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Lampanyctophilus wisneri gen. et sp. n. (Monogenea: Diclidophoridae), a Gill Parasite of *Lampanyctus ritteri* (Myctophidae) from the Eastern Pacific and an Emended Description of *Myctophiphilus sprostonae* (Martin, 1973) comb. n.

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ABSTRACT: *Lampanyctophilus wisneri* gen. et sp. n. (Monogenea: Diclidophoridae, Diclidophorinae) is proposed for specimens found on the gills of the mesopelagic fish, *Lampanyctus ritteri* Gilbert (Myctophidae), collected from the Pacific Ocean off central and southern California. The new genus differs from previously described genera of the subfamily by having haptoral clamps with peripheral sclerites of the anterior jaw fused and the lamellate extension of sclerite b reduced to two transverse sclerites that support the posterior jaw; and by being the only monogenean reported from mesopelagic fishes of the Pacific Ocean. *Diclidophora sprostonae* Martin, 1973, was transferred to *Myctophiphilus* Mamaev, 1976, becoming *M. sprostonae* (Martin, 1973) comb. n., because of the fused sclerites of anterior jaw and presence of prostatic vesicle. *Myctophiphilus sprostonae* differs from *M. arabicus* Mamaev, 1976, in detail of clamp morphology, shape of body, testes, and ovary.

The family Myctophidae is well represented in the mesopelagic fish fauna of the eastern Pacific Ocean (Wisner, 1976), but myctophids rarely harbor adult helminths (Collard, 1970; Noble and Collard, 1970). An undescribed monogenean was collected from the gills of *Lampanyctus ritteri* Gilbert (Myctophidae) during a survey of mesopelagic fishes for parasites.

Materials and Methods

Between December 1966 and December 1969, 108 specimens of *L. ritteri* were collected off the Pacific coast of California with an Isaacs-Kidd midwater trawl at depths between 100 and 1,000 m. Immediately after capture, and aboard ship, gills were removed and examined with a dissecting microscope. Monogeneans were fixed in AFA (alcohol–formalin–acetic acid) under slight coverglass pressure, and stored in 70% ethanol. In the laboratory, specimens were stained in Semichon's acetocarmine or Van Cleave's hematoxylin, dehydrated, cleared in methyl benzoate, and mounted in Histoclad. Observations were made using standard light microscopy and Nomarski differential interference contrast; figures were drawn with the aid of a drawing tube. Measurements are in micrometers unless otherwise stated; ranges are followed by means in parentheses. Clamp nomenclature is that of Llewellyn (1958). Representative specimens have been deposited in the United States National Museum (USNM) Helminthological Collection, Beltsville, Maryland, and the Harold W. Manter Laboratory (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska; balance of specimens in author's collection.

Results

Diclidophoridae Cerfontaine, 1895

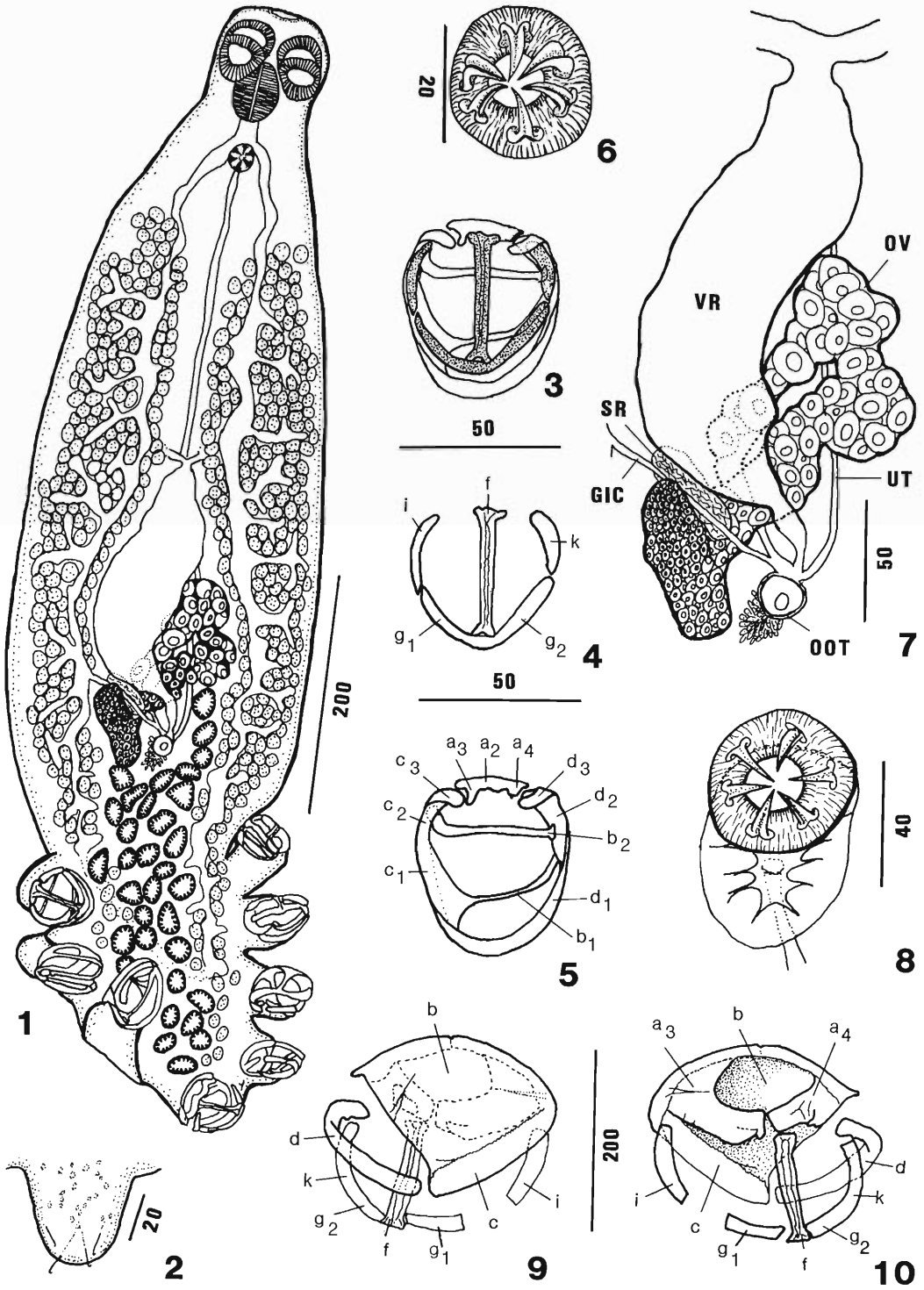
Diclidophorinae Cerfontaine, 1895

Lampanyctophilus gen. n.

GENERIC DIAGNOSIS: Diclidophoridae sensu Beverley-Burton, 1984, Diclidophorinae sensu Mamaev, 1976. Body proper undivided. Buccal suckers paired, septate. Haptor not set off from body, bearing 4 pairs of pedunculate clamps. Clamps asymmetrical with opposable jaws; anterior jaw composed of 5 sclerites including 2 peripheral, 2 transverse and 1 proximal; median sclerite absent; posterior jaw composed of 5 sclerites including 1 median and 2 pairs of peripheral sclerites. Terminal lappet with 2 pairs of marginal hooks. Ceca diverticulate, extending into haptor. Testes numerous, para- and postovarian. Genital corona armed with 6 grooved and recurved spines. Prostatic vesicle absent. Ovary C-shaped. Genito-intestinal canal present. Vaginae rudimentary, or not visible. Parasites on gills of Myctophidae. Type and only species: *L. wisneri.*

Lampanyctophilus wisneri sp. n. (Figs. 1–7)

DESCRIPTION (based on 10 specimens, 6 measured): With characters of the genus. Body total length 0.655–1.256 (0.995) mm, maximum width



Figures 1-7. ***Lampanyctophilus wisneri*** **gen. et sp. n., holotype, unless otherwise stated. 1. Ventral view. 2. Terminal lappet (paratype). 3. Entire clamp, posterior view. 4. Posterior jaw of clamp, posterior view. 5. Anterior jaw of clamp, posterior view. 6. Genital corona. 7. Detail, female reproductive system. Figures 8-10.** ***Myctophiphilus sprostonae*** **(Martin, 1973) comb. n., holotype. 8. Genital corona and prostatic vesicle. 9. Clamp, anterior**

0.183–0.576 (0.338) mm near midbody. Buccal suckers 44–65 (55) long by 26–43 (39) wide, ovoid. Haptor 141–380 (278) long by 183–441 (304) wide. Terminal lappet with marginal hooks 9 long. Clamps 48–65 (55) long by 37–57 (48) wide; posterior jaw formed by median sclerite f, sclerite i, sclerites g_1 and g_2 fused distally, and sclerite k; anterior jaw formed by sclerite a ($=a_2a_3a_4$), sclerite c ($=c_1c_2c_3$), sclerite d ($=d_1d_2d_3$); median sclerite a_1 absent; sclerites c_1 and d_1 fused distally; sclerites a_3 and c_3 approaching each other medially, as do sclerites a_4 and d_3; lamellate extension b reduced to separate sclerites b_1 and b_2; b_1 broadly fused to c_1 distally, narrowing to an obliquely transverse bar fused to d_2 proximally; b_2 narrow and transverse, articulating at junction of c_2 and a_3, and with d_2 near proximal portion of anterior jaw.

Mouth small, subterminal, ventral. Pharynx 35–61 (48) long by 36–48 (42) wide. Esophagus 38–44 (40) long. Ceca laterally diverticulate, extending to level of third pair of clamps.

Testes subspherical, 34–62 in number, intercecal, extending into haptor to level of fourth pair of clamps. Vas deferens 475–665 (561) long, extending anteriorly along midline and joining genital corona. Genital corona 21–27 (24) in diameter, muscular, immediately posterior to cecal bifurcation; spines 10–13 (11) long by 4 wide.

Ovary 219–433 (334) long by 29–95 (53) wide, more or less C-shaped, with or without distal extension filled with ova, median in third quarter of body. Seminal receptacle 42–87 (60) long by 11–42 (23) wide, immediately anterior to proximal portion of ovary; genito-intestinal canal dextral. Vitelline follicles numerous, extending from near cecal bifurcation to level of fourth pair of clamps. Vitelline reservoir 106–266 (189) long by 57–76 (68) wide, anterodextral to ovary. Vaginal pores, if visible, symmetrical, on lateral margins near level of genital corona.

HOST: *Lampanyctus ritteri* Gilbert; Myctophidae.

HABITAT: Gills.

LOCALITIES (south to north): southwest of Ensenada, Baja California, Mexico (31°30′N, 117°55′W); south of San Juan Seamount off Santa Barbara, California (32°40′N, 121°W, locality of holotype); south of Santa Rosa–Cortez Ridge off Santa Barbara, California (33°20′N, 119°50′W); West Cortez Basin off Santa Barbara, California (32°10′N, 119°10′W); south of Davidson Seamount off central California (34°40′N, 122°45′W); south southeast of Davidson Seamount off central California (35°N, 122°W); southwest of Farallon Islands, west of Pioneer Seamount off central California (37°N, 117° 15′W).

PREVALENCE: On 10 of 108 fish examined, 9.3%.

INTENSITY: One per host.

TYPE SPECIMENS: Holotype, USNM Helm. Coll. No. 79178; paratypes, USNM Helm. Coll. No. 79179, HWML No. 30572, HWML No. 30573.

ETYMOLOGY: The generic name indicates the affinity of this parasite for fish of the genus *Lampanyctus*. The specific name honors Robert L. Wisner, University of California, San Diego, Scripps Institution of Oceanography, for his extensive work on the myctophid fishes of the eastern Pacific Ocean.

REMARKS: *Lampanyctophilus* most closely resembles *Flexophora* Prost and Euzet, 1962, in the shape and number of genital corona spines; the absence of median sclerite a_1; the persistence of sclerites a_2, a_3, and a_4; the distal fusion of sclerites c_1 and d_1; and the presence of a terminal lappet with marginal hooks. *Lampanyctophilus* differs from *Flexophora* by having septate buccal suckers, sclerites g_1 and g_2 fused, lamellate extension b reduced to b_1 and b_2; by lacking a prostatic vesicle; and by parasitizing fishes in a different order (Myctophiformes). *Lampanyctophilus* resembles *Myctophiphilus* Mamaev, 1976 in shape of body, septate buccal suckers, presence of parovarian testes, and shape and number of genital corona spines. *Lampanyctophilus* differs from *Myctophiphilus* in clamp morphology, the absence of a prostatic vesicle, and the presence of a terminal lappet with marginal hooks.

Because *Diclidophora sprostonae* Martin, 1973, was collected from a myctophid (*Diaphus watasei* Jordan and Starks) in the Indian Ocean, the holotype was examined for comparison with *L. wisneri*. This study revealed that *D. sprostonae* belongs in the genus *Myctophiphilus* Mamaev,

←

view, sclerite g_1-i broken. 10. Clamp, posterior view, sclerite g_1-i broken. Abbreviations: GIC, genito-intestinal canal; OOT, ootype with ovum surrounded by Mehlis' gland; OV, ovary; SR, seminal receptacle; UT, uterus; VR, vitelline reservoir. Scales in micrometers.

1976, and that the species description (Martin, 1973) should be emended to include description of buccal suckers, prostatic vesicle, and detailed clamp morphology.

Myctophiphilus sprostonae (Martin, 1973) comb. n.

syn. *Diclidophora sprostonae* Martin, 1973 (Figs. 8-10)

EMENDED DESCRIPTION (based on holotype, Allan Hancock Foundation No. 639): Buccal suckers 63–68 long by 51–59 wide, septate. Genital corona 44 in diameter. Prostatic vesicle 68 long by 48 wide; prostate cells large but indistinct. Clamps 140–220 long by 112–260 wide; asymmetrical with opposable jaws; posterior jaw formed by median sclerite f, peripheral sclerites i and g_1 fused, and peripheral sclerites g_2 and k fused; anterior jaw formed by a_3, a_4, lamellate extension b, c, and d; median sclerites a_1 and a_2 absent; lamellate extension b contiguous with c along entire inner border; a_3 and a_4 approaching each other medially, forming ring with proximal portion of lamellate extension b.

REMARKS: *Myctophiphilus sprostonae* resembles *M. arabicus* Mamaev, 1976, in clamp and genital corona morphology, presence of a prostatic vesicle, and host family (Myctophidae), but differs in details of clamp morphology (a_3 and a_4 not fused medially), fan-like arrangement of testes, ribbon-like ovary, and host genus.

Discussion

Llewellyn and Tully (1969) in a study of the subfamily Diclidophorinae found that parasitological evidence, in general, supported a classification of fishes that split Gadiformes into several distinct groups. Subsequently, the diclidophorine genera *Lampanyctophilus, Myctophiphilus, Polyipnicola* Mamaev and Paruchin, 1975, and *Imbjumia* Mamaev and Paruchin, 1984, have been described. They are noteworthy in that their hosts are mesopelagic and bathypelagic fishes in the families Argentinidae, Myctophidae, Sternoptychidae, and Gnathostomatidae, respectively, and most of the remaining Diclidophorinae are parasitic on benthopelagic fishes primarily in the families Gadidae and Macrouridae (Gadiformes). Rohde (1982), in discussing ecological niches of marine parasites, distinguishes between ecological and phylogenetic host specificity. With the exception of *Diclidophora embiotocae* Hanson, 1979, members of Diclidophorinae exhibit an ecological specificity for hosts inhabiting deep bottom waters (benthopelagic) or deep open ocean waters (meso- and bathypelagic), in addition to exhibiting distinct phylogenetic host specificity.

Lampanyctophilus wisneri is the only monogenean reported from a Pacific mesopelagic fish. Preliminary reports of an "unidentified Macravalvitrematidae" from *Lampanyctus ritteri* by Collard (1968), Love and Moser (1983), and Noble and Collard (1970) were based on two specimens in the type series of *L. wisneri.*

Mamaev (1976) proposed a system of phylogeny for the Diclidophoridae based on clamp morphology and the structure of the terminal male genitalia. Because the clamps of *Lampanyctophilus* lack sclerite a_1, and have a highly modified lamellate extension b, forming two transverse bars in the anterior jaw, in addition to agreeing with the terminal male genitalia of the diclidophorine trunk, *Lampanyctophilus* can be added to Mamaev's (1976) phylogeny as a branch from the *Flexophora–Polyipnicola–Myctophiphilus* line.

Addendum

Since the acceptance of this manuscript for publication, it has come to the attention of the author that *Myctophiphilus* was synonymized with *Polyipnicola* (Mamaev, Y. L., T. N. Mordvinova, and A. M. Paruchin, 1985, Monogenea in the genus *Polyipnicola* (Diclidophoridae). Vestnik Zoologii, No. 5:8–14). As reported in Helminthological Abstracts (1986, 55:11), "*Diclodophora sprostonae* Martin, 1973 was transferred to *Polyipnicola* as *P. sprostonae* (Martin, 1973) n. comb. *species inquirenda.*" Therefore, *Myctophiphilus sprostonae* (Martin, 1973) comb. n. (present work) becomes a synonym of *P. sprostonae* (Martin, 1973) Mamaev, Mordvinova, and Paruchin, 1985. The present work verifies *P. sprostonae* as a valid and distinct species.

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I wish to thank Dr. Carl L. Hubbs, Robert L. Wisner, and the captains and crews of the R/V *Melville* and the R/V *Thomas Washington,* University of California, San Diego, Scripps Institution of Oceanography, for their assistance in collecting and identifying the fishes. Thanks are also due my colleagues at the University of California, Santa Barbara, Sneed B. Collard and Janet Schlechte, for collecting additional fishes; to Dr. Elmer R. Noble, Professor Emeritus,

U.C.S.B., for overall encouragement and support; to Dr. Janet Haig, Allan Hancock Foundation, for loaning the holotype of *D. sprostonae.* Special thanks are extended to Professor Mary Hanson Pritchard of the H. W. Manter Laboratory, University of Nebraska State Museum, for her counsel and helpful suggestions. Supported in part by U.S.P.H.-N.I.H. Trainee Grants 5 TI-GM 900-02 and 5 T01 AI 00327-02, and N.S.F. Grant GB4868.

Literature Cited

Beverley-Burton, M. 1984. Monogenea and Turbellaria. Pages 5–209 *in* L. Margolis and Z. Kabata, eds. Guide to the Parasites of Fishes of Canada. Part I. Canadian Special Publication of Fisheries and Aquatic Sciences, No. 74, Ottawa.

Collard, S. B. 1968. A study of parasitism in mesopelagic fishes. Doctoral Dissertation, University of California, Santa Barbara. 251 pp.

———. 1970. Some aspects of host-parasite relationships. Pages 41–56 *in* S. F. Snieszko, ed. A Symposium on Diseases of Fishes and Shellfishes. American Fisheries Society Special Publication, Number 5, Washington, DC.

Llewellyn, J. 1958. The adhesive mechanisms of monogenetic trematodes: the attachment of species of the Diclidophoridae to the gills of gadoid fishes. Journal of the Marine Biological Association of the United Kingdom 37:67–79.

———, **and C. M. Tully.** 1969. A comparison of speciation in diclidophorinean monogenean gill parasites and in their fish hosts. Journal of the Fisheries Research Board of Canada 26:1063–1074.

Love, M. S., and M. Moser. 1983. A checklist of parasites of California, Oregon and Washington marine and estuarine fishes. National Oceanic and Atmospheric Administration Technical Report, National Marine Fisheries Service, Special Scientific Report Fisheries 777, Washington, DC. 576 pp.

Mamaev, Yu. L. 1976. The system and phylogeny of monogeneans of the family Diclidophoridae [in Russian]. Proceedings of the Institute for Biology and Pedology, Far East Science Center, Academy of Sciences of the U.S.S.R., New Series 35:57–80.

Martin, W. E. 1973. A new species of trematode (Trematoda: Monogenea) from the gills of *Diaphus watasei* Jordan and Starks, 1904. Bulletin of the Southern California Academy of Sciences 72:109–111.

Noble, E. R., and S. B. Collard. 1970. The parasites of midwater fishes. Pages 57–68 *in* S. F. Snieszko, ed. A Symposium on Diseases of Fishes and Shellfishes. American Fisheries Society Special Publication, Number 5, Washington, DC.

Rohde, K. 1982. Ecology of Marine Parasites. University of Queensland Press, New York. 245 pp.

Wisner, R. L. 1976. The taxonomy and distribution of lanternfishes (family Myctophidae) of the eastern Pacific Ocean. Naval Oceanographic Research and Development Activity, Report 3, Bay St. Louis, MS. 229 pp.

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Ecological Aspects of *Multicalyx cristata* (Aspidocotylea) Infections in Northwest Atlantic Elasmobranchs

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ABSTRACT: Sixteen species of elasmobranchs were collected between Cape Fear, North Carolina, and Long Island, New York. Specimens of *Multicalyx cristata* were recovered only from the bullnose ray, *Myliobatis freminvillei,* a new host record (prevalence 10.7%, 15 of 140), and the cownose ray, *Rhinoptera bonasus* (prevalence 1.3%, 1 of 76). Only bullnose rays with a disc width greater than 68 cm were infected, but no differences in prevalence were found with sex or season. Intensity of infection was 1 or 2 worms per host.

Multicalyx cristata (Faust and Tang, 1936) Stunkard, 1962 was originally described from the spiral valve of the cownose ray (*Rhinoptera bonasus*) collected near Biloxi Bay, Mississippi. Since then, *M. cristata* has been collected from gall bladders of 10 elasmobranch species (Dollfus, 1958; Stunkard, 1962; Hendrix and Overstreet, 1977; Bray, 1984) and 2 teleost species (Hendrix and Overstreet, 1977) from both the Atlantic and Pacific oceans. The small number of elasmobranch host species examined in previous studies has precluded meaningful interpretation of such factors as intensity and prevalence, especially in relation to host size or collection season. The availability of elasmobranchs in samples made along the Atlantic coast of the United States permitted investigation of the distribution and ecology of *M. cristata* in several host species.

Materials and Methods

Elasmobranchs from continental shelf waters of the eastern U.S.A. from Cape Fear, North Carolina, to Long Island, New York were examined for *M. cristata* during the spring (March) and fall (October) (1982–1984) National Marine Fisheries Service Ground Fish Surveys aboard the R/V *Albatross IV.* Cownose rays examined within Chesapeake Bay were obtained during June and July 1984 by sampling pound nets at the mouth of the York River, Virginia. A few sharks including single specimens of *Odontaspis taurus, Nagaprion brevirostris,* and *Carcharhinus limbatus,* and five of *C. plumbeus* were collected in July and August 1984 by long-line in the Atlantic Ocean off the Eastern Shore of Virginia. Gall bladders and bile ducts of all hosts were carefully examined for *M. cristata.* Specimens of *M. cristata* were relaxed in a saturated chlorobutanol–seawater mixture and fixed in 10% formalin and seawater (Hargis, 1953) or placed in elasmobranch physiological saline as formulated by Bapkin et al. (1933) and chilled on ice for transport back to VIMS.

Results

The host species examined and the prevalence of *M. cristata* within those hosts are presented in Table 1. The bullnose ray (*Myliobatis freminvillei*), a new host record for *M. cristata,* had the highest prevalence of infection. Of the 140 bullnose rays examined, 15 were infected. Only those with a disc width greater than 68 cm were infected, and among those animals the prevalence was 14.7% (15 of 102). Prevalence did not increase with host size beyond 68 cm, and prevalence did not differ between sexes of large rays. Intensity of infection was 1 or 2 worms, with 38% of the infected hosts having 2 worms. No differences in intensity or prevalence of infection were detected between spring and fall samples, even though the majority of bullnose rays were south of Cape Hatteras, North Carolina, in spring and north of the Cape in fall, suggesting a seasonal migration pattern for the host. The single infected *R. bonasus* from Chesapeake Bay was an 88-cm disc width male. Total length of both relaxed and preserved worms ranged from 72 to 129 mm.

Discussion

Only 2 species examined, the type host *Rhinoptera bonasus* and the confamiliar *Myliobatis freminvillei,* were infected. Infection of these hosts is probably a consequence of their similar distributions and feeding habits. Almost all reported aspidocotyleans require a mollusc as the first or only host in the life cycle (Yamaguti, 1963; Rohde, 1972). The intermediate host of *M. cristata* is not known, but rays feed extensively on bivalve molluscs, which probably serve as the intermediate host.

Table 1. Prevalence of *Multicalyx cristata* in elasmobranch hosts from the western North Atlantic.

	Infected/examined	Size range (cm)
Sharks		Length
Odontaspididae		
**Odontaspis taurus* (Rafinesque)	0/2	92–223
Carcharhinidae		
Carcharhinus limbatus (Valenciennes)	0/1	156
C. obscurus (Lesueur)	0/1	106
C. plumbeus (Nardo)	0/7	90–174
**Mustelus canis* (Mitchill)	0/42	51–119
Negaprion brevirostris (Poey)	0/1	258
Rhizoprionodon terraenovae (Richardson)	0/8	88–99
Squalidae		
Squalus acanthias Linnaeus	0/14	71–92
Squatinidae		
Squatina dumerili Lesueur	0/7	40–112
Rays		Disc width
Rajidae		
Raja eglanteria Bosc	0/1	
Dasyatidae		
Dasyatis americana Hildebrand and Schroeder	0/1	59
**D. centroura* (Mitchill)	0/3	91–183
**D. sayi* (Lesueur)	0/29	38–104
Gymnura altavela (Linnaeus)	0/9	59–295
Myliobatidae		
**Rhinoptera bonasus* (Mitchill)	1/49 (bay)	33–99
	0/27 (ocean)	50–87
Myliobatis freminvillei Lesueur	15/140	29–120

* Infected hosts previously cited by other investigators.

Hendrix and Overstreet (1977) collected juvenile worms from 2 teleost fish hosts, *Menticirrhus americanus* and *Sphoeroides testudineus,* and Manter (1931) also found juvenile worms in *M. americanus.* Teleosts may act as paratenic hosts, which may be a route for infection of neritic predators, such as many of the sharks, as suggested by Hendrix and Overstreet (1977). Rays also occasionally feed on benthic teleosts. Infection of rays larger than 68 cm suggests that they do not feed on either infected mollusc intermediate hosts or teleost paratenic hosts until they reach a particular size. However, infection of relatively small teleosts requires that the intermediate host be small. It is possible that teleosts and rays are infected via different intermediate hosts, as Rohde (1972) has indicated that there is no strict specificity with regard to the mollusc host in those species where the life cycle is known. Smith (1982) found that small cownose rays were unable to reach deep burrowing bivalves and fed instead on shallow infauna or epifauna such as *Mytilus edulis.* Adult cownose rays fed primarily on *Mya arenaria* and *Macoma balthica* in Chesapeake Bay. Two other eagle rays, *Myliobalis aquila* and *M. californica,* feed occasionally on teleosts as adults (Capape, 1976; Talent, 1982, respectively). Such dietary shifts may be responsible for the observed infection pattern. It is also possible that the aspidocotylean infecting teleosts is not *M. cristata* but some other species; however, infection experiments are required to determine this with certainty.

The large size of *M. cristata* within host gall bladders may lead to intraspecific competition which restricts infection intensity to 1 or 2 worms as has been suggested for the digenean *Hirudinella ventricosa* in *Acanthocybium solanderi,* the gyrocotylidean *Gyrocotyle* in *Chimaera montrosa,* and the eucestode *Abothrium gadi* in *Gadus morhua* (Iversen and Yoshida, 1957; Halvorsen and Williams, 1968; Williams and Halvorsen,

1971, respectively). Intensity of the much smaller *Taeniocotyle elegans* Olsson, 1869 (Stunkard, 1962) in the gall bladder of *Chimaera monstrosa* reaches 6 worms per host (Brinkmann, 1957). *Multicalyx cristata* found individually had developing eggs within the uterus indicating self-fertilization. The absence of seasonality between fall and spring sampling indicates that *M. cristata* may have a long life-span or that rays are continually exposed to infective stages, although juvenile stages were not found.

Absence of worms in other elasmobranchs examined, especially those that have been cited previously as hosts, may be a consequence of sample size. *Multicalyx cristata* has been collected from *Mustelus canis, Rhinobatos cemiculus,* and *Scoliodon terrae-novae* near Senegal (Dollfus, 1958), from *M. canis* from New Zealand (Manter, 1954), and from *Cephaloscyllium ventriosum* off California (Hendrix and Overstreet, 1977). Bray (1984) described specimens from *Sphyrna lewini* and *Odontaspis taurus* collected off South Africa. Stunkard (1962) also described specimens from *Dasyatis centroura* near Woods Hole, Massachusetts, and Hendrix and Overstreet (1977) collected specimens from *Pristis pectinata* and *D. sayi* in the Gulf of Mexico. Therefore, *M. cristata* has been collected from both sides of the Pacific and Atlantic oceans from a large number of definitive hosts and this wide zoogeographical range indicates that *M. cristata* probably has more than 1 intermediate host. It is unlikely that a single mollusc intermediate host would be found in such wide-ranging and varying habitats. Likely, *M. cristata* will be found in more elasmobranch species around the world as more hosts are examined.

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Literature Cited

Bapkin, B. P., D. J. Bowie, and J. V. V. Nicholls. 1933. Structure and reactions to stimuli of arteries (and conus) in the elasmobranch genus *Raja.* Contributions to Canadian Biology and Fisheries N.S. 8:207–225.

Bray, R. A. 1984. Some helminth parasites of marine fishes and cephalopods of South Africa: Aspidogastrea and the digenean families Bucephalidae, Haplosplanchnidae, Mesometridae and Fellodistomidae. Journal of Natural History 18:271–292.

Brinkmann, A. 1957. Fish trematodes from Norwegian waters. IIa. The Norwegian species of the orders Aspidogastrea and Digenea (Gasterostomata). Universitetet i Bergen, Arbok 1957, Naturvitenskapelig Rekke 4:1–31.

Capape, C. 1976. Study of the diet of the eagle ray *Myliobatis aquila* from the Tunisian coast. Journal du Conseil, Conseil International pour l'Exploration de la Mer 37:29–35.

Dollfus, R. P. 1958. Sur *Macraspis cristata* (E.-C. Faust et C.-C. Tang 1936) H.-W. Manter 1936 et sur une emendation necessaire a ma definition de la famille des Aspidogastridae (Trematoda). Annales de Parasitologie Humaine et Comparee 33: 227–231.

Halvorsen, O., and H. H. Williams. 1968. Studies on the helminth fauna of Norway IX. *Gyrocotyle* (Platyhelminthes) in *Chimaera monstrosa* from Olso Fjord, with emphasis on its mode of attachment and regulation in the degree of infection. NYTT Magasin for Zoologi (Oslo) 15:130–142.

Hargis, W. J., Jr. 1953. Chlorotone as a trematode relaxer, and its use in mass-collecting techniques. Journal of Parasitology 39:224–225.

Hendrix, S. S., and R. M. Overstreet. 1977. Marine aspidogastrids (Trematoda) from fishes in the northern Gulf of Mexico. Journal of Parasitology 63:810–817.

Iversen, E. S., and H. O. Yoshida. 1957. Notes on the biology of the wahoo in the Line Islands. Pacific Science 11:370–379.

Manter, H. W. 1931. Some digenetic trematodes of marine fishes of Beaufort, North Carolina. Parasitology 23:396–411.

———. 1954. Some digenetic trematodes from fishes of New Zealand. Transactions of the Royal Society of New Zealand 82:475–568.

Rohde, K. 1972. The Aspidogastrea, especially *Multicotyle purvisi* Dawes, 1941. Advances in Parasitology 10:78–151.

Smith, J. W. 1982. The life history of the cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), in lower Chesapeake Bay with notes on the management of the species. Unpublished Masters Thesis, College of William and Mary. 151 pp.

Stunkard, H. W. 1962. *Taeniocotyle nom. nov.* for *Macraspis* Olsson, 1869, preoccupied, and systematic position of the Aspidobothrea. Biological Bulletin, Marine Biological Laboratory, Woods Hole 122:137–148.

Talent, L. C. 1982. Food habits of the gray smoothhound *Mustelus californicus,* the brown smoothhound *Mustelus henlei,* the shovelnose guitarfish *Rhinobatos productus,* and bat ray *Myliobatis californica* in Elkhorn Slough California. California Fish and Game 68:224–234.

Williams, H. H., and O. Halvorsen. 1971. The incidence and degree of infection of *Gadus morhua* L. 1758 with *Abothriium gadi* Beneden 1871 (Cestoda: Pseudophyllidea). Norwegian Journal of Zoology 19:193–199.

Yamaguti, S. 1963. Systema Helminthum. Vol. IV. Monogenea and Aspidocotylea. Interscience Division, John Wiley & Sons, Inc., New York. 699 pp.

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Observations on the Life Cycle of *Ornithodiplostomum ptychocheilus* (Trematoda: Diplostomatidae)

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ABSTRACT: The experimental life cycle of *Ornithodiplostomum ptychocheilus* involves a physid snail (*Physa gyrina*) as first intermediate host; cyprinid fish (*Pimephales promelas, P. notatus*) as second intermediate hosts; and chicks, ducklings, sparrows, and mice as definitive hosts. Encysted neasci develop only on the brain of experimentally infected fish. Visceral metacercariae reported here and in the literature probably represent a distinct species differing in fish host(s), preferred site and site specificity in the fish host(s), as well as geographic distribution. Striking similarities in life histories and in morphologies, particularly of miracidia and cercariae, suggest that *Ornithodiplostomum* and *Posthodiplostomum* are synonymous.

Ornithodiplostomum ptychocheilus (Faust, 1917) Dubois, 1936 is a nearctic parasite of anatids (primarily *Mergus* spp.) and an ardeid (*Botaurus lentiginosus*) (Van Haitsma, 1930; Hoffman, 1960). However, probably due to their small size, adults have only rarely been reported. Metacercariae are more commonly seen and have been described on a number of occasions (Faust, 1917, 1918; Hughes and Piszczek, 1928). Metacercariae have been reported primarily in 1 of 3 situations: in the cranial cavity (brain) of cyprinids, in the body cavity of cyprinids, or in the body cavity of catostomids (Hoffman, 1954, 1958; Amin, 1969).

Van Haitsma (1930) first established the relationship between metacercarial and adult stages of *O. ptychocheilus.* Hoffman (1958) completed the life cycle with very brief descriptions of miracidium, mother and daughter sporocysts, and cercariae. Cyprinid fish were infected with cercariae from the snail *Physa anatina.* Metacercariae from the viscera and brain of cyprinids were fed to 1-day-old unfed chicks in which adults developed. *Physa gyrina* and *Physa integra* have also been reported as suitable first intermediate hosts (Sankurathri and Holmes, 1976; Radabaugh, 1980).

Sudarikov and Kurochkin (1968) observed a similar life cycle in the European *Ornithodiplostomum scardinii* Schulman *in* Dubinin, 1952. They fed brains of rudd, *Scardinius erythrophthalmus,* to 16 avian species and recovered one adult from a grey heron and numerous adults from pigeons and ducklings. Natural infections occurred in the merganser, *Mergus albellus.* Cercariae from naturally infected *Physa fontinalis* were similar to those of *Posthodiplostomum cuticola* (v. Nordmann, 1832).

My study was undertaken to re-examine the life cycle of *Ornithodiplostomum ptychocheilus* and to determine if morphology of larval stages, particularly miracidium and cercariae, was consistent with Dubois' (1970) treatment of this genus and species in his scheme of classification for the family Diplostomatidae.

Materials and Methods

Laboratory-reared snails (*Helisoma trivolvis, Lymnaea catascopium, Lymnaea palustris, Physa gyrina,* and *P. integra*) were maintained in artificial spring water (Ulmer, 1970) at 22–26°C with crushed oyster shell. They were fed leaf lettuce supplemented once weekly with fish food (Tetramin®). Physid snails used for experimental exposures were less than 2 mo old (usually less than 1 mo). Ages of other species varied. Snail cultures originated with wild snails collected in the vicinity of Iowa Lakeside Laboratory, Milford, Iowa, except for *L. catascopium,* which was originally collected near Alanson, Michigan. Uninfected fathead minnows from Neosho National Fish Hatchery (Neosho, Missouri), other uninfected fish from Squaw Creek, Iowa (Story County, R-24W, T-84N, S-33), and naturally infected fathead minnows from Highway 53 Frontage Road Pond, Wisconsin (Eau Claire County, R-9W, T-27N, S-35) were kept in artificial spring water at 22–26°C and fed fish food and frozen brine shrimp. Fathead minnows used in experimental infections were less than 1 yr old (20–37 mm total length). Ages and sizes of other species varied.

To obtain eggs for life history studies, 1-day-old unfed chicks were force-fed whole brains of infected fathead minnows and maintained without food at 22–26°C. Feces from infected chicks were collected in artificial spring water, decanted 2–3 times in a 2,000-ml graduate, homogenized in a Waring blender, strained through 4 layers of cheesecloth and 3 graded sieves (300, 250, and 150 μm mesh), and decanted 2–3 more times in a 250-ml graduate. Eggs were incubated in the dark at 30°C. Some eggs were stored at 10°C for up to 3 mo with no apparent effect on hatchability or miracidial infectivity. Miracidia (10 per vial) were pipetted into plastic vials (2.5 × 1.2 cm) containing enough

water to cover the bottom. One laboratory-reared snail was placed in each vial for 2–3 hr. Then, vials were filled with water, corked so as to allow an air bubble and left overnight. Subsequently, snails were kept in lots in 1-gallon aquaria for about 2 wk and then isolated in 5-cm-diameter stender dishes.

Whole mounts of various stages were prepared using standard staining techniques. Miracidia were also fixed in hot (70°C) 0.5% silver nitrate, rinsed in distilled water, exposed to light, and mounted in glycerine to determine ciliated epidermal cell boundaries. Larval stages were studied alive with both light and phase-contrast microscopy. Intra vitam dyes (neutral red, nile blue sulfate) aided in examination of miracidia and cercariae. Measurements are in micrometers and from specimens fixed in hot (70°C) 10% formalin unless noted otherwise; means are followed by ranges in parentheses.

Adult
(Fig. 1)

DESCRIPTION (measurements from 11 specimens unless noted otherwise from unfed chicks): Body 432–702 (509), indistinctly bipartite when fixed. Forebody 236–389 (328) by 336–452 (393), concave ventrally. Hindbody 162–294 (241) long, bluntly rounded. Oral sucker 30–45 (37) by 20–46 (28). Acetabulum 25–48 (31) by 32–38 (36). Holdfast 70–110 (89) by 102–133 (117). Prepharynx 0–10 long by 10–18 ($N = 5$) wide. Pharynx 30–43 (35) by 16–27 (22). Esophagus 10–48 (20) by 7–15 (12). Ceca 7–14 (9) wide, extending to near posterior hindbody margin. Anterior testis 50–125 (93) by 67–208 (171), elliptical to triangular, in anterolateral portion of hindbody. Posterior testis 220–315 (273) wide, bilobed, lobes connected by a narrow isthmus. Larger lobe 37–155 (101) in diameter. Smaller lobe 47–115 (71) in diameter. Seminal vesicle large, posterior to posterior testis. Ejaculatory pouch 20–35 (30) by 20–53 (39) ($N = 4$), arises anteromedially from seminal vesicle. Ovary 21–52 (37) by 55–113 (86), elliptical, opposite anterior testis. Mehlis' gland lateral to ovary. Uterus with short ascending and longer descending limbs. Vitelline reservoir fills space between testes and ovary. Vitelline follicles abundant in forebody, reaching anterior margin of ventral sucker; less abundant and concentrated laterally in hindbody, extending to posterior margin of vitelline reservoir. Laurer's canal joining oviduct near midline just posterior to vitelline reservoir. Copulatory bursa 43–100 (69) by 105–178 (141), evaginable, with subterminal pore. Uterus and ejaculatory duct converge near genital pore. Eggs few (usually 1–2) in utero, when fixed 74–87 (80) by 49–56 (54). Freshly passed eggs 78–94 (88) by 62–74 (67), golden brown, transparent, with operculum 16–24 (21) ($N = 17$) in diameter.

HOSTS: *Gallus gallus,* domestic chick (unfed), experimental; *Anas platyrhynchos,* domestic mallard (Rouen strain), experimental; *Passer domesticus,* English sparrow (nestling), experimental; *Mus musculus,* albino mouse, experimental.

SITE OF INFECTION: In chicks, duodenum (posterior one-third), ileum (anterior one-half).

VOUCHER SPECIMENS: USNM Helm. Coll. No. 79025.

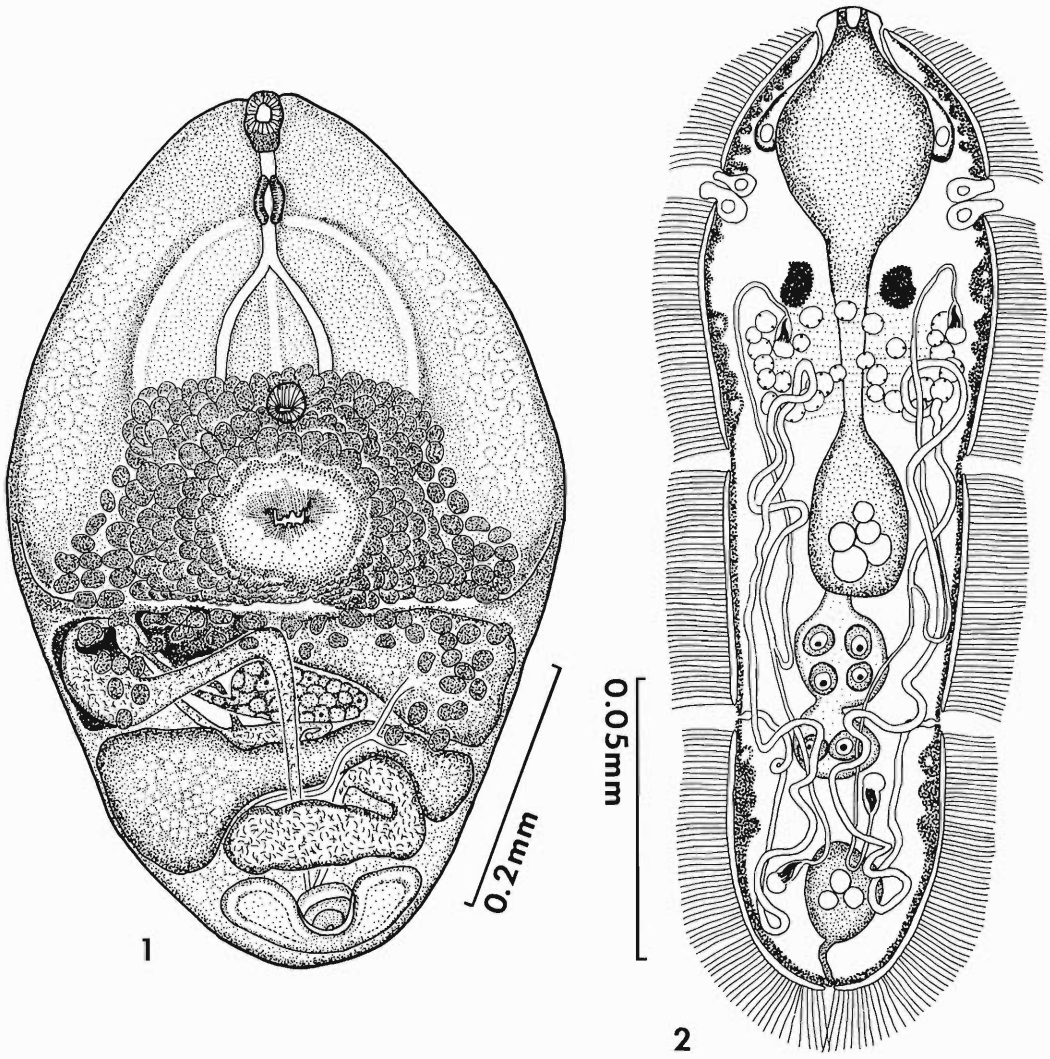
Remarks

Amphitypy was common, the ovary being on the right in 66 and on the left in 52 of 118 specimens. Other reproductive structures were correspondingly reversed. The relative size of the hindbody was greater in living than in fixed specimens and was greater in specimens fixed in hot fixative than in specimens fixed in cold fixative.

Van Haitsma (1930) previously described adult *O. ptychocheilus.* However, all of his specimens were either from naturally infected ducks or from ducks fed metacercariae from the peritoneum and mesentery of *Notropis deliciosus stramineus.* Adults described herein were reared in domestic chicks fed metacercariae from the brain of *Pimephales promelas.* There is no apparent morphological difference between body cavity-derived and brain-derived adults.

Miracidium
(Fig. 2)

DESCRIPTION: Body 95–127 (109) by 31–44 (37). Ciliated epidermal cells in 4 tiers in 6:9:4:3 = 22 pattern. First tier of 2 dorsal, 2 ventral, 2 lateral cells. Second tier of 2 dorsal, 2 dorsolateral, 2 lateral, 2 ventrolateral, 1 ventral cells. Third tier of 2 dorsolateral, 2 ventrolateral cells. Fourth tier of 1 dorsal, 2 ventrolateral cells. Apical papilla 2–6 (4) by 6–10 (8), lacking cilia. Apical gland bilobed with 4–5 nuclei in posterior lobe. Cytoplasm of apical gland coarsely granular, densely staining with neutral red or nile blue sulfate. Apical gland terminating in 4 ducts that traverse apical papilla. Cephalic glands paired, unicellular, with dense nuclei, finely granular cytoplasm, evident only under phase contrast microscopy, opening to exterior at base of apical papilla. Caudal sac syncytial with up to 4 nuclei lacking nucleoli; apparently attached to body wall posteriorly but not opening to exterior. Eyespots 7–9 (8) by 5–8 (7), pigmented, concave anterolaterally, near junction of first and second tier of



Figures 1, 2. *Ornithodiplostomum ptychocheilus*. 1. Adult, ventral view, from unfed chick (*Gallus gallus*), drawn from stained whole mount. 2. Miracidium, composite based on photograph of living specimen.

epidermal cells. Cerebral ganglion just posterior to eyespots. No commissures observed. Germinal cells about 6, in 2 clusters posterior to apical gland, with large nuclei and prominent nucleoli. Flame cell formula 2(1+1). Excretory pores near junctions of dorsolateral and ventrolateral epidermal cells of third tier on each side.

Remarks

Early cell divisions and development of the miracidium were similar to that as described by Pearson (1961) for *Neodiplostomum intermedium* Pearson, 1959. Many miracidia hatched when eggs incubated in the dark at 30°C for 8 days were brought into the light. However, the peak of hatching occurred after 9 days of incubation, some eggs requiring as many as 14 days.

Dubois (1970) characterized strigeid miracidia as relatively large, with 2 pairs of flame cells, and 22 (or 21?) ciliated epidermal cells arranged in 4 tiers (6:9[or 8?]:4:3). Pearson (1956) observed considerable variation in miracidia of *Alaria arisaemoides* Augustine and Uribe, 1927 (6:9:4: 3—8 specimens, 6:8:4:3—3 specimens, 6:10:4: 2—1 specimen). I found 16 *O. ptychocheilus* miracidia with the standard 6:9:4:3 pattern and 1

with a 6:8:4:3 pattern. In this specimen, 2 plates of the second tier were dorsal, 2 dorsolateral, 2 ventrolateral, and 2 ventral. Such variation within a species suggests more variation within a higher taxon. Pearson's (1961) contention that 6:9:4:3 = 22 is the correct formula and will be found throughout the strigeate trematodes seems unwarranted.

Forty-nine of 61 (80.3%) *Physa gyrina* exposed to 10 miracidia each became infected. Exposures were negative for 2 *Helisoma trivolvis,* 6 *Lymnaea catascopium,* 17 *L. palustris,* and 65 *Physa integra.* Radabaugh (1980), however, experimentally infected 5 of 26 *P. integra* with *O. ptychocheilus* miracidia. A careful re-examination of snail hosts is called for.

Sporocyst development in the snail host followed general patterns observed in other strigeoid trematodes (Pearson, 1956, 1961; Dönges, 1964, 1965; and others). At 22°C, mother sporocysts localized in the hepatopancreas within 2 days PI (postinfection). Daughter sporocyst embryos were in mothers by 6–9 days PI, and were released by 9–12 days PI. By 12 days PI, daughter sporocysts with early cercarial embryos were throughout the hepatopancreas. Shedding of cercariae began 21–32 days PI (usually on or near 24 days PI).

Cercaria
(Figs. 3, 4)

DESCRIPTION (based on measurements from 10 specimens fixed in hot [70°C] 10% formalin): Freshwater, bioculate, monostomate, longifurcocercaria. Body 112–169 (147) by 25–44 (32). Tailstem 153–199 (168) by 24–38 (30). Furcae 147–204 (166) along greatest curvature by 11–20 (16) at base. Eyespots pigmented, 3–5 in diameter, about two-thirds body length from anterior end, evident only in living specimens. Penetration organ 56–86 (72) by 17–35 (25), armed with scattered spines over anterior one-third. Penetration gland cells 6, of a single kind. Excretory bladder bipartite. Primary excretory tubules with 2 or 3 pairs ciliated patches. Eight pairs flame cells in body, 2 pairs in tailstem. Flame cell formula probably 2[(2+2)+(2+2)+(2)] = 20. Excretory duct terminating at excretory pore on margin of each furca. Genital primordium anterior to bladder. Acetabular primordium between second and third pairs of penetration gland cells. Tailstem aspinose but with "flagellets" of Hoffman (1958). Five to 6 pairs caudal bodies, evident only in young (<24 hr) cercariae. Distal half of each furca with fin fold continuous around tip.

Remarks

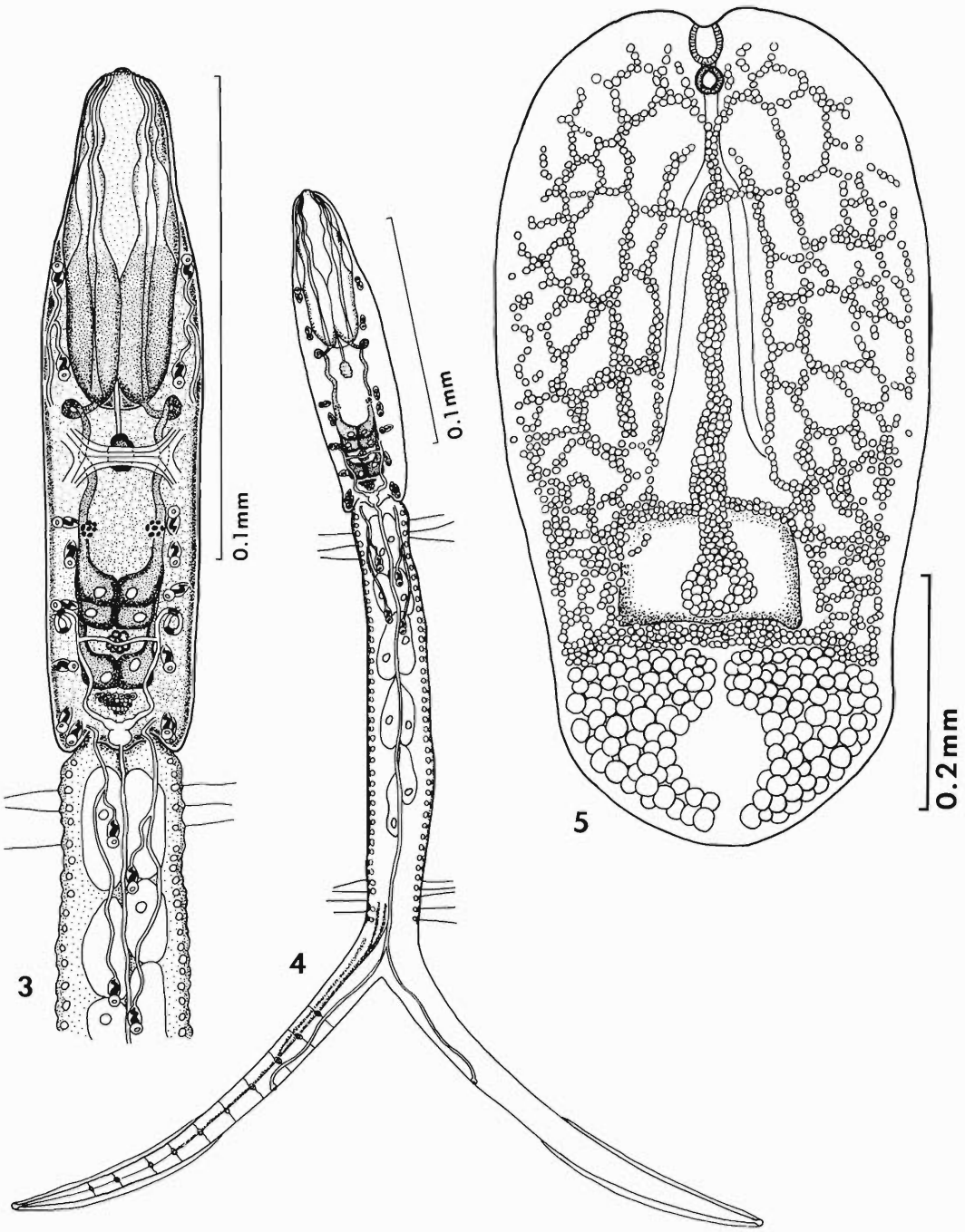
Resting cercariae hung at various angles to water's surface with furcae at about 80° to the tailstem. Cercariae alternate periods of sinking for 30–45 sec with active swimming upward for about 2 sec. Ascent was not perpendicular but upward and outward towards the walls of the container. Cercariae emerged during morning hours from snails maintained under a "normal" photoperiod (0800–2200 light, 2200–0800 dark). Cercariae were positively phototactic.

Hoffman (1958) observed 6 pairs of flame cells in the body and 2 pairs in the tailstem of *O. ptychocheilus* cercariae. Sudarikov and Kurochkin (1968) observed the same in *O. scardinii.* In this study on *O. ptychocheilus,* I observed 8 pairs of flame cells in the body and 2 pairs in the tailstem (probably in the formula 2[(2+2)+(2+2)+(2)] = 20). I found that overnight refrigeration of cercariae considerably enhanced distinguishing flame cells from ciliated patches in the primary excretory tubules. If my flame cell formula is correct, *O. ptychocheilus* cercariae bear a striking resemblance to those of *Posthodiplostomum* spp., particularly as described by Miller (1954) for *P. minimum* (MacCallum, 1921) and by Dönges (1965) for *P. brevicaudatum* (v. Nordmann, 1832).

Metacercaria
(Fig. 5)

DESCRIPTION (based on measurements from 12 specimens obtained from the brains of experimentally infected fathead minnows): Cyst double layered, 504–735 (639) by 299–426 (325). Larva typical neascus. Body length 388–499 (438). Forebody foliaceous, 261–364 (308) by 136–200 (165). Hindbody short, 110–153 (130) by 126–173 (145). Oral sucker 20–30 (26) by 17–30 (23). Acetabulum 22–30 (25) by 22–30 (28). Holdfast 52–93 (67) by 62–105 (81), opening by a median slit. Prepharynx short, 5 or less. Pharynx 23–30 (28) by 15–19 (17). Esophagus short. Ceca extending to near posterior end of hindbody. Rudimentary ovary and testes evident only in stained specimens. Copulatory bursa 30–43 (36) by 42–50 (46), rarely everted.

HOSTS AND LOCATION IN HOSTS: *Pimephales promelas,* fathead minnow; brain, cranial cavity,



Figures 3, 4. *Ornithodiplostomum ptychocheilus* cercaria. 3. Body and anterior tailstem showing internal organs, composite based on specimen fixed in hot (70°C) 10% formalin. 4. Cercaria, newly emerged from *Physa gyrina,* composite based on photograph of living specimen. Figure 5. *Ornithodiplostomum ptychocheilus* metacercaria. Composite based on photograph of living specimen.

eyes (natural and experimental). *Pimephales notatus,* bluntnose minnow; brain, cranial cavity, eyes (natural and experimental).

LOCALITIES: Iowa: Dickinson County, Garlock Slough, Jemmerson Slough, Pillsbury Creek; Lyon County, Big Sioux River. Arkansas: Prairie County, DeValls Bluff, R. L. Hart Minnow Farm. Wisconsin: Eau Claire County, Eau Claire, Highway 53 Frontage Road Pond.

VOUCHER SPECIMENS: USNM Helm. Coll. No. 79026.

Remarks

Faust (1917, 1918) and Hughes and Piszczek (1928) described the metacercaria of *O. ptychocheilus* based on mesenteric cysts obtained from *Ptychocheilus oregonensis* and *Notropis deliciosus stramineus,* respectively. Metacercariae described herein were obtained from the brains of experimentally infected *Pimephales promelas* and *Pimephales notatus.* There is no apparent morphological difference between body cavity and brain metacercariae.

Metacercariae were recovered from experimentally exposed *Pimephales promelas* (86/86) and *P. notatus* (5/5), and all experimentally developed metacercariae were recovered from the cranial cavity, brain, and eyes. Experimental infections were negative for *Castostomus commersoni* (0/2), *Notropis cornutus* (0/7), *N. dorsalis* (0/6), and *Semotilus atromaculatus.* However, natural infections of a morphologically identical parasite were observed in *Hybognathus hankinsoni*—brain (Iowa); and *Notropis cornutus*—body cavity, brain, eyes (Iowa).

Discussion

The relationship between "visceral" and "cranial" "*O. ptychocheilus*" metacercariae requires further investigation. Radabaugh's (1980) suggestion of cranial and visceral races is oversimplified and does not explain the fact that, in this study, *Notropis cornutus* harbored natural infections of both cranial and visceral metacercariae but were refractory to experimental cranial infection. It seems likely that there are several races or species but that these races or species vary in fish host(s), preferred site, and site specificity in the fish host, as well as geographic distribution.

Defining the species *Ornithodiplostomum ptychocheilus* is further complicated by the striking similarity between it and the European *O. scardinii.* Strong similarities in morphology at all stages (excepting perhaps the flame cell formula of the cercaria), and identical life histories suggest the possibility that they are specifically identical.

Niewiadomska (1973) defined a genus as ". . . a systematic unit with similar morphology at the stages of cercariae, metacercariae, and the adult." By her definition, *Ornithoplostomum* and *Posthodiplostomum* are congeneric. However, it seems unwise to redefine a genus (*Ornithodiplostomum*) until we can more adequately define the type species (*O. ptychocheilus*).

Ornithodiplostomum ptychocheilus has a cercaria of the rhabdocaeca group of fork-tailed cercariae (Miller, 1926). These cercariae are longifurcate monostomes with 3 pairs of penetration glands and a rudimentary digestive system. The metacercaria is a typical neascus. Rhabdocaecan cercariae and neascus metacercariae occur in *Ornithodiplostomum* and *Posthodiplostomum* (both tribe Diplostomatini), and in *Crassiphiala* and *Uvulifer* (both tribe Crassiphialini). Based on larval characters *Ornithodiplostomum* and *Posthodiplostomum* (both Diplostomatini) are more closely aligned to *Crassiphiala* and *Uvulifer* (both Crassiphialini) than either is to *Diplostomum* (Diplostomatini). *Diplostomum* possess cercariae that are longifurcate distomes with four or six pairs of penetration glands and a well-developed digestive system, and diplostomulum-type metacercariae. Dubois' (1970) separation of the tribes of the subfamily Diplostomatinae is based largely on distribution of vitellaria in adult worms. Such a separation is inconsistent with our present knowledge of life cycles and larval stages.

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Literature Cited

Amin, O. M. 1969. Helminth fauna of suckers (Catostomidae) of the Gila River System, Arizona. II.

Five parasites from *Catostomus* spp. American Midland Naturalist 82:429–443.

Dönges, J. 1964. Der Lebenszyklus von *Posthodiplostomum cuticola* (v. Nordmann 1832) Dubois 1936 (Trematoda, Diplostomatidae). Zeitschrift für Parasitenkunde 24:169–248.

———. 1965. Der Lebenszyklus von *Posthodiplostomum brevicaudatum* (Trematoda), eines Parasiten in den Augen von Süsswasserfischen. Zoologica (Stuttgart) 40:1–39.

Dubois, G. 1970. Synopsis des Strigeidae et des Diplostomatidae (Trematoda). Mémoires de la Société Neuchâteloise des Sciences Naturelles 10:259–727.

Faust, E. C. 1917. Notes on the cercariae of the Bitter Root Valley, Montana. Journal of Parasitology 3: 105–123.

———. 1918. Life history studies on Montana trematodes. Illinois Biological Monographs 4:1–120.

Hoffman, G. L. 1954. The occurrence of *Ornithodiplostomum ptychocheilus* (Faust) (Trematoda: Strigeida) in fish and birds. Journal of Parasitology 40:232–233.

———. 1958. Studies on the life-cycle of *Ornithodiplostomum ptychocheilus* (Faust) (Trematoda: Strigeoidea) and the "self cure" of infected fish. Journal of Parasitology 44:416–421.

———. 1960. Synopsis of Strigeoidea (Trematoda) of fishes and their life cycles. United States Fish and Wildlife Service, Fishery Bulletin 175 60:439–469.

Hughes, R. C., and F. R. Piszczek. 1928. Studies on the trematode family Strigeidae (Holostomidae) No. XI *Neascus ptychocheilus* (Faust). Journal of Parasitology 15:58–62.

Miller, H. M., Jr. 1926. Comparative studies on furcocercous cercariae. Illinois Biological Monographs 10:1–112.

Miller, J. H. 1954. Studies on the life history of *Posthodiplostomum minimum* (MacCallum 1921). Journal of Parasitology 40:255–270.

Niewiadomska, K. 1973. Some aspects of the biology and evolution of Strigeata La Rue and their importance in systematics of this group of Trematoda. Acta Parasitologica Polonica 21:21–62.

Pearson, J. C. 1956. Studies on the life cycles and morphology of the larval stages of *Alaria arisaemoides* Augustine and Uribe, 1927 and *Alaria canis* LaRue and Fallis, 1936 (Trematoda: Diplostomidae). Canadian Journal of Zoology 34:295–387.

———. 1961. Observations on the morphology and life cycle of *Neodiplostomum intermedium* (Trematoda: Diplostomatidae). Parasitology 51:133–172.

Radabaugh, D. C. 1980. Encystment site selection in the brain-inhabiting metacercariae of *Ornithodiplostomum ptychocheilus* (Trematoda: Strigeoidea). Journal of Parasitology 66:183–184.

Sankurathri, C. S., and J. C. Holmes. 1976. Effects of thermal effluents on parasites and commensals of *Physa gyrina* Say (Mollusca: Gastropoda) and their interactions at Lake Wabamun, Alberta. Canadian Journal of Zoology 54:1742–1753.

Sudarikov, V. E., and I. V. Kurochkin. 1968. Systematic position and development of metacercaria of *Neodiplostomulum scardinii* S. Schulman—parasitic in brain of fish (in Russian). Sbornik Gel'mintologicheskoĭ Rabot, Trudy Astrakhanskogo Zapovednika 11:255–273.

Ulmer, M. J. 1970. Notes on rearing of snails in the laboratory. Pages 143–144 *in* A. J. MacInnis and M. Voge, eds. Experiments and Techniques in Parasitology. W. H. Freeman and Co., San Francisco.

Van Haitsma, J. P. 1930. Studies on the trematode family Strigeidae (Holostomidae) No. XX. *Paradiplostomum ptychocheilus* (Faust). Transactions of the American Microscopical Society 49:140–153.

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Infectivity, Growth, and Distribution of *Echinostoma revolutum* in Swiss Webster and ICR Mice

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ABSTRACT: Swiss Webster (SW) and ICR mice were each fed 25 metacercariae of *Echinostoma revolutum* and at necropsy worms were recovered from the small intestine, but not the colon or cecum. At 2 weeks postinfection, 75% of the worms were recovered from SW mice, but only 22% from ICR mice. Flukes were not recovered from ICR mice at 3 weeks, nor from SW mice at 4 weeks postinfection. Host sex had no effect on worm establishment. Beyond 1 week, worm weight and body area were variable in the 2 strains of mice. Most worms were ovigerous at 2 and 3 weeks, but not at 1 week. Eggs teased from the uteri of flukes and embryonated in tap water produced miracidia. Metacercariae excysted mainly in the last 10 cm of the ileum and then migrated anteriorly to localize 20–30 cm from the ileocecal valve.

Little information is available on the infection of mice with *Echinostoma revolutum,* and there are no studies on the body area, weight, and distribution of this trematode in male and female mice. Beaver (1937) infected 5 of 15 mice with this fluke, but the mouse strain and number of metacercariae used were not given. Moravec et al. (1974) experimentally infected white mice, but gave no infectivity data. Female albino mice given a primary infection of 4–15 metacercariae by Sirag et al. (1980) showed a 63–100% infection rate 2–3 weeks postinfection. However, mice fed fewer than 15 metacercariae gave lower worm recoveries, and eliminated worms between 34 and 49 days postinfection. Recently, Bindseil and Christensen (1984) infected albino and nude mice each with 20 metacercariae, and recovered 15–20 adults per mouse.

The purpose of our study was to determine body area, weight, and distribution of *E. revolutum* in Swiss Webster (SW) and ICR mice. Also, the effects of mouse sex on infectivity were studied.

Materials and Methods

Metacercariae of *E. revolutum* were obtained from experimentally infected *Physa heterostropha* snails. Only cysts with formed walls and transparent contents were used. They were stored at 4°C in half-strength Locke's solution and used within 4 weeks to infect 42 outbred SW and 36 ICR mice (Ace Animals, Boyertown, Pennsylvania) 7–8 weeks old. Each was given 25 cysts in 3% $NaHCO_3$ by stomach tube and then fed ad libitum. Mice were killed 1, 2, 3, and 4 weeks postexposure, and their small intestines were divided into 10-cm segments beginning at the ileocecal junction. Each segment was turned inside out on a wooden applicator and placed in a petri dish of Locke's solution to count any worms present. The cecum and colon of 10 randomly selected mice were also examined. Twelve additional mice were each fed 25 cysts and killed 2 and 24 hr later to determine where metacercariae excysted and to obtain 24-hr-old worms for body area measurements.

For body area measurements (length times midacetabular width), 143 worms were fixed in hot AFA, dehydrated in ethanol, cleared in toluene, and mounted unstained in Permount. In addition, 236 were dried for 48 hr at 60°C and weighed. Eggs teased from the uteri of 12 gravid specimens were permitted to develop in tap water at 24 or 30°C.

The Student's t-test was used to analyze differences between means and $P < 0.05$ was considered significant.

Results

The results of infectivity and growth studies (Exp. A–M) are summarized in Table 1.

From the total of 300 cysts fed to male SW and female ICR mice, 187 (62%) worms were recovered 1 week postinfection (Exp. A, J). The average number of worms recovered in the 2 groups (16.7 vs. 14.5) was not significantly different.

At 2 weeks postinfection, 75% of the flukes were recovered from SW mice (Exp. B, E), but only 22% from ICR mice (Exp. H, K). There was no significant difference in worm recovery from SW males vs. SW females (Exp. B vs. E), or from ICR males vs. ICR females (Exp. H vs. K). There were significantly fewer flukes ($P < 0.01$) in ICR than SW males (Exp. H vs. B). ICR females (Exp. K) contained fewer worms than SW females (Exp. E), but the difference was not significant ($P =$

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Table 1. Infectivity, dry weight, and body area of *Echinostoma revolutum* in mice fed 25 cysts.

Exper.	Strain and sex of mouse	Weeks post-infection	No. of infected mice*	Avg. no. ± SE and (%) of worms	No. of worms weighed	Avg. dry weight per worm (mg)	No. of worms measured	Avg. body area ± SE (mm²)
A	SW male	1	6	16.7 ± 1.1 (67)	48	0.11	30	1.4 ± 0.05
B	SW male	2	6	22.0 ± 2.5 (88)	37	0.70	30	3.2 ± 0.10
C	SW male	3†	2	4.2 ± 4.0 (17)	10	0.49	9	4.3 ± 0.37
D	SW male	4	0	0	0	0	0	0
E	SW female	2	5	15.7 ± 3.6 (63)	51	0.88	20	2.8 ± 0.08
F	SW female	3	2	2.0 ± 1.3 (8)	6	1.00	5	2.4 ± 0.25
G	SW female	4	0	0	0	0	0	0
H	ICR male	2	3	4.7 ± 2.5 (19)	24	0.75	4	1.6 ± 0.07
I	ICR male	3	0	0	0	0	0	0
J	ICR female	1	5	14.5 ± 3.6 (58)	50	0.11	30	1.6 ± 0.04
K	ICR female	2	3	6.0 ± 2.5 (24)	10	0.65	15	3.0 ± 0.12
L	ICR female	3	0	0	0	0	0	0
M	ICR female	4	0	0	0	0	0	0

* Six mice in each group were exposed.
† This does not include 1 infected host that died 9 days postexposure.

0.064). There was a significant decline in worm recoveries in both SW males and females between weeks 2 and 3 (Exp. B vs. C, $P < 0.01$; Exp. E vs. F, $P < 0.01$). Echinostomes were not found in ICR mice at 3 weeks (Exp. I, L) nor in SW mice at 4 weeks (Exp. D, G).

Worms in each experiment were weighed as a group and could not be analyzed statistically. Average dry weights of week-old worms from SW males and ICR females were the same (Exp. A, J). At 2 weeks, worms from ICR females (Exp. K) weighed less than those from ICR males (Exp. H), but worms from SW males (Exp. B) weighed less than those from SW females (Exp. E). Worms from ICR females (Exp. K) weighed less than those from SW females (Exp. E), but worm weights from ICR (Exp. H) and SW males were similar. From 2 to 3 weeks, fluke weight from SW males (Exp. B, C) decreased, but that from SW females (Exp. E, F) increased. At 3 weeks, worms from SW females (Exp. F) weighed more than those from SW males (Exp. C).

Ten 24-hr-old flukes from a single ICR female had an average body area of 0.035 ± 0.001 mm². The body area of week-old worms from ICR females (Exp. J) and SW males (Exp. A) was significantly larger ($P < 0.01$) than that of 24-hr-old flukes. The average body area of worms from ICR females (Exp. J) was not significantly larger than that of worms from SW males (Exp. A). Data from week-old ICR males and SW females were not collected. At 2 weeks, flukes from SW males and ICR females were significantly larger than those from week-old SW males and ICR females ($P < 0.01$). Worms from SW females (Exp. E) were smaller than those from SW males (Exp. B, $P < 0.02$) at 2 weeks, but the worms from ICR females (Exp. K) were larger than those from ICR males (Exp. H, $P < 0.01$). There was no difference in body area in echinostomes from SW and ICR females (Exp. E vs. K), but flukes from SW males were significantly larger than those from ICR males (Exp. B vs. H, $P < 0.01$). In SW males, 3-week-old worms were larger than 2-week-old worms (Exp. C vs. B, $P < 0.01$), but in SW females, worms at 2 weeks were larger than those at 3 weeks (Exp. E vs. F, $P < 0.05$).

None of the week-old worms were ovigerous, but 170 of 176 (97%) 2- and 3-week-old worms were. At 2 weeks, the number of eggs in worms from SW male and female mice ranged from 200 to 250. The number of eggs in worms from ICR males and females at 2 weeks and from SW males and females at 3 weeks ranged from 20 to 100. Eggs from worms embryonated and released miracidia in 7–10 days at 30°C and 14–18 days at 24°C.

Worms were never found in the colon or cecum. Distribution data for both strains of mice are given in Table 2. Except for 2-week-old worms from SW males that were mainly 10–20 cm from the ileocecal valve, and 3-week-old worms from SW females that were distributed throughout the intestine, worms were usually located 20–30 cm anterior to the valve. Of 6 SW males exposed to

Table 2. Distribution of *Echinostoma revolutum* in the mouse small intestine.*

Strain and sex of mouse	Weeks post-infection	No. of infected mice	Average no. ± SE of worms recovered (segment no.)					
			1	2	3	4	5	6
SW male	1	6	0	4.8 ± 2.5	11.8 ± 3.4	0	0	—
SW male	2	6	0.5 ± 0.5	14.0 ± 4.9	2.2 ± 2.1	0.8 ± 0.5	0.5 ± 0.5	—
SW male	3	2	0.2 ± 0.2	0.8 ± 0.6	2.8 ± 2.8	0.4 ± 0.4	0	—
SW female	2	5	1.0 ± 1.0	3.0 ± 1.2	9.7 ± 3.0	2.0 ± 1.0	0	—
SW female	3	2	0.5 ± 0.5	0.7 ± 0.5	0.2 ± 0.2	0.7 ± 0.5	—	—
ICR male	2	3	1.2 ± 0.7	1.0 ± 0.8	2.0 ± 1.3	0.3 ± 0.3	0.2 ± 0.2	—
ICR female	1	5	0	3.2 ± 3.0	10.3 ± 3.6	0.8 ± 0.5	0	0.2 ± 0.2
ICR female	2	3	0.2 ± 0.2	1.0 ± 0.6	2.7 ± 1.3	1.2 ± 0.5	0.8 ± 0.8	0.2 ± 0.2

* Successive 10-cm segments are numbered from the cecum.

25 cysts and killed 2 hr postinfection, a total of 25 excysted metacercariae were found, 17 within 10 cm of the valve, and 8 within 10–20 cm. Of 6 ICR females each exposed to 25 cysts and killed 24 hr postinfection, a total of 17 excysted metacercariae were found, 8 within 10 cm of the valve and 9 within 10–20 cm.

Discussion

Our results show that ICR and SW mice can be infected with metacercariae of *E. revolutum*. The worm burden of SW mice was reduced at 3 weeks postinfection and eliminated by 4 weeks. Recoveries in female ICR mice were reduced at 2 weeks and eliminated by 3 weeks. Our findings are in contrast to Christensen et al. (1981) who reported that inbred albino mice exposed to more than 15 metacercariae retained consistent numbers of *E. revolutum* up to 70 days, and that infections with less than 15 metacercariae resulted in reduced worm numbers by 34 days. In their studies, mice exposed to 5 or 15 metacercariae eliminated worms by days 47 and 54, respectively. Recently, Christensen et al. (1984) showed that mice exposed to 6 metacercariae reduced their worm burden at 28 days and eliminated flukes by day 35 postexposure. The infective dose in our study was 25 metacercariae, and mice reduced their worm burdens and eliminated worms more rapidly than reported by Christensen et al. (1981, 1984). Our study also showed that mice eliminated *E. revolutum* more rapidly than chickens. Longevity of worms in chickens averaged 35 (Senger, 1954), and 44 days (Fried, 1984).

We recovered up to 88% of the worms in mice, whereas Christensen et al. (1981) and Sirag et al. (1980) reported recoveries as high as 94% and 100%, respectively. These recoveries are higher than the 12–25% worm burden in chickens reported by Fried and Freeborne (1984) and Fried (1984).

In our study male and female mice were equally susceptible to *E. revolutum* infection. This is in contrast to the recent study of Molan and James (1984), which showed that female mice were more resistant than males to infection with the intestinal trematode, *Microphallus pygmaeus*.

The mean body area of *E. revolutum* recovered from chickens (1.3 mm^2 at 1 week and 3.1 mm^2 at 2 weeks; see Fried and Freeborne, 1984) and SW mice were comparable after 1 and 2 weeks postinfection, but the area of worms from ICR mice were comparable only for 1-week postinfection. After 2 weeks in ICR mice and 3 weeks in SW mice, worms were smaller than those in the chicken.

Dry weights of week-old worms from mice and chickens were comparable, but differed at 2 and 3 weeks. Sleckman and Fried (1984) reported average body weights for worms in chickens of 0.13, 1.43, and 1.68 mg at 1, 2, and 3 weeks, respectively. Worms from our mice averaged 0.11 mg at 1 week and 0.74 mg at 2 and 3 weeks.

Worms from mice produced eggs capable of hatching as did worms in chickens (Fried and Weaver, 1969). However, the number of eggs in worms from chickens (about 250 to 500/worm; see Fried and Freeborne, 1984) was greater than that in mice. Even though our worm recoveries were higher than in the chicken, body area, weight, and egg data suggest that worm growth and fecundity were suboptimal in ICR and SW mice.

E. revolutum in the mouse and chicken have different distribution patterns. All worms re-

covered from the mouse were located in the small intestine, mainly in the ileum. In contrast, 59% of ovigerous worms in the chickens were in the rectum–cloaca and 32% in the ileum (Fried, 1984). In the mouse, *E. revolutum* excysts mainly in the last 10 cm of the ileum, but during development flukes migrate anteriorly, and most gravid worms were found 20 to 30 cm anterior to the ileocecal valve.

Acknowledgments

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Literature Cited

Beaver, P. C. 1937. Experimental studies on *Echinostoma revolutum* (Froelich), a fluke from birds and mammals. Illinois Biological Monographs 15: 1–96.

Bindseil, E., and N. Ø. Christensen. 1984. Thymus-independent crypt hyperplasia and villous atrophy in the small intestine of mice infected with the trematode *Echinostoma revolutum.* Parasitology 88:431–438.

Christensen, N. Ø., B. O. Fagbemi, and P. Nansen. 1984. *Trypanosoma brucei*-induced blockage of expulsion of *Echinostoma revolutum* and of homologous *E. revolutum* resistance in mice. Journal of Parasitology 70:558–561.

———, **R. Nydal, F. Frandsen, and P. Nansen.** 1981. Homologous immunotolerance and decreased resistance to *Schistosoma mansoni* in *Echinostoma revolutum*-infected mice. Journal of Parasitology 67:164–165.

Fried, B. 1984. Infectivity, growth and development of *Echinostoma revolutum* (Trematoda) in the domestic chick. Journal of Helminthology 58:241–244.

———, **and N. E. Freeborne.** 1984. Effects of *Echinostoma revolutum* (Trematoda) adults on various dimensions of the chicken intestine, and observations on worm crowding. Proceedings of the Helminthological Society of Washington 51:297–300.

———, **and L. J. Weaver.** 1969. Effects of temperature on the development and hatching of eggs of the trematode *Echinostoma revolutum.* Transactions of the American Microscopical Society 88: 253–257.

Molan, A. L., and B. L. James. 1984. The effects of sex, age and diet of mice and gerbils on susceptibility to *Microphallus pygmaeus* (Digenea: Microphallidae). International Journal for Parasitology 14:521–526.

Moravec, F., V. Barüs, B. Ryšavý, and F. Yousif. 1974. Observations on the development of two echinostomes, *Echinoparyphium recurvatum,* and *Echinostoma revolutum,* the antagonists of human schistosomes in Egypt. Folia Parasitologica 21:107–126.

Senger, C. M. 1954. Notes on the growth, development, and survival of two echinostome trematodes. Experimental Parasitology 6:491–496.

Sirag, S. B., N. Ø., Christensen, F. Frandsen, J. Monrad, and P. Nansen. 1980. Homologous and heterologous resistance in *Echinostoma revolutum* infections in mice. Parasitology 80:479–486.

Sleckman, B. P., and B. Fried. 1984. Glycogen and protein content in adult *Echinostoma revolutum* (Trematoda). Proceedings of the Helminthological Society of Washington 51:355–356.

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The Biology of *Cyclocoelum mutabile* (Trematoda) Infections in American Coots

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ABSTRACT: Adult coots arriving at the Delta Marsh, Manitoba, Canada in April and May harbored ovigerous *Cyclocoelum mutabile.* Recruitment of new infections was low during this period and infections virtually disappeared by July. Young coots apparently acquired their flukes from infective pools established in young snails each spring. Prevalence of *C. mutabile* in young coots increased steadily throughout the summer and fall. Based on the presence of immature flukes, the peak period of recruitment occurred between July to mid-August.

C. mutabile survived between 16 and 20 weeks in experimentally infected coots. Flukes acquired during the summer apparently do not survive the winter in the coot host. Populations seen in adult coots in the spring represent new infections acquired during the winter. Previously unexposed 2-year-old, 1-year-old, and 3- to 4-week-old coots were equally susceptible to infection. Existing infections had no significant effect on the number of coots that became infected when challenged 4 weeks later nor on the mean intensity of the infections that developed. The lack of an age resistance and an effective acquired resistance results in the acquisition of new infections on the wintering grounds that are carried to the breeding grounds in the spring.

The helminth fauna of the American coot, *Fulica americana* is well known from several localities throughout North America (Roudabush, 1942; Gullion, 1952; Colbo, 1965; Kinsella, 1973; Eley, 1976, and others). One frequently encountered species is *Cyclocoelum mutabile,* a large digenean found in the air sacs. Colbo (1965) found this species in adult coots returning to Alberta in the spring but also found that the infections disappeared by early summer. Both adult and young coots acquired new infections later in the summer. The prevalence of infection increased throughout the fall and a large proportion of the population was infected at the onset of southward migration.

Buscher (1965) suggested that helminths, which occupied protected sites within migrating waterfowl (i.e., sites other than the intestinal lumen), might persist for extended periods. If sufficiently long lived, individuals acquired on the breeding grounds could survive the winter within the host and return with it to contaminate the area the following year. *C. mutabile* occupies such a site and the seasonal prevalence reported by Colbo (1965) suggests that it might survive the winter in the coot host.

Between 1971 and 1979, a long-term study on the seasonal dynamics of helminths found in coots was conducted in the Delta Marsh, Manitoba near the eastern limit of the coots' breeding range on the prairies. The present study examines the seasonal dynamics of *C. mutabile,* its longevity to determine whether it can overwinter in the coot host, and the effects of acquired resistance and age resistance on its acquisition by coots.

Materials and Methods

Collecting periods varied from year to year. Collection of adults (>1-year-old) began between mid-April and mid-May and generally concluded by mid-August. Young-of-the-year were collected from early July until the end of August except in 1971 and 1979 when collections continued through October. An attempt was made to collect 20 individuals per month. Live flukes were found in the livers and air sacs; dead flukes were normally found in fibrotic nodules on the air sac membranes. Confirmation of presumptive flukes involved examination of these nodules for eggs in temporary squash preparations.

Details concerning the rearing of *C. mutabile* metacercariae have been described previously (McLaughlin, 1976). In this study, however, 1–2-week-old laboratory-reared *Stagnicola elodes* were used as intermediate hosts rather than *Promenetus exacuous.* Coots were hatched from eggs collected in the Delta Marsh. They were maintained in parasite-free conditions in specially built pens at Concordia University. They were kept in outdoor pens from mid-April to mid-November and in heated indoor pens under ambient light conditions for the remainder of the year. All experiments were conducted under ambient conditions in outdoor pens.

To determine longevity, 31 3–4-week-old coots were each given 20 metacercariae by oral intubation. Six groups of 5 individuals each were randomly assigned for necropsy at 4-week intervals. The experiment was originally designed to last for 24 weeks but was terminated at 20 weeks. Each coot was thoroughly ex-

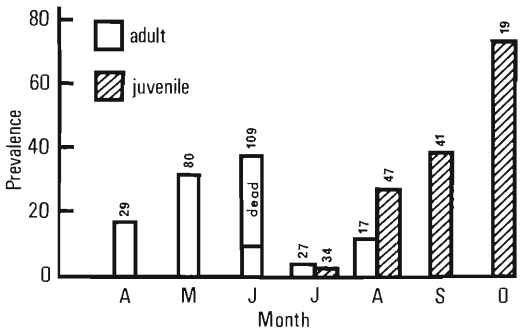

Figure 1. Monthly prevalence of natural *Cyclocoelum mutabile* infections in coots collected at Delta, Manitoba. Numbers above each column indicate sample size.

amined for living and dead flukes. In some cases dead flukes were clearly recognizable. In others, their remains occurred in fibrotic nodules usually on the air sac membranes.

To determine whether age affects the susceptibility of coots to *C. mutabile,* 6 2-year-old coots, 16 1-year-old coots and 16 3–4-week-old coots were each given 20 metacercariae by oral intubation. None of the coots had been previously exposed to *C. mutabile.* The coots were necropsied 35–37 days postinfection (PI) and the flukes counted.

To determine whether existing infections provide protection against subsequent infections, 30 3–4-week-old coots were randomly assigned to either an experimental group or control group consisting of 15 birds each. Each member of the experimental group received 20 metacercariae per bird. Both groups received 30 metacercariae per bird 4 weeks later. Necropsies were performed 12 days postchallenge, while the challenge flukes were still in the liver (McLaughlin, 1977). Flukes from the livers of experimental and control coots were stained routinely in acetocarmine and mounted in Permount to permit comparisons of growth and development. Statistical analyses included *G*-tests and analysis of variance carried out on raw or square-root-transformed data (Sokal and Rohlf, 1969). The significance level was set at 5%.

A voucher specimen of *C. mutabile* has been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM No. 79029).

Results

Figure 1 shows the monthly prevalence of *C. mutabile* in coots collected in the Delta Marsh. All flukes found in adult coots examined during April, July, and August and in all but 2 coots examined in May were alive and ovigerous. One coot examined in May had only immature specimens in the air sacs; flukes found in the other coot were dead. When living and dead flukes were considered, the highest prevalence occurred in June; however, only 10 of 41 coots infected with *C. mutabile* harbored living worms and in 6 of these, dead flukes were also present in the air sacs. All of the living flukes were ovigerous.

Table 1. Longevity of *Cyclocoelum mutabile* in experimentally infected American coots.

Weeks PI	Number of coots Exposed	Number of coots Infected	Number of *C. mutabile* Total	Number of *C. mutabile* Living	Number of *C. mutabile* Dead	% survival
4	5	4	14	14	0	100
8	5	5	11	7	4	63.6
12	6	4	23	15	8	65.2
16	5	4	13	4	9	30.7
20	10	7	15	0	15	0

The few young coots infected in July had immature specimens in the liver or in the air sacs. In August, 8 of the infected coots had some or all of their flukes in the liver; the remaining immature and ovigerous flukes were found in the air sacs. The first ovigerous specimen was found on August 11. All but 3 coots examined in September and October had live, ovigerous, flukes in the air sacs. Two coots examined in September had immature specimens in the air sacs along with ovigerous flukes; one coot examined in October had only dead flukes in the air sacs.

Results of the longevity experiment are presented in Table 1. One coot died 13 weeks PI and was included with the 12-week group. After an initial drop in survival between 4 and 8 weeks PI, survivorship remained relatively constant until weeks 12–16. At 16 weeks PI approximately one-third of the flukes recovered were still alive. No living flukes were found in the first 5 coots examined at 20 weeks PI. Accordingly, the remaining coots were also necropsied and, similarly, no live flukes were found.

Results of the exposure of 2- and 1-year-old and of 3–4-week-old coots are summarized in Table 2. No significant differences occurred in either the proportion of each group that became infected ($G = 3.376$; 2 df) or in the mean intensity of infection in each group ($F = 1.249$; 2,28 df). One 3-week-old coot died shortly after exposure and was not included in the analysis.

Nine of the 14 experimental coots exposed in the acquired resistance experiment became infected (Fig. 2). However, only 4 of the 9 infected coots in the experimental group developed infections postchallenge. One individual died 2 days PI and was not included. Neither the number of

Table 2. Summary of *Cyclocoelum mutabile* infections in experimentally exposed coots of different ages.

Age of coots	Number of coots		Mean intensity ± SD
	Exposed	Infected	
2 years	6	4	4.5 ± 6.3
1 year	16	15	4.4 ± 3.2
3–4 weeks	15	12	2.5 ± 1.5

coots that became infected postchallenge (G = 0.58; 1 df) nor the mean intensity of the challenge infection (F = 0.05; 1,15 df) differed significantly between the groups.

The mean length, testis diameter and ovarian diameter of 12-day-old flukes from the experimental and control infections (Table 3) did not differ significantly (F = 2.0, 0.61, 1.29; 1,19 df, respectively).

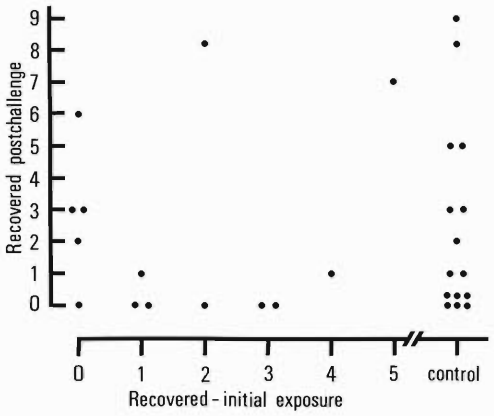

Figure 2. The number of *Cyclocoelum mutabile* found in each experimentally exposed coot (x axis) plotted against the number of *C. mutabile* recovered from that coot postchallenge. The number of *C. mutabile* found in each control coot postchallenge is presented for comparison.

Discussion

The monthly prevalence of live *C. mutabile* in coots collected in the Delta Marsh followed the same pattern described by Colbo (1965), with the prevalence of living flukes approximately 3 times greater in May than in June. The apparent increase in prevalence between April and May was not due to recruitment of *C. mutabile* on the breeding grounds. Only a single coot harboring immature flukes was found during this period and Colbo (1965) reported identical findings during the same period in Alberta. As the prepatent period for *C. mutabile* is 28 days (McLaughlin, 1983), immature flukes should have been found more frequently if new infections were common at this time. Limited recruitment apparently occurred on arrival in the spring (perhaps accounting for the infections seen in adults in July and August) but it was not sufficient to increase the prevalence to the extent seen in the present study. A more probable explanation for this increase lies in staggered arrival times of different cohorts of the coot population. Eley (1976) found that first-year coots, collected between October and January, had a significantly higher prevalence of *C. mutabile* than older coots. The increase seen between April and May was likely the result of early arrival of older coots followed by an influx of more heavily infected yearling coots somewhat later. In addition, older experienced breeders are likely to set up territories earlier and may be more susceptible to collection in early spring.

Ovigerous flukes were abundant during May when large numbers of young lymnaeid, physid, and helisome snails hatch and become available as intermediate hosts. Eggs of *C. mutabile* hatch within a few hours of being shed, intramolluscan development is completed within a single snail, and metacercariae may appear as soon as 4–5 weeks PI (McLaughlin, 1976). Hence young snails exposed in May could produce metacercariae by late June or early July and still be small enough for young coots to eat.

Prevalence in young coots increased steadily throughout the summer and fall. Recruitment, however, was most intense during July and early August. Most flukes found in late August, September, and October were ovigerous. The paucity of immature flukes suggests that few new infections were acquired during this period.

Although a high proportion of coots harbored *C. mutabile* infections in late fall the flukes are unable to survive the winter in the host. Their life span is only 16–20 weeks and flukes acquired during the summer or early fall would perish by the end of January. Thus, flukes found in coots returning in the spring represent new populations acquired on the wintering grounds. The source of such infections remains speculative. Perhaps coots establish new infections in snails on arrival in the wintering areas. The developmental time required in the snail host could bridge the time between the onset of attrition in the coot host and the recruitment of new infections later in the winter. A second possibility is that wintering coots

Table 3. A comparison of the mean length, testis diameter, and ovarian diameter of 12-day-old *Cyclocoelum mutabile* from experimental and control coots. All measurements are in mm.

	Experimental $\bar{x} \pm$ SD	Control $\bar{x} \pm$ SD
Body length	3.77 ± 0.38	3.85 ± 0.30
Testis diameter	0.11 ± 0.02	0.12 ± 0.02
Ovarian diameter	0.09 ± 0.01	0.10 ± 0.01

acquire their infections from pools of metacercariae maintained in local snails by resident hosts (e.g., gallinules) that also harbor *C. mutabile* (Kinsella et al. 1973). The predominance of dead flukes found in June suggests that the winter infections were likely acquired sometime between early January and late February.

Eley (1976) found that the prevalence of *C. mutabile* was significantly higher in first-year birds than in second- or third-year birds. However, neither prevalence nor mean intensity varied significantly among coots of different ages experimentally exposed to this fluke. In previously unexposed coots, the age of the bird by itself has no effect on its susceptibility to *C. mutabile*. Age-related factors, however, may operate to limit exposure of otherwise susceptible coots under natural conditions, producing the differences between age groups noted by Eley (1976).

Little information is available on acquired resistance to avian digeneans. Rau et al. (1975), and Macy (1973) found that *Trichobilharzia ocellata* and *Sphaeridiotrema globulus* provided some protection against challenge infections, but neither Fried (1963) nor Nollen (1971) were able to demonstrate any resistance in chicks to infections of either *Philophthalmus hegeneri* or *P. megalurus,* respectively. Examination for possible acquired resistance to *C. mutabile* in the coot host was limited to the liver phase of infection. It was reasoned that any adverse effects on challenge flukes resulting from reaction on the part of the host or from damage to the liver caused by previous fluke migration would occur during this period when the flukes were small and in a tissue site. Although few experimental coots with existing infections harbored challenge flukes, the number that became infected did not differ significantly from that in the controls. Furthermore, the mean length of the flukes in each group was similar and flukes from both groups exhibited a similar level of development. Thus, for the duration of the liver phase of *C. mutabile* at least, existing infections have no apparent effect on the growth or development of challenge flukes. It is unlikely that challenge flukes would be affected upon entering the body cavity or air sacs a few days later. It appears, therefore, that coots harboring *C. mutabile* at levels similar to those found in nature are not resistant to subsequent infections by the parasite. The absence of an effective resistance, particularly in the heavily infected first-year cohort, results in the acquisition of new infections during the winter, which the coots carry to the breeding areas in the spring.

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Literature Cited

Buscher, H. N. 1965. Dynamics of the intestinal helminth fauna in three species of ducks. Journal of Wildlife Management 29:772–781.

Colbo, M. A. 1965. Taxonomy and ecology of the helminths of the American coot in Alberta. M.Sc. Thesis, University of Alberta, Edmonton. 155 pp.

Eley, T. 1976. Helminth parasites in American coots from the lower Colorado River. California Fish and Game 62:156–157.

Fried, B. 1963. Reinfection of chicks with an ocular trematode *Philophthalmus hegeneri* Penner and Fried, 1963. Journal of Parasitology 49:981–982.

Gullion, G. W. 1952. Some diseases and parasites of American coots. California Fish and Game 38: 421–423.

Kinsella, J. M. 1973. Helminth parasites of the American coot, *Fulica americana americana,* on its winter range in Florida. Proceedings of the Helminthological Society of Washington 40:240–242.

———, L. T. Hon, and P. B. Reed. 1973. A comparison of the helminth parasites of the common gallinule (*Gallinula chloropus cachinnans*) and the purple gallinule (*Porphyrula martinica*) in Florida. American Midland Naturalist 89:467–473.

Macy, R. A. 1973. Acquired resistance in ducks to infection with the psilostome trematode *Sphaeridiotrema globulus* (Rudolphi, 1814). Journal of Wildlife Disease 9:44–46.

McLaughlin, J. D. 1976. Experimental studies on the life cycle of *Cyclocoelum mutabile* (Zeder) (Trematoda: Cyclocoelidae). Canadian Journal of Zoology 55:1565–1567.

———. 1977. The migratory route of *Cyclocoelum mutabile* (Zeder) (Trematoda: Cyclocoelidae) in

the American coot, *Fulica americana* (Gm.). Canadian Journal of Zoology 55:274–279.

———. 1983. Growth and development of *Cyclocoelum mutabile* in coots, *Fulica americana* (Gm.). Journal of Parasitology 69:617–620.

Nollen, P. M. 1971. Studies on growth and infection of *Philophthalmus megalurus* (Cort, 1914) (Trematoda) in chicks. Journal of Parasitology 57:261–266.

Rau, M. E., T. K. R. Bourns, and C. J. Ellis. 1975. Egg production by *Trichobilharzia ocellata* (Trematoda: Schistosomatidae) after initial and challenge infection in ducks. Canadian Journal of Zoology 53:642–650.

Roudabush, R. K. 1942. Parasites of the American coot (*Fulica americana*) in Iowa. Iowa State College Journal of Science 41:437–441.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman, San Francisco. 776 pp.

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Cestodes of Some Ecuadorian Amphibians and Reptiles

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ABSTRACT: Three species of amphibians (*Bufo typhonius, Bufo marinus,* and *Hyla geographica*) and 1 reptile (*Bothrops atrox*) collected in Ecuador were examined for cestodes. *Hyla geographica* was infected with *Ophiotaenia olseni, Bufo typhonius* and *Bufo marinus* with *Cylindrotaenia americana,* and *Bothrops atrox* with *Ophiotaenia calmettei. Bufo typhonius* represents a new host record for *C. americana* and Ecuador a new locality record for *O. calmettei* and *C. americana.*

Few references are available on the cestodes of amphibians and snakes of South America and especially so on those of the Republic of Ecuador. In conjunction with an ecological study on amphibians and reptiles of Ecuador conducted during the summer of 1968 under the direction of Dr. William E. Duellman, Museum of Natural History, University of Kansas, an opportunity became available to study the helminths of some of these animials. Included in this report are new host and locality records for three species of tapeworms found in *Bufo typhonius* (Linnaeus, 1758), *Bufo marinus* (Linnaeus, 1758), *Hyla geographica* Spix, 1824, and *Bothrops atrox* (Linnaeus, 1758) collected from 3 localities in the Republic of Ecuador.

Materials and Methods

Helminths were recovered in situ by autopsy from the amphibians and reptile shortly after capture. The cestodes were fixed in AFA, stained with either alcoholic borax carmine or Harris' hematoxylin, dehydrated, cleared in beechwood creosote and mounted in Canada balsam. Notations for deposited specimens are: USNM Helm. Coll. for United States National Museum Helminthological Collection, USDA, Beltsville, Maryland and MNHUK for Museum of Natural History, University of Kansas, Lawrence, Kansas.

Results and Discussion

A single specimen of *Ophiotaenia calmettei* (Barrios, 1898) La Rue, 1911 (Proteocephala, Proteocephalidae La Rue, 1911) was found in a fer-de-lance, *Bothrops atrox* (Linnaeus, 1758), collected at an elevation of 340 m in Santa Cecilia, Napo Province, Ecuador. It agrees with the description given by La Rue (1914) supplemented by data from the reports of Marotel (1898) and Schwarz (1908) but differs from the information given in possessing 128–195 testes (average 161) located 27–49 preporally, 25–49 postporally, and 68–105 antiporally rather than 130–160 and in having a cirrus pouch 200–407 μm long by 88–105 μm wide rather than 250–290 μm long by 100–130 μm wide. Further, in gravid segments, the uterus gives off 30–39 uterine branches rather than 24–35 on each side. *Ophiotaenia calmettei* was originally described from the jararaca, *Bothrops jararac* (Wied, 1824), from Martinique, Argentina, and Brazil and has since been reported in *Bothrops atrox* in Venezuela. Ecuador constitutes a new locality record for this tapeworm.

Four slides containing the entire cestode have been deposited in USNM Helm. Coll., No. 78838. Host specimen has been deposited in MNHUK, No. 121934.

Two specimens of *Ophiotaenia olseni* Dyer and Altig, 1977 were found in the intestines of 2 of 5 *Hyla geographica* collected at an elevation of 340 m in Santa Cecilia, Napo Province, Ecuador. These specimens agree with the description of *Ophiotaenia olseni* as given by Dyer and Altig (1977). The finding of *O. olseni* in *H. geographica* from Ecuador constitutes the second report of this cestode in *H. geographica* from this locality.

Cestode specimens have been deposited in USNM Helm. Coll., No. 78868. Host specimens have been deposited in MNHUK, No. 122729 and the Werner C. A. Bokermann Collection, São Paulo, Brazil.

One specimen of *Cylindrotaenia americana* Jewell, 1916 (Cyclophyllidea, Nematotaeniidae Lühe, 1910) was found in the intestine of 1 of 13 *Bufo typhonius* collected at an elevation of 1,150 m on the south slope of the Cordillera del Dué above Rio Coca, Napo Province, Ecuador. Another was found in the intestine of 1 of 2 *Bufo marinus* collected at an elevation of 340 m in Santa Cecilia, Napo Province, Ecuador. This cylindroid tapeworm was originally described by Jewell (1916) from the intestines of several species of anurans in the contiguous United States,

namely, *Acris gryllus* (Le Conte, 1825) in Illinois, *Rana pipiens* Schreber, 1782 from Michigan and Illinois, *Rana virescens* Cope, 1889 in Nebraska, and *Bufo lentiginosus* Shelford, 1913 of unknown locality. *Cylindrotaenia americana* has since been reported from anurans and caudate amphibians as well as a single reptile in North America and anurans in South America. Harwood (1932) reported it from *Acris gryllus, Hyla squirella* Sonnini and Latreille, 1802, *Pseudacris triseriata* (Wied, 1838), and *Leiotopisma laterale* Cope, 1900. Joyeux (1924) reported *C. americana* from *Arthroleptis ogoensis* Boulenger, 1906 in Mozambique, Africa. However, Harwood (1932) pointed out that the discrepancies that Joyeux noted on comparing his specimens from African amphibians with Jewell's description are so great that specific identity of the African form with the American form seems unlikely, particularly because a form examined by Harwood taken from an American lizard, namely, *Scincella lateralis* Say, 1823 is similar to Jewell's description. Mann (1932) reported it from *Desmognathus f. fuscus* (Green, 1818) in North Carolina. Ulmer and James (1976) reported *C. americana* in *Rana pipiens* Schreber, 1782, *Bufo americanus* Holbrook, 1836, and *Acris crepitans* Baird, 1854 from Iowa. More recently, this cestode was reported in *Plethodon jordani* Blatchley, 1901 from North Carolina by Dyer (1983). According to Wardle and McLeod (1952), this species has been reported in *Leptodactylus ocellatus* (Linnaeus, 1758) in Brazil and Argentina. It has also been reported in *Bufo marinus* from Colombia by Brooks (1976). *Bufo typhonius* represents a new host record and Ecuador a new locality record for *C. americana.*

Although neotropical *Cylindrotaenia* and the North American ones are indistinguishable morphologically, they are probably not conspecific. Neotropical specimens are almost entirely found in bufonids, occasionally in leptodactylids, and not in hylids whereas the North American situation is almost the reverse, with hylids being the primary hosts and a variety of other frogs, and some salamanders, being occasional hosts.

Tapeworm specimens have been deposited in USNM Helm. Coll., Nos. 78869 and 78870. Host specimens have been deposited in MNHUK, Nos. 123949 and 123998.

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Literature Cited

Brooks, D. R. 1976. Five species of platyhelminths from *Bufo marinus* L. (Anura: Bufonidae) in Colombia with descriptions of *Creptotrema lynchi* sp. n. (Digenea: Allocreadiidae) and *Glypthelmins robustus* sp. n. (Digenea: Macroderoididae). Journal of Parasitology 62:429–433.

Dyer, W. G. 1983. A comparison of the helminth fauna of two *Plethodon jordani* populations from different altitudes in North Carolina. Proceedings of the Helminthological Society of Washington 50:257–260.

———, **and R. Altig.** 1977. Helminths of some Ecuadorian anurans. Herpetologica 33:293–296.

Harwood, P. D. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. Proceedings of the United States National Museum 81:1–71.

Jewell, M. E. 1916. *Cylindrotaenia americana* nov. spec. from the cricket frog. Journal of Parasitology 2:181–192.

Joyeux, C. 1924. Récherchés sur le cycle évolutif des *Cylindrotaenia.* Annales de Parasitologie Humaine et Comparée, France 2:74–81.

La Rue, G. R. 1914. A revision of the cestode family Proteocephalidae. Illinois Biological Monographs 1:1–350.

Mann, D. R. 1932. The ecology of some North Carolina salamanders with special reference to their parasites. M.Sc. Thesis, Duke University. 50 pp.

Marotel, G. 1898. Sur une téniadé du *Bothrops lanceolatus* (note preliminairé). Comptes Rendus de la Société de Biologie, France 50:99–101.

Schwarz, R. 1908. Die Ichthyotaenien der Reptilien und Beiträge zur Kenntnis der Bothriocephalen. Inaug. Diss. University of Basel. 52 pp.

Ulmer, M. J., and H. A. James. 1976. Studies on the helminth fauna of Iowa II. Cestodes of amphibians. Proceedings of the Helminthological Society of Washington 43:191–200.

Wardle, R. A., and J. A. McLeod. 1952. The Zoology of Tapeworms. University of Minnesota Press, Minneapolis. 750 pp.

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Acanthocephala from Lake Fishes in Wisconsin: The Giant Nuclei Pattern in *Neoechinorhynchus robertbaueri* and *N. prolixoides* (Neoechinorhynchidae)

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ABSTRACT: Numerical and structural variations in the giant nuclei of *Neoechinorhynchus robertbaueri* Amin, 1985 and *N. prolixoides* Bullock, 1963 collected from *Erimyzon sucetta* (Lacépède) in Silver Lake (Kenosha County), Wisconsin were systematically studied. Both species normally had 6 giant subcuticular nuclei and 3 giant lemniscal nuclei but younger *N. prolixoides* had more than the usual 8 giant nuclei in the cement gland. Numerical variations were not related to season or worm sex. *Neoechinorhynchus robertbaueri* apparently do not reach the older adult stage. In most older adult *N. prolixoides,* the giant nuclei do not assume the pre-reproductive condition. Reproductive activity and growth proceed simultaneously in both species. Structural changes of giant nuclei of the various developmental stages are described. Some overlap of stages of giant subcuticular nuclei was observed in *N. robertbaueri* and attributed to the compression of its reproductive season into 2–4 months. No positional changes in the giant subcuticular nuclei occurred in either species. Qualitative findings support the hypothesis that changes in all 3 sets of giant nuclei are associated with the initiation and maintenance of reproductive activity of worms. The relationship between giant nuclei patterns and the synchronization of reproductive and generation cycles appear to be common in neoechinorhynchid acanthocephalans.

One of the earliest and most important studies of Eoacanthocephala in North America is that of Van Cleave (1914), which included careful but limited observations of the giant nuclei of 5 species. Structural and functional relationships of the giant nuclei of the eoacanthocephalan *Neoechinorhynchus cylindratus* (Van Cleave, 1913) Van Cleave, 1919 was studied by Amin and Vignieri (1986). Findings indicated that changes in all giant nuclei were associated with the initiation, continuation and termination of reproductive activity of worms. Comparative studies of *N. robertbaueri* Amin, 1985 and *N. prolixoides* Bullock, 1963 collected from the same lake (Silver Lake) in southeastern Wisconsin are reported herein. Present results support the hypothesis produced by the *N. cylindratus* study and emphasize the importance of the generation span in the fish host and of worm reproductive vs. growth phases in the ultimate expression of the giant nuclei pattern.

Materials and Methods

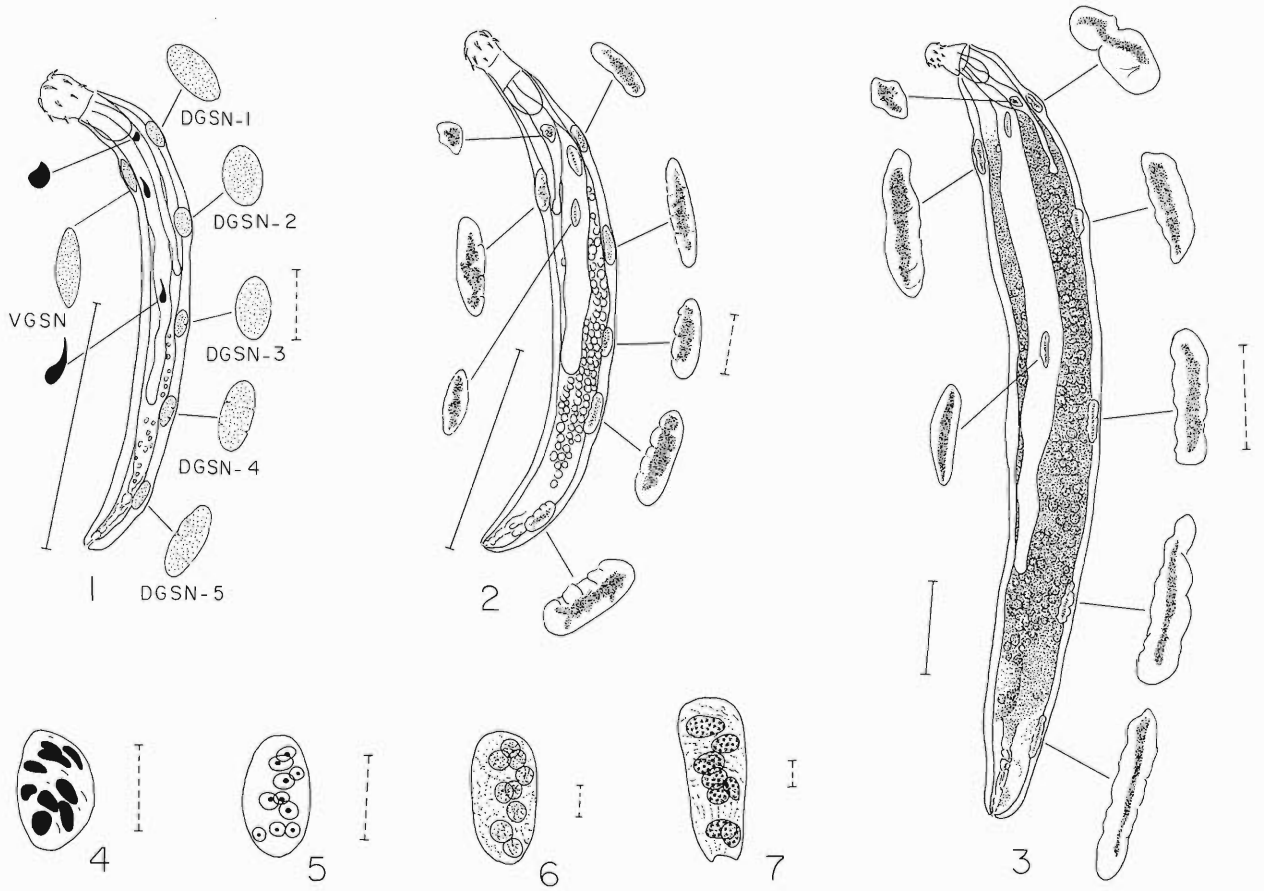
Most of the *N. robertbaueri* and *N. prolixoides* material reported by Amin (1985, 1986) and collected from the lake chub sucker, *Erimyzon sucetta* (Lacépède), in Silver Lake (Kenosha County, Wisconsin) constituted the study material. Specimens were stained in Mayer's acid carmine, cleared in ascending concentrations of terpineol (in 100% ethyl alcohol), and mounted in Canada balsam after initial fixation in AFA.

For purposes of the present investigation, worms were classed in 5 categories according to their developmental states. (1) *Juveniles:* small worms with incompletely developed reproductive system. (2) *Post-juveniles:* small worms with reproductive system developed but with no sperm or eggs; ovarian balls small or absent. (3) *Young adults:* medium-sized worms. Males with sperm; females fertilized, with many ovarian balls (some breaking up). Eggs being produced, unripe when present. (4) *Adults* (Adult I in *N. robertbaueri*): long worms. Males mature, with sperm production at its peak. Females gravid with mostly unripe eggs mixed with many ovarian balls. (5) *Adults II* (in *N. robertbaueri* only): long worms. Females gravid with mostly ripe eggs mixed with many ovarian balls. (6) *Older adults* (only in *N. prolixoides*): longest worms. Males most slender with very large testes and cement glands. Giant nuclei of latter tend to coalesce with cytoplasm. Sperm in sperm duct but its active production may have ceased. Females gravid with ripe eggs; remnants of ovarian balls or unripe eggs may be present in some. Production of new eggs may have ceased.

Structural variations in the giant subcuticular nuclei (GSN) were categorized as ovoid, elongating, or lobulating-splitting with central or dispersed chromatin.

Results and Discussion

The *N. prolixoides* studied were mostly recruited during late summer–early autumn, reached sexual maturity by the spring, and continued its simultaneous reproductive activity and growth through the summer into the following autumn (Amin, 1986). The life span of *N. robertbaueri* in its fish host was, however, very short



Figures 1-7. Developmental stages and associated changes in the giant nuclei of *Neoechinorhynchus robertbaueri* females (Figs. 1-3) and in male cement glands (Figs. 4-7). 1. Post-juvenile collected on April 24. 2. Young adult collected on May 12. 3. Adult (II) collected on July 5. 4-7. Giant nuclei in cement gland of a juvenile (Fig. 4), of an older post-juvenile (Fig. 5), of a young adult (Fig. 6), and of an adult (Fig. 7). Ovarian balls and/or eggs overlapping the lemnisci (Figs. 1-3) are not shown. Solid measurement bar = 1 mm, dotted bars = 100 μm. All giant nuclei in each of Figures 1-3 are to same scale.

Table 1. Variations in the number of giant subcuticular nuclei of male and female *Neoechinorhynchus robertbaueri* collected from *Erimyzon sucetta* in Silver Lake.

			No. of worms with specified no. of giant subcuticular nuclei (%)			
			Dorsal			Ventral
			Normal	<Normal*	>Normal	Normal
Worm sex	Developmental stage	*N*	(5)	(4)	(6)	(1)
Female	Juvenile, post-juvenile	19	16 (84)	2 (11)	1 (5)	19 (100)
	Young adult	43	36 (84)	6 (14)	1 (12)	43 (100)
	Adult (I, II)	21	18 (86)	3 (14)	—	21 (100)
	Total	83	70 (84)	11 (13)	2 (3)	83 (100)
Male	Juvenile, post-juvenile	26	22 (85)	4 (15)	—	26 (100)
	Young adult	24	19 (80)	5 (20)	—	24 (100)
	Adult (I)	21	17 (81)	4 (19)	—	21 (100)
	Total	71	58 (82)	13 (18)	—	71 (100)

* One female not included in this sample had 3 dorsal giant subcuticular nuclei.

(April–July; males are lost after May) (Amin, 1985). This brief period appeared to have ontogenetically enhanced the simultaneous growth and sexual development of *N. robertbaueri* resulting in (1) overlap in the GSN stages in many reproductively active worms, and (2) virtual absence of older adults in which the production of sex cells has ceased. Materials of both species examined were all collected from 1 host species, *E. sucetta,* in 1 lake, Silver Lake, in southeast Wisconsin. Variables examined include season, worm sex, and developmental stage, number, shape, and position of giant nuclei. Changes in the GSN and giant lemniscal nuclei (GLN) were similar in both sexes but only shown in females (Figs. 1–3, 8–11).

I. Quantitative Variations

The giant lemniscal nuclei (GLN) and giant subcuticular nuclei (GSN)

Seventy-one male and 83 female *N. robertbaueri,* and 94 male and 149 female *N. prolixoides* were studied quantitatively. The normal number of GLN in both species was 3 (2 and 1 in the longer and shorter lemniscus, respectively) in 100% of all males and females examined. Five dorsal and 1 ventral GSN were present in 82% of male and 84% of female *N. robertbaueri* (Table 1) and in 90% of male and 89% of female *N. prolixoides* (Table 2). These percentages are more or less comparable to those observed in *N. cylindratus* but the magnitude of variation in the dorsal giant subcuticular nuclei (DGSN) (usually ±1) was less than that of the more variable *N. cylindratus* (see Amin and Vignieri, 1986). These variations did not appear to be related to worm sex or developmental state. Misallocation, shuffling, and rearrangement as suggested by Van Cleave (1949) were not evident.

The giant cement gland nuclei (GCGN)

Eight male *N. robertbaueri,* in which the number of GCGN was ascertained, had 8 nuclei in the cement gland of each (100%) in addition to the normal 6 GSN and 3 GLN. Three other males with 9, 10, and 11 GCGN were, however, encountered. *Neoechinorhynchus prolixoides* had a wide range of 6–14 GCGN. Ninety-five percent of 43 immatures (post-juveniles) had more than 8 GCGN; the most common number was 11 nuclei (in 58% of worms) (Table 3). The number of GCGN was somewhat fewer in young adults and adults but reached 8 in most (85% of 34) older adults.

In the latter worms, the nuclei tended to coalesce with the cement gland cytoplasm. The large percentage of male *N. prolixoides* (78% of 65) having a total of more than 17 giant nuclei during the spring was directly related to the presence of 43 post-juvenile males in that sample. The summer and autumn samples included only adult and older males with only 30–33% of worms having a total of more than 17 nuclei (Table 2). It is concluded that the number of GCGN in *N. prolixoides* is not related to season but to devel-

Table 2. Summary of quantitative variations in giant nuclei of male and female *Neoechinorhynchus prolixoides* collected from *Erimyzon sucetta* in Silver Lake during the spring (April), summer (June, July, early August) and autumn (late October, November).

			No. of worms with specified no. of giant nuclei (%)							
	N		Usual*		Usual/ reallocated†		Less than usual‡		More than usual§	
Season	♂	♀	♂ (17)	♀ (9)	♂ (17)	♀ (9)	♂ (15–16)	♀ (8)	♂ (18–23)	♀ (10–11)
Spring	65	79	13 (20)	70 (89)	0	1 (1)	1 (1)	5 (6)	51 (78)	3 (4)
Summer	23	45	14 (61)	39 (87)	1 (4)	0	1 (4)	6 (13)	7 (30)	0
Autumn	6	25	4 (67)	23 (92)	0	0	0	2 (8)	2 (33)	0
Total	94	149	31 (33)	132 (89)	1 (1)	1 (1)	2 (2)	13 (9)	60 (64)	3 (2)

* Subcuticular: 5 dorsal and 1 ventral; lemniscal: 2 (in longer lemniscus) and 1 (in shorter); cement gland: 8.
† Subcuticular 4 and 1–2; lemniscal 2 and 1; cement gland 8–9.
‡ Subcuticular 4–5 and 1; lemniscal 2 and 1; cement gland 6–8.
§ Subcuticular 5–7 and 1; lemniscal 2 and 1; cement gland 8–14.

opmental stage. It is not clear whether changes in the number of GCGN is related to reproductive activity or if the "normal" number is 8 as characteristically found in most older adults. Whether the initiation and continuation of sperm production might require more than the usual eight GCGN in *N. prolixoides* is unknown.

II. Qualitative Variations

Seventy-one male and 83 female *N. robertbaueri* and 117 male and 149 female *N. prolixoides* were studied qualitatively. Observations were made to determine if giant nuclei changes were related to reproductive activity or to season.

The giant subcuticular nuclei (GSN)

As in *N. cylindratus,* sexually immature *N. robertbaueri* and *N. prolixoides* males and females had ovoid GSN (Figs. 1, 8) that began to elongate upon initiation of reproductive activity irrespective of the time of year. In reproductively active young adult and adult (I, II) *N. robertbaueri* the GSN elongate then lobulate-split and the chromatin becomes more deeply stained and central (Table 4, Figs. 2, 3). Most young adult and adult *N. prolixoides* have elongate GSN that persist in older adults (Figs. 9–11) with the exception of a few in which the nuclei reassume the ovoid shape characteristic of the sexually immature juveniles and post-juveniles (Table 5). In the latter older adults (not observed in the autumn), the production of new sex cells appeared to have ceased. A similar stage was observed in only one male *N. robertbaueri* (Table 4) but was the rule in *N. cylindratus* (see Amin and Vignieri, 1986). In *N. cylindratus* reproductive activity appeared to have ceased in males and females by the end of the spring supposedly to channel metabolic energy into growth in order to accommodate more ripe sex cells in older summer adults (Amin and Vignieri, 1986). This *N. cylindratus* pattern would not be expected to prevail in species with simultaneous reproductive activity and growth like *N. robertbaueri* and *N. prolixoides.*

The very brief generation span of *N. robertbaueri* in *E. sucetta* apparently effected the following. (1) Females do not reach the older adult stage in which sex cell production ceases. (2) The GSN shapes characteristic of reproductively active females did not usually occur in a distinct

Table 3. Quantitative variations in the cement gland giant nuclei of *Neoechinorhynchus prolixoides* of different developmental stages.

Developmental stage	*N*	No. (%) of worms with specified number of giant nuclei in cement gland								
		6	7	8	9	10	11	12	13	14
Post-juvenile	43	—	—	2 (5)	3 (7)	9 (21)	25 (58)	1 (2)	2 (5)	1 (2)
Young adult	5	—	—	—	—	2 (40)	1 (20)	—	1 (20)	1 (20)
Adult	12	—	—	2 (17)	2 (17)	3 (25)	3 (25)	1 (8)	—	1 (8)
Older adult	34	1 (3)	—	29 (85)	4 (12)	—	—	—	—	—

Table 4. Variations in the shape of the giant subcuticular nuclei of 83 female and 71 male *Neoechinorhynchus robertbaueri* examined from *Erimyzon sucetta* in Silver Lake.

Worm sex	Developmental stage	*N*	No. of worms whose giant subcuticular nuclei were shaped as below*		
			Ovoid	Elongate	Elongate-lobulate
Female	Juvenile, post-juvenile	18	12 (67)†	—	6 (33)
	Young adult	43	—	26 (60)	17 (40)
	Adult (I, II)	22	—	11 (50)	11 (50)
Male				—	
	Juvenile, post-juvenile	26	19 (73)	—	7 (27)
	Young adult	24	—	19 (79)	5 (21)
	Adult (I)	21	1 (15)	15 (71)	5 (24)

* Often in same worm DGSN-5 or DGSN-4, 5 are almost split and the other GSN are elongated and/or lobulated; see text for other combinations.

† Number (%).

sequence, e.g., elongating to lobulating-splitting, but were often mixed in the same worm. In these cases, the DGSN 4 or 4 and 5 were almost splitting, whereas others in the same worm were elongated or lobulated (Fig. 2). Worms with 4 or 5 "splitting" GSN and 1 lobulating nucleus, and other intermediate combinations were also observed. Compression of *N. robertbaueri* reproductive activity in the short time available was clearly associated with overlap in the stages (Fig. 2) of the GSN in individual worms. In another expression of this compression, about one-third of the nonreproductive stages (mostly post-juveniles) were observed to have elongating-lobulating GSN like those observed in reproductive stages. It may be that sex cell production had already been initiated in these worms but that detectable anatomical evidence was not yet readily evident.

Chromatin granules were centrally located in practically all sexually immature, many young adult, and some adult *N. prolixoides,* but were dispersed in most older adults (Figs. 8–11). The significance of this observation is yet to be determined. Unlike *N. cylindratus,* no positional changes in the *N. robertbaueri* or *N. prolixoides* GSN associated with reproductive state was observed.

The giant lemniscal nuclei (GLN)

In *N. robertbaueri* males and females, the GLN underwent certain changes as the worms became reproductively active (Table 6). In all juveniles and post-juveniles, the GLN of the binucleate lemniscus that were homogeneously deeply stained were invariably tear-drop-shaped (Fig. 1). In young adults and adults, they consistently elongated like the GSN, with the chromatin granules clearly removed from the nuclear margin to a central position (Figs. 2, 3). The nuclei did not assume the tear-drop form in the older adults. The nuclei of the considerably smaller uninucleate lemnisci of *N. robertbaueri* were truncated tear-drop-shaped in immatures (Fig. 1), which changed to ameboid-amorphous oblong (Figs. 2, 3).

In *N. prolixoides,* the shape of the GLN was similar in both lemnisci and showed a definite relationship with the developmental state of worms, irrespective of season or worm sex. Almost all (98%) of the 91 juveniles (45 males, 46 females) examined had ovoid GLN which changed into amorphous-elongate with the onset of reproductive activity in young adults ($N = 16$) (Figs. 8–11). The only exceptions were 3 juveniles with amorphous-elongate nuclei and 3 young adults with ovoid nuclei. In all 51 adults (13 males, 38 females) and 104 older adults (53 males, 51 females), the GLN invariably remained amorphous-elongate; none assumed the ovoid shape characteristic of immature worms even though the GSN in some older adults (11% or 110) did so (Table 5).

The above data thus indicate that, like the GSN, changes in the GLN of *N. robertbaueri* and *N. prolixoides* are also associated with the synchronization of reproductive activity. The pattern in both species was similar to that observed in *N. cylindratus* by Amin and Vignieri (1986)

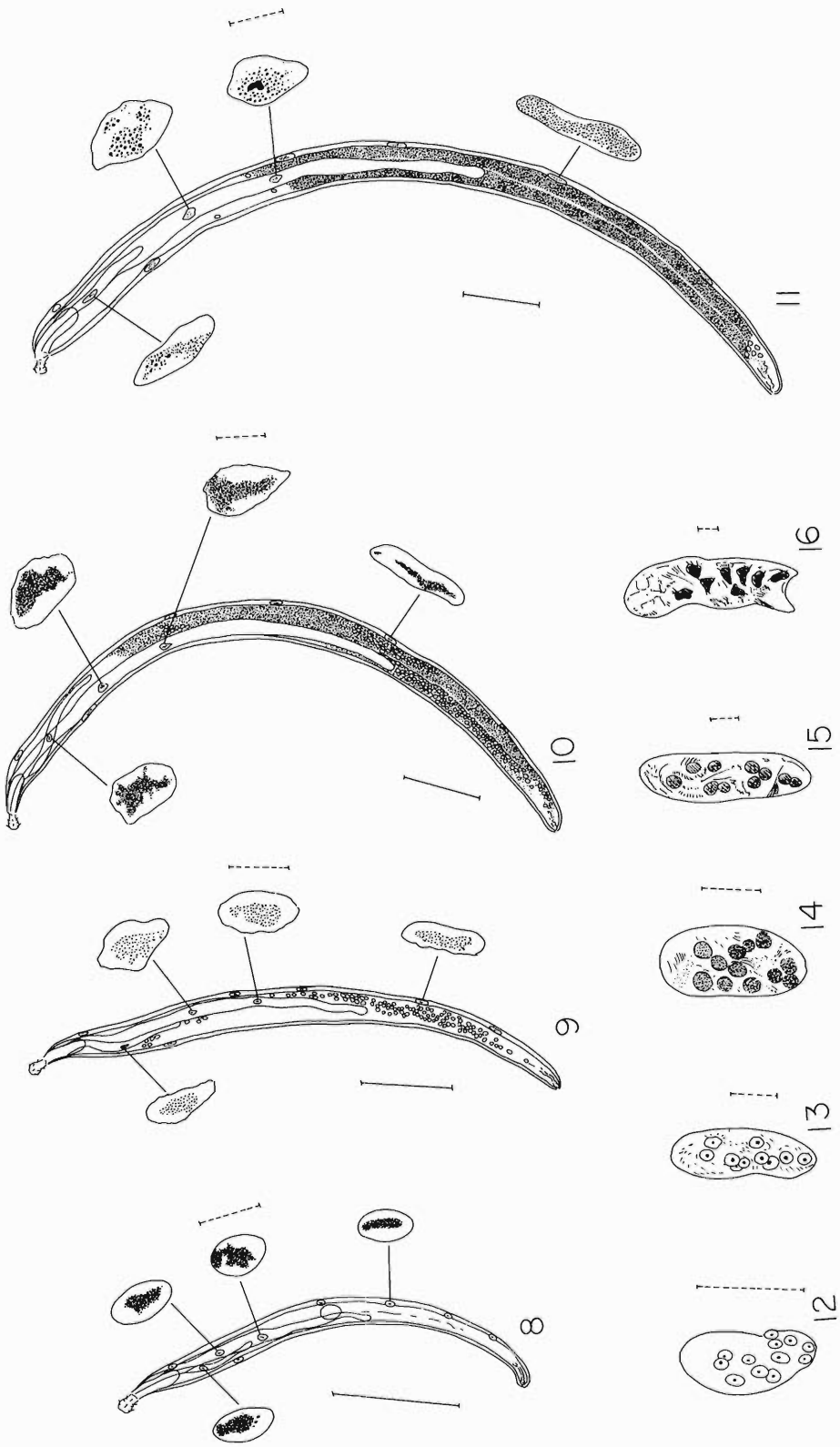
Table 5. Variations in the shape of the giant subcuticular nuclei of *Neoechinorhynchus prolixoides* examined from *Erimyzon sucetta* in Silver Lake during the spring (79 females, 78 males), summer (45, 26), and autumn (25, 13).

		No. of worms whose giant subcuticular nuclei are shaped as below*									
		Females					Males				
			Ovoid		Elongate			Ovoid		Elongate	
Season	Developmental stage*	*N*	Chromatin central	Chromatin dispersed	Chromatin central	Chromatin dispersed	*N*	Chromatin central	Chromatin dispersed	Chromatin central	Chromatin dispersed
Spring	Juvenile	45	39 (87)†	3 (7)	3 (7)	—	45	44 (98)	1 (2)	—	—
	Young adult	6	—	2 (33)	4 (67)	—	5	1 (20)	—	4 (80)	—
	Adult	14	—	1 (7)	—	13 (93)‡	5	—	2 (40)	3 (60)	—
	Older adult	14	—	3 (21)	—	11 (79)	23	—	6 (26)	7 (30)	10 (43)
Summer	Young adult	6	—	—	3 (50)	3 (50)	0	—	—	—	—
	Adult	17	—	—	14 (82)‡	3 (18)	3	—	—	3 (100)	—
	Older adult	22	—	3 (14)	9 (41)‡	10 (45)‡	23	—	—	18 (78)	5 (22)
Autumn	Juvenile	1	1 (100)	—	—	—	0	—	—	—	—
	Young adult	3	2 (67)	—	1 (33)	—	0	—	—	—	—
	Adult	3	—	—	3 (100)	—	3	—	—	2 (67)	1 (33)
	Older adult	18	—	—	—	18 (100)	10	—	—	—	10 (100)

* See Figures 8–11.

† Number (%).

‡ Giant subcuticular nuclei were exceptionally slender and long in 1 of 14 (7%) adult females in the spring, 3 of 17 adult females in the summer and 10 of 22 older adult females in the summer.



Figures 8-16. Developmental stages and associated changes in the giant nuclei of *Neoechinorhynchus prolixoides* (Figs. 8-11) and in male cement glands (Figs. 12-16). 8. Juvenile. 9. Young adult. 10. Adult. 11. Older adult. Giant nuclei in cement gland of a juvenile (Fig. 12), of a post-juvenile (Fig. 13), of a young adult (Fig. 14), of an adult (Fig. 15), and of an older adult (Fig. 16). Ovarian balls and/or eggs overlapping the lemnisci (Figs. 8-11) are not shown. Solid measurement bars = 1 mm, dotted bars = 100 μm. All giant nuclei in each of Figures 8-11 are to same scale.

Table 6. Variations in the shape of the giant nuclei of the binucleate lemniscus of *Neoechinorhynchus robertbaueri* examined from *Erimyzon sucetta* in Silver Lake.

Worm sex	Developmental stage	*N*	No. of worms whose giant lemniscal nuclei were shaped as below: Tear drop	Elongated
Female	Juvenile, post-juvenile	15	15 (100)*	—
	Young adult	25	—	25 (100)
	Adult	9	—	9 (100)
Male	Juvenile, post-juvenile	13	13 (100)	—
	Young adult	15	—	15 (100)
	Adult	13	—	13 (100)

* Number (%).

Table 7. Variations in the shape of the giant nuclei of the cement gland in 48 male *Neoechinorhynchus robertbaueri* examined from *Erimyzon sucetta* in Silver Lake.

Developmental stage	*N*	No. of males whose giant cement gland nuclei were shaped as below: Amorphous-rounded (Figs. 4, 5)	Rounded (Fig. 6)	Oblong (Fig. 7)
Juvenile, post-juvenile	18	18 (100)*	—	—
Young adult	20	—	10 (50)	10 (50)
Adult	10	—	—	10 (100)

* Number (%).

even though the structural forms assumed by the GLN were different.

The giant cement gland nuclei (GCGN)

Changes in the GCGN appear to correspond to the developmental state of males. In juvenile and post-juvenile *N. robertbaueri,* the GCGN are small, homogeneously deeply stained and amorphous (Fig. 4), then round up retaining the deeply stained chromatin in the center (Fig. 5). The latter (Fig. 5) were similar to those observed in *N. emydis* (Leidy, 1851) sensu Fisher, 1960 by Van Cleave (1914, figs. 41, 42). In young adult *N. robertbaueri,* they remain round, increase in size, and their chromatin granules become lightly dispersed; the largest nuclei begin to assume an oblong shape (Fig. 6). The oblong form predominates in adults (Fig. 7, Table 7).

In *N. prolixoides,* immatures invariably have ovoid-rounded nuclei with predominantly central chromatin. The very few young adults and adults examined showed mixed stages, but the older adults consistently had amorphous nuclei or ones that were at various stages of coalescing with cement gland cytoplasm (Figs. 12–16, Table 8). The above changes in the GCGN of both species were more distinct than those observed in *N. cylindratus* by Amin and Vignieri (1986). The pattern in the GCGN was similar to that in the GLN of *N. prolixoides* in the following 2 respects. (1) The nuclei in older adults did not assume the shape characteristic of the immature stages. (2) Chromatin granules were central in immatures but became dispersed in older worms. The pattern in these 2 sets of giant nuclei thus agrees with that of the GSN, which shows significant synchrony with the developmental state of worms, irrespective of season.

Conclusions

Findings on the giant nuclei pattern in *N. robertbaueri* and *N. prolixoides* generally agree with, and are best viewed in conjunction with, those observed in *N. cylindratus* (see Amin and Vignieri, 1986). Normally, 6 (5 + 1) GSN, 3 GLN (2 + 1), and 8 GCGN are present in all 3 species except for younger *N. prolixoides,* which had markedly more GCGN (Table 3). The reasons for this exception are not known. The same normal number of nuclei was observed by Van Cleave (1914) in *N. emydis* and *N. tenellus* (Van Cleave, 1913) Van Cleave, 1919, which showed no variation. Later studies documenting numerical variations include that of Lynch (1936) on *N. cristatus* Lynch, 1936, *N. crassus* Van Cleave, 1919, and *N. venustus* Lynch, 1936.

All three species had ovoid GSN that change to an elongating or lobulating-splitting shape with the onset of reproductive activity; the changes were not essentially identical but were rather species specific. Corresponding changes in the shape of the GLN and the GCGN were also evident in all species. Only Van Cleave (1914) made the isolated observation that the GSN of *N. tenellus* "assumes a rounded outline in immature forms as contrasted with the more elongated and less regular outline of each nucleus in the fully mature animal." Van Cleave (1914) also observed that in some species of *Neoechinorhynchus* "the nuclei of the lemnisci . . . closely resembled those of the subcuticula in structure . . . ." It is thus concluded that changes in all 3

Table 8. Variations in the shape of the giant cement gland nuclei in male *Neoechinorhynchus prolixoides* examined from *Erimyzon sucetta* in Silver Lake during the spring (72 specimens), summer (26), and autumn (12).

Season	Developmental stage*	*N*	No. of males whose giant cement gland nuclei were shaped as below*			
			Ovoid-rounded		Amorphous	Coalesced with cytoplasm
			Chromatin central	Chromatin dispersed	Chromatin dispersed	
Spring	Post-juvenile	43	39 (91)†	4 (9)	—	—
	Young adult	3	1 (33)	2 (67)	—	—
	Adult	3	2 (67)	1 (33)	—	—
	Older adult	23	—	—	12 (52)	11 (48)
Summer	Adult	2	1 (50)	—	1 (50)	—
	Older adult	24	1 (4)	—	18 (75)	5 (21)
Autumn	Adult	1	—	—	1 (100)	—
	Older adult	11	—	—	4 (36)	7 (64)

* See Figures 12–16.
† Number (%).

sets of giant nuclei are closely associated with initiation and retention of the reproductive state of neoechinorhynchid acanthocephalans. Only in *N. cylindratus* were there additional and corresponding positional changes in the GSN (Amin and Vignieri, 1986). Where the processes of reproduction and growth are biphasic, e.g., in *N. cylindratus,* the production of new sex cells appears to come to a halt in late spring simultaneously with the reversal of all giant nuclei back to the pre-reproductive condition (in shape and position) and with the initiation of the growth phase. The fact that the giant nuclei in older *N. cylindratus* adults reassume the pre-reproductive ovoid shape having less surface area calls into question Van Cleave's (1951) assumption that "changes in form of the original embryonic nuclei seems to be basically a response toward attainment of increased nuclear surface to compensate for increased volume or activity of the cytoplasm" in adults. In worms that have a generation span similar to that of *N. cylindratus* but which have simultaneous reproductive and growth phases, e.g., *N. prolixoides,* such shape reversal is scarcely evident. A possible regulatory role of the giant nuclei may be worthwhile investigating. It is not certain which of the above two species has the "better" strategy for a high reproductive potential; both species had a similar proportion of reproductive females (ca. 50%) among their summer populations (Amin, 1986). The overlap in the GSN stages observed only in *N. robertbaueri* was primarily due to its short generation span compressed in 2–4 months.

The above conclusions provide morpho-ecological foundation for the associations of giant nuclei with the reproductive, seasonal, and growth cycles of some neoechinorhynchid acanthocephalans. Additional studies in other species should produce patterns that would be compatible with the ones studied above. Studies exploring the biochemical nature of these giant nuclei in their different structural states and relating it to corresponding reproductive phases of worms are underway. The ultimate contribution would be an attempt to explain "the mechanisms turning certain reproductive activities on and off and thus synchronizing reproductive and generation cycles which yield different, but associated, information obtainable at the ecological and population levels" (Amin and Vignieri, 1986).

Literature Cited

Amin, O. M. 1985. Acanthocephala from lake fishes in Wisconsin: *Neoechinorhynchus robertbaueri* n. sp. from *Erimyzon sucetta* (Lacépède) with a key to species of the genus *Neoechinorhynchus* Hamann, 1892, from North American freshwater fishes. Journal of Parasitology 71:312–318.

———. 1986. Acanthocephala from lake fishes in Wisconsin: host and seasonal distribution of species of the genus *Neoechinorhynchus,* Hamann, 1892. Journal of Parasitology 72:111–118.

———, **and J. C. Vignieri.** 1986. Acanthocephala from lake fishes in Wisconsin: numerical and structural-functional relationships of the giant nu-

clei in *Neoechinorhynchus cylindratus* (Neoechinorhynchidae). Journal of Parasitology 72:88–94.

Lynch, J. E. 1936. New species of *Neoechinorhynchus* from the western sucker, *Catostomus macrocheilus* Girard. Transactions of the American Microscopical Society 55:21–43.

Van Cleave, H. J. 1914. Studies on cell constancy in the genus *Eorhynchus*. Journal of Morphology 25: 253–298.

———. 1949. The acanthocephalan genus *Neoechinorhynchus* in the catostomid fishes of North America, with descriptions of two new species. Journal of Parasitology 35:500–512.

———. 1951. Giant nuclei in the subcuticula of the thorny-headed worm of the hog (*Macracanthorhynchus hirudinaceus*). Transactions of the American Microscopical Society 70:37–45.

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Helminth Parasites of the Steller Sea Lion, *Eumetopias jubatus,* in Alaska

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ABSTRACT: Sixty-seven Steller sea lions from the Gulf of Alaska and 7 from the Bering Sea were examined for helminth parasites. The Gulf of Alaska sea lions harbored 11 species of helminths in the digestive tract: *Diplogonoporus tetrapterus* (von Seibold, 1848) Ariola, 1896, *Diphyllobothrium pacificum* (Nybelin, 1931) Margolis, 1956, *Anophryocephalus ochotensis* Delyamure and Krotov, 1955, *Phocanema decipiens* (Krabbe, 1878) Myers, 1959, *Contracaecum osculatum* (Rudolphi, 1802) Baylis, 1920, *Anisakis similis* (Baird, 1853) Baylis, 1920, *Pricetrema zalophi* (Price, 1932) Ciurea, 1933, *Phocitrema fusiforme* Goto and Ozaki, 1930, *Corynosoma strumosum* (Rudolphi, 1802) Luhe, 1904, *C. villosum* Van Cleave, 1953, and *Bolbosoma bobrovoi* Krotov and Delyamure, 1952. The sea lions collected in the Bering Sea contained all of the above species except *P. zalophi, P. fusiforme, C. strumosum,* and *B. bobrovoi.* In addition, they harbored *Corynosoma semerme* (Forssell, 1904) Luhe, 1911, and *C. validum* Van Cleave, 1953. *Phocitrema fusiforme, C. semerme,* and *C. validum* are new host records for the Steller sea lion.

In the course of life history investigations of marine mammals from areas of potential petroleum development in the state of Alaska, personnel of the Alaska Department of Fish and Game made available Steller sea lions collected from hauling grounds throughout the Gulf of Alaska (location 1) and from the southern edge of the seasonal pack ice in the Bering Sea (location 2) (Fig. 1). Because reports of helminths from Alaskan Steller sea lions are rare (Neiland, 1962; Rausch, 1964) and because of the considerable sample size of these collections, the results of this study are reported here.

Materials and Methods

A total of 67 Steller sea lions from the Gulf of Alaska and 7 from the Bering Sea were necropsied to determine the kinds and incidence of helminths present.

The lungs, liver, heart, stomach, and intestine were examined for helminths. In order to determine stratification of helminth species within the intestine, 3.3-m sections of the anterior, middle, and posterior parts of the small intestine, as well as the entire large intestine were examined. Each section was split open and allowed to stand in a container of fresh water for several hours before the helminths, freed from the mucosa, were decanted and relaxed in tap water containing several drops of pentabarbitol sodium (Nembutal). After relaxation, the helminths were fixed in hot 10% formalin. Cestodes, trematodes, and acanthocephalans were stained in Semichon's acetocarmine, dehydrated in ethanol, cleared in terpineol, and mounted in permount. Nematodes were stained as above, destained in 70% acetic ethanol, and mounted directly into Hoyer's modified Berlese solution (Goldstein, 1955). Tissues were not checked for the presence of *Trichinella spiralis.*

Results and Discussion

Helminths were found only in the stomach and the small and large intestine. Gross visual examination indicated that most of these appeared to have caused little or no pathological changes in those organs. The prevalence of each helminth species is summarized in Table 1. Representative specimens of each have been deposited in the National Parasite Collection, Agricultural Research Service, Beltsville, Maryland (USNM Helm. Coll. Nos. 74448, 76200–76211). The helminths recovered are summarized below.

Cestoda

1. *Anophryocephalus ochotensis* Delyamure and Krotov, 1955. This was the most frequently found cestode in Steller sea lions and was the most abundant cestode in the individuals where found. It generally was represented only by scolices with a short germinal portion and was located in the duodenal portion of the small intestine. Only a few individuals with gravid proglottids were found. This cestode has been reported from Steller sea lions in the Okhotsk Sea (Delyamure, 1955) but had not been found previously in this host in the eastern Pacific.

2. *Diphyllobothrium pacificum* (Nybelin, 1931) Margolis, 1956. *Diphyllobothrium pacificum* was present together with *Diplogonoporus tetrapterus* in sea lions from both areas of collection. It was found in the ilial portion of the small intestine. It has been reported from Steller sea lions in British Columbia, Canada (Margolis, 1956).

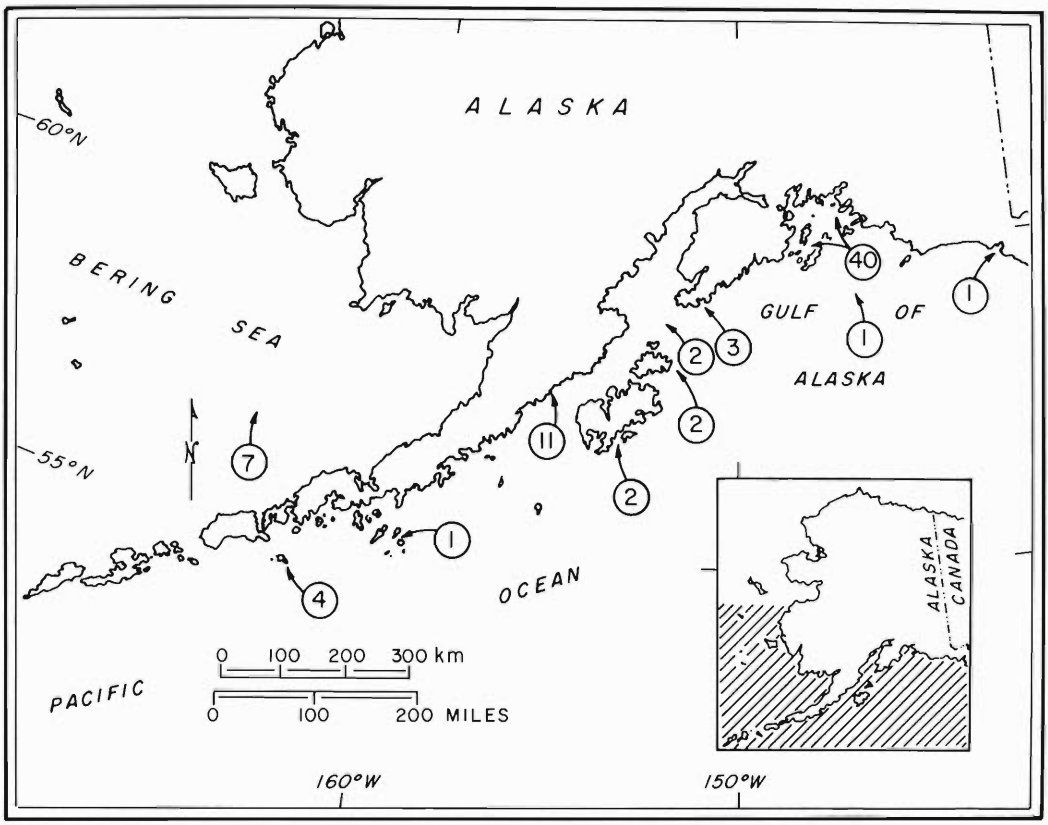

Figure 1. Geographical locations of Steller sea lion collections. Circled numbers indicate sample size at each collecting locality.

3. *Diplogonoporus tetrapterus* (von Siebold, 1848) Ariola, 1896. This cestode was found in the ilial portion of the small intestine and large intestine in sea lions from both locations. It has been reported previously from this host by Rausch (1964) and it exhibited morphologic variations similar to those described by him in having from 1 to 4 sets of reproductive organs per proglottid.

Digenea

4. *Phocitrema fusiforme* Goto and Ozaki, 1930. This trematode, found in the jejunal portion of the small intestine, occurred only in sea lions from the Gulf of Alaska. It has been recorded from *Phoca vitulina* collected at St. Lawrence Island in the Bering Sea (Rausch and Locker, 1951), and from Steller sea lions in northern Japan (Machida et al., 1981).

5. *Pricetrema zalophi* (Price, 1932) Ciurea, 1933. *Pricetrema zalophi* was the most common trematode encountered in this study. It was found in hosts only from the Gulf of Alaska and occurred in the jejunal portion of the small intestine. This species has been reported from *E. jubatus* in California (Dailey and Hill, 1970) and northern Japan (Machida et al., 1981).

Nematoda

6. *Anisakis similis* (Baird, 1853) Baylis, 1920. Nematodes of this species occurred in the stomach but were not associated with any pathological lesions in the animals examined from either locality. They have been reported previously from this host in California by Schroeder and Wegeforth (1935).

7. *Contracaecum osculatum* (Rudolphi, 1802) Baylis, 1920. *Contracaecum osculatum* was present in the stomach and pylorus of sea lions from the Gulf of Alaska. This species has been reported from *E. jubatus* in Canada (Margolis, 1956).

Table 1. Helminth parasites of Steller sea lions from the Gulf of Alaska and the Bering Sea.

Species	Gulf of Alaska $N = 67$		Bering Sea $N = 7$	
	No. inf	% inf	No. inf	% inf
Diplogonoporus tetrapterus	63	94	2	28
Diphyllobothrium pacificum	21	31	1	14
Anophryocephalus ochotensis	64	96	5	71
Pricetrema zalophi	39	58	—	—
Phocitrema fusiforme	7	10	—	—
Phocanema decipiens	39	58	5	71
Anisakis similis	51	76	4	57
Contracaecum osculatum	50	75	1	14
Corynosoma semerme	—	—	1	14
C. strumosum	39	58	—	—
C. validum	—	—	1	14
C. villosum	65	97	7	100
Bolbosoma bobrovoi	3	4	—	—

8. *Phocanema decipiens* (Krabbe, 1878) Myers, 1959. In a few cases, clumps of these nematodes caused ulcerations of the gastric mucosa in sea lions from both the Gulf of Alaska and Bering Sea. It has been reported from Steller sea lions in British Columbia (Margolis, 1956) as well as the Commander Islands in the western Bering Sea by Delyamure (1955). Dailey and Hill (1970) did not find it in 9 *E. jubatus* examined in California.

Acanthocephala

9. *Bolbosoma bobrovoi* Krotov and Delyamure, 1952. This helminth was found in the ilial segment of the small intestine of a small portion of Steller sea lions from the Gulf of Alaska. It has been previously reported from this host in the Okhotsk Sea (Delyamure, 1955).

10. *Corynosoma semerme* (Forssell, 1904) Luhe, 1911. *Corynosoma semerme* occurred in the ilial portion of the small intestine of Steller sea lions from the Bering Sea. This is the first report of this species from the Steller sea lion although it has been reported from spotted seals *Phoca largha* Pallas, 1811 from the Bering Sea (Shults, 1982) and other phocid seals by Delyamure (1955).

11. *Corynosoma strumosum* (Rudolphi, 1802) Luhe, 1904. This acanthocephalan was found in the ilial portion of the small intestine of Steller sea lions from the Bering Sea. It has been reported in this host from California (Dailey and Hill, 1970). Margolis and Dailey (1972) reported it from Alaska, British Columbia, and Washington in other pinnipeds as well as sea lions.

12. *Corynosoma validum* Van Cleave, 1953. This helminth was found only in hosts from the Bering Sea where it also occurred in the ilial portion of the small intestine. It has been reported from bearded seals *Erignathus barbatus* Erxleben, 1777 and walruses *Odobenus rosmarus* (Linnaeus, 1758) by Margolis and Dailey (1972) but is a new host record for the Steller sea lion.

13. *Corynosoma villosum* Van Cleave, 1953. This species also occurred in the ilial portion of the small intestine of Steller sea lions from both the Gulf of Alaska and the Bering Sea. It was the most common helminth encountered from both areas and has been reported previously in this host from Canada by Margolis (1956).

Examination of the jejunal, duodenal, and ilial portions of the small intestine of Steller sea lions from the Gulf of Alaska and Bering Sea showed marked stratification of helminths. *Anophryocephalus ochotensis* was found mainly in the anterior (duodenal) part, and the jejunal portion was the principal site for the trematodes *Phocitrema fusiforme* and *Pricetrema zalophi.* The acanthocephalans *Corynosoma strumosum, C. villosum, C. semerme, C. validum,* and *Bolbosoma bobrovoi* were found primarily in the ilial portion of the small intestine. The ilial portion of the small intestine was also the usual site occupied by *Diphyllobothrium pacificum. Diplogonoporus tetrapterus* was found with about equal frequency in both the ilium and the large intestine.

The helminth fauna of Steller sea lions from the Gulf of Alaska was very similar to that reported from this host in the southwestern and northwestern parts of the Pacific (Delyamure, 1955; Margolis and Dailey, 1972). Exceptional was the absence of nematodes of the genus *Parafilaroides* in the Alaskan hosts and the presence of *Phocitrema fusiforme, Corynosoma semerme,* and *C. validum* not previously reported from this host. Steller sea lions collected in the Gulf of Alaska harbored 4 species of helminths not found in those from the Bering Sea and the Bering Sea hosts had 2 species not found in the Gulf of Alaska. Although the life histories of these helminths are generally not completely known, it would seem that the different species composition between the 2 localities is probably due to sea lions utilizing different intermediate hosts as food items in each locality.

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Literature Cited

Dailey, M. D, and B. L. Hill. 1970. A survey of metazoan parasites infecting the California (*Zalophus californianus*) and Steller (*Eumetopias jubatus*) sea lion. Bulletin of the Southern California Academy of Sciences 69:126–132.

Delyamure, S. L. 1955. Helminthofauna of marine mammals (ecology and phylogeny). Izdatel'stvo Akademii Nauk SSSR, Moscow. 517 pp. Israel Program for Scientific Translations, Jerusalem. 552 pp.

Goldstein, H. L. 1955. A novel medium for mounting nematodes. Journal of Agriculture of the University of Puerto Rico 1:46–47.

Machida, M., N. Seki, and K. Yamaguchi. 1981. Trematodes of Steller sea lions caught off Hokkaido, northern Japan. Bulletin of the National Science Museum, Tokyo, Series A (Zoology) 7: 147–154.

Margolis, L. 1956. Parasitic helminths and arthropods from pinnipeds of the Canadian Pacific Coast. Journal of the Fisheries Research Board of Canada 13:489–505.

———, **and M. D. Dailey.** 1972. Revised annotated list of parasites from sea mammals caught off the west coast of North America. United States Department of Commerce Technical Report NMFS SSRF-647. 23 pp.

Neiland, K. A. 1962. Alaskan species of acanthocephalan genus *Corynosoma* Luehe, 1904. Journal of Parasitology 48:69–75.

Rausch, R. L. 1964. Studies on the helminth fauna of Alaska. XLI. Observations on cestodes of the genus *Diplogonoporus* Lonnberg, 1892 (Diphyllobothriidae). Canadian Journal of Zoology 42: 1049–1069.

———, **and B. Locker.** 1951. Studies on the helminth fauna of Alaska. II. On some helminths parasitic in the sea otter, *Enhydra lutris* (L.). Proceedings of the Helminthological Society of Washington 18:77–81.

Schroeder, C. R., and H. M. Wegeforth. 1935. The occurrence of gastric ulcers in sea mammals of the California coast, their etiology and pathology. Journal of the American Veterinary Medical Association 87, N.S. 40:333–342.

Shults, L. M. 1982. Helminths of the spotted seal *Phoca largha* from the Bering Sea. Journal of Wildlife Diseases 18:59–62.

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Experimental Transmission of *Trypanosoma theileri* to Bison[1]

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ABSTRACT: Bloodstream trypomastigotes were recovered from a bison experimentally exposed to cryogenically preserved culture forms of *Trypanosoma theileri* of bovine origin. The bison trypomastigotes were compared statistically with bloodstream *Trypanosoma theileri* trypomastigotes from cattle and bloodstream *Trypanosoma cervi* trypomastigotes from North American deer. The trypanosomes from bison retained the morphological features of *T. theileri* suggesting that cattle and bison share this parasite.

Wrublewski (1908) first reported trypanosomes from bison; he found these parasites in blood cultures from the wisent, or European bison, *Bison bonasus* (L.), in Puszcza Białowieska in eastern Poland. Trypanosomes were reported in North American bison, *Bison bison* (L.), in 1981 (Kingston et al., 1981). Clotted blood samples were collected in 1979 from 39 bison that were imported into Wyoming. Samples were cultured in veal infusion medium (VIM) and eight (20.5%) were positive for trypanosomes. Culture forms were grown in cell culture through several passages using the procedures of McHolland-Raymond et al. (1978) and frozen at −70°C. Attempts to recover bloodstream trypomastigotes by the concentration methods of Kingston and Morton (1975a) and Kingston and Crum (1977) from four of the known positive animals failed. Of 79 other bison from various herds examined in Wyoming between 1979 and 1982, 2 animals were positive by VIM culture. In 1983 blood samples from 73 additional bison in Albany County, Wyoming were examined by the direct method and VIM culture. Results of these studies are reported herein. Also, in the spring of 1983, 3 yearling bison were purchased from the Durham Meat Company, Gillette County, Wyoming in order to infect them with cryopreserved material and recover bloodstream stages of bison trypanosomes. This report details the results of this experiment.

Materials and Methods

Two yearling bison and 2 bovine calves were inoculated IV per jugular with 8th passage, cryogenically preserved, culture-form bison trypanosomes obtained from the initial 1979 isolation. One yearling bison and 1 bovine calf were inoculated with cryogenically preserved, culture-form *Trypanosoma theileri* Laveran, 1902, 3rd isolate, passage 23, of bovine origin (McHolland-Raymond et al., 1978). Inoculum size was 5×10^6 trypanosomes/animal. Animals were bled on day 4 following exposure and periodically thereafter. Blood was cultured (VIM) and centrifuged blood was examined directly (DE) in microhematocrit tubes for the presence of swimming trypanosomes. Positive microhematocrit tubes were scored, broken, and thin films prepared of some of the contents (Kingston and Crum, 1977; Kingston et al., 1985). The slides were fixed, stained with Giemsa, and scanned for trypanosomes. When found these were photographed on 35-mm transparency film. Transparencies were projected and the trypanosomes traced; tracings were measured using a calibrated map-wheel reader. Measurements were analyzed and compared, using a computer programmed one-way ANOVA, with similar mensural values derived from trypanosomes from bovines (Matthews et al., 1979; McKenzie and Kingston, unpubl. data) and values derived from trypanosomes from North American Cervidae (Kingston et al., 1985).

Forty-four ml of heparinized blood that contained about 400 trypanosomes per ml from a positive experimental bison (#23) were collected (day 11 PI) and one-half inoculated IV per jugular into a bison and one-half into a bovine calf.

Hematocrit (HT) and hemoglobin (Hb) determinations, total RBC, WBC, and differential counts were carried out on the animals studied.

One positive bison and 1 positive bovine calf were

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Table 1. Results of trypanosome inoculation of bison and cattle, including subinoculation (S.I.).

Inoculum	Preinoculation culture	Recipient #	Results DE day examined							
			4	7	11	12	17	21	25	28
Cultured bison trypanosomes	–	Bis 21	–	–	–	S.I.	–	–	+	–
	–	Bov 473	–	–	–		–	ND	ND	–
	–	Bis 22	–	–	–		–	ND	ND	–
	–	Bov 198	–	–	–	S.I.	–	–	–	–
Cultured bovine trypanosomes	–	Bis 23	+	+	+		–	ND	ND	–N
(*Trypanosoma theileri*)	–	Bov 541	+	–	–		–	ND	ND	–N

N = necropsied.
ND = not done.

killed for tissue collection; necropsies were performed immediately following. Tissue imprints were made and tissues preserved and routinely handled for histopathological examination.

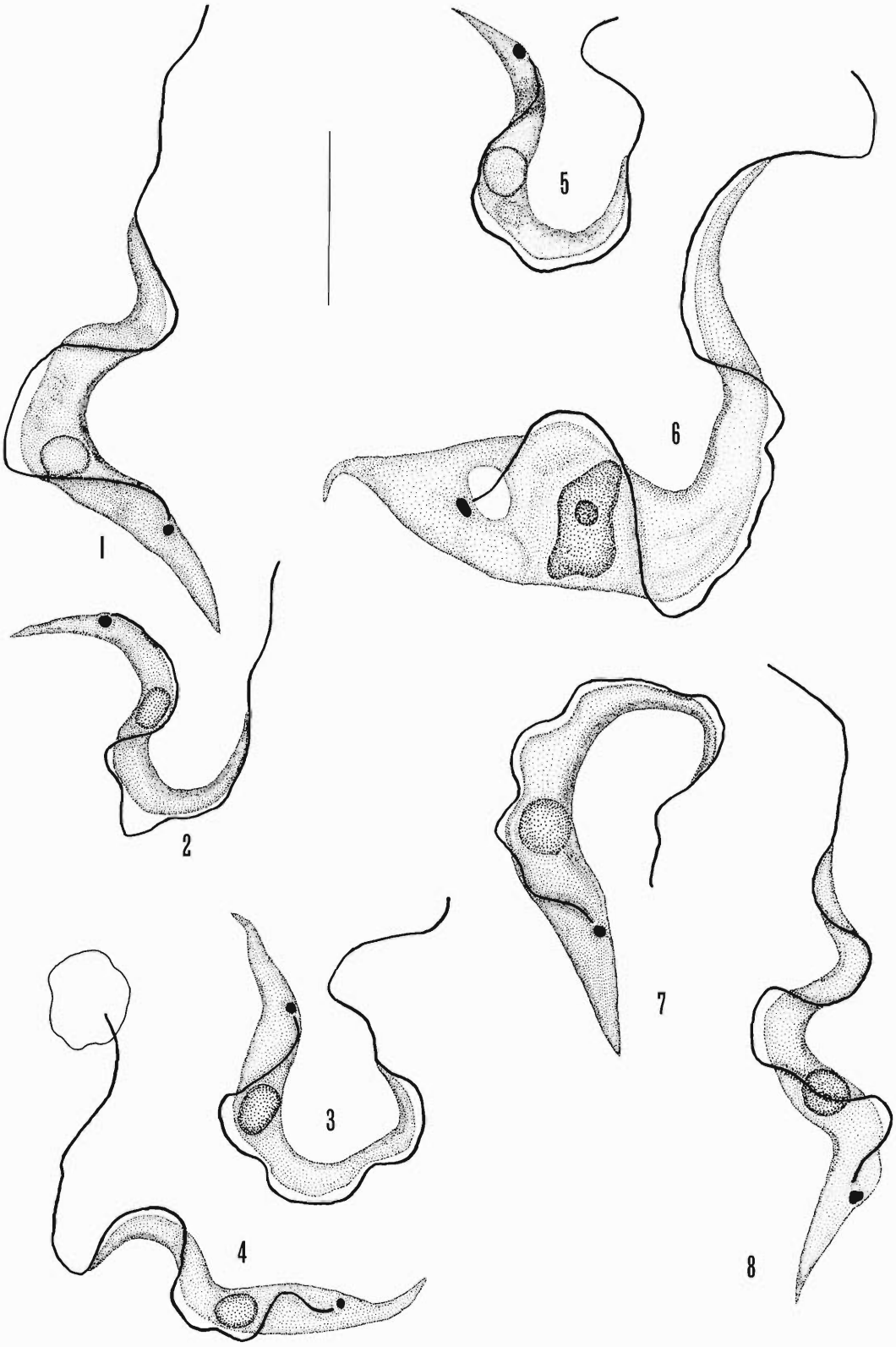
Results

TRANSFER EXPERIMENTS: The 2 yearling bison and the 2 bovine calves that received bison culture trypanosomes were uniformly negative when examined by DE and by VIM culture beginning on day 4 PI and continuing through day 17 PI (maximum of 4 examinations). The yearling bison (#23) that received bovine culture trypanosomes became patent on day 4 PI with ca. 90 trypanosomes seen per ml. On day 7 ca. 1,200 trypanosomes per ml were present and the infection declined on day 11 when there were about 400 trypanosomes per ml. No trypanosomes were observed thereafter. The bovine calf (#541) that received bovine culture trypanosomes was seen to be positive with ca. 10 trypanosomes per ml only on day 4 PI (Table 1). About 45 ml of heparinized blood from the positive bison was collected on day 11 PI and half of this amount was inoculated into 1 of the bison (#21) previously exposed to bison trypanosomes and half into a bovine calf (#198) previously exposed to bison trypanosomes. The bison (#21) became positive on day 13 PI (5 trypanosomes/ml) but infection was not detected thereafter; the bovine calf (#198) similarly exposed did not become patent (Table 1). Preinoculation cultures (VIM) were negative for all animals.

TRYPANOSOMES RECOVERED FROM BISON BLOOD: Trypanosomes were recovered from the blood of the bison (#23) (Figs. 1–8) inoculated with cultured *T. theileri* of bovine origin. Measurements of 27 trypanosomes were made and compared with bloodstream bovine *T. theileri* and bloodstream cervid *Trypanosoma cervi* (Table 2). Greater similarities were noted between trypanosomes recovered from cattle and bison than between trypanosomes recovered from the latter host and deer. Kinetoplast indices (KI) derived from trypanosomes from cattle and bison were identical (1.9) and varied widely from that value for deer trypanosomes (2.7). Comparisons of free flagellum to body length ratios (FF:BL) showed bison and cattle to be closely associated (1:2.4 and 1:2.8, respectively) and distinctly different from deer (1:6.1). Other values measured showed similar correspondence between bison and cattle trypanosomes and differences with deer trypanosomes. Measurement data for each mensural value showed an essentially normal distribution when comparing bison, cattle, and deer trypanosomes.

TISSUE IMPRINTS FROM BISON (#23): Examination of Giemsa-stained imprints of spleen, liver, brain, kidney, pituitary, and lymph nodes (hepatic, mediastinal, tracheobronchial, and mesenteric) revealed no trypanosomes.

HISTOPATHOLOGICAL EXAMINATION, BISON (#23): Sections of lung were characterized by peribronchial cuffing and submucosal infiltration of lymphocytes, plasma cells, and macrophages; bronchial and bronchiolar epithelial hyperplasia; and mild hypertrophy of bronchial smooth muscle. Scattered airways contained neutrophils and necrotic debris. Lungworms were found in bronchi on gross examination. Neutrophils were numerous in the splenic pulp and surrounding germinal centers and lymphoid follicles. Lymphoid tissue in spleen and all lymph nodes were hyperplastic and germinal centers were active. Significant lesions were not observed in liver, kidney, adrenal, ovary, uterus, and heart. Try-



panosomes were not observed in any tissue section. Acute suppurative splenitis, lymphoid hyperplasia of the spleen and lymph nodes, and mild verminous bronchitis were diagnosed.

TISSUE IMPRINTS FROM BOVINE CALF (#541): Trypanosomes were not seen in tissue imprints (made from the same organs examined as noted above) from the trypanosome-positive bovine calf.

HISTOPATHOLOGICAL EXAMINATION, BOVINE (#541): Lymphocytes lightly cuffed many airways in the lung and occasional lymphoid follicles were present. Lymph nodes had thick active cortices and many germinal centers. Other tissues were essentially normal. No trypanosomes were seen in histological sections of tissues. Lymphoid hyperplasia was diagnosed.

HEMATOLOGIC EXAMINATION—RBC, PCV AND HB: There was a decline of ~33% (day 21 PI) in the number of RBC's in the trypanosome infected bison (#23). The RBC count rose thereafter to a more normal level. Packed cell volumes (PCV) paralleled RBC counts in the infected bison (#23) and dropped 26% by day 11 PI. This was followed by a return to the day 0 value by day 28 PI. Erythrocytic parameters remained constant in the other bison and bovines.

WBC: White blood cell (WBC) counts decreased 47.5% ($4.0 \times 10^3/mm^3$ to $1.9 \times 10^3/mm^3$) in the first infected bison (#23) during the 11-day period when trypanosomes could be recovered (DE) from the blood of this animal. The leucopenia then reversed and WBC's increased sharply to $11.0 \times 10^3/mm^3$ by day 21. By day 28 PI the numbers of WBC's had declined to approximately normal values. WBC numbers in the challenge bison (#21) showed only a moderate decline (19%) on day 25 PI following inoculation and this decline was reversed at the termination of the experiment. The WBC response in the infected bovine calf (#541) was markedly different from that seen in the bison (#23) with the numbers of WBC's fluctuating sharply upwards through day 17 and returning to approximately normal by day 28.

DIFFERENTIAL WHITE BLOOD CELL COUNTS: Differential WBC counts in the trypanosome-infected animals remained relatively unchanged throughout infection except that the bovines exhibited a 21–27% increase in the number of lymphocytes between 4–11 days PI.

SURVEY OF BISON FOR TRYPANOSOMES, ALBANY COUNTY, WYOMING (1983): Examination (VIM) of blood samples from 73 bison in Albany County, Wyoming in late 1983 revealed 26 (35.6%) infected with trypanosomes; no trypanosomes were recovered by DE. Twelve bulls (46%) and 14 cows (54%) were infected. They were 1–2 years of age with the majority (85%) being yearlings.

Discussion

Previous reports (Kingston et al., 1981) refer to trypanosomes in North American bison as *Trypanosoma* sp. because culture forms only were examined and it is not possible to use such material for species identification (Trager, 1975). The trypanosomes recovered from European bison were designated *T. wrublewskii* (Vladimirov and Yakimov, 1908). Yakimov (1915) concluded later that the parasite was actually *T. theileri.*

The present report provides additional information on the identity of trypanosomes from bison. These trypanosomes were of bovine origin. Measurements of specimens (27) from the acutely infected bison when compared with the measurements of (304) trypanosomes from cattle (*Bos taurus*) and (174) trypanosomes from North American cervids indicate that the bison trypanosomes retain *T. theileri* morphology after passage through bison, especially with regard to flagellar length (FF), flagellar length to body length ratios (FF:BL), and kinetoplast index (KI) (Table 2).

Trypanosomes of the stercorarian subgenera *Megatrypanum* and *Herpetomonas* are considered to be relatively to strictly host specific (e.g., *T. melophagium* in sheep and *T. lewisi* in rat). This feature has been used in the identification of such trypanosomes (Hoare, 1972) even when bloodstream stages have not been available. The

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Figures 1–8. Representative bloodstream trypomastigotes from bison (#23) experimentally infected with *Trypanosoma theileri* of bovine origin. 1. BL 30, FF 13, FF:BL 1:2.05, W 4, KI 1:2.2. 2. BL 25, FF 9, FF:BL 1:2.8, W 2.5, KI 1:1.8. 3. BL 28, FF 15, FF:BL 1:1.9, W 3, KI 1:1.9. 4. BL 23, FF 17, FF:BL 1:1.4, W 3.5, KI 1:1.8. 5. BL 23, FF 11.5, FF:BL 1:2.0, W 2.5, KI 1:1.6. 6. BL 47, FF 11, FF:BL 1:4.3, W 9, KI 1:2.4. Largest form seen, considered predivision stage. 7. BL 30, FF 7, FF:BL 1:4.2, W 3.5, KI 1:2.2. 8. BL 30, FF 12, FF:BL 1:2.5, W 3, KI 1:2.3. Approximately average trypomastigote. See legend for Table 2 for definition of abbreviations used here.

Table 2. Comparison of mensural values of bloodstream trypomastigotes recovered from bison and *Trypanosoma theileri* from bovines and *T. cervi* from Cervidae in North America.

	PK*	KN	PN	NA	BL	FF	L	W	FF:BL	NI	KI
Bison	5.7	6.4	12.4	15.9	28.0	12.4	40.3	3.1	1:2.43	0.8	1.9
$N = 27$											
SD	2.3	0.97	2.5	3.9	6.0	3.1	6.5	1.5	0.92	0.2	0.4
Range	2–11	4–9	8–18	9–29	17–47	7–17	24–58	2–9	1:1.35–4.71	0.542–1.143	1.286–3
Bovine	7.4	8.9	16.2	20.2	36.4	14.2	50.5	3.3	1:2.8	0.8	1.9
$N = 304$											
SD	3.3	2.6	5.1	6.3	10.5	4.5	12.7	2.0	2.3	0.2	0.4
Range	0–17	2–20	5–33	7–36	13–59	1–37	16–90	1–13	1:0.89–39.0	0.4–1.7	1–4
All deer†	11.5	7.1	18.5	23.3	42.0	8.2	50.1	5.5	1:6.1	0.8	2.7
$N = 174$											
SD	5.6	2.1	6.3	7.3	12.4	3.4	13.6	2.5	3.8	0.2	0.9
Range	3–27	2–14	8–36	10–43	21–74	1–21	26–83	1–15	1:0–27	0.4–1.6	1.2–7

* PK = posterior end to kinetoplast distance, KN = kinetoplast-to-nucleus distance, PN = posterior end-to-nucleus distance, NA = nucleus-to-anterior end distance, BL = body length, FF = length of free flagellum, L = overall length, FF:BL = free flagellum to body length ratio, W = width, NI = PN/NA (nuclear index).

† All deer, see Kingston et al. (1985).

course of infection in the bison was similar to that seen in experimentally infected bovines (Matthews et al., 1979) differing mainly in the brevity of infection (trypanosomes not detected in blood of bison #23 after 11 days PI) compared with experimental infections in the bovine (where peak infections were achieved on day 12 PI and were terminated on days 20–26 PI) (Matthews et al., 1979). Differences in duration of infection of *T. theileri* in these 2 hosts might result because the bison is an unusual host for this parasite, of this strain of parasite, or to other undiscernible factors. On reflection, infection of bison with *T. theileri* of bovine origin might be expected. The 2 hosts are closely related members of the Bovidae. This is indicated by reproduction between cattle and bison, and some mammalogists place bison in the genus *Bos* along with cattle. Although it is not possible to state precisely that trypanosomes from naturally infected bison are, actually, *T. theileri,* it is highly probable that bison and cattle share this parasite.

The lack of infectivity of trypanosomes of bison origin to bison yearlings or bovine calves cannot be presently explained. The trypanosomes inoculated were alive, but had been cryogenically preserved from 1979 to the time of their use in 1983 when they were re-cultured (McHolland-Raymond et al., 1978) to provide the numbers used in this experiment. These trypanosomes appeared to have lost infectivity, which may be inferred from the fact that bison #21 became patent when challenged with trypanosomes from bison #23. A transitory infection was detected in bison #21 on day 13 PI, but the low intensity of infection and its delayed patency may be ascribed to the small numbers of trypanosomes inoculated (about 9,000 total to each of the 2 recipients) or the anamnestic response of bison #21 to *T. theileri* organisms after exposure to bison trypanosomes. This would suggest that the 2 organisms share similar antigenic determinants.

If, indeed, bison trypanosomes are *Trypanosoma theileri* Laveran, 1902 then, Wrublewski (1908) was remarkably astute in assigning the culture forms he observed from the wisent to that species.

The effect of trypanosomes on the infected bison (#23) appears to be reflected in an anemia and a transitory leucopenia, the significance of which is unknown.

The histological examination revealed immune activation in both bison #23 and bovine calf #541. No trypanosomes were found in any organs, but the neutrophil infiltration in the spleen of bison #23 suggests splenic involvement during infection perhaps similar to that seen in elk (Kingston and Morton, 1975b).

Literature Cited

Hoare, C. A. 1972. The Trypanosomes of Mammals. Blackwell, Oxford and Edinburgh. Pp. 3–749.

Kingston, N., and J. Crum. 1977. *Trypanosoma cervi*

Kingston and Morton, 1975 in white-tailed deer, *Odocoileus virginianus,* in the Southeastern United States. Proceedings of the Helminthological Society of Washington 44:179–181.

———, **and J. K. Morton.** 1975a. *Trypanosoma cervi* n. sp. from elk in Wyoming. Journal of Parasitology 61:17–23.

———, **and** ———. 1975b. Recovery of multiplication stages of *Trypanosoma cervi* Kingston and Morton, 1975, in elk spleen. Proceedings of the Helminthological Society of Washington 42:179–181.

———, **A. Franzmann, and L. Maki.** 1985. Redescription of *Trypanosoma cervi* (Protozoa) in moose, *Alces alces,* from Alaska and Wyoming. Proceedings of the Helminthological Society of Washington 52:54–59.

———, **E. T. Thorne, G. Thomas, L. McHolland, and M. S. Trueblood.** 1981. Further studies on trypanosomes in game animals in Wyoming. II. Journal of Wildlife Diseases 17:539–564.

Matthews, D. M., N. Kingston, L. Maki, and G. Nelms. 1979. *Trypanosoma theileri* Laveran, 1902, in Wyoming cattle. American Journal of Veterinary Research 40:623–629.

McHolland-Raymond, L. E., N. Kingston, and M. S. Trueblood. 1978. Continuous cultivation of *Trypanosoma theileri* at 37C in bovine cell culture. Journal of Protozoology 25:388–394.

Trager, W. 1975. Remarks made in Presidential address, American Society of Parasitologists, Kansas City, Mo. Aug. 7, 1974. On the cultivation of *Trypanosoma vivax*: a tale of two visits in Nigeria. Journal of Parasitology 61:3–11.

Vladimirov, A. A., and V. L. Yakimov. 1908. Bemerkung zur vorstehenden Mitteilung Wrublewski's. Zentralblatt fur Bakteriologie Parasitenkunde und Infektionskrankheiten 48:164.

Wrublewski, K. I. 1908. Ein Trypanosoma des Wisent von Bielowesch. Zentralblatt fur Bakteriologie, Parasitenkunde und Infektionskrankheiten 48: 162–163.

Yakimov, V. L. 1915. A propos du *Trypanosoma wrublewskyi.* Bulletin de la Societe de Pathologie Exotique 8:431–433.

Obituary

GERTRUDE E. MCINTOSH
November 1, 1905–April 30, 1986
Elected Member January 20, 1934

The Allen and Gertrude McIntosh Biology Student Enrichment Fund
Belmont Abbey College
Belmont, North Carolina 28012

Mrs. Gertrude E. McIntosh, wife of Allen McIntosh (Member 1930–1977), established the memorial fund at the Belmont Abbey College in 1983. The fund has now been renamed.

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Ultrastructural Surface Interaction of Serum or Hybridoma Antibodies with the Pellicle of *Eimeria tenella* Sporozoites

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ABSTRACT: A monoclonal antibody (Ab), which caused clumping of sporozoites of *Eimeria tenella* and inhibited penetration of the parasite into primary chicken kidney cells, and serum from immunized chickens were utilized to study Ab–antigen (Ag) interaction on the surface of the sporozoites. The parasites were exposed to either immune chicken serum (ICS) for 5–30 min or the monoclonal Ab (designated B10) for 45 min at room temperature (RT) and then fixed in 2% glutaraldehyde. Other B10 Ab-pretreated sporozoites were incubated with normal chicken serum (NCS) for 5–30 min at RT before fixation or subjected to labeling with ferritin antiserum after fixation. Sporozoites exposed to ICS had large clumps of material present on the surface of the pellicle at 5–10 min postincubation (PI). By 20 min PI, most of the ICS-treated parasites had lysed. Sporozoites exposed to the monoclonal Ab had a fuzzy coat on the surface of the pellicle when compared to untreated controls and no lysis occurred. The presence of complement applied by the exposure to NCS caused lysis of the Ab-pretreated sporozoites within 30 min. Ab-pretreated sporozoites exposed to NCS that were fixed at 5 and 10 min had a thick coat of material on the surface. The B10 Ab-treated parasites that underwent the ferritin antiserum labeling procedure demonstrated a uniform layer of ferritin on the surface. The results indicate that this monoclonal Ab reacts with antigenic determinants present on the surface of *E. tenella* sporozoites.

The production of hybridoma antibodies (Ab) directed against *E. tenella* sporozoites (Danforth, 1982) has made it possible to study the inhibitory effects of these Ab on the in vitro penetration and intracellular development of the parasites. Danforth (1983) pretreated sporozoites with one of these hybridomas (designated B10) and showed that the clumping of the Ab-pretreated sporozoites occurred and that host cell penetration was reduced by approximately 80%. Because these results indicated that the B10 Ab may be interacting with the surface of the sporozoite, an ultrastructural study of the pellicle of sporozoites exposed to fetal calf serum, normal and immune chicken serum, and P3X or B10 Ab ascites was undertaken. Complement–B10 interaction on the pellicle of the sporozoites and the surface ferritin labeling of Ab-pretreated parasites were also studied.

Materials and Methods

SPOROZOITES: *Eimeria tenella* sporozoites (API L.S. #24) were excysted and fiber-cleaned as previously described (Doran and Vetterling, 1967; Bontemps and Yvore, 1974). Parasites of this strain were also used in an earlier study (Danforth, 1982) for the production of the B10 hybridoma cell line.

HYBRIDOMA AB PRODUCTION: The development of the B10 Ab-secreting hybridoma cell line has been described (Danforth, 1982). This cell line was injected into pristane (2,6,10,14-tetramethyl pentadecane; Aldrich Chemical Company)-primed mice to produce ascites tumors. The B10 ascites fluid collected from these mice was stored at −80°C until used. Ascites fluid was also collected from mice injected with the original parent line of P3-X63-Ag8 mouse myeloma cells for use as controls to insure that no coat formation or labeling of sporozoite surface occurred with mouse ascites that did not contain Ab. All ascites fluid was heat inactivated at 56°C for 30 min before sporozoite pretreatment.

NORMAL AND IMMUNE CHICKEN SERUM PRODUCTION: White Leghorn chickens raised coccidia free were bled at 4 weeks of age and the serum (NCS) was recovered and stored in 2-ml aliquots at −80°C until used (Witlock and Danforth, 1982). The immune chicken serum (ICS) was obtained from the same strain of chickens immunized against *E. tenella* (API L.S. #24) with 6 oral inoculations of 20,000 oocysts within a 3-week interval. These birds were bled 7 days after the final inoculation and the serum was stored at −80°C.

SPOROZOITE PRETREATMENT: Approximately 3×10^6 fiber-cleaned sporozoites were used in each treatment group and were subjected to the following procedures. All dilutions were made with Dulbecco's Modified Eagles Medium (DMEM, Gibco). Sporozoites were exposed to a 1:100 dilution of B10 Ab ascites for 45 min at room temperature (RT) and either fixed in 2% phosphate (PO_4)-buffered glutaraldehyde, or treated with a 1:10 dilution of NCS for time periods ranging from 5 to 30 min before glutaraldehyde fixation. Other parasites were treated with a 1:10 dilution of ICS for the 5–30-min time periods before fixation. In other groups, sporozoites were fixed for 25 min in 2.5% cacodylate-buffered glutaraldehyde at 4°C, and exposed to dilute B10 ascites for 30 min at 37°C. These parasites were then treated (30 min at 37°C) with a predetermined dilution of rabbit anti-mouse IgG followed by ferritin-conjugated goat anti-rabbit IgG (Miles-Yeda) before a

final fixation in the cacodylate-buffered glutaraldehyde. Appropriate fetal calf serum (FTCS), NCS, or P3-X63-Ag8 ascites (1:100 dilution) pretreated sporozoites were included with all treatment groups. With the ferritin labeling procedure, sporozoites were exposed to either the rabbit anti-mouse IgG followed by the ferritin-conjugated goat anti-rabbit IgG, or to ferritin conjugate alone to insure that no nonspecific ferritin labeling occurred. The FTCS treated control parasites were included because this serum was used in previous in vitro penetration and development studies (Danforth, 1983).

ELECTRON MICROSCOPY: After fixation in the PO_4- or cacodylate-buffered glutaraldehyde, the sporozoites were washed in the appropriate buffer, post-fixed in 1% (w/v) PO_4-buffered OsO_4, dehydrated in a graded series of ethanols, and embedded in Epon. The specimens were sectioned on a Porter-Blum MT-2 ultramicrotome and examined in a Philips 200 electron microscope.

Results

The FTCS treated control and NCS pretreated sporozoites did not show any type of surface coat or precipitate on the pellicle (Fig. 1). In contrast, parasites exposed to the ICS for 5–10 min before fixation had large clumps of material closely associated with the parasite (Fig. 2). On other areas of the pellicle, only a thin coat of material was present. By 15 min incubation, the ICS-treated sporozoites demonstrated a breakdown of the pellicle, and after 30 min incubation most of these parasites had undergone lysis.

Sporozoites exposed to the P3X ascites without Ab did not demonstrate any type of coat on the pellicle, and were similar in appearance to the FTCS control parasites (Fig. 1). Sporozoites incubated with the B10 Ab ascites had a uniform fuzzy coat present on the surface pellicle (Fig. 3). There was no apparent migration or clumping of this material toward either the anterior or posterior end of the parasite, and the treatment had no ultrastructural effect on the pellicle of the sporozoite (Fig. 3).

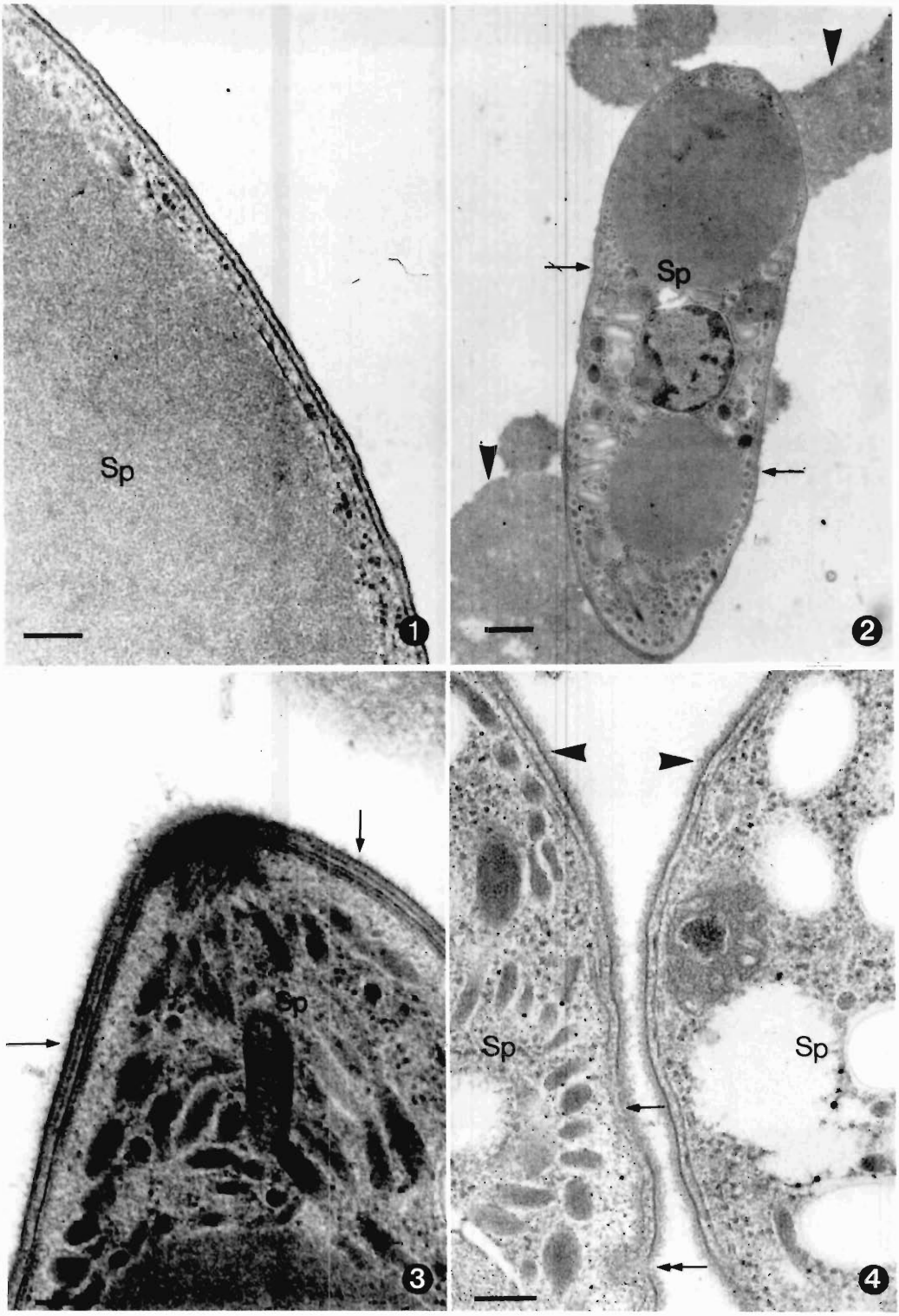
Parasites exposed to NCS after the B10 ascites pretreatment had a thick uniform layer of material on the pellicle at 5 and 10 min postincubation (Fig. 4). With some of these specimens, there were areas where both the inner and outer membrane were incomplete, suggesting that complement–B10 Ab interaction caused a degeneration of the pellicle. In some areas of pellicular degeneration, there appeared to be an influx of the coat material into the cytoplasm of the sporozoite (Fig. 4). After 30 min incubation with the NCS, most of the Ab-pretreated sporozoites had undergone lysis.

Sporozoites pretreated with the B10 Ab had uniform ferritin labeling as demonstrated by longitudinal and oblique cuts along the entire area of the pellicular surface (Figs. 5, 6). No internal cytoplasmic labeling by the ferritin was seen with any of the sporozoites examined, and no migration or aggregation of the ferritin was observed. A layer of material was seen between the pellicle of the sporozoite and the ferritin granules (Fig. 5) and this may have been formed with the exposure of the Ab-pretreated parasite to 2 antisera during the labeling procedure. No ferritin labeling occurred with parasites exposed to the rabbit anti-mouse IgG followed by the ferritin-conjugated IgG or to the ferritin conjugate alone. Sporozoites pretreated with P3X ascites before ferritin labeling did show a few small clumps (5 granules or less) randomly scattered along the surface of the pellicle.

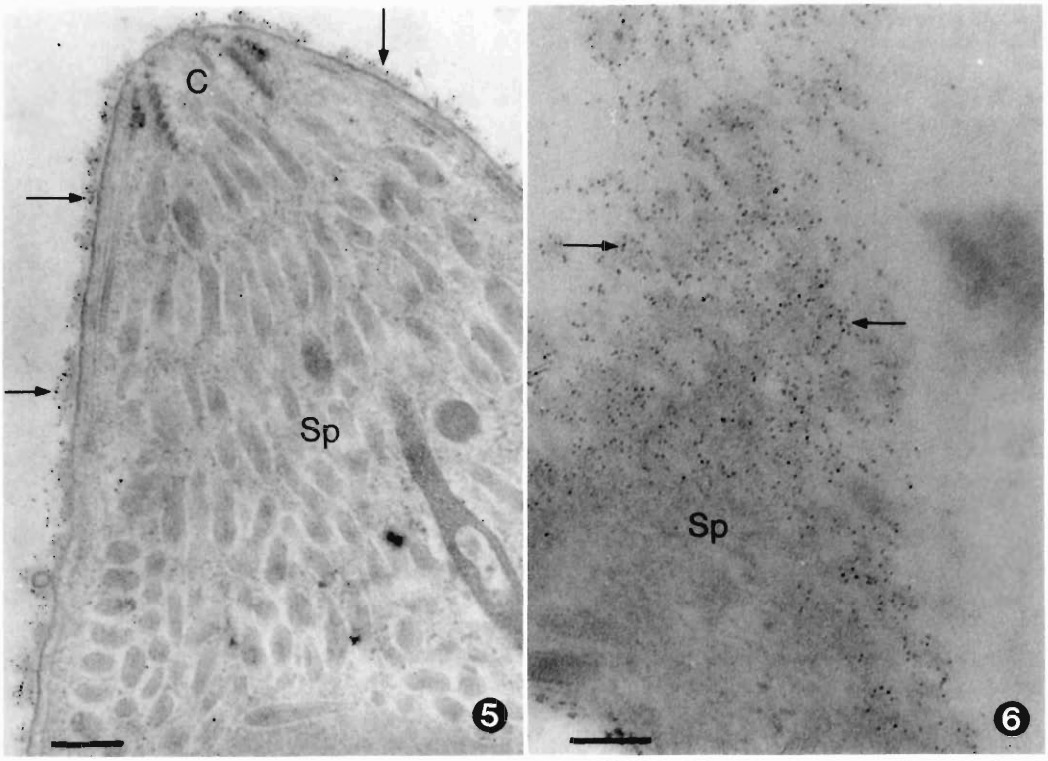
Discussion

The present study has demonstrated that a monoclonal antibody, which has been shown to clump viable *E. tenella* sporozoites and inhibit in vitro host cell penetration (Danforth, 1983), and ICS will both interact with antigenic determinants on the surface of these parasites. No such visible uniform interaction occurred with parasites exposed to FTCS, NCS or P3X ascites.

A previous scanning electron microscopy investigation reported that sporozoites of *E. tenella* exposed to ICS for 10–15 min demonstrated surface swelling or bulges, were covered with a thick fibrinous coat, and underwent complement-mediated lysis (Witlock and Danforth, 1982). In the present study, large clumps of material, that may have been part of the fibrinous coat, were observed next to the pellicle of the ICS-treated sporozoite at 5–10 min PI. This would be expected because the ICS would contain Ab directed against all life cycle stages of the parasite, including sporozoites, as well as other serum constituents that could nonspecifically interact with the surface of the parasite. This same type of clumped material has been seen with 4 different samples of immune serum in 3 different experiments. Therefore, it is probably not the result of serum contamination. The clumping may represent a type of patch formation caused by aggregation of surface antigens that have reacted with multivalent polyclonal ICS. In contrast, a fuzzy coat rather than clumps of material was seen on the surface of sporozoites pretreated with



Figures 1–4. Sporozoites (Sp) of *E. tenella*. **1.** Pellicle of Sp exposed to a 1:10 dilution of FTCS. Note absence of coat material on the surface, which was also similar to that seen for NCS and P3X ascites pretreated Sp.



Figures 5, 6. Sporozoites (Sp) of *E. tenella* pretreated with B10 Ab and subjected to ferritin (Fe) labeling. 5. Anterior longitudinal section of Sp with conoid (C) showing ferritin (Fe) labeling (arrows) of the surface pellicle. ×46,000. Bar = 0.2 μm. 6. Oblique cut of ferritin labeled Sp showing uniform distribution of ferritin particles (arrows) over surface pellicle. ×52,000. Bar = 0.2 μm.

the B10 Ab. The absence of clumps on these specimens is probably a reflection of either specificity of the Ab for the antigenic determinants on the surface of the parasite, or a lack of multivalency of the cloned B10 Ab.

There have been reports of fibrillar coats on developmental stages of *Plasmodium* parasites exposed to monoclonal antibodies (Aikawa et al., 1981b), and one investigation (Speer et al., 1985) has described the presence of a thin hybridoma antibody layer on the plasma membrane of Ab-treated *E. tenella* sporozoites. In an earlier study Speer et al. (1983) did not observe an antibody layer on sporozoites treated with 4 different hybridoma Ab, including the one that showed such a layer in the later report. It is possible that all of these hybridoma Ab produced against *E. tenella* sporozoites were directed against different antigenic determinants than that recognized by the B10 Ab. Therefore, these Ab would not demonstrate the same type of antigen–antibody interaction on the pellicle as was seen in this study with the B10 Ab-pretreated sporozoites.

The exposure of sporozoites to a mouse-derived B10 monoclonal Ab in the presence of complement supplied by NCS resulted in the formation of a thick coat on the pellicle of the parasite, and caused degeneration of the pellicular

←

×46,000. Bar = 0.2 μm. 2. Sp exposed to 1:10 dilution of ICS at 5 min PI. Note clumps of material (large arrows) next to some areas of the pellicle and other areas with a thin coat of material (small arrows). ×15,000. Bar = 0.5 μm. 3. Sp pretreated with heat-inactivated B10 Ab diluted 1:100. Note fuzzy coat (arrows) on the surface of the pellicle including conoidial area (C). ×46,000. Bar = 0.2 μm. 4. Two Sp pretreated with heat-inactivated B10 Ab and exposed to NCS. Note uniform coat on the surface of the parasites (large arrows), areas of the pellicle that have degeneration of the inner and outer membrane (small arrows), and an apparent influx of coat material into the Sp cytoplasm (small double arrows). ×46,000. Bar = 0.2 μm.

membranes with subsequent lysis. This is similar to what occurs when sporozoites are treated with ICS and complement, but it is not known if a single or similar Ab in the ICS would cause this type of reaction. Speer et al. (1983) has also demonstrated that monoclonal antibodies directed against *E. tenella* sporozoites, in the presence of mouse complement, will cause lysis of the parasite. In the present study complement was supplied by treatment with NCS, and the presence of other constituents in this serum, which may bind nonspecifically to the pretreated parasite, may account for the formation of a thicker coat on these sporozoites than that seen with parasites exposed to only the B10 ascites. It is also possible, as was suggested by Speer et al. (1983), that antibody plus complement may expose additional receptor sites on the pellicle so that they are more accessible to antibody binding.

The immunocytochemical labeling of the surface of various developmental stages of protozoan parasites by monoclonal Ab has been well documented (Aikawa et al., 1981a, b; Epstein et al., 1981; Johnson et al., 1983; Speer et al., 1983, 1985). In these reports, the hybridoma Ab were directed against surface receptor sites (antigen), and it was expected that the Ab would react with antigen localized on the surface. The B10 hybridoma Ab was originally described as reacting with the cytoplasm of the anterior half of air-dried sporozoites (Danforth, 1982), and showed only a faint reaction with the surface pellicle. However, by exposing B10 Ab-pretreated sporozoites to a variety of procedures including ferritin labeling, and examining them with electron microscopy, interaction of the Ab with antigen determinants on the surface of the parasite was clearly visualized. The antigen(s) may be a secretion of the sporozoite that originates from the cytoplasm of the parasite and is then expressed on the surface, hence the brighter internal ½ IFA pattern seen with air-dried sporozoites. Internal localization of the B10 Ab as determined by ferritin labeling was not seen in this study, but this is probably because the Ab was unable to penetrate the intact glutaraldehyde-fixed pellicle.

In other investigations (Aikawa et al., 1981a; Epstein et al., 1981), it was demonstrated that surface monoclonal Ab–Ag interactions, which caused agglutination of *Plasmodium* merozoites and gametes, will inhibit erythrocyte penetration and gamete fertilization. Sporozoites of *Plasmodium,* when incubated with monoclonal Ab directed against surface antigen, have shown a precipitation (CSP response) on the surface of the parasite and were inhibited from penetrating cultured cell lines (Hollingdale et al., 1983). Because the B10 Ab pretreatment also causes clumping of viable parasites and inhibits in vitro host cell penetration, the surface Ag–Ab interaction seen in this study may play a major factor in blocking host cell invasion. Efforts are now currently underway to isolate and characterize what may be an important surface antigen or antigens specific for the B10 hybridoma antibody.

Literature Cited

Aikawa, M., J. Rener, R. Carter, and L. H. Miller. 1981a. An electron microscopical study of the interaction of monoclonal antibodies with gametes of the malarial parasite *Plasmodium gallinaceum.* Journal of Protozoology 28:383–388.

———, N. Yoshida, R. S. Nussenzweig, and V. Nussenzweig. 1981b. The protective antigen of malarial sporozoites (*Plasmodium berghei*) is a differentiation antigen. Journal of Immunology 126: 2494–2495.

Bontemps, M., and P. Yvore. 1974. Technique de purification de suspensions de sporozoites d'*Eimeria* sur colonne de fibres synthetiques. Annales de Recherches Veterinaires 5:109–113.

Danforth, H. D. 1982. Development of hybridoma-produced antibodies directed against *Eimeria tenella* and *E. mitis.* Journal of Parasitology 68: 392–397.

———. 1983. Use of monoclonal antibodies directed against *Eimeria tenella* sporozoites to determine stage specificity and in vitro effect on parasite penetration and development. American Journal of Veterinary Research 44:1722–1727.

Doran, D. J., and J. M. Vetterling. 1967. Cultivation of the turkey coccidium *Eimeria meleagrimitis* Tyzzer, 1929, in mammalian kidney cell cultures. Proceedings of the Helminthological Society of Washington 34:59–65.

Epstein, N., L. H. Miller, D. C. Kaushel, I. J. Udeinya, J. Rener, R. J. Howard, R. Asofsky, M. Aikawa, and R. L. Hess. 1981. Monoclonal antibodies against a specific surface determinant on malarial (*Plasmodium knowlesi*) merozoites block erythrocyte invasion. Journal of Immunology 127: 212–217.

Hollingdale, M. R., P. Leland, J. L. Leef, and A. L. Schwartz. 1983. Entry of *Plasmodium berghei* sporozoites into cultured cells and their transformation into trophozoites. American Journal of Tropical Medicine and Hygiene 32:685–690.

Johnson, A. M., W. D. Haynes, P. J. Leppard, P. J. McDonald, and S. H. Neoh. 1983. Ultrastructural and biochemical studies on the immunochemistry of *Toxoplasma gondii* antigens using monoclonal antibodies. Histochemistry 77:209–215.

Speer, C. A., R. B. Wong, J. A. Blixt, and R. H. Schenkel. 1985. Capping of immune complexes by sporozoites of *Eimeria tenella.* Journal of Parasitology 71:33–42.

———, ———, **and R. H. Schenkel.** 1983. Ultrastructural localization of monoclonal IgG antibodies for antigenic sites of *Eimeria tenella* oocysts, sporocysts, and sporozoites. Journal of Protozoology 30:548–554.

Witlock, D. R., and H. D. Danforth. 1982. Surface changes induced by immune serum on *Eimeria tenella* sporozoites and merozoites. Journal of Protozoology 29:441–445.

Obituary Notice

DONALD K. MCLOUGHLIN
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Proc. Helminthol. Soc. Wash.
53(2), 1986, pp. 210–217

Redescription of *Ochoterenella digiticauda* Caballero, 1944 (Nematoda: Filarioidea) from the Toad, *Bufo marinus,* with a Redefinition of the Genus *Ochoterenella* Caballero, 1944

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ABSTRACT: *Ochoterenella digiticauda* Caballero, 1944, from *Bufo marinus* in Mexico has been redescribed from specimens in the type collection. On the basis of certain morphological features (e.g., lateral cuticularized parastomal structures, annular bands of longitudinally oriented bosses, lack of lateral and caudal alae) the genus was redefined. Eight species previously included in *Waltonella* (*W. convoluta, W. scalaris, W. vellardi, W. guyanensis, W. royi, W. dufourae, W. oumari, W. albareti*) have been transferred to *Ochoterenella.* A filaria from a racophorid frog in Madagascar, *O. guibei,* was removed from *Ochoterenella,* and *Paramadochotera* gen. n. has been created for it. *O. papuensis* from New Guinea has been removed from the genus and is considered incertae sedis. Features of *Ochoterenella digiticauda* (the type species) distinguishing it from the other members of the genus include the size and arrangement of the cuticular bosses on the body of both sexes, the appearance of the median ventral preanal cuticularized plaque and the structural details of the spicules in the male, and the shape and size of the microfilaria. *Ochoterenella* as presently constituted appears to be a morphologically compact group of species restricted to the neotropical region. Most of the currently accepted members have been recovered from *Bufo marinus,* but previous reports of worms misidentified as *O. digiticauda* indicate that the host range of the genus includes other toads as well as frogs, particularly leptodactylids.

Caballero (1944) recovered and described filarial nematodes from the body cavity of *Bufo marinus* examined in Mexico. A new genus, *Ochoterenella,* was created for them, and the species was named *O. digiticauda.* Lent et al. (1946) redescribed this species from worms obtained from *Bufo paracnemis* in Paraguay, and re-examined Caballero's specimens. Subsequently, worms presumed to be *O. digiticauda* have been reported from a variety of toads and frogs in the neotropical region by several investigators (Brenes and Hollis, 1959; Travassos and de Freitas, 1960; Marinkelle, 1970; Masi Pallares and Maciel, 1974; Vicente and dos Santos, 1976; Dyer and Altig, 1977; Vicente and Jardim, 1980). This genus was treated as being monotypic until Johnston (1967) described *O. papuensis* from a frog in New Guinea and Bain and Prod'hon (1974) described *O. guibei* from a frog in Madagascar. In spite of the inadequacies of the original descriptions of *O. digiticauda* in the 1940's, since that time no detailed re-examination of this species has been undertaken. The subsequent description of other filariae (*Waltonella, Madochotera*) from anurans, and the erection of the subfamily Waltonellinae to accommodate the "*Foleyella*"-like filariae of amphibians has made a reconsideration of *Ochoterenella* imperative. The present investigation is concerned with the further characterization of *O. digiticauda* based on Caballero's type collection and a reassessment of the status of the genus *Ochoterenella.*

Materials and Methods

Several lots of specimens from *Bufo marinus* were examined. Most, all identified as *Ochoterenella digiticauda,* were obtained from the Helminth Collection of the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, D.F. (noted hereafter as IBUNM). These, as well as others, are listed below. The first three (IBUNM 106-3, 107-1, II-64) constitute Caballero's type collection and form the basis for the present redescription.

IBUNM 106-3: Female "type" and 24 female paratypes. Collected 1943, Huixtla, Chiapas, Mexico; IBUNM 107-1: Male "type" and 17 male paratypes (same collection data as 106-3); IBUNM II-64: 2 slides blood films with microfilariae (same collection data as 106-3); IBUNM 106-4: 4 females; 106-5, 10 females; 107-2, 12 females (same collection data as 106-3); IBUNM 128-4: 29 females. Collected 1945, "Olas de Moca," Atitlan, Guatemala; IBUNM II-71: 2 slides blood films with microfilariae (same collection data as 128-4); IBUNM 128-5: 2 males, 28 females. Collected 1947, Tuxtepec, Oaxaca, Mexico; IBUNM II-72: 2 slides blood films with microfilariae (same collection data as 128-5); IBUNM 144-5: 8 females, one posterior fragment of male. Collected 1953, San José, Costa Rica; IBUNM 148-5: 22 females. Collected 1957, San José, Costa Rica (see Brenes and Hollis, 1959); IBUNM II-73: 2 slides blood films with microfilariae (same collection data as 148-5); USNM Helm. Coll. No. 36893: *Ochoterenella digiticauda.* Paratypes (2 females, 1 male) deposited by E. Caballero (from IBUNM 106-3 and 107-1 above).

Waltonella oumari Bain, Kim, and Petit, 1979. Holotype female, allotype male. Collected from *Bufo marinus* (body cavity), Maripasoula, French Guiana. Loaned by A. Chabaud and O. Bain from the helminth collection, Laboratoire de Zoologie (Vers), Muséum National d'Histoire Naturelle, Paris.

Additional collections of unidentified filariae from *Bufo marinus* provided for this study are:

ACR: 8 females, blood in 2% aqueous formalin, 6 slides blood films with microfilariae. Collected 1984 by A. Cruz-Reyes from Ejido Cardenas, Las Tuxtlas, Veracruz, Mexico; JAA: 8 females, 2 males, 2 slides blood films with microfilariae. Collected 1985 by J. Almeyda-A. from Catemaco, Veracruz, Mexico.

All adult worms were examined in pure glycerin following slow evaporation from 70% ethanol containing 5% glycerin. Microfilariae were examined within the vagina and uterus through the body wall, or were removed from broken specimens and were examined in glycerin, either unstained or stained with Azure II or methylene blue.

Illustrations were made with the aid of a Wild drawing apparatus and measurements were made with an ocular micrometer. In the following descriptions, all measurements (ranges, with means in parentheses) unless otherwise stated are in micrometers. Locations of structures in the microfilariae are given as the distance from the anterior extremity of the body.

Redescription

Ochoterenella Caballero, 1944

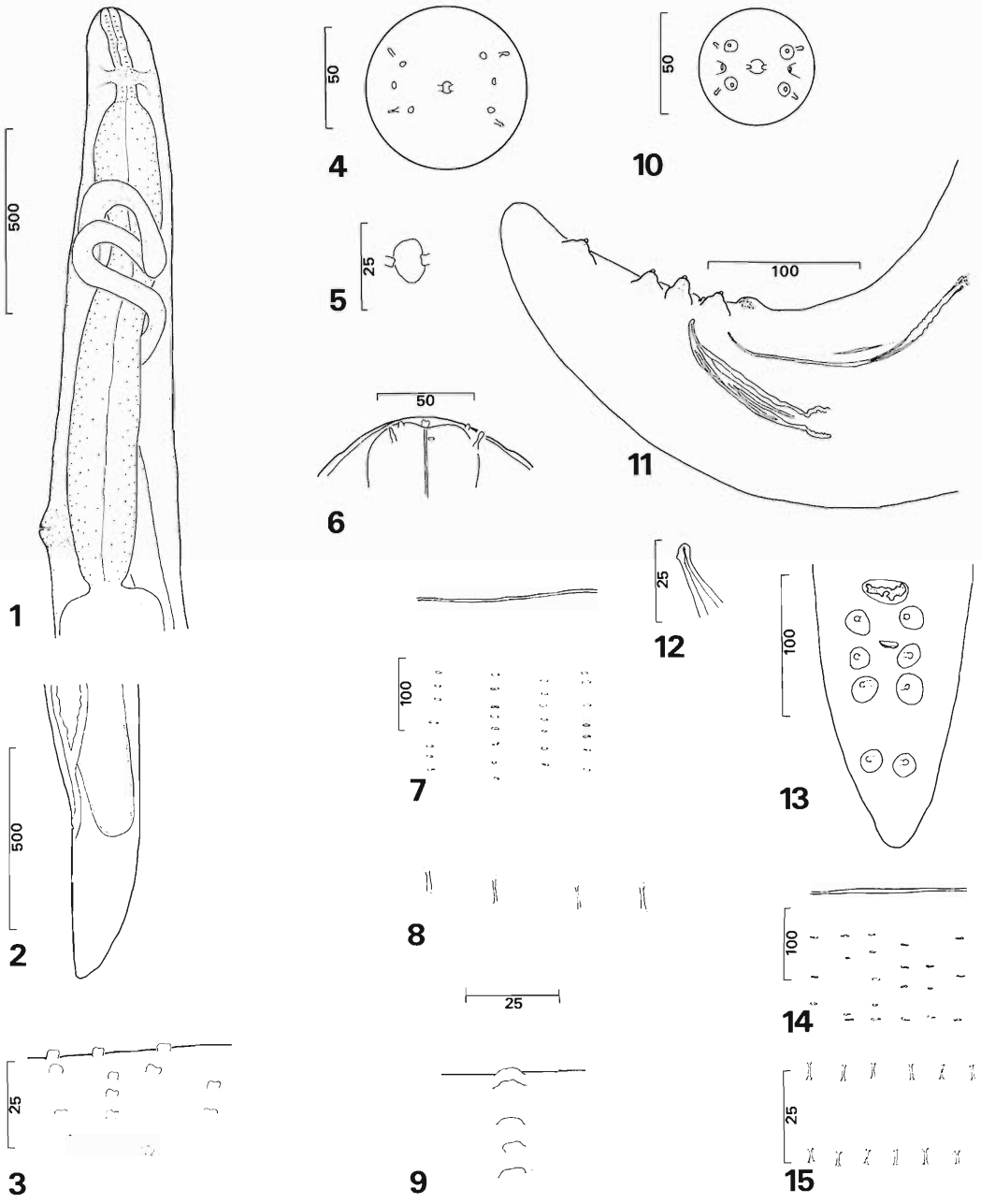
GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974. Cephalic extremity with pair of lateral flaplike cuticular parastomal structures. Cephalic plate with lateral axis longer than dorsoventral axis; 4 pairs papillae, each papilla often appearing to be comprised of a broad basal portion and a slender distal portion. Body cuticle, except at extremities, provided with annular bands of minute, longitudinally oriented bosses, often bacillary in appearance. Lateral and caudal alae lacking. Vulva in region of glandular portion of esophagus. Microfilariae sheathed. Parasites of body cavity of anuran amphibians; predominantly in neotropical Bufonidae.

Ochoterenella digiticauda Caballero, 1944 (Figs. 1-27)

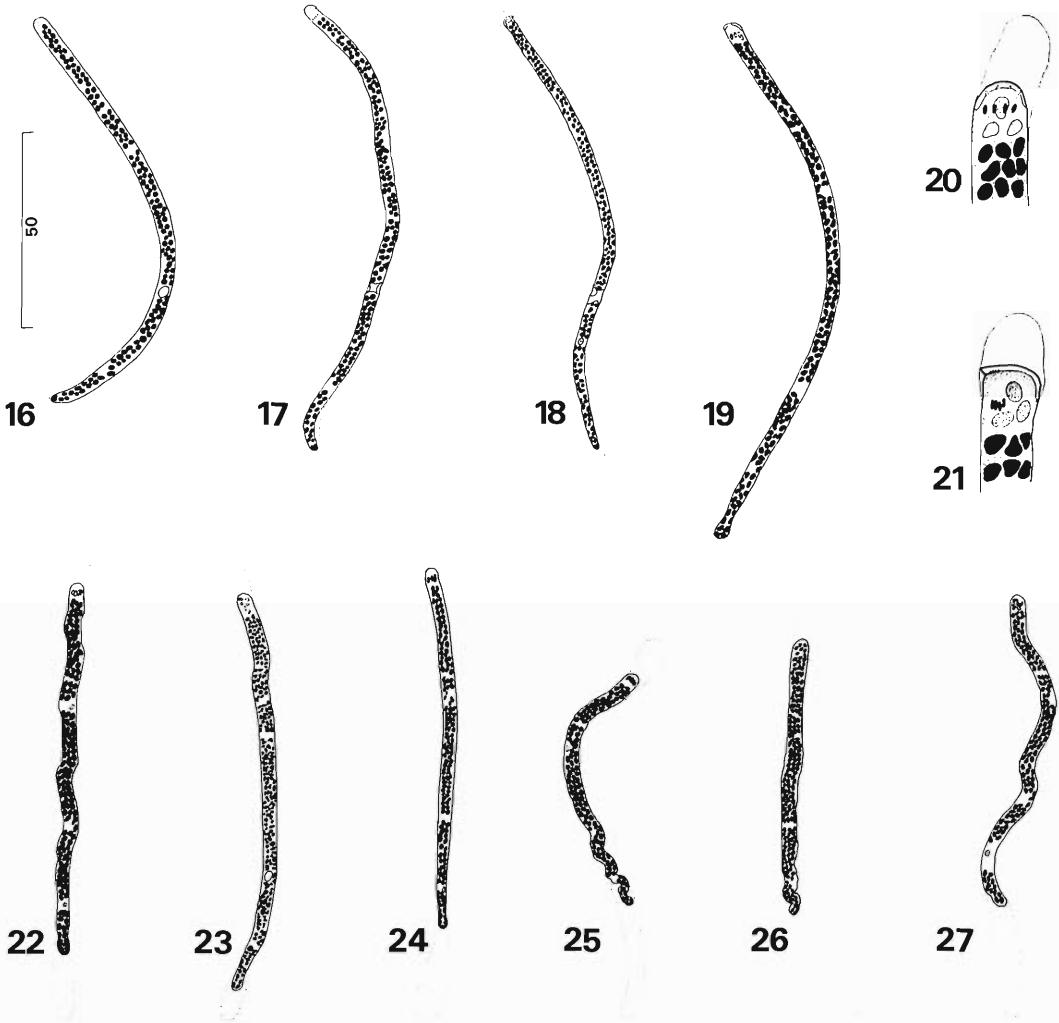
GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974; *Ochoterenella* Caballero, 1944. Males one-third to one-half length of females. Body gradually attenuated at both extremities (Fig. 1), widest just posterior to esophagointestinal junction. Posterior extremity of male helically coiled with 1–4 turns; that of female (Fig. 2) slightly flexed ventrad, digitiform. Cephalic extremity rounded (Figs. 1, 8); cephalic plate usually not salient. Parastomal structures (Figs. 4, 5, 10) distinct, truncate to slightly bilobed distally, approximately 4–5 wide, 4 high. Cuticle of both sexes with annular bands of longitudinally oriented, low, bacillary bosses; bands discontinuous along lateral margins of worm (Figs. 7–9, 14). Esophagus (Fig. 1) clearly divided; posterior glandular portion approximately three times width of anterior muscular part.

MALE (9 specimens; Figs. 10–15): Body length 16.5–24.1 (20.4) mm, maximum width 267–366 (314). Width body at nerve ring 104–170 (145), at junction of muscular and glandular portions of esophagus 112–182 (157), at esophagointestinal junction 225–376 (285). Esophagus total length 1,287–1,832 (1,603); muscular portion 182–281 (237) long, 27–41 (34) wide; glandular portion 1,138–1,584 (1,367) long, 73–134 (115) wide; ratio length glandular to muscular 5.0–7.3 (5.4). Nerve ring 120–228 (180) from anterior extremity. Tail length 117–180 (141); dorsoventral thickness of body at level of anus 87–116 (106); ratio of tail length to thickness at anus 1.07–1.65 (1.34). Caudal papillae (Figs. 11, 13) large, sessile, mammiform; 1 pair preanal, 3 pairs postanal, the posteriormost separated from the second postanal papillae and lying midway between them and posterior extremity at 55–69 (61) from tip of tail. Distinct median ventral preanal cuticularized plaque, with configuration as illustrated, located just anterior to caudal papillae. Spicules as illustrated (Fig. 11); right 119–146 (131) long, 10–14 (12) wide in midportion, with expanded cuplike base approximately 20 in diameter, tip (Fig. 12) closed, slightly bulbous; left 167–240 (202) long, proximal portion weakly cuticularized, often distorted. Spicular ratio (left to right) 1.23–1.73 (1.50). Area rugosa well developed, consisting of transverse bands of raised, longitudinally oriented cuticular bosses as illustrated (Fig. 15). Measured at midportion of area rugosa individual bosses 3–5 (4) in length, 5.5–8.5 (7.0) apart within a band, bands 16–30 (25) apart. Cuticular bosses on body (Fig. 14) measured at distance of 3 times length of esophagus from anterior extremity; length individual bosses 8.1–9.6 (8.7), distance between bosses 19–29 (22), between bands 21–34 (28).

FEMALE (13 gravid specimens; Figs. 1–9): Body length 44–57 (51) mm, maximum width 564–673 (605). Width body at nerve ring 190–307 (242), at junction of muscular and glandular por-



Figures 1–15. ***Ochoterenella digiticauda.*** **1. Anterior of female, lateral view. 2. Posterior of female, lateral view. 3. Bosses on dorsal surface of female at level of anus. 4. Cephalic extremity of female, en face view. 5. Detail of parastomal structures of female. 6. Cephalic extremity of female, lateral view. 7. Bands of cuticular bosses on female, midbody region, showing interruption of bands at lateral cord (top). 8. Detail of midbody bosses of female, surficial view. 9. Detail of midbody bosses of female, lateral view. 10. Cephalic extremity of male, en face view. 11. Posterior of male, lateral view. 12. Tip of right spicule. 13. Posterior of male, ventral view. 14. Bands of cuticular bosses of male, region adjacent to lateral cord. 15. Area rugosa, detail of bosses in surficial view.**



Figures 16–27. ***Ochoterenella digiticauda*** **microfilariae (all figures except 20 and 21 to same scale). 16. Immature, ex utero, paratype specimen. 17. Nearly mature, ex utero, paratype specimen. 18. Mature, ex vagina uterina, paratype specimen. 19. Blood in 2% aqueous formalin, ACR. 20, 21. Anterior extremity, semidiagrammatic, based on ex vagina uterina specimens. 22, 23. Blood film, IBUNM II-64. 24. Blood film, JAA. 25–27. Blood film, ACR.**

tions of esophagus 194–277 (258), at vulva 386–515 (443), at esophagointestinal junction 391–634 (486). Cephalic plate (Figs. 4, 6) 53–79 (61) by 32–45 (37). Esophagus total length 1,486–2,474 (1,896); muscular portion 228–416 (332) long, 39–56 (47) wide; glandular portion 1,238–1,683 (1,537) long, 134–190 (157) wide; ratio length glandular to muscular 4.0–6.1 (4.8). Nerve ring 218–337 (266) from anterior extremity. Vulva slightly salient, 1,020–1,782 (1,420) from anterior extremity, usually in region of posterior one-third of glandular part of esophagus. Vagina uterina usually extending anteriad, coiling around esophagus (Fig. 1). Tail (Fig. 2) 371–639 (456) long; dorsoventral thickness at level of anus 163–248 (203); ratio of tail length to thickness at anus 1.92–2.60 (2.25). Anus often difficult to discern. Cuticular bosses on dorsal surface of tail near anus (Fig. 3) usually well developed, 4–6 wide, 2–4 high. Cuticular bosses on body (Figs. 7–9) measured on dorsal and ventral surface in mid-body region; length individual bosses 7–10 (8.7),

distance between bosses 13–20 (17), between bands 62–93 (76).

Additional female specimens (6 ACR, 7 JAA) from Veracruz, Mexico, yielded measurements compatible with the above.

MICROFILARIAE: A, in blood films (27 specimens from IBUNM II-64, Giemsa, Figs. 22, 23; 40 specimens from ACR, hematoxylin and eosin, Figs. 25–27. Measurements of ACR specimens within brackets): Body slender, cylindrical, with gradual attenuation of posterior third; tip of tail rounded, usually bulbous, set off by slight constriction. Sheath present. Cephalic extremity rounded, with inconspicuous cephalic hook. Somatic nuclei ovoid to spheroid, column 3–4 nuclei wide; cephalic space often with 3 large nuclei, transverse row of 4 minute bodies; tip of tail with 2 or 3 nuclei. Body attitude usually slightly curved, often with posterior portion sinuous (Fig. 25), seldom looped. Body length 78–108 (90) [73–98 (82)], maximum width near nerve ring 3.0–4.0 (3.4) [3.4–4.8 (4.2)]. Cephalic space 3–5 (4.4) [3–5 (4.2)]; nerve ring 17–23 (20) [18–30 (22)]; excretory space 26–34 (31) [23–35 (29)]; Innenkörper 48–76 (60) [48–65 (55)], often ovoid; anal space 67–96 (78) [62–84 (71)].

Another sample (20 specimens from JAA, thin film) yielded the following measurements: Body length 99–110 (105), width 2.8–3.5 (3.1). Cephalic space 4.3–5.3 (4.6); nerve ring 21–34 (26); excretory space 29–40 (35); Innenkörper 66–75 (72); anal space 88–100 (93).

B, in blood fixed in 2% aqueous formalin (80 specimens from ACR, wet preparations, Fig. 19): Body cylindrical, straight, or assuming gentle curve; sheath extending only slightly beyond body. Tip of tail usually with weakly developed bulb. Cephalic extremity with hyaline cap, small cephalic hook. Body length 125–164 (149), maximum width 3.2–4.8 (3.6). Cephalic space 4.3–6.7 (5.3); nerve ring 29–41 (35); excretory space 44–56 (51); Innenkörper usually oval, hyaline, 88–108 (98); anal space 115–142 (129).

C, extracted from vagina vera of fixed gravid females observed in glycerin with Azure II (45 specimens from broken worm in IBUNM 106-3, Figs. 18, 20, 21; 20 specimens from ACR. Measurements of ACR specimens within brackets): Body slender, cylindrical, gradually attenuated in posterior third, tip of tail sometimes with inconspicuous bulb. Cephalic extremity as illustrated (Figs. 20, 21), cephalic space with 3 large vesicular nuclei, row of 4 minute bodies. Body length 96–134 (115) [92–106 (102)], maximum width 2.6–3.1 (2.8) [2.9–3.6 (3.3)]. Cephalic space 1.0–3.0 (2.1) [3.8–5.8 (4.7)]; nerve ring 13–24 (20) [16–28 (22)]; excretory space 38–48 (42) [23–35 (29)]; Innenkörper short, ovoid to irregular, 58–77 (72) [59–80 (67)]; anal space 86–107 (97) [81–103 (89)].

Taxonomic Summary

Diagnosis

The combination of morphological features that separates the genus *Ochoterenella* from the other genera in the subfamily Waltonellinae includes the presence of cuticularized flaplike parastomal structures, annular bands of longitudinally oriented minute bosses in the midbody region, and the lack of lateral and caudal alae in both sexes. *O. digiticauda* is distinguished from the other species in the genus by the size and arrangement of the cuticular bosses on the body of both sexes, the appearance of the median ventral preanal cuticularized plaque and the dimensions and structural details of the spicules in the male, and the appearance and size of the microfilaria.

SPECIMENS DEPOSITED: Except where noted, all specimens are deposited in the Helminth Collection of the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico.

TYPE SPECIMENS: IBUNM 107-1 (male), IBUNM 106-3 (female) [these specimens are labeled "typo" and correspond to the holotype and allotype], IBUNM II-64 (2 slides blood films with microfilariae).

PARATYPES: IBUNM 107-1-1 (9 males selected by JHE), IBUNM 106-3-1 (13 females selected by JHE). USNM Helm. Coll. No. 36893 (1 male, 2 females; deposited by Caballero from IBUNM 107-1 and 106-3, respectively).

TYPE HOST: *Bufo marinus* Linn., 1758.

SITE OF INFECTION: Body cavity.

TYPE LOCALITY: Mexico, Chiapas, Huixtla.

Discussion

A major difficulty encountered in the present study was that Caballero's paratype collections contained more than a single species of *Ochoterenella*. Fortunately, the type male and female (IBUNM 107-1, 106-3) as well as the paratypes deposited in the U.S. National Museum Helminth Collection are apparently all of the same species and conform reasonably well to his orig-

inal description of *O. digiticauda.* It should be added that Caballero's other collections (see Materials and Methods) are likewise mixtures. It is for this reason that certain specimens were selected as special paratypes with distinct numbers.

In his original description of *O. digiticauda,* Caballero (1944) either did not observe or misinterpreted some important features. The parastomal structures were apparently included among the "eight internal papillae," and the preanal cuticularized plaque in the male was interpreted as a pair of papillae; the latter was corrected by Lent et al. (1946). Microfilariae, characterized as unsheathed, were reported as being of 2 sizes. Caballero's blood films (IBUNM II-64) did show a variation in size (contraction), but some of the microfilariae appear to be a different species altogether. Although the original description of the microfilaria is misleading, Caballero's photograph is identical to the microfilariae described in the present report.

The "redescription" of *O. digiticauda* by Lent et al. (1946) adds further confusion. The specimens were obtained from a different host (*Bufo paracnemis*) in a different geographic location (Paraguay). These investigators compared their specimens with some of Caballero's and considered them to be identical. The description, however, has several discrepancies (configuration of cuticular bosses, appearance of left spicule, shape of microfilaria) which suggest that the Paraguayan worms are a distinct species. Masi Pallares and Maciel (1974) later reported worms from *B. paracnemis* in Paraguay that they identified as *O. digiticauda,* but they neither described nor figured the specimens.

Subsequent investigators (Anderson, 1968; Anderson and Bain, 1976) considered *Ochoterenella* to lack the parastomal structures, and the separation of this genus from other members of the subfamily has been based in part on their presumed absence. As a result of the findings in the present study, it is necessary to modify the definition of the genus *Ochoterenella* to include the presence of cuticularized flaplike parastomal structures and annular bands of longitudinally oriented bosses, and the absence of lateral and caudal alae.

Only 2 species other than *O. digiticauda* have been hitherto included in the genus. Johnston (1967) described *O. papuensis* from a frog (*Cornufer papuensis*) in New Guinea, although the reasons for its determination as *Ochoterenella* were tenuous. This identification seems highly unlikely, and because of the inadequacy of the description, *O. papuensis* should be considered incertae sedis.

Bain and Prod'hon (1974) described *O. guibei* from a racophorid frog (*Mantidactylus redimitus*) in Madagascar. Its features exclude it from *Ochoterenella* as here defined. Although the geographic location and type of host are the same as for *Madochotera,* a genus erected by Bain and Brunhes (1968), *O. guibei* is morphologically distinct, and the generic name *Paramadochotera* is proposed to accommodate it. The distinguishing features of *Paramadochotera* gen. n. are: Cephalic extremity lacking cuticularized flaplike parastomal structures, distinctly cuticularized buccal capsule present, papillae with broad base and salient apical portion; lateral alae lacking; body in both sexes abruptly attenuated at both extremities; cuticle of female with transversely oriented ridges or bosses on dorsal and ventral surfaces.

Five species of *Waltonella* were described by Bain and Prod'hon (1974) and Bain et al. (1979) from *Bufo marinus* in the neotropical region: *W. guyanensis, W. royi, W. oumari, W. dufourae,* and *W. albareti.* All of these conform to the characteristics of *Ochoterenella*; moreover, these same features have set them apart from the rest of the *Waltonella,* an otherwise reasonably uniform group. The 5 species are consequently transferred to the genus *Ochoterenella.* The status of the genus *Waltonella* is considered elsewhere (Esslinger, 1986).

Travassos (1929) described 3 species of filariae from Brazilian anurans: *Foleyella convoluta* (Molin, 1858) from *Leptodactylus pentadactylus, F. scalaris* from *L. ocellatus,* and *F. vellardi* from *Bufo marinus.* All were reported as having bands of longitudinally oriented cuticular bosses and measurements of these were given, but the descriptions were otherwise very cursory and none of the worms was illustrated. These, considered to be *Waltonella* by Bain and Prod'hon (1974) are also included within the genus *Ochoterenella.* Vicente and Jardim (1980) held that *F. vellardi* was identical to *O. digiticauda* and proposed synonymy, but striking differences in the measurements and arrangement of the cuticular bosses between the 2 species strongly suggest that this is not justified.

The general similarities between species of *Ochoterenella* have contributed to the confusion

in the initial descriptions as well as in subsequent identifications and host records. Certain features are reliable for differentiating species and were emphasized by Bain et al. (1979). Experience in the present study has shown that the cuticular bosses in both sexes can be quite useful, but the selection of the level of the body where measurements are made must be considered. The general midbody region of females was preferred, because in that area there are no apparent differences between the dorsal and ventral surfaces. Such differences are seen in the posterior portion of male worms (as the area rugosa is approached); hence, an acceptable level at approximately three lengths of the esophagus from the anterior extremity was selected.

The appearance and length of individual bosses is notably consistent within a species. This affords an indication of the identity of males with corresponding females. The distance between adjacent bosses within a band is of less use in this respect, and the distance between consecutive bands cannot be used because it is considerably greater in the females. All of these parameters are useful in distinguishing species, but dimensions tend to be less in the smaller individuals, particularly immature and nongravid females. Other features of special importance include the detail of spicular structure and the ratio of the lengths of the muscular to the glandular portions of the esophagus. Because often few or no males are recovered, female worms must be used as the principal means of identification.

Microfilariae are valuable in distinguishing species, but their reliability largely depends upon the type of material available. The most useful appears to be host blood fixed in 2% aqueous formalin. Blood films are less valuable because dimensions and appearance may vary widely (contracting, stretching). The identification of microfilariae within or extracted from preserved worms is possible, but care must be taken to select specimens from as close to the vagina as possible, because those from the uterus may be immature and will differ from those found in the host's blood (see Figs. 16–18).

As redefined, the genus *Ochoterenella* now includes *O. digiticauda* Caballero, 1944 (type species); *O. guyanensis* (Bain and Prod'hon, 1974) comb. n.; *O. royi* (Bain et al., 1979) comb. n.; *O. dufourae* (Bain et al., 1979) comb. n.; *O. oumari* (Bain et al., 1979) comb. n.; *O. albareti* (Bain et al., 1979) comb. n.; *O. convoluta* (Molin, 1858) comb. n.; *O. scalaris* (Travassos, 1929) comb. n.; and *O. vellardi* (Travassos, 1929) comb. n.

The structure of the left spicule (proximal portion weakly cuticularized, distorted) readily distinguishes *O. digiticauda* from all other species for which the male has been described (*O. guyanensis, O. royi, O. oumari*). The median ventral preanal cuticularized plaque of *O. digiticauda* appears to differ in its configuration from the comparable structures illustrated for the males of *O. royi, O. oumari,* and *O. guyanensis.* The muscular to glandular esophageal ratio of the female of *O. albareti* (9.6) is much greater than that of *O. digiticauda* (4.0–6.1). The arrangement and dimensions of the cuticular bosses of females of *O. digiticauda* are distinct from those of most of the remaining species, including those of Travassos. Although the bosses of *O. dufourae* (4–7 long) approach the lower limit of *O. digiticauda,* the tail of the former is shorter and truncated.

The genus *Ochoterenella* as presently constituted appears to be a morphologically compact group of species restricted in distribution to the neotropical region. Even though most of the currently accepted members have been recovered from *Bufo marinus,* other toads and some frogs, particularly leptodactylids, are also infected.

It is evident that specimens identified as *O. digiticauda* in existing collections need re-examination. Studies of Caballero's worms are currently in progress, and it is likely that previously undescribed species of *Ochoterenella* will be disclosed.

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Literature Cited

Anderson, R. C. 1968. The comparative morphology of cephalic structures in the superfamily Filarioidea (Nematoda). Canadian Journal of Zoology 46: 181–199.

———, **and O. Bain.** 1976. Diplotriaenoidea, Aproctoidea and Filarioidea. Pages 59–116 *in* R. C. An-

derson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 3. Keys to the Genera of the Order Spirurida. Farningham Royal, Bucks, England, Commonwealth Agricultural Bureaux.

Bain, O., and J. Brunhes. 1968. Un nouveau genre de filaire parasite de grenouilles malgaches. Bulletin du Muséum National d'Histoire Naturelle (2nd ser.) 40:797–801.

———, **D. C. Kim, and G. Petit.** 1979. Diversité spécifique des filaires du genre *Waltonella* coexistant chez *Bufo marinus.* Bulletin du Muséum National d'Histoire Naturelle, Paris (4th ser.) 1, sect. A:199–212.

———, **and J. Prod'hon.** 1974. Homogenéité des filaires de batracien des genres *Waltonella, Ochoterenella* et *Madochotera*; création des Waltonellinae n. subfam. Annales de Parasitologie, Humaine et Comparee 49:721–739.

Brenes, R. R., and M. B. Hollis. 1959. Helmintos de la Republica de Costa Rica VIII. Nematoda 2. Algunos nématodes de *Bufo marinus marinus* (L.), y algunas consideraciones sobre los géneros *Oxysomatium* y *Aplectana.* Revista de Biología Tropical 7:35–55.

Caballero, E. 1944. Estudios helmintologicos de la region oncocercosa de Mexico y de la Republica de Guatemala. Nematoda: la parte. Filarioidea. I. Anales del Instituto de Biología, Mexico 15:87–108.

Dyer, W. G., and R. Altig. 1977. Helminths of some Ecuadorian anurans. Herpetologica 33:293–296.

Esslinger, J. H. 1986. Redescription of *Foleyellides striatus* (Ochoterena and Caballero, 1932) (Nematoda: Filarioidea) from a Mexican frog, *Rana montezumae,* with reinstatement of the genus *Foleyellides* Caballero, 1935. Proceedings of the Helminthological Society of Washington 53:218–223.

Johnston, M. R. L. 1967. *Icosiella papuensis* n. sp. and *Ochoterenella papuensis* n. sp. (Nematoda: Filarioidea), from a New Guinea frog, *Cornufer papuensis.* Journal of Helminthology 41:45–54.

Lent, H., J. F. T. de Freitas, and M. C. Proença. 1946. Alguns helmintos de batráquios colecionados no Paragui. Mémorias do Instituto Oswaldo Cruz 44: 195–214.

Marinkelle, C. J. 1970. Observaciones sobre la periodicidad de las microfilarias de *Ochoterenella* en *Bufo marinus* de Colombia. Revista de Biología Tropical 16:145–152.

Masi Pallares, R., and S. Maciel. 1974. Helminthes en batracios del Paraguay (1ra Parte), con descripcion de una nueva especie, *Aplectana pudenda* (Oxyuridae: Cosmocercinae). Revista Paraguaya de Microbiologia 9:55–60.

Travassos, L. 1929. Filaridés des batraciens du Brésil. Comptes rendus des séances de la Societé de Biologie, Paris 100:967–968.

———, **and J. F. T. de Freitas.** 1960. Excursão a Maicuru, Estado do Pará. Atas de Sociedade de Biologia do Rio de Janeiro 4:13–15.

Vicente, J. J., and C. R. Jardim. 1980. Filarídeos da coleção helmintologica do Instituto Oswaldo Cruz. I. Peixes, anfibios e répteis. Atas da Sociedade de Biologia do Rio de Janeiro 21:45–57.

———, **and E. dos Santos.** 1976. Fauna helmintologica de *Leptodactylus ocellatus* (L., 1758) de Volta Redonda, Estado do Rio de Janeiro. Atas da Sociedade de Biologia do Rio de Janeiro 18:27–42.

Proc. Helminthol. Soc. Wash.
53(2), 1986, pp. 218–223

Redescription of *Foleyellides striatus* (Ochoterena and Caballero, 1932) (Nematoda: Filarioidea) from a Mexican Frog, *Rana montezumae,* with Reinstatement of the Genus *Foleyellides* Caballero, 1935

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ABSTRACT: With subsequent additions of filariae known to infect anuran amphibians, the original descriptions of *Foleyellides striatus,* the type species of the genus, are now inadequate. *F. striatus* from *Rana montezumae* in Mexico is herein redescribed from the syntypes and the genus is redefined. The name *Waltonella* Schacher, 1975, falls as a junior synonym of *Foleyellides* Caballero, 1935, but the subfamily name Waltonellinae Bain and Prod'hon, 1974, is retained in accordance with the International Code of Zoological Nomenclature. The subfamily contains the 4 genera *Foleyellides* Caballero, 1935, *Ochoterenella* Caballero, 1944, *Madochotera* Bain and Brunhes, 1968, and *Paramadochotera* Esslinger, 1986. *Foleyellides* is distinguished from the others by the presence in both sexes of cuticularized parastomal structures and both lateral and caudal alae, and by the lack of a distinct cuticularized buccal capsule and annular bands of longitudinally oriented bosses on the cuticle of the midbody region. The genus *Foleyellides* is considered to include *F. striatus* (Ochoterena and Caballero, 1932) Caballero, 1935 [type species]; *F. duboisi* (Gedoelst, 1916) comb. n.; *F. ranae* (Walton, 1929) comb. n.; *F. americana* (Walton, 1929) comb. n.; *F. dolichoptera* (Wehr and Causey, 1939) comb. n.; *F. brachyoptera* (Wehr and Causey, 1939) comb. n.; *F. confusa* (Schmidt and Kuntz, 1969) comb. n.; *F. flexicauda* (Schacher and Crans, 1975) comb. n.; and *F. malayensis* (Petit and Yen, 1979) comb. n. These species are all parasitic in *Rana* spp. and inhabit the body cavity of the host with the exception of *F. confusa,* which is subcutaneous. Although most are known from the western hemisphere, 3 of the species have been described from central Africa, Palestine, Malaysia, and the Philippines.

Ochoterena and Caballero (1932) recovered filarial nematodes from the body cavity of a Mexican frog, *Rana montezumae,* and described them as representing a new species of the genus *Chandlerella* Yorke and Maplestone, 1926, *C. striata.* After further study, Caballero (1935) redescribed the worm, erecting the genus *Foleyellides* to accommodate it, and renaming it *F. striatus.* Caballero stated that although the species had many of the characters of the genus *Foleyella* Seurat, 1917, ". . . the differences are so great as to induce me to create the new genus *Foleyellides* . . . ." Evidence that this worm has been recovered subsequently or that specimens have been re-examined is lacking. The cursory and now inadequate original descriptions have made a restudy of this worm mandatory before its systematic position among the filariae occurring in anuran amphibians can be properly assessed.

In the present investigation the "type" collection (actually syntypes, because no single specimen had been selected as the holotype) was examined and the redescription of *Foleyellides striatus* is presented.

Materials and Methods

SPECIMENS EXAMINED: *Foleyellides striatus* "types," Museum No. 107-3 (20 males, 19 females) in Helminth Collection of the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico, deposited by E. Caballero; *Foleyellides striatus* "cotypes" (1 male, 1 female), USNM Helm. Coll. No. 8911, deposited by E. Caballero; *Foleyella brachyoptera* Wehr and Causey, 1939, paratypes (2 males, 3 females), USNM Helm. Coll. No. 40391; *Foleyella dolichoptera* Wehr and Causey, 1939, paratypes (2 males, 2 females), USNM Helm. Coll. No. 40393; *Foleyella americana* Walton, 1929, paratypes (2 females), USNM Helm. Coll. No. 50696; *Foleyella confusa* Schmidt and Kuntz, 1969, paratypes (2 males), USNM Helm. Coll. No. 70479; *Foleyella flexicauda* Schacher and Crans, 1973, allotype (female) and paratypes (15 males), USNM Helm. Coll. Nos. 72556, 72557.

LECTOTYPE SPECIMENS DESIGNATED (by author): *Foleyellides striatus.* Museum numbers, with suffix added, correspond to catalog of the Helminth Collection of the Instituto de Biología where specimens are deposited. Lectotype: (male), No. 107-3-1. Paralectotypes: selected female No. 107-3-2; other paralectotypes (19 males, 18 females), No. 107-3-3.

PROCEDURES: All specimens were removed to 70% ethanol containing 5% glycerin and slowly evaporated to pure glycerin in which they were examined. Microfilariae were examined within the vagina through the body wall or were removed from the vagina uterina of a broken specimen.

Illustrations were made with the aid of a Wild drawing apparatus and measurements were made with an ocular micrometer. In the following descriptions, all measurements (ranges, with means in parentheses), unless otherwise stated, are in micrometers. Locations of structures in the microfilariae are given as the distance from the anterior extremity of the body.

Redefinition

Foleyellides Caballero, 1935

[=*Waltonia* Schacher and Crans, 1973, subgenus; *Waltonella* Schacher, 1975, subgenus; *Waltonella* (Schacher, 1975) Bain and Prod'hon, 1974, genus.]

Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974. Cephalic extremity with pair of laterally disposed cuticularized flaplike parastomal structures. Four pairs cephalic papillae, at least outer member of each having broad base and slender distal portion. Distinct cuticularized buccal capsule lacking. Lateral and caudal alae present on both sexes. Cuticle of midbody region lacking annular bands of minute, longitudinally oriented bosses. Esophagus comprised of short, muscular anterior portion and long, glandular posterior portion, the latter being distinctly wider. Vulva in region of esophagointestinal junction. Microfilaria sheathed. Parasites of anuran amphibians; predominantly in Ranidae. Type species: *Foleyellides striatus* (Ochoterena and Caballero, 1932) Caballero, 1935.

Redescription

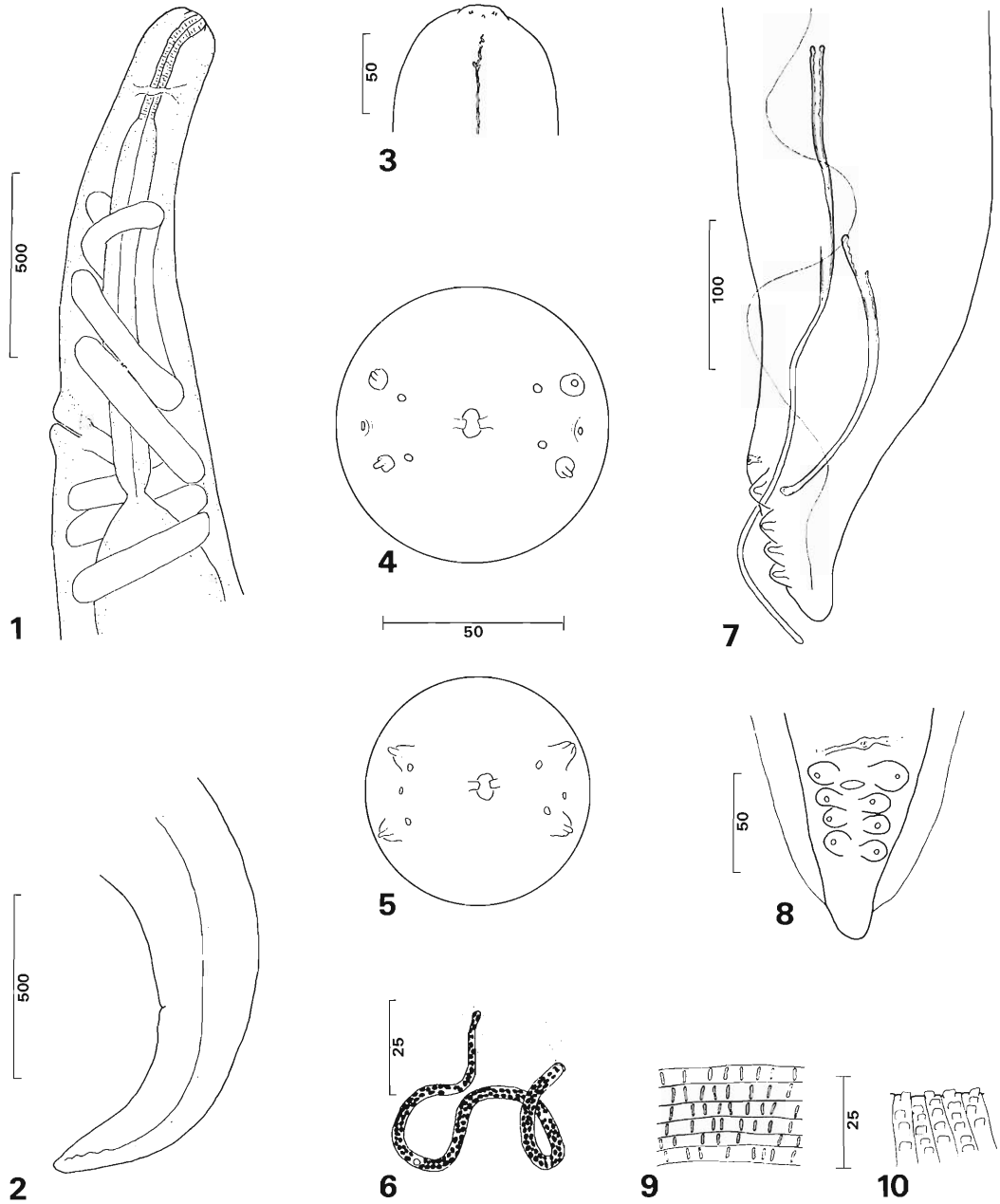
Foleyellides striatus (Ochoterena and Caballero, 1932) Caballero, 1935 (Figs. 1-10)

[=*Chandlerella striata* Ochoterena and Caballero, 1932; *Foleyella striatus* (Ochoterena and Caballero, 1932) Witenberg and Gerichter, 1944; *Waltonella striatus* (Ochoterena and Caballero, 1932) Bain and Prod'hon, 1974.]

GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974; *Foleyllides* Caballero, 1935. Male approximately one-third length of female. Body with both anterior and posterior extremities gradually but distinctly attenuated, particularly so with the more robust females (Figs. 1, 2), widest slightly posterior to esophagointestinal junction. Cuticle smooth. Cephalic plate (Figs. 3–5) with long axis laterally oriented, measuring approximately twice as much laterally as dorsoventrally, slightly salient at lateral margins; 4 pairs papillae, the outer member of each having broad base and slender distal portion. Parastomal structures (Figs. 4, 5) distinct, truncate, 3–4 wide, often extending over stomatal margin. Esophagus distinctly divided into short anterior muscular and long posterior glandular portions; glandular part approximately twice as wide as muscular.

MALE (20 specimens; Figs. 5, 7–10): Body length 9.8–19.2 (15.3) mm, maximum width 216–366 (277). Width body at nerve ring 102–168 (143), at junction of muscular and glandular portions of esophagus 113–181 (155), at esophagointestinal junction 151–282 (236). Cephalic plate 33–50 (40) by 19–26 (22). Esophagus total length 977–1,297 (1,149); muscular portion 178–347 (274) long, 26–43 (34) wide; glandular portion 713–980 (873) long, 60–96 (77) wide; ratio length glandular to muscular 2.51–4.09 (3.20). Nerve ring 132–228 (204) from anterior extremity. Tail length 46–85 (73); dorsoventral thickness of body at level of anus 35–67 (51); ratio of tail length to thickness at anus 1.07–1.88 (1.45). Caudal papillae (Figs. 7, 8) large, sessile, mammiform; 1 pair preanal, 3 pairs postanal, the posteriormost 29–48 (41) from tip of tail. Distinct median ventral preanal cuticularized plaque with appearance as figured, located just anterior to caudal papillae. Spicules as illustrated (Fig. 7); right 144-218 (189) long, 7–12 wide at base; left 336–465 (383) long, divided; proximal portion 108–172 (137) long, 5–7 wide at base; distal 185–305 (242) long; ratio distal to proximal 1.35–2.19 (1.78). Spicular ratio 1.71–2.67 (2.05). Area rugosa well developed, consisting of distinct transverse bands of raised cuticular processes as illustrated (Figs. 9, 10). Lateral alae (Figs. 7, 8) distinct, low, becoming higher in region of tail.

FEMALE (18 gravid specimens; Figs. 1–4): Body length 38–74 (55) mm, maximum width 416–811 (583). Width body at nerve ring 130–234 (181), at junction of muscular and glandular portions of esophagus 154–248 (190), at esophagointestinal junction 282–604 (424), at vulva 257–644 (423). Cephalic plate (Fig. 4) 38–56 (46) by 19–29 (24). Esophagus total length 1,242–1,604 (1,422); muscular portion 235–356 (307) long, 28–60 (40) wide; glandular portion 980–1,297 (1,113) long, 70–108 (90) wide; ratio length glandular to muscular 3.03–4.67 (3.66). Nerve ring 156–248 (209) from anterior extremity. Vulva slightly salient, 574–2,624 (1,275) from anterior extremity. Vagina uterina usually extending anteriad coiling around glandular portion of esophagus (Fig. 1). Tail (Fig. 2) 156–718 (489) long; dorsoventral thickness at level of anus 89–376 (205); ratio of tail length to thickness at anus 1.91–3.20 (2.41). Lateral alae (Figs. 2, 3) distinct, low.



Figures 1–10. ***Foleyellides striatus.*** **1. Anterior of female, lateral view. 2. Posterior of female, lateral view. 3. Cephalic extremity of female, lateral view. 4. Cephalic extremity of female en face. 5. Cephalic extremity of male en face. 6. Microfilaria from vagina. 7. Posterior of male, lateral view. 8. Posterior of male, ventral view. 9. Detail of area rugosa, ventral view. 10. Detail of area rugosa, lateral view.**

MICROFILARIA (25 specimens from vagina uterina of broken preserved female, unstained; Fig. 6): Body slender, cylindrical with posterior half gradually attenuated, tip of tail rounded, often slightly bulbous. Sheath present. Cephalic extremity with distinct hyaline cap, cephalic hook only slightly developed. Somatic nuclei ovoid to spheroid, extending to tip of tail, column 2–3

nuclei wide. Cephalic space with anterior transverse row of four minute bodies, posterior pair of large elongate vesicular nuclei. Tail region with nuclei in single file, extremity usually with distinct pair. Body length 95–159 (118), maximum width near nerve ring 3.2–3.8 (3.7). Cephalic space 3.8–6.8 (5.1); nerve ring 15–39 (26); excretory space 22–49 (35); Innenkörper 51–107 (73), ovoid, hyaline; anal space 75–139 (97).

TYPE HOST: *Rana montezumae* Baird, 1854.

SITE OF INFECTION: Body cavity.

TYPE LOCALITY: Mexico, Mexico, D.F., Xochimilco.

Discussion

With the more restrictive definition of *Foleyellides,* certain species of "frog filariae" previously listed within *Foleyella* (sensu lato) and its successor *Waltonella* must be excluded. Many of the filariae of anurans reported in the early part of the century and before are so insufficiently described that there is little hope of identifying them beyond unsubstantiable speculation. These are listed by Witenberg and Gerichter (1944). It is felt that *Foleyella bouillezi* Witenberg and Gerichter, 1944 (=*Filaria* sp. of Bouillez, 1916) and *Foleyella leiperi* (Railliet, 1916 [=?*Filaria bufonis* Leiper, 1908]) should also be considered unidentifiable.

Seven species previously included by Bain et al. (1979) in *Waltonella* (*W. guyanensis, W. royi, W. oumari, W. dufourae, W. albareti, W. scalaris, W. convoluta,* and *W. vellardi*) were transferred to *Ochoterenella* by Esslinger (1986).

Most of the species herewith placed in the genus *Foleyellides* have been reported from *Rana* spp. in the Americas. *F. striatus,* the type species, was found in *R. montezumae* and *R. halecina* in Mexico (Ochoterena and Caballero, 1932; Caballero, 1935). Walton (1929) described *F. ranae* comb. n. from a Louisiana bullfrog (*R. catesbeiana*), and females of *F. americana* comb. n. from *R. pipiens* in Illinois. He (Walton, 1935) later described the male from the type host obtained from Wisconsin. Wehr and Causey (1939) described *F. dolichoptera* comb. n. and *F. brachyoptera* comb. n. from *R. sphenocephala* in Florida. Causey (1939) provided a redescription of the microfilariae of these 2 species and that of *F. ranae,* and descriptions of all 3 were further augmented by Kotcher (1941). It should be noted that Cowper's (1946) presumed redescription of "*Foleyella leiperi*" from *R. sphenocephala* collected in Florida was in fact based on specimens of *F. dolichoptera.* Schacher and Crans (1973) described *F. flexicauda* comb. n. from *R. catesbeiana* in New Jersey.

Three species included in *Foleyellides* have been found outside of the western hemisphere. *F. duboisi* (Gedoelst, 1916) comb. n. was originally described from a "big toad" in Leopoldville, Belgian Congo. Witenberg and Gerichter (1944) redescribed this species from worms recovered from the body cavity of *Rana esculenta ridibunda* in northern Palestine. Schmidt and Kuntz (1969) described *F. confusa* comb. n. from male specimens recovered from *Rana limnocharis vittigera* in the Philippines. Although the general features of the worm conform to those of *Foleyellides,* its site of infection (subcutaneous) and its pronounced spinelike outer cephalic papillae seem unusual. Confirmation of its correct placement in the genus awaits further investigation and description of the female and microfilaria. *F. malayensis* comb. n. described by Petit and Yen (1979) occurs in Malaysia and was recovered from the body cavity of *Rana glandulosa.* The files of minute bosses on the middorsal and midventral lines of the female are unique.

The dimensions of the adults of *F. striatus* place this worm among the larger members of the genus, but size alone is of limited use. Males afford the most reliable features for distinguishing species. *F. striatus* has 4 pairs of caudal papillae as does *F. duboisi*; however, *F. confusa* has 5, *F. flexicauda* and *F. brachyoptera* 6, *F. americana* and *F. malayensis* 7, and *F. ranae* 8. The distinctive cuticularized preanal plaque (or comparable structure) is absent in *F. duboisi, F. confusa, F. dolichoptera,* and *F. brachyoptera.* The spicular ratio of *F. duboisi* (4.0) is approximately twice that of *F. striatus* (2.1). Differences between microfilariae are not striking, although that of *F. dolichoptera* is nearly twice as long as the microfilaria of *F. striatus.*

Caballero (1935) erected the genus *Foleyellides* to exclude his worms from *Foleyella,* which at that time contained species from both reptiles and amphibians. This was based, in part, on the apparent absence of cephalic structures (papillae, lips) and lateral alae. Witenberg and Gerichter (1944) considered *Foleyellides* to be synonymous with *Foleyella,* Schacher and Crans (1973) divided the genus *Foleyella* Seurat, 1917, into two morphologically distinct subgenera, *Fole-*

yella and *Waltonia,* found in reptiles and amphibians, respectively. Schacher (1975) replaced the name *Waltonia,* which was preoccupied, with *Waltonella,* but left *Foleyellides* as a separate genus as proposed by Sonin (1968). Bain and Prod'hon (1974) elevated *Waltonella* to the generic level, created the subfamily Waltonellinae to accommodate the 3 genera *Waltonella, Ochoterenella,* and *Madochotera,* and included *Foleyellides* in the first, renaming *F. striatus, Waltonella striatus.*

Because the name *Foleyellides* Caballero, 1935, takes precedence over *Waltoniella* Schacher, 1975, the latter name falls as a junior synonym, and *Foleyellides* is accordingly reinstated. *Foleyellides striatus* thus becomes the type species of the genus by original designation, but the subfamily name Waltonellinae is nevertheless retained in conformity with the International Code of Zoological Nomenclature (Article 40, Section a).

Although the definition of the subfamily Waltonellinae remains the same as stated by Bain and Prod'hon (1974), its composition reflects changes in the genus *Ochoterenella* Caballero, 1944, proposed by Esslinger (1986). In this revision *O. papuensis* Johnston, 1967, and *O. guibei* Bain and Prod'hon, 1974, were excluded from *Ochoterenella* and a new genus, *Paramadochotera,* was created for the latter.

The subfamily Waltonellinae now contains four genera that are distinguished by the features listed:

Foleyellides Caballero, 1935; cuticularized parastomal structures, lateral and caudal alae present in both sexes. Distinct buccal capsule, annular bands of longitudinally oriented bosses in midbody region lacking. Parasites of Ranidae, distribution worldwide.

Ochoterenella Caballero, 1944; cuticularized parastomal structures, bands of longitudinally oriented bosses in midbody region present in both sexes. Distinct buccal capsule, lateral and caudal alae lacking. Parasites of Bufonidae and Leptodactylidae of neotropical region.

Madochotera Bain and Brunhes, 1968; cuticularized parastomal structures, lateral alae present. Cuticle sometimes with transversely oriented ridges or bosses. Vulva markedly posterior to esophagus. Distinct buccal capsule lacking. Parasites of Racophoridae, Madagascar.

Paramadochotera Esslinger, 1986; distinct cuticularized buccal capsule present; cuticle of female with transversely oriented ridges or bosses on dorsal and ventral surfaces. Body in both sexes abruptly attenuated at extremities. Cuticularized parastomal structures, lateral and caudal alae lacking. Parasites of Racophoridae, Madagascar.

Members of the genus *Foleyellides* appear to constitute a reasonably uniform group. These filariae are characteristically parasitic in the body cavity of ranid frogs. The apparent predominance of species in the western hemisphere probably reflects areas where investigations have been undertaken rather than any valid indication of geographic range. Certain morphological features (e.g., parastomal structures, position of vulva, median ventral preanal plaque or papilla) suggest a much closer affinity with *Ochoterenella* than with the other genera in the Waltonellinae.

Acknowledgments

I wish to express my appreciation to Rafael Lamothe-Argumedo and Alejandro Cruz-Reyes of the Instituto de Biología, Universidad Nacional Autónoma de México, for providing me with the specimens of *Foleyellides striatus.*

Literature Cited

Bain, O., and J. Brunhes. 1968. Un nouveau genre de filaire, parasite de grenouilles malgaches. Bulletin du Muséum National d'Histoire Naturelle (2nd ser.) 40:797–801.

———, **D. C. Kim, and G. Petit.** 1979. Diversité spécifique des filaires du genre *Waltonella* coexistant chez *Bufo marinus.* Bulletin du Muséum National d'Histoire Naturelle, Paris (4th ser.) 1, sect. A:199–212.

———, **and J. Prod'hon.** 1974. Homogenéité des filaires de batracien des genres *Waltonella, Ochoterenella* et *Madochotera*; création des Waltonellinae n. subfam. Annales de Parasitologie Humaine et Comparee 49:721–739.

Caballero, E. 1935. Nematodos parasitos de los batracios de Mexico III. Curata contribucion al conocimiento de la parasitologia de *Rana montezumae.* Anales del Instituto de Biología México 6: 103–117.

———. 1944. Estudios helmintologicos de la region oncocercosa de Mexico y de la Republica de Guatemala. Nematoda: 1a. parte. Filarioidea. I. Anales del Instituto de Biología, México *15:87–108.*

Causey, O. R. 1939. Description of three species of frog microfilariae, with notes on staining methods. American Journal of Hygiene 30(Ser. D):117–121.

Cowper, S. G. 1946. Some observations on a filaria, *Foleyella leiperi* (Railliet, 1916), of the North American leopard-frog. Annals of Tropical Medicine and Parasitology 39:119–124.

Esslinger, J. H. 1986. Redescription of *Ochoterenella digiticauda* Caballero, 1944 (Nematoda: Filarioidea) from the toad, *Bufo marinus,* with a re-

definition of the genus *Ochoterenella* Caballero, 1944. Proceedings of the Helminthological Society of Washington 53:210–217.

Kotcher, E. 1941. Studies on the development of frog filariae. American Journal of Hygiene 34(Sect. D): 36–64.

Ochoterena, I., and E. Caballero. 1932. Una nueva filaria parasita de las ranas. Anales del Instituto de Biología, Mexico 3:29–32.

Petit, G., and P. Yen. 1979. *Waltonella malayensis* n. sp., une nouvelle filaire de batracien, en Malaisie. Bulletin du Muséum National d'Histoire Naturelle, Paris 1, sect. A:213–218.

Schacher, J. F. 1975. *Waltonella,* nom. n. for subgenus *Waltonia* (Nematoda: Filarioidea) Schacher and Crans, 1973, preoccupied by *Waltonia* Davidson, 1850 (Brachiopoda). Journal of Parasitology 61:58.

———, **and W. J. Crans.** 1973. *Foleyella flexicauda* sp. n. (Nematoda: Filarioidea) from *Rana catesbeiana* in New Jersey, with a review of the genus and erection of two new subgenera. Journal of Parasitology 59:685–691.

Schmidt, G. D., and R. E. Kuntz. 1969. Nematode parasites of Oceanica. VI. *Foleyella confusa* sp. n., *Icosiella hoogstraali* sp. n. (Filarioidea), and other species from Philippine amphibians. Parasitology 59:885–889.

Sonin, M. D. 1968. Filariata of animals and man and diseases caused by them. Part 2. Diplotraenoidea. *In* K. I. Skrjabin, ed. Essentials of Nematology. Edition Nauka, Moscow. Translated from Russian, Israel Program for Scientific Translations, Jerusalem, 1975.

Walton, A. C. 1929. Studies on some nematodes of North American frogs. I. Journal of Parasitology 15:227–249.

———. 1935. The Nematoda as parasites of amphibia. II. Journal of Parasitology 2:27–50.

Wehr, E. E., and O. R. Causey. 1939. Two new nematodes (Filarioidea: Dipetalonematidae) from *Rana sphenocephala.* American Journal of Hygiene 30(Sect. D):65–68.

Witenberg, G., and C. Gerichter. 1944. The morphology and life history of *Foleyella duboisi* with remarks on allied filariids of amphibia. Journal of Parasitology 30:245–256.

Proc. Helminthol. Soc. Wash.
53(2), 1986, pp. 224–231

Ultrastructure of the Body Wall of Larval Stages of *Dirofilaria immitis* (Nematoda: Filarioidea) in the Mosquito Host

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ABSTRACT: Transmission electron microscopy is used to describe the morphogenesis of the cuticle, hypodermis, and musculature of first-, second-, and third-stage larvae of *Dirofilaria immitis* from the Malpighian tubules of the mosquito *Aedes aegypti*. The cuticle of first-stage larvae (L_1) consists of a trilaminate epicuticle and finely particulate exocuticle similar to that of microfilariae. The developing L_2 cuticle is evident by days 4–6 postingestion (PI) of an infective blood meal and consists of a thin striated band possessing occasional cuticular projections. This cuticle increases in thickness by days 8–10 PI when the L_3 cuticle begins to form. The L_3 cuticle at days 10–12 PI is composed of a trilaminate epicuticle, homogenous exocuticle, and dense fibrous layer. The hypodermis of L_1 consists of large undifferentiated cells. An increase in mitochondria, ribosomes, GER, and Golgi bodies is evident in the hypodermis as molting from L_1 to L_2 and L_2 to L_3 stages occurs. A thinning of the hypodermis and formation of hypodermal cords are present in the L_3. Development of myofilaments is evident at day 4 PI and numbers of myofilaments increase as molting occurs. Muscle cells of the L_3 consist of apical myofibrillar and basal myofibrillar portions.

Several ultrastructural studies on *Dirofilaria immitis* have been published, but only light microscopy has been used to evaluate larval development in the mosquito host. Of these light level studies, Taylor (1960) provided the most detailed account of *D. immitis* development in the vector. Recently, Lichtenfels et al. (1985) published a light microscopic description of the larval stages in the vertebrate host, and included an ultrastructural assessment of the body wall of the fourth-stage larva. Hendrix et al. (1984) examined the external morphology of third-stage larvae using scanning electron microscopy. Although Bradley et al. (1984) reported on the ultrastructural changes in the Malpighian tubules of *Aedes taeniorhynchus* at <1 to 48 hr following *D. immitis* infection, they did not assess the fine structure of developing larvae.

Ultrastructural studies on the cuticle of developing stages of filarial worms within the thoracic musculature of an arthropod vector include those of Weber (1984) on *Wuchereria bancrofti* and Collin (1971), Lehane (1978), and Tongu et al. (1978) on *Brugia pahangi*. The purpose of this study was to examine the ultrastructure of the body wall of *D. immitis* during its development in the Malpighian tubules of the mosquito *Aedes aegypti*.

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Materials and Methods

The black-eyed Liverpool strain of *A. aegypti* used in this study was from a laboratory colony originally obtained from the University of London in 1977. Mosquito rearing followed procedures previously described (Christensen and Sutherland, 1984). Four- to 6-day-old mosquitoes were exposed to either an infected (>500 microfilariae/20 μl blood) or uninfected beagle and then maintained on 0.3 M sucrose in an environmental chamber at 26.5 ± 1°C, 75 ± 10% RH, and under a 16-hr photoperiod. Dogs were anesthetized with a Ketamine-HCl/Rompun mixture before blood feeding mosquitoes. The infected dog was obtained from J. W. McCall, University of Georgia, through a program supported by the U.S.–Japan Cooperative Medical Sciences Program—NIAID.

Both infected and uninfected mosquitoes were dissected at 2, 4, 6, 8, 10, and 12 days postingestion (PI) of a blood meal, and Malpighian tubules were immediately fixed in Karnovsky's fixative (4% formalin and 6% glutaraldehyde in sodium cacodylate buffer) overnight. Specimens were then rinsed in buffer, postfixed in 1% osmium tetroxide in cacodylate buffer, dehydrated in a graded ethanol/propylene oxide series, and embedded in Poly-bed 812 (Polysciences, Warrington, Pennsylvania). Sections cut with a diamond knife were stained with uranyl magnesium acetate and lead citrate and examined with a Hitachi HS-9 transmission electron microscope.

Terminology describing the cuticle is after Maggenti (1979).

Results

The body wall of larval stages of *Dirofilaria immitis* consists of the outer cuticle, the underlying hypodermis, and muscle cells.

Cuticle

The first-stage larva (L_1) of *D. immitis* at 2 days postingestion (PI) of an infective blood meal possessed a double-layered cuticle approximately 100 nm in thickness. The epicuticle consisted of a trilaminate membrane, 15 nm in thickness, and an exocuticle, 85 nm thick, was composed of a uniformly stained, finely particulate material (Fig. 1). In sagittal section, the epicuticle was divided into annulations which averaged 500 nm in length (Fig. 2).

At days 4–6 PI, the cuticle of the L_1 had increased to 150 nm in thickness and was composed of the L_1 cuticle which had separated from the hypodermis and the newly forming L_2 cuticle which was evident above the hypodermal membrane. The L_1 epicuticle remained trilaminate (Fig. 3), but the L_1 exocuticle was beginning to thin and light areas were apparent between the L_1 exocuticle and newly forming L_2 cuticle (Fig. 4). The new L_2 cuticle appeared as a striated band when cut in sagittal section, which rested directly on the ridges of the underlying hypodermal cells (Fig. 5).

By days 6–8 PI the L_1 cuticle had been ecdysed. The new L_2 cuticle was 30–50 nm thick and consisted of a thin epicuticle (Fig. 6) and a striated exocuticle (Fig. 7). In some sections, the L_2 cuticle possessed thick projections approximately 400 nm in height (Figs. 6, 8).

At days 8–10 PI, an L_3 cuticle was beginning to form beneath the L_2 cuticle. The L_3 epicuticle appeared as a densely stained line beneath the L_2 exocuticle, and a lightly stained newly forming L_3 cuticle rested directly upon the hypodermis (Fig. 8).

In the molting L_2 at days 8–10 PI, the L_3 cuticle possessed a fibrous appearance and measured 200 nm in thickness (Fig. 9). The thin L_3 epicuticle separated the L_2 and L_3 cuticles. Numerous thin dense structures, possibly remnants of the molted L_1 epicuticle, were present above some worms (Fig. 9).

By days 10–12 PI, the L_2 cuticle had been shed; the remaining L_3 cuticle (Fig. 10), 260 nm in thickness, consisted of a thin trilaminate epicuticle resting on a lightly stained homogenous exocuticle and dense basal fibrous layer.

Hypodermis and muscle cells

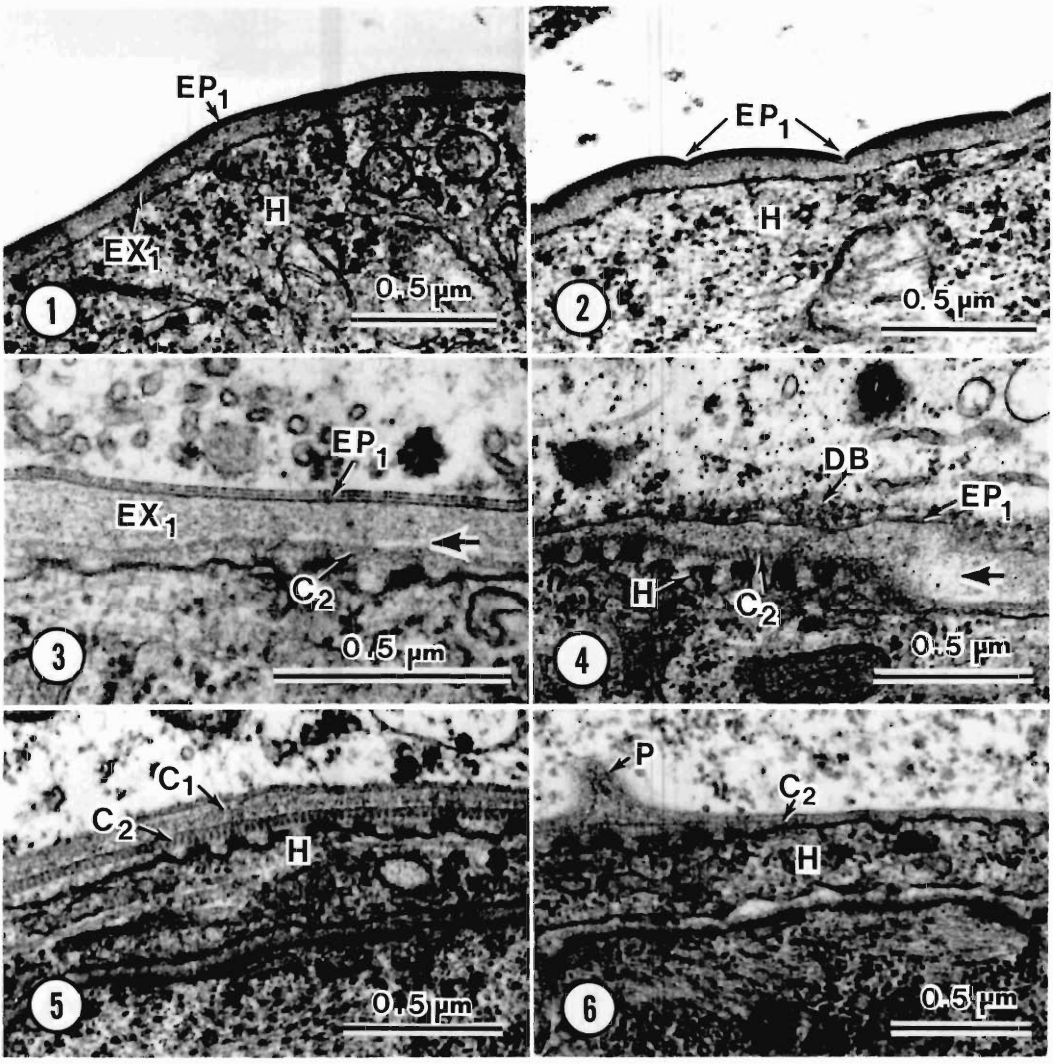
Hypodermal cells of the L_1 at days 2–4 PI each contain a large circular nucleus with dense central nucleolus and an undifferentiated cytoplasm containing a few mitochondria and ribosomes (Fig. 11). By days 4–6 PI numerous ribosomes, granular endoplasmic reticulum (GER), and lysosomes were observed in the hypodermis as worms were molting from the L_1 to L_2 stage (Fig. 12). Body wall musculature was evident in a larva fixed on day 4 PI, and thick and thin myofilaments were present (Fig. 12). Many Golgi bodies and mitochondria were observed in the hypodermis of larvae at days 6–8 PI (Fig. 13). By days 8–10 PI the hypodermal cytoplasm of L_2 worms was compressed between the cuticle and developing muscle cells (Fig. 14). The hypodermal membrane at the cuticle–hypodermis interface was ridged, and large deposits of α-glycogen occurred in the hypodermal cytoplasm (Fig. 14). By days 10–12 PI the hypodermis of the L_3 consisted of a narrow band of cytoplasm between the cuticle and muscle cells. Hypodermal nuclei were concentrated in expanded areas between the muscle cells to form hypodermal cords (Fig. 15). Muscle cells of the L_3 contain a greater number of myofilaments and were differentiated into apical myofibrillar and basal amyofibrillar portions. Occasional lipid inclusions were present in the basal portion of these muscle cells (Fig. 15).

Discussion

According to Lumsden (1975), the nematode cuticle has 3 primary functions: (1) it serves as a protective barrier from outside elements, (2) it (and the hypodermal membrane) is differentially permeable and regulates the passage of substances into and out of the worm, and (3) it is an antagonist to the underlying somatic muscles during movement.

The basic structure of the cuticle of all nematodes is similar (Lumsden, 1975). According to the terminology used by Maggenti (1979), the cuticle consists of an outer trilaminate epicuticle, an exocuticle and underlying fibrous layer.

The cuticle of the L_1 of *D. immitis* resembles that of the microfilaria from the bloodstream of the definitive host as described by Johnson and Bemrick (1969), Kozek (1971), and McLaren (1972). There are annulations in the epicuticle, and the trilaminate structure is present. However, the exocuticle, which appears as a fibrous region in the microfilaria, is composed of a finely particulate substance. Our observations concur with reports by Lehane (1978) and Weber (1984)

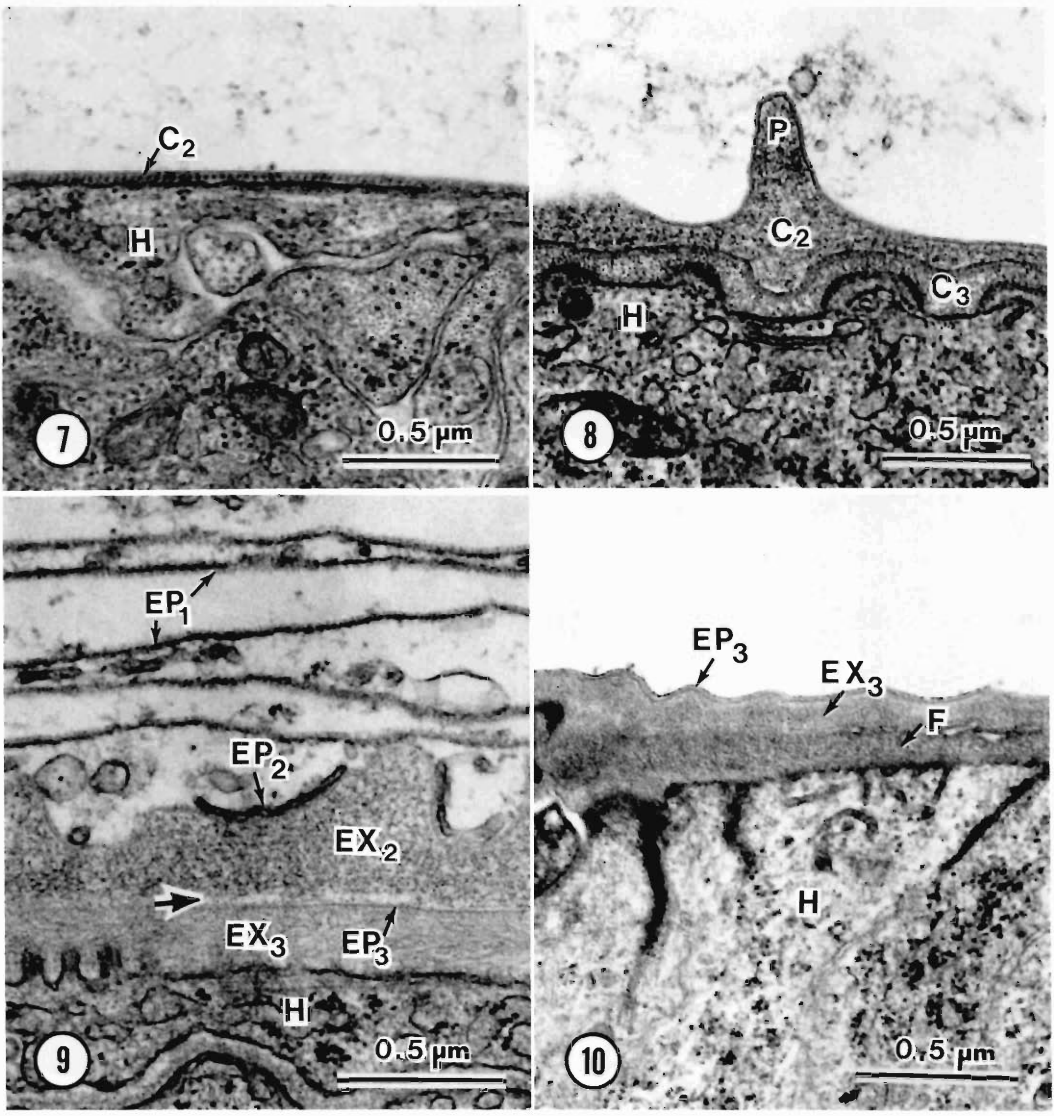


Figures 1-6. Transmission electron microscopy of the cuticle of larval *Dirofilaria immitis*. 1. Cross section of cuticle of first-stage larva (L_1) at 2 days PI showing epicuticle (EP_1), exocuticle (EX_1), and hypodermis (H). 2. Sagittal section showing annulations in L_1 epicuticle (EP_1). 3. Molting L_1 cuticle at days 4-6 PI is composed of trilaminate epicuticle (EP_1) and finely particulate exocuticle (EX_1). Note light areas (arrow) above newly forming L_2 cuticle (C_2). 4. Molting L_1 at days 4-6 PI showing concentration of debris (DB) on surface of L_1 epicuticle (EP_1) and light area (arrow) where L_1 exocuticle is separating from underlying hypodermis and new cuticle. Note appearance of the striated L_2 cuticle (C_2) above the folded hypodermal membrane (H). 5. Cuticle of molting L_1 at days 4-6 PI. Note L_1 cuticle (C_1) and striated layer of L_2 cuticle (C_2) forming above hypodermis (H). 6. Cross section of L_2 cuticle at days 6-8 PI. Cuticle (C_2) is very thin and possesses occasional projections (P).

in studies of the cuticle of other first-stage filarial worms.

At days 4–6 and 8–10 PI, juveniles begin to molt from L_1 to L_2 and L_2 to L_3, respectively. According to the model of nematode molting proposed by Bird and Rogers (1965), the new cuticle is secreted by the hypodermis at the same time the old cuticle is being reabsorbed into the new cuticle. In the reabsorption process, the fibrous layer breaks down immediately adjacent to the new epicuticle. Eventually, the fibrous layer and the exocuticle are reabsorbed, and the epicuticle is shed.

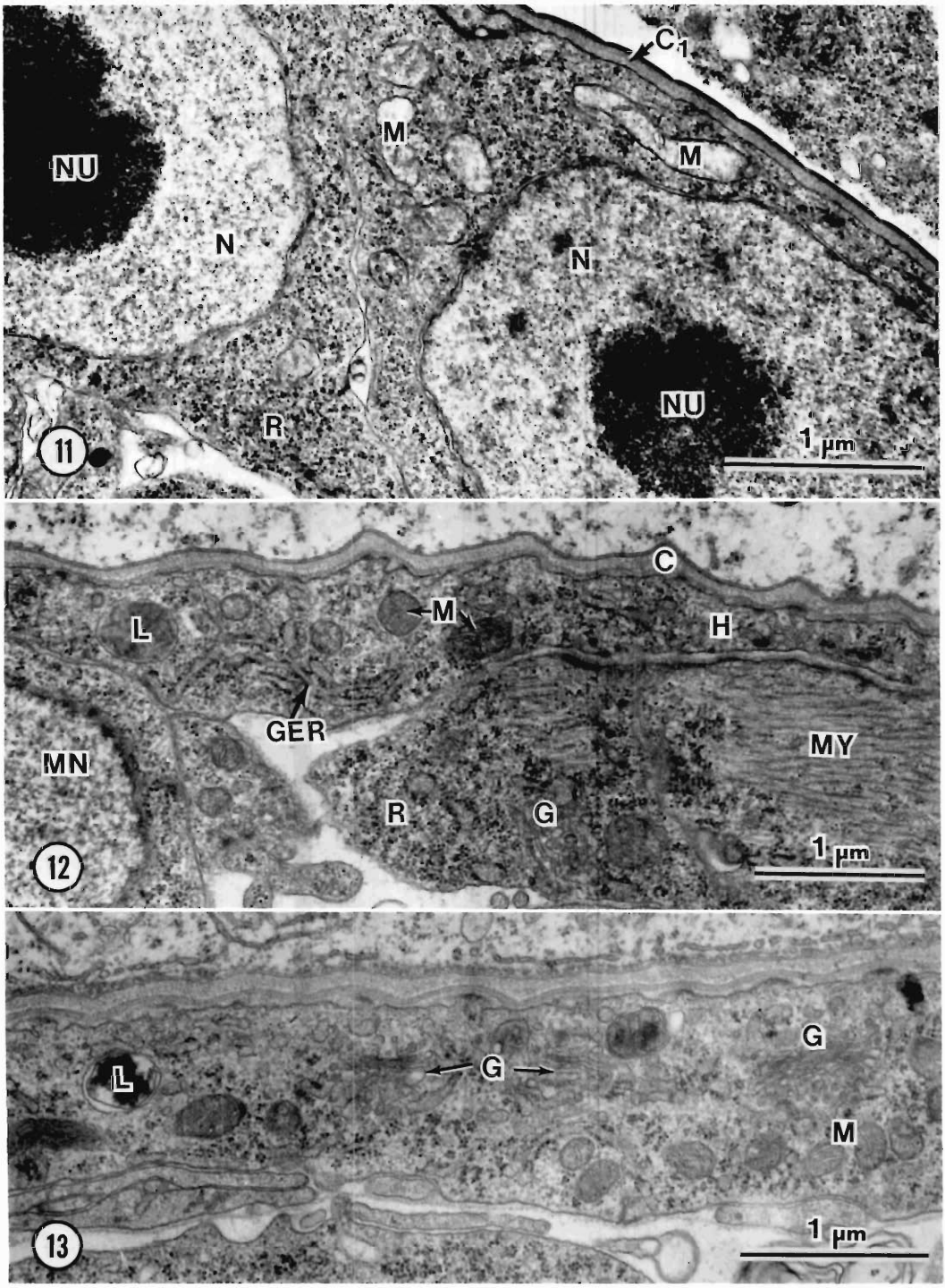
The exocuticle of *D. immitis* on days 4–6 and



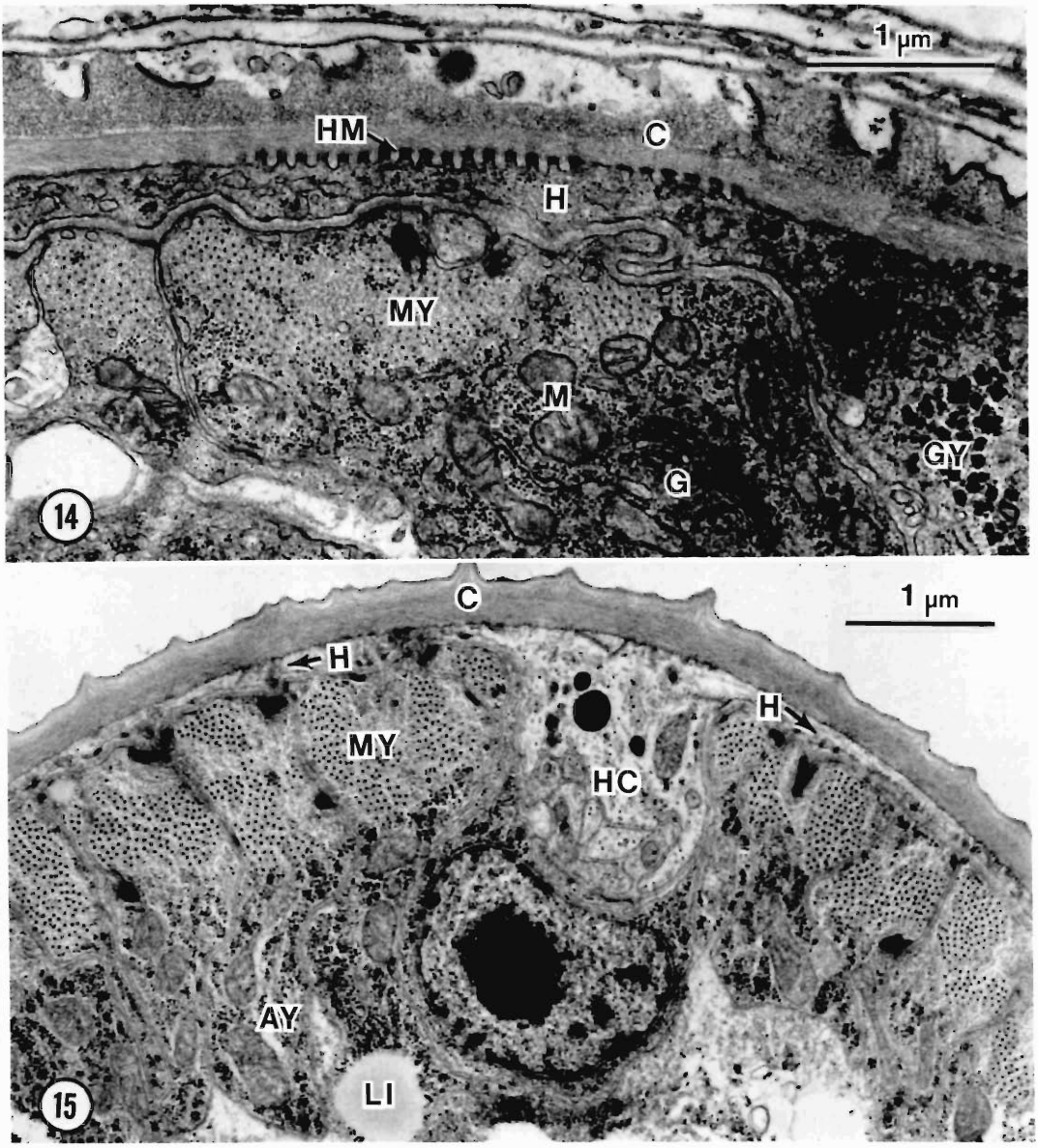
Figures 7–10. Transmission electron microscopy of the cuticle of larval *Dirofilaria immitis*. 7. Sagittal section showing striated appearance of thin L_2 cuticle (C_2). 8. Cuticle of L_2 at days 8–10 PI showing development of L_3 cuticle (C_3) between L_2 cuticle (C_2) and hypodermis (H). A cuticular projection (P) is formed by L_2 cuticle. 9. Molting L_2 at days 8–10 PI showing light areas (arrow) separating L_2 exocuticle (EX_2) and thin L_3 epicuticle (EP_3). Structures above worm are wrinkled remnants of the shed L_1 epicuticle (EP_1). 10. L_3 cuticle at days 10–12 PI showing epicuticle (EP_3), finely particulate exocuticle (EX_3), and densely stained fibrous region (F).

8–10 PI appears to be separating from the hypodermis and is broken down to allow for formation of the new cuticle. Breaks between the L_2 exocuticle and L_3 epicuticle are evident where the cuticle is being reabsorbed. Below the fibrous layer, the L_3 cuticle is being formed by the hypodermis and appears as a striated band. The epicuticle does not break down but is shed. Remnants of epicuticle from the previous molt could be observed above an L_2 on day 8 PI. Several layers are present because the shed epicuticle has wrinkled and the section was cut through these layers.

The cuticle of the L_2 is much thinner than the L_1 cuticle. This is consistent with observations made by Tongu et al. (1978), Lehane (1978), and



Figures 11-13. Transmission electron microscopy of the hypodermis and muscle of larval *Dirofilaria immitis*. 11. Hypodermal cells of L_1 at days 2-4 PI. Note large nucleus (N) with central nucleus (NU) and cytoplasm with mitochrondria (M) and ribosomes (R). 12. Hypodermis (H) at days 4-6 PI containing granular endoplasmic reticulum (GER), free ribosomes, mitochrondria (M), and lysosomes (L) is a thin layer between cuticle (C) and



Figures 14, 15. Transmission electron microscopy of the hypodermis and muscle of larval *Dirofilaria immitis*. 14. By days 8-10 PI hypodermis (H) of molting L_2 is compressed against cuticle (C). Foldings of hypodermal membrane (HM) are evident. Areas of α-glycogen (GY) occur in hypodermis. Note myofilaments (MY), Golgi body (G), and mitochondria (M) in developing muscle cell. 15. Body wall of L_3 at days 10-12 PI consists of cuticle (C), thin hypodermis (H), hypodermal cord (HC), and muscle cells. Note apical myofibrillar region (MY) and basal amyofibrillar region (AY) with lipid inclusions (LI) of muscle cells.

←

developing muscle cells. Note nucleus (MN), thick and thin myofilaments (MY), ribosomes (R), and Golgi body (G) in developing muscle cell. 13. Golgi bodies (G), mitochrondria (M), and lysosome (L) in hypodermis at days 6-8 PI.

Weber (1984) of larval stages of other filarial worms. The thin cuticle at this stage is attributed to the sedentary existence of the worm within a protective intracellular environment (Weber, 1984). Collin (1971) has suggested that the alimentary tract of the larval stages of *Brugia pahangi* is not functional because of occlusion at the esophagointestinal junction. If the same condition exists in the larval stages of *D. immitis,* the thin cuticle may facilitate the absorption of nutrients (Tongu et al., 1978).

The cuticle of the molting L_2 at days 8–10 PI has increased in thickness compared to the thin cuticle of the L_2 at day 6 PI. The L_2 cuticle at day 6 PI was only 30 nm in thickness compared to a thickness of 210 nm in some molting L_2 at day 10 PI. The reasons for this increase in thickness are not known.

The cuticle of the L_3 of *D. immitis* is increased in thickness and resembles the thick protective cuticle of adult filarial worms (Vincent et al., 1975; Franz, 1980, 1982; Franz and Buttner, 1983). The cuticle is composed of a trilaminate epicuticle, a lightly stained exocuticle, and thick fibrous layer.

Weber (1984) described the formation of the hypodermis and muscle cells of juvenile stages of *Wuchereria bancrofti.* In the L_1, the 4 hypodermal cells are large with an abundance of cytoplasmic organelles. As the worm develops, the hypodermal cells increase in volume and number and form characteristic hypodermal cords. The region of the cell containing the nucleus extends between muscle cells in 4 areas dividing the worm into quadrants. Muscle cells of the L_1 are small but begin to multiply, possibly by fission of germ cells (Weber, 1984). As the muscle cells increase in number, myofilaments become more abundant and the cells differentiate into myofibrillar and amyofibrillar portions.

Our observations of hypodermal cells were limited. Serial cross sections of larvae must be obtained to properly observe the structure of hypodermal cells. Serial section analysis was not possible because of the random orientation of larvae within Malpighian tubules. However, our observations of the development of the hypodermis and muscle cells suggest a progression similar to that observed by Weber (1984) in *Wuchereria bancrofti.* Large hypodermal cells are evident in the L_1. In the L_2, hypodermal cells appear to compress against the cuticle. In L_3 the hypodermis appears as a thin layer beneath most of the cuticle except in areas of the hypodermal cords. In some nematodes the hypodermis has been described as being multinucleate (Lehane, 1978; Weber, 1984); however, no determination of a multinucleate condition was made in this study of *D. immitis.*

Throughout the development of the larval stages, hypodermal cells contain numerous mitochondria, ribosomes, GER, and Golgi bodies, all of which indicate high levels of synthetic activity involving cuticle formation, and lysosomes, which may be involved in the breakdown of portions of the old cuticle. Foldings of the outer hypodermal membrane were observed in late L_1 and L_2 stages. These infoldings may serve to allow for expansion of the worm after molting.

Formation of myofilaments is evident in later stages of L_1. In L_2 worms at days 8–10 PI, muscle cells are well developed with thick and thin myofilaments present along the apical surface of each muscle cell. However, cells have not yet differentiated into myofibrillar and amyofibrillar portions. In the L_3 the muscle cells are present beneath the compressed hypodermis and are separated by the hypodermal cords. Muscle cells are composed of an apical myofibrillar and amyofibrillar portion and resemble those of adult *D. immitis* as observed by Lee and Miller (1967), and also those of other larval filarial worms (Collin, 1971; Weber, 1984).

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Literature Cited

Bird, A. F., and G. E. Rogers. 1965. Ultrastructure of the cuticle and its formation in *Meloidogyne javanica.* Nematologica 11:224–230.

Bradley, T. J., D. M. Sauerman, and J. K. Nayer. 1984. Early cellular responses in the mosquito *Aedes taeniorhynchus* to infection with *Dirofilaria immitis.* Journal of Parasitology 70:82–88.

Christensen, B. L., and D. R. Sutherland. 1984. *Brugia pahangi*: exsheathment and midgut penetration in *Aedes aegypti.* Transactions of the American Microscopical Society 103:423–433.

Collin, W. K. 1971. Ultrastructural morphology of the infective larvae of *Brugia pahangi* (Nematoda: Filarioidea). Journal of Parasitology 57:449–468.

Franz, M. 1980. Electron microscope study of the cuticle of male and female *Onchocerca volvulus* from various geographic areas. Tropenmedizin und Parasitologie 31:149–164.

———. 1982. The fine structure of adult *Onchocerca volvulus*. I. The cuticle, the hypodermis and the muscle cell of the male worm. Tropenmedizin und Parasitologie 33:69–75.

———, **and D. Büttner.** 1983. The fine structure of adult *Onchocerca volvulus*. III. The cuticle, the interchordal hypodermis and the muscle cells of the female worm. Tropenmedizin und Parasitologie 34:61–69.

Hendrix, C. M., M. J. Wagner, W. J. Bemrick, J. C. Schlotthauer, and B. E. Stromberg. 1984. A scanning electron microscope study of the third-stage larvae of *Dirofilaria immitis*. Journal of Parasitology 70:149–151.

Johnson, K. H., and W. J. Bemrick. 1969. *Dirofilaria immitis* microfilaria: EM examination of cuticle and muscle cells. American Journal of Veterinary Research 30:1443–1450.

Kozek, W. J. 1971. Ultrastructure of the microfilariae of *Dirofilaria immitis*. Journal of Parasitology 57:1052–1067.

Lee, C. C., and J. H. Miller. 1967. Fine structure of *Dirofilaria immitis* body-wall musculature. Experimental Parasitology 20:334–344.

Lehane, M. J. 1978. The first-stage larvae of *Brugia pahangi* in *Aedes togoi*: an ultrastructural study. International Journal for Parasitology 8:202–218.

Lichtenfels, J. R., P. A. Pilitt, T. Kotani, and K. G. Powers. 1985. Morphogenesis of developmental stages of *Dirofilaria immitis* (Nematoda) in the dog. Proceedings of the Helminthological Society of Washington 52:98–113.

Lumsden, R. D. 1975. Surface ultrastructure and cytochemistry of parasitic helminths. Experimental Parasitology 37:267–339.

Maggenti, A. R. 1979. The role of cuticular strata nomenclature in the systematics of Nemata. Journal of Nematology 11:94–98.

McLaren, D. J. 1972. Ultrastructural studies on microfilariae (Nematoda: Filarioidea). Parasitology 65:317–332.

Taylor, A. E. R. 1960. The development of *Dirofilaria immitis* in the mosquito, *Aedes aegypti*. Journal of Helminthology 34:27–38.

Tongu, Y., A. L. Vincent, and L. R. Ash. 1978. The ultrastructure of early larval morphogenesis in *Brugia pahangi* (Nematoda: Filarioidea). Japanese Journal of Parasitology 27:245–260.

Vincent, A. L., L. R. Ash, and S. P. Frommes. 1975. The ultrastructure of adult *Brugia malayi* (Brug, 1927) (Nematoda: Filarioidea). Journal of Parasitology 61:499–512.

Weber, P. 1984. Electron microscope study on the developmental stages of *Wuchereria bancrofti* in the intermediate host: structure of the body wall. Tropenmedizin und Parasitologie 35:221–230.

PROPOSED MEETING SCHEDULE HELMINTHOLOGICAL SOCIETY OF WASHINGTON 1986-1987

(Wed) 15 Oct 1986	Uniformed Services University of the Health Sciences, Bethesda, MD (with Oxford Biological Laboratory)
(Wed) 12 Nov 1986	Animal Parasitology Institute, U.S. Department of Agriculture, Beltsville, MD
(Wed) 10 Dec 1986	Plant Protection Institute, U.S. Department of Agriculture, Beltsville, MD
(Wed) 14 Jan 1987	National Institutes of Health, Bethesda, MD
(Wed) 11 Feb 1987	Naval Medical Research Institute, Bethesda, MD (with Food and Drug Administration)
(Wed) 18 Mar 1987	Walter Reed Army Institute of Research, Washington, DC (with Armed Forces Institute of Pathology)
(Wed) 15 Apr 1987	Johns Hopkins University, Baltimore, MD
(Sat) 9 May 1987	University of Pennsylvania, New Bolton Center, PA

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53(2), 1986, pp. 232–236

Rhabditis myriophila sp. n. (Rhabditidae: Rhabditida), Associated with the Millipede, *Oxidis gracilis* (Polydesmida: Diplopoda)

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ABSTRACT: A new species, *Rhabditis myriophila* (Rhabditidae: Rhabditida), is described as an associate of the garden millipede, *Oxidis gracilis* (Polydesmida: Diplopoda). This hermaphrodite can be distinguished from other members of the genus *Rhabditis* by the structure of the spicules and bursa and the size of the stoma, metacorpus, tail, and rectum. The dauer juveniles of *R. myriophila* enter the alimentary tract and body cavity of the host and can develop to fourth-stage juveniles in both locations. Reproduction only occurs when the millipede dies and becomes colonized by bacteria.

During routine dissections of the garden millipede, *Oxidis gracilis,* one species of rhabditoid nematode was consistently found inside the gut lumen, Malpighian tubules and hemocoel of the host. This nematode matured in living millipedes but only reproduced on dead hosts. It could be cultured on nutrient agar plates containing bacteria isolated from dead or dying millipedes.

The nematode was an unknown species of *Rhabditis* and is described below, along with observations on the nature of its association with *O. gracilis.*

Materials and Methods

Millipedes, *Oxidis gracilis* (Koch) (Polydesmida: Paradoxosomatidae), were collected and sent to the author by Gary Phipps of the Monrovia Nursery in Azusa, California. They were maintained in a plastic shoe box containing sterile damp soil and lettuce as a food source. Live millipedes were washed and dissected for rhabditoid nematode associates. During dissections, the entire alimentary tract was first removed by severing the head and then pulling on the posterior 2 segments. The removed gut and remaining body column were examined separately for associated nematodes. The numbers, stages, and location of nematodes were noted. These collected nematodes, together with reproducing stages recovered from millipedes that had died in the container, were placed on nutrient agar plates for later systematic studies.

All nematodes recovered from both living and dead millipedes belonged to the same species and were protandrous hermaphrodites. When they were cultivated at room temperature (20°C) on nutrient agar plates with a mixed population of bacteria, males appeared at a ratio of 250 hermaphrodites to 1 male.

When the colonies were placed at a higher temperature (32°C), males appeared at a ratio of about 100 hermaphrodites to 1 male. It is presumed that the higher temperature increased the rate of nondisjunction of the sex chromosome. In order to obtain a still higher ratio of males for taxonomic studies, 3 males obtained from the heat-treated plates were removed and placed with a single hermaphrodite on a nutrient agar plate at 20°C. The males mated with the hermaphrodite and the resultant F_1 population consisted of a 1:1 ratio (equal males and hermaphrodites).

Nematodes were fixed in TAF and processed to glycerin for identification and characterization.

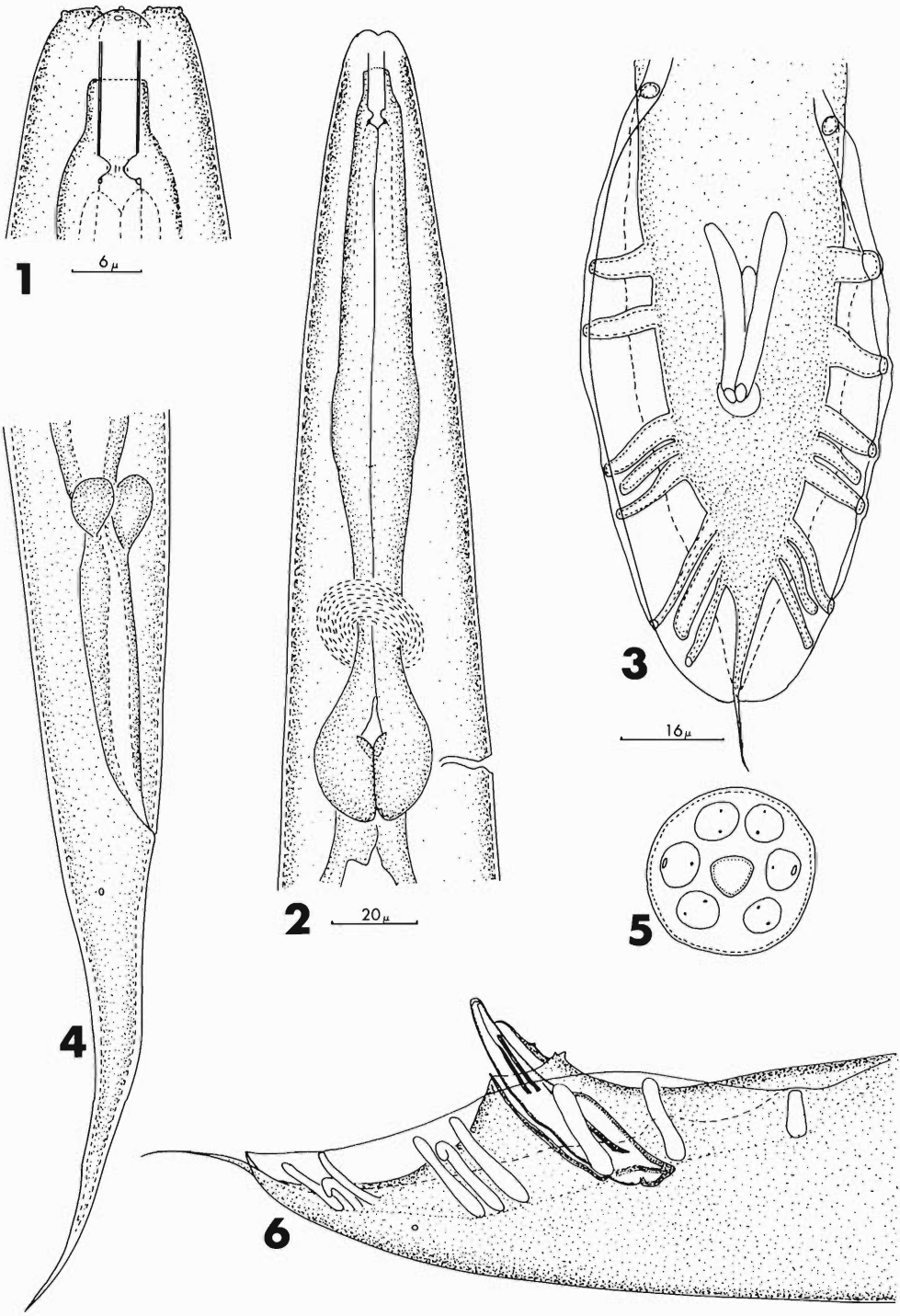
Results

The nematodes removed from both dead and living specimens of *O. gracilis* belonged to the same unknown species and are described below. All measurements are given in micrometers unless otherwise specified. The number following the character is the average value and the figures in parentheses represent the range of the characters.

Rhabditis myriophila sp. n. (Figs. 1-6)

Rhabditida Chitwood, 1933; Rhabditina Chitwood, 1933; Rhabditoidea (Örley, 1880) Travassos, 1920; Rhabditidae Örley, 1880; *Rhabditis* Dujardin, 1845 as defined by Andrássy (1983).

GENERAL CHARACTERISTICS: Cuticle smooth, with fine longitudinal striations. Head continuous with neck. Lips closed, partially fused, each containing 1 inner labial papilla and another outer labial papilla except for lateral lips that contain elliptical-shaped amphidial openings. Stoma cylindrical, distinct, cheilorhabdions not noticeably cuticularized, metarhabdions isomorphic, each bearing 2–3 minute tubercles, pharyngeal collar present. Corpus cylindrical, slightly enlarged to form a median bulb; isthmus surrounded by nerve ring. Basal bulb large, with well-formed valve plates. Excretory pore in adults opening at the vicinity of the basal bulb; phasmids distinct.



Figures 1-6. ***Rhabditis myriophila.*** **1. Lateral view of hermaphrodite head. 2. Lateral view of hermaphrodite pharyngeal region. 3. Ventral view of male tail. 4. Lateral view of hermaphrodite tail (mag. same as Fig. 2). 5. En face view of male (mag. same as Fig. 1). 6. Lateral view of male tail (mag. same as Fig. 3).**

HERMAPHRODITES ($N = 10$): Length, 1.32 (1.20–1.50) mm; greatest width, 62 (57–70); length of stoma, 20 (18–21); width of stoma, 3.2; head width, 10 (9–13); distance from head to excretory pore, 179 (165–190); distance from head to nerve ring, 139 (126–146); length of pharynx, 185 (174–193); length of tail, 117 (108–135); width at anus 25 (22–28); % vulva, 50 (49–51); length of eggs (in situ), 60 (56–72); width of eggs (in situ), 32 (25–40); reproductive system amphidelphic, ovaries reflexed; length of rectum, 79 (56–96); tail conical, tapering to a fine point. a = 19.1–21.3; b = 6.8–7.7; c = 2.2–3.5. Ratio of length of rectum to anal width, 2.2–3.5; ratio of length of tail to anal width, 4.2–5.3.

MALES ($N = 10$): Length, 1.27 (0.83–1.47) mm; greatest width, 63 (38–80); length of stoma, 17 (16–19); width of stoma, 3.2; head width, 11 (9–13); distance from head to excretory pore, 198 (149–229); distance from head to nerve ring, 150 (117–165); length of pharynx 187 (161–200); reflexion of testis, 191 (101–228); length of tail, 65 (56–72); body width at cloacal opening, 39 (32–45). Bursa open, leptoderan (Andrássy [1983] describes this condition as pseudopeloderan because the portion of the tail that protrudes outside of the bursa is small and fragile, measuring 17 (12–19) in length). The bursa is 105 (84–117) long and composed of a double membrane which encloses the bursal papillae. A shorter membrane or fold reaches slightly less than half way up the bursal papillae (represented by a dotted line in Fig. 3). Nine pairs of bursal papillae are present, 3 pairs precloacal and 6 pairs postcloacal. The 3 precloacal pairs are separate, although pair numbers 2 and 3 are closer together than pairs 1 and 2. Pairs 4, 5, and 6 form 1 group and pairs 7, 8, and 9 form the second postcloacal group. The tips of the fifth and eighth pairs are turned outwards. The spicules are paired and separate. They are slightly curved and have a triangular head and a rounded tip. Three longitudinal ribs occur on the outer surface of each spicule. The length and width of the spicules are 47 (32–54) and 10 (8–11), respectively. The gubernaculum is dorsal-ventrally flattened and follows the contour of the spicules. The proximal tip is curved upward. The length and width of the gubernaculum are 28 (19–32) and 0.87 (0.64–1.20), respectively. a = 18.4–21.9; b = 5.16–7.36; c = 14.8–20.4.

DAUER JUVENILES ($N = 6$): Length, 564 (504–611); greatest width, 23 (19–26); distance from the head to the excretory pore, 107 (97–114); distance from the head to the nerve ring, 89 (83–96); length of pharynx, 129 (120–136); length of tail, 78 (75–80); width at anus, 15 (14–16). This stage is the third-stage juvenile enclosed in a second-stage cuticle that surrounds the nematode like a sheath.

TYPE LOCALITY: The Monrovia Nursery in Azusa, California.

TYPE LOCATION: Found in the intestine and body cavity of the garden millipede, *Oxidis gracilis*. This species was originally introduced into North America from Southeast Asia.

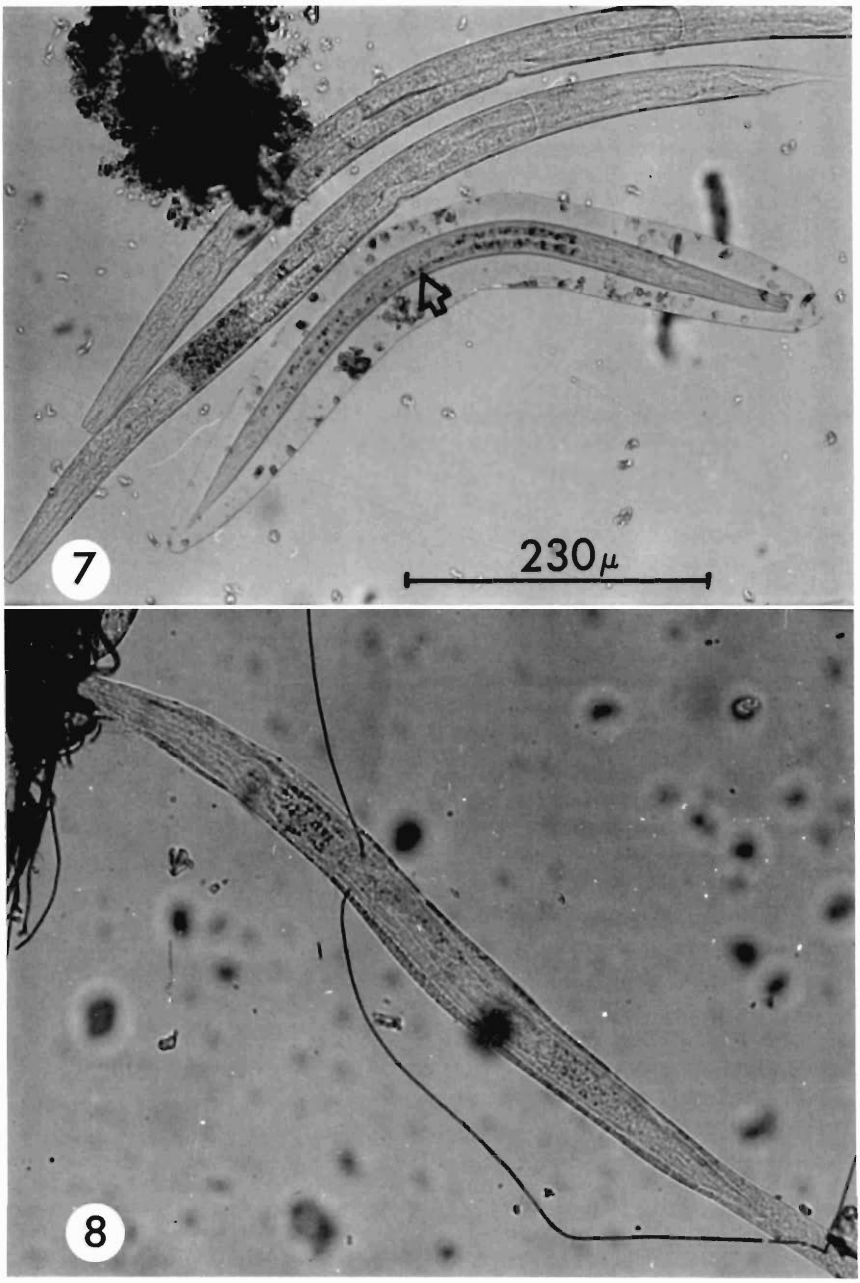
TYPE SPECIMEN: Holotype (female) and allotype (male) deposited in the nematology collection at the University of California, Davis.

DIAGNOSIS: The well-developed stoma and open bursa places *R. myriophila* in the genus *Rhabditis* sensu Andrássy, 1983. The pseudopeloderan bursa, three pairs of precloacal bursal papillae, long stoma (1.5 or more times longer than the head diameter), long female tail (between 4 and 8 anal body diameters long), and female rectum between 2.2 and 3.5 anal body diameters long separate this species from all existing members of *Rhabditis* except *R. wohlgemuthi* Völk, 1950. However, the latter species possesses a more distinct median pharyngeal bulb than in *R. myriophila*, a shorter pharyngeal collar covering half or less of the stoma (always more than half in *R. myriophila*), a shorter stoma length (2.5 times longer than wide) than *R. myriophila* (5.0–6.0 times longer than wide), and has all bursal papillae reaching the rim of the bursa (the fifth and eighth bursal papillae clearly do not reach the rim in *R. myriophila*). In addition, *R. wohlgemuthi* is an amphimictic species whereas *R. myriophila* is hermaphroditic.

HOST RELATIONSHIP: The dauer juveniles of *R. myriophila* enter the alimentary tract and body cavity of developing millipedes. Inside the gut and body cavity the dauers may exsheath and develop to fourth-stage hermaphrodites (Fig. 7). They remain very active, and may enter the Malpighian tubules of the millipede (Fig. 8). These fourth-stage juveniles never complete the final molt to adults in association with a living millipede. Further development into egg-laying hermaphrodites occurs only when the host dies and the body is colonized by bacteria.

Discussion

The association between *R. myriophila* and the millipede, *O. gracilis*, is similar to that of



Figures 7, 8. ***Rhabditis myriophila*** **in association with** ***Oxidis gracilis*****. 7. Juvenile nematodes removed from the millipede's alimentary tract. Arrow shows dauer juvenile. The other 2 are fourth-stage juveniles. 8. A fourth-stage juvenile inside a Malpighian tubule of the millipede.**

Rhabditis pellio (Schneider) and earthworms (Poinar and Thomas, 1975). The dauer stages of both nematodes enter the host's external openings (the excretory system with *R. pellio* and alimentary tract with *R. myriophila*) and body cavity, but they are unable to multiply until the host succumbs and is invaded by bacteria. Studies showed that the dauer juveniles of *R. myriophila* develop only to fourth-stage juveniles in the host's hemolymph. Bacteria are apparently required as

a food source. Encapsulated juveniles of *R. myriophila* were sometimes found in the body cavity of living millipedes. These dead nematodes appeared to be subjected to the same defense reactions reported earlier in this host against entomophagous rhabditoid nematodes (Poinar and Thomas, 1985). However, most nematodes found in the body cavity of *O. gracilis* were not encapsulated; thus the millipedes' defense system may be less efficient than the brown bodies formed in earthworms against *R. pellio* as reported by Poinar and Hess (1977). Similar to other autotokous rhabditids, *R. myriophila* is a protandric hermaphrodite, with males occurring rarely. In the hermaphrodite, sperm are produced in the fourth-stage juvenile and ova are produced after the final molt. Both gametes are produced in the syngonic gonad, the ovotestis.

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Literature Cited

Andrássy, I. 1983. A taxonomic review of the suborder Rhabditina (Nematoda: Secernentia). ORSTOM, Paris. 241 pp.

Poinar, Jr., G. O., and Roberta Hess. 1977. Immune responses in the earthworm, *Aporrectodea trapezoides* (Annelida) against *Rhabditis pellio* (Nematoda). Pages 69–84 *in* L. A. Bulla, Jr. and T. C. Cheng, eds. Comparative Pathology. Plenum Press, New York.

———, **and G. M. Thomas.** 1975. *Rhabditis pellio* (Schneider) from the earthworm *Aporrectodea trapezoides* Dugés (Annelida). Journal of Nematology 7:374–379.

———, **and** ———. 1985. Effect of neoaplectanid and heterorhabditid nematodes (Nematoda: Rhabditoidea) on the millipede *Oxidis gracilis*. Journal of Invertebrate Pathology 45:231–235.

Völk, J. 1950. Die Nematoden der Regenwürmer und aasbesuchenden Käfer. Zoologisch Jahrbuch für Systematiks 79:1–70.

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Predacious Activity of the Nematode-destroying Fungus, *Arthrobotrys oligospora*, on Preparasitic Larvae of *Cooperia oncophora* and on Soil Nematodes

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ABSTRACT: Comparisons were made among larval stages of *Cooperia oncophora* and the soil nematodes *Rhabditis wohlgemuthi* and *Panagrellus redivivus* in their abilities to induce the nematode-destroying fungus *Arthrobotrys oligospora* Fres. to form traps in vitro. No difference in the potential of the various nematodes to induce fungal traps were found. Regardless of the nematode species or stage, the fungus trapped with same efficiency. In all tests the soil nematodes and first- and second-stage *C. oncophora* larvae were immobilized and killed soon after entrapments. However, the third-stage *C. oncophora* larvae continued to struggle vigorously in the traps for more than 20 hours. This is explained possibly by the inability of the fungus to penetrate the nematode's outer cuticle from the previous molt.

The nematode-destroying fungus, *Arthrobotrys oligospora* Fres, is possibly the most commonly encountered of the predacious fungi in Danish agricultural soils (Shepherd, 1961). To ensnare nematodes the predacious fungi must develop the characteristic organs of capture, which in the case of *A. oligospora* are three-dimensional networks of sticky hyphal anastomosing loops.

Undoubtedly, the capturing devices of the predacious fungi have primarily evolved to trap soil nematodes, but it has been demonstrated that some species and stages of animal-parasitic nematodes may be effectively trapped as well (Descazeaux, 1939; Roubaud and Deschiens, 1941; Soprunov, 1958; Pandey, 1973; Grønvold et al., 1985; Gruner et al., 1985). This is perhaps not surprising in the light of prevailing views on the evolution of parasitism, which suggest that most zooparasitic nematodes have derived from free-living ancestors belonging to the soil-dwelling rhabditids (Anderson, 1984). Furthermore, as already pointed out by Drechsler (1941), capturing organs of many fungi are such that they allow little discrimination in their choice of prey.

In pure culture, and probably also in a nematode-free natural environment, many predacious fungi do not form traps and they behave as saprophytes. In *A. oligospora* the transition from a saprophytic to a predacious phase of growth may be induced by the presence of live nematodes, nematode metabolites, peptides and amino acids, etc. Factors effecting trap formation and predacity in this particular nematode-destroying fungus have been intensively studied by Nordbring-Hertz and her group (cf. Nordbring-Hertz, 1977; Nordbring-Hertz and Jansson, 1984).

The ability of animal-parasitic nematodes to induce the capturing devices in the fungi has not been studied in detail. In these experiments the morphogenic potentials of the 3 external larval stages of *Cooperia oncophora*, a trichostrongylid nematode parasite of cattle, will be compared with that of nonparasitic, free-living nematodes, i.e., *Rhabditis wohlgemuthi* and *Panagrellus revividus*. Also, the experiments allowed for a direct comparison of the efficiency of the fungus to capture animal-parasitic nematodes as opposed to the free-living nematodes. We included all 3 developmental stages of *C. oncophora*: The first 2 stages (L_1 and L_2) are bacteria feeders with a rhabditoid esophagus, and in many respects they are comparable with their free-living ancestors. However, third and infective larval stage (L_3) is encased in the cast cuticle of the second molt and cannot feed. The filariform esophagus and the ensheathing cuticle may to some extent protect the stage from the surrounding environment.

Materials and Methods

Arthrobotrys oligospora

The strain of *A. oligospora* Fres. (ATCC 24927) was grown and kept on corn meal agar (CMA) adjusted to

pH 7 as described by Lysek and Nordbring-Hertz (1981). This medium allowed good mycelial growth and conidial formation but not trap development. Test petri dishes, 3.2 cm in diameter, were each filled with approximately 4 ml of CMA and inoculated with 3–5-week-old fungal cultures. Using a metal cork borer (5 mm in diameter), circular agar plugs were cut from the fungal lawn and placed with mycelium down in the center of the CMA petri dish.

Both stock and test cultures were maintained at 100% humidity at room temperature (20–23°C) in daylight. Under these conditions the mycelium usually reached the periphery of the agar in 4–5 days. Grid lines on the bottom of each petri dish facilitated the counting of nematodes and measuring trap development.

Free-living nematodes

Panagrellus redivivus was cultured in flasks containing a soy peptone–liver extract medium (Nordbring-Hertz, 1972). *Rhabditis wohlgemuthi* was cultivated on serum agar plates (Monrad, pers. comm.). These nematodes were harvested from approximately 1-week-old cultures, using the Baermann funnel technique, and washed several times by alternate centrifugations and resuspensions in sterile water. The resulting suspensions contained both adult and juvenile nematodes.

Parasitic nematodes

Eggs of *Cooperia oncophora* were harvested from the feces of a calf carrying an experimental monospecific infection of the nematode. Larvae were allowed to develop in the feces by a cultivation procedure of Henriksen and Korsholm (1983) and were isolated by a modified Baermann technique. By starting cultures at different intervals it was possible to have batches of L_1, L_2, and L_3 larvae simultaneously available. The state of development was checked in each case by microscopical examination. Prior to use in the experiments the larvae were washed by serial centrifugations and resuspensions in sterile water.

Experimental procedure

To compare the ability of the nematodes to induce capture organs (hyphal loops) and to become trapped in such organs, their suspensions were added to 4-day-old cultures of *A. oligospora*. A drop of each nematode suspension, which had been adjusted to 100–150 per drop, was added to each of 3 test and 3 control dishes. Counts of free-living nematodes included both adults and juveniles. To check whether any nematode-free substance of the inoculum would induce traps, fungus CMA dishes were exposed to 1 drop of the supernatant of each of the final nematode suspensions.

Starting with 3-hour intervals and ending with 6-hour intervals, for a 27-hour period, test dishes were examined at 100× using a binocular microscope. Trap formation began as a stout branch erecting from a vegetation hypha. Subsequently it grew and curled back so its tip anastomosed with the parent hypha or with an adjacent trap already formed. Only traps that formed completely closed loops, either in isolated position or more commonly as part of complex, three-dimensional networks, were counted. Five randomly selected fields were counted in each dish for a total of 15 fields. The average number of traps per mm^2 was calculated.

At each time interval nematodes were also counted (×20). Only the normal, freely moving individuals were enumerated. In order to account for "natural" deaths among the nematodes the average numbers of such free individuals recorded in the test dishes were expressed as a percentage of those in fungus-free control petri dishes. In addition, on several occasions the contact between individual worms and traps was studied in closer detail with higher magnification (100×).

Two series of experiments were conducted: Series A included *R. wohlgemuthi* and the 3 developmental stages of *C. oncophora*. Series B differed in that *P. redivivus* replaced *R. wohlgemuthi*. In addition, at the end of series B, the L_3 of *C. oncophora* larvae were added to the dishes where traps were already present and induced by the other nematodes.

Results

Figure 1 (Series A) and Figure 2 (Series B) show that the rates of trap development were virtually independent of the type of nematode added. After 3–6 hours traps were formed in some nematode treatments; by 6 hours traps were formed in all nematode–fungus combinations. Over subsequent hours all treatments exhibited an almost parallel increase in number of traps. The treatment receiving nematode-free supernatants had no traps.

Also, Figures 1 and 2 show that the decline in the numbers of the free migratory nematodes started 3–6 hours after they were added to the dishes. The observation coincided with the initiation of trap formation. After 9 hours the majority of the nematodes were trapped and at 15 hours there was an almost complete absence of freely migrating individuals. Checks using higher magnification (100×) revealed that the majority of immobile nematodes were trapped. However, *R. wohlgemuthi* (Series A) seemed to present an exception in that a few migratory juveniles were observed at the end of the experiment.

During the experiments, the L_1 and L_2 stages of *C. oncophora* on the fungus-free control dishes decreased 10–25% in numbers. Both species of the free-living nematodes increased slightly in numbers towards the end of the experiment.

On close examination all treatments showed nematodes migrating over the entire agar surfaces of the dishes. On the fungus dishes they migrated in close physical contact with the hyphal networks and occasionally caused slight movements of the hyphal system. After the traps had developed it was noticed that casual nematode contacts did not necessarily result in immediate capture. Sometimes nematodes were seen to move into the loops and then escape by sud-

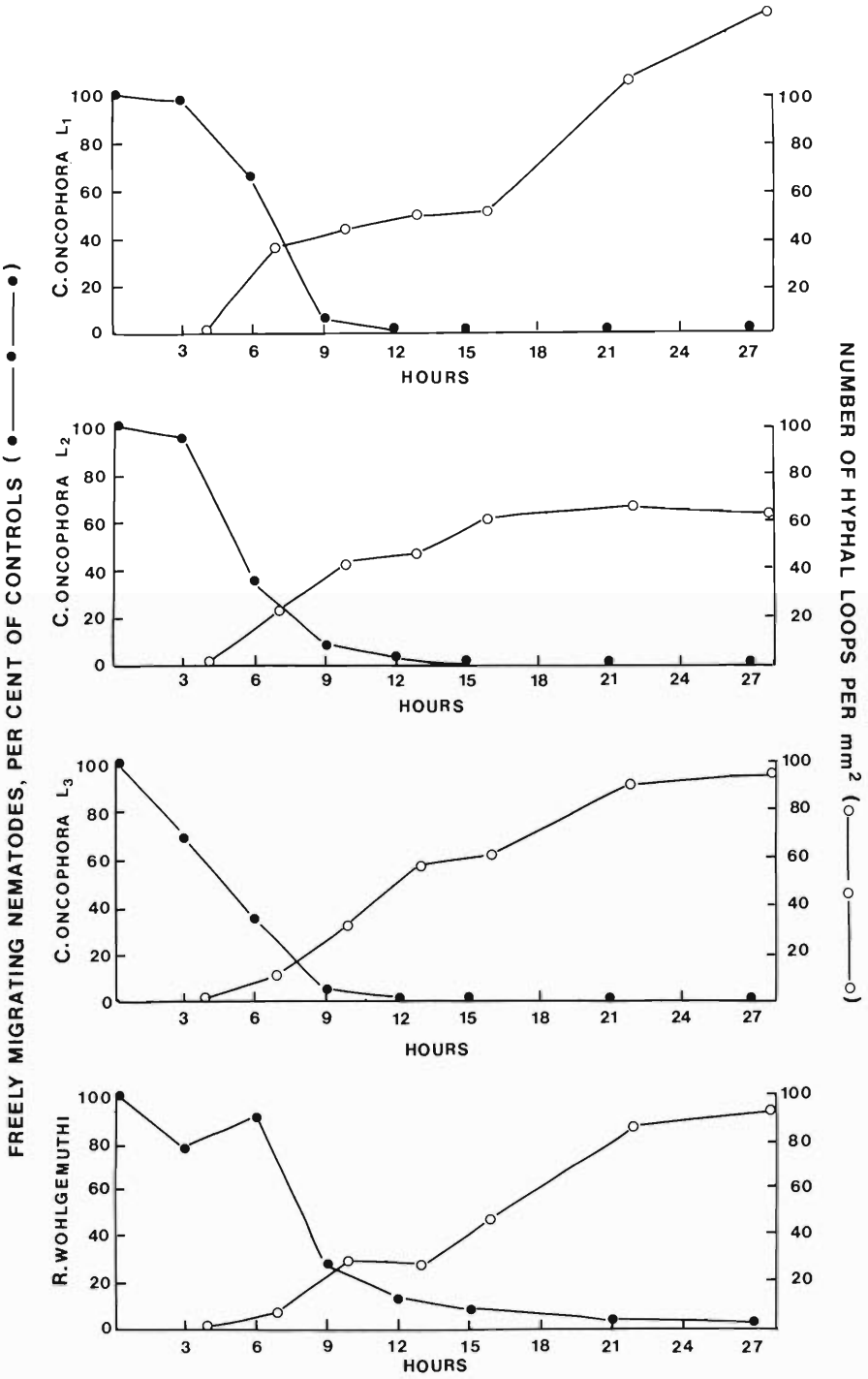

Figure 1. Series A: Loops per mm² and trapping efficiency of *A. oligospora* exposed to first-, second- and third-stage *C. oncophora* larvae and to *R. wohlgemuthi* (juveniles and adults). Average number of free individuals on fungus dishes expressed as a percentage of that on fungus-free control dishes.

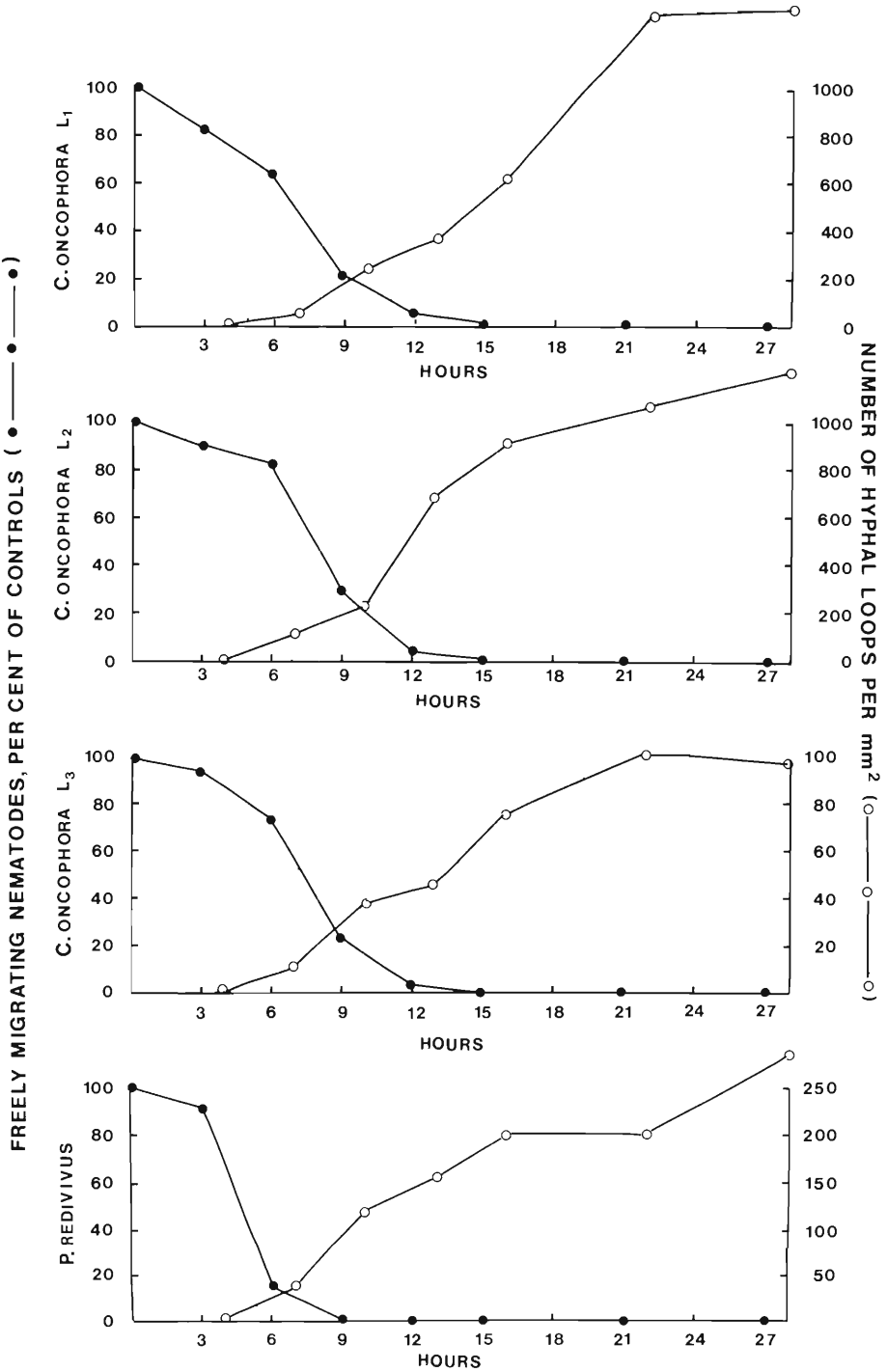

Figure 2. Series B: Loops per mm² and trapping efficiency of *A. oligospora* exposed to first-, second- and third-stage *C. oncophora* larvae and to *P. redivivus* (juveniles and adults). Average number of free individuals on fungus dishes expressed as a percentage of that on fungus-free control dishes. Notice that figures on the scale for *P. redivivus*-induced loops are higher than those of the other scales.

denly retracting, curling up, and circumventing the trap. All nematodes exhibited this interesting behavior but inevitably most were trapped.

However, once the prey were ensnared their subsequent fate was sealed. Free-living nematodes and L_1 and L_2 stages of *C. oncophora* wriggled up to a few hours, after which they became paralyzed, and fungal hyphae could be seen inside their bodies. The L_3 stage of *C. oncophora,* on the other hand, struggled vigorously for a much longer period of time and some few of them even succeeded in breaking the hyphal nets. Single individuals were seen freely moving on the substrate with fungal loops around their bodies with attached hyphal branches. Before long these nematodes were recaptured in other traps. The L_3 of *C. oncophora* continued to wriggle in their traps, some for more than 20 hours after their capture.

In Series B when the original nematodes were all caught, a suspension of L_3 *C. oncophora* was added to 1 of each type of test dish and to 1 control dish. Figure 3 shows that all test dishes possessed a rapid and high trapping efficiency in that all nematodes were caught within approximately 1 hour. In the previously *P. redivivus*-exposed dish, traps were particularly numerous and the capture of L_3 was instantaneous. On closer inspection it was noticed again that larvae struggled violently, but after 24 hours roughly only 15% remained active.

Discussion

In these experiments the capability of *C. oncophora* larvae to induce traps in the predacious hyphomycete, *A. oligospora,* was comparable with that of 2 free-living soil nematodes, *R. wohlgemuthi* and *P. redivivus.* The loops developed only in the presence of living nematodes, but our observations do not allow us to make conclusions as to the nature of the morphogenic stimulus. Nevertheless, others have suggested that the direct physical contact of nematodes with the fungal hyphae, or influence from metabolic products excreted by the living nematodes, or both, are responsible (cf. Nordbring-Hertz, 1977; Nordbring-Hertz and Jansson, 1984). Among the developmental stages of *C. oncophora* the morphogenic capability of the ensheathed L_3 was comparable with that of the preceding and metabolically more active stages (L_1 and L_2). This is noteworthy and may perhaps suggest that the predominant stimulus of this animal parasitic

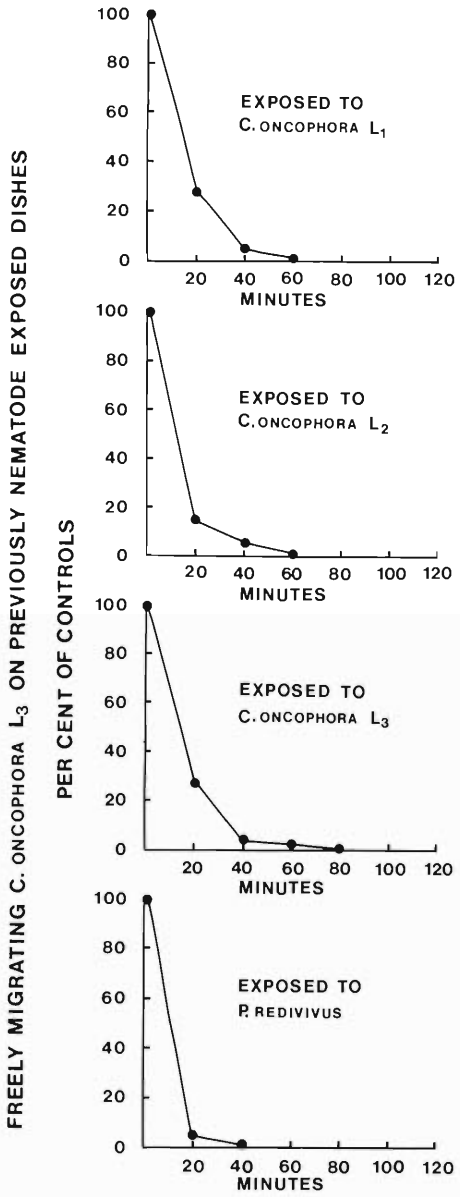

Figure 3. Trapping of third-stage *C. oncophora* larvae on *A. oligospora* dishes previously exposed to first-, second- and third-stage *C. oncophora* larvae, or to *P. redivivus* (juveniles and adults). The figure presents number of free individuals on fungus dishes expressed as a percentage of that on fungus-free control dishes.

nematode was the direct intimate contact with the hyphae. In fact, this was our observation under the microscope.

Drechsler (1941) studied the specificity of a particular fungus to a particular nematode and

stated that as a rule a predacious fungus traps and digests nematodes to a large extent independently of their species or genus position. This has later been confirmed by others (Soprunov, 1958; Barron, 1977). Our finding that various stages of two soil and one animal parasitic nematode are trapped with comparable efficiency is, therefore, not surprising. In *A. oligospora* and related species the firmness of attachment of soil nematodes to the trap is believed to be highly dependent on an adhesive produced by the fungus, and experimentally a lectin has been shown to mediate the binding of nematodes (Nordbring-Hertz and Mattiasson, 1979). In our study *A. oligospora* was just as effective in trapping *C. oncophora* as soil nematodes; thus, we might suspect that an adhesive was also important for capture of the parasite.

A. oligospora invades and digests free-living nematodes within a few hours after capture. Initially after dissolving the cuticle a mycelial branch penetrates into the body of the nematode and forms a so-called infection bulb from which hyphae grow and fill the entire length of the body. Death of the nematode often seems faster than can be explained by the hyphal growth alone and consequently it has been suggested that a toxin secreted by the fungus paralyzes the prey (Shepherd, 1955; Olthof and Estey, 1963). In the present study hyphal branches were observed both in free-living nematodes and in L_1 and L_2 *C. oncophora,* and presumably the fungus handled these nematodes in the same way.

L_3 larvae of *C. oncophora,* on the other hand, continue to wriggle in their traps over an extended period of time and at least within our observation period hyphal growth was not seen inside them. The cast cuticle from the previous molt may protect the larvae. Because neither mycelia nor paralyzing toxins can enter the worm, it continues to struggle. This hypothesis finds support in the observations by Nordbring-Hertz and Stålhammar-Carlemalm (1978) that *A. oligospora* does not penetrate the cuticle of dead *P. redivivus.* An interesting parallel may also be drawn to a recent study by Jansson et al. (1985) on the endoparasitic fungus *Meria coniospora.* They found that adhesion of conidia to the first-stage larvae of newly hatched eggs of the rodent trichostrongylid *Nematospiroides dubius* resulted in infection whereas adhesion to third-stage larvae did not. However, if the ensheathing cuticle of the latter was removed, infection succeeded. In line with this adhesion to third-stage larvae of 4 species of ruminant, trichostrongylids did not in any case result in infection.

The present and previous results suggest that under natural conditions in organic matters *A. oligospora* and related fungi may be capable of trapping free-living stages of animal parasitic nematodes such as trichostrongylids. This may take place in the soil surrounding cow pats where parasitic larvae may be abundant and persist over longer periods of time (Al Saqur et al., 1982; Grønvold, 1984), or it may take place even in the dung itself since *A. oligospora* has also been isolated from such material (Shepherd, 1956; Soprunov, 1958; Duddington, 1962). Studies should be made to elucidate possible, already existing relationships between nematode-destroying fungi and animal-parasitic nematode larvae in the natural feces/soil ecosystems, although we are aware that such studies involve great technical and interpretative difficulties.

In a recent paper we discussed the potential use of the fungus in controlling nematodes of ruminants (Grønvold et al., 1985).

Literature Cited

Al Saqur, I., K. Bairden, J. Armour, and G. Gettinby. 1982. Population study of bovine *Ostertagia* spp. infective larvae on herbage and in soil. Research in Veterinary Science 32:332–337.

Anderson, R. C. 1984. The origins of zooparasitic nematodes. Canadian Journal of Zoology 62:317–328.

Barron, G. L. 1977. The nematode-destroying fungi. Canadian Biological Publications. Pages 1–140.

Descazeaux, J. 1939. Action des champignons *Hyphomycètes* prédateur sur les larves de certain nematodes parasites des ruminants. Bulletin de la Société de Pathologie Exotique 32:457–459.

Drechsler, C. 1941. Predacious fungi. Biological Reviews of the Cambridge Philosophical Society 16: 265–290.

Duddington, C. L. 1962. Predacious fungi and the control of eelworms. *In* Duddington, C. L. and J. D. Carthy, eds. Viewpoints in Biology. Vol. 1. Butterworths, London.

Grønvold, J. 1984. The influence of earthworms on the transmission of infective larvae of bovine trichostrongyles. Ph.D. Thesis. Royal Veterinary and Agricultural University, Copenhagen.

———, **H. Korsholm, J. Wolstrup, P. Nansen, and S. A. Henriksen.** 1985. Laboratory experiments to evaluate the ability of *Arthrobotrys oligospora* to destroy infective larvae of *Cooperia* species, and to investigate the effect of physical factors on the growth of the fungus. Journal of Helminthology 59:119–125.

Gruner, L., M. Peloille, C. Sauvé, and J. Cortet. 1985. Survie et conservation de l'activité predatrice

vis-à-vis de nématodes trichostrongylides après ingestion par des Ovins de trois hyphomycètes prédateurs. Comptes Rendus de l'Academie des Sciences, Paris 300(III):525–528.

Henriksen, S. A., and H. Korsholm. 1983. A method for culture and recovery of gastrointestinal strongyle larvae. Nordisk Veterinærmedicin 35:429–430.

Jansson, H.-B., A. Jeyaprakash, and B. M. Zuckermann. 1985. Differential adhesion and infection of nematodes by the endoparasitic fungus *Meria coniospora* (*Deuteromycetes*). Applied and Environmental Microbiology 49:552–555.

Lysek, G., and B. Nordbring-Hertz. 1981. An endogenous rhythm of trap formation in the nematophagous fungus *Arthrobotrys oligospora.* Planta 152:50–53.

Nordbring-Hertz, B. 1972. Scanning electron microscopy of the nematode-trapping organs in *Arthrobotrys oligospora.* Physiologia Plantarum 26: 279–284.

———. 1977. Nematode-induced morphogenesis in the predacious fungus *Arthrobotrys oligospora.* Nematologica 23:443–451.

———, **and H.-B. Jansson.** 1984. Fungal development, predacity, and recognition of prey in nematode-destroying fungi. Pages 327–333 *in* M. J. Klug and C. A. Reddy, eds. Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington, DC.

———, **and B. Mattiasson.** 1979. Action of a nematode-trapping fungus shows lectin-mediated host-microorganism interaction. Nature 281:477–479.

———, **and M. Stålhammar-Carlemalm.** 1978. Capture of nematodes by *Arthrobotrys oligospora,* an electron microscope study. Canadian Journal of Botany 56:1297–1307.

Olthof, T. H. A., and R. H. Estey. 1963. A nematoxin produced by the nematophagous fungus *Arthrobotrys oligospora* Fres. Nature 197:514–515.

Pandey, V. S. 1973. Predatory activity of nematode trapping fungi against the larvae of *Trichostrongylus axei* and *Ostertagia ostertagi*: a possible method of biological control. Journal of Helminthology 47:35–48.

Roubaud, E., and R. Deschiens. 1941. Essais relatifs à la prophylaxie de l'anguillulose du mouton par l'usage des hyphomycètes prédateur de sol. Comptes Rendus des Séances de la Société de Biologie, Paris 135:687–690.

Shepherd, A. M. 1955. Formation of the infection bulb in *Arthrobotrys oligospora* Fres. Nature 175: 475.

———. 1961. Nematode-trapping fungi in Danish agricultural soils. Horticultura 15:94–96.

Soprunov, F. F. 1958. Predacious hyphomycetes and their application in the control of pathogenic nematodes. Academy of Sciences of the Turkmen SSR, Ashkhabad. Pages 1–292. (English translation 1966 by Israel Program for Scientific Translations, Jerusalem.)

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Influence of Cold Temperatures upon Development and Survival of Eggs of Washington Isolates of *Haemonchus contortus* and *Ostertagia circumcincta*

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ABSTRACT: Eggs of isolates of *Ostertagia circumcincta* and *Haemonchus contortus* from sheep in eastern Washington were examined for their ability to develop at 10°C, and to survive exposure to −18°C for 15 hr. Eggs of *O. circumcincta* were first larvated after 48 hr of incubation at 10°C, and 95% of the eggs were larvated after 120 hr of incubation. Eggs of *H. contortus* developed less rapidly with only 32% larvated after 120 hr of incubation. Survival of eggs (determined by their ability to hatch) after exposure to −18°C was >87% for *O. circumcincta* and <4% for *H. contortus*. These results indicate that the eggs of the strains of *O. circumcincta* and *H. contortus* examined differed in their ability to tolerate cold temperatures and that those of the former should be more successful than those of the latter in overwintering in colder climates.

The capacity of free-living stages of trichostrongylid nematode parasites to survive exposure to cold temperatures has been examined by many investigators (Furman, 1944; Kates, 1950; Crofton, 1965; Gibson and Everett, 1972, 1976; Todd et al., 1976; Le Jambre, 1981); however, strains of parasites from the northwestern United States have not been included in these observations.

The present study examines differences in survival of eggs and larval maturation of eastern Washington isolates of *Haemonchus contortus* (Rudolphi, 1803) and *Ostertagia circumcincta* (Stadelmann, 1894) subjected to cold temperatures. Observations included the ability of eggs to develop at 10°C and to hatch after exposure to −18°C. The amount of lipid present in eggs also was measured to determine whether this energy source (Passey and Fairbairn, 1957; Ward and Fairbairn, 1970; Kahn and McFadden, 1980; Womersley et al., 1982) could be correlated with cold survival.

Materials and Methods

Nematode eggs were obtained from donor lambs infected with strains of either *H. contortus* or *O. circumcincta* isolated from naturally infected sheep from the Palouse region of eastern Washington. The lambs were raised in confinement until 10 weeks of age. Prior to experimental infection, it was demonstrated by fecal examination that the lambs were free of nematode infection. Lambs were infected by oral administration of approximately 2,000 L_3 of *H. contortus* or 10,000 L_3 of *O. circumcincta*. Donor lambs were maintained in raised stanchions and given free access to pelleted alfalfa hay (WSU sheep ration 7008) and water. Specificity of infection was monitored by examination of larvae from fecal cultures obtained when infections became patent and at 1- to 2-week intervals thereafter.

The development of eggs from fecal pellets incubated at 10°C was evaluated by determining when nuclear indentations and larval stages first appeared and subsequently increased in numbers. The percentage of such eggs in these cultures was recorded at 24- to 48-hr intervals for 168 hr (Table 1). A sugar flotation technique (Cox and Todd, 1962) was used to separate eggs from feces for microscopic examination, which was used to assign them into 3 categories based on the appearance of their nuclei: (1) oval nucleus, with 8–500 cells; (2) indented nucleus, with a visible ventral indentation; (3) larvated, with tadpole or prehatch larva (Christie and Jackson, 1982). Two to 4 replicates were performed for each interval reported.

The ability of eggs to survive intense cold was evaluated by determining the percentage of eggs that hatched after exposure to −18°C for 15 hr. In the procedure used, feces containing eggs were incubated at −18°C, and eggs were removed from the feces by sugar flotation and then incubated in distilled water at 30°C for 24 hr to stimulate hatching. The percentage of hatching then was determined immediately by viewing the samples with a dissecting microscope and counting intact eggs and hatched larvae. At least 3 aliquots each containing >100 eggs and larvae were counted for all samples. Two replicates were performed for both *H. contortus* and *O. circumcincta* in all treatment and control groups (Table 2).

Both fresh and developing eggs were tested. Developing eggs were incubated at 10°C in feces for 48 hr (*O. circumcincta*) to 96 hr (*H. contortus*) prior to exposure to −18°C. The incubation periods were based on the approximate times when larvated eggs first ap-

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peared in 10°C cultures (Table 1). Controls included both fresh and developing eggs that were not exposed to −18°C.

The lipid content of fresh eggs was measured in 4 experiments. Eggs of *H. contortus* and *O. circumcincta* were cleaned of fecal debris by sugar flotation and washing in distilled water and were used immediately in paired experiments under identical conditions. Lipid content of each sample tested was determined as follows: (1) a known number (>1,000) of eggs was placed in a test tube and treated with 0.5% hypochlorite for 10 min, rinsed twice in distilled water, centrifuged at 5,000 rpm for 1 min, rinsed twice in 70% ethanol, and transferred to glass stender dishes; (2) eggs were stained for 2–3 hr in 70% ethanol saturated with Sudan IV; (3) excess stain was removed with a pipet, the eggs were rinsed twice in distilled water, and the absorbed stain was extracted by disrupting the eggs in 100% ethanol in a Heat Systems-Ultrasonics sonicator at 50 W for 3 min; (4) micrograms of lipid per milliliter of ethanol were estimated by measuring absorbance of the supernatant at 515 nm with a Varian DMS 80 spectrophotometer; (5) absorbance was compared with a standard absorbance curve produced with corn oil saturated with Sudan IV, and total lipid per sample was converted to μg of lipid per 1,000 eggs. Egg size of each species was determined by measuring length and width of 25 eggs obtained from fresh fecal pellets using a compound microscope equipped with an ocular micrometer at 400×.

The Student's *t*-test (Steel and Torrie, 1980) was used to test significance of differences. Data from all experiments were treated as unpaired observations, except those involving lipid measurements, which were treated as paired observations.

Table 1. Development of eggs of *Haemonchus contortus* and *Ostertagia circumcincta* at 10°C.

Incubation (hr)	Replicates (no.)	Eggs examined	Appearance of eggs (%)		
			Oval nucleus	Indented nucleus	Larvated
Haemonchus contortus					
0	4	4,727	100	0	0
24	5	3,958	>99	<1	0
48	5	2,580	78	22	0
72	4	2,121	44	55	1
96	2	765	9	87	4
120	2	752	11	57	32
168	2	1,659	16	41	43
Ostertagia circumcincta					
0	4	1,803	100	0	0
24	5	1,528	98	2	0
48	5	1,538	59	37	4
72	4	1,413	15	56	29
96	2	609	2	14	84
120	2	513	2	3	95
168	2	611	1	4	95

Results

Eggs of both *H. contortus* and *O. circumcincta* developed at 10°C (Table 1); however, larval stages of *O. circumcincta* were present earlier and in much higher proportions than those of *H. contortus* in samples taken after 24 hr of incubation. Fresh and developing eggs of *O. circumcincta* also were superior in their ability to withstand exposure to −18°C for 15 hr (Table 2). Mean percentage of hatching for *O. circumcincta* was from 88 to 93%, whereas that of *H. contortus* was from 1 to 4%. After 24 hr of incubation at 30°C, most (86–95%) fresh eggs of both parasites hatched, but significantly ($P < 0.01$) fewer developing eggs of *H. contortus* hatched than did those of *O. circumcincta.*

Other results indicated that: (1) there was significantly ($P < 0.05$) more lipid in eggs of *O. circumcincta* (126 μg/1,000 eggs) than in eggs of *H. contortus* (75 μg/1,000 eggs); and (2) eggs of *O. circumcincta* were significantly ($P < 0.05$) larger than those of *H. contortus,* measuring 87.5 ± 3.4 × 47.7 ± 2.2 μm and 73.2 ± 5.0 × 43.0 ± 1.7 μm, respectively.

Routine monitoring of larvae from fecal cultures from donor lambs indicated that infections of *H. contortus* and *O. circumcincta* were essentially monospecific. Over 99% of larvae in all cultures examined were those of the intended species.

Discussion

The results of the present experiments demonstrate differences in response to cold temperatures in fresh and developing eggs of strains of *O. circumcincta* and *H. contortus* isolated from eastern Washington. Most (>87%) eggs of the former developed rapidly at 10°C and hatched readily after exposure to −18°C. Eggs of the latter developed slowly and incompletely at 10°C and few (<4%) fresh or developing eggs hatched after exposure to −18°C. Although the mechanism responsible for cold tolerance was not determined, it seems unlikely that the egg shell could be an effective insulator at the temperatures tested. It is more probable that the eggs of *O. circumcincta* and *H. contortus* contain differing amounts of a cryoprotectant, such as trehalose, which serves this purpose in free-living stages of *Nematodirus battus* as reported by Ash and Atkinson (1982).

Sustained viability of eggs subjected to cold also may depend upon their content of energy stores. The fact that the larger eggs of *O. circumcincta* contained nearly 40% more lipid than did the smaller eggs of *H. contortus* suggests that this

Table 2. Percentage of eggs of *Haemonchus contortus* and *Ostertagia circumcincta* that hatched after exposure to −18°C for 15 hr.

Group	Mean percentage hatching	
	H. contortus	*O. circumcincta*
Fresh eggs → −18°C → 30°C	1.3[a] ± 1.8*	93.0[a] ± 3.5
Developing eggs → −18°C → 30°C	4.0[a] ± 2.4	87.9[a] ± 7.4
Fresh eggs → 30°C	85.9[c] ± 2.2	95.2[a] ± 2.0
Developing eggs → 30°C	46.2[b] ± 23.8	94.4[a] ± 3.7

* Means in columns that do not share superscript letters differ significantly ($P < 0.01$). ± = standard deviation.

energy source enhances cold survival. This hypothesis is supported further by observations of Ash and Atkinson (1982), who have shown that the large eggs of *Nematodirus* spp. are extremely cold-resistant.

The practical importance of the results is that they advance understanding of the role eggs of *H. contortus* and *O. circumcincta* play in the epizootiology of these parasites in the Northwest. Development at 10°C of the strain of *H. contortus* used in the present study was more like that observed by Le Jambre (1981) for a strain (*H. contortus cayugensis*) of this parasite from New York than for strains of *Haemonchus* from warmer areas. However, the degree of cold resistance demonstrated was not sufficient to allow the egg stages of this parasite to make a major contribution to overwintering on pastures in eastern Washington, where temperatures frequently fall below −18°C. On the other hand, the egg stages of the strain of *O. circumcincta* tested were extremely resistant to cold temperatures and appear to be capable of surviving local winters.

Acknowledgments

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Literature Cited

Ash, C. P. J., and H. J. Atkinson. 1982. The possible role of trehalose in the survival of the eggs of *Nematodirus battus* during dormancy. Parasitology 85: lv.

Christie, M., and F. Jackson. 1982. Specific identification of strongyle eggs in small samples of sheep faeces. Research in Veterinary Science 32:113–117.

Cox, D. D., and A. C. Todd. 1962. Survey of gastrointestinal parasitism in Wisconsin dairy cattle. Journal of the American Veterinary Medical Association 141:706–709.

Crofton, H. D. 1965. Ecology and biological plasticity of sheep nematodes. I. The effect of temperature on the hatching of eggs of some nematode parasites of sheep. Cornell Veterinarian 55:242–250.

Furman, D. P. 1944. Effects of environment upon the free-living stages of *Ostertagia circumcincta* (Stadelmann) trichostrongylidae: I. Laboratory experiments. American Journal of Veterinary Research 5:79–86.

Gibson, T. E., and G. Everett. 1972. The ecology of the free-living stages of *Ostertagia circumcincta.* Parasitology 64:451–460.

———, **and** ———. 1976. The ecology of the free-living stages of *Haemonchus contortus.* British Veterinary Journal 132:50–59.

Kates, K. C. 1950. Survival on pasture of free-living stages of some common gastrotintestinal nematodes of sheep. Proceedings of the Helminthological Society of Washington 17:39–58.

Khan, F. R., and B. A. McFadden. 1980. Embryogenesis and the glyoxylate cycle. FEBS Letters 115: 312–314.

Le Jambre, L. F. 1981. Hybridization studies of Australian *Haemonchus placei* (Place, 1893), *Haemonchus contortus cayugensis* (Das & Whitlock, 1960) and *Haemonchus contortus* (Rudolphi, 1803) from Louisiana. International Journal for Parasitology 11:323–330.

Passey, R. F., and D. Fairbairn. 1957. The conversion of fat to carbohydrate during embryonation of ascaris eggs. Canadian Journal of Biochemistry and Physiology 35:511–525.

Steel, R. G. D., and H. J. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill Book Company, New York. Pages 95–111.

Todd, K. S. Jr., N. D. Levine, and P. A. Boatman. 1976. Effect of temperature on survival of free-

living stages of *Haemonchus contortus*. American Journal of Veterinary Research 37:991–992.

Ward, C. W., and D. Fairbairn. 1970. Enzymes of β-oxidation and their function during development of *Ascaris lumbricoides* eggs. Developmental Biology 22:366–387.

Womersley, C., S. N. Thompson, and L. Smith. 1982. Anhydrobiosis in nematodes II: carbohydrate and lipid analysis in undesiccated and desiccate nematodes. Journal of Nematology 14:145–153.

New Index of Literature on Nematoda Parasitic in Animals

The last of the publications of the Index-Catalogue of Medical and Veterinary Zoology, "Special Publication No. 6: Nematoda and Nematode Diseases," is now available free of charge to those willing to pay the shipping costs. Higher taxa are listed alphabetically to genus level, but specific and subspecific names are listed only under the generic alphabetization. References to the literature include only author and date. The Author Catalogues of the Index-Catalogue of Medical and Veterinary Zoology must be consulted to obtain complete references. The publication period covered in Special Publication No. 6 is 1920–1964. Nematode literature published prior to 1920 was indexed in the Roundworm Catalogue compiled by C. W. Stiles and A. Hassall (published as Hygienic Laboratory Bulletin No. 114). This 1920 Roundworm Catalogue has been reprinted and is also available for the cost of shipping charges. References published after 1964 are available in the Nematoda sections of the Index-Catalogue (Supplements 15–24). Supplements 15–23 are available as above. Supplement 24 is available only from Oryx Press, 2214 North Central at Encanto, Phoenix, Arizona 85004 (Telephone 602-254-6156).

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Acetylcholinesterase Release by the Sexes of *Trichostrongylus colubriformis* (Nematoda)

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ABSTRACT: Both sexes of *Trichostrongylus colubriformis* released an enzyme during incubation that was characterized as acetylcholinesterase, based on substrate optima and specificity. The feeding stimulants, histamine or dopamine, and feeding inhibitors, octopamine and immune serum, had no effect on enzyme release by the nematode. The anthelmintic, ivermectin, which reduces in vitro feeding, had no effect on release of enzyme. Incubation of worms over 6 hours showed that the release of enzyme declined as did enzyme activity under reduced osmolarity. Crowding of both sexes of the nematode reduced the in vitro release of enzyme, but incubation of various ratios of both sexes had no effect. The results suggest that certain in vitro conditions influence ingestion and enzyme release by nematodes differently. Thus, enzyme release may not be directly related to the helminth's physiology of feeding.

Acetylcholinesterase is released by a number of nematode species (Ogilvie et al., 1973); however, the function of this enzyme is unclear. Proposed functions for the enzyme are a role in feeding through digestion of tissue and resistance to expulsion from the host through inhibition of local muscular contractions (Lee and Atkinson, 1977). *Trichostrongylus colubriformis* contained high levels of acetylcholinesterase that was localized in the esophageal and excretory glands (Rothwell et al., 1973).

Recent investigations of feeding activity by *T. colubriformis,* based on the in vitro and in vivo uptake of dye, have examined the effects of various environmental, biological, and chemical parameters (Bottjer and Bone, 1984; Bone and Bottjer, 1985). This study investigated the influences of selected factors that modulate feeding activity on the release of acetylcholinesterase by *T. colubriformis.*

Materials and Methods

Third-stage larvae of *Trichostrongylus colubriformis* were recovered from fecal-sphagnum moss cultures with a Baermann apparatus (Ciordia et al., 1966). Crossbred male goats that averaged 20 kg in weight were inoculated per os with 50,000 larvae and maintained in clean pens. Nematodes were collected in groups of 2,000 worms of each sex at 21 days postinfection and incubated in 2 ml of glucose-free Tyrode's solution at 37°C for 4 hr, unless stated otherwise. Then, the solution was removed and assayed for acetylcholinesterase according to Sigma Technical Bulletin No. 420 (1983). The following experiments were done to determine any relation between nematode feeding activity and the release of enzyme by *T. colubriformis.*

Males and females of *T. colubriformis* were incubated in the feeding stimulants, histamine or dopamine, at concentrations from 10^{-10} to 10^{-2} M and the feeding inhibitors, octopamine (10^{-6}–10^{-2} M) or immune serum (5–100%) from goats at 90 days or more postinfection. Serum from nonimmune animals at 21 days postinfection was tested similarly. Enzyme activity in these sera was eliminated by heating to 60°C for 1 hr. Incubates from untreated worms in Tyrode's solution were used as controls.

Worms were incubated in 0, 0.1, 1, and 10 μg/ml concentrations of ivermectin. Acetylcholinesterase was assayed subsequently to determine any alteration of release by the anthelmintic.

The influence of osmolarity on enzyme release was examined by incubation of worms in 100, 75, 50, 25, and 0% Tyrode's solution. The release rate of enzyme was evaluated by withdrawing and replacing the incubation medium at 1, 2, 4, 6, and 8 hr and assaying for enzyme activity. Helminths were incubated in Tyrode's solution with 2, 4, 12, 25, and 50 mM concentrations of acetylcholine to ascertain if enzyme secretion was induced by the presence of substrate.

Selected biological parameters were studied. Effects of crowding were tested by incubation of each sex of *T. colubriformis* in groups of 500, 1,000, 1,500, 2,000, and 3,000 worms in 2 ml of Tyrode's solution for 4 hr. Worms were incubated also in the following female: male ratios: 250:1,750, 500:1,500, 1,000:1,000, 1,250: 750, and 1,750:250.

Several experiments were done to characterize the helminth's enzyme. Substrate specificity was determined by using 25 mM butyrylcholine or acetyl-β-methylcholine as substrates by comparison to an identical concentration of acetylcholine. Effects of substrate concentration on hydrolysis by the enzyme were studied with a range of acetylcholine from 0.25 to 98 mM. Ionic effects on enzyme activity were examined by addition of 0.0015, 0.015, and 0.15 mM concentrations of sodium chloride, calcium chloride, zinc chloride, manganese chloride, and magnesium chloride to the enzyme–substrate mixture.

Data were analyzed by analysis of variance or linear regression. The 0.05 probability level was considered significant. Triplicate determinations were done for any trial, so that data are given as the mean ± SEM.

Results

Incubation of males and females of *T. colubriformis* in the feeding stimulants, histamine or dopamine, had no effect on the release of acetylcholinesterase. Similarly, the feeding inhibitors, octopamine or sera from immune goats, had no influence on the level of enzymatic activity. Serum from nonimmune hosts also had no effect. Decreased osmolarity caused a decline in enzyme release by the nematodes (Fig. 1). Enzyme activity declined 3-fold in less than 50% concentrations of Tyrode's solution. Similar quantities of acetylcholinesterase were found after 1, 2, or 4 hr of incubation, but decreased after 8 hr by 54 and 37% in male and female nematodes, respectively. Ivermectin had no effect on enzymatic activity. Male and female nematodes released 33–37 and 31–35 Rappaport Units/ml, respectively, as the drug concentration was increased from 0 to 10 μg/ml.

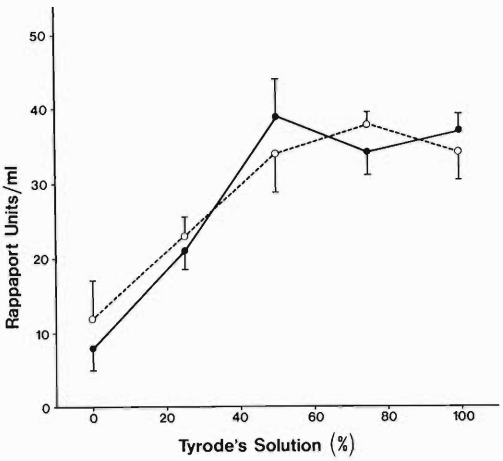

Figure 1. Release of acetylcholinesterase (mean ± SEM of Rappaport Units/ml) by male (●) and female (○) *Trichostrongylus colubriformis* during incubation in Tyrode's solution at various concentrations.

Acetylcholinesterase release varied little in incubates of various sex ratios of the nematode. Enzyme activity ranged from 28 to 34 Rappaport Units/ml as the sex ratio was varied over a 7-fold range of males to females. In contrast, enzyme release in individual worms declined 5-fold as the number of incubated worms was raised from 500 to 3,000 (Fig. 2). Incubation of *T. colubriformis* in various concentrations of acetylcholine had no discernable effect on the release of enzyme.

The enzyme from *T. colubriformis* hydrolyzed butyrylcholine less rapidly (19%) than acetylcholine; however, acetyl-β-methylcholine was hydrolyzed more rapidly (26%) than acetylcholine. The chlorides of sodium, calcium, zinc, manganese, and magnesium had no significant effect on substrate hydrolysis at the 0.0015–0.15 mM concentrations. More hydrolysis (57 and 53 Rappaport Units/ml, respectively) occurred at substrate concentrations of 4.1 and 6.1 mM than at greater or lesser concentrations of substrate, which averaged less than 40 Units/ml.

Discussion

The activity of acetylcholinesterase from *T. colubriformis* agreed with that described by Nachmansohn and Wilson (1955). Greater hydrolysis at a 4–7 mM concentration of substrate or reduced hydrolysis of butyrylcholine and increased hydrolysis of acetyl-β-methylcholine are characteristic of this enzyme.

Histamine and dopamine stimulated feeding activity by both sexes of nematodes, whereas octopamine inhibited ingestion by males (Bone and Bottjer, 1985). However, these compounds had little influence on the release of enzyme by *T. colubriformis.*

There was no significant difference in the release of enzyme by male and female nematodes, based on numbers of worms. However, males may produce more enzyme on a weight basis since their dry body weight is 22% less than the female (Bottjer and Bone, 1984). Sanderson (1969) found more acetylcholinesterase activity in male *Nippostrongylus brasiliensis* than in females. Crowding of worms in groups of 500–1,500 nematodes caused a 40% decline in enzyme release. Previous results indicate that in vitro feeding activity is diminished by about 60% during incubation of similar numbers of helminths (Bottjer and Bone, 1984). Assay of released enzyme may be a suitable procedure for determination of crowding effects in other studies.

The decrease in enzyme release by *T. colubriformis* after 6 hr confirmed the results of Rothwell et al. (1973). However, in vitro ingestion by the nematode was increased by about 33% after 8 hr of incubation based on uptake of dye (Bottjer and Bone, 1984). Thus, the timed pattern of enzyme release and feeding differ. Additionally, decreases in osmolarity caused a 4-fold elevation of in vitro ingestion of dye, whereas the results of this study indicate that, in contrast, acetyl-

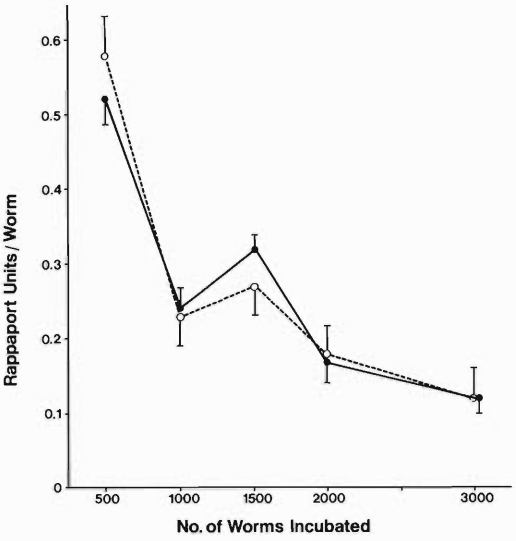

Figure 2. Release of acetylcholinesterase (mean ± SEM of Rappaport Units/ml) by individual males (●) and females (○) of *Trichostrongylus colubriformis* during incubation at various densities of worms.

cholinesterase release diminished with reduced concentrations of solute. Exposure to ivermectin at 0.001 μg/ml decreased feeding in vitro by 39 and 21% in males and females to *T. colubriformis,* respectively (Bottjer and Bone, 1985). However, in this study, the release of enzyme was not affected by even higher concentrations of drug.

Host immunity altered the electrophoretic pattern and level of acetylcholinesterase in *N. brasiliensis* (Jones and Ogilvie, 1972), but not *T. colubriformis* (Rothwell et al., 1973). In the present study, immune serum had no discernable effect on enzyme release. Immune serum caused a 50% reduction of in vitro feeding activity by *T. colubriformis* (Bottjer et al., 1985).

These results suggest, collectively, that the nematode's feeding activity via pharyngeal pumping and enzyme release are affected differently by the same experimental conditions. Thus, the role of acetylcholinesterase release in *T. colubriformis* and possibly other nematodes may be more likely as a "biochemical holdfast" to resist expulsion from the host rather than as a function of ingestive physiology.

Literature Cited

Bone, L. W., and K. P. Bottjer. 1985. Stimulation of ingestion in *Trichostrongylus colubriformis* (Nematoda). Proceedings of the Helminthological Society of Washington 52:80–84.

Bottjer, K. P., and L. W. Bone. 1984. Ingestion of dye by the sexes of *Trichostrongylus colubriformis.* Journal of Parasitology 70:671–676.

———, **and** ———. 1985. *Trichostrongylus colubriformis*: effect of anthelmintics on ingestion and oviposition. International Journal for Parasitology 15:501–503.

———, **P. H. Klesius, and L. W. Bone.** 1985. Effects of host serum on feeding by *Trichostrongylus colubriformis* (Nematoda). Parasite Immunology 7:1–9.

Ciordia, H., W. E. Bizzell, O. A. Porter, and C. F. Dixon. 1966. The effect of culture temperature and age on the infectivity of the larvae of *Trichostrongylus axei* and *T. colubriformis* in rabbits and guinea pigs. Journal of Parasitology 52:866–870.

Jones, V. E., and B. M. Ogilvie. 1972. Protective immunity to *Nippostrongylus brasiliensis* in rat. III. Modulation of worm acetylcholinesterase by antibodies. Immunology 22:119–129.

Lee, D. L., and H. J. Atkinson. 1977. Physiology of Nematodes. Columbia University Press, New York. 215 pp.

Nachmansohn, D., and I. B. Wilson. 1955. Acetylcholinesterase. Pages 642–651 *in* S. P. Colowick and N. O. Kaplan, eds. Methods in Enzymology, Vol. 1. Academic Press, New York.

Ogilvie, B. M., T. L. W. Rothwell, K. C. Bremner, H. J. Schnitzerling, J. Nolan, and R. K. Keith. 1973. Acetylcholinesterase secretion by parasitic nematodes—I. Evidence for secretion of the enzyme by a number of species. International Journal for Parasitology 3:589–597.

Rothwell, T. L. W., B. M. Ogilvie, and R. J. Love. 1973. Acetylcholinesterase secretion by parasitic nematodes—II. *Trichostrongylus* spp. International Journal for Parasitology 3:599–608.

Sanderson, B. E. 1969. Acetylcholinesterase activity in *Nippostrongylus brasiliensis* (Nematoda). Comparative Biochemistry and Physiology 29:1207–1213.

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Prevalence of *Dictyocaulus arnfieldi* (Nematoda) in Equids and Clinical Problems with *Strongylus vulgaris* (Nematoda) Mainly in Donkeys on a Farm in Central Kentucky

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ABSTRACT: Prevalence of lungworms was investigated in equids on a farm in central Kentucky. Lungworm larvae were found in fecal samples of 93% of 14 donkeys, in 50% of 6 Thoroughbreds, and in none of 2 ponies. Five of the donkeys and 1 Thoroughbred suckling died from *Strongylus vulgaris*-related blockage, extensive or partial, of the cranial mesenteric artery and branches. Lungworms, 2–213 specimens, were recovered from the lungs of the 5 dead donkeys at necropsy. Lungs of the dead horse suckling were not examined. All donkeys, dead and live, showed evidence of poor nutrition.

The present authors have been studying lungworms (*Dictyocaulus arnfieldi*) in equids in Kentucky in recent years (Lyons et al., 1982b, 1985a, b, c). Our research has included only limited data on this parasite in donkeys. Data on *D. arnfieldi* in equids are sparse in this country. Several publications on lungworms are limited to clinical problems in horses and ponies associated with donkeys. One publication does indicate that, in an examination of fecal samples from part of a donkey herd, 85 of 90 donkeys were positive for lungworm larvae (Thomas and Jones, 1960). Recently, there was an opportunity to determine the prevalence of lungworm larvae in fecal samples from equids, including donkeys, on a farm in central Kentucky. Coincidentally, acute *Strongylus vulgaris* infections and deaths were documented in equids, mainly donkeys, on this farm. The dead donkeys provided the chance to enumerate *D. arnfieldi* in the lungs at necropsy.

Materials and Methods

The prevalence of lungworms was investigated in an equid population consisting of 15 donkeys, 11 Thoroughbreds, and 3 ponies (Shetland-type) on a farm in central Kentucky. Fecal samples were collected on November 9, 1984 from the rectums of 22 of the 29 equids (Table 1). One of the donkeys, a yearling, was dead at the time of sampling. On the day of collection of fecal samples, the Thoroughbreds and ponies were in good physical condition and were on pastures with lush vegetation. The donkeys were in generally poor physical condition and were located in 2 dry lots (estimated to be about 0.30 ha total). Management problems, relative to nutrition and parasite control, were evident, particularly in the donkeys. Apparently, there was occasional intermixing of the donkeys and the other equids on the farm. The background of anthelmintic treatment of the equids and management practices were not possible to reconstruct because of vagueness of recollection of the owner. Supposedly, ivermectin (Eqvalan®, Merck), which is highly effective on *D. arnfieldi* (Britt and Preston, 1985; Lyons et al., 1985a) and *S. vulgaris* including migratory stages (Slocombe and McCraw, 1980, 1981; Slocombe et al., 1982; Lyons et al., 1982a; Drudge et al., 1984; Klei et al., 1984), was administered to some of the horses previously but not to the donkeys. Febantel paste (Rintal®, Haver) was administered to all equids on the day of our visit to the farm.

Routine necropsy of equids that died on the farm was done by pathologists in the Department of Veterinary Science, University of Kentucky. The cranial mesenteric arteries were examined grossly for *S. vulgaris* and damage caused by them. Specimens of *S. vulgaris* found were not counted. Examination was made of the lungs from dead donkeys for *D. arnfieldi,* which were counted, and for pneumonia.

Techniques have been published for examination of fecal samples for *D. arnfieldi* larvae (Lyons et al., 1985a), for nematode egg counts per gram of feces (EPG) (Drudge et al., 1963; Lyons et al., 1976), and for recovery of *D. arnfieldi* from the lungs at necropsy (Lyons et al., 1985a).

Results and Discussion

Lungworm larvae were found in fecal samples of 93% of 14 donkeys, in 50% of 6 Thoroughbreds, and in none of 2 ponies (Table 1).

Nematode eggs per gram of feces (EPG) were recorded for 8 equids (Table 2). Strongyle EPG varied from 90 to 1,780; other nematode eggs found were ascarids in a Thoroughbred yearling and strongyloides in a donkey weanling. The EPG can only be considered as evidence of parasites being present but not the extent of worm burden. However, EPG did indicate that probably dewormers had not been recently used in the equids.

Five of the 14 donkeys, from which fecal samples were collected, died over the ensuing 2-month period. Death occurred for 1 (a yearling) on the day of our visit to the farm, for 3 (a suckling and

Table 1. Prevalence of lungworm (*Dictyocaulus arnfieldi*) larvae in feces of equids* on a farm in Kentucky.

	Donkeys					Thoroughbreds				Ponies		
	Adult		Yearling	Suckling		Adult	Yearling†	Weanling†		Adult		
Item	♂	♀	♀	♀	All	♀			All	♂	♀	All
No. infected	1	8	2	2	13	1	1	1	3	0	0	0
No. examined	1	9	2	2	14	3	2	1	6	1	1	2
(% infected)	(100)	(89)	(100)	(100)	(93)	(33)	(50)	(100)	(50)	(0)	(0)	(0)

* Of the 29 equids on the farm, fecal samples were collected from 22: 14 of 15 donkeys (1 weanling not examined), 6 of 11 Thoroughbreds (5 mares not examined), and 2 of 3 ponies (1 mare not examined).

† Sexes not recorded.

2 jennies) within 9 days later and during severe cold weather, and for 1 (a jack) about 2 months later (Table 3). The deaths of all 5 donkeys were attributed to *S. vulgaris* by pathologists at the University of Kentucky Department of Veterinary Science. The first donkey to die had a large verminous aneurysm with extensive thrombosis of the cranial mesenteric artery and its major branches; also, a bronchial pneumonia, possibly related to infection of *D. arnfieldi,* was present. The other 4 dead donkeys had aneurysms in the cranial mesenteric arteries but blockage of these blood vessels and branches was incomplete and gross infarctions of the digestive tract were not present. The numbers of *D. arnfieldi* recovered from the lungs of each of the 5 dead donkeys varied from 2 to 213 (Table 3). The physical condition of all dead donkeys was poor and probably related to arterial infections of *S. vulgaris* and dietary insufficiencies. The vague history available from the owner did not indicate respiratory distress or colic in the 5 donkeys prior to death or in any of the other equids on the farm.

Review of the necropsy records in the department revealed that a 5-month-old Thoroughbred weanling colt from the same farm died about a month before our visit there. Death was due to blockage of the cranial mesenteric artery and its branches by thrombotic deposits from the presence of migrating *S. vulgaris.* The lungs were not examined for lungworms.

The present investigation increases the body of data on the prevalence of lungworms in equids, particularly in donkeys, in this geographical area. Also, this situation provided a unique occasion to document acute *S. vulgaris* infection in equids, particularly in donkeys, for which published data on deaths from this parasite or even its prevalence are sparse in this country. The number of deaths of the donkeys due to migrating *S. vulgaris* during the 2-month investigation period is especially interesting because of the relatively high mortality (33%) during such a short period of time. Poor nutrition, however, certainly appeared to be a contributing factor to the deaths of the donkeys.

The findings of lungworm larvae in 93% of the fecal samples from the live (including 1 dead) donkeys and specimens of *D. arnfieldi* in 100%

Table 2. Nematode eggs per gram of feces (EPG) for 8 equids on a farm in central Kentucky.

Type of Equid	Identification no.	EPG		
		Ascarid	Strongyle	*Strongyloides*
Donkey—adult ♀	4	0	90	0
Donkey—adult ♀	5	0	270	0
Donkey—18-month-old ♀	13	0	1,780	0
Donkey—weanling ♂	22	0	400	840
Thoroughbred—yearling*	6	60	140	0
Thoroughbred—adult ♀	9	0	310	0
Thoroughbred—adult ♀	10	0	500	0
Thoroughbred—adult ♀	11	0	990	0

* Sex not recorded.

Table 3. Data on lungworms recovered from lungs of 5 dead donkeys from a farm in Kentucky.

Donkeys				*Dictyocaulus arnfieldi**		
Identification no.	Date of death	Age or group category	Sex	♂	♀	All
1884	1984: November 9	Yearling	♀	107	106	213
1892	November 12	Suckling	♀	2	2	4
1910	November 15	Adult	♀	62	66	128
1920	November 18	Adult	♀	13	11	24
86	1985: January 18	Adult	♂	0	2	2

* Most were adult.

of the lungs from the dead donkeys were higher than those recently reported for donkeys in this geographical area (Lyons et al., 1985b, c). Probably, the lungworms in the Thoroughbreds were derived from the donkeys (Round, 1976; Clayton, 1980; Lyons et al., 1982b, 1985a, b, c); however, horse-to-horse transmission also has been reported (Round, 1976; Lyons et al., 1982b, 1985b).

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Literature Cited

Britt, D. P., and J. M. Preston. 1985. Efficacy of ivermectin against *Dictyocaulus arnfieldi* in ponies. Veterinary Record 116:343–345.

Clayton, H. M. 1980. Clinical aspects of ascarid and lungworm infections in horses. Proceedings of the Annual Meeting of American Equine Practitioners 26:29–32.

Drudge, J. H., E. T. Lyons, and S. C. Tolliver. 1984. Controlled tests of activity of ivermectin against natural infections of migratory strongyles and other internal parasites of equids. American Journal of Veterinary Research 45:2267–2271.

———, **Joseph Szanto, Z. N. Wyant, and George Elam.** 1963. Critical tests of thiabendazole as an anthelmintic in the horse. American Journal of Veterinary Research 24:1217–1222.

Klei, T. R., B. J. Torbert, M. R. Chapman, and M. A. M. Turk. 1984. Efficacy of ivermectin in injectable and oral paste formulations against eight-week-old *Strongylus vulgaris* larvae in ponies. American Journal of Veterinary Research 45:183–185.

Lyons, E. T., J. H. Drudge, and S. C. Tolliver. 1976. Studies on the development and chemotherapy of larvae of *Parascaris equorum* (Nematoda: Ascaridoidea) in experimentally and naturally infected foals. Journal of Parasitology 62:453–459.

———, ———, **and** ———. 1982a. Ivermectin: activity against larval *Strongylus vulgaris* and adult *Trichostrongylus axei* in experimental infections in ponies. American Journal of Veterinary Research 43:1449–1450.

———, ———, **and** ———. 1985a. Ivermectin: treating for naturally occurring infections of lungworms and stomach worms in equids. Veterinary Medicine 80:58–64.

———, ———, **S. M. Zygmont, J. L. Twehues, R. G. Downing, and H. H. Sutton.** 1982b. Clinical history of lungworm disease in a foal. Veterinary Medicine & Small Animal Clinician 77:1533–1537.

———, **S. C. Tolliver, J. H. Drudge, T. W. Swerczek, and M. W. Crowe.** 1985b. Lungworms (*Dictyocaulus arnfieldi*): prevalence in live equids in Kentucky. American Journal of Veterinary Research 46:921–923.

———, ———, ———, ———, **and** ———. 1985c. Parasites in lungs of dead equids in Kentucky: emphasis on *Dictyocaulus arnfieldi*. American Journal of Veterinary Research 46:924–927.

Round, M. C. 1976. Lungworm infection (*Dictyocaulus arnfieldi*) of horses and donkeys. Veterinary Record 99:393–395.

Slocombe, J. O. D., and B. M. McCraw. 1980. Evaluation of pyrantel pamoate, nitramisole, and avermectin B_1 against *Strongylus vulgaris* larvae. Canadian Journal of Comparative Medicine 44:93–100.

———, **and** ———. 1981. Controlled tests of ivermectin against migrating *Strongylus vulgaris* in ponies. American Journal of Veterinary Research 42:1050–1051.

———, ———, **P. W. Pennock, and J. Vasey.** 1982. Effectiveness of ivermectin against 4th-stage *Strongylus vulgaris* in ponies. American Journal of Veterinary Research 43:1525–1529.

Thomas, R. E., and L. P. Jones. 1960. Lungworm infection in the burro. Veterinary Medicine 55: 38–40.

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A Water Agar En Face Technique

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ABSTRACT: A method is described whereby en face views of live or fixed nematodes are prepared using water agar suitable for study, photography, or camera lucida drawings. Specimens subjected to this method can be fixed and remounted permanently.

A number of methods have been devised to prepare nematodes for en face examination (Cobb, 1920; Buhrer, 1949; Tromba and Douvres, 1953; Anderson, 1958; Lee, 1964). The most prevalent mode entails fixation, decapitation, and finally, orientation in glycerine-gelatin.

Workers who have used the glycerine-gelatin technique are very likely familiar with the agony and frustrating experiences associated with this procedure. Problems include cutting the head too long, or at an acute angle that prevents vertical presentation, or losing the head entirely in the cutting process. New problems arise when the head is placed in the glycerine-gelatin. Premature hardening may occur prior to orienting the head properly. Correct orientation can take considerable time, and sometimes the substrate must be melted several times to correct improper orientation.

A new method was devised to prevent some of the cumbersome problems inherent in the glycerine-gelatin technique.

Method

Subject fixation: Nematodes were fixed in either 2% formalin or lacto-phenol (Esser, 1973). In some cases, live immobile females or males were used.

Procedure: A 12 × 12 × 3-mm square of 1.7% water agar is cut very evenly with a razor blade (Fig. 1A) and placed on a microscope slide. A 3- to 4-mm piece is precisely cut from the square (Fig. 1A) and laid with the outer face down (Fig. 1B). Nematodes (at least 3 specimens per subject taxon) are placed on the upper side of the cut piece with the longitudinal axis of the head parallel with the outer edge of the cut piece (Fig. 1B). The cut piece is then placed back into the same orientation it occupied in Figure 1A, then gently pushed into its original position against the parent back (Fig. 1C). A small (4-mm) drop of water is applied to a 15-mm cover slip that is then placed waterside down over the cut line (Fig. 1C). A drop of immersion oil is applied to the center of the cover slip at the junction of the cut pieces. When the body of the nematode is properly aligned, the en face appears as in Figure 1D. If the en face is off-center or below the field of focus, the cover slip is removed, the cut piece placed backside down, and the specimen reoriented. Water must be added to the cover slip each time it is placed on the agar block. It takes 10–15 min to prepare an en face ready for viewing using this technique. Locating the en face is rather easy because it lies within the cut line.

Discussion

The method has been employed successfully and camera lucida drawings made using males, females, and larvae of *Verutus volvingentis* Esser, 1981; females of *Butlerius* sp., *Criconema* sp., *Criconemoides xenoplax* Raski, 1952, *Fictor* sp., *Helicotylenchus* sp., *Labronema* sp., *Mononchus* sp., *Rhabditis* sp., *Tripyla* sp., *Xiphinemella es-*

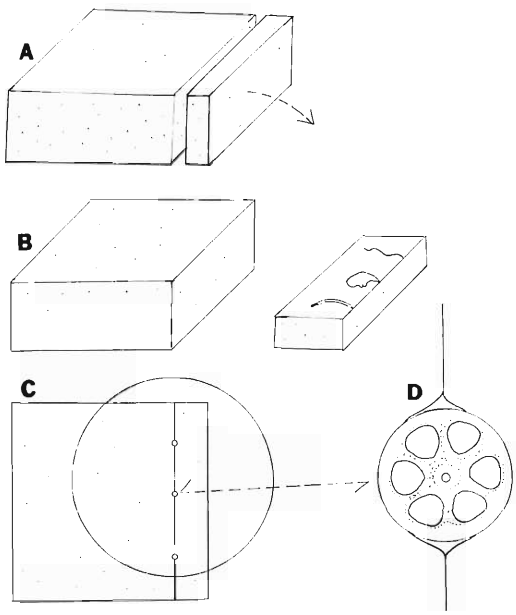

Figure 1. Water agar en face method: A) 12 × 12 × 3-mm square of water agar with a 3- to 4-mm piece cut off; B) specimens aligned on outer edge of the inner face of the cut piece; C) re-alignment of the separated agar pieces, with cover slip in place; D) close-up of en face in junction line of re-aligned agar.

seri Chitwood, 1957, and males only of *Meloidogyne* sp. Since initiation of the method, it has been 100% effective. An en face has been demonstrated at each trial of a subject taxon. Three specimens or more per trial insures success of at least one excellent en face. If the slide on which the en faces are mounted is placed in a petri dish with a small, moist piece of absorbent tissue, it will remain in good condition, ready for re-examination, for several days. When desirable, the specimens can be recovered for permanent mounting.

Swollen females and acutely curved forms in which the head lies far below the tail tip in the death curvature may have to be severed below the esophageal gland area to employ this technique.

Advantages of this method are basically its simplicity, speed, and high potential for success. En face drawings are also possible from live immobile specimens which preclude fixation artifacts. The total specimen can also be permanently mounted after the en face drawings are complete. The principal disadvantage of this method is that the en face mount cannot be permanently preserved.

Literature Cited

Anderson, R. C. 1958. Methode pour l'examen des nématodes en vue apicale. Annales de Parasitologie 33:173–175.

Buhrer, E. M. 1949. Technique for beheading and *en face* examination of nematodes and similar animal types. Proceedings of the Helminthological Society of Washington 16:3–6.

Cobb, N. A. 1920. Micro-technique, Sec. III. To obtain an end view of a nema, rotifer, or other similar small object. Transactions of the American Microscopical Society 39:236–237.

Esser, R. P. 1973. A four minute lactophenol fixation method for nematodes. Plant Disease Reporter 57: 1045–1046.

Lee, S. H. 1964. A technique for studying *en face* view of moderate-sized nematodes without decapitation. Proceedings of the Helminthological Society of Washington 31:137–138.

Tromba, F. G., and F. W. Douvres. 1953. A modified *en face* technique. Proceedings of the Helminthological Society of Washington 20:59.

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Development of *Ascaris suum* from In Vivo-derived Third-stage Larvae to Egg-laying Adults In Vitro

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ABSTRACT: A 2-step roller culture system was designed that supported optimally the development of late-third-stage larvae (L3) of *Ascaris suum,* obtained from rabbit lungs, to mature adults in vitro. The culture system consisted of 1) medium API-18 for 7 days, and 2) thereafter, medium API-1 supplemented with bovine hemin. Cultures were gassed with 85% nitrogen/5% oxygen/10% carbon dioxide and incubated at 39°C. Under these conditions, L3 developed to fourth molt, young adults, and mature adults at 14, 20, and 53 days in culture, respectively. Morphogenesis of larval and adult stages in vitro was similar to that of worms of a comparable stage obtained from swine. The rate of development of late L3 to late fourth stage (L4) was similar to that of larvae developing in vivo, but development of late L4 to adults in vitro was delayed. Fertilized eggs were produced by females in cultures containing mature males. The eggs were comparable morphologically to eggs from females isolated from swine, but somewhat smaller. Late L3 developed into mature males and females ovipositing fertilized eggs in 3 other 2-step culture systems and a 1-step culture system. Medium API-1 plus hemin or a modification of API-1 that contained its complex mammalian tissue extracts and peptide digests (medium API-23 plus hemin) was essential. Copulation was observed for the first time between *A. suum* mature adult males and females in an extant culture of the 1-step culture system.

Attempts to grow advanced stages of *Ascaris suum* in vitro have had varying degrees of success (Taylor and Baker, 1968; Stromberg et al., 1977; Hansen and Hansen, 1978; Urban and Douvres, 1981; Douvres and Urban, 1983; and Urban et al., 1984). The most successful cultivation method was a 3-step roller culture system containing complex, cell-free media KW-2, API-1, and API-18 with appropriately added supplements of reducing agents and bovine hemin and precisely applied gas phases that supported the development of second-stage larvae (L2), hatched from eggs, to mature adult males and egg-laying females (Douvres and Urban, 1983).

Other investigators have used a variety of culture conditions to obtain high yields of fourth-stage larvae (L4) of *A. suum* in vitro from third-stage larvae (L3) obtained from the lungs of experimentally infected animals (Sylk et al., 1974; Stromberg et al., 1977; Urban and Douvres, 1981; Urban et al., 1984). The most effective of these was a stationary multi-well system that used RPMI 1640 medium plus serum (Urban et al., 1984). Urban and Douvres (1981) observed that more than 95% of the larvae obtained from *A. suum* infected rabbits at 7 days after oral inoculation with eggs were in the late phase of L3. This phase predominates as larvae re-enter the intestinal milieu of the natural host and begin development to mature adults in situ (Douvres et al., 1969). These findings suggested that late L3 might require a simpler culture system for in vitro development to mature adults than the L2, and that conditions used to cultivate advanced stages of other intestinal nematodes from infective L3 might effectively support the development of *A. suum* L3 obtained from rabbit lungs. This concept was tested by inoculating in vivo-grown L3 of *A. suum* into a modified 1-step roller culture system used to grow *Oesophagostomum radiatum* from infective larvae to young adults (Douvres, 1983). The system consisted of medium API-1 plus L-glutathione (reduced) for 7 days followed by medium API-1 plus bovine hemin. It supported the development of *A. suum* L3 to mature adults. However, enhanced development of *A. suum* L3 to mature adults also was attained with a 2-step culture system that used medium API-18 (Douvres and Urban, 1983) followed by API-1 plus hemin.

This report describes the preparation of roller culture systems with combinations of 1 or 2 media (KW-2, API-18, API-1, RPMI 1640) with or without supplements (reducing agents, bovine hemin, or serum) that were used to grow *A. suum* L3. In addition, 2 new media derived from API-1 (API-22 and API-23) were prepared and

[1] Retired, January 1986.

tested to determine the active ingredients in API-1 necessary to promote development of *A. suum* to mature adults.

Materials and Methods

PREPARATION OF MEDIA: Medium API-1 (Douvres and Malakatis, 1977) was used alone or supplemented with 10 mM glutathione (reduced). Media KW-2 and API-18 (Douvres and Urban, 1983) and API-1 were used with and without bovine hemin (Hemin Type I: Bovine, Sigma Chemical Company, St. Louis, Missouri) at a final concentration of 24 μg/ml.

Medium RPMI 1640+S was prepared with RPMI 1640 (M. A. Bioproducts, Walkersville, Maryland) plus a supplement of 25% (v/v) bovine calf serum (obtained from 4-mo-old calves maintained helminth-free at the Animal Parasitology Institute) that had been heat-inactivated for 30 min at 56°C. Media API-22 and API-23 were new formulations (Table 1) that evolved from modifications of medium API-1; they were used freshly prepared or after storage at −20°C for up to 6 months. These media were used alone or with a supplement of 24 μg/ml bovine hemin.

All media contained 1,000 units/ml of penicillin G potassium, 1 mg/ml of streptomycin sulfate and 10 μg/ml Fungizone, and were adjusted to pH 6.8 with the addition of either 1 N HCl or 1 N NaOH as needed.

ANIMAL INFECTIONS AND PREPARATION OF LARVAL INOCULUM: New Zealand white rabbits (males and females) of approximately 2–3 kg were obtained locally and maintained in wire-based cages. Rabbits were inoculated orally with 75,000 decoated, embryonated eggs of *A. suum* (Urban and Douvres, 1981), and lungs were removed 7 days after infection. Larvae were recovered from the lungs of infected rabbits, freed of host tissue, and prepared for inoculation of cultures (Urban and Douvres, 1981). The stage of development of larvae was determined by the morphological characters described by Douvres et al. (1969). The recovered larval population was composed of greater than 95% mid to late L3; the remainder were early L3.

PREPARATION AND HANDLING OF CULTURES: Protocols for preparation and handling of 10 different roller culture systems (A through J) and data on the number of trials and cultures involving each system are given in Table 2. Systems A, B, and C each used a distinct medium for the first 7 days of culture that was then altered only by the addition of hemin from day 7 to termination. Systems D and E contained the same medium from day 0 to 7 in culture, but it was altered subsequently by the removal of glutathione (reduced) (system D) or the removal of glutathione (reduced) and the addition of hemin (system E). These systems were considered as involving a single basic medium composition and, for the purposes of discussion, were described as 1-step systems. Systems F through J each involved marked changes in medium composition after the first 7 days of culture and were, therefore, described as 2-step systems.

The roller bottle cultures were prepared and gassed with a mixture of 85% N_2/5% O_2/10% CO_2 (Douvres and Malakatis, 1977). Inocula were transferred to fresh media and a new culture bottle, on days 7, 14, 21, 28, and 35 (Douvres et al., 1966). Large larval stages or adults were transferred with a pick to avoid damage (Douvres and Urban, 1983).

Table 1. Comparison of media composition of API-1, API-22, and API-23.

API-1 stock solutions*	API-22	API-23
Chemically undefined		
Bovine calf serum	+	+
Rabbit embryo extract	−	+
Calf liver extract	−	+
Bactopeptone	−	+
Trypticase	−	+
Yeast extract	−	+
Casein	−	+
Chemically defined		
MEM essenital amino acids	+	−
MEM nonessential amino acids	+	−
L-cysteine-HCl, ascorbic acid	+	−
L-glutamine	+	−
N-acetyl-D-glucosamine	+	−
Vitamin mixture	+	−
Nucleic acids	+	−
Sugar mixture	+	+
Carboxylic acids	+	+
Salt mixture	+	+

* Groups of stock solutions used to prepare medium API-1 (Douvres and Malakatis, 1977).

Checks for sterility of all stocks of media and evaluation of development of the cultured nematodes were performed (Douvres et al., 1966). Cultures were judged free of contamination if there was no visible evidence of fungi or bacteria. Adults were killed and fixed in hot buffered 5% formalin, cleared in phenol–alcohol, and were studied in temporary wet mounts. The larval stages and adults were classified according to Douvres et al. (1969), Pilitt et al. (1981), Tromba (1978), and from original observation. Fertilized and unfertilized eggs oviposited by adult females were identified according to Alicata (1936) and Foor (1967).

Results

Advanced development from L3 was obtained in all trials of the 10 culture systems (Table 2). Development from L3 to mature adult males and ovipositing females was obtained in 5 systems (E, G, H, I, J), but was best in systems E and J. Only results from systems E and J will be described in detail.

MORPHOGENESIS, HEALTH, AND GROWTH: Third-stage larvae obtained after 7 days in rabbits developed to mature adult males and egg-laying females by day 53 in culture (systems E and J, Table 3). In system E, L3 advanced to early L4 in medium API-1 + glutathione (reduced) and completed development to adults in medium

Table 2. *Ascaris suum*: Protocols used to obtain advanced development from third-stage larvae* in roller culture systems consisting of 1 or 2 media with and without supplements of serum (S), glutathione (reduced) (G), or hemin (H), at 39°C.†

Culture system‡	No. of trials (and cultures)§	Nonsupplemented and supplemented media used during following periods of incubation:		Age (days) when cultures terminated
		Days 0–7	Day 7 on	
A	2 (4)	RPMI 1640+S	RPMI 1640+S+H	21, 35
B	2 (4)	KW-2	KW-2+H	30
C	3 (6)	API-18	API-18+H	21, 40, 48
D	2 (4)	API-1+G	API-1	32, 35
E	7 (40)	API-1+G	API-1+H	26, 32, 45, 54, 65, 80, 112
F	2 (4)	API-1+G	API-22+H	30, 40
G	4 (9)	API-1+G	API-23+H	30, 77, 80, 97
H	2 (5)	RPMI 1640+S	API-1+H	49, 54, 123
I	2 (4)	KW-2	API-1+H	30, 123
J	6 (16)	API-18	API-1+H	32, 42, 49, 70, 108, 116, 123, 140

* Recovered from the lungs of rabbits 7 days after oral inoculation with eggs.

† Cultures were prepared with 40 ml medium for first 7 days and 80 ml medium from day 7 on, with a gas phase of 85% nitrogen/5% oxygen/10% carbon dioxide, in roller bottles that were rotated at 1 rev./1.5 min.

‡ Systems A through J were tested with inocula of 5,000 L3; systems E and J were also tested with inocula of 2,500 and 10,000 L3.

§ A trial consisted of two or more cultures/trial.

API-1 + hemin. In system J, L3 advanced to early L4 in medium API-18 (Step 1) and completed development to adults in medium API-1 + hemin (Step 2).

The morphogenesis of larval and adult stages was similar in the 2 systems and identical to that obtained previously when L2 were used as a starting inoculum (Douvres and Urban, 1983). There was no apparent sex difference in the rate of development. Growth occurred in all stages of development (Table 4). In system E, the largest mature male and female were 90.0 and 150.0 mm long, respectively. In system J, the largest male and female were 110.0 and 140.0 mm long, respectively.

In cultures that contained mature males and females, both unfertilized and fertilized eggs were found in the medium, whereas only unfertilized eggs were found in cultures that contained only females. In 1 culture of system J, containing 1 mature female and 2 mature males, approximately 73,000 eggs had been produced by day 70 in culture and 73% of these were fertilized. The unfertilized eggs were identical to those produced previously by *A. suum* females grown in vitro from L2 (Douvres and Urban, 1983). They appeared in the medium singly or paired, but usually in clumps. Single eggs appeared elongated, ovoid, and somewhat asymmetrical, and were encased by a thin shell and outer refractive, sticky mammilated coat. Fertilized eggs appeared spherical or elliptical and were encased by a thick, mammilated shell and outer refractive, sticky (uterine) coat. They contained an unsegmented cell with condensed cytoplasm enveloped by a distinct membrane and an extruded polar body. In some eggs, the polar body was not visible.

Generally, morphogenesis of larval and adult stages in vitro was identical to that observed in vivo. The rate of development in vitro of late L3 to late L4 is similar to that obtained in vivo (Table 3). However, the continued development of L4 to adults was slower and the growth of larval and adult stages were retarded. Spermatogenesis, oogenesis, and egg-laying were also delayed in vitro (Tables 3 and 4). The in vitro-derived fertilized eggs were similar to in vivo-derived fertilized eggs (Alicata, 1936; Foor, 1967) except that they were somewhat smaller. Eggs obtained from in vitro-grown worms were 44–84 μm (average 64 μm) by 40–80 μm (average 53 μm), and eggs recovered from swine are 68–84 μm by 50–76 μm (Alicata, 1936).

Cultures included live, moribund, and dead nematodes in all phases of development, as well as L4 and young adults that showed incomplete ecdysis, i.e., part of the sheath remained attached to the body.

Copulation between mature adult males and females was observed on days 60 and 61 in a culture of system E. One mature male was completely wound or coiled about the body of a mature female (90 mm long) at the level of her vulva. The male's tail end, with the proximal portions of the spicules partially visible, was pressed against the vulva. The female's vigorous motility prevented confirmation of spicule insertion into the vulva. The pair remained in this position, for about 1.5 hr. At the end of this period, the male disengaged from the female by unwinding from the anterior to posterior end. There were no eggs visible in the culture medium at this time; however, on the next day, following a second observed copulation of a male and female, a few clumps of unfertilized eggs were present in the culture medium. Copulation was not observed subsequently, but both unfertilized and fertilized eggs were detected in the medium. The number of eggs increased from a few to several thousand by day 97 in culture. A total of 28,100 eggs were recovered on day 77 in culture; 33% of these were fertilized. Close examination of mature females from cultures containing fertilized eggs revealed an absence of a genital girdle or body construction at the level of the vulva. Mature females from culture with no mature males had a genital girdle.

Growth and development in systems G, H, and I were comparable to that obtained in systems E and J (Table 3). In systems H and I, development to mature adult males and females was obtained by day 53 in culture, but the females began egg-laying 4–10 days later. In system G, development to mature adults was slower by 6 days and egg-laying slower by 8–17 days than that obtained in systems E and J. In the 5 other systems tested, development was limited to larvae in fourth molt (4M) in systems C, D, and F, and to late L4 in systems A and B (Table 3). These stages were attained at the same rate as in systems E and J except development to 4M in system D occurred 4 days later. There were no apparent differences in the morphogenesis and growth of the larval and adult stages among the 10 systems.

SURVIVAL AND YIELDS OF ADVANCED STAGES: Data on survival and yields of advanced stages obtained in systems E and J at 7–28 days were similar (Table 5). Survival and yields of advanced stages were generally comparable between cultures within the same trial, but there were some differences between trials.

Table 3. *Ascaris suum*: Development from in vivo-grown late-third-stage larvae* to advanced stages in 10 1- and 2-step roller culture systems† and development in swine.‡

Culture system	Time (days) of earliest appearance to§:				
	Fourth stage		Fourth molt‖	Adult‖	
	Early	Late‖		Young	Mature
One-step system					
A	10	15	—¶	—	—
B	10	16	—	—	—
C	10	18	22#	—	—
D	10	15	25#	—	—
E	10	15	20	33	60
Two-step system					
F	10	15	20	—	—
G	10	15	20	31	66#
H	10	15	20	33	60
I	10	15	21	35	60
J	10	15	21	27	60
In swine	10	14	21	22	40

* Recovered from lungs of rabbits 7 days after oral inoculation with eggs.
† On basis of data obtained from 1 to 4 cultures/system.
‡ Data from Douvres and Urban (1983).
§ Time includes 7 days growth of larvae in vivo.
‖ Data apply to males and females.
¶ Denotes stage not attained.
# Stage rarely attained.

At day 7, survival rates were about 85%, but decreased to about 31–44% at day 28. The yields of advanced stages of surviving worms consisted of about 82–92% early L4 at day 7. At day 28, 30–41% of the surviving worms were early L4, 28–53% were late L4, 15–28% were 4M, and 2–4% were young adults (approximately 90–125 young adults/culture). Survival decreased to about 2–11% at day 42 and the worm population consisted of 8% and 32% early and late phases of L4, respectively, 44% 4M, and 16% young adults. Between 54 and 70 days, survival was less than 1% but the yields of advanced stages were generally comparable to those obtained at day 42. However, these cultures also contained a single mature male or female, or 1–3 mature adult males and 1–3 mature adult females.

The remarkable growth and development of 4M to adults in systems E and J after day 35 required a splitting of the worm population to overcome crowding and the massive build-up of organic acids released by 4M completing ecdysis to young adults (Douvres and Urban, 1983).

An inoculum of 10,000 L3 in system E generally had lower viability than cultures contain-

Table 4. *Ascaris suum*: Comparison of growth of advanced stages that developed from third-stage larvae* in the 2-step roller culture system J and development in swine.†

Stage of development‡	In vitro-grown worms		In vivo-grown worms‖	
	Age (days)§	Body length (mm)	Age (days)	Body length (mm)
Fourth: Early	14	1.7–4.2 (2.6)	10	1.9–2.5 (2.3)
Late	21	6.0–13.0 (9.3)	21–24	13.4–27.0 (19.7)
Fourth molt	28	9.0–17.0 (14.0)	24	27.2
Young adult: Early	35	17.0–25.0 (21.0)	23–24	22.0–35.6 (27.5)
Late	48	20.0–60.0 (38.4)	30	38.0–82.0 (61.4)
Mature adult: Males	64	65.0–67.0 (66.0)	43	116.0–152.0 (130.5)
	147	85.0–110.0 (98.0)		
Females	64	90.0–92.0 (90.0)	43	147.0–198.0 (168.4)
	147	95.0–140.0 (118.0)		

* Recovered from lungs of rabbits 7 days after oral inoculation with eggs.

† Data for each measurement based on range (and averages) for 20–30 specimens per stage up to young adults and 2 mature adult males and females grown in vitro.

‡ Include measurements of males and females.

§ Age in vitro includes 7 days growth of larvae in vivo.

‖ Data from Douvres and Urban (1983).

ing 2,500 or 5,000 L3 from day 7 through 28, but the yields of advanced stages were marginally greater. Survival and yields of advanced stages in systems H and I were comparable to systems E and J from day 7 through 28, but lower in system G. After day 7, survival rates and yields of advanced stages in systems A through D and F were comparable to systems E and J, but much lower thereafter.

Discussion

A 1-step roller culture system (Table 2, E) and four 2-step roller culture systems (Table 2, G, H, I, and J) supported the development of in vivo-derived *A. suum* late L3 to mature adults. Copulation between mature adults in vitro and the subsequent production of fertilized eggs were observed for the first time. Previously, the in vitro development of mature *A. suum* adult males, and females ovipositing unfertilized eggs was achieved using a starting inoculum of L2 hatched from eggs (Douvres and Urban, 1983). This development required a 3-step roller culture system consisting of 1) medium KW-2 plus 10 mM L-cysteine for the first 4 days and 5 mM L-cysteine for the next 7 days; 2) followed by medium API-18 for 7 days; and 3) thereafter, by medium API-1 plus 24 μg of bovine hemin/ml. A gas phase of 95% nitrogen/5% carbon dioxide was required for the first 4 days in culture, and thereafter 85% nitrogen/5% oxygen/10% carbon dioxide. Under these conditions L2 advanced to L3 and 3M in step 1, then to early L4 in step 2, and to young adults and mature adults in step 3. One of the critical elements in this system was the requirement for medium API-1 plus hemin to enhance development of *A. suum* L4 to adults. Likewise, medium API-1 plus hemin was essential to the development of mature adults from in vivo-derived late L3. For example, the transfer of early L4 that had developed in medium API-1 plus glutathione to API-1 plus hemin resulted in the development of mature adults (system E); but, when API-1 was substituted for API-1 plus hemin, development was limited to 4M (system D). Similarly, the transfer of early L4 that had developed in media RPMI 1640 plus serum, KW-2, or API-18 to medium API-1 plus hemin produced mature adults (systems H, I, and J, respectively); but, when medium API-1 plus hemin was replaced by either RPMI 1640 plus serum and hemin, KW-2 plus hemin, or API-18 plus hemin, development was no greater than 4M (systems A, B, and C, respectively). The critical components of medium API-1 plus hemin necessary for triggering the development of late L4 to adults were apparently part of medium API-1's complex mammalian tissue extracts and peptide digests, which were used to formulate medium API-23, and not in the defined components of API-1, which were used to prepare medium API-22 (Table 1). This conclusion is based on the fact that only API-23 plus hemin but not API-22 plus hemin could support development of in vivo-derived L3 to mature adults (systems G and F).

Table 5. *Ascaris suum*: Survival and yield of advanced stages that developed from in vivo-grown third-stage larvae* in 1-step (E) and 2-step (J) roller culture systems, at 7–28 days.†

Culture system	Media	Percent of total inoculum alive	Percent of live worms in stage‡:			
			Fourth			
			Early	Late	Fourth molt	Young adult
At 7 days						
E	API-1+G§	80–88 (86)‖	71–90 (82)	0	0	0
J	API-18	67–100 (85)‖	69–100 (92)	0	0	0
At 14 days						
E	API-1+H¶	60–69 (65)‖	62–77 (72)	19–37 (25)	+	0
J	API-1+H	44–82 (65)‖	46–100 (66)	0–39 (25)	0–16 (7)	0
At 21 days						
E	API-1+H	57–63 (60)	66–82 (74)	17–31 (24)	1–3 (2)	+
J	API-1+H	35–56 (47)	36–80 (64)	12–57 (30)	4–8 (6)	++
At 28 days						
E	API-1+H	32–50 (44)	3–48 (30)	45–63 (53)	3–35 (15)	1–5 (2)
J	API-1+H	20–49 (31)	16–65 (41)	19–35 (28)	10–35 (28)	0–11 (4)

* Recovered from lungs of rabbits 7 days after oral inoculation with eggs.

† Data based on ranges (and averages) of estimated percentages or actual counts from 4 to 8 cultures begun with 10,000 larvae/culture.

‡ + = 5 or fewer worms/culture; ++ = 6 to 10 worms/culture.

§ G = supplement of glutathione (reduced).

‖ Includes larvae in third stage and third molt.

¶ H = supplement of bovine hemin.

Optimal development of in vivo-derived L3 to mature adults was obtained in a 2-step system (system J) composed of the same media sequence and gas phase as the last 2 steps of the 3-step system that supported the development of L2 to mature adults (Douvres and Urban, 1983). Thus, mature adult worms of *A. suum* can be obtained in vitro from either L2 cultured in a 3-step roller culture system using two gas phases or from in vivo-derived L3 using a simplified 2-step system and a single gas phase. The other advantage of the latter system is that it can provide 4M, young adults, and mature adults in 14, 20, and 53 days in culture, respectively (Table 3). Similar development in the 3-step system takes 31, 34, and 67 days in culture, respectively (Douvres and Urban, 1983). Interestingly, in vivo-derived L3 can also develop to mature adults in a 1-step system (system E), which is essentially the same as that used to support the in vitro development of infective larvae of *Oesophagostomum radiatum* to adults (Douvres, 1983). These results indicate that common principles might be applied to the in vitro cultivation of phylogenetically distinct nematode parasite species that inhabit the mammalian intestinal milieu.

Copulation or mating of adult parasitic nematodes of vertebrates in vitro has only been observed in *Nematospiroides dubius* by Sommerville and Weinstein (1964). Others have attributed the in vitro production of segmented or early cleavage eggs by *Hyostrongylus rubidus* females (Leland, 1969) and by *Haemonchus contortus* females (Stringfellow, 1986) to parthenogenetic development because mating was not observed. Normal sexual reproduction of *A. suum* occurred in vitro in this study.

Mueller (1930) described copulation of a pair of *A. suum* adults obtained from the intestine of swine that had been fixed: "The tail end of the male grasped the female at the level of the vulva, making one circuit of the body. It then bent sharply and the rest of the worm extended forward along the anterior end of the female." In the present report, observations on mating between live mature adults occurring on 2 successive days were made. Mueller's description of worm copulation can be used to describe copulation of *A. suum* adults in vitro except that the male coiled or wound itself completely about the female at the level of the vulva. Based on histological preparations of the copulating pair of worms, Mueller found that the spicules were not inserted in the vulva and vagina of the female. He suggested that spicules may ". . . play the role of excitant organs, or enable the male in some

way to maintain proper position during copulation." Whether spicules were inserted into the vulva in vitro could not be determined because of the vigorous motility of the females.

Beaver and Little (1964) described the presence of a genital girdle or body constriction at the level of the vulva of *A. suum* females collected from swine with no adult males. Greater than 80% of these females had infertile eggs in the uterus. Females obtained from swine that also had mature males in the intestine lacked a genital girdle, and the majority had fertile eggs or abundant spermatozoa in the uterus. In the present study, females from a culture containing mature males lacked a genital girdle and laid infertile and fertile eggs. Females from cultures lacking mature males possessed a genital girdle and laid only infertile eggs. In a previous study (Douvres and Urban, 1983) only mature females passing infertile eggs in vitro had developed from a starting inoculum of L2. A creaselike indentation at the vulva of one of these females was identified as a morphologic abnormality. It is now apparent that this structure should have been identified as a genital girdle. Thus, copulation and production of fertile and infertile eggs by *A. suum* females in vitro is essentially identical to that of in vivo-derived worms.

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Literature Cited

Alicata, J. E. 1936. Early developmental stages of nematodes occurring in swine. Technical Bulletin Number 489, U.S. Department of Agriculture, December 1935. 96 pp.

Beaver, P. C., and M. D. Little. 1964. The genital girdle in relation to estrus and mating in *Ascaris lumbricoides*. Journal of Parasitology 50:128–130.

Douvres, F. W. 1983. The in vitro cultivation of *Oesophagostomum radiatum*, the nodular worm of cattle. III. Effects of bovine heme on development to adults. Journal of Parasitology 69:570–576.

———, **and G. M. Malakatis.** 1977. In vitro cultivation of *Ostertagia ostertagi*, the medium stomach worm of cattle. I. Development from infective larvae to egg-laying adults. Journal of Parasitology 63:520–527.

———, **and F. G. Tromba, and D. J. Doran.** 1966. The influence of NCTC 109, serum, and swine kidney cell cultures on the morphogenesis of *Stephanurus dentatus* to fourth stage in vitro. Journal of Parasitology 52:875–889.

———, ———, **and G. M. Malakatis.** 1969. Morphogenesis and migration of *Ascaris suum* larvae developing to fourth stage in swine. Journal of Parasitology 55:689–712.

———, **and J. F. Urban, Jr.** 1983. Factors contributing to the in vitro development of *Ascaris suum* from second-stage larvae to mature adults. Journal of Parasitology 69:549–558.

Foor, W. E. 1967. Ultrastructural aspects of oocyte development and shell formation in *Ascaris lumbricoides*. Journal of Parasitology 53:1245–1261.

Hansen, E. L., and J. W. Hansen. 1978. In vitro cultivation of nematodes parasitic in animals and plants. Pages 227–277 *in* A. E. R. Taylor and J. R. Baker, eds. Methods of Cultivating Parasites in Vitro. Academic Press Inc., New York.

Leland, S. E., Jr. 1969. Cultivation of the parasitic stages of *Hyostrongylus rubidus in vitro*, including the production of sperm and development of eggs through five cleavages. Transactions of the American Microscopical Society 88:246–252.

Mueller, J. F. 1930. The mechanism of copulation in the nematode *Ascaris lumbricoides*. Transactions of the American Microscopical Society 49: 42–45.

Pilitt, P. A., J. R. Lichtenfels, F. G. Tromba, and P. A. Madden. 1981. Differentiation of late fourth and early fifth stages of *S Ascaris suum* Goeze, 1782 (Nematoda: Ascaridoidea) in swine. Proceedings of the Helminthological Society of Washington 48:1–7.

Sommerville, R. I., and P. P. Weinstein. 1964. Reproductive behavior of *Nematospiroides dubius* in vivo and in vitro. Journal of Parasitology 50:401–409.

Stringfellow, F. 1986. Cultivation of *Haemonchus contortus* (Nematoda: Trichostrongylidae) from infective larvae to the adult male and egg-laying female. Journal of Parasitology 72: (In press).

Stromberg, B. E., P. B. Khoury, and E. J. L. Soulsby. 1977. Development of larvae of *Ascaris suum* from the third to the fourth stage in a chemically defined medium. International Journal of Parasitology 7:149–151.

Sylk, S. R., B. E. Stromberg, and E. J. L. Soulsby. 1974. Development of *Ascaris suum* larvae from the third to the fourth stage, in vitro. International Journal of Parasitology 4:261–265.

Taylor, A. E. R., and J. R. Baker. 1968. The Cultivation of Parasites in Vitro. Blackwell Scientific Publ., Oxford. Pp. 249–329.

Tromba, F. G. 1978. Immunization of pigs against experimental *Ascaris suum* infection by feeding ultraviolet attenuated eggs. Journal of Parasitology 64:651–656.

Urban, J. F., Jr., and F. W. Douvres. 1981. In vitro development of *Ascaris suum* from third- to fourth-stage larvae and detection of metabolic antigens in multi-well culture systems. Journal of Parasitology 67:800–806.

———, ———, **and Shoutai Xu.** 1984. Culture requirements of *Ascaris suum* larvae using a stationary multi-well system: increased survival, development and growth with cholesterol. Veterinary Parasitology 14:33–42.

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Survival of *Haemonchus contortus* Adults in Defined, Semi-defined, and Complex Cell-free Media

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ABSTRACT: Viable mature adult males and females of *Haemonchus contortus* recovered from sheep 25–30 days postinfection were maintained in 4 defined media, 6 semi-defined media, and 2 complex cell-free media, with a gas phase of 85% N_2/5% O_2/10% CO_2, in a roller culture system, for periods of 3–7 days. Optimal survival was obtained in 2 new semi-defined media, API-27 and API-28, when supplemented with bovine hemin. Survival in complex media KW-2 + hemin and API-1 + hemin and defined media RPMI 1640, API-24, API-25, and API-26, with and without hemin were inferior to survival obtained in semi-defined media RPMI 1640+25CS, API-22, API-27, and API-28, with and without hemin. These semi-defined media, when supplemented with hemin, supported 100% survival on day 1, 75–80% on day 2, and 57–79% on day 3. In contrast to the enhancing influence of hemin in semi-defined media, hemin had a slightly adverse effect on survival when defined media were used. These results show that there are media now available in which *H. contortus* adults can be maintained with high viability.

Haemonchus contortus (Rudolphi, 1803) is a blood-sucking nematode parasite of the abomasum of ruminants. Development of *H. contortus* in laboratory hosts is limited to fourth-stage larvae in mice and rabbits (Mapes and Gallie, 1977; Sommerville, 1977) and to young adults in rabbits (Wood and Hansen, 1960).

Previous attempts to grow advanced stages of *H. contortus* in vitro, from infective larvae have had varying degrees of success (Taylor and Baker, 1968; Hansen and Hansen, 1978; Stringfellow, 1984, 1986). Optimal development of a few *H. contortus* infective larvae to mature adult males and ovipositing females was obtained in a roller bottle system containing complex cell-free medium API-1 (Douvres and Malakatis, 1977) with supplements of bovine heme and ovine gastric juice (Stringfellow, 1986).

Survival of adult parasitic nematodes in vitro for more than 2 weeks has been demonstrated with species such as *Dirofilaria immitis* (Leidy, 1856), *Angiostrongylus cantonensis* (Chen, 1935), and *Stephanurus dentatus* (Diesing, 1839) as reported by Taylor and Baker (1968), Tromba and Douvres (1969), and Hansen and Hansen (1978). In contrast, adult *H. contortus* obtained from sheep can remain viable in vitro for only brief periods, i.e., 6–24 hr (Ward, 1974; Ward and Huskisson, 1978, 1980; Ward et al., 1981; Kaur and Sood, 1982; Rew et al., 1982).

Current interest in the biochemistry and immunochemistry of *H. contortus* warrants the need for in vitro techniques that could provide for extended survival of adults. The objective of the present study was to test defined, semi-defined, and complex media for their ability to support the survival of adult *H. contortus*.

Materials and Methods

Animal infection

Neutered Polled Dorset lambs (2–6 months old) raised helminth-free except for minimal infection with *Strongyloides papillosus* (Weld, 1856), were artificially infected with *H. contortus* (BPL isolate). Adult males and females of *H. contortus* were obtained from the abomasum of sheep that were orally inoculated with 5,000 or 10,000 infective larvae and killed 25–30 days after infection.

Preparation of adult inocula

At slaughter, the abomasum of sheep was opened, and the adult worms were removed from the mucosal surface with forceps and immersed in pre-warmed (37–39°C) 0.85% saline. Under aseptic conditions, the worms were washed extensively in Earle's Balanced Salt Solution containing antibiotics and an antimycotic agent (EBSSA) (Douvres and Malakatis, 1977). Freshly isolated worms incubated in warm EBSSA are almost always intertwined or clumped together. Accordingly, at each change of EBSSA the clumped worms were gently agitated to free them of abomasal mucus and ingesta. The tendency of the worms to clump makes it difficult to obtain inocula with a precise number of worms. However, preliminary work showed that worm counts of 100, 200, 300, etc., formed clumps of a particular size. Accordingly, before use as inocula, collections of cleaned worms were incubated in fresh EBSSA, in a covered Petri dish, for 1 hr, at 39°C and clumps

[1] Retired, January 1986.

judged to contain approximately 100 or 250 worms, were transferred to a culture vessel containing a test medium. Approximately 2.5 hr were required to recover the adult worms from the host and to prepare them for inoculation into the test media.

Preparation of media

Complex cell-free media API-1 (Douvres and Malakatis, 1977) and KW-2 (Douvres, 1970; Douvres and Urban, 1983) and semi-defined medium API-22 (Douvres and Urban, 1986) were prepared as described previously. Media API-1 and KW-2 were supplemented with bovine hemin (Hemin Type I: Bovine, Sigma Chemical Company, St. Louis, Missouri) at a final concentration of 24 mcg/ml. Medium API-22 was used as is, or supplemented with bovine hemin at 24 mcg/ml. Defined media NCTC 135 (GIBCO, Grand Island, New York) and Dulbecco's Modified Eagle's Medium (DMEM) (M. A. Bioproducts, Walkersville, Maryland), were each supplemented with 25% (v/v) heat-inactivated bovine calf serum (obtained from 4-month-old calves maintained helminth-free at the Animal Parasitology Institute) and bovine hemin at 24 mcg/ml (NCTC 135+25CS+H and DMEM+25CS+H). Defined medium RPMI 1640 (M. A. Bioproducts, Walkersville, Maryland) was used with or without a supplement of 25% (v/v) heat-inactivated bovine calf serum, and with and without 24 mcg bovine hemin/ml (RPMI 1640, RPMI 1640+H, RPMI 1640+25CS, RPMI 1640+25CS+H).

Defined media API-24, API-25, and API-26, and semi-defined media API-27 and API-28 are new formulations that evolved from modification of medium API-22 (Douvres and Urban, 1986) by eliminating serum (API-24), or by eliminating serum and 3 to 7 of its defined components (API-25, API-26, and API-27), or by eliminating 3 to 7 of its defined components (API-28).

Media API-22, API-24, API-26, API-27, and API-28 were selected for use in this study because preliminary data (F. W. Douvres, unpubl.) indicated that these media with the addition of hemin could support development of in vivo-derived (sheep) late-fourth-stage larvae of *H. contortus* to young adult males and females.

Media API-24, API-25, API-26, API-27 and API-28 when prepared as follows, provided for working media (1×). The sources of ingredients and the methods used to prepare sterile stocks of culture media were the same as those described previously (Douvres and Malakatis, 1977).

Fraction A: 720 ml of an aqueous mixture of 53 ingredients prepared by combining the following sterile stock solutions:

Solution 1: 50 ml of an aqueous solution that contained adenosine 5′-triphosphate-2-Na, 95 mg; yeast adenylic acid, 87 mg; cytidine 2′- and 3′-monophosphate (free acid), 77 mg; guanosine 2′- and 3′-monophosphate (Na salt), 101 mg; and thymine, 24.5 mg.

Solution 2: 20 ml of MEM-essential amino acids, 50×.

Solution 3: 10 ml of MEM-nonessential amino acids, 100×.

Solution 4: 20 ml of BME-vitamin solution, 100×.

Solution 5: 1 ml of aqueous 1% cyanocobalamine.

Solution 6: 0.75 ml of 0.5% p-aminobenzoic acid in aqueous triethanolamine.

Solution 7: 0.6 ml of a fat soluble vitamin mixture that contained vitamin A, 0.103 mg; vitamin D_2, 1.03 mg; vitamin E, 0.01 mg. The mixture is prepared by combining sterile stocks of vitamins A, K, and E (dissolved in aqueous 10% triethanolamine), and vitamin D_2 (dissolved in aqueous 4% Tween 80).

Solution 8: 1.5 ml of 5% N-acetyl-D-glucosamine.

Solution 9: 10 ml of aqueous 2.92% L-glutamine.

Solution 10: 25 ml of aqueous solution that contained dextrose, 700 mg; fructose, 180 mg; maltose, 850 mg; and trehalose, 350 mg.

Solution 11: 10 ml of an aqueous solution that contained L-cysteine $HCl \cdot H_2O$, 125 mg; and ascorbic acid, 50 mg.

Solution 12: 100 ml of an aqueous solution that contained NaCl, 3,774 mg; KCl, 222 mg; $NaH_2PO_4 \cdot H_2O$, 77.7 mg; $MgSO_4$ (separate), 55.5 mg; $CaCl_2$ (separate), 111 mg; and $NaHCO_3$, 1,680 mg. Aqueous solutions of required amounts of $MgSO_4$ and $CaCl_2$ were sterilized separately and then added to solution 12.

The 12 solutions are combined and the volume is adjusted to 720 ml with sterile, distilled water. The resulting solution can be used when freshly prepared or after storage for up to 6 mo at −20°C.

Fraction B: 20 ml of an aqueous solution that contained sodium pyruvate, 500 mg; malic acid, 300 mg; alpha-ketoglutaric acid, 200 mg; and fumaric acid, 0.36 mg.

Fraction C: 10 ml of an aqueous solution that contained sodium acetate, 350 mg; propionic acid, 100 mg; and butyric acid, 50 mg.

Fraction D: 250 ml of bovine calf sera, inactivated at 56°C for 30 min.

Medium API-25 (1×) was prepared with Fraction A to which distilled water was added to a volume of 1 liter. The 4 other media (1×) were prepared by combining the following fractions and adjusting the volume to 1 liter by adding distilled water: API-24, by combining Fractions A, B, and C; API-26, by combining Fractions A and B; API-27, by combining Fractions A and D; and API-28, by combining Fractions A, B, and D. Each of the 5 media was used as is, or supplemented with 24 mcg of bovine hemin/ml.

It is noted that the fractions described above can also be used to prepare medium API-22 (1×) (Douvres and Urban, 1986) by combining Fractions A, B, C, and D and adjusting the volume to 1 liter with distilled water.

All media contained 1,000 units/ml of penicillin G potassium, 1 mg/ml of streptomycin sulfate and 10 mcg/ml of Amphotericin B (Fungizone). All media were adjusted to pH 6.8 with the addition of either 1 N HCl or 1 N NaOH as needed.

Preparation and handling of cultures

Inocula of approximately 100 or 250 adults were used in a culture system that consisted of 20 ml of test medium with a gas phase of 85% nitrogen/5% oxygen/10% carbon dioxide, in roller bottles (205 mm long × 15 mm diameter, supplied with teflon-lined screw caps, Bellco Glass, Inc., Vineland, New Jersey) and rotated at 1 rev./1.5 min, at 39°C. The method of Douvres and Malakatis (1977) was used to gas the cultures.

Eleven maintenance trials involving different collections of adult worms were used to study the test media. Trials of similar design were repeated 2 or more times to evaluate the effectiveness of each test medium with inocula of approximately 100 adults (Tables 1 and 2). However, the effectiveness of media NCTC 135+25CS+H, DMEM+25CS+H, API-1+H, and KW-2+H were evaluated in duplicate cultures of 250 adults each in a single cultivation trial (Table 3); this trial included 2 additional test media (API-28+H and RPMI 1640+25CS+H). Another trial that tested the effectiveness of media API-28+H, RPMI 1640+25SC+H, API-25+H, API-26+H, API-27+H utilized inocula of 25 adults (Table 4). No changes of culture media were made prior to terminating a trial.

Criteria for evaluating survival and condition of *H. contortus* adults in vitro were similar to those described previously for *S. dentatus* (Douvres et al., 1966; Tromba and Douvres, 1969). Observations were made with an inverted microscope at 4 hr after inoculation, and thereafter daily to estimate worm condition, i.e., color and integrity of organs and body form, survival, motility, and degree of clumping. Survival was determined by loss of motility in body and head of the worms. Values for these observations were expressed as percent total. Cultures were terminated after 3–7 days (Tables 1–4) and the total inoculum from each culture was examined to quantitatively determine condition, degree of clumping, motility, and survival. Survival was the most definitive and least subjective criterion of a test medium on the adults. Therefore, prolongation of survival is considered the most important criterion of the effectiveness of a test medium on maintenance of adults.

In addition to the foregoing, the number of eggs and hatched larvae in extant cultures were estimated. Quantitative determinations of eggs and hatched larvae were made from cultures of media API-27 and API-28, with and without hemin, with inoculum of 100 adults, in 2 trials terminated on day 3 (Table 2).

Results

Mature adult male and female *H. contortus* that had been subjected to the washing procedure using EBSSA were identical to freshly isolated worms from sheep in that they were clumped and vigorously motile with pink to red pseudocoelomic fluid. The examination of 11 different collections of adult worms showed that the ratio of males to females was generally about 1:1.

Effectiveness of media on survival of adult worms

Survival of adult males and females was obtained in all maintenance trials of the test media (Tables 1–4) and the females survived better than the males. Optimal survival with inocula of 100 adults was obtained in semi-defined medium API-27+H (Tables 1 and 2) and with inocula of 250 adults in semi-defined medium API-28+H (Tables 3 and 4). Only results from these trials are described in detail.

The effectiveness of defined and semi-defined media, with and without hemin, on inocula of 100 worms is assessed from data given in Table 1 that were selected as representative from 2 or more replicate trials of the test media. Compar-

Table 1. *Haemonchus contortus* adults: Effects on survival, clumping, and motility with an inoculum of 100 adults per culture using defined and semi-defined media, with and without a supplement of hemin (H),† in a roller culture system.‡

Media§	Total inoculum alive (%)	Clumping of adults (%)	Motility of adults (%)‖ V	S
At day 0	100	99	100	0
At day 2				
*API-24	30	25	50	50
API-24+H	45	60	90	10
*API-25	20	0	50	50
API-25+H	20	25	50	50
*API-26	35	50	25	75
API-26+H	25	0	25	75
API-27	80	75	100	0
API-27+H	85	65	100	0
API-28	75	85	100	0
API-28+H	80	85	100	0
API-22	90	75	100	0
API-22+H	80	75	100	0
RPMI 1640+25CS	85	90	100	0
RPMI 1640+25CS+H	75	50	75	25
At day 3				
*API-24	8	0	0	100
API-24+H	20	0	0	100
*API-25	11	0	0	100
API-25+H	19	0	10	90
*API-26	16	0	25	75
API-26+H	11	0	10	90
API-27	70	25	90	10
API-27+H	79	75	100	0
API-28	58	50	100	0
API-28+H	66	75	100	0
API-22	66	75	100	0
API-22+H	70	75	100	0
RPMI 1640+25CS	54	75	100	0
RPMI 1640+25CS+H	57	50	75	25

† Hemin Type I: Bovine, at a concentration of 24 mcg/ml of medium.

‡ Based on duplicate cultures of approximately 100 adults each; worms obtained at 28–30 days after infection of sheep.

§ Media identified with (*) are defined; all others are semi-defined.

‖ Motility identified as V = vigorous and S = sluggish.

Table 2. *Haemonchus contortus*: Effects on survival, clumping, and motility of adults and yield of oviposited eggs and hatched larvae in roller cultures using semi-defined media API-27 and API-28, with and without a supplement of hemin (H),* after 3 days.†

Media	Total inoculum alive (%)	Clumping of adults (%)	Motility of adults (%)‡		Total number of eggs (and % normal)	Total number hatched larvae (and % alive)
			V	S		
At day 0	100	99	100	0	some present	none
With inoculum from sheep, 30 DAI§						
API-27+H	55	95	100	0	6,140 (67%)	2,480 (72%)
API-27+H	58	95	100	0	8,645 (55%)	2,880 (79%)
With inoculum from sheep, 25 DAI						
API-27+H	60	60	100	0	17,100 (79%)	9,900 (5%)
API-27+H	56	70	100	0	14,700 (55%)	7,500 (15%)
API-28	71	30	90	10	17,000 (46%)	9,100 (15%)
API-28+H	44	40	90	10	10,500 (35%)	6,700 (10%)

* Hemin Type I: Bovine, at a concentration of 24 mcg/ml of medium.
† Based on duplicate cultures of approximately 100 adults each.
‡ Motility identified as V = vigorous and S = sluggish.
§ DAI = days after infection.

isons of the data given in Table 1 showed that survival rate, degree of clumping, and motility were best in medium API-27+H. Up to the end of the first day of maintenance, the inoculum of 100 worms in medium API-27+H showed survival rates of 100%, maximal clumping (99%), and 100% motility; by day 3 (when cultures were terminated), survival decreased to 79%, clumping decreased to 75%, and motility remained vigorous (Table 1). Comparisons with these results in medium API-27+H showed that in API-27, without hemin, there were no differences in survival, clumping, and motility of worms up to the end of day 1; however, thereafter, the worms showed 5–10% less survival, 50% less clumping, and 10% less motility (Table 1). During the same periods, survival, clumping, and motility of worms in the other media were either comparable or lower than those obtained in medium API-27+H. Of these media, survival, clumping, and motility of worms were, with and without hemin, better in semi-defined media than in defined media, and best in semi-defined media API-22, API-28, and RPMI 1650+25CS, in that order (Table 1).

With all test media, differences in survival occurred occasionally between cultures within the same cultivation trial and between trials. These differences are exemplified by comparisons of data on survival, clumping, and motility of worms obtained in different trials with media API-27 and API-28, with and without hemin, given in Tables 1 and 2.

Comparisons of data obtained with inocula of 250 worms in two separate trials given in Tables 3 and 4 showed that survival rate, degree of clumping, and motility were best in medium API-28+H. These data show that there were differences in survival and clumping of worms in medium API-28+H during comparable periods between the 2 cultivation trials. Despite these differences, in both trials, up to the end of the first day, the worms showed survival rates of 100%, maximal clumping (99%), and 100% vigorous motility. The data given in Tables 3 and 4 show that by days 2 and 3 survival decreased to 80–90%, clumping was 25–99%, and motility was 100% and vigorous; and by days 6 or 7 (cultures terminated) survival decreased to 12–38%, clumping was absent or 50%, and motility was 75–95% vigorous and the remainder sluggish. During the same periods described for AP-28+H, survival, clumping, and motility of worms in the other media were either comparable or lower (Tables 3 and 4). Of these media, survival, clumping, and motility of worms were best in semi-defined media (RPMI 1640+25CS+H, NCTC 135+25CS+H, DMEM+25CS+H, and API-27+H), complex media (API-1+H and KW-2+H), and defined media (RPMI 1640+H, API-26+H, and API-25+H) in the order given.

Effectiveness of media on egg deposition by females and hatching of larvae

In all maintenance trials, with all test media, adult females deposited eggs in the media im-

Table 3. *Haemonchus contortus* adults: Effects on survival, clumping, and motility with an inoculum of 250 adults per culture using semi-defined and complex media with a supplement of hemin (H),† in a roller culture system.‡

Media§	Total inoculum alive (%)	Clumping of adults (%)	Motility of adults (%)‖ V	S
At day 0	100	99	100	0
At day 3				
*NCTC 135+25CS+H	65	75	50	50
*DMEM+25CS+H	50	25	75	25
*RPMI 1640+25CS+H	80	90	100	0
*API-28+H	90	99	100	0
API-1+H	75	90	90	10
KW-2+H	80	75	100	0
At day 7				
*NCTC 135+25CS+H	19	0	0	100
*DMEM+25CS+H	26	50	95	5
*RPMI 1640+25CS+H	27	0	95	5
*API-28+H	38	50	95	5
API-1+H	12	0	50	50
KW-2+H	23	50	75	25

† Hemin Type I: Bovine, at a concentration of 24 mcg/ml of medium.

‡ Based on duplicate cultures of approximately 250 adults each; worms obtained at 28 days after infection of sheep.

§ Media identified with (*) are semi-defined; others are complex.

‖ Motility identified as V = vigorous and S = sluggish.

Table 4. *Haemonchus contortus* adults: Effects on survival, clumping, and motility with an inoculum of 250 adults per culture using defined and semi-defined media with a supplement of hemin (H),† in a roller culture system.‡

Media§	Total inoculum alive (%)	Clumping of adults (%)	Motility of adults (%)‖ V	S
At day 0	100	99	100	0
At day 2				
*API-25+H	40	0	25	75
*API-26+H	50	0	100	0
API-27+H	65	25	100	0
API-28+H	80	25	100	0
*RPMI 1640+H	55	25	100	0
RPMI 1640+25S+H	50	25	100	0
At day 6				
*API-25+H	1	0	0	100
*API-26+H	5	0	25	75
API-27+H	7	25	90	10
API-28+H	12	0	75	25
*RPMI 1640+H	1	0	0	100
RPMI 1640+25S+H	6	0	0	100

† Hemin Type I: Bovine, at a concentration of 24 mcg/ml of medium.

‡ Based on duplicate cultures of approximately 250 adults each; worms obtained at 30 days after infection of sheep.

§ Media identified with (*) are defined; others are semi-defined.

‖ Motility identified as V = vigorous and S = sluggish.

mediately following introduction of worms; most of the egg deposition was generally completed by days 1–2. Almost all of the eggs were fertile and in various stages of cleavage when deposited. First-stage larvae appeared as early as day 1. In general, more eggs were deposited and more larvae hatched in defined media than in semi-defined media; however, survival of the larvae was considerably better in semi-defined media than in defined media. Some of the larvae developed to first molt (ensheathed), and ecdysis to second-stage larvae appeared to have occurred in semi-defined media such as API-27, API-28, and RPMI 1640+25CS.

Data on the actual number of eggs deposited and larvae that hatched from them in 2 trials with media API-27 and API-28, with and without hemin, at 3 days are given in Table 2. These data show that age of worms had an effect on the yield of eggs deposited by the females; the 25-day-old females deposited almost twice as many eggs as 30-day-old females (Table 2). However, essentially the same percentage (about 50%) of the eggs hatched with both age worms and there were minor differences in the number of eggs or yield of larvae between media.

Discussion

In the present study, viable mature adult male and female *H. contortus* from sheep have been maintained in defined, semi-defined, and complex media, in a roller culture system, for periods of 3–7 days. These media include 6 (NCTC 135, DMEM, RPMI 1640, KW-2, API-1, and API-22) that have been previously used for in vitro growth of nematodes such as *Oesophagostomum radiatum* (Rudolphi, 1803), *Ascaris suum* (Goeze, 1792), and *H. contortus* (Douvres, 1983; Douvres and Urban, 1983, 1986; Urban et al. 1984; Stringfellow, 1984, 1986, respectively). Five others (API-24, API-25, API-26, API-27, and API-28) are new formulations that evolved from modification of medium API-1 (Douvres and Malakatis, 1977). Our results show that optimal survival of adult *H. contortus* was obtained in semi-defined media API-27 + hemin and API-

28 + hemin, for a period of 3 days. Previous biochemical studies of *H. contortus* that required the maintenance of adults have shown that survival was limited to 6–24 hr, and optimal for 6 hr, when a ". . . medium similar to Tyrode's saline . . . to which extra NaCl was added . . ." was used (Ward, 1974; Ward and Huckisson, 1978, 1980). Ward et al. (1981) used the same medium for the maintenance of adult *Ostertagia circumcincta* (Stadelmann, 1894) and *H. contortus* and found that optimal survival was 72 hr for the former and 9 hr for *H. contortus*.

The beneficial effects of serum on survival and growth of advanced stages of parasitic nematodes are well established (Weinstein and Sawyer, 1961; Taylor and Baker, 1968; Tromba and Douvres, 1969; Hansen and Hansen, 1978). Although a rigorous analysis of the effects of serum on *H. contortus* survival was not performed it is clear that in the present study the in vitro survival of adult *H. contortus* is enhanced by the presence of serum. The survival rate of parasites in 4 serum-containing media (semi-defined media; API-22, API-27, API-28, and RPMI+25CS) was about 4 times higher than survival rate of adult worms maintained in comparable media that lacked serum (defined media; API-24, API-25, API-26, and RPMI 1640). It should be noted, however, that the positive effect of serum on *H. contortus* survival may be due to interaction of serum with one or more components of the various media rather than because of a direct effect of the serum.

The importance of heme, in some form, for the growth, reproduction, and development of nematodes is also well known (Bolla, 1979; Douvres, 1983; Douvres and Urban, 1983, 1986). In studies on the in vitro development of *H. contortus* from infective larvae in medium API-1, Stringfellow (1984, 1986) found that advancement from L4 to young adults required bovine heme in the form of Filde's reagent (peptic digest of defibrinated bovine blood). The present experiments indicate that survival of *H. contortus* adults after 3 days in either defined or semi-defined media is on average about 5% greater when hemin is present in the media. Although hemin was observed to have a relatively small effect on enhancing survival it seems reasonable to include hemin in the semi-defined media API-27 and API-28 as the best available media for in vitro survival of adult *H. contortus*. The observation that *H. contortus* is a blood-feeding parasite gives an additional reason for including hemin in the culture media. It is suggested that API-27 and API-28 with hemin supplements serve as the starting point for future experiments to extend the in vitro survival of *H. contortus*.

In the present study, we have described roller culture systems in which semi-defined media such as API-27 and API-28 can provide for survival of adult *H. contortus* from sheep for periods up to 7 days, but optimally for 3 days. Because our results show that worm populations maintained in this system included survivors that were similar in many respects to freshly isolated adults from sheep, it may be possible to use populations such as these for nutritional, biochemical, anthelmintic, or immunologic studies.

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Literature Cited

Bolla, R. I. 1979. Developmental nutrition of nematodes: the biochemical role of sterols, heme compounds, and lysosomal enzymes. Journal of Nematology 11:250–259.

Douvres, F. W. 1970. Cultivation of *Oesophagostomum radiatum* developmental stages in cell culture systems. Journal of Parasitology 56(Sect. II, Part I):83–84.

———. 1983. The in vitro cultivation of *Oesophagostomum radiatum,* the nodular worm of cattle. III. Effects of bovine heme on development to adults. Journal of Parasitology 69:570–576.

———, **and G. M. Malakatis.** 1977. In vitro cultivation of *Ostertagia ostertagi,* the medium stomach worm of cattle. I. Development from infective larvae to egg-laying adults. Journal of Parasitology 63:520–527.

———, **F. G. Tromba, and D. J. Doran.** 1966. The influence of NCTC 109, serum, and swine kidney cell cultures on the morphogenesis of *Stephanurus dentatus* to fourth stage in vitro. Journal of Parasitology 52:875–889.

———, **and J. F. Urban, Jr.** 1983. Factors contributing to the in vitro development of *Ascaris suum* from second-stage larvae to mature adults. Journal of Parasitology 69:549–558.

———, **and** ———. 1986. In vitro development of *Ascaris suum* from in vivo-derived third-stage larvae to egg-laying adults. Proceedings of the Helminthological Society of Washington 53:263–269.

Hansen, E. L., and J. W. Hansen. 1978. In vitro cultivation of nematode parasites in animals and plants. Pages 227–277 *in* A. E. R. Taylor and J. R. Baker, eds. Methods of Cultivating Parasites In Vitro. Academic Press Inc., New York.

Kaur, R., and Sood, M. L. 1982. *Haemonchus contortus*: the in vitro effects of DL-tetramisol and

rafoxanide on glycolytic enzymes. International Journal of Parasitology 12:585–588.

Mapes, C. J., and G. J. Gallie. 1977. The development of *Haemonchus contortus,* a nematode parasite of the ovine abomasum, in the laboratory rabbit. Parasitology 74:235–242.

Rew, R. S., C. Smith, and M. L. Colglazier. 1982. Glucose metabolism of *Haemonchus contortus* adults: effects of thiabendazole on susceptible versus resistant strain. Journal of Parasitology 68: 845–850.

Sommerville, R. I. 1977. Development of *Haemonchus contortus* to the fourth stage in an abnormal host, the laboratory mouse. Journal of Parasitology 63:952–954.

Stringfellow, F. 1984. Effects of bovine heme on development of *Haemonchus contortus in vitro.* Journal of Parasitology 70:989–990.

———. 1986. Cultivation of *Haemonchus contortus* (Nematode: Trichostrongylidae) from infective larvae to the adult male and egg-laying female. Journal of Parasitology 72: (In press).

Taylor, A. E. R., and J. R. Baker. 1968. The Cultivation of Parasites In Vitro. Blackwell Scientific Publication, Oxford. Pp. 249–329.

Tromba, F. G., and F. W. Douvres. 1969. Survival of juvenile and adult *Stephanurus dentatus* in vitro. Journal of Parasitology 55:1050–1054.

Urban, J. F., Jr., F. W. Douvres, and Shoutai Xu. 1984. Culture requirements of *Ascaris suum* larvae using a stationary multi-well system: increased survival, development and growth with cholesterol. Veterinary Parasitology 14:33–42.

Ward, P. F. V. 1974. The metabolism of glucose by *Haemonchus contortus, in vitro.* Parasitology 69: 175–190.

———, **W. J. Coadwell, and N. S. Huskisson.** 1981. The glucose metabolism of adult *Ostertagia circumcincta, in vitro.* Parasitology 82:17–22.

———, **and N. S. Huskisson.** 1978. The energy metabolism of adult *Haemonchus contortus, in vitro.* Parasitology 77:255–271.

———, **and** ———. 1980. The role of carbon dioxide in the metabolism of adult *Ostertagia circumcincta, in vitro.* Parasitology 80:73–82.

Weinstein, P. P., and T. K. Sawyer. 1961. Survival of adults of *Dirofilaria uniformis* in vitro and production of microfilaria. Journal of Parasitology 47(Suppl.):23–24.

Wood, I. B., and M. F. Hansen. 1960. Experimental transmission of ruminant nematodes of the genera *Cooperia, Ostertagia,* and *Haemonchus* to laboratory rabbits. Journal of Parasitology 46:775–776.

Proc. Helminthol. Soc. Wash.
53(2), 1986, pp. 270–276

Pseudaprocta samueli sp. n. (Nematoda: Aproctoidea) from the Blue Jay (*Cyanocitta cristata*) in Canada, with Comments on the Genus

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ABSTRACT: *Pseudaprocta samueli* sp. n. is described from the thoracic air sac of 1 of 20 blue jays (*Cyanocitta cristata* (L.)) collected near Guelph, Ontario, Canada and this apparently represents the first report in the literature of *Pseudaprocta* in the New World. Specimens of *Pseudaprocta,* unidentifiable to species, from a blue jay in Alabama, U.S.A., had, however, been deposited in the United States National Museum in 1954. *Pseudaprocta samueli* belongs to that group of species within the genus that have short spicules (<500 μm) and 10–11 pairs of caudal papillae. It is distinguished by the irregular spacing of the cephalic spines and the presence of inflated cuticle in the caudal region of the female. An annotated list of and key to species in *Pseudaprocta* are given. The identities of many of the species reported in Eurasia require clarification.

Aproctoids are oviparous nematode parasites of the air sacs and orbital sinuses of birds. Their relationship to other superfamilies in the order Spirurida is not well understood although an origin from Seuratoidea (Ascaridida) has been suggested (Chabaud, 1974; Bain and Mawson, 1981). Among the 7 recognized aproctoid genera, the genus *Pseudaprocta* Shikhobalova, 1930 is easily distinguished by the presence of small spines on the cephalic extremity. These spines form a lobed pattern that Anderson and Bain (1976) referred to as "cordons" although they are not homologous to the cordons found in Acuarioidea. Species of *Pseudaprocta* have been reported in Europe, Asia, Madagascar, and Australia but seldom in large numbers. The transmission of species of *Pseudaprocta* has not been studied although presumbly it is similar to that of species of *Aprocta* (see Quentin et al., 1976).

The present study describes a new species from blue jays (*Cyanocitta cristata*) in Canada and this apparently represents the first report of the genus in the New World. A brief taxonomic review of *Pseudaprocta* is also presented herein.

Materials and Methods

Blue jays collected near Guelph, Ontario, Canada, from 1979 to 1983 were examined for nematodes. Nematodes found were fixed in hot 5% glycerin–70% alcohol and cleared for examination in glycerin; those found in the thoracic air sacs represented a new species of *Pseudaprocta* described herein.

Specimens of *Pseudaprocta* in the WHO Collaborating Centre for Filarioidea Collection at the Commonwealth Institute of Parasitology (CIP) in St. Albans, England, and in the Helminth Collection of the United States National Museum (USNM) in Beltsville, Maryland, U.S.A., which were borrowed and examined included the type specimens of *P. buckleyi* (CIP Nos. B11 and H16) and specimens identified as *P.* ? *decorata* (USNM No. 49305), *P. decorata* (USNM. No. 70593), *P. gubernacularia* (USNM No. 70597), and *P. sichotealinensis* (USNM No. 70596).

Results

Pseudaprocta samueli sp. n. (Figs. 1–19)

GENERAL: Spirurida, Aproctoidea, Aproctidae, Aproctinae, *Pseudaprocta* Shikhobalova, 1930. Stout nematodes, width uniform over most of length but tapering towards bluntly rounded extremities. Cuticle with delicate transverse striations. Cephalic extremity (Fig. 12) with inconspicuous amphids, 4 large double papillae, 1 obscure papilla beside each amphid, and large oral opening (Fig. 13). Cephalic spines delicate, forming lobed but variable pattern around papillae and amphids (Figs. 2, 10, 11); distance between spines variable. Delicate cuticular line present along posteriormost aspects of lobes (not visible in en face view). Buccal cavity present (Fig. 9), walls of cavity formed of cuticle similar in appearance to that of body. Esophagus well developed, muscular throughout (Figs. 1, 8). Esophageal–intestinal junction distinct. Excretory pore and vesicle conspicuous, excretory canals in lateral chords (Fig. 19) readily visible. Deirids obscure, posterior to excretory pore, 1 10–20 μm anterior to other.

MALE (2 specimens, measurements of holo-

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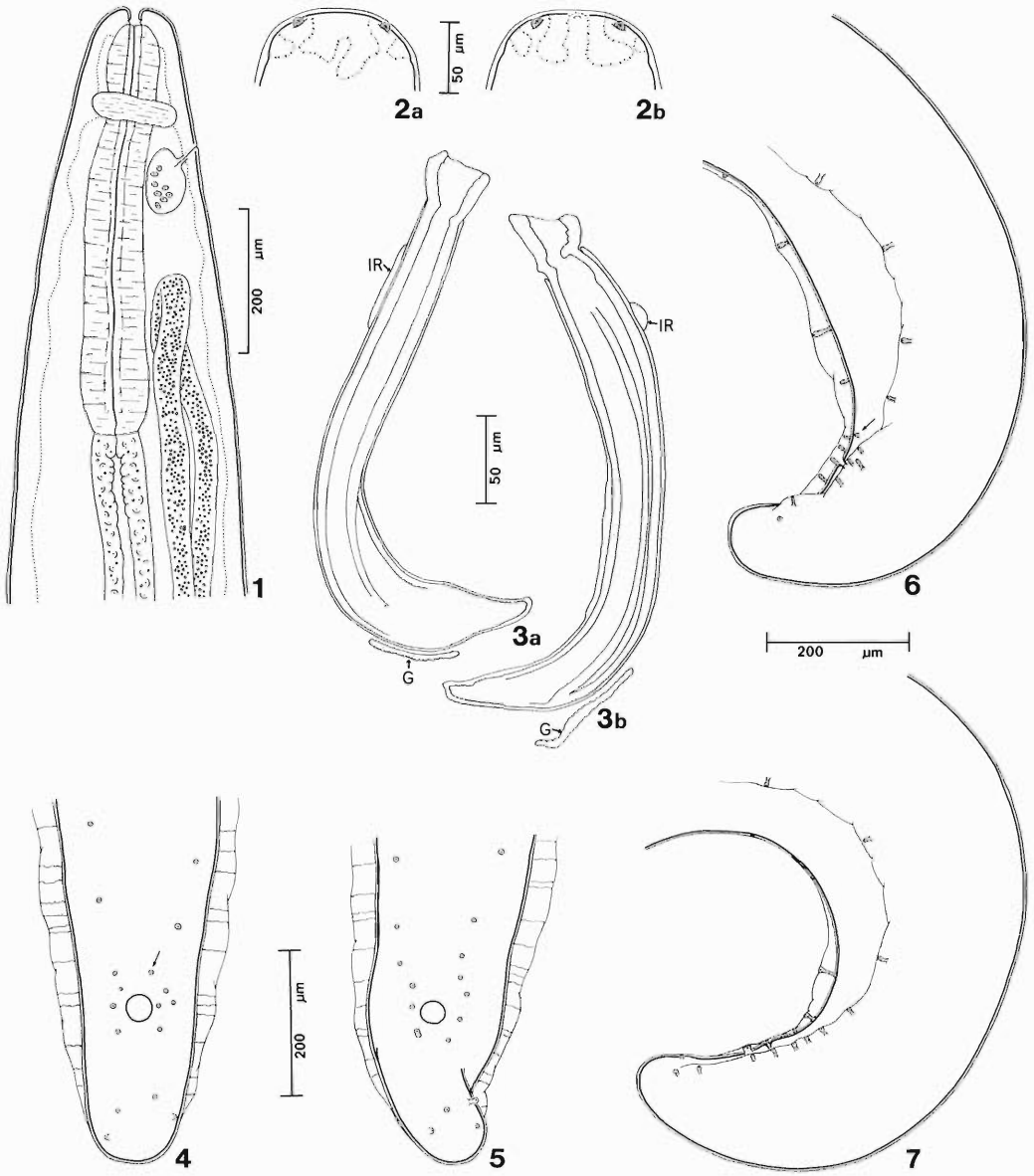
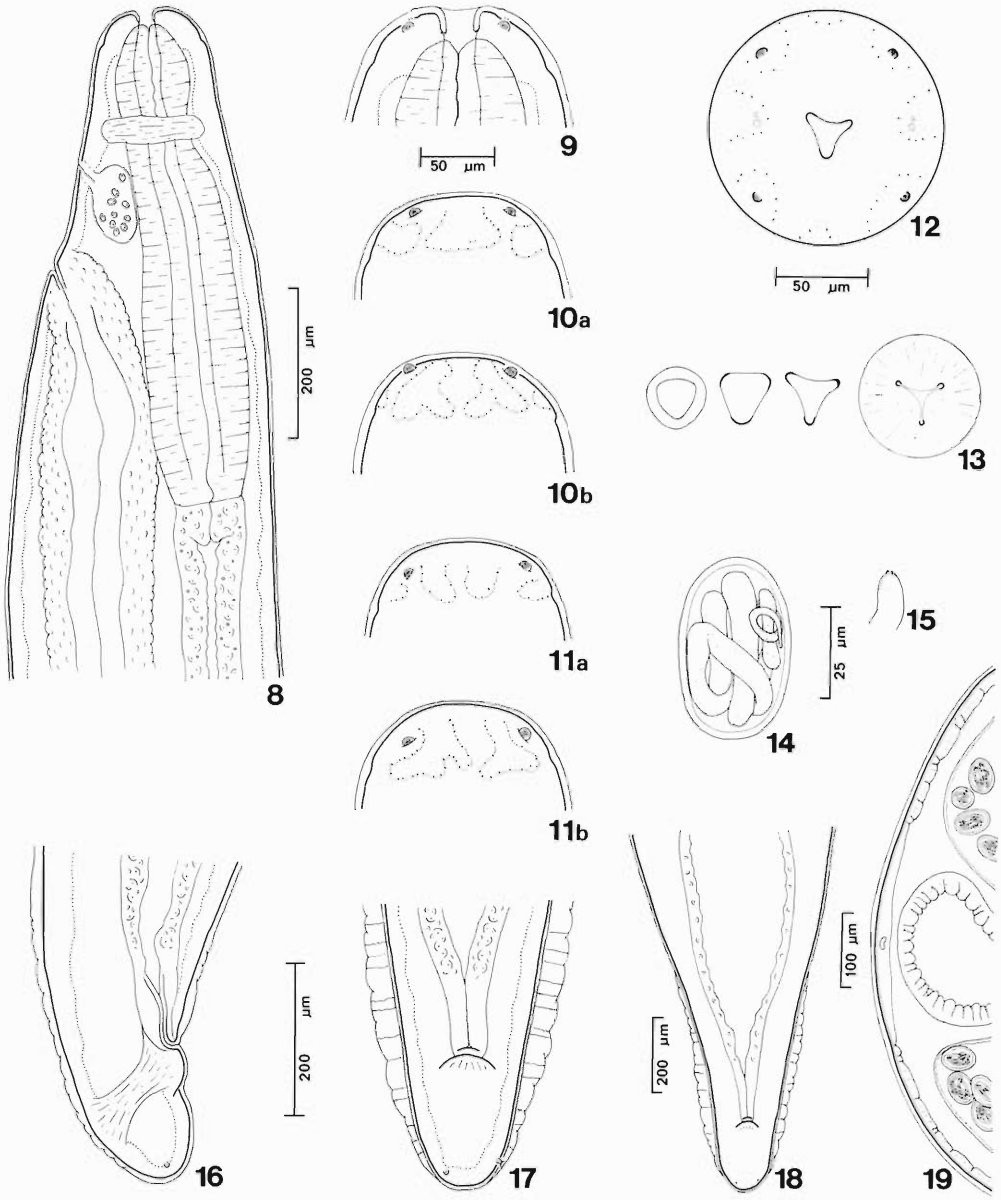


Figures 1–7. ***Pseudaprocta samueli*** **sp. n., male (2 specimens). 1. Anterior end, lateral view (holotype). 2a, b. Anterior extremity, ventral (a) and lateral (b) surface views (holotype). 3a, b. Spicules, right (a) and left (b), each with associated portion of gubernaculum (G); note irregular thickenings (IR) adjacent to proximal portions of spicules (paratype). 4. Posterior end, ventral view, arrow indicates unpaired papilla (holotype). 5. Posterior end, ventral view (paratype). 6. Posterior end, lateral view, arrow indicates unpaired papilla (holotype). 7. Posterior end, lateral view (paratype).**

type followed by paratype): Length 14, 11 mm. Maximum width 520, 380 µm. Taper of body gradual at both ends. Cuticle thin over anterior 1–2 mm of body, becoming gradually inflated over remainder of body. Inflation greatest in lateral, ventrolateral, and dorsolateral regions. Inflation conspicuous in caudal region where it resembles caudal alae (Figs. 6, 7). Numerous radial striations running between inner and outer portion of inflated cuticle (Figs. 4, 5) and appearing as minute dots at surface. Buccal cavity 20, 20 µm long and 15, 18 µm wide. Nerve ring 120,



Figures 8–19. ***Pseudaprocta samueli*** **sp. n., female (2 gravid specimens). 8. Anterior end, lateral view (allotype). 9. Anterior extremity, ventral view (allotype). 10a, b. Anterior extremity, ventral (a) and lateral (b) surface views (allotype). 11a, b. Anterior extremity, ventral (a) and lateral (b) surface views (paratype). 12. Anterior extremity, en face view (paratype). 13. Oral opening (left) and views at increasing depths in *buccal* cavity to level of esophagus (right) (paratype). 14. Egg from uterus of paratype. 15. Anterior end of larva within egg from uterus of paratype. 16. Posterior extremity, lateral view (allotype). 17. Posterior extremity, ventral view (allotype). 18. Posterior end showing extent of cuticular inflation in caudal region (allotype). 19. Partial transverse section at midbody (paratype).**

155 µm and excretory pore 190, 180 µm from anterior extremity. Excretory vesicle voluminous with numerous oval inclusions (Fig. 1). Deirids 250–285 µm from anterior extremity. Length of esophagus 580, 520 µm; maximum width 100, 100 µm. Monorchic, testis located in anterior ¼–⅓ of body. Tail in 1–2 spiral turns. Anus 215, 200 µm from posterior extremity. Pre-,

ad-, and post-anal papillae present; 10 ventrolateral pairs and 1 median unpaired in holotype (Fig. 4), 11 ventrolateral pairs in paratype (Fig. 5) (note: the variation occurs in the number and position of the papillae near the anus, and papillae 8 and 9 on the right side are fused in the paratype). Pair of papillae closest to caudal extremity generally more lateral in position than others (note: this is not true of the posteriormost papilla on the left side in the paratype). Spicules slightly dissimilar in morphology and length, left (Fig. 3b) 330, 340 μm long, right (Fig. 3a) 320, 335 μm long; strongly cuticularized over most of length but distal region lightly cuticularized. Irregular thickenings sometimes present in proximal portions of spicular pouches (Fig. 3). Gubernaculum present, formed by irregular thickening of distal and common portions of spicular pouches (Fig. 3), readily visible.

FEMALE (3 specimens, measurements of allotype (gravid) followed by 2 paratypes [gravid and nongravid, respectively]): Length 26, 27, 26 mm. Maximum width 730, 670, 480 μm. Taper of body gradual at anterior end, abrupt at posterior end of gravid females but gradual at posterior end of nongravid female. Cuticle thin over most of body (Fig. 19), becoming conspicuously inflated in lateral regions of posterior ½ mm of body (Fig. 18) and resembling, as in male, caudal alae with numerous radial striations (Fig. 17). Inflation more marked in gravid females than in nongravid female. Buccal cavity 18, 20, 18 μm long and 18, 18, 10 μm wide. Nerve ring 150, 125, 130 μm and excretory pore 225, 180, 220 μm from anterior extremity. Excretory vesicle voluminous with numerous oval inclusions in gravid females (Fig. 8) but deflated without inclusions in nongravid female. Deirids 240–280 μm from anterior extremity. Length of esophagus 670, 680, 620 μm; maximum width 140, 125, 100 μm. Vulva conspicuous, forming swelling 375, 275, 400 μm from anterior extremity (Fig. 8). Vagina 3.5 mm long in allotype. Didelphic and opisthodelphic. One ovary located in posterior ¼ of body, other in anterior ¼. Loop of one oviduct present near vulva (presence not illustrated in Fig. 8). Anus 180, 180, 200 μm from posterior extremity. Large swelling present immediately posterior to anus (Fig. 16). One pair small, subterminal papillae present.

EGG (5 specimens, from anterior vagina of allotype): Shell smooth, thick, oval in shape 48–52 μm long by 30–34 μm wide (Fig. 14). Larva in numerous coils, extremities bluntly rounded, anterior end (Fig. 15) difficult to locate, tail long and tapering.

TYPE HOST: Blue jay, *Cyanocitta cristata* (L.) (Corvidae).

LOCATION IN HOST: Thoracic air sac.

TYPE LOCALITY: Guelph, Ontario, Canada.

INTENSITY AND PREVALENCE: Five worms in 1 of 20 blue jays.

SPECIMENS: Deposited in the Helminth Collection of the United States National Museum, Beltsville, Maryland: USNM Nos. 79135 (holotype), 79136 (allotype), 79137 (paratypes).

ETYMOLOGY: The species is named in honor of Professor W. M. Samuel, Department of Zoology, University of Alberta, Edmonton, Alberta, Canada.

DIAGNOSIS: *Pseudaprocta samueli* is closely related to *P. decorata, P. buckleyi,* and *P. ungriai.* However, in *P. samueli* the cephalic spines are numerous and the interval between them is irregular (Figs. 2, 11, 12), in *P. decorata* they are also numerous but the interval between them is regular (see fig. 1 in Li [1933] and figs. 1c and 1d in Jaroń [1967]), and in *P. buckleyi* they are few and obscure. Inflated cuticle occurs at the posterior end of the female in *P. samueli* but this has not been reported in *P. decorata* and it is absent in *P. buckleyi. Pseudaprocta ungriai* was described on the basis of 1 damaged male specimen and the number and spacing of the cephalic spines were not determined.

Species in *Pseudaprocta*

The 11 known species on *Pseudaprocta,* their type hosts, and type localities are listed below. Additional hosts are listed only if they were given by the author(s) who described the species, if the report was accompanied by an adequate description, or if we were able to confirm the identification by examining specimens. Other reports are discussed later (see Discussion).

1. *P. gubernacularia* Shikhobalova, 1930 (type species)

TYPE HOST: *Garrulus glandarius krynicki* Kaleniczenko (Corvidae).

TYPE LOCALITY: Vladikavakase, Caucasus, U.S.S.R.

COMMENTS: The females described by Shikhobalova (1930) were not gravid.

2. *P. decorata* Li, 1933

TYPE HOST: *Corvus corax* L. (by virtue of first listing) (Corvidae).

TYPE LOCALITY: Beijing, China.

ADDITIONAL HOSTS: Li (1933): "*Pica caudata,*" presumably *Pica pica* (L.) (Corvidae), China; Jaroń (1967): *Delichon urbica* (L.) (Hirundinidae), Poland.

COMMENTS: Sonin (1966) considered *P. buckleyi* a synonym of *P. decorata*; however, Jaroń (1967) redescribed *P. decorata* and considered *P. buckleyi* distinct. Also, see comments under *P. buckleyi.*

3. *P. myzanthae* Johnston and Mawson, 1940

TYPE HOST: *Manorina flavigula* (Gould) (=*Myzantha flavigula*) (Meliphagidae).

TYPE LOCALITY: "75 miles north" of Renmark, Australia.

COMMENT: The male has not been described.

4. *P. buckleyi* (Singh, 1949) Yeh, 1957

TYPE HOST: *Copsychus saularis* (L.) (Muscicapidae).

TYPE LOCALITY: Hyderabad State, India.

COMMENTS: Re-examination of the type specimens (CIP) Nos. B11 and H16 confirmed Yeh's (1957) statement that cephalic spines are present. They are, however, minute, difficult to discern, and not numerous (cf. *P. decorata*; cf. Yeh, 1957). The cuticle at the posterior end of the females is not inflated.

5. *P. sichotealinensis* Oshmarin and Belous, 1951

TYPE HOST: *Oriolus chinensis* L. (Oriolidae).

TYPE LOCALITY: Maritime Territory, U.S.S.R.

ADDITIONAL HOST: Schmidt and Kuntz (1970): *Oriolus chinensis diffusus* Sharpe (Oriolidae), Taiwan (USNM No. 70596).

6. *P. skrjabini* (Ali, 1956) Yamaguti, 1961

TYPE HOST: *Oriolus oriolus* (L.) (Oriolidae).

TYPE LOCALITY: Hyderabad, India.

7. *P. leiperi* (Rasheed, 1960) Chabaud, Brygoo, and Richard, 1964

TYPE HOST: *Dicrurus macrocerus* (Vieillot) (Dicruridae).

TYPE LOCALITY: Hyderabad, India.

8. *P. mirzai* Sultana, 1964

TYPE HOST: *Motacilla maderaspatensis* Gmelin (Motacillidae).

TYPE LOCALITY: Hyderabad, India.

9. *P. ungriai* Chabaud, Brygoo, and Richard, 1964

TYPE HOST: *Dicrurus forficatus* (L.) (Dicruridae).

TYPE LOCALITY: Périnet, Madagascar.

COMMENT: The female has not been described.

10. *P. copemani* Bain and Mawson, 1981

TYPE HOST: *Petroica multicolor* (Gmelin) (Muscicapidae).

TYPE LOCALITY: Maggs Mt., Tasmania, Australia.

ADDITIONAL HOST: Bain and Mawson (1981): *Pachycephala pectoralis* (Latham) (Muscicapidae), Australia.

11. *P. samueli* sp. n.

TYPE HOST: *Cyanocitta cristata* (L.) (Corvidae).

TYPE LOCALITY: Guelph, Ontario, Canada.

COMMENTS: In *P. samueli* it is apparent that neither the pattern of cephalic spines nor the number and pattern of papillae near the anus can be assumed to be stable intraspecific characters. Specimens of *Pseudaprocta* in the USNM (No. 49305) from *C. cristata* collected near Auburn (?), Alabama, U.S.A., in 1954 by R. E. Thorson may be conspecific with *P. samueli.* Unfortunately, however, their poor condition precludes specific identification.

Key to Species[2]

1 (8). Spicules <500 μm.

2 (5). Terminal nipple-like structure (="papilla" or "spine") present on tail of male.

3 (4). Five pairs caudal papillae; caudal alae[3] not reported in male ..... *P. gubernacularia*

4 (3). Thirteen pairs caudal papillae; caudal alae present in male, delicate and narrow ..... *P. mirzai*

5 (2). Terminal nipple-like structure absent on tail of male; caudal alae present in male, delicate and narrow.

6 (7). Twelve pairs caudal papillae of which 3 pairs occur near end of tail; caudal alae conspicuous considerably anterior to anteriormost caudal papillae ... *P. leiperi*

[2] The male of *P. myzanthae* is not known and the species is not included.

[3] The term "alae" has been used throughout the *Pseudaprocta* literature, but these "alae" may be inflated cuticle as described in *P. samueli.*

7 (6). Ten to 11 pairs caudal papillae of which 2 pairs occur near end of tail; caudal alae first becoming readily apparent near anteriormost caudal papillae ..... 4 closely related species:

(i) *P. decorata:* Cephalic spines numerous and readily visible, interval between spines tending to be regular; spicules subequal, 339–390 μm long; caudal papillae near anus tending to be slightly spaced; gubernaculum strongly cuticularized; caudal alae not reported in female.

(ii) *P. buckleyi:* Cephalic spines few in number and difficult to discern; spicules subequal, 386–422 μm long; caudal papillae near anus tending to be close together; gubernaculum weakly cuticularized; caudal alae absent in female.

(iii) *P. ungriai:* Anterior end of only specimen damaged but cephalic spines present; caudal papillae near anus slightly spaced; spicules equal, 400 μm long; gubernaculum strongly cuticularized; female not known.

(iv) *P. samueli:* Cephalic spines less numerous than in *P. decorata* but readily visible, interval between spines variable; caudal papillae near anus slightly spaced or close together; spicules slightly subequal 320–340 μm long; gubernaculum strongly cuticularized; caudal alae present in female.

8 (1). Spicules >500 μm.

9 (12). Caudal papillae near anus tending to be close together.

10 (11). Caudal alae present in male, delicate and narrow; spicules 566–599 μm long ..... *P. skrjabini*

11 (10). Caudal alae not reported in male; spicules 520–580 μm long ..... *P. sichotealinensis*

12 (9). Caudal papillae near anus relatively well spaced; spicules 550–640 μm long ..... *P. copemani*

Discussion

There are, in addition to the references listed previously, reports of *P. gubernacularia* in Corvidae in Czechoslovakia (Skarda, 1964) and the U.S.S.R. (Kurashvili, 1983) and in Muscicapidae in Taiwan (Schmidt and Kuntz, 1970), of *P. decorata* in Corvidae in the U.S.S.R. (Oshmarin, 1950; Bashkirova, 1960; Shumilo, 1966; Kurashvili, 1983), in Sturnidae in the U.S.S.R. (Sonin, 1961; Kurashvili, 1983), and in Muscicapidae in Malaysia (Schmidt and Kuntz, 1970), and of *P. sichotealinensis* in Oriolidae in the U.S.S.R. (Sonin, 1963) and in Motacillidae in the U.S.S.R. (Bashkirova, 1960). It seemed prudent, however, not to include these reports in the annotated list of species given previously because the identifications may not be correct. For example, the 3 anteriormost pair of papillae on the tail of the male illustrated and identified as "*P. decorata*" in Sonin (1961) are much closer to the anus than indicated in the original species description. Also, we examined the "*P. decorata*" and "*P. gubernacularia*" specimens mentioned by Schmidt and Kuntz (1970) (USNM Nos. 70593 and 70597, respectively) and found that the caudal morphology of the males did not correspond to that in the original species description; a terminal nipple-like structure was present on the tail of the male in the "*P. decorata*" specimens, but not in the "*P. gubernacularia*" specimens. Specimens or illustrations are not available for the other reports of *Pseudaprocta* from Eurasia.

Jaroń (1967) noted that the prevalence of species of *Pseudaprocta* in wild birds is frequently low; his finding of *P. decorata* in 1 of 46 swallows in Poland is similar to that of *P. samueli* in 1 of 20 blue jays in Canada. From an epizootiological point of view, it is difficult to understand how parasites can exist in an avian population in such low prevalences. Species of *Pseudaprocta* possibly are not highly host specific (this is suggested for example by the fact that *P. decorata* has been conclusively shown to occur in Corvidae and Hirundinidae) and they might occur in avian communities rather than being restricted to a particular host species. Bartlett and Anderson (1980) suggested an avian community hypothesis to explain the variable levels of prevalence of filarioid nematodes frequently observed in birds and stated that in such a community there may be both reservoir hosts and sporadic hosts. Low prevalences of infection would be expected in the sporadic hosts and it may be that swallows and blue jays are simply sporadic hosts of *P. decorata* and *P. samueli,* respectively. The reservoir hosts remain to be identified.

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Literature Cited

Ali, S. M. 1956. Studies on the nematode parasites of fishes and birds found in Hyderabad State. Indian Journal of Helminthology 8:1–83.

Anderson, R. C., and O. Bain. 1976. Keys to the genera of the order Spirurida. Part 3. Diplotriaenoidea, Aproctoidea, and Filarioidea. Pages 59–116 *in* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 3. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England.

Bain, O., and P. M. Mawson. 1981. On some oviparous filarial nematodes mainly from Australian birds. Records of the South Australian Museum 18(13):265–284.

Bartlett, C. M., and R. C. Anderson. 1980. Filarioid nematodes (Filarioidea: Onchocercidae) of *Corvus brachyrhynchos bracyrhynchos* Brehm in southern Ontario, Canada and a consideration of the epizootiology of avian filariasis. Systematic Parasitology 2:77–102.

Bashkirova, E. Y. 1960. The nematode fauna of birds of the Primorsk region. (In Russian.) Trudy Gel'mintologicheskoi Laboratorii. Akademiya Nauk SSR 10:46–57.

Chabaud, A. G. 1974. Keys to subclasses, orders and superfamilies. Pages 6–17 *in* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England.

———, **E. R. Brygoo, and J. Richard.** 1964. Filaires d'oiseaux malgaches (Deuxième note). Annales de Parasitologie Humaine et Comparée 39:69–94.

Jaroń, W. 1967. A new species in Poland of a nematode, *Pseudaprocta decorata* Li, 1933, in *Delichon urbica* (new host). Acta Parasitologica Polonica 14: 219–224.

Johnston, T. H., and P. M. Mawson. 1940. Some filarial parasites of Australian birds. Transactions of the Royal Society of South Australia 64:355–361.

Kurashvili, B. E. 1983. Nematoda and Acanthocephala of Birds from Black Sea and Caspian Sea Areas. (In Russian.) "Metsniereba," Tbilisi, U.S.S.R.

Li, H. C. 1933. Report on a collection of parasitic nematodes, mainly from North China. I. Filarioidea. Parasitology 25:192–224.

Oshmarin, P. G. 1950. Filariae in animals in the southern zone of the Far East. (In Russian.) Trudy Gel'mintologicheskoi Laboratorii. Akademiya Nauk SSR 3:180–190.

———, **and E. V. Belous.** 1951. Notes on the filariae of wild animals. (In Russian.) Trudy Gel'mintologicheskoi Laboratorii. Akademiya Nauk SSR 5:121–127.

Quentin, J. C., P. M. Troncy, and N. Barre. 1976. *Aprocta cylindrica* Linstow, 1883, filaire ovipare d'oiseaux plocéidés au Tchad. Morphogénèse larvaire du nématode. Annales de Parasitologie Humaine et Comparée 51:83–93.

Rasheed, S. 1960. The nematode parasites of the birds of Hyderabad (India). Biologia, Lahore 6:1–116.

Schmidt, G. D., and R. E. Kuntz. 1970. Nematode parasites of Oceanica. VIII. Filariids of birds, with a new genus and four new species. Parasitology 60:313–326.

Shikhobalova, N. 1930. Sur une nouvelle filaire d'oiseaux: *Pseudaprocta gubernacularia* n. g., n. sp. Annales de Parasitologie Humaine et Comparée 8:624–627.

Shumilo, R. P. 1966. Helminths of synanthropic birds of central Moldavia. Parazity Zhivotnykh i Rastenii. Akademiia Nauk Moldavskoi SSR. Institut Zoologii 2:120–138.

Singh, S. N. 1949. Studies on the helminth parasites of birds in Hyderabad State. Nematoda IV. Journal of Helminthology 23:39–56.

Skarda, J. 1964. The helminthofauna of some wild birds in Czechoslovakia. (In Czechoslovakian.) Sbornik Vysoké Školy Zemědělské v Brne. Řada B. Spisy Fakulty Veterinární 12:269–293.

Sonin, M. D. 1961. Filariae of birds in the Turkmen SSR. (In Russian.) Helminthologia 3:322–339.

———. 1963. Filariae of birds in Far-Eastern USSR. (In Russian.) Trudy Gel'mintologicheskoi Laboratorii. Akademiya Nauk SSR 13:227–249.

———. 1966. Filariata of animals and man and diseases caused by them. Part I. Aproctoidea. *In* K. I. Skrjabin, ed. Essentials of Nematology, Vol. XVII. Izdatelstvo "Nauka," Moscow. (Translated by the Israel Program for Scientific Translations, Jerusalem, 1974.)

Sultana, A. 1964. A new species of *Pseudaprocta* Shikhobalova 1930 from the large pied wagtail in India. Indian Journal of Helminthology 16:71–74.

Yamaguti, S. 1961. Systema Helminthum. Vol. 3. The Nematode Parasites of Vertebrates. Interscience Publishers, Inc., New York.

Yeh, L. S. 1957. On *Chandlerella braziliensis* n. sp. from a green-billed toucan and a discussion on some related genera. Journal of Helminthology 31:33–38.

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Coccidiosis in the Gallbladder of a Goat

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ABSTRACT: Cocciodiosis, probably due to *Eimeria* sp., was found in the gallbladder of a goat. Meronts, gamonts, and oocysts were found in the epithelium of villous and submucosal glands. Meronts were 11.7 × 7.7 μm and contained 3–20 merozoites; merozoites were 8–12 μm long and 1.5–2.3 μm wide. Macrogamonts and unsporulated oocysts were 9.6 × 8.7 μm and 11.6 × 9.5 μm, respectively. There was generalized cholecystitis characterized by necrosis and infiltration by mononuclear cells. Liver was normal.

Several species of *Eimeria* parasitize the intestines of goats, sheep, and cattle, but none is known to invade the gallbladder (Levine, 1973; Lima, 1980). This report describes severe coccidiosis in the gallbladder of a dairy goat.

Materials and Methods

The goat was a 118-day-old purebred Alpine female from a commercial goat dairy in Darby, Montana and had been fed 50,000 sporocysts of *Sarcocystis capracanis* 33 days prior to necropsy. The goat showed clinical signs of acute sarcocystosis between 23 and 30 days postinoculation (Dubey et al., 1981).

The goat was killed by electrocution, exsanguinated, and necropsied immediately. Portions of gallbladder and other tissues were fixed in 10% neutral-buffered formalin (NBF). A small piece of the gallbladder was also fixed in Helly's fixative. Paraffin-embedded sections were examined after staining with hematoxylin and eosin (HE). After finding coccidian stages in the gallbladder, several pieces of gallbladder fixed in NBF for 18 months were embedded in glycol methacrylate for light microscopy and also processed for transmission electron microscopy. Methacrylate-embedded sections were cut at 3 μm, and examined after staining with hematoxylin and eosin, Giemsa, iron-hematoxylin (IH) or periodic acid Schiff (PAS) hematoxylin. For transmission electron microscopy, sections were examined under a JEOL 100 cx microscope.

Results

Histologic examination of the gallbladder revealed an unidentified coccidium different from *Sarcocystis*. Because the unidentified coccidium, which may be *Eimeria* sp., has not been reported previously from the gallbladder of goats, the parasite is described below.

Parasitic stages

Meronts and gamonts were found in the epithelium of villous and submucosal glands of the gallbladder (Figs. 1–7). More parasites were found in glands than in the villous. Meronts were more numerous than gamonts and the intensity of infection varied; some sections were heavily infected, whereas others had few parasites.

Meronts were in various stages of development (Figs. 1–4). Mature meronts were 9.3 × 6.7 μm (5–15 × 4–11 μm; $N = 58$) and contained 1–17 merozoites. The merozoites were 8–11 μm long and 1–2 μm wide; they occupied the entire length of the meront (Fig. 4). Merozoites were curved at the broader posterior end and pointed at the thin anterior end; the nucleus was usually located toward the posterior end. The nucleus stained prominently with hematoxylin and occupied the entire width of the merozoite (Fig. 4). A few fine granules were seen just anterior to the merozoite nucleus in sections stained with iron hematoxylin. Merozoites contained several PAS-positive granules; the intensity of reaction varied among merozoites. Merozoites within a given meront were arranged randomly often head to tail (Figs. 3, 4); thus, it was difficult to count them accurately or determine their length. Free merozoites or meronts were seen in the lumen of the glands and the gallbladder.

Most gamonts were in glands. Macrogamonts had a large nucleus with a prominent nucleolus, even in the youngest (3 × 2 μm) macrogamont (Fig. 1). Mature macrogamonts were 9.6 × 8.3 μm (7–14 × 6–12 μm; $N = 21$) and contained PAS-positive wall-forming bodies (Fig. 5). Microgamonts were not identified with certainty. However, few 2- to 8-nucleated bodies (Fig. 2) interpreted as microgamonts were 7 × 5.7 μm (5–9 × 4–7 μm; $N = 5$); their nuclei were much smaller than those of macrogamonts. Unsporulated oocysts were 11.6 × 9.5 μm (9–15 × 7–12 μm; $N = 10$) and were seen in glands (Figs. 6, 7).

Ultrastructurally, meronts were located within a parasitophorous vacuole (PV) in the cytoplasm of epithelial cells (Figs. 8–12). Of the 28 meronts studied, 5 were immature and 23 contained merozoites. The youngest meront observed was 5.7 ×

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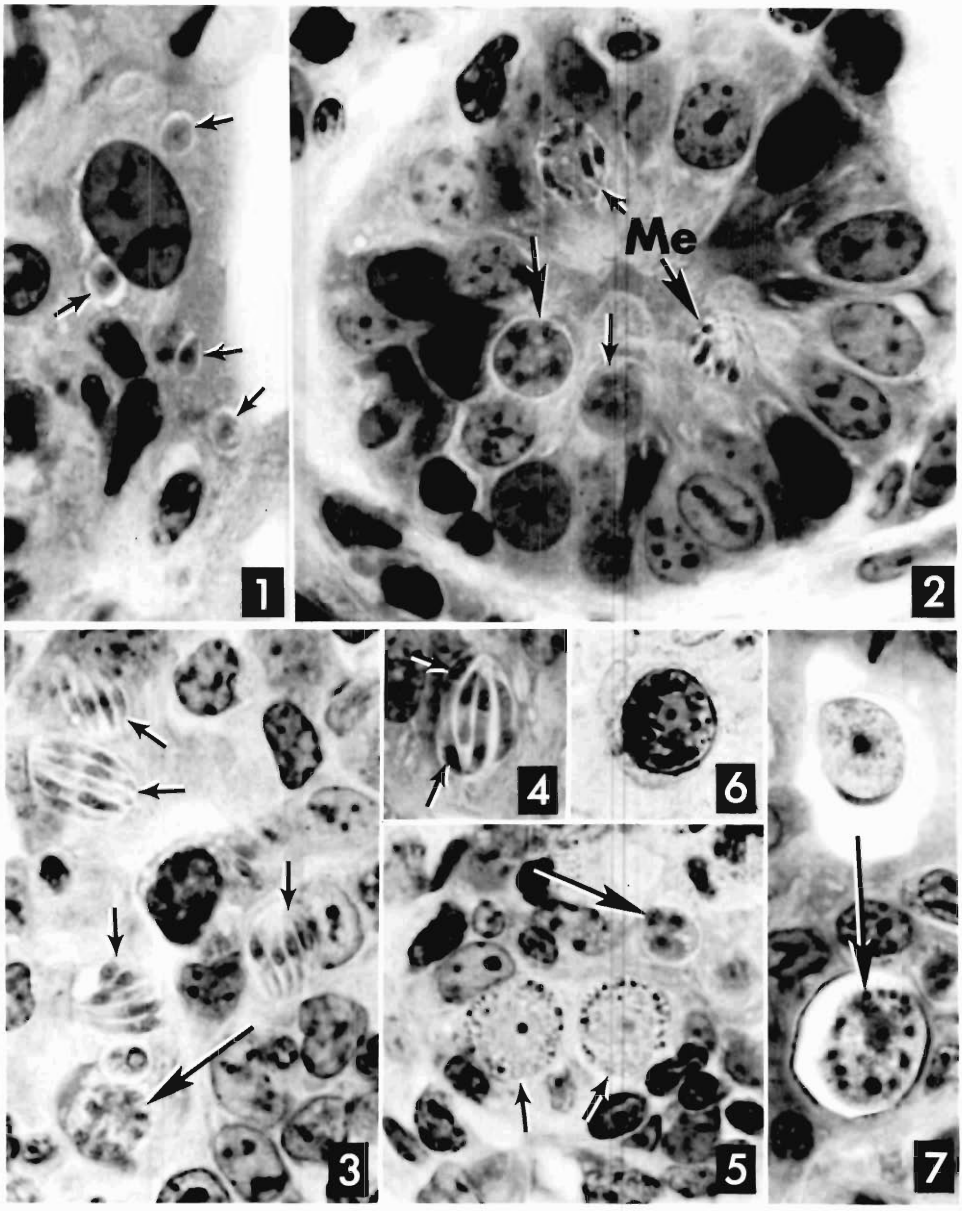

Figures 1-7. Stages of the coccidium in gallbladder of goat (×1,000). Figures 1-5 and 7, 3 μm, methacrylate; Figure 6, 1 μm, epoxy resin. 1. Uninucleate zoites (arrows) in parasitophorous vacuoles in epithelial cells of the villous. HE. 2. Two immature meronts (Me), a binucleate (small arrow) and a 5-nucleate (large arrow) meront or microgamont. IH. 3. Four mature meronts (small arrows) and an immature meront (large arrow) in epithelial cells of a submucosal gland. HE. 4. Meront with 6 merozoites. Arrows point to the nucleus of the merozoite. HE. 5. Two macrogamonts (small arrow) each with a central nucleus, and a 3-nucleate meront (large arrow). HE. 6. Partly formed oocyst with a central nucleus and wall-forming bodies. Toluidine blue. 7. Two unsporulated oocysts with wrinkled walls. Well-forming bodies are present in 1 oocyst (arrow) and absent in the other. IH.

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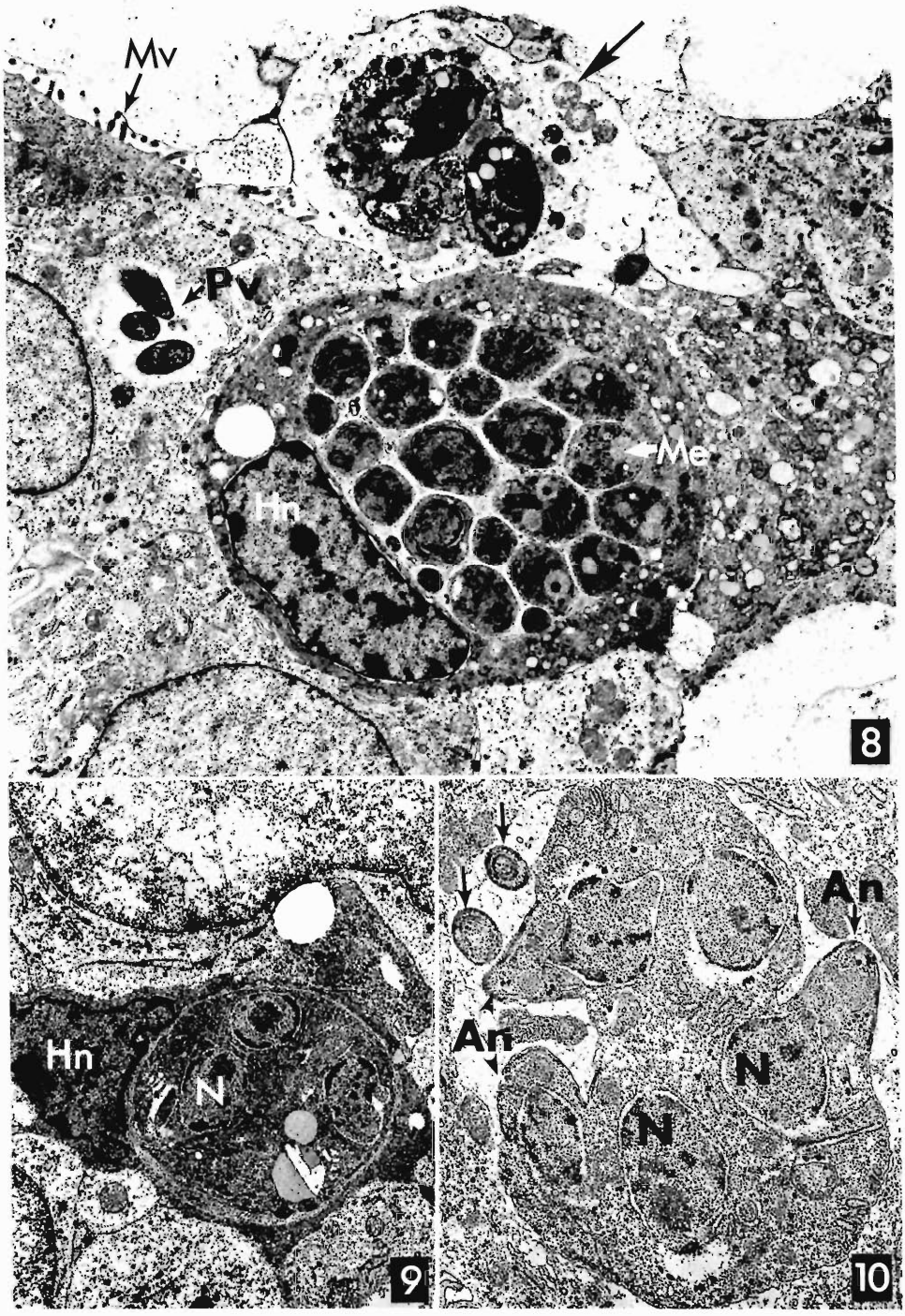
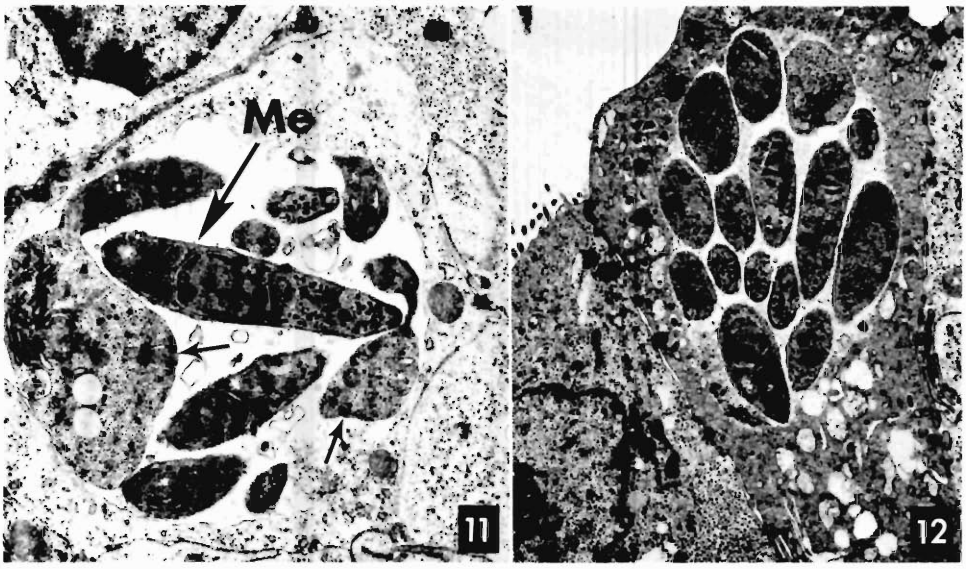
Figures 8-10. Transmission electron micrographs of the coccidium in epithelial cells of the gallbladder of the goat. Abbreviations: Hn = host cell nucleus, Pv = parasitophorous vacuole, Me = merozoites, N = nucleus



of the parasite, Mv = microvilli of the host cell, An = anlagen of the merozoite. 8. Three meronts in villus. The meront toward the far left contains the anterior ends of 3 merozoites. The meront toward the lumen (arrow) is being extruded into the lumen. The meront in the center contains 23 cross sections of merozoites. Note spaces among merozoites and absence of residual body (×5,775). 9. Three-nucleate meront (×7,656). 10. Developing merozoites in a meront. Note nuclei in close proximity of merozoite anlagen (arrows). Small arrows point to anterior ends of merozoites that have separated from the meront (×9,628).



Figures 11, 12. Transmission electron micrographs of meronts of the coccidium in the gallbladder of a goat. 11. Meront with merozoites separating from residual bodies (arrows). Note the position of the nucleus in a longitudinally cut merozoite (Me) (×7,656). 12. Mature meront with 15 merozoites (×5,800).

4.9 μm and contained 3 nuclei (Fig. 9). One immature meront contained 7 nuclei without evidence of merozoite formation. Prior to merozoite formation, the nuclei moved towards the periphery of the meront and each nucleus was incorporated into a merozoite anlage (Fig. 10). Merozoites separated asynchronously from the mother cell leaving 1 or 2 residual bodies (Fig. 11). The residual body was present in 10 of 23 meronts and was located usually toward one side of the meront; the residual bodies were 4.5 × 2.4 μm (2.5–5.7 × 1.1–3 μm; $N = 10$). Mature meronts were 11.7 × 7.7 μm (5.7–16 × 3.9–8.3 μm; $N = 23$) and contained 3–20 merozoites. Out of 23 meronts, 1 each had 3, 4, 5, 6, 15, 16, and 20 merozoites and 16 meronts had between 7 and 12 merozoites. Of more than 100 merozoites studied, only 3 were cut longitudinally and the position of the nucleus varied from terminal to central. The longest merozoite was 7.9 μm. Merozoites were 1.7 μm (1–2.3 μm; $N = 46$) wide. In all but 2 meronts, merozoites were arranged randomly within the meront with spaces among them. In 2 meronts, merozoites were tightly packed.

Gamonts were not identified with certainty under the electron microscope because of their rarity. Uninucleate organisms interpreted as macrogamonts were seen in epithelial cells; these contained more storage granules than those in meronts.

Lesions

The entire gallbladder was thickened due to edema and infiltration by mononuclear cells. The mononuclear cell infiltration was seen throughout the width of the gallbladder but was most pronounced in the lamina propria. There were minute hemorrhages in the lamina propria. The villi were stunted and occasionally fused. The villous epithelial cells were flat to low cuboidal. Focal necrosis was seen in the lamina propria, submucosal glands, and villous epithelium, and there was a focus of ulceration in 1 villus (Fig. 13). The epithelium in glands and the villi was hyperplastic as indicated by increased mitotic figures, and the crypts of the submucosal glands contained desquamated epithelial cells and parasites.

Neither lesions nor parasites were seen in bile ducts, liver, intestines, mesenteric lymph nodes, or other tissues.

Discussion

Lesions in the present study were considered to be due to the associated coccidium because the infection was localized in the gallbladder and parasites were seen in the lesions. Although species of *Eimeria* occasionally invade mesenteric lymph nodes of goats (Lotze et al., 1964; Lima, 1979), infection of the gallbladder in goats or other mammals has not been previously reported (Levine, 1973; Levine and Ivens, 1981).

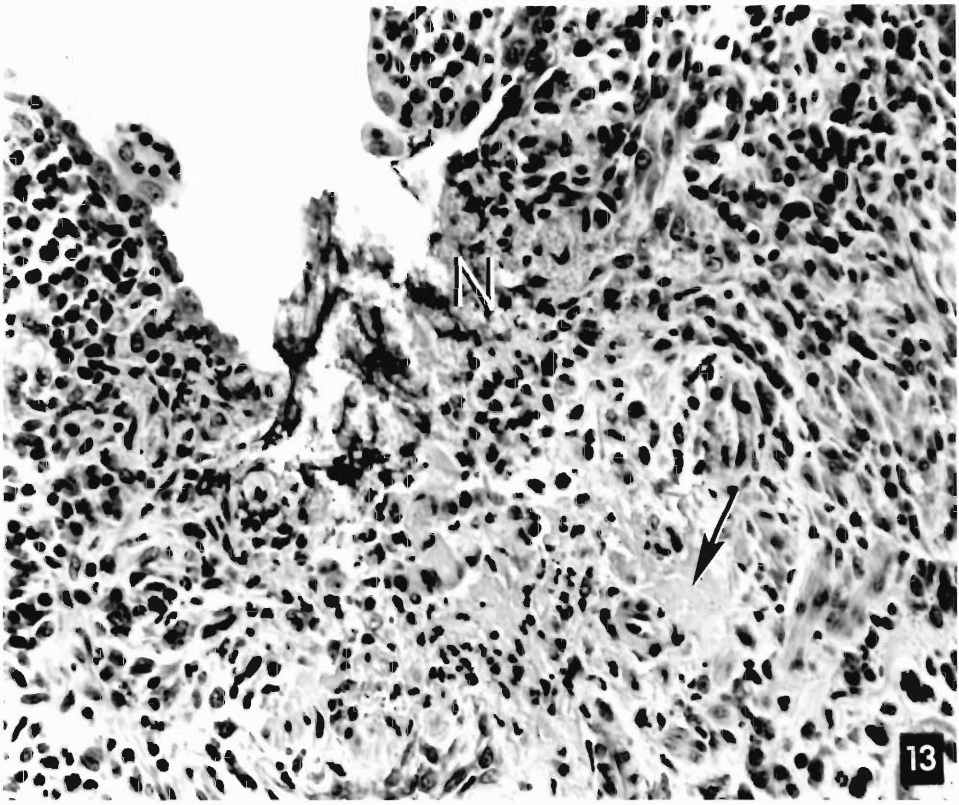


Figure 13. Edema (arrow), necrosis (N), and mononuclear cell infiltration in the lamina propria (×280). IH, paraffin-embedded, 5 μm.

The coccidian parasite could not be identified further. Of the many species of *Eimeria* that are known to occur in goats, the oocysts of *Eimeria parva* (14–23 μm) are the smallest (Levine, 1973; Lima, 1980). The longest oocyst in the gallbladder in the present case was 15 μm long. The endogenous stages of *E. parva* in goats are unknown. Also, the meronts were the smallest of any eimerian species in goats (Levine, 1973). The parasite was different from *Sarcocystis*, although *S. capracanis* meronts are known to occur in the gallbladder of goats (Dubey et al., 1981); however, they occur in the vascular endothelium and are in direct contact with host cytoplasm whereas meronts in the present study were located in a parasitophorous vacuole. Eimerian stages were not identified in sections of intestines.

Acknowledgments

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Literature Cited

Dubey, J. P., S. E. Weisbrode, C. A. Speer, and S. P. Sharma. 1981. Sarcocystosis in goats: clinical signs and pathologic and hematologic findings. Journal of the American Veterinary Medical Association 178:683–699.

Levine, N. D. 1973. Protozoan Parasites of Domestic Animals and of Man, 2nd ed. Burgess, Minneapolis. IX + 406 pp.

———, **and V. Ivens.** 1981. The Coccidian Parasites (Protozoa: Apicomplexa) of carnivores. Illinois Biological Monographs 51, University of Illinois Press, Urbana, Illinois. 248 pp.

Lima, J. D. 1979. Development of *Eimeria* species in mesenteric lymph nodes of goats. Journal of Parasitology 65:976–978.

———. 1980. Prevalence of coccidia in domestic goats from Illinois, Indiana, Missouri and Wisconsin. International Goat and Sheep Research 1:234–241.

Lotze, J. C., W. T. Shalkop, R. G. Leek, and R. Behin. 1964. Coccidial schizonts in mesenteric lymph nodes of sheep and goats. Journal of Parasitology 50:205–208.

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Research Note

Evidence for Mannose as a Terminal Saccharide in the Lectin Binding Site of a Surface Membrane Glycoprotein of *Schistosoma mansoni*[1]

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A number of investigators have demonstrated the presence of lectin receptors on the tegumental surface of both schistosomula and adult *Schistosoma mansoni* and have used immobilized lectins to isolate surface antigens of parasites (Hayunga et al., 1983, Proceedings of the Helminthological Society of Washington 50:219–235; Aronstein and Strand, 1984, The Journal of Parasitology 70:545–557). The information from lectin affinity chromatography and from metabolic labeling using hexose and hexosamine precursors has allowed a preliminary characterization of over a dozen surface membrane glycoproteins from *S. mansoni* adult worms (Hayunga and Sumner, 1986, Journal of Chemical Ecology, in press). In the present study, enzymatic degradation of radiolabeled glycoproteins by exoglycosidases was used to further characterize these molecules.

Adult worms obtained by portal perfusion of 7-week infected mice were thoroughly washed in Earle's balanced salt solution, quickly frozen by plunging into a dry ice–methanol bath, then thawed to yield a standard freeze–thaw antigen (AFT). The AFT antigen preparation was radioiodinated to a specific activity of 40–65 μCi/mg using the Bolton-Hunter procedure, then applied to a Concanavalin A–Sepharose 4B chromatography column, as described previously (Hayunga et al., 1983, loc. cit.) and the α-methylmannoside eluate analyzed by electrophoresis to yield a reproducible SDS-PAGE profile as depicted in the control figure. Carbohydrate arrangement of the labeled glycoproteins was examined by incubation of the labeled antigen in the presence of specific exoglycosidases, to remove the terminal saccharide, prior to affinity chromatography. Approximately 3.0 μCi (=4,000,000 cpm as detected by a scintillation counter with 60% effi-

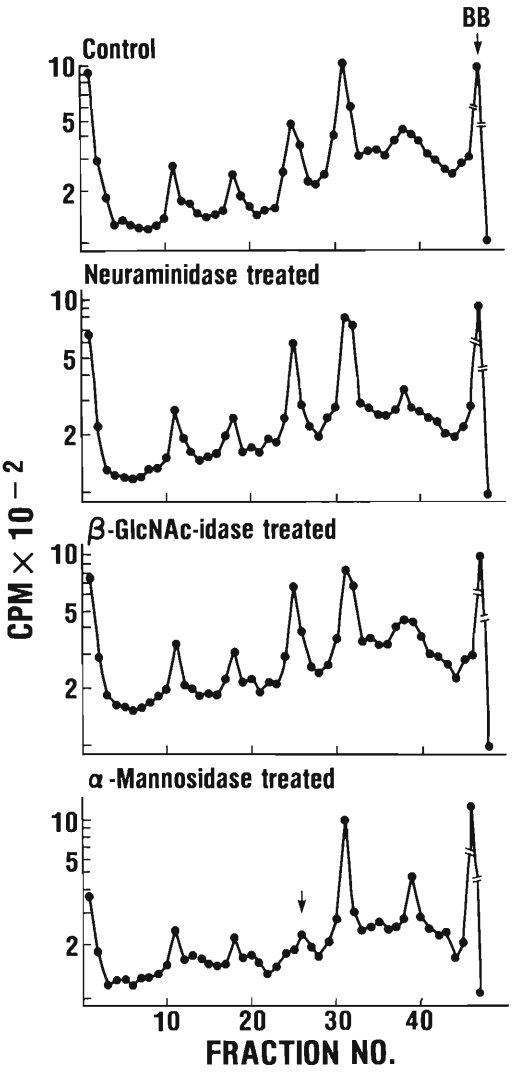

Figure 1. SDS-PAGE analysis of the α-methylmannoside eluates from Concanavalin A-Sepharose 4B columns following the application of Bolton-Hunter labeled AFT antigen that had been incubated with saline (control) or one of several exoglycosidases as indicated. Arrow indicates decreased lectin binding of the 58,000 mol. wt. glycoprotein after incubation with α-mannosidase.

[1] Presented at the 573rd meeting of the Helminthological Society of Washington in Bethesda, Maryland, October 1985.

ciency) of AFT antigen was incubated for 2 hr at 37°C with 5 units/ml of each of the following enzymes: neuraminidase, β-N-acetylglucosaminidase, α-mannosidase, α-galactosidase, and β-galactosidase. Enzymes were obtained from Sigma Chemical Company.

As shown in Figure 1, incubation of the labeled AFT antigen preparation with α-mannosidase resulted in a significant decrease in lectin binding of the 58,000 mol. wt. glycoprotein. Because the exoglycosidase cleaves only terminal saccharides, the change in lectin binding indicates the presence of a terminal mannoside on this molecule as originally proposed by Hayunga et al. (1983, loc. cit.). Furthermore, the magnitude of the decrease in lectin binding would suggest the presence of more than one mannose residue in the binding site, either in a linear sequence (mannobiosylmannose) or in a branched arrangement with terminal mannose residues attached to another saccharide, possibly N-acetylglucosamine. Concanavalin A binding sites are generally believed to accommodate structures as large as di- or trisaccharides; thus, the removal of a single saccharide would not be expected to result in the virtually complete loss of lectin binding depicted in the figure. For this reason we conclude that mannose is not only present as a terminal saccharide, but that mannose residues constitute a significant portion of the lectin binding site. In contrast, incubation of the AFT preparation with other exoglycosidases failed to reduce lectin binding of the 58,000 mol. wt. component or any of the other labeled glycoproteins. This may indicate the absence of these saccharides in terminal position. Alternatively, they may be present along portions of the molecule other than the binding sites, and thus, their removal would not alter lectin binding.

The presence of sialic acid on parasite surfaces is well documented (McDiarmid and Podesta, 1983, Molecular and Biochemical Parasitology 10:33–43), so that it was surprising that neuraminidase treatment of the labeled glycoproteins had little apparent effect on lectin binding. Simpson and Smithers (1980, Parasitology 81:1–15) reported an increase in the binding of soybean agglutinin and peanut agglutinin to adult worms following neuraminidase treatment, and speculated that the removal of sialic acid might expose additional binding sites on tegumental glycoproteins and thus increase their affinity for the lectins. We did observe a slight increase in the relative size of the 58,000 mol. wt. peak, but no additional Con A binding glycoproteins were detected following neuraminidase treatment (Fig. 1). However, direct comparison of our work with that of Simpson and Smithers should be guarded because the presence of sialic acid on intact worms may impose other steric hindrances that are not apparent when working with glycoprotein extracts in solution.

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The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Animal Resources, National Research Council, DHEW Pub. No. (NIH) 78-23.

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Research Note

Histochemical Localization of Cholinesterase in the Excysted Metacercaria of *Cloacitrema michiganensis* (Trematoda)

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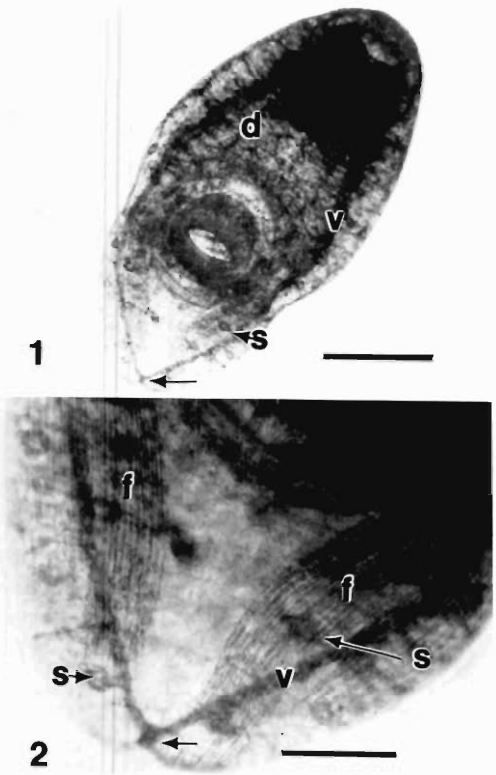
Except for the study on the localization of cholinesterase in the cercaria of *Cloacitrema michiganensis* by LeFlore et al. (1980, Transactions of the American Microscopical Society 99:201–206), information is not available on this enzyme in other larval stages of this parasite. The localization of cholinesterase in whole mounts of larval trematodes is important in the broader context of the evolution of the helminth nervous system and the mechanism of nervous transmission. Moreover, such studies using larval stages can be used to elucidate similarities and differences in the nervous system of different stages in the life cycle of trematodes. Smyth and Halton (1983, the Physiology of Trematodes, Cambridge University Press, Cambridge, 446 pp.) have reviewed the histochemical demonstration of cholinesterases in a number of trematodes. We report here our observations on the histochemical localization of acetylcholinesterase (AChE) in the chemically excysted metacercaria of *C. michiganensis*.

Cercariae of *C. michiganensis* were collected from naturally infected *Cerithidea californica* snails maintained in finger bowls containing artificial sea water. Metacercariae were scraped from the bottom and sides of the bowls and chemically excysted according to LeFlore and Bass (1983, Journal of Parasitology 69:200–204). Whole worms were fixed at 1°C for 30 min in 10% formalin buffered to pH 7.0 with sodium phosphate. The specimens were flattened during fixation by applying gentle pressure on the coverslips. After fixation, they were washed with cold distilled water at 1°C to remove all traces of the fixative and treated for 12 hr at room temperature by the acetylthiocholine iodide method for AChE (Gomori, 1952, Microscopic Histochemistry, University of Chicago Press, 273 pp.). Controls consisted of worms heat inactivated at 90°C for 5 min prior to incubation, medium in which the substrate was omitted, and medium in which 10^{-3} M and 10^{-4} M eserine were included. All control worms were negative for AChE.



Figures 1, 2. Photomicrographs of *Cloacitrema michiganensis*. 1. Photomicrograph of a chemically excysted metacercaria of *Cloacitrema michiganensis* stained for cholinesterase. Arrow points to area where nerve trunks meet. Scale bar = 125 μm. 2. Photomicrograph of the posterior region of an excysted metacercaria showing longitudinal subtegumental fibers. Scale bar = 45 μm. Abbreviations: d = dorsal nerve trunk; f = longitudinal fibers; s = sensory cell; v = ventral nerve trunk.

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The morphology of the nervous system of the excysted metacercaria is essentially similar to that of the cercaria of *C. michiganensis* as described by LeFlore et al. (1980, loc. cit.). In brief, there are cerebral ganglia connected by a commissure in the region of the pharynx; 3 pairs of nerves extend from the ganglia into the oral sucker; and 3 pairs of posterior longitudinal trunks pass from the ganglia to the posterior region of the body where they join the trunks of the opposite side (Fig. 1). These trunks possess transverse commissures along their length. Secondary branches innervate the suckers and pharynx. Numerous sensory cells "Senneszellen" of Bettendorf (1897, Zoologische Jahrbücker, Abteilung für Anatomie und Ontogenie der Tiere 10:307–358) are present along the length of the longitudinal trunks (Figs. 1, 2). Two bands of fine subtegumental fibers pass from the mid-acetabular region to the posterior part of the body of the metacercaria (Fig. 2).

The sensory cells in the metacercaria of *C. michiganensis* appear similar to those reported by Dixon and Mercer (1965, Journal of Parasitology 51:967–976) for the cercaria of *Fasciola hepatica,* by LeFlore (1979, Transactions of the American Microscopical Society 98:225–232) for the cercaria of *Plagiorchis elegans,* and by Niewiadomoska and Moczón (1984, Zeitschrift für Parasitenkunde 70:537–548) for the metacercaria of *Diplostomum pseudospathaceum.* Sensory cells were not seen in the cercaria of *C. michiganensis.*

The cholinesterase-staining subtegumental fibers of the metacercaria of *Cloacitrema michiganensis* differ in morphology from those described by Bruckner and Voge (1974, Journal of Parasitology 60:437–446) for the cercaria of *Schistosoma mansoni* and LeFlore (1979, loc. cit.) for the cercaria of *Plagiorchis elegans.* The cholinesterase-staining fibers in the other 2 studies were both circular and longitudinal, producing an apparent nerve net. In *C. michiganensis* the fibers were only longitudinal. Subtegumental fibers were not seen in the cercaria of *C. michiganensis.*

We have no evidence of non-neuronal AChE in *C. michiganensis,* although Davies (1979, International Journal for Parasitology 9:553–564) demonstrated this enzyme in the forebody glands of *Microphallus similis* and observed that it may interfere with local peristalsis in the gut of the host and thus prevent the expulsion of these digeneans. Barrett (1981, Biochemistry of Parasitic Helminths, University Park Press, Baltimore, 308 pp.) described secretory cholinesterases for a number of parasitic nematodes, the significance of which is unknown, but they have been postulated to function as a biological holdfast to resist peristalsis of the host intestine.

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Research Note

Comparative Infectivity of *Trichinella spiralis spiralis* (from Domestic Swine) and *T. spiralis nativa* (from Polar Bear) for Arctic Foxes and Domestic Swine

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Trichinellosis exists as 2 separate entities, the sylvatic cycle in wild animals and the domestic or synanthropic cycle involving domestic swine and commensal rodents (Campbell, 1983, pages 425–444 *in* W. C. Campbell, ed. *Trichinella* and Trichinosis. Plenum Press, New York). Although the sylvatic infections are widely regarded as forming a potentially important reservoir for domestic swine (Andrews et al., 1969, Proceedings of the 73rd Annual Meeting of the U.S. Animal Health Association, pages 336–353; Zimmermann and Brandly, 1965, Public Health Reports 80:1061–1066), there is evidence that they typically have low infectivity for the domestic pig (Rausch et al., 1956, Journal of Parasitology 42: 759–771; Nelson and Mukundi, 1963, Journal of Helminthology 37:329–338; Kruger et al., 1969, Wiadomosci Parazytologiczne 15:540–554; Bessonov et al., 1975, Wiadomosci Parazytologiczne 21:561–575; Murrell et al., 1985, pages 301–305 *in* C. W. Kim, ed. *Trichinellosis.* State University of New York Press, Albany; Smith, 1985, Canadian Journal of Comparative Medicine 49:88–90). However, a recent epidemiological investigation of an outbreak of swine trichinellosis (Murrell et al., 1986, Journal of Parasitology, 72: In press) suggested that porcine *T. spiralis spiralis* has spread from the farm's herd to wild furbearing mammals associated with the farm. This was supported by isozyme and DNA restriction enzyme analyses, and experimental infections of pigs using isolates from the farm's furbearing mammals. The results raised the question of the potential importance in the epidemiology of swine trichinellosis of invasion of the sylvatic biotope by porcine *T. spiralis spiralis.* The experiments described were designed to assess the infectivity of *T. spiralis spiralis* from domestic swine and *T. s. nativa* from a polar bear, for arctic foxes, and for domestic swine.

Infectivity trials were conducted on a *T. s. nativa* isolate recovered from a polar bear (*Ursus maritimus*) (Pb-1) (58°00′N, 95°00′W) in 1976, provided by Dr. T. Dick, University of Manitoba. The isolate had been passed in albino laboratory mice (CFW) for 24 generations prior to these experiments. For comparison, the Beltsville pig isolate of *T. s. spiralis* was used. This isolate had been recovered from an infected slaughter hog in Illinois (40°N, 88°W) at least 50 years ago and has been maintained at the Animal Parasitology Institute by numerous passages through pigs/mice and laboratory rats. Comparison of this pig isolate with recent pig isolates from New Jersey and Maine by DNA restriction enzyme analysis has not revealed any significant differences (J. Dame, unpubl.). The procedures for recovering and counting muscle larvae from infected muscle have been described previously (Alizadeh and Murrell, 1984, Journal of Parasitology 70:767–773).

Captive-bred arctic foxes (*Alopex lagopus*) purchased from a commercial breeder were used at the experimental host; the foxes were less than 1 year of age and were housed at the School of Veterinary Medicine, University of Pennsylvania. The foxes were fed chicken muscle and chicken parts. Starved foxes were exposed to the *Trichinella* isolates by feeding them ground infected mouse muscle containing a predetermined number of muscle larvae. Eight weeks later, the foxes were necropsied, and the anterior muscles of the carcass, including the tongue, masseter, and diaphragm, were pooled for recovery of muscle larvae. To inoculate pigs with the isolates, muscle larvae were suspended in warm (37°C)

Table 1. Comparative infectivity of *T. spiralis spiralis* (pig) and *T. spiralis nativa* (polar bear) for arctic foxes and domestic pigs.

	Primary passage				Secondary passage			
Isolate	Host	Number larvae inoculated (×1,000)	Number larvae recovered/ gram muscle	Infectivity index*	Host	Number larvae inoculated (×1,000)	Number larvae recovered/ gram muscle	Infectivity index*
Experiment 1								
T. s. spiralis	fox 1	48.0	17.10	0.04	pig 1	8.4	35.40	0.42
T. s. nativa	fox 2	26.4	148.10	0.56	pig 1	20.0	3.08	0.02
					pig 2	60.0	16.46	0.03
Experiment 2								
T. s. spiralis	fox 1	32.5	27.00	0.08				
	fox 2	32.5	46.00	0.14†	pig 1	38.8	2,272.00	5.85
	fox 3	32.5	28.00	0.09	pig 2	38.8	864.00	2.22
T. s. nativa	fox 1	32.5	588.00	1.80				
	fox 2	32.5	528.00	1.60†	pig 1	40.5	35.00	0.09
	fox 3	32.5	336.00	1.00	pig 2	40.5	6.00	0.01

* Infectivity indices were derived by dividing the number of larvae recovered from 100 g of pooled, ground host muscle by the total number of muscle larvae inoculated.

† Infected muscle from all 3 foxes were pooled to provide inoculum for pigs.

0.2% agar and administered orally with a mechanical pipette. Pigs used in these studies were crossbreds from a *Trichinella*-free herd at the Animal Parasitology Institute. and weighed between 9 and 14 kg. Six weeks later, the pigs were necropsied and the muscle larval densities determined by digesting pooled tongue, diaphragm, and masseter muscles. In the first of 2 experiments, the *T. s. spiralis* isolate was considerably less infective for the fox than was the *T. s. nativa* isolate (Table 1), but its infectivity for a pig increased markedly after the fox passage. The opposite results were obtained for *T. s. nativa.* Because the variability between experimental hosts was great, a second experiment was conducted with 3 foxes per group. The second experiment (Table 1) confirmed previous results, showing again that the porcine isolate, *T. s. spiralis* has a much lower infectivity for foxes in comparison to *T. s. nativa.* In this instance, the infectivity index of the porcine isolate, on passage back to pigs, returned to the normal range for *T. s. spiralis* in swine (Murrell et al., 1985, loc. cit.). Again, the reverse occurred for the polar bear isolate, which displayed a much greater reproductive capacity in the fox than in the pig. Passage through foxes appeared to have little affect on the intrinsic infectivity of either subspecies for swine.

These results support the concept that there are major differences between some *Trichinella* isolates, with regard to their host specificities. Although there is published evidence that isolates of *Trichinella* from wild animals are characteristically poorly infective for swine, we are aware of only one other report on the comparative ability of *T. s. spiralis* to infect carnivores (Dick, 1983, Journal of Wildlife Diseases 19:333–336). Two raccoons were inoculated, each receiving 1 of 2 different pig isolates. Although "pig isolate-one" had a low infectivity index, "pig isolate-two" infectivity was relatively high.

The importance of these findings to the epidemiology of trichinellosis lies in the question of wildlife reservoirs for porcine trichinellosis. A case might be made that because of their high host specificity, sylvatic *Trichinella* isolates do not constitute an important reservoir for domestic trichinellosis, but the ability of *T. s. spiralis* to enter the sylvatic cycle would alter the significance of a sylvatic reservoir. Although the experimental results show that the infectivity of *T. s. spiralis* for the fox is low, infection did occur, and the muscle larvae recovered were in turn infective for domestic pigs. Murrell et al. (1985, loc. cit.) have recovered isolates from bears, raccoons, an oppossum, and a skunk that exhibit high infectivity for pigs. Because these isolates exhibit isozyme and DNA restriction analysis profiles identical with *T. s. spiralis,* they provide evidence that this potential is real (Mur-

rell et al., 1986, Journal of Parasitology 72:In press).

Further research is needed to determine the host specificity of porcine *T. s. spiralis* over a wider range of furbearing mammals, to assess its ability to adapt to such wild animal hosts through repeated passage, and to determine whether hybridization with *T. s. nativa* would lead to higher infectivity for both domestic swine and carnivores. Comprehensive and effective programs for the control of swine trichinellosis will require this information.

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Research Note

Validity of Tongue Muscle Digestions for Prevalence Surveys on Rat Trichinellosis

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The predilection of larval *Trichinella spiralis* for certain host muscles has been well documented. In many animal species the diaphragm and the masseters are the most heavily infected muscles (Weatherly, 1983, pages 173–208 *in* W. C. Campbell, ed. *Trichinella* and Trichinosis. Plenum Press, New York). Additionally, Olsen et al. (1964, Journal of Parasitology 50:489–495) and Kotula et al. (1984, Journal of Animal Science 58:94–98) reported the tongue to have the highest muscle larvae densities in experimentally infected pigs. In field investigations on trichinellosis among wild rat populations the recovery of *T. spiralis* by digestion of complete carcass musculature is inconvenient, time consuming, and expensive. Hence, we carried out an evaluation of the accuracy of rat tongue digestions compared with body musculature digestions for the determination of *T. spiralis* prevalence among wild rat populations.

Female Sprague Dawley rats weighing between 175 and 200 g were inoculated with muscle-stage larvae of *T. spiralis* obtained from an experimentally infected rat by digestion in 1% Pepsin–1% HCl for 4 hours at 37°C. Inocula of up to 10 larvae were prepared by drawing the desired number of larvae directly into a syringe under a dissecting microscope. Larger inocula were prepared by suspending the larvae to the desired concentration in warm 0.2% agar and administered by an animal feeding needle. Five weeks after inoculation, the rats were killed, skinned, and eviscerated. The tongue was removed and digested separately from the rest of the carcass. The number of larvae recovered was recorded for both digests. The average weight of the rats at the time of necropsy was 250 g, with the tongue weighing 1–2 g.

The results (Table 1) show that in all rats inoculated with 10 or more muscle larvae the infection could be detected consistently in both body and tongue. In rats given less than 10 larvae, the muscle larvae density varied between 0.01 and 6.7 larvae per gram. Infections were

Table 1. Muscle larvae recoveries from body and tongue of rats inoculated with varying doses of *Trichinella spiralis*.

No. larvae inoculated/ rat	Rats infected/ inoculated	Mean larvae recovered	Minimum larvae recovered	% larvae recovered	
				Tongue	Body
2	3/20	162	52	3.7*	96.3
4	12/15	188	9	7.9†	92.1
8	11/15	352	3	7.1†	92.9
10	3/3	821	728	5.8	94.2
25	3/3	1,957	1,731	6.0	94.0
50	3/3	3,740	2,203	7.7	92.3
100	3/3	4,322	2,686	5.8	94.2

* Including 1 infected rat with no larvae recovered from the tongue.

† One rat each with 100% larvae recovered from the tongue not included.

Table 2. *T. spiralis* muscle larvae recovery from wild rats caught on a hog farm.

No. rats examined	Average rat weight (g) ± SD	% infected	Geometric mean and range of larvae recovered by complete carcass digestion	% infected by tongue digestion	Geometric mean and range of larvae recovered from tongue
64	352 ± 94	12.5	2,557 (165–454,050)	12.5*	242 (10–22,050)

* The same rats were found infected as revealed by total muscle digestion.

detected by tongue digestion in 49 of 50 rats inoculated with less than 10 larvae. In the 1 rat from which no larvae were recovered from the tongue, digestion of the body muscles yielded 0.2 larvae per gram. Importantly, in 3 of the rats receiving less than 10 larvae, the total larval recovery for combined tongue and body was less than 10 muscle larvae, yet, the infectivity was detected in all three by tongue digestion. In 2 of these rats, the body musculature failed altogether to yield muscle larvae. The observed large variability in the total number of larvae produced and the level of progenies recovered are in agreement with those reported by Edeny et al. (1953, Tennessee Academy of Science 58:62–68).

In order to determine the validity of these observations for a wild rat population, a field survey was carried out on a hog farm with endemic swine trichinellosis in Gloucester County, New Jersey. Rats were caught during 3 nights using snap traps and live traps baited with peanut butter. The animals caught were brought into the laboratory and processed as described above. The results are summarized in Table 2. The presence of *T. spiralis* among all naturally infected rats as determined by total muscle digestion was also revealed by tongue digestion. The percentage of larvae in the tongue varied between 4.1% and 14.6% of the total carcass recovery. Two rats had less than 1 larvae per gram of whole carcass muscle, yet both were detected by tongue digestion.

We conclude that digestion of only the tongue represents a simple, sufficiently reliable technique for field studies designed to determine the prevalence of *T. spiralis* in rat populations. However, for data on the intensity of infection, digestion of the whole carcass is preferable, because the variations of the percentage of recovered muscle larvae in the tongue in natural infections are too large to give reliable estimates of the total number of muscle larvae.

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Research Note

Parasites of Wolves, *Canis lupus*, in Wisconsin, as Determined from Fecal Examinations

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The wolf is an endangered species in Wisconsin. Five packs (Moose Lake [MLP], State-Line Flowage [SFP], Bear Lake [BLP], Tripod [TP], and St. Croix [SCP]) exist in northwestern Wisconsin at 92°00′ west longitude, 46°25′ north latitude and 2 packs (Bootjack [BJP], and Averill Creek [ACP]) exist in the north-central part of the state at 90°00′ west longitude, and 45°30′ north latitude (Thiel, 1982, Unpublished Report of Wisconsin Department of Natural Resources, 18 pp.). The total number of wolves varies from 20 to 25 annually. Because the timber wolf is nearly extinct within the state it was decided to monitor their parasites, primarily by checking fecal samples. Seventy-one fecal samples less than 24 hours old were collected in the wild, from May 1982 to March 1984, fixed in formalin, and examined using the formalin–ether sedimentation technique for protozoan cysts and helminth eggs. Table 1 indicates cysts and eggs recovered.

Byman et al. (1977, Canadian Journal of Zoology 55:376–380) found 74 of 204 wolf scats in Cook and Lake counties of Minnesota harboring oocysts, eggs, or larvae including *Eimeria, Isospora, Alaria, Moniezia,* Taeniidae, *Ancylostoma, Uncinaria, Trichuris, Capillaria, Toxocara,* and *Filaroides.* In our study 68 of 71 scats were positive. We found *Giardia, Entamoeba hartmanni, Alaria,* Taeniidae, *Diphyllobothrium latum,* hookworms, and *Capillaria.*

The difference in parasites found in the 2 studies could be related to sample size, time of year samples were collected, concentration techniques ($ZnSO_4$ vs. formalin–ether), and geographical area collected.

From limited samples it appears that 2 common parasites, *Alaria* and taeniids are present in wolf populations throughout the year. *Alaria* was collected in all months but April and September, taeniids in all months but April, September, November, and December. Hookworms were seen only during April, August, October, and November. It has been shown that larval *Ancylostoma* may suspend development under unfavorable environmental conditions according to Schad et al. (1973, Science 180:502–504). The apparent absence of hookworm eggs in Wisconsin wolves during winter may be due to a cessation of egg production by adult worms during months unfavorable for egg development.

Parasitic amoebae and flagellates have been reported in canids by numerous authors and cited by Burrows and Lillis (1967, Journal of the American Veterinary Medical Association 150: 880–883). Two protozoans, *Entamoeba hartmanni* and *Giardia canis,* were recovered by them

Table 1. Parasite cysts and eggs recovered from scats of 7 Wisconsin wolf packs.

	Prevalence (number infected/number examined)						
Wolf packs	BLP	MLP	SCP	SFP	TP	BJP	ACP
Giardia	0/6	0/28	0/2	1/15	0/3	0/14	0/3
Entamoeba hartmanni	0/6	3/28	0/2	0/15	0/3	0/14	0/3
Alaria	6/6	16/28	2/2	11/15	3/3	6/14	0/3
Taeniidae	2/6	7/28	0/2	3/15	2/3	4/14	0/3
Diphyllobothrium latum	1/6	1/28	0/2	0/15	0/3	1/14	0/3
Hookworms	1/6	19/28	0/2	2/15	1/3	1/14	0/3
Capillaria	0/6	2/28	1/2	1/15	0/3	0/14	0/3

from necropsied central New Jersey dogs. *E. hartmanni* and *Giardia* are reported herein for the first time from Wisconsin wolves.

Giardia has been recovered from a number of wild animals including beaver (Center for Disease Control, 1977, Morbidity and Mortality Weekly Report 26:169–175). The presence of *Giardia* in beaver is of interest because 16.8% of a wolf's diet in Wisconsin consists of beaver (Mandernack, 1983, M.S. Thesis, University of Wisconsin–Eau Claire). It is possible that *Giardia* is cycling between wolves and beaver in Wisconsin.

Giemsa-stained blood samples from 7 live-trapped wolves contained no protozoans or microfilariae.

A total of 43 ticks, *Dermacentor variabilis,* was collected from 2 live-trapped wolves. Two female voucher specimens are deposited at the USNM, accession numbers RML 117978 and RML 117979.

The Wisconsin wolf populations are endangered and continuous monitoring of all aspects of their health is necessary to insure their survival.

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Research Note

Helminth Parasites of Six Lizard Species from Southern Idaho

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The Helminth fauna of lizards from the western states has been studied in southern California (Telford, 1970, American Midland Naturalist 83: 516–554); Arizona and Nevada (Babero and Kay, 1967, Journal of Parasitology 53:168–175; Babero and Matthias, 1967, Transactions of the American Microscopical Society 86:173–177); Utah (Woodbury, 1934, Copeia 1:51–52; Grundmann, 1957, Proceedings of the Utah Academy of Sciences 34:147–148; Grundmann, 1959, Journal of Parasitology 45:394; Pearce and Tanner, 1973, Great Basin Naturalist 33:1–18); central Oregon (White and Knapp, 1979, Proceedings of the Helminthological Society of Washington 46:270–272); and Idaho (Waitz, 1961, Journal of Parasitology 47:51). The present study reports the helminths infecting 5 species of Iguanidae, *Sceloporus graciosus, S. occidentalis, Uta stansburiana, Phrynosoma platyrhinos,* and *Crotaphytus wislizeni,* and 1 species of Teiidae, *Cnemidophorus tigris* from southern Idaho. Idaho represents the northern limits in the range of these 6 lizard species. Host and locality records for the 6 helminth species reported in this study are presented in Table 1.

Lizards were collected from 10 arid localities on the Snake River Plain between May and September 1982 and 1983. Lizards were collected by noosing and either drowned in warm water for immediate dissection or preserved in 70% ethanol for later necropsy. The nares, mouth, pharynx, coelom, mesenteries, and intact organs were examined for helminths. All internal organs were removed separately to individual dishes for microscopic examination. Examination of gut contents revealed that the hosts had fed chiefly on arthropods. Recovered worms were preserved in 70% ethanol. Nematodes were cleared and mounted in glycerine; cestodes were stained with Grenacher's alcoholic borax-carmine.

Three specimens of the cestode *Oochoristica scelopori* Voge and Fox, 1950 were recovered from the duodenum of 1 adult female sagebrush lizard, *Sceloporus graciosus.* It also was collected from the duodenum of 2 adult female western fence lizards, *S. occidentalis,* which harbored 14

Table 1. Host and locality records for 6 helminth species of lizards in the western United States.

Parasite	Host	Locality	Reference
Nematoda			
Physalopteridae			
Skrjabinoptera phrynosoma (Ortlepp, 1922)	*Sceloporus graciosus*	California	Stebbins and Robinson (1946; cited by Pearce and Tanner, 1973)
	S. occidentalis	Utah	Pearce and Tanner, 1973
		Idaho	Waitz, 1961
	Cnemidophorus tigris	California	Telford, 1970
	Phrynosoma platyrhinos	California	Telford, 1970
		Nevada	Babero and Kay, 1967
		Utah	Grundmann, 1959; Woodbury, 1934
		Idaho	Waitz, 1961; *nobis*
	Crotaphytus wislizeni	Idaho	*nobis*
Atractidae			
Cyrtosomum readi Gambino, 1958	*Sceloporus graciosus*	Utah	Pearce and Tanner, 1973
	Phrynosoma platyrhinos	California	Telford, 1970
		Nevada	Babero and Kay, 1967
		Idaho	Waitz, 1961; *nobis*
	Crotaphytus wislizeni	California	Telford, 1970
		Idaho	Waitz, 1961; *nobis*
Pharyngodonidae			
Pharyngodon giganticus Read and Amrein, 1953	*Sceloporus graciosus*	California	Telford, 1970
		Oregon	White and Knapp, 1979
	S. occidentalis	California	Telford, 1970
		Utah	Pearce and Tanner, 1973
		Idaho	*nobis*
	Uta stansburiana	California	Telford, 1970
Cestoda			
Anoplocephalidae			
Oochoristica scelopori Voge and Fox, 1950	*Sceloporus graciosus*	California	Telford, 1970
		Utah	Pearce and Tanner, 1973
		Idaho	Waitz, 1961; *nobis*
	S. occidentalis	California	Telford, 1970
		Oregon	White and Knapp, 1979
		Idaho	*nobis*
	Crotaphytus wislizeni	California	Telford, 1970
O. bivitellobata Loewen, 1940	*Cnemidophorus tigris*	California	Telford, 1970
		Nevada	Babero and Matthias, 1967
		Utah	Grundmann, 1959
		Idaho	*nobis*
O. phrynosomatis (Harwood, 1932)	*Phrynosoma platyrhinos*	Nevada	Babero and Kay, 1967
		Utah	Grundmann, 1959
		Idaho	*nobis*

and 9 cestodes, respectively. The latter finding represents the first record of *O. scelopori* in *S. occidentalis* from Idaho. One and 4 *Pharyngodon giganticus* Read and Amrein, 1953 were recovered from the large intestine of 1 adult male and 1 adult female *S. occidentalis,* respectively. All 5 *P. giganticus* were gravid. Idaho represents a new locality record for *P. giganticus.* Differences in helminth prevalences among lizard populations at the 10 collection localities are recorded in Table 2.

Oochoristica bivitellobata Loewen, 1958 was removed from the duodenum of 6 adult female and 4 adult male western whiptailed lizards, *Cnemidophorus tigris.* An additional 3 *C. tigris* (1 male and 2 female) each harbored one immature tapeworm, tentatively identified as *O. bivitellobata.* The number of *O. bivitellobata* within an individual *C. tigris* ranged from 1 to 27 with a median of 2 cestodes per host. Idaho constitutes a new locality record for *O. bivitellobata.*

Table 2. Prevalence of helminths in 6 species of lizards from 10 localities in southern Idaho, USA.

	Total (by locality number*)	Helminth habitat
Oochoristica scelopori		
S. graciosus	1/118 (0/39 loc. 1; 0/8 loc. 2; 1/50 loc. 3; 0/21 loc. 4)†	Small intestine
S. occidentalis	2/19 (0/4 loc. 5; 0/1 loc. 7; 2/14 loc. 9)	Small intestine
Pharyngodon giganticus		
S. occidentalis	2/19 (0/4 loc. 5; 0/1 loc. 7; 2/14 loc. 9)	Large intestine
Oochoristica bivitellobata		
C. tigris	13/32 (2/11 loc. 4; 3/6 loc. 8; 8/15 loc. 10)	Small intestine
Oochoristica phrynosomatis		
P. platyrhinos	4/10 (1/3 loc. 9; 3/7 loc. 10)	Small intestine
Skrjabinoptera phrynosoma		
P. platyrhinos	6/10 (1/3 loc. 9; 5/7 loc. 10)	Stomach
C. wislizeni	2/14 (0/1 loc. 6; 0/1 loc. 8; 0/2 loc. 9; 2/10 loc. 10)	Stomach
Cyrtosomum readi		
P. platyrhinos	4/10 (0/3 loc. 9; 4/7 loc. 10)	Cecum
C. wislizeni	3/14 (0/1 loc. 6; 0/1 loc. 8; 0/2 loc. 9; 3/10 loc. 10)	Cecum

* Expressed as number of hosts infected/number examined.

† The 10 collection localities are numbered as follows: 1, Idaho Falls; 2, Ammon; 3, Pocatello; 4, Massacre Rocks; 5, Twin Falls; 6, Filer; 7, Malad Gorge; 8, Bruneau Dunes; 9, Swan Falls; 10, Murphy.

Twenty-seven male and 20 female specimens of the side-blotched lizard, *Uta stansburiana,* were examined. No helminths were found in these lizards.

Three helminth species were recovered from the desert horned lizard, *Phrynosoma platyrhinos*: *Oochoristica phrynosomatis* (Harwood, 1932), *Skrjabinoptera phrynosoma* (Ortlepp, 1922), and *Cyrtosomum readi* Gambino, 1958. The number of *O. phrynosomatis* per male host was 8, 23, and 38; 1 female horned lizard harbored 5 tapeworms. Idaho represents a new locality record for *O. phrynosomatis. Skrjabinoptera phrynosoma* was present in the stomach of 3 male and 1 female *P. platyrhinos.* A single gravid female *S. phrynosoma* was found in the large intestine of one horned lizard. *Cyrtosomum readi* occupied the cecum of 3 female and 1 male *P. platyrhinos.* Six *P. platyrhinos* were infected with helminths. Four lizards each harbored 2 species and 2 *P. platyrhinos* were infected with all 3 species of helminths.

Recovered from the leopard lizard, *Crotaphytus wislizeni,* were 2 nematode species. *Cyrtosomum readi* was collected from the cecum of 2 male and 1 female *C. wislizeni. Skrjabinoptera phrynosoma* was present in the stomach and large intestine of 1 male *C. wislizeni,* and in the stomach, small intestine, and large intestine of another. One male *C. wislizeni* harbored both *C. readi* and *S. phrynosoma.* The discovery of *S. phrynosoma* in *C. wislizeni* represents a new host record. All previous reports indicate the stomach as the habitat for *S. phrynosoma.* Presumably, the female *S. phrynosoma* recovered from the small and large intestines of *Phrynosoma platyrhinos* and *C. wislizeni* were worms migrating to the rectum to be expelled with host's feces (see Lee, 1955, Journal of Parasitology 41:70–74). As these lizards were dissected immediately after death, the presence of *S. phrynosoma* outside of the stomach is not likely due to postmortem migration. No differences in prevalence of helminths due to host sex were observed in the 6 lizard species.

This study is part of the author's thesis for the M.S. degree in Biology at Idaho State University. Representative specimens of the 6 helminth species reported in this study have been deposited in the Harold W. Manter Laboratory at the University of Nebraska State Museum and correspond to the accession numbers HWML 22984 through HWML 22991. I thank Dr. Gerald D. Schmidt for confirming identifications of the helminths and am grateful to Bethany and Angeline Lyon for their assistance in the field.

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Research Note

Notes on Leeches (Hirudinea) of the Connecticut Lakes Region of Northern New Hampshire

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The freshwater leech (Hirudinea) fauna of northern New England, particularly New Hampshire, is poorly known when compared to southern parts of the region. The only report giving distribution data on New Hampshire leeches is by Kendall and Goldsborough (1908, Report Commission Fisheries (U.S.), 1907, Special Papers (633):1–77) in which *Mooreobdella* (their *Dina*) fervida, *Glossiphonia* (their *Glossyphonia*) *complanata,* and *Batracobdella* (their *Placobdella*) *picta* (Verrill) are listed. Klemm's (1985, pages 70–173 *in* D. J. Klemm, ed. A Guide to the Freshwater Annelida of North America. Kendall-Hunt, Dubuque) comprehensive synthesis of North American leech distribution lists only 5 species (including *B. picta*) known to occur in New Hampshire. Klemm (1985, loc. cit.), whose nomenclature is followed, did not include Kendall and Goldsboroughs' (1908, loc. cit.) data which would have added 2 species (*M. fervida* and *B. picta*) to the New Hampshire fauna, bringing the total number of species to 7. In a recent survey conducted during August 1985, of the Connecticut Lakes district of northernmost New Hampshire, leech collections were made that resulted in many new records for New Hampshire and documented the first New England record for 1 species (Table 1).

The Connecticut Lakes and associated streams comprise the headwaters of the Connecticut River, a major south-flowing river in New England. A few of the species collected during this survey are northern in their distribution, known elsewhere in New England only in Maine, or Canada, and are unknown from the more temperate southern parts of the Connecticut River system

Table 1. Species of leeches collected during this study and previously in New Hampshire. NR indicates not recorded in this study.

Species	Sites of collection
Glossiphoniidae	
Actinobdella inequiannulata Moore, 1901§	NR
Batracobdella picta (Verrill, 1872)‡	1, 8, 10
Glossiphonia complanata (Linn., 1758)‡§	1, 2, 3, 8, 10, 12
Helobdella stagnalis (Linn., 1758)*	1
Helobdella triserialis (E. Blanchard, 1849)§	NR
Placobdella ornata (Verrill, 1872)*	1, 10, 12
Theromyzon biannulatum Klemm, 1977†	7
Hirudinidae	
Haemopis grandis (Verrill, 1874)*	8
Haemopis marmorata (Say, 1824)*	1, 3, 6, 8, 11
Macrobdella decora (Say, 1824)§	NR
Erpobdellidae	
Dina dubia Moore and Meyer, 1951*	7, 12
Erpobdella p. punctata (Leidy, 1870)§	7, 9, 10
Mooreobdella fervida (Verrill, 1871)‡	NR
Nephelopsis obscura (Verrill, 1872)*	3, 4, 5, 8, 10, 12

* First New Hampshire record.
† First New England record.
‡ Kendall and Goldsborough (1908).
§ Klemm (1985).

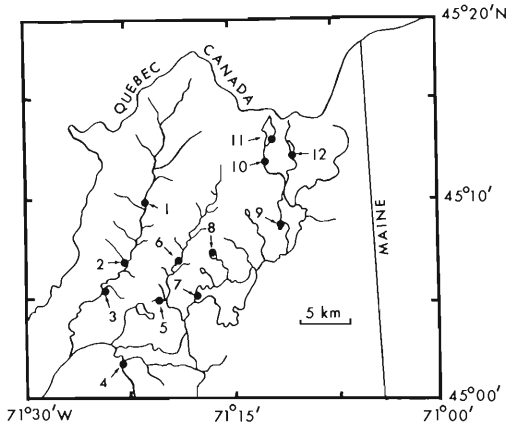

Figure 1. Map showing Connecticut Lakes region of New Hampshire and collection sites. Numbers refer to sites listed in text.

in Massachusetts and Connecticut (Klemm, 1985, loc. cit.; Smith, unpubl. records). The occurrence of these northern species in the upper Connecticut River watershed possibly represents the extent of their southward range in this portion of New England.

Leeches were collected at 12 locations corresponding to numbers in Figure 1. The locations are: 1, Indian Stream; 2, flooded meadow holes along Indian Stream; 3, beaver ponds along Indian Stream; 4, Dead Water Stream near Lake Francis; 5, Scott Brook; 6, Perry Stream below Lamb Valley Brook; 7, First Connecticut Lake near its dam; 8, Round Pond; 9, Second Connecticut Lake along its west shore; 10, Connecticut River below Third Connecticut Lake; 11, Third Connecticut Lake along U.S. Route 3; 12, Scott Bog at its outlet. All collection sites are in Pittsburgh, Coos County except site 4 which is in Clarksville, Coos County. Specimens were collected by hand or dip net and were identified by the author. Preserved specimens have been placed in the Invertebrate Division of the Museum of Zoology, University of Massachusetts at Amherst.

In addition to the 10 species collected, representing 3 families, the other 4 species previously known from New Hampshire are included in Table 1. Among the 10 species collected, *Glossiphonia complanata, Nephelopsis obscura,* and *Haemopis marmorata* were most common; *Theromyzon biannulatum, Haemopis grandis,* and *Helobdella stagnalis* were least common.

An interesting discovery was *T. biannulatum,* which is poorly understood both taxonomically and biogeographically. The 2 specimens collected were free-living adults, 1 greatly gorged with blood, the other typically translucent and gelatinous (Sawyer, 1972, Illinois Biological Monographs 46:1–146), and brooding 37 young. Annulation was difficult to discern (see Klemm, 1977, Michigan Academician 9:397–418), however, in 1 specimen, the gonopores were clearly separated by 2 annuli. The pigmentation was chiefly made up of a green background with small orange-yellow spots distributed throughout the dorsal surface. The more distinctive spots were arranged roughly in 4 longitudinal rows. The body margins were extensively spotted with alternating orange-yellow patches as well. Black chromatophores were not apparent.

Dina dubia is rare in New England, having been reported previously in New England only from Maine (Klemm, 1985, loc. cit.). *Dina dubia* has been considered a species with a more westerly distribution in North America (Sawyer, 1972, loc. cit.; Davies, 1973, Canadian Journal of Zoology 51:531–545). Prior to Klemm's (1985, loc. cit.) account no published records existed of this species east of the Great Lakes region.

Batracobdella picta was found often in association with *H. marmorata.* In 2 instances, specimens of *B. picta* were collected while attached to *H. marmorata* and were apparently feeding on the *Haemopis* individuals as evidenced by the enlarged digestive ceca and the tenacity and duration of attachment of the *B. picta* specimens.

I thank Karsten Hartel and Frank Ross for their assistance in the field and the Connecticut River Watershed Council for providing financial assistance.

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Research Note

Experimental Infection of the Brown Water Snake, *Nerodia taxispilota*, with *Sebekia mississippiensis* (Pentastomida)

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The brown water snake, *Nerodia taxispilota* (Holbrook), is a nonpoisonous, semi-aquatic species characteristic of lakes, ponds, swamps, and marshes from coastal Virginia through Florida to southwest Alabama (Behler and King, 1979, The Audubon Society Field Guide to North American Reptiles and Amphibians, Chanticleer Press, Inc., New York). Its diet is composed chiefly of fish (Camp et al., 1980, Journal of Herpetology 14(3):301–304; Collins, 1980, Brimleyana 4:157–159). Five specimens of *N. taxispilota,* newly born in captivity and unfed, were used to determine this snake's potential as a paratenic host of *Sebekia mississippiensis* Overstreet, Self, and Vliet, a lung pentastomid of the American alligator, *Alligator mississippiensis* Daudin (Overstreet et al., 1985, Proceedings of the Helminthological Society of Washington 52:266–277).

The snakes were randomly allocated to 2 groups, "infected" ($N = 3$) and "control" ($N = 2$), and kept in separate aquaria. Each day for 21 days "infected" snakes were offered live, infected mosquitofish, *Gambusia affinis* (Baird and Girard), collected from a man-made lake in Hillsborough County, Florida, known to harbor fish infected with *S. mississippiensis* (Boyce, 1985, Proceedings of the Helminthological Society of Washington 52:278–282). Dissection of 24 fish revealed a prevalence of 58% with a mean intensity of 4.8 nymphs of *S. mississippiensis* per infected fish. "Control" snakes were offered commercially reared specimens of the guppy, *Poecilia reticulata* Peters. Dissection of 15 guppies revealed these to be free of *S. mississippiensis* nymphs.

One snake in the "infected" group died on day 12. Upon its necropsy 7 nymphs of *S. mississippiensis* were found distributed randomly in subcutaneous tissues and 2 nymphs within the abdominal cavity (intra-abdominally). The cause of death, however, was most likely attributable to a larva of *Eustrongylides* sp. that had perforated the esophagus at the level of the heart.

The 4 remaining snakes were necropsied on day 21. Both surviving "infected" snakes harbored nymphs of *S. mississippiensis.* One specimen contained 12 nymphs, 7 distributed intra-abdominally, 4 in subcutaneous tissues, and 1 within the stomach wall. Thirty nymphs were recovered from the second snake, 13 distributed intra-abdominally, 11 in subcutaneous tissues, and 6 within the musculature of the body wall. No evidence of infection by *S. mississippiensis* was observed in the 2 "control" snakes. There was no evidence of development of *S. mississippiensis* beyond the nymphal stage in infected snakes and no pathologic lesions attributable to migration of the nymphs were observed.

Natural infection by nymphs of *S. mississippiensis* has been reported from 5 species of *Nerodia*—*N. rhombifera* (Hallowell), *N. sipedon* (Linnaeus), *N. cyclopion* (Dumeril and Bibron), *N. fasciata confluens* Blanchard, and *N. erythrogaster* Forster (Overstreet et al.). However, Dukes et al. (1971, Journal of Parasitology 57:1028) failed to experimentally transmit *S. mississippiensis* to 3 of these species (*N. sipedon, N. rhombifera,* and *N. erythrogaster*) by force feeding tissues of the largemouth bass, *Micropterus salmoides* (Lacépède), containing viable "larvae" (presumably nymphs), although infection was successfully transmitted to a turtle, *Chelydra serpentina* (Linnaeus), by this technique.

Water snakes have been reported in the diet of free-ranging adult alligators (Giles and Childs, 1949, Journal of Wildlife Management 13:6–28; McNease and Joanen, 1977, Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 31:36–40; Valentine et al., 1972, Journal of Wildlife Management 36:809–815). Because young snakes in this experimental study readily ate live infected mosquitofish and became infected, it is likely

that *N. taxispilota* could transmit *S. mississippiensis* to alligators feeding upon them under natural conditions.

We wish to thank Dr. Jack M. Gaskin, University of Florida College of Veterinary Medicine, and Tracy Howell and personnel of Gator Jungle, Dover, Florida, for their cooperation in this project. Published as University of Florida Agricultural Experiment Stations Journal Series No. 6976.

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Research Note

Infection of Sheep with Frozen Sporocysts of *Sarcocystis ovicanis*

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Sarcocystis sporocysts from feces in the large intestine of frozen coyote carcasses were infective for calves (Fayer and Johnson, 1975 Journal of Infectious Diseases 131:189–192). Heydorn (1980, Berliner und Münchener Tierärztliche Wochenschrift 90:267–270) reported infection of a calf with *S. bovicanis* sporocysts that had been stored at −22°C for 10 days. To test the viability and infectivity of frozen sporocysts, *Sarcocystis ovicanis* sporocysts from dogs fed experimentally infected lamb meat were cleaned of fecal debris, refrigerated at 7–10°C in HBSS-PSMF (Leek and Fayer, 1979, Proceedings of the Helminthological Society of Washington 46:151–154) for 19 mo and then stored frozen for 23 mo at −15–−20°C. The culture was thawed at room temperature, examined microscopically and estimated to contain approximately 50% normal appearing sporocysts, and then was tested for excystation as described by Fayer and Leek (1973, Proceedings of the Helminthological Society of Washington 40:294–296). Approximately 50% of the sporocysts either completely excysted or had active sporozoites therein.

Other sporocysts from the same refrigerated, frozen, and then thawed batch were inoculated in gelatin capsules by balling gun into 2 weaned 3-month-old Polled Dorset lambs. The lambs were born in the Animal Parasitology Institute flock which has had no history or problems of sarcocystis infection. One lamb (LL) received approximately 1.8 and the other (HL) 3.2 million sporocysts and an additional lamb (C) served as an uninoculated control. Feces were collected in fecal collection bags for 4 days to prevent infection via sporocysts that passed through the gastrointestinal tract intact. Lambs were housed in a barn and maintained on pelleted alfalfa, grain mix, trace mineralized salt, and water. Temperatures were recorded daily, weights were recorded weekly, and venous blood was collected weekly in vacuum tubes containing EDTA anticoagulant for determination of hematocrit levels and total counts of red and white blood cells by Coulter Counter.

Temperatures of the infected lambs were elevated on days 15–17, from days 23–26 when lamb HL died and from days 23–30 when moribund lamb LL was killed. Peak temperatures were 40.8°C (16 days) and 42.2°C (24 days) for lamb LL and 40.7°C (15 days) and 42.5°C (24 days) for lamb HL. Both lambs became anorectic from day 23. A 19% weight gain by the control, 23% by lamb HL until death at day 26 contrasted sharply to the 8% loss by lamb LL after day 26. Blood values were relatively unchanged through day 21. Lamb LL values at day 30 compared to day 1 were as follows: RBC 42%, WBC 28%, HCT 40%, and HGB 50%. No blood was collected from lamb HL after day 21. Comparable values on day 28 to day 1 for the control lamb were: RBC 127%, WBC 88%, HCT 134%, and HGB 88%. Postmortem examination of the in-

[1] Retired, October 1984.

fected lambs revealed hemorrhage of heart, skeletal muscle, subcutaneous and visceral fat, spleen, and stomach but dose-related differences could not be discerned. Schizonts were found in hematoxylin-eosin-stained tissue slides of heart, liver, lung, kidney, spleen, etc., as previously reported (Leek et al., 1977, Journal of Parasitology 63(4):642–650).

Previous reports have shown that frozen *Sarcocystis* in the tissues of the intermediate host did not infect the final host. Gestrich (1974, Proceedings of the 3rd International Congress for Parasitology, Munchen 1:117) obtained no infections in cats fed infected bovine diaphragm stored at −20°C for 3 days. Infections were not produced in dogs fed hamburger kept frozen for 7 days after purchase from a supermarket though control dogs fed unfrozen meat from the same batch did become infected (Fayer, 1975, Proceedings of the Helminthological Society of Washington 42:138–140). The question arises as to the viability of the less resistant tissue stages in infected carcasses frozen under natural conditions.

The infectivity of frozen sporocysts reported here might be likened to the natural freezing on the ground of sporocysts in the feces of predators. That cleaned sporocysts survived 1 freezing and thawing leaves open the question of their resistance to repeated freezing and thawing under either experimental (cleaned sporocysts) or natural conditions. The high prevalence of *Sarcocystis* in sheep and cattle indicates that some sporocysts, possibly a low percentage, do survive on the ground following extremes of hot and cold temperatures.

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Research Note

Taenia ovis krabbei from Grizzly Bears, *Ursus arctos*, in Montana and Adjacent Areas

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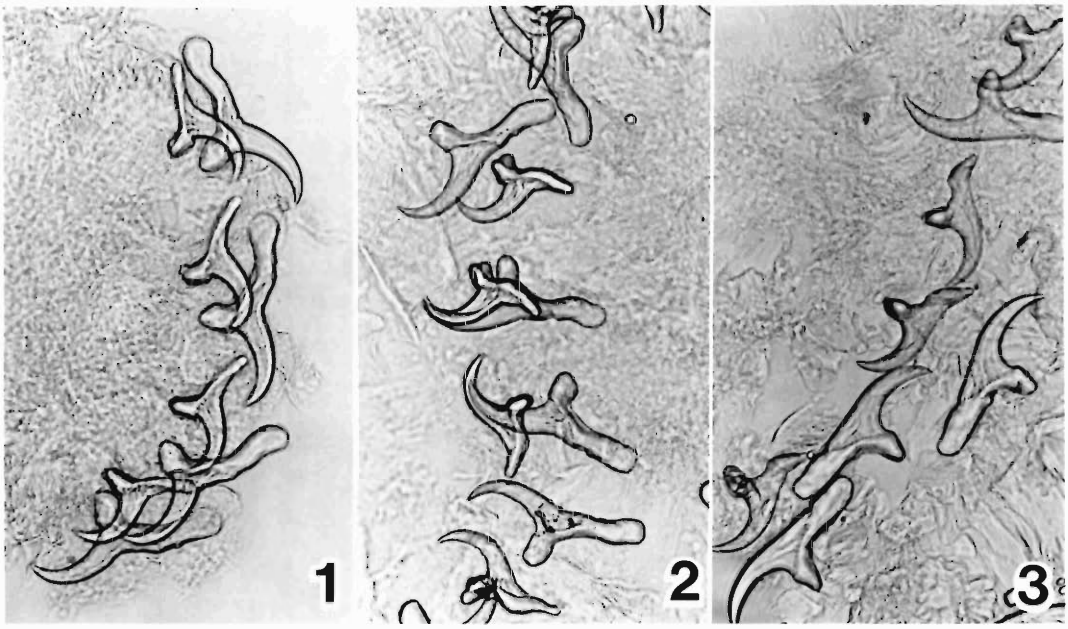
Taenia sp. found in 2 of 30 black bears, *Ursus americanus,* and in 14 of 66 grizzly bears, *Ursus arctos,* from Montana and adjacent areas could not be identified to species due to lack of fresh specimens bearing rostellar hooks (Worley et al., 1976, Third International Conference on Bears, International Union for Conservation of Nature No. 40, Morges, Switzerland, pp. 455–464). More recently, however, additional specimens with hooks have been obtained from the intestines of other grizzly bears from the same general area and these have facilitated identification of this species as *Taenia ovis krabbei* (Moniez, 1879) Verster, 1969. One of these bears contained 48 strobilate worms, most with complete sets of rostellar hooks. The cestodes were fixed in 10% buffered formalin, stained in Semichon's acetocarmine, and mounted in Permount. Individual scolices with hooks were squashed under a coverslip and examined at 170×. Hook shape, length, and number per worm were then compared with hooks dissected from cysticerci in musculature of mule deer, *Odocoileus hemionus,* and moose, *Alces alces,* from Montana. Voucher specimens of *T. ovis krabbei* from the grizzly bears were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, No. 79053.

Hooks from the 3 sources—grizzly bears, moose, and mule deer—are shown in Figures 1–3. Those from adult tapeworms in the grizzly bears closely resemble those from the juveniles in cysts from the moose but are different from those of the mule deer. The blade is much straighter in hooks from the mule deer and the handle is not as sinuous as in those from the grizzly bear and moose, although handles of large hooks from some grizzly bears were not sinuous. Hook length and number per worm, however, were nearly identical in all 3 groups (see Table 1). Small hooks from adult worms in the grizzly bears ranged in length from 94 to 117 μm. Those



Figures 1-3. Rostellar hooks of the cestode *Taenia ovis krabbei*, ×170. 1. From an adult worm in the intestine of a grizzly bear. 2. From a cysticercus dissected from the muscles of a moose. 3. From a cysticercus in muscles of a mule deer.

from cysticerci of moose ranged from 83 to 122, and those from mule deer ranged from 97 to 115. Large hooks ranged from 155 to 169 in the grizzly bears, 151 to 167 in moose, and 155 to 170 in the mule deer. Number of hooks per worm ranged from 29 to 34 in the grizzly bears, 27 to 34 in the moose, and 28 to 32 in mule deer.

Previous records of *Taenia ovis krabbei* in

Table 1. Rostellar hooks from *T. ovis krabbei* found in grizzly bears, moose, and mule deer from Montana and adjacent areas.

	Length of hooks (μm)							
	Small				Large			
Host species	$\bar{x}$*	SD†	Range	No. hooks/ worm	$\bar{x}$*	SD†	Range	No. hooks/ worm
Grizzly bear 105-85	107	4.4	101–113	15	159	1.6	158–162	16
	103	1.7	101–106	?	158	1.4	155–160	?
	113	3.8	108–117	16	167	3.5	162–171	16
	105	1.9	101–106	17	159	2.6	158–165	16
Grizzly bear 350-83	101	1.4	94–104	17	162	2.8	158–167	17
	108	8.9	101–112	13	165	4.0	158–169	16
Moose 221-84	97	1.7	94–99	16	158	3.2	153–162	16
	111	6.6	101–122	14	162	1.4	160–165	17
	94	8.3	83–104	12	152	1.8	151–155	15
	100	4.2	92–104	17	162	3.7	158–167	17
Mule deer 17256	99	3.0	97–104	15	160	2.2	158–162	15
	106	1.7	104–108	14	162	1.4	160–165	14
	109	3.1	106–113	16	170	2.3	167–174	16
	112	2.2	110–115	14	159	2.7	155–162	14

* $\bar{x}$ based on measurement of 6 rostellar hooks per scolex.
† SD = standard deviation.

grizzly bears are limited to a single observation by Choquette et al. (1969, Canadian Journal of Zoology 47:167–170) in northern Canada. Two of 29 grizzly bears examined harbored *T. ovis krabbei*. One contained 3 specimens, the other 6. Records from black bears are as follows: in captive animals in Alaska (Rausch, 1954, Journal of Parasitology 40:540–563); in 4% of 55 bears collected in Quebec (Frechette and Rau, 1977, Journal of Wildlife Diseases 13:432–434); in 2 of 148 in central Ontario (Addison et al., 1978, Canadian Journal of Zoology 56:2122–2126); in a large number of bears from the Chapleau Game Preserve, Ontario (Addison et al., 1979, Canadian Journal of Zoology 57:1619–1623); and in 10 of 91 bears from the Peace River Region of northwestern Alberta (Dies, 1979, Journal of Wildlife Diseases 15:49–51).

Differences in the shape of *Taenia ovis krabbei* hooks from cysticerci in moose versus those in mule deer probably represent intraspecific variation. Hook relationships suggest a grizzly bear–moose cycle. In North America, *T. ovis krabbei* in its adult host stage is primarily a parasite of wolves (Rausch and Williamson, 1959, Journal of Parasitology 45:395–403). There is apparently no previous record of *T. ovis krabbei* in moose that live outside of the range of wolves (Samuel, 1972, Transactions of the Eighth North American Moose Conference, Queens Printer, Ontario, Canada, pp. 18–41). The present records of *T. ovis krabbei* are from areas where moose are relatively common in grizzly bear habitat in and around Yellowstone and Glacier National parks where wolves are rare or absent. In these locations the grizzly bear may be an important alternate definitive host for this cestode.

We would like to acknowledge the technical assistance of Merrie Mendenhall in preparation of the figures and plate. Published as Journal Series No. 1807.

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MINUTES

Five Hundred Seventy-Third Through Five Hundred Eightieth Meetings

573rd Meeting: Uniformed Services University of Health Sciences, Bethesda, MD, 16 October 1985, Cosponsor, Tropical Medicine Association of Washington, D.C. Willis A. Reid presided over the business meeting. The following slate of officers was presented to the membership for consideration: Ralph P. Eckerlin, President; Patricia A. Pilitt, Vice President; Michael D. Ruff, Corresponding Secretary-Treasurer; Jeffrey W. Bier, Recording Secretary. Bryce C. Redington presided over the scientific session of three papers: Ronald Neafie discussed *Baylisascaris* as a human parasite; Eugene G. Hayunga and Mary P. Sumner described surface glycoprotein antigens of *Schistosoma mansoni*; and John H. Cross reviewed intestinal capillariasis.

574th Meeting: Dinner meeting in commemoration of the Society's 75th Anniversary, Chevy Chase Holiday Inn, Bethesda, MD, 22 November 1985. Willis A. Reid presided over the business meeting to elect officers for the 1986 calendar year (above). Bryce C. Redington presented the Anniversary Award to A. Morgan Golden for his contributions to the field of nematology and service to the Society. Gilbert F. Otto reminisced about significant characters in the Society's history.

575th Meeting: Animal Parasitology Institute, USDA, Beltsville, MD, 11 December 1985, Cosponsor, Nematology Laboratory, USDA, Beltsville, MD. Officers for the upcoming year were announced. Ron Fayer presided over the scientific session where Robin Huettel explained the reorganization of Nematology at Beltsville; John Dame described the relation between the DNA and infectivity in *Trichinella*; Hyun S. Lillehoj discussed the immune response of chickens to coccidiosis; and posters by J. R. Lichtenfels, P. A. Pilitt and L. F. Le Jambre illustrated cuticular ridges in *Haemonchus* spp. and by R. H. Fetterer and J. F. Urban on effects on growth and development of *Ascaris suum* by proline analogs.

576th Meeting: National Institutes of Health, Bethesda, MD, 15 January 1986. President Eckerlin presented a constitutional amendment to permit the Executive Committee to elect new members of the Society. Frank Neva presided over the scientific session. Anita Aggarwal described two antigenetically distinct *Giardia lamblia*; James A. Sherwood discussed *Plasmodium falciparum* infected erythrocytic binding of thrombosporin; and Frank Klotz related findings on the relationship of the 140 kd merozoite protein to antigen expression in chronic *Plasmodium knowlesi* infections.

577th Meeting: Naval Medical Research Institute, Bethesda, MD, 12 February 1986, Cosponsor, Food and Drug Administration. The constitutional amendment regarding membership was passed. Richard Beaudoin presided over the scientific session. Herbert Koch described coinfection with *Giardia lamblia* and *Candida albicans*; Yupin Charoenvit characterized antigens of *Plasmodium yoelii*; and Monte P. Bawden related experiences of a parasitologist in Panama. David R. Lincicome demonstrated a poster of past poetic efforts by Society members.

578th Meeting: Armed Forces Institute of Pathology, 19 March 1986, Cosponsor, Walter Reed Army Institute of Research, Washington, D.C., 19 March 1986. M. D. Ruff presented the audited Treasurer's report for 1985. The membership commended M. D. Ruff for exemplary service as Corresponding Secretary-Treasurer. The scientific meeting was presided over by J. Kevin Baird and Willis A. Reid. Gladys H. George described microcirculation in an *Onchocerca* nodule; Dennis E. Kyle discussed the regulatory effects of some peptides on *Eimeria tenella*; and Max Grogl characterized the schizodeme of *Leishmania braziliensis.*

579th Meeting: Johns Hopkins University, 16 April 1986, Baltimore, MD, Everett L. Schiller presided over the scientific session. Wesley Tamashiro talked about the proteolytic enzymes of *Dirofilaria immitis*; Victoria Han discussed immunoregulation of *Nippostrongylis*; David Fryauff described *Onchocerca volvulus* in Li-

berian blackflies; James K. Lovelace detailed activities of acid phosphatases of *Leishmania.*

580th Meeting: University of Pennsylvania, New Bolton Center, Kennett Square, PA, 10 May 1986, Cosponsor, New Jersey Society of Parasitologists and Royal Society of Tropical Medicine and Hygiene. Paul C. Beaver and Raymond M. Cable were elected life members. G. A. Schad presided over a symposium on the new interest in intestinal nematodes. Samuel Ward discussed *Caenorhabditis elegans*; Peter J. Hotez hookworms; and Gary Smith population biology.

Members elected between the 565th and 580th meetings are listed below since last year's list was omitted: *565th:* Carter T. Atkinson, J. W. Bernstein, Vincent A. Conners, Martin D. Crosby, Alejandro Cruz-Reyes, Rosemary Kelly, Hyun S. Lillehoj, William K. Richey, Lora G. Rickard, Ronald B. Rosen, Daniel E. Snyder, Jeffrey D. Stamper. *566th:* Harold C. Gibbs. *576th:* Robert J. Chinnis. *568th:* Joan K. Lunney, Catherine A. Palmer, Yurin Matte Ryuntyu. *569th:* Thomas H. Cribb. *570th:* Pauline M. Huber. *571st:* Cheryl Courtney, John B. Dame, Frank Sherwin, May Yun Yue. *572nd:* Donald W. Hosier, Deborah T. Hanfman, Alan M. Johnson. *573rd:* John Bratty, Douglas G. Cloutman, Douglas D. Colwell, P. Illiscas Gomez, Malcom K. Jones, Michael Kogut, Steve J. Upton. *574th:* David S. Lindsay, Hideo Hasegawa. *575th:* J. Kevin Baird, Walter M. Boyce, Ernest Kamanga-Sollo, Terry A. Wheeler. *576th:* Ruth Kulstad, Raphael R. Payne. *578th:* P. C. Gupta. *579th:* Neriede G. Ellis. *580th:* Cheryl M. Bartlett, Jean B. Blythe, R. C. Krecek, Tammy Roberts, L. G. Sellers.

Respectfully submitted,
JEFFREY W. BIER

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ANNOUNCEMENT

The *Ostertagia* Workshop
December 3–5, 1986

A workshop on *Ostertagia* will be held at the University of Maryland, University College Center of Adult Education, College Park, MD. The scientific program is intended to be comprehensive on all aspects of research on *Ostertagia ostertagi* and its diseases. The workshop will include invited speakers and roundtable discussions on topics to promote research and control. An abridged agenda and scientific program follows.

December 3 (Wednesday evening)
Registration and social

December 4 (Thursday morning)
Introductions and welcome
Session 1: *Systematics and Biology of Ostertagia*
Systematics; Biology.
Session 2: *Epidemiology—U.S.A.*
Southern & western regions; Northern region; Roundtable discussion.

December 4 (Thursday afternoon)
Session 3: *Economic Aspects of Ostertagiasis*
Beef production; Economics, infection, disease modeling; Roundtable discussion.
Session 4: *Selected Research Papers*
Importance and control of *Ostertagia* in Australia; Epidemiology & control of ostertagiasis in South America; In vitro cultivation of *Ostertagia*; Experimental ostertagiasis in goats and rabbits; Drug resistant *Ostertagia* in cattle.

December 5 (Friday morning)
Session 5: *Approaches to Control*
Anthelmintics and control; Integrated control; Importance of diagnostic aspects; Epidemiological parameters—herbage larval sampling; Novel approach to control; Roundtable discussion.

December 5 (Friday afternoon)
Session 6: *Immunology and Pathology*
Review of immunity to *O. ostertagi*; Pathology; Immunobiology; Roundtable discussion.
Session 7: *Summation and Recommendation for Research*

For further information about the workshop, agenda and scientific sessions, please write or call: Dr. P. H. Klesius, USDA, ARS, Animal Parasite Research Laboratory, P.O. Box 952, Auburn, AL 36830 (U.S.A.); telephone no. (205)887-3741.

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)

ANNIVERSARY AWARD RECIPIENTS

Edna M. Buhrer	1960	E. J. Lawson Soulsby	1974
Mildred A. Doss	1961	David R. Lincicome	1975
* Allen McIntosh	1962	Margaret A. Stirewalt	1975
* Jesse R. Christie	1964	* Leo A. Jachowski, Jr.	1976
Gilbert F. Otto	1965	Horace W. Stunkard	1977
* George R. LaRue	1966	Kenneth C. Kates	1978
* William W. Cort	1966	* Everett E. Wehr	1979
* Gerard Dikmans	1967	O. Wilford Olsen	1980
* Benjamin Schwartz	1969	Frank D. Enzie	1981
* Willard H. Wright	1969	Lloyd E. Rozeboom	1982
Aurel O. Foster	1970	Leon Jacobs	1983
Carlton M. Herman	1971	Harley G. Sheffield	1984
May Belle Chitwood	1972	A. Morgan Golden	1985
* Elvio H. Sadun	1973		

HONORARY MEMBERS

* George R. LaRue	1959	Justus F. Mueller	1978
Vladimir S. Ershov	1962	John F. A. Sprent	1979
* Norman R. Stoll	1976	Bernard Bezubik	1980
Horace W. Stunkard	1977	Hugh M. Gordon	1981

CHARTER MEMBERS 1910

* W. E. Chambers	* Philip E. Garrison	* Maurice C. Hall	* Charles A. Pfender
* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	Carlton M. Herman	1975
* Albert Hassall	1931	Lloyd E. Rozeboom	1975
* Charles W. Stiles	1931	Albert L. Taylor	1975
* Paul Bartsch	1937	David R. Lincicome	1976
* Henry E. Ewing	1945	Margaret A. Stirewalt	1976
* William W. Cort	1952	* Willard H. Wright	1976
* Gerard Dikmans	1953	* Benjamin Schwartz	1976
* Jesse R. Christie	1956	Mildred A. Doss	1977
* Gotthold Steiner	1956	* Everett E. Wehr	1977
* Emmett W. Price	1956	Marion M. Farr	1979
* Eloise B. Cram	1956	John T. Lucker, Jr.	1979
* Gerald Thorne	1961	George W. Luttermoser	1979
* Allen McIntosh	1963	John S. Andrews	1980
Edna M. Buhrer	1963	* Leo A. Jachowski, Jr.	1981
* Benjamin G. Chitwood	1968	Kenneth C. Kates	1981
Aurel O. Foster	1972	Francis G. Tromba	1983
Gilbert F. Otto	1972	A. James Haley	1984
* Theodor von Brand	1975	Paul C. Beaver	1986
May Belle Chitwood	1975	Raymond M. Cable	1986

* Deceased.

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