects.

Attempts to transmit the virus from T. martini swarmers to nonswarmers have failed, but since it now appears probable that we are working with a CPV of nematodes, progress can be anticipated on transmission through the egg and from one nematode species to another in soil. The occurrence of swarming in Dorylaimus pusillus Cobb, 1893 was observed in populations extracted from greenhouse crocks of soil planted to rice and infested with T. martini swarmers.

There have been two reports of viruses or virus diseases on individual nematode species in the laboratory (Loewenberg et al., 1959; Boyce-Thompson Inst. Annu. Rep., 1971) but the swarming disease (or diseases) exhibits a worldwide occurrence and bears significance in nematode ecology.

The discovery of nematodes, a class of cutelous animals, as hosts for cytoplasmic polyhedroses enriches the opportunities to attack problems of plant-parasite nematode control, virus latency, and the induction and chemotherapy of viral cancer in animals.

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Redescription of Truttaedacnitis stelmioides (Vessichelli, 1910) (Nematoda: Cucullanidae) from Lampetra lamottenii (Lesueur, 1827)

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ABSTRACT: Truttaedacnitis stelmioides (Nematoda: Cucullanidae) from the intestine of Lampetra lamottenii from Ontario is described and recognized as a valid species. It is distinguished from T. alpinus (Mudry and McCart, 1974) n. comb. by its funnel-shaped pseudobuccal capsule, conspicuous oral collarette with a dentigerous ridge, lateral alae, and a small terminal spine. Truttaedacnitis stelmioides is distinguished from T. truttae (Fabricius, 1794) by the lack of a dorsal cephalic ridge and a marked swelling of the body anterior to the nerve ring and by the presence of lateral alae.

Truttaedacnitis stelmioides has been reported from various species of lampreys (Lampetra fluviatilis, L. mariae, L. planeri, Petromyzon marinus) in Europe and North America (Vessichelli, 1910; Tornquist, 1931;

Zekhnov, 1956; Shul'man, 1957; Wilson, 1967; Wilson and Ronald, 1967). The species has been incompletely described, however, and is considered a species inquirendum by some authors (Campana-Rouget, 1957; Maggenti,

1971; Petter, 1974). Adult *T. stelmioides* are commonly found in the intestine of adult *L. lamottenii* in Ontario. In the present study *T. stelmioides* is redescribed and distinguished from related species.

Materials and Methods

All stages of *L. lamottenii* were collected with a portable electrofishing unit. Lampreys were transported alive to the University of Guelph. After immersion in a 0.2% solution of MS222 (tricaine methane sulphonate, Kent Laboratories, Ltd.), the heart, kidney, liver and intestine were examined separately for cucullanids. Worms recovered were washed in 0.6% saline and fixed in hot glycerine alcohol. Specimens were cleared and mounted in glycerine for study. Measurements and drawings were made with the aid of a drawing tube. Specimens examined with the scanning electron microscope were dehydrated, dried by the critical point method and coated with gold.

Specimens studied have been placed in the following collections: USNM No. 73130; National Museum of Canada—Parasites 1977, 607.

Truttaedacnitis stelmioides (Vessichelli, 1910) (Figs. 1–14, Table 1)

General

Slender worms with blunt anterior end and pointed, conical tail with small cuticular spine. Maximum body width slightly posterior to esophagus. Cuticle smooth or finely striated transversely; maximum thickness of cuticle 10 μ m. Cuticle with lateral alae extending from level of nerve ring to posterior border of preanal sucker. Deirids behind nerve ring either directly opposite each other or one slightly anterior to other; in latter case, right usually anterior



Figure 1. Scanning electron micrograph of cephalic extremity of male *T. stelmioides*.

to left. Excretory pore at same level or anterior to at least one deirid. Minute papillae present in lateral fields near midbody.

Cephalic end lacking dorsal cephalic ridge and often inclined dorsally. Slitlike or triangular oral opening dorsoventral in position, surrounded by thin collarette containing 100– 120 cuticular teeth, each 6 μ m in length. Cephalic papillae consisting of four prominent double submedian papillae approximately 22 μ m wide and 15 μ m high. Fused papillae of unequal size; larger papilla containing central cuticular rod. Amphids lateral, terminating below plane of submedian papillae. Six small papillae 3–4 μ m high located near oral margin.

Esophagus clavate and muscular, expanding anteriorly around conical pseudobuccal capsule. Dorsal esophageal gland nucleus prominent, near posterior end esophagus; gland opening

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Figures 2-5. Cephalic extremity of male T. stelmioides. 2. Dorsal view. 3. Ventral view. 4. Lateral view. 5. En face view.

Figures 6-11. Male *T. stelmioides.* 6. Lateral view. 7. Gubernaculum, ventral view (distal end to the reader's left). 8. Posterior extremity showing adanal and postanal caudal papillae, ventral view. 9. Posterior region, lateral view. 10. Cross section of left spicule, midway along shaft. 11. Left spicule, lateral view.







in dorsal angle of oral opening. Subventral esophageal gland nuclei inconspicuous; glands opening at level of nerve ring. Cuticle lining each angle of triradiate esophagus thickened into paired longitudinal rods extending from near oral margin to immediately anterior to esophageal-intestinal valve. Rods changing in size and appearing interrupted at level of nerve ring. Single thin, cuticular extension fringed with numerous serrations projecting outward into esophageal muscle between paired rods along entire length of esophagus.

Esophageal rods diverging anteriorly to form funnel-shaped pseudobuccal capsule. Cuticle lining pseudobuccal capsule thickened to form roughened ventral, ventrolateral, dorsolateral and dorsal pseudobuccal plates separated by thin sutures. Ventral plate consisting of small, single, median oval plate (v) anterior to ventral esophageal rods. Ventrolateral plates consisting of (1) large, paired, posterior triangular plates (vl 1) located between dorsolateral and ventral esophageal rods; (2) paired, crescent-shaped plates (vl 2) extending posterolaterally from ventral plate to dorsolateral esophageal rods and located anterior to posterior triangular plates; (3) paired, triangular plates (vl 3) extending ventrally from dorsolateral esophageal rods and lying anterior to crescent-shaped plates; (4) paired, triangular plates (vl 4) extending ventrally along oral margin from dorsolateral plate to vl 5; and (5) paired, triangular plates (vl 5) extending ventrally along oral margin from vl 4 to ventral plate. Dorsolateral plates consisting of small, narrow, paired plate (dl) extending posteriorly from oral margin to anterior end dorsolateral esophageal rods. Dorsal plates consisting of (1) large, single posterior triangular plate (d 1) located between dorsolateral esophageal rods, and (2) small, single, bilaterally symmetrical plate in form of pair of wings (d 2) located anterior to posterior triangular plate. Posterior ventrolateral triangle tapering anteriorly to join ventral esophageal rods. Dorsolateral rods tapering to point at lateral edge of dorsal winged plate; ventral rods connecting to ventral plate. Dorsal groove not present.

Two to four coelomocytes noted in body cavity, varying positions. Numerous hypodermal extensions from body wall to all internal organs. Posterior part of intestine with many elongated cells crisscrossing outer surface, number of cells increasing posteriorly.

Male

Monorchic, testis in posterior third of body looping anteriorly then reversing and expanding into posteriorly directed seminal vesicle. Posterior limit seminal vesicle marked by muscular valve extending into vas deferens. Vas deferens filled with spherical sperm $12-16 \mu m$ in diameter. Left midbody papilla anterior to junction seminal vesicle and vas deferens; right papilla posterior to junction. Muscular preanal sucker present but lacking cuticularized rim. Spicules equal or subequal in size, relatively short and thick with knobbed anterior and pointed posterior ends. Cross section of spicules showing three longitudinal hollow areas running entire length. Spicules not reaching posterior border of preanal sucker when fully retracted. Gubernaculum Y-shaped. Eleven pairs large, dome-shaped caudal papillae present; 3 pairs preanal and subventral; 3 pairs adanal and subventral; 5 pairs postanal of which 2 pairs subventral and 3 pairs lateral.

Female

Slightly larger than males. Didelphic, amphidelphic. One ovary located in middle third of body (anterior ovary), other in posterior third of body (posterior ovary). Oviducts looping beyond vulva then reversing and

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Figures 12–14. Female T. stelmioides. 12. Lateral view. 13. Posterior region of tail, ventral view. 14. Egg in two-celled stage.

Abbreviations used in Figures 2-14: AO, anteriory ovary; C, coelomocyte; CE, cuticular extension; d, dorsal esophageal plate; DE, deirid; DGN, dorsal esophageal gland nucleus; dl, dorsolateral esophageal plate; EP, excretory pore; G, gubernaculum; H, hemizonid; I, intestine; IV, intestinal valve; LR, longitudinal rod; NR, nerve ring; OE, esophagus; P, phasmid; PO, posterior ovary; PS, preanal sucker; SP, spicule; V, vulva; v, ventral esophageal plate; vl, ventrolateral esophageal plate.

	M a (2)	le 0)	Fema (20	le)
	Mean \pm S.D.	Range	Mean \pm S.D.	Range
Length (mm) Maximum width Width head L. pseudobuccal capsule Nerve ring† % body length Hemizonid† Deirids-left† Deirids-left† Deirids-right† Excretory pore† % body length D. es. gland nucleus† L. esophagus % body length Anus (mm)† Tail % body length Terminal spine Post, end seminal ves. (mm)† L. testis (mm) Preanal sucker (mm)† Spicules Gubermaculum	$\begin{array}{c} 10.2\pm1.2\\ 310\pm37\\ 182\pm20\\ 229\pm23\\ 370\pm40\\ 4\pm0.5\\ 421\pm40\\ 570\pm49\\ 560\pm52\\ 573\pm50\\ 6\pm0.5\\ 934\pm80\\ 970\pm80\\ 10\pm0.9\\ 97\pm1.2\\ 287\pm31\\ 97\pm0.6\\ 7\pm1\\ 6.3\pm0.8\\ 10.0\pm1.7\\ 8.8\pm1.1\\ 502\pm48\\ 87\pm19 \end{array}$	$\begin{array}{c} 8.1 \\ -12.9 \\ 260 \\ -367 \\ 160 \\ -235 \\ 189 \\ -260 \\ 324 \\ -465 \\ 3-4 \\ 368 \\ -528 \\ 455 \\ -670 \\ 465 \\ -665 \\ 465 \\ -650 \\ 5-6 \\ 810 \\ -1,120 \\ 861 \\ -1,120 \\ 861 \\ -1,150 \\ 9-11 \\ 7.9 \\ -12.6 \\ 237 \\ -367 \\ 95 \\ -98 \\ 4-10 \\ 4.9 \\ -7.6 \\ 7.5 \\ -13.9 \\ 7.1 \\ -11.3 \\ 382 \\ -650 \\ 7.4 \\ -104 \end{array}$	$\begin{array}{c} 11.6 \pm 1.4 \\ 336 \pm 84 \\ 180 \pm 14 \\ 245 \pm 20 \\ 397 \pm 32 \\ 3 \pm 0.5 \\ 467 \pm 28 \\ 586 \pm 47 \\ 589 \pm 42 \\ 597 \pm 43 \\ 5 \pm 0.6 \\ 1,010 \pm 70 \\ 1,040 \pm 70 \\ 1,040 \pm 70 \\ 9 \pm 0.7 \\ 11.2 \pm 1.4 \\ 382 \pm 66 \\ 97 \pm 0.7 \\ 8 \pm 2 \end{array}$	$\begin{array}{c} 9.5{-}14.3\\ 256{-}437\\ 158{-}220\\ 218{-}300\\ 350{-}470\\ 3{-}4\\ 430{-}523\\ 530{-}695\\ 530{-}695\\ 530{-}695\\ 4{-}6\\ 850{-}1,150\\ 937{-}1,200\\ 9{-}1,20\\ 9{-}500\\ 9{-}500\\ 9{-}50\\$
Vulva (mm) [†] End ant. ovary (mm) [†] End post. ovary (mm) [†] Phasmids to tail tip			7.2 ± 0.8 305 ± 70 $1,091 \pm 142$ 161 ± 30	$\begin{array}{r} 6.4-8.9\\ 206-474\\ 885-1,365\\ 85-192\end{array}$

Table 1. Dimensions of adult Truttaedacnitis stelmioides (Vessichelli, 1910) from Lampetra lamottenii (Lesueur) in Ontario.*

* All measurements in micrometers unless indicated otherwise. + Distance from anterior extremity.

expanding into uteri. These join, forming single uterus leading into muscular vagina. Vulva located behind midbody; anterior lip larger than posterior. Uterus filled with ovoid, relatively thin-shelled eggs in various stages of early cleavage. Eggs near vagina often in morula stage; length 63–87 μ m, width 51–68 μ m. Left midbody papilla anterior to vulva; right papilla posterior to vulva. Tail bearing one pair lateral, papilliform phasmids.

Discussion

Petter (1974) proposed the genus Truttaedacnitis for cucullanids in which the anterior extremity was markedly inclined dorsally and included the following species in the genus: T. stelmioides (which she noted was insufficiently described), T. heterodonti, T. squali, T. clitellarius, T. lebedeva, T. sibiricus, T. sphaerocephala, T. truttae, T. ampullastoma, T. scotti, T. laevis, and T. australis. She was apparently unaware of T. alpinus (Mudry and McCart, 1974) n. comb. (=Bulbodacnitis alpinus), the types of which were examined in the present study. Truttaedacnitis stelmioides most closely resembles T. alpinus of arctic char (Salvelinus alpinus) but can be distinguished by its funnelshaped pseudobuccal capsule, well-developed oral collarette with a dentigerous ridge and lateral alae. In addition, T. alpinus lacks the conspicuous nucleus of the dorsal esophageal gland and the terminal spine found in T. stelmioides.

Zekhnov (1956) and Shul'man (1957) were unable to distinguish females of T. stelmioides from those of T. truttae (Fabricius, 1794) but males of the former could be distinguished by their unequal spicules, larger gubernaculum and somewhat shorter tail. Berland (1970) and Mudry and McCart (1974) gave more detailed descriptions of the morphology of T. truttae. Unlike T. truttae, T. stelmioides lacks a cephalic dorsal ridge and a marked swelling of the body anterior to the nerve ring and has lateral alae. In T. truttae the excretory pore is always behind the deirids (Berland, 1970; Mudry and McCart, 1974). The positions of the excretory pore and deirids relative to each other are variable in T. stelmioides but generally the excretory pore is at the same level or anterior to the deirids.

Truttaedacnitis stelmioides is apparently the only cucullanid presently known from Petromyzoniformes. Moravec (1976) described cucullanid larvae from an ammocoete of L. *planeri* as T. *truttae* but these may have been T. *stelmioides*.

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Centenary of Academician

K. I. Skrjabin

In December 1978 the Soviet Union and other countries will celebrate the centenary of the distinguished Soviet helminthologist Academician K. I. Skrjabin who died just six years ago. The Proceedings will mark this occasion by featuring a special article in the January 1979 issue.—Ed.

On Narsingiella narsingi, a New Genus and Species of Aspidoderid Nematode from Bufo viridis Found in Berhampur, India

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Narsingiella narsingi n. g., n. sp.

On one occasion the writer collected specimens of an aspidoderid nematode which, on detailed study, proved to belong to a new species and a new genus. The material studied consists of 15 males and 20 females recovered from the intestine of the green toad, Bufo viridis Laurenti, procured from Berhampur. The body of the worm is cylindrical with rounded anterior ends and conical tails in both sexes. The mouth is surrounded by three lips which bear two pairs of submedian papillae and a pair of laterally situated amphids arranged as in the typical oxyuroid worms with three cephalic lips. The head bears cervical cordons characteristic of worms belonging to the Aspidoderidae Freitas, 1956. The cordons, which are continuous, run parallel to the anterior borders of the lips following a sinuous course, while they form loops between the adjacent lips; they are also armed with spines. Circular rows of spines also occur on the cuticle between the striae present on the body, but are discernible only under high magnification. Projecting from the inner surface of the lips are found three distinct onchia as illustrated in Fig. 3. The mouth opens into a small somewhat swollen pharynx. The esophagus which follows consists of a long cylindrical portion with a terminal valvate bulb preceded by a small pyriform second bulb continuous with the esophageal corpus. The following measurements are all in millimeters and the mean value is in parenthesis.

MALE: The males are smaller than the females and measure 3.15-5.38 (4.45) in length and 0.22-0.28 (0.24) in maximum thickness. The esophagus, including the two bulbs, is 0.65-0.85 (0.75) long, its total length occupying $\frac{1}{6}$ to $\frac{1}{5}$ of the body length. The excretory pore is 0.34-0.47 (0.41) from the head end. The tail is short and conical, measuring 0.16-0.25(0.23) in length; it forms roughly $\frac{1}{20}$ of the body length. A precloacal ventral sucker is present close to the cloacal opening. Welldeveloped caudal alae are present which originate slightly anteriorly to the sucker and extend down the tail, enclosing its tip. There are 12 pairs of caudal papillae, of which six are preanal and six postanal. The first three pairs of preanals, which are pedunculate, are situated laterally to the sucker; the next three pairs are sessile, lying between the sucker and the cloacal opening. The first pair of postanal papillae are pedunculate and stand out prominently, projecting into the alae. The next two pairs lie close behind the cloaca, while the last three pairs are arranged down the attenuated region of the tail as illustrated in Fig. 5. An additional minute pair of papillae is found between the last pair of postanal papillae as shown in the figure. The spicules are equal and similar, measuring 0.30-0.52 (0.44) in length. A gubernaculum is present which is 0.05-0.08 (0.065) long and 0.035-0.05 (0.043) wide; its hind border is marked by a cleft.

FEMALE: The females, which are slightly larger in size than the males, measure 4.14–6.56 (5.03) in length and 0.25-0.40 (0.30) in maximum width. The esophagus, including its two bulbs, has a length of 0.83-1.00(0.91); it constitutes about 1/6 of the body length. The excretory pore is 0.45-0.60 (0.49) from the head end. The tail tapers evenly to a pointed tip; it is 0.30-0.36 (0.33) long, occupying roughly 1/6 of the body length. The vulva, situated anterior to the middle of the body, is 2.40-4.25 (2.90) from the tail end; its position divides the body in the ratio of 1: 1.4-1: 1.8. The eggs are 0.055–0.074 (0.058) long and 0.03-0.05 (0.04) wide; those contained in the vagina are embryonated.

Discussion of the systematic position

The heterakoid nematode described above belongs to the family Aspidoderidae Freitas,



0.2 mm.

Figures 1-8. Narsingiella narsingi n. g., n. sp. 1. Anterior end, female, lateral view. 2. Head end, ventral view. 3. Head end-on view showing lips, cephalic papillae, amphids, onchia and first anterior row of circularly disposed spines and cordons. 4. Terminal portion of male tail. 5. Posterior end, male, ventral view. 6. Posterior end, female, lateral view. 7. Gubernaculum. 8. Embryonated egg.

1956, since it possesses cervical cordons and the male has the characteristic precloacal sucker. As revealed by the presence of cordons and absence of cuticular plaques, it shows close affinities to the subfamily Spinaspidoderinae Freitas, 1956, members of which are parasites of birds. Skrjabin and Schikhobalova raised the genus Spinaspidodera in 1947 for the species Pseudaspidodera spinosa Maplestone, 1932. The worm under discussion being parasitic in an amphibian host is excluded from this subfamily because of the presence of two esophageal bulbs in both sexes and the presence, in the male, of two equal spicules. These are features which it shares with the worms belonging to the genus Cheloniheterakis Yamaguti, 1961. The writer feels it necessary to create not only a new genus and a new species, but also a new subfamily for the accommodation of the newly discovered worm. It is therefore proposed to name the new genus Narsingiella n. g., with the new species Narsingiella narsingi n. sp. as its type. The new genus, parasitic in the intestine of Bufo viridis, is proposed to be the type of the new subfamily Narsingiellinae. The genus Cheloniheterakis Yamaguti, 1961 is transferred to this subfamily owing to its possession of cordons-figured though not mentioned-and also because there are two bulbs in its esophagus.

Diagnostic features of the new subfamily Narsingiellinae

Aspidoderidae possessing cervical cordons without plaques; esophagus with 2 esophageal

bulbs; males having 2 equal spicules. Parasites of amphibians and reptiles.

Diagnostic Key to the Genera of Narsingiellinae

- 1. With gubernaculum and parasites of amphibians Narsingiella n. g.

The type-species of the new genus Narsingiella parasitic in a toad.

HOST: Bufo viridis.

HABITAT: Intestine.

LOCALITY: Berhampur, India.

Type specimens are deposited in the Museum of the Department of Zoology, Osmania University, Hyderabad, India.

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Parasite Fauna of Splake (Salvelinus fontinalis \times S. namaycush)¹

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ABSTRACT: During 1962–74, recaptured splake from plantings in Lake Huron were parasitized by 21 species, with an incidence of infection of 92.7%. The predominant parasites were the acanthocephalans *Metechinorhynchus salmonis*, *Acanthocephalus jacksoni*, and *Pomphorhynchus bulbocolli* (in 56.8, 5.7 and 5.7% of the specimens, respectively); the trematodes *Diplostomulum flexicaudum* and *Tetracotyle intermedia* (9.9 and 5.7%, respectively) and the cestode *Eubothrium salvelini* (9.9%). The only heavy infection occurred with *Metechinorhynchus salmonis* (more than 50 parasites per host). No mortalities of splake were recorded attributable to the effects of parasites in Lake Huron.

A program to reestablish trout populations of Lake Huron following the decimation of lake trout (Salvelinus namaycush) by the sea lamprey (Petromyzon marinus) (Fry, 1953; Eschmeyer, 1957; Lawrie, 1970) has involved the introduction of splake into Lake Huron and Georgian Bay by the Province of Ontario (Budd, 1957, Berst and Spangler, 1972). Studies on the population dynamics of the planted hybrids in Lake Huron and Georgian Bay (Berst and Spangler, 1970; Berst and Payne, 1974; Spangler and Berst, 1976) provided the opportunity to study the parasites acquired by splake. Accumulation of information on the host-parasite relationship is required as an aid in understanding the biotic factors in the biology of the planted trout in the lake (Polyanski, 1957; Margolis, 1960; Pippy, 1969; Hoffman and Bauer, 1971).

Methods

During this study (1962–74), 220 adult splake of various age groups were examined for external and internal parasites (except for blood protozoans). Splake were captured by the Lake Huron Research Unit primarily from experimental gill nets and pound nets fished in South Bay. Additional specimens were obtained by sampling commercial catches from the North Channel, Burnt Island Bay, Providence Bay and southern Georgian Bay. Splake were examined fresh or after freezing. Standard procedures were used to examine the fish for parasites and to classify the incidence and intensities of the infections encountered (Dechtiar, 1972). All parasites from this study have been retained in the authors' collection at the Fish and Wildlife Research Branch, Ontario Ministry of Natural Resources, Maple, Ontario.

Parasites Found and Biological Implications

Splake were infected by parasites of six groups (Table 1). No protozoans were found. Wounds attributed to sea lamprey were present on 2.3% of the splake examined compared with 8% of the splake specimens examined by Berst and Spangler (1970). Intensity of infection by parasites and, where available, information on life cycles of the parasites, are given below.

The monogenean, Discocotyle sagittata (syn. D. salmonis), was found on splake with a low incidence and intensity of infection on the gills. This parasite was reported from coregonines (bloater, Coregonus hoyi; lake herring, Coregonus artedi; and round whitefish, Prosopium cylindraceium) by Bangham (1955) and from lake whitefish (Coregonus clupeaformis) by Dechtiar (unpublished data). It is considered to be a typical oligotrophic form of parasite fauna (Wisniewski, 1958; Chubb, 1963).

Larval and adult stages of three species of digenetic trematodes parasitized the splake: Diplostomum flexicaudum, Tetracotyle intermedia and Crepidostomum farionis; metacercariae of Diplostomulum flexicaudum oc-

¹Contribution No. 77-16 of the Fish and Wildlife Research Branch, Ontario Ministry of Natural Resources, Box 50, Maple, Ontario L0J 1E0.

	No. of fish	% fish infected	Infection intensity†	Organs infected	Intermediate hosts
Fungi					
Saprolegnia sp.	4	1.8	L, M	skin, gills	none
Monogenea					
Discocotyle sagittata	2	0.9	L	gills	none
Digenea					
*Tetracotylc intermedia *Diplostomulum flexicaudum Crepidostomum farionis	$\begin{smallmatrix} 11\\ 20\\ 6\end{smallmatrix}$	5.7 9.9 2.7	L, M L, M L, M	heart, mesenteries eye intestine	gastropods gastropods gastropods and mayflies
Cestoda					
*Triaenophorus nodulosus *Proteocephalus sp. *Diphyllobothrium sp. Eubothrium saloelini Cyathocephalus truncatus	$\begin{array}{c}2\\6\\4\\19\\3\end{array}$	$0.9 \\ 2.7 \\ 1.8 \\ 9.9 \\ 1.4$	L L, M L, M L, H	liver intestine pyloric caeca intestine pyloric caeca	copepods copepods copepods copepods amphipods
Nematoda					
Rhabdochona sp. Capillaria salvelini Contracaecum brachyurum Spinitectus gracilis Cystidicola stigmatura	5 3 7 5 8	$2.3 \\ 1.4 \\ 3.2 \\ 2.3 \\ 3.6$	L L, M L, M L, M	intestine intestine intestine intestine swimbladder	mayfly nymphs mayfly nymphs copepods mayfly nymphs
Acanthocepl	nala				
Metechinorhynchus salmonis Acanthocephalus jacksoni Neoechinorhynchus tumidus Pomphorhynchus bulbocolli	$125 \\ 12 \\ 8 \\ 12$	$56.8 \\ 5.7 \\ 3.6 \\ 5.7$	L, M L L	intestine intestine intestine intestine	amphipods isopods ostracods amphipods
Arthropoda					
Ergasilus caerulus	5	2.3	L	gills	none

Table 1.	Incidence	e of parasi	tes (%,	intensity	of i	nfection,	organs	infected,	and	intermediate	hosts)	in	220
splake fr	om Lake	Huron du	ring 19	62-74.									

* References to these parasites in table and text are to larval stage. + L (light) represents 1–9 parasites per host, M (medium), 10–49 and H (heavy), 50 or more.

curred predominantly in the external layers of the eyes and less in the vitreous body of the eyes of 20 splake. Incidence of infection was 9.9% and intensity from light to medium. This parasite may cause a worm cataract of the eye, and blindness. Metacercariae of Tetracotyle intermedia occurred on the surface of the heart and in the pericardium and infrequently on the surface of the liver. Incidence of infection was 5.7% and intensity from light to medium. An intensive infection by this parasite may cause mass mortality of fish (Petrushevski and Kogteva, 1954).

In the adult stage only one species of digenean Crepidostomum farionis infected the splake. This trematode is cosmopolitan; it was recorded in Europe, U.S.A. and Canada (Cooper, 1915; Brown, 1927; Sandeman and Pippy, 1967; Bangham and Adams, 1954). This parasite was found in the pyloric region

of the intestine of the fish, with incidence of infection 2.8% and intensity ranging from light to medium. Splake acquire infection by ingestion of nymphs of mayflies (Ephemeroptera spp.) that serve as the second intermediate host and carry the stages which mature in fish. First intermediate hosts are snails of the genera Pisidium and Sphaerium. An intensive infection of this parasite may cause mortality of the fish (Wales, 1958).

Cestodes parasitizing splake, were as follows: Triaenophorus nodulosus, Proteocephalus sp., Diphyllobothrium sp., Eubothrium salvelini and Cyathocephalus truncatus. The first three species were plerocercoid larvae or sexually immature forms, the last two, adult. Infections by the parasites above were acquired by ingestion cyclopid and diaptomid copepods and of amphipods. All tapeworms occurred in splake with a low intensity and incidence of infection

Parasites of splake	Lake Whitefish (90)†‡	Rainbow smelt (328)	Yellow perch (134)	White sucker (147)	Northern sucker (62)	Bloater (59)	Cisco (101)	Alewife (93)	No. of species parasitized
Saprolegnia sp. Discocotyle sugittata Petracotyle intermedia Diplostomulum flexicaudum Crepidostomum farionis Triaenophorus nodulosus Proteocephalus sp. Diphyllobothrium sp. Eubothrium saloelini Cyathocephalus truncatus Rhabdochona	+++++++++++++++++++++++++++++++++++++++	+ + + +	+++++++++++++++++++++++++++++++++++++++	+	+ + +	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+ +	6 3 3 8 0 2 5 3 1 5 1
Contracaecum brachyurum Spinitectus gracilis Cystidicola stigmatura Metechinorhynchus salmonis Acanthocephalus jacksoni Neoechinorhynchus tumidus *Pomphorhynchus bulbocolli Ergasilus caeruleus	+ + +	+ + + +	+ + +	+ + +	+	+ + +	+ +	+ +	0 3 4 7 1 2 3 3 3
Total	9	0	10	4	4	10	10	4	

Table 2. Occurrence of parasites of splake and major associated fish in Lake Huron 1961-74.

* References to these parasites in table and text are to larval stage.
† The scientific names from left to right are: Coregonus clupeaformis, Osmerus mordax, Percu flavescens, Catostomus commersoni, Catostomus catostomus, Coregonus hoyi, Coregonus artedi, Alosa pseudoharengus.
‡ Numerals are numbers examined.

with the exception of Eubothrium salvelini. Incidence of infection in the latter was 9.9% and intensity ranged from light to medium. This parasite may cause damage to young salmonid fishes (Smith and Margolis, 1970). Vik (1954, 1958) reported that Cyathocephalus truncatus caused serious damage to salmonid fishes and also showed experimentally that only a few worms were needed to cause severe inflammation and ruptures of the gut wall, leading to death. Plerocercoids of Triaenophorus nodulosis occur in cysts in the liver of fish, causing tissue damage (Lawler, 1969) and may cause mortality (Matthey, 1963). The larval stage of *Diphyllobothrium* sp. may cause epizootics among trout (Duguid and Shepard, 1944). Hoffman and Dunbar (1961) reported mortality of Salvelinus fontinalis (U.S.A.) caused by Diphyllobothrium sp. Vik (1965) considered that the plerocercoids of this species caused a major decline of Salmo trutta and Salvelinus alpinus in Norway.

Acanthocephalans of four genera occurred in Metechinorhynchus salmonis (syn. splake. Echinorhynchus salmonis) was the most common parasite in the splake with highest incidence of infection (56.8%). Intensity of infections ranged from medium to extremely heavy. About 50% of the infected splake had

a heavy infection, about 20% had extremely heavy (200–500 parasites per fish), and the rest of the infected fish had a medium infection. In most cases the parasites occurred in the posterior part of the intestine, where pronounced hyperemia laceration and inflammation were observed (Bauer, 1953). According to DeGuisti and Budd (1959), Brownell (1970), and Hoffman (1967) the intermediate host for this parasite is *Pontoporeia affinis*. The splake acquires infection by ingestion of Pontoporeia affinis and fishes (rainbow smelt, *Osmerus mordax*; lake herring) which carry the parasite (Hnath, 1968). This acanthocephalan is detrimental to the nutrition of the host and can cause a lower rate of growth (Petrushevski and Kogteva 1954).

second species of acanthocephalan, A Acanthocephalus jacksoni, with incidence of infection 5.7% in the splake may cause thickening of the lamina propria, destruction of the epithelium at the point of attachment, and even mortality of the host (Bullock, 1963).

The nematodes (roundworms) occurring in splake belong to five genera: Cystidicola stigmatura, Rhabdochona sp., Capillaria salvelini, Contracaecum brachyurum, and Spini*tectus gracilis*. All parasites occurred with low incidence and intensity of infection. Two of them, Cystidicola stigmatura and Contracaecum brachyurum, are more frequent, with incidence of infection of 3.6% and 3.2%, respectively. Cystidicola stigmatura were found in the swimbladder of splake. Little is known about the life history and pathology of this parasite. Mamayev (1971) found that Pontoporeia affinis and Anisogammarus sp. were intermediate hosts for this parasite in Europe and far eastern USSR, respectively. It is likely that Pontoporeia and Gammarus are intermediate hosts in Canada.

The adult stage of Contracaecum brachyurum is potentially dangerous; it lives in the stomach and intestine of the splake and a wide range of fishes (smallmouth bass, Micropterus dolomieui; burbot, Lota lota; and northern pike, Esox lucius). The life cycle of this parasite is unknown and its assignment to the genus Contracaecum is provisional. Salmonids and other fishes have been shown to acquire infections of Rhabdochona sp. and Spinitectus gracilis by ingestion of mayfly nymphs (Ephemeroptera) (Gustafson, 1942).

It is generally accepted that the salmonid community of coldwater fish is characteristic of oligotrophic lakes in Europe and North America (Colby et al., 1972; Numann, 1972). Lake Huron proper may be placed in an oligotrophic category (Beeton, 1965), but considerable eutrophication has occurred in bays, estuaries and streams (Berst and Spangler, 1972). The presence of parasite species on or in fishes including splake are determined by ecological conditions which influence the possibility of infection of fish and intermediate hosts such as molluscs, ephemeropter, crustaceans, and the specificity of parasites, in particular the monogeneans (most of them are adapted to one host species or a few related fish species). Digenea, Cestoda, Acanthocephala and Nematoda do not seem to be narrowly specific; they can live in a wide range of fish hosts. Of the 21 species of parasites found in splake, 10 of them may be regarded as typical oligotrophic forms. These are: Discocotyle sagittata, Tetracotyle intermedia, Crepidostomum farionis, Diphyllobothrium sp., Eubothrium salvelini, Cyathocephalus truncatus, Capillaria salvelini, Cystidicola stigmatura, Neoechinorhynchus tumidus and Metechinorhynchus salmonis. All other parasites which were found in the splake such as Diplostomulum flexicaudum, Triaenophorus nodulosus, Contracaecum brachyurum, Spinigracilis. tectus Acanthocephalus jacksoni, Pomphorhynchus bulbocolli and Ergasilus *caeruleus* have been recorded from salmonid and warm-water fishes. These seven species of parasites may occur in fish in all types of lakes, unless the absence of the intermediate hosts in the environment is a limiting factor. The most typical eutrophic forms of the parasite fauna of the splake are Diplostomulum flexicaudum, Triaenophorus nodulosus, Acanthocephalus jacksoni, Pomphorhynchus bulbocolli and Ergasilus caeruleus. According to the parasitological data above, Lake Huron still has an abundant population of Pontoporeia affinis, Gammarus spp., Cyclops spp. (C. bicuspidatus, C. vernalis) Diaptomus spp., and Hexagenia limbata, all of which appear to be suitable as food for the splake.

Most of the parasite species found in the splake were also found in one or more species of fish associated with the splake (Table 2). The common parasites of salmonids such as *Triaenophorus crassus* (larval stage in the muscle); *Proteocephalus exiguus*, *P. laruei* (intestine); *Salmonicola* spp., and *Tetraonchus variabilis* (gills) (Bangham, 1955; Dechtiar, unpublished data) were not found in or on the splake.

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Obituary Notice

Jessie R. Christie

Sept. 17, 1889-April 21, 1978

Member since 1922 Recording Secretary 1926–1927 President 1930–1931 Editor 1934–1947 Life Member 1956
Research Note

A Report of Polymorphus paradoxus (Acanthocephala) in Microtus pennsylvanicus from Hastings Lake, Alberta (Canada)

Twelve specimens of the genus Polymorphus Lühe, 1911 identified as P. paradoxus Connell and Corner, 1957 were found at necropsy in three of 29 Microtus pennsylvanicus trapped at Hastings Lake, Alberta from May through July 1974. Five worms, including specimens from each of the three hosts, were encysted in the mesentaries and could not be identified at the specific level. The remaining specimens $(2 \delta \delta, 2 9 9$ and three recently excysted cystacanths) were found in the small intestine of a single animal. The testes and cement glands of the males were fully formed but sperm were not evident in the seminal vesicle. The female specimens were robust. One had an intact ovary, while the second female contained a large number of ovarian balls.

Identification was made on the basis of the number of hooks per row (8-9), the size of the largest hook ($65-81 \mu m$), and the number of hook rows (16-18), as well as the structure of the musculature of the cystacanth (Denny, 1969, Parasitology 59: 795-827). Additional measurements of the males were smaller than those given in the original description (Connell and Corner, 1957, Can. J. Zool. 35: 525–533). This may be the result of immaturity, being in an abnormal host, or a combination of both factors. This is the first report of a natural infection of a terrestrial mammal with an acanthocephalan of the genus Polymorphus. A male, female, and cystacanth have been deposited in the USNM Helminth Collection (No. 73131).

Polymorphus contains approximately 30 species, almost all reported exclusively from avian hosts (Schmidt, 1965, J. Parasitol. 51: 809–813). Three species have been reported from mammals. Connell and Corner (loc. cit.) originally described *P. paradoxus* from beaver (*Castor canadensis*) and muskrat (*Ondatra zibethicus*) in Alberta. Polymorphus magnus has been reported from muskrats in the Soviet Union on two occasions (Lavrent'ev, 1957, Tezisy Dokl. Nauchn. Konf. Vses. Obsh. Gel'- mintol., Chast 1: 169–170; Gubanov, 1964, Gel'minthofauna promysolvykh mlekopitayuschikh Yakutii, Izdvo AN-SSSR). Mituch (1964, Stud. Helminthol. 1: 83–100) reported *P. minutus* from the small intestine of the water shrew (*Neomys fodiens*) in Czechoslovakia. This species has also been reported from the muskrat in the Soviet Union (Lavroff, 1953, Vnutrenniye i naruzhnye parazity ondatry, Vol. XII, 132). Unidentified species of *Polymorphus* have been reported from muskrats in British Columbia (Knight, 1951, Can. J. Zool. 29: 188–214) and Alaska (Van Cleave, 1953, Ill. Biol. Monogr. 23, 179 p.).

Microtus pennsylvanicus probably becomes infected with *P. paradoxus* by ingesting amphipods (Gammarus lacustris) harboring fully developed cystacanths, which are particularly abundant during the spring at Hastings Lake (Denny, 1967, unpubl. Ph.D. thesis, Univ. of Alberta). Above average snowfall during the winter of 1973-74 and heavy rains during the spring resulted in flooding of a large number of rodent burrows which may have resulted in displacement and subsequent feeding stress in the rodent population. These factors, combined with the altered behavior of G. lacustris infected with *P. paradoxus*, as described by Bethel and Holmes (1973, J. Parasitol. 59: 945-956), would result in the necessary overlap of the parasite and host feeding range required for the successful transmission of the parasite (Holmes and Bethel, 1972, J. Linn. Soc. London, Suppl. No. 1, Zool. 51: 123-149). Microtus pennsylvanicus cannot be considered a primary host of P. paradoxus; however, under unusual conditions, this and other terrestrial rodents may become infected with this acanthocephalan.

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Research Note

Determination of Dorsal and Ventral Surfaces in Histological Preparations of Archiacanthocephala

Anyone that has tried to determine the dorsal and ventral surface in Acanthocephala has surely wished that these worms possessed some superficial structure on which they could key. Unfortunately, external criteria are nonexistent in many species and restricted to hook patterns or body curvature in others. In addition, Rauther (1930, Handbuch der Zoologie, 2. Band, 1. Hälfte, p. 452) stated that the genital opening in both sexes should be regarded as occurring on the ventral surface. These latter characteristics may distinguish surfaces in whole worms but are of little use in histological preparations. Worms that have no recognizable surface features that provide a clue to orientation must be examined internally. Hyman (1951, The Invertebrates, Vol. III, McGraw-Hill, N.Y.) suggested that the position of the cerebral ganglion be used for determining dorsoventrality. This ganglion is presumed to always be located along the ventral wall of the proboscis receptacle. Regrettably, the cerebral ganglion is not visible through the praesomal musculature in many species and therefore must be located by careful dissection or via histological methods. This is an added burden to that investigator who is only interested in components other than the ganglion. Additional structures such as the main lacunar channels have been described as located in the tegument (T) dorsally and ventrally in Archiacanthocephala and Eoacanthocephala and laterally in Palaeacanthocephala (Hyman, 1951, loc. cit.). Since these two large channels occur along the entire length of the body, they can be used for general orientation in histological preparations. However, they cannot be used to separate the dorsal and ventral surface. The difference in size between these two channels has been suggested as a means of separating these two surfaces. Indeed, some difference in size of the two channels may occasionally be observed, but in our experience this has not been a consistent feature. Components not associated with the body wall are also unusable because of their restriction to specific areas or regions of the worm or because they may change position following muscle contraction.

We suggest that the medial longitudinal channels (MLC) (Miller and Dunagan, 1976, Proc. Helminthol. Soc. Wash. 43: 99-106) be used for orientation. They are easily observed (Fig. 1) but histologically complex internal structures which are located closer to the dorsal lacunar channel (DLC) than to the ventral lacunar channel. As shown (Fig. 1), the label points to the lumen of the MLC. The lateral posterior nerves (LPN) are between the MLC and longitudinal muscles (LM) and not in the lumen of the MLC. The LPN are overly emphasized in Figure 1 to illustrate how they may be used to help identify the MLC. Indeed, the LPN could also be used to identify worm surfaces, except that they are not always



Figure 1. Illustration of a cross section through the region of the bursal muscle of a male Moniliformis moniliformis. Note the position of the medial longitudinal channels (MLC) in the dorsal half of the body wall. BD, bursal depressor muscle; CM, circular muscle; DLC, dorsal lacunar channel; GG, genital ganglion; LM, longitudinal muscle; LPN, lateral posterior nerve; P, protrusor muscle; T, tegument.

readily observed. If there is any question as to whether one is observing the MLC or a tubular longitudinal muscle, this can be determined by the presence of the LPN associated with the MLC and the thicker wall of the muscles compared to the thin walled MLC. The LM may vary in size and those LM associated with the MLC and LPN are frequently much larger than others at the level shown in Figure 1. Only these larger LM are depicted as patent, whereas

all LM are believed to be tubular muscles (Miller and Dunagan, 1977, Proc. Helminthol. Soc. Wash. 44: 201–205). Thus the investigator has a marker available in any cross section of the worm other than the praesoma.

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Research Note

Aspidogaster conchicola in St. Croix River, Wisconsin Clams

Aspidogaster conchicola Von Baer, 1827 parasitizes North American and European unionid clams. Its distribution in North America was summarized by Hendrix (1968, J. Parasitol. 54: 179–180), and it was also reported from Columbia River unionids by Pauley and Becker (1968, J. Parasitol. 54: 917–920). In North American studies, A. conchicola para-

					Loc	ation				
	Presco (Piero	ett, Wis. če Co.)	Hudso (St. C	on, Wis. roix Co.)	St. Croix (Poll	Falls, Wis. k Co.)	8 k Grantsb (Burn	m W urg, Wis. ett Co.)	Gordo (Doug	n, Wis. las Co.)
Host	No. ex.	No. inf. (%)	No. ex.	No. inf. (%)	No. ex.	No. inf. (%)	No. ex.	No. inf. (%)	No. ex.	No. inf. (%)
Alasmodonta marginata Say							2	0		
Anodonta grandis Say A. imbecilis (Say)	2	1(50)	$\frac{4}{1}$	$3(75) \\ 1(100)$						
rariplicata (Lam.) Eliptio dilatatus (Baf.)	31 8	$\frac{1(3)}{1(12)}$	62	38(61)			14	1(7)		
Fusconaia ebenus (Lea) F. flava (Raf.)		- (,	2	2(100)		0			3	0
F. undata (Barnes) Lampsilis	18	1(6)	15	1(7)	2	0				
siliquoidea (Barnes)	11	0	8	5(62)			20	1(5)	26	0
<i>complanata</i> (Barnes) L. costata Raf.			1	0			11	0		
Leptodea fragilis Raf. L. laevissma (Lea)	1	0	$\frac{1}{1}$	0 1(100)					7	0
Ligumia recta Lam. Obliquaria reflexa Raf. Protera alata (Say)	2	0	$\frac{1}{5}$	$ 0 \\ 5(100) $					1	0
Quadrula pustulosa (Lea) Strophitis			3	2(67)	1	0	0	0		
Tritogonia verrucosa Baf.					2	1(50)	2	0		
Truncilla truncata Raf.			1	1(100)						
Totals	73	4(5)	105	59(56)	5	1(20)	49	2(4)	36	0

Table 1. Aspidogaster conchicola from St. Croix River Clams.*

* All new locality records.

sitized 40% of 1,610 unionids from Illinois, Iowa, and Pennsylvania (Kelly, 1899, Bull. Ill. State Lab. Nat. Hist. 5: 401–418), 46% of 26 Pacific Northwest unionids (Pauley and Becker, 1968 loc. cit.), 28% of 155 Texas unionids (Gentner and Hopkins, 1966, J. Parasitol. 52: 458–461), and 51% of 72 Tennessee River unionids (Hendrix, 1968, loc. cit.).

Two hundred and sixty-nine clams representing 20 species of the Unionidae from five locations along the St. Croix River (collection data are summarized in Table 1) were collected in July 1977 at depths of 0.2 to 3 m. *Aspidogaster conchicola* were fixed in hot formalin and stained in Mayer's paracarmine. Four specimens have been deposited in the USNM Helm. Coll. (No. 74295). In this study, the first account of A. conchicola from the upper Mississippi River drainage, 25% of 269 clams were parasitized, with from 1 to 19 ($\bar{x} = 2.3$) worms. The parasites were obtained from the pericardial and, occasionally, renal cavities. No pathology was observed. The highest percentage of infected clams (59%) (Table 1) was at Hudson, a lenticlike habitat. No clams were parasitized at Gordon, a lotic habitat with the fastest current (4–5 km/hr, determined by velocity of flow studies).

Appreciation is expressed to Dr. S. S. Hendrix, Gettysburg College, for supplying information about aspidogastrids.

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Research Note

Scanning Electron Microscopy of the Head Region of *Physaloptera felidis* Ackert, 1936

The use of scanning electron microscopy (SEM) has been utilized by numerous investigators to assess the cephalic arrangement and sensory structures of various nematodes (Madden et al., 1970, J. Parasitol. 56: 202–203; Ubelaker and Allison, 1972, 30th Annu. Proc. Electron Microsc. Soc. Am., Los Angeles, p. 392; Allison et al., 1973, Trans. Am. Microsc. Soc. 92: 291–297; Ansel et al., 1974, Int. J. Parasitol. 4: 17–23; Margolis et al., 1975, Can. J. Zool. 53: 960–966). For comparative purposes and because of the taxonomic importance of the cephalic structures on the classification of the Spiruroidea (Chitwood and Wehr, 1933, Z. Parasitenkd. 7: 273–335), the microtopographical features of the head region of *Physaloptera felidis* (Ackert, 1936, Trans. Am. Microsc. Soc. 55: 250–254), a stomach worm of cats and dogs, are presented.

Living adult specimens of *P. felidis* were collected from feral and domestic cats (*Felis domesticus*) in and around Provo, Utah. All

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Figures 1-6. Scanning electron micrographs of *Physaloptera felidis*, female. 1. Ventrolateral view of head and neck region showing three of the four cervical alae (A) and the excretory pore (*EP*). $\times 300$. 2. Frontal view of head with collarette (C), the two triangular pseudolabia (PL), and oral opening (O). $\times 640$. 3. *En face* view of head region with papillae (PA), ampliid (AM), and teeth (T) visible. Note the fissures (double arrows) on the internolateral surface of the pseudolabia. $\times 1,760$. 4. Four internolateral teeth. Two sublateral teeth (TS), one lateral (TL), and one externolateral tooth (TE). Note the porelike openings (arrows) below the two sublateral teeth and the small slitlike opening containing a septum (double arrows) off center and below the lateral tooth. $\times 6,240$. 5. Amplid showing external pore (P) and cuticular collar (CC). $\times 11,200$. 6. Fused compound papillae. $\times 8,060$.



specimens were fixed in alcohol-formalin-acetic acid (AFA) and stored in 70% ethanol for several months before use. Twelve specimens were prepared for SEM as described by Allison et al. (1972, J. Parasitol. 58: 414–415). The specimens were coated with gold-palladium (60: 40) in a vacuum evaporator and viewed with an ETEC autoscan scanning electron microscope at 10–20 Kv.

Scanning electron micrographs (Figs. 1–6) illustrate the characteristic features of the anterior end of P. felidis. The overall ventrolateral view shows the position of the excretory pore and four cervical alae which reflect partially over the lips, forming a large cephalic collarette (Fig. 1). The collarette is rectangular and resembles a suctoral apparatus probably adapted for attachment and adhesion to the stomach or duodenal lining of its host (Fig. 2). The cervical alae and collarette were not as prominent in some male and female specimens as others. Cuticular projections reported to be lining the depression between the lips and the collarette were not observed (Ackert, 1936, loc. cit.). Although not evident on the cuticle, transverse striations are seen on the inner surface of the collarette (Fig. 2).

The oral opening is surrounded by two triangular lateral pseudolabia (Figs. 2, 3). The inner face of the pseudolabia is somewhat pointed medially, and two fissures or clefts are reminiscent of the once unfused set of three lips (Fig. 3). Each pseudolabia bears an amphid and two completely fused compound papillae (Fig. 3). The pseudolabia are armed with four serrated, roughly pyramidal teeth on the internolateral surface (Fig. 3). The teeth are arranged as an internal group of three, consisting of a central lateral and two sublateral teeth and a prominent single externolateral tooth (Fig. 4). The two sublateral teeth of the internal group of three are larger and extend well above the central tooth. Also, two porelike openings of unknown function, presumed to be formed from the fusion of the lips, are seen below the two sublateral teeth (Fig. 4). A small slitlike opening containing a septum is seen off center and below the central tooth (Fig. 4). The single external tooth is more robust than the two sublateral teeth and extends above the central tooth (Figs. 3, 4). The large external tooth probably helps anchor

the worm, while the internal group of three function in a rasping manner. Because of the size of the central tooth and its position in relation to the other teeth, it appears that this tooth is less functional than the sublateral teeth.

The amphid with its external pore is situated on the externolateral surface of the pseudolabia, directly behind the large external tooth and medial to the papillae (Fig. 3). The amphid appears spherical to oblong and sits atop a paddle-shaped swelling of the cuticle (Figs. 3, 5). The amphid appears as intertwining folds and is surrounded by a thin cuticular collar (Fig. 5). Amphids in other nematodes have been assumed to be chemoreceptors because they open to the exterior via a pore (Bird, 1971, The Structure of Nematodes, Academic Press, N.Y.; Croll, 1970, The Behavior of Nematodes, E. Arnold Ltd., London). McLaren (1976, In Dawes (ed.), Advances in Parasitology, Academic Press, N.Y. 14: 195–265), however, emphasizes that nematode amphids are variable in form and size. Therefore, fine-structural studies of the amphidial neurons, cilia and associated glands are necessary to determine their functional type.

The papillae appear dome shaped with a rugose surface of cuticular folds (Fig. 6). No central pore was observed and the papillae arise from a depression on the surface of the pseudolabia (Fig. 6). Papillae which open to the external environment by a pore are considered to be more involved with chemoreception than with tactile reception (McLaren, 1976, loc. cit.). Therefore, the absence of a visible pore in the papillae is suggestive of a tactoreceptive function.

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Research Note

Improved Bioassay for the Female Sex Pheromone in *Pelodera strongyloides*

Stringfellow (1974, Proc. Helminthol. Soc. Wash. 41: 4–10; 1976, ibid. 43: 206–211) reported three pheromones produced by *Pelodera strongyloides*: alkaline pH, male and female sex pheromones. In the present studies those conclusions were further tested by an evaluation of the worms' behavior under different conditions. The results reported herein support the previous findings, present a simpler and faster bioassay for the female sex pheromone when live worms are used, and show attraction curves characteristic of receptor fatigue (habituation).

In the first experiment, the migrating male and female worms were placed in Falcon spacesaver petri plates at a constant distance under restrained conditions. The media were either nutrient agar only, nutrient agar with 0.1 M sodium phosphate buffer incorporated, nutrient agar with 0.001 M acetazolamide (Diamox) incorporated (all three at pH 6.8), or nutrient agar with the pH adjusted to 9.0 with 0.1 N NaOH. Each isolation apparatus was inoculated with 20 males, or 20 females, or 10 males: 10 females, or their associated, unidentified bacteria grown separately from the worms. A ring of 30 males or females, placed equidistantly 2 cm from each isolation apparatus, migrated freely for 6 hr. A + was recorded if a worm tagged an isolation apparatus. The worm was then removed from the petri plate. The data were tested with chi square at the 0.05 level of significance by a comparison of the experimental and theoretical (dotted lines in figures) values. The critical value for a 1-tailed test at 3 degrees of freedom is 7.81. If chi square exceeded this value, the null hypothesis (random migration) was rejected, and the alternative hypothesis (nonrandom migration) was accepted.

The purpose of the following test was to see whether the effects of the inhibitors on the worms were reversible. The isolated worms were exposed to the inhibitor for 12–24 hr on a petri plate, then inoculated into isolation apparatuses on nutrient agar petri plates (pH: 6.8). The worms were then surrounded with the freely migrating worms.

Figure 1A-J summarizes the data of the first experiment. Histograms A, C, E, G, and I present evidence that males were sensitive to an alkaline pH and that the females produced a sex pheromone. Possibly, males were sensitive to other products produced by the males. Adjacent males, mutually attracted to each other (Fig. 1A, E), except in the presence of a buffer or an elevated pH (Fig. 1C, I), caused the pattern of random migration by the male (Fig. 1A). If a buffer was used, males migrated significantly to the females (Fig. 1C), in contrast to their migration to the bacterial control. Presumably, the buffer eliminated the alkaline pH produced by the males, so that adjacent males were attracted to isolated females producing a sex pheromone. The test for the female sex pheromone, shown previously with different methods (Stringfellow 1974, ibid.), shortened the assay time fivefold. If the isolated worms were exposed to the buffer then reinoculated into isolation apparatuses on nutrient agar only, the random pattern of migration (Fig. 1E) was similar to that of Fig. 1A. The effects of the phosphate buffer on the isolated females apparently were reversible. Again, free males were attracted to each other rather than the isolated worms. The males migrated randomly (Fig. 1G) when acetazolamide, irreversibly bound to the female (unpublished data), was incorporated into the nutrient agar. Presumably, acetazolamide inhibited the female sex pheromone here as well as with the old assay (1976, ibid. 43: 206-211). A significant migration pattern (Fig. 1I) similar to that of Fig. 1C resulted if the pH of the nutrient agar was adjusted to 9. Apparently, the effects of masking on the migrating males produced patterns of migration similar to that of Figure 1C. In both cases, males found the females because the alkaline pH factor, eliminated either directly by the buffer or indirectly by masking, did not compete with the female sex pheromone. Female worms served



Figure 1A-J. Histograms: A. Males migrated randomly on nutrient agar only (pH 6.8) because adjacent males were attracted to eath other (chi square: 1.44). C. Males migrated significantly (chi square: 19.82) to females when assayed on nutrient agar plus 0.1 M phosphate buffer (pH 6.8). Presumably the buffer eliminated the alkaline pH produced by the males so that males were attracted to females producing a sex pheromone. E. Males migrated randomly on nutrient agar after isolated worms were exposed to phosphate buffer-nutrient agar then reinoculated onto nutrient agar. Effects of phosphate buffer on isolated worms (Fig. 1C) were reversible (chi square: 0). G. Males migrated randomly on nutrient agar plus 0.001 M acetazolamide and thus indicated that the female sex pheromone was in-

as useful negative controls (Fig. 1B, D, F, H, and J). Adjacent females placed around isolation apparatuses were not generally attracted to each other.

In the second experiment, the effects of distance on mating under unrestrained conditions were investigated. One pair of worms, a male and a virgin female and their associated, unidentified bacteria were placed on each 100 mm nutrient agar petri plate (pH 6.8). Fifty petri plates were run for each of the 9 intervals (0-80 mm)-450 for each test. The same experiment was repeated with 0.1 M phosphate buffer or 0.001 M acetazolamide incorporated into the nutrient agar (pH 6.8), or with the pH of the nutrient agar adjusted to 9.0. Adjacent petri plates were oriented opposite to randomize outside influences. The criterion of mating was offspring produced. A + wasscored if offspring were produced after incubation for 3 days. A - was scored if offspring were not produced and both male and female lived after 3 days. If the adults died before mating the score was unrecorded.

Figure 1K summarizes the results of the second experiment. The fact that fewer matings resulted with increased distance between the two sexes indicated that the attractant(s) were less concentrated with increasing distance from their sources. If only distance influenced mating, then the curves should have increased between 10 and 0 mm; instead, they decreased. These types of curves result when slowly adapting receptors (habituation) are fatigued. The following summarizes the data for each test: nutrient agar (NA)-73 (16%) matings and 102 (23%) nonmatings, with 39% scored data; nutrient agar and 0.1 M phosphate buffer (NA : B)—106 (24%) matings and 77 (17%) nonmatings, with 41% scored data; nutrient agar and 0.001 M acetazolamide (NA:D)-

235 (52%) matings and 33 (7%) nonmatings, with 60% scored data; nutrient agar at pH 9.0 (pH 9)-302 (67%) matings and 33 (7%) nonmatings, with 74% scored data. In general, mating increased when the worms were cultured on nutrient agar plus 0.1 M phosphate buffer (NA : B) or nutrient agar plus 0.001 M acetazolamide (NA:D). Mating was higher when alkaline pH (NA : B), or it and the female sex pheromone (NA:D) were inhibited than when all three pheromones could operate (NA). These data indicate that a male sex pheromone operated because phosphate buffer, acetazolamide, and elevated pH enhanced the ability of the two sexes to find each other under unrestrained conditions. These data also indicate that up to a point the more pheromones eliminated the more accurately the opposite sexes find one another. Unfortunately, no inhibitor for the male sex pheromone is known as yet, so no experiments were run with all three inhibited. Presumably, mating would drop precipitously. When the pH of the nutrient agar was 9, a very high number of matings as well as the total scored data resulted. These results may have been caused by masking as well as an excitation effect of alkaline pH on the male (Beroza, 1975, J. Chromatogr. Sci. 13: 314–321).

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hibited (chi square: 5.2). I. If pH of nutrient agar was 9, males migrated significantly (chi square: 10.68) to females. Presumably the alkaline pH factor was eliminated indirectly by the effects of masking on the migrating males. B, D, F, H, and J show patterns of random migration by females to males in parallel experiments. All experiments run at pH 6.8 except those for Figures I and J. K. Line Chart: Mating (+) and nonmating (-) of unrestrained male and female worms on nutrient agar (NA), nutrient agar plus 0.1 M phosphate buffer (NA : B), nutrient agar plus 0.001 M acetazolamide (NA : D) all at pH 6.8; and where pH of the nutrient agar was adjusted to 9 (pH 9). In general (1) Mating decreased with increased distance between the sexes; and (2) if mating was influenced only by distance, then the curves should have increased between 10 and 0 mm; instead, they decreased. Abbreviations: NA, nutrient agar; B, bacteria; D, acetazolamide (Diamox); M, Male; F, Female.

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Research Note

The Influence of Mating and a Protein Inhibitor on the Response of *Nippostrongylus brasiliensis* to Sex Pheromone

Our initial investigation of precopulatory pheromonal communication in *Nippostrongylus brasiliensis* revealed both male to female and female to male sexual attraction (Bone et al., 1977, J. Parasitol. 63: 364–367). However, quantitative differences were observed in the response of males vs. females to various pheromone dosage levels of the opposite sex, with fewer than 50% of the females responding to pheromone released by 100 males in a laboratory bioassay, whereas over 70% of the males responded to pheromone released by 100 females.

The only comparative study of virgin vs. mated nematodes concerned pheromone release by gravid or virgin female *Panagrellus silusiae* (Cheng and Samoiloff, 1971, Can. J. Zool. 49: 1443–1448). Therefore, further study of *N. brasiliensis* appeared warranted to quantify the locomotor responses of males and females to various pheromone dosages and also to examine the effect of mating on these responses. Additionally, the effects of the growth inhibitor actidione (cycloheximide) on female pheromone release and on the male response were tested since this compound blocked pheromone communication in *P. silusiae* (Cheng and Samoiloff, 1972, Can. J. Zool. 50: 333–336).

Quantitative bioassay procedures were conducted as previously reported (Bone et al., 1977, Exp. Parasitol. 42: 82–86). Zero, 10, 35, or 100 worms of one sex were used as pheromone sources. Each bioassay replicate utilized a single, responding worm of the opposite sex. Forty replicates were performed for the 0-, 10-, and 35-worm pheromone sources, while 20 replicates were obtained for the 100-worm source. Results were analyzed with linear regression, and the 0.05 probability level was considered statistically significant.

Pheromone-source helminths of both sexes were obtained at 5 days of age from sexually mixed infections and were at various levels of reproductive maturity, although most members of the population presumably had mated, based on our observations. Males and ovigerous females were selected from these heterosexual populations for usage as mated responders.

Virgin responders were obtained by establishing homosexual infections of 92-hour-old helminths in recipient mice through intraduodenal transfers. Thus, these responders were unmated when recollected for bioassay usage 24 hr later at 5 days of age.

The growth inhibitor actidione (Calbiochem, Inc.) was added to animal water bottles at 0.2 g/liter. Mice were given this solution ad lib. from 5 days postinfection to 7 days postinfection. Seven-day-old treated helminths were used for experimental purposes, while untreated worms served as controls. Pheromone sources of 35 treated or normal females were utilized to test the response of normal or treated responding males, according to our bioassay protocol.

Figure 1 indicates the mean distances traveled by mated and virgin male *N. brasiliensis* toward various dosages of female sex pheromone. Both mated ($F_{98}^2 = 3.66$) and virgin ($F_{98}^2 = 5.59$) males were significantly dosage dependent in their response. No statistically significant difference was found between the regression lines for these two male groups, indicating that male helminths respond equally to female sex pheromone by moving toward the pheromone source, regardless of their mating history.

The equal responses by virgin or mated males indicate that males are responsive to female sex pheromone at most, if not all, times. Several reports indicate that male nematodes copulate readily even after successful mating. *Rhabditis pellio* (Somers, et al., 1977, J. Nematol. 9: 143–148) mated an average of 5.8 times during a 4-hour interval when 3 days of age, but only three copulations produced larvae. Continual responsiveness to sex pheromone may result in such multiple matings, thus reducing the loss in fecundity from occasional infertile





NO. OF FEMALES

Figure 1. Mean distances traveled by mated and virgin male *N. brasiliensis* toward female sex pheromone sources (Φ = mated, \bigcirc = virgin).

inseminations and possibly offering an additional advantage of genetic heterozygosity in the succeeding nematode population.

The responses of ovigerous and virgin females to varying male sex pheromone sources are shown in Figure 2. Virgin females ($F_{98}^2 =$ 3.23) were significantly dosage dependent in their locomotor response toward male sex pheromone, while no constant dosage-dependent response by mated females was found. However, the within-treatment variability of virgin and mated females was the same (SE = ±0.16). No explanation for this difference in response

Figure 2. Mean distances traveled by mated and virgin female *N. brasiliensis* toward male sex pheromone sources (Φ = mated, \bigcirc = virgin).

between the two female groups is apparent presently.

A comparison of Figures 1 and 2 indicates that male helminths tend to be more responsive in our attraction bioassay to female pheromone than vice versa, although the difference is slight. In our previous experiments (Bone et al., 1977, J. Parasitol. 63: 364–367) gravid females were found considerably less responsive to male pheromone than were mated males to female pheromone. The reason for this difference in the response of gravid females in our present vs. our earlier experiments is not apparent.

The in vivo exposure of *N*. *brasiliensis* to actidione resulted in a significant reduction in

Table 1. Response of normal or actidione-treated male N. brasiliensis to normal or treated females used as pheromone sources.

Male responder	Female pheromone source	Distance traveled (cm)* Mean \pm SE
Normal	Normal	$0.900 \pm 0.26*$
Actidione-treated	Normal	$0.725 \pm 0.29^{*b}$
Normal	Actidione-treated	$0.075 \pm 0.21^{ m b}$

* Means followed by the same letter are not significantly different at the 0.05 probability level according to Duncan's Multiple Range Test.

sex pheromone release by female worms (Table 1). On the other hand, actidione-treated males remained normally responsive to female pheromone sources. These results suggest that female *N. brasiliensis*, like *P. silusiae*, may require protein or enzymatic syntheses for direct or indirect control of pheromonal attractiveness since actidione is known to inhibit these syntheses.

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Research Note

Hematozoa of Passeriform Birds from Eagle Lake, California

Considering the geographical diversity of the state of California, the number of avian blood parasite surveys conducted in this area is relatively few. Early surveys (Wood and Wood, 1937, J. Parasitol. 23: 197-201; Herms et al., 1939, J. Parasitol. 25: 511-522; Whonus and Ryerson, 1941, J. Parasitol. 27: 540-541; Wood and Herman, 1943, J. Parasitol., 29: 187-196; Herman, 1944, Bird-Banding 15: 89-112) included blood smears obtained from birds in northern and southern California but failed to mention where each was obtained. Herman et al. (1954, Am. J. Trop. Med. Hyg. 3: 676-695) reported an extensive survey of avian Plasmodium from Kern County in southern California. More recent surveys (Clark, 1964, J. Protozool. 11: 581-584; Clark, 1966, J. Protozool. 13: 108–110; Clark and Swinehart, 1966a, J. Protozool. 13: 395-397; Clark and Swinehart, 1966b, Bull. Wildl. Dis. Assoc. 2: 53-54) included data obtained exclusively from the Sacramento Valley region, and are the only data representative of the northern portion of the state. As Greiner et al. (1975, Can. J. Zool. 53: 1762–1787) have mentioned, surveys that are cognizant of seasonal, geographic, and environmental factors are particularly useful in evaluating the regional distribution of avian hematozoa.

Birds were captured using mist nets at the Eagle Lake Field Station, Lassen County, California. Sampling was done from mid-June through mid-August in 1973 through 1976. Captured birds were released after blood was obtained by clipping the left middle claw, which aided in the recognition of recaptures. During the summer of 1976, several birds were retained in wire cages, while their respective blood smears were examined for gametocytes. Subsequently, 13 birds were killed and tissue impression smears made of heart, kidney, liver, lung and spleen. Blood and tissue smears were air dried, fixed in 100% methyl alcohol and stained with Giemsa's stain.

Examination of 334 blood smears prepared from 33 species (13 families, 28 genera) revealed 116 (34.7%) birds harbored one or more species of *Haemoproteus*, *Trypanosoma*, microfilaria, and *Plasmodium*. Table 1 summarizes our findings for all four years. Also indicated in Table 1 are previously unrecorded host-parasite associations for North America.

Several microfilariae were observed in tissue impression smears in the lung of one *Piranga ludoviciana*, though no microfilariae were observed in peripheral blood. Schizonts were observed in spleen reticuloendothelial cells of a *Pipilo erythrophthalmus* and a single schizont was observed in a cell from the spleen. Several liver schizonts were observed in *Spizella passerina*. Schizonts in *P. erythrophthalmus* had numerous distinct nuclei with one to several nuclei inside parasitophorous vacuoles. "Compartmentalized foci" were not evident. Schiz-

Bird family Genus and species	# collected	# infected	Trypanosoma	Haemoproteus	Plasmodium	Microfilaria	% infected
Certhiidae Certhia familiaris	21	1	1*				4.8
Corvidae Cyanocitta stelleri	1	1	1	1	1		100.0
Fringillidao Pheucticus melanocephalus Passerina amoena Carpodacus purpureus Carpodacus cassinii Carpodacus mexicanus Chlorura chlorura Pipilo erythrophthalmus Junco hyemalis Spizella passerina	$3 \\ 1 \\ 17 \\ 2 \\ 2 \\ 4 \\ 1 \\ 23$	1 1 2 2 1 17	1 1 2	$1 \\ 10 \\ 1 \\ 2 \\ 1 \\ 1 \\ 14$	4* 3	3	$\begin{array}{r} 33.3\\ 0.0\\ 0.0\\ 88.2\\ 50.0\\ 100.0\\ 50.0\\ 100.0\\ 73.9\end{array}$
Hirundinidae Hirundo rustica Iridoprocene bicolor	$\frac{5}{28}$	4	3	1*			$\begin{array}{c} 0.0\\ 14.3\end{array}$
Icteridae Xanthocephalus xanthocephalus Euphagus cyanocephalus Molothrus ater Icterus galbula	3 9 2 23	$1\\1\\17$	1 3	12	2	$1 \\ 1 \\ 3$	$0.0 \\ 11.1 \\ 50.0 \\ 73.9$
Paridae Parus gambelli Psaltriparus minimus	$\frac{44}{22}$	13 1	4 1	7*	2*		$29.5 \\ 4.5$
Parulidae Vermivora celata Dendroica auduboni	$\frac{1}{2}$						0.0 0.0
Sittidae Sitta carolinensis Sitta canadensis Sitta pygmaea	9 2 9	3	1	1*		2*	0.0 0.0 33.3
Sylviidae Regulus satrapa	2						0.0
Thraupidae Piranga ludoviciana	14	11	4	6	3*	1	78.6
Troglodytidae Troglodytes aedon	20	4	1			3*	20.0
Turdida o Turdus migratorius Myadestes townsendi Sialia mexicana	$\begin{array}{c} 17\\ 4\\ 9\end{array}$	$\frac{7}{2}$	1 1	$\frac{2}{1*}$	1	4 1* 1	$41.2 \\ 50.0 \\ 22.2$
Tyrannidae Empidonax wrightii Empidonax sp. (difficilis?) Contopus sordidulus	$3\\28$	2 7	1 4	1* 2*	1*	1	$\begin{array}{c} 66.7 \\ 0.0 \\ 25.0 \end{array}$
TOTALS	334	116	32	64 10-2	16	23	
<u>%</u> of 10tal Con.		04.1	9.0	13.4	4.0	0.9	

Table 1.	Hematozoa	infecting	passeriform	birds	at	Eagle	Lake,	California.
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* New host (N. America).

onts in *S. passerina* had more small nuclei without parasitophorous vacuoles.

Our findings suggest a similarity in the prevalence of avian hematozoa from Eagle Lake and the Sacramento Valley area where Clark and Swinehart (1966b, loc. cit.) found 134 (34.9%) of 383 passeriform birds infected. Though similar prevalences of *Haemoproteus* (19.2%) are apparent, we found substantially higher prevalences of *Trypanosoma* and microfilaria and a slightly higher prevalence of *Plasmodium*.

Although *Leucocytozoon* has been reported in passeriforms throughout California (Wood and Wood, 1937, loc. cit.; Whonus and Ryerson, 1941, loc. cit.; Wood and Herman, 1943, loc. cit.; Clark and Swinehart, 1966a, b, loc. cit.), we found no birds infected with this parasite.

Double infections were *Haemoproteus*-microfilaria combinations (8), and *Haemoproteus*-*Trypanosoma* combinations (6).

Exoerythrocytic schizonts were seen in tissue impression smears in the spleen of *Pipilo* erythrophthalmus and the liver of *Spizella* passerina. Based on the morphology of erythrocytic and exoerythrocytic stages, these infections were diagnosed as *Haemoproteus* and *Plas*modium, respectively. As a result of this study, 14 new hostparasite associations are recorded for North America.

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Research Note

An Intestinal Helminth Survey of Three Species of Centrarchidae from Bald Eagle Creek, Centre County, Pennsylvania

The study of helminth parasites of fish from Pennsylvania has been neglected when compared to that of many other states. White suckers (*Catostomus commersoni*) from Forest and Blair Counties and from Northampton County were examined for intestinal helminths (Mackiewicz and McCrae, 1962, J. Parasitol. 48: 798–806; Fried et al., 1964, Proc. Penn. Acad. Sci. 38: 95–98). Mackiewicz and Deutsch (1976, Proc. Helminthol. Soc. Wash. 43: 9–17) described two new carophyllid genera from the quillback carpsucker (*Carpiodes cyprinus*) from Luzerne County. The purpose of this paper is to report the presence and numbers of intestinal helminths found in three species of the family Centrarchidae. Since this is the first

Helminths	Number	Descent	Numl infect		
	infected	infected	mean	range	Total
Trematoda Acolpenteron sp. Crepidostomum cornutum	$1 \\ 22$	$\frac{4}{88}$	128.7	1-128	$\begin{smallmatrix}&1\\632\end{smallmatrix}$
Bothriocephalus sp. Proteocephalus fluviatilis	$1 \\ 24$	4 96	112.1	1-75	$1 \\ 302$
Nematoda Spinitectus carolini	5	20	1.6	1-2	8

	Noushau	Dansant	Num infec		
Helminths	infected	infected	mean	range	Total
Trematoda Crepidostomum cooperi C. cornutum Castoda	$1 \\ 16$	4.3 69.6	$\overset{1}{_{12.3}}$	1-89	$1\\199$
Proteocephalus fluviatilis	3	13.0	3.7	1-7	11
Spinitectus carolini	1	4.3	3		3

Table 2. Prevalence and intensity of helminth infections in 23 rock bass (Ambloplites rupestris).

published helminth survey of this type performed in Pennsylvania, all helminths found represent a new locality.

Twenty-five smallmouth bass (Micropterus dolomieui). 23 rock bass (Ambloplites rupestris). and 19 redbreast sunfish (Lepomis auritus) were collected from Bald Eagle Creek in Centre County, Pennsylvania during the spring, summer, and fall of 1974. Helminths recovered from the intestinal tracts including pyloric ceca were killed and preserved according to Meyer and Olsen (1971, Essentials of Parasitology, William C. Brown Co., Dubuque, Iowa, 305 p.). The nematodes were mounted unstained in glycerin jelly. Due to the small size of the trematodes, standard methods of staining proved too tedious. Through trial and error, excellent results were obtained by placing the trematodes on a microscope slide containing a combination of melted glycerin jelly and safranin, at an approximate 10:1 ratio. Best results at staining the cestodes were obtained with the Giemsa stain. The AFIP procedure of Giemsa staining for bacteria, fungi, and inclusion bodies as described by Luna (1968, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, McGraw-Hill Book Co., New York, 258 p.) was used (with increased time at each step to compensate for the relative thickness of the cestodes), without the differentiating stain step of rosin alcohol.

Eight species of helminths were recovered from the three species of fish examined: three Trematoda, two Cestoda, three Nematoda.

All of the 25 smallmouth bass examined were infected with at least one species of helminth. Table 1 lists the number of helminths recovered and their prevalence and intensity of infection. A single specimen of the trematode genus Acolpenteron was taken from the intestine of a smallmouth bass and-being somewhat damaged-was not identified beyond the genus level. Of the two species described belonging to the genus, A. ureteroecetes was found on the gills and in the ureters and urinary bladder of Micropterus species (Fischthal and Allison, 1941, J. Parasitol., 27: 517-524; Meade and Bedinger, Jr., 1972, Southwest. Nat. 16: 281-295). This specimen may have been originally on the gills and after accidental swallowing may have undergone partial digestion. This would explain the lack of sensory hairs on its tegument and its presence in the intestine. The single specimen of the cestode genus Bothriocephalus was represented by a scolex only, therefore, specific identification could not be made.

Seventeen (73.9%) of 23 rock bass were in-

		D	Num infec		
Helminths	infected	infected	mean	range	Total
Trematoda	-02		35	022227	202
Crepidostomum cooperi	16	84.2	6.1	1-21	97
C. cornutum	3	15.8	1.3	1-2	4
Bothriocenhalus sn	1	53	2		2
Nematoda	-	0.0	_		
Spinitectus micracanthus	19	100	15.1	1-39	286
S. carolini	9	47.4	4.1	1-15	37
Rhabdochona sp.	1	5.3	1		1

Table 3. Prevalence and intensity of helminth infections in 19 redbreast sunfish (Lepomis auritus).

fected with one or more species of helminth (Table 2). *Crepidostomum cooperi* was represented by a single immature specimen. All three *Spinitectus carolini* recovered were also immature.

All of the 19 redbreast sunfish collected were infected with at least one species of helminth (Table 3). The two *Bothriocephalus* specimens and the *Rhabdochona* specimen were immature and therefore specific identification could not be made. This is apparently the first reported occurrence of *Bothriocephalus* sp., *Rhabdochona* sp., and *Spinitectus micracanthus* from the redbreast sunfish.

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Research Note

Parasites of the Fox Sparrow (Passerella iliaca) and Northern Waterthrush (Seiurus noveboracensis) in Newfoundland, Canada

The fox sparrow [Passerella iliaca (Merrem)] and northern waterthrush [Seiurus noveboracensis (Gmelin)] are found throughout much of the Nearctic region and both species are common summer residents in Newfoundland. The fox sparrow feeds primarily on vegetable matter (Terrill, 1968, In O. L. Austin, Jr. (ed.), Life Histories of North American Cardinals, Grosbeaks, Buntings, Towhees, Finches, Sparrows, and Allies. Order Passeriformes, Family Fringillidae, Pt. 3, U.S. Nat. Mus. Bull. 237: 1395–1415), while the northern waterthrush relies heavily on animal matter (Griscom and Sprunt, 1957, The Warblers of North America, Devin-Adair Co., New York, 356 p.)

Although certain aspects of the natural history of these birds has been documented, few people have carried out research on their parasite fauna. Records of parasites from these passerines are, for the most part, merely host records obtained when small numbers of these birds were examined in broadly based surveys.

The purpose of this study was to determine the parasite burdens of these two passerines in Newfoundland, and to correlate this burden with their habits.

Forty-four birds (20 fox sparrows and 24 northern waterthrushes) were examined for metazoan parasites and haematozoa, using con-

ventional parasitological techniques (Bain and Threlfall, 1977 Proc. Helminthol. Soc. Wash. 44: 219–221. Eveleigh and Threlfall, 1976, Can. J. Zool. 54: 1694–1711). All the birds, except one, were caught during the summer of 1974 in Japanese mist nets at two localities on the Avalon Peninsula, namely, Pickavance Creek (47°29'30"N, 52°52'00"W) on the Trans Canada Highway near St. John's, and Gull Island (47°15'30"N, 52°46'30"W) in the Witless Bay Sea Bird Sanctuary. The other specimen, an adult male fox sparrow was found freshly dead on 4 May 1975 in St. John's.

Since immediate examination was not possible, specimens taken at Pickavance Creek were transported back to the laboratory and deep-frozen within 2 hr of death, while specimens captured on Gull Island were placed in freezer chests with ice packs and transported back to the laboratory for freezing within 24 hr.

During necropsy any parasites found were fixed and stained as outlined by Andrews and Threlfall (1975, Proc. Helminthol. Soc. Wash., 42: 24–28). Representative specimens have been deposited in the collection of the junior author.

A total of 13 genera of helminths, 9 of ectoparasites and 2 haematozoa were recovered from the two host species during the present study (Table 1), including 17 new host records.

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			I	Intensity/infected bird		T
		Parasite	No. of birds (%) infected	Mean no.	Range of nos.	Location in/on bird
Host:	fox sparrow	Conspicuum icteridorum [†] Denton and Byrd, 1951	3 (15)	3	1 - 7	3, 4, 6‡
		Pande, 1939	4 (25)	4	1 - 7 +	6
		Zonorchis alveyi* (Martin and Gee, 1949) Tangina (Tangrania) zarudnui*	1 (5)	1	1	7
		Skrjabin, 1924 Schistosomatid	$\begin{array}{c} 4 & (25) \\ 1 & (5) \end{array}$	$\frac{2}{1}$	$_{1}^{1-2}$	
		Paricterotaenia passerellae (Cooper, 1921) Aploparaksis elisae	6 (30)	10	1 - 28	3, 4a
		Skrjabin, 1914	9 (45)	4	1 - 13	3,4
		(Montagu, 1811) Capillaria contorta*	4 (25)	3	2-4	9
		(Creplin, 1839)	3(15)	3	1-7	1
		Porrocaecum brevispiculum Webster, 1943 Philosterus fringillust	1 (5)	1	1	4b
		(Scopoli, 1772) Mureidea incertat	10 (50)	19	1 - 62	11, 14, 15, 16
		(Kellogg, 1896)	9 (45)	4	1-9	11, 14, 15
	deGeer, 1778 Brueelia vulgata* (Kellogg, 1896) Carotabullus gazai*	2(10)	8	7 - 8	12, 14, 16	
		2 (10)	5	2-7	12, 13	
		Rothschild, 1902 Haemaphysalis leporispalustris† Packard, 1867 Broctochulldage sp	4 (20)	1	1	11
			1 (5)	1	1	16
		(brevicaudatus?)† Analges sp.*	$12(60) \\ 1(5)$	$ \begin{array}{c} 61\\ 4 \end{array} $	5-250 4	$\begin{smallmatrix}&14\\12,15\end{smallmatrix}$
Host:	northern waterthrush	Conspicuum icteridorum* Denton and Byrd, 1951 Dilenis undula*	1(4)	1	1	6
		(Schrank, 1788)	1(4)	2	2	4c, 5
		(Goeze, 1782)	9 (38)	8	1-33	3,4
		(Montagu, 1811)	8 (33)	5	2 - 12	9
		(van Cleave, 1918) Ceratophullus garei*	4 (17)	2	1 - 3	2, 4a
		Rothschild, 1902 Proctophyllodes sp.* Montesauria sp.*	$egin{array}{c} 3 & (13) \\ 17 & (71) \\ 9 & (38) \end{array}$	$\begin{array}{c}2\\34\\57\end{array}$	$^{1-3}_{2-91}_{4-245}$	$\begin{array}{c}11\\14\\14\end{array}$

Table 1. Details of infection/infestation of 20 fox sparrows and 24 northern waterthrushes with metazoan parasites.

New host record.

New host record for Canada.
+ New host record for Canada.
+ 1, oesophagus; 2, gizzard; 3, duodenum; 4, small intestine (a) anterior (b) mid (c) posterior; 5, large intestine;
6, bile ducts; 7, gall bladder; 8, kidneys; 9, trachea; 10, blood vessel; 11, body; 12, back; 13, breast; 14, wings; 15, neck; 16, head.

The fox sparrow was host to 20 genera of parasites (10 helminth, 8 arthropod ectoparasite, 2 haematozoan), while the northern waterthrush hosted relatively fewer genera (5 helminth, 3 arthropod ectoparasite, 1 haematozoan). All the fox sparrows examined were parasitised, the most lightly infected bird being an AHY (after hatching year) female which bore a moderate infection of *Haemoproteus* fringillae (Labbé, 1894) and Haemoproteus orizivora Anschutz, 1909. Eighteen sparrows (90%) were parasitised by helminths (5 genera of Trematoda, 2 of Cestoda, 3 of Nematoda), 19 (95%) with ectoparasites (4 genera of Mallophaga, 1 genus of Siphonaptera, 3 genera of Acarina) and 6 (32%), of the 19 birds from which blood smears were taken, with haematozoa (2 genera). Three (13%) of the northern waterthrushes were parasite free (all AHY birds), 19 (79%) being host for helminths (1 genus of each of the Trematoda, Nematoda, and Acanthocephala; 2 genera of Cestoda), 21 (87%)

for ectoparasites (1 genus of Siphonaptera, 3 genera of Acarina), and 2 (8%) for haematozoa (1 genus).

An examination of Table 1 reveals that only a few genera of metazoan parasites are common to both host species, despite the fact that the birds were all caught in nets in the same area. This fact points to the birds occupying different ecological niches within the habitat, with different diets, or differences in anatomy and/or physiology of the two hosts that preclude the development of certain parasites on or in a given host species.

As previously noted eight of the 44 birds (18%) examined during this study were found to contain haematozoa. Leucocytozoon fringillinarum Woodcock, 1910 was found in two AHY male northern waterthrushes and one AHY male fox sparrow. In the two cases where it was found in the former species only a single gametocyte was observed. In the fox sparrow L. fringillinarum was found in association with Leucocytozoon majoris (Laveran, 1902). L. fringillinarum is probably the most prevalent haematozoan in passerines of Newfoundland. A study done during 1969–1972 recorded this parasite as occurring in 1,335 (49.9%) of 2,675 passeriformes examined (Bennett et al., 1974, Can. J. Zool. 52: 765–772). The occurrence of this species in association with *L. majoris* is also quite common in birds of Atlantic Canada (Bennett and Cameron, 1975, J. Parasitol. 61: 1091–1095).

Five other fox sparrows (2 AHY females and 3 AHY males) contained mixed infections of H. fringillae and H. orizivora; Bennett et al. (1974, op. cit.) stated that these two parasites occurred consistently as mixed infections in passerines of six families.

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Research Note

Notes on *Dina lineata* (O. F. Müller) (Hirudinea: Erpobdellidae) from the Gut of Some Nile Fishes in Egypt

The examination of 10 species of Nile fishes for parasites from Giza, Egypt during June, 1975 (Amin, 1978, J. Parasitol. 64: 93–101) revealed the presence of *Dina lineata* (O. F. Müller, 1774) in the gut of two fish species. Three hundred and thirty-two *D. lineata* were recovered, mostly from the large intestinal ceca (74%) of eight Nile eel, *Anguilla vulgaris*, as well as from the esophageal (22%) and the intestinal (4%) regions. Only one specimen was recovered from the gut of Norus sardine, *Alestes nurse*. A maximum of 105 leeches per eel was recorded and many more were found but not all recovered. Some were hanging down the mouth. *D. lineata* is a free-living freshwater leech which is widely reported in the Palaearctic region including North Africa and the Middle East. The present geographical and site records are new and unique. *A. vulgaris* must have been feasting on dense aggregations of this leech shortly before capture.

Structural Observations

All leeches were apparently freshly dead, pink to dark brown in color with no stripes and uniformly "relaxed" when recovered. Segmentation and annulation became mostly faint or inapparent in whole mounts. All subsequent measurements (in mm; means in parentheses) and observations were made from stained whole mounts. Body 12–43 (23.9) long by 0.6–2.4 (1.5) at widest point, usually at prominent clitellum (n = 219). Posterior sucker ventrally produced into a round to slightly oval disc occasionally with somewhat straight or invaginated anterior margin; 0.7–2.0 (1.2) long by 0.7–1.9 (1.1) wide (n = 105).

Eves were rarely absent; a new observation. The number and position of eyes were documented from 122 specimens. With the exception of two specimens (Figs. 5, 6), all had the larger anterodorsal pair, rarely alone (in two specimens) (Fig. 4). In all other specimens eye variations were of two major categories. In the first, all eyes behind the anterodorsal pair are transversely arranged; commonly four eves in two lateral pairs (Fig. 7) (n = 70,57%). This form was previously observed in Italian and German material by Blanchard (1894, Boll. Mus. Zool. Anat. Comp. Univ. Torino 9: 1-84) and Johansson (1914, Sitzungsber. K. Akad. Wiss. Wien Math.-Naturwiss. Kl. Abt. 1, 123: 837-852), respectively. Variants in this category (Figs. 8-10) were observed in six specimens (5%). In the second category, all eyes posterior to the first pair are arranged in two longitudinal rows; commonly eight eyes in two rows with the distance between the eyes widening posteriorly (Fig. 11) (n = 27, 22%). Variants in this category (Figs. 12-16) were observed in 16 specimens (13%). Second category forms were not previously reported.

Known literature on *D. lineata* deals with distributional records, segmentation/annulation, and ocular arrangements. Only Mann (1952 and 1959, J. Zool. 122: 395–405 and 132: 369–379) referred to the shape of its genital atrium

and ovisacs, respectively; and the key by Soós (1963, Acta Univ. Szegediensis, Acta Biol. N.S. 9: 253–261) also included the position of these structures and the sperm duct. A complete description of internal structures is provided below.

Male reproductive system (Figs. 1-3) includes 72–128 (100.33) testes arranged in two lateral rows extending from near the posterior end of body to shortly posterior to its middle (n = 49). Testes are spherical to oval; 0.12-0.56 (0.28) by 0.08-0.52 (0.21) (n = 105). A straight sperm duct is connected to each testes row ventrally; slightly enlarging anteriorly. Each duct abruptly enlarges directly anterior to the anteriormost testis to form a sperm sac which becomes gradually smaller as it forms the looped ejaculatory ducts more anteriorly. The latter empty to the outside through the male genital pore via the genital atrium and its median chamber. The female reproductive system (Figs. 1–3) includes two relatively large and variably constricted ovisacs located dorsal to the ejaculatory duct. They narrow anteriorly to jointly empty into the female gonopore posterior to that of the male's; both on segment XII. In my specimens the ejaculatory duct did not extend anterior to the inverted V-shaped atrium and the ovisacs were unlike those reported by Mann (1959, loc. cit.). He showed the arms of the "V" to be directed anteriorly with the distal ends of ejaculatory ducts extending slightly anterior to it. Digestive tract is a simple tube; pouches are occasionally seen in posterior half of engorged and/or incompletely relaxed specimens.

SPECIMENS: Seventeen leeches (11 whole mounts and 6 in AFA) deposited in Smithsonian Institution, Division of Worms, USNM No. 55150.

Dr. Marvin C. Meyer, University of Maine, Orono identified *D. lineata*. Drs. Harry Hoogstraal and W. F. Miner, U.S. NAMRU-3, Cairo, Egypt kindly provided laboratory space

→

Figures 1-16. D. lineata. 1. Adult (dorsal view). 2. Details of reproductive region (dorsal view). 3. Details of reproductive region (lateral view). 4-16. Occular variations. 4. With only anterodorsal pair. 5-6. Without this pair. 7. Common arrangement of first category. 8-10. Variants of first category. 11. Common arrangement of second category. 12-16. Variants of second category (at: anterior testis; cl: clitellum; ed: ejaculatory duct; fa: female aperture; ga: genital atrium; gn: ganglion; gt: gut; os: ovisac; ps: posterior sucker; sd: sperm duct; ss: sperm sac; ts: testis). Drawings were made by the author with the aid of a microprojector.

ga 11 ga gn 1 e d fa fa e d gn cl - 0 S 0 5 E a l m m 2 mm - gt S 5 a t a t 2 s d t s 3 5 6 7 4 8 9 p s 13 10 11 12 14 1 15 16

in the Department of Medical Zoology to process specimens. O'Brien C. Smith, University of Wisconsin-Milwaukee Medical School helped with field and laboratory aspects. Mark J. Redlin, University of Wisconsin-Parkside, reproduced original line drawings. I am grateful for all these colleagues' significant contributions.

> Омак М. Амін University of Wisconsin–Parkside Kenosha, Wisconsin 53141

PRESENTATION

1977 Anniversary Award of the HelminthologicalSociety of Washington509th Meeting, 21 October 1977Professor Horace Wesley Stunkard



The Anniversary Award is presented to Dr. Horace W. Stunkard by Dr. Aurel O. Foster.

Mr. President, Guests and Members of the Helminthological Society of Washington:

It is my happy errand to be the instrument for consummating in behalf of the Society something that our Society has never done before. On this occasion, we confer honorary membership upon a long-time member; this has been done only twice before since Honorary Members, although few, have more commonly been foreign parasitologists and nonmembers, as is our only surviving Honorary Member, namely, V. S. Ershov of the USSR. Unprecedentedly, however, we are also presenting the Anniversary Award on this date to that same member!

I shall add only, by way of introduction, that I am functioning rather particularly in behalf of our Awards Committee and our Committee on Honorary and Life Members, the respective chairmen being Drs. E. A. Steck and Harley Sheffield. Assuredly, I am personally honored by this assignment but feel also humbled by the fact that I can scarcely do justice either to it or to our distinguished candidate. Our particularly honored member at this 67th Anniversary Meeting had become "of age" (but scarcely so) when our Society (probably the oldest parasitological society in America, possibly the world) held its first meeting on 8 October 1910; he was born on August 23, 1889 in Monmouth, Iowa, which is now as then a modest town toward the Mississippi side of the state. His "roots" must be highly significant, but at this point we have learned nothing about them. Nevertheless, we know that we are dealing with a man whose credo from his earliest days has been the pursuit of excellence, and he has indeed excelled in everything he has undertaken.

Our uniquely honored awardee was formally educated at Coe College from which he received the B.S. degree in 1912 and at the University of Illinois from which he received the A.M. degree in 1914 and Ph.D. degree in 1916. He is one of that illustrious group of parasitologists turned out by that Father of Parasitology in the United States, the redoubtable Henry Baldwin Ward. He has vivid and revealing recollections of his memorable experiences with "H.B." and fellow students.

The man of whom I speak is our eminently distinguished and long-time member, Horace Wesley Stunkard. Dr. Stunkard became a member of the Helminthological Society 21 January 1922. Records of about this time are seemingly incomplete, yet my personal papers and recollections suggest that he was probably made a Corresponding Member on that date and the occasion may have been the 57th meeting. Today's Anniversary Meeting, 21 October 1977, is our 509th. Can anyone else claim to have been a member of this Society over an elapsed period of 452 meetings?

The man we honor was a friend, contemporary, and closely working associate of Charter Members of our Society—Stiles, Hassall, Ransom, Hall, Cobb et al. I have heard him say, albeit not in these words, that W. W. Cort did much to assist him in getting started at the University of Illinois when both were graduate students there. He was a close associate of Eloise Cram, Benjamin Schwartz, and Robert Hegner and was undoubtedly among the small group that in 1924 conspired to establish the American Society of Parasitologists. He entered Illinois in 1912, a year after George R. LaRue had completed his degree work there and be-

came an Instructor at Michigan. Incidentally, LaRue, Cort, Stunkard, and Cram-all close associates, all past presidents of the American Society of Parasitologists, all with strong ties to "Helm. Soc."-were among Iowa's contribution to parasitology. He was a long-time friend of Norman Stoll. The researches of Stoll and Krull, perhaps more than those of others, influenced his own highly significant work on the life history of anoplocephaline tapeworms. Indeed, the man we recognize this day has known and worked with many, if not most, of our members throughout the 67 years of our Society's existence. He has attended many of our decennial and other special celebrations. Throughout the years he has rendered invaluable services to our journal, which today has achieved an enviable status of respectability.

In mentioning illustrious names like LaRue and Stoll, it may be parenthetically appropriate to mention also that these two individuals are apparently the only active members of our Society who have heretofore been given the status of honorary membership.

Perhaps best at this point, something should be said, albeit brief, about Dr. Stunkard's lifework. His extensive published researches and some of the many recognitions along the way are well known. Having reflected on this during recent weeks probably somewhat perforce, I feel constrained to emphasize something more than "eminent scholar, dedicated and inspiring teacher, outstanding investigator and effective administrator"; he was and is all of these, to be sure. He is also a gifted, respected leader; he is untiring in his work and assistance to others; he is unusually familiar with the places, people, literature and languages of science; he is devoted to the ethic of hard work and the pursuit of excellence; and above all, he is an extraordinarily humble, human person.

As a research investigator, Stunkard has contributed steadily and regularly since 1915. Indeed, he has been continuously active in biological research since at least as early as 1912, a period of 65 years to date and there is no current sign of diminishing productivity. Since my own retirement, I have come to rely heavily upon the *Index Catalogue of Medical* and Veterinary Zoology and to appreciate something of its worth. These volumes now come close to the Holy Scriptures in their revelations! Dr. Stunkard is listed therein a principal author of over 200 publications and a collaborating author of many more. His own distributed reprints, apparently successively numbered, run to at least 273. No one would venture to guess the number of publications that have stemmed directly from his teaching, counseling, and ready assistance to associates and junior colleagues!

The work of this man, moreover, in every aspect and instance, has the earmarks of quality! These statements are descriptive of the man we honor, and it is worthy of thought that one can make them with no fear whatever that anyone will demur.

Most of Prof. Stunkard's publications have dealt with life-cycle and descriptive aspects of trematodes. Some, however, have pertained to other areas of biology, including general parasitology, zoonoses, invertebrate zoology, and related subjects. Having emphasized his fluke studies, I must comment, in passing, upon his cestode studies, especially those leading to solution of life histories of the Anoplocephalidae. Here was an enigma of more than a half-century and of rather concentrated study by some of the best minds in parasitology. Stunkard seems to have called his shot in 1934 and given the solution in 1937. I am awed to reflect that I may have been personally among the earliest parasitologists to see the larval stages; in 1937, I went to Dr. Stunkard's laboratory and his technician dissected mites and showed me the cysticercoids. Only within the last few days I have been privy to a taped interview involving Dr. Stunkard and, among others, a brilliant and aggressive young parasitologist with the appealing name of Hugo James, in which Dr. Stunkard states that his anoplocephaline studies were a high point among his researches.

The year 1934 was significant in another way. Starting with a single specimen of trematode (*Typhlocoelum* sp.), the life history was determined and published. Reference is made to this in his ASP Presidential Address of 1939, published in 1940. From what I have heard him say, other critical or determinative years during his research and teaching were those involving sabbatical leaves in England, France and Germany. These exposures of as much as 15 months each provided close contact with some of the best parasitologists of the day —Nuttall, Brumpt, Fülleborn, Baer and others. They also contributed greatly to the education of a multilinguist.

The researches of which I speak were carried on at New York University where Dr. Stunkard was instructor, professor, and head of the Department of Biology, 1916 until his retirement in 1954, but he has pursued a significant share of his studies at the Woods Hole Marine Biological Laboratory and the American Museum of Natural History, with both of which he has been associated for many years and is still operating at both institutions. In addition to his two full time jobs in teaching and research, he has contributed heavily of his time to many scientific organizations and societies. In the American Society of Parasitologists, as most of us know, he has held every major office, served on innumerable committees, and carried the burdens of editor and secretary over many years. He also carried the work of editorship for the Proceedings of the Second International Congress of Parasitology. There is much more to be said about the varied work of this indefatigable man, but you will have to consult your reference volumes.

Honors, Awards, and other forms of recognition and tribute, here and abroad, have come to Dr. Stunkard along the way. I shall mention only a few that I know about but which seem not to have been widely publicized. A few years ago, the American Museum of Natural History recognized Dr. Stunkard's 50 years' (1921–71) distinguished service to that institution with its gold medal. In 1973, the New York Academy of Sciences gave him the Pregel Award, a gold medal and \$500.00, for Research in Biology in that year. Another award came to my knowledge through "in-house" communications of the World Federation of Parasitologists. At the closing plenary session of the Third International Congress of Parasitology 31 August 1974 in Munich, the Rudolph Leuckart Medal of the Deutsche Gesellschaft was awarded to Dr. Stunkard. Although a matter of record, I should probably mention that Dr. Stunkard has received honorary Sc.D. degrees from his Alma Mater, Coe College, and also the institution that he served so long, New York University.

Two additional, wholly extraneous items ought to be cited. He served in the U.S.A.A.F. 1917–19. From what I've heard, the sheer joy of survival must have transcended that of any fame or glory. In his spare time in college, I'm told that he was a hurdler; indeed, a champion hurdler, both high and low. He also played football and remembers the thrill of his last college game when he caught a pass and scored against Drake. He also enjoyed debating and public speaking. His debating team was a champion in the area and, in his senior year, Stunkard was the representative of his college in the area public speaking contest. Throughout, there was emphasis on scholarship. He graduated *magna cum laude*. This is a sketchy profile of one of today's outstanding leaders in parasitology. The awardee has brought much honor and credit to our Society. We now have the privilege and pleasure of giving a little of the same to him.

Other members of committees, of which chairmen have already been mentioned, are Richard L. Beaudoin and Sherman S. Hendrix. I thank them all for asking me to make these presentations to Professor Stunkard.—A. O. FOSTER

MINUTES

Five Hundred Ninth Through Five Hundred Sixteenth Meetings

509th Meeting: University of Maryland, College Park, Maryland, October 21, 1977. The 1977 Anniversary Award and honorary membership in the Society was presented to Dr. Horace Stunkard by Dr. A. O. Foster. President, Dr. Powers, announced that Mildred Doss and Everett Wehr had been selected for Life Membership in the Society. Slate of officers for 1978 presented: Harley Sheffield (President), Ronald Fayer (Vice President), Ralph Lichtenfels (Corresponding Secretary-Treasurer), Larry Hendricks (Recording Secretary). Dr. Lichtenfels presented a report prepared by Dr. Gilbert Otto, Chairman of the Ad Hoc Advisory Committee. Reports from three members of the Society on their recent attendance to the 8th International Congress of the World Association for the Advancement of Veterinary Parasitology (WAAVP), held in Sydney, Australia, July 11-15, 1977, was the theme of the presentations. Papers presented: "An overview of the organization of the WAAVP and the Sydney meetings and impressions of helminthology research in Australia and New Zealand," G. F. Otto; "Impressions of research on protozoan diseases of livestock in Australia and New Zealand," R. Fayer; "Approval procedures and new animal drugs presented at the Congress," K. Powers.

510th Meeting: Animal Parasitology Institute, Beltsville, Maryland, November 19, 1977. Papers presented: "Activity of diamfenetide against Fasciola hepatica in sheep," R. S. Rew, M. L. Colglazier, F. D. Enzie; "Effect of polypeptides on host cell penetration by coccidian sporozoites," P. Augustine; "Intestinal malabsorption of nutrients during coccidiosis," M. L. Ruff. It was announced that anyone not associated with a hosting institute but desiring to present a paper may contact the Program Chairman (Vice President). Fifteen minutes will be set aside at the end of all 1977–78 meetings for unscheduled reports from the floor.

511th Meeting: Animal Parasitology Institute, Beltsville, Maryland (Sponsored by the Biological Laboratory, National Marine Fisheries Service, Oxford, Maryland), December 9, 1977. Officers elected at the 510th meeting were installed. The membership voted to accept an increase in the annual dues beginning in 1978 from \$8.00 to \$12.00. Papers presented: "New information on the larval anisakid nematode of marine Mollusca," J. R. Lichtenfels, J. W. Bier, P. A. Madden; "Monogenetic trematodes of fish in the New York bight," Sherman Hendrix; "Experimental Phocanema decipiens infections in seals and pigs: Course of infection and microscopic pathology,'

J. W. Bier and G. McClelland; "Introductions of shellfish disease through indiscriminate transport," A. Rosenfield.

512th Meeting: Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, Bethesda, Maryland, January 20, 1978. J. R. Lichtenfels, Corresponding Secretary-Treasurer, gave the results of the audit and a brief review of the financial statement for 1977. Papers presented: "Clonal growth of *Entamoeba histolytica* in agar," F. D. Gillin; "Lymphocyte transformation to *Trypanosoma* cruzi antigens in Chagas disease," R. Gusmâo; "Role of lysozyme in the defense mechanism of *Biomphalaria glabrata* to *Schistosoma mansoni* infection," K. Kassim; "Further studies on immunogenic surface polysaccharides of hemoflagellates," M. Gottlieb.

513th Meeting: Nematology Laboratory, Plant Protection Institute, U.S. Department of Agriculture, Beltsville, Maryland, February 15, 1978. Papers presented: "Life and death of a nematicide: DBCP and EPA's RPAR," J. Feldmesser; "Feeding plug formation in soybean roots infected with the soybean cyst nematode, *Heterodera glycines*," B. Y. Endo; "Ascaris eggs as indicator organisms for regulating sewage disposal," G. J. Jackson; "New World onchocerciasis," E. Shiller.

514th Meeting: Walter Reed Army Institute of Research, Washington, D.C., March 21, 1978. The theme of the presentation was "Malaria: Drug Development Program." Papers presented: "Vulnerable morphological stages to drug pressure," J. C. Burke; "Drug screening in animals," D. E. Davidson; "Preclinical and clinical considerations," M. H. Heiffer; "Chemotherapeutics and prophylacis of humans," M. S. Wolfe; "New drug development," C. J. Canfield. Presentations were followed by a panel discussion of the malaria drug development program. 515th Meeting: Naval Medical Research Institute, Bethesda, Maryland, April 19, 1978. The theme of the presentation was the U.S. Navy Malaria Vaccine Program. Dr. R. Beaudoin presented an overview entitled "Current aspects of the U.S. Navy Malaria Vaccine Program." The remainder of the program consisted of a poster session on the subject of malaria vaccine and the Navy program. Poster presentations were by J. Armstrong, M. Bowden, N. Pacheco, W. Pryor, Jr., L. Smirkovski, C. Strome, and D. Wood.

516th Meeting: University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, May 13, 1978. President Sheffield opened the meeting. A moment of silence was observed in memory of Dr. Jesse R. Christie, a life member of the Society recently deceased. Papers presented: "Life history and control of Filaroides hirthi lungworm infection in commercially reared beagles," J. Georgi; "The effect of albendazole on the metacestodes of T. saginata in cattle," S. Lloyd; Immunopathology of ocular Ascaris suum in the guinea pig," J. Donnelly; "Electron microscopic and cytochemical studies of the surface of microfilariae of Dirofilaria immitis," P. Cherian; "The SD antigen: A protective antigen, an allergen and immunopotentiator isolated from Ascaris suum," B. Stromberg. President Sheffield presented Dr. E. J. L. Soulsby with a gift from the Society as a token of its appreciation for his contributions and service and as a farewell present prior to his departing the U.S. The gift consisted of back issues of the Proceedings from the first issue published thru the issue at the time that Dr. Soulsby became a member of the Society.

During the 1977–78 meetings of the Society, 32 new members were elected to membership.

LARRY D. HENDRICKS Recording Secretary

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