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CONTENTS

(*Continued on Outside Back Cover*)

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Cosmocerca panamaensis sp. n. (Nemata: Cosmocercidae) from the Panamanian Poison-arrow Frog, *Dendrobates pumilio* Schmidt, 1857, with a Discussion of Prodelphy, the Type Species and Family Authorship in *Cosmocerca* Diesing, 1861

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ABSTRACT: *Cosmocerca panamaensis* sp. n. (Nemata: Cosmocercidae) is described. The nematode is a parasite in the small intestine of *Dendrobates pumilio* Schmidt, 1857, a poison-arrow frog from Panama. Males of *C. panamaensis* sp. n. differ from all other described *Cosmocerca* males by having 3 pairs of tuberculate papillae surrounding the anal opening. The amphidelphic condition is described for the females of *C. panamaensis* sp. n. and it is noted that the prodelphic condition is an erroneous character of the genus. *Paracosmocerca spinocerca* Rao, 1979, is recognized as *Cosmocerca spinocerca* comb. n.

In the western hemisphere, *Cosmocerca* has been hitherto only reported from South America; *C. panamaensis* sp. n. thus represents the first species from Central America. This is the second species of *Cosmocerca* parasitizing an anuran of the family Dendrobatidae.

The superfamily, family, and subfamily names Cosmocercoidea, Cosmocercidae, and Cosmocercinae, respectively, are credited to the authorship Railliet, 1916.

KEY WORDS: nematode, animal parasitic, poison-arrow frog, taxonomy, morphology, SEM, *Cosmocerca panamaensis*.

The genus *Cosmocerca* is found among the parasitofauna of amphibians, especially frogs and newts; however, in the western hemisphere reports are limited to South America and now with this report, Central America. Historically, the genus goes back to Dujardin, 1845, who described *Cosmocerca ornata* (=*Oxyuris ornata*) from the intestine of *Rana esculenta* and *Rana temporaria*. The genus name was proposed by Diesing in 1861. Currently, there are 25 nominal species recognized in the genus (Table 1). Only 8 of these have been recorded from anurans occurring in the western hemisphere. They are *C. brasiliensis* Travassos, 1925, *C. chiliensis* Lent and Freitas, 1948, *C. commutata* Diesing, 1851, *C. cruzi* Oliveira and Fabio, 1970, *C. parva* Travassos, 1925, *C. rara* Freitas and Vincente, 1966, *C. travassoi* Oliveira and Fabio, 1970, and *C. uruguayensis* Lent and Freitas, 1948. These species all occur in South America. The present report is a description of a new species of *Cosmocerca* from Panama and is the first species of this genus found outside South America in the western hemisphere. It is also the second species to be described from the anuran genus *Dendrobates*. Dyer and Altig (1976) first reported *Cosmocerca* parasitizing *D. parvulus* in their redescription of *C. braziliensis*.

Cosmocerca panamaensis sp. n. is named after the country in which it was found.

Materials and Methods

Frog specimens were collected in the Bocas del Toro area of the Isla Bastimentos, Panama on 21 November 1968, 20 January 1971, and 4 May 1977. The frogs were killed in the field within 24 hr after capture using Chloretone (1,1,1-trichloro-2-methyl-2-propanol), fixed in 10% formalin for 24 hr, and subsequently preserved in 70% ethanol. Specimens were deposited in the American Museum of Natural History.

The nematodes were removed from the small intestine and preserved in 70% ethanol and 5% glycerol. Clearing of the worms was carried out in U.S. Bureau of Plant Industry Model watch glasses (BPI) using the standard glycerol-dehydration technique. The BPI watch glasses were placed individually in covered petri dishes containing filter paper saturated with 80% ethanol to control the rate of evaporation of the alcohol in the BPI watch glass. Glycerol was added on a daily basis to the BPI watch glasses in order to replace the evaporating alcohol. After approximately 1.5 wk the BPI watch glasses containing the nematodes (now in 100% glycerol medium) were placed in a desiccator for 1 wk to remove any excess water from the glycerol. Following this procedure, the nematodes were permanently mounted in glycerol with glass rods to prevent compression of the worms, and they were subsequently studied with the aid of a light microscope. Measurements were made with the aid of a camera lucida. Specimens were prepared for scanning electron microscopy by dehydrating to 100% ethanol with increasing concentrations of amyl acetate (25%, 50%, 70%, and 100% amyl acetate). Specimens then underwent critical point drying (Anderson, 1951), sputtered with 400–500 Å thickness of gold, and viewed with a Cambridge Mark II Scanning Electron Microscope using an accelerating voltage of 10 kV.

Table 1. List of nominal species.

Type:	*Cosmocerca ornata* (Dujardin, 1845) Diesing, 1861
	C. trispinosa Railliet and Henry, 1916, species inquirenda
	C. commutata (Diesing, 1851) Diesing, 1861
	C. longicauda (Linstow, 1885) Railliet and Henry, 1916
	C. brasiliensis Travassos, 1925
	C. parva Travassos, 1925 (Syn. *C. freitasi* Jorge de Silva, 1954)
	C. minuscula Travassos, 1931
	C. japonica Yamaguti, 1938
	C. pulcherrima Ivanitzky, 1940
	C. limnodynastes Johnston and Simpson, 1943
	C. chiliensis Lent and Freitas, 1948
	C. uruguayensis Lent and Freitas, 1948
	C. timpohejevoi Skarbilovitch, 1950
	C. banyulensis Chabaud and Campana-Rouget, 1955
	C. kashmirensis Fotedar, 1959
	C. rara Teixeira de Freitas and Vicente, 1986
	C. cruzi de Oliveira Rodrigues and de Fabio, 1970
	C. travassosi de Oliveira Rodrigues and de Fabio, 1970
	C. crenshawi Fotedar, 1973
	C. indica Nama and Khichi, 1973
	C. mucronata (King and Wu, 1945) Chabaud, 1978
	C. macrogubernaculum Rao, 1979
	C. spinocerca (Rao, 1979) comb. n.
	C. panamaensis Martinez and Maggenti sp. n.
	C. podicipinus Baker and Vaucher, 1984

All measurements are in millimeters unless otherwise stated.

Specimens identified as *Cosmocerca parva* from Paraguay (Baker and Vaucher, 1984) were obtained from the Muséum d'Histoire naturelle, Genéva, Switzerland, through the courtesy of Dr. Vaucher. Specimens of *C. parva* Travassos, 1925, were obtained from the Helminthological Collection of Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

Description

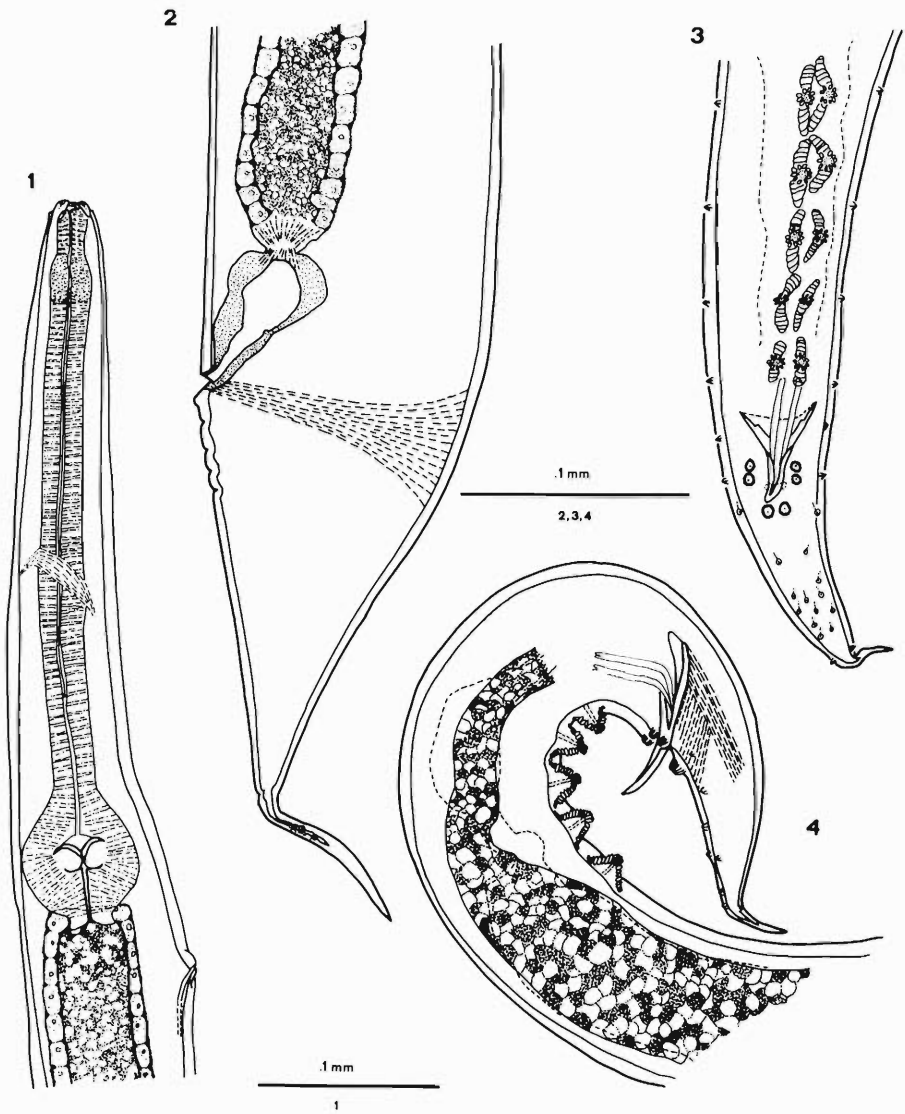
Cosmocerca panamaensis sp. n. (Figs. 1–8)

GENERAL: Cosmocercoidea Railliet, 1916; Cosmocercidae Railliet, 1916; Cosmocercinae Railliet, 1916; *Cosmocerca* Diesing, 1861. Small, slender, spindle-shaped worms. Cosmocercoid type esophagus present, excretory pore situated at level of or slightly posterior to posterior bulb. Posterior bulb valvate. Female amphidelphic, anterior gonad outstretched, posterior gonad reflexed. Vulva approximately at midbody. Males approximately one-half size of females. Cuticle whitish in color with transverse striae. Papillae arranged in longitudinal rows, with 2 spicules weakly sclerotized. Gubernaculum well developed, Y-shaped. Tubercles arranged in 6 circular patterns surrounding anal opening. Ten (5 pairs) plectanate supplementary organs with tubercles arranged in a rosette pattern, present along ventrocaudal surface of male.

DIMENSIONS: MALES (11): Length 1.51–2.16; width 0.07–0.16. Stoma length 1.63–3.00 μm; vestibule (cheilostome) 0.18–0.45 μm, esophastome 1.45–2.36 μm. Esophageal length (including bulb) 0.21–0.26; diameter of posterior bulb 0.03–0.05. Excretory pore from anterior extremity 0.20–0.26. Anterior margin of nerve ring from anterior extremity 0.13–0.20. Gubernaculum length 0.07–0.10; spicule length 0.02–0.04. Tail length (anus to caudal filament terminus) 0.09–0.15.

FEMALES (5 gravid specimens): Length 3.46–5.61; width 0.09–0.18. Stoma length 3.09–4.74 μm; vestibule (cheilostome) 0.55–0.73 μm, esophastome 2.36–3.73 μm. Esophageal length (including bulb) 0.40–0.46; diameter of posterior bulb 0.06–0.07. Excretory pore from anterior extremity 0.41–0.46. Anterior margin of nerve ring from anterior extremity 0.19–0.35. Vulva from anterior extremity 1.89–3.10, distance from vulva to posterior end (caudal filament terminus) 1.52–2.51. Vulva position in percent of total body length 51–63%. Egg size 0.05–0.06 × 0.03–0.06. Range due to compression of the egg mass due to egg numbers and membrane stretching of eggs containing embryos. Tail length (anus to caudal filament terminus) 0.24–0.33. Uteri amphidelphic.

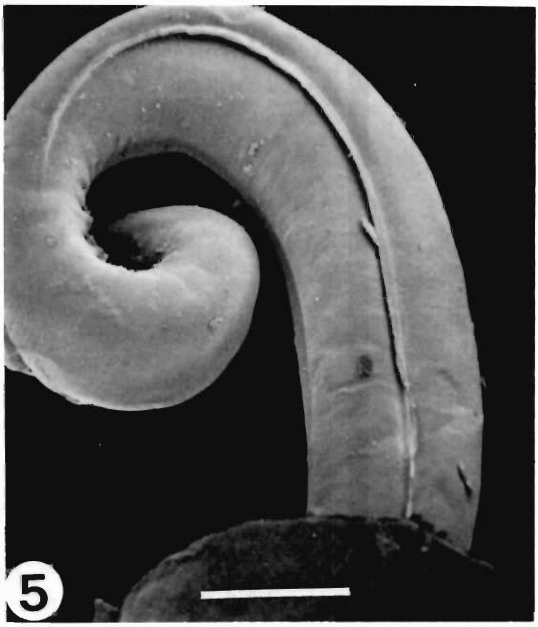
HOLOTYPE (male): Length 1.53; width 0.09. Stoma length 2.09 μm; vestibule (cheilostome) 0.36 μm, esophastome 1.73 μm. Esophageal length (including bulb) 0.26; diameter of posterior bulb 0.04. Excretory pore from anterior extremity 0.24. Anterior margin of nerve ring from anterior extremity 0.20. Gubernaculum length 0.09; spicule length 0.04. Protractor muscles of gubernaculum distinct; 1 set situated ventrally, 1 set situated posteriorly. Testis extends anteriorly and is flexed posteriad near the terminal end. Tail length (anus to caudal filament terminus) 0.14. Ten preanal plectanes (plates) arranged in 5 oblique pairs along the ventral surface with central tuberculate papillae with tubercles arranged in a rosette pattern, with a pore situated in the center (Figs. 6, 7). Six tuberculate papillae, with tubercles arranged in a circular pattern (rosette but lacking plectanes)



Figures 1–4. ***Cosmocerca panamaensis*** **from the poison-arrow frog** ***Dendrobates pumilio.*** **1. Lateral view of anterior end of female. 2. Lateral view of female tail showing anal musculature. 3. Ventral view of male tail. 4. Lateral view of male tail with gubernacular muscles.**

surround the anal opening; 2 pair adanal or just anterior to anal opening, and 1 pair just posterior to anal opening (Figs. 3, 4, 8). Eight pairs of caudal papillae located ventrolaterally, posterior to the anus. Lateral alae (lateral wings) begin at level of posterior stoma region and terminate at the first anterior most plectane. Somatic papillae arranged in 6 longitudinal rows: 1 dorsal, 1 ventral, 2 subdorsal, and 2 subventral to the lateral alae.

ALLOTYPE (female): Length 4.75; width 0.18. Stoma length 3.19 μm; vestibule (cheilostome) 0.55 μm, esophastome 2.64 μm. Esophageal length (including bulb) 0.40; diameter of posterior bulb 0.06. Excretory pore from anterior extremity 0.45. Distance from vulva to anterior extremity 2.44, distance from vulva to posterior end (caudal filament terminus) 2.31. Vulva position in percent of total body length 51%. Egg size 0.05 × 0.06. Tail length (anus to caudal filament terminus) 0.26. Uteri amphidelphic; anterior ovary outstretched anteriorly with a flex-



Figures 5–8. Scanning electron micrographs of *Cosmocerca panamaensis.* **5.** Lateral view of male tail. **6.** Lateral view of male tail. **7.** Ventral view of plectane and supplement (note pore in center). **8.** Ventral surface view of gubernaculum and anal opening and tuberculate papillae surrounding anal opening. (Figures 5, 6: scale = 100 μm; Figs. 7, 8: scale = 10 μm.)

ure directed posteriorly near its terminus. Posterior ovary reflexed, outstretched anteriorly. Two pairs of caudal papillae associated with caudal filament.

DIAGNOSIS: Males of *C. panamaensis* resemble males of *C. ornata* (Dujardin, 1845), a species parasitizing several species of *Rana, Bufo,* and *Tartura* in Europe; *C. parva* Travassos, 1925, a species parasitizing *Elosia nasus* in Brazil; *C. limnodynastes* Johnston and Simpson, 1943, parasitizing *Limnodynastes dorsalis* in Australia; *C. minuscula* Travassos, 1931, parasitizing *Rana temporaria*; *C. indica* Nama and Khichi, 1973, parasitizing *Rana cyanophyctis*; *C. macrogubernaculum* Rao, 1979, parasitizing *Rana cyanophyctis* in India; and *C. cruzi* Oliveira et al., 1970, parasitizing *Leptodactylus ocellatus* in Brazil.

Males of *C. panamaensis* can be distinguished from other *Cosmocerca* males with 5 pairs of plectanes by the presence of 3 pairs of rosette-like tubercles surrounding the cloacal opening. It can be further distinguished by its shorter body length (1.5–2.16) from the longer *C. parva* (3.5+), *C. indica* (2.29–3.02), and *C. cruzi* (2.99); it can on length also be distinguished from the shorter *C. minuscula* (0.60–0.91). In addition, *C. panamaensis* has a longer esophageal length (0.21–0.26) than *C. minuscula* (0.11–0.15) and a shorter esophageal length than *C. macrogubernaculum* (0.29–0.30). *Cosmocerca cruzi* is nearly twice the body length of *C. panamaensis*; however, the excretory pore is far more anterior (0.10 from the anterior extremity vs. 0.20–0.26 for *C. panamaensis*). In addition to differing from *C. parva* by the presence of the paired rosettes and body length, it also differs by the length of the spicules (0.03–0.04 in *C. panamaensis* vs. 0.08 in *C. parva*) and the length of the gubernaculum (0.07–0.10 vs. 0.12–0.14 in *C. parva*). *Cosmocerca limnodynastes* can be separated by the presence of a single median plectane anterior to the anal opening that is absent on *C. panamaensis.*

Females of *C. panamaensis* differ from *C. ornata* by having a shorter body length (3.46–5.61 vs. 5.05–10.27); they also differ by the length of the esophagus: *C. ornata* (0.44–0.66), *C. panamaensis* (0.40–0.46). Among female *Cosmocerca* of similar size range, *C. panamaensis* has the excretory pore more posteriorly located (0.41–0.46) than *C. longicauda* (0.35), *C. spinocerca* (0.28–0.39), and *C. macrogubernaculum* (0.30–0.34). The tail of *C. panamaensis* is also considerably shorter (0.24–0.33) than *C. longicauda* (0.87–1.19), *C. spinocerca* (0.44–0.54), and *C. macrogubernaculum* (0.39–0.47).

The above distinguishing characteristics of the males and females warrant the designation of *Cosmocerca panamaensis* as a new species.

Validity of Baker and Vaucher's 1984 redescription of *C. parva* Travassos, 1925

Eleven samples from Paraguay and identified as *C. parva* Travassos, 1925, by Baker and Vaucher (1984) were obtained from Vaucher. These specimens were compared to our species (*C. panamaensis*) and to specimens collected by Travassos on 10 April 1924 from the type locality (Angra dos Reis-Estado do Rio) and type host *Elosia* (=*Helosia*) *nasus* Licht.

The redescription of *C. parva* from Paraguay specimens we consider invalid for *C. parva* for the following reasons: Baker and Vaucher's description was based on specimens from 4 species of frogs (3 reported as new hosts, none were the type host) collected in 9 separate localities in Paraguay. We do not accept the reported, and our personally observed, variations among their specimens as within the limits of a single species of *Cosmocerca.* They report that esophageal lengths varied in males from 236 to 405 μm and the nerve ring from the anterior extremity ranged from 89 to 210 μm; our examination of their specimens confirmed this. In our diagnosis, *C. panamaensis* is distinguished from *C. parva* Travassos, 1925, and all other male *Cosmocerca* with 5 pairs of plectanes by the presence of 3 pairs of rosette-like tubercles surrounding the cloacal opening. This difference was confirmed by examination of Travassos' specimens. Baker and Vaucher described their *C. parva* as having the "... subventral adanal region with two or four (usually three) pairs of relatively broad, flat papillae which are commonly (but not always) surrounded by a small rosette of punctations." Because of the foregoing inconsistencies confirmed on actual specimens, we reject Baker and Vaucher's 1984 emendation as valid for *C. parva* Travassos, 1925.

SPECIMENS DEPOSITED: University of California Nematode Collection (UCDNC), Davis, California 95616.

HOLOTYPE: Male, Slide No. 1606, UCDNC.
ALLOTYPE: Female, Slide No. 1607, UCDNC.
PARATYPES: Slide Nos. 1608, 1609, UCDNC.

HOST: *Dendrobates pumilio* Schmidt, 1861. Poison-arrow frog. Uncatalogued, American Museum of Natural History. Collectors: 21 November 1968, Daly; 20 January 1971, Meyers and Daly; 4 May 1977, Meyers and Jaslow.

LOCALITY: Bocas del Toro, Isla Bastimentos, Panama.

SITE OF INFECTION: Small intestine.

Discussion

The genus *Cosmocerca* has been plagued with confusion and inconsistency as regards the designation of the type species. Dujardin (1845) described *Oxyuris ornata* from the intestine of *Rana esculenta* and *Rana temporaria*. In 1861, Diesing proposed the genus *Cosmocerca* to contain 2 nominal species: *C. ornata* formerly *Oxyuris ornata* Dujardin, 1845, and *C. commutata* formerly *Ascaris commutata* Diesing, 1850. Diesing was not sure that *C. commutata* was a valid species and therefore designated it species inquirenda. Stiles and Hassall in 1905 designated *C. ornata* (Dujardin, 1845) Diesing, 1861, as the type species of *Cosmocerca* because they reasoned that Diesing in 1861 had only 1 valid species in the genus, hence the type.

In 1916, Railliet and Henry rejected Stiles and Hassall's designation of *C. ornata* and proposed that *C. trispinosa,* their new species based on descriptions published by Walter (1857) and Diesing (1861), be accepted as the type species. They erred on 2 counts by this proposal: first, in accordance with the rules of the International Code of Zoological Nomenclature (ICZN) (Ride et al., 1985), Stiles and Hassall clearly have priority (1905 vs. 1916). Secondly, according to the "type by designation" clause of the ICZN (Article 69, sec. a, part IV), *C. trispinosa* cannot take precedence. Furthermore, Article 70 of the ICZN states: "It is to be assumed that an author correctly identifies the nominal species that he . . . refers to a new genus when he establishes it." Therefore, *Oxyuris ornata* Dujardin, 1845, now cited as *Cosmocerca ornata* (Dujardin, 1845) Diesing, 1861, is the valid type species of *Cosmocerca* Diesing, 1861.

There is further confusion throughout the literature as concerns the proper author of the superfamily, family, and subfamily. Travassos, 1925, is credited with the superfamily Cosmocercoidea and the family Cosmocercidae. The subfamily Cosmocercinae is credited to Railliet, 1916. According to Article 36,"Categories coordinate" of the ICZN, all categories in the family group are of coordinate status in nomenclature . . . available with its original date and author. . . ." Therefore, the correct author for all family categories is Railliet, 1916.

The spicules of *C. panamaensis* are weakly sclerotized, and therefore are very difficult to distinguish from the rest of the internal anatomy. Similar findings are also reported in other species of *Cosmocerca* (Skrjabin et al., 1961). Reports of the spicules being absent or rudimentary in *C. minuscula* Travassos, 1931, may be explained by their weak development.

The juxtaposition of the gubernaculum and the spicules of *C. panamaensis* makes it difficult to discern if the spicules are free or fused with the gubernaculum. The morphology of these structures suggests that the distal tip of the gubernaculum, at least in part, may functionally substitute for the distal end of a "spicule" and is probably inserted into the vulva during copulation. It may be that the spicules are not functional at all in *C. panamaensis.* The relationship of the spicules with the gubernaculum requires further study.

Chabaud (1978) synonymized the genus *Paracosmocerca* Kung and Wu, 1945, with the genus *Cosmocerca* because they mistook the gubernaculum for fused spicules, the only character difference. We accept Chabaud's synonymy, and therefore *Paracosmocerca spinocerca* Rao, 1979, is proposed as *Cosmocerca spinocerca* comb. n.

The females of *Cosmocerca* have uteri that are amphidelphic. This morphology has been mistakenly reported as prodelphic in previous literature. The mistake arises from the fact that the posterior ovary is reflexed; therefore, both ovaries travel anteriorly in the body. In the past, the ovaries of *Cosmocerca* have been used to discern the prodelphic condition rather than the uteri. The uteri are opposed; therefore, the females are amphidelphic (Seurat, 1920).This is clearly shown by Grabda-Kazubska (1974) in his paper on gonad development; however, he also terms them as prodelphic. Baker (1980) attempted to address the problem as it exists in Cosmocercinae; unfortunately, he was unable to resolve the issue and added to the complexity by extending the discussion to *Haemonchus* (Trichostrongylidae). Baker was correct in his observation that the reproductive systems of *Haemonchus* and *Aplectana* are similar; however, *Aplectana* is designated as prodelphic and *Haemonchus* is amphidelphic. The terminology proposed by Seurat is

not ambiguous, only its usage. When these terms are limited to the uteri as Seurat clearly intended, then *Haemonchus, Aplectana, Oxysomatium,* and *Cosmocerca* are all amphidelphic. Confusion arises when the terms are misused by applying them to the ovaries. The etymology is clear: amphidelphic (Gr. amphi, on both sides; delphys, womb, uterus); prodelphic (Gr. pro, forward; delphys, womb, uterus). This misunderstanding causes dangerous taxonomic interpretations, for example, in Skrjabin et al. (1961) the subfamily Oxysomatinae is separated from the subfamily Cosmocercinae by the former being amphidelphic and the latter being prodelphic; in reality both are amphidelphic with the posterior ovary reflexed forward. This should be noted in future works.

Cosmocerca of the western hemisphere have been found only in South America (Brazil, Uruguay, Chile, and Ecuador). *Cosmocerca panamaensis* is the first species of *Cosmocerca* found outside South America in the western hemisphere. This is also the second time that the genus has been reported parasitizing the anuran genus *Dendrobates.* Dyer and Altig (1976) first reported *Cosmocerca* parasitizing *D. parvulus* in their redescription of *C. brasiliensis.*

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Epizootiology of *Nematodirus battus, N. filicollis,* and *N. spathiger* (Nematoda: Trichostrongyloidea) in Western Oregon[1]

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ABSTRACT: The epizootiology of the 3 species of *Nematodirus* commonly infecting sheep in Oregon is described. From January 1986 through March 1988, 22 groups of 3–4 tracer lambs each were allowed to graze for a period of 28 days on a pasture known to be contaminated with several species of *Nematodirus*. After grazing, the animals were held in isolation for 21 days, then euthanized and necropsied for recovery of nematodes. Also during 1987, 4 groups of 3 tracer calves each were allowed to graze the pasture at times corresponding to winter, spring, summer, and fall. In tracer lambs, 3 species of *Nematodirus* (*N. battus, N. filicollis,* and *N. spathiger*) were commonly found throughout the trial while a fourth species (*N. helvetianus*) was only occasionally found. Both *N. battus* and *N. filicollis* were transmitted to the tracers during the entire year. Major peaks in transmission were confined to late fall and winter. Low levels of transmission occurred during the rest of the year with minor increases in abundance occasionally in summer. A more seasonal pattern of transmission was demonstrated for *N. spathiger* with transmission largely confined to summer and low levels sporadically present during winter. Transmission of both *N. battus* and *N. filicollis* appears to be dependent on precipitation while that of *N. spathiger* is more closely linked to temperature. A re-examination of published data from England indicates that, rather than strictly spring transmission, year-round transmission of *N. battus* occurs in that region. Consequently, the only real differences in the epizootiology of *N. battus* in Oregon and England is in the timing of the major peaks of transmission and the period of time during which the greatest risk of disease exists. In addition, transmission of *N. battus* to tracer calves in Oregon demonstrates cattle may also play a role in the epizootiology of this parasite in the United States as they do elsewhere.

KEY WORDS: *Nematodirus battus, Nematodirus filicollis, Nematodirus spathiger,* epizootiology, sheep.

Nematodirus battus Crofton and Thomas, 1951, was originally described from domestic sheep (*Ovis aries* L.) in Great Britain (Crofton and Thomas, 1951, 1954). For most of the subsequent decade, it was thought to be restricted to the British Isles where it had been reported as the most pathogenic of helminth parasites infecting sheep (Kingsbury, 1953; Thomas and Stevens, 1956; Baxter, 1957; Dunn, 1978). Then, in 1961, *N. battus* was recovered from sheep in southwestern Norway, and has since become established both on lowland farms and the mountain grazing areas (Helle, 1969; Overaas, 1976). Currently, this parasite has been identified in sheep in The Netherlands, France, Italy, the United States, Canada, and possibly Yugoslavia (Cvetkovic et al., 1963; Lepojev, 1963; Nardi et al., 1974; Borgsteede et al., 1978; Borgsteede and Konig, 1979; Hubert and Kerboeuf, 1985; Hoberg et al., 1986; Smith and Hines, 1987). The foci in Norway, The Netherlands, and Canada have been linked to the importation from Britain of adult sheep chronically infected with *N. battus* (Helle, 1969; Borgsteede et al., 1978; Smith and Hines, 1987). However, the origin and abrupt appearence of this parasite in Great Britain and elsewhere have never been adequately explained (Jansen, 1973).

In North America, the known distribution of this parasite includes the Pacific Northwest (Oregon and Washington), the Eastern Seaboard (Maryland, New York, and Vermont), and Canada (Prince Edward Island and Novia Scotia) (Zimmerman et al., 1986; Smith and Hines, 1987); however, with the rapid transport of sheep from 1 area to another, it is considered that *N. battus* is probably widely distributed throughout North America. Although *N. battus* has been implicated in the deaths of lambs 8–20 weeks of age in Canada (Smith and Hines, 1987), no such connection has been made in the United States.

The life cycles of *Nematodirus* spp. are different from those of the other common trichostrongyloids typical of sheep. The epizootiology of the 3 primary species of *Nematodirus* (*N. battus, N. filicollis* (Rudolphi, 1802), and *N. spathiger* (Railliet, 1896)) found in sheep has been the focus of much research in Great Britain (see Thomas

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and Stevens, 1956, 1960; Baxter, 1957; Thomas, 1959b; Gibson, 1963; Gibson and Everett, 1976, 1981, 1982; and others). Among *Nematodirus* spp. the first 2 larval molts are completed within the egg. At 21°C, development to the infective third stage (L_3) requires approximately 4 wk for *N. battus,* 3–4 wk for *N. filicollis,* and 3 wk for *N. spathiger* (Thomas, 1959a). After ingestion of the L_3, the minimum prepatent period for *N. battus, N. filicollis,* and *N. spathiger* is 2 wk for the former and 2–3 wk for the latter 2 (Kates and Turner, 1955; Thomas, 1959a). A marked seasonality in the transmission, mediated by precipitation and/or temperature, has been observed for these species in Great Britain (Thomas, 1959b; Thomas and Stevens, 1960; Christie, 1962; Parkin, 1976; Gibson and Everett, 1976, 1981, 1982; Mitchell et al., 1985).

With the apparent recent introduction of *N. battus* into North America and the tendency for inadequate differentiation of *N. spathiger* from *N. filicollis* in previous studies, epizootiological data for these species are not available in North America. However, such information is essential for development of sound control programs and, as pointed out by Graham et al. (1984) in regard to *N. battus,* the results of epizootiological studies from 1 region cannot automatically be applied to other areas. This was exemplified by Rickard et al. (1987) who demonstrated that late fall transmission of *N. battus* occurred in western Oregon and postulated year-round transmission of this parasite, a pattern quite different from that considered typical in Britain (Thomas and Stevens, 1956; Baxter, 1957; Thomas, 1959b; Gibson, 1963; Mitchell et al., 1985). Additionally, it has been demonstrated that cattle (*Bos taurus* L.) are capable of acquiring and maintaining infections of this parasite even in the absence of sheep and, therefore, their presence should also be considered in the development of control programs (Parfitt and Michel, 1958; Helle, 1981; Coop et al., 1988). Consequently, a study was initiated to determine the pattern of transmission of those species of *Nematodirus* commonly present in sheep in western Oregon. Results are reported herein.

Materials and Methods

The pasture on which the study occurred is located at the Veterinary Medical Animal Isolation Laboratory (VMAIL) at Oregon State University (OSU). The 1.2-ha fenced pasture consists of fescue (*Festuca* spp.) bentgrass (*Agrostis* spp.), rye-grass (*Elymus* spp.), and subclover (*Trifolium* spp.). Contamination with eggs and larvae of *N. battus* probably occurred in 1984 (Hoberg et al., 1986). From April 1985 to December 1986 the pasture was grazed continually by 4–17 sheep. Beginning in 1986, the pasture was grazed only by the animals in the study with the exception of January, February, and April–May 1986 when 4, 4, and 40 additional sheep, and November and December 1986 when 2 and 2 horses (*Equus caballus* L.) grazed on the pasture, respectively. The 40 additional sheep grazing the pasture were purchased from a local producer for use in an anthelmintic trial. They were not passing eggs of *N. battus* at the time they were placed on pasture (14 April 1987). They were allowed to graze for 30 days to become infected with *N. battus.* Neither the very low numbers of eggs passed while these animals were on pasture nor the removal of low numbers of infective larvae by the animals is thought to have influenced the subsequent results (see Zimmerman et al., 1988, for details).

Lambs (primarily Hampshire-Suffolk crosses, 5–9 mo old) were bought at auction and housed in indoor isolation stalls at VMAIL. Individual fecal samples were taken and counts of eggs per gram (EPG) were performed. Any animals found to be passing eggs of *N. battus* were replaced, with the exception of 1 lamb in group 3, 2 lambs in group 10, and 2 lambs in group 14. These latter animals were not removed from the trial as replacements were not available and the continuity of the experiment would have been severely comprised. Prior to inclusion in the study, all animals were treated twice, at weekly intervals, with fenbendazole at 5 mg/kg. This specific anthelmintic and dosage was chosen as it has been shown to be highly effective in reducing levels of infection by larval and adult stages of *Nematodirus* as well as other species of trichostrongyle (Kennedy and Todd, 1975; Ross, 1975). Posttreatment fecal samples were examined 1 wk following the second anthelmintic treatment.

Beginning in January 1986, 4 (groups 1 and 2) or 3 (all remaining groups) lambs were turned out onto the contaminated pasture (day 0). The animals were allowed to graze for 28 days after which they were returned to the isolation stalls and housed an additional 21 days prior to necropsy in order to determine levels of larval inhibition. Individual fecal samples were taken beginning on day 17 and at weekly intervals thereafter beginning on day 21.

At necropsy the small intestine was ligated in situ, separated from the remaining viscera, and removed from each animal. It was then opened longitudinally and the mucosal surface stripped 3 times and washed. The contents and washings were placed into a bucket from which 2 5% duplicate aliquots were taken. Each aliquot was sieved using a 400-mesh (37.5-μm) screen with the material remaining on the screen backwashed into a dish and fixed in 10% neutral buffered formalin. The small intestine was then incubated overnight at room temperature in tap water (except for groups 2 and 3). After incubation the small intestine was handled as described above; however, duplicate 50% aliquots were taken rather than 5%. All nematodes were recovered for identification from 1 each of the aliquots from the contents and incubates of the small intestine. In addition to identifying adult male *Nematodirus* to

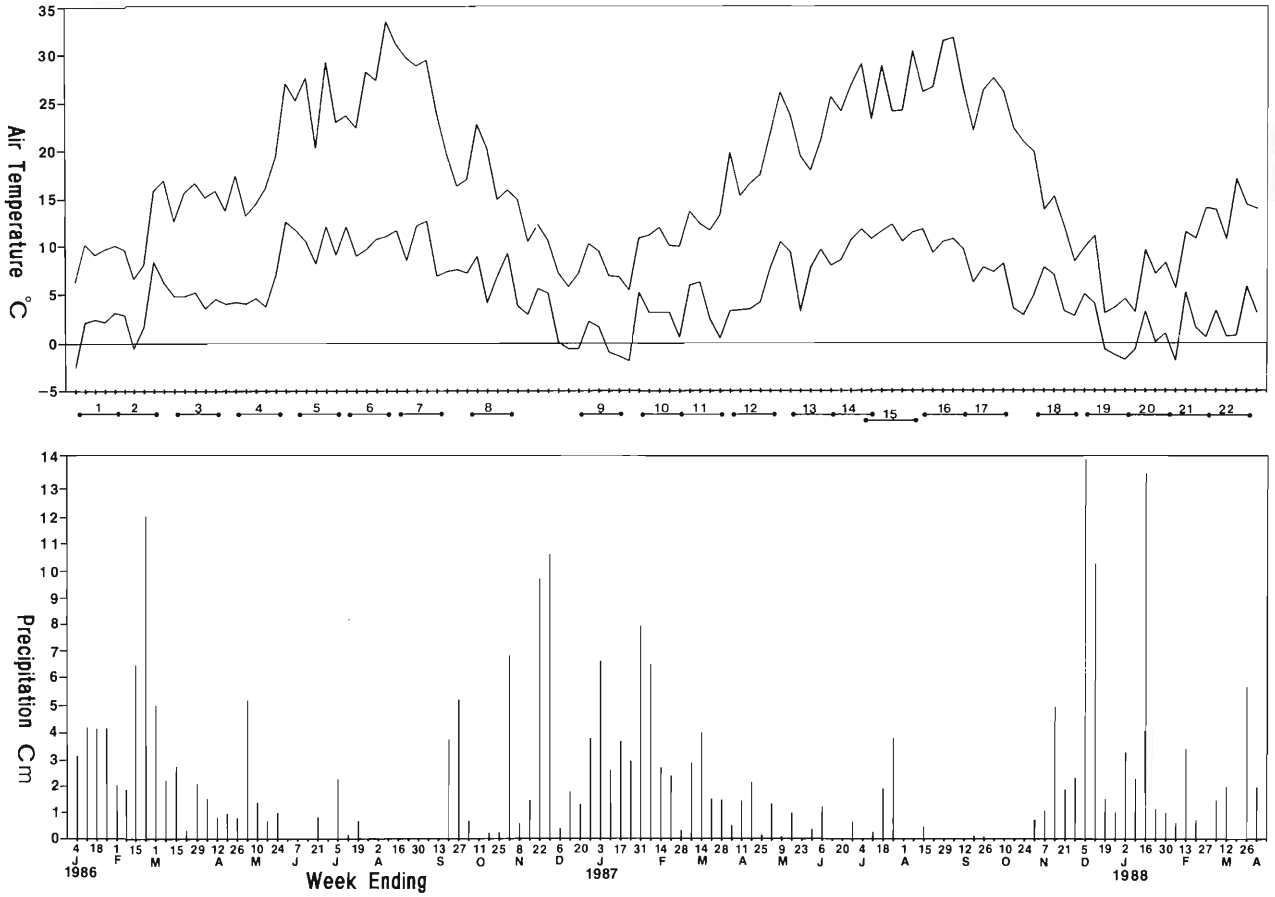

Figure 1. Mean weekly minimum and maximum air temperature and total weekly precipitation. Bars indicate time period in which tracer sheep were on the pasture. Numbers above bars correspond to the group number.

species, adult female *Nematodirus* present were also identified based on the synlophe (Lichtenfels and Pilitt, 1983). Larvae were differentiated according to Herlich (1954), Kates and Turner (1955), and Thomas (1959a). All numerical means presented are abundance as defined by Margolis et al. (1982). Although a mathematical model describing the data is not presented, data points in Figures 2 and 3 are connected by lines for ease of visualization.

In addition to the tracer lambs, 4 groups of tracer calves were allowed to graze the pasture; group A during the late winter (day 0 = 16 March), group B during spring (day 0 = 26 May), group C during summer (day 0 = 31 August), and group D during late fall (day 0 = 16 November) of 1987. Tracers were male Holstein calves (3–7 mo old) raised from birth in confinement at the OSU dairy barn. Length of time on pasture, in isolation, and necropsy techniques were the same as for the lambs.

Meteorological data were supplied by the OSU Climatic Research Institute, Corvallis, Oregon. These data were recorded at Hyslop Field Laboratory, approximately 12.5 km from the study site.

Results

The weekly mean minimum and maximum air temperatures and total weekly precipitation are illustrated in Figure 1. During the course of the study, the mean weekly temperatures rarely fluctuated more than 10°C from the 30-yr average. However, precipitation fluctuated quite extensively about its 30-yr average. February 1986 was atypically wet (mean monthly precipitation a minimum of 5 cm above 30-yr average) as were September and November 1986 and December 1987. December 1986 was atypically dry (mean monthly precipitation a minimum of 5 cm below 30-yr average) which was followed by 5 mo of slightly below normal precipitation, beginning in February 1987. October and November 1987 and February 1988 were again atypically dry.

Day 0 for each group of tracer lambs as well as the day 28 mean counts of EPG are presented in Table 1. Only eggs of *N. battus* were differentiated on fecal examinations. While eggs of *N. filicollis* and *N. spathiger* may be differentiated, the presence of *N. abnormalis* May, 1920, on the pasture prior to the initiation of the trial (Rickard et al., unpubl. data) precluded exact identification of eggs for all species. Eggs of *N. abnormalis* cannot be reliably distinguished from those of *N. spathiger* or *N. helvetianus* May, 1920 (see Onar, 1975). All lambs were negative for eggs of *N. battus* on the posttreatment fecal examination. By day 28, when the animals were returned to isolation, 17 of the 22 groups were passing eggs of *N. battus* (Table 1). Of the 5 negative groups, 3 (groups 5, 7, and 16) did have positive fecal samples at times other than day 28 (first positive sample, $\bar{x} \pm$ SE: group 5—day 42, 6.7 ± 3.4; group 7—day 35, 3.3 ± 3.3; group 16—day 17, 3.3 ± 3.3). Thus, only 2 groups (groups 9 and 17) remained negative on fecal examination throughout the sampling period.

Table 1. Day 28 mean counts of *N. battus* eggs per gram of feces.

Group no.	Day 0	$\bar{x}$ ± SE*
1	6 Jan 86	20.0 ± 9.1
2	3 Feb 86	92.5 ± 8.5
3	17 Mar 86	40.0 ± 11.5
4	28 Apr 86	3.3 ± 3.3
5	9 June 86	0.0 ± 0.0
6	14 July 86	43.3 ± 8.8
7	18 Aug 86	0.0 ± 0.0
8	6 Oct 86	60.0 ± 60.0
9	22 Dec 86	0.0 ± 0.0
10	2 Feb 87	13.3 ± 8.8
11	2 Mar 87	13.3 ± 13.3
12	6 Apr 87	16.7 ± 8.8
13	18 May 87	3.3 ± 3.3
14	15 June 87	3.3 ± 3.3
15	6 July 87	16.7 ± 8.8
16	17 Aug 87	0.0 ± 0.0
17	14 Sept 87	0.0 ± 0.0
18	2 Nov 87	35.0 ± 35.3
19	7 Dec 87	30.0 ± 25.2
20	4 Jan 88	10.0 ± 5.8
21	1 Feb 88	10.0 ± 5.8
22	29 Feb 88	3.3 ± 3.3

* Mean ± standard error.

At necropsy, 3 species of *Nematodirus* were commonly recovered from the tracer sheep: *N. battus, N. filicollis,* and *N. spathiger* (Table 2; Figs. 2, 3). Transmission of *N. battus,* as indicated by abundance of nematodes, occurred yearround with all groups becoming infected. In 1986, transmission remained high during winter, decreased slightly in spring (group 4), increased in early summer (group 5), and then decreased from midsummer through early fall (group 8). Although peaks were not as high in 1987 as in 1986, transmission followed essentially the same pattern: high over winter and early spring, decreasing from late spring through summer (except in group 15 which had considerably higher levels of *N. battus*), increasing again over the fall to peak in early winter (group 19). Transmission then decreased slightly but remained at a relatively high, constant level over the remainder of the study.

The numbers of *N. battus* in the lambs which

Table 2. Abundance of *N. battus*, *N. filicollis*, and *N. spathiger* recovered from tracer sheep.

Group no.	*N. battus*			*N. filicollis*			*N. spathiger*		
	Adults	L_4*	E_4†	Adults	L_4	E_4	Adults	L_4	E_4
1	524 ± 236‡	0	21 ± 20	296 ± 276	0	64 ± 34	0	0	0
2	2,175 ± 758	0	0	555 ± 194	15 ± 15	10 ± 10	0	0	0
3	1,380 ± 630	0	0	180 ± 95	0	0	27 ± 27	0	0
4	457 ± 119	0	0	53 ± 44	0	0	0	0	0
5	755 ± 133	0	0	81 ± 54	0	5 ± 3	265 ± 24	0	0
6	584 ± 169	0	7 ± 7	230 ± 61	0	7 ± 7	141 ± 50	0	0
7	243 ± 133	0	34 ± 33	27 ± 14	0	1 ± 1	117 ± 18	0	0
8	20 ± 20	0	53 ± 29	20 ± 20	0	7 ± 7	20 ± 20	0	0
9	105 ± 60	1 ± 1	21 ± 21	96 ± 59	0	1,663 ± 827	7 ± 7	0	20 ± 20
10	252 ± 196	14 ± 14	0	67 ± 67	80 ± 80	68 ± 68	27 ± 18	0	0
11	134 ± 70	0	0	161 ± 151	0	18 ± 7	0	0	0
12	304 ± 155	0	0	34 ± 18	0	0	7 ± 7	0	0
13	40 ± 12	0	0	7 ± 7	0	0	0	0	0
14	7 ± 7	0	0	0	0	0	0	0	0
15	759 ± 216	0	9 ± 9	13 ± 7	0	0	27 ± 18	0	0
16	31 ± 10	0	0	40 ± 31	0	0	0	0	0
17	13 ± 13	0	0	0	0	0	0	0	0
18	421 ± 33	7 ± 7	38 ± 13	34 ± 18	0	0	0	0	0
19	742 ± 339	14 ± 7	203 ± 56	34 ± 34	0	9 ± 9	68 ± 41	0	3 ± 3
20	363 ± 51	0	1 ± 1	0	0	19 ± 7	20 ± 0	0	1 ± 1
21	333 ± 94	0	1 ± 1	0	0	4 ± 4	27 ± 26	0	0
22	325 ± 241	20 ± 11	2 ± 2	0	0	1 ± 1	0	0	0

* L_4 = Late fourth-stage larvae.
† E_4 = Early fourth-stage larvae.
‡ Abundance ± standard error.

harbored infections prior to the study and their appropriate group mates are presented in Table 3. Comparisons of the numbers of nematodes recovered apparently indicate that, for those animals previously infected, some acquired resistance did exist. However, the variation in numbers in these groups was no greater than in many other groups which utilized the apparent *N. battus*-naive lambs. Additionally, deletion of data from these previously infected lambs does not substantially alter the pattern of transmission.

Essentially year-round transmission of *N. filicollis* occurred with 20 of the 22 groups of tracer lambs becoming infected (Figs. 2, 3). Although the abundance of *N. filicollis* was usually lower than *N. battus,* the transmission pattern was basically the same with peak transmission during the late fall or winter months. Low levels of transmission were present during the rest of the year with minor peaks occasionally during the summer.

The transmission pattern of *N. spathiger* was quite different from that of the previous 2 species (Figs. 2, 3). Only 13 of the 22 groups became infected with this parasite. In 1986, peak transmission occurred over the summer followed by low levels in the fall through the early winter of 1987. Low levels of transmission were present again the following summer with slightly higher levels during the winter.

The abundance of adults, late fourth-stage larvae (L_4) and early fourth-stage larvae (E_4) for each of these 3 species of *Nematodirus* is presented in Table 2 and Figure 3. Although both L_4's and E_4's of *N. battus* and *N. filicollis* were recovered from some groups of lambs, no L_4's of *N. spathiger* were present. Developmental arrest did occur in all 3 species with inhibited larvae present during the early winter months.

In addition to the above 3 species of *Nematodirus,* small numbers of *N. helvetianus* were recovered from tracer lambs in groups 19 (adults = 55 ± 30; L_4 = 7 ± 7), 20 (adults = 7 ± 7; L_4 = 7 ± 7), and 21 (adults = 33 ± 33; L_4 = 1 ± 1). Other trichostrongylid nematodes recovered from the tracer lambs were not enumerated but included *Cooperia* spp. and *Trichstrongylus* spp.

Of the 4 groups of tracer calves, only the first exhibited eggs of *N. battus* on fecal flotation. This group first became positive on day 17 ($\bar{x}$ ± SE: 13.0 ± 3.0) and remained positive throughout the sampling period. However, at necropsy, both

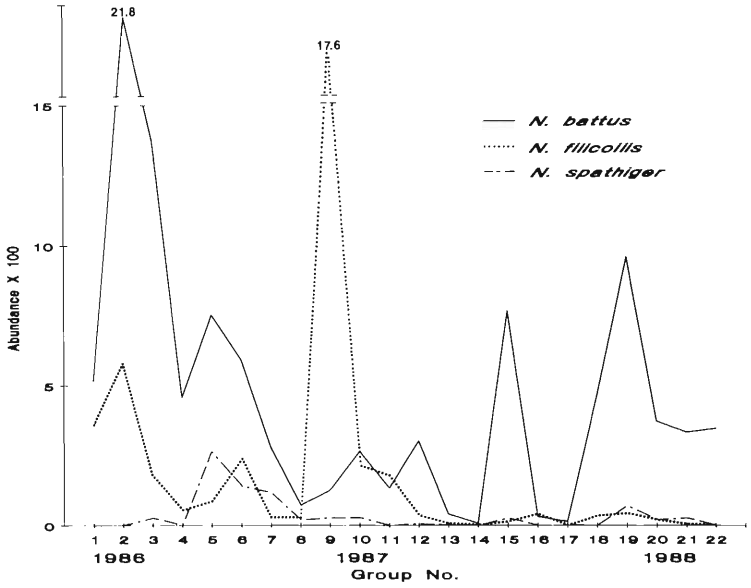

Figure 2. Total abundance of *Nematodirus* spp. present in tracer lambs.

groups 1 and 2 harbored infections of *N. battus* (Table 4). Also present in the calves were *N. filicollis, N. spathiger,* and *N. helvetianus.* All nematodes recovered were adults with the exception of E_4's of *N. filicollis* in group 1 and L_4's of *N. helvetianus* in group 4.

Discussion

The most striking result obtained in this study was the presence of year-round transmission of *N. battus* despite limited recontamination of the pasture and the use of older tracer lambs. It has long been considered that age immunity and acquired resistance is in operation in lambs older than 3–4 mo of age, thus explaining a lack of heavy infections of *N. battus* and clinical disease in older animals (see Dunn, 1978). Adult sheep have been thought to play only a minor role in the maintenance of the parasite (Gibson, 1963; Dunn, 1978). However, results of the presnt study and those reported by Rickard et al. (1987) do suggest, at least in western Oregon, older lambs may acquire relatively heavy burdens of *N. battus,* particularly during times of peak transmission. Although, in the present study, acquired resistance may have occurred in lambs previously infected with *N. battus,* the normal variation in numbers seen among individuals within a group and the high numbers in some older lambs may be explained by the presence of responder vs. non-responder lambs, as suggested by Taylor and Thomas (1986). An overall lower abundance of *N. battus* in 1987 than in 1986 could be attributable to the experimental design which allowed few animals other than those in the experiment to graze the pasture, thus limiting recontamination.

The pattern of transmission observed in the present study apparently deviates substantially from that considered to be typical in northern England. There, the sequence of events are such that usually only a single parasitic generation occurs per year (Thomas, 1959b). Development to infective third-stage larvae (L_3) proceeds irrespective of the time of year eggs are deposited on pasture (Gibson and Everett, 1981). However, hatching usually does not occur until the larvae are preconditioned by exposure to cold temperatures followed by warming above 10°C in moist conditions (Thomas and Stevens, 1960; Christie, 1962; Parkin, 1976). These conditions lead to a synchronous hatch of larvae concentrated into a few weeks during the spring (Parkin, 1976) with subsequent transmission being concentrated during that period (Thomas and Stevens, 1956; Baxter, 1957; Thomas, 1959b; Gibson, 1963). Although autumn transmission has been recently reported in England, Scotland, and Norway (Borgsteede, 1983; McKellar et al., 1983; Rodger, 1983; Hollands, 1984; Hosie, 1984), the

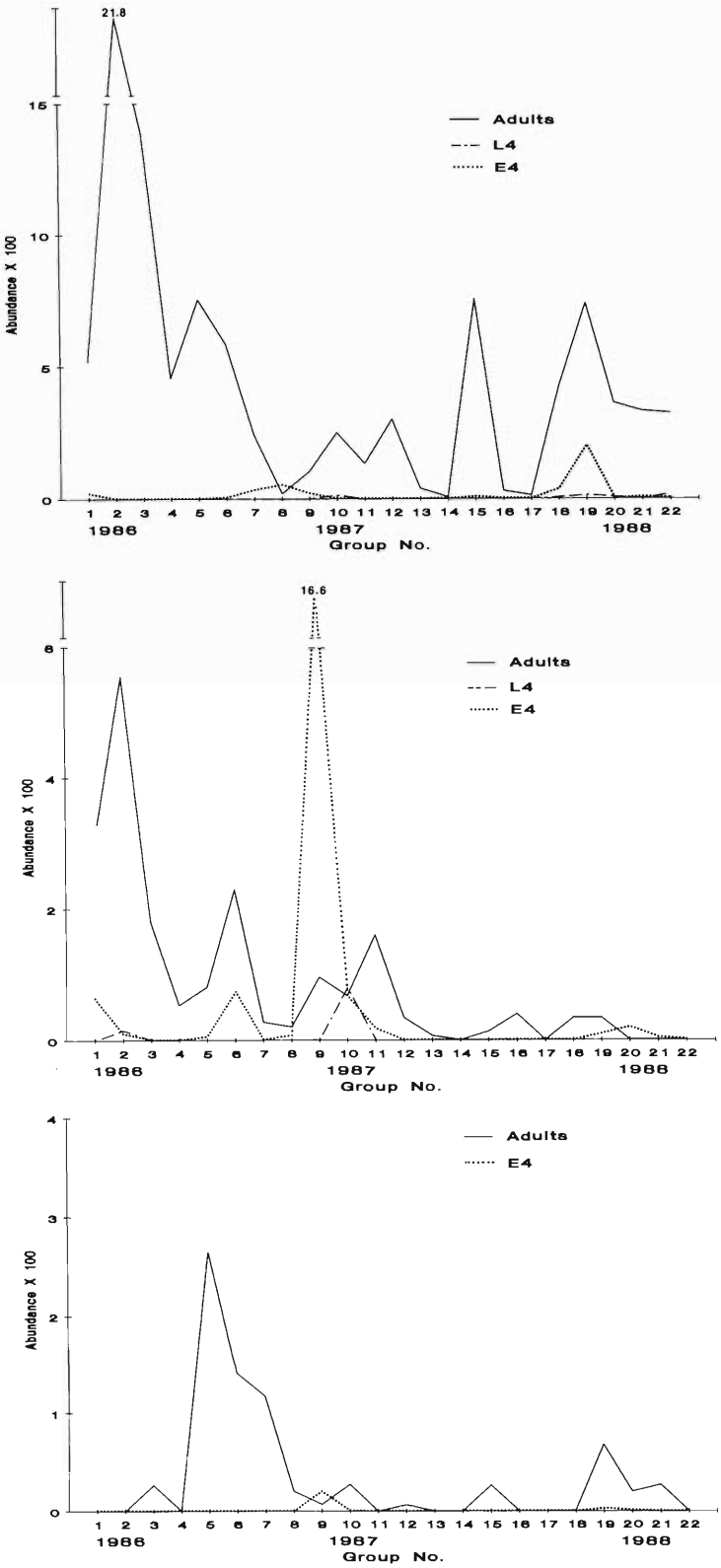

Figure 3. Abundance of adults, late fourth-stage larvae (L_4) and early fourth-stage larvae (E_4) for *N. battus* (top), *N. filicollis* (middle), and *N. spathiger* (bottom).

Table 3. Numbers of *N. battus* in individual lambs in groups containing animals previously infected.

Group no.	Animal no.	*N. battus**
3	303†	120
3	304	2,000
3	305	2,020
10	389†	70
10	390	644
10	391†	42
14	401	20
14	402†	0
14	403†	0

* Numbers represent adults; no larval stages were recovered from these sheep.

† Indicates animal previously infected with *N. battus* as demonstrated on pre-treatment counts of EPG.

Table 4. Abundance of *N. battus, N. filicollis, N. spathiger,* and *N. helvetianus* recovered from tracer calves.

	Group no.			
	A	B	C	D
N. battus				
Adults	511 ± 79‡	20 ± 12	0	0
L_4*	0	0	0	0
E_4†	0	0	0	0
N. filicollis				
Adults	7 ± 7	7 ± 7	0	0
L_4	0	0	0	0
E_4	9 ± 8	0	0	0
N. spathiger				
Adults	13 ± 7	16 ± 6	0	0
L_4	0	0	0	0
E_4	0	0	0	0
N. helvetianus				
Adults	20 ± 12	0	136 ± 36	31 ± 17
L_4	0	0	0	1 ± 1
E_4	0	0	0	0

* L_4 = Late fourth-larvae.

† E_4 = Early fourth-larvae

‡ Abundance ± standard error.

spring pattern previously described is still considered typical (Mitchell et al., 1985).

Detailed examination of the studies on which this typical pattern is defined allows expanded interpretation of the published information. Few of the studies used tracer lambs (in the sense of the present study) to define actual periods of transmission. Rather, larval availability, as indicated by herbage larval counts, was monitored. This, combined with previous reports on outbreaks of nematodiriasis, fecal egg counts, and necropsy of permanent grazing animals at various times of the year were used to define the pattern of transmission (see Thomas and Stevens, 1956; Baxter, 1957; Thomas, 1959b; Gibson, 1963; Boag and Thomas, 1975; Graham et al., 1984). Yet those studies that did use tracer lambs at specific times of the year (Connan, 1968; Waller and Thomas, 1983; Mitchell et al., 1985) have shown that transmission of *N. battus* does occur at times other than that of maximum larval abundance. One such study demonstrated transmission during periods when no larvae could be detected on pasture, thus emphasizing the limitations of pasture larval counts as an indicator of nematode challenge (Mitchell et al., 1985). When these studies are combined with those previously cited, the period of transmission in England appears to extend almost the year-round. Peak transmission occurs during spring–early summer followed by low levels over summer. Transmission then either increases in the autumn or remains low. This is followed by little or no transmission during the winter. Emphasis on spring transmission actually emphasizes the period at which the risk of disease is greatest as this is the time when large numbers of larvae are available to young, susceptible lambs. Therefore, the primary difference between the pattern of transmission demonstrated in the present study and that in England is in the timing of the period during which peak transmission occurs and the risk of disease is greatest. In Oregon, this period extends from late fall through winter while in England it is in the spring.

In the present study, correlation of transmission with climatic conditions is problematic. Unlike England, the major peaks of transmission occurred during the winter months when precipitation was high but ambient temperature was generally below 10°C (Fig. 1). The mechanism responsible for increased larval availability during this time is unknown as the actual timing of larval development and hatching of *N. battus* in the Pacific Northwest has yet to be determined. It is possible that in this region the mass larval hatch actually occurs during the winter rather than the spring as in England. There transmission during other periods, aside from those of maximum larval abundance, has been accounted for by several mechanisms: 1) eggs hatching within the same year as deposited (Gibson and Everett, 1981), 2) eggs hatching in spring followed by long-term survival of the larvae (Borgsteede, 1983), and 3)

eggs overwintering and hatching the following fall (Hollands, 1984).

We suggest that the determinant for the peak periods of transmission in western Oregon is precipitation rather than the temperature. The highest levels of parasite abundance coincided with high levels of precipitation even when the ambient temperature was below 10°C. Although our data are somewhat equivocal, patterns associated with levels of abundance in group 15 during the summer of 1987 further suggest that precipitation is the limiting factor for emergence of larvae from eggs present on pasture. During the few months prior to the release of this group onto the pasture, precipitation had been continually decreasing and was also below normal; concomitantly, transmission had decreased reaching the lowest levels recorded during the study. Subsequently, levels of precipitation increased during 2 of the 4 wk group 15 was on pasture and high numbers of nematodes were recovered from the tracer lambs. Precipitaion then decreased again with transmission being reduced until November, when both again increased. This hypothesis is further supported by observations in England and Norway of increases in larval abundance, levels of transmission, and numbers of clinical cases of nematodiriasis at times other than spring, when immediate prior exposure of eggs to cold temperatures did not occur (Gibson, 1959; Gibson and Everett, 1981; Borgsteede, 1983; McKellar et al., 1983; Rodger, 1983; Waller and Thomas, 1983; Hollands, 1984; Hosie, 1984). The increase in larval abundance in autumn described by Gibson and Everett (1981) was attributed to hatching of larvae at this time brought about by high levels of precipitation following a period of low precipitation.

The continuity of transmission of *N. battus* in the present study, particularly during drier months, is considered to be due to long term survival of the larvae on pasture. The temperatures and moisture levels present in the study area appear to be well within the extremes that larvae of *N. battus* are capable of surviving (Gibson and Everett, 1981; Boag and Thomas, 1985).

Early experimental studies on *N. filicollis* in England suggested a pattern of transmission similar to that of *N. battus* (Thomas, 1959b; Thomas and Stevens, 1960). However, later work showed that while this pattern was evident in northern England, it was different in southern England. Larval hatching occurred over a more extended period of time thereby resulting in infections during seasons other than spring (Gibson, 1963; Boag and Thomas, 1975; Gibson and Everett, 1976). In this regard, the pattern of transmission for *N. filicollis* seen in the present study most closely resembles that of southern England.

There is minimal epizootiological data available for comparison for *N. filicollis* in North America as early studies generally did not distinguish between *N. filicollis* and *N. spathiger*, thus effectively eliminating their use in establishing epizootiological patterns for either species (see Kates, 1943, 1950; Hawkins et al., 1944; Ayalew and Gibbs, 1973). However, some studies have demonstrated that both *N. filicollis* and *N. spathiger* are capable of overwintering and establishing infections in sheep the following spring (Griffiths, 1937; Herd et al., 1984; Coles et al., 1986). Herd et al. (1984), using permanent tracer lambs, demonstrated continuous transmission of *N. filicollis* in Ohio between May and November. There, low levels of transmission occurred during early summer and fall with high levels occurring in late summer (August–September). The differences in the timing of peak transmission of *N. filicollis* in Ohio and Oregon is most likely explained by the presence of a more moderate climate in Oregon. Furthermore, those factors (as outlined above) responsible for controlling larval availability of *N. battus* which results in peak transmission during late fall and winter in Oregon are also probably responsible for the transmission pattern of *N. filicollis*.

The variance in the transmission pattern of *N. spathiger* compared with the other 2 species is likely due to differences in the timing of development of the L_3 and the stimulus for hatching. Eggs of *N. spathiger* hatch much more quickly than those of *N. filicollis* and *N. battus* (Kates and Turner, 1955; Marquardt et al., 1959; Gibson and Everett, 1976, 1981, 1982). In England, eggs deposited on pasture from early spring through late summer hatch during the summer and early fall. Those deposited in fall and winter overwinter to hatch the following spring and summer. Consequently, peak periods of larval availability are during the summer months (Gibson and Everett, 1982). In the United States, as in England, development of eggs of *N. spathiger* to the infective L_3 appears to be dependent on temperature rather than precipitation (Kates and Turner, 1955; Marquardt et al., 1959). In Montana, development of eggs occurred during any season of the year except the coldest months of winter. The most rapid development occurred

during late spring through early fall with little or no development occurring from late fall through the winter and early spring. Larvae were also shown to be very resistant to cold temperatures but were quite susceptible to high temperatures; thus, the highest rate of survival of the free-living stages of *N. spathiger* occurred from fall to spring. The combination of relatively slow development and high survival during colder months with rapid development and short survival during hot months indicates peak larval availability occurs during spring and summer with a decrease over fall and winter. In the present study, transmission of *N. spathiger* was largely confined to the summer with low levels sporadically present during winter. Therefore, it appears that, as in Montana, temperature rather than precipitation controls larval availability in Oregon.

In Ireland, England, and Norway, *N. battus* is capable of cycling among cattle even in the absence of sheep; thus, cattle may play an important role in the transmission of this parasite to sheep (Parfitt and Michel, 1958; Taylor and Cawthorne, 1972; Downey, 1973; Helle, 1981; Bairden and Armour, 1987; Coop et al., 1988). The transmission of this parasite to the tracer calves in the present study indicates that cattle in this region are probably equally as important in maintenance of *N. battus* as they are elsewhere.

To date, clinical cases of nematodiriasis attributable to infections of *N. battus* have yet to be documented in Oregon. Three possible explanations for this exist. First, this parasite may not have been present long in this country as it has been shown that it must accumulate on a given pasture over several years before severe outbreaks will occur (Gibson, 1963; Helle, 1969; Boag and Thomas, 1975). Second, a less pathogenic strain of *N. battus* may be present. Preliminary analyses have demonstrated the existence of biochemical differences between isolates of this parasite from Oregon and England (Weybridge) (Rickard, unpubl. data). The implication of this is currently under study. The third explanation combines the transmission pattern with management practices common to the area. Although it was demonstrated during the present study and that of Rickard et al. (1987) that older lambs of a breed common to the area can acquire relatively high numbers of *N. battus,* they did not acquire the massive infections needed to produce clinical disease. Consequently, young lambs are probably more at risk. Lambing in the Willamette Valley of western Oregon is usually conducted between January and April. During this time, transmission of *N. battus* is peaking and subsequently declining. Thus, lambs will probably only be at risk if high levels of *N. battus* are present late into spring. After lambing, the animals are moved to pastures which are routinely burned the previous summer and remain fallow until the following spring. Consequently, little or no contamination is present when the animals enter the pasture after lambing. By the time transmission increases again in the fall, the lambs are old enough to tolerate relatively high numbers of worms. Consequently, severe clinical disease may not become common in western Oregon as the timing of peak transmission is not exactly coincidental with the presence of young, susceptible lambs on the pasture. Studies on the pathogenicity of different isolates of *N. battus* are needed to elucidate which factor(s) is/are responsible for this apparent lack of clinical disease.

The formulation of control measures for these 3 species of *Nematodirus* must take into account the variations in epizootiology as well as the presence of alternative hosts, such as cattle (present study) or llamas (Bishop and Rickard, 1987), which aid in perpetuation of the parasites on a pasture. Control measures in a region must also be based on the transmission patterns and the management practices present in that region as differences in both occur from 1 area to the next.

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Obituary Notice

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Influence of Host Age and Sex on Nematode Populations in the Wild Rabbit (*Oryctolagus cuniculus* L.)

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ABSTRACT: Forty-seven wild rabbits (*Oryctolagus cuniculus*) were collected during March 1987 from an area of sand dunes in eastern Scotland, and examined for gastrointestinal nematode parasites to determine how these parasite burdens related to the age and sex of the rabbits. *Graphidium strigosum* numbers increased significantly with age while those of *Trichostrongylus retortaeformis* tended to decline with age. Male rabbits aged 1–3 yr had greater *G. strigosum* populations than comparably aged female rabbits. Female rabbits also had fewer *T. retortaeformis* than male rabbits, but the differences in all age groups were less than those for *G. strigosum*. The relationship between *G. strigosum* and rabbit age confirmed previously published results. Those of *T. retortaeformis* with age and the effect of host sex and *G. strigosum* and *T. retortaeformis* worm burdens were at variance with results obtained from New Zealand and Australia, which found no evidence of age resistance on the size of the population of *T. retortaeformis* and that male rabbits had nematode populations similar to or lower than those in female rabbits.

KEY WORDS: nematode, *Graphidium strigosum, Trichostrongylus retortaeformis,* rabbit, *Orcytolagus cuniculus,* age.

Results from laboratory experiments have indicated that gastrointestinal nematodes may reduce the fecundity and survival of rabbits (Dunsmore, 1980) and under certain conditions may limit the size of natural populations (Dunsmore, 1971), but the influence of the age of the rabbits on their worm burden is poorly documented. Bull and Taylor (1956) suggested that the incidence of different parasites altered with the age of the rabbit, but used paunched rabbit weight as an index of host age, a criterion known to be unsatisfactory after 3–4 mo (Dudzinski and Mykytowcz, 1960). More reliable techniques are available for determining the age of rabbits up to 33 mo (Morris, 1972). In New Zealand and Australia, results of investigations into the relationship between the size of the parasite population and the sex of the rabbit host have suggested that nematode numbers in pregnant female rabbits are generally greater than in males during summer, while the reverse is the case in winter (Bull, 1959, 1964; Dunsmore, 1966a, b, c, 1971). However, surveys in Great Britain (Boag, 1972, 1985, 1987; Preston, 1976) found no similar relationship. The objective of the present paper is to investigate the relationship between 2 of the most common gastrointestinal parasites of the rabbit and host sex and age.

Materials and Methods

Forty-seven adult rabbits were cage trapped at the end of March 1987 on 10 ha of established coastal sand dunes in East Lothian. The area varied between 25 and 50 m a.s.l. and was mainly covered by mature Marram grass (*Ammophila arenaria*). The population was relatively undisturbed, and rabbit sign was frequent over the whole area, suggesting a very high density of animals. Quadrat counts gave an average of around 250 active burrow entrances per ha and a total entrance count of nearly 500 per ha (Kolb, unpubl. data). The incidence of myxomatosis was low.

Rabbits were removed to the laboratory where they were weighed, measured, and their reproductive state noted. The age of each animal was determined from the weight of the eye lens (Myers and Gilbert, 1968; Wheeler and King, 1980; Wallage-Drees, 1986) and the degree of fusion of the epiphyses of the lumbar vertebrae (Taylor, 1959). The abdominal contents were removed and frozen. The viscera were later thawed separated into 3 regions: stomach, small intestine, and large intestine. These were opened separately and the contents washed over a 100-mesh (125-μm) sieve. The residues were collected and stored in 2% formalin. Unless nematode numbers were low, no fewer than 20 worms were counted, the dilution never exceeding 1 part in 25. Helminth counts were transformed by log(n + 1) (Dunsmore and Dudzinski, 1968; Dunsmore, 1972) before being statistically analyzed using ANOVA regression methods for unbalanced sample sizes.

Results

Of the 47 rabbits collected during the survey, 19 were estimated to be 1 yr old, 13 were 2 yr old, 14 were 3 yr old, and only 1 was 4 yr old (Table 1). Nineteen of the rabbits were female. Of these, 11 were in the early stages of pregnancy, the majority being 3 yr old. However, an initial analysis using a t-test indicated no significant

Table 1. Relationship between age, sex of host, and numbers of *Trichostrongylus retortaeformis* and *Graphidium strigosum*.

Age (yr)	Sex (*N*)*	*Trichostrongylus retortaeformis*		*Graphidium strigosum*	
		Mean intensity ± SEM†	Range	Mean intensity ± SEM	Range
1	F (6)	1,420 ± 435	120–2,700	152 ± 41	44–295
	M (13)	3,275 ± 628	240–6,300	231 ± 66	28–540
	Total (19)	2,689 ± 515	120–6,300	206 ± 47	28–540
2	F (5)	798 ± 504	120–2,800	452 ± 180	23–925
	M (8)	2,443 ± 1,027	0–8,100	677 ± 109	385–1,200
	Total (13)	1,810 ± 683	0–8,100	590 ± 97	23–1,200
3	F (8)	364 ± 160	0–1,400	903 ± 192	375–2,000
	M (6)	2,725 ± 843	1,050–7,200	1,068 ± 269	200–2,300
	Total (14)	1,424 ± 502	0–7,200	974 ± 167	200–2,300
4	M (1)	0	0	870	0
1–4	F (19)	874 ± 213	0–2,800	547 ± 118	23–2,000
	M (28)	2,803 ± 458	0–8,100	561 ± 99	0–2,300
	Total (47)	2,012 ± 318	0–8,100	555 ± 146	0–2,300

* Sex: F = female; M = male. *N* = number in category.
† Standard error of the mean.

differences in nematode populations existed between pregnant and non-pregnant females, and consequently all female rabbits were considered together for further analysis and comparison with male rabbits.

The mean intensity of *T. retortaeformis* in 1-yr-old rabbits was 2,689 and generally decreased in the second and third year although numbers in male rabbits in the third year were slightly greater than in the second year. Only 1 rabbit in the second, third, and fourth year had no detectable *T. retortaeformis*. *Trichostrongylus retortaeformis* numbers were significantly greater in male rabbits than in female rabbits (Table 2), the accumulated average in the first 3 yr in male rabbits being 2,814 nematodes compared with 861 in female rabbits.

In contrast to *T. retortaeformis*, the mean *G. strigosum* numbers per rabbit increased significantly from the first to third year. Maximum numbers also showed a similar trend, the highest population recorded being 2,300. Mean *G. strigosum* populations were (although not significantly at 5% level) greater in male rabbits than in female rabbits each year, the accumulated mean in the first 3 yr for male and female rabbits being 659 and 502, respectively.

Discussion

The study demonstrates a differential effect of host age on the population dynamics of the 2 most common nematode parasites of the wild rabbit. The results confirm the positive relationship between numbers of *G. strigosum* and host age reported by Dudzinski and Mykytowycz (1963) and which Dunsmore and Dudzinski (1968) suggested was due to a gradually increasing natural susceptibility with age. However, there has been some controversy as to the effect of age on the population dynamics of *T. retortaeformis*. Bull and Taylor (1956) found the prevalence of *T. retortaeformis* was less in heavier animals than in juvenile animals. Dudzinski and Mykytowycz (1963) found the size of *T. retortaeformis* worms and number of eggs per female nematode de-

Table 2. Statistical significance of the relationships between nematode numbers and the age and sex of the rabbit population.

Age (yr) or sex*	*Trichostrongylus retortaeformis*		*Graphidium strigosum*	
	Mean	SEM†	Mean	SEM
Age yr 1	7.304	0.422	4.939	0.212
2	6.127	0.500	6.027	0.251
3	6.646	0.505	6.676	0.254
F	6.070	0.426	5.58	0.214
M	7.265	0.353	5.928	0.177
Variance ratio (*F*) determined by ANOVA tests‡				
Age	2.51	NS	16.11	***
Sex	4.87	*	1.81	NS
Interaction	1.45	NS	0.91	NS

* Sex: F = female; M = male.
† Standard error of the mean.
‡ Significant differences: * = $P < 0.05$; *** = $P < 0.001$; NS = not significant.

creased with age, but neither Dudzinski and Mykytowycz (1963) nor Dunsmore and Dudzinski (1968) found any evidence of age resistance reducing the size of the population. Michel (1952), however, showed that in domestic rabbits an immune reaction significantly reduced the population size of *T. retortaeformis,* whereas Boag (1985) found in a 6-yr study of wild rabbits that adult rabbits had fewer worms than juvenile rabbits. The present results indicate that where *T. retortaeformis* populations are naturally high there is a decline in number of *T. retortaeformis* with age.

The differences in parasite intensities reported by Bull (1959, 1964) and Dunsmore (1966a, b, c, 1971) resulting from the female breeding condition have not been confirmed from previous surveys in Great Britain (Boag, 1972, 1985, 1987; Preston, 1976). The numbers of female rabbits in the present survey were relatively small and only in the early stages of pregnancy, but again no significant differences were obtained between pregnant and barren females possibly because the sample size was too small to detect any difference.

The average and maximum population size from the rabbits in this study are far higher than previously recorded in Great Britain, particularly in the males. Previous samples have been taken from arable farmland and pasture where pest populations are usually controlled and rabbit densities are naturally lower than on marginal sandy ground. The high nematode counts, therefore, probably reflect the high density of the host population. On sand dunes male rabbits are more mobile than females during the winter and spring, and their ranges overlap with a greater number of consexuals (Cowan and Garson, 1985; Kolb, unpubl. data). This may lead to a greater exposure to infection for males rather than for females. There is no evidence from the age structure of the population that these higher parasite populations play much of a role in the survival of rabbits up to 3 yr old, but the sudden truncation at this age may reflect some effect of accumulated *Graphidium strigosum* populations on the health of older animals. Nothing is known about the epidemiology of myxomatosis in this population of rabbits, but it is likely that this disease may have reduced the life expectancy of the rabbits directly or by interacting with the parasites (Boag, 1988).

Acknowledgments

We would like to express our thanks to W. Storrie and Tyninghame Estates for permission to catch rabbits, and to K. D. Annand for assistance in the field.

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STUDENT PRESENTATION COMPETITION

The first student presentation competition was the main event of the 602nd meeting of the Helminthological Society of Washington. The Walter Reed Army Institute of Research in Washington, D.C. was the host on 15 March 1989. Each of the six students gave excellent presentations of their research results. First prize ($300) was awarded to Melanie E. Small, Department of Biology, Georgetown University. Second prize ($200) went to George W. Benz, Life Sciences Group, University of Connecticut. Third Prize ($100) was won by Sansanee Changkasiri, Department of Biology, Georgetown University. Other fine papers were presented by Marina Rodrigues-del Valle, and Grant Hayashi, both from Georgetown University, and Ann M. Barse of the University of Maryland. Titles of all the papers presented are listed in the Minutes of the 602nd Meeting (see p. 218).

Sincere thanks are extended to all the competitors, the judges, Willis A. Reid, Jr. who coordinated the competition, and to WRAIR for making the event such a success. A second student competition is tentatively planned for October 1990. Get your students ready!

Proc. Helminthol. Soc. Wash.
56(2), 1989, pp. 120–124

Rabbium paradoxus sp. n. (Seuratidae: Skrjabinelaziinae) Maturing in *Camponotus castaneus* (Hymenoptera: Formicidae)

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ABSTRACT: *Rabbium paradoxus* sp. n. (Seuratidae: Skrjabinelaziinae) is described from workers of the ant *Camponotus castaneus* (Hymenoptera: Formicidae) from Florida. This is the first report of males of the genus *Rabbium* and the first report of a member of the Seuratidae that develops to the adult stage inside an invertebrate host. All other members of this family occur in the alimentary tract of reptiles, especially lizards. Males and females of *R. paradoxus* occurred together in the abdominal body cavity of worker ants. Female parasites contained completely embryonated eggs.

KEY WORDS: *Rabbium,* Nematoda, *Camponotus,* ant.

Specimens of *Camponotus castaneus* parasitized by nematodes were collected in Florida by James C. Trager. These nematodes, which were submitted to the senior author for identification, showed strong affinities with the Spirurida and represent a new species of *Rabbium* M. B. Chitwood. The genus *Rabbium* contains a single species, *R. caballeroi* M. B. Chitwood (1960), which is a parasite in the stomach of the lizard *Leiocephalus carinatus* in the Bahama Islands. The genus *Rabbium* was originally described in the family Thelazeidae of the superfamily Spiruroidea, but is now placed in the subfamily Skrjabinelaziinae of the family Seuratidae. This new species of *Rabbium* is unique because it is the first representative of the Seuratoidea known to mature and apparently complete its life cycle in invertebrates, in this case, ants of the genus *Camponotus.*

Materials and Methods

Preserved workers of the ant species *Camponotus castaneus* infected with *Rabbium* nematodes were submitted in a solution of 1% propionic acid, 10% formalin, and 89% water by volume. The specimens were either processed and mounted in glycerin or placed in lactophenol for 3 wk at 32°C, then mounted in lactophenol on standard microscope slides. The latter method of mounting proved helpful in relaxing and partially clearing the specimens, permitting easier viewing of the stoma, excretory pore, rectal area, and genital system.

The infected worker ants were collected on 22 July 1987 by J. C. Trager in the San Felasco Hammock State Preserve, Alachua Co., Florida. The parasites occurred in the body cavity of the host, and 1 ant contained 5 oviparous females and 3 males in its abdominal cavity (Fig. 1).

Results

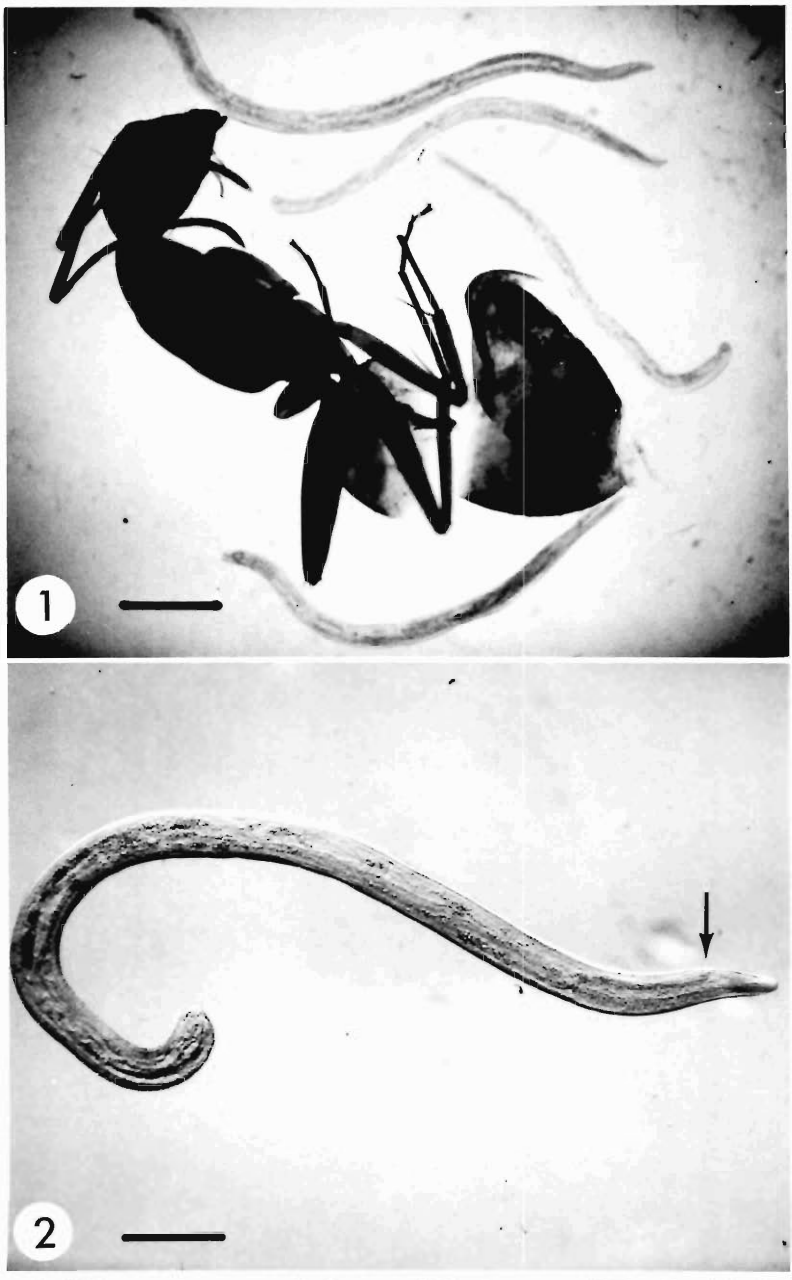
Male and gravid female nematodes were collected from the abdominal cavities of parasitized ants (Figs. 1, 2). A comparison of the females with the description of *Rabbium caballeroi* M. B. Chitwood (1960) showed that both nematodes were congeneric but belonged to different species. Dimensions are given as means followed by range in parentheses.

Rabbium paradoxus sp. n. (Figs. 1–10)

Description

GENERAL: *Rabbium* M. B. Chitwood, 1960; Skrjabinelaziinae Chabaud, Campona-Routeg, and Brygoo; Seuratidae (Hall); Seuratoidea (Hall); Ascaridida.

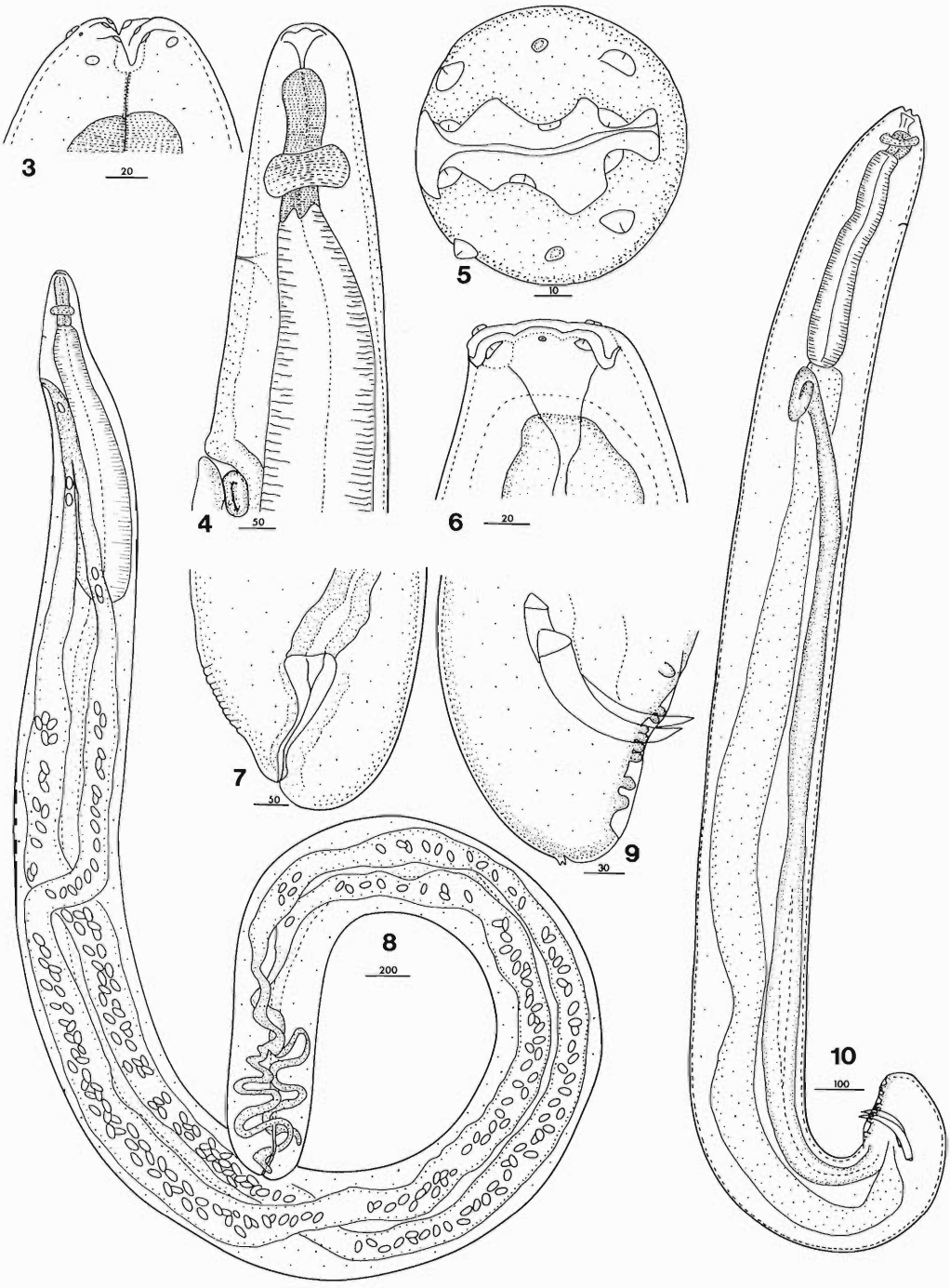
ADULTS (Figs. 1–10): Medium-sized, rather stout nematodes with blunt anterior end and bluntly rounded posterior end; cuticle with distinct transverse striations ranging from 12 to 19 μm in thickness, pseudolabia bearing 6 labial papillae, 4 cephalic papillae, and paired amphids located further posteriorly; mouth and stoma dorsoventrally elongated; pharynx divided into a short muscular anterior and a long glandular posterior portion; intestine reduced, seemingly degenerate; rectum distinct, anus terminal or subterminal. Females didelphic and opisthodelphic with the vulva located posterior to the excretory pore in the cervical region; ovaries extend tortuously to the posterior end, filled with relatively thin walled, smooth shelled eggs containing fully developed juveniles that possess a small apical thorn; males smaller than females, similar in gen-



Figures 1, 2. ***Rabbium paradoxus*** **sp. n. 1. Four gravid females removed from the abdominal cavity of the figured worker ant,** ***Camponotus castaneus*** **(bar = 1.7 mm). 2. Gravid female removed from the abdominal cavity of a** ***Camponotus castaneus*** **worker (arrow denotes position of vulva) (bar = 0.9 mm).**

eral structure; testis single, may or may not be once or double reflexed at tip; spicules paired, separate, subequal, slightly curved, triangular in cross section, pointed at tip, with the left one slightly longer than the right; narrow bursa supported by 8–9 small knoblike genital papillae, gubernaculum absent. Parasites of ants.

FEMALES (Figs. 1–8) (N = 10): All measurements are in micrometers unless otherwise stated. Length 8.0 (5.1–9.3) mm; greatest width 440



Figures 3–10. ***Rabbium paradoxus*** **sp. n. (all measurements in micrometers). 3. Dorsal view of female head. 4. Lateral view of female pharyngeal region. 5. En face view of female. 6. Lateral view of female head. 7. Lateral view of female tail. 8. Lateral view of female. 9. Lateral view of male tail. 10. Lateral view of male.**

(350–610); length of stoma 35 (25–54); greatest width of stoma (dorsoventrally) 54 (41–63); length from head to excretory pore 259 (176–409); from head to nerve ring 170 (151–202); from head to vulva 440 (315–599); from head to base of muscular pharynx 193 (157–252); from head to base of glandular portion of pharynx 1,385 (1,070–1,575); length of tail 32 (0–88); width at anus 124 (75–220); length of vagina 372 (252–454); length of eggs 61 (54–63); width of eggs 32 (25–38).

MALES (Figs. 9, 10) ($N = 3$): Length 2.6 (2.4–2.7) mm; greatest width 214 (201–233); length of stoma 12 (12–13); greatest width of stoma (dorsoventrally) 29 (25–32); distance from head to excretory pore 174; from head to nerve ring 85 (79–95); from head to base of muscular portion of pharynx 86 (70–95); from head to base of glandular portion of pharynx 517 (473–542); length of tail 96 (94–101); width at cloaca 145 (132–158); length of right spicule 115 (79–139); length of left spicule 147 (104–178); greatest width of right spicule 18 (6–25); greatest width of left spicule 25 (19–32).

TYPE SPECIMENS: Holotype female and allotype male deposited in the Nematology Collection at the University of California, Davis, California. Paratype females at the Laboratoire de Zoologie (Vers), Paris.

TYPE HOST: *Camponotus castaneus* (Latreille) (Hymenoptera: Formicidae).

TYPE LOCALITY: San Felasco Hammock State Preserve, Alachua County, Florida, U.S.A.

Diagnosis

The new species *R. paradoxus* can be distinguished from the only other species in the genus, *R. caballeroi,* by the larger egg size (30 μm wide by 40 μm long in *R. caballeroi* and 30 μm wide by 60 μm long in *R. paradoxus*), the egg shell texture (rugose in *R. caballeroi* and smooth in *R. paradoxus*), and overall length (11–12 mm in *R. caballeroi* and 5–9 mm in *R. paradoxus*). Males of *R. caballeroi* are unknown.

Redescription of subfamily

With the addition of *Skrjabinelazia galliardi* Chabaud, 1973, and *Rabbium paradoxus,* the subfamily Skrjabinelaziinae is hereby redefined as follows: Seuratidae; Seuratoidea; Ascaridida; mouth triangular without lips or modified lips on the inner faces of paired pseudolabia; buccal capsule varies from cylindrical to triangle-shaped, sometimes with a crown of small teeth; normally 10 anterior papillae and paired amphids present; pharynx may be simple or divided into a muscular anterior and glandular posterior portion; cuticle varies from smooth to bearing transverse striations; tail variable, may be absent in some females; bursa present or absent, genital papillae present, mostly post anal; spicules mostly present, usually unequal; gubernaculum present or absent; female with vulva placed anteriorly at the level of the pharynx, eggs become completely embryonated in female; oviparous or ovoviviparous; adults occur in the alimentary tract of reptiles and rarely in the body cavity of insects.

Discussion

Rabbium paradoxus is the first member of the Seuratoidea with adults which mature and develop in invertebrates. The females of *R. paradoxus* are closely related to those of *R. caballeroi,* which occur in the stomach of the lizard *Leiocephalus carinatus* from the Bahamas. Although the life cycle of *R. caballeroi* is not known, the immature stages could be in ants. *Rabbium paradoxus* may have a vertebrate host (possibly a lizard) in some portion of its range, but in Florida the nematode is able to complete its development in the intermediate host. The selection pressure to achieve this could have been the decline or possible extinction of the original vertebrate host.

In the present case, the ant *Camponotus castaneus* has taken over the role of the vertebrate host. This ant species is native to the eastern portion of the United States and southeastern portion of Canada. Parasitized workers were easily recognized in the field by their swollen gasters and unusual behavior of daytime movement. Normally, *C. castaneus* will forage only at night. It is a generalist feeder with a likeness for sweets (fruits), but does also feed at vertebrate feces (Trager, pers. comm.).

A study on the life history of another member of this subfamily, *Skrjabinelazia galliardi,* recently has been undertaken (Chabaud et al., 1988). This nematode is a parasite of a sphaerodactyline lizard (*Gonatodes humeralis* Guichenot) in Brazil. The females produce eggs that contain third-stage juveniles. These eggs are voided with the feces, and when ingested by insects, hatch, and the nematodes enter the insect's body cavity. They

remain as third-stage juveniles without further development, and the cycle is completed when the insect is eaten by a lizard.

In the case of *R. paradoxus,* it appears that at least with this population, the cycle could continue in the invertebrate line if worker ants ingest the eggs by consuming the bodies of their parasitized nestmates. Eggs of *R. paradoxus* containing juveniles were found throughout the abdominal cavity of parasitized ants. Since the parasites are located in the body cavity of the insect, the eggs would be unable to pass out of the ant naturally, as they would with *R. caballeroi* in the lizard. Therefore, if the parasite is restricted to ants, transmission must occur after the host's death. It is not known if the nematodes bring about the ant's death from a depletion of nutrients or possible rupture of the abdominal cavity, but considering the parasite burden in some instances, the latter is a definite possibility.

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MEETING SCHEDULE
HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1989–1990

(Wed) 11 Oct 1989	"Selected Epidemiology Topics," Uniformed Services University of the Health Sciences, Bethesda, MD
(Wed) 15 Nov 1989	"*Haemonchus* and Haemonchiasis—Old Questions and New Answers," Animal Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD
(Wed) 6 Dec 1989	"To Be Announced," Plant Protection Institute, U.S. Department of Agriculture, Beltsville, MD
(Wed) 10 Jan 1990	"To Be Announced," Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD
(Wed) 14 Feb 1990	"Recent Advances in Pre-erythrocytic Stage Malaria Vaccine Development," Department of Immunoparasitology, U.S. Naval Medical Research Institute, Bethesda, MD
(Wed) 14 Mar 1990	"Antiparastic Diseases Drug Development," Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC
(Wed) 11 Apr 1990	"To Be Announced," School of Hygiene and Public Health, The Johns Hopkins University; and Medical College, University of Maryland, Baltimore, MD
(Sat) 5 May 1990	"To Be Announced," Department of Pathobiology, Veterinary School, University of Pennsylvania, New Bolton, PA; Royal Society of Tropical Medicine and Hygiene; and New Jersey Society for Parasitology
(Sat) August 1990	"To Be Announced," Crabfest, Easton, MD

Members that are in the area and could attend meetings who do not receive announcements by mail should contact Dr. Leonard Francl, Nematology Laboratory, USDA ARS, BARC-West, Building 11A, Beltsville, MD 20705.

Proc. Helminthol. Soc. Wash.
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Scleroductus yuncensi gen. et sp. n. (Monogenea) from *Pimelodella yuncensis* (Siluriformes: Pimelodidae) in Peru

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ABSTRACT: *Scleroductus yuncensi* gen. et sp. n. (Gyrodactylidea: Gyrodactylinae) is described from *Pimelodella yuncensis* Steindachner, 1912 (Siluriformes; Pimelodidae), from fresh water in Peru. The new material is unique in having a bulbous penis with 2 sclerotized ribs within the ejaculatory duct and a sclerotized partial ring surrounding the opening. The hamuli are slender and with thin, continuously curved blades. The superficial bar has 2 posteriorly directed filamentous processes in place of the typically broad gyrodactylid ventral bar membrane. *Scleroductus* is the fourth genus of the Gyrodactylidea to be described from South America, all of which occur on siluriform fishes.

KEY WORDS: *Scleroductus yuncensi* gen. et sp. n., Gyrodactylidea, Monogenea, Peru, *Pimelodella yuncensis.*

During a parasite survey of freshwater fishes of Peru, one of us (C.A.J.) found a previously undescribed viviparous monogenean from the external surface of *Pimelodella yuncensis* that could not be classified into any known genus within the Gyrodactylidea (Baer and Euzet, 1961). The present study describes the new material as *Scleroductus yuncensi* gen. et sp. n.

Materials and Methods

Forty-eight host specimens measuring approximately 10 cm long were collected by netting tributaries of the Chicama River, Ascope Province, Peru. Parasites were collected by the dislodgement method of Mizelle and Kritsky (1967) and fixed in 5% formalin. The specimens were mounted unstained in glycerine jelly. Measurements of the holotype are followed in parentheses by those of the paratypes. All measurements are in micrometers. Descriptive terminology follows Malmberg (1970).

Results

Scleroductus gen. n.

DIAGNOSIS: Gyrodactylidae Cobbold, 1864; Gyrodactylinae Monticelli, 1892. Body elongate with 2 cephalic lobes. Haptor with 16 marginal hooks evenly distributed around lateral and posterior margins of haptor. Bulbous pharynx, short esophagus, and intestinal crura ending blindly. Hamuli slender, with poorly developed deep root. Single superficial and deep bars. Viviparous reproduction. Bulbous penis with 2 sclerotized ribs along length of ejaculatory duct and a sclerotized partial ring surrounding the opening. Parasites of freshwater teleosts.

TYPE SPECIES: *Scleroductus yuncensi.*

Scleroductus yuncensi sp. n. (Figs. 1–5)

DESCRIPTION (12 specimens studied; 6 specimens measured): Partially flattened holotype 460 (400–470) long, 90 wide at midlength. Bulbous pharynx 42 (35–45) wide. Penis bulbous, 15 (15–16) in diameter; armed with 2 sclerotized ribs 16 (15–16) long within wall of ejaculatory duct, sclerotized, horseshoe-shaped spinous ring on the surface of the bulb at the duct opening. Gland cells of penis not seen. Hamuli relatively slender, 75 (76–78) long; root 33 (32–35), shaft 60 (59–62), point 32 (30–35). Hamulus point curved on both inner and outer surfaces. Superficial bar dumbbell-shaped, 16 (12–18) wide, 4 (4–5) long; anterolateral processes absent; bar membrane in form of 2 longitudinal filamentous processes, 23 (20–30) long. Deep bar simple, without median notch, 13 (13–18) wide. Marginal hooks 20 (20–21) long. Sickle small, 5 (4–5) long, 5 (5–6) wide distally, 4 (4–5) wide proximally, with round upturned "toe." Handle relatively thick, 16 (16–17) long. Marginal hook filament 15 (15–16) long.

HOST: *Pimelodella yuncensis* Steindachner, 1912 (Pimelodidae), "Bagre."

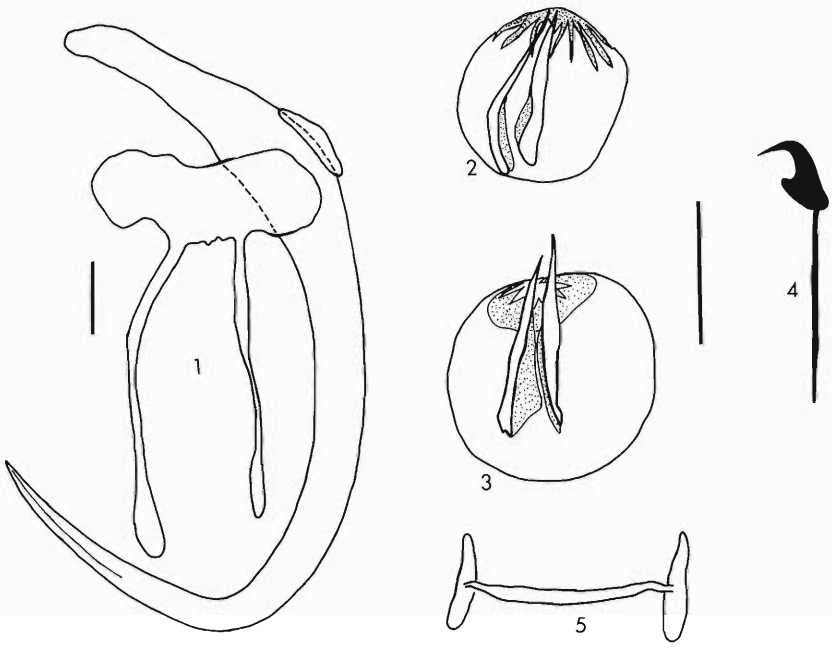
LOCALITY: Tributaries of the Chicama River, Peru, 79°21′16″W longitude, 7°46′54″S latitude.

SITE OF INFECTION: Present in body washings.

HOLOTYPE: USNM Helm. Coll. No. 80629.

PARATYPES: USNM Helm. Coll. Nos. 80630.

Scleroductus closely resembles species of *Gyrodactylus* Nordmann, 1832. The haptor contains slender hamuli devoid of a prominent deep root, a small single superficial bar, and 16 mar-



Figures 1–5. Sclerotized body parts of *Scleroductus yuncensi* gen. et sp. n. from *Pimelodella yuncensis* Steindachner in Peru. The figures are a composite prepared from the holotype and paratypes. 1. Hamulus and superficial bar. 2. Penis in lateral view. 3. Oblique view of flattened penis showing the horseshoe-shaped form of the terminal sclerotized ring and an alternative view of the 2 ribs. 4. Marginal hook. 5. Deep bar. Scale bar = 10 μm.

ginal hooks spaced evenly around the lateral and posterior margins of the simple disk. Initially we suspected that the Peruvian material was a member of a unique Asian lineage of *Gyrodactylus* (*G. afghanensis* Ergens, 1979, *G. luckyi* Ergens, 1970, *G. moraveci* Ergens, 1979, *G. parvus* Bychowsky, 1936, and *G. tibetanus* Dzhalilov, 1981) parasitizing cypriniform fishes and having the superficial bar membrane as 2 posteriorly directed filamentous processes (Ergens, 1970, 1979; Dzhalilov, 1981; Gussev, 1985). However, the morphology of the bulbous penis with the sclerotized terminal ring and 2 sclerotized ribs within the ejaculatory duct proved significantly different from that of all known members of the Gyrodactylidea to warrant establishment of a new genus.

Gyrodactylideans most frequently have a penis in the form of a muscular bulb armed with varying numbers of small sclerites or spines and a large beaklike sclerite at the opening. Small gland cells can occur at the base of certain of the sclerites. The duct passing through the penis is nonsclerotized, but may be dilated into a saclike structure. This form is found in the genera *Anacanthocotyle* Kritsky and Fritts, 1970, *Archigyrodactylus* Mizelle and Kritsky, 1967, *Fundulotrema* Kritsky and Thatcher, 1977, *Gyrdicotylus* Vercammen-Grandjean, 1960 (see Harris and Tinsley, 1987), *Gyrodactyloides* Bychowsky, 1947, *Gyrodactylus* Nordmann, 1832, *Isancistrum* de Beauchamp, 1912 (see Llewellyn, 1984), *Laminiscus* Palsson and Beverley-Burton, 1983, *Macrogyrodactylus* Malmberg, 1957, *Metagyrodactylus* Yamaguti, 1963, *Paragyrodactylus* Gvozdev and Martechov, 1953, *Paragyrodactyloides* (Szidat, 1973) Ostowski de Nunez, 1975, *Polyclithrum* Rogers, 1967, and *Swingleus* Rogers, 1969. In *Afrogyrodactylus* Paperna, 1968, the penis is an elongated muscular tube containing a duct studded with minute rods or spines (Paperna, 1968). In *Oogyrodactylus* Harris, 1983, the penis is a muscular tube with a basal bulb; the expressible tip bears a sclerotized ring and gland cells are present distally and basally (Harris, 1983). In *Phanerothecium* Kritsky and Thatcher, 1977, the penis is described as an unarmed muscular tube that has a lightly sclerotized duct and incorporates a coiled portion of the vas deferens (Kritsky and Thatcher, 1977).

Scleroductus yuncensi is the fourth species of the Gyrodactylidea to be described from South American freshwater fishes. The others are *Oogyrodactylus farlowellae* Harris, 1983, from body surface of the loricarid siluriform *Farlowella amazonum* imported into Britian; *Phanerothecium caballeroi* Kritsky and Thatcher, 1977, from the gills of the pimelodid siluriform *Cephalosilurus zungaro* in 2 locations in Colombia; and *Paragyrodactyloides superbus* (Szidat, 1973) from the callichthyid siluriform *Corydoras paleatus* held captive in the Museum of Natural History in Buenos Aires. Thus, information to date suggests that neotropical representatives of this particular parasite lineage have successfully radiated among a variety of siluriform fishes.

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Neotropical Monogenea. 15. Dactylogyrids from the Gills of Brazilian Cichlidae with Proposal of *Sciadicleithrum* gen. n. (Dactylogyridae)

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ABSTRACT: *Sciadicleithrum* gen. n. is proposed for dactylogyrids possessing overlapping gonads, a coiled cirrus with clockwise rings, unmodified anchors, a ventral bar with 2 umbelliform membranes or cavities on the anterior bar margin, and similar hooks with undilated shanks and erect thumbs. The following species are described from neotropical cichlids: *Gussevia asota, G. astronoti,* and *G. rogersi* spp. n. from *Astronotus ocellatus* (Cuvier); *Sciadicleithrum uncinatum* (type), *S. umbilicum,* and *S. ergensi* spp. n. from *Cichla ocellaris* Bloch and Schneider; *S. tortrix* sp. n. from *Uaru amphiacanthoides* Heckel; *S. iphthimum* sp. n. from *Pterophyllum scalare* (Lichtenstein); and *S. geophagi* sp. n. from *Geophagus surinamensis* (Bloch). *Urocleidoides variabilis* Mizelle and Kritsky, 1969, *Urocleidus aequidens* Price and Schlueter, 1967, and *Urocleidus cavanaughi* Price, 1966, are transferred to *Sciadicleithrum. Ancyrocephalus kostomarovi* Lucky, 1973, is considered a junior subjective synonym of *S. variabilum* comb. n.

KEY WORDS: Monogenea, taxonomy, morphology, systematics, *Gussevia asota* sp. n., *Gussevia astronoti* sp. n., *Gussevia rogersi* sp. n., *Sciadicleithrum uncinatum* sp. n., *Sciadicleithrum tortrix* sp. n., *Sciadicleithrum geophagi* sp. n., *Sciadicleithrum iphthimum* sp. n., *Sciadicleithrum umbilicum* sp. n., *Sciadicleithrum ergensi* sp. n., *Sciadicleithrum variabilum* comb. n., *Sciadicleithrum aequidens* comb. n., *Sciadicleithrum cavanaughi* comb. n., *Ancyrocephalus kostomarovi, Sciadicleithrum* gen. n., *Astronotus ocellatus, Cichla ocellaris, Uaru amphiacanthoides, Pterophyllum scalare, Geophagus surinamensis,* Cichlidae.

Prior to the revision of *Urocleidoides* by Kritsky et al. (1986), the genus as emended by Mizelle et al. (1968) contained 30 neotropical species from fishes representing 4 teleost orders. Kritsky et al. (1986) considered the taxon polyphyletic and proposed limiting the genus to monogeneans possessing a sinistral vaginal sclerite, overlapping gonads, counterclockwise cirral rings, simple anchors, and hooks (pairs 1 and 5 usually reduced) with enlarged shanks. *Gussevia* Kohn and Paperna, 1964, was resurrected from synonymy with *Urocleidoides* for some dactylogyrids from neotropical cichlid hosts. This revision resulted in 22 (1 from a cichlid host) of the original *Urocleidoides* species without final generic placement (status incertae sedis). In the present paper, 9 new ancyrocephalines from cichlid hosts are described, *Sciadicleithrum* gen. n. is proposed, and *Urocleidoides variablis* Mizelle and Kritsky, 1969, *Urocleidus aequidens* Price and Schlueter, 1967, and *Urocleidus cavanaughi* Price, 1966, are transferred to *Sciadicleithrum.* As a result, only *Gussevia* Kohn and Paperna, 1964, *Trinidactylus* Hanek, Molnar, and Fernando, 1974, and *Sciadicleithrum* gen. n. currently include species infesting neotropical cichlids; however, the monotypic *Trinidactylus* from *Cichlasoma* of Trinidad may be a synonym of *Gussevia* (see Kritsky et al., 1986).

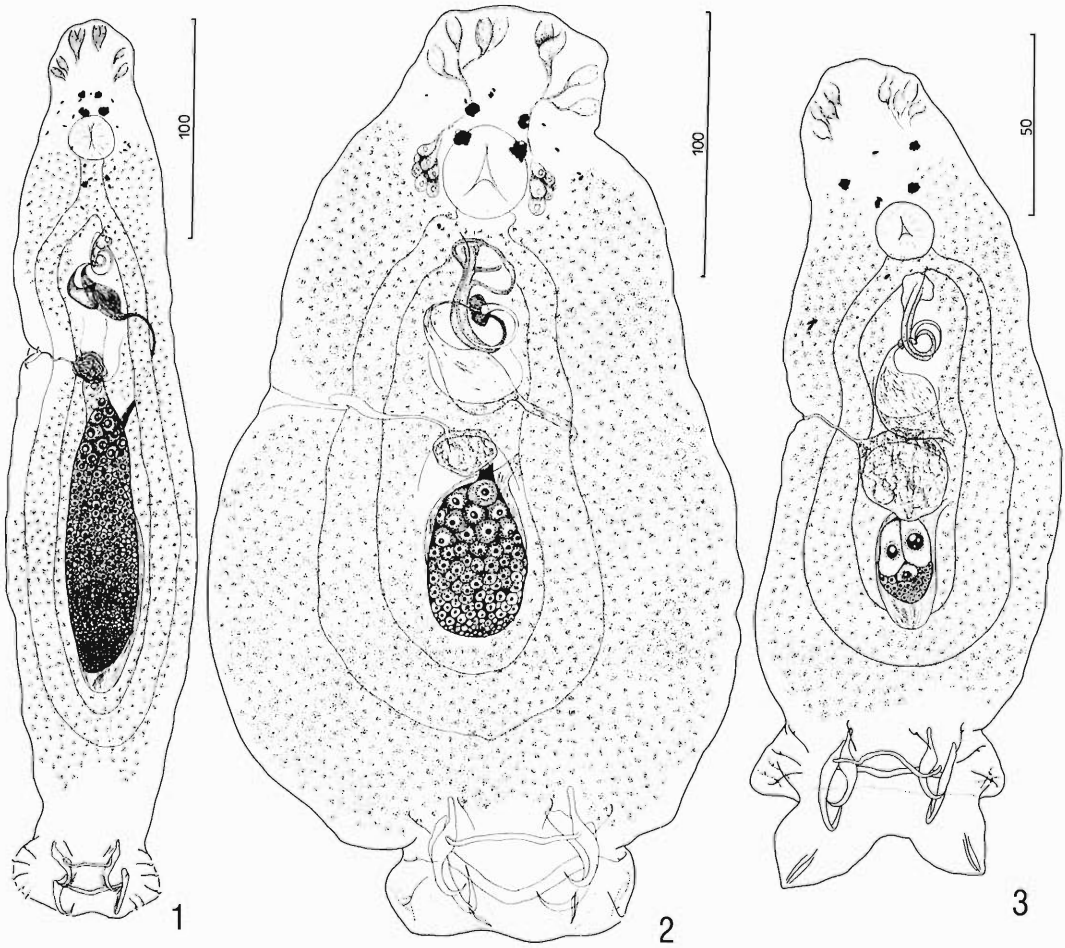
Materials and Methods

Fish were collected near Manaus, Amazonas, Brazil, during the period 1978–1984. Methods of collection, preparation, measurement, and illustration were those described by Kritsky et al. (1986). Measurements are in micrometers; the mean is followed by the range in parentheses. Type specimens are deposited in the helminthological collections of the Instituto Nacional de Pesquisas da Amazônia (INPA), the U.S. National Museum (USNM), and University of Nebraska State Museum (HWML) as indicated in the respective descriptions. In addition, the following type specimens were examined: *Urocleidoides variabilis* Mizelle and Kritsky, 1969 (USNM 71011, holotype), *Ancyrocephalus kostomarovi* Lucky, 1973 (USNM 78793, cotype), *Urocleidus aequidens* Price and Schlueter, 1967 (USNM 60894, holotype), and *Urocleidus cavanaughi* Price, 1966 (USNM 61204, holotype).

Gussevia asota sp. n. (Figs. 1, 4–12)

HOST: Acara, *Astronotus ocellatus* (Cuvier), Cichlidae.

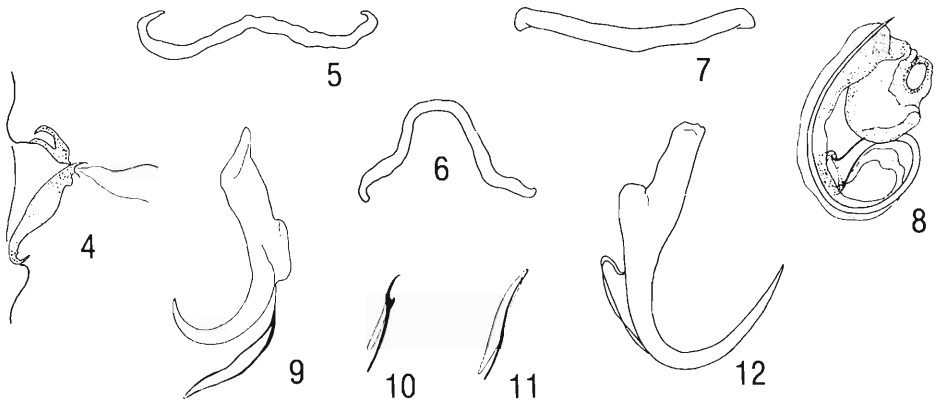
TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (8 August 1978); also collected from the same host held in an aquarium, Pocatello, Idaho (June 1977).



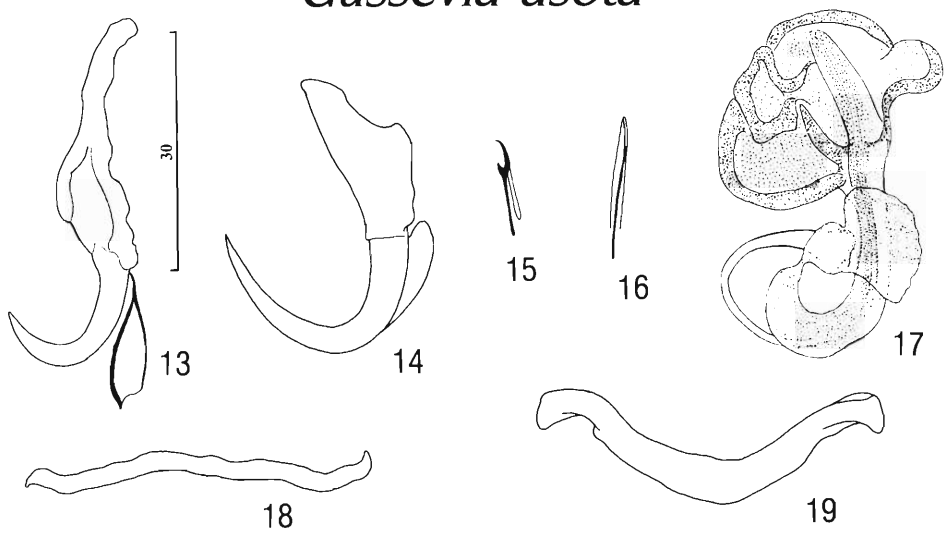
Figures 1–3. Whole mount illustrations of *Gussevia* spp. (holotypes, ventral). 1. *Gussevia asota*. 2. *Gussevia astronoti*. 3. *Gussevia rogersi*.

TYPE SPECIMENS: Holotype, INPA PA 316-1; paratypes, INPA PA 316-2 to 316-10, USNM 80401, 80402, HWML 20726.

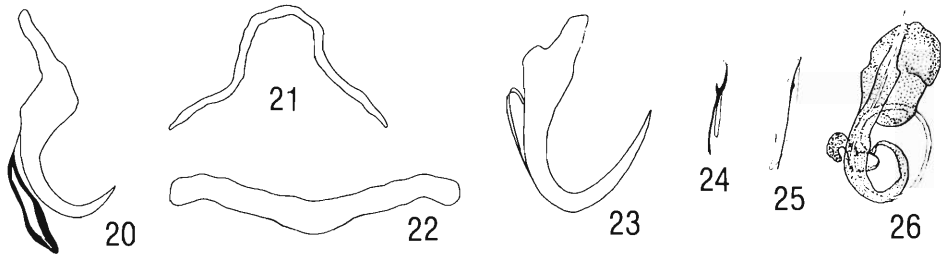
DESCRIPTION (based on 94 specimens, 20 measured): With characters of the genus as emended by Kritsky et al. (1986). Body 394 (380–462) long, fusiform, with narrow cephalic region; greatest width 88 (73–111) at level of gonads. Cephalic lobes poorly developed, usually 2 terminal, 2 bilateral. Four eyes, equidistant; posterior pair larger; accessory granules absent or scattered throughout anterior trunk, cephalic region; granules small, elongate ovate. Mouth ventral to pharynx; pharynx ovate, greatest pharyngeal diameter 25 (21–30); esophagus long. Peduncle moderately long, broad; haptor 42 (34–54) long, 66 (61 –78) wide, subhexagonal to subcircular in outline, with poorly developed posteroventral lobe. Ventral anchor 26 (25–28) long, with subrectangular base, short shaft, acute point, short anterior projection arising from tip of superficial root; roots poorly differentiated; base 14 (12–16) wide. Dorsal anchor 27 (24–30) long, with superficial root variable in length, short deep root, curved shaft, elongate point; base 12 (10–13) wide. Ventral bar 31 (24–35) long, filamentous, with ends curling around ventral anchor base; dorsal bar 31 (23–36) long, rod-shaped with slightly expanded ends. Hook pairs 1, 2, 3, 4, 6, 7 are 11 (10–12) long, with delicate point, erect thumb, slender shank; hook pair 5 is 14 (13–15) long, splinter-like. FH loop 0.6 –1.0 shank length. Gonads ovate; testis 90 (71–108) × 40 (33–51), ovary 109 (85–132) × 45 (36–57). Seminal ves-



Gussevia asota



Gussevia astronoti



Gussevia rogersi

Figures 4–26. Sclerotized parts of *Gussevia* spp. 4–12. *Gussevia asota.* 4. Vagina. 5, 6. Ventral bars. 7. Dorsal bar. 8. Copulatory complex. 9. Ventral anchor. 10. Hook. 11. Hook (pair 5). 12. Dorsal anchor. 13–19. *Gussevia astronoti.* 13. Ventral anchor. 14. Dorsal anchor. 15. Hook. 16. Hook (pair 5). 17. Copulatory complex. 18. Ventral bar. 19. Dorsal bar. 20–26. *Gussevia rogersi.* 20. Ventral anchor. 21. Ventral bar. 22. Dorsal bar. 23. Dorsal anchor. 24. Hook. 25. Hook (pair 5). 26. Copulatory complex. All figures are drawn to the 30-μm scale.

icle sigmoid; cirrus a coil of about 1½ rings, cirrus 61–62 long, proximal ring diameter 14 (10–16). Accessory piece 27 (23–29) long, basally articulating with cirrus, with terminal flabellate piece possessing sclerotized ring. Vagina dextral, armed with external sclerotized shield, opening in anterior half of trunk; seminal receptacle immediately anterior to ovary, overlying ootype; vitellaria dense, coextensive with gut.

REMARKS: Based on morphology of the copulatory complex and haptoral armament, species of *Gussevia* parasitizing *Astronotus ocellatus* are more closely related to each other than to any other described species in the genus. Features distinguishing *G. asota* from *G. astronoti* and *G. rogersi* include a less robust body, an external sclerotized vaginal shield, and a distinct ring on the terminal portion of the accessory piece. *Gussevia asota* apparently was a contributing cause of death of the specimen of *A. ocellatus* held in an aquarium in Pocatello. After death of the fish, several hundred specimens of this helminth were collected from its gills. Hosts collected from Janauaca Lake in Brazil supported low infestation levels of this species (<10 worms/fish). The specific name is from Greek (*asotas* = destruction).

Gussevia astronoti sp. n. (Figs. 2, 13–19)

HOST: Acara, *Astronotus ocellatus* (Cuvier), Cichlidae.

TYPE LOCALITY: Janauacá Lake near Manaus, Amazonas, Brazil (8 August 1978).

TYPE SPECIMENS: Holotype, INPA PA 317-1; paratypes, USNM 80400, HWML 20725.

DESCRIPTION (based on 4 specimens): With characters of the genus as emended by Kritsky et al. (1986). Body 347 (318–373) long, broad, robust, foliform; greatest width 178 (131–226) in posterior trunk. Cephalic margin rounded or possessing 2 terminal, 2 bilateral cephalic lobes poorly developed. Four eyes, equidistant; members of posterior pair with lens, larger than those of anterior pair; accessory granules scattered in cephalic, anterior trunk region; granules elongate ovate to fusiform. Mouth ventral to pharynx; pharynx 28–29 in diameter, subspherical; esophagus short; intestinal crura dilated. Haptor 59 (52–65) long, 103 (91–111) wide, with ventroposterior lobe containing ventral anchors, hook pair 5. Ventral anchor 37 (36–39) long, with evenly curved shaft and point, enlarged base with elongate projection arising from proximal end; base width 11 (10–12); filament well developed. Dorsal anchor 35 (33–37) long, with well-developed superficial root, short deep root, elongate point, short shaft; base 13 (12–15) wide. Ventral bar 41 (38–45) long, filamentous; dorsal bar 44 (43–45) long, broadly U- or V-shaped, with subterminal narrowing at each end. Hook pairs 1, 2, 3, 4, 6, 7 are 13 (12–15) long, with erect thumb, slender shank; hook pair 5 is 16–17 long, filamentous; FH loop approximately 0.8 shank length. Gonads ovate; testis 42–43 × 38–39, ovary 47–48 × 43–44. Seminal vesicle pyriform; cirrus 76–77 long, a coil of about 1¼ rings, proximal ring diameter 16 (14–18). Accessory piece 41 (40–42) long, articulated to cirral base, with terminal flap showing several sclerotized ridges. Vagina dextral, lightly sclerotized, with sigmoid bend near midlength as it traverses intestine. Seminal receptacle lying immediately anterior to ovary, variable; vitellaria dense, distributed throughout trunk except absent in region of gonads and copulatory complex.

REMARKS: *Gussevia astronoti* resembles *G. rogersi,* from which it differs by being noticeably larger in dimensions of the body, haptoral and copulatory sclerites. Our worms are contracted; apparently body length would increase significantly in relaxed specimens. The species is named for its host.

Gussevia rogersi sp. n. (Figs. 3, 20–26)

HOST: Acara, *Astronotus ocellatus* (Cuvier), Cichlidae.

TYPE LOCALITY: Rio Solimões near Marchantaria Island, Manaus, Amazonas, Brazil (November 1983).

TYPE SPECIMENS: Holotype, INPA PA 318-1; paratypes, USNM 80399, HWML 20724.

DESCRIPTION (based on 8 specimens): With characters of the genus as emended by Kritsky et al. (1986). Body 220 (218–222) long, broad, flattened dorsoventrally, tapering anteriorly; greatest width 80 (78–82) in posterior trunk. Cephalic lobes poorly developed, 2 bilateral, 2 terminal. Four eyes, subequal, members of anterior pair usually farther apart than those of posterior pair; granules elongate ovate; accessory granules sparse in anterior trunk, cephalic region. Mouth ventral to pharynx; pharynx spherical, 15–16 in diameter; esophagus short to nonexistent. Peduncle broad; haptor 53 (50–57) long, 75 (74–77) wide, with well-developed posteroventral

lobe. Ventral anchor 26 (24–29) long, lacking roots, with elongate anterior basal projection, evenly curved shaft, acute point; base 7 (6–9) wide. Dorsal anchor 24 (21–27) long, with elongate point, well-developed superficial root variable in length, small deep root; base 10 (8–12) wide. Ventral bar 33 (26–46) long, filamentous; dorsal bar 36 (33–40) long, broadly V-shaped. Hook pairs 1, 2, 3, 4, 6, 7 are 11–12 long, with delicate point, erect thumb, slender shank; pair 5 is 13–14 long, delicate; FH loop 0.8–1.0 shank length. Gonads ovate; testis 24–25 × 18–19, ovary 20–21 × 13–14. Seminal vesicle fusiform or pyriform; cirrus a coil of about 1½ rings, cirral length 58–59, ring diameter 11 (10–13). Accessory piece 22 (20–24) long, basally articulated to cirrus, distal portion flabellate. Vagina dextral, comprising a simple unsclerotized tube opening near midlength; seminal receptacle large, lying ventral to ootype. Vitellaria dense, distributed throughout trunk except absent in region of reproductive organs.

REMARKS: *Gussevia rogersi* most closely resembles *G. astronoti*. Features distinguishing *G. rogersi* include its smaller size, the comparatively short basal projection of the ventral anchor, the well-developed haptoral lobe, the elongate filamentous ventral bar, and delicate vaginal tube. *Gussevia rogersi* is named for Dr. Wilmer A. Rogers, Auburn University, Auburn, Alabama, in recognition of his work on Monogenea.

Sciadicleithrum gen. n.

DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Four pairs of head organs; 2 terminal, 2 bilateral cephalic lobes; unicellular cephalic glands comprising 2 groups posterolateral to pharynx. Eyes present. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to gonads, lacking diverticula. Gonads overlapping, intercecal; testis dorsal to ovary. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens; 1 or 2 prostatic reservoirs. Cirrus comprising a base from which a coiled tube arises, tube with less than 1 to several clockwise rings (see Kritsky et al., 1985); accessory piece present. Genital pore midventral at level of intestinal bifurcation. Oviduct short; uterus delicate, extending anteriorly along midline; seminal receptacle ventral to anterior end of ovary; vagina sinistral, dextral, or ventral. Vitellaria well developed, scattered throughout trunk. Haptor armed with dorsal and ventral pairs of unmodified anchors, ventral and dorsal bars, 7 pairs of similar hooks with ancyrocephaline distribution. Ventral bar with 2 variably developed umbelliform membranes or cavities on anterior bar margin; hook shanks slender, thumb erect. Parasites of gills of neotropical Cichlidae.

TYPE SPECIES AND HOST: *Sciadicleithrum uncinatum* sp. n. from *Cichla ocellaris* Bloch and Schneider, Cichlidae.

OTHER SPECIES: *Sciadicleithrum tortrix* sp. n. from *Uaru amphiacanthoides* Heckel; *S. geophagi* sp. n. from *Geophagus surinamensis* (Bloch); *S. iphthimum* sp. n. from *Pterophyllum scalare* (Lichtenstein); *S. umbilicum* sp. n. from *Cichla ocellaris* Bloch and Schneider; *S. ergensi* sp. n. from *Cichla ocellaris* Bloch and Schneider; *S. aequidens* (Price and Schlueter, 1967) comb. n. from *Aequidens maroni* (Steindachner); *S. cavanaughi* (Price, 1966) comb. n. from *Aequidens maroni* (Steindachner); and *S. variabilum* (Mizelle and Kritsky, 1969) comb. n. from *Symphysodon discus* Heckel.

REMARKS: Species of *Sciadicleithrum* have several features that suggest relationship with *Gussevia* Kohn and Paperna, 1964 (as emended by Kritsky et al., 1986). Among these characters are delicate hooks with slender shanks and upright thumbs, a coiled cirrus with clockwise rings, vaginae occurring either ventrally or laterally near the trunk midlength, general morphology of the dorsal bar, and overlapping gonads. These genera are differentiated by *Sciadicleithrum* species having 1) an unmodified hook pair 5 (usually with reduced thumb and base in *Gussevia*), 2) a ventral bar with anterior umbelliform membranes or cavities (absent in *Gussevia*), 3) normal anchors (ventral anchors modified in *Gussevia*), 4) normally developed ventral anchor filaments (well-developed filaments in *Gussevia*), and 5) a haptor lacking a posteroventral lobe (present in *Guessvia*). The generic name is from Greek (*skiados* = a canopy or umbel + *kleithron* = bar).

Sciadicleithrum uncinatum sp. n. (Figs. 27, 33–38)

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

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (13 May 1980; 27 June 1983; December 1983).

TYPE SPECIMENS: Holotype, INPA PA 321-1; paratypes, INPA PA 321-2, USNM 80396, HWML 20730.

DESCRIPTION (based on 11 specimens): Body fusiform, 478 (406–541) long; greatest width 77 (70–84) in posterior half. Cephalic lobes poorly developed. Eyes 4, equidistant; members of posterior pair with lens, larger than anterior pair; eye granules small, generally elongate ovate; accessory granules sparse in cephalic, anterior trunk regions. Pharynx spherical, 26 (22–29) in diameter; esophagus moderately long. Peduncle broad; haptor subhexagonal, 85 (79–97) wide, 54 (46–63) long. Ventral anchor 29 (26–31) long, with exaggerated roots, short shaft, rapidly tapering point; base width 20 (18–23). Dorsal anchor 30 (26–33) long, with well-developed roots, evenly curved shaft and point; base width 16 (14–17). Ventral bar 32 (29–36) long, with well-developed anterior cavities, enlarged ends; dorsal bar 41 (37–46) long, broadly U-shaped, with enlarged terminations. Hooks 12–13 long; each with delicate point, upright thumb, robust shank; FH loop 0.8 shank length. Cirrus a coil of about 1½ rings; cirral length 84 (83–85), ring diameter 22 (19–26). Accessory piece 24 (23–25) long, a variable sheath surrounding distal ½ ring of cirrus, articulating to cirral base. Gonads fusiform; testis 49 (41–50) × 17 (16–18), ovary 55 (37–75) × 19 (16–22). Vas deferens conspicuous; seminal vesicle small; prostatic reservoir large, spherical. Oviduct, ootype, uterus not observed; vagina opening dextrally, comprising a distal funnel, proximal delicate tube uniting with irregular seminal receptacle; vitellaria absent in region of reproductive organs.

REMARKS: *Sciadicleithrum uncinatum* is the type species for the genus. Its specific name is derived from Latin (*uncinatus* = barbed).

Sciadicleithrum tortrix sp. n. (Figs. 28, 39–44)

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

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

TYPE SPECIMENS: Holotype, INPA PA 320-1; paratypes, INPA PA 320-2, USNM 80397, HWML 20722.

DESCRIPTION (based on 10 specimens): Body fusiform, 360 (311–398) long; greatest width 57 (51–64) at various points along trunk. Cephalic lobes well developed. Eyes 2, lying anterior to pharynx; eye granules large, elongate ovate; accessory granules occurring posteriorly to level of testis. Pharynx spherical, 15 (14–17) in diameter; esophagus moderately long. Peduncle broad; haptor subhexagonal, 96 (78–111) wide, 60 (50–71) long. Ventral anchor 31 (29–32) long, lacking roots, with small base, bent shaft, short point; base width 15 (14–16). Dorsal anchor 32 (29–33) long, with elongate superficial root, short deep root, bent shaft, short point; base width 14–15. Ventral bar 50 (37–57) long, robust, with large anterior membranes, slightly enlarged terminations; dorsal bar 51 (41–57) long, broadly U-shaped, ends enlarged. Hooks 14 (13–15) long, similar; each with truncate erect thumb; uniform shank; FH loop 0.8 shank length. Cirrus a U-shaped coil of about 3 rings; cirral length 64–65, ring diameter 8 (6–10). Accessory piece 32 (30–36) long, articulating to cirral base, enclosing distal 2 rings of cirrus. Testis pyriform, 43 (34–55) × 16 (14–19); seminal vesicle fusiform; prostatic reservoir reduced, not greatly differentiated from duct originating from prostate. Ovary irregular, 40 (35–45) × 12 (10–14); oviduct elongate; ootype not observed; uterus delicate; vagina opening on dextral margin, comprising an expanded coiled tube originating from small seminal receptacle; vitellaria coextensive with gut.

REMARKS: Based on the comparative morphology of the haptoral armament and copulatory complex, *S. tortrix* most closely resembles *S. iphthimum.* These species are differentiated by the positions of the vaginal aperture (dextral in *S. tortrix,* ventral in *S. iphthimum*), shape of the ventral bar (straight in *S. tortrix,* broadly U-shaped in *S. iphthimum*), and diameter of the cirral shaft (larger in *S. tortrix*). The specific name is from Latin (*tortrix* = twisted) and refers to the shape of the cirrus.

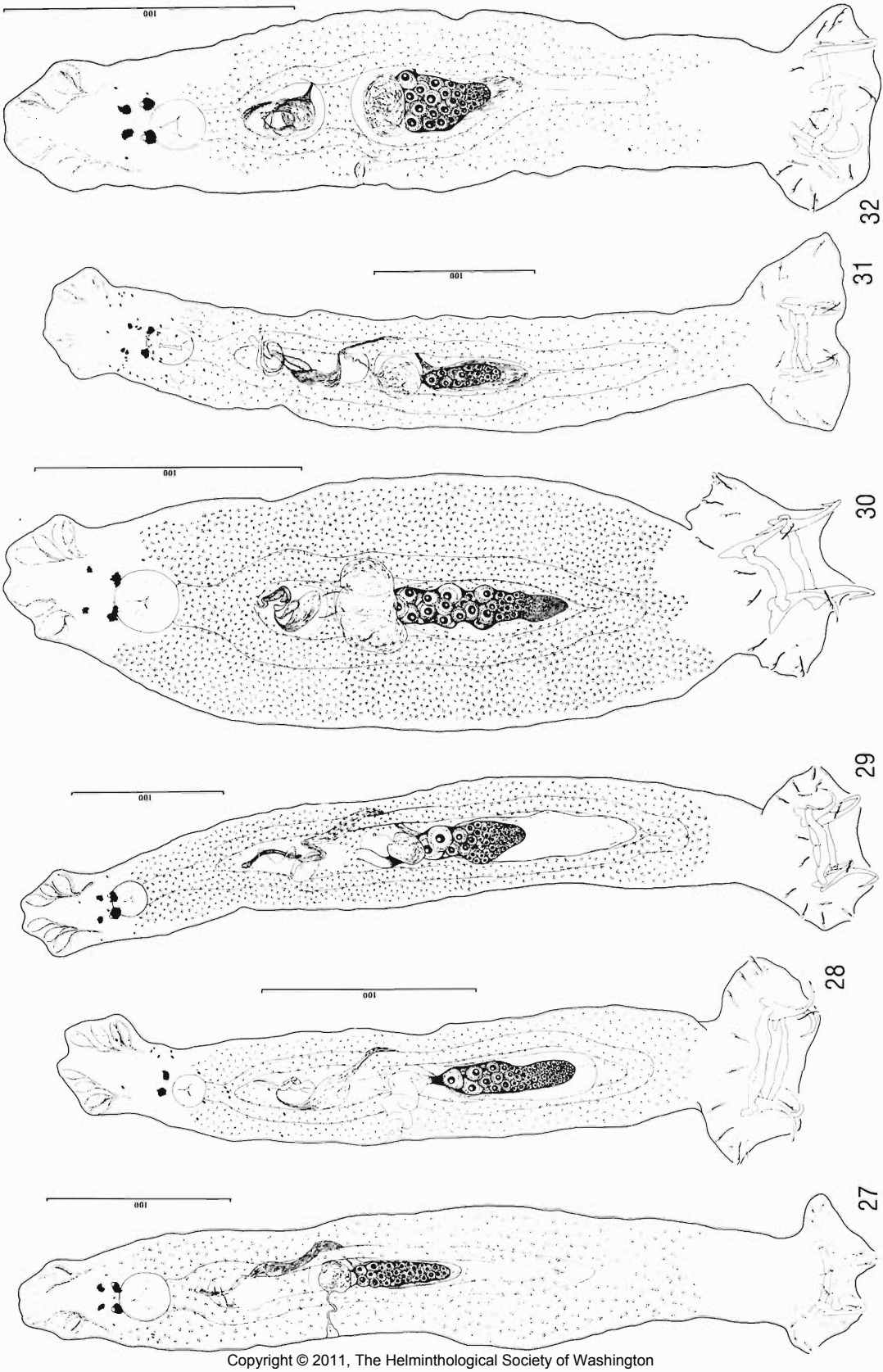
Sciadicleithrum umbilicum sp. n. (Figs. 29, 45–53)

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

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (13 May 1980; 27 June 1983; December 1983).

TYPE SPECIMENS: Holotype, INPA PA 319-1; paratypes, INPA PA 319-2, 319-3, USNM 80398, HWML 20723.

DESCRIPTION (based on 18 specimens): Body 515 (419–586) long, fusiform, slender; greatest width 70 (57–82) at various points along trunk.



Cephalic lobes moderately to well developed. Eyes 4, members of posterior pair with lens, larger, closer together than members of anterior pair; eye granules small, usually elongate ovate; accessory granules sparse in cephalic area. Pharynx spherical, 19 (16–24) in diameter; esophagus elongate. Peduncle broad; haptor subhexagonal, 88 (72–109) wide, 77 (57–89) long. Ventral anchor 40 (30–49) long, with large base, well-developed roots, curved shaft, short point; base width 23 (19–26). Dorsal anchor 45 (34–54) long, with elongate superficial root, poorly developed deep root, evenly curved shaft, point; base width 23 (21–25). Ventral bar 51 (40–62) long, robust, with umbelliform cavities; dorsal bar 45 (33–54) long, with enlarged ends, posteriorly saddle-shaped. Hooks 15 (13–17) long; each with erect thumb, relatively robust point, shank; FH loop ¾ shank length. Cirrus a coil of about 2 rings, base variable; accessory piece rodlike with terminal ring-shaped aperture; cirral length 69–70, ring diameter 19 (13–28); accessory piece 45 (40–47) long. Testis elongate, fusiform, 95 (77–111) × 30 (23–42); vas deferens conspicuous; seminal vesicle an inconspicuous dilation of vas deferens; prostatic reservoir subellipsoidal. Ovary extending posteriorly to about midlength of testis, irregular in outline, 58 (48–69) × 22 (18–26); oviduct elongate; ootype, uterus not observed; vagina opening ventrally, composed of short delicate sclerotized tube; seminal receptacle variable; vitellaria dense, coextensive with gut.

REMARKS: Although the shape of the haptoral sclerites and copulatory complex of *S. umbilicum* is relatively constant, size of these structures varied considerably among specimens, more so than generally occurs among individual species of Dactylogyridae. The variants are considered conspecific since specimens with sclerites of intermediate sizes were found in our collection.

The haptoral armament of *S. umbilicum* resembles those of *S. iphthimum* and *S. tortrix*. *S. umbilicum* is the only speices of the genus with a rod-shaped accessory piece provided with a terminal ring. The specific name is from Latin (*umbilicus* = the navel) and refers to the position and nature of the vaginal aperture.

Sciadicleithrum iphthimum sp. n. (Figs. 30, 54–59)

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

TYPE LOCALITY: Rio Solimões near Marchantaria Island, Manaus, Amazonas, Brazil (1 November 1984).

TYPE SPECIMENS: Holotype, INPA PA 324-1; paratypes, INPA PA 324-2 to 324-9, USNM 80394, HWML 20727.

DESCRIPTION (based on 19 specimens): Body 329 (270–364) long, foliform, robust; greatest width 92 (71–104) near midlength. Cephalic margin narrow; cephalic lobes moderately developed. Eyes 4; members of posterior pair larger, closer together than members of anterior pair; eye granules elongate ovate; accessory granules uncommon in cephalic, anterior trunk regions. Pharynx spherical, 24 (20–27) in diameter; esophagus moderately long. Peduncle broad; haptor subhexagonal, 79 (57–88) wide, 59 (52–65) long. Ventral anchor 32 (27–35) long, with poorly differentiated roots, evenly curved shaft and point; base width 19 (16–21). Dorsal anchor 35 (33–37) long, with elongate superficial root, short deep root, curved shaft, point; base width 17 (15–19). Ventral bar 29 (27–32) long, yoke-shaped, with delicate umbelliform membranes; dorsal bar 31 (28–33) long, straight, with enlarged ends. Hook 12 (11–14) long, with upright thumb, delicate point, shank; FH loop ¾ shank length. Cirrus a loose coil of about 1½ poorly defined rings, frequently appearing U-shaped, cirral base with sclerotized margin; cirral length 24 (23–25). Accessory piece comprising variable sheath enclosing cirral shaft. Margins of testis obscured by vitellaria; vas deferens not observed; seminal vesicle, prostatic reservoir pyriform. Ovary with irregular margin, elongate, 55 (42–64) × 17 (12–24); oviduct elongate; ootype not observed; uterus delicate; vagina ventral, comprising a short delicate tube opening into large irregular seminal receptacle; vitellaria dense throughout trunk, except absent in regions of reproductive organs.

REMARKS: *Sciadicleithrum iphthimum* re-

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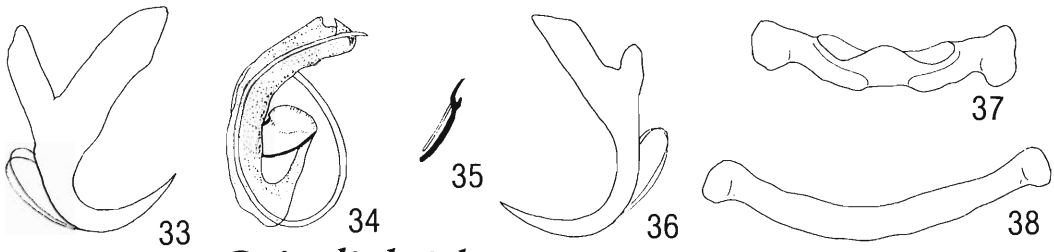
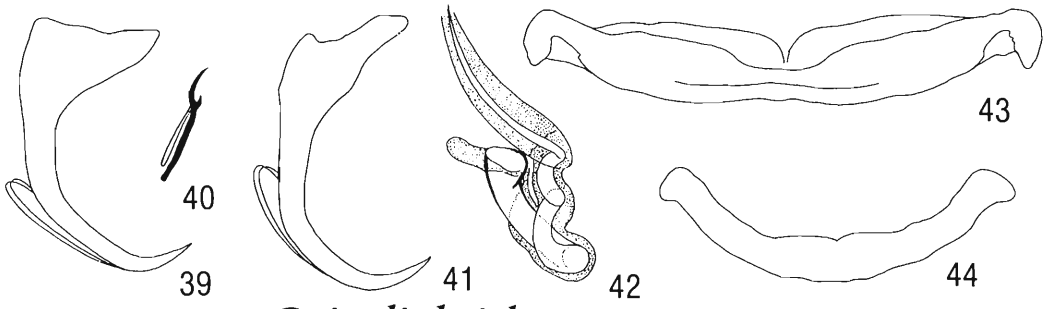
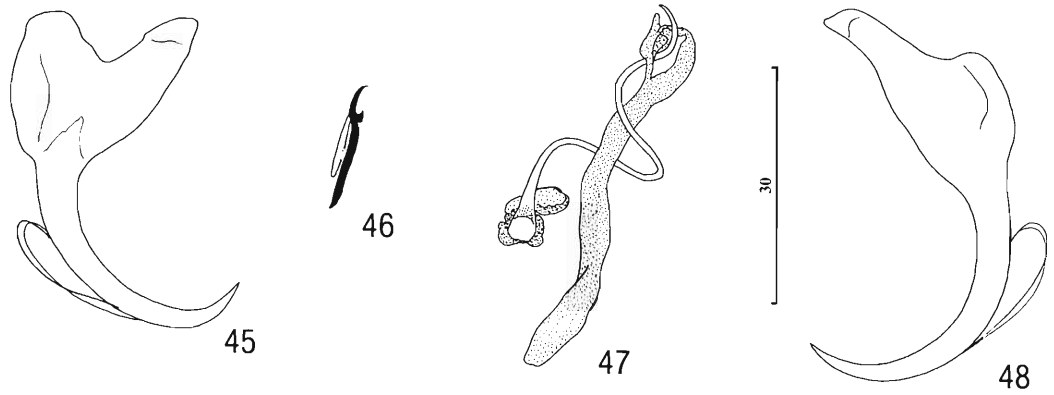
Figures 27–32. Whole mount illustrations of *Sciadicleithrum* spp. (holotypes, ventral). 27. *Sciadicleithrum uncinatum*. 28. *Sciadicleithrum tortrix*. 29. *Sciadicleithrum umbilicum*. 30. *Sciadicleithrum iphthimum*. 31. *Sciadicleithrum geophagi*. 32. *Sciadicleithrum ergensi*.

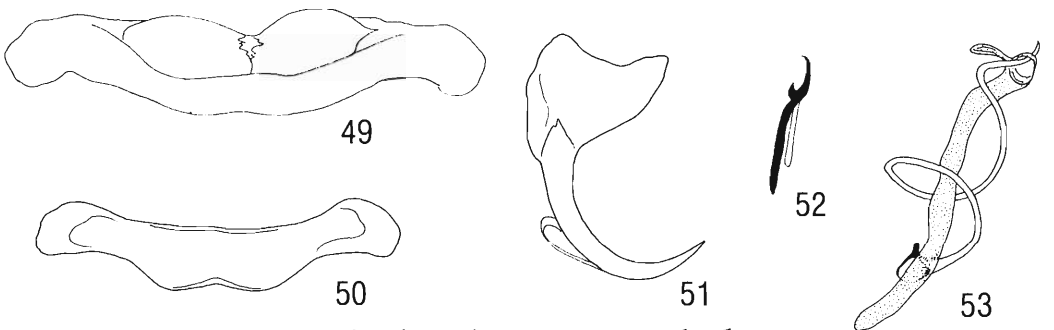


Sciadicleithrum uncinatum



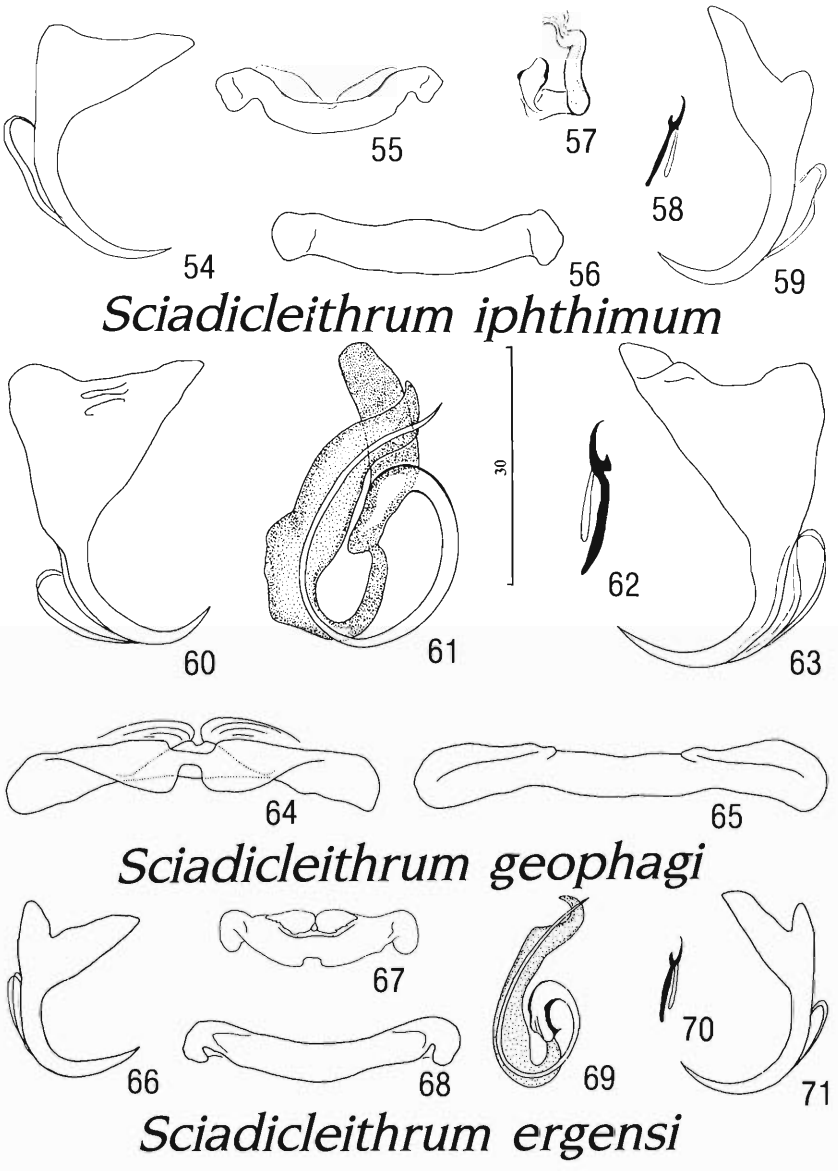
Sciadicleithrum tortrix





Sciadicleithrum umbilicum

Figures 33–53. Sclerotized parts of *Sciadicleithrum* spp. 33–38. *Sciadicleithrum uncinatum*. 33. Ventral anchor. 34. Copulatory complex. 35. Hook. 36. Dorsal anchor. 37. Ventral bar. 38. Dorsal bar. 39–44. *Sciadicleithrum tortrix*. 39. Ventral anchor. 40. Hook. 41. Dorsal anchor. 42. Copulatory complex. 43. Ventral bar. 44. Dorsal bar. 45–50. *Sciadicleithrum umbilicum* (large form). 45. Ventral anchor. 46. Hook. 47. Copulatory complex. 48. Dorsal anchor. 49. Ventral bar. 50. Dorsal bar. 51–53. *Sciadicleithrum umbilicum* (small form). 51. Ventral anchor. 52. Hook. 53. Copulatory complex. All are drawn to the 30-μm scale.



Figures 54–71. Sclerotized parts of *Sciadicleithrum* spp. 54–59. *Sciadicleithrum iphthimum*. 54. Ventral anchor. 55. Ventral bar. 56. Dorsal bar. 57. Copulatory complex. 58. Hook. 59. Dorsal anchor. 60–65. *Sciadicleithrum geophagi*. 60. Ventral anchor. 61. Copulatory complex. 62. Hook. 63. Dorsal anchor. 64. Ventral bar. 65. Dorsal bar. 66–71. *Sciadicleithrum ergensi*. 66. Ventral anchor. 67. Ventral bar. 68. Dorsal bar. 69. Copulatory complex. 70. Hook. 71. Dorsal anchor. All drawings are to the 30-μm scale.

sembles *S. tortrix,* from which it differs in morphology of the haptoral bars and copulatory complex. The vagina opens dextrally and is expanded in *S. tortrix,* whereas in *S. iphthimum* it is a slender tube opening on the ventral surface. The specific name is from greek (*iphthimos* = strong) and reflects the robust body shape.

Sciadicleithrum geophagi sp. n. (Figs. 31, 60–65)

HOST: Juquia, *Geophagus surinamensis* (Bloch), Cichlidae.

TYPE LOCALITY: Rio Negro near Manaus, Amazonas, Brazil (19 October 1984).

TYPE SPECIMENS: Holotype, INPA PA 323-1;

paratypes, INPA PA 323-2 to 323-5, USNM 80393, HWML 20728.

DESCRIPTION (based on 22 specimens): Body fusiform, 497 (448–526) long; greatest width 81 (68–87) near midlength. Cephalic lobes well developed. Eyes 4, equidistant; members of posterior pair larger than anterior pair; eye granules ovate, small; accessory granules numerous, scattered from cephalic area to level of gonads. Pharynx ovate, 27 (25–29) wide; esophagus moderately long. Peduncle broad; haptor subtrapezoidal, 121 (96–147) wide, 70 (61–78) long. Anchors similar; each with poorly developed roots, enlarged base, delicate evenly curved shaft and point; ventral anchor 38 (34–41) long, base 25 (21–28) wide; dorsal anchor 41 (38–44) long, base 25 (23–28) wide. Ventral bar 45 (42–48) long, rod-shaped with flap reflected posteriorly, possessing medial notch, umbelliform membranes delicate. Dorsal bar 48 (42–53) long, rod-shaped with anterolateral folds. Hook 15 (13–17) long, with delicate point, truncate and erect thumb, shank tapering proximally; FH loop ¾ shank length. Cirrus a coil of one ring, base with large anterior flap; cirral length 81–82, ring diameter 22 (18–25). Accessory piece 32 (30–34) long, articulating to cirrus base, serving as cirral guide distally. Gonads fusiform; testis 54 (39–72) × 18 (12–24); ovary 58 (54–61) × 16 (14–17). Vas deferens delicate; seminal vesicle sigmoid; prostatic reservoir elongate, with thickened walls. Oviduct, ootype, uterus not observed; vagina ventral, comprising a delicate tube opening to large spherical seminal receptacle; vitellaria scattered throughout trunk except absent in regions of reproductive organs.

REMARKS: This species resembles *S. ergensi,* from which it is distinguished by possessing poorly developed anchor roots, hooks with truncate thumbs, and a ventral instead of dextral vaginal aperture. The species is named for its host.

Sciadicleithrum ergensi sp. n. (Figs. 32, 66–71)

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

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

TYPE SPECIMENS: Holotype, INPA PA 322-1; paratypes, USNM 80395, HWML 20729.

DESCRIPTION (based on 7 specimens): Body fusiform, 356 (296–436) long; greatest width 70 (63–81) near midlength. Cephalic lobes moderately developed. Eyespots 4; members of posterior pair larger, slightly farther apart than those of anterior pair, possessing conspicuous lens; eye granules ovate; accessory granules sparse but may occur posteriorly to termination of gut. Pharynx spherical, 19 (14–26) in diameter; esophagus moderately long. Peduncle broad; haptor subhexagonal, 77 (69–87) wide, 52 (44–59) long. Ventral anchor 23 (22–24) long, with elongate roots, short straight shaft, curved point; base 16 (15–17) wide. Dorsal anchor 30 (26–33) long, with elongate roots, evenly curved shaft and point; base 14 (12–16) wide. Ventral bar 29 (26–34) long, yoke-shaped, with conspicuous umbelliform cavities; dorsal bar 33 (30–36) long, rod-shaped, ends slightly enlarged. Hook 11 (10–12) long, with delicate point, upright thumb, shank varying in diameter along length; FH loop ¾ shank length. Cirrus a coil of about 1½ rings; cirral length 50–51, ring diameter 15 (13–18). Accessory piece 23 (19–26) long, articulating with cirral base, serving as guide distally, with terminal hook. Testis fusiform, 36 (34–38) × 12 (11–14); vas deferens not observed; seminal vesicle sigmoid; prostatic reservoirs pyriform. Ovary variable, 32 (24–40) × 17 (16–18); oviduct, ootype, uterus not observed; vagina opening on right margin, distally a sclerotized funnel, proximally a delicate tube uniting with ovate seminal receptacle. Vitellaria coextensive with gut.

REMARKS: *Sciadicleithrum ergensi* resembles *S. geophagi.* Differentiation of the 2 species is presented in the remarks for *S. geophagi.* This species is named for Dr. Radim Ergens, Czechoslovak Academy of Science, in recognition of the support he has provided for our studies on Monogenea.

Sciadicleithrum variabilum (Mizelle and Kritsky, 1969) comb. n. (Figs. 72–76)

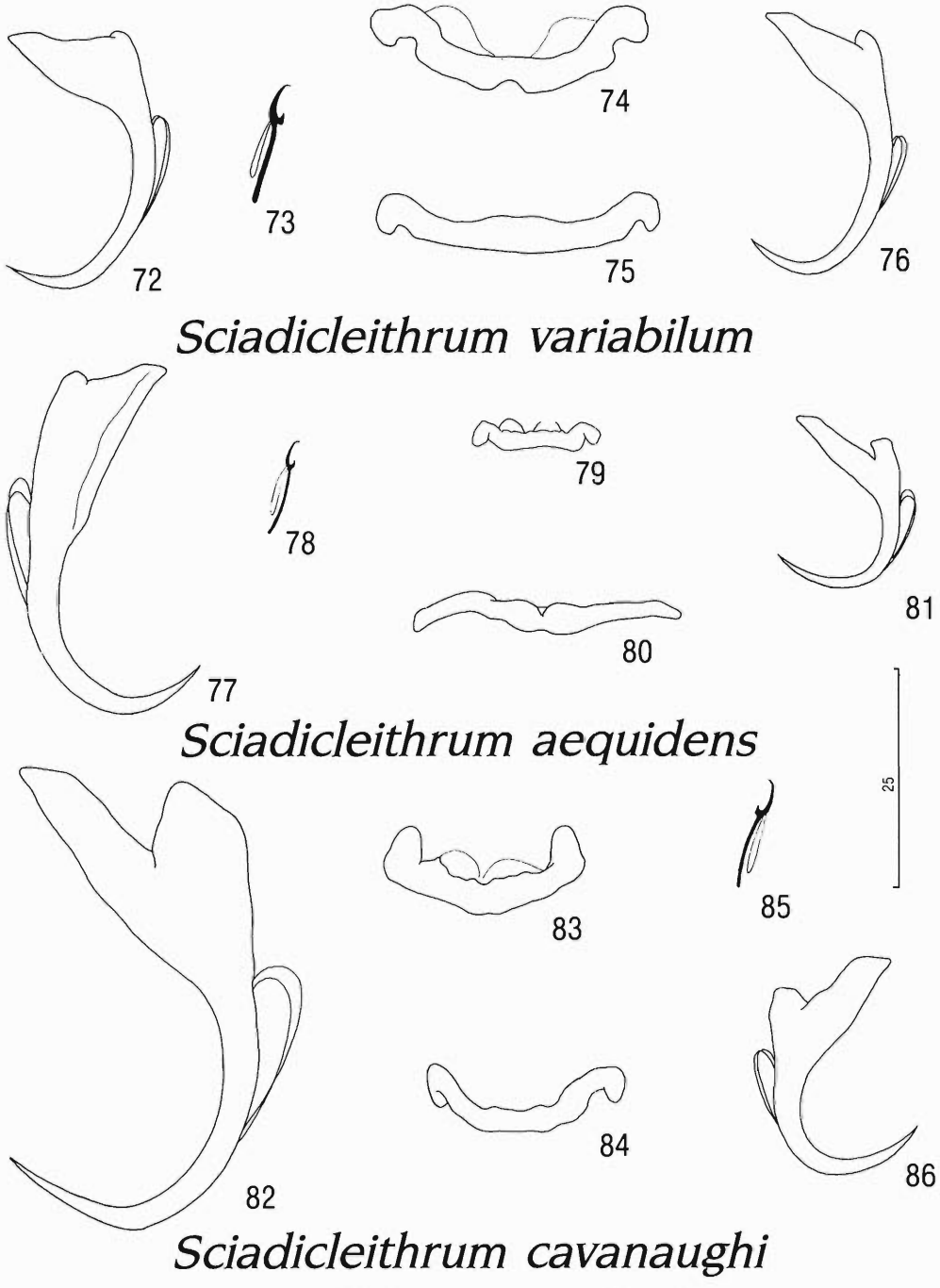
SYNONYMS: *Urocleidoides variabilis* Mizelle and Kritsky, 1969; *Ancyrocephalus kostomarovi* Lucky, 1973.

HOST: Cará disco, *Symphysodon discus* Heckel, Cichlidae.

TYPE LOCALITY: Amazon River Basin, Brazil.

SPECIMENS STUDIED: Holotype, USNM 71011. Cotype, *Ancyrocephalus kostomarovi* Lucky, 1973, USNM 78793.

REMARKS: This species, originally placed in *Urocleidoides* by Mizelle and Kritsky (1969),



Figures 72–86. Sclerotized parts of *Sciadicleithrum* spp. 72–76. *Sciadicleithrum variabilum.* 72. Ventral anchor. 73. Hook. 74. Ventral bar. 75. Dorsal bar. 76. Dorsal anchor. 77–81. *Sciadicleithrum aequidens.* 77. Ventral anchor. 78. Hook. 79. Ventral bar. 80. Dorsal bar. 81. Dorsal anchor. 82–86. *Sciadicleithrum cavanaughi.* 82. Ventral anchor. 83. Ventral bar. 84. Dorsal bar. 85. Hook. 86. Dorsal anchor. All figures are drawn to the same scale (25 μm).

possesses a ventral bar with bilateral umbelliform membranes, overlapping gonads, and a cirrus comprising a loose coil of clockwise rings. Therefore, we propose the transfer of this species to *Sciadicleithrum* as a new combination (*S. variabilum* comb. n.). Our study of the cotype of *Ancyrocephalus kostomarovi* Lucky, 1973, shows that it is conspecific with *S. variabilum* based on comparative morphology of the haptoral and copulatory sclerites. *Ancyrocephalus kostomarovi* is thus considered a junior subjective synonym of *S. variabilum* comb. n.

Sciadicleithrum aequidens (Price and Schlueter, 1967) comb. n. (Figs. 77–81)

SYNONYM: *Urocleidus aequidens* Price and Schlueter, 1967.

HOST: *Aequidens maroni* (Steindachner), Cichlidae.

TYPE LOCALITY: British Guiana (host from ichthyology collection of Mississippi College, Clinton, Mississippi).

SPECIMEN STUDIED: Holotype, USNM 60894.

REMARKS: Our examination of the holotype of *Urocleidus aequidens* reveals that this species is a member of *Sciadicleithrum* based on morphology of the haptoral sclerites. Although not depicted by Price and Schlueter (1967), the ventral bar has delicate anterior membranes, and the morphology of the hook shank and thumb is consistent with that of other members of *Sciadicleithrum*. Therefore, transfer of the species to *Sciadicleithrum* is proposed. The holotype is unstained, which precluded determination of internal anatomy and confirmation of the morphology of the copulatory complex.

Sciadicleithrum cavanaughi (Price, 1966) comb. n. (Figs. 82–86)

SYNONYM: *Urocleidus cavanaughi* Price, 1966.

HOST: *Aequidens maroni* (Steindachner), Cichlidae.

TYPE LOCALITY: British Guiana (host from ichthyology collection of Mississippi College, Clinton, Mississippi).

SPECIMEN STUDIED: Holotype, USNM 61204.

REMARKS: *Urocleidus cavanaughi* Price, 1966, is transferred to *Sciadicleithrum* based on morphology of the haptoral sclerites. While the haptoral bars of the holotype are deformed as a result of coverslip pressure, the indistinct membranes of the ventral bar and basic morphology of the dorsal bar clearly suggest affinity of this species with others included in *Sciadicleithrum*. The morphology of the hook (upright thumb, nondilated shank) also supports the transfer. We were not able to confirm distribution of the gonads, which Price (1966) states are tandem. The cirrus comprises a coil of about 2 clockwise rings; the accessory piece is not totally visible in the unstained and cleared specimen but is articulated to the cirral base.

Acknowledgments

The authors are grateful to Dr. J. Ralph Lichtenfels, USNM, for providing type specimens of *Ancyrocephalus kostomarovi, Urocleidoides variabilis, Urocleidus aequidens,* and *Urocleidus cavanaughi*. The Max Planck Institute, Plön, Germany, kindly provided technical support for collection of hosts.

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Proc. Helminthol. Soc. Wash.
56(2), 1989, pp. 141–144

A Vaginal Ganglion in Female *Echinopardalis atrata* (Acanthocephala)

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ABSTRACT: The vaginal ganglion consists of 4 cells. Two of these cells (central pair) are located on the dorsal surface of the outer syncytial muscle of the vagina near the posterior terminus of the inner syncytial muscle. Each of the remaining 2 cells is located in the pseudocoel near the central pair with a portion of each respective cell inserted along the dorsolateral surface of the longitudinal muscles. Each cell has a single, well defined, oval nucleus with a perinuclear ring. Nerve processes from the central pair of neurons innervate the vaginal muscles as well as the body wall musculature. Nerve processes from the remaining neurons join the lateral posterior nerves as well as extend toward the central neurons. The ultimate target of these is unknown, but it may be the same as the central neurons.

KEY WORDS: vaginal ganglion, *Echinopardalis atrata,* Acanthocephala.

A single cerebral ganglion has been presumed to occur in all female acanthocephalans. This ganglion is located in the posterior half of the praesoma where it is surrounded by thick receptacle wall muscles. Male worms have long been known to contain a cerebral and a genital ganglion, the later being a bipartite structure connected by commissures. A third ganglion (bursal ganglion) associated with the posterior portion of the reproductive system was described (Dunagan and Miller, 1977) in male *Moniliformis moniliformis.* We are unaware of additional reports of a similar ganglion in males of other species of this phylum. However, it seems unlikely that occurrence of a bursal ganglion in *M. moniliformis* is an isolated event.

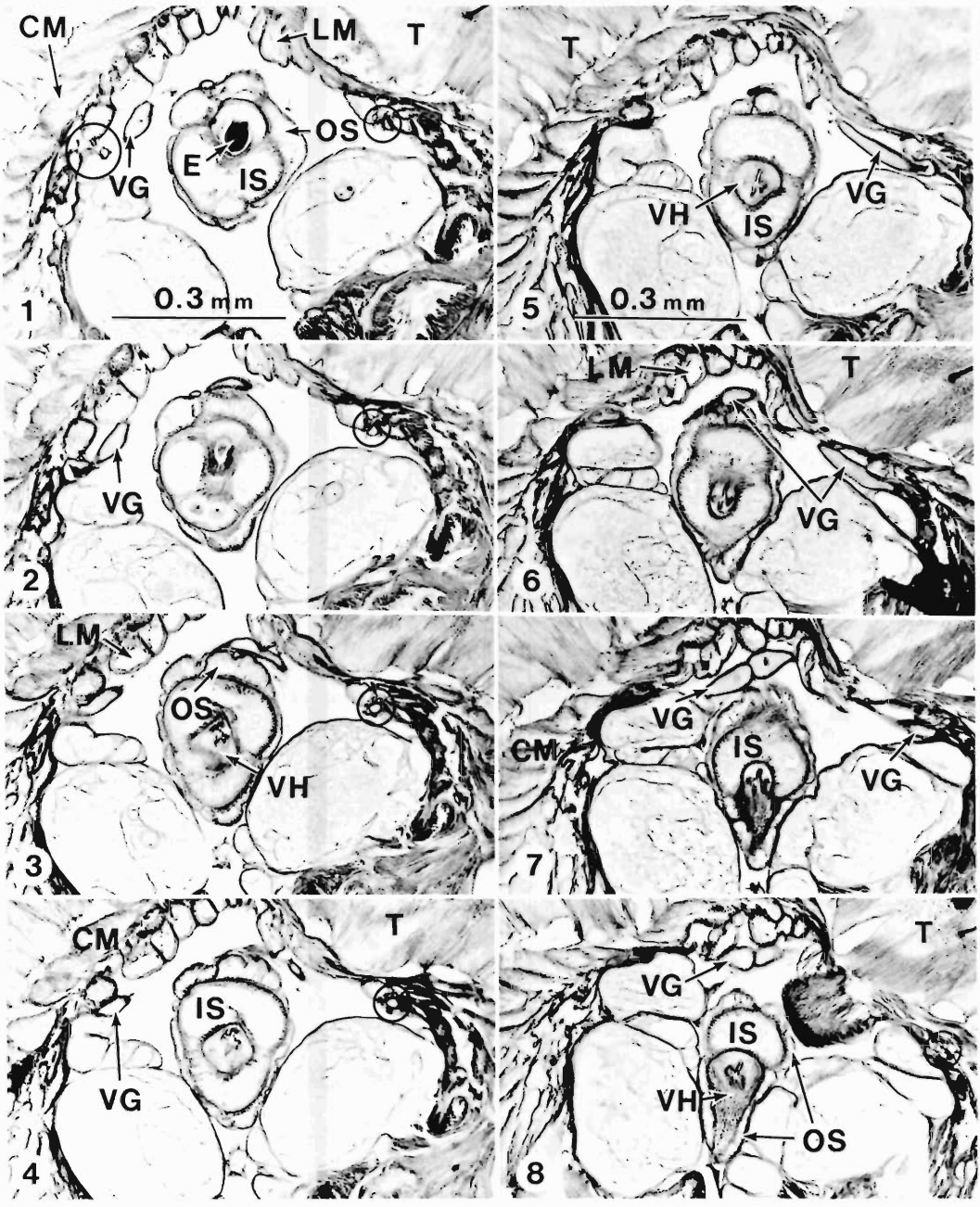
This paper describes a second ganglion, associated with the vagina, in female *Echinopardalis atrata* Meyer, 1931.

Materials and Methods

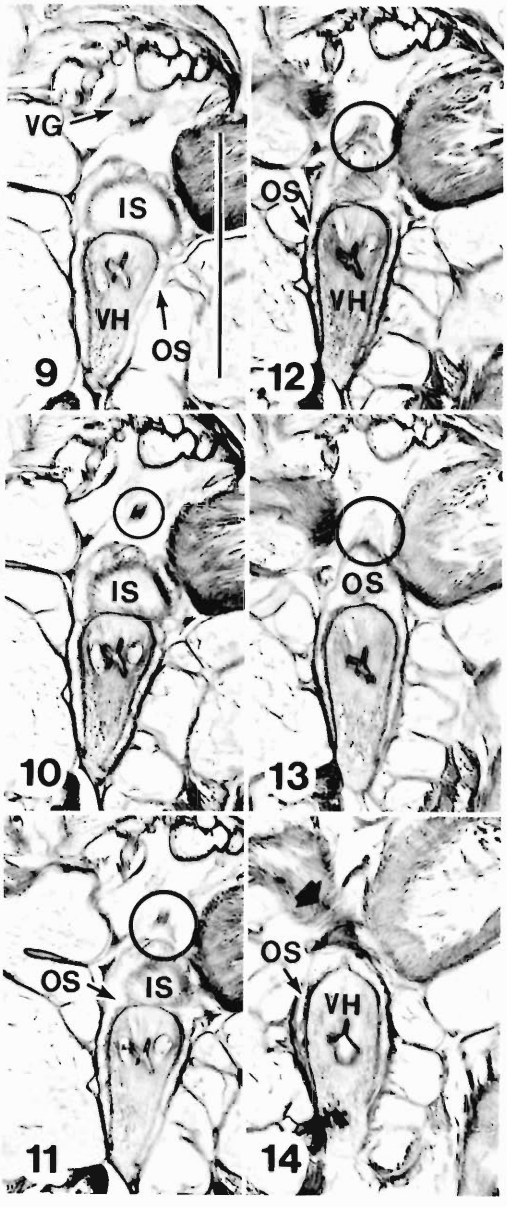
Echinopardalis atrata were collected from the small intestine of 5 feral dogs (*Canis familiaris*) of 5,360 dogs examined at postmortem in Cairo, Egypt during 1986 and spring 1987. Infected dogs were taken from the districts of Torah, El Basateen, and Maasara. After washing briefly in tap water, live worms were fixed in AFA or 2% gluteraldehyde. Specimens were prepared for routine paraplast embedding and sectioned at 8 μm. Staining was accomplished by standard methods for H&E. Two sets of serial sections were prepared and form the basis of this report. All photographs are organized with dorsal at the top.

Results

The vaginal ganglion (Figs. 1–19) consists of 4 large mononucleated cells arranged in 2 pairs. The lateral pair is located in the pseudocoel along the dorsolateral surface of the longitudinal muscles and near the anterior margin of the vagina. Position varied considerably in specimens studied, but 1 cell was always near the anterior margin of the vagina (Fig. 1). In the figures presented here, the second cell was in the posterior half of the vagina near the second set of cells (Figs. 5, 6). The second medial pair of cells lie adjacent to one another on the outer dorsal surface of the outer syncytial muscle in the posterior half of the vagina (Fig. 6). However, rather than remaining in this position throughout their length, their posterior ends extend into the pseudocoel (Figs. 7, 8). Thus all 4 cells have flexibility in their position near the vagina. Each cell appears to have a nerve process extending from its respective poles. We have been unable to verify interfacing of nerve processes between any of the 4 cells. Rather it appears that each cell contributes a process that extends to the lateral posterior nerve (black circles in Figs. 1–4, 8). The remaining process from each bipolar cell extends posteriorly along the outer dorsal surface of the outer syncytial muscle of the vagina. Figures 10–13 have this group of processes enclosed in a black circle. The targets for these processes are the vaginal musculature near the gonopore and the surrounding body wall musculature. Figure 14 (arrow) shows some branches from these processes as they extend into adjacent body wall muscles. The cell bodies of these neurons are very large with uniform, finely granulated cytoplasms. Their nuclei have perinuclear rings and the nucleoli are compact and well defined (Figs. 15–18).



Figures 1–8. Cross sections (not serial) from anterior to posterior through posterior terminus of female *Echinopardalis atrata*. Dorsal is top of each picture. Solid black circles enclose lateral posterior nerve. CM, circular muscle; E, egg; IS, inner sphincter muscle; LM, longitudinal muscle; OS, outer sphincter muscle; T, tegument; VG, vaginal ganglion; VH, vaginal hypodermis. Scale (Figs. 1, 5) is the same for all figures.



Figures 9–14. Cross sections showing nerve processes crossing (black circles) from vaginal ganglion (VG) to vaginal musculature (OS) in female *Echinopardalis atrata*. Dorsal is top of each picture. IS, inner sphincter muscle; OS, outer sphincter muscle; VG, vaginal ganglion; VH, vaginal hypodermis. Scale bar (Fig. 9) is 0.3 mm and applies to all figures.

Discussion

The nervous system in male Acanthocephala has been described for very few species. These include but are not limited to *Macracanthorhynchus hirudinaceus, Bolbosoma turbinella, Polymorphus phippsi, Oligacanthorhynchus microcephala,* and *Moniliformis moniliformis.* Recent reviews (Miller and Dunagan, 1985) indicate that several additional species have been examined, particularly regarding the cerebral ganglion, but also including the genital ganglion. A third ganglion, the bursal ganglion, has been described (Dunagan and Miller, 1977) in *M. moniliformis.* This 4-cell ganglion, in coordination with the genital ganglion, was said to participate in the eversion and retraction of the bursa muscle. Thus the male worm was presumed to have 2 ganglia and in some species 3. Only the cerebral ganglion has previously been described in the female worm, although Harada (1931) credited Leuckart (1876) as having reported observing 2 genital ganglia at the posterior end of the genital passage in female worms. We are unaware of anyone verifying this report. Crompton (1985), in a review of acanthocephalan reproduction, indicated that he was also unaware of any such "neural tissue in females."

The position of the vaginal ganglion in *E. atrata* and the location of the posterior nerve processes strongly suggest that this ganglion may have a role in the control of vaginal musculature. In addition, because of its position, it may control body wall muscles associated with the region of the gonopore. Thus movement of eggs through these sphincters may be under the control of these cells. It is also possible that in this species the relaxation of the vaginal muscles during copulation would aid in this process as well as in sperm movement.

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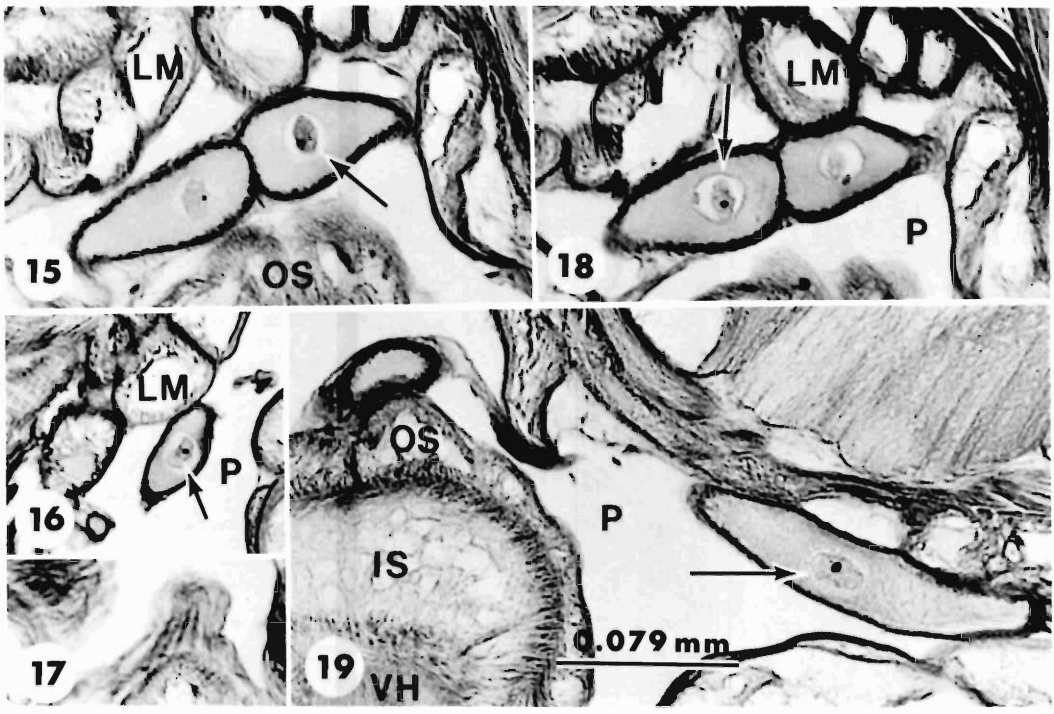

Figures 15–19. 15–18. Cross sections of cells of vaginal ganglion enlarged 3.8× those of Figures 1-8. Nuclei (arrows) of each of the 4 cells. 19. Enlargement (3.8×) of Figure 12. IS, inner sphincter muscle; LM, longitudinal muscle; OS, outer sphincter muscle; P, pseudocoel; VH, vaginal hypodermis. Same scale applies to all figures.

mander in Chief of the Cairo mounted police, and his staff in the Ministry of Interior, Cairo, Egypt. *Echinopardalis atrata* was identified by Dr. B. B. Nickol, School of Biological Sciences, University of Nebraska, Lincoln, Nebraska.

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Neoentomelas asatoi gen. et sp. n. (Nematoda: Rhabdiasidae) and *Hedruris miyakoensis* sp. n. (Nematoda: Hedruridae) from Skinks of the Ryukyu Archipelago, Japan

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ABSTRACT: *Neoentomelas asatoi* gen. et sp. n. (Rhabdiasidae) and *Hedruris miyakoensis* sp. n. (Hedruridae) are described from skinks of the Ryukyu Archipelago, Japan. *Neoentomelas asatoi* from the lung of *Ateuchosaurus pellopleurus* on Okinawa and Amami-oshima islands is distinguished from all other rhabdiasids in having well-developed dorsoventral lips with posteriorly directed lobulate formations. *Hedruris miyakoensis* from the stomach of *Scincella boettgeri* on Miyako Island differs from other species of the genus by the simple lateral cuticular structures of the interlabia, eggs without peripheral swellings, absence of preanal unpaired papilla, stout spicules with prominent accessory structure, simple hook of the holdfast, and/or difference in the measurements.

KEY WORDS: *Neoentomelas asatoi,* Rhabdiasidae, new genus, *Hedruris miyakoensis,* Hedruridae, Nematoda, new species, taxonomy, skink, *Ateuchosaurus pellopleurus, Scincella boettgeri,* Scincidae, Ryukyu Islands, Japan.

There have been only a few records of skink parasites in the Ryukyu Archipelago, which connects the mainland of Japan and Taiwan. During a survey of the helminth fauna in this region, a rhabdiasid species of unknown genus and an undescribed species of the genus *Hedruris* were detected from skinks. The new genus and new species are described herein.

Materials and Methods

Skinks were hand-collected, killed with ether, and their viscera were examined under a dissecting microscope. Nematodes were fixed in hot 70% ethanol, cleared in glycerin-alcohol solution, and mounted with 50% glycerin jelly for microscopical observation. Figures were made with the aid of a drawing tube. Measurements given are for the holotype or the allotype followed by range for the paratypes in parentheses, and are in micrometers unless otherwise indicated.

Results

Neoentomelas gen. n.

DIAGNOSIS: Nematoda, Rhabditoidea, Rhabdiasidae. Parasitic female: Cephalic end subspherical. Massive dorsoventral lips with well-developed muscular and hypodermal tissues present. Each lip with 2 double papillae and 2 minute single papillae; amphids near corners of mouth. Posterior extremity of each labium projected outwards forming 1 median and 2 lateral round lobes. Buccal capsule globular, well-developed, thick-walled, and slightly indented laterally. Onchia absent. Esophagus club-shaped. Vagina weakly developed. Amphidelphic. Eggs thin-shelled and uncleaved at deposition. Parasitic in the lung of reptiles.

TYPE AND ONLY SPECIES: *Neoentomelas asatoi.*

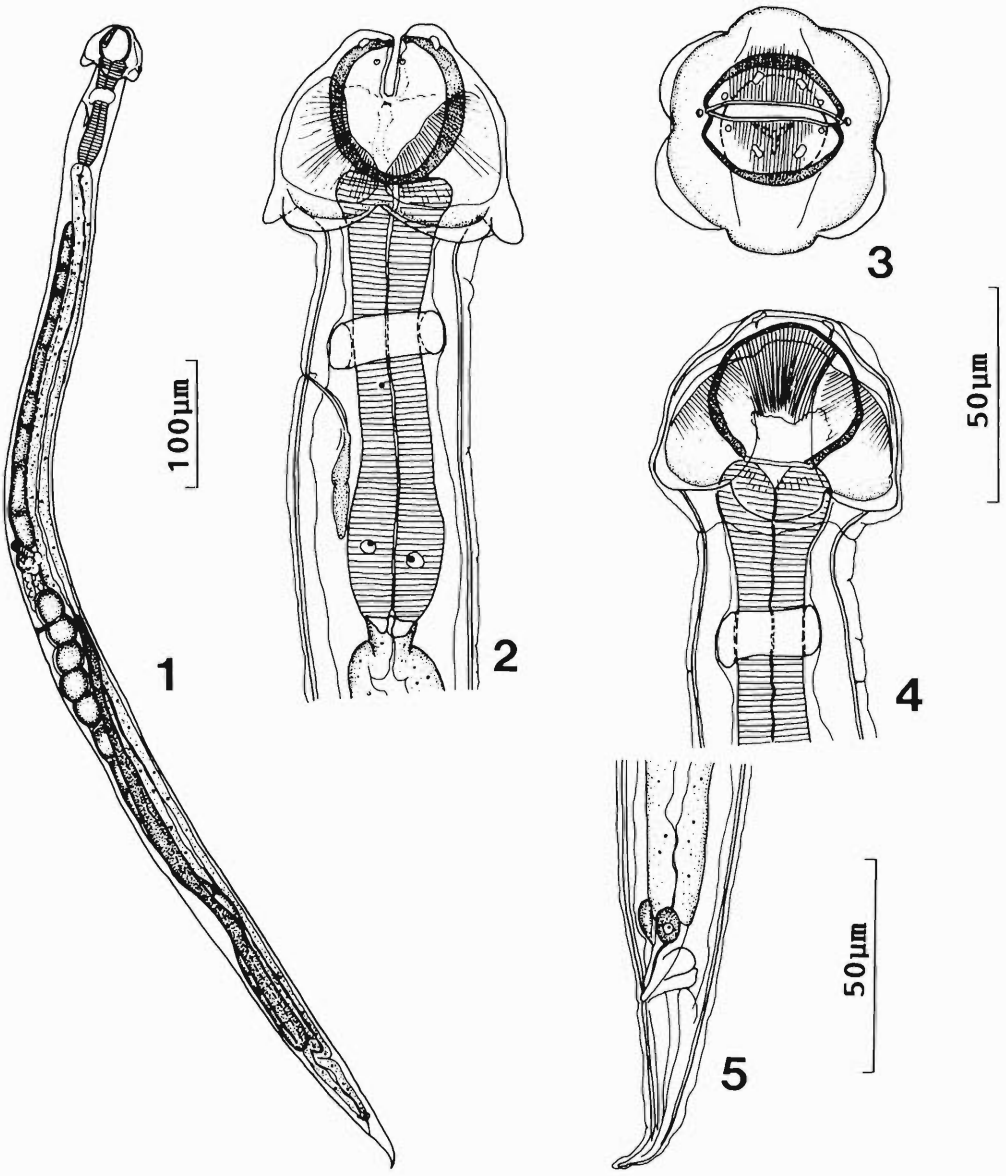
Neoentomelas asatoi sp. n. (Figs. 1–5)

DESCRIPTION: Parasitic females (holotype and 5 paratypes): With characters of the genus outlined above. Body small with reddish intestine. Length 2.00 (1.21–2.36) mm, width 68 (55–58) at nerve ring and 83 (62–88) at vulva (Fig. 1). Cuticle delicate, with adhesive outer surface and easily separated from subcuticular tissue. Cephalic end globular, 88 (73–90) long by 101 (85–99) wide. Buccal capsule 55 (48–60) long by 42 (42–60) wide; wall of buccal capsule thick especially dorsoventrally, and slightly indented laterally (Figs. 2–4). Esophagus with constricted middle portion, 182 (163–185) long, anterior portion 47 (39–49) wide, middle portion 26 (23–29) wide and posterior portion 38 (34–42) wide (Figs. 1, 2). Nerve ring 113 (104–120), excretory pore 151 (128–169), and deirids 137 (137–172) from anterior extremity (Fig. 2). Vulva slightly posterior to middle of body, 1.01 (0.64–1.17) mm from anterior extremity (Fig. 1). Anterior oviduct directed anteriorly, then flexed posteriorly to join anterior ovary; posterior oviduct directed posteriorly and then flexed anteriorly to join posterior ovary. Both ovaries ending near vulva. Tail conical, 70 (55–75) long (Fig. 5). Eggs elliptical, 52–73 by 39–48.

HOST: *Ateuchosaurus pellopleurus.*

SITE IN HOST: Lung.

LOCALITY: Afuso, Onna-son, Okinawa Island (type locality); Seiphah-Utaki, Chinen-son,



Figures 1–5. ***Neoentomelas asatoi*** **gen. et sp. n., parasitic female. 1. Holotype, general view. 2. Anterior extremity of paratype, lateral view. 3. Anterior extremity of paratype, apical view. 4. Anterior extremity of paratype, ventral view. 5. Tail of holotype, lateral view.**

Okinawa Island; Mt. Yuwan-dake, Amami-oshima Island.

DATE OF COLLECTION: 6 May 1987 (at Afuso); 23 May 1987 (at Seiphah-Utaki); 15 July 1981 (at Mt. Yuwan-dake).

SPECIMENS DEPOSITED: Holotype, USNM Helm. Coll. No. 80623; paratypes, National Science Museum, Tokyo, Japan, NSMT As-1910.

REMARKS: *Neoentomelas* is distinguished from other genera of the family Rhabdiasidae in having well-developed dorsoventral lips. The species name is dedicated to Mr. Ryuji Asato,

Okinawa Prefectural Institute of Public Health, who collected the material from Amami-oshima Island.

Hedruris miyakoensis sp. n. (Figs. 6–16)

GENERAL: Nematoda, Habronematoidea, Hedruridae. Body cuticle thick, transversely striated. Cephalic end with 2 large lateral pseudolabia each bearing 2 sessile and 2 digitiform papillae and amphid (Figs. 7–9). Base of each pseudolabium supported by large, posteriorly directed cuticular ridge (Figs. 8, 9). Dorsal and ventral interlabia present between pseudolabia, each supported by large, posteriorly directed ridge. Each interlabium with a blunt anteriorly directed lobe and 2 simple lateral cuticular structures (Figs. 8, 9). Esophagus indistinctly divided at nerve ring into anterior muscular and posterior glandular portions (Fig. 6). Deirids small, spinelike, located slightly posterior to nerve ring (Fig. 6).

MALES (holotype and 3 paratypes): Length 8.90 (8.67–8.92) mm, maximum width 0.19 (0.19–0.20) mm. Caudal end of body forming 3 or 4 coils. Cephalic diameter 85 (80–88), pseudolabium 63 (60–65) long. Esophagus 0.73 (0.69–0.81) mm long and 78 (63 –75) wide. Nerve ring 0.22 (0.18–0.21) mm, excretory pore 0.37 (0.31–0.33) mm, and deirids 0.27 (0.22–0.23) mm from anterior extremity. Caudal alae absent. Tail 0.47 (0.43–0.47) mm long. Caudal papillae 10–12 pairs distributed as follows: 1 or 2 preanal, 1 adanal (absent in 1 specimen), and 7–10 postanal pairs arranged on subventral lines, 1 subdorsal pair just anterior to terminal pair of papillae. Preanal surface with about 25 rows of scalelike bosses extending from near anus to beginning of caudal coils of body (Figs. 10, 11). Spicules robust, fused in posterior two-thirds, distal ends twisted around each other, 135 (133–158) long (Figs. 10–12). Accessory sclerotized structure prominent on ventral side of spicules (Figs. 10, 12). Anus markedly large (Fig. 11).

FEMALES (allotype and 3 paratypes): Length 9.61 (9.71–9.82) mm, width at midbody 0.30 (0.29–0.35) mm, at vulva 0.37 (0.34–0.38) mm. Cephalic diameter 95 (93–107), pseudolabium 80 (88–93) long. Esophagus 0.95 (0.82–0.90) mm long and 78 (78–88) wide. Nerve ring 0.23 (0.23–0.26) mm, excretory pore 0.34 (0.33–0.40) mm, and deirids 0.28 (0.28–0.31) mm from anterior extremity. Vulva 0.55 (0.39–0.62) mm from anus (Fig. 13). Tail 0.51 (0.43–0.49) mm long, with eversible posterior holdfast armed with hook and supported by large musculature (Fig. 13). Hook 145 (143–150) long (Fig. 14). Surface of holdfast with numerous transverse rows of pointed spines (Fig. 15). Eggs elliptical, 48–53 by 23–25, with plugs on both poles, lacking peripheral swellings, and containing fully developed larvae (Fig. 16).

HOST: *Scincella boettgeri.*

LOCALITY: The Miyako Plant Garden, Hirara-shi, Miyako Island, Okinawa, Japan.

SITE IN HOST: Stomach. Females attached to mucosa with holdfast, males coiled around females.

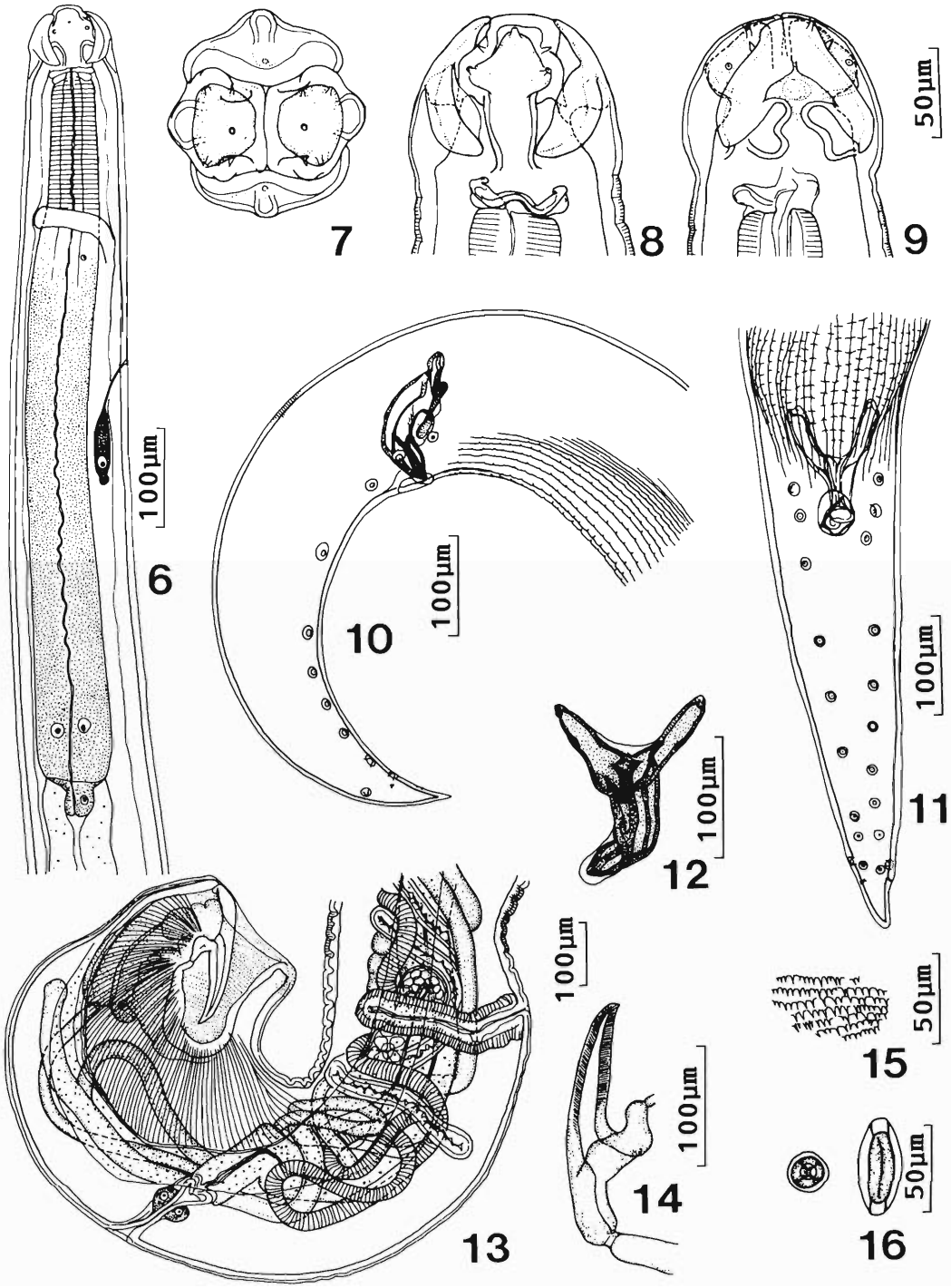
DATE OF COLLECTION: 11 June 1988.

SPECIMENS DEPOSITED: Holotype and allotype, USNM Helm. Coll. No. 80624; paratypes, National Science Museum, Tokyo, Japan, NSMT As-1911.

REMARKS: *Hedruris miyakoensis* resembles *H. pendula* (Leidy, 1851) Chandler, 1919, *H. longispicula* Thomas, 1959, and *H. minuta* Andrews, 1974, among the genus representatives parasitic in terrestrial and freshwater vertebrates in that the eggs lack peripheral swellings and the lateral cuticular structures of the interlabia are simple. *Hedruris miyakoensis* differs from *H. pendula* in having a much smaller body (over 15 mm long in *H. pendula*) and in lacking unpaired papilla on the anterior anal lip (cf. Baker, 1986). *Hedruris miyakoensis* is also distinguished from *H. longispicula* and *H. minuta* by the much larger body (male 3.0 mm long and female 4.0–5.0 mm long in *H. longispicula*; male 2.55–3.20 mm long and female 2.05–2.85 mm long in *H. minuta*). Besides the body size, *H. miyakoensis* differs in that *H. longispicula* has slender spicules and *H. minuta* has a two pronged hook on the holdfast.

Discussion

The representatives of the family Rhabdiasidae Railliet, 1916, are heterogonic parasites found in the lungs, body, and pericardial cavities of amphibians and reptiles (Anderson and Bain, 1982). In this family only 4 genera have been recognized: *Rhabdias* Stiles and Hassall, 1905, *Acanthorhabdias* Pereira, 1927, *Entomelas* Travassos, 1930, and *Pneumonema* Johnston, 1916 (Baker, 1980). All of them lack well-developed lips. *Neoentomelas* is an aberrant rhabdiasid in that it has well-developed dorsoventral lips (cf.



Figures 6–16. ***Hedruris miyakoensis*** **sp. n. 6. Anterior part of holotype male, lateral view. 7. Anterior extremity of paratype female, apical view. 8. Anterior extremity of paratype female, lateral view. 9. Anterior extremity of paratype female, ventral view. 10. Posterior part of holotype male, lateral view. 11. Posterior part of paratype male, ventral view. 12. Spicules, ventral view. 13. Posterior part of allotype female, lateral view. 14. Hook of holdfast of allotype female, lateral view. 15. Inner surface of holdfast of allotype female. 16. Eggs.**

Anderson and Bain, 1982). *Neoentomelas* is closest to *Entomelas* Travassos, 1930, in having a large buccal capsule with a chitinized wall of which the lateral sides are slightly indented (cf. Baker, 1980). The morphological similarity between *Neoentomelas* and *Entomelas* suggests that both were derived from a common ancestor. *Neoentomelas* seems to be more specialized than *Entomelas* since it has well-developed lips with posterior lobulate formations.

Hedruris species have been recorded from marine and freshwater fishes, urodeles, anurans, turtles, snakes, and lizards (Baker, 1987). Most species are parasitic in aquatic and semiaquatic vertebrates while some have been described from terrestrial reptiles. Baker (1982) considered that the genus *Hedruris* was established when the Pangea continent began to split into Laurasia and Gondwanaland. He also pointed out that slender spicules are characteristic of the typical Gondwanaland group of *Hedruris*. The poorly developed accessory sclerotized structure on the ventral side of the spicules, and the bilobed lateral cuticular structures of the interlabia, are found only in *Hedruris* species in South America, Africa, and New Zealand. These structures are therefore considered to be characteristic of the Gondwanaland group.

Although the Australian Region was derived from the Gondwanaland, the skinks in this area harbor *Hedruris* showing characteristics of the Laurasian group. Namely, *Hedruris longispicula* Thomas, 1959, from *Lampropholis challengeri* (=*Lygosoma challengeri*) of Australia has simple lateral cuticular structures of the interlabia and prominent accessory structure of the spicules (Thomas, 1959), and *Hedruris minuta* Andrews, 1974, from *Leiolopisma smithi* of New Zealand has stout spicules with prominent accessory structure besides the simple lateral cuticular structures of the interlabia (Andrews, 1974). However, the distribution of *Hedruris* species of the Laurasian group in this region is reasonable since the Australian and New Zealand skinks have their origins in Asia (Greer, 1979; Robb, 1980). It is thus estimated that the skink ancestors extended their distribution to the Australian Region with *Hedruris*.

Some *Hedruris* species have been poorly described and some have been described only on the basis of immature females. Because of the extreme precocity of *Hedruris* (Leuckart, 1876; Petter, 1971; Hasegawa and Otsuru, 1979; Anderson, 1988), accidental infections may occur in vertebrates feeding on arthropods. Thus, critical re-examination or re-collection of these inadequately known species is especially necessary to elucidate the evolutionary line of *Hedruris*.

Acknowledgments

Thanks are tendered to Mr. N. Iwatsuki and Mr. H. Moriguchi, the Okinawa Herpetological Society, for their kind help in collecting materials. Thanks are also due to Dr. H. Ota, University of the Ryukyus, for helpful discussion on the phylogeny of hosts, and to Dr. M. Machida, National Science Museum, Tokyo, Mr. A. Ichihara, Meguro Parasitological Museum, Tokyo, for their kindness in providing copies of some papers.

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ANNOUNCEMENT

The William C. Campbell Endowment Fund for Visiting Scientists at the Harold W. Manter Laboratory

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Henry Baldwin Ward introduced parasitology into the curriculum at the University of Nebraska in 1893, doubtless the first laboratory course in parasitology in the Western Hemisphere. Ward (1892–1909) was succeeded by Franklin D. Barker (1909–1926) and Manter (1926–1966). Now Research Associates J. Janovy, Jr., B. B. Nickol, T. O. Powers, and Curator M. H. Pritchard continue the unbroken tradition at UN–L. Research Affiliates include W. L. Bullock, D. W. Duszynski, D. Heyneman, N. D. Levine, J. R. Lichtenfels, J. S. Mackiewicz, R. M. Overstreet, R. L. Rausch, G. D. Schmidt, and F. Sogandares-Bernal. Research Affiliates Emeriti include M. B. Chitwood, M. F. Hansen, J. T. Self, H. W. Stunkard, and M. J. Ulmer.

The William C. Campbell Endowment Fund was established by Merck & Co., Inc., when Dr. Campbell was honored with the 1987 Directors' Scientific Award for his contributions to the development of the avermectin/ivermectin family of antiparasitic compounds. Dr. Campbell designated the endowment for a Visiting Scientist Program so that HWML resources can be shared with a larger number of parasitologists, enable individual research projects, and provide an even more creative environment at HWML.

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56(2), 1989, pp. 151–155

Experimental and Natural Infection of Planktonic and Benthic Copepods by the Asian Tapeworm, *Bothriocephalus acheilognathi*

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Department of Biology, Wake Forest University, Winston–Salem, North Carolina 27109

ABSTRACT: The life cycles of pseudophyllidean tapeworms include a procercoid stage that inhabits the hemocoel of copepods. A variety of copepods from Belews Lake, North Carolina, were individually exposed to 5–10 coracidia of the Asian fish tapeworm, *Bothriocephalus acheilognathi*. The planktonic cyclopoid species *Diacyclops thomasi, Mesocyclops edax,* and *Tropocyclops prasinus* proved susceptible to infection, whereas a sympatric calanoid copepod (*Skistodiaptomus pallidus*) could not be infected experimentally. In addition, lab experiments revealed that the benthic cyclopods *Eucyclops agilis* and *Paracyclops fimbriatus poppei* could also be infected. All cyclopoids appear to be susceptible to infection regardless of developmental stage. Natural infections during the period of fall recruitment of the parasite by the fish hosts were highest among the planktonic *T. prasinus*. Although no benthic copepods were found infected during summer months in the field, these organisms may be important in transmitting the cestode to the detritivorous fathead minnow (*Pimephales promelas*) during the spring and fall periods of recruitment.

KEY WORDS: cyclopoid copepods, benthic copepods, *Bothriocephalus acheilognathi, Diacyclops thomasi, Mesocyclops edax, Tropocyclops prasinus, Eucyclops agilis, Paracyclops fimbriatus poppei,* experimental infection.

Bothriocephalus acheilognathi Yamaguti, 1934 (*B. gowkongensis* Yeh, 1955), commonly referred to as the Asian fish tapeworm, has spread from Asia throughout Europe and parts of North America. This parasite is known to infect over 40 species of fish, mainly cyprinids (Riggs and Esch, 1987). In Belews Lake, North Carolina, the cestode is commonly found in mosquitofish (*Gambusia affinis*), red shiners (*Notropis lutrensis*), and fathead minnows (*Pimephales promelas*), and sporadically in green sunfish (*Lepomis cyanellus*).

Numerous intermediate hosts have been experimentally infected, but research has been confined to Asia and Europe. Species of *Bothriocephalus* are generally non-specific for intermediate hosts (Liao and Shih, 1956; Jarecka, 1964; Korting, 1975; Jarroll, 1979; Dupont and Gabrion, 1987). Thus, *B. claviceps, B. cuspidatus,* and *B. acheilognathi,* all parasites of freshwater fish, infect a variety of cyclopoid copepods. *Bothriocephalus scorpii,* a common marine form, infects calanoid copepods. The only cestode in the genus for which unsuccessful copepod infection attempts have been reported is *B. rarus* (Jarroll, 1979), which is unusual in that it infects newts. Jarroll (1979) was unable to infect either *Eucyclops speratus* or *Paracyclops fimbriatus poppei* with coracidia of *B. rarus.*

Still fewer studies have attempted to determine which species serve as natural intermediate hosts. Jarroll (1979) found *Macrocyclops ater* to be the only copepod to be naturally infected with *B. rarus,* and prevalence was very low (0.82%). Liao and Shih (1956), however, stated that prevalence of *B. acheilognathi* in cyclopoid copepods can reach 7%.

Cyclopoid copepods and other microcrustaceans from Belews Lake were exposed under laboratory conditions to coracidia of *B. acheilognathi* in an effort to determine which species may serve as an intermediate host. Both planktonic and benthic copepods from Belews Lake were also examined for evidence of infection.

Materials and Methods

Planktonic copepods were obtained from Belews Lake by vertical and/or horizontal tows using a No. 20 (76-μm) Wisconsin net. Benthic copepods and ostracods were collected with a 15.1 × 15.1-cm Ekman grab. On return to the laboratory, organisms were isolated from the sediments using the method of Elgmork (1959). Water was added to the sediments and the sample was agitated. After approximately 1 hr, the water above the settling sediments was filtered through bolting cloth fixed within a hoop. Animals trapped on the cloth hoop were transferred to a finger bowl. This process was repeated at least 3 times, or until no more copepods were recovered.

Gravid tapeworms were removed from red shiners

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Table 1. Experimental infections of individual copepods with *Bothriocephalus acheilognathi*.

Potential host	No. exposed	No. infected	%
Diacyclops thomasi	28	12	42
Mesocyclops edax	54	15	28
Tropocyclops prasinus	5	2	40
Eucyclops agilis	45	13	29
Paracyclops fimbriatus poppei	13	6	46

Table 2. Experimental infections of groups of organisms with *Bothriocephalus acheilognathi*.

Potential host	No. exposed	No. infected	%
Skistodiaptomus pallidus	8	0	0
Ostracods	7	1	14.3
Eucyclops agilis nauplii	4	3	75

and fathead minnows that were trapped or seined at Belews Lake. Worms were placed in water at room temperature to induce release of eggs. Coracidia hatched after a period of development (approximately 24 hr). Cyclopoid copepods were isolated in 2 ml of filtered lake or artificial pond water and exposed to 5 or 10 coracidia each. Calanoid copepods, copepod nauplii, and ostracods were placed in 20 ml of filtered lake or conditioned tap water and exposed in groups of 4–7 to 100–200 coracidia per group. All experiments were performed at room temperature (25°C). The cyclopoid copepods were identified according to Yeatman (1959), and the calanoids according to Wilson (1959). Ostracods were not identified.

After 5 days live animals were fixed in 70% ethanol and stained in acetocarmine (Shostak et al., 1985). They were then examined under ordinary light microscopy at 400×; or, alternatively, copepods were examined live under phase contrast. Examination of live copepods proved to be the better method for detection of procercoids. Copepods that died during the experiments were examined for infections but omitted from data analysis, unless otherwise indicated. No deaths of heavily infected copepods were observed.

In order to determine parasite prevalence in natural infections, zooplankton were sampled by towing the Wisconsin net for approximately 4 min during daylight hours in late October/early November 1986, a period of active parasite recruitment by the fish hosts. Night samples were also taken during the same period. Animals were fixed for 1 hr in 70% ethanol, stained in acetocarmine (Shostak et al., 1985), and examined microscopically for infection. Benthic copepods were captured during the summer of 1986 using the method described above, and fixed and stained as per the planktonic copepods, except that the duration of fixation and storage was not limited to 1 hr, but extended for a period of a few weeks.

Results

All cyclopoid copepods tested were susceptible to infection with coracidia of *Bothriocephalus acheilognathi* (Table 1). The planktonic copepods included *Diacyclops thomasi* (S. A. Forbes, 1892), *Mesocyclops edax* (S. A. Forbes, 1891), and *Tropocyclops prasinus* (Fischer, 1860). The numbers of *T. prasinus* were limited because the animals were very difficult to maintain in the laboratory, and most died early in the experiments. The benthic copepods *Eucyclops agilis* (Koch, 1838), and *Paracyclops fimbriatus poppei* (Rehberg, 1880), also proved susceptible to infection.

In the group exposures, 1 of 7 ostracods developed an infection, and 3 of 4 copepod nauplii were found to harbor procercoids (Table 2). The nauplii were early larval stages of the benthic copepod *E. agilis*. No calanoid copepods (*Skistodiaptomus pallidus* (Herrick), 1879) became infected on exposure to coracidia.

The copepod infection data were further analyzed to determine which developmental stages were susceptible to infection by *B. acheilognathi*. All stages examined proved vulnerable to infection (Table 3); development of the parasite seemed to be hindered by the presence of egg masses in females. For example, 3- and 5-day-old worms remained small in the fifth-stage copepodite female *M. edax* with developing eggs, as compared to procercoids in other copepod species.

The majority of the copepods captured during the night-time surface tows in the autumn were *M. edax* (97%), a strong vertical migrator. Few of these were infected (0.6%) (Table 4). The majority of the copepods taken during daylight hours were *T. prasinus* (92%). A much higher proportion of these were parasitized (7.1%) (Table 4). All benthic copepods sampled during the summer proved negative for infections (Table 4).

Discussion

All cyclopoid copepod species tested in the present study could be experimentally infected with *B. acheilognathi*. Thus, this tapeworm is not restricted to certain species of intermediate host in North America, an observation which confirms those of others in Europe and Asia who have successfuly infected numerous cyclopoid species (Liao and Shih, 1956; Shcherban et al., 1963 *in* United States Department of Agricul-

Table 3. Copepod stages experimentally exposed.*

Potential host	Developmental stage								
		Copepodites					Adults		Female + eggs
	Nauplii	C1	C2	C3	C4	C5	Male	Female	
Diacyclops	−	−	+	+	+	+	−	+	−
Mesocyclops	−	−	−	+	+	+	−	−	−
Tropocyclops	−	−	−	−	−	+	−	+	−
Eucyclops	+	−	−	−	+	+	†	−	+
Paracyclops	−	−	−	−	+	+	†	−	−

* − = not exposed; + = exposed, became infected; † = exposed, became infected, and died.

ture, 1972; Körting, 1975; Esinenko-Marits et al., 1968 *in* United States Department of Agriculture, 1976).

The successful infection of benthic copepods has important implications for the population dynamics of the parasite in the definitive host. Mosquitofish and red shiners are planktivorous (Riggs, 1986), and most likely acquire the parasite by ingesting infected planktonic copepods (*D. thomasi, M. edax,* and *T. prasinus*). The fathead minnows are benthic detritivores (Riggs, 1986). The eggs of *B. acheilognathi* sink and stick to the substratum (pers. obs.). When coracidia hatch from eggs on the substratum, benthic copepods, such as *E. agilis* and *P. f. poppei,* are thus exposed to infection. These species in turn transmit the parasite to the fathead minnows feeding on the bottom.

Because all of the developmental stages of cyclopoid copepods that were examined could be experimentally infected with *B. acheilognathi* (Table 3), it appears that the potential of a copepod becoming infected is not dependent on the host's developmental state. Inasmuch as molting of infected copepods was observed, parasitism also does not seem to affect development of the copepods, a situation that is consistent with that described by Dupont and Gabrion (1987), who worked with *B. claviceps* in copepods. However, we have no data regarding the impact of parasitism on the rate of copepod development or the effect of copepod age on procercoid development, except the observation that parasite development seemed to be impeded by the presence of eggs in the body cavity of the fifth copepodite female *M. edax.* Michajlow (1953) was able to infect fourth and fifth copepodites and adults of *Cyclops strenuus* with *Triaenophorus lucii,* but noted development was retarded in the juvenile forms. Together with Halvorsen's (1966) observations that the development of *Diphyllobothrium latum* is retarded in juvenile *C. strenuus,* these various observations suggest that although infection by cestodes can occur in various developmental stages of the copepod intermediate hosts, the subsequent development of the parasite may be influenced by host maturity (Humes, 1950; Guttowa, 1956; Watson and Lawler, 1965; Kuperman and Kireev, 1976).

It is possible that adult male copepods are poor hosts for *B. acheilognathi* inasmuch as males of *E. agilis* and *P. f. poppei* became infected but died soon afterward (Table 3). Michajlow (1938) and Guttowa (1956) also found that males and females of the calanoid *D. gracilis* exhibited differing abilities to serve as hosts for the cestode *T. lucii,* although Humes (1950) and Michajlow (1938) found no sex-specific differences in susceptibility of a variety of diaptomids to *Dibothriocephalus latus.*

There are few estimates of the extent of infection in copepods by procercoids in nature. Jarroll

Table 4. Natural copepod infections recorded from Belews Lake.

Sampling period	Species	*N*	No. infected	Prevalence (%)
Night (fall)	*Mesocyclops*	704	4	0.6
	Tropocyclops	8	0	0
	Diacyclops	7	0	0
	Eucyclops	7	0	0
Day (fall)	*Tropocyclops*	85	6	7.1
	Mesocyclops	5	0	0
	Paracyclops	2	0	0
Benthic (summer)	*Eucyclops*	46	0	0
	Paracyclops	12	0	0
	Mesocyclops	9	0	0

(1979) found *Macrocyclops ater* to be the only copepod naturally infected with *Bothriocephalus rarus,* and prevalence was exceedingly low (0.82%). Liao and Shih (1956) stated that prevalence of *B. acheilognathi* may reach 7%. Prevalence of 1% or 2% were recorded for *Triaenophorus* spp. in *D. thomasi* at a number of different localities over a number of years (Watson and Lawler, 1965). Because infected *D. thomasi* were more abundant in inshore, shallow waters as compared with offshore sites (Watson and Lawler, 1965), and because prevalence of *Triaenophorus* spp. in *D. thomasi* has been shown to vary from 43% in early June to 0 in July (Miller, 1952), such seasonal and spatial heterogeneity complicates the determination of prevalence in the field.

Tropocyclops prasinus was the dominant planktonic copepod during the autumn recruitment of *B. acheilognathi* by the fish hosts in Belews Lake and harbored the parasite at a prevalence of 7.1%, which is comparable to that observed by Liao and Shih (1956) in China. Because *M. edax* is not an important component of the zooplankton community during the autumn and is parasitized at a very low level (0.6%), it probably is not important in the transmission of *B. acheilognathi* in the fall. Furthermore, strong vertical migrators such as *M. edax* (Williamson and Magnien, 1982) experience reduced vulnerability to fish predation (Zaret and Suffern, 1976; Zaret, 1980; Stich and Lampert, 1981; Gliwicz, 1986), which in turn reduces transmission rates of parasites utilizing migrants as intermediate hosts.

Despite the fact that no benthic copepods harbored natural infections, these zooplankters may still be important in transmission of the cestode to detritivores such as fathead minnows. Sample sizes are low and the benthic animals were collected in the summer when no recruitment occurs (Riggs and Esch, 1987); thus, it is not surprising that none was parasitized in the present study. On the other hand, benthic copepods are susceptible to infection by coracidia in the laboratory, and may indeed acquire the parasite during the spring and fall periods of transmission. It is unlikely that organisms other than cyclopoid copepods serve as intermediate hosts for *B. acheilognathi* in nature since only 1 ostracod and no *S. pallidus* became infected after extensive exposure to coracidia in the group experiments (Table 2).

The ubiquitous distribution of this parasite and its continued spread to new areas (Heckman et al., 1987; Riggs and Esch, 1987) are in part attributable to the lack of specificity not only for its definitive host, but for its intermediate host as well. *Bothriocephalus acheilognathi* infects over 40 species of fish belonging to a variety of families (Riggs and Esch, 1987). Because the cestode is found primarily in cyprinids, and because all cyclopoid copepods that have been tested may serve as intermediate hosts, this cestode is likely to expand its present range in North America and probably other parts of the world as a result of the cosmopolitan distribution of both cyclopoid copepods and cyprinid fishes.

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A Redescription of *Simhatrema simhai* Chattopadhyaya, 1970 (Trematoda: Exotidendriidae), with Comments on its Pathogenesis in Sea Snakes (Serpentes: Hydrophiidae)

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ABSTRACT: *Simhatrema simhai* is redescribed from material from 7 species of sea snakes from Malaysian and northern Australian waters. Most worms were found in connective tissue cysts in the wall of the large intestine near the ileocecal valve. In 3 snakes, hundreds of such cysts were bound together with connective tissue to form a large mass which protruded into the body cavity and in 1 case occluded the intestine.

KEY WORDS: *Disteira kingi, D. major,* Exotidendriidae, *Enhydrina schistosa, Hydrophis cyanocinctus, H. elegans, H. ornatus, Lapemis hardwickii,* pathology, *Simhatrema simhai,* variation.

A survey of the parasites of marine snakes yielded several hundred specimens representing the genus *Simhatrema.* On the basis of only 7 specimens, Chattopadhyaya (1970) erected the genus and described the 2 known species, *S. simhai* and *S. osmaniae,* both from *Cherysdrus* (=*Acrochordus*) *granulatus.* Srivastava (1981) synonymized the 2 species after examining the type specimens. This study describes my new material, with special attention to its variability, and supports that synonymy. The extensive pathogenesis in a few snakes is also described briefly.

Materials and Methods

Sea snakes were captured in the Gulf of Carpentaria as a by-product of trawling for a long-term study of penaeid prawns by the Commonwealth Scientific and Industrial Research Organization (see Redfield et al., 1978, for details). Snakes were quick-frozen immediately after capture, returned to the laboratory where most were partially thawed, eviscerated, and the viscera refrozen and shipped to the University of Alberta for examination. Two *Hydrophis cyanocinctus* from Malaysia were obtained from fishermen and were examined fresh; other material from Malaysia was obtained as preserved specimens from Dr. Leong Tak Seng, Universiti Sains Malaysia.

Living specimens from fresh snakes were fixed in hot alcohol–formaldehyde–acetic acid (AFA), dead ones from frozen snakes in cold AFA. They were stained in Harris' hematoxylin, dehydrated in alcohols, cleared in methyl salicylate, and mounted in permount. Fourteen specimens were embedded in wax, serially sectioned at 7 μm, stained with hematoxylin and eosin, and mounted in permount. Portions of cysts were fixed in Bouin's fixative, embedded, sectioned, and stained as above.

Measurements were made with an ocular micrometer at magnifications of 100–400×. Locations were measured from the anterior end of the specimen to the anterior edge of the organ, and were expressed as percentages of body length.

Representative specimens have been deposited in the following museums: U.S. National Museum Helminthological Collection (No. 80563), National Museum of Canada, Invertebrate Collection (No. 1989-0001), Queensland Museum (No. GL10282), University of Nebraska State Museum Manter Lab. (No. 31063); the remaining specimens are in the University of Alberta Parasitology Collection (Nos. 10360–10367, 10368–10371, and 11305–11307).

Results

Taxonomy

Over 1,900 specimens were found in 25 sea snakes belonging to 7 species (Table 1). The following description is based on whole mounts of 42 specimens, plus sectioned material of an additional 56 specimens (42 sectioned in situ within cysts), from 5 species of snakes. The large number of eggs present frequently obscured the internal organs; actual sample sizes for each organ measured can be determined from Table 2. Unless otherwise stated, all measurements are in micrometers, and are given as the range, followed by the mean (in parentheses).

Simhatrema simhai Chattopadhyaya, 1970 (Fig. 1)

S. osmaniae Chattopadhyaya, 1970 (Srivastava, 1981).

REDESCRIPTION: Exotidendriidae: *Simhatrema.* Body elongate-oval, 0.99–2.54 (1.81) mm long, 0.21–1.03 (0.69) mm wide. Cuticle spinose; spines lost in frozen specimens. Oral sucker spherical, sub-terminal, 85–171 (128) wide; prepharynx narrow, thin-walled, 51–156 (99) long; pharynx globular, muscular, 65–122 (94) wide, 57–112 (89) long; ratio of prepharynx length to pharynx length 0.81–1.68 (1.18); esophagus thin-

walled, 65–188 (123) long, bifurcating at 18–31% (23%) body length into two slender ceca, which extend to testicular zone. Walls of ceca thick, glandular. Additional glands associated with pharynx. Scattered cuticular glands throughout forebody. Acetabulum spherical, larger than oral sucker, 117–236 (182) in diameter, anterior edge at 28–40% (33%) body length. Testes oval, entire, opposite, subequal; anterior edge at 35–74% (55%) body length; right testis 136–608 (374) long by 85–440 (224) wide; left testis 128–612 (350) long by 85–316 (195) wide. Cirrus pouch large, 377–1,171 (737) long, muscular, curved around right side of acetabulum to vicinity of ovary and anterior edge of right (occasionally, left) testis; basal portion 71–901 (291) long, contains saccular seminal vesicle; middle portion contains well-developed pars prostatica; distal portion contains muscular cirrus, 298–790 (462) long; cirrus thickly set with thin, curved spines, 19 long, with expanded base 4 wide. Genital pore pre-acetabular, median, at 21–33% (26%) body length, in or posterior to bifurcal zone. Ovary entire, anterior to right testis, 77–233 (142) long by 63–210 (106) wide. Vitellaria consisting of large follicles, in a band across body from testes to acetabular zone; occasionally limited to lateral fields (medial follicles often obscured by uterus in whole mounts). Uterus voluminous, occupying most of the space in the hindbody, often obscuring most of the organs. Metraterm muscular, located dorsal or to left or right of acetabulum, 270–525 (396) long; inner lining sparsely set with thick, sharp-pointed spines, 14 long, with expanded base 8 wide. Eggs in uterus numerous, light brown, thin-walled, operculate, without polar filament, 24–31 (28) long by 14–20 (17) wide. Excretory vesicle Y-shaped (with short stem) or V-shaped.

HOSTS: *Acrochordus granulatus* (type host; Chattopadhyaya, 1970), *Disteira kingi, D. major, Enhydrina schistosa, Hydrophis cyanocinctus, H. elegans, H. ornatus, Lapemis hardwickii* (this study).

LOCATION: In pits or cysts in wall of large intestine near junction with small intestine; occasionally in intestinal lumen.

LOCALITY: Arabian Sea near Bombay (type locality; Chattopadhyaya, 1970). Australia, eastern Gulf of Carpentaria; Malaysia, sea near Penang (this study).

According to the original description, *Simhatrema osmaniae* differed from *S. simhai* in having more anteriorly placed testes, a shorter prepharynx, a genital pore located posterior to the cecal bifurcation rather than in the zone of the bifurcation, and large vitellaria located laterally rather than in a median field. The measurements of the 2 species also indicated that *S. osmaniae* was longer, with a larger oral sucker, larger gonads, and a more anteriorly placed bifurcation (Table 2). In addition, the drawings of the 2 worms indicated a difference in the position of the cirrus pouch. In *S. osmaniae,* the cirrus pouch is shown extending to the anterior border of the right testis. In *S. simhai,* the cirrus pouch is shown extending to the anterior border of the left testis.

Table 1. Hosts of *Simhatrema simhai.*

Species	Inf./ exam.	No. of worms
Gulf of Carpentaria		
Disteira kingi	6/30	432, 21, 17, 16, 8, 2
Disteira major	2/36	2, 1
Enhydrina schistosa	1/93	7
Hydrophis elegans	2/146	4, 1
Hydrophis ornatus	3/11	763, 580, 3
Penang, Malaysia		
Enhydrina schistosa	6/22	4, 3, 2, 2, 1, 1
Hydrophis cyanocinctus	2/2	4, 1
Lapemis hardwickii	3/12	136, 1, 1

In the present material, both the position of the cirrus pouch and the distribution of the vitellaria are variable. In most of the present specimens, the cirrus pouch ends anterior to the right testis, and the vitellaria extend in a continuous band across the body; however, in a few specimens, the cirrus pouch ends anterior to the left testis, and in a few others, the vitellaria are lateral in position, with no medial follicles. These 2 characters are not correlated. In about ⅔ of the specimens, the genital pore was clearly posterior to the bifurcation, but in the other ⅓, the genital pore was in the same zone (within 1% of the body length) as the bifurcation. The variation in measurements of the present material encompasses the variation in both species, except for the large ovary of specimens described as *S. osmaniae.* There were no differences between the material from the Gulf of Carpentaria and that from Malaysia (Table 2). I conclude that Srivastava (1981) was correct in deciding that the differences are intraspecific variation, and that *S. osmaniae* is a synonym of *S. simhai.*

Neither Chattopadhyaya nor Srivastava de-

Table 2. Comparison of measurements (in millimeters) of *Simhatrema* species.

	Chattopadhyaya, 1970		Present study	
	S. simhai	*S. osmaniae*	Gulf of Carpentaria	Malaysia
No. examined	3	4	29	13
Body length	1.06–1.20	1.76–1.82	1.455–2.537 (29)	0.993–1.695 (4)
Body width	0.52–0.70	0.61–0.71	0.450–1.028 (29)	0.210–0.841 (7)
Oral sucker width	0.08–0.11	0.13–0.14	0.104–0.171 (29)	0.085–0.156 (7)
Acetabulum width	0.15–0.18	0.16–0.17	0.128–0.236 (28)	0.117–0.196 (5)
Acetabulum position (%)	39*	31*	28–37 (26)	32–40 (3)
Prepharynx length	0.04–0.14	0.05	0.074–0.156 (27)	0.051–0.105 (3)
Pharnyx length	0.06–0.09	0.09–0.11	0.071–0.110 (27)	0.057–0.112 (6)
Prepharynx/pharynx	1.69*	0.63*	0.813–1.677 (25)	0.859–1.364 (3)
Esophagus length	0.06–0.08	0.80–0.11	0.071–0.188 (27)	0.065–0.136 (6)
Position of bifuraction (%)	33*	20*	18–31 (27)	21–25 (2)
Right testis length	0.19–0.24	0.49–0.50	0.233–0.608 (20)	0.136–0.391 (11)
Right testis width	0.09–0.16	0.23–0.35	0.128–0.440 (20)	0.085–0.321 (11)
Pos. right testis (%)	65*	58*	35–69 (22)	48–65 (3)
Left testis length	0.19–0.33	0.41–0.44	0.134–0.612 (20)	0.128–0.467 (11)
Left testis width	0.11–0.19	0.23–0.31	0.117–0.316 (21)	0.085–0.291 (10)
Pos. left testis (%)	78*	53*	47–71 (21)	59–74 (3)
Spinose cirrus length			0.324–0.549 (9)	0.298–0.790 (5)
Seminal vesicle length	0.13*	0.29–0.30	0.139–0.901 (6)	0.071–0.485 (6)
Cirrus sac length	0.71–0.73	0.73–0.81	0.543–1.171 (9)	0.377–0.944 (6)
Metraterm length	0.34–0.46	0.40–0.42	0.310–0.525 (11)	0.270–0.508 (3)
Ovary length	0.12–0.14	0.26–0.27	0.094–0.233 (8)	0.077–0.128 (8)
Ovary width	0.13*	0.12–0.21	0.085–0.210 (9)	0.063–0.091 (8)
Pos. genital pore (%)	36*	23*	21–33 (27)	28–29 (2)
Egg length	0.025–0.037	0.025–0.038	0.024–0.031 (29)	0.026–0.028 (12)
Egg width	0.014–0.019	0.014–0.018	0.014–0.020 (29)	0.016–0.019 (12)

* From measurements of figures.

scribed the cutaneous glands or the glandular lining of the intestinal ceca (although these features were considered characteristic of the family Exotidendriidae by Srivastava). Both are present in my hot fixed material, but not in my frozen material. In addition, both Chattopadhyaya and Srivastava indicated that the eggs were non-operculate; in my material, opercula are not obvious in whole mounts, but are clearly present in sectioned material. Thus *Simhatrema* resembles *Exotidendrium* in all 3 of these features. (See Blair et al., in press, for a review of the family, including a description of a third genus.)

Pathology

The precise location of the worms was recorded in 15 snakes. In 1 (a *D. major*) a single worm was free in the lumen of the large intestine. In 2 others (*H. cyanocinctus*) the worms were located in fibrous connective tissue cysts that opened into the lumen of the large intestine. In the other snakes, the worms were apparently completely enclosed in fibrous connective tissue cysts. In most snakes, these cysts were clustered around the large intestine close to the ileocecal junction. In 3 snakes (1 *D. kingi,* 2 *H. ornatus*) with large numbers (>400) of worms, clusters of cysts were enclosed in a connective tissue capsule attached to the large intestine and extending into the body cavity, and forming a mass 11 × 4.5 × 3 cm in 1 snake and only slightly smaller in the other 2. In *D. kingi* a relatively small number of worms (34) were found free in this mass, not in a cyst. Most worms were enclosed in large, soft, spherical cysts (1–5 mm in diameter) surrounded by a thin fibrous connective tissue wall. These cysts contained from 0 to 64 worms, trematode eggs, and unconsolidated tissue debris. In addition, there were smaller, oblong, hard cysts containing consolidated caseous material, trematode eggs, and in rare cases a trematode. The distributions of worms in the 2 types of cysts are shown in Table 3. Worms were obviously aggregated (overall variance/mean ratio 5.5), and there were relatively few cysts with only a single worm.

In *D. kingi* the lumen of the large intestine was completely occluded by fibrotic material; in the other 2 snakes with the large masses of cysts, the

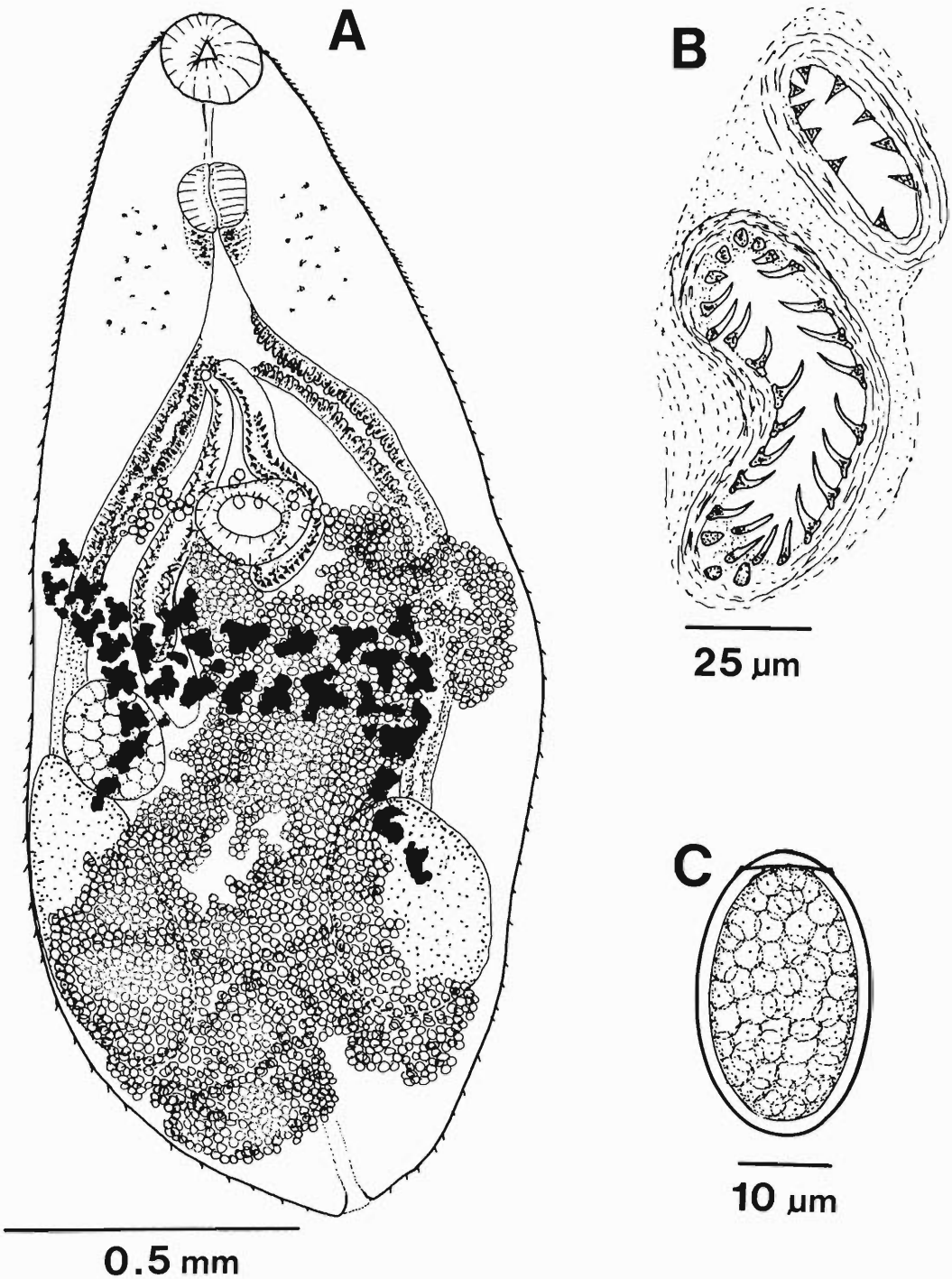

Figure 1. *Simhatrema simhai.* **A. Whole mount, ventral view. B. Section through invaginated cirrus (lower left) and metraterm (upper right), showing spines. C. Egg, showing operculum.**

Table 3. Distribution of *Simhatrema simhai* in small and large cysts in sea snakes.

	Host number and species							
	G78.7 *D. kingi*		G78.14 *H. ornatus*		G79.73 *H. ornatus*		Others (11) (6 spp.)	
No. of worms not in cysts	34		0		0		1	
	Small	Large	Small	Large	Small	Large	Small	Large
No. in cyst								
0	135	11	7	62	92	263	25	25
1	1	23		28		26	1	8
2		43		40		61		20
3		42		19		46		7
4		14		14		28		2
5		9		17		13		
6		5		6		12		1
7		2		6		12		
8		1		4		4		
9		1				2		
10				4		2		
11						1		
12				1		1		
13				1				
14+				2 (17, 64)		3 (14, 14, 23)		
Var/mean	2.25		9.53		4.27			

intestinal lumen was open. None of these 3 snakes had food in the stomach; 9/23 other *D. kingi* (including snakes with up to 17 *S. simhai*) and 6/9 other *H. ornatus* taken in this study did have food in the stomach.

The material had been frozen and thawed at least twice, and was not suitable for detailed histological examination. Sections did reveal trematode eggs in all of the cysts (both large and small) examined; many (including most of the small cysts examined) also contained what appeared to be fragments of trematode tegument. The trematodes were frequently found flattened against the cyst wall, but were sometimes found in the center of the cellular debris.

Discussion

This parasite apparently infects a small proportion of individual sea snakes, but can reach high numbers in some infected snakes, suggesting that its metacercariae are highly aggregated in the intermediate hosts. Many host snakes (8 of 15 for which I have good records) also had cysts containing eggs, but no adults, suggesting that additional worms, present earlier, had died and disintegrated. Cysts of this type were not found in any of the snakes that did not harbor *S. simhai*.

The very large masses found in 3 of the snakes occupied a high proportion of the space in the posterior part of the body cavity. Such a mass might be expected to interfere with feeding or with reproduction. One snake did have an occluded gut, and neither of the other 2 contained food in the stomach, suggesting that the masses may have interfered with feeding. However, there was no evidence of interference with reproduction. Two of the 3 snakes were collected during the breeding season (based on data on reproductive conditions in 492 females of 13 species, in prep.); they had the expected enlarged ova and testes, respectively. Snakes with fewer worms and smaller clusters of cysts showed no evidence that their infections had influenced either feeding or reproduction. Although *S. simhai* has the potential to be pathogenic in sea snakes, realizing that potential apparently requires a large number of worms, and appears to be relatively infrequent.

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The Brayton H. Ransom Memorial Trust Fund was established in 1936 to "encourage and promote the study and advance of the Science of Parasitology and related sciences." Income from the Trust currently provides token support of the *Proceedings of the Helminthological Society of Washington* and limited support for publication of meritorious manuscripts by authors lacking institutional or other backing. Contributions may be directed to the Secretary-Treasurer.

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Balance on hand, 1 January 1988	$ 9,876.73
Receipts:	
Net interest received in 1988	954.45
Donation*	878.40
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Disbursements:	
Grant to the Helminthological Society of Washington for 1988	($ 50.00)
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A Comparative Study of Endoparasites in Three Species of Sympatric *Bufo* (Anura: Bufonidae), from Texas

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ABSTRACT: Seventy immature and adult toads, *Bufo* spp., including 27 *B. debilis debilis,* 23 *B. valliceps valliceps,* and 20 *B. woodhousii woodhousii* from 4 counties of northcentral Texas, were examined for endoparasites. Forty-nine (70%) of the toads were infected with 1 or more species of protozoan or metazoan parasites. New host records are reported for *Nyctotherus cordiformis* Ehrenberg, 1838, *Myxidium serotinum* Kudo and Sprague, 1940, *Distoichometra bufonis* Dickey, 1921, and *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931, in *B. d. debilis*; *M. serotinum* and *Mesocestoides* sp. Vaillant, 1863, in *B. v. valliceps*; and *N. cordiformis* and *M. serotinum* in *B. w. woodhousii.* A new geographic locality record is reported for *D. bufonis.* Prevalence of infection and parasite intensities varied among the 3 host taxa and differences were associated with breeding season, microhabitat selection, and climatological conditions.

KEY WORDS: toads, *Bufo* spp., *Bufo debilis debilis, Bufo woodhousii woodhousii, Bufo valliceps valliceps, Opalina* sp., *Nyctotherus cordiformis, Myxidium serotinum, Distoichometra bufonis, Adelina* sp., *Eimeria* sp., *Mesocestoides* sp., metacestode, *Cosmocercoides variabilis,* prevalence, intensity, survey, anurans.

Toads of the genus *Bufo* are represented by 10 species distributed throughout various regions of Texas. These taxa inhabit diverse habitats, including marshes, river bottoms, mountain canyons, desert streams, coastal prairies, irrigation ditches, and upland cedar glades. Because bufonids are an integral part of the anuran community in many areas, comparative study of their parasites may lead to better understanding of patterns of host specificity, prevalence of infection, and possible effects of parasitism among the different species.

The few previous studies on parasites of *Bufo* spp. have been conducted on single species inhabiting widely separated geographic localities. We chose to compare the endoparasites of 3 of the more common bufonids of the state: the green toad, *B. debilis debilis* Girard, 1854, Gulf Coast toad, *B. valliceps valliceps* Wiegmann, 1833, and Woodhouse's toad, *B. woodhousii woodhousii* Girard, 1854. Only a few studies are available concerning the endoparasites of the latter 2 species (see Harwood, 1932; Trowbridge and Hefley, 1934; Walton, 1940, 1946; Kuntz, 1941; Kuntz and Self, 1944; Little, 1966); nothing is known of the parasites of the former.

Materials and Methods

Between May 1986 and September 1987, and again during June 1988, 70 toads, including 27 *B. d. debilis* ($\bar{x}$ ± SE snout–vent length [SVL] = 33.9 ± 1.1, range 28–40 mm), 23 *B. v. valliceps* (85.1 ± 4.8, 35–122), and 20 *B. w. woodhousii* (59.1 ± 3.3, 35–91), were collected in Denton, Hood, Johnson, and Somervell counties of northcentral Texas. Specimens were collected by hand either by overturning rock shelters, excavating shallow retreats, or by spotlighting during rainfall at night on roadways. Toads were placed in individually labeled plastic bags, transported to the laboratory, and examined within 24 hr for endoparasites. These anurans were killed by pithing or by overdosing with sodium pentobarbital. Blood samples were obtained by heart puncture and smears stained with Giemsa and examined for hematozoa. The digestive tract, lungs, heart, gall bladder, liver, coelomic cavity, and overlying muscle tissues of each individual were examined for parasites. A portion of the large intestine, along with feces, was placed in vials containing tap water supplemented with antibiotic (100 I.U./ml penicillin-G–100 μg/ml streptomycin) and mailed to Kansas State University for examination of coccidia. Samples were examined microscopically following flotation in a modified Sheather's sugar solution (sp. gr. 1.30). Gall bladder and intestinal contents were smeared on glass slides, placed in hot Schaudinn's fixative, and stained with Gomori trichrome for examination of certain protozoans. Some tissues containing metacestodes were removed from the hosts for parasite identification and histopathological studies. This material was fixed in 10% formalin, embedded in Paraplast, sectioned at 7 μm, affixed to glass slides, stained with Harris' hematoxylin and eosin counterstain, and mounted in damar. Non-encapsulated immature and adult cestodes were relaxed and killed by slowly warming them in petri dishes containing 0.6% saline, fixed in alcohol–formaldehyde–acetic acid (AFA) for 24 hr, and trans-

Table 1. Endoparasites of 3 species of toads, *Bufo* spp., in Texas.

Parasite	Host species and prevalence of infection		
	B. d. debilis	*B. v. valliceps*	*B. w. woodhousii*
Protozoa			
Sarcomastigophora			
Opalina sp.	0/27 (0%)	2/23 (8.7%)	1/20 (5.0%)
Ciliophora			
Nyctotherus cordiformis	2/27 (7.4%)*	0/23 (0%)	1/20 (5.0%)*
Apicomplexa			
Eucoccidiorida			
Adelina sp.-like coccidian	0/27 (0%)	2/23 (8.7%)	0/20 (0%)
Eimeria sp.	0/27 (0%)	1/23 (4.3%)	0/20 (0%)
Myxozoa			
Bivalvulida			
Myxidium serotinum	2/27 (7.4%)*	6/23 (26.1%)*	7/20 (35.0%)*
Cestoidea			
Cyclophyllidea			
Unidentified metacestode	0/27 (0%)	0/23 (0%)	1/20 (5.0%)
Mesocestoides sp.	0/27 (0%)	3/23 (13.0%)*	0/20 (0%)
Distoichometra bufonis	4/27 (14.8%)*	0/23 (0%)	10/20 (50.0%)
Nematoda			
Ascaridida			
Cosmocercoides variabilis	14/27 (51.9%)*	16/23 (69.6%)	0/20 (0%)

* New host record.

ferred to 70% ethanol. They were later stained in Mayer's hematoxylin or Semichon's acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in permount. Nematodes were killed in hot AFA, placed in 70% ethanol, and transferred to lactophenol or glycerol for clearing and study.

Voucher specimens are deposited in the U.S. National Museum Helminthological Collection, USDA, Beltsville, Maryland 20705 as follows: *Opalina* sp. (USNM 80385); *Nyctotherus cordiformis* (USNM 80386); *Myxidium serotinum* (USNM 80382-80384); *Mesocestoides* sp. tetrathyridia (USNM 80381); tetracetabulate anacanthocysticercus (USNM 80380); *Distoichometra bufonis* (USNM 80387-80391); and *Cosmocercoides variabilis* (USNM 80378-80379).

Results and Discussion

Forty-nine (70%) of the toads were infected with 1 or more endoparasites, including 14 (51.9%) *B. d. debilis,* 20 (87.0%) *B. v. valliceps,* and 15 (75.0%) *B. w. woodhousii* (Table 1). Blood samples were negative for apicomplexans or trypanosomes. New host records are reported for several endoparasites (see Table 1).

An unidentified species of *Opalina* Purkinje and Valentin, 1840, was found in the rectum of an immature (35 mm SVL) and adult (83 mm SVL) male *B. v. valliceps* and in a single adult (73 mm SVL) male *B. w. woodhousii.* Specific identification was impossible because of a low prevalence of infection, an absence of a series of infections, and a range of forms. Although we did not find opalinids in any of the 27 *B. d. debilis,* Metcalf (1923) noted "a very few cysts of some species of Opalinidae" in 1/9 (11.1%) *B. debilis* from an unknown locality near the Texas-Mexico border. In addition, Walton (1946) listed *O. obtrigonoidea* Metcalf, 1923, and *O. triangularis* Ghosh, 1918, in *B. valliceps* from Texas, and *O. woodhousii* Metcalf, 1923, and an unidentified *Opalina* sp. (of Trowbridge and Hefley, 1934) in *B. woodhousii* from Utah, Arizona, and Oklahoma.

Walton (1946) reported *Nyctotherus cordiformis* Ehrenberg, 1838, in *B. valliceps.* However, we did not recover this endosymbiont from any Gulf Coast toads; only 2 subadult (29 and 30 mm SVL) male *B. d. debilis* and an immature (55 mm SVL) female *B. w. woodhousii* were infected. In addition, Frandsen and Grundmann (1960) reported *N. cordiformis* in the boreal toad, *B. boreas,* from Utah.

In the gall bladder contents of all 3 species of

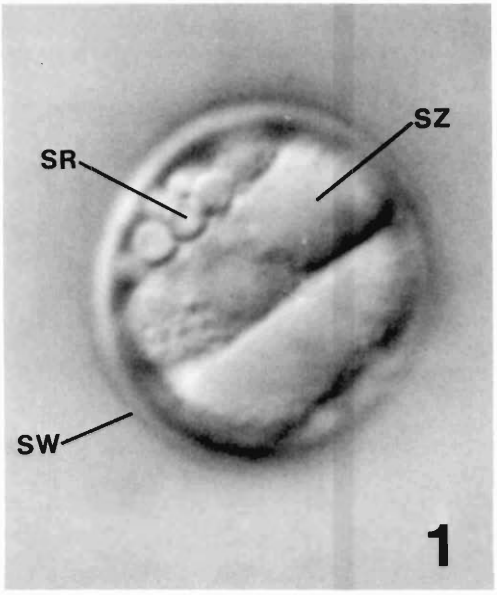


Figure 1. Nomarski interference-contrast photomicrograph of adelid-like sporocyst (*Adelina* sp.?) from *Bufo valliceps valliceps*. ×2,688. SR, sporocyst residuum; SW, sporocyst wall; SZ, sporozoite.

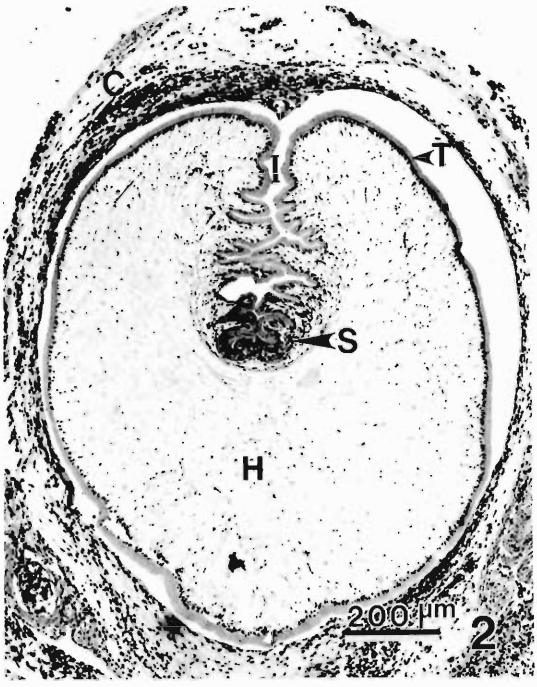
toads, spores and trophozoites of *Myxidium serotinum* Kudo and Sprague, 1940, were present. Only 2 adult (33 and 38 mm SVL) male *B. d. debilis* were infected; however, prevalence was higher in *B. v. valliceps* and *B. w. woodhousii* (Table 1). This myxosporean has previously been reported in the bile of toads, *Bufo* sp. (Kudo, 1943), and southern toads, *B. terrestris* (Kudo, 1966) from Florida, and in frogs and salamanders (see McAllister, 1987; McAllister and Upton, 1987a).

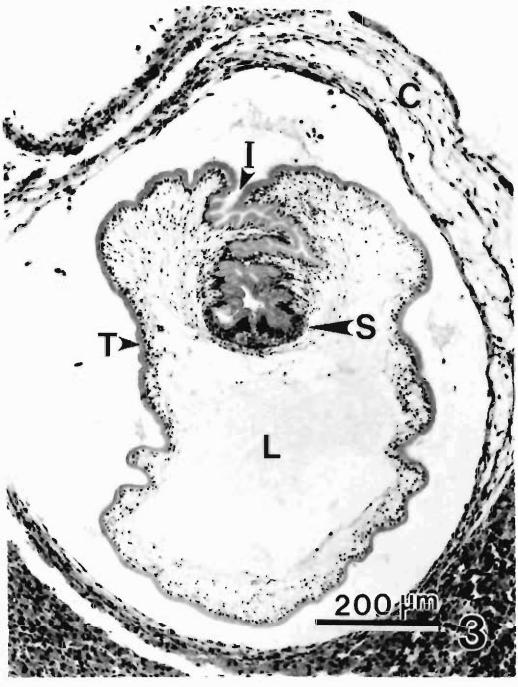
In the feces of a single *B. v. valliceps,* we observed unsporulated coccidian oocysts. Upon sporulation in 2.5% (w/v) aqueous $K_2Cr_2O_7$ for 1 wk at ca. 23°C, oocysts were determined to be those of an *Eimeria* sp. Schneider, 1875. Oocysts had a moderately pitted oocyst wall, measured ca. 20 × 16 μm, and possessed a single globular oocyst residuum, polar granule, and ovoid sporocysts with Stieda bodies. Oocysts were of the "type A" morphology (see Reduker et al., 1987) characteristic of rodent coccidia. Since this toad was, at the time of capture, inhabiting an abandoned rodent's nest, these oocysts were judged to be pseudoparasites of *B. v. valliceps* (Upton and McAllister, 1988). It is known that bufonid toads are quite opportunistic in their food habits and, perhaps, as suggested by this observation, are also coprophagic.

Two *B. v. valliceps* were found to be passing moderate numbers of what appeared to be adelid sporocysts (Apicomplexa: Adeleidae). Each sporocyst was spherical or subspherical, had a relatively thick wall, 2 sporozoites, and a coarse, granular residuum (Fig. 1). Because all known genera (*Adelina* Schneider, 1875) are found in invertebrates (annelids, chilopods, priapulids, and insects), we hypothesized that these coccidia were pseudoparasites. To test this hypothesis, we placed 2 wild-caught *B. v. valliceps* into 38-liter glass aquaria, examined feces for 3 wk to assure the absence of coccidia, and inoculated each toad orally with 2.0×10^4 3-wk-old sporocysts. Feces were collected continually for 2 wk after the inoculations and microscopic examination revealed no additional coccidia. In addition, microscopic examination of the intestinal tract, liver, gall bladder, and kidneys from each of the toads at the end of the 2-wk period revealed no coccidial developmental stages, thus supporting our hypothesis.

Three *B. v. valliceps* were infected with numerous tetrathyridia of *Mesocestoides* sp. Vaillant, 1863. These occurred in the liver, intestines, and musculature, where they frequently were localized under the peritoneal capsules and in adjacent mesenteries (Fig. 2). Presence of worms was accompanied by a moderate amount of localized inflammation. All the worms appeared viable, but had been encapsulated by the host. Each tetrathyridium possessed a single deeply invaginated scolex, and showed no sign of asexual proliferation.

Species of the genus *Mesocestoides* can be identified only from adult characteristics, but generic identity of metacestodes can be determined by their tetracetabulate scolex, lack of an apical organ or armed rostellum, and solid cellular hindbody that results from primitive rather than neoteric development (Fig. 2). Thus, in terms of comparative metacestode structure, tetrathyridia are invaginated anacantho-tetracetabulo-plerocercoids (Freeman, 1973). The possible taxonomic significance of asexual proliferation in tetrathyridia is unclear; however, worms reported here are of the far more common non-proliferative type (Conn, 1988). Tetrathyridia have been reported previously from American toads, *B. americanus,* and Great Plains toads, *B. cognatus,* in Iowa and South Dakota (James and





Figures 2, 3. Brightfield light micrographs of mid-longitudinal sections of metacestodes from *Bufo* spp. Each is surrounded by a fibrous host capsule (C) and possesses a deep invagination canal (I), distinct tegument (T), and well-developed tetracetabulate scolex (S). 2. *Mesocestoides* sp. tetrathyridium in the mesenteries of *B. v. valliceps*. Note the solid cellular hindbody (H). 3. Unidentified metacestode in the liver of *B. w. woodhousii*. The hindbody is occupied by a primary lacuna (L) filled with amorphous non-cellular material.

Ulmer, 1967). The only other anurans reported to serve as hosts in North America are green frogs, *Rana clamitans,* leopard frogs, *R. pipiens,* and Strecker's chorus frogs, *Pseudacris streckeri streckeri* (James and Ulmer, 1967; Williams and Taft, 1980; McAllister, 1987). Various mammals and reptiles are also naturally infected (McAllister, 1988).

A single *B. w. woodhousii* harbored several unidentified metacestodes in the liver parenchyma (Fig. 3). Little inflammatory exudate surrounded these worms, but most had been encapsulated by the host. Both worms and host liver tissue appeared healthy.

The identity of these metacestodes could not be determined, but they clearly were not *Mesocestoides* tetrathyridia. The most obvious distinguishing feature is the presence of a primary lacuna filled with amorphous non-cellular material in the unidentified worm (Fig. 3). According to the scheme of Freeman (1973), these can be described as invaginated anacantho-tetracetabulo-cysticerci or possibly -precysticerci, and apparently belong to the order Cyclophyllidea. Cyclophyllidean, proteocephalidean, and pseudophyllidean metacestodes have been reported from amphibians (Prudhoe and Bray, 1982). In addition, Thomas et al. (1984) reported unidentifiable tetrathyridia in the gall bladder, mesenteries, heart, and liver of Houston toads, *B. houstonensis,* from southeastern Texas, and Brandt (1936) found proteocephalid cysts in Fowler's toad, *B. w. fowleri.* Unfortunately, lack of accurate life cycle information on many amphibian cestodes, and a paucity of detailed information on comparative metacestode structure, makes species identification impossible.

Immature pre-stobilar metacestodes and adult nematotaeniid tapeworms, *Distoichometra bufonis* Dickey, 1921, were found in the small intestine of 14 (20.6%) toads, including 3 male and 1 female *B. d. debilis* (adults, 37.0 ± 1.2 mm) and 6 male and 4 female *B. w. woodhousii* (juveniles and adults, 56.3 ± 4.0 mm). Mean intensities were 5.5 ± 1.3 (range = 2–8) and 24.2 ± 9.5 (range = 4–105) worms in *B. d. debilis* and

B. w. woodhousii, respectively. Interestingly, none was found in any *B. v. valliceps.* This cestode has been previously reported from southern toads, *B. lentiginosus* (=*terrestris*) in Georgia (Dickey, 1921); *B. w. fowleri* and eastern spadefoot toads, *Scaphiopus holbrookii* in North Carolina (Brandt, 1936; Douglas, 1958); and Texas toads, *B. speciosus,* Great Plains toads, *B. cognatus,* Couch's spadefoot toads, and *S. couchii* in Oklahoma (Kuntz, 1941); and *B. w. woodhousii* in Oklahoma (Kuntz, 1941) and Nebraska (Hardin and Janovy, 1988).

Since its original description as *Oxysomatium variabilis* from the intestine of *B. valliceps, Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931, has been reported in numerous amphibians and reptiles of southern Texas and in wood frogs, *R. sylvatica* from New York (Baker, 1987). In our study, this nematode was the most common parasite of both *B. d. debilis* and *B. v. valliceps* (combined prevalence = 60%); mean intensity was 11.0 ± 1.8 (range = 3–25) and 13.9 ± 3.6 (range 1–51), respectively. However, none of the *B. w. woodhousii* was infected.

Recently, evidence provided by Vandenburgh and Anderson (1987) has shown that a related species, *C. dukae* (Holl, 1928) Travassos, 1931, formerly thought to be a ubiquitous parasite of amphibians (Baker, 1978; McAllister and Upton, 1987b), is primarily a roundworm parasite of molluscs and represents an accidental parasite of amphibians.

In summary, differences in prevalence of infection and parasite intensities among the 3 species of *Bufo* were evident in our study and we offer the following observations which may account for these findings. Of the 3 taxa, *B. d. debilis,* a more arid-adapted toad, was seldom seen in open areas except during and after periods of heavy rainfall; it most often remained inactive in burrows beneath limestone rocks. Its narrow temporal niche may explain the lower prevalence and diversity of parasites in the species. Likewise, *B. w. woodhousii,* which was moderately parasitized, is not as widely distributed throughout the southwest and is highly restricted to localized stream-edge habitat (Axtell, 1963; Wiest, 1982). However, the species showing the highest prevalence and diversity of parasites, *B. v. valliceps,* occurs in a wide variety of habitats, even in altered regions such as garbage dumps and storm sewers. Since toads are restricted to breeding in aquatic sites (i.e., suggesting an increased opportunity for exposure to diversity of parasites), fluctuations in seasonal weather conditions may alter the pattern of parasitism as observed herein. Precipitation during the spring and early summer months initiates calling and male territoriality. The reproductive seasons of all 3 bufonids overlap, even to some degree with other anurans in Texas (Wiest, 1982), and this would permit contact between larvae and possible exchange of parasitic stages.

Finally, our interpretation of the data was based on information gathered over a period of several months. In addition, the number of hosts examined was limited and the numbers of parasites recovered are insufficient to permit statistical comparisons between host species. We suggest that comparative parasitological data must be accumulated for several years and additional hosts should be examined before conclusive statements may be made on parasitism among the species.

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In Vitro Cultivation of *Echinostoma revolutum* (Trematoda) Metacercariae in an Extract of Chick Mucosal Epithelium

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ABSTRACT: *Echinostoma revolutum* metacercariae cultivated in NCTC 135 supplemented with 40% chicken serum and 10% mucosal extract from the lower ileum of the domestic chick increased their body area 3× by 8 days and showed genital development. Growth of these worms was equivalent to growth and development of worms in domestic chicks for 2 days. Metacercariae cultivated in NCTC 135 plus 50% chicken serum showed minimal somatic growth and no genital development. Worms stained in Oil Red O from chicks showed sparse accumulation of neutral lipid in the excretory system. Accumulation of neutral lipid was intense in worms grown in the medium containing chicken serum and moderate in worms grown in the medium containing a mucosal extract. Accumulation of lipid may serve as an indicator of the suitability of the habitat in supporting growth of this parasite; worms with the least accumulation of neutral lipid grew the best and vice versa.

KEY WORDS: Trematoda, *Echinostoma revolutum,* in vitro culture, growth and development, lipid histochemistry, thin-layer chromatography.

Relatively few studies of in vitro cultivation of non-progenetic hermaphroditic digeneans appear in the literature (Fried, 1978; Clegg and Smith, 1987). Davies and Smyth (1978) cultivated *Fasciola hepatica* metacercariae to a six-fold increase in length but with little genital development. Recently, metacercariae of 2 species of *Paragonimus* were cultivated to adults with well-developed genitalia, but lacking eggs or with abnormal eggs (Hata et al., 1987). Howell (1968) reported limited development of *Echinoparyphium serratum* metacercariae cultivated in a yolk-albumen-saline medium supplemented with yeast extract. *Echinostoma revolutum* metacercariae doubled their mean body area in NCTC 135 supplemented with 20% hen's egg yolk within 14 days (Butler and Fried, 1977).

The effect of gastrointestinal mucus on the in vitro cultivation of helminths is poorly understood, and Miller (1987) has reviewed the role of gastrointestinal mucus as a medium for the survival of parasitic nematodes. Wisnewski et al. (1986) reported that *E. revolutum* adults feed on the mucosa of the lower ileum of the domestic chick. NCTC 135 supplemented with 40% chicken serum and 20% chick mucosal extract was used to culture tetracotyles of 3 species of *Cotylurus* (Basch et al., 1973; Fried et al., 1978; Magnus and Johnson, 1985). Studies on the use of chick mucosal extract media to culture non-progenetic digeneans are not available.

The present study compares the development of excysted metacercariae of *E. revolutum* metacercariae cultivated in vitro in a defined medium supplemented with either chicken serum alone or chicken serum plus mucosal extract from the lower ileum of the domestic chick. Comparisons of cultivated worms with those grown in domestic chicks for 2 days were also made.

Materials and Methods

Metacercarial cysts of *Echinostoma revolutum* were removed from the kidneys of experimentally infected *Biomphalaria glabrata* snails (Anderson and Fried, 1987), excysted in an alkaline trypsin-bile medium (Fried and Emili, 1988), and used for in vitro cultivation studies within 30 min of excystation. Excysted metacercariae were rinsed rapidly in 3 changes of sterile Locke's solution containing penicillin (200 units/ml) and streptomycin (200 μg/ml) (Fried and Contos, 1973), inoculated into capped culture tubes (20 metacercariae per 8 ml of medium), and incubated in an upright position at 37.5°C. Three media were used: the defined medium NCTC 135 (NCTC 135) alone, NCTC 135 supplemented with 50% chicken serum (NCTC 135 + 50 CS), and NCTC 135 supplemented with 40% chicken serum and 10% mucosal extract from the lower ileum of the domestic chick (NCTC 135 + 50 CS + 10 M). The defined medium NCTC 135, chicken serum, and antibiotics were purchased from Grand Island Biological Company (Grand Island, New York). The gas phase was air. One-half of the medium was removed daily and replaced with fresh medium. Experiments were arbitrarily terminated on day 8.

Preparation of the mucosal extract was modified from Basch et al. (1973). About 20 cm of the lower ileum from each of 3 freshly killed 14-day-old domestic chicks were removed, slit longitudinally, and rinsed briefly in

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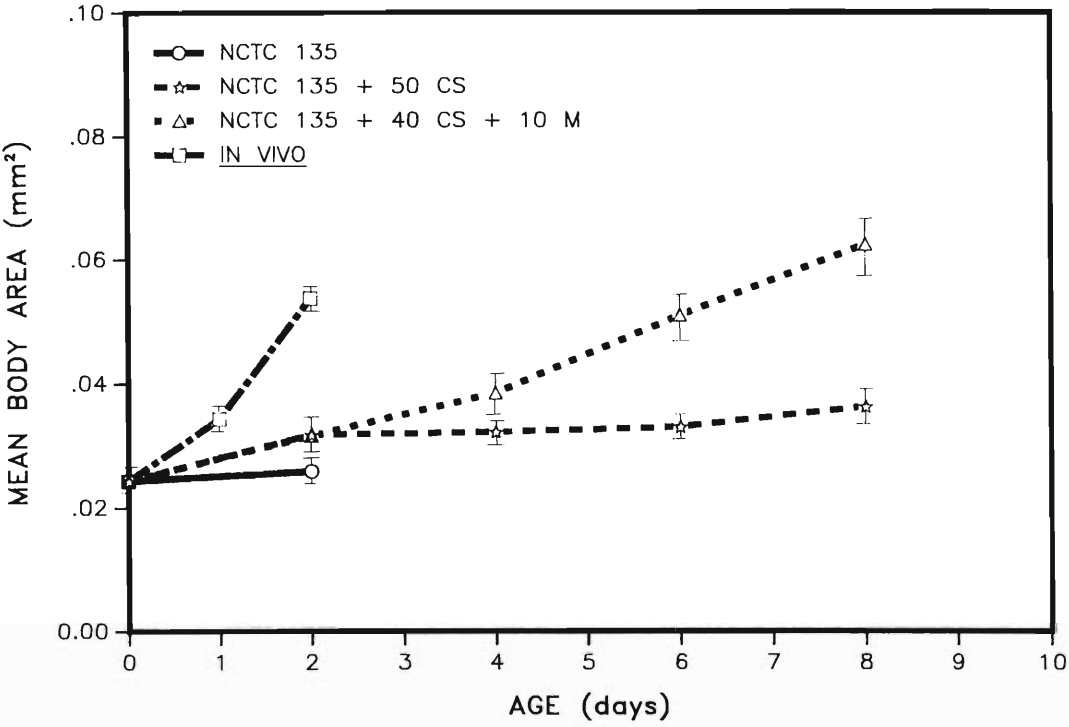


Figure 1. Body area measurements of *Echinostoma revolutum* cultivated in vitro and in vivo. Each bar represents the mean ± SE of 15 worms for the in vivo study and 24 worms for the in vitro studies.

Locke's solution. The mucosal layer was scraped off with a glass microscope slide, collected, and homogenized in a tissue grinder. The homogenate was made up to 50 ml in sterile Locke's solution and then centrifuged at 2,000 *g* for 60 min. The supernatant was filtered first through glass wool and then through a 0.45-μm Acrodisc filter from Gelman Scientific, Inc. (Ann Arbor, Michigan) for sterilization. The extract was either used immediately or after storage at −20°C for up to 3 mo with no apparent differences in culture.

For in vivo growth studies 6 1-day-old domestic chicks were each fed about 300 metacercarial cysts and necropsied 1 or 2 days postinfection. Preovigerous worms recovered from the lower ileum were prepared for morphological studies as described below.

To assess worm growth, organisms cultivated in vitro ($N = 24$ per day/culture medium for up to 8 days) and those grown in vivo ($N = 15$ per day for 1 and 2 days) were placed in depression slides, relaxed at 4°C, and measured live with a calibrated ocular micrometer. The length and width of each worm was measured to determine the relative body area (Berntzen and Macy, 1969). Some worms grown in vitro and in vivo were fixed in cold 10% neutral buffered formalin and stained with either Gower's carmine for general morphology or Oil Red O for localization of neutral lipids.

Thin-layer chromatography (TLC) was used to analyze neutral lipids in 40 excysted metacercariae and 40 worms cultivated in each of the 3 media for 2, 4, and 6 days. In addition, 0.5-ml samples of each medium removed on days 2, 4, and 6 were examined by TLC. Worms and media were extracted in 2 ml chloroform/methanol (2:1) and applied along with neutral lipid standards to the origins of Whatman LK6D, 20 × 20-cm, silica gel plates (Fried and Sherma, 1986). Chromatograms were developed in hexanes/diethyl ether/acetic acid (80:20:1) and neutral lipids were detected by dipping the chromatograms in 5% phosphomolybdic acid (PMA) in 95% ethanol and drying them at 110°C for 5 min.

Results

Worms cultivated in NCTC 135 did not survive beyond 2 days. Almost 100% of the worms in supplemented media survived for 8 days at which time the cultures were arbitrarily terminated. From 30 to 50 juvenile worms were recovered from each chick at 1 and 2 days postinfection, and used to compare body area measurements of worms grown in vitro (see Fig. 1). Worms were not grown in chicks beyond 2 days, because the growth of older in vivo worms far exceeded that of echinostomes grown in vitro.

Figure 1 shows the mean body area (±SE) of worms cultivated in vitro in the 3 media and in vivo for 1 and 2 days. Worms cultivated in NCTC 135 + 40 CS + 10 M for 8 days showed an approximate 3× increase in mean body area

compared to excysted metacercariae (day 0), and an approximate 2× increase compared to worms cultivated in NCTC 135 + 50 CS for 8 days. Worms grown in NCTC 135 + 40 CS + 10 M for 8 days were slightly larger than those grown in chicks for 2 days, whereas worms grown in NCTC 135 + 50 CS for 8 days were slightly smaller than worms grown in chicks for 1 day.

Compared to worms grown in NCTC for 2 days, there was a considerable increase in worm growth from days 2 to 8 for worms cultivated in either NCTC 135 + 50 CS or NCTC 135 + 40 CS + 10 M. Moreover, there was a considerable increase in growth of worms from days 4 to 8 in NCTC 135 + 40 CS + 10 M compared to those in NCTC 135 + 50 CS. Figure 2 shows a live worm (top) cultivated for 6 days in the mucosal extract medium compared to a newly excysted metacercaria.

Only genital anlagen were observed in excysted metacercariae stained in Gower's carmine (see fig. 1 *in* Fried and Pentz, 1983). Worms cultivated in NCTC 135 + 40 CS + 10 M for 8 days showed enhanced genital development both anterior and posterior to the acetabulum (Fig. 3). The area of the genital anlagen in these cultured worms was about 5× that seen in excysted metacercariae. Similar development was observed in worms grown in vivo for 2 days. Worms grown in media lacking mucosal extract showed no genital development.

Gut contents were clearly seen in the intestinal ceca of worms cultivated in the mucosal extract medium (Fig. 4). This was not the case for worms grown in the other media or for excysted metacercariae.

Histochemical studies on excysted metacercariae stained in Oil Red O and worms grown for 1 and 2 days in vivo showed only traces of neutral lipids in the excretory system (Fig. 5). Localization of neutral lipids in the excretory system was greater in worms grown in NCTC 135 + 40 CS + 10 M and such worms also showed abundant neutral lipid droplets in the oral collar zone (Fig. 6). The localization of neutral lipids was intense in the excretory system, parenchyma, and suckers and oral collar of worms cultivated in NCTC 135 + 50 CS for 8 days (Fig. 7).

TLC analysis showed that the major neutral lipid fraction of excysted metacercariae was composed of free fatty acids, with lesser amounts of triacylglycerols and free sterols. Worms cultivated in NCTC 135 alone for 2 days contained minimal amounts of free fatty acids and free sterols and a trace of sterol esters. Worms cultivated in NCTC 135 + 50 CS for 2, 4, and 6 days showed mainly sterols and triacylglycerols. Worms cultivated in NCTC 135 + 40 CS + 10 M showed sterol esters at 2 days, and sterol esters and triacylglycerols at 4 and 6 days. Unsupplemented NCTC 135 was neutral lipid negative. Both NCTC 135 + 50 CS and NCTC 135 + 40 CS + 10 M showed mainly free fatty acids, free sterols, and sterol esters, with lesser amounts of triacylglycerols.

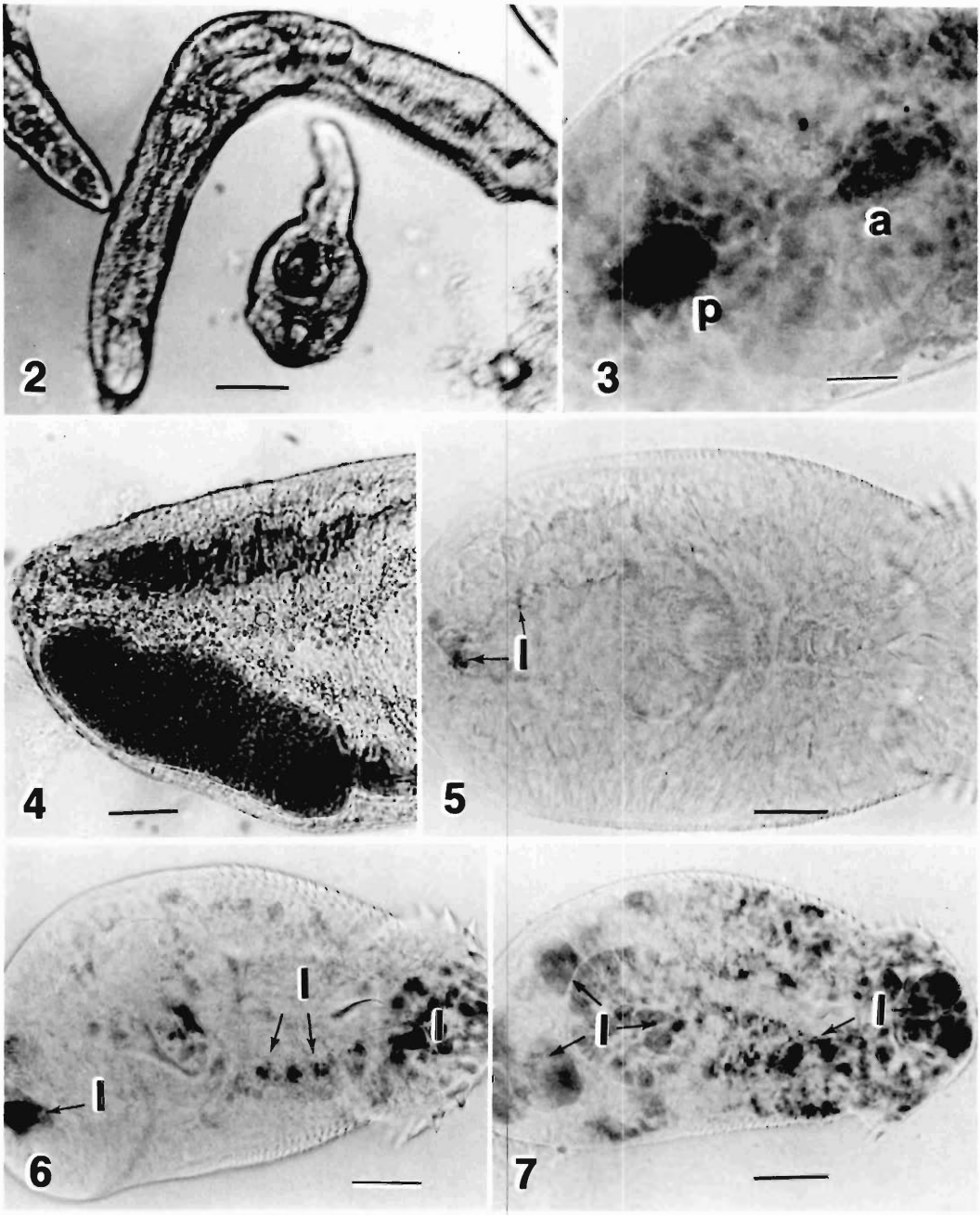
Discussion

Basch et al. (1973) found that tetracotyles of *Cotylurus lutzi* became ovigerous adults in NCTC 135 supplemented with 40% chicken serum and 20% chicken duodenal mucosal extract. In the present study, a significant increase in body area and enhanced genital development were observed in *E. revolutum* metacercariae cultivated in NCTC 135 + 40 CS + 10 M.

Echinostoma revolutum metacercariae have been cultivated to the preovigerous uterine coiling stage on the chick chorioallantois (strictly speaking, not an in vitro site), a stage about equivalent to worms grown in chicks for 7 days (Fried and Pentz, 1983). On the chorioallantois, this echinostome feeds on blood, whereas those grown in chicks feed on the intestinal mucosa (Wisnewski et al., 1986). In the present study, worm feeding was also apparent in the mucosal extract medium.

In the present study, growth and development of worms in NCTC 135 + 40 CS + 10 M was about equivalent to that seen in 2-day-old adults from chicks. Such growth and development was better than the 2× increase in somatic growth in the absence of gonadal development when *E. revolutum* metacercariae were cultivated in NCTC 135 supplemented with 20% hen's egg yolk (Butler and Fried, 1977). Presumably in that study, physical and chemical factors associated with yolk lipids enhanced growth.

In the present study, histochemical observations on distribution of lipids suggest that in situations that promote the best growth and development of *E. revolutum*, i.e., the chick ileum, localization of lipids in the worm is minimal, whereas in the least successful medium, i.e., defined medium supplemented with chicken serum, localization of lipids in worms is maximal.



Figures 2–7. Photomicrographs of *E. revolutum* cultivated in vitro and in vivo. 2. Live worm (top) cultivated for 6 days in mucosal extract medium compared to a newly excysted metacercaria. Scale bar = 76 μm. 3. Posterior (p) and anterior (a) genitalia of 6-day-old worm cultivated in mucosal extract and stained in carmine. Scale bar = 17 μm. 4. Live worm cultured in mucosal extract for 6 days showing ingesta in the intestinal ceca. Scale bar = 30 μm. 5. Worm grown for 2 days in chick and stained with Oil Red O. Note sparse lipid (l) droplets in the excretory system. Scale bar = 29 μm. 6. Worm cultivated for 8 days in mucosal extract medium and stained with Oil Red O. Note moderate lipid (l) droplets in excretory system and oral collar. Scale bar = 38 μm. 7. Worm cultivated for 8 days in chicken serum medium and stained with Oil Red O. Note heavy lipid (l) droplets in suckers, excretory system, parenchyma, and oral collar. Scale bar = 28 μm.

Accumulation of lipids in these worms may suggest that culture conditions are suboptimal for growth and development.

Neutral lipid profiles, as determined by TLC, were quite variable in worms cultivated in the different environments. Fewer classes of major neutral lipids were demonstrated in worms grown in vivo than in vitro. The neutral lipid profile as determined by TLC was qualitatively similar in both NCTC 135 + 50 CS and NCTC 135 + 40 CS + 10 M. Probably factors other than or in addition to mucosal lipids contribute to the enhanced growth and development of *E. revolutum* metacercariae in the mucosal extract medium.

Acknowledgments

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Note Added in Proof

The nomenclature on 37-collar-spined echinostomes in the *Echinostoma revolutum* group based on I. Kanev's Unpublished Doctoral Thesis (University of Sofia, Bulgaria, 1985) is gaining widespread acceptance among trematodologists who work with echinostomes. According to Kanev the correct name of the echinostome used in this study is *Echinostoma trivolvis.*

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Cuticular Ridge Patterns of *Marshallagia marshalli* and *Ostertagia occidentalis* (Nematoda: Trichostrongyloidea) Parasitic in Ruminants of North America

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ABSTRACT: Surface cuticular ridge patterns (synlophes) are among the most useful characteristics for identifying trichostrongyloid nematodes to species. Because *Ostertagia occidentalis* occurs only in the presence of *Marshallagia marshalli,* the former species is suspected of being a polymorphic form of the latter. This study compared the synlophes of the 2 species from *Ovis aries, Ovis canadensis, Ovis dalli, Ovibos moschatus, Antilocapra americana, Odocoileus* sp., and *Oreamnos americanus.* One synlophe with 3 lateral ridges and parallel adjacent ridges was found in both nematode species from all hosts except *Oreamnos americanus.* A unique synlophe, with 1 lateral ridge and swirling adjacent ridges, was present in both nematode species from *Oreamnos americanus* indicating that these nematodes in this host may be a separate species from those in the other ruminants. The failure to find synlophe differences between *Marshallagia marshalli* and *Ostertagia occidentalis* provides additional evidence that the latter may be only a polymorphic form of the former species.

KEY WORDS: Nematoda, Trichostrongyloidea, *Marshallagia marshalli, Ostertagia occidentalis, Ovis aries, Ovis canadensis, Ovis dalli, Ovibos moschatus, Antilocapra americana, Oreamnos americanus,* nematode morphology and taxonomy, synlophes, ruminants.

The Ostertagiinae are among the most severe pathogens of domesticated ruminants (American Association of Veterinary Parasitologists, 1983). Proper management of ostertagiasis is hampered because we do not know how many species of nematodes are involved. Recently, we (Lichtenfels et al., 1988a) described the synlophes (pattern of surface, longitudinal, cuticular ridges) of 7 species of Ostertagiinae. Those results provided new evidence to support the proposal of Lancaster et al. (1983) that polymorphism is common in the subfamily. Moreover, differences in the synlophes have been found to be one of the most useful characteristics for separating species of Trichostrongyloidea (Lichtenfels, 1977, 1983; Lichtenfels and Pilitt, 1983a, b; Measures and Anderson, 1983; Fukumoto, 1986; Lichtenfels et al., 1986; Hoberg and Rickard, 1988).

The objective of the present study was to describe the synlophes of 2 species of the Ostertagiinae, *Marshallagia marshalli* (Ransom, 1907) Orloff, 1933, and *Ostertagia occidentalis* Ransom, 1907. When Ransom (1907) described these 2 species from *Ovis aries* in the genus *Ostertagia,* he noted that *O. occidentalis* was found only in the company of *M. marshalli.* Again in his later monograph, Ransom (1911) noted that the 2 species appeared to be confined to the Rocky Mountain region, and *O. occidentalis* was always associated with *M. marshalli.* Much later, in developing their proposal of polymorphism in the Ostertagiinae, Lancaster and Hong (1981) pointed out that the same 2 nematode species are found together in sheep in the Middle East and that they may be morphotypes of 1 species. Our hypothesis was that if *M. marshalli* and *O. occidentalis* were different species they would probably have different synlophes.

Materials and Methods

Nematodes

All specimens were obtained from the USDA Parasite Collection maintained in this Laboratory. Host and locality data (Table 1) were obtained from the records of the collection. Common and scientific names of hosts and synonymies of nematodes are provided (Table 1). The species identities of male nematodes were confirmed prior to study of their synlophes on the basis of spicule morphology (Ransom, 1907; Shul'ts and Andreeva, 1953; Skrjabin et al., 1954; Andreeva, 1958). Female *Marshallagia marshalli* could not be distinguished from female *Ostertagia occidentalis,* which have never been described.

Microscopy

Specimens were studied either as 1) temporary whole mounts cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) and examined with ordinary light microscopy or interference-contrast light microscopy; or 2) critical point dried, coated with gold palladium, and viewed at 5–20 kV with scanning electron microscopy (SEM) (Madden and Tromba, 1976).

Cross sections were studied in free-hand cuts made

Table 1. Specimens of *Marshallagia marshalli, Ostertagia occidentalis, Marshallagia* sp., and *Ostertagia* sp. studied by host and locality.

Species and synonyms	No. of lots/no. of male and female specimens by host and locality
Marshallagia marshalli (Ransom, 1907) Orloff, 1933. Synonyms: *Ostertagia marshalli* Ransom, 1907; *O. brigantiaca* Blanchard, 1909; *O. tricuspis* Marotel, 1912; *Haemonchus* sp. Marshall, 1904; *Ostertagia orientalis* Bhalero, 1932	*Ovis aries,* sheep Alaska 5/20, 4 Colorado 4/4, 8 Montana 1/1, 2 *Ovis canadensis,* big horned sheep Montana 1/6, 6 Wyoming 1/5, 8 *Ovis dalli,* Dall sheep Alaska 1/2, 0 *Antilocapra americana,* pronghorn South Dakota 1/0, 1 *Odocoileus* sp., deer Wyoming 1/4, 0 *Ovibos moschatus,* muskox Alaska 2/6, 0
Ostertagia occidentalis Ransom, 1907. Synonyms: *Grosspiculagia occidentalis* of Jensen, 1958; not *Ostertagia trifida* Guille, Marotel, and Panisset, 1911; not *O. skrjabini* Kamenskii, 1929	*Ovis aries,* sheep Alaska 3/7, 0 Colorado 1/1, 0 Montana 1/1, 0 *Ovis canadensis,* big horned sheep Canada 2/7, 0 Idaho 1/1, 0 Montana 2/5, 0 *Ovis dalli,* Dall sheep Alaska 2/3, 0 *Odocoileus* sp., deer Wyoming 1/2, 0 *Ovibos moschatus,* muskox Alaska 2/7, 0
Marshallagia sp.	*Oreamnos americanus,* mountain goat Canada 4/12, 3 Wyoming 1/2, 3
Ostertagia sp.	*Oreamnos americanus,* mountain goat Canada 4/11, 0 Alaska 1/1, 0 Washington 1/5, 0

with a cataract knife, and embedded in glycerine jelly. Measurements are in millimeters unless indicated otherwise.

Characters studied

In addition to the synlophes of the nematodes, several morphometric characteristics were studied (Table 2).

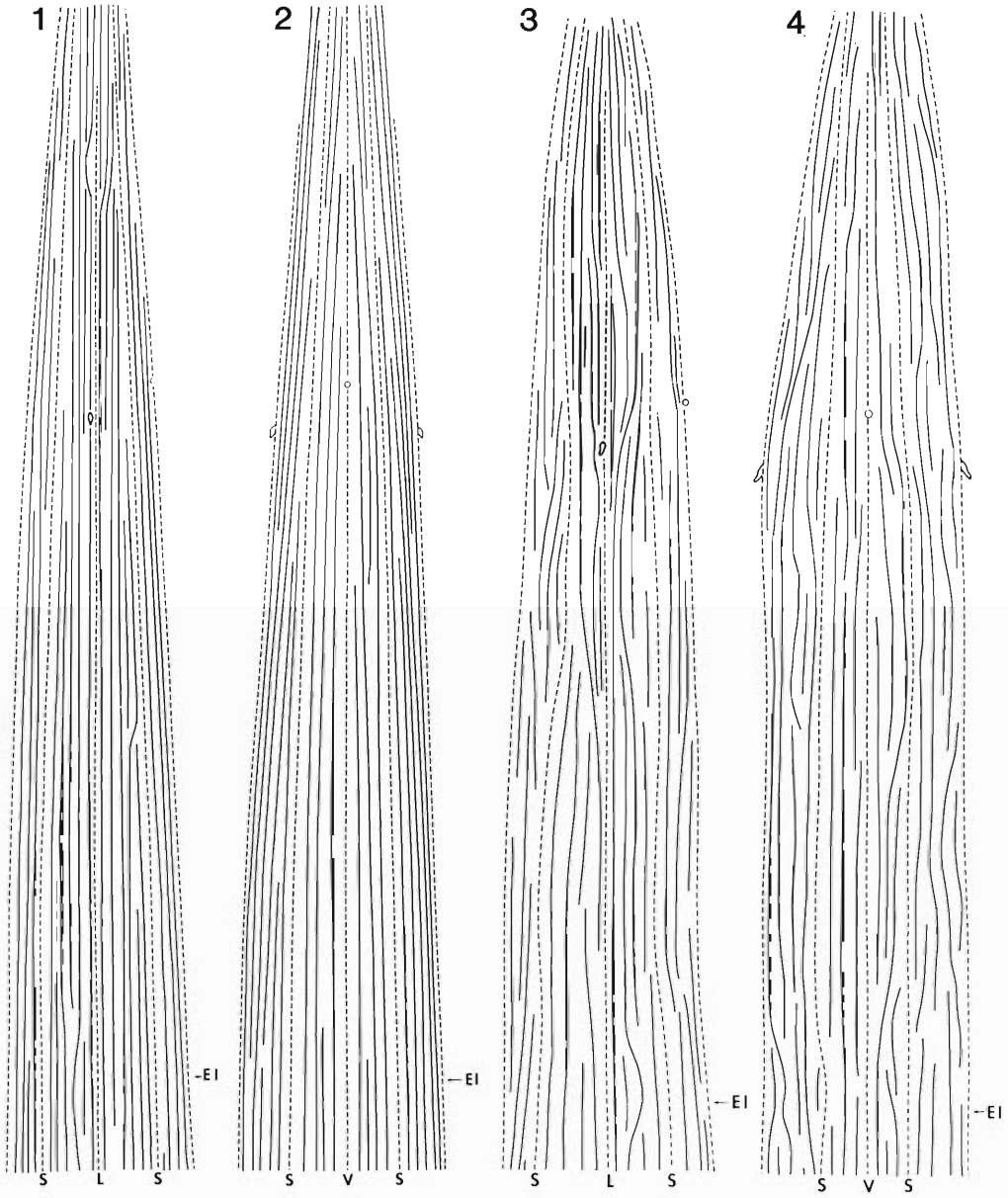
Results

The synlophes of *Marshallagia marshalli* and *Ostertagia occidentalis* were found to be identical. However, 2 different synlophes were found: 1) a 3 lateral ridge synlophe (Figs. 1, 2, 5) in both nematode species from *Ovis aries, Ovis cana-*

Table 2. Morphometrics (in micrometers; range with mean in parentheses) of males and females of *Marshallagia marshalli, Ostertagia occidentalis, Marshallagia* sp., and *Ostertagia* sp. in North American ruminants.

Characteristics	Species			
	Marshallagia marshalli	*Ostertagia occidentalis*	*Marshallagia* sp. (ex. *Oreamnos americanus*)	*Ostertagia* sp. (ex. *Oreamnos americanus*)
Males: number measured	*N* = 43	*N* = 28	*N* = 13	*N* = 15
Body length	8,400–12,000 (10,870)	7,800–15,600 (11,775)	9,240–12,800 (10,870)	11,200–14,800 (12,790)
Esophagus length	676–1,020 (838)	684–995 (866)	628–908 (716)	680–836 (772)
Esophageal–intestinal valve length	100–180 (143)	100–172 (137)	108–188 (127)	100–144 (122)
Subventral esophageal gland orifices*	236–368 (301)†	260–400 (319)	252–364 (283)	232–324 (288)
Nerve ring*	200–324 (264)†	260–380 (298)	220–280 (262)	200–300 (274)
Excretory pore*	280–424 (333)	292–440 (360)	248–340 (301)	280–380 (348)
Cervical papillae*	300–460 (363)	320–480 (396)	248–380 (328)	312–420 (369)
Spicule length	224–340 (267)	260–360 (309)	240–292 (268)	292–348 (325)
Genital cone (dorsal part) enlarged	absent	present	absent	present
Bursal ray pattern	2-1-2	2-1-2	2-1-2	2-1-2
Females: number measured	*N* = 20		*N* = 7	
Body length	12,100–16,800 (14,500)		11,400–18,900 (14,900)	
Esophagus length	720–1,020 (870)		760–796 (773)	
Esophageal–intestinal valve length	120–168 (146)		112–144 (133)	
Subventral esophageal gland orifices*	280–360 (315)		220–332 (286)	
Nerve ring*	232–368 (283)		220–284 (262)	
Excretory pore*	280–396 (330)		232–364 (293)	
Tail length	192–280 (249)‡		216–324 (276)	
Egg length × width	151–188 (172) × 62–95 (82)‡		172–193 (182) × 82–93 (88)§	
Vulva position*	71.8–80.5 (76.2%)		74.4–79.6 (76.9%)	
Anterior ovejector length	420–680 (552)‖		480–804 (656)§	
Posterior ovejector length	440–628 (525)¶		400–760 (608)§	

* From anterior end.
† *N* = 42.
‡ *N* = 19.
§ *N* = 5.
‖ *N* = 17.
¶ *N* = 18.



Figures 1–4. Diagrammatic drawings of 2 synlophe patterns, cervical regions. 1. Three lateral ridge system found in *Marshallagia marshalli* and *Ostertagia occidentalis* from various hosts, lateral view. 2. Same as preceding figure, ventral view. 3. Single lateral ridge system found in *Marshallagia* sp. and *Ostertagia* sp. from *Oreamnos americanus*, lateral view. 4. Same as preceding figure, ventral view.

densis, Ovis dalli, Odocoileus sp., and *Ovibos moschatus*; and 2) a 1 lateral ridge synlophe (Figs. 3, 4, 8) in lots identified as *M. marshalli* and *O. occidentalis* from *Oreamnos americanus.*

The 3 lateral ridge system will be described as the synlophe of *M. marshalli* and *O. occidentalis.* This is the synlophe found in all lots from *Ovis aries,* the type host of both nematode species. We also found the same 3 lateral ridge synlophe in the type specimens of both species. Specimens with the 1 lateral ridge synlophe will be described separately below and will be referred to herein as *Marshallagia* sp. and *Ostertagia* sp.

Some characteristics of the 2 synlophes ob-

served in this study may be general throughout the subfamily. Like other members of the Ostertagiinae, the longitudinal cuticular ridges are distributed in 4 relatively equal and symmetrical fields of 9–11 ridges each at the level of the cervical papillae. The lateral ridge (dashed line L in Figs. 1, 3) is thinner than adjacent ridges (Figs. 5, 8), is ventral to the cervical papilla, and is continuous throughout the length of the nematode. The ventral ridge (dashed line V in Figs. 2, 4) intersects with the excretory pore and is continuous for the nematode length. Subventral and subdorsal ridges (dashed lines S in Figs. 1–4) are also usually continuous for the length of the nematode and are counted as part of the ventral and dorsal fields, respectively. The remaining ridges in the interior of each field (solid lines in drawings) are usually somewhat more variable in length and interchangeable in position in individual specimens than the ridges that border the fields.

Synlophe of *M. marshalli* and *O. occidentalis*

The lateral fields included 11 ridges each and dorsal and ventral fields included 9 ridges each for a total of 40 ridges in the region just posterior to the cervical papillae (Figs. 1, 2, 4). Bilaterally the thin lateral ridge had an additional ridge adjacent to it both dorsally and ventrally, resulting in 3 parallel lateral ridges that extended for almost the entire length of the nematode. In most specimens, the ridges adjacent to the 3 lateral ridges were short and extended posteriorly only to the area of the cervical papillae. In some specimens, 1 of these short ridges extended the length of the nematode and the next adjacent ridge moving away from lateral was short and ended near the cervical papilla. Moving posteriorly from the cephalic region, ridges were added, anterior and posterior to the cervical papillae (Figs. 1, 2), resulting in a total of 40–44 ridges in cross sections through this region. In the region from just posterior to the esophageal–intestinal junction through the anterior quarter of the nematode, the number of ridges counted in cross sections was 40–54 (Figs. 11, 13). Through the second quarter of the nematodes the number of ridges increased slightly to 51–56 (Figs. 12, 14). In the posterior half of male nematodes the number of ridges increased from 53 at midbody to 56 just anterior to the copulatory bursa where the ventral and dorsal ridges disappeared. The lateral ridges in this region numbered 15–19 in each lateral field and extended posteriorly to the level of the prebursal papillae. The synlophe of female *M. marshalli* was almost identical to that of males of *M. marshalli* and *O. occidentalis*. Females of *O. occidentalis* are unknown. In female *M. marshalli* there were a few more ridges in the region of the vulva and ovejectors (59–61) than the maximum observed in males (56). Posterior to the middle of the postvulvar body the number of ridges decreased gradually, but some extended within 100 μm of the tip of the tail.

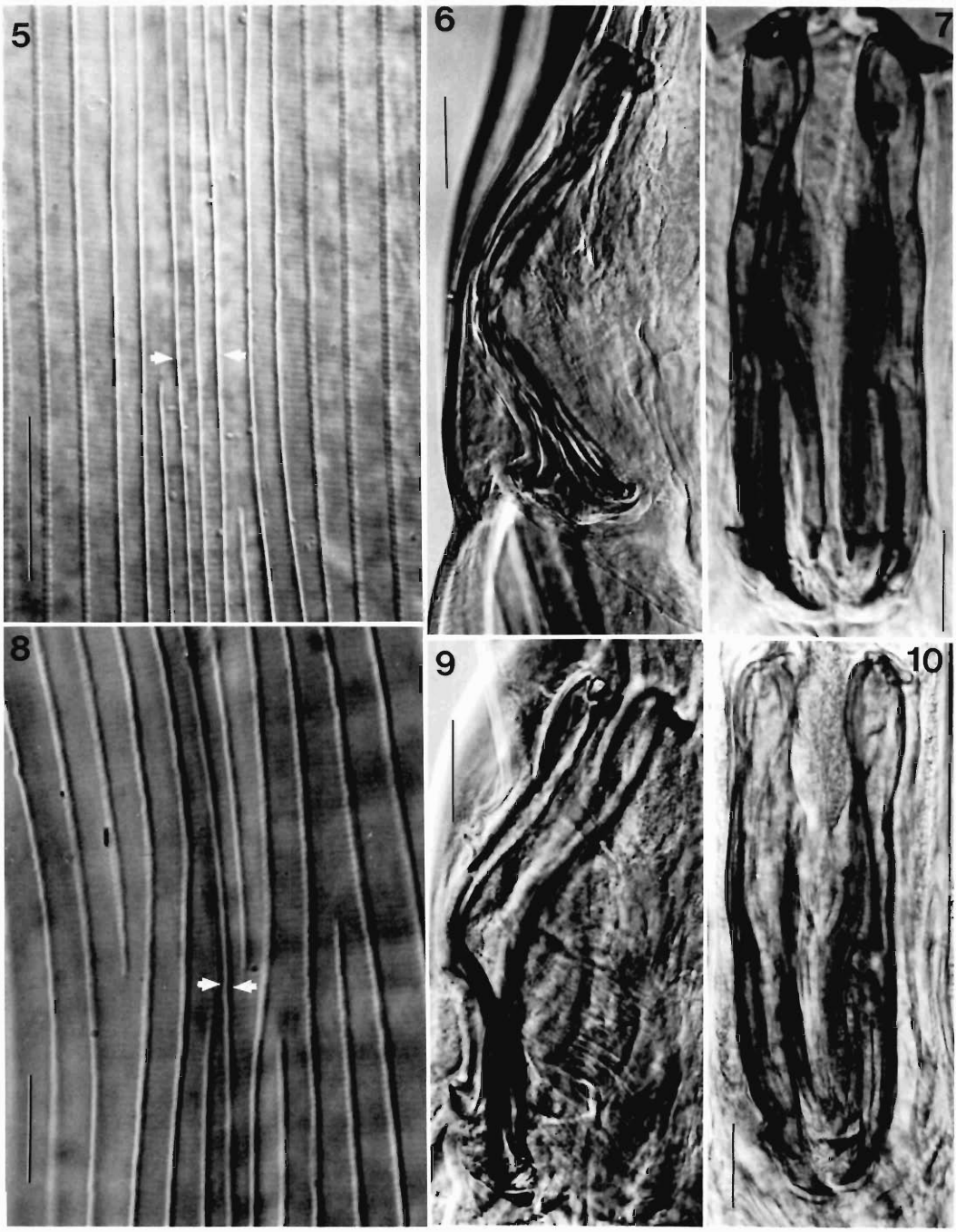
Synlophe of *Marshallagia* sp. and *Ostertagia* sp. from *Oreamnos americanus*

The synlophes of specimens identified as *M. marshalli* and *O. occidentalis* (referred to herein as *Marshallagia* sp. and *Ostertagia* sp.) were identical to each other, but were unique compared to the synlophe of those species from all other hosts. Each of the lateral fields and the dorsal and ventral fields included 9 ridges just posterior to the cervical papillae (Figs. 3, 4) for a total of 36. Laterally there was a single continuous ridge on each side, and excepting the lateral ridges and the dorsal, ventral, subdorsal and subventral continuous ridges bordering the 4 fields, many ridges were short and angled (rather than running parallel) in relation to the continuous ridges. The shorter, angled ridges gave the appearance of a swirling system of ridges rather than the straight parallel ridges usually seen in the Ostertagiinae.

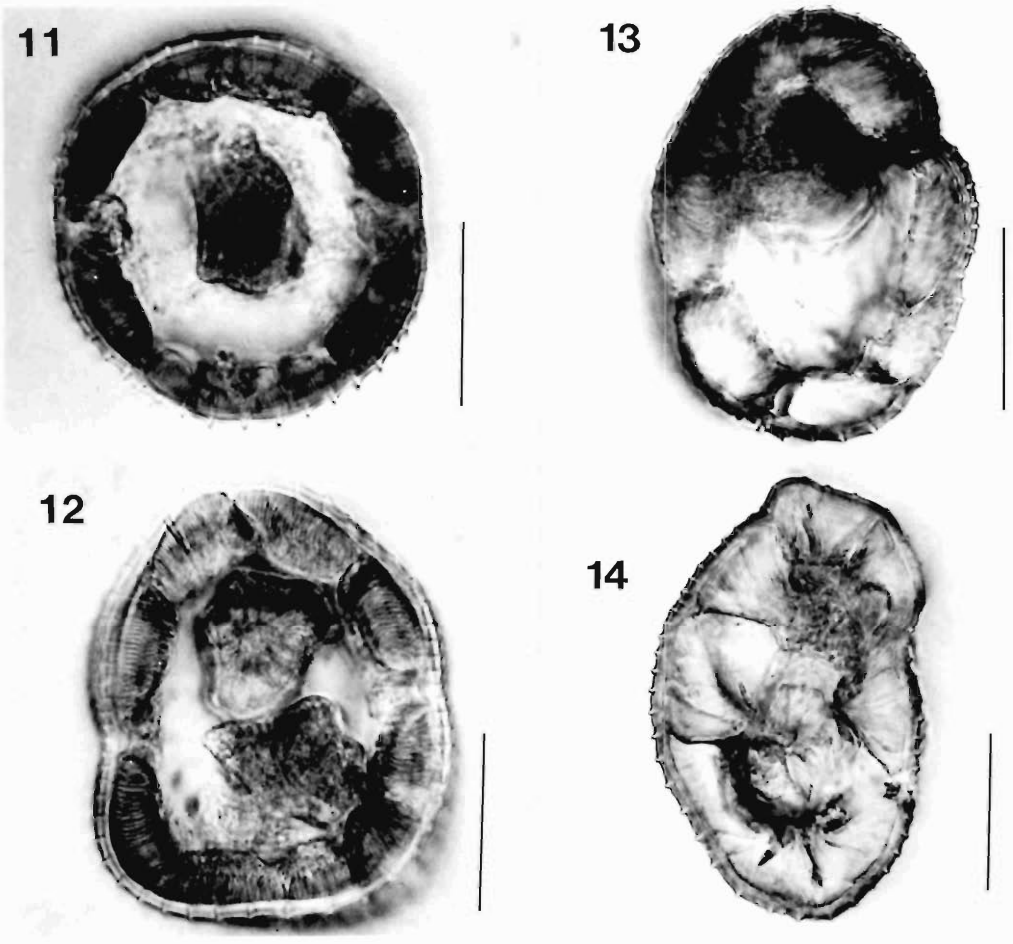
The numbers of ridges in various body regions were counted in cross sections to verify counts made on whole mounts (Figs. 15–18). Because few specimens were available, only 1 or 2 could be sectioned. Counts on whole specimens are not as accurate as those in cross sections, especially posterior to the region of the esophagus. In the region just posterior to the esophageal–intestinal junction through the first quarter of the nematode, the number of ridges was 40–50 in *Marshallagia* sp. males, 39–58 in *Marshallagia* sp. females, and 40–55 in *Ostertagia* sp. males. The number of ridges did not increase substantially beyond the end of the first quarter of the body. Even in female *Marshallagia* sp. the number of ridges in the region of the ovejectors was not greater than in more anterior regions.

Other characteristics

In addition to the synlophes, other characteristics of the nematodes were studied including the length of the esophageal valve, the position of the openings of the subventral esophageal gland



Figures 5–10. Synlophe and spicule characteristics of *Marshallagia marshalli, Ostertagia occidentalis,* and an unknown related species. 5. Synlophe with 3 lateral ridges (arrows) near midbody of *M. marshalli* from *Ovis canadensis.* Scale bar = 25 μm. 6. Spicules of *M. marshalli* from *Ovis canadensis,* lateral view. Scale bar = 50 μm. 7. Spicules of *O. occidentalis* from *Ovis dalli,* ventral view. Scale bar = 50 μm. 8. Synlophe with 1 lateral ridge (arrows) near midbody of *Marshallagia* sp. from *Oreamnos americanus.* Scale bar = 25 μm. 9. Spicules of *Marshallagia* sp. from *Oreamnos americanus,* lateral view. Scale bar = 50 μm. 10. Spicules of *Ostertagia* sp. from *Oreamnos americanus,* ventral view. Scale bar = 50 μm.



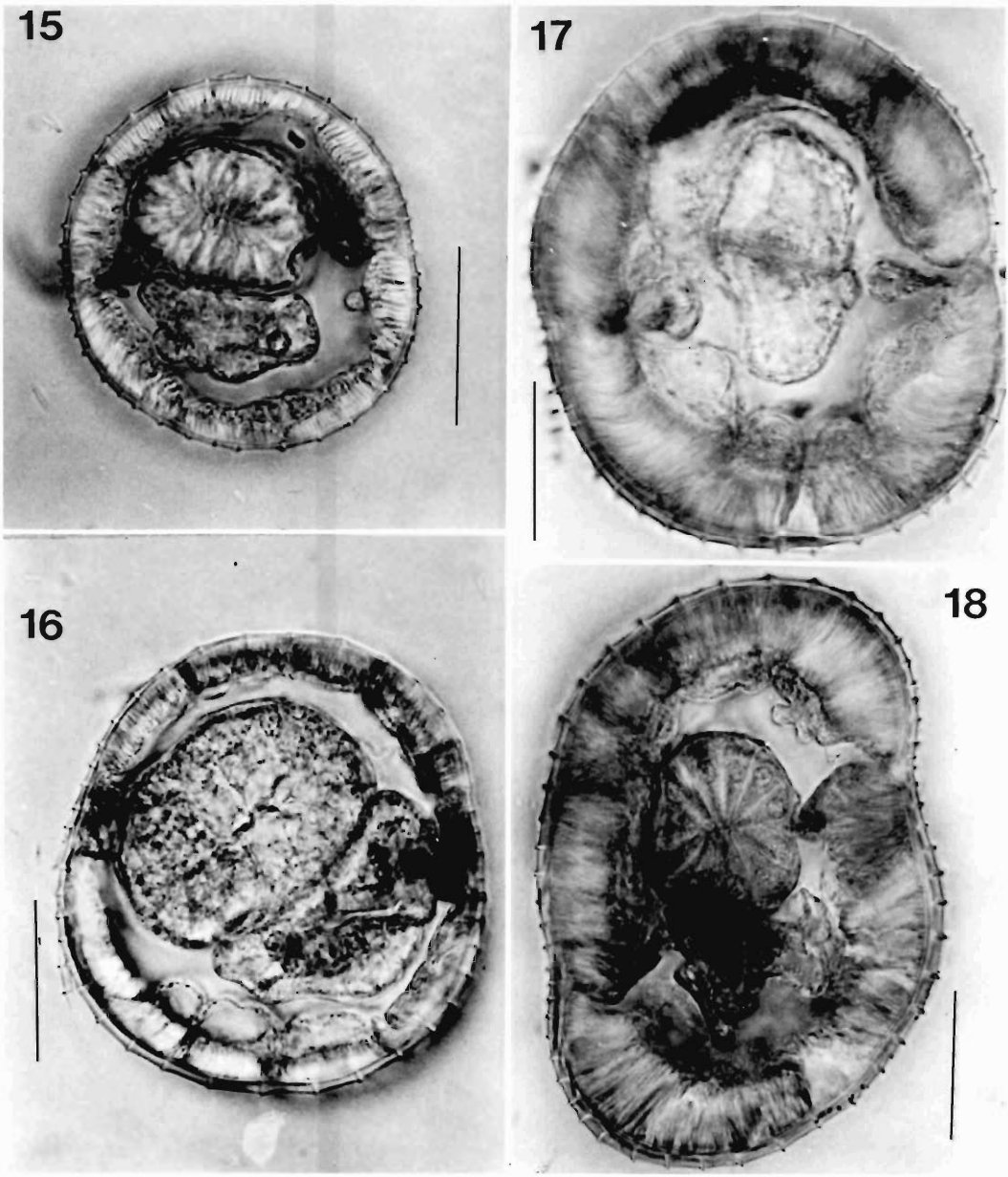
Figures 11–14. Photomicrographs of free-hand cross sections of *Marshallagia marshalli* and *Ostertagia occidentalis*. Scale bars = 50 μm. 11. *M. marshalli* male from *Ovis canadensis*, one-fourth of body length from anterior end, showing 51 ridges. 12. *M. marshalli* male from *Ovis canadensis*, near midbody, showing 53 ridges. 13. *O. occidentalis* male from *Ovis canadensis*, one-fourth of body length from anterior end, showing 52 ridges. 14. *O. occidentalis* male from *Ovis canadensis*, near midbody, showing 53 ridges.

orifices (SVGO) in the lumen of the esophagus, and numerous other meristic values. No differences among nematode species were found in these characteristics (Table 2).

Discussion

Our results support, or at least are consistent with, the polymorphism proposal of Lancaster and Hong (1981). In *M. marshalli* and *O. occidentalis* from 6 host species, a single synlophe with 3 lateral ridges was present. In addition, the study of other characteristics (Table 2) revealed no differences between the 2 nematode species except in the size and shape of the spicules (Figs. 6, 7, 9, 10) and the genital cone. These 2 characteristics are present in the same pattern between all major and minor species as described by Lancaster and Hong (1981) and Lichtenfels et al. (1988b). The results of the synlophe comparisons are consistent with those of an earlier study by Lichtenfels et al. (1988a) in which no differences were found between pair members of 3 pairs of species of Ostertagiinae, but were found among the 3 pairs.

The results of the study of nematodes from *Oreamnos americanus* provided additional evidence to support the polymorphism proposal. All nematode lots from *O. americanus* that had been



Figures 15–18. Photomicrographs of free-hand cross sections of *Marshallagia* sp. and *Ostertagia* sp. from *Oreamnos americanus*. Scale bars = 50 μm. 15. *Marshallagia* sp. male, one-fourth of body length from anterior end, showing 46 ridges. 16. *Marshallagia* sp. male, near midbody, showing 44 ridges. 17. *Ostertagia* sp. male, one-fourth of body length from anterior end, showing 55 ridges. 18. *Ostertagia* sp. male, near midbody, showing 54 ridges.

identified previously on the basis of spicule morphology as *M. marshalli* or *O. occidentalis* were found to have a unique synlophe. We interpret this to indicate that nematodes of this species are from a different gene pool than those from the other ruminants in this study. We chose to identify specimens from *O. americanus* as *Marshallagia* sp. and *Ostertagia* sp. herein because a species or subspecies determination must await the study of similar species from Palearctic ru-

minants. *Marshallagia marshalli* and *Osteragia occidentalis* have been reported from a wide range of geographic regions and hosts (Levine, 1980), and because of the international shipment of *Ovis aries,* a wide geographic distribution is possible. However, because species identifications are especially difficult in the genus *Marshallagia,* which includes at least 9 described species (Boev et al., 1963; Hu and Jiang, 1984), and because of the difficult separation of species similar to *O. occidentalis* (e.g., *O. trifida* Guille, Marotel, and Panisset, 1911; see Shul'ts and Andreeva, 1953; Skrjabin et al., 1954, p. 666), the host and geographic range of these species must be regarded to be unsettled. We believe a study of the synlophes of Palearctic species will be of great value in solving these problems.

Whatever the ultimate identity of the nematodes from *O. americanus,* their importance in this study is that they provide additional evidence on 2 important points: 1) they provide another example of a unique population of a pair of species very similar to *M. marshallagia* and *O. occidentalis* in which no differences were found between the 2 species or morphotypes; and 2) they provide evidence that in these species (like others we have studied) variation does occur in synlophes among apparently different gene pools; i.e., 1 pool in *O. americanus* and a different pool in all the other hosts. In all previous studies of synlophes of various species, there was no evidence that a change in host immediately affected the synlophe (Lichtenfels, 1974; Lichtenfels and Pilitt, 1983a, b; Lichtenfels et al., 1988a). The lack of differences in synlophes among lots of *M. marshalli* and *O. occidentalis* from 6 different hosts also supports the conclusion that the environmental effects of a different host species does not change the synlophe, and that when synlophe differences are found they can be expected to result from genetic differences. Speculation on how *O. americanus* came to have a different population of these nematodes is premature until more is learned about the synlophes of Palearctic species. It is interesting to note, however, that *O. americanus* is also parasitized by unique species of the genera *Nematodirus* (*N. maculosus* Becklund, 1965) and *Trichuris* (*T. oreamnos* Knight, 1974).

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Habronema malani sp. n. and *Habronema tomasi* sp. n. (Nematoda: Habronematidae) from the Burchell's Zebras and Hartmann's Mountain Zebras in Southern Africa

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ABSTRACT: *Habronema malani* sp. n. is described from the stomachs of 44 Burchell's zebras, *Equus burchelli antiquorum,* in the Etosha and Kruger national parks and 6 Hartmann's mountain zebras, *Equus zebra hartmannae,* from the Etosha National Park in southern Africa. *Habronema tomasi* sp. n. is described from the small intestines of 35 Burchell's zebras in the Kruger National Park. *Habronema malani* is distinguished from other members of the genus by its deep buccal capsule with walls that are narrower anteriorly than posteriorly and have projections in the anterior end; spicule length ratio (right : left) ranging 1:2.3 to 1:3.7; a short, stout, and striated right spicule; and a long and slender left spicule with a pointed projection. *Habronema tomasi* is differentiated from the other species by buccal capsule walls that are wider anteriorly than posteriorly; a distance between the anterior wall of the buccal capsule and the inner surface of the lateral lips that is almost equal to the buccal capsule depth; an ovejector with spiral-shaped muscles; and a spicule length ratio (right : left) ranging 1:1.5 to 1:2.95. The right spicule of *H. tomasi* is short and cross striated except at the distal fourth where the tip is flanged. The left spicule is long and cross striated in the first one-third and partially cross striated in the second one-third. A key to the equine *Habronema* species is also included.

KEY WORDS: taxonomy, Nematoda, *Equus burchelli, Equus zebra,* key to genera of equine *Habronema,* zebras, equids, southern Africa.

Members of the nematode family Habronematidae parasitize the stomachs of mammals and birds. There are 6 species found in equids and these are frequently pathogenic. These include 5 belonging to the genus *Habronema* and 1 to the genus *Draschia* (Yamaguti, 1961). The larval stages develop in flies (e.g., *Musca domestica*), and if the infective larvae of some of these habronematids (e.g., *Draschia megastoma* (Rudolphi, 1819), *Habronema majus* (Creplin, 1849) Ransom, 1911, *Habronema muscae* (Carter, 1861) Diesing, 1861) are deposited in wounds, they cause "summer sores" (Lichtenfels, 1975; Arundel, 1978). The adults of *Draschia megastoma* (syn. *Habronema megastoma* (Rudolphi, 1819) Railliet, 1923) often form abscesses that can range in size from 2.5 to 10.0 cm in diameter and are found in both the glandular and non-glandular portions of the stomach in both domestic and wild equids (Lichtenfels, 1975; Scialdo-Krecek, 1984).

During parasitological investigations on Burchell's zebras and Hartmann's mountain zebras (Scialdo et al., 1982; Scialdo-Krecek, 1983; Scialdo-Krecek et al., 1983), 2 new species of *Habronema* were recovered. These are described below.

Materials and Methods

Adult worms were recovered from the stomachs and small intestines of 35 Burchell's zebras (*Equus burchelli antiquorum* H. Smith, 1841) in the Kruger National Park (KNP), Republic of South Africa, and 9 Burchell's zebras and 6 Hartmann's mountain zebras (*Equus zebra hartmannae* Matschie, 1898) in the Etosha National Park, South West Africa/Namibia. These nematodes agree with the generic description of *Habronema* by Lichtenfels (1975), but they could not be assigned to known species.

The zebras were processed for parasitological studies and the nematodes were killed in Lugol's iodine and fixed in 10% formalin (Malan et al., 1981a, b). The specimens were cleared in lactophenol and examined with a Nikon Optiphot light microscope fitted with disc interference contrast. The anterior end of some of the specimens was cut transversely in the region of the buccal capsule. This cut end was mounted en face, which enabled the structures of the head region to be examined.

For scanning electron microscopy (SEM) the formalinized nematodes were dehydrated in ethanol and critical point dried in liquid CO_2 (Humphreys, 1975). The dried nematodes were oriented onto a stub bearing adhesive and coated with gold/palladium. They were examined by SEM at 5–20 kV.

Specimens of *Habronema* from the United States National Museum Helminthological Collection (USNM Helm. Coll.), Beltsville, Maryland, U.S.A., which were borrowed and examined, included *H. majus* (USNM Helm. Coll. Nos. 31066, 31091), *H. muscae* (USNM

Helm. Coll. Nos. 33300, 43258, 58493), *H. zebrae* (USNM Helm. Coll. No. 79007), and type specimens of *H. longistoma* (USNM Helm. Coll. No. 61415).

Description of Species

GENERAL: Spirurida, Habronematoidea, Habronematidae, Habronematinae, *Habronema*. Long, filiform worms. Deep buccal capsule, walls narrower anteriorly than posteriorly with projections in anterior end. Head with 2 lateral trilobed pseudolabia with 6 inner labial papillae. Four cephalic papillae each with 1 outer labial papilla next to it, and 2 amphids with 1 outer labial papilla next to each. Anterior portion of esophagus muscular and narrower than posterior glandular portion.

Habronema malani sp. n. (Figs. 1–7, Table 1)

DESCRIPTION: Dimensions given as range (mean in μm ± 1 standard deviation) unless otherwise indicated.

MALES (10 specimens): Length 16.3–19.6 (18 ± 1.0) mm. Width 305–332 (316 ± 22.5). Depth of buccal capsule 52–70 (59 ± 6.9). Width of buccal capsule 20–42 (29 ± 7.6). Base of buccal capsule to outer lip 72–90 (79 ± 6.6). Esophagus 2.1–4.2 (3 ± 0.6) mm long and 120–240 (166 ± 32) wide. Nerve ring 217–303 (273 ± 27.8) from base of stoma. Right spicule stout and striated, 580–900 (772 ± 98.0). Left spicule slender, 1.9–2.4 (2 ± 0.2) mm with a pointed projection. Spicule length ratio ranging (right : left) 1:2.3 to 1:3.7. Four pairs of pedunculated preanal papillae, 4 on the right slightly anteriorly placed relative to the cloaca. Four postanal papillae, 1 next to lip of cloaca, 1 single and 1 pair ⅔ distance from anus to posterior extremity.

FEMALES (10 specimens): Length 17.8–24.6 (22 ± 2.0) mm. Width 305–492 (354 ± 56.0). Depth of buccal capsule 52–88 (67 ± 12.4). Width of buccal capsule 20–30 (26 ± 3.0). Base of buccal capsule to outer lip 80–112 (91 ± 10.4). Esophagus 2.8–3.9 (3 ± 0.3) mm long and 104–200 (156 ± 41.0) wide. Nerve ring 239–332 (294 ± 65.3) from base of stoma. Vagina short 72–104 (78 ± 15.2). Ovejector long 700–1,000 (887 ± 102.4). Vulva 8.7–12.6 (11.0 ± 1.2) mm from anterior extremity. Anus 240–340 (286 ± 38.2) from tip of tail. Egg 40–80 (66 ± 12.3) long and 4–16 (10 ± 4.1) wide.

HOST RECORD INFORMATION: Total burdens (3–1,244) of this species were recovered from the stomachs of both Hartmann's mountain zebras and Burchell's zebras in the KNP and Etosha National Park.

TYPE SPECIMENS: Three intact males and females (T-2169) in the Onderstepoort Helminthological Collection, Veterinary Research Institute, Onderstepoort, South Africa; 3 intact males and females (1985. 2185–2190) in the British Museum, London, U.K.; and 4 intact males and females (79803) in the United States National Museum Helminthological Collection (USNM Helm. Coll., USDA), Beltsville, Maryland, U.S.A.

HOST AND LOCALITY: *Equus burchelli antiquorum* H. Smith, 1841. Kruger National Park, Republic of South Africa (25°12′–24°24′S, 31°36′–32°2′E). Etosha National Park, South West Africa/Namibia (19°15′S, 14°31′E).

Equus zebra hartmannae Matschie, 1898. Etosha National Park, South West Africa/Namibia (19°15′S, 14°31′E).

SITE OF INFECTION IN HOST: Stomach.

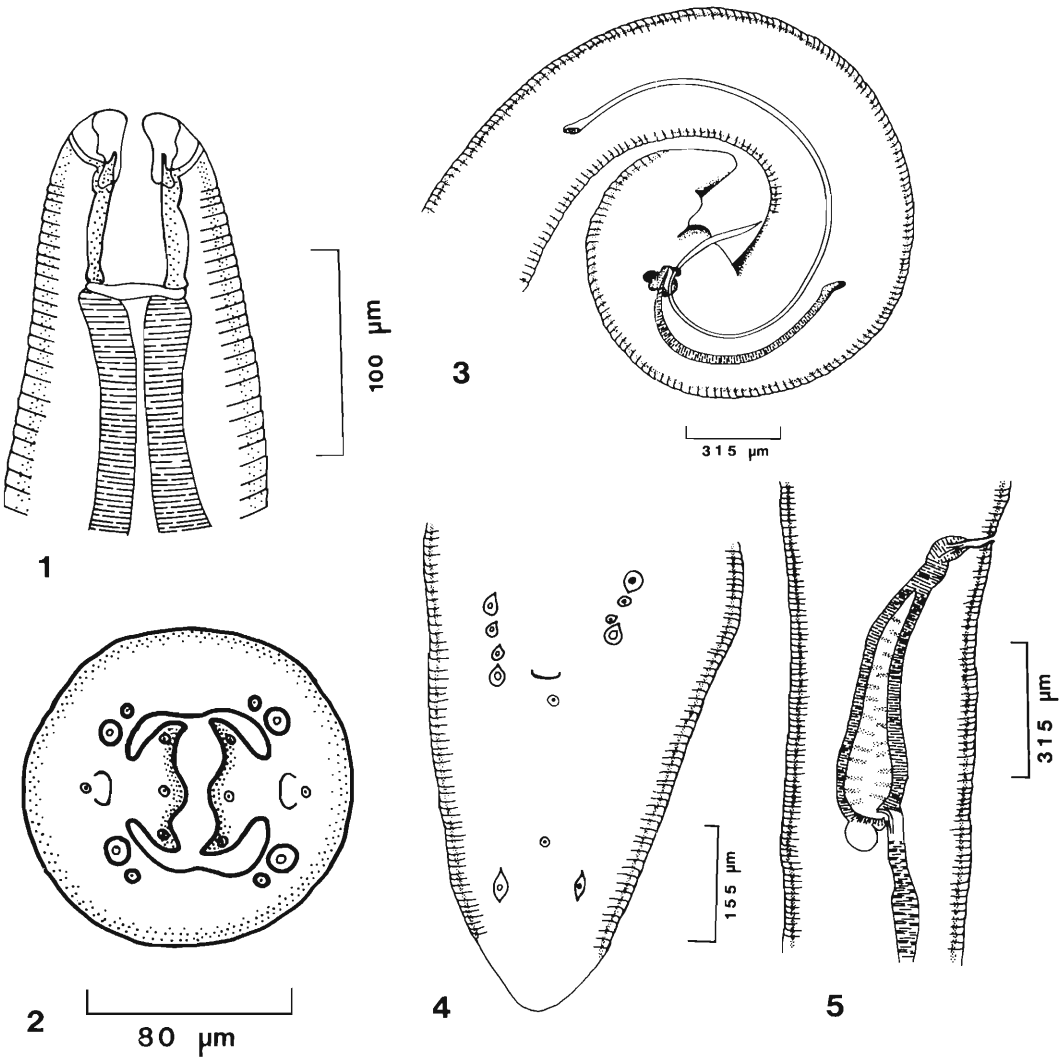
ETYMOLOGY: This species is named after Dr. F. S. Malan, a South African parasitologist.

Habronema tomasi sp. n. (Figs. 8–16, Table 1)

GENERAL: Anteriorly in buccal capsule, and interlabially, is a pair of medial ridges with a slit-like opening between them. Each has 3 teeth. Posteriorly there is a larger tooth on either side of the ridges. Two lateral pseudolabia with 4 inner labial papillae. Four cephalic papillae with 1 outer labial papilla next to each, and 2 amphids with 1 outer labial papilla next to each.

DESCRIPTION: Dimensions given as range (mean in μm ± 1 standard deviation) unless otherwise indicated.

MALES (10 specimens): Length 7.9–10.9 (9.1 ± 0.9) mm. Width 144–208 (174 ± 20.2). Depth of buccal capsule 28–52 (40 ± 7.4). Width of buccal capsule 12–32 (21 ± 6.2). Base of buccal capsule to outer lip 76–82 (80 ± 2.4). Esophagus 2.2–2.8 (2.4 ± 0.2) mm long and 72–112 (86 ± 12.1) wide. Nerve ring 180–240 (204 ± 15.8) from base of stoma. Right spicule stout and striated, 336–480 (410 ± 49.5) and distal fourth with flanged tip. Left spicule slender, 690–992 (851 ± 108.9). Spicule length ratio ranging (right : left) 1:1.5 to 1:2.95. Four pairs of pedunculated preanal papillae, 4 on the right slightly anteriorly placed relative to the cloaca, and 1 single preanal papilla next to the cloaca. Two postanal pairs of papillae, 1 posterior to the cloaca and the other separated so 1 on either side, approximately half



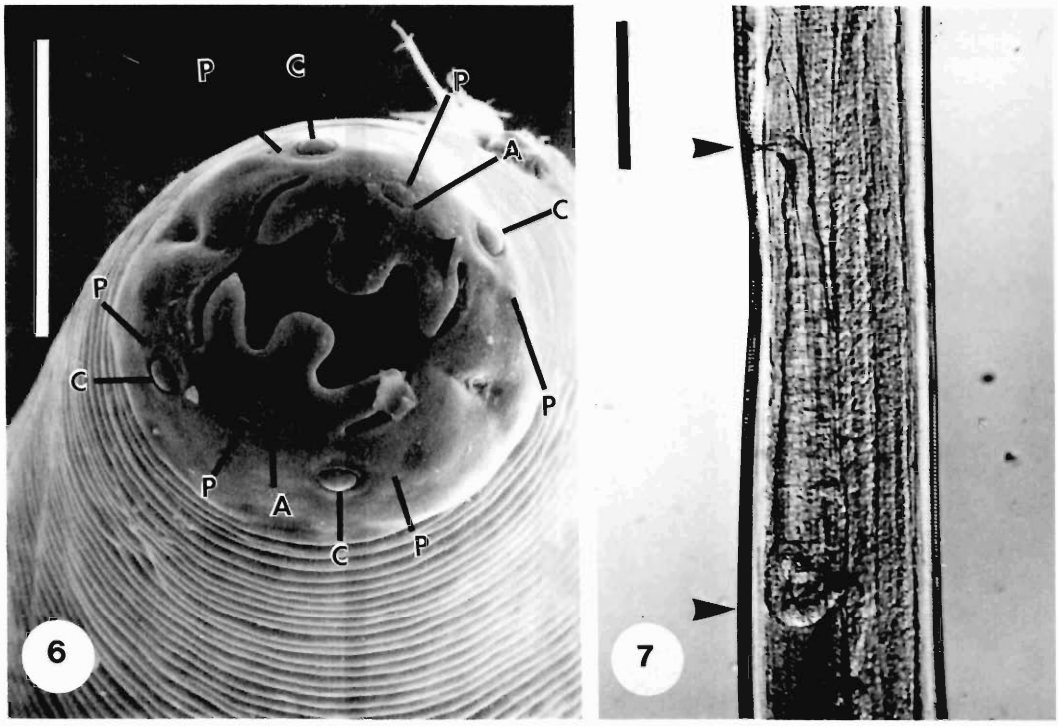
Figures 1–5. ***Habronema malani*** **sp. n. 1. Lateral view of head. 2. En face view of head. 3. Lateral view of male tail showing spicules. 4. Ventral view of male tail with papillae. 5. Lateral view of female vulva, vagina, and ovejector.**

of the distance between the tip of the tail and the cloaca.

FEMALES (10 specimens): Length 13.2–16.4 (14.9 ± 1.1) mm. Width 192–260 (229.2 ± 20.9). Depth of buccal capsule 48–92 (67.2 ± 14.6). Width of buccal capsule 12–32 (20.8 ± 5.9). Base of buccal capsule to outer lip 72–100 (86.0 ± 8.7). Esophagus 2.3–4.2 (3.0 ± 0.6) mm long and 80–128 (105.6 ± 17.6) wide. Nerve ring 176–360 (251.6 ± 61.0) from base of stoma. Vagina very short, 20–40 (22.2 ± 6.7). Ovejector long and straight, 300–400 (346.6 ± 33.5) with spiral-shaped muscles. Vulva 5.0–7.0 (6.2 ± 0.7) mm from anterior extemity. Anus 136–200 (176.4 ± 19.3) from tip of tail. Egg 96–120 (110.4 ± 10.4) long and 8–12 (8.4 ± 4.1).

HOST RECORD INFORMATION: Total burdens (1–1,243) of this species were recovered from the small intestines of 35 Burchell's zebras in the KNP.

TYPE SPECIMENS: Three intact males and females (T-2170) in the Onderstepoort Helminthological Collection, Veterinary Research Institute, Onderstepoort, South Africa; 3 intact males and females (1985. 2191–2196) in the British Museum, London, U.K.; and 5 intact males



Figures 6, 7. ***Habronema malani*** **sp. n. 6. Scanning electron micrograph of en face view of head. A, amphids; P, outer labial papillae; C, cephalic papillae; arrows, inner labial papillae. Scale bar = 40 μm. 7. Light micrograph of female vulva (top arrow) with long ovejector (ends at bottom arrow). Scale bar = 300 μm.**

and females (79804) in the United States National Museum Helminthological Collection, USDA, Beltsville, Maryland, U.S.A.

HOST AND LOCALITY: *Equus burchelli antiquorum* H. Smith, 1841. Kruger National Park, Republic of South Africa (25°12′–24°24′S, 31°36′–32°2′E).

SITE OF INFECTION IN HOST: Small intestine.

ETYMOLOGY: This species is named after my husband, Mr. Tomas E. Krecek.

Discussion

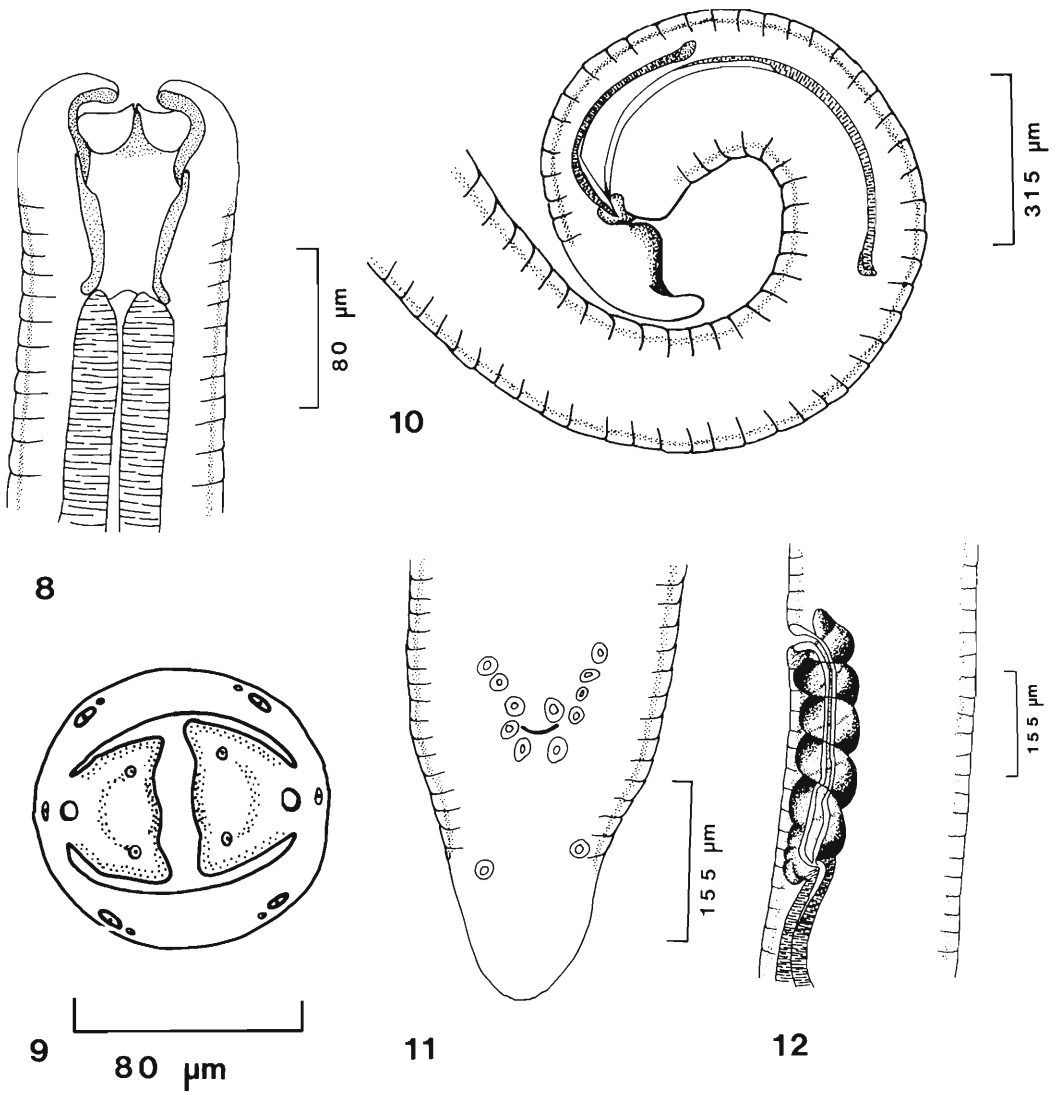
The 6 habronematid species reported from equids are: *H. longistoma* van den Berghe, 1943; *H. majus*; *H. muscae*; *H. tyosenense* Yamaguti, 1943; *H. zebrae* Theiler, 1923; and *D. megastoma*. With the exception of *H. tyosenense*, all these species have been reported from Hartmann's mountain zebras and Burchell's zebras (Theiler, 1923; Round, 1968; Scialdo et al., 1982; Scialdo-Krecek, 1983; Scialdo-Krecek et al., 1983).

Although *H. muscae* is reported from a different host than *H. malani* sp. n., this new species most closely resembles *H. muscae*. Between these 2 species, *H. malani* has a shorter vagina. This structure crosses the body transversally in both species before reaching a long muscular ovejector. Further, the spicule ratio of *H. malani* is smaller than that of *H. muscae*. *Habronema malani* is a large worm surpassed only by *H. majus* in total body length. *Habronema malani* is further distinguished by the buccal capsule shape, which is narrower anteriorly than posteriorly, and by the anterior projections on the buccal capsule walls.

Although *H. tomasi* sp. n. does not closely resemble any of the other known species, its closest possible congener in terms of ovejector shape is *H. majus*. *Habronema tomasi* differs from Theiler's (1923) *H. majus* in the distance between the anterior wall of the buccal capsule and inner surface of the lateral lips, which is almost equal to the buccal capsule depth. *Habronema tomasi* has 1 more postanal papilla near the cloaca than *H. majus*. The ovejector of *H. tomasi* is muscular and spiral-shaped but elongated, while that of *H. majus* is muscular and rounded. Further, *H. tomasi* is a small worm and delicate in appearance. This may be because of its short

Table 1. Principal measurements of *Habronema muscae, H. majus, H. malani,* and *H. tomasi* (all measurements in μm unless otherwise indicated).

	H. muscae		*H. majus*		*H. malani*		*H. tomasi*	
	♂	♀	♂	♀	♂	♀	♂	♀
Total length (mm)	9.5–13.0	11.2–19.7	15.2–18.4	22.6–23.2	16.3–19.6	17.8–24.6	7.9–10.9	13.2–16.4
Width	199–279	199–319	360–400	440–500	305–332	305–492	144–208	192–260
Buccal capsule								
Length	42–70	40–60	48–64	54–64	52–70	52–88	28–52	48–92
Width	12–20	20–30	16–30	36–54	20–42	20–30	12–32	12–32
Base buccal capsule to outer lip	60–80	60–80	66–88	84–88	72–90	80–112	76–82	72–100
Esophagus								
Length (mm)	1.9–2.5	1.8–2.8	3.1–4.0	3.3–3.8	2.1–4.2	2.8–3.9	2.2–2.8	2.3–4.2
Width	88–144	96–140	140–160	160–200	120–240	104–200	72–112	80–128
Distance nerve ring from base of stoma	191–244	198–297	120–280	240–280	217–303	239–332	180–240	176–360
Spicule lengths								
Right spicule	360–520	–	340–380	–	580–900	–	336–480	–
Left spicule (mm)	2.0–2.8	–	750–810	–	1.9–2.4	–	690–992	–
Length of vagina	–	176–400	–	136–140	–	72–104	–	20–40
Length of ovejector	–	0.8–1.0	–	200–250	–	0.7–1.0	–	300–400
Distance vulva from anterior end (mm)	–	5.5–8.1	–	9.2–9.6	–	8.7–12.6	–	5.0–7.0
Distance anus to tip of tail	–	280–376	–	220–240	–	240–340	–	136–200
Egg								
Length	–	48–60	–	15–18	–	40–80	–	96–120
Width	–	8–12	–	6–8	–	4–16	–	8–12

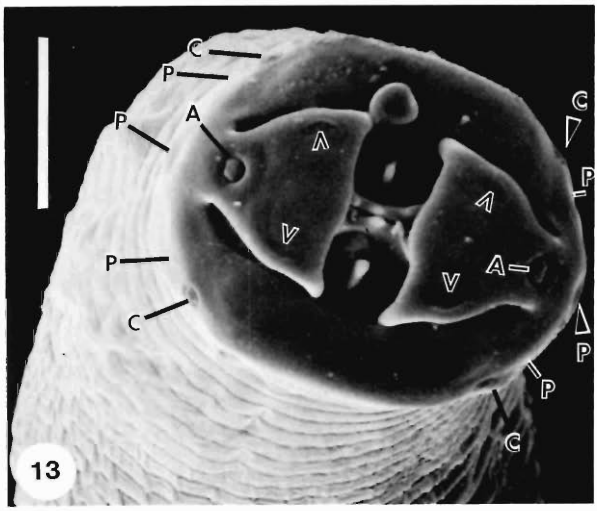


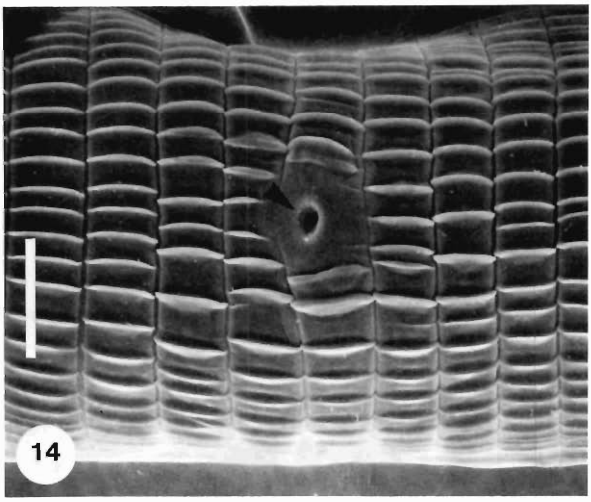
Figures 8–12. ***Habronema tomasi*** **sp. n. 8. Lateral view of head. 9. En face view of head. 10. Lateral view of male tail with spicules. 11. Ventral view of male tail showing papillae. 12. Lateral view of female vulva, vagina, and ovejector.**

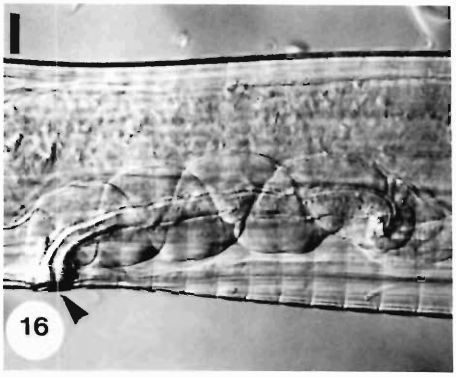
body length but widely separated transverse striations of the cuticle.

The medial interlabial ridges of the buccal capsule of *H. tomasi* may be an extension of the capsule walls and of the interlabia, and appear to separate the buccal capsule into a lower and an upper chamber. This buccal capsule differs considerably from all of the other equine species of this genus. However, there is some morphologic similarity with a free-living marine enoplid *Pontonema yaena* Inglis, 1964. This species has ventro-lateral and dorsal tooth-like structures (onchia) that develop from the lining of the buccal capsule (Inglis, 1964). Inglis speculated on the feeding functions of these structures. *Habronema tomasi* inhabits the small intestine of the zebra, and in the equid this organ is rarely inhabited by helminths. The viscous character of the small intestinal contents contrasts markedly with the dry, coarse ingesta of the stomach of equids. In the present study, it appears that the interlabial ridges could be of assistance during feeding in this viscous environment.

The description of the genus *Habronema* ac-







Figures 13–16. ***Habronema tomasi*** **sp. n. 13. Scanning electron micrograph (SEM) of en face view of head with teeth on interlabial ridges and teeth either side. A, amphids; P, outer labial papillae; C, cephalic papillae; arrows, inner labial papillae. 14. SEM of vulvar opening (arrow) and widely separated transverse striations. 15. SEM of male tail showing papillae near cloaca and a spicule partially extruded. 16. Light micrograph of spiral-shaped, muscular female ovejector with vulvar opening (arrow). Scale bars = 20 μm, Figure 13; 40 μm, Figures 14–16.**

cording to Lichtenfels (1975) should be revised to accommodate the total body length as 7–35 mm and not 8–35 mm.

The location in the host for the *Habronema* species known in the horse is the stomach (Lichtenfels, 1975). It is of interest to note than *H. tomasi* sp. n. was recovered only from the small intestine in the zebras. Contrastingly, in horses it is uncommon to recover any nematodes from this organ.

The absence of the 2 new species from Theiler's (1923) study may be attributable to several factors. *Habronema tomasi* is a much smaller nematode than the others of its genus. The mean total length is almost half the length if compared with *H. malani*. Hence, such a nematode could be easily missed using conventional recovery techniques. Furthermore, few nematodes appear to inhabit the small intestine of the equid as compared with the other organs (Lichtenfels, 1975). It remains though a sizeable organ, and if not sampled adequately, a small nematode such as *H. tomasi* may be overlooked. Theiler's study was only semi-quantitative, and presumably

techniques used for nematode recovery were not as advanced as those of today. The high prevalence of *H. malani* in recent reports, 96% in 25 Burchell's zebras (Krecek et al., 1987), suggests that this nematode may have been present but was not detected in Theiler's small sample size of 3 zebras. Alternatively, it is possible that conditions required for the presence of a suitable intermediate host for these nematodes were absent in the habitat of Theiler's zebras, and therefore, the life cycle of this nematode was not completed.

A key to distinguish all equine species of the genus *Habronema* follows.

1a. Very long cylindrical buccal capsule 2
1b. Short buccal capsule 3
2a. Cuticular collar present at anterior end of stoma ..... *H. longistoma*[1]
2b. Cuticular collar absent; left spicule 4 times length of right; vagina long and narrow, crosses body transversally before reaching long muscular ovejector ..... *H. zebrae*
3a. Left spicule 2–3 times length of right; vagina very short before reaching rounded part of muscular ovejector 4
3b. Left spicule 2–5 times length of right; vagina crosses body transversally before reaching long muscular ovejector 5
4a. Vagina short (136–140 μm) with large rounded ovejector (200–250 μm); cuticle without widely separated transverse striations; teeth present on ventral and dorsal walls of buccal capsule ..... *H. majus*
4b. Vagina very short (20–40 μm); ovejector 300–400 μm long with spiral-shaped muscles; cuticle with widely separated transverse striations; pair of medial ridges interlabially with slit-like opening and 3 small teeth on each ridge, also, larger tooth either side of the ridges ..... *H. tomasi*
5a. Vagina short (72–104 μm); left spicule 2½–3½ times length of right ..... *H. malani*
5b. Vagina long and narrow (176–400 μm); left spicule 5 times length of right ..... *H. muscae*

H. tyosenense is not included in the preceding key because type specimens were not available. Based on Yamaguti (1961), *H. tyosenense* resembles *H. majus* and *H. tomasi* with a 1:2 (right: left) spicule ratio. *Habronema tyosenense* differs from all other species in the arrangement and number of genital papillae. The number of papillae are fewest and are the most symmetrically arranged, with 4 pairs of preanal and 2 pairs of postanal papillae. All other *Habronema* species have extra papillae post- or preanally as well as an asymmetric arrangement.

[1] Type specimens are all females; males were not included in original description, and are still unknown.

Acknowledgments

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Further Studies on the Population Regulation in *Echinostoma caproni* Infections in NMRI Mice

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ABSTRACT: The population regulation (establishment, survival, fecundity) was studied in *Echinostoma caproni* infections in outbred, 8-wk-old female NMRI mice. The pattern of expulsion was negatively infection–dose dependent. Survival was prolonged in response to concurrent infection with *Nematospiroides dubius,* and a marked resistance to secondary infection persisted for a period of at least 12–14 wk. The capacity to mount an effective regulatory response to both high and low level repeated infections was marked, reflecting that a marked concomitant resistance to superimposed infection became pronounced by week 2 in primary infections with 8–10 worms. Resistance to superimposed infection was markedly impaired in 4-wk-old mice. The egg production capacity/fluke/day was infection–dose independent and unaffected by concurrent *N. dubius* infection. The overall reproductive capacity/fluke increased, however, in response to increasing worm burden and to concurrent *N. dubius* infection as a result of the delayed expulsion. The heterogeneity in the capability to mount an effective regulatory response to *E. caproni* infection was marked. The *E. caproni*/NMRI mouse system represents a model in which homologous and heterologous immunomodulation may affect markedly the reproductive potential and thereby the parasite population dynamics.

KEY WORDS: *Echinostoma caproni,* outbred female NMRI mice, population regulation, challenge infection, repeated infection, concurrent infection, *Nematospiroides dubius.*

Essential findings from the *Echinostoma caproni*/NMRI mouse model comprise an infection–dose independency of primary worm establishment, a negatively infection–dose dependent pattern of worm expulsion, and an infection–dose independency of individual worm fecundity in infections with 6 and 25 metacercariae per mouse (Odaibo et al., 1988). Thus, the density-dependent constraints on parasite survival and fecundity reported for other host–parasite models (Keymer, 1982; Anderson and Medley, 1985) play no major role in the *E. caproni*/NMRI mouse model. Immunomodulation associated with high infection intensity is suggested to be responsible for the negatively infection–dose dependent pattern of expulsion (Christensen et al., 1981). An overdispersed pattern of distribution of intestinal helminth infections, combined with the growing awareness of the potential effect of immunomodulation on parasite transmission dynamics (Bundy and Golden, 1987; Christensen et al., 1987), make the *E. caproni*/NMRI mouse system valuable for studying the regulatory response to intestinal helminth infection. The aim of the experiments reported in this paper was to study the regulatory response to secondary, superimposed, and repeated *E. caproni* infections in outbred female NMRI mice and to elucidate possible effects of concurrent infection with *Nematospiroides dubius.*

Materials and Methods

Unless otherwise stated, 8-wk-old, outbred albino female NMRI mice (Gl. Bomholtgård, Ry, Denmark) weighing 20–25 g were used. Metacercariae of *E. caproni* (Egyptian strain) were obtained from *Biomphalaria glabrata* as described by Christensen et al. (1980), and infection of mice with metacercariae was made with a stomach tube. The echinostome population is the same as that used by Odaibo et al. (1988), as that initially named *E. revolutum* by Christensen and coworkers (Sirag et al., 1980; Christensen et al., 1981, 1984, 1985, 1986), and as that named *E. liei* by Christensen et al. (1980) and Hosier et al. (1988). Infections with *N. dubius* (strain from Institute of Internal Medicine, Royal Veterinary and Agricultural University, Copenhagen, Denmark) were established by peroral inoculation of 200 larvae/mouse. *Echinostoma caproni* was recovered by the procedure described by Christensen et al. (1986). In experiments where the mice were given challenge infections, animals were necropsied on day 7 postchallenge. Body length measurements were taken using a calibrated ocular micrometer on worms fixed by the method described by Hosier and Fried (1986). For fecundity determinations, feces from the individual mouse were collected over a 12-hr (series 1–3, 5) or 24-hr (series 4) period according to the method described by Keymer and Hiorns (1986). Eggs per gram of feces were determined according to Brindley and Dobson (1981). The time pattern of expulsion was determined by weekly examination for eggs in feces using the direct smear technique. The

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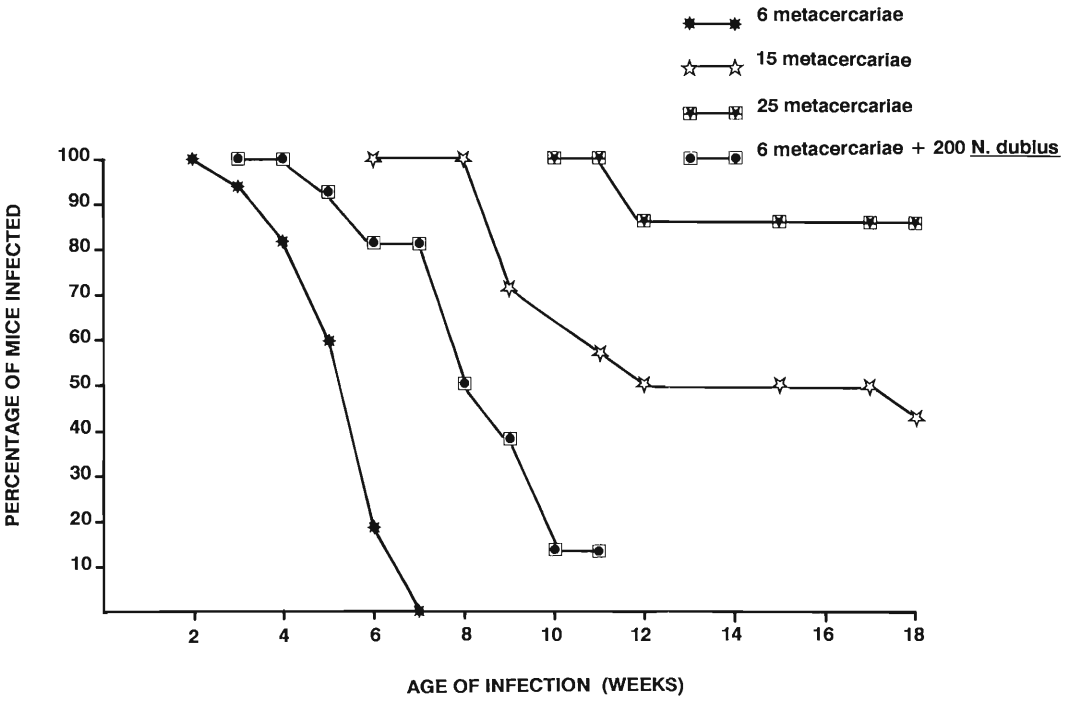

Figure 1. Time pattern of expulsion in infections in female, albino NMRI mice with 6, 15, and 25 metacercariae per mouse and in mice concurrently infected with 6 *Echinostoma caproni* metacercariae and 200 larvae of *Nematospiroides dubius* (20–22 mice per group, week 2 following infection).

statistical tests used were the Student's *t*-test and analyses of variance, and a value of $P > 0.05$ was considered non-significant. This study was designed in 5 series of experiments (series 1–5).

Series 1 comprised experiments on the pattern of expulsion of primary, non-challenged infections. Groups of mice (20–22 mice per group) were inoculated with 6, 15, or 25 metacercariae per mouse, respectively, or concurrently with 6 *E. caproni* metacercariae and 200 larvae of *N. dubius*. Mice becoming negative for eggs in feces were necropsied to confirm expulsion based on adult worm recovery.

Series 2 comprised a study on resistance to secondary infection following termination of a primary infection. Mice with naturally terminated infections with 6 metacercariae, together with corresponding previously non-infected mice, were given a challenge infection of 10 metacercariae per mouse from 2 to 16 wk following termination of the primary infection.

Series 3 comprised a comparison of the reproductive potential in groups of mice (20 mice per group) given either 6 *E. caproni* metacercariae or 6 metacercariae and 200 larvae of *N. dubius* concurrently. At regular intervals following infection, the population size and fecundity of *E. caproni* were determined in 3–4 randomly selected mice from each group.

Series 4 was designed to elucidate the regulatory response to repeated infections. Groups of mice (25 mice per group) were given either 2 or 10 metacercariae per mouse 3 times weekly for a period of 6 wk. At weekly intervals from week 1 following the start of the experiment, parasite numbers and fecundity were determined in 4–7 mice randomly selected from each group.

Series 5 attempted to elucidate parameters affecting resistance to superimposed *E. caproni* infection. Groups of 4-, 8-, and 16-wk-old mice harboring primary 11-day-old infections with 10 metacercariae per mouse, and groups of 8-wk-old mice harboring 8-, 10-, and 14-day-old primary infections with 8 metacercariae per mouse were, together with corresponding groups of non-infected mice, challenged with 10 metacercariae per mouse. Also included was an experiment in which a group of mice was given the *E. caproni* challenge infection on day 18 following primary *E. caproni* infection of mice harboring 6-day-old *N. dubius* infections.

Results

The pattern of expulsion of *E. caproni* was negatively infection–dose dependent (series 1, Fig. 1). Necropsy of 5 mice from each group 1 wk following infection revealed worm burdens of 4–6, 10–15, and 18–25 worms per mouse in infections with 6, 15, and 25 metacercariae per mouse, respectively. Expulsion in infections with 6, 15, and 25 metacercariae started weeks 3, 9, and 12 following infection, respectively. All mice har-

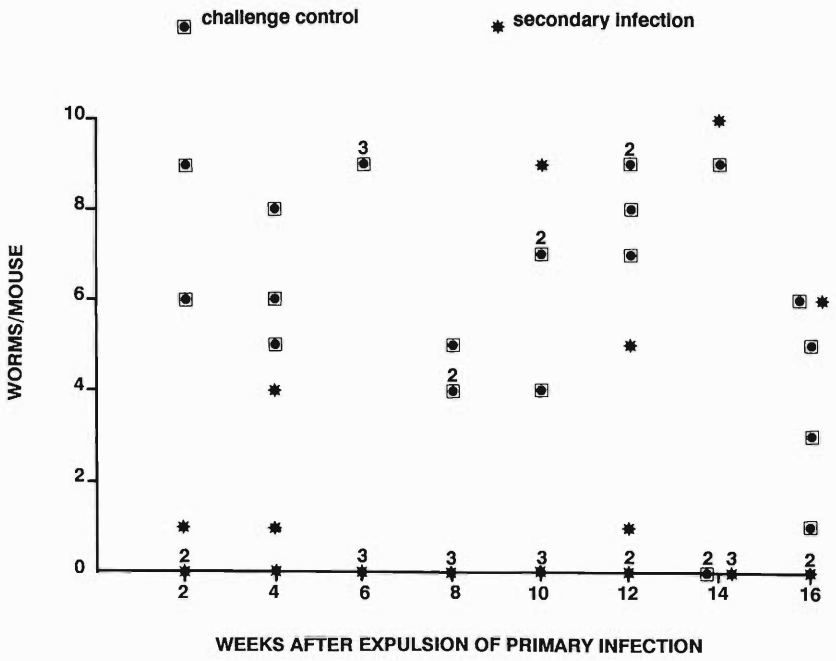

Figure 2. ***Echinostoma caproni*** **challenge worm recovery in individual female, albino NMRI mice challenged with 10 metacercariae/mouse at increasing time intervals (weeks) following natural expulsion of primary infections with 6 metacercariae per mouse. Necropsy was performed on day 7 following the challenge infection. Figures in parentheses show number of mice with worm burden indicated.**

boring infections with 6 metacercariae had expelled the worms prior to the start of expulsion in mice harboring infections with 15 and 25 metacercariae, and the percentage of positive mice with infections with 25 metacercariae from week 12 onwards exceeded that of mice with infections with 15 metacercariae ($P < 0.05$). From Figure 1 it also appears that mice concurrently infected with *E. caproni* and *N. dubius* experienced a markedly impaired expulsion capability. Thus, 80% of mice harboring infections with both *E. caproni* and *N. dubius* remained positive for *E. caproni* infection by week 8 when all mice harboring *E. caproni* infection only had expelled all worms (Fig. 1). The mice infected with *N. dubius* developed chronic, heavy infections with pronounced inflammatory reactions in the proximal part of the small intestine. Generally, the heterogeneity in the expulsion capability was pronounced, with the time taken to mount an effective expulsion response differing markedly.

Resistance to secondary infection was very marked (series 2, Fig. 2). The challenge control worm recovery exceeded consistently the secondary worm recovery for up to week 12 following termination of the primary infection ($P < 0.05$). Most mice remained fully resistant to the secondary infection. Comparison of challenge control and secondary worm recoveries from week 14 onwards was hampered by the development of an age-related resistance to primary infection in the challenge control mice (Fig. 2). The heterogeneity in the capability to mount an effective response to secondary infection was moderate.

Series 3 experiments showed that the echinostome expulsion capability in concurrently *N. dubius*- and *E. caproni*-infected mice was markedly impaired. Results from series 1 were thus confirmed. The mean percentage of *E. caproni* worm recovery remained stable throughout ($P > 0.05$) in concurrently *N. dubius*-infected mice, whereas that in mice only infected with *E. caproni* became gradually reduced ($P < 0.05$) (Fig. 3). The mean number of eggs/g feces/*E. caproni* worm was comparable throughout ($P > 0.05$) in single and concurrently infected mice (Fig. 3). However, the prolonged persistence in response to concurrent *N. dubius* infection resulted in the mean number of *E. caproni* eggs/g feces in con-

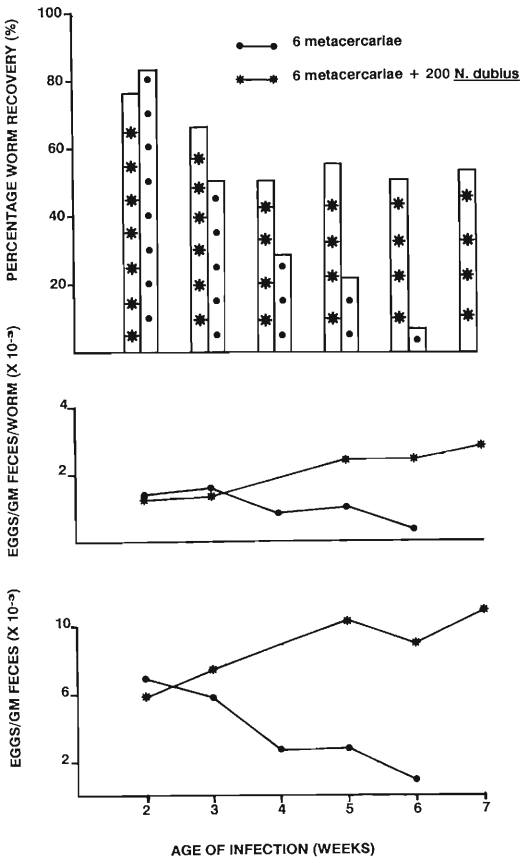

Figure 3. Percentage *Echinostoma caproni* worm recovery, eggs/g feces, and eggs/g feces/worm ($\bar{x} \pm 2$ SE) at increasing age (weeks) in *E. caproni* and in *E. caproni* plus *Nematospiroides dubius*-infected female, albino NMRI mice (results based on 5–7 mice from each group at each killing).

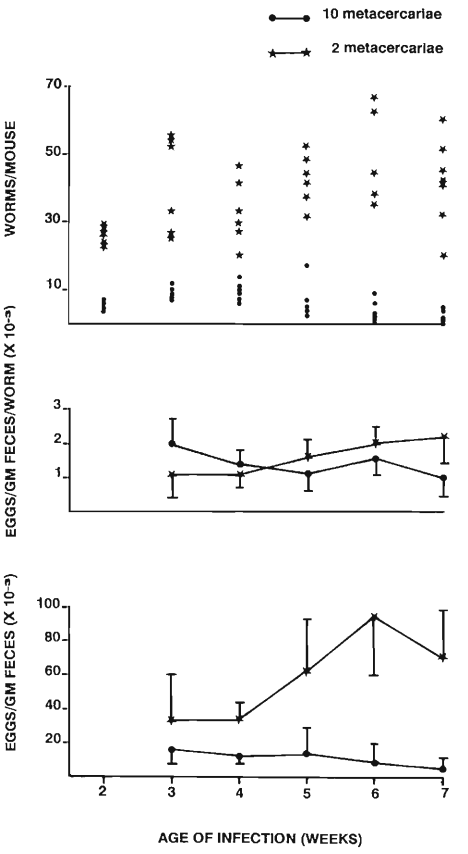

Figure 4. *Echinostoma caproni* worm recovery, eggs/g feces, and eggs/g feces/worm ($\bar{x} \pm 2$ SE) at increasing age (weeks) in repeated infections with 2 and 10 metacercariae 3 times weekly for 6 wk in female, albino NMRI mice.

currently infected mice exceeding markedly ($P < 0.05$) that in single *E. caproni*-infected mice from week 3 onwards (Fig. 3).

The regulatory response to repeated infection with either 2 or 10 metacercariae/mouse 3 times weekly for 6 wk was pronounced (Fig. 4). Thus, at both infection levels, peak of worm establishment was reached by week 2. The worm burden at the high infection level subsequently remained stable ($P > 0.05$), whereas that at the low infection level became gradually reduced ($P < 0.05$) (Fig. 4). Immature worm recovery following week 2 was negligible reflecting that recruitment of new worms following week 2 was insignificant. Thus, population turnover did not take place. The marked variation in worm burdens in individual mice at the high infection level reflects that a marked individual variation in the onset of the regulatory response exists. Eggs/g feces/worm were strictly comparable throughout ($P > 0.05$) at the 2 infection levels resulting in eggs/g feces at the high infection level exceeding markedly ($P < 0.05$) that at the low infection level.

Resistance to superimposed infection (series 5, Table 1) was affected both by the age of the mouse host and the age of the primary infection (Table 1). In 8-wk-old mice, complete resistance to superimposed infection was induced by primary 14- and 18-day-old infections with 8–12 metacercariae (Table 1, exp. 2 and 3). Concurrent *N. dubius* infection failed to interfere with this resistance (Table 1, exp. 3). In infections with 8–10 metacercariae of an age of only 8–11 days in 8- and 16-wk-old mice, resistance to super-

Table 1. Effect of age of mouse, age of primary infection, and of concurrent infection with *Nematospiroides dubius* (200 larvae/mouse) on resistance to superimposed *Echinostoma caproni* infections in outbred, female, albino NMRI mice (5–8 mice per group; necropsy day 7 following challenge).

Experiment no.	Age of mouse at primary infection (weeks)	Age of primary infection at challenge (days)	No. of metacercariae administered (primary/challenge)	*E. caproni* worm recovery ($\bar{x}$ ± SE)		Variance/mean ratio of challenge worm recovery	% resistance when significant ($P < 0.05$)	*E. caproni* challenge worm length ($\bar{x}$ ± SE) ($N = 10$)	
				Primary	Challenge			Challenge worm	Challenge control worm
1	4	11	10/10	9.7 ± 0.2	5.7 ± 1.4	2.1	—	3.5 ± 0.1	
	8	11	10/10	9.6 ± 0.4	2.2 ± 1.3	4.1	76	1.7 ± 0.0†	
	16	11	10/10	8.8 ± 0.4	1.0 ± 0.8	2.9	89	1.5 ± 0.1†	
	8	11	—/10	—	9.2 ± 0.4	0.1	—	—	4.1 ± 0.1
2	8	8	8/10	5.8 ± 0.4	5.3 ± 0.9	0.9	—	2.5 ± 0.1†	
	8	10	8/10	7.8 ± 0.2	4.0 ± 1.2	2.3	56	2.3 ± 0.2†	
	8	14	8/10	6.8 ± 0.8	0.0 ± 0.0	—	100	—	
	8	—	—/10	—	9.0 ± 0.3	0.1	—		4.8 ± 0.2
3*	8	18	12/8	10.2 ± 0.6	0.0 ± 0.0	—	100	—	
	8	18	12/8	9.9 ± 0.7	0.0 ± 0.0	—	100	—	
	8	—	—/8	—	6.3 ± 0.5	0.3	—	—	—
4	4	11	10/10	9.2 ± 0.4	7.2 ± 0.6	0.2	—	5.0 ± 0.1	
	4	20	10/10	7.8 ± 0.4	6.0 ± 1.9	3.1	—	4.9 ± 0.2	
	4	—	—/10	—	8.4 ± 0.7	0.3	—	—	5.7 ± 0.2

* Primary *E. caproni* infections were superimposed on 6-day-old *N. dubius* infection.
† Level of significance = $P < 0.05$.

imposed infection was less marked although still significant ($P < 0.05$) (Table 1, exp. 1 and 2). The high variance to mean ratio shows that an effective resistance response was mounted in some but not in all mice. Heterogeneity in response to superimposed infection was thus marked. Established superimposed worms experienced a marked stunting ($P < 0.05$). In contrast to 8- and 16-wk-old mice, 4-wk-old mice harboring primary 11- or 20-day-old primary infections with 10 metacercariae failed on a group basis to mount an effective resistance response to superimposed infection with mean challenge worm and mean challenge control worm establishment being comparable ($P > 0.05$) (Table 1, exp. 4). The high variance to mean ratio reflects that some mice are in fact responder mice. However, most mice were non-responder mice and established superimposed worms did not experience stunting ($P > 0.05$).

Discussion

Previous studies on the *E. caproni*/NMRI and on the *E. caproni*/SVS mouse models have demonstrated interesting host–parasite relationships like concomitant immunity, rapid expulsion of superimposed infections, infection–dose independency of primary worm establishment, a negatively dose-dependent pattern of expulsion of primary infections, and a positively dose-dependent reproductive potential in infections within the range of 6–25 metacercariae per mouse (Christensen et al., 1986; Odaibo et al., 1988). This study has elucidated further aspects of the population regulation in *E. caproni* infections in outbred, female albino NMRI mice. Attention is given to the regulatory response to secondary, superimposed, and repeated infections and to the effects of concurrent *N. dubius* infection on the population regulation.

The characteristics of the resistance response to superimposed *E. caproni* infection in NMRI mice (present study) agree well with those in SVS mice (Christensen et al., 1986). The response, however, expresses mouse strain dependency. Thus, the ability to mount an effective response develops more quickly in NMRI than in SVS mice (Sirag et al., 1980; Christensen et al., 1986; present study), and the ICR mouse strain is a low responder strain with an only moderate capability to mount an effective response to superimposed challenge infection (Hosier et al., 1988). The unresponsiveness of young animals in the *E. caproni*/NMRI mouse model agrees

well with that demonstrated in other intestinal helminth/rodent models (see Behnke, 1987). Slow maturation of the cellular components of the immune system and enhanced suppressor cell activity have been suggested to be responsible for this immunological unresponsiveness (Dineen and Kelly, 1973; Strober, 1984). The marked capability to mount an effective response to repeated *E. caproni* infection is explained by the early onset of an effective resistance to incoming juveniles. The repeated exposure to juvenile worm antigen did not interfere with the resistance to incoming worms. However, as stated by Behnke (1987), carefully controlled and appropriate experimentation is needed to elucidate further the regulatory response to repeated infection with intestinal helminths in various host–parasite relationships.

The marked resistance to secondary infection in the *E. caproni*/NMRI mouse model (present study) for up to at least 12–14 wk following expulsion of primary infections with 6 metacercariae extends earlier observations on the *E. caproni*/SVS mouse model in which the resistance was shown to persist for at least 6 wk (Christensen et al., 1986). Such acquired resistance combined with development of an age-related reduced susceptibility to primary infection (Odaibo et al., 1988; present study) result in the responder part of the mouse population remaining resistant throughout. However, heterogeneity in the regulatory response to secondary infection in NMRI mice exists, and the low responder part of the population regains the susceptibility to infection. A further elucidation of the immunological reactivity governing the course of primary, superimposed and repeated *E. caproni* infections should be carried out using genetically and immunologically well defined inbred strains of mice.

The prolonged persistence of heavy as compared with light infections and the prolonged persistence of infections with 6 *E. caproni* metacercariae in response to concurrent *N. dubius* infection are suggested induced by immunomodulation. Prolonged survival of intestinal helminths in response to concurrent *N. dubius* infection has been demonstrated previously (see Behnke, 1987). As demonstrated in the present study, such prolonged persistence may increase markedly the overall reproductive potential of parasites. The dose-dependent suppression of immunity, initially demonstrated by Christensen et al. (1981) working on the *E. caproni*/SVS mouse model, has recently been demonstrated in rodent infections with *N. dubius* (Dobson and Cayzer, 1982; Hannah and Behnke, 1982; Dobson et al., 1985; Sitepu et al., 1985) and with *Trichinella spiralis* (Bell et al., 1983, 1984; Wassom et al., 1984). However, the immunological background for these events of immunomodulation remains to be fully elucidated (see Behnke, 1987).

Immunomodulatory events induced by factors like malnutrition, concurrent infection, and dose-dependent suppression of immunity presumably exert a major regulatory influence on the overall population dynamics (Behnke, 1987; Bundy and Golden, 1987; Christensen et al., 1987). The *E. caproni*/NMRI mouse system represents a model in which homologous and heterologous immunomodulation may affect synergistically the reproductive potential and thereby the parasite population dynamics.

Acknowledgments

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Research Note

The Development of *Quadriplotriaena hypsokysta* (Nematoda: Diplotriaenoidea) in Grasshoppers (Orthoptera)

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ABSTRACT: *Quadriplotriaena hypsokysta* Crites, 1964, from western meadowlarks (*Sturnella neglecta* Audubon), developed to the infective stage in delicate capsules in fat body tissue of the nymphs of *Aeropedellus clavatus* (Thomas). Infective larvae, found 30 days after the grasshoppers were exposed to eggs, resembled those of species of *Diplotriaena*.

KEY WORDS: Nematoda, *Quadriplotriaena hypsokysta*, development, grasshoppers.

During the summer of 1987 the air sacs of a western meadowlark (*Sturnella neglecta* Audubon) collected near Brooks, Alberta, Canada, contained mature *Quadriplotriaena hypsokysta* Crites, 1964. The genus is closely related to *Diplotriaena*, but is characterized by the presence of 4 trident-like structures with apical pores on the anterior extremity rather than 2 found in the latter genus (Anderson and Bain, 1976).

Eggs containing first-stage larvae were removed from the reproductive tract, mixed with water and flour, then spread on blades of grass and fed to locally caught grasshopper nymphs of *Aeropedellus clavatus* (Thomas) considered to be free of parasitic nematodes (10 examined prior to the experiment were uninfected). The exposed grasshoppers were kept at ambient temperatures in a plexiglass cage in an unheated garage and 30 days later examined for larvae. Fifteen of the 20 grasshoppers fed eggs contained infective third-stage larvae.

Larvae, which were in delicate capsules in fat body tissue, were stout and slightly bowed dorsally when fixed in glycerin alcohol (Fig. 1). Prominent lateral alae extended from near the cephalic extremity to the region of the anus (Fig. 1). The tail was short and ended in a delicate nipple-like swelling (Fig. 2). The muscular esophagus was short and rather irregular in outline (Fig. 4). The glandular esophagus was broad and extended to within a short distance of the anus (Fig. 1). The intestine was compressed into a small space between the terminal end of the esophagus and the anus (Fig. 2). The oral opening was surrounded by thickened cuticle which was elevated in the dorso- and ventrolateral corners (Fig. 3). The amphids were tiny and there were 4 pairs of cephalic papillae, the inner group being close to the cuticular structure surrounding the oral opening.

The dimensions (in micrometers, mean followed by range) of the infective larva ($N = 5$) were as follows: length 733 (678–820); maximum width (in posterior third of body) 48 (45–52); length of muscular part of esophagus 107 (92–120); length of glandular part of esophagus 620 (582–662); total length of esophagus 725 (694–782); nerve ring and excretory pore 79 (78–82) and 87 (81–92), respectively, from anterior extremity. Tail 29 (28–32) in length.

Only 2 species of *Quadriplotriaena* are known. In addition to *Q. hypsokysta*, *Q. dolichodema* Wehr, 1939, has been described from *Pica pica* (L.) in Montana, U.S.A. The present report is the first to reveal the mode of transmission of members of the genus. Development in fat body tissue of grasshoppers and the morphology of the infective larva confirm the close affinities between the genus *Quadriplotriaena* and the much more common genus *Diplotriaena* (for information on the development of *D. tricuspis* see Cawthorn and Anderson, 1980). It has been suggested that the former genus originated as a mutant of the latter (Anderson, 1968).

Dr. V. R. Vickery of the Lyman Entomological Museum and Research Laboratory, Macdonald College of McGill University, kindly identified the grasshoppers used in this study.

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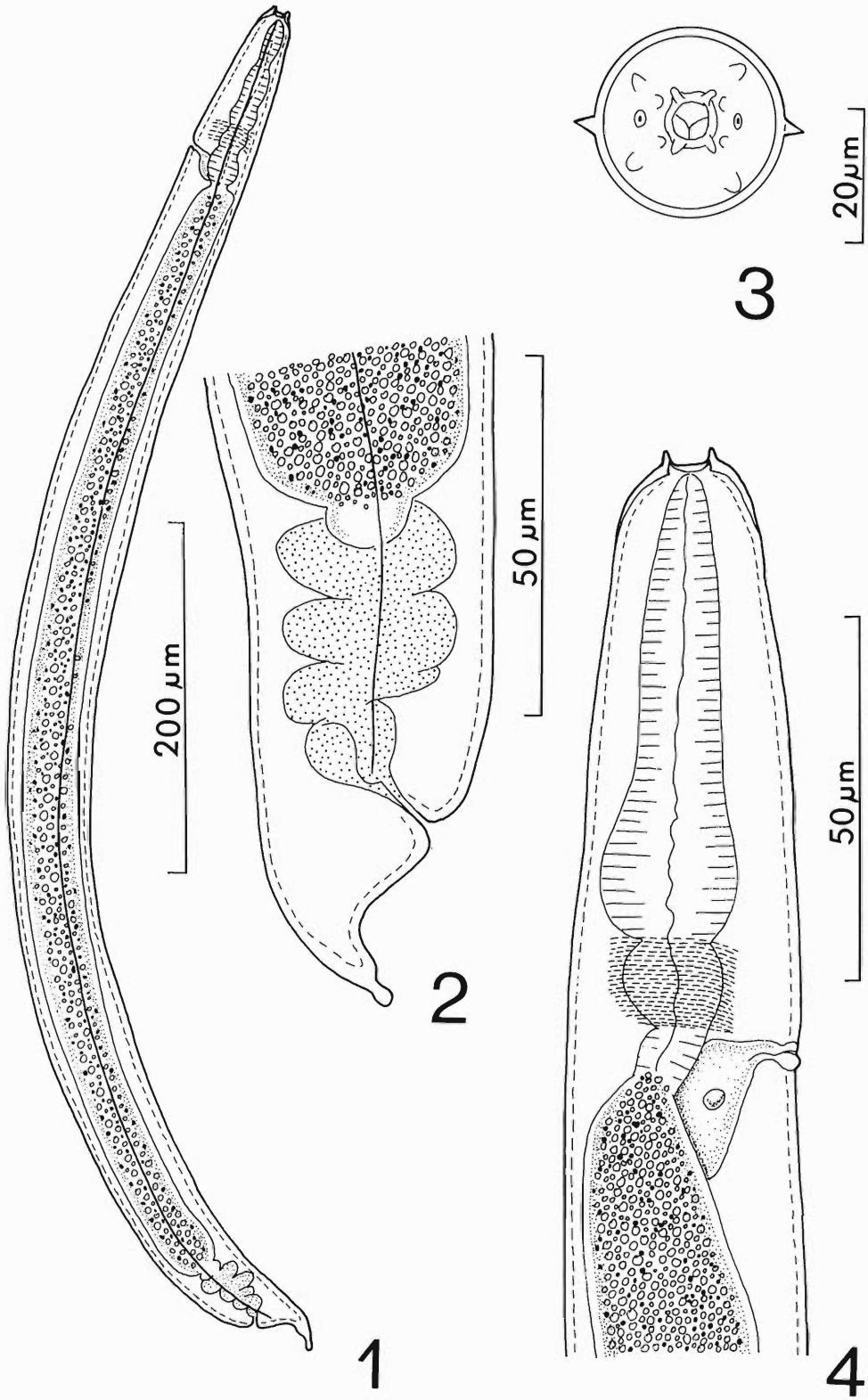

Figures 1–4. Third-stage larva of *Quadriplotriaena hypsokysta*. 1. Lateral view of entire larva. 2. Posterior extremity, lateral view. 3. Cephalic extremity, en face view. 4. Anterior extremity, lateral view (alae not included).

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Research Note

Procedure for the Recovery of Excretory–Secretory Products from Molting Fourth-stage Larvae of *Ostertagia ostertagi*

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ABSTRACT: A procedure is described that allows the collection of secreted antigens from *Ostertagia ostertagi* undergoing the molt from fourth-stage larvae to young adults. Calves were experimentally infected with infective larvae of *O. ostertagi* and then killed at various times after infection. The abomasa were aseptically removed and allowed to incubate in physiological saline, and worms which had migrated from the tissues were collected and placed in culture. Materials released by the worms in culture were collected. The results of the study indicated that the optimal time to collect worms in the molt to young adult was 9–10 days after infection of the donor calf. Overnight incubation of the abomasa resulted in the largest percentage of worms recovered, while a culture time of 72 hr gave the highest amount of material recovered. As expected, as culture time increased the viability of the worms decreased, with mortality rates of about 30% at 72 hr.

KEY WORDS: nematode, cattle, stomach worm, *Ostertagia ostertagi*, excretory–secretory products.

Nematode excretory–secretory (ES) products have been shown to be an excellent source of protective antigens (Lloyd, 1981). The major obstacle in working with such preparations has been the small quantities of material that are usually obtainable.

One of the most important applications of in vitro cultivation of parasitic nematodes is the potential recovery of these antigens from the culture media. In spite of the progress made in recent years with in vitro culture techniques, the development of nematodes in vitro is significantly delayed compared to that of worms developing in vivo, resulting in increased labor and materials required to raise the parasites successfully in culture. In addition, as the worms grow in vitro, the populations tend to become more and more asynchronous making it impossible to collect stage-specific antigens. The highest rate of in vitro development of *Ostertagia ostertagi* has been reported by Douvres and Malakatis (1977) using a 2-step roller culture system. After 30 days the cultures contained estimated percentages of 35% mature adults (MA), 23% young adults (YA), and over 40% fourth-molt (4M), fourth-stage (L4), and third-molt (3M) larvae. The authors also reported the majority of the worms required 16–18 days to reach the young adult stage (Douvres and Malakatis, 1977). *Ostertagia ostertagi* develop from the parasitic third stage to the YA in the gastric glands of the abomasal tissue, and recovery of larval *O. ostertagi* from the abomasal tissues has traditionally been accomplished by pepsin–hydrochloric acid digestion of the abomasal mucosa (Herlich, 1956). However, methods have been recently described for the recovery of larval *Ostertagia* spp. using incubation of the abomasa in saline or tap water (Jackson et al., 1984; Snider et al., 1985; Gasbarre, 1987). Urban and Douvres (1981) and Nelson and Douvres (1984) utilized modified baermanization of homogenized lungs or entire intestine to recover *Ascaris suum* or *Trichostrongylus colubriformis* larvae and adults from

Table 1. In vitro development of *O. ostertagi* recovered from calves at various times after infection.

Trial number	Number of calves	Days in vivo	Percent of inoculum recovered	Hours in culture	Percent of worms motile	Percent of motile worms				Protein concentration of ES
						Late fourth	Fourth molt	Loose sheath	Young adults	
1	2	9	43	0	100	9	90	1	—	400 mg/ml
				72	73	7	9	47	37	
2	1	9	30	0	100	17	79	5	—	380 mg/ml
				72	68	3	19	43	35	
3	1	10	10	0	100	7	39	49	8	156 mg/ml
				48	87	—	12	23	65	
4	1	11	6	0	100	—	47	45	8	40 mg/ml
				48	80	—	6	14	80	

tissue taken from infected animals. Using a combination of these methods, we have designed a procedure that results in the recovery of *O. ostertagi* at the time the larvae are undergoing the molt from the fourth to the fifth stage inside the abomasal mucosa. The recovered worms are then placed in cultures for the isolation of specific ES products of the molt.

Five Holstein calves, 4–5 wk old, were each orally inoculated with 2.5×10^5 infective larvae of *O. ostertagi*. Animals were killed 9 days (trials 1 and 2), 10 days (trial 3), or 11 days (trial 4) after infection. The abomasa were aseptically removed, opened longitudinally, and the mucosal surface was rinsed with physiological saline. In trial 1 the abomasum was placed in a sterile bucket containing 1 liter of physiological saline with antibiotics (1,000 U/ml penicillin G potassium, 1 mg/ml streptomycin sulfate, and 5 μg/ml amphotericin B) and incubated at room temperature for 17 hr, after which the bucket was placed at 37°C and incubated for an additional 3 hr. The abomasum was recovered from the bucket and the mucosal surface gently rubbed and rinsed with saline plus antibiotics. The resulting washings of the 2-step incubation process were added to a Baermann apparatus consisting of a glass funnel filled with warm saline plus antibiotics, and containing a wire screen insert to which 2 layers of cheesecloth were attached. After an additional 5 hr of incubation at 37°C, the worms that had settled in the collection vessel were removed. In trial 2, to minimize handling and potential contamination of the recovered worms, the abomasum was opened, rinsed with saline, cut into 3 pieces, and each piece was placed directly on the screens of the Baermann apparatus. In this trial the abomasum was again taken 9 days after infection, and incubated overnight at 37°C before harvest of the worms. To optimize the recovery of molting larvae, and to reduce the time of incubation, in trials 3 and 4 the animals were killed 10 and 11 days after infection, and the abomasa were incubated for 8 hr at 37°C.

Worms collected from the abomasa in each of these experiments were washed 6 times by centrifugation at 200 *g* for 2 min in Dulbecco's phosphate buffered saline (DPBS) containing the same concentrations of antibiotics as used in the isolation of the worms. The worms were then transferred to roller bottles (205 mm long × 25 mm diameter) containing 40 ml of RPMI-1640, supplemented with 10 mM L-glutamine, 25 mM HEPES, and the antibiotics described above. A gas phase of N_2, O_2, CO_2 (85-5-10) was used, and the bottles were rotated at 1 rev/1.5 min, at 39°C.

Culture supernates were collected after 48 hr (trials 3 and 4) or 72 hr (trials 1 and 2) by centrifugation to remove the worms and the resulting supernates were concentrated to a final volume of 10 ml using an ultrafiltration cell (Amicon Corporation, Danvers, Massachusetts) fitted with a 10,000 MW cut-off membrane. The concentrates were dialyzed overnight against DPBS at 4°C. The dialysate was filter sterilized (0.2-μm filter) and the protein concentration estimated by the Bio-Rad coomassie blue dye-binding assay (Bradford, 1976).

Samples of the worm populations were taken before and after culture to estimate the number, viability, and stage of development of the worms recovered. The resultant recoveries, developmental stages, and protein concentration of the ES products are shown in Table 1. Recovery of larvae 10 days postinfection appeared to result in the highest percentage of larvae undergoing

the final molt, while larvae recovered at 9 days postinfection were less able to complete the fourth molt. Larvae recovered 11 days after infection (trial 4) were at the same developmental stages as worms recovered after 10 days. This observation, and the smaller number of worms recovered, raises the possibility that later stages are lost during processing of the mucosa, even though the majority of the worms will not emerge into the abomasal lumen until day 16 under normal circumstances (Ritchie et al., 1966). The stage of development of the worms at the times of recovery was in agreement with the observations of Douvres (1956). According to Douvres (1956) and Rose (1969) the molt to the young adult was completed by the twelfth day.

The final protein concentration of the ES products isolated using the 4 different protocols was related to the number of worms placed in culture. The highest concentrations were achieved in trials 1 and 2, and the lowest in trial 4 where just 6% of the initial dose was recovered.

Worm recovery increased with the length of time that the abomasa were incubated. However, the survival rate of the recovered larvae decreased when the abomasa were incubated for 72 hr. High mortality in culture has been implied to be the cause of contamination of ES with somatic material (Rothwell and Love, 1974; Zimmerman and Leland, 1974). Since handling and hence problems of contamination were reduced by direct baermanization of the abomasal pieces, and overnight incubation enhanced total recovery, the procedures outlined in trial 2 offered the best opportunity for antigen recovery.

In summary the protocols described offer the potential for recovery of ES products released during the molt from L4 to young adult of *O. ostertagi*. This information should allow further characterization of potentially important antigens released during a period of intense metabolic activity by the worm, at a time of intimate contact with host tissues.

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Research Note

Endoparasites of the White-tailed Prairie Dog, *Cynomys leucurus*, at Meeteetse, Park County, Wyoming[1]

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ABSTRACT: Endoparasites of 8 species were recovered from 17 white-tailed prairie dogs, *Cynomys leucurus*, examined from colonies near Meeteetse, Wyoming in 1986, viz., 4 protozoans: *Eimeria cynomysis, E. larimerensis, E. ludoviciani,* and *Sarcocystis* sp.; 2 species of cestodes: *Hymenolepis citelli* and *Taenia mustelae*; and 2 nematodes: *Physaloptera* sp. and *Capillaria* sp. Six of these parasites are reported here for the first time from this host with only *E. larimerensis* and *E. ludoviciani* previously known from white-tailed prairie dogs.

KEY WORDS: *Cynomys leucurus*, white-tailed prairie dog, endoparasites, survey, Protozoa, Nematoda, Cestoda.

Few reports concerning parasites of the white-tailed prairie dog (*Cynomys leucurus* Merriam) exist in the literature. The purpose of this paper is to report results of an investigation of the endoparasites of white-tailed prairie dogs from northwestern Wyoming. Interest in this particular population of prairie dogs was stimulated by the discovery of the endangered black-footed ferret (*Mustela nigripes* Audubon and Bachman) in this area in 1981. Prairie dogs are the primary prey of black-footed ferrets (Sheets et al., 1972).

Seventeen prairie dogs were live trapped near Meeteetse (44°0.5′–44°15′N, 108°55′–109°15′W) from a population comprised of 33 colonies covering approximately 30,000 ha. These animals were transported to the Wyoming State Veterinary Laboratory, Laramie, Wyoming, where they were held for 3–4 days on a diet of laboratory rodent chow and carrots prior to necropsy. Fecal samples and tissues from stomach, intestine, liver, and muscle were collected and examined for parasites.

For identification of the coccidian oocysts, fecal specimens were stored at room temperature (approximately 25°C) for 2–3 wk in 2% potassium dichromate to allow oocyst sporulation. Fecal floatations using Benbrook's sugar solution (specific gravity = 1.2) were performed to concentrate oocysts. Striated muscle from the lumbar and caudal thigh regions and a variety of other tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 6 μm for examination by light microscopy. Stomach and intestines were opened longitudinally and the contents scraped into finger bowls and washed several times with tap water. The material was then poured into petri dishes and examined under a dissecting microscope (12×) for helminths. Cestodes were relaxed and killed in hot 10% formalin and later stained with Ehrlich's hematoxylin and mounted on slides for identification. Nematodes were relaxed and killed in hot 70% EtOH plus 5% glycerol and later cleared in lactophenol for identification. Livers containing cystlike structures were dissected and examined for parasites. Hook mounts of cysticerci were prepared in Hoyer's medium for examination and identification.

Four protozoan, 2 cestode, and 2 nematode species were found infecting white-tailed prairie dogs in this study. A list of species and their distribution by age class and sex are shown in Table 1. For ease of comprehension each species will be considered separately.

Voucher specimens of the following helminths have been deposited in the USNM Helminthological Collection under the following accession numbers: *Physaloptera* sp. (80540), *Capillaria* sp. (80541), *Taenia mustelae* (80542), and *Hymenolepis citelli* (80543).

Eimeria cynomysis Andrews, 1928, was first reported infecting prairie dogs by Andrews (1928), but he did not specify what species of prairie dogs he had examined. Vetterling (1964) reported and redescribed *E. cynomysis* from black-tailed prairie dogs (*Cynomys ludoviciani* Ord) from northern Colorado. This is the first report of *E. cynomysis* infecting white-tailed prairie dogs.

Eimeria larimerensis Vetterling, 1964, was re-

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Table 1. Results of survey for endoparasites of white-tailed prairie dogs, *Cynomys leucurus*, near Meeteetse, Wyoming.

Species of parasite	Adult (N = 4)		Juvenile (N = 13)		Total infected/ prevalence*
	Male	Female	Male	Female	
Eimeria cynomysis	0	0	1	3	4/23.5%
E. larimerensis	0	0	1	3	4/23.5%
E. ludoviciani	1	1	3	6	11/64.7%
Sarcocystis sp.	1	1	0	0	2/11.8%
Hymenolepis citelli	2	1	1	0	4/23.5%
Taenia mustelae	0	1	0	0	1/5.8%
Physaloptera sp.	2	2	2	0	6/35.3%
Capillaria sp.	1	0	1	0	2/11.8%

* Prevalence = number infected with parasite species/total number examined (Margolis et al., 1982).

ported and described from black-tailed prairie dogs from northern Colorado by Vetterling (1964). Todd and Hammond (1968b) reported *E. larimerensis* infecting *C. leucurus* and *Spermophilus tridecemlineatus* Mitchill from Wyoming, *S. armatus* Kennicott from Utah and Montana, *S. variegatus* Erxleben and *S. lateralis* Say from Utah, and *S. beecheyi* Richardson from California.

Eimeria ludoviciani Vetterling, 1964, was initially reported and described from black-tailed prairie dogs from northern Colorado. Todd and Hammond (1968a) reported *E. ludoviciani* from white-tailed prairie dogs from Wyoming.

Mixed infections of 2 or more species of *Eimeria* were found in 5 prairie dogs. One adult female was infected with both *E. larimerensis* and *E. ludoviciani.* Two juvenile prairie dogs (1 female, 1 male) were infected with *E. cynomysis* and *E. ludoviciani.* One juvenile female was infected with both *E. cynomysis* and *E. larimerensis.* One juvenile male was infected with all 3 species of *Eimeria.*

The finding of these 3 species of *Eimeria* in white-tailed prairie dogs, and previous reports of their occurrence in black-tailed prairie dogs and ground squirrels of the genus *Spermophilus,* is consistent with the findings of Todd and Hammond (1968a, b) who found that while host restriction for *Eimeria* in the Rodentia usually occurs at the genus level, the close taxonomic relationship of *Cynomys* and *Spermophilus* allows for crossover of some eimerian species.

Two adult prairie dogs were infected with muscle cysts that appeared to be of the genus *Sarcocystis* (Lankester, 1888). These cysts were ≤ 1 mm in length, rod-shaped, and oriented parallel to the muscle fibers. Further work is required before identification of this organism can be completed. No reports of *Sarcocystis* infecting members of the genus *Cynomys* exist in the literature.

The tapeworm *Hymenolepis citelli* McLeod, 1933, was recovered from the intestine of 4 (23%) prairie dogs. This species has never been reported infecting members of the genus *Cynomys.* McLeod (1933) first reported and described *H. citelli* from ground squirrels, *Spermophilus* (syn. *Citellus*), from Manitoba, Canada. Buscher and Tyler (1975) reported this tapeworm from thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) collected from active black-tailed prairie dog towns in Oklahoma.

Cysticerci of *Taenia mustelae* Gmelin, 1790, were recovered from the liver of 1 adult female prairie dog. Hook numbers and measurements were consistent with those reported by Freeman (1956) in an extensive treatment of *T. mustelae.* This is the first report of larval *T. mustelae* from white-tailed prairie dogs.

Both mature and immature forms of *Physaloptera* sp. (Rudolphi, 1819) were attached to the gastric mucosa of 6 prairie dogs. An ulcerative gastritis was present at the point of attachment of these nematodes to the mucosa. The finding of *Physaloptera* sp. in white-tailed prairie dogs constitutes a new host record for this genus.

Capillaria sp. (Zeder, 1800) was found in liver sections of 2 prairie dogs. While whole specimens were not recovered, the location, size, and morphology of the recovered material leads us to believe this worm is most likely *Capillaria hepatica* Bancroft, 1893. The nematodes were found associated with a granulomatous inflammation. This is the first report of the genus *Capillaria* infecting members of the genus *Cynomys.*

The endoparasites infecting white-tailed prairie dogs in this study were similar to those reported from black-tailed prairie dogs (Vetterling, 1962, 1964) and ground squirrels of the genus *Spermophilus* (McLeod, 1933; Morgan, 1943; Todd and Hammond, 1968a, b). The occupation of similar habitat types (Vaughan, 1978) and utilization of similar food types, along with the close taxonomic position of these 2 genera (Bryant, 1945), may account for this similarity in parasite fauna. Definitive hosts for the larval *Taenia mustelae* reported here may be the black-footed ferret, *Mustela nigripes* (N. Kingston and J. Rockett, unpubl. data), and other mustelids; mustelids may also serve as a host for *Capillaria hepatica* (Levine, 1980).

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Research Note

Muscular *Sarcocystis* in a Dog

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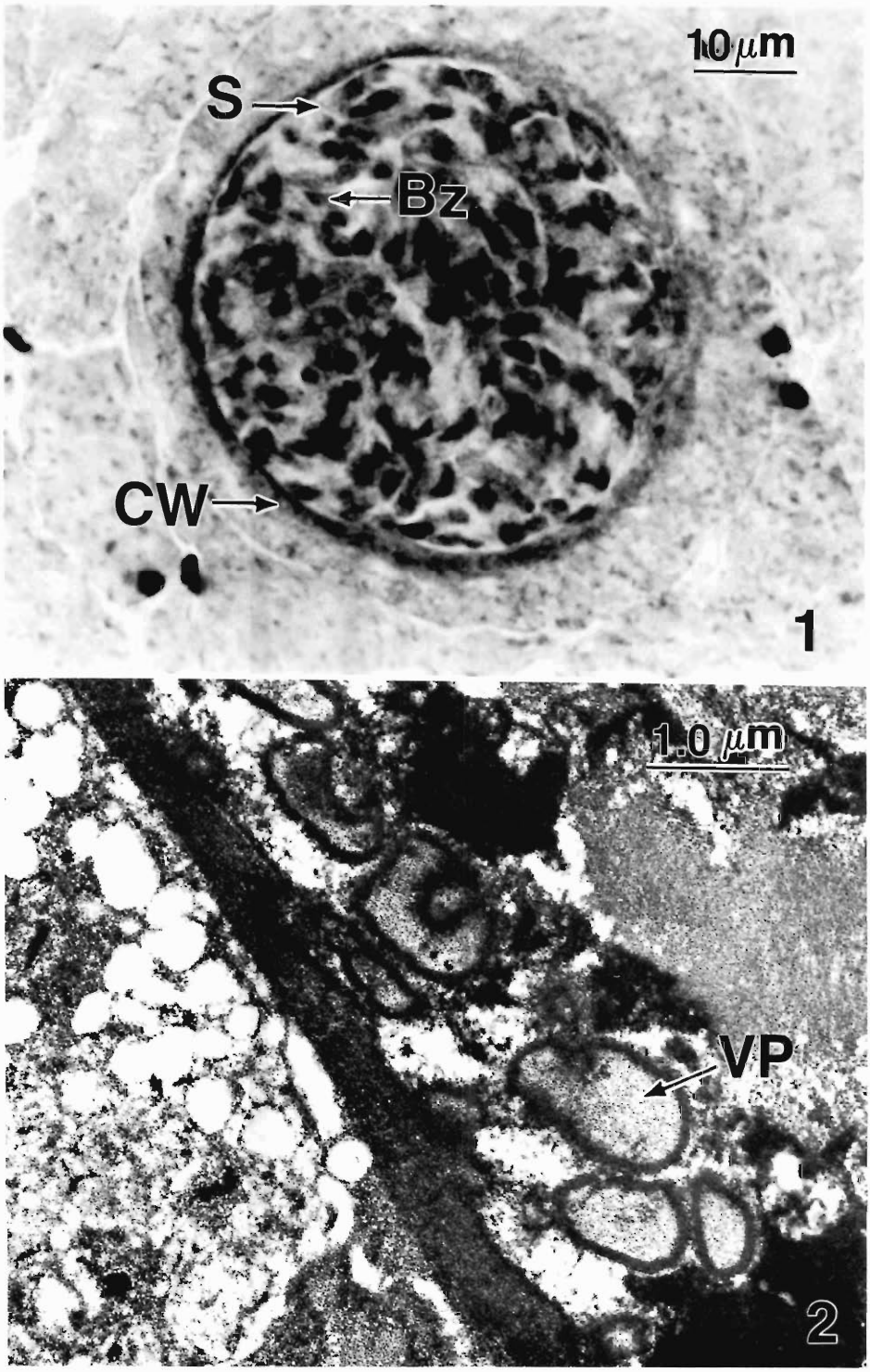
ABSTRACT: A sarcocyst is described from the biceps femoris muscle of a dog using both light and transmission electron microscopy. The sarcocyst was spherical to ovoid and measured 47 × 52 μm inclusive of the wall. The wall was palely eosinophilic, approximately 2.3 μm at its widest margin, and 0.9 μm at its narrowest margin. Cyst wall projections were barely visible in histological sections. Ultrastructurally, they appeared as irregularly spaced electron dense projections, measuring up to 1.5 μm long and 0.9 μm wide. The electron dense granular layer of the wall was approximately 0.7 μm thick. Interior septa were visible as electron dense lines that appeared to compartmentalize numerous irregularly arranged bradyzoites. Bradyzoites were elongate with discernible apical complexes and with posterior nuclei. Metrocytes were not seen. The sarcocyst did not appear to elicit an inflammatory response and was considered an incidental finding.

KEY WORDS: canine, dog, *Sarcocystis*, muscle.

Sarcocystis spp. are traditionally viewed as obligatorily heteroxenous coccidia that normally undergo vascular merogony and muscular cystogenesis in intermediate hosts, and gametogony, sporogony, and sporulation in definitive hosts (Fayer, 1980). The presence of *Sarcocystis* cysts in the muscles of a carnivore, normally a definitive host, is perplexing. This unusual and infrequent occurrence is the basis of the present report.

A 4-yr-old male (castrated) dog was presented to a veterinary clinic in the southeastern United States for physical and neurological examination because of ataxia and limb stiffness. Biopsies of biceps femoris (BF), triceps brachii, and temporalis muscles were submitted to 1 of the authors (K.G.B.) for histomorphological and histochemical evaluations. Frozen sections were prepared as previously described (Braund et al., 1978) and stained with a battery of stains including the periodic acid-Schiff (PAS) reaction. Following the discovery of what appeared to be a cyst of *Sarcocystis* species in a section of BF, additional frozen samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for additional light microscopic examination. Formalin-fixed BF muscle was prepared for electron microscopy using the following procedure: frozen sections were air-dried at room temperature, fixed in 10% formalin, stained with H&E, and left in distilled water. Sections were examined using a light microscope and the area containing the sarcocyst demarcated using a diamond pen. Sections were then postfixed in 1% osmium tetroxide in Millonig's buffer, pH 7.3, for 1 hr, dehydrated in an ethanol series, and embedded in Spurr resin (Polysciences Inc., Warrington, Pennsylvania). Resin flooded slides, elevated by toothpicks, were oven-cured overnight at 70°C. The area of the section containing the sarcocyst was detached using a single-edge razor blade. This portion of the section was then glued onto a Spurr dummy block (Aron Alpha Quick Setting Adhesive, Ted Pella Inc., Tustin, California). Thin sections were cut using an Ultratome III, LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined using a Philips 301 electron microscope.

The single cyst observed in an H&E-stained cross section of the BF using light microscopy was spherical to ovoid and measured approximately 47 × 52 μm inclusive of the cyst wall (Fig. 1). The wall was palely eosinophilic, approximately 2.3 μm at its widest margin, and 0.9 μm at its narrowest margin. Outer cyst wall projections were barely visible in histological sections. Septate projections of the cyst wall into the interior of the cyst were visible as clear or dark lines in PAS- and H&E-stained sections, respectively. Bradyzoites were moderately to intensely eosinophilic in H&E-stained sections, and were irregularly arranged in groups bordered by septa. The same cyst stained using the PAS reaction revealed a cyst wall and interior septa that were PAS-negative. Bradyzoites were magenta and were arranged as described for H&E-stained



Figures 1, 2. Cyst of *Sarcocystis* from a frozen biopsy of biceps femoris muscle of a dog. 1. Photomicrograph of cyst showing primary cyst wall (CW), septum (S), and bradyzoites (Bz). Hematoxylin and eosin. 2. Electron micrograph of the cyst wall prepared from formalin-fixed, frozen biopsy of biceps femoris muscle. Note villous projections (VP) from the primary cyst wall.

sections. Ultrastructurally, the primary cyst wall contained numerous irregularly spaced villous projections up to 1.5 μm long and 0.9 μm wide (Fig. 2). An electron dense granular layer, approximately 0.7 μm thick, was present just beneath the villous projections. This electron dense granular substance was also present in many of the villous projections. In some areas, a parasitophorous vacuole was present adjacent to the primary cyst wall. Septate projections of the cyst wall were visible as electron dense lines that appeared to compartmentalize the numerous irregularly arranged bradyzoites. Bradyzoites were elongate with discernible apical complexes and with posterior nuclei. Metrocytes were not seen. The presence of the sarcocyst in the BF muscle was considered an incidental finding.

Morphological characteristics of the observed cyst are consistent with those of *Sarcocystis* spp. (Dubey, 1977). The presence of sarcocysts in the muscles of a dog is intriguing. Previous reports of canine muscular sarcocystiasis included recovery of an apparent sarcocyst in esophageal muscles of a dog from India (Sahasrabudhe and Shah, 1966) and in the myocardium of a dog from the United States (Hill et al., 1988). In neither case was an inflammatory reaction associated with the sarcocysts. Carnivorous hosts from which muscular cysts of *Sarcocystis* spp. have been recovered include: humans, domestic dogs, domestic cats, leopards, raccoons, whales, black bears, viperid snakes, pythons, buzzards, weasels, skunks, badgers, genets, and mongooses (Bhatavdekar and Purohit, 1963; Sahasrabudhe and Shah, 1966; Tadros and Laarman, 1982; Kirkpatrick et al., 1986; Everitt et al., 1987; Kirkpatrick et al., 1987; Somvanshi et al., 1987; Hill et al., 1988). Several explanations may be forwarded for the presence of muscle cysts in what would be considered a definitive host for *Sarcocystis* spp. Lack of rigid host specificity for *Sarcocystis* stages in the intermediate host would allow sporocysts of *Sarcocystis* species to infect more than a single intermediate host species. Recent evidence supports decreased intermediate host specificity for certain *Sarcocystis* species (Box and Duszynski, 1978). *Sarcocystis* spp. may form intestinal and extraintestinal stages in the same host. This has been reported recently for *Sarcocystis galloti* infecting Canarian lizards (Matuschka and Bannert, 1987). In addition, domestic canids may serve as intermediate hosts for *Sarcocystis* spp. whose definitive host preys upon canids. Certain canids might prey upon homologous members of the species or upon closely related canids. It was reported, for example, that coyotes will prey upon other coyotes (Andrews and Boggess, 1978). Further, over an 8-yr period, the Patuxent Wildlife Research Center, Laurel, Maryland, confirmed 24 depredations of domestic dogs by wolves. Of 19 carcasses that were subsequently checked, 14 were partially or fully eaten. Wolves are also known to kill and consume other wolves (Dr. Steven H. Fritts, Patuxent Wildlife Research Center, Laurel, Maryland, pers. comm.).

Because muscle cyst morphology is not considered taxonomically definitive, it was not possible to determine the species to which the sarcocyst belongs. The cyst described herein is morphologically similar to those described previously from cats (Everitt et al., 1987; Kirkpatrick et al., 1987; Hill et al., 1988). Comparisons cannot be made with the cysts previously described from dogs, because electron micrographs were not included with either report.

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Research Note

The Growth of *Hymenolepis diminuta* in Five Strains of Mice

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ABSTRACT: The kinetics of growth and subsequent rejection pattern of the intestinal cestode, *Hymenolepis diminuta,* were examined in 5 different inbred strains of mice. Six 8-wk-old females from the strains C57/Bl, A, Balb/c, DBA, and C3H/He were infected with 5 cysticercoids via a stomach tube. The effects of infection were examined by autopsy on days 6, 8, 10, 12, and 14 postinfection. Parameters examined were worm number, worm length, worm biomass, spleen weight, liver weight, and white blood cell differentials. There were no significant differences in spleen or liver weights or white blood cell differentials among the different strains. However, there were significant differences in development as assessed by worm number, length, and biomass. Autopsy indicated that worms established and grew normally in all mice by day 6, but between days 10 and 14 most worms were lost. Worms recovered from different strains demonstrated different growth profiles. For example, worms that were isolated from C3H/He mice exhibited a high recovery, long length, and large biomass when compared to worms isolated from C57/Bl mice.

KEY WORDS: *Hymenolepis diminuta,* cestode, growth, development, mice, inbred strains.

The rat cestode, *Hymenolepis diminuta,* is a non-invasive intestinal tapeworm that lives as an adult in a variety of rodent hosts. When mice are given a primary infection of *H. diminuta,* they respond by rejecting the worms. These events are known to be immunologically mediated (Hopkins, 1980). The influence of the strain of mouse on rejection of *H. diminuta* has not been fully explored. Limited studies have examined Swiss albino, CFLP, Porton, Balb/c, C57, and nude mice (Weinmann, 1966; Hopkins et al., 1972; Isaak et al., 1975; Bland, 1976; Andreassen et al., 1978; Isaak, 1983). In separate trials, each of these strains was shown under different circumstances independently to reject *H. diminuta.* However, simultaneous infections were not performed within 1 laboratory, so it is difficult to assess if there is differential growth and rejection of *H. diminuta* among mouse strains.

The purpose of this investigation was to examine the kinetics of growth and subsequent rejection pattern of *H. diminuta* in 5 strains of mice. Parameters examined were white blood cell differentials, spleen and liver weights, and worm number, length, and biomass.

Tapeworm-free, 6- to 8-wk-old female mice of the inbred strains designated A/J, Balb/cBYJ, C3H/HeJ, C57/Bl/6J, and DBA/2J were obtained from the Jackson Laboratory (Bar Harbor, Maine). They were maintained under conventional laboratory conditions. They were supplied with mouse breeding diet (Purina mouse chow) and water ad libitum.

Cysticercoids of *H. diminuta* approximately 32 days old were dissected from adult flour beetles, *Tribolium confusum,* in distilled water as described by Insler and Roberts (1976). Five cysticercoids in 0.3 ml of distilled water were administered to each of 25 mice of each strain by intragastric intubation. Control mice received 0.3 ml of distilled water.

Table 1. Percentage of mice infected with *Hymenolepis diminuta* on different days.

Strain	H2 haplotype	% of mice with worms on day*: 6	8	10	12	14
C57/Bl	H-2^b	40	60	40	40	0
A	H-2^a	100	100	100	20	0
DBA	H-2^d	60	80	80	20	0
Balb/c	H-2^d	20	80	80	60	0
C3H/He	H-2^k	100	100	100	60	0

* Twenty-five mice of each strain were infected with 5 cysticercoids on day 0 and sacrificed in groups of 5 as indicated.

To perform differential blood counts, mice were bled through the retroorbital sinus and cardiac puncture prior to autopsy. The small intestine was then removed and cut into thirds. Each section was flushed 4 times with 0.1 M Krebs Ringer Tris-HCl solution (pH 7.4). The intestine was then incubated at 37°C for 1½ hr and flushed again as described by Hopkins (1982).

The intestinal lavage was examined under transmitted light (10× magnification) to ensure that all worms were recovered. Worms with scolices, and greater than 1 mg in weight, were counted and measured in length in 70% ethanol. "Worm biomass per mouse" results were obtained as described by Elowni (1982). Briefly, all the worms from each mouse were counted and placed in preweighed foil cups and dried at 100°C for 24 hr for biomass and dry weight determinations.

Spleens and livers of infected and uninfected controls were removed and weighed at the time of autopsy.

There were no significant differences in spleen or liver weights or white blood cell differentials when the experimentals were compared to the controls (data not shown). There were, however, some significant differences in growth and rejection patterns as assessed by worm number, length, and biomass.

Previous reports have demonstrated an increase in eosinophils in some cestode infections (Ansari and Williams, 1976; Shinkai et al., 1985). In the present study, eosinophilia was not observed. Furthermore, no increase of peripheral blood lymphocytes was detected. Liver and spleen weights were also examined. Shimoda et al. (1983) demonstrated liver pathology and subsequent increase of alkaline phosphatase in mice infected with *Hymenolepis nana*. We did not see an increase in liver weights in mice infected with *H. diminuta*. Unlike *H. diminuta*, *H. nana* undergoes a mucosal or tissue phase of development before its adult lumen phase, which may contribute to the liver pathology evident in *H. nana*. Although Judson et al. (1987) demonstrated that *H. diminuta* produces material with mitogenic activity when cultured in vitro, in the present study, the mice did not display splenomegaly. The difference may be due to the difficulty of demonstrating mitogenicity in vivo.

The data in the present study demonstrate that *H. diminuta* exhibits different recoveries, lengths, and biomasses according to the strain of the mouse used. Table 1 demonstrates that worms were evident in all strains by day 6 and were rejected in all strains by day 14. The pattern of growth and rejection was different in the 5 strains. The data suggest that A, DBA, Balb/c, and C3H/He mice begin to reject worms between days 10 and 12. C3H/He mice exhibited a high infection rate (100%) on days 6, 8, and 10. On day 12, the infection rate for C3H/He dropped to 60%. In contrast, the infection rate was consistently low (40%–60%) on days 6–12 for C57/Bl mice.

Table 2 demonstrates statistically significant worm length differences (analyzed by 2-factor

Table 2. Mean length of *Hymenolepis diminuta* recovered from different strains of mice.

Strain	Mean length of worms on day*: 6	8	10	12	14
C57/Bl	0.28 ± 0.05	1.5 ± 0.54	2.6 ± 0.54	7.8 ± 2.4	—
A	0.82 ± 0.12	3.9 ± 0.44	2.4 ± 0.46	7.2†	—
DBA	0.42 ± 0.01	0.8 ± 0.24	3.2 ± 0.52	2.6†	—
Balb/c	1.2†	2.4 ± 0.44	4.2 ± 0.55	11.6 ± 2.4	—
C3H/He	1.8 ± 0.38	3.2 ± 0.39	5.1 ± 1.3	11.4 ± 2.4	—

* Mean length of worms in centimeters ± standard error. A 2-factor analysis of variance (ANOVA) demonstrates a significant difference in days ($F = 20.80$, $P = 0.0001$) and in strains ($F = 6.06$, $P = 0.0002$).

† One worm was recovered from 1 mouse.

Table 3. Mean biomass of *Hymenolepis diminuta* recovered from different strains of mice.

Strain	Mean biomass of worms on day*: 6	8	10	12	14
C57/Bl	3.5 ± 0.002	4.4 ± 0.001	7.7 ± 0.002	9.7 ± 0.003	–
A	4.7 ± 1.1	7.6 ± 0.001	11.4 ± 0.002	13.9†	–
DBA	4.8 ± 4.1	5.1 ± 2.1	7.8 ± 0.001	6.2†	–
Balb/c	4.5†	6.3 ± 0.001	12.3 ± 0.002	35.5 ± 0.005	–
C3H/He	6.7 ± 0.001	9.1 ± 0.001	10.9 ± 0.003	15.6 ± 0.007	–

* Mean biomass of worms in milligrams ± standard error. A 2-factor analysis of variance (ANOVA) demonstrates a significant difference in days ($F = 20.30$, $P = 0.0001$) and in strains ($F = 9.34$, $P = 0.0001$).

† One worm was recovered from 1 mouse.

analysis of variance—ANOVA) among the strains. Previous studies indicated the presence of worms as long as 6 cm in mice (Hopkins, 1980). In our experiments we demonstrated the presence of 11-cm worms in Balb/c mice and C3H/He mice. On days 6 and 10 the worms recovered from C3H/He were significantly longer in comparison to each of the other strains. On day 12, worms recovered from C3H/HeJ were very long (11.4 ± 2.4 cm) and similar in length to those recovered from Balb/c mice (11.6 ± 2.4 cm). Worms recovered from C57/Bl were significantly shorter than worms recovered from Balb/c mice and C3H/He mice.

Table 3 demonstrates statistically significant worm biomass differences (analyzed by 2-factor ANOVA) in 5 different strains of mice. On day 6, there was no significant difference in worm biomass among all the strains examined. On day 8, however, worms recovered from C3H/He had a significantly larger biomass than worms recovered from C57/Bl. Worms recovered from A, Balb/c, and C3H/He on day 10 possessed a similar biomass and were significantly larger than worms recovered from C57/Bl and DBA. On day 12, worms recovered from Balb/c were significantly larger than worms recovered from all other strains of mice examined.

In the present study, the most obvious difference occurs when worms recovered from C57/Bl mice are compared to worms recovered from C3H/He mice. C57/Bl mice appear to have an enhanced immune system. They have a low incidence of tumors and they are widely used as resistant animals in radiation research (Green, 1975). Alternatively, C3H/He mice express a B cell function abnormality in response to certain T independent antigens (Longo et al., 1981). These reported differences may contribute to the different growth patterns of *H. diminuta* in C57/Bl and C3H/He mice.

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Research Note

Lintaxine cokeri (Monogenea: Microcotylidae) on Freshwater Drum in the Kanawha River, West Virginia

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ABSTRACT: A total of 46 *Lintaxine cokeri* (Linton, 1940) individuals was collected from 22 of 173 freshwater drum, *Aplodinotus grunniens,* in the Kanawha River, West Virginia. Intensity of *L. cokeri* infection was inversely correlated with host length, but the coefficient of correlation was not significant ($r = -0.230$). This is the first report of *L. cokeri* from West Virginia.

KEY WORDS: *Aplodinotus grunniens, Lintaxine cokeri,* Monogenea, West Virginia.

While studying length class frequencies of *Microcotyle spinicirrus* MacCallum, 1918, on freshwater drum (*Aplodinotus grunniens* Rafinesque, 1820) in the Kanawha River, West Virginia, another microcotylid species, *Lintaxine cokeri* (Linton, 1940), from the same host sample population was collected. *Lintaxine cokeri* has been reported in the literature only on freshwater drum from Fairport, Iowa (Linton, 1940; Hoffman, 1967). Kritsky (1988, pers. comm.) recalls recovering *L. cokeri* from drum in Oahe Reservoir, Missouri River, South Dakota (Walworth County) in 1972, and Rogers (1988, pers. comm.) states that this monogenean can generally be found on drum in southern states. Thus, *L. cokeri* is more widely distributed than previously recognized and appears to be host specific for freshwater drum. In addition to reporting a more widespread geographic range for *L. cokeri,* this paper describes the prevalence, mean intensity, intensity related to host total length, and length class frequency of *L. cokeri* on freshwater drum from the Kanawha River, West Virginia. Prevalence and intensity are as defined by Margolis et al. (1982) and procedures are outlined in Joy (1988). Voucher specimens of *L. cokeri* have been deposited in the USNM Helminthological Collection, Accession No. 80472.

Overall prevalence of *Lintaxine cokeri* on Kanawha River drum (see Table 1) at 13% (22/173) is low, especially when considering that another microcotylid, *Microcotyle spinicirrus,* was recovered from 79% (137/173) of the same host

Table 1. Prevalence (P) and number of *Lintaxine cokeri* individuals by length class on the gills of *Aplodinotus grunniens* from 2 Kanawha River, West Virginia, collection sites, 1986.

	Winfield				Marmet			
		Length classes (mm)				Length classes (mm)		
	P	1–3	3–5	>5	P	1–3	3–5	>5
May	1/20			1	0/20			
Jun	4/25	3		3	3/20	1	1	1
Jul	4/19		4	2	0/6			
Aug	3/23	1	5		1/5		1	
Sep	3/6		6	7	0/4			
Oct	3/23		6	4	0/2			
Totals	18/116	4	21	17	4/57	1	2	1

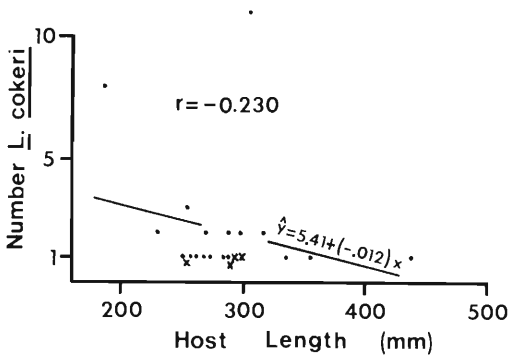

Figure 1. Scatter diagram plotting intensity of *L. cokeri* individuals against total length of host. Closed circles and x's represent drum taken from Winfield and Marmet, respectively. Each point represents a single host. All months combined.

sample population (Joy, 1988). Since differences in prevalence rates were recorded at the 2 collection sites, separated by a distance of 37 mi, a Chi-square 2 × 2 contingency table corrected for continuity (Clayton, 1984) was constructed to consider the null hypothesis (H_0) that the proportion of infected drum at Winfield (P_w) was the same as the proportion infected at Marmet (P_m). H_0: $P_w = P_m$ was not rejected ($\chi^2 = 1.78$, $P = 0.182$).

The 46 *L. cokeri* individuals collected during this study ranged from 1.7 to 6.3 mm in length with half of those 46 individuals in the 3.0 to 5.0 mm length class (Table 1). Intensity of infection was low at both sites: 2.33 at Winfield and 1.00 at Marmet. Joy (1988) demonstrated that a positive, and significant, correlation existed between intensity of *M. spinicirrus* and length of host. In the present study, however, *L. cokeri* intensity was inversely, but insignificantly, correlated with host length (Fig. 1). The slope, b, of the regression equation was not significantly different from zero. Calculations for testing H_0: b = 0 by an ANOVA (Zar, 1974) resulted in an *F*-value of 1.12, which did not exceed the critical value of $F_{0.05[1,20]} = 4.35$. Still, the regression line must be viewed with caution, because 41 of the *L. cokeri* individuals were obtained from a relatively limited length range of hosts (250–300 mm). The above data indicate that *L. cokeri* is relatively uncommon on freshwater drum in the Kanawha River, and that intensity of infection is not significantly related to host length.

I wish to thank Drs. Delane Kritsky, Idaho State University; Wilmer Rogers, Auburn University; and Donald Cloutman, Duke Power Company for sharing their records and observations with me.

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Proc. Helminthol. Soc. Wash.
56(2), 1989, pp. 215–216

PRESENTATION OF THE 1988 ANNIVERSARY AWARD TO MILFORD N. LUNDE

Dr. Leon Jacobs (left) presenting the Anniversary Award to Milford N. Lunde.

I wish to introduce you to the recipient of this year's Anniversary Award of the Helminthological Society of Washington.

It has been my pleasure to have been associated with this man for 34 years. His name is Milford N. Lunde.

Mil Lunde is a product of the country which "Lake Wobegone" depicts, except that he is the best character ever mentioned in that series.

After starting his collegiate education at Luther College in 1942, Mil entered the U.S. Navy in 1944. He served on the hospital ship Repose from 1944 through 1946. During the latter part of this duty, he saw his ship wander up and down the Yangtze River in China. This may have stimulated his interest in parasitology.

After WWII, Mil finished his undergraduate work at Luther College (1947) and went on to graduate work at the University of North Carolina under Dr. John Larsh. Then he went to work, in 1948, with the West Virginia State Department of Health, from 1948 to 1951. After that, he was recruited to a program in tropical medicine engineered by Dr. Thomas P. MacKie, at Bowman-Gray College of Medicine. He stayed with this group until 1953, when he joined the Parsons Co., an organization running the biomedical research laboratories at Fort Detrick, Maryland.

Fortunately for me, that organization folded in 1955, and Dr. Willard Wright, then Chief of the Laboratory of Parasitic Diseases, recruited Mil to work with me. I am very grateful to Willard Wright, because he introduced me to a great collaborator and a friend.

Mil Lunde is a self-starter, a diligent and effective worker with a knack for the laboratory. It was a very fruitful time we had together. The hemagglutination test for toxoplasmosis was one significant result. A latex agglutination test followed later. Information on different antigenic components of *Toxoplasma* to which antibodies revealed by the dye and hemagglutination tests responded was obtained. Mil was always in there pitching. He even acquired toxoplasmosis in the laboratory and became the subject in a very important paper on the acquired disease.

After I left LPD in 1964, Mil continued to

work not only on the serology of toxoplasmosis, but also of schistosomiasis, filariasis, amebiasis, and other parasitoses. He collaborated with many of the investigators in the Laboratory whose focus was on these different infections, and the results were very useful. In his last few years at NIH, he worked with clinical care people on AIDS, and this work was very significant in demonstrating the importance of *Toxoplasma* as a principal cause of encephalitis in AIDS patients. He and I also collaborated again, later, in demonstrating different antigens on the surface of different stages of *Toxoplasma.*

Mil has been active in the American Society of Parasitologists and especially in the Helminthological Society of Washington. He has served on various committees of Helmsoc and as Secretary and President. He is a man who gives of himself thoroughly in efforts to help people and organizations in which he finds merit.

Indeed, I have often noted the friendliness and gentility with which he has responded to the numerous people who have come into his laboratory to seek his help or his collaboration. He is just, by his very nature, a helpful man. Albert Einstein was quoted as saying, in response to some question about important facets of life, that probably the best aim a man could have would be to be helpful to others. Like the rest of us here, Mil may not come up with an equation as powerful as $E = mc^2$, but he indeed does fulfill the requirements that Einstein identified.

It is a great pleasure to participate in recognizing Milford N. Lunde as this Society's anniversary awardee.

LEON JACOBS, Chair
Awards Committee

Proc. Helminthol. Soc. Wash.
56(2), 1989, pp. 217–218

MINUTES

Five Hundred Ninety-Seventh Through Six Hundred Fourth Meetings

597th Meeting: Uniformed Services University of the Health Sciences, Bethesda, MD, 12 October 1988. Robin N. Huettel presided over the business meeting. A slate of candidates was presented for Society offices and plans for a student presentation competition in March 1989 were announced. Llewellyn J. Legters presided over the scientific program during which the following papers were presented: USUHS overseas research and training programs in Pakistan, Zambia, and Belize, by Llewellyn J. Legters; Remote sensing and malaria prediction program in Mexico, by Donald R. Roberts; Malaria epidemiology and drug development programs in the People's Republic of China, by Richard G. Andre.

598th Meeting: Animal Parasitology Institute, USDA, Beltsville, MD, 16 November 1988. The meeting was presided over by Vice-President Jeffrey W. Bier. A moment of silence was held in memory of Merritt P. Sarles. The following individuals were elected to Society offices by the membership: Jeffrey W. Bier, President; John H. Cross, Vice-President; David J. Chitwood, Corresponding Secretary-Treasurer; Leonard J. Francl, Recording Secretary and Ralph P. Eckerlin, Editor. A change in the name of the journal from "Proceedings of the Helminthological Society of Washington" to "Journal of the Helminthological Society of Washington" was proposed. The proposal involves a constitutional change. The scientific portion of the program was presided over by Joseph F. Urban. Kathleen B. Madden discussed a novel activity against the encysted muscle larvae of *Trichinella spiralis* in swine; Eldin A. Leighton presented a talk on the strength of the link between parasitism and host bovine genetics; and Dominic A. Strohlein discussed the use of live *E. coli* containing a gene sequence encoding an *Eimeria acervulina* merozoite surface protein as a means of protecting against coccidiosis.

599th Meeting: Plant Sciences Institute, USDA, Beltsville, MD, 8 December 1988, Robin N. Huettel, presiding. Life Member Awards were presented to Glenn L. Hoffman, Robert F. Kuntz, and Raymond V. Rebois. New officers were installed. Robin N. Huettel presided over the scientific program: Analogs of the sex pheromone of the soybean cyst nematode, by Robin N. Huettel; Production of fungal mutants for biocontrol of the soybean cyst nematode, by Susan L. F. Meyer; and Gross and microscopic observations of copepod parasitism of some marine fish larvae, by J. E. Bodammer, Northeast Fisheries Center, Oxford, MD.

600th Meeting: National Institutes of Health, Bethesda, MD, 11 January 1989. Jeffrey W. Bier presided over the meeting. The 1988 Anniversary Award was presented to Milford N. Lunde by Leon Jacobs. The change in the name of the journal was discussed. Proceedings were turned over to Frank Neva who presided over the scientific portion of the program. Nithya Raghavan discussed the isolation and partial characterization of recombinant clones from a genomic library of *Wuchereria bancrofti.* Steven Heath presented a talk on a cAMP-inducible gene expressed during the development of infective stages of *Trypanosoma cruzi.* David S. Peterson discussed how a point mutation in dihydrofolate reductase–thymidylate synthase confers resistance in Falciparum malaria.

601st Meeting: Naval Medical Research Institute, Bethesda, MD, 15 February 1989, Jeffrey W. Bier, presiding. The constitutional change necessitated by substituting the word "Journal" for "Proceedings" in the title "Proceedings of the Helminthological Society of Washington" was voted on by the members present. The change was accepted in a show of hands. Proceedings were turned over to Richard L. Beaudoin of NMRI who conducted the scientific session: Immunogenicity in mice with different major histocompatibility background, by Walter Weiss, NMRI; Malaria sporozoites escape from the circumsporozoite precipitation reaction: Potential implication for the malaria vaccine development, by Ana Szarfman, FDA; A *Plasmodium yoelii* antigen whose amino acid sequence contains a region that is similar to a conserved motif

found in thrombospondin and properdin, by Richard Hedstrom, NMRI.

602nd Meeting: Walter Reed Army Institute of Research, Washington, DC, 15 March 1989. Jeffrey W. Bier presided over the business meeting. The proposed budget for 1989 ($36,522) was accepted. It was announced that the next student competition is tentatively scheduled for October 1990. The Society hosted a special scientific program, the Student Presentation Competition, Willis A. Reid, Jr., presiding. Six students presented papers: Malarial antigens in urine of individuals with *Plasmodium falciparum* infection; by Marina Rodriguez-del Valle, Department of Biology, Georgetown University. Identification of *Plasmodium yoelii* DNA sequence encoding an antigen (Py117) that circulates in sera during acute infection; by Sansanee Changkasiri, Department of Biology, Georgetown University. A new measure of host specificity; by George W. Benz, University of Connecticut. Helminth parasites of white marlin (*Tetrapterus albidus*) from Maryland and Delaware waters; by Ann M. Barse, Horn Point Environmental Laboratories, University of Maryland. C-reactive protein levels in patients with *Plasmodium falciparum*; by Grant Hayashi, Department of Biology, Georgetown University. Characterization and localization of a *Plasmodium falciparum* membrane-associated polypeptide; by Melanie E. Small, Department of Biology, Georgetown University. Judges awarded first place ($300) to Melanie E. Small, second place ($200) to George W. Benz, and third place ($100) to Sansanee Changkasiri.

603rd Meeting: School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD, 12 April 1989. Cosponsored by the University of Maryland–Baltimore School of Medicine. The meeting was called to order by Jeffrey W. Bier and turned over to Michael Goettlieb who presided over the scientific portion of the meeting. Characterization of murine T-cell clones reactive with *Trypanosoma cruzi* antigens; by Dr. Steve Nickell, Department of Immunology and Infectious Diseases, Johns Hopkins. Effect of host immunization with a malaria vaccine on mosquito transmission; by Dr. Abdu Farhang-Azad, Department of Microbiology, University of Maryland. Ecological epidemiology of onchocerciasis in Liberia, West Africa; by Dr. Milan Trpis, Department of Immunology and Infectious Diseases, Johns Hopkins.

604th Meeting: New Bolton Center, Kennett Square, PA, 6 May 1989; joint meeting with the New Jersey Society for Parasitology and the Royal Society of Tropical Medicine and Hygiene and cosponsored by Smith Kline Animal Health Products and the Laboratory of Parasitology, University of Pennsylvania. Jeffrey W. Bier presided over the business meeting. Frank W. Douvres and Thomas K. Sawyer were selected as Life Members and J. Ralph Lichtenfels will receive the Anniversary Award. Michael Kemp, President of the American Society of Parasitologists, spoke about the activities of ASP. Robert S. Rew and Gerhard A. Schad presided over the scientific portion of the meeting. Mark Crane, Merck Institute for Therapeutic Research, discussed coccidiosis vaccines, Mario Philipp, New England Biolabs, talked about filarial vaccines, and Steve Wikel, University of North Dakota, discussed tick vaccines.

The following 17 new members were elected at the meetings indicated: *597th:* Emmet A. Dennis, Mark A. De Fries, Ralene R. Mitschler, Christine Sunderman, Motomi Torii, Minoru Yamada, Dante S. Zarlenga; *598th:* John M. Hawdon; *599th:* Mark F. Feldlaufer, William J. Wardle; *600th:* Shigehiko Uni; *601st:* Christine Hakenkamp, Sergei E. Spiridonov; *602nd:* Dilip K. Ghosh, Joaber Pereira, Jr.; *603rd:* None; *604th:* Faten A. Al-Zamel, Hong-Kean Ooi.

Respectfully submitted,

LEONARD FRANCL,
Recording Secretary

Proc. Helminthol. Soc. Wash.
56(2), 1989, p. 219

AUTHOR INDEX FOR VOLUME 56

KEY WORD AND SUBJECT INDEX FOR VOLUME 56

The Helminthological Society of Washington

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Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Proceedings is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues are payable on notification of election. Send this completed form to:

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Print name: _____ Date of birth: _____

Mailing address: _____
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Degree and year received: _____

Present position: _____

Field of interest: _____
_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
(a member)

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	David R. Lincicome	1975
Mildred A. Doss	1961	Margaret A. Stirewalt	1975
* Allen McIntosh	1962	* Leo A. Jachowski, Jr.	1976
* Jesse R. Christie	1964	Horace W. Stunkard	1977
Gilbert F. Otto	1965	Kenneth C. Kates	1978
* George R. LaRue	1966	* Everett E. Wehr	1979
* William W. Cort	1966	O. Wilford Olsen	1980
* Gerard Dikmans	1967	Frank D. Enzie	1981
* Benjamin Schwartz	1969	Lloyd E. Rozeboom	1982
* Willard H. Wright	1969	Leon Jacobs	1983
Aurel O. Foster	1970	Harley G. Sheffield	1984
Carlton M. Herman	1971	A. Morgan Golden	1985
May Belle Chitwood	1972	Louis S. Diamond	1986
* Elvio H. Sadun	1973	Everett L. Schiller	1987
E. J. Lawson Soulsby	1974	Milford N. Lunde	1988

HONORARY MEMBERS

* George R. LaRue	1959	Justus F. Mueller	1978
Vladimir S. Ershov	1962	John F. A. Sprent	1979
* Norman R. Stoll	1976	Bernard Bezubik	1980
Horace W. Stunkard	1977	Hugh M. Gordon	1981

CHARTER MEMBERS 1910

* W. E. Chambers	* Philip E. Garrison	* Maurice C. Hall	* Charles A. Pfender
* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	* Albert L. Taylor	1975
* Albert Hassall	1931	David R. Lincicome	1976
* Charles W. Stiles	1931	Margaret A. Stirewalt	1976
* Paul Bartsch	1937	* Willard H. Wright	1976
* Henry E. Ewing	1945	* Benjamin Schwartz	1976
* William W. Cort	1952	Mildred A. Doss	1977
* Gerard Dikmans	1953	* Everett E. Wehr	1977
* Jesse R. Christie	1956	Marion M. Farr	1979
* Gotthold Steiner	1956	John T. Lucker, Jr.	1979
* Emmett W. Price	1956	George W. Luttermoser	1979
* Eloise B. Cram	1956	* John S. Andrews	1980
* Gerald Thorne	1961	* Leo A. Jachowski, Jr.	1981
* Allen McIntosh	1963	Kenneth C. Kates	1981
* Edna M. Buhrer	1963	Francis G. Tromba	1983
* Benjamin G. Chitwood	1968	A. James Haley	1984
Aurel O. Foster	1972	Paul C. Beaver	1986
Gilbert F. Otto	1972	Raymond M. Cable	1986
* Theodor von Brand	1975	Harry Herlich	1987
May Belle Chitwood	1975	Glenn L. Hoffman	1988
Carlton M. Herman	1975	Robert E. Kuntz	1988
Lloyd E. Rozeboom	1975	Raymond V. Rebois	1988

* Deceased.

CONTENTS

(*Continued from Front Cover*)

RESEARCH NOTES

ANNOUNCEMENTS

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