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CONTENTS

(*Continued on Outside Back Cover*)

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Visceral Helminths from an Expanding Insular Population of the Long-nosed Armadillo (*Dasypus novemcinctus*)

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ABSTRACT: Long-nosed armadillos (*Dasypus novemcinctus*) ($N = 117$) from Cumberland Island, Georgia were infected with encysted cystacanths of *Macracanthorhynchus ingens* and *Centrorhynchus* sp. and larvae of *Physaloptera* sp. Ninety-five, 56, and <3% of the hosts had 1, 2, and 3 species of helminths, respectively. The frequency distribution pattern of each helminth species was aggregated; overdispersion was homogeneous across host sex and season variables. Abundances of all helminth species collectively, and *Physaloptera* sp. individually, were significantly greater in the warm versus cool seasons. As a corollary to Brown's (1984) theory, we propose that a host population at the periphery of its geographic range may have fewer species and lower abundances of helminths and/or lack a defined helminth community when compared to a conspecific population at the host's epicenter of origin. This may partially explain why a community of species of adult helminths has not developed in the recently colonized population of armadillos on Cumberland Island. Other reasons for this vacant niche may include (1) the unique physiology of armadillos may preclude them as a suitable definitive host for helminths already on the island; (2) there are no other related hosts on the island with a community of helminth species that could infect the armadillo; and (3) the founders of this armadillo population were not infected prior to colonizing the island.

KEY WORDS: Brown's theory, *Dasypus novemcinctus,* geographic barrier, helminth community ecology, helminth survey, host colonization, insular host population, long-nosed armadillo, range periphery, physiological barrier, unsuitable host, vacant niche.

The range of the long-nosed armadillo (*Dasypus novemcinctus* Linnaeus) extends from the southern United States through Mexico and Central America into South America as far south as Uruguay (McBee and Baker, 1982). Armadillos were first reported in the United States in 1854 from southern Texas (Bailey, 1905). Subsequently, they have expanded their range northward and eastward from the Texas population and northward from expanding populations introduced in Florida in the early 1920's (Cleveland, 1970). Active invasion at a rate of 4–10 km/yr has extended the present distribution of armadillos from central Kansas eastward to South Carolina (Humphrey, 1974; Mayer, 1989).

Invasion of a species into a new locality may be more successful if the colonizing species is free from specific pathogenic parasites infecting it in the original habitat. Possible examples of this in species that have invaded North America include house sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*), both of which have fewer species of parasites than their European counterparts (Dobson and May, 1984). Although the long-nosed armadillo recently has colonized a large area in the southeastern United States, the few helminth surveys indicate that the invading population has only a fraction of the helminth species (Taber, 1945; Chandler, 1946, 1954) reported in this host from its native range in Central and South America.

Although islands are favored study-sites of ecologists, there are few studies (Kisielewska, 1970) on the acquisition of helminths by invading insular vertebrate hosts. Specimens collected in conjunction with other studies on the long-nosed armadillo population of Cumberland Island, a barrier island on the coast of Georgia, provided a unique opportunity to determine the recruitment and establishment of helminths in a recently colonized insular host population. Our objectives were to examine the structure (composition and abundances of species) and pattern (frequency distribution, species richness, effects of extrinsic and intrinsic variables) of the assemblage of species of helminths that have been acquired by this armadillo population since it was introduced to the island; specifically, if a community of helminth species has become established within this host.

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Materials and Methods

Study area

The 25-km-long × 1–9-km-wide, or 9,400-ha (including salt marshes) Cumberland Island, is the most southern and largest of Georgia's barrier islands. Located in Camden County, Georgia (30°48′N, 81°26′W), Cumberland Island is separated by a linear distance of about 2 km from the mainland by the Cumberland River and Cumberland Sound (Hillestad et al., 1975). In 1972, Cumberland Island was designated a National Seashore and incorporated into the National Park System; prior to this, it was privately owned. The island's natural history and ecology have been described in detail by Hillestad et al. (1975) and summarized by Pence et al. (1988b).

Armadillos were first reported on Cumberland Island in 1973 (Hillestad et al., 1975); they are now abundant in most vegetated upland habitats. This insular population of armadillos became established either through human introduction or by natural invasion from the nearby mainland where armadillos were present at least since 1954 (Humphrey, 1974). Other introductions to the island during the past 10–20 yr include feral populations of swine, horses, and cattle (Hillestad et al., 1975).

Collection of data

In conjunction with other physiological and ecological studies by the junior authors (R.J.W. and M.N.), 117 armadillos (67 males and 50 females; 110 adults, 7 juveniles <1 yr old) were collected from Cumberland Island by shooting during 4–7-day collection periods in late March ($N = 28$), mid-June ($N = 31$), late August ($N = 29$) and mid-December ($N = 29$) 1987. The June and August collections were the warm season sample, while the March and December collections were the cool season sample. About 80% of the armadillos were collected in oak-palmetto and oak-pine habitats. These habitats comprise 61% of the island's forested area (Hillestad et al., 1975). Carcasses were weighed, sexed, and immediately frozen (Pence et al., 1988a). Age was based on the assumption that individuals weighing <3.0 kg were <1 yr old (McNab, 1980). All internal organs were examined; helminths were removed, identified, and quantified. Thin blood smears, prepared in duplicate immediately following death of the armadillos, were stained 10 min with phosphate-buffered Giemsa (pH 7.2) after fixation in 100% methanol for 1 min; they were examined microscopically for filariid nematodes. Necropsy techniques followed the procedures outlined by Wobeser and Spraker (1980), except that visual examination of organ contents was supplemented by repeated washings and sedimentation in conical glasses followed by examination of the sediment with a dissecting microscope. Contents of the stomach were removed for studies on food habits and nutritional ecology.

Nematodes were fixed briefly in glacial acetic acid, stored in a mixture of 70% ethyl alcohol with 8% glycerine, and examined in glycerine wet mounts. Acanthocephalans were fixed and stored in alcohol–formalin–acetic acid (AFA) solution, stained with Semichon's acetocarmine, cleared in oil of wintergreen, and mounted in Canada balsam. Representative specimens of the helminth species collected in this study are deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705, USA; accession numbers 80826–80828).

Analysis of data

Overdispersion was indicated when the variance was significantly larger than the mean (by chi-square analysis) in the frequency distribution of the respective helminth species, and was defined by the negative binomial parameter k (Bliss and Fisher, 1953). Homogeneity in the values of k generated from the helminth species' frequency distributions across host and seasonal variables were calculated by the method outlined in Wallace and Pence (1986) as modified from Bliss and Fisher (1953).

The main and interactive effects of 2 independent host variables (sex and season) on the numbers of individuals (abundances) of each species of helminth were examined with a factorial ANOVA, and for all species collectively with a subsequent MANOVA (PROC GLM, SAS; SAS Institute, Inc., 1985). Potential factors influencing abundances of these helminths were sex, season, and sex-season. Age could not be considered as a variable in the analysis because the sample size of the cohort of animals <1 yr old collected during the warm season was too small.

Definitions

The terms prevalence, intensity, mean intensity, and abundance follow the definitions of Margolis et al. (1982). Overdispersion is defined by Bliss and Fisher (1953); the term is used herein to describe aggregated helminth frequency distributions as outlined by Wallace and Pence (1986). Helminth community herein refers to an assemblage of helminth species occupying a certain site (habitat) within the host.

Results

The helminth fauna

Two larval acanthocephalans (*Macracanthorhynchus ingens* (Linstow, 1879) Meyer, 1933 and *Centrorhynchus* sp.) encysted as cystacanths on the serosal surface of the gastrointestinal tract and in the mesenteries, and a larval nematode (*Physaloptera* sp.) from the lumen of the small and large intestines, were found. This is the first report of *Centrorhynchus* sp. from the long-nosed armadillo. Table 1 lists the prevalences, intensities, and abundances of these helminth species. Armadillos were infected with none to 3 ($\bar{x}$ = 2.2) species of helminths. The number of armadillos infected with 1, 2, and 3 species of helminths were 111 of 117 (95%), 66 of 117 (56%), and 3 of 117 (<3%), respectively. There were 6,363 helminths collected from the armadillos; abundances ranged from 0 to 481 ($\bar{x}$ = 14.6 ± 5.0). Microfilariae were not observed in the blood smears.

Table 1. Visceral helminths of an insular population of long-nosed armadillos from Cumberland Island, Georgia.

Species of helminths	Prevalence: Number infected/ Number examined	Intensity: %	Intensity: $\bar{x} \pm$ SE*	Abundance: Range	Abundance: $\bar{x} \pm$ SE*	Total
Macracanthorhynchus ingens	11/117	95	47.6 ± 7.3	0–432	41.1 ± 6.4	4,628
Centrorhynchus sp.	5/117	4	2.2 ± 0.7	0–5	0.1 ± 0.3	11
Physaloptera sp.	75/117	65	22.5 ± 7.6	0–481	14.6 ± 5.0	1,724

* Mean ± standard error.

Helminth dispersion patterns

As characteristic of an overdispersed distribution (Bliss and Fisher, 1953), the variance was significantly larger than the mean for the frequency distributions of helminth individuals in each of the 3 species (Table 2). As indicated by Bliss and Fisher (1953) and outlined by Wallace and Pence (1986), the low values (<1.0) for the negative binomial parameter k in each of these species, indicated aggregation within the host population and within each of the 2 host subpopulations delineated by host sex and season variables. However, values of k were not significantly different across these host subpopulations when compared to the average (expected) value for the entire dataset (Table 2); thus, the degree of overdispersion was homogeneous across these subgroups of the host population.

Effects of intrinsic and extrinsic variables

The main effect of season accounted for significant differences in the numbers of individuals of all helminth species collectively (MANOVA) and individually (factorial ANOVA) for a single species (Table 3). Respectively, this resulted from the greater collective abundance of both *M. ingens* and *Physaloptera* sp. and the significantly greater abundance of *Physaloptera* sp. in the warm versus cool seasons. The number of helminth individuals collected during the warm and cool seasons were 4,148 and 2,364, respectively. Mean abundances were 48.2 ± 9.5, 0.1 ± 0.1, and 20.9 ± 8.6 versus 33.7 ± 8.8, 0.1 ± 0.1, and 8.0 ± 4.8 for *M. ingens, Centrorhynchus* sp., and *Physaloptera* sp., respectively.

The number of individuals of *M. ingens* varied across host sexes. There were significantly greater abundances of *M. ingens* in females (61.6 ± 12.7) than males (25.9 ± 5.6).

Discussion

All the helminths in armadillos from Cumberland Island were larvae, most of which were encysted and frequently were dead and calcified. The adults of *M. ingens* occur in carnivores, especially raccoons (*Procyon lotor*) (Petrochenko, 1958). The definitive hosts of *Centrorhynchus* spp. are terrestrial birds, especially raptors (Petrochenko, 1958). Adult *Physaloptera* spp. occur in many species of wild and domestic mammals (Levine, 1968). The frequency distributions and abundances of these larval species tend to follow

Table 2. Values of k as an inverse measure of overdispersion for 3 species of helminths across 4 major category variables delineated by host sex and season from the 117 sample dataset of the long-nosed armadillo from Cumberland Island, Georgia.

Species of helminth	Total (117)	Season: Cool, Male (31)	Season: Cool, Female (26)	Season: Warm, Male (36)	Season: Warm, Female (24)	Heterogeneity: Total χ^2	Heterogeneity: P
Macracanthorhynchus sp.	0.36*	0.47*	0.36*	0.35*	0.35*	0.05	>0.05
Centrorhynchus sp.	0.47	0.16	—	—	0.10	0.51	>0.05
Physaloptera sp.	0.07*	0.06*	0.26*	0.12*	0.12*	0.55	>0.05

* Variance significantly larger than mean as determined by chi-square analysis of the frequency distribution.

Table 3. *F* values generated by MANOVA and factorial ANOVA for main and interactive effects of host sex and season factors across the 117 sample dataset of rank abundances for 3 species of helminths in the long-nosed armadillo from Cumberland Island, Georgia.

	Sex	Season	Sex × Season
MANOVA			
Total helminth species	1.68	3.59*	0.93
Factorial ANOVA			
Macracanthorhynchus ingens	4.75*	3.71*	0.02
Centrorhynchus sp.	0.01	0.04	2.57
Physaloptera sp.	0.41	6.73*	0.17

* Significant at $P < 0.05$.

similar patterns to those described for populations of adult helminths in other host species (i.e., Wallace and Pence, 1986). Obviously, the armadillo is a paratenic host for these helminths, which probably were acquired incidentally through ingestion of arthropod intermediate hosts and after immigration to the island. Because just 3 species of larval helminths were found, and only 2 of these were encysted at the same site, the armadillo population on Cumberland Island has not yet acquired a defined helminth community.

The theory of Brown (1984), which interprets the relationship between abundance and distribution of animal species, was extrapolated to helminth communities by Fedynich et al. (1986) in order to help explain the disparity of helminth species in a beaver (*Castor canadensis*) population at the southern periphery of its native range. While the stable high population densities at the host's epicenter of origin tend to support larger numbers of more numerous species of helminths (a well-developed helminth community), the unstable lower population densities of a host species at the periphery of its range may support fewer numbers and species of helminths (diminished or no helminth community). This may partially explain the lack of species diversity and low numbers of helminths in the long-nosed armadillo from the United States, and, specifically, the absence of a helminth community in the armadillo population from Cumberland Island, which is at the extreme northeastern periphery of its range.

Vertebrate hosts at the extreme periphery of their range may outdistance many of their helminth parasites; however, some hosts such as raccoons in Saskatchewan (Hoberg and McGee, 1982) and coyotes (*Canis latrans*) in Tennessee (Pence, 1989) can acquire new species from taxonomically related host species in the new locality as replacements for those lost from their original helminth community. Because adult helminths were not found, it appears that the armadillo is not a suitable definitive host for any helminth species already established on Cumberland Island. The long-nosed armadillo is the only extant representative of the mammalian order Xenarthra (Edentata) in North America (Cleveland, 1970). Lack of closely related host species with established helminth communities greatly reduces the chances for acquisition of adult helminths by an invading species (Pence, 1989). This we define as (1) the unsuitable host hypothesis.

There are at least 52 species of adult helminths in the long-nosed armadillo and related species from Central and South America (Chandler, 1946). Although only 3 species of adult helminths were found in this host from Texas, there were several encysted larval acanthocephalan and nematode species (Chandler, 1946). Unfortunately, we could not sample armadillos from the mainland adjacent to Cumberland Island. However, if a helminth community or even individual species of adult helminths did occur in armadillos on the mainland, then those hosts that emigrated to the island must not have been infected with sufficient numbers of helminths to establish and/or maintain a helminth community. This we propose as (2) the geographic barrier hypothesis.

A final tentative explanation for the absence of a helminth community in armadillos on Cumberland Island is that the unique physiological features of the Dasypodidae may prevent establishment, growth, and/or reproduction of certain common helminth species that have little host specificity and are otherwise widely distributed across many different mammalian host taxa. These unique features include low body temperatures (31°–38°C) and a physiology similar to poikilotherms (Chandler, 1954) in which thermoregulatory mechanisms include shivering, changing posture, and probably vasoconstriction (Galbreath, 1982). This we term (3) the physiological barrier hypothesis.

The ramifications of a host population without

a defined helminth community lends itself to many potential studies. The armadillos on Cumberland Island are unique because this host population coexists on an island with other defined faunal components that may be reservoirs for helminths that could potentially infect them. Certainly, further studies are warranted to determine which of the above hypotheses (the corollary to Brown's theory, or our hypotheses 1, 2, and/or 3), or combination thereof, are valid explanations of why a helminth community failed to establish in an invading host population at the periphery of its native range.

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Neotropical Monogenea. 16. New Species of Oviparous Gyrodactylidea with Proposal of *Nothogyrodactylus* gen. n. (Oogyrodactylidae)

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ABSTRACT: Four new species of oviparous Gyrodactylidea (Oogyrodactylidae) are described from siluriform fishes from an unnamed stream flowing through the Bairro de São Jorge, Manaus, Amazonas, Brazil: *Phanerothecium harrisi* sp. n. from *Plecostomus plecostomus* (Linnaeus) (Loricariidae); and *Nothogyrodactylus clavatus, N. amazonicus,* and *N. plaesiophallus* spp. n., all from *Ancistrus* sp. (Loricariidae). *Nothogyrodactylus* gen. n. is proposed for oviparous forms possessing 1 or more accessory sclerites associated with the male copulatory organ. The diagnosis of *Phanerothecium* Kritsky and Thatcher, 1977, is emended. Notes on hatching and hatched larvae of *P. harrisi* are included, and the haptoral sclerites, egg, and copulatory organ of *Oogyrodactylus farlowellae* Harris, 1983, are figured.

KEY WORDS: Monogenea, taxonomy, morphology, systematics, Oogyrodactylidae, Gyrodactylidea, *Phanerothecium harrisi* sp. n., *Phanerothecium caballeroi, Nothogyrodactylus clavatus* sp. n., *Nothogyrodactylus amazonicus* sp. n., *Nothogyrodactylus plaesiophallus* sp. n., *Oogyrodactylus farlowellae, Nothogyrodactylus* gen. n., *Ancistrus* sp., *Plecostomus plecostomus,* Loricariidae.

Harris (1983) first recognized oviparity in members of the Gyrodactylidea. He proposed the Oogyrodactylidae for his new species, *Oogyrodactylus farlowellae* and *Phanerothecium caballeroi* Kritsky and Thatcher, 1977, both from Neotropical siluriform fishes. Four new species of oviparous gyrodactylideans, collected from loricariid catfishes, are described herein, and *Nothogyrodactylus* gen. n. is proposed for those possessing 1 or more accessory sclerites supporting the copulatory organ.

Materials and Methods

Loricariid hosts, *Ancistrus* sp. and *Plecostomus plecostomus* (Linnaeus), were collected by throw-net from a small unnamed stream within the Bairro de São Jorge, Manaus, Amazonas, Brazil, on 8 January 1989. Specimens of individual host species were treated collectively with a 1:4,000 formalin solution for removal of gyrodactylideans (Putz and Hoffman, 1963). Helminths were collected from host washings and prepared for study according to procedures of Mizelle and Kritsky (1967). Some specimens were mounted in Malmberg's medium (Malmberg, 1956) or Gray and Wess' medium (Humason, 1979) for study of sclerotized structures; Gomori's trichrome was used to stain haptoral bars (Kritsky et al., 1978) and features of internal organs. Illustrations were prepared with the aid of a camera lucida or microprojector. Measurements, all in micrometers, were made with a filar micrometer according to procedures of Mizelle and Klucka (1953); the average is followed by the range and number (*N*) of specimens measured in parentheses. Type specimens were deposited in the helminth collections of the Instituto Nacional de Pesquisas de Amazônia (INPA) (holotypes only), the Instituto Oswaldo Cruz (IOC) (1 paratype of each species), the U.S. National Museum (USNM) (balance of paratypes), the University of Nebraska State Museum (HWML) (1 paratype each of *Nothogyrodactylus amazonicus* and *N. plaesiophallus*; 2 each of *N. clavatus* and *Phanerothecium harrisi*), the British Museum (Natural History) (BM[NH]) (1 paratype of each species), and the Zoological Institute, U.S.S.R. Academy of Sciences, Leningrad (ZIAC) (1 paratype of each species). For comparative purposes, the following type specimens were examined: holotype, 4 paratypes, *Oogyrodactylus farlowellae* Harris, 1983 (BM[NH] 1982.3.30.1–9); 5 paratypes, *O. farlowellae (USNM 77095); and holotype, 9 paratypes, Phanerothecium caballeroi* Kritsky and Thatcher, 1977 (USNM 73407, 73408, 73409, 73410).

Phanerothecium Kritsky and Thatcher, 1977

EMENDED DIAGNOSIS: Gyrodactylidea, Oogyrodactylidae. Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Two cephalic lobes terminal, each containing portions of head organs, spike sensilla (Lyons, 1969). Two bilateral groups of unicellular cephalic glands posterolateral, dorsal to level of pharynx. Eyes absent. Mouth ventral, subterminal; pharynx muscular, glandular, comprising 2 subhemispherical bulbs, anterior bulb with extrusive papillae; esophagus short; intestinal ceca (2) lacking diverticula, terminating blindly in posterior trunk. Worms oviparous, protandrous; testis becoming nonfunctional as female reproductive system develops. Gonads tandem, in-

tercecal; testis postovarian (when present). Two seminal vesicles: primary (proximal) vesicle lying anterolateral to uterus; secondary (distal) vesicle with delicate wall, situated immediately proximal to cirral sac. Cirral sac containing terminal coiled portion of vas deferens, serving as "prostatic" reservoir; free prostatic reservoir lying ventral to cirral sac, draining large prostatic mass dorsal to terminal male genitalia, opening at male genital pore by short duct. Cirrus extrusive, may be sclerotized. Ovary submedian; seminal receptacle incorporated into ovary, frequently with marginal pouches, usually filled with sperm; oviduct short; ootype surrounded by large glandular mass; uterus delicate, lying diagonally in anterior trunk; uterine pore dextroventral, separate from male pore; vagina absent. Uterus containing 1 or more eggs; egg with single proximial filament; egg filaments embedded in amorphous cap. Vitellaria comprising numerous follicles in trunk; each follicle emptying individually into ventral bilateral collecting ducts by short follicular duct; follicles in 4 longitudinal rows in posterior trunk, anterior follicles reduced in number. Haptor gyrodactyloid, ventrally concave, with pair of ventral anchors lying on central lobe; deep bar, superficial bar present; superficial bar lacking shield. Hooks 16, marginal, similar in shape, size, arranged radially in haptor. Parasitic on external surfaces of Neotropical siluriform fishes.

TYPE SPECIES: *Phanerothecium caballeroi* Kritsky and Thatcher, 1977 (syn. *P. caballeroi* forma minor of Kritsky and Thatcher, 1977), from *Cephalosilurus zungaro* (Humboldt), Pimelodidae, from Colombia, South America.

OTHER SPECIES: *Phanerothecium harrisi* sp. n. from *Plecostomus plecostomus* (Linnaeus), Loricariidae, from unnamed stream in the Bairro de São Jorge, Manaus, Amazonas, Brazil; *Phanerothecium* sp. (syn. *P.caballeroi* forma major of Kritsky and Thatcher, 1977), from *Cephalosilurus zungaro* (Humboldt), Pimelodidae, from Colombia, South America.

REMARKS: Harris (1983) utilized characters of the cirrus and seminal vesicle to separate *Oogyrodactylus* from *Phanerothecium*. Included among these were the "unarmed" nature of the cirrus except for a terminal sclerotized ring in *Oogyrodactylus* (cirrus sclerotized throughout in *Phanerothecium*), the absence of a "cirral sac" in *Oogyrodactylus* (present in *Phanerothecium*), presence of a "penis bulb" in *Oogyrodactylus* (absent in *Phanerothecium*), presence of a uniformly elongate seminal vesicle in *Oogyrodactylus* (irregularly saccate in *Phanerothecium*), and a vas deferens emptying into the center of the seminal vesicle in *Oogyrodactylus* (unknown in *Phanerothecium*).

Our finding of *P. harrisi* and examination of type specimens of *O. farlowellae* and *P. caballeroi* suggest that these characters may not be reliable for separation of the 2 genera. We confirm the presence of a ring (sclerotized?) at the tip of the cirrus in some specimens of *O. farlowellae,* while cirral sclerotization is absent in paratypes of *P. caballeroi* designated "forma major" by Kritsky and Thatcher (1977). Presence or absence of cirral sclerotization becomes even less important diagnostically with the discovery of *P. harrisi,* which also lacks the feature.

Species of *Phanerothecium* possess a pyriform cirral sac (as described by Kritsky and Thatcher, 1977) containing various amounts of the terminal vas deferens and prostatic secretions. While this structure is reported to be absent in *O. farlowellae,* the types of this species clearly show that the "penis" (term used by Harris, 1983) possesses essentially the same, but modified, structure as that of *Phanerothecium.* In *Oogyrodactylus,* the proximal diameter of the bulb is reduced, prostatic secretions are absent, and a thick external wall is present; the terminal coiled portion of the vas deferens is enclosed within an optically light central cavity in the cirral sac of *O. farlowellae* (Fig. 28). Absence of a cirral sac, therefore, cannot be used to differentiate the 2 genera, although some of the morphologic differences (absence of prostatic secretions, thickness of the wall, and proximal diameter) appear to be of generic importance.

Harris (1983) described a thick-walled "penis bulb" in *O. farlowellae* lying perpendicular to the cirral sac and into which the vas deferens empties by an expanded diameter of its lumen; the "penis bulb" connects to the cirral sac by a narrowed or constricted segment of the wall of the male duct. This structure undoubtedly is homologous to the secondary (distal) seminal vesicle described in species of *Phanerothecium* and *Nothogyrodactylus* (nobis). In *Oogyrodactylus farlowellae,* the wall of the homologue is relatively thick (Fig. 28) and may represent a character for distinguishing this genus from *Phanerothecium* and *Nothogyrodactylus.*

Because point of entry of the proximal vas deferens into the seminal vesicle is unknown in all species of *Phanerothecium,* the last 2 char-

acters used by Harris (1983) to differentiate the genera are not useful. Determination of a saccate or nonsaccate seminal vesicle depends on the course of the proximal vas deferens and its point of entry into the seminal vesicle. Furthermore, shape of the seminal vesicle has questionable diagnostic merit in this case since all available specimens of *O. farlowellae* are excessively flattened, which may have resulted in the deformation of this soft internal structure.

Kritsky and Thatcher (1977) described 2 morphologic forms of *Phanerothecium caballeroi* based primarily on size of the anchor. Our examination of the type specimens, many of which have been damaged from previous use, suggests that these authors were dealing with more than 1 species. This is supported by the finding that specimens of the form they designated "minor" possess extensive sclerotization of the cirrus (see figs. 1, 2 *in* Kritsky and Thatcher, 1977), while those of "major" apparently lack this attribute (fig. 5 *in* Kritsky and Thatcher, 1977). In addition, haptoral sclerites of all members of the Oogyrodactylidae are remarkably similar, and relatively small differences may constitute specific traits. Kritsky and Thatcher (1977) designated "minor" as the type form for *P. caballeroi.* We propose that "major" not be included in this taxon since it is likely a distinct species of *Phanerothecium* on the basis of cirral and anchor morphology. However, description and naming of "major" should await new collections that would provide differential information on morphology of the egg, haptoral and copulatory structures.

Harris' (1983) statement that the types of *Phanerothecium caballeroi* are likely immature specimens is justified. In most, the testis is well developed while the vitellaria are poorly to not developed. Both features would be expected in young individuals of a protandrous species.

Phanerothecium harrisi sp. n. (Figs. 1–6)

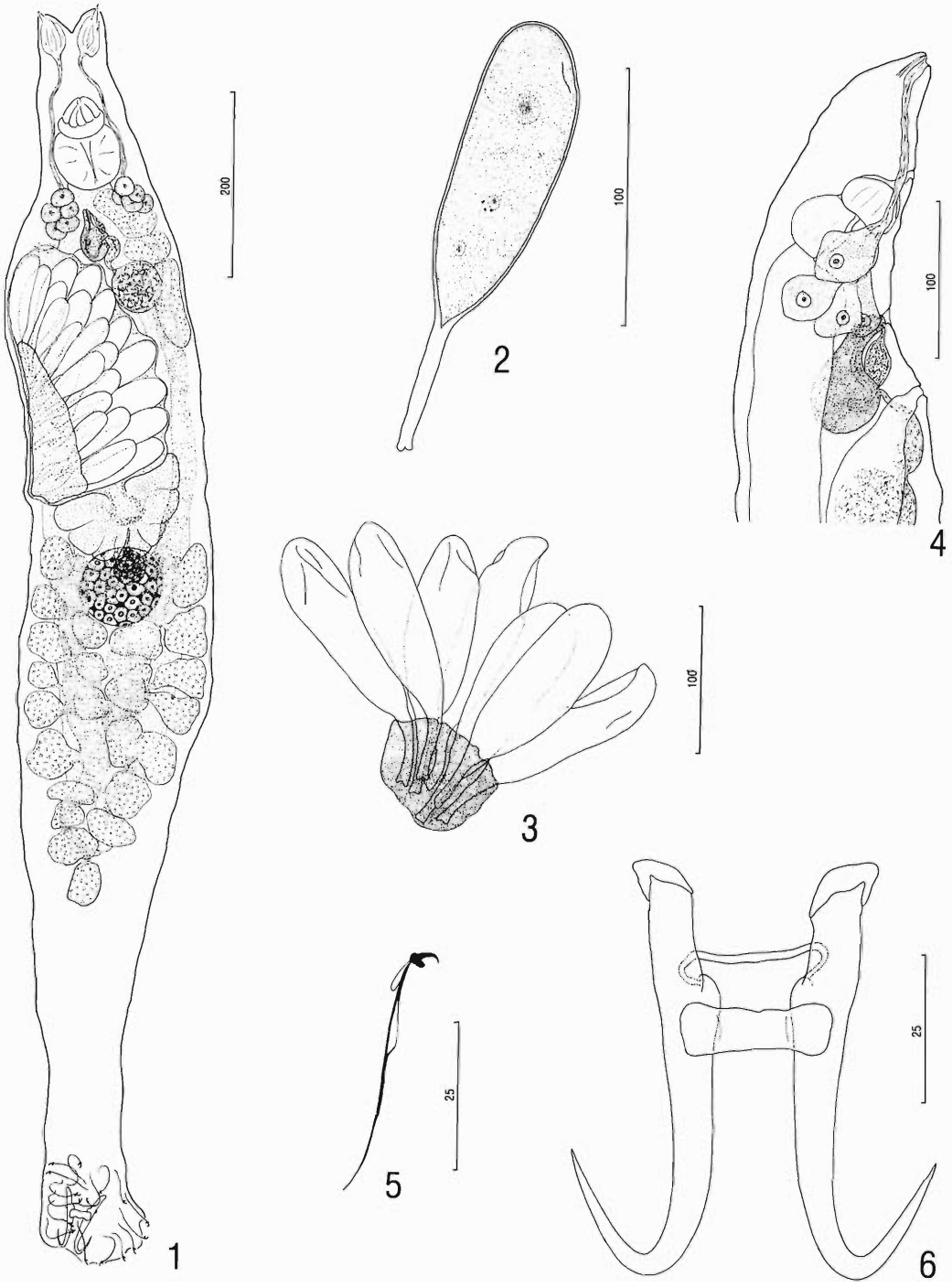
HOST: *Plecostomus plecostomus* (Linnaeus), Loricariidae.

TYPE SPECIMENS: Holotype, INPA PA 333; paratypes, IOC 32704, USNM 81042, 81044, HWML 32809, BM(NH) 1990.2.20.4, ZIAC N11681. An egg cluster is deposited as a voucher in the USNM (81043).

DESCRIPTION (based on 46 specimens): Body fusiform, 1,203 (954–1,502; $N = 13$) long; greatest width 189 (152–230; $N = 14$) near midlength or in anterior half. Cephalic lobes well developed; head organs conspicuous; each group of cephalic glands comprising about 5 cells. Greatest pharyngeal width 66 (55–83; $N = 13$). Testis spherical, variable in size; primary seminal vesicle large, spherical; secondary seminal vesicle sigmoid; cirral sac pyriform; cirrus lacking sclerotization. Ovary spherical, 87 (69–104; $N = 8$) in diameter; seminal receptacle spherical, marginal pouches usually visible; oviduct indistinct. Uterus with a maximum of 22 eggs in 1 or 2 groups defined as egg groups with coalesced filament caps; egg 162 (146–179; $N = 7$) long, 44 (33–51; $N = 4$) wide, elongate ovate, with moderately long filament slightly expanded proximally; filament cap encompassing filaments of several to many eggs. Vitelline follicles absent dextrally anterior to ootype; single vitelline collecting duct extending anteriorly on left side. Haptor 108 (87–125; $N = 9$) long, 104 (83–124; $N = 5$) wide. Anchor 70 (67–74; $N = 13$) long, with elongate robust superficial root, short deep root, relatively straight shaft, recurved point; base width 17 (15–18; $N = 5$). Superficial bar 25 (23–28; $N = 9$) long, slightly expanded on each end; deep bar 30 (24–34; $N = 10$) long, filamentous, inserting near or into deep root. Hooklet 5–6 ($N = 7$) long, with slanted shaft, blunt thumb; shank slender, with delicate ventral keel; hook 33 (31–36; $N = 13$) long; shank ligament present; FH loop ⅕ shank length.

REMARKS: *Phanerothecium harrisi* differs from *P. caballeroi* in morphology of the haptoral armament and in the relative length of the vas deferens enclosed in the cirral sac (long in *P. caballeroi*; short in *P. harrisi*). In addition, the cirrus of *P. harrisi* lacks sclerotization (present in *P. caballeroi*). This species is named for Dr. P. D. Harris, University of Birmingham, Birmingham, U.K., in recognition of his work on the Gyrodactylidea.

When mature worms with eggs in utero were placed in small petri dishes containing stream water, parent worms readily deposited egg clusters that adhered to the glass surface by the sticky filament caps. In near-term eggs, the larva is situated in an inverted U with cephalic and haptoral ends directed toward the proximal (filamented) end of the egg. After 5–6 days (room temperature–air conditioned), eggs hatched releasing nonciliated larvae with 16 fully developed hooks arranged radially in the haptor. The anchor points were present, but other features of the anchor, including the shaft, base and roots, and the bar, showed no development. The newly



Figures 1–6. ***Phanerothecium harrisi*** **sp. n. 1. Ventral view of holotype. 2. Egg. 3. Egg cluster. 4. Lateral view of cephalic and anterior trunk (paratype). 5. Hook. 6. Anchor/bar complex. All figures are drawn to respective scales.**

hatched larva possessed a pharynx and lacked eyes; a primordium of the intestinal ceca was present, but the remaining internal organs, including those of the reproductive system, were not visible. The life cycle and post-hatching development of *P. harrisi* are similar to those reported for *Oogyrodactylus farlowellae* by Harris (1983). In addition, sequential development of the haptoral sclerites of *P. harrisi* is concordant with that reported for *Gyrodactylus* spp. by Mizelle and Kritsky (1967).

Nothogyrodactylus gen. n.

DIAGNOSIS: Gyrodactylidea, Oogyrodactylidae. Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Cephalic lobes (2) terminal, each containing portions of head organ, spike sensilla. Cephalic glands unicellular, in 2 bilateral groups posterolateral, dorsal to level of pharynx. Eyes absent. Mouth ventral, subterminal; pharynx muscular, glandular, comprising 2 subhemispherical bulbs; esophagus short; intestinal ceca (2) lacking diverticula, terminating blindly in posterior trunk. Worms oviparous, protandrous. Gonads tandem, intercecal; testis postovarian. Primary (proximal) seminal vesicle large, lying sinistral to uterus; secondary (distal) vesicle proximal to cirral sac, with thin wall. Proximal vas deferens, prostate not observed. Cirral sac poorly defined; cirrus extrusive, muscular; 1–3 accessory copulatory sclerites present. Ovary submedian; spherical seminal receptacle filled with sperm, lying on or just beneath surface of ovary; ootype surrounded by large glandular mass; uterus with thin wall; uterine pore dextroventral at level of cirral sac; vagina absent. Uterus containing a maximum of 1 egg; egg with single proximal filament; egg filament embedded in amorphous cap. Vitellaria comprising numerous follicles in trunk, each emptying ventrally into bilateral vitelline collecting duct by short follicular duct; follicles lying in 4 longitudinal rows in posterior trunk, anterior follicles reduced in number especially on right side. Haptor gyrodactyloid, ventrally concave, with pair of ventral anchors, deep bar, superficial bar, 16 hooks; superficial bar lacking shield; hooks marginal, similar in shape and size, arranged radially in haptor. Parasitic on external surfaces of Neotropical siluriform fishes.

TYPE SPECIES: *Nothogyrodactylus clavatus* sp. n. from *Ancistrus* sp., Loricariidae, from unnamed stream, Bairro de São Jorge, Manaus, Brazil.

OTHER SPECIES: *Nothogyrodactylus amazonicus* and *N. plaesiophallus* spp. n., both from *Ancistrus* sp., Loricariidae, from unnamed stream in Bairro de São Jorge, Manaus, Amazonas, Brazil.

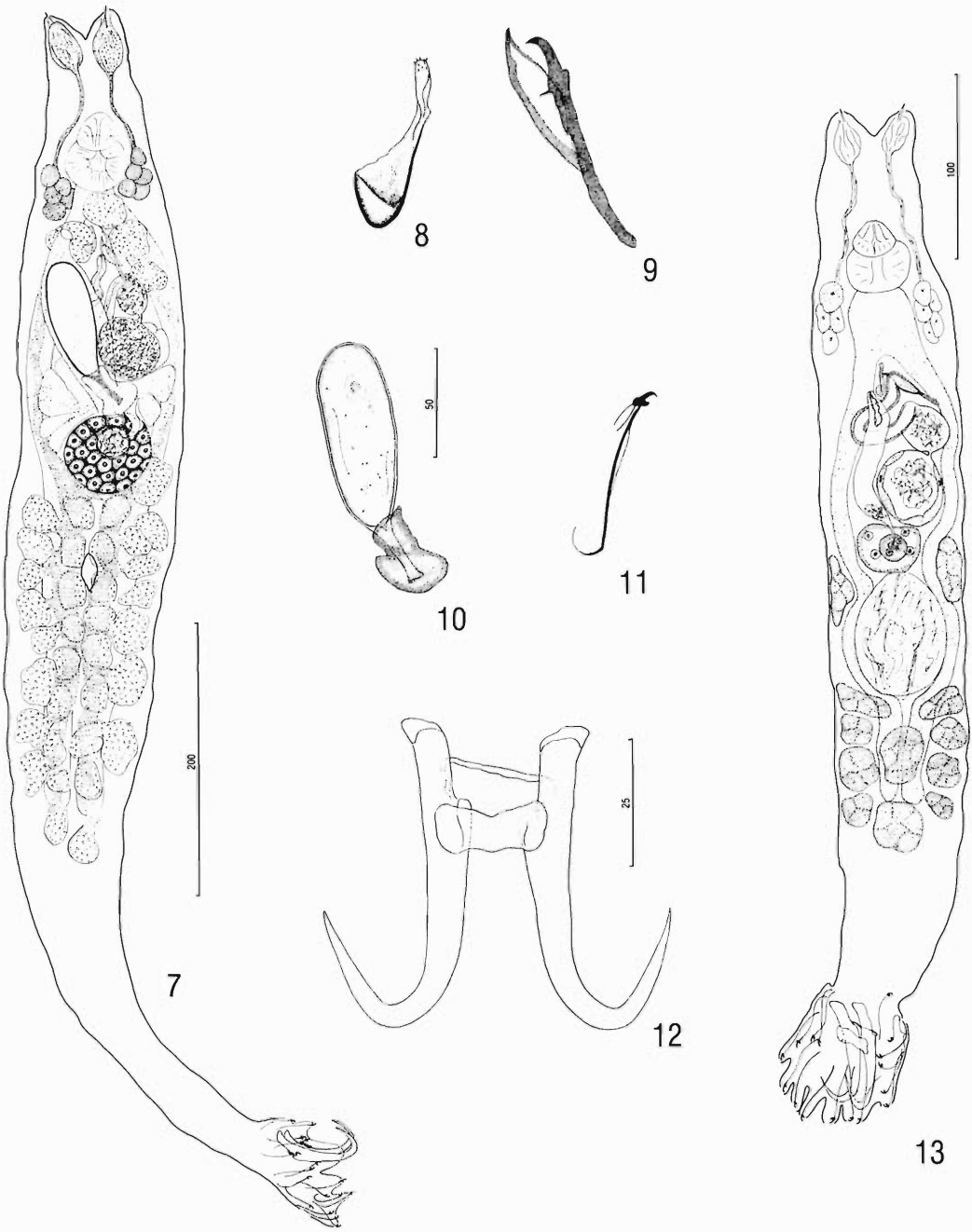
REMARKS: This genus differs from *Oogyrodactylus* and *Phanerothecium* by possessing 1 or more accessory copulatory sclerites associated with the cirral sac. The cirral sac is poorly defined, and the cirrus comprises an elongate muscular organ lacking sclerotization. The generic name is from Greek (*nothos* = spurious or false).

Nothogyrodactylus clavatus sp. n. (Figs. 7–13)

HOST: *Ancistrus* sp., Loricariidae.

TYPE SPECIMENS: Holotype, INPA PA 330; paratypes, IOC 32705, USNM 81039, HWML 32808, BM(NH) 1990.2.20.3, ZIAC N11680.

DESCRIPTION (based on 30 specimens): Body fusiform, 823 (724–938; $N = 10$) long; greatest width 133 (110–158; $N = 14$) in anterior half. Cephalic lobes, head organs well developed; cephalic glands comprising 2 groups of about 5 cells each. Greatest pharyngeal width 52 (43–64; $N = 13$). Testis subovate, degenerated to small fusiform sac in older individuals; primary seminal vesicle spherical, large; secondary vesicle fusiform; cirral sac elongate, coiled or bent dextral to secondary seminal vesicle. Three accessory sclerites: first 34 (28–41; $N = 5$) long, cup- or club-shaped with terminal spined handle; second 44 (39–49; $N = 11$) long, rod-shaped, with terminal hook, subterminal spine; third 29 (24–33; $N = 9$) long, foliform. Ovary spherical, 65 (50–98; $N = 11$) in diameter; seminal receptacle with thick wall; oviduct short. Egg elongate ovate, 121 (113–129; $N = 5$) long, 40 (37–43; $N = 5$) wide, with moderately long proximal filament slightly expanded proximally; filament cap urn-shaped. Bilateral vitelline collecting ducts extend into anterior trunk both sinistrally and dextrally; vitelline follicles further reduced on anterior right side. Haptor 90 (79–108; $N = 9$) long, 101 (82–116; $N = 7$) wide. Anchor 60 (52–63; $N = 15$) long, robust, with elongate superficial root, short deep root, bent shaft, straight recurved point; base 16 (13–19; $N = 8$) wide. Superficial bar 24 (22–26; $N = 11$) long, platelike, with slightly enlarged ends; deep bar 24 (21–27; $N = 9$) long, filamentous, uniting with or near deep anchor root. Hooklet 5–6 ($N = 17$) long, with slanted shaft, blunt thumb; shank slender, ventral keel delicate; ligament delicate; hook 32 (28–35; $N = 19$) long; FH loop about ⅛ shank length.



Figures 7–13. ***Nothogyrodactylus clavatus*** **gen. et sp. n. 7. Holotype (ventral view). 8, 9. Accessory copulatory sclerites. 10. Egg. 11. Hook. 12. Anchor/bar complex. 13. Ventral view of young specimen (paratype). Figures 8–12 are drawn to the 25-μm scale; others as indicated.**

REMARKS: *Nothogyrodactylus clavatus* is the type species of the genus. The specific name is from Neolatin (*clavatus* = club-shaped) and refers to the shape of 1 of the accessory copulatory sclerites.

Nothogyrodactylus amazonicus sp. n. (Figs. 14–18)

HOST: *Ancistrus* sp., Loricariidae.

TYPE SPECIMENS: Holotype, INPA PA 331; paratypes, IOC 32706, USNM 81040, HWML 32806, BM(NH) 1990.2.20.1, ZIAC N11678.

DESCRIPTION (based on 8 adult specimens): Body fusiform, 681 (625–753; $N = 3$) long; greatest width 109 (99–142; $N = 5$) usually in posterior trunk. Cephalic lobes, head organs well developed; cephalic glands comprising 2 bilateral groups of 4–5 cells each. Greatest pharyngeal width 43 (30–49; $N = 6$). Testis, primary seminal vesicle not observed; secondary seminal vesicle indistinct; cirral sac short, with thickened wall near midlength; single accessory sclerite 31 (28–34; $N = 4$) long, rod-shaped, with hinged hooklike termination. Ovary spherical, 54 (48–58; $N = 5$) in diameter; seminal receptacle with thick wall, oviduct indistinct. Egg 109 (102–116; $N = 4$) long, 34 (31–35; $N = 4$) wide, ellipsoidal, with short proximal filament noticeably flared proximally; filament cap urn-shaped. Vitelline follicles absent on right side of anterior trunk; dextral collecting duct absent anterior to ovary. Haptor 71 (67–74; $N = 3$) long, 78 (76–79; $N = 3$) wide. Anchor 51 (48–53; $N = 3$) long, delicate, with slightly curved shaft, recurved point, elongate superficial root, short deep root; base 14 (11–16; $N = 2$) wide. Superficial bar 17 (16–18; $N = 5$) long, platelike, with slightly enlarged ends; deep bar 21 (19–23; $N = 5$) long, filamentous. Hooklet 5–6 ($N = 2$) long, with slanted shaft, truncate thumb; shank slender, keel absent; ligament delicate; hook 62 (60–65; $N = 4$) long; FH loop approximately ⅛ shank length.

REMARKS: This species differs from *N. plaesiophallus* and *N. clavatus* by possessing a single hook-shaped copulatory sclerite. The specific name reflects the drainage basin from which the species was collected.

Nothogyrodactylus plaesiophallus sp. n. (Figs. 19–24)

HOST: *Ancistrus* sp., Loricariidae.

TYPE SPECIMENS: Holotype, INPA PA 332; paratypes, IOC 32707, USNM 81041, HWML 32807, BM(NH) 1990.2.20.2, ZIAC N11679.

DESCRIPTION (based on 9 adults, 1 subadult): Body 993 (870–1,301; $N = 7$) long, fusiform; greatest width 162 (144–179; $N = 7$) at level of ootype. Cephalic lobes, head organs well developed; bilateral groups of cephalic glands comprising about 5 cells each. Greatest pharyngeal width 62 (39–74; $N = 7$). Testis subovate. Primary seminal vesicle spherical, with thick wall; secondary seminal vesicle fusiform; cirral sac muscular, with proximal loop. Two accessory sclerites: first 41 (32–47; $N = 9$) long, bar-shaped, with bladelike termination at right angle to bar; second 38 (32–49; $N = 9$) long, foliform, with thickened margins and spined termination. Ovary pyriform, 61 (57–66; $N = 5$) in greatest diameter; seminal receptacle with thick wall, spherical; oviduct short. Egg 137 (122–147; $N = 4$) long, 46 (44–48; $N = 4$) wide, ellipsoidal, with short proximal filament; filament flared proximally; filament cap urn-shaped. Vitelline follicles, collecting ducts well developed in both anterior and posterior trunk. Haptor 129 (113–152; $N = 7$) long, 135 (112–155; $N = 4$) wide. Anchor 66 (51–72; $N = 10$) long, delicate, with robust superficial root, short deep root, slightly bent shaft, elongate recurved point; base 19 (16–23; $N = 6$) wide. Superficial bar 29 (27–33; $N = 5$) long, subrectangular; deep bar 36 (33–37; $N = 4$) long, filamentous, uniting with anchor near deep root. Hooklet 6–7 ($N = 4$) long, with slanted shaft, blunt thumb; shank slender, keel narrow; ligament delicate; hook 39 (35–42; $N = 9$) long; FH loop approximately ¼ shank length.

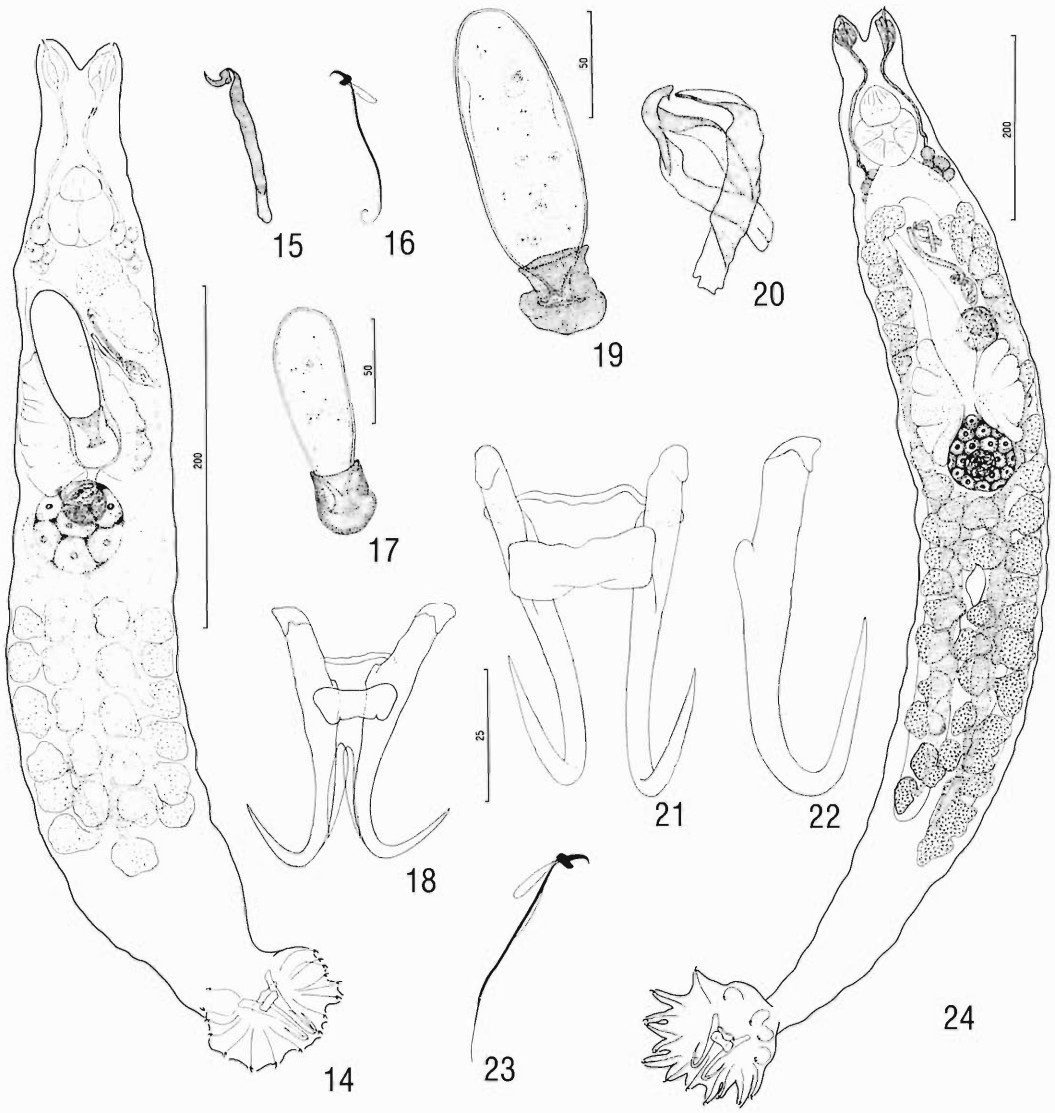
REMARKS: *Nothogyrodactylus plaesiophallus* differs from *N. clavatus* and *N. amazonicus* by possessing 2 copulatory sclerites. It resembles *N. clavatus* by having a foliform copulatory sclerite and *N. amazonicus* by having a short flared filament on the egg. The specific name is from Greek (*plaisos* = crooked or bent + *phallos* = penis) and refers to the shape of the cirral sac.

Oogyrodactylus farlowellae Harris, 1983 (Figs. 25–28)

HOST: *Farlowella amazonum* Gunther, Loricariidae.

SPECIMENS STUDIED: Holotype, BM(NH) 1982.3.30.1; 9 paratypes, BM(NH) 1982.3.30.2–9, USNM 77095.

REMARKS: Morphologic details of the egg, anchor, and hook of *O. farlowellae* are presented in Figures 25–27 for comparative purposes. The egg possesses a short pointed proximal filament

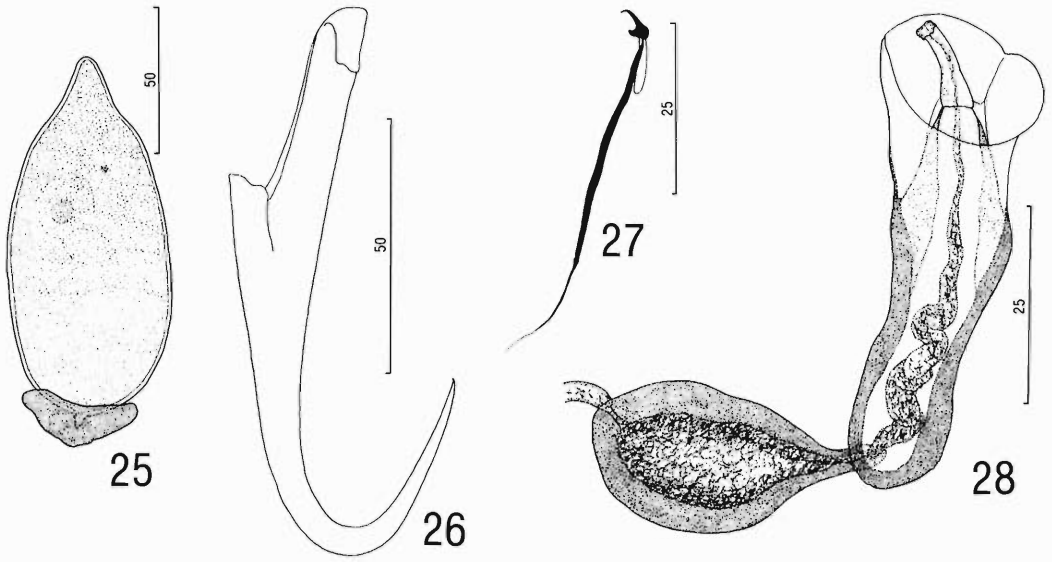


Figures 14–24. 14–18. *Nothogyrodactylus amazonicus* sp. n. 14. Holotype (ventral view). 15. Accessory copulatory sclerite. 16. Hook. 17. Egg. 18. Anchor/bar complex. 19–24. *Nothogyrodactylus plaesiophallus* sp. n. 19. Egg. 20. Accessory copulatory sclerites. 21. Anchor/bar complex. 22. Anchor. 23. Hook. 24. Ventral view of holotype. All figures are drawn to respective scales except Figures 15, 16, 18, 20–23 (25-μm scale).

embedded in a reduced filament cap; the distal margin of the egg is tapered to a blunt point. The superficial anchor root of *O. farlowellae* is comparatively longer than those of all other oogyrodactylids. While hook shanks are relatively robust, they apparently lack a ventral keel.

Discussion

Harris (1983) utilized the following to diagnose the Oogyrodactylidae: presence of 1) protandry and oviparity, 2) separate male and female genital pores, 3) well-developed vitellaria, 4) a tubular, weakly sclerotized cirrus, 5) unciliated larvae, and absence of 6) a vagina. These features either represent symplesiomorphies of the Gyrodactylidea or are primitive characters shared by the Gyrodactylidea and its sister taxon, the Dactylogyridea (see Boeger, 1988). Thus, it does not appear that the Oogyrodactylidae is supported by any apomorphic characters, suggesting that this taxon is paraphyletic. If the accessory copulatory sclerites in *Nothogyrodactylus* are



Figures 25–28. ***Oogyrodactylus farlowellae*** **Harris, 1983. 25. Egg. 26. Anchor. 27. Hook. 28. Cirral sac and secondary seminal vesicle (holotype). Figures are drawn to respective scales.**

homologues of the accessory piece of dactylogyrideans, their absence in the Gyrodactylidae, *Oogyrodactylus* and *Phanerothecium* provides further support for paraphyly of the Oogyrodactylidae. Thus, our acceptance of the Oogyrodactylidae as defined by Harris (1983) is provisional pending phylogenetic analysis of the entire order.

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Redescription of *Eimeria escomeli* (Rastegaieff, 1930) from *Myrmecophaga tridactyla*, and a First Report from Bolivia

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ABSTRACT: *Eimeria escomeli* (Rastegaieff, 1930) Levine and Becker, 1933, is redescribed from the giant anteater, *Myrmecophaga tridactyla* (L.), from the departamento de La Paz, Bolivia. This is the first report of parasites from *M. tridactyla* from Bolivia and only the third time that coccidians have been recorded from this host.

KEY WORDS: *Eimeria escomeli*, Coccidia, Apicomplexa, giant anteater, *Myrmecophaga tridactyla*, departamento de Beni, Bolivia, Xenarthra.

During a survey of the parasites of mammals of Bolivia from 1984 to 1986, several xenarthrans were examined for coccidian parasites. Of 2 individual armadillos examined, neither *Chaetophractus vellerosus* (Gray) or *C. nationi* (Thomas) had coccidians in their feces. Of representatives of 2 species of anteaters examined (*Cyclopes didactylus* (L.) and *Myrmecophaga tridactyla* L.), only the feces of *M. tridactyla* contained oocysts at the time of sampling. These oocysts conform most closely in size and shape to (gen. ?) *escomeli* Rastegaieff, 1930, originally described from *M. tridactyla* (see Rastegaieff, 1930). The specimens described by Rastegaieff (1930) were unsporulated and could not be properly identified. Although Levine and Becker (1933) tentatively placed *E. escomeli* in the genus *Eimeria*, Pellerdy (1974) confirmed the placement of this species in the genus *Eimeria*. During our fieldwork in Bolivia, we obtained sufficient numbers of sporulated oocysts from the feces of an individual of *M. tridactyla* to allow a complete description. In the present paper, we redescribe *E. escomeli* and properly confirm its place in the genus *Eimeria*.

Materials and Methods

Methods and procedures of study follow McAllister and Upton (1988) and Lambert et al. (1988). Measurements are in micrometers, with the mean given first followed by the range. Unless stated otherwise, $N = 30$.

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Description

Eimeria escomeli (Rastegaieff, 1930) Levine and Becker, 1933 (Figs. 1–4)

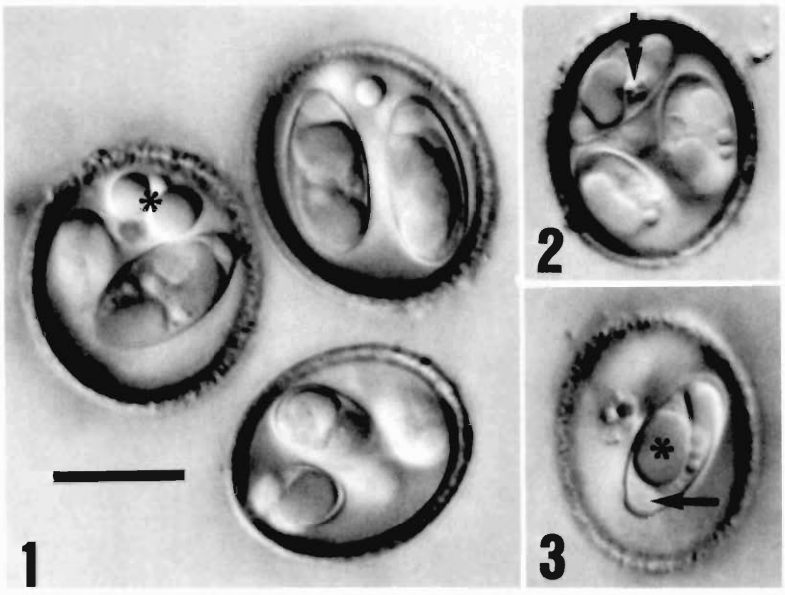
Oocysts subspherical to ellipsoidal, 21.6 × 19.0 (17.6–26.4 × 15.2–23.2), with bi-layered wall 1.6 (1.2–2.0) thick; shape index (length/width ratio) 1.15 (1.0–1.3). Outer layer of wall about 1.0 thick and slightly pitted externally; inner layer thin and smooth. Micropyle and polar granule usually absent; many oocysts containing debris, perhaps remnants of a polar granule. Oocyst residuum present, formed from 1 to several homogeneous globules (Fig. 1), each 5.0 (3.2–8.0) in diameter ($N = 18$). Sporocysts 13.2 × 6.5 (10.8–15.2 × 5.6–7.4), with a smooth, single-layered wall. Stieda body present, consisting of a flattened dome at 1 end of sporocyst about 0.8 high × 2.4 wide; substieda body present (Fig. 3), about 1.6 high × 2.2 wide and homogeneous. Sporocyst residuum present, formed of either a loose mass of homogeneous granules of various sizes (Fig. 2) or as scattered granules. Sporozoites elongate, lying parallel and head-to-tail. Posterior ends of sporozoites curved back along poles of sporocysts. Each sporozoite contains a large, ellipsoidal posterior refractile body (Fig. 3) and 3/30 (10%) contained a smaller spherical anterior refractile body. Nucleus rarely seen.

TYPE HOST: *Myrmecophaga tridactyla* L.

TYPE LOCALITY: Bolivia, La Paz, North Bank of the Rio Beni (long. 13°16′S, lat. 67°17′E), 240 m elevation.

DATE COLLECTED: 8 September 1985.

AGE OF OOCYSTS WHEN MEASURED: 1,010 days.



Figures 1–3. Photomicrographs of sporulated oocysts of coccidia recovered from the feces of *Myrmecophaga tridactyla*. Bar = 10.8 μm. 1. Sporulated oocyst showing oocyst residuum consisting of several homogeneous globules (*). 2. Note sporocyst residuum composed of a loose mass of homogeneous granules. 3. Sporocysts with homogeneous substieda body (arrow), and large ellipsoid posterior refractile body (*). Note thick, externally pitted outer wall.

MATERIAL DEPOSITED: Phototype (=photographs, see Bandoni and Duszynski, 1988) of sporulated oocysts USNM Helm. Coll. No. 81056.

COLLECTOR: O. C. Jordán, personal field catalog number OCJ/85-169.

Discussion

Little information is available concerning coccidian parasites of Xenarthra in the Neotropics. Up to the present time, only 3 species of *Eimeria* have been described from anteaters, 1 from the sloth (*Bradypus* sp.) and 4 from armadillos. Representatives of this group of mammals occur only in the Nearctic and Neotropical regions (Simpson, 1980); thus, no comparisons will be made with coccidians described from any of the pholidote anteaters occurring in other biogeographic regions.

Eimeria escomeli differs from *E. tamanduae* Lainson, 1968, from *Tamandua tetradactyla* (L.) in being smaller and without a highly refractile spherule in the stieda body (Lainson, 1968). The oocysts of *E. escomeli* are smaller than *E. cyclopei* Lainson and Shaw, 1982, from *Cyclopes didactylus* (L.), and possess an oocyst residuum.

Eimeria escomeli can be recognized as distinct from *E. travassoi* Da Cunha and Muniz, 1928, from *Euphractus* and *Dasypus* spp. and from *E. dacunhai* Levine, 1984, from *Cabassous* sp. and

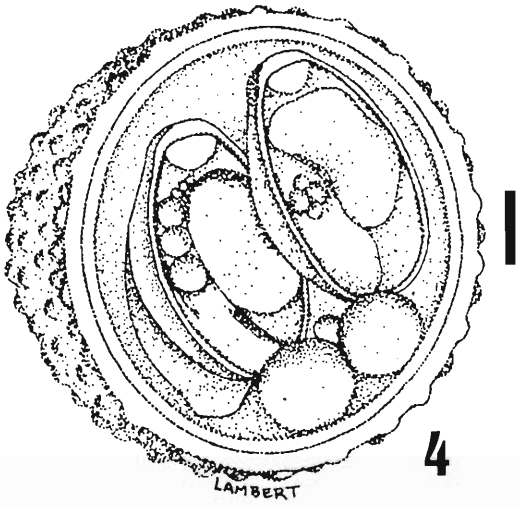


Figure 4. Line drawing of *Eimeria escomeli* recovered from feces of *Myrmecophaga tridactyla*. Bar = 3 μm.

Chaetophractus sp. in having much smaller oocysts and smaller sporocysts (Da Cunha and Muniz, 1928). *Eimeria escomeli* differs from *E. cabassusi* Carini, 1933, from *Cabassous* sp. in having ellipsoidal to subspherical oocysts and a well-developed oocyst residuum, and also differs from *E. choloepi* Lainson and Shaw, 1982, from *Choloepus didactylus* (L.) in having an oocyst residuum and well-developed stieda and substieda bodies (Carini, 1933; Lainson and Shaw, 1982).

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The Identity and Prevalence of Trypanosomes in White-tailed Deer (*Odocoileus virginianus*) from Southern Florida

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ABSTRACT: *Trypanosoma cervi* Kingston and Morton, 1975, is reported from white-tailed deer, *Odocoileus virginianus* (Zimmermann), in Collier, Dade, and Monroe counties of southern Florida. Annual prevalences from thin blood smears averaged 21% (range 7–41%) from 1984 to 1989. Fall prevalence averaged 93% (range 85–100%) when determined by culture of blood in a monophasic liquid medium, whereas examination of thin films averaged only 18% in these years. Examination of thick blood smears proved comparably effective when compared to blood culture, resulting in the detection of 98% of infections diagnosed by blood culture.

KEY WORDS: *Trypanosoma cervi*, white-tailed deer, *Odocoileus virginianus*, prevalence, diagnostic techniques, Florida.

The trypanosome of white-tailed deer in the United States has been identified as *Trypanosoma cervi* Kingston and Morton, 1975. Infected deer have been found from central Florida north to West Virginia and west to Arkansas (Kingston and Crum, 1977; Davidson et al., 1983). However, no data are available on the prevalence of this parasite in deer populations from the southernmost portion of the range in Florida.

Since 1984 we have participated in an ongoing cooperative study of white-tailed deer populations in southern Florida with the Florida Game and Fresh Water Fish Commission (FGFWFC) and the National Park Service (NPS). Trypanosomes were found often in high prevalence and this study describes the identity and prevalence of the trypanosomes as determined by examination of blood films and blood cultures.

Materials and Methods

Blood samples were obtained from a total of 238 white-tailed deer collected during 1984–1989 in Collier, Dade, and Monroe counties, Florida. Deer samples comprised 50 males and 188 females, representing 39 fawns (age 3–11 mo), 51 yearlings (12–23 mo), and 148 adults (over 23 mo). Between October 1984 and October 1986, 9–25 deer were sampled during each of 3 seasons: spring (March), summer (June–August), and fall (October–November). From 1987 to 1989, 27–50 deer were obtained during fall only. Cardiac blood samples were taken immediately after the deer were killed by gunshot at night by FGFWFC or NPS biologists and were refrigerated in anticoagulant tubes (EDTA or sodium citrate) until the following morning. Several thin blood films were prepared from whole blood of each deer, air-dried, and fixed in absolute methanol. Additionally, in 1989 standard thick films consisting of a circular drop of blood 5–8 mm in diameter and thin films of blood centrifuged for hematocrit determinations were prepared. These latter films, obtained from a limited number of deer, made both from the vicinity of the buffy coat and from the packed end of the hematocrit tube, were fixed and stained as standard thin films. All 1989 thin films were stained on the day of preparation for 1 hr with Giemsa (Harleco, EM Diagnostic Systems, Inc., Gibbstown, New Jersey) at a dilution of 1:9, pH of 7.0 or 7.2. Thick films were air-dried for at least 24 hr and then placed face side down for 1 hr in a dish containing dilute Giemsa stain (1:20), which hemolyzed the erythrocytes. Slides were rinsed gently with distilled or bottled drinking water and air-dried. An additional thin film from each deer was stained by the Leukostat procedure (Fisher Scientific, Orangeburg, New York), which gave results comparable to Giemsa staining when done on the day of preparation. The thin blood films collected from 1984 to 1988 were generally poorly stained, and most required destaining and restaining when unstained duplicates were not available. Poorly stained films were destained for 10 min in 95% acid-ethanol, neutralized by immersion in 95% alkaline-ethanol, and washed for a few minutes in running tap water. Old, unstained and newly destained thin films were then immersed in Giemsa stain in an acetone–methanol–distilled water solution buffered to pH 6.4 for 2 hr, using an unpublished procedure developed by R. B. Kimsey, University of California, Davis. This generally gave results comparable to material stained when fresh. All smears were screened for trypanosomes at 160×, covering the entire smear with special attention paid to the "tails" and edges of the films. In infections of high intensity, trypanosomes could be found anywhere on the film, but in infections of low intensity they were most often on the periphery. A 5-ml sample of blood from each deer collected in October 1985, 1986, 1988, and 1989 was inoculated into 2–5 ml of a monophasic liquid medium (Sadigursky and Brodskyn, 1986). The cultures, maintained at room temperature, were examined 5–7 days following inoculation, and in the case of ap-

Table 1. A comparison of mensural values for trypanosomes of white-tailed deer from southern Florida with those of *Trypanosoma cervi*.

Source	Measurements in μm							
	PK	KN	PN	NA	BL	FF	L	W
S. Florida								
Mean	12.6	7.6	20.2	28.6	47.8	6.1	53.9	9.4
Range	6–22	5–16	13–31	19–39	32–65	0–12	39–76	4–14
SE USA*								
Mean	13.5	8.3	22.0	26.3	48.2	9.5	57.5	5.6
Range	5–27	2–14	14–34	12–41	31–74	3–21	40–83	3–15
Type host (elk)†								
Mean	12.2	7.0	19.4	24.8	45.4	6.6	52.0	4.6
Range	5–20	4–9	11–32	20–30	32–56	3–11	40–61	3–8

* From Kingston and Crum (1977).
† From Kingston and Morton (1975).

parent negatives, several times for up to 3 mo thereafter. Thin films of the culture forms were prepared from 3 positive samples collected in October 1989, and fixed and stained similarly to thin blood films.

A series of 25 trypomastigotes with clear diagnostic characters were selected from slides collected from 1986 to 1989. These were measured by calibrated ocular micrometer at 1,000× under oil immersion. Taxonomic characters utilized were those of Kingston and Morton (1975) and are abbreviated in Tables 1 and 2 as follows: PK, posterior end to kinetoplast; KN, nucleus to kinetoplast; PN, posterior end to nucleus; NA, nucleus to anterior end; BL, total length minus free flagellum; FF, free flagellum; L, total length; W, width; NI, nuclear index, PN/NA; and KI, kinetoplast index, PN/KN. Nucleus location was measured to the center of the nucleus, while width refers to the greatest breadth of the trypanosome exclusive of the undulating membrane. This latter character may differ in its measurement by other authors. The free flagellum was not measured unless its entire length was evident. All trypanosomes measured were trypomastigote forms and are compared below only with bloodstream trypomastigote data of other authors. Chi-square tests were used to compare prevalences. Representative blood films have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (accession nos. 81000–81002).

Results

Identity of the trypanosomes

Comparison of mensural data of the trypanosomes from the population of deer in southern Florida (Table 1) with those published by Kingston and Morton (1975) and Kingston and Crum (1977) demonstrated clearly that they were conspecific with *Trypanosoma cervi,* and distinct from *Trypanosoma theileri* (Table 2). In *T. cervi* there was a smaller ratio of the distance from the posterior end–nucleus to the kinetoplast–nucleus distance (KI), a lower mean ratio between the distance posterior end–kinetoplast to body length (PK/BL), and a greater mean ratio of the nucleus–anterior end to the body length (NA/BL). Given the difference in measurement technique, i.e., the use of an ocular micrometer (present study) instead of projected photomicrographs measured with a calibrated map reader, the concordance of the data was remarkable. Only the mean width measurement (W) differed appreciably, and this was probably a combination of fixation or configuration artifacts and the inclusion of both slender and broad forms (Kingston and Crum, 1977) in the sample. Three trypanosomes of the measured 25 could be described as long, slender forms; the remainder of the sample was broad in appearance. The critical diagnostic characters of the southern Florida sample (Table 2) either were completely within the range of variation described for *T. cervi* or overlapped at one end of the range. No attempt was made to measure bloodstream or cultural epimastigote forms because of their variability in shape. The ratio of trypomastigote (T) to epimastigote (E) forms indicated highly significant ($P < 0.0001$) seasonal differences: in summer 1985, 6 T : 31 E; in pooled fall samples from 1984 to 1988, 25 T : 6 E; and pooled samples from 5 deer in fall 1989, 53 T : 2 E.

Prevalence

Thin films were the only samples available for every sampling period (Table 3). Using only the data obtained from those films, we found significant differences between the prevalence of

Table 2. A comparison of diagnostic characters for trypanosomes of white-tailed deer from southern Florida with those of *Trypanosoma cervi* and *Trypanosoma theileri*.

Source	FF:BL	NI	KI	PK/BL	NA/BL
			T. cervi		
S. Florida					
Mean	1:6.8	0.71	2.77	0.26	0.60
Range	4.8–12.6	0.5–1.1	1.9–4.6	0.2–0.4	0.5–0.9
SE USA*					
Mean	1:5.7	0.88	2.86	0.28‡	0.55‡
Type host (elk)†					
Mean	1:6.9	0.78	2.77	0.27	0.55
Range	—	0.5–1.3	2.8–3.6	0.2–0.4	0.5–0.6
			T. theileri§		
Broad forms					
Mean	1:8.5	0.91	5.68	0.39	0.51
Range	7.0–9.3	0.7–1.1	4.4–5.0	0.3–0.5	0.5–0.6
Slender forms					
Mean	1:8.6	0.84	4.00	0.35	0.52
Range	6.3–13.8	0.5–1.1	3.6–4.1	0.2–0.5	0.5–0.6

* From Kingston and Crum (1977).
† From Kingston and Morton (1975).
‡ Calculated from data of Kingston and Crum (1977).
§ Saisawa et al. (1933), quoted by Kingston and Morton (1975).

trypanosomes during fall of 1984 (44%) and spring of 1985 (0%, $P = 0.04$), between spring of 1985 (0%) and summer of 1985 (56%, $P = 0.04$), and between summer of 1985 (56%) and summer of 1986 (8%, $P = 0.05$). There were no significant differences ($P = 0.15$) when all 3 summer samples were compared with each other. Spring samples from 1985 (0%) and 1986 (15%) did not differ significantly ($P = 0.50$), and there were no differences among the samples collected in fall of each year ($P = 0.37$). The seasonal and annual prevalence of *T. cervi* in southern Florida, as determined from the examination of thin blood films (Table 3), was likely a considerable underestimate as suggested by the results of blood culture (Table 4). In the fall of 1989, when thick films and centrifuged thin films were prepared, 4 diagnostic methods were employed. Both thick films and culture methods were very sensitive for detecting infection by *T. cervi* and resulted in prevalences of 98 and 100% in comparison to 16 and 15%, respectively, detected by normal and centrifuged thin blood films. The latter did not prove to be better for the detection of try-

Table 3. Seasonal and annual prevalences of *Trypanosoma cervi* determined from thin blood films of white-tailed deer from southern Florida, 1984–1989.

	Spring (Mar.)			Summer (June–Aug.)			Fall (Oct.–Nov.)			Totals		
Year	No. deer exam.	% pos.	95% C.I.	No. deer exam.	% pos.	95% C.I.	No. deer exam.	% pos.	95% C.I.	No. deer exam.	% pos.	95% C.I.
1984	0	—	—	13	38	16–68	9	44	17–76	22	41	21–64
1985	12	0	0–30	25	56	35–75	15	20	6–49	52	33	20–48
1986	20	15	5–40	12	8	1–40	14	7	1–39	46	11	4–25
1987	0	—	—	0	—	—	27	7	2–26	27	7	2–26
1988	0	—	—	0	—	—	41	22	11–39	41	22	11–39
1989	0	—	—	0	—	—	50	16	8–31	50	16	8–31
Totals	32	9	3–27	50	40	26–56	156	17	12–25	238	21	16–28

Table 4. Prevalences of *Trypanosoma cervi* in white-tailed deer in southern Florida during October as determined by thin blood films vs. blood culture techniques.

Year	No. deer examined	% positive by	
		Thin blood film	Blood culture
1985	15	20	93
1986	14	7	92
1988	41	22	85
1989	50	16	100*
Totals	120	18	93

* This value represents results of cultures from 46 deer; cultures from 4 other deer were contaminated.

panosome infection than thin films of uncentrifuged whole blood. More importantly, the use of thick films can provide an adequate survey method when cultures cannot be used, as they resulted in the diagnosis of 98% of the 46 infections found by culture of fresh blood and all 4 of the positive samples for which the cultures were contaminated. By using thin films or centrifuged blood sample techniques, only 15–16% of the positive samples were detected, which proved to be highly significant ($P < 0.001$) from either thick film or culture methods.

Discussion

The identification of *T. cervi* from white-tailed deer in southern Florida was not surprising given the broad distribution of this parasite in North American cervids (Kingston and Morton, 1975). Because deer in southern Florida intermingle freely with range cattle in the Collier County study area, it was possible that infection by *Trypanosoma theileri,* which is known in Florida (Ristic and Trager, 1958), might occur in the deer. However, none of the trypanosomes found on blood smears was *T. theileri. Trypanosoma cervi* appears to be host specific to cervids, as suggested by the failure of Kingston and Morton (1975) to infect cattle with *T. cervi* from elk. It is unlikely that deer are infected from transmission of *T. theileri* among sympatric cattle herds.

In 1985 the very high prevalence (56%) of trypanosomes on thin blood films from the summer samples (June and August) indicates that transmission probably occurred following the March sample, when no infections were found. Ten of 14 (71%) June–August infections had bloodstream epimastigotes present, while only 5 had trypomastigotes. The ratio of trypomastigote to epimastigote forms in this summer sample, when compared to pooled fall samples from 1984 to 1988 and the 1989 fall sample, also supports summer transmission of *T. cervi* in southern Florida. Epimastigotes are the multiplicative stage of *T. cervi* in the bloodstream and in culture (Kingston and Morton, 1975; Kingston and Crum, 1977). The presence of epimastigotes in numbers greater than those of trypomastigotes should indicate either a recently acquired active infection or recrudescence of a chronic infection. In the related *T. theileri,* reproduction also occurs in the epimastigote stage, and when infection begins, fairly high parasitemias may be apparent (Hoare, 1972). After 2–4 wk, parasitemias cannot be detected by microscopic examination of blood films, but the blood remains infective for up to 1 yr. There is no lasting immunity. In the temperate zone, reinfection may occur in spring when horse-flies again appear. The available data for *T. cervi* suggest similar epidemiology. In 1986, prevalence of *T. cervi* in spring was twice that of the subsequent summer and fall. This could reflect annual variation in the seasonal abundance of the presumed tabanid fly vectors (Kingston, 1981), with tabanid appearance earlier in spring that year than usual. Thirty-five of the 118 species of tabanid flies known from Florida (Jones and Anthony, 1964) are considered to be economically important on the basis of abundance, and 26 of these reach peak densities between April and July, the period during which increased prevalence of *T. cervi* and epimastigotes was found. The small sample from the summer of 1986 ($N = 12$) may have been partially responsible for the discrepant prevalence pattern observed that year. The October samples for 1985–1989 show prevalences, based on thin blood smears, varying from 7% to 22%, and by culture from 86% to 100%. Utilizing thin blood films, Davidson et al. (1983) found maximum prevalence (61%) of *T. cervi* from July to September, with a drop to one-half that level from October to December (30%). They also reported the highest prevalence of epimastigote forms on blood films taken from July to September. Their samples came from central Florida north to West Virginia and Arkansas, where the climatic pattern differs considerably from subtropical southern Florida. Our data are consistent with those of Davidson et al. (1983) and suggest that the relatively insensitive thin blood film technique

may provide better evidence of fluctuations in intensity due to transmission and infection phase than do the highly sensitive methods of culture or thick blood films. The prevalence of *T. cervi* appears to be high wherever adequate samples have been examined (Kistner and Hanson, 1969; Stuht, 1975; Davidson et al., 1983).

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Life Cycle of *Amphimerus elongatus* (Trematoda: Opisthorchiidae)

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ABSTRACT: Life cycle of *Amphimerus elongatus* and seasonal occurrence of cercariae and metacercariae were studied in Half Moon Lake, Eau Claire, Wisconsin from September 1982 to November 1984. Oculate pleurolophocercous cercariae shed from naturally infected *Amnicola limosa* were positively phototactic. Cercariae possessed 14 penetration glands with ducts terminating anteriorly in a 3:4:4:3 pattern, similar to that described for *Clonorchis sinensis*. Peak of cercarial production occurred in midsummer. Nearly all centrarchid fishes collected from Half Moon Lake were infected with metacercariae of *A. elongatus*. *Lepomis macrochirus, L. gibbosus,* and *Micropterus salmoides* exposed to cercariae in laboratory experiments proved to be the most susceptible second intermediate hosts. Prevalence in experimentally infected juvenile bluegills was 100% with an average intensity of 10.5 metacercariae. Two cyprinids, *Pimephales promelas* and *Rhinichthys cataractae,* were less suitable as hosts for metacercariae, and no other fishes exposed to cercariae in the laboratory could be infected. Metacercariae were infective 1 month postexposure. Adult *A. elongatus* were recovered from liver and pancreas of chickens and house sparrows fed metacercariae from naturally and experimentally infected fishes.

KEY WORDS: *Amphimerus elongatus,* Opisthorchiidae, *Amnicola limosa,* Hydrobiidae, life cycle, seasonal dynamics, natural infections, experimental infections, cercaria, metacercaria, *Lepomis macrochirus, Micropterus salmoides,* fish, waterfowl.

Amphimerus elongatus Gower, 1938, was originally described from specimens obtained from liver and pancreas of wild, captive, and experimentally infected ducks and swans at the W. K. Kellogg Bird Sanctuary, Michigan (Gower, 1938a). Gower infected mallards, *Anas platyrhynchos,* by feeding them various species of fish but was not successful in determining specifically which fish served as second intermediate hosts. Subsequently, Gower (1938b) reported briefly the seasonal prevalence of *A. elongatus* in ducks from the wildlife sanctuary. He found prevalences of 11.5%, 2%, and 30% in summer, fall, and winter, respectively.

Wallace (1939) described the metacercaria of *A. elongatus* from naturally infected minnows, *Notropis deliciosus stramineus,* in Minnesota. Adult worms which he obtained from experimentally infected chicks and ducks were morphologically similar to specimens described by Gower. Cameron (1944) incidentally reported the occurrence of metacercariae in suckers in Canada. Boyd and Fry (1971) reported adult *A. elongatus* from the common loon, *Gavia immer,* and the belted kingfisher, *Megaceryle alcyon.* Pence and Childs (1972) described the tissue damage to the liver of a double-crested cormorant, *Phalacrocorax auritus,* caused by a massive infection of *A. elongatus.* Nothing has been published on the mollusc first intermediate host or cercaria of *A. elongatus* except for a single statement in an abstract by Wallace (1940): "pleurolophocercous distome cercariae with 14 penetration glands from *Amnicola limosa portata* develop in fish into metacercariae identical with those of *A. elongatus.*"

Weil et al. (1986) reported the pathophysiology of *A. elongatus* in experimentally infected chicks. Their study was made possible by the discovery of *A. elongatus* metacercariae in bluegills, *Lepomis macrochirus,* in Half Moon Lake, Eau Claire, Wisconsin. Because only limited life history information has been reported for this parasite, a study of the life cycle of *A. elongatus* was initiated at this study site and is reported in this paper.

Materials and Methods

Natural infections

Hydrobiid snails identified as *Amnicola limosa* (Say) were collected with dipnets and by hand from Half Moon Lake, Eau Claire, Wisconsin, and maintained in 38-liter aerated aquaria. Voucher specimens of *A. limosa* were relaxed with menthol crystals, preserved in 70% ethanol, and deposited in the Malacology Collection, Florida State Museum, Gainesville (UF 40463). Snails were isolated individually in 37-mm diameter Stender dishes and induced to shed cercariae by exposure to bright sunlight or artificial light.

Bluegills, *Lepomis macrochirus* Rafinesque, large-

mouth bass, *Micropterus salmoides* (Lacépède), white crappie, *Pomoxis annularis* Rafinesque, black bullheads, *Ictalurus melas* (Rafinesque), and northern pike, *Esox lucius* Linnaeus, were collected from Half Moon Lake with dipnets, castnets, seines, or by ice fishing in winter. Fins and musculature were examined for metacercariae. Survival of metacercariae in dead fish maintained at 4°C was determined by microscopic observation of movement of the whole worm within the cyst and by observation of flame cell motility.

A limited search for natural definitive hosts of *A. elongatus* was made by examining 9 hunter-killed ducks from north central Wisconsin; however, no birds from Half Moon Lake, located in the city park, were collected. Observations on birds foraging in the lake were made in all seasons of the year.

Experimental infections

Fish collected from locations that were determined to be free from *A. elongatus* by examination of at least 10 specimens of each fish species, were exposed in groups of 20 to more than 1,000 cercariae in 4-liter aquaria for 24 hr. Bluegill, *Lepomis macrochirus,* pumpkinseed, *L. gibbosus* (Linnaeus), black bullhead, *Ictalurus melas,* longnose dace, *Rhinichthys cataractae* (Valenciennes), and emerald shiner, *Notropis atherinoides* Rafinesque, were collected in Fall Creek, town of Fall Creek, Wisconsin. Fantail darter, *Etheostoma flabellare* Rafinesque, Johnny darter, *E. nigrum* Rafinesque, and mudminnow, *Umbra limi* (Kirtland), were collected in O'Neil Creek, 5 km N Chippewa Falls, Wisconsin. Fathead minnow, *Pimephales promelas* Rafinesque, and largemouth bass, *Micropterus salmoides,* were collected in Frontage Road Pond, 3 km S of Eau Claire. Brook stickleback, *Culaea inconstans* (Kirtland), and creek chub, *Semotilis atromaculatus* (Mitchell), were collected in Little Niagara Creek, Eau Claire. After exposure, fish were maintained in aerated 38-liter aquaria and fed flake fish food. Fish were examined for metacercariae at various intervals from day 1 through day 68 postexposure (PE).

Metacercariae from both naturally infected and experimentally infected fishes were fed to young chicks, adult chickens, house sparrows, mice, rats, and cats in the laboratory.

Parasite morphology

Cercariae were studied alive with the aid of bright field, phase contrast, and differential interference contrast optics. Neutral red and Nile blue were employed as vital stains. Twenty cercariae were fixed in steaming 10% formalin, mounted under a floating coverslip, and measured using an ocular micrometer.

Metacercarial cysts from naturally infected fish were measured alive under floating coverslip ($N = 20$). Over 100 metacercariae from experimentally infected fish were measured at various intervals postexposure. Metacercariae were excysted artificially at 38°C in a solution of trypsin and bile salts (Irwin, 1983). Excysted metacercariae and adults were killed with steaming (160°C) AFA, stained with Semichon's carmine, and mounted in Permount. Unless otherwise indicated, measurements are expressed in micrometers, the range followed by mean in parentheses. Drawings were made with a microprojector.

Results

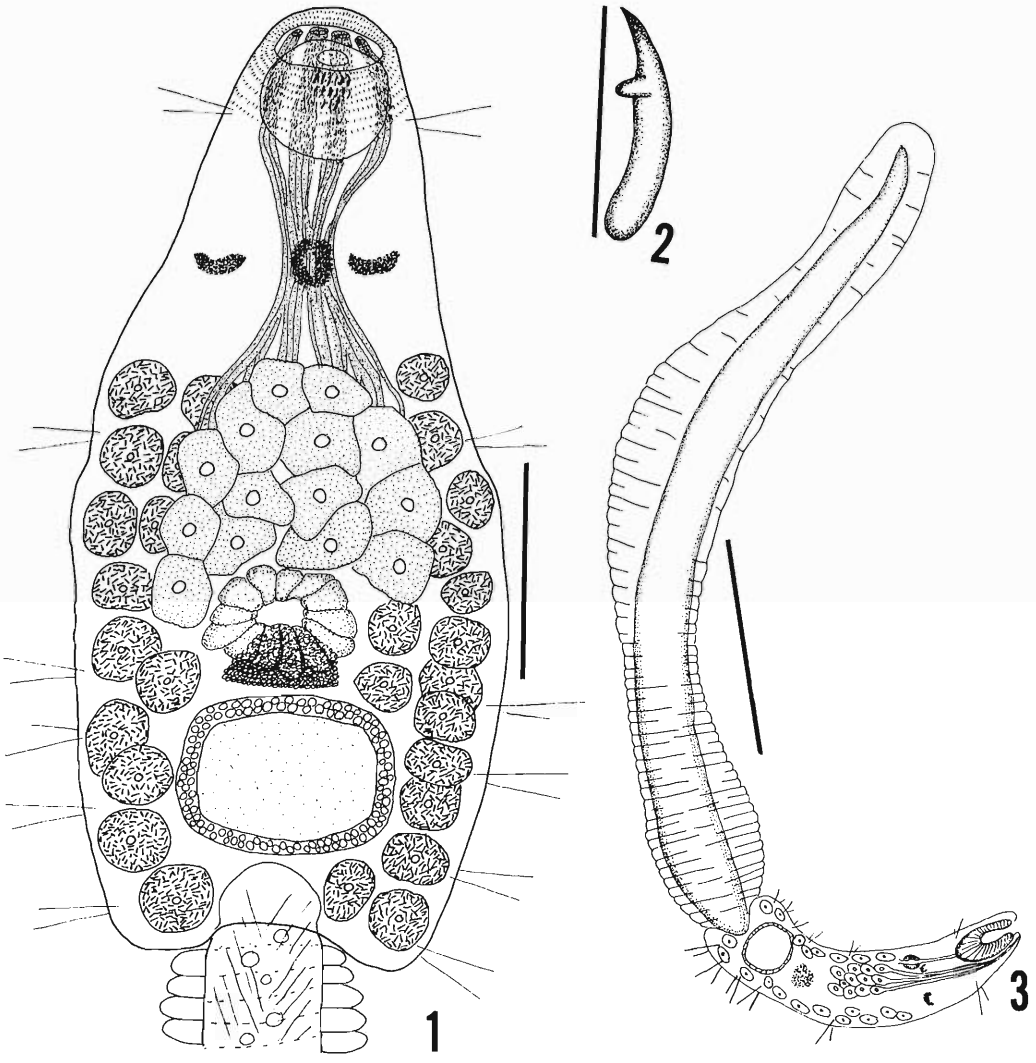
Cercaria (Figs. 1–3)

Body spinose anteriorly, oculate, pleurolophocercous, 193–243 (215) long by 53–75 (66) wide; with paired sensillae on lateral margins, 1 pair near oral sucker, 1 pair near midbody, 4 pairs in hindbody (Fig. 1). Oral sucker, protrusible from tegumental hood 28–38 (35) long by 25–33 (29) wide bearing 3 rows of small teeth (Fig. 2) on oral sucker, 8 per row anteriormost to 3 per row posteriormost. Acetabulum rudimentary, in tegumental fold at midbody. Genital anlage overlapping acetabulum. Pharynx situated medially between eyespots; esophagus and ceca undifferentiated. Fourteen penetration glands, staining weakly, clustered medially between ventral sucker and pharynx; ducts terminating anterior to oral sucker forming 3:4:4:3 pattern. Thirteen to 17 pairs of granular cystogenous glands staining intensely with vital stains, occupying dorsolateral margins of mid- and hindbody. Wall of translucent excretory vesicle epithelial with 2 rows of conspicuous spherical nuclei; ovoid, bounded anteriorly by genital anlage and posteriorly by tail insertion. Excretory ducts and flame cell pattern obscured by coarse granules of cystogenous gland cells. Tail elongate 400–475 (435) long by 28–38 (33) wide, entirely covered by dorsoventral finfold with numerous transverse striations (Fig. 3). Finfold widest in midregion of dorsal margin.

Cercariae emerged from the snail, *Amnicola limosa* (Hydrobiidae), in the laboratory under the stimulus of bright light, with emergence commencing within 15 min of stimulation. Cercariae were positively phototactic. They remained motionless, suspended with body oriented downward, and slowly settled in the water column. They rose in the water column by occasional bursts of swimming activity, simultaneously moving toward the light. Cercariae were able to swim actively for approximately 24 hr. Moribund cercariae lost their tails and crawled weakly on the bottom prior to death.

Metacercaria

Measurements of metacercariae encysted in naturally infected bluegills averaged 185 by 170 with a parasite cyst wall 12 thick. Dimensions of cysts and morphology of excysted metacercariae were similar to those described by Wallace (1939).



Figures 1–3. Cercaria of *Amphimerus elongatus* from *Amnicola limosa*, Half Moon Lake, Eau Claire, Wisconsin. 1. Body of cercaria, ventral view. Scale = 50 μm. 2. Rasping tooth from oral hood. Scale = 4 μm. 3. Resting posture of cercaria, lateral view. Scale = 110 μm.

Adult

No naturally infected definitive hosts were found during this study. Confirmation of the identity of *Amphimerus elongatus* was based upon adult specimens obtained from experimentally infected hosts.

Natural infections in snails

Amnicola limosa from Half Moon Lake initially shed *Amphimerus elongatus* cercariae in fall 1982. *Amnicola limosa* displayed a seasonal population cycle in Half Moon Lake similar to that reported for the species in Rhode Island (Kesler, 1980). Most snails lived for 1 year. Snails present after ice melted in April were hatched in July of the previous summer. As lake temperatures rose (18°C in mid-May), egg laying commenced and continued for 2 mo. Snails ceased laying eggs and began dying in July when temperatures reached 26°C. Few adult snails survived into the fall. Juvenile snails grew rapidly

Table 1. Prevalence of *Amnicola limosa* from Half Moon Lake, Eau Claire, Wisconsin shedding *Amphimerus elongatus* cercariae.

Date	Number examined	Number shedding	Prevalence (%)
27 Sep 1982	100	3	3
24 Apr 1983	200	0	0
25 May 1983	72	4	6
6 Jun 1983	200	15	8
6 Jul 1983	341	60	18
9 Jul 1983	50	10	20
21 Jul 1983	144	39	27
30 Jul 1983	54	26	48
12 Aug 1983	72	10	14
4 Sep 1983	72	1	1
2 Oct 1983	72	0	0
7 Apr 1984	10	0	0
29 May 1984	164	0	0
4 Jun 1984	119	5	4
13 Jun 1984	134	6	4
19 Jun 1984	137	8	6
26 Jun 1984	139	16	12
6 Jul 1984	200	13	7
19 Jul 1984	63	5	8
26 Jul 1984	147	12	8
15 Aug 1984	108	8	7
15 Sep 1984	72	1	1
8 Oct 1984	144	2	1

and by late fall some attained adult size. Final growth of this generation resumed in spring and egg laying by this generation was initiated in May.

The seasonal dynamics of cercarial production was closely related to the 1-yr life span of *Amnicola limosa*. Few snails shed *Amphimerus elongatus* cercariae in April, May, and June of both 1983 and 1984, but by late July prevalence of snails shedding *A. elongatus* peaked (Table 1). The remaining adult snails continued to shed cercariae in August and into the fall; however, so few adult snails were still alive at this time that cercarial production was greatly reduced. By fall the size of the smallest specimens of remaining adults overlapped the size of the largest snails belonging to the new generation. Therefore, prevalence of snails shedding cercariae could not be determined separately for the 2 generations. It could be ascertained qualitatively, however, that the only snails that were shedding cercariae were unequivocally old adults. These were the largest specimens and had heavily eroded and encrusted shells. Similarly, none of the snails belonging to the most recent generation shed *A. elongatus*. The pattern of cercarial shedding by *Amnicola limosa*, i.e., emergence beginning in late May and peaking in July, was similar in both years of this study, although prevalence of infected snails was much higher in 1983 than in 1984.

Table 2. Distribution of *Amphimerus elongatus* metacercariae in one bluegill, *Lepomis macrochirus* (standard length = 45 mm) collected in Half Moon Lake, Eau Claire, Wisconsin on 15 October 1983.

Site in fish	Number of metacercariae	Percentage
Caudal fin	85	49
Dorsal fin (soft)	37	21
Dorsal fin (spiny)	8	5
Anal fin	19	11
Pectoral fin (left)	2	1
Pectoral fin (right)	3	2
Pelvic fins	0	0
Muscle (caudal peduncle)	9	5
Muscle (all other)	11	6
Total	174	100

Natural infections in fishes

Bluegills, *Lepomis macrochirus*, and largemouth bass, *Micropterus salmoides*, were the only fishes in Half Moon Lake that harbored *Amphimerus elongatus* metacercariae. Half Moon Lake was not a typical locality to determine the range of host specificity of *A. elongatus* in a truly natural environment, because the fishes of the lake were exterminated with rotenone by the Wisconsin Department of Natural Resources (DNR). Black bullheads, *Ictalurus melas* ($N = 12$), northern pike, *Esox lucius* ($N = 5$), and white crappie, *Pomoxis annularis* ($N = 1$), restocked in the lake by DNR, were not infected. No fish of the families Cyprinidae or Catostomidae were collected in Half Moon Lake despite extensive effort.

Adult bluegills and bass typically were heavily infected with metacercariae. Prevalence in bluegills was 97% ($N = 185$) and bass was 92% ($N = 25$). Intensities of 100–300 or more metacercariae were common. Because of the large numbers of metacercariae, only estimates were made for most fish. However, careful count of cysts in 1 bluegill (45-mm standard length) harboring a typical infection was made to show the actual number and distribution of metacercariae (Table 2).

In July and August, adult bluegill and bass harbored both fully developed metacercariae and newly encysted, incompletely developed meta-

cercariae, indicating that reinfection was possible. Newly hatched bluegill and bass in July had an average prevalence of approximately 20% with typically only 1 or 2 small, incompletely developed metacercariae per infected fish. By October, young-of-the-year bluegill and bass harbored metacercariae considered to be fully developed, although prevalence and intensity remained low.

The effect of temperature on the viability of metacercariae encysted in dead bluegills was determined in order to ascertain whether infections might be acquired by scavengers as well as predatory definitive hosts. Metacercariae did not survive freezing at −10°C for 24 hr. After 48 hr, 100% of metacercariae in dead fish refrigerated at 4°C remained viable, and 50% were able to survive for 12 days at this temperature.

Natural infections in birds

Incidental to this study 5 mallards, *Anas platyrhynchos* Linnaeus, and 4 blue-winged teal, *A. discors* Linnaeus, collected within 50 mi of Half Moon Lake, were examined but were not infected with *A. elongatus*. Because this lake was located in a city park in the center of Eau Claire, Wisconsin, it was not possible to obtain birds for necropsy. However, observations of bird populations were made in order to understand better the ecology of *A. elongatus*. The lake was used by piscivorous summer residents such as herons and kingfishers, and numerous waterfowl considered spring and fall migrants were observed. Furthermore, the city aerated the center of the lake during the winter, and this ice-free area attracted huge flocks of waterfowl, which would ordinarily not use the lake from November through early April.

Geographic distribution of *Amphimerus elongatus*

Although the determination of the geographic distribution of *Amphimerus elongatus* in Wisconsin was beyond the scope of this study, a few bluegills in backwaters of the Red Cedar River, where *Amnicola limosa* also occurred, were infected with metacercariae. Low prevalence and intensity of metacercarial infections precluded an analysis of host specificity in other fishes from the Red Cedar River. Several thousand individuals of the only other hydrobiid snail in the area, *Somatogyrus depressus* (Tryon), were examined for cercariae, but none was infected with *A. elongatus*. Species of fish from numerous localities where hydrobiid snails were absent were not infected.

Experimental infections in fishes

Of the various species of fishes exposed to cercariae in the laboratory, only centrarchid fishes were readily infected with *A. elongatus*. Viable metacercariae were also obtained from several fathead minnows. Only 1 of 20 longnose dace harbored 1 viable metacercaria when examined 24 days postexposure. No other species of fish exposed to cercariae of *A. elongatus* hosted viable metacercariae for more than 7 days. Most insusceptible fish hosts showed no evidence of infection after exposure to over 50 cercariae per fish, but a small percentage of creek chubs, dace, and emerald shiners harbored a few dead, encysted specimens surrounded by a host cellular capsule 1 or more days PE. To illustrate differences in susceptibility, in one experiment 20 specimens each of fathead minnows, juvenile bluegills, and juvenile black bullheads were exposed, in separate containers, to equal numbers of all cercariae shed by 35 *Amnicola limosa*. All bluegills became infected, with a mean intensity of 10.5 metacercariae. Prevalence in fathead minnows was 20% with a mean intensity of 2.3. No black bullheads became infected.

Development of metacercariae was investigated in experimentally infected juvenile bluegills maintained at 22°C. On day 5 PE, cysts measured 123–152 (137) by 62–82 (71) with a parasite cyst wall 7.5 thick. A host-induced cellular capsule was already present around the cyst. The brownish pigmented worms moved actively within the cyst. Eyespots, oral sucker, and empty excretory bladder were clearly visible through the cyst wall. By day 15 PE, excretory concretions were apparent in the bladder, and the brown pigmentation of the body was markedly reduced. On day 20 PE, the ceca were completely developed and filled with cecal platelets (="disk-shaped concretions" of Wallace). The excretory bladder was made conspicuous by black concretions that completely filled the lumen. Cysts measured 180–225 (210) by 100–113 (103). On day 29 PE, metacercariae were identical with fully developed specimens from naturally infected bluegills and bass. Anatomical features, such as the pharynx and oral and ventral suckers, could be observed and measured in encysted specimens. Eyespots were no longer visible. These 29-day-old laboratory-reared metacercariae, when

fed to chicks, developed into adults in liver and pancreas that were morphologically identical to specimens described by Gower (1938a). Viable cysts were found in centrarchids and fathead minnows up to 68 days PE at which time experiments were terminated.

Experimental infections in birds and mammals

Only avian hosts became infected when birds and mammals were fed metacercariae from naturally infected Half Moon Lake bluegills in the laboratory. Mice, rats, and cats were completely refractory to infection. Adult specimens were obtained in chickens and in house sparrows. One-day-old unfed chicks typically attained higher levels of infection than older chickens, but all age groups were successfully infected. Adults were recovered from both liver and pancreas in all hosts harboring more than 1 worm. Adult worms conformed to the description of Gower (1938a), and ontogenetic changes from metacercariae were similar to those described by Wallace (1939).

Discussion

The occurrence of 14 penetration glands in the cercaria of *Amphimerus elongatus* confirms the preliminary description of the cercariae from *Amnicola limosa* in Minnesota (Wallace, 1940). The 3:4:4:3 pattern of ducts formed by the 14 penetration glands indicates that the cercaria of *A. elongatus* is most similar to that of *Clonorchis sinensis* (Komiya and Suzuki, 1964).

The life cycle of *Amphimerus elongatus* is similar to that of most members of the family Opisthorchiidae in which fishes serve as second intermediate hosts and piscivorous birds or mammals are definitive hosts for adults parasitizing the liver and pancreas. Both natural and experimental infections of *A. elongatus* indicated that adults are specific to avian hosts. Thus, it is unlikely that the species represents a public health threat, in spite of its abundance in fishes commonly consumed by humans. Susceptibility of house sparrows to infection was in agreement with the broad host specificity reported by other workers for avian hosts (Gower, 1938a; Boyd and Fry, 1971; Pence and Childs, 1972). Infection of nonpiscivorous birds, like house sparrows and chickens, indicated that natural host range may be limited by avian feeding ecology rather than by host physiology.

Seasonal dynamics in Half Moon Lake were closely associated with the seasonal population cycle of the first intermediate host, *Amnicola limosa*. The large size of the population of *A. limosa*, and the high prevalence of snails infected with *Amphimerus elongatus*, resulted in high cercarial production in the lake. Peak cercarial emergence, however, was confined to a relatively brief but intense period in July, immediately prior to death of the large majority of adult snails. It was interesting that the timing of maximum cercarial emergence meant that most young-of-the-year centrarchids in the lake receive only light infections during their first summer of life. Reinfection of older age class fishes is responsible for most metacercarial infections. These dynamics may be driven by extrinsic factors such as snail ecology and water temperature. However, if juvenile fishes are killed by heavy infections of metacercariae during their first few months of life, this intense selective pressure may be the most important factor responsible for the evolution of the seasonal pattern that exists in Half Moon Lake.

The high prevalence and intensity of *A. elongatus* in Half Moon Lake may be a natural phenomenon, or may be exacerbated by human intervention. Because a portion of the lake was kept ice-free during winter, and birds were fed throughout this period by city residents, the lake supported a much larger waterfowl population for a longer duration than would occur naturally. Greater than normal abundance of metacercariae in fishes may be a result of increased use of the lake by waterfowl, and concomitantly greater amounts of egg-laden feces entering the lake to infect snails. Because the potential pathogenicity of *A. elongatus* adults in birds is of serious concern (Gower, 1938a; Pence and Childs, 1972; Weil et al., 1986), the possible consequences of increased exposure of birds to this parasite due to modification of natural conditions, such as limiting the diversity of fish species in a lake or increasing waterfowl population density, must be considered.

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Cercaria chilkaensis II, a New Zoogonid Cercaria from the Snail, *Nassarius orissaensis,* from Chilka Lake, India

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ABSTRACT: A new zoogonid cercaria, *Cercaria chilkaensis* II sp. n., from the snail, *Nassarius orissaensis* (Preston), from Chilka Lake, a brackish water habitat in India, is described. Comparison of its characteristics with those of the 7 previously described zoogonid cercariae indicates the closest resemblance to the cercaria of *Diphtherostomum brusinae* Stossich, 1904.

KEY WORDS: *Cercaria chilkaensis* II sp. n., Chilka Lake, Zoogonidae, cercaria, *Nassarius orissaensis,* India.

Larval stages of the family Zoogonidae have so far not been reported from the Indian Ocean area, although adult zoogonids are known from marine fishes of the Bay of Bengal (Madhavi, 1979). The present report provides a description of a new zoogonid cercaria, *Cercaria chilkaensis* II, from the prosobranch snail, *Nassarius orissaensis* (Preston), collected from Chilka Lake, a brackish water habitat in Orissa state, India. *C. chilkaensis* I was described as a new cystophorous cercaria from the same locality (Shameem et al., 1990).

Materials and Methods

Monthly samples of *N. orissaensis* were collected from Chilka Lake and examined for larval trematode infections. The snails were isolated individually in petri dishes containing diluted seawater and examined on the following day for the presence of cercarial infections.

Details of cercariae were studied by examining live, freshly liberated cercariae mounted on a slide. Neutral red was used as a vital stain. Measurements were taken from cercariae killed in nearly boiling seawater of salinity 10‰ and mounted freely floating under a coverglass. Measurements, given in micrometers, are based on 8 specimens and specify the range and mean, the latter given in parentheses. The figure was drawn with the help of a camera lucida from a heat killed cercaria and details were added freehand.

Results

Infection with the zoogonid cercaria was found only once during August 1986 when 1 of 30 snails emitted tailless cercariae. Salinity of the lake at that time was 5‰. None of the 200 snails examined during the period April 1986 to July 1986 was infected. The cercariae emerged from the snail during daytime, and were found firmly adhering to the bottom of the glass container by their sticky posterior ends. They were very active and remained alive for 48 hr at room temperature in water of 10‰ salinity.

Cercaria chilkaensis II sp. n. (Fig. 1)

DESCRIPTION: Cercariaeum, body oval, 280–300 (290) long, 80–100 (90) wide. Tegument thick with large, thornlike, retrorse spines. Suckers well developed, oral sucker terminal, 56–60 × 42–44 in size, with small triangular stylet 10–12 (11) long. Ventral sucker posterior to midlevel of body, protrusible 56–60 (58) in diameter. Prepharynx short, pharynx 36–40 × 20–24 in size, esophagus narrow, 48–50 long, gut bifurcating anterior to ventral sucker, ceca terminating at midlevel of hindbody. Six penetration glands in 2 groups of 3 each located near intestinal bifurcation, each gland irregular in shape with prominent nucleus and fine granular cytoplasm, staining deeply with neutral red. Penetration gland ducts in 2 narrow bundles, opening through pores situated on either side of stylet. Excretory bladder globular, with sphincter adjacent to excretory pore, bladder wall cells if present, indistinct. Two pairs of main excretory tubules enter bladder anterolaterally. Flame cell formula 2[(2 + 2) + (2 + 2)] = 16. Two genital primordia present, 1 anterior to and another posterior to ventral sucker. Body containing abundant refringent granules, rendering body opaque.

HOST: *Nassarius orissaensis.*

LOCALITY: Chilka Lake.

PREVALENCE: 3.3% in August 1986.

SPECIMENS DEPOSITED: Zoological Survey of India, Calcutta accession number W1/7756.

Discussion

The cercaria here described is the eighth species of larval zoogonids reported worldwide (Table 1). It most closely resembles the cercaria of

Table 1. List of reported zoogonid cercariae with their hosts and localities.

Cercaria	Hosts	Locality	Authority	Name employed
Zoogonus rubellus (Olsson, 1868)	*Nassarius reticulatus*	Roscoff, France	Stunkard, 1932	*Cercariaeum reticulatum*
Odhner, 1902	*Nassarius reticulatus*	Roscoff, France	Stunkard, 1941	as *Z. rubellus*
Zoogonus lasius (Leidy, 1891) Stunkard, 1940	*Illyanassa mutabilis*	New Jersey, USA	Leidy, 1891	*Distomum lasium*
	Illyanassa mutabilis	Massachusetts, USA	Linton, 1915	*Cercaria* sp.
	Illyanassa mutabilis	Massachusetts, USA	Miller and Northup, 1926	*Cercariaeum lintoni*
	Illyanassa mutabilis	New York, USA	Africa, 1930	*Cercariaeum lintoni*
	Illyanassa mutabilis	California, USA	Glading, 1935	*Cercariaeum lintoni*
	Illyanassa mutabilis	Massachusetts, USA	Stunkard, 1940	*Z. lasius* (Leidy, 1891)
Zoogonoides viviparus (Olsson, 1868)	*Buccinum undatum*	Boulogne, France	Pelseneer, 1906	*Cercaria giardi*
Odhner, 1902	*Buccinum undatum*	Northumberland, UK	Lebour, 1911	*Cercaria buccini*
	Buccinum undatum	Northumberland, UK	Lebour, 1918	*Z. viviparus*
	Buccinum undatum	Denmark	Koie, 1971, 1976	*Z. viviparus*
Diphtherostomum brusinae	*Nassarius reticulatus*	Black Sea	Sinitzin, 1911	*C. inconstans*
Stossich, 1904	*Nassarius reticulatus*	Black Sea	Dolgikh, 1970	*D. brusinae*
	Nassarius reticulatus	Naples, Italy	Palombi, 1930, 1934	*D. brusinae*
	Nassa mutabilis	Banyuls, France	Bayssade-Dufour and Maillard, 1974	*D. brusinae*
Cercaria crispata Pelseneer, 1906	*Natica alderi* (=*N. poliana*)	Boulogne, France	Pelseneer, 1906	*C. crispata*
Zoogonoides laevis Linton, 1940	*Mitrella lunata*	Massachusetts, USA	Stunkard, 1943	*Z. laevis*
Cercaria brachycaeca Shimura and Ito, 1980	*Batillus cornutus*	Japan	Shimura and Ito, 1980	*C. brachycaeca*
Cercaria chilkaensis II	*Nassarius orissaensis*	Orissa, India	Present report	*C. chilkaensis* II n. sp.

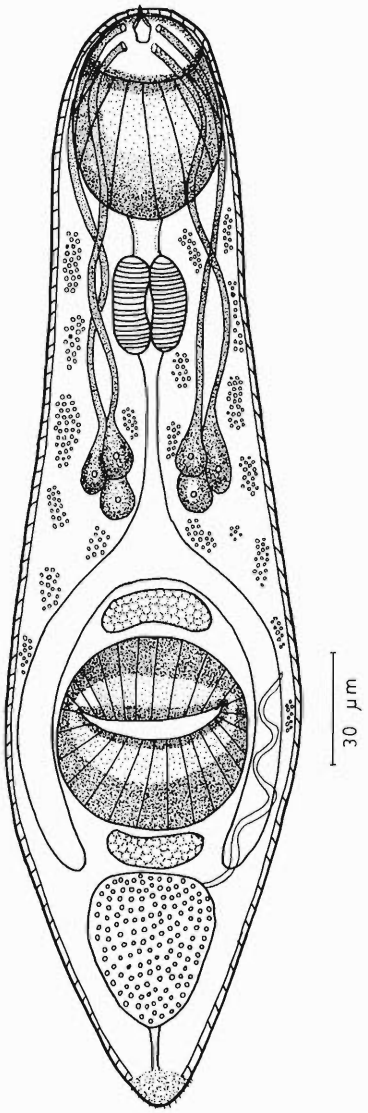

Figure 1. ***Cercaria chilkaensis*** **II sp. n. from the snail,** ***Nassarius orissaensis,*** **ventral view.**

D. brusinae, especially in having 3 pairs of penetration glands. The number is much higher in the remaining species; there are 6–8 pairs of penetration glands in the cercariae of *Z. rubellus* and *Z. laevis,* the former of which has well organized rudiments of testes and ovary. The cercaria of *Z. viviparus* has numerous penetration glands, and the posterior part of the body forms a prominent disc covered with spines. In *Cercaria brachycaeca* described by Shimura and Ito (1980) there are 6 pairs of penetration glands, and ceca are short and stout, terminating in the equatorial plane of the body.

The above comparison suggests that the present form may represent a species congeneric with *D. brusinae.* Adults of such a species occur in the cat fish, *Tachysurus thalassinus,* from the lake where infected snails were collected, and metacercarial cysts referable to the genus *Diphtherostomum* were found attached to the mantle fold of *N. orissaensis.* Experimental work is needed to link the various stages in the life cycle.

Acknowledgments

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Scientist of the Year

From the Director, United States Department of Agriculture, Agricultural Research Services

Jitender P. Dubey, Research Microbiologist, Zoonotic Diseases Laboratory, Livestock & Poultry Sciences Institute, has been selected as the Beltsville Area Scientist of the Year for 1990. Dr. Dubey has distinguished himself as a leader in parasitology research nationally and internationally. He is especially well known for his research on *Toxoplasma,* a protozoan parasite that infects many domestic animals, cattle, swine, sheep, goats, and horses, resulting in disease and substantial economic losses. He has documented the complex life cycle of the parasite, studied its epidemiology in livestock, and developed methods for rendering meat products noninfectious from the standpoint of public health.

Dr. Dubey's research extends broadly beyond his contributions on toxoplasmosis. For example, he recently discovered a new genus of parasites (*Neospora*) of potentially great economic importance. He has developed a diagnostic test to detect infections in animals and man and is assisting the State of California in diagnosing an outbreak in dairy cattle.

Dr. Dubey received a Ph.D. from the School of Medicine, University of Sheffield, England, in 1966. He worked at Montana State University prior to joining ARS at Beltsville in 1982. His research is documented in over 350 publications. Dr. Dubey has received a number of prestigious awards, including the Distinguished Veterinary Parasitologist Award from the American Association of Veterinary Parasitology.

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Anapalaeorchis hamajimai gen. et sp. n. (Trematoda: Monorchiidae) from the Loach, *Cobitis biwae,* in Japan

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ABSTRACT: *Anapalaeorchis hamajimai* gen. et sp. n. (Digenea: Monorchiidae: Asymphylodorinae) is described from gravid specimens from the digestive tract of a freshwater fish, *Cobitis biwae,* collected in the Tokigawa River, Saitama Prefecture, central Japan. *Anapalaeorchis* gen. n. is proposed for a species possessing ceca terminating midway in the hindbody, an acetabulum larger than the oral sucker, 2 tandem, nearly contiguous testes, a bipartite seminal vesicle, a trilobed ovary, and vitellaria in paired compact bunches.

KEY WORDS: *Anapalaeorchis* gen. n., *Anapalaeorchis hamajimai* sp. n., Digenea, Monorchiidae, Asymphylodorinae, *Cobitis biwae.*

In the course of examination of the digestive tract of the freshwater fish, *Cobitis biwae* Jordan and Snyder, taken from the Tokigawa River, Saitama Prefecture, central Japan, we found a monorchiid trematode with characters that did not agree with any existing genus. This trematode was found to be new and is here described as a new genus.

Materials and Methods

A total of 128 specimens were collected from the digestive tract of the loach, *Cobitis biwae,* in the Tokigawa River, Saitama Prefecture, central Japan from 29 April 1985 to 27 August 1988. Of these, 46 gravid specimens were designated as type specimens. Descriptions and measurements were based on these specimens. The specimens were fixed in 70% ethanol under the pressure of a cover glass, stained with carmine, or eosin and hematoxylin, or only hematoxylin, and mounted. Measurements, given in micrometers unless otherwise stated, were made with the aid of a filar micrometer. The figure was drawn using a Nikon profile projector (6CT2). For scanning electron microscopy (SEM), worms and eggs were rinsed well with Ringer's saline and fixed for 4 hr in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) and postfixed for 3 hr in phosphate-buffered osmium tetroxide. After dehydration with a graded series of ethanol, the specimens were dried in a critical point drying apparatus, coated with gold in a JEOL FC-1100 ion-sputtering apparatus, and photographed with a JEOL JSM-U3 scanning electron microscope operated at 15 kV.

Results

Anapalaeorchis gen. n.

DIAGNOSIS: Body fusiform or spatulate, spinous. Oral sucker subterminal. Esophagus fairly long, slender. Ceca extending halfway through hindbody. Acetabulum circular, larger than oral sucker, in forebody. Testes double, tandem, nearly contiguous, ovoid to subtriangular, in hindbody. Cirrus eversible, spined. Cirrus pouch claviform. Seminal vesicle bipartite, pars prostatica well differentiated. Ovary pretesticular, trilobed. Uterus filling space in hindbody. Terminal organ receiving metraterm terminally. Vitellaria laterally paired postacetabular compact bunches, equatorial.

TYPE SPECIES: *Anapalaeorchis hamajimai* sp. n.

Anapalaeorchis hamajimai sp. n. (Figs. 1–8)

DESCRIPTION WITH CHARACTERS OF THE GENUS: Body fusiform or spatulate, 910 (700–1,110) in length and 290 (240–330) in maximum width (Figs. 1–4); covered entirely with minute spines, which become thinner and smaller posteriorly (Figs. 5, 6). Oral sucker circular, subterminal, 122 (106–144) in diameter. Prepharynx not present. Pharynx oval, 65 (52–76) by 67 (58–79). Esophagus elongate, 205 (200–210) long. Ceca terminating at level of anterior testis or posterior end of ovary. Acetabulum greatly larger than oral sucker, 168 (132–199) by 162 (136–189), situated one-third of body length from anterior extremity; sucker width ratio, 1:1.3–1.4. Testes nearly touching, tandem; anterior testis ovoid to subtriangular, 104 (89–124) long by 90 (76–101) wide; posterior testis ovoid, elongate, 127 (87–145) long by 94 (69–110) wide. Cirrus eversible, spined. Cirrus pouch club-shaped, seminal vesicle bipartite, pars prostatica well differentiated. Ovary trilobed, 110 (79–143) long by 114 (73–162) wide, slightly posterior to middle of body, closely located to anterior testis. Genital pore near left margin of body, slightly anterior to level of posterior edge of acetabulum. Uterus sur-

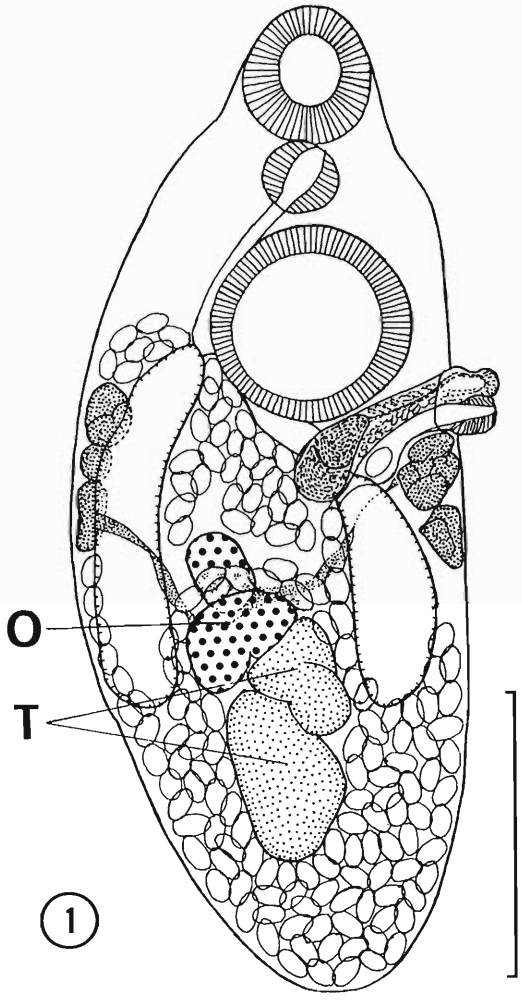


Figure 1. ***Anapalaeorchis hamajimai,*** **holotype, ventral view. Bar = 200 μm. O, ovary; T, testes.**

rounding ovary and testes, filling hindbody. Vitellaria in lateral clusters of several follicles at midbody; right vitelline mass longer than left. Terminal organ receiving metraterm terminally. Eggs elliptical, operculate, embryonated, 29.3 (26.4–31.2) × 16.6 (15.4–17.2) (N = 100, live eggs) (Fig. 7). Egg shell surface coarse with shallow irregular pits or depressions (Fig. 8). Excretory system not observed.

TYPE MATERIALS: Holotype and 6 paratypes are deposited in the collection of the National Science Museum, Tokyo (NSMT, P1 3776); 4 paratypes in the United States National Museum Helminthological Collection (USNM Helm. Coll. No. 81141); 35 paratypes in the collection of the Department of Parasitology, Faculty of Medicine, Kyushu University (PDKU T001–T035).

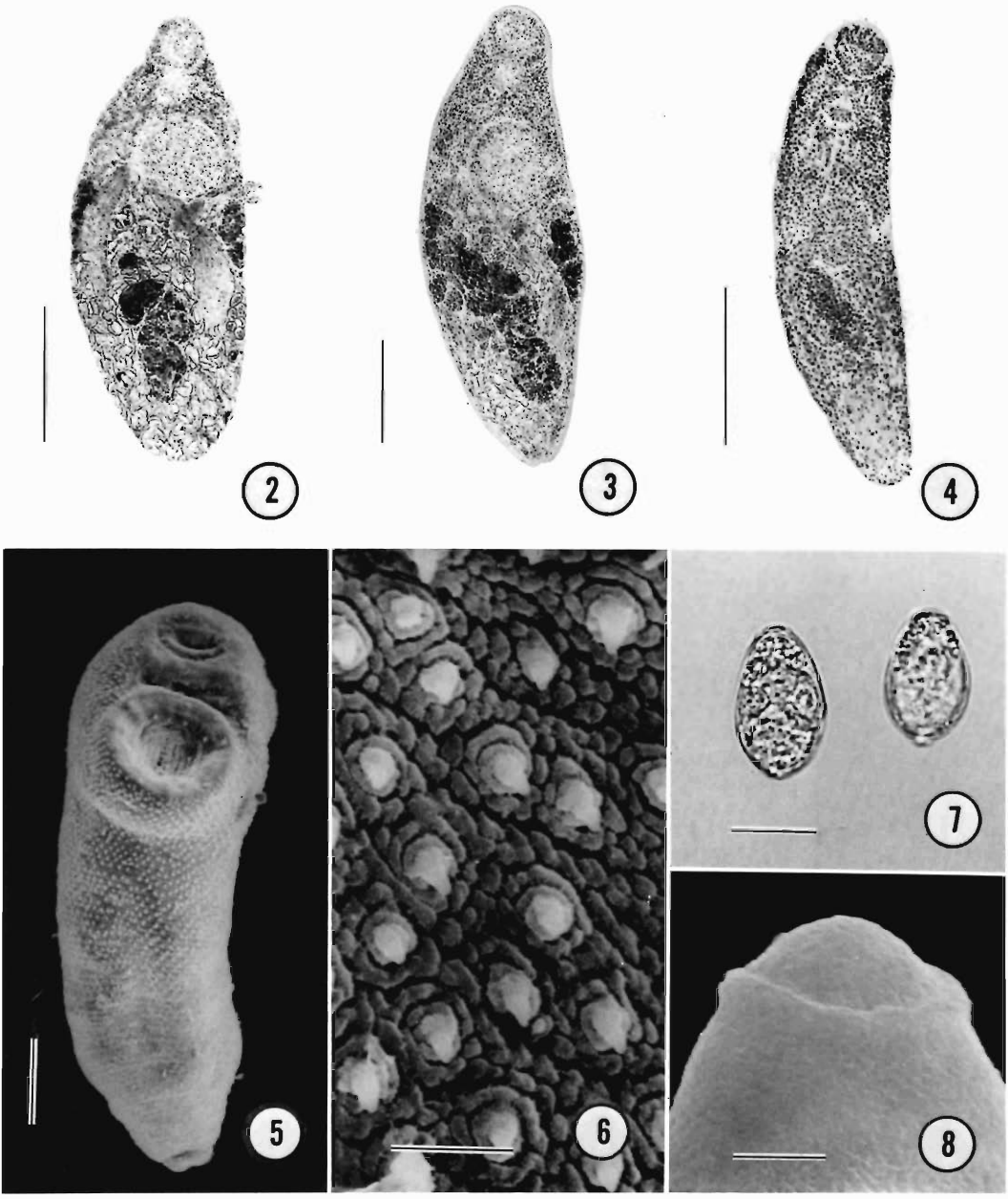
HOST: Sand loach, *Cobitis biwae* Jordan and Snyder.

HABITAT: Stomach (juvenile worms) and intestine (adult worms).

ETYMOLOGY: This species is named after Prof. Fusanori Hamajima who collected and donated the present material.

Discussion

According to Yamaguti (1971), the monorchiid subfamily Asymphylodorinae includes 3 genera, *Palaeorchis* Szidat, 1943, *Asymphylodora* Looss, 1899, and *Triganodistomum* Simer, 1929. The new genus *Anapalaeorchis* is similar to *Palaeorchis* in that the double testes are unequal in size and the vitellaria are compact, but is easily separable in tandemly arranged testes, a trilobed ovary, and an acetabulum that is larger than the oral sucker. The testes of *A. hamajimai* are in tandem and almost touching, whereas all the species of *Palaeorchis* have testes that are juxtaposed and clearly separated. *Anapalaeorchis* is also similar to *Asymphylodora* in its large acetabulum and long esophagus. *Asymphylodora,* however, is different from *Anapalaeorchis* in having a single testis. Yamaguti (1938) erected a new species, *Asymphylodora japonica,* parasitic in the freshwater fish, *Cyprinus carpio.* He included some worms from the small intestine of *Cobitis biwae,* which were much smaller than those from *C. carpio.* We had the loan of Yamaguti's specimens from *Cobitis biwae* preserved in Meguro Parasitological Museum, Tokyo (No. 22034). Although these specimens mounted on 1 slide are too old to make sure whether the testis is single or separated, the specimens are found to have some features identifiable to *A. japonica*: the longer ceca extending over the posterior end of the testis and the more extended vitellaria than in *Anapalaeorchis hamajimai.* The main similarity of *Anapalaeorchis* to *Triganodistomum* is that in both genera the testes are arranged in tandem. The testes in *Triganodistomum,* however, are distinctly separated, whereas those of *Anapalaeorchis* are close to each other. The ceca reach to near the posterior extremity of the body in *Triganodistomum,* whereas the ceca terminate in the mid-hindbody in *Anapalaeorchis. Triganodistomum* has far more extensive vitellaria than has *Anapalaeorchis.*



Figures 2–8. 2–4. ***Anapalaeorchis hamajimai,*** **H&E stain. Bar = 200 μm. 2. Holotype, gravid adult. 3. Paratype, gravid adult. 4. Juvenile. 5, 6. *A. hamajimai,* SEM of body. 5. Whole body in ventral view. Bar = 50 μm. 6. Tegumental spines, ventral aspect in middle region. Bar = 5 μm. 7, 8. *A. hamajimai,* eggs. 7. Light micrograph of whole eggs. Bar = 20 μm. 8. SEM of operculum and part of egg shell. Bar = 1 μm.**

Schell (1973) erected the genus *Neopaleorchis* based upon the new species, *N. catostomi,* which inhabits the intestine of a coarsescale sucker, *Catostomus macrocheilus. Anapalaeorchis* bears superficial resemblance to *Neopaleorchis* in having tandem testes; however, in *Neopaleorchis,* the seminal vesicle is folded, but not bipartite, the oral sucker and acetabulum are nearly equal, vitellaria are postcecal and contiguous dorsal to uterus and testes, and eggs are small and numerous.

Acknowledgments

We are greatly indebted to Prof. Fusanori Hamajima of National Defense Medical College for the collection and donation of the present material. Our sincere thanks are also due to Dr. Shunya Kamegai of Meguro Parasitological Museum, Tokyo, and Dr. Susumu Saito of Yamagata University for their interests and valuable suggestions; to Dr. Koichi Fukuda of National Defense Medical College for his help in preparing specimens; and to Dr. Atsuo Ichihara of Meguro Parasitological Museum, Tokyo, for the loan of Yamaguti's specimens of *Asymphylodora japonica.*

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Observations on the Life Cycle of *Proteocephalus tumidocollus* (Cestoda: Proteocephalidae) in Steelhead Trout, *Oncorhynchus mykiss*

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ABSTRACT: The life cycle of *Proteocephalus tumidocollus* in a brackish water, aquacultural pond involves steelhead trout (*Oncorhynchus mykiss*) as definitive hosts, and freshwater (*Diaptomus* sp., *Paracyclops fimbriatus poppei*) or benthic marine (*Halicyclops* sp.) copepods as intermediate hosts. Steelhead trout do not feed on copepods directly. Instead, they consume the macroalga *Enteromorpha* sp. with epiphytic copepods attached. Between May 1981 and July 1982, there were no statistically significant monthly variations in prevalence, mean intensity, or relative density of *P. tumidocollus* in steelhead trout. However, there were marked decreases in mean intensity and relative density and a marked increase in the percentage of cestodes that were gravid in January to March 1982 when *Enteromorpha* sp. was absent from the pond.

KEY WORDS: *Proteocephalus tumidocollus,* Proteocephalidae, Cestoda, life cycle, seasonality, steelhead trout, *Oncorhynchus mykiss,* California.

Wagner (1953) described *Proteocephalus tumidocollus* from rainbow and brook trout from the California Trout Company, Mentone, California. Five reports of *P. tumidocollus* have since been published (Hicks and Threlfall, 1973; Hanek and Molnar, 1974; Arthur et al., 1976; Mamer, 1978; Arai and Mudry, 1983).

Wagner (1954) studied the life cycle of *P. tumidocollus* in freshwater. He fed its eggs to the copepods *Cyclops vernalis, Eucyclops agilis, Eucyclops speratus,* and *Tropocyclops prasinus,* which became infected. He was unable to infect the copepods *Macrocyclops albidus* and *Paracylops fimbriatus poppei* nor several other invertebrates. Procercoids developed rapidly at 20°C and became infective to trout at 9 to 19 days postinfection (PI). Trout definitive hosts became infected by eating copepod intermediate hosts. Second intermediate hosts were not required, although large trout could become infected by eating small infected trout.

Proteocephalus tumidocollus is common in steelhead trout, *Oncorhynchus mykiss* (Walbaum), in the brackish water ponds of the Arcata Wastewater Aquaculture Project, Arcata, California. While freshwater species of *Proteocephalus* are common and well studied, brackish water species are rare and poorly known (Wardle and McLeod, 1952; Yamaguti, 1959; Freze, 1965; Schmidt, 1986). The Arcata system offered an unusual opportunity to examine the seasonality and life cycle of a *Proteocephalus* sp. in brackish water.

Materials and Methods

Infected steelhead trout were obtained from South Pond (0.6 ha, avg. depth 2 m, avg. salinity 14.5 ppt), Arcata Wastewater Aquaculture Project, Arcata, California. Uninfected steelhead trout fry were obtained from the Humboldt State University Fish Hatchery. Fish were held in 19–38-liter aquaria supplied with 11°–17°C aerated, filtered water. Potential invertebrate hosts were sieved from South Pond (brackish water), the adjacent oxidation pond of the Arcata sewage treatment plant (freshwater), or from the adjacent Butcher's Slough on Humboldt Bay (seawater). Invertebrates were cultured in 2–19-liter aquaria (11°–17°C) and fed yeast and flaked fish food.

Seasonal occurrence of *P. tumidocollus* in steelhead trout from South Pond was determined from fish collected by angling at weekly intervals from 12 May 1981 to 31 July 1982. Seven to 20 fish were obtained at each sampling. Fish total length and weight were recorded prior to necropsy. Necropsy procedures were standard (Hoffman, 1967). Food items in stomachs of fish hosts were analyzed quantitatively using percent composition by weight (Bowen, 1983).

Adult cestodes were placed in 0.7% saline and allowed to shed eggs for use in life history studies. Eggs were used to expose cultured invertebrates, which were examined for developing procercoids from 30 min to 86 days after initial exposure. In addition, 150 wild *Anisogammarus confervicolus* (Stimpson) (Amphipoda) from South Pond were examined for natural procercoid infections.

To infect experimental fish definitive hosts, invertebrates from cultures were fed to hatchery-reared (cestode-free) steelhead trout fry. Twenty-five fish were fed *Anisogammarus confervicolus* (Amphipoda), and 25 fish were fed a combination of *Corophium spinicorne*

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Table 1. Levels of infection of *Proteocephalus tumidocollus* and percentage of cestodes gravid in steelhead trout, *Oncorhynchus mykiss*, from South Pond, Arcata Wastewater Aquaculture Project, California, May 1981 to July 1982.

Month	No. of fish examined	No. of fish infected (%)	Mean intensity*	Relative density†	Range in nos. per infection	Percentage of cestodes gravid
1981						
May	14	6 (42.8)	8.5	3.6	2–28	74.5
June	12	3 (25.0)	7.7	1.9	6–7	13.0
July	8	4 (50.0)	5.8	2.9	2–4	13.0
August	16	2 (12.5)	1.5	0.2	1–2	0
September	18	8 (44.4)	7.5	3.3	1–37	25.0
October	18	8 (44.4)	4.8	2.1	1–10	92.1
November	20	6 (30.0)	17.7	5.3	2–84	19.8
December	7	2 (28.6)	5.0	1.4	4–6	70.0
1982						
January‡	13	4 (30.8)	6.0	1.8	1–11	100
February‡	12	6 (50.0)	2.5	1.2	1–5	93.3
March‡	8	1 (12.5)	2.0	0.3	2	100
April	16	7 (43.8)	30.0	13.1	1–66	96.2
May	20	10 (50.0)	19.7	9.8	1–125	29.4
June	18	7 (38.9)	10.7	4.2	1–48	57.3
July	16	4 (25.0)	8.2	2.1	1–25	0

* Total number of cestodes in sample ÷ number of infected hosts in sample.
† Total number of cestodes in sample ÷ total number of hosts in sample.
‡ No *Enteromorpha* sp. in South Pond.

Stimpson (Amphipoda) and *Gnorimosphaeroma oregonensis* (Dana) (Isopoda). Twenty-five fish were fed a mixture of small Copepoda, including *Diaptomus* sp., 1 planktonic and 1 benthic species of *Halicyclops, Paracyclops fimbriatus poppei* (Rehberg), and *Tigriopus californicus* (Baker). Seventy-two fish were held as controls and were fed a commercial trout pellet. After 8 wk, all fish were killed and examined for cestodes. In addition, 26 three-spined sticklebacks (*Gasterosteus aculeatus* L.) from South Pond were examined for cestodes to see if they might be capable of passing juvenile cestodes on to steelhead trout via predation.

Results

Proteocephalus tumidocollus was recovered from the pyloric ceca of 78 of 216 (36.1%) steelhead trout examined from South Pond during the seasonal survey. It was present in all months of the year. There was no marked seasonal variation in prevalence (Table 1). Relative density and mean intensity seemed to vary seasonally with peaks in November 1981 and May 1982; however, these variations were not statistically significant by analysis of variance ($P < 0.05$). There was a marked decrease in mean intensity and relative density in January to March 1982 when *Enteromorpha* sp. was absent from South Pond. Prevalence, mean intensity, and relative density all increased as fish total length increased. Gravid cestodes occurred in all but 2 mo and were most prevalent in winter, particularly in those months when *Enteromorpha* sp. was absent from South Pond (Table 1).

Analysis of stomach contents from 68 steelhead trout from South Pond showed that on a percent composition by weight basis, they ate: *Corophium spinicorne* (Amphipoda) 36.70%, *A. confervicolus* (Amphipoda) 29.90%, detritus 11.90%, *Enteromorpha* sp. (macroalga) 4.80%, mysids (Crustacea) 3.60%, *G. aculeatus* (fish) 2.50%, *G. oregonensis* (Isopoda) 2.12%, filamentous algae 1.90%, earwigs (terrestrial insects) 1.70%, Diptera 1.41%, unidentifiable fish larvae 1.20%, annelids 1.60%, unidentifiable invertebrates 0.36%, crabs 0.30%, and copepods 0.01%. *Enteromorpha* sp. was eaten whenever it was available in South Pond (May to December 1981, April to July 1982).

Experimental infections were established in the copepods *Diaptomus* sp., *P. fimbriatus poppei,* and a benthic species of *Halicyclops.* The freshwater copepod *Acanthocyclops* (*A.*) *vernalis* (Fischer) was also experimentally infected. This species enters South Pond when the pond is filled using a mixture of treated sewage effluent and seawater. However, it cannot tolerate the salinity in South Pond for very long. *Anisogammarus confervicolus* (Amphipoda), *C. spinicorne* (Am-

phipoda), *G. oregonensis* (Isopoda), *T. californicus* (Copepoda), and a planktonic species of *Halicyclops* (Copepoda) were refractory to experimental infection. None of 150 wild *A. confervicolus* examined from South Pond was infected.

Three of 25 hatchery-reared steelhead trout fed copepods (*Diaptomus* sp., 1 planktonic and 1 benthic species of *Halicyclops, P. fimbriatus poppei, T. californicus*) from exposed cultures had immature *P. tumidocollus* at 56 days PI. One fish had 3 cestodes and 3 fish had 1 immature cestode each. All cestodes were attached to the pyloric ceca. None of the 25 fish fed exposed *A. confervicolus,* none of the 25 fish fed exposed *C. spinicorne* and *G. oregonensis,* and none of the 72 control fish was infected. None of 26 three-spined sticklebacks examined from South Pond harbored cestodes even though smaller sticklebacks fed almost exclusively on copepods.

Discussion

Both marine (benthic *Halicyclops* sp.) and freshwater (*Diaptomus* sp., *P. fimbriatus poppei*) copepods are suitable intermediate hosts for *Proteocephalus tumidocollus* in South Pond. In addition, the entire life cycle takes place in brackish water. This utilization of a marine host and brackish water is unusual among species of *Proteocephalus* (Wardle and McLeod, 1952; Yamaguti, 1959; Freze, 1965; Schmidt, 1986).

Copepod intermediate hosts of *P. tumidocollus* are not an important item in the diet of steelhead trout definitive hosts in South Pond (0.01% by weight). However, the benthic *Halicyclops* sp. and *P. fimbriatus poppei* are epiphytic on the macroalga *Enteromorpha* sp. *Enteromorpha* sp. accounted for up to 30.1% by weight of the diet of steelhead trout in some months (4.8% over the entire study). Thus, steelhead trout in South Pond most likely become infected by incidentally ingesting copepods as they feed on *Enteromorpha* sp. This incidental mode of infection has not to our knowledge been reported previously.

There were no statistically significant monthly variations in prevalence, mean intensity, or relative density of *P. tumidocollus* in steelhead trout in South Pond. However, there was a marked decrease in both mean intensity and relative density, and a marked increase in the percentage of cestodes recovered from steelhead trout that were gravid in January to March 1982 when *Enteromorpha* sp. was absent from South Pond. In the absence of *Enteromorpha* sp., steelhead trout do not eat significant numbers of copepods. Hence, new infections do not occur. Measures of infection intensity are reduced and cestodes from older infections tend to become gravid. Thus, seasonal cycles in abundance or maturity of *P. tumidocollus* in steelhead trout in South Pond depend largely on the seasonal occurrence of *Enteromorpha* sp. *Enteromorpha* sp. is normally present in South Pond as long as South Pond remains sufficiently brackish. When there is sufficient rain to dilute South Pond (most likely in the winter rainy season), *Enteromorpha* sp. dies back and in some years disappears altogether.

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Sterol Composition of the Corn Root Lesion Nematode, *Pratylenchus agilis,* and Corn Root Cultures

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ABSTRACT: Sterols from mixed stages of the corn root lesion nematode, *Pratylenchus agilis,* and uninfected corn root cultures were analyzed by gas chromatography-mass spectrometry. Twenty-eight sterols were identified in *P. agilis,* including 9 not previously detected in nematodes. The major sterols were 24-ethylcholest-22-enol, 24-ethylcholesta-5,22-dienol, 24-methylcholestanol, 24-ethylcholestanol, isofucostanol, and 24-ethylcholesterol. The principal corn root sterols were 24-ethylcholesta-5,22-dienol, 24-methylcholesterol, 24-ethylcholesterol, isofucosterol, and cycloartenol. Therefore, the major metabolic transformation of sterols by *P. agilis* was saturation of the sterol nucleus. In addition, very small amounts of 4α-methylsterols were biosynthesized by *P. agilis.* The 4-methylation pathway is unique to nematodes and was previously demonstrated only in free-living Rhabditida. The sterol composition of *P. agilis* is remarkably different from that of other nematodes in its very small relative percentage of cholesterol (0.4% of the total nematode sterol).

KEY WORDS: corn, lesion nematode, lipid, maize, nematode, *Pratylenchus agilis,* sterol, *Zea mays.*

The development of new control strategies for parasitic nematodes could exploit biochemical differences between them and their vertebrate or plant hosts. One of the few such areas is steroid metabolism; unlike higher animals and plants, nematodes possess a nutritional requirement for sterol as a result of their lack of de novo sterol biosynthesis (for review: Chitwood et al., 1986). Sterols have 2 major functions; they are integral components of cell membranes as well as metabolic precursors of steroid hormones (Nes and McKean, 1977). Interest in nematode sterol biochemistry has intensified in recent years because of the discovery that several azasteroids and aliphatic amines strongly disrupt growth and development and also inhibit sterol metabolism in nematodes (Chitwood, 1987).

Because free-living nematodes are easily propagated in artificial media, they have been utilized in most investigations involving metabolism of radiolabeled dietary sterols by nematodes (Chitwood et al., 1986). The difficulty in in vitro culture of parasitic nematodes has restricted investigation of sterol metabolism in these organisms to comparison of sterol compositions of host and parasite. As expected, cholesterol is the major sterol of mammalian parasites (Cole and Krusberg, 1967b; Castro and Fairbairn, 1969; Barrett et al., 1970; Fulk and Shorb, 1971; Chung et al., 1986).

A significant biochemical difference between higher animals and plants is that plant sterols usually contain alkyl (i.e., methyl or ethyl) substituents at C-24 of the sterol side chain (Nes and McKean, 1977). Comparison of the sterols of plant-parasitic nematodes and their hosts has indicated that plant parasites are similar to free-living nematodes in that both groups can remove the C-24 alkyl groups of plant sterols (Chitwood, 1987). Unequivocal proof in the case of plant parasites, however, would involve experiments with radiolabeled plant sterols in an in vitro system. Because large quantities of the corn root lesion nematode, *Pratylenchus agilis* Thorne and Malek, 1968, can be readily obtained from corn root explant cultures, we examined the sterols of this plant parasite and its host in order to determine if it was a suitable candidate for radiotracer studies of dealkylation of plant sterols by phytoparasitic nematodes.

Materials and Methods

Seeds of *Zea mays* cv. Iochief were surface sterilized by immersion in distilled water for 2 hr and then 1.3% sodium hypochlorite containing 0.1% (v:v) Tween 80 for 25 min. The sterilized seeds were germinated for 4 days on 1.5% water agar in culture dishes, and then 2.5-cm root pieces were transferred to culture dishes containing Gamborg's B5 medium formulated with 1.5% agar and 2.0% sucrose, and lacking cytokinins or auxins (Huettel, 1990). The agar had been extracted previously with methanol and chloroform : methanol 2:1 (v:v, twice each) to reduce the high amounts of endogenous cholesterol in commercial agar (Nes, 1987) to an acceptable level (20.6 ng of cholesterol per gram dry weight as determined by subsequently described

Table 1. Sterol content of *Pratylenchus agilis* and root cultures of *Z. mays*.

	% dry weight		% sterol esterified
	Lipid	Sterol	
P. agilis	14.6	0.03	26.8
Z. mays	3.9	0.83	—*

* Not determined.

methods). One-week-old root cultures were inoculated with agar plugs from similar cultures containing sterile *P. agilis*. Nematodes from 8-week-old cultures maintained at 25°C were harvested with modified Baermann funnels, floated on 30% sucrose, rinsed 5 times with distilled water, frozen immediately, and lyophilized.

Lipids were extracted from lyophilized nematodes (2.0–2.2 g dry wt) by homogenization in a Ten-Broeck tissue grinder 3 times with chloroform : methanol 2:1 and were purified by partition against 0.85% NaCl (Folch et al., 1957). Neutral lipids were separated from polar lipids and were fractionated into various lipid classes by column chromatography on silica (Chitwood et al., 1985). The lipid fractions containing steryl esters and free sterols were saponified separately in methanolic KOH (Chitwood et al., 1985); the liberated 4-desmethylsterols and 4-methylsterols were purified by Florisil column chromatography (Chitwood et al., 1987c).

Roots from uninfected cultures were gently removed with forceps, frozen, and lyophilized. Extraction of roots (0.3–0.5 g dry wt) was identical to that of nematodes, except a Virtis homogenizer was used. The root lipids were saponified directly without prior fractionation on a silica column. The 4-methylsterols and 4-desmethylsterols were isolated as described above. As a precautionary measure, any potentially tightly bound sterols were removed from the extraction residue by saponification of the residue and analyzed identically to the root extracts.

Sterols were analyzed by gas-liquid chromatography (GLC) on a DB-1 fused silica capillary column and an OV-17 packed glass column as described previously (Chitwood et al., 1987c). Following preliminary analysis, the sterols were acetylated, the steryl acetates were purified on Florisil columns and analyzed by GLC, and then tentative identifications were confirmed by gas chromatography-mass spectrometry (GC-MS, Chitwood et al., 1987c). The steryl acetates were also fractionated according to degree and position of unsaturation by chromatography on columns of silica impregnated with $AgNO_3$ and analyzed by GLC (Chitwood et al., 1987c).

Results are the means of two replicates.

Results

Gravimetric analysis indicated that lipid constituted approximately 15% of the dry weight of *P. agilis*; subsequent quantification of sterols by GLC revealed that sterol comprised only a very small proportion of the lipid extract (Table 1).

Table 2. Gas-liquid chromatographic relative retention times (RRT's) of sterols from *Pratylenchus agilis* and *Zea mays*, expressed relative to cholesterol. GLC was performed on a temperature-programmed DB-1 fused silica capillary column (14 m × 0.25-mm i.d., 0.25-μm film) and on an isothermally operated packed glass column containing 2.0% OV-17 stationary phase.

Sterol	DB-1	OV-17
Cholesterol	1.00	1.00
Cholestanol	1.02	1.02
24-Methylcholesta-5,22-dienol	1.11	1.14
Lathosterol	1.12	1.19
24-Methylcholest-22-enol	1.14	1.17
24-Methylcholesta-5,23-dienol	1.25	1.38
24-Methylenecholesterol	1.26	1.37
24-Methylenecholestanol	1.30	1.39
24-Methylcholesterol	1.30	1.33
24-Methylcholestanol	1.33	1.35
24-Ethylcholesta-5,22E-dienol	1.40	1.45
24-Ethylcholest-22E-enol	1.43	1.47
24-Methylcholesta-5,24(25)-dienol	1.47	1.67
24-Ethylcholesta-5,23-dienol	1.53	1.67
24-Ethylcholesta-7,22-dienol	1.57	1.70
Fucosterol	1.59	1.76
24-Ethylcholesterol	1.60	1.67
24-Ethylcholestanol	1.63	1.69
Isofucosterol	1.66	1.85
Isofucostanol	1.69	1.88
24-Ethylcholesta-5,24(25)-dienol	1.77	1.98
24-Ethyllathosterol	1.81	1.96
24-Ethylcholest-24(25)-enol	1.81	2.04
24Z-Ethylidenelathosterol	1.89	2.20
Obtusifoliol	1.52	1.56
	(1.49)*	(1.50)
4α,24-Dimethylcholestanol	1.58	1.52
	(1.53)	(1.48)
4α-Methyl-24-ethylcholest-22-enol	1.71	1.66
	(1.67)	(1.61)
4α-Methyl-24-ethylcholestanol	2.10	1.87
	(1.91)	(1.87)
4α-Methylisofucostanol	2.07	2.11
	(1.95)	(2.06)
4α-Methyl-24Z-ethylidenelathosterol	2.26	2.52
	(2.12)	(2.44)
Cycloartanol	1.71	1.67
	(1.60)	(1.53)
Cycloeucalenol	1.74	1.88
	(1.69)	(1.78)
Cycloartenol	1.86	2.05
	(1.73)	(1.85)
24-Methylenecycloartanol	2.14	2.28
	(1.99)	(2.06)

* Values in parentheses are RRT's of steryl acetates relative to cholesteryl acetate. Trivial and systematic names: cholesterol, cholest-5-en-3β-ol; cholestanol, 5α-cholestan-3β-ol; lathosterol, 5α-cholest-7-en-3β-ol; fucosterol, 24E-ethylidenecholest-5-en-3β-ol; isofucosterol, 24Z-ethylidenecholest-5-en-3β-ol; isofucostanol, 24Z-ethylidene-5α-cholestan-3β-ol; obtusifoliol, 4α,14-dimethyl-24-methylene-5α-cholest-8-en-3β-ol; cycloartanol, 4,4,14-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol; cycloeucalenol, 4α,14-dimethyl-24-methylene-9β,19-cyclo-5α-cholestan-3β-ol; cycloartenol, 4,4,14-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol.

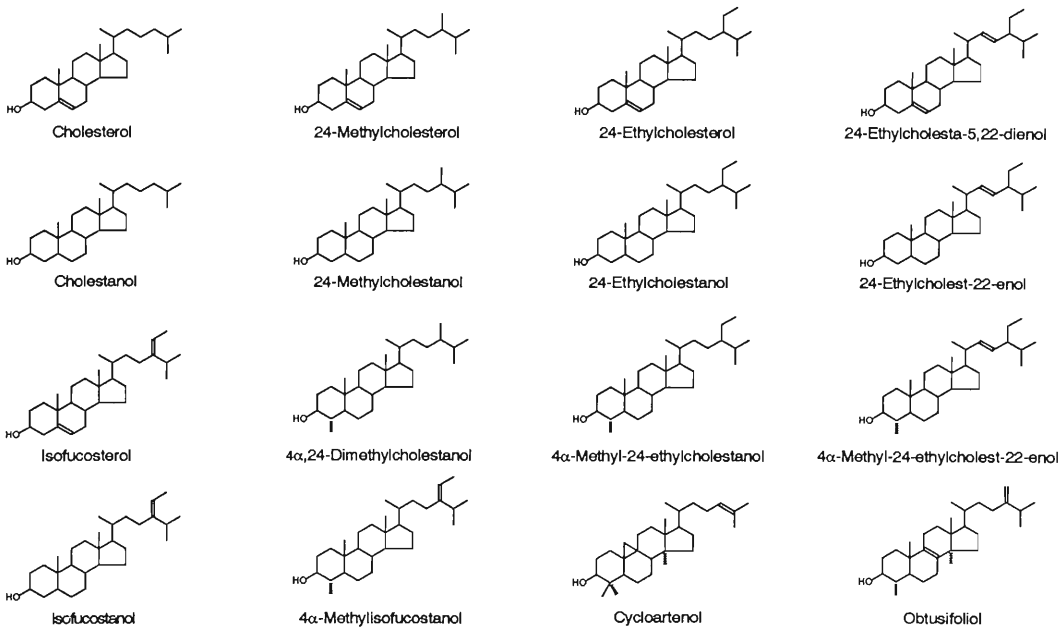

Figure 1. Some representative sterols from *Pratylenchus agilis* or *Zea mays*.

Most of the nematode sterol was unesterified. Total sterols were much more abundant in *Z. mays*. Saponification of the root extraction residue yielded only an additional 0.1% sterol.

All sterols from *P. agilis* and *Z. mays* (Fig. 1) were identical to authentic standards (when available) by GLC relative retention times (RRT's, Table 2) of both the free sterols and steryl acetate derivatives, argentation column chromatographic behavior, and GC-MS. Authentic 24-methylcholesta-5,24(25)-dienol was prepared by isomerization of 24-methylenecholesterol by iodine (Salt et al., 1986). Its GLC RRT's were similar to literature values (Itoh et al., 1982) and the mass spectrum of the steryl acetate (mass/charge m/z [structure and relative intensity]: 380 [molecular ion M^+-CH_3COOH, 27], 365 [M-CH_3COOH-CH_3, 5], 296 [M-CH_3COOH-C_6H_{11}-H, 53], 281 [296-CH_3, 17], 253 [M-CH_3COOH-C_9H_{17} side chain-2H, 19], 213 [M-CH_3COOH-side chain-C_3H_6, 17], and 83 [C_6H_{11}, 100]) was nearly identical to that of 24-methylenecholesterol but contained an abundant side chain fragment at m/z 83 produced by allylic cleavage at C-22 due to the $\Delta^{24(25)}$-bond. We lacked authentic standards for several compounds, including 24-ethylcholest-22E-enol, 24Z-ethylidenelathosterol, 4α,24-dimethylcholestanol, 4α-methyl-24-ethylcholestanol, 4α-methyl-24Z-ethylidenelathosterol, cycloartanol, and 24-methylenecycloartanol. These were indistinguishable by GLC and GC-MS from the same compounds previously identified by us in *Heterodera zeae* and *Meloidogyne incognita* (Chitwood et al., 1985, 1987c).

Other sterols for which we lacked authentic standards were 24-methylcholest-22-enol, 24-ethylcholesta-5,23-dienol, 24-ethylcholesta-5,24(25)-dienol, 24-ethylcholest-24(25)-dienol, obtusifoliol, 4α-methyl-24-ethylcholest-22-enol, 4α-methylisofucostanol, and cycloeucalenol. These were identified as follows.

As is typical for other sterols with saturated ring systems (Patterson, 1971), the GLC RRT's for 24-methylcholest-22-enol were characteristically slightly greater than those of the corresponding sterol containing a Δ^5-bond, i.e., 24-methylcholesta-5,22-dienol. The mass spectrum of the steryl acetate (442 [M^+, 18], 344 [M-C_7H_{13}-H, 29], 329 [344-CH_3, 3], 315 [M-C_9H_{17} side chain-2H, 36], 257 [M-CH_3COOH-side chain, 57], 215 [M-CH_3COOH-side chain-C_3H_6, 14], and 55 [C_4H_7, 100]) was very similar to that of 24-ethylcholest-22-enyl acetate (Chitwood et al., 1985), but all ions containing an intact side chain were 14 mass units smaller. The GLC RRT's of 24-ethylcholesta-5,23-dienol were similar to literature values (Itoh et al., 1982); the mass

spectrum of the acetate (394 [M-CH_3COOH, 100], 379 [M-CH_3COOH-CH_3, 7], 283 [M-CH_3COOH-C_8H_{15}, 16], 253 [M-CH_3COOH-$C_{10}H_{19}$ side chain-2H, 22], and 213 [M-CH_3COOH-side chain-C_3H_6, 11]) included a diagnostic loss of C_8H_{15} resulting from allylic cleavage of the C-20(22) bond. The GLC RRT's for 24-ethylcholesta-5,24(25)-dienol were similar to literature values (Itoh et al., 1982). The mass spectrum of the steryl acetate (394 [M-CH_3COOH, 32], 379 [M-CH_3COOH-CH_3, 6], 296 [M-CH_3COOH-C_7H_{13}-H, 100], 281 [296-CH_3, 23], 253 [M-CH_3COOH-$C_{10}H_{19}$ side chain-2H, 18], and 213 [M-CH_3COOH-side chain-C_3H_6, 9]) was similar to a literature spectrum (Kim et al., 1988), contained a more abundant side chain fragment at m/z 97 (analogous to the m/z 83 fragment in the spectrum of 24-methylcholesta-5,24(25)-dienyl acetate), but was otherwise indistinguishable from the spectra of fucosteryl and isofucosteryl acetates. The RRT's of 24-ethylcholest-24(25)-enol were slightly greater than RRT's of 24-ethylcholesta-5,24(25)-dienol. The mass spectrum of the $\Delta^{24(25)}$-acetate was indistinguishable from that of its $\Delta^{24(28)}$-isomer, i.e., authentic isofucostanyl acetate (Chitwood et al., 1987c).

The fact that the RRT's of the newly identified 4α-methylsterols were greater as free sterols than as the acetate derivatives is indicative of the presence of a 4α-methyl substituent (Patterson, 1971). The RRT's of obtusifoliyl acetate (Table 2) and its mass spectrum (468 [M^+, 28], 453 [M-CH_3, 64], 393 [M-CH_3COOH-CH_3, 22], 369 [M-CH_3-C_6H_{11}-H, 9], 343 [M-C_9H_{17} side chain, 4], 309 [M-CH_3COOH-CH_3-C_6H_{11}-H, 11], 287 [M-side chain-CH_3-D ring, 19], 227 [287-CH_3COOH, 14], and 55 [C_4H_7, 100]) were similar to literature values (Itoh et al., 1978, 1982; Staphylakis and Gegiou, 1985; Rahier et al., 1989). The mass spectrum included ions produced by loss of a C_6H_{11} fragment resulting from allylic cleavage of the C-22 bond. The mass spectrum of 4α-methyl-24-ethylcholest-22-enyl acetate (470 [M^+, 28], 455 [M-CH_3, 2], 427 [M-C_3H_7, 2], 367 [M-CH_3COOH-C_3H_7, 17], 358 [M-C_8H_{15}-H, 24], 343 [M-C_8H_{15}-H-CH_3, 4], 329 [M-$C_{10}H_{19}$ side chain-2H, 23], 271 [M-CH_3COOH-side chain, 33], 229 [M-CH_3COOH-side chain-C_3H_6, 8], and 55 [C_4H_7, 100]) included ions produced by the side chain fragmentations characteristic of Δ^{22}-sterols (Lenfant et al., 1967; Hutchins et al., 1970) and nuclear ions (229, 271) characteristic of 4α-methylsterols. The mass spectrum of 4α-methylisofucostanyl acetate (470 [M^+, 4], 455 [M-CH_3, 1], 372 [M-C_7H_{13}-H, 100], 357 [M-C_7H_{13}-H-CH_3, 12], 329 [M-$C_{10}H_{19}$ side chain-2H, 6], 312 [M-CH_3COOH-C_7H_{13}-H, 4], 269 [M-CH_3COOH-side chain-2H,3],230[M-CH_3COOH-sidechain-C_3H_5, 27], and 229 [M-CH_3COOH-side chain-C_3H_6, 18]) was similar to a literature spectrum (Piretti and Viviani, 1989) and virtually identical to that of authentic isofucostanyl acetate (Chitwood et al., 1987b), but all ions containing an intact steroid nucleus were 14 mass units higher. The GLC RRT's of cycloeucalenyl acetate (Table 2) and its mass spectrum (468 [M^+, 4], 453 [M-CH_3, 7], 408 [M-CH_3COOH, 62], 393 [M-CH_3COOH-CH_3, 37], 300 [M-CH_3COOH-A ring, 6], 283 [M-CH_3COOH-C_9H_{17} side chain, 8], 281 [M-CH_3COOH-side chain-2H, 7], and 55 [C_4H_7, 100]) agreed with literature values (Itoh et al., 1978, 1982; Staphylakis and Gegiou, 1985; Rahier et al., 1989). The mass spectrum included the loss of the A ring characteristic of 9β,19-cyclopropyl sterols (Rahier and Benveniste, 1989).

The major sterols of corn root cultures were 24-ethylcholesta-5,22E-dienol, 24-methylcholesterol, and 24-ethylcholesterol (Table 3). In addition to these 3 sterols, *P. agilis* contained 24-ethylcholest-22E-enol as its major sterol and substantial quantities of 24-methylcholestanol, 24-ethylcholestanol, and isofucostanol. The free and esterified sterol fractions of *P. agilis* were qualitatively similar but quantitatively different; the steryl esters contained a much greater proportion of cycloartenol and 24-methylenecycloartenol.

Discussion

The lipid content of *P. agilis* (15%) was less than that found by Krusberg (1967) in *Pratylenchus penetrans* (25%) propagated on alfalfa callus. The small percentage of dry weight of *P. agilis* as sterol (0.03%) is within the range reported in other phytoparasitic nematodes: *Globodera tabacum solanacearum* (0.01%), *Meloidogyne incognita* (0.01%), *M. arenaria* (0.01%), *Rotylenchulus reniformis,* (0.02%), *Heterodera zeae* (0.05%), and *Ditylenchus dipsaci* (0.06%) (Cole and Krusberg, 1967a; Svoboda and Rebois, 1977; Orcutt et al., 1978; Chitwood et al., 1985, 1987c). These values are lower than the range of 0.2%–0.6% reported in the animal-par-

Table 3. Relative percentages of sterols in root cultures of *Zea mays* and in free sterol and steryl ester fractions from *Pratylenchus agilis*.

	P. agilis		
	Free sterols	Steryl esters	*Z. mays*
Cholesterol	0.3	0.7	0.2
Cholestanol	0.4	0.4	Trace*
24-Methylcholesta-5,22-dienol	0.1	Trace	0.3
Lathosterol	0.1	Trace	Trace
24-Methylcholest-22E-enol	0.1	Trace	—†
24-Methylcholesta-5,23-dienol	—	—	0.1
24-Methylenecholesterol	0.2	0.1	1.2
24-Methylenecholestanol	0.4	0.2	0.1
24-Methylcholesterol	2.3	4.7	14.2
24-Methylcholestanol	11.0	4.8	0.5
24-Ethylcholesta-5,22E-dienol	24.6	21.9	60.3
24-Ethylcholest-22E-enol	34.2	18.4	1.4
24-Methylcholesta-5,24(25)-dienol	—	—	0.1
24-Ethylcholesta-5,23-dienol	—	—	0.2
24-Ethylcholesta-7,22E-dienol	0.4	0.1	0.3
Fucosterol	—	—	0.1
24-Ethylcholesterol	4.5	4.8	9.0
24-Ethylcholestanol	10.2	11.9	0.6
Isofucosterol	1.8	2.1	4.3
Isofucostanol	5.2	5.1	0.1
24-Ethylcholesta-5,24(25)-dienol	Trace	—	0.2
24-Ethyllathosterol	0.3	0.9	0.1
24-Ethylcholest-24(25)-enol	0.1	—	—
24Z-Ethylidenelathosterol	0.8	3.0	0.2
Obtusifoliol	0.1	0.2	0.1
4α,24-Dimethylcholestanol	0.6	0.3	—
4α-Methyl-24-ethylcholest-22E-enol	0.4	0.4	—
4α-Methyl-24-ethylcholestanol	0.6	0.2	—
4α-Methylisofucostanol	0.2	0.4	—
4α-Methyl-24Z-ethylidenelathosterol	0.1	Trace	0.1
Cycloartanol	—	—	0.3
Cycloeucalenol	0.1	—	0.4
Cycloartenol	0.7	16.1	3.9
24-Methylenecycloartanol	0.2	3.3	1.7

* Less than 0.05%.
† None detected.

asitic nematodes *Ascaris suum, Nippostrongylus brasiliensis, Strongyloides ratti,* and *Trichostrongylus colubriformis* (Cole and Krusberg, 1967b; Barrett, 1968, 1969; Tarr and Fairbairn, 1973; Chung et al., 1986). A small fraction of the sterols in *P. agilis* are esterified, as in *H. zeae, Meloidogyne* spp., and the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* (Chitwood et al., 1984, 1985, 1987c; Salt et al., 1989). The composition of the free and esterified sterol fractions from *P. agilis* were qualitatively identical but quantitatively different. Most notably, the steryl esters were rich in cycloartenol and 24-methylenecycloartanol, 2 important intermediates in de novo sterol biosynthesis in higher plants (Nes and McKean, 1977). These two 4,4,14-trimethylsterols are probably not utilized by nematodes, as a related 4,4,14-trimethylsterol, lanosterol, does not support growth and reproduction in *C. elegans* or the insect-parasitic nematode, *Steinernema feltiae* (Chitwood et al., 1987a; Ritter, 1988). Perhaps *P. agilis* and other nematodes isolate nonutilizable sterols by sequestering them as steryl esters.

The major sterols of *P. agilis* are 24-ethylcholest-22-enol, 24-ethylcholesta-5,22-dienol, 24-methylcholestanol, 24-ethylcholestanol, isofucostanol, and 24-ethylcholesterol. The principal sterols of corn root cultures are 24-ethylcholesta-5,22-dienol, 24-methylcholesterol, 24-ethylcholesterol, and isofucosterol. Our results support other reports of 24-ethylcholesta-5,22-dienol

(stigmasterol) as the predominant sterol of *Z. mays* roots and 24-ethylcholesterol (sitosterol) and 24-methylcholesterol as other abundant sterols (Rohmer et al., 1972; Bladocha and Benveniste, 1983). The most striking difference between *P. agilis* and its host is in the percentage of stanols (i.e., sterols without double bonds in the ring system). Less than 3% of the corn root sterols were stanols, whereas approximately two-thirds of the sterols from *P. agilis* were stanols. Although selective uptake of the small quantities of plant stanols cannot be excluded, it seems likely that *P. agilis* can hydrogenate the Δ^5-bonds of corn sterols to produce a variety of stanols. Two free-living nematodes, *Turbatrix aceti* and *P. redivivus,* reduce radiolabeled dietary cholesterol to form cholestanol (Chitwood et al., 1987b), and comparison of sterols of hosts and parasite indicate that *S. feltiae, G. tabacum solanacearum, H. zeae,* and *Meloidogyne* spp. also saturate Δ^5-bonds (Chitwood et al., 1985, 1987b; Morrison and Ritter, 1986; Orcutt et al., 1978).

One interesting aspect of sterol metabolism in free-living Rhabditida is their production of significant quantities of 4α-methylsterols (Chitwood et al., 1984, 1987b). This 4-methylation pathway does not occur in any other class of organisms. Interestingly, we identified very small amounts of several 4α-methylstanols in *P. agilis*: 4α-methyl-24-ethylcholest-22-enol, 4α,24-dimethylcholestanol, 4α-methyl-24-ethylcholestanol, and 4α-methylisofucostanol. Their small relative percentage (less than 2% of the total *P. agilis* sterol) makes any conclusion about their biosynthetic origin speculative. However, their endogenous biosynthesis by *P. agilis* is suggested by the facts that we were unable to isolate them from corn roots and that they are the 4-methyl derivatives of the 4 major products of sterol biosynthesis in *P. agilis,* i.e., the 4 abundant stanols.

Many compounds that inhibit sterol metabolism and growth and development in free-living nematodes inhibit the C-24 dealkylation pathway (Chitwood, 1987). Because free-living nematodes remove the C-24 methyl and ethyl groups of plant sterols and because the sterols of all other plant-parasitic nematodes examined consist of 5%–50% cholesterol, plant-parasitic nematodes are thought similarly to dealkylate phytosterols (Chitwood, 1987). *P. agilis,* however, is distinguished from other nematodes by the small (0.4%) relative percentage of cholesterol and may lack the ability to dealkylate phytosterols. The following sterols previously undetected in nematodes were identified in *P. agilis* in this investigation: 24-methylcholest-22-enol, 24-methylcholesta-5,24(25)-dienol, 24-ethylcholesta-5,23-dienol, 24-ethylcholesta-5,24(25)-dienol, 24-ethylcholest-24(25)-enol, 4α-methyl-24-ethylcholest-22-enol, 4α-methylisofucostanol, obtusifoliol, and cycloeucalenol. We are unaware of identification in *Z. mays* of the following: 24-methylenecholestanol, 24-methylcholesta-5,24(25)-dienol, 24-ethylcholesta-5,23-dienol, and 24-ethylcholesta-5,24(25)-dienol. Sterols with $\Delta^{24(25)}$- or Δ^{23}-bonds have been implicated in the biosynthesis of the major sterols of corn (Zakelj and Goad, 1983), although few of these have been identified previously in *Z. mays.*

The sterol composition of *P. agilis* reflects the diversity of sterol metabolism in nematodes. There is no evidence for phytosterol dealkylation in *P. agilis,* in contrast to other plant-parasitic and free-living nematodes. Instead, *P. agilis* saturates the nucleus of its dietary sterols; the specific value to nematodes of stanol production is presently unknown. Further examination of sterol metabolism in taxonomically distant orders could reveal even greater diversity in sterol biochemistry between nematodes and their hosts.

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Protozoan, Helminth, and Arthropod Parasites of the Spotted Chorus Frog, *Pseudacris clarkii* (Anura: Hylidae), from North-central Texas

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ABSTRACT: Thirty-nine juvenile and adult spotted chorus frogs, *Pseudacris clarkii,* were collected from 3 counties of north-central Texas and examined for parasites. Thirty-three (85%) of the *P. clarkii* were found to be infected with 1 or more parasites, including *Hexamita intestinalis* Dujardin, 1841, *Tritrichomonas augusta* Alexeieff, 1911, *Opalina* sp. Purkinje and Valentin, 1840, *Nyctotherus cordiformis* Ehrenberg, 1838, *Myxidium serotinum* Kudo and Sprague, 1940, *Cylindrotaenia americana* Jewell, 1916, *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931, and *Hannemania* sp. Oudemans, 1911. All represent new host records for the respective parasites. In addition, a summary of the 36 species of amphibians and reptiles reported to be hosts of *Cylindrotaenia americana* is presented.

KEY WORDS: Anura, *Cosmocercoides variabilis, Cylindrotaenia americana, Hannemania* sp., *Hexamita intestinalis,* Hylidae, intensity, *Myxidium serotinum, Nyctotherus cordiformis, Opalina* sp., prevalence, *Pseudacris clarkii,* spotted chorus frog, survey, *Tritrichomonas augusta.*

The spotted chorus frog, *Pseudacris clarkii* (Baird, 1854), is a small, secretive, hylid anuran that ranges from north-central Kansas southward through central Oklahoma and Texas to northeastern Tamaulipas, Mexico (Conant, 1975). The species inhabits marshy areas of open prairie grasslands and edges of woodland. Little information is available on the ecology and natural history of *P. clarkii* (Pierce and Whitehurst, 1990), and even less is known about its parasites. Kuntz and Self (1944) examined 3 *P. clarkii* from Comanche County, Oklahoma, for helminth parasites and reported *Glypthelmins quieta* (Stafford, 1900) Stafford, 1905, in a single frog. To my knowledge, nothing else has been published regarding either ecto- or endoparasites of this frog. The purposes of this paper are to report the identity, prevalence, and intensity of parasites infecting *P. clarkii* in north-central Texas, and to provide a summary of the amphibians and reptiles of the world known to be hosts of *Cylindrotaenia americana.*

Materials and Methods

Between May 1986 and April 1988, and again during March 1990, 39 juvenile and adult *P. clarkii* (24 males, 15 females; $\bar{x} \pm$ SE snout–vent length [SVL] = 28.7 ± 0.4, range = 24–33 mm) were collected from Dallas (N = 14), Hood (N = 12), and Somervell (N = 13) counties of north-central Texas and examined for parasites. Specimens were either taken by hand by overturning limestone rocks or were captured with dipnets in temporary ponds during spring breeding activities. Frogs were placed in plastic freezer bags on ice and transported to the laboratory where they were killed with an overdose of Nembutal®. Necropsy and parasite techniques are identical to the methods of McAllister (1987) and McAllister and Upton (1987a, b), except that cestodes were stained with Semichon's acetocarmine and larval chiggers were fixed in situ with 10% formalin, sectioned at 7 μm, and stained with hematoxylin and eosin counterstain. Voucher specimens of frogs have been deposited in the Arkansas State University Museum of Zoology (ASUMZ 5948, 5966, 5969, 5977–5979, 6015–6016, 7069–7072, 8515, 8578, 8628–8634, 8637, 8683–8688, 8691). Parasites have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705 as follows: *Hexamita intestinalis* (USNM 81062), *Tritrichomonas augusta* (USNM 81063), *Opalina* sp. (USNM 81059), *Nyctotherus cordiformis* (USNM 81060), *Myxidium serotinum* (USNM 81061), *Cylindrotaenia americana* (USNM 81057-81058), *Cosmocercoides variabilis* (USNM 81065), *Hannemania* sp. (USNM 81064).

Results and Discussion

Thirty-three (85%) of the *P. clarkii* harbored 1 or more parasites (Table 1); all are new host records. None of the *P. clarkii* was found to be infected with coccidial parasites in the feces and the blood was negative for intraerythrocytic or trypanosomal hematozoa. Of frogs from the 3 separate county locations, 14 (100%) from Dallas, 11 (92%) from Hood, and 8 (62%) from Somervell counties were infected.

The cosmopolitan flagellates, *Hexamita intestinalis* Dujardin, 1841, and *Tritrichomonas augusta* Alexeieff, 1911, were the most common parasites of *P. clarkii.* Both species have been reported previously from a number of amphib-

Table 1. Parasites found in *Pseudacris clarkii* from north-central Texas.

Parasite	Site of infection*	Prevalence†
Protozoa		
Mastigophora		
Hexamita intestinalis	CO, RE	33/39 (85%)
Tritrichomonas augusta	CO, RE	33/39 (85%)
Opalinata		
Opalina sp.	CO	17/39 (44%)
Ciliophora		
Nyctotherus cordiformis	CO	2/39 (5%)
Myxozoa		
Myxidium serotinum	GB	23/39 (59%)
Platyhelminthes		
Cestoidea		
Cylindrotaenia americana	SI	3/39 (8%)
Nematoda		
Cosmocercoides variabilis	RE	2/39 (5%)
Acari		
Hannemania sp. (larvae)	DE	11/39 (28%)

* Abbreviations: CO, colon; DE, dermis; GB, gall bladder; RE, rectum; SI, small intestine.
† Number infected/number examined (Margolis et al., 1982).

ians (Buttrey, 1954; Frank, 1984), including Brimley's chorus frogs, *P. brimleyi,* from North Carolina (Brandt, 1936) and western chorus frogs, *P. triseriata triseriata,* from Ohio (Odlaug, 1954).

The endocommensal *Opalina* sp. Purkinje and Valentin, 1840, was found in nearly half of the frogs examined. McAllister (1987) reported that 51 of 52 (98%) Strecker's chorus frogs, *P. streckeri streckeri,* from Dallas County, Texas, were infected with *Opalina* sp. Interestingly, the opalinids noted herein were morphologically indistinguishable from those of sympatric *P. s. streckeri.* However, as noted by McAllister (1987), specific identification was not possible.

Only 5% of the *P. clarkii* were found to be infected with *Nyctotherus cordiformis* Ehrenberg, 1838. However, a 10-fold higher prevalence of *N. cordiformis* was reported for *P. s. streckeri* (McAllister, 1987), as 54% were infected. This ciliate has been reported from other *Pseudacris* spp. (Walton, 1964).

Spores and trophozoites of *Myxidium serotinum* Kudo and Sprague, 1940, were found in more than half of the frogs. A moderately high prevalence of this myxozoan was also reported for *P. s. streckeri* (McAllister, 1987). Additional amphibians from north-central Texas have been reported previously to harbor *M. serotinum,* including smallmouth salamanders, *Ambystoma texanum* (McAllister and Upton, 1987b), and 3 species of toads, *Bufo* spp. (McAllister et al., 1989).

Adult nematotaeniid tapeworms, *Cylindrotaenia americana* Jewell, 1916, were found in 3 frogs, 1 each collected on 16 March and 10 April 1987 and on 22 June 1986 from Dallas (30 mm SVL male, ASUMZ 8684), Hood (25 mm SVL male, ASUMZ 7071), and Somervell (30 mm SVL male, ASUMZ 6016) counties, respectively. The mean intensity was 2.7 (range = 1–6) worms per host. This cestode has been reported previously from *Gastrophryne olivacea* (McAllister and Upton, 1987b) and *Ambystoma texanum* (McAllister and Upton, 1987a) from north-central Texas. None of the *P. s. streckeri* examined by McAllister (1987) was found to be infected with *C. americana,* although sympatric salamanders and other frogs harbored the parasite. *Cylindrotaenia americana* has been reported from other amphibians and 2 reptiles from North and South America, Europe, and Asia, including 9 species of salamanders, 25 species of frogs and toads, 1 skink, and 1 snake (Table 2).

Joyeux (1924) reported *C. americana* from the puddle frog, *Phrynobatrachus* (syn. *Arthroleptis*) *ogoensis* Boulenger, 1906, and mascarene grass frog, *Ptychadena mascareniensis* (syn. *Rana aequiplicata*) Werner, 1898, from Mozambique, Africa. Harwood (1932) questioned the identity of Joyeux's material and Joyeux (*in* Baer, 1933)

Table 2. Amphibians and reptiles of the world reported to be hosts of *Cylindrotaenia americana* Jewell, 1916.

Host	Locality	Reference
Amphibia		
Caudata		
Ambystomatidae		
Ambystoma texanum	Texas	McAllister and Upton, 1987b
Plethodontidae		
Desmognathus fuscus	North Carolina	Mann, 1932
	New York	Fischthal, 1955
D. monticola	Tennessee	Dunbar and Moore, 1979
	North Carolina	Goater et al., 1987
D. quadramaculata	North Carolina	Goater et al., 1987
D. ochrophaeus	Tennessee	Dunbar and Moore, 1979
	North Carolina	Goater et al., 1987
Plethodon cinereus	Tennessee	Dunbar and Moore, 1979
P. glutinosus	Tennessee	Dunbar and Moore, 1979
P. jordani	North Carolina	Dyer, 1983
P. richmondi	Tennessee	Dunbar and Moore, 1979
Anura		
Pelobatidae		
Scaphiopus multiplicatus	Mexico	Walton, 1940
Leptodactylidae		
Leptodactylus ocellatus	Argentina, Brazil	Savazzini, 1929
Hylidae		
Acris crepitans	Illinois, Michigan	Jewell, 1916
	Nebraska	Brooks, 1976a
	Iowa	Ulmer and James, 1976
	Texas	McAllister and Upton, 1987a
A. gryllus	Texas	Harwood, 1932
	Oklahoma	Trowbridge and Hefley, 1934
Hyla arborea	Czechoslovakia	Prokopic, 1957
H. arenicolor	Utah	Parry and Grundmann, 1965
H. squirella	Texas	Harwood, 1932
Pseudacris clarkii	Texas	McAllister, this study
P. triseriata	Texas	Harwood, 1932
Bufonidae		
Bufo americanus	Iowa	Ulmer and James, 1976
B. canorus	California	Ingles, 1936; Walton, 1941
B. compactilis	Mexico	Walton, 1940
B. ictericus	Brazil	Stumpf, 1981/1982a, 1981/1982b
B. marinus	Colombia	Brooks, 1976b
	Ecuador	Dyer, 1986
B. microscaphus	Utah	Parry and Grundmann, 1965
B. terrestris	SE United States	Jewell, 1916
B. typhonius	Ecuador	Dyer, 1986
B. woodhousii	Virginia	Campbell, 1968
Melanophryniscus stelzneri	Uruguay	Mane-Garzon and Gonzalez, 1978
Ranidae		
Rana aurora	Oregon, Washington	Lehmann, 1965
R. catesbeiana	Massachusetts	Rankin, 1945
	Virginia	Campbell, 1968
R. pipiens	Illinois, Michigan, Nebraska	Jewell, 1916
	Oregon, Washington	Lehmann, 1965
	Iowa	Ulmer and James, 1976
R. pretiosa	Oregon, Washington	Lehmann, 1965
R. septentrionalis	Maine	Bouchard, 1951
Microhylidae		
Gastrophryne olivacea	Texas	McAllister and Upton, 1987a
Reptilia		
Sauria		
Scincidae		
Scincella lateralis	Texas	Harwood, 1932
	Florida	Brooks, 1972
Serpentes		
Colubridae		
Ptyas mucosus	Burma	Meggitt, 1931

synonymized it with *Barietta jaegerskioeldi* (Janicki, 1928) Hsü, 1935. However, Mettrick (1953) assigned Joyeux's specimens to *B. janicki* (Hilmy, 1936) Douglas, 1958 (see also Fischthal and Asres, 1970). Nevertheless, Yamaguti (1959) recognizes them as *B. jaegerskioeldi,* while Schmidt (1986) lists *Arthroleptis* as a host of *C. americana.* Dyer (1986) was apparently unaware of this confusion although he did suggest that the identity of the Joyeux's African form of *C. americana* with the American form seemed unlikely. In view of this controversy over Joyeux's material, neither of the above hosts is listed in Table 2.

Two nematodes, *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931, were each found in 2 *P. clarkii* (26 mm SVL male, ASUMZ 8691; 28 mm SVL female, uncatalogued) collected on 7 March 1987 and 18 March 1990 in Somervell and Hood counties, respectively. *Pseudacris clarkii* could represent an accidental host since other sympatric anurans (i.e., *Bufo* spp. and *G. olivacea*) have been reported to have a higher prevalence of *C. variabilis* (McAllister and Upton, 1987a; McAllister et al., 1989), whereas the more closely related *P. s. streckeri* has not been found to be infected (McAllister, 1987).

Larval intradermal mites, *Hannemania* sp. Oudemans, 1911, infested 8 (57%) *P. clarkii* from Dallas, 1 (8%) from Hood, and 2 (15%) from Somervell counties. It is not known why there is such a great disparity in prevalence among the 3 localities. Unengorged or partially engorged larvae were encapsulated by host dermal connective tissue. The majority of capsules was found on the undersides of legs, on the venters, and near the cloacal openings of frogs. Because only larvae were found, it was not possible to determine specific identity. However, *H. multifemorala* Loomis, 1956, has been reported previously from neighboring Erath County on Great Plains narrowmouth toads, *Gastrophryne olivacea, H. dunni* Sambon, 1928, is known from eastern Texas on dusky salamanders, *Desmognathus auricularus* and *G. olivacea,* and *H. eltoni* (syn. *H. penetrans* Ewing, 1931) has been reported from Bexar County on southern leopard frogs, *Rana sphenocephala* (Loomis, 1956). In addition, Kuntz and Self (1944) reported *H. eltoni* on *G. olivacea* from Comanche County, Oklahoma, and Duszynski and Jones (1973) reported 9 species of frogs and toads from New Mexico harboring *Hannemania* sp.

In conclusion, most of the protozoan parasites of *P. clarkii* are shared with sympatric *P. s. streckeri,* while neither the helminth nor arthropod parasites are shared. Furthermore, a common helminth of the region in other anurans (*Cylindrotaenia americana*) is reported to have a low prevalence in *P. clarkii* but apparently is not harbored by *P. s. streckeri.* An ecological explanation for these comparative data is elusive at present; however, it is similar to that reported by Upton and McAllister (1988) who noted that several *Eimeria* spp. infect *P. s. streckeri* but not *P. clarkii* from the same aquatic environment.

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Lists of Larval Worms from Marine Invertebrates of the Pacific Coast of North America

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ABSTRACT: Immature stages of 73 digenetic trematodes are listed by their families, marine invertebrate hosts, and localities and then cross listed according to their molluscan hosts. The list contains many new host records, and larval stages of 15 digenetic trematodes were newly recorded from gastropods from British Columbia and California. A list of immature stages of 14 cestodes, acanthocephalans, and nematodes is also included.

KEY WORDS: Digenea, Cestoda, Acanthocephala, Nematoda, marine mollusks, invertebrate hosts, Pacific coast, North America.

Although immature stages are recognized to be as important as adults in the descriptions of parasites, few studies focus on larval helminths in marine invertebrates. Stunkard (1983) pointed out the difficulties in conducting studies on marine parasites but the necessity to do so should concern all biologists interested in making inventories of parasites and in conserving biodiversity (Pritchard, 1989). The marine ecosystem has become particularly vulnerable to physical changes which in turn can affect both host and parasite populations. My studies of larval digenetic trematodes began in 1957 at Friday Harbor, Washington. Since then, many parasites were found in marine invertebrates from British Columbia to California. Additional sampling was not possible, or when recent collections were attempted, snails and clams were found to be scarce due to oil pollution, or successive changes in the physical environment, or the use of lead-based paints on boats. In this paper I provide lists of larval stages of digenetic trematodes, cestodes, acanthocephalans, and nematodes along with their hosts and localities.

Methods and Materials

Snails, clams, and other invertebrates have been collected by the author at Friday Harbor, Washington (WA), and Vancouver, British Columbia (BC). Eric Cabot collected subtidal snails at Saturnina Island, BC; W. P. Sousa provided infected *Cerithidea* snails from Bolinas Lagoon, California (CA), and G. M. Ruiz gave specimens from clams from Bodega and San Francisco bays, CA. Mollusks were isolated to find free-swimming parasites and then dissected to obtain larval stages. The parasites were studied alive with the use of vital dyes. For permanent preparations, they were usually fixed in hot seawater, transferred to alcohol–formalin–acetic acid and stored in 70% ethanol. They were stained with Semichon's carmine and mounted in permount. Most of the measurements were made on preserved specimens. Measurements are given in micrometers unless otherwise indicated with the mean in parentheses. Drawings were made with the aid of a camera lucida.

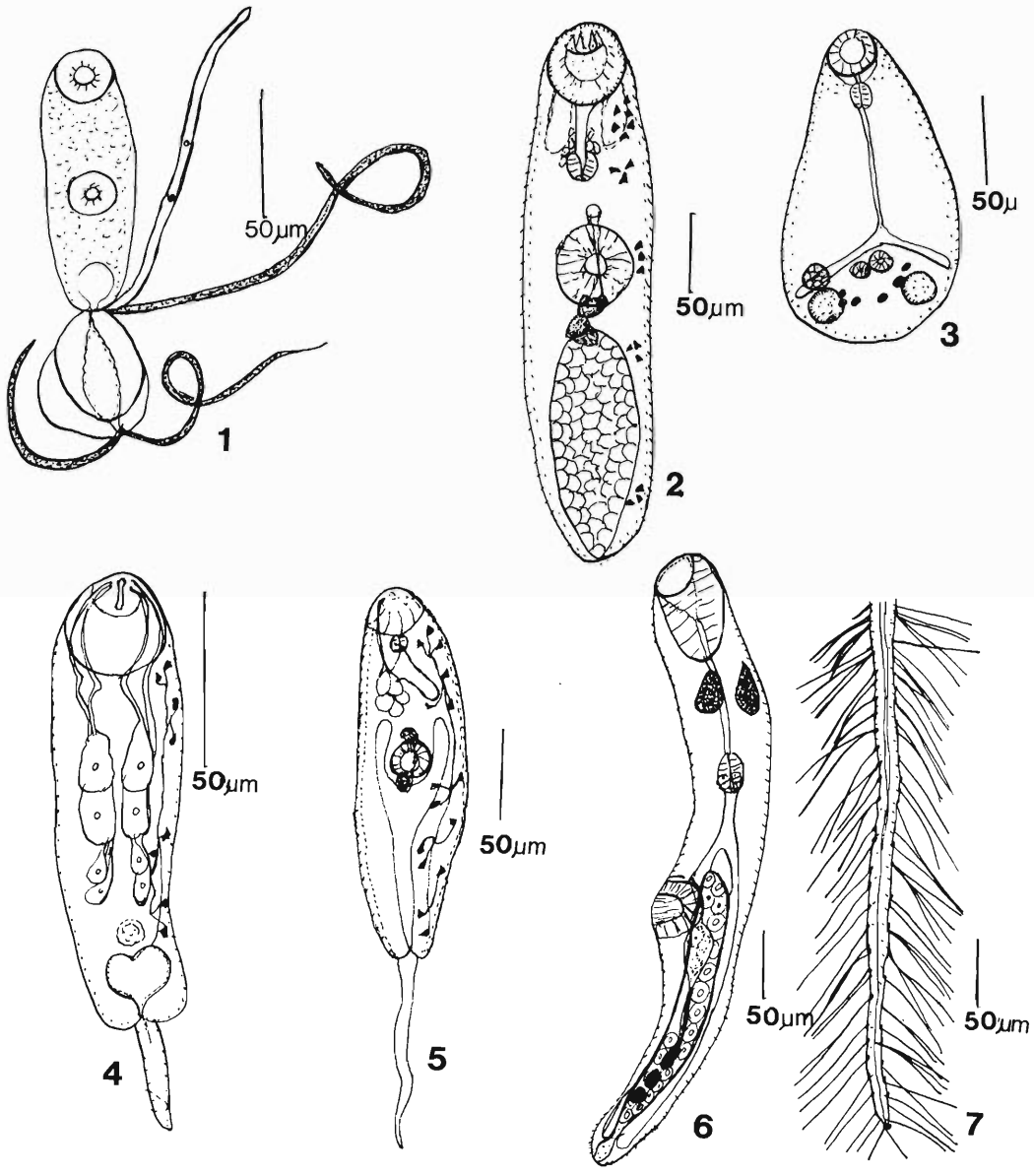
Stages that I have reported for the first time are designated with *mihi*. Host and distribution records not previously published are indicated in the list with asterisks. The names of the molluscan hosts and common names when given are cited according to Abbott (1974) and those of other invertebrates are cited according to Austin (1985). Accession numbers are given for specimens in the United States National Museum Helminthological Collections.

List 1. Larval Digeneans from Marine Invertebrates from the North American Pacific Coast

Class TREMATODA
Subclass DIGENEA
Order STRIGEIFORMES

Family CYATHOCOTYLIDAE

1. *Mesostephanus appendiculatus* (Ciurea, 1916) Lutz, 1935
   Stage: Furcocercous cercaria
   Location: Sporocyst in mantle
   Host: *Cerithidea californica* Haldeman, California Horn Shell
   Localities: Upper Newport Bay, Bolinas Lagoon, CA
   Records: Martin (1961, 1972), Ching and Sousa, 1986*
   USNM Helm. Coll. No. 81066
2. Cyathocotylid species
   Stage: Furcocercous cercaria
   Location: Sporocyst in digestive gland
   Host: *Cerithidea californica*
   Localities: Upper Newport Bay, Bolinas Lagoon, CA



Figures 1–7. Marine digeneans from gastropod hosts. 1. Hemiuroid cercaria from *Alvania compacta*. 2. Enenterid cercaria from *A. compacta*. 3. Metacercaria of *Microphallus* sp. from *A. compacta*. 4. Microphallid cercaria from *Littorina scutulata*. 5. Renicolid cercaria from *Cerithidea californica*. 6. Body of lepocreadiid cercaria from *Nitidella carinata*. 7. Tail of lepocreadiid cercaria.

Records: Martin (1972), Ching and Sousa, 1986*
USNM Helm. Coll. No. 81067

Family SCHISTOSOMATIDAE

3. *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953
Stage: Furcocercous cercaria
Location: Sporocyst in gonads
Host: *Ilyanassa obsoleta* (Say), Eastern Mud Nassa
Locality: Vancouver, BC
Record: Ching, 1964*, identification according to Stunkard and Hinchliffe (1952)

4. *Austrobilharzia* sp.
Stage: Furcocercous cercaria
Location: Sporocyst in digestive gland
Host: *Cerithidea californica*
Locality: Upper Newport Bay, CA
Record: Martin (1972)

5. *Cercaria littorinalinae* Penner, 1950
   Stage: Furcocercous cercaria
   Location: Not mentioned, sporocyst in digestive gland?
   Host: *Littorina planaxis* Philippi, Eroded Periwinkle
   Locality: Southern California
   Record: Penner (1950)

Order HEMIURIFORMES

Family HEMIURIDAE

6. *Cercaria B* Miller, 1925
   Stage: Cystophorous cercaria
   Location: Redia in digestive gland
   Host: *Nucella lamellosa* (Gmelin), Frilled Dogwinkle
   Locality: Friday Harbor, WA
   Records: Miller (1925), Ching (1960)
7. *Cercaria C* Ching, 1960
   Stage: Cystophorous cercaria
   Location: Redia in digestive gland
   Host: *Dentalium dalli* Pilsbry and Sharp
   Locality: Friday Harbor, WA
   Record: Ching (1960)
8. Hemiuroid Metacercaria
   Stage: Metacercaria
   Location: Hemocoel
   Host: *Mesamphiascus parvus*
   Locality: Friday Harbor, WA
   Record: Ching (1960). The generic name of the host is not listed by Austin (1985), and the copepod may be *Amphiaccus parvus.*
9. Hemiuroid Metacercaria
   Stage: Metacercaria
   Location: Unknown
   Host: *Bolinopsis microptera*
   Locality: Puget Sound, WA
   Record: Lloyd (1938). *Bolinopsis microptera* is a synonym of the ctenophore, *B. infundibulum* (Muller), according to Austin (1985).
10. Hemiuroid Cercaria *mihi 1* (Fig. 1)
    Stage: Cystophorous cercaria
    Location: Redia in digestive gland
    Host: *Alvania compacta* Carpenter, Compact Alvania
    Locality: Saturnina I., BC
    Record: Ching, 1977*
    USNM Helm. Coll. No. 81068
    Notes: The prevalence was 3 of 487 of the subtidal snails. The rediae are elongate about 1 mm in length. The cercaria has a delivery tube and 3 appendages.
11. *Lecithaster salmonis* Yamaguti, 1934
    Stage: Cystophorous cercaria
    Location: Redia in digestive gland
    Hosts: *Nucella canaliculata* Duclos, Channeled Dogwinkle; *N. emarginata* (Deshayes), Emarginate Dogwinkle
    Localities and records: Friday Harbor, WA, by Miller as *Cercaria A* (1925) and Ching (1960); BC, by Boyce (1966) as *L. gibbosus.*

Family BUCEPHALIDAE

12. *Cercaria noblei* Giles, 1962
    Stage: Furcocercous cercaria
    Location: Sporocyst in digestive gland
    Host: *Mytilus californianus* Conrad, Californian Mussel
    Locality: Dillon Beach, CA
    Record: Giles (1962)
13. Bucephalid Cercaria *mihi 2*
    Stage: Furcocercous cercaria
    Location: Sporocyst in digestive gland
    Host: *Lyonsia* sp.
    Locality: Friday Harbor, WA
    Record: Ching, 1958*
    Note: The cercaria and host species are similar to those of *Rhipidocotyle* spp. described by Stunkard (1976).

Family FELLODISTOMIDAE

14. *Pronoprymna petrowi* (Layman, 1930) Bray and Gibson, 1980
    Stage: Trichocercous cercaria
    Location: Sporocyst in digestive gland
    Host: *Macoma inconspicua* (Broderip and Sowerby), Inconspicuous Macoma
    Locality: Vancouver, BC
    Records: Ching, 1963*, 1974*, 1989*
    USNM Helm. Coll. No. 81069
    Notes: The prevalence in 1963 was 2.7% or 27 of 1,138 clams. The cercaria resembled young forms found in smelt hosts in the arrangement and development of the gonads (Margolis and Ching, 1965).
15. Fellodistomid Metacercaria *mihi 3*
    Stage: Metacercaria
    Location: Digestive gland
    Host: *Tapes philippinarum* (Adams and Reeve), Japanese Littleneck
    Locality: Vancouver I., BC
    Record: Ching, 1987*
    Notes: About 150 clams from 15 areas were sampled with 1–2 metacercariae found in 7 of 15 clams in 1 area.

Family GYMNOPHALLIDAE

16. *Cercaria margaritensis* Ching, 1982
    Stage: Furcocercous germinal sac
    Location: Germinal sac in extrapallial cavity
    Hosts: *Margarites costalis* (Gould), Northern Rosy Margarite; *M. helicinus* (Phipps), Helicina Margarite; *M. pupillus* (Gould), Puppet Margarite
    Locality: Saturnina I., BC
    Record: Ching (1982)
    USNM Helm. Coll. No. 81070
17. *Gymnophallus deliciosus* (Olsson, 1893) Odhner, 1900
    Stage: Metacercaria
    Location: Extrapallial cavity
    Host: *Mytilus edulis* L., Blue Mussel
    Locality: Vancouver, BC
    Record: Ching, 1963*, identified from Stunkard and Uzmann (1958)
    USNM Helm. Coll. No. 81071
18. *Gymnophallus somateriae* (Levinson, 1881) Odhner, 1900
    Stage: Furcocercous cercaria
    Location: Sporocyst in digestive gland
    Stage: Metacercaria
    Location: Extrapallial cavity

Host for 2 stages: *Macoma inconspicua*
Locality: Vancouver, BC
Records: Ching (1973), 1963*, 1976* for cercaria
USNM Helm. Coll. Nos. 81072, 81073

19. *Gymnophallus* sp.
Stage: Metacercaria
Location: Extrapallial cavity
Hosts: *Cryptomya californica* (Conrad), California Glass Mya; *Mya arenaria* (L.), Soft-shell Clam
Record: Ching, 1962*, identified from Stunkard and Uzmann (1958)
USNM Coll. No. 81074

20. *Lacunovermis conspicuus* Ching, 1965
Stage: Furcocercous cercaria
Location: Sporocyst in digestive gland
Stage: Metacercaria
Host for 2 stages: *Macoma inconspicua*
Locality: Vancouver, BC
Record: Ching (1965a)

21. *Meiogymnophallus multigemmulus* Ching, 1965
Stage: Metacercaria
Location: Sporocyst in digestive gland
Host: *Macoma inconspicua*
Locality: Vancouver, BC
Record: Ching (1965a)

22. *Parvatrema borealis* Stunkard and Uzmann, 1958
Stage: Furcocercous cercaria
Location: Sporocyst in digestive gland
Stage: Metacercaria
Location: Extrapallial cavity
Hosts for 2 stages: *Nuticola tantilla* (Gould), Tantilla Transennella; *Gemma gemma* (Totten), Amethyst Gem Clam
Localities and records: Friday Harbor, WA, by Ching, 1959*; San Francisco Bay, CA, by Oglesby (1965); Bodega Bay, CA, by Ching and Ruiz, 1986*
USNM Helm. Coll. No. 81075

23. *Parvatrema obscurus* (Ching, 1960) James, 1964
Stage: Metacercaria
Location: Digestive gland
Hosts: "*Collisella*" *scabra* (Gould), Rough Limpet; *Lottia digitalis* (Rathke), Fingered Limpet
Localities: Bodega Bay to San Nicholas I., CA
Record: Ching and Grosholz (1988)
USNM Helm. Coll. No. 79923

24. Gymnophallid Metacercaria
Stage: Metacercaria
Location: Gills, mantle
Host: *Crassostrea gigas* (Thunberg), Giant Pacific Oyster
Localities: Broughton I., Fanny Bay, Vancouver I., BC
Record: Bower, 1987, pers. comm., Pacific Biological Station, Nanaimo, BC

Order PLAGIORCHIIFORMES

Family ACANTHOCOLPIDAE

25. *Cercaria foliatae* Miller, 1925
Stage: Oculate cercaria
Location: Sporocyst in digestive gland
Host: *Ceratostoma foliatum* (Gmelin), Foliated Thorn Purpura
Locality: Friday Harbor, WA
Record: Miller (1925). This cercaria is placed in this family because of its resemblance to the cercaria of *Stephanostomum baccatum* described by Wolfgang (1955) and the close relationship of the hosts.

Family ENENTERIDAE

26. Enenterid Cercaria *mihi 4* (Fig. 2)
Stage: Tail-less cercaria
Location: Redia in digestive gland
Host: *Alvania compacta*
Locality: Saturnina I., BC
Record: Ching, 1978*
USNM Helm. Coll. No. 81076
Notes: A single infection was found in 487 snails. The cercaria appears to be similar to that of *Neophasis lagenformis* as described by Koie (1973). Some opecoelid-like genera with body spination including *Neophasis* have been placed in this family by Gibson and Bray (1982).

Family MICROPHALLIDAE

Tribe MARITREMATIDI

27. *Maritrema gratiosum* Nicoll, 1907
Stage: Metacercaria
Location: Hemocoel
Host: *Balanus glandula* Darwin
Locality: Vancouver, BC
Record: Ching (1978)

28. *Maritrema laricola* Ching, 1962
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Hosts: *Littorina scutulata* Gould, Checkered Periwinkle; *L. sitkana* Philippi, Sitka Periwinkle
Stage: Metacercaria
Location: Digestive gland
Hosts: *Hemigrapsus nudus* (Dana), *H. oregonensis* (Dana)
Localities: Vancouver, BC; Bodega Bay, CA
Records: Ching (1962b), Ching, 1986*

29. *Maritrema megametrios* Deblock and Rausch, 1968
Stage: Metacercaria
Location: Hemocoel
Hosts: *Telorchestia traskiana* (Simpson), *Gnorimosphaeroma oregonense* Dana
Locality: Vancouver, BC
Record: Ching (1974)
USNM Helm. Coll. No. 81077

30. *Maritrema pacifica* Ching, 1974
Stage: Metacercaria
Location: Hemocoel
Host: *Megalorchestia corniculata* (Stout)
Locality: Santa Barbara, CA
Record: Ching (1974)
USNM Helm. Coll. No. 81078

31. *Probolocoryphe uca* (Sarkisian, 1957) Yamaguti, 1971
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Cerithidea californica*
Stage: Metacercaria
Location: Muscles
Host: *Uca crenulata* (Lockington)
Localities: Upper Newport Bay, Bolinas Lagoon, CA

Records: Maxon and Pequegnat (1949), Sarkisian (1957), Martin (1972) as the larger of 2 stylet cercariae, Sousa (1983) as a microphallid cercaria, Ching and Sousa for cercarial stage, 1986*
Cercaria, USNM Helm. Coll. No. 81079

32. *Pseudospelotrema japonicum* Yamaguti, 1939
Stage: Metacercaria
Location: Thoracic ganglion
Host: *Cancer magister* Dana
Localities: Willapa Bay, WA; Vancouver, BC
Records: Sparks and Hibbert (1981), identified by Ching, 1988*

Tribe MICROPHALLIDI

33. *Ascorhytis charadriformis* (Young, 1949) Ching, 1965
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Hosts: *Littorina scutulata, L. sitkana*
Stage: Metacercaria
Hosts: *Hemigrapsus oregonensis, H. nudus*
Location: Thoracic ganglion
Localities: Bodega Bay, CA to Vancouver, BC
Records: Ching (1962c, 1965b)

34. *Microphallus nicolli* (Cable and Hunninen, 1940) Baer, 1943
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Olivella plicata* (Sowerby), Purple Dwarf Olive
Stage: Metacercaria
Location: Hemocoel
Host: *Emerita analoga* (Stimpson)
Locality: Santa Barbara, CA
Records: Young (1938). Yoshino (1975) reported this species in *Cerithidea californica* but the cercaria in this host is *Probolocoryphe uca* according to Sarkisian (1957).

35. *Microphallus pirum* (Afanassew, 1941) Belopolskaia, 1952
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Nucella emarginata*
Stage: Metacercaria
Location: Abdomen
Hosts: *Pagurus hirsutiusculus* (Dana), *Telmessus cheiragonus* (Tilsius)
Locality: Alaska
Records: Schiller (1954, 1959)

36. *Microphallus pygmaeus* (Levinsen, 1881) Baer, 1943
Stage: Metacercaria
Location: Sporocyst in digestive gland
Hosts: *Littorina scutulata, L. sitkana*
Localities: Friday Harbor, WA; Vancouver, BC
Record: Ching (1961)
USNM Helm. Coll. No. 81080

37. *Microphallus similis* (Jagerskiold, 1900) Baer, 1943
Stage: Metacercaria
Location: Digestive gland
Host: *Cancer magister*
Locality: Vancouver, BC
Record: Ching, 1988*; many specimens from avian hosts have been recovered

38. *Microphallus* sp. *mihi 5* (Fig. 3)
Stage: Metacercaria
Location: Sporocyst in digestive gland
Host: *Alvania compacta* Carpenter, Compact Alvania
Locality: Saturnina I., BC
Record: Ching, 1977*
USNM Helm. Coll. No. 81081
Notes: The prevalence was 5.4% or 26 of 487 snails. Measurements on 10 metacercariae: Body length by width, 152–180 by 76–112 (168 by 90). Oral sucker diameter 26–30 (30), ventral sucker diameter 16–20 (18). Pharynx as large as ventral sucker, male papilla diameter, 8–12 (10).

39. Microphallid Cercaria *mihi 6*
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Lacuna marmorata* Dall
Locality: Friday Harbor, WA
Record: Ching, 1959*. Three of 6 snails were infected.

40. Microphallid Cercaria *mihi 7*
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Trichotropis cancellata* (Hinds), Cancellate Hairy-shell
Locality: Friday Harbor, WA
Record: Ching, 1959*. Two of 108 snails were infected.

41. Microphallid Cercaria *mihi 8* (Fig. 4)
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Littorina scutulata*
Locality: Vancouver, BC
Record: Ching, 1974*
Notes: I have found only 2 infections in many years of sampling littorine snails. Measurements on 10: Body length by width, 120–171 by 26–42 (132 by 37). Tail short, used for creeping, 26–39 by 5–9 (37 by 7).

Family OPECOELIDAE

42. *Cercaria searlesiae* Miller, 1925
Stage: Cotylocercous cercaria
Location: Redia in digestive gland
Host: *Searlesia dira* (Reeve)
Locality: Friday Harbor, WA
Record: Miller (1925)

43. Metacercaria A Thompson and Margolis, 1987
Stage: Metacercaria
Location: Somatic musculature
Host: *Pandalus jordani* Rathburn, Smooth Pink Shrimp
Locality: Vancouver I., BC
Record: Thompson and Margolis (1987)

44. *Neolebouria tinkerbelli* Thompson and Margolis, 1987
Stage: Metacercaria
Location: Heart
Host: *Pandalis jordani*
Locality: Vancouver I., BC
Record: Thompson and Margolis (1987)

45. *Podocotyle enophrysi* Park, 1937
Stage: Cotylocercous cercaria
Location: Redia in digestive gland

Host: *Lacuna marmorata* Dall
Locality: Saturnina I., BC
Record: Ching (1979)

46. Opecoelid Cercaria *mihi 9*
Stage: Stumpy-tailed cercaria
Location: Sporocyst in digestive gland
Hosts: *Margarites* sp., *M. helicinus*
Localities: Friday Harbor, WA; Saturnina I., BC
Records: Ching, 1957*, 1977*
Note: The cercaria has a short tail with 5–6 glands forming a striated appearance at the tip which is unlike the tails of the cotylocercous cercariae listed above.

47. Opecoelid Metacercaria
Stage: Metacercaria
Location: Hemocoel
Host: *Traskorchestia traskiana*
Locality: Vancouver, BC
Record: Ching, 1977*

48. Opecoelid Metacercaria
Stage: Metacercaria
Location: Abdominal muscles
Host: *Crangon alaskensis* Lockington
Locality: Sinclair Inlet, WA
Record: Morado and Sparks (1983). I have placed this metacercaria in the family Opecoelidae.

Family MONORCHIIDAE

49. *Telolecithus pugetensis* Lloyd and Guberlet, 1932
Stage: Brevifurcate cercaria
Location: Sporocyst in gonads
Host: *Nutricola tantilla* (Gould)
Stage: Metacercaria
Location: Extrapallial cavity
Hosts: *N. tantilla*; *Macoma nasuta,* Conrad Bentnose Macoma; *Tellina nuculoides* (Reeve), Salmon Tellin
Localities: Yaquina Bay, OR; Friday Harbor, WA
Record: DeMartini and Pratt (1964)

Family RENICOLIDAE

50. *Renicola buchanani* Martin and Gregory, 1951
Stage: Magnacercous cercaria
Location: Sporocyst in mantle
Host: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin and Gregory (1951), Martin (1955), Yoshino (1975) as *Renicola* sp., Ching and Sousa, 1986*
USNM Helm. Coll. No. 81082

51. *Renicola cerithidicola* Martin, 1971
Stage: Gymnocephalous cercaria
Location: Sporocyst in digestive gland
Host: *Cerithidea californica*
Record: Martin (1971)

52. *Renicola thaidus* Stunkard, 1964
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Nucella lamellosa*
Stage: Metacercaria
Location: Digestive gland
Host: *Mytilus edulis*
Locality: Vancouver, BC
Records: Ching, 1957*, 1961*, 1969*, Stunkard (1964)

53. Renicolid Cercaria *mihi 10* (Fig. 5)
Stage: Xiphidiocercaria
Location: Sporocyst in digestive gland
Host: *Cerithidea californica*
Locality: Bolinas Lagoon, CA
Record: Ching and Sousa, 1986*
USNM Helm. Coll. No. 81083
Notes: Sporocyst with yellow pigment, sparsely filled with 2–14 (8) cercariae, 676 by 298 in length and width. Cercarial body finely spined, 179–230 by 58–78 (199 by 69). Suckers equal in size, about 30 in diameter. Short stylet present. Excretory bladder U-shaped with long stem, arms lateral to ventral sucker. Flame cells 2[(3 + 3 + 3) + (3 + 3 + 3)]. Tail length by width, 110–150 by 13–16 (133 by 15).

Order LEPOCREADIIFORMES

Family LEPOCREADIIDAE

54. Lepocreadiid Cercaria *mihi 11* (Figs. 6, 7)
Stage: Trichocercous cercaria
Location: Redia in digestive gland
Host: *Nitidella carinata* (Hinds), Carinate Doveshell
Locality: Saturnina I., BC
Record: Ching, 1979*
USNM Helm. Coll. No. 81084
Notes: The prevalence was 5% or 4 of 73 snails. The cercaria has a trumpet-shaped oral sucker, prominent eye spots, and excretory bladder reaching to the ventral sucker. The tail has 36 pairs of setae.

Order ECHINOSTOMATIFORMES

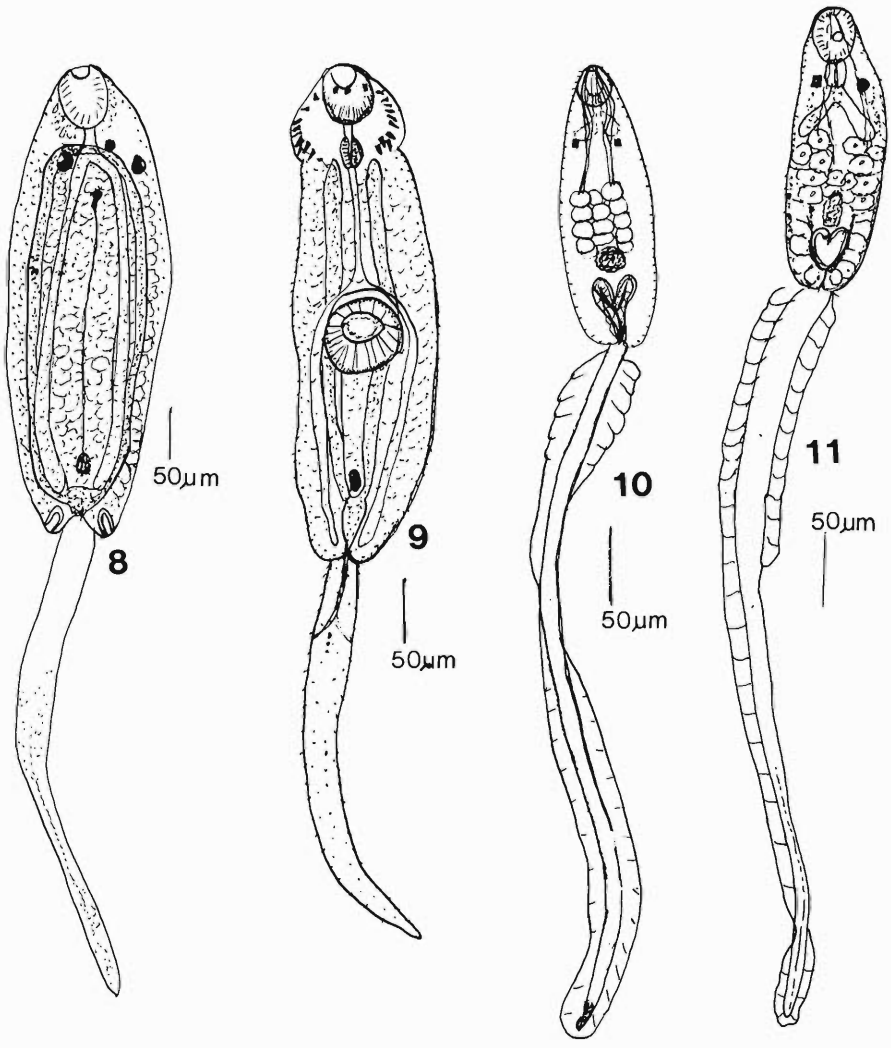
Family NOTOCOTYLIDAE

55. *Catatropis johnstoni* Martin, 1956
Stage: Oculate, monostome cercaria
Location: Redia in digestive gland
Host: *Cerithidea californica*
Locality: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin (1955, 1956), Yoshino (1975), Sousa (1983)

56. Notocotylid Cercaria *mihi 12* (Fig. 8)
Stage: Oculate, monostome cercaria
Location: Redia in digestive gland
Host: *Littorina scutulata*
Locality: Vancouver, BC
Records: Ching (1962a), 1977*
USNM Helm. Coll. No. 81085
Notes: The prevalence was 0.49% or 21 of 4,322 snails sampled in 1977–1978 from 3 locations in Vancouver. Measurements on 10 cercariae: Body length by width, 330–595 by 145–244 (419 by 216). Tail length by width, 376–508 by 33–66 (422 by 43). Three eye spots present.

Family ECHINOSTOMATIDAE

57. *Acanthoparyphium spinulosum* Johnston, 1917
Stage: Cercaria with collar spines
Location: Redia in gonads
Host: *Cerithidea californica*
Stage: Metacercaria



Figures 8–11. Marine digeneans from gastropod hosts. 8. Notocotylid cercaria from *Littorina scutulata*. 9. Echinostomatid cercaria of *Himasthla* sp. from *L. scutulata*. 10. Heterophyid cercaria from *Batillaria zonalis*. 11. Heterophyid cercaria from *Bittium escherichtii*.

Location: Radular pockets
Host: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin and Adams (1960, 1961), Yoshino (1975), Sousa (1983), Ching and Sousa, 1986*
USNM Helm. Coll. No. 81086

58. *Acanthopharyphium* sp.
Stage: Cercaria with collar spines
Location: Redia in gonads
Host: *Cerithidea californica*
Locality: Upper Newport Bay, CA
Record: Martin (1972)

59. "*Echinoparyphium*" sp.
Stage: Cercaria with collar spines
Location: Redia in gonad, digestive gland
Host: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin (1972), Sousa (1983), Ching and Sousa, 1986*. Martin (1972) placed this echinostome in this genus, but the 31 spines around the head collar are arranged in a single ring dorsally and laterally, with 2 clustered at each side ventrally.
USNM Helm. Coll. No. 81087

60. *Himasthla rhigedana* Dietz, 1909
Stage: Oculate cercaria with collar spines
Location: Redia in digestive gland
Host: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA

Records: Adams and Martin (1960, 1963), Yoshino (1975), Sousa (1983), Ching and Sousa, 1986*
USNM Helm. Coll. No. 81088

61. *Himasthla* sp. Cercaria *mihi 13* (Fig. 9)
Stage: Cercaria with collar spines
Location: Redia in digestive gland
Host: *Littorina scutulata*
Localities: Friday Harbor, WA; Vancouver, BC
Records: Ching (1962a as echinostome I), 1975–1978*, 1988*
USNM Helm. Coll. No. 81089
Notes: The prevalence was 2.5% or 21 of 835 snails at Vancouver and Friday Harbor from 1975 to 1978. At 1 lagoon site at Friday Harbor in 1988, 11% or 23 of 207 snails were infected. Cercarial body spined, with head collar of 29 spines in single continuous row, with 2 ventrally in each corner. Body length by width, 352–454 by 123–217 (400 by 158). Oral sucker 51–59 by 51–56 (57 by 52), ventral sucker, 64–70 by 66–102 (75 by 82). Tail length by width, 237–409 by 33–57 (319 by 41).

62. Echinostomatid Metacercaria
Stage: Metacercaria with collar spines
Location: Gut, mantle, base of gills
Host: *Protothaca staminea* (Conrad), Common Pacific Littleneck
Locality: Sooke, BC
Record: Bower, 1986* pers. comm., Pacific Biological Station, Nanaimo, BC

Family PHILOPHTHALMIDAE

63. *Cloacitrema michiganensis* McIntosh, 1938
Stage: Megalurous cercaria
Host: *Cerithidea californica*
Locality: Upper Newport Bay, CA
Records: Robinson (1952), Martin (1955, 1972), LeFlore et al. (1985)

64. *Parorchis acanthus* (Nicoll, 1906) Nicoll, 1907
Stage: Megalurous cercaria
Location: Redia in digestive gland
Host 1: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin (1955, 1972), Yoshino (1975), Sousa (1983), Ching and Sousa, 1986*
USNM Helm. Coll. No. 81090
Host 2: *Nucella lamellosa, N. emarginata*
Locality: Thetis I., BC
Record: Ching (1978)
USNM Helm. Coll. No. 81091

Family PSILOSTOMIDAE

65. *Psilostomum magniovum* Ching, 1980
Stage: Cercaria with caudal fin fold
Location: Redia in digestive gland
Host: *Littorina scutulata*
Stage: Metacercaria
Location: Extrapallial cavity
Host: *Mytilus edulis* L., Blue Mussel
Locality: Vancouver, BC
Record: Ching (1980)

Order OPISTHORCHIIFORMES

Family HETEROPHYIDAE

66. *Cercaria purpuracauda* Miller, 1925
Stage: Magnacercous cercaria
Location: Redia in digestive gland
Host: *Bittium escherichtii* (Middendorf), Giant Pacific Coast Bittium
Locality: Friday Harbor, WA
Record: Miller (1925)

67. *Cryptocotyle lingua* (Creplin, 1825) Fischoeder, 1903
Stage: Pleurolophocercous cercaria
Location: Redia in gonads
Host: *Littorina scutulata*
Locality: Vancouver, BC
Record: Ching (1978)
USNM Helm. Coll. No. 81092

68. *Euhaplorchis californiensis* Martin, 1950
Stage: Pleurolophocercous cercaria
Location: Gonad (redial stage in digestive gland)
Host: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin (1950a, 1955), Yoshino (1975), Sousa (1983), Ching and Sousa, 1986*
USNM Helm. Coll. No. 81093

69. *Parastictodora hancocki* Martin, 1950
Stage: Pleurolophocercous cercaria
Location: Redia in gonads
Host: *Cerithidea californica*
Locality: Upper Newport Bay, CA
Records: Martin (1950b, 1955)

70. *Phocitremoides ovale* Martin, 1950
Stage: Pleurolophocercous cercaria
Location: Redia in gonads
Host: *Cerithidea californica*
Localities: Upper Newport Bay, Bolinas Lagoon, CA
Records: Martin (1950c), Ching and Sousa, 1986*
USNM Helm. Coll. No. 81094

71. *Pygidiopsoides spindalis* Martin, 1951
Stage: Pleurolophocercous cercaria
Location: Redia in gonads
Host: *Cerithidea californica*
Locality: Upper Newport Bay, CA
Record: Martin (1951)

72. Heterophyid Cercaria *mihi 14* (Fig. 10)
Stage: Pleurolophocercous cercaria
Location: Redia in gonads
Host: *Batillaria zonalis* (Bruguiere) Cumings, False Cerith
Locality: Vancouver, BC
Records: Ching, 1978*, 1989*
USNM Helm. Coll. No. 81095
Notes: The prevalence was 10% or 8 of 77 in 1978 and 22% or 12 of 54 in 1989 in 2 sites in BC. Measurements of 10 cercariae: Body finely-spined, length by width, 139–211 by 66–99 (184 by 80). Tail with finfold ending in small fan around the tip, length by width, 297–475 by 17–36 (366 by 27). Oral sucker with subterminal opening with glandular openings, crenulated walls, 25–46 by 31–37 (31 by 36). Eyespots al-

most square. Penetration glands 14 in total. A pleurolophocercous cercaria reported from this host in California by Whitlatch (1974) may be the same species.

73. Heterophyid Cercaria *mihi 15* (Fig. 11)
Stage: Pleurolophocercous cercaria
Location: Redia in gonads
Host: *Bittium escherichtii*
Locality: Vancouver, BC
Record: Ching, 1974*
USNM Helm. Coll. No. 81096
Notes: The prevalence was 9.6% or 5 of 52 snails. Measurements on 9 cercariae: Body length by width, 164–188 by 60–66; tail with short anterior folds, fin folds continuing around bent tip, length by width, 248–409 by 25–30. Oral sucker with 8 spines on the dorsal edge followed by 5 tubular spines in the opening of the mouth. Penetration glands filling body, about 16 in total.

List 2. Molluscan Hosts of Larval Digeneans of the North American Pacific Coast

(Name of mollusc: number of larval digenean in List 1)

GASTROPODA:

Alvania compacta: 10, 26, 38
Batillaria zonalis: 72
Bittium escherichtii: 66, 73
Ceratostoma foliatum: 25
Cerithidea californica: 1, 2, 4, 31, 50, 51, 53, 55, 57, 58, 59, 60, 63, 64, 68, 69, 70, 71
"*Collisella*" *scabra*: 23
Ilyanassa obsoleta: 3
Lacuna marmorata: 39, 45
Littorina planaxis: 5
Littorina scutulata: 28, 33, 36, 41, 56, 61, 65, 67
Littorina sitkana: 28, 33, 36
Lottia digitalis: 23
Margarites costalis: 16
Margarites helicinus: 16, 46
Margarites pupillus: 16
Margarites sp.: 46
Nitidella carinata: 54
Nucella canaliculata: 11
Nucella emarginata: 11, 35, 64
Nucella lamellosa: 6, 52, 64
Olivella plicata: 34
Searlesia dira: 42
Trichotropis cancellata: 40

SCAPHOPODA:

Dentalium dalli: 7

BIVALVIA:

Crassostrea gigas: 24
Cryptomya californica: 19
Gemma gemma: 22
Lyonsia sp.: 13
Macoma inconspicua: 14, 18, 20, 21
Macoma nasuta: 49
Mya arenaria: 19
Mytilus californianus: 12
Mytilus edulis: 17, 52, 65
Nuticola tantilla: 22, 49
Protothaca staminea: 62
Tapes philippinarum: 15
Tellina nuculoides: 49

List 3. Larval Tapeworms, Acanthocephalans, and Roundworms from Marine Invertebrates of the North American Pacific Coast

Class CESTODA
Order TETRAPHYLLIDEA
Family PHYLLOBOTHRIIDAE

1. *Anthobothrium* sp.
Stage: Larva
Location: Foot
Hosts: *Tresus nuttalli* (Conrad), Pacific Gaper; *Macoma nasuta*, Bent-nose Macoma; *Protothaca staminea* (Conrad), Common Pacific Littleneck
Locality: Elkhorn Slough, CA
Record: MacGinitie and MacGinitie (1968)
2. *Echeneibothrium* sp.
Stage: Plerocercoid larva
Location: Mantle, foot
Host: *Protothaca staminea*
Locality: Humboldt Bay, CA
Record: Sparks and Chew (1966)
3. *Echeneibothrium* sp.
Stage: Larva
Location: Foot
Host: *Protothaca laciniata*
Locality: Morro Bay, CA
Record: Warner and Katkansky (1969a)
4. *Echeneibothrium* sp.
Stage: Smaller larva
Location: Peripheral and mantle area
Host: *Protothaca staminea*
Locality: Humboldt Bay, CA
Record: Warner and Katkansky (1969b)
5. *Echeneibothrium* sp.
Stage: Larger larva
Location: Viscera
Host: *Protothaca staminea*
Locality: Humboldt Bay, CA
Record: Warner and Katkansky (1969b)

Order TETRARHYNCHIDEA

Family TETRARHYNCHIDAE

6. *Tetrarhynchus* sp. 1 and 2
   Stage: Larvae
   Location: Muscle
   Host: *Dosidicus gigas*
   Locality: Newport Bay, CA
   Record: MacGinitie and MacGinitie (1968)

Order CYCLOPHYLLIDEA

Family HYMENOLEPIDAE

7. Hymenolepid Cysticercoid *mihi 16*
   Stage: Cysticercoid
   Location: Hemocoel
   Host: *Traskorchestia traskiana*
   Locality: Newport Bay, OR
   Record: Ching, 1974*

Phylum ACANTHOCEPHALA
Order PALAEACANTHOCEPHALA

Family POLYMORPHIDAE

1. *Polymorphus kenti* Van Cleave, 1947
   Stage: Cystacanth
   Location: Hemocoel
   Host: *Emerita analoga* (Stimpson)
   Locality: Oregon
   Record: Reish (1950)
2. *Profilicollis botulus* (Van Cleave, 1916) Meyer, 1913
   Stage: Cystacanth
   Location: Rectal ceca
   Host: *Hemigrapsus oregonensis*
   Localities: Bodega Bay, CA to Vancouver, BC
   Record: Ching (1989)
3. Unidentified Cystacanth
   Stage: Cystacanth
   Location: Midgut
   Host: *Paralithodes camtschaticus* (Tilesius)
   Locality: Alaska
   Record: Sparks (1987)

Phylum NEMATODA
Order ASCARIDIDA

Family ANISAKIDAE

1. *Contracaecum* sp.
   Stages: Juvenile and adult
   Location: Hemocoel
   Host: *Pandalis borealis*
   Locality: Open ocean, BC
   Record: Margolis and Butler (1954)

Order SPIRURIDA

Family GNATHOSTOMATIDAE

2. *Echinocephalus pseudouncinatus* Millemann, 1963
   Stage: Juvenile
   Location: Foot
   Host: *Haliotis corrugata* Wood
   Locality: San Clemente I., CA
   Record: Millemann (1963)
   Host: *Centrostephanus coronatus*
   Location: Gonads
   Locality: Southern CA
   Record: Pearse and Timm (1971)
   Location: Adductor muscle
   Host: *Argopecten aequisulcatus* (Carpenter)
   Locality: Baja California, Mexico
   Record: McLean (1983)

Family CYSTIDICOLIDAE

3. *Ascarophis* sp. 1 and 2
   Stage: Juveniles
   Location: Hemocoel
   Hosts: *Pagurus samuelis* (Stimpson), *P. granosimanus* (Stimpson)
   Locality: Bodega Bay, CA
   Record: Poinar and Thomas (1976)
4. *Ascarophis* sp.
   Stage: Juvenile
   Location: Hemocoel
   Hosts: *Pachycheles pubescens* Holmes, *P. rudis*, *Pugettia producta* (Randall), *Hemigrapsus oregonensis*
   Locality: Bodega Bay, CA
   Records: Poinar and Thomas (1976), Poinar and Kuris (1976)

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Light and Electron Microscopical Study of a Bacterial Parasite from the Cyst Nematode, *Heterodera glycines*

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ABSTRACT: The host range and morphology of a mycelial and endospore-forming bacterium (CNP) that parasitizes the cyst nematode, *Heterodera elachista,* differentiates it from 2 related species, *Pasteuria penetrans* and *Pasteuria thornei,* parasites of *Meloidogyne* spp. and *Pratylenchus* spp., respectively. In cross-inoculation experiments with the 3 bacteria, the CNP parasitized the nematodes *Heterodera glycines* and *Globodera rostochiensis* but not species in either *Meloidogyne* or *Pratylenchus.* The rhomboidal shape of the sporangia of *Pasteuria thornei* distinguishes this species from the other 2 bacteria, which have cup-shaped sporangia. The results of the cross-inoculation studies and differences in the fine structure of the cup-shaped sporangia suggest that CNP should be assigned to a new species within the genus *Pasteuria.*

KEY WORDS: plant-parasitic nematodes, soil bacterium, *Pasteuria* spp., soybeans, morphology, scanning electron microscopy (SEM), transmission electron microscopy (TEM).

A recent revision of the genus *Pasteuria* delineated 2 species, *Pasteuria penetrans* Starr and Sayre, 1988, sensu stricto and *Pasteuria thornei* Starr and Sayre, 1988 (Starr and Sayre, 1988; Sayre and Starr, 1989), which are parasites of the root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and the root-lesion nematode, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, respectively. Recently, another possible member of the genus *Pasteuria* was found by Nishizawa (1984) parasitizing *Heterodera elachista* Oshima, 1974. Following his initial observation, Nishizawa (1986) associated the field presence of the bacterium with a decline in the population of the cyst nematode. Confirmation of this phenomenon was noted at the Tochigi Agricultural Experiment Station where soybean breeding lines were being screened for cyst nematode resistance (Nishizawa, 1987).

The present study uses light and electron microscopy to compare the morphology of this recently discovered bacterium, which parasitizes cyst nematodes with that of *Pasteuria penetrans* and *Pasteuria thornei,* 2 species of bacteria that parasitize some species in the genera *Meloidogyne* and *Pratylenchus,* respectively. Although the nomenclature of the new bacterium will be presented at a later time, in this study the isolate that parasitizes cyst nematodes in the genera *Heterodera* and *Globodera* will be referred to as the "cyst nematode parasite" or CNP.

Materials and Methods

Terminology

The fine structure of the spore wall is used to delineate the species of *Pasteuria* taxonomically. To avoid confusion, in this study we have used the terminology of Sussman and Halvorson (1966) who described *Neurospora* spp., and that of Iterson (1988) who discussed and clarified the terminology of Gram-positive bacteria. Briefly, the following terms will be used: (1) "endospore" refers to the single asexual spore that develops within a sporangium and is surrounded by an exosporium; (2) "endospore wall" is the structure that surrounds the central protoplast or core; (3) "plasma membrane" is the innermost structure that apposes the wall; (4) "cortex" is the multilayered inner spore wall and the outer spore wall; and (5) "perispore" refers to the limiting structures that include the wall layer formed outside the exosporium.

Sources of nematodes

For laboratory bioassays, pure populations of several different species of plant-parasitic nematodes were needed. These populations had the following characteristics: (1) the second stage juveniles (J_2) were healthy, vigorous, and capable of root penetration; (2) the majority of the J_2 were the same physiological age; and

[5] Deceased 29 April 1989.

(3) the juveniles belonged to identified species and races of root-knot or soybean cyst nematodes. Populations with these characteristics were obtained from aseptic cultures of root explants (Lauritis et al., 1982, 1983a, b; Huettel and Rebois, 1985). They consisted of *Meloidogyne incognita, Pratylenchus brachyurus, Heterodera zeae* Koshy, Swarup, and Sethi, 1971, and 4 races of *Heterodera glycines* Ichinohe, 1952. The nematodes were harvested from the cultures using a modified Baermann funnel technique (Niblack and Huang, 1985). In addition to the aseptic cultures, the following nematodes were maintained in greenhouse pot cultures: *M. incognita* on tomato cultivars (cvs.), Marglobe or Tiny Tim; *M. hapla* on a strawberry cv., Sunrise; *M. javanica* on a tomato cv., Marglobe; *H. glycines* on a soybean cv., Kent; *H. zeae* on a corn cv., Iowa Chief; and *P. brachyurus* on a peanut cv., Florarunner. Details for rearing *M. incognita* and *P. brachyurus* were reported previously (Sayre and Wergin, 1977; Sayre et al., 1988). *H. glycines* was maintained in the greenhouse on either soybeans (cv. Kent) or adzuki beans, *Vigna angularis* (Willd.) Ohwi and H. Ohashi (USDA accession 416742). Cysts were extracted from the soil (Ayoub, 1980) and placed on fine-mesh nylon in a Baermann funnel. The hatching J_2 were collected from the funnel over a 2- to 4-day period. Also, examinations of soil samples from previously reported field plot studies of Nishizawa (1987) had isolated 10 additional, commonly occurring, genera or species of plant-parasitic nematodes. These nematodes, which included *Aphelenchoides* sp., *Aphelenchus* sp., *Helicotylenchus* sp., *Globodera rostochiensis, Paratylenchus* sp., *Paratrichodorus porosus, Rotylenchulus reniformis, Tylenchorhynchus* sp., *Tylenchulus semipenetrans,* and *Tylenchus* sp., were also examined for the presence of CNP sporangia on their cuticular surfaces.

Sources of the *Pasteuria* spp.

Methods for the axenic cultivation of *Pasteuria penetrans* have not been reported; however, all isolates of the bacteria were maintained on their respective nematode hosts by the senior author. The cultivation procedure of Stirling and Wachtel (1980) was used to maintain *Pasteuria penetrans* on *M. incognita* infecting tomato plants (cv. Tiny Tim). Tomato roots, which were infested with root-knot nematodes parasitized by *Pasteuria penetrans,* were air-dried and ground into a fine powder. Samples of the powder were rewetted and ground with a pestle and mortar to release the endospores. The bacteria were separated from the plant debris by filtering the suspension through a 500-mesh screen that allowed passage of the sporangia and subsequent collection by centrifugation of the *Pasteuria penetrans* endospores. The source of *Pasteuria thornei* endospores was parasitized J_2 and adults of *P. brachyurus* (Sayre et al., 1988; Starr and Sayre, 1988). The infected nematodes were extracted from the roots of peanut (cf. Florarunner) in a mist chamber (Chapman, 1957). A second source of these endospores was air-dried soil from pots containing peanut roots that were infected with nematodes parasitized by *Pasteuria thornei.* When healthy J_2 and adults of *P. brachyurus* migrated through the contaminated soil, a high proportion of the nematodes were encumbered with endospores of the bacterium.

CNP endospores were obtained from cysts of *H. glycines* that had been imported from Japan (Nishizawa, 1986). To initiate a greenhouse culture in Beltsville, the cysts were crushed in tap water. The number of endospores in this aqueous suspension was measured with a hemocytometer and adjusted to about 1,000 per ml. Juveniles of *H. glycines* (race 3), which were obtained from soybean (cv. Kent) root explant cultures, were suspended in tap water at a concentration of 100 per ml. A 5-ml suspension of J_2 was then filtered through a 47-mm diameter membrane filter (Millipore 0.3-μm pore size). The filter, which retained the J_2, was inverted onto the surface of a 3-ml endospore suspension in a 50-mm diameter Petri dish (Falcon; 1006). The dish was then shaken (50 rpm) for 24–48 hr to allow the endospores to attach to the J_2. The suspension, consisting of the J_2 and their attached endospores, was then pipetted around the roots of soybean seedlings that were grown in 40-mm diameter Cone-Tainers (Ray Leacher Cone-Tainers, Canby, Oregon 97013) containing autoclaved Norfolk loamy sand. Forty-five to 60 days later, the entire plant was removed from the container and the soil was washed from the root system. A dissecting microscope was used to examine the root system for parasitized immature females and mature cysts (Ayoub, 1980). Visual selection and removal of parasitized cysts were difficult because less than 10% of the cysts were diseased. However, the diseased females and cysts were generally smaller, duller, or more opaque than healthy cysts. The opacity resulted from bacterial and/or fungal colonizers of cysts. In addition, the diseased cysts usually sank to the bottom of the sampling dishes while the healthy cysts generally floated on the surface. Finally, the presence of the normal reproductive structures in adult females (i.e., uterus, ovaries, and eggs), or the presence of eggs in cysts, indicated that the bacterial parasite CNP was not present.

Light microscopy of CNP

Cysts parasitized by CNP were crushed and mounted on a microscope slide in a drop of tap water (Southey, 1986). Photomicrographs of CNP were taken with an automatic exposure 35-mm camera attached to a compound microscope (NIKON Microphot F-X) fitted with an interference contrast system. Negatives were obtained on Kodak Tri-X Pan film that was processed in Microdol-X. Morphometric data were obtained by measuring the images in printed enlargements of the photomicrographs.

Previous studies (Sayre and Starr, 1985; Starr and Sayre, 1988) indicated that the measurements of endospores observed by scanning electron microscopy (SEM) were smaller than those obtained by light microscopy. This difference resulted from shrinkage that occurred during fixation, dehydration, and critical-point drying procedures that are required for SEM observation. Consequently, sources of the morphometric data in the present study are designated by the instruments used to obtain the values, e.g., light microscopy data (LM), scanning electron microscopy data (SEM), and transmission electron microscopy data (TEM) (Table 1). All measurements are in micrometers (μm) unless stated otherwise.

Table 1. Comparison of *Pasteuria penetrans, Pasteuria thornei,* and the CNP from cyst nematodes. All measurements are in micrometers.

Characteristic	*Pasteuria penetrans*	*Pasteuria thornei*	CNP
Colony shape	Spherical, to clusters of elongated grapes	Small, elongate clusters	Spherical, to clusters of elongated grapes
Sporangia			
Shape	Cup-shaped	Rhomboidal	Cup-shaped
Diameter			
LM	4.5 ± 0.3	3.5 ± 0.2	5.3 ± 0.3
TEM	3.4 ± 0.2	2.4 ± 0.2	4.4 ± 0.3
Height			
LM	3.6 ± 0.3	3.1 ± 0.2	4.3 ± 0.3
TEM	2.5 ± 0.2	2.2 ± 0.2	3.1 ± 0.3
Episporic structures			
Exosporium	Present, relatively smooth surface	Present, smooth	Present, velutinous to hairy surface
Stem cell	Rarely seen; attachment of a second sporangium sometimes observed	Neither stem cell nor second sporangium seen	Attachment of a second sporangium regularly seen
Central body			
Shape	Oblate spheroid, an ellipsoid sometimes almost spherical, narrowly elliptic in section	Oblate spheroid, an ellipsoid sometimes almost spherical, narrowly elliptic in section	Oblate spheroid, an ellipsoid narrowly elliptic in section
Diameter			
LM	2.1 ± 0.2	1.6 ± 0.1	2.1 ± 0.2
TEM	1.4 ± 0.1	1.3 ± 0.1	1.6 ± 0.2
Height			
LM	1.7 ± 0.2	1.5 ± 0.1	1.7 ± 0.1
TEM	1.1 ± 0.1	1.1 ± 0.2	1.3 ± 0.1
Partial epicortical wall	Surrounds protoplast laterally, not in basal or polar areas	Surrounds protoplast somewhat sublaterally	Entirely surrounds protoplast
Characteristics of pore	Basal annular opening formed from thickened outer wall	Basal cortical wall thins to expose inner central body	Thickness of basal wall constant and is the depth of pore
Pore diameter (TEM)	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Episporal structures			
Fibers, origin and orientation	Fibers arise directly from cortical wall, gradually arching downward to form an attachment layer of numerous shorter fibers	Long fibers arise directly from cortical wall, bending sharply downward to form an attachment layer of numerous shorter fibers	Same as *P. penetrans,* but additional layer is formed on the obverse surface of endospore
Matrix, at maturity	Becomes coarsely granular; lysis occurs; sporangial wall collapses; base is vacuolate	Persists, but more granular; some strands are formed and partial collapse may occur	Persists, numerous strands are formed and partial collapse may occur
Host	Root-knot nematodes: *Meloidogyne incognita*	Root-lesion nematodes: *Pratylenchus brachyurus*	Cyst nematodes: *Heterodera* spp., *Globodera rostochiensis*
Completes life cycle in juveniles	No, only in adults	Yes, in all stages	No, only in adults

Scanning electron microscopy of CNP

Nematodes that had been encumbered or parasitized by CNP were chemically fixed, dehydrated, critical point-dried, and coated for SEM observation. The specimens were placed in a 3.0% solution of glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for at least 1.5 hr, dehydrated in an ethanol series, critical point-dried, and mounted on aluminum stubs. Stubs bearing the dried specimens were sputter coated with 200–300

A of gold-palladium and then examined with a Hitachi S430 or S530 scanning electron microscope operating at 15 or 20 kV. In addition, other specimens were coated with about 30 A of platinum (Wergin and Sayre, 1988; Wergin et al., 1988) and observed in a Hitachi S900 low voltage field emission SEM at 1–2 kV.

Measurements of endospores from SEM micrographs were made either from anaglyphs of digitized images that were collected and viewed with a Kevex 8000 energy dispersive X-ray analyzer equipped with an image-analysis software program, or from stereographic images that were viewed with a 4-mirror stereoscope having a floating light-point attachment. A 10° tilt was used to record the stereo images. The image-analysis program or use of the formula $Z = P/2M \sin(Q/2)$ (where P = parallax value, M = magnification, and Q = tilt angle) was used to obtain the Z measurements (Wergin, 1985).

Transmission electron microscopy (TEM) of CNP

For TEM, single developing females of *H. glycines* parasitized by CNP were hand-picked from soybean roots and placed in a small drop of tap water. The water was mixed with molten 3.0% water-agar at 50°C. After the agar solidified, 0.3–0.5-mm³ blocks of agar, each containing a single diseased individual, were transferred to a vial of 3.0% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8). Following fixation for 3 hr, the specimens were washed in several changes of fresh buffer, postfixed in 2.0% osmium tetroxide for 2 hr, and dehydrated in an acetone series. Finally, the agar blocks were infiltrated with a low-viscosity resin (Spurr, 1969). Silver-gray sections of selected nematodes were cut on a Sorvall MT2 ultramicrotome with a diamond knife and then mounted on uncoated copper grids (75 by 100 mesh). The sections were stained for 10 min with 2.0% aqueous uranyl acetate and then for 5 min with 3.0% lead citrate. The stained thin sections were viewed with a Hitachi H500 transmission electron microscope operating at 75 kV with a 30-μm aperture.

Micrographs of the CNP were compared to those of *Pasteuria penetrans* and *Pasteuria thornei,* which had been prepared by the same procedures as described in earlier studies (Sayre et al., 1983; Sayre and Starr, 1985; Sayre et al., 1988; Starr and Sayre, 1988).

Host specificity

Host specificity was determined by the attachment of bacterial endospores to the cuticles of the J_2 species indicated in Table 2. The J_2 from cultures were counted in a Hawksley chamber, adjusted to 300 per ml, and added to the endospore suspension in a 50-mm Petri dish. To prepare suspensions of the infective endospores of CNP, diseased cysts were hand-picked from the roots of soybean and crushed in 5 ml of water to liberate the sporangia. The resulting suspension was filtered through a 500-mesh wire sieve to remove cuticular debris. The number of sporangia in the suspension was adjusted with a hemocytometer to 5×10^5 per ml. Finally, 5-ml portions of the suspension were added to equal volumes of tap water containing 1,000 J_2.

A shallow pan containing the J_2 and spores was placed on a rotary shaker (50 rpm) and aerated for 24–48 hr. Then, the J_2 were examined with a Leitz inverted microscope at 250× to determine whether endospores had attached to the cuticles of the J_2. When 3–10 endospores had attached to 10% or more of the J_2, the larvae were pipetted around the roots of host plants to determine whether the bacterium would complete its life cycle on a particular nematode host. After 45 days, the seedlings were harvested and their roots were examined for diseased nematode cysts.

Results

Light microscopy

The original Japanese isolate of CNP, which was initially found parasitizing *H. elachista,* was used in all experiments. This Japanese isolate also parasitized 2 additional hosts: *G. rostochiensis* and *H. glycines,* which were reinfected with CNP to maintain a source of the bacterium. Crushing diseased cysts of the soybean nematode, *H. glycines,* in tap water on microscope slides released various stages of the life cycle of CNP, which could be examined and photographed (Fig. 1). LM measurements of the developing bacterium (i.e., immature through mature sporangia) were made from positive photographic prints and compared to those obtained from the 2 other species in the genus *Pasteuria* (Table 1). Generally, 25 separate measurements were taken of a particular stage, and are presented as the mean plus or minus the standard deviation. The mature sporangia exhibited the most distinguishing characteristics of the bacterial isolates; therefore, their features were measured and described (Table 1).

Sporangia were usually found in the opaque cysts (Fig. 2). The mean number of mature sporangia extracted per female was 4.4×10^5. The highly refractile central spore body was oblate spheroid, with axes of 2.1 ± 0.2 by 1.7 ± 0.1. The enveloping lenticular-shaped endospores, which were found attached to the J_2 of *H. glycines* (Fig. 3), measured 5.3 in diameter. Their height, from the margin of the dome of the central body to the base of the sporangium, was 4.0 (Fig. 2). The early, but less frequently found, quartet stage of a developing sporangium had a diameter of 1.8 and measured 3.2 in height from the point of attachment to its distal end (Fig. 4). Similarly, the more mature quartets (Fig. 5) measured 3.0 in diameter and were 3.5 high. In the doublet configuration, the individual sporangium had a diameter of 4.1 and a height of 3.9.

Scanning electron microscopy

Two types of scanning electron microscopes (SEM), a conventional SEM and a low-voltage

field-emission (LVFE) SEM, were used in this study. The LVFE SEM has a resolution approximately 10 times greater than the conventional SEM (Wergin et al., 1988). Mature endospores of CNP, including their associated perisporic remnants and episporal structures, were found adhered to the surfaces of J_2 (Figs. 6, 7) and also to the adult males of *H. glycines* (Figs. 8, 9). The endospores measured about 4.2 in diameter. Two distinct forms of endospores have been observed for CNP (Figs. 6, 7); this observation is similar to that existing in *P. penetrans*. In 1 form, the surface of the endospore is covered by perisporic walls; in the other, the episporic wall structure is exposed. In CNP, the surface appears to be covered by a velutinous membrane, the exosporium. This membrane may be the remnants of the sporangium that had encompassed the surface of the endospore but was not sloughed (Figs. 6, 7). When this material is shed, the exposed surface can be resolved into 2 distinct components: a central body, averaging 1.5 in diameter and 1.2 in height, and a surrounding episporic matrix composed of fine fibers (Fig. 7). In *P. penetrans*, the central body and the related structures measured about 3.3 in diameter and 2.3 in height. Wrinkled perisporic walls covered the *P. penetrans* endospores. The central body in *P. penetrans* was 1.3 in diameter and 1.0 in height; the episporic fibers measured approximately 1.0 in width.

Table 2. Host specificity of RKP (the *Pasteuria* species from root-knot nematodes) and CNP (the cyst nematode parasite from cyst nematodes) as scored by attachments of endospores to nematode J_2.

Nematode	Stage*	*Meloidogyne*-isolate (RKP)	*Heterodera*-isolate (CNP)
Aphelenchoides sp.	J & A	–	–
Aphelenchus sp.	J & A	–	–
Helicotylenchus sp.	J & A	–	–
Heterodera elachista	J_2	–	+
H. glycines	J_2	–	+
H. zeae	J_2	–	–
Globodera rostochiensis	J_2	–	+
Meloidogyne hapla	J_2	+	–
M. incognita	J_2	+	–
M. javanica	J_2	+	–
Paratylenchus sp.	J & A	–	–
Paratrichodorus porosus	J & A	–	–
Pratylenchus brachyurus	J & A	–	–
P. coffeae	J & A	–	–
P. penetrans	J & A	–	–
P. vulnus	J & A	–	–
Rotylenchulus reniformis	J & A	–	–
Tylenchorhynchus sp.	J & A	–	–
Tylenchulus semipenetrans	J & A	–	–
Tylenchus sp.	J & A	–	–

* J, Juveniles; J_2, juveniles, second stage; A, adults.

Transmission electron microscopy of CNP—vegetative growth

The developmental stages (vegetative mycelium, early and late stages of endospore formation, and mature sporangia) of CNP may occur simultaneously in the infected soybean nematode host (Fig. 10). The dichotomously branching hyphal colonies of CNP were septate; the hyphae were bounded by a compound wall, about 0.04 thick, that had outer and inner membranes (Fig. 11). The inner membrane, which opposed the septa, delineated individual cells. Mesosomes were frequently found associated with septa during the vegetative growth (Fig. 11).

Transmission electron microscopy of CNP—endospore formation

In CNP, the sporulation process involved the terminal cells of the microcolonies. These ter-

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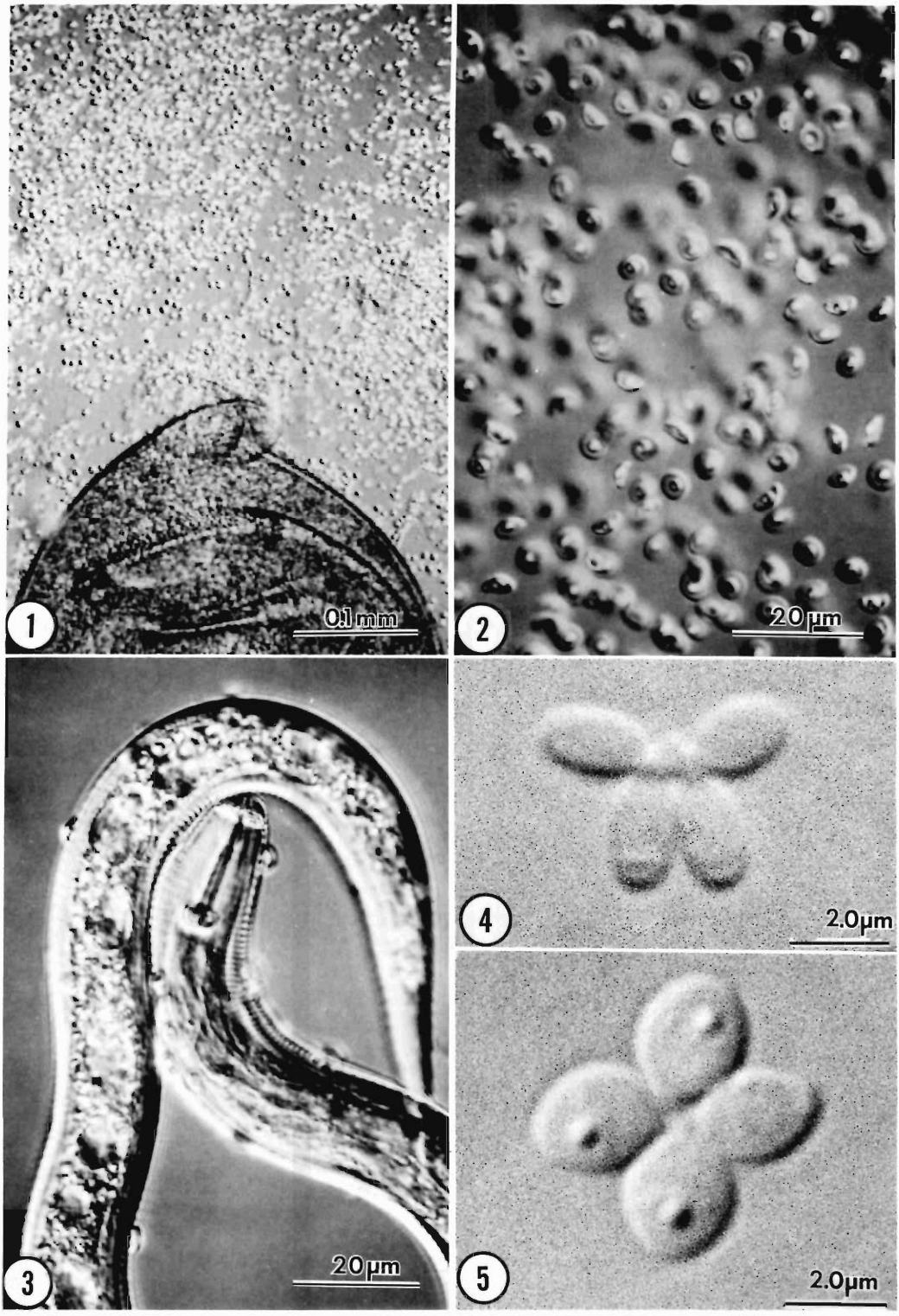
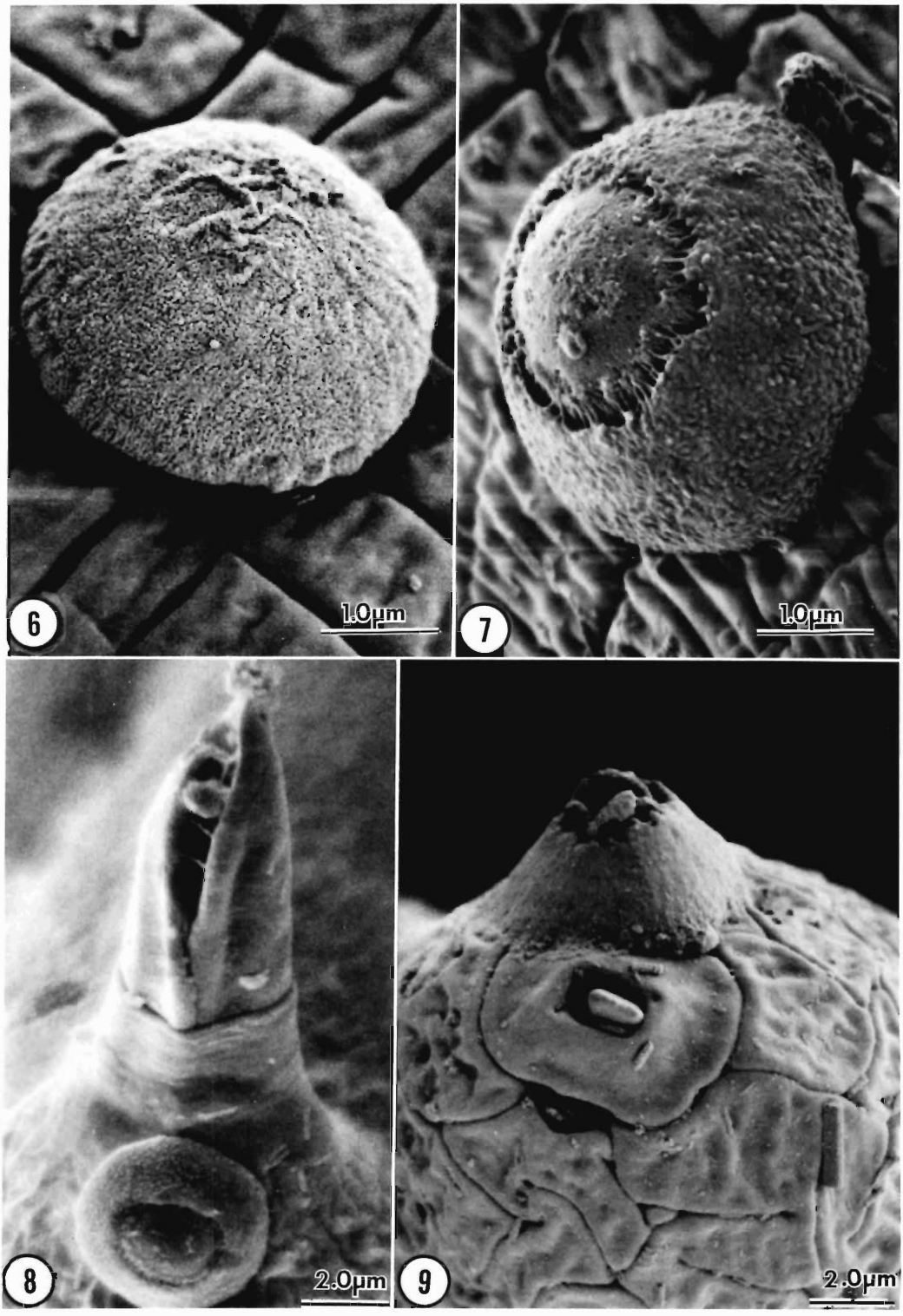
Figures 1–5. Light photomicrographs of the cyst nematode parasite (CNP) from the soybean cyst nematode, *Heterodera glycines*. 1. Numerous sporangia of the CNP released from a crushed cyst. 2. Sporangia of CNP showing their highly refractile central bodies. 3. A juvenile of *H. glycines* with several attached endospores of the CNP. 4. Quartets of early vegetative sporangia. 5. Quartets of late vegetative sporangia that are undergoing endosporogenesis.

Figures 6–9. Scanning electron micrographs of endospores of the cyst nematode parasite (CNP) that are attached to *Heterodera glycines*. 6. CNP endospore attached to a nematode juvenile. The velutinous exosporium covers the central body and its surrounding peripheral fibers. 7. CNP endospore attached to a juvenile of *H. glycines*. The endospore has lost its exosporium revealing the central endosporal body and the surrounding peripheral fibers. 8. CNP endospore attached near the extended copulatory spicules of a male nematode of *H. glycines*. 9. CNP endospore attached to the anterior of an adult male nematode. The endospore blocks the amphidial opening near the slightly protruding stylet.





minal cells enlarged and became more ovate (Fig. 12). The protoplasts of the ovate cells, which initially consisted of a granular matrix of ribosomes, gradually exhibited well-defined organelles. During this early transformation, transverse membranes formed within the developing sporangia (Fig. 12). These membranes separated one-third of the upper/distal end of the sporangium, or the developing forespore, from the lower/basal perisporic portion (Fig. 13).

In the later stages of CNP endosporogenesis, the upper end condensed into an electron-dense central core that became encircled by the multilayered wall (Fig. 14). The electron-translucent peripheral region developed laterally with respect to the central body; in later stages, the fibers emerged within these lateral structures, the multilayered walls of the endospore thickened (Fig. 14), and the central body of the endospore became more ellipsoidal. Because the basal matrix of *P. thornei* does not degrade, its sporangium retains a rhomboidal shape at maturity when observed in cross section (Fig. 17).

Transmission electron microscopy of CNP—mature sporangia

In the basal peripheral region of the CNP sporangia, differentiated fibrous strands intermingled with electron-dense granules. Subsequently, the CNP sporangia retained a somewhat lenticular shape in lateral views (Fig. 15). This shape persisted until the outer sporangial wall degraded, thereby exposing the velutinous exosporium (Fig. 6). As a result, the endospore with its accompanying fibers becomes crescent-shaped (Fig. 16) and similar in appearance to the endospores of *P. penetrans.* The mature central body of the CNP endospore is broadly elliptical with axes measuring 1.6 ± 0.2 by 1.3 ± 0.1. One difference between CNP and the other 2 species is the occurrence in the former of an electron-opaque granular layer, or epicortical wall, that surrounds the cortical layer except for an interruption at the basal pore. This structure is not present in the basal or polar areas in *P. penetrans,* and only occurred in the sublateral areas of *P. thornei* en-

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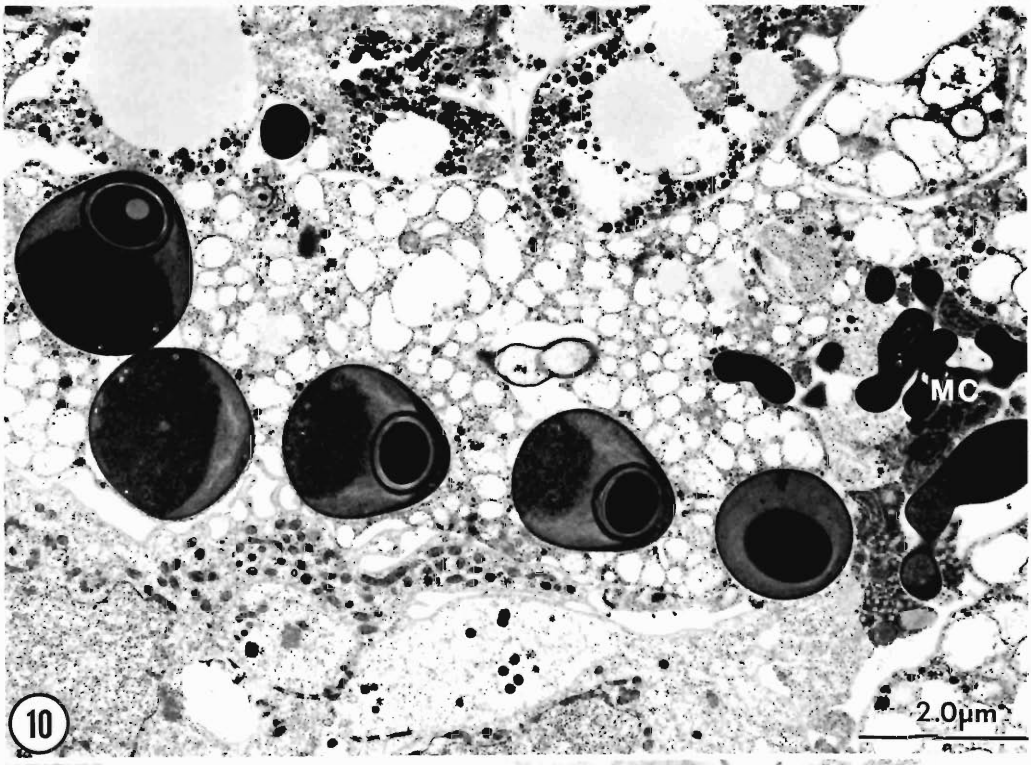
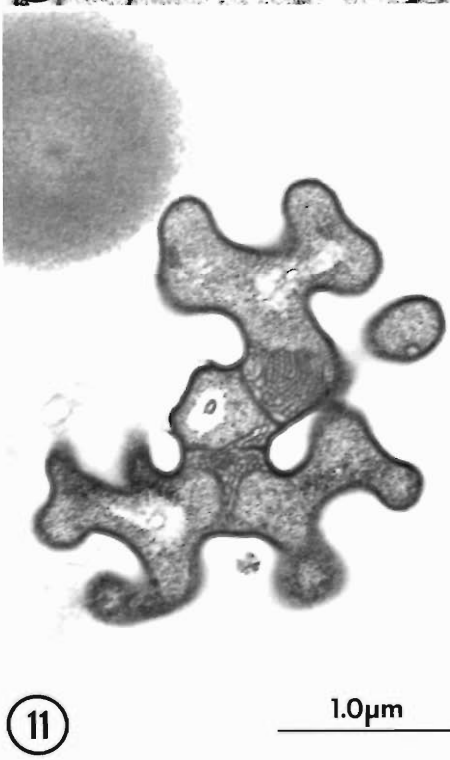
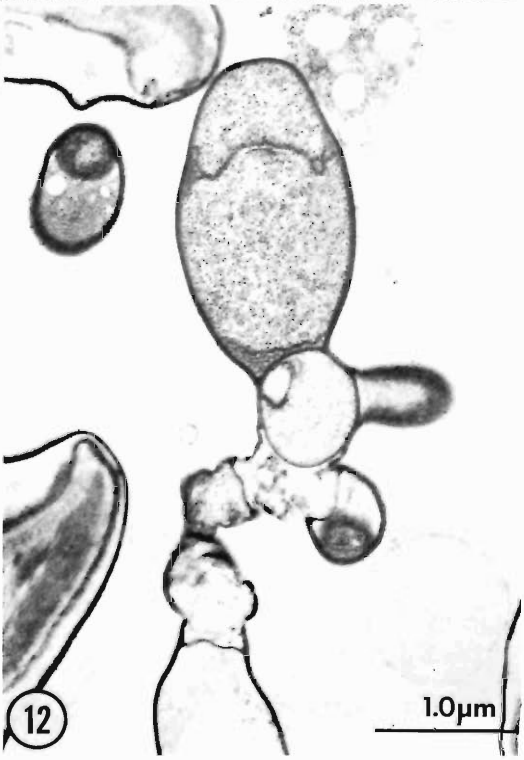
Figures 10–12. Transmission electron micrographs of the cyst nematode parasite (CNP) from the cyst of the soybean cyst nematode, *Heterodera glycines.* 10. Mycelial colonies (MC) and sporangial stages of the CNP extruded from a cyst of *H. glycines.* Note the simultaneous occurrence of all life stages of the bacterium in this nematode. 11. A portion of a mycelial colony showing early vegetative stages of the CNP. The hyphal walls, which are septate, appear to bifurcate at the margins of the colony. Mesosomes are associated with the septa. 12. An early sporangial stage of the CNP extruded from a cyst of *H. glycines.* A septum delineates the apical forespore of the sporangium from the basal parasporal cell. The compound cell wall of the developing sporangium is composed of an outer and inner membrane.

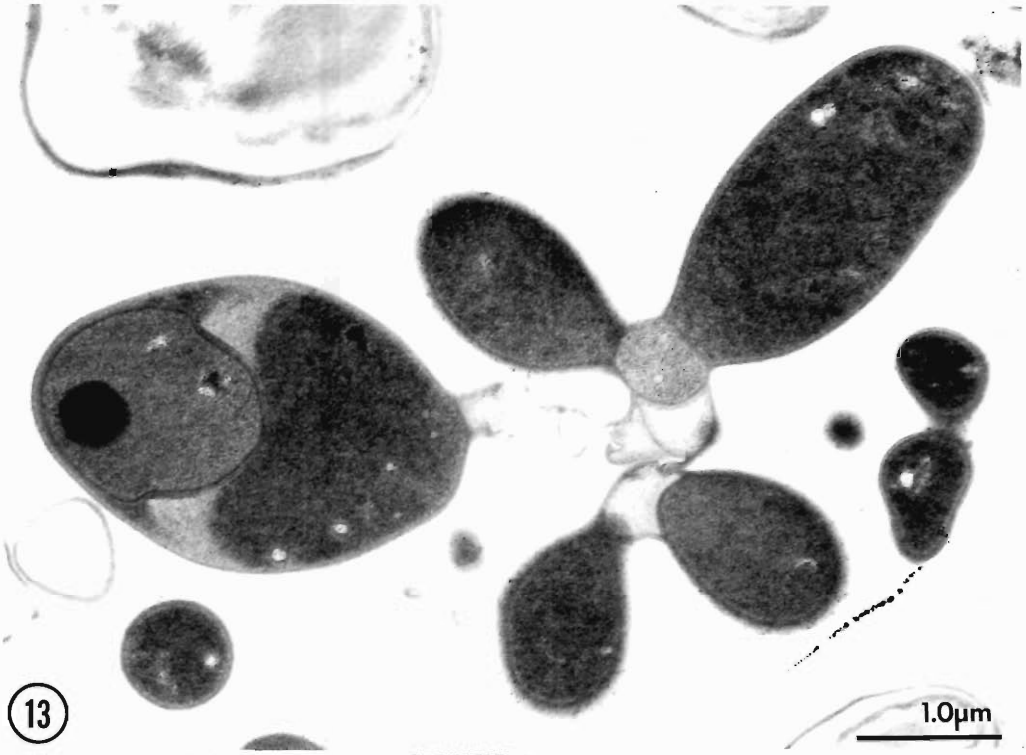
Figures 13–15. Transmission electron micrographs of the cyst nematode parasite (CNP) from the soybean cyst nematode, *Heterodera glycines.* 13. An electron-opaque body has formed within the forespore. Surrounding the spherical body is a membrane that will condense and contribute to the multilayered wall of the mature endospore. Note the 2 lateral electron-translucent areas that will develop into peripheral fibers. Lysis of cells between the sporangium and the rest of the microcolony allows separation. 14. A sporangium with nearly mature endospore. This endospore, whose multilayered wall has formed, has discernable peripheral fibers. 15. A cross section of a mature sporangium from *H. glycines.* This section shows final stages in differentiation of the endospore that include the formation of an encircling membrane or exosporium and the emergence of the peripheral fibers. Granular material fills the basal portion of the sporangium. The sporangium retains a somewhat lenticular shape in lateral view until the old sporangial wall is fully degraded.

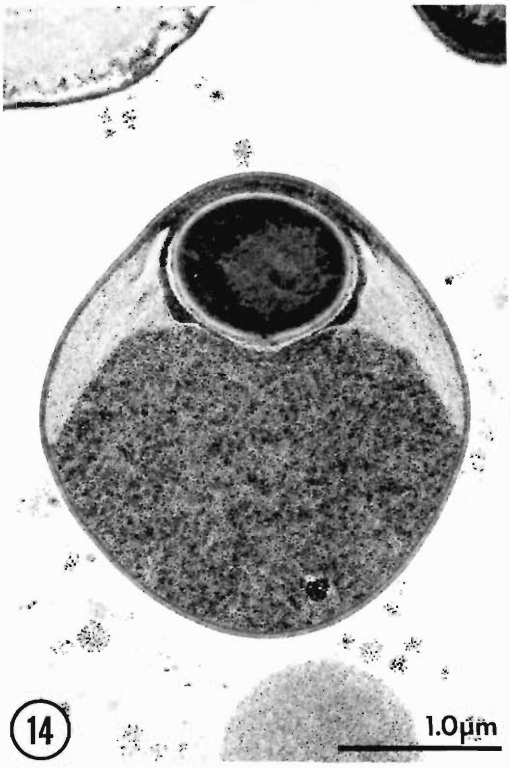
Figures 16, 17. Transmission electron micrographs comparing the endospores of the cyst nematode parasite (CNP) and *Pasteuria thornei.* 16. Cross section of an endospore of the CNP in which the sporangial wall has completely degraded leaving the central body and peripheral fibers surrounded by the velutinous exosporium (EX) and a partial hirsute layer (HC), which have separated from the attachment layers (AL). 17. Cross section of a mature sporangium of *P. thornei,* found parasitizing *Pratylenchus brachyurus,* a root-lesion nematode. The central body of the endospore is nearly spherical. The partial electron-opaque layer (arrows) on the obverse surface of the endospore is unique to this bacterial species. The cortical walls gradually narrow at the base to form a pore unlike that found in *Pasteuria penetrans* or that found in parasites of the cyst nematodes.

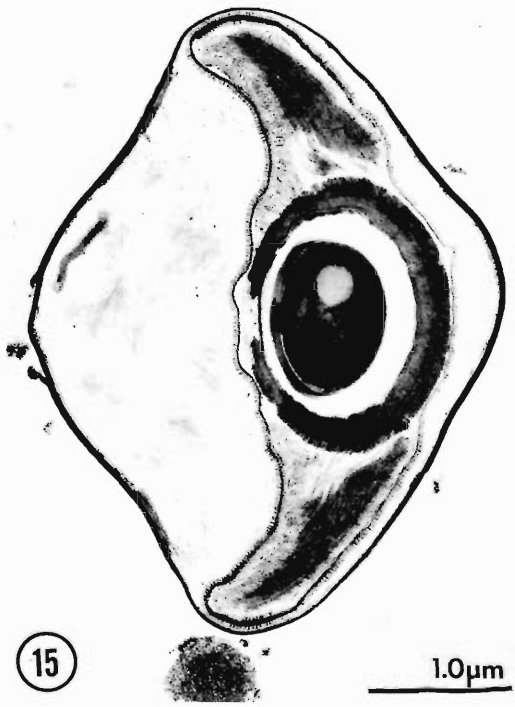


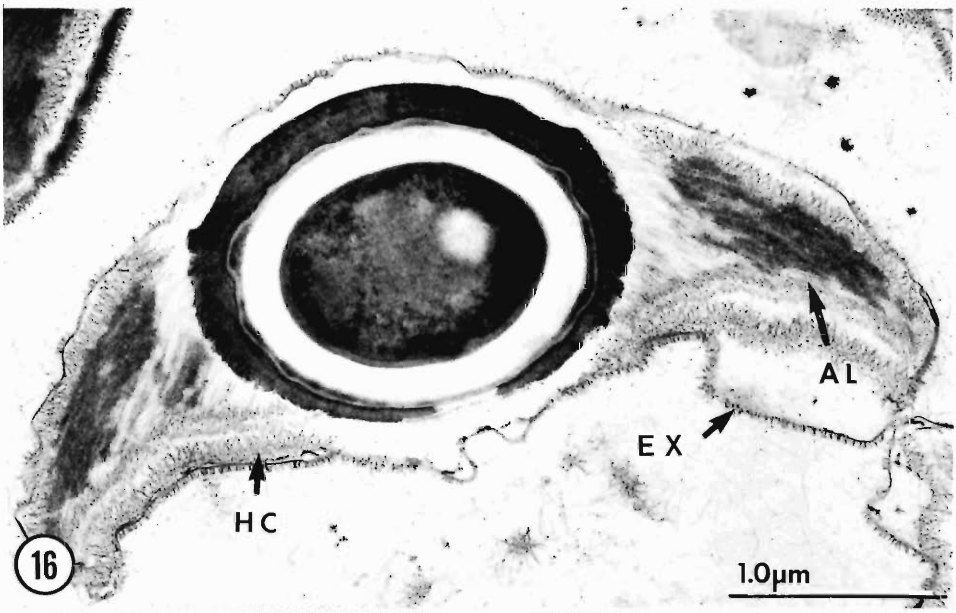


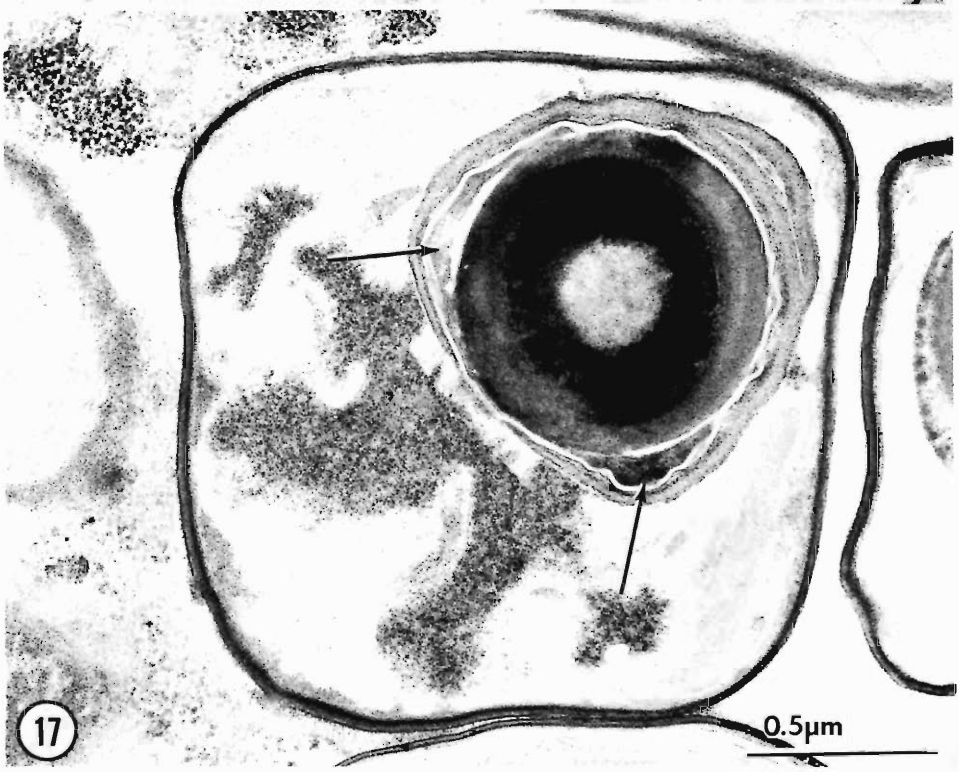












dospores. The outer wall of CNP at the endospore equator measured 0.2 in thickness, and became somewhat thinner toward the base of the central body. A partial layer, hirsute in appearance and originating from the basal adhesion layer of the central body, was unique to CNP (Fig. 16); it has not been observed in members of the genus *Pasteuria.*

Life cycle of CNP

The stages in the life cycle of CNP are similar to those of *P. penetrans* (Table 1). The morphology of all stages of CNP, except the mature sporangium, was similar to that of *P. penetrans.* In addition, the stages in CNP development are in synchrony with those of its cyst nematode host, a relationship that was also found in *P. penetrans.* This synchrony was absent in *P. thornei,* which completed its life cycle in all stages of the host nematode.

CNP also differed from *P. penetrans* in that endospores of the former attached to the adult males of *H. glycines,* but males of *M. incognita* were never observed encumbered by endospores. However, the completion of the life cycle of the bacterium within the males of either nematode species has not been observed.

Host range studies of CNP

In the host range studies of CNP, the attachment of endospores to the respective host J_2 was used as the criterion for susceptibility to the bacterial disease (Fig. 3). The hosts were restricted to species of root-knot and cyst nematodes (Table 2). The endospores of CNP attached to the cyst nematodes but not to root-knot nematodes or to other plant-parasitic or free-living species.

Discussion

Preparatory methods for light microscopic (LM) observations caused least shrinkage to endospores compared to those used in scanning (SEM) and transmission electron microscopy (TEM). Consequently, LM measurements were considered more indicative of the true morphological values. Unfortunately, the small size of the endospore (i.e., 3.5–5.3), and its inherent resistance to staining, limited the LM observations to a few external measurements (Table 1). Nevertheless, these few measurable differences, plus the distinct host range of the bacterial species as expressed by attachment to specific nematode species, were sufficient indicators of isolate differences and helped to delineate and characterize the CNP isolate. Another characteristic that distinguished bacterial isolates at the LM level was their differential abilities to develop in the juvenile stages of nematodes. *Pasteuria thornei* completed its entire life cycle in all stages of the nematode *P. brachyurus* (Sayre et al., 1988). The other species examined could not. Also, the number of sporangia of *P. penetrans* in females of the root-knot nematode was 4 times greater than the number of CNP sporangia in the cyst nematodes. Only a few hundred sporangia were formed in each stage of *P. brachyurus.* These differences are partly due to the smaller volume of vermiform and cyst nematodes compared to that of the swollen root-knot nematode female.

The preparatory methods for SEM caused more shrinkage than those for LM; however, resolution and definition of surface features were far superior with SEM compared to LM. In particular, LVFE SEM revealed details of the external morphology of sporangia and endospores of CNP that had not been resolved in the earlier SEM studies of the other isolates. The episporic wall coverings of *P. penetrans* were found to be relatively smooth, while those of CNP were velutinous to hairy. Additionally, SEM revealed differences in the attachment of episporic wall to the endospore that were not evident in the LM photomicrographs.

In TEM investigations, some fine structural features were common to all species of *Pasteuria* and the CNP (Table 1). Mycelial cell walls of all isolates were typical of the Gram-positive bacteria. During vegetative cell division, mesosomes were often associated with developing septa in all species. The stages of endogenous spore formation, which were typical of Gram-positive rod bacteria, also occurred in all members of *Pasteuria* that have been examined.

Some qualitative differences associated with spore coats of CNP separated this isolate from *P. penetrans* and *P. thornei.* The epicortical spore coat of CNP completely surrounded its central protoplast, but analogous coats were only partially complete in the other 2 species.

Other differences among the endospores of the CNP and *Pasteuria* spp. were the shape and depth of the basal pore. In *P. penetrans,* the wall thickened to form a doughnut-shaped structure that surrounded the pore. In *P. thornei,* the wall tapered to form the opening at the base of the endospore. In CNP, the wall of the endospore neither tapered nor appreciably thickened; thus, the depth of the pore reflected the thickness of

the wall. In addition to this feature, the CNP had a partial wall, which formed on the obverse face of the endospore, that appeared to arise from the endospore attachment layer. These morphologic differences, as revealed by LM, SEM, and TEM, suggest that the CNP isolate is a new species of *Pasteuria.*

Acknowledgments

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On the Geographical Distribution and Parasitism of *Rhabditis* (*Pelodera*) *orbitalis* (Nematoda: Rhabditidae)

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ABSTRACT: Successful crossings between Californian and European populations proved the nematode *Rhabditis* (*Pelodera*) *orbitalis* to be of holarctic distribution. Larvae of this species occur in the lachrymal fluid of small rodents, whereas its free-living (microbivorous) stages live in the nesting material. No pathogenic effects were observed in rodents infected by the nematode larvae. Interfertility of the 2 separated populations is discussed on the basis of stabilizing conditions in the nematode's microhabitat in both the Old and the New Worlds.

KEY WORDS: nematodes, *Rhabditis* (*Pelodera*) *orbitalis*, larval parasitism, nest fauna, hybridization, biogeography.

Rhabditis (*Pelodera*) *orbitalis* Sudhaus and Schulte, 1986, are microbotrophic nematodes in the nests of arvicolid and, to a lesser extent, murid rodents. Adults, developmental stages, and dauer juveniles can easily be extracted from that habitat and grown in the laboratory (Schulte, 1988). Third-stage infective juveniles, however, enter the orbital fluid of the rodents and remain as parasites for several days (Schulte, 1989). The phenomenon has attracted interest since its first discovery (Morozov, 1955; Stammer, 1956) and additional reports have documented similar eye nematodes resembling *R. orbitalis* (often confused with *R. strongyloides* [Schneider, 1860]) from numerous different hosts and sites in the Northern Hemisphere.

During the summer of 1989, the authors were able to establish laboratory cultures of 2 Californian and 1 European isolates of this species. The present paper reports on the results of experimental crosses between Californian and German isolates.

Materials and Methods

Adults of the suspected *R. orbitalis* were extracted using a Baermann funnel from material collected from nests of voles (*Microtus* sp.) at the University of California Field Station in Hopland, California (about 700 m elevation).

Cultures of the nematode were established on 2% agar plates containing small pieces of calf liver and maintained at room temperature. Additional nematodes were removed from the conjunctival sacs of a specimen of *Microtus californicus* (Peale) livetrapped near the Sagehen Creek Field Station near Truckee, California (elevation approx. 2,000 m) in the Sierra Nevada. Nematodes from the lachrymal fluid of both eyes (left eye 43, right eye 56) were transferred to agar plates. Two mature females survived and were crossbred with males from the Hopland strain.

The European isolate was collected by the Baermann method from nest material of the European meadow vole, *Microtus arvalis* (Pallas), at the Attendorn area (Northrhine-Westfalia, Western Germany; elevation about 350 m). It contained adults and developmental stages of *R. orbitalis* in great numbers that were used to start a laboratory population.

To test for interfertility, males and females of each strain were raised separately and males of one strain were combined with females of another strain in the ratio of 5 females to 10 males on an agar dish in a droplet of bacterial suspension. Mating and egg-laying were monitored with a dissecting microscope. Five replicates were conducted of each of 2 reciprocal crosses: American strain males × European strain females and European strain males × American strain females.

Results

Appearance of eye infection

With a little experience it is possible to determine the presence of the nematodes in situ in the field. Eyes parasitized by rhabditid nematode larvae in California have exactly the same appearance as those of European voles: the moving nematodes appear as an undulating glistening mass on the eye's surface and can be seen readily with a low power hand lens, especially when the skin at the corner of the eye is drawn back. Occasionally, some larvae near the inner angle of the eye leave the lachrymal fluid and wave one-third of their bodies back and forth in the air. These larvae nearly always resume development when removed from the eye and transferred to an agar plate seeded with bacteria. In general, "older" larvae (i.e., living in the orbital fluid for more than 5 days) show a tendency to stay at the

Table 1. Reports of parasitic larvae of *Rhabditis orbitalis* in the lachrymal fluid of small rodents.

Locality	Host species	Author(s)
A) North America		
1. Eskimo Point (Hudson Bay)	*Dicrostonyx groenlandicus* (Traill), *Lemmus sibiricus* (Kerr)	Cliff et al. (1978)
2. Missoula (Montana)	*Clethrionomys gapperi* (Vigors), *Microtus longicaudus* (Merriam)	Kinsella (1967)
3. California	*Microtus californicus* (Peale)	Poinar (1965), present study
4. Point Barrow (Alaska)	*Lemmus sibiricus*	Rausch (1952)
5. Black Hills (S. Dakota)	*Clethrionomys* sp.	Trapido, pers. comm.
B) Europe		
6. Central Europe (Western Bohemia [CSSR]; Berlin, Erlangen, Vogelsberg, Freiburg, Sauerland [Germany])	*Apodemus flavicollis* (Melchior), *A. sylvaticus* (L.), *A. agrarius* (L.), *Arvicola terrestris* (L.), *Clethrionomys glareolus* (Schreber), *Mus musculus* (L.), *Pitymus subterraneus* (de Selys-Longchamps), *Microtus agrestis* (L.), *M. arvalis* (Pallas)	Helm (1974), Prokopič et al. (1974), Stammer (1956), Schulte (1989), present study
7. Great Britain (Oxford, Slapton Ley, Ascot)	*Apodemus sylvaticus, Clethrionomys glareolus, Microtus agrestis, M. arvalis*	Canning et al., 1973, Hominick and Aston (1981), Poinar (1965), Trapido, pers. comm.
C) Asia		
8. Nantou Hsien (Taiwan)	*Apodemus sylvaticus, Rattus norvegicus* (Berkenhout)	Cross and Santana (1974)
9. Novosibirsk Province (USSR)	*Apodemus flavicollis, Arvicola terrestris, Clethrionomys glareolus, Lagurus lagurus* Pallas, *Micromys minutus* Pallas, *Microtus agrestis, M. arvalis, M. oeconomus* (Pallas), *Pitymus subterraneus*	Morozov (1955)

bottom of the conjunctival sac while "younger" larvae can be seen moving across the surface of the eye.

Under natural conditions, there is no sign of inflammation of the eyes or surrounding tissues caused by the nematode larvae even in hosts with hundreds of nematodes present. Since the nematode microhabitat is the "outside world" of the orbital fluid surface film rather than the host tissue, even high numbers of larvae may be tolerated after being sterilized by exsheathment (Schulte, 1989). It is not known whether the host's vision is handicapped by the nematodes themselves or their movements. However, no noticeable changes were observed in the behavior of infected voles or mice kept in the laboratory.

Cross-mating experiments

Females reared from eye larvae from the Sagehen Creek area were fertilized by males from Hopland, resulting in a laboratory culture which is now referred to as the "American strain." Individually raised males and females were crossbred with the European *R. orbitalis*. All crosses were successful except for one of American males with European females where worms were overcome by fungal infection.

These results confirm the conspecificity of the different strains and, furthermore, the holarctic distribution of the species, *Rhabditis* (*Pelodera*) *orbitalis*.

Geographical distribution by earlier records

Unidentified small nematode "eye larvae" were first briefly mentioned by Rausch (1952) in a survey on the parasites of lemmings, *Lemmus sibiricus* (Kerr). First detailed descriptions came from Morozov (1955), who found similar larvae in 9 different species of small rodents in the Novosibirsk Province (USSR). Further records hitherto published are listed in Table 1.

With reference to the successful crossings between European and North American populations presented above, we would further expect to find *R. orbitalis* throughout the Northern Hemisphere wherever environmental conditions are suitable.

Discussion

Rhabditis orbitalis is a larval parasite that is strictly associated with a rodent host: without the opportunity of entering the orbital fluid, populations of this nematode would become "trapped" in the decaying nesting material and sooner or later perish. However, its highly specialized third-stage (infective) larvae allow this species to use the orbital fluid as an unexploited nutrient source and become distributed to new habitats (i.e., rodents' nests) at the same time (Schulte, 1989).

Although the parasitism of *R. orbitalis* is obligatory in this particular respect, the species can be grown indefinitely on any bacteria in the laboratory like many other nematodes of the genus *Rhabditis*. This offers the rare opportunity to cross-breed populations from widely separated localities and/or different hosts. Apart from morphological identification characters, conspecificity can thus be tested directly. This becomes a matter of particular interest as several species closely related to *R. orbitalis* proved to be morphologically nearly identical sibling species (Sudhaus et al., 1987).

Because *R. orbitalis* is found in both parts of the Northern Hemisphere, Eurasia and North America, the association must have evolved before the continents were separated by the Bering strait. As in many other patterns of holarctic animal distribution, closely related species of arvicolid rodent species (*Microtus* spp., *Lemmus* spp., *Clethrionomys* spp.), occur in both the Old and the New Worlds (Thenius, 1980). Since ecological conditions in the separated continents are very much the same, there is no noticeable divergence in the evolution of the 2 populations of *R. orbitalis*, as shown by the hybridization studies reported here.

The place of origin of *R. orbitalis* remains unknown, but a possible hypothesis is that this parasitic association may have evolved among colonies of arctic lemmings. This is probable on the basis of a continuously high level of humidity offered there for the microbotrophic stages.

Acknowledgments

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Dactylogyrids (Platyhelminthes: Monogenea) of *Labeo* (Teleostei: Cyprinidae) from West African Coastal Rivers

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ABSTRACT: Dactylogyrids from *Labeo parvus* Boulenger, 1902, *L. alluaudi* Pellegrin, 1933, and *L. rouaneti,* Daget, 1962, were studied in Atlantic coastal basins in West Africa. Nine species (6 new) of Dactylogyridae were found: *Dactylogyrus longiphallus* Paperna, 1973, *D. falcilocus* Guégan, Lambert, and Euzet, 1988, and *Dogielius kabaensis* sp. n. from *L. parvus* populations in coastal rivers of Guinea, Sierra Leone, and Liberia; *Dactylogyrus longiphalloides* sp. n. and *Dogielius kabaensis* sp. n. from *L. alluaudi* in the river Bagbwe in Sierra Leone; *Dactylogyrus sematus* sp. n., *D. jucundus* sp. n., *D. omega* sp. n., and *Dogielius rosumplicatus* sp. n. from *L. rouaneti* in the Konkouré system in Guinea. *Dactylogyrus brevicirrus* Paperna, 1973, characteristic of *L. parvus* in the large Sahel-Sudan basins, was not found in coastal rivers of Guinea, Sierra Leone, and Liberia. *Labeo alluaudi* from the rivers Cavally and Nipoué in Côte d'Ivoire and Liberia were not parasitized.

Comparison of branchial monogeneans in different populations of *L. parvus* in West Africa shows that there are 2 host groups. The first consists of host populations in Guinean coastal basins, characterized by *Dactylogyrus longiphallus, D. falcilocus,* and *Dogielius kabaensis* sp. n. The second comprises the other populations in adjacent basins, marked by *Dactylogyrus brevicirrus,* whose presence is interpreted as a host switching. Importance of isolation phenomena in monogeneans and their cyprinid hosts is discussed.

KEY WORDS: West Africa, Guinean ridge, Dactylogyridae, *Dactylogyrus longiphallus, Dactylogyrus brevicirrus, Dactylogyrus falcilocus, Dactylogyrus longiphalloides* sp. n., *Dactylogyrus sematus* sp. n., *Dactylogyrus jucundus* sp. n., *Dactylogyrus omega* sp. n., *Dogielius kabaensis* sp. n., *Dogielius rosumplicatus* sp. n., Cyprinidae, *Labeo,* parasite specificity, parasite communities, host switching, endemism, refugia, biogeography.

Monopisthocotylean monogeneans on species of *Labeo* (Teleostei: Cyprinidae) are known in Africa through the studies of Price and Yurkiewicz (1968) in Rhodesia, Price et al. (1969) in South Africa, Paperna (1969, 1973, 1979) in Uganda, Kenya, Tanzania, and Ghana, and in Mali (Guégan et al., 1988, 1989), in Guinea, Sierra Leone, and Côte d'Ivoire (Guégan and Lambert, 1990). *Labeo* is represented in West Africa by 7 species (Lévêque and Daget, 1984). Some have a large distribution: *L. senegalensis* Valenciennes, 1842, in Chad, Niger, Senegal, Gambia, Volta river basins and several short coastal basins of West Africa; *L. coubie* Rüppell, 1832, from the Nile to the Senegal, in the Volta, in the Sahel basins, and in several coastal systems; *L. parvus* Boulenger, 1902, in Zaïre, Chad, Senegal, Gambia, Volta, Niger, Ouémé, Mono, and numerous small Atlantic systems. Other species have a more limited distribution: *L. roseopunctatus* Paugy, Guégan, and Agnèse, 1990, from the middle Niger in Mali and the upper Baoulé in the Senegal basin; *L. rouaneti* Daget, 1962, from the Konkouré basin in the Republic of Guinea; *L. alluaudi* Pellegrin, 1934, from the Cavally (Cess) and the Nipoué in Côte d'Ivoire and Liberia: distribution of this species has been extended to the Bagbwe (Sewa) in Sierra Leone by Paugy (pers. comm.); *L. djourae* Blache and Miton, 1960, from the upper Bénoué (Niger basin) in Cameroon. Finally, 2 species are known only by the types: *L. curriei* Fowler, 1919, in the Saint Paul river in Liberia and *L. brachypoma* Günther, 1863 (type locality unknown), probably from western Nigeria (Reid, 1985).

Monogeneans from *Labeo coubie, L. senegalensis, L. roseopunctatus,* and from populations of *L. parvus* in the Niger and the Senegal basins, have been discussed by Guégan et al. (1988, 1989). In the present paper, the parasites of *L. alluaudi, L. rouaneti,* and *L. parvus* in coastal basins in West Africa are reported. The first 2 hosts are well defined taxonomically. The third, *L. parvus,* consists of individuals belonging to a species complex (Reid, 1985). Jégu and Lévêque (1984) and Daget and Iltis (1965) stressed the affinities between the different species described as synonymous while accepting that the variations observed in the different populations might be caused by morphological or geographical variability (Jégu and Lévêque, 1984). However, we recently recognized a new species of *Labeo, L.*

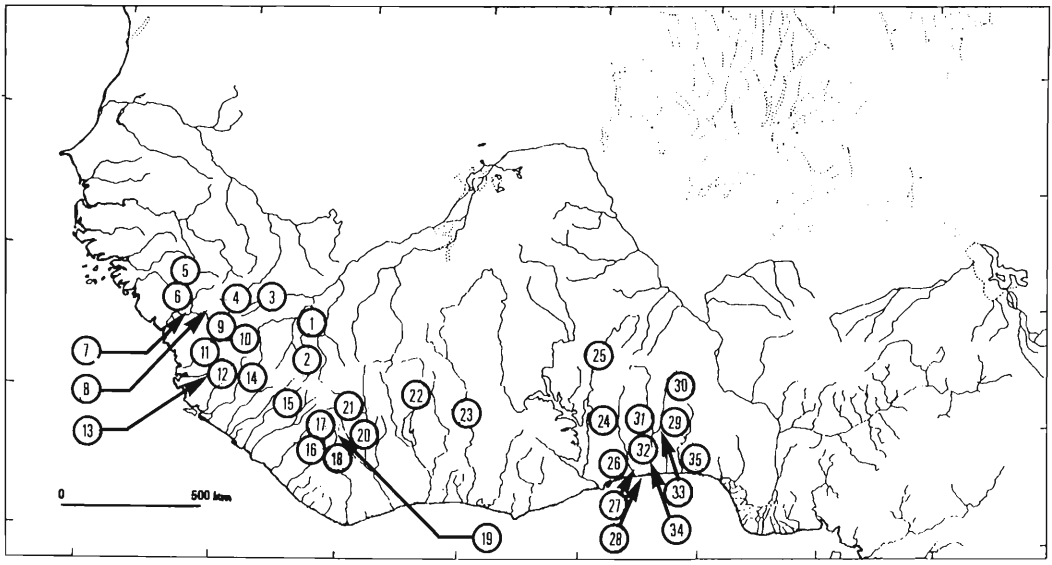


Figure 1. Distribution of the different stations sampled in West Africa (1–35). Niger basin: 1 and 2 Milo; 3 Tinkisso. Senegal basin: 4 Bafing. Tominé basin: 5 Tominé (or Rio Corubal). Fatala basin: 6 Fatala. Konkouré basin: 7 Konkouré; 8 Kakrima. Little Scarcies basin: 9 Kaba; 10 and 11 Mongo. Rokel basin: 12 and 13 Rokel. Bagbwe basin: 14 Bagbwe. Loffa basin: 15 Loffa. Nipoué basin: 16, 17 Nipoué (or Cess); 19 Boan. Cavally basin: 18 Cavally. Sassandra basin: 20 Sassandra; 21 Feredougouba. Bandama basin: 22 Bandama blanc. Comoé basin: 23 Comoé. Volta basin: 24 Wawa; 25 Oti. Todjie basin: 26 Todjie. Togo lake system: 27 Haho; 28 Sio. Mono basin: 29 and 30 Mono; 31 Anié; 32: Amou; 33: Aou; 34: Na. Ouémé basin: 35 Ouémé.

roseopunctatus Paugy et al., 1990, based on 3 simultaneous and independent approaches: parasite, allozyme, and morphometric data.

The coastal rivers in West Africa which flow from the Guinean ridge to the Atlantic Ocean form a separate catchment unit (Grove, 1985) characterized by a large number of endemic fishes (Daget, 1962). Isolation of fish populations in these basins is a good model for studying evolutionary biology of host–parasite relationships and may provide answers to the following questions: Are branchial monogeneans of endemic species of *Labeo* vicarious forms of those found in species of *Labeo* in peripheral basins? Do small endemic host populations affect the branchial parasite communities? These questions are appropriate with regard to the strict host specificity exhibited by the Dactylogyridae (Guégan et al., 1988, 1989).

Geographical Framework, Materials and Methods

Collection sites were in several river systems in West Africa (Fig. 1). The systems consisted of the upper Niger basin (Milo and Tinkisso rivers in Guinea), and upper Senegal basin (Bafing, Baoulé, and Bakoye rivers in Guinea), and of a series of Atlantic coastal basins. Rivers and tributaries are located on the coastal slope of the Guinean ridge which runs from the Fouta Djalon mountains (Guinea) to the Nimba mountains (frontier between Guinea, Côte d'Ivoire, and Liberia). A site on the Mariti Iga river, an endorheic basin in the Sudan, is included but not illustrated on the map (Fig. 1).

In addition the following cyprinid specimens were examined for parasites from the collection of the Ichthyology Laboratory of the MNHN (Museum National d'Histoire Naturelle, Paris): *Labeo alluaudi* (MNHN 1979-128) Cavally river, Boan tributary near Danané (19), Côte d'Ivoire; *Labeo rouaneti* (MNHN 1988-1889) Konkouré system, Kakrima river near Kasseri (8), Republic of Guinea; *Labeo parvus* (MNHN 1988-1888) Niger system, upper Milo near Konsankoro (2), Republic of Guinea, (MNHN 1981-902) Tominé river at Gaoual (5), Republic of Guinea, (MNHN 1988-1887) Konkouré basin, Kakrima tributary near Kasseri (8), Republic of Guinea, (MNHN 1988-1886) Little Scarcies basin, Mongo river near Marela (10), Republic of Guinea, (MNHN 1988-1885) Little Scarcies system, Kaba river near Kouloundala (9), Republic of Guinea, (MNHN 1982-1170, 1982-1172) Loffa river near Macenta (15), Republic of Guinea, (MNHN 1981-900) Comoé system, Comoé (23), Côte d'Ivoire; Wawa river (24), lake Volta system, Ghana, (MNHN 1981-912) Oti river (25), lake Volta system, Ghana; Todjie river (26), Ghana; Haho river (27), Lake Togo system, Togo, (MNHN 1982-959) Sio at Kali (28), Lake Togo system, Togo, (MNHN 1981-909) Mono system, Mono at Kpessi (29), Togo, (MNHN 1989-1033) Mono system, Mono near Tchamba (30), Togo, (MNHN 1989-1036)

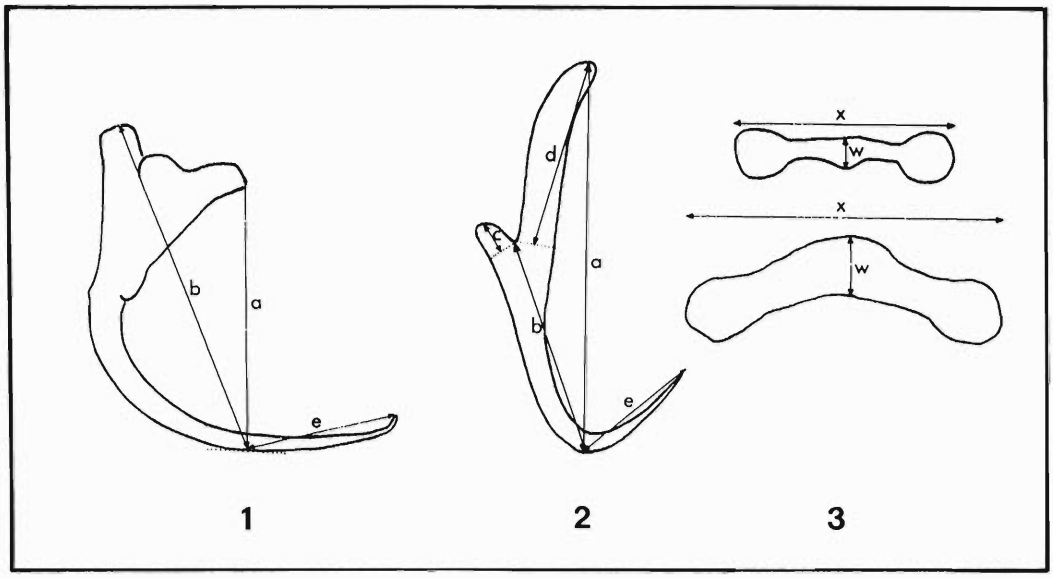


Figure 2. Measurements of haptoral pieces used in this work. 1. *Dogielius* hamuli. 2. *Dactylogyrus* hamuli. 3. Transverse bars. a. Overall hook length. b. Length of shaft and inner root (1). b. Length of shaft (2). c. Length of outer root. d. Length of inner root. e. Length of the tip. W, width of transverse bar; X, length of transverse bar.

Mono system, Anié river at Anié (31), Togo, (MNHN 1989-1060) Mono system, Amou river at Amou-Oblo (32), Togo, (MNHN 1989-1058) Mono system, Aou at Aou-Losso (33), Togo, Mono system, Na river at Paratao (34), Togo, (MNHN 1989-1061) and (MNHN 1981-957) Mariti Iga river near Juba, Sudan. Monogeneans from these hosts were mounted directly in Berlèse liquid and observed with a microscope.

Five field missions were carried out in West Africa and specimens of the following species were collected: *Labeo alluaudi* (MNHN 1988-1949) Nipoué river at Gbé-Nda (17), Côte d'Ivoire; Bagbwe river at Yfin (14), Sierra Leone, no collection code; *Labeo rouaneti* (MNHN 1988-1898, 1988-1899), Konkouré system, Konkouré near Télimélé (7), Republic of Guinea; *Labeo parvus,* Niger system, Milo at Boussoulé (1), Republic of Guinea, Fish not deposited, (MNHN 1988-1892) Niger system, upper Tinkisso near Dabola (3), Republic of Guinea, (MNHN 1988-1894, 1989-980) Senegal system, upper Bafing near Sokotoro (4), Republic of Guinea, (MNHN 1989-984) Fatala system, Fatala near Mabé (6), Republic of Guinea, (MNHN 1988-1895, 1989-985) Konkouré system, Konkouré near Télimélé (7), Republic of Guinea, (MNHN 1989-983) Little Scarcies system, Mongo at Moussaïa (11), Sierra Leone, (MNHN 1988-1896, 1988-1897) Little Scarcies system, Kaba river near Kouloundala (9), Republic of Guinea, (MNHN 1988-1893) Little Scarcies system, Mongo at Marela (10), Republic of Guinea, (MNHN 1989-982) Rokel river at Bumbuna (12), Sierra Leone, (MNHN 1989-1001) Rokel river at Kondembaya (13), Sierra Leone, (MNHN 1989-1002, 1989-1003) Bagbwe river at Yfin (14), Sierra Leone, Loffa river near Macenta (15), Guinea, Fish not conserved, (MNHN 1989-1045) Nipoué at Toyébli (16), Côte d'Ivoire, (MNHN 1989-1047) Nipoué at Gbé-Nda (17), Côte d'Ivoire (MNHN 1989-1044, 1989-1051) Cavally at Sahoubli (18), Côte d'Ivoire, (MNHN 1989-1025) Sassandra system, Sassandra at Sémien (20), Côte d'Ivoire, Sassandra system, Férédougouba tributary near Touba (21), Côte d'Ivoire, Fish not conserved, (MNHN 1989-1024) Bandama blanc near Marabadiassa (22), Côte d'Ivoire, Ouémé near Porto-Novo (35), Bénin, Fish not conserved. These fish were caught with gill nets and cast-nets. Various species of *Labeo* were identified by ORSTOM (Institut Français de Recherche pour le Développement en Coopération) ichthyologists. Nomenclature of Lévêque and Daget (1984) was used. The various individuals studied were deposited as vouchers at the Laboratoire d'Ichtyologie Générale et Appliquée, MNHN, Paris. The gills of fishes caught during field missions were placed in tubes (Eppendorf type), labelled, referenced, and then placed in liquid nitrogen in a cryogenic container. Liver and skeletal muscle were sampled in the same fish to study the polymorphism of enzyme proteins. Transport of the samples to France was effected either in an insulated box or a cryogenic container. In the laboratory, the detached gill arches were immersed in fresh water for examination under a binocular microscope. The monogeneans collected were fixed between slide and cover slip in Malmberg's mixture (glycerine and ammonium picrate). The preparations were sealed, and the parasites examined under a microscope. The sclerotized haptoral parts and the copulatory apparatus were drawn with the aid of a drawing tube. Naming and numbering of haptoral parts were in conformity with those adopted at ICOPA IV (Euzet and Prost, 1981).

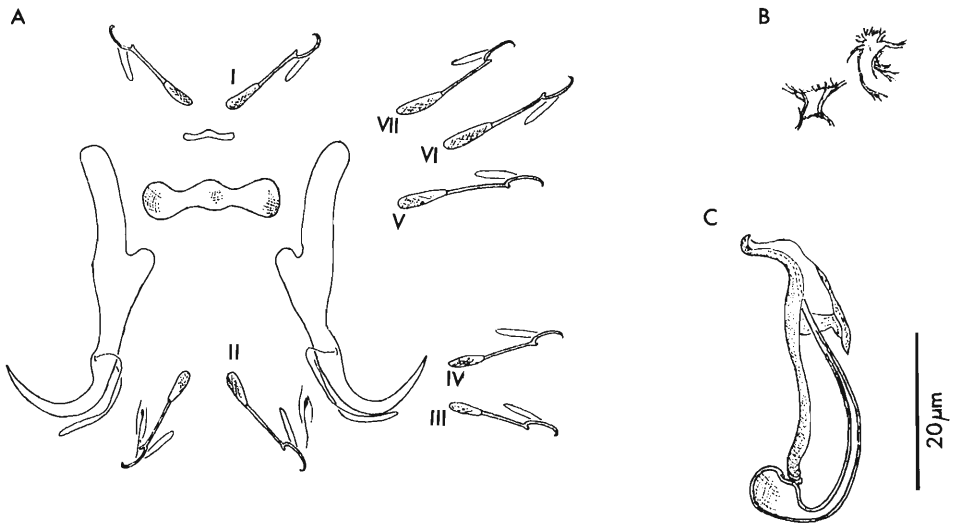


Figure 3. ***Dactylogyrus longiphallus*** **Paperna, 1973. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.**

Hamuli measurements of *Dactylogyrus* were those proposed by Gussev (1962) (Fig. 2). Slide b measurements of *Dogielius* species were defined in a slightly different manner (Fig. 2). All measurements are in micrometers (µm). In all cases in which there was a doubt as regards to the value of the morpho-anatomical criterion, analysis of variance (ANOVA) was applied to a set of quantitative variables corresponding to measurements of haptoral parts (a, b, c, d, e, X, W) (Fig. 2) and of the copulatory apparatus (accessory piece length, penis length). The results obtained for 2 discriminant variables to within the threshold of security are shown by a scatter diagram. Analyses were carried out using BIOMECO Version 3.2 supplied by CEPE-CNRS Biometry Group, route de Mende, BP 5051, 34033 Montpellier CEDEX, France.

Results and Descriptions

Dactylogyrus longiphallus Paperna, 1973 (Fig. 3)

TYPE HOST AND LOCALITY: *Labeo victorianus*; Victoria lake, Uganda and Nzoia river, Victoria lake system, Kenya (Paperna, 1973).

OTHER RECORDS: *L. forskali,* Albert lake, Uganda; *Barbus* cf. *kersteni,* Mobuku river, Monts Ruwenzori, Lake Georges system, Uganda; *B. altianalis,* Kazinga channel, Uganda; *Labeo* sp. 1 and *Labeo* sp. 2, Ruaha river, Tanzania (Paperna, 1973, 1979).

NEW RECORDS: *Labeo parvus,* localities 2–4, 6–7, 9–16, 18, 20–26, 29, 31–32, 34–35, and Mariti Iga river, Sudan.

SPECIMENS: Syntypes M.T. 35.702 (Paperna, 1973).

REMARKS (based on 30 specimens in toto): Body length 390 (280–480); greatest width 70 (40–80) usually at level of ovary. Hamuli lengths: a: 38 (35–41); b: 23 (20–26); c: 2–3; d: 16 (14–18); e: 11 (10–13). Dorsal transverse bar 16–19 long, vestigial ventral bar 5–8 long. Hook lengths: I, II, III, IV: 14–17; V, VI, VII: 16–20; 4A: 7–9. Male copulatory apparatus (38–45) long, consisting of basal ampulla (diameter 6–8) followed by narrow, tubular penis tapering distally. Accessory piece (31–40) fixed to basal ampulla, forming a fork, 1 branch of which continued by finely sclerotized sheath, serving as penis guide. Vagina slightly and irregularly sclerotized near vaginal pore.

This species occurs on *Labeo parvus* in numerous basins in West Africa. Paperna (1973, 1979) reported *Dactylogyrus longiphallus* on *L. victorianus* in Uganda and Kenya, on *L. forskali, Barbus* cf. *kersteni, B. altianalis* in Uganda and on 2 unclassified *Labeo* in Tanzania. Paperna revealed considerable morphometric variation in haptoral parts and in the copulatory apparatus. The individuals presented here are of similar size to the parasites of *L. victorianus* from the Nzoia river (Lake Victoria system) in Kenya. The specific diversity of the hosts studied (sometimes unclassified), and their varied biogeographical origins, suggest that a complex of parasite species exists. However, no criterion appeared to us to be sufficiently weighty to discriminate a new species.

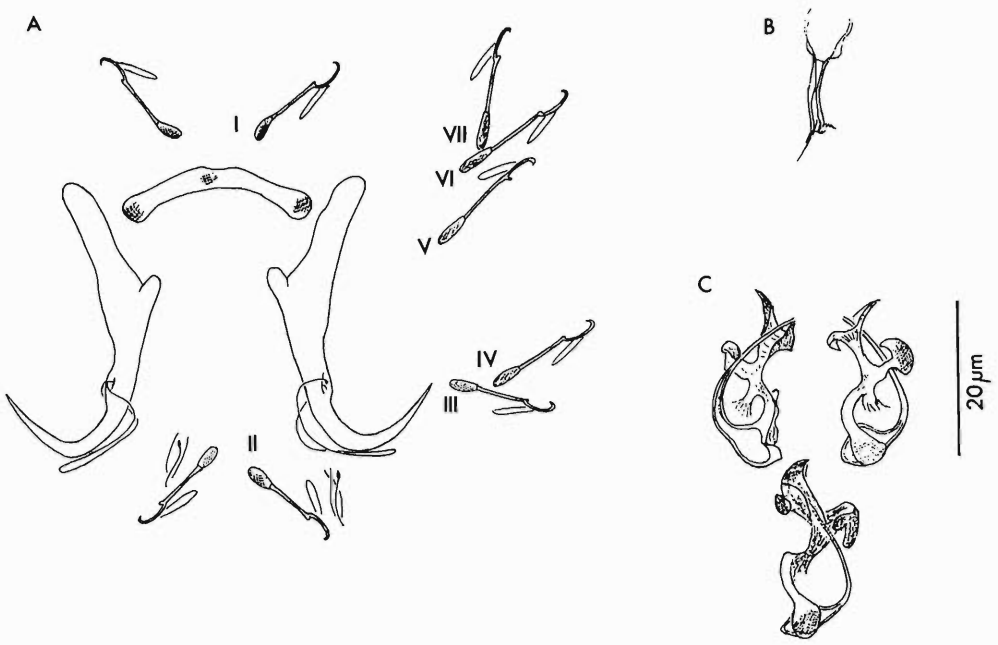


Figure 4. ***Dactylogyrus falcilocus*** **Guégan, Lambert, and Euzet, 1988. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets. Drawn after specimens from** ***Labeo parvus*** **from the Little Scarcies.**

Dactylogyrus brevicirrus Paperna, 1973

TYPE HOST AND LOCALITY: *Labeo victorianus*; Victoria lake, Uganda (Paperna, 1973).

OTHER RECORDS: *L. victorianus,* Nzoia river, lake Victoria system, Kenya; *L. forskali* and *Barilius niloticus,* lake Albert (Mobutu), Uganda and Zaïre; *Barbus perince,* Sonso river, lake Albert system, Uganda; *B. neglectus,* lake Edward, Uganda; *B. kersteni,* Jinja bay, lake Victoria, Uganda; *B. altianalis,* Nzoia river, Kenya; *Labeo cylindricus,* Ruaha river, Tanzania (Paperna, 1973, 1979).

NEW RECORDS: *Labeo parvus,* localities 3–5, 18, 20, 22–23, 25–26, 29, 31, 35.

SPECIMENS: Holotype, paratypes M.T. 35.706 (Paperna, 1973).

Dactylogyrus falcilocus Guégan, Lambert, and Euzet, 1988 (Fig. 4)

TYPE HOST AND LOCALITY: *Labeo coubie*; Niger system at Bamako, Mali.

OTHER RECORDS: *Labeo coubie,* Senegal system, Baoulé river at Missira and Dlaba, Mali (Guégan et al., 1988).

NEW RECORDS: *Labeo parvus,* localities 1, 3, 4, 7, 9–14, 22; *Barbus wurtzi,* localities 9–11.

SPECIMEN: Holotype MNHN 264 HC.

DESCRIPTION (based on 20 specimens in toto): Body length 400 (320–520); greatest width 80 (60–100) usually at level of ovary. Hamuli lengths: a: 36 (34–39); b: 23 (22–24); c: 1–3; d: 16 (14–18); e: 12–14. Dorsal transverse bar 23–27 long, 2–4 wide. Hook length (all pairs) 14–17; 4A: 7–10. Male copulatory apparatus (20–24) consisting of basal ampulla followed by a fine penis (18–23). Sclerotized bifid accessory piece (15–20) fixed to ampulla: proximal branch forming elbow; distal part in shape of small gutter with raised edges. Vagina with finely sclerotized wall, forming a pocket prolonged by hollow tube (10–14).

REMARKS: Specimens collected on *Labeo parvus* in numerous basins are similar to *Dactylogyrus pseudanchoratus micronchus* Paperna, 1979, from unidentified *Labeo* in the Ruaha river in Tanzania, except for the presence of a sclerotized vagina. These parasites are identified as *D. falcilocus* Guégan, Lambert, and Euzet, 1988, reported previously as specific to *L. coubie* in the middle Niger and upper Senegal basins. *Dactylogyrus falcilocus* is absent from *L. parvus,* which is sympatric with *L. coubie* in these 2 basins (Guégan et al., 1988). This monogenean is found on another cyprinid, *Barbus wurtzi,* in the Little

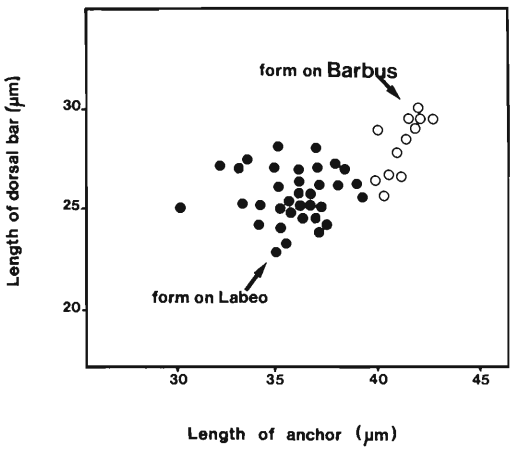


Figure 5. Scatter diagram representing length of anchor versus length of dorsal bar for 2 forms of *Dactylogyrus falcilocus*: form on *Labeo coubie* (Niger and Senegal basins) and *Labeo parvus* (short coastal rivers), and form on *Barbus wurtzi* (Little Scarcies basin) ($P < 0.05$).

Scarcies basin of Guinea and Sierra Leone (Guégan and Lambert, 1990; Lévêque and Guégan, 1990). A biometric analysis carried out on the parasites occurring on *L. coubie, L. parvus,* and *B. wurtzi* resulted in 2 distinct morphotypes: the first is that of individuals found on *Labeo* and the second on *Barbus* (Fig. 5).

Dogielius kabaensis sp. n. (Fig. 6)

TYPE HOST AND LOCALITY: *Labeo parvus*; Little Scarcies system, Kaba at Kouloundala, Republic of Guinea (locality 9).

OTHER RECORDS: *L. parvus,* localities 6–7, 10–11, 13–15; *Labeo alluaudi,* locality 14.

SPECIMENS: Holotype MNHN 454 HC, slide Tj 241; paratype MNHN 454 HC, slide Tj 241 bis.

DESCRIPTION (based on 19 specimens in toto): See Table 1. Male copulatory apparatus short, with double accessory piece: first piece initially thin, spoon-shaped with edges slightly raised; second piece more sclerotized, bifid distally. Penis slightly incurvate, tubular slides in elbow formed by outermost branch of accessory piece. Vagina forming small pocket, with finely sclerotized walls.

ETYMOLOGY: The specific name is derived from the name of the tributary where the species was initially found.

REMARKS: Paperna (1979) characterized 2 subspecies of *Dogielius junorstrema* Price and

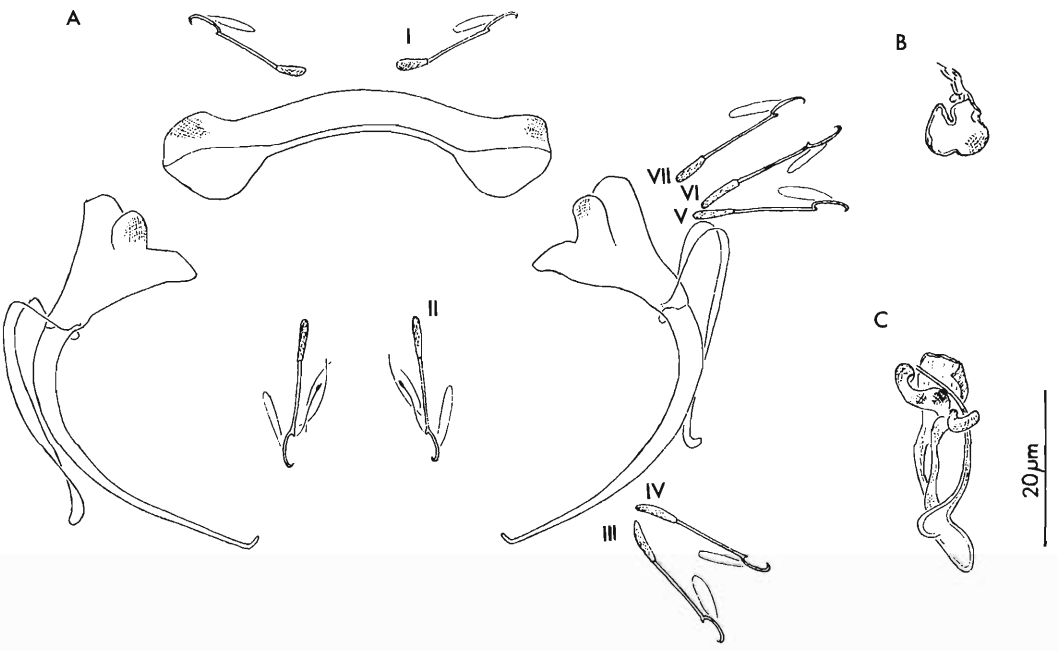


Figure 6. *Dogielius kabaensis* sp. n. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.

Table 1. Measurements of *Dogielius kabaensis* sp. n. in relation to the host species.

Locality:	Form on *Labeo parvus* ($N = 14$) Coastal rivers	Form on *Labeo alluaudi* ($N = 5$) Bagbwe river
Body length	370 (330–420)	320 (280–380)
Body width	70 (60–80)	60 (50–80)
Anchor lengths a	33 (30–36)	28–30
b	43 (41–45)	34–37
e	16 (14–17)	17–20
Dorsal bar length	50–56	40–42
Dorsal bar width	5–7	4–5
Hook lengths I	16–18	16–18
II	16–18	16–18
III	16–18	16–18
IV	16–18	16–18
V	20–22	18–20
VI	20–22	18–20
VII	20–22	18–20
4A	8–10	7–10
Copulatory organ length	28–31	30–32
Accessory piece length	19–22	20–24
Penis length	22–30	25–30

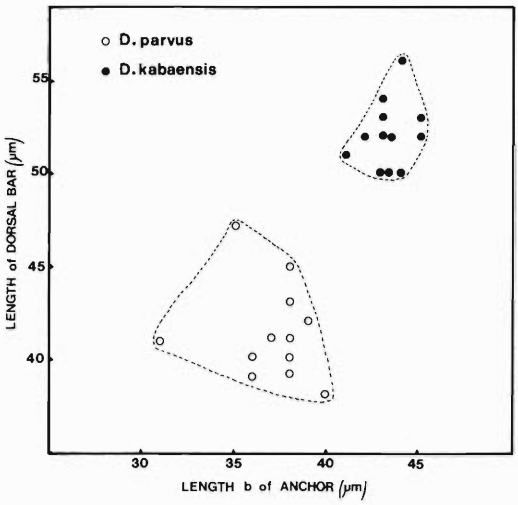


Figure 7. Scatter diagram of length b of anchor versus length of dorsal bar for 2 species of *Dogielius*: *D. parvus* from *Labeo parvus* (Niger and Senegal basins) and *D. kabaensis* sp. n. from *L. parvus* in short coastal rivers (Guinea, Sierra Leone, and Liberia) ($P < 0.05$).

Yurkiewicz, 1968: *D. junorstrema victorianus* from *L. victorianus* and *Barbus altianalis* in Kenya; *D. junorstrema ruahae* from *L. cylindricus* and an unclassified *Labeo* in Tanzania. Similarities of morphology and size of individuals described here and *D. junorstrema victorianus* Paperna, 1979, are apparent. Paperna (1979) felt that these morphs belong to the same species and that they are encountered in different hosts in different basins. These worms also have affinities with *Dogielius parvus* Guégan, Lambert, and Euzet, 1989, from *Labeo parvus* in the Niger and the Senegal basins (Guégan et al., 1989). Present specimens differ from *D. parvus* in the morphology of the accessory piece, the presence of a sclerotized vagina and size of the haptoral pieces (Fig. 7). Distinction is made between forms of *D. kabaensis* sp. n. occurring on *L. parvus* and *L. alluaudi*. We are of the opinion that they represent a complex of species which is difficult to characterize with usual anatomical criteria.

Dactylogyrus longiphalloides sp. n. (Fig. 8)

TYPE HOST AND LOCALITY: *Labeo alluaudi*; Bagbwe river near Yfin, Sierra Leone (locality 14).

SPECIMENS: Holotype MNHN 80 HF slide 95 Ti; paratype MNHN 80 HF slide 96 Ti.

DESCRIPTION (based on 7 specimens in toto): Body length 370 (250–460); greatest width 60 (40–80) usually at level of ovary. Hamuli lengths: a: 43 (40–45); b: 25 (24–28); c: 1–3; d: 20 (19–21); e: 12–14. Dorsal transverse bar 18 (17–20) long, 3–5 wide; vestigial ventral bar length 8–10 long. Hook lengths: I, II: 16 (15–18); III, IV: 15–16; V, VI, VII: 18 (17–19); 4A: 8–10. Male copulatory apparatus 50 (45–52), forming a tubular penis 40–46 long which becomes thinner towards its extremity. Bifid accessory piece 48–54 long, 2 branches connected by a fine sclerotized formation. Vagina finely sclerotized near the pore.

ETYMOLOGY: The species name is derived from the similarity of the copulatory organ to that of *Dactylogyrus longiphallus*.

REMARKS: *Dactylogyrus longiphalloides* found in *Labeo alluaudi* from the Bagbwe in Sierra Leone resemble *Dactylogyrus longiphallus* Paperna, 1973, from *L. parvus* in numerous basins in West Africa. Although they are of the same morphological type, the specimens presented differ from *D. longiphallus* insofar as they have larger haptoral features (length of a, length of d, lateral hooklets V, VI, VII), and copulatory apparatus. A scatter diagram is presented in which distinction can be made between the 2 groups

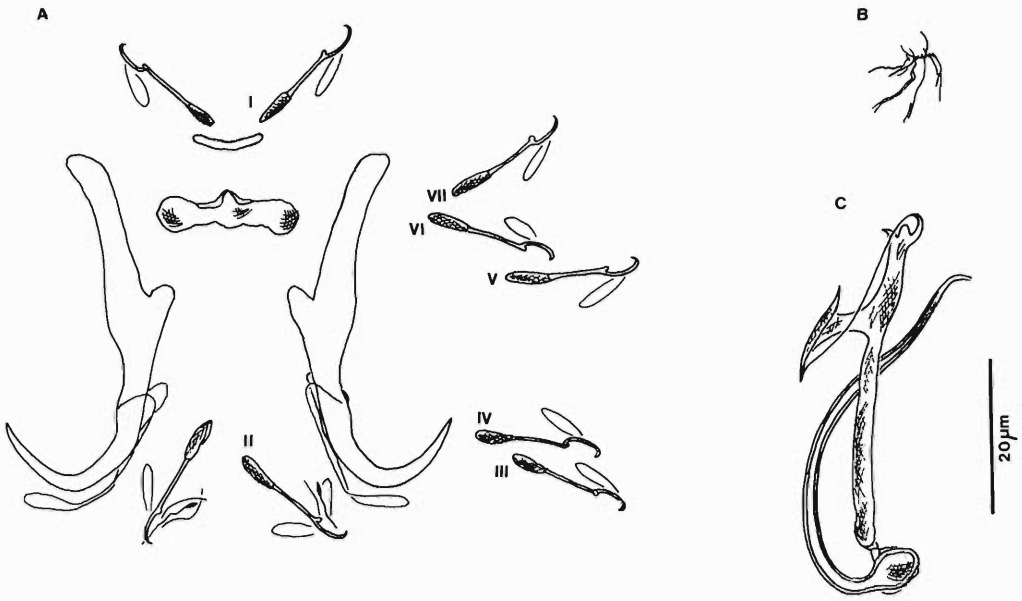


Figure 8. ***Dactylogyrus longiphalloides*** **sp. n. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.**

(*D. longiphallus* from *L. parvus* and *D. longiphalloides* from *L. alluaudi*) (Fig. 9). The fact that there is no overlapping between measurements of the genital parts leads us to consider that the 2 groups are well isolated.

Paperna (1979) observed similar variations according to host species and basin. He deduced that individuals of *D. longiphallus* from different isolated populations belong to a species complex (cf. discussion on *Dactylogyrus longiphallus*). The simultaneous presence of *D. longiphallus* on *L. parvus* and of *D. longiphalloides* on *L. alluaudi* in the Bagbwe basin in Sierra Leone suggests to us that there are 2 distinct but related species, each associated with a different but related host species. This monogenean was not found in Nipoué and Cavally *L. alluaudi* populations at the Liberia-Côte d'Ivoire border.

Dactylogyrus sematus sp. n. (Fig. 10)

TYPE HOST AND LOCALITY: *Labeo rouaneti*; Konkouré system, Konkouré river near Télimélé, Republic of Guinea (locality 7).

OTHER RECORDS: *L. rouaneti,* locality 8.

SPECIMENS: Holotype MNHN 450 HC, slide Tj 237; paratype 450 HC, slide Tj 237 bis.

DESCRIPTION (based on 26 specimens in toto): Body length 370 (220–670); greatest width 70 (40–110) usually at level of ovary. Hamuli lengths: a: 41 (39–43); b: 25 (23–27); c: (3–5); d: 20 (18–21); e: 14 (13–15). Dorsal transverse bar 19–23 long, 3–5 wide; vestigial transverse ventral bar 8–10 long. Hook lengths: I, II: 16–19; III, IV:

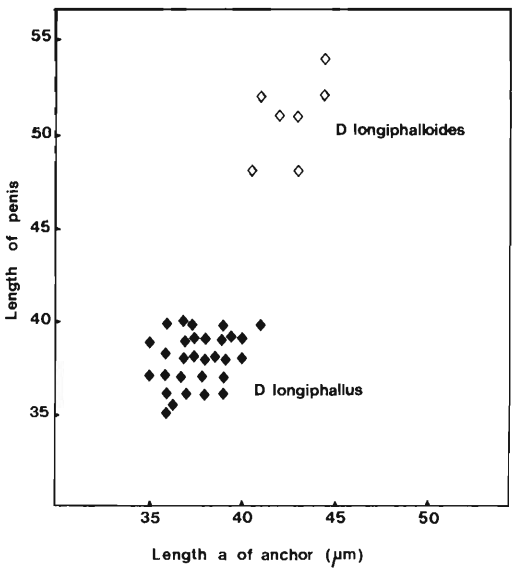


Figure 9. Scatter diagram representing length a of anchor versus length of penis for 2 species of ***Dactylogyrus*****:** ***D. longiphallus*** **from** ***Labeo parvus*** **(short coastal rivers) and** ***D. longiphalloides*** **sp. n. from** ***L. alluaudi*** **(Bagbwe river) (*P* < 0.01).**

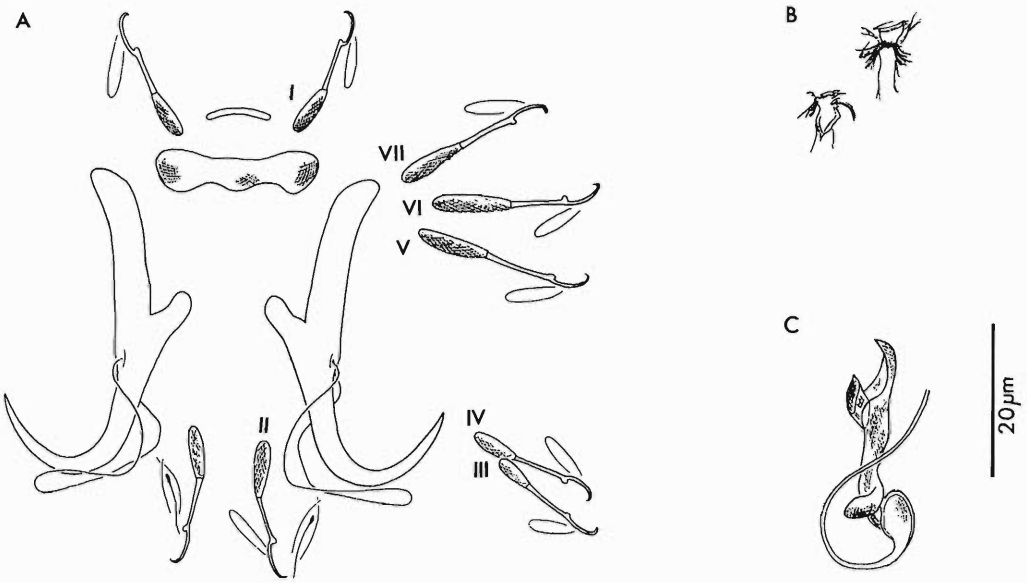


Figure 10. ***Dactylogyrus sematus*** **sp. n. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.**

15–17; V, VI, VII: 19–23; 4A: 8–10. Male copulatory apparatus starts with oval basal ampulla from which long, fine, convoluted penis originates (35–42). Sclerotized accessory piece attached to basal ampulla by stem, possessing proximal elbow, distal bifid expansion. Vagina is finely sclerotized near vaginal pore.

ETYMOLOGY: The species name is derived from the accessory piece, which is simple and bare in comparison to other species (Latin *sematus,* half full, half empty).

REMARKS: The male copulatory apparatus of this species is of the same morphotype as that of species of *Dactylogyrus* from *Barbus* spp. in southern Cameroon (Birgi and Lambert, 1987). However, details of morphology and size differ in parasites from *Labeo rouaneti. Dactylogyrus sematus* do not resemble any known parasite from *Labeo* or from great *Barbus* in West Africa.

Dactylogyrus jucundus sp. n. (Fig. 11)

TYPE HOST AND LOCALITY: *Labeo rouaneti*; Konkouré system, Konkouré river near Télimélé, Republic of Guinea (locality 7).

OTHER RECORDS: *L. rouaneti,* locality 8. *L. parvus,* localities 4, 7, and 14.

SPECIMENS: Holotype MNHN 451 HC, slide Tj 238; paratype MNHN 451 HC, slide Tj 238 bis.

DESCRIPTION (based on 15 specimens in toto): Body length 490 (250–630), greatest width 90 (40–120) usually at level of ovary. Hamuli lengths: a: 50 (47–55); b: 30 (26–33); c: 4 (3–6); d: 26 (22–29); e: 17–18. Dorsal transverse bar 22–26 long; vestigial ventral transverse bar 8–10 long. Hook lengths: I, II, III, IV: 18–20; V, VI, VII: 20–22; 4A: 8–10. Male copulatory apparatus (26–30) has an oval basal ampulla with long (40–47), curved penis. Sclerotized accessory piece (26–30) attached to ampulla by stem, finely sclerotized first shaft and characteristic main swelling forming a beak at the distal extremity. Vagina finely sclerotized near the pore.

ETYMOLOGY: The species name is derived from Latin, *jucundus,* charming, appealing.

REMARKS: The general morphology of the male copulatory apparatus is similar to that of *Dactylogyrus helicophallus* Paperna, 1973, found on *Labeo forskali* (type host) in Uganda, *L. victorianus* in Kenya and in 2 unclassified species of *Labeo* from the Ruaha river in Tanzania (Paperna, 1973, 1979). However, penis and accessory piece morphology and hooklet size are different. The genital morphology of these parasites also resembles that of *D. longiphallus gracilis* Paperna, 1979, from *Barbus ablabes* in Ghana (Paperna, 1979). However, *D. helicophallus* and *D. longiphallus gracilis* are distinctly different from *D. jucundus* in the size of the male copulatory apparatus and hamuli morphology and size. We report a sclerotized vagina, a feature not

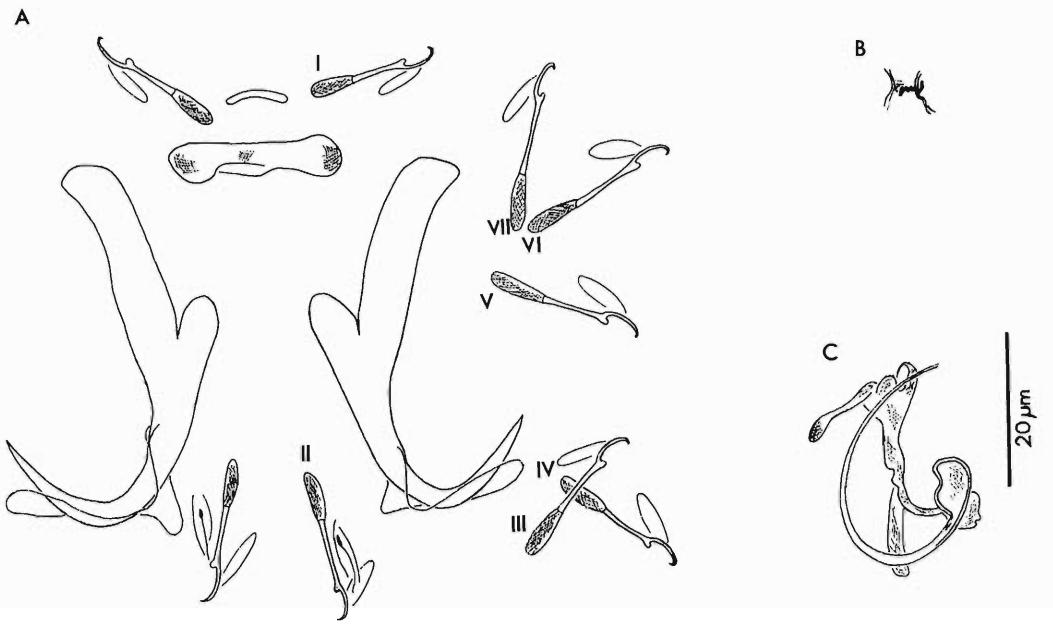


Figure 11. ***Dactylogyrus jucundus*** **sp. n. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.**

mentioned in the original descriptions of *D. helicophallus* and *D. longiphallus gracilis. Dactylogyrus jucundus* possesses a copulatory apparatus accessory piece of the same type as *D. decaspirus* Guégan, Lambert, and Euzet, 1988, a parasite of *L. coubie,* and *D. senegalensis* Paperna, 1969, found on *L. senegalensis* from the Niger and Senegal basins (Paperna, 1969; Guégan et al., 1988). However, individuals examined in this study differed in that they had a shorter, non-spiral penis.

Dactylogyrus omega sp. n. (Fig. 12)

TYPE HOST AND LOCALITY: *Labeo rouaneti*; Konkouré system, Konkouré river near Télimélé, Republic of Guinea (locality 7).

OTHER RECORDS: *L. rouaneti,* locality 8. *L. parvus*, localities 14 and 20.

SPECIMENS: Holotype MNHN 452 HC, slide Tj 239; paratype MNHN 452 HC, slide Tj 239 bis.

DESCRIPTION (based on 31 specimens in toto): Body length 530 (360–830); greatest width 100 (70–120) usually at level of ovary. Hamuli lengths: a: 35 (32–36); b: 28 (26–30); c: 5 (3–6); d: 12 (10–14); e: 14 (13–15). Dorsal transverse bar 24–28 long, 4–6 wide. Small longitudinal ventral sclerification (4–6). Hook lengths: I: 18–20; II: 20–22; III, IV, V, VI, VII: 15–16; 4A: 8–10. Male copulatory apparatus (35–42) has large basal ampulla from which runs a hollow penis (diameter 2–3), forming a loose single spiral. Ampulla bears finely sclerotized accessory piece in the shape of an elongated ring lying on either side of the cirrus. No sclerotized vagina observed.

ETYMOLOGY: The species name is derived from the similarity of the penis to the Greek letter Ω.

REMARKS: This parasite is found on *Labeo rouaneti,* mainly in the Konkouré basin. A low prevalence is found on *L. parvus.* Morphology of male copulatory apparatus is similar to that of *Dactylogyrus cyclocirrus* on *L. cylindricus* (type host) in Tanzania, on *L. victorianus* in Kenya, and in *L. coubie* and *L. senegalensis* in Ghana (Paperna, 1973). The haptoral pieces of our specimens are very similar to measurements of worms described on *L. victorianus* from the Nzoia river in Kenya. Paperna (1979) reported that individuals of *D. cyclocirrus* varied morphometrically depending on collection site and host species. *D. cyclocirrus* also was observed on *Labeo senegalensis* in West Africa (Guégan et al., 1988), but the parasite was specific to this host species. Monogeneans from *L. rouaneti* (Konkouré ba-

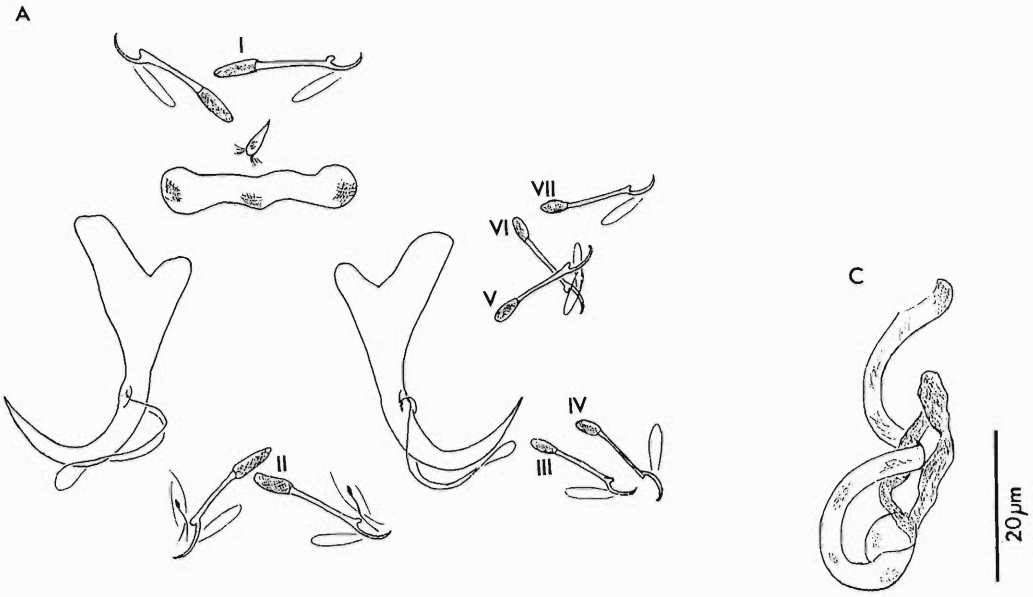


Figure 12. ***Dactylogyrus omega*** **sp. n. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.**

sin) and *L. senegalensis* (Niger and Senegal basins) are distinguished based on length of transverse bars versus total length of hamulus (Fig. 13). Although the individuals described here are morphologically similar to *D. cyclocirrus,* they are distinguished by a smaller penis diameter, different size of hamuli and geographical distribution.

Dogielius rosumplicatus sp. n. (Fig. 14)

TYPE HOST AND LOCALITY: *Labeo rouaneti*; Konkouré system, Konkouré river near Télimélé, Republic of Guinea (locality 7).

OTHER RECORDS: *L. rouaneti* and *L. parvus,* locality 8.

SPECIMENS: Holotype MNHN 453 HC, slide Tj 240; paratype MNHN 453 HC, slide Tj 240 bis.

DESCRIPTION (based on 24 specimens in toto): Body length 330 (250–450); greatest width 80 (70–90) usually at level of ovary. Hamuli lengths: a: 34 (32–36); b: 43 (41–45); e: 16 (15–18). Dorsal transverse bar 50–57 long, 8–10 wide. Hook lengths: I, II, III, IV, V, VI, VII: 20–22; 4A: 7–8. Male copulatory apparatus (30–36), with slightly incurvate penis, 25–30 long. Distal part of accessory piece is palette-shaped with lateral extensions, one of which is an elbow in which the penis slides. Vagina is well sclerotized around its opening, walls becoming less distinct.

ETYMOLOGY: The species name is derived from the combination of 2 Latin words that reflect the aspect of the accessory piece (*plico,* to bend, and *rosum* from *rodo,* deckle-edged, shabby).

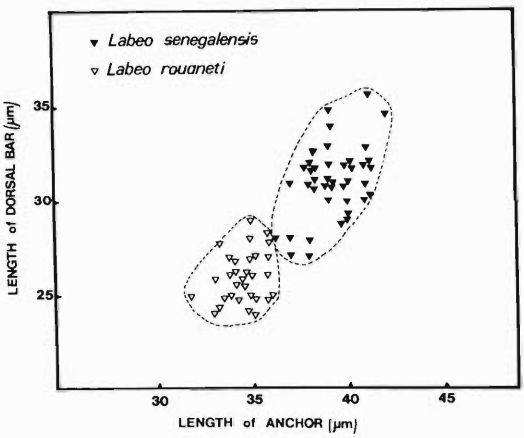


Figure 13. Scatter diagram of length of anchor versus length of dorsal bar representing ***D. cyclocirrus*** **from** ***Labeo senegalensis*** **(Niger and Senegal basins) and** ***D. omega*** **sp. n. from** ***L. rouaneti*** **(Konkouré basin) (*P* < 0.01).**

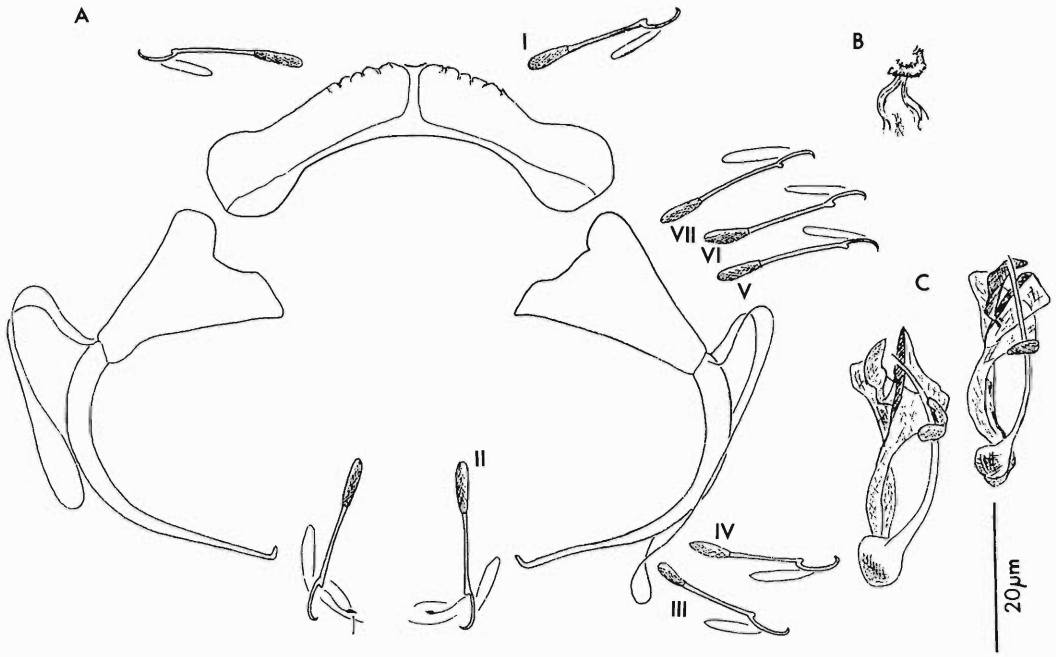
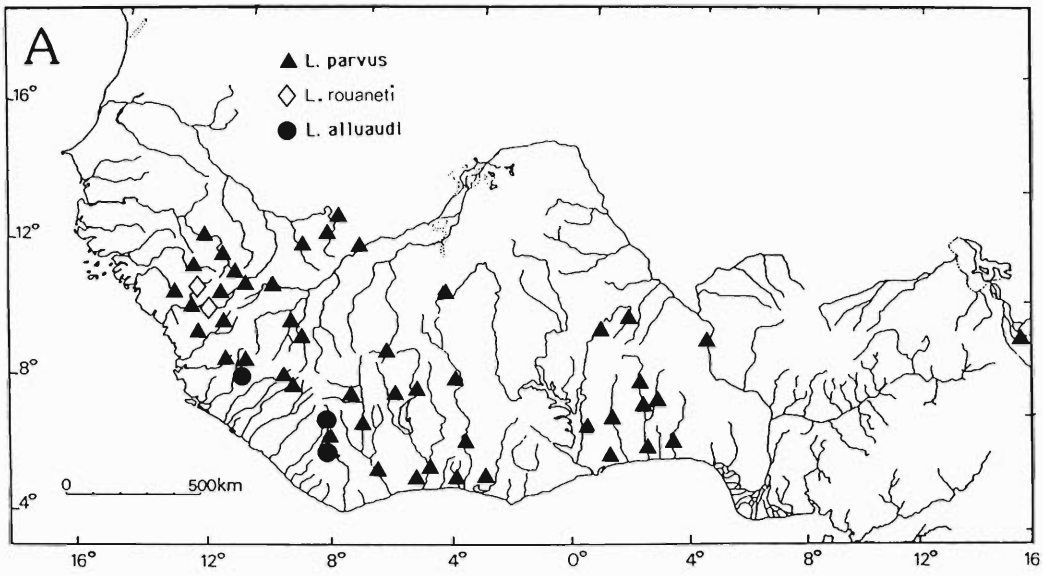


Figure 14. ***Dogielius rosumplicatus*** **sp. n. A. Haptoral apparatus. B. Vagina. C. Male copulatory organ. I to VII. Marginal hooklets.**

REMARKS: This monogenean resembles *Dogielius harpagatus* Guégan, Lambert, and Euzet, 1989, and *D. clavipenis* Guégan, Lambert, and Euzet, 1989, from *Labeo coubie* in the Niger and Senegal basins (Guégan et al., 1989). It displays the same morphological type of accessory piece but differs in hamuli size and morphology of the copulatory apparatus.

Discussion

Dactylogyridae described herein from *Labeo* (Teleostei: Cyprinidae) in coastal river basins in West Africa are different from those of *Labeo* in Sahel-Sudan basins in Niger and in the Senegal basin in Mali (Guégan et al., 1988, 1989). Affinities between the different species of *Labeo* and those observed in their parasitofauna are compared. With regard to biogeographical features, we noted presence of monogeneans in populations of *L. parvus* in the large Sahel-Sudan basins, in Côte d'Ivoire systems, in Ghana, Togo and Benin systems, and their absence in the short coastal basins running from the Guinean ridge in Guinea, Sierra Leone, and Liberia. The distribution of *Labeo parvus* (Fig. 15a) and 2 species of dactylogyrids, *Dactylogyrus longiphallus* and *D. brevicirrus* (Fig. 15b), are compared. The monogenean populations of *L. parvus* from the Guinean short coastal basins differ from populations of adjacent basins in that *D. brevicirrus* is absent. It is presumed that this is the result of a "host switching" followed by speciation on a new host (Guégan and Agnèse, 1990). A comparable phenomenon is observed for monogeneans on cyprinid species, genus *Barbus,* where there is exclusion of very primitive parasite forms on *B. petitjeani, B. sacratus,* and *B. parawaldroni* from small coastal basins and their replacement by more derived species in barbels of the *B. bynni* group in peripheral basins (Guégan and Lambert, 1990; Lévêque and Guégan, 1990). The variations in parasitism observed between the different populations of the *L. parvus* complex suggest that fishes in the Guinean zone have not been affected by invasion of *D. brevicirrus.* The parasitism observed is certainly similar to the original parasitofauna of the ancestor(s) of *L. parvus.* Parasite distribution defines 2 different biogeographical regions marked by isolated river systems. Our study concluded that there are considerable differences in the parasitofauna in these 2 host populations which are separated by a natural barrier. This supports Pellegrin's (1908) hypothesis distinguishing *L. obscurus* limited to a few basins in Guinea and Sierra Leone, from all the other populations of *L. parvus.* However, it



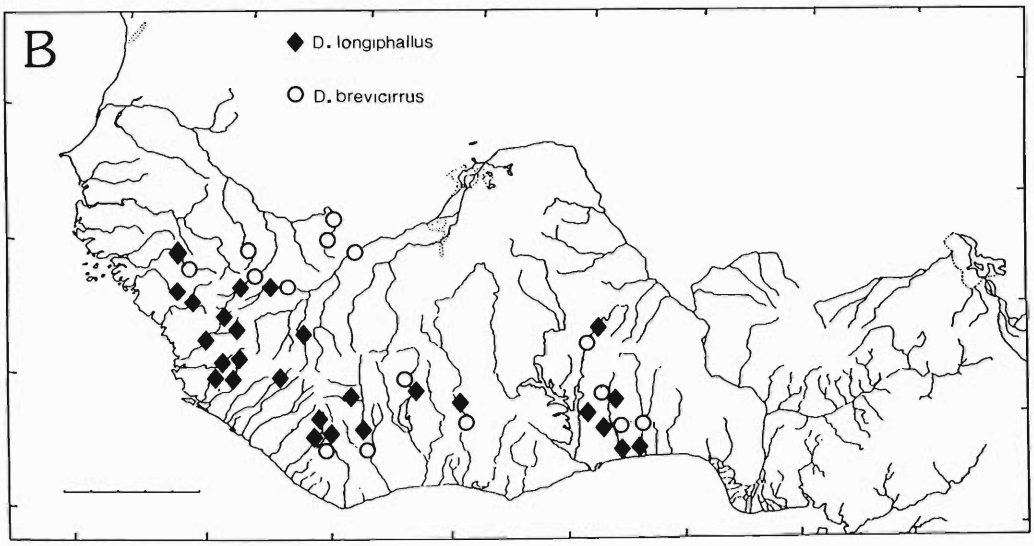


Figure 15. Area of distribution of 2 species and 1 complex of species of *Labeo*: *L. rouaneti, L. alluaudi,* and *L. parvus* complex in West Africa (Fig. 15a), and distribution of 2 species of *Dactylogyrus*: *D. longiphallus* and *D. brevicirrus* identified on *L. parvus* complex (Fig. 15b).

is impossible to specify the degree of divergence acquired by the various populations of the *L. parvus* complex since only a study of breeding would enable evaluation of their taxonomic levels.

Labeo rouaneti, which is considered to be phylogenetically close to *L. coubie* (Reid, 1985), displays a branchial parasitofauna that was not a precise reflection of the assumed affinity of these hosts. *Dactylogyrus jucundus* sp. n. and *Dogielius rosumplicatus* sp. n. are similar to 2 derived parasite forms found on *L. coubie.* The same does not apply to the other monogeneans present on *L. rouaneti*: *Dactylogyrus omega* sp. n. is morphologically close to *D. cyclocirrus* described on several cyprinids in East Africa (Paperna, 1973, 1979), and which we report in West Africa on *L. senegalensis* (Guégan et al., 1988); *D. sematus* sp. n. belongs to a completely original type of dactylogyrid monogenean from West African species of *Labeo* since it is similar to certain *Dactylogyrus* on small *Barbus* in southern Cameroon (Birgi and Lambert, 1987). In a biogeographical study on the ichthyological communities of the various basins in West Africa, Hugueny (1989) demonstrated the faunistic similarities between Guinea and the Cameroon-Gabon area. The presence of *D. sematus* sp. n. in a Guinean basin supports this hypothesis.

The monogeneans found on *L. alluaudi* in Sierra Leone are closely related to those on *L. parvus* populations in short coastal basins. It can be imagined that the parasites evolved in a concomittant way, speciation of the fish being followed by that of the monogeneans. On the basis of the parasite criterion, we accept the theory put forward by Reid (1985) who classified *L. alluaudi* as part of the *L. parvus* group. The absence of monogeneans in the *L. alluaudi* populations of the rivers Cavally and Nipoué might be explained by the ecological conditions of this fish (small rapids in the upper course of the river).

Acknowledgments

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Early Development of Larval *Taenia polyacantha* in Experimental Intermediate Hosts

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ABSTRACT: The early larval development and migration route of *Taenia polyacantha* were examined using oral inoculation of oncospheres into red-backed voles and Mongolian gerbils. The larvae were recovered in the wall of the small intestine and in the mesenteric lymph nodes by 5 days postinfection (PI) and from the peritoneal cavity after 6 days PI. These results suggest that the larval cestodes developed initially in the wall of the small intestine and the mesenteric lymph nodes, and later migrated to the peritoneal cavity. Although the development of the parasite was quite similar in the 2 host species, pathological changes were different. In Mongolian gerbils, these changes were slight, but in red-backed voles, they were marked and fatal. In addition to oral inoculation, hatched oncospheres were injected intraperitoneally and subcutaneously into red-backed voles, Mongolian gerbils, and AKR/J mice. Larval development took place at the injection sites in gerbils and mice, but was delayed and abnormal. Some of the parasites in the injection site showed abnormal numerous budding. High pathogenicity was shown after subcutaneous and intraperitoneal injection as well as after oral inoculation.

KEY WORDS: *Taenia polyacantha,* rodents, migration route, larval development, abnormal development, histopathology, pathogenicity.

The larval *Taenia polyacantha* Leuckart, 1856, proliferates asexually and the metacestodes are found in the peritoneal and pleural cavities of rodents (Schiller, 1953; Rausch, 1959a; Murai and Tenora, 1973; Slais, 1973; Wiger et al., 1974; Tenora et al., 1979). Rausch and Fay (1988a) reported that the migration of postoncospheral stages was via the portal vein to the liver, and that initial larval development and formation of the primary vesicle took place in the liver. The production of secondary vesicles occurred after migration to the peritoneal cavity. Fujita et al. (1990) reported on the susceptibility and mortality of various small mammals to infection by *T. polyacantha* after oral inoculation of eggs. Red-backed voles and cotton rats showed high mortality at the early phase of infection. In those hosts, the sites of early larval development and the production of primary and secondary vesicles were different from those reported by Rausch and Fay (1988a).

In this study we examined the route of migration and development of the larvae, and assessed pathological changes in red-backed voles and Mongolian gerbils. In order to determine whether the postoncospheral development of *T. polyacantha* is pathogenic, oncospheres were inoculated parenterally in red-backed voles, AKR/J mice, and Mongolian gerbils.

Materials and Methods

CESTODE: Metacestodes were isolated from northern voles, *Microtus oeconomus,* trapped at Savoonga, St. Lawrence Island, Alaska, in 1988 and 1989. In the laboratory, they were then transplanted surgically into the peritoneal cavity of Mongolian gerbils, *Meriones unguiculatus,* which were maintained for 3 months. Eight mature metacestodes were then administered orally to a cestode-free mongrel dog. Gravid segments expelled in feces of the dog were collected daily and stored at 4°C in saline containing penicillin, streptomycin, and fungizone (Mitchell et al., 1977; Williams et al., 1981). Eggs from the gravid segments were used within 1 month of collection.

EXPERIMENTAL ANIMALS: Mongolian gerbils and AKR/J inbred mice were bred and maintained in our laboratory. Red-backed voles, *Clethrionomys rufocanus bedfordiae,* were trapped in a shelter-belt near Sapporo City, Hokkaido, and maintained in our laboratory at 22–24°C.

EXPERIMENT 1 (observations on early larval migration): Red-backed voles (18 ♂; age not determined) and Mongolian gerbils (15 ♂, 9 ♀; 4–8 wk old) were used. Individual hosts were inoculated with 5,000 to 100,000 eggs by stomach-tube under light ether anesthesia, and killed by exsanguination under ether anesthesia at 12 hr postinfection (PI) and at 24-hr intervals up to 6 days PI; animals that died were examined at different intervals. Washings from the peritoneal

cavity were examined for larval cestodes by means of a dissecting microscope (at 35×). The recovered larvae were fixed in 10% formalin and mounted in glycerin jelly. The small intestine was divided into 5 equal parts, and the middle portion of each, and all abdominal and thoracic organs, were fixed in 10% buffered neutral formalin and embedded in paraffin by standard methods. Paraffin sections, cut at 4–6 μm, were stained with hematoxylin-eosin (HE) and Alcian blue-periodic acid-Schiff (AB-PAS) for microscopic examination. Sixteen to 20 serial sections of organs in which larval cestodes were found were studied, and the maximal lengths and widths of the vesicles were recorded. For electron microscopy, the small intestines of rodents of both species were fixed on day 5 PI in 3.0% glutaraldehyde in 0.1 M cacodylate buffer, and then postfixed in 1.0% osmium tetroxide in 0.1 M cacodylate buffer. The segments of the intestines were dehydrated in graded ethanol and embedded in Quetol-812. Ultrathin sections were cut and stained with uranyl acetate and lead citrate, and subsequently observed and photographed with an HITACHI HU-12A electron microscope.

EXPERIMENT 2 (parenteral injection of oncospheres): Mongolian gerbils (6 ♂, 18 ♀; 6–8 wk old), AKR/J mice (8 ♂, 7 ♀; 4 wk old), and red-backed voles (5 ♂, 3 ♀; age not determined) were used. The experimental design is shown in Table 1. The oncospheres were hatched with artificial intestinal fluid (Silverman, 1954); 1,000 or 20,000 oncospheres were injected subcutaneously or intraperitoneally in sterile saline. Individual animals were killed by exsanguination on days 19 and 21 PI and autopsied immediately after death. Sites of injection were washed with saline and examined for presence of vesicles using a dissecting microscope. Vesicles recovered were fixed in 10% formalin and stained with Schneider's aceto-carmine for morphological examination. If gross lesions were observed in the tissues, sections were examined microscopically.

To compare larval development in the subcutaneous tissue and in the peritoneal cavity, and to define relative pathogenicity, Mongolian gerbils also were inoculated orally with 300 or 500 eggs.

Results

Experiment 1

Early larval development was discerned only in the wall of the small intestine and in the mesenteric lymph nodes in voles and gerbils. Average size of the developing larvae and their locations are shown in Table 2.

RED-BACKED VOLES: Developing larvae were found in the lamina propria of the small intestine between 12 hr PI and 5 days PI. They were present in the submucosa or passing through the walls of the lymphatic lacteal on the third day PI (Figs. 1, 2). On day 4 PI, they had penetrated further and were recovered mainly in the submucosa and muscularis, as well as having reached the afferent lymph vessels and the peripheral and marginal sinuses of the mesenteric lymph nodes. After 5 days PI, some larvae were found in the peritoneal cavity, and from the sixth day PI they were no longer observed in the wall of the small intestine or mesenteric lymph nodes.

Table 1. The number of eggs or oncospheres of *Taenia polyacantha* in each inoculation route.

	Dose of inoculum	No. of hosts examined
Oral inoculation (shelled eggs)		
Mongolian gerbils	300	6
	500	6
Subcutaneous injection (oncospheres*)		
Mongolian gerbils	1,000	4
AKR/J inbred mice	1,000	8
Red-backed voles	20,000	4
Intraperitoneal injection (oncospheres*)		
Mongolian gerbils	1,000	6
AKR/J inbred mice	1,000	7
Red-backed voles	20,000	4

* Oncospheres were hatched artificially.

By 2 days PI, the early vesicle contained several prominent nuclei and PAS-positive granules, and was always surrounded by a halo-like, amorphous area, about 4–8 μm in width, within which was AB-positive material. Concurrent with a great increase in size of larva between days 2 and 3 PI, the amorphous area faded out almost completely. At this time, marked cellular differentiation occurred; a central cavity began to develop and the primary vesicle was formed. A few primary vesicles with buds were recovered in the wall of the small intestine on day 5 PI. On day 6 PI, the vesicles in the peritoneal cavity had produced some buds, and numerous host cells covered their surfaces (Fig. 3).

Between days 2 and 3 PI, slight hemorrhage and infiltration by inflammatory cells, mainly neutrophils and mononuclear cells, were observed in the wall of the small intestine and, at this time, blood-tinged ascitic fluid began to accumulate. These changes, especially extensive hemorrhage and purulent inflammation mainly surrounding the vesicles, became prominent in the wall of the small intestine, and thereafter in the mesenteric lymph nodes (Figs. 4, 5). Electron microscopically, it was determined that numerous neutrophils adhered closely to the surface of the tegument of the vesicles, and the number of cytoplasmic granules decreased (Fig. 6). The volume of ascitic fluid increased. After 4 days PI, inflammatory cells, mainly neutrophils accumulated in the mesenteric lymph nodes and

Table 2. Size and location of early larval stage of *Taenia polyacantha* in gerbils and voles.

Host		Days postinfection			
		0.5	1	2	3
Gerbil	Size (μm)*	21.0 × 14.8	22.2 × 17.8	38.0 × 25.2	61.5 × 35.5
	Location**	SI (LP) MLN	SI (LP) MLN	SI (LP) MLN	SI (LP ~ SM) MLN
Vole	Size (μm)*	22.2 × 14.6	23.8 × 16.3	36.8 × 27.8	85.7 × 62.8
	Location**	SI (LP)	SI (LP)	SI (LP)	SI (LP ~ SM)

* Average maximum length and width of each parasite measured in histological sections.
** SI = small intestine; LP = lamina propria; SM = submucosa; M = muscularis; MLN = mesenteric lymph node; PC = peritoneal cavity.
*** External budding.

spleen, along with hemorrhage, and the number of lymphocytes, decreased in these lymphatic organs.

MONGOLIAN GERBILS: By 3 days PI, developing larvae were recovered in the lamina propria and submucosa of the small intestine, especially in the jejunum and ileum. After 12 hr PI, they were found in the afferent lymph vessels and marginal sinuses of the mesenteric lymph nodes. On day 6 PI, vesicles were found in the peritoneal cavity.

The early larval development in gerbils was similar to that in the red-backed voles, with the larvae showing great enlargement between days 2 and 3 PI, and with the beginning development of a central cavity and primary vesicles. On day 6 PI, vesicles recovered from the peritoneal cavity displayed some buds.

Microscopically, a slight infiltration by inflammatory cells was evident in the lamina propria and submucosa of the small intestine after day 1 PI. Small accumulations of inflammatory cells were scattered focally in the dilated marginal sinus and capsule of the mesenteric lymph nodes, especially around the larvae, between days 4 and 6 PI (Fig. 7). A slight accumulation of turbid ascitic fluid with fibrin occurred on day 5. After 4 days, a few neutrophils adhered to the surfaces of the vesicles, and on day 5, their pseudopods were loosely attached to the fragments of microvilli (Fig. 8).

Experiment 2

Almost all of the Mongolian gerbils inoculated orally with 300 and 500 eggs were alive until the scheduled date of autopsy, but almost all those injected parenterally with 1,000 and 20,000 oncospheres died before 21 days PI. Development and pathological changes associated with the routes of inoculation are described below.

Oral inoculation

MONGOLIAN GERBILS: Ten to 58 ($\bar{x} = 31$) secondary vesicles were recovered from the peritoneal cavity in 5 of 12 infected gerbils necropsied on days 19 and 21 PI. Most of the larvae on day 19, ranging from 1.8 to 2.4 mm in length and 1.6 to 1.8 mm in diameter, had developed rostellar cones, and about half of them exhibited 2 rows of developing rostellar hooks (Fig. 9). Macroscopically, the infected gerbils exhibited a slight accumulation of turbid ascitic fluid, with fibrous adhesions on the serosa of abdominal organs.

Subcutaneous injection

MONGOLIAN GERBILS: Larval cestodes were recovered from the injection site of 3 of 4 gerbils that died between 12 and 19 days PI. Most of those by day 19 PI had formed single secondary vesicles, 88–272 μm in length and 80–152 μm in diameter; some still consisted of aggregations of secondary vesicles with less than 35 buds, and ranged from 368 to 552 μm in length by 208 to 360 μm in diameter (Fig. 10). An accumulation of nuclei was observed at the distal ends of detached secondary vesicles. At the site of injection, marked hemorrhage and edema were noted, and microscopically extensive necrosis and infiltration by eosinophils and neutrophils were observed in the dermis, subcutaneous tissues, and underlying skeletal muscles.

AKR/J MICE: Four mice died between 9 and 19 days PI. Vesicles were recovered from the injection sites of 6 of 8 animals. On days 9 and 11 PI all had formed single vesicles, apparently

Table 2. Continued.

Days postinfection		
4	5	6
99.0 × 54.6 MLN	123.4 × 65.0 MLN	990 × 340*** MLN PC
100.6 × 65.6 SI (LP ~ M), MLN	159.2 × 80.8*** SI (LP ~ M), MLN, PC	335 × 212*** PC

a primary vesicle, and most of those on days 19 and 21 PI had abnormally developed aggregations of secondary vesicles, with 32 to more than 50 buds; the aggregations ranged from 440 to 1,180 μm in length, and 200 to 980 μm in diameter. Nuclei had accumulated at the distal ends of almost all secondary vesicles, and a few were developing invaginated canals (Fig. 11). Gross lesions at sites of injection were similar to those in gerbils: extensive necrosis, hemorrhage, and a slight accumulation of inflammatory cells between the epidermis and underlying skeletal muscles were observed microscopically.

RED-BACKED VOLES: Two of 4 infected voles died between 5 and 8 days PI. They exhibited mild ascites with blood; extensive edema and slight necrosis were observed in the subcutaneous tissue. Larval cestodes were found in only 1 vole, which died on day 5 PI. In that animal, numerous ecchymoses were present on the serosa of the small intestine. Microscopically, the larvae consisted of primary vesicles ranging from 60 to 112 μm in length, and 40 to 64 μm in diameter. They were found mainly in the hemorrhagic lesions between the lamina propria and the muscularis. Slight hemorrhage and focal degenerative changes also occurred in the mesenteric lymph nodes.

Intraperitoneal injection

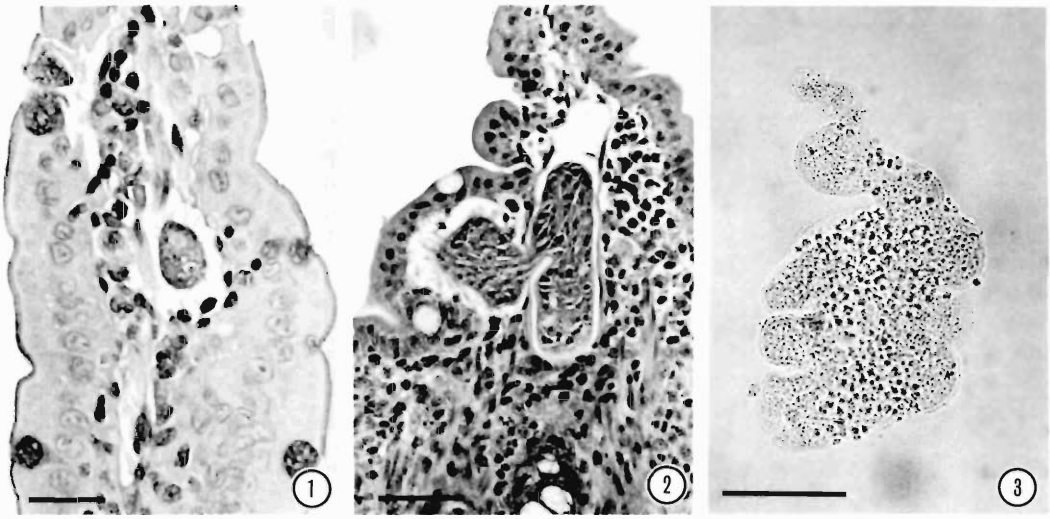
MONGOLIAN GERBILS: All gerbils died between 15 and 17 days PI, and larval cestodes were recovered from the peritoneal cavities. Almost all of the larval cestodes on day 17 formed single primary vesicles or 2 secondary vesicles, which were rounded and subspherical in form, and ranged from 112 to 416 μm in length and 96 to 224 μm in diameter. Some had formed abnormally minute aggregations of secondary vesicles, with 16 to more than 50 buds, which ranged from 384 to 584 μm by 240 to 442 μm in diameter; their surfaces were covered with numerous host cells (Fig. 12). Macroscopically, slight yellowish or turbid ascitic fluid was present, and all abdominal organs were congested.

AKR/J INBRED MICE: Six of the 7 infected mice died between 11 and 15 days PI; the last was necropsied on day 21 PI. Developing larvae were recovered from the peritoneal cavities of 6 mice. By day 15, the larvae had formed single or numerous secondary vesicles, all of similar size, with the aggregations ranging from 384 to 1,096 μm in length and 252 to 704 μm in diameter. Accumulations of nuclei at the distal ends of the vesicles had formed on day 12 PI. On day 21 PI, about half of the vesicles had detached, and some had developed invaginated canals which ranged from 184 to 216 μm in length by 104 to 128 μm in diameter.

RED-BACKED VOLES: Three of the 4 infected voles died by the seventh day PI. They exhibited mild ascites, with the fluid stained by blood, and all of the abdominal organs were congested. Slight hemorrhages had occurred in the mesenteric lymph nodes, but no larvae could be found microscopically in those lesions. Primary vesicles with central cavities were found under the phrenic serosa of the liver in only 1 vole dead on day 5 PI; they ranged from 136 to 200 μm in length by 72 to 112 μm in diameter. Focal accumulations of inflammatory cells and necrosis surrounded the larvae (Fig. 13). In the hepatic lymph nodes, a decrease in the number of lymphocytes had occurred, caused by focal degenerative change and hemorrhage.

Discussion

After the eggs of taeniids are ingested, the oncospheres hatch and become activated in the small intestine, after which they penetrate into the mucosal epithelium. The usual route of migration of the oncospheres is via the portal vein or lymphatic vessels. For example, it is well known that the oncospheres of *Taenia hydatigena, T. taeniaeformis, T. pisiformis,* and *Echinococcus multilocularis* migrate to the liver via the portal vein (Olsen, 1974). Concerning *T. polyacantha* in *Microtus oeconomus,* Rausch and Fay (1988a) reported that oncospheres migrate from the small intestine to the liver via the portal vein, and that early larval development takes place in the liver. Furthermore, Dorosz (1968) and Tenora et al. (1988) reported that metacestodes of *T. polyacantha* are localized under the



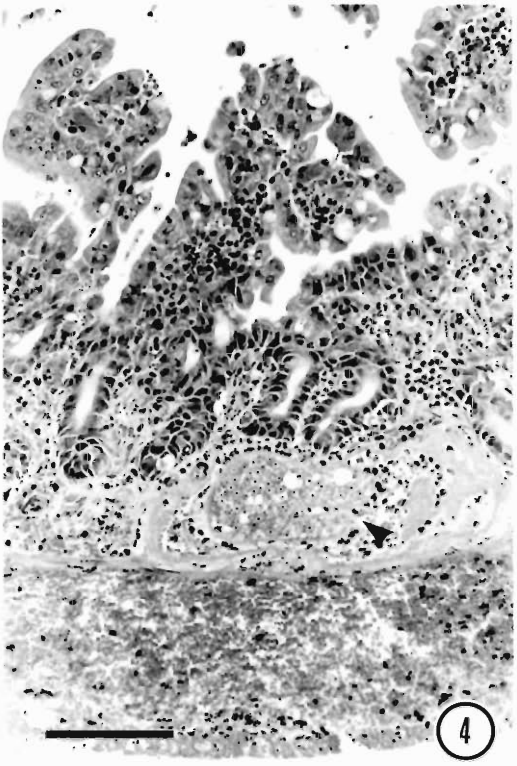
Figures 1–3. Larval *Taenia polyacantha* in red-backed voles. 1. Oncospheres situated in the lamina propria of the small intestine at 12 hr PI. AB-PAS stain. Scale bar = 20 μm. 2. The embryo passing through the wall of the lymphatic lacteal from the lamina propria, day 3. HE stain. Scale bar = 50 μm. 3. Developing secondary vesicles in the peritoneal cavity, day 6. Scale bar = 100 μm.

hepatic serosa of naturally infected hosts. These reports suggest that the liver is the typical site of early development of the larval cestode. However, in the rodents used in the present study, early development of the larvae was found to take place in the wall of the small intestine and in the mesenteric lymph nodes. Fujita et al. (1990) reported that larval *T. polyacantha* in cotton rats, *Sigmodon hispidus,* were recovered from the same organs as in gerbils and red-backed voles on days 8 and 10 PI. It is suggested that the oncospheres had a predilection for the wall of the small intestine and the mesenteric lymph nodes, and that those sites were peculiar to *T. polyacantha.* Since a few larvae were recovered from the liver of a red-backed vole inoculated intraperitoneally, the liver of that rodent appears also to be a site of development of the metacestode. Many larvae were situated in the afferent lymph vessels and marginal sinus of the mesenteric lymph nodes in gerbils and red-backed voles. We considered that the larvae could not migrate farther via the lymphatic system, because their size was too great relative to the diameter of the lymph vessels. The migration route of oncospheres in the present study was different from that reported by Rausch and Fay (1988a) in *Microtus oeconomus.* The isolates of *T. polyacantha* used in both investigations were evidently the same, having been obtained from voles, *M. oeconomus,* collected on St. Lawrence Island, in the Bering Sea. The differences in migration routes of the oncospheres appear to be related to differences in species of rodents used as experimental intermediate hosts.

Budding of the primary vesicles in the wall of the small intestine and in the mesenteric lymph nodes was observed by us from day 5 PI. Rausch and Fay (1988a) suggested that budding occurred after the early-stage vesicles had migrated from the liver into the peritoneal cavity; they reported that the larvae in the liver on day 5 PI were only

→

Figures 4–6. Pathological changes caused by larval *Taenia polyacantha* in the red-backed vole, day 5 PI. 4. Extensive hemorrhage and accumulation of numerous inflammatory cells surrounding the budding larva (arrowhead) in the wall of the small intestine. HE stain. Scale bar = 100 μm. 5. Marked decrease in the number of lymphocytes caused by extensive hemorrhage and degenerative changes in tissues around the larval cestode (arrowhead) in the mesenteric lymph node. AB-PAS stain. Scale bar = 100 μm. 6. Transmission electron micrograph of the larval cestode in the injured muscularis of the small intestine. Numerous neutrophils (N) and macrophages (M) closely attached to the surface of the vesicle (P). Scale bar = 5 μm.



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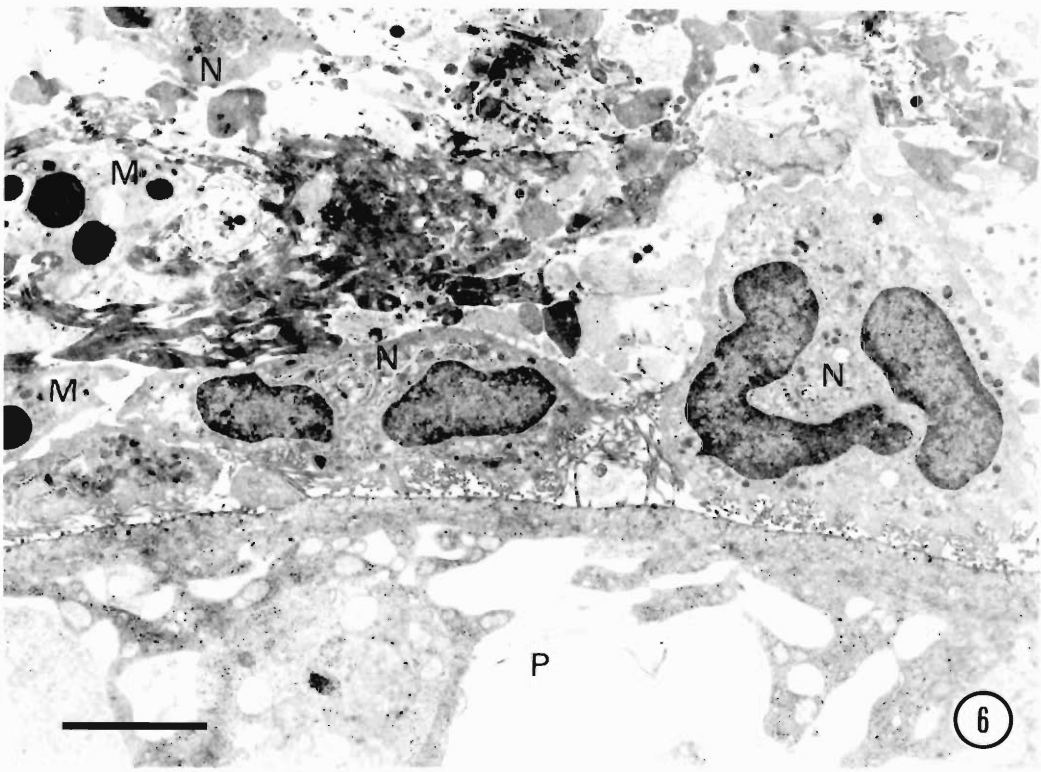

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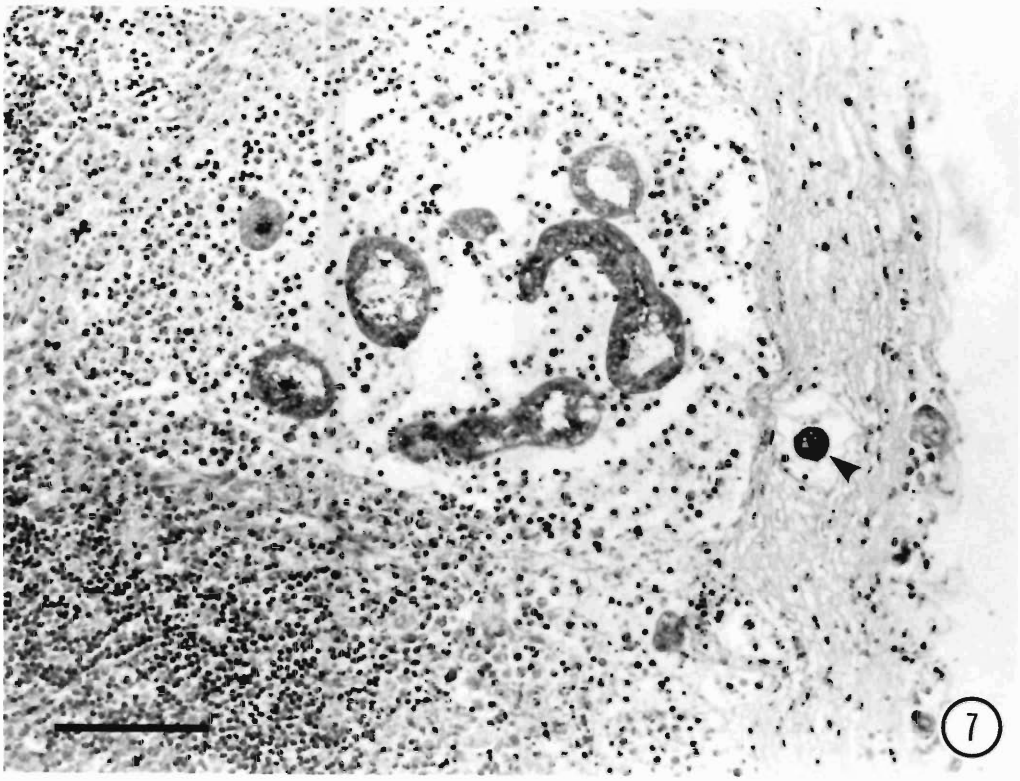

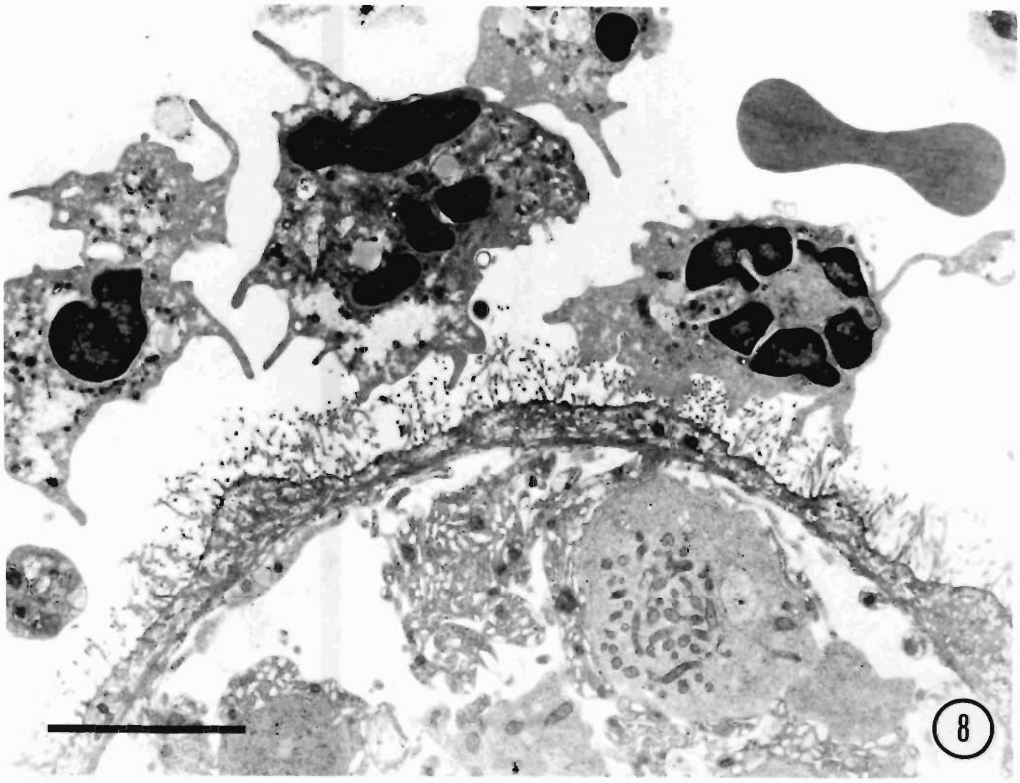

19 and 22 μm in greater diameter. In the present study, the average size of larvae on day 5 PI was 123 μm in length and 65 μm in diameter; thus, they were much larger than those described by Rausch and Fay (1988a).

Although the early development of the larval cestode was quite similar in gerbils and red-backed voles, it produced different pathological changes in the 2 hosts. In the gerbils, the tissue-response was slight, but in red-backed voles, marked purulent inflammation occurred, and numerous neutrophils attached closely to the tegumental surface of the larvae. Such changes in red-backed voles were not proportional to the number of eggs inoculated. Rausch and Fay (1988b) suggested that infections involving more than 5 eggs might be fatal, because of the inflammatory response evoked by the migration of the vesicles into the peritoneal cavity. The pathogenicity of other taeniid cestodes, i.e., *T. twitchelli* and *T. mustelae* is proportional to the number of eggs inoculated and the lesions caused by migrations of the larvae (Freeman, 1956; Rausch, 1959b). We considered from the present results that the high degree of pathogenicity and the high mortality in red-backed voles might be due to the greater susceptibility of that host, and marked pathological changes caused by the development and migrations of the larvae were the main cause of death. Since larvae developing in the subcutaneous tissues also were quite pathogenic, they possibly secrete some deleterious substances. Shield et al. (1973) reported that 8-day larvae of *T. pisiformis* exhibited PAS-positive secretory cells of large size, which had some effect in the migration of the larvae. In the larval stage of *T. polyacantha,* numerous PAS-positive cells of small size were observed, but the larger cells described by Shield were not observed. The mechanisms of pathogenesis in the case of *T. polyacantha* remain unclear.

When oncospheres of *T. hydatigena, T. saginata, T. ovis,* and *Echinococcus granulosus* were inoculated subcutaneously and intraperitoneally into the intermediate hosts, development of the larvae took place at the site of inoculation (Gem-

Figure 9. Advanced stage of secondary vesicle, showing the rostellar cone (arrow) and developing hooks, day 19 PI, from a Mongolian gerbil inoculated orally. Schneider's aceto-carmine stain. Scale bar = 100 μm.

mell, 1962; Williams and Colli, 1970; Slais and Machnicka, 1976). In the present investigation, the development of the larval *T. polyacantha* took place at the injection sites in Mongolian gerbils and AKR/J mice, and involved the abnormal budding of numerous vesicles of small size. In *M. oeconomus,* fewer than 16 buds were usually produced (Rausch and Fay, 1988a). Parenteral inoculation of oncospheres may deprive the larva of appropriate stimuli and nutrients for normal development. A few larvae recovered from the peritoneal cavity of the intraperitoneally inoculated AKR/J mouse, however, were similar on day 21 PI to those from orally inoc-

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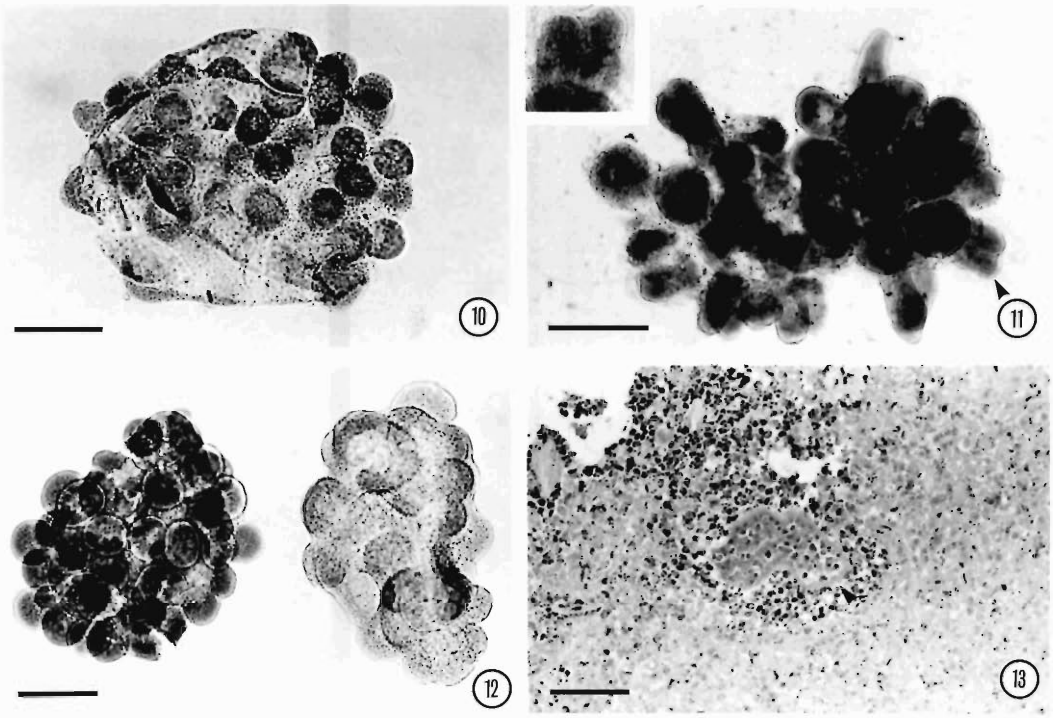
Figures 7, 8. Larval *Taenia polyacantha* in the mesenteric lymph node of Mongolian gerbils, day 5 PI. 7. Focal accumulation of inflammatory cells surrounding the vesicles in the marginal sinus and capsule. Vesicle in the afferent lymph vessel (arrowhead). AB-PAS stain. Scale bar = 100 μm. 8. Transmission electron micrograph of the vesicle in the marginal sinus. A few neutrophils cover the surface of the vesicle, and their pseudopods extend to fragments of the microvilli. Scale bar = 5 μm.



Figures 10–13. Larval *Taenia polyacantha* from parenterally inoculated rodents. 10. Proliferation of minute secondary vesicles, subcutaneous tissue of a Mongolian gerbil on day 19 PI. Schneider's aceto-carmine stain. Scale bar = 50 μm. 11. Vesicle with numerous buds, subcutaneous tissue of AKR/J mouse, day 19 PI. Schneider's aceto-carmine stain. Scale bar = 30 μm. Inset shows early formation of an invaginated canal in a secondary vesicle (arrowhead). 12. Proliferation of secondary vesicles, peritoneal cavity of a Mongolian gerbil, day 17 PI. Schneider's aceto-carmine stain. Scale bar = 50 μm. 13. Accumulation of inflammatory cells, with focal necrosis, around an early vesicle (arrowhead), beneath the phrenic serosa of the liver of a red-backed vole that died on day 5 PI. HE stain. Scale bar = 50 μm.

ulated hosts (Fujita et al., 1990). Many larvae were recovered in the wall of the small intestine of a red-backed vole that had been inoculated subcutaneously. This finding cannot now be explained, but the possibility of the migration of the oncospheres cannot be excluded.

Acknowledgments

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Helminth Parasites of Llamas (*Lama glama*) in the Pacific Northwest[1]

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ABSTRACT: A survey of helminth parasites of 18 llamas from the Pacific Northwest was conducted between February 1986 and September 1989. A total of 16 species (9 genera) of nematodes and 1 species of trematode was recovered. The most prevalent species of nematodes were in the genera *Camelostrongylus* and *Trichostrongylus* followed by *Nematodirus, Trichuris, Capillaria,* and *Cooperia.* Species in the genera *Haemonchus, Ostertagia,* and *Oesophagostomum* were rare. The only trematode species present was *Fasciola hepatica.* This is the first study of the helminth fauna of llamas in North America, and it defines what may be the typical species composition present in these animals.

KEY WORDS: llama, *Lama glama,* survey, helminths, *Camelostrongylus, Trichostrongylus, Nematodirus, Trichuris, Capillaria, Cooperia, Haemonchus, Ostertagia, Oesophagostomum, Fasciola hepatica.*

Information available from around the world indicates the llama (*Lama glama* (L.)) is host to a wide variety of external and internal parasites (Zawadowsky and Zvaguintzev, 1933; Vasquez et al., 1956; Jansen, 1959; Chavez and Guerrero, 1960; Guerrero and Rojas, 1970; Gorman et al., 1986). However, scientific knowledge regarding the health care of llamas in the United States has not kept pace with the explosion in the animals' popularity. This is particularly true with regard to parasitic diseases. *Giardia* sp., *Toxoplasma gondii,* and 4 species of *Eimeria* (Riemann et al., 1974; Kiorpes et al., 1987; Rickard and Bishop, 1988) are the only protozoan parasites identified thus far in llamas. *Fasciola hepatica* and *Fascioloides magna* are the only species of trematodes currently known to infect llamas (Conboy et al., 1988; Cornick, 1988). The nematode parasites include *Parelaphostrongylus tenuis,* which has been associated with neurologic disease in both guanacos (*Lama guanicoe*) and llamas (Brown et al., 1978; Baumgartner et al., 1985; Krogdahl et al., 1987), and at least 5 genera of gastrointestinal nematodes (Bishop and Rickard, 1987). However, other than *Nematodirus battus,* the actual species composition of these helminths is unknown. This study reports the results of postmortem examinations of 18 llamas from the Pacific Northwest providing basic information on the helminths present in these animals.

Materials and Methods

Between February 1986 and September 1989, necropsies for parasites were performed on 18 llamas at the Oregon State University Diagnostic Laboratory. All animals were either donated or admitted to the College of Veterinary Medicine, Oregon State University for reasons unrelated to parasitism. Most of the animals were from the Willamette Valley of western Oregon. The remainder were from northern and central Oregon and western Washington. Animals ranged in age from 6 mo to approximately 15 yr.

Necropsy procedures were as follows: the gastrointestinal tract was ligated in situ and the component parts (compartment 3 of the forestomach, small intestine, and cecum with 1 m of large intestine) removed. Each was opened into separate containers and the contents collected. The mucosal surface was then stripped by hand 3 times and the washings added to the contents which were brought to known volumes and appropriate duplicate aliquots (1 to be examined, the second for backup) taken. For compartment 3 and the small intestine, each aliquot was washed with tap water through a 400-mesh (37.5-μm opening) sieve. The material retained on the screen was backwashed into a dish and preserved in 10% neutral buffered formalin. For the cecum/large intestine, a 100-mesh (150-μm opening) sieve was used. Compartment 3 was further processed by soaking it in tap water for a minimum of 14 hr at room temperature. The mucosal surface was stripped again and the washings added to the incubation fluid. Aliquots were collected as per compartment 3 contents. The major airways and smaller bronchioles of the lungs were opened and visually examined for the presence of nematodes. The liver was sliced into large chunks and squeezed to express any flukes that may have been present. The chunks were then cut into approximately 1-cm^3 pieces and incubated a minimum of 12 hr in tap water. The pieces were then washed under running tap water over a 400-mesh sieve. The fluid from the incubation was then washed through the same sieve and the material retained was backwashed into a dish and preserved as above.

The small intestine and cecum/large intestine were examined from all 18 animals. Compartment 3 from 17 of the 18 animals was examined with 14 of these incubated in tap water. Livers from only 8 and lungs from only 6 animals were available for examination.

Parasites were removed from 1 of the 2 aliquots from each organ and from each liver sample, identified to

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species, and the total number of each species calculated. Liver flukes were enumerated by counting the whole flukes, anterior ends, and posterior ends separately. Total numbers were then calculated by adding the numbers of whole flukes to the number of anterior or posterior pieces, whichever was greater. Identifications of helminths were based on the descriptions and keys provided in the following: Orloff, 1933; Skrjabin et al., 1954; Sommerville, 1956; Lichtenfels, 1977; Levine, 1980; Soulsby, 1982; Durette-Desset, 1983; Lichtenfels and Pilitt, 1983; Lichtenfels et al., 1986, 1988; Rickard and Bishop, 1991. Identification of female *Camelostrongylus mentulatus* was also based on the synlophe (Rickard, unpubl. data).

Representative specimens of adult nematodes recovered have been deposited with the USNM Helminthological Collection (Nos. 80824, 81324–81335).

Results

Sixteen species of nematodes comprising 9 genera were recovered from the llamas along with 1 species of trematode (Table 1). The most prevalent nematodes were in the genera *Camelostrongylus* and *Trichostrongylus,* whereas species of *Nematodirus, Trichuris, Capillaria,* and *Cooperia* were less often encountered. Species referable to *Haemonchus, Ostertagia,* and *Oesophagostomum* were rare.

Discussion

It is apparent from the present study that llamas are host to a wide variety of nematode species. *Camelostrongylus mentulatus* has not been considered to be common in the United States and has only been reported from camels (*Camelus dromedarius* and *C. bactrianus*) in zoos and blackbuck (*Antilope cervicapra*) on private ranches in Texas (Canavan, 1929; Jaskoski and Williamson, 1958; Thornton et al., 1973a; Averbeck et al., 1981). However, this parasite has a wide host range throughout the rest of the world having been reported in both species of camels, domestic sheep (*Ovis aries*), domestic goats (*Capra hircus*), Saiga antelope (*Saiga tartarica*), goitred gazelle (*Gazella subgutturosa*), red deer (*Cervus elaphus*), as well as alpacas (*Lama pacos*) and llamas (Rogers, 1939; Bhalerao, 1942; Jansen, 1959; Guerrero, 1960; Copland, 1965; Guerrero and Chavez, 1967; Dunn, 1968; Ermolova, 1968; Hernandez et al., 1980). Given the high prevalence of *C. mentulatus* in the present study and the widespread transportation of llamas within the United States, this parasite may actually be more common here than previously believed.

The pathogenicity and potential for cross-transmission of *C. mentulatus* has also been documented (Thornton et al., 1973b; Beveridge et al., 1974; Hilton et al., 1978; Flach and Sewell, 1987). The pathogenic effects of this parasite in blackbuck include chronic emaciation and death. Examination of infected abomasa revealed lesions consistent with parasitic gastritis. Transmission experiments indicate that *C. mentulatus* can be transferred from blackbuck and camels to domestic sheep and goats but not to cattle (Thornton et al., 1973b; Beveridge et al., 1974). Pathologic changes present in the sheep were similar to those caused by *Teladorsagia* spp. with extensive mucous metaplasia, loss of parietal cells, elevated abomasal pH, elevated Na^+ concentrations in the abomasal contents, and elevated plasma pepsinogen levels. Although it was not within the scope of this study to determine the effects of parasites on llamas, gross lesions were noticed in compartment 3 of some animals infected with *C. mentulatus.* Small, raised nodules up to 5 mm in diameter, often containing a central depression, were observed on the mucosal surface. The lesions were generally confined to the distal portion of compartment 3. These observations, although limited, may indicate that *C. mentulatus,* which parallels *Teladorsagia* spp. in pathogenicity and development in sheep, may cause a similar disease syndrome in the llama.

It is interesting to note that in those areas of South America in which alpacas have been examined, the prevalence of *Ostertagia* spp. is much greater than that for *C. mentulatus* (23.1%–59% and 2.3%–3%, respectively; Chavez and Guerrero, 1965; Chavez et al., 1967). However, in the llamas in the present study, the reverse was found to occur. The reasons for this reversal cannot be determined at this time, but possible causes include: 1) lack of exposure of the llamas to *Ostertagia*; 2) competitive inhibition by *C. mentulatus*; 3) host factors favoring 1 parasite over the other; 4) lack of exposure of the alpacas to *C. mentulatus* due to an overall low prevalence in South America; and 5) differences in husbandry including grazing practices and anthelmintic treatment.

The predominant species of *Trichuris* present in the llamas was *T. tenuis.* This nematode was first described from a camel in the Houston Zoo (Chandler, 1930). We recently redescribed this nematode from llamas and noted that the report was the second of this species in North America and the first in these free-ranging animals (see Rickard and Bishop, 1991). In contrast to *T.*

Table 1. Species composition, intensity, range, and prevalence of the helminths recovered from llamas.

Parasite (USNM No.)	Intensity*	Minimum	Maximum	Prevalence*
Nematodes				
Camelostrongylus mentulatus ♀	1,781	20	11,890	76%
C. mentulatus ♂	1,346	2	9,850	76%
C. mentulatus adults, total (81324)	3,127	24	21,740	76%
C. mentulatus L_4†	1,158	2	7,440	47%
C. mentulatus E_4‡	45,773	22	182,710	27%
Ostertagia ostertagi ♀	254	8	500	12%
O. ostertagi ♂	109	6	280	18%
O. ostertagi adults, total (81325)	278	14	780	18%
Ostertagia L_4	—	—	100	6%
Ostertagia/Camelostrongylus E_4§	8,545	70	17,020	12%
Ostertagia/Camelostrongylus L_3§‖	—	—	80	6%
Haemonchus sp. ♂	—	—	20	6%
Trichostrongylus spp. ♀ (81329)	419	2	2,920	72%
T. axei ♂ (81332)	233	4	548	33%
T. vitrinus ♂ (81331)	160	20	561	22%
T. longispicularis ♂ (81330)	330	20	1,060	22%
Trichostrongylus adults, total	629	2	4,641	78%
Trichostrongylus L_4	127	2	480	22%
Nematodirus spp. ♀	1,239	20	11,440	61%
N. helvetianus ♂ (81326)	1,268	20	10,180	56%
N. spathiger ♂ (81334)	136	20	340	28%
N. filicollis ♂	—	—	160	6%
Nematodirus adults, total	2,468	60	21,860	61%
Nematodirus L_4	780	20	3,600	33%
Nematodirus E_4	264	20	880	28%
Nematodirus L_3	—	—	120	6%
Cooperia spp. ♀	300	40	480	22%
C. oncophora ♂ (81335)	240	20	500	22%
C. surnabada ♂ (81327)	50	20	80	11%
Cooperia adults, total	452	20	860	28%
Cooperia L_4	50	20	80	11%
Cooperia E_4	—	—	20	6%
Capillaria sp. adults (81333)	46	1	120	56%
Capillaria sp. larvae	—	—	10	6%
Trichuris tenuis adults (80824)	84	1	569	61%
T. discolor ♀	—	—	1	6%
T. skrjabini ♀	—	—	1	6%
Trichuris spp., adults¶	7	3	11	11%
Trichuris adults, total	85	3	569	61%
Trichuris larvae	38	1	102	17%
Oesophagostomum venulosum adults (81328)	4	1	7	11%
Trematodes				
Fasciola hepatica	—	—	38	11%

* Intensity = mean number of parasites per infected host; prevalence = percent of individuals of a host species infected with a particular parasite.

† L_4 = late fourth-stage larvae.

‡ E_4 = early fourth-stage larvae.

§ In mixed *Ostertagia–Camelostrongylus* infections, third-stage and early fourth-stage larvae of the two species could not be reliably differentiated.

‖ L_3 = third-stage larvae.

¶ Species identification could not be determined due to the condition of the specimens.

tenuis, T. ovis is the most commonly reported species in South American camelids (Guerrero, 1960; Chavez and Guerrero, 1960, 1965); however, we did not recover this species from any of the animals examined. The predominance of *T. tenuis* indicates that this species is probably the typical trichurid present in llamas in the Pacific Northwest and possibly the United States as well.

Both *O. venulosum* and *Haemonchus* sp. were

found infrequently in the llamas, paralleling observations of the parasites in alpacas (Chavez and Guerrero, 1965; Chavez et al., 1967). The lack of infection with *Haemonchus* in alpacas was attributed to the poor survival of larvae in the areas where the animals lived. Poor larval survival was considered to be due to adverse climatic conditions (low temperatures and inadequate humidity). However, *Haemonchus* spp. and *O. venulosum* are present in sheep and cattle from the same areas in which the llamas originated (Malczewski et al., 1975; Richards et al., 1987a; Hoberg et al., 1988). Consequently, host factors may play an important role in limiting infections with these parasites.

Neither *Strongyloides* spp. nor *N. battus* were found in the llamas examined in the present study although eggs of both have been recorded on standard fecal examinations from llamas in the study area (Bishop and Rickard, 1987). *Nematodirus lamae,* the typical nematodirid species of South American camelids, was not among the species of *Nematodirus* found in the present survey, nor was *Lamanema chavezi,* which is considered to be the most pathogenic nematode present in llamas and alpacas. Lungworms were also absent even though *Dictyocaulus* sp. is often found in lamoids in South America (Chavez and Guerrero, 1960, 1965); however, their absence is likely due to the small sample size.

South American camelids are host to a variety of cestodes, including both metacestodes and adult stages (*Taenia hydatigena, Echinococcus granulosus, Moniezia expansa, M. benedeni*) (Chavez and Guerrero, 1960, 1965). Fecal examinations of over 500 animals in Oregon indicated *Moniezia* sp. can be found in llamas but is very rare (<1% positive) (Rickard, unpubl. data). In the present study, neither adults nor cysts of tapeworms were found in the animals examined. However, the role of llamas as an intermediate host for *E. granulosus* should be carefully considered in those areas of North America in which this parasite is found.

Fasciola hepatica was present in only 1 of the 9 livers examined. The overall prevalence of liver flukes in llamas in North America is currently unknown. Sedimentation of fecal samples from approximately 250 llamas in Oregon showed <1% to be positive for eggs of *F. hepatica,* whereas an earlier survey showed 6% of 180 fecal samples to be positive (Rickard, unpubl. data). The only other report of *F. hepatica* in llamas in the United States was from Texas (Cornick, 1988). Development of more accurate serologic tests, such as the ELISA or dot-ELISA, would aid in delineating the overall prevalence of liver flukes in llamas and their potential impact on the llama industry.

With the exceptions of *C. mentulatus* and *T. tenuis,* the nematodes encountered in the llamas are also common parasites of cattle and sheep in the study area (Malczewski et al., 1975; Richards et al., 1987a, b; Hoberg et al., 1988; Rickard et al., 1989) as well as across the United States (Levine, 1980). This indicates that the llama must be considered in management and control practices where these animals share common ground. In addition, the exchange of parasites among llamas and other exotic ruminants such as the blackbuck must also be considered. This is especially important in game parks and private ranches where free-ranging, susceptible animals share common habitat.

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58(1), 1991, pp. 116–117

Research Note

Synonymy of *Hofmonostomum* Harwood, 1939, with *Paramonostomum* Lühe, 1909 (Digenea: Notocotylidae)

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ABSTRACT: The genus *Hofmonostomum* Harwood, 1939, is shown to have been established on the basis of characters derived from misinterpretation of the anatomy of the sole species, *H. himantopidis* Harwood, 1939. The species is consistent with the anatomy of *Paramonostomum* species. *Hofmonostomum* is placed in synonymy with *Paramonostomum* and the new combination *P. himantopidis* (Harwood, 1939) Cribb, 1991, is proposed.

KEY WORDS: Digenea, Notocotylidae, *Hofmonostomum, Paramonostomum,* taxonomy, synonymy.

The author recently had the opportunity to examine the holotype (United States National Museum Helminth Collection No. 30037) and the 3 paratypes (USNM No. 40615) of *Hofmonostomum himantopidis* Harwood, 1939. This species was described by Harwood (1939) on the basis of the 4 specimens mentioned above (2 mounted, 2 unmounted) from a black-necked stilt, *Himantopus mexicanus,* from Puerto Rico. Harwood recognized the new genus for 2 features. First, he recorded that, uniquely for the Notocotylinae, the vitelline follicles extended posterior to the anterior margin of the testes to near the end of the body. In other genera the vitelline follicles terminate at the anterior margin of the testes. Second, he recorded a weakly developed median ventral ridge such as is seen in *Catatropis* Odhner, 1905, but without accompanying lateral ventral papillae. On the basis of these characters the genus *Hofmonostomum* has been accepted by subsequent authors (e.g., Yamaguti, 1971; Groschaft and Tenora, 1981; Schell, 1985).

Inspection of the holotype and mounted paratype using Nomarski interference microscopy showed that the vitelline follicles in both specimens in fact terminate at or near the anterior margin of the testes. In the holotype the follicles on one side extend just posterior to the anterior margin of the testis but on the other side and in

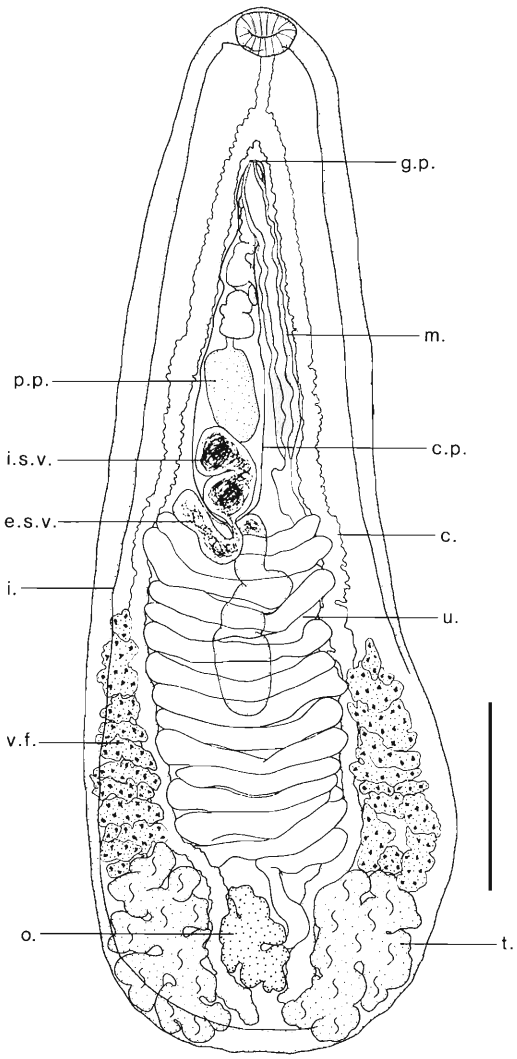

Figure 1. ***Paramonostomum himantopidis*** **comb. n. c., cecum; c.p., cirrus pouch; e.s.v., external seminal vesicle; g.p., genital pore; i., infolding of lateral margin; i.s.v., interval seminal vesicle; m., metraterm; o., ovary; p.p., pars prostatica; t., testis; u., uterus; v.f., vitelline follicles. Scale bar = 200 μm.**

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the mounted paratype they terminate close to the anterior margin of the testes. In the holotype, and to a lesser extent in the paratype, the lateral margins of the specimen are curved inwards and include portions of the lobed margin of the testes. This evidently produced the impression of vitelline follicles lateral to the testes which is, however, clearly not the case. The mounted paratype is figured here (Fig. 1) as it is the less ambiguous specimen.

In neither mounted specimen was a median ventral ridge of the type seen in *Catatropis* species discerned. In the holotype the ventral surface is somewhat folded, and presumably as an artifact of fixation, has an irregular ridge visible in places. This does not appear to be a genuine structure. No ridge was visible at all on the mounted paratype. Given that Nomarski microscopy was used, it is reasonable to believe that a ridge would have been observed if present. There was no sign of a median ridge on either of the unmounted paratypes which were examined under a dissecting microscope.

In view of the observations outlined above, the 2 characters that were used to distinguish *Hofmonostomum* are not valid. Alternative distinguishing characters were not found. Consequently, the genus cannot be distinguished from the large, cosmopolitan genus *Paramonostomum* Lühe, 1909. This genus is characterized by the complete absence of ventral surface ridges and papillae and has the vitelline follicles terminating at the anterior margin of the testes. I therefore propose that *Hofmonostomum* should be considered a junior synonym of *Paramonostomum* and that the new combination *Paramonostomum himantopidis* (Harwood, 1939) Cribb, 1991, be recognized.

The question of the validity of *P. himantopidis* with respect to other *Paramonostomum* species is beyond the scope of the present study. The genus contains about 50 nominal species, many of which are separated by only the most superficial of characters. It seems not at all improbable that critical review will show *P. himantopidis* to be either a junior or senior synonym of existing *Paramonostomum* combinations. It is noteworthy that *P. himantopidis* appears not to have been recorded since its original description (see Hinojos and Canaris, 1988).

Thanks are due to Dr. J. R. Lichtenfels of the USNM for kindly lending type specimens.

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Research Note

The Response of *Tubifex tubifex* (Oligochaeta: Tubificidae) to a Second Infection with *Glaridacris catostomi* (Cestoidea: Caryophyllaeidae)

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ABSTRACT: The response of the aquatic oligochaete, *Tubifex tubifex,* to primary and secondary infection by the caryophyllaeid cestode, *Glaridacris catostomi,* was investigated by comparing rates of parasite mortality between the 2 infections. *Tubifex tubifex* that lost the first infection of *G. catostomi* became infected after second exposure to the cestode, but rate of parasite mortality did not increase. Over an 8-day period, both cumulative parasite mortality and percentage of hosts that lost their caryophyllaeid infection were greater for the first infection (90% and 59%) than for the second (46% and 2%). A cellular response, involving the encapsulation of metacestodes by host coelomocytes, accounted for part of the high caryophyllaeid mortality observed.

KEY WORDS: Cestoidea, Caryophyllidea, Tubificidae, metacestode.

Much of the literature on annelid immunity has involved the response of terrestrial earthworms to tissue grafts (Cooper, 1986), the coelomocytes active in the phagocytosis process (Stein et al., 1977), and characterization of antibacterial molecules (Valembois et al., 1986). Additionally, studies have been conducted on the encapsulation of parasitic organisms in annelids. Poinar and Thomas (1975) described the encapsulation of the nematode, *Rhabditis pellio,* in the coelom of the earthworm, *Aporrectodea trapezoides,* which involves the encasement of the parasite in host coelomocytes and formation of multiple capsules or "brown bodies." A similar reaction was observed by Calentine et al. (1970) in tubificid oligochaetes infected with caryophyllaeid cestodes. Host coelomocytes attached to the metacestodes, but no "brown bodies" developed. This cellular response against caryophyllaeids was reported for a number of tubificids including *Tubifex tubifex* Müller, 1774. This species was used in the present study to compare rates of parasite mortality between a first infection with *Glaridacris catostomi* Cooper, 1920, and a second infection with the same caryophyllaeid species.

Laboratory reared oligochaetes were experimentally infected by 24-hr exposure to embryonated cestode eggs in mud-free water (1,200–1,800 eggs per 25 ml of water), under continuous aeration, at a temperature range of 18–21°C. Cestode eggs were obtained from the laboratory of Robert Calentine, University of Wisconsin–River Falls. Immediately after exposure to cestode eggs, infected annelids were separated from uninfected ones. Infected annelids were identified by the presence of oncospheres in the coelom. All annelids used in experiments were maintained under aeration in 100-ml glass beakers containing initially sterile mud and fed a diet of Tetramin® flaked fish food. At various days postexposure (PE), wet mounts of infected annelids were examined microscopically at 100× and/or 430× with bright field or phase contrast optics and the number of cestodes present in the coelom counted.

In 2 separate trials, 1-wk-old *T. tubifex* were exposed for the first time to *G. catostomi.* The individuals that became infected were designated as group 1 (first infection). Approximately 2½-wk-old oligochaetes in group 1 that lost their cestodes (the cestodes died) were reexposed a second time to *G. catostomi.* The oligochaetes that became reinfected were designated as group 2 (second infection). At the same time as group 2, another group of approximately 2½-wk-old annelids, designated the control group, was exposed to *G. catostomi* for the first time. Data were combined from both trials and rates of parasite mortality were compared among all 3 groups.

Cumulative host and parasite mortality and percentage of hosts that lost their caryophyllaeid infection were compared among the 3 groups using R × C contingency tables with chi-square analysis. Student's *t*-test was used to compare group means at the various days PE. Differences in each of these analyses were considered significant at $P < 0.05$.

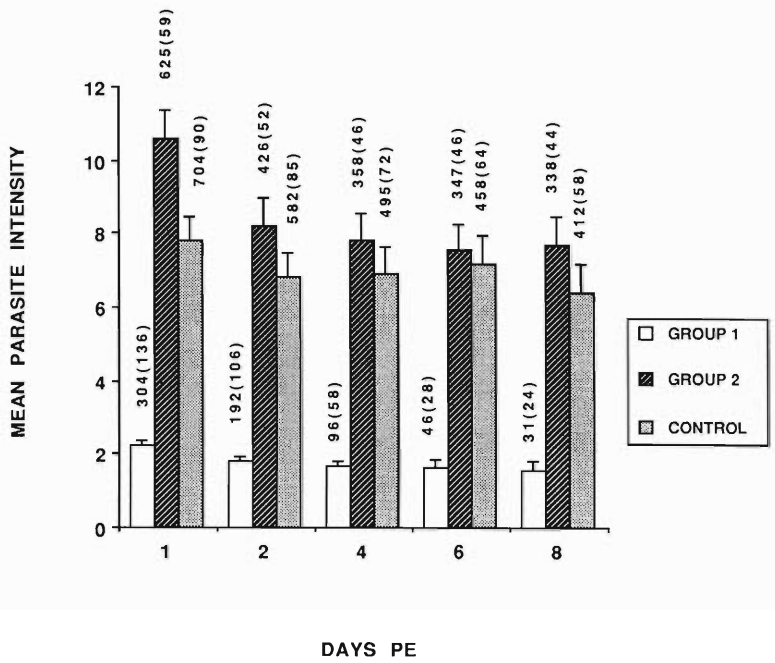

Figure 1. Mean parasite intensities of *Glaridacris catostomi* in *Tubifex tubifex* for group 1 (first infection), group 2 (second infection), and a control group (same age as group 2). Bars represent the standard error of the mean. The numbers above each bar represent total number of parasites followed by total number of infected hosts in parentheses.

Overall, mean parasite intensity (Fig. 1) decreased over time for each group (e.g., from 2.23 at 1 day PE to 1.55 at 8 days PE in group 1, from 10.59 at 1 day PE to 7.68 at 8 days PE in group 2, and from 7.82 at 1 day PE to 6.38 at 8 days PE in the control group). Mean parasite intensities were higher in the second infection compared to the first infection. Only at 1 day PE was the mean parasite intensity for the second infection significantly larger than for the control group.

By 8 days PE, 90% of the cestodes were dead in group 1 hosts and 46% in group 2 (Fig. 2A). A significantly higher number of *G. catostomi*-infected *T. tubifex* lost their metacestodes in group 1 compared to group 2, 59% and 2%, respectively (Fig. 2C). Only 2% of control oligochaetes were no longer infected at 8 days PE (Fig. 2C). Host mortality was similar for all 3 groups (Fig. 2B).

Evidence of immunologic memory in annelids was provided by Cooper (1968) in his studies on tissue graft rejection in terrestrial earthworms. Secondary rejection of allografts and xenografts by earthworms was specific and enhanced (Cooper, 1968) and this memory was also transferrable with "sensitized" coelomocytes (Hostetter and Cooper, 1974). We attempted to measure the potential for an adaptive immune response in aquatic oligochaetes by using a natural parasite of the annelid. However, on second exposure to *G. catostomi* in *T. tubifex* we did not find that the rate of parasite death increased. Thus, we concluded that the response exhibited by this host to the parasite is non-adaptive. Even after 35 days, percentage of hosts that lost their infection after second exposure was still lower than that for controls (5% vs. 18%). Parasite mortality was significantly higher in controls (73%) than in group 2 (61%) at 35 days PE. There appears to be no difference between susceptibility to infection on second exposure to *G. catostomi* for *T. tubifex* that previously lost the infection and those still infected with the parasite (see Calentine, 1967).

The high rate of parasite mortality seen in this study can be partially explained by a cellular response. We observed the encapsulation reaction in *G. catostomi*-infected *T. tubifex* that was reported by Calentine et al. (1970). Host coelomocytes attached to the metacestode and eventually encased it. Because of the infrequency of the response (observed 4 times), and because we observed the death of older and larger metacestodes

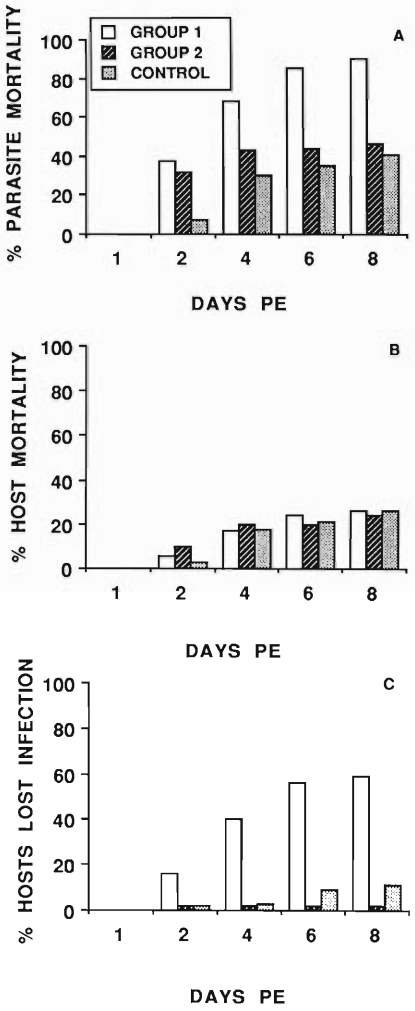

Figure 2. Percent cumulative parasite mortality (A), host mortality (B), and hosts that lost their caryophyllaeid infection (C) in *Glaridacris catostomi*-infected *Tubifex tubifex* for group 1 (first infection), group 2 (second infection), and a control group (same age as group 2).

without attachment of host cells, we believe that the encapsulation reaction alone is not the cause of the high parasite mortality seen in this study. Other mechanisms may be operating in this host–parasite system. The possible role of humoral immune components interacting with the coelomocyte population should be investigated.

Terrestrial earthworms possess humoral factors that are capable of agglutination and opsonization. A natural hemolysin of *Eisenia fetida andrei* shows lytic activity against sheep red blood cells (Roch, 1979) as well as antibacterial activity toward pathogenic soil bacteria (Lassègues et al., 1981; Valembois et al., 1982). Valembois et al. (1986) characterized the antibacterial molecules in this earthworm that are responsible for neutralization of pathogenic bacteria. Four proteins mediated hemolytic activity in the coelomic fluid but only 1 protein was involved in this process in the cocoon albumen. Stein and Cooper (1981) found that phagocytosis of yeast by neutrophils of *Lumbricus terrestris* was enhanced when the yeast were first washed with cell-free coelomic fluid. Whether the coelomic fluid of aquatic oligochaetes has similar immune capabilities is not known.

Age is an important component of the *G. catostomi–T. tubifex* association (Courtney and Christensen, 1988). Most infected tubificids do not become sexually mature (Sekutowicz, 1934; Kulakovskaya, 1962; Calentine, 1965, 1967) or the breeding period is delayed (Kennedy, 1969). Since young annelids are more susceptible to infection, the caryophyllaeid may be applying some mechanism that delays sexual maturity (e.g., liberating molecules that inhibit maturation of the gonads). A delay in host maturity could also be related to the depletion of host nutrients by the parasites. As a consequence of this, there is not enough energy available for reproductive growth. Additionally, the amount of space utilized by these cestodes may physically obstruct the development of the gonads. Thirty-day-old *T. tubifex* can have a body length of 25 mm or more (Kosiorek, 1974). The average length of an infective metacestode of *G. catostomi* is 1.2 mm (Calentine, 1967).

Equally important in the present study is the initial parasite intensity that can enhance the suitability of *T. tubifex* as a host (see Courtney and Christensen, 1987). When *T. tubifex* was subjected to a second exposure to *G. catostomi,* the initial parasite burden was higher and the percentage of hosts that lost their infection lower. If this phenomenon also occurs in tubificids in nature, then caryophyllaeids that parasitize previously infected hosts have a much higher probability of surviving to the infective stage. An increased susceptibility to infection can result in a higher parasite burden.

The increased susceptibility to caryophyllaeid infection could be a consequence of a reduction in the number of coelomocytes that occurred in the first infection. A reduced coelomocyte pop-

ulation in conjunction with depletion of humoral factors may account for a greater susceptibility to infection in group 2 compared to age-matched controls. In the snail, *Helix pomatia,* a decrease in the number of circulating blood cells was seen after injection of foreign particles. Additionally, the number of circulating blood cells can differ in snails of different ages with the number increasing in larger snails (Siminia, 1981). Further research is needed to determine the physiological differences between younger (more susceptible) annelids and older (less susceptible) annelids, and the immunocompetence of each.

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Research Note

Differences between the Energy Metabolism of *Trichinella spiralis* and *Trichinella pseudospiralis*

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ABSTRACT: Comparative biochemical investigations showed the existence of differences between the enzyme kinetics of *Trichinella spiralis* and *Trichinella pseudospiralis*. The higher affinity of succinate dehydrogenase-fumarate reductase complex to fumarate in *Trichinella pseudospiralis* is discussed in relation to the branched anaerobic-type electron transport system suggested in this parasite.

KEY WORDS: Nematoda, *Trichinella spiralis, Trichinella pseudospiralis,* enzyme kinetics.

At present, the genus *Trichinella* appears more complex than was thought in the past, and comparative biochemical investigations could be helpful in understanding its existing diversity.

The larvae of *Trichinella pseudospiralis,* which in contrast to *Trichinella spiralis* larvae do not become "trapped" in host muscles due to encapsulation, move continously, thus altering the entire muscle fiber of the host (Hulińska et al., 1984). So the differences in bioenergetic metabolism between the motile, unencapsulated *T. pseudospiralis* larvae and motionless encapsulated *T. spiralis* seem to be especially interesting.

Considering the electrophoretic analysis of tissue protein as a taxonomic tool, a comparative study of the soluble protein profile of 7 *Trichinella* isolates was performed (Fukumoto et al., 1987). They observed that the pattern of 6 of the isolates clearly differed from that of *T. pseudospiralis.* From the zymograms of 8 enzymes investigated in the same study, 7 of them in *T. pseudospiralis* were clearly different from other isolates. More recently, Pozio et al. (1989) presented a study of a gene-enzyme system and it showed a high genetic discontinuity in the genus *Trichinella* with 7 distinct gene pools. In addition, analysis of the principal components of 51 *Trichinella* isolates and 5 biological and physical variables confirmed the presence of specific clusters among species in the genus *Trichinella* (La Rosa et al., 1989).

Generally speaking, *T. pseudospiralis* larvae seem to stimulate a weaker immunological response by the host, show a lower metabolic activity of some glycolytic enzymes (Boczoń, 1986), and their excretory-secretory products uncouple the host muscle mitochondria during the encystation phase of muscle infection to a lesser extent than *T. spiralis* excretory-secretory products (Boczoń et al., 1989).

Recent biochemical investigations on metabolic differences between *T. spiralis* and *T. pseudospiralis* indicated similarities in their susceptibility to chemotherapeutic attack (Boczoń et al., 1984) and in their prostaglandin content (Hadaś and Staude-Adamczewska, 1990), but differences in their bioenergetic metabolism. For example, *T. pseudospiralis* mitochondria, in contrast to *T. spiralis,* have a cytochrome oxidase (OX) that is partially insensitive to KCN, but mitochondria that are poorly coupled and able to oxidize exogenous NADH. All these features may suggest the presence of an alternative pathway in the electron transport system of *T. pseudospiralis* (Boczoń, 1985). Qualitative and quantitative comparative determinations of quinones revealed about 4 times greater amount of both ubiquinone and rhodoquinone in *T. pseudospiralis* than in *T. spiralis* (Boczoń, 1985).

This report presents the results of kinetic measurements of succinate dehydrogenase-fumarate reductase (SDH-FR) complex and some cytochrome c oxidoreductases in mitochondria of *T. pseudospiralis* larvae and compares these data with those obtained in *T. spiralis.*

Larvae of *T. pseudospiralis* Garkavi, 1974, originally isolated in USSR from a raccoon, were isolated from rat muscles by pepsin digestion (Gursch, 1948) lasting 1 hr. After several washings in water and 0.9% NaCl, larvae were homogenized in sucrose (0.25 M, Tris-HCl 0.03 M, pH 7.3) medium. The mitochondrial fraction was obtained and the activities of cytochrome c oxidoreductases were measured as described previously (Hryniewiecka et al., 1970; Boczoń and Michejda, 1978). For the measurements of SDH and FR activity the mitochondria were frozen and thawed 3 times. The SDH (EC 1.3.99.1) ac-

Table 1. The activities and apparent K_m values for cytochrome c oxidoreductases (NADH-cytochrome c oxidoreductase—NC, succinate-cytochrome c oxidoreductase—SC) and cytochrome c oxidase (OX) in *Trichinella pseudospiralis* mitochondria.

$V_{max} \pm SD$					$K_m \pm SD$	
SC	NC_t	NC_r	OX	OX:NC_r	SC	NC
164 ± 82	324 ± 68	81 ± 15	280 ± 50	3.5	40 ± 5	1 ± 0.5

Enzyme activity expressed in nmoles of appropriate substrate/min/mg of protein. NC_t = total activity of NC. NC_r = portion of activity sensitive to rotenone (concentration of rotenone—100 nM/mg of protein). Number of experiments 5–6.

tivity was measured with dichloroindophenol according to King (1963) and the activity of FR (EC 1.3.99.1) was determined in aerobic conditions according to Prichard (1970). All enzyme activities were assayed at various concentrations of substrates and the apparent K_m and V_{max} determined from a double-reciprocal Lineweaver-Burk plot.

As presented in Table 1, the succinate-cytochrome c oxidoreductase (SC) activity in *T. pseudospiralis* mitochondria resembled the activity of the same enzyme in rat muscle mitochondria and in *T. spiralis* mitochondria (135 nmoles/min/mg of protein and 129, respectively); the NADH-cytochrome c oxidoreductase (NC), however, showed a significantly higher total activity than in rat muscle mitochondria and in *T. spiralis* (166 nmoles/min/mg of protein and 101, respectively). The activity of rotenone-sensitive NC (NC_r), which amounts to 25% of total NC activity only, is lower than the activity of the same enzyme in rat muscle mitochondria (133 nmoles/min/mg of protein).

The cytochrome oxidase activity in *T. pseudospiralis* was 7 times lower than that in rat muscle mitochondria (1,960 nmoles/min/mg of protein) being at the same time 2 times lower than the sum of the activities of SC and NC. In rat tissue mitochondria this sum is 6 times lower than the OX activity and it suggests that the OX might become a rate limiting factor in the electron transport chain.

NC_r enzyme is localized in the inner mitochondrial membrane in mammalian mitochondria. Significantly lower participation of inner mitochondrial membrane in a structure of *T. pseudospiralis* mitochondria is evident from the ratio of OX:NC_r in *T. pseudospiralis* which is 4 times lower than in rat muscle mitochondria (14.7) and even 2 times lower than in *T. spiralis* mitochondria (7.0).

SDH and FR activities (Table 2) were similar in *T. pseudospiralis* and *T. spiralis* mitochondria (Boczoń, 1976), and the SDH activity in both nematodes did not differ significantly from that in rat liver mitochondria; however, the apparent K_m values with succinate for SDH were almost 4 times higher than the K_m value for SDH in rat liver mitochondria and increased the ratio K_{mSDH}: K_{mFR} (R) in *T. pseudospiralis* to 7.2. The value of about 1 for this ratio measured by us in rat liver mitochondria is typical of all aerobic organisms and *Escherichia coli* grown aerobically (Singer, 1971). A similar value of R was obtained in *T. spiralis* larvae (Boczoń, 1976), which suggests some characteristics of an aerobic type of metabolism in this parasite. The value of R calculated in our experiments in larvae of *T. pseudospiralis* differs from that of *T. spiralis,* on the other hand it is similar to the values presented for some organisms that are characterized by anaerobic (e.g., Propionic bacterium R = 3.1 or anaerobically grown *Escherichia coli* E = 59, Singer, 1971) or anaerobic-aerobic switching type of metabolism (e.g., adult *Nippostrongylus brasiliensis* R = 5.0 or adult *Ascaridia galli* E = 4.4, Fry and Branzeley, 1984).

Our results confirm the R value in *T. spiralis* which might be calculated from the results of Rodriguez-Caabeiro et al. (1985). The SDH-FR complex in many parasitic helminths is a point of particular interest since it is considered as one of the points of chemotherapeutic attack of some benzimidazoles (Prichard, 1986); whether it is a primary site of action of mebendazole or thiabendazole in *T. spiralis* and *T. pseudospiralis* larvae is an open question (Boczoń, 1976; Boczoń et al., 1984; Rodriguez-Caabeiro et al., 1985; Criado-Fornelio et al., 1987). Recently the direct, stimulatory effect of levamisole, thiabendazole and mebendazole on mitochondrial ATPase in *Trichinella spiralis* and *Trichinella pseudospiralis* larvae was proved (Boczoń et al., 1990).

Although both nematodes of the genus *Trich-*

Table 2. The comparison of the V_{max} and apparent K_m values of succinate dehydrogenase (SDH) and fumarate reductase (FR) in *Trichinella pseudospiralis*.

	SDH ± SD	FR ± SD	K_{mSDH}:K_{mFR}
V_{max}	51 ± 13	7.0 ± 1.8	
K_m	29 ± 6	4.0 ± 1.0	7.2

All explanations as in Table 1.

inella live in the same well-oxygenated biotope muscles, their mitochondria seem to differ in a number of bioenergetic features: 1) *T. pseudospiralis* mitochondria in comparison to *T. spiralis* mitochondria revealed lower participation of the inner mitochondrial membrane in their function, which may result in loose coupling of oxidative phosphorylation, 2) in *T. pseudospiralis* a partial insensitivity of OX to KCN and oxidation of the exogenous NADH may suggest the participation of an alternative pathway in energy generation (Boczoń, 1985), 3) a high affinity of SDH-FR complex to fumarate and low affinity to succinate may be indicative of the greater involvment in *T. pseudospiralis* than in *T. spiralis* in the generation of some $\Delta\psi$ by site I of oxidative phosphorylation due to the activity of FR, despite the same activity of FR in both nematodes, and malic enzyme (Ward et al., 1969; Boczoń, 1986).

Some participation of fumarate reductase in the generation of the transmembrane potential $\Delta\psi$ by site I of oxidative phosphorylation without involvment of oxygen, as proved in *Ascaris* by Chojnicki et al. (1987), was suggested recently in *T. spiralis* by Michejda et al. (1989). Those results, obtained with *T. spiralis*, support our previous view (Boczoń, 1985) that the respiratory metabolism of *T. spiralis* larvae, although being much more aerobic than that of *Ascaris*, may also partially involve the anaerobic generation of $\Delta\psi$ by site I of oxidative phosphorylation due to the activity of FR and malic enzyme, the system operating anaerobically in most helminths.

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Research Note

Differentiation between Mixed Infections of *Ancylostoma caninum* and *Ancylostoma duodenale* in Dogs Using an In Vitro Assay for the Resumption of Feeding by Third-stage Infective Larvae

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ABSTRACT: The identification of 2 mixed hookworm infections in dogs by using differences of larval in vitro feeding behavior is reported. In the first case, infective third-stage larvae (L_3) from a putative *Ancylostoma duodenale* infection were compared to larvae of known *A. duodenale* and *A. caninum* infections in a resumption of feeding time course experiment. The second case involved comparison of feeding behavior of L_3's from a second suspect infection with L_3's from a known *A. caninum* infection at 24 hr. In both cases, the suspect larvae exhibited feeding behavior similar to known *A. caninum* larvae, suggesting accidental infection with *A. caninum*. Upon necropsy, the suspect infections contained adults of both species. This technique has potential application in differentiation of closely related nematode species in several areas of parasitology.

KEY WORDS: *Ancylostoma caninum, Ancylostoma duodenale,* infective larvae, in vitro feeding.

As long ago as 1924, epidemiologists had hoped to distinguish among the infective larvae of the dog hookworm, *Ancylostoma caninum* (Ercolani, 1859), and the human hookworms, *Ancylostoma duodenale* (Dubini, 1843) and *Necator americanus* (Stiles, 1903). They concluded that although *N. americanus* is distinguishable from the others on morphological grounds, the 2 *Ancylostoma* species are not (Schuurmans Stekhoven and Schuurmans Stekhoven-Meyer, 1924; Komiya and Yasuraoka, 1966). In our laboratory, both species of *Ancylostoma* have been maintained in dogs for more than 15 yr (Schad, 1979), and on rare occasions donor animals have inadvertently been infected with larvae of the wrong species, or with a mixed inoculum. Until recently, dogs harboring putatively pure infections, but with atypical prepatent periods or egg counts, had to be sacrificed, and the adult hookworms recovered and examined morphologically to determine the true nature of the infection. This is a very expensive procedure due to the high cost of purpose-bred dogs. A reliable method of distinguishing between the larvae of the 2 hookworms would obviate the unnecessary termination of an infection. We have found that

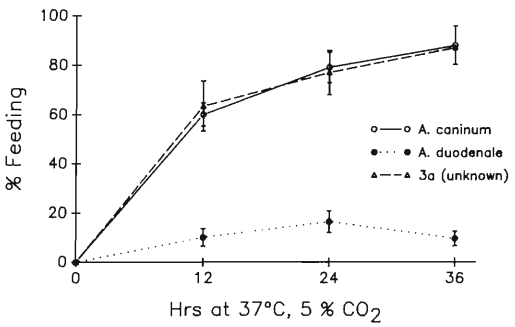

Figure 1. Differentiation of hookworm species using an in vitro assay of L_3 feeding. Larvae are assayed for feeding by the method of Hawdon and Schad (1990). Larvae cultured from the feces of donor dog 3a were compared to larvae cultured from known *A. caninum* and *A. duodenale* donors. Each point is the mean ± standard deviation of 3 replicates.

Table 1. Resumption of feeding by third-stage larvae of a known *Ancylostoma caninum* infection, and infective larvae from donor F151, a potentially mixed infection of *A. caninum* and *A. duodenale*.*

Strain	10% canine serum	Mean % feeding ± SD†
A. caninum	+	73.8 ± 8.0[a]
	−	11.4 ± 2.4[b]
F151	+	74.6 ± 9.5[a]
	−	1.4 ± 1.3[b]

* L_3's incubated in 0.1 ml RPMI ± 10% normal canine serum at 37°C, 5% CO_2 for 24 hr. After incubation, feeding larvae are labelled as described elsewhere (Hawdon and Schad, 1990).

† Mean of 3 replicates ± standard deviation (SD). Values with different superscripts are significant at $P < 0.01$ using the Student's *t*-test. Statistics are done on arcsin transformed data, and the retransformed means expressed as percentages.

third-stage larvae (L_3) of the 2 species behave differently in an in vitro feeding assay (Hawdon and Schad, 1990), and have employed this assay to determine the species identity of suspect hookworm infections on 2 occasions.

In the first infection, donor beagle 3a was inoculated with approximately 4,000 L_3, believed to be *A. duodenale*. Fecal egg counts were not performed between days 13 and 18 postinfection, by which time the infection was patent. Although generally 25–30 days, a prepatent period of 18 days was close to the range reported for *A. duodenale* infections (Leiby et al., 1987). However, the initial egg output of 5,200 eggs per gram (epg), and a peak egg output on day 11 postpatency of 29,600, were extraordinarily high for a pure *A. duodenale* infection in our canine model system, casting further doubt as to the purity of the infection. Consequently, the suspect donor's feces were collected and cultured separately from those of a known *A. duodenale* infected dog. After 10 days incubation, L_3's were harvested from known pure cultures of *A. caninum* and *A. duodenale* and from cultures of the suspect infection. Larvae were assayed for feeding at 12, 24, and 36 hr incubation as described elsewhere (Hawdon and Schad, 1990). The results are shown in Figure 1. The *A. caninum* larvae showed characteristic species-specific feeding behavior, with a maximum proportion of the larvae having resumed feeding by 24 hr incubation. In sharp contrast, few larvae from the pure *A. duodenale* larvae initiated feeding by 24 hr. Larvae from the suspect donor 3a exhibited feeding behavior identical to that of the known *A. caninum* larvae, suggesting accidental contamination of the infection with this species. At necropsy, 110 adult hookworms were recovered, and 22 of the smallest worms (i.e., in the size range of *A. duodenale* in the dog) were identified by counting the number of ventral teeth in the buccal cavity. Nineteen of the examined worms were *A. caninum,* and 3 were *A. duodenale,* confirming the suspected mixed nature of the infection.

A second helminth-naive beagle (donor F151) was inoculated with approximately 1,000 putative *A. duodenale* larvae. The prepatent period was 18–22 days, and the initial egg output was 4,600 epg at day 22 postinfection, reaching a peak of 17,400 epg on day 37. These parameters suggested contamination of the infection with *A. caninum.* Again, feces from the suspect donor were cultured separately, and L_3's were recovered after 10 days. These larvae, together with 10-day known *A. caninum* larvae, were assayed for feeding after 24 hr incubation. At the time of comparison there were insufficient *A. duodenale* larvae to assay. The results suggested that the suspect larvae were *A. caninum,* since the known *A. caninum* larvae were feeding at 73.8 ± 8.0%, and the unknown larvae at 74.6 ± 9.5% (Table 1). Examination of 215 of a total of 248 adult hookworms recovered from the small intestine at necropsy yielded 198 *A. caninum* and 17 *A. duodenale* adults.

These results indicate that it is possible to distinguish between pure and contaminated infections involving 2 closely related species of hookworm in experimentally infected dogs using in

vitro feeding as an assay. Since, to our knowledge, *A. duodenale* is maintained in dogs at only 2 locations worldwide (the other being the Institute of Parasitic Diseases, Hangzhou, Peoples' Republic of China), the need for such a technique in a laboratory situation is probably uncommon. However, this technique may prove useful in epidemiological and epizootological studies. For example, it may be possible to identify mixed infections of the sympatric human hookworms *A. duodenale* and *A. ceylanicum* (Looss, 1911), whose larvae are morphologically very similar (Yoshida, 1971), based on in vitro differences in feeding behavior. In the cases reported here, where 1 species was more successful in establishing in the host than the other, feeding behavior resembled that of the dominant species. However, when co-occurring parasites are similarly vigorous, the feeding behavior of larval populations derived from a mixed infection should be intermediate. Secondly, this technique may be useful in field situations where soil ecologists or epidemiologists have no practical method to distinguish among the common congeneric ancylostomes (*A. duodenale, A. caninum, A. tubaeforme* Zeder, 1800, and *A. braziliense* de Faria, 1910) of humans and their usual companion animals. In industrialized countries, this is particularly important in parks, playgrounds, and other public open spaces, where irresponsible pet ownership with attendant fecal pollution raises the possibility of invasion by nematode larvae associated with cutaneous and visceral larva migrans in humans. Finally, adaptation of this technique to other parasites may aid in the nondestructive differentiation of closely related species inhabiting the same host.

These experiments also suggest that the 2 *Ancylostoma* species may resume feeding in response to different host signals during infection, or more likely, that the larvae respond to a species-specific host stimulus. Although this strain of *A. duodenale* is adapted to the dog (Leiby et al., 1987), larvae may require a signal specific to human serum in order for a large proportion of them to resume feeding in vitro. Comparative investigations of the resumption of feeding in larvae of various hookworm species are currently underway in this laboratory.

We thank Lan Han for expert technical assistance in maintaining the parasites. This work was supported in part by NIH grant AI-22662.

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58(1), 1991, p. 128

Research Note

Treatment of Bertiellosis in *Macaca fascicularis* with Praziquantel

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ABSTRACT: A single *Macaca fascicularis* recently received from the Philippines was found to harbor *Bertiella studeri*. To eliminate the infection, the monkey was treated with praziquantel in a single oral dose at 5 mg/kg of body weight. Two strobilae but no scolices were found in the feces following treatment. *Bertiella studeri* were not present at necropsy 1 mo after treatment. It appears that praziquantel can be added to the list of drugs which can be used to control bertiellosis.

KEY WORDS: *Macaca fascicularis, Bertiella studeri,* praziquantel, monkey, tapeworm.

During routine fecal examination of a newly received group of monkeys (*Macaca fascicularis*) from the Philippines, 1 animal was found to harbor *Bertiella studeri* (Blanchard, 1891) Stiles and Hassall, 1902. The monkey was treated with praziquantel to eliminate the infection. Praziquantel was used because it has become the drug of choice in treating cestode infections over the last several years (Harnett, 1988; King and Mahmoud, 1989). It is highly efficacious against both adult and larval tapeworms and is extremely well tolerated (Andrews et al., 1983). To our knowledge, however, the drug has never been tried against *B. studeri*.

Praziquantel (Droncit canine tablets) was administered to the infected monkey in a single oral dose at 5 mg/kg of body weight. Feces were monitored for the next 48 hr following treatment, during which time 2 strobilae were observed to have been passed. Scolices were not found in the feces. No additional proglottids were observed in the feces at any later time after treatment. About 1 mo following treatment, the monkey was found to have tuberculosis and was sacrificed by an overdose of sodium pentobarbital to protect the colony and caretakers. At necropsy, no *B. studeri* were found. Thus, it appears that praziquantel was effective in eliminating *B. studeri* from the monkey. No adverse effects of treatment with praziquantel were observed.

A number of drugs have been reported to be effective in treating bertiellosis in animals and humans (Dissanaike et al., 1977; Schutze, 1977; Duwel et al., 1981; Graber and Gevrey, 1981; Jueco, 1982; Imamkuliev et al., 1983; Bandyopadhyay and Manna, 1987). It appears that praziquantel can be added to the list of drugs which can be used to control bertiellosis.

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Research Note

Intensity of *Neobenedenia girellae* (Monogenea: Capsalidae) on the Halfmoon, *Medialuna californiensis* (Perciformes: Kyphosidae), Examined Using a New Method for Detection

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ABSTRACT: The intensity and prevalence of infestation of *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963, were examined in the halfmoon, *Medialuna californiensis* (Steindachner). The halfmoon is a new host for this parasite, and its presence on this fish near Santa Catalina Island, California, extends its known geographical distribution. Two methods of examination were compared, 1 of which was a far more effective way of detecting non-microscopic *N. girellae*. Our results suggest that female *M. californiensis* have a significantly higher intensity of infestation than males. There appears to be no relationship between adult host size and intensity of infestation.

KEY WORDS: Monogenea, Capsalidae, intensity, *Neobenedenia girellae*, Kyphosidae, *Medialuna californiensis*, prevalence, California.

The halfmoon, *Medialuna californiensis* (Steindachner), is a kyphosid fish common off southern California. It ranges from Vancouver Island to the Gulf of California, but is rare north of Point Conception, California (Eschmeyer et al., 1983). The halfmoon is often found in rocky areas and in association with kelp forests, and ranges in depth from the surface to 40 m (Eschmeyer et al., 1983). Little is known about the occurrence of helminth parasites, particularly monogeneans, in northeastern Pacific kyphosids (Hargis, 1955). Herein we report the first finding of a monogenean on the halfmoon.

Forty-one halfmoons were obtained by hook and line and gill net between 19 and 29 October 1988, from 3 locations off Santa Catalina Island, California: Big Fisherman Cove, Blue Cavern Cove, and off Bird Rock (all at approximately 33°27′N, 118°29′W). All specimens were transferred in buckets to the Catalina Marine Science Center where they were held in tanks (for up to 8 hr) until examined for monogeneans.

Each fish was killed by severing the spinal cord behind the head, and then measured and sexed. The skin, fins, buccal cavity, and opercular cavity were examined for monogeneans. Two different methods of examination were employed on the freshly killed fish. The first 24 fish obtained were examined visually (without magnification), while the next 17 fish obtained were examined visually after the application of alcohol–formalin–acetic acid (AFA), a fixative listed by Cailliet et al. (1986). The AFA was applied via pipette onto the entire external surface of the fish. The AFA turned the monogeneans opaque, facilitating their discovery. All monogeneans were fixed in AFA for 24 hr and then transferred to 70% ethanol. Forty voucher specimens from 9 different hosts were deposited in the collection of the Harold W. Manter Laboratory, University of Nebraska State Museum (HWML 32711–32719). The external body surface of each fish was examined for any gross, physical damage caused by the parasites.

All monogeneans found were the same species, *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963, according to criteria presented by Crane (1972). *Neobenedenia girellae* is a capsalid monogenean that parasitizes a variety of marine teleosts, including *Girella nigricans* (Ayres), *Leptocottus armatus* Girard, and *Semicossyphus pulcher* (Ayres) off La Jolla, California and *Myctoperca pardalis* (=*Mycteroperca rosacea* (Streets)) off Baja California (Yamaguti, 1963). The occurrence of *N. girellae* on *Medialuna californiensis* constitutes a new host and geographic record (69 km northern extension of range).

We compared the 2 methods of examination for parasites to determine whether they were significantly different in terms of number of parasites detected per fish. A Mann-Whitney *U*-test comparing the AFA and visual-only methods showed a highly significant difference between them ($U_s = 336.5$, $P < 0.001$). Figure 1 shows the number of fish having a given number of parasites for the 2 methods of examination. A mean intensity of 2.6 parasites per fish and a prevalence of 75% was found using the visual-only method, whereas a mean of 11.8 parasites per fish and a prevalence of 94% was found using the AFA method. These results indicate that the

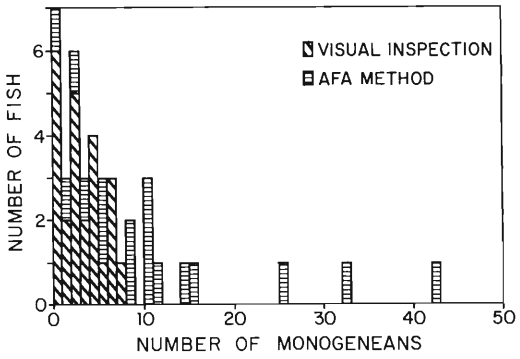

Figure 1. Comparison of numbers of *Neobenedenia girellae* found on *Medialuna californiensis* using the visual-only and AFA methods of examination.

AFA method is more effective in detecting *N. girellae* than is the visual-only method. The visual-only method is less efficient than the AFA method for 2 reasons. First, these parasites are naturally transparent, allowing individuals to blend in with the transparent mucus covering of the fish. Second, many of the parasites occurred underneath the scales of the fish, making them virtually impossible to detect without the aid of AFA. We suggest that future studies utilize the AFA method of detection for *N. girellae*. This method should be used in conjunction with a microscope in order to detect juvenile (100–200 μm) *N. girellae*. The AFA method may be useful for the detection of other species of monogeneans parasitic on fishes, but this warrants further study.

Because of the significant difference between the 2 methods, only the data from the AFA method were analyzed statistically. A Mann-Whitney *U*-test revealed that female fish had a significantly higher intensity of infestation than males ($U_s = 50.5$, $P < 0.025$). Prevalence was 92% for males and 100% for females. No significant correlation between size and intensity of infestation was found using Spearman's coefficient of rank correlation ($df = 15$, $P > 0.05$). Size related differences in intensity of infestation due to dietary and immunity changes have often been reported for parasite–host interactions (Noble et al., 1989). Future studies on the diet and immunity of the halfmoon are therefore in order. A relationship does indeed exist between host sex and intensity of infestation. Females have a significantly greater intensity of infestation than do males. Differences may result from behavioral differences between males and females that make females more vulnerable, or females may be exuding a chemical attractant.

Visual examination of the external surface of the specimens revealed no apparent adverse effects due to the presence of *Neobenedenia girellae*. Nigrelli (1947) found that death occurred in many marine teleosts due to heavy infestations of *Neobenedenia melleni*. In this study, halfmoons that were heavily infested (up to 42 parasites) showed no obvious adverse effects.

This study was conducted during the 1988 UCLA Catalina Marine Biology Quarter. We thank the Catalina Marine Science Center staff for the use of their facilities. R. Gibson provided statistical assistance and D. G. Buth provided comments on an earlier draft of the note.

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Research Note

Adherence of Eosinophils to the Epicuticle of Infective Juveniles of *Anisakis simplex* (Nematoda: Anisakidae)

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ABSTRACT: We previously have observed that eosinophils were prominent in chronic granulomas around third-stage juveniles of *Anisakis simplex* surgically implanted in laboratory mice and destruction of the juveniles within the host tissues was initiated by day 14 postinfection (PI). Based on histology, the mechanism underlying destruction of these juveniles appeared to involve the interaction of eosinophils on the helminth's cuticle. To test this hypothesis, eosinophils were harvested by bronchoalveolar lavage from CBA/J mice previously infected with a closely related ascaridoid and added to cultures of juvenile worms in the presence or absence of sera from *A. simplex*-immune mice. Adherence of eosinophils occurred only in the presence of *A. simplex*-immune serum taken 14 or 21 days PI from mice infected with 10 worms. Transmission electron microscopy revealed active degranulation of eosinophils onto the epicuticle of the parasite. Destruction of the cuticular surface of the juveniles by eosinophils was not evident after 24 hr of in vitro culture. Apparently, eosinophils lack the ability to kill the juveniles. Damage to the cuticle, however, was observed in areas associated with macrophage adherence.

KEY WORDS: parasitic nematode, *Anisakis simplex, Toxocara canis,* CBA/J mice, eosinophil adherence, epicuticle, juvenile–eosinophil interactions, antibody.

Eosinophils represent a prominent cellular component of the inflammatory response surrounding tissue-invading helminths and have been demonstrated in vivo to adhere to infective juvenile parasites. Linkage of eosinophils to infective juveniles leads to degranulation of the toxic contents of the cells and often results in damage to the surface or killing of the parasites (James and Colley, 1978; Kazura and Grove, 1978; Ramalho-Pinto et al., 1978; McLaren and Ramalho-Pinto, 1979; Kazura and Aikawa, 1980; Desakorn et al., 1987).

One ascaridoid parasite, *Toxocara canis,* the canine roundworm, apparently can avoid the deleterious effects of the immune-mediated adherence of eosinophils. Badley et al. (1987) suggested that infective juveniles of this nematode avoided the toxic effects of eosinophils by rapidly sloughing areas of its epicuticle in contact with the granulocyte; thus, the juveniles continually were releasing surface antigens. This method of evading the host's immune attack might explain the ability of juvenile *T. canis* to migrate with relative impunity through the visceral organs of the body. In contrast, Jones et al. (1990) showed that juvenile *Anisakis simplex* did not survive in vivo beyond 3 weeks postinfection (PI). Using SEM and TEM, we analyzed the ability of eosinophils to adhere to the epicuticle of *A. simplex.*

Third-stage juveniles (L_3) of *A. simplex* were removed from the viscera of several species of Pacific rockfishes (*Sebastes* spp.) collected from the Pacific Northwest in 1989. L_3 were isolated from fish viscera by the pepsin-hydrochloric acid process (Deardorff and Throm, 1988), rinsed several times in sterile PBS, and maintained at ambient temperature in RPMI-1640 tissue culture medium (Sigma Chemical Co., St. Louis, Missouri) containing 50 μg gentamicin sulfate/ml (Sigma).

Eggs, isolated from mature *T. canis,* were cultured in 0.1 N H_2SO_4 for 28 days in the dark at room temperature to allow for embryonation. Following microscopic verification that eggs contained infective second-stage juveniles, the cultures were stored at 4°C until needed. Three mice were placed under mild ether anesthesia and infected with 250 eggs by gastric intubation. These procedures are described in detail by Kayes (1984) and Kayes et al. (1986).

Female CBA/J mice were selected for our experiments because previous reports demonstrated this species is a good experimental model for human anisakiasis (Jones et al., 1990) and provides an excellent source of eosinophils (Kayes et al., 1987). CBA/J mice, obtained from the

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Jackson Laboratories (Bar Harbor, Maine), were maintained on standard rodent Chow and water ad libitum in the Animal Health and Resources facility of the College of Medicine, University of South Alabama. These facilities meet the guidelines established by the National Institutes of Health in the *Guide for the Care and Use of Laboratory Animals.* All mice were obtained as weanlings (weight, 12–15 g) and allowed a minimum of 7 days to acclimate to these facilities prior to inclusion into the experiments.

L_3 of *A. simplex* were rinsed in sterile RPMI-1640 without antibiotics prior to placement into the abdominal cavity of the mice. Mice were anesthetized with ether vapor; a surgical incision was made over the lateral peritoneal cavity; 2, 5, or 10 viable and undamaged L_3 were placed in the abdominal cavity; and the wound was closed with 9-mm stainless steel wound clips. Surgical implantation was selected over gavage techniques as the method of infection because of the size of the mice in relation to the large L_3. Mice were necropsied at days 14 and 21 PI and sera were collected and pooled for each group of mice. Antibody titers for the immune mouse sera (IMS) and normal mouse serum (NMS) were determined using an ELISA assay specific to IgG- and IgM-*A. simplex* excretory–secretory products (ASEX) following the methods adopted by Kayes et al. (1985).

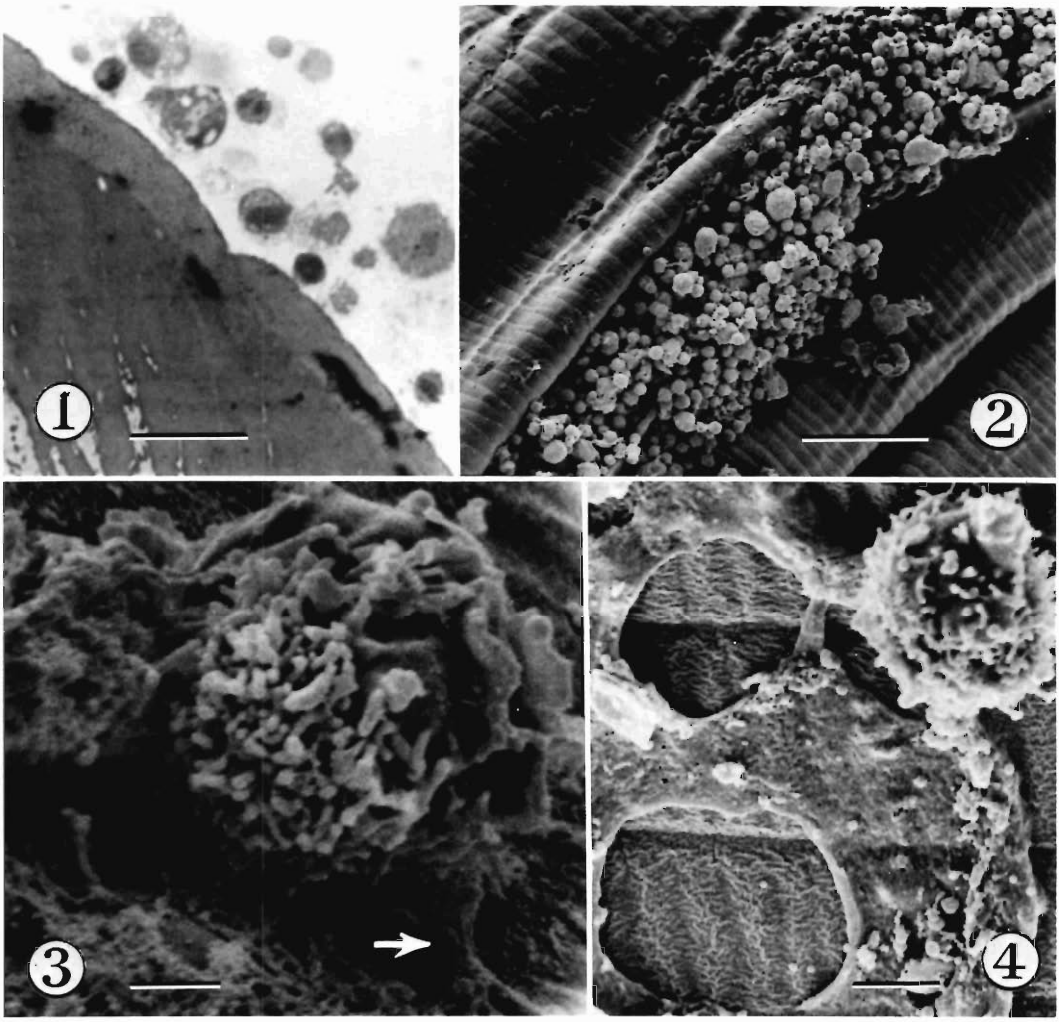
For removal of complement, the procedure of Capron et al. (1981) was followed. Briefly, 100 mM EDTA was added to tubes from each group and the tubes were incubated at 37°C. After 1 hr, sera were removed, dialyzed against Dulbecco balanced saline solution (DBSS; GIBCO, Long Island, New York) for 2 hr at 4°C, and subsequently dialyzed twice against 0.85% NaCl for 2 and 12 hr, respectively.

On day 14 PI, eosinophils were isolated from the lungs of infected mice following the procedures for bronchoalveolar lavage (BAL) as outlined by Kayes et al. (1986, 1987) with slight modification. Briefly, mice were exsanguinated under ether vapor, the thoracic cavity was opened, and the trachea exposed. A MINISET® 21-gauge, 1.9-cm needle with 9-cm-long tubing (Baxter Laboratories, Inc., McGaw Park, Illinois) was inserted into the trachea and a silk ligature was secured around both the trachea and needle. A 1-cc syringe containing 0.5 ml of DBSS with 60 U of heparin/ml was attached to the secured needle, and the contents were infused into the lungs and immediately aspirated back into the syringe. The syringe was detached from the needle and the infusate collected in a 5-ml sterile culture tube maintained at 0–2°C. The process was repeated until the culture tube was full. Collected infusate was centrifuged at 400 *g* for 10 min and resuspended in RPMI-1640. The BAL procedure was selected over peritoneal lavage because it yields relatively pure populations of eosinophils and does not need to be elicited with chronic irritants such as proteose peptone.

An aliquot of 9.0×10^6 cells collected by BAL was treated as described by James and Colley (1978). Briefly, the eosinophils were exposed to a 5-mg/ml solution of bovine pancreatic trypsin (Type 1, 2X crystallized, Sigma) in DBSS, pH 7.6. Cells were suspended at a ratio of 0.1 ml of packed cells to 2 ml of enzyme solution and were incubated at 37°C for 45 min. Soybean trypsin inhibitor (Type 1-S, Sigma), mixed at 15 mg of inhibitor to 5 mg trypsin, terminated the trypsinization process. Eosinophils were washed 3 times in RPMI-1640 prior to use. Three viable juvenile *A. simplex* were introduced into separate 16-mm-diameter tissue culture wells (Costar, Cambridge, Massachusetts) containing 1.5×10^6 cells in 300 μl of RPMI-1640. Each well then received an additional 200 μl of IMS, NMS, or RPMI-1640. Three additional culture wells were set up with similar components as mentioned above but with frozen worms substituted for viable worms.

Alternatively, cytophilic antibodies were removed from a second aliquot of 9.0×10^6 cells by incubation in DBSS, pH 4, for 1 min and washed 3 times in RPMI-1640. Viable worms were exposed to identical conditions as group 1. All cultures were placed in a 37°C incubator with 5% CO_2 in air for 24 hr; after incubation they were removed and fixed.

Worms were fixed directly in the culture wells with cold 0.1 M phosphate-buffered 3% (v/v) glutaraldehyde and refrigerated for 24 hr. Evidence of cellular adherence to *A. simplex* was observed using an inverted microscope. Specimens for scanning electron microscopy (SEM) were dehydrated, critical point-dried in liquid carbon dioxide, mounted on a specimen stub, coated with gold palladium, and examined with a Philips 501 scanning electron microscope. For transmission electron microscopy (TEM), L_3 with attached eosinophils were cut with a razor blade into small pieces, postfixed in 1% osmium te-



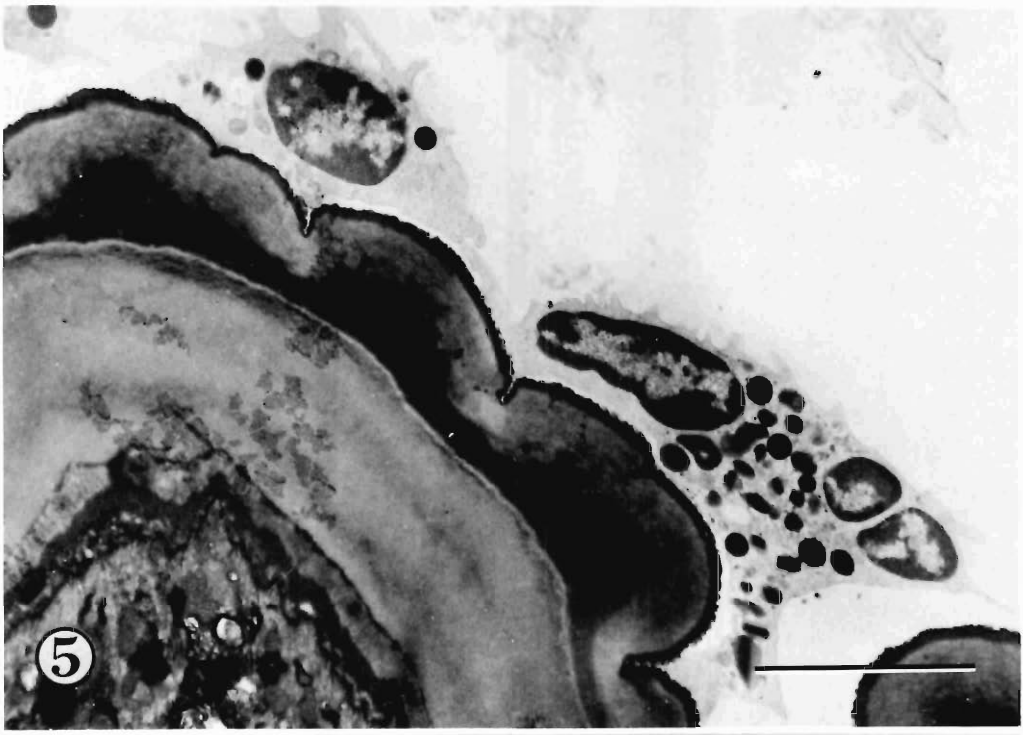
Figures 1–4. 1–3. Adherence of eosinophils on epicuticle of *Anisakis simplex* third-stage juvenile. 1. A cross section of worm showing stratification of cells on cuticle. Bar represents 20 μm. 2. Scanning electron photomicrograph showing aggregation of cells. Bar represents 50 μm. 3. Scanning electron photomicrograph of an eosinophil showing extrusion of pseudopod (arrow). Bar represents 2 μm. 4. Scanning electron photomicrograph showing normal surface topography in areas where eosinophils were removed. Cuticle on right shows normal cuticle without eosinophil contact. Bar represents 4 μm.

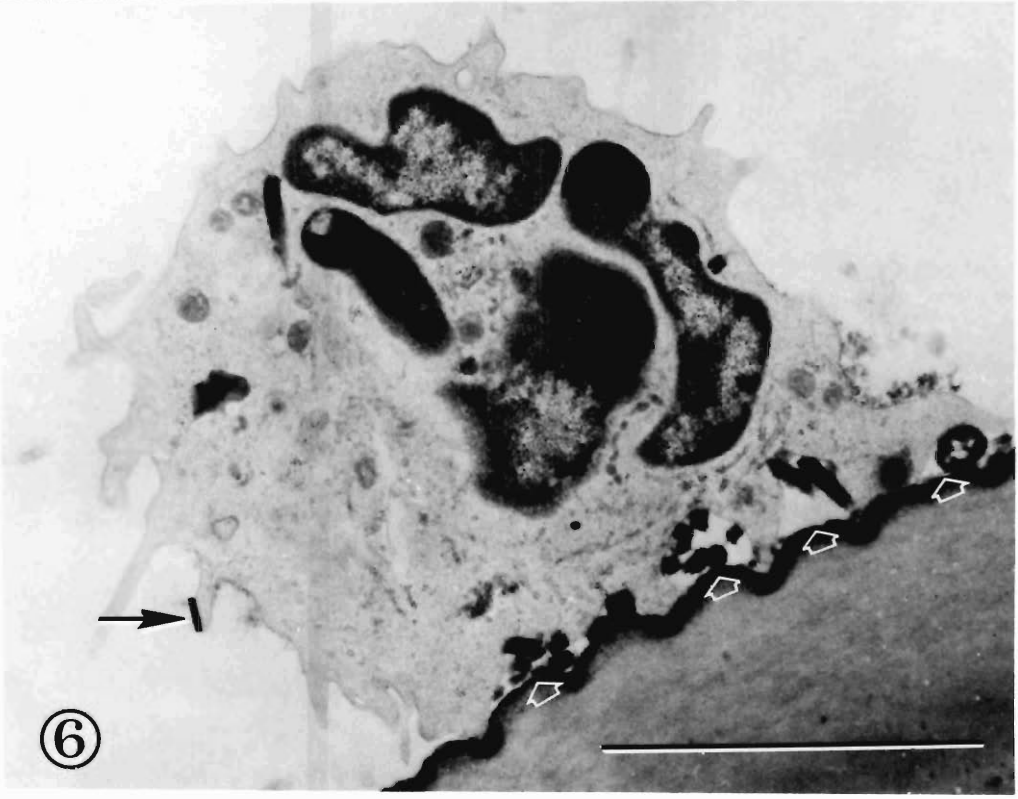
troxide for 2 hr, dehydrated in ethanol, treated with acetone, and embedded in Spurr's low viscosity resin. Thin sections were cut with a diamond knife, mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined with a Philips 301 electron microscope.

For light microscopic analysis, 1-μm thick orientation sections were mounted on glass slides and stained with toluidine blue.

Cells recovered by BAL were approximately 95% eosinophils, as determined by light microscopic observation of stained smears. Macrophages represented a small portion of the remaining 5% of the cell population. These findings were subsequently confirmed by SEM and TEM examination.

Eosinophils attached to the surface of the L_3 in the presence of *A. simplex*-immune serum regardless of the method used to remove the cytophilic antibodies (Figs. 1–3). Eosinophil attachment was predominantly on 1 side of viable





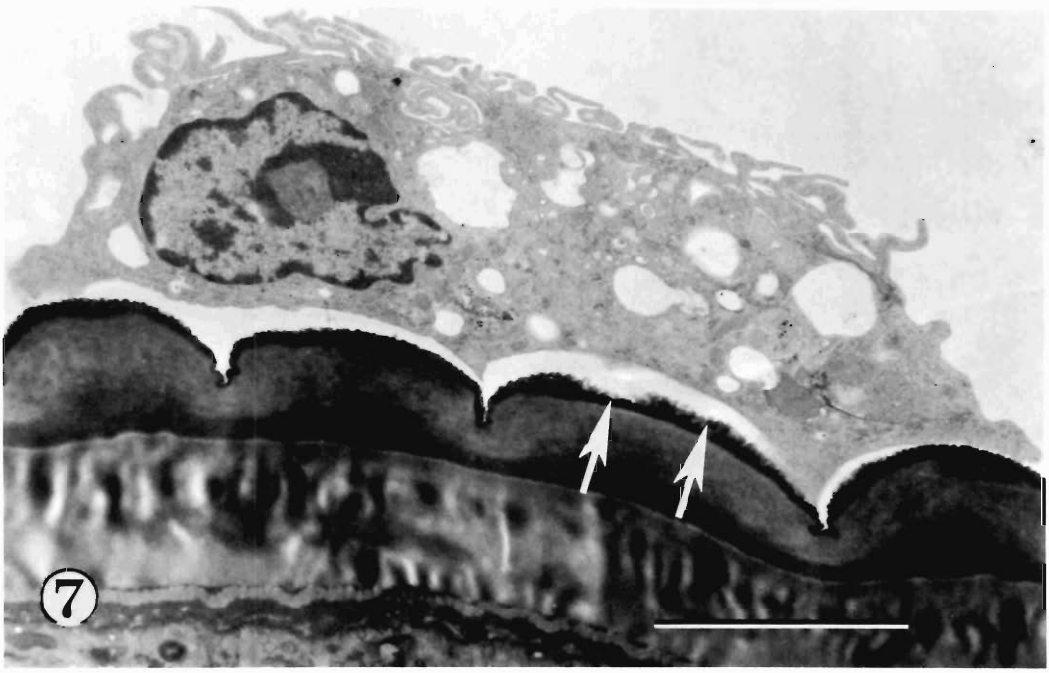


Figure 7. Cross section of juvenile *Anisakis simplex* showing intimate contact with macrophage and areas of cuticular alteration (arrows). Bar represents 6 μm.

worms with the preponderance of the cells affixed near the anterior extremity of the L_3. The vigorous motility of these worms in culture, as viewed through an inverted microscope, removed attached cells on the surface. Adherence was reduced in cultures with NMS and lacking in cultures deficient in IMS. Cells generally attached to the epicuticle in large aggregations, often several layers thick (Figs. 1, 2). No damage to the surface could be seen in areas where cells had been attached but were no longer present (Fig. 4). TEM revealed attached eosinophils and macrophages strictly conforming to the surface topography of the juveniles (Fig. 5). Following attachment, eosinophil granules were seen in vacuoles of plasma membranes juxtaposed to the epicuticle (Fig. 6). Some cuticular damage was evident at the site of macrophage attachment (Fig. 7).

The anterior extremity of the L_3, where large numbers of eosinophils were attached, corresponds with the areas where ASEX are released. Our findings are consistent with those of Sakanari et al. (1988) who showed that the excretory pore and opening of the dorsal gland of the esophagus were involved in the in vivo release of ASEX in humans.

Even though we observed no damage to the cuticle attributable to eosinophils and immune serum, we did notice cuticular alterations associated with the attachment of antibody-sensitized macrophages. This suggest that these cells could be involved in the killing of the juveniles. Several researchers have observed damage or cytocidal events to parasites mediated by macrophages (Haque et al., 1980; Ouaissi et al., 1981; Yen et al., 1986a, b).

Eosinophils that were recovered from *T. canis*-

←

Figures 5, 6. TEM micrographs of eosinophils attached to epicuticle of juvenile *Anisakis simplex*. 5. Cellular adherence closely conforming to the annulated contours of cuticle. Bar represents 5 μm. 6. Eosinophil in process of degranulation. Hollow arrows indicate eosinophil granules being released into extracellular pockets and solid arrow shows crystalloid core apparently being released at tip of pseudopod. Bar represents 7 μm.

infected mice and used in adherence experiments with *A. simplex* without first removing cytophilic anti-*T. canis* antibodies were minimally adherent. They displayed specificity for their anisakid targets only after their surfaces were stripped of cytophilic antibodies and reconstituted with anti-*A. simplex* antibodies. Whether the same antibodies that mediate eosinophil adherence also mediate eosinophil cytotoxicity remains unknown.

Sera from mice infected with *T. canis* mediate adherence of eosinophils to the surface of second-stage juveniles of *T. canis* and the adsorption of these sera with homologous excretory–secretory (ES) products completely abolished this ability (Badley et al., 1987). Even though electron microscopy showed these cells to be tightly adherent to the parasite surface, they were unable to mediate a cytotoxic reaction against these juveniles. Because a diffusely granular material was formed on the surface of the epicuticle, it was postulated that the release of ES-like products prevented the killing of juveniles.

We observed no comparable granular product on the worm surface. This observation is peculiar in light of our previous finding that surgically implanted juveniles are rapidly killed (Jones et al., 1990) and our present electron microscopic images which indicate essentially no damage to the epicuticle after eosinophil degranulation on the surface of juvenile *A. simplex*. We conclude, therefore, that the eosinophil is not the major assassin of juvenile ascaridoids as seen with infections of juvenile schistosomes (Ramalho-Pinto et al., 1978; McLaren and Ramalho-Pinto, 1979; Vadas et al., 1980) and *Trichinella spiralis* (Kazura and Grove, 1978; Kazura and Aikawa, 1980; Butterworth and Richardson, 1985).

In conclusion, we determined that eosinophils attach to and degranulate on juveniles of *A. simplex,* IMS was required to induce maximum attachment, and the epicuticle lacked damage at the site of eosinophil adherence. These reports are similar to those of Badley et al. (1987) for *T. canis*. Our findings, however, principally differ from Badley et al. (1987) because juveniles of *A. simplex* did not detach areas of their epicuticle that were in contact with the eosinophils, even though our worms were exposed to the cells 47 times longer than theirs. *Toxocara canis* apparently has evolved this cuticular-sloughing phenomenon to avoid the host's immune response. Thus, in naturally occurring infections in humans or experimental infections in our mouse model, juveniles of *T. canis* can complete their complex tissue migration with relative impunity. In contrast, juveniles of *A. simplex,* whether infecting humans or CBA/J mice, do not survive. This may be the result of their inability to slough their cuticle and the cytotoxic effects associated with the adherence of macrophages.

We gratefully acknowledge Ms. Barbara Hyde (University of South Alabama EM Center, Mobile) for technical assistance. Fishes were collected by FDA inspector Mr. Dick Throm (Seattle District) with the cooperation of Mr. Mike Kinkade and the staff at Bornstein Seafood Inc., Bellingham, Washington, and Mr. John W. McCallum and the staff at The Pacific Salmon Co., Inc., Seattle, Washington. This study was supported in part by N.I.H. Grant AI 19968 and American Heart Association, Alabama Affiliate, Inc., Grant No. 870034 awarded to S.G.K.

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Research Note

Sphyranura euryceae (Monogenea) on *Eurycea* spp. (Amphibia: Caudata), from Northcentral Arkansas

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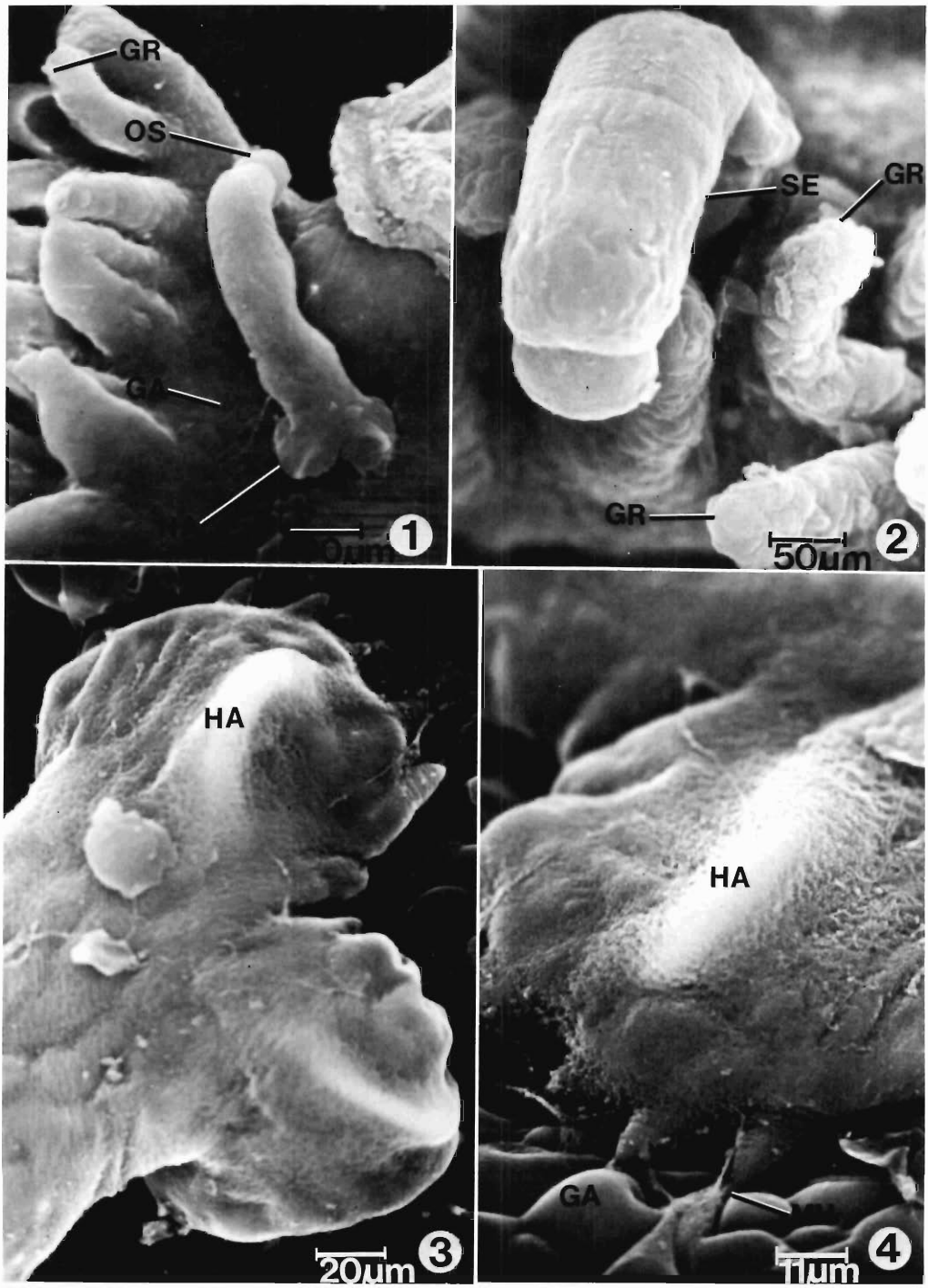
ABSTRACT: *Sphyranura euryceae* Hughes and Moore, 1943 (Polystomatoidea: Sphyranuridae) was found on the external gills, skin, and gular folds of 10/10 larval cave salamanders, *Eurycea lucifuga,* and 10/10 neotenic graybelly salamanders, *E. multiplicata griseogaster,* in northcentral Arkansas. This represents the second time *S. euryceae* has been reported and constitutes new host and distributional records for the parasite. A summary is presented on the *Sphyranura* spp. from caudate amphibians. Based on morphological similarities and the opinions of earlier workers, the synonymy of *S. polyorchis* Alvey, 1936, with *S. osleri* Wright, 1879, is provisionally supported.

KEY WORDS: cave salamander, *Eurycea lucifuga, E. multiplicata griseogaster,* graybelly salamander, Monogenea, Polystomatoidea, prevalence, *Sphyranura euryceae, S. oligorchis, S. osleri, S. polyorchis,* Sphyranuridae, synonymy.

Wright (1879) described *Sphyranura osleri* from the gills and mouth cavity of mudpuppies, *Necturus lateralis* (syn. of *N. maculosus*). Wright and Macallum (1887) provided additional information about the species. Since then, additional *Sphyranura* spp. have been described or reported from North American hosts (Alvey, 1933a, b, 1936; Hughes and Moore, 1943; Coggins and Sajdak, 1982). The purpose of this note is to report *Sphyranura euryceae* Hughes and



Figures 1–4. Scanning electron micrographs of *Sphyranura euryceae* infesting neotenic *Eurycea multiplicata griseogaster* from Arkansas. 1. Worm attached to gill arch (GA) and gill rakers (GR) showing oral sucker (OS) and haptor (HA). 2. Closer view of worm (SE) on gill rakers (GR). 3. Attachment of haptor (HA) on gill arch. 4. Higher magnification of haptor (HA); note marginal hook (MH) attached to gill arch (GA).

Table 1. Species of *Sphyranura* reported from North American Caudata.

Sphyranura spp.	Host(s)	Localities	Reference(s)
S. euryceae	*Eurycea tynerensis*; *E. multiplicata griseogaster*, *E. lucifuga*	Oklahoma; Arkansas	Hughes and Moore, 1943; McAllister et al., this report
S. oligorchis	*Necturus maculosus*	Pennsylvania	Alvey, 1933a, b, 1936
S. osleri	*N. maculosus*	Canada; Wisconsin	Wright, 1879; Wright and Macallum, 1887; Coggins and Sajdak, 1982
*S. polyorchis**	*N. maculosus*	Pennsylvania	Alvey, 1936

* May be a synonym of *S. osleri*.

Moore, 1943, on 2 species of plethodontid salamanders from Arkansas and to comment on possible synonymy of *S. polyorchis* Alvey, 1936.

During January 1990, 10 larval cave salamanders, *Eurycea lucifuga* Rafinesque, 1822, and 10 neotenic graybelly salamanders, *E. multiplicata griseogaster* Moore and Hughes, 1941, were collected 2.4 km W Lakeway off Hwy 14 at Chapman Spring, Marion County. Salamanders were captured by hand, placed in individual plastic bags, and on return to the laboratory (within 24 hr), were killed by immersion in a dilute chloretone solution, fixed in 10% formalin, and stored in 70% ethanol. Monogeneans were noted on all salamanders and some were teased from gill filaments, gular folds, or skin to be flattened under gentle cover glass pressure. Specimens were later stained with acetocarmine, dehydrated through an ethanol series, cleared in xylene, and mounted in Permount®. Techniques used to prepare tissues of other infested salamanders for SEM followed routine procedures and included dehydration in an ethanol series with amyl acetate transition solvent. Specimens were dried in a Samdri critical point dryer and coated with gold/palladium in a Hummer IV sputter coater from 2 to 5 min. A JEOL 100 CXII TEM-SCAN electron microscope was used to view gills and skin at an accelerating voltage of 20 kV. Representative specimens of *S. euryceae* have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as USNM 81047. Voucher specimens of salamanders have been deposited in the Arkansas State University Museum of Zoology (ASUMZ) as follows: *E. lucifuga* (ASUMZ 15348–15357) and *E. m. griseogaster* (ASUMZ 15338–15347, 15358–15359).

Of the 20 salamanders examined, all were harboring at least 1 polystomatid monogenean identified as *Sphyranura euryceae* (Hughes and Moore, 1943). Numerous worms were observed attached to gill arches and rakers of individual salamanders and are shown in Figures 1–4. Five *S. euryceae* had the following characteristics and measurements (mean followed by the range in micrometers [μm] in parentheses): total body length 1,620 (800–2,400); maximum width 420 (300–600); haptor 463 (269–767) wide by 259 (191–355) in maximum length; caudal sucker diameter 137 (111–197); oral sucker width 203 (155–284) by 186 (153–225) long ($N = 4$); spheroid to subspheroidal testes 7 (4–8) in number, 102 (78–120) wide by 77 (53–98) long ($N = 6$); uterus obscured in 2 specimens and occupied by a single, heavy-shelled, egg 190 (145–217) wide by 268 (254–282) long ($N = 3$).

Hughes and Moore (1943) reported *S. euryceae* was found on 45 of 90 Oklahoma salamanders, *Eurycea tynerensis* Moore and Hughes, 1939, from Cherokee County, Oklahoma. The type locality of *S. euryceae* (Pea Vine Creek near Tahlequah) is approximately 225 km WSW of the locality reported herein.

Although there have been several studies on the helminth parasites of *Eurycea lucifuga* involving 370 salamanders (Landewe, 1963; Dyer and Brandon, 1973; Dyer and Peck, 1975; Castle et al., 1987), *Sphyranura* spp. was not reported. Perhaps this is a consequence of surveying only metamorphosed terrestrial adults, since species of *Sphyranura* have been reported previously to infest only aquatic larval or neotenic salamanders having external gills (Table 1).

Four species of *Sphyranura* are known from North American caudate amphibians (Table 1). However, the validity of *S. polyorchis* Alvey, 1936, was questioned by Price (1939) who noted that the subtle differences between *S. osleri* and *S. polyorchis*, claimed by Alvey (1936), probably do not justify the recognition of 2 separate species. This view was later reiterated by Hughes

and Moore (1943). According to Price (1939) there are no specimens of *S. polyorchis* available and except for differences in number of testes (20–23 in *S. polyorchis* and 12–16 in *S. osleri*) and in the supposed absence of spines on large haptoral hooks of *S. polyorchis*, the 2 species are essentially the same in all other characteristics and measurements. Compelling evidence for recognizing distinct species is not evident and it is possible that the differences noted by Alvey (1936) may be due to individual variation. Therefore, the synonymy originally proposed by Price (1939) is provisionally supported until specimens of *S. polyorchis* can be rediscovered and examined.

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Research Note

Long-term Storage of Hookworm Infective Larvae in Buffered Saline Solution Maintains Larval Responsiveness to Host Signals

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ABSTRACT: Third-stage larvae (L_3's) of *Ancylostoma caninum* stored in water exhibited a decline in the number of larvae that resumed feeding in response to canine serum, whereas those stored in copro-culture for the same amount of time failed to show this decline. When L_3's were stored for 39 days in BU, a *Caenorhabditis elegans* handling buffer, they retained the ability to resume feeding. Short (<24 hr) storage in water had no effect on feeding.

KEY WORDS: *Ancylostoma caninum*, host signals, hookworm, infective larvae.

During investigations of the resumption of feeding by infective hookworm larvae (L_3's) when exposed to host-mimicking conditions in vitro (Hawdon and Schad, 1990), we have observed a marked decrease in the proportion of larvae responding to a feeding stimulus when the larvae were first stored in water. Larvae that remained in copro-culture for the same length of time failed to exhibit this decline in the proportion feeding,

Table 1. Effect of storage conditions on the feeding of third-stage larvae of the hookworm *Ancylostoma caninum*.

Storage conditions	Mean % feeding ± SD*
None	61.9 ± 8.9[a]
39 days in culture	70.1 ± 4.1[a]
39 days in dH_2O	23.3 ± 0.6[b]
39 days in BU†	68.0 ± 2.6[a]

* Mean of 3 replicates; SD = standard deviation. Values with different superscripts are significantly different at $P < 0.05$ using Student's *t*-test on arcsin transformed data.

† BU = 50 mM Na_2HPO_4/22 mM KH_2PO_4/70 mM NaCl. See text for details.

Table 2. Effect of axenization in distilled water and BU on the resumption of feeding by third-stage larvae of *Ancylostoma caninum* in vitro.

Axenization medium	Serum	Mean % feeding ± SD*
dH_2O	+	60.4 ± 4.1[a]
	−	3.3 ± 0.6[b]
BU	+	61.4 ± 4.1[a]
	−	1.7 ± 1.6[b]

* SD = standard deviation; $N = 3$. Values with different superscripts are significantly different at $P < 0.05$.

suggesting that conditions in culture were more favorable for maintaining responsiveness to the host signals encountered during infection. One possible explanation for these observations was the difference in solute concentration in the storage media. Therefore, an experiment was designed to test this hypothesis.

Ancylostoma caninum L_3's were recovered from copro-cultures by a Baermann technique, and washed 3 times in sterile distilled water (dH_2O). The pellet was suspended in 20 ml of sterile dH_2O containing penicillin (100 U/ml), streptomycin (100 μg/ml), and tetracycline (1 mg/ml) for axenization. The worms were transferred to sterile glass petri dishes and incubated overnight at 25°C. After axenization, the larvae were counted and divided into 3 equal groups. One group was washed 3 times with sterile dH_2O, while a second was washed 3 times with sterile BU, a buffer used in the handling of the free-living nematode, *Caenorhabditis elegans* (50 mM Na_2HPO_4/22 mM KH_2PO_4/70 mM NaCl, pH 6.8; Clokey and Jacobson, 1986). Antibiotics were not included in the wash solutions. The final pellets were suspended in 20 ml of the appropriate solution supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (5 μg/ml), and transferred to sterile glass petri plates. The plates were incubated for 39 days at 25°C, with a light : dark cycle of 11:13. To establish a baseline feeding activity, the third group was incubated under host-like conditions immediately after axenization and assayed for feeding 24 hr later as described previously (Hawdon and Schad, 1990). After the 39-day incubation period, the dH_2O- and BU-groups were also incubated and assayed for feeding as above. Larvae harvested from 39-day-old copro-cultures (i.e., cultured from the same fecal sample) served as controls. The results are shown in Table 1. The proportion of larvae stored in BU that were feeding did not differ ($P > 0.05$) from that observed among larvae assayed immediately (i.e., no treatment), or those recovered directly from 39-day copro-cultures. In sharp contrast, a markedly smaller proportion of larvae fed after storage in dH_2O. There also appeared to be higher mortality in water-stored larvae, although this was not evaluated quantitatively.

To determine if even the short axenization in water adversely affected feeding, larvae from a single batch of copro-cultures were either axenized in dH_2O or BU for 14 hr and then assayed for feeding. There was no significant difference ($P > 0.05$) in the proportion of larvae feeding (Table 2), indicating that processing larvae in dH_2O for a short period (<24 hr) had no effect on their ability to resume feeding.

These results suggest that storage in the buffer BU instead of distilled water may maintain the infectivity of hookworm larvae stored for extended periods of time. Third-stage larvae of hookworms lose infectivity with storage, generally thought to be the result of decreased metabolic rates and lipid reserves associated with aging (Clark, 1969; Croll and Matthews, 1973). Storage in near-isotonic solutions may conserve energy by decreasing the activity of the excretory ampulla, a structure used in maintaining water balance (Weinstein, 1952; Croll et al., 1972). However, Croll (1972) suggests that maintenance of water balance requires minimal energy expenditure, and that lipid use in *Ancylostoma tubaeforme* L_3's is approaching maximum at NaCl concentrations equivalent to that of BU. Alternatively, storage in distilled water may induce a quiescent or moribund state in which larvae are unable to respond to host signals upon infection.

Conversely, storage in BU may keep the larvae within their "activity sphere" (Croll, 1972), i.e., the optimum physiological parameters for activity and survival, thereby allowing them to remain responsive to environmental signals. Indeed, larvae of *A. ceylanicum* and *Necator americanus* stored in BU for 3–4 wk were capable of causing patent infections in hamsters, although their infectivity compared to water-stored larvae was not examined. The percentage of hookworm L_3's that resume feeding in vitro has not been correlated directly with infectivity, but the level of feeding exhibited by larvae stored in BU suggests that these larvae retain the ability to respond to the host signals encountered during infection.

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Research Note

Helminths of Three Toads, *Bufo alvarius, Bufo cognatus* (Bufonidae), and *Scaphiopus couchii* (Pelobatidae), from Southern Arizona

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ABSTRACT: The gastrointestinal tracts and lungs of 3 toad species were examined for helminths. Examination of 95 *Bufo alvarius* revealed the presence of the nematodes *Aplectana itzocanensis* Bravo Hollis, 1943, *Physaloptera* sp. Rudolphi, 1819, *Physocephalus* sp. Diesing, 1861, *Oswaldocruzia pipiens* Walton, 1929, the cestode *Nematotaenia dispar* (Goeze, 1782) Lühe, 1899, in the gastrointestinal tract, and the nematode *Rhabdias americanus* Baker, 1978, in the lungs. *Bufo cognatus* ($N = 21$) had the nematodes *A. itzocanensis, O. pipiens, Physaloptera* sp., and the cestode *Distoichometra bufonis* Dickey, 1921, in the gastrointestinal tract. The nematode *R. americanus* was found in the lungs. *Scaphiopus couchii* ($N = 76$) had the nematodes *Aplectana incerta* Caballero, 1949, and *O. pipiens,* and the cestode *D. bufonis* in the digestive trace. No helminths were found in the lungs of *S. couchii.*

KEY WORDS: Nematoda, *Aplectana incerta, Aplectana itzocanensis, Physaloptera* sp., *Physocephalus* sp., *Oswaldocruzia pipiens, Rhabdias americanus,* Cestoda, *Distoichometra bufonis, Nematotaenia dispar,* prevalence, intensity, survey, Bufonidae, *Bufo alvarius, Bufo cognatus,* Pelobatidae, *Scaphiopus couchii.*

The Colorado River toad, *Bufo alvarius,* the Great Plains toad, *Bufo cognatus,* and Couch's spadefoot, *Scaphiopus couchii,* occur sympatrically in southern Arizona. *Bufo alvarius* ranges across southern Arizona and extreme southwestern New Mexico to northwest Sinaloa, Mexico from sea level to 1,610 m. *Bufo cognatus* has a geographic range extending from extreme

Table 1. Prevalence (%), intensity, range, and location of helminths from *Bufo alvarius, Bufo cognatus,* and *Scaphiopus couchii.*

Parasite	*Bufo alvarius*			*Bufo cognatus*			*Scaphiopus couchii*		
	Prevalence	$\bar{x}$ Intensity (range)	Location*	Prevalence	$\bar{x}$ Intensity (range)	Location*	Prevalence	$\bar{x}$ Intensity (range)	Location*
Cestoidea									
Distoichometra bufonis	—	—	—	19	4 (1–6)	c, d	20	3 (1–11)	c, d
Nematotaenia dispar	67	7 (1–39)	c, d	—	—	—	—	—	—
Nematoda									
Aplectana incerta	—	—	—	—	—	—	82	74 (2–447)	d
Aplectana itzocanensis	52	33 (1–371)	c, d	5	1 (1)	d	—	—	—
Oswaldocruzia pipiens	49	3 (1–9)	b, c, d	38	6 (1–17)	b, c, d	11	21 (1–13)	b, c
Physaloptera sp.	38	7 (1–77)	a, b, d	14	1 (1)	b	—	—	—
Physocephalus sp.	2	9 (1–12)	b, c	—	—	—	—	—	—
Rhabdias americanus	65	7 (1–34)	e	38	7 (1–21)	e	—	—	—

* a = esophagus; b = stomach; c = small intestine; d = large intesstine; e = lung.

southern Canada to San Luis Potosi, Mexico from near sea level to 2,440 m. *Scaphiopus couchii* ranges from southwestern Oklahoma, central New Mexico, and south central Arizona to the tip of Baja California, Nayarit, and southern San Luis Potosi, Mexico (Stebbins, 1985). To our knowledge there are no reports on the helminth fauna of *B. alvarius.* There are 2 reports of *B. cognatus* as a host (Kuntz, 1940, 1941) and 5 reports for *S. couchii* (Kuntz, 1940, 1941; Tinsley and Jackson, 1986, 1988; Baker, 1987). The purpose of this note is to describe the prevalences and intensity of the helminth fauna from southern Arizona populations of these 3 toad species.

Ninety-five *B. alvarius* (mean snout–vent length, SVL = 126 ± 21 mm, range 71–162 mm) and 21 *B. cognatus* (mean SVL = 79 ± 12 mm, range 55–98 mm) were hand collected during July 1985 at Robles Junction, Pima County, Arizona (32°04′N, 111°18′W, 712 m elevation). Seventy-six *S. couchii* (mean SVL = 63 ± 4 mm, range 55–77 mm) were hand collected along Drexel Road at Arizona Highway 89, Pima County, Arizona (32°09′N, 110°59′W, 743 m elevation). Toads were fixed in neutral, buffered 10% formalin. The body cavity was opened by a longitudinal incision from vent to throat and the gastrointestinal tract was excised by cutting across the anterior esophagus and the rectum. The lungs were also removed for examination; no other organs were removed. Esophagus, stomach, small intestine, large intestine, and lungs were examined separately. Each organ was slit longitudinally and examined under a dissecting microscope. Each helminth was removed and identified utilizing a glycerol wet mount. Representative cestodes were stained with hematoxylin and mounted in balsam. Selected intact specimens were placed in vials of alcohol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *Bufo alvarius–Aplectana itzocanensis,* 81007; third-stage *Physaloptera* sp., 81009; third-stage *Physocephalus* sp., 81010; *Oswaldocruzia pipiens,* 81008; *Rhabdias americanus,* 81011; *Nematotaenia dispar,* 81012. *Bufo cognatus–Aplectana itzocanensis,* 81013; third-stage *Physaloptera* sp., 81015; *Oswaldocruzia pipiens,* 81014; *Rhabdias americanus,* 81016; *Distoichometra bufonis,* 81017. *Scaphiopus couchii–Aplectana incerta,* 81018; *Oswaldocruzia pipiens,* 81019; *Distoichometra bufonis,* 81020.

Prevalence, location, and mean intensity for each host are given in Table 1. The parasite load, as well as prevalence and intensity of helminths, were different for each host. For *B. alvarius,* 92 of 95 (97%) contained helminths. There were 56 male toads (55 infected; 98%) and 39 female toads (37 infected; 95%) in the sample. The cestode *N. dispar* had the highest prevalence (67%). Greatest mean intensity was recorded for the nematode *A. itzocanensis* (33). There was no significant difference in intensity of infection between male and female toads for *Physaloptera* sp., *O. pipiens, R. americanus,* or *N. dispar* (Kruskal-Wallis statistic = 1.35, 0.5, 0, and 0.37, respectively, 1 df, $P > 0.05$ for each case). A similar statistic was not calculated for *Physocephalus* sp. since only 2 female toads were infected. Significant difference in intensity of infection between male and female toads was found for *A. itzocanensis* (Kruskal-Wallis statistic = 9.7, 1 df, $P < 0.001$). There

was no correlation between total number of helminths and SVL ($r = -0.08$).

For *B. cognatus,* 14 of 21 (67%) contained helminths. There were 6 male toads (4 infected; 67%) and 15 female toads (10 infected; 67%) in the sample. Mean intensity for total helminth load was 10 (1–65). There was no significant difference in intensity of total infection between male and female toads (Kruskal-Wallis statistic = 0.5, 1 df, $P > 0.05$). Parasite species statistics were not calculated because of our small sample size: 1 female infected with *A. itzocanensis*; 1 male, 2 females infected with *Physaloptera* sp.; 1 male, 7 females infected with *O. pipiens*; 1 male, 3 females infected with *D. bufonis*; 2 males, 6 females infected with *R. americanus.* The nematodes *O. pipiens* and *R. americanus* had equally high prevalences (38%). Greatest mean intensity was recorded for *R. americanus* (7). There was no correlation between total number of helminths and SVL ($r = 0.04$).

For *S. couchii,* 69 of 76 (91%) contained helminths. There were 49 male toads (43 infected, 88%) and 27 female toads (26 infected, 96%) in the sample. Highest prevalence and greatest mean intensity were recorded for *A. incerta* (82%, 74, respectively). In contrast to *B. alvarius* and *B. cognatus,* no nematodes were found in the lungs of *S. couchii.* There was no significant difference in intensity of infection between male and female toads for *A. incerta, O. pipiens,* or *D. bufonis* (Kruskal-Wallis statistic = 1.4, 0.35, and 1.46, respectively, 1 df, $P > 0.05$ for each case). There was no correlation between total helminth numbers and SVL ($r = 0.04$).

The most prevalent parasite was the ascarid nematode, *A. incerta.* It was originally described by Caballero (1949) from *Bufo marinus* from Chiapas, Mexico and has been redescribed from type specimens by Baker (1985). It has not been previously reported from other hosts. Likewise, *A. itzocanensis* was originally described by Bravo Hollis (1943) from *Scaphiopus multiplicatus* from Puebla, Mexico. It has also been redescribed by Baker (1985) from *Bufo woodhousii woodhousii* and has been reported in *B. marinus* from Costa Rica (Brenes and Bravo Hollis, 1959) and Veracruz, Mexico (Caballero Deloya, 1974).

Species of the genus *Physaloptera* occur in the stomachs of a variety of terrestial vertebrates. However to our knowledge, no cases of parasitism of toads by adult physalopterans have been reported. We noted third-stage physalopterans in both *B. alvarius* and *B. cognatus* but not in *S. couchii.* Third-stage physalopterans have also been reported from the toads *Bufo microscaphus* and *B. woodhousii* from Utah (Parry and Grundmann, 1965) and *Bufo americanus* from Ohio (Ashton and Rabalais, 1978). Kuntz (1940) reported the family Physalopteridae as occurring in *Bufo speciosus* (=*compactilis*) and *B. cognatus* but did not name the stage.

Alicata (1935) reported species of the genus *Physocephalus* occur in the stomachs of swine, mice, and rats, in the esophagus and rumen of ruminants, and in the crop of chickens. They are of accidental occurrence in other mammals, birds, reptiles, and amphibians as third-stage larvae (Alicata, 1935). They have not been previously reported in toads. *Physocephalus* sp. requires an insect intermediate host.

Oswaldocruzia pipiens has frequently been encountered in amphibians and is widely distributed in North America. Baker (1978a) studied the life cycle of *O. pipiens* in the frog *Rana sylvatica* and reported prevalence to vary between 61% and 62%. He considered 33% of the *R. sylvatica* to have patent infections. Intensity varied from 1 to 45, but most infected frogs contained 1–5 worms. Our prevalence and intensities fall within Baker's ranges. *Oswaldocruzia pipiens* is infective as third-stage larvae which migrate out of fecal masses into the water column. *Bufo americanus* exposed to contaminated frog feces have become infected (Baker, 1978a). In addition, infection has been reported in *Bufo woodhousii fowleri* by Brandt (1936), Rankin (1945), Campbell (1968); *B. americanus* by Ashton and Rabalais (1978); *Bufo houstonensis* by Thomas et al. (1984); and *Scaphiopus holbrookii* by Brandt (1936).

According to Baker (1987), North American bufonids are apparently infected by a single species of *Rhabdias, R. americanus,* which was described from *B. americanus* by Baker (1978b). Baker (1987) suggested that reports of *Rhabdias bufonis* in *B. americanus* and *B. woodhousii fowleri* from eastern North America (Reiber et al., 1940; Fantham and Porter, 1948; Campbell, 1968; Williams and Taft, 1980) should be referred to *R. americanus. Rhabdias* sp. was listed for *B. speciosus* (=*compactilis*) by Kuntz (1940).

The cestode *N. dispar* is listed in Schmidt (1986) as occurring in the Bufonidae, Ranidae, Sala-

mandridae, Hylidae, and Gekkonidae. It has been reported from the North American toads *B. americanus* by von Linstow (1899), *Bufo terrestris* by Walton (1939), and *B. speciosus* (=*compactilis*) and *S. couchii* by Kuntz (1940).

Distoichometra bufonis was originally described from *B. terrestris* (=*lentiginosus*) by Dickey (1921). It has been reported from *Scaphiopus* sp., *B. terrestris,* and *B. woodhousii fowleri* by Douglas (1958), from *S. holbrookii* by Brandt (1936), from *B. speciosus* (=*compactilis*) and *S. couchii* by Kuntz (1940), and from *Bufo debilis debilis* and *B. woodhousii woodhousii* by McAllister et al. (1989). Hardin and Janovy (1988) reported prevalences of 70–100%, with mean intensities of 2.7–14.8 for *D. bufonis* from samples of *B. woodhousii* from Nebraska. The prevalence reported in our study was much lower, 29% and 20%, respectively, for *B. cognatus* and *S. couchii.* The mean intensities for these toads, 4.0 and 3.0, respectively, are within the values established by Hardin and Janovy (1988).

None of the parasites found in this study were unique to *B. alvarius, B. cognatus,* or *S. couchii,* but those from *B. alvarius* are new host records. Kuntz (1940) reported both *D. bufonis* and *N. dispar* to occur in *S. couchii* and only *N. dispar* in *B. cognatus.* We found *D. bufonis* to occur in both *B. cognatus* and *S. couchii. Nematotaenia dispar* was reported only from *B. alvarius.* We cannot explain these host differences.

The toads in this study were collected from localities within the Sonoran desert where their activity is tied to the summer rains which normally begin in July and end in late August. They are inactive and remain underground the rest of the year. Whether the differential gender infection rates in *B. alvarius* by *A. itzocanensis* are an artifact of this sample or a reflection of microhabitat differences remains to be determined.

From Baker (1987) and McAllister et al. (1989) we calculated an average of 4.0 (±5.1 SD) species of nematodes (1–22) for 52 species of the family Bufonidae and a mean of 1.7 (±1.9 SD) species of nematodes (1–7) for 10 species of the family Pelobatidae. The toads in this study fell within the range of what might be expected for members of their respective families. Additional work will be required to determine the reasons for the differences that we report in the helminth fauna of these sympatric species.

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Research Note

Gastrointestinal Helminths of the Reticulate Gila Monster, *Heloderma suspectum suspectum* (Sauria: Helodermatidae)

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ABSTRACT: Examination of the gastrointestinal tract of 110 *Heloderma suspectum suspectum* Cope, 1869, revealed the presence of 1 cestode, *Oochoristica whitentoni* Steelman, 1939, and 2 nematode species, *Oswaldocruzia pipiens* Walton, 1929, and *Skrjabinoptera phrynosoma* Ortlepp, 1922. Helminth prevalence and mean intensity were 12% and 9.9, respectively. These findings represent new host records.

KEY WORDS: Cestoda, *Oochoristica whitentoni*, Nematoda, *Oswaldocruzia pipiens*, *Skrjabinoptera phrynosoma*, Helodermatidae, *Heloderma suspectum suspectum*, Gila monster.

The Gila monster, *Heloderma suspectum* Cope, 1869, is found from extreme southwestern Utah and southern Nevada through southern Arizona and southwestern New Mexico to northern Sinaloa, Mexico from sea level to about 1,520 m (Stebbins, 1985). The few reports of parasitism in this species mostly concern filariae. Smith (1910) recovered 4 adult filariae which he named *Filaria mitchelli*; they were reassigned to the genus *Piratuba* by Chabaud and Frank (1961a). Hannum (1941) described adult and microfilaria of *Chandlerella corophila* which were subsequently reassigned to the genus *Splendidofilaria* by Yamaguti (1961). Ryerson (1949) reported but did not identify microfilariae from 2 Gila

Table 1. Number, prevalence, and intensity of gastrointestinal helminths in 110 *Heloderma suspectum suspectum*.

	H. suspectum				*S. phrynosoma*	
Year	Male	Female	*Oo. whitentoni*	*Os. pipiens*	Adult	Third stage
1964	6	5	—	4	—	1
1965	13	11	1	67	1	—
1966	12	12	—	20	8	9
1967	3	1	—	—	—	—
1969	1	—	—	—	—	—
1985	34	12	4	26	—	—
Total	69	41	5	117	9	10
Prevalence			2%	5%	5%	
Mean intensity (range)			2.5 (1–4)	19.5 (1–67)	3.2 (1–8)	

monsters, 1 of which had adult filariae in the heart and aorta. Stabler and Schmittner (1958) recovered sheathed microfilariae which they named *Microfilaria stahnkei* (synonymized with *Piratuba mitchelli* by Chabaud and Frank [1961a]). In addition, Chabaud and Frank (1961b) described *Macdonaldius andersoni* from adult filariae found in abdominal arteries. Mahrt (1979) found microfilariae in *H. suspectum* that resembled *Macdonaldius seetae* which occur in snakes. Griner (1983) observed but did not identify filariae in the ventricle of a Gila monster. Goldberg and Bursey (1990a) redescribed the microfilaria of *Piratuba mitchelli.* The only other helminths that have been reported are an unidentified cestode and an unidentified acanthocephalan by Bogert and Del Campo (1956). The purpose of this report is to describe the prevalence and intensity of gastrointestinal helminths in an Arizona population of *H. suspectum suspectum.*

One hundred ten specimens were examined (mean snout–vent length, SVL = 244 ± 51 mm SD; range 110–344 mm). All were from the vicinity of Tucson, Pima County, Arizona (32°13′N, 110°58′W, elevation 701 m) and were collected over several years (Table 1). The body cavity was opened and the esophagus, stomach, small intestine, and large intestine were slit longitudinally and examined under a dissecting microscope. Nematodes were identified using glycerol wet mounts; proglottids of cestodes were stained with hematoxylin. One cestode species, *Oochoristica whitentoni,* and 2 nematode species, *Oswaldocruzia pipiens* and *Skrjabinoptera phrynosoma,* were recovered. Representative specimens were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): *Oochoristica whitentoni* (2 strobilae in alcohol), USNM Helm. Coll. No. 81198; *Oswaldocruzia pipiens* (6 males, 6 females in alcohol), 81199; *Skrjabinoptera phrynosoma* (3 males, 3 females, 81200, and 6 third-stage larvae, 81201, all in alcohol).

Prevalence, location, and intensity for each species are given in Table 1. *Oochoristica whitentoni* was found in the small intestines of 1 male and 1 female lizard. *Oswaldocruzia pipiens* was recovered from the small intestines of 4 male and 2 female lizards. Adult *Skrjabinoptera phrynosoma* occurred in the stomachs of 2 male and 1 female lizards; while third-stage larvae were recovered from 1 male and 2 female lizards. The frequency of infection was the same for female and male lizards (female, 5/41 = 12%; male, 8/69 = 12%) but there was significant difference in parasite load (Kruskal-Wallis statistic = 3.9, 1 df, $P < 0.05$); mean intensity for females was 1.6 and for males, 15.1. There was no significant difference in infection rates between the lizards of the 1964–1967 collection and the 1985 collection (Kruskal-Wallis statistic = 1.17, 1 df, $P > 0.05$).

Thirteen of the 66 described species of *Oochoristica* occur in North America; 8 have been recovered from lizards (Schmidt, 1986). Based upon our update of the key developed by Hughes (1940), only 1 species, *O. whitentoni,* has characteristics similar to the specimens that we recovered. The strobilae of our specimens averaged 300 by 2.0 mm with 230 proglottids and 115 testes compared to 275 by 1.3 mm with 211 proglottids and 100–150 testes for *O. whitentoni. Oochoristica whitentoni* was originally described from a land tortoise, *Terrapene triunguis* (=*ornata*), collected near Stillwater, Oklahoma (Steelman, 1939) and has also been reported from

a false iguana, *Ctenosaura pectinata,* from Mexico (Schmidt, 1986). Insects and mites serve as intermediate hosts (Schmidt, 1986).

Oswaldocruzia pipiens is the only trichostrongyloid to infect reptiles in the United States (Baker, 1987). It is a common parasite occurring mainly in amphibians, but has also been found in lizards: *Anolis carolinensis* by Conn and Etges (1984); *Leiolopisma laterale, Eumeces fasciatus, Sceloporus undulatus* by Harwood (1932); *Gerrhonotus multicarinatus* by Goldberg and Bursey (1990b), and the turtles: *Terrapene carolina* and *Terrapene ornata* by Ernst and Ernst (1975).

Skrjabinoptera phrynosoma has been recovered from a number of lizard species from North America (Baker, 1987). Its life cycle in the Texas horned lizard, *Phrynosoma cornutum,* has been elucidated by Lee (1957); the intermediate host is the ant, *Pogonomyrmex barbatus.*

None of the helminths reported here is host specific for helodermatids, but all are new host records. The low levels of infection and what is known about the life cycles of these helminths suggests accidental infections. Bogert and Del Campo (1956) noted that compared with other lizards, helodermatids appeared remarkably free of intestinal parasites and suggested that diet may be a factor in their lack of gastrointestinal helminths. Since eggs, fledgling birds and nestling mammals make up much of their diet, Bogert and Del Campo (1956) reasoned that these young animals would be less likely to be infected with larval stages and subsequently helodermatids would ingest fewer parasites. Because their diet is relatively insect free, helodermatids have few chances of infections by helminths with insect intermediate hosts. However, the inclusion of carrion in their diet (Stebbins, 1985) increases the probability of ants and beetles being consumed which may contain larval helminths.

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Research Note

Attraction of Amoebocytes to *Cyclocoelum oculeum* Rediae Entering the Snail Host

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ABSTRACT: Sixteen-millimeter cinephotomicrography was used to study the attraction of amoebocytes to *Cyclocoelum oculeum* rediae entering the snail host, *Gyraulus parvus.* As the redia pushed against the lining of the peripheral blood sinus, amoebocytes emerged from among epithelial cells lining the sinus 10–14 μm away and migrated to the area of penetration within 7–10 sec. When the redia broke through the sinus, the amoebocytes attached.

KEY WORDS: Trematoda, Cyclocoelidae, *Cyclocoelum oculeum, Gyraulus parvus,* redia, amoebocytes, 16-mm cinephotomicrography.

Cyclocoelum oculeum miracidia each contain a fully-formed redia (Palm, 1963). After attachment of the miracidium to the snail host, the activated redia leaves the miracidium within an hour and enters the snail. While studying this process using cinephotomicrography, it was noted that amoebocytes were attracted to the site of redial penetration.

Even though there is an extensive literature on amoebocytes and their relationship to helminths (Bayne, 1983; Cheng et al., 1969; Joky et al., 1985; Sullivan, 1988), there has never been any in vivo filming of the attraction and attachment of these cells to entering parasites.

Adult *C. oculeum* miracidia were collected from the nasal cavities and orbits of American coots (*Fulica americana*) and placed in sterilized aquarium water. Their uteri were dissected out and teased apart to release miracidia. Miracidia were then placed along with the recently dissected head-foot of *Gyraulus parvus* on a clean microscope slide in a drop of water and mounted with a coverslip. Filming was accomplished using a Bolex 16-mm movie camera with attached Nikon Cine Autotimer mounted on a Zeiss Universal Microscope. Photographs were taken at 1-sec intervals using Kodak Plus-X positive film. Selected frames were printed as negatives to retain detail. Line drawings from the same frames were produced to further clarify the relationships.

The most common attachment site of the miracidium was to the snail's tentacle. This organ is covered by ciliated epithelium underlain by dense connective tissue, peripheral blood sinuses, and a central artery. After attachment, the apical papilla of the miracidium elongated and pushed against the connective tissue surrounding the peripheral blood sinus. Following this the enclosed redia became very active. Eventually the apical papilla retracted into the miracidium and the redia broke through the miracidial membranes, moved past the retracted papilla, and pushed up against the peripheral blood sinus causing it to invaginate. Apparently aiding in the breakdown of the miracidial membranes and snail tissues were substances produced by the redial esophageal glands. These glands can be seen decreasing in volume as the redia escaped the miracidium and entered the snail. On numerous occasions while filming, and also when making observations, 1–3 amoebocytes could be seen emerging from among epithelial cells lining the blood sinus. One such example is depicted in Plate I, Figures 1–6 and Plate II, Figures 1–6

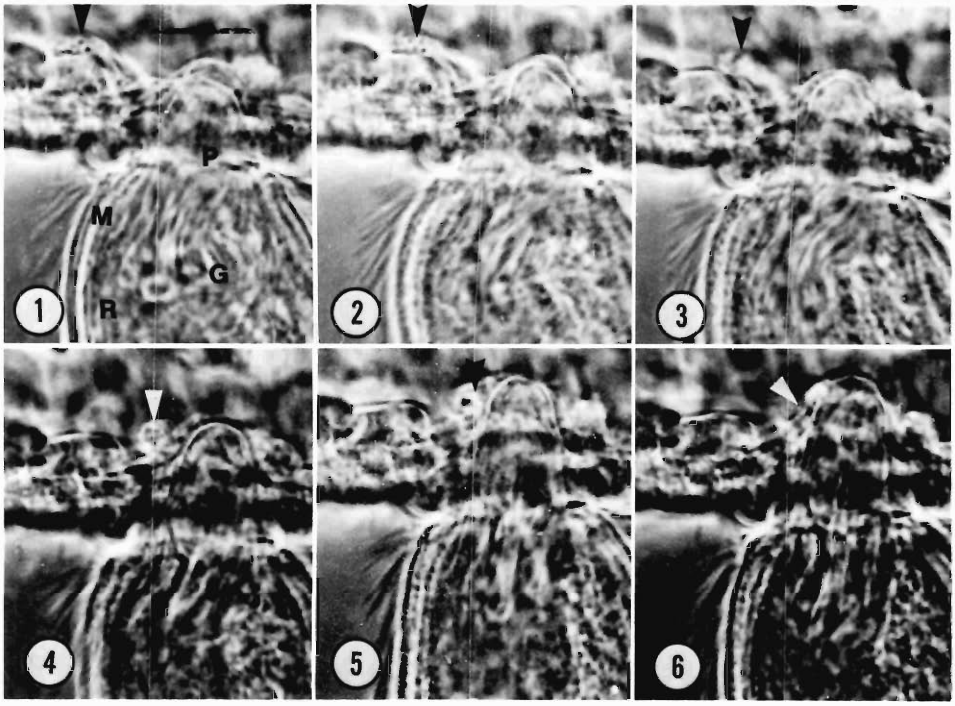

Plate I. Figures 1–6. ***Cyclocoelum oculeum*** **miracidium (m) containing a redia (r) with a pharynx (p) and esophageal glands (g). This sequence of Figures 1-6, reproduced from 16-mm film, shows the movement of, and attachment by an amoebocyte (arrow) to the entering redia. A decrease in size of the esophageal glands is noted. The gland contents may aid the redia during the penetration process. Scale bar = 14 μm.**

where amoebocytes at a distance of 10–14 μm from the redia migrated to the area of penetration within 7–10 sec. As soon as the redia broke through the blood sinus, amoebocytes attached. According to Cheng et al. (1969), there is no universal consensus in what tissues or organs the phagocytic cells originate. They may come from epithelial tissue of various organs, from mantle or lung connective tissue, from cells budding off the hepatopancreas, from fibroblasts in the mantle blood sinuses, and from the cellular reticulum found in the wall of the kidney near the pericardium. Some believe, according to Cheng et al. (1969), that connective tissue cells and those of the epithelium are capable of differentiating into amoebocytes. This study indicated that amoebocytes emerged from among epithelial cells lining the blood sinus. Whether they originated here is unclear. According to Bayne (1983), snail responses to trematode larval invasion may take 1 of 3 pathways: encapsulation with destruction; no cellular response; benign association of hemocytes with parasite surfaces. It appears that invasion of *G. parvus* by *C. oculeum* rediae follows the latter response.

It is difficult to assess the role the amoebocytes may play in protecting the snail from invasion by *C. oculeum* rediae because in the laboratory one can infect snails with large numbers (up to 30 or more) and the snails survive. No cellular reactions can be observed against this stage as determined by histochemistry (Taft, unpubl.). *Cyclocoelum oculeum* larvae are unusual among trematodes in that they are not very host specific, developing in snails of other genera including *Physa, Lymnaea,* and *Helisoma* (Taft, 1972). Whether or not a greater or lesser amoebocyte response occurs in snails of these genera to invading *C. oculeum* rediae might be tested in the laboratory.

The cyclocoelid–*Gyraulus* system is a useful model system in studying attraction and attachment of amoebocytes to rediae for the following reasons: 1. The snails are small and translucent;

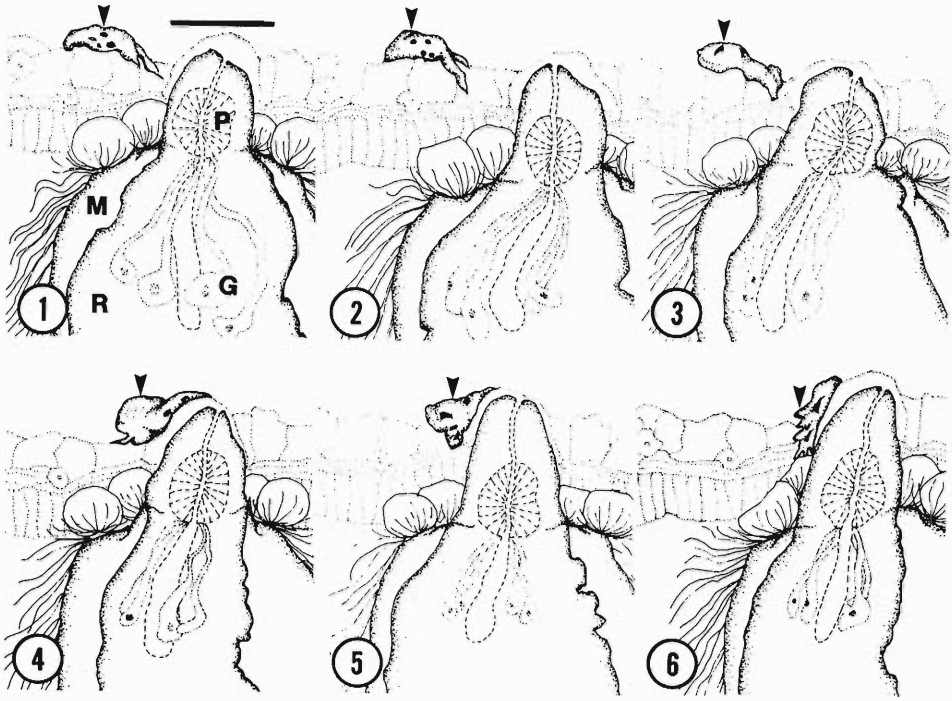

Plate II. Figures 1–6 are a series of line drawings of Plate I depicting the same events for greater clarity. Scale bar = 14 μm.

2. The rediae are large, thus making photography relatively easy; 3. Large numbers of miracidia can easily be obtained from 1 worm.

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Research Note

A Survey for Trichinosis in Selected Predatory and Scavenger Birds in Montana, with an Evaluation of the Infectivity of Two Mammalian *Trichinella spiralis* Isolates in Birds

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ABSTRACT: Muscle samples from 103 free-ranging predatory and scavenger birds of 27 species from western Montana were digested and examined for *Trichinella* larvae during the period from 1971 to 1988. Families represented included accipiters, falcons, and strigids; golden and bald eages, red-tailed and Cooper's hawks, and great horned owls predominated. Additional specimens of fish- or carrion-feeders such as herons, loons, ospreys, crows, ravens, and turkey vultures also were examined. No infected individuals were found. Attempts to induce experimental infections in a short-eared owl, long-eared owl, and crow with *T. spiralis* isolates from a grizzly bear and fisher also were unsuccessful. The apparent absence of avian trichinosis in the wild bird population and the inability to induce experimental infections in captive birds suggest that birds play little or no obvious role in the epizootiology of sylvatic trichinosis in the high plains/Rocky Mountain region of western Montana.

KEY WORDS: *Trichinella spiralis,* avian infections, cross-transmission.

Although *Trichinella spiralis* (Owen, 1835) probably has the broadest host range of any helminth (Gould, 1970; Despommier, 1983), under natural conditions the various *T. spiralis* subspecies are thought to be infective only in mammals (Gould, 1970; Despommier, 1983). The related species *T. pseudospiralis,* originally described from the raccoon *Procyon lotor* in Dagestan, Northern Caucasus (Garkavi, 1972), is believed to be a normal parasite of birds (Miroshnichenko, 1976; Shaikenov, 1980), although under experimental conditions mice and other rodents are susceptible (Tomašovičová, 1975). Subsequent avian records of *T. pseudospiralis* have extended the known distribution of the species to Spain (Calero et al., 1978) and the United States (Wheeldon et al., 1983).

Although the natural occurrence of *T. pseudospiralis* has been demonstrated on 3 continents, its distribution and the species involved in transmission are virtually unknown. The primary purpose of the present study was to screen tissues from a series of raptorial and scavenger birds for the presence of *Trichinella* larvae in a region of high trichinosis endemicity in mammals (Worley et al., 1974, 1982). A secondary objective was to evaluate the infectivity of 2 mammalian *T. spiralis* isolates in birds likely to be exposed to this infection via their normal feeding behavior as predators or scavengers on known mammalian reservoirs of *T. spiralis* in western Montana, northern Wyoming, and eastern Idaho.

Samples of breast, thigh, or other muscle were obtained from eagles, hawks, owls, and other raptors submitted to the Raptor Rehabilitation Program at Montana State University but found unsuitable for release. Other avian specimens were provided by the Montana Department of Fish, Wildlife and Parks or were obtained from road kills. The classification used was that of the A.O.U. Check-list of North American Birds, 6th ed. One 25-g muscle sample was examined in most instances. Occasionally, tissues from 2 sites were screened for larvae.

Tissues were removed from some birds shortly after death. In most instances, carcasses were frozen for several months before muscle samples were removed and processed. Tissues were cut into small pieces with scissors prior to comminuting with an Omnimixer. Homogenized tissue then was digested in 1% pepsin–0.7% HCl for 5–12 hr at 37°C under constant agitation. The digested material was washed on a 200-mesh screen to remove soluble debris prior to examination of the retained material for *Trichinella* larvae with a dissecting microscope.

The infectivity of 2 mammalian *T. spiralis* isolates in birds was tested by oral inoculation of 2 owls and a crow with larvae originally isolated from a grizzly bear (*Ursus arctos*) and a fisher (*Martes pennanti*) in western Montana. Both biotypes were maintained in laboratory-reared deer mice (*Peromyscus maniculatus*) at the Montana State University Animal Resources Center. Larval suspensions obtained by peptic digestion of skinned, eviscerated mouse carcasses were ad-

Table 1. Wild birds negative for *Trichinella spiralis* larval tissue infections in western Montana (1971–1988).

Family	Species	No. examined
Gaviidae	Common loon (*Gavia immer*)	1
Ardeidae	Great blue heron (*Ardea herodias*)	1
Cygnidae	Trumpeter swan (*Olor buccinator*)	1
Cathartidae	Turkey vulture (*Cathartes aura*)	1
Accipitridae	Goshawk (*Accipiter gentilis*)	1
	Sharp-shinned hawk (*Accipiter striatus*)	1
	Cooper's hawk (*Accipiter cooperii*)	6
	Red-tailed hawk (*Buteo jamaicensis*)	7
	Rough-legged hawk (*Buteo lagopus*)	2
	Ferruginous hawk (*Buteo regalis*)	1
	Golden eagle (*Aquila chrysaetos*)	17
	Bald eagle (*Haliaeetus leucocephalus*)	5
	Marsh hawk (*Circus cyaneus*)	3
Pandionidae	Osprey (*Pandion haliaetus*)	1
Falconidae	Gyrfalcon (*Falco rusticolus*)	1
	Prairie falcon (*Falco mexicanus*)	3
	Peregrine falcon (*Falco peregrinus*)	2
	American kestrel (*Falco sparverius*)	4
Strigidae	Great horned owl (*Bubo virginianus*)	34
	Great gray owl (*Strix nebulosa*)	3
	Long-eared owl (*Asio otus*)	2
	Short-eared owl (*Asio flammeus*)	1
	Saw-whet owl (*Aegolius acadicus*)	1
Caprimulgidae	Common nighthawk (*Chordeiles minor*)	1
Picidae	Northern flicker (*Colaptes auratus*)	1
Corvidae	Common raven (*Corvus corax*)	1
	Common crow (*Corvus brachyrhynchos*)	1

ministered via stomach tube in physiological saline solution. Recipient birds were maintained in individual cages for 26–45 days postinoculation prior to euthanasia. At necropsy, tissue samples were collected from wing, thigh, gastrocnemius, and pectoralis muscle, when available, for evaluation for larvae.

A total of 103 birds was examined for *Trichinella* muscle larvae between 1971 and 1988 (Table 1). Included in this series were 27 species representing 11 families: 9 accipiters, 4 falcons, and 5 owls. Additional fish or carrion feeders such as herons, loons, ospreys, crows, ravens, and vultures were examined when available. A few insectivorous or planktonic feeders also were included, i.e., trumpeter swan, nighthawk, and flicker on the basis of possible involvement of insects or other invertebrates in transmission of *T. spiralis*.

No evidence of *Trichinella* muscle larvae was seen in any of the material examined. Because of the limited availability of most species, the status of avian trichinosis in western Montana remains unclear. However, the absence of infection in 65 individuals of 5 major raptorial species (golden and bald eagle, red-tailed hawk, Cooper's hawk, and great horned owl) suggests that these birds appear to play no demonstrable role in biological transmission of *Trichinella* sp. in a region where this parasite occurs widely as a sylvatic infection in mammalian hosts (Worley et al., 1974; Worley and Greer, 1982). The absence of infection in the Cooper's hawk is pertinent also in view of the first North American report of *T. pseudospiralis* in this species in California (Wheeldon et al., 1983). Further, the absence of the parasite in species such as the great horned owl, *Bubo virginianus,* which is known to feed on the common skunk, *Mephitis mephitis* (Bent, 1938), a known host of *Trichinella* in western Montana (Worley and Greer, 1982), suggests that skunk-to-owl transmission may be unlikely to occur as a result of natural exposure via predation or scavenger feeding behavior. On the other hand, Zimmermann and Hubbard (1963) found that trichinosis occurred as a low level infection in the great horned owl in Iowa.

Limited experimental evidence from the present study confirmed the inability of 2 mammalian isolates to develop in birds (Table 2). No

Table 2. Experimental inoculation of birds with mammalian *Trichinella spiralis* larvae.

Host	Larval dose	Source of isolate	Muscles examined postmortem	Tissue larvae recovered*
Short-eared owl (*Asio flammeus*)	865	fisher (*Martes pennanti*)	wing	0
			pectoralis	0
			thigh	0
			gastrocnemius	0
Long-eared owl (*Asio otus*)	8,100	grizzly bear (*Ursus arctos*)	wing	0
			pectoralis	0
			thigh	0
			gastrocnemius	0
Crow (*Corvus brachyrhynchos*)	10,000	fisher (*Martes pennanti*)	wing	0
			thigh	0
			gastrocnemius	0

* Via peptic digestion of muscle samples 26–45 days postinoculation.

tissue invasive stages were found in either of 2 owls or 1 crow given single oral doses of 865–10,000 larvae. The inability to induce infections that progressed past the enteral stage in birds given *T. spiralis* isolates from mammals is consistent with most previous observations. Doerr and Schmidt (1930) attempted unsuccessfully to transmit *T. spiralis* from rodents to hens. Augustine (1933) found that some *Trichinella* larvae given to baby chicks survived in the intestinal tract to produce migratory tissue larvae, but they died in the musculature within a short time. Matoff (1938) reported that pigeons were also an unsuitable host for rodent-derived *Trichinella.* On the other hand, Nemeseri (1968) found that by lowering the natural resistance of chickens by starvation or by feeding an inadequate diet, it was possible to induce disseminated tissue infections, and these larvae were infective to white rats. Overall, the preponderance of experimental evidence suggests that *T. spiralis* isolates derived from pigs or rodents are not infective to galliform or columbiform birds.

Results of the present experimental study complement these findings by demonstrating that some strigiform and passeriform species also are refractory to infection with *T. spiralis* isolates from wild carnivores. The possibility that raptors such as eagles and certain hawks that travel extensively during seasonal migrations could transport muscle larvae mechanically to establish new sylvatic foci is an additional consideration that was outside the scope of the present study. Hence, the role of birds in the epizootiology of trichinosis in the western U.S.A. may be associated with their ability to disseminate infective larvae of mammalian *Trichinella* biotypes rather than as biological reservoirs in the traditional predator/scavenger–prey cycle.

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IN MEMORIAM

J. ALLEN SCOTT
1898–1990

James Allen Scott is among the last of a group of distinguished parasitologists who studied under Hegner, Stoll, Root, and Cort of the Johns Hopkins University School of Hygiene and Public Health in the first quarter of this century. He died at home after an extended illness on 18 August 1990.

Scotty was born in Binghamton, New York, on 2 June 1898. He attended high school in Binghamton and after graduation spent 2 years at Wyoming Seminary in Kingston, Pennsylvania. He spent a short time in the U.S. Army as a Chief Bugler (he enjoyed telling of his experiences as a bugler) and then enrolled in Wesleyan University, Middleton, Connecticut, and graduated with a B.A. in 1922 and an M.A. in 1924. He taught for a brief time at the University of Vermont before entering Johns Hopkins University in 1925. He received his Sc.D. in 1927 under the tutelage of W. W. Cort and carried out monumental studies on hookworm in dogs and cats. He remained at Johns Hopkins as an instructor and research associate in helminthology, and in 1929 he became a member of the field staff in the International Health Division of the Rockefeller Foundation and carried out field research in Egypt. He worked in Egypt until 1936 and carried out extensive studies on hookworm and schistosomiasis. Upon his return from Egypt, he spent a year at Johns Hopkins writing up his research. His work in Egypt has become classic and is often referred to today in that country, especially his comprehensive studies on schistosomiasis. In 1937 he was called again to an overseas assignment where he worked on schistosomiasis in Venezuela until 1940. Again he returned to Johns Hopkins to write up his work. From 1941 to 1944 he was a Visiting Professor at Ohio State University, worked on malaria and hookworm in Georgia, and then put his statistical expertise to use for awhile at the U.S. Census Bureau.

In 1944 Scotty joined the faculty at the University of Texas Medical Branch in Galveston, Texas, where he became a Professor of Statistics and Epidemiology in the Department of Preventive Medicine. He established the Laboratory of Helminth Research and carried out continuous studies on the cotton rat filaria *Litomosoides carinii.* These studies were some of the earliest to be done on filarial immunity. He established a graduate program in helminthology, and among his students were Leroy J. Olson, N. Ted Briggs, and John H. Cross. A number of post-doctoral students also had the opportunity to work with Scotty: Etta Mae MacDonald, Haig Najarian, Ahmed Zien Eldin, among the many.

In 1962 and after the publication of over 100 papers, Scotty left academics and bench research to join the staff of the National Institutes of Health. He became a Health Science Administrator and Chief of the Special Research Grants

J. Allen Scott in Egypt in the 1930's.

Program and later Chief of the Parasitology and Medical Entomology Branch. In this latter capacity, he helped many budding, as well as established, investigators in their quest for NIH grant support.

Scotty served on committees of many national and international organizations. He was a consultant on Inter-American Affairs for the State Department, a member of the WHO Expert Advisory Panel on Parasitic Diseases and a WHO Consultant on Schistosomiasis. He belonged to many scientific societies and served on the councils of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitology, and was active in the Helminthological Society of Washington.

Those of us who studied or were associated with J. Allen Scott were very fortunate for the experience. He gave us a plethora of knowledge of parasites and parasitic diseases, as well as an in depth exposure to international health. He will always be remembered by us and all who had the opportunity to have been associated with him.

He is survived by his daughter, Lois Scott Dean of Alexandria, Virginia, 4 grandchildren, and 8 great-grandchildren.

JOHN H. CROSS
Uniformed Services University
of the Health Sciences
Bethesda, Maryland

CALL FOR PAPERS

Rescheduled Student Presentation Competition

The Helminthological Society of Washington is sponsoring the second Student Presentation Competition during its monthly meeting on 13 March 1991, at the Walter Reed Army Institute of Research, Washington, D.C.

Eligibility: An undergraduate or graduate student registered in a college or university degree program at the time of the presentation is eligible to compete for this award.

Conditions: Although multiple authorship is allowed, the project on which the paper is based must be substantially that of the student. The student must be the senior author and present the paper.

The presentation must be on a parasitological subject.

A student may compete with only a single presentation.

An abstract, which is limited to a single, double-spaced, typewritten page, must be provided. The abstract page also must contain the title, author(s), and institutional affiliation(s).

Presentation will be limited to 10 minutes. There will be approximately 5 minutes for questions and discussion between each presentation.

Membership in the Helminthological Society of Washington is not required.

Deadlines:

1 Feb 1991 Submission of abstract as described above together with a completed application form signed by an advisor or university official certifying the student status of the proposed presenter of the paper.

14 Feb 1991 Notification of acceptance of paper for presentation at the 13 March 1991 meeting.

13 March 1991 Student Presentation Competition at the 618th Meeting of the Helminthological Society of Washington.

Selection of Presentations: A maximum of 8 student presentations will be selected for the competition. The selection of an abstract will be based on the organization of the abstract, originality of the work described, and its potential contributions to parasitology.

Judging of Presentations: The presentations will be evaluated by a panel of judges on the following bases: organization, techniques, originality, contribution, interpretation of results, and knowledge of the subject.

Awards: Monetary awards in the amounts of $300, $200, and $100 will be presented to the first, second, and third place winners, respectively. In addition, the Society will waive page charges if the first place manuscript is accepted for publication in the *Journal of the Helminthological Society of Washington.* Further, the first place winner will receive a 1-year subscription to the *Journal of Parasitology.*

Application: Submit abstract and completed application form to:

W. Patrick Carney, Ph.D.
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20889-4799
(202) 295-3701
FAX (202) 295-3431

The application form should include the title of the paper, the name, address, and telephone number of the student competitor, the student's signature, and the signature of an advisor or university official. The student's signature verifies that the research is original work performed by a graduate or undergraduate student.

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	Margaret A. Stirewalt	1975
Mildred A. Doss	1961	* Leo A. Jachowski, Jr.	1976
* Allen McIntosh	1962	* Horace W. Stunkard	1977
* Jesse R. Christie	1964	Kenneth C. Kates	1978
Gilbert F. Otto	1965	* Everett E. Wehr	1979
* George R. LaRue	1966	O. Wilford Olsen	1980
* William W. Cort	1966	Frank D. Enzie	1981
* Gerard Dikmans	1967	Lloyd E. Rozeboom	1982
* Benjamin Schwartz	1969	Leon Jacobs	1983
* Willard H. Wright	1969	Harley G. Sheffield	1984
Aurel O. Foster	1970	A. Morgan Golden	1985
Carlton M. Herman	1971	Louis S. Diamond	1986
May Belle Chitwood	1972	Everett L. Schiller	1987
* Elvio H. Sadun	1973	Milford N. Lunde	1988
E. J. Lawson Soulsby	1974	J. Ralph Lichtenfels	1989
David R. Lincicome	1975	A. James Haley	1990

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
		E. J. Lawson Soulsby	1990

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	Margaret A. Stirewalt	1976
* Albert Hassall	1931	* Willard H. Wright	1976
* Charles W. Stiles	1931	* Benjamin Schwartz	1976
* Paul Bartsch	1937	Mildred A. Doss	1977
* Henry E. Ewing	1945	* Everett E. Wehr	1977
* William W. Cort	1952	Marion M. Farr	1979
* Gerard Dikmans	1953	John T. Lucker, Jr.	1979
* Jesse R. Christie	1956	George W. Luttermoser	1979
* Gotthold Steiner	1956	* John S. Andrews	1980
* Emmett W. Price	1956	* Leo A. Jachowski, Jr.	1981
* Eloise B. Cram	1956	Kenneth C. Kates	1981
* Gerald Thorne	1961	Francis G. Tromba	1983
* Allen McIntosh	1963	A. James Haley	1984
* Edna M. Buhrer	1963	Paul C. Beaver	1986
* Benjamin G. Chitwood	1968	Raymond M. Cable	1986
Aurel O. Foster	1972	Harry Herlich	1987
Gilbert F. Otto	1972	Glenn L. Hoffman	1988
* Theodor von Brand	1975	Robert E. Kuntz	1988
May Belle Chitwood	1975	Raymond V. Rebois	1988
Carlton M. Herman	1975	Frank W. Douvres	1989
Lloyd E. Rozeboom	1975	Thomas K. Sawyer	1989
* Albert L. Taylor	1975	Leon Jacobs	1990
David R. Lincicome	1976	* J. Allen Scott	1990
		Judith H. Shaw	1990

* Deceased.

VOLUME 58 JANUARY 1991 NUMBER 1

CONTENTS

(*Continued from Front Cover*)

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