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Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae)

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ABSTRACT: *Urocleidoides* Mizelle and Price, 1964, is restricted to species possessing a sinistral vaginal sclerite, overlapping (tandem?) gonads, counterclockwise cirral rings, simple anchors, and hooks (pairs 1 and 5 usually reduced) with enlarged shanks. *Urocleidoides* contains the type species, *U. reticulatus* Mizelle and Price, 1964, *U. anops* Kritsky and Thatcher, 1974, and *U. curimatae* Molnar, Hanek, and Fernando, 1974. In addition, *U. eremitus* sp. n. and *U. paradoxus* sp. n., herein described from *Hoplias malabaricus* (Bloch) and *Rhytiodus microlepis* Kner, respectively, are included in the genus. Fish hosts of the superfamily Characoidea (Cypriniformes) are considered to be the natural hosts of *Urocleidoides* species. *Ancyrocephalus dyki* Lucký, 1972, and *Gussevia minuta* Kohn and Paperna, 1964, are junior synonyms of *U. reticulatus*. *Vancleaveus* gen. n. is proposed for ancyrocephaline species infesting the gills of siluriform fishes and possessing a ventral vagina, overlapping gonads, an elongate seminal vesicle and prostatic vesicle, dorsal anchors with conspicuous folds on the superficial roots, and hooks with shanks inflated along their entire length. The genus includes *V. janauacaensis* sp. n. (type) from *Pterodoras granulosus* (Valenciennes), *V. cicinnus* sp. n. from *Phractocephalus hemiliopterus* (Bloch and Schneider), *V. fungulus* sp. n. from *Pseudoplatystoma tigrinum* (Cuvier and Valenciennes) and *P. fasciatum* (Linnaeus), and *V. platyrhynchi* sp. n. from *Hemisorubim platyrhynchos* (Valenciennes). *Cosmetocleithrum* gen. n. is proposed from siluriform fishes and is characterized by species possessing a dorsal bar with two submedian posterior projections, a sinistral vagina, tandem gonads, and hooks with undilated shanks. The following new species of *Cosmetocleithrum* are described: *C. gussevi* (type), *C. confusus, C. parvum, C. rarum,* and *C. sobrinus,* all from *Oxydoras niger* (Valenciennes), and *C. bulbocirrus* from *Pterodoras granulosus* (Valenciennes). *Gussevia* Kohn and Paperna, 1964 is resurrected for *G. spiralocirra* Kohn and Paperna, 1964 (type) from *Pterophyllum scalare* (Lichtenstein); *G. alii* (Molnar, Hanek, and Fernando, 1974) comb. n., *G. cichlasomatis* (Molnar, Hanek, and Fernando, 1974) comb. n., and *G. dobosi* (Molnar, Hanek, and Fernando, 1974) comb. n. from *Cichlasoma bimaculatum* (Linnaeus); *G. obtusa* sp. n. and *G. elephus* sp. n. from *Uaru amphiacanthoides* (Heckel); *G. longihaptor* (Mizelle and Kritsky, 1969) comb. n., *G. undulata* sp. n., *G. arilla* sp. n., and *G. tucunarense* sp. n. from *Cichla ocellaris* Bloch and Schneider; and *G. alioides* sp. n., *G. dispar* sp. n., and *G. disparoides* sp. n. from *Cichlasoma severum* (Heckel). *Gussevia* is characterized by having overlapping gonads, a haptor with anterior and posterior lobes, modified ventral anchors with well-developed anchor filaments, modified (reduced) hook pair 5, and a clockwise cirrus coil. All known species of *Gussevia* occur on fishes of the family Cichlidae. *Ancyrocephalus pterophylii* Lucký, 1970 is a junior synonym of *G. spiralocirra*; and *Longihaptor* Mizelle and Kritsky, 1969 is considered a junior synonym of *Gussevia*. *Urocleidoides affinis* Mizelle, Kritsky, and Crane, 1968, *U. amazonensis* Mizelle and Kritsky, 1969, *U. carapus* Mizelle, Kritsky, and Crane, 1968, *U. catus* Mizelle and Kritsky, 1969, *U. chavarriai* (Price, 1938) Molnar, Hanek, and Fernando, 1974, *U. corydori* Molnar, Hanek, and Fernando, 1974, *U. costaricensis* (Price and Bussing, 1967) Kritsky and Leiby, 1972, *U. gymnotus* Mizelle, Kritsky, and Crane, 1968, *U. heteroancistrium* (Price and Bussing, 1968) Kritsky and Leiby, 1972, *U. kabatai* Molnar, Hanek, and Fernando, 1974, *U. lebedevi* Kritsky and Thatcher, 1976, *U. mamaevi* Kritsky and Thatcher, 1976, *U. margolisi* Molnar, Hanek, and Fernando, 1974, *U. megorchis* Mizelle and Kritsky, 1969, *U. microstomus* Mizelle, Kritsky, and Crane, 1968, *U. robustus* Mizelle and Kritsky, 1969, *U. stictus* Mizelle, Kritsky and Crane, 1968, *U. strombicirrus* (Price and Bussing, 1967) Kritsky and Thatcher, 1974, *U. travassosi* (Price, 1938) Molnar, Hanek, and Fernando, 1974, *U. trinidadensis* Molnar, Hanek, and Fernando, 1974, *U. variabilis* Mizelle and Kritsky, 1969, and *U. virescens* Mizelle, Kritsky, and Crane, 1968 are considered incertae sedis based on the generic revision provided herein.

Urocleidoides Mizelle and Price, 1964 represents the second genus of Monogenea proposed from Neotropical freshwater fishes. As frequently happens in investigations of new regions, subsequent workers realized difficulty in understanding morphologic limits of the taxon, with Mizelle et al. (1968) greatly expanding the generic bounds in their emended diagnosis. Kritsky and Thatcher (1983) listed 30 species of *Urocleidoides* (all Neotropical) from fishes repre-

senting four teleost orders. Based on this host occurrence and the fact that most Dactylogyridae exhibit relatively high host specificity, Gussev (1978) suggested that species currently assigned to *Urocleidoides* represent several genera and possibly subfamilies. Our collections from Brazil, Colombia, Peru, and El Salvador, made over a 15-year period (see also Kritsky and Thatcher, 1974, 1976), have provided a large number of species that fall into the broad generic definition proposed by Mizelle et al. (1968). Studies on the comparative morphology of this material have allowed the revision of *Urocleidoides* presented herein; a historical account of the genus is included.

Historical Review

Urocleidoides was proposed by Mizelle and Price (1964) for their new species, *U. reticulatus,* collected from the gills of *Poecilia reticulata* (Poeciliidae). The genus was characterized by possessing a sinistral vagina and an articulated cirrus and accessory piece and was considered to be intermediate to the North American *Urocleidus* Mueller, 1934 (as emended by Mizelle and Hughes, 1938) and *Cleidodiscus* Mueller, 1934. The generic revision by Mizelle et al. (1968) allowed inclusion of their new species: *U. affinis* from *Creatochanes affinis* (Characidae), *U. carapus* and *U. gymnotus* from *Gymnotus carapo* (Gymnotidae), *U. microstomus* from *Hemigrammus microstomus* (Characidae), *U. stictus* from *Hyphessobrycon stictus* (Characidae), and *U. virescens* from *Eigenmannia virescens* (Gymnotidae). All subsequent reports on the genus have followed the generic boundaries established by the latter authors.

Mizelle and Kritsky (1969) described five additional species: *U. amazonensis* and *U. catus* from *Phractocephalus hemiliopterus,* Pimelodidae; *U. megorchis* from *Sorubim lima,* Pimelodidae; *R. robustus* from *Rhamdia* sp., Pimelodidae; and *U. variabilis* from *Symphysodon discus,* Cichlidae. Within 5 years of the original proposal of the genus, the taxon contained species infesting fishes of the orders Atheriniformes, Cypriniformes, Perciformes, and Siluriformes.

In a report on Monogenea of *Astyanax fasciatus* (Characidae), Kritsky and Leiby (1972) synonymized *Palombitrema* Price and Bussing, 1968 with *Urocleidoides* and transferred its type species, *P. heteroancistrium* Price and Bussing, 1968 and *Cleidodiscus costaricensis* Price and Bussing, 1967 to the genus. Molnar et al. (1974) described eight new species (*U. alii, U. cichlasomatis,* and *U. dobosi* from *Cichlasoma bimaculatum,* Cichlidae; *U. corydori* and *U. margolisi* from *Corydoras aeneus,* Pimelodidae; *U. curimatae* from *Curimata argentea,* Curimatidae; and *U. kabatai* and *U. trinidadensis* from *Astyanax bimaculatus,* Characidae) and transferred *Cleidodiscus chavarriai* Price, 1938 and *C. travassosi* Price, 1938 both from *Rhamdia* spp. (Pimelodidae) into *Urocleidoides.* Kritsky and Thatcher (1974) described *U. anops* from *Characidium caucanum* (Characidae) and placed *Cleidodiscus strombicirrus* Price and Bussing, 1967 from *Astyanax fasciatus* (Characidae) in the genus.

Considerable diversity in the structure of the internal organ systems of species of *Urocleidoides* was indicated by Kritsky and Thatcher (1976), who presented whole-mount illustrations of their new species, *U. lebedevi* from *Pimelodus grosskopfi* (Pimelodidae) and *U. mamaevi* from *Cephalosiluris zungaro* (Pimelodidae). In 1983, Kritsky and Thatcher listed 30 species in *Urocleidoides,* which included their transfer of *Gussevia spiralocirra* Kohn and Paperna, 1964 from *Pterophyllum eimekei* (Cichlidae) and *G. minuta* Kohn and Paperna, 1964 from *Poecilia reticulata* (Poeciliidae) into the genus. Kritsky and Thatcher (1983) considered *Gussevia* Kohn and Paperna, 1964 a junior synonym of *Urocleidoides* as emended by Mizelle et al. (1968).

In a series of papers, Lucký (1970, 1972, 1973) reported on the following ancyrocephalines from aquarium fishes in Czechoslovakia: *Ancyrocephalus xiphophori* from *Xiphophorus maculatus* (Poeciliidae); *A. pterophylli* and *A.* sp. from *Pterophyllum eimekei* (Cichlidae); *A. kostomarovi* from *Symphysodon discus* (Cichlidae); and *A. dyki* from *Poecilia reticulata* (Poeciliidae). Investigators in the western hemisphere have not commented on these species even though they clearly show close resemblance to *Urocleidoides* spp.

Materials and Methods

Fish hosts were collected by hook-and-line, seine, or net from locations in Brazil and Peru during the period 1977–1984. Gills were removed, placed in finger bowls, and covered with a 1:4,000 formalin solution. After ½ hour, gills were agitated in this liquid and then removed from the bowl. Helminths were allowed to settle to the bottom and were subsequently removed with the aid of a small probe and dissecting microscope. They were immediately fixed and stored in AFA. Some were mounted unstained in Gray and Wess' medium for study of sclerotized structures. Other specimens were

stained with Semichon's carmalum, Mayer's acid carmalum, or Gomori's trichrome to determine internal structures. Illustrations were prepared with the aid of a camera lucida or microprojector. Measurements, all in micrometers, were made according to the procedures of Mizelle and Klucka (1953) except as described below. The measurements of the cirrus include: (1) the diameter of the proximal ring of the coil, depicted on the respective drawings as the interval between the solid straight lines, and (2) an approximation of total length of the cirrus obtained by using a Minerva curvimeter on camera lucida drawings. Dimensions of organs and other structures represent the greatest measurement in dorsoventral view; lengths of curved structures (bars, accessory piece) represent a straight-line measurement between extreme ends; the hook measurement represents the total hook length; greatest body width is that of the trunk region (excluding the haptor); and values for the ovary and testis represent the length followed by width, respectively. Average measurements are followed by ranges in parentheses. Haptoral terminology is that of Kritsky and Mizelle (1968) and Mizelle et al. (1968).

Numbering of hook pairs follows that recommended by Mizelle (1936). This sequencing is preferable because it is the only proposed method currently in use that considers both anteroposterior and dorsoventral positions of respective hook pairs in the adult haptor. An ancyrocephaline distribution of haptoral hooks refers to the usual distribution of hook pairs in the Ancyrocephalinae described by Mizelle (1936). Direction of the cirrus coil (counterclockwise vs. clockwise) was determined using the procedure proposed by Kritsky et al. (1985). Type specimens were deposited in the collections of the Instituto Nacional de Pesquisas da Amazônia (INPA), the U.S. National Museum Helminthological Collection (USNM), and the University of Nebraska State Museum (HWML) as indicated in the respective descriptions.

For comparative purposes, type specimens of the following species were examined: 3 cotypes(?), *Ancyrocephalus dyki* Lucký, 1972 (USNM 78794); cotype(?), *A. kostomarovi* Lucký, 1973 (USNM 78793); cotype(?), *A. pterophylli* Lucký, 1970 (USNM 78801); paratype, *Cleidodiscus bulbus* Rogers and Rawson, 1969 (USNM 71363); holotype, *C. microcirrus* Price and Schlueter, 1967 (USNM 60890); 3 paratypes, *Longihaptor longihaptor* Mizelle and Kritsky, 1969 (USNM 71000); paratype, *Trinidactylus cichlasomatis* Hanek, Molnar, and Fernando, 1974 (USNM 73181); 2 paratypes, *Urocleidoides affinis* Mizelle, Kritsky, and Crane, 1968 (HWML 22936); 2 paratypes, *U. alii* Molnar, Hanek, and Fernando, 1974 (USNM 73163); 2 paratypes, *U. amazonensis* Mizelle and Kritsky, 1969 (HWML 22932); holotype, *U. anops* Kritsky and Thatcher, 1974 (USNM 72841); 10 paratypes, *U. carapus* Mizelle, Kritsky, and Crane, 1968 (HWML 22934); 15 paratypes, *U. catus* Mizelle and Kritsky, 1969 (HWML 22942); 2 paratypes, *U. cichlasomatis* Molnar, Hanek, and Fernando, 1974 (USNM 73165); 2 paratypes, *U. curimatae* Molnar, Hanek, and Fernando, 1974 (USNM 73169); 2 paratypes, *U. dobosi* Molnar, Hanek, and Fernando, 1974 (USNM 73171); 6 paratypes, *U. megorchis* Mizelle and Kritsky, 1969 (HWML 22935); 4 paratypes, *U. microstomus* Mizelle, Kritsky, and Crane, 1968 (HWML 22939); 3 paratypes, *U. reticulatus* Mizelle and Price, 1964 (HWML 22938); 11 paratypes, *U. robustus* Mizelle and Kritsky, 1969 (HWML 22941); 4 paratypes, *U. stictus* Mizelle, Kritsky, and Crane, 1968 (HWML 22937); 2 paratypes, *U. trinidadensis* Molnar, Hanek, and Fernando, 1974 (USNM 73177); 15 paratypes, *U. variabilis* Mizelle and Kritsky, 1969 (HWML 22943); 6 paratypes, *U. virescens* Mizelle, Kritsky, and Crane, 1968 (HWML 22933); holotype, *Urocleidus aequidens* Price and Schlueter, 1967 (USNM 60894); and holotype, *U. cavanaughi* Price, 1966 (USNM 61204).

Urocleidoides Mizelle and Price, 1964

EMENDED DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Cephalic lobes, head organs, cephalic glands present. Eyes present or absent. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to testis, lacking diverticula. Gonads intercaecal, overlapping or (?) tandem; testis dorsal or posterior to ovary. Vas deferens looping left intestinal caecum; seminal vesicle an inconspicuous dilation of vas deferens; copulatory complex comprising a coiled cirrus and accessory piece; cirrus coil counterclockwise; accessory piece serving as cirrus guide distally. Oviduct short, uterus delicate; vagina dextral or sinistral; seminal receptacle present. Vaginal sclerite present, sinistral. Vitellaria well developed. Haptor armed with dorsal and ventral pair of unmodified anchors, dorsal and ventral bars, seven pairs of hooks with ancyrocephaline distribution. Hook pairs 1, 5 usually reduced in size. Parasites primarily of gills of freshwater cypriniform fishes.

TYPE SPECIES AND HOST: *Urocleidoides reticulatus* Mizelle and Price, 1964 from the guppy, *Poecilia* (*Lebistes*) *reticulata* (Peters), Poeciliidae.

OTHER SPECIES: *U. anops* Kritsky and Thatcher, 1974 from *Characidium caucanum* Eigenmann, Characidae; *U. curimatae* Molnar, Hanek, and Fernando, 1974 from *Curimata argentea* (Gill), Curimatidae; *U. eremitus* sp. n. from *Hoplias malabaricus* (Bloch), Erythrinidae; *U. paradoxus* sp. n. from *Rhytiodus microlepis* Kner, Anostomidae.

REMARKS: *Urocleidoides* is herein restricted to species possessing a sinistral vaginal sclerite, overlapping (tandem?) gonads, counterclockwise cirral rings, unmodified anchors, and hooks (pairs 1, 5 usually reduced) with enlarged shanks. Ex-

cept for the type species, members of the genus have been reported only from fishes of the superfamily Characoidea (Cypriniformes), which appear to be their natural hosts.

Reports of the type species, *U. reticulatus,* from the atheriniform host, *Poecilia* (*Lebistes*) *reticulata* by Mizelle and Price (1964), Kohn and Paperna (1964), and Lucký (1972) may represent spurious infestations of the guppy. In the above reports, the hosts were obtained from aquaria in California, Israel, and Czechoslovakia, respectively, and the parasite has never been recorded from guppies collected from native habitats in Trinidad (listed as the type locality). The guppy, as well as many species of Characoidea, is a common aquarium fish kept in community-type tanks where interspecific transfer of monogeneans could easily occur. Because our examination of numerous guppies collected from the Arouca River in Trinidad during 1982 (hosts provided by Dr. M. Beverley-Burton) failed to show infestation by the parasite, we suggest that studies of the gill parasites of other characoid fishes commonly found in community-type aquaria may be necessary to demonstrate the natural host of *U. reticulatus.*

Urocleidoides reticulatus Mizelle and Price, 1964

SYNONYMS: *Ancyrocephalus dyki* Lucký, 1972; *Gussevia minuta* Kohn and Paperna, 1964; *Urocleidoides minuta* (Kohn and Paperna, 1964) Kritsky and Thatcher, 1983.

HOST: Guppy, *Poecilia* (*Lebistes*) *reticulata* (Peters), Poeciliidae.

TYPE LOCALITY: Trinidad; aquarium fish descended from Trinidad stock in California.

SPECIMENS STUDIED: Three paratypes, HWML 22938; three cotypes(?) of *Ancyrocephalus dyki* Lucký, 1972, USNM 78794.

REMARKS: The original specimens on which this species is based are unstained and mounted in glycerine jelly, and details of the anatomy of the reproductive system could not be verified with certainty. Although Mizelle and Price (1964) state that the gonads are tandem (testis postovarian), the gonads in one specimen available for study appeared to be overlapping under phase contrast microscopy. However, we cannot state with certainty that this is the case and suggest that examination of living specimens or fresh material stained to show internal features will be necessary to verify this character. Nonetheless, the comparative morphology of the sclerotized structures of the haptor, vagina, and copulatory complex strongly suggests a close relationship of this species with others we presently include in the genus.

Examination of three cotype specimens (on one slide and mounted in Malmberg's Ammonium Picrate Solution) of *Ancyrocephalus dyki* Lucký, 1972 has shown this species to be conspecific with *U. reticulatus.* Sclerotized structures of the haptor and copulatory complex of *A. dyki* are indistinguishable from those of the paratypes of the type species; details of the reproductive system could not be determined. Although type material of *Gussevia minuta* Kohn and Paperna, 1964 (*U. minuta* of Kritsky and Thatcher, 1983) was not available for study, this species is also undoubtedly a synonym of *U. reticulatus* based on the comparison of the original drawings provided by Kohn and Paperna (1964) and available type specimens of *U. reticulatus* and *A. dyki.*

Urocleidoides anops Kritsky and Thatcher, 1974

HOST: *Characidium caucanum* Eigenmann, Characidae.

TYPE LOCALITY: Rio Pance, Cali, Valle, Colombia.

SPECIMEN STUDIED: Holotype, USNM 72841.

REMARKS: *Urocleidoides anops* possesses a sinistral vaginal sclerite, counterclockwise cirral rings, unmodified anchors, and hooks with enlarged shanks. Kritsky and Thatcher (1974) state that the gonads were indistinct, and we were not able to determine their limits in the unstained holotype. Nonetheless, we consider this species a member of *Urocleidoides* because it possesses the primary characters distinguishing the genus as emended herein.

Urocleidoides curimatae Molnar, Hanek, and Fernando, 1974

HOST: *Curimata argentea* (Gill), Curimatidae.

TYPE LOCALITY: Arouca River near D'Abadie, Trinidad.

SPECIMENS STUDIED: Two paratypes, USNM 73169.

REMARKS: Based on the presence of a sinistral vaginal sclerite and the morphology of the haptoral armament and copulatory complex, this species is considered a member of *Urocleidoides.* Molnar et al. (1974) consider the sclerite as the vagina in this species. However, our examination

of the paratypes confirms that it is a hook-shaped structure morphologically similar to those of *U. eremitis* and *U. paradoxus* spp. n. The type specimens are unstained and details of the internal anatomy could not be confirmed; the original authors state that the gonads are ovate and the testis is postovarian.

Urocleidoides eremitus sp. n. (Figs. 1-9)

HOST: Traíra, *Hoplias malabaricus* (Bloch), Erythrinidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (April 18, 1980).

TYPE SPECIMENS: Holotype, INPA PA260-1; paratypes, INPA PA260-2, PA260-3, USNM 78764, HWML 22940.

DESCRIPTION (based on 13 specimens): Body fusiform; cephalic margin with two terminal, two bilateral cephalic lobes poorly developed. Eyes 2–4, poorly developed, subequal; members of posterior pair usually farther apart than those of anterior pair; eye granules frequently dissociated, small, usually ovate; accessory granules (granules not associated with the eyes) present in cephalic region and anterior trunk. Pharynx subovate; esophagus moderately long. Peduncle broad; haptor hexagonal. Ventral anchor with large superficial root, small deep root, curved shaft, short point. Dorsal anchor with elongate superficial root, incipient deep root, slightly curved shaft, point moderate in length. Ventral bar with bulbous terminations, anteromedial indentation; dorsal bar broadly U-shaped, with terminations directed laterally. Hooks similar, each with delicate shaft and point, protruding thumb, dilated shank; hook pairs 1, 5 reduced in size; FH loop ⅓ shank length (pairs 2, 3, 4, 6, 7), ½ shank length (pairs 1, 5). Cirrus a coil of about 2¼ rings, base with lateral flange, tube delicate; accessory piece flabellate. Vagina sinistral, a tortuous tube; vaginal sclerite a flexible rod with distal hook, subterminal short projection, proximal portion with longitudinal groove.

MEASUREMENTS: Body 581 (480–681) long, greatest width 83 (75–106) in posterior half of trunk. Pharyngeal diameter 23 (21–24). Haptor 73 (65–84) long, 102 (85–140) wide. Ventral anchor 45 (44–47), base width 28 (26–30); dorsal anchor 40 (38–42), base width 21 (19–23). Ventral bar 36 (32–39); dorsal bar 33 (30–36). Hook pairs 2, 3, 4, 7—26 (25–27); hook pairs 1, 5—18 (17–19); hook pair 6—24 (23–25). Cirrus 136 long, ring diameter 16 (13–18); accessory piece 20 (18–21) long. Testis 106 × 20–21; ovary 64 × 20–21. Vaginal sclerite 35 (30–40) long.

REMARKS: Based on the comparative morphology of the anchors and copulatory complexes, the closest relative of this species is apparently *U. reticulatus* Mizelle and Price, 1964. *Urocleidoides eremitus* differs from this species by possessing hook shanks inflated along their entire length and anchors with distinct angular unions of the points and shafts. The specific name is from Latin (*eremitus* = solitary).

Urocleidoides paradoxus sp. n. (Figs. 10-18)

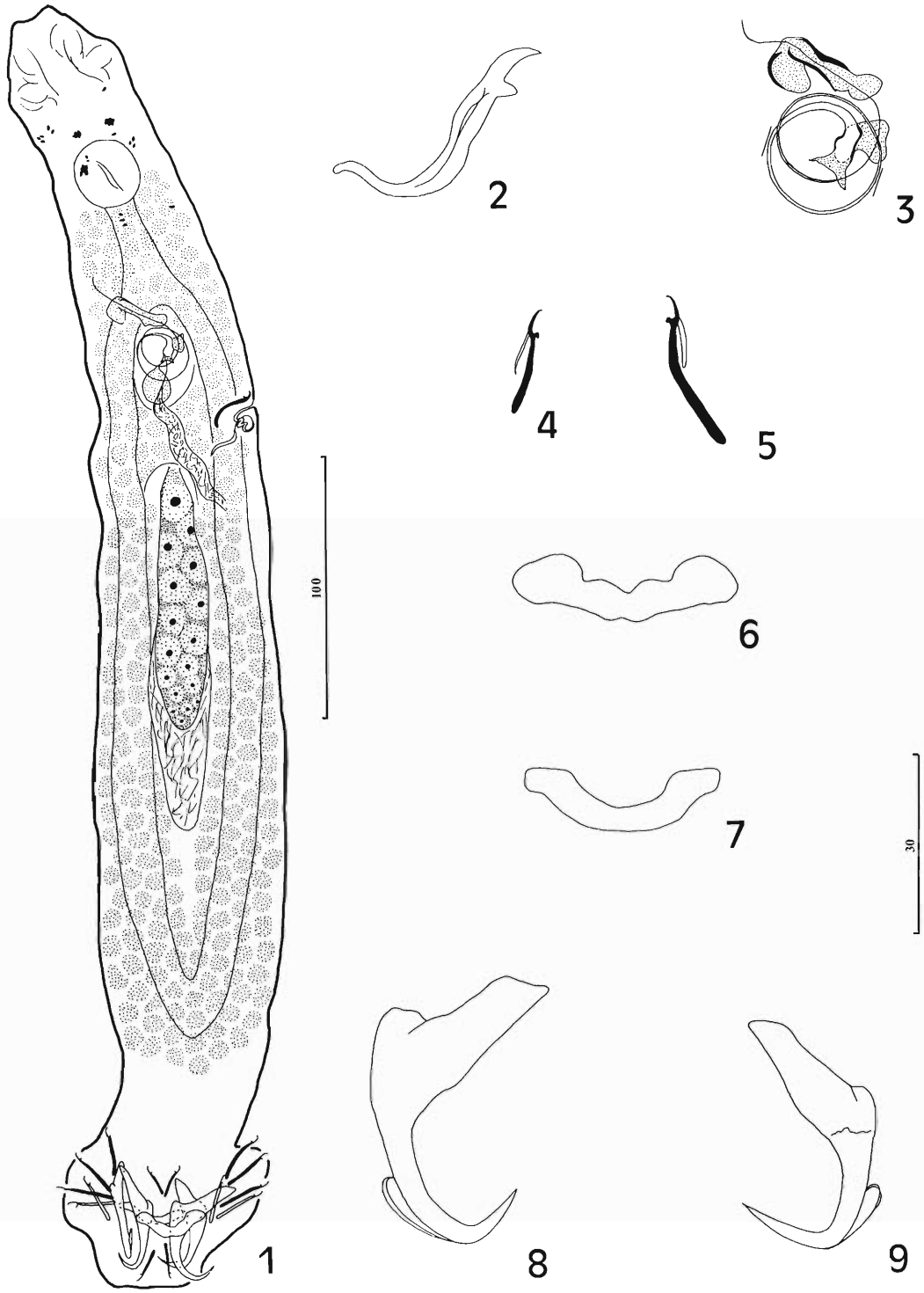
HOST: Aracu pau de negro, *Rhytiodus microlepis* Kner, Anostomidae.

TYPE LOCALITY: Rio Solimões near Ilha Marchantaria, Manaus, Amazonas, Brazil (June 8, 1983); also collected from same host at Furo do Catalão, near Encontro das Águas, Manaus, Amazonas, Brazil (October 16, 1982).

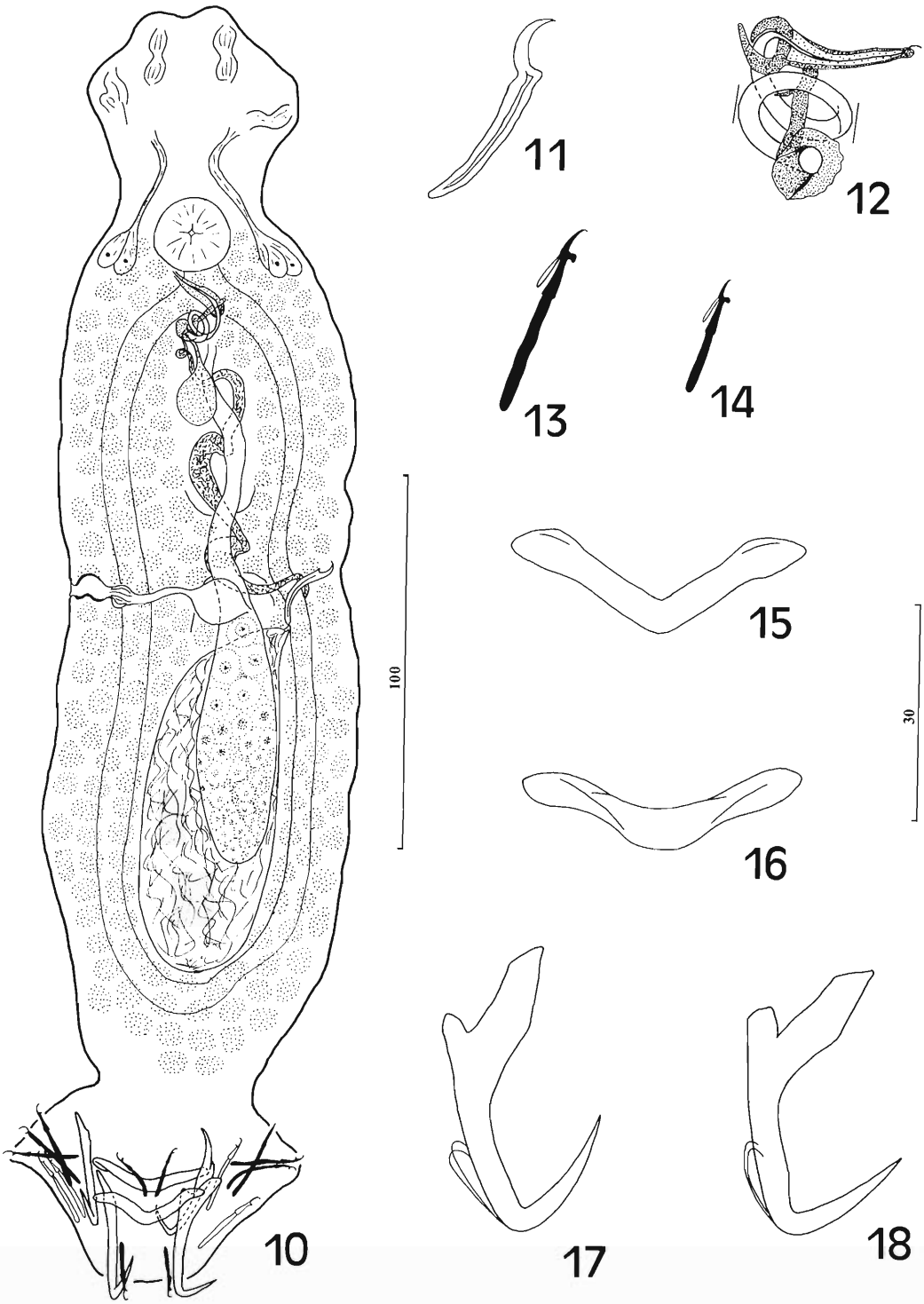
TYPE SPECIMENS: Holotype, INPA PA261-1; paratypes, INPA PA261-2 to PA261-4, USNM 78765 and 78766, HWML 22944, 22945.

DESCRIPTION (based on 28 specimens, 20 measured): Body robust; cephalic margin usually expanded or with subterminal narrowing; two terminal, two bilateral cephalic lobes poorly developed. Eyes absent; accessory granules absent or widely scattered throughout trunk and cephalic region, variable in size, ovate. Pharynx spherical; esophagus short. Peduncle board, haptor hexagonal. Anchors similar; each with elongate superficial root, small deep root, elongate straight shaft, sharply recurved point. Ventral bar broadly V-shaped, ends slightly expanded; dorsal bar broadly U-shaped, with ends directed laterally. Hooks similar; each with delicate point and shaft, depressed thumb, inflated shank comprising two distinct parts; hook pairs 1, 5 reduced in size; FH loop ¼–⅓ shank length. Cirrus coiled, with about two rings; base with anteriorly directed process which may articulate with accessory piece, tube with large diameter sharply attenuated distally. Accessory piece grooved, with two proximal arms. Vagina dextral, a lightly sclerotized tube of varying diameter, possessing internal sclerotized ridges proximal to distal funnel; vaginal sclerite lying near left body margin, composed of grooved rod with sickle-shaped termination.

MEASUREMENTS: Body 353 (295–463) long,



Figures 1–9. ***Urocleidoides eremitus*** **sp. n. 1. Ventral view of holotype. 2. Vaginal sclerite. 3. Copulatory complex. 4. Hook (pairs 1, 5). 5. Hook (pairs 2, 3, 4, 6, 7). 6. Ventral bar. 7. Dorsal bar. 8. Ventral anchor. 9. Dorsal anchor. All figures are drawn to the same scale (30 micrometers) except Figure 1 (100 micrometers).**



Figures 10–18. ***Urocleidoides paradoxus*** **sp. n. 10. Composite drawing of whole mount (ventral). 11. Vaginal sclerite. 12. Copulatory complex. 13. Hook (pairs 2, 3, 4, 6, 7). 14. Hook (pairs 1, 5). 15. Ventral bar. 16. Dorsal bar. 17. Ventral anchor. 18. Dorsal anchor. All illustrations are to the same scale (30 micrometers) except Figure 10 (100 micrometers).**

greatest width 83 (67–94) in anterior or posterior half. Pharyngeal diameter 19 (17–21). Haptor 59 (53–67) long, 75 (66–83) wide. Ventral anchor 38 (36–40), base width 18 (17–19); dorsal anchor 39 (37–40), base width 17 (16–18). Ventral bar 39 (37–40); dorsal bar 37 (35–40). Hook pair 1—21–22; hook pairs 2, 3, 4, 6—27 (25–30); hook pair 5—18–19; hook pair 7—31 (29–32). Cirrus 77 long, ring diameter 16 (14–17); accessory piece 26 (21–28) long. Testis 79 (73–85) × 29 (24–34); ovary 59 (52–66) × 24 (23–26). Vaginal sclerite 31 (28–32) long.

REMARKS: This is the only described species of *Urocleidoides* with a dextral vagina, sinistral vaginal sclerite, and hooks bearing a shank of two distinct parts. Based on the comparative morphology of the anchors and bars and the absence of eyes, this species most closely resembles *U. anops* Kritsky and Thatcher, 1974. In addition to the respective positions of the vagina, *U. paradoxus* is differentiated from this species by lacking a subterminal branch on the vaginal sclerite. The specific name, from Greek (*paradoxus* = incredible), refers to the fact that this species differs significantly from other congeneric forms by having a dextral vagina and sinistral vaginal sclerite.

Vancleaveus gen. n.

DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Head organs, cephalic lobes present; cephalic glands unicellular, comprising two bilateral groups posterolateral to pharynx. Eyes incipient or absent. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to testis, lacking diverticula. Gonads overlapping, intercaecal; testis dorsal to ovary. Vas deferens looping left intestinal caecum; seminal vesicle an elongate dilation of vas deferens; prostatic reservoir elongate, with proximal and distal terminations directed anteriorly. Cirrus comprising a base from which a coiled tube arises, tube with less than one to several rings, rings counterclockwise; accessory piece not articulated to cirrus, a fleshy rod serving as cirrus guide distally. Common genital pore midventral at level of intestinal bifurcation. Oviduct short; uterus delicate, extending anteriorly along midline; seminal receptacle near anterior end of ovary; vagina ventral, weakly sclerotized. Vitellaria well developed, coextensive with gut. Haptor armed with dorsal and ventral pairs of anchors, ventral and dorsal bars, seven pairs of hooks with ancyrocephaline distribution. Superficial root of dorsal anchor with conspicuous basal fold; hooks with inflated shank along entire length. Parasites of gills of siluriform fishes.

TYPE SPECIES AND HOST: *Vancleaveus janauacaensis* sp. n. from *Pterodoras granulosus* (Valenciennes), Doradidae.

OTHER SPECIES: *Vancleaveus fungulus* sp. n. from *Pseudoplatystoma tigrinum* (Cuvier and Valenciennes) (type host) and *P. fasciatus* (Linnaeus), Pimelodidae; *V. cicinnus* sp. n. from *Phractocephalus hemiliopterus* (Bloch and Schneider), Pimelodidae; *V. platyrhynchi* sp. n. from *Hemisorubim platyrhynchos* (Valenciennes), Pimelodidae.

REMARKS: Including the fact that members of *Vancleaveus* are parasitic on the gills of siluriform fishes, features distinguishing the genus include the combined presence of (1) a ventral vagina comprising a tube with an inconspicuous distal funnel, (2) overlapping gonads, (3) an elongate seminal vesicle, (4) a conspicuous, elongate prostatic vesicle, (5) dorsal anchors with conspicuous basal folds on the superficial roots, and (6) hooks with shanks inflated along their entire length. There are no other species in *Urocleidoides,* sensu Mizelle, Kritsky, and Crane (1968), that may be included in this genus. The genus is named for the late Dr. Harley J. Van Cleave who provided the first description of a monogenean from freshwater fishes of North America.

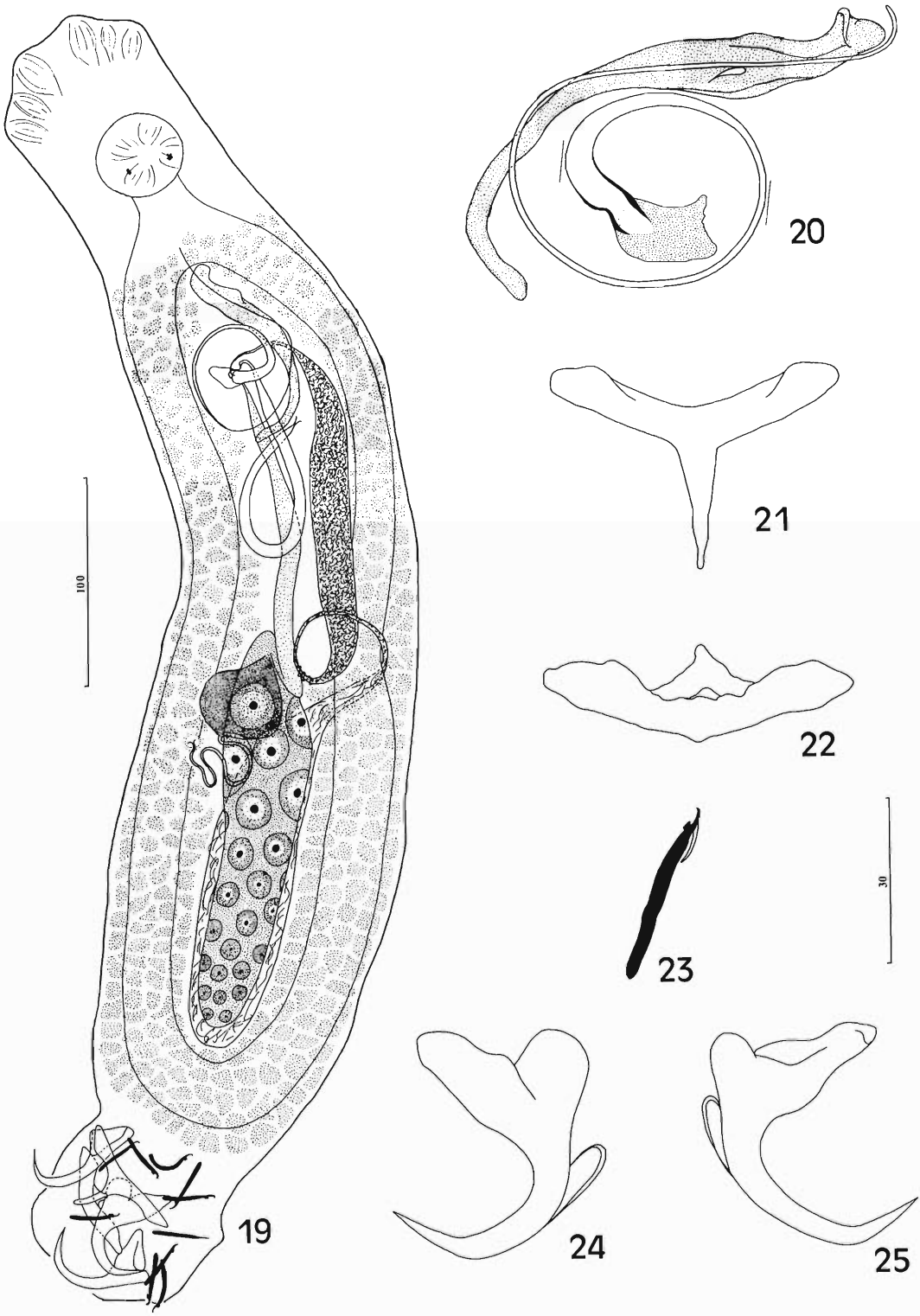
Vancleaveus janauacaensis sp. n. (Figs. 19–25)

HOST: Bacú liso, *Pterodoras granulosus* (Valenciennes), Doradidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (March 22, 1978; May 4, 1978).

TYPE SPECIMENS: Holotype, INPA PA262-1; paratypes, INPA PA262-2, PA262-3, USNM 78767, HWML 22946.

DESCRIPTION (based on 23 specimens; 20 measured): Body fusiform; cephalic area rounded or with two pairs of poorly developed lobes. Eyes 2, 3, or absent, dorsal to pharynx when present; eye granules usually dissociated, small, irregular; accessory granules few in anterior trunk. Pharynx subovate, with long axis oriented dorsoventrally; esophagus short. Peduncle broad; haptor hexagonal. Ventral anchor robust, with short shaft and point, well-developed deep and superficial roots. Dorsal anchor with bent shaft, short point,



Figures 19-25. ***Vancleaveus janauacaensis*** **sp. n. 19. Composite illustration of whole mount (ventral). 20. Copulatory complex (dorsal). 21. Ventral bar. 22. Dorsal bar. 23. Hook. 24. Ventral anchor. 25. Dorsal anchor. Illustrations are drawn to the 30-micrometer scale, except Figure 19 (100 micrometers).**

superficial root with conspicuous inner hump. Ventral bar broadly V-shaped with large posteromedial projection; dorsal bar with expanded ends, short anteromedial projection. Hooks similar, pair 5 somewhat reduced; each with recurved point, terminally flattened thumb, expanded shank; FH loop ¼ shank length. Gonads bacilliform. Cirrus coil with about 1½ rings, base with subrectangular flange; accessory piece variable, flattened distally. Vas deferens with external spiral filament at junction with seminal vesicle. Seminal receptacle pyriform; vagina dextroventral.

MEASUREMENTS: Body 606 (547–661) long, greatest width 138 (105–197) near midlength. Greatest diameter of pharynx 41 (33–48). Haptor 90 (72–118) long, 115 (83–138) wide. Ventral anchor 45 (41–48), base width 32 (29–36); dorsal anchor 43 (40–47), base width 30 (27–33). Ventral bar 57 (53–60); dorsal bar 52 (48–56). Hook pairs 1, 2, 3, 4, 6, 7—33 (30–36); hook pair 5—25 (24–26). Cirrus 230 long, ring diameter 43 (36–48); accessory piece 81 (69–90). Testis 144 (127–153) × 30 (24–35); ovary 115 (82–144) × 32 (29–34).

REMARKS: *Vancleaveus janauacaensis* sp. n. is the type species for the genus. It most closely resembles *V. fungulus* sp. n., from which it differs by possessing a cirrus with 1½ rings (about one ring in *V. fungulus*) and a ventral bar with an elongate posteromedial process (absent in *V. fungulus*). The specific name is derived from the type locality.

Vancleaveus cicinnus sp. n. (Figs. 26–32)

HOST: Pirarara, *Phractocephalus hemiliopterus* (Bloch and Schneider), Pimelodidae.

TYPE LOCALITY: Solimões River near Manaus, Amazonas, Brazil (October 16, 1982).

TYPE SPECIMENS: Holotype, INPA PA263-1; paratypes, INPA PA263-2, PA263-3, USNM 78768, HWML 22947.

DESCRIPTION (based on 11 specimens): Body flat, robust; cephalic lobes poorly developed, usually two terminal, two bilateral. Eyes absent. Pharynx subspherical; esophagus moderately long. Peduncle broad, short; haptor hexagonal. Ventral anchor with large roots, angular bend near junction of curved shaft and point. Dorsal anchor with curved shaft and point, incipient deep root, large basal fold on superficial root. Ventral bar with enlarged ends, elongate anteromedial process; dorsal bar with dorsal keel on each termination, anteromedial knob. Hooks similar, each with inflated shank, robust point and shaft, strongly depressed thumb; FH loop about ⅓ shank length. Gonads bacilliform; cirrus appearing sigmoid, representing a loose coil of about one ring; accessory piece increasing in breadth distally with ornate termination. Seminal receptacle irregular, vagina midventral.

MEASUREMENTS: Body 491 (427–585) long, greatest width 91 (75–113) at various points along trunk. Pharyngeal diameter 32 (27–38). Haptor 88 (75–107) long, 110 (87–130) wide. Ventral anchor 47 (43–54), base width 32 (28–35); dorsal anchor 46 (43–48), base width 32–33. Ventral bar 56 (51–65); dorsal bar 56 (47–61). Hook pairs 1, 2, 3, 4, 6, 7—33 (31–38); hook pair 5—21–22. Cirrus 89 long, ring diameter 24 (22–27); accessory piece 59 (54–66) long. Testis 122 × 26; ovary 162 × 33–34.

REMARKS: *Vancleaveus cicinnus* sp. n. most closely resembles *V. platyrhynchi* sp. n., from which it differs in the comparative size and morphology of the anchors and the position of the gonads. It differs from *V. janauacaensis* sp. n. by having a small sigmoid cirrus and a dorsal bar with a short median protuberance. Characters which separate it from *V. fungulus* sp. n. include the relative positions of the gonads and the presence of an elongate anteromedial projection on the ventral bar. The specific name is from Greek (*cicinn/o* = a curl of hair) and refers to the shape of the cirrus.

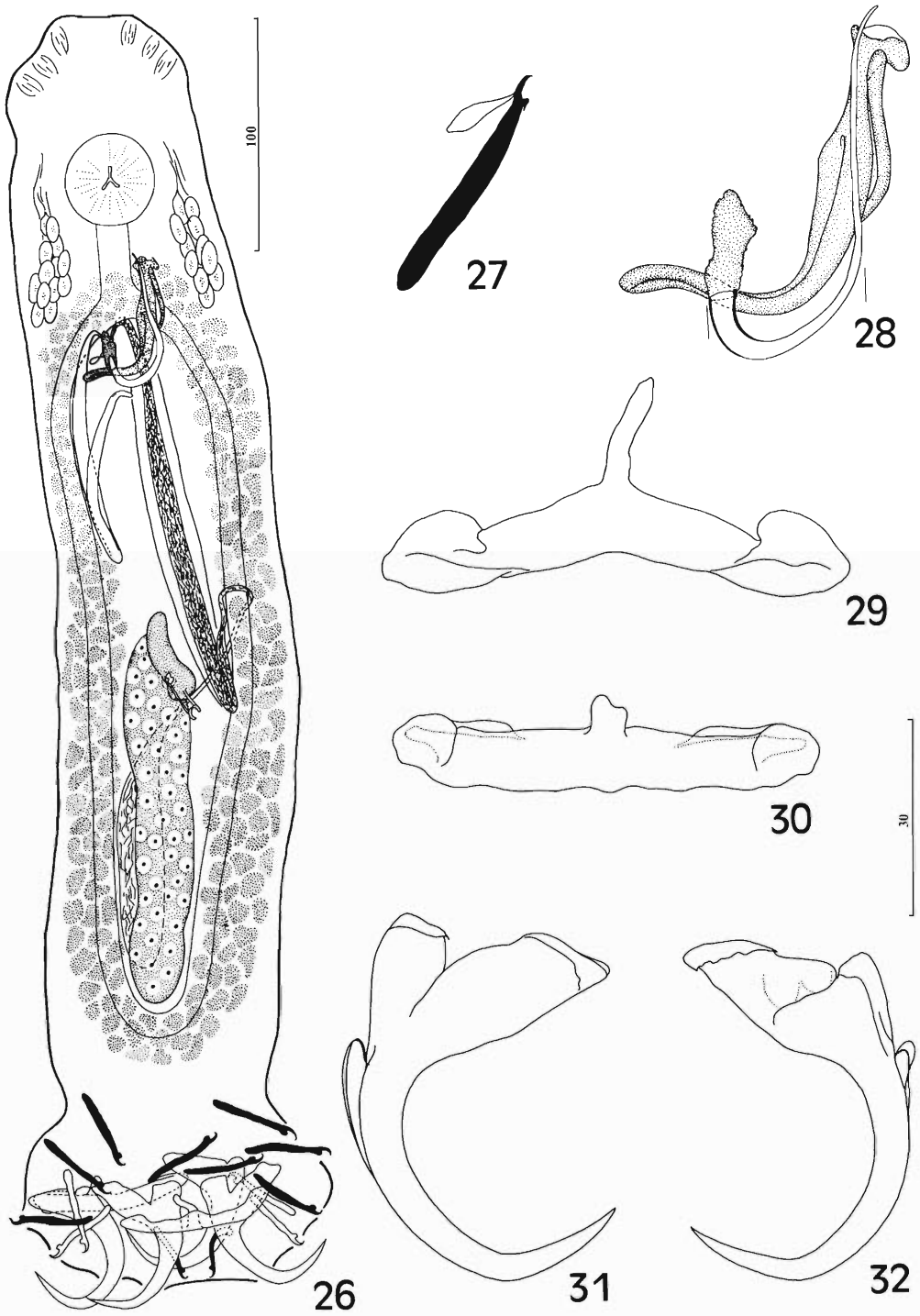
Vancleaveus fungulus sp. n. (Figs. 33–39)

HOST: Caparari, *Pseudoplatystoma tigrinum* (Cuvier and Valenciennes) (type) and sorubim, *P. fasciatum* (Linnaeus), Pimelodidae.

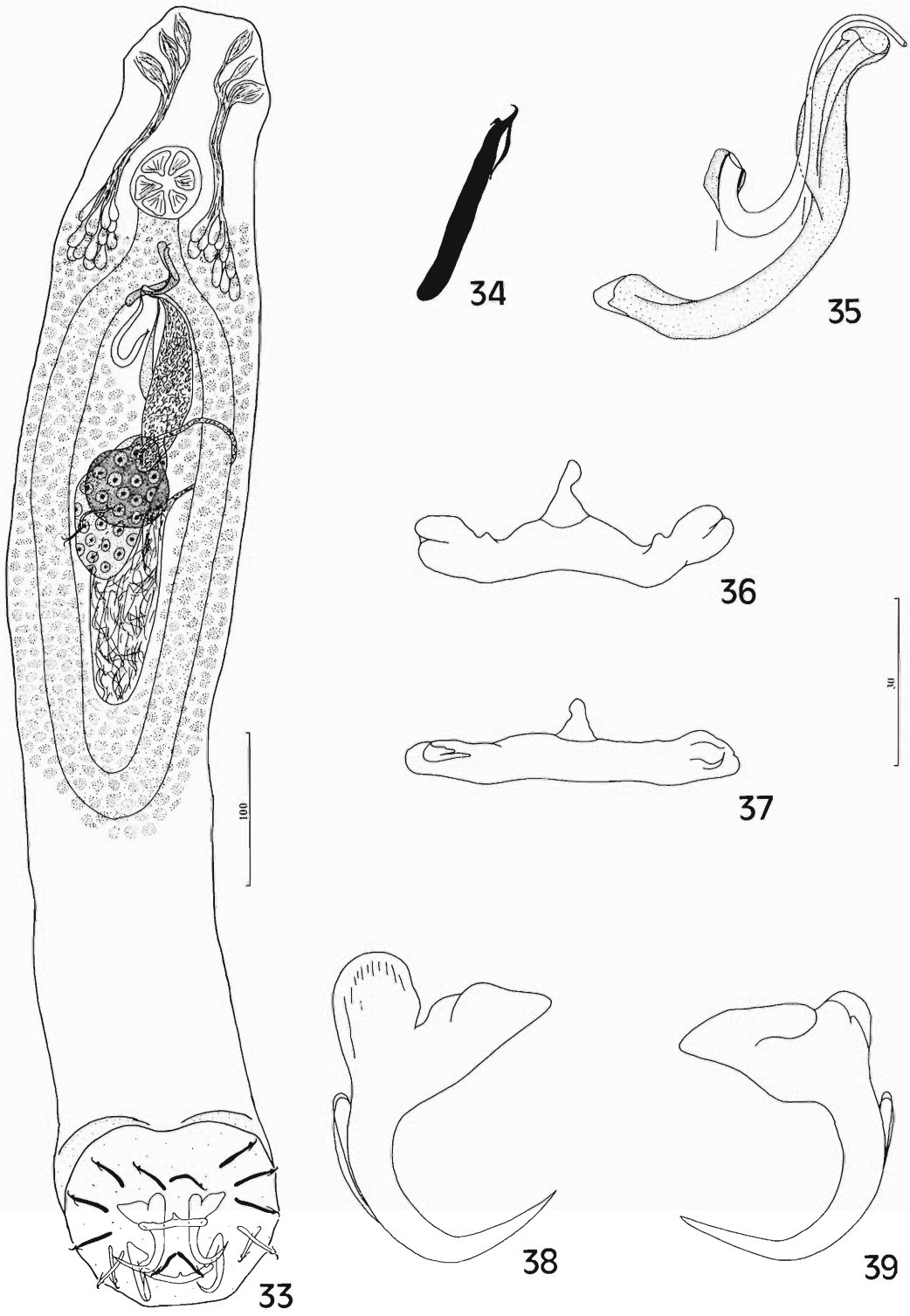
TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (April 7, 1978; April 23, 1980; June 7 and 8, 1983).

TYPE SPECIMENS: Holotype, INPA PA264-1; paratypes, INPA PA264-2 to PA264-5, USNM 78769 and 78770, HWML 22948.

DESCRIPTION (based on 41 specimens; 20 measured): Body elongate, fusiform; cephalic lobes 4, two terminal, two bilateral. Eyes absent; accessory granules small, subspherical, variable in size, scattered in cephalic area and anterior trunk. Pharynx subovate, with long axis oriented dorsoventrally; esophagus short. Peduncle elongate, broad; haptor circular to hexagonal. Ventral anchor with well-developed roots, curved shaft, short point; dorsal anchor with poorly developed



Figures 26-32. ***Vancleaveus cicinnus*** **sp. n. 26. Composite drawing, whole mount (ventral). 27. Hook. 28. Copulatory complex. 29. Ventral bar. 30. Dorsal bar. 31. Ventral anchor. 32. Dorsal anchor. Drawings of sclerotized parts are drawn to 30-micrometer scale; Figure 26 to 100-micrometer scale.**



Figures 33–39. ***Vancleaveus fungulus*** **sp. n. 33. Ventral view of holotype. 34. Hook. 35. Copulatory complex. 36. Ventral bar. 37. Dorsal bar. 38. Ventral anchor. 39. Dorsal anchor. All drawings are to the same scale (30 micrometers) except Figure 33 (100 micrometers).**

roots, curved shaft, short point. Bars similar, each variable with medial anterior projection. Hooks similar, pair 5 slightly reduced; each with recurved point, depressed thumb, inflated shank; FH loop flabellate, ⅓ shank length. Testis bacilliform; ovary pyriform. Cirrus sigmoid or a coil of about one ring; accessory piece variable, originating proximal to base of cirrus. Seminal receptacle fungulate; vagina ventral, slightly dextral.

MEASUREMENTS: Body 757 (440–1,104) long, greatest width 127 (81–179) in anterior half at level of gonads. Greatest diameter of pharynx 46 (41–54). Haptor 103 (74–147) long, 128 (102–160) wide. Ventral anchor 49 (47–53), base width 34 (32–37); dorsal anchor 47 (45–49), base width 32 (30–35). Ventral bar 55 (49–60); dorsal bar 53 (47–61). Hook pairs 1, 2, 3, 4, 6, 7—39 (32–43); hook pair 5—26–27. Cirrus 72 long, ring diameter 24 (21–28); accessory piece 69 (58–78). Testis 109 (85–131) × 48 (32–57); ovary 71 (59–85) × 33 (28–40).

REMARKS: *Vancleaveus fungulus* sp. n. is closely related to *V. cicinnus* sp. n. as shown by similarities of the copulatory complex, hooks, and bars. They are easily distinguished by the comparative morphology of the dorsal and ventral anchors, the positions of the gonads, and the nature of the peduncle. The specific name is from Latin (*fungulus* = a mushroom).

Vancleaveus platyrhynchi sp. n. (Figs. 40–46)

HOST: Braço de moça, *Hemisorubim platyrhynchos* (Valenciennes), Pimelodidae.

TYPE LOCALITY: Rio Solimões near Marchantaria Island, Manaus, Amazonas, Brazil (January 1984).

TYPE SPECIMENS: Holotype, INPA PA265-1; paratypes, INPA PA265-2 to PA265-4, USNM 78771, *HWML* 22949.

DESCRIPTION (based on 10 specimens): Body flat, robust; cephalic lobes poorly developed, usually two terminal, two bilateral. Eyes absent; accessory granules small, subovate, widely scattered in cephalic region and anterior trunk. Pharynx broad, elongate; haptor subcircular. Ventral anchor robust, with well-developed roots, angular bend near base of shaft and at junction of point and shaft; dorsal anchor with conspicuous superficial root, large basal fold, curved shaft, short point. Ventral bar with enlarged terminations, short anteromedial process; dorsal bar with flattened ends, anteromedial delicate keel. Hooks similar, each with inflated shank, delicate point and shaft, terminally flattened thumb; FH loop ¼ shank length. Testis bacilliform; ovary elongate, irregular. Cirrus appearing sigmoid, representing a loose coil of about one ring; accessory piece expanded distally, with ornate termination. Seminal receptacle irregular, vagina midventral.

MEASUREMENTS: Body 559 (462–716) long, greatest width 124 (108–142) usually in anterior half. Pharyngeal diameter 33 (32–36). Haptor 84 (75–96) long, 102 (87–120) wide. Ventral anchor 41 (40–42), base width 28 (26–29); dorsal anchor 38 (37–39), base width 26–27. Ventral bar 49 (47–52); dorsal bar 41 (39–46). Hook pairs 1, 2, 3, 4, 6, 7—31 (30–33); hook pair 5—22–23. Cirrus 96 long, ring diameter 22–23; accessory piece 64–65 long. Testis 56 × 26–27; ovary 146 (127–177) × 32 (30–33).

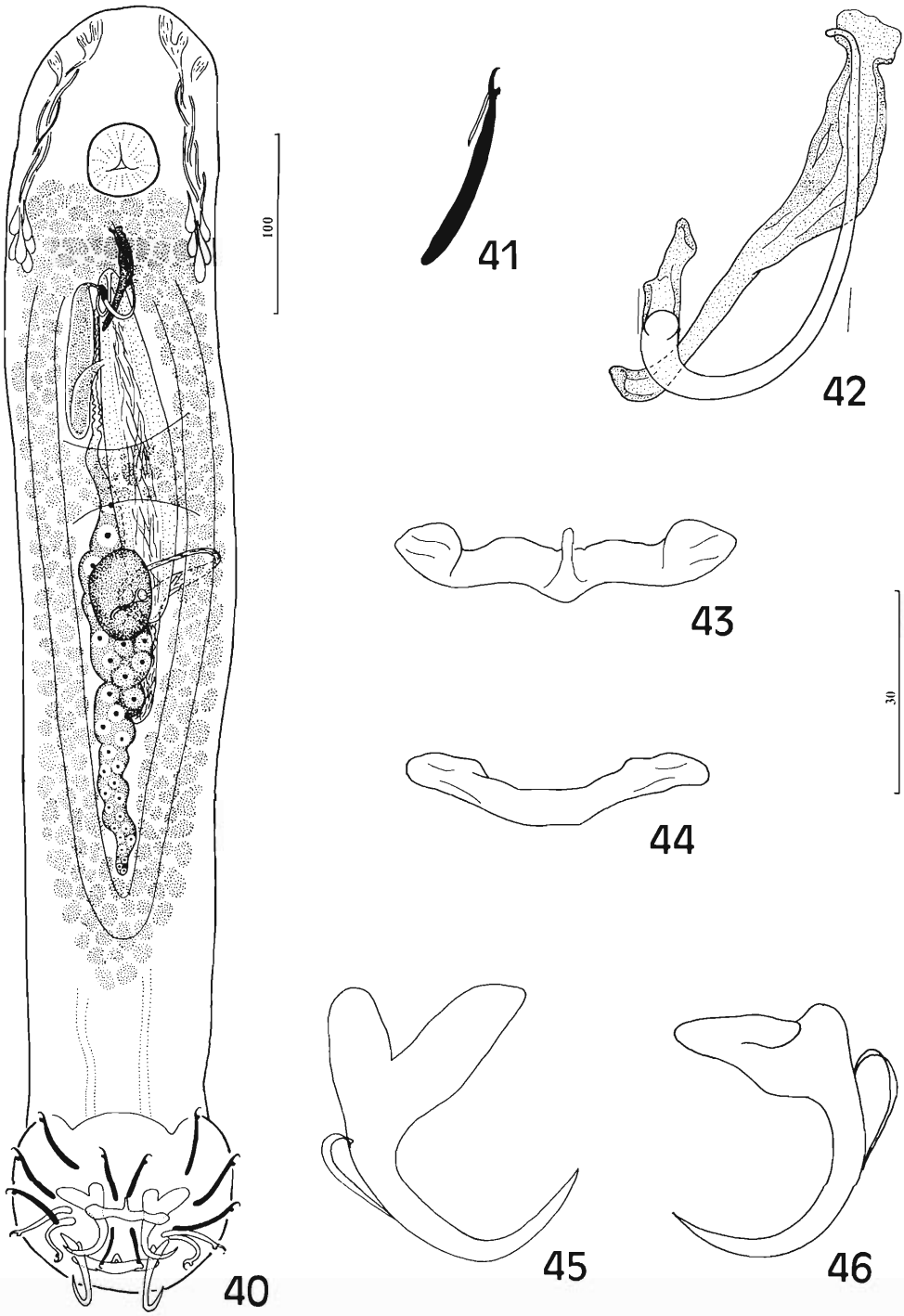
REMARKS: The closest relative of this species is *V. cicinnus* sp. n., from which it differs by possessing more robust but smaller anchors. The species is named for its host.

Cosmetocleithrum gen. n.

DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Head organs, cephalic lobes present; cephalic glands unicellular, comprising two bilateral groups posterolateral to pharynx. Eyes incipient or absent. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to testis, lacking diverticula. Gonads tandem, intercaecal; testis postovarian. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens; prostatic reservoir present. Copulatory complex comprising a variably coiled cirrus with counterclockwise rings, elaborate accessory piece not articulated to cirrus base. Common genital pore midventral, immediately posterior to intestinal bifurcation. Oviduct short; uterus delicate, extending anteriorly along midline; seminal receptacle inconspicuous; vagina sinistral, weakly sclerotized. Vitellaria well developed, coextensive with gut. Haptor armed with two pairs of anchors (dorsal and ventral), 14 hooks with ancyrocephaline distribution, ventral and dorsal bars. Dorsal bar with two submedial projections arising from anterodorsal surface of bar, directed posteriorly or posterolaterally. Parasites of the gills of fishes of the Order Siluriformes.

TYPE SPECIES AND HOST: *Cosmetocleithrum gussevi* sp. n. from *Oxydoras niger* (Valenciennes), Doradidae.

OTHER SPECIES: *Cosmetocleithrum bulbocirrus*



Figures 40–46. ***Vancleaveus platyrhynchi*** **sp. n. 40. Holotype (ventral view). 41. Hook. 42. Copulatory complex. 43. Ventral bar. 44. Dorsal bar. 45. Ventral anchor. 46. Dorsal anchor. Figure 40 is drawn to the 100-micrometer scale; all others to the 30-micrometer scale.**

sp. n. from *Pterodoras granulosus* (Valenciennes), Doradidae; and *C. confusus, C. parvum, C. rarum,* and *C. sobrinus* sp. n. all from *Oxydoras niger* (Valenciennes), Doradidae.

REMARKS: *Cosmetocleithrum* gen. n. resembles the African genus *Cichlidogyrus* Paperna, 1960 in that members of both possess dorsal bars with two submedian projections. These genera are differentiated by the species of *Cosmetocleithrum* possessing (1) tandem gonads (overlapping in *Cichlidogyrus*), (2) undilated hook shanks (basally dilated or often modified in *Cichlidogyrus*), (3) a sinistral vagina (ventral, sinistroventral, or dextroventral in *Cichlidogyrus*), and (4) a vas deferens looping the left intestinal caecum (intercaecal in *Cichlidogyrus*). See the generic diagnosis of *Cichlidogyrus* provided by Yamaguti (1963).

Although these genera phenotypically appear to be related, caution must be taken in proposing that the finding of *Cosmetocleithrum* species suggests common ancestry of Neotropical and Ethiopian Monogenea. Because members of the African genus occur naturally only on fishes of the family Cichlidae (Order Perciformes) and species of *Cosmetocleithrum* occur on doradid hosts of the Order Siluriformes, it is likely that these taxa represent ecomorphs (White and Keller, 1984) whose morphologic resemblances have resulted from convergence owing to the tracking of similar ecological resources.

Although *Cosmetocleithrum* is clearly a part of the *Urocleidoides* complex defined by Mizelle et al. (1968), there are no previously described species that could be included in the new genus. The generic name is from Greek (*cosmet/o* = adorned + *cleithrum* = bar) and refers to the characteristic dorsal bar.

Cosmetocleithrum gussevi sp. n. (Figs. 47–54)

HOST: Cuiú-cuiú, *Oxydoras niger* (Valenciennes), Doradidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (June 3, 1978; August 1982).

TYPE SPECIMENS: Holotype, INPA PA266-1; paratypes, INPA PA266-2, USNM 78772, HWML 22950.

DESCRIPTION (based on eight specimens): Body fusiform; two terminal, two bilateral cephalic lobes moderately developed. Eyes, eye granules absent. Pharynx ovate; esophagus elongate. Peduncle elongate, broad; haptor ellipsoidal. Anchors similar; each with poorly developed roots, large base, evenly curved shaft and point. Ventral bar broadly V-shaped, with posteromedial rounded keel; dorsal bar with pointed posteromedial protuberance, posterior projections flattened. Hooks similar; each with tapered shaft and point, erect thumb, proximally tapered shank; FH loop ¾ shank length; hook pair 1 peduncular, hook pairs 5 and 6 apparently absent. Gonads subovate; seminal vesicle elongate. Cirrus a coil of 2–3 rings; accessory piece variable, usually Y-shaped. Vagina a sclerotized tube. Egg with moderately long proximal projection, end of projection flattened.

MEASUREMENTS: Body 1,012 (894–1,182) long, greatest width 160 (139–213) at level of testis in posterior half of trunk. Pharyngeal diameter 46 40–52). Haptor 88 (86–92) long, 129 (117–151) wide. Ventral anchor 49 (44–53), base width 34 (28–38); dorsal anchor 46 (39–48), base width 31 (24–33). Hook (all pairs) 16 (15–17). Cirrus 122 long, ring diameter 24 (22–26); accessory piece 45 (41–48). Testis 237 (221–252) × 119–120; ovary 63 (59–67) × 43–44. Egg 97 × 58.

REMARKS: This species is unique in that adults lack hook pairs 5 and 6 ; all other species in the genus possess seven pairs of haptoral hooks. *Cosmetocleithrum gussevi* sp. n. is the type species for the genus and is named in honor of Dr. A. V. Gussev, U.S.S.R. Academy of Sciences, Leningrad, a friend, in recognition of his important studies on Monogenea.

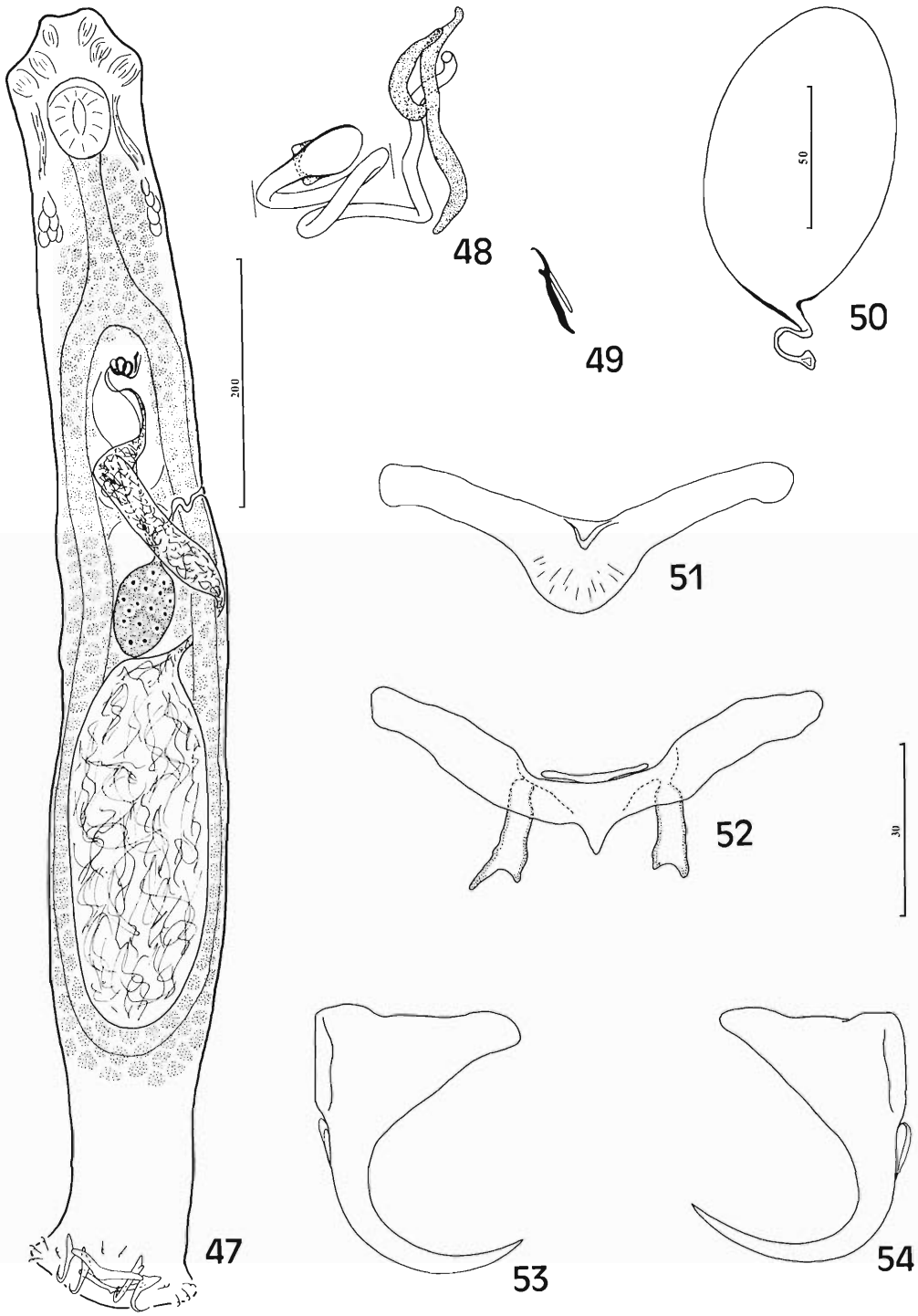
Cosmetocleithrum confusus sp. n. (Figs. 55-62)

HOST: Cuiú-cuiú, *Oxydoras niger* (Valenciennes), Doradidae.

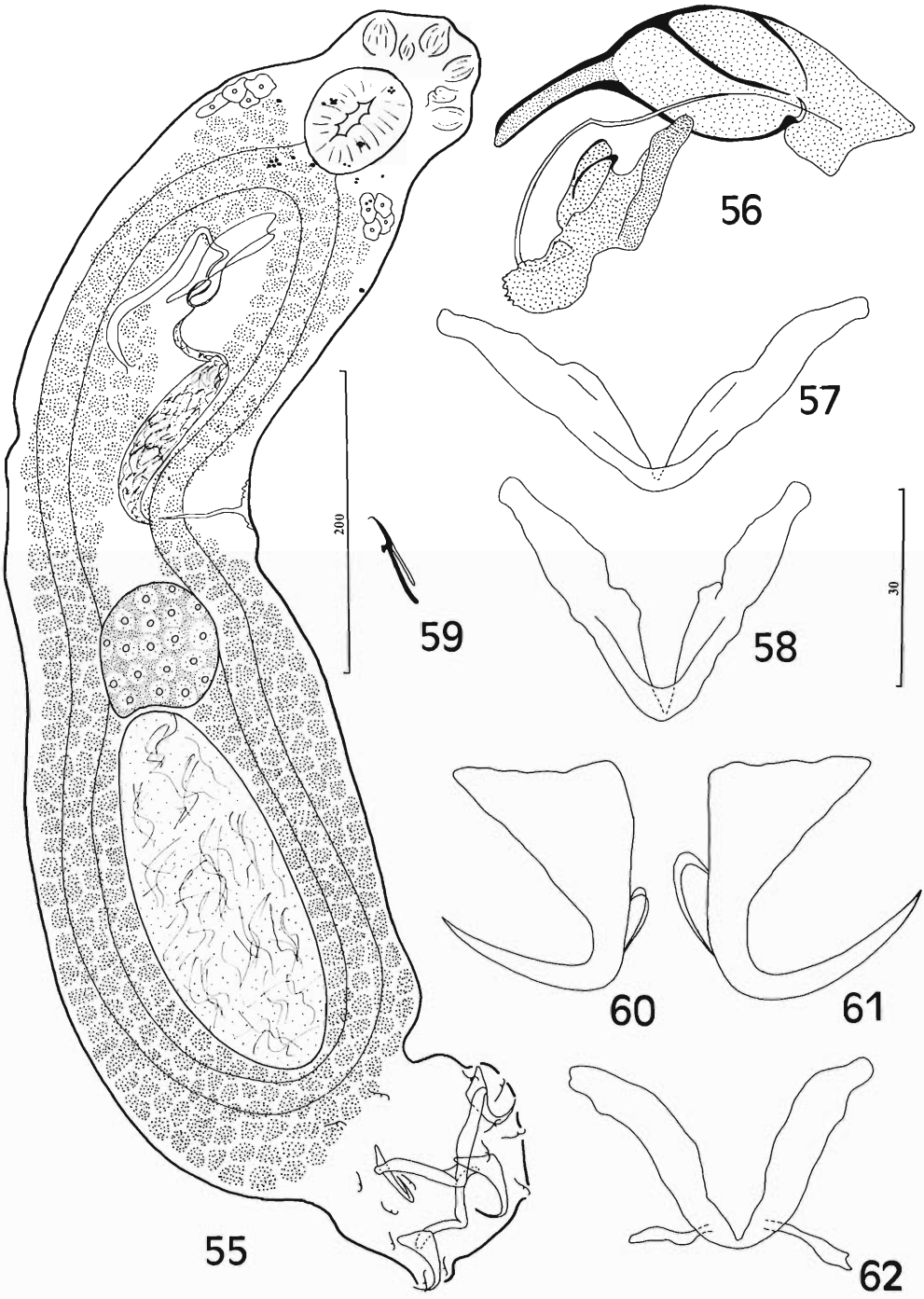
TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (June 3, 1978; May 6, 1983; August 1982).

TYPE SPECIMENS: Holotype, INPA PA267-1; paratypes, INPA PA267-2, PA267-3, USNM 78773, HWML 22951.

DESCRIPTION (based on 20 specimens): Body robust; cephalic lobes poorly developed, two terminal, two bilateral. Eyes 2 or absent; eye granules small, subspherical; accessory granules scattered throughout cephalic area and anterior trunk. Pharynx subovate; esophagus short to absent. Peduncle short, broad; haptor hexagonal. Anchors similar; each with poorly developed roots, large base, short shaft, elongate point. Bars V-shaped; dorsal bar projections variable. Hooks



Figures 47–54. ***Cosmetocleithrum gussevi*** **sp. n. 47. Holotype (ventral). 48. Copulatory complex. 49. Hook. 50. Egg. 51. Ventral bar. 52. Dorsal bar. 53. Ventral anchor. 54. Dorsal anchor. All drawings are to the same scale (30 micrometers) except Figure 47 (200 micrometers) and Figure 50 (50 micrometers).**



Figures 55–62. ***Cosmetocleithrum confusus*** **sp. n. 55. Ventral view of holotype. 56. Copulatory complex. 57, 58. Ventral bars. 59. Hook. 60. Ventral anchor. 61. Dorsal anchor. 62. Dorsal bar. All figures are drawn to the same scale (30 micrometers) except Figure 55 (200 micrometers).**

similar, each with tapered shaft and point, depressed thumb, slender shank; FH loop ¾ shank length; hook pair 1 peduncular. Testis ovate; ovary subspherical, closely appressed to anterior margin of testis; seminal vesicle elongate; prostatic reservoir narrow, elongate. Cirrus a loose, poorly defined coil of about 1–1½ rings; shaft delicate, base large. Accessory piece appearing as a hollow structure with sclerotized walls and truncate termination. Vagina lightly sclerotized, with wide lateral opening.

MEASUREMENTS: Body 564 (449–706) long, greatest width 158 (81–185) in anterior or posterior half. Greatest diameter of pharynx 46 (45–47). Haptor 84 (70–95) long, 107 (97–123) wide. Ventral anchor 34 (31–36), base width 25 (21–27); dorsal anchor 38 (36–43), base width 24 (22–26). Ventral bar 59 (47–74), dorsal bar 53 (47–62). Hook (all pairs) 15 (14–16). Cirrus 75 long, accessory piece 61 (53–64). Testis 155 (103–193) × 62 (39–96); ovary 59 (46–92) × 57 (46–76).

REMARKS: *Cosmetocleithrum confusus* sp. n. is closely related to *C. parvum* and *C. sobrinus* spp. n. as shown by the comparative morphology of the copulatory complexes. It differs from *C. parvum* by possessing anchors with comparatively short shafts and poorly developed roots and by having a delicate cirral tube with enlarged base. It is differentiated from *C. sobrinus* by being significantly smaller, lacking anchor roots and the exaggerated baglike accessory piece.

Cosmetocleithrum confusus exhibits a great deal of variation in size of its sclerotized haptoral parts, although morphology is relatively stable. Variation in the distance between the ends of each haptoral bar reflects differences in the angles of the V-shaped structures (compare Figs. 57 ,58). In individual specimens, differences in sizes between the ventral and dorsal anchors ranged from 1.4 to 6.5 micrometers, with the dorsal anchor always being the larger. No useful measurement of the cirral ring diameter could be made because the coil was extremely loose and frequently distorted as a result of coverslip pressure.

The specific name reflects the possible confusion of the accessory piece for the cirrus.

Cosmetocleithrum bulbocirrus sp. n. (Figs. 63–69)

HOST: Bacú liso, *Pterodoras granulosus* (Valenciennes), Doradidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (March 22, 1978).

TYPE SPECIMENS: Holotype, INPA PA268-1; paratypes, INPA PA268-2, USNM 78774, HWML 22952.

DESCRIPTION (based on 14 specimens): Body fusiform; cephalic lobes poorly developed, two terminal, two bilateral. Eyes absent; accessory granules large, subspherical, present in anterior trunk and cephalic area. Pharynx subspherical to ovate; esophagus short. Peduncle short, broad; haptor subspherical. Anchors similar, each with elongate point, straight shaft, well-developed roots. Ventral bar broadly V-shaped, with expanded terminations; dorsal bar V-shaped, with medial narrow region, projections delicate usually directed laterally. Hooks similar, each with recurved point, erect thumb, slender shank; FH loop $^{9}/_{10}$ shank length; hook pair 1 peduncular. Testis ovate, ovary pyriform; seminal vesicle an indistinct dilation of vas deferens; prostatic reservoir gourd-shaped, with smooth wall. Cirrus a coil of about two rings, with terminal bulbous expansion; accessory piece a variable fleshy rod. Vagina unsclerotized, a tube with distal sphincter.

MEASUREMENTS: Body 544 (452–617) long, greatest width 99 (81–132) in posterior half. Greatest diameter of pharynx 32 (25–35). Haptor 68 (54–82) long, 79 (77–82) wide. Ventral anchor 32 (31–34), base width 18 (17–19); dorsal anchor 31 (29–34), base width 16 (14–18). Ventral bar 44 (41–49), dorsal bar 44 (37–50). Hook (all pairs) 16 (14–17). Cirrus 136 long, ring diameter 26 (24–31); accessory piece 21 (20–23). Testis 102 (93–107) × 41 (36–48); ovary 51 × 28.

REMARKS: This species is distinct from other species in the genus in that the dorsal bar projections are delicate and generally directed laterally. It most closely resembles *Cosmetocleithrum rarum* sp. n. in the morphology of the copulatory complex and anchors, but is readily separated from it by the comparative shapes of the bars and the terminal portion of the cirrus shaft. The specific name, *bulbocirrus,* refers to the terminal inflation of the cirrus shaft.

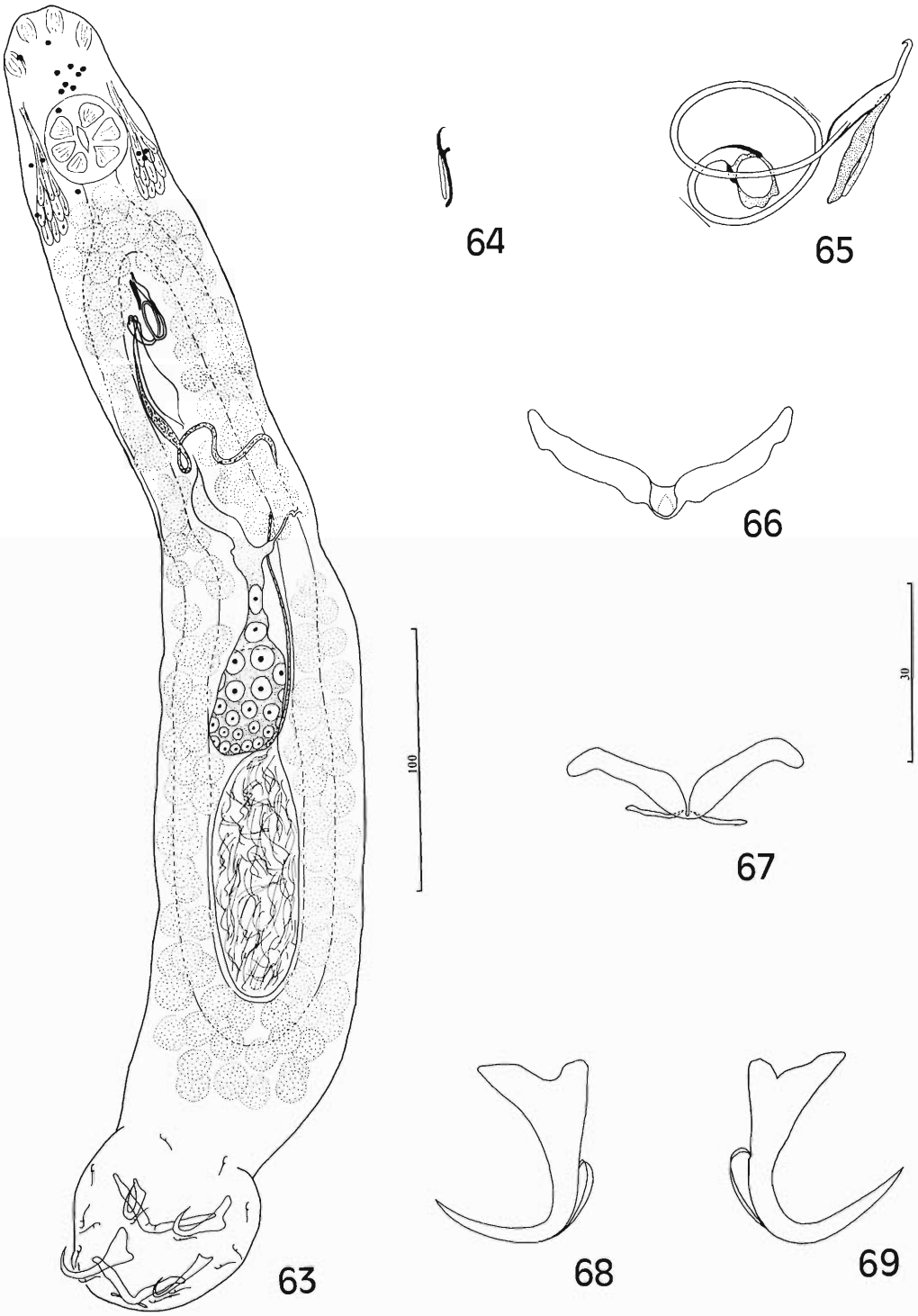
Cosmetocleithrum parvum sp. n. (Figs. 70–75)

HOST: Cuiú-cuiú, *Oxydoras niger* (Valenciennes), Doradidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (February 9, 1979; August 1982; May 6, 1983).

TYPE SPECIMENS: Holotype, INPA PA269-1; paratypes, INPA PA269-2, PA269-3, USNM 78775, HWML 22953.

DESCRIPTION (based on 20 specimens): Body



Figures 63–69. ***Cosmetocleithrum bulbocirrus*** **sp. n. 63. Ventral view of holotype. 64. Hook. 65. Copulatory complex. 66. Ventral bar. 67. Dorsal bar. 68. Ventral anchor. 69. Dorsal anchor. All figures are to the same scale (30 micrometers) except Figure 63 (100 micrometers).**

bacilliform or fusiform; cephalic margin usually rounded or with two terminal, two bilateral incipient lobes. Eyes absent; accessory granules varying in size from minute to small, subovate, occasionally scattered throughout cephalic and trunk regions. Pharynx subspherical; esophagus moderately long. Peduncle short, frequently constricted; haptor subpyramidal. Anchors similar, each with elongate point, short shaft, small base, well-developed roots. Ventral bar V-shaped, with narrowed terminations; dorsal bar U-shaped, with elongate projections directed posteriorly. Hooks similar, each with tapered shaft and point, slightly depressed thumb, slender shank; FH loop ½ shank length; hook pair 1 peduncular. Testis elongate ovate, ovary pyriform; seminal vesicle indistinct. Cirrus a poorly defined coil of about one ring; accessory piece with proximal arm, hollow bulbous portion distally. Vagina comprising a relatively large bag distally with numerous infoldings into lumen.

MEASUREMENTS: Body 475 (338–573) long, greatest width 86 (63–116) in trunk. Pharyngeal diameter 28 (27–29). Haptor 73 (65–84) long, 89 (70–107) wide. Ventral anchor 25 (24–27), base width 16 (14–18); dorsal anchor 27 (25–29), base width 15 (13–18). Ventral bar 43 (34–49); dorsal bar 34 (26–40). Hook pairs 1, 2, 3, 4, 6, 7—15 (14–16), hook pair 5—16 (15–18). Cirrus 54 long, ring diameter 13 (12–15); accessory piece 32 (30–34) long. Testis 111–112 × 39–40; ovary 43–44 × 25–26.

REMARKS: *Cosmetocleithrum parvum* sp. n. most closely resembles *C. sobrinus* sp. n., from which it is distinguished by having a significantly smaller size, a smaller copulatory complex, and in the comparative morphology of the ventral and dorsal bars. The species name is from Latin (*parvus* = small).

Cosmetocleithrum rarum sp. n. (Figs. 76-81)

HOST: Cuiú-cuiú, *Oxydoras niger* (Valenciennes), Doradidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (August 1982).

TYPE SPECIMEN: Holotype, INPA PA270-1.

DESCRIPTION (based on one specimen): Body fusiform; cephalic lobes well developed, two terminal, two bilateral. Single eyespot present; granules variable in size and shape; accessory granules absent. Pharynx subspherical; esophagus short. Peduncle short, broad; haptor hexagonal. Anchors similar, delicate; each with well-developed base, evenly curved point and shaft, well-developed roots. Ventral bar with posteromedial rotund projection; dorsal bar V-shaped, projections elongate. Hooks similar; each with finely tapered shaft and point, erect thumb, narrow shank; hook pair 1 subpeduncular; FH loop ¾ shank length. Gonads subovate; seminal vesicle conspicuous. Cirrus a coil of 2–3 rings, base small; accessory piece variable with ventral oblique groove. Vagina a funnel with small irregular protuberances in lumen of expanded portion.

MEASUREMENTS: Body 594 long, greatest width 115 in posterior trunk at level of testis. Pharyngeal diameter 34. Haptor 103 long, 108 wide. Ventral anchor 42, base width 24; dorsal anchor 38, base width 20. Ventral bar 37; dorsal bar 27. Hook (all pairs) 14–15. Cirrus 138 long, ring diameter 18; accessory piece 25 long. Testis 109 × 35; ovary 45 × 57.

REMARKS: The delicate anchors with evenly curved points and shafts distinguish this species from all others in the genus. Based on the morphology of the copulatory complex, it most closely resembles *C. bulbocirrus* sp. n., but *C. rarum* lacks the terminal inflation of the cirrus shaft. The specific name is from Latin (*rarum* = rare) and refers to the fact that it was found only once in several collections of the host from the Manaus area in Brazil.

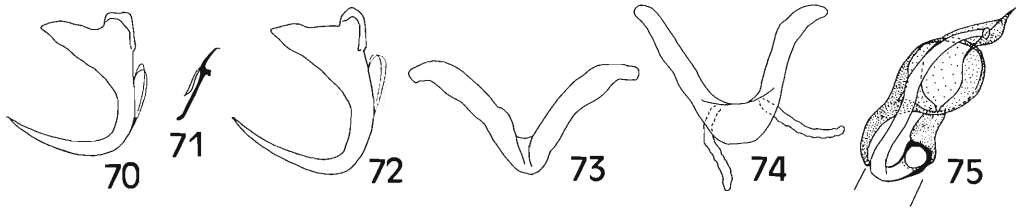
Cosmetocleithrum sobrinus sp. n. (Figs. 82-88)

HOST: Cuiú-cuiú, *Oxydoras niger* (Valenciennes), Doradidae.

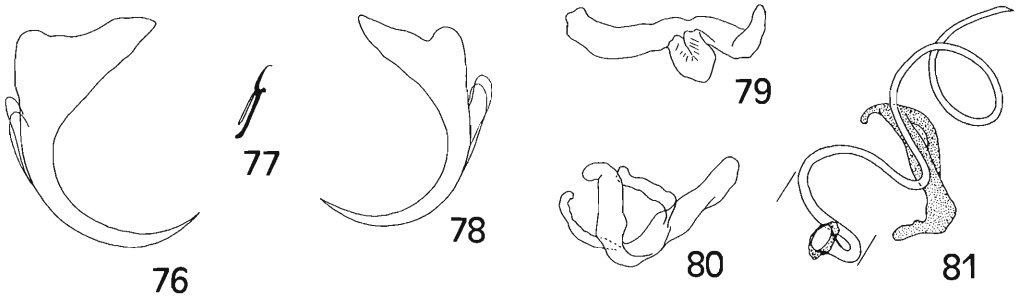
TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (February 9, 1979; August 1982).

TYPE SPECIMENS: Holotype, INPA PA271-1; paratypes, INPA PA271-2, USNM 78776, HWML 22954.

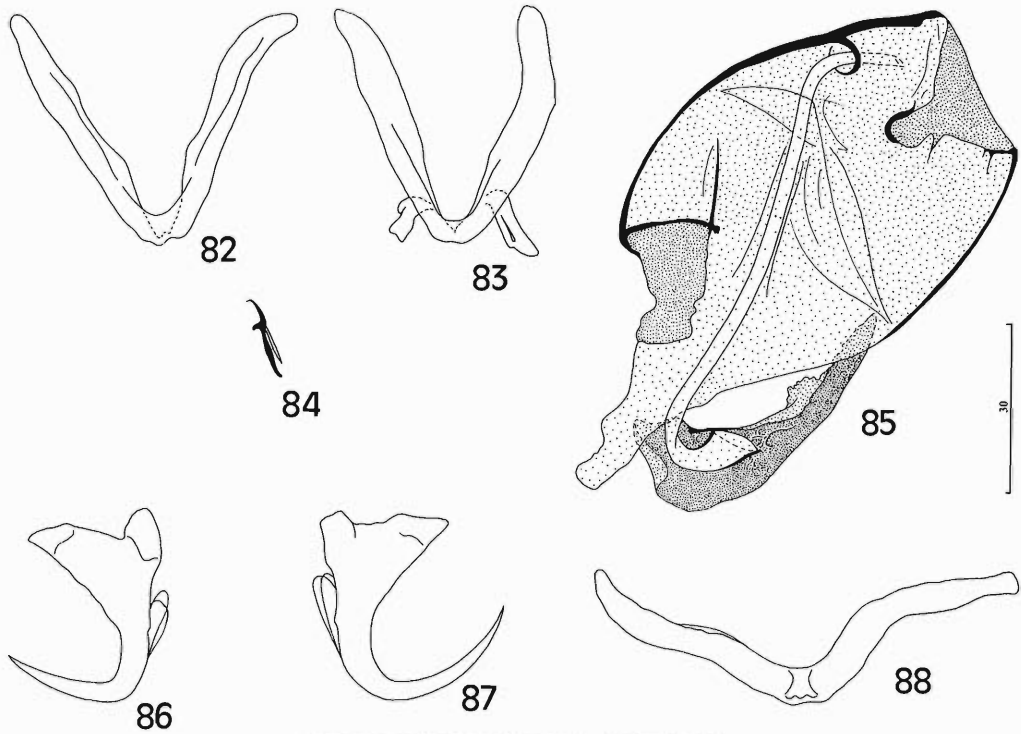
DESCRIPTION (based on 11 specimens): Body robust, fusiform; cephalic margin usually rounded or with two terminal, two bilateral lobes. Eyes 2, comprised of small spherical granules; accessory granules frequently absent, occasionally scattered in cephalic region. Pharynx subspherical; esophagus short. Peduncle broad, elongate; haptor bulbar. Ventral anchor with large deep root, broad superficial root, short straight shaft, slightly curved point; dorsal anchor with well-developed roots, short shaft, curved elongate point. Ventral bar broadly V-shaped, with ventral posterior fold along most of its length; dorsal bar V-shaped, projections short. Hooks similar;



Cosmetocleithrum parvum



Cosmetocleithrum rarum



Cosmetocleithrum sobrinus

Figures 70-88. Sclerotized parts of *Cosmetocleithrum* species. Figures 70-75. *Cosmetocleithrum parvum* sp. n. 70. Ventral anchor. 71. Hook. 72. Dorsal anchor. 73. Ventral bar. 74. Dorsal bar. 75. Copulatory complex. Figures 76-81. *Cosmetocleithrum rarum* sp. n. 76. Ventral anchor. 77. Hook. 78. Dorsal anchor. 79. Ventral bar. 80. Dorsal bar. 81. Copulatory complex. Figures 82-88. *Cosmetocleithrum sobrinus* sp. n. 82, 88. Ventral bars. 83. Dorsal bar. 84. Hook. 85. Copulatory complex. 86. Ventral anchor. 87. Dorsal anchor. All drawings are to the same scale (30 micrometers).

each with delicate point, straight tapered shaft, depressed thumb, shank swollen near midlength; FH loop ¾ shank length. Cirrus a conspicuously extended coil of about one ring, appearing as a straight tube with proximal and distal ends bent ventral; accessory piece large, globose, apparently hollow. Vagina a weakly sclerotized, irregular tube.

MEASUREMENTS: Body 1,088 (752–1,344) long, greatest width 264 (231–307) in anterior half. Pharyngeal diameter 95 (76–103). Haptor 94 (76–127) long, 129 (103–143) wide. Ventral anchor 34 (32–35), base width 23 (22–25); dorsal anchor 35 (33–37), base width 22 (21–23). Ventral bar 58 (45–75); dorsal bar 47 (39–53). Hook (all pairs) 17 (16–20). Cirrus 134 long; accessory piece 99 (82–122) long. Testis 121 × 86; ovary 115 (98–132) × 97 (67–127).

REMARKS: *Cosmetocleithrum sobrinus* sp. n. is closely related to *C. parvum* sp. n. and *C. confusus* sp. n. as indicated by the morphology of their accessory pieces. It is a larger worm than either species and can be further separated from them in the comparative morphology of the cirrus, anchors, bars, and hooks. The specific name is from Latin (*sobrinus* = a cousin) and refers to the relationships with the above named species.

Gussevia Kohn and Paperna, 1964

EMENDED DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle and haptor. Tegument thin, smooth. Head organs, cephalic lobes present; cephalic glands unicellular, comprising bilateral groups posterolateral to pharynx. Eyes present, frequently dissociated. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to gonads. Gonads overlapping, intercaecal; testis dorsal to ovary. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens; prostatic reservoirs indistinct or apparently absent. Cirrus comprising a base from which a coiled tube arises; tube with less than one to several clockwise rings. Accessory piece distally ornate or complex. Common genital pore midventral near level of intestinal bifurcation. Oviduct short; uterus delicate, extending anteriorly along midline; seminal receptacle usually conspicuous immediately anterior to ovary; vagina sinistral, ventral, or usually dextral; vitellaria well developed, coextensive with intestinal caeca. Haptor developed into anterior and posterior lobes, armed with dorsal and ventral pairs of anchors, 14 hooks, and ventral and dorsal bars. Ventral anchors lying on posterior haptoral lobe, modified in shape, possessing a conspicuous anchor filament. Hook pairs 1, 2, 3, 4, 6, 7 similar, with slender shanks, lying on anterior haptoral lobe; pair 5 modified, usually elongate, delicate, associated with ventral anchors. Parasites of the gills of Neotropical cichlid fishes.

TYPE SPECIES AND HOST: *Gussevia spiralocirra* Kohn and Paperna, 1964 from *Pterophyllum scalare* (Lichtenstein) (=*P. eimekei* Ahl), Cichlidae.

OTHER SPECIES: *G. alii* (Molnar et al., 1974), *G. cichlasomatis* (Molnar et al., 1974), and *G. dobosi* (Molnar et al., 1974) combs. n. from *Cichlasoma bimaculatum* (Linnaeus); *G. obtusa* and *G. elephus* spp. n. from *Uaru amphiacanthoides* (Heckel); *G. longihaptor* (Mizelle and Kritsky, 1969) comb. n., *G. undulata, G. arilla,* and *G. tucunarense* spp. n. from *Cichla ocellaris* Bloch and Schneider; and *G. alioides, G. dispar,* and *G. disparoides* spp. n. from *Cichlasoma severum* (Heckel).

OTHER POSSIBLE INCLUSIONS: *Trinidactylus cichlasomatis* Hanek, Molnar, and Fernando, 1974 from *Cichlasoma bimaculatum* (Linnaeus).

REMARKS: Features which distinguish *Gussevia* from other genera in the *Urocleidoides* complex include the combined presence of (1) overlapping gonads, (2) a haptor with anterior and posterior lobes, (3) a modified ventral anchor with well-developed anchor filament, (4) a modified hook pair 5 usually delicate and lying on the posterior haptoral lobe with the ventral anchors, and (5) a cirrus coil with clockwise rings. All known species of *Gussevia* are parasitic on cichlid fishes, whereas *Urocleidoides* (as defined herein) species occur primarily on characoid hosts; *Vancleaveus* and *Cosmetocleithrum* species are found only on hosts of the Order Siluriformes.

Kohn and Paperna (1964) proposed *Gussevia* for ancyrocephaline species characterized primarily by having a dextral vagina, a cirrus with a long spiral (coiled) tube, and accessory piece attached to the distal end of the cirrus (not basally articulated). Designating their new species, *G. spiralocirra,* as the type species, they included *G. minuta* Kohn and Paperna, 1964, from the guppy (*Poecilia reticulata*) and two species described previously by Jain (1958) from India, *G. rhynchobdelli* (=*Urocleidus r.*) and *G. xenentodoni* (=*Urocleidus x.*). From their definition of the genus, their remarks, and the fact that *G. minuta*

(considered herein as a junior synonym of *Urocleidoides reticulatus* Mizelle and Price, 1964, type species of *Urocleidoides*) was included in their genus, it was obvious to Kritsky and Thatcher (1983) that the original authors had considered the configuration of *Gussevia* identical to that previously defined for *Urocleidoides* as emended by Mizelle et al. (1968). As a result, Kritsky and Thatcher (1983) considered the two genera synonymous, with *Urocleidoides* having priority; without comment, these authors transferred *G. spiralocirra* and *G. minuta* to this genus. *G. rhynchobdelli* and *G. xenentodoni* were excluded from *Urocleidoides* by Thatcher and Kritsky (1983), thus returning them provisionally to *Urocleidus*.

Mizelle and Kritsky (1969) proposed *Longihaptor* for what appeared as a unique species (*L. longihaptor*) from the gills of the aquarium fish *Cichla ocellaris*. The monotypic *Longihaptor* was characterized by having the ventral anchors situated on a conspicuous posterior haptoral lobe and adorned with a heavy anchor filament; modification of hook pair 5 was also considered a distinguishing character and the vagina was not observed in their specimens. These authors were concomitantly working with several *Urocleidoides* species, which enhanced the apparent distinction of the two genera. Collection of numerous species of Ancyrocephalinae for the present study, including *Gussevia spiralocirra* Kohn and Paperna, 1964 and other species from cichlids in South America, has shown that the group proposed by Mizelle and Kritsky (1969) is likely valid but that *Gussevia* has priority because its type species is clearly a member. Thus, *L. longihaptor* Mizelle and Kritsky, 1969 is transferred to *Gussevia* as a new combination. Based on comparison of type specimens, *Cleidodiscus bulbus* Rogers and Rawson, 1969, under study simultaneously with the work of Mizelle and Kritsky (1969), is considered a junior subjective synonym of *G. longihaptor* comb. n.

Our study of paratypes of *Urocleidoides alii, U. cichlasomatis,* and *U. dobosi,* all described by Molnar et al. (1974), confirms that these species are members of *Gussevia* as emended herein. Although paratypes of all three species are unstained and therefore unsuitable for study of internal features, they are clearly members of *Gussevia* based on the morphology and configuration of the haptor and its armament. Ventral anchors of all three species possess blunt points similar to those of *G. spiralocirra* Kohn and Paperna, 1964 and *G. alioides* sp. n. (Figs. 95, 119). Thus, the following new combinations are proposed: *G. alii* (Molnar et al., 1974) comb. n., *G. cichlasomatis* (Molnar et al., 1974) comb. n., *G. dobosi* (Molnar et al., 1974) comb. n.

In addition, *Trinidactylus cichlasomatis* Hanek, Molnar, and Fernando, 1974 is likely a member of *Gussevia*. Our study of two paratype specimens has revealed that at least one of them possesses two pairs of anchors. The dorsal "hooklike structures," described for this species and one of the characters used to establish the monotypic *Trinidactylus,* apparently represent the point and shaft of one pair of anchors whose bases are difficult to observe in the cleared and unstained paratypes. Because two pairs of anchors were definitely observed only in one paratype, we hesitate to formally transfer this species to *Gussevia*; examination of fresh material will probably be necessary to determine the valid generic placement of this species.

Gussevia spiralocirra
Kohn and Paperna, 1964
(Figs. 89-96)

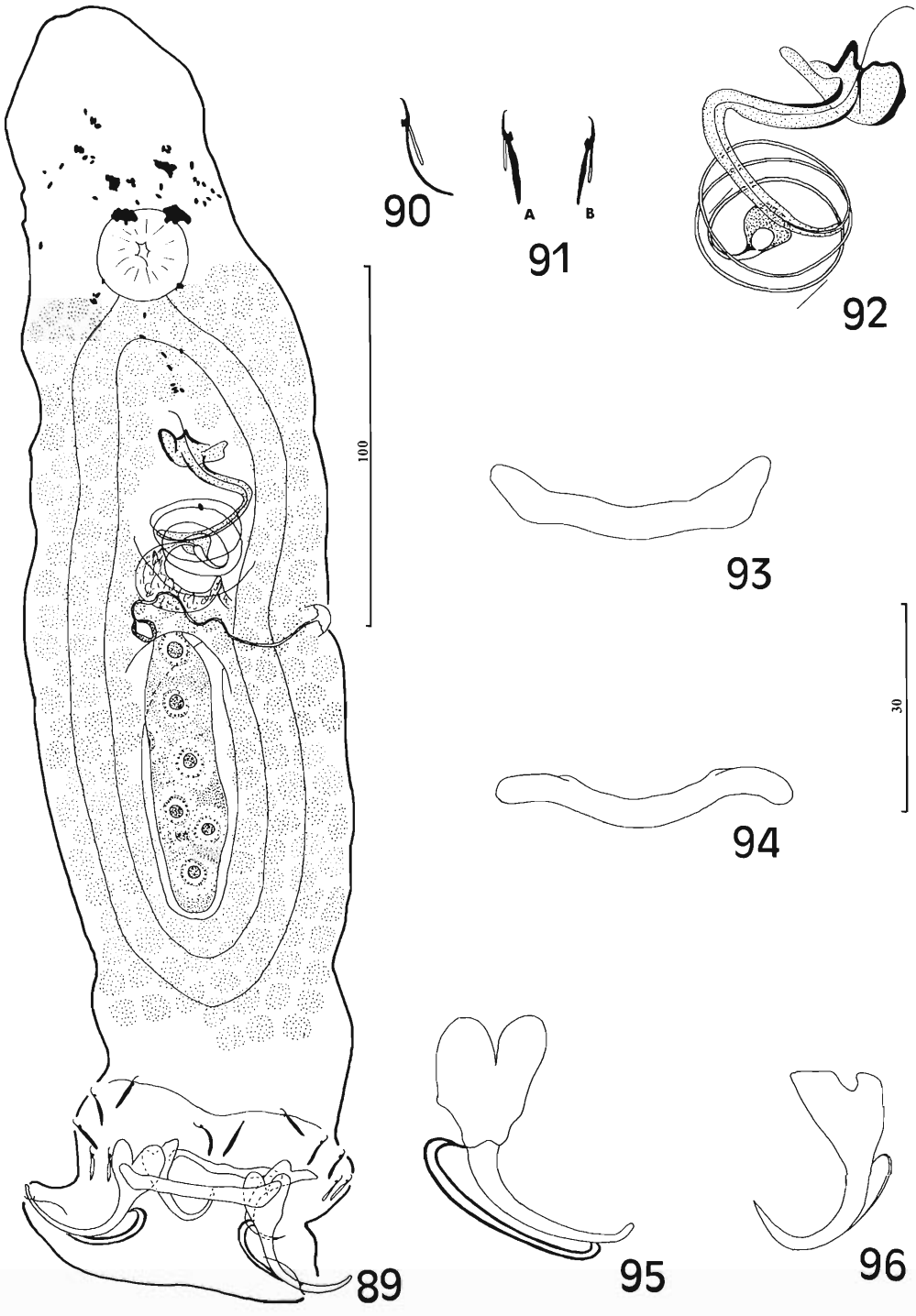
SYNONYMS: *Ancyrocephalus pterophylli* Lucký, 1970; *Urocleidoides spiralocirra* (Kohn and Paperna, 1964) Kritsky and Thatcher, 1983.

HOST: Cará bandeira, *Pterophyllum scalare* (Lichtenstein), Cichlidae.

LOCALITY: Rio Atacuari near its confluence with the Amazon River, East of Iquitos, Peru (March 1977).

SPECIMENS STUDIED: Vouchers, INPA PA272-1 to PA272-3, USNM 78778, HWML 22955; cotype (?), *Ancyrocephalus pterophylli* Lucký, 1970, USNM 78801.

REDESCRIPTION (based on 24 specimens, 20 measured): Body robust, fusiform; cephalic lobes poorly developed, usually two terminal, two bilateral. Four eyes, equidistant, members of posterior pair larger; eye granules dissociated, variable in size, generally ovate; accessory granules present in cephalic region and anterior trunk. Pharynx spherical; esophagus short. Peduncle broad; posterior haptoral lobe wide, poorly differentiated from anterior portion of haptor. Ventral anchor with equal, large roots; anchor point blunt, sharply recurved near termination. Dorsal anchor with large superficial root, evenly curved shaft and point. Ventral bar with enlarged ends, variable; dorsal bar rod-shaped, undulating. Hook pairs 1, 2, 3, 4, 6, 7 with enlarged thumb, slender or slightly inflated shank; hook pair 5 delicate



Figures 89-96. ***Gussevia spiralocirra*** **Kohn and Paperna, 1964. 89. Whole mount (ventral view). 90. Hook pair 5. 91A, B. Two forms of remaining hook pairs. 92. Copulatory complex. 93. Ventral bar. 94. Dorsal bar. 95. Ventral anchor. 96. Dorsal anchor. All figures are to the 30-micrometer scale except Figure 89 (100 micrometers).**

with well-developed thumb; FH loop ½ shank length. Gonads bacilliform; seminal vesicle lunate. Cirrus a coil of 3–4 rings, small base; accessory piece closely associated with terminal ring of cirrus, terminally ornate. Vagina sinistral, a tube opening into thick-walled "seminal receptacle."

MEASUREMENTS: Body 342 (264–416) long, greatest width 94 (68–143) in anterior or posterior trunk. Pharyngeal diameter 21 (18–26). Haptor 59 (54–64) long, 91 (88–94) wide. Ventral anchor 35 (31–39), base width 17–18; dorsal anchor 23 (17–27), base width 12 (10–14). Ventral bar 38 (27–44); dorsal bar 36 (27–42). Hook pairs 1, 2, 3, 4, 6, 7—13 (12–14); hook pair 5—17 (15–18). Cirrus 233 long, ring diameter 21 (17–25); accessory piece 36 (31–42) long. Testis 50–51 × 17–18; ovary 48 (35–56) × 19 (12–29).

REMARKS: *Gussevia spiralocirra* is the type species of the genus. Our specimens were morphometrically variable, with two semidistinct forms being present. In most specimens, the shanks of hooks 1, 2, 3, 4, 6, and 7 are slightly inflated (Fig. 91A), whereas those in other specimens possessed slender shanks (Fig. 91B). Although measurements of both forms overlapped, those of specimens with slender hook shanks tended to occupy the smaller values of the ranges for anchors and bars. All of our specimens possessed a sinistral vagina, whereas Kohn and Paperna (1964) report it as dextral in their specimens. We were not able to examine type specimens of this species; however, based on the comparison of our specimens with drawings provided by these authors, we consider our collection to be conspecific with this species. Verification of the position of the vagina will depend on study of the type material deposited in the Museum of the Oswaldo Cruz Institute, Brazil.

Our examination of one cotype(?) of *Ancyrocephalus pterophylli* Lucký, 1970 has shown this species to be conspecific with our collection. The cotype is unstained and verification of the features of the internal organs was not possible. However, the shapes of the sclerotized structures of the haptor and copulatory complex fall within variation observed in our series. Thus, *A. pterophylli* is considered a junior subjective synonym of *Gussevia spiralocirra*.

Gussevia spiralocirra is closely related to *G. alii* (Molnar et al., 1974) comb. n., *G. alioides* sp. n., *G. cichlasomatis* (Molnar et al., 1974) comb. n., and *G. dobosi* (Molnar et al., 1974) comb. n. based on the comparative morphology of the haptoral armament and copulatory complex. These species are easily differentiated by the number of rings in the cirral coil, the position of the vagina, and the morphology of the accessory piece.

Gussevia elephus sp. n. (Figs. 97–104)

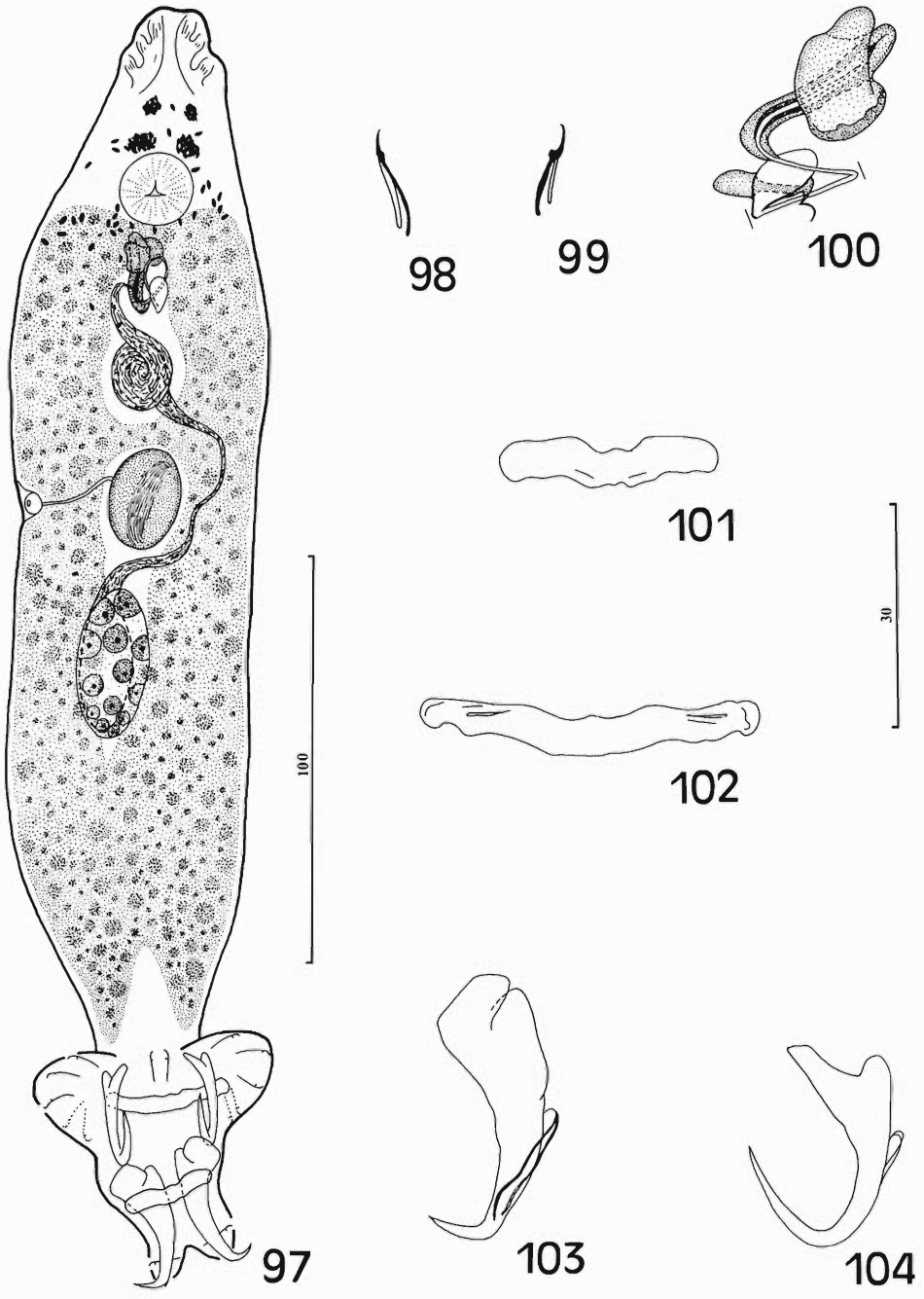
HOST: Cará bararuá, *Uaru amphiacanthoides* (Heckel), Cichlidae.

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (June 27, 1983).

TYPE SPECIMENS: Holotype, INPA PA273-1; paratypes, USNM 78779, HWML 22956.

DESCRIPTION (based on one immature and six adult specimens, adults measured): Body foliform, robust; cephalic margin narrow, with two terminal and two bilateral cephalic lobes poorly developed. Eyes 4; members of posterior pair larger, slightly closer together than members of anterior pair; eye granules elongate ovate; accessory granules present in cephalic and anterior trunk regions. Pharynx spherical; gut obscured by dense vitellaria. Peduncle tapered; haptor (ventral view) shaped like the head of an African elephant, posterior lobe narrow. Ventral anchor with superficial root depressed on deep root, shaft expanded, point straight and aculeate; dorsal anchor with well-developed roots, curved shaft, elongate point. Ventral bar with medial anterior depression; dorsal bar rod-shaped with slight terminal enlargements. Hook pairs 1, 2, 3, 4, 6, 7 similar, with delicate point, conspicuous thumb, slightly enlarged shank; hook pair 5 delicate; FH loop ¾ shank length. Gonads subovate; seminal vesicle coiled posterior to cirrus base. Cirrus a coil of about 1½ rings, enlarged base; accessory piece enclosing distal ½-ring of coil, with flabellate termination. Vagina dextral, a short delicate tube connecting with large medial seminal receptacle showing local regions of spermatozoa.

MEASUREMENTS: Body 419 (317–529), greatest width 98 (65–123) in anterior or posterior trunk. Pharyngeal diameter 23 (20–26). Haptor 71 (64–81) long, 66 (53–93) wide. Ventral anchor 33 (32–34), base width 13 (12–14); dorsal anchor 27 (25–28), base width 14 (12–15). Ventral bar 27 (24–29), dorsal bar 38 (33–42). Hook pairs 1, 2, 3, 4, 6, 7—13–14; hook pair 5—15 (14–16). Cirrus 60 long, ring diameter 19 (16–24); accessory piece 23 (22–25) long. Testis 38–39 × 20–21; ovary 39 (32–43) × 24 (23–26).



Figures 97-104. ***Gussevia elephus*** **sp. n. 97. Ventral view of holotype. 98. Hook pair 5. 99. Remaining hook pairs. 100. Copulatory complex. 101. Ventral bar. 102. Dorsal bar. 103. Ventral anchor. 104. Dorsal anchor. All figures are to the same scale (30 micrometers) except the whole-mount drawing (100 micrometers).**

REMARKS: This species is most closely related to *G. obtusa* and *G. dispar* spp. n. They are easily distinguished by the comparative morphology of the haptoral armament, copulatory complex, and vagina. The haptor, shaped as the head of an African elephant, distinguishes this species from all others in the genus and is the characteristic from which the specific name is derived (Latin, *eleph/o* = elephant).

Gussevia obtusa sp. n. (Figs. 105–112)

HOST: Cará bararuá, *Uaru amphiacanthoides* (Heckel), Cichlidae.

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (June 27, 1983).

TYPE SPECIMENS: Holotype, INPA PA274-1; paratypes, USNM 78780, HWML 22957.

DESCRIPTION (based on five specimens): Body fusiform; cephalic lobes poorly developed, usually two terminal, two bilateral. Four eyes, usually compact; members of posterior pair larger, closer together than members of anterior pair; eye granules small, ovate; accessory granules infrequent in cephalic and anterior trunk regions. Pharynx spherical; esophagus moderately long. Peduncle moderate; haptor deeply incised bilaterally forming well-developed anterior and posterior lobes. Ventral anchor with appressed roots, evenly curved shaft and point; tip of point bent, blunt. Dorsal anchor with well-developed roots, curved shaft, elongate point. Ventral bar with enlarged ends, medial swelling; dorsal bar rod-shaped, with tapered ends. Hook pairs 1, 2, 3, 4, 6, 7 with delicate point, enlarged thumb, slender shank; pair 5 delicate; FH loop ¾ shank length. Gonads elongate; seminal vesicle pyriform. Cirrus a coil of 2–3 rings; accessory piece closely associated with terminal cirral ring, distal portion flabellate, clavate. Vagina dextral, comprising a terminal bulbous structure, moderately elongate tube opening into conspicuous seminal receptacle with local regions of spermatozoa.

MEASUREMENTS: Body 349 (288–393) long, greatest width 72 (59–86) usually in posterior trunk. Pharyngeal diameter 16 (15–17). Haptor 67 (51–79) long, 68 (62–77) wide. Ventral anchor 35 (34–37), base width 11–12; dorsal anchor 24–25, base width 12–13. Ventral bar 27 (23–29); dorsal bar 32 (30–34). Hook pairs 1, 2, 3, 4, 6, 7—14 (13–15); hook pair 5—18–19. Cirrus 112 long, ring diameter 19 (15–21); accessory piece 21 (19–24) long. Ovary 46 (39–52) × 14 (13–15).

REMARKS: *Gussevia obtusa* most closely resembles *G. disparoides* sp. n. as shown by the comparative morphology of the bases of the anchors and of the copulatory complex. These species are distinguished by the presence of a smooth origin of the ventral anchor shaft from the base in *G. obtusa* (shaft with distinct proximal bend in *G. disparoides*), small terminal enlargements of the ventral bar in *G. obtusa,* and the comparative morphology of the distal portion of the vagina. The specific name from Latin (*obtus* = blunt) refers to the tip of the ventral anchor point.

Gussevia alioides sp. n. (Figs. 113–120)

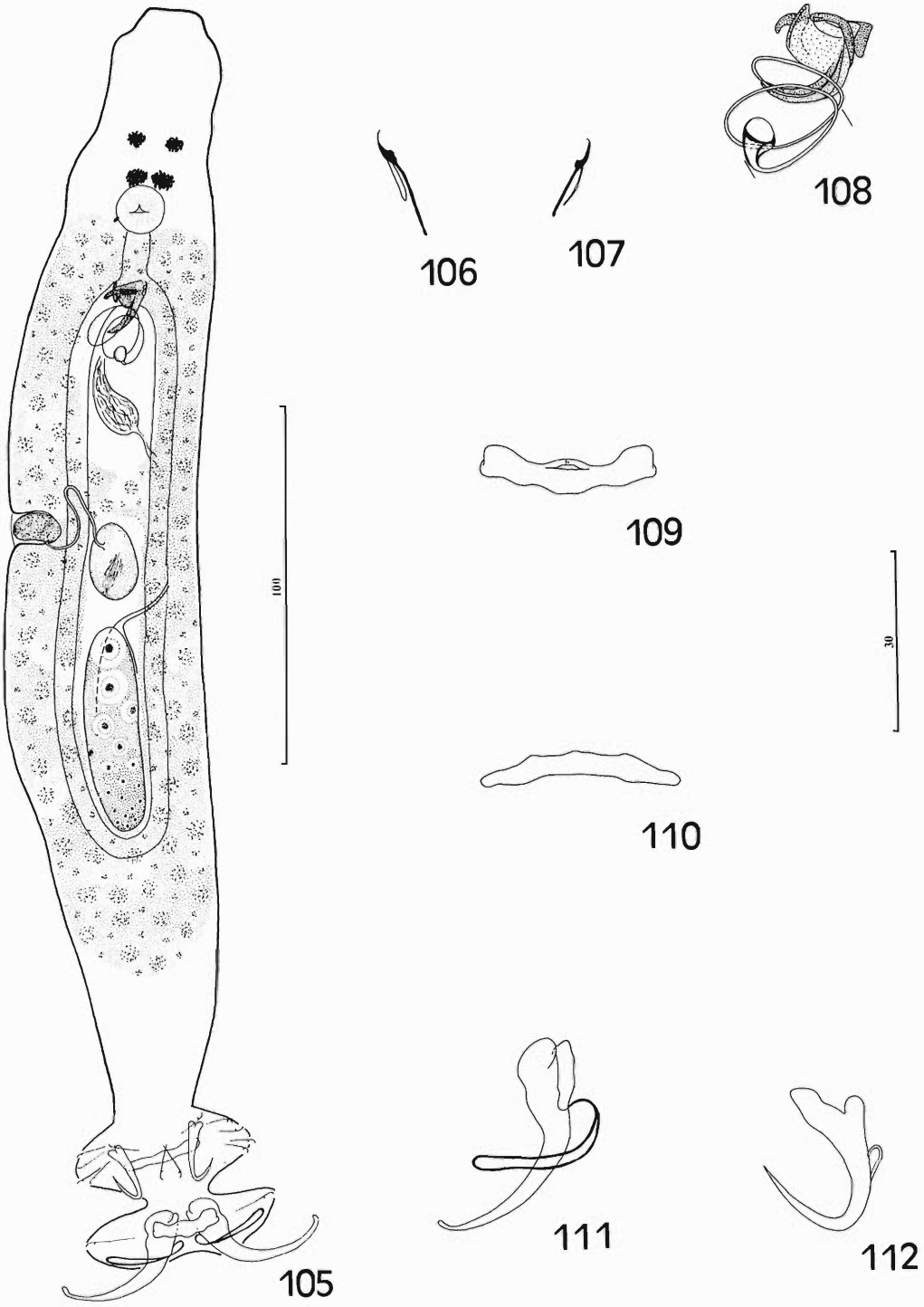
HOST: Cará roxo, *Cichlasoma severum* (Heckel), Cichlidae.

TYPE LOCALITY: Rio Solimões, near Marchantaria Island, Manaus, Amazonas, Brazil (January 2, 1984).

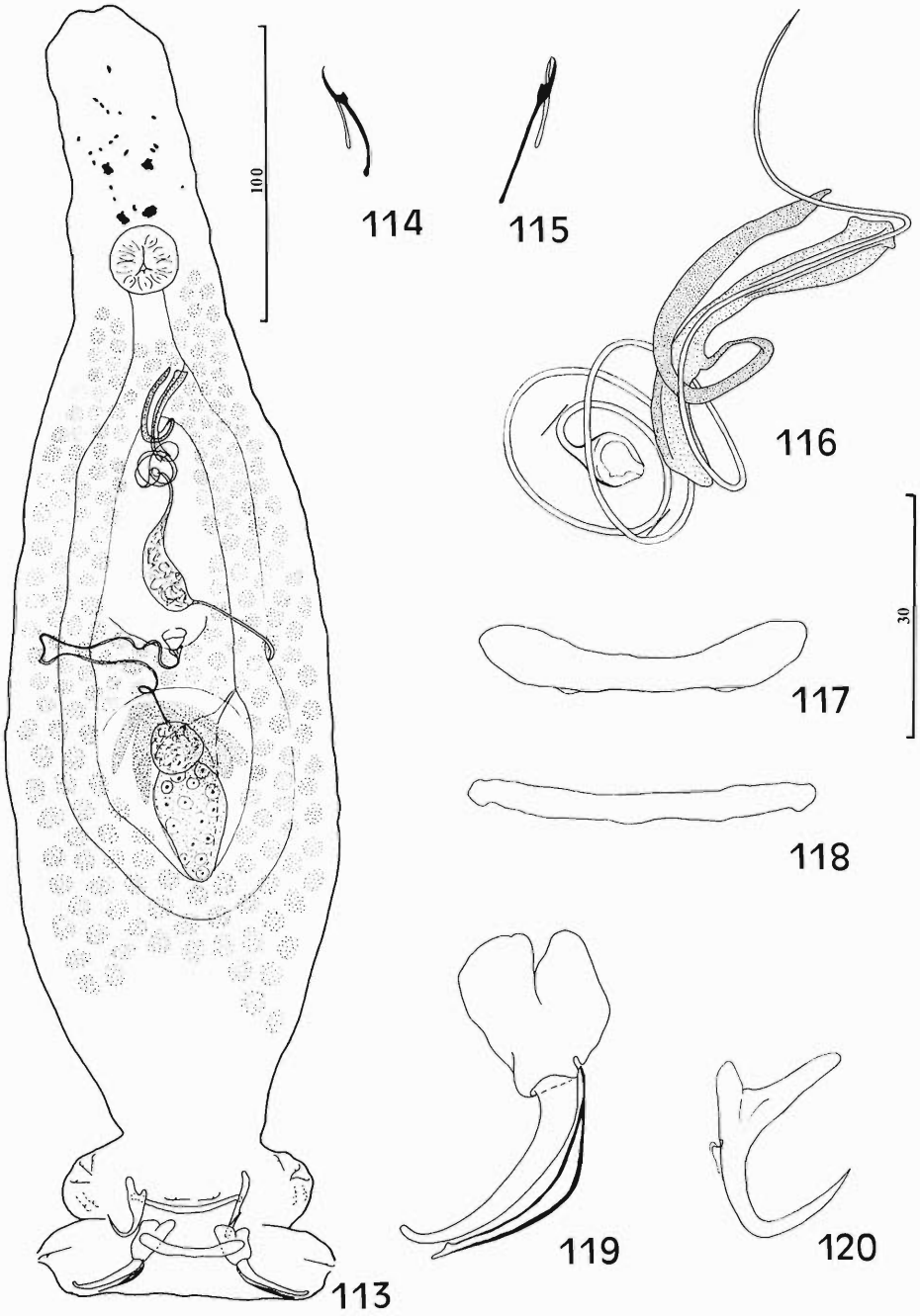
TYPE SPECIMENS: Holotype, INPA PA275-1; paratypes, INPA PA275-2, USNM 78781, HWML 22958.

DESCRIPTION (based on 19 specimens): Body robust, with narrow anterior trunk and cephalic regions; cephalic lobes poorly developed, usually two terminal, two bilateral. Eyes 4, subequal; members of posterior pair closer together; eye granules ovate, large; accessory granules scattered in cephalic area. Pharynx spherical; esophagus moderately long. Peduncle broad; posterior haptoral lobe well developed, large. Ventral anchor with large base, evenly curved shaft and point, tip of point recurved and blunt. Dorsal anchor with well-developed roots, slightly bent shaft, elongate point. Ventral bar with enlarged ends; dorsal bar usually straight, rod-shaped, with slightly enlarged ends. Hook pairs 1, 2, 3, 4, 6, 7 with delicate point, large thumb, slender shank having slight basal enlargement; hook pair 5 delicate, with large thumb; FH loop ½–¾ shank length. Gonads pyriform; seminal vesicle a conspicuous dilation of vas deferens. Cirrus a coil of 4–5 rings; accessory piece closely associated with distal cirral ring, with elongate projection arising near midlength. Vagina midventral, with dextral loop of tube lying ventral to right intestinal caecum. Egg ovate to subspherical, with short irregular proximal filament.

MEASUREMENTS: Body 381 (321–441), greatest width 101 (85–126) in posterior half. Pharyngeal diameter 21 (19–23). Haptor 65 (58–77) long, 93



Figures 105-112. ***Gussevia obtusa*** **sp. n. 105. Holotype (ventral view). 106. Hook pair 5. 107. Hook of remaining pairs. 108. Copulatory complex. 109. Ventral bar. 110. Dorsal bar. 111. Ventral anchor. 112. Dorsal anchor. All figures are to the same scale (30 micrometers) except Figure 105 (100 micrometers).**



Figures 113–120. ***Gussevia alioides*** **sp. n. 113. Ventral view of holotype. 114. Hook of pairs 1, 2, 3, 4, 6, 7. 115. Hook pair 5. 116. Copulatory complex. 117. Ventral bar. 118. Dorsal bar. 119. Ventral anchor. 120. Dorsal anchor. All are drawn to the same scale (30 micrometers) except Figure 113 (100 micrometers).**

(80–101) wide. Ventral anchor 39 (33–42), base width 12 (10–18); dorsal anchor 25 (23–26), base width 15 (12–17). Ventral bar 37 (27–45); dorsal bar 39 (27–43). Hook pairs 1, 2, 3, 4, 6, 7—14 (13–15); hook pair 5—20 (19–21). Cirrus 253 long, ring diameter 19 (17–21); accessory piece 39 (34–44) long. Testis 44 (30–60) × 24 (20–27); ovary 44 (38–53) × 19 (18–21). Egg 77 × 66.

REMARKS: *Gussevia alioides* sp. n. is the only described species in the genus having a midventral vaginal opening with a dextral loop of the vaginal tube. It is closest to *G. alii* (Molnar et al., 1974) as shown by the morphology of the haptoral armament and copulatory complex. The vagina in *G. alii* is dextroventral. The specific name refers to the relationship of these species.

Gussevia tucunarense sp. n. (Figs. 121-128)

HOST: Tucunaré, *Cichla ocellaris* Bloch and Schneider, Cichlidae.

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (June 12, 1978; June 27, 1983; December 1983).

TYPE SPECIMENS: Holotype, INPA PA276-1; paratypes, INPA PA276-2 to PA276-5, USNM 78782, HWML 22959.

DESCRIPTION (based on 28 specimens; 20 measured): Body fusiform; cephalic margin rounded or with poorly developed lobes, usually two terminal, two bilateral. Eyes 4, frequently dissociated, subequal, equidistant; eye granules large, subspherical; accessory granules present in cephalic and anterior trunk regions. Pharynx spherical; esophagus apparently short; gut obscured by dense vitellaria. Peduncle broad, posterior haptoral lobe reduced. Ventral anchor with truncate superficial root, well-developed deep root, angular bends at junctions of base and shaft and shaft and point; point tip obtuse, slightly recurved. Dorsal anchor with elongate superficial root, short deep root, bent shaft, elongate point. Ventral bar with slightly enlarged ends, anteromedial indented plate; dorsal bar rod-shaped with slightly enlarged terminations. Hook pairs 1, 2, 3, 4, 6, 7 with delicate point, erect thumb, slender shank; hook pair 5 delicate; FH loop extending to near proximal end of shank. Gonads elongate; seminal vesicle inconspicuous. Cirrus a coil of about 1½ rings, base with fleshy projection apparently following proximal ⅓ coil; accessory piece flabellate, enclosing terminal portion of cirral tube. Vagina dextral, with sinuous tube.

MEASUREMENTS: Body 292 (219–416), greatest width 66 (48–84) near midlength. Pharyngeal diameter 18 (15–21). Haptor 52 (32–72) long, 55 (43–74) wide. Ventral anchor 25 (23–27), base width 12 (11–13); dorsal anchor 26 (23–28), base width 10 (9–11). Ventral bar 20 (17–24); dorsal bar 28 (19–32). Hook pairs 1, 2, 3, 4, 6, 7—11 (10–12); hook pair 5—14–15. Cirrus 63 long, ring diameter 19 (14–30); accessory piece 27 (17–37) long. Testis 33 (27–39) × 17 (13–20); ovary 41 (31–52) × 17 (13–19).

REMARKS: Based on the morphology of the ventral anchor, *G. tucunarense* sp. n. is closely related to *G. longihaptor* (Mizelle and Kritsky, 1969) comb. n., *G. arilla* sp. n., and *G. undulata* sp. n., all from *Cichla ocellaris*. Structures which best distinguish these species include the vagina, copulatory complex, and ventral anchor shafts and points. The species name is derived from the local name of the fish host, tucunaré.

Gussevia longihaptor (Mizelle and Kritsky, 1969) comb. n. (Figs. 129-136)

SYNONYMS: *Cleidodiscus bulbus* Rogers and Rawson, 1969; *Longihaptor longihaptor* Mizelle and Kritsky, 1969.

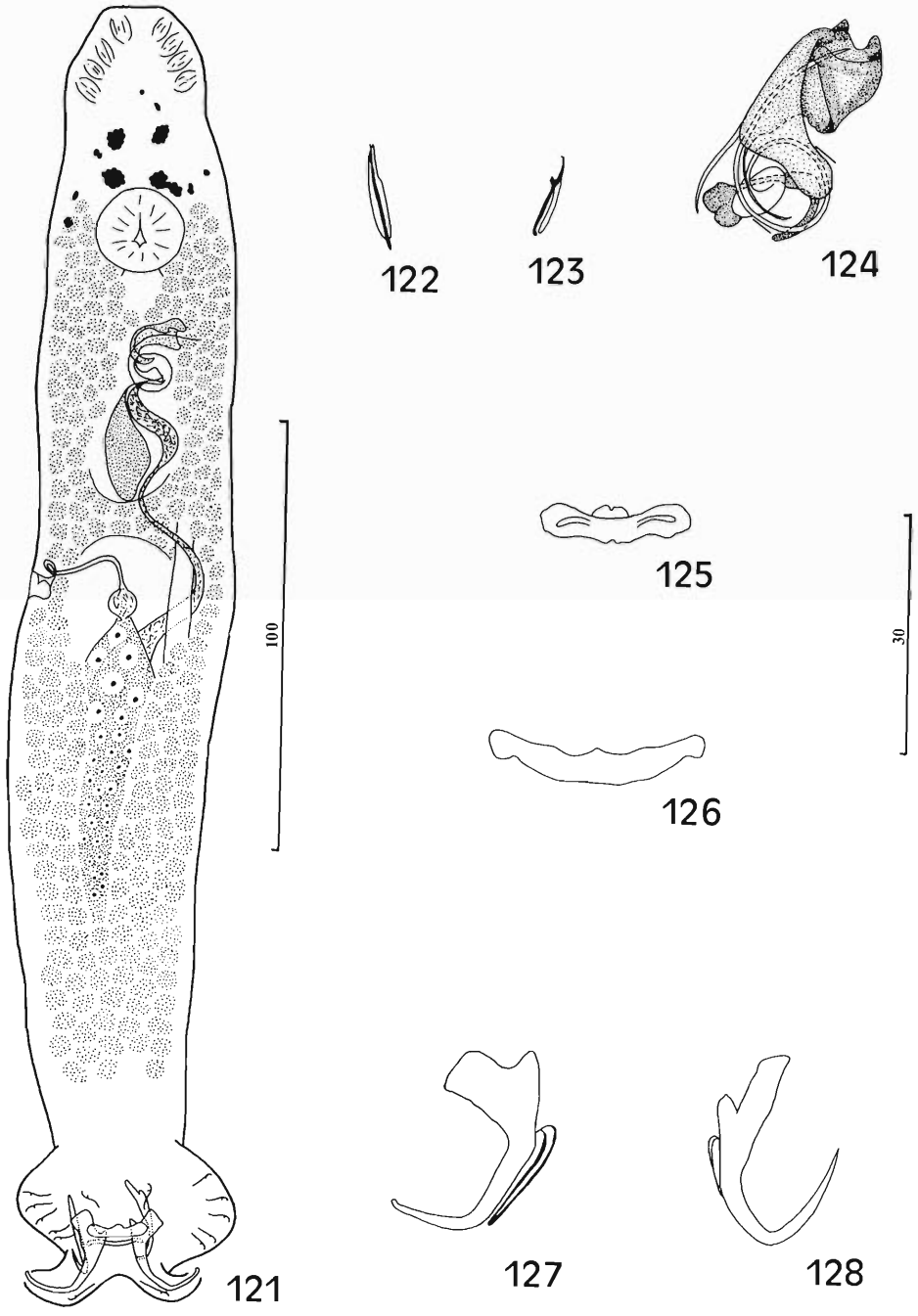
HOST: Tucunaré, *Cichla ocellaris* Bloch and Schneider, Cichlidae.

TYPE LOCALITY: Amazon River Basin, Brazil (Mizelle and Kritsky, 1969).

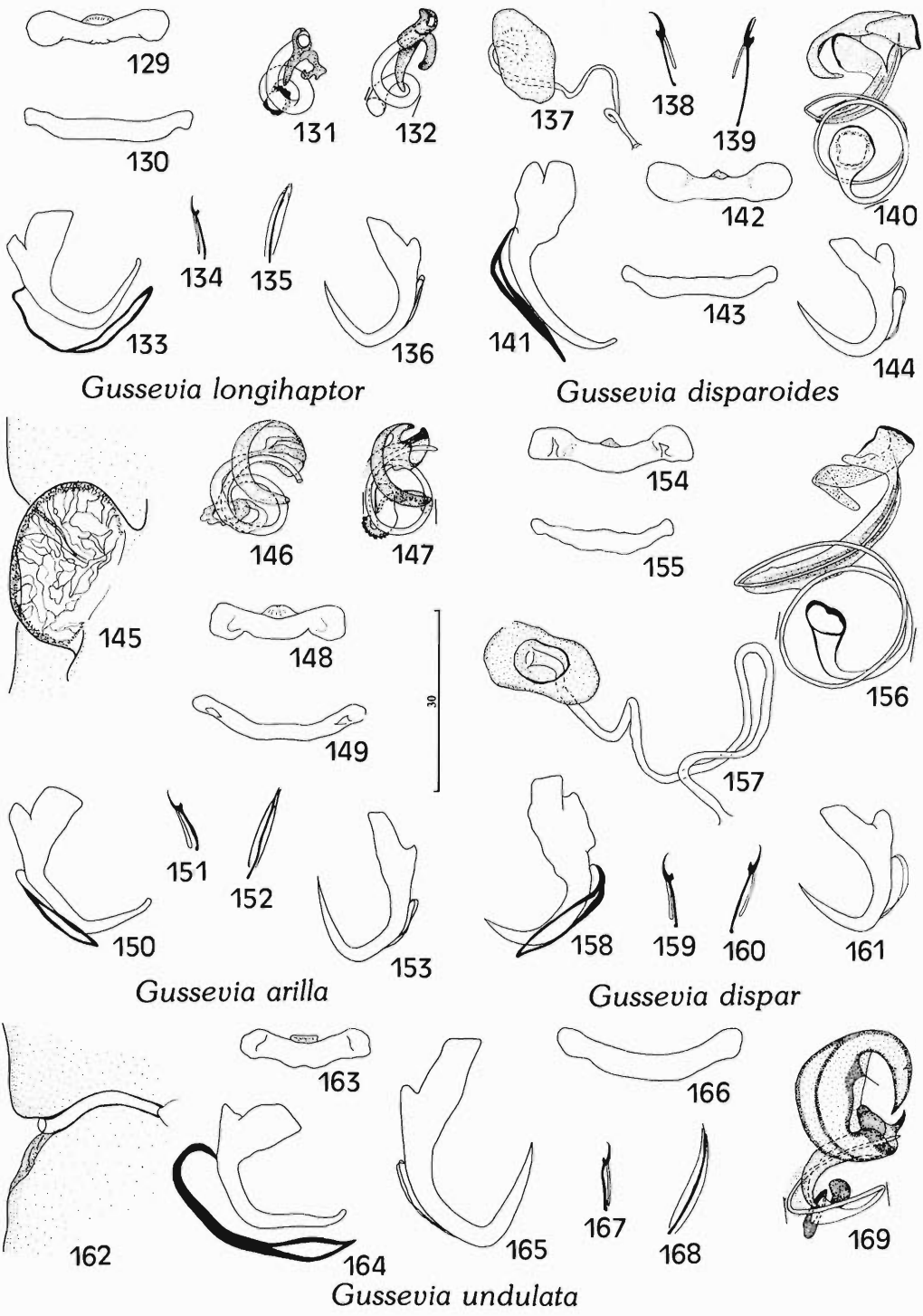
PRESENT LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (June 27, 1983).

SPECIMENS STUDIED: Vouchers, INPA PA277-1, USNM 78783, HWML 22960; three paratypes, *Longihaptor longihaptor* Mizelle and Kritsky, 1969, USNM 71000; paratype, *Cleidodiscus bulbus* Rogers and Rawson, 1969, USNM 71363.

REDESCRIPTION (based on three specimens): Body robust, fusiform; cephalic margin rounded or with two terminal, two bilateral cephalic lobes. Eyes 4, equidistant; members of posterior pair larger than those of anterior pair; eye granules variable in size and shape; accessory granules rare in cephalic area. Pharynx spherical; esophagus short to nonexistent. Peduncle tapered posteriorly, broad; haptoral lobe well developed. Ventral anchor with large superficial root, conspicuous deep root, short shaft, tip of point obtuse. Dorsal anchor with elongate superficial root, short deep root, curved shaft, acute point. Ventral bar with enlarged ends, small anteromedial plate; dorsal bar rod-shaped with slight terminal



Figures 121-128. ***Gussevia tucunarense*** **sp. n. 121. Holotype, ventral view. 122. Hook pair 5. 123. Hook of remaining pairs. 124. Copulatory complex. 125. Ventral bar. 126. Dorsal bar. 127. Ventral anchor. 128. Dorsal anchor. Figures are drawn to the same scale (30 micrometers) except Figure 121 (100 micrometers).**



Figures 129-169. Sclerotized structures of *Gussevia* spp. Figures 129-136. *Gussevia longihaptor* (Mizelle and Kritsky, 1969) comb. n. 129. Ventral bar. 130. Dorsal bar. 131, 132. Copulatory complexes. 133. Ventral anchor. 134. Hook of pairs 1, 2, 3, 4, 6, 7. 135. Hook pair 5. 136. Dorsal anchor. Figures 137-144. *Gussevia disparoides* sp. n. 137. Vagina. 138. Hook of pairs 1, 2, 3, 4, 6, 7. 139. Hook pair 5. 140. Copulatory complex.

enlargements. Hook pairs 1, 2, 3, 4, 6, 7 with erect thumb, delicate point and shaft, slender shank; hook pair 5 with poorly developed thumb, slender shank; FH loop $^{9}/_{10}$ shank length. Gonads elongate; seminal vesicle indistinct. Cirrus a coil of about 1½ rings, small base, tube dilated; accessory piece with short proximal portion giving rise to two terminal branches. Vagina not observed, apparently unsclerotized.

MEASUREMENTS: Body 414 (373–439) long, greatest width 84 (49–106) near midlength. Pharyngeal diameter 35 (21–40). Haptor 51 (49–52) long, 63 (43–76) wide. Ventral anchor 22–23, base width 12–13; dorsal anchor 21–22, base width 11–12. Ventral bar 21 (19–23); dorsal bar 28 (27–29). Hook pairs 1, 2, 3, 4, 6, 7—11–12; hook pair 5—15–16. Cirrus 33 long, ring diameter 10 (9–11); accessory piece 12 (9–14) long. Testis 49–50 × 8–9; ovary 70 (62–78) × 14 (13–16).

REMARKS: This species was originally described as *Longihaptor longihaptor* by Mizelle and Kritsky (1969) from the gills of *Cichla ocellaris,* an aquarium fish in the United States. Examination of three paratype specimens of *L. longihaptor* and comparison of these specimens with ours confirms their conspecificity. Major differences in the depictions of the copulatory complex are due to the fact that Mizelle and Kritsky (1969) drew a specimen that had been severely flattened with the cirrus lying in lateral view. Nonetheless, these authors do depict an expanded cirrus tube with about 1½ coils. Similarly, examination of the paratype of *Cleidodiscus bulbus* described by Rogers and Rawson (1969 publication date: Aug. 21, 1969) confirmed that this species is conspecific with *L. longihaptor* (publication date: Apr. 16, 1969), and it is relegated to junior subjective synonomy because of a later publication date.

Gussevia disparoides sp. n. (Figs. 137–144)

HOST: Cará roxo, *Cichlasoma severum* (Heckel), Cichlidae.

TYPE LOCALITY: Rio Solimões near Marchantaria Island, Manaus, Amazonas, Brazil (January 2, 1984).

TYPE SPECIMENS: Holotype, INPA PA278-1; paratypes, INPA PA278-2, USNM 78784, HWML 22961.

DESCRIPTION (based on 11 specimens): Body fusiform, stout; cephalic margin rounded or with two terminal, two bilateral cephalic lobes. Eyes 4; members of posterior pair larger, closer together than members of anterior pair; eye granules subovate, variable in size; accessory granules few in cephalic region. Pharynx subovate; esophagus short to nonexistent. Peduncle broad, tapered posteriorly; posterior haptoral lobe with divergent arms. Ventral anchor with appressed roots, proximal bend of shaft, obtuse point; dorsal anchor with well-developed roots, short shaft, elongate point. Ventral bar with enlarged ends and short, acute anteromedial projection. Dorsal bar rod-shaped, with slightly enlarged ends. Hook pairs 1, 2, 3, 4, 6, 7 with curved shaft and point, erect thumb, slender shank; hook pair 5 similar except for elongate shank; FH loop ½–¾ shank length. Gonads small, bacilliform; seminal vesicle pyriform. Cirrus a coil of about 2½ rings; accessory piece closely associated with distal ring of cirrus, with terminal lamellar projections. Vagina dextral with terminal fleshy funnel and winding tube.

MEASUREMENTS: Body 352 (303–405), greatest width 80 (62–101) near midlength. Pharyngeal diameter 17 (16–18). Haptor 56 (51–60) long, 69 (63–73) wide. Ventral anchor 32 (29–34), base width 11 (9–12); dorsal anchor 21 (20–23), base width 11 (10–12). Ventral bar 26 (22–28), dorsal bar 27 (23–31). Hook pairs 1, 2, 3, 4, 6, 7—13 (12–14); hook pair 5—18 (16–19). Cirrus 122 long, ring diameter 18 (17–20); accessory piece 25 (21–38) long. Testis 30 (25–35) × 13 (10–16); ovary 40 (38–42) × 15 (14–17).

REMARKS: *Gussevia disparoides* sp. n. is closest to *G. dispar* sp. n., also from *Cichlasoma severum.* They are easily distinguished by the mor-

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141. Ventral anchor. 142. Ventral bar. 143. Dorsal bar. 144. Dorsal anchor. Figures 145–153. *Gussevia arilla* sp. n. 145. Vagina. 146, 147. Copulatory complexes. 148. Ventral bar. 149. Dorsal bar. 150. Ventral anchor. 151. Hook of pairs 1, 2, 3, 4, 6, 7. 152. Hook pair 5. 153. Dorsal anchor. Figures 154–161. *Gussevia dispar* sp. n. 154. Ventral bar. 155. Dorsal bar. 156. Copulatory complex. 157. Vagina. 158. Ventral anchor. 159. Hook of pairs 1, 2, 3, 4, 6, 7. 160. Hook pair 5. 161. Dorsal anchor. Figures 162–169. *Gussevia undulata* sp. n. 162. Vagina. 163. Ventral bar. 164. Ventral anchor. 165. Dorsal anchor. 166. Dorsal bar. 167. Hook of pairs 1, 2, 3, 4, 6, 7. 168. Hook pair 5. 169. Copulatory complex. All figures are given to the same scale (30 micrometers).

phology of the ventral anchor (anchor point acute in *G. dispar*; obtuse in *G. disparoides*). The specific name reflects the apparent close relationship of these species.

Gussevia arilla sp. n. (Figs. 145–153)

HOST: Tucunaré, *Cichla ocellaris* Bloch and Schneider, Cichlidae.

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (June 27, 1983).

TYPE SPECIMENS: Holotype, INPA PA279-1; paratypes, USNM 78785, HWML 22962.

DESCRIPTION (based on seven specimens): Body robust, tapered at both extremities; cephalic lobes poorly developed, two terminal, two bilateral. Eyes 4, equidistant; members of posterior pair larger or subequal in size to members of anterior pair; eye granules variable in size, subovate; accessory granules usually present in cephalic region. Pharynx spherical; posterior haptoral lobe with divergent arms. Ventral anchor with large truncate superficial root, well-developed deep root, straight shaft with proximal angular origin, straight point with obtuse tip. Dorsal anchor with elongate superficial root, small deep root, bent shaft, elongate point. Ventral bar with enlarged terminations, anteromedial flap with small indentation. Dorsal bar rod-shaped. Hook pairs 1, 2, 3, 4, 6, 7 with erect thumb, delicate point and shaft, shank with slight variation in diameter; hook pair 5 elongate, delicate, with poorly developed thumb; FH loop $^{9}/_{10}$ shank length. Gonads short, bacilliform; seminal vesicle indistinct. Cirrus a coil of 1½ rings, base articulated to accessory piece. Accessory piece complex with proximal arm flared distally into a sheath wrapped around termination of cirral tube. Vagina dextral, with fleshy lobe ventral to lateral opening (Fig. 145); lobe with internal ridges imparting a cerebral appearance.

MEASUREMENTS: Body 248 (199–305), greatest width 79 (57–93) near midlength. Pharyngeal diameter 20 (14–23). Haptor 48 (41–62) long, 66 (61–72) wide. Ventral anchor 26 (25–27), base width 12 (10–14); dorsal anchor 26 (24–27), base width 11 (9–13). Ventral bar 22 (20–23); dorsal bar 28 (27–29). Hook pairs 1, 2, 3, 4, 6, 7—11–12; hook pair 5—15–16. Cirrus 83 long, ring diameter 12 (11–14); accessory piece 15 (14–16) long. Ovary 44–45 × 21–22.

REMARKS: *G. arilla* sp. n. is easily distinguished from all other known species in the genus by the characteristic fleshy lobe ventral to the dextral opening of the vagina. Based on haptoral morphology, it most closely resembles *G. tucunarense* sp. n. The specific name is from Neolatin (*arilla* = a wrapper) and refers to the complex structure of the terminal portion of the accessory piece.

Gussevia dispar sp. n. (Figs. 154–161)

HOST: Cará roxo, *Cichlasoma severum* (Heckel), Cichlidae.

TYPE LOCALITY: Rio Solimões near Marchantaria Island, Manaus, Amazonas, Brazil (January 2, 1984).

TYPE SPECIMENS: Holotype, INPA PA280-1; paratypes, USNM 78786, HWML 22963.

DESCRIPTION (based on six specimens): Body fusiform, gently tapered posteriorly; cephalic lobes poorly developed, two terminal, two bilateral. Eyes 4, subequal; members of posterior pair slightly closer together than members of anterior pair; eye granules variable in size, subovate to bacilliform; accessory granules present in cephalic and anterior trunk regions. Pharynx spherical, esophagus moderately elongate. Peduncle narrow, elongate; haptor with small posterior lobe. Ventral anchor with truncate superficial root, knoblike deep root, short shaft, curved and acute anchor point. Dorsal anchor with elongate superficial root, small deep root, bent shaft, elongate point. Ventral bar with enlarged terminations, small anteromedial triangular projection. Dorsal bar rod-shaped with slightly enlarged terminations. Hook pairs 1, 2, 3, 4, 6, 7 with curved shaft and point, erect thumb, slender shank; hook pair 5 similar, with longer shank and small proximal enlargement; FH loop ¾ shank length. Gonads elongate; seminal vesicle stout, fusiform. Cirrus a coil of about 2½ rings; accessory piece with well-developed proximal arm flaring distally with folded lamellar projection. Vagina dextral, with terminal fleshy funnel, winding delicate tube. Egg ovate, with short anterior filament and posterior pointed elevation.

MEASUREMENTS: Body 540 (500–587), greatest width 85 (82–88) in anterior half near midlength. Pharyngeal diameter 24 (23–26). Haptor 61 (54–66) long, 73 (66–77) wide. Ventral anchor 29 (28–30), base width 12 (10–13); dorsal anchor 21 (20–22), base width 11–12. Ventral bar 28–29, dorsal bar 25 (24–26). Hook pairs 1, 2, 3, 4, 6, 7—13–14; hook pair 5—16–17. Cirrus 166

long, ring diameter 25 (23–26); accessory piece 41 (37–44) long. Ovary 97–98 × 13–14. Egg 87–88 × 39–40 wide.

REMARKS: Based on the structure of the vagina, bars, copulatory complex, hooks and dorsal anchor, this species most closely resembles *G. disparoides* sp. n. They are easily distinguished by the morphology of the ventral anchors and relative body shapes. The species name is from Latin (*dispar* = different) and refers to the unique ventral anchor.

Gussevia undulata sp. n. (Figs. 162-169)

HOST: Tucunaré, *Cichla ocellaris* Bloch and Schneider, Cichlidae.

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (June 27, 1983); also collected from the same host purchased at the Manaus Fish Market (December 1983).

TYPE SPECIMENS: Holotype, INPA PA281-1; paratypes, INPA PA281-2, USNM 78787 and 78788, HWML 22964.

DESCRIPTION (based on eight specimens): Body fusiform, tapered gently posteriorly; cephalic margin rounded or with two terminal, two bilateral cephalic lobes poorly developed. Four eyes, equidistant; members of posterior pair larger than those of anterior pair; eye granules elongate ovate to bacilliform; accessory granules present in cephalic and anterior trunk regions. Pharynx subspherical; esophagus short. Peduncle short, broad; haptoral lobe with divergent elongate arms. Ventral anchor with large truncate superficial root, small deep root, straight shaft originating at an angle from anchor base, undulating point with obtuse tip. Dorsal anchor with large superficial root, small deep root, curved shaft, sharply recurved elongate point. Ventral bar small, with slightly enlarged ends and anteromedial truncate process. Dorsal bar rod-shaped, with slightly enlarged terminations. Hook pairs 1, 2, 3, 4, 6, 7 with delicate point and shaft, erect thumb, slender shank; hook pair 5 apparently lacking thumb; FH loop nearly equals shank length. Gonads fusiform; seminal vesicle large. Cirrus a coil of about 1½ rings; base with lateral flange. Accessory piece with short proximal arm from which complex terminal branch arises, accessory piece flared distally. Vagina dextral, a short tube; posterior surface sclerotization present at vaginal opening.

MEASUREMENTS: Body 420 (366–526), greatest width 84 (71–111) near midlength. Pharyngeal diameter 23 (17–27). Haptor 74 (67–81) long, 77 (70–82) wide. Ventral anchor 24 (22–26), base width 13 (12–15); dorsal anchor 34 (31–39), base width 15 (12–18). Ventral bar 24 (21–25); dorsal bar 31 (30–33). Hook pairs 1, 2, 3, 4, 6, 7—12–13; hook pair 5—17 (15–19). Cirrus 58 long, ring diameter 21 (17–27); accessory piece 31 (29–33) long. Testis 46 (39–53) × 19 (17–22); ovary 30 (18–41) × 17 (16–18).

REMARKS: *Gussevia undulata* sp. n. is most closely related to *G. longihaptor* (Mizelle and Kritsky, 1969) as shown by the comparative morphology of the ventral anchor, hooks, and bars. *Gussevia undulata* is separated from this species by having (1) an undulating ventral anchor point, (2) a complex accessory piece of the copulatory complex, (3) a slender cirral tube (expanded in *G. longihaptor*), and (4) a sclerotized vagina. The specific name, from Latin (*undulata* = wavy), refers to the shape of the ventral anchor point.

Discussion

Although Kritsky and Thatcher (1983) listed 30 described species belonging to *Urocleidoides* sensu Mizelle et al. (1968), the present revision does not consider the generic status of 22 of them. This remaining group of species is undoubtedly polyphyletic. However, its members are morphologically redundant with regard to the haptoral and copulatory sclerites, and most available specimens (mostly types) of the species are unstained and cleared, which precludes the study of their internal organ systems. It was not possible to assign at the generic level even those for which the internal organ systems are known because general organizational patterns could not be determined. Therefore, with regard to the present revision, we consider the following species incertae sedis: *Urocleidoides affinis* Mizelle, Kritsky, and Crane, 1968, *U. amazonensis* Mizelle and Kritsky, 1969, *U. carapus* Mizelle, Kritsky, and Crane, 1968, *U. catus* Mizelle and Kritsky, 1969, *U. chavarriai* (Price, 1938) Molnar, Hanek, and Fernando, 1974, *U. corydori* Molnar, Hanek, and Fernando, 1974, *U. costaricensis* (Price and Bussing, 1967) Kritsky and Leiby, 1972, *U. gymnotus* Mizelle, Kritsky, and Crane, 1968, *U. heteroancistrium* (Price and Bussing, 1968) Kritsky and Leiby, 1972, *U. kabatai* Molnar, Hanek, and Fernando, 1974, *U. lebedevi* Kritsky and Thatcher, 1976, *U. mamaevi* Kritsky and Thatcher, 1976, *U. margolisi* Molnar,

Hanek, and Fernando, 1974, *U. megorchis* Mizelle and Kritsky, 1969, *U. microstomus* Mizelle, Kritsky, and Crane, 1968, *U. robustus* Mizelle and Kritsky, 1969, *U. stictus* Mizelle, Kritsky, and Crane, 1968, *U. strombicirrus* (Price and Bussing, 1967) Kritsky and Thatcher, 1974, *U. travassosi* (Price, 1938) Molnar, Hanek, and Fernando, 1974, *U. trinidadensis* Molnar, Hanek, and Fernando, 1974, *U. variabilis* Mizelle and Kritsky, 1969, and *U. virescens* Mizelle, Kritsky, and Crane, 1968.

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MEETING SCHEDULE OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON FOR 1986

(Wed.)	15 Jan.	National Institutes of Health, Bethesda, MD
(Wed.)	12 Feb.	Naval Medical Research Institute, Bethesda, MD (*with* Food and Drug Administration)
(Wed.)	19 Mar.	Walter Reed Army Institute of Research, Washington, D.C. (*with* Armed Forces Institute of Pathology)
(Wed.)	16 Apr.	Johns Hopkins University, Baltimore, MD
(Sat.)	10 May	University of Pennsylvania, New Bolton Center, PA

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The Proceedings needs papers. There is no backlog and publication time for good quality well-written manuscripts is relatively short. Manuscripts in any and all areas of parasitology are welcomed. Material in final form for the January issue is sent to Allen Press in September and that for the July issue in March.

The Editors

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The Early Embryology of *Hymenolepis diminuta* (Cestoda)

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ABSTRACT: The early embryology of *Hymenolepis diminuta,* from the primary oocyte to the formation of the first mesomere, is followed by means of scanning electron microscopy, histochemistry, and light microscopy using both paraffin and glycol methacrylate sections. Fertilization of the primary oocyte begins with the attachment of the narrow end of the sperm. Meiosis of the oocyte occurs, the pronuclei form, and the zygotic cleavage results in blastomeres of unequal size. The larger blastomere retains the shell granules. The first mesomere arises at about the 8-blastomere stage and the macromere divides equally at about the 15-cell stage; the shell granules are divided equally between the two macromeres.

Hymenolepis diminuta (Rud. 1819), as now conceived, is a species exhibiting relatively little host-specificity. As an adult it has been reported from several species of rodents as well as humans. The cysticercoid infects a wide range of hosts including several species of insects representing a number of orders. The ever-growing fund of knowledge about this parasite derives from the fact that it can be maintained for years in the laboratory rat or the cysticercoid can be reared in easily cultured grain beetles. The recent publication of a book devoted solely to this worm (Arai, 1980) is a measure of the interest in this nonpathogenic and noneconomic worm.

Certain aspects of the embryology of *H. diminuta* have been described and it appears that this worm does not vary greatly from other cyclophyllideans in this regard. The use of this worm as a model system in both research and teaching requires well-illustrated, detailed publications dealing with the basic features of the life cycle. The recent development of new techniques and the application of other systems to tapeworm embryology allows such a publication at this time.

The embryology of *H. diminuta* has a combination of characteristics shared either collectively or singly with other cyclophyllidean tapeworms: (1) The first cleavage of the zygote results in two blastomeres of unequal size. (2) Early cleavage results in the production of two macromeres and three mesomeres, cells which later form the outer and inner envelopes respectively. (3) The oocyte contains shell granules that are carried to the macromeres and they are released in the space between the embryo and the outer capsule. (4) The vitelline cells contain glycogen as well as shell granules. (5) The embryophore (inner capsule) is of a generalized type seemingly exhibiting no unique features in either composition or confirmation. The results of this study will add to our understanding of fertilization, the formation of the first, outer capsule, oogenesis, and early cleavage.

Methods

Specimens were from the Carolina Biological Supply strain obtained through the courtesy of Dr. Donal Myer at Southern Illinois University at Edwardsville. Rats were infected with 6–8 cysticercoids. Later they were killed with chloroform or CO_2 and the worms were removed from the intestine immediately and placed in Hanks' BSS adjusted to a pH of 7.4.

Smears were prepared by macerating a 1-cm piece of worm, removing the large pieces, and then staining the residue with a drop of FLP orcein. The coverglass was sealed and stabilized with clear fingernail polish. These preparations were studied and photographed using Heine phase microscopy.

Specimens used in cryofracture were prepared the following ways:

(1) Fixative: 3% glutaraldehyde, 3% sucrose in cacodylic buffer at a pH of 7.4 for 3 hr on ice. Post-fixed in 1% OsO_4 for 2 hr on ice. Dehydration was in an ethanol series. Specimens in 100% ethanol were fractured after coming to temperature equilibrium (absence of bubbles) in liquid nitrogen.

(2) A second series of specimens was processed as above, but they were fractured after a 2-day infiltration with L. R. White resin (a hydrophilic acrylic resin produced by London Resin Co. Basingstoke, Hants, U.K.). Fractured worm fragments were removed from liquid nitrogen and placed in a large volume of 100% ethanol to remove the resin.

(3) A third series of worms for ethanol cryofracture was fixed in AFA and dehydrated in an ethanol series as in No. 1 above.

All specimens from the three series were critical-point dried using CO_2 and, after mounting on stubs with silver paste, they were coated with a thin layer of gold-palladium. The mounted worms were stored in a desiccator over silica gel until examined. Scanning electron micrographs (SEM) were taken on a Philips 501 microscope. Other specimens, fixed in AFA, were infiltrated with paraffin or glycol methacrylate, sectioned and stained. All light microscope (LM) photographs

were taken with a Leitz Ortholux microscope with a 70× apochromat oil immersion lens and Heine phase. Kodak Tech Pan 2415 film was developed in Kodak HC110, diluted 1:20.

Results

Sperm are similar to those described for other cyclophyllidean tapeworms. They are elongate (0.25–0.30 mm) with a narrow end (0.16–0.2 μm) and a wider flattened end (0.8 μm). The long filamentous nucleus extends from the narrow end where it forms a loose coil or spiral, to near the other end of the sperm (Figs. 6, 7). The sperm nuclei are Feulgen positive for DNA. FLP orcein is also DNA positive as used here (note dark-staining nuclei at bottom of Fig. 7). Glycogen is present in sperm as evidenced by the use of PAS with and without saliva. Sperm are highly active in Hanks' BSS, forming loops and spirals and vibrating at a high rate of speed at the thin end. Sperm mixed with oocytes in Hanks' BSS begin penetration within 3 min by attachment of the narrow end to the oocyte (Figs. 1, 2, 12, 18).

The primary oocyte (Fig. 5) released from the ovary is about 15–18 μm (SEM) in diameter and it contains shell granules (1.0–3.0 μm, LM). During the early cleavages in the uterus, the granules are carried to the macromeres from which they are released later.

Oocyte shell granules are positive to the following tests: bromphenol blue, malachite green, and very slightly positive to proprionic orcein. There is a thin PAS-positive layer around the oocyte.

The process of fertilization begins in the oviduct when the sperm attaches to the primary oocyte (Figs. 1, 2, 18) and it is completed in the uterus when the male and female pronuclei (Figs. 9, 22) fuse to complete the formation of the zygote (Fig. 10). Under natural conditions only a single sperm attaches to an ovum; in no instance was there more than one sperm observed in any oocyte. However, during the preparation of smears, the ducts and ovaries were torn, releasing oocytes and large numbers of sperm together (Figs. 6, 7) resulting in multiple attachments of sperm on the oocyte (Fig. 2). As many as six to eight sperm were observed attached to a single oocyte.

The precise time of sperm penetration was not observed, but the long, filamentous sperm nucleus can be seen in the oocyte cytoplasm during the diakinesis stage of the first meiotic division (Fig. 3). During the process of penetration, the sperm nucleus apparently separates from the rest of the sperm, leaving behind the tail on the outside of the oocyte (Fig. 1). The nucleus shortens and forms a characteristic shape that remains during the following meiotic divisions (Figs. 6–8). Ultimately, the male pronucleus is formed (Figs. 9, 22), a structure not easily differentiated from the female pronucleus.

These hosts were killed between 10:00 and 14:00 hr and numerous examples of the pronuclear stage were observed; this is not always the case, see below.

The vitelline gland is about 65–75 μm in diameter and individual vitelline cells are about 5–6 μm (SEM) (Fig. 4) or 5–12 μm with the LM (Figs. 9, 10). Vitelline cells in the gland have discrete glycogen granules, but later, when the vitelline cell is in the uterus, attached to an oocyte, the PAS-positive reaction product (glycogen) becomes diffuse with no granules visible (LM), but with a greater PAS reaction. In addition, shell granules are present in the vitelline cell which are released into the lumen of the ootype. The granules are positive to bromphenol blue and malachite green. They cannot be demonstrated in the vitelline cell in the uterus.

The outer capsule (OC) is a thin, PAS-positive capsule surrounding the sperm–oocyte–vitelline cell complex (Figs. 3–5). The OC is PAS positive, due to the PAS-positive contribution from the Mehlis gland.

The cleavage of the zygote (Figs. 10, 23) results in two blastomeres (Figs. 11, 24) not only of unequal size (the one being 3–4 times larger in diameter), but the larger retains the shell granules seen earlier in the oocyte. After the first, unequal cleavage, a series of micromeres is produced (Figs. 14–16, 24–26) until the 8-cell stage when the macromere divides to give rise to the first mesomere (Figs. 13, 16, 26). These cells can be differentiated on the basis of size and the presence of shell granules in the macromere.

Discussion

The entry of the sperm nucleus into the oocyte is associated with the early stages of meiosis (Figs. 19–21). It may be that sperm penetration stimulates this process, however, the sperm nucleus condensation and the stages of meiosis are not uniformly synchronized. For example, in Figure 6, the sperm nucleus is still partly filamentous and the chromosomes are in leptotene, whereas in Figure 3 the sperm nucleus is more filamen-

tous and the chromosomes are in either pachytene or diplotene. The sperm nucleus is completely condensed before the first meiotic division (Figs. 7, 20). The characteristic shape of the sperm nucleus is retained through the second meiotic division. The condensed sperm nucleus in *Gyrocoelia* in Coil (1972) has a characteristic shape, but different from that seen in *H. diminuta.*

Host animals utilized here were maintained on a standard light cycle (12 and 12) and their worms, processed between 10:00 and 14:00 hr, had large numbers of pronuclear stages. As a comparison, in a study of 42 *Gyrocoelia* collected from bird hosts at 6:00 to 6:30 (about sunrise), I found a single pronuclear stage. This interesting disparity might be due to a short pronuclear stage in the latter species (and a corresponding long pronuclear stage in *H. diminuta*) or it might be due to a circadian response to the availability of nutrients in the lumen of the small intestine. The rats fed at night and the birds fed beginning at daylight.

The morphology of the sperm has received much attention from the TEM viewpoint (Lumsden, 1965b; Silveira, 1974; Kelsoe, 1977). In these and LM studies there is discussion regarding which end of the sperm is the head (narrow or flat). Motility is presented as one criterion. In *H. diminuta* it is the narrow end that is highly motile and it is this end that attaches to the oocyte, and the filamentous, helical nucleus can be seen trailing from that site. Featherstone (1971) did not detect Feulgen-positive (DNA) material in the sperm of *Taenia hydatigena,* but in the present study Feulgen-positive sperm were observed in the seminal vesicle. Furthermore, whereas FLP orcein is not absolutely specific for DNA, in the tapeworm sperm it stains only the nucleus (Fig. 6). Sperm are rich in glycogen as first noted by Hedrick and Daugherty (1957) and since corroborated by many other reports.

The difficulty in studying fertilization in tapeworms is reflected by the paucity of substantial reports concerning this event. Douglas (1963) re-

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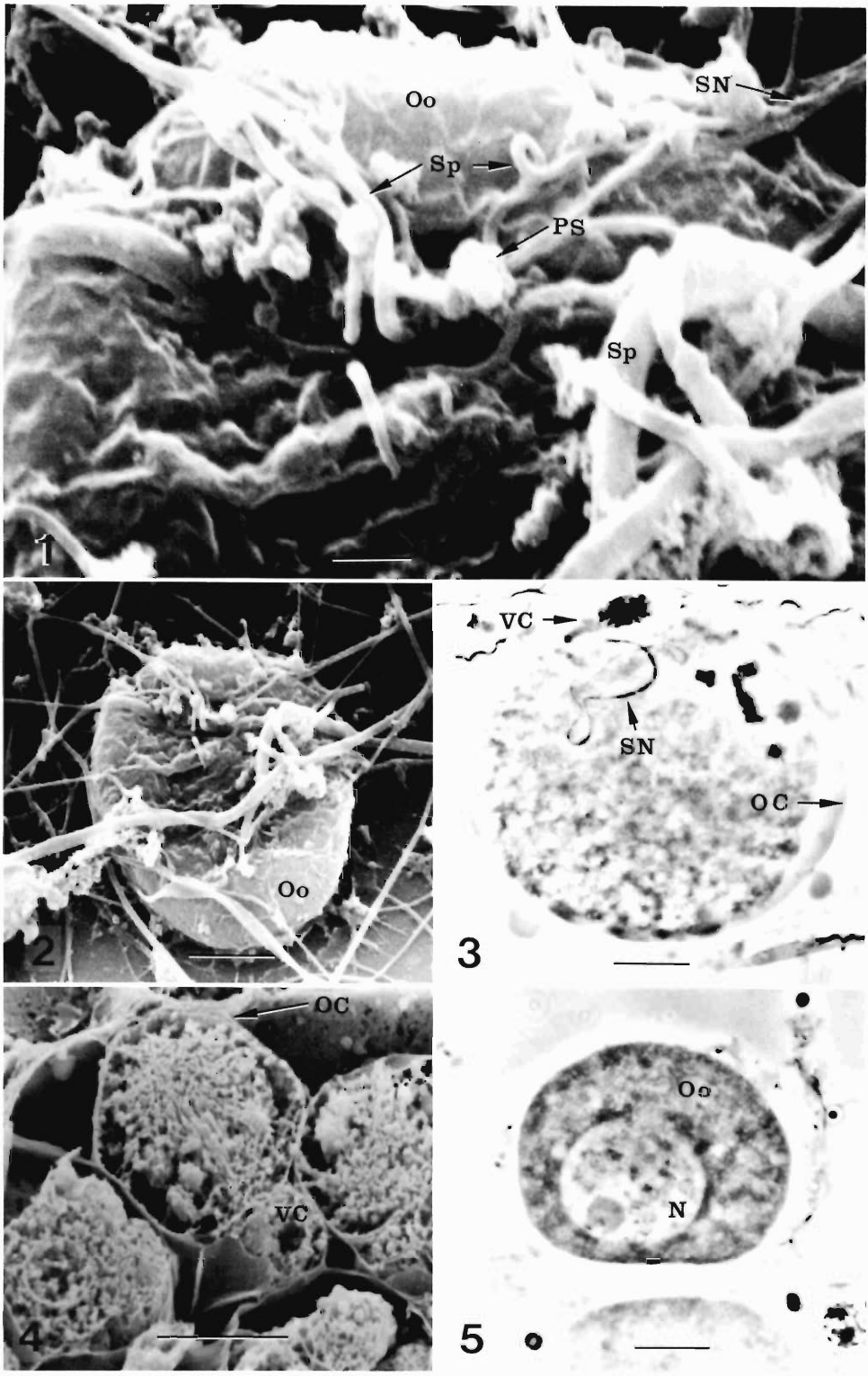
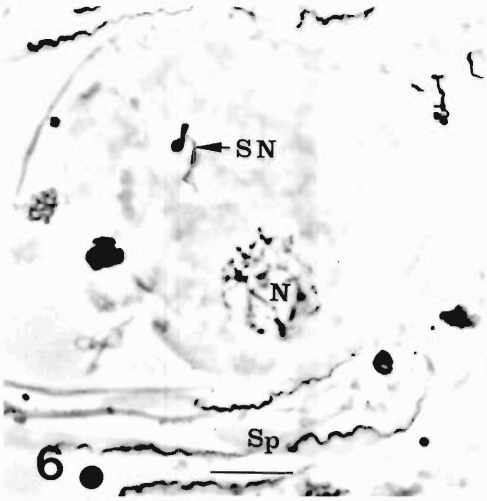
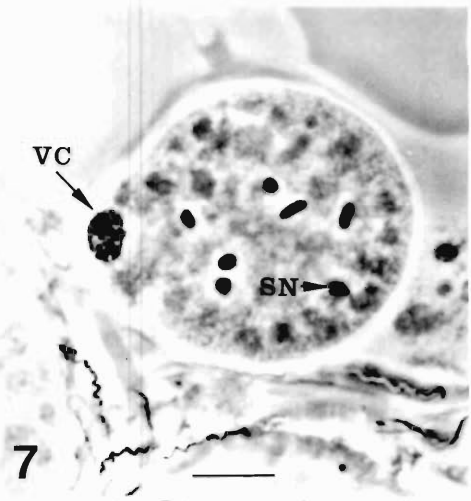
Figures 1-5. ***Hymenolepis diminuta.*** **Labeled structures include nucleus (N), outer capsule (OC), oocyte (Oo), penetration site (PS), sperm nucleus (SN), sperm (Sp), and vitelline cell (VC). 1. SEM of oocyte showing sperm penetration (arrow) after 3 min exposure. Preparation was made by macerating several proglottids of the proper age on an albuminized coverglass, thus several sperm appear to be attached. Fixation was in glutaraldehyde 3 min after maceration. Scale bar, 1 μm. 2. SEM of same specimen seen in Figure 1 showing the whole oocyte trapped in a web of sperm. Note the attachment of several sperm by the narrow end. Scale bar, 5 μm. 3. LM of oocyte I with filamentous sperm nucleus, vitelline cell, and outer capsule. The oocyte is in the diakinesis stage of oogenesis. Scale bar, 10 μm. All LM photographs (Figs. 3, 5-11, 14, 16) are smear preparations stained with proprionic orcein. Each specimen was photographed at the same magnification and later enlarged to the same extent. The differences in size seen here are due to natural variation, the stage of development, and the amount of coverglass pressure. 4. SEM of ethanol cryofracture of an early embryo in utero showing the oocyte with shell granules, the vitelline cell, and the outer capsule. AFA fixation. Scale bar, 10 μm. 5. LM of oocyte before sperm entry. Scale bar, 10 μm.**

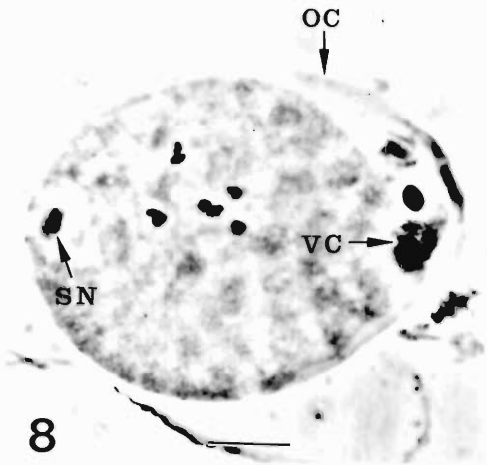
Figures 6-11. ***Hymenolepis diminuta.*** **Labeled structures include blastomere (Bm), nucleus (N), outer capsule (OC), polar body (PB), pronuclei (PN), sperm nucleus (SN), sperm (Sp), and vitelline cell (VC). 6. LM of oocyte in early prophase. Note the condensation of sperm nucleus (compare with Figs. 3 and 8). Scale bar, 10 μm. 7. LM of oocyte I in the first reduction division. Scale bar, 10 μm. 8. LM of oocyte in second reduction division. Note first polar body. Scale bar, 10 μm. 9. Male and female pronuclei ready to fuse. Scale bar, 10 μm. 10. LM of first, zygotic cleavage with chromosomes aligned at anaphase. Both polar bodies lie next to the vitelline cell. Scale bar, 10 μm. 11. LM of macromere in the process of second cleavage. Note the three polar bodies and the two blastomeres of unequal size. Scale bar, 10 μm.**

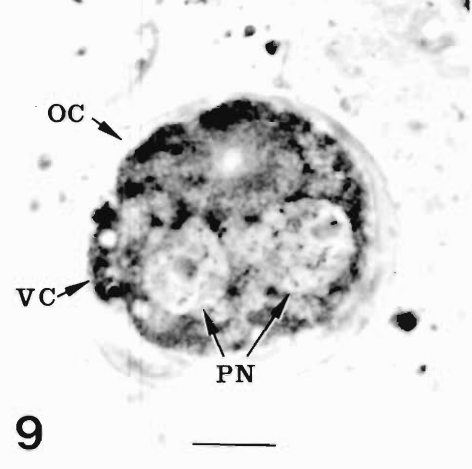
Figures 12-17. ***Hymenolepis diminuta.*** **Labeled structures include macromere (Ma), mesomere (Me), micromere (Mi), outer capsule (OC), uterus (U), and vitelline cell (VC). 12. SEM of oocyte showing sperm attachment. Scale bar, 10 μm. 13. SEM of ethanol cryofracture of early embryo. AFA fixation. By comparing the sizes of the blastomeres in Figures 13 and 16, one can infer there are about eight blastomeres present in the whole embryo shown in Figure 13. Scale bar, 10 μm. 14. LM of embryo at the 4-blastomere stage. The mesomere has not yet formed. Scale bar, 10 μm. 15. SEM of L. R. White resin cryofracture. Glutaraldehyde fixation. This stage of development is similar to that seen in Figure 13. Note differences in detail revealed in the macromere by these two techniques. Note what appears to be extracellular secretory material in Figure 15 (arrow) not seen in Figure 13. Scale bar, 5 μm. 16. LM of early embryo showing eight blastomeres including one macromere and one mesomere, and several micromeres. Scale bar, 10 μm. 17. SEM of ethanol cryofracture of early embryo. Note blastomere (arrow) revealing chromosomes and spindles during mitosis. The individual blastomeres show great variation in the organization of organelles. Scale bar, 5 μm.**

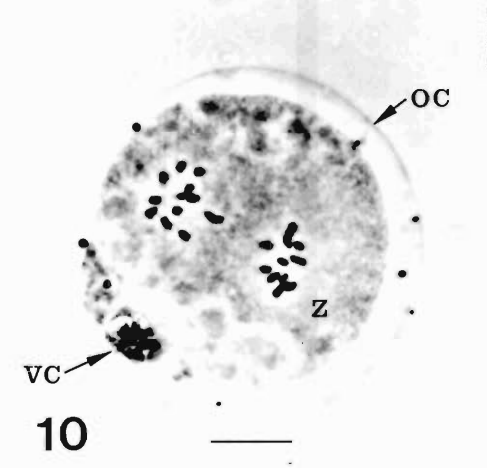


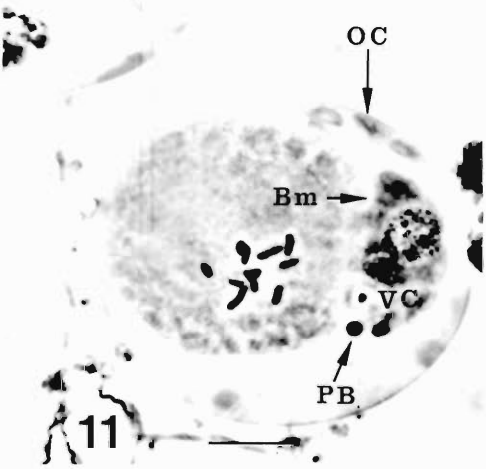


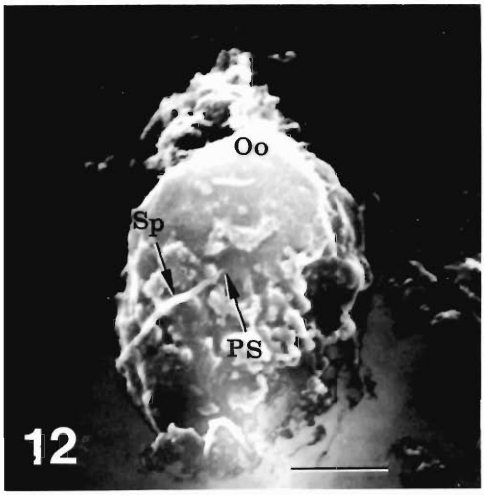


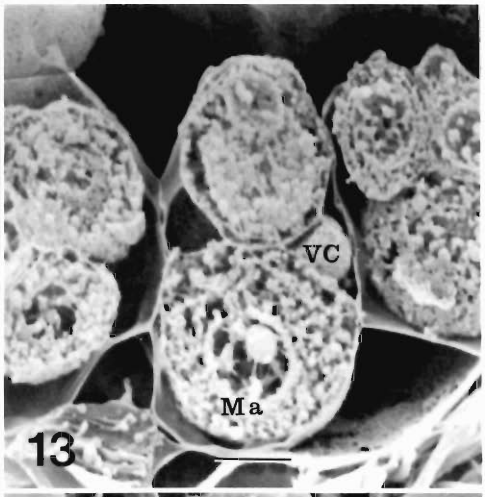


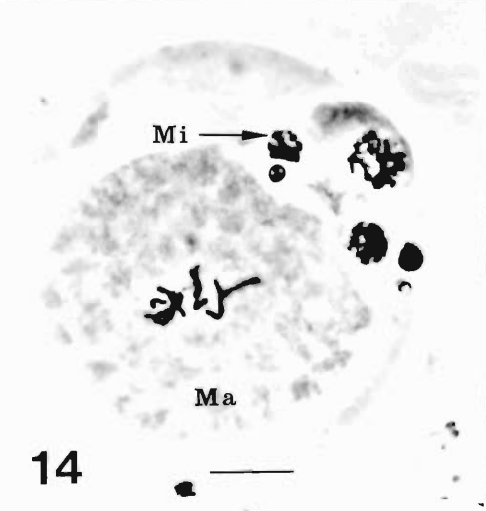


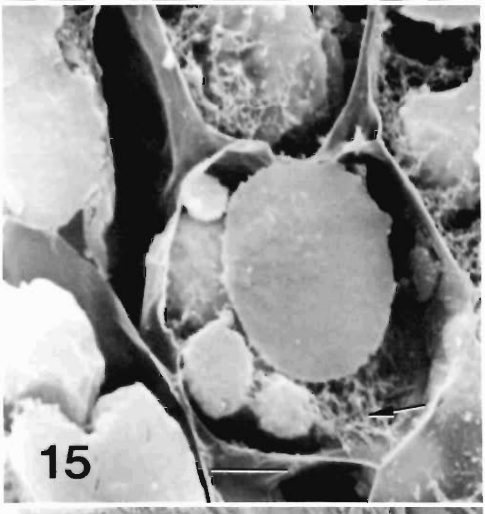


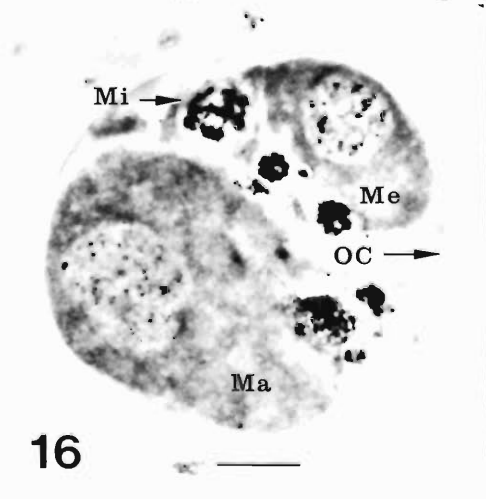


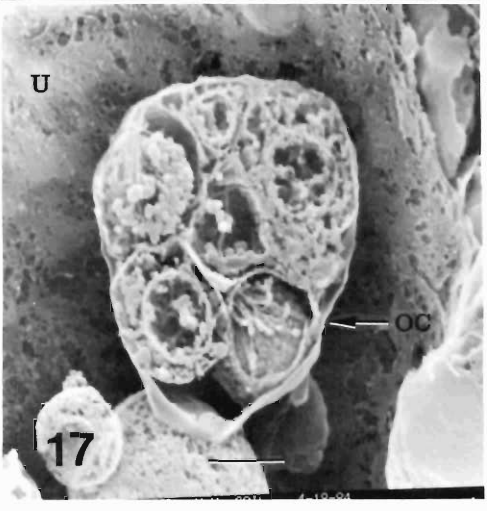


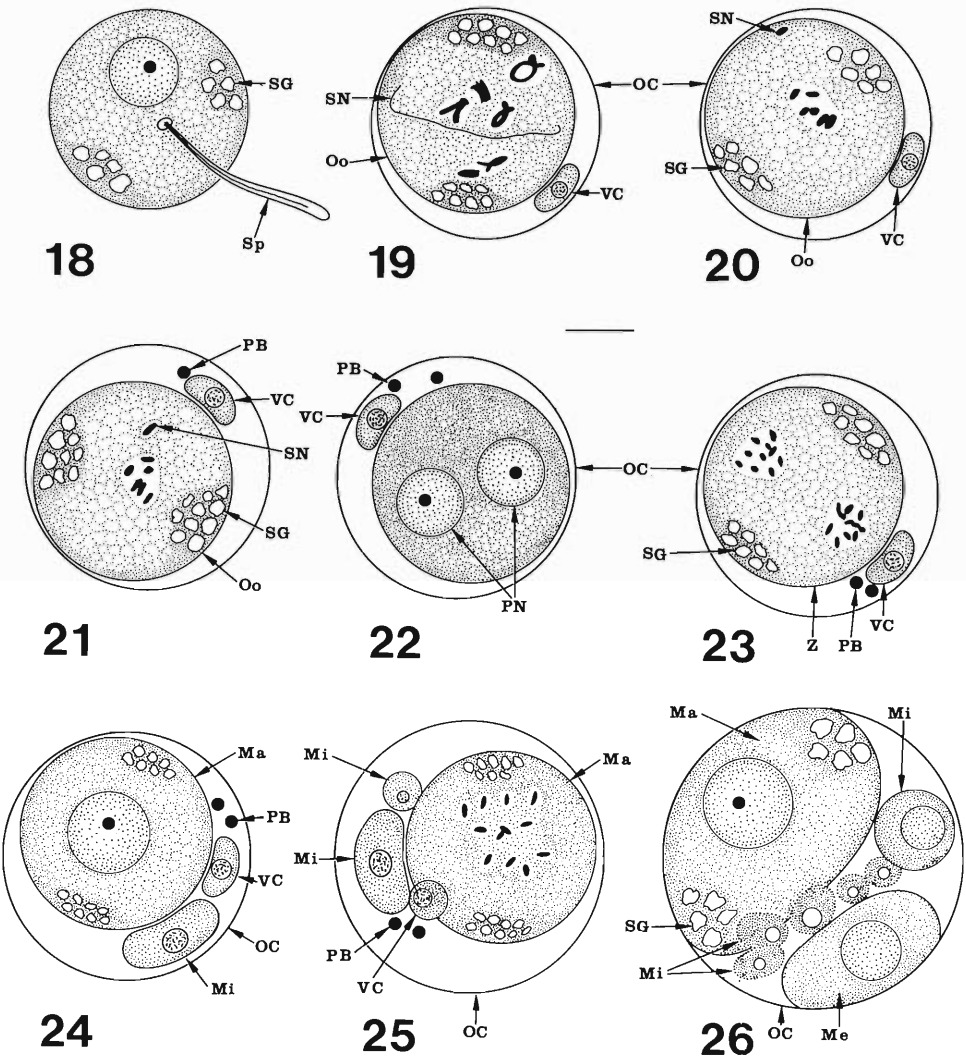












Figures 18-26. ***Hymenolepis diminuta.*** **Sketches made from specimens studied under coverglass pressure. Some details observed in other specimens added freehand. The scale is approximate, bar equals 10 μm. Labeled structures include macromere (Ma), mesomere (Me), micromere (Mi), outer capsule (OC), oocyte (Oo), polar body (PB), pronuclei (PN), shell granules (SG), sperm nucleus (SN), sperm (Sp), vitelline cell (VC), and zygote (Z). 18. Oocyte with a single sperm attached. Compare with Figures 1 and 12. 19. Oocyte in prophase I, early diplotene. Note filamentous sperm nucleus. 20. Oocyte in prophase I, Pachytene. Sperm nucleus has condensed and it is peripheral. 21. Oocyte in metaphase II with a single polar body extruded from the oocyte. 22. Oocyte with male and female pronuclei. The cytoplasm stains densely and the shell granules cannot be discerned. Frequently three polar bodies are present at this stage. 23. Zygote in anaphase with diploid chromosomes in each group. Division will be unequal. 24. A micromere and a macromere result from the zygotic cleavage. The macromere can be identified by its large size, shell granules, and large nucleus. Micromeres have a smaller nucleus and the amount of cytoplasm is much smaller, comparatively. 25. Three-blastomere stage with two micromeres and the macromere with the chromosomes in anaphase. 26. Late cleavage with large macromere containing shell granules, micromeres, and the first mesomere. The mesomere can be differentiated from the micromeres by its large amount of cytoplasm and the larger nucleus. The vitelline cell and the polar bodies could not be discerned here.**

viewed the older papers and his observations on fertilization in *Baerietta diana* appear to parallel the events observed here. It is clear from my observations that the narrow end of the sperm attaches to a specific site on the oocyte. This fact is revealed when several sperm attach to the same site at the same time, a phenomenon easily demonstrated by LM, but I was unable to show it by SEM. The filamentous nucleus penetrates the oocyte by methods still unknown, leaving behind the cytoplasmic part of the sperm (Fig. 1). Child (1907) reported that the sperm in *Moniezia* wrapped around the oocyte. In *H. diminuta,* the size of the oocyte (about 18 μm) and the length of the sperm (250–300 μm) would make this feasible. However, micrographs of attached sperm show these sperm to be shorter than those reported. One might conclude that the sperm contracts during the process of penetration. The mechanisms of sperm attachment and penetration are unknown, therefore both problems are ripe for further study with SEM and TEM. For example, even though there are several TEM studies on tapeworm sperm, none of them addresses the problem of the nature of a specialized organelle for attachment nor do they describe organelles that serve in penetration (such as an acrosome). SEM to 15,000 diameters did not reveal a "penetration organelle" on the narrow end of the sperm in this study.

In spite of the studies on *H. diminuta* of Rybicka (1966), Löser (1965), Moczon (1972), Douglas (1962), and review articles (Lumsden and Specian, 1983; Ubelaker, 1983), the significance of the oocyte shell granules is not made clear. In general, oocyte granules, if present, can be detected while oocytes are still in the ovary, and their depletion can be followed by the judicious use of bromphenol blue or malachite green, which stain what we assume are shell precursors as well as the early shell. Thus, the granules may be released into the ootype [*Shipleya inermis* in Coil (1970b) and *Mesocestoides corti* in Ogren (1956)] or they are carried with the oocyte into the uterus where they are released either from the oocyte (Coil, 1968) or from the macromeres to which they were carried during cleavage (Coil, 1979).

It should be noted that the RNA granules described by Rybicka (1967) can be differentiated from the shell granules (she calls them vitelline granules) on the basis of staining (azure B for RNA and bromphenol blue for shell granules) and on the basis of size in *H. diminuta,* the shell granules being much larger.

The oocyte shell granules are from 1.0–3.0 μm in diameter and of irregular shape. The size reported here overlaps sizes reported for *Cittotaenia* in Coil (1979) and *Dioecocestus* in Coil (1984), but they stain more intensely in these latter two genera and they approach a more spherical shape. Furthermore, the granules in *H. diminuta* tend to be widely placed in the ooplasm, whereas in contrast, the granules in *Cittotaenia* in Coil (1979) and *Dioecocestus* in Coil (1984) occur in more discrete clumps.

It seems very likely, then, that the shell granules carried by the oocyte and macromeres are utilized to contribute to the outer capsule. Later the outer envelope and the uterus both contribute to the formation of a heavy, granular outer capsule. Although the nature of the outer capsule is different, the events that lead to its formation are similar in *H. diminuta, Gyrocoelia* in Coil (1972), and *Cittotaenia* in Coil (1979). Clearly the oocyte granules question needs to be investigated with more sophisticated techniques.

The vitelline cells in the vitelline gland are rich in alpha-glycogen rosettes (Lumsden, 1965a) discernible with PAS. Shell granules also present in the vitelline cells are secreted into the lumen of the ootype where they contribute to the formation of the OC in a process not yet understood, but widely documented. The vitelline cell remains attached to the oocyte and both are surrounded by the first-formed OC when the embryo passes to the uterus. Ultimately, the vitelline cell becomes absorbed.

The glycogen granules seen early in the vitelline cell metamorphose into an amorphous (LM), PAS-positive mass. Although not yet proven, this event seems to follow a generalized explanation for glycogen mobilization (Lumsden, 1965a) in which the alpha particles break down into lower molecular weight polymers of beta glycogen. Rybicka (1960) described granular glycogen in *Diorchis ransomi* oocytes in the uterus using Carnoy's fixative.

Glycogen in the vitelline cell has been reported by Hedrick and Daugherty (1957) and Lumsden (1965a); however, the function of glycogen and the significance of its apparent morphometric change during the cell cycle of the vitelline cell are vexing problems for the future. Curiously,

the micrographs of *H. diminuta* vitelline cells (Swiderski et al., 1970) did not show the presence of glycogen.

The contribution of the vitelline cell shell granules to the formation of the first outer capsule can be inferred from the loss of the granules from the cell during its passage through the ootype. Also, the fact that the shell granules are bromphenol blue-positive and the first OC is also bromphenol blue-positive is strong evidence for their contribution to the OC. This is consistent with observations on several other cestodes [*Infula* in Coil (1968), *Dioecocestus* in Coil (1970a), and *Cittotaenia* in Coil (1979)]. Swiderski et al. (1970) showed with TEM the contribution of the vitelline cell to the OC in the ootype.

Rybicka (1966) described the first cleavage in *H. diminuta* as resulting in a macromere and a mesomere. A comparison of her figure 3C with Figures 11 and 14 printed here shows that the cell she purports as the mesomere is larger than the comparable cell shown here. Two explanations can be offered to account for this disparity: (1) the first cleavage is not a consistent event, or (2) the use of sectioned material was misleading. It is my opinion that the use of smears, and therefore the study of the whole, intact embryo, is the best way to reveal early cleavage in the cyclophyllidean cestodes.

The first cleavage of the zygote in cyclophyllideans, in those species studied at this writing, is generally unequal. The larger blastomere (macromere) receives the shell granules that are carried by the female gamete. *Baerietta* in Douglas (1963), *Dipylidium* in Rybicka (1964), *Mesocestoides* in Ogren (1956), and *Taenia* in Leuckart (1856) are genera reported to have equal cleavage. The four genera above have three macromeres, rather than two, forming the outer envelope. Two more genera, *Catenotaenia* in Swiderski (1972) and *Drepanidotaenia* in Swiderski (1967), have three macromeres forming the outer envelope, but their first cleavage is unequal.

Tapeworms reported to have both unequal cleavage and two macromeres forming the outer envelope are listed below (unfortunately, some of these studies did not include either photographs or adequate drawings and therefore the type of cleavage pattern could not be verified): *Anoplocephala magna* in St. Remy (1900), *Cittotaenia variabilis* in Coil (1979), *Dioecocestus acotylus* in Coil (1984), *Diorchis inflata* in Spätlich (1925), *Diplophallus polymorphus* in Coil (1967), *Gyrocoelia pagollae* in Coil (1972), *Hymenolepis diminuta* in Rybicka (1966), *Infula macrophallus* in Coil (1968), *Moniezia expansa* in Rybicka (1964), *Paricterotaenia porosa* in Bona (1957), *Schistotaenia* (Coil, unpubl. research), and *Shipleya inermis* in Coil (1970b).

One of the intriguing problems associated with meiosis in tapeworms is the amount of time required for the various stages. One might infer, on the basis of the high fecundity, that meiosis takes place very quickly resulting in the production of hundreds of eggs in each proglottid per unit time. However, textbook examples of meiosis cite figures for the duration of the process from a few hours to 12 days (Dyer, 1979). Obviously, the events associated with cell division in *Plasmodium* and *Eimeria* take place very rapidly resulting in astronomical numbers of progeny in a short length of time.

Our knowledge of the cyclophyllidean embryology allows for the recognition of three, possibly significant, groups based on the possession of equal (or unequal) cleavage and the presence of either two or three macromeres in the outer envelope.

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Caryophyllaeidae (Cestoda) from Lake Fishes in Wisconsin with a Description of *Isoglaridacris multivitellaria* sp. n. from *Erimyzon sucetta* (Castostomidae)

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ABSTRACT: Thirteen species in eight genera of caryophyllaeid cestodes were recovered from three catostomid and one cyprinid fish species in two southeastern Wisconsin lakes. The diversity of species was considerably greater (11 species) but prevalence was much lower in the larger river-connected Tichigan Lake than in the smaller land-locked Silver Lake (four species). Conditions in the latter lake enhanced denser populations of some species, e.g., *Glaridacris catostomi* Cooper, 1920 and *G. laruei* (Lamont, 1921) Hunter, 1927 and the acceleration of sexual development and/or early elimination of gravid specimens of others, e.g., *G. catostomi* and *Isoglaridacris agminis* Williams and Rogers, 1972. *Isoglaridacris multivitellaria* sp. n. is described from *Erimyzon sucetta* (Lacépède) in Silver Lake. It is most similar to *I. agminis*, e.g., in number of testes and length of ovarian arms, but is distinguished from it and other species of the genus by its extensive postovarian vitellaria and the long distance between its cirrus sac and ovary as well as by its follicular ovary. New state records for Wisconsin include *I. folius* Fredrickson and Ulmer, 1965 and *Rowardleus pennensis* Mackiewicz and Deutsch, 1976. The recovery of the first species from *Catostomus commersoni* (Lacépède) represents a new host record. Seasonal and host relationships are explored in the commonly encountered species and the morphometric characteristics of all species were assessed and compared with other published accounts.

This is the first in a series of reports on the cestode parasites of fishes from two southeastern Wisconsin lakes in Racine and Kenosha counties. Some species were not previously reported in Wisconsin and most expressed significant morphometric variations from original and/or subsequent descriptions. Ecological information particularly pertaining to seasonality and concurrent infections are also documented along with the description of a new caryophyllaeid species of the genus *Isoglaridacris* Mackiewicz, 1965.

Materials and Methods

Seasonal parasitological collections yielding the reported material were made from Silver Lake (Kenosha County) and Tichigan Lake and canal (Racine County) between 1976 and 1984. The 188-ha [revised from 200 ha reported in earlier papers, e.g., Amin (1982)] Silver Lake has a maximum depth of 13.4 m. It is a eutrophic natural land-locked lake of glacial origin lying within the lateral moraine of the Lake Michigan lobe of the Wisconsin glacier. A small outlet historically permitted the intermittent discharge of overflow waters (until dammed in 1932) into the Fox River, a tributary of the Mississippi River drainage system. The size of Tichigan Lake, originally a natural lake on the above-mentioned Fox River, increased to 458 ha [not 108 ha as reported in Amin (1982) and earlier papers] as a result of an impoundment of the Fox River in 1830; it has a maximum depth of 19.2 m.

Fishes were collected by electro-shocking and systematically examined within 24 h. Recovered worms were refrigerated in distilled water overnight, fixed in cold AFA, stained in Semichon's carmine, cleared in oil of winter green (methyl salicylate), and mounted in Canada balsam.

Observations and measurements were made of mature adults that were usually gravid. In each studied specimen, both ovarian arms and at least two testes and two eggs were measured. All measurements are in micrometers, unless otherwise noted, with averages in parentheses. Figures were drawn with the aid of a carbon arc microprojector.

Mean per host is the number of cestodes recovered/number of fish examined. The site of infection was determined in the following six intestinal regions of each of the fish species examined (see fig. 2 in Amin, 1975b): stomach (A), first straight postgastric limb (B), the second limb (C1), two coils (C2,3), and the posteriormost limb leading to the vent (C4); the C regions were usually similar in size. This system is preferred over the percentile method, used by some authors, as it takes into account regional differences in intestinal topography and biochemistry. Fish size is expressed in total length (cm). Representative specimens were deposited in the U.S. National Museum Helminthological Collection (USNM Helm. Coll.) and the Milwaukee Public Museum Collection (MPM Coll.).

Results and Discussion

Caryophyllaeid cestodes were found in: quillback sucker, *Carpiodes cyprinus* (LeSueur), white sucker, *Catostomus commersoni* (Lacépède), lake chubsucker, *Erimyzon sucetta* (Lacépède) (Catostomidae), and carp, *Cyprinus carpio* (Linn.) (Cyprinidae). A total of 382 fishes of the above four species were examined from Silver and Tichigan lakes during the spring (April), summer

Table 1. Prevalence and intensity of caryophyllaeid cestodes in fishes of Silver and Tichigan lakes, 1976–1984.

		Silver Lake					Tichigan Lake				
		Fish		No. of cestodes			Fish		No. of cestodes		
Host species	Cestode species	*N*	Infect. (%)	Total	Mean	Max.	*N*	Infect. (%)	Total	Mean	Max.
Carpiodes cyprinus	*Rowardleus pennensis*	—	—	—	—	—	19	2 (10)	2	0.10	1
Catostomus commersoni	*Biacetabulum biloculoides*	10	—	—	—	—	105	1 (1)	2	0.02	2
	B. macrocephalum	10	—	—	—	—	105	1 (1)	3	0.03	3
	Biacetabulum sp.	10	—	—	—	—	105	1 (1)	5	0.05	5
	Glaridacris catostomi	10	5 (50)	139	13.90	96	105	8 (8)	141	1.34	108
	G. laruei	10	6 (60)	376	37.60	263	105	7 (7)	21	0.20	8
	Hunterella nodulosa	10	—	—	—	—	105	4 (4)	18	0.17	12
	Isoglaridacris folius	10	—	—	—	—	105	1 (1)	10	0.09	10
	Monobothrium hunteri	10	—	—	—	—	105	1 (1)	1	0.01	1
Cyprinus carpio	*Atractolytocestus huronensis*	52	—	—	—	—	78	2 (3)	5	0.06	4
	Khawia iowensis	52	—	—	—	—	78	2 (3)	2	0.03	1
Erimyzon sucetta	*Glaridacris laruei*	116	3 (3)	5	0.04	3	2	1 (50)	1	0.50	1
	Isoglaridacris agminis	116	28 (24)	139	1.20	38	2	—	—	—	—
	I. multivitellaria	116	1 (1)	4	0.03	4	2	—	—	—	—
Total		178	43 (24)	663	3.72	263	204	31 (15)	211	1.03	108

(June, July, early August), and autumn (late October and November) between 1976 and 1984 (Table 1). No caryophyllaeids were found in carpsucker, *Carpiodes carpio* (Raf.) (3 fish); silver redhorse, *Moxostoma anisurum* (Raf.) (4); river redhorse, *M. carinatum* (Cope) (3); and golden redhorse *M. erythrurum* (Raf.) (18) (Catostomidae) from Tichigan Lake and canal.

Of the 382 fish listed in Table 1, 74 were infected with one or more of 13 species of caryophyllaeids from eight genera and two families in the order Caryophyllaeidea Van Beneden (in Carus, 1863).

Family Caryophyllaeidae Leuckart (in Luhe, 1910)

Biacetabulum biloculoides Mackiewicz and McCrae, 1965

One juvenile and one gravid adult were recovered from the stomach of a 36-cm-long female *C. commersoni* collected from Tichigan Lake during the autumn. The same fish also harbored one gravid *Hunterella nodulosa* Mackiewicz and McCrae, 1962, three juvenile *Glaridacris catostomi* Cooper, 1920, and five juvenile *Biacetabulum* sp. in the stomach and postgastric region B. *Biacetabulum biloculoides* was first reported in Wisconsin from some Kenosha and Racine County streams (Pike and Root rivers which drain into Lake Michigan) by Amin (1974) and further studied by Amin (1975a, b, 1977). Those two streams are not connected to either Silver or Tichigan lakes.

Dimensions of the gravid worm fell within the range of those described by Mackiewicz and McCrae (1965) with the eggs being more similar to those from New York than from Colorado.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 78811.

Biacetabulum macrocephalum McCrae, 1962

One gravid and two juvenile *B. macrocephalum* and one gravid *Monobothrium hunteri* Mackiewicz, 1963 infected the stomach of a 16-cm-long *C. commersoni* from Tichigan Lake canal on June 14, 1978. White suckers were more frequently and heavily infected with *B. macrocephalum* in the Pike and Root rivers (Amin, 1974, 1977). The adult specimen was 7.6 mm long and 0.56 mm wide at gonopore and at scolex and had 110 testes measuring 90–138 in diameter. Cirrus sac was 168 in diameter and eggs were 38–51 by 26–32. McCrae's (1962) specimens were shorter (4.0–6.5 mm) and had fewer (75–95) and larger (156–224) testes and larger eggs (50–presumably 55 by 30–presumably 35;

Table 2. Seasonal distribution of *Glaridacris catostomi, G. laruei,* and *Isoglaridacris agminis* from *Catostomus commersoni* and *Erimyzon sucetta* in Silver and Tichigan lakes.

			Autumn (late October, November)						
Cestode	Lake	Host	Juv.*	Adult	Grav.	*N*	Inf./ exam.	%	Mean
G. catostomi	Silver	*C. commersoni*	2	4	0	6	1/3	33	2.00
	Tichigan	*C. commersoni*	29	2	0	31	6/42	14	0.74
G. laruei	Silver	*E. sucetta*	0	0	2	2	2/42	5	0.05
	Tichigan	*E. sucetta*	—	—	—	—	0/1	—	—
	Silver	*C. commersoni*	0	2	1	3	2/3	67	1.00
	Tichigan	*C. commersoni*	4	5	6	15	4/42	9	0.36
I. agminis	Silver	*E. sucetta*	1	65	19	85	21/42	50	2.02

* Juv. = no. of juveniles; adult = no. of nongravid adults; grav. = no. of gravid adults; *N* = total; inf./exam. = no. of fish infected/no. examined; % = prevalence; mean = total no. of worms recovered/no. of fish examined.

reported as "0.05 to 0.55 long by 0.03 to 0.085 wide").

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 78659.

Biacetabulum sp.

Five juvenile *Biacetabulum* sp. were recovered from the stomach (3 specimens) and postgastric region B (2) of the white sucker from Tichigan Lake, which was also infected with *B. biloculoides* (above). Worms were 2.56 to 3.20 mm (2.89) long and had a prominent rectangularly shaped scolex clearly set off from the neck. The scolex had one pair of large well-developed dorsal and ventral acetabular suckers and four moderately developed lateral loculi. Two rows of primordial reproductive units in the body were variably recognizable in all specimens. These specimens resembled the immature *B. meridianum* reported by Grimes and Miller (1975, figs. 1, 2) from North Carolina.

DEPOSITED SPECIMENS: USNM Helm. Coll. Nos. 78658, 78811.

Glaridacris catostomi Cooper, 1920

A total of 280 *G. catostomi* infected 13 of 115 (11%) *C. commersoni* examined from both lakes. Prevalence and intensity of infections were considerably heavier in the land-locked Silver Lake (50% and 13.90) than in Tichigan Lake (8% and 1.34) (Table 1). In both lakes, recruitment appears to begin in late summer and early autumn; only a few worms, mostly juveniles, were recovered during the autumn. Maturation and population buildup progressed until the spring before worms were eliminated; none was recovered during the summer (Table 2). These results agree with those reported for *G. catostomi* from the same host species in Iowa (Calentine and Fredrickson, 1965), Maine (Lawrence, 1970), Wisconsin (Williams, 1979), and New Hampshire (Muzzall, 1980). In addition, results also show earlier maturation of *G. catostomi* in suckers of Silver Lake compared to those from Tichigan Lake (Table 2). The relatively warmer waters of the smaller, land-locked and shallower Silver Lake probably enhanced the acceleration of worm maturation there. Suckers were not infected with *G. catostomi* and scarcely infected with other helminth species during the summer.

The anterior localization of *G. catostomi* and *Hunterella nodulosa* Mackiewicz and McCrae, 1962 and the posterior distribution of *G. laruei* (Lamont, 1921) Hunter, 1927 and *Pomphorhynchus bulbocolli* (Linkins, 1919) Van Cleave, 1919 in the intestine of *C. commersoni* from both Silver and Tichigan lakes did not significantly vary by season (Figs. 1, 2). Muzzall (1980) observed similar distribution of *G. catostomi* and *G. laruei* in *C. commersoni* from New Hampshire. Similar and other observations of *G. catostomi* in the same host species from some southeastern Wisconsin streams were noted by Amin (1975a, b, 1977).

In Silver and Tichigan lakes, white suckers were usually concurrently infected with *G. catostomi* and *G. laruei* as well as with *P. bulbocolli* and/or *H. nodulosa* and infrequently with *Acanthocephalus dirus* (Van Cleave, 1931) VanCleave and Townsend, 1936, *B. biloculoides, Biacetabulum* sp., or with other cestode species singly (Table 1). This is a large assortment of helminths infecting one species of fish.

Morphologically, these *G. catostomi* (Table 3)

Table 2. Continued.

Spring (April)							Summer (June, July, early August)						
Juv.	Adult	Grav.	*N*	Inf./ exam.	%	Mean	Juv.	Adult	Grav.	*N*	Inf./ exam.	%	Mean
0	17	116	133	4/5	80	26.60	—	—	—	—	0/2	—	—
29	80	1	110	2/37	5	2.97	—	—	—	—	0/26	—	—
0	1	2	3	1/49	2	0.06	—	—	—	—	0/25	—	—
—	—	—	—	0/0	—	—	0	0	1	1	1/1	100	1.00
33	252	87	372	3/5	60	74.40	0	0	1	1	1/2	50	0.50
0	0	2	2	1/37	3	0.03	0	0	4	4	2/26	8	0.15
0	8	43	51	6/49	12	1.04	0	0	3	3	1/25	4	0.12

were similar to those described by Cooper (1920) and Mackiewicz (1965) but varied in the following respects. Cooper (1920) reported fewer (150–160) and smaller testes (135–227 by 100–145 by 127–181) and shorter ovarian arms (800–900), and Mackiewicz (1965) reported shorter ovarian arms (700–900) and larger eggs (55–77 by 50–57).

DEPOSITED SPECIMENS: USNM Helm. Coll. Nos. 78657, 78811; MPM Coll. No. IZ985-01C.

Glaridacris laruei (Lamont, 1921) Hunter, 1927

A total of 403 *G. laruei* were collected mostly from *C. commersoni* in Silver Lake. White suckers from Tichigan Lake and *E. sucetta* from both lakes were very lightly infected (Table 1). Recruitment and seasonal development (Table 2) appear to be similar to those described for *G. catostomi* above. The persistent distribution of *G. laruei* in posterior intestinal locations of *C. commersoni* and its interrelationships with other helminths concurrently infecting that host species during the autumn and the spring were also discussed under *G. catostomi* (above) and expressed in Figures 1 and 2. Grey and Hayunga (1980) demonstrated, however, that *G. laruei* becomes displaced anteriorly when posterior intestinal locations of *C. commersoni* are heavily infected with *P. bulbocolli.*

Except for one protogynous specimen with no testes, all adult *G. laruei* examined were protandrous. Local material exhibited the usual variations in the shape of scolex and number of testes and postovarian vitellaria (Table 3) as previously reported by Mackiewicz (1960) and Williams (1980). The ovary was, however, invariably H-shaped. Specimens from *C. commersoni* had a relatively smaller cirrus sac and ovarian arms, shorter postgonopore distance and fewer postovarian vitellaria compared to those from *E. sucetta* (Table 3). The above material from *C. commersoni* was similar to specimens reported from the same host species by Mackiewicz (1960) but varied from those of Hunter (1927) and Williams (1980) who reported fewer testes [60–80 and 0–82 (mean 9.3), respectively] and smaller cirrus sac [108–120 and 94–141 (mean 115), respectively].

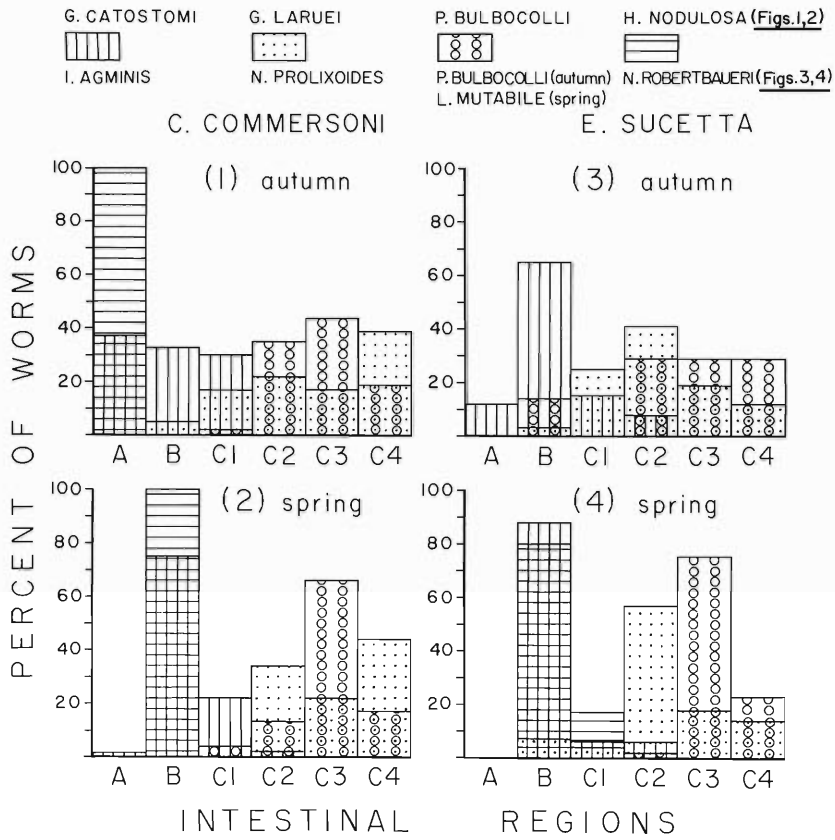
DEPOSITED SPECIMENS: USNM Helm. Coll. Nos. 78655, 78656; MPM Coll. No. IZ985-01B.

Hunterella nodulosa Mackiewicz and McCrae, 1962

Four *C. commersoni* were infected with 18 gravid *H. nodulosa,* three with four worms in the stomach during the autumn and one with 12 worms in intestinal region B during the spring, only in Tichigan Lake (Tables 1, 2). Figure C (far right) in Amin (1974) is of *H. nodulosa* as reported by Amin (1977) from *C. commersoni* in the Pike and Root rivers, southeastern Wisconsin. The intestinal distribution of *H. nodulosa* in concurrent infections did not show appreciable seasonal changes. Worms cohabited with *G. catostomi* in the anterior intestinal locations of *C. commersoni* (Figs. 1, 2).

My worms were similar to those originally described by Mackiewicz and McCrae (1962) except for having fewer and larger testes as well as a relatively larger cirrus sac (Table 3). In the original description, worms had 95–175 testes measuring 50–100 and cirrus sac diameter was 114–205.

DEPOSITED SPECIMENS: USNM Helm. Coll. Nos. 78660, 78811.



Figures 1-4. Concurrent infections of *Glaridacris catostomi, G. laruei, Pomphorhynchus bulbocolli,* and *Hunterella nodulosa* in the intestine of *Catostomus commersoni* from Silver and Tichigan Lakes during the autumn (Fig. 1) and the spring (Fig. 2) and of *Isoglaridacris agminis, Neoechinorhynchus prolixoides, P. bulbocolli, Lissorchis mutabile,* and *N. robertbaueri* in the intestine of *Erimyzon sucetta* from Silver Lake during the autumn (Fig. 3) and the spring (Fig. 4). 1. Based on 40 *G. catostomi,* 18 *G. laruei,* 205 *P. bulbocolli,* and 6 *H. nodulosa* from 11 hosts; 4 *A. dirus* from regions C1-C3, 2 *Biacetabulum biloculoides* from region A, and 5 *Biacetabulum* sp. from regions A and B were also recovered (only from Tichigan Lake) but not shown. 2. Based on 243 *G. catostomi,* 374 *G. laruei,* 88 *P. bulbocolli,* and 12 *H. nodulosa* from 8 hosts; 2 *A. dirus* from region C2 and 10 *I. folius* from regions A and B were also recovered (only from Tichigan Lake) but not shown. 3. Based on 85 *I. agminis,* 32 *N. prolixoides,* and 7 *P. bulbocolli* from 21 hosts; 1 *N. cylindratus* from region C1 and 4 *I. multivitellaria* from region C4 were also recovered but not shown. 4. Based on 51 *I. agminis,* 28 *N. prolixoides,* 84 *L. mutabile,* and 133 *N. robertbaueri* from 6 hosts; 1 *Leptorhynchoides thecatus* from region C1 was also recovered but not shown. A = stomach; B = first straight postgastric limb; C1 = second limb; C2, 3 = two coils; C4 = posteriormost limb leading to the vent.

Isoglaridacris agminis Williams and Rogers, 1972

A total of 139 worms infected 28 of 116 (24%) *E. sucetta* examined from Silver Lake (Table 1). The percent of gravid worms was 22 in the autumn, 84 in the spring, and 100 in the summer (Table 2), thus suggesting a seasonal reproductive cycle similar to that of *G. catostomi* or *G. laruei* (above). However, the fact that virtually no juveniles were recovered in the autumn (with 22% of the worms being gravid then) and that the prevalence and intensity of infection progressively decreased in spring and summer also suggests accelerated reproductive development early in the infectious cycle as well as earlier loss of gravid worms in the spring. Recruitment of *I. agminis* appears to cease altogether by the spring (no juveniles were recovered then) in contrast to *G. catostomi* or *G. laruei* (Table 2).

During all seasons, *I. agminis* primarily infected postgastric region B, with only 10 specimens extending into region A during the autumn

Table 3. Morphometric characteristics of some caryophyllaeid cestodes collected from *Catostomus commersoni* and *Erimyzon sucetta* in Silver and Tichigan lakes.

	Glaridacris catostomi from *C. commersoni* ($N = 10$)	*Glaridacris laruei* from *C. commersoni* ($N = 22$)	*Glaridacris laruei* from *E. sucetta* ($N = 5$)	*Hunterella nodulosa* from *C. commersoni* ($N = 10$)	*Isoglaridacris agminis* from *E. sucetta* ($N = 17$)	*Monobothrium hunteri* from *C. commersoni* ($N = 1$)
Length (L) (mm)	16.8–39.0 (27.1)*	3.8–9.6 (6.2)	5.9–12.2 (9.5)	2.6–5.3 (4.1)	3.0–13.2 (6.4)	17.4
Width at gonopore (mm)	0.92–1.76 (1.41)	0.40–0.84 (0.64)	0.48–1.04 (0.74)	0.52–1.16 (0.84)	0.40–0.72 (0.51)	1.08
Width of scolex (mm)	0.68–1.56 (1.07)	0.36–0.92 (0.67)	0.48–0.76 (0.63)	0.56–1.12 (0.78)	0.32–0.72 (0.49)	0.68
Testes (T) number	160–401 (296)	0–122 (99)†	70–112 (97)	90–120 (106)	17–68 (42)	106
T diameter	77–416 (226)	80–208 (133)	122–208 (150)	77–182 (127)	80–192 (124)	96–154 (137)
First T to ant. tip (mm)	1.60–3.32 (2.39)	0.64–1.56 (1.03)	0.84–1.88 (1.29)	0.36–0.96 (0.57)	0.80–4.20 (2.14)	6.80
Cirrus sac diameter	350–728 (589)	126–168 (137)	126–224 (171)	98–266 (173)	140–238 (180)	210
First preovarian vitellarium to ant. tip (mm)	1.60–3.00 (2.05)	0.52–1.52 (0.94)	0.68–1.64 (1.19)	0.40–1.20 (0.75)	0.68–2.80 (1.56)	2.40
% of worm L	6–11 (8)	12–27 (16)	7–13 (11)	14–23 (18)	11–38 (27)	14
Postgonopore distance (mm)	2.00–5.88 (3.73)	0.60–1.60 (0.92)	0.84–1.76 (1.41)	0.80–1.36 (1.06)	0.84–1.52 (1.14)	2.64
% of worm L	12–17 (14)	11–18 (15)	12–14 (13)	19–31 (26)	10–17 (13)	15
No. of postovarian vitellaria	22–56 (38.6)	0–5 (2.6)	1–15 (5.4)‡	14–40 (26.4)	5–16 (9.7)	0
Ovarian arms length	686–1,820 (1,275)	350–1,400 (720)	728–1,162 (933)	168–392 (293)	350–840 (522)	1,162–1,232 (1,197)
Egg length	48–61 (54)	38–58 (45)	38–48 (43)	48–61 (56)	35–54 (46)	64–83 (74)
Egg diameter	35–51 (40)	26–38 (32)	26–35 (32)	32–42 (37)	29–38 (34)	42–64 (50)
Eggs measured	13	24	7	20	17	7

* Range (mean).

† The range becomes 60–122 if one protogynous specimen with no testes is excluded.

‡ The range becomes 1–4 if the one specimen with 15 vitellaria is excluded; Hunter (1927) showed one specimen (fig. 36) with 17 vitellaria.

and a few more into regions C1 and C2 during the autumn (13 and 7) and spring (3 and 3) (Figs. 3, 4). The short-lived *Neoechinorhynchus robertbaueri* Amin, 1985 shared the same anterior intestinal locations with *I. agminis* during the spring; it is not present in its fish hosts during the autumn. More posterior intestinal sites were usually concurrently infected with *P. bulbocolli* during the autumn or with *Lissorchis mutabile* (Cort, 1919) during the spring and with *N. prolixoides* Bullock, 1963 (Figs. 3, 4).

My worms varied from those originally described by Williams and Rogers (1972) by having longer bodies, more but smaller testes, larger cirrus sac, and longer (or shorter?) distance between first vitellarium and anterior tip of scolex (Table 3). Worms in the original description were 3.77–7.73 mm (5.52) long and had 28–40 tests measuring 101–221 (166) in diameter. The cirrus sac was 120–162 (138) by 85–138 (118) and two different measurements for the distance between the first vitellarium and anterior tip were given: 1.390–2.910 mm (1.968) and 0.630–2.230 (1.160) (Williams and Rogers, 1972). The measurements of a larger sample of *I. agminis* from *Minytrema melanops* (Raf.) by Williams (1975) were similar to those in the original description except for relatively wider range values. Grimes and Miller (1975) reported *I. agminis* from *E. oblongus* (Mitchill) in North Carolina with even a greater number of testes, 56–77 (69) ($N = 5$).

DEPOSITED SPECIMENS: USNM Helm. Coll. Nos. 78653, 78654; MPM Coll. No. IZ985-01A.

Isoglaridacris folius Fredrickson and Ulmer, 1967

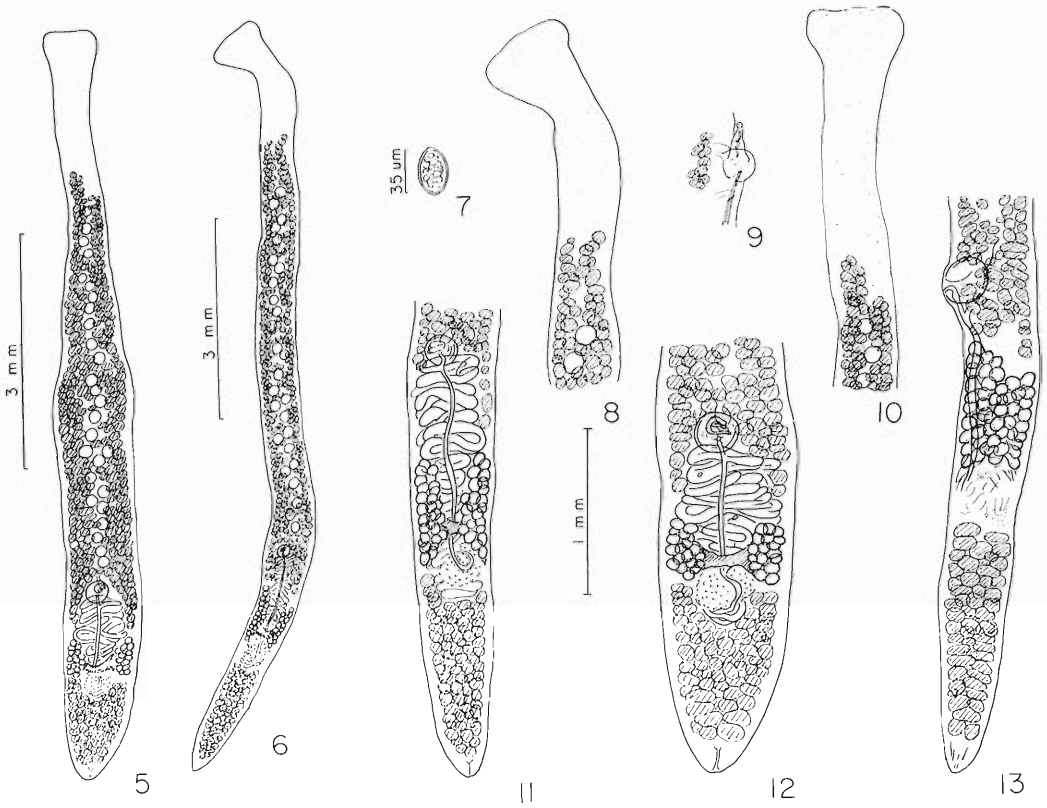
Ten gravid 7.0–12.0-mm-long worms exclusively infected the stomach (6 specimens) and postgastric region B (4) of a 40-cm-long male *C. commersoni* in Tichigan Lake on April 7, 1977. This represents new host and state records. The fact that all worms were gravid, even though with a few eggs each, indicates that this may not be an accidental infection. *Isoglaridacris folius* normally infects *Moxostoma erythrurum* (Raf.) just posterior to the stomach (Fredrickson and Ulmer, 1967) and has not been reported from any other host species since its description. None of 18 *M. erythrurum* examined from Tichigan Lake was, however, infected with this or any other helminth species. Measurements of local material fell within the range of those in the original description except for the longer ovarian arms (1.68–2.18 mm long); Fredrickson and Ulmer's (1967) corresponding measurements were 0.8–1.65 (1.19).

DEPOSITED SPECIMENS: USNM Helm. Coll. No. 78665.

Isoglaridacris multivitellaria sp. nov.

Four worms, three of which were gravid with up to five eggs each, were recovered from posteriormost intestinal region C4 of a 20-cm-long female *E. sucetta* in Silver Lake on November 26, 1978; 116 lake chubsuckers were examined. The same fish was infected with two *I. agminis* in region C1 and two *Neoechinorhynchus prolixoides* in C3. It is interesting to note that in a clearly rare species like this one, all four specimens were found in one individual host. Similarly, the description of *I. erraticus* Williams, 1975 was based on seven specimens recovered from one *Moxostoma* sp. and that of *I. etowani* Williams, 1975 on 50 specimens (of 154) from one (of 87) *Hypentelium etowanum* (Jordan). The mechanisms affecting the concentration of uncommon species in very few hosts (thus insuring the availability of breeding partners) should be exciting to explore.

DESCRIPTION (based on four specimens, Figs. 5–13): With characteristics of the genus *Isoglaridacris*. Worms 7.68–10.80 mm (9.53) long by 560–960 (690) wide at gonopore. Scolex fan-shaped with three pairs of very shallow loculi, 640–720 (680) wide. Neck intermediate in length. Outer longitudinal muscles unpronounced. Testes spheroid, begin 2.08–2.48 mm (2.28) from scolex apex, extend to near anterior border of cirrus sac, number 25–50 (34), measure 102–176 (132) in diameter ($N = 25$). External seminal vesicle dorsal. Cirrus sac unarmed, eversible, 210–294 (245) in diameter, well anterior of ovary. Gonopore large and conspicuous. Preovarian vitelline follicles round, oval, or laterally elongate, smaller than testes, 56–182 (115) in diameter ($N = 24$), extensive, entirely medullary in two lateral rows that irregularly extend and often merge medially, begin anterior to testes, 1.20–1.72 mm (1.55) from scolex apex or 12–21% (17) of worm length, extend past cirrus sac to near anterior border of ovary. Postovarian vitellaria not continuous with preovarian vitellaria, extensive, cover an area about as long as that occupied by cirrus sac, uterus and ovary, 1.00–1.48 mm (1.25) long. Postgonopore distance 2.28–3.32 mm (2.79) or 24–32% (29) of worm length. Ovary H-shaped, arms



Figures 5–13. ***Isoglaridacris multivitellaria*** **sp. nov. 5. Holotype. 6. Paratype. 7. Egg. 8. Scolex and neck of paratype in Figure 6. 9. Lateral view of genital area of another paratype. 10. Scolex and neck of holotype. 11. Reproductive structures of a paratype. 12. Reproductive structures of holotype. 13. Lateral view of the posterior region of paratype in Figure 6. All are ventral views except Figures 9 and 13 (lateral). Figures 8–13 to same scale.**

distinctly follicular 420–882 (678) long. Vagina straight, without seminal receptacle. Eggs ovoid, operculated, with smooth thin shell, 45–54 (49) long by 35–38 (38) in diameter ($N = 8$).

HOLOTYPE: USNM Helm. Coll. No. 78651.

PARATYPE: USNM Helm. Coll. No. 78652.

TYPE HOST: *Erimyzon sucetta* (Lacépède) (Catostomidae).

TYPE LOCALITY: Silver Lake, Kenosha County, Wisconsin.

ETYMOLOGY: Named for its extensive vitellaria.

Remarks

The new species has six loculi on the scolex, a single gonopore, uterine coils not extending anterior to cirrus sac, preovarian vitellaria beginning anterior to first testis and not continuous with postovarian vitellaria, and an external seminal vesicle. These features place it in the genus *Isoglaridacris*. In addition, the ovary is H-shaped, which is structurally not unlike the open-apexed inverted A-shaped ovary present in many members of this genus. The distance between the posterior branches of the ovarian arms may be related to proximity to posterior body tip, e.g., narrowest where ovary is nearest posterior extremity.

The body of *I. multivitellaria* has nearly parallel sides (Fig. 6), but greater disparity between its width anteriorly and posteriorly was expressed in one specimen (Fig. 5). Length of ovarian arms was also somewhat variable (Figs. 11–13). When the cirrus is extended (Fig. 9) two gonopores may appear to be present.

The most unique features of *I. multivitellaria* are the extent of development and distribution of its vitelline follicles, ovarian structure, and

location of cirrus sac. The postovarian vitellaria are most extensive, cover an area about as long as the distance between the cirrus sac and the posterior border of ovarian arms, and include more than 100 follicles. In none of the 11 species of *Isoglaridacris* known from North America does the number of these follicles exceed 20; they are absent in *I. calentinei* Mackiewicz, 1974 and *I. jonesi* Mackiewicz, 1972. The two lateral rows of preovarian vitellaria are also extensive; they extend and often merge medially. This condition is not known in any of the other species; one medial and two lateral rows of preovarian vitellaria are present in *I. etowani, I. chetekensis* Williams, 1977, and *I. wisconsinensis* Williams, 1977. The extreme follicular structure of the ovarian arms of *I. multivitellaria* is unique to that species; the arms are parallel and short. In the other North American species, the arms are longer (except in *I. agminis* Williams, 1972, 420–840 long) and are either H-shaped with the arms invariably merging posteriorly (in *I. agminis, I. calentinei, I. chetekensis, I. erraticus, I. folius, I. wisconsinensis*), inverted A-shaped with fused apex (in *I. etowani*), or both (in *I. bulbocirrus* Mackiewicz, 1965, *I. hexacotyle* (Linton, 1897), Mackiewicz, 1968, *I. jonesi, I. longus* Fredrickson and Ulmer, 1965). In *I. multivitellaria,* the cirrus sac is anteriorly distant from the ovary; a distance about equal to the length of the ovarian arms separates it from their anterior border. The postgonopore distance is uniquely large (24–32% of body length). In the other 11 species, the cirrus sac lies within the ovarian arms, at or near their anterior border. Among these species, *I. jonesi* has the most anteriorly located cirrus sac but its postgonopore distance is only 7.6–11.6% of body length (Mackiewicz, 1972).

The small number of testes (25–50) in *I. multivitellaria* distinguishes it from all the other species except *I. agminis,* which has 28–40 testes (Williams and Rogers, 1972); *I. agminis* from Wisconsin (this paper) has 17–68 testes. The minimum number of testes in the other 10 species is greater than 100 with the exception of *I. etowani,* which has 80–105 testes (Williams, 1975). The fan-shaped scolex of *I. multivitellaria* also distinguishes it from the other species that have either wedge- or cuneiform-shaped scolices, which may be rounded. The shape of the scolex may, however, be labile to variations due to prefixation methods.

Monobothrium hunteri Mackiewicz, 1963

One gravid worm and three *B. macrocephalum* were recovered from the stomach of a 16-cm-long *C. commersoni* from Tichigan Lake canal on June 14, 1978. Measurements of that specimen (Table 3) fit within the normal range of variation of those in the original description except that the latter had slimmer bodies (0.03–0.70 mm), smaller testes (55–133), shorter ovarian arms (400–1,200), and smaller eggs (62–70 by 42–47) (Mackiewicz, 1963).

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 78664.

Rowardleus pennensis Mackiewicz and Deutsch, 1976

Two gravid specimens were found in the intestine of two (24- and 26-cm-long) *Carpiodes cyprinus* (LeSueur) from Tichigan Lake during August 1978. This is a new record for Wisconsin and the first outside of Pennsylvania from where the species was originally described. These two worms differed from the type material (Mackiewicz and Deutsch, 1976) in having relatively longer bodies (>21.0 mm compared to 8.9–18.4), more testes (>90 compared to 53–86) measuring 154–192, and longer distance between first testis and anterior tip (>9.0 mm compared to 4.0–7.3) and between first vitelline follicle and anterior tip (>5.0 mm compared to 2.7–5.1); eggs were 32–54 by 22–26 ($N = 5$).

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 78663.

Family Lytocestidae Wardle and Mcleod, 1952

Atractolytocestus huronensis Anthony, 1958

Five worms (four were gravid) infected the stomach of two female *Cyprinus carpio* from Tichigan Lake during July 1978 and 1983. Characteristics of the 4.0–13.8-mm-long gravid worms fit those in the original description except that the two vitelline clear areas at the cirrus sac and at the ovary (Anthony, 1958, fig. 2) were replaced by one continuous and larger clearing between those two spots in my specimens. The posterior end of the local material also abruptly narrowed behind the ovary to a blunt tip.

DEPOSITED SPECIMENS: USNM Helm. Coll. No. 78662.

Khawia iowensis Calentine and Ulmer, 1961

Two adults (one was gravid) were recovered from two *C. carpio* from Tichigan Lake during July 1983. The 4.5- and 13.0-mm-long specimens fell within the normal range of variation of that species as described by Calentine and Ulmer (1961).

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 78661.

Conclusions

Thirteen species of eight genera of caryophyllaeid cestodes were recovered from three catostomid and one cyprinid fish species in two southeastern Wisconsin lakes. The diversity of species was considerably greater in the larger Tichigan Lake (with 11 species), which is connected to the Fox River, a tributary of the Mississippi River, than in Silver Lake (with 4 species). Only *G. catostomi* and *G. laruei* infected fishes from both lakes. The closed system in the smaller landlocked Silver Lake clearly enhanced the population density of these two cestodes compared to Tichigan Lake (Table 1) even though their definitive host, *C. commersoni,* appeared to be more common in the latter lake (unpubl.). The other two species from Silver Lake include the new species *I. multivitellaria,* which is herein described from *E. sucetta,* as well as *I. agminis,* which has been previously reported only in certain southern states. *Isoglaridacris multivitellaria* is a rare species that is characterized by its uniquely extensive postovarian vitellaria and by the anterior location of the cirrus. All the other species from the open Tichigan Lake system were present in very low numbers. Some showed unusual host records, e.g., *I. folius* from *C. commersoni.*

Catostomus commersoni was infected with the most helminth species (8 cestodes, 2 acanthocephalans). The anterior localization of *G. catostomi* and *H. nodulosa* (in *C. commersoni*) and of *I. agminis* and *N. robertbaueri* (in *E. sucetta*) as well as the posterior localization of *G. laruei* and *P. bulbocolli* (in *C. commersoni*) and of *N. prolixoides, P. bulbocolli,* and *L. mutabile* (in *E. sucetta*) did not undergo any significant seasonal changes. Recruitment of *Glaridacris* spp. occurred in late summer and early autumn, population buildup and maturation through spring, and virtual loss of gravid adults during the summer. Acceleration of development and/or early loss of gravid *G. catostomi* and *I. agminis* appear to be enhanced by the relatively warmer temperatures of the smaller land-locked Silver Lake. Morphometric variations in the local material were documented and compared to those reported for the same species elsewhere.

Acknowledgments

I gratefully appreciate the help of Professor John S. Mackiewicz, State University of New York at Albany, in the determination of *I. multivitellaria* as a new species and for reviewing its description, the identification of *Biacetabulum* sp., *B. biloculoides, B. macrocephalum, H. nodulosa,* and *I. agminis,* and the verification of other determinations.

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Neocyclustera ralli gen. et sp. n. (Cestoidea: Dilepididae) and Other Endohelminths from Clapper Rails, *Rallus longirostris*, from a Marsh in Galveston County, Texas

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ABSTRACT: Ten clapper rails, *Rallus longirostris*, from a salt marsh in Galveston County, Texas were examined for helminth parasites. Fourteen species of digenetic trematodes, one cestode, and three nematodes were recovered. The cestode is described herein as *Neocyclustera ralli* gen. et sp. n. The new genus is differentiated by the dorsal position of the genital ducts to the osmoregulatory canals, placement of reproductive organs, and structure of the uterus. Prevalence and mean intensity of infection are given for all helminths recovered.

Clapper rails, *Rallus longirostris* Boddaert, inhabit *Spartina* marshes from southern Maine to southern Texas and along the California coast (Mangold, 1977). These birds feed on a variety of organisms including small crabs, gastropods, bivalves, insects, and small fish (Bateman, 1965). Considering the habitat and feeding habits, *R. longirostris* should harbor a diverse helminth fauna and this assumption is supported in the literature. Heard (1970) reported 26 species of digenetic trematodes and 2 cestodes from 146 clapper rails collected along the Atlantic and Gulf coasts (exclusive of Florida and Texas). Bates and Meade (1972) examined king and clapper rails from Bolivar Peninsula on the Texas coast and recovered eight species of digeneans, three cestodes, and one nematode from clapper rails. An acanthocephalan has also been reported from clapper rails (Nickol and Heard, 1970). The clapper rail is the type host for five helminth species, all Digenea: *Levinseniella byrdi, Maritrema prosthometra, Renicola ralli, R. glandoloboides,* and *Notocotylus schmidti* (see Heard, 1968; Deblock and Heard, 1969; Byrd and Heard, 1970; Brooks and Heard, 1977).

The purpose of this paper is to report helminth species, including a new cestode, that were recovered from clapper rails on the Texas coast.

Materials and Methods

Nine adult (seven male and two female) and one juvenile clapper rails were collected from *Spartina* salt marshes along the Intercoastal Waterway in Galveston County, Texas. Birds were collected by shotgun between July 1978 and February 1980. Birds were placed on ice and transported to the laboratory where all internal organs and systems were examined for helminths by standard techniques. Platyhelminth parasites were heat killed, fixed in AFA, stained with Semichon's Carmine, and mounted in Kleermount. Nematodes were killed in warm 70% ethanol, cleared in glycerol, and mounted in glycerine jelly. Representative specimens were deposited in the U.S. National Parasite Collection in Beltsville, Maryland and in the Texas A&M University Regional Invertebrate Collection, Department of Biology, Texas A&M University, College Station, Texas. Measurements in the description are given in micrometers unless otherwise indicated, with ranges followed by means in parentheses.

Results

Fourteen species of digeneans, one cestode, and three nematodes were recovered during the study (Table 1).

Neocyclustera gen. n.

DESCRIPTION: Dilepididae Railliet and Henry, 1909, Dilepidinae Fuhrmann, 1907. Scolex with an armed rostellum bearing two circles of hooks. Suckers unarmed. Proglottids craspedote, wider than long. Genital pores alternating regularly. Genital ducts pass dorsal to osmoregulatory canals. Cirrus pouch thick-walled, in anterior half of proglottid, crossing osmoregulatory canals. Testes dorsal to ovary. Ovary lobate, median. Vitelline gland posteromedial to ovary, compact. Vagina posterior to cirrus pouch. Seminal receptacle present. Uterus at first saclike, becoming an inverted U-shape with thick arms and internal trabeculae.

Neocyclustera ralli sp. n. (Figs. 1–5)

DESCRIPTION (based on 7 complete specimens): Scolex (Fig. 2) rounded, 266–422 (327) long, 310–508 (382) at greatest width. Suckers unarmed, oval, 131–203 (154) long, 93–161 (125) wide. Rostellar sac 331–518 (459) long, extending into 5th to 7th proximal proglottid. Rostellar hooks (Fig. 3) in two circles of 10 hooks each; anterior hooks 120–133 (128) in length, posterior hooks

Table 1. Endohelminth species, accession numbers, prevalence, mean intensity of infection, and site of infection for helminths recovered from 10 clapper rails from the Texas coast.

Endohelminth	USNM number	Prevalence	Mean intensity	Site
Trematoda				
Ascocotyle pachycystis	78465	60%	73.5	Small intestine
Athesmia heterolecithodes	78466	10%	20.0	Liver and bile ducts
*Austrobilharzia penneri**	78467	30%	20.3	Hepatic and mesenteric vessels
Echinochasmus schwartzi	78468	30%	11.7	Small intestine
Echinostoma attenuatum	78469	30%	3.0	Small intestine
Levinseniella byrdi	78470	50%	13.4	Ceca
Lypersomum sinuosum	78471	10%	8.0	Pancreas
Maritrema prosthometra	78472	90%	152.8	Small intestine
Parorchis acanthus	78473	50%	3.4	Cloaca
Phagicola diminuta	78474	10%	11.0	Small intestine
Probolocoryphe glandulosa	78475	70%	70.0	Small intestine
Prosthogonimus ovatus	78476	40%	3.5	Cloaca and bursa
*Renicola hydranassae**	78477	50%	42.0	Kidney
Tanaisia fedtschenkoi	78478	10%	84.0	Kidney
Cestoda				
Neocyclustera ralli	78463	40%	8.8	Small intestine
Nematoda				
Capillaria sp.	78479	70%	16.4	Ceca
*Skrjabinoclava horrida**	78480	60%	4.7	Proventriculus
Syncuaria sp.*†	78481	50%	1.8	Esophagus

* New host record.

† All specimens of *Syncuaria* sp. were immature.

101–113 (107) in length (measurements based on 15 hooks of each type). Blade and handle long, guard short. Blade of anterior hooks curved near tip, blade of posterior hooks also curved near tip but to a lesser extent. Neck absent.

Strobila (Fig. 1) 2.3–4.5 mm (3.2 mm) long, 322–405 (366) wide at level of last mature proglottid. Total number of proglottids 27–39 when uterus is present. Genital pores at level of anterior two-fifths of segment, regularly alternating. Genital ducts pass dorsal to osmoregulatory canals (Fig. 4). Reproductive systems protandrous. Ventral osmoregulatory canals united near posterior margin of proglottid by a single transverse canal; ventral canals 7–9 (8) wide, dorsal canals 2–5 (3) wide in last mature proglottid. Genital atrium simple, 24–36 (30) deep.

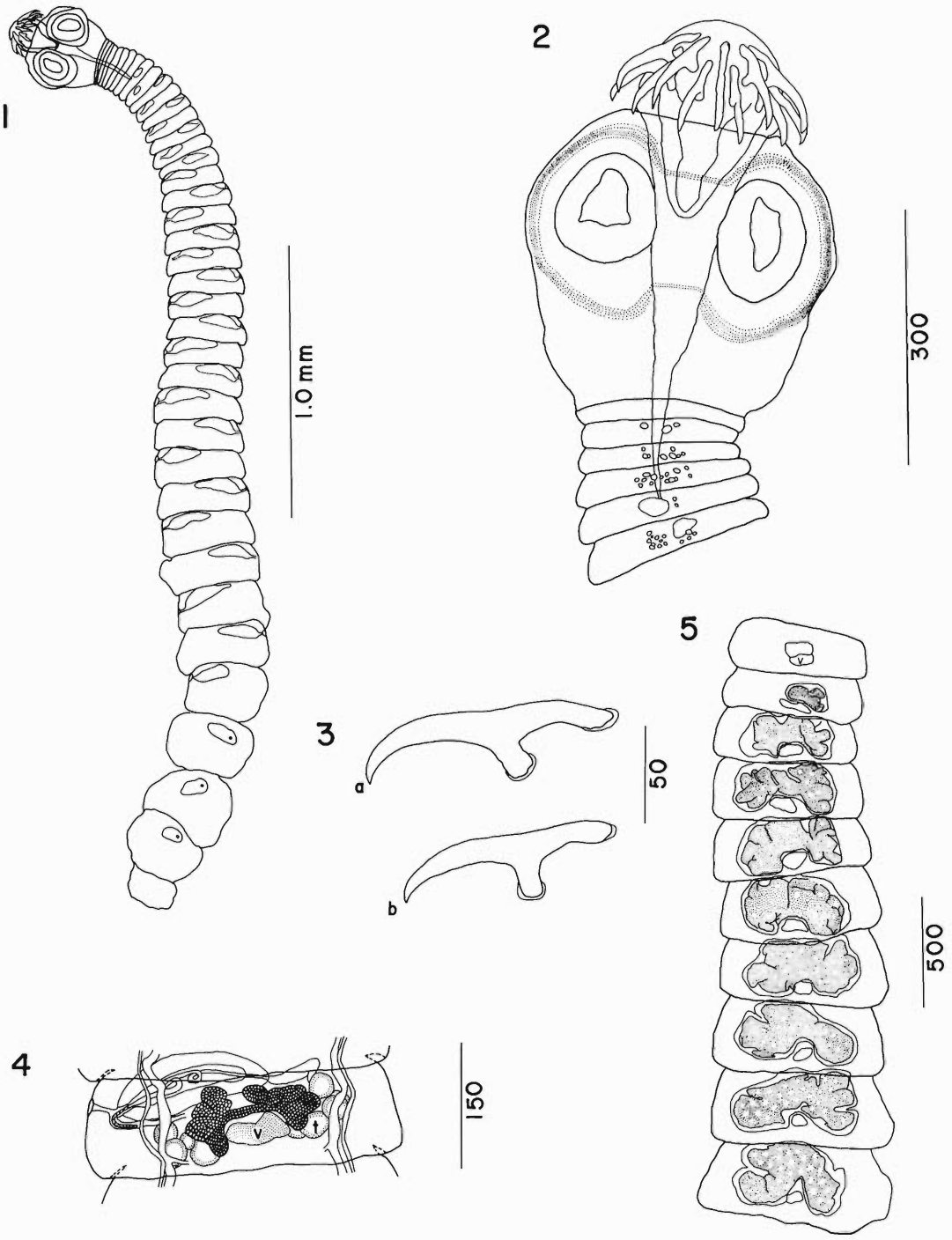
MALE GENITALIA: Fifteen to 18 testes, in a single field dorsal to ovary and extending from anterior margin of proglottid to posterior of ovary; each 35–44 (40) wide in mature segments. Testes first appearing in two fields separated by distal end of rostellar sac, 4–6 proglottids from scolex. External seminal vesicle absent; vas deferens highly coiled in anterior, medial field. Ejaculatory duct convoluted within cirrus pouch. Cirrus slender, 54–62 (59) long, with many small spines along length; cirrus, when everted, usually inserted into vagina of same proglottid. Cirrus pouch oval, transverse, crossing dorsal to osmoregulatory canals; 134–219 (196) long, 44–65 (61) wide. Walls of cirrus pouch 10–23 (19) thick.

FEMALE GENITALIA: Ovary median with 12–14 lobes, filling center of proglottid but not extending to osmoregulatory canals (Fig. 4), 40–110 (72) long, 92–209 (152) wide. Ovary appears 11–17 proglottids from scolex. Vitellarium compact, posteromedial to ovary, 19–41 (28) long, 47–146 (86) wide. Vagina simple, distal end posterior to cirrus pouch. Vagina curves ventral to cirrus pouch and extends to seminal receptacle, which measures 34–113 (72) wide in mature proglottids. Uterus appears 20–28 proglottids posterior to scolex as a single saclike structure, which becomes a broad inverted U-shaped structure with wide arms and several internal divisions when filled with eggs (Fig. 5). Individual eggs within the uterus could not be measured. Terminal proglottids showing a state of atrophy.

TYPE HOST: Clapper rail, *Rallus longirostris* Boddaert (Gruiformes: Rallidae).

SITE: Small intestine.

TYPE LOCALITY: Galveston County, Texas, U.S.A.



Figures 1–5. ***Neocyclustera ralli*** **gen. et sp. n. from** ***Rallus longirostris.*** **1. Scolex and strobila. 2. Scolex and anterior proglottids. 3. Hooks. a—anterior hook. b—posterior hook. 4. Mature proglottid (seminal receptacle omitted for clarity). Ventral view. 5. Posterior region of strobila showing development of uterus. v = vitellarium, t = testes. (Scale bars are in micrometers unless otherwise indicated.)**

TYPE SPECIMENS: USNM Helm. Coll. holotype No. 78463, paratype No. 78464. TAMU Paratype Nos. 78-A-108-4, 79-A-36, 80-A-14.

ETYMOLOGY: The generic name indicates the close association with the genus *Cyclustera*: the specific epithet refers to the type host.

Remarks

Neocyclustera gen. n. most closely resembles the genus *Cyclustera* Fuhrmann, 1901 in hook morphology, shape of the ovary, and shape of the uterus as described by Bona (1975). The new genus differs from *Cyclustera* in that the genital ducts pass dorsal to the osmoregulatory canals, a characteristic utilized by Yamaguti (1959) at the generic level, and the uterus does not form a complete circle but retains a horseshoe-shape in gravid proglottids. The position of the genital ducts relative to the osmoregulatory canals is similar to that found in *Liga* (Weinland, 1856) Weinland, 1857, *Neoliga* Singh, 1952, and *Thaparea* Johri, 1953, however, this genus differs from those genera in several ways. *Neocyclustera* differs from *Liga* in the more median position of the ovary, the presence of testes anterior and dorsal to the ovary, and the absence of a strongly lobed uterus. The new genus differs from both *Neoliga* and *Thaparea* in not possessing a saclike uterus. *Neocyclustera* differs further from *Neoliga* in that the posterior hooks are smaller than the anterior and *Neocyclustera* does not possess a vaginal sphincter as does *Neoliga*. *Neocyclustera* also differs from *Thaparea* in possessing a single field of testes whereas *Thaparea* has separate groups of testes situated anterior and posterior to the ovary.

Heard (1970) reported *Cyclustera* sp. from clapper rails, a red-breasted merganser (*Mergus serrator*), and a glossy ibis (*Plegadis falcinellus*) from the Atlantic Coast. Bona (1975) reviewed these materials and determined that specimens from *M. serrator* were *Cyclustera ibisae* (Schmidt and Bush, 1972) Bona, 1975 and that those specimens from the clapper rails represented a different genus. We have been unable to obtain those specimens from clapper rails and thus can not make a determination as to their relationship to *Neocyclustera ralli*.

Acknowledgments

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Bioassay Comparisons for Pheromone Detection in *Heterodera glycines*, the Soybean Cyst Nematode

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ABSTRACT: Comparisons of two bioassays, an agar plate method and a sand slide method, demonstrated that the gradient established by the pheromone could travel 30 mm, with a net positive movement of males to the pheromone at 27–66% and 85–90%, respectively. Both bioassays required 36 hr or longer to demonstrate positive movement of males to an attraction source. Observations of mating behavior and associated courting behavior provided a more rapid bioassay for future screening of crude and purified chemical fractions of soybean cyst nematode sex pheromones than previous bioassays. A sample can be tested within 1 to 2 min by behavior analysis. Criteria for measuring pheromone activity are generally the distance and the time required for a nematode to travel to the source of a pheromone; but behavioral displays are indicative also of some nematode responses to stimulants or arrestants.

Recent losses of some efficient soil fumigants and nematicides and the potential banning of others by regulatory agencies has made the search for new approaches to management of plant parasitic nematodes of foremost importance. Renewed interest in the potential of bioregulators as novel management mechanisms is evident (Huettel, 1986). Studies were conducted during the late sixties and early seventies (Green, 1966, 1967; Greet et al., 1968; Green and Plumb, 1970) that showed that sex pheromones occurred in plant parasitic nematodes. Over the last decade, little emphasis was placed on isolation and identification of chemicals that influenced nematode mate finding. Recently, Rende et al. (1982) rekindled interest by demonstrating new bioassay techniques for attraction of males to female *Heterodera glycines*, the soybean cyst nematode, race 3. They verified work by Green and Plumb (1970) on attractance of soybean cyst nematode males to females and established a dosage response curve of the optimal number of females needed to observe the best response of males of this species. They also determined pH, the temperature, age of female and male, and mated status of females.

The most important aspect of testing for potential bioregulators is the development of bioassays (Shorey, 1970). Currently used bioassays require about 36 hr for maximum attraction and are time-consuming to use. The purpose of this study is to (1) compare different types of bioassays that were used in previously reported studies with newly developed or improved bioassays and (2) develop a bioassay to determine if behavioral characteristics could be used to detect sex pheromones.

Material and Methods

STOCK CULTURES: *Heterodera glycines* Ichinohe, races 3, 4, and 5, were obtained from previously established cultures of monoxenic root explant cultures (Lauritis et al., 1982). Nematodes were maintained on excised root tips of *Glycine max* (L.) Merr. CV Kent, grown on Gamborg's B-5 medium (Gamborg et al., 1976; Rebois et al., 1984).

AGE-CONTROLLED EXPERIMENTS: Gravid females were aseptically transferred to sterile vials containing 4–5 ml of sterile water and the eggs were allowed to hatch for 4 days at 28°C. A sterile 0.2-ml aliquot of second-stage juveniles was removed and the number of viable nematodes counted. From the remaining supernatant, petri plates were inoculated with ca. 100 second-stage juveniles. Each petri plate contained 3-day-old root explants of Kent soybean, two per plate on Gamborg's B-5 medium. The petri plates were sealed with Parafilm and incubated at 28°C.

Virgin females appeared first at day 10 (Lauritis et al., 1983). Mating was never observed until day 11, so 10-to 11-day-old females were considered DAI virgins (days after inoculation). Females from day 12 to day 14 were yellow, contained eggs, and were called DAI yellow. Females from day 15 to day 20 were completely tanned, observed to have large egg masses, and were called DAI brown. Egg masses were isolated initially from 11–15-DAI females.

Males were collected from cultures by soaking the agar and roots in water on a modified Baermann funnel for 24 hr. The males were isolated from the water by hand and aerated in fresh water ca. 2 hr to remove any effects of previous exposure to pheromone. Ten males were used in each trial run. The following combinations

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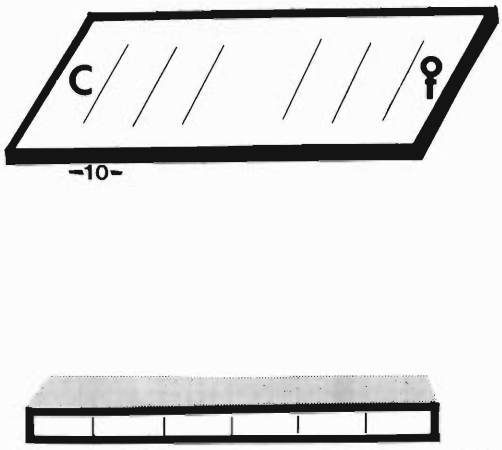

Figure 1. Diagram of a sand slide bioassay (C = control, ♀ = females).

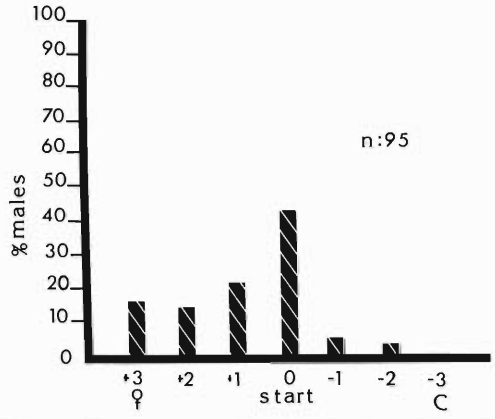

Figure 3. Attraction of males of *Heterodera glycines*, race 3, to virgin females on agar bioassays (significant for positive movement, χ^2, df = 1, $P < 0.05$).

were observed: all possible combinations of males and females of races 3, 4, and 5.

BIOASSAY: Because dose levels were already established by Rende et al. (1982) and by the authors (unpubl.), the optimum level of 12 females was used for each of the two methods of bioassay. The first was the agar dish method described by Green and Plumb (1970) and modified by Huettel et al. (1982). The second, a sand slide bioassay, was developed by Nordmeyer and Dickson (pers. comm., University of Florida). Quartz sand was sieved to give a particle size less than 600 micrometers. The sand was washed, autoclaved, and placed on a microscope slide to give a depth of 2 mm while still moist. The slide was marked off in 10-mm intervals (Fig. 1). For each test, females of the same age were placed at the end designated as (♀) and a root tip was placed as a control at the end designated (C). The slide was incubated for 24 hr in a moisture chamber to prevent desiccation. Then 10 males were placed at the center area and the slide was incubated another 12 hr, after which time 10-mm portions of the sand were scraped off the slide into 15 ml 1 M sucrose. The sand was gently agitated to suspend the males in the sucrose. The supernatant was decanted onto a 500-mesh sieve, washed, and number of males counted.

BEHAVIOR BIOASSAYS: Behavioral bioassays were conducted on 15 × 60-ml petri plates with 1.5% Agar Noble and observed on an inverted Nikon PIC LWD microscope and a videomonitor with a Gyyr Video Recorder and a UFX Nikon stereomicroscope. All photographs were taken with a Nikon FX-35A camera.

ANALYSIS: In the two dose-response bioassay tests, net positive movement was determined from the total percent males that moved to a pheromone source (Rende et al., 1982). This was obtained by counting the number of males that moved towards the females, minus the number of males that moved toward the control, divided by the total trials [(no. moving toward − no. moving toward C)/no. trials]. Chi-square analysis was made on all the data.

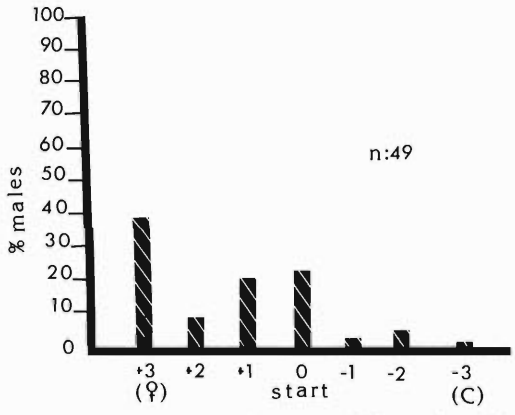

Figure 2. Attraction of males of *Heterodera glycines*, race 3, to egg masses from females on an agar bioassay (significant for positive movement, χ^2, df = 1, $P < 0.05$).

Results

The movement studies of the agar plate bioassay resulted in significant positive movement of males to all age-classes of females ($P < 0.05$). In agar plate bioassays of 12 females of race 3 to 10 males of race 3, the highest percent net positive movement (66%) from males was observed to isolated egg masses from DAI 11–15 females (Fig. 2). There was 40% net positive movement to DAI virgin females (Fig. 3) and 43% net positive movement to DAI yellow females (Fig. 4). In females that were over 15 days old (DAI brown), there was a 27% net positive movement (Fig. 5). Even though this was significant, it is not known whether this was due to pheromone entrapped in the gelatinous matrix or to active pheromone production.

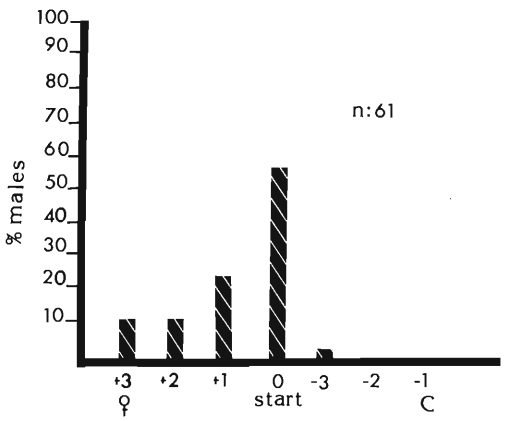

Figure 4. Attraction of males of *Heterodera glycines*, race 3, to yellow females with egg masses on agar bioassays (significant for positive movement, χ^2, df = 1, $P < 0.05$).

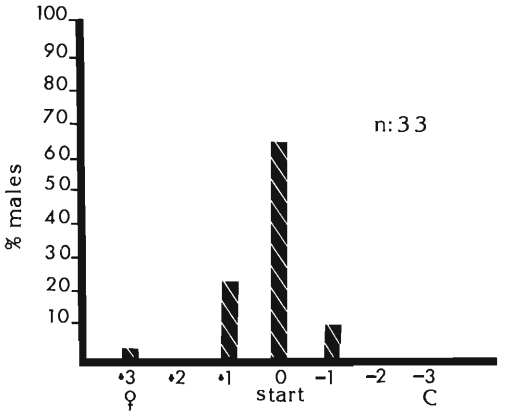

Figure 5. Attraction of males of *Heterodera glycines*, race 3, to brown females on agar bioassays (significant for positive movement, χ^2, df = 1, $P < 0.05$).

The recovery from the sand bioassay was much higher than from the agar plate method with 85–90% net positive movement to the pheromone source when DAI virgins were compared. About 75% of the total number of males placed on the sand slides were recovered at the pheromone source. In all movement studies of all possible combinations of races 3, 4, and 5 (3 ♂ to 4 ♀; 3 ♂ to 5 ♀; 4 ♂ to 3 ♀; 4 ♂ to 5 ♀; 4 ♂ to 4 ♀; 5 ♂ to 5 ♀; 5 ♂ to 3 ♀; 5 ♂ to 4 ♀) the attraction was approximately the same percent net positive movement observed in the 3 ♂ to 3 ♀ (Table 1).

During precopulatory behavior on root explant cultures, a characteristic male behavior consisted of backward coiling, a tight coiling of the tail, occasional protrusion of the spicules, and tail raising in a C-shape (Fig. 6). Isolated females placed on agar also elicited the same effect on males. When the isolated females were allowed to remain on the agar plates for up to 24 hr and then removed, this same behavior occurred in males placed directly on the spot where the females were located previously (Fig. 7).

Discussion

Our bioassay results verified the existence of sex pheromones used for mate finding in the soybean cyst nematode (Green and Plumb, 1970; Rende et al., 1982). The existence of the coiling behavior stimulant may just be an effect of one of several actual sex behavioral stimulants. We will refer to the coiling pheromone as the encirclement pheromone. In our tests males moved up to 30 mm toward the pheromone source as compared to 5 mm in the study of Rende et al. (1982). Chemical cues in the soil would have to travel a great enough distance to attract males, especially at low population densities. In in vitro root explant cultures, males are seldom observed developing immediately adjacent to females. On plates receiving low inoculation numbers, sometimes no more than five males and females establish the first generation. However, the males are able to find the females anywhere on the petri plate.

The number of males that moved in a positive direction was greatly increased in the sand slide bioassay when compared to the agar plate method or the method reported by Rende et al. (1982). The percent net positive movement was improved from 48 to 66% for the agar plate bioassay and 67% (Rende et al., 1982) to 85–90% for the sand slide bioassay. Males are difficult to recover

Table 1. Percent net positive movement of various combinations of males and females of *Heterodera glycines* of races 3, 4, and 5 on agar plate and sand slide bioassays with 10–11-day-old virgin females.

Males/race	Females/race	Agar plate	Sand slide
3	3	66%	93%
3	4	60%	
3	5	56%	87%
4	4	41%	
4	3		96%
4	5		85%
5	5	42%	
5	3	42%	
5	4	38%	

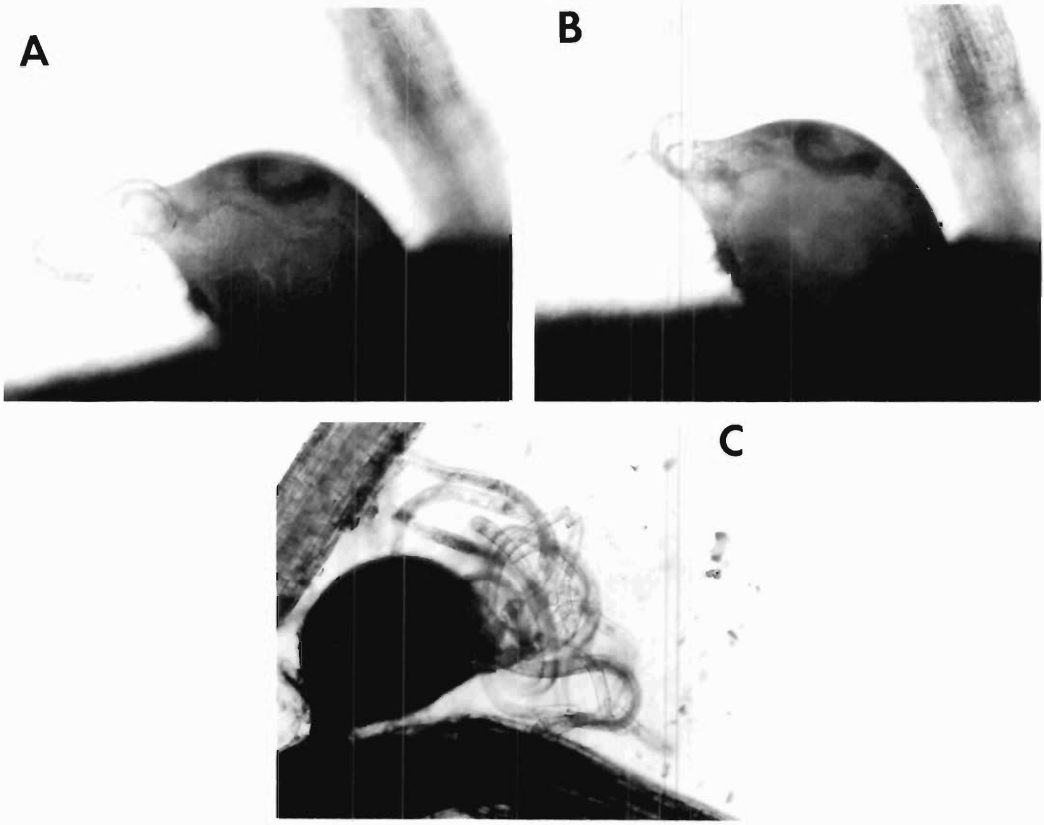

Figure 6A-C. Photograph of typical coiling and encirclement behavior of males of *Heterodera glycines*, race 3, around a female on a root explant culture.

due to their small size and are also very subject to desiccation if an agar plate is slightly dry. Not all the males used in a single trial could be recovered from the agar plate. They often moved to the side of the petri plate or into the agar making it impossible to observe them. The sand slide method prevents the nematode from desiccating if the sand remains moist. Further, it closely resembles the natural habitat of nematodes in the soil.

The time required to conduct either type of bioassay was 36 hr, which is about the same time reported by Rende et al. (1982). In order to have a pheromone gradient set up from the source, at least 8 hr are required; but on agar plate and sand slide bioassays, which allow for movement of 30 mm, at least 24 hr were required. The behavioral bioassay required only about 2–3 min to conduct. It also required little preparation and is a rapid method that allows for crude and purified samples to be tested in a short period.

Behavior analysis was demonstrated as an important approach to understanding mating behavior in *Radopholus similis* and *R. citrophilus* (Huettel et al., 1982). Golden and Riddle (1984) have isolated pheromones that induce dauer larva formation from *Caenorhabditis elegans* and have used the behavior associated with dauer formation as an identification technique for the pheromone. Our behavior analysis also shows that actual behavioral observations are as valuable for some pheromone identification. The behavioral characteristics provide rapid identification of close-range pheromonal activities (encirclement pheromones) that require little preparation of bioassay techniques and are also very time saving when compared to movement bioassays. However, further testing is needed to determine if the compound(s) that causes the encirclement is the same as the long-range attractant used by males to locate females from a distance.

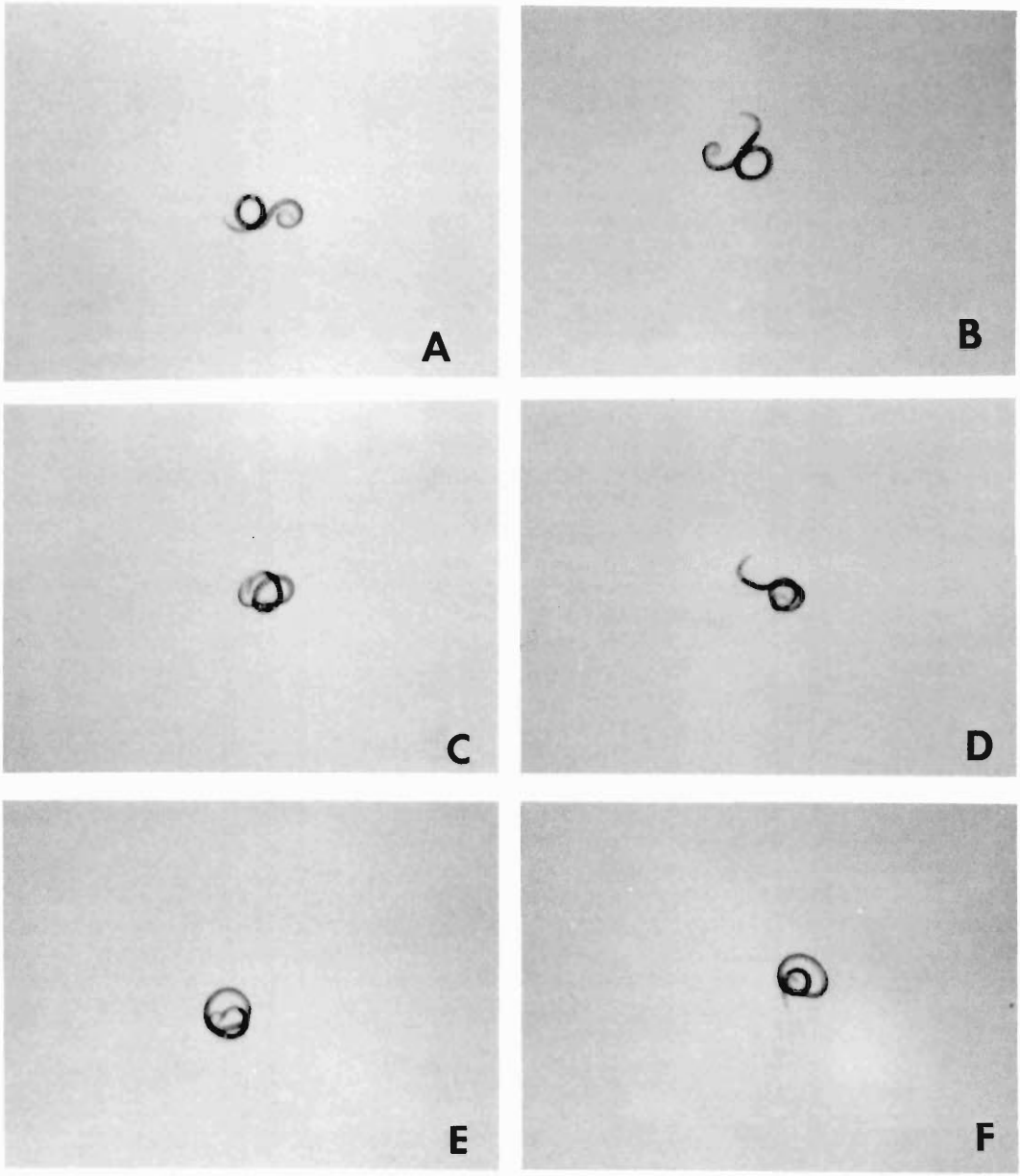

Figure 7A–F. Typical coiling behavior of males of *Heterodera glycines*, race 3, in the presence of isolated females of this species on agar plates.

Actual in vitro studies of plant parasitic nematodes offer new insight into understanding the processes of bioregulation. Once these processes are understood then attempts can be made to alter these mechanisms to provide new management strategies that could be used to disrupt normal behavior.

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Comparative Morphology of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* (Heteroderidae) with Scanning Electron Microscopy

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ABSTRACT: Comparative scanning electron microscopy (SEM) of second-stage juveniles (J2) and adults of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* revealed surface characteristics of the en face pattern, lateral field, phasmid, cuticle, and cone. En face pattern and cone morphology may be particularly useful for identification and interpreting phylogeny. J2 of *S. hydrophila* and *R. sequoiae* share a derived en face pattern in which adjacent submedial lips are fused, and lateral lips are distinct. En face patterns of males of *S. hydrophila* and *R. sequoiae* also have fused submedial lips. However, males of *S. hydrophila* are polymorphic; in the most common en face pattern all lips are fused, whereas in variants, lateral lips remain separate as in *R. sequoiae.* The en face patterns of females of these two genera share elevated submedial corners on the labial disc and protuberances with *Hylonema ivorense,* features not known to occur in other Heteroderidae. In addition, the cone regions of females of *S. hydrophila* and *R. sequoiae* have lacelike cuticular patterns; in both species the vulva occurs in a depression and the anus is on the dorsal lip within the depression. These shared derived characters may indicate that *Sarisodera* and *Rhizonema* are monophyletic. SEM observations support the hypothesis that *S. hydrophila* and *A. africana* are polyphyletic. The en face pattern of J2 and adults of *A. africana* is distinct from *S. hydrophila* but resembles the derived pattern of certain *Heterodera* species. In *A. africana* adjacent submedial lips are fused with one another and with the labial disc. As in *Heterodera,* the cone of *A. africana* lacks a vulval depression and the anus is dorsal to the cone; unlike *Heterodera,* the cone does not develop fenestrae. These SEM observations, together with additional characters, may indicate that *Afenestrata* and certain *Heterodera* are sister-groups.

Sarisodera Wouts and Sher, 1971 was originally introduced to accommodate *Sarisodera hydrophila* Wouts and Sher, 1971, a heteroderid with females that develop into cysts (Wouts and Sher, 1971). Subsequently, *Sarisodera africana* sensu Luc et al., 1973 was added to *Sarisodera.* On the other hand, *Rhizonema sequoiae* Cid Del Prado-Vera et al., 1983, although similar in many respects to *S. hydrophila,* lacks a cyst, and was placed in a new genus (Cid Del Prado-Vera et al., 1983). Recently, Baldwin and Bell (1985) concluded that females of *S. hydrophila* do not form cysts, contrary to the original description, and redescribed *Sarisodera* to exclude this character. In addition, Baldwin and Bell (1985) described *Afenestrata* to accommodate the cyst-forming species, *Afenestrata africana* (Luc et al., 1973) Baldwin and Bell, 1985 (=*S. africana*). These changes are supported by new characters including comparative host responses (Mundo and Baldwin, 1983; Baldwin and Bell, 1985), cuticle layering in females (Baldwin, 1983; Cliff and Baldwin, 1985), and scanning electron microscopy (SEM) of second-stage juveniles (J2) and adults. The SEM observations are reported in this study and include en face patterns, lateral fields, tails, and sensory openings of J2, males, and females of *S. hydrophila, R. sequoiae,* and *A. africana* as well as the terminal cone of females and cysts. These comparable studies expand on previous more limited observations of *R. sequoiae* (Cid Del Prado-Vera et al., 1983) and also are related to similar studies on heteroderids, *Meloidodera* and *Verutus,* which demonstrated the value of SEM in revealing new characters (Othman and Baldwin, 1985). SEM characters can be used for identification as well as in testing and refining hypotheses of phylogeny of Heteroderidae as a basis for developing a natural and more stable classification.

Materials and Methods

Second-stage juveniles, males, and females of all species were collected from type localities (Table 1). These stages of *S. hydrophila, R. sequoiae,* and *A. africana* were prepared for SEM by either critical-point drying (cpd) (Othman and Baldwin, 1985) or glycerin infiltration (gly) (Sher and Bell, 1975) as previously described. In most cases both methods were used for comparison, and to identify possible artifacts; results of the two methods were as reported by Othman and Baldwin (1985). All specimens were fixed in 3.5% glutaraldehyde or 5% formalin with the exception of fe-

[1] A portion of the senior author's Ph.D. Thesis.

Table 1. Species and numbers of *Sarisodera, Rhizonema,* and *Afenestrata* examined and their source.

Nematode	Host	Location	Number examined
Sarisodera hydrophila Wouts and Sher, 1971	Willow *Salix lasiolepis* Benth.	Temecula, California	300 juveniles 200 males 80 females
Afenestrata africana (Luc et al., 1973) Baldwin and Bell, 1985	Guinea grass *Panicum maximum* Jacq.	Ivory Coast, Africa	300 juveniles 80 males 40 females 40 cysts
Rhizonema sequoiae Cid Del Prado-Vera et al., 1983	Coast Redwood *Sequoia sempervirens* (D. Don) Endl.	Lagunitas Lake, California	100 juveniles 4 males 40 females

males and cysts of *A. africana,* which were only available to us in lactophenol. For cpd, the fixed nematodes were postfixed with 1% osmium tetroxide (OsO_4), treated with 1% thiocarbohydrazide (TCH), again fixed in 1% OsO_4, dehydrated with a graduated acetone series, infiltrated with Freon 113, and critical-point dried with carbon dioxide. The specimens were mounted on stubs, sputter coated with 20 nm gold-palladium, and examined with a Jeol 35C SEM at 15 kV.

Voucher specimens from collections made for this study were processed to glycerin, mounted on Cobb slides, and deposited in the University of California Riverside Nematode Collection (UCRNC) as follows: *S. hydrophila,* V1112 8 J2, V1113 3 males, V1114 3 females; *R. sequoiae,* V1115 5 J2, V1116 3 females; *A. africana,* V1117 5 J2, V1118 4 males, V1119, 1 female.

Results

HEAD MORPHOLOGY: The en face patterns of J2, males, and females of *Sarisodera, Rhizonema,* and *Afenestrata* species are variable. However, these variations are modifications of a basic pattern. This basic or primitive pattern occurs in other Heteroderidae genera (e.g., Ferris, 1979) and consists of a labial disc surrounded by the first head annule consisting of four submedial (two subdorsal and two subventral) and two lateral lips. The rationale for the en face terminology used in this paper has been previously discussed (Othman and Baldwin, 1985).

The en face patterns of J2 of *S. hydrophila* and *R. sequoiae* are similar in that the labial disc is dorsoventrally ovoid, both adjacent submedial lips are fused, and lateral lips are separated or partially fused with submedial lips (Figs. 1, 4). The head includes two additional annulations (Figs. 2, 3, 5, 6). However, in *S. hydrophila,* unlike *R. sequoiae,* the lip annule is broader in the area of fusion of adjacent submedial lips than in the mediolateral position (Figs. 1, 4). In J2 of *R. sequoiae,* fused adjacent submedial lips are the rule, but variant individuals occur in which there is separation (Fig. 7). In the en face pattern of J2 of *A. africana* adjacent submedial lips are fused with each other, with the labial disc, and partially with the second head annule (Fig. 8). Lateral lips are separate and the head region includes one additional complete annulation (Figs. 9, 10). In J2 of *A. africana* variant individuals occur in which adjacent submedial lips are separate from one another, although they are fused with the labial disc and second head annule (Fig. 11).

The en face pattern of *S. hydrophila* males consists of a circular to ovoid labial disc surrounded by a first head annule in which all lips are fused (Fig. 12), but variant individuals occur in which the lateral lips are separate (Fig. 13). The head region includes four additional annulations (Figs. 14, 15). In males of *R. sequoiae* the labial disc is circular. The first head annule con-

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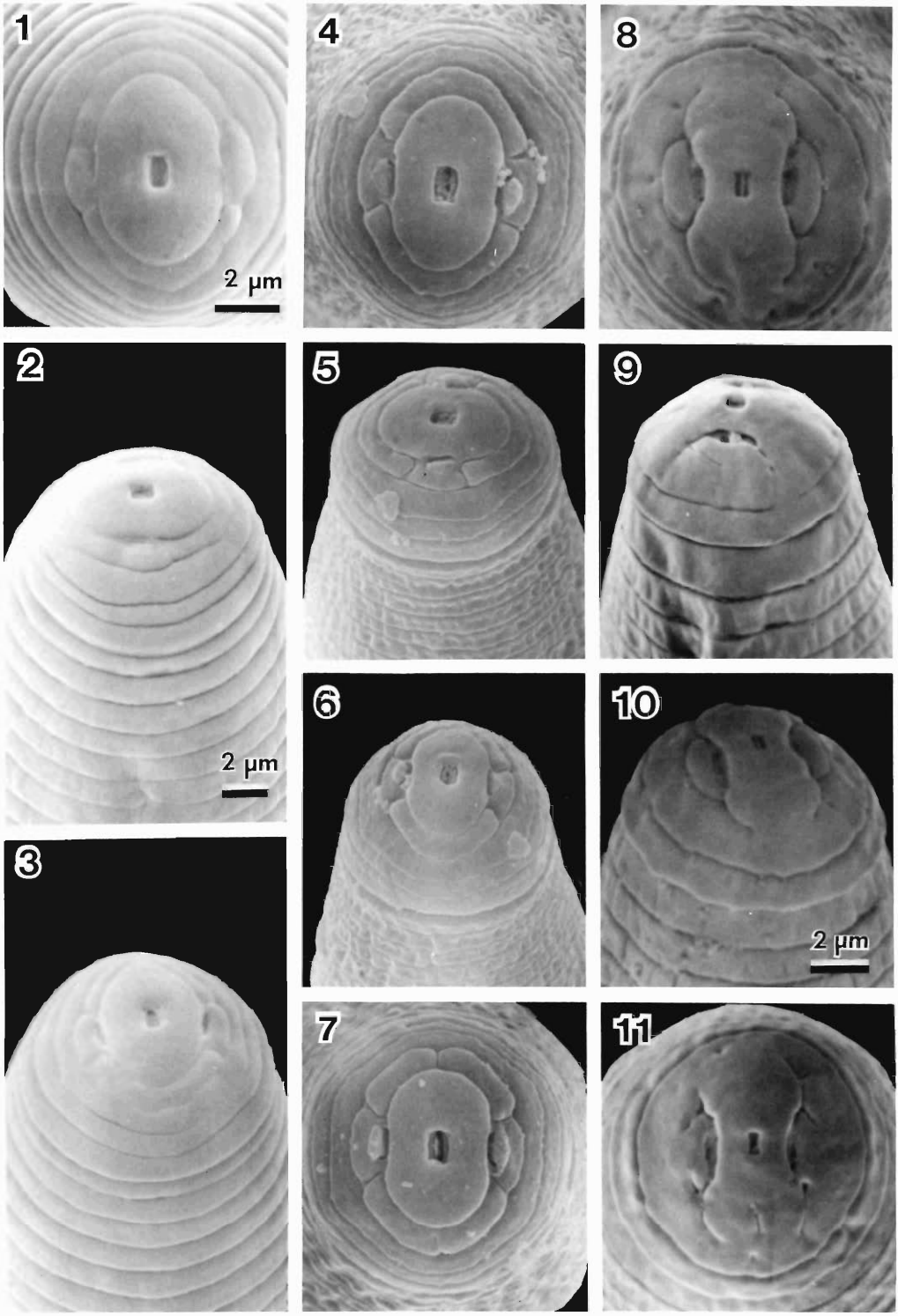
Figures 1-11. Head region of second-stage juveniles of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* prepared by glycerin (gly) or critical-point drying (cpd). Figures 1-3. *S. hydrophila* (gly). 1. En face. 2. Lateral. 3. Medial. Scale as in 2. Figures 4-7. *R. sequoiae* (cpd). 4. En face (common). Scale as in 1. 5. Lateral. Scale as in 2. 6. Medial. Scale as in 2. 7. En face (variant). Scale as in 1. Figures 8-11. *A. africana* (gly). 8. En face (common). Scale as in 1. 9. Lateral. Scale as in 2. 10. Medial. 11. En face (variant). Scale as in 1.



sists of separate lateral lips, but adjacent submedial lips are fused. The two areas of fusion are narrower than the rest of the annule (Fig. 16). The head includes three additional annulations, at least two of which are complete (Figs. 17, 18). The en face pattern of *A. africana* males is different. The labial disc and submedial lips fuse forming a unit, and the lateral lips partially fuse with this unit (Fig. 19). The head region includes one additional complete and at least two incomplete annulations (Figs. 20, 21).

The en face patterns of females of *Sarisodera, Rhizonema,* and *Afenestrata* species are variable. However, in each case the labial disc includes a raised ring at the periphery occupying about half of the diameter of the disc; the first head annule is not separated into lip sectors and the amphid openings generally are not clearly marked (Figs. 22, 23, 26, 27, 30, 31). In *S. hydrophila,* the labial disc has elevated submedial corners and lateral lips faintly delimited in a few individuals (Figs. 22, 23). Head annulations posterior to first annule are diminutive, but annulations do occur in the neck region (Fig. 23). In addition, a few faint protuberances are scattered over the head surface (Figs. 22, 23). In *R. sequoiae* females, as in *S. hydrophila,* the labial disc has elevated submedial corners and a centrally located depression surrounded by the raised peripheral ring (Figs. 22, 23, 26, 27). The submedial and lateral lips form a circular plate, but lateral lips are clearly marked from the rest of the plate by indentations (Figs. 26, 27). This plate extends posteriorly to follow the contour of the head region. Annulations begin immediately posterior to the plate but there is no clear demarcation between the head region and the rest of the body annulations (Fig. 27). Protuberances are scattered over the surface of the plate and are most abundant dorsoventrally. The head region includes at least three additional complete annulations (Fig. 27).

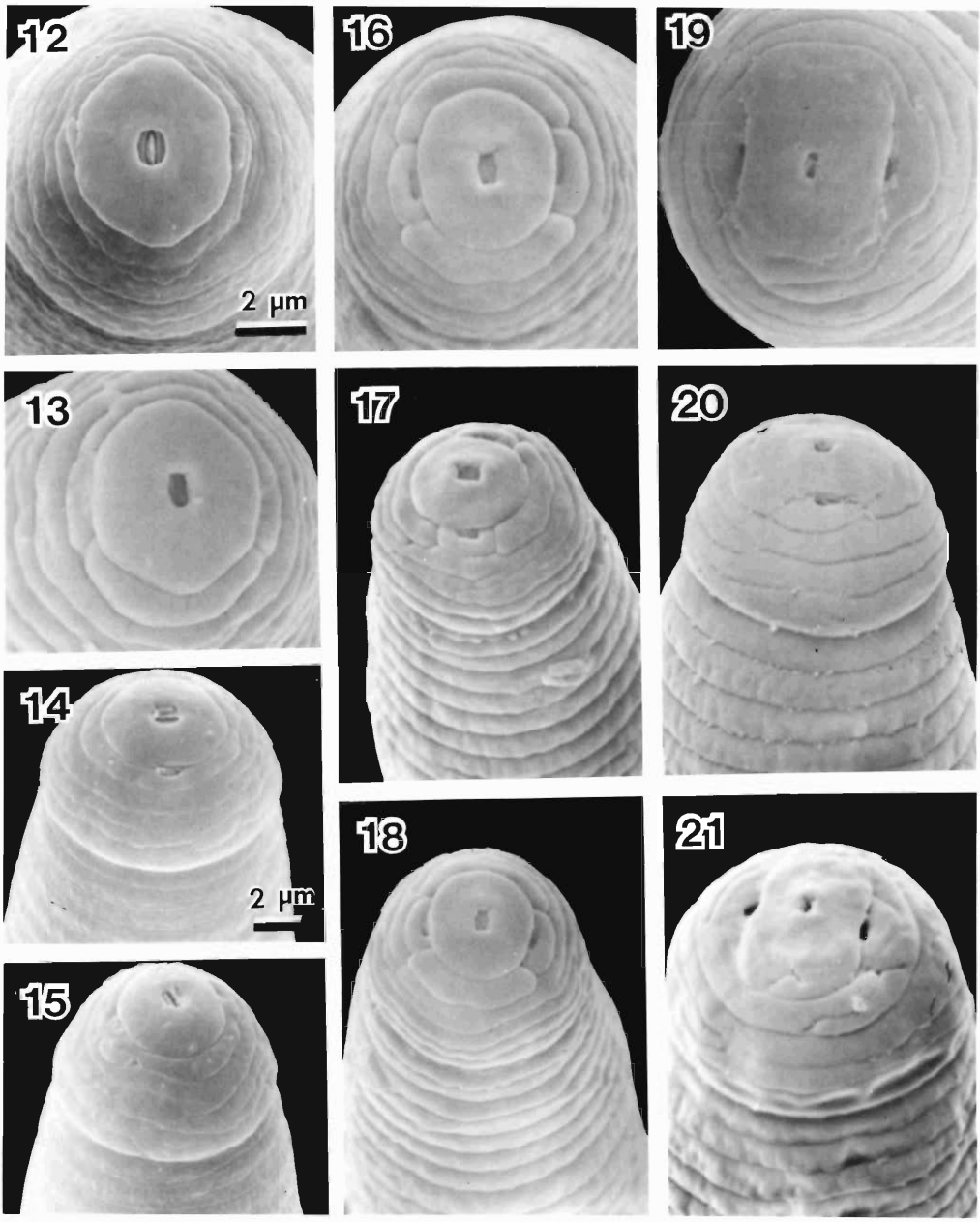
In females of *A. africana,* elevated submedial corners on the labial disc, lateral lips, and protuberances are not observed (Figs. 30, 31). Furthermore, at least three deeply grooved annulations occur posterior to the first head annule (Fig. 31).

CONE OF FEMALES AND CYSTS: In females of *S. hydrophila* and *R. sequoiae* the cone terminus is divided into two large outer vulva lips by a deep depression that includes the vulva slit (Figs. 24, 28). In *S. hydrophila,* the slit is surrounded by two inner vulva lips of equal length but the dorsal lip is slightly wider (Fig. 24). The lips have a lacelike cuticular pattern similar to the rest of the body (Fig. 25). The cone of *R. sequoiae* is similar to *S. hydrophila* except the vulva slit is located in a more shallow depression and the inner vulva lips are more clearly delimited (Fig. 28). The entire cone has a lacelike cuticular pattern with very deep furrows; unlike *S. hydrophila,* the rest of the body is annulated (Figs. 29, 38).

The cone in females and cysts of *A. africana* is divided by the vulva slit into two outer vulva lips of equal size that are broadest at the vulva slit. However, no depression or inner vulva lips are observed (Figs. 32, 33). The lacelike cuticular pattern of the body partially extends to the vulva lips. Although the anus is located on the inner side of dorsal lip in both *S. hydrophila* and *R. sequoiae,* its precise position differs between the two (Figs. 24, 28). In *S. hydrophila* the anus occurs in the boundary delimiting the inner and the outer dorsal lip (Fig. 24), whereas in *R. sequoiae* the anus is located more externally, on the dorsal outer lip (Fig. 28). In *A. africana,* the anus is located dorsal to the cone. A lacelike cuticular pattern surrounds the anus in all species (Figs. 25, 29). However, in *S. hydrophila* the region is further modified by small pits (Fig. 25).

CUTICULAR PATTERN IN FEMALES AND CYSTS: Although the cuticle pattern is lacelike in females of *Sarisodera* and females and cysts of *Afenestrata,* it varies in detail between the two species, being pitted and slightly coarser in *S. hydrophila* (Figs. 34, 42). The cuticle of *R. sequoiae* females is composed of wavy annules that extend over the entire body excluding the cone (Fig. 38).

LATERAL FIELDS AND PHASMIDS: The lateral field in J2 of *Sarisodera, Rhizonema,* and *Afenestrata* species includes four incisures delineating three longitudial bands of equal diameter; only the outer two bands are areolated (Figs. 36, 40, 44). In each case the lateral field originates anteriorly about 7–8 annules from the labial disc (Figs. 35, 39, 43). In *S. hydrophila,* the lateral field begins as a single incisure, and remains single for about the anterior 30% of the body length (Fig. 35). Then three additional incisures begin simultaneously forming the three bands. The lateral field in J2 of *R. sequoiae* originates as two incisures forming one narrow band, which divides, 3–9 annules posteriorly, into two wide (about 7.0 μm) bands (Fig. 39). The two bands remain wide for about 30 annules, and then become narrow (about 4.0 μm) throughout the rest of the body. The third band begins about 30% of the body length



Figures 12-21. Head region of males of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* prepared by glycerin (gly) or critical-point drying (cpd). Figures 12-15. *S. hydrophila* (cpd). 12. En face (common). 13. En face (variant) (gly). Scale as in 12. 14. Lateral. 15. Medial. Scale as in 14. Figures 16-18. *R. sequoiae* (gly). 16. En face. Scale as in 12. 17. Lateral. Scale as in 14. 18. Medial. Scale as in 14. Figures 19-21. *A. africana* (gly) 19. En face. Scale as in 12. 20. Lateral. Scale as in 14. 21. Medial. Scale as in 14.

from the anterior end of the nematode. In J2 of *A. africana* the lateral field occurs anteriorly as two bands (Fig. 43). A third band appears about 10 annules from the origin of the lateral field. The lateral field changes little throughout the length of the nematode, except just anterior to the phasmid opening (Figs. 37, 41, 45). In *S. hydrophila* this posterior region of the lateral field

includes two bands (Fig. 37), whereas in *R. sequoiae* and *A. africana* it includes only one band (Figs. 41, 45). The distinct phasmid pore in *S. hydrophila* occurs on or between either of the two bands about 8–12 annules anterior from the tail end (Fig. 37). In *A. africana,* the phasmid pore tends to be smaller than in the other species and is located in the center of the single band about 9 annules from the tail end (Fig. 45). In contrast, the phasmid pore of J2 of *R. sequoiae* is located in the center of the single band about 25 annules anterior to the tail end (Fig. 41).

The lateral field in males of *Sarisodera, Rhizonema,* and *Afenestrata* species also includes four incisures delineating three longitudinal bands; the middle band is not areolated (Figs. 47, 51, 55); the two outer bands, however, are clearly areolated in *S. hydrophila* and *R. sequoiae* (Figs. 47, 51) but are only faintly areolated in *A. africana* males (Fig. 55). In *S. hydrophila* and *R. sequoiae* the lateral field originates as a single band about 12 annules posterior to the labial disc (Figs. 46, 50) whereas in *A. africana* it originates as two bands about 10 annules from the labial disc (Fig. 54). The lateral field of all three species changes little throughout the length of the nematode. The three bands of the lateral field of males of *Sarisodera* and *Afenestrata* species extend to the tail terminus (Figs. 49, 57). The tail end view in *Sarisodera, Rhizonema,* and *Afenestrata* shows a triangular shape including the spicular sheath (Figs. 48, 52, 56). In *Sarisodera* and *Rhizonema* the dorsal side has irregular indentations that do not occur in *Afenestrata* (Figs. 48, 52, 56). In *R. sequoiae* the lateral field bands fade to wavy indentions about 50 annules from the tail terminus (Fig. 53). Phasmid openings were not observed in any of the species (Figs. 48, 49, 52, 53, 56, 57).

Discussion

SEM of J2, males, and females of *Sarisodera, Afenestrata,* and *Rhizonema* species revealed new characters, which may be used with additional characters in identification as well as in testing hypotheses of phylogenetic relationships. For example, J2 of *S. hydrophila* and *R. sequoiae* share a derived en face pattern in which adjacent submedial lips are fused, but there is no fusion with other lips or the labial disc. This shared derived pattern may indicate that *Sarisodera* and *Rhizonema* have a common ancestor and are mono-

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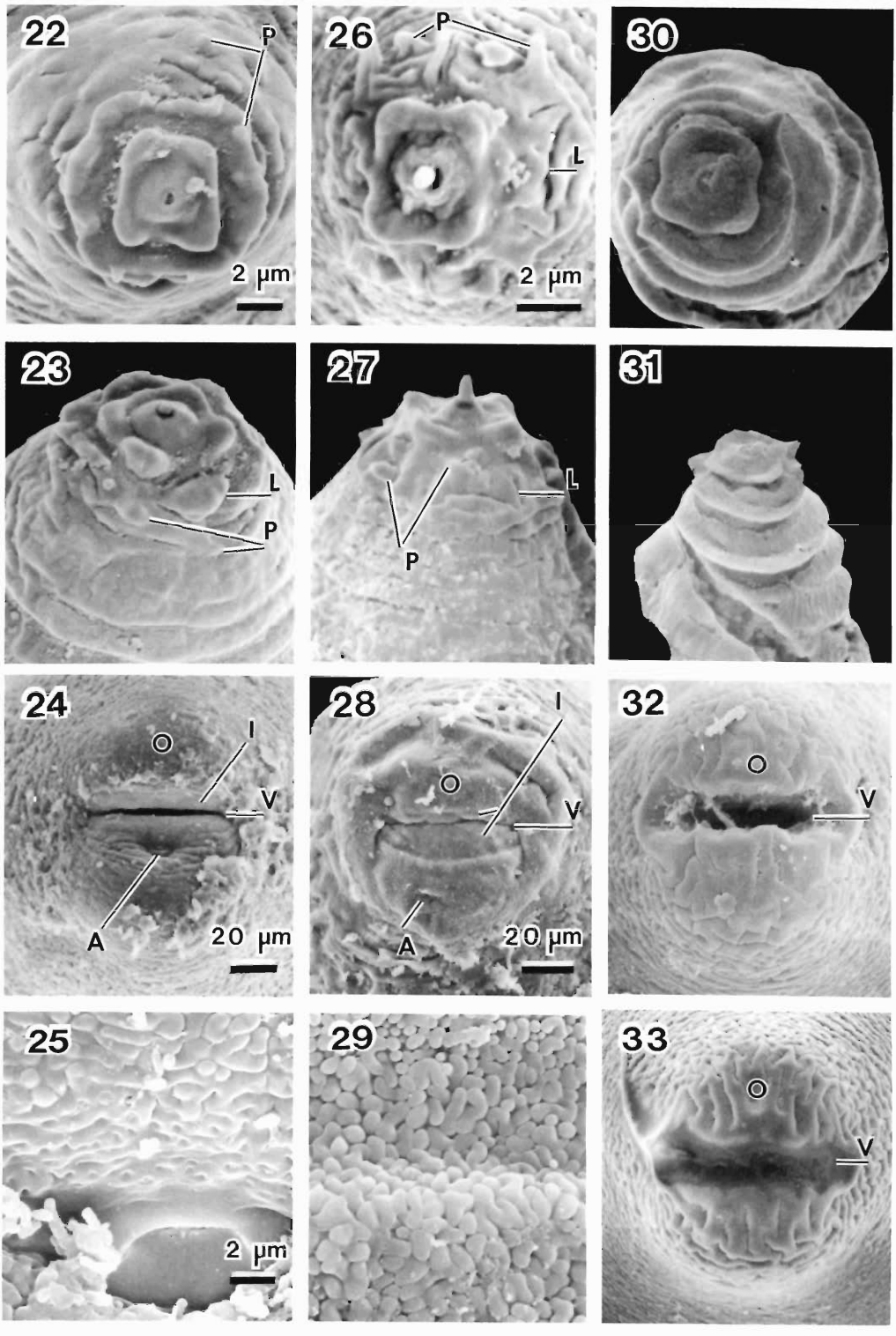
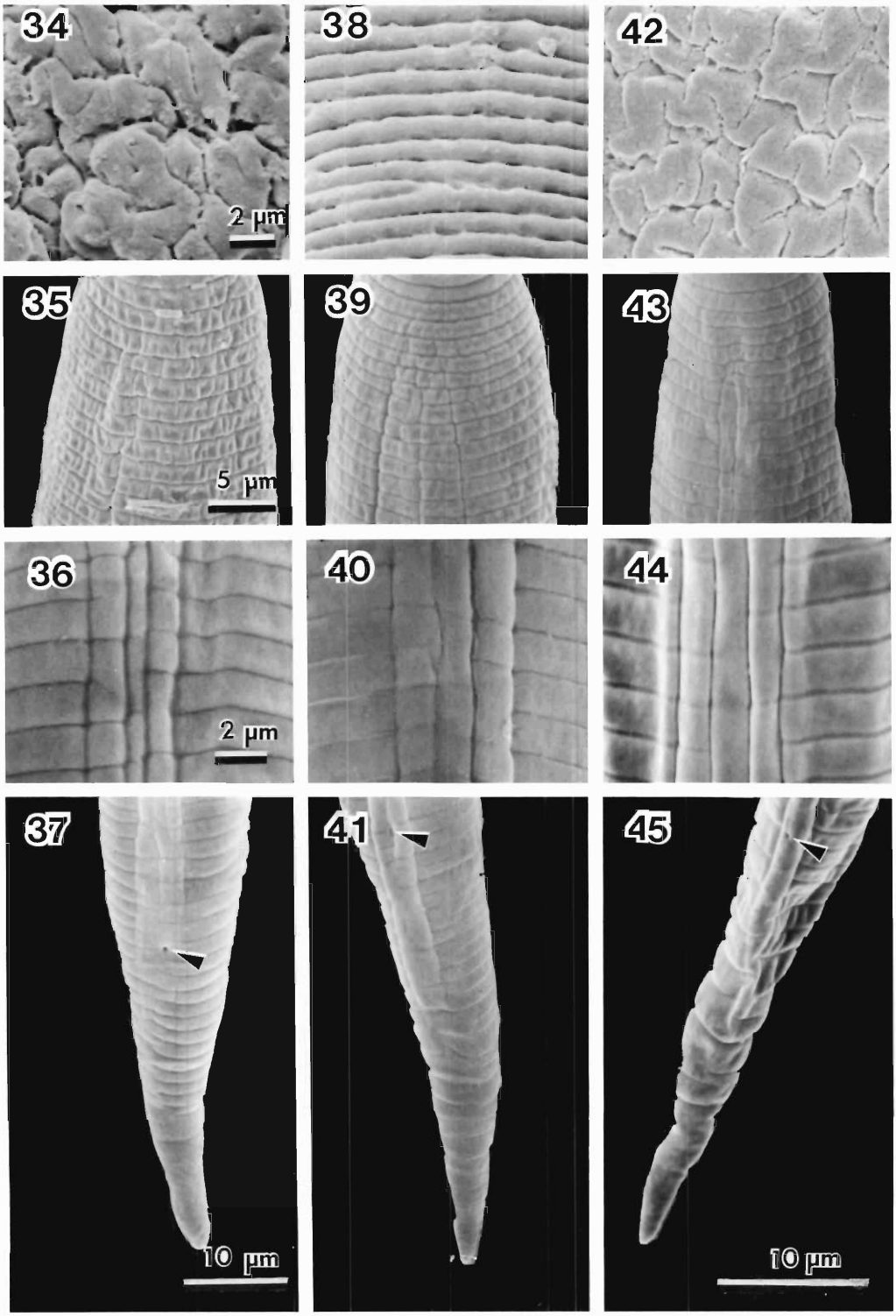
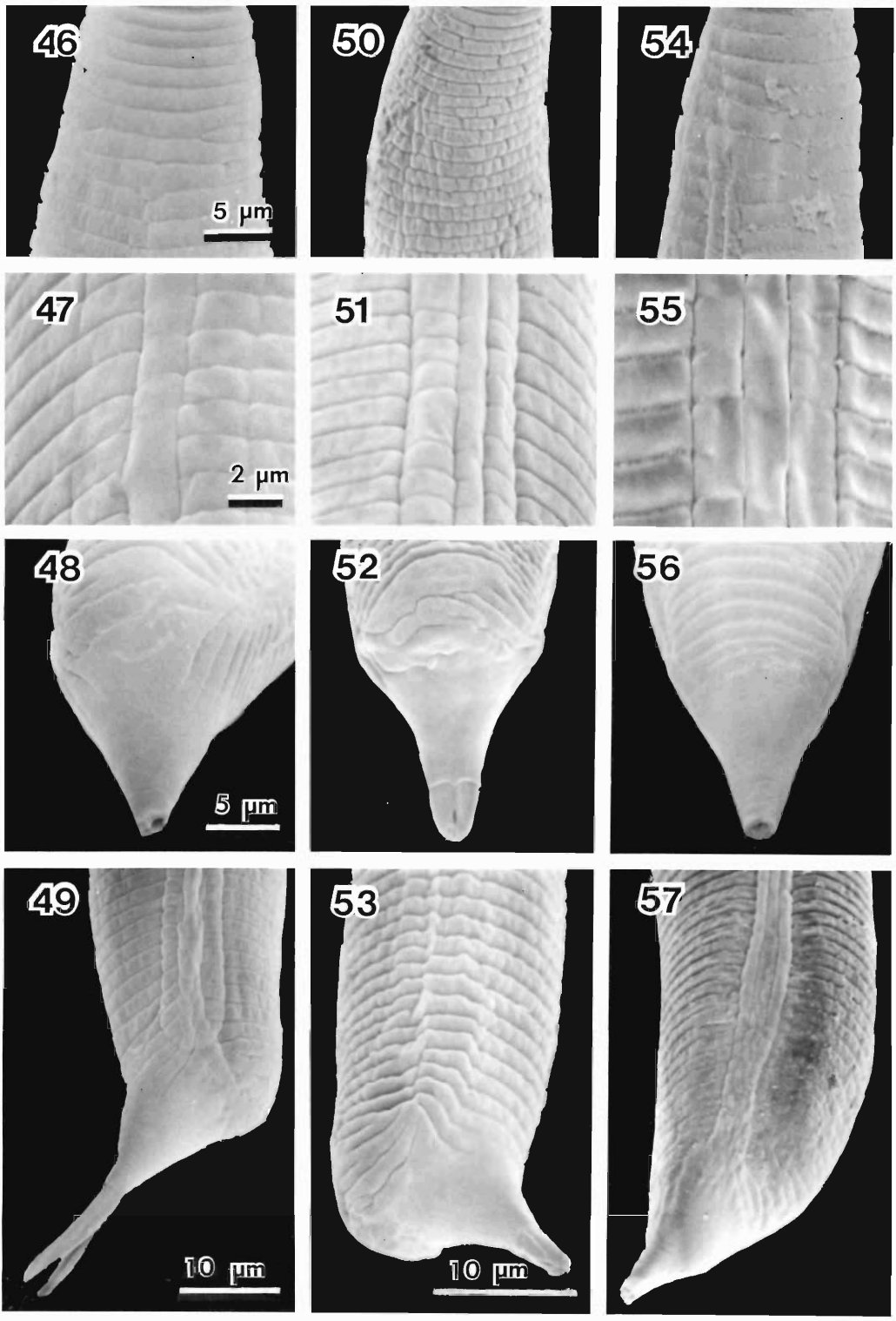
Figures 22-33. Head region and cone of females and cysts of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* prepared by glycerin (gly) or critical point drying (cpd). Figures 22-25. *S. hydrophila.* Figures 22-23. Head (gly). 22. En face showing lateral lips (L) and protuberances (P). 23. Medio-lateral showing lateral lips (L) and protuberances (P). Figures 24-25. Cone (cpd). 24. Cone terminus showing vulva (V), inner vulva lips (I), outer vulva lips (O), and anus (A). 25. Cuticle pattern of the area between the anus and the vulva. Figures 26-29. *R. sequoiae.* Figures 26-27. Head (gly). 26. En face showing lateral lips (L) and protuberances (P). Scale as in 22. 27. Lateral showing lateral lips (L) and protuberances (P). Scale as in 22. Figures 28-29. Cone (cpd). 28. Cone terminus showing the vulva (V), inner vulva lips (I), outer vulva lips (O), and anus (A). Scale as in 24. 29. Cuticle pattern in the area between the anus and the vulva. Scale as in 25. Figures 30-33. *A. africana.* Figures 30-31. Head (gly). 30. En face. Scale as in 23. 31. Mediolateral. Scale as in 23. Figures 32-33. Cone. 32. Female cone terminus (cpd) showing vulva (V) and outer vulval lips (O). Scale as in 24. 33. Cyst cone terminus (gly) showing vulva (V) and outer vulva lips (O). Scale as in 24.

Figures 34-45. Cuticular pattern of females, lateral field, and tail region of second-stage juveniles of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* prepared by glycerin (gly). Figures 34-37. *S. hydrophila.* 34. Female cuticular pattern. 35. Anterior end of lateral field. 36. Lateral field at midbody. 37. Tail region showing phasmid (arrow). Figures 38-41. *R. sequoiae.* 38. Female cuticular pattern. Scale as in 34. 39. Anterior end of lateral field. Scale as in 35. 40. Lateral field at midbody. Scale as in 36. 41. Tail region showing phasmid (arrow). Scale as in 37. Figures 42-45. *A. africana.* 42. Female cuticular pattern. Scale as in 34. 43. Anterior end of lateral field. Scale as in 35. 44. Lateral field at midbody. Scale as in 36. 45. Tail region showing phasmid (arrow).

Figures 46-57. Lateral field and tail region of males of *Sarisodera hydrophila, Rhizonema sequoiae,* and *Afenestrata africana* prepared by glycerin (gly). Figures 46-49. *S. hydrophila.* 46. Anterior end of lateral field. 47. Lateral field at midbody. 48. End of view of tail. 49. Tail region. Figures 50-53. *R. sequoiae.* 50. Anterior end of lateral field. Scale as in 46. 51. Lateral field at midbody. Scale as in 47. 52. End of view of tail. Scale as in 48. 53. Tail region. Figures 54-57. *A. africana.* 54. Anterior end of lateral field. Scale as in 46. 55. Lateral field at midbody. Scale as in 47. 56. End of view of tail. Scale as in 48. 57. Tail region. Scale as in 49.







phyletic, a hypothesis that is subject to falsification by other characters. It must also be considered that en face patterns that approximate those of *Sarisodera* and *Rhizonema* occur in J2 of *Verutus volvingentis* (Othman and Baldwin, 1985) and *Atalodera* spp. (unpubl. obs.). However, the size and shape of the labial disc, lips, and amphid openings of *Verutus* and *Atalodera* are highly distinctive, and the en face patterns of these genera may not be homologous with those of J2 of *Sarisodera* and *Rhizonema.*

The en face patterns of adults also indicate certain similarities between *Sarisodera* and *Rhizonema.* Generally, all the lips of males of *S. hydrophila* are fused; yet this character is polymorphic in the species, with some individuals having distinct lateral lips. En face patterns of these variants of *S. hydrophila* are identical to the consistent pattern in males of *R. sequoiae.* In addition, the en face patterns of females of *S. hydrophila* and *R. sequoiae* both have elevated corners on the labial disc and protuberances on the head region, characters only known to be shared with *Hylonema ivorense* (Luc et al., 1978). Certain shared derived characters between *S. hydrophila and R. sequoiae* might indicate monophyly. However, the character of body wall layering of *S. hydrophila* and *R. sequoiae* appears to be incongruent with the many shared characters. In *S. hydrophila* the lacelike cuticle is thick and elaborately layered (Baldwin, 1983) whereas in *Rhizonema* the striated (annulated) cuticle is thin with few layers (Cliff and Baldwin, 1985). However, we have shown that the cone areas of the two species have similar surface patterns; thus, examination of cuticle layering in the cone might be useful in interpreting this character for phylogeny, including identifying possible evolutionary reversals.

En face patterns of *S. hydrophila* and *A. africana* do not support monophyly of *Sarisodera* and *Afenestrata.* The en face pattern of J2 and males of *A. africana* resembles that of some species of *Heterodera* (e.g., group 4; Stone, 1975) and includes fusion of the submedial lips with the labial disc. In addition, the en face pattern of females of *A. africana* does not have elevated corners on the labial disc and the head region lacks protuberances.

This evidence suggesting that *Sarisodera* and *Afenestrata* are polyphyletic is further substantiated by the presence of cysts in *A. africana* (Luc et al., 1973) and their absence in *S. hydrophila* and *R. sequoiae* (Cid Del Prado-Vera et al., 1983; Baldwin and Bell, 1985). Polyphyly is also supported by the presence of a pore-shaped phasmid in J2 of *A. africana* (Luc et al., 1973) and lenslike phasmid in J2 of *S. hydrophila* and *R. sequoiae* (Wouts and Sher, 1971; Cid Del Prado-Vera et al., 1983). In *A. africana* the anus of females is located dorsal to the cone (Luc et al., 1973), whereas in *S. hydrophila* and *R. sequoiae* it occurs on the inner side of the dorsal lip (Wouts and Sher, 1971; Cid Del Prado-Vera et al., 1983). Furthermore, as with other cyst nematodes, *A. africana* induces formation of a syncytium (Baldwin and Bell, 1985), whereas *S. hydrophila* and *R. sequoiae* induce single uninucleate giant cells in their hosts (Mundo and Baldwin, 1983. Cid Del Prado-Vera and Lownsbery, 1984). *Afenestrata* shares these and other derived characteristics with certain *Heterodera.* On this basis *Afenestrata* might be proposed as a sister-group of some *Heterodera* species distinguished primarily on the basis of the absence of fenestrae in cysts of *Afenestrata* and presence of fenestrae in *Heterodera* and other cyst-forming genera.

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First Report of *Nematodirus battus* (Nematoda: Trichostrongyloidea) in North America: Redescription and Comparison to Other Species

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ABSTRACT: *Nematodirus battus* Crofton and Thomas, 1951 from sheep is reported for the first time in North America. Nematodes of this species were recovered from the small intestine of sheep born and raised in the Willamette Valley of western Oregon. Previously, *N. battus* was believed to have a geographic distribution limited to the British Isles and smaller foci in Europe where it is recognized as a significant pathogen in lambs. The presence of this nematode, exotic to North America, is consequently of great importance because of its potential negative economic impact. Specimens of *N. battus* can be distinguished from those of related species found in North America by the structure of the synlophe in both sexes, the form of the copulatory bursa and terminal portion of the spicules in males, and the form of the tail in females. It is likely that *N. battus* in sheep will most often occur in mixed infections with *N. filicollis* and *N. spathiger*.

Nematodes identified as *Nematodirus battus* Crofton and Thomas, 1951 were recovered from the small intestines of sheep (mixed breed), *Ovis aries* Linnaeus, at Oregon State University during a routine anthelminthic trial. Animals found to be infected had been locally raised in the Willamette Valley of western Oregon, thus establishing the endemic source of infection. Prior to the present study, *N. battus* had not been found outside of the western Palearctic. The presence of this species, exotic to North America and the Western Hemisphere, is notable and of significance beyond the apparent new geographic record.

Nematodirus battus was originally described from sheep, in Great Britain (Crofton and Thomas, 1951, 1954). Until 1969, it was thought that *N. battus* was restricted to the British Isles where it had been reported as a significant cause of disease and mortality in lambs (documented losses from 5 to 30%) (Kingsbury, 1953; Thomas and Stevens, 1956; Baxter, 1957; Dunn, 1978). Isolated populations of this nematode have since been recognized from several foci in Western Europe (Lepojev, 1963; Helle, 1969; Nardi et al., 1974a; Borgsteede et al., 1978). Foci in Norway and the Netherlands developed following the importation of infected sheep from Britain.

The pathogenicity of *N. battus* in lambs has been well documented (Thomas, 1959; Mapes and Coop, 1972; Coop et al., 1973; Dunn, 1978; Martin and Lee, 1980). The parasite is capable of causing serious clinical disease and death in lambs, and its presence in North America could pose a serious problem to the sheep production industry.

The present paper deals with several aspects concerning the occurrence of *N. battus* in North America. We present a redescription of the species based on North American material. Specimens from Oregon are compared with those reexamined by us from Weybridge, England, and are compared to previous redescriptions (Crofton and Thomas, 1954; Durette-Desset, 1979). Additionally, *N. battus* is distinguished from morphologically similar species, and from those known to occur in domestic and wild ruminants in North America. Aspects of potential epidemiology and future research are briefly discussed.

Materials and Methods

Specimens of *Nematodirus* spp., including *N. battus*, were recovered from the small intestine of sheep that were part of an anthelminthic trial. Thirty sheep purchased from a local producer in Philomath, Oregon on June 14, 1984, were maintained on pastures of the Veterinary Medical Animal Isolation Laboratory at Oregon State University until October 5, 1984, when they were transferred to indoor isolation stalls. On November 5, 20 of these sheep were treated with SCH 32481 (Schering Corporation) (10 at 7.5 mg/kg and 10 at 20 mg/kg) and the 10 untreated animals were utilized as controls. Necropsies were conducted on November 12 and 19 when equal numbers of animals were examined. Major sections of the gastrointestinal tract (abomasum, small intestine, large intestine, and caecum) were ligated in situ and later processed separately. Small

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intestines were opened longitudinally and the mucosa was stripped, with all washings and contents being brought to a known volume from which two 5% aliquots were saved. Aliquots were sieved through a 400-mesh (37.5-μm) screen and all material retained, along with parasites, was preserved with 70% ethanol/iodine. In the laboratory, nematodes were removed from aliquots of intestinal contents for later identification.

Specimens of *Nematodirus* spp. were transferred to 70% ethanol/5% glycerin and later cleared in phenol-alcohol, glycerin, or glycerin jelly. The synlophe was studied using light and scanning electron microscopy. The description of the synlophe follows the methods and terminology described previously for the species of *Nematodirus* parasitic in domestic ruminants of North America (Lichtenfels and Pilitt, 1983). The redescription is based on 28 male and 21 female specimens. Fifteen male and five female specimens have been deposited in the National Parasite Collection, USDA, Beltsville, Maryland as USDA Par. Coll. No. 69984. All measurements are in micrometers unless stated otherwise; ranges are followed by mean values in parentheses and sample sizes for some measurements are given as (N =).

Other specimens examined: *Nematodirus battus,* 12 males and 9 females from *Ovis aries,* representing the Weybridge strain, in Great Britain. USNM Helm. Coll. No. 69359, deposited by M. Lancaster.

Results

Nematodirus spp. were only found in the small intestine of control animals. *Nematodirus battus* was present in 4 of 10 sheep (range in intensity = 40–640; $\bar{x}$ = 250 ± 268), whereas *N. filicollis* (Rudolphi, 1802) occurred in 9 of 10 (range = 40–1,560; $\bar{x}$ = 400 ± 495) and *N. spathiger* (Railliet, 1896) was found in 7 of 10 (range = 40–200; $\bar{x}$ = 80 ± 61). *N. battus* was always found in association with these species of *Nematodirus.*

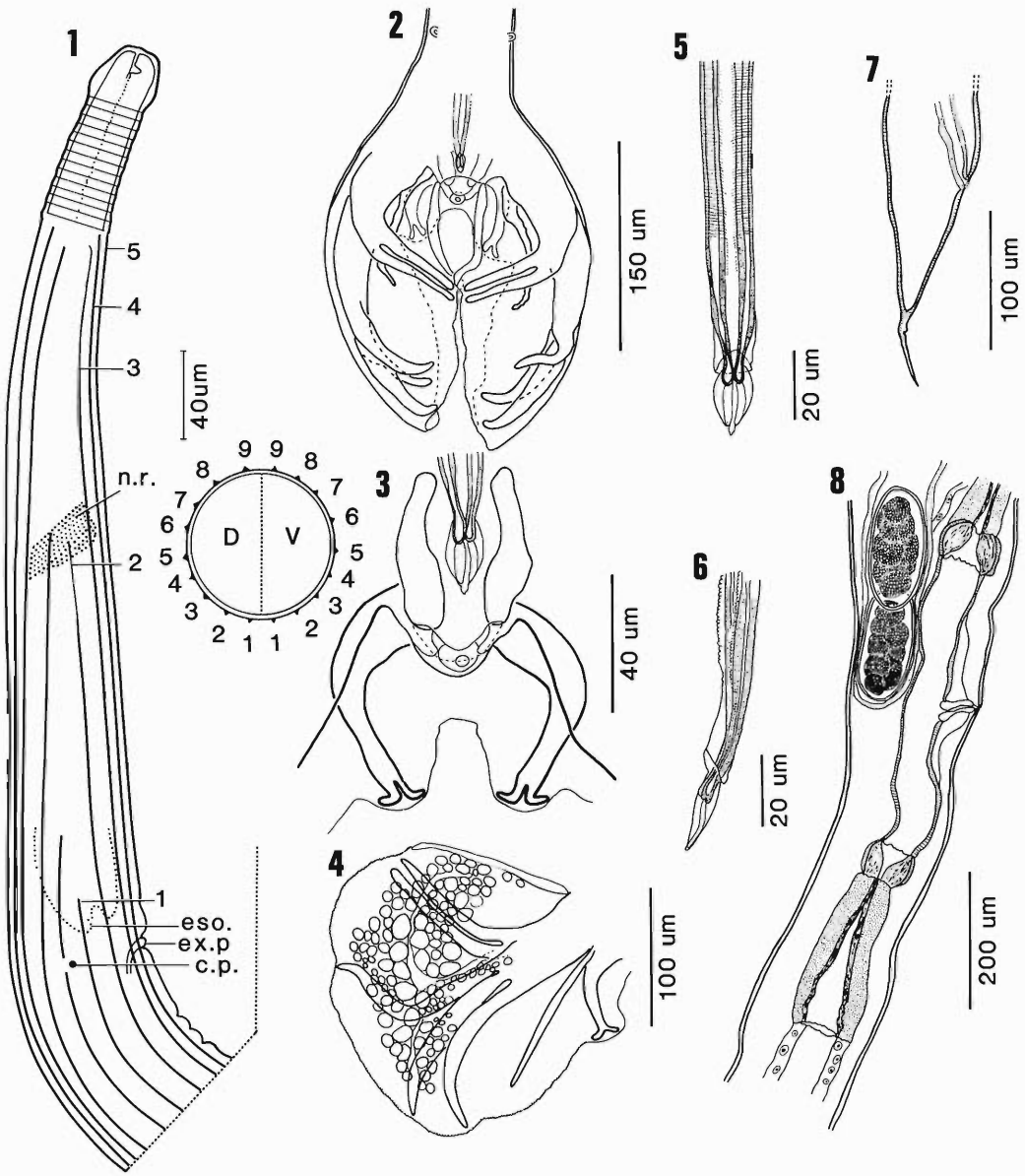
Nematodirus battus Crofton and Thomas, 1951 (Figs. 1–17)

REDESCRIPTION: Cephalic structures. Cephalic expansion with swelling anteriorly and transverse striations covering remaining portions. Six papillae of internal circle with sclerotized semicircular supports. Because of the poor condition of the specimens, papillae of the external circle were not seen. Perioral denticles number 37–40.

SYNLOPHE: The synlophe of *N. battus* consists of an 18-ridge bilaterally symmetrical system in the region of the esophagus (Fig. 1). The ridges are numbered 1–9 in ventral and dorsal sets beginning at the right cervical papilla (Figs. 1–2). Ridges numbered 1 and 9 extend only slightly anterior to the cervical papillae and excretory pore (Fig. 10). In a few specimens ridges numbered 1–9 do not extend anterior to the cervical papilla but end just posterior to it. Ridges numbered 2 and 8 extend anteriorly to the level of the nerve ring and ridges numbered 3 and 7 extend almost to the cephalic expansion. The anterior portions of ridges 2, 3, 7, and 8 are thinner than more posterior portions of the same ridges or than the anterior portions of ridges 4, 5, and 6, which extend to the cephalic expansion.

A few additional irregular and discontinuous ridges are present in each lateral field between the pair of ridges numbered 1 and between the pair numbered 9 in the postcervical region. Usually a pair of discontinuous ridges are present in the lateral fields but one to three have been observed in the lateral postcervical region. Therefore, cross sections posterior to the esophagus may show 18–22 ridges (rarely 23 or 24 ridges). The ridges are continuous, except for those in the lateral fields, and they extend posteriorly to within about 190–220 μm anterior to the bursa in the male and about 1.0–1.10 mm posterior to the vulva in the female.

MALE: Body length 10.8–13.0 mm, width at excretory pore 70–78, at prebursal papillae 85–104. Cephalic expansion (N = 17) 94–108 (101) long by 41–60 (48) in maximum width. Esophagus (N = 21) 429–535 (486) long by 28–37 (33) wide at base. Nerve ring (N = 10) 276–317 (288) and excretory pore (N = 12) 455–558 (492) from anterior extremity. Excretory pore either anterior or posterior to base of esophagus. Spicules equal (N = 28) 805–990 in length; enveloped by delicate membrane, with two alae bearing minute transverse striations ventrally, terminating just proximal to tip. Fused terminal portion of spicules, including tip (N = 25) 28–39 (32) long, bent ventrally. Tip, bluntly pointed, delicate, heart-shaped (N = 25) 14–21 (17) long by 11–14 (12) wide. Ratio of fused portion:tip 1:0.41–0.67. Copulatory bursa symmetrical, with lobes 180–250 long by 270–305 wide. One pair of prebursal papillae. Genital cone well developed. Dorsal rays 48–62 long, bifurcate distally, reaching margin of bursa. Slender externodorsal rays 92–119 long not attaining margin. Lateral rays arising on common trunk. Anterolateral rays 131–154 long; mediolateral rays 141–168; posterolateral 126–154. Lateral rays approaching and often reaching margin of bursal membrane; antero- and posterolateral rays diverge from mediolateral distally. Ventral rays arising on common trunk; anteroventral 110–133 long, posteroventral 110–133. Bursal membrane well-developed, bosses and



Figures 1–8. ***Nematodirus battus*** **from Oregon sheep. 1. Cephalic extremity of male specimen, showing detail of synlophe. 2. Copulatory bursa of male, ventral view. 3. Genital cone, ventral view. 4. Copulatory bursa, lateral view, showing distribution of bosses and position of bursal rays. 5. Terminal portion of spicules, ventral view. 6. Spicules, lateral view. 7. Female tail, lateral view. 8. Ovejectors, lateral view, showing vestibula, *sphincters*, and infundibulum.**

striations evident. Bosses generally oval, 4–18 in maximum diameter, distributed in three groups across lateral and ventral rays.

FEMALE: Body length 16.6–17.0 mm, maximum width anterior to vulva 270. Cephalic expansion (N = 10) 81–104 (91) long by 39–53 (46) wide. Esophagus (N = 11) 496–610 (529) long by 32–55 (44) wide. Nerve ring (N = 10) 209–297 (266) and excretory pore (N = 9) 320–504 (440) from cephalic extremity. Excretory pore either anterior or posterior to esophagus. Anus (N = 18) 145–207 (168) anterior to end of tail;

tail not attenuated, tapering to a blunt conical point followed by a whiplike ventral process with a sharp point. Vulva ($N = 13$) 5,010–6,940 (5,971) anterior to end of tail. Ovejectors well developed; anterior and posterior vestibula generally equal ($N = 23$), 184–253 (221) in length; anterior and posterior infundibula unequal, with latter generally greater in length, respectively ($N = 8$) 150–276 (221) and ($N = 12$) 219–253 (232) long. Sphincters strongly developed ($N = 29$), 46–69 (59) long by 62–83 (73) wide. Eggs thick-shelled ($N = 40$), 161–196 (179) long by 69–96 (82) wide, with irregular thickenings at each end.

HOST: *Ovis aries* Linnaeus.

LOCALITY: Willamette Valley, Oregon.

HABITAT: Small intestine.

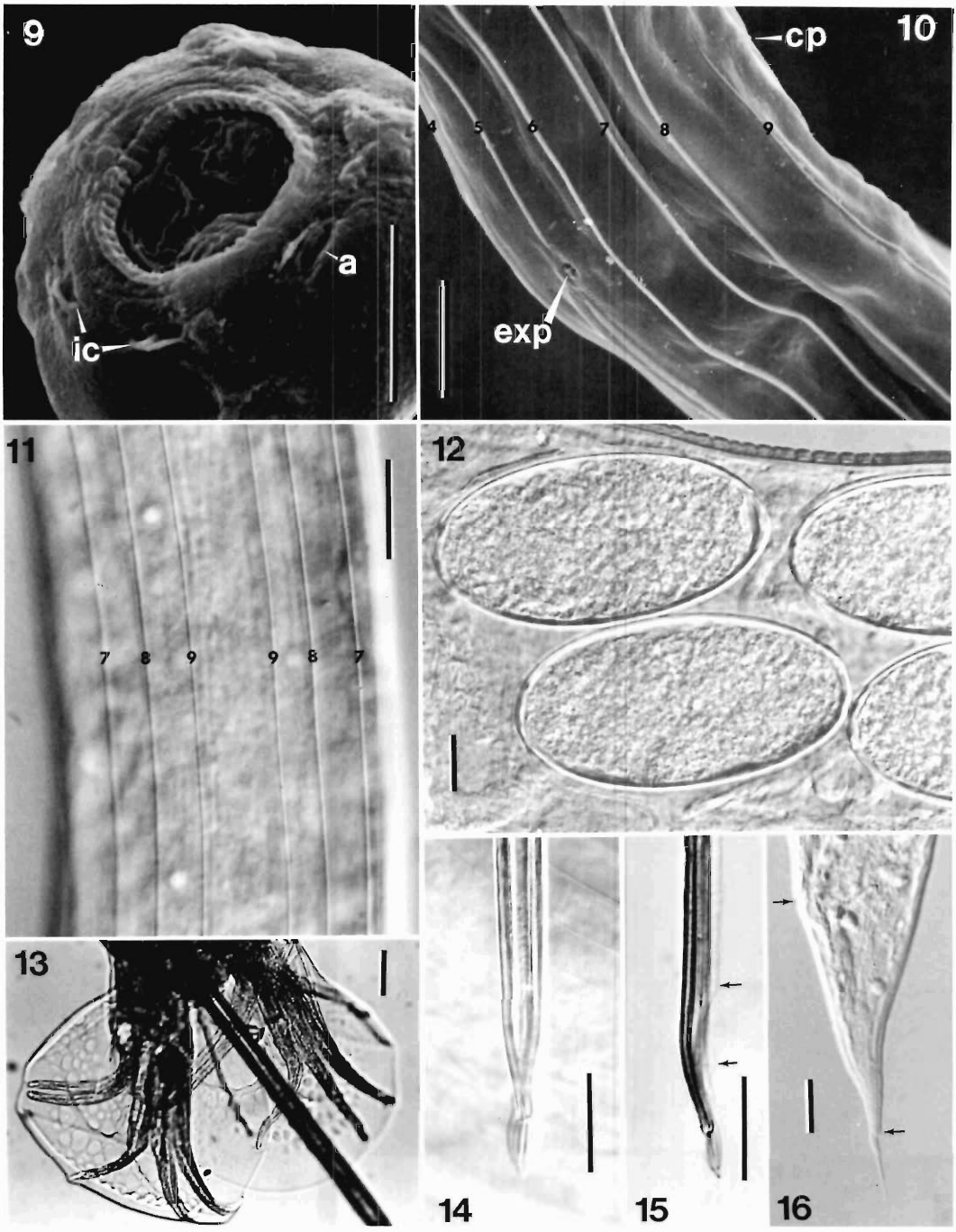
VOUCHER SPECIMENS: USDA Par. Coll. No. 69984.

COMPARISONS: Specimens of *N. battus* from Oregon, and those studied from England, did not differ in any major morphological characters (Table 1) from the original description and subsequent redescriptions (Crofton and Thomas, 1951, 1954; Dunn, 1978; Durette-Desset, 1979). Type specimens of *N. battus* could not be located in England (David Gibson, pers. comm.). The bursa and spicules of males and the form of the female tail, ovejectors, and eggs were identical. Polar sculpturing, mentioned by Jansen (1973) and Dunn (1978), was observed only as irregular thickenings on an internal layer of the shells of eggs in utero in both lots of specimens that were studied. This agrees with observations of smooth-shelled eggs by Thomas (1959). The synlophe in specimens from Oregon, composed of 18–24 ridges was similar to the 18–22 ridges described by Durette-Desset (1979). However, photographs of specimens examined by Martin and Lee (1983), although not described, clearly show 25 ridges in the midbody region of female *N. battus*.

Among species of *Nematodirus, N. battus* is similar to *N. urichi* Cameron, 1935, and *N. roscidus* Railliet, 1911 from cervids and *N. triangularis* Boughton, 1932 and *N. arizonensis* Dikmans, 1937 from lagomorphs. Females of *N. battus* differ from these and other species of *Nematodirus,* except *N. urichi* and possibly *N. lamae* Becklund, 1963, in having a conical, rather than attenuated tail with a single terminal spine (Crofton and Thomas, 1954; Becklund, 1963). Eggs of both *N. battus* and *N. roscidus* apparently have sculptured poles. Males of these four species, except for *N. roscidus,* were adequately distinguished from *N. battus* by Crofton and Thomas (1954). They generally differ in the form of the bursa and terminal portion of the spicules (Boughton, 1932; Cameron, 1935; Dikmans, 1937; Crofton and Thomas, 1954). Males of *N. battus* most closely resemble those of *N. roscidus* in having divergent lateral rays that approach the margin of the bursa. However, they can be distinguished by the synlophe (18–24 ridges in *N. battus*; 34 in *N. roscidus*), number of perioral denticles (35–40 versus 46), length and form of the spicule tips (14–21 μm versus 48 μm), and distribution and size of bosses on the bursa (Kotrlá and Kotrlý, 1973; Durette-Desset, 1979; Rossi, 1983). The morphology of the synlophe of species of *Nematodirus* from lagomorphs was found by Durette-Desset (1979) to differ from those in ruminants sufficiently to justify erection of a new genus, *Rauschia* Durette-Desset, 1979.

Host and geographic distributions further differentiate these species. *Rauschia triangularis* and *R. arizonensis* are apparently limited to lagomorphs in North America (Boughton, 1932; Dikmans, 1937). *Nematodirus urichi* may be restricted to Trinidad in *Mazama americana* (Erxleben) (=*M. simplicicornis*) (Cameron, 1935, 1936), whereas *N. roscidus* has a broad host distribution in the western Palearctic, having been reported from *Cervus elaphus* Linnaeus, *Cervus dama* Linnaeus, *Capreolus capreolus* (Linnaeus), *Ovis musimon* Pallas, *Rupicapra rupicapra* (Linnaeus), and *Cervus nippon* Temminck but not apparently *Ovis aries* (Kotrlá and Kotrlý, 1973; Rossi, 1983).

Additionally, *N. battus* can be distinguished from those species of *Nematodirus* known from domestic and wild ruminants in North America. In this geographic region six species, including *N. filicollis, N. davtiani* Grigorian, 1949, *N. helvetianus* May, 1920, *N. oiratianus interruptus* Lichtenfels and Pilitt, 1983, *N. abnormalis* May, 1920, and *N. spathiger* are characteristic of domestic hosts, particularly sheep and cattle (Becklund, 1964; Becklund and Walker, 1967a; Stringfellow, 1968; Knight and Vegors, 1970; Lichtenfels and Pilitt, 1983; and others). These and an additional three species, including *N. odocoilei* Becklund and Walker, 1967, *N. maculosus* Becklund, 1965, and *N. archari* Sokolova, 1948 are found in wild ruminants, particularly deer, mountain sheep, and goats (Becklund, 1965; Becklund and Senger, 1967; Becklund and



Figures 9-16. ***Nematodirus battus*** **from Oregon sheep. Scale bars: 10 μm, Figure 9; 25 μm, Figures 10-16. 9. SEM of anterior extremity of male showing perioral denticles, six papillae of the internal circle (ic), and a lateral amphid (a). 10. SEM of synlophe at level of excretory pore (exp) and left cervical papilla (cp), showing ventral ridges numbered four through nine. 11. Interference-contrast light micrograph of synlophe, just posterior to region of esophagus showing left lateral view. 12. Eggs in uterus showing irregular polar thickenings in the shells. 13. Copulatory bursa of male, dorsolateral view, showing unique pattern of lateral bursal rays. 14. Spicule tip, dorsoventral view, showing heart-shaped tip. 15. Spicule tip, lateral view, showing delicate membrane (lower**

Table 1. Comparison of *Nematodirus battus* Crofton and Thomas, 1951, from Oregon and localities in Britain.

	Oregon		Weybridge			Crofton and Thomas, 1954
Male: Total (length)	10.7–12.9 mm		7.4–12.6			10–16
Cephalic expansion (length)	94–108	(101)	90–108	(102)	(N = 10)	120 × 60
(width)	41–60	(48)	41–48	(46)		
Esophagus (length)	429–535	(486)	430–476	(449)	(N = 10)	350–540
(width)	28–37	(33)	35–44	(37)		
Excretory pore*	455–558	(492)	312–515	(471)	(N = 10)	–
Nerve ring*	276–317	(288)	223–273	(255)	(N = 6)	–
Spicules (length)	805–990	(908)	720–1,000	(918)	(N = 12)	850–950
Spicule tip (length)	14–21	(17)	14–21	(16)	(N = 8)	–
(width)	11–14	(12)	11–14	(12)		
Female: Total (length)	16.6–17.0 mm		12.8–17.2			15–24
Cephalic expansion (length)	81–104	(91)	99–115	(106)	(N = 9)	–
(width)	39–53	(46)	46–51	(49)		
Esophagus (length)	496–610	(529)	483–541	(498)	(N = 9)	–
(width)	32–55	(44)	39–48	(44)		
Excretory pore*	320–504	(440)	389–564	(473)	(N = 9)	–
Nerve ring*	209–297	(266)	236–299	(272)	(N = 6)	–
Anus–tail†	145–207	(168)	127–156	(141)	(N = 9)	90–140
Vulva–tail†	5,010–6,940	(5,971)	4,480–6,018	(5,367)	(N = 8)	–
Vestibula (length)	184–253	(221)	207–322	(262)	(N = 10)	–
Infundibula A‡	150–276	(221)	230–239			
P	219–253	(232)	196–257	(220)	(N = 7)	550–850§
Sphincters (length)	46–69	(59)	48–61	(56)		
(width)	62–83	(73)	69–74	(71)	(N = 9)	
Eggs (length)	161–196	(179)	143–196	(166)	(N = 50)	150–195
(width)	69–96	(82)	65–87	(75)		80–95
Cephalic:						
No. denticles	37–40		–			–

* Distance from anterior extremity.
† Distance to tail.
‡ Anterior = A; posterior = P.
§ Overall length of ovejectors.

Walker, 1967b; Pursglove et al., 1976; and others). Differentiation of *N. battus* from these nine species is based on an array of characters including the synlophe and number of perioral denticles in both sexes, the structure of the bursa (only in *N. battus* are the lateral rays divergent), distribution of bosses, and form of the fused portion and tip of the spicules in males, and the form of the tail in females (Skrjabin et al., 1954; Becklund, 1965; Becklund and Senger, 1967; Becklund and Walker 1967a, b; Stringfellow, 1968; Durette-Desset, 1979; Lichtenfels and Pilitt, 1983).

HOST DISTRIBUTION: Host records for *N. battus* suggest that it is primarily a parasite of sheep (Crofton and Thomas, 1954; Thomas and Stevens, 1956; Thomas, 1959; Dunn, 1978; and others) although there have been several reports from cattle (Parfitt and Michel, 1958; Taylor and Cawthorne, 1972). Jansen (1973) suggested that sheep have only recently become a host for *N. battus* and that some species of wild ruminant or lagomorph may be the typical definitive host. This argument was supported by records of *N. battus* in roe deer (Dunn, 1965) and in wild rabbits (Boag, 1972). As previously mentioned, the

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arrow) that covers spicule just anterior to the tip, and one of two alae (upper arrow) with minute transverse striations. 16. Female tail, lateral view, showing anus (upper arrow), conical tail with a bluntly pointed tip (lower arrow) and a ventral terminal whiplike process with a sharp tip.

morphological attributes of *N. battus* suggest its greatest affinities are to species of *Nematodirus* from cervids (e.g., *N. roscidus* and *N. urichi*) but the origin of *N. battus* remains a mystery.

GEOGRAPHIC DISTRIBUTION: Records indicate that *N. battus,* in domestic ruminants, originally may have been restricted to the British Isles (Crofton and Thomas, 1951, 1954; Baxter, 1957; Dunn, 1978; and others). Generally, *N. battus* is considered to have been present in Britain for a relatively long period prior to its recognition by Crofton and Thomas (1951). However, Jansen (1973) has suggested that *N. battus* did not occur in Britain prior to 1940.

Apparently *N. battus* has become established only recently in several limited foci in western Europe. The introduction and subsequent development of isolated populations of *N. battus* in Norway (Helle, 1969) and the Netherlands (Borgsteede et al., 1978; Borgsteede and Konig, 1979) can be attributed to the importation of infected sheep from Britain. In Italy, *N. battus* has been present since at least 1954 (Nardi et al., 1974a, b) and may also occur in Yugoslavia (Lepojev, 1963; Cvetkovic et al., 1963).

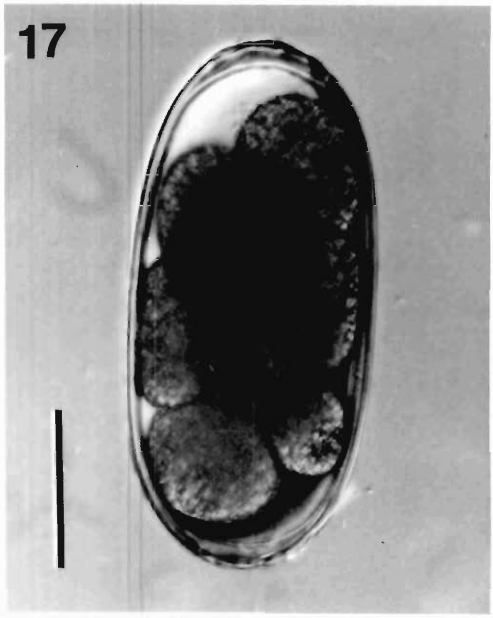

Figure 17. ***Nematodirus battus*** **egg from feces of lamb. Scale bar: 50 μm.**

Discussion

Prior to the present study, *Nematodirus battus* was not known from domestic or wild ruminants in North America, or the Western Hemisphere. The extent of its geographic distribution and mode of introduction in North America has yet to be elucidated. This species may have been: (1) present in North America but not previously recognized; (2) imported recently with animals brought into the United States from an area where the parasite is endemic; or (3) introduced via fomites. Currently, we can only speculate about the potential source of infection.

It is possible that *N. battus* was only recently introduced to North America. The morphological attributes that characterize this nematode clearly distinguish it from other *Nematodirus* spp. Therefore, it is unlikely that specimens of such a distinctive species would have been misidentified during the relatively extensive surveys of parasites in domestic sheep over the past 50 years (Becklund, 1964). Detailed studies in systematics of nematodes from both domestic and wild ruminants further suggest that *N. battus* has not previously been present for an extended period in North America (Becklund, 1966; Becklund and Walker, 1967a; Samson, 1968; Stringfellow, 1968; Lichtenfels and Pilitt, 1983; and others).

In other areas where *N. battus* has been introduced, it became the most prominent species in lambs and apparently displaced *N. filicollis* and *N. spathiger* (Kingsbury, 1953; Thomas and Stevens, 1956; Helle, 1969). In our studies *N. filicollis* was the most adundant species in sheep, followed by *N. spathiger* and *N. battus.* Clinical disease associated with infections of *N. battus* had not been confirmed in Oregon at the time the present redescription was prepared. However, on May 29, 1985, a lamb of 12–16 weeks in age (from a farm adjacent to the original source of infected sheep) and exhibiting some clinical signs of the disease (see Dunn, 1978) was necropsied by us (GLZ and EPH) following death. Upon examination, 1,552 specimens of *Nematodirus* spp. were found in the small intestine, in addition to a few *Ostertagia* spp., *Cooperia* spp., and numerous *Moniezia expansa* Rudolphi, 1810. Specimens of *N. battus* accounted for 95% of the *Nematodirus* spp. present (6% represented by fourth-stage larvae and immature adults; 89% represented by mature males and gravid females). In more typical cases of nematodiriasis the proportion of larvae and immature adult *N. battus* was generally greater than mature and gravid adults, and infections were often of greater intensity than that observed by us (Kingsbury, 1953; Thomas and Stevens, 1956; Baxter, 1957; Dunn, 1978).

It has been shown that *N. battus* must accumulate on a given pasture over several years, be coincidental with the presence of susceptible lambs, and an optimum climatological regime which promotes survival of eggs and hatching of larvae, for severe outbreaks to occur (Thomas and Stevens, 1960; Helle, 1969; Smith and Thomas, 1972; Boag and Thomas, 1975; Gibson and Everett, 1981; Borgsteede, 1983; and others). The potential for spread of this nematode in North America, analogous to the situation in the British Isles, warrants concern and indicates the need to elucidate the current geographic distribution and epidemiology of *N. battus* in Oregon. The development of monospecific isolates of *N. battus* would allow detailed studies of pathogenesis in sheep, and may provide a means to determine the degree to which populations of *N. battus* in Europe and North America are related.

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DISTINGUISHED VETERINARY PARASITOLOGIST AWARD
Request for Nominations

The American Association of Veterinary Parasitologists is seeking nominations of outstanding scientists for its annual Distinguished Veterinary Parasitologist Award. This is an international award that seeks to honor an individual whose contributions to veterinary parasitology are widely recognized as significant and important to the understanding and control of parasitic diseases of animals. The 1986 recipient will be honored at the annual meeting of AAVP in Atlanta, Georgia, July 1986; all expenses of the recipient will be paid. Nominations should include a curriculum vitae, list of publications, and letters of support and should be sent by February 1, 1986 to Dr. K. D. Murrell, Animal Parasitology Institute, Bldg. 1040, Room 2, BARC-East, Beltsville, Maryland 20705. Members of the Awards Committee are Drs. G. Conder, J. Hansen, P. Klesius, D. Murrell, and J. C. Williams.

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Oxyspirura youngi sp. n. (Nematoda: Thelaziidae) from the Patas Monkey, *Erythrocebus patas*[1,2]

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ABSTRACT: *Oxyspirura youngi* sp. n. from the conjunctival sacs of two Patas monkeys, *Erythrocebus patas*, born in the Jacksonville Zoological Park is described. *Oxyspirura youngi* sp. n. is readily distinguished from other species of *Oxyspirura* by the presence of a divided buccal capsule, the presence and size of a gubernaculum, the number of caudal papillae, and by the length of the left spicule. This is only the second species from mammals to be ascribed to *Oxyspirura*.

Eyeworms recovered from the conjunctival sacs of two Patas monkeys belonged in the genus *Oxyspirura* but did not conform to descriptions of species previously ascribed to the genus. A new species is described and distinguished from other species of *Oxyspirura*. The species is named in honor of Dr. Martin D. Young in recognition of his significant contributions to parasitology, especially malariology of primates.

Materials and Methods

Worms were collected from the conjunctival sacs of two Patas monkeys. Monkey number one was an 18-month-old female and monkey number two was a 29-month-old male. Both were born at the Jacksonville Zoological Park in Jacksonville, Florida and were housed in the same cage. The parents of both monkeys were free of eyeworms. Monkey number one exhibited no ocular signs; the eyeworms were incidental findings at necropsy. This monkey had been euthanized following a non-union of a radius and ulna fracture. Approximately 100 worms were found free floating in the conjunctival sac, mainly behind the third eyelid. Worms were present in both eyes. Monkey number two exhibited mild conjunctivitis of the left eye with swollen eyelids (blepharedema). The animal was immobilized and several worms were removed from near the fornix in the conjunctival sac. The eye was treated with an antibiotic-corticosteroid ophthalmic ointment. The eye was examined again five days later and the conjunctivitis was considerably improved. No worms were seen at this time, nor were any seen seven months later.

The specimens were fixed in a solution of glycerine and 70% ethanol and were cleared and studied in glycerine or lactophenol. Cephalic extremities of worms were dehydrated in increasing concentrations of ethanol, dried by CO_2 substitution, and coated with gold for study of en face preparations using the scanning electron microscope. Dimensions of holotype and allotype are followed in parentheses by the range and mean values of paratypes. Measurements are in micrometers unless stated otherwise.

Description of Species

Oxyspirura youngi sp. n. (Figs. 1-6)

GENERAL: Spirurida, Thelazioidea, Thelaziidae, Oxyspirurinae, *Oxyspirura* (*Oxyspirura*) Drasche in Stossich, 1897. Slender worms with bluntly rounded anterior extremity and pointed tail in both sexes. Oral opening round and surrounded by an inner circle of six cephalic papillae and an outer circle of four individual cephalic papillae (Fig. 2). Amphids are lateral. Buccal capsule well developed, divided into anterior and posterior portions with small teeth (Fig. 1). Deirids present. Lateral alae absent. Division of esophagus into muscular and glandular portions not apparent. Vulva in posterior half of worm. Spicules uneven and dissimilar. Gubernaculum present.

MALE (holotype and nine paratypes): Length 8.7 (6.3–9.4, 7.6) mm. Maximum width 260 (260–310, 282). Anterior portion of buccal capsule 14 (10–15, 12.2) long, 19 (18–22, 20.5) wide. Posterior portion of buccal capsule 20 (18–27, 20.8) long, 19 (14–22, 18.7) wide. Esophagus 700 (565–740, 670) long. Nerve ring 200 (180–205, 194), excretory pore 295 (195–295, 265), and deirids 250 and 265 (200–295, 249) from anterior extremity. Right spicule stout, 180 (145–195, 170) long, 26 (19–35, 28) maximum width (Fig. 3). Left spicule slender, 1,300 (1,125–1,400,

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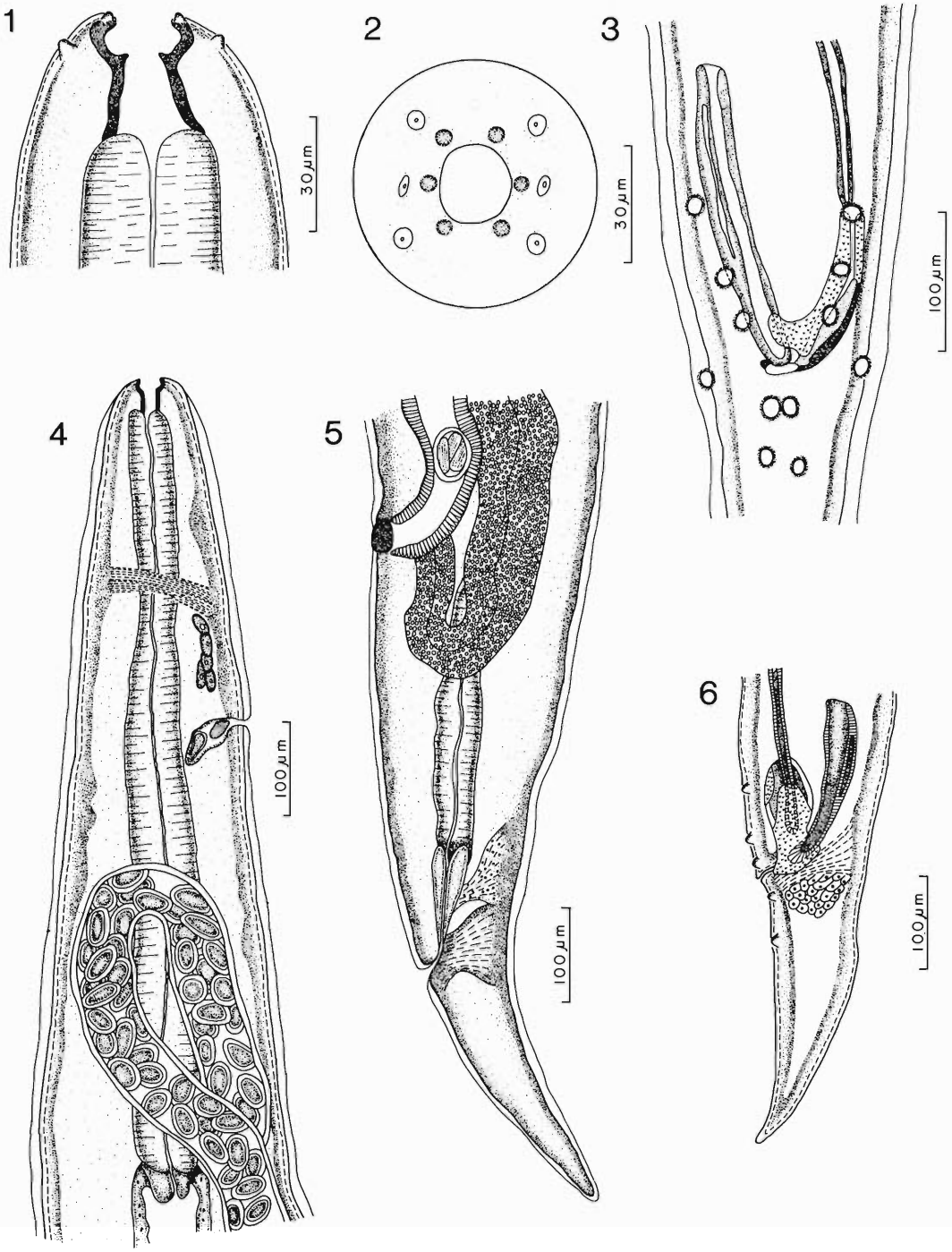

Figures 1–6. ***Oxyspirura youngi*** **sp. n. 1. Cephalic extremity of female, lateral view. 2. Cephalic extremity of female, apical view. 3. Anal region of male showing caudal papillae, ventral view. 4. Anterior end of female, lateral view. 5. Caudal end of female, lateral view. 6. Caudal end of male, lateral view.**

1,290) long, 25 (15–25, 19.5) wide at capitulum, 8 (6–8, 6.9) wide near posterior end. Gubernaculum complex, 108 (95–113, 104) maximum length (Figs. 3, 6). Holotype and six of nine paratypes in which three pairs of preanal caudal papillae, one pair of adanal papillae, and two pairs of postanal papillae observed (Fig. 3). The remaining paratypes with three pairs preanal, one pair adanal, and three pairs postanal papillae; three pairs and a single preanal, one pair adanal, and three pairs postanal papillae; and three pairs and two single preanal papillae, one pair adanal, and three pairs postanal papillae. Papillae distributed between 105 anterior and 72 posterior to anus. Phasmids 84 and 92 (55–102, 79) from posterior extremity. Tail 290 (265–335, 301) long.

FEMALE (allotype and 19 paratypes): Length 11.1 (10.3–12.6, 11.5) mm. Maximum width 400 (320–430, 387). Anterior portion of buccal capsule 12 (10–18, 13.6) long, 23 (21–27, 24.0) wide. Posterior portion of buccal capsule 22 (16–29, 21.4) long, 21 (11–26, 19.9) wide (Fig. 1). Esophagus 800 (710–930, 799) long (Fig. 4). Nerve ring 205 (188–235, 214), excretory pore 280 (280–390, 332), and deirids 265 (240–330, 270) from anterior extremity. Vulva 840 (680–1,020, 809) from posterior extremity (Fig. 5). Vagina 300 (200–330, 254) in length. Eggs (N = 5/worm) 41–44 (40–50, 44.8) long, 29–31 (28–32, 29.8) wide (Fig. 4). Phasmids 40 (40–98, 74) from posterior extremity. Tail 330 (250–400, 329) long.

HOST: *Erythrocebus patas* (born in captivity).

LOCATION: Conjunctival sac.

LOCALITY: Jacksonville Zoological Park, Jacksonville, Florida.

SPECIMENS: Invertebrate Collection (Parasites), National Museum of Natural Sciences (Canada) Nos. 1983-0001 (holotype), 1983-0002 (allotype), and 1983-0003 (paratypes).

United States National Museum Helm. Coll. No. 77396 (paratypes).

Discussion

Division of the esophagus into anterior muscular and posterior glandular portions is a subfamily characteristic of the Oxyspirurinae (Chabaud, 1975). The present species is placed within the Oxyspirurinae in the absence of an apparently divided esophagus because of its being within the family Thelaziidae and in all other features being similar to *Oxyspirura* and dissimilar to genera within the Thelaziinae. Division of the esophagus is not discernible in whole specimens of many other species of *Oxyspirura* (see Hsü, 1933; Wehr and Hwang, 1957; Yeh, 1957; Pence, 1972).

Species of *Oxyspirura* having a divided buccal capsule are placed in the subgenus *Caballeroispirura* if lateral alae are present, and in the subgenus *Oxyspirura* if lateral alae are absent (Chabaud, 1975). *Oxyspirura youngi* sp. n. differs from *O. octopapillata* Caballero, 1942 and *O. navali* Caballero, 1936 in the absence of lateral alae and in having a left spicule approximately three times longer. *Oxyspirura youngi* sp. n. differs from *O. altensis* Oliveira Rodrigues, 1962, *O. tanasijtchuki* Skrjabin, 1916, and *O. tsingchengensis* Hsü, 1933 by the presence of a gubernaculum and a left spicule either much longer or much shorter.

Oxyspirura youngi sp. n. can be distinguished from *O. chauvancyi* Diaz-Ungria, 1963, *O. centropusi* Gupta and Kumar, 1977, *O. diazungria* Guerrero, 1969, *O. guriensis* Guerrero, 1969, *O. hispanica* Yeh, 1957, *O. mansoni* (Cobbold, 1879) Ransom, 1904, and *O. pusillae* Wehr and Hwang, 1957 by the presence of a much shorter (0.49–4.6 mm) left spicule. Many species with a divided buccal capsule are similar to *O. youngi* sp. n. in having three pairs of preanal, one pair of adanal, and two pairs of postanal caudal papillae. However, *O. youngi* sp. n. is readily distinguished from *O. cephaloptera* (Molin, 1860) Stossich, 1897 which has seven pairs of preanal and six pairs of postanal caudal papillae (see Drasche, 1884).

Oxyspirura youngi sp. n. is most similar to *O. conjunctivalis* (von Linstow, 1907) Baer, 1935. However, *O. youngi* sp. n. has a much longer (81–95% longer) gubernaculum (95–113 μm) than does *O. conjunctivalis* (58 μm). This difference in length of gubernacula far exceeds intraspecific variation within the genus. There are six species of *Oxyspirura* for which measurements of gubernacula from four or more specimens are available. Intraspecific variation in the length of gubernacula in these species varies from 6.1% for *O. mansoni* (Cobbold, 1879) Ransom, 1904 (see Ybarra, 1948) to 22.2% for *O. turcottei* Addison and Prestwood, 1978 and 33.3% for *O. diazungria* Guerrero, 1969.

Oxyspirura conjunctivalis is the only one of approximately 100 species previously ascribed to *Oxyspirura* that was from a mammal. *Oxyspirura conjunctivalis* was recovered from a lemur (*Microcebus murinus*) in the Berlin Zoolog-

ical Garden. Baer (1935) redescribed the original material and other specimens from lemurs (*Stenops gracilis*). Barus (1963) considered *O. conjunctivalis* a questionable species, and Addison and Anderson (1969) did not include *O. conjunctivalis* as a valid species of *Oxyspirura* largely because it was from a mammal rather than a bird. Similarly, Chabaud (1965) considered species of *Oxyspirura* to be from birds.

The description of *O. youngi* sp. n. from primates supports the view that *Oxyspirura* legitimately encompasses parasites capable of maturing in mammals as well as in birds. Infection of primates with *O. youngi* sp. n. and *O. conjunctivalis* might be considered incidental acquisitions of bird parasites, because the infections were from zoo animals in an artificial environment. This suggestion cannot be assessed because of the time which has passed since recovery of the worms. However, *O. conjunctivalis* in addition to *O. youngi* sp. n. is distinguishable by the presence of divided buccal capsule and gubernaculum, the length of the left spicule and the number of caudal papillae from all species of *Oxyspirura* presently described from birds.

Acknowledgments

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Cuticular Ridge Patterns of *Haemonchus contortus* and *Haemonchus placei* (Nematoda: Trichostrongyloidea)

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ABSTRACT: Biological differences have been reported between the large abomasal nematodes *Haemonchus contortus* of sheep and *Haemonchus placei* of cattle, but reliable morphological characteristics for identifying the species are unknown. The synlophes (pattern of surface cuticular ridges) of *Haemonchus* from natural and experimental infections of cattle and sheep were studied to determine whether this characteristic might be used to differentiate the two species. Initially *H. placei* from its type locality (Australia) was compared with *H. contortus* from Australia; both species were raised experimentally in sheep and *H. contortus* was also raised in cattle. Additionally, both species from numerous other localities and hosts were studied. No differences were found in the pattern of ridges in the anterior half of the body. A difference was found between the species, however, in the percentage of the nematode body length covered by the synlophe. In both sexes of *H. contortus* the synlophe extended significantly beyond midbody, but in both sexes of *H. placei* the synlophe ended near midbody. All Australian specimens of the two species were distinguishable by the proportionate lengths of the synlophes. Experimentally derived hybrids of the two species had synlophes of intermediate lengths that overlapped both species. Specimens from naturally acquired infections in cattle, sheep, and feral ruminants from North and South America and China were found to have synlophes identical to the populations from Australia. Populations from cattle were usually identified as *H. placei* and those from sheep as *H. contortus*. Eighty-eight percent of the specimens from North and South America and China fell within 95% confidence limits established for synlophe proportionate lengths of *H. contortus* and *H. placei* from Australia and only 4% were intermediate between the confidence bands of the two species. This characteristic can be observed in whole specimens with light microscopy. Passing the nematodes through unusual hosts did not affect the synlophe.

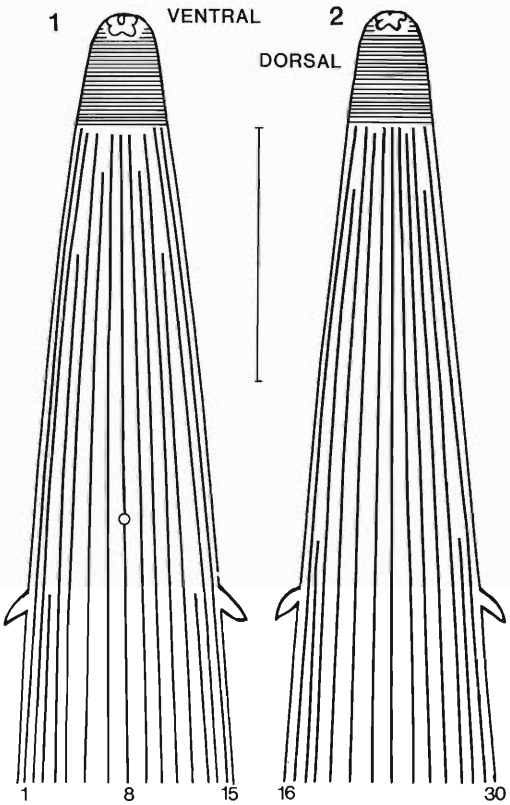
Haemonchus contortus (Rudolphi, 1803) was described from sheep and has a worldwide distribution. *Haemonchus placei* (Place, 1893) was described from Australian cattle. Herlich et al. (1958) found similar morphological differences between *Haemonchus* from cattle and sheep in North America to those reported earlier between *H. contortus* and *H. placei* in Australia by Roberts et al. (1954). Both species will develop in cattle and sheep and they are so similar morphologically that Gibbons (1979), in a revision of the genus, synonymized the two nematode species. Recently, however, Le Jambre (1979, 1981) provided strong evidence for the recognition of both species in Australia by determining that, although crossbreeding occurred between *H. contortus* and *H. placei,* the hybridization produced sterile males by the F_1 or F_2 generation and hybrid females had a low level of fertility. Le Jambre (1983a, b) provided additional evidence of differences between the two species in Australia by demonstrating that *H. contortus* will exclude or displace *H. placei* in sheep. The specific status of populations of *Haemonchus* in cattle outside of Australia, however, was uncertain and some from cattle fron North America were apparently *H. contortus* based on hybridization studies (Le Jambre, 1981). This evidence, along with that of earlier workers (Roberts et al., 1954; Herlich et al., 1958) of biological and cytological differences (Bremner, 1955) between the species, prompted this study of synlophe patterns (the system of longitudinal surface cuticular ridges) in an attempt to find a morphological characteristic useful for identifying the species.

Materials and Methods

The sources, hosts, and numbers of specimens studied are listed in Table 1. Each lot of specimens was assigned a unique number (1–43) in Table 1 to facilitate identification of specimen data in Figures 7 and 8. Scientific names of hosts are given once in Table 1 and not repeated in the text. Whole specimens were studied in temporary mounts cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute alcohol). Standard light microscopy, interference-contrast light microscopy (Leitz), and scanning electron microscopy were used when sufficient specimens were available. Scanning electron micrographs were ob-

Table 1. Specimens of *Haemonchus* studied with assigned lot number, locality and host, USDA Parasite Collection number, and number of specimens.

Lot no.	Locality and host	USDA parasite collection no.	No. of specimens	
			Males	Females
1	Maryland, U.S.A.; cattle (*Bos taurus*)	47205	10	10
2	Maryland, U.S.A.; cattle	29346	7	9
3	Maryland, U.S.A.; cattle	24820	3	2
4	Washington, D.C., U.S.A.; cattle	3679	—	5
5	Georgia, U.S.A.; cattle	69985	12	—
6	Georgia, U.S.A.; cattle	33990	10	9
7	Florida, U.S.A.; cattle	39902	12	11
8	Florida, U.S.A.; cattle	69986	10	—
9	Florida, U.S.A.; cattle	17388	10	12
10	Mississippi, U.S.A.; cattle	19472	10	10
11	Louisiana, U.S.A.; cattle	47217	12	12
12	Louisiana, U.S.A.; cattle	69987	10	10
13	Louisiana, U.S.A.; cattle	58801	—	10
14	Colorado, U.S.A.; cattle	16025	—	4
15	Hawaii, U.S.A.; cattle	49243	5	10
16	New Mexico, U.S.A.; antelope (*Antilocapra americana*); experimental in sheep (*Ovis aries*)	69988	10	10
17	Maine, U.S.A.; sheep	33156	5	5
18	Maryland, U.S.A.; sheep, experimental	69989	10	10
19	Maryland, U.S.A.; sheep, experimental	69990	10	10
20	West Virginia, U.S.A.; sheep	56711	12	11
21	North Carolina, U.S.A.; sheep	40174	10	—
22	North Carolina, U.S.A.; sheep	40172	10	10
23	Georgia, U.S.A.; sheep	69991	10	10
24	Florida, U.S.A.; sheep	39960	5	5
25	Mississippi, U.S.A.; sheep	24577	10	10
26	Nebraska, U.S.A.; sheep	47825	12	12
27	South Dakota, U.S.A.; sheep	45558	12	10
28	New Mexico, U.S.A.; sheep	32152	10	5
29	Vermont, U.S.A.; muskox (*Ovibos moschatus*)	56320	10	11
30	Maryland, U.S.A.; cattle	69992	3	1
31	Georgia, U.S.A.; cattle	69993	10	—
32	Georgia, U.S.A.; goat (*Capra hircus*)	69994	10	10
33	Texas, U.S.A.; deer (*Odocoileus virginianus*)	66540	3	3
34	Guyana, S.A.; cattle	59090	10	9
35	Peru, S.A.; sheep	56897	7	10
36	Uruguay, S.A.; sheep	65775	11	6
37	N.S.W., Australia; sheep, experimental	69995	60	60
38	N.S.W., Australia; sheep, experimental	69996	30	30
39	China; sheep	18682	8	10
40	N.S.W., Australia; cattle, experimental	69997	30	30
41	N.S.W., Australia; sheep, experimental	69998	20	20
42	N.S.W., Australia; sheep, experimental	69999	20	20
43	N.S.W., Australia; sheep, experimental	70000	20	20



Figures 1, 2. Diagrammatic drawings of the anterior extremity of *Haemonchus* spp. showing the synlophe, scale bar 100 μm. 1. Ventral view. 2. Dorsal view.

tained by the methods of Madden and Tromba (1976). Cross sections were studied in either freehand cuts made with a cataract knife or in paraffin embedded sections. Measurements of specimens were obtained with the aid of a calibrated field diameter of a 12.5× objective on a compound microscope after the ends of the ridges were located with a 40× objective. Measurements are in micrometers unless indicated otherwise. Drawings were made with the aid of a camera lucida.

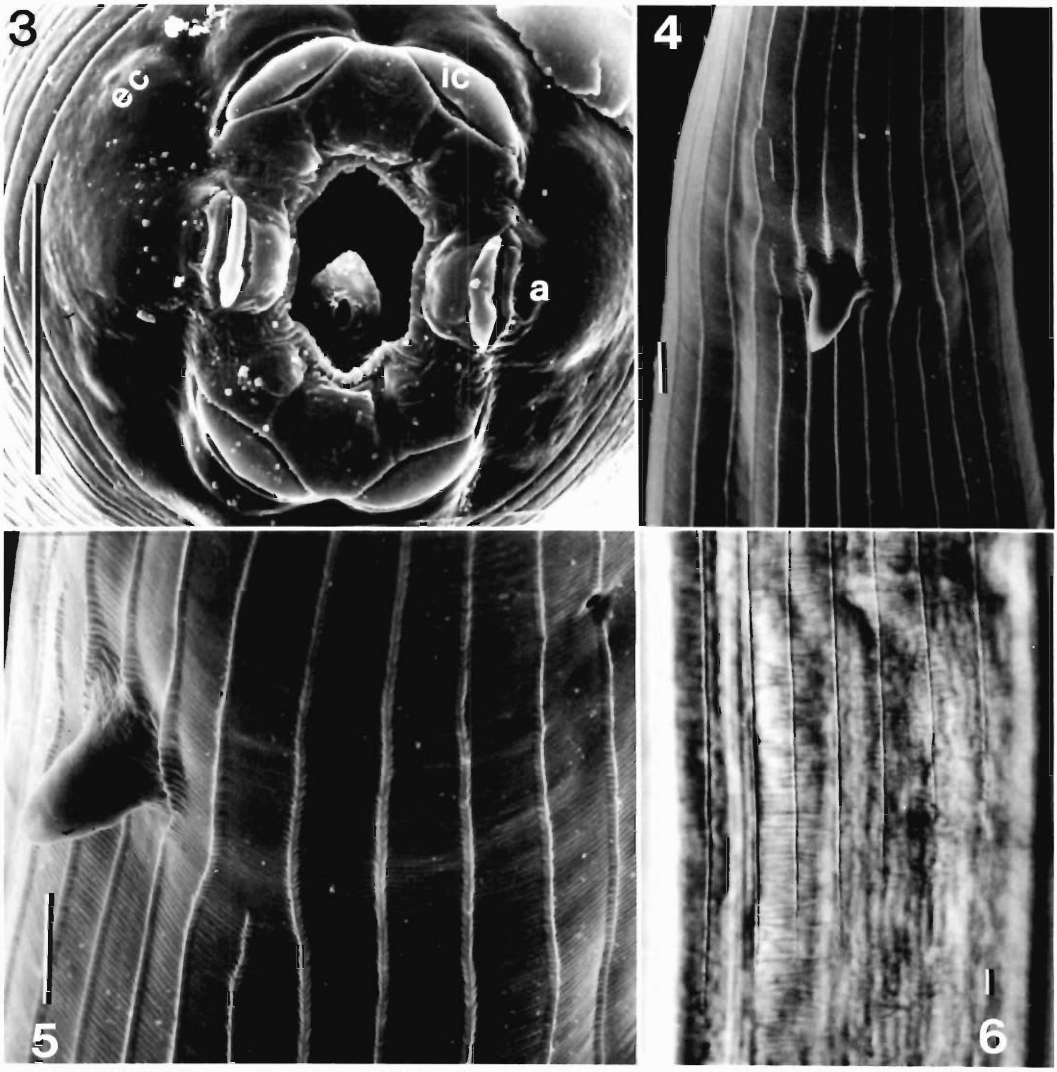
The approach to the problem of comparing the two species was to study pure populations of *H. placei* experimentally raised in sheep (Lot 37) and *H. contortus* experimentally raised in sheep (Lot 38) and in cattle (Lot 40). Additionally, experimentally produced hybrids (Lots 41–43) were studied. Lots 41 and 43 were the F_1 and F_2 generations of the mating of male *H. placei* and female *H. contortus* and Lot 42 was the F_1 generation of the reciprocal cross, all raised in sheep. These populations (Lots 37, 38, 40–43), of known species composition, were used to provide statistical parameters to be used in identifying 37 additional populations of *Haemonchus* from natural or feral infections. Most of these 37 lots of specimens were obtained from the United States Department of Agriculture Parasite Collection (USDA), Agricultural Research Service, Beltsville, Maryland. USDA Parasite Collection numbers are listed in Table 1. The calculation of two standard deviations on each side of the means of the known Australian populations of *H. contortus* and *H. placei* provided theoretical confidence limits within which 95% of the individuals of that species should fall (Steel and Torrie, 1960). Two standard errors of the means were calculated to provide 95% confidence limits of the sample means.

Results

Experimental Australian populations of *H. placei* (Lot 37) differed from those of *H. contortus* (Lots 38 and 40) in characteristics of the synlophe. Although the pattern of 30 ridges in the cervical region was similar in all populations studied (Figs. 1–6), the percentage of the nematode body length covered by the synlophe was found to differ significantly between the two species (Figs. 7, 8). Both males and females of *H. placei* had a proportionately shorter synlophe than *H. contortus*. In males the synlophe percentage is defined as the percentage of the total body length covered by longitudinal cuticular ridges from the anterior end posteriorly. In females, however, the synlophe percentage is defined as the percentage of the body, from the anterior end to the vulva, that is covered by longitudinal cuticular ridges. The percentage of the nematode covered by the synlophe was not different between populations of *H. contortus* raised in sheep (Lot 38) and those raised in cattle (Lot 40) (Figs. 7, 8).

A study of experimentally derived populations of hybrids (Lots 41–43) found them to be intermediate between *H. contortus* and *H. placei* in proportionate length of the synlophe (Figs. 7, 8). The hybrids had a greater range of variation than either *H. contortus* or *H. placei* and, at least the females appeared to have a bimodal distribution of synlophe percentages closer to one or the other of the two species (Figs. 7, 8). The three hybrid populations (Lots 41–43) did not differ among themselves in synlophe percentages (Figs. 7, 8).

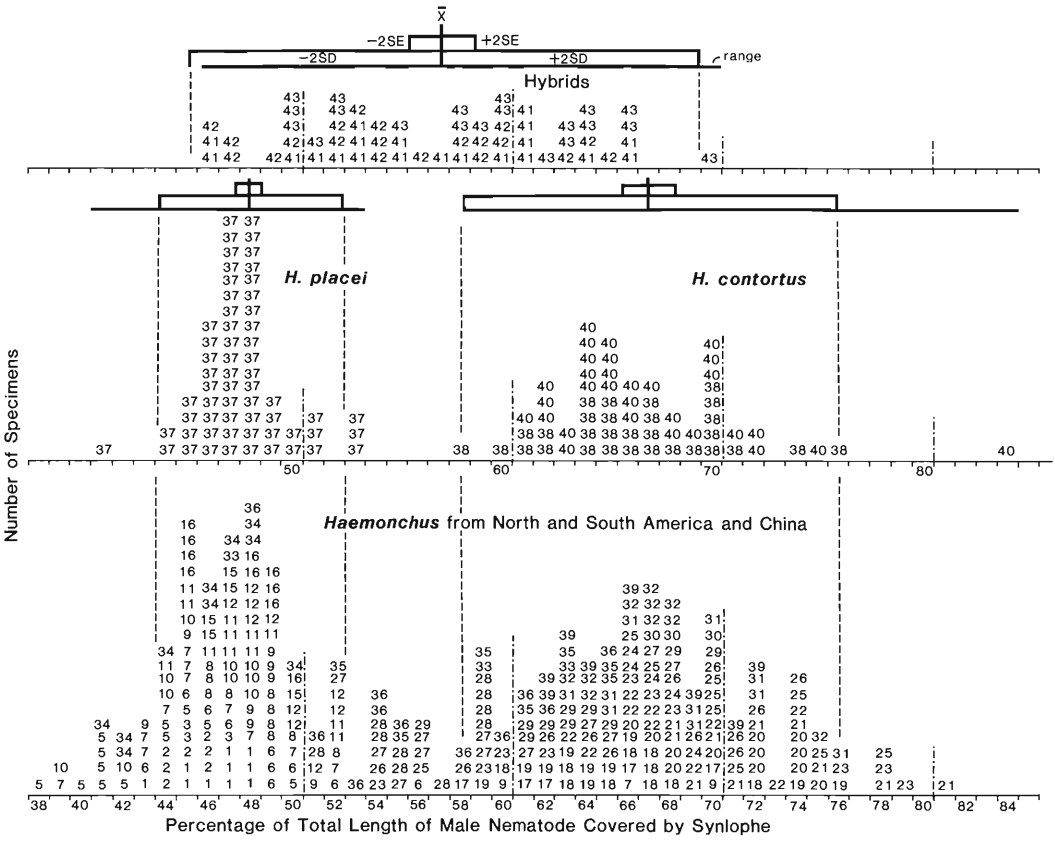
The distribution of synlophe percentages observed in individuals from the 37 naturally occurring populations was very similar to that observed in the Australian populations (Figs. 7, 8). Of 309 males studied from the 37 populations, the synlophe percentages of 273 (88%) fell within the 95% confidence limits estimated for males of the Australian populations. The synlophe percentages of only 6% of the males fell between the confidence limits estimated for Australian pop-



Figures 3-6. ***Haemonchus placei,*** **scale bars 10 μm. 3. En face view, scanning electron micrograph showing dorsal buccal lancet with oval orifice of duct of dorsal esophageal gland on its ventral surface, hexagonal mouth, six sclerotized semicircular supports for the six papillae of the inner circle (ic), lateral amphids (a), and papillae of the external circle (ec). 4. Lateral view, scanning electron micrograph of cervical region showing right cervical papilla and the anterior end of ridges numbered 3 and 28 in the system of 30 ridges illustrated in Figures 1 and 2. 5. Lateroventral view, scanning electron micrograph of synlophe in region of cervical papilla and excretory pore showing anterior ends of ridges numbered 3 (ventral) and 28 (dorsal). 6. Lateroventral view, light micrograph showing the posterior ends of ridges near midbody.**

ulations of *H. contortus* and *H. placei* (Figs. 7, 8). Of 282 females studied from the 37 naturally occurring populations, 250 (88%) had synlophe percentages within the 95% confidence limits estimated for Australian populations of *H. contortus* and *H. placei* (Figs. 7, 8). Only 4% of the females had synlophe percentages between the confidence limits estimated for Australian populations of *H. contortus* and *H. placei.*

The synlophe characteristics determined in the study of the Australian populations were used to identify the naturally occurring populations from North and South America and from China. Most populations from sheep were identified as *H.*



Figures 7, 8. Histograms, by lot number, of synlophe percentages for individual nematodes from various populations (see Table 1 for key to lot numbers). The statistical parameters represented on the figures were

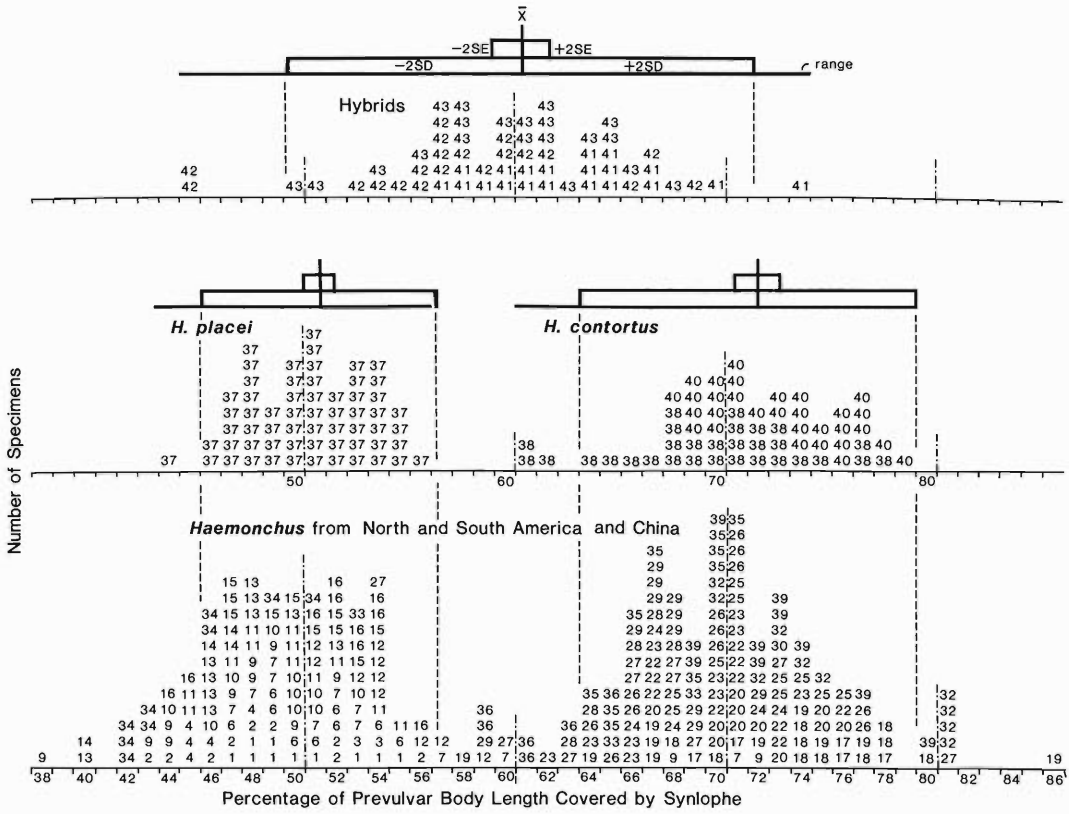
contortus (Lots 17–26, 39 in Table 1 and Figs. 7, 8), and most lots from cattle were identified as *H. placei* (Lots 1–15, 34 in Table 1 and Figs. 7, 8). However, two lots from cattle were identified as *H. contortus* (Lots 30 and 31), and both *H. contortus* and *H. placei* were identified in two populations from cattle (Lots 7 and 9), one population from sheep (Lot 27), and one population from a whitetail deer (Lot 33). A few populations (Lots 35, 36, 27, and 28) consisted mostly of specimens intermediate between the confidence limits estimated for *H. placei* and *H. contortus* (Figs. 7, 8).

In addition to populations from sheep and cattle, several populations from other ruminants were examined. Specimens from a domestic goat (Lot 32) and muskox (Lot 29) were *H. contortus,* but specimens from a whitetail deer (Lot 33) included both *H. contortus* and *H. placei* (Table 1, Figs. 7, 8).

Discussion

The results of this study clearly show that characteristics of the synlophe can be used to identify both males and females of *H. contortus* and *H. placei.* Because a few specimens with synlophe characteristics intermediate between those of *H. contortus* and *H. placei* can be expected to occur, and hybrids may occasionally be found, it will not be possible to identify every individual, but a population from a host can be identified confidently by examining 10 specimens of each sex. Furthermore the overall pattern of distribution by host found in this study indicates that most populations in cattle will be *H. placei* and most in sheep will be *H. contortus.*

The study of populations from Australia of known species composition provided a reliable estimate of the synlophe pattern present in *H. placei* and *H. contortus* from other localities. The 95% confidence limits for synlophe percentages



calculated only for the *H. placei* (37), *H. contortus* (38, 40), and hybrid (41-43) populations from Australia. 7. Males. 8. Females.

of *H. placei* are probably slightly underestimated because they are based on only one population. But 88% of specimens from 22 populations from other continents fell within the 95% confidence limits estimated from the Australian population. When only specimens intermediate between the confidence limits estimated for the two species were considered to be unidentifiable, 96% of the females and 94% of the males from three continents were identified with this method. When one considers that most of the unidentifiable specimens came from only four populations (that may have contained hybrids), the reliability of this diagnostic character is exceptional. The four populations with mostly intermediate specimens were from small lots deposited in the USDA Parasite Collection and may represent samples selected by their collectors as unusual specimens.

The uniformity of the characteristics of the synlophes of the two species of *Haemonchus* from four continents agrees with the results of earlier studies of *Nippostrongylus* and *Cooperia* spp. by Lichtenfels (1974, 1977) and *Nematodirus* spp. and *Nematodirella* spp. by Lichtenfels and Pilitt (1983a, b). All of these studies showed the synlophe to be a uniform character with little variation among populations from different regions of the world.

The apparent lack of host effect on the synlophes of *H. placei* and *H. contortus* also agrees with earlier studies of this character in other trichostrongylids (Lichtenfels, 1974, 1977; Lichtenfels and Pilitt, 1983a, b; Measures and Anderson, 1983).

Finding *H. contortus* in most populations from sheep and *H. placei* in most populations from cattle, despite all the opportunities for mixing populations provided by grazing practices in modern farming, indicates a degree of host specificity for the two species. Earlier workers (Herlich et al., 1958) experimentally documented that a greater percentage of infective larvae of *H. placei*

developed in cattle than in sheep, but the inverse was true for *H. contortus*. Earlier workers in Australia (Roberts, 1942; Roberts et al., 1954) found that *H. placei* could be established in sheep more readily than *H. contortus* could be in cattle. Among the 37 lots from naturally occurring infections in the present study two from cattle were all *H. contortus* rather than the usual species *H. placei*; and two from cattle, one from sheep, and one from a whitetail deer included both nematode species. We did not find a pure infection of *H. placei* in sheep among the naturally occurring populations. The pattern of host–parasite species associations observed in the naturally occurring infections in this study appear to be consistent with the pre-mating barriers described by Le Jambre (1983a) in which established *H. contortus* infections in sheep could exclude *H. placei* and incoming *H. contortus* larvae could dislodge established *H. placei* infections in sheep. Similar experimental studies using cattle instead of sheep would determine whether the dominance of *H. contortus* in Le Jambre's experiments depends on being in its normal host.

The results of the study of the hybrid populations (Lots 41–43) (Figs. 7, 8) clearly show that if large numbers of hybrids were present in natural populations species identification would be impossible using synlophe percentages. However, large numbers of hybrid specimens were not found in the natural populations studied and the use of synlophe percentages proved to be a reliable character for identifying populations and 94–96% of the individual specimens. The pre-mating barriers to species hybridization described by Le Jambre (1983a), the complete sterility of hybrids by the F_2 generation (Le Jambre, 1979), and the chromosome abnormalities and meiotic disturbances reported by Le Jambre (1981) are consistent with the observation in the present study of very few specimens with the intermediate characteristics of the hybrids.

The results of the present study indicate that *H. placei*, indistinguishable from the population from its type locality in Australia, is distributed widely in North and South America and Hawaii. Characteristics of the synlophe support the conclusions of Herlich et al. (1958) that the *Haemonchus* of cattle and sheep in North America are comparable to *H. placei* and *H. contortus* as differentiated by Australian workers. In light of these conclusions, one must suspect that the Louisiana strain of *Haemonchus* from cattle that failed to produce fertile offspring when crossed with *H. placei* in tests conducted by Le Jambre (1981) may have been derived from a cow infected with *H. contortus*. In the future synlophe characteristics can provide a morphological character for identifying both males and females in such experiments.

The utility of the proportional length of the synlophe for identifying species of *Haemonchus* deserves some discussion. Living or freshly frozen specimens are best for examining the synlophe and determining the region of the body where it ends. Specimens fixed for later study must be cleared in order to see the ridges. Phenol–alcohol appears to be the ideal clearing agent and interference-contrast microscopy improves the visibility of the ridges, but standard light microscopy can be used satisfactorily.

The significance of *Haemonchus* of cattle and sheep being recognized as two separate species should not be overlooked. Previously these populations were believed to be a single species outside of Australia (Le Jambre, 1981). Control programs for these highly pathogenic and economically important stomach worms of cattle and sheep, whether chemically, environmentally, or immunologically based, must take into account that they are two separate genetic entities. If for example genetic engineering is used to produce an antigen for a vaccine against *Haemonchus,* an antigen common to both species or separate antigens from both species should be included. Now that an additional morphological characteristic has been provided for identifying both males and females of *H. contortus* and *H. placei,* prospects for further differentiating and characterizing the two species are greatly improved.

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Goat and Sheep Research, as a courtesy, to protect the confidentiality of the review process and to avoid any appearance of conflict of interest because the first two authors edit the Proceedings.

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Ultrastructure of Flame Bulbs in Male *Macracanthorhynchus hirudinaceus* (Acanthocephala)

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ABSTRACT: An electron microscopic examination of the flame bulbs in the protonephridium of male *Macracanthorhynchus hirudinaceus* indicated two regions. The distal region consists of a spongy layer encompassing a matrix containing the basal bodies. This shelf of basal bodies is anchored to the walls of the spongy layer as well as to the external membrane of the flame bulb. Cavities in the spongy layer appear to be continuous from the external (adjacent to pseudocoel) membrane to the lumen. The proximal region is very fibrous with large numbers of filaments and microtubules. The latter may occur in singlet or doublet form and are imbedded in a matrix that appears highly disrupted. Evidence for endocytosis occurs throughout the surface of the layer. Cilia arise from one or more shelflike layers of basal bodies that are embedded in an amorphous matrix found in the distal end of the spongy layer. The layer with the basal bodies is attached to some of the walls of this spongy layer as well as to the plasma membrane. Basal bodies give rise to cilia. The average number of cilia per flame bulb is 253. The basal bodies are constructed of doublet not triplet combinations of microtubules.

The discovery of nephridia in Acanthocephala was made by Bojanus in 1821, according to Kaiser (1892). However, Schepotieff (1908) credited Andres (1878) with this discovery. Each author indicated that the material examined was *Macracanthorhynchus hirudinaceus*. Other species with nephridia were reported by Meyer (1931) and it was Meyer who first reported that the dendritic design of the nephridial organ was different in *M. hirudinaceus* than in *Oligacanthorhynchus taenioides*, which he was then studying. Meyer designated this second type as capsular. Von Haffner (1942b) described a "rudimentäres Exkretionsorgan" in *Gigantorhynchus echinodiscus*.

The general design and some information on ciliary activity was provided by Kaiser (1892, 1893) for *M. hirudinaceus*. Schepotieff (1908) confirmed Kaiser's work and suggested that the protonephridia had phyletic importance showing the relationship between Acanthocephala and Priapulida. Although a few additional reports mentioned the presence of protonephridia in *M. hirundinaceus*, no new information was provided until a recent study (Dunagan and Miller, 1985).

The purpose of this study was to examine the protonephridial organs of male *M. hirudinaceus* using transmission electron microscopy.

Materials and Methods

Macracanthorhynchus hirudinaceus were obtained through the courtesy of Swift Fresh Meats Company in East St. Louis, Illinois. Artificial seawater (30%) was used to clean debris from worms and then used as the retention medium until fixation. Protonephridia were removed by an inversion technique (Dunagan and Miller, 1985). For scanning electron microscopy inverted males were fixed in phosphate-buffered (pH 7.2) 3% glutaraldehyde for 2 hr at 4°C. They were postfixed in 1% osmium tetroxide at room temperature for 2–4 hr. Specimens were dehydrated using a graded ethanol series and critical-point dried with liquid carbon dioxide as the transitional fluid. After mounting, the protonephridia were coated with 40 nm palladium/gold and examined in a Hitachi S-570 SEM at an acceleration voltage of 20 kV. For transmission electron microscopy, the protonephridia were fixed in 3% glutaraldehyde buffered with sodium cacodylate (pH 6.8) for 2 hr at 4°C. Specimens were then washed in the same buffer and postfixed in 1% osmium tetroxide for 1 hr at room temperature, dehydrated through graded ethanol, and embedded in Epon-araldite. Sections were collected on nitrocellulose- or formvar-coated grids, stained in uranyl acetate and lead citrate, and examined with an Hitachi H500H electron microscope. Proximal and distal regions of the flame bulbs are those proposed by Kaiser (1892).

Results

The flame bulbs (Fig. 1) belong to the dendritic type. The branching pattern varies from cluster to cluster but primary, secondary, and tertiary branches are evident. This SEM view shows the flame bulbs to be club-shaped with a smooth external surface. TEM sections show this surface to be covered with a coat similar if not identical to a glycocalyx.

Longitudinal sections (Figs. 2–3) through the free ends of the flame bulbs indicate that the structural organization consists of two parts: (1) a distal terminal area(s) that looks like a sponge,

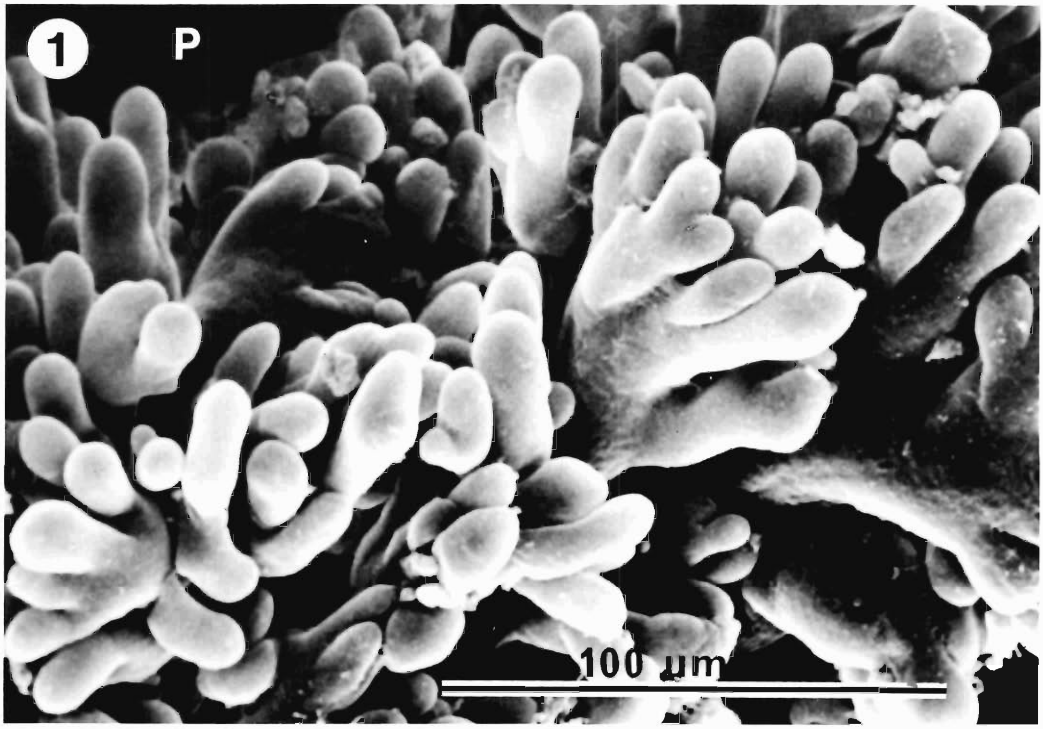


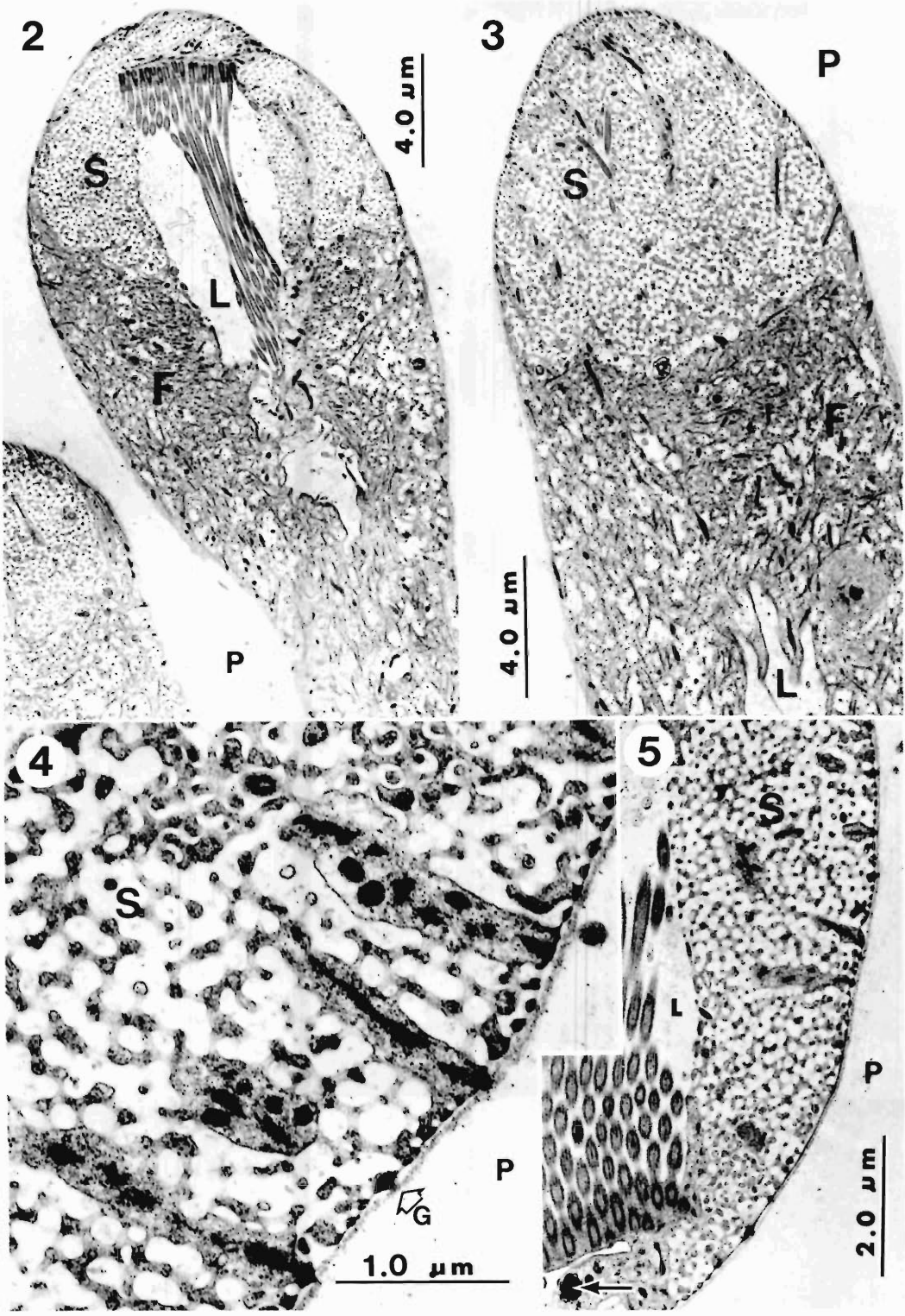
Figure 1. Scanning electron micrograph of a portion of the protonephridium of *M. hirudinaceus*. The clublike nephrostomes show the dendritic-type branching pattern.

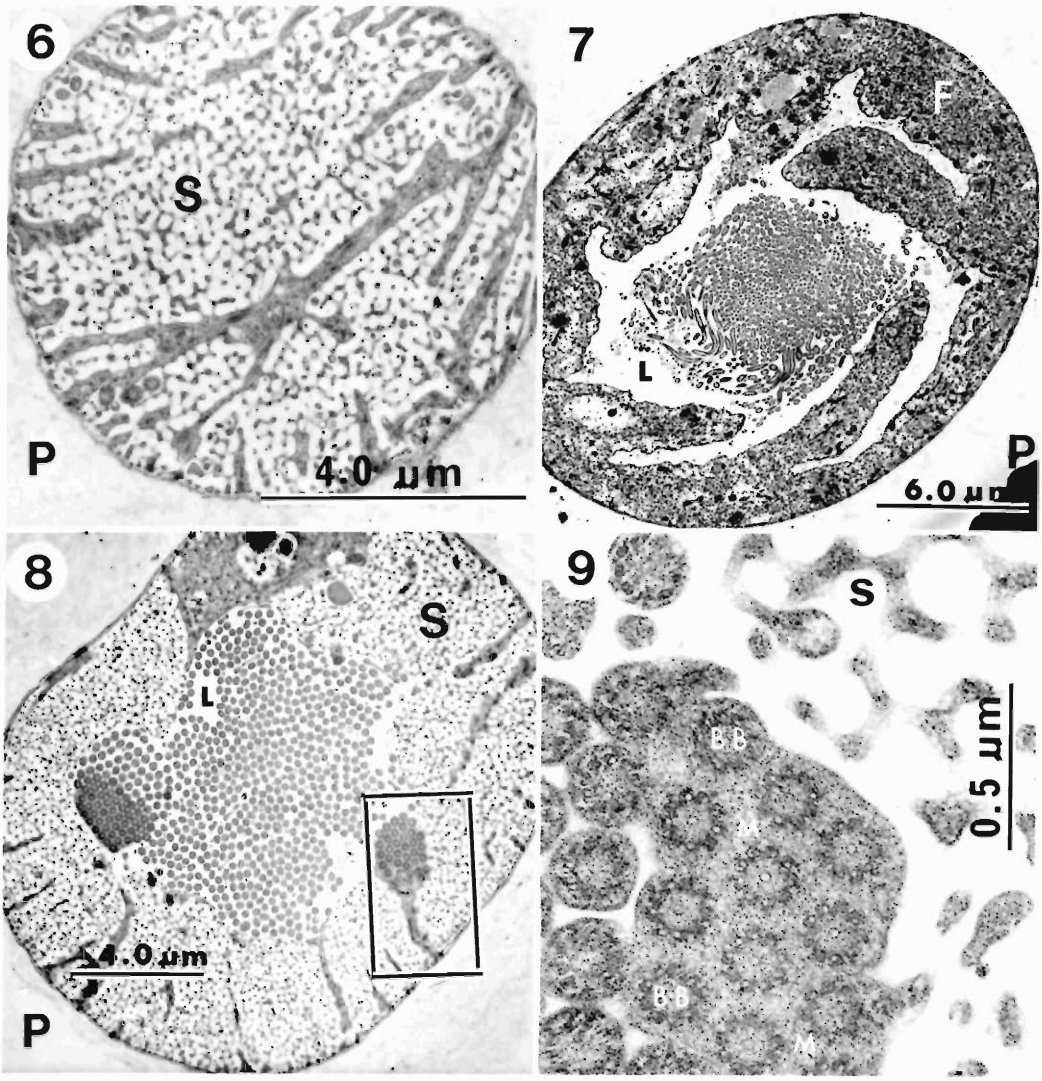
and (2) a proximal fibrous layer (F) forming the tube of the flame bulb. The interface of these two areas is very distinct although the exact position of the border varies somewhat from unit to unit. Notice (Fig. 2) that the fibrous layer extends farther toward the terminus on the left side than on the right. In some instances this extention occurred to the level of the basal bodies but only along the outer wall. The spongy layer extends to approximately the same level on the lumen surface. A closer examination of the spongy layer (Figs. 4–5) indicates that the spaces open into the lumen and suggests that they are all continuous with one another forming tortuous channels between the pseudocoel and the lumen. The walls of these channels contain numerous microfilaments and microtubules that aid in the formation of beadlike accumulations that anchor this layer along the outer membrane surface of the flame bulb. Notice also that the spongy layer is continuous around the distal surface. Occasionally, circular accumulations of numerous membranes (Fig. 5, arrow) are present between the basal bodies and outer membrane.

The outer surface is coated by a layer (G, Figs. 4, 10–11) that we assume is similar to a glycocalyx. Occasionally a ciliary shaft is embedded in the lateral surface of the spongy layer but otherwise we are unable to recognize other organelles.

A cross section through the spongy layer (Fig. 6) beneath the basal bodies shows numerous fibrous elements, some of which extend from one surface to another. Many of the others probably do so at levels outside this section.

The fibrous layer (F, Figs. 2, 3, 7, 11) has a very irregular outer surface (Fig. 11) with endocytosis evident. This surface is also coated with a glycocalyx (G). The invaginations result in numerous vesicles and channels near this surface that disappear toward the lumen. Irregular spaces that do not appear membrane-bound are scattered throughout giving a very disorganized appearance to this layer. Fibrous elements are conspicuous. These consist of various combinations of microfilaments and microtubules. Figure 11 shows microtubular singlets and doublets (some disrupted) near the outer surface. There does seem

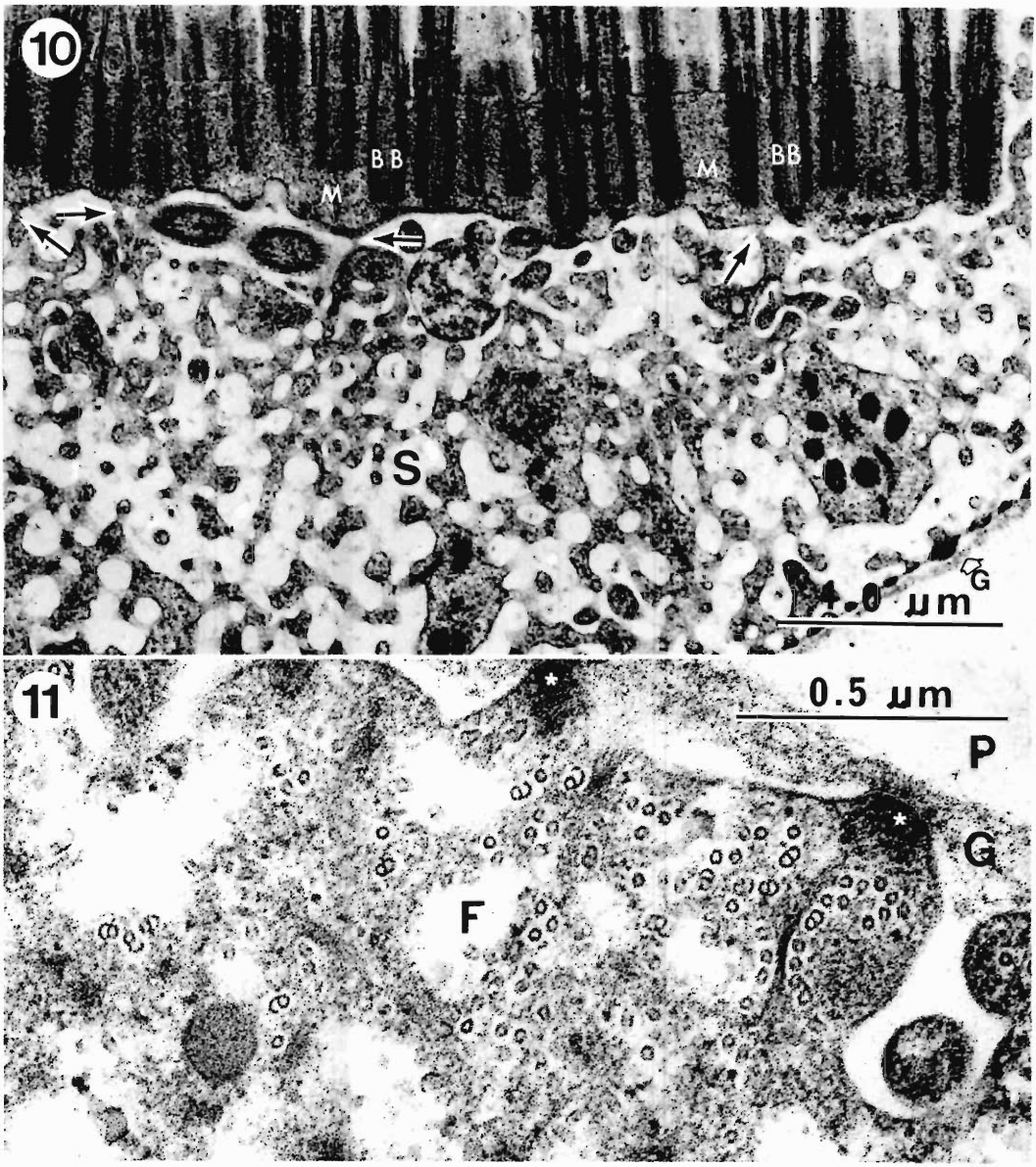




Figures 6-9. TEM cross sections of *M. hirudinaceus*. 6. Nephrostome distal to the layer of basal bodies. Notice the large collections of filaments and microtubules that traverse this region. 7. Section proximal to the basal body in the region of the fibrous layer (F). Notice how the walls of this layer form spiral-like extensions adjacent to the lumen (L). Cilia occupy lumen. 8. Section proximal to the main shelf of basal bodies showing two additional shelves of basal bodies and associated cilia. Notice that these additional shelves are also in spongy layer(s). 9. Enlargement of basal bodies (BB) from an area similar to that outlined in Figure 8. Notice the internal structure of these BB that are further enlarged in Figure 13. M = matrix.

←

Figures 2-5. TEM longitudinal sections of *M. hirudinaceus* nephrostomes. L, lumen; P, pseudocoel. 2. Section through basal bodies and cilia showing well-defined spongy (S) and fibrous (F) layers. 3. Section cut outside the lumen. Notice the vesicular nature of the fibrous layer. 4. Section shows area distal to basal bodies. Notice the well-defined coat (glycocalyx) (G). 5. Spongy layer showing openings into lumen. Arrow indicates ball of membranes.



Figures 10–11. TEM longitudinal sections of *M. hirudinaceus* nephrostome. 10. Section through basal bodies (BB). Notice the distinct layering of these bodies and the attachment (arrows) of this matrix (M) to the walls of the spongy layer (S). 11. Higher magnification of outside margins of fibrous layer (F). A large number of microtubules evident—doublets and singlets. Stars identify periodic dense areas on membrane surface. Membrane undercoat (G) shows much vesiculation. P = pseudocoelom.

to be a loose organization of these fibers. Those nearest the lumen are predominantly circular and those nearer the outer surface are more longitudinal. A hint of this is observed in Figures 2 and 3. Periodic areas of dense materials (star) are irregularly located in the outer membrane surface (Fig. 11). We have been unable to recognize other organelles in this layer. The luminal surface of the fibrous section is cylindrical near the spongy layer but may be broken into a variety of spiral folds (Fig. 7) proximally. Some of these folds are incompletely separated from the wall

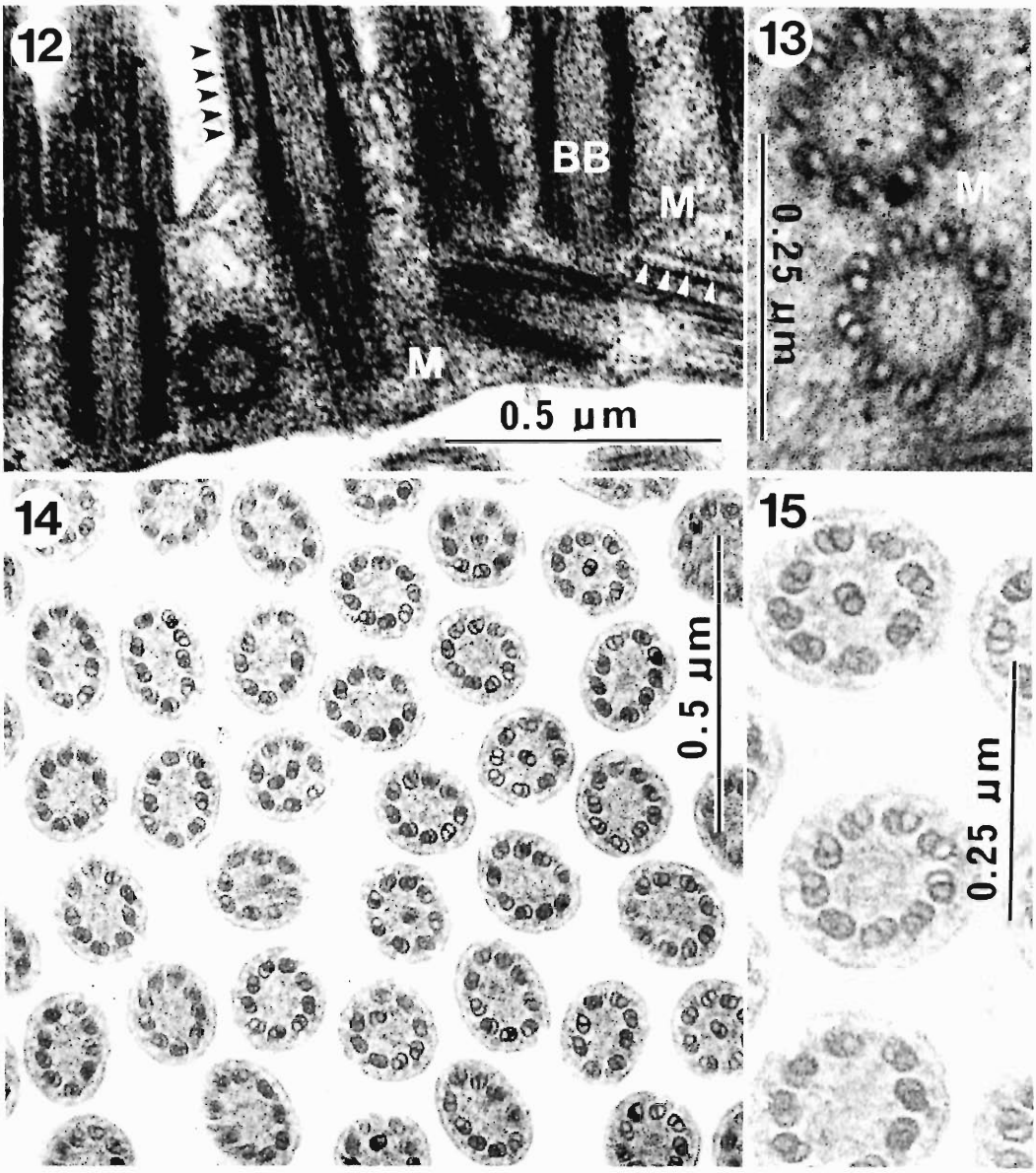


Figure 12. TEM longitudinal section of *M. hirudinaceus* nephrostome. Arrowheads indicate rows of anchor granules evident at the base of many of the cilia. Figures 13-15. TEM cross sections. 13. Shows doublet nature of basal body (BB) and matrix (M). 14. A field of cilia with several different patterns of microtubules. 15. An enlarged cross-sectional view of cilia.

at either end and form separate compartments. Notice that these spirals are abundant in the area of cilia (Fig. 7).

The lumen (L) of the flame bulb begins at the base of the ciliary shaft (Fig. 2) and continues through the different degrees of dendrite branching. At present we have not observed any sphincter that would restrict the movement of materials occupying this space. It is common to see vesicles and "cellular debris" (Figs. 2, 3, 5) in this area, not so much between the cilia as in the area between the outer cilia and the spongy or fibrous layers.

The cilia typically originate from a single shelf

of basal bodies (BB) located near the terminal area of the spongy layer (Fig. 2). However, other shelves of cilia may also occur (Fig. 8) more proximal than this primary and largest group. Figure 8 shows two such shelves (one of which is enclosed by a rectangle) cross-sectioned at a slight angle with some basal bodies embedded in their matrix (M). Figure 9 is an enlarged view of a similar section. These basal bodies differ from a typical centriole by having an outer ring of nine doublets (Fig. 13) with a single centrally located hub. Some sections suggest that other components may also be in the core of the basal body, such as radial spokes. The basal bodies are restricted to a distinct layer (Figs. 10, 12) and have the same general orientation; however, some basal bodies (Fig. 12) occur at right angles to the others but in the same matrix. The lumen surface of this matrix is rather flat when viewed in cross section but the surface adjacent to the spongy layer is undulating with frequent attachments to fibrous strands that may in turn attach to the outer membrane. Additional connections with the "walls" forming the spaces in this layer are observed (Fig. 10, arrows). No top-to-bottom asymmetry has been observed and terminal plates seem to be missing. There does appear to be a direct continuum between the microtubules in the basal body and those in the axoneme.

A cross section of the ciliary shaft taken immediately above the basal body (Figs. 14–15) reveals a pattern different from the 9+2 microtubular design observed in many organisms. Most of these cilia have an 8+0 or a 9+0 pattern, although several other combinations also occur. The subfibrils of each doublet are of unequal size with the larger slightly closer to the center of the cilium (Fig. 15). The central fibers always appear as microtubular doublets and never as singlets. The number of cilia per flame bulb varies from 166 to 284 (average 253).

Discussion

Kaiser (1892) was the first investigator to recognize that the nephridium in *M. hirudinaceus* was not a solid tissue but hollow with cilia in the "Nephrostomen" (flame bulbs). He also recognized that the primary function of these bodies was excretion. Kaiser (1892) credited Leuckart with recognizing the porous nature of the distal end of the flame bulb. Both observed these "Porenkanalchen" across the entire width of the distal end. Von Haffner (1942a) reviewed their distribution and described the pattern of canals leading to the outside in *Oligacanthorhynchus thumbi.*

Although results of transmission electron microscopy have not been published on the protonephridial system of Acanthocephala, axonemes in spermatozoa have been studied. Whitfield (1971) reported that axonemes in *Polymorphus minutus* had a 9+2 tubular organization. Marchand and Mattei (1977b) stated that the eoacanthocephalan families Quadrigyridae and Tenuisentidae had a flagellar organization of 9+n where n varied from 0 to 4. However, in Neoechinorhynchidae n varied from 0 to 5 with a prevalence of 3. They also examined the other two orders of Acanthocephala and concluded "the number of central fibers of the axoneme in the acanthocephalan sperm cell is never absolutely fixed." Afzelius (1963), in a discussion of aberrant cilia and flagella, stated that the cylinder of peripheral filaments rarely had eight fibers. Because this report demonstrates that eight fibers are common in flame bulbs in *M. hirudinaceus,* this must be another feature in which Acanthocephala are the exception to the rule. Furthermore, the basal body (centriole) at the base of the axoneme has nine peripheral doublets, which is in agreement with flagellar organization in the sperm (Marchand and Mattei, 1977a).

The material in the lumen of the flame bulb includes vesicles of many sizes, shapes, and forms plus granular material organized into different-sized clumps. The amount of this "debris" varies from site to site but has been observed in all of our sections of this area. This material is also observed between individual cilia extending as far as the basal bodies. Meyer (1931) observed similar but much larger vesicles in the excretory bladder (nephridialsack) and canal leading into it (nephridialkanal) in *Oligacanthorhynchus taenioides.* Meyer did not mention the content of these vesicles or their origin nor can we clarify this.

The function of the protonephridium has been stated (Meyer, 1931) to be primarily excretory and secondarily osmoregulatory, the latter being a primary function of the tegument. Meyer injected *M. hirudinaceus* with indigo carmine, carmin acidic ammonia, and methylene blue but failed to observe any accumulation in the nephridial organs. We are unaware of other attempts to study the physiology of these organs.

Two types of protonephridia are recognized in

Acanthocephala. The dendritic type protonephridia are found in species of *Tchadorhynchus, Macracanthorhynchus,* and some species of *Oligacanthorhynchus.* The remaining members of the Oligacanthorhynchidae have protonephridia of the capsular type.

Acknowledgments

The TEM micrographs were taken in the Southern Illinois University Center for Electron Microscopy, formerly supervised by Dr. Robert Price, presently supervised by Dr. John Bozzola. The project was supported by financial aid from the Southern Illinois University School of Medicine.

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53(1), 1986, pp. 110–112

Eimeria halleri sp. n. (Apicomplexa: Eimeriidae) from the Round Stingray, *Urolophus halleri* (Rajiformes: Dasyatidae)

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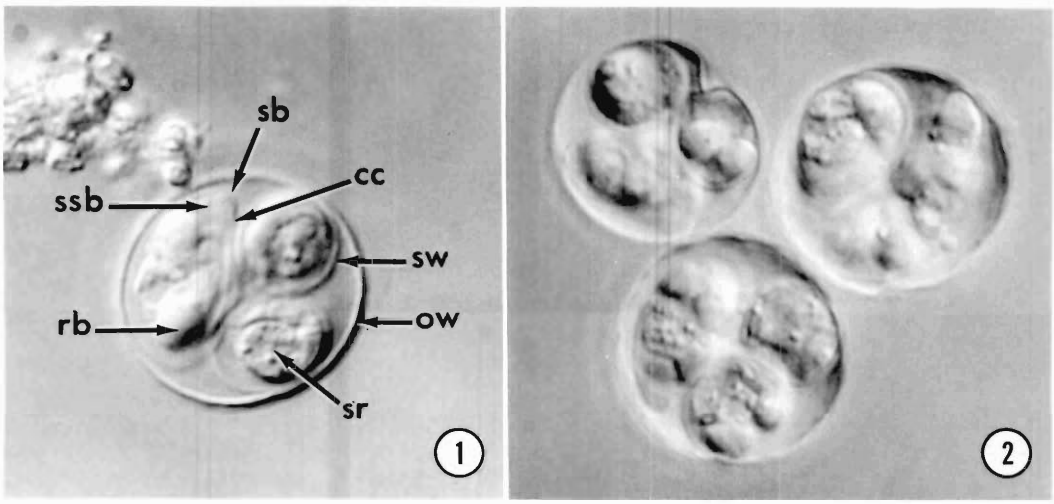
ABSTRACT: *Eimeria halleri* sp. n. (Apicomplexa: Eimeriidae) is described from the rectal contents of the round stingray, *Urolophus halleri* Cooper (Rajiformes: Dasyatidae) from Puerto Peñasco, Mexico. Oocysts are spherical or subspherical, 16.9 × 16.8 (15.0–18.0 × 15.0–18.0) μm, with a smooth, thin wall. Micropyle, polar granule, and oocyst residuum are absent. Sporocysts are ovoid, 11.1 × 6.8 (10.0–13.0 × 6.0–7.5) μm and possess Stieda and substieda bodies. Sporozoites are comma-shaped, 9.9 × 3.2 (9.0–11.0 × 2.8–4.0) μm and contain an ovoid posterior and a spherical anterior refractile body. The sporocyst residuum consists either of numerous finely granular particles scattered among the sporozoites or as a spherical mass.

During a survey of marine fish for parasites at Puerto Peñasco, Mexico, we noted many round stingrays, *Urolophus halleri* Cooper, to be passing unsporulated coccidian oocysts in the feces. Further examination of these oocysts revealed a previously unknown species of *Eimeria*. This paper describes the morphological characteristics of this new species of coccidian.

Materials and Methods

Both male and female round stingrays, *Urolophus halleri,* were collected either with a Hawaiian sling or by seining along the coastline at Puerto Peñasco, Mexico, in October 1984. Spiral valve contents from each stingray were divided into four equal portions and each portion placed within one of the following solutions: 1) 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) in tap water; 2) 1.0% (w/v) $K_2Cr_2O_7$ in 1:1 seawater–tap water; 3) 1.0% (v/v) H_2SO_4 in tap water; or 4) seawater supplemented with 100 IU/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml Actidione. Samples were stored in screw-top vials at room temperature (~25°C) for three days, placed in petri dishes at room temperature for five days so that oocysts could sporulate, and examined by brightfield and Nomarski interference contrast microscopy for parasites. All measurements were made with a calibrated ocular micrometer and are reported in micrometers (μm), with the mean followed by the range in parentheses. Fifty parasites were used for each measurement.



Figures 1–2. Nomarski interference contrast photomicrographs of oocysts of *Eimeria halleri* sp. n. ×2,000. 1. Sporulated oocyst. Note concave side of sporocyst wall (CC), oocyst wall (OW), refractile body (RB), Stieda body (SB), sporocyst residuum (SR), substieda body (SSB), and sporocyst wall (SW). 2. Three sporulated oocysts.

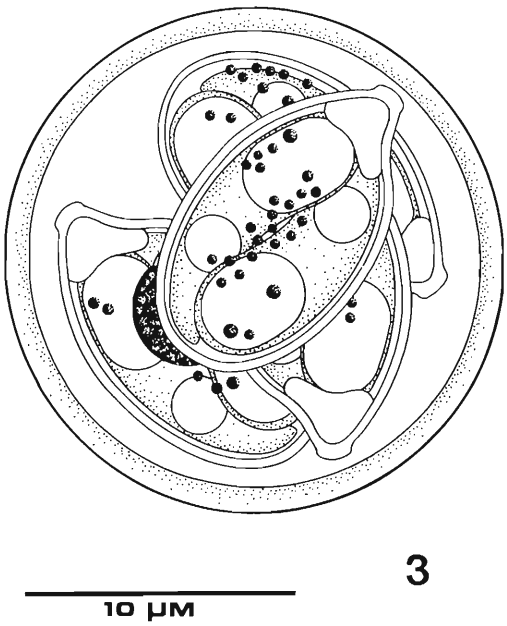


Figure 3. Composite line drawing of sporulated oocyst of *Eimeria halleri* sp. n.

Results

Seven of 21 (33%) *Urolophus halleri* were found to be passing a previously undescribed species of *Eimeria* in the feces. Only oocysts placed in seawater supplemented with antibiotics sporulated. Below is the description of the form that we saw.

Apicomplexa: Eimeriidae

Eimeria halleri sp. n. (Figs. 1-3)

DESCRIPTION: Oocysts spherical or subspherical, 16.9 × 16.8 (15.0–18.0 × 15.0–18.0); shape index (length/width) 1.0 (1.0–1.1). Wall smooth, composed of a single colorless layer <1.0 thick (confirmed by crushing oocysts between slide and coverslip). Micropyle, polar granule, and oocyst residuum absent. Sporocysts ovoid, 11.1 × 6.8 (10.0–13.0 × 6.0–7.5); shape index 1.7 (1.4–2.0). Wall smooth and thin, and appears to be composed of a single colorless layer. The pointed end of the sporocyst is often curved to one side (Figs. 1, 3) and has a thin, knoblike Stieda body; substieda body present, large and homogenous, ~2.5 wide × 2.0 high. Sporozoites comma-shaped, with the anterior end distinctly more pointed than the posterior end, 9.9 × 3.2 (9.0–11.0 × 2.8–4.0) in situ. Each sporozoite contains an ovoid posterior refractile body 4.0 long × 3.0 wide (3.0–5.0 × 2.0–3.5) and, usually, a spherical anterior refractile body, 2.1 (1.0–3.0). Sporocyst residuum present, consisting of numerous fine granules, ~0.2–0.5 in diameter, scattered among the sporozoites or (sometimes) as a compact sphere.

TYPE HOST: *Urolophus halleri* Cooper "round stingray" (Rajiformes: Dasyatidae).

TYPE LOCALITY: Puerto Peñasco, Mexico.

SITE OF INFECTION: Unknown. Oocysts found in feces and contents of spiral valve.

SPORULATION: Exogenous. All oocysts recovered from the feces and spiral valve were unsporulated but became fully sporulated after 5 days in seawater supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml Actidione at ~22°C.

PREVALENCE: 7/21 (33%) stringrays.

TYPE SPECIMENS: Syntypes (sporulated oocysts in 10% formalin) USNM Helm. Coll. No. 78490.

REMARKS: Only four species of coccidia have been described previously from stingrays: *Eimeria ottojiroveci* Dyková and Lom, 1983 from *Raja clavata*; *E. raiarum* van den Berghe, 1937 from *Raja batis*; and *E. quentini* Boulard, 1977, and *E. southwelli* Halawani, 1930 from *Aetobatis narinari* (see Halawani, 1930; van den Berghe, 1937; Boulard, 1977; Lom and Dyková, 1981; Dyková and Lom, 1983). *Eimeria halleri* differs from these species by the following characteristics: Oocysts and sporocysts of *E. ottojiroveci* are smaller, the Stieda and substieda bodies are structurally different, and the sporocyst residuum is compact and coarse, rather than fine grained and often dispersed; oocysts of *E. raiarum* are larger, the sporocysts smaller, and an oocyst residuum is present; oocysts of *E. quentini* and *E. southwelli* are larger and far more elongate and an oocyst residuum is sometimes present.

Acknowledgments

We wish to thank Mr. Kevin D. Roy and Mr. Russell R. Broaddus for aiding in the collection of many of the stingrays represented in this study.

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Obituary Notice

MYRON G. RADKE

September 26, 1928–July 9, 1985

Obituary Notice

LAWRENCE R. PENNER

March 29, 1913–June 28, 1985
Elected Member April 18, 1952

Proc. Helminthol. Soc. Wash.
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Parasites of the Bobcat (*Lynx rufus pallescens*) in Central and Southern Utah

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ABSTRACT: Carcasses of 28 bobcats, *Lynx rufus pallescens* Merriam, 1899 (10 adult and 2 juvenile males, 11 adult and 5 juvenile females) were surveyed for helminths. In addition, six live-trapped bobcats were examined for helminths and sporozoa. Thirty-three (97.1%) of the 34 bobcats harbored one or more species of parasite. No parasites were found in one of the bobcat carcasses. Parasite species identified and their prevalence from 28 bobcat carcasses included *Taenia macrocystis* (Diesing, 1850) Lühe, 1910 (75.0%), *T. rileyi* Loewen, 1929 (35.7%), *Taenia* spp. (17.9%), *Mesocestoides* spp. (7.1%), and *Toxocara cati* (Schrank, 1788) Brumpt, 1927 (42.9%). No trichinae were found in diaphragm samples from these animals. Examination of fecal samples from six live-trapped bobcats showed the presence of *Isospora felis* (Wasielewski, 1904) Wenyon, 1923 (16.7%), *Sarcocystis* spp. (33.3%), *Taenia* spp. (16.7%), *Toxocara cati* (50.0%), and eggs of an unidentified trematode (16.7%). One of six (16.7%) serum samples was positive for antibodies to *Toxoplasma gondii* Nicolle and Manceaux, 1908 as determined by the indirect hemagglutination test. Identified intestinal contents and their occurrence in the bobcat carcasses showed diet selections of rabbits (32.1%), mule deer (25.0%), rodents (17.9%), and birds (7.1%). In addition, vegetation (39.3%) as well as soil, rocks, and unidentified materials (100%) were found in the intestinal tracts of the 28 bobcat carcasses.

During a study of home ranges and movements of bobcats (*Lynx rufus pallescens* Merriam, 1899) in central and southern Utah (Karpowitz, 1981), the opportunity arose to conduct a limited survey for parasites of this carnivore. This report details data obtained on the endoparasites of 34 bobcats.

Materials and Methods

Twenty-eight bobcat carcasses were collected from trappers in four central Utah counties (Juab, Millard, Sanpete, and Utah) and one southern county (Iron) during November and December 1979. In addition, six live bobcats were trapped in Diamond Fork Canyon, Utah County.

Each carcass was grossly examined to determine the sex, and the lower canines from each animal were removed for age determination. Canines from bobcats less than one year of age retain an open apical foramen. Canine teeth with a closed foramen were sectioned, stained, and examined for cementum annuli (Crowe, 1972). All bobcats older than one year of age were considered to be adult because bobcats are sexually mature by the second winter of life (Fritts and Sealander, 1978).

The small and large intestines were removed from the carcasses, most of which had been frozen in the field. A sample of the contents of the large intestine was removed to determine diet selection. These samples were air-dried at 22°C for at least 30 days, crumbled, and examined under a dissecting microscope. Identification of intestinal contents was accomplished by examination of hair, bones, feathers, and vegetation aided by reference collections and identification keys (Moore et al., 1974).

The small and large intestines of each animal were fixed in 10% formalin and later examined in the laboratory for helminths. Cestodes were fixed in formol-alcohol (70%) for 24 hr and placed in 70% ethyl alcohol (EtOH) until they could be stained in Semichon's acetic carmine. After staining, each specimen was dehydrated to 100% EtOH, cleared in methyl salicylate or xylene, and mounted in Permount®. In order to obtain flat hooks for measurements, each rostellum of all *Taenia* specimens was mounted separately en face.

Nematodes recovered from the intestines were fixed in 70% EtOH, stored in glycerine-alcohol, and mounted in lactophenol. A sample of the diaphragm from each of the bobcat carcasses was examined microscopically for the presence of *Trichinella* larvae.

Fecal and serum samples from the six live-trapped bobcats were also examined. Each fecal sample was placed in 3% potassium dichromate and later examined for helminth eggs and coccidia using a standard sugar flotation method. Serum samples were tested for antibodies to *Toxoplasma gondii* by the indirect hemagglutination method.

Results and Discussion

Twelve (10 adult, 2 juvenile) male and 16 (11 adult, 5 juvenile) female bobcat carcasses were examined for intestinal helminths (Table 1). *Taenia macrocystis* (Diesing, 1850) Lühe, 1910 was the most common cestode occurring in 21

Present addresses:
[1] SDS Biotech Corporation, P.O. Box 348, Painesville, Ohio 44077.
[2] Utah State Division of Wildlife Resources, 455 West Railroad Avenue, Price, Utah 84501.
[3] The Upjohn Company, 7923-190-41, Kalamazoo, Michigan 49001.

Table 1. Prevalence of helminths in bobcat (*Lynx rufus pallescens* Merriam, 1899) carcasses from central and southern Utah.

Parasite	Number of bobcats infected (%)				
	Male		Female		
	Adult ($N = 10$)	Juvenile ($N = 2$)	Adult ($N = 11$)	Juvenile ($N = 5$)	Total ($N = 28$)
Cestodes					
Mesocestoides sp.	1 (10.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (7.1)
Taenia macrocystis (Diesing, 1850) Lühe, 1910	9 (90.0)	2 (100.0)	7 (63.6)	3 (60.0)	21 (75.0)
Taenia rileyi Loewen, 1929	4 (40.0)	1 (50.0)	4 (36.4)	1 (20.0)	10 (35.7)
Taenia spp.*	3 (30.0)	0 (0.0)	2 (18.2)	0 (0.0)	5 (17.9)
Nematodes					
Toxocara cati (Schrank, 1788) Brumpt, 1927	5 (50.0)	2 (100.0)	3 (27.3)	2 (40.0)	12 (42.9)

* Specimens could not be identified due to deterioration and loss of rostellar hooks.

(75.0%) of the 28 bobcats with numbers ranging from 1 to 46 ($\bar{x} = 8$). Adult and juvenile male bobcats exhibited a 90.0 and 100% prevalence of *T. macrocystis,* respectively, compared to 63.6 and 60.0% in adult and juvenile female bobcats, respectively.

Adult cestodes of *T. macrocystis* have been reported from *Lynx rufus pallescens* Merriam, 1899 in Utah and the metacestodes have been found by Grundmann (1958) in black-tailed jackrabbits, *Lepus californicus deserticola* Mearns, 1896. Identification of the intestinal contents from the bobcat carcasses is given in Table 2. Nine (32.1%) of the bobcats contained rabbit remains in their intestinal tracts. Such a selection and diet preference would favor transmission of the metacestode of *T. macrocystis* to the definitive host.

Taenia rileyi Loewen, 1929 was present in 10 (35.7%) of the 28 bobcat carcasses with numbers ranging from 1 to 23 ($\bar{x} = 7$): 40.0 and 50.0% in adult and juvenile males, respectively; 36.4 and 20.0% in adult and juvenile female bobcats, respectively (Table 1). Although the morphology of the rostellar hooks resembles *T. rileyi,* measurements of the small hooks were less than previous reports of *T. rileyi* (Riser, 1956; Verster, 1969; Rausch, 1981). Measurements of the large hooks ranged in length from 210 to 255 μm ($\bar{x} = 225$), whereas the small hooks were 122–173 μm ($\bar{x} = 147$). These measurements are in agreement with those obtained by Kenneth L. Tiekotter

Table 2. Identification of intestinal contents and occurrence in bobcat (*Lynx rufus pallescens* Merriam, 1899) carcasses.

Contents	Number of bobcats positive (%)				
	Male		Female		
	Adult ($N = 10$)	Juvenile ($N = 2$)	Adult ($N = 11$)	Juvenile ($N = 5$)	Total ($N = 28$)
Birds	0 (0.0)	0 (0.0)	1 (9.1)	1 (20.0)	2 (7.1)
Mule deer	3 (30.0)	0 (0.0)	2 (18.2)	2 (40.0)	7 (25.0)
Rabbits	4 (40.0)	1 (50.0)	4 (36.4)	0 (0.0)	9 (32.1)
Rodents	2 (20.0)	0 (0.0)	1 (9.1)	2 (40.0)	5 (17.9)
Vegetation	4 (40.0)	1 (50.0)	5 (45.5)	1 (20.0)	11 (39.3)
Other*	10 (100.0)	2 (100.0)	11 (100.0)	5 (100.0)	28 (100.0)

* Soil, rocks and unidentified materials.

(pers. comm.) from our voucher specimen USNM Helm. Coll. No. 76921. Adult cestodes of *T. rileyi* have been collected from *Lynx rufus baileyi* Merriam, 1890 in Skull Valley, Utah (Riser, 1956). Grundmann (1958) reported the antelope ground squirrel, *Citellus leucurus leucurus* (Merriam) (=*Ammospermophilus leucurus leucurus* Barnes, 1927) and the desert wood rat, *Neotoma lepida lepida* Thomas, 1893, as intermediate hosts for the metacestode of *T. rileyi* in Utah. In addition, metacestodes of *T. rileyi* have been reported from the black-tailed jackrabbit in Utah (Butler and Grundmann, 1954). Raw materials from the intestinal contents of five (17.9%) and nine (32.1%) of the bobcat carcasses contained rodent and rabbit remains, respectively (Table 2). Five (17.9%) of the 28 bobcat carcasses harbored *Taenia* species that could not be identified due to deterioration and loss of rostellar hooks.

One specimen of *Mesocestoides* sp. was found in each of the intestinal tracts from the carcasses of one adult (10.0%) and one juvenile (50.0%) male bobcat from Millard County (Table 1). Although a characteristic midventral genital pore and bilobed vitellarium were visible in the mature proglottids of the specimens, the internal structural details were highly distorted, probably due to freezing of the specimens. Therefore, the specimens of *Mesocestoides* could not be identified to species. Grundmann (1956) described the adults of *M. carnivoricolus* Grundmann, 1956 from the small intestines of *Lynx rufus pallescens* Merriam, 1899 and reported the tetrathyridia in the lungs, liver, and body cavity of the canyon mouse, *Peromyscus crinitus pergracilis* Goldman, 1939, and the deer mouse, *P. maniculatus sonoriensis* (LeConte) Osgood, 1909, in Utah. *Mesocestoides kirbyi* Chandler, 1944 has been reported in coyotes, *Canis latrans* Say, 1823, from Utah (Butler and Grundmanan, 1954; Conder and Loveless, 1978).

Toxocara cati (Schrank, 1788) Brumpt, 1927 was recovered from the intestines of 12 (42.9%) of the bobcat carcasses (50.0 and 100% in adult and juvenile males, respectively; 27.3 and 40.0% in adult and juvenile female bobcats, respectively) with numbers ranging from 1 to 11 ($\bar{x} = 3$) (Table 1). Eggs of *T. cati* were found in the feces of three (50.0%) live-trapped bobcats (Table 3). No trichinae were found in the diaphragm samples.

Representative specimens of *Taenia macrocystis, T. rileyi, Mesocestoides* sp., and *Toxocara cati* have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705 (USNM Helm. Coll. Nos. 76919, 76921, 76920, and 76922, respectively).

Table 3. Prevalence of parasites in live-trapped bobcats (*Lynx rufus pallescens* Merriam, 1899) from Diamond Fork Canyon, Utah County.

Parasite	Number of bobcats infected (%)
Protozoa	
Isospora felis (Wasielewski, 1904) Wenyon, 1923*	1/6 (16.7)
Sarcocystis spp.*	2/6 (33.3)
Toxoplasma gondii Nicolle and Manceaux, 1908†	1/6 (16.7)
Cestode	
Taenia spp.*	1/6 (16.7)
Nematode	
Toxocara cati (Schrank, 1788) Brumpt, 1927*	3/6 (50.0)
Trematode	
Unidentified sp.*	1/6 (16.7)

* Fecal sample.
† Serum sample, indirect hemagglutination test.

Trematode eggs in a fecal sample of one (16.7%) live-trapped bobcat could not be identified microscopically with certainty to species (Table 3). The mean egg dimensions were 63 × 113 μm, which differentiates them from either *Diphyllobothrium* or *Spirometra*. The trematode species of *Heterobilharzia americana* Price, 1927, *Paragonimus kellicotti* Ward, 1908, *P. rudis* (Diesing, 1850) Stiles and Hassel, 1900, and *Alaria marcianae* (LaRue, 1917) Walton, 1949, have been reported from bobcats by Price (1929); Jordan and Byrd (1958), McKeever (1958), Watson et al. (1981); Miller and Harkema (1968); and Stone and Pence (1978), respectively. The eggs we observed most closely resemble *Paragonimus* in size.

Two coccidian species were identified from the fecal samples of three live-trapped bobcats, and an additional species was identified serologically (Table 3). The importance of the bobcat as a definitive host in the sylvatic cycle of *Toxoplasma gondii* Nicolle and Manceaux, 1908 has been reported previously (Riemann et al., 1975; Marchiondo et al., 1976; Oertley and Walls, 1980). Animals with persisting cysts are the main source of *T. gondii* and *Sarcocystis* spp. infection for

carnivores (Marchiondo et al., 1976; Watson et al., 1981). In addition, Frenkel and Dubey (1972) reported that rodents can serve as transport hosts for *Isospora felis* (Wasielewski, 1904) Wenyon, 1923.

Acknowledgments

We thank Ms. Sue deGraff for conducting the serological tests reported herein. We also thank Dr. Robert L. Rausch, University of Washington, Seattle, Washington; Dr. Gerald D. Schmidt, University of Northern Colorado, Greeley, Colorado; and Dr. Kenneth L. Tiekotter, Good Samaritan Hospital and Medical Center, Portland, Oregon for their helpful suggestions regarding the identification of the specimens of *Taenia rileyi.*

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Scanning Electron Microscope Studies on *Hammerschmidtiella diesingi* (Nematoda: Oxyuroidea)

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ABSTRACT: Both males and females of *H. diesingi* were studied with the scanning electron microscope. The en face views of the female worm showed two amphids but no external papillae. Eight lips and eight interlabia were arranged in a specific radially symmetrical pattern. Males had two amphids but lacked other labial structures. The large caudal papillae of the male worm showed a pattern of 10 small papules circling one medial papule on the top of each large papilla. The female worms had two phasmids, but no phasmids were found in male worms. The excretory pore of the female worm showed a different surface topography from that of the male worm.

Hammerschmidtiella diesingi (Hammerschmidt, 1838) is a thelastomatid nematode parasitic in the hind gut of cockroaches. Hammerschmidt (1838) first described this species os *Oxyuris diesingi* and Chitwood (1932) revised the description and put the species into a new genus, *Hammerschmidtiella.* The morphology of adult and fourth-stage larva were further described by Lee (1958). In 1981, Trett and Lee described the cephalic sense organs of the adult female *H. diesingi.* They included a scanning electron micrograph of the en face view. No other anatomical structures of males or females of this species have been scanned. In the present paper, male and female *H. diesingi* were studied with the SEM in order to reveal details of surface topography.

Materials and Methods

Both male and female worms of *H. diesingi* were collected from the hind gut of naturally infected American cockroaches, *Periplaneta americana.* The nematodes were briefly washed in "cold" Ringer's solution three or four times. The worms were then washed in acetic acid for 30 sec and fixed in 2% buffered osmium tetroxide (OsO_4) at 4°C overnight. The fixed worms were washed in 0.001 M Millonig's phosphate buffer and dehydrated in an ascending series of ethyl alcohol dilutions (20–100%). The dehydrated specimens were critical-point dried from absolute ethyl alcohol with CO_2 in an Autosamdri-810 Critical Point Dryer. The dried specimens were mounted on double sticky tape on 13-mm-diameter aluminum stubs, coated with gold using a Technics Sputtering System, and examined with a Hitachi S-500 SEM at an accelerating voltage of 20 kV. Twenty female and five male worms were examined.

Results

Figures 1, 2 and 7, 8 show the en face and lateral views of the female and male of *H. diesingi.* Both male and female specimens have two salient amphids but no external papillae around the mouth. The mouth of the female worm is surrounded by eight similar lips that are symmetrically arranged on the first cephalic ring. The interlabia are not equal in size; large ones alternate regularly with small ones (Figs. 1, 2). Amphids are located on two large lateral interlabia. The male worm is without lips. There is a second cephalic ring, which is narrow and has a crinkled appearance between the first cephalic ring and the first somatic annule in both male and female worms (Figs. 1, 2, 8).

There is an irregularly folded flangelike ring around the female excretory pore (Fig. 3). The

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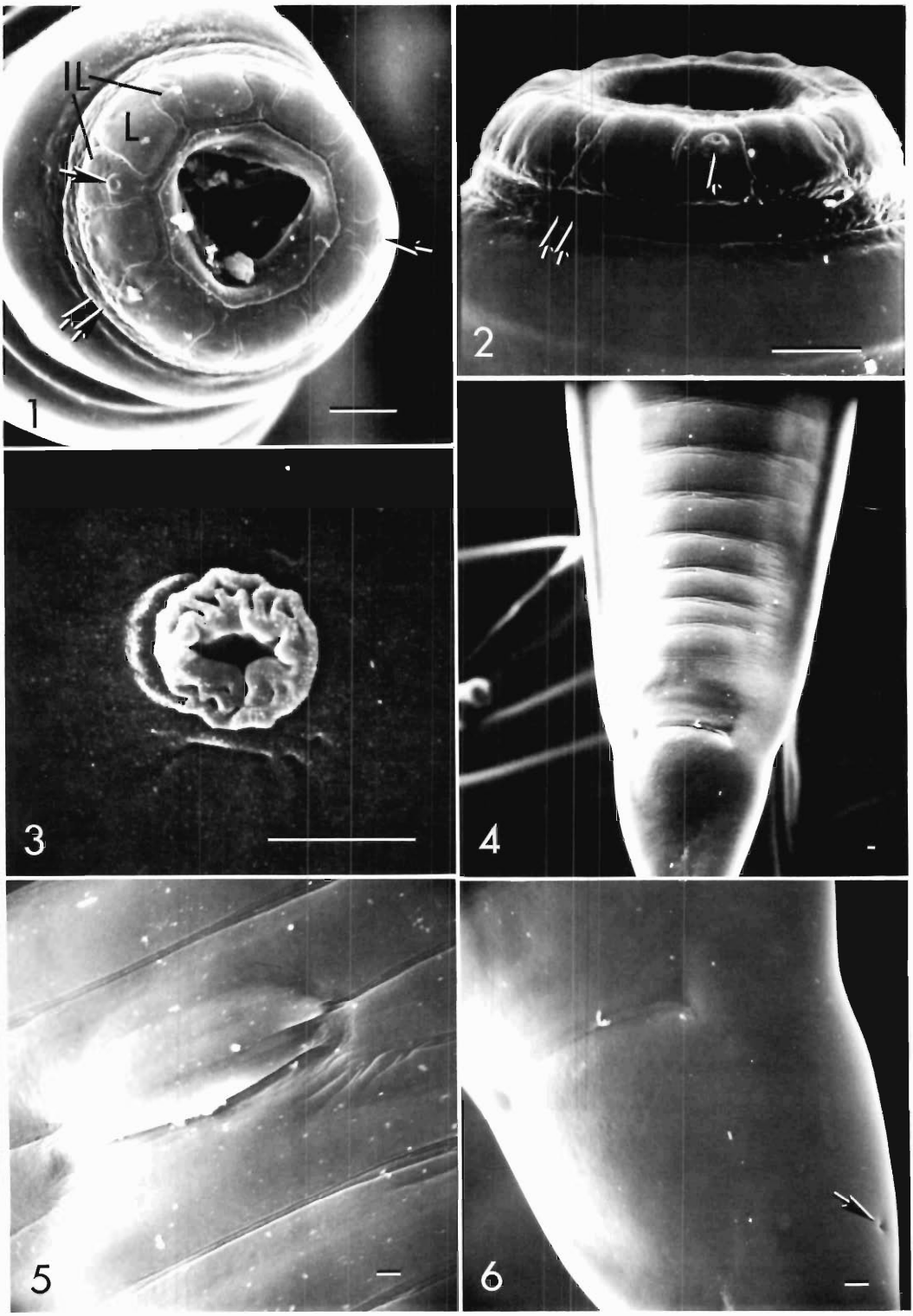
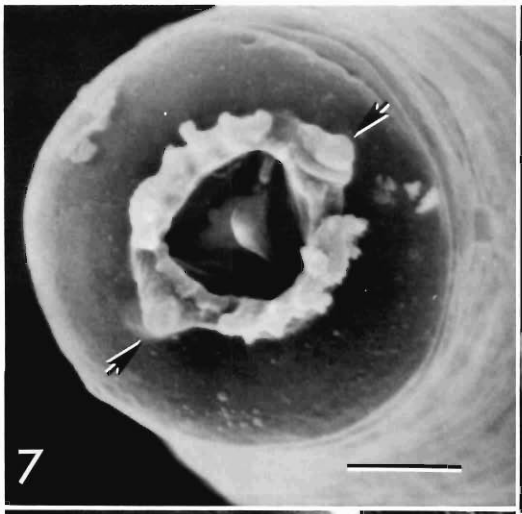
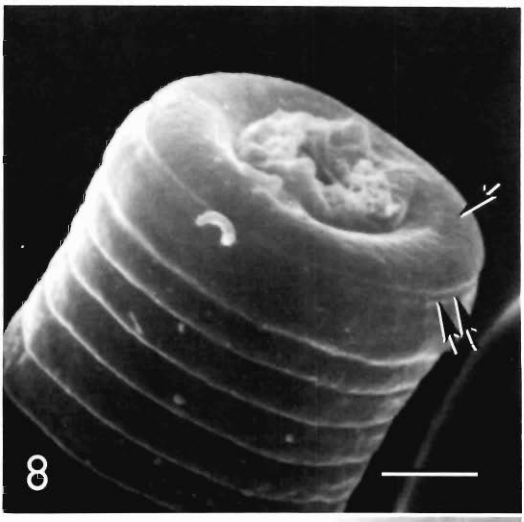
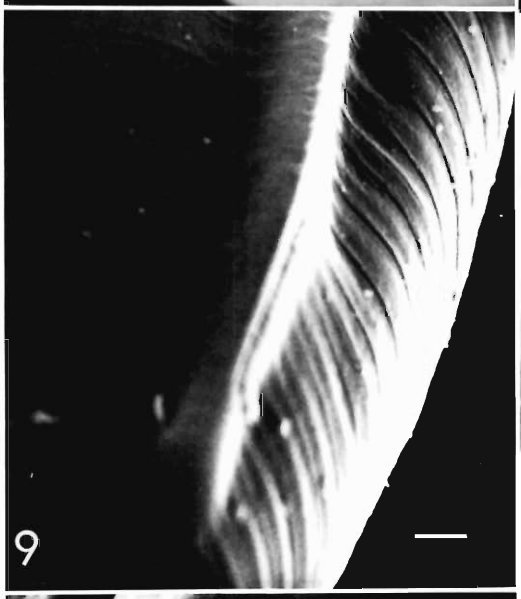
Figures 1–6. ***Hammerschmidtiella diesingi*** **females, scanning electron micrographs. All scale bars 3 μm. 1. En face view. Single arrows indicate amphids. Double arrows indicate second cephalic ring. IL = interlabium, L = lip. 2. Lateral view of anterior end. Arrow indicates amphid. Double arrows indicate second cephalic ring. 3. Excretory pore. 4. Ventral view, posterior end. 5. Vulva. 6. Anus and phasmid. Arrow indicates phasmid.**

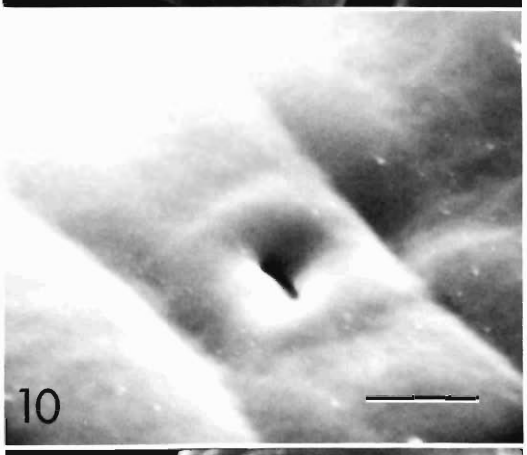
Figure 7–12. ***Hammerschmidtiella diesingi*** **males, scanning electron micrographs. All scale bars 2 μm. 7. En face view. Arrows indicate amphids. 8. Lateral view of anterior end. Single arrow indicates first cephalic ring, double arrows indicate second cephalic ring. 9. Lateral ala towards posterior end of the body. 10. Excretory pore. 11. Large caudal papilla. 12. Posterior extremity of body. Single arrows indicate large caudal papillae, double arrows indicate small adanal papilla.**

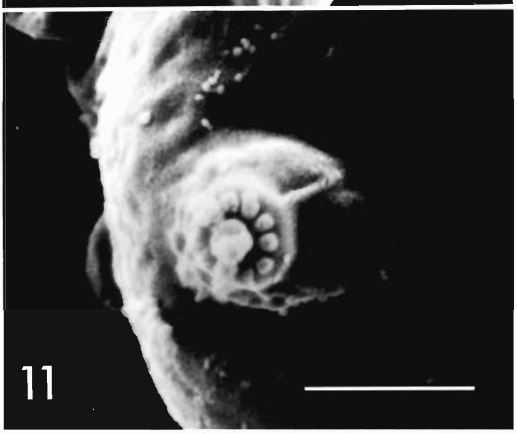


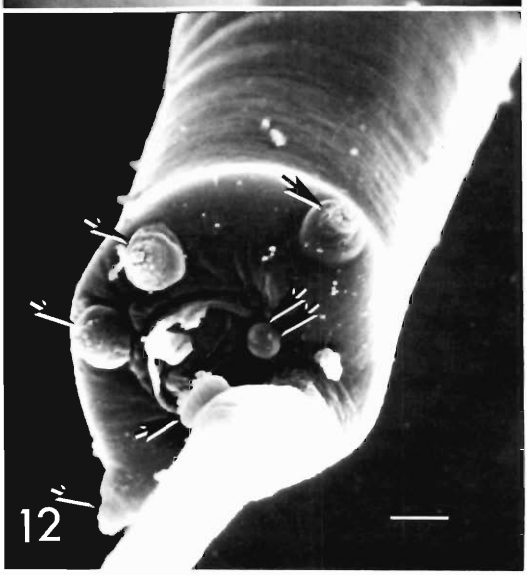












excretory pore of the male worm is much simpler (Fig. 10). There is a low cuticular ring around the opening.

Two phasmids open posterolateral to the anus on the female tail (Fig. 6). Males have one pair of large preanal papillae, one pair of large adanal papillae, and one pair of small adanal papillae around the cloacal opening, as well as one single ventral papilla at the base of the tail, but no phasmids were found (Fig. 12). All of the five large papillae have a similar structure, i.e., on the top of each large papilla, there are 10 small papules organized into a circle surrounding a central papule (Fig. 11). The pair of small adanal papillae lack any particular surface features (Fig. 12).

The vulva and anus are simple transverse slits on the ventral surface, but the anus is larger than the vulva (Figs. 5, 6). The ventral part of the body immediately posterior to the anus is slightly swollen (Figs. 4, 6). The lateral alae of the female worm terminate close to the anus. The male worm also possesses narrow lateral alae that terminate anterior to the anus (Fig. 9).

Discussion

Our SEM micrographs of en face views of adult female *H. diesingi* clearly show only amphids on the first cephalic ring. These findings coincide with those of Trett and Lee (1981). However, the amphids of our female specimens are more lateral than those of Trett and Lee (1981) and the amphids of our specimens are clearly surrounded by a cuticular ring (Fig. 1).

This paper describes two structures not previously described, the "first cephalic ring," which is the anteriormost ring around the mouth and on which the lips are located, and the "second cephalic ring," which is located between the first cephalic ring and the first somatic annule. We believe that it is necessary to distinguish these two rings from the somatic annules because they are morphologically different.

Phasmids are important characteristics of the class Secernentea but we were able to see them only in the female. It may be that the phasmids of the male are internal without external openings as is the case with the internal cephalic papillae of this species (Trett and Lee, 1981).

The difference in appearance of the male and female excretory pores is striking. Because this difference can only be seen with SEM and very little has been done with SEM in other closely related species one cannot say whether it is unique to this species or is common among other species. It has not been noted in light microscopic studies.

The large caudal papillae of the male, which were described as setalike papillae with light microscopy by Chitwood (1932), have a quite unique structure. Ten small papules circling a medial papule on a single large papilla is different from that of *Cosmocercoides* (Ascaridida), which has rosette papillae circling a single papilla on the cuticle rather than on a large papilla (Wilkie, 1930). We speculate that, in this case, the small papules might have different internal structure and even have different function from the medial papule. It would be interesting to see whether they are special papillae by using transmission electron microscopy. The results of our SEM studies have demonstrated that the sexual dimorphism in *H. diesingi* is not limited to size and shape but also includes details of the surface topography.

Acknowledgment

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Recognition of *Neoaplectana* Species (Steinernematidae: Rhabditida)

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ABSTRACT: Morphological characters are presented to separate the males and infective stage juveniles of the four commercially available species of *Neoaplectana* (Steinernematidae: Rhabditidae), namely *N. glaseri, N. bibionis, N. carpocapsae,* and *N. intermedia.* Diagnostic characters in males include the presence and length of the cuticular tail spine and the curvature, nature of the capitulum (manubrium), and tips of the spicules. The infective juveniles can be distinguished by total body length, distance from head to excretory pore, and the ratio distance from head to excretory pore divided by length of tail. A list of the various geographical strains of the above four species is presented.

There are now four well-defined species of *Neoaplectana* that are commercially available and being investigated for use as biological control agents. These are *Neoaplectana glaseri* Steiner, 1929 (redescribed by Poinar, 1978), *N. bibionis* Bovien, 1937 (redescribed by Wouts, 1980), *N. carpocapsae* Weiser, 1955 (redescribed by Poinar, 1967), and *N. intermedia* Poinar, 1985b. The author prefers to utilize the generic name *Neoaplectana* in place of *Steinernema* and *carpocapsae* in place of *feltiae* for reasons presented elsewhere (Poinar, 1984).

The above four species are well established and species determination is based on reproductive isolation, morphological features (Poinar, 1979, 1985b), and DNA analysis (Curran et al., 1985). The previously described species of *Neoaplectana* that are presently not represented by living material will not be considered here because they all require redescriptions.

The need for identification of the above four species has become apparent in commercial operations where more than one species is being produced and accidental mixing can occur. Also in field situations there is often a need to verify the identity of nematodes used. The two stages in the development of *Neoaplectana* that contain diagnostic characters are males and infective juveniles. The present paper describes characters that can be used to separate these stages of the four commercially available species of *Neoaplectana.*

Materials and Methods

The 42 strain of *N. carpocapsae,* the SN strain of *N. bibionis,* the FL strain of *N. glaseri,* and the SC strain of *N. intermedia* were used in this study (Table 1).

The nematodes were grown in larvae of the wax moth (*Galleria mellonella*) maintained at room temperature (~20°C). Males were obtained by dissecting infected insects 5 days after infection. Infective stage juveniles were collected as they emerged from the insect cadavers 10–14 days after infection. All worms were heat killed (50°C), fixed in TAF for 72 hr, and then processed to glycerin. Measurements and photographs were made with a Nikon Orthophot microscope equipped with differential interference contrast optics. For measurements of the infective stage juveniles, 25 individuals of each species were chosen at random from a plate containing several thousand specimens. The ratios used were the standard A (total length divided by width), B (total length divided by distance from head to base of pharynx), and C (total length divided by length of tail). In addition, two other ratios were included; D (distance from head to excretory pore divided by distance from head to base of pharynx) and E (distance from head to excretory pore divided by length of tail).

Results

Three of the four *Neoaplectana* species discussed here are composed of a number of strains (Table 1). These strains interbreed and thus occupy an intraspecific rank. Although it is often impossible to distinguish between strains of the same species on the basis of morphology, it is important to keep them separate because they may differ in host preference, physiology, and behavior. Strains represent populations from a particular host and/or locality. Although strains have been called after the collector (e.g., ALL strain) and the accession number (DD-136), it is preferable to use a name representing the locality or host insect.

The variation found among the adults of a single population from a single insect host prohibits the use of most quantitative characters in separating *Neoaplectana* species. Certain qualitative characters clearly separate the males of the four *Neoaplectana* species being considered. They include the presence and size of the cuticular spine on the end of the male tail and the shape

Table 1. Strains of *Neoaplectana* species.

Species	Strain	Original source	Geographical area	Reference
N. carpocapsae	Czechoslovakian	*Laspeyresia pomonella* (L.)	Czechoslovakia	Weiser, 1955
	DD-136	*L. pomonella* (L.)	Virginia, U.S.A.	Dutky and Hough, 1955
	Mexican	*L. pomonella* (L.)	Allende, Chihuahua, Mexico	Collected by L. Caltagirone
	Sierra	*L. pomonella* (L.)	California, U.S.A.	Collected by A. Berlowitz
	Agriotos (Leningrad)	*Agriotes lineatus* L.	Leningrad, U.S.S.R.	Poinar and Veremtchuk, 1970
	All	*Vitacea polistiformis* (Har.)	Georgia, U.S.A.	Collected by All (in Poinar, 1979)
	XI	*L. pomonella* (L.)	Poland	Stanuszek, 1974
	X-III	*Agrotis segetum* (Schiff)	Poland	Stanuszek, 1974
	X-IV (Pieridarum)	*Pieris brassicae* (L.)	Poland	Stanuszek, 1974 and Akhurst 1983b
	Breton	*Otiorhynchus sulcatus* Fab.	France	Collected by C. Laumond
	Umea	Soil	Sweden	Collected by A. Pye
	42	Cross between Breton and DD-136		Poinar
	Italian	Soil	Italy	Collected by A. Kovec
	Hopland	Soil	California, U.S.A.	Collected by R. S. Lane
	Quebec	*Listronotus oregonensis* (LeConte)	Quebec, Canada	Belair et al., 1984
	N.C.	Soil	North Carolina, U.S.A.	Akhurst and Brooks, 1984
	Nelson	*Vespula* sp.	Tasmania, Australia	Akhurst, 1980
	Powranna	Soil	Tasmania, Australia	Akhurst, 1983a
	Murrumbateman	Soil	New South Wales, Australia	Akhurst, 1983a
	P7	Soil	Tasmania, Australia	Akhurst, 1980
	N55	Soil	Tasmania, Australia	Akhurst, 1980
	Argentinian	*Graphognathus leucoloma* Boh.	Argentina	Ahmad, 1974
	Rhagolites	*Rhagolites pomonella* (L.)	Massachusetts, U.S.A.	Poinar et al., 1977
N. glaseri	N.J.	*Popillia japonica* (L.)	New Jersey, U.S.A.	Glaser, 1932
	N.C.	*Strigoderma arboricola* (Fab.)	North Carolina, U.S.A.	Poinar and Brooks, 1977
	FL.	Soil	Florida, U.S.A.	Collected by O. Sosa
N. bibionis	KL	*Bibio* spp.	Denmark	Bovien, 1937; Poinar and Lindhardt, 1971
	SN	Soil	France	Collected by C. Scotto la Massese
	NZ	*Graphognathus leucoloma* Boh.	New Zealand	Wouts, 1980
	N60	Soil	Canberra, Australia	Molyneux et al., 1983
	T335	*Otiorhynchus sulcatus* Fab.	Tasmania, Australia	Molyneux et al., 1983
	Dutch	Soil	Holland	Galle, 1983
	Murrumbateman	Soil	New South Wales, Australia	Akhurst, 1983a
	Dover	Soil	Tasmania, Australia	Akhurst, 1983a
	T231 (Risdon)	Soil	Tasmania, Australia	Akhurst, 1983b
	Nive strain	Soil	Tasmania, Australia	Akhurst, 1983a
	T298 (Plenty)	Soil	Tasmania, Australia	Akhurst, 1983b
	Bruny strain	Soil	Tasmania, Australia	Akhurst, 1983a
	T319 (Wellington)	Soil	Tasmania, Australia	Akhurst, 1983b
	VI	Soil	Victoria, Australia	Akhurst, 1980
N. intermedia	S.C.	Soil	South Carolina, U.S.A.	Poinar, 1985b

Table 2. Measurements of the infective stages of *N. carpocapsae*.

Character	42 strain (N = 25) (present study)	Czechoslovakian strain (N = 25) (Poinar, 1967)	Polish strain (Stanuszek, 1974)	DD-136 strain (N = 25) (Poinar, 1967)
Total length	544 (502–608)	572 (488–613)	572 (498–650)	547 (438–625)
Greatest width	25 (22–29)	26 (25–28)	23 (20–30)	24 (22–28)
Distance: head to excretory pore	33 (30–37)	42 (39–56)	42 (38–51)	36 (34–40)
Distance: head to nerve ring	80 (76–91)	88 (84–93)	87 (80–99)	85 (81–90)
Distance: head to pharynx base	126 (118–133)	—	115 (103–190)	—
Length tail	55 (51–59)	53 (47–59)	50 (46–61)	53 (50–59)
Anal diameter	13 (11–14)	—	14 (10–16)	—
Ratio A	21 (19–24)	—	—	—
Ratio B	4.4 (4.0–4.8)	—	—	—
Ratio C	10.0 (9.1–11.2)	—	—	—
Ratio D	0.26 (0.23–0.28)	—	—	—
Ratio E	0.60 (0.54–0.66)	—	—	—

and character of the spicules. Characters used to separate males of *Neoaplectana* are presented in the following key and shown in Figures 9–12.

Infective stage juveniles of *Neoaplectana* are less variable in size and shape and quantitative measurements can be used for specific diagnosis. Measurements of the infective stages of one strain of the four studied *Neoaplectana* species, together with previous measurements from the literature, are presented in Tables 2–4. The four species can be recognized by the length of the infective stage juveniles if at least 10 individuals are measured and the average value used. Another important measurement is the distance from the head to the excretory pore. This value is relatively short in *N. carpocapsae* in comparison with the other species and relatively long in *N. glaseri*. Other figures, such as the distance from the head to nerve ring, head to pharynx base, anal diameter, length of tail and greatest width can be used in some instances. The ratios A, B, and C were too variable to be of assistance. However, ratio E showed no overlapping in the strains of four species studied here and is considered of value in separating these and possibly other *Neoaplectana* species. On the basis of characters evaluated in the present study, the following keys to infective stage juveniles and males are presented. Available values reported in the literature for these four species are also incorporated into the present data in the keys.

Key to Infective Stage Juveniles

1. Average length of infective stages (N = 10) greater than 725 μm (Figs. 1, 2) 2
   Average length of infective stages (N = 10) less than 725 μm (Figs. 3, 4) 3
2. Length generally around 1,200 μm but can vary from 860 to 1,500; distance from head to the excretory pore 85–110 μm; distance from head to base of pharynx 158–168 μm; E ratio from 1.22 to 1.38 ..... *N. glaseri* (Figs. 1, 5)
   Length generally around 800 μm but can vary from 700 to 1,000; distance from head to excretory pore, 53–67 μm; distance from head to base of pharynx 115–150 μm; E ratio from 0.69 to 0.86 ..... *N. bibionis* (Figs. 2, 6)
3. Length generally around 550 μm but can vary from 438 to 650; distance from head to excretory pore 30–56 μm; E

Table 3. Measurements of the infective stages of *N. bibionis*.

Character	SN strain (N = 25) (present study)	NZ strain (N = 20) (Wouts, 1980)
Total length*	817 (736–896)	880 (750–950)
Greatest width	26 (25–29)	25 (22–27)
Distance: head to excretory pore	61 (56–66)	62 (53–67)
Distance: head to nerve ring	98 (88–112)	100 (89–108)
Distance: head to pharynx base	136 (128–147)	135 (115–150)
Length tail	79 (70–88)	83 (71–92)
Anal diameter	17 (16–19)	16 (15–17)
Ratio A	31 (29–33)	—
Ratio B	6.0 (5.3–6.4)	—
Ratio C	10.4 (9.2–12.6)	—
Ratio D	0.45 (0.42–0.51)	—
Ratio E	0.78 (0.69–0.86)	—

* Bovien (1937) gave a value of 700–1,000 for the KL strain of *N. bibionis*.

Table 4. Measurements of the infective stages of *N. glaseri* and *N. intermedia*.

Character	*N. glaseri*		*N. intermedia*	
	FL. strain ($N = 25$) (present study)	N.C. strain ($N = 10$) (Poinar, 1978)	S.C. strain ($N = 25$) (present study)	S.C. strain ($N = 25$) (Poinar, 1985b)
Total length	1,200 (1,000–1,300)	1,060 (864–1,448)	661 (608–704)	680 (608–800)
Greatest width	40 (31–45)	45 (35–50)	29 (25–32)	28 (25–32)
Distance: head to excretory pore	104 (97–110)	100 (87–108)	64 (59–68)	65 (61–69)
Distance: head to nerve ring	120 (112–126)	—	94 (88–99)	92 (85–96)
Distance: head to pharynx base	162 (158–168)	—	125 (112–133)	121 (110–131)
Length tail	80 (72–86)	76 (62–87)	67 (60–74)	64 (53–72)
Anal diameter	23 (19–24)	—	15 (13–18)	16 (13–18)
Ratio A	29 (26–35)	—	23 (20–26)	—
Ratio B	7.3 (6.3–7.8)	—	5.3 (5.0–6.0)	—
Ratio C	14.7 (13.6–15.7)	—	10.0 (9.3–10.8)	—
Ratio D	0.65 (0.58–0.71)	—	0.51 (0.48–0.58)	—
Ratio E	1.31 (1.22–1.38)	—	0.96 (0.89–1.08)	—

ratio, 0.54–0.66 ..... *N. carpocapsae* (Figs. 4, 8)

Length generally around 650 μm but can vary from 608 to 800 μm; distance from head to excretory pore 59–69 μm; E ratio, 0.89–1.08 ..... *N. intermedia* (Figs. 3, 7)

Key to Males

1. Tip of tail lacking a cuticular spine (Figs. 9, 11) 2
   Tip of tail bearing a cuticular spine (Figs. 10, 12) 3
2. Tip of spicules with a ventral notch, hook or scar; spicules moderately curved (angle between calomus and lamina between 50° and 70°) ..... *N. glaseri* (Fig. 9)
   Tip of spicules bluntly rounded; spicules strongly curved (angle between calomus and lamina between 70° and 90°) ..... *N. intermedia* (Fig. 11)
3. Tail spine 1–4 μm long; spicules grey-yellow; capitulum distinct ..... *N. carpocapsae* (Fig. 12)
   Tail spine 4–13 μm long; spicules yellow-orange; capitulum indistinct ..... *N. bibionis* (Fig. 10)

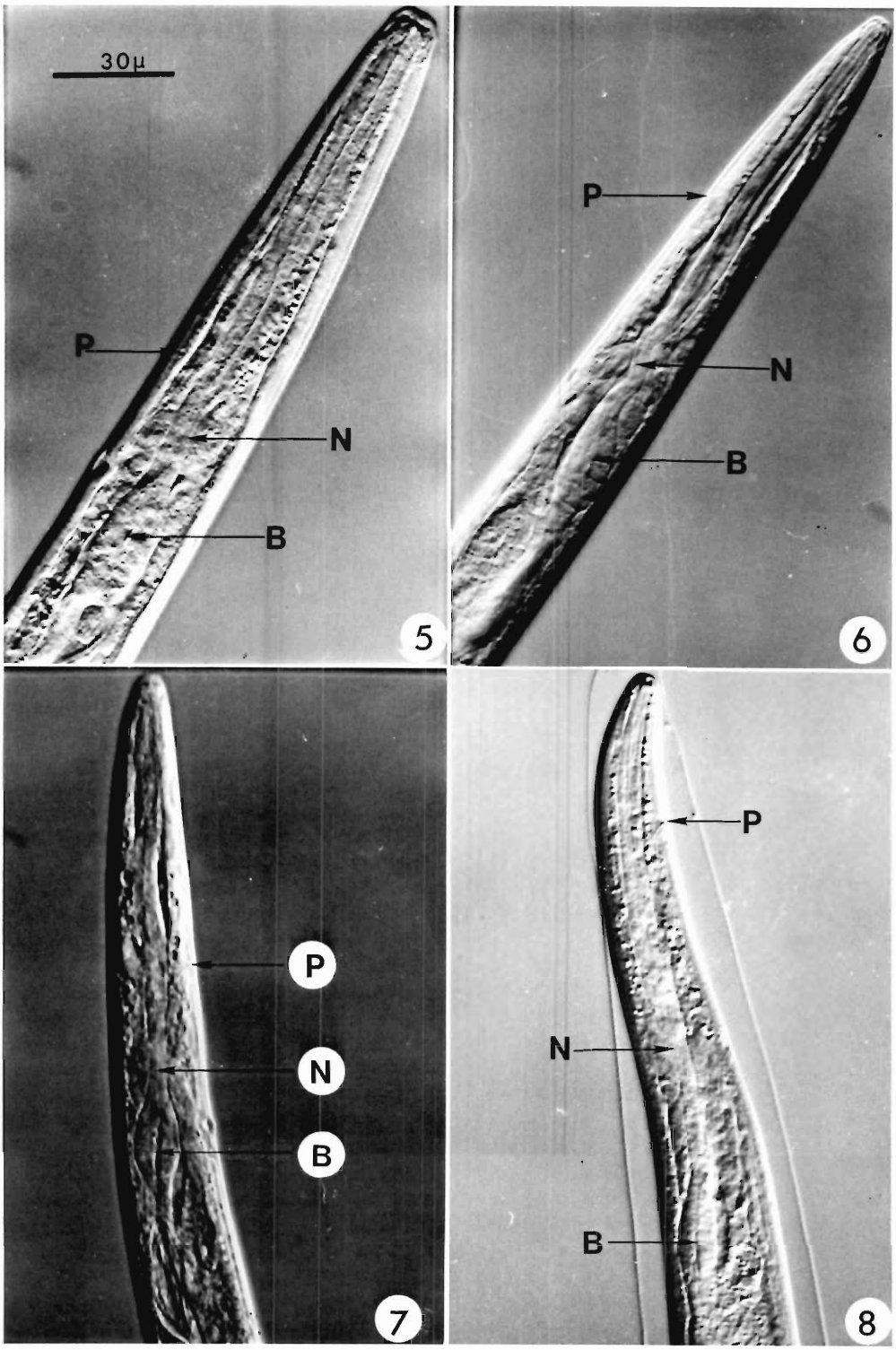
Discussion

With the commercialization and widespread field testing of nematodes belonging to the genus *Neoaplectana* (Poinar, 1985a), there is a growing need for quick methods of identification of the species now in use. The first described species, *N. glaseri,* has been recovered from North America and possibly the Soviet Union (Poinar, 1979). The present distribution of *N. bibionis* includes Europe, Australia, and New Zealand and that of *N. intermedia,* North America. The greatest distribution of the four species is found in *N. carpocapsae,* which has been recovered from North America, Europe, Mexico, Australia, and South America.

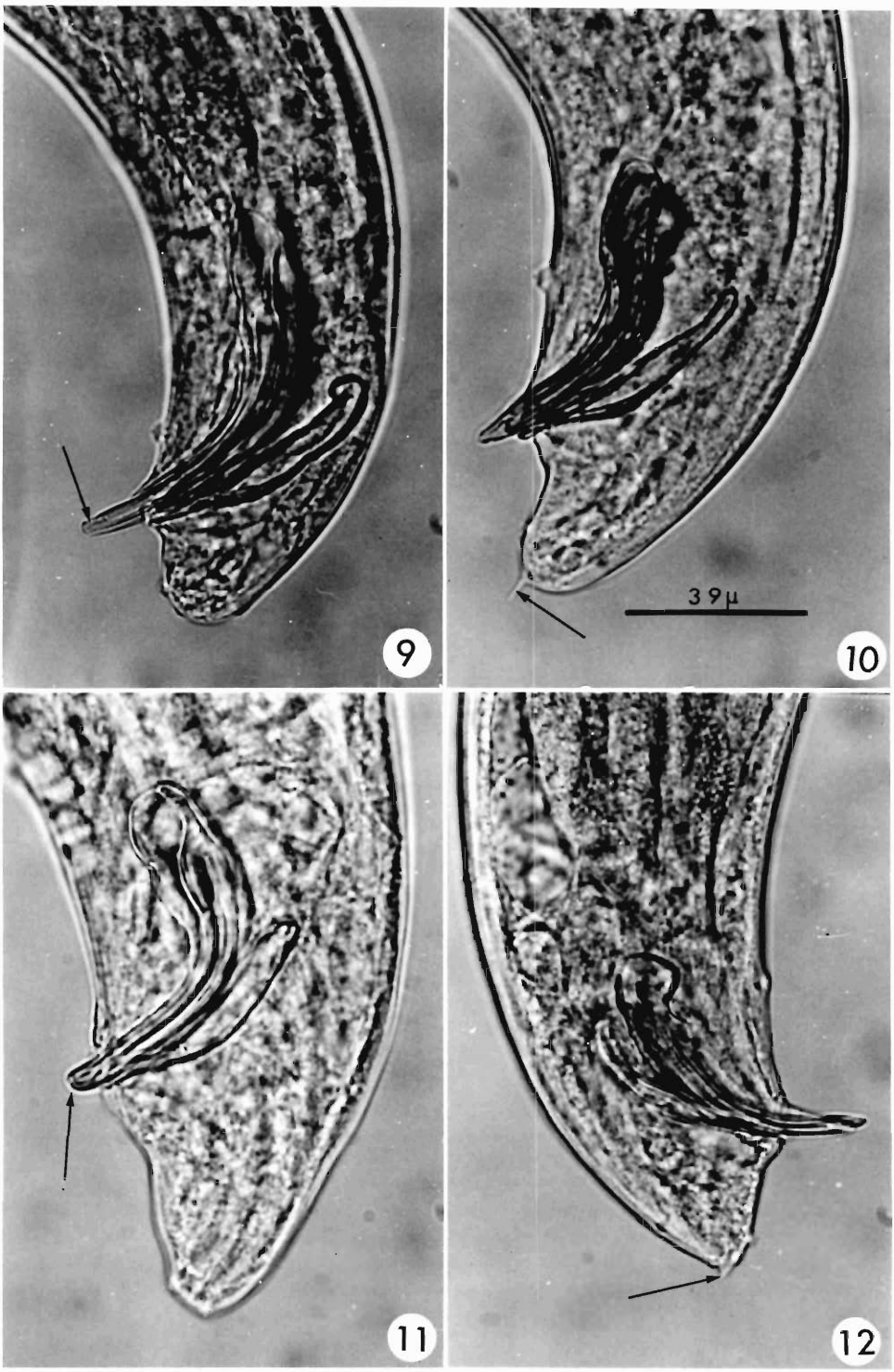
Previous studies (Poinar, 1967, 1978, 1985b; Wouts, 1980) showed that the variability in size found amongst adults of the same *Neoaplectana* species prohibits the use of quantitative characters in distinguishing these stages. Ratio D (distance from the head to the excretory pore divided by the distance from the head to the base of the pharynx) has been used as a specific character for adults (Poinar, 1979) and can be reliable if the sex and generation of the specimen is also known (Poinar, 1985b). It can also be used to separate the infective juveniles of some neoaplectanids. The most reliable ratio, which has shown no overlap in the populations of infective juveniles compared in the present paper, is the distance from the head to the excretory pore divided by the tail length (ratio E).

Although the total length is a fairly reliable character for distinguishing infective juveniles of the four species, there can be some overlap and the range will undoubtedly increase when measurements of the various strains are present. Another possible source of variation arises when the nematodes are cultivated on artificial media. Populations on spent media produce smaller in-

Figures 1–4. Infective stage *Neoaplectana*. *N. glaseri* (1), *N. bibionis* (2), *N. intermedia* (3), and *N. carpocapsae* (4). All photos at same magnification.



Figures 5–8. Pharyngeal regions of infective stage *Neoaplectana. N. glaseri* (5), *N. bibionis* (6), *N. intermedia* (7), and *N. carpocapsae* (8). Note excretory pore (P), nerve ring (N), and basal bulb of pharynx (B). All photos at same magnification.



Figures 9–12. Male tails of *Neoaplectana* species. 9. *N. glaseri* without tail spine, with moderately curved spicules, and scar on spicule tips (arrow). 10. *N. bibionis* with indistinct capitulum and long tail spine (arrow). 11. *N. intermedia* without tail spine, with strongly curved spicules, and blunt spicule tips (arrow). 12. *N. carpocapsae* with minute tail spine (arrow), pointed spicule tips, and well-developed capitulum. All photos at same magnification.

fectives than those on fresh media (pers. obs.). The range of the infectives under these conditions still require investigation.

The infectives of *Neoaplectana* are easily distinguished from those of the related insect parasite, *Heterorhabditis,* by the position of the excretory pore in relation to the nerve ring. The pore is posterior to the nerve ring in *Heterorhabditis* and anterior in *Neoaplectana.* Other characters such as the paired rhabdions in the stoma area, the longitudinal cuticular situations, and the hook on the tip of the head in *Heterorhabditis* are lacking in *Neoaplectana.*

Distinguishing features in male neoaplectanids are restricted to the secondary sex characters although sperm morphology still requires investigation. The presence, absence, and length of a cuticular tail spine is a reliable character although some variation occurs in the length. Variation also occurs in the shape of the spicules and gubernaculum; however, characters such as the presence of a capitulum and arch and the degree of curvature in the spicules are fairly constant. The nature of the spicule tips (blunt, pointed, or notched) is another reliable character. The gubernaculum also shows some variation in lateral view, yet the dorsal or ventral aspect may prove to be a more reliable character.

Female neoaplectanids have no easily recognizable characters to separate them. Some reliability can be given to the location of the excretory pore and shape of the tail but because of their great variability in size, even these characters should be used with caution.

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Research Note

Prevalence of Larval *Proctoeces maculatus* (Trematoda: Fellodistomatidae) Infection in Hooked Mussels from a Louisiana Estuary

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The hooked mussel, *Ischadium recurvum* (Rafinesque), occurs in estuaries from North Carolina to Texas where it attaches to oyster shells and other substrate by means of fibrous byssus threads (Emerson and Jacobson, 1976, The American Museum of Natural History Guide to Shells, Alfred A. Knopf, New York, 482 pp.).

Hopkins (1954, Journal of Parasitology 40:29–31) described the larval digenetic trematode *Cercaria brachidontis* from infections of the gonads and mantles in 2 of 40 *I. recurvum* examined in July 1951 from Barataria Bay, Louisiana. Although he recognized the larva as a member of the family Fellodistomatidae Nicoll, which occur as adults in the intestines of marine fish, Hopkins was unable to provide a valid generic designation.

Wardle (1980, Bulletin of Marine Science 30: 737–743) reported *C. brachidontis* infection in 1 of 17 hooked mussels examined from Galveston Bay, Texas. He also described a metacercaria from the digestive glands in five of those mussels. Based upon morphological similarity he concluded that both cercaria and metacercaria were larval stages of adult *Proctoeces maculatus* (Looss) that resided in the hindgut of sheepshead, *Archosargus probatocephalus* (Walbaum), inhabiting Galveston Bay.

Winstead and Couch (1981, Transactions of the American Microscopical Society 100:296–305) found *Proctoeces* sp. metacercariae unencysted in the gonads and associated ducts of eastern oysters, *Crassostrea virginica* Gmelin, from Gulf Coast estuaries in Florida, Alabama, and Mississippi. Although they believed the worms were *P. maculatus,* which were using oysters as surrogate hosts, the authors did not illustrate or study whole mounts of the parasite.

In a recent taxonomic review of *P. maculatus,* Bray (1983, Journal of Natural History 17:321–329) listed 21 synonyms and indicated that the species had a worldwide distribution and employed a variety of hosts and life cycle patterns.

As part of a survey of parasites in molluscs inhabiting subtidal oyster reefs, I examined 653 hooked mussels from a southwestern Louisiana estuary. From October 1983 through September 1984 mussels were collected monthly from a shallow reef located in the West Cove area of Calcasieu Lake in Calcasieu Parish. Medium to large (35–60 mm) specimens were removed from oyster shells and taken to the laboratory. They were opened and placed individually in glass petri dishes containing tap water. Soft parts were dissected and examined with a stereomicroscope. Trematode specimens were removed and studied live or fixed in hot AFA solution, stained in Semichon's acetic-carmine, dehydrated in alcohol, cleared in xylene, and mounted in Permount for study. Heat-fixed specimens were measured with an ocular micrometer. Representative whole-mount voucher specimens were deposited in the U.S. National Parasite Collection, Beltsville, Maryland.

In October, 8 of 40 mussels were found harboring one to several metacercariae encapsulated in their mantles, gonads, and digestive glands. These larvae (USNM Helm. Coll. No. 78757) were morphometrically identical to the metacercariae of *P. maculatus* as described by Wardle (1980, loc. cit.). Monthly prevalence of infection remained stable through May (Fig. 1) but declined to about two percent in June. In July, 5 of 60 mussels had gonads and mantles infected with orange-pigmented sporocysts releasing tailless cercariae (USNM Helm. Coll. No. 78756). Observations of living cercariae and average measurements of 10 heat-fixed specimens established them as identical to *Cercaria brachidontis.*

By August, prevalence of infection with cercariae declined whereas prevalence of metacercariae increased. Most of those metacercariae were small, had undifferentiated gonads and cirrus sacs, and resembled enlarged cercariae.

Infections with sporocysts disappeared from the sampled mussel population by September. Hindguts of three sheepshead examined from the area in February 1985 contained fragments of *I.*

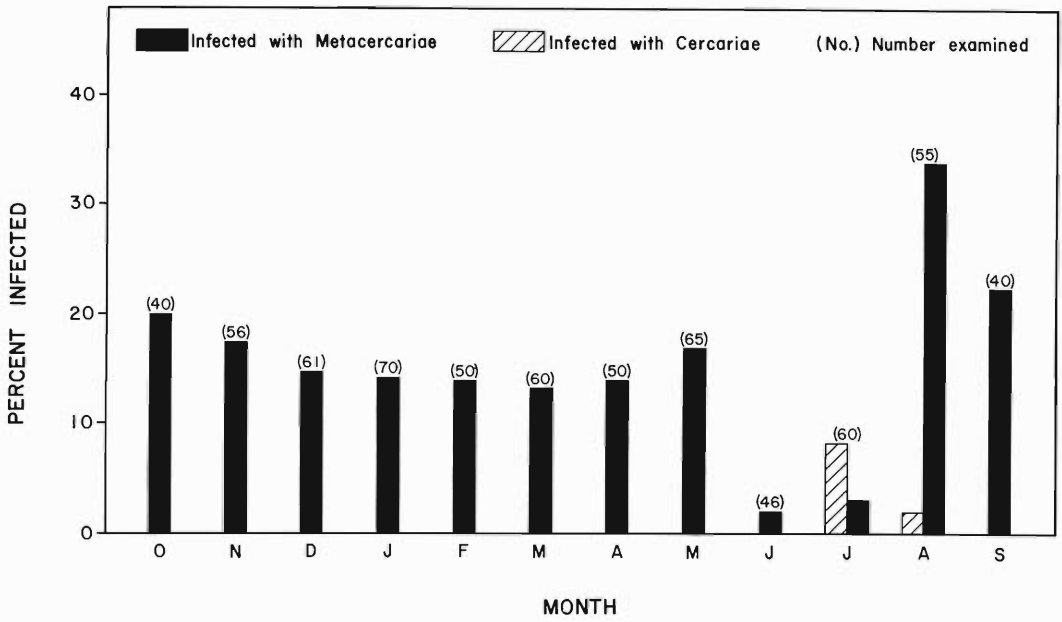

Figure 1. Monthly prevalence of larval *Proctoeces maculatus* infection in hooked mussels from Calcasieu Lake during October 1983 through September 1984.

recurvum shells as well as adult *P. maculatus* (USNM Helm. Coll. No. 78758).

Prevalence of infection with metacercariae was over 10 times greater than that noted by Winstead and Couch (1981, loc. cit.) for oysters. However, seasonality was similar with prevalences lowest in June and highest in August.

In coastal waters of the northeast U.S., which are beyond the range of hooked mussels or sheepshead, *P. maculatus* apparently completes its life cycle by reproducing sexually in the kidney and pericardium of the mussel *Mytilus edulis* Linnaeus (Stunkard and Uzmann, 1959, Biological Bulletin 116:184–193; Lang and Dennis, 1976, Ophelia 15:65–75). According to Wardle (1980, loc. cit.), such progenetic development did not occur in *P. maculatus* from Galveston Bay. The metacercariae noted in the present study were not progenetic to the extent of ova production; however, they exhibited precocious protandry in that seminal vesicles of larger individuals contained active sperm.

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Research Note

Taenia ovis krabbei Cysticerci in the Pronghorn Antelope, *Antilocapra americana*

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The cysticercus of *Taenia ovis krabbei* (Moniez, 1879) Verster, 1969, is commonly found as a parasite of a variety of cervids, including the mule deer, *Odocoileus hemionus* (Rafinesque) and the elk, *Cervus elaphus* L., and in a bovid, Rocky Mountain bighorn sheep, *Ovis canadensis* Shaw, in much of western North America (Thorne et al., 1982, Diseases of Wildlife in Wyoming, Wyoming Game and Fish Department, Laramie, 353 pp.). This metacestode may be ubiquitous in many kinds of cervids but it may be frequently overlooked due to the small size of the cysticercus and the fact that the preferred site of infection is within the striated and cardiac muscles of the intermediate host.

It is the purpose of this note to report the occurrence of cysticerci of *Taenia ovis krabbei* in the pronghorn antelope, *Antilocapra americana* (Ord). Two cases of this parasite have been found in the past two years. The first, found in a host collected near Laramie, Wyoming, was identified on the basis of a single cysticercus. The second, from a hunter-killed animal, came from east-central Wyoming and again was identified from a single parasite. The two specimens had 26 and 28 hooks respectively. Large hooks measured 143 to 162 μm and small hooks measured 100 to 115 μm. These measurements are well within the ranges for this species (Verster, 1969, Onderstepoort Journal of Veterinary Research 36(1):3–58). These are the first records of this parasite from the pronghorn antelope. Representative specimens of prepared scolices have been deposited in the National Parasite Collection, Agricultural Research Service, Beltsville, Maryland (USNM Helm. Coll. No. 78640).

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Research Note

Fractionation of the Female's Pheromone of the Soybean Cyst Nematode, *Heterodera glycines*

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Limited information is available about the pheromones of cyst nematodes despite the major economic importance of cyst nematodes as phytoparasites. Females of *Heterodera schachtii* released a less labile or more concentrated pheromone than females of *H. rostochiensis* (Green, 1966, Annals of Applied Biology 58:327–339). The pheromone from *Heterodera* females was reported as both nonvolatile and volatile (Green, 1967, Nematologica 13:172–174; Greet et al., 1968, Annals of Applied Biology 61:511–519). The female's pheromone was stable after drying, moderate heating, and ultraviolet irradiation (Greet et al., loc. cit.). Six or seven attractants accounted for the pattern of cross-specific pairing during in vitro assay of ten species of *Heterodera* (Green and Plumb, 1970, Nematologica 16:39–46). Huettel et al. (1984, Proceedings of First

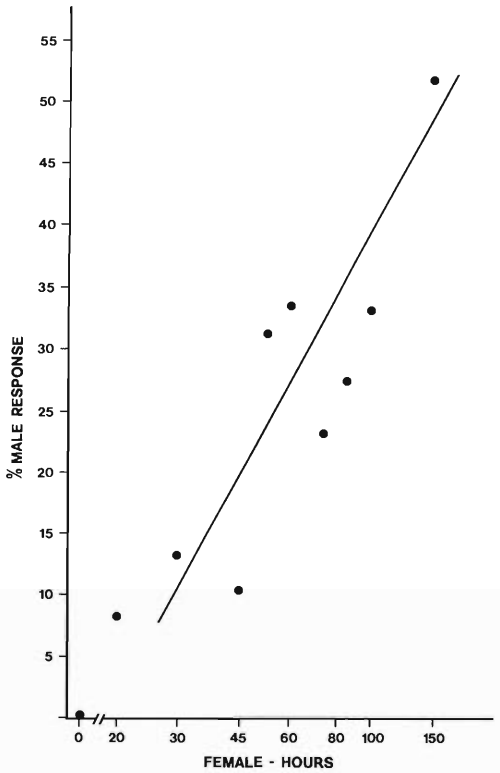

Figure 1. Male responses (%) to the indicated doses of female-hours from incubation of female *H. glycines* (r = 0.88, SEE = 3.71).

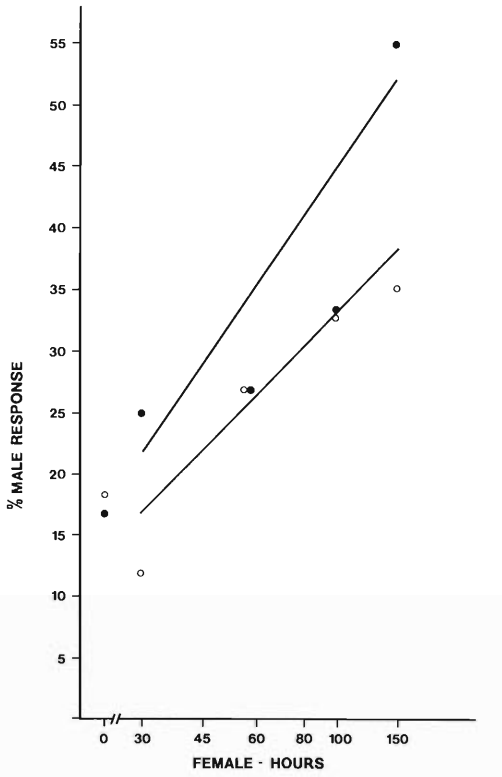

Figure 2. Male responses (%) to aqueous- (●; r = 0.95, SEE = 3.36) and methanol-soluble (○; r = 0.85, SEE = 3.10) doses of female-hours from incubation of *H. glycines*.

International Congress of Nematology, pp. 36–37) have isolated an active pheromone component from females of *H. glycines*.

Males of *H. glycines* moved toward various numbers of living females during bioassay (Rende et al., 1982, Journal of Chemical Ecology 8:981–991). The male's response was influenced by pH, temperature, helminth age, period of pheromone diffusion, and length of time that was allowed for male response. Light intensity had no effect, and a nitrogen atmosphere eliminated the male's response in bioassay. Additional knowledge of the pheromones of *H. glycines* may facilitate chemical isolation and characterization of their components and enable the development of hypotheses for pheromone-based control strategies. Accordingly, this study quantified the response of male *H. glycines* to crude and fractionated pheromone from incubation of female worms.

Females of *H. glycines* were collected according to previous procedures (Rende et al., loc. cit.) and incubated in Tyrode's solution to produce a solution containing various female-hours of pheromone (one female/hr/μl = 1 female-hour). The responses of males to various doses of female-hours were tested by in vitro assay (Papademetriou and Bone, 1983, Journal of Chemical Ecology 9:387–396). Briefly, plastic petri dishes (10 × 1.5 cm) were coated with a thin film of 1.5% agar and a 7-mm-diameter filter paper disc (Whatman No. 1) was placed in the center. Solutions were placed on the disc and an 8-hr diffusion period was allowed. Then, two males were placed equidistantly in the dish and 2 cm from the pheromone source. After 24 hr at 25°C, male response was determined based on contact with the filter paper disc. Males and females were obtained at 3 days postemergence for bioassay. The solution in which females were incubated was fractionated by using reverse-phase Sep-Pak cartridges (Waters Associates) with reagent-grade water (Milli-Q, Millipore) and methanol (HPLC grade, Fisher) as eluants according

to previous procedures (Ward and Bone, 1983, Journal of Parasitology 69:302–306). A 5-fold volume of mobile phase and air-purge were used to eliminate residues. Biological activity of the aqueous and organic fractions was determined by bioassay. The response of 50 males was determined for each dosage of crude or fractionated incubate. The 0.05 probability level was considered significant. Data are given as the means of the individual male responses. The standard error of estimate (SEE) is the regression variance (Steel and Torrie, 1960, Principles and Procedures of Statistics, McGraw-Hill, New York, 481 pp.).

Responses of male *H. glycines* to female-hours of pheromone were dosage-dependent (F^{9}_{499} = 9.73) (Fig. 1). Responses increased linearly (r = 0.88) as the amount of pheromone in female-hours was raised during bioassay. Separation of the incubate from females yielded aqueous- and methanol-soluble fractions that significantly (F^{9}_{249} = 11.71, 7.87, respectively) attracted males during in vitro assay (Fig. 2). Doses of the aqueous- and methanol-soluble fractions elicited linear responses (r = 0.95, 0.85, respectively) from males of *H. glycines*.

These results indicate that the pheromone from females of *H. glycines* is composed of at least two components of different solubility. The more polar compound was not retained by the reverse-phase cartridge and, thus, eluted with water. The second compound was less polar and eluted with methanol. Investigations of other nematodes have revealed multiple components in their female's pheromone. The pheromone of the free-living *Panagrellus redivivus* contained two compounds of different solubility and biological activity (Balakanich and Samoiloff, 1974, Canadian Journal of Zoology 52:835–845). Females of *Nippostrongylus brasiliensis* produced two components of different solubility in their pheromone (Ward and Bone, loc. cit.). Each component from *N. brasiliensis* accounted for 50% of the activity of crude incubate. A similar situation may occur in the pheromone of female *H. glycines*. Addition of the males' responses to the two fractions after correction for the higher control responses, which may result from some constituent of the cartridge or residual methanol, yielded a 61% response. This additive response approximated the 52% response elicited by crude incubate from females. Thus, the two eluants were not synergistic and represented the total biological activity in the crude solution from incubation of females. Investigation of other species may reveal that most, if not all, nematodes have a medley of compounds in their pheromone, although the behavioral significance of the individual components remains unknown.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other products that may also be suitable. Research was supported in part by the Illinois Soybean Program Operating Board.

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Research Note

In Vitro Ingestion by *Trichostrongylus colubriformis* (Nematoda) in Sera, Bacteria, and Media

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Limited information is available concerning in vitro ingestion by zooparasitic nematodes. The incorporation of glucose by *Ancylostoma caninum* was stimulated in vitro by dog serum (Warren et al., 1962, Journal of Parasitology 48:25 [Abstract]; Fernando and Wong, 1964, Experimental Parasitology 15:284–292). Roberts and Fairbairn (1965, Journal of Parasitology 51:119–138) found that dog serum contained an unknown stimulus for feeding by adults of *A. caninum*. In contrast, rabbit serum was not an effective stimulus for feeding activity by *Nippostrongylus brasiliensis*.

Ingestion by *Trichostrongylus colubriformis* in

vitro was stimulated by doses of histamine and dopamine whereas host bile or chyme and selected sugars, amino acids, and enzymes had no effect (Bone and Bottjer, 1984, Proceedings of the Helminthological Society of Washington 52: 80–84). Serum from neonatal goats slightly elevated ingestion by *T. colubriformis* in vitro, but feeding by nematodes was depressed in sera from goats that were infected with *T. colubriformis* (Bottjer et al., 1985, Parasite Immunology 7:1–9). This inhibition of feeding activity by serum was associated with immunoglobulin G_1, based on fractionation of crude serum and indirect immunofluorescent studies of binding (Bottjer et al., loc. cit.).

The present study examined the effect of sera from various mammalian and avian species on in vitro ingestion by *T. colubriformis*. An indication of the specificity of inhibition or stimulation of helminth feeding activity by serum may expand our understanding of helminth ingestion. Additionally, ingestion by *T. colubriformis* in various media, bacteria, and cell lines was studied to assess their potential usefulness as feeding sources for in vitro cultivation.

Trichostrongylus colubriformis was maintained in cross-bred male goats and recovered at 21 days postinfection for determination of feeding activity in vitro by the uptake of the fluorescent dye Rhodamine B as described previously (Bone and Bottjer, loc. cit.). Triplicate samples of 250 worms of each sex were used to determine ng of ingested dye/μg dry body weight of worms for comparison to a standard curve constructed by mixing dye with macerated, undyed worms.

Blood was obtained from various animals by venipuncture, allowed to clot, and stored overnight at 4°C. Serum was collected after centrifugation and warmed to 37°C prior to incubation of the helminths in 1-ml aliquots. Blood was taken from the following: rabbit, pig, human, chicken, cow, dog, and rat. Goat serum was used as a control.

Worms were incubated also in various culture media. These solutions included: Tyrode's solution (control), *Caenorhabditis briggsae* Maintenance Medium (Gibco), phosphate-buffered saline, Hanks' balanced salt solution (Sigma), Earle's Minimum Essential Medium (Sigma), Earle's balanced salt solution (Sigma), Neuman and Tytell Medium (Gibco), Low Serum Medium (LoSM) (Hybridoma Science), Roswell Park Memorial Institute Medium 1640 (RPMI 1640) (Gibco), and Tissue Culture Medium 199 (Gibco). Tyrode's solution was supplemented with mycostatin (500 IU/ml) (Sigma) and penicillin/streptomycin (1,000 IU:1,000 μg/ml) (Sigma) to determine the influence of antibiotics on helminth ingestion.

Selected cell lines were tested also for any effect on feeding activity by *T. colubriformis*. Cells were harvested, frozen, thawed, treated with trypsin, and washed repeatedly prior to use as a medium in Tyrode's solution for determination of the helminth's feeding. Cell lines and their concentrations were as follows: bovine embryonic lung (2.9×10^5/ml), mouse hybridoma (4.3×10^5/ml), and Green Monkey kidney (8.3×10^3/ml). Bacteria were obtained from Dr. Gerald Wilt, Dept. of Microbiology, Auburn University School of Veterinary Medicine and tested also as a food source for *T. colubriformis*. Cells were harvested from their media and suspended in Tyrode's solutions. Suspensions were rinsed three times by centrifugation. A final concentration of approximately 10^6 cells/ml was used as a medium for ingestion by the nematodes. Tyrode's solution was used as a control in the above trials. The following bacteria were tested: *Bacillus* sp., *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella* sp., *Staphylococcus aureus, Streptococcus epidemicus.*

The percent difference in feeding for each treatment was compared to its control by Chi-square distribution. The 0.05 probability level was considered significant.

Comparison of feeding values in serum from the other animals to the helminths' feeding in goat serum revealed no significant differences, according to the Chi-square distribution. The percent difference in feeding by males and females of *T. colubriformis* ranged from +1.6 to −16.9 and +6.5 to −14.7, respectively, in the various sera.

The tested salt solutions and culture media had no significant effect on ingestion by either sex of helminth. However, ingestion by both sexes of the nematode was reduced considerably in Neuman-Tytell, Low Serum and RPMI 1640 media, which suggests that these media are unsatisfactory. Supplementation of Tyrode's solution with mycostatin or penicillin/streptomycin at the tested concentrations had no influence on helminth feeding during incubation.

Ingestion by *T. colubriformis* in bovine em-

bryonic lung, mouse hybridoma, and Green Monkey kidney cells was similar to their controls, according to the Chi-square distribution. However, a tendency of reduced feeding was evident. No significant change occurred in the helminths' feeding in the seven bacterial suspensions that were tested. Percent differences in ingestion by males and females ranged from +13.8 to −17.1 and +10.6 to −16.6, respectively.

Feeding by the sexes of *T. colubriformis* was similar in serum from various mammalian species, selected bacterial species, and a number of media. These results suggest that many media that are used for cultivation of nematodes may have little effect on the feeding physiology of the helminth. However, the more complex media that were tested reduced pharyngeal pumping by the worms during the incubation period. Thus, selection of media for in vitro cultivation should consider the ingestive physiology of the helminth in addition to the nutritional content of the media.

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Research Note

First Report of *Ostertagia kolchida* (Nematoda: Trichostrongyloidea) from North America

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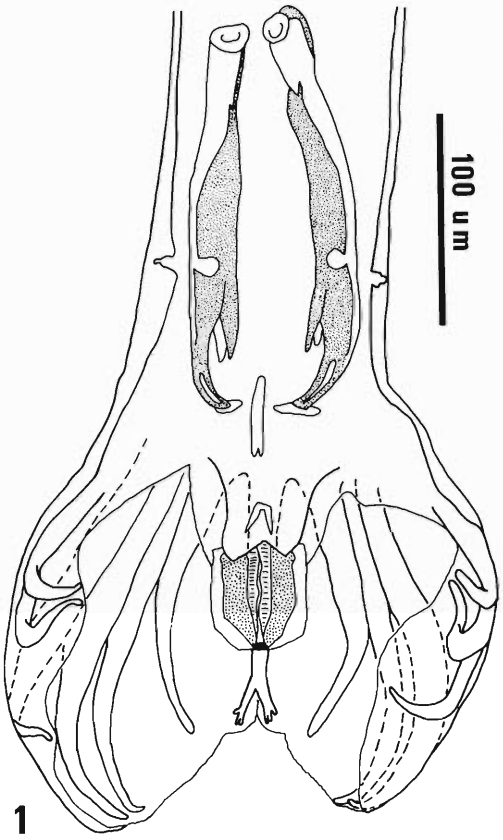
Ostertagia kolchida Popova, 1937 is an abomasal nematode parasite found in some members of the Cervidae and Bovidae (Swiestra et al., 1959, Tijdschrift voor Diergeneeskunde 84: 892–900). This nematode is distributed throughout the Palearctic region and was recently introduced from Europe into New Zealand (Andrews, 1973, New Zealand Veterinary Journal 21:43–47). Common hosts within the Palearctic region include elk, *Cervus elaphus* L. (Dróżdż, 1966, Acta Parasitologica Polonica 14:1–13; Kutzer and Hinaidy, 1969, Zeitschrift für Parasitenkunde 32: 354–368), Sika deer, *Cervus nippon* Temminck (Dróżdż, 1966, op. cit.), roe deer, *Capreolus capreolus* (L.) (Dunn, 1965, Parasitology 55:739–745; Dróżdż, 1966, op. cit.; Kutzer and Hinaidy, 1969, op. cit.), fallow deer, *Cervus dama* L. (Dróżdż, 1966, op. cit.), moose, *Alces alces* (L.) (Dróżdż and Bylund, 1970, Acta Parasitologica Polonica 17:259–260), and chamois, *Rupricapra rupricapra* (L.) (Kutzer and Hinaidy, 1969, op. cit.). Other hosts include mouflon, *Ovis musimon* Pallas (Kutzer and Hinaidy, 1969, op. cit.), sheep, *Ovis aries* L. (Swiestra et al., 1959, op. cit.), cattle, *Bos taurus* L. (Rose, 1968, Veterinary Record 82:615–617; Hong et al., 1981, Veterinary Record 109:12–14), and a llama, *Lama glama* (L.) from a zoo in the Netherlands (Jansen, 1959, Journal of Parasitology 45:509). Hosts in New Zealand include elk (Andrews, 1973, op. cit.), white-tailed deer, *Odocoileus virginianus* (Zimmermann) (Andrews, 1973, op. cit.), and cattle (Bisset, 1980, New Zealand Veterinary Journal 28:54).

Previous classifications of *O. kolchida* have placed this species in either the genus *Ostertagia* or *Skrjabinagia* (see Dróżdż, 1965, Acta Parasitologica Polonica 13:445–481). Recently, Durette-Desset (1982, Annales de Parasitologie Humaine et Comparée 57:375–381) considered *Skrjabinagia* to be a junior synonym of *Ostertagia*. It is this classification that we are following.

Recently, during an anthelmintic trial conducted at Oregon State University, two specimens of *O. kolchida* (Figs. 1–3) were found in an abomasal sample from 1 of 10 calves (comprising the untreated control group) at necropsy. The calves (less than 12 months of age) originated from a ranch near Newberg in northwest Oregon and were transported to the Veterinary Medical Animal Isolation Laboratory in January 1984 where they were placed in isolation. They were necropsied 3 weeks later.

Based upon the identification of male specimens, *O. kolchida* was one of three species of



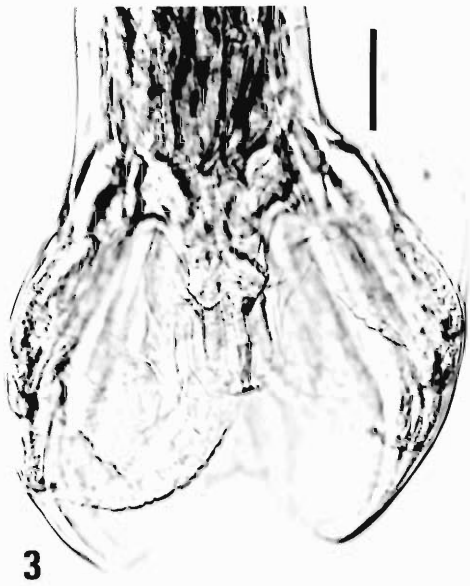
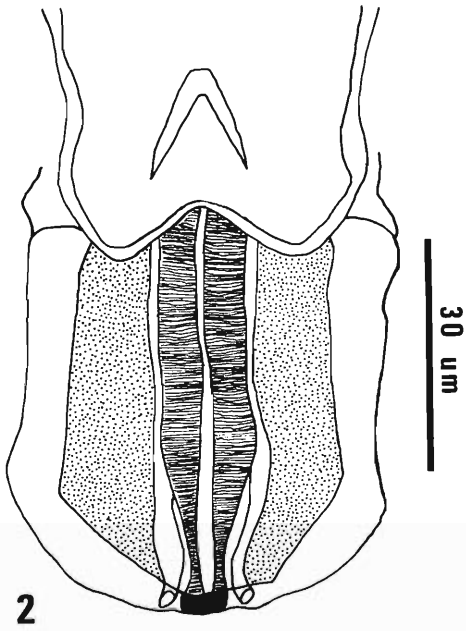


Figure 3. Caudal end of male *Ostertagia kolchida*, ventral view. Nomarski interference contrast. Scale bar = 50 μm.



Figures 1, 2. *Ostertagia kolchida*. 1. Line drawing of copulatory bursa, ventral view. 2. Line drawing of Sjöberg's organ, ventral view.

Ostertagia present in the sample, representing 0.6% of the total *Ostertagia* burden. The remaining species were *O. ostertagi* (Stiles, 1892) and *O. lyrata* Sjöberg, 1926 which, respectively, accounted for 98.8% and 0.6% of the sample. *Ostertagia kolchida* is a rare parasite of cattle and, when present, is usually found in low numbers, comprising less than 5% of the total *Ostertagia* spp. present (Bisset, 1980, op. cit.; Hong et al., 1981, op. cit.). The finding of *O. kolchida* in cattle from Oregon represents the first record of this nematode from North America.

Morphologically, the present specimens did not differ substantially from those described from hosts in the Palearctic (Jansen, 1958, Lebmaagtrichostrongyliden bij Nederlandse Herten, Thesis, Utrecht). A short description of the two males follows with measurements expressed as length versus width in micrometers, unless otherwise stated: Total length, 7.2 and 7.6 mm. Esophagus, 810 and 820 by 35 and 32 at the base. Excretory pore, 283 and 306 and cervical papillae 301 and 334 from the anterior extremity. Width, at prebursal papillae, 113 and 97. Spicules, 205 and 207 long; split into three branches beginning 127

and 136 from the anterior end. Gubernaculum, 44 and 48 long. Sjöberg's organ, 39 and 44 by 32 and 32. Specimens have been deposited in the U.S. National Parasite Collection, USDA, ARS, Beltsville, Maryland (No. 78821).

Ostertagia kolchida and *O. leptospicularis* Assadov, 1953 are considered by some to represent two morphological variants of a single polymorphic species with the latter occurring as the dominant partner (Hong et al., 1981, op. cit.; Lancaster and Hong, 1981, Systematic Parasitology 3:29–31; Lancaster et al., 1983, pages 293–302 *in* Stone et al., eds. Concepts in Nematode Systematics. Academic Press, New York). However, based on the morphological characteristics used to identify species of *Ostertagia* (i.e., morphology and length of spicules, gubernaculum, genital cone, and Sjöberg's organ), *O. leptospicularis* was not present in samples from any calves in this study.

The ranch from which the calves originated is an area populated by black-tailed deer, *Odocoileus hemionis* (Rafinesque) and utilized occasionally by elk. Whether these species represent reservoir hosts for this parasite is unknown.

Thanks to Eric P. Hoberg for advice on the illustrations.

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Research Note

Nematode Parasites of the Parthenogenetic Whiptail Lizard, *Cnemidophorus laredoensis* (Sauria: Teiidae) from South Texas

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The Laredo striped whiptail (*Cnemidophorus laredoensis*) was described by McKinney et al. (1973, Herpetologica 29:361–366) as an all-female teiid lizard restricted to a small area of urban habitat at Chacon Creek Arroyo in Laredo, Webb County, Texas. This diploid parthenogenetic species is considered to have originated from a single hybridization between the Texas spotted whiptail, *C. gularis,* and the six-lined racerunner, *C. sexlineatus* (Bickham et al., 1976, Herpetologica 32:395–399; Cole, 1984, Scientific American 250:94–100).

Aside from the original description, additional information on karyology (Bickham et al., loc. cit.), mitochondrial DNA (Wright et al., 1983, Herpetologica 39:410–416), and a recent paper by Walker et al., 1986 (Southwestern Naturalist, in press) on the ecology of *C. laredoensis* have extended our understanding of this unusual lizard. Since endoparasites are known from one of the parental species (*C. sexlineatus*), specimens of *C. laredoensis* were examined to determine if parasites were present.

Thirty-four female whiptails (including both blue and white color morphs) were collected alive from individual burrows or shot with a BB gun on July 16 and 17, 1983 and June 16, 1984 in the vicinity of Chacon Creek. Neonates, juveniles, and adults were separated into size and age-classes on the basis of snout–vent length, SVL (Walker et al., op. cit.). Individuals were euthanized with an overdose of sodium pentobarbital and fixed in 10% formalin. Helminths were later removed from the gastrointestinal tract of these specimens. Two nematode species were recovered and transferred to vials containing 3% paraformaldehyde for several days; then they were placed in 70% ethanol. The nematodes were cleared in warm lactophenol for identification.

Specimens of *C. laredoensis* are deposited in the University of Kansas Museum of Natural History (KU 199775–199804). Representative samples of helminths are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705.

Five of 22 (22.7%) adult *C. laredoensis* (SVL

range 68–81 mm, $\bar{x}$ = 76.8 ± 3.1 mm) were heavily infected in the colon and rectum with an oxyurid nematode, *Pharyngodon warneri* Harwood, 1932. Neonate and juvenal whiptails (N = 12, 34–48 mm SVL) did not harbor infections of *P. warneri.* The total combined infection rate was 14.7% (5 of 34) lizards examined. Specimens are deposited as USNM Helm. Coll. 78614.

Selected measurements of *P. warneri* recovered from *C. laredoensis* are as follows: male 2.3–2.5 long by 0.14–0.16 mm wide; female 2.9–4.2 long by 0.11–0.18 mm wide; thick-shelled eggs 140–150 long by 45–50 μm wide. These measurements are similar to those reported in the original description of *P. warneri* (Harwood, 1932, Proceedings of the United States National Museum 81:1–71).

Harwood (loc. cit.) described *P. warneri* in *C. sexlineatus* from Huntsville, Walker Co., Texas. Additionally, this species has been reported from *C. sexlineatus* in South Dakota (Dyer, 1971, Proceedings of the Helminthological Society of Washington 38:256), from *C. inornatus* in southwestern Texas (Specian and Ubelaker, 1974, Proceedings of the Helminthological Society of Washington 41:46–51), and from *C. tigris* in Utah (Grundmann, 1959, Journal of Parasitology 45: 394), and in Arizona and Nevada (Babero and Matthias, 1967, Transactions of the American Microscopical Society 86:173–177). Numerous *Pharyngodon* species have been reported from various whiptail lizards of the genus *Cnemidophorus* from North and South America (Pereira, 1935, Archivos do Instituto Biologico 6:5–27; Hannum, 1942, Publications of the University of Washington Theses Series 7:229–231; Calvente, 1948, Revista Iberica de Parasitologia 8:367–410; Read and Amrein, 1953, Journal of Parasitology 39:365–370; Bostic, 1965, Southwestern Naturalist 10:313; Walker and Matthias, 1973, Proceedings of the Helminthological Society of Washington 40:168–169; Specian and Ubelaker, loc. cit.; Specian and Whittaker, 1980, Proceedings of the Helminthological Society of Washington 47:275–276).

The other nematode, a third-stage larval *Physaloptera* sp. (USNM Helm. Coll. No. 78615), was recovered from the stomach of an adult *C. laredoensis* (SVL = 81 mm). This whiptail lizard may have served as a paratenic or second intermediate host in the life cycle scheme and the definitive host could have been a predator (i.e., snake, bird, or mammal). In addition, many *Pharyngodon warneri* were removed from the rectum of this specimen. Babero and Matthias (loc. cit.) found a single female of *P. retusa* in the stomach of *C. tigris.* Nematodes of the genus *Physaloptera* are relatively common helminths of lizards of the Western Hemisphere (Babero and Kay, 1967, Journal of Parasitology 53:168–175; Telford, 1970, American Midland Naturalist 83:516–554; Pearce and Tanner, 1973, Great Basin Naturalist 33:1–18; Prieto, 1980, Journal of Herpetology 14:190–192; Specian and Whittaker, loc. cit.; McKee and Martinez, 1981, Southwestern Naturalist 26:75–76; and others).

In summary, this report represents new host and distribution records for two endoparasites infecting *C. laredoensis* in southern Texas. Although *P. warneri* occurs in the biparental species, *C. sexlineatus,* it is unknown from *C. gularis.* The presence of *P. warneri* in *C. laredoensis* does not provide aid as yet in supporting the hypothesis that *C. laredoensis* has a closer genetic identity to *C. gularis.* Because oxyurid nematodes have direct life cycles and species often exhibit host specificity, it would be necessary to survey the parasites of *C. gularis* in order to possibly delineate coevolutionary patterns in these three species of *Cnemidophorus* lizards. Finally, Hannum (loc. cit.) reported *P. papillocauda* in *C. gulae* (sic) from Arizona; however, the range of *C. gularis* only includes southern Oklahoma, Texas, southeastern New Mexico, and northern Veracruz, México.

We thank Dr. J. Martin Walker and James M. Britton, University of Arkansas, for their aid in collecting whiptails. Appreciation is also extended to Drs. Jon D. Blachley, VA Medical Center, Lawrence W. Hinck, Arkansas State University, and two anonymous reviewers for improving the manuscript.

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Research Note

Pomphorhynchus rocci (Acanthocephala: Echinorhynchidea) from the Freshwater Drum, *Aplodinotus grunniens,* in West Virginia

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Food preferences of the freshwater drum, *Aplodinotus grunniens,* captured from the Ohio River (Gallipolis Dam, river mile point 279.1, U.S. Army Corps of Engineers Ohio River Navigation Charts, January 1975) were studied from April through November 1982. During that investigation 2,006 acanthocephalans were found in 161 (86.6%) of the 186 drum examined with

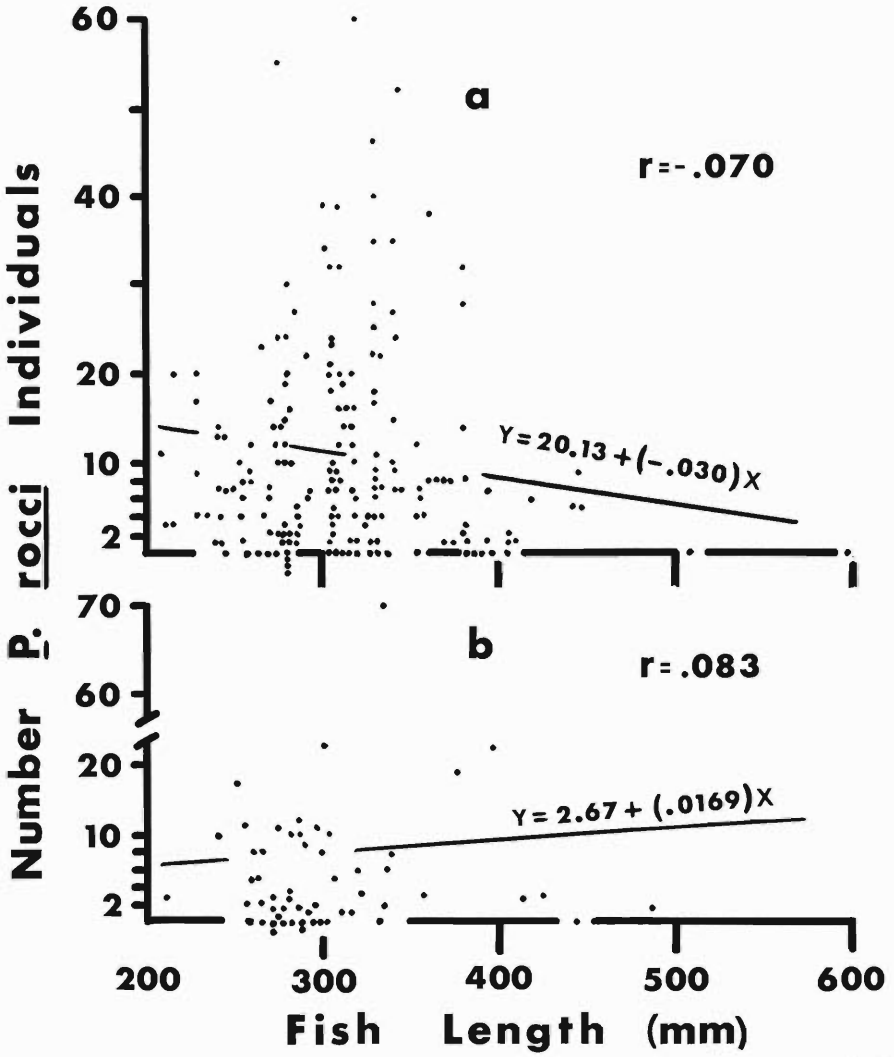

Figure 1. Scatter diagrams depicting relationship between host length (*Aplodinotus grunniens*) and number of *P. rocci* individuals present. Each dot represents a single fish. a = Gallipolis Dam; b = Greenup Dam.

a mean intensity of 12.5. The parasites were tentatively identified—based upon a dozen well-prepared specimens—as *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919. However, after employing the diagnostic procedure developed by Huffman and Nickol (1978, Journal of Parasitology 64:851–859) for differentiating *P. bulbocolli* from *P. rocci* Cordonnier and Ward, 1967, it was determined that all 12 specimens belonged to the latter species. Two specimens were forwarded to Dr. Huffman who confirmed the *P. rocci* identification. One of these, a gravid female, has been deposited in The Ohio State University, Museum of Zoology Helminthology Collection, cat. no. OSUM-1.

An additional 57 freshwater drum were collected during July and August 1983 from a second Ohio River location (Greenup Dam, river mile point 341.1, loc. cit.). Of these drum, 43 (75.4%) were infected with a mean intensity of 7.5. Seventy-two parasites were prepared well enough for accurate diagnoses and all were *P. rocci.* Two of these, a male and a female whose identifications were also confirmed by Dr. Huffman, have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland, under Coll. No. 78616.

Muzzall (1980, Journal of Parasitology 66:127–133) noted that as mean length of white suckers increased, there was a corresponding, and significant, increase in both prevalence and mean intensity of *P. bulbocolli.* He felt these increases might be associated with higher feeding levels and/or change in feeding habits of hosts as they increased in size. Hine and Kennedy (1974, Journal of Fish Biology 6:665–679) and Rumpus (1975, Journal of Fish Biology 7:469–483) found that mean intensity of *P. laevis* increased with host age. Lawrence (1970, Journal of Parasitology 56:567–571) found that prevalence of *P. bulbocolli* in white suckers remained high (≥74%) through the year. Moreover, he added (p. 569), ". . . these parasites equally infect all age groups of fishes and remain at fairly constant levels." Results of the present study paralleled Lawrence's findings in that prevalence was always high (≥75%), and there was little correlation between fish length and number of *Pomphorhynchus* individuals present from either Ohio River location (Fig. 1A and B). It should be added that Sheridan (1983, M.S. Thesis, Marshall University) found little difference in feeding habits of drum by season or length class.

In conclusion, only 84 of 2,329 *Pomphorhynchus* specimens recovered in the combined investigations were suitably removed from hosts and prepared for critical examination. Even though all 84 have been identified as *P. rocci,* the occurrence of *P. bulbocolli* cannot be discounted. At any rate, a significant invasion of inland freshwaters is recorded for *P. rocci,* a parasite previously known only from marine or coastal freshwater fishes (fide Huffman and Nickol, loc. cit.). It remains to be seen if both acanthocephalan species occur in drum or other fishes of the Ohio River contiguous with West Virginia.

We thank Dr. Huffman for his encouragement, and for providing meristogram analyses. The ecological terms "prevalence" and "mean intensity" follow the definitions of Margolis et al. (1982, Journal of Parasitology 68:131–133).

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Research Note

Parasites of Mottled Sculpins, *Cottus bairdi*, from the Au Sable River, Crawford County, Michigan

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Although various reports of parasites infecting the mottled sculpin, *Cottus bairdi* Girard, are scattered througout the literature (Bangham and Hunter, 1939, Zoologica; New York Zoological Society, 24:385–448; Fischthal, 1945, Transactions of the Wisconsin Academy of Sciences, Arts and Letters 37:275–278; Fischthal, 1953, Transactions of the Wisconsin Academy of Sciences, Arts and Letters 42:83–108; Bangham, 1955, American Midland Naturalist 53:184–194; Dechtiar, 1972, Journal of the Fisheries Research Board of Canada 29:275–283), there has been no extensive study on the parasites of this fish from one locality. This study reports on the parasites of a population of mottled sculpins from the Keystone area of the Au Sable River, Crawford Co.,

Table 1. Parasites of *Cottus bairdi* from the Au Sable River.

Parasite	No. infected (%)	Mean no. ± 1 SD (range)	Site of infection
Trematoda			
Monogenea			
*Gyrodactylus bairdi** Wood and Mizelle, 1957	13 (6)		Gills
Digenea			
Crepidostomum cooperi Hopkins, 1931	2 (1)	4.5 ± 2.1 (3–6)	Anterior intestine
Diplostomum spathaceum† (Rudolphi, 1819)	3 (2)	4.3 ± 5.8 (1–11)	Eyes (lens)
Diplostomum sp.†	4 (2)	1.0	Mesenteries
Neascus sp.†	8 (4)	2.2 ± 3.2 (1–10)	Skin, muscle
Tetracotyle sp.†	61 (30)	5.6 ± 6.7 (1–27)	Muscle, mesenteries, eye orbit; on surface of gonads, heart, kidney, liver, urinary bladder
Cestoda			
Proteocephalus sp.‡	4 (2)	1.3 ± 0.5 (1–2)	Intestine
Nematoda			
Cystidicoloides tenuissima‡ (Zeder, 1800)	2 (1)	1.0	Stomach
§*Rhabdochona cotti* Gustafson, 1949	42 (21)	3.5 ± 4.4 (1–24)	Intestine
§*Spinitectus gracilis* Ward and Magath, 1916	17 (8)	3.0 ± 3.7 (1–13)	Stomach, intestine
Protozoa			
Epistylis sp.	79 (39)		Gills
Unidentified sporozoan	31 (15)		Gonads, hemocoel, mesenteries
Trichodina sp.	59 (29)		Gills

* No postscript indicates adult parasites.
† Larval stages.
‡ Immature parasites.
§ New state record.

located in the north-central portion of the lower peninsula of Michigan.

Two hundred five mottled sculpins were collected by electrofishing in July, September, and November 1982; March, April, July, September, and November 1983; and April 1984. Fish were killed and preserved in 20% formalin in the field; they were sexed, measured for total length, and necropsied in the laboratory. Parasites found were processed using conventional parasitological techniques. Prevalence is the percentage of fish infected and mean number is the number of worms per host. Representative specimens of *Crepidostomum cooperi* (78736), *Diplostomum spathaceum* (78737), *Cystidicoloides tenuissima* (78738), *Rhabdochona cotti* (78739), and *Spinitectus gracilis* (78740) have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705.

One hundred sixty-three (80%) of the 205 mottled sculpins examined were infected with one or more parasites. In Table 1, the prevalence, mean number, and site of infection are presented for the 13 species of parasites (1 Monogenea, 5 Digenea, 1 Cestoda, 3 Nematoda, 3 Protozoa) recovered. *Cystidicoloides tenuissima* and *S. gracilis* are reported from mottled sculpins for the first time; these species and *R. cotti* are reported for the first time from Michigan. *Tetracotyle* sp. and *R. cotti* had the highest prevalence and mean number of trematodes and nematodes found, respectively. *Epistylis* sp. had the highest prevalence of protozoans.

Individuals of nine parasitic species infecting mottled sculpins in the present study were counted. Five hundred ninety-one individuals of these species were recovered. The parasite community composed of these species consisted of (number of individuals, percentage of community): *C. cooperi* (9, 1.5%), *D. spathaceum* (13, 2%), *Diplostomum* sp. (4, 1%), *Tetracotyle* sp. (339, 57%), *Neascus* sp. (22, 4%), *Proteocephalus* sp. (4, 1%), *C. tenuissima* (2, 0.5%), *R. cotti* (147, 24%), *S. gracilis* (51, 9%). Taxonomically, 387 (65%) trematodes, 200 (34%) nematodes, and 4 (1%) cestodes were found. Larval trematodes numbered 378, comprising 64% of the total parasite community.

Tetracotyle larvae have been reported from several piscine species in Michigan (Hughes, 1928, Transactions of the American Microscopical Society 47:414–433). *Tetracotyle* metacercariae found in mottled sculpins in the present study were very difficult to free from their cysts mechanically. Digestion of the cyst wall with pepsin followed with trypsin (Hoffman, 1959, Transactions of the American Fisheries Society 88:96–99) resulted in a few larvae becoming excysted. All excysted larvae exhibited sluggish movement.

Although sculpins were examined during different seasons of the year, data were not treated statistically because of the small sample size. However, *R. cotti* and *S. gracilis* primarily were found in sculpins in July and September 1983 and 1984. The other species encountered occurred sporadically. No significant differences in prevalence (Chi-square analysis) and mean number (Student's *t*-test) were found for *R. cotti, S. gracilis, Tetracotyle* sp., and the protozoan species between male and female mottled sculpins, or between different length classes.

The authors acknowledge Dr. C. Robert Peebles, MSU, for help in identifying some of the parasitic species and Mr. Craig L. Milewski for his technical assistance. We also thank Dr. Glenn L. Hoffman for his correspondence on *Tetracotyle* sp. This study was supported by BRSG Grant #2-S07-RR070490-15, Biomedical Research Support Grant Program, NIH.

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Research Note

Eustrongylides sp. (Nematoda: Dioctophymatoidea): Differentiation of Third- and Fourth-Stage Larvae from Killifish, *Fundulus* sp., Collected in Chesapeake Bay Area, U.S.A.

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Parasitic nematodes of the genus *Eustrongylides* reach maturity in piscivorous birds. Larval stages will invade a wide variety of vertebrate hosts including man. In the Chesapeake Bay area of the United States killifish of the genus *Fundulus* are frequently intermediate hosts (Cullinan, 1945, Journal of Parasitology 31:109–112; Shirazian et al., 1984, Journal of Parasitology 71:803–806).

The identification of *Eustrongylides* species is based on characteristics of adults so we must refer to our larval specimens as *Eustrongylides* sp. However, von Brand and Simpson (1944, Journal of Parasitology 30:121–129) obtained an adult male *Eustrongylides ignotus* Jägerskiöld, 1909 from an in vitro culture of larval specimens from *Fundulus heteroclitus* (L.) collected in the Chesapeake Bay area. This appears to be the only species that has been identified from this area.

Most larval *Eustrongylides* reported from vertebrates have been in the fourth stage of development. The few reports of third-stage larvae of *Eustrongylides* have been: by Karmanova (1965, Trudy Gel'mintologicheskaya Laboratoriya, Akademiya Nauk SSSR 15:86–87; 1968, Osnovy Nematodologii XX, Nauka Publishers, Moscow, SSSR, pp. 92–151); by Sprinkle (1973, Thesis, Ohio State University, 60 pp.); by Crites (1982, Clear Technical Report No. 258, Ohio State University, Center for Lake Erie Area Research, Columbus, Ohio, July 1982, 83 pp.); by Fagerholm (1982, Acta Academiae Aboensis, Series B, 40: 11–19); and by Lichtenfels and Stroup (1985, Proceedings of the Helminthological Society of Washington 52:320–323). Only that by Lichtenfels and Stroup (1985, loc. cit.) concerned a species in the Chesapeake Bay area and it was a larva collected from an invertebrate. This report describes third- and fourth-stage *Eustrongylides* sp. from *Fundulus* sp. from the Chesapeake Bay area and characteristics useful for separating third and fourth stages.

Two third-stage larvae were collected along with two fourth-stage larvae from *Fundulus* sp. from the Chesapeake Bay area by Professor A. James Haley and Dr. Beverly A. Mock, University of Maryland. In addition, a fourth-stage larva from *Fundulus heteroclitus* from the Chesa-

Table 1. Morphometrics (in mm) of third- and fourth-stage larval *Eustrongylides* sp. collected in the Chesapeake Bay area.

Characteristics	Third stage		Fourth stage		
USNM Helm. Coll. No.	69458*	69458*	69459*	69459*	77118†
Sex	Male	Female	Male	Female	Male
Total length	8.90	11.5	115.0	15.2	70.0
Buccal capsule depth	0.119	0.122	0.177	0.128	0.120
Distance from anterior end to:					
Papillae of internal circle	0.030	0.029	—	0.016	0.015
Papillae of external circle	0.060	0.072	—	0.047	0.051
Nerve ring	0.122	0.178	—	0.179	0.195
Esophagus length	1.94	—	18.20	4.47	12.64
Width at external circle	0.080	0.107	—	0.115	0.108
Width at esophagus base	0.178	0.387	—	0.328	0.456

* Collected by A. J. Haley and B. A. Mock from *Fundulus* sp., September 8, 1982.
† Collected by E. L. Schiller from *Fundulus heteroclitus*, April 21, 1982.

Table 2. Morphometrics (in mm) of third- and fourth-stage larval *Eustrongylides* spp. from various hosts and localities.

Characteristics	*Eustrongylides tubifex*				*Eustrongylides* sp.	
	Third stage		Fourth stage		Fourth stage	
USNM Helm. Coll. No.	72963*	72963*	72963*	72963*	34744†	47290‡
Sex	Male	Female	Female	Male	Male	Male
Total length	17.6	18.24	36.96	66.00	30.74	25.2
Buccal capsule depth	0.082	0.080	0.096	0.100	0.126	0.120
Distance from anterior end to:						
Papillae of internal circle	0.008	0.008	0.018	0.024	0.028	0.028
Papillae of external circle	0.038	0.040	0.054	0.068	0.050	0.052
Nerve ring	0.126	0.124	0.148	0.188	0.204	0.168
Esophagus length	5.4	6.0	10.2	15.8	7.3	8.0
Width at external circle	0.064	0.080	0.079	0.124	0.216	0.136
Width at esophagus base	0.176	0.172	0.300	0.442	0.450	0.466

* 72963 *Eustrongylides tubifex* (exper.) *Anas platyrhynchos* collected by Sprinkle-Fastzkie, Lake Erie.
† 34744 *Eustrongylides* sp. from *Fundulus diaphanus* collected by G. A. MacCallum, Long Island, New York.
‡ 47290 *Eustrongylides* sp. from *Catostomus commersoni* collected by M. Meyer, Maine.

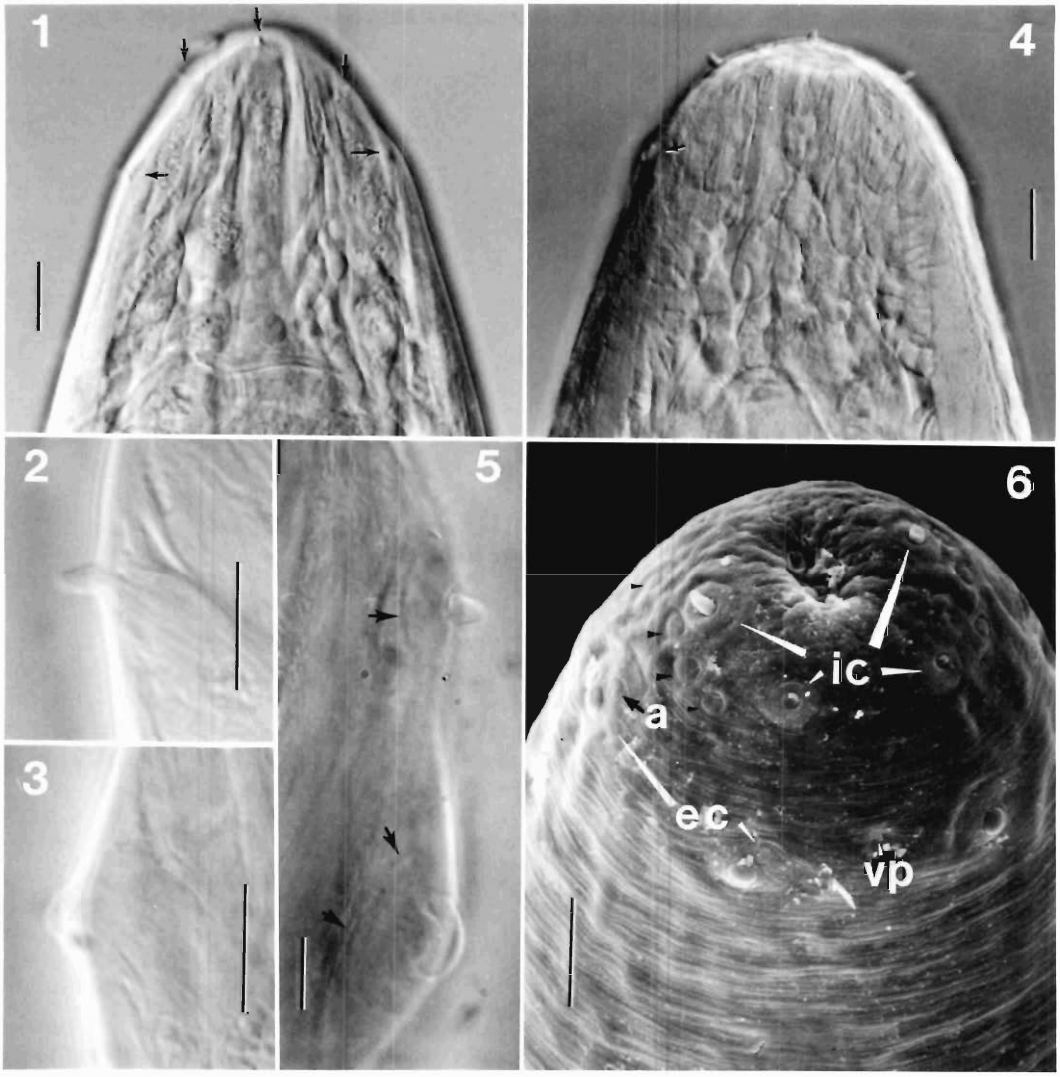
peake Bay area was provided by Professor E. L. Schiller, Johns Hopkins University. Specimens were studied by light and scanning electron microscopy (SEM) using standard techniques. Nematodes fixed and stored in alcohol–glycerine were first measured and photographed by light microscopy in phenol–alcohol before being prepared for SEM. Specimens were deposited (Tables 1, 2) in the U.S. National Museum Helminthological Collection, USDA, Agricultural Research Service, Beltsville, Maryland 20705.

The most useful characteristic for distinguishing third- from fourth-stage *Eustrongylides* sp. larvae is body length because this characteristic is most easily observed. However, characteristics of head shape and cephalic papillae can also be used to distinguish between third- and fourth-stage larvae of *Eustrongylides* species. Because molting specimens were not seen, characteristics of cephalic papillae were especially useful for separating stages.

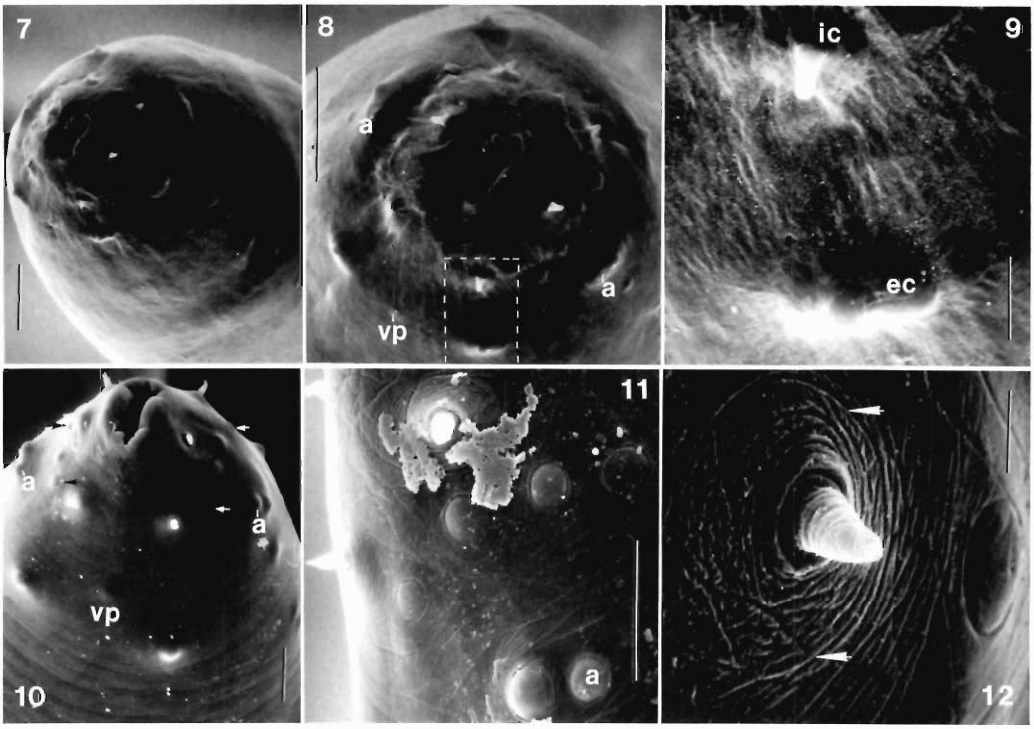
BODY LENGTH: The two third-stage larvae from *Fundulus* sp. were 8.9 and 11.5 mm long. The smallest fourth-stage larva from the same collection and host was 15.2 mm long (Table 1). Because we had only a few third- and early fourth-stage larvae we searched the records of the U.S. National Museum Helminthological Collection for additional larval specimens for comparison. We found (Table 2) only three collections containing larval *Eustrongylides* sp. about 30 mm or less in length. In addition, we examined two third- and two fourth-stage *Eustrongylides tubifex* (Nitzsch, 1819) from *Anas platyrhynchos* (L.) and found (Table 2) the third-stage larvae of that species to be slightly larger than the specimens from *Fundulus*.

HEAD SHAPE: The heads of the third-stage larvae were conical (Fig. 1) compared to the more bluntly rounded heads (Fig. 4) of the fourth stage. Because of differences in contraction due to fixation, seen with numerous specimens of this species and other nematodes, it is expected that this characteristic may be of limited value in poorly fixed specimens. The head shape of a third-stage *Eustrongylides* sp. reported from an oligochaete by Lichtenfels and Stroup (1985, loc. cit.) and third-stage *Eustrongylides tubifex* was also markedly conical.

LABIAL AND CEPHALIC PAPILLAE: Fourth-stage larvae of *Eustrongylides* sp. have a distinct ring or halo around the base of each labial and cephalic papilla with a cuticular depression marking the perimeter. Labial and cephalic papillae of third-stage larvae lack these rings or haloes and depressions. Nematodes of the genus *Eustrongylides* have 20 papillae, two amphids and a ventral pore around the dorsoventrally elongated mouth (Lichtenfels and Madden, 1980, Proceedings of the Helminthological Society of Washington 47:55–62). The 20 papillae include an internal circle of six and an external circle of six labial papillae, and two sets of four cephalic papillae located laterally between the two circles of labial papillae. The lateral papillae of both internal and external circles of both stages are slightly anterior to the subdorsal and subventral papillae of their corresponding circles (Figs. 1, 4,



Figures 1–6. ***Eustrongylides*** **sp. third- and fourth-stage larvae. 1. Anterior end of third-stage female larva (11.5 mm) from** ***Fundulus*** **sp. from Chesapeake Bay, showing conical head shape and labial papillae of internal circle (vertical arrows) and external circle (horizontal arrows). Scale bar 25 μm. 2. Labial papilla of the internal circle, third stage (same specimen as in Fig. 1) showing spinelike process as seen with oil immersion light microscopy. Scale bar 10 μm. 3. Labial papilla of external circle of the third stage (same specimen as in Fig. 1; oil immersion). Scale bar 10 μm. 4. Anterior end of fourth-stage male larva (70 mm) from** ***Fundulus heteroclitus*** **from Chesapeake Bay, showing bluntly rounded head, three spinelike labial papillae of the internal circle anteriorly, and one papilla of external circle in profile (arrow). Scale bar 25 μm. 5. Papillae of the internal and external circles of a fourth-stage male larva (30 mm) from** ***Catostomus commersoni*** **collected in Maine (oil immersion). Arrows indicate cuticular depression at perimeter of large, flat, round base surrounding each papilla. Scale bar 10 μm. 6. Head of fourth-stage larva (same specimen as in Fig. 4; as seen with SEM), showing labial papillae of internal circle (ic) and external circle (ec), four cephalic papillae on right side of specimen between circles of labial papillae (small black arrows), amphid (a), and a ventral pore (vp). White arrows indicate the cuticular depression at the perimeter of large, flat, round base surrounding each labial papilla. Scale bar 25 μm. Note: The spinelike processes of all except one of the papillae of the internal circle were broken or collapsed during processing.**



Figures 7–9. ***Eustrongylides*** **sp. third-stage female larva (11.5 mm) from** ***Fundulus*** **sp. collected in the Chesapeake Bay area. 7. Head showing labial papillae of the internal and external circles, amphids, and a lateral row of cephalic papillae. Scale bar 25 μm. 8. En face view, higher magnification, showing dorsoventrally elongated mouth, six papillae of the internal circle with spinelike tips, six papillae of the external circle, amphids (a), ventral pore (vp), and eight cephalic papillae (arrows) between the internal and external circles. Scale bar 25 μm. 9. Enlargement of area in box of Figure 8 of a single internal (ic) and an external papilla (ec). (Note the absence of a cuticular depression around papillae of the third stage.) Scale bar 5 μm.**

Figures 10–12. ***Eustrongylides*** **sp. fourth-stage male larva (115 mm) from** ***Fundulus*** **sp. collected from the Chesapeake Bay area. 10. Head, showing labial papillae of the internal and external circles, amphids (a), ventral pore (vp), and two rows of cephalic papillae (between sets of arrows) between the internal and external circles (one spinelike tip of the left lateral internal papilla is broken). Scale bar 25 μm. 11. Higher magnification showing three papillae of the internal circle with spinelike tips, one papilla of the external circle with amphid (a), and a row of four cephalic papillae. (Note the cuticular depressions that surround all papillae of the fourth stage.) Scale bar 25 μm. 12. A single papilla of the internal circle and one cephalic papilla at higher magnification. Arrows indicate perimeter of broad flat base surrounding the spinelike papilla. Scale bar 5 μm.**

6–8, 10). The amphids are located slightly anterior and ventral to the lateral papillae of the external circle (Figs. 6–8, 10, 11). In both third- and fourth-stage larvae the six papillae of the internal circle have a long, tapering, spinelike central process (Figs. 4, 6, 8, 10). In fourth-stage larvae the six papillae of the internal circle each have a large, flat, round base delimited by a cuticular depression surrounding the spinelike central process (Figs. 5, 6, 10–12). In third-stage larvae the six papillae of the internal circle lack the large, flat, round base and cuticular depression, and consist of the spinelike central process only (Figs. 2, 7–9). In fourth-stage larvae the six large umbonate papillae of the external circle are delimited both by distinct circular depressions in the cuticle at the base of the papilla and by a large, flat, round base similar to the one that surrounds the papillae of the internal circle (Figs. 5, 6). The perimeter of the large, flat, round base of the papillae of the external circle is not evident in some specimens (Figs. 10, 11). In third-stage larvae papillae of the external circle are devoid of circular depressions or other ornamentation (Figs. 3, 7–9).

As with the labial papillae, the cephalic papillae (in lateral rows of four each between the internal and external circles) differ between third

and fourth stages: cephalic papillae and the amphids are delimited by circular grooves or depressions in fourth-stage larvae (Figs. 6, 10–12), but the grooves are absent in third-stage larvae (Figs. 7, 8)

Three previous reports have described the morphology of labial and cephalic papillae of larval *Eustrongylides* spp. with the aid of SEM. Lichtenfels and Madden (1980, loc. cit.) described the labial and cephalic papillae of fourth-stage *Eustrongylides* sp. from frogs collected in Nevada. Fagerholm (1982, loc. cit.) described the labial and cephalic papillae of fourth-stage larvae of *Eustrongylides mergorum* (Rudolphi, 1809) from fish collected in Finland. Crites (1982, loc. cit.) described with SEM both third- and fourth-stage *E. tubifex* from fish collected in Ohio. All three previous studies and the present study described similar morphology for the labial and cephalic papillae of the fourth stage. The only previous SEM study of third-stage *Eustrongylides* was by Crites (1982, loc. cit.). We examined with light microscopy specimens of *E. tubifex* collected and identified as third stage by Sprinkle (1973, loc. cit.), and through the courtesy of Professor John L. Crites we examined the original prints of his (Crites, 1982, loc. cit.) SEM micrographs of third-stage *E. tubifex.* We determined that the morphology of labial and cephalic papillae of third-stage *E. tubifex* was identical to our third-stage larvae in lacking the cuticular depression marking the large basal ring that surrounds each labial papilla in fourth-stage larvae.

The practical value of the morphology of labial and cephalic papillae for separating third- and fourth-stage *Eustrongylides* larvae is limited because it is difficult to observe with light microscopy. This is compounded by the fact that the papillae of the fourth stage can be seen through the third-stage cuticle prior to the third molt (Fig. 3). However, this character can be used to determine the size range of the two larval stages, after which body length can be used to identify the larva for most purposes. In the present study molting specimens were not available, but the morphological differences in papillae indicated that a molt occurred in female specimens between 11.5 and 15.2 mm long. For *E. mergorum,* Fagerholm (1982, loc. cit.) observed a single specimen 14.5 mm long in the third ecdysis. Karmanova (1968, loc. cit.) reported a much greater body length for *E. excisus* Jägerskiöld, 1909 in the third molt (30.1–31.7 mm). Because of Karmanova's observation, Sprinkle (1973, loc. cit.) arbitrarily identified specimens larger than 32 mm as fourth stage and those smaller than 31 mm as third stage. Crites (1982, loc. cit.) identified specimens 8.9–31 mm as third stage.

Three characters have therefore been found to be useful for separating third- and fourth-stage larval *Eustrongylides* spp. in this study. Body length (Tables 1 and 2) and head shape were the most easily observed, but the characteristics of the labial and cephalic papillae, although more difficult to observe, were the most definitive.

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Book Review

Handbook of Trematodes of North America North of Mexico. By Stewert C. Schell.

This 1985 revision of Dr. Schell's 1970, "How to Know the Trematodes," updates the knowledge of trematodes. The key to monogeneans has been revised and clarified to make diagnosis easier. The family Rugogastridae Schell, 1973 has been added to the Subclass Aspidogastrea to represent *Rugogaster hydrolagi* from the marine ratfish, *Hydrolagus collei.*

The author has added the digenetic families; Acanthostomidae, Accacoeliidae, Bathycotylidae, Botulisaccidae, Cephaloporidae, Lampritrematidae, Monascidae, Ptychogonimidae, and Sclerodistomidae representing one or more genera present in marine fishes. He also added the families Rhytidodidae and Pachypsolidae from marine turtles and the families Nasitrematidae from the nasal passages of whales and Opisthotrematidae from other marine mammals.

He also removed the genera: *Collyriculum, Nanophyetus,* and *Paragonimus* from the family Troglotrematidae to separate families as follows: *Collyriculum* to family Collyriclidae Ward, 1917, *Nanophyetus* to family Nanophyetidae Dollfus, 1939 and *Paragonimus* to the family Paragonimidae Dollfus, 1939. He also removed the genus *Haematoloechus* from the family Haplometridae and with the genus *Ostoliolum* placed it in the family Haematoloechidae Odening, 1964. He also removed the genera *Cephalophallus, Allassogonoporus,* and *Myotitrema* from the family Lecithodendriidae (Lühe, 1901) and placed them in the family Allassogonoporidae Odening, 1964. Additionally, the brachylaemid genus *Hasstilesia* was placed in the family Hasstilesiidae Hall, 1916 and the brachylaemid genera *Leucochloridiomorpha* and *Ptyalincola* were placed in the family Leucochloridiomorphidae Travassos and Kohn, 1966. Other similar changes reflect additional knowledge since his original publication.

These changes, in some instances, reflect new life-cycle information. Most changes, however, do not indicate phylogenetic relationships, because in several instances life-cycles are still not known. They do, however, facilitate identification of adult stages by way of keys. This is the main objective of this revision. Undoubtedly, as additional life-cycles are completed, further revisions will be necessary. This is especially true of those families where no life-cycles are presently known.

One cannot help but wonder what the intention of the author was, in his discussion of progenesis on page 22, where he uses the genus *Pseudallocreadium* for *P. neotenicum* and *P. alloneotenicum* from the body cavities of diving beetles and caddisflies respectively. In his treatment of the family Allocreadiidae (Looss, 1902) he places both species in the genus *Allocreadium* Looss, 1900 (page 217).

Typographical errors are minimal throughout the manual and do not cause misunderstanding of the intended meaning.

The listing of the majority of published keys to species, where available, will be very helpful to beginning students in gaining an understanding of species diversity within genera and families.

Future taxonomists in this field, with aid of computers, will undoubtedly make advances in our understanding of phylogenetic relationships in the trematodes. The relative value of adult trematode morphology versus the larval stages still poses many unresolved problems.

The author has accomplished his objective in publishing a well-illustrated revision of his original set of keys to include additional trematodes from marine coastal hosts. The use of this manual will be very helpful to students of parasitology, at all levels, as well as to practicing parasitologists.

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In Memoriam

SEWELL HEPBURN HOPKINS
1906–1984

Sewell Hepburn Hopkins was born at Waverly on the North River in Gloucester County, Virginia in March 1906. He was taught at home until 1920 when he enrolled in public school as a high school sophomore. He was graduated from high school in 1923 and entered William and Mary for the fall semester at age 17. He completed a degree in English and Biology in 1927 and it was during his senior year that he was elected to Phi Beta Kappa. He entered graduate school at Johns Hopkins the fall semester 1927, attending the Homewood campus where zoology was located. He studied with Drs. S. O. Mast, H. S. Jennings, and E. A. Andrews. However, finances were insufficient and it was only with the help of $150 borrowed from an uncle that the academic year was completed. During this time of financial stress, the Zoology Department of Johns Hopkins received a letter from an alumnus, Justus Mueller, stating that he was finishing at the University of Illinois and that if anyone wanted to apply for his vacated research assistantship, they should write to Dr. Henry B. Ward regarding this opening. Mr. Hopkins was notified of this opening but was concerned about the quality of education at the University of Illinois. He discussed this matter with Dr. W. W. Cort, an alumnus of the University of Illinois who was then a faculty member on the Medical School campus. Dr. Cort assured him that Illinois was a good school and parasitology was a good field. Thus reinforced, Sewell Hopkins applied for and received the research assistantship offered by Dr. Ward. He entered Illinois in 1928 and received his M.S. degree in 1929 and the Ph.D. in 1933. His dissertation title was "The Papillose Allocreadiidae" which was published in the "Illinois Biological Monographs" in 1934.

Dr. Hopkins was married during the Christmas of 1929 to Pauline Cole of Macon, Illinois who was a senior undergraduate Home Economics major at the University of Illinois when he met her in the spring of 1929. Two sons were born to this marriage.

Upon graduation Dr. Hopkins joined the faculty at Danville Junior College in Danville, Illinois, which was only in its first year of operation. However, in 1934 he took a better paying job with the USDA in Beltsville, Maryland. Here he met Benjamin Schwartz and E. W. Price who later introduced him to L. A. Spindler who was his immediate supervisor and who explained his responsibilities on a trichinosis project. The project was to last only about one year. At its completion he was asked to stay at the USDA, but he had decided that teaching was what he enjoyed most, so in 1935 he joined the faculty at Texas A&M College. He remained at Texas A&M until his retirement in 1972 except for a brief interlude with the Virginia Fisheries Laboratory in 1944–1946. He directed dissertations on everything from Myxomycetes to Scyphozoa and took pride in his versatility. Speaking for myself, and I am sure for his other graduate students as well, he was a tireless worker who knew his subject well and who asked no more of others than he asked of himself. He died in Gloucester, Virginia on November 15, 1984.

T. T. Dunagan, Professor of Physiology and Pharmacology, Southern Illinois University, Carbondale, Illinois 62901.

Editor's Note: Key Words

Key words or index descriptors help readers determine if they wish to read an article in full. Authors should provide 3 to 10 key words or short phrases not included in the title that can be used by indexers in preparing the index. Scientific names of all parasites and hosts should be included. Authors are requested to add the key words to the title page of manuscripts.

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53(1), 1986, pp. 151–154

PRESENTATION OF THE 1985 ANNIVERSARY AWARD TO A. MORGAN GOLDEN

By Bryce C. Redington, 22 November 1985
Helminthological Society of Washington

Figure 1. Dr. Redington (left) presenting Anniversary Award to Dr. Golden.

Mr. President, members of the Helminthological Society of Washington, and guests: This evening, as we mark the 75th Anniversary of our Society, we honor one of our members by conferring upon him the 1985 Anniversary Award. We are proud to present Dr. A. Morgan Golden with the Helminthological Society of Washington's Anniversary Award for 1985.

The Awards Committee has been charged by the Executive Committee to recommend a candidate for the Anniversary Award according to the Bylaws of the Society. This award is made to a present or past member of the Society who is honored for one or more of the following achievements:

(a) outstanding contributions to parasitology or related sciences that bring honor and credit to the Society,
(b) an exceptional paper read at a meeting of the Society or published in its Proceedings,
(c) outstanding service to the Society, and
(d) other achievement or contribution of distinction that warrants highest and special recognition by the Society.

Morgan Golden clearly qualifies for this award based on these criteria.

Morgan Golden was born on July 13, 1920, near the farming community of Milledgeville, Georgia. He received his B.S. Degree in 1950 from the University of Georgia and followed with his M.S. Degree from the same institution in 1951. His thesis on the subject of root-knot nematodes represented a preview of the subject which Morgan was to pursue in his life's work and which would lead to his becoming a world expert in his field.

Morgan served as an Assistant Plant Pathologist at the University of Georgia from 1950 to 1951. He also worked for the Vanderbilt Company of New York as a Plant Pathologist in the development of fungicides in 1951.

From 1952 to the present, Morgan Golden has been employed as a zoologist by the USDA, Agricultural Research Service. From 1952 to 1956, while at Beltsville, he worked primarily on spiral and root-knot nematodes which included research involving their taxonomy, host relationships, and methods of controlling them chemically. During this time, he had enrolled at the University of Maryland, College Park, where he earned his Ph.D. Degree in 1956. His dissertation provided the first comprehensive treatise of the taxonomy and biology of the spiral nematodes.

Morgan then transferred to Salinas, California in 1956, where he was to establish a new ARS laboratory which concentrated on root-knot nematodes and nematode parasites of sugar beets. He made valuable contributions in this position, including the characterization of resistance to the sugar beet cyst nematode.

Three years later in 1959, Morgan was reassigned to Beltsville to plan and establish the present ARS research program on nematode taxonomy and morphology. In 1960, Morgan assumed responsibility for the USDA Nematode Collection and since then has worked to revitalize and expand this valuable collection which also includes material of earlier USDA workers. At present, this collection is one of the largest and most valuable of its kind worldwide. Currently, it boasts of over 25,000 cataloged slides and vials with specimens which originate from around the world. Of particular note is the Type Collection which currently contains the designated type specimens of more than 1,200 species.

Dr. Golden became a member of this Society on March 18, 1953. During the past 22 years, he has served the Society with distinction in numerous capacities. Beginning in 1973, he served in the role of Recording Secretary for two years. In 1975, he held the office of Vice President and in 1976 served as the 59th President of the Society. He presently is a member of the Editorial Board of our Proceedings. It should not go unsaid that in addition to these official duties, Morgan Golden lobbied for about 10 years in an attempt to have our Society incorporated. This important objective was finally accomplished in 1981 and was largely due to Morgan's persistence.

Dr. Golden has more than 140 publications recorded to his credit which have provided valuable contributions to our knowledge of plant parasitic nematology. Thirteen of these publications have appeared in our own Proceedings. In addition to his description of many new genera and species, his monograph of the Order Tylenchida represents a landmark contribution to the systematics of plant-parasitic nematodes. In 1983, Morgan and a co-worker published a second monograph which is an illustrated key to the cyst-forming genera and species of Heteroderidae in the Western Hemisphere. This particular family of nematodes includes the soybean cyst nematode which alone is estimated to cause 400 million dollars worth of damage annually to soybean crops. Also, over the years, Morgan has provided nematode identification for many colleagues and agencies for research, control, and regulatory purposes.

Morgan Golden also has served with distinction as an elected officer and on numerous committees of other professional and honorary societies. These include the Society of Nematologists and the Brayton H. Ransom Memorial Trust Fund.

Dr. Golden has been the recipient of numerous notable awards. In 1982, he received the "International Honor Award" which is given annually by the U.S. Department of Agriculture to a deserving recipient who has made significant contributions to the development, conduct, and evaluation of programs directed by the Office of International Cooperation and Development. Also in 1982, Morgan was awarded Honorary Membership to the Florida Nematology Forum in recognition of outstanding service to the science of nematology. Earlier this year, he was elected as a "Fellow of the Society of Nematologists" in recognition for his many years of scientific achievements.

It should not go unsaid that Morgan's wife, Thelma, also has been a longtime supporter of the science of nematology and served for several years as the first Archivist of the Society of Nematologists.

This evening we are celebrating the 75th Anniversary of the establishment of the Helminthological Society of Washington. It seems very appropriate, therefore, that we honor Dr. Golden, a plant nematode-oriented scientist to receive

Figure 2. Anniversary Award recipients in attendance at dinner meeting to mark the 75th Anniversary of the Heminthological Society of Washington. Seated (left to right) Dr. Margaret A. Stirewalt (1975), Ms. Mildred A. Doss (1961), Mrs. Virginia Jachowski representing Dr. Leo A. Jachowski, Jr. (deceased) (1976); standing (left to right) Dr. Leon Jacobs (1983), Dr. David R. Lincicome (1975), Dr. Gilbert F. Otto (1965), Dr. Harley G. Sheffield (1984) and Dr. A. Morgan Golden (1985).

this award as we recall that one of the Society's founders, Dr. N. A. Cobb, is recognized as the father of American nematology.

On behalf of the Helminthological Society of Washington and the members of the Awards Committee (Margaret Stirewalt and Ronald Fayer), I am pleased and honored to present the 1985 Anniversary Award to Dr. A. Morgan Golden.

Acceptance of the 1985 Anniversary Award By A. Morgan Golden

President Reid, Members of the Executive Committee, and Ladies and Gentlemen:

My remarks tonight will be very brief. I would first like to thank Dr. Redington and his Awards Committee, the Executive Committee, and others who might have been involved in honoring me with this prestigious Anniversary Award. Throughout my career I have had a close, special feeling for HelmSoc. When I came to Beltsville to work in Nematology with Dr. Steiner, Edna Buhrer, A. L. Taylor and others, an important event each month was to go to the HelmSoc meeting. During those earlier times and since then, I have found our meetings stimulating, and have never failed to learn something new and of interest. I think this may be due not only to the excellence and quality of the presentations given, but also to the diversity of the scientific areas represented by the membership of HelmSoc. This diversity is of course reflected also in our superb Journal, the *Proceedings of the Helminthological Society of Washington.*

In plant nematode research, I have always felt right at home, and recognized common bonds, with my friends and colleagues in the various areas of parasitology and related sciences. As we know, these bonds of strength, friendship, and commonality had been long established in

HelmSoc by various outstanding workers, including in my own area: N. A. Cobb, a Charter member of HelmSoc and Father of Nematology in the United States; G. Steiner, President and active in many society activities; J. R. Christie, first Editor of the Proceedings (served 14 years); and Edna Buhrer, Corresponding Secretary-Treasurer for 38 years (1934–1971). I think my own research has been enhanced and enriched by my associations in HelmSoc; and it is indeed gratifying to have it recognized by this great Society.

I view my opportunities to have served HelmSoc in some ways over the years as representing a privilege, a pleasure and an honor. Thus, I am especially delighted to be honored with the 1985 Anniversary Award tonight. I accept it with humility, deep gratitude, and much pride—Thank you very much.

REQUEST FOR OUT-OF PRINT BACK ISSUES

Your Society is very short on the following issues of the Proceedings: 27(1); 28(2); 30(1); 37(1); and the 1975 special issue on Parasites of Equines. If you have these issues and would like to donate them, please contact the Corresponding Secretary/Treasurer (M. D. Ruff, API, BARC-East, Beltsville, MD 20705) or send the back issues to Helminthological Society of Washington, % Allen Press, Inc., 1041 New Hampshire St., Lawrence, KS 66044. We will be glad to reimburse you for the shipping. You can claim a tax deduction for their value and at the same time help your Society meet its orders for complete back issues.

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Proceedings is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues are payable on notification of election. Send this completed form to:

The Recording Secretary
Helminthological Society of Washington
Post Office Box 368
Lawrence, Kansas 66044

Print name: _____ Date of birth: _____

Mailing address: _____

_____

_____

Degree and year received: _____

Present position: _____

Field of interest: _____

_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
(a member)

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