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CONTENTS

Two New Monogenea (Macrovalvitrematidae) from Eastern Pacific Ocean Fishes[1]

RAPHAEL R. PAYNE[2]
Harold W. Manter Laboratory, University of Nebraska State Museum and
School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0514

ABSTRACT: Llewellyn's nomenclature for Diclidophoridae clamp sclerites is modified for Macrovalvitrematidae. *Papillopseudotagia hubbsi* gen. et sp. n. (Monogenea: Macrovalvitrematidae: Macrovalvitrematinae) from gills of *Citharichthys sordidus* (Bothidae) from the Pacific Ocean off Monterey Bay, California is described. *Papillopseudotagia* gen. n. differs from previously described genera of its subfamily in having short-stalked, subspherical to concave papillae on the ventral jaw tegument of the 3 posterior pairs of haptoral clamps; in details of clamp morphology, presence of proximally oblique sclerite b; and in ordinal host status. *Pseudohargisia cortesi* gen. et sp. n. (Pterinotrematoidinae) from gills of *Micropogon megalops* (Sciaenidae) from the Gulf of California, Mexico, is described. *Pseudohargisia* gen. n. differs from previously described genera in its subfamily in details of clamp morphology, presence of oblique transverse sclerite b in posterior clamp pair, and in having 8–9 genital corona spines. The diagnosis of Pterinotrematoidinae is emended to include a genital corona with circlet of similar spines, or 2 lateral groups of dissimilar spines.

KEY WORDS: Macrovalvitrematinae, Pterinotrematiodinae, *Papillopseudotagia hubbsi* gen. et sp. n., *Pseudohargisia cortesi* gen. et sp. n., central California, Gulf of California, *Citharichthys sordidus*, *Micropogon megalops*.

Bravo-Hollis (1982) reviewed Macrovalvitrematidae Yamaguti, 1963, and recognized 2 subfamilies and 8 monotypic genera. Most of the species are restricted to sciaenid fishes inhabiting temperate and subtropical waters of the Western Hemisphere. This report describes 2 new genera and species from gills of fishes collected from the eastern Pacific Ocean.

Materials and Methods

Fishes were collected by hook and line or otter trawl from localities along the central California coast and in the Gulf of California between 1967 and 1968. Immediately after capture, gills were removed and examined with a dissecting microscope. Monogeneans were fixed in AFA (alcohol–formalin–acetic acid) under slight coverglass pressure, and stored in 70% ethanol. In the laboratory, specimens were hydrated and then stained with Van Cleave's hematoxylin, dehydrated, cleared in methyl benzoate, and mounted in Permount. Observations were made using standard light microscopy and Nomarski differential interference contrast; figures were drawn with the aid of a drawing tube. Measurements are in micrometers unless otherwise stated; ranges are followed by means in parentheses. Clamp nomenclature is modified from Llewellyn's (1958) designations for Diclidophoridae. Representative specimens have been deposited in the United States National Museum (USNM) Helminthological Collection, Beltsville, Maryland, and the Harold W. Manter Laboratory (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln; the balance of the specimens are in the author's collection.

Results

Macrovalvitrematidae Yamaguti, 1963

Macrovalvitrematinae Yamaguti, 1963

Papillopseudotagia gen. n.

GENERIC DIAGNOSIS: Body elongate, subcylindrical. Haptor bearing 4 pairs asymmetrical oval clamps with opposable jaws; anterior pair reversed dorsoventrally. Clamps composed 10 sclerites; dorsal jaw with 1 proximal, 1 median, and 5 peripheral sclerites; ventral jaw with 1 proximal oblique, and 2 peripheral sclerites. Papillae present on tegument ventral jaws. Tegumental bars present on dorsal and ventral jaws. Terminal lappet with marginal hooks present. Mouth wide, buccal suckers paired. Pharynx ovoid, between or posterior to buccal suckers; ceca simple. Testes numerous. Genital corona with curved grooved spines. Prostatic vesicle present. Ovary somewhat convoluted. Genitointestinal canal present. Seminal receptacle absent. Parasites on gills of marine teleosts. Type and only species: *P. hubbsi*.

Papillopseudotagia hubbsi sp. n.
(Figs. 1–8)

DESCRIPTION (based on 17 specimens, 5 measured): Body total length 1.959–2.988 (2.497)

169
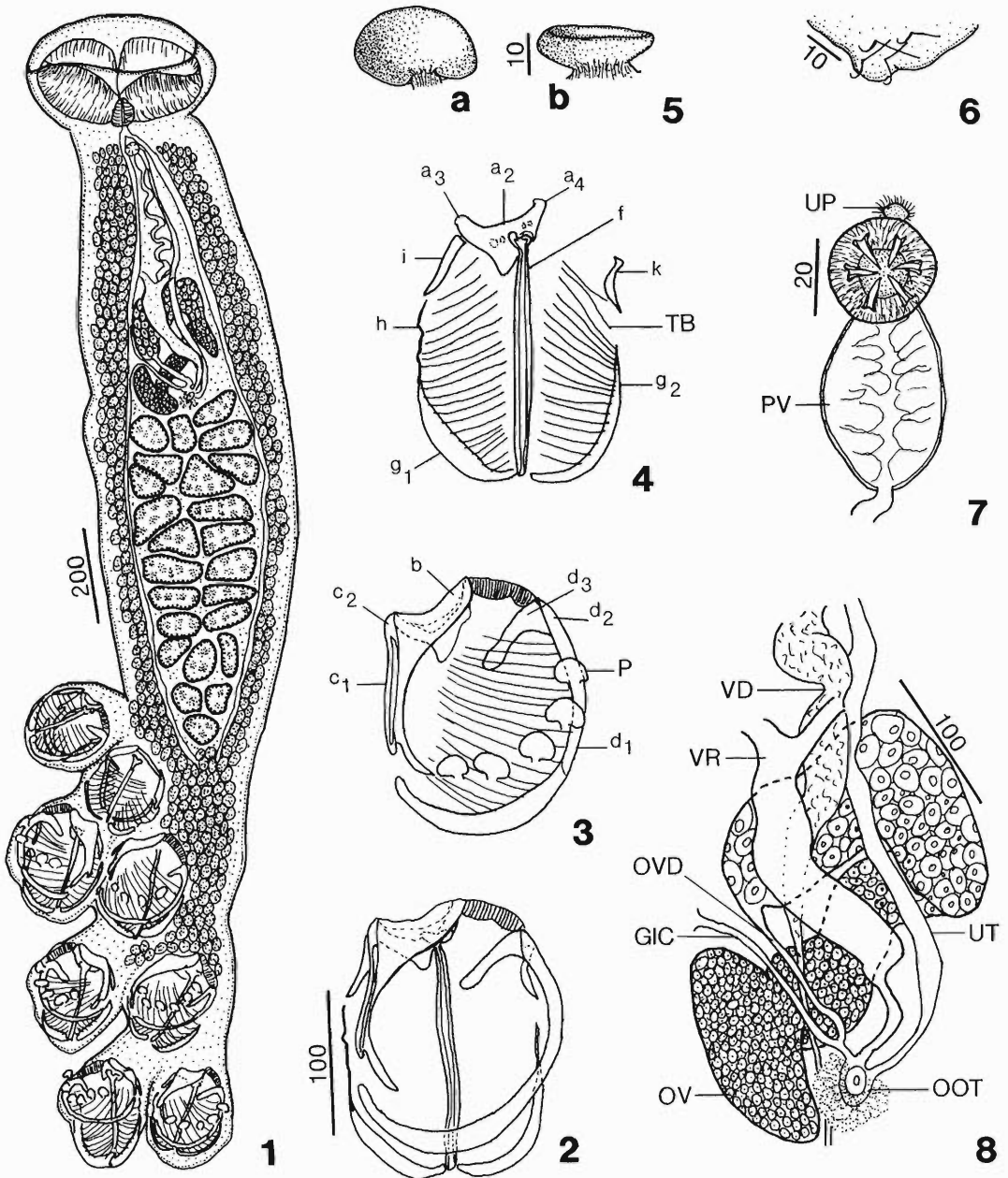
Figures 1–8. *Papillopseudotagia hubbsi* gen. et sp. n.; all holotype and ventral view unless otherwise stated. 1. Whole mount. 2. Entire clamp. 3. Ventral jaw. 4. Dorsal jaw. 5a. Papilla, subspherical form. 5b. Papilla, concave form; paratype. 6. Terminal lappet; paratype. 7. Genital corona; paratype. 8. Female reproductive system; paratype. Abbreviations: GIC, genitointestinal canal; OOT, ootype; OV, ovary; OVD, oviduct; P, papilla; PV, prostatic vesicle; TB, tegumental bars; UP, uterine pore; UT, uterus; VD, vas deferens; VR, vitelline reservoir. Scales in micrometers.

mm, maximum width 396–465 (424) near mid-body. Buccal suckers 132–195 (165) long by 114–174 (149) wide. Haptor 840–1,083 (931) long. Clamps 132–240 (195) long by 123–171 (146)

wide, pedunculate. Anterior clamp pair reversed dorsoventrally. Dorsal jaw posterior 3 clamp pairs and ventral jaw anterior pair formed by proximal sclerite a (=$a_2a_3a_4$), median sclerite f, peripheral

sclerites g_1, g_2, i, and k, and accessory sclerite h; sclerites g_1 and g_2 approaching each other medially, lightly sclerotized, embedded in muscle; sclerite h threadlike, lightly sclerotized, contiguous with i proximally and g_1 distally; median sclerite f articulating with a_2 proximally, a_1 absent. Ventral jaw posterior 3 clamp pairs and dorsal jaw anterior pair formed by proximal oblique sclerite b, and peripheral sclerites c ($=c_1c_2$), and d ($=d_1d_2d_3$); sclerite b fused with a_4 proximally, and fused along mediolateral margin sclerite c; sclerite d becoming contiguous with a curving muscle proximally; sclerite c_2 fused with a_3 proximally; sclerite i dorsal jaw articulating at fusion c_2 and a_3; sclerite k dorsal jaw articulating with d_2. Five to 6 short-stalked papillae, 21–39 (29) wide, varying from subspherical to concave, on tegument ventral jaw 3 posterior clamp pairs. Numerous tegumental bars present in dorsal and ventral jaws all clamps. Terminal lappet with 2 pairs marginal hooks, 20 long; not seen on holotype.

Mouth 132–324 (218) wide, terminal. Pharynx 54–67 (60) long by 34–48 (44) wide. Ceca simple, presumably confluent in anterior region haptor.

Testes 39–123 (74) long by 75–153 (115) wide, 18–25 (22) in number, intercecal, in middle third body. Genital corona 23–29 (25) in diameter, muscular, immediately posterior to cecal bifurcation; spines 8–9 long, 6–7 in number. Prostatic vesicle 38–57 (44) long by 32–38 (35) wide.

Ovary greater than 350 long, convoluted, in anterior third of body; genitointestinal canal dextral. Vitelline follicles numerous, extending into haptor to third pair clamps. Ootype and Mehlis' gland medial to posterior part ovary. Uterine pore immediately anterior to genital corona. Vaginae not observed.

HOST: *Citharichthys sordidus* (Girard); Bothidae.

HABITAT: Gills.

LOCALITIES: Pacific Ocean off central California, west of Pt. Pinos (36°38′N, 122°09′W), holotype locality; south of Santa Cruz, outside Monterey Bay (36°53′N, 122°05′W). Depth 67–123 m.

PREVALENCE AND INTENSITY: On 3 of 6 fishes examined, 1–10 per host.

TYPE SPECIMENS: Holotype, USNM Helm. Coll. No. 79500; paratypes, USNM Helm. Coll. No. 79501, HWML No. 23640.

ETYMOLOGY: The generic name refers to the haptoral clamp papillae, and to the resemblance

to *Pseudotagia* Yamaguti, 1963. The specific epithet honors the late ichthyologist, Dr. Carl L. Hubbs (Scripps Institution of Oceanography, University of California, San Diego).

REMARKS: *Papillopseudotagia* gen. n. most closely resembles *Pseudotagia* Yamaguti, 1963, in displaying: a dorsoventral reversal of the anterior clamp pair, similar clamp shape, and presence of a terminal lappet. *Papillopseudotagia* gen. n. differs from *Pseudotagia* by having: papillae on the ventral jaw tegument, 10 versus 7 clamp sclerites, simple rather than diverticulate ceca, presence versus absence of prostatic vesicle, vitelline follicles extending into the haptor, and lacking toothlike serrations on the clamps.

Pterinotrematoidinae Bravo-Hollis, 1982

Pseudohargisia gen. n.

GENERIC DIAGNOSIS: Body elongate, subcylindrical. Haptor bearing 4 pairs asymmetrical clamps with opposable jaws. Anterior 3 pairs clamps elongate, "fire-tong"-shaped, each composed 11 sclerites: dorsal jaw with 1 proximal and 2 peripheral sclerites, ventral jaw with 1 proximal, 1 median, and 6 peripheral sclerites. Posterior pair clamps rounded, each composed 10 sclerites: dorsal jaw with 1 proximal, 1 median, and 4 peripheral sclerites; ventral jaw with 1 proximal, 1 oblique transverse, and 2 peripheral sclerites. Terminal lappet absent. Buccal suckers paired. Pharynx ovoid, between or posterior buccal suckers; ceca diverticulate. Testes few. Genital corona with recurved grooved spines. Prostatic vesicle present. Ovary cylindrical, inverted U-shaped. Genitointestinal canal present. Seminal receptacle absent. Eggs in utero pyriform, filamented. Vagina median. Parasites on gills of marine teleosts. Type and only species: *P. cortesi*.

Pseudohargisia cortesi sp. n.
(Figs. 9–17)

DESCRIPTION (based on 2 specimens): Body total length 1.457–1.780 (1.619) mm, maximum width 353–360 (357) near midbody. Buccal suckers 96–102 (98) long by 66–78 (72) wide. Haptor 450–676 (563) long. Clamps pedunculate. Anterior 3 pairs "fire-tong" clamps 118–152 (132) long by 78–91 (84) wide; dorsal jaw formed by proximal sclerite a ($=a_2a_3a_4$), and peripheral sclerites c ($=c_1c_2c_3$) and d ($=d_1d_2d_3$) with sclerites c and d approaching each other medially, curving
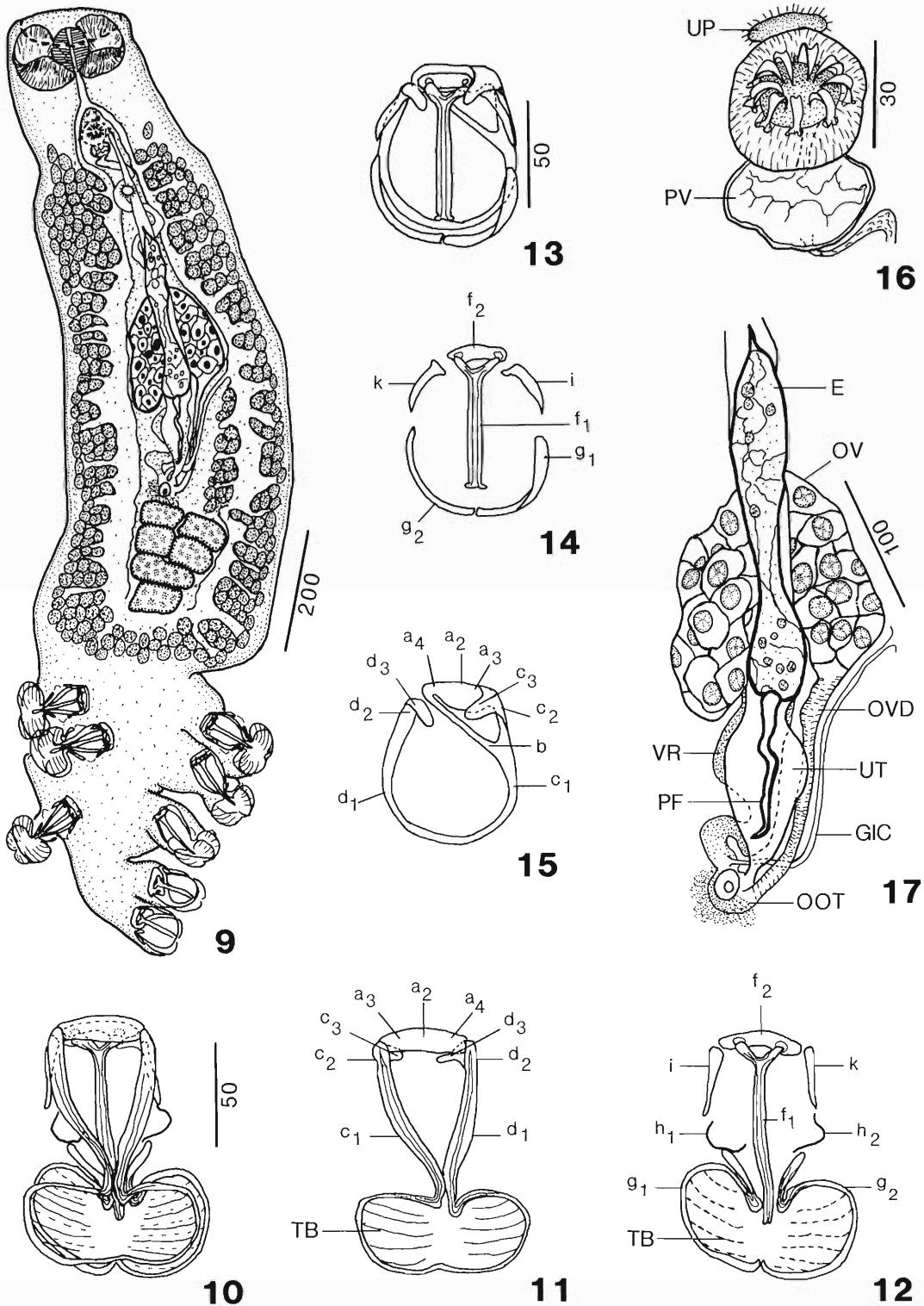
Figures 9–17. *Pseudohargisia cortesi* gen. et sp. n., holotype; all dorsal view unless otherwise stated. 9. Whole mount. 10. Anterior clamp. 11. Dorsal jaw anterior clamp. 12. Ventral jaw anterior clamp. 13. Posterior clamp. 14. Dorsal jaw posterior clamp. 15. Ventral jaw posterior clamp. 16. Genital corona, ventral view. 17. Female reproductive system. Abbreviations: E, egg in utero; PF, posterior filament; other abbreviations as in Figures 1–8. Scales in micrometers.

172

laterally, and fusing distally; a_1 absent; ventral jaw formed by proximal sclerite f_2, median sclerite f_1, peripheral sclerites g_1, g_2, i, and k, and accessory sclerites h_1 and h_2; sclerites g_1 and g_2 approaching each other medially, curving laterally, and nearly contiguous distally; sclerites h_1 and h_2 threadlike, lightly sclerotized; sclerite i articulating with c_2 proximally; sclerite k articulating with d_2 proximally; median sclerite f_1 bifurcated proximally, articulating with f_2, and extending distally just beyond constriction g_1 and g_2; sclerite f_2 articulating with a; 5–6 lightly sclerotized tegumental bars in distal quadrants dorsal and ventral jaws. Posterior pair clamps 82–95 (88) long by 67–76 (72) wide; dorsal jaw formed by proximal sclerite f_2, median sclerite f_1, and peripheral sclerites g_1, g_2, i, and k; sclerites g_1 and g_2 nearly contiguous distally; ventral jaw formed by proximal sclerite a ($=a_2a_3a_4$), obliquely transverse sclerite b, and peripheral sclerites c ($=c_1c_2c_3$) and d ($=d_1d_2d_3$); sclerite a_1 absent; sclerite b fused with a_4 proximally and c_1 distally; sclerites c_1 and d_1 fused distally; sclerites c_2 and d_2 articulating with i and k respectively; sclerite f_2 articulating with a. The ventral jaws posterior pair clamps are structurally analogous to dorsal jaws anterior 3 pairs.

Mouth wide, terminal. Pharynx 63–67 (56) long by 48–55 (52) wide. Ceca diverticulate laterally, occasionally medially, not extending into haptor.

Testes 34–48 (44) long by 48–78 (66) wide, 5–6 in number, intercecal. Vas deferens 585 long by 36 wide (seen in holotype only), extending anteriorly first dextrally then along midline and joining genital corona. Genital corona 40 in diameter, muscular, immediately posterior to cecal bifurcation; spines 8–9, 13 long by 4 wide. Prostatic vesicle 26 long by 44 wide.

Ovary 250–288 (269) long by 78–90 (84) wide, at midbody; genitointestinal canal dextral. Vitelline follicles numerous, coextensive with ceca; vitelline reservoir 75–87 (81) long by 42–45 (44) wide. Uterus 540–615 (577) long, median, dorsal to vitelline reservoir; uterine pore immediately anterior to genital corona. Egg (partially collapsed in holotype) 255 long with anterior filament 15 long, and posterior filament 108 long. Vagina pore dorsomedian, between genital corona and ovary.

HOST: *Micropogon megalops* Gilbert; Sciaenidae.

HABITAT: Gills.

LOCALITY: Gulf of California, southeast of San Felipe, Mexico (30°29′N, 114°14′W). Depth 75–86 m.

PREVALENCE AND INTENSITY: On 2 of 5 fishes examined, 40%, 1 per host.

TYPE SPECIMENS: Holotype, USNM Helm. Coll. No. 79499; paratype, HWML No. 23641.

ETYMOLOGY: The generic name indicates morphological similarity to *Hargisia* Yamaguti, 1963. The specific epithet refers to the type locality, Sea of Cortez (Gulf of California).

REMARKS: *Pseudohargisia* gen. n. most closely resembles *Hargisia* Yamaguti, 1963, in arrangement of the 3 anterior pairs of "fire-tong" and posterior pair of rounded clamps, shape of genital corona spines, and by parasitizing the same host family. *Pseudohargisia* gen. n. differs from *Hargisia* by having: more testes (5–6 versus 1) and more genital corona spines (8–9 versus 6), a prostatic vesicle (ejaculatory bulb of Bravo-Hollis, 1982), an oblique transverse sclerite b in ventral jaw of posterior clamps, lightly sclerotized tegumental bars in distal quadrants of "fire-tong" clamps, a dorsomedian vaginal pore, and by lacking a terminal lappet and anterolateral plicated placodes. The latter were interpreted by Hargis (1956) as ornamentation of the vaginal opening regions.

Bravo-Hollis (1982) included *Hargisia* in Pterinotrematoidinae, and diagnosed the subfamily as having a cirrus bulb with 3 pairs of dissimilar spines arranged bilaterally. Both *Hargisia* and *Pseudohargisia* gen. n. have a genital corona with spines arranged in a circle. The subfamily diagnosis is, therefore, emended to include a genital corona with circlet of similar spines, or 2 lateral groups of dissimilar spines.

Discussion

The "fire-tong" clamp shape is not unique to Macrovalvitrematidae because it also is found in Microcotylidae (*Rhinecotyle* Euzet and Trilles, 1960), Pyragraphoridae (*Pyragraphorus* Sproston, 1946), and Pterinotrematidae (*Pterinotrema* Caballero, Bravo-Hollis, and Grocott, 1954). Sproston (1946) proposed the homology of diclidophorid clamp sclerite with those of less complex clamps. Hargis (1955, 1956) emphasized that it is the details of clamp sclerite structure, sclerite arrangement, and number of sclerites that are the important taxonomic characters rather than general clamp shape. He also extended the homology of diclidophorid clamps to those of Discocotylidae. Yamaguti (1963) estab-

174

lished Macrovalvitrematidae from genera previously included in Discocotylidae. The ease with which Llewellyn's (1958) nomenclature for diclidophorid clamp sclerites can be modified and applied to Macrovalvitrematidae supports the views of Mamaev (1976) and Mamaev and Lebedev (1979) that the 2 families are closely related phylogenetically. While parts of major sclerites may be absent, e.g., sclerite a_1 of *Papillopseudotagia* and *Pseudohargisia,* the major sclerite is identifiable.

Papillopseudotagia hubbsi is the first macrovalvitrematid to be described from the gills of *C. sordidus* (a bothid flatfish) and from the eastern Pacific Ocean north of Mexico. Fourteen additional specimens of *Citharichthys* spp. examined from the Gulf of California, Mexico, and the waters off La Jolla, California, were uninfected. *Pseudotagia cupida* (Hargis, 1956) Yamaguti, 1963, from the gills of *Orthopristus chrysopterus* (L.), a haemulid pigfish from Florida, is the nearest known relative to *P. hubbsi.* Although the hosts are in different orders, they may be bentholittoral ecological equivalents. *Pseudohargisia cortesi* from the Gulf of California is most closely related to *Hargisia bairdiella* (Hargis, 1956) Yamaguti, 1963, from Florida, and their hosts are both sciaenids.

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Resurrection of *Characidotrema* Paperna and Thurston, 1968 (Monogenea: Dactylogyridae) with Description of Two New Species from Togo, Africa

D. C. KRITSKY,[1] S.-D. KULO,[2] AND W. A. BOEGER[3]

[1] Department of Allied Health Professions and Idaho Museum of Natural History,
Idaho State University, Pocatello, Idaho 83209
[2] Laboratoire de Parasitologie, Ecole des Sciences, Université du Bénin,
B.P. 1515, Lomé, Togo
[3] Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brasil, and
Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209

ABSTRACT: *Characidotrema* Paperna and Thurston, 1968, is resurrected and emended for African species previously included in *Jainus* Mizelle, Kritsky, and Crane, 1968. The haptoral armament and copulatory complex of each species of *Characidotrema* are figured, and 2 new species, *C. undifera* and *C. zelotes* spp. n., are described from *Alestes* cf. *nurse* (Rüppell) collected in Togo. *Characidotrema brevipenis* Paperna, 1969, is redescribed from material collected from *A.* cf. *nurse* in Togo, which represents a new locality record for the helminth. *Alestes jacksoni* Boulenger is considered the type host for *C. elongata* Paperna and Thurston, 1968. *Jainus longipenis* Paperna, 1973, and *J.* cf. *longipenis* of Paperna (1979) are considered junior synonyms of *C. nursei* Ergens, 1973. *Jainus brevipenis nzoiae* Paperna, 1979, *J. b. ruahae* Paperna, 1979, and *J. spinivaginus* Paperna, 1973, are transferred to *Characidotrema* as *C. nzoiae* (Paperna, 1979), *C. ruahae* (Paperna, 1979), and *C. spinivaginus* (Paperna, 1973) combs. n., respectively.

KEY WORDS: Monogenea taxonomy, morphology, systematics, *Characidotrema undifera* sp. n., *Characidotrema zelotes* sp. n., *Characidotrema brevipenis* redescribed, *Characidotrema elongatus*, *Characidotrema nursei*, *Characidotrema nzoiae* comb. n., *Characidotrema ruahae* comb. n., *Characidotrema spinivaginus* comb. n., *Jainus* spp. as synonyms, *Alestes* spp., characoid fishes.

The present study represents the first in a series dealing with selected dactylogyrid genera of African freshwater fishes. This series was initiated to develop the basis for eventual analysis of biogeographic relationships of the Ethiopian and Neotropical monogenean faunas. Similar studies on Neotropical Dactylogyridae are currently underway (see Kritsky and Thatcher, 1976, 1983; Kritsky et al., 1979, 1980, 1985, 1986a, b; Thatcher and Kritsky, 1983).

Materials and Methods

Fish hosts (5) were collected from the Mono River near Kolokopé, Togo, during November 1985. Gills were removed and placed in vials containing a 1:4,000 formalin solution; after about 1 hr, gills were agitated by vigorous shaking, and formalin concentration was increased to about 5% for preservation. Hosts were immediately preserved in 10% formalin after removal of the gills. Fish hosts and vials containing helminths were labeled and shipped to Idaho. Dactylogyrids were removed from vial sediments with the aid of a small probe and dissecting microscope and prepared for microscopy. Some specimens were mounted unstained in Gray and Wess' medium for study of sclerotized structures; others were stained with Semichon's carmalum or Gomori's trichome and mounted in Harleco synthetic resin to determine features of the internal organ systems. Illustrations were prepared with the aid of a camera lucida or microprojector. Measurements, in micrometers, were made with a filar micrometer according to the procedures of Mizelle and Klucka (1953), except that cirrus length is an approximation by using a Minerva curvimeter on camera lucida drawings; average measurements are followed by ranges in parentheses.

In addition to the parasites collected from Togo, type and voucher specimens of all previously described species were examined as follows: *Characidotrema elongata* Paperna and Thurston, 1968 (MRAC 35.569, holotype, 2 paratypes); *C. brevipenis* Paperna, 1969 (MRAC) 35.913, holotype, 3 paratypes); *Jainus brevipenis nzoiae* Paperna, 1979 (MRAC 35.715, holotype, 3 paratypes); *J. brevipenis ruahae* Paperna, 1979 (MRAC 35.716, holotype, 2 paratypes, vouchers); *J. longipenis* Paperna, 1973 (MRAC 35.918, holotype, 2 presumed paratypes, 1 voucher); *J.* cf. *longipenis* of Paperna (1979) (MRAC 35.907, voucher); *C. nursei* Ergens, 1973 (ČSAV M-282, holotype; MRAC 35.504, paratype); and *J. spinivaginus* Paperna, 1973 (MRAC 35.942, holotype). Acronyms are MRAC (Musee Royal de l'Afrique Centrale, Tervuren, Belgium) and ČSAV (Institute of Parasitology, Czechoslovak Academy of Sciences, Prague). Type specimens of new species and vouchers of *C. brevipenis* Paperna, 1969, collected during the present study were deposited in the helminthological collections of the U.S. National Museum (USNM), the University of Nebraska State Museum (HWML), the Instituto Nacional de Pesquisas da Amazônia (INPA), and the Musee Royal de l'Afrique Centrale as indicated in the respective descriptions. Fish

175

hosts were deposited in the American Museum of Natural History (AMNH 57075).

Results

Characidotrema Paperna and Thurston, 1968

EMENDED DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body robust, divisible into cephalic region, trunk, peduncle, haptor. Tegument variably developed, smooth or with ciliated tufts. Usually 2 terminal cephalic lobes poorly developed; head organs present in cephalic lobes and adjacent cephalic zones; cephalic glands present. Eyes present, usually 2 pairs. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; intestinal ceca (2) confluent posterior to testis, lacking diverticula. Gonads intercecal, partially overlapping; testis dorsoposterior to ovary. Vas deferens looping left intestinal cecum; seminal vesicle a sigmoid dilation of vas deferens; copulatory complex comprising a tubular cirrus with variably developed base and accessory piece articulated to cirrus base; prostatic reservoir anteroventral to seminal vesicle. Oviduct short; uterus delicate; vagina dextral or dextroventral in anterior trunk; seminal receptacle immediately anterior to ovary. Genital pore midventral. Vitellaria well developed into 2 bilateral bands in trunk, confluent posterior to gonads. Peduncle short; haptor poorly developed, armed with dorsal and ventral pairs of anchors, dorsal and ventral bars, 7 pairs of hooks with ancyrocephaline distribution (Mizelle, 1936). Ventral anchor with diagonally truncate point, elongate deep and superficial root; dorsal anchor shaft slightly enlarged proximally. Ventral bar with 2 bilateral anterior arms and 1 posteromedial process. Hooks similar, with undilated shanks, poorly developed thumb. Parasites of gills of African characoid fishes of the genus *Alestes* (Alestidae).

TYPE SPECIES, HOST, AND LOCALITY: *Characidotrema elongata* Paperna and Thurston, 1968, from *Alestes nurse* (Rüppell), Jinga, Lake Victoria, Uganda; also reported from *A. leuciscus* Günther, Mawli River, Volta Lake, Ghana.

OTHER SPECIES: *Characidotrema brevipenis* Paperna, 1969, from *A. nurse* and *A. baremose* (Joannis) (Ghana), from *A.* cf. *nurse* (Togo); *C.*

nursei Ergens, 1973, from *A. nurse* (Egypt, Uganda) and *A. leuciscus* Günther (Ghana); *C. nzoiae* (Paperna, 1979) comb. n. from *A. jacksoni* Boulenger (Kenya); *C. ruahae* (Paperna, 1979) comb. n. from *A. imberi* Peters (Tanzania); *C. spinivaginus* (Paperna, 1973) comb. n. from *A. nurse* (Uganda); and *C. undifera* and *C. zelotes* spp. n., both from *A.* cf. *nurse* (Togo).

Characidotrema elongata Paperna and Thurston, 1968 (Figs. 10–14)

SYNONYM: *Jainus elongatus* (Paperna and Thurston, 1968) Paperna, 1979.

HOSTS AND LOCALITIES: *Alestes jacksoni* Boulenger, Jinja, Lake Victoria, Uganda (type host and locality); *A. leuciscus* Günther, Mawli River and Volta Lake at the Black and White Volta confluence, Ghana (Paperna, 1969).

SPECIMENS STUDIED: MRAC 35.569 containing holotype and 2 paratypes.

REMARKS: The microscope slide containing the type specimens was invaded by bubbles which had filled with the dark ringing medium. As a result, the sclerotized structures of the holotype could not be observed. Thus, the figures and following observations are based on a paratype specimen present in slide ring "D".

Originally indicated by monotypy, *C. elongata* is the type species of the genus. It is characterized by possessing a short robust accessory piece which encircles the shaft of the cirrus with short terminal projections (Fig. 10). It closely resembles *C. nursei* from which it differs by lacking a well-developed distal flange on the cirral base. The presence of a posteromedial projection of the ventral bar (dorsal bar of Paperna and Thurston, 1968) could not be confirmed, although a suggestion of this structure is apparent in the paratype. Measurements of paratype "D" follow: cirrus 41; accessory piece 15; ventral anchor length 16, base width 10; dorsal anchor length 23, base width 8; ventral bar 12; dorsal bar 16.

Because of the poor condition of the slide, the vagina could not be observed in the paratype, but Paperna and Thurston (1968) indicate a sinistral vagina in the original diagnosis of *Characidotrema*. However, these authors mistakenly

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Figures 1–9. *Characidotrema brevipenis* Paperna, 1969. 1. Whole mount (ventral). 2. Vagina. 3. Hook. 4, 5. Copulatory complexes. 6. Ventral bar. 7. Dorsal bar. 8. Ventral anchor. 9. Dorsal anchor. All figures are based on specimens collected from Togo and are drawn to the same scale (30 μm) except Figure 1 (75 μm).

C. elongata

C. brevipenis

C. nursei

C. nursei

C. nursei

C. nzoiae

C. ruahae

C. spinivaginus

C. zelotes
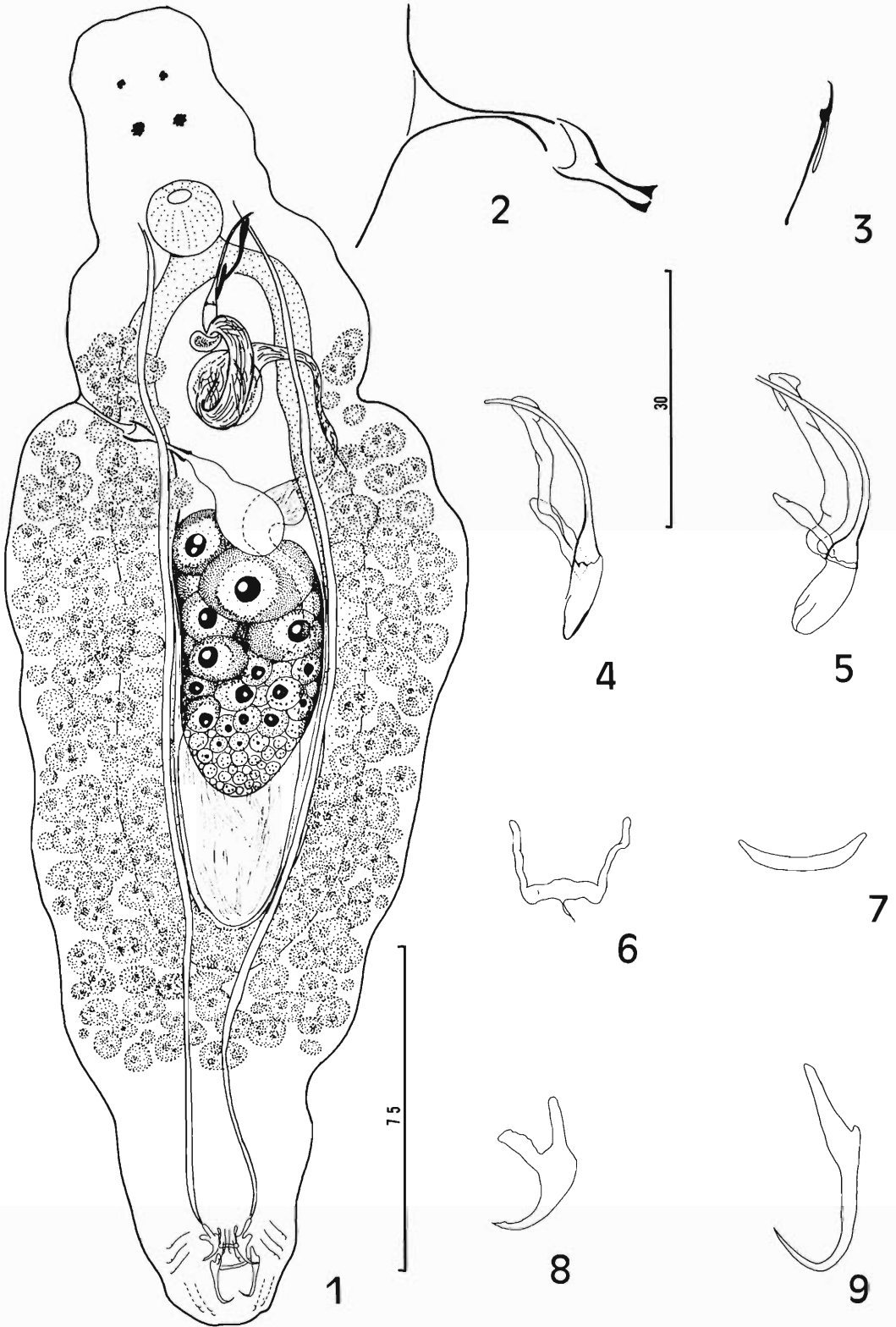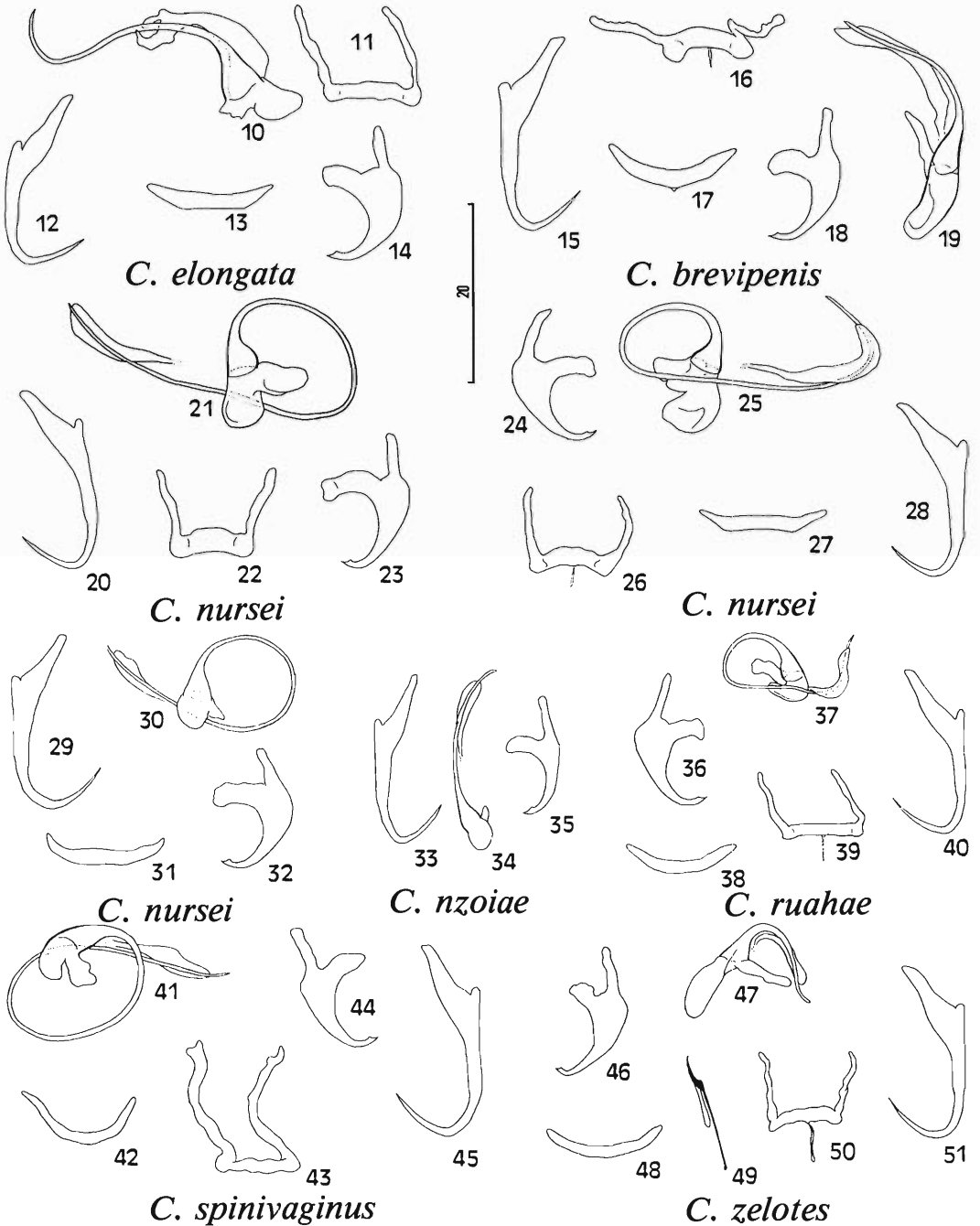
Figures 10–51. Sclerotized parts of *Characidotrema* species. Figures 10–14. *Characidotrema elongata* Paperna and Thurston, 1968, based on paratype (MRAC 35.569). 10. Copulatory complex. 11. Ventral bar. 12. Dorsal anchor. 13. Dorsal bar. 14. Ventral anchor. Figures 15–19. *Characidotrema brevipenis* Paperna, 1969, based on holotype (MRAC 35.913). 15. Dorsal anchor. 16. Ventral bar. 17. Dorsal bar. 18. Ventral anchor. 19. Copulatory complex. Figures 20–23. *Characidotrema nursei* Ergens, 1973, based on holotype (ČSAV M-282). 20. Dorsal anchor. 21. Copulatory complex. 22. Ventral bar. 23. Ventral anchor. Figures 24–28. *Characidotrema nursei* Ergens, 1973, based on holotype of *Jainus longipenis* Paperna, 1973 (MRAC 35.918). 24. Ventral anchor. 25. Copulatory complex. 26. Ventral bar. 27. Dorsal bar. 28. Dorsal anchor. Figures 29–32. *Characidotrema nursei*

had the dorsoventral axis reversed as indicated in their description of the haptor of *C. elongata.* This suggests that the vagina actually opens on the right body margin in *C. elongata,* as it does in all other species of *Characidotrema* in which type material permitted verification.

Paperna and Thurston (1968) list *Alestes nurse* as the type host for this species. However, Paperna's (1979) report of *C. elongata,* which is based on the type specimens, gives *A. jacksoni* as its host, and the slide containing the holotype and paratypes indicates the latter host. Greenwood (1959) has shown that the *Alestes* populations previously referred to *A. nurse* from Lake Victoria comprise the species *A. jacksoni,* while Géry (1977) suggests that *A. jacksoni* is a possible synonym of *A. imberi* (=*Brycinus imberi*). In either case, we consider the type host of *C. elongata* to be the *Alestes* species from Lake Victoria and its drainages assigned to *A. jacksoni* by Greenwood (1959).

Characidotrema brevipenis Paperna, 1969
(Figs. 1–9, 15–19)

SYNONYM: *Jainus brevipenis* (Paperna, 1969) Paperna, 1979.

HOSTS AND LOCALITIES: *Alestes nurse* (Rüppell) (type host), Volta Lake at Kete Krachi, at Yeji (type locality), and at the Black and White Volta confluence, Ghana; *A. baremose* (Joannis), Volta Lake at Yeji, Ghana (Paperna, 1969); *A.* cf. *nurse,* Mono River, Kolokopé, Togo (new locality record).

SPECIMENS STUDIED: MRAC 35.913 containing holotype and 3 paratypes; 22 vouchers from Togo (USNM 79408; HWML 23555; INPA PA289-1,2; MRAC 37.112).

REDESCRIPTION (based on specimens from Togo): Body foliform, 317 (217–425) long; greatest width 91 (59–125) near midlength or in anterior half. Tegument smooth. Cephalic margin rounded or truncate, lobes poorly developed or absent; head organs, cephalic glands indistinct. Eyes equidistant, members of posterior pair larger than anterior pair; eye granules ovate to subspherical; accessory granules present in cephalic region and anterior trunk. Pharynx spherical, 19 (13–22) in diameter. Peduncle tapered posteriorly, broad; haptor subhemispherical, 27 (22–32) long, 32 (22–37) wide. Ventral anchor 19 (17–21) long, base 10–11 wide; dorsal anchor 26 (24–27) long, base 9 (8–10) wide. Bilateral arms of ventral bar delicate, posteromedial projection small; ventral bar 9–10 long. Dorsal bar 17 (16–19) long, simple, with tapered ends. Hook point delicate, thumb subtriangular; hook 17 (15–18) long; FH loop 0.5 shank length. Cirrus comprising curved shaft, ellipsoidal base with elongate distal projection; cirrus 39 (38–40) long; accessory piece 22 (20–24) long, variable, with slight terminal expansion. Testis subovate, 52 (49–56) × 30 (24–36); seminal vesicle with thick muscular wall proximally. Ovary subovate, 84 (45–144) × 25 (17–32); vagina at level of seminal vesicle, comprising a dumbbell-shaped tubular sclerite; vitellaria composed of relatively large cellular masses extending from level of copulatory complex to peduncle.

REMARKS: Comparison of our specimens with the holotype of *C. brevipenis* confirms that all are conspecific. Measurements of the sclerites of the holotype fall within ranges reported herein for specimens collected from Togo, while ranges reported by Paperna (1969, 1979) for the ventral and dorsal anchors and the ventral and dorsal bars of the species do not include the corresponding values of the holotype. Dimensions of the sclerites of the holotype follow (Paperna's [1979] values are in parentheses): cirrus 38 (20–40); ventral anchor length 18 (30–40), base width 10 (none provided); dorsal anchor length 24 (35–40), base width 8 (none provided); ventral bar 13 (18); dorsal bar 16 (20).

Characidotema brevipenis is related to *C. nzoiae*

←

Ergens, 1973, based on a voucher identified as *Jainus* cf. *longipenis* by Paperna (1979) (MRAC 35.907). 29. Dorsal anchor. 30. Copulatory complex. 31. Dorsal bar. 32. Ventral anchor. Figures 33–35. *Characidotrema nzoiae* (Paperna, 1979) comb. n. based on holotype of *Jainus brevipenis nzoiae* Paperna, 1979 (MRAC 35.715). 33. Dorsal anchor. 34. Copulatory complex. 35. Ventral anchor. Figures 36–40. *Characidotrema ruahae* (Paperna, 1979) comb. n. based on holotype of *Jainus brevipenis ruahae* Paperna, 1979 (MRAC 35.716). 36. Ventral anchor. 37. Copulatory complex. 38. Dorsal bar. 39. Ventral bar. 40. Dorsal anchor. Figures 41–45. *Characidotrema spinivaginus* (Paperna, 1973) comb. n. based on holotype of *Jainus spinivaginus* Paperna, 1973 (MRAC 35.942). 41. Copulatory complex. 42. Dorsal bar. 43. Ventral bar. 44. Ventral anchor. 45. Dorsal anchor. Figures 46–51. *Characidotrema zelotes* sp. n. 46. Ventral anchor. 47. Copulatory complex. 48. Dorsal bar. 49. Hook. 50. Ventral bar. 51. Dorsal anchor. All drawings are to the 20-μm scale.

comb. n., *C. undifera* sp. n., and *C. zelotes* sp. n. It differs from *C. nzoiae* by having a more elongate cirrus base, and from *C. undifera* and *C. zelotes* by possessing a cirral shaft with a generally smooth curve.

Characidotrema nursei Ergens, 1973
(Figs. 20–32)

SYNONYMS: *Jainus longipenis* Paperna, 1973; *Jainus* cf. *longipenis* of Paperna (1979); *Jainus nursei* (Ergens, 1973) Paperna, 1979.

HOSTS AND LOCALITIES: *Alestes nurse* (Rüppell), Nile River, Cairo, Egypt (type host and locality); *A. nurse,* Lake Albert, Uganda (Paperna, 1973, 1979); *A. leuciscus* Günther, Volta Lake and Mawli River, Ghana (Paperna, 1979).

SPECIMENS STUDIED: ČSAV M-282, holotype; MRAC 35.504, paratype; MRAC 35.918, holotype, 2 paratypes, 1 voucher of *Jainus longipenis* Paperna, 1973; MRAC 35.907, voucher of *Jainus* cf. *longipenis* of Paperna (1979).

REMARKS: Independently and apparently without knowledge of the other, Ergens (1973) described *Characidotrema nursei* from Egypt and Paperna (1973) proposed *Jainus longipenis* from Uganda. Examination of the holotypes of each of these species confirms their conspecificity (compare Figs. 20–23, 24–28). Since *Characidotrema nursei* (30 March 1973) has priority over *longipenis* (28 September 1973), *J. longipenis* is considered a junior subjective synonym of *C. nursei.*

Ergens' (1973) description of the sclerites of this species is accurate and is the first to have depicted the nature of the point of the ventral anchor. Indeed, our examination of all previously described species in the genus and the 2 new species described herein indicates that the unique ventral anchor point could be sufficiently constant to be considered a generic character. The diagonally truncate or scoop-shaped point of the ventral anchor, therefore, has been incorporated as a diagnostic trait in the emended diagnosis. Measurements of the haptoral sclerites of the types of *C. nursei* and *J. longipenis* fall within the ranges provided by Ergens (1973) except for the total length of the cirrus (68–71, nobis).

Paperna (1979) reported *Jainus* cf. *longipenis* from *Alestes leuciscus* in Ghana. The specimen studied indicates that it is similar to *C. nursei* in morphology of haptoral and copulatory sclerites;

it differs from *C. nursei* in being somewhat smaller (compare Figs. 20–23, 29–32). The tubular vagina, characteristic of *C. nursei,* is absent. Measurements of the voucher include: cirrus 50; dorsal anchor length 21, base width 8; ventral anchor length 16, base width 10; dorsal bar 14. Because of these differences, we provisionally include this specimen in *C. nursei* until the form is restudied from *A. leuciscus.*

Characidotrema nzoiae
(Paperna, 1979) comb. n.
(Figs. 33–35)

SYNONYM: *Jainus brevipenis nzoiae* Paperna, 1979.

HOST AND LOCALITY: *Alestes jacksoni* Boulenger, Nzoia River (Lake Victoria system), Kenya.

SPECIMENS STUDIED: MRAC 35.715 containing holotype and 3 paratypes.

REMARKS: The microscope slide containing the type specimens of this form was provided with 3 circular coverslips, each overlying 1 or 2 specimens of the species. The second (center) coverslip had a typed label partially overlying it which indicated the center specimen as the holotype. This specimen most closely conforms to the body shape of the species depicted in the wholemount figure (Plate XL) of Paperna (1979), except that the haptor is folded ventrally over the trunk, imparting a foreshortened specimen (not shown in the original drawing). All type specimens available for study were stained and mounted in resin, which precluded complete determination of the sclerotized parts. The ventral anchor of the holotype was not visible in a single microscopic plane, resulting in a foreshortened basal width (Fig. 35). The vagina opens on the right margin of the anterior trunk.

Although the copulatory complex shows some similarity to that of *Characidotrema brevipenis,* the size and shape of the base indicates that these specimens should be considered a separate species. Its closest relative is likely *C. brevipenis* as shown by the morphology of the cirral shaft, but the small cirral base is considered sufficiently different to warrant elevation of the form to separate specific status. This species is in need of redescription, which will depend on the collection of fresh material prepared to show internal anatomy and morphology of the haptoral and copulatory sclerites.
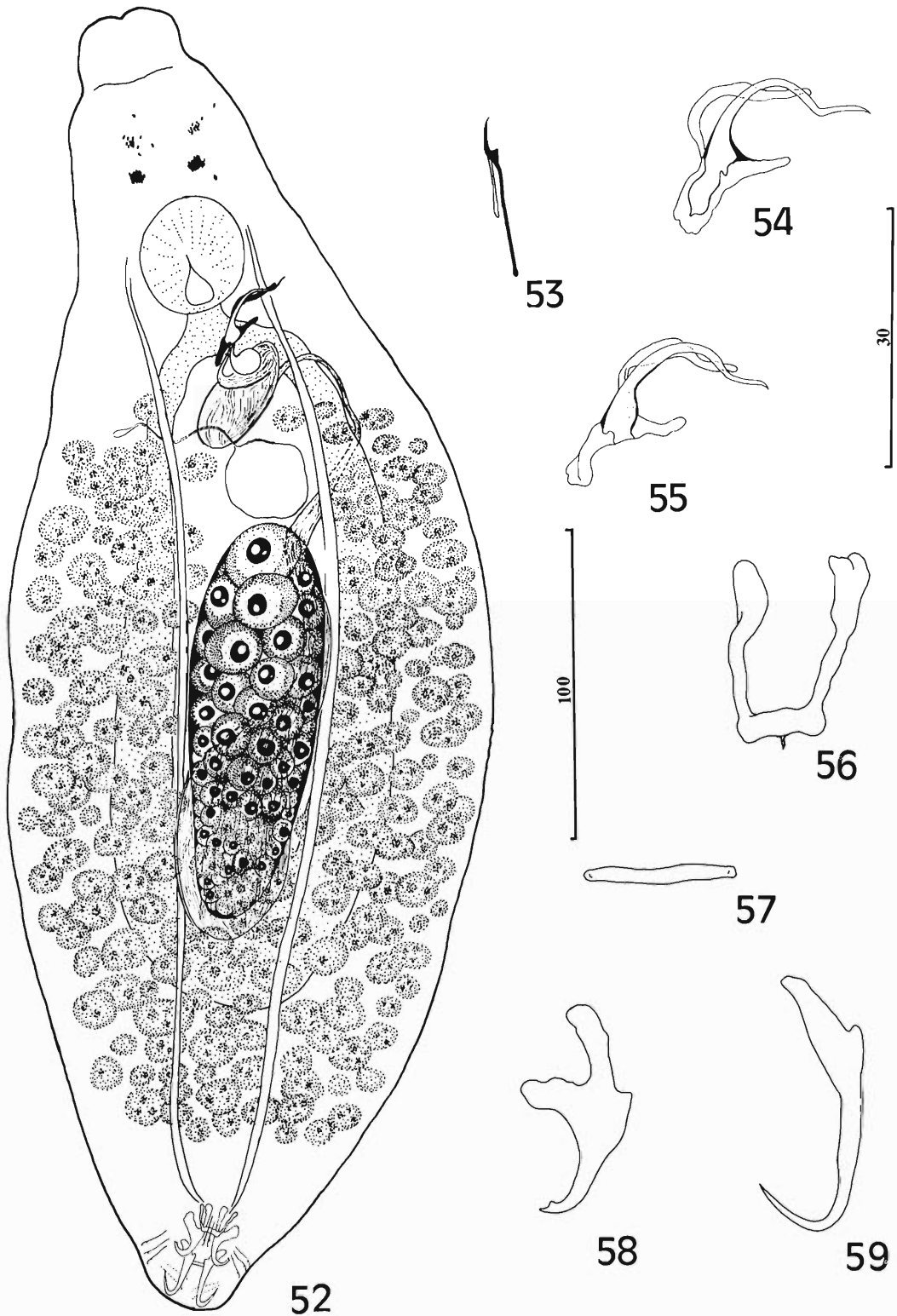
Figures 52–59. *Characidotrema undifera* sp. n. 52. Holotype (ventral). 53. Hook. 54, 55. Copulatory complexes. 56. Ventral bar. 57. Dorsal bar. 58. Ventral anchor. 59. Dorsal anchor. All figures are drawn to the 30-µm scale except Figure 52 (100 µm).

181

Characacidotrema ruahae
(Paperna, 1979) comb. n.
(Figs. 36–40)

SYNONYM: *Jainus brevipenis ruahae* Paperna, 1979.

HOST AND LOCALITY: *Alestes imberi* Peters, Ruaha River, Tanzania.

SPECIMENS STUDIED: MRAC 35.716 containing the holotype and 2 paratypes.

REMARKS: This species is characterized by possessing a small cirrus with a base provided with 2 sclerotized flanges. The proximal flange is bent anteriorly, and the distal flange is elongate. Based on the morphology of the cirrus, this species is intermediate between *C. nursei* and *C. brevipenis* by having a coiled cirral shaft like that of *C. nursei* and a cirral base with 2 well-developed flanges similar to those of *C. brevipenis*. The differences in the cirral base are considered sufficient to raise this form to specific rank, since cirral morphology is the most apparent morphological character distinguishing species in the genus. Measurements of the haptoral and copulatory sclerites of the holotype fall within the ranges provided by Paperna (1979); the vagina was not observed.

Characacidotrema spinivaginus
(Paperna, 1973) comb. n.
(Figs. 41–45)

SYNONYM: *Jainus spinivaginus* Paperna, 1973.

HOST AND LOCALITIES: *Alestes nurse* (Rüppell), Lake Albert, Uganda (type) and Volta Lake, Ghana.

SPECIMEN STUDIED: MRAC 35.942 containing the holotype.

REMARKS: The spinous vaginal aperture depicted by Paperna (1973) distinguishes *C. spinivaginus* from all other species in the genus. The species is most closely related to *C. nursei* as shown by the comparative morphology of the copulatory complex. However, it can be separated further from *C. nursei* by possessing longer anterior projections on each end of the ventral bar. Paperna did not present drawings of the haptoral sclerites which are presented here for the first time (Figs. 42–45); measurements of the holotype fall within ranges presented by Paperna (1973). Although visible, the position of the vagina could not be determined in the twisted holotype. The microscope slide containing the holotype had numerous specimens of *C. nursei*,

which could easily be separated from *C. spinivaginus* by the morphology of the vagina.

Characacidotrema undifera sp. n.
(Figs. 52–59)

HOST AND LOCALITY: *Alestes* cf. *nurse* (Rüppell), Mono River, Kolokopé, Togo.

TYPE SPECIMENS: Holotype, USNM 79404; paratypes, USNM 79405, HWML 23553, INPA PA291-1,2, MRAC 37.111.

DESCRIPTION (based on 18 specimens): Body spindle-shaped, 401 (303–499) long; greatest width 163 (140–238) near midlength. Cephalic region with 2 terminal, poorly developed cephalic lobes; head organs, cephalic glands indistinct. Eyes equidistant, anterior pair frequently dissociated; eye granules ovate; accessory granules present in cephalic region. Pharynx spherical, 34 (27–41) in diameter. Peduncle rapidly tapering posteriorly; haptor indistinct, appearing as simple extension of peduncle. Ventral anchor with robust basal projection; anchor 26 (23–29) long, base 13 (11–14) wide. Dorsal anchor 30 (28–31), base 10 (9–12) wide. Bilateral arms of ventral bar elongate, well developed; posteromedial projection short, indistinct; ventral bar 15–16 long. Dorsal bar rod-shaped, 15 (14–16) long. Hook delicate, with curved point, subtriangular thumb; hook 18 (14–20) long; FH loop 0.5 shank length. Cirrus comprising a curved shaft with subterminal angular bend, large base with well-developed anterior and posterior flanges; cirrus 34 (33–35) long. Accessory piece 15 (13–18) long, curved, variable. Gonads subovate; testis 72 (53–85) × 38 (27–39); ovary 123 (75–150) × 39 (28–45). Vagina dextroventral, a delicate sclerotized tube with slight distal enlargement; vitellaria comprising large cellular masses extending in 2 bilateral zones from level of seminal vesicle to peduncle.

REMARKS: *Characacidotrema undifera* most closely resembles *C. zelotes* sp. n. in the general morphology of the copulatory complex. It differs from *C. zelotes* by possessing (1) larger haptoral sclerites, (2) an obvious subterminal bend of the cirrus shaft, and (3) a larger body size. The specific name is from Latin (*undifera,* =wave bearing), and refers to the shape of the cirral shaft.

Characacidotrema zelotes sp. n.
(Figs. 46–51)

HOST AND LOCALITY: *Alestes* cf. *nurse* (Rüppell), Mono River, Kolokopé, Togo.

TYPE SPECIMENS: Holotype, USNM 79406; paratypes, USNM 79407, HWML 23554, INPA PA290-1,2, MRAC 37.110.

DESCRIPTION (based on 15 specimens): Body foliform, 216 (169–250) long; greatest width 111 (92–136) near midlength or in posterior half. Cephalic margin rounded or with 2 poorly developed terminal lobes; cephalic glands, head organs indistinct. Eyes equidistant, posterior pair larger; eye granules elongate ovate to subspherical; accessory granules occasionally present in cephalic area and anterior trunk. Pharynx spherical, 13–14 in diameter. Peduncle almost nonexistent; haptor an extension of peduncle or trunk. Ventral anchor 16 (13–18) long, base 9 (7–10) wide; dorsal anchor 22 (20–23), base 8 (7–9) wide. Bilateral arms of ventral bar delicate, posteromedial projection elongate; ventral bar 10 (9–11) long. Dorsal bar 15 (14–17) long, rod-shaped, with slightly tapered ends. Hook 15 (13–17) long, delicate, with fine point, indistinct thumb; FH loop 0.5 shank length. Cirrus 28–29 long, comprising a tapered shaft in shape of interrogation point, enlarged base with large proximal and distal flanges. Accessory piece 13 (11–14) long, club-shaped, apparently articulated to cirrus base. Gonads overlapping, ovate to pyriform; testis 49 (45–53) × 33 (25–40); ovary 72 (64–79) × 34 (29–38). Seminal vesicle bulbous; prostatic reservoir with heavy wall. Vagina unsclerotized, a simple tube opening dextroventrally at level of seminal vesicle; vitellaria well developed, absent from cephalic and haptoral regions.

REMARKS: The closest relative of this species is *Characidotrema undifera* sp. n., based on comparative morphology of the copulatory complex and haptoral sclerites. Features distinguishing these species are given in the remarks for *C. undifera*. The specific name is from Greek (*zelotes*, =an emulator) and refers to the similarity of this diminutive species with the larger *C. undifera*.

Discussion

Soon after its proposal, *Characidotrema* Paperna and Thurston, 1968, was placed in synonymy with the neotropical *Jainus* Mizelle, Kritsky, and Crane, 1968, by Paperna (1973). This synonymy is not without merit since members of both genera possess many similar and somewhat unique characteristics: (1) robust bodies with poorly developed peduncles and haptors; (2) modified ventral anchor-bar complexes; (3) overlapping gonads; (4) strongly developed vitellaria; and (5) both taxa are restricted as parasites of characoid fishes. The synonymy has gone unchallenged in the literature, with Gussev (1976a, b, 1978) using Paperna's (1973) proposal of congeneric Neotropical and Ethiopian species as evidence for an ancient evolutionary relationship between the monogenean faunas of the 2 biogeographical regions. Indeed, Gussev (1976b) points, in part, to *Jainus* (*Characidotrema* + *Jainus* sensu stricto) as evidence that monogenean distributions are better explained by mechanisms of continental drift than by the "landbridge" theories of Darlington (1957).

Our resurrection of *Characidotrema* does not challenge Gussev's ideas on monogenean biogeography. At most, it suggests that vicariant speciation has occurred since the breakup of Gondwanaland with speciation events in this group of Monogenea progressing at a similar or slightly slower pace that that of their hosts. *Characidotrema* and *Jainus* will likely be shown to be sister groups that developed from a common ancestral group present in Gondwanaland prior to separation of the African and South American continents.

Our rationale for recognizing *Characidotrema* rests primarily on information obtained from the reexamination of the type specimens of previously described species. All of these species as well as the new species described herein possess a relatively uniform morphology of the haptoral sclerites, which is fundamentally different from that of Neotropical species of *Jainus*. Although the ventral anchor is highly modified in both genera, that of *Characidotrema* species possesses a diagonally truncate or scoop-shaped point and an anterior projection developed from the deep root of the base. In *Jainus* species, the ventral anchor point is never developed into a diminutive scoop, although it is frequently modified into a bladelike structure; modification of the ventral anchor base usually includes both the superficial and deep roots. Further, Neotropical *Jainus* species do not show the development of bilateral anterior projections of the ventral bar found in *Characidotrema* species, although some do exhibit anteromedial and/or posteromedial processes. The hooks of *Characidotrema* species lack a well-developed thumb and dilated shanks, while those of *Jainus* species possess a protruding thumb and shanks may be dilated. Features of the internal organ systems of species of both genera are strikingly similar, but the vagina of *Jainus*

is always sinistral and that of *Characidotrema* species is dextral.

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Dermophthirius penneri sp. n. (Monogenea: Microbothriidae) an Ectoparasite of Carcharhinid Sharks, Carcharhinus brevipinna and Carcharhinus limbatus

GEORGE W. BENZ

Department of Zoology, 6270 University Blvd., The University of British Columbia,
Vancouver, British Columbia V6T 2A9, Canada

ABSTRACT: *Dermophthirius penneri* sp. n. (Monogenea: Microbothriidae) is described from specimens collected from 2 species of carcharhinid sharks, *Carcharhinus brevipinna* (Müller and Henle, 1839) in the eastern Gulf of Mexico off Sarasota County, Florida, and *C. limbatus* (Valenciennes, 1839) in the western North Atlantic off New Jersey. *Dermophthirius penneri* sp. n. is most easily distinguished from its congeners in having a cirrus equipped solely with proximal armature.

KEY WORDS: Microbothriinae, taxonomy, morphology, spinner shark, blacktip shark, Gulf of Mexico, North Atlantic.

The genus *Dermophthirius* MacCallum, 1926 (Microbothriidae Price, 1936) contains 3 known species; *D. carcharhini* MacCallum, 1926, *D. maccallumi* Watson and Thorson, 1967, and *D. nigrellii* Cheung and Ruggieri, 1983. The genus has been recorded from the Gulf of Mexico (Thatcher, 1959; Benz, unpubl. data); Florida Keys (Cheung and Ruggieri, 1983; Benz, unpubl. data); Rio San Juan at San Carlos, Nicaragua (Watson and Thorson, 1976); western North Atlantic (MacCallum, 1926; Cheung and Ruggieri, 1983; Rand et al., 1986; Benz, unpubl. data); and eastern Central Atlantic (Euzet and Maillard, 1967). *Dermophthirius* species are ectoparasites of sharks, and typically purchase the crown of a placoid scale. This paper describes a new species of *Dermophthirius* collected from the spinner shark by Dr. L. R. Penner and from the blacktip shark by myself.

Materials and Methods

Sharks were captured by hook and line. Parasites were removed with forceps, fixed in warm AFA (alcohol–formalin–acetic acid) under light coverslip pressure, and stored in 70% ethanol. Fixed parasites were stained with Mayer's acid carmine or Delafield's hematoxylin, dehydrated in a graded ethanol series, cleared in toluene, and mounted in neutral Canada balsam. Three specimens were serially sectioned (at 8-μm intervals; frontal, sagittal, and transverse planes) on a rotary microtome after being dehydrated through a graded ethanol series and embedded in paraffin. After processing, sectioned material was stained with Mayer's hematoxylin and eosin, cleared, and permanently mounted through use of standard histological techniques. Prepared specimens were examined under a compound microscope, and illustrations were made with the aid of a camera lucida. Common and scientific names of hosts are in accordance with Compagno (1984).

Dermophthirius penneri sp. n.
(Figs. 1–5)

MATERIAL EXAMINED: Fifteen whole mounts and 3 serially sectioned individuals. En toto holotype (No. 79664) and 2 paratypes (No. 79665) deposited in the U.S. National Museum (USNM) Helminthological Collection. Additional paratypes in author's collection.

HOSTS AND LOCALITIES: *Carcharhinus brevipinna* (Müller and Henle, 1839), spinner shark (3 specimens)—captured eastern Gulf of Mexico off Sarasota County, Florida, during summer 1965; *C. limbatus* (Valenciennes, 1839), blacktip shark (2 specimens)—captured western North Atlantic off New Jersey during summer 1980. Holotype from spinner shark.

ATTACHMENT SITE: Body surface, mostly along dorsum just below trailing tip of first dorsal fin, few individuals found on sides of caudal fin. All specimens purchasing crowns of placoid scales.

ETYMOLOGY: The species epithet honors my good friend and advisor, the late Dr. Lawrence R. Penner (Professor of Parasitology, University of Connecticut).

DESCRIPTION: Body (Fig. 1) elongate, ovoid. Holotype 3.5 mm long, 2.1 mm wide; paratypes 2.0–2.7 mm long, 1.0–1.4 mm wide. Eyes absent. Nervous system not seen. Oral aperture opens subterminally, flanked by prohaptor consisting of 2 small bothridia. Prepharynx short. Pharynx muscular, residing in pharyngeal atrium and seemingly capable of being protruded through
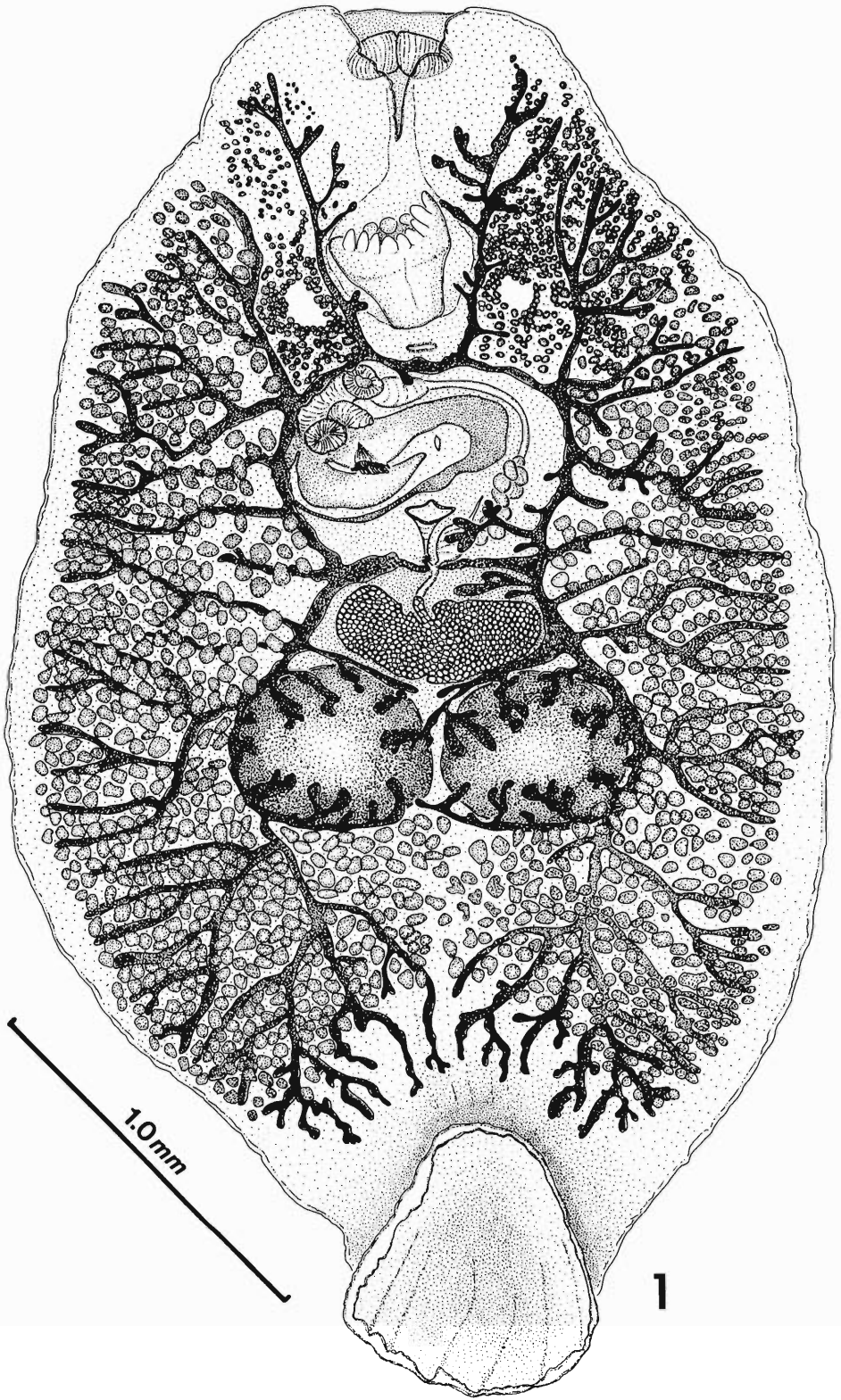
185

Figure 1. *Dermophthirius penneri* sp. n. Holotype en toto, ventral view.

186

oral aperture onto feeding surface. Anterior portion of pharynx spacious and rimmed with approximately 12–14 digitiform papillae. Pharynx leads to short esophagus, then to intestine. Intestine composed of 2 main longitudinal crura, each usually with 11 dendritic lateral diverticula and several smaller medial diverticula. An excretory vesicle lies at level of pharynx between first and second lateral diverticula on each side of body. Follicular vitellaria densely fill space between lateral diverticula and extend just short of body periphery, but are not found about opisthaptor, oral aperture, or overlying testes, ovary, genital region, or excretory vesicles. Opisthaptor unarmed, shallow cuplike structure located ventrally at posterior of body and often exhibiting several furrows presumably corresponding to region of former contact with ridges on crown of host's placoid scale. Genital region consisting of female and male reproductive tracts (Fig. 2), bounded anteriorly and laterally by intestinal crura and posteriorly by ovary. Ovary median, transversely elongate. Oviduct leaves ovary anteriorly, joining bulblike seminal receptacle and common transverse vitelline duct, then proceeding anteriorly as ootype to uterus. Mehlis' gland found at base of ootype. Uterus triangular in cross section, eggs tripolar (approximately 189 μm long tip to tip). Vagina a thin tube leading to seminal receptacle. Two ovoid testes juxtaposed just posterior to ovary. Vas deferens tortuously coiled just anterior and sinistral to ovary, then continuing as straight tube to prostate gland. Prostate gland (Fig. 3) a multi-atrial structure exhibiting striated appearance due to presence of numerous large columnar cells. Cirrus sac present (Fig. 3). Cirrus (Fig. 3) a muscular papilla, thicker proximal region armed with what appears to be 2 ranks of blunt spines (Fig. 4). Exact number of spines in each rank difficult to determine due to tight packing, however, at least 33 and 9 spines, respectively, compose ventral and dorsal ranks. In some views, optical interference created by the 2 closely applied ranks causes the entire proximal armature to appear as an unorganized cluster of many blunt spines. Genital aperture unarmed, located sinistrally beyond cirrus tip.

Discussion

The most recent authoritative review (Price, 1963) considers Microbothriidae to contain 3 subfamilies: Pseudocotylinae, Microbothriinae,

and Dermophthiriinae. The subfamily Dermophthiriinae contains 2 genera, *Dermophthirius* and monotypic *Neodermophthirius* Price, 1963. *Dermophthirioides* Cheung and Nigrelli, 1983, shares many Dermophthiriinae characteristics (e.g., highly convoluted vas deferens, uterus triangular in cross section, compressed ovary), although it is regarded as a member of Microbothriinae based on its total lack of cirrus armature (Cheung and Nigrelli, 1983). *Dermophthirius penneri* sp. n. differs most notably from its congeners, *Neodermophthirius* and *Dermophthirioides,* regarding cirrus structure. Only *D. penneri* sp. n. and *Dermophthirioides pristidis* lack distal cirrus armature. *Dermophthirioides pristidis,* however, also lacks proximal cirrus armature whereas *D. penneri* sp. n. has proximal armature in the form of 2 ranks of blunt-tipped spines. Additionally, the proximal cirrus armature of *D. penneri* sp. n. differs markedly from those of *Neodermophthirius harkemai* (see Price, 1963) and other *Dermophthirius* species (see MacCallum, 1926; Watson and Thorson, 1976; Cheung and Ruggieri, 1983) regarding number of spines and overall shape.

Microbothriid species tend to be host specific, and they remain stenoxenous even under confined captive conditions presumably affording ample opportunity to infest nonnatural, but seemingly closely related, elasmobranchs (e.g., see Cheung and Ruggieri, 1983). It is, therefore, interesting when a microbothriid species is reported from more than 1 host species. Such is the case for *D. penneri* sp. n., however, the hosts (*Carcharhinus brevipinna* and *C. limbatus*) must be considered closely allied congeners amongst the some 29 species comprising the genus *Carcharhinus* (see Garrick, 1982) as evidenced by the general difficulty in distinguishing these 2 species (Branstetter, 1982; Castro, 1983; Compagno, 1984). *Dermophthirius carcharhini* (the only other *Dermophthirius* species reported from more than 1 host species) has been collected from 5 *Carcharhinus* species: *C. altimus* (Springer, 1950) (Benz, unpubl. data), *C. galapagensis* (Snodgrass and Heller, 1905) (see Rand et al., 1986), *C. limbatus* (see Thatcher, 1959), *C. brevipinna* (see Euzet and Maillard, 1967), and *C. obscurus* (LeSueur, 1818) (see Cheung and Ruggieri, 1983; also Benz, unpubl. data). *Carcharhinus altimus, C. galapagensis,* and *C. obscurus* are easily confused with one another (Castro, 1983), and presumably have close phylogenetic
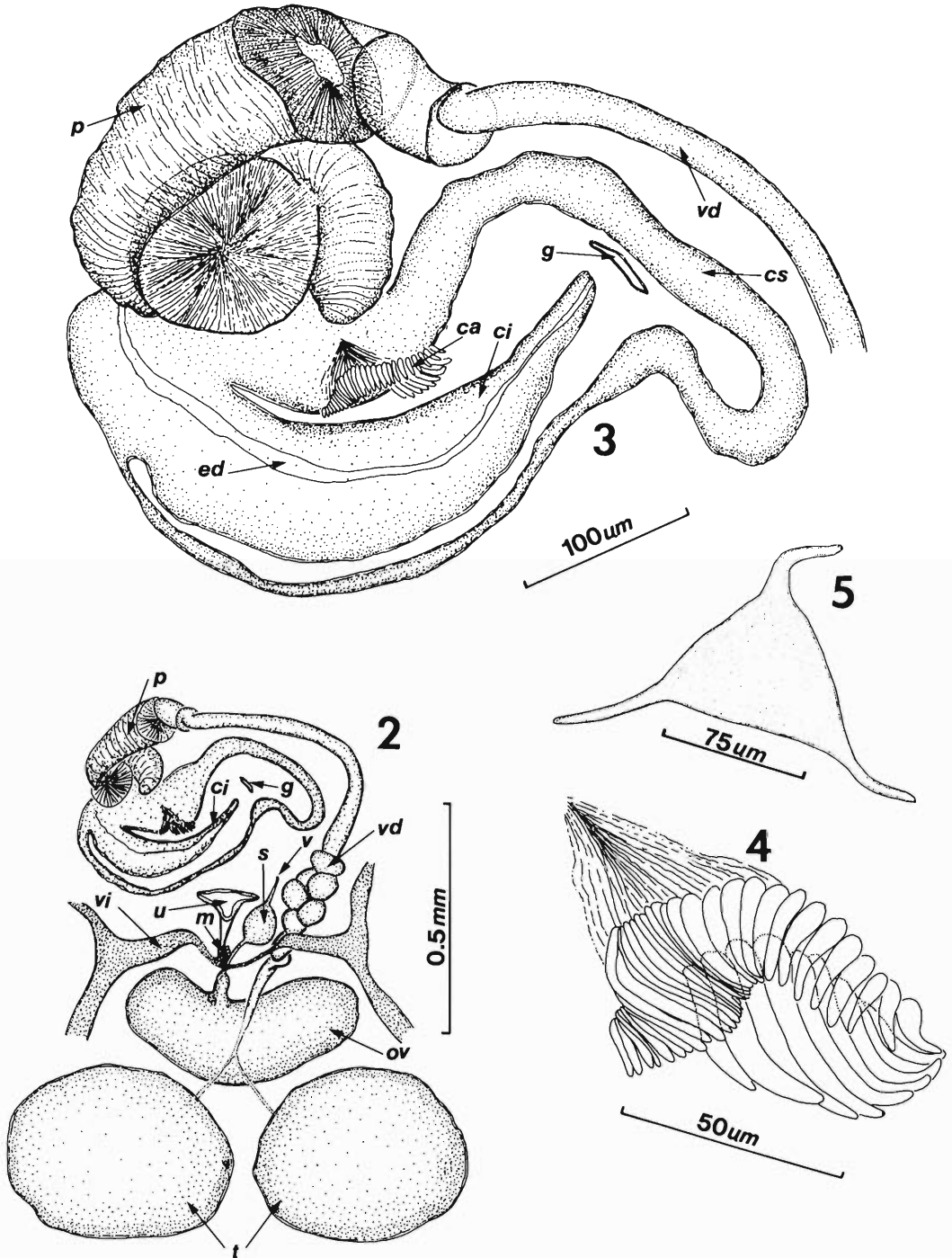
Figures 2–5. *Dermophthirius penneri* sp. n. 2. Male and female reproductive systems (ventral view). 3. Cirrus sac (ventral view). 4. Proximal cirrus armature (ventral view). 5. Egg. ci = cirrus, ca = cirrus armature, cs = cirrus sac wall, ed = ejaculatory duct, g = genital aperture, m = Mehlis' gland, ov = ovary, p = prostate gland complex, s = seminal receptacle, t = testes, u = uterus, v = vaginal aperture, vd = vas deferens, vi = vitelline duct. Drawn from holotype with minor interpretation from en toto and sectioned paratypes.

affinities. *Carcharhinus limbatus* and *C. brevipinna,* however, appear taxonomically distinct from the foregoing species (see Garrick, 1982; Castro, 1983; Compagno, 1984), and while host taxonomic distinction may have little influence on host specificity it is interesting that Cheung and Ruggieri (1983) noted that infestation with *D. carcharhini* was confined to *Carcharhinus obscurus* even though *C. limbatus* resided in the same aquarium tank. This situation becomes even more problematic given the propensity for *Dermophthirius* infestations to rapidly spread to all individuals of susceptible host species under captive situations (Cheung et al., 1982; Cheung and Ruggieri, 1983). Because of the apparent incongruence between Thatcher's (1959) host record and Cheung and Ruggieri's (1983) aquarium observations, and in light of this report and the high degree of host specificity exhibited by other microbothriids, I suggest the specimens identified as *D. carcharhini* by Thatcher (1959) may have been *D. penneri* sp. n. Similarly, in their description of *D. carcharhini* from *C. brevipinna,* Euzet and Maillard (1967) stated that the cirrus had but a single group of spines and surface mammilations. Given the specific distinctiveness of cirrus armature within the genus *Dermophthirius* and the notable similarity of their description to that given herein, I also suggest that the record by Euzet and Maillard (1967) may refer to *D. penneri* sp. n.

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New Format for Research Notes

Several problems associated with Research Notes have become apparent over the years. Because they lack an abstract, some indexing and abstracting services ignore them. Incomplete literature citations provided in the text hamper reading and literature retrieval. We have decided to adopt a format for Research Notes that will avoid these deficiencies. Accordingly, beginning in the January, 1988 Proceedings, Research Notes will include an Abstract and a Literature Cited section. Literature citations will be handled as in full papers; journal titles will be unabbreviated. Key words will follow the abstract. The format will be unchanged otherwise without the major headings used in full papers.

Revised Format for Key Words

In order to expedite the computerized preparation of the Subject Index for the Proceedings, authors are requested to include 6–12 key words or short phrases following the Abstract (on the same page) in all manuscripts (full papers as well as research notes). Key words included in the title should be repeated in the Key Word list. Useful words for the subject index include: scientific and common names of parasites and hosts; taxonomic group names; name or class name of chemical or biological reagent; scientific field such as systematics or immunology; type of study such as survey, case report or redescription; geographic region; or any other useful term or label.

Partial Life Cycle and Fish Hosts of *Bolbogonotylus corkumi* gen. et sp. n. and *Cryptogonimus chyli* (Digenea: Cryptogonimidae) in Wisconsin

WILLIAM F. FONT

Department of Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana 70402

ABSTRACT: *Bolbogonotylus corkumi* gen. et sp. n. (Digenea: Cryptogonimidae: Cryptogoniminae) is proposed for gravid worms found in the intestinal tract of *Micropterus dolomieui* in O'Neil Creek, Chippewa County, Wisconsin. The subfamily Cryptogoniminae (sensu Greer and Corkum, 1979) is emended to include genera possessing tandem testes. The genus *Bolbogonotylus* differs from all other genera in the family in having a gonotyl consisting of 2 distinct lobes, 1 anterior and 1 posterior to the acetabulum. *Bolbogonotylus* most nearly resembles *Textrema,* but differs in lacking gonotyl support rods, position of acetabulum, length of ejaculatory duct, shape of oral sucker, and extent of vitellaria. Metacercariae were found encysted in the musculature of several species of darters, *Etheostoma flabellare, E. nigrum, E. caeruleum, E. zonale, E. asprigene, Percina maculata,* and *P. caprodes* from O'Neil Creek. *Micropterus salmoides* fed infected darters harbored adult *B. corkumi* which exhibited limited egg production prior to expulsion. In contrast, *Micropterus salmoides* fed naturally infected darters containing metacercariae of *Cryptogonimus chyli* yielded adults of *C. chyli* as gravid as those occurring naturally in *M. dolomieui* from O'Neil Creek.

KEY WORDS: Trematode taxonomy, morphology, metacercariae, *Micropterus dolomieui, Micropterus salmoides,* bass, *Etheostoma* spp., darters, *Percina maculata, Percina caprodes,* perch, *Salvelinus fontinalis,* trout, *Textrema, Multigonotylus, Allacanthochasmus,* fishes.

Several species of cryptogonimid trematodes have been described from predominately piscivorous centrarchid fishes, especially the freshwater basses in the genus *Micropterus*. In the few life cycles of these cryptogonimids from freshwater hosts that have been determined by experimentation, small fish serving as intermediate hosts and harboring encysted metacercariae are preyed upon by bass (Lundahl, 1941; Greer and Corkum, 1979; Cribb, 1986). Metacercariae of other cryptogonimid species have been identified by morphological comparison with adults, rather than by experimental infection (Fischthal, 1945; Chandler, 1951).

Metacercariae of an undescribed cryptogonimid trematode were found in the musculature of several species of darters (*Etheostoma flabellare, E. nigrum, E. caeruleum, E. zonale, E. asprigene, Percina maculata,* and *P. caprodes*) from O'Neil Creek, Wisconsin. Fishes that prey upon darters in that creek were necropsied in search of the natural definitive host (Table 1). It proved to be smallmouth bass, *Micropterus dolomieui,* which yielded gravid specimens agreeing in morphology with excysted metacercariae. Largemouth bass, *M. salmoides,* were used as experimental definitive hosts to verify the metacercarial and adult stages.

Dual infections with the undescribed crypto-gonimid metacercaria and the metacercaria of *Cryptogonimus chyli* Osborn, 1903, in darters from O'Neil Creek provided an opportunity to demonstrate experimentally the transmission of *C. chyli* to the definitive host.

Materials and Methods

Fishes were collected at a study site in O'Neil Creek, approximately 1.6 km southwest of Eagleton, Chippewa County, Wisconsin (town of Eagle Point, range 8W, township 30N, section 30). Darters collected and examined for cryptogonimid metacercariae were *Etheostoma flabellare* ($N = 100$), *E. nigrum* (30), *E. caeruleum* (25), *E. zonale* (50), *E. asprigene* (3), *Percina maculata* (10), and *P. caprodes* (10). Other fishes examined for cryptogonimids are listed in Table 1. Habitat description and collection techniques for darters and other small fishes are provided by Kuntz and Font (1984). Large fishes were obtained with a backpack, battery-powered electrofisher.

Metacercaria removed from the musculature of naturally infected darters were excysted mechanically with fine needles. Adult specimens from experimentally and naturally infected fishes were fixed in either steaming or cold AFA, 10% neutral buffered formalin, and in Berland's fixative. Coverslip pressure varied from none to heavy. All specimens used for measurements were killed by pipetting them into Berland's fixative (1 part formalin: 9 parts acetic acid) and immediately transferring them to AFA. Whole mounts were stained with Van Cleave's hematoxylin or Semichon's carmine. Gravid specimens fixed in steaming 10% buffered formalin were sectioned longitudinally or transversely at

191

8 μm and stained with Harris' hematoxylin and eosin. Living specimens were studied with brightfield, phase-contrast, and Nomarski differential interference phase-contrast to observe features not apparent in stained specimens (i.e., excretory system, gland cells, and motility of gonotyl). Drawings were made with a camera lucida. Measurements in micrometers are given as ranges, followed by averages in parentheses. Type specimens and voucher specimens have been deposited in the U.S. National Museum Helminthological Collection and the National Museum of Canada Invertebrate Collection (Parasites).

Experimental life cycle studies

Experimental infections were conducted using uninfected centrarchids obtained with gill nets from Fort Bayou, Ocean Springs, Mississippi, as potential definitive hosts. Necropsy of several centrarchids (*Micropterus salmoides* [15], *Lepomis microlophus* [10], *L. microchirus* [10]) was used to determine that these fishes were free of adult cryptogonimid infections. Approximately 40 living *E. flabellare* from O'Neil Creek, Wisconsin, were shipped to Gulf Coast Research Laboratory, Ocean Springs, Mississippi. Seven *M. salmoides* and 2 *L. microlophus* were force-fed 3 darters each and were then maintained in a 500-gallon outdoor tank at 18–21°C. Experimentally infected centrarchids were given grass shrimp and uninfected killifish as food until necropsied.

Results

Bolbogonotylus gen. n.

GENERIC DIAGNOSIS: Cryptogonimidae (Ward, 1917) Cirurea, 1933; Cryptogoniminae Ward, 1917. Body elongate, spinous, with eyespots. Oral sucker terminal, funnel-shaped, larger than acetabulum. Prepharynx and pharynx present. Ceca extending to near posterior end. Gonotyl occurring as 2 bulbous lobes, the larger muscular lobe preacetabular and second lobe formed from the posterior margin of the acetabulogenital sac. Acetabulum small, round, in anterior fourth of body. Acetabulum and gonotyl protruded or enclosed within acetabulogenital sac. Testes oval, tandem. Seminal vesicle saccate, bipartite. Genital atrium formed by union of ejaculatory duct and uterus. Cirrus and cirrus sac absent. Genital pore median, between anterior bulb of gonotyl and acetabulum. Ovary pretesticular. Seminal receptacle between ovary and seminal vesicle. Laurer's canal present. Vitellaria follicular, in lateral fields between acetabular and ovarian levels. Uterine coils descending sinistrally to near posterior end and ascending dextrally, median in preovarian region. Excretory vesicle Y-shaped, bifurcating at ovariotesticular level, arms extending anteriorly. Eggs operculate, not filamented.

Bolbogonotylus corkumi sp. n.
(Figs. 1, 2)

DESCRIPTION (based upon measurements of 15 fixed gravid specimens and observation of living and stained specimens):   With characters of the genus. Body 1,941–2,561 (2,340) long by 193–230 (204) wide at level of acetabulum; entirely covered with spines. Eyespot remnants lateral to pharynx. Oral sucker 168–186 (177) by 175–223 (193). Prepharynx 41–99 (62). Pharynx 69–85 (76) by 64–74 (71) muscular. Esophagus 35–81 (54), bifurcating anterior to gonotyl. Ceca terminating 35–58 (48) from posterior end. Anterior lobe of gonotyl 48–69 (57) by 69–83 (79) subspherical. Acetabulum 81–92 (87) by 74–87 (80), posterior margin contiguous with posterior lobe of gonotyl. Testes contiguous, dorsal to uterus and ceca. Anterior testis 99–122 (111) by 74–115 (93), posterior testis 81–138 (115) by 69–104 (88). Seminal vesicle 190–331 (241) by 58–97 (77) bipartite. Ejaculatory duct narrow, surrounded by gland cells, extending from anterior end of seminal vesicle to genital atrium. Ovary 97–150 (125) by 173–207 (191) transversely elongate with irregular lateral lobes. Seminal receptacle 69–104 (82) by 133–184 (154) ovoid, overlapping dorsally anterior margin of ovary. Mehlis' gland overlying ovary. Laurer's canal elongate, opening dorsally at level of anterior testis. Vitellaria follicular, dorsal and ventral to ceca, extending from posterior margin of acetabulum to ovary. Uterus pattern invariant; descending sinistrally from ovary to 99–175 (130) from posterior end of body 99–175 (130) with individual coils alternately overlying and underlying ceca, then ascending dextrally. Uterine coils anterior to ovary larger, less numerous, median. Uterus joining ejaculatory duct to form genital atrium. Common genital pore located between acetabulum and gonotyl. Eggs 17–19 (18) by 8–10 (9), numerous, operculate, embryonated. Excretory vesicle bifurcating at level of anterior testis, arms reaching pharyngeal level, pore subterminal.

HOST: *Micropterus dolomieui* Lacépède, smallmouth bass. Prevalence = 83% (5 of 6 infected); intensity = 0–150.

TYPE LOCALITY: O'Neil Creek, near Eagleton, Chippewa County, Wisconsin.

LOCATION IN HOST: Intestine.

TYPE SPECIMENS: USNM Helm. Coll.: holotype No. 78726; paratype No. 78727. NMCIC(P):

paratype No. NMCP1985-0055 to NMCP1985-0057.

ETYMOLOGY: The generic name refers to the bulbous lobes of the gonotyl. The specific epithet was chosen to acknowledge the contributions to parasite systematics made by Dr. Kenneth C. Corkum of Louisiana State University.

Metacercaria (Fig. 3)

DESCRIPTION (based upon measurements and observations of 10 living encysted and 15 stained excysted worms): Enclosed within subspherical parasite cyst wall, surrounded by thin layer of host inflammatory tissues; cyst diameter 480–720 (600) by 450–540 (489). Similar to adult except as follows. Body, 1,505–2,278 (1,891) long by 156–296 (216) wide. Eyespots conspicuous. Oral sucker 133–175 (151) by 129–209 (167). Prepharynx 29–63 (44). Pharynx 49–80 (65) by 48–70 (58). Gland cells numerous, lateral to pharynx; ducts terminating at base and anterior margins of oral sucker. Esophagus 5–105 (33) long. Ceca terminating 19–53 (32) from posterior end. Anterior lobe of gonotyl 38–67 (50) by 51–80 (67). Anterior testis 105–171 (140) by 95–143 (115); posterior testis 122–205 (154) by 95–139 (115). Seminal vesicle and ejaculatory duct fully developed, devoid of sperm. Ovary 44–86 (60) by 91–171 (129). Seminal receptacle empty. Vitellaria distributed as in adult, less developed. Uterus completely developed, forming pattern identical to adult, eggs absent. Excretory vesicle filled with refractile inclusions appearing densely black in living specimens.

HOSTS: *Etheostoma flabellare* Rafinesque, fantail darter; *E. nigrum* Rafinesque, johnny darter; *E. caeruleum* Storer, rainbow darter; *E. zonale* (Cope), banded darter; *E. asprigene* (Forbes), mud darter; *Percina maculata* (Girard), blackside darter; *P. caprodes* (Rafinesque), logperch.

LOCALITY: O'Neil Creek, near Eagleton, Chippewa County, Wisconsin.

LOCALITY IN HOST: Generally distributed in somatic musculature.

VOUCHER SPECIMENS: USNM Helm. Coll. No. 78728; NMCIC(P): NMCP1985-0058 to NMCP1985-0061.

Remarks

Bolbogonotylus corkumi is assigned to the subfamily Cryptogoniminae Ward, 1917, as emended by Greer and Corkum (1979). The nar-row, elongate hindbody of *B. corkumi,* however, restricts the testes to a tandem arrangement. The subfamily diagnosis is thus further emended to include cryptogonimids possessing tandem testes.

Cryptogoniminae Ward, 1917

Cryptogonimidae. Body more or less elongate. Circumoral spines absent. Oral sucker funnel- to saucer-shaped; prepharynx present or absent; pharynx present; esophagus short or long; cecal bifurcation pre- or postacetabular; ceca short or long. Acetabulum median usually contained within an acetabulogenital sac. Gonotyl present or absent. Testes tandem, slightly oblique, opposite, spherical, or divided into longitudinal series of lobes. Ovary compact or lobate. Vitellaria follicular to dendritic, acetabular and more extensive, or clumped pre- or postacetabular.

Bolbogonotylus is distinguished from all other genera of Cryptogoniminae by its unique gonotyl which consists of 2 distinct lobes, 1 anterior and 1 posterior to the acetabulum. *Bolbogonotylus* most nearly resembles *Textrema* but can be distinguished by the lack of gonotyl support rods, possession of a more anteriorly positioned acetabulum, longer ejaculatory duct, funnel-shaped oral sucker, and the posterior extent of the vitellaria. *Bolbogonotylus* differs from all species of *Cryptogonimus* in the bilobed gonotyl, position of testes, shape of ovary, and vitelline distribution. Genera assigned to other subfamilies within the Cryptogonimidae also bear morphological similarities to *Bolbogonotylus.* Among these, *Multigonotylus* (Multigonotylinae) appears most similar, but can readily be distinguished by its serial arrangement of multiple gonotyl lobes. Within the Neochasminae, *Allacanthochasmus,* particularly *A. artus,* displays many morphological similarities but is readily separated by the possession of circumoral spination.

Natural fish hosts of *Bolbogonotylus corkumi* and *Cryptogonimus chyli*

All 6 species of darters that occurred at the type locality, *Etheostoma flabellare, E. niger, E. caeruleum, E. zonale, Percina maculata,* and *P. caprodes,* harbored *B. corkumi* metacercariae. Additionally, *E. asprigene,* which occurred 3 km upstream but not at the type locality, was also infected. *Etheostoma flabellare* were more heavily infected than other darters, although quantitative data were not recorded. The most heavily infected darters harbored over 150 metacercar-
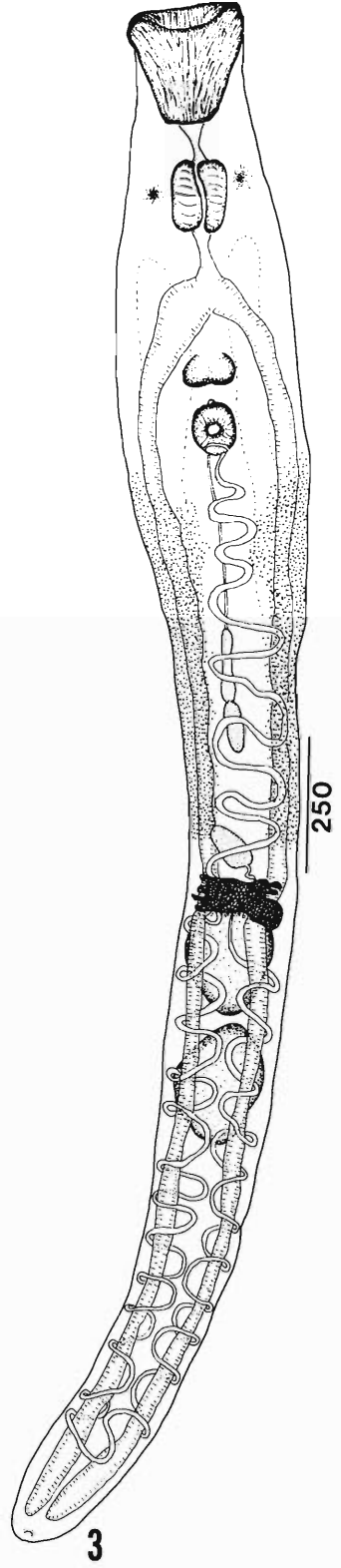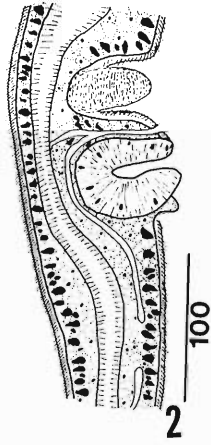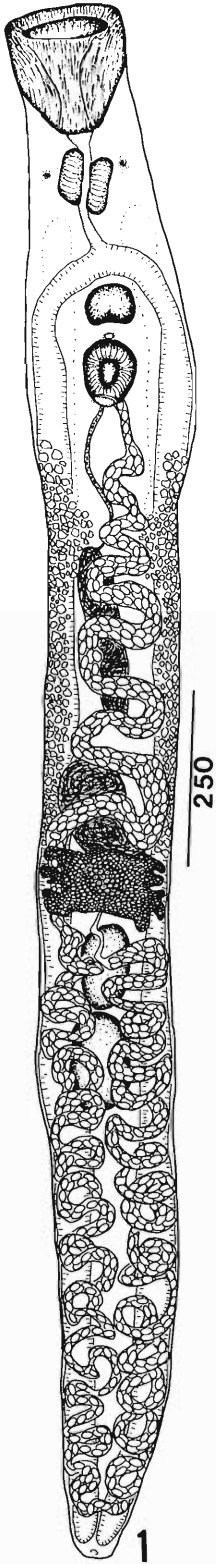
Table 1.   Fishes from O'Neil Creek examined for the presence of *Bolbogonotylus corkumi.*

Scientific name	Common name	Adults no. infected/ no. examined	Metacercariae no. infected/ no. examined
Micropterus dolomieui Lacépède	Smallmouth bass	5/6	0/6
Micropterus salmoides (Lacépède)	Largemouth bass	0/2	0/2
Salvelinus fontinalis (Mitchell)	Brook trout	2/2	0/2
Stizostedion vitreum (Mitchell)	Walleye	0/10	0/15*
Perca flavescens (Mitchell)	Yellow perch	0/4	0/6*
Lepomis macrochirus Rafinesque	Bluegill	0/10	0/15*
Ambloplites rupestris (Rafinesque)	Rockbass	0/15	0/23*
Esox masquinongy Mitchell	Muskellunge	0/1	0/1
Ictalurus melas (Rafinesque)	Black bullhead	0/4	0/10*
Cottus bairdi Girard	Mottled sculpin	0/15	0/15
Semotilus atromaculatus (Mitchell)	Creek chub	0/15	0/29*
Nocomis biguttatus (Kirtland)	Hornyhead chub	0/15	0/21*
Lota lota (Linnaeus)	Burbot	0/1	0/1
Notropis cornutus (Mitchell)	Common shiner	0/5	0/13*
Rhinichthys cataractae (Valenciennes)	Longnose dace	0/5	0/10*
Culaea inconstans (Kirtland)	Brook stickleback	0/12	0/12

* Includes juvenile fish.

iae. Preferred sites within the somatic musculature of infected darters were not discerned. Other species of fishes were examined for the presence of *B. corkumi* metacercariae, but no infected fish were found (Table 1).

Gravid specimens of *B. corkumi* were found only in smallmouth bass. Five of 6 specimens of smallmouth bass were infected; the largest bass harboring over 100 gravid worms in its intestine. Two brook trout, *Salvelinus fontinalis* (Mitchell), harbored 1 and 2 worms, respectively. All 3 specimens of the parasite, although excysted, contained refractile granules in the excretory bladder and were otherwise similar to metacercariae in that they were not gravid and contained no sperm in the seminal vesicle or seminal receptacle. Stomachs of the 2 brook trout contained the partly digested remnants of small fish, possibly darters. All other O'Neil Creek fishes examined for the presence of adult specimens of *B. corkumi* were negative (Table 1). *Bolbogonotylus corkumi* occupied the middle third of the intestine, although some overlap between the 2 species did occur. *Bolbogonotylus corkumi* metacercariae and adults have not been found in darters and centrarchids in other tributaries of the Chippewa

River drainage, and in the Red Cedar drainage of north-central Wisconsin.

All 7 species of darters from O'Neil Creek harbored concurrent infections of *Cryptogonimus chyli* metacercariae in addition to metacercariae of *B. corkumi.* Typically, darters harbored heavier infections of the smaller cysts of *C. chyli* with intensity being greatest in *E. flabellare.* Gravid specimens of *C. chyli* were recovered from all 6 smallmouth bass, *M. dolomieui,* but not from any other fish listed in Table 1. In the most heavily infected bass, several thousand *C. chyli* inhabited the pyloric ceca and anterior one-third of the intestine.

Experimental infections of *Bolbogonotylus corkumi* and *Cryptogonimus chyli*

Experimental transmission of metacercariae to 2 species of centrarchids was attempted prior to the discovery of the natural definitive host of *B. corkumi.* Living darters shipped from O'Neil Creek were force-fed to 7 uninfected *M. salmoides* and 2 *L. microlophus* obtained from Fort Bayou, Ocean Springs, Mississippi, and maintained at Gulf Coast Research Laboratory. Each

←

Figures 1–3.   *Bolbogonotylus corkumi* gen. et sp. n. 1. Adult from *Micropterus dolomieui,* ventral view. 2. Terminal genitalia, sagittal section. 3. Excysted metacercaria from *Etheostoma flabellare,* ventral view. Scale bars in micrometers.

fish received 3 live *E. flabellare* and was carefully checked for possible regurgitation. Neither sunfish became infected, but all bass harbored cryptogonimids. Three bass contained *B. corkumi* and all 7 contained *C. chyli*. Specimens of *B. corkumi* were not gravid until day 10, when 84 worms containing a maximum of 100 eggs per worm were recovered. Bass examined after day 10 were free of *B. corkumi* and harbored only *C. chyli*. *Cryptogonimus chyli* became gravid on day 10, but unlike *B. corkumi,* heavily gravid specimens occurred abundantly until day 17 when the last bass was examined. The intestinal distribution of both cryptogonimids was similar to that seen in natural infections and *C. chyli* did not migrate posteriorly in the absence of *B. corkumi.*

Discussion

The natural definitive host of *Bolbogonotylus corkumi* in O'Neil Creek is *Micropterus dolomieui.* The suitability of *M. salmoides,* as a definitive host, however, is problematic. An insufficient number of largemouth bass from O'Neil Creek have been examined, and results of experimental infections are equivocal. In spite of the fact that lightly gravid specimens were obtained, infections did not persist beyond 10 days. The temperature in which bass were maintained in outdoor tanks may have been suboptimal and did not reach the maximum summer water temperature of 25°C in O'Neil Creek reported by Kuntz and Font (1984). Yet fully gravid specimens of *C. chyli* were produced and persisted under these same experimental conditions.

Smallmouth bass in O'Neil Creek were also the only fish found to be infected with *C. chyli*, although Hoffman (1967) and Margolis and Arthur (1979) report its occurrence in largemouth bass, rock bass, and bluegill in North America. In O'Neil Creek, the rock bass and bluegills that were examined were mainly small specimens. The preponderance of insects and crustaceans in their stomachs indicates that these smaller fish may have been uninfected due to lack of exposure to metacercariae in fish hosts.

The number of species of cryptogonimids described from centrarchids has increased greatly in recent years (see Greer and Corkum, 1979).

The richness of this fauna should provide a particularly good opportunity for systematists to study host–parasite coevolution and for ecologists to build upon the pioneering work of Greer and Corkum (1980) and investigate infrapopulation and suprapopulation structures.

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Ophioxenos microphagus (Ingles, 1936) comb. n. (Digenea: Paramphistomidae) from Ectotherms in Western North America with Comments on Host-Parasite Relationships

M. BEVERLEY-BURTON

Department of Zoology, College of Biological Science, University of Guelph,
Guelph, Ontario N1G 2W1, Canada

ABSTRACT: Paramphistomes from western brook lamprey (*Lampetra richardsoni*) taken on Vancouver Island were found to be conspecific with *Megalodiscus microphagus* Ingles, 1936, from frogs taken in the same locality. However, having reviewed 46 paramphistome genera known to parasitize ectotherms, the species is assigned to *Ophioxenos* rather than *Megalodiscus*. Thus, *Ophioxenos microphagus* (Ingles, 1936) comb. n., is proposed and *O. lampetrae* Beverley-Burton and Margolis, 1982, is regarded as a junior subjective synonym.

KEY WORDS: digenean taxonomy, synonym, *Rana aurora, Taricha granulosa, Lampetra richardsoni,* frog, newt, lamprey, fishes, amphibians, Vancouver Island.

Ophioxenos lampetrae Beverley-Burton and Margolis, 1982, was proposed for paramphistomes found in the western brook lamprey (*Lampetra richardsoni* Vladykov and Folett) taken on Vancouver Island, British Columbia. Moravec (1984) reported the finding of paramphistomes, identified as *Megalodiscus microphagus* Ingles, 1936, from the red-legged frog (*Rana aurora* Baird and Gerard) and the rough-skinned newt (*Taricha granulosa* Skilton), also taken on Vancouver Island. Material from both collections was reexamined and considered to be conspecific. Forty-six genera of paramphistomes known to occur in ectotherms were reviewed. It was concluded that the species should be assigned to *Ophioxenos* rather than *Megalodiscus.*

Materials and Methods

SPECIMENS EXAMINED: *Ophioxenos lampetrae* U.S. National Museum (USNM) Helminthological Collection, Beltsville, Maryland, Nos. 76584 (holotype), 76585 (paratypes), and 76586 (juveniles); *Megalodiscus microphagus* USNM Helm. Coll. No. 8923 (holotype) and Czechoslovak Academy of Sciences, Institute of Parasitology, 370 05 České Budějovice, Branišovská 31, Czechoslovakia (material collected by Moravec).

Results

Morphologic comparison of the above-listed material and figures of Ingles (1936), Beverley-Burton and Margolis (1982), and Moravec (1984) revealed no distinctly different features, although differences in testicular and egg size are evident in the recorded morphometric data (Table 1). Nevertheless, because the sample size from each host is small and the reproductive status of the

worms is not known, the specimens are regarded as conspecific and the name *O. lampetrae* Beverley-Burton and Margolis, 1982, becomes a junior subjective synonym of *O. microphagus,* Ingles, 1936. It is, however, evident that this species cannot be included in the genus *Megalodiscus* Chandler, 1923, sensu Yamaguti (1971). Reexamination of morphologic generic characters (from Yamaguti, 1971; Khalil, 1981; Beverley-Burton and Margolis, 1982) of all 46 paramphistome genera known from ectotherms led to the recognition of 3 similar genera: *Cleptodiscus* Linton, 1910, *Schizamphistomoides* Skunkard, 1924, and *Ophioxenos* Sumwalt, 1926. These can be separated on the basis of host "preference," ecology (marine or freshwater), and geographic distribution. Known species of *Ophioxenos* occur in freshwater or terrestrial ectotherms from North America and the material from lampreys and amphibians taken on Vancouver Island is therefore assigned to *Ophioxenos* as defined by Beverley-Burton and Margolis (1982). *Ophioxenos microphagus* (Ingles, 1936) comb. n. is proposed and *O. lampetrae* Beverley-Burton and Margolis, 1982, is regarded as a junior subjective synonym.

Discussion

Yamaguti (1971) provided a separate key for Digenea parasitizing each class of vertebrates. However, it proved impossible to locate the appropriate taxon (either subfamily or genus) for the brook lamprey material by using Yamaguti's key to piscine paramphistomes. Our material was

Table 1. Comparative measurements (in mm, except where indicated) of *Ophioxenos microphagus* (Ingles, 1936) comb. n. from various ectotherms.

Source of data	Beverley-Burton and Margolis (1982)	Moravec (1984)		Ingles (1936)
Body length	3.02 (1.92–3.80)*	1.56–1.84	3.22–4.42	3.7–5.2
Body width (max)	1.18 (0.80–1.33)	0.67–0.88	1.07–1.29	1.4
Oral sucker:				
length	0.38 (0.25–0.44)	—	0.31–0.38	0.25–0.30
width	0.34 (0.27–0.39)	0.16–0.22	0.29–0.35	0.28–0.36
Ventral sucker:				
length	0.83 (0.59–0.95)	0.53–0.71	0.87–1.06	—
width	0.83 (0.56–1.01)	0.50–0.64	0.86–0.98	0.77–1.1
Ratio of transverse diameters,				
oral : ventral suckers	1:2.44 (1:2.07–2.8)	1:2.5 (1:2.4–2.6)†	1:2.60†	1:2.5–2.7‡
Anterior testis:				
length	0.37 (0.32–0.41)	0.22–0.25	0.20–0.25	—
width	0.30 (0.13–0.44)	0.16	0.14–0.20	0.63–0.68
Posterior testis:				
length	0.41 (0.32–0.46)	0.19–0.27	0.15–0.27	—
width	0.27 (0.14–0.43)	0.15–0.18	0.14–0.32	0.50–0.72
Ovary:				
length	0.26 (0.21–0.29)	0.11–0.16	0.19–0.31	—
width	0.26 (0.19–0.32)	0.06–0.11	0.18–0.27	0.18–0.27
No. vitelline follicles on each side	9–16	9–10	14	15–18§
Eggs:				
length (μm)	112–131	96–111	105–135	88
width (μm)	53–70	54–60	57–84	48
No. specimens measured	7	3	3	?
Host	*Lampetra richardsoni*	*Rana aurora*	*Taricha granulosa*	*Bufo boreas*
Site	Intestine	Intestine	Intestine	Intestine, rectum, bladder‖

* Mean followed by range in parentheses.

† Calculated by present author from specimens collected by Moravec.

‡ Calculated by present author from extremes of ranges quoted by Ingles (1936).

§ Approximation by present author based on holotype and figure in Ingles (1936).

‖ According to Ingles (1936), mature specimens were found only in the bladder of 1 toad. Immature worms were taken from the intestine.

eventually assigned to *Ophioxenos* Sumwalt, 1926, the previously described species of which (*O. dienteros* Sumwalt, 1926, and *O. singularis* Parker, 1941) parasitize reptiles (snakes and terrapins) and amphibians (toads).

In contrast, Moravec (1984), unaware of the paper by Beverley-Burton and Margolis (1982), reviewed the paramphistome species previously reported from amphibians and concluded that the material from *R. aurora* and *T. granulosa* taken in British Columbia was identical with *Megalodiscus microphagus.* Although the specific designation of Moravec (1984) appears to be valid, the inclusion of this species in *Megalodiscus* cannot be upheld.

The reported host spectrum for *Ophioxenos* spp. is broad: *O. dienteros* was recorded by Sumwalt (1926) from garter snakes (*Thamnophis sirtalis conicinnus* (type host) and possibly *T.*

ordinoides ordinoides, T. ordinoides biscutalus) and *Bufo boreas boreas,* all from San Juan Island, Puget Sound, Washington, and by Thatcher (1954) from terrapin (*Clemmys marmorata*) taken in Oregon; *O. singularis* was recorded by Parker (1941) from *T. sirtalis sirtalis* (type host) and *Rana catesbeiana* taken in Florida and Tennessee, respectively; *O. microphagus* was recorded by Ingles (1936) from *Bufo boreas* (type host) taken in California, by Macy (1960) from *Dicamptodon ensatus, Hyla regilla, Rana aurora,* and *Taricha granulosa,* by Beverley-Burton and Margolis (1982) from *Lampetra richardsoni* taken on Vancouver Island, B.C., and by Moravec (1984) from *R. aurora* and *T. granulosa* from the same locality. Thus, the speculative comment of Beverley-Burton and Margolis (1982) concerning the low host specificity of both *O. lampetrae* and *O. microphagus* appears to be substantiated

and support the conclusion that *O. lampetrae* is a junior subjective synonym of *O. microphagus*.

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Aspidogastrid and Digenetic Trematode Single and Double Infections in the Gastropod, *Elimia livescens,* from the Upper Cuyahoga River

MARTIN K. HUEHNER

Biology Department, Hiram College, Hiram, Ohio 44234

ABSTRACT: *Elimia livescens* were collected from the Upper Cuyahoga River, Ohio, from July 1978 to December 1983. *Aspidogaster conchicola,* plagiorchid, heterophyid, opecoelid, strigeid, and philophthalmid single and double infections occurred in 844 snails. Larger snails (≥ 16 mm) had significantly greater prevalence of digenean infections. *Aspidogaster conchicola* was significantly more prevalent and intense during colder months of November through April and in snails with digenean parthenitae. The number of gravid *A. conchicola* showed the same distribution. This and previous studies of experimental aspidogastrid and digenean infections in *E. livescens* suggest that concurrent digenean infections may be beneficial to the establishment and development of *A. conchicola.*

KEY WORDS: Trematoda, Digenea, Aspidogastrea, *Aspidogaster conchicola, Elimia livescens,* polyspecific infections, parasite seasonality, cercaria, snails, Ohio.

Gastropods are very frequently parasitized by asexual reproductive stages of digenetic trematodes, but several operculate snail species are also known to serve as definitive or intermediate hosts for various aspidogastrid trematodes (Rohde, 1972, 1975; Hendrix et al., 1985). Rohde and Sandland (1973) reported the aspidogastrid *Lobatostoma manteri* to occur more frequently in an Australian prosobranch snail infected with digenean parthenitae and this is the sole record of naturally occurring aspidogastrid–digenean double infections to date. Using *Elimia livescens* as hosts, Huehner (1975) experimentally demonstrated that *Aspidogaster conchicola* could establish itself and successfully develop in snails with preexisting plagiorchid, opecoelid, and strigeid infections, and that these infections could occur in nature. The present study reports seasonality of such infections in *E. livescens* from the Upper Cuyahoga River, where *A. conchicola* also parasitizes the snail *Cipangopaludina chinensis* and several unionid mussel species.

Materials and Methods

Elimia livescens were collected by hand or dip net from a 25-m section of the Cuyahoga River 0.3 km upstream from the Ohio State Route 82 bridge (Mantua, Ohio) on 7 August 1978, 24 May 1981, and 31 August and 14 December 1982, and on the first day of each month during 1983. Snails were maintained in aerated river water at 10°C and examined not more than 7 days after collection. Each snail was measured for spire height and anesthetized with menthol prior to dissecting out the viscera, which were teased apart on a glass microscope slide. The tissues were first searched, by dissecting microscopy, for larger *A. conchicola* and digenean parthenitae, and were subsequently squashed between microscope slides to search for smaller worms by compound microscopy. Aspidogastrids were isolated, heat-fixed under minimal coverslip pressure, examined for the presence of eggs, measured with an ocular micrometer, and later stained with acetocarmine for whole mounts. Digenean infections, identified by type and cercariae, were treated as above. Chi-square 2×2 contingency tables and 2-tailed values of Chi-square were used to evaluate statistical significance of data.

Results

Of a total of 1,887 *E. livescens* examined, 844 were found to contain either single or double trematode infections. Snails with single infections consisted of 652 plagiorchid (xiphidiocercous cercariae; 2 sizes possibly representing 2 species), 58 *A. conchicola,* 58 heterophyid (pleurolophocercous cercariae; probably *Apophallus* sp.), 11 strigeid (pharyngeate furcocercous cercariae), and 1 each of an opecoelid (microcercous cercariae) and a philophthalmid (gymnocephalous cercariae quickly producing lightbulb-shaped cysts). *Aspidogaster conchicola* occurred in conjunction with digeneans to produce 62 double infections (48 plagiorchid, 12 heterophyid, 1 strigeid, and 1 opecoelid). Only a single strictly digenean double infection (plagiorchid and strigeid) was observed. The remaining 1,043 snails were uninfected.

Snails examined ranged in spire height from 8 to 24 mm, with an average of 15.4 mm and a median of 15.8 mm. Size of collected snails did not vary significantly with season ($\chi^2 = 21.3$ for 16 df; $P > 0.05$), but infection prevalence varied with snail size. Frequency of trematode infections of all kinds was 79% for snails at and above

Table 1. Shell spire height and trematode infections of *Elimia livescens*.

	No. snails infected/ uninfected spire height		Chi-square	
	<16 mm	≥16 mm	χ^2	P
All infections*	177/483	560/667	131.7	<0.001
Plagiorchid	134/526	566/661	122.7	<0.001
Heterophyid	13/647	57/1,170	8.6	<0.01
Aspidogastrid	38/622	82/1,145	0.6	>0.05
All double infections	11/649	52/1,175	8.8	<0.01

* Includes groups (opecoelid, etc.) not individually listed. Plagiorchid, heterophyid, and aspidogastrid numbers include double infections.

and 21% below the rounded median of 16 mm spire height (Table 1). Plagiorchid infections accounted for the majority of this distribution, whereas heterophyids and all double infections were of lesser significance. No host size preference was noted for *A. conchicola*.

Aspidogaster conchicola displayed significant changes in infection prevalence and intensity between otherwise uninfected snails and those with concurrent digenean infections (Table 2). Although *A. conchicola* prevalence was not significantly greater in 2 of the groups (all digenean pooled and plagiorchid), intensity of infection was. Intensity ranged from 1 to 2 worms in single infections, from 1 to 12 worms in snails with concurrent plagiorchid infections, and from 1 to 2 worms in snails with concurrent heterophyid infections. Fifty-eight percent of *A. conchicola* found came from the 786 (41.7%) snails with digenean parthenitae, while the remaining 1,101 snails (58.3%) contained only 42%. Not only was the greater portion of the *A. conchicola* population found in those snails with digenean infections, but this group also carried the greatest number of gravid worms (nearly triple that of single *A. conchicola* infections).

Significant seasonal differences in infection prevalence were noted by comparing infection frequency of snails collected during warm months (8/78, 8/82, and 5/83–10/83) to that of snails collected in cold months (11/82, 1/83–4/83, and 11/83–12/83). With the exception of heterophyids, infection prevalence showed significant seasonality (Table 3). Plagiorchids reached a maximum in July 1983 (102/128 snails) and a minimum in March 1983 (16/125 snails), show-

Table 2. Prevalence and intensity of *A. conchicola* in *E. livescens* snails with concurrent digenean infections.

	Infection prevalence and intensity*			
	Aspido-gastrid alone	Aspido-gastrid + digenean	Chi-square	
			χ^2	P
Prevalence				
All digenean	58/1,101	62/786	5.3	<0.05
Plagiorchid	58/1,101	48/700	2.0	<0.2
Heterophyid	58/1,101	12/70	16.5	<0.001
Intensity				
All digenean	62/1,101	90/786	17.7	<0.001
Plagiorchid	62/1,101	72/700	11.5	<0.001
Heterophyid	62/1,101	16/70	24.1	<0.001
Gravid *A. conchicola* intensity				
All digenean	9/1,101	26/786	15.0	<0.001
Plagiorchid	9/1,101	19/700	9.7	<0.01
Heterophyid	9/1,101	6/70	28.6	<0.001

* Prevalence = number of infected snails out of total snails in each group; intensity = number of *A. conchicola* found in each group of snails.

ing a distribution that was almost inversely mirrored by that of *A. conchicola*. Although heterophyid prevalence showed no significant seasonality, aspidogastrid/heterophyid and plagiorchid/aspidogastrid double infections were more common in cold months (Table 3). Intensity of *A. conchicola* infections also varied with season (Table 3), being greater in cold than warm months for all single and double infections. Interestingly, *A. conchicola* showed the least seasonality of intensity alone and the greatest seasonality in plagiorchid-infected snails that were least abundant during cold months. The number of gravid *A. conchicola* was also significantly greater in cold months.

Discussion

The strong correlation between larger *E. livescens* and their increased probability of digenetic trematode infection is difficult to explain. Cheng (1971) showed that digenean infections sometimes caused snails to grow exceptionally large shells by modifying their calcium metabolism. Although this phenomenon may occur in the host–parasite systems presently studied, it is of uncertain consequence to *E. livescens*, which has an estimated life in the field of up to 4 yr (Dazo, 1965). During such a lifespan, several infections could be gained and lost. The present observations could simply result from older,

Table 3. Seasonality of infection prevalence and intensity in *E. livescens.*

	Infection prevalence and intensity*			
			Chi-square	
	Warm months†	Cold months	χ^2	P
Prevalence				
Aspidogastrid	51/993	69/894	5.3	<0.05
Plagiorchid	472/993	228/894	97.8	<0.001
Heterophyid	39/993	31/894	2.8	<0.1
Aspidogastrid/plagiorchid	25/472	23/228	4.6	<0.05
Aspidogastrid/heterophyid	3/39	9/31	5.5	<0.05
Aspidogastrid/all digenean	28/518	34/268	12.9	<0.001
Intensity				
Aspidogastrid/all snails	56/993	96/894	14.0	<0.001
Gravid aspidogastrid/all snails	11/993	24/894	6.2	<0.02
Aspidogastrid/all digenean	32/518	58/268	31.8	<0.001
Aspidogastrid alone	27/521	53/666	3.1	<0.1
Aspidogastrid/plagiorchid	29/472	43/228	21.1	<0.001
Aspidogastrid/heterophyid	4/39	18/31	9.5	<0.01

* Prevalence = number of infected snails out of total snails in each group; intensity = number of *A. conchicola* found in each group of snails.
† May–October.

larger snails gaining greater exposure to trematode transmission stages because they may eat or move more.

Rohde and Sandland (1973) reported a significant tendency for the aspidogastrid, *Lobatostoma manteri,* to occur more frequently in snails with digenean infections and suggested that reduced resistance to a second infection may be responsible. Present findings demonstrate more strongly that digenean infection significantly increases the probability of aspidogastrid infection or vice versa. The mechanism that produces this relationship is not known but could be either physiological (reduced resistance) or behavioral (i.e., increased feeding or exposure to eggs or miracidia).

The presently observed high frequency and thereby apparently low antagonism between aspidogastrid and digenean double infections may be explained by the different microhabitats they occupy within the snail host. *Aspidogaster conchicola* is coelozoic within digestive diverticulae and digeneans are histozoic in either hepatopancreas hemolymph spaces or the gonads. No direct contact between parasites is therefore possible as long as excessive damage does not occur. By experimentally infecting digenean-bearing snails with *A. conchicola,* Huehner (1975) found that *E. livescens* with strigeid infections produced significantly larger ($P < 0.01$) worms and that maturity was reached 60 days earlier than in control

snails with only *A. conchicola* present (see Huehner and Etges, 1977 for *A. conchicola* development studies). The presence of plagiorchid sporocysts had no significant effect on aspidogastrid growth, but a triple infection (opecoelid/plagiorchid/aspidogastrid) produced significantly ($P < 0.01$) larger *A. conchicola.* These results suggest that some digeneans may produce beneficial effects on concurrent aspidogastrid infections and that they were not harmful in any instance. These findings are consistent with the present report of greater *A. conchicola* prevalence, intensity, and frequency of gravid worms in snails with digenean infections.

Infection seasonality reported here for *A. conchicola* is opposite that reported for *L. manteri* (Rohde and Sandland, 1973). Increased prevalence, intensity, and frequency of gravid *A. conchicola* during cold months has no explanation at this time although cycles other than seasonal ones may be involved. Steinberg (1931) reported that *A. conchicola* from the unionid *Pseudoanodonta anatina* produced eggs from mid-May to October, and infection prevalence did not fluctuate during the year. Such seasonality was not evident in the present study. It is possible that the *E. livescens* infrapopulation of *A. conchicola* in the Cuyahoga River is not representative of its suprapopulation in other hosts from this and other localities.

While profound complexities cloud our un-

derstanding of host–parasite ecology, the interactions between polyspecific infections and their hosts are even further veiled from scrutiny. Previous studies of intramolluscan intertrematode interactions (Joe et al., 1965; Lim and Heyneman, 1972) have focused on digenean parthenitae in either direct or indirect antagonism. The present report is based on an obviously more indirect relationship between trematodes in separate microhabitats of the same host and sometimes the same host organ. This novel system offers numerous opportunities for further investigation.

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Studies on the Location of Adult Fringed Tapeworms, *Thysanosoma actinioides*, in Feeder Lambs

R. J. Boisvenue and J. C. Hendrix

Animal Science Division, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, Indiana 46140

ABSTRACT: One hundred seven of 151 feeder lambs used in parasite location studies were infected with fringed tapeworms acquired during the summer of the year of slaughter. Location data were compared between 29 ligated and 78 nonligated infected lambs at slaughter. All tapeworms were found in the duodenum of lambs whose bile ducts and small intestine were ligated within 5 min following euthanasia. The duodenal area was 5 cm anterior to and 10 cm posterior to the common bile duct opening. Cestodes in the nonligated slaughtered lambs were located mainly in the duodenum (78.6%) in addition to bile, hepatic, and pancreatic duct areas. These limited data suggest that the most preferred location of the tapeworm in young lambs may be the duodenum rather than the distal end of the common bile duct.

KEY WORDS: cestoda, habitat, duodenum, bile ducts, *Ovis aries,* sheep.

Although the existence of the parasite, *Thysanosoma actinioides,* has been known for over a century (Curtice, 1890), little information is present in the literature regarding its life cycle, location, and pathogenicity (Allen, 1973). This cestode has been reported as occurring in the duodenum, bile, and pancreatic ducts of sheep and a number of other ruminants in the western United States (Stiles and Hassall, 1893; Porter and Kates, 1956). At one time, *T. actinioides* was thought to be responsible for considerable losses in sheep, and it was assumed that its pathogenicity was associated with the clogging of the bile passages. Christenson (1931), Newsom and Cross (1934), and Newsom and Thorp (1938) concluded that the fringed tapeworm is only slightly, if at all, pathogenic in sheep. Allen and Kyles (1950) stated that the question of whether *T. actinioides* is the primary or secondary cause of pathological changes has not been determined. However, the presence of the worm is of decided economic importance due to liver condemnations of infected animals (Porter and Kates, 1956; Ordaz, 1980). As many as 60% of the livers from lambs originating in endemic areas are condemned. Allen et al. (1962) suggested that in the control of this helminth, attention should be given to chemicals that are eliminated in bile because the fringed tapeworms inhabit the biliary system. In a later paper of Allen et al. (1967), no mention of the location of fringed tapeworms found in dewormed sheep was made. The considerable volume of data accumulated over a long period by Allen and other workers relate to the effect that the fringed tapeworm prefers the distal end of the common bile duct, and may also occur in the duodenum. The purpose of this paper is to present data on the preferred location of adult fringed tapeworms acquired mainly by feeder lambs during the summer of the year of slaughter.

Materials and Methods

The 121 feedlot lambs used in 2 trials were obtained from range flocks in the endemic tapeworm area near Gillette, Wyoming. The studies were conducted at the University of Nebraska's Scottsbluff Experiment Station. Test lambs were fed ad libitum a basal ration consisting of 50% corn, 40% alfalfa hay, 3% soybean meal, and 7% liquid cane molasses in a pelleted form.

In the first trial, 83 market lambs were processed through a local abattoir. On the processing line the small intestine was separated from the pancreas and liver at a point slightly above the common duct opening. Collected viscera were brought to the laboratory and stored at room temperature while the worms were counted and their location in the alimentary canal established. The worm analysis was completed within 8 hr following the collection of viscera. For the second trial, 38 feeder lambs, weighing approximately 35 kg each, were selected, brought into the laboratory, and euthanized by electrocution. Within 5 min after death, the ends of the bile and common ducts and the first 1.22 m of the small intestine were ligated in 34 animals. The first ligation was at the distal end of the common duct, i.e., the area immediately above the opening of the common duct. Then the area of the bile duct (extrahepatic) close to the serosal surface of the liver was ligated. The hepatic portion of the bile duct or the cystic duct could not be ligated because the ducts are under the serosal surface of the liver. A third ligation was made at the junction of the abomasum and the duodenum, and a fourth was approximately 1.22 m from the proximal end of the small intestine. Four lambs were nonligated and served as controls.

Results

Sixty of the 83 lambs (72.3%) in the first trial were infected with mature fringed tapeworms

204

Table 1. Number and location of *Thysanosoma actinioides* in feeder lambs.

No. lambs	No. infected lambs	Total no. tapeworms	Extrahepatic					Hepatic		Undetermined free
			Small intestine			Common duct	Pancreas	Bile duct	Gall bladder	
			Anterior to	Duct opening*	Posterior to					
Trial No. 1: Nonligated abattoir lambs										
83	60	794	140	0	484	37	9	30	0	94
Trial No. 2: Ligated/nonligated euthanized lambs										
34 L†	25	348	76‡	25	247§	0	0	0	0	0
4 NL	4	89	8	20	35	22	0	4	0	0

* Opening of the common bile duct.
† L = ligated; NL = nonligated.
‡ Ligated at junction of the abomasum and the duodenum.
§ Ligated at 1.22 m posterior to the common duct opening.

(Table 1). No tapeworms were located in the abomasum or gall bladder. However, live *T. actinioides* were found attached to the wall of the cystic, hepatic, and bile ducts of the liver and the ducts of the pancreas. The small intestine contained 78.6% of the total tapeworm population, with the majority (77.6%) in an area 10 cm posterior to the common duct opening. Approximately 10% of the cestodes were located in the common, bile, and hepatic ducts. Tapeworms labeled as undetermined (11.8%) were those found free outside these locations due to the separation of viscera at slaughter immediately above the common duct opening.

Twenty-nine of the 38 lambs (76.3%) in the second trial were infected with tapeworms. No cestodes were present in the abomasum, gall bladder, or in the hepatic, common bile, cystic, or pancreatic ducts of ligated animals. Again the majority of the cestodes (71%) were in an area 10 cm posterior to the common duct opening. Of the 89 adult tapeworms found in the 4 nonligated (control) lambs, 63 (70.8%) were in the small intestine. Cestodes located in the common and bile ducts made up 29.2% of the population. The cestode specimens collected in these trials were identified by C. Hibler (Colorado State University, Fort Collins, Colorado) and determined to be mature in development.

Discussion

Unpublished data on feeder lambs slaughtered at the University of Nebraska's Scottsbluff Experiment Station in 1957 were obtained from Dr. G. W. Kelley (pers. comm.). In the 1957 experiment, 30 lambs were processed in a manner similar to that used in the present authors' first trial. Eighteen of the lambs (60%) were infected with the fringed tapeworms. Of a total population of 190 tapeworms, 148 (77.9%) were in the small intestine. According to Dr. Kelley, those tapeworms found in areas other than the small intestine were categorized as hepatic. This interpretation would include those cestodes in the hepatic and extrahepatic bile ducts. This may account for the higher percentage (22.1%) of worms in the hepatic locations. Nevertheless, Dr. Kelley's data are in agreement with our findings in that the majority of worms in feeder lambs were found in the small intestine.

A review of the literature indicates that the fringed tapeworm is only slightly, if at all, pathogenic for feedlot lambs. However, Gassner and Thorp (1940) reported that because this worm occurs in the bile duct of the liver of slaughtered lambs, this organ is condemned as unfit for human consumption. Liver condemnations may be as high as 65% in lambs within the high plains region. Kelley et al. (1959) stated that examination of viscera on several occasions has shown that the worm was present in the small intestines when livers were not condemned. Therefore, liver condemnation is not an exact measure of the extent of infestation of lambs. However, the high percentage of wormy livers reported indicates that the infestation of lambs is large.

The main issue raised in this paper is the authors' contention that adult *T. actinioides* in young lambs reside mainly in the duodenal area of the small intestine near the opening of the common bile duct. Allen (1973) showed that adult tapeworms were most commonly found in the

206

distal end of the common duct, in the duodenum or with a part of the worm extending across the junction. This location appears to be the most common in older lambs and breeding sheep. Immature or juvenile forms of 2–4 cm in length were not found by the authors deep in the hepatic ducts of the ligated lambs. These forms have been reported in these ducts 2.5–30 min after the death of the lamb (Allen, 1973). In the present study we did not look for senile adults which detach from the duodenal mucosa and reattach in the jejunum and ileum before being excreted as necrotic debris.

Fibrosis and hyperplasia of the distal one-half of the common duct were not observed grossly in lambs of the second study. Though the data were sparse, the 4 nonligated lambs had 22 of 89 adult tapeworms in the common bile duct, whereas the 25 infected ligated lambs did not have any worms in that duct. A possible explanation for the rather large number of adult tapeworms near the opening of the common bile duct in the small intestine of slaughtered lambs is that the worms move from the duct to the duodenum after death. This explanation would account for the pathological changes at the distal end of the common duct, though few or no worms are found in the duct at slaughter.

The authors realize that their data concern only adult worms in lambs of a given age so that only a small portion of the total problem is examined.

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Use of Monoclonal Antibodies to Study Surface Antigens of *Eimeria* Sporozoites

P. C. AUGUSTINE AND H. D. DANFORTH

Protozoan Diseases Laboratory, Animal Parasitology Institute,
Agricultural Research Service, USDA, Beltsville, Maryland 20705

ABSTRACT: Air-dried and glutaraldehyde-fixed sporozoites of *Eimeria meleagrimitis, E. adenoeides, E. tenella,* and *E. acervulina* were exposed to monoclonal antibodies (McAb) and either fluorescein-conjugated rabbit anti-mouse IgG for epifluorescence microscopy or ferritin-conjugated goat anti-rabbit IgG for transmission electron microscopy. Seven McAb appeared to react with the surface of air-dried sporozoites. Six of the 7 reacted only with the species of *Eimeria* against which they were elicited; 1, McAb 1227, cross-reacted with all 4 species. Five of the McAb produced similar fluorescent and ferritin labels on air-dried and glutaraldehyde-fixed sporozoites, suggesting that the epitopes recognized by these antibodies were exposed on the sporozoite surface and were not markedly altered by glutaraldehyde fixation. In contrast, 2 McAb, 42E11 and D2, reacted strongly with the surface of air-dried sporozoites but only weakly or in localized areas with glutaraldehyde-fixed sporozoites. These 2 McAb probably recognized epitopes that were either located within or on the cytosolic side of the sporozoite surface membrane or were cross-linked by glutaraldehyde fixation and no longer capable of binding the McAb. By Western blot analysis, all 7 McAb recognized antigens in sodium dodecyl sulfate-solubilized sporozoites that had relative molecular weights of ~24,000.

KEY WORDS: *Eimeria meleagrimitis, Eimeria adenoeides, Eimeria tenella, Eimeria acervulina,* Western blot analysis, ferritin immuno-label, transmission electron microscopy.

Previous studies have shown that sporozoites of *Eimeria* contain a number of surface membrane proteins ranging in molecular weight from 20,000 to >200,000 (Wisher, 1983). Antiserum from *Eimeria*-infected chickens reacted with 7 of these proteins, suggesting that sporozoite surface antigens might be important in the immune control of the parasite (Wisher, 1983). In our laboratory, monoclonal antibodies (McAb) that appear to react with the surface of sporozoites of 8 species of chicken and turkey *Eimeria* have been produced (Danforth, 1982; Danforth and Augustine, 1983). Studies with these McAb showed that several were capable of inhibiting the invasion of cultured cells by the sporozoites, whereas others were not inhibitory (Augustine and Danforth, 1985). The objects of the present study were to characterize the antigens recognized by some of these McAb with regard to relative molecular weight (Mr) and location on or within the sporozoite surface membrane.

Materials and Methods

Sporozoites

Sporozoites of *E. meleagrimitis, E. adenoeides, E. tenella,* and *E. acervulina* were excysted and separated from sporocyst debris (Danforth, 1982). The sporozoites were air-dried on 12-well immunofluorescent slides (10⁴ sporozoites/well) at room temperature (RT, 22 ± 2°C), or fixed in glutaraldehyde (2% in cacodylate buffer, pH 7.4) at RT for 30 min, or suspended in sample buffer containing sodium dodecyl sulfate (SDS) (Laemmli, 1970). The glutaraldehyde-fixed (G-F) sporozoites were washed twice after fixation and resuspended in phosphate-buffered saline (PBS).

Monoclonal antibodies

Hybridoma cell lines that elicited antibodies reacting with sporozoite surface and internal antigens were produced as reported earlier (Danforth, 1982; Danforth and Augustine, 1983). The cell lines used in this study were then cloned by the limiting dilution technique (Danforth, 1982).

Labeling procedures

Indirect fluorescent antibody (IFA) procedures were conducted on both air-dried and G-F sporozoites. Sporozoites in the experimental groups were treated with surface-reacting McAb in culture supernatant (undiluted) or ascitic fluid (1:320). Controls were treated with culture supernatant or ascitic fluid from the parent myeloma cell line (P3-X63-Ag), with nonreacting fused cell lines, or with McAb 91C7, 1209, and 1223, that react specifically with internal structures of air-dried sporozoites. The sporozoites were exposed to the McAb or control solution for 30 min, washed twice (10 min) in PBS, exposed to fluorescein-conjugated rabbit anti-mouse IgG (H+L; Miles Laboratories, Elkhart, Indiana) for 30 min, and washed twice in PBS. Glutaraldehyde-fixed sporozoites were centrifuged at 11,600 g for 1.5 min between each step and the excess reagents were aspirated. All steps were conducted at RT. The sporozoites were mounted in buffered glycerol (pH 8.0) and examined by epifluorescence microscopy. For transmission electron microscopy, glutaraldehyde-fixed sporozoites were exposed to McAb, rabbit anti-mouse IgG, and ferritin-conjugated goat anti-rabbit IgG, pro-

207
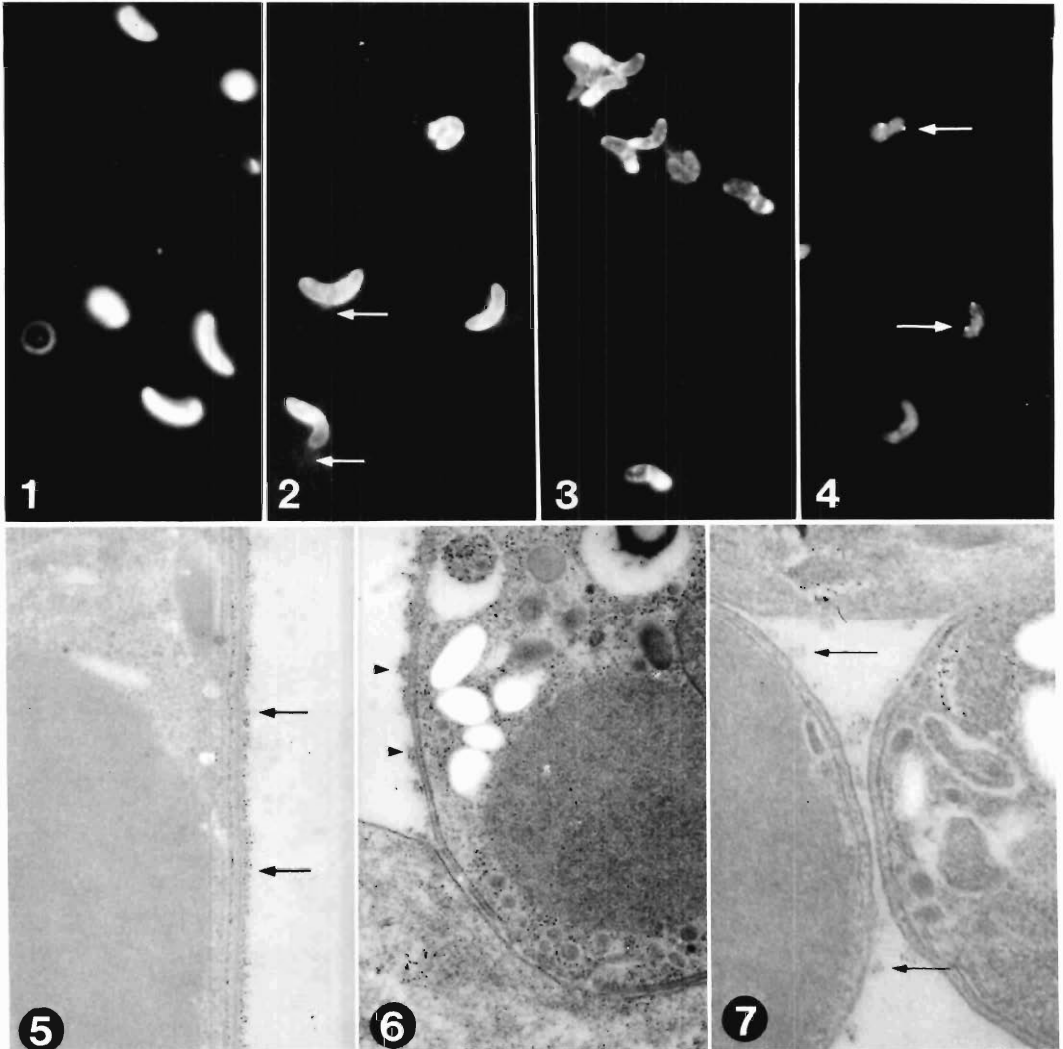
Figures 1–7. Fluorescence and ferritin labeling of *Eimeria* sporozoite surfaces. 1. Air-dried sporozoites labeled with McAb 1227 and fluorescein conjugate showing smooth surface reaction (×2,250). 2. Air-dried sporozoites labeled with McAb 43A6 and fluorescein conjugate showing irregular surface reaction. Note reactive material around sporozoites (arrows) (×2,250). 3. Air-dried sporozoites labeled with McAb 42E11 showing surface and anterior tip label (×2,250). 4. Glutaraldehyde-fixed sporozoites labeled with McAb 42E11. Strongest fluorescence is in pinpoint areas on the surface (arrows) (×2,250). 5. Transmission electron micrograph of sporozoite surfaces treated with McAb 1227 and a ferritin conjugate showing uniform layer of ferritin (arrows) (×36,000). 6. TEM of sporozoite surface treated with McAb 43A6 and a ferritin conjugate showing aggregates of ferritin (arrowheads) (×21,000). 7. TEM of sporozoite surfaces treated with McAb 42E11 and a ferritin conjugate. Note small separated clusters of ferritin granules (arrows) (×64,000).

cessed, and examined as reported earlier (Augustine and Danforth, 1985).

Electrophoresis and Western blot procedures

Sporozoites were solubilized in sample buffer containing SDS (Laemmli, 1970) by vortexing with 100-μm glass beads for 3 min. The sporozoite samples, containing 2×10^7 sporozoites per ml of buffer, were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) at 100 V/60 mA for 1.5 hr at 4°C, using 11% polyacrylamide (SDS-PA; 33:1 acrylamide : bisacrylamide) gels and 5% stacking gels. Prestained protein standards (BRL, Gaithersburg, Maryland), which included myosin ($Mr = 200,000$), phosphorylase B ($Mr = 97,400$), bovine serum albumin ($Mr = 68,000$), ovalbumin ($Mr = 43,000$), chymotrypsinogen ($Mr = 25,700$), B lactoglobulin ($Mr = 18,400$), and lysozyme ($Mr = 14,300$),

Table 1. Indirect fluorescent antibody (IFA) and ferritin labeling of antigens recognized by monoclonal antibodies (McAb) directed against *Eimeria* sporozoite surface membrane and interior organelles.

McAb	Specificity*	*Eimeria* species†	IFA‡			Isotype (IgG)
			Air-dried	Glutaral-dehyde-fixed	Ferritin label	
B10	S	*tenella*	S—even	S—even	Uniform	2b
1227	X	*acervulina*	S—even	S—even	Uniform	3
43A6	S	*meleagrimitis*	S—irreg.	S—irreg.	Irregular	1
33A11	S	*adenoeides*	S—irreg.	S—irreg.	ND	2b
42E11	S	*meleagrimitis*	S—even	Localized	Clusters	2a
D2	S	*tenella*	S—even	NR	ND	M
91C7	X	*adenoeides*	RB	NR	NR	2a
1209	X	*acervulina*	RB	NR	NR	2a
1223	S	*acervulina*	Tip	NR	NR	ND
P3-X63-Ag8			NR	NR	NR	

* Specificity of reaction; S = reacts only with species against which McAb was elicited; X = cross-reactive with 2 or more species.

† Species against which McAb was produced.

‡ Reaction with *Eimeria* species against which McAb was elicited; S = surface; RB = refractile body; NR = no reaction; ND = not done.

were electrophoresed along with the sporozoite protein. The separated proteins were transferred onto nitrocellulose paper and subjected to Western blot procedures using a modification of the method described by Towbin et al. (1979). Briefly, the gels were equilibrated in transfer buffer, placed in contact with nitrocellulose paper (Hoefer Scientific Instruments, San Francisco, California), and sandwiched between sheets of blotting paper. The proteins were transferred (Mini Transphor, Hoefer Scientific) for 2 hr at 35–39 V/20 mA. The nitrocellulose paper was blocked in gelatin (3% in Tris-buffered saline, pH 7.5) overnight at RT. The paper was then rinsed twice with distilled water, cut into strips, and exposed to the McAb or control solutions for 5 hr at RT. The McAb in culture supernatants were used undiluted; the ascitic fluid was diluted 1:100 in antibody buffer (pH 7.4). The strips were rinsed twice in phosphate-buffered saline with Tween 20 (PBS/Tween 20) and exposed to biotinylated anti-mouse IgG (H+L; Cooper Biomedical-Scientific Division, Malvern, Pennsylvania; 1:1,000 in antibody buffer) for 2 hr at RT. After washing in PBS/Tween 20, the strips were labeled with avidin–peroxidase conjugate (Cooper Biomedical-Scientific Division, Malvern, Pennsylvania; 1:1,000 in antibody buffer) for 2 hr at RT. The nitrocellulose paper was then developed using Peroxidase Substrate System (4-chloro-1-naphthol) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland) for 5–15 min, and rinsed with distilled water.

Results

Antibody labeling

Seven McAb produced either even or irregular fluorescent reactions (Figs. 1, 2) on the surface of air-dried sporozoites. Five of these McAb produced similar fluorescent labels on G-F sporozoites (Table 1). However, antibody 42E11 re-

acted strongly with the entire surface and anterior end of air-dried sporozoites (Fig. 3) but primarily with localized areas on G-F sporozoites (Fig. 4), while antibody D2 labeled the surface of air-dried sporozoites but failed to react with G-F sporozoites. With the control monoclonal antibodies, 91C7, 1209, and 1223, faint internal fluorescence was observed only in damaged sporozoites. With supernatants from the parent myeloma and fused cell lines, no fluorescent labeling of intact G-F sporozoites was observed. Ferritin labeling was generally similar to the fluorescent labeling, occurring as uniform layers or as aggregates (Figs. 5, 6). With McAb 42E11, scattered clusters of ferritin granules were observed (Fig. 7). Few ferritin granules were observed on sporozoites treated with supernatants or ascitic fluid from parent myeloma and fused cell lines, or with McAb specific for internal structures.

Relative molecular weight

Western blots of SDS-PA gels of SDS-solubilized sporozoites of 4 species of *Eimeria* are shown in Figure 8. Seven McAb that reacted with the surface membrane of *Eimeria* sporozoites labeled antigens having Mr bands of ~24,000. There were slight variations in that the Mr of the antigens recognized by *E. tenella*-specific McAb (B10—lane A5, and D2—lane B4) appeared to be slightly greater than those recognized by the other McAb.
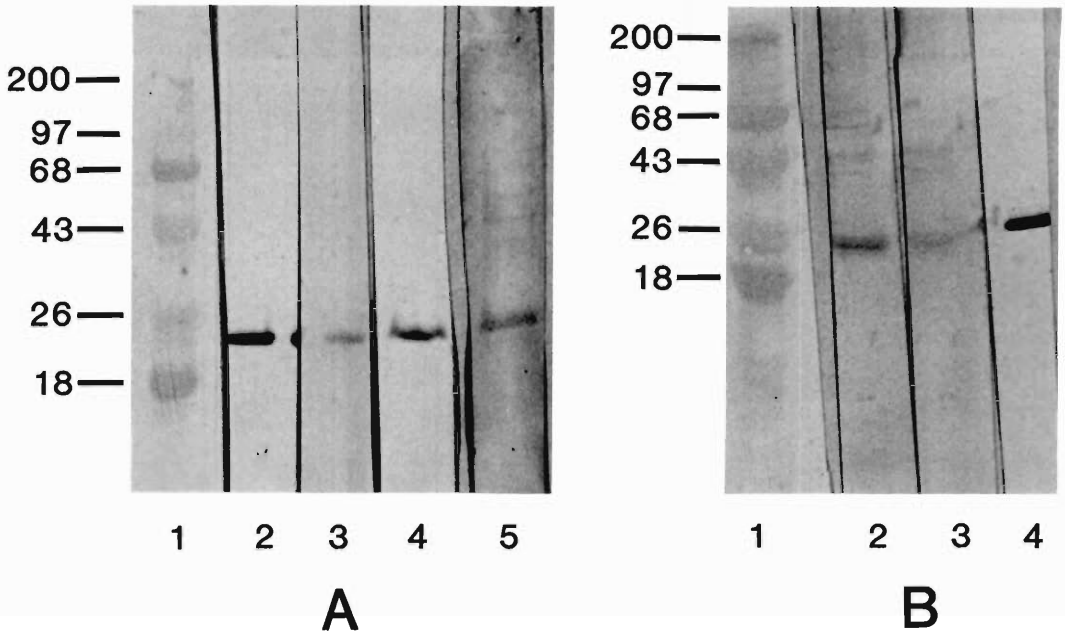
Figure 8. Western blots of *Eimeria* sporozoite antigens recognized by monoclonal antibodies elicited against various *Eimeria* species. The McAb were reacted with the species of *Eimeria* against which they were produced. Lanes A1 and B1—molecular weight standards from 18,400 to 200,000; lane A2—*E. meleagrimitis* + 42E11; lane A3—*E. acervulina* + 1227; lane A4—*E. meleagrimitis* + 43A6; lane A5—*E. tenella* + B10; lane B2—*E. adenoeides* + 33A11; lane B3—*E. meleagrimitis* + 42E11; lane B4—*E. tenella* + D2.

Discussion

In the present study, McAb elicited by different *Eimeria* species reacted with sporozoite surface antigens having similar Mr (~24,000) (Fig. 8). However, 6 of these antibodies reacted only with the *Eimeria* species against which they were elicited, suggesting differences among the specific epitopes recognized by the McAb. Apparently, these epitopes are unique to the eliciting species although they occur on antigens having similar Mr. In contrast to the specificity of the other 6 McAb, the seventh McAb, 1227, recognized an ~24,000-Mr antigen in SDS-solubilized *E. meleagrimitis, E. adenoeides,* and *E. tenella* sporozoites, as well as in the eliciting species, *E. acervulina.* This epitope is apparently different from those recognized by the species-specific McAb, and is more widely distributed among the *Eimeria.*

Fluorescence and ferritin labeling suggested differences in the location and distribution of the antigens recognized by the 7 McAb. Three of the McAb, B10, 1227, and 42E11, labeled the surface of air-dried sporozoites. Two, B10 and 1227, also labeled the surface of G-F sporozoites, suggesting that the epitopes recognized by B10 and 1227 are predominantly on the sporozoite surface and that the McAb–epitope binding was not markedly reduced by glutaraldehyde fixation. The species-specificity of McAb B10 for *E. tenella* antigen and the cross-reactivity of McAb 1227 for several *Eimeria* species (Table 1) suggest that the epitopes recognized by these 2 McAb are different. In contrast, McAb 42E11 labeled the entire surface and anterior end of air-dried sporozoites but only localized areas of G-F sporozoites. The change in labeling pattern suggests that the recognized epitopes are either altered by glutaraldehyde fixation or that the epitope is intramembranous and glutaraldehyde fixation of the membrane prevented contact with the McAb.

Monoclonal antibodies 33A11 and 43A6, which produced irregular fluorescent and ferritin labels, also reacted with substances surrounding air-dried sporozoites, suggesting that the recognized antigens are loosely associated with the sporozoite surface and/or are secretory products. However, 33A11 reacted only with *E. adenoeides* sporozoites and 43A6 only with *E. meleagrimitis,* suggesting that the recognized epitopes are not identical.

Two of the McAb, B10 and 33A11, were previously shown to inhibit the invasion of cells by the sporozoites; a third McAb, 43A6, had no effect on invasion (Augustine and Danforth, 1985). In the studies presented here, neither surface labeling by IFA or ferritin, nor SDS-PAGE and Western blots revealed characteristics that would differentiate the invasion-inhibiting McAb from noninhibitors. Therefore, continued study is required to define the specific characteristics of sporozoite surface antigens that are involved in the invasion of host cells by the *Eimeria*.

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Errata

In recent issues of this journal, the following corrections should be made:

January 1985, 52(1):111, in the article by Lichtenfels et al.:

In Figures 60–62, the cuticle of *Dirofilaria immitis* early and middle fourth stages, the scale bars should equal 2 μm instead of 5 as indicated.

January 1987, 54(1):133, in the article by Lichtenfels et al.:

In the last line, measurements of the external surface layer of *Dirofilaria immitis* infective larval cuticle given as 10–20 μm should be 0.1–0.2 μm.

January 1987, 54(1):136, same article:

In Figure 5, the cuticle of mid-fourth-stage larval *Dirofilaria immitis* 15 DAI, the scale bar is missing. It should be 17.9 μm long.

Fine Structure of a Bacterial Community Associated with Cyathostomes (Nematoda: Strongylidae) of Zebras

R. C. Krecek,[1] R. M. Sayre,[2] H. J. Els,[3]
J. P. Van Niekerk,[3] and F. S. Malan[4]

[1] Department of Parasitology, Faculty of Veterinary Science, University of Pretoria,
P.O. Box 12580, Onderstepoort 0110, Republic of South Africa
[2] Nematology Laboratory, Agricultural Research Service, USDA,
Beltsville, Maryland 20705
[3] Electron Microscope Unit, Medical University of Southern Africa,
0204 Medunsa, Republic of South Africa and
[4] Hoechst Research Farm, P.O. Box 124, 1320 Malelane, Republic of South Africa

ABSTRACT: Microorganisms attached to the posterior and anterior extremities of zebra cyathostomes are studied by scanning electron (SEM) and transmission electron microscopy (TEM). The predominant constituent of the microbial community is a filamentous prokaryotic organism, which bears resemblance to *Arthromitus* Leidy, 1849, and is designated *Arthromitus*-like organism (ALO). ALO is associated with the vulvar and anal openings of the female's posterior end. The other organisms include those with a filamentous cross wall, a distinct double cell wall, a blunt end, and spiral shape. These microbes are not observed to cause harm to the cyathostome host.

KEY WORDS: morphology, SEM, TEM, filamentous prokaryotes, nematodes, equine, strongyles, bacteria, wildlife, cuticle, South Africa.

During recent dry periods in southern Africa, individual Burchell's zebras (*Equus burchelli antiquorum*) were randomly culled from herds in the Kruger and Etosha National Parks to reduce population pressures. This action provided the opportunity to survey this wild equid for its helminth populations.

The large intestine of wild and domestic equids is the habitat for the majority of more than 109 known species of helminth parasites, principally nematodes (Theiler, 1923; Lichtenfels, 1975; Scialdo-Krecek, 1984). Filamentous prokaryotic organisms were noted attached to the anterior and posterior extremities, particularly to the vulvar and anal openings of female strongyles, and to the cuticle of both sexes of worms that were recovered from the large intestines of these zebras. During investigations, members of the Strongylidae, the largest family of nematodes in this equid, were identified and frozen for future biochemical studies. Our purposes here are (1) to report on the fine structure, based on scanning and transmission electron microscopy, of bacterial organisms associated with the cyathostomes (Strongylidae: Cyathostominae) from zebras, and (2) to present evidence of a microbial community associated with these nematodes.

Materials and Methods

Helminth collection

Five zebras were killed and processed at necropsy according to published methods (Malan et al., 1981a, b; Scialdo-Krecek, 1984). Live cyathostomes were recovered from the large intestine at necropsy and maintained in physiological saline (0.85% NaCl solution) in a water bath at 37°C.

Preparation for electron microscopy

The cyathostomes were examined under a stereomicroscope and those nematodes with filamentous microorganisms attached were identified. Between examination and fixation, the cyathostomes were maintained in a water bath at 37°C. Those worms intended for transmission electron microscopy (TEM) were transferred to 3% glutaraldehyde (GA) in Millonig's phosphate buffer at pH 7.2 (room temperature) for 7 days, washed twice for 15 min in Millonig's buffer, and postfixed with 1% OsO₄ in 0.15 M sodium cacodylate buffer. The specimens were washed in 0.15 M sodium cacodylate buffer, dehydrated in 50%, 70%, 90%, and 100% (twice) ethanol, cleared in propylene oxide, and embedded in Polarbed 812 resin. Thin sections through the cyathostome's posterior extremity, including a few mm of the anus and vulva, were cut on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and photographed in a JEOL 100 CX transmission electron microscope at 100 kV.

In preparation for scanning electron microscopy (SEM), the nematodes were fixed in glutaraldehyde as

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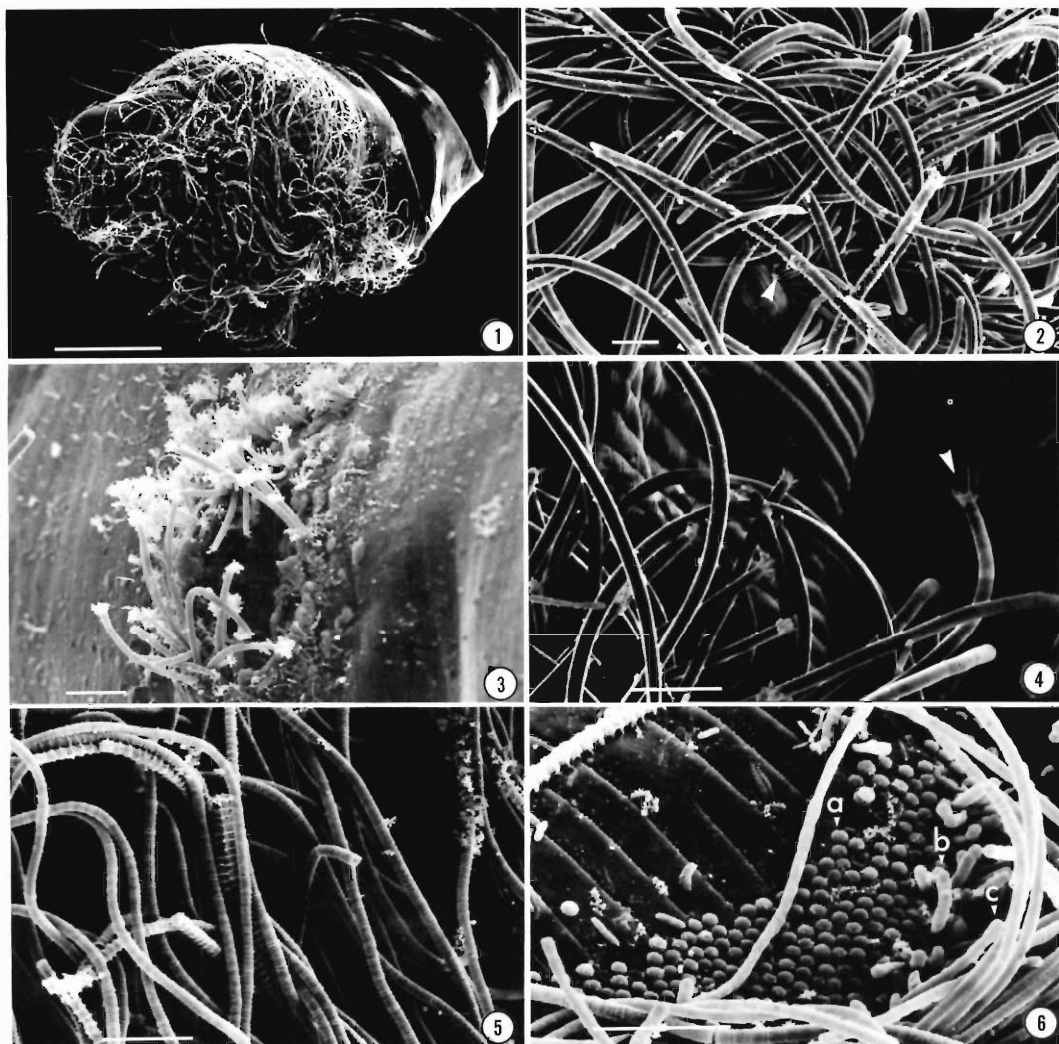
Figures 1–6.   Scanning electron micrographs of the filamentous microorganisms associated with zebra cyatho-stomes which resemble *Arthromitus* Leidy, 1849, and were therefore labeled ALO (*Arthromitus*-like organism). 1. Posterior extremity of female cyathostome showing mass of filaments covering tip of tail, and anal and vulvar openings. Scale bar 100 μm. 2. The organisms extrude from the anal opening (arrow). Scale bar 10 μm. 3. Short filaments surround the vulvar opening. Scale bar 10 μm. 4. Fingerlike tips (arrow) of free end of organism suggests a stage of growth. Scale bar 10 μm. 5. Segmentation evident in what may be older filaments. Scale bar 10 μm. 6. Coccoid bodies (a) on cyathostome cuticle which may give rise to longer segments (b) and eventually filaments (c). Scale bar 10 μm.

above, as well as in 70% ethanol or in 1% picric acid in Karnovsky's, washed in sodium cacodylate buffer, and subsequently dehydrated in 50%, 70%, 90%, and 100% (twice) ethanol followed by critical point drying in CO_2. Individual specimens were mounted on stubs, previously coated with a thin carbon layer. This was followed by sputter-coating with an Au-Pd layer. They were then viewed in a JEOL 35C scanning electron microscope at 8–12 kV.

Results

Filamentous microorganisms were found attached to the posterior and anterior extremities and along the cuticle of several cyathostome species recovered from the large intestines of the zebras. In addition, these organisms were attached inside the vulvar and anal openings of
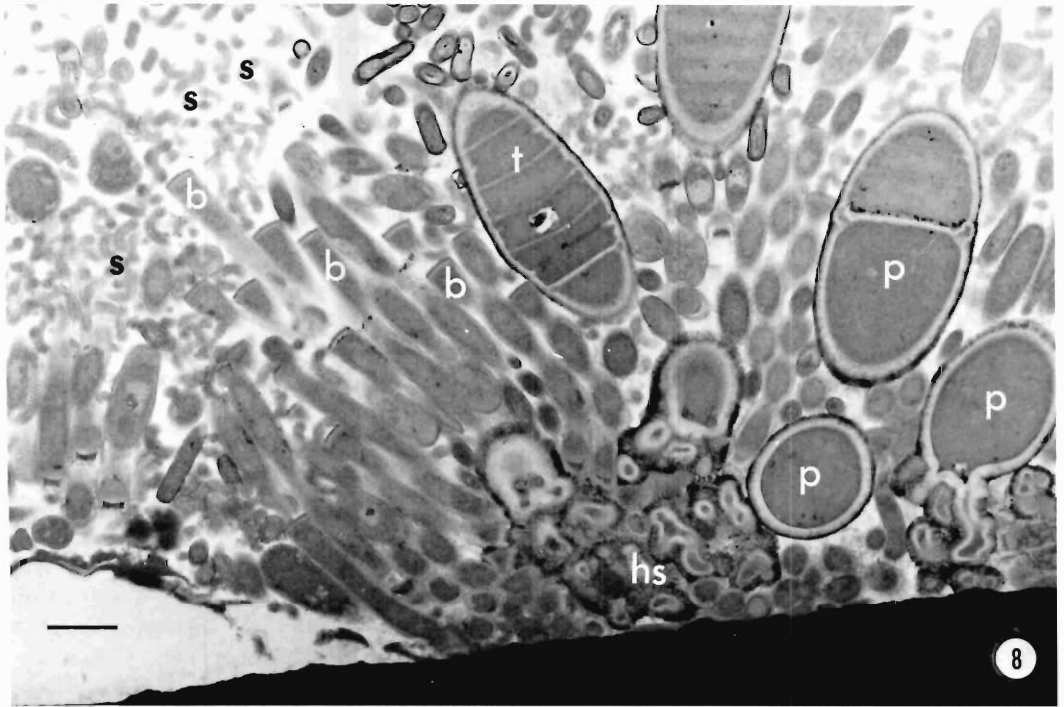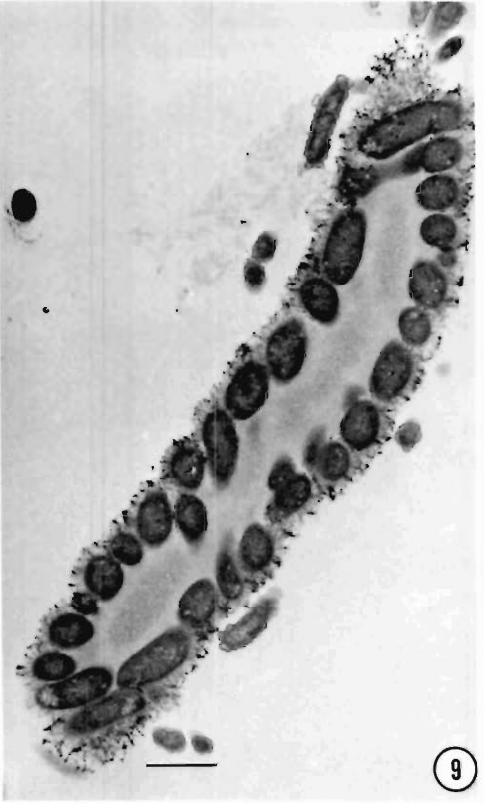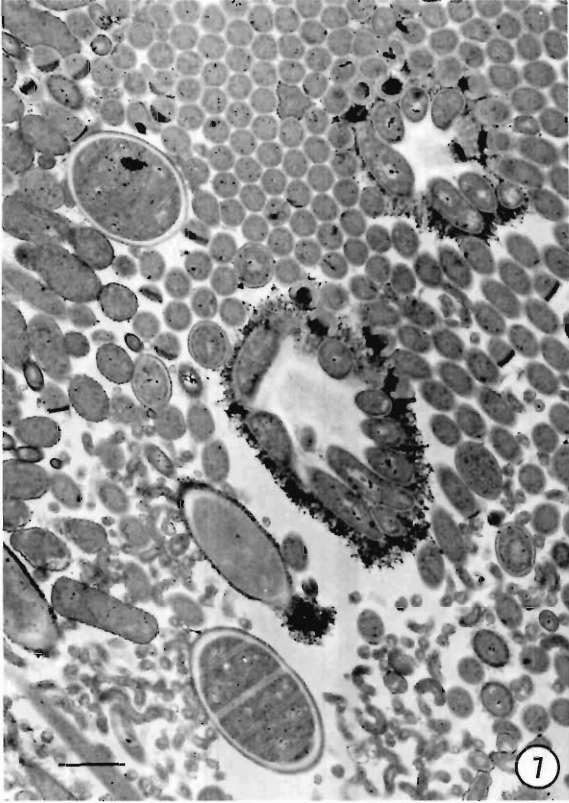
Table 1. Nematode and animal hosts of *Arthromitus cristatus* and *Arthromitus*-like organisms (ALO).

Microbe	*Arthromitus cristatus*	*Arthromitus*-like organisms (ALO)
Animal host	Millipede: *Narceus annularis* (Rafinesque)	Burchell's zebra: *Equus burchelli antiquorum* Smith, 1841
Nematode hosts	Rhigonematidae:	Strongylidae: Cyathostominae
	Thelastoma attenuatum (Leidy, 1849)	*Cylicocyclus auriculatus* (Looss, 1900) Chaves, 1930
	Aorurus agile (Leidy, 1849)	*Cylicocyclus triramosus* (Yorke and Macfie, 1920) Chaves, 1930
	Rhigonema infectum (Leidy, 1849)	*Cylindropharynx* sp. (?*C. intermedia* Theiler, 1923)

female cyathosomes. These microbes were associated with cyathostomes from all 5 zebras examined at 2 geographic locations, the Kruger National Park, South Africa, and the Etosha National Park, South West Africa/Namibia. One type of filamentous microorganism that resembled *Arthromitus* Leidy, 1849, was labeled ALO (*Arthromitus*-like organism) (Figs. 1–8) and shared characteristics with the prokaryotic Actinomycetales (Williams et al., 1973) — namely, the absence of a nuclear membrane, mitochondria, and a polyribosomal reticulum — but resembled gram-positive bacteria in having a plasma membrane with a parallel appearance, relatively dispersed fine fibrils of the nuclear membrane, and numerous ribosomal particles.

A community of microbes comprised of at least 4 more bacterial organisms was observed. In addition to ALO another filamentous microbe was observed and designated FCO (filamentous cross wall organism) because of the manner in which the cross walls formed. The numerous cross walls suggest the possibility of an elaborate branching (Fig. 12) which might result in the types of organisms demonstrated in Figures 17F or 15b.

A blunt-ended organism (BEO), the second microbe noted, was frequently present (Fig. 8) and because of its consistency was not considered an artifact. A third spiral-shaped organism (SSO) was regularly present (Fig. 8). The fourth microorganism observed had a distinct double cell wall (DWO) (Fig. 10).

The hosts reported to be associated with *Arthromitus* and those observed as ALO in this study are listed in Table 1. Table 2 provides a comparison of *Arthromitus* as described by Leidy, 1853, and the predominant filamentous microorganism, ALO.

Scanning electron micrographs (Figs. 1–6) demonstrate the association of filamentous microorganisms with the posterior extremity and cuticle of female cyathostomes. Transmission electron micrographs of these microorganisms attached to the nematode cuticle and female reproductive tract are depicted in Figures 7–13. Leidy's (1853) drawings of a microbial community in which both bacteria and fungi are associated with 3 nematodes of a millipede are shown in Figures 14–18.

Discussion

The *Arthromitus*-like organism (ALO) was the predominant constituent of the microbial community associated with cyathostomes of zebras. Its conspicuous length (92–1,200 μm) allowed easy designation with the naked eye and served to separate a number of the cyathostome genera from the large strongyles by its attachment.

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Figures 7–9. Transmission electron micrographs of the filamentous microorganisms attached externally to the cyathostome cuticle as well as internally to reproductive and digestive tracts. Scale bar 1.0 μm. **7.** Section through cuticle, resembles the coccoid bodies (c) in Figure 6. **8.** Aggregate of holdfast structures (hs) for organism attached to nematode, typical prokaryotes (p), and development of thallus (t). Note other organisms that may form a microbial community; spiral-shaped (s) and blunt-ended (b) organisms. **9.** Cross section of finger-like tips in free end of filamentous organism, which resemble finger-like tips in Figure 4.
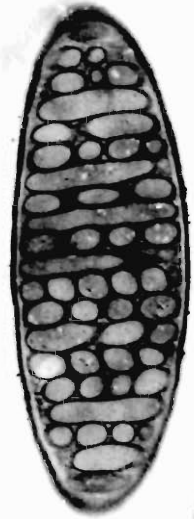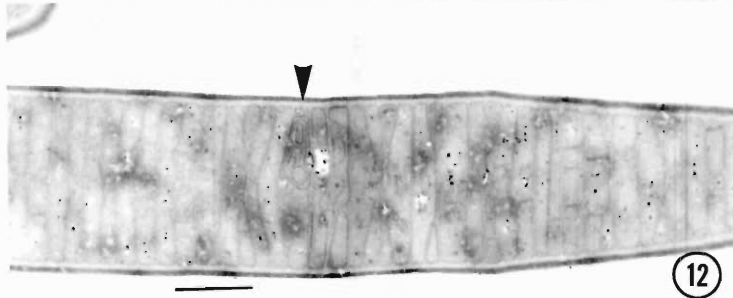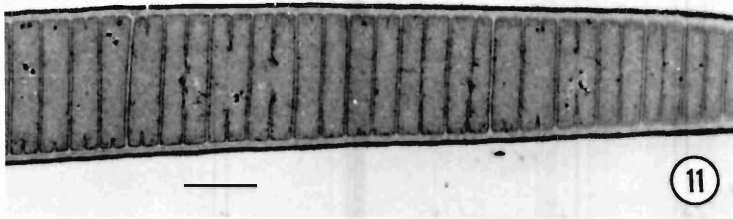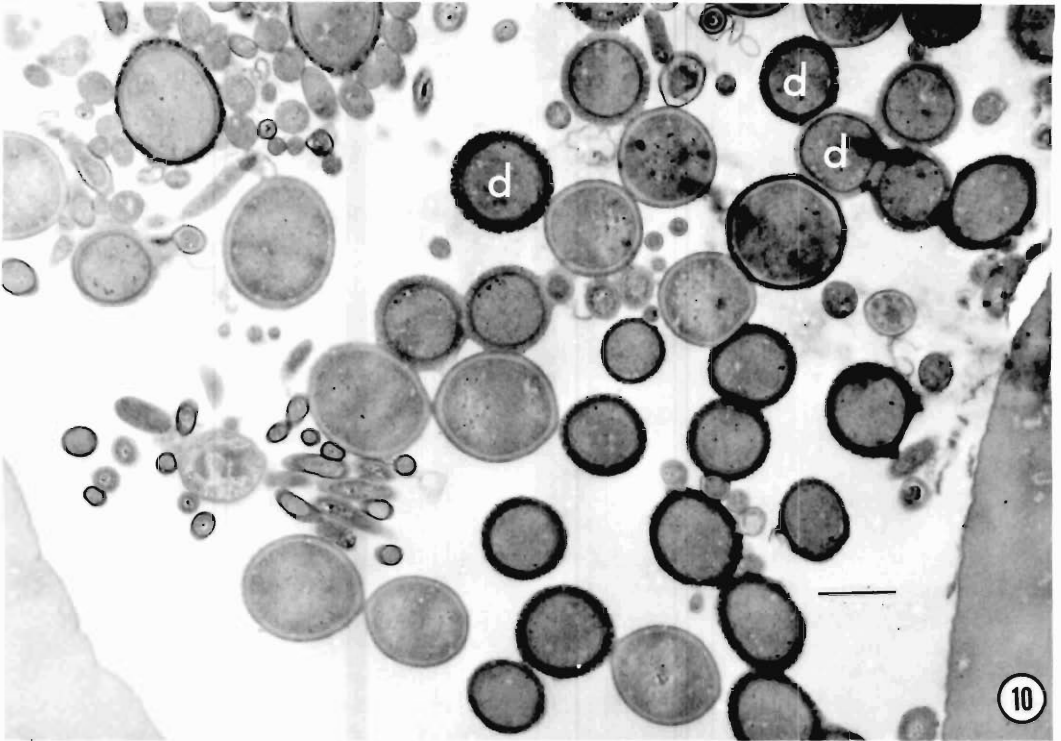
Figures 10–13.   Further organisms of the microbial community and cross wall development. Scale bars 1.0 μm. 10. Microorganisms include double wall (d). 11. Different stages (arrows) of cross wall formation. 12. A second organization of cross wall development is evident (arrow). 13. A third type of cross wall.

Table 2. A comparison of *Arthromitus* Leidy, 1849 (Leidy, 1853) with the *Arthromitus*-like organisms (ALO) of present study.

Arthromitus (after Leidy, 1853)	ALO
(1) Thallus—delicate, filamentous, linear, straight or inflected, flexible, colorless, translucent, obtusely rounded at free end.	(1) Same, except free end with finger-like projection.
(2) Pedicle of attachment 1 or more amber-colored round or oval granules or in aggregations of several granules.	(2) Attachment site usually aggregation.
(3) Articuli indistinct, but becoming well marked after the development of the interior sporular body.	(3) Articuli distinct and indistinct.
(4) Spore oval, simple, faintly yellowish, translucent, highly refractive, usually lying oblique and alternating in position in different articuli.	(4) Spores not evident.
(5) Length 17–2,083 μm; width 0.15 μm.	(5) Length 92–1,200 μm; width 3 μm.
(6) Habitat: Mucous membrane of ventriculus and large intestine of *Narceus annularis,* and also *Enterobryus elegans, Rhigonema infectum, Aorurus agile,* and *Thelastoma attenuatum;* from the mucous membrane and its appendages of the ventriculus of *Passalus cornutus* and *Polydesmus virginiensis* and *Eccrina longa.* Usually from lips of anal and generative apertures.	(6) Habitat: Usually of cuticle, anal and vulvar openings of the posterior extremity of females. Sometimes associated with anterior extremities and with males. Only associated with the genera, *Cylicocyclus* and *Cylindropharynx* (Strongylidae: Cyathostominae).

Though these filamentous microorganisms bear similarities to *Arthromitus* described by Leidy (1853) (Table 2), they did not exhibit evidence of endospores, a characteristic of *Arthromitus.* The possibility exists, however, that our samples were observed in a nonendospore-forming phase of growth. Leidy's (1853) observations took place over a period of time (i.e., hours or days), whereas our specimens were collected and fixed at one moment in time.

No correlation between the presence of eggs in the uteri of female cyathostomes and the occurrence of ALO was observed. A closer examination may reveal if young females (nongravid) or spent females (beyond egg-laying age) were more frequently associated with the presence of ALO, or whether ALO affects the production or expulsion of eggs at all. Such an effect is probably unlikely because the penetration of ALO appeared to be superficial and not more than 100–300 μm deep into the reproductive tract.

We feel that the relationship of these microorganisms to their host is probably of a commensal nature and therefore agree with the definition proposed by Noble and Noble (1961)— i.e., the association of 2 species in which only 1 species may benefit. We can assume 1 of the 2 associants in the present study, either the nematode or the microbes, is dependent on the other; however, we have no evidence that either could define the nature of the apparent commensalism. Poinar (1979) cites several instances of potential pathogenic bacteria in nematodes and Anderson et al. (1978), for instance, observed microbes in

association with cuticular lesions. *Strongylus edentatus,* recovered from horses, belongs to the same nematode family as the cyathostomes (Lichtenfels, 1975). Anderson et al. (1978) considered the microbes harmful and described 4 types of lesions. According to these authors, the association of the lesions with the nematode genitalia suggests that "some lesions of helminth cuticles may be a venereal disease."

The cyathostome species with which ALO are associated in this study may not be a complete list (Table 1). Those included reflect a random sampling (400–500 cyathostomes) that was fixed at the zebra postmortem examination. Identification of further specimens to species level should reveal whether host species restriction or prevalence on a seasonal basis occurs. Cyathostome species preference will also relate to intestinal site distribution since it is a characteristic of these nematodes (Lichtenfels, 1975). Furthermore, the ALO appears to be associated more often with the posterior and less frequently with the anterior extremities of the cyathostomes. Closer examination of more specimens will reveal whether other nematode sites are involved.

Leidy (1853) observed a community in which bacteria and fungi inhabited millipede nematodes (Figs. 14–18). More extensive microbial communities exist consisting of 4–30 protists as in the paunch of wood-eating termites (Margulis et al., 1986). Perhaps the extent of the microbial communities in the millipede, termites, and zebras is related in some way to the ingestion of organic matter and the appearance of ALO in 2
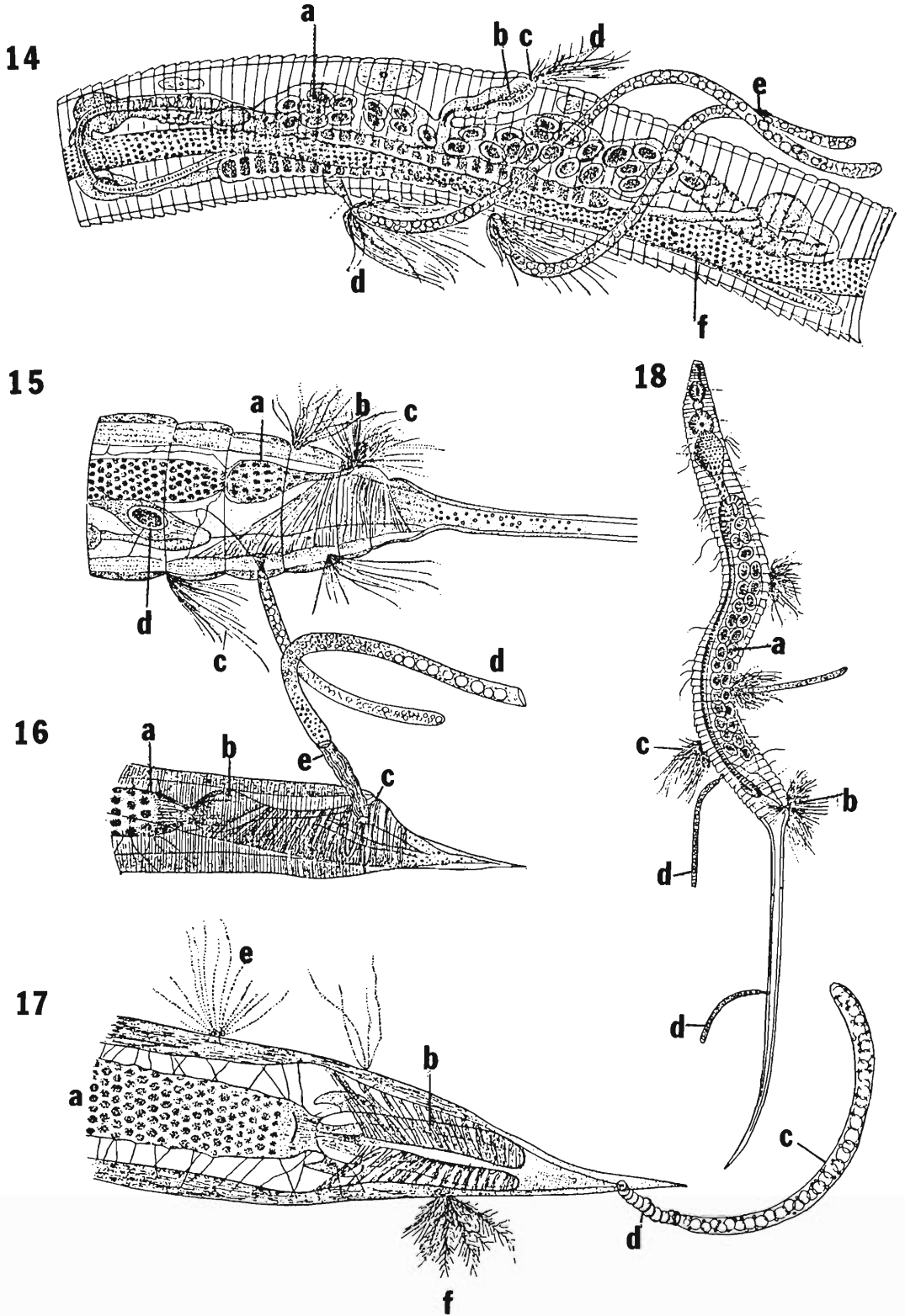
of the 3 hosts. Certainly, given the milieu, it would be surprising if the nematode's cuticle would be free of bacteria. It is unlikely that all adherent bacteria are harmful; some may not be pathogenic, and others may benefit the nematode (Sayre and Starr, 1987). Likewise, filamentous bacterial organisms have frequently been reported from the gut of mice, dogs, cats, sheep, horses, and pigs (Anderson et al., 1971, 1978; Davis and Savage, 1974; Davis et al., 1976, 1977; Gregory et al., 1985). The present study is the first known report of a microbial community believed to be nonpathogenic and associated with strongyles inside a mammalian gut.

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Figures 14–18. Drawings of a microbial community associated with nematodes from the intestinal tract of the millipede *Narceus annularis* (Rafinesque) according to Leidy (1853). 14. Middle portion of the body of *Thelastoma attenuatum* Leidy, 1849, with 2 thalli of *Enterobryus* growing from it. a, Uterus; b, vagina; c, vulva; d, *Arthromitus*; e, *Enterobryus*; f, intestine. 15. Posterior extremity of *Aorurus agile.* a, Rectum; b, anus; c, tufts of *Arthromitus*; d, uterus with egg. 16. Lateral view of the posterior extremity of *Rhigonema infectum.* a, Intestine; b, rectum; c, anus; d, *Enterobryus*; e, pedicle of attachment of *Enterobryus.* 17. Posterior extremity of *Rhigonema infectum.* a, Intestine; b, rectum; c, young thallus of *Enterobryus*; d, long pedicle of *Enterobryus*; e, *Arthromitus cristatus*; f, *Cladophytum comatum.* 18. *Aorurus agile* with *Enterobryus elegans* and *Arthromitus cristatus.* a, Uterus with eggs; b, anus; c, *Arthromitus cristatus*; d, *Enterobryus elegans.*

Hammerschmidtiella andersoni sp. n. (Thelastomatidae: Oxyurida) from the Diplopod, Archispirostreptus tumuliporus, in Saudi Arabia with Comments on the Karyotype of Hammerschmidtiella diesingi

MARTIN L. ADAMSON[1] AND ABDUL K. NASHER[2]

[1] Department of Zoology, University of British Columbia,
Vancouver, British Columbia V6T 2A9, Canada and
[2] King Saud University, Abha Branch, College of Education,
P.O. Box 919, Abha, Saudi Arabia

ABSTRACT: Hammerschmidtiella andersoni sp. n. (Thelastomatidae: Oxyurida) is described from the posterior gut of Archispirostreptus tumuliporus (Spirostreptida: Diplopoda) from Saudi Arabia. The new species is distinguished from all previously described species in the genus except for H. manohari by its slender shape and by the fact that the cephalic annules decrease abruptly in size after the first few annules. The 2 species are distinguished by the fact that the anterior, long annules alternate with short annules in H. andersoni but not in H. manohari. The new species further differs from H. manohari in having an unflexed testis, a much shorter tail in the male, and by the form of cytoplasmic processes surrounding the oral opening of the female. Finally, the new species is the only species in the genus in which a gubernaculum has been reported. The karyotype of H. diesingi is shown to be the same as that of H. andersoni, namely 5 in males and 10 in females.
KEY WORDS: Nematoda, Spirostreptida, haplodiploidy, chromosome complement.

In an earlier article (Adamson, 1984) cytological aspects of gametogenesis were studied in a species of Hammerschmidtiella collected from Archispirostreptus tumuliporus from Saudi Arabia. The material represents a new species and is herein described as Hammerschmidtiella andersoni sp. n., in honor of Professor R. C. Anderson (Department of Zoology, University of Guelph, Canada). In addition H. diesingi was studied cytologically to determine whether the karyotype was similar to that in H. andersoni. These data are reported herein.

Materials and Methods

Diplopods collected from Asir, Saudi Arabia, were fixed in 70% ethanol before dissection. Hosts were identified by Dr. J. P. Mauries of the Museum national d'Histoire naturelle (Laboratoire des Arthropodes) in Paris. Nematodes recovered from the posterior gut were stored in 70% ethanol before being cleared and studied in lactophenol and glycerin.

Cytological studies of H. diesingi were carried out on male and female worms recovered from Periplaneta americana from a colony housed in the Zoology Department (University of British Columbia, Vancouver, British Columbia). Worms were dissected in 0.066 M phosphate buffer and reproductive tracts were fixed for 5 min in a solution containing 70 parts ethanol to 25 parts acetic acid and 5 parts formalin. Preparations were squashed between slide and coverslip and chromosomes were drawn with the aid of a drawing tube attached to a microscope equipped with phase and Nomarski differential interference contrast.

Hammerschmidtiella andersoni sp. n.

Description (Figs. 1–15)

GENERAL: Slender worms with marked sexual dimorphism with respect to size.

MALE: Cephalic extremity pointed. Mouth opening hexagonal, surrounded by 4 submedian pairs of nerve endings, presumably representing outer labial papillae, and 2 pedunculate amphids. Inner papillae not observed.

Cuticle just posterior to cephalic extremity with tiny transverse striations about 2 μm apart disappearing near level of anus. Narrow lateral alae extending from just posterior to level of base of esophagus to just anterior to anus.

Buccal capsule short, in form of narrow ring. Esophagus consisting of clavate corpus distinctly set off from cylindrical isthmus and elongate pear-shaped bulb. Nerve ring encircling isthmus. Testis outstretched, its anterior extremity just posterior to level of excretory pore. Caudal extremity truncate at level of anus bearing slender caudal appendage.

Five pairs caudal papillae, 1 pair subventral and 1 pair lateral preanal raised on fleshy lobes; 1 pair lateral adanal; 1 pair represented by 2 inconspicuous nerve endings on posterior anal lip; 1 pair at base of caudal appendage. Phasmids on fleshy lobes supporting lateral preanal papillae. Spicule short, simple. Small gubernaculum present.

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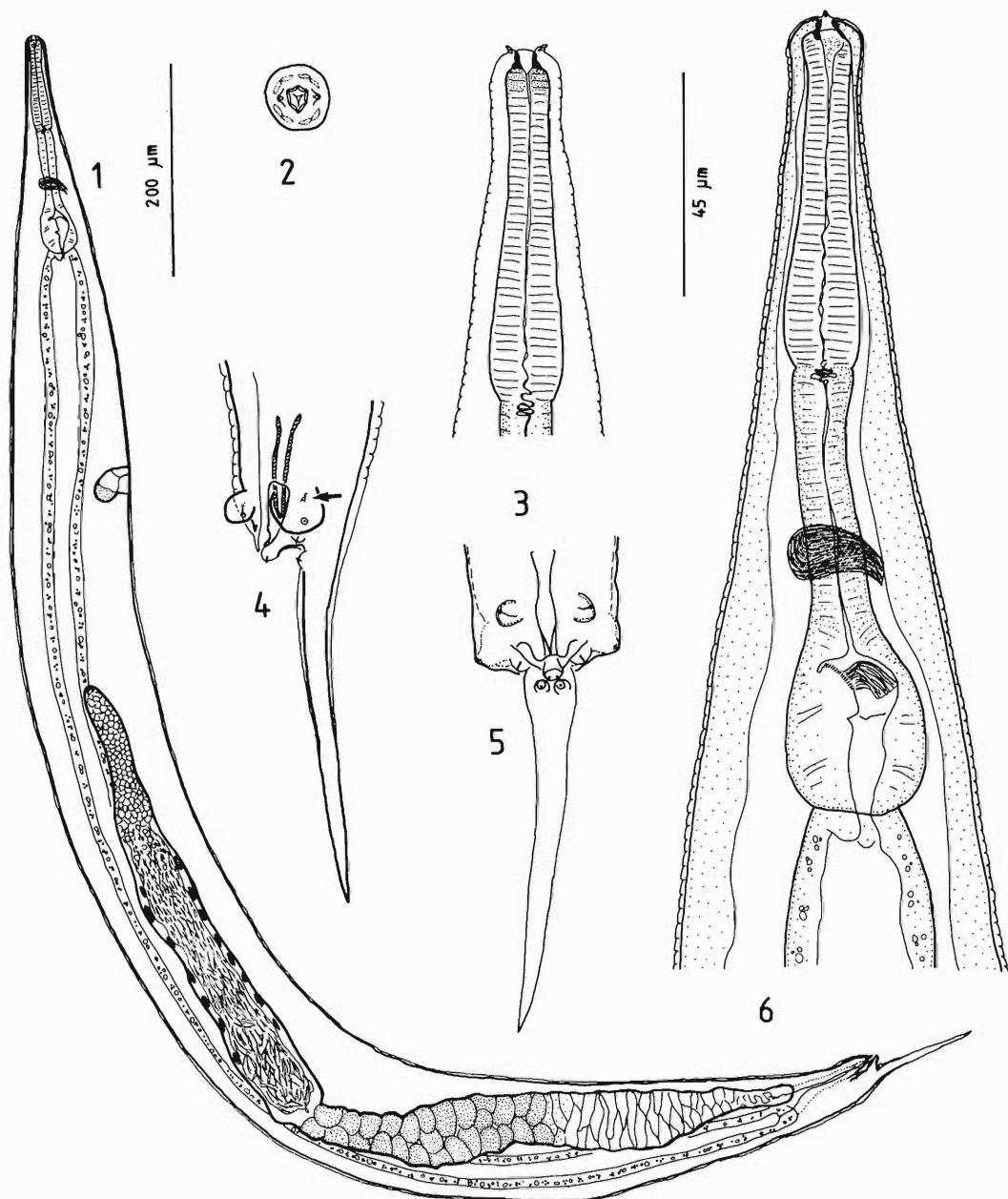
Figures 1–6. Male *Hammerschmidtiella andersoni* sp. n. 1. Entire worm, lateral view. 2. Apical view. 3. Anterior extremity, ventral view; note pedunculate amphids. 4, 5. Caudal extremity, lateral and ventral views respectively; note phasmid (arrow). 6. Esophageal region, lateral view. Scale bars: 1 = 200 μm; 2–6 = 45 μm.

FEMALE: Body increasing in width gradually posteriorly, reaching maximum width at midbody and ending in long attenuate tail.

Oral opening subtriangular, surrounded by 6 inner papillae and 8 pairs of digitiform cyto-plasmic processes, perhaps representing nerve endings of outer labial papillae.

Cuticle in anterior region bearing large cephalic annule 22–28 μm long followed by 4 an-nules about 1.5 μm long alternating with 4 an-
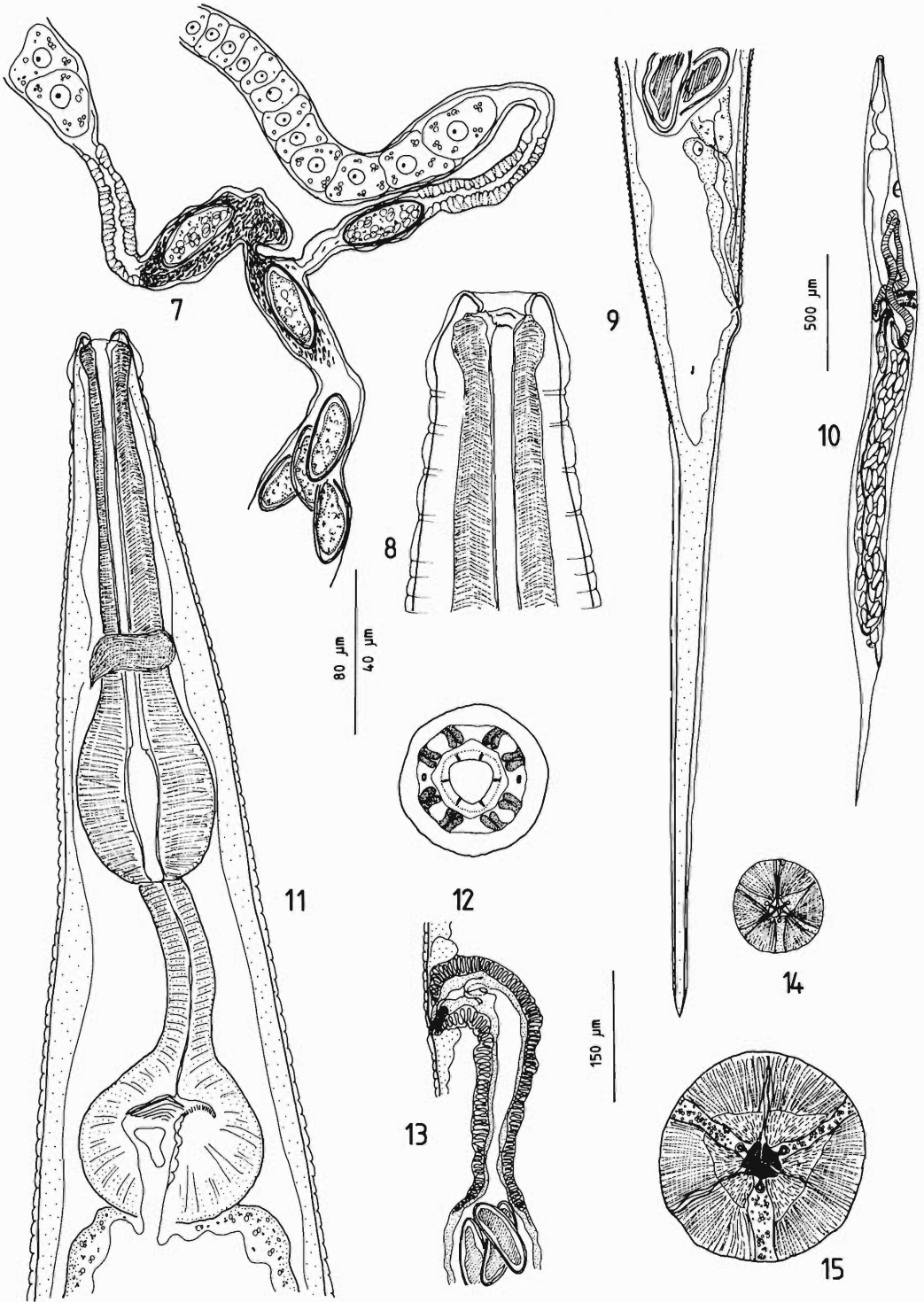
Figures 7–15. Female *Hammerschmidtiella andersoni* sp. n. 7. Junction of oviduct with common uterus. 8. Cephalic extremity, lateral view. 9. Caudal extremity, lateral view. 10. Entire worm, lateral view. 11. Esophageal region, lateral view. 12. Apical view. 13. Vulvar region, lateral view. 14, 15. Cross sections through corpus and metacorpus, respectively. Scale bars: 7, 9, 13 = 150 μm; 8, 12, 14, 15 = 40 μm; 10 = 500 μm; 11 = 80 μm.

nules about 8 μm long; annules posterior to these about 4 μm long, disappearing just posterior to anus.

Ovaries, their blind ends just posterior to level of excretory pore, leading anteriorly, flexing posteriorly, and then flexing anteriorly before emptying into oviducts near level of vulva. Oviducts emptying into short paired uteri, fusing to form common uterus; common uterus leading posteriorly, flexing anteriorly about 100 μm from anus, and emptying into vagina.

Measurements

MALE (range of 5 paratypes): Length 1.11–1.44 mm. Maximum width 65–86 μm near midbody. Buccal cavity 2–3 μm and esophagus 148–168 μm long with corpus 62–71 μm long, isthmus and bulb 83–99 μm long and bulb 26–29 μm wide. Nerve ring 91–103 μm, excretory pore 290–384 μm long and anterior extremity of testis 408–562 μm from anterior extremity. Spicule 22–24 μm, gubernaculum 14–18 μm, and caudal appendage 71–83 μm long.

FEMALE (range of 5 paratypes): Length 3.00–3.53 mm. Maximum width 141–188 μm near midbody. Buccal capsule 3–6 μm and esophagus 391–457 μm long with corpus 240–275 μm, isthmus 73–99 μm and bulb 78–83 μm long. Maximum width of corpus 54–61 μm and bulb 75–81 μm. Nerve ring 132–160 μm, excretory pore 483–611 μm, anterior extremity of ovaries 674–769 μm, and vulva 877–1,041 μm from anterior extremity. Tail 579–679 μm long. Eggs 86–100 μm long and 32–43 μm wide (range of 12 specimens from all females).

Specimens

Type and other specimens are deposited in the parasite collection of the Museum national d'Histoire naturelle (Laboratoire de Zoologie des Vers RA 143, Paris, France).

Gametogenesis in *H. diesingi* (Figs. 16–18)

Five chromosomes were observed in the germinative zone of the testis. Typical stages in meiosis were not observed and cells in the transformation zone of the testis contained 5 chromosomes with irregular, fuzzy outlines.

Ten chromosomes were observed in the germinative zone of the ovaries. Meiosis appeared normal. Ova nearest the oviduct contained 5 bivalents and figures representing the 2 meiotic divisions were observed. Ova developed only as far as the pronuclear stage in utero.
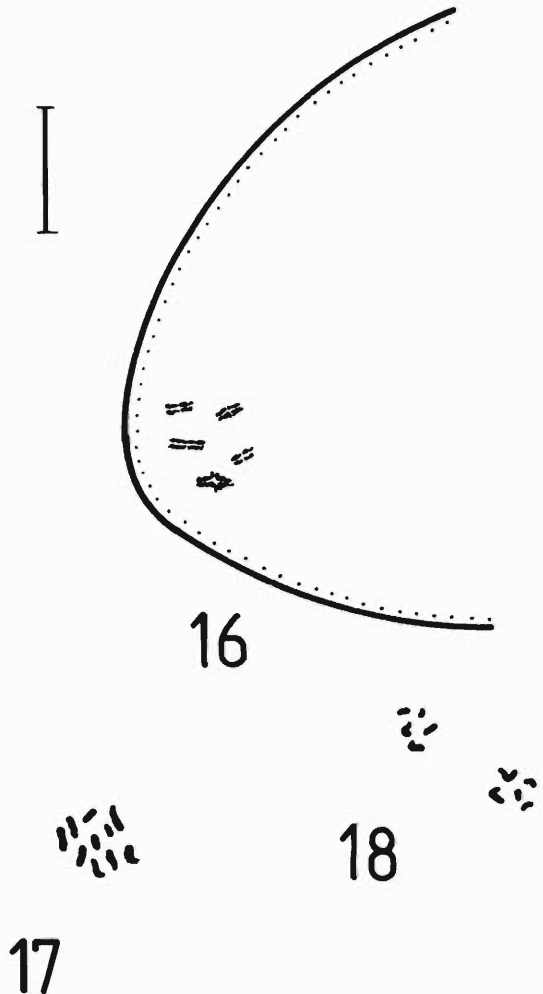
Figures 16–18. Chromosomes of *Hammerschmidtiella diesingi*. 16. Metaphase of meiosis I in ovum showing 5 bivalents. 17. Metaphase plate from germinative zone of ovary showing 10 chromosomes. 18. Two metaphase plates from germinative zone of testis. Scale bar = 10 μm.

Discussion

In addition to the new species, there are 11 nominal species of *Hammerschmidtiella*: *H. diesingi* (Hammerschmidt, 1838), the type species, *H. blatta orientalis* (Hammerschmidt, 1847), and *H. macrura* Diesing, 1850, from *Blatta orientalis* in Europe; *H. neyrae* Sanchez, 1947, from *Periplaneta orientalis* (=?*Periplaneta americana* or *Blatta orientalis*) in Spain; *H. gracile* (Leidy, 1850) from *Periplaneta americana* in North America; *H. periplaneticolae* (Singh and Singh, 1955), *H. aspiculus* Biswas and Chakravarty, 1963, and *H. bareillyi* Sharma and Gupta, 1983, from *Peri-*

224

planeta americana, H. singhi Rao and Rao, 1965, from *Corydia* sp. (Blattoidea), and *H. manohari* Rao, 1958, from *Spirostreptus* sp. (Diplopoda) in India; *H. acreana* Kloss, 1966, from *Eublaberus* sp. in Brazil (Basir, 1956; Rao, 1958; Kloss, 1966).

Chitwood (1932) considered *H. blatta orientalis, H. macrura,* and *H. gracile* to be synonyms of *H. diesingi. Hammerschmidtiella periplaneticola* was considered a synonym of *H. diesingi* by Kloss (1966). *Hammerschmidtiella bareillyi* and *H. singhi* are poorly known and the characters used to distinguish them from the type species (see Rao and Rao, 1965; Sharma and Gupta, 1983) are of dubious value. They may be synonyms of *H. diesingi.* The species is apparently nearly cosmopolitan in *Periplaneta americana* and *Blatta orientalis.*

Hammerschmidtiella andersoni sp. n. most closely resembles *H. manohari*; both are slender worms with a de Man value, V, of about 0.30, and in both, the size of annules on the cephalic extremity of females decreases abruptly after the first few annules. In other species in the genus, annule length decreases gradually as one moves posteriorly. The arrangement of annules in female *Hammerschmidtiella* spp. is constant and an excellent diagnostic character. In *H. andersoni* there is a long cephalic annule followed by 4 short (1.5 μm) annules alternating with 4 longer (about 8 μm) annules before annules decrease abruptly in length to about 4 μm. The arrangement is similar in *H. manohari* except that the long anterior annules do not alternate with short annules. Aside from differences in the cephalic annules, the new species differs from *H. manohari* in having an unflexed testis, a much shorter tail in the male, and by the form of the cytoplasmic processes surrounding the oral opening and visible in the apical view of females; these latter form 8 heart-shaped masses in *H. manohari* and 8 pairs of digitiform masses in *H. andersoni.* Finally, *H. andersoni* is the first species in the genus in which a gubernaculum has been reported.

Most thelastomatids are amphidelphic and the uteri fuse at the vagina. In *Hammerschmidtiella,* however, the ovaries are parallel and oviducts lead through paired uteri of variable length, fusing to form a long common uterus. The paired uteri are extremely short in *H. andersoni* but are over a millimeter long in *H. diesingi.* Unfortunately, this character has not been recorded in other species in the genus.

The Thelastomatidae are considered the most primitive family of the Oxyurida. In a previous study, Adamson (1984) reported on the chromosome complement of *H. andersoni* and an undescribed species of *Thelastoma.* Both were found to be haplodiploid. *Hammerschmidtiella diesingi* is only the third Thelastomatidae that has been examined cytologically. Its chromosome complement, like that of *H. andersoni,* is 5 in males and 10 in females. This supports the hypothesis that haplodiploidy is the primitive form of reproduction in the Oxyurida.

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Molossinema wimsatti gen. et sp. n. (Nematoda: Onchocercinae) from the Brain of Molossus ater (Chiroptera: Molossidae)

J. R. GEORGI,[1] M. E. GEORGI,[1] J. JIANG, [2] AND M. FRONGILLO[1]
[1] New York State College of Veterinary Medicine, Cornell University,
Ithaca, New York 14853 and
[2] College of Veterinary Medicine, Beijing Agricultural University,
The People's Republic of China

ABSTRACT: Molossinema wimsatti gen. et sp. n. from the brain of the free-tailed bat Molossus ater is described. Molossinema gen. n. differs from Litomosa Yorke and Maplestone, 1926, and from Litomosoides Chandler, 1931, in lacking a well-developed buccal capsule, in the form of the distal end of the left spicule which is gougelike instead of lashlike, and in the position of the vulva relative to the esophago–intestinal junction. Molossinema gen. n. also differs from Litomosa in lacking spines on the tail of the female. This parasite was found in the cerebral ventricles of virtually all bats trapped in a Trinidad warehouse.
KEY WORDS: nematode taxonomy, morphology, scanning electron microscopy, bats, Trinidad.

Several hundred free-tailed bats, *Molossus ater,* were captured in a warehouse in Trinidad, West Indies, and transported to Cornell University Medical School, New York City, U.S.A. for reproductive studies being conducted there under NIH Grant No. R01 HD17739. The brains of virtually all of these bats were found to be infected with a hitherto undescribed filarioid nematode.

Materials and Methods

The nematodes in question were first encountered by Dr. John Rasweiler and Dr. Hai Nguyen of Cornell Medical School in histologic sections of *Molossus ater* brain tissue, which they forwarded to the New York State Diagnostic Laboratory, Cornell University, Ithaca, New York, for identification. The histological characteristics of nematode sections in these tissues accorded well with those of Case 3 of Lichtenfels et al. (1981). Dr. Rasweiler later supplied the authors with formalin-fixed brain tissue from which 3 intact male and 5 female specimens were recovered. Dr. Rasweiler also supplied glutaraldehyde–osmium-fixed worm specimens for scanning electron microscopic examination. Microfilariae were not detected in the circulating blood; only uterine microfilariae of uncertain maturity were available for study. All measurements in the following description and illustrations are in millimeters. Ranges of all measurements are presented except for those related to the circumoral features, which were based on the best SEM of a good specimen.

Description

Molossinema wimsatti gen. et sp. n.

GENERAL: Onchocercinae Leiper, 1911. Stoma a simple dorsoventral slit 0.002 long, surrounded by elliptical zone of nonstriated cuticle 0.04 by 0.02 with long axis running from side to side and with 1 pair of outer ventral submedian papillae lying 0.02 apart just within its borders. Amphids lie 0.005 each side of stoma. Four very small inner submedian papillae located at corners of a trapezoid with base and sides 0.008 and top 0.005 (Figs. 1, 6). Buccal cavity inconspicuous (Figs. 4, 7). Esophagus long, slender, cylindrical, not divided into muscular and glandular portions, surrounded by nerve ring at first quarter of its length (Fig. 4).

MALE: Body length 13–22, maximum width 0.10–0.16. Esophagus 0.8–1.0. Caudal end coiled, caudal alae weakly developed (Fig. 2). Anus flanked by 2 pairs of sessile papillae lying in the caudal alae (Figs. 2, 8). Rugose area of ventral cuticle consisting of many transverse rows of tiny projections and extending from 0.05 to 0.5 anterior to anus (Figs. 2, 8). Spicules arcuate, unequal in size and shape (Fig. 2). Left spicule 0.11–0.13, divided into a proximal more or less cylindrical portion representing 2/3–3/4 of the total length and a distal gougelike portion. Right spicule 0.04–0.05, terminating in a small knob (Fig. 2). Anus to tip of tail 0.05–0.07.

FEMALE: Body length 48–58, maximum width 0.18–0.20. Esophagus 1.0–1.5. Vulva well posterior to esophago–intestinal junction (Figs. 5, 9). Caudal 0.5 of lateral lines bearing about 60 very small papilloid elevations at an average interval of 0.008, this interval decreasing to 0.002 near the tail. Phasmids surmount small hemispherical bases flanking tip of tail; lateral cords displaced dorsally at tip of tail (Figs. 3, 10). Anus to tip of tail 0.14–0.24 (Fig. 3).

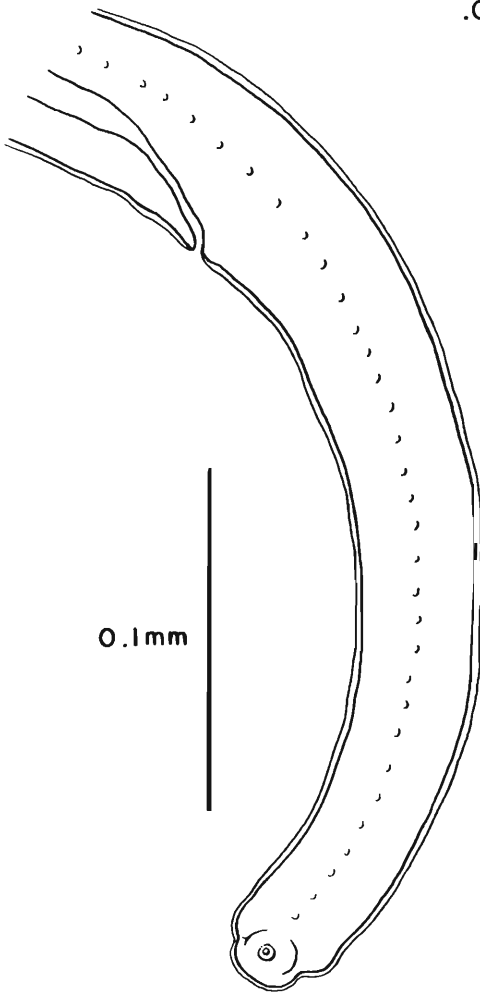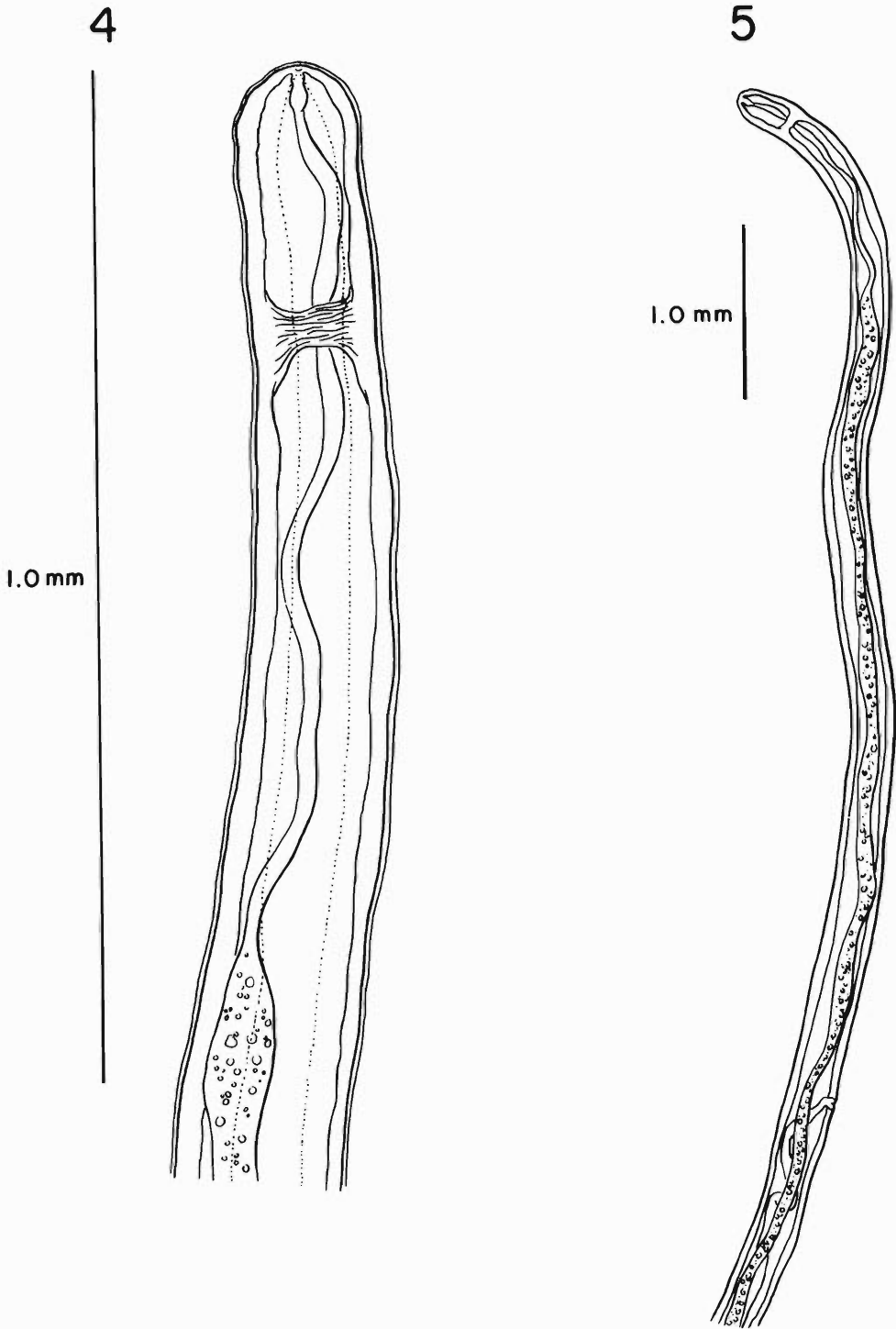MICROFILARIA (Fig. 11): Uterine microfilaria

225
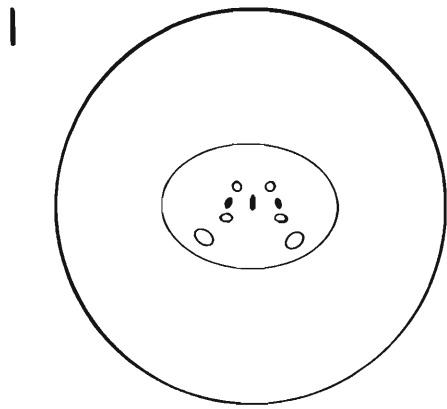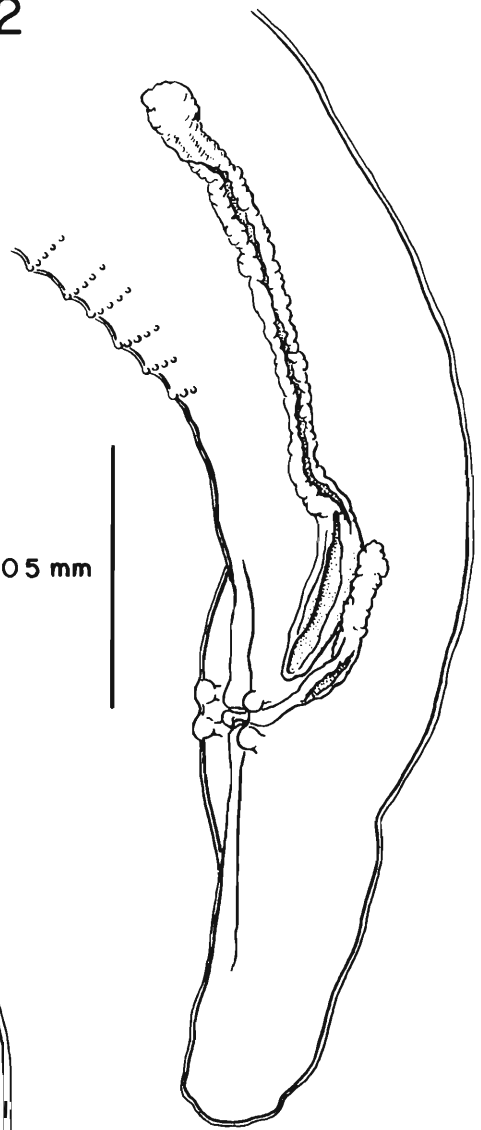
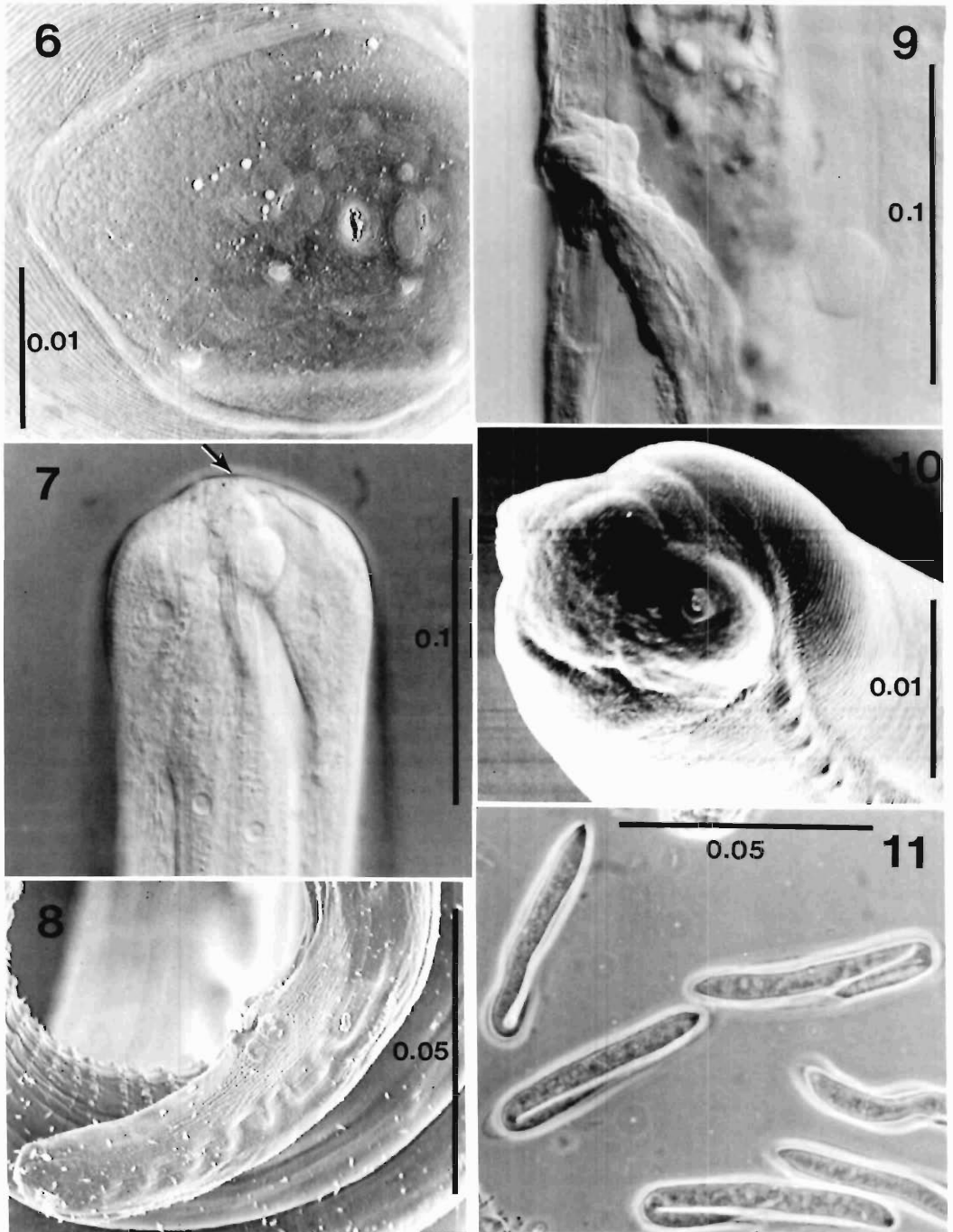Figures 1–3. *Molossinema wimsatti* gen. et sp. n. 1. Circumoral structures. 2. Tail of male. 3. Tail of female.

4

5

1.0 mm

1.0 mm

1.0 mm

Figures 4, 5. *Molossinema wimsatti* gen. et sp. n. 4. Lateral view of the esophageal region of a female. A lateral cord is represented by dotted lines. 5. Anterior end of female showing the distance from the esophago-intestinal junction to the vulva.

Figures 6–11. *Molossinema wimsatti* gen. et sp. n. 6. Circumoral structures. SEM. 7. Stomal end of female showing inconspicuous buccal cavity. 8. Tail of male. SEM. The caudal alae are less apparent in this electron micrograph than indicated in Figure 2, which was based on camera lucida drawings and photomicrographs. 9. Vulva. 10. Caudal extremity of female. SEM. 11. Microfilariae from uterus.

folded once within delicate sheath; anterior end tapering to a rounded extremity from which a cephalic hook projects, posterior end tapering to a pointed extremity; excretory pore 0.3 body length from anterior end; no area except excretory pore lacking nuclei; length 0.060–0.076, width 0.005–0.006.

CROSS-SECTIONAL ANATOMY (Fig. 12): Sections of females with 2 reproductive tubes. Uteri contain many microfilariae. Muscles coelomyarian, polymyarian, weakly developed, and divided into dorsal and ventral fields by lateral cords. Lateral cords broad at base in most sections. Esophagus slender. Intestine slender; intestinal cells frequently vacuolate. Cuticle thin.

Taxonomic Summary

DIAGNOSIS: Egg thin-shelled, containing microfilaria. Vulva well posterior to esophago–intestinal junction. Spicules arcuate, unequal, and dissimilar; distal end of left (longer) spicule gougelike. Buccal cavity inconspicuous. Esophagus undivided. Anus of male flanked by weakly developed caudal alae and 2 pairs of small sessile papillae. Parasite of the cerebral ventricular system of bats.

SPECIMENS DEPOSITED: USNM Helminthological Collection, USDA, Beltsville, Maryland 20725, holotype (male), No. 79153; allotype (female), No. 79154; paratypes (2 males, 4 females), No. 79155.

HOST: *Molossus ater* (Chiroptera: Molossidae).

LOCALITY: Trinidad, West Indies.

SITE OF INFECTION: Ventricles of brain.

ETYMOLOGY: Named in honor of Dr. William A. Wimsatt for his lifelong pioneering investigations of the biology of bats.

Remarks

The zone of nonstriated circumoral cuticle, amphidial pores, and ventral pairs of inner and outer submedian papillae were observed by both light microscopy and by scanning electron microscopy. The dorsal pair of inner submedian papillae were observed only by scanning electron microscopy; these were slightly closer together than the ventral pair. No cephalic structure projected sufficiently to be visible when the lateral aspects of specimens were studied with the light microscope. The outer submedian papillae were determined to be ventral by reference to the location of the excretory pore. The asymmetrical
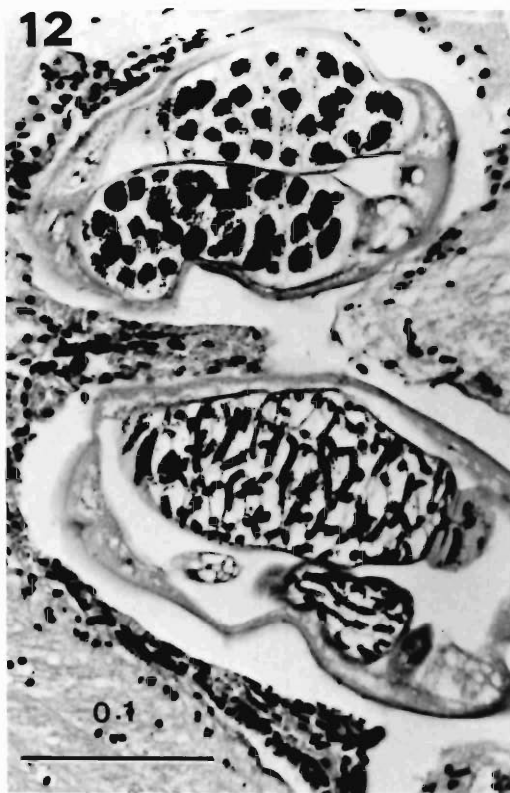
Figure 12. Cross sections of *M. wimsatti* gen. et sp. n. in histologic sections of bat brain. H&E.

circumoral pattern of *Molossinema* gen. n. resembles that presented for *Litomosoides* by Anderson (1968, fig. 59) except that the outer ventral submedian papillae are spaced very much further apart on *Molossinema* than on *Litomosoides*.

The long, slender esophagus is somewhat difficult to demonstrate. Only 1 histologic cross section of esophagus was observed. The esophagus of this section was nonmuscular, lacked a triradiate lumen, and lacked secretory granules. The lateral cords are broad and prominent in the neck region and, in viewing the lateral aspect of whole mounts, care must be exercised to avoid mistaking lateral cord for esophagus. In some histologic cross sections, the lateral cords stain more intensely at their bases, suggesting the presence of a supporting structure.

We consider the characters represented in Figures 1–5 sufficient to differentiate *M. wimsatti* gen. et sp. n. from other species of bat filariids by routine microscopic techniques. However, we

have included characters requiring special techniques in the hope that these may prove helpful in further investigation of related filariid taxa.

Molossinema gen. n. most nearly resembles *Litomosa* Yorke and Maplestone, 1926, and *Litomosoides* Chandler, 1931. *Molossinema* gen. n. differs from *Litomosa* in the greater length of its esophagus, in the postesophageal location of its vulva, in lacking a "small buccal cavity with thickened walls infundibular with apex anteriorly," in lacking a "short subterminal point" on the tail of the male, in lacking "two small diverging processes between which are two minute spines" on the tail of the female, and in the structure of the left male spicule which has a gougelike distal portion instead of a lashlike distal portion. *Molossinema* gen. n. differs from *Litomosoides* in lacking a thick-walled, tubular buccal cavity and in the structure of the left male spicule which

has a gougelike distal portion instead of a lashlike distal portion.

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MEETING SCHEDULE
HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1987–1988

(Wed) 14 Oct 1987	"Changing Patterns of Parasitic Disease," Uniformed Services University of the Health Sciences, Bethesda, MD (with Food and Drug Administration)
(Wed) 11 Nov 1987	"Protective Immunity and Immunodiagnosis in Helminths," Animal Parasitology Institute, U.S. Department of Agriculture, Beltsville, MD
(Wed) 9 Dec 1987	"Disease Ecology," Plant Protection Institute, U.S. Department of Agriculture, Beltsville, MD (with Oxford Biological Laboratory)
(Wed) 12 Jan 1988	"Interactions Between Parasites and Their Hosts," National Institutes of Health, Bethesda, MD
(Wed) 17 Feb 1988	"To be announced," Naval Medical Research Institute, Bethesda, MD
(Wed) 16 Mar 1988	"Anti-parasitic Diseases—Drug Development," Walter Reed Army Institute of Research, Washington, D.C. (with Armed Forces Institute of Pathology)
(Wed) 13 Apr 1988	Special Meeting with Johns Hopkins University and Tropical Medicine Dinner Club, Baltimore, MD
(Sat) 9 May 1988	"To be announced," University of Pennsylvania, New Bolton, PA

Prevalence of Trichinellosis in the North-Central United States

B. E. Stromberg and S. M. Prouty

Department of Veterinary Pathobiology, College of Veterinary Medicine,
University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT: Diaphragms from 3,245 pigs slaughtered in the north-central states (Minnesota, Wisconsin, Iowa, South Dakota, North Dakota) were digested and examined for the larvae of *Trichinella spiralis* from 1983 to 1985. The animals examined originated from small family farms that raised pigs for home consumption as well as from large commercial operations. None of the animals sampled were positive for trichinae. During the same period of time, diaphragm samples were obtained from 413 bear, 222 bobcats, 21 coyotes, 749 fishers, 1 gray fox, 2 red fox, 23 martens, 260 otters, and 2 wolves. All samples were from Minnesota and were examined for larvae of *T. spiralis*. Two wild animals were positive for *Trichinella,* a bear with 1.4 larvae per gram of muscle (LPG) and a fox with 2.4 LPG. These data demonstrate a low prevalence of trichinellosis in both swine and wild animals in this region of the country.

KEY WORDS: *Trichinella spiralis,* swine, bear, fox, *Ursus* sp., *Vulpes vulpes.*

There has been no recent information pertaining to the prevalence of *Trichinella spiralis* in either swine or wildlife in the north-central U.S. (Minnesota, Wisconsin, Iowa, South Dakota, North Dakota). Zimmerman and Zinter (1971) reported the prevalence in pigs to be 0.15% over the period 1966–1970 for their east and west north-central regions of the country. Recent studies from other areas of the country have reported prevalences of 0.58% in the mid-Atlantic region (Schad et al., 1985a), 0.73% in New England (Schad et al., 1985b), and 0.08% in Louisiana (Hugh-Jones et al., 1985). The prevalence of trichinellosis in furbearing animals was 3.2% in Pennsylvania (Schad et al., 1984). With larvae of *T. spiralis* present in several food sources, including pork and wild game, and the incidence in the human population still of general concern (Campbell, 1983; Schantz, 1983), this study was undertaken to determine if the prevalence of trichinellosis in pigs had changed since 1971 in this region. This study also determined the prevalence of *T. spiralis* in wildlife in Minnesota.

Materials and Methods

We arranged with several commercial abattoirs to collect muscle samples (1/pig) from the pillars of the diaphragms shortly after slaughter. Some samples (<10) were obtained from small farms where the pigs were slaughtered for home consumption. Most animals sampled were slaughter hogs, however, some were older sows. Samples were transported back to the laboratory in an ice cooler and kept under refrigeration until digested. Diaphragm samples from bears were sent to the Minnesota Department of Natural Resources (DNR) by the hunters when they registered the bears they had shot. Other wildlife samples from bobcats, coyotes, fishers, foxes (red and gray), martens, otters, and wolves were collected by the DNR from cooperating trappers. All samples were kept under refrigeration (the coyote and fox samples were frozen) until transported to our laboratory. Some muscle samples were collected from animals submitted to the College of Veterinary Medicine for necropsy. All samples were maintained under refrigeration in our laboratory prior to digestion.

The pooled digestion technique was used to digest the muscle, freeing the larvae for identification as described by Schad et al. (1985a). Sample pools, composed of either 10 10-g samples (most hogs) or 20 5-g samples, were minced with a scissors or laboratory blender and digested in artificial gastric juice (1% pepsin–HCl) in a Stomacher 3500 Lab-Blender® (Tekmar Co., Cincinnati, Ohio) for 10 min. Digestion was completed by agitating the pools on a shaker for 4 hr at 37°C. Each sample pool was then sedimented in an Imhoff cone for 30 min. Fifty ml of sediment was then drawn off, washed several times by sedimentation with tap water, and the sediment examined under 30× magnification.

When a pooled sample was positive (i.e., contained larvae), a 10-g sample (from each sample in a pool) was digested individually using a smaller blender (Stomacher 400®). This procedure was also followed for samples too small to pool (i.e., remaining sample less than 5 g). All larval counts are reported as larvae per gram of muscle (LPG).

Results

No larvae of *T. spiralis* were recovered from the 3,245 pigs sampled. Samples were obtained from 413 bear (*Ursus* spp.), 222 bobcats (*Lynx rufus*), 21 coyotes (*Canis latrans*), 749 fishers (*Martes pennanti*), 1 gray fox (*Urocyon cinereoargenteus*), 2 red fox (*Vulpes vulpes*), 23 martens (*Martes americana americana*), 260 otters (*Lutra* spp.), and 2 wolves (*Canis lupus*). One bear had 1.4 LPG and 1 red fox had 2.4 LPG of *T. spiralis*. All the other animals were negative for larvae of *Trichinella*.

Discussion

This study demonstrated the very low prevalence of trichinellosis in the north-central U.S. None of the swine samples were positive, which may be an artificially low number because of the small number of samples evaluated. Schad et al. (1985a, b) reported that the most frequent sources of infected hogs were small commercial slaughterhouses that killed 1,000 hogs per day or less. No infected hogs were found in medium to large slaughterhouses nor were any associated with the small custom packer, who killed 3,000 or more per day or 150 per week, respectively. Most of the samples in this study were obtained from medium to large abattoirs, which may also bias these results. However, pigs were purchased both directly from the supplier and via brokers at the abattoirs we sampled. A larger sampling would perhaps be the only way to verify the low prevalence in the north-central region.

Because 13.9% of human infections in the U.S. have been considered to be the result of the ingestion of meat of wild animals (Schantz, 1983), we examined several species of furbearing animals. We found only 1 bear (0.2%) and 1 of 2 red foxes infected with *T. spiralis*. Small numbers of larvae of *T. spiralis* may have gone undetected in the frozen samples (coyote and fox) using the digestion–sedimentation technique. However, 1 of the 2 positive wildlife samples had been frozen, the red fox. Minnesota has not reported any human cases of trichinellosis since 1976, indicating that either the infection pressure was low or all potential sources of infection were processed adequately to kill the parasite in infected meat.

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Epizootiology of Internal Parasites in Lambs and Ewes During the Periparturient Period in Kentucky in 1986

EUGENE T. LYONS, J. HAROLD DRUDGE, AND SHARON C. TOLLIVER

Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40546-00761

ABSTRACT: Lambing in 2 small flocks of ewes ($N = 14$) in Kentucky occurred between 28 January and 2 March 1986. Selected lambs ($N = 11$), born to these ewes, were euthanized at periodic intervals from 1 April to 22 July 1986 for worm egg counts in feces (EPG) and helminth count determinations at necropsy. For the lambs, EPG and number of helminths, in general, increased progressively throughout the investigation. EPG in lambs were over 1,600 by mid-June and attained a high of over 8,000 by late July. Number of helminths in the lambs were very low at the outset, increased to more than 1,500 by the third week of May (1 of 2 lambs), and exceeded 14,000 by late July. EPG of the lambing ewes were determined biweekly from 28 January to 1 July 1986. The ewes had significantly ($P > 0.01$) greater mean EPG at 7–10 wk (mean EPG = 1,627) postpartum than at 0–3 wk (mean EPG = 301) prepartum. The major source of the nematodes acquired by the lambs was deemed to be the infections in the ewes. The exposure of the lambs apparently was markedly increased by the periparturient rises of EPG's in the ewes and subsequent buildup of infectious stages on pastures.

KEY WORDS: Trichostrongyles, *Moniezia* spp. relation of lambing and increased EPG, worm burdens, statistical analysis.

Michel (1974) and Herd et al. (1983) have reviewed and discussed the periparturient increase in nematode eggs per gram of feces (EPG) in ewes and also the seasonal or "spring rise." The major internal parasites in spring lambs are acquired from environmental sources of larvae from nematode eggs passed in feces of ewes during the periparturient period. Increase in nematode EPG at this time is related to lactation. The major purpose of the present investigation was to follow the acquisition of internal parasites in neonatal spring lambs in 2 small flocks in Kentucky in 1986. Also, determinations of nematode EPG in ewes were made during the periparturient period.

Materials and Methods

Sheep in the present investigation in 1986 were of predominant Cheviot bloodlines. At the Department of Veterinary Science, University of Kentucky, research farm ewes were pasture-bred and the lambs were from 2 small flocks maintained over 20 yr with natural infections of internal parasites. There has been limited usage of anthelmintics in recent years in the breeding flocks. For about the last 10 yr, the only anthelmintic usage was occasional treatment of rams and replacement females. No anthelmintic was administered to any of the flock during the gestation period or course of the present investigation.

Lambing ewes ($N = 14$) in 2 flocks (Field 21 — $N = 10$; and Field 20 — $N = 4$) and some of their lambs ($N = 11$), designated as testers, were investigated. Lambs were born between 28 January and 2 March 1986. Tester lambs were euthanized at periodic intervals from 1 April through 22 July 1986; at necropsy, EPG were determined, and helminths were counted and identified. An additional lamb (No. 8600), from a field adjacent to Field 20, was found in extremis on 13 June 1986; for this lamb, the EPG was determined, and the gastrointestinal tract was examined for internal parasites to obtain supplementary data on the helminth infections. Data for lambs, including dates for necropsy examinations and ages at necropsy, are recorded (Table 1).

For the ewes, worm egg counts for fecal samples (EPG) were determined biweekly from 28 January through 1 July 1986. Occasionally, EPG were not determined for some ewes because inadequate amounts of feces were collected. The times for collecting fecal samples from the various ewes ranged from 0 to 5 wk before lambing and from 15 to 22 wk after lambing. Arrangement of data on EPG was made according to the week of lambing of the ewes; this represented 1–11 EPG relative to week of lambing (Fig. 1).

The necropsy examination of the gastrointestinal tract for immature and adult parasites included the abomasum, small intestine, cecum, and large intestine. Worm egg counts on fecal samples (EPG) and recovery of internal parasites were done according to methods used previously (Drudge and Szanto, 1963).

Statistical analysis (standard errors of means [SEM]) of the EPG data for the ewes was done; comparing the highest mean EPG for a 4-wk period (7–10 wk) after lambing with the mean EPG for the 4-wk period (0–3 wk) before lambing.

Representative specimens of all helminths found in the lambs have been deposited in the USNM National Parasite Collection (Nos. 79605–79626).

Results

EPG for the 11 tester lambs at necropsy are tabulated (Table 1). In the mid-June sampling period, EPG began increasing and reached a high of over 8,000 in late July for the last lamb killed. In the tester lambs, parasites found at necropsy
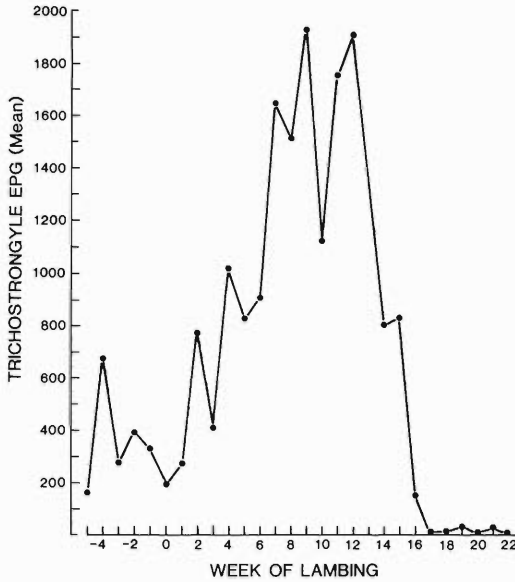
233

Figure 1. Periparturient data on mean trichostrongyle eggs per gram of feces (EPG) in ewes (N = 14) relative to time of lambing, which occurred between 28 January and 2 March 1986.

in mid-June of numbers of *H. contortus* and some of the other species paralleled that of a similar high increase in EPG at the same time. Findings regarding EPG and helminth burden from an additional lamb, found near death in a third field on 13 June, were similar to the tester lamb (No. 8680) killed and examined on 3 June.

Pre- and posttreatment EPG for 14 ewes in 2 fields are recorded (Fig. 1). The highest EPG in a ewe before lambing (-1 wk) was 1,270 and after lambing ($+9$ wk) was 7,680. Mean EPG was highest at 9 wk after lambing. April was the month with the highest average EPG. There was a dramatic decline in EPG characteristically beginning a short time after the highest worm egg count was ascertained, with EPG for the ewes during the last 6-wk sampling period ranging from 0 to 120. Statistical analysis (SEM) of the data, comparing the highest mean EPG (1,627) for a 4-wk period (7–10 wk) postlambing with the mean EPG (301) for the 4-wk period (0–3 wk) prelambing, indicated a significant difference ($P >$ 0.01).

Discussion

The present investigation, although using a low number of lambs and ewes, did provide insight into the dynamics of internal parasites in 2 small lambing flocks in Kentucky in the first 6 mo of 1986.

Lambs, born on pastures, were killed periodically and served as indicators of parasite infestations there. The finding of very few internal parasites in lambs (about 2 mo or more old),

included 14 species of nematodes and 1 species of tapeworm (Table 2). The trichostrongyles (*Haemonchus, Trichostrongylus, Ostertagia, Nematodirus,* and *Cooperia*), in general, increased progressively in number in the later-killed lambs which were usually the oldest at the time of euthanasia. Numbers of *Haemonchus contortus* were present in highest numbers with *Nematodirus* being next highest. The great increase

Table 1. Nematode eggs per gram of feces (EPG) for tester lambs (N = 11) in 2 fields (20 and 21) in 1986.

	Lamb		EPG*			
I.D. No.	Date killed	Age (days)	Tricho-strongyle†	*Nematodirus*	*Strongyloides*	All types
11 (A)‡	4/1	58	0	10	10	20
71 (B)	4/8	62	10	0	0	10
72 (B)	4/22	74	120	0	0	120
13 (A)	5/6	84	10	0	0	10
15 (A)	5/20	94	60	10	0	70
74 (B)	5/20	92	30	20	0	50
80 (B)	6/3	95	290	10	0	300
83 (B)	6/17	76	1,610	50	20	1,680
78 (B)	7/1	127	1,020	20	10	1,050
12 (A)	7/8	156	5,280	70	150	5,500
82 (B)	7/22	142	7,960	160	20	8,140

* Feces collected on day lamb killed.

† Excluding *Nematodirus.*

‡ A = Field 20 and B = Field 21.

Table 2. Data on species and numbers of helminths* recovered at necropsy of tester lambs (N = 11) in 2 fields in 1986.

Parasite species‡	Lamb No. (date killed: age in days)										
	11A† (4/1:58)	71B (4/8:62)	72B (4/22:74)	13A (5/6:84)	15A (5/20:94)	74B (5/20:92)	80B (6/3:95)	83B (6/17:76)	78B (7/1:127)	12A (7/8:156)	82B (7/22:142)
Haemonchus contortus (imm.)	0	0	0	0	40	0	100	3,160	1,260	3,270	1,160
Haemonchus contortus	15	0	4	1	20	3	142	2,465	3,700	4,790	5,080
Ostertagia spp. (imm.)	0	7	20	0	0	40	67	1,220	400	20	300
Ostertagia circumcincta ♂	0	47	126	10	0	80	240	1,060	740	40	390
Ostertagia trifurcata ♂	0	0	7	0	0	50	0	20	60	0	0
Ostertagia spp. ♀	7	53	137	10	30	210	353	1,000	920	10	460
Trichostrongylus spp. (imm.)	0	0	0	0	0	0	0	80	150	20	170
Trichostrongylus axei	0	0	0	10	0	0	7	40	170	100	440
Trichostrongylus colubriformis	0	0	20	0	0	0	20	180	860	0	1,400
Trichostrongylus vitrinus	50	0	0	0	20	0	0	40	40	250	60
Nematodirus spp. (imm.)	60	10	10	10	40	290	180	240	380	440	160
Nematodirus spathiger ♂	30	20	20	80	40	290	660	1,240	700	1,220	2,040
Nematodirus spp. ♀	0	0	70	150	60	310	690	1,560	840	1,660	1,800
Cooperia spp. (imm.)	0	0	0	0	0	0	0	20	0	0	20
Cooperia curticei	10	0	0	0	0	0	20	80	280	40	820
Cooperia oncophora	10	0	10	0	0	20	0	0	20	0	0
Strongyloides papillosus	87	0	0	0	0	0	0	20	0	100	20
Moniezia spp.	0	0	7	5	2	16	18	0	2	0	105
Trichuris spp. (imm.)§	0	50	30	100	20	160	40	120	0	0	0
Trichuris ovis	0	80	157	39	158	109	65	553	90	69	27
Oesophagostomum columbianum	0	0	0	0	0	0	0	0	0	2	2
Capillaria spp.	0	0	0	0	20	0	0	0	0	0	0
All helminths	267	267	618	415	450	1,578	2,602	13,098	10,612	12,031	14,454

* All helminths are nematodes except for the flatworm, Moniezia spp.

† A = Field 20 and B = Field 21.

‡ All nematodes are mature except where noted as immature (imm.); number of nematodes (mature) includes both ♂ and ♀ unless otherwise stated.

§ The immature category for Trichuris spp. is arbitrary and based only on being much smaller in size than the other specimens.

236

killed in April and early May, indicated probably very little infection from overwintering stages on pastures. Previous research in this geographical area has shown that several species of ruminant nematodes can live on pastures throughout the winter (Todd et al., 1949; Drudge et al., 1958; Lyons et al., 1983). The great increase in EPG and worm burdens in lambs in June was about 2 mo after the highest EPG in the ewes. Delay of large numbers of parasites being found in lambs verified that the periparturient increase in EPG in ewes was probably the major source of parasitic infections in the lambs. This was, no doubt, due to the buildup of infective stages on pastures after the ewes lambed (Michel, 1974; Herd et al., 1983).

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Obituary Notice

WILLIAM B. LeFLORE

22 February 1930–6 December 1986
Member since 22 October 1971

The Society wishes to express
its deepest sympathy
to Gilbert F. Otto and family
on the death of
MRS. LOU OTTO
10 October 1898–8 June 1987

Meteterakis ishikawanae sp. n. (Nematoda: Heterakidae) from the Frog, *Rana ishikawae,* on Okinawa Island, Japan

HIDEO HASEGAWA

Department of Parasitology, School of Medicine, University of the Ryukyus,
Nishihara, Okinawa, 903-01, Japan

ABSTRACT: *Meteterakis ishikawanae* sp. n. from the rectum of the frog, *Rana ishikawae,* collected in the mountainous forest of Okinawa Island, Japan, is described. *Meteterakis ishikawanae* is readily distinguished from related species of the genus in having spicules with slightly widened proximal ends and well-developed heavily tessellated alae. This is the first species of the genus *Meteterakis* that utilizes a ranid frog as the primary definitive host.

KEY WORDS: Amphibia, taxonomy, morphology, host specificity.

The nematodes of the genus *Meteterakis* Karve, 1930 (Heterakoidea: Meteterakidae: Meteterakinae), are parasites of amphibians and reptiles of the Oriental region, and 17 species have been described. The hosts are mainly toads and lizards, rarely gymnophionian amphibians, frogs other than bufonids, and snakes and turtles (Inglis, 1958; Biswas and Chakravarty, 1963; Oshmarin and Demshin, 1972; Crusz and Ching, 1975; Crusz and Santiapillai, 1982). *Bufonerakis* Baker, 1980, parasitic in toads and snakes of South America (Baker, 1980), and *Gireterakis* Lane, 1917, parasitic in porcupines of India (Chabaud, 1978), are the only other genera in the subfamily Meteterakinae. During a study on the helminth fauna of the Ryukyu Archipelago, Japan, an undescribed species of *Meteterakis* was recovered from the frog, *Rana ishikawae,* of Okinawa Island, and is described herein.

Materials and Methods

From July 1981 to January 1985, a total of 66 frogs belonging to 5 species were collected by hand in the mountainous forest of Kunigami-son, Okinawa Island, Japan. They were autopsied after being killed with chloroform or ether inhalation. Recovered nematodes were fixed in hot 70% ethanol, cleared in a glycerin–alcohol solution, and mounted on slides with pure glycerin for microscopical study. Figures were made with the aid of a drawing apparatus, Olympus BH-DA-LB. Measurements are in micrometers unless stated otherwise, with the range being followed by the mean. Specimens of *Meteterakis japonica* (Wilkie, 1930) from *Bufo gargarizans miyakonis* of Miyako Island, Japan, were also examined.

Results

A species of *Meteterakis* was detected in all of the 4 *Rana ishikawae* examined: 34, 15, 39, and 74 worms, respectively, were recovered. On the other hand, *Meteterakis* was not found in the other frogs examined, namely 40 *Rana narina,* 2 *Rana holsti,* 8 *Rana namiyei,* and 12 *Rhacophorus viridis viridis.*

Meteterakis ishikawanae sp. n.
(Figs. 1–11)

GENERAL: Ascaridida, Heterakoidea, Heterakidae, Meteterakinae, *Meteterakis* Karve, 1930. Small worms with tapered extremities (Fig. 1). Cuticle finely striated transversely, with narrow lateral alae commencing from level of nerve ring and ending at precloacal region in male and at posterior end in female (Figs. 2, 7, 10). Minute somatic papillae present on cuticle of both sexes, especially prominent in female (Figs. 5, 6, 9, 10). Cephalic end with 3 lips separated from each other by deep grooves (Figs. 2–4) of which posterior margin extends to about midlevel of pharynx; dorsal lip with 2 lateral double papillae and 2 minute apical papillae; subventral lips each with a subventral double papillae, a sublateral single papilla, 2 minute apical papillae, and an amphid (Fig. 3). Anterior extremity of pharynx with 3 pharyngeal teeth projecting anteriorly (Figs. 3, 4). Esophagus consisting of long narrow cylindrical portion and bulbous portion (Fig. 1). Nerve ring at junction between anterior and middle third of esophagus (Fig. 1). Excretory pore at midlevel of esophagus (Figs. 1, 5); excretory vesicle large and lobulated (Figs. 5, 6).

MALE (holotype and 12 paratypes): Length 4.13–6.27 (5.08) mm. Maximum width 150–230 (180). Caudal end bent ventrally (Figs. 1, 7, 8). Pharynx 53–63 (58) long; cylindrical portion of esophagus 590–700 (650) long by 40–48 (45)
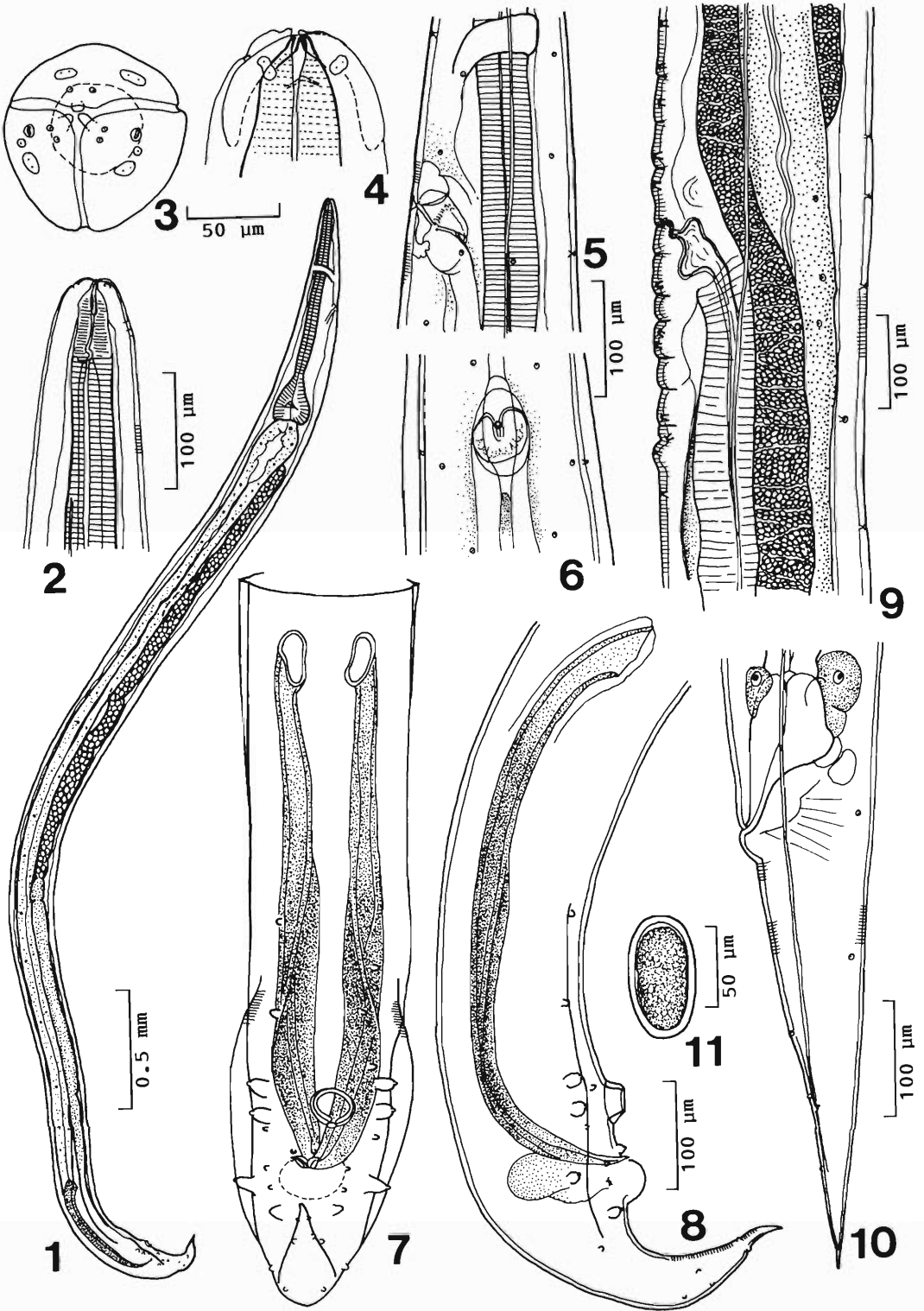
wide; esophageal bulb 143–180 (160) long by 118–150 (153) wide. Cephalic apex to nerve ring 240–290 (260), to excretory pore 410–510 (450). Spicules almost equal, stout, alate, heavily tessellated including alae but except distal tip, bent ventrally, 520–650 (590) long (Figs. 7, 8). Gubernaculum absent. Precloacal sucker 33–43 (38) in diameter, 26–40 (33) from cloacal aperture (Figs. 7, 8). Narrow caudal alae supported by 3 pairs of large papillae present (Figs. 7, 8); in addition, a pair of large papillae present posteroventrally to cloacal aperture; a pair of small papillae present just in front of cloacal aperture; about 10 pairs of small sessile papillae also present in caudal region (Figs. 7, 8). Tail conical, 168–230 (192) long, with pointed tip (Figs. 7, 8). Ventral surface of posterior half of tail with distinct striae (Fig. 8).

FEMALE (allotype and 20 paratypes): Length 4.53–6.43 (5.57) mm. Maximum width 180–240 (210). Pharynx 53–70 (60) long; cylindrical portion of esophagus 640–800 (720) long by 40–55 (49) wide; esophageal bulb 175–213 (185) long by 133–175 (158) wide. Cephalic apex to nerve ring 260–320 (290), to excretory pore 410–490 (460), to vulva 1.93–2.59 (2.24) mm. Vulva slitlike; cuticle anterior and posterior to vulva striated distinctly and with deep grooves (Fig. 9). S-shaped cuticular channel present between vulva and muscular portion of vagina (Fig. 9); vagina, 0.90–1.55 (1.25) mm long, running posteriorly and splitting into 2 parallel uteri; uteri elongate and joining oviducts near anus; oviducts directed anteriorly; ovaries long, directed anteriorly, then flexed posterior to esophageal bulb and terminating posterior to vulva. Tail conical 260–420 (350) long, with pointed tip (Fig. 10). Eggs elliptical, relatively thick-shelled, 61–78 (72) by 40–48 (44), containing morula-stage embryo at deposition (Fig. 11).

HOST: *Rana ishikawae* (Stejneger, 1901).

LOCATION: Rectum.

LOCALITY: Kunigami-son, Okinawa Island, Japan.

SPECIMENS: National Science Museum, Tokyo, NSMT—As 1806 (holotype and allotype);

Meguro Parasitological Museum, Tokyo, MPM—Coll. No. 19424 (paratypes).

Discussion

The present species has every morphological feature of the genus *Meteterakis* except the vulval flap, which has been considered to be a key characteristic of the genus (Inglis, 1958). However, the S-shaped cuticular channel between the vulva and the muscular portion of the vagina suggests that the vulval flap may be retracted into the body. It is therefore reasonable to put the present species into the genus *Meteterakis*.

Meteterakis ishikawanae sp. n. resembles *M. govindi* Karve, 1930, *M. baylisi* Inglis, 1958, *M. longispiculata* (Baylis, 1929), *M. louisi* Inglis, 1958, and *M. sinharajensis* Crusz and Ching, 1975, in having alae on the spicules. However, *M. ishikawanae* is readily distinguished from *M. govindi* and *M. louisi* in that these alae are much more prominent, and from *M. baylisi* by the absence of the caplike formation at the distal tip of the spicules (Inglis, 1958). *Meteterakis ishikawanae* sp. n. has a slightly widened proximal portion of the spicule in contrast to the strongly expanded and funnel-shaped appearance in *M. longispiculata* and *M. sinharajensis* (Inglis, 1958; Crusz and Sanmugasunderam, 1973; Crusz and Ching, 1975). Other distinguishing characteristics are as follows: *M. govindi* has shorter spicules (180–270) in males 4.0–5.4 mm long (Inglis, 1958); *M. baylisi* has tessellated spicules, but the alae are nontessellated (Inglis, 1958); *M. longispiculata* has a somewhat longer tail (270–310) in males 7.0–7.5 mm long (Inglis, 1958); 210–290 in males 3.5–5.7 mm long (Crusz and Sanmugasunderam, 1973); *M. louisi* has longer spicules (0.97–1.10 mm) in males 5.0–7.4 mm long (Inglis, 1958); *M. sinharajensis* has 2 pairs of large fleshy papillae lateral to the cloaca in males (Crusz and Ching, 1975). The presence or absence of alae on the spicules has not been sufficiently described in *M. singaporensis* (Sandosham, 1954) and *M. varani* (Maplestone, 1931). The latter species was considered to be a synonym of *M. govindi* by Inglis (1958) but Skrjabin

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Figures 1–11. *Meteterakis ishikawanae* **sp. n. 1. Male (holotype), lateral view. 2. Anterior end of female (paratype), lateral view. 3. Anterior end of female (paratype), apical view. 4. Anterior end of female (paratype), dorsal view. 5. Excretory pore of female (allotype), lateral view. 6. Excretory pore of female (allotype), ventral view. 7. Posterior end of male (paratype), ventral view. 8. Posterior end of male (paratype), lateral view. 9. Vulval part of female (allotype), lateral view. 10. Posterior end of female (paratype), lateral view. 11. Egg.**

et al. (1961) retained its validity. However, *M. singaporensis* has very long spicules (740–960) in males 5.2–5.7 mm long (Sandosham, 1954) and *M. varani* has spicules with a whiplike distal half (Maplestone, 1931) that are clearly different from those of *M. ishikawanae.*

Meteterakis ishikawanae sp. n. was recovered only from *Rana ishikawae* in the locality surveyed, and thus it may be suggested that it has a strict host-specificity to this frog. *Rana ishikawae* is a relict species, being distributed only on Okinawa and Amami-oshima islands in the Ryukyu Archipelago (Frost, 1985). It is believed that most frog species of the Ryukyu Archipelago have their origins in continental China (Kuramoto, 1979). *Rana ishikawae* (or its ancestor) might have extended its distribution to this island chain in the late Miocene or Pleistocene periods, when there was a land connection of the Ryukyus to the continent.

There have been only a few reports of *Meteterakis* from anurans other than bufonids. Wilkie (1930) described *Meteterakis japonica* from the "bull frog," which he suggested may be *Rana japonica.* However, this nematode is a common parasite of bufonids in Japan (Yamaguti, 1935, 1941), and Ichikawa (1951) considered that the "bull frog" of Wilkie (1930) might be *Bufo bufo japonicus.* On the other hand, *M. govindi* was recovered from a "tree frog" in China (Inglis, 1958) and Biswas and Chakravarty (1963) recorded *Meteterakis varani* from *Rana hexadactyla* in India. However, the former nematode is commonly a parasite of bufonids (Karve, 1930; Inglis, 1958), and the latter was first described from a lizard (Maplestone, 1931) and its prevalence and intensity in *R. hexadactyla* were relatively low (Biswas and Chakravarty, 1963). It is possible that these frogs were only accidental hosts for the parasites. Therefore, *M. ishikawanae* sp. n. is considered to be the first described species of the genus that utilizes a ranid frog as the primary definitive host.

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Financial Report for 1986

Balance on hand, 1 January 1986	$8,534.14
Receipts: Net interest received in 1986	730.78
	$9,264.92
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Grant to the Helminthological Society of Washington for 1986	$ 50.00
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Infectivity of *Trichostrongylus axei* for *Bos taurus* Calves After 25 Years of Passages in Rabbits, *Oryctolagus cuniculus*

Eugene T. Lyons, J. Harold Drudge, Sharon C. Tolliver, and Thomas W. Swerczek

Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40546-00761

ABSTRACT: Monospecific infections of 2 strains of the minute stomach worm, *Trichostrongylus axei,* were established in rabbits (*Oryctolagus cuniculus*) at the University of Kentucky in 1953 (equid strain A) and in 1954 (bovid strain O); these isolates have been maintained in rabbits since the original isolations. In the 1950's, cross-species infections were investigated among gerbils, rabbits, calves, sheep, and horses. Accurate records on passages of both strains in rabbits have been maintained since 1962 and are summarized here. Both strains of *T. axei,* after over 25 yr maintenance in rabbits, matured in all of 19 exposed *Bos taurus* calves.

KEY WORDS: methodology, in vivo maintenance, infectivity rates, patency, Nematoda, Trichostrongyloidea.

A series of publications from the University of Kentucky from 1955 to 1963 elucidated several aspects of the biology of *Trichostrongylus axei,* including the ease of establishing infections in various hosts such as rabbits (Drudge et al., 1955; Leland and Drudge, 1957; Leland et al., 1959a, b, 1960a, b, 1961; Leland, 1963).

The present paper includes information accumulated on 2 strains (equid and bovid origin) of *T. axei,* utilized in the foregoing investigations, and perpetuated in rabbits for over 25 yr. Also, tests were made of the infectivity of both strains in calves in 1986 after over 25 yr maintenance in rabbits.

Materials and Methods

For 2 strains (A and O) of *T. axei,* isolated in rabbits since 1953 (strain A) and 1954 (strain O), exact numbers of passages, recorded only since 31 July 1962 to the present, are presented herein.

The origin of strain A was from a horse in 1953 (Drudge et al., 1955). Strain A has been maintained in rabbits from 1953 to the present. In the 1950's, there was occasional passage of strain A and strain O from rabbits to calves and sheep and back to rabbits. Data are presented for all rabbits (now dead) that were infected with strain A of *T. axei* during the period 31 July 1962 through 25 October 1985.

Strain O (bovid origin) was isolated in 1954. It was maintained in rabbits from 1954 to 24 September 1959 at which time it was inadvertently lost because of the unexpected death of the last infected donor rabbit. This strain had been sent in the mid-1950's to Dr. Dale Porter (at that time with USDA, Auburn, Alabama) who maintained it in calves. Cultures of strain O infective larvae (L₃) from the Auburn calves were obtained and given to donor rabbits on 5 November 1959. This restarted strain has been maintained here continuously in rabbits since that time. Data are included for all rabbits (now dead) that were infected with strain O of *T. axei* during the period 31 July 1962 through 21 December 1984.

For both strains, the period of patency was based on the time of the last positive *T. axei* egg count per gram of feces (EPG). Rabbits that died while still passing *T. axei* eggs are included along with those that became negative when still alive. The shortest period of patency was for rabbits that died before *T. axei* egg-negative feces were observed.

From 740 to 9,400 *T. axei* infective larvae (L₃) were given to each donor rabbit. The number of larvae given donors was much higher in the earlier than later years. Feces from infected rabbits were collected approximately every 2 wk for determining EPG. Usually, a maximum of 3–4 infected rabbits were kept with each strain of *T. axei.* When EPG fell in the donors, infection of new donor rabbits was commonly done. Attempts were not made to recover adult *T. axei* from stomachs of rabbits at any time during this investigation.

Trichostrongylus axei larvae (L₃) (10,000–49,500), derived from strains A and O from rabbit donors, were administered to each of 19 male calves (5 Holsteins, 1 Holstein–Angus crossbred, and 13 Jerseys), raised worm-free, at 3–4 months old (May through September 1986). Sources of *T. axei* (L₃) were from infections maintained in rabbits for 32 yr for strain A (3 calves) and for either 26 or 27 yr for strain O (16 calves). Periodic examinations (qualitative or EPG) of calf feces for *T. axei* eggs were made including 1 at the time of necropsy, 24–73 days after administration of larvae. At necropsy, the abomasum was examined for *T. axei.* Exact worm counts were made for 15 calves and a few specimens (5–11) were examined from the 4 other remaining calves (with low to 0 EPG) to verify identification of *T. axei.*

Details have been published on methods for culturing and administering the larvae (Leland et al., 1959a), determining EPG (Drudge et al., 1963; Lyons et al., 1976), and recovering *T. axei* at necropsy (Leland and Drudge, 1957).

Results and Discussion

Data compiled on donor rabbits, infected with *T. axei* strain A (equid origin) or strain O (bovid origin), revealed several aspects about this parasite.

242

For strain A, 11 serial passages were made since 31 July 1962. During this period, a total of 30 rabbits (including the 11 for serial passages) were infected with this strain. The highest EPG for the resultant infections in the 30 rabbits varied from 30 to 3,600 ($\bar{x} = 420$) and were found at 30–808 ($\bar{x} = 299$) days after administration of larvae. The period of patency varied from 154 to 2,055 ($\bar{x} = 1,141$) days in the 30 rabbits.

Fourteen serial passages of strain O have been done since 31 July 1962. A total of 32 rabbits (including the 14 for serial passages) were infected with this strain during that period of time. Highest EPG of resultant infections in the 32 rabbits ranged from 90 to 1,350 ($\bar{x} = 451$) and were recorded at 30–807 ($\bar{x} = 297$) days postadministration of larvae. Patent periods of 71–1,810 ($\bar{x} = 913$) days were found for infections in the 32 rabbits.

For both strains, there did not appear to be a clear relationship between the number of larvae given and EPG. In a great number of instances, eggs were present until the rabbits died. The fact that several rabbits died a relatively short time after administration of larvae shortened the figures on patency. It is of interest that relatively few passages were necessary to perpetuate the strains of *T. axei* in rabbits.

The data presented herein verify what an excellent donor model the laboratory rabbit is for maintaining strains of *T. axei*. This host is ideal because of its small size compared to larger animals. Also, because of the long patency (over 5 yr in several rabbits) of *T. axei,* only occasional transfer to new donors is necessary to perpetuate this parasite. It should be noted that there was virtually no chance of natural infection of donor rabbits with *T. axei* larvae because of frequent (once or twice a week) cleaning of cages and water and feed containers. Also, the size of the openings of the cage bottom were large enough for drop-through of feces.

The 19 calves to which *T. axei* (L$_3$) strain A (equid origin) and strain O (bovid origin) were given all developed patent infections (Table 1). For the calves (6) killed a short time (24 days) after administration of larvae, EPG were negative at necropsy but *T. axei* eggs were found upon qualitative examination of feces. At necropsy, the other 13 calves, killed at 30–73 days after administration of *T. axei* L$_3$, had EPG varying from 10 to 1,080.

Specimens of *T. axei* were recovered from all 19 calves that were examined at necropsy. Fif-

Table 1. Data on 19 calves with experimental infections of *Trichostrongylus axei* derived from 2 strains (A and O) maintained for over 25 yr in rabbits.

Calf no.	Strain* identification	No. of larvae administered	EPG	No. of specimens recovered from abomasum
				Trichostrongylus axei — Findings at necropsy
253 (30)†	A	32,600	40	19,190
257 (50)	A	32,600	150	18,110
262 (50)	A	32,600	80	12,280
229 (65)	O	34,600	10	10,500
233 (65)	O	34,600	30	5,330
250 (41)	O	23,960	440	12,990
252 (41)	O	23,960	110	8,030
259 (30)	O	17,500	1,080	7,230
261 (34)	O	17,500	20	5,960
234 (24)	O	12,700	0 (+)‡	(+)§
236 (24)	O	12,700	0 (+)‡	1,860
239 (24)	O	12,700	0 (+)‡	(+)§
240 (24)	O	12,700	0 (+)‡	1,350
242 (24)	O	12,700	0 (+)‡	(+)§
244 (24)	O	12,700	0 (+)‡	(+)§
268 (50)	O	49,500	190	23,260
274 (73)	O	10,000	10	5,510
280 (73)	O	10,000	40	5,160
281 (73)	O	10,000	120	5,000

* Strain A (maintained in rabbits for 32 yr) is of equid origin and strain O (maintained in rabbits for 26 or 27 yr) is of bovid origin.

† The value in parentheses is the number of days from the time the larvae were administered to the calves until the calves were killed.

‡ EPG negative but eggs present on qualitative examination.

§ Specimens were not counted but 4–11 from each calf were examined to verify that they were *T. axei.*

teen calves, from which exact worm counts were made, harbored from 1,350 to 23,260 specimens of *T. axei*. For the other 4 calves, from 5 to 11 specimens were examined from each and verified to be *T. axei*. Eleven percent (12,700 larvae given) to 59% (32,600 larvae given) (average of 39%) of *T. axei,* given as larvae, were recovered as adult specimens at necropsy of the 15 calves for which exact counts were made. Previously (Leland et al., 1959b), in experimental infections with strain A in 11 calves, recovery of adult *T. axei* ranged from 12.3% (1,500,000 larvae given) to 59.5% (41,000 larvae given) with an average of 34.4%.

The present investigation in 1986 revealed no loss of infectivity of calves with 2 strains of *T. axei* maintained for over 25 yr in rabbits. Infection of calves with 1 of these strains (A), using

244

rabbits as a source of larvae, last occurred in 1956 (Leland et al., 1959a, b). The last infection of an equid with 1 of the strains, maintained in rabbits, was in 1979 when strain A developed in ponies (Lyons et al., 1982).

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Studies on the Life Cycle and Host Specificity of *Parastrongylus schmidti* (Nematoda: Angiostrongylidae)[1]

J. M. KINSELLA[2]

Department of Infectious Diseases, College of Veterinary Medicine, University of Florida,
Gainesville, Florida 32610

ABSTRACT: First-stage larvae of *Parastrongylus schmidti* from the lungs of rice rats, *Oryzomys palustris,* were used to infect land snails, *Polygyra septemvolva,* and aquatic snails, *Biomphalaria glabrata.* Third-stage larvae were recovered from *P. septemvolva* 26 days after exposure and from *B. glabrata* after 28 days. In experimental infections in the rice rat, 50% of the larvae recovered reached the lungs and heart within 12 hr, and 80% within 24 hr. Eggs were first noted in the lungs of rice rats 26 days after infection and larvae in the feces on day 31. Cotton rats, deer mice, white mice, white rats, gerbils, and golden hamsters were experimentally infected with *P. schmidti.* Only white-footed mice were refractory to infection. All white mice, hamsters, and gerbils died from the infections. In hamsters dosed with from 10 to 50 larvae, survival time increased with decreasing dose, but all hamsters died by 25 days postinfection. The life cycle of *P. schmidti* appears to most closely resemble that of *Parastrongylus dujardini* from European rodents.

KEY WORDS: *Oryzomys palustris, Sigmodon hispidus, Peromyscus maniculatus, Peromyscus leucopus, Mus musculus, Rattus norvegicus, Mesocricetus auratus, Meriones unguiculatus,* rice rat, cotton rat, deer mouse, white-footed mouse, white mouse, white rat, golden hamster, gerbil, *Polygyra septemvolva, Biomphalaria glabrata,* snails.

The discovery that *Parastrongylus cantonensis* (Chen, 1935), a metastrongylid lungworm of rats, was the cause of human eosinophilic meningitis in the South Pacific and Asia (Alicata, 1962) caused a renewal of interest in this genus, with the result that 8 new species were described between 1968 and 1973. One of these species, *P. costaricensis* (Morera and Céspedes, 1971), was found to be the cause of abdominal angiostrongylosis of humans in Central America (Morera and Céspedes, 1971). After the discovery of *P. schmidti* (Kinsella, 1971) in rice rats (*Oryzomys palustris*) in Florida, studies were undertaken to determine the pattern of larval migration and experimental host specificity of this species.

The systematics of species previously referred to the genus *Angiostrongylus* has been recently revised (Ubelaker, 1986) and this taxonomy is followed here.

Materials and Methods

In initial experiments, land snails, *Polygyra septemvolva,* were collected on the campus of the University of Florida. Later, laboratory-raised aquatic snails, *Biomphalaria glabrata,* were obtained through the courtesy of the Geographic Medicine Branch of the National Institutes of Health. Laboratory-born rice rats and cotton rats (*Sigmodon hispidus*) were the offspring of pregnant females trapped on Paynes Prairie, Alachua County, Florida. Deer mice (*Peromyscus maniculatus*) and white-footed mice (*Peromyscus leucopus*) were from the laboratory colonies of Dr. Donald J. Forrester, University of Florida. White mice (*Mus musculus*), white rats (*Rattus norvegicus*), golden hamsters (*Mesocricetus auratus*), and gerbils (*Meriones unguiculatus*) were purchased from commercial sources.

First-stage larvae of *P. schmidti* were obtained originally from rice rats trapped on Paynes Prairie. Fecal pellets from infected rats were teased apart in distilled water and the larvae concentrated using the Baermann funnel technique. To obtain larger numbers of larvae, lungs from both naturally and experimentally infected rats were teased apart and the larvae concentrated by the same method. Land snails were infected by feeding them on lettuce on which a drop of water containing concentrated larvae had been placed. Aquatic snails were isolated individually for 24 hr in finger bowls containing dilution counts of 200–500 larvae.

Third-stage larvae were obtained from infected snails using the method described by Wallace and Rosen (1969). Snails were digested in an acid-pepsin solution and the larvae concentrated in a Baermann funnel. Larvae in distilled water were then isolated mechanically into small groups under the microscope, drawn into a syringe, and the total amount of water adjusted to 1 ml. The larvae were then administered to rodents using a metal esophageal tube.

During studies of larval migration in the definitive host, rodents were killed at intervals, various organs teased apart, and then digested in acid-pepsin solution. The resulting fluid was filtered in Baermann funnels and the larvae obtained were counted under the microscope. All measurements are in micrometers unless otherwise indicated.

[2] Address for reprints: 2108 Hilda Avenue, Missoula, Montana 59801.

Table 1. Numbers of larvae recovered from tissues of rice rats experimentally infected with *Parastrongylus schmidti.*

Tissue	12 hr ($N = 300$)	24 hr ($N = 500$)*	96 hr ($N = 100$)
Lungs	27	35	30
Liver	24	5	0
Heart	1	1	0
Stomach	2	3	0
Small intestine	0	0	0
Large intestine	0	0	0
Mesentery	2	0	0
Brain	0	0	0
Total	56	44	30

* Data from 2 hosts combined.

Results

First-stage larvae of *P. schmidti* were recovered from lung tissue of infected rice rats. Measurements are based on 15 larvae, with means in parentheses. The larvae were 239–326 (282) long and 12–19 (16) in maximum width. The esophagus was rhabditoid, 132–151 (143) long. The nerve ring was slightly posterior to the middle of the esophagus, 72–79 (76) from the anterior end, and the excretory pore was slightly posterior to the nerve ring. The distance from the anus to the end of the tail was 22–26 (24) and the tail had a distinct notch on the dorsal surface. The genital primordium was at about the midpoint of the intestine.

Small numbers of third-stage larvae were recovered from *Polygyra septemvolva* 26 days after exposure to first-stage larvae. From 8 to 25 larvae were given to each of 4 rice rats, and 3 of the 4 were later found to be infected with 1–3 adult worms. Since these snails were not laboratory-raised, the larvae were not used for measurements.

Ten third-stage larvae recovered from *Biomphalaria glabrata* 28 days after exposure were measured. These larvae were about twice the size of first-stage larvae, 500–568 (529) long by 21–24 (23) in maximum width. The stoma had prominent sclerotized rhabdions. The esophagus was 158–180 (169) long with the nerve ring slightly anterior to the midpoint, and the excretory pore slightly posterior to the nerve ring. The distance from the anus to the end of the tail was 31–36 (33), and the genital primordium was near the midpoint of the intestine.

Large numbers of larvae were given to rice rats to study the migration of larvae within the host (Table 1). At 12 hr after infection, 50% of the larvae recovered had already reached the lungs and heart, and 43% of the remaining larvae were found in the liver. At 24 hr after infection, 80% of the larvae recovered were in the lungs, with a few found in the liver and stomach. By 4 days after infection, larvae were found only in the lungs. A similar pattern was seen in a white mouse killed 18 hr after infection; 80% of the larvae were in the lungs and the rest in the liver.

Eggs were first noted in the lungs of rice rats on day 26 and larvae were first found in the feces on day 31. No rice rats died from infections, although 1 animal, killed on day 35, contained 37 adult worms and the lung tissue in both lobes was almost completely obliterated by eggs and larvae. This animal was exceptional; the usual number of adult worms found was from 1 to 5. No direct relationship was found between the number of larvae given and number of adult worms recovered.

To determine the host specificity of *P. schmidti,* 7 other species of rodents were exposed

Table 2. Results of transmitting *Parastrongylus schmidti* to various rodents.

Host	No. exposed	No. infected	No. larvae dosed	No. adults*	Larvae in lungs
Rice rats	16	14	50–300	9 (1–35)	+
Cotton rats	4	4	35–50	7 (4–13)	+
Deer mice	2	2	25	6 (1, 11)	−
White-footed mice	3	0	25	—	−
White mice†	9	9	20–25	10 (3–22)	−
White rats	3	3	50–100	3 (1–7)	+
Hamsters†	18	18	10–50	9 (4–19)	−
Gerbils†	2	2	25	14 (12, 15)	+

* Mean (range in parentheses).

† All infected animals died.

to third-stage larvae (Table 2). All rice rats in this study are included in Table 2 for comparison. Only white-footed mice were refractory to infection. The prevalence of infection in cotton rats, deer mice, white mice, white rats, hamsters, and gerbils was 100%, but all white mice, hamsters, and gerbils died from the infections. Besides the rice rats, only cotton rats, white rats, and gerbils developed patent infections.

In an attempt to find a suitable host to maintain a laboratory strain of *P. schmidti,* groups of 3-wk-old, female hamsters were dosed with 10, 20, 30, and 50 larvae respectively (Table 3). Despite the small numbers of larvae used, all hamsters died by day 25 postinfection. Survival time increased slightly with decreasing dose, but as few as 4 adult worms were still fatal. Several hamsters were observed to be in acute respiratory distress shortly before dying. Clumps of 4 to 6 worms were found commonly in the lumen of the pulmonary artery surrounded by clotted blood. A control group of 5 hamsters showed no signs of disease at the end of the experiment.

Infections in white mice followed the same pattern. The longest surviving mouse died 35 days postinfection and had 1 female and 2 male worms in the lung. No larvae were found in the lung, although the female worm had eggs in the uterus.

One gerbil, which died 31 days postinfection, was infected with 15 mature worms and larvae were present in lung tissue, but not in the feces.

Four cotton rats were infected. One cotton rat, killed 26 days postinfection, had mature worms, but no larvae were found in the lung. A second rat, which died on day 29, was found to have larvae in the lungs, the earliest date larvae were found in any host. Larvae were first found in the feces of cotton rats on day 30. Young white rats were also suitable hosts, with larvae first found in the feces on day 30.

Discussion

Three types of migration within the definitive host are known for larvae of the genus *Parastrongylus.* The migration of *P. schmidti* appears to be closest to that of *P. dujardini* (Drozdz and Doby, 1970). Larvae were found in the lungs within 12 hr after infection, apparently migrating by way of the hepatic portal system, since most of the remaining larvae were found in the liver. No larvae were found in the brain at any time, nor were larvae found in the wall of the small

Table 3. Results of transmitting *Parastrongylus schmidti* to hamsters.

No. hamsters exposed	No. larvae per hamster	No. hamsters infected	Day hamsters died*	No. worms recovered*
5	10	5	23 (22–25)	5 (4–7)
6	20	6	20 (18–23)	12 (8–13)
5	30	5	19 (18–22)	14 (11–16)
2	50	2	17 (17)	17 (15–19)

* Mean (range in parentheses).

intestine, indicating that the intestinal lymph nodes are not a site for larval molting. By 4 days postinfection, larvae were found only in the lung.

There appeared to be some resistance to infection in the rice rat. The number of larvae recovered in comparison to the number dosed was low, ranging from 9% to 30%. This resistance to infection may be the reason for the lack of mortality in rice rat infections, in marked contrast to other hosts.

Species of the genus *Parastrongylus* do not exhibit strict host specificity. Drozdz and Doby (1970), in a study on *P. dujardini,* infected 8 rodent hosts, including white rats, white mice, and hamsters. Natural infections of *P. costaricensis* ae known from 5 species from 3 different families of rodents, as well as man (Morera, 1973). Only 1 host, *Peromyscus leucopus,* was not infected in this study with *P. schmidti.* The prevalence of infection in most hosts was 100%; only 2 rice rats failed to become infected.

The mortality in hamsters and white mice appeared to be related both to the smaller size of the animals and to the number of adult worms that developed. Survival time in both species increased as the number of worms decreased. Drozdz and Doby (1970) also found that *P. dujardini* killed white mice between 16 and 31 days postinfection, and that survival time increased with decreasing dose. White mice with single sex infections or single worm infections of *P. dujardini* survived up to 2 mo, indicating that larvae released into the lung may be the cause of mortality. Interestingly, a deer mouse infected with a single male *P. schmidti* survived 41 days. This study indicated that *P. schmidti* could be a significant pathogen in smaller rodents such as *Peromyscus* spp. if they ingested larvae in the wild.

Natural infections of *P. schmidti* are known only from the rice rat trapped in freshwater and

saltwater marshes in Florida (Kinsella, 1971). Nine of 108 rice rats were found infected. No natural infections of *P. schmidti* were found in 86 cotton rats, many of which were trapped in the same 2 areas (Kinsella, 1974). The cotton rat, however, appears to be the major reservoir host for *P. costaricensis,* the cause of human abdominal angiostrongylosis in Costa Rica and Panama (Morera, 1973; Tesh et al., 1973). Ubelaker and Hall (1979) found 2 of 419 cotton rats infected with *P. costaricensis* in Texas. This parasite has not been reported in a number of other surveys of cotton rats in the southeastern United States (Kinsella, 1974).

Because the cotton rat was easily infected experimentally with *P. schmidti,* the absence of natural infections may be due to the fact that the cotton rat is much less omnivorous than the rice rat as noted by Sharp (1967). Morera (1973) reported that contamination of lettuce leaves with third-stage larvae through the mucous secretion of infected slugs can take place in *P. costaricensis.* This may account for infections of this species in the more herbivorous cotton rat, as well in as humans.

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Motility Response of Benzimidazole-resistant *Haemonchus contortus* Larvae to Several Anthelmintics

S. D. Folz,[1] R. A. Pax,[2] E. M. Thomas,[1] J. L. Bennett,[3]
B. L. Lee,[1] and G. A. Conder[1]

[1] The Upjohn Company, Kalamazoo, Michigan 49001
[2] Department of Zoology, Michigan State University, East Lansing, Michigan 48824 and
[3] Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824

ABSTRACT: The effects of several benzimidazoles and levamisole on the motility of 2 *Haemonchus contortus* (L-3) populations (in vitro) were determined. At 100 μg/ml, the motility of a cambendazole-resistant population of *H. contortus* was significantly affected by all treatments. Treatment with 10 μg/ml of cambendazole, fenbendazole, oxfendazole, or thiabendazole did not significantly affect the motility of the cambendazole-resistant larval population. Albendazole, oxibendazole, and levamisole (10 μg/ml) significantly reduced the motility of the larvae. At the 1.0 or 0.1 μg/ml levels, none of the treatments had a significant effect on larval motility.

At 100 and 10 μg/ml, the larval motility of an Upjohn *H. contortus* (L-3) population was significantly affected by all drug treatments. At 1.0 μg/ml, albendazole, fenbendazole, thiabendazole, oxibendazole, and levamisole significantly affected the motility of larvae; the effects of the drugs were low and comparable. No effect was observed with cambendazole or oxfendazole at 1.0 μg/ml. Only levamisole and thiabendazole significantly impacted on motility at 0.1 μg/ml.

At 100 μg/ml the effects of cambendazole, oxfendazole, thiabendazole, and oxibendazole on the motility of the Upjohn strain were significantly greater than on the cambendazole-resistant population; the effects of fenbendazole, albendazole, and levamisole on the 2 larval populations were comparable. The 10 μg/ml concentration of oxfendazole, thiabendazole, fenbendazole, oxibendazole, and albendazole also had a significantly greater effect on the motility of the Upjohn strain of larvae. Treatment with 1.0 μg/ml of thiabendazole, oxibendazole, and albendazole or 0.1 μg/ml thiabendazole also resulted in a significantly greater effect on the Upjohn strain.

KEY WORDS: Nematoda, Trichostrongylidae, resistance, paralysis, assay.

Thiabendazole, the first of the benzimidazoles marketed in the U.S., was approved in 1963. Structural modifications of this drug led to the development of several new products with improved spectrum, potency, and pharmacodynamic characteristics. This class of compounds has broad-spectrum anthelmintic activity (nematodes, cestodes, trematodes) and also antifungal and antitumor activities.

Widespread resistance to the benzimidazoles is a potentially serious problem, as these drugs are frequently used on a worldwide basis. There are indications that anthelmintic resistance to the benzimidazoles continues to expand (Prichard et al., 1980; Donald, 1983; Vlassoff and Kettle 1985). Furthermore, detecting the initial development of anthelmintic resistance is difficult (Le Jambre et al., 1978). Consequently, there is a continuing need for new and improved assays that detect subtle changes in sensitivity to anthelmintics.

Benzimidazole anthelmintics affect embryonation and hatching of nematode ova. Hence, in vitro egg-hatch assays to detect benzimidazole resistance have been developed (Le Jambre, 1976; Coles and Simpkin, 1977; Hall et al., 1978; Whitlock et al., 1980). A biochemical test (tubulin binding) also has been developed for *Trichostrongylus colubriformis* by Sangster (1983), and differences between susceptible and resistant nematode populations have been reported. The effects of levamisole and morantel on larval motility led to the development of the larval paralysis test for *Ostertagia circumcincta* (Martin and Le Jambre, 1979). The recent development of the micromotility meter for monitoring parasite motor function (Bennett and Pax, 1986; Folz et al., 1987a) has provided a unique opportunity to extend the paralysis test. We can now accurately quantitate parasite motor function, and thereby more precisely assess the effects of drugs on helminth motor activity. The effects of several benzimidazoles and levamisole on the motility of *Haemonchus contortus* larvae were determined (in vitro). The larval motor activities of cambendazole-resistant and Upjohn-derived *H. contortus* populations were measured and compared.

Materials and Methods

The resistant population of *H. contortus* used for the experiment was the U.S.D.A. cambendazole-resistant strain obtained from G. C. Coles (University of Massachusetts). A second *H. contortus* population (Upjohn strain) was obtained from K. S. Todd (University of Illinois) approximately 6 yrs ago. The parasites have been maintained in lambs without exposure to anthelmintic treatment.

After obtaining a clean, homogeneous, clump-free sample of ensheathed larvae, the collection was diluted with distilled water to a concentration of 20 larvae per 10 μl. To prevent bacterial growth, 1,000 units each of penicillin and streptomycin were added per milliliter of larval suspension. The larval samples (50 ml maximum volume) were then stored at 4°C, in 16-oz amber jars.

Twenty-four hours before use, a batch of larvae was removed from the refrigerator, warmed to ambient temperature (22°C), and mixed by vortexing and stirring. Twelve 10-μl samples of the larval suspension were removed with a microliter pipette, placed on microscope slides, and examined under a stereomicroscope. All larvae were counted in each sample; separate tallies were compiled for motile and nonmotile larvae. For calculation of the mean larval counts per batch of larval suspension, the highest and lowest tallies from the 12 samples were eliminated, and the remaining 10 tallies were averaged. If the larval suspension had a mean count of more than 3% nonmotile larvae, it was discarded, and a new culture was processed and counted. If the mean counts showed less than 3% nonmotile larvae, the culture was considered acceptable and was diluted with distilled water to a concentration of 500 larvae per ml.

A 3-channel micromotility meter (Folz et al., 1987a) was used to quantitatively determine the motility of the target nematodes after exposure to treatment. The micromotility meter uses a light directed upward through the tube containing the larval suspension. Movement of the helminths changes the angle of refraction of the light rays from the meniscus of the suspension, which in turn modulates an electrical signal emanating from a photodetector. The numerical representation of the modulated electrical signal is the motility index; this provides a quantitative measurement of the helminth movement. Greater larval movement yields a higher motility index, whereas dead larvae generate motility index readings comparable to those obtained from blank samples.

Each drug was tested at 4 concentrations, and 6 replicate samples were run for each concentration. The replicate 1 samples for the various concentrations of all drugs were run first, followed by replicate 2 samples, and then replicate 3 samples. Within each block of replicates, the samples were tested in a random sequence, and blocked by meter channel. This process was again repeated for the remaining 3 replicates. These procedures assured that each of the replicate samples for a given drug and concentration was analyzed in a different meter channel, thus lessening the likelihood of bias in results due to small differences in readings between channels or over time. After the tubes were placed in the meter, a 60-sec acclimation period was automatically initiated, followed immediately by the 60-sec test.

Six benzimidazole ruminant anthelmintics were evaluated for activity in the in vitro assay: albendazole (Smith Kline), cambendazole (Merck), fenbendazole (Hoescht), oxibendazole (Smith Kline), oxfendazole (Syntex), and thiabendazole (Merck). Levamisole hydrochloride (American Cyanamid) also was included in the study. All of these drugs have in vivo *H. contortus* activity.

For each concentration of drug tested, 450 μl of distilled water containing L-3 *H. contortus* (500/ml) was added to each of 6 culture tubes (10 × 75 mm). In a separate tube, 1 mg of technical (nonformulated) drug was weighed and dissolved in a mixture of: 200 μl acetone, 50 μl of a 1:1 mixture of Tween 20 (polysorbate monolaurate) and distilled water, and 750 μl distilled water. Six replicates were prepared at each of 4 concentrations (100, 10, 1.0, 0.1 μg/ml) for each drug tested. To achieve the 100 μg concentration of active drug per milliliter of vehicle, 50 μl of the acetone–drug solution was transferred to each of 6 culture tubes containing 450 μl of distilled water and larvae. The stock drug solution was then diluted (10-fold) with the blank vehicle solution to achieve the other test concentrations (10, 1.0, 0.1 μg/ml); again, for each concentration, 50 μl of the acetone–drug solution was transferred to each of 6 culture tubes containing 450 μl of water and larvae. Controls were prepared by combining (in culture tubes) 50 μl of the blank vehicle solution and 450 μl of the distilled water and larval preparation. A background index was obtained by including distilled water samples (500 μl) in the experiment.

The drug preparations and larvae were maintained in the culture tubes at room temperature (22°C) for 24 hr. Each rack of tubes was covered with Parafilm® to preclude evaporation. After a 24-hr incubation period, the tubes were analyzed in the micromotility meter.

Data were analyzed according to the randomized restricted design method. The experimental unit was a single tube. Differences between experimental treatments were tested for statistical significance ($P \leq 0.05$) by the analysis of variance procedure using the general linear models method. The Waller-Duncan k-ratio t-test was used for separation of means. Motility indices were transformed to percent reductions (motility) by means of the following formula: [(vehicle control index − treatment index)/(vehicle control index − background index)] × 100.

Results

The effects of 6 benzimidazoles and levamisole on the motor function (motility) of 2 *H. contortus* third-stage, ensheathed larval populations were determined. For the cambendazole-resistant population, 100 μg/ml of all 6 benzimidazoles significantly affected helminth motility (reductions ≥12.2%; Table 1). Cambendazole had a significantly lesser effect on helminth motility than any other treatment. Albendazole and levamisole caused comparable and significantly

Table 1. Effects of several anthelmintics on the motility of cambendazole-resistant (CBZ-R) and Upjohn *Haemonchus contortus* L-3 populations, at 100 μg/ml.

Treatment (100 μg/ml)	Mean reduction in motility ± SE (%)		
	CBZ-R strain	Upjohn strain	Strain difference
Cambendazole	12.3 ± 5.0	48.1 ± 6.3	35.8*
Oxfendazole	31.4 ± 9.1	68.9 ± 2.9	37.5*
Thiabendazole	34.7 ± 7.4	66.1 ± 4.7	31.4*
Fenbendazole	64.8 ± 2.4	75.5 ± 6.8	10.7
Oxibendazole	66.2 ± 4.7	82.7 ± 2.9	16.5*
Albendazole	82.5 ± 7.2	91.2 ± 2.9	8.7
Levamisole	89.6 ± 3.3	95.5 ± 2.1	5.9

* Significant difference ($P \leq 0.05$); 12.2 = least significant difference value.

Table 3. Effects of several anthelmintics on the motility of cambendazole-resistant (CBZ-R) and Upjohn *Haemonchus contortus* L-3 populations, at 1.0 μg/ml.

Treatment (1.0 μg/ml)	Mean reduction in motility ± SE (%)		
	CBZ-R strain	Upjohn strain	Strain difference
Cambendazole	8.3 ± 5.6	9.2 ± 6.8	0.9
Oxfendazole	1.4 ± 1.4	6.8 ± 4.4	5.4
Thiabendazole	0 ± 0	22.3 ± 5.1	22.3*
Fenbendazole	10.7 ± 4.1	20.6 ± 5.6	9.9
Oxibendazole	1.6 ± 1.6	19.5 ± 4.7	17.9*
Albendazole	6.1 ± 3.9	30.8 ± 7.3	24.7*
Levamisole	9.8 ± 6.5	20.9 ± 8.7	11.1

* Significant difference ($P \leq 0.05$); 12.2 = least significant difference value.

greater reductions in motility than all of the remaining benzimidazoles. At 10 μg/ml, cambendazole, fenbendazole, oxfendazole, and thiabendazole had no significant effect ($< 12.2\%$) on the motility of the cambendazole-resistant larval population (Table 2). The remaining treatments (levamisole, albendazole, oxibendazole) significantly affected motility, with levamisole having a significantly greater effect than any other treatment. None of the treatments (including levamisole) caused a significant reduction in helminth larval motility at 1.0 or 0.1 μg/ml (Tables 3, 4).

An Upjohn *H. contortus* population was also assayed. The same benzimidazole compounds and levamisole were again evaluated for effects on larval motility. Treatment at 100 μg/ml resulted in a significant reduction in larval motility for all treatments (reductions ≥ 12.2%; Table 1).

The effect on helminth motility from cambendazole was significantly less than any of the other treatments. Albendazole and levamisole were significantly more active than other treatments, with the exception of oxibendazole, which was comparable to albendazole. At the 10 μg/ml level, all treatments again significantly reduced larval motility (Table 2). Cambendazole had a significantly lesser effect than all other treatments, and levamisole was significantly more effective. Treatment at 1.0 μg/ml resulted in a significant reduction in motility for albendazole, fenbendazole, oxibendazole, thiabendazole, and levamisole, but not cambendazole or oxfendazole (Table 3). At 0.1 μg/ml, only thiabendazole and levamisole significantly affected the motility of the larvae; the activities of these 2 drugs were comparable.

Differences between the larval strains are also

Table 2. Effects of several anthelmintics on the motility of cambendazole-resistant (CBZ-R) and Upjohn *Haemonchus contortus* L-3 populations, at 10.0 μg/ml.

Treatment (10.0 μg/ml)	Mean reduction in motility ± SE (%)		
	CBZ-R strain	Upjohn strain	Strain difference
Cambendazole	7.9 ± 3.8	19.5 ± 7.3	11.6
Oxfendazole	4.5 ± 4.5	37.3 ± 7.2	32.8*
Thiabendazole	6.7 ± 3.4	40.6 ± 9.2	33.9*
Fenbendazole	7.2 ± 6.8	33.4 ± 8.1	26.2*
Oxibendazole	18.7 ± 6.8	49.0 ± 8.4	30.3*
Albendazole	22.7 ± 7.4	52.6 ± 10.3	29.9*
Levamisole	61.8 ± 4.9	69.5 ± 4.6	7.7

* Significant difference ($P \leq 0.05$); 12.2 = least significant difference value.

Table 4. Effects of several anthelmintics on the motility of cambendazole-resistant (CBZ-R) and Upjohn *Haemonchus contortus* L-3 populations, at 0.1 μg/ml.

Treatment (0.1 μg/ml)	Mean reduction in motility ± SE (%)		
	CBZ-R strain	Upjohn strain	Strain difference
Cambendazole	2.3 ± 1.5	12.1 ± 6.5	9.8
Oxfendazole	0 ± 0	11.8 ± 3.9	11.8
Thiabendazole	0.5 ± 0.5	22.3 ± 6.2	21.8*
Fenbendazole	5.6 ± 4.7	10.1 ± 6.6	4.5
Oxibendazole	2.9 ± 2.9	8.1 ± 4.9	5.2
Albendazole	4.2 ± 4.1	2.6 ± 1.5	1.6
Levamisole	5.1 ± 3.2	15.8 ± 5.7	10.7

* Significant difference ($P \leq 0.05$); 12.2 = least significant difference value.

depicted in Tables 1–4. At the 100 µg/ml concentration, the effects (reductions in motility) of cambendazole, oxfendazole, thiabendazole, and oxibendazole on the Upjohn strain were significantly greater than on the cambendazole-resistant population (Table 1). The effects of fenbendazole, albendazole, and levamisole on the 2 *H. contortus* strains were comparable (differences <12.2%). The 10 µg/ml level of oxfendazole, thiabendazole, fenbendazole, oxibendazole, and albendazole also had a significantly greater effect on the motility of the Upjohn strain of larvae (Table 2); both nematode strains responded comparably to cambendazole and levamisole. At 1.0 µg/ml, thiabendazole, oxibendazole, and albendazole had a significantly greater effect on the motility of the Upjohn strain, whereas at 0.1 µg/ml the strain difference was noted only for thiabendazole (Tables 3, 4).

Discussion

The cambendazole-resistant strain of *H. contortus* utilized in our studies was experimentally developed by Kates et al. (1973). Cross-resistance (currently referred to as side-resistance) to other benzimidazoles (thiabendazole, oxibendazole, mebendazole) was later reported by Colglazier et al. (1975); however, the cambendazole-resistant population was susceptible to treatment with levamisole. These data were generated from in vivo studies, and involved primarily the adult stage of the parasite. The cambendazole-resistant larval data reported herein appear to correlate with the adult data. At the 100 µg/ml concentration, the benzimidazoles affected motility in the following rank/order: (albendazole > oxibendazole = fenbendazole > thiabendazole = oxfendazole > cambendazole). Only albendazole demonstrated activity that was comparable to levamisole. At 10 µg/ml, albendazole and oxibendazole were the only benzimidazoles significantly affecting the motility of the larvae (comparable activity); as expected, levamisole was significantly more active than either of the 2 active benzimidazoles (levamisole > albendazole = oxibendazole). Hence, benzimidazole side-resistance was also observed with the larvae. The differentiation between the various benzimidazoles in terms of the effect on motility is of interest, in that albendazole (100 and 10 µg/ml) was significantly more active than any of the other benzimidazoles tested, with the exception of oxibendazole (10 µg/ml).

The Upjohn *H. contortus* larval population appeared consistently more susceptible to treatment with the benzimidazoles and also levamisole (Tables 1–4). At 100 and 10 µg/ml, cambendazole had a significantly lesser effect on larval motility than any of the other treatments. Albendazole and levamisole (100 µg/ml) caused significantly greater reductions in motility than did the other treatments, with the exception of oxibendazole. Unlike the cambendazole-resistant population, all 6 benzimidazoles (and levamisole) significantly affected larval motility at 10 µg/ml. Again, unlike the cambendazole-resistant strain, 1.0 µg/ml of albendazole, fenbendazole, oxibendazole, thiabendazole, and levamisole significantly impacted on *H. contortus* motility (comparable activity). Cambendazole and oxfendazole (1.0 µg/ml) did not significantly affect motility of the Upjohn larval population. At the lowest drug level tested (0.1 µg/ml), thiabendazole and levamisole continued to significantly affect the larvae (minimal effect).

The effects of anthelmintics on the motility of ensheathed third-stage larvae and the use of the micromotility meter to accurately quantitate these effects (Bennett and Pax, 1986; Folz et al., 1987a, b) was the basis for extending the larval paralysis test of Martin and Le Jambre (1979). The assay described herein may have application for determining side-resistance, cross-resistance, multiple-resistance, reversion, and/or counter selection. The assay could be easily expanded to generate data that can be used to predict a dose–response curve, and also LC_{50} and LC_{90} concentrations. In conclusion, the micromotility meter method offers several advantages as an adjunct to the other techniques used to evaluate resistance or susceptibility to anthelmintic treatment.

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A Unique Postganglionic Cell in the Praesoma of the Genus *Neoechinorhynchus* (Acanthocephala)

RANDALL J. GEE

Department of Biology, The Cleveland State University, Cleveland, Ohio 44115

ABSTRACT: A characteristic ganglionic cell between the posterior end of the proboscis receptacle and the posterior end of the cerebral ganglion was found in 6 of the 8 species studied in the family Neoechinorhynchidae and is described herein. This cell was observed only in members of the genus *Neoechinorhynchus*. It was not found in *Paulisentis fractus* Van Cleave and Bangham, 1948, or *Octospinifer macilentus*, Van Cleave, 1919, 2 other species of Neoechinorhynchidae, and neither was it found in representatives of the classes Palaeacanthocephala and Archiacanthocephala. This postganglionic cell is characterized by the presence of large granules in the cytoplasm and 2 elongated extensions that extend anteriorly to become associated with the nerve fibers of the cerebral ganglion. The function of this cell has not been determined. Photomicrographs of this cell showing its cytoplasmic granules, its location, and its lateral extensions are presented.

KEY WORDS: acanthocephalan nervous system, *Neoechinorhynchus chrysemydis*, *N. cylindratus*, *N. emydis*, *N. emyditoides*, *N. magnapapillatus*, *N. pseudemydis*, *Octospinifer macilentus*, *Paulisentis fractus*.

The literature contains little information concerning the individual cells in the cerebral ganglia in species of the class Eoacanthocephala, especially the genus *Neoechinorhynchus*. There are descriptions concerning the numbers of cells in the cerebral ganglion, but few describe their functions and the nerves extending from them. Crompton (1963) described cholinesterase activity in the cells of the cerebral ganglion of *Polymorphus minutus* (Goeze, 1782). Dunagan and Miller (1975, 1981) enumerated the cells and reconstructed the cerebral ganglion in *Moniliformis moniliformis* (Bremser *in* Rudolphi, 1819) and *Oligacanthorhynchus tortuosa*. Dunagan and Miller (1975) in a review found that most descriptions are of members of the classes Archiacanthocephala and Palaeacanthocephala. Bone (1976), Budziakowski and Mettrick (1985), and Budziakowski et al. (1983, 1984) described the ultrastructure and possible neurosecretory activity of the cells in the cerebral ganglion of *M. moniliformis*. Golubev and Sal'nikov (1979) described the ultrastructure of the cerebral ganglion in *Echinorhynchus gadi* Zoega *in* Müller, 1776. During an extensive study on the morphology of the nervous system in the praesoma of 9 species of Acanthocephala, Gee (1969) observed a uniquely different cell on the posterior end of the cerebral ganglion of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) and *Neoechinorhynchus emydis* (Leidy, 1851).

Since its original discovery in these 2 species, it has been observed in 4 additional species belonging to the genus *Neoechinorhynchus*. At the present time, it appears that this cell may be characteristic for only the genus *Neoechinorhynchus*. Because it may be of taxonomic, evolutionary, or physiological importance, it is described in this paper.

Materials and Methods

Eight species from the family Neoechinorhynchidae were studied. These species and their hosts are: (1) *Neoechinorhynchus chrysemydis* Cable and Hopp, 1954; hosts: *Chrysemys picta picta*, *Pseudemys scripta scripta*; (2) *N. cylindratus*; hosts: *Micropterus salmoides*, *Ambloplites rupestris*; (3) *N. emydis*; host: *C. picta picta*; (4) *Neoechinorhynchus emyditoides* Fisher, 1960; hosts: *P. scripta scripta*, *P. scripta elegans*; (5) *Neoechinorhynchus magnapapillatus* Johnson, 1969; host: *P. scripta scripta*; (6) *Neoechinorhynchus pseudemydis* Cable and Hopp, 1954; hosts: *P. scripta scripta*, *P. scripta elegans*; (7) *Octospinifer macilentus* Van Cleave, 1919; host: *Catostomus commersoni commersoni*; and (8) *Paulisentis fractus* Van Cleave and Bangham, 1948; host: *Semotilus atromaculatus atromaculatus*.

Adult specimens were collected alive from their hosts and fixed according to the following method: (1) Worms collected from the intestine of the host were placed in tap water and refrigerated overnight to evert the proboscis. (2) Worms were fixed in warm, 50–60°C, AFA or 10% formalin for 24–48 hr. During fixation, the body wall was punctured with fine needles to facilitate flow of fixative into the pseudocoel. (3) AFA-fixed specimens were washed in several changes of 70% isopropanol. Worms fixed in 10% formalin were washed in tap water for 8–12 hr and stored in 5% formalin to prevent excessive hardening of tissues. (4) Fixed worms were infiltrated with paraffin according to the isopropanol method of Doxtader (1948). Transverse and sagittal sections were cut from 4 to 6 μm in thickness. (5) Sections were stained progressively in 10% Ehrlich's acid-hematoxylin according to Guyer (1936).

Neoechinorhynchus from turtles were identified based on the shape of the posterior end of females and size and shape of eggs. Because there are no known reliable

taxonomic characters to distinguish males of the various species of the genus *Neoechinorhynchus* from turtles, females were used as study specimens. Males were included to compare possible differences between male and female praesoma structure. Both sexes of *N. cylindratus* from fish were studied.

Measurements based on 30 specimens of each species were determined with an ocular micrometer.

Results

In *Neoechinorhynchus cylindratus, N. chrysemydis, N. emydis, N. emyditoides, N. magnapapillatus,* and *N. pseudemydis* there is a large postganglionic cell (PGC) located between the posterior end of the cerebral ganglion (CG) and the musculature of the posterior end of the proboscis receptacle (PR) which appears to be different from other cerebral ganglion nerve cells (Fig. 5). In *N. chrysemydis, N. emydis, N. emyditoides, N. magnapapillatus,* and *N. pseudemydis* this cell measures 20–30 μm wide and 15–18 μm long ($N = 150$). In *N. cylindratus* it is 12–23 μm wide and 12–18 μm long ($N = 30$). Differences in size appear to be due to sizes of worms studied. The cytoplasm of this ganglion cell contains numerous dark-staining granules located around the inner surface of the cell membrane (Figs. 1, 2, 5; PGC). The nucleus contains a large dark-staining nucleolus. The cytoplasm appears as light-staining granulated material when compared to the compact dark gray-staining cytoplasm of other cells in the cerebral ganglion. In some specimens a large clear area, possibly an empty vesicle, was observed in the cytoplasm. Two elongated lateral processes (LPGC) of the cell extend anteriorly along the lateral surface of the cerebral ganglion to the point where the retinacular nerves leave the cerebral ganglion (Figs. 2–4, 6; LPGC). At this point they appear to extend into the neuropile of the cerebral ganglion where they become indistinguishable from other fibers in this region (Fig. 4; LPGC). This ganglion cell was not demonstrable in either *Paulisentis fractus* or *Octospinifer macilentus,* 2 other species of Neoechinorhynchidae studied.

Discussion

In their descriptions, based on light microscopy, of the cells in the cerebral ganglion of *Moniliformis moniliformis* and *Oligacanthorhynchus tortuosa,* Dunagan and Miller (1975, 1981) do not mention a cell such as described in this investigation. Bone (1976), Golubev and Sal'nikov (1979), Budziakowski et al. (1984), and Budziakowski and Mettrick (1985), using transmission electron microscopy, described 5–6 possible cellular types within the cerebral ganglion of *M. moniliformis* and *E. gadi.* Their studies do not describe a granulated cell on the posterior surface of the cerebral ganglion. Harada (1931) described a tripolar postganglionic cell between the proboscis invertor muscles in the posterior region of the proboscis receptacle of *Bolbosoma turbinella* (Dies., 1851). This cell has nerve fibers originating from it that innervate the proboscis invertor muscles in the posterior region of the proboscis receptacle. Gee (1969) observed a postganglionic cell, similar to the one described by Harada (1931), in *Echinorhynchus salmonis* Müller, 1784, *Leptorhynchoides thecatus* Linton, 1891, and an undescribed species of Echinorhynchidae.

In *Neoechinorhynchus cylindratus, N. chrysemydis, N. emydis, N. emyditoides, N. magnapapillatus,* and *N. pseudemydis* this ganglion cell differs from the postganglionic cell in *E. salmonis, L. thecatus,* and *B. turbinella* by the absence of nerve fibers extending from it to terminate in the proboscis invertor muscles. It should be noted that in species of *Neoechinorhynchus* the cerebral ganglion is situated at the posterior end of the proboscis receptacle. In *E. salmonis, L. thecatus,* and *B. turbinella* it is located in the midregion of the proboscis receptacle. Gee (1969) did not observe a postganglionic cell in *Pomphorhynchus bulbocolli* (Linkins, 1919), a species of Palaeacanthocephala in which the cerebral ganglion is located at the posterior end of the proboscis receptacle. If the ganglion cell in these species of *Neoechinorhynchus* proves to be homologous to the postganglionic cell in the Palaeacanthocephala, the posterior position of the cerebral ganglion may explain why there are no nerve fibers associated with the postganglionic cell in the species of *Neoechinorhynchus* studied in this investigation. I have observed nerve fibers that originate from cells in the cerebral ganglion that appear to innervate the invertor muscles of the proboscis in this area (Gee, 1969, 1987a).

The granules may contain neurotransmitter substances, may be neurosecretory, neurohormonal, or may have another function. Crompton (1963) demonstrated cholinesterase activity in cells of the cerebral ganglion of *Polymorphus minutus.* Bone (1976), Budziakowski et al. (1983, 1984), and Budziakowski and Mettrick (1985) have described vesicles and granules that appear to contain biogenic amines in the cerebral gan-
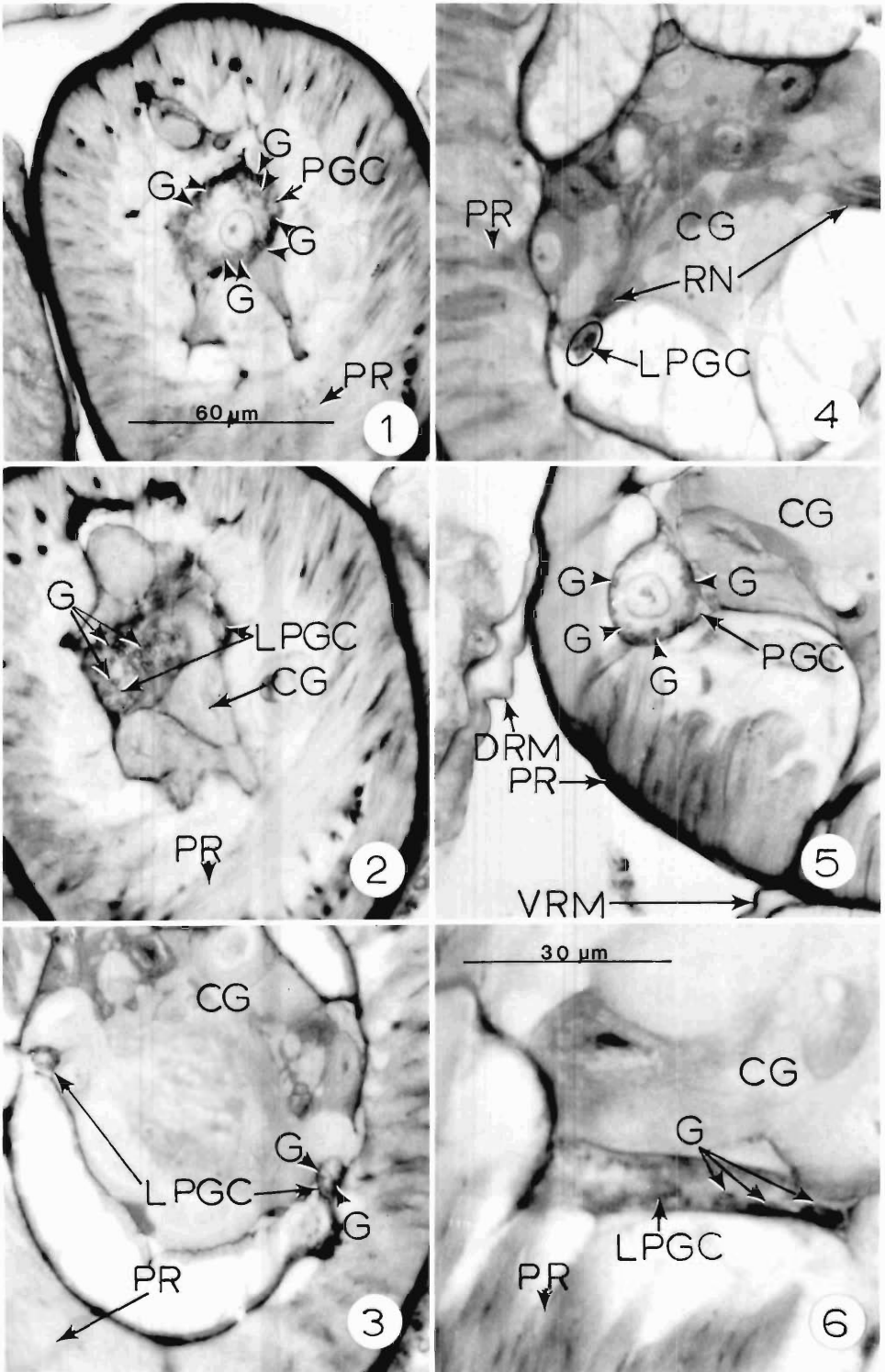
Figures 1–6. 1–4. Transverse sections of posterior region of proboscis receptacle (PR) and cerebral ganglion (CG) of *Neoechinorhynchus cylindratus* illustrating postganglionic cell (PGC) and lateral extensions of postgan-

glion of *M. moniliformis*. Granules were widely distributed in all cells that they observed in the cerebral ganglion. I have studied *M. moniliformis* and *Macracanthorhynchus hirudinaceus* Pallas, 1781, using light microscopy, and observed small granules in various cells in the cerebral ganglion. I could not find an isolated cell containing granules as distinctive as described in this report in either of these Archiacanthocephala or in *E. salmonis, L. thecatus,* and *Pomphorhynchus bulbocolli,* species of Palaeacanthocephala.

This postganglionic cell should not be confused with the Stützzelle (support cell). In these species of *Neoechinorhynchus* the Stützzelle is a binucleate structure located on the inner surface of the dorsal wall of the proboscis receptacle between the anterior end of the cerebral ganglion and the neck region (Gee, 1969, 1987a, b).

At present, it appears that this postganglionic cell is unique to the genus *Neoechinorhynchus.* Further studies on other members of the Eoacanthocephala are needed to establish whether this is truly a characteristic cell found only in the genus *Neoechinorhynchus* and to elucidate the function of this cell type.

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glionic cell (LPGC). Granules (G) in cytoplasm and association of lateral extensions of postganglionic cell with retinacular nerve (RN) can be seen. Lateral extensions become indistinguishable from fibers in neuropile in sections anterior to Figure 4. Sections proceed anteriorly from posterior end of proboscis. Relative position of sections in Figures 1 and 2 can be obtained by comparing with Figure 5. Figure 3 is approximately midway through cerebral ganglion and Figure 4 about 36 μm posterior to anterior end of cerebral ganglion. 5, 6. Sagittal section through proboscis receptacle and cerebral ganglion of *Neoechinorhynchus emyditoides* showing position of postganglionic cell (PGC) between posterior end of cerebral ganglion (CG) and posterior end of proboscis receptacle (PR) and granules (G) around the inner surface of the cell membrane. The dorsal and ventral retractor muscles of the proboscis receptacle are shown (DRM, VRM). Figure 6. Sagittal section showing granules (G) in cytoplasm of the lateral extensions (LPGC). Figures 1–5 are of the size shown in bar graph in Figure 1. Figure 6 has been enlarged photographically to demonstrate granules in 1 of the lateral extensions.

Endoparasites of the Smallmouth Salamander, *Ambystoma texanum* (Caudata: Ambystomatidae) from Dallas County, Texas

CHRIS T. MCALLISTER[1,2] AND STEVE J. UPTON[3]

[1] Renal-Metabolic Lab (151-G), Veterans Administration Medical Center,
4500 South Lancaster Road, Dallas, Texas 75216
[2] Department of Biological Sciences, North Texas State University, P.O. Box 5218,
Denton, Texas 76203-5218 and
[3] Division of Biology, Ackert Hall, Kansas State University, Manhattan, Kansas 66506

ABSTRACT: Thirty-seven adult and 15 immature smallmouth salamanders, *Ambystoma texanum,* from a farm pond located in Dallas County, Texas, were examined for endoparasites. Seventy-three percent of the salamanders were infected with 1 or more species of endoparasite. New host records are reported for the protozoa, *Hexamastix batrachorum* Alexeieff, 1911, *Myxidium serotinum* Kudo and Sprague, 1940, and *Eimeria ambystomae* Saxe, 1955, and for the cestode, *Cylindrotaenia americana* Jewell, 1916. New geographic records are reported for *E. ambystomae* and *H. batrachorum.* Prevalence was not significantly different among sexes or size classes of adults; however, only 20% of immature salamanders were infected with endoparasites.

KEY WORDS: Cestoda, *Cylindrotaenia americana,* Coccidia, *Eimeria ambystomae, Hexamastix batrachorum, Myxidium serotinum,* oocysts, residuum, spores, trophozoites, gilled larvae, transforming larvae, prevalence.

The smallmouth salamander, *Ambystoma texanum* (Matthes, 1855), is a moderately large caudate amphibian that ranges from extreme southeastern Michigan and Pelee Island, Ontario, west to southern Iowa and south to the Gulf coasts of Texas, Louisiana, and Mississippi. It occurs in various habitats throughout the range, including tall-grass prairie, moist pine woodlands, flood plain forest, oak woodland, dense hardwood forest, and intensively farmed areas (Anderson, 1967).

A great deal of information is available on various aspects of the natural history of this salamander (see Anderson, 1967, for review); however, few published reports exist dealing with parasites of *A. texanum.* Harwood (1932) examined 4 *A. microstomum* (=*A. texanum*) from southern Texas and reported 2 helminth species in 1 specimen. Walton (1942) summarized both protozoan and helminth fauna of various *Ambystoma* spp., including *A. texanum.* Rosen and Manis (1976) reported a new host record for *Brachycoelium ambystomae* in a single *A. texanum* from Arkansas and, recently, Price and St. John (1980) examined 57 *A. texanum* from Williamson County, Illinois, and reported helminths in 54 specimens. Although unpublished, the most comprehensive investigation was by Landewe (1963), who reported several helminths in 61 *A. texanum* from southern Illinois.

The purpose of the present survey was to (1) examine a generous sample of various age and size classes of *A. texanum* for the prevalence of endoparasites from a single locality near the southern portion of their range, and (2) compare the results of our survey with previous reports on parasites of *A. texanum* from other parts of their range.

Materials and Methods

Measurements of salamanders are in millimeters (mm), with the mean ± standard error of the snout–vent length (SVL) followed by the range. Fifteen gilled larvae and transforming (immature) smallmouth salamanders (32.0 ± 0.9; 29–34) were collected with dipnets from a temporary farm pond on 12 and 16 March 1986 in Dallas County, Texas, 2.4 km west of DeSoto off FM 1382. Thirty-seven adult *A. texanum* (24 males, 13 females; 75.1 ± 1.3; 54–90) were captured during December 1986 and early January 1987 at the same locale. These adults had apparently congregated at the pond by traveling overland for initiation of courtship and breeding activities. Salamanders were rendered immobile by complete immersion in a 1:2,000 dilution of MS-222 (tricaine methanesulfonate) and blood smears were obtained from the exposed ventricle. Thin blood smears were fixed in absolute methanol, stained with Giemsa for 1 hr, and rinsed briefly in phosphate-buffered tap water (pH = 7.1). Gastrointestinal tracts were removed from anesthetized specimens, placed in 0.9% NaCl, and examined for helminths. Both the body cavity and other organs, including the urinary bladder, gall bladder, lungs, and liver, were examined similarly. Thin smears of intestinal scrapings were fixed in warm Schaudinn's fluid and stained with Gomori trichrome. Contents from the gall bladder were smeared on slides and treated in a similar manner. Portions of feces and additional gall bladder contents were collected and examined by microscopy following flotation in Sheather's sugar solution (spec. grav. 1.18). Cestodes were rinsed in ringers, placed in water-filled petri dishes to be slow-

258

ly heated until the worms relaxed and died in an extended condition, then placed in AFA for 24 hr, and transferred to 70% ethanol until studied. Cestodes were stained in a 1:15 dilution of Mayer's hematoxylin for 24 hr, dehydrated in a series of alcohols, cleared in xylene, and whole-mounted in permount.

Voucher specimens of *A. texanum* are deposited in the Arkansas State University Museum of Zoology (ASUMZ 5894.0–.12, 5895, 6279–6318). Representative samples of parasites are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 as follows: *Hexamastix batrachorum* (USNM 79551), *Myxidium serotinum* (USNM 79550), *Eimeria ambystomae* (USNM 79549) in 10% formalin, and *Cylindrotaenia americana* (USNM 79552).

Results and Discussion

Results revealed 38 of the 52 (73.1%) *A. texanum* to be infected with 1 or more species of endoparasite. Blood was negative for intraerythrocytic or trypanosomal hematozoans. New host records are reported for *Hexamastix batrachorum* Alexeieff, 1911, *Myxidium serotinum* Kudo and Sprague, 1940, *Eimeria ambystomae* Saxe, 1955, and *Cylindrotaenia americana* Jewell, 1916 (Table 1). Texas represents a new geographic locality for *E. ambystomae* and *H. batrachorum.*

The most common protozoan found in *A. texanum* was the trichomonad, *H. batrachorum.* All but 1 of the adult smallmouth salamanders were infected; however, only a single immature (transformed larvae, SVL = 35 mm) specimen was found to harbor this flagellate. Rankin (1937) reported *H. batrachorum* in the intestinal tract of 13 species of salamanders from North Carolina, including the closely related marbled salamander, *A. opacum.* Honigberg and Christian (1954) found 1 of 6 (16.7%) spotted salamanders, *A. maculatum,* to harbor *H. batrachorum.*
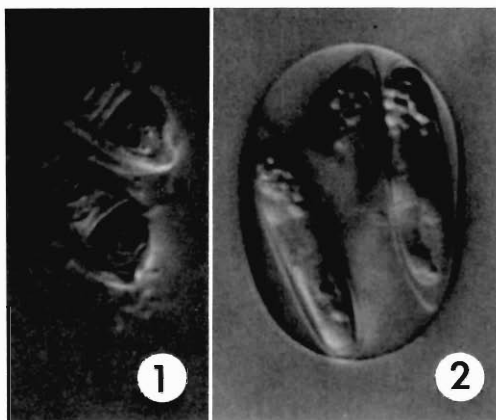
Figures 1, 2.   Nomarski interference contrast photomicrographs of *Myxidium serotinum* spores (Fig. 1) and *Eimeria ambystomae* oocyst (Fig. 2) from *Ambystoma texanum.* ×1,400.

In North America, myxozoans of the genus *Myxidium* occur primarily in the gall bladder, hepatic ducts, kidneys, gills, and muscle of freshwater and marine fishes; however, a few amphibians (mostly anurans) and reptiles (turtles) also serve as hosts. Kudo and Sprague (1940) reported *M. serotinum* in northern leopard frogs, *Rana pipiens,* and *Rana* sp. from the midwestern United States and Louisiana, respectively. In addition, Kudo (1943) found *M. serotinum* in green frogs (*R. clamitans*) from Louisiana and in southern leopard frogs (*R. sphenocephala*) and southern toads (*Bufo terrestris*) from Florida, and McAllister (1987) reported 32 of 52 (61.5%) Strecker's chorus frogs (*Pseudacris streckeri streckeri*) from the same locality mentioned herein for *A. texanum* to be infected with *M. serotinum.*

Table 1.   Endoparasites of 52 smallmouth salamanders (*Ambystoma texanum*) from Dallas County, Texas.

Parasite	Site of infection	Number*: adults, immatures	Prevalence (%): adults, immatures
Zoomastigophorea Calkins, 1933			
Monoceromonadidae Honigberg, 1963			
Hexamastix batrachorum	Colon, rectum	36/37, 1/15	97.3, 6.7
Myxosporea Bütschlii, 1881			
Myxidiidae Thélohan, 1892			
Myxidium serotinum	Gall bladder	24/37, 1/15	64.9, 6.7
Sporozoasida Leuckart, 1879			
Eimeriidae Minchin, 1903			
Eimeria ambystomae	Intestinal mucosa, feces	8/37, 3/15	21.6, 20.0
Cyclophyllidea			
Nematotaeniidae Lühe, 1910			
Cylindrotaenia americana	Small intestine	5/37, 0/15	13.5, 0.0

* Number = number infected/number examined.

Clark and Shoemaker (1973) reported *M. serotinum* in 51 of 58 (87.9%) two-lined salamanders (*Eurycea bislineata*) from West Virginia. They further noted that *E. bislineata* appeared to be the normal host for *M. serotinum* while anurans represent secondary or incidental hosts. Apparently, the basis for Clark and Shoemaker's (1973) hypothesis was the higher prevalence of *M. serotinum* in *E. bislineata* and because there were no infections of *M. serotinum* observed in other salamanders, frogs, and toads from the study area. However, on the basis of total number of anuran hosts reported for *M. serotinum,* we disagree with their conclusion. It is apparent that there is little host specificity in amphibians for the myxosporean. In our survey of *A. texanum,* the overall prevalence of *M. serotinum* (Fig. 1) was 48.1% (Table 1) and prevalence was not significantly different among the size classes or sexes of adult *A. texanum,* although only 1 immature *A. texanum* (SVL = 35 mm) possessed *M. serotinum* spores and trophozoites in its gall bladder. A slightly smaller species, *Myxidium immersum* Lutz, 1880, occurs in the gall bladder of frogs of the genera *Bufo, Leptodactylus,* and *Atelopus* in Brazil and Uruguay (Lee, 1985).

Eimeria ambystomae (Fig. 2) was originally described by Saxe (1955) from *A. tigrinum, Desmognathus monticola,* and *D. quadramaculatus* from Iowa. Duszynski et al. (1972) extended the geographical range of the coccidium when they reported it from 17 of 17 (100%) *A. tigrinum* from Colorado and northern New Mexico. The only discrepancy between the 2 accounts is the shape and size of the oocyst residuum. Saxe (1955) described the oocyst residuum from freshly sporulated oocysts as a large, hyaline structure surrounded by small granules. Duszynski et al. (1972), who studied oocysts that were 1–24 mo old, reported the residuum as composed of numerous scattered granules and hypothesized that the residuum may have become dispersed as oocysts aged. We examined oocysts from the 8 infected adult *A. texanum* (5 females, 3 males; 72.8 ± 3.4, 54–82 mm) that ranged in age from 1 wk to 4 mo and can confirm this hypothesis. Newly sporulated oocysts tended to have a compact residuum whereas older forms had residua that became dispersed throughout the oocyst.

Cyclophyllidean cestodes, identified as *Cylindrotaenia americana* Jewell, 1916, were found in the small intestine of 5 of 52 (9.6%) *A. texanum.* The hosts (4 males, 1 female) ranged in size from 54 to 90 mm SVL ($\bar{x}$ = 72.6 ± 6.3 mm); mean intensity was 9.0 (range 1–15) worms. Also, 1 of the *A. texanum* infected with this tapeworm was passing numerous terminal proglottids and eggs in fecal contents from the rectum. Salamanders from the United States that have been reported to be hosts of *C. americana* include Jordans salamander (*Plethodon jordani*) from North Carolina (Dyer, 1983), seal salamanders (*D. monticola*), mountain dusky salamanders (*D. ochrophaeus*), slimy salamanders (*P. glutinosus*), ravine salamanders (*P. richmondi*), and redback salamanders (*P. cinereus*) from Tennessee (Dunbar and Moore, 1979), and northern dusky salamanders (*D. fuscus fuscus*) from North Carolina (Mann, 1932).

In summary, new host and locality records are reported for 3 species of protozoa and 1 tapeworm found in *A. texanum* collected from a single locality in Dallas County, Texas. Although Landewe (1963) reported *Neodiplostomum* sp. (later identified as *Diplostomulum ambystomae* by Price and St. John [1980]), *Gorgoderina bilobata, Pseudopisthodiscus* sp., *Cosmocercoides dukae,* and *Rhabdias* sp. in smallmouth salamanders from southern Illinois, and Price and St. John (1980) found *D. ambystomae, G. bilobata, Brachycoelium* sp., *C dukae,* and *Rhabdias* sp. in *A. texanum* from Illinois, we recovered none of the above taxa. Several factors may account for the absence of trematodes and nematodes in *A. texanum* in our study, including limited access to suitable molluscan intermediate hosts, geographic location, lack of systematic collections throughout the year, habitat constraints (i.e., drying up of farm pond in summer), and seasonal changes in food habits.

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Research Note

Excystment in the Plerocercus Metacestode of *Otobothrium insigne* (Cestoda: Trypanorhyncha)

MICHAEL B. HILDRETH[1] AND ROBERT R. LAZZARA

Department of Biology, Tulane University, New Orleans, Louisiana 70118

KEY WORDS: blastocyst digestion, blastocyst function.

The physico-chemical factors that cause evagination or excystment of the tapeworm scolex from its metacestode bladder have been studied for several different metacestodes from the order Cyclophyllidea (e.g., cysticercoid and cysticercus metacestodes). Yet, little is known of the factors responsible for evagination or excystment in other types of metacestodes. Because members of the order Trypanorhyncha utilize a very different life cycle than cyclophyllidians, we chose to examine the factors necessary for excystment in the trypanorhynch metacestode (termed a plerocercus) of *Otobothrium insigne* Linton, 1905.

The adult of *O. insigne* has been reported from the spiral valve of *Carcharhinus obscurus* sharks (Linton, 1905, Bulletin of the Bureau of Fisheries [for 1904] 24:321–428); the plerocercus stage has been reported from the skeletal musculature of *Arius felis* catfish (Hildreth and Lumsden, 1985, Proceedings of the Helminthological Society of Washington 52:44–50). This plerocercus consists of a juvenile scolex surrounded by a blastocyst. The blastocyst consists of a thick outer wall, a fluid-filled blastocyst cavity, and a thin inner wall (Hildreth and Lumsden, 1987, Journal of Parasitology 73:400–410). The juvenile scolex lies within a second cavity, the receptaculum scolecis, formed by the blastocyst's inner wall. Once the plerocercus is transferred to its definitive host, the juvenile scolex must excyst from the blastocyst before it can attach to the spiral value.

We tested 2 digestive agents present in the stomach (low pH and pepsin) and 2 digestive agents present in the spiral valve (trypsin and bile salts) in order to determine if 1 or more of these factors may cause excystment in vitro. Plerocerci were obtained from naturally infected catfish as described by Hildreth and Lumsden (1985,

loc. cit.). The various incubations were conducted at 37°C in fish saline (Hanks' basal salt solution plus 0.3% [w/v] NaCl as recommended by Wolf and Quimby [1969, pages 253–305 *in* W. S. Hoar and D. J. Randall, eds., Fish Physiology, Vol. 3, Academic Press, New York]. Because we were unable to acquire shark pepsin, trypsin, and bile salts, we used commercially prepared mammalian enzymes and bile salts (crystalline porcine pepsin, crystalline bovine trypsin, and porcine bile salts; from Sigma Chemical Co.). In the absence of available data on concentrations of trypsin and bile salts in the spiral valve of sharks, we used concentrations (0.5% trypsin and 0.3% bile salts [w/v]) found to be optimum for scolex evagination in hymenolepid cysticercoids (Rothman, 1959, Experimental Parasitology 8:336–364).

The results of the excystment study are summarized in Table 1. A 2.0% solution of pepsin at a pH of 2.0 failed to excyst any of the scoleces; however, a 0.5% solution of trypsin (pH 7.6) produced excystment of all the scoleces within 90 min. The addition of bile salts into the trypsin solution decreased the time needed for 100% excystment from 90 min to 60 min. Excystment did not occur in control plerocerci incubated in fish saline at a pH of either 2.0 or 7.6.

The process of excystment in the trypsin/bile salt-treated plerocerci initially involves partial

[1] Present address: Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin 53706.

Table 1. Effect of digestive agents on excystment of *Otobothrium insigne* plerocerci.

"Digestive" conditions	% excysted at each time period			
	30 min	60 min	90 min	120 min
2.0% Pepsin at pH 2.0 (N = 25)	0	0	0	0
0.5% Trypsin at pH 7.6 (N = 15)	0	27	100	100
0.5% Trypsin and 0.3% bile salts at pH 7.6 (N = 25)	52	100	100	100

Table 2. Effect of pH on juvenile scolex viability of *Otobothrium insigne.*

Scolex condition*	pH	% viability at each time period			
		30 min	60 min	90 min	120 min
Manually excysted	2.0	0	0	0	0
Manually excysted	2.5	100	40	40	20
Within blastocyst	2.0	100	100	100	100

* N = 25 scoleces/group.

digestion of the blastocyst outer wall; the juvenile scolex then eventually penetrates through the inner wall and weakened portions of the outer wall. Histological observations of paraffin sections from pepsin/HCl-treated, trypsin/bile salt-treated, and untreated plerocerci showed that the pepsin/HCl solution caused no apparent change in the blastocyst wall; in contrast, the trypsin/bile salt solution digested away areas of the blastocyst-wall tegument and portions of the subtegumental muscle.

We also tested the ability of juvenile scoleces to survive pH values approximating those found in the shark stomach (i.e., pH less than 2.5; Williams, 1971, Symposia of the British Society for Parasitology 8:43–77). Plerocerci and manually excysted juvenile scoleces were incubated at 18°C in fish saline with pH values of 2.0 and 2.5 (pH adjusted with HCl). Results for this study are summarized in Table 2. None of the juvenile scoleces that lacked blastocysts survived the 30-min incubation at a pH of 2; a few survived for 2 hr at a pH of 2.5. One hundred percent of the blastocyst-enclosed scoleces survived the 2-hr incubation period at pH 2. These scoleces were then transferred to fish saline and removed from their blastocysts; all but 3 of these scoleces remained viable after 30 days in fish saline plus a mixture of amino acids (20 ml/liter; 50× MEM Essential Amino Acid Solution; Grand Island Biological Co.).

Because pepsin/pH 2 treatment does not cause excystment of the scoleces in vitro, and because manually excysted scoleces do not survive in a low pH in vitro, we speculate that in vivo excystment occurs after the plerocerci leave the stomach. The ability of trypsin/bile salt treatment to digest the blastocyst outerwall and thereby cause excystment additionally suggests that excystment occurs in the spiral valve. We also speculate that one function of the blastocyst is to shield the juvenile scolex from the acidic environment of the shark's stomach during the scolex's passage to the spiral valve.

Research Note

Observations on the Surface of *Taenia solium* Following Treatment with Niclosamide

Paz María Salazar-Schettino,[1] Irene de Haro Arteaga,[1] Marietta Voge,[2] and Adela Ruiz Hernández[1]

[1] Departmento de Ecología Humana, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510 México, D. F. and
[2] Deceased, School of Medicine, University of California, Los Angeles

KEY WORDS: Cestoda, treatment, scanning electron microscopy.

Studies on the effect of niclosamide on cestodes have shown that the effect of the administration of a curative dose produces the partial digestion of scolex and proglottids (Goodman et al., 1980, The Pharmacological Basis of Therapeutics, MacMillan, New York, 1,019 pp.). Histological studies of *Taenia solium* proglottids after exposure to niclosamide have revealed vacuolization of the segments (Martínez et al., 1971, Revista de Investigacion en Salud Publica, Mexico 31:152–162). Vacuolization of the te-
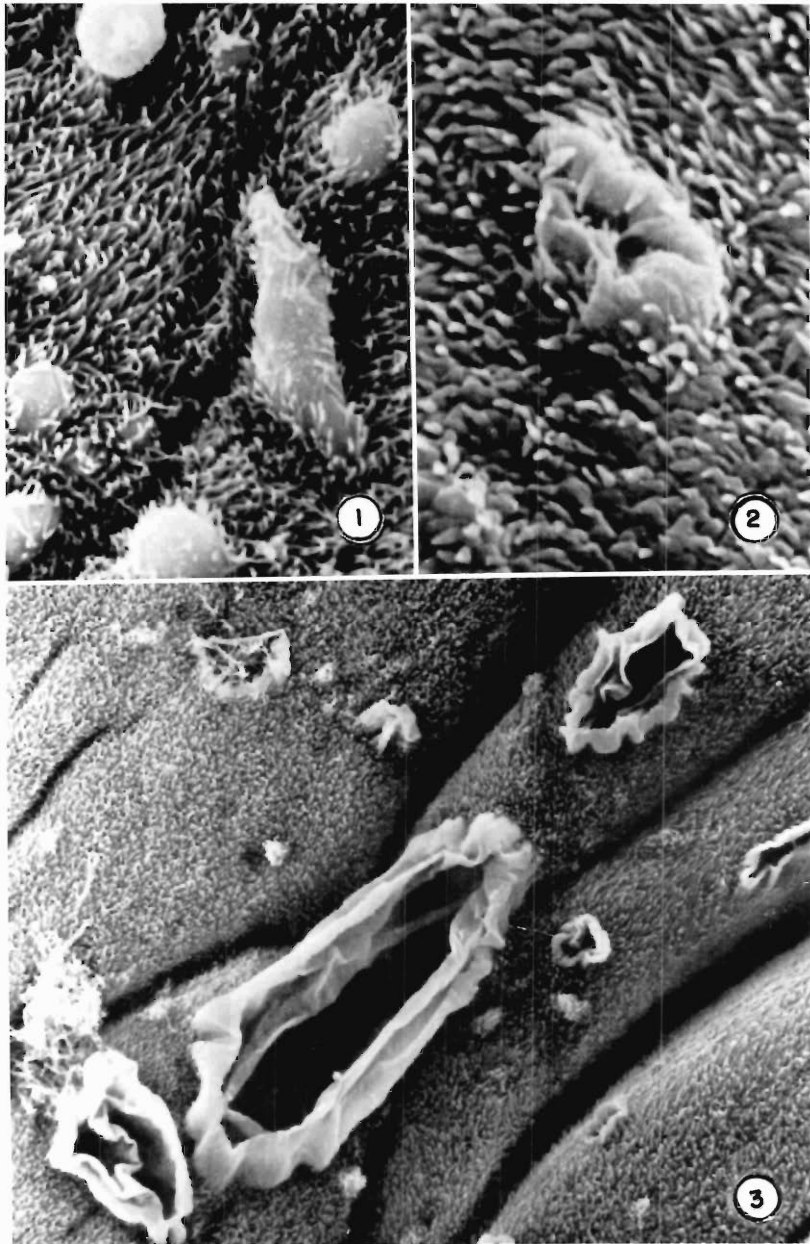
Figures 1–3. Effect of niclosamide on microtriches. 1. Lifting of superficial layer of tegument with bubble formation and attached host cell (at top) (×4,000). 2. Microtriches within bubble (×8,000). 3. Rupture of bubbles, resulting in small or large craters on the surface (×1,600).

gument of *Hymenolepis nana* after treatment with praziquantel and major effects on the scolex and early segments, without apparent effect on gravid segments, was described by Becker et al. (1980, Zeitschrift für Parasitenkunde 61:121–133).

In the present study the normal surface of im-

mature, mature, and gravid proglottids of *T. so-lium* are described, and the changes observed with niclosamide treatment are reported.

An untreated patient, infected with *T. solium*, spontaneously passed gravid proglottids, which were washed briefly in 0.85% NaCl solution and

fixed in cacodylate-buffered 2% glutaraldehyde solution. The patient was then treated with a single 2-g dose of niclosamide (Yomesan).

The worm was eliminated by the patient 8 hr after the administration of the drug. The cestode included immature, gravid, and semigravid proglottids. The proglottids were washed in saline solution, fixed as above, and processed for scanning electron microscopy as described by Voge et al. (1978, Journal of Parasitology 64: 368–372).

The observed damage to the scolex caused by the drug was: (1) The scolex could not be found, apparently being completely destroyed. (2) In immature proglottids, host cells were observed on the surface, as well as a lifting of the worm's surface layer, with numerous blebs of variable size, which had lost their microtriches (Fig. 1). Some of these blebs were involuted and collapsed (Fig. 2). Eventually, the blebs ruptured (Fig. 3), forming small or large craters.

Niclosamide induces drastic changes in the surface of *T. solium.* We observed or demonstrated that the most pronounced effects are seen in immature segments, with a progressive disappearance of the microtrichal layer, which lifts from the surface and then peels off. The disappearance of this layer is more extensive on the lateral edges of the segments, and it is possible that the gravid segments, which normally lose the microtrichal layer, are not measurably affected by niclosamide, and that the eggs within the gravid segments remain viable.

Research Note

In Vitro Maintenance of the Pentastome *Sebekia mississippiensis*

W. M. Boyce,[1] C. H. Courtney, S. R. Wing, and E. W. Kurose
Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610

KEY WORDS: Pentastomida, culture methods, *Alligator mississippiensis,* mosquitofish, *Gambusia affinis.*

Adults of the pentastome *Sebekia mississippiensis* occur in the lungs of American alligators (*Alligator mississippiensis*) and shed eggs that are passed with the feces (Deakins, 1971, Journal of Parasitology 57:1197). Nymphs have been reported from a variety of hosts (reviewed by Overstreet et al., 1985, Proceedings of the Helminthological Society of Washington 52:266–277) and hamsters, rats, and turtles have been shown to serve as paratenic hosts under experimental conditions (Boyce, 1985, Proceedings of the Helminthological Society of Washington 52:278–282). As with many other pentastomes, the life cycle of *S. mississippiensis* is incompletely known, in part because of the difficulty of maintaining live specimens in the laboratory. This report describes the use of 2 simple in vitro maintenance techniques for nymphs and adults of *S. mississippiensis.*

Nymphs and adults of *S. mississippiensis* were obtained by dissection of naturally infected mosquitofish (*Gambusia affinis*) and alligators, respectively. Two different culture systems were tested: (1) Eagle's minimum essential medium with Earle's salts and 10% fetal calf serum (MEM, Grand Island Biological Co., New York), and (2) Dulbecco's modified Eagle's medium without

[1] Correspondence address: Department of Veterinary Microbiology, Pathology, and Public Health, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907.

serum (DMEM, GIBCO). Physiologic saline (0.85% NaCl) was utilized as a control medium for maintaining nymphs and eggs. Penicillin (10,000 IU/mml) and streptomycin (10,000 μg/ml) were included in both systems (MEM and DMEM) and cultures were maintained at 30°C in 5% CO_2 and the medium changed weekly.

Nymphs maintained in either MEM with serum or DMEM without serum remained viable and infective to hamsters or mice for at least 6 wk. Nymphs maintained in physiologic saline at 30°C died over a 1-wk period. Adult females survived for similar periods of time in both MEM and DMEM (3–6 wk) and produced from 75 to 4,200 eggs/wk in MEM. Egg production was not assessed in DMEM nor were sufficient adults available for control cultures in saline. Adults were more difficult to maintain than nymphs, possibly because they were removed from the lungs of alligators that had died several hours earlier as opposed to nymphs that were recovered by dissection of freshly killed mosquitofish. However, the possibility that adults are more fastidious in their culture requirements than nymphs was not ruled out.

Eggs deposited in vitro contained a quadruped larva and the morphology of both the egg and larva strongly resembled that described by Esslinger for *Porocephalus crotali* (1962, Journal of Parasitology 48:457–462). Eggs kept in saline at 4°C contained live larvae for periods as long as 2 mo. Attempts to infect hatchling alligators with eggs obtained from in vitro cultures were unsuccessful.

These relatively simple culture techniques have allowed us to maintain both nymphs and adults of *S. mississippiensis* and to obtain large numbers of eggs and larvae. Exploitation and modifications of these methods may provide other workers with a tool for further investigations of *S. mississippiensis* as well as other species of pentastomes.

Research Note

Trematodes of Cuban Brown Anoles, *Anolis sagrei sagrei,* from Florida

LaRue George Sellers and Genie Graham
Santa Fe Community College, Unit 2 A 31, 3000 N.W. 83 Street, Gainesville, Florida 32602

KEY WORDS: *Urotrema scabridum, Mesocoelium monas,* new locality record.

King and Krakauer (1966, Quarterly Journal of the Florida Academy of Sciences 29:144–154) reported that the Cuban brown anole, *Anolis sagrei sagrei* Dumeril and Bibron, was an accidental introduction into Florida through 3 ports—Key West prior to 1931; Port of Palm Beach, 1960; Port Everglades (Broward County), 1964. Since those introductions, it has rapidly increased its range to become one of the most abundant reptiles in south Florida (King and Krakauer, 1966, loc. cit.). Although it is a very common species throughout its range, only 2 studies have been conducted to determine to what extent it is parasitized by intestinal helminths. Otero (1970, Ciencias 4:1–51) examined 21 *A. s. sagrei* collected in Cuba and found 2 nematodes, *Cyrtosomum scelopori* Gedoelst, 1919, and *Skrjabinoptera phrynosoma* (Ortlepp, 1922), and 2 trematodes, *Urotrema scabridum* Braun, 1900, and *U. wardi* Perez Vigueras, 1940. Price and Underwood (1984, Florida Scientist 47:205–207) examined 100 anoles collected in residential areas of Tampa, Florida, and reported 2 nematodes, *Physaloptera squamatae* Harwood, 1932 and *C. scelopori,* and 1 trematode, *Mesocoelium monas* (Rudolphi, 1819) Freitas, 1958.

Eighty-two adult Cuban brown anoles, *Anolis*

Locality*	Hosts examined/ hosts infected	Trematodes	Number of worms recovered
Broward	2/1	*Urotrema scabridum*	2
Dade	17/4	*U. scabridum*	44
Lee	2/0		
Monroe	22/0		
Polk	4/1	*U. scabridum*	1
Sarasota	35/3	*Mesocoelium monas*	4
		U. scabridum	1

* Counties in Florida.

sagrei sagrei, were examined for intestinal platy-helminths between August 1977 to March 1986. All anoles were collected alive by hand from 6 Florida counties, and necropsied within a week after capture. Trematodes were fixed in luke-warm alcohol–formalin–acetic acid (AFA) with slight coverslip pressure, stained in Semichon's carmine or Harris' hematoxylin, dehydrated in ethanol, cleared in xylene, and mounted in Kleer-mount. Two species of trematodes were recovered; 1 a new locality record (Table 1).

Forty-eight specimens of the digenetic trematode *Urotrema scabridum* representing the first report for *Anolis sagrei sagrei* from the United States were removed from the small intestines of 7 anoles. Measurements and morphology of these worms correspond with those given by Otero (1970, loc. cit.). *Urotrema* spp. are normally parasites of bats. *Anolis carolinensis* and *A. s. sagrei* represent the reptilian hosts for *Urotrema* spp. (Otero, 1970, loc. cit.). Four specimens of *Mesocoelium monas* were recovered from the small intestines of 2 *A. s. sagrei*. Measurements and morphology of these specimens agree with the redescription presented by Nasir and Diaz (1971, Rivista di Parassitologia 32:149–158). This is the second report of *M. monas* parasitizing a Cuban brown anole. It should also be noted that all specimens of *U. scabridum* and *M. monas* contained numerous ova. Representative specimens of *U. scabridum* (No. 79345) and *M. monas* (No. 79344) have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705.

We thank Grady Knight and Shawn Mack for help in collecting anoles.

Research Note

Excystation of *Echinostoma revolutum* Metacercariae (Trematoda) in the Domestic Chick

BERNARD FRIED AND KARA KLETKEWICZ
Department of Biology, Lafayette College, Easton, Pennsylvania 18042

KEY WORDS: *Gallus domesticus,* in vivo excystation, metacercarial cysts, echinostomes.

Although information is available on chemical excystation of *Echinostoma revolutum* metacercarial cysts (Fried and Butler, 1978, Journal of Parasitology 64:175–177), there are no detailed studies on the in vivo excystation of this parasite in the domestic chick. This note reports our observations on in vivo excystation of *E. revolutum* metacercariae in the domestic chick.

Encysted metacercariae were obtained from the kidneys of experimentally infected *Physa heterostropha* snails as described in Fried and Weaver (1969, Proceedings of the Helminthological Society of Washington 36:153–155) and fed by pipet approximately 400 per chick in 3% $NaHCO_3$ to each of 14, day-old unfed domestic White Leghorn chicks. Groups of 2 chicks each were necropsied at 0.25, 0.5, 1.0, 2.0, 3.0, 6.0, and 24 hr postinfection and the number of encysted and excysted metacercariae in the gizzard, upper

Table 1. Number (%) and location of encysted (EN) and excysted (EX) metacercariae of *Echinostoma revolutum* recovered from 14 chicks,* each fed approximately 400 cysts.

Group	Time in hours	Metacer-cariae	Gizzard	Upper ileum	Lower ileum	Rectum	Total
A	0.25	EN	170 (21.3)	5 (0.6)	0	0	175 (21.9)
		EX	0	0	0	0	0
B	0.5	EN	160 (20.0)	2 (0.3)	0	0	162 (20.3)
		EX	0	3 (0.4)	15 (1.8)	2 (0.3)	20 (2.5)
C	1.0	EN	75 (9.3)	2 (0.3)	0	8 (1.0)	85 (10.6)
		EX	0	8 (1.0)	147 (18.4)	0	155 (19.4)
D	2.0	EN	63 (7.9)	5 (0.6)	1 (0.1)	0	69 (8.6)
		EX	0	2 (0.3)	107 (13.3)	0	109 (13.6)
E†	3.0	EN	80 (10.0)	2 (0.3)	2 (0.3)	0	84 (10.6)
		EX	0	0	107 (13.3)	4 (0.5)	114 (14.2)
F	6.0	EN	81 (10.1)	0	0	0	81 (10.1)
		EX	0	3 (0.4)	85 (10.6)	0	88 (11.0)
G	24.0	EN	0	0	0	0	0
		EX	0	0	88 (11.0)	2 (0.3)	90 (11.3)

* Two chicks at each time period.
† Three excysted metacercariae recovered from the ceca of 1 host at 3 hr postinfection.

ileum, lower ileum, ceca, and rectum was counted. Relatively few metacercariae were found in the crop, proventriculus, and duodenum and data from these sites were not tabulated.

The results of the experiment are presented in Table 1. Of the 5,600 cysts fed to all chicks, a total of 1,232 (22%) were recovered as either encysted or excysted metacercariae; of the 1,232 metacercariae recovered, 656 (53.2%) were encysted and 576 (46.8%) excysted. There was considerable variation in recovery between groups, and total recovery ranged from a high of 240/800 or 30% in Group C to a low of 90/800 or 11.3% in Group G. Only organisms fully emerged from all cyst walls were scored as excysted.

More cysts were in the gizzard at 0.25 and 0.5 hr (Groups A, B) than at any other time. Excysted metacercariae were first seen in the upper ileum at 0.5 hr (Group B). However, from 0.5 to 24 hr (Groups B–G), most excysted metacercariae were in the lower ileum. Excysted metacercariae were in the rectum at 0.5 hr (Group B) and encysted metacercariae were there by 1 hr (Group C). Some of these organisms in the rectum were undoubtedly eliminated during defecation, since intestinal emptying is very rapid in the chick. Macy, Berntzen, and Benz (1967, Journal of Parasitol-

ogy 54:28–38) reported that total intestinal emptying time for the domestic chick was 1.15 hr. Excysted metacercariae were recovered from the ceca of one host (Group E) at 3 hr postinfection which was not unexpected since adults of *E. revolutum* have been reported previously from the cecum of the domestic chick (Fried, 1984, Journal of Helminthology 58:241–244).

To test cyst viability, some organisms were removed from the gizzard at 1–6 hr postinfection and placed in the trypsin–bile salts excystation medium of Fried and Butler (1978, loc. cit.) at 41°C. Most cysts removed from the gizzard at 1 and 2 hr excysted in the medium within 10 min. Most cysts removed from the gizzard at 3 and 6 hr were opaque, and did not excyst in the medium.

In conclusion, excystation can occur within 0.5 hr in the lower ileum of the domestic chick. The lower ileum is a preferred site of *E. revolutum* in the domestic chick. Cysts retained in the gizzard for more than 3 hr are adversely affected, presumably by the acidic environment of that organ or by other factors such as mechanical disruption.

Research Note

Helminth Parasites of the Cave Salamander, *Eurycea lucifuga,* from Western Kentucky

MARC D. CASTLE,[1] DOMINIC A. STROHLEIN,[2] AND BRUCE M. CHRISTENSEN[1]

[1] Department of Veterinary Science, 1655 Linden Drive, University of Wisconsin,
Madison, Wisconsin 53706 and
[2] Southeast Cooperative Wildlife Disease Study, College of Veterinary Medicine, Department of Parasitology,
University of Georgia, Athens, Georgia 30602

KEY WORDS: Trematoda, Nematoda, prevalence, intensity, Amphibia, Plethodontidae.

The cave salamander, *Eurycea lucifuga* Rafinesque, 1822 (Plethodontidae), is found from western Virginia in the east to eastern Oklahoma in the west, and from central Indiana southward to northern Georgia. Although occurring most often in the twilight zone of limestone caves, the cave salamander also can be found in the entrance and dark zones (Hutchison, 1958, Ecological Monographs 28:1–21). Studies on helminths of cave salamanders have been limited in number. Prior to this study, 296 cave salamanders from 3 surveys had been examined for helminth parasites (Landewe, 1963, Master of Science Thesis, Southern Illinois University at Carbondale, 47 pp.; Dyer and Brandon, 1973, Transactions of the Illinois Academy of Science 66:23–29; Dyer and Peck, 1975, Canadian Journal of Zoology 53:52–54).

Salamanders were collected from 2 limestone caves, Rimcrest (RMCR) and Taylor Bluff (TBFC) in Trigg County, Kentucky, from October of 1978 through October of 1979. Hosts were captured by hand, placed in moist plastic bags, and transported to the laboratory, where they were refrigerated (4°C) until killed and necropsied (within 3–6 hr). Salamanders were killed in 17% isopropyl alcohol and the snout–vent length (SVL) was measured to the nearest 0.1 mm. Digestive tracts were removed and separated into esophagus, stomach, small intestine, large intestine, and cloaca, placed into physiological saline (0.65% NaCl), and examined with the aid of a stereomicroscope. Nematodes were fixed in hot 70% glycerin–alcohol, and trematodes were fixed in hot AFA and then stained with Mayer's paracarmine. Representative specimens were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705, USA) as USNM Coll. Nos. 79486–79490.

Prevalence data were analyzed using Chi-square analysis of 2×2 contingency tables and intensity data by Student's *t*-test and ANOVA. Regression analysis was used to ascertain any correlation between intensity of infection and size of the host. These tests were part of statistical packages for a Hewlett-Packard HP 86 microcomputer system. Differences were considered significant at $P < 0.05$.

Seventy-four adult *Eurycea lucifuga* ranging in size from 43–71 mm (SVL) were collected from 2 caves in Trigg County, Kentucky. Five species of helminths were collected, and included: *Brachycoelium salamandrae* (Froelich, 1789); *Capillaria inequalis* Walton, 1935; *Oswaldocruzia pipiens* Walton, 1929; *Thelandros magnavulvaris* (Rankin, 1937) Schad, 1960; and *Trichoskrjabinia* sp. Travassos, 1937. Prevalence and mean intensity of the recovered helminths are presented in Table 1. Nematodes were the most commonly encountered helminth, with at least 1 species found in 77% of the hosts, 28% were infected with 2 species, and 4% harbored 3 species of nematodes. With the exception of *Trichoskrjabinia* sp., which marks a new host record for the genus and will be described elsewhere, all recovered helminths have been found previously in *E. lucifuga.*

Several significant differences occurred in the helminth fauna of the salamanders from the 2 caves. The nematode population of salamanders from RMCR was dominated by *C. inequalis,* whereas no nematode species dominated the helminth fauna of salamanders from TBFC. With the exception of *C. inequalis,* significant differences were seen in the prevalence with all recovered nematodes from the 2 caves. Although salamanders from TBFC exhibited a consistently heavier mean intensity of infection, only the difference in intensity of *C. inequalis* was statistically significant.

Comparisons of mean intensities between in-

269

Table 1. Prevalence and mean intensity of gastrointestinal helminths collected from cave salamanders (*Eurycea lucifuga*) from Rimcrest Cave (RMCR) and Taylor Bluff Cave (TBFC) in Trigg County, Kentucky, 1978-1979.

| Parasite | Cave site | % prevalence (mean intensity*; range) | | |
		Males $N = 41$	Females $N = 33$	Total $N = 74$
	RMCR†	7 (1; 1)	26 (2; 1–4)	15 (2; 1–4)
	TBFC‡	25 (2; 1–4)	0	12 (2; 1–4)
Brachycoelium salamandrae	Total	12 (2; 1–4)	15 (2; 1–4)	14 (2; 1–4)
	RMCR	59 (3; 1–7)	63 (4; 1–18)	60 (3; 1–18)
	TBFC	33 (11; 3–24)	21 (1; 1–2)	27 (7; 1–24)
Capillaria inequalis	Total	51 (4; 1–24)	45 (3; 1–18)	49 (4; 1–24)
	RMCR	17 (2; 1–4)	0	6 (2; 1–4)
	TBFC	42 (6; 1–11)	64 (5; 1–9)	46 (5; 1–11)
Oswaldocruzia pipiens	Total	24 (4; 1–11)	29 (5; 1–9)	26 (4; 1–11)
	RMCR	14 (2; 1–3)	5 (3; 3)	10 (2; 1–3)
	TBFC	0	0	0
Thelandros magnavulvaris	Total	10 (2; 1–3)	3 (3; 3)	7 (2; 1–3)
	RMCR	7 (4; 4)	0	4 (4; 4)
	TBFC	67 (9; 1–23)	64 (7; 1–14)	65 (8; 1–23)
Trichoskrjabinia sp.	Total	24 (8; 1–23)	29 (7; 1–14)	26 (7; 1–23)

* No. parasites/infected host; if ≥ 0.5, then rounded to next highest integer.

† Twenty-nine males, 19 females.

‡ Twelve males, 14 females.

fected males from the 2 caves showed significant differences of both *C. inequalis* and *O. pipiens,* with males of TBFC having the greater mean intensity (Table 1). No significant differences of mean intensity were seen between infected female salamanders, but females from the 2 caves were very different in prevalence of all helminths, particularly with *O. pipiens* and *Trichoskrjabinia* (Table 1). No correlations between size of the hosts and infection by any parasite species were evident.

Comparisons of these findings with those of the studies mentioned already show a much greater prevalence of helminths in the salamanders from our 2 cave sites. Landewe (1963, loc. cit.) reported *B. salamandrae* and *Oswaldocruzia* sp. in 21% of 24 hosts. Dyer and Brandon (1973, loc. cit.) found *Brachycoelium* sp. in only 6% of 17 hosts. Dyer and Peck (1975, loc. cit.) reported *B. salamandrae* (9.4%), *C. inequalis* (5.1%), *O. pipiens* (9.8%), and *T. magnavulvaris* (0.8%) from

255 hosts. One possible reason for this discrepancy is that, in our study, a far greater number of salamanders were taken from one site rather than a few salamanders taken from many cave sites, as in the study of Dyer and Peck (1975, loc. cit.). Consequently, a truer evaluation of the helminth fauna of the salamanders within one area might be achieved through a greater sample size.

One point of interest concerning *Thelandros magnavulvaris* is that all specimens were males, and were keyed to species using the information supplied by Schad (1963, Canadian Journal of Zoology 41:943–946), where *T. magnavulvaris* and *T. salamandrae* are separated on the basis of spicule length.

Research Note

Protozoan and Metazoan Parasites of Strecker's Chorus Frog, *Pseudacris streckeri streckeri* (Anura: Hylidae), from North-Central Texas

CHRIS T. MCALLISTER

Renal-Metabolic Lab (151-G), Veterans Administration Medical Center,
4500 S. Lancaster Road, Dallas, Texas 75216

KEY WORDS: Cyclophyllidea, *Mesocestoides* sp., *Myxidium serotinum, Nyctotherus cordiformis, Opalina* sp., *Oswaldocruzia* sp., prevalence, tadpoles, tetrathyridia, trophozoites.

Strecker's chorus frog (*Pseudacris streckeri streckeri*) is a robust toadlike hylid whose range extends from north-central Oklahoma and western Arkansas south through Texas to the Gulf of Mexico. Much information is available on the ecology of this anuran (see Smith, 1966, Catalogue of American Amphibians and Reptiles 27.1–27.2, for account); however, little is known regarding its endoparasites. In an annotated record of parasites of the Hylidae, Walton (1946, Journal of Parasitology 32[Suppl.]:19) listed 2 species of Protozoa from *P. s. streckeri*. Also, Walton (1947, Transactions of the Illinois State Academy of Science 40:205–214) summarized the known parasites from North American *Pseudacris* spp. This paper presents information from a survey that examined a large sample of *P. s. streckeri* from 1 locality in north-central Texas to determine the prevalence and identity of parasites.

Forty-two adult chorus frogs (35 males, 7 females; $\bar{x} \pm$ SEM snout–vent length (SVL) = 40.6 ± 0.3 mm, range 38–46 mm) were collected from a temporary pond on 9 December 1985 in Dallas County, Texas, 2.4 km west of DeSoto off FM 1382. In addition, various stages of tadpoles and metamorphosing *P. s. streckeri* ($N = 10$) were collected on 12 March 1986 from the same locale and examined for parasites. Frogs were anesthetized with a 0.2% solution of ethyl-m-amino-benzoate (tricaine methanesulfonate, Sigma Chemical Company, St. Louis, Missouri) and blood was obtained by cardiac puncture, then stained with Giemsa following conventional methods for examination of intraerythrocytic hematozoa. A portion of the gastrointestinal tract and feces from the cecum and colon were placed in vials containing standard hard water plus 1% penicillin–streptomycin for isolation of coccidian oocysts. Thin smears of intestinal scrapings were fixed in warm Schaudinn's fluid and stained with Gomori trichrome for examination of intestinal protozoans. Bile contents from the gall bladder were treated in a similar manner. Tissues that appeared to be infected with encapsulated helminths were fixed in AFA, sectioned at 8 μm, and stained with Harris' hematoxylin and eosin counterstain. A single nematode was recovered, fixed in AFA, and examined as a temporary mount in glycerol.

One or more species of endoparasites were found in 51 of the 52 (98.1%) *P. s. streckeri* examined (Table 1). Hematozoa or coccidia were absent. New host records are reported for *Myxidium serotinum* Kudo and Sprague, 1940, *Mesocestoides* sp. Vaillant, 1863, and *Oswaldocruzia* sp. Travassos, 1917. All but 1 of the frogs were found to be infected with opalinids. Metcalf (1923, Bulletin of the United States National Museum 120:1–484) found the endocommensal *Opalina chorophili* in *Chorophilus ornatus* (=*P. s. streckeri*) from Cooke County, Texas. In addition, Walton (1946, loc. cit.) reported *Nyctotherus cordiformis* Ehrenberg, 1838, in Strecker's chorus frog. Metcalf (1940, Proceedings of the United States National Museum 87:465–634) provided a detailed summary of opalinids from anuran hosts.

Shaw (1967, Journal of Parasitology 14:38) reported that 50% and 90–100% of several species of amphibians from northern Minnesota are infected with *Nyctotherus* sp. and *Opalina* sp., respectively. Further, Evans et al. (1977, Proceedings of the West Virginia Academy of Sciences 49:23–24) noted that 88% of 5 species of frogs from West Virginia harbor infections of *O. ranarum* Metcalf, 1923. The present survey reports similar trends in prevalence for these taxa from the *P. s. streckeri* of north-central Texas.

271

Table 1. Parasites found in *Pseudacris streckeri streckeri* from Dallas Co., Texas.

Parasite	Site of infection	Number* adults; tadpoles	Prevalence (%) adults; tadpoles
Protozoa:			
Opalinida Poche, 1913			
Opalinidae Claus, 1874			
Opalina sp.	Colon	41/42; 10/10	97.6; 100.0
Heterotrichida Stein, 1854			
Plagiotomidae Bütschli, 1885			
Nyctotherus cordiformis	Colon	24/42; 4/10	57.1; 40.0
Myxosporidia Bütschli, 1885			
Myxidiidae Thélohan, 1892			
Myxidium serotinum	Gall bladder	30/42; 2/10	71.4; 20.0
Cestoidea:			
Cyclophyllidea Braun, 1900			
Mesocestoididae Perrier, 1897			
Mesocestoides sp.	Coelom, intestinal wall, liver, muscles, mesonephros	3/42; 0/10	7.1; 0.0
Nematoda:			
Strongylida Diesing, 1851			
Trichostrongylidae Leiper, 1912			
Oswaldocruzia sp.	Small intestine	1/42; 0/10	2.4; 0.0

* Number = number infected/total examined.

Specific identification of *Opalina* sp. Purkinje and Valentin, 1840, from *P. s. streckeri* was not attempted; rather, I follow the advice of Metcalf (1909, Archiv für Protistenkunde 13:195–375) and recent suggestions by Sandon (1976, Transactions of the American Microscopical Society 95:357–366) who noted that identifications of *Opalina* sp. should be based on a series of infections and a whole range of forms rather than on selected individuals. Since their original descriptions, many species of opalinids have been suppressed or revised due to inadequacy of definition (Earl, 1973, Publicaciones Biologicas Instituto de Investigaciones Cientificas Universidad Autonoma de Nuevo Leon, Mexico 1:25–33).

Kudo (1943, Journal of Morphology 72:263–271) reported that 30 transforming toads (*Bufo* sp.) did not have infections of young *M. serotinum* trophozoites in their gall bladder. However, in the present study, 2 young *P. s. streckeri* (SVL = 19 mm) contained many small (20–30 μm in diameter) unsporulated *M. serotinum* trophozoites in bile smears from the gall bladder. Previously, this myxosporidian has been reported in the gall bladder of southern toads (*B. terrestris*), northern leopard frogs (*Rana pipiens*), southern leopard frogs (*R. sphenocephala*), and green frogs (*R. clamitans*) from the United States

(Kudo, 1966, Protozoology, 5th ed., C. C Thomas Publishers, Springfield, Illinois).

The *Mesocestoides* sp. tetrathyridia from *P. s. streckeri* were present in 3 adult male frogs ($\bar{x}$ SVL = 41.3 ± 1.2 mm, range 39–43 mm). Host response was minimal with little or no inflammatory tissue (Fig. 1). Specific identification of *Mesocestoides* sp. in *P. s. streckeri* is not possible until experimentally obtained adults can be examined from infected definitive hosts. In the life cycle, the first intermediate host of *Mesocestoides* spp. has not been determined although asexual multiplication of tetrathyridia was reported by Hanson and Widmer (1985, Journal of Wildlife Diseases 21:20–24) in prairie rattlesnakes, *Crotalus viridis viridis*. *Mesocestoides* is a common genus of cyclophyllidean tapeworm in the metacestode stage in amphibians (Prudhoe and Bray, 1982, British Museum [Natural History], Oxford University Press, London) and particularly reptiles (Voge, 1953, The American Midland Naturalist 49:249–251; Specht and Voge, 1965, Journal of Parasitology 51:268–272; Telford, 1970, The American Midland Naturalist 83:516–554; Dyer, 1971, Proceedings of the Helminthological Society of Washington 38:256; Mankau and Widmer, 1977, Japanese Journal of Parasitology 26:256–259; Widmer and Hanson, 1983, Journal of Parasitology 69:788–789; Gold-

Figure 1. Encapsulated *Mesocestoides* sp. tetrathyridia in the liver of a Strecker's chorus frog; S = sucker, IH = invaginated holdfast, C = capsule.

berg, 1985, Journal of Wildlife Diseases 21:310–312), which reaches sexual maturity in falconiform birds and various carnivorous mammals (Webster, 1949, Journal of Parasitology 35:83–90; Voge, 1955, University of California Publications in Zoology 59:125–155; James, 1969, Dissertation Abstracts 29:3541-B). Anurans from North America previously reported to be paratenic hosts of *Mesocestoides* spp. include: *R. clamitans* and *R. pipiens* from northwestern Wisconsin (Williams and Taft, 1980, Proceedings of the Helminthological Society of Washington 47: 278) and American toads (*B. americanus*), great plains toads (*B. cognatus*), and *R. pipiens* from northwestern Iowa, southeastern South Dakota, and Minnesota (James and Ulmer, 1967, Journal of Parasitology 53:59).

The only nematode recovered, an adult gravid female *Oswaldocruzia* sp., was found in an adult female *P. s. streckeri* (SVL = 39 mm). Because adult male specimens were not found, specific identification could not be determined. Nematodes of the genus *Oswaldocruzia* in amphibians and reptiles are cosmopolitan in distribution

(Baker, 1978, Canadian Journal of Zoology 58: 1026–1031) and are relatively common helminths of anurans of the western hemisphere. *Oswaldocruzia* spp. have been reported in the western chorus frog (*P. triseriata triseriata*) from southern Ontario, Canada (Baker, 1977, Canadian Journal of Zoology 55:104–109), in upland chorus frogs (*P. t. feriarum*) from Virginia (Walton, 1933, Proceedings of the United States National Museum 82:1–5), in *B. americanus* and *R. pipiens* from southern Ontario (Baker, 1977, op. cit.), in southern cricket frogs (*Acris gryllus*) from Indiana (Walton, 1941, The American Midland Naturalist 25:418–419), and in wood frogs from New York (Harwood, 1932, Proceedings of the United States National Museum 81: 1–71) and southern Ontario (Baker, 1977, op. cit.). In southeastern Texas, Harwood (1932, op. cit.) found *O. pipiens* Walton, 1929, in *B. terrestris,* gulf coast toads (*B. valliceps*), pickerel frogs (*R. palustris*), and *R. sphenocephala* from Houston, Harris County, and in green treefrogs (*Hyla cinerea*) and *R. sphenocephala* from Huntsville, Walker County.

Specimens of *P. s. streckeri* obtained in the present study are deposited in the Arkansas State University Museum of Zoology (ASUMZ 5272–5313). Representative samples of parasites are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 as follows: *Opalina* sp. (USNM 79353); *Nyctotherus cordiformis* (USNM 79355); *Myxidium serotinum* (USNM 79354); *Mesocestoides* sp.

(USNM 79356); *Oswaldocruzia* sp. (USNM 79357).

Research Note

Human Anisakiasis: Two Case Reports from the State of Washington

THOMAS L. DEARDORFF,[1] JEFF ALTMAN,[2] AND CHARLES M. NOLAN[3]

[1] Fishery Research Branch, U.S. Food and Drug Administration, Box 158,
Dauphin Island, Alabama 36528,
[2] Student Health Center and Department of Family Medicine, University of Washington GS-10,
Seattle, Washington 98195 and
[3] Infectious Disease Section, Seattle–King County Department of Public Health,
1200 Public Safety Building, Seattle, Washington 98104

KEY WORDS: *Anisakis,* case reports, nematode, human infections.

The change in U.S. dietary habits to include more raw seafoods is exposing the public to a greater risk of parasitic infection. One such zoonotic disease, transmitted from fish to humans, is anisakiasis, which is acquired by consuming raw or undercooked seafoods and involves the penetration of a larval anisakid nematode into or through the gastrointestinal tract. Of the 2 cases that occurred in Seattle, Washington, 1 represents the first reported occurrence of anisakiasis in the United States in which food served at a restaurant was implicated in the transmission of a larval *Anisakis* sp.

Case Histories

Case 1

A 46-yr-old male awoke on the morning of 12 April 1985 to find 2 larval nematodes wriggling in the posterior oropharynx. The previous evening the patient had eaten smoked salmon and tuna sushi at a restaurant. He experienced no symptoms on the evening following the meal. He coughed-up and manually extricated the helminths. Prior to eating at the restaurant, it had been several weeks since the patient had eaten any seafood products. Histological slides of one nematode showed it to be a third-stage larva belonging to the genus *Anisakis* (U.S. National Museum, Helminthology Collection No. 79656).

Case 2

On 3 December 1981 a 22-yr-old man felt a tickle in the back of his throat and removed a viable 5-cm-long worm. No other symptoms were noted. The patient frequently ate raw tuna, rock cod, and sea bass. He had never observed worms in the flesh of these fish. His last meal of sashimi was 2 wk prior to finding this parasite. Hematologic findings on 9 December 1981 were a total leukocyte count of 7,300, of which 8% were eosinophils. The worm was tentatively identified as the third-stage larva of *Anisakis* sp. by the State Health Department Laboratory. The specimen was not available to confirm this identification. Based on the size of the worm, however,

it seems more likely that it was the third-stage larva of *Pseudoterranova* (=*Phocanema*), which may be 5-cm long, rather than *Anisakis,* which is rarely over 2-cm long.

In recent years, the number of cases of human anisakiasis in the United States has increased. Including these case reports from the Seattle area, at least 37 suspected or confirmed cases of anisakiasis have been reported from the United States and additional human infections are known to have occurred (J. W. Bier, Division of Microbiology, FDA, Washington, D.C., pers. comm.). Where geographical information is known, 32 (86%) of individual infections with a larval *Anisakis* nematode occurred in the western U.S. (including Alaska and Hawaii) and 5 (14%) were from the eastern United States.

The substantially higher number of cases reported from the western United States can be attributed to at least 3 factors: a greater concentration of various ethnic groups (e.g., Eskimos, Japanese, Chinese), who are known to consume raw foods; the recent trend of eating more raw seafoods (e.g., sushi, sashimi, lomi lomi, ceviche), either at home or at restaurants; and the large number of commercially important fishes, infected with larval ascaridoid nematodes, caught in the Pacific ocean (e.g., Myers, 1979, Journal of Food Protection 42:380–384; Dailey et al., 1981, California Fish and Game 67:240-245; Deardorff et al., 1982, Pacific Science 36:187–201). Infected fishes are usually associated with large numbers of marine mammals, which are the definitive host for this nematode. Such large concentrations of marine mammals are found along the U.S. west coast.

This case history represents the first confirmed report in the United States to implicate a restaurant in the transmission of third-stage larval *Anisakis*. It demonstrates that *Anisakis* larvae are capable of surviving some commercial procedures associated with the preparation of these types of foods. Two other human cases have been suspected following meals at different west coast restaurants. One undocumented case involved a Japanese tourist who experienced gastrointestinal upset after eating raw fish; and the other case involved a native Californian, who experienced nausea, vomiting, diarrhea, and pleural effusion following his meal of raw salmon and shellfish (Kobayaski et al., 1985, American Journal of Tropical Medicine and Hygiene 34:310–313). No worms were recovered from the patients in either of these cases.

Public health authorities are concerned about the problem of human infection resulting from parasites in seafood products (e.g., the recent increase in case reports of anisakiasis) and the possible involvement of restaurants in the transmission process. *Anisakis* larvae are sensitive to the temperature extremes of thorough cooking or freezing. Because heating is not always desirable, freezing is presently regarded as the most promising preventive measure (e.g., cost effective, ease of regulation) against infection with anisakid nematodes (Deardorff, 1986, Proceedings of the Eleventh Annual Tropical and Subtropical Fisheries Conference of the Americas, pages 285–291).

There is some confusion in the literature as to a suitable time and temperature relationship to inactivate ascaridoid larvae. The recommended time and temperature found in Japanese and European literature for freezing fish to kill anisakine larvae is −20°C for 24 hr; however, some North American species survive after 52 hr at this temperature (Bier, 1976, Journal of Milk and Food Technology 39:132–137). For example, Jackson and Bier (1981, FDA By-lines, No. 3, pages 152–156) recommended freezing fish intended to be consumed raw or partially cooked to −20°C for 60 hr and Deardorff et al. (1984, Journal of Food Protection 47:49–52) demonstrated that subjecting Hawaiian snappers to at least −20°C for 24 hr and rockfishes to at least −20°C for 120 hr was necessary to inactivate the living anisakines. The safe freezing period appears to vary, based on the product and type of larvae being tested. The effectiveness of commercial and domestic freezing and various time/temperature ratios for killing *Anisakis* larvae in whole salmon and rockfish, fishes that are commonly implicated in the transmission of anisakiasis in the United States, are currently being studied.

Research Note

First Report of *Hedruris siredonis* (Nematoda: Hedruridae) from North American Frogs

Patrick M. Muzzall[1] and Michael R. Baker[2]

[1] Department of Natural Science, North Kedzie Laboratory, Michigan State University,
East Lansing, Michigan 48824 and
[2] Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

KEY WORDS: bullfrog, cold blooded vertebrates, green frog, New Hampshire, *Rana* spp.

Muzzall (1981, Proceedings of the Helminthological Society of Washington 48:91–92) found *Hedruris* sp. attached to the mucosa of the stomachs of green frogs, *Rana clamitans,* and bullfrogs, *R. catesbeiana,* from the Oyster River, New Hampshire. The specimens were not identified to species because the taxonomy of North American *Hedruris* was confused. Recently, Baker (1986, Canadian Journal of Zoology 64:1567–1572) reviewed North American *Hedruris* spp. and demonstrated that there were only 2 valid species, *H. pendula* and *H. siredonis,* occurring in aquatic vertebrates. This note reports that the *Hedruris* in frogs of New Hampshire is *H. siredonis* Baird, 1858, a species hitherto reported only from salamanders. Measurements of *H. siredonis* from green frogs are given.

Frogs were speared from the Oyster River (Strafford County, Durham, New Hampshire) in July and August 1975, brought to the laboratory, and examined within 3 hr. Nematodes were fixed in hot glycerin–alcohol (9 parts 70% ethanol, 1 part glycerin) and cleared for study in glycerin. Voucher specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, No. 79520. The terms prevalence and mean intensity follow the definitions of Margolis et al. (1982, Journal of Parasitology 68:131–133).

The measurements of *H. siredonis* from green frogs (Table 1) are within the known range for the species. Morphologically, the specimens also conform closely to the redescription of *H. siredonis* (Baker, 1986, loc. cit.), with cephalic structures and eggshell morphology being the most easily observed features. Specimens measured were all mature adults and females contained fully developed eggs, suggesting that parasitic existence proceeds normally in green frogs. Collection records show that female *H. siredonis* with fully developed eggs were also present in bullfrogs from the Oyster River.

Seven (41%) of the 17 green frogs and 5 (42%) of the 12 bullfrogs from the Oyster River were infected with *H. siredonis.* The mean intensities ± 1 SD (range in parentheses) of *H. siredonis* in green frogs and bullfrogs were 13.0 ± 6.7 (4–27) and 4.2 ± 2.3 (2–8), respectively. All *H. siredonis* were sexed. In green frogs, 59 (65%) were females and 32 (35%) were males. In bullfrogs, 15 (71%) were females and 6 (29%) were males.

It is clear from the literature that *H. siredonis* is frequently parasitic in salamanders, but its status as a parasite of frogs is unclear. One may postulate that frogs acquired this parasite by eating infected salamanders. However, the prevalences and mean intensities of *H. siredonis* in green frogs and bullfrogs are relatively high, and infections therefore do not appear to be accidental. Also, 11 dusky salamanders, *Desmognathus*

Table 1. Major dimensions of *Hedruris siredonis* Baird, 1858, from *Rana clamitans* of New Hampshire.

	Male	Female
No. of worms	9	10
Total length (mm)	10.7 (8.3–11.8)*	10.3 (7.5–11.6)
Esophagus length (μm)	1,223 (995–1,380)	1,399 (1,200–1,680)
Nerve ring (μm)†	267 (235–290)	310 (265–365)
Excretory pore (μm)†	441 (345–480)	465 (380–610)
Spicules (μm)	219 (194–247)	—
Tail length (μm)‡	426 (340–480)	—
Vulva (μm)§	—	613 (455–715)

* Mean (range).
† Distance from anterior extremity.
‡ Not given for female worms because specimens had a retracted hook.
§ Distance from anus.

fuscus, collected along the edge of the Oyster River in August 1975 where infected frogs were sampled, were negative for *H. siredonis.* This information suggests that transmission of *H. siredonis* to green frogs and bullfrogs in the Oyster River was by the usual cycle observed for *Hedruris,* i.e., by predation on aquatic isopods that were observed to be infected with larval *Hedruris* in the study area (Muzzall, 1981, loc. cit.).

Research Note

Activities of Isocitrate Lyase and Malate Synthase During the Development of Free-living Stages of *Haemonchus contortus* (Nematoda)

MICHAEL J. CARRINGTON,[1] DOUGLAS P. JASMER,[2] AND BRUCE A. MCFADDEN
Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164-4660

KEY WORDS: biochemistry, enzymes of eggs and larvae, glyoxylate cycle, *Ascaris lumbricoides, Caenorhabditis elegans.*

Good evidence indicates that the conversion of lipid to carbohydrate in nematodes depends upon a functional glyoxylate cycle. In 2 nematodes, *Ascaris lumbricoides* and *Caenorhabditis elegans,* the synthesis of carbohydrates during early development is correlated with decreasing levels of lipid and an increase in specific activities of isocitrate lyase and malate synthase, 2 key enzymes of the glyoxylate cycle (Barrett et al., 1970, Comparative Biochemistry and Physiology 35:577–586; Khan and McFadden, 1980, FEBS Letters 115:312–314). Glycogen and trehalose are the major constituents of the synthesized carbohydrate in *A. lumbricoides* (Barrett et al., 1970, loc. cit.).

Although these observations suggest that the glyoxylate cycle and accumulating carbohydrates play a critical role in *C. elegans* during embryogenesis, the importance of this process is not understood. In addition, this pathway may occur in postembryonic stages since glyoxylate cycle enzyme activities increased in first-stage larvae of *C. elegans* after culturing without a bacterial food source (Khan and McFadden, 1982, Experimental Parasitology 54:47–54). Thus, the correlation between developmental stages and the glyoxylate cycle in other nematode species may provide an insight into the role of this pathway in these organisms. Here we report the occurrence of isocitrate lyase and malate synthase in *Haemonchus contortus* throughout the course of free-living development.

Eggs were removed at the morula stage from feces of a monospecifically infected sheep by the sugar flotation technique (Cox and Todd, 1962, Journal of the American Veterinary Medical Association 141:706–709) and were then rinsed in distilled water. Isolated eggs were sterilized by addition of fungizone (2.5 μg/ml) and tetracycline (10 μg/ml) for 6 hr at 30°C, washed with sterile distilled water, plated on a lawn of *E. coli* deficient in isocitrate lyase (Patel and McFadden, 1978, Nematologica 24:51–62), and incubated at 30°C which produced third-stage larvae after 72 hr. Samples of eggs or larvae were harvested by a previously described method (Colonna and McFadden, 1975, Archives of Biochemistry and Biophysics 170:608–619) that reduces background enzyme activities from bacteria to undetectable levels. Eggs and larvae were sonically disrupted in 2 ml of buffer (0.1 M MOPS, pH

Table 1. Isocitrate lyase and malate synthase in *H. contortus* incubated with *E. coli* at 30°C.

Incubation time (hr)	Predominant developmental stage	Specific activity*	
		Malate synthase	Isocitrate lyase
0	Morula	$28.2 \pm 10.8^{a,b}$	12.3 ± 6.2^a
4	Gastrula	35.1 ± 5.1^b	12.1 ± 0.4^a
9	Prehatch larval	33.8 ± 8.9^b	13.4 ± 2.2^a
24	First larval	19.7 ± 5.7^a	17.0 ± 3.5^a
48	Second larval	$28.7 \pm 5.1^{a,b}$	22.4 ± 1.6^b
72	Third larval	$28.2 \pm 3.4^{a,b}$	29.9 ± 4.7^c

* Three replicate samples were measured at each time point. Means ($\pm$SD) that differ significantly ($P < 0.05$), using the least significant difference test, have different superscripts (a, b, or c). Highly significant differences ($P < 0.01$) were detected for isocitrate lyase between: means at time points 0, 4, and 9 hr, and time points 48 and 72 hr; and means at time points 24 and 48 hr, and time point 72 hr.

7.6, containing 5 mM $MgCl_2$ and 1 mM EDTA) containing glass beads in a glass vessel immersed in ice. Sonic treatment was conducted at a power setting of 4.5 for eggs and first- and second-stage larvae or 7 for third-stage larvae, by using a microprobe coupled to a transducer (Heat Systems, Ultrasonics, Inc.). Suspensions were then centrifuged at 10,000 g for 20 min at 2°C prior to assay of the supernatant.

For isocitrate lyase measurements, 200-μl samples were incubated with 100 μl of 20 mM D,L-isocitrate for 60 min at 30°C and the glyoxylate measured by a modification of the method of McFadden (1969, Methods in Enzymology 13:163–170) after quenching the reaction by addition of 100 μl of 1 M oxalic acid. One hundred μl of 3.3% phenylhydrazine-HCl was then added and the mixture shaken and incubated at ambient temperature for 10 min. Next 0.55 ml of concentrated HCl and 100 μl of 16.7% $K_3Fe(CN)_6$ were sequentially added, the solution was thoroughly mixed, and the absorbance at 535 nm measured after 15 min. For malate synthase activity, the glyoxylate-dependent liberation of CoASH from acetyl CoA was measured at 30°C by assaying the stoichiometric formation of 2-nitro-4-thiobenzoate from DTNB after quenching the reaction (Colonna and McFadden, 1975, loc. cit.). Protein in extracts was determined using the Folin method (Lowry et al., 1953, Journal of Biological Chemistry 193:265–275). For both enzymes, the specific activity is given as munits/mg protein, where a milliunit is that

amount of enzyme catalyzing the production of 1 nanomole of product per minute at 30°C.

Enzyme activities were generally too low to measure in assays lasting less than 1 hr. To determine the proportion of each enzyme which is inactivated during the assay, samples were assayed after 1 and 2 hr. The percentage of the first activity remaining after 2 hr was 9.5 $\pm$ 3.1% ($N = 12$) for malate synthase and 76.0 $\pm$ 15.2% ($N = 12$) for isocitrate lyase. Neither of the observed losses of activities could be accounted for by substrate disappearance. Measurements of both enzymes were routinely conducted after 60-min incubations.

Malate synthase and isocitrate lyase were both detectable in all free-living stages of *H. contortus* (Table 1). The enzyme activities were worm-specific since the supernatant of the final wash contained 3 $\pm$ 2% of the total isocitrate lyase and malate synthase, whereas the pellet containing nematodes (and no detectable bacteria) accounted for 97 $\pm$ 2% of the total activities. The specific activities for these enzymes exhibited differences that were statistically significant during development. For instance, that for malate synthase was relatively high throughout embryogenesis, decreased after hatching of eggs, and returned to a relatively high value in subsequent stages. In contrast, the specific activity for isocitrate lyase was initially relatively low but gradually increased to a maximum level in the third larval stage.

In previous studies the presence of isocitrate lyase in third-stage larvae of *H. contortus* was inferred and malate synthase was not directly detected (Moon and Schofield, 1968, Comparative Biochemistry and Physiology 24:581–590). The results presented here suggest that isocitrate lyase and malate synthase are active throughout all free-living stages of this species. The activity profile of these enzymes during the free-living development of *H. contortus* differs from that of *C. elegans* and *A. lumbricoides*. In both of the latter species, enzyme activity levels are low early in embryogenesis, but rapidly increase as development proceeds. Peak levels are reached about the time of hatching in eggs of *C. elegans* and then decrease in first-stage larvae (Khan and McFadden, 1980, loc. cit.), whereas in eggs of *A. lumbricoides,* peak levels are reached and then decrease to negligible values while larvae are still within the egg (Barrett et al., 1970, loc. cit.). We cannot discount the possibility that malate syn-

thase and isocitrate lyase are initially low during embryogenesis of *H. contortus* because changes in enzyme activity may occur during passage through the alimentary tract of the host. Although the changes in enzyme activity that were observed during the free-living development of *H. contortus* may reflect regulation of the glyoxylate cycle, these changes are minor and whether they represent biologically significant differences requires further study.

The level of activity of glyoxylate cycle enzymes in all free-living stages of *H. contortus* suggests that the importance of this pathway is not restricted to embryonic development. Some evidence suggests that trehalose resulting from the lipid to carbohydrate conversion may protect free-living stages from freezing and desiccating (Ash and Atkinson, 1982, Parasitology 85:LV), and the highest levels of glyoxylate cycle enzyme activities occur in the environmentally resistant stages just prior to hatching of eggs of *C. elegans*

and *A. lumbricoides* (Khan and McFadden, 1980, loc. cit.). In this context, developing egg stages and third-stage larvae of *H. contortus* are resistant to freezing at temperatures as low as −25°C (Jasmer et al., 1986, Proceedings of the Helminthological Society of Washington 53:244–247; Jasmer et al., 1987, Proceedings of the Helminthological Society of Washington 54:48–52). However, a strong connection between the glyoxylate cycle and environmentally resistant nematode stages remains to be demonstrated. It is also possible that the function of the glyoxylate cycle differs among the various nematodes in which it is active. Additional studies concerning the function of carbohydrates produced via the glyoxylate cycle and the comparative activities of glyoxylate cycle enzymes during the life cycles of other nematodes will be useful in analyzing these possibilities.

Research Note

Sarcocystis sp. in the Striated Muscle of Domestic Cats, *Felis catus*

JEFFREY I. EVERITT,[1] EDWARD J. BASGALL,[2] STEPHEN B. HOOSER,[2]
AND KENNETH S. TODD, JR.[1]

[1] Department of Veterinary Pathobiology, University of Illinois, Urbana, Illinois and
[2] Department of Veterinary Biosciences, University of Illinois, Urbana, Illinois

KEY WORDS: Protozoa, Coccidia, skeletal and cardiac muscle, transmission electron microscopy.

Coccidian parasites in the genus *Sarcocystis* have a heteroxenous life cycle, with gametogony and sporogony taking place in the intestinal tissue of suitable definitive hosts. Asexual development occurs within the internal organs and, at a later stage, by endodyogeny within the sarcocysts in the striated musculature, of the specific intermediate host (Tadros and Laarman, 1982, Advances in Parasitology 20:293–468). Predatory species serve as definitive hosts.

Domestic cats and other carnivores have been reported infrequently to have sarcocysts in their

striated muscles (Eisenstein and Innes, 1956, Veterinary Reviews and Annotations 2:61–78). This report describes the light microscopic and ultrastructural features seen in feline muscular sarcocysts in a group of domestic cats (*Felis catus*).

Twelve young domestic cats from a farm in southeastern Indiana were given complete necropsy examinations following termination of toxicologic study. The cats were obtained and necropsied during a 3-mo period. All animals were clinically normal. Tissues were fixed in 10% phosphate-buffered formalin, embedded in Tissue Prep®, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Selected sections
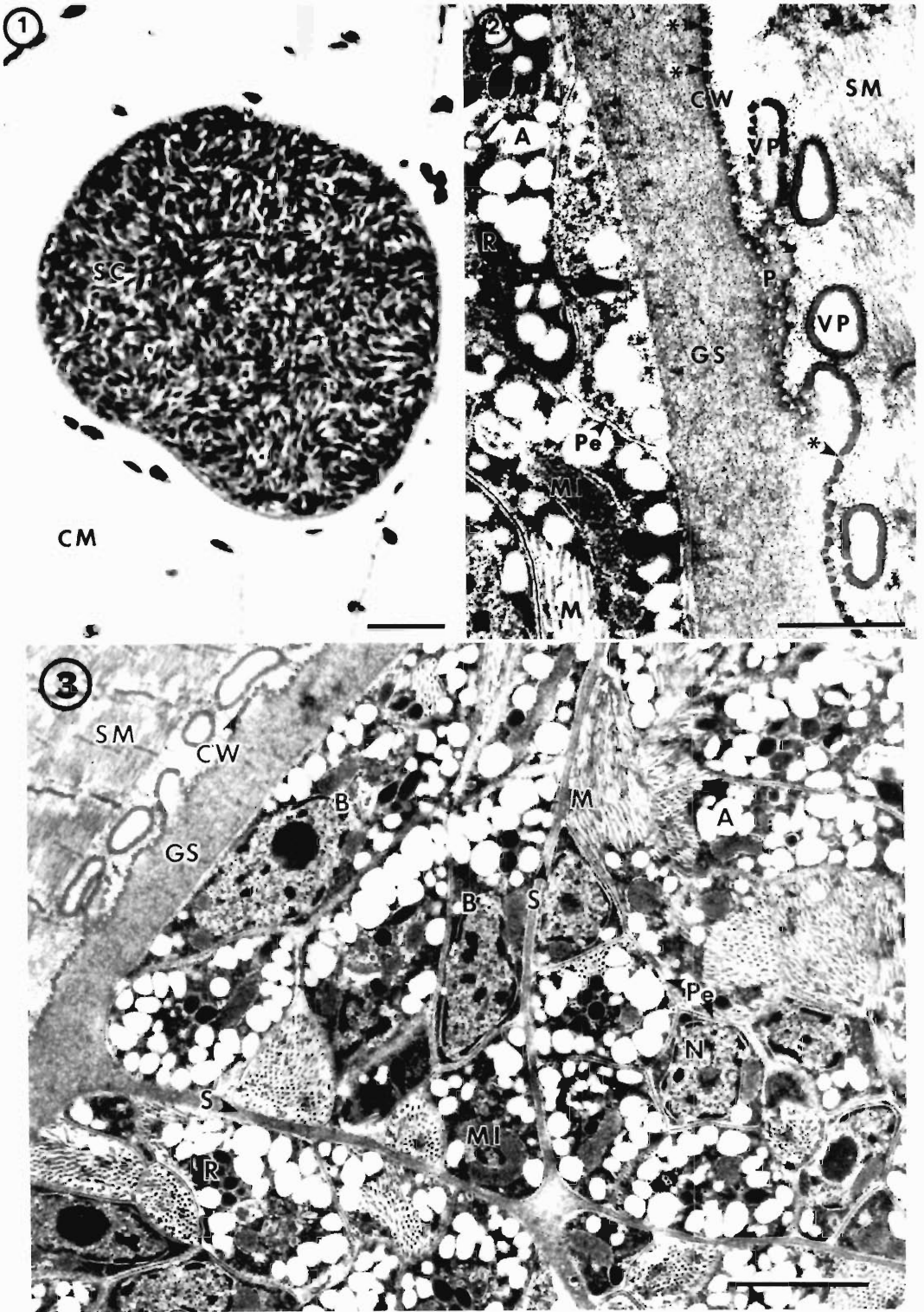
were stained with periodic acid-Schiff reagent. Tissues examined included the left ventricular free wall, quadriceps femoris skeletal muscle, brain, and spinal cord.

Sarcocysts were found in the skeletal muscle of 3 cats and the cardiac muscle of a fourth (Fig. 1). Sections of brain and spinal cord revealed no significant lesions or evidence of organisms. PAS-positive granules were present in bradyzoites within the sarcocysts. The sarcocysts were round in cross section and elongate in longitudinal section. They measured 28–270 × 24–700 μm (mean 58 × 198 μm). Their walls did not stain with the periodic acid-Schiff reaction. No inflammatory cell reaction or muscle degeneration was noted around them.

A sarcocyst was located on a paraffin block face from an H&E-stained slide. The sarcocyst plus surrounding muscle tissue was excised with a razor blade and immersed in a capped vial containing 100% xylene. The paraffin was dissolved at 60°C, and the sample was rinsed several times to remove excess paraffin. The sarcocyst was rehydrated through an ethanol : distilled H_2O series and then postfixed in 1% OsO_4 in distilled H_2O. After redehydration to 100% ethanol and propylene oxide substitution, the sample was infiltrated and embedded in Epon 812. Thin sections were double stained with uranyl acetate and lead citrate prior to examination in a JEOL 100 CX transmission electron microscope.

Ultrastructural examination from the skeletal muscle confirmed the typical structural features of *Sarcocystis* sp. infection. The cyst was mature, with a well-differentiated cyst wall, had no metrocysts, but had numerous compartments filled with mature bradyzoites. An electron-dense pri-mary cyst wall, approximately 60 nm thick, contained numerous irregularly spaced villous protrusions (Fig. 2), which varied from 0.4 to 1.0 μm in length and from 0.3 to 0.6 μm in width. The primary cyst wall appeared serrated except in the regions that capped the villous projections. The serrations appeared to be due to numerous small pores (Fig. 2).

Beneath the primary cyst wall was a zone of fine granular ground substance approximately 1 μm thick. This zone extended as septa into the interior of the cyst, dividing it into compartments (Fig. 3). Tubular structures were not found in the ground substance at the base of the villous projections. Bradyzoites within the cyst contained numerous granules, a conoid, micronemes, anterior rhoptries, and prominent nuclei. The bradyzoites were 0.7–1.3 μm wide.

The fine structure of the sarcocysts in the striated muscle of these cats closely corresponds to the description of *Sarcocystis* sp. reported from cats by Kirkpatrick et al. (1986, Veterinary Pathology 23:88–90).

Feline muscular sarcocystiasis has been reported in the domestic cat (Eisenstein and Innes, 1956, loc. cit.; Kirkpatrick et al., 1986, loc. cit.) and the Indian lion (Bhatavdekar and Purchit, 1963, Indian Veterinary Journal 40:44–45). The cat has been reported to be the definitive host for several *Sarcocystis* spp. (Levine and Ivens, 1981, Illinois Biological Monographs 51:1–248).

No muscle degeneration or inflammation was present in conjunction with parasitic encystation in the cats described here.

←

Figures 1–3. 1. Light micrograph of sarcocyst (SC) in H&E-stained paraffin section from cardiac muscle (CM) of cat. Bar = 50 μm. 2. Enlarged view of primary sarcocyst wall (CW) within skeletal muscle (SM). Irregularly spaced villous projections (VP) are shown in both longitudinal and cross section. The primary sarcocyst wall appears serrated (arrows) except near tips of villous projections. In cross section the serrations appear to be small pores (P) that pass through the sarcocyst wall (*). Beneath the ground substance (GS), portions of mature bradyzoites are seen, each bounded by a pellicle (Pe). Electron-lucent amylopectin granules (A), rhoptries (R), micronemes (M), and mitochondria (MI) are evident. Bar = 1.0 μm. 3. Low-power micrograph of sarcocyst containing mature bradyzoites (B). Delineation between skeletal muscle (SM) and sarcocyst wall (CW) clearly indicates noninvolvement. Ground substance (GS) extends into septa (S), compartmentalizing groups of bradyzoites. Each parasite has a sharply defined pellicle (Pe), a nucleus (N), mitochondria (MI), amylopectin granules (A), rhoptries (R), and micronemes (M). Bar = 1.0 μm.

MINUTES

Five Hundred Eighty-First Through
Five Hundred Eighty-Eighth Meetings

581st Meeting: Uniformed Services University of Health Sciences, Bethesda, MD, Cosponsor Oxford Biological Laboratory, Oxford, MD, 15 October 1986. Ralph P. Eckerlin presided over the business meeting at which Dr. Louis Diamond was announced as recipient of the Anniversary Award. The following new members were elected: Mrs. Batra, Robin M. Giblin-Davie, Chris Gardiner, R. D. Klann, Jong Yil Chai, M. Kamiya, Leslie S. Uhazy, and David E. Bean-Knudsen. The following slate of officers was announced: Patricia A. Pilitt, President; Robin N. Huettel, Vice President; Michael D. Ruff, Corresponding Secretary-Treasurer; Jeffrey W. Bier, Recording Secretary. Bryce C. Redington presided over the scientific meeting. Eugene G. Hayunga described an antigen assay for the early detection of schistosomiasis. Austin Farley evaluated the status of sarcomas in Chesapeake Bay soft shell clams. Roy G. Taylor presented an analysis of amoebicidal activity of related hydroxyquinones correlated with chelation.

582nd Meeting: Animal Parasitology Institute, U.S.D.A., Beltsville, MD, 12 November 1986. Ralph P. Eckerlin presided over the election of officers (above). Bryce C. Redington, Chairman of the Awards Committee gave a professional biographical sketch of Dr. Louis Diamond, recipient of the Anniversary Award, and presented the award. A change in the December meeting date to coincide with the *Ostertagia* Workshop was announced. Hyun Lillehoj introduced Jong Yil Chai who described research on trematodiases in Korea. Dante S. Zarlenga described the immunodetection of diagnostic antigens of *Trichinella spiralis* derived from a C-DNA expression library, and J. R. Lichtenfels described cuticular ridge patterns in the Ostertagiinae.

583rd Meeting: Nematology Laboratory, Systematic Botany, Nematology, and Mycology Laboratory, and Insect and Nematode Hormone Laboratory, U.S.D.A., Beltsville, MD, 4 December 1986. Ralph P. Eckerlin presided over the business meeting where the following new members were announced: Steven Plotka, Warren Shaffer, and Dennis Kyle. Richard Sayre presided over the scientific meeting where Phil Klesius presented an overview of the *Ostertagia* Workshop. G. A. Schad discussed hookworm development and arrest. Robin N. Huettel described a sex phermone of the soybean cyst nematode. Kevin Baird and Ron Neafie presented posters on unusual human nematode infections. President Eckerlin installed the new officers and turned over the presidency to Patricia A. Pilitt.

584th Meeting: Laboratory of Parasitic Diseases, NIH, Bethesda, MD, 14 January 1987. Patricia A. Pilitt presided over the business meeting, where new members Molly Fitzmaurice, Laureen Peters, and Charles Whitehill were announced as elected to membership. In addition to the officers (above) the following appointments were announced: Executive Committee Members-at-Large: J. Kevin Baird, John H. Cross, Robert J. Chinnis, Dennis E. Kyle; Representative to Washington Academy of Sciences, Kendall G. Powers; Representative to American Society of Parasitologists, Willis A. Reid; Custodian of Back Issues, Gerhard A. Schad; Archivist Librarian, David R. Lincicome; Awards Committee, Margaret A. Stirewalt, Leon Jacobs, Sherman S. Hendrix; Business Advisory Committee, Harley G. Sheffield, Gilbert F. Otto, J. R. Lichtenfels, and M. D. Ruff; Honorary and Life Membership Committee, Everett L. Schiller, Thomas K. Sawyer, Lawrence Lightner; Audit Committee: Willis A. Reid and Hyun S. Lillehoj; Membership Committee: Louis S. Diamond, Eugene G. Hayunga and Roy G. Taylor. Franklin Neva presided over the scientific session. Altaf Lal discussed vaccine trials in rodent malaria. Vidal de la Cruz related some effects of variation in circum-sporozoite protein on vaccine efficacy. Paul J. Brindley described a role of antibody response in efficacy of praziquantel against *Schistosoma mansoni.* Patricia Romans and Louis Miller described the stable integration of foreign DNA into a malaria vector.

585th Meeting: Naval Medical Research Institute, Bethesda, MD, 11 February 1987. Patricia A. Pilitt presided over the business meeting. Suggestions for program changes were solicited. Richard Beaudoin presided over the scientific meeting. Monte Bawden discussed the mouse's antibody response to one dose of irradiated sporozoites. Martha Sedegah described the spleen cell of the mouse after vaccination with murine malaria. Pat Rogers characterized three cloned genes of sporozoite antigens.

586th Meeting: Walter Reed Army Institute of Research, Cosponsor Armed Forces Institute of Pathology, Washington, DC, 18 March 1987. Patricia A. Pilitt presided over the business meeting where the following new members were announced as elected: Robert Seville, Afzal A. Siddiqui, Karin Gerber, D. J. Patel, Beckey L. Brown, and R. Madhavi. The proposed budget for 1987 of $39,463 was distributed and approved. Willis A. Reid presided over the scientific meeting. G. Childs compared two quinones against falciparium malaria. A. Oduala discussed the use of chloroquin to treat malaria. J. Tally and J. Jackson discussed development of media and micro procedures for in vitro evaluation of antileshmanial agents. Posters dealing with the epidemiology of drug resistance in malaria were presented by the WRAIR staff.

587th Meeting: Johns Hopkins University, Joint Meeting with the Tropical Medicine Society of Baltimore Dinner Club, Baltimore, MD, 15 April 1987. Patricia A. Pilitt presided over the business meeting where the following new members were announced: Warren B. Shaffer, Ana Szarefman, James Campbell, Djamshid Shirasian, George E. Childs, James Bailey, and Colin Dobson. The audited balance sheet for 1986 was distributed and accepted by the members present. Michael Gottlieb presided over the scientific session. M. S. Ibrahim discussed vector host parasite relationships in brugian filariasis. M. Gottlieb talked about trypanosome enzyme regulation. J. Glenn Morris explained multiple antibiotic resistance in *Vibrio cholerae* as studied genetically.

588th Meeting: New Bolton Center, The University of Pennsylvania, Kennet Square, PA, 9 May 1987. Joint meeting with the New Jersey Society of Parasitologists. Patricia A. Pilitt presided over the business meeting where Everett L. Schiller was announced as the recipient of the 1987 Anniversary Award. G. A. Schad presided over the scientific session, a symposium, "How do parasites reach their predilection sites in hosts." Bernard Salafsky discussed entering the host, Michael Sukedo discussed finding the predilection site, and Bernard Fried, discussed the interaction between parasites.

Respectfully submitted,
JEFFREY W. BIER,
Recording Secretary

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Munson, Donald A.
Murrell, K. Darwin
Neva, Franklin A.
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Olsen, John L.
*Otto, Gilbert F.
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284

Pilitt, Patricia Ann
Porter, Clarence A.
Powers, Kendall G.
Quakyi, Isabella
Rebois, Raymond V.
Redington, Bryce C.
Rhoads, Marcia L.
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SUBJECT INDEX FOR VOLUME 54

292

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* Jesse R. Christie	1964	* Leo A. Jachowski, Jr.	1976
Gilbert F. Otto	1965	Horace W. Stunkard	1977
* George R. LaRue	1966	Kenneth C. Kates	1978
* William W. Cort	1966	* Everett E. Wehr	1979
* Gerard Dikmans	1967	O. Wilford Olsen	1980
* Benjamin Schwartz	1969	Frank D. Enzie	1981
* Willard H. Wright	1969	Lloyd E. Rozeboom	1982
Aurel O. Foster	1970	Leon Jacobs	1983
Carlton M. Herman	1971	Harley G. Sheffield	1984
May Belle Chitwood	1972	A. Morgan Golden	1985
* Elvio H. Sadun	1973	Louis S. Diamond	1986

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Gilbert F. Otto	1972	A. James Haley	1984
* Theodor von Brand	1975	Paul C. Beaver	1986
May Belle Chitwood	1975	Raymond M. Cable	1986

* Deceased.

CONTENTS

(Continued from Front Cover)

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