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Vertical Transmission in the Trematoda

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ABSTRACT: Vertical transmission is defined as contagious distribution of a symbiotic, sometimes infectious, agent from one generation of host to the next. With respect to helminths, vertical transmission is not common because contagion (i.e., direct transmission of an infectious agent between 2 members of the same host species) is rare; the overwhelming majority of helminth life cycles, even those that are called direct, require some form of additional development outside the host before the infective stage is reached and a return to the host is possible. Of 38 helminth species for which there is some evidence of vertical transmission, only 6 are trematodes. Two species, *Fasciola hepatica* and *Schistosoma japonicum,* have been reported to undergo vertical transmission through prenatal routes. Although the metacercariae and schistosomules of these respective species undergo somatic migration in mammals, there is neither evidence that their movement is directed to the fetus nor that they can become hypobiotic and capable of queuing for a future pregnancy, characteristics common to other species that rely on vertical transmission for a substantial part of their maintenance. Consequently, these infections are interpreted as aberrant migrations during pregnancy. Four species, *Alaria alata, A. marcianae, Pharyngostomoides adenocephala,* and *P. procyonis,* undergo vertical transmission postnatally via the milk and there is ample evidence available to suggest that this is a significant mechanism in their life cycles. The infective agent transmitted through the milk is the mesocercaria and this stage is capable of prolonged hypobiosis in the tissues of the female. Lactating females that are infected with mesocercariae serve primarily as paratenic hosts. This is true even in those host species that are usually regarded as definitive hosts. The term "amphiparatenesis" is used to describe the pattern of parasite transmission between 2 members of the same host species, 1 serving as a paratenic host (the mother) and the other as a definitive host (the offspring), and in which vertical transmission is the mechanism.

KEY WORDS: *Alaria* spp., endosymbiosis, milk-borne transmission, *Pharyngostomoides* spp., vertical transmission.

Infectious agents usually have limited mobility and must rely to a large part on the habits of their hosts for transmission. Some rely on "horizontal transmission" and infect contemporary hosts by employing vectors, seeding migratory pathways, or intertwining themselves in the food web of the target host. Others use "vertical transmission" and infect noncontemporary hosts. Vertical transmission connotes passage in time and is defined herein as contagious distribution of a symbiotic, sometimes infectious, agent from one generation of host to the next.

Vertical Transmission and Endosymbiosis

Although many believe that vertical transmission is rare and utilized by few organisms, it was noted in an earlier review on the subject that this is not so (Shoop, 1991). In fact, it could be argued that vertical transmission is the most common form of transmission in nature. The endosymbiotic theory suggests that mitochondria are the offspring of bacteria that were phagocytized by our ancestors eons ago. Some of these bacteria possessed mechanisms by which they survived in our ancestors and became symbionts. Some even possessed mechanisms by which they contributed to the survival of our ancestors. At some

point, rather than relying on phagocytosis to enter the host, a mechanism developed whereby these bacteria were vertically transmitted from generation to generation in our ancestors. This intimate symbiosis and transmission has now proceeded to such a point that we hardly recognize the bacteria as distinct entities any longer. Covertly, vertical transmission of the bacteria/mitochondria lineage(s) occurs every time a eukaryote, whether plant, animal, or fungus, reproduces.

Vertical Transmission and Helminths in General

With respect to helminths, vertical transmission is rare because it entails true contagion, i.e., direct transmission of an infectious agent between 2 members of the same host species. The overwhelming majority of helminth life cycles require some form of additional development outside the host. For some, this additional development takes place in a prolonged free-living state, and for many, intermediate hosts, paratenic hosts, or vectors are required before returning to the same host species. Nonetheless, evidence for vertical transmission has been presented for 38 helminth species and has been re-

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viewed by Baer (1972), Stone and Smith (1973), Hubbert et al. (1975), Stoye (1976), Miller (1981), Loke (1982), Macchioni and Tosi (1984), Shoop (1991), Conn (1994), and Lyons (1994).

Although not all of the evidence for the 38 species is convincing, 5 phylogenetic lines of helminths employ vertical transmission as a significant mechanism in their life cycles and include the hookworms, ascarids, protostrongylid lungworms, intestinal threadworms, and diplostomid flukes. Despite great differences in the life cycles of these helminths, the following 6 generalities with regard to vertical transmission can be made: (1) it is known to occur only in mammalian hosts; (2) transmission is strictly from the maternal side; (3) only larval parasites are involved; (4) there is an obligate somatic migration on the part of the parasite; (5) the transmitted stage shows substantial capacity for hypobiosis (temporary arrested development); and (6) the same stage infective to the mother is the stage that passes to the offspring.

Vertical Transmission and Trematodes

Of the 38 helminth species for which there is some evidence of vertical transmission, only 6 are trematodes (Shoop, 1991). Four of these species, *Alaria alata, A. marcianae, Pharyngostomoides adenocephala,* and *P. procyonis,* undergo postnatal transmission through milk and there is ample evidence available to suggest that this is a significant mechanism in their life cycles.

Two other species, *Schistosoma japonicum* and *Fasciola hepatica,* have been found in fetuses and neonates at times that, if based on the theoretical prepatent period of these worms, would have ruled out all but prenatal infection. Narabayashi (1914) was able to show experimental vertical transmission in several species of laboratory hosts with *S. japonicum* and the migrating schistosomule was the infective agent. However, prenatal infection among the schistosomes has not been found to be a significant pathway in nature even though it is not uncommon for schistosomules to migrate to maternal vessels associated with the placenta (Bittencourt et al., 1980). Prenatal infection of young calves with *F. hepatica* has also been documented, but the reported prevalences are usually quite low and variable. Stoye (1976) reviewed the subject and found most prevalences to be less than 2%. For example, Rees et al. (1975) examined the livers of 16,667 1–3-wk-old calves in Australia and found *F. hepatica* in 84 livers (prevalence of 0.5% and a total of 108 worms). Interestingly, a more recent report by Pecheur (1984) stated that 40% of 3–8-wk-old calves were positive for *F. hepatica* eggs from several farms in Belgium. The disparity between these prevalences is presently inexplicable. Metacercariae of *F. hepatica* are known to migrate to sites other than the liver (Boray, 1969), but there is neither evidence that their movement is directed to the fetus nor that they can become hypobiotic and capable of queuing for a future pregnancy. Consequently, these infections are categorized tentatively as aberrant migrations of the metacercariae during pregnancy; but it is important to recognize that we may be observing the primitive explorations of a parasite whose behavior could become genetically fixed should it provide fitness advantages.

At this time, however, trematode life cycles in which vertical transmission is both a clear strategy and significant part of the maintenance of the organism in nature are known only from species that undergo milk-borne transmission.

Phylogeny of *Alaria* and *Pharyngostomoides*

In a previous review of trematode transmission patterns, it was indicated that the only known cases of milk-borne transmission occur in the order Strigeiformes (Shoop, 1988). In a subsequent study, this was examined in greater detail (Shoop, 1989), and it was shown that *Alaria* and *Pharyngostomoides* are closely related genera in the family Diplostomidae. Among the characters that these genera share is the presence of a mesocercarial stage. This stage is known from only 4 genera: *Alaria, Pharyngostomoides, Procyotrema,* and *Strigea.* There is yet no evidence for milk-borne transmission in *Procyotrema* and no possibility of such in *Strigea,* a genus whose members infect birds.

Mesocercaria

The mesocercaria is the infective agent in the Trematoda that is vertically transmitted in the milk (Fig. 1). As the name indicates, it is a stage intercalated between the cercaria and metacercaria. Morphologically, it appears as an enlarged cercarial body that is characterized by a large number of small, posteriorly oriented surface spines and by 4 large, unicellular penetration glands. Temporally, the mesocercaria can live for years in a hypobiotic state in paratenic hosts and may be shuttled through various trophic levels.

The obligative somatic migration that occurs in mammals combined with the extraordinary capacity to undergo prolonged hypobiosis contribute to the singular ability of the mesocercaria to utilize milk-borne transmission as a strategy in the Trematoda.

Vertical Transmission in *Pharyngostomoides* Spp.

The life cycle of *Pharyngostomoides* spp. includes raccoons, *Procyon lotor,* as definitive hosts (Fig. 2). Within the definitive host there is a complex intestine–lung–intestine migration whereby the ingested mesocercaria migrates to the lungs and develops to the metacercarial stage. The metacercaria then migrates up the trachea, is swallowed, and matures to the adult worm in the small intestine. Eggs produced by adult worms are defecated into the environment, embryonate, and the resulting miracidia seek and penetrate aquatic snails of the genus *Menetus.* Several sporocyst generations ensue in the mollusc with the culmination of free-swimming cercariae. The cercariae penetrate or may be ingested by branchiobdellid annelids, which are symbiotic on crayfish, and develop to mesocercariae. When crayfish are ingested by raccoons, the symbiotic branchiobdellids are carried into the digestive tract of the vertebrate as well. The complex intestine–lung–intestine migration then completes the life cycle (Miller, 1981). Historically, it has been believed that paratenic hosts play no part in these life cycles.

A peculiar twist to the *Pharyngostomoides* spp. life cycle was presented in 1967 when it was suggested that vertical transmission from captive raccoons to their young had occurred (Harris et al., 1967). Three raccoons were both naturally infected with *Pharyngostomoides* spp. and pregnant when captured. In the laboratory, they were fed a diet devoid of any stages of *Pharyngostomoides* spp. and their young were nursed by them. The observations showed that a single pup born to and nursed from a female in 1961 had 78 adult *P. procyonis* in the intestine by the third month of life. In 1964, 3 pups were born to and nursed from a second female and again all were infected with adult worms by the third month of life; 1 pup was euthanized and 1,753 adult worms were found in the small intestine. And in 1966, a third female gave birth to a dead pup that was uninfected. In this latter case, the mother was infected and live mesocercariae were observed in her
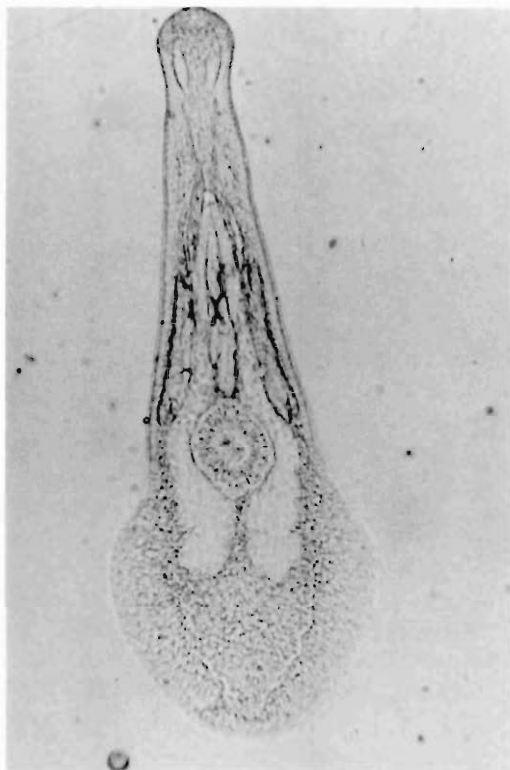
Figure 1.   Living mesocercaria of *Alaria marcianae* after recovery from a reptilian paratenic host, *Agkistrodon piscivorus.*

mammary gland secretions. The authors' conclusions from these 3 cases were that vertical transmission had occurred, and that it most likely involved a milk-borne route.

In studying the life cycle of *Pharyngostomoides* spp., Beckerdite et al. (1971) reported that 2 species, *P. procyonis* and *P. adenocephala,* occurred in the genus and that both were probably vertically transmitted. However, they neither offered any further data for this belief nor mentioned the mesocercarial stage.

No further publication on vertical transmission of trematodes was released until 1981 when data were presented showing that multiple litters born to 2 captured, naturally infected raccoons became infected with *Pharyngostomoides* spp. (Miller, 1981). In 1 female, all neonates in the first 7 litters born to her which nursed became infected, while 3 of 5 neonates became infected in the eighth litter, and none was infected in the ninth litter. A similar pattern was observed for the second naturally infected female and her lit-
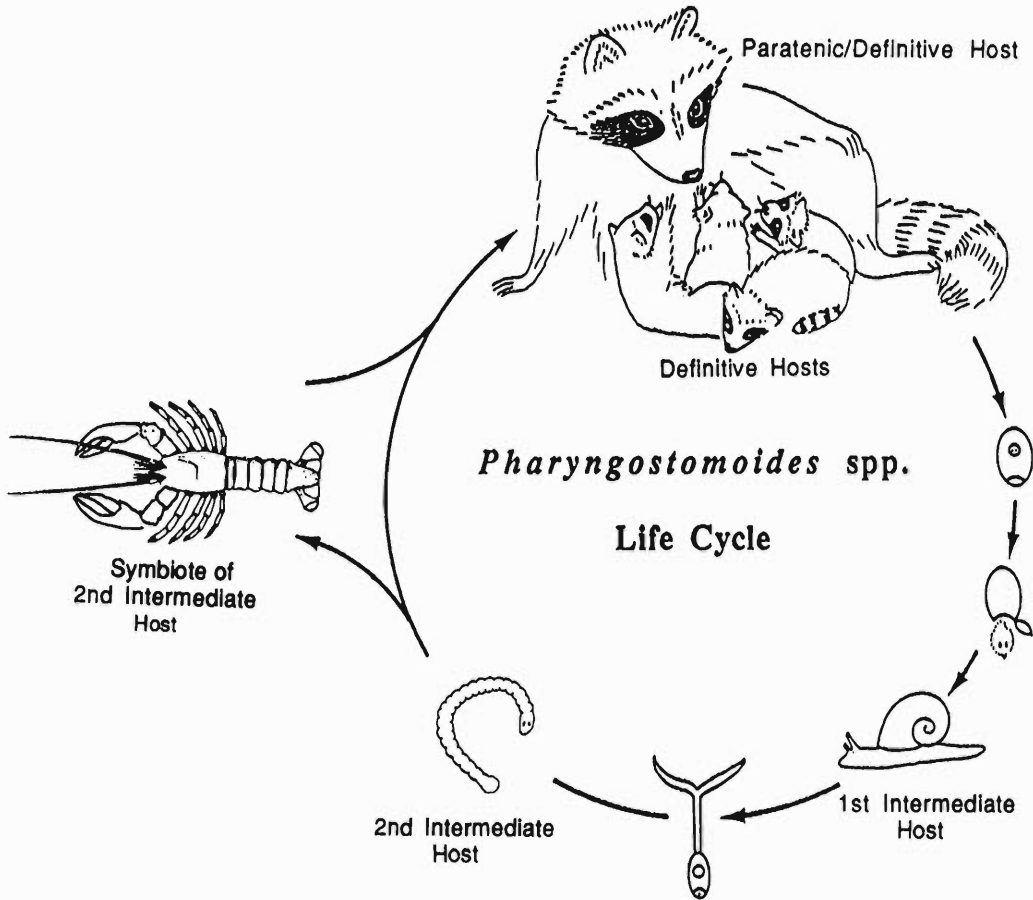
Figure 2.   Life cycle of *Pharyngostomoides* spp.

ters. These data established unequivocally that (1) some form of vertical transmission had occurred, (2) the mechanism producing 100% infection of sequential litters was not trivial, (3) the mesocercariae had survived in the tissues of the females for many years, and (4) once a female was infected with mesocercariae, she was most probably infected for life. Although prenatal infection could not be ruled out as a possible source of these infections, the author clearly felt that they were the result of milk-borne transmission and cited the fact that several late-term fetuses and neonatal raccoons from other infected mothers were not infected, thus negating any form of prenatal transmission.

Although paratenic hosts have not been cited in previous work on *Pharyngostomoides* spp., these data suggest that they do play a major role in their life cycles, but not in the usual sense. It should be noted that the lactating raccoon is a paratenic host because mesocercariae ingested by her are transmitted without any further development in the milk. It is in the neonatal raccoons where development of these transmitted stages occurs.

Vertical Transmission in *Alaria* Spp.

Life cycles of the genus *Alaria,* although fundamentally similar to *Pharyngostomoides,* are more complicated because of the different species that can be definitive hosts and, more importantly, because of the number of paratenic hosts that may be involved in the life cycles (Pearson, 1956; Johnson, 1968) (Fig. 3). Usually, domestic or wild species of felines or canines are definitive hosts and, as in *Pharyngostomoides,* there is a complex intestine–lung–intestine migration (Shoop and Corkum, 1983a, 1984a). The

Alaria marcianae Life Cycle

Paratenic/Definitive Host

Paratenic Hosts

2nd Intermediate Host

1st Intermediate Host
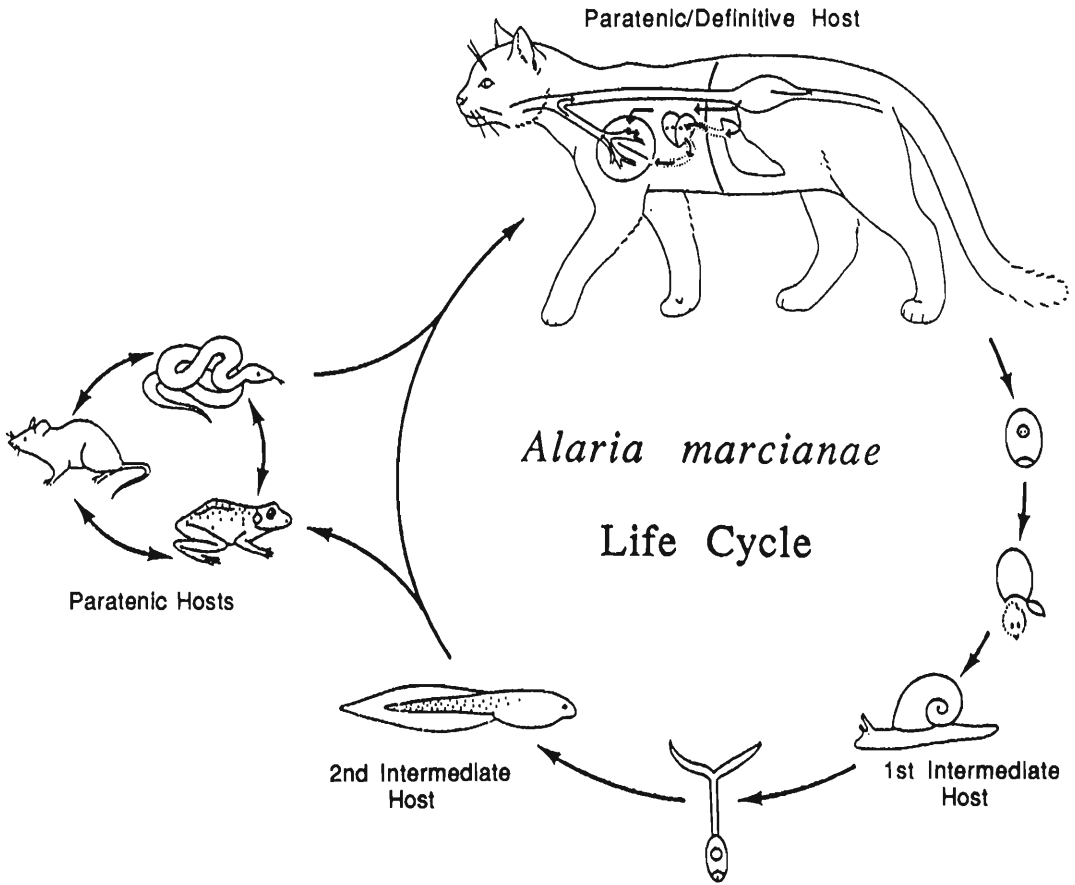
Figure 3.   Life cycle of *Alaria marcianae.*

ingested mesocercaria migrates to the lung where development to the metacercaria takes place. The metacercaria then migrates up the trachea, is swallowed, and maturation to the adult worm occurs in the small intestine. Eggs produced by the adult worm are defecated into the environment, embryonate, and the resulting miracidia infect aquatic snails such as *Helisoma.* After several sporocyst generations in the mollusc, cercariae are released. The free-swimming cercariae penetrate the epidermis of tadpoles and develop to mesocercariae. At this point, 2 pathways exist. The tadpole could be ingested by the definitive host and the complex intestine–lung–intestine migration would lead to the adult worm. However, because few feline or canine hosts feed on tadpoles, it is more common for the mesocercariae to enter the paratenic pathway and be shuttled from 1 host to another without any development. The paratenic spectrum involves amphibians, reptiles, birds, and mammals, including man. Ultimately, the mesocercariae along with the paratenic host is ingested by the feline or canine definitive host and the intestine–lung–intestine migration then results in adult worms. Until the discovery of vertical transmission in *Alaria* spp., infection of man in modern times had usually been regarded as a dead-end for the mesocercaria.

My work on *Alaria* spp. began as a result of a human infection in Louisiana (Beaver et al., 1977) and culminated in an epidemiological study in 1981 (Shoop and Corkum, 1981). Numerous hosts were field-collected and examined for natural infections of *A. marcianae*. Experimental infections were also conducted in the laboratory to determine the range of possible hosts. One of those experimental infections included a female cat that was subsequently discovered to be pregnant. She was removed from study and returned

to a sixth floor vivarium to give birth. Several months after parturition, routine check of feces of all cats in the vivarium revealed eggs typical of *A. marcianae* in the feces of 2 kittens. At the time, the infection of these kittens was inexplicable, but records showed them to be the offspring of the female that had been given the experimental infection. Subsequent necropsy confirmed that both of these kittens were infected with adult *A. marcianae* in their small intestines. Necropsy of the female revealed mesocercariae in her mammary glands and adult worms in the small intestine. Although the source of infection for the neonates appeared to be from the milk, the possibility of prenatal infection could not be ignored.

At that point, it was decided to attempt an experiment in mice rather than to continue in cats (Shoop and Corkum, 1983b). The short gestation and suckling time as well as the ability to examine greater numbers of mice were primary reasons. The experiment was comprised of 3 major groups. Group 1 was designed to examine only the possibility of prenatal transmission. Five females were mated and infected with 150 mesocercariae each. Immediately at birth, the neonates were removed before suckling and both they and their dams were examined for mesocercariae. Group 2 was designed to examine the possibility of postnatal transmission. Five females were mated, allowed to give birth, and immediately after birth they were infected with 150 mesocercariae each and then returned to their litters to nurse. At weaning, both the dams and their offspring were examined for mesocercariae. Group 3 was composed of 5 virgin female mice each infected with 150 mesocercariae. These mice remained unmated and were examined for the presence of mesocercariae at the same time the females from the other groups were examined.

Results from Group 1 showed that none of 53 young born to the 5 prenatally infected females became infected. When the dams were examined it was found that all had become infected but there was no development beyond the mesocercarial stage. The majority of mesocercariae from the dams were found in the anterior trunk musculature and fat. No worms were found in the uterus or surrounding organs. Results from the females from Group 3 were essentially the same.

To the contrary, all 38 young nursed by dams infected postpartum in Group 2 became infected. When their dams were examined, it was found that the preponderance of worms occurred in the

mammary glands. Histologically, worms were not only documented in the mammary glands of the dams, but also in their milk. As in the previous groups, however, there had been no development beyond the mesocercarial stage.

In summary, prenatal transmission had been negated as a factor in the mice; no infection of any offspring occurred in Group 1 and there was no association between the mesocercariae and the uterus or surrounding organs of the dams. Instead, it was found that 100% infection of offspring from 5 litters that suckled from infected dams had resulted. Moreover, there was a predilection of mesocercariae for the mammary glands of the lactating mice and the fact that these stages were also observed in the milk was incontrovertible evidence for milk-borne transmission in these paratenic hosts. Since those initial data, we have repeated these experiments in mice as well as rats and have found the same results each time (Shoop and Corkum, 1984b, c). Independent tests conducted in Russia on a closely related species, *A. alata,* in mice and rats have verified these findings (Sharpilo and Tkach, 1989).

Subsequently, similar experiments were conducted by us in cats, albeit with lesser numbers. To date, no prenatal transmission has been observed in these hosts either. Contrarily, every kitten nursed from infected female cats has become infected and mesocercariae have been recovered from the mammary glands of the females that nursed them. The only difference relative to the rodents is that the vertically transmitted mesocercariae develop to metacercariae in the lungs and then to adult worms in the small intestines of the kittens. What the stimulus is that draws the mesocercariae from the normal intestine–lung–intestine migration and to the mammary glands in the lactating cat, or the lactating female of any species, is not presently understood. However, the dependability of the transmission mechanism would have to be great for the parasite to forego reproductive maturity in what would otherwise appear to be a "good" definitive host only to risk dissemination to a new group of hosts.

This peculiar ability of a parasite to use an otherwise capable definitive host as a paratenic host and the young as definitive hosts is not unique to either *Alaria* or *Pharyngostomoides.* This pattern also occurs in species such as *Toxocara canis, T. cati, T. pteropodis, T. vitulorum, Strongyloides stercoralis, S. ransomi, S. ratti, S. westeri, Ancylostoma caninum,* and *Uncinaria*

lucasi. The process that produces these life cycle patterns is vertical transmission. We considered the pattern and process important enough to name. "Amphiparatenesis" was defined as the pattern of parasite transmission between 2 members of the same host species, 1 serving as a paratenic host (the mother) and the other as a definitive host (the offspring), and in which vertical transmission is the mechanism.

To understand more about amphiparatenesis in *A. marcianae,* we (Shoop and Corkum, 1987) conducted a study in cats similar to that reported for *Pharyngostomoides* spp. in raccoons (Miller, 1981). A female cat was infected experimentally postpartum with 800 mesocercariae of *A. marcianae* and allowed to return to her litter and nurse. After 21 days of nursing, all 5 of her kittens became infected and adult worms were found in their small intestines. There was no possibility of prenatal infection in this first litter. This female was then mated by males in our vivarium and produced 7 additional litters over 3 yr. All 5 kittens in the second litter, all 5 in the third litter, all 5 in the fourth litter, 1 of 4 in the fifth litter, and none of 5 in the eighth litter which nursed became infected. The female destroyed her sixth and seventh litter shortly after birth before they could be examined. However, kittens born dead in the second, third, and fourth litters were examined and none was infected. All infections in kittens that nursed resulted in either metacercariae in the lungs or adult worms in the small intestine. Over 25% of the initial inoculum given to the female cat was recovered from the first litter, and then lesser amounts were recovered through the course of litters until it appeared that the female was exhausted of worms. The amphiparatenic strategy of the worms resulted in the contagious infection of 21 offspring over the course of 3 yr. The female served primarily as a paratenic host and her offspring as definitive hosts.

Studies by Pence and Windberg (1984) have illustrated the significance of this amphiparatenic strategy in nature. They examined 177 coyotes, *Canis latrans,* in Texas and found 2 of the most common parasites to be *A. marcianae* and *A. caninum*; 128 coyotes were infected with the former and 175 by the latter. Furthermore, these species were the only 2 of a total of 20 observed that showed age-dependent overdispersion in young hosts. Rank abundances for *A. marcianae* and *A. caninum* were greater in pups than they were in juveniles, and greater in juveniles than

they were in adult coyotes. The authors clearly believed this pattern resulted from vertical transmission and stated that "it is particularly significant that both of the helminth species demonstrating marked age dependent overdispersion . . . are capable of transmammary transmission."

Later, Pence et al. (1988) demonstrated that vertical transmission did occur in coyotes. A captured female coyote with natural infections of both *A. marcianae* and *A. caninum* was bred in captivity and gave birth to 5 pups. The pups nursed for 22 days and were then necropsied. All 5 became infected with both *A. marcianae* and *A. caninum.* Consequently, the population patterns of these 2 helminths, which had been observed previously in the 177 coyotes, could be clearly attributed to vertical transmission.

In the normal life cycles of species of *Alaria,* 3 orders of mammals can be infected by mesocercariae: rodents, carnivores, and primates. Rodents, as have been shown, usually serve as paratenic hosts and carnivores usually serve as definitive hosts, that is as long as they are not lactating. Primates, specifically man, have been infected on only a few occasions and although little information is known, it does appear they are paratenic hosts. Because of our epidemiological interests in *A. marcianae,* we investigated the nature of infection in primates to determine if vertical transmission could occur. We used callitrichid monkeys (Shoop et al., 1990). A single female was inoculated with 600 mesocercariae of *A. marcianae* 10 days after parturition and she was returned to her litter of 2. She showed signs of severe mesocercariasis in the first few days after infection and euthanasia was considered. She recovered, however, without intervention after several days, but neglected her infants, only feeding them occasionally. The offspring were necropsied 4 wk postinoculation of the mother and both were infected with a total of 16 mesocercariae. This same female was then mated again without further infection and produced triplets. One died the first day without nursing and was found uninfected at necropsy. The other 2 nursed normally for 5 wk before they were necropsied. Both had become infected and a total of 115 mesocercariae were recovered. Interestingly, 1 metacercaria was discovered in the lungs and 3 young adults were found in the small intestine of these young monkeys.

When the female monkey was necropsied 246 mesocercariae were recovered from numerous tissues. Histological examination of her mam-

mary glands revealed 36 mesocercariae. Many were in pools of milk and near lactiferous ducts in proximity to the nipple. Thus, the adult monkey served only as a paratenic host, as does man, and vertical transmission occurred to all offspring of 2 sequential litters that nursed. Prenatal transmission was not possible in the first litter and did not occur in the infant born dead in the second litter. What is curious about this infection is that although >99% of worms in the offspring remained mesocercariae, there was a small number that was apparently undergoing, or that had already undergone, a somatic migration leading to adulthood. How or why these few made it to adulthood is not presently understood. Nonetheless, the significance of these results was clear; that is, vertical transmission of these worms in humans is a distinct possibility. Therefore, an infected human is not necessarily a dead-end for this parasite!

Perhaps the most startling discovery in vertical transmission of trematodes occurred when it was found that vertical transmission of *A. marcianae* mesocercariae from lactating mice to their offspring did not stop at the F_1 generation (Shoop and Corkum, 1984b). It was observed that females of the F_1 generation, which had acquired infection through nursing from their mothers, were capable, in turn, of infecting their own offspring, the F_2, during lactation. This ability to transmit an infectious agent from generation to generation to generation in paratenic hosts represents the ultimate in paratenesis. It was also found that vertical transmission of the same agents in cats stopped at the F_1 generation because the worms developed to maturity in the kittens and were spontaneously passed some months thereafter.

Acknowledgment

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Directory of Parasitologists

Electronic communications provide a rapid means of obtaining information from or sending information to colleagues involved in parasitological research. However, there is currently no centralized "directory" that provides the e-mail addresses and FAX numbers of parasitologists. Dr. Peter Pappas is beginning to put together such a directory and invites all individuals involved in parasitological research to communicate with him in order to be included.

In addition to e-mail addresses and FAX numbers, this new directory will also include mailing addresses, telephone numbers, research interests, etc. As the directory grows and is updated, it will be sent electronically to everyone whose name appears in it (and who has provided an e-mail address), thus providing parasitologists with the most current available. For those individuals not currently using electronic mail, Dr. Pappas plans to make the directory available on disk at a later date.

Interested persons can contact Dr. Pappas via e-mail and he will forward additional information (a questionnaire) to them. Readers who do not have an e-mail address but wish to be included in the directory can contact Dr. Pappas by mail or FAX.

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Cestode Infections of Mammary Glands and Female Reproductive Organs: Potential for Vertical Transmission?

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ABSTRACT: A widely studied aberrant strain of tetrathyridia of *Mesocestoides vogei* infecting laboratory mice is the only cestode for which vertical transmission has been verified experimentally. Vertical transmission has been reported for *Taenia saginata* in cattle and *Echinococcus* sp. in humans, but the validity of these cases has proved difficult to verify. However, metacestode stages of *Taenia solium, Echinococcus granulosus, Echinococcus multilocularis, Multiceps* sp., *Diphyllobothrium mansoni, Spirometra erinacei,* and *Proteocephalus ambloplitis* have been reported from the mammary glands, uterus, placenta, and/or ovary of several naturally infected hosts. Such infection foci, particularly the mammary glands, suggest a potential for vertical transmission in these species. Such possibilities warrant further study in these and other cestodes. Field surveys of hosts should be conducted to elucidate the prevalence of cestode localization in female organs in which there is potential for transmission.

KEY WORDS: Cestoda, *Echinococcus,* mammary glands, maternal transmission, *Mesocestoides,* placenta, prenatal, sparganosis, *Taenia,* uterus.

In recent years, interest in vertical transmission of parasitic organisms has grown substantially. This has resulted in the elucidation of several fascinating examples of transmammary and intrauterine transmission of parasites as reviewed by Shoop (1991). Increased research activity over the past 2 decades has revealed that vertical transmission is common among many nematodes and trematodes. Conversely, the limited data available seem to suggest that vertical transmission is not common among cestodes. However, efforts to identify cases of vertical transmission among cestodes have been fewer than those involving nematodes and trematodes. Many cestodes have been reported to localize in female organs, which suggests the potential for vertical transmission. The present review summarizes the published cases of vertical transmission and invasion of female organs by cestodes and suggests directions for future research in this area.

The Concept of Vertical Transmission

In this review, vertical transmission is defined as the transmission of parasites directly (i.e., without any intervening period either outside a host or in a host of another species) from a living parent (usually a mother) to its viable offspring in which parasite reproduction can occur. This may occur in parasites having either monoxenous or heteroxenous life cycles and restricts the concept of vertical transmission to those situations involving specific parental/filial relationships.

Living parent condition

By including only living parents, transmission by cannibalism as proposed by Mead and Olsen (1971) for *Ophiotaenia filaroides* plerocercoids and by Kroeze and Freeman (1982) for *Taenia crassiceps* cysticerci can be eliminated. As these authors suggested, cannibalism may have important epizootiological implications. However, cannibalism may occur among siblings and frequently involves a parent eating its young rather than vice versa. Thus, it is more accurate to regard this as a type of paratenesis that happens to involve intra- rather than interspecific exchange.

Reproduction in offspring condition

The inclusion in the preceding definition of only parasites having the potential to reproduce within the filial host ensures that the only cases recognized are those in which transmission results in the establishment of a new source of parasite dissemination. This epizootiologically relevant condition excludes those cases in which microfilariae are transmitted transplacentally to unborn young as has been described for several species of filarial nematodes (Eberhard et al., 1993). Transplacental transmission of microfilariae obviously involves a specific parental/filial relationship but does not actually result in permanent establishment of parasites in the young. The only epizootiological significance of this would be the chance that the total number of reservoirs available to insect vectors would be increased temporarily. However, even this may

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be unimportant inasmuch as the intensity of congenital microfilaremia in most cases is probably so low that the potential for transmission to insect intermediate hosts is negligible (Mantovani and Jackson, 1966).

Vertical transmission of helminths has been demonstrated conclusively only among placental mammals. In the case of prenatal transmission this is not surprising given the lack of transmission routes available in oviparous hosts. Among insect hosts, Fay (1961) stated that some evidence existed for occasional passage of *Leidynema appendiculata* eggs within the oothecae of cockroaches, though she presented no data. Among avian hosts, trematodes of the genus *Prosthogonimus* inhabit the oviducts and occasionally the shelled eggs (Schell, 1985) but have not been reported to invade the developing chicks. All known cases of postnatal vertical transmission of helminths involve transmammary passage of the helminth into the nursing young. However, postnatal vertical transmission of protozoan symbionts is known to occur among termites during trophallaxis and proctodeal feeding (Cleveland, 1928). There may be some potential for postnatal vertical transmission among esophageal nematodes of birds that feed their young by regurgitation from the crop. Potential candidates for further study of this possibility might include parasites such as species of *Capillaria* that are capable of autoinfection of their avian hosts. In such cases, both paternal and maternal relationships might be involved.

Vertical Transmission of Cestodes

All helminths that are known to undergo vertical transmission are characterized by a histozoic migratory stage in the life cycle. Such a migratory habit possibly provided an essential preadaptation for those species that penetrate the placenta, uterine wall, or mammary glands. Considering this, it is not surprising that most vertically transmitted helminths are nematodes, particularly strongylates and ascaridoids (Miller, 1981; Lyons, 1994) or strigeoid trematodes (Shoop, 1991, 1994).

Vertical transmission appears to be rare among cestodes in comparison with nematodes and trematodes. This may be related to the fact that virtually all adult tapeworms inhabit the intestinal lumen of their vertebrate hosts. This is also true of adult strigeoids, strongylates, and ascarids, but the juveniles of these nematodes generally undergo more extensive histozoic migration than do the larvae and juveniles of cestodes. The only histozoic migratory stages of cestodes are the oncospheres and, in some species, the metacestodes. In most cestode taxa, these stages are restricted to oviparous arthropod intermediate hosts where, as discussed above, vertical transmission is unlikely. Notable exceptions to this include the pseudophyllideans, the proteocephalideans, and the cyclophyllidean families Taeniidae and Mesocestoididae. Each of these groups has oncospheres and/or metacestodes that migrate through the tissues of vertebrate intermediate hosts, so it is not surprising that some species of each group have been reported to infect mammary glands or female reproductive organs (Table 1).

Taeniids have received more attention than other cestodes, probably because of their veterinary and medical importance. Vertical transmission of taeniids would be possible only at the oncosphere or metacestode stages. If it occurred, transmission of oncospheres could result in cysticercosis, coenurosis, or hydatidosis (depending on the taeniid species) in the young of infected mothers. *Taenia solium* might present an interesting case because humans can serve as both intermediate and definitive host, thereby resulting in either cysticercosis or adult taeniasis in the young of infected mothers.

Shillinger and Cram (1923) cited several authors whose early reports of congenital *E. granulosus* hydatidosis in humans were thought to have been misdiagnoses of nonparasite fetal anomalies. Shillinger and Cram's (1923) opinion was cited in a more recent review by Loke (1983), who neither agreed nor disagreed with their interpretation. Similarly, Gluhovschi et al. (1970) reported the occurrence of 6 large but "sterile" hydatid cysts of *Echinococcus granulosus* in the viscera of an unborn calf whose mother had an abnormal placenta. The authors regarded this as a rare instance of prenatal hydatidosis in which transmission of oncospheres had been facilitated by placental pathology. However, although the cysts looked like hydatids upon gross examination, the fact that they were "incompletely developed" and "sterile" made absolute identification impossible.

Several authors have reported evidence for prenatal transmission of *Taenia saginata* (=*Cysticercus bovis*) among cattle. McManus (1960) reported a 3.07% prevalence of cysticercosis bovis among 14,855 Kenyan calves, some of which

Table 1. Cestode infections reported from female organs. All are metacestodes except where noted.

Cestode species	Mammary	Uterus	Placenta	Ovary	References*
Cyclophyllidea: Mesocestoididae					
Mesocestoides vogei	Mouse	Mouse	Mouse		7, 11, 23
Cyclophyllidea: Taeniidae					
Echinococcus granulosus	Human	Human		Human	3, 10, 15
Echinococcus multilocularis		Human			18
Echinococcus sp.	Human	Human		Human	2, 4, 8, 9, 16, 19, 20
Multiceps sp.	Human				12
Taenia crassiceps	Mouse				6
Taenia solium	Human				1, 13, 21, 22
Taenia sp. (gravid proglottid)		Human			17
Proteocephalidea: Proteocephalidae					
Proteocephalus ambloplitis				Bass	14
Pseudophyllidea: Diphyllobothriidae					
Diphyllobothrium mansoni	Human				5
Spirometra erinacei	Human				24

* References: 1 = Alagaratnam et al., 1988; 2 = Alvarez et al., 1985; 3 = Atasü et al., 1988; 4 = Chiva et al., 1987; 5 = Chuen-Fung and Alagaratnam, 1991; 6 = Conn, present report; 7 = Conn and Etges, 1983; 8 = Epstein, 1969; 9 = Gaspa and Eusebi, 1973; 10 = Georgakopoulos et al., 1980; 11 = Hess, 1972; 12 = Kurtycz et al., 1983; 13 = Leggett, 1983; 14 = McCormick and Stokes, 1982; 15 = Ouedraogo, 1985; 16 = Prokopenko, 1989; 17 = Schacher and Hajj, 1970; 18 = Semchyshyn, 1974; 19 = Thurairatnam, 1992; 20 = Turan and Kücüksorgulu, 1987; 21 = Viratchai and Jimakorn, 1981; 22 = Vuong, 1989; 23 = Williams and Conn, 1985; 24 = Yamane et al., 1975.

were as young as 2 days postpartum. Urquhart (1961) reported bovine cysticercosis in 18 young Kenyan calves; it was his opinion that only 1 of these was acquired prenatally, the others being acquired shortly after birth. Haas (1967) speculated that generalized cysticercosis in a 3-wk-old calf was acquired prenatally. Šlais and Mann (1976) reported cysticerci of *T. saginata* from 2 calves in Kenya; the calves were 21 and 27 days old when necropsied, but the scolex anlagen of the cysticerci conformed morphologically to stages that develop only after 4–6 mo. Each of these authors concluded that the infections occurred prenatally. However, because no experimental infections were involved these reports do not constitute definitive proof of prenatal transmission.

Some attempts have been made to verify experimentally the occurrence of prenatal transmission of taeniid cestodes. No infections were found among calves born to 8 pregnant cows that had been exposed experimentally to 500–150,000 eggs of *T. saginata* by Urquhart (1961). In a similar experimental study, Kozakiewicz (1975) infected 15 cows with cysticerci of *T. saginata* by exposing them to 500,000 eggs each; the cows ranged from 5 to 8 mo of pregnancy at the time of exposure. When euthanized and necropsied

within 10–14 days postpartum, their calves lacked cysticerci.

The only experimentally verified cases of vertical transmission of cestodes have involved laboratory rodents infected with the aberrant tetrathyridia of *Mesocestoides vogei* (=*Mesocestoides corti* of Specht and Voge, 1965). Eckert (1970) was the first to report tetrathyridia in very young rats but gave no direct evidence of vertical transmission. Definitive experimental work was done by Hess (1972), who provided strong evidence for transmammary transmission of tetrathyridia in mice but expressed uncertainty as to whether the worms entered the milk ducts or migrated directly through the teats. Hess (1972) further provided equivocal evidence for in utero transmission but failed to use adequate controls to eliminate the possibility of transmammary transmission immediately postpartum. These results were summarized by Baer (1972), Miller (1981), and Stoye (1976) in reviews of milk-borne transmission of helminths.

Because of the uncertainties of earlier studies, Conn and Etges (1983) performed a detailed set of experiments using prenatal and early postnatal examinations of mice from mothers infected experimentally with tetrathyridia; they also used reciprocal cross-fostering techniques with neo-

nates of infected and uninfected mothers. The resulting data from 132 fetuses and 32 neonates showed no evidence of prenatal transmission, although a few tetrathyridia did penetrate into the uterine lumen and 1 was found in the maternal portion of a placenta. Conversely, the data conclusively demonstrated a 62% rate of transmammary transmission. Besides clarifying the basic mode of vertical transmission of this species, Conn and Etges (1983) showed that tetrathyridia entered the milk ducts of the mother mice, thus becoming positioned for immediate transmission to the young at the first nursing event. In a follow-up study, Williams and Conn (1985) provided some data on mammary gland histopathology associated with infection by tetrathyridia. They also presented quantitative data on the distribution of tetrathyridia within the mammary gland fat pads; these data suggested that the parasites did not localize preferentially in the mammary tissue but probably occurred there as a result of subcutaneous migration related to other unknown factors. Thus, it appears that transmammary transmission in this species is fortuitous. This is quite unlike the highly regulated vertical transmission of many nematode and trematode species (Shoop, 1991).

Cestodiases of Mammary Glands and Female Reproductive Organs

Despite the few confirmed cases of vertical transmission among cestodes, there are numerous reports of cestodes occurring in female organs from which such transmission might be possible (Table 1). All but 1 of these cases involved metacestode stages. The single exception was a gravid proglottid of *Taenia* sp. that apparently had crawled from the anal area through the vagina and into the uterus of a woman (Schacher and Hajj, 1970).

Table 1 shows that most of the reports of metacestodes occurring in female organs have resulted from experimental infections of laboratory mice or clinical cases of human infections. Most of the latter have involved the mammary glands, where cysticerci, hydatid cysts, and plerocercoids (spargana) have been encountered during routine cancer-screening examinations for breast lumps. Uterine cestodiases have been discovered primarily during obstetrical or gynecological examinations. Inasmuch as these cestodiases were reported because of their clinical significance to individual patients, estimates of the prevalence of such infections among women based on a survey of the literature undoubtedly would underestimate the true prevalence. Even among humans, the host species most studied in this respect, it is likely that subclinical cases of mammary, uterine, placental, or ovarian cestodiases are quite common. This seems particularly likely when one considers that the vast majority of human cestodiases occur in areas of the world where mammograms and modern obstetrical examination tools are not readily available or affordable.

Other than the case reports cited above, few studies have examined the clinical consequences of cestodiases of female organs. Williams and Conn (1985) described the histopathology of mouse mammary glands infected by tetrathyridia of *M. corti*. They reported host responses that increased in severity as parasite density increased; responses ranged from localized inflammation with little or no loss of lactogenic tissue, to generalized fibrous hyperplasia resulting in complete destruction of lactogenic tissue. Among human patients, occasional reports have described the co-occurrence of hydatid cysts and mammary carcinomas in women (Gaspa and Eusebi, 1973; Alvarez et al., 1985). However, no study has demonstrated a causal relationship between the 2 conditions. Such situations are very rare and are noteworthy only because of the complications they cause in diagnosis of cancer. Schacher and Hajj (1970) reported an isolated case of postmenopausal bleeding associated with the presence of a proglottid of *Taenia* sp. in the uterus of 1 woman. Hydatid cysts in the uterine wall and around the uterus during pregnancy have been reported to cause difficulties in labor among women (Semchyshyn, 1974).

Directions for Future Research

The fact that few cestodes have been reported to employ vertical transmission in their life cycles does not necessarily mean that such transmission is rare. Vertical transmission among other helminth and protozoan parasites was overlooked for many decades and is difficult to demonstrate in most cases (Miller, 1981; Shoop, 1991). Likewise, the rarity of reports of cestode infestations of female organs does not necessarily imply that cestodes do not frequently invade those organs. Conversely, it is possible that many cestode species invade the mammary glands of their hosts. Such cases may be reported simply (and
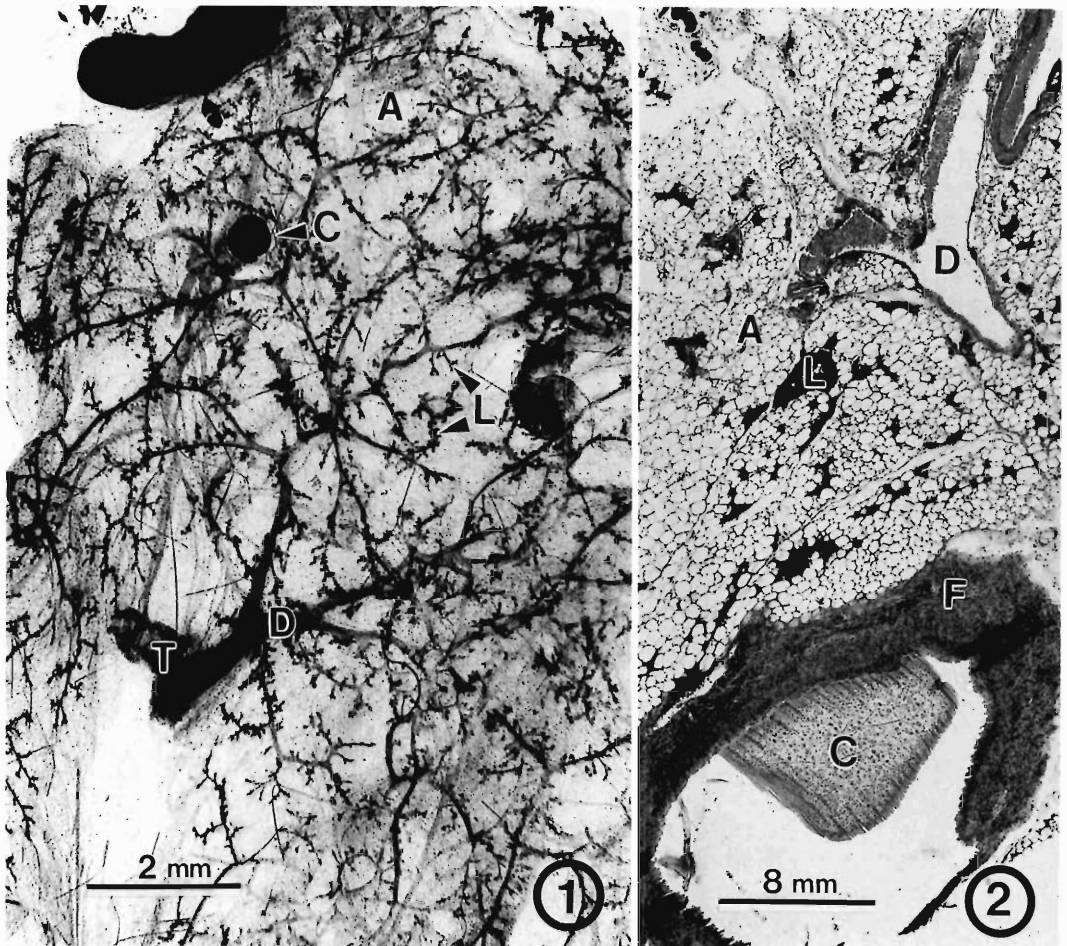
Figures 1, 2. Brightfield light micrographs showing cysticerci of *Taenia crassiceps* (KBS strain) in the mammary glands of laboratory mice experimentally infected by intraperitoneal inoculation. 1. Whole mount of a pressed posterior mammary gland stained with Semichon's acetocarmine. An encapsulated cysticercus (C) is present among the lactogenic alveoli (L) that branch throughout the adipose tissue (A) of the fat pad. The teat (T) and lactiferous ducts (D) are clearly discernible. 2. Histological section (10 μm thick) showing a cysticercus (C) within a host-derived fibroblastic capsule (F) within a posterior mammary gland. The adipose tissue (A), lactiferous ducts (D), and lactogenic alveoli (L) appear unaffected by the presence of the encapsulated worm.

ambiguously) as involving subcutaneous foci, when in fact the mammary glands, because of their location and configuration, are almost certainly included in many such examinations. Most reports of parasite surveys do not mention subcutaneous examinations, so mammary glands and other subcutaneous foci are probably underreported as sites of parasite localization. Based on this paucity of information, the following 2 suggestions for future research hold promise for uncovering cases of vertical transmission among cestodes.

First, future parasite surveys of mammalian hosts should incorporate examination of mammary glands as a routine part of their protocols. Many taeniid and diphyllobothriid metacestodes are known to occur commonly or even predominantly in subcutaneous regions near mammary tissues (Delvalle, 1989; Whittington et al., 1992; Keeling et al., 1993). Additionally, studies should be initiated with the primary focus of searching for mammary helminthiases in natural host populations. This has not been done for any group of helminths, including cestodes. A good starting place would be to look at small rodents whose mammary glands can be removed in toto and

processed as whole mounts such as those prepared by Conn and Etges (1983) and Williams and Conn (1985). Such whole mounts allow not only relatively rapid screening for mammary helminths but also determination of specific locations of the helminths in relation to lactiferous ducts, lactogenic alveoli, and other organ components. Additional information on basic host responses to the parasites can be obtained if half the mammary glands from each host are mounted whole for rapid screening, while half are prepared for routine histology. Examples of these complementary techniques are shown from experimental infections of *Taenia crassiceps* in mouse mammary glands in Figures 1 and 2. Searching for the presence of cestodes in mammary glands of larger mammals might be accomplished using enzymatic digestion of excised glands. This technique would not give as much information as the former but would at least allow an assessment of the prevalence of mammary helminthiases among larger host species.

Second, some research should be focussed on experimental work with individual cestode species that seem particularly well suited for vertical transmission. The best candidates would be the species listed in Table 1, or any other species with oncospheres and/or metacestodes that undergo histozoic migration in mammalian hosts. *Taenia crassiceps* might provide an ideal experimental model because it can live in laboratory rodents. Also, *T. crassiceps* undergoes histozoic migration in both oncosphere (Freeman, 1962) and cysticercus (Kroeze and Freeman, 1982, 1983) stages. Furthermore, this species localizes preferentially in subcutaneous sites, many of which are near mammary glands (Freeman, 1962; Delvalle, 1989). This species also has the advantage of undergoing asexual proliferation in the cysticercus stage; this would result in more rapid buildup and, thus, easier detection in newly infected fetal or newborn hosts.

In conclusion, metacestode stages of cestodes frequently occur in female organs of mammalian hosts where there is a distinct potential for vertical transmission. Few cases of vertical transmission have been demonstrated conclusively, but little research effort has been made in this area (Mackiewicz, 1988). Recently, increased awareness of the epizootiological importance of vertical transmission among some nematodes and trematodes should encourage more rigorous searching for evidence of this phenomenon among cestodes.

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Vertical Transmission of Nematodes: Emphasis on *Uncinaria lucasi* in Northern Fur Seals and *Strongyloides westeri* in Equids

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ABSTRACT: A review of vertical transmission of nematodes is presented. Emphasis is on the life cycles of the hookworm, *Uncinaria lucasi* Stiles, 1901, in northern fur seals (*Callorhinus ursinus* Linn.) and the intestinal threadworm, *Strongyloides westeri* Ihle, 1917, in equids. A tabular summary is given for species of nematodes with reported prenatal/transmammary transmission.

KEY WORDS: review, vertical transmission, nematodes, *Uncinaria lucasi, Strongyloides westeri,* transmammary transmission, northern fur seals, equids.

Most of this discourse will be on the life cycles of the hookworm (*Uncinaria lucasi*) in northern fur seals (*Callorhinus ursinus*) and the intestinal threadworm (*Strongyloides westeri*) in equids (*Equus caballus*). The author investigated the transmission aspect of the life cycle of these 2 species of parasites. Additionally, other nematode species reported to have vertical transmission are summarized.

Periods and Places of Investigation

Research by the author on *U. lucasi* included 5 trips to Alaska. Three trips, in 1960, 1961, and 1962, were as a graduate student working with O. Wilford Olsen, his major professor, and totalled about 13 months (Lyons and Olsen, 1960, 1962a, b; Lyons, 1963; Olsen and Lyons, 1962, 1965). Two other trips to Alaska involved working with Mark Keyes in 1977 and 1978, totalling about 6 wk (Lyons and Keyes, 1978; Lyons et al., 1978, 1980). Also, there was cooperative research with Mike Bigg in Canada (Bigg and Lyons, 1981; Lyons and Bigg, 1983) and Mark Keyes in Seattle (Lyons and Keyes, 1984). For the life cycle of *S. westeri,* research was conducted in central Kentucky, mostly over the 10-yr period 1963–1973 (Lyons et al., 1969, 1973, 1977).

Uncinaria lucasi

Discovery of the mode of transmission of *U. lucasi* will be discussed first. Before presenting details on this parasite, a few basic features of the life cycle of the host, the northern fur seal (Anonymous, 1977), are appropriate for an understanding of the intricacies of the hookworm life cycle.

Northern fur seals usually come on land only during the breeding season. They breed mainly on the Pribilof Islands, Alaska, in the Bering Sea.

Breeding bulls begin arriving in May and establish territories on rookeries for their harems. Pregnant cows start coming on land in June, and the main breeding season is from about mid-June through early August. Typically, cows stay on land about a week after first arriving. During this time, they give birth (within about 2 days), nurse their pups, and are rebred. The cows then go to sea to feed for several days, after which they return for a couple of days to nurse their pups. This routine is repeated during the nursing period, which lasts 3 or 4 mo.

When the author began investigations on the life cycle of *U. lucasi* in 1960, Dr. Olsen had already established several significant features (Olsen, 1958, 1959): (1) older seals did not have intestinal infections of adult hookworms; (2) pups had about 90% prevalence of adult hookworms; (3) pups, if they survived the effects of the parasite, "lost" intestinal infections at about 3 mo of age; (4) fourth stage larvae were found in very young pups born on rocks washed by the sea; (5) only 1 size or stage, i.e., L_4 or L_5 were found in pups; (6) no patent experimental infections could be produced in pups by administration of free-living L_3; (7) hookworm larvae could not be recovered from lungs of pups born on rookeries; and (8) pups died from hookworm disease after being born on areas of rookeries: (a) sprayed with cresylic acid known to kill free-living L_3, and (b) devoid of free-living L_3, such as on rocks or soil where no overwintering of these larvae occurred. After all of these findings were evaluated, it was concluded that possibly the source of larvae for development to adult hookworms in pups was from prenatal infection.

The focus of initial research in 1960 by the author, working closely with Dr. Olsen, was to determine whether prenatal infection occurred.

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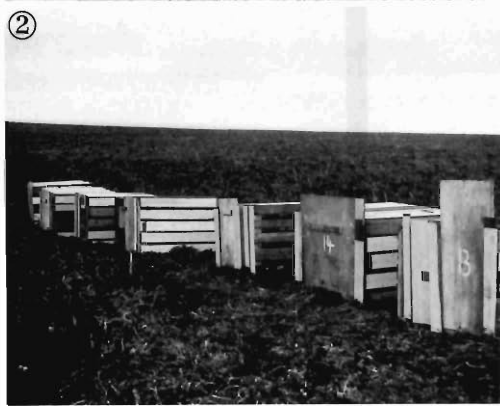
Figures 1, 2. St. Paul Island, Alaska. Cages for containment of pregnant northern fur seal cows (not visible) in the experiment with 30 pregnant cows. 1. Cages on a rookery (Group I) with 2 bulls nearby. 2. Cages on "clean ground" (Groups II and III) where no free-living L_3 *Uncinaria lucasi* were present.

First, marked newborn pups, born on rookeries, were found to begin passing hookworm eggs in their feces at about 2 wk of age. Next, various tissues of 26 neonatal pups (19 taken by cesarean section, 6 stillborns, and 1 prematurely born), which never nursed, were examined, but no hookworms were found. Belly blubber only was examined from 12 additional fetal pups and it was also negative for larvae. Live pups ($N = 12$), taken by cesarean section, were isolated in cages for periods ranging from 2 to 62 days (most for 10 days) and hookworms did not develop in them. Thus, there was no evidence of prenatal infection in the 50 pups examined. Because the natural infection rate of adult *U. lucasi* in pups born on rookeries was known to be about 90%, there appeared to be some other route of infection than prenatal.

In spite of the lack of evidence of prenatal

infection, a "mother factor" seemed to be involved because free-living L_3 would not mature in pups. An experiment was set up in 1961 to determine if infection of pups involved (1) the mother only, (2) the mother plus rookery, and/or (3) a specific time period postpartum. Thirty pregnant fur seal cows were collected, assigned to 3 groups (10 per group), and placed in individual cages. The protocol was: Group I—put on rookery soil (Fig. 1) and, on each of days 1–5 postpartum, take 2 pups to "clean ground" (an area inland, uninhabited by fur seals, where free-living L_3 were not present on soil or vegetation) for 1 day and then return them to their mothers; Group II—put on "clean ground" (Fig. 2) and, on each of days 1–5 postpartum, take 2 pups to rookery soil for 1 day and then return them to their mothers; and Group III—put on "clean ground" (Fig. 2) as controls and leave the pups with their mothers. This experiment provided the first evidence of the manner of transmission of *U. lucasi* to pups. The breakthrough occurred on 4 July 1961.

From this experiment with the 30 pregnant cows, some of the following aspects of the life cycle were derived: (1) 9 of 10 control pups (Group III) were positive for gastrointestinal infections of hookworms and (2) examination of young pups, 1 only 2 hr old, revealed hookworm larvae in milk in their stomachs. Subsequent research revealed that (1) parasitic L_3 were present in a mixture of mammary tissues, belly blubber, and milk from dead pregnant cows; (2) intestinal infections developed in pups, taken by cesarean section, after administration, by stomach tube, of parasitic L_3 derived from milk/belly tissues of pregnant cows; the prepatent period was about 2 wk; (3) hookworm larvae (1–135/cow) were recovered from milk samples from 7 of 8 pregnant cows; and (4) parasitic L_3 were also found in belly tissues of fur seal nonpregnant cows, bulls, bachelors, 2-yr-old males, yearlings, and pups, and Steller seal lion subadults. Thus, it was discovered that infections of adult *U. lucasi* in pups are derived from larvae passed through the mammary system of their mothers.

There were several indications that parasitic L_3 were present in milk of cows for only a short time postpartum: (1) only 1 size or stage of hookworms could be found in the gastrointestinal tract of pups; (2) postpartum cows ($N = 8$) were milked in late summer and no larvae were found in their milk; (3) examination of milk from the stomachs of pups nursing cows that just returned from the

Table 1. Development of *Uncinaria lucasi* parasitic third stage larvae from various sources of belly tissues in intestines of experimentally exposed (via stomach tube) northern fur seal pups taken by cesarean section.

Source of larvae	No. of pups infected*/exposed
Northern fur seals	
Pregnant cows	21/23
Nonpregnant cows	0/2
Bulls	0/3
Bachelors	0/8
Pup†	0/1
Stellar sea lions	
Bachelors	0/2

* Intestinal stages of *U. lucasi* recovered.

† From experimental exposure of pup taken by cesarean section to free-living L_3.

sea revealed no larvae; any larvae present should have accumulated in the mammary system of the cows during the periods of several days at sea; (4) foster pups ($N = 4$), taken by cesarean section and unfed, nursed 2 cows (their pups were removed soon after birth) in cages, and 3 of 4 became infected; also, 2 of these pups, at necropsy, 13 days after placement with foster mothers, harbored only adult hookworms; and (5) superinfections were attempted in 5 pups taken by cesarean section; 2 doses of parasitic L_3 from belly tissues/milk of pregnant cows were administered, the second dose 8–12 days after the initial dose. At necropsy, 2–4 days after the second exposure, 3 of the 5 pups had both immature (L_4) and adult hookworms in their intestines, which indicated that pups were susceptible to more than 1 exposure to larvae. However, the reason naturally infected pups presented only 1 stage resulted from the short time postpartum, probably a few hours, that parasitic L_3 are in the milk.

Infectivity of parasitic L_3 from various sources of belly tissues was studied in fur seal pups taken by cesarean section (Table 1). Intestinal infections of hookworms developed in 21 of 23 pups given larvae from pregnant cows. However, intestinal infections did not develop in 16 pups given larvae from belly tissues of fur seal nonpregnant cows, bulls, bachelors, and a pup (experimentally infected with free-living L_3), and Steller sea lion bachelors.

Measurements were made of parasitic L_3 in belly tissues from various sources. Only total lengths are discussed in this paper. Most larvae from adult female fur seals were larger (802 and 939 μm long for nonpregnant and pregnant cows, respectively) than those (641–732 μm long) from a fur seal bull, a bachelor, 2-yr-old males, both sexes of yearlings and pups, and Steller sea lion subadults. In belly blubber separated from mammary tissue of 2 pregnant cows, larvae were much shorter than those from a mixture of mammary tissue, belly blubber, and milk of other pregnant cows. The latter indicated that all larvae in tissues of pregnant cows are not depleted at pregnancy and all are not affected by the "growth factor." Possibly, hormones or some other factor(s) cause parasitic L_3 in milk/belly tissues of pregnant cows to be larger and infectious. Adult hookworms can develop in pups whether born on rocky (Fig. 3) or sandy (Fig. 4) rookeries because the source of infection is parasitic L_3 in the milk.

Longevity and viability of parasitic L_3 in tissues of northern fur seal cows were evaluated in 3 situations by examining belly tissues of cows for larvae and/or feces of their pups for eggs (Table 2). This research indicated that parasitic L_3 live and are infective in cow tissues for at least 6 yr.

Research on free-living *U. lucasi* larvae indicated that there is development to L_3 within the egg. At room temperature, the time for development for the various stages was (1) L_1 by about 24 hr, (2) L_2 by about 40 hr, and (3) L_3 by about 60 hr. Hatching of L_3 occurred at about 100 hr. On rookeries, hatching does not occur until several months after deposition of eggs. Parasitic L_3, passed in the milk, develop to L_4 about 24 hr after ingestion by pups. The final molt to L_5 occurs during the 4th and 5th days after infection.

The life cycle of *U. lucasi* can be summarized as follows (Lyons, 1963, Lyons and Keyes, 1984; Olsen, 1962; Olsen and Lyons, 1962, 1965):

A. Pups
  1. Parasitic L_3 are acquired in the "first milk" of the mother and mature into adult hookworms in about 2 wk.
  2. Adult female hookworms lay eggs that are passed in the feces of pups.
  3. Adult hookworm infections are spontaneously eliminated after about 3 mo and are never present again.
B. Rookery soil
  1. Free-living L_3 hatch from eggs in late summer.
  2. Free-living L_3 enter seal tissues after oral/percutaneous infection.
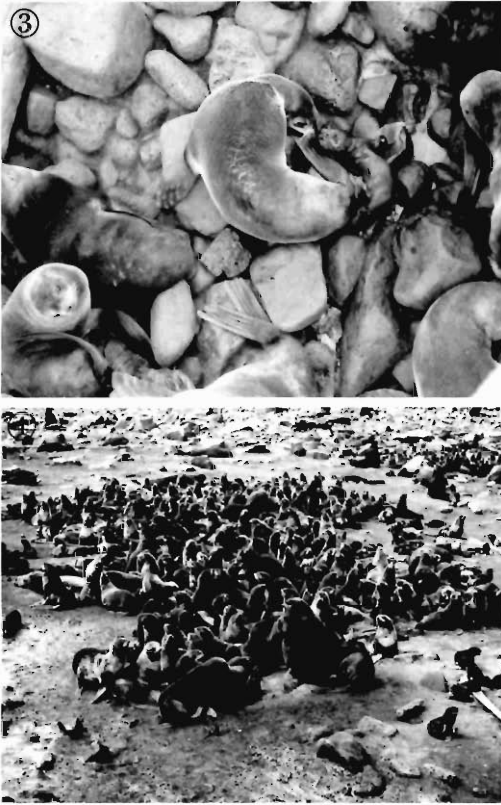
Figures 3, 4. St. Paul Island, Alaska. Northern fur seals. 3. Cows and pups. *Note* cow in the center looking at her still wet, newborn pup. Whether pups are born on rocky (in this scene) or sandy terrain, they can develop infections of adult *Uncinaria lucasi* because the source of infective larvae is from milk stages of parasitic L_3 stemming from free-living L_3 previously acquired by their mothers. 4. Harems, consisting of bulls, cows, and pups. Hookworm disease is generally a greater problem in pups born on sandy rookeries (as in this scene) than on rocky rookeries. This is because seals tend to return to the area of birth, and, therefore, females inhabiting sandy rookeries have a greater chance of acquiring higher numbers of L_3 in their tissues to pass in milk to their pups than do females on rocky rookeries.

C. Fur seals—all ages/both sexes
  1. Parasitic L_3 are found in belly and possibly other tissues.
  2. Parasitic L_3 remain in tissues except for parturient cows.
D. Parturient cows
  1. Parasitic L_3 pass in "first milk" to pups for a short time postpartum and these stages are the only ones that mature in pups.
  2. Parasitic L_3 in tissues live and are infective for several years.

Lucas (1899) believed that adult hookworms in northern fur seal pups that originated from hookworm larvae (free-living L_3) located on fur around teats of cows and ingested while nursing. He was very close to being correct on the manner of infection of the pups.

After our initial publication of the life cycle of *U. lucasi*, 2 other reports of transmammary transmission of nematodes were found in the literature. They were on natural infections of *Trichinella spiralis* in humans (Salzer, 1916) and on *Ancylostoma caninum* and *Toxocara canis* in experimentally infected guinea pigs (Kotake, 1928, 1929). The potential significance of transmammary transmission in these reports was not recognized at the time of their publication.

The discovery of transmammary transmission of *U. lucasi* led to interest by several researchers to determine if this manner of infection occurred for other parasite species. Some of the earlier literature was, and still is, clouded because it was assumed in many instances that early-infected neonatal mammals could have been infected only by intrauterine infection.

Strongyloides westeri

Research experience on the life cycle of *U. lucasi* would seem to have eased the unraveling of the life cycle of *S. westeri* in equids. However, it took 6 yr to discover the major way that foals are infected with this parasite. At the beginning (1963) of the concerted effort on research on the life cycle of *S. westeri*, knowledge on this parasite consisted mainly of the following (J. H. Drudge, pers. comm.; Russell, 1948; Todd et al., 1949): (1) prevalence of adult *S. westeri* in foals was about 90%, (2) most infected foals were passing eggs in their feces at about 2 wk of age, (3) foals were infected regardless of the time of year and place of birth, (4) indications were that infection of foals was at birth or soon after, (5) enteric infections were spontaneously eliminated by foals at a few months of age, and (6) older equids do not usually have adult enteric infections.

In the first major effort to investigate the life cycle of *S. westeri*, foals ($N = 158$) were removed from their dams at a young age (0–11 days of age). Only 8 foals were positive for eggs in their feces during periodic examinations. Of the 8 infected foals, only 2 were less than 4 days old when removed from their dams. These observations seemed to contradict the theory of early infection of foals. However, the research also appeared to

Table 2. Longevity and infectivity of *Uncinaria lucasi* parasitic third stage larvae in tissues of northern fur seal cows.

Location	Cows			No. of pups infected†/ examined
	Time		No. infected*/ examined	
	At sea	Captivity		
Bering Sea (Olsen and Lyons, 1965)	≥6 mo	—	20/27	—
Nanaimo, Canada (Lyons and Bigg, 1983)	—	4 yr	2/2‡	3/3
Seattle Aquarium (Lyons and Keyes, 1984)	—	6 yr	—§	2/2

* Parasitic L_3 recovered from belly tissues at necropsy.
† *Uncinaria lucasi* eggs found in feces.
‡ Three gave birth, but only 2 examined.
§ Two gave birth, but neither examined.

substantiate the theory that prenatal infection did not occur.

Worm-free foals ($N = 10$) were experimentally exposed to free-living L_3 *S. westeri*. All foals began passing eggs at 10–14 days postinfection. This prepatent period was similar to the age when eggs first appeared in the feces of naturally infected foals. This was more evidence that foals are infected at an early age, but the environmental source was not necessarily excluded in natural settings.

Placing pregnant mares ($N = 7$) in separate isolated areas, devoid of free-living L_3, provided the first strong indication that the dam was involved in infecting her foal. Foals born to 6 of the 7 mares began passing eggs at about 14 days of age. Other attempts were made to demonstrate the dams as a source of initial infections. Examination of mare tissue/excretions/secretions, including colostrum, revealed no *S. westeri* larvae. Additionally, tissues of fetuses and newborn foals were negative for *S. westeri* larvae. The latter observations indicated absence of prenatal infection.

At this point in the investigation, the source of the primary infection of foals was a mystery. It was evident that foals were definitely infected at or soon after birth. The question was: What could be the main source of infection if not prenatal, colostral, or environmental?

Definite evidence that the mare does infect her foal was demonstrated in a simple experiment. A foal was removed from its dam at birth and placed in a cage where an environmental source of larvae was not possible. The mare was milked 5 times daily for 20 days. A portion of each colostrum/milk sample was examined for larvae and the remainder fed to her foal. One larva was recovered from the milk at 11 days postpartum. The foal was passing eggs at 15 days of age. At 20 days of age, the foal was euthanatized and 8 adult *S. westeri* were found in its small intestine.

Subsequently, milk was collected from 82 mares and 32 were positive for *S. westeri* parasitic L_3, first at 4 days and last at 47 days postpartum. Usually, a very low number of larvae were recovered from milk samples. Larvae were found in the mammary glands of 2 mares at necropsy. Periodicity of larvae in milk samples was found for 5 mares milked every 4 hr for 24-hr periods. The highest numbers of larvae (66–89%) were found in milk in the AM, mostly between midnight and 8 AM.

Parasitic L_3 from milk of mares were administered via stomach tube to worm-free foals ($N = 7$) to determine if maturation occurred. The first eggs found in the feces of the foals appeared 8 or 9 days later in 6 foals and at 12 days for the other foal. This established, under experimental conditions, that parasitic L_3 in the milk were capable of maturing in foals.

There were several indications that parasitic L_3 of *S. westeri* require a shorter period for maturation than free-living L_3. These were (1) foals, experimentally infected with parasitic L_3, began passing eggs in feces 1 or 2 days earlier than foals infected with free-living L_3; (2) larvae were not found in colostrum nor in milk until 4 days postpartum, even though eggs are found in feces of naturally infected foals by about 14 days of age; (3) some measurements for parasitic L_3 were greater than those for free-living L_3; and (4) early

weaned foals, such as in the experiment where 158 foals were removed from their dams at 0–11 days of age, do not become infected because larvae are apparently not transmitted through the mammary system until a few days postpartum.

Although not present in colostrum, parasitic L_3 begin passing in the milk at a few days postpartum and, as previously mentioned, are capable of maturing quicker than free-living L_3. Perhaps for *S. westeri,* as indicated for some other species of *Strongyloides* (Katz, 1969; Nolan and Katz, 1981), parasitic L_3 acquired through the milk do not undergo hepatopulmonary migration, which is apparently required by free-living L_3.

The life cycle of *S. westeri* can be summarized as follows (Lyons et al., 1973, 1977):

A. Foals
1. Parasitic L_3 passing in the milk of dams are the probable major source of infective larvae that mature in foals.
2. Free-living L_3, derived from eggs passed in foal feces, can infect orally and/or percutaneously and mature, but also can migrate to tissues; this stage probably is a minor source of infection resulting in adult worms in foals.
3. Adult *S. westeri* lay eggs that are first voided in the feces at about 2 wk of age.
4. Adult *S. westeri* usually are permanently eliminated when the foal is a few months of age.
B. Mares
1. Parasitic L_3 are transmitted to foals in milk, beginning a few days postpartum, continuing for several weeks.
2. Parasitic L_3 are stored in tissues and only mobilized during lactation.

Summary of Vertical Transmission of Several Species of Nematodes

By the time that transmammary transmission of *S. westeri* was confirmed in 1969, it had been reported for a few other nematode species besides *U. lucasi,* including *Strongyloides ransomi* in swine (Moncol and Batte, 1966), *A. caninum* in dogs (Stone and Girardeau, 1966), and *T. canis* in dogs (Stone and Girardeau, 1967). Subsequently, several other nematode species were discovered to transmit larval stages through the mammary system of their hosts (Table 3).

Vertical transmission, both prenatal and trans-mammary, is summarized for several nematode species (Table 3). Most of the references were derived from the excellent publication on this subject by Shoop (1991). The present summary is comprehensive regarding literature but may not include all nematode species with vertical transmission. The literature cited includes both natural and experimental infections. In some instances, experimental infections were in other than definitive hosts. For the table, question marks are placed in the "prenatal" or "trans-mammary" columns for some species because the exact type of transmission seems unclear.

Some of the research citations did not necessarily show that one or the other means of vertical transmission did not occur. For instance, a paper may relate that prenatal infection occurred, based on earlier than usual maturation. However, transmammary transmission, and possibly an environmental source, may not have been eliminated from consideration. It is believed that for each species of nematode, the references include the first, or at least an early, report of transmammary transmission; this is possibly not true for all species relative to prenatal infection.

Two lungworm species (*Dictyocaulus viviparus* and *Muellerius capillaris*) are not included in the table. This is because proof of vertical transmission is not apparent, according to Soulsby (1965) for *D. viviparus* and to Runge (1974) and Cabaret (1988) for *M. capillaris.*

Vertical transmission varies in its importance as a source of infective stages that develop to adult nematodes in neonatal mammals. For example, it is nonsignificant for filariids with microfilariae passing prenatally. However, it is highly significant for *U. lucasi* because parasitic L_3, passed by transmammary transmission, are the only stages capable of maturation. Definite importance of prenatal/transmammary infection has not been determined for most nematode species for which vertical transmission has been reported.

Table 3. Summary of vertical transmission of nematodes.

Nematodes	Transmission		References*
	Prenatal	Transmammary	
Rhabditoidea			
Strongyloides spp. (*fulleborni*?)	−	+	6
S. papillosus	−	+	7, 30, 35, 40, 53, 54
S. ransomi	+	+	11, 34, 48, 49
S. ratti	?	+	18, 19, 59, 60
S. stercoralis	−	+	15
S. venezuelensis	−	+	37
S. westeri	−	+	29, 31
Ascaridoidea			
Ascaris lumbricoides	+	−	8
Toxascaris leonina	−	+	47
Toxocara canis	+	+	13, 21, 51
T. cati	−	+	55
T. pteropodis	−	+	41
T. vitulorum	−	+	7, 56–58
Ancylostomatoidea			
Ancylostoma caninum	+	+	10, 12, 22, 50, 52
A. tubaeforme	−	+	46
Gaigeria pachyscelis	?	+	2
Necator americanus	+	−	1
Uncinaria lucasi	−	+	25–28, 38, 39
U. stenocephala	−	+	10
Metastrongyloidea			
Protostrongylus stilesi	+	−	17
Filarioidea			
Brugia pahangi	?	−	20
Dipetalonema perstans	?	−	61
D. viteae	+	−	16
Dirofilaria immitis	+	−	14, 24, 33
D. repens	+	−	32
Loa loa	?	−	61
Onchocerca volvulus	+	−	5
Setaria cervi	+	−	42
Wuchereria bancrofti	?	−	4, 9
Trichostrongyloidea			
Dictyocaulus filaria	+	−	36
Nippostrongylus brasiliensis	−	?	59
Strongyloidea			
Stephanurus dentatus	?	−	3
Trichuroidea			
Trichinella spiralis	+	+	23, 43–45

* 1, Ackert and Payne (1923); 2, Ansari (1981); 3, Batte et al. (1966); 4, Bloomfield et al., (1978); 5, Brinkman et al. (1976); 6, Brown and Girardeau (1977); 7, Chauhan et al. (1974); 8, Chu et al. (1972); 9, Eberhard et al. (1993); 10, Enigk and Stoye (1967) (cited by Stoye, 1973); 11, Enigk et al. (1974); 12, Foster (1932); 13, Fülleborn (1921); 14, Galeb and Pourquier (1877) (cited by Beaver, 1970); 15, Haines et al. (1992); 16, Haque and Capron (1982); 17, Hibler et al. (1972); 18, Katz (1964); 19, Katz (1969); 20, Kimmig (1979); 21, Kotake (1928); 22, Kotake (1929); 23, Kuitunen-Ekbaum (1941); 24, Lewis (1879); 25, Lyons (1963); 26, Lyons and Olsen (1960); 27, Lyons and Olsen (1962a); 28, Lyons and Olsen (1962b); 29, Lyons et al. (1969); 30, Lyons et al. (1970); 31, Lyons et al. (1973); 32, Mantovani (1966); 33, Mantovani and Jackson (1966); 34, Moncol and Batte (1966); 35, Moncol and Grice (1974); 36, Neveu-Lemaire (1912) (cited by Soliman, 1953); 37, Nolan and Katz (1981); 38, Olsen and Lyons (1962); 39, Olsen and Lyons (1965); 40, Pfeiffer and Supperer (1969); 41, Prociv (1983); 42, Refuerzo (1952); 43, Roth (1935); 44, Roth (1936) (cited by Gould, 1945); 45, Salzer (1916); 46, Setasuban (1975); 47, Steffe (1983); 48, Stewart et al. (1969); 49, Stewart et al. (1976); 50, Stone and Girardeau (1966); 51, Stone and Girardeau (1967); 52, Stone et al. (1970); 53, Sukhapesna et al. (1975a); 54, Sukhapesna et al. (1975b); 55, Swerczek et al. (1971); 56, Tongson (1971); 57, Warren (1969); 58, Warren (1971); 59, Wilson et al. (1976); 60, Zamirdin and Wilson (1974); 61, Zanetti and Lambrecht (1948) (cited by Beaver, 1970).

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Helminths of the Roseate Spoonbill, *Ajaia ajaja,* in Southern Florida

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ABSTRACT: One hundred and thirty-six nestling, juvenile, and adult roseate spoonbills, *Ajaia ajaja,* were collected from southern Florida and examined for parasitic helminths. One hundred and twenty-one (89%) of the birds were infected with at least 1 of 28 species of helminths including 15 trematodes, 7 nematodes, 3 cestodes, and 3 acanthocephalans. Twenty species are new host records, while 7 are reported from Florida for the first time. Of the parasites studied, the prevalence of *Echinochasmus dietzevi* and the intensity of infection of *Contracaecum multipapillatum* showed statistically significant differences between sexes and amount of body fat, respectively. Generally, the prevalence and intensity of helminths were significantly higher in older roseate spoonbills and in birds from eastern Florida Bay colonies. In addition, the prevalence and/or intensity of infection of the trematodes *Phagicola longa, Microphallus turgidus,* and *Ascocotyle mcintoshi* and of the nematode *Contracaecum multipapillatum* varied significantly between years. These differences were attributed to hydropattern changes on the roseate spoonbill's feeding grounds through the years.

KEY WORDS: roseate spoonbill, *Ajaia ajaja,* Florida, helminths, prevalence, intensity.

The roseate spoonbill, *Ajaia ajaja* (Allen, 1942), ranges from southern Florida to central Chile and Argentina (American Ornithologists' Union, 1983). This species was very abundant in Florida Bay, but the population was drastically reduced by human harvesting and plume hunting (Powell et al., 1989). Even though roseate spoonbills in Florida have experienced a notable recovery through the years, they are considered specially susceptible to habitat disturbances in southern Florida due to their small population and high trophic position in relatively complex food webs (Powell et al., 1989).

The present study constitutes the first systematic survey of the parasites of the roseate spoonbill. Previously, a small number of spoonbills from South, Central, and North America have been examined and a total of 18 species of helminths have been found (Brandes, 1888; Cram, 1927; Freitas and Almeida, 1935; Caballero, 1939; Petrochenko, 1958; Dubois and Macko, 1972; Huey and Dronen, 1981; Dronen, 1985).

The objectives of this study were to survey the helminthofauna of the roseate spoonbill in southern Florida and to relate statistically prevalence and intensity of infection of these helminths to host age, sex, body fat, locality, and year.

Materials and Methods

One hundred thirty-three roseate spoonbills, found dead in 12 colonies on mangrove islands in Florida Bay, were collected during the breeding seasons (December–March) of 1986–1987 through 1991–1992. Severely autolyzed birds were not included in the study. Colonies consisted of those in eastern Florida Bay (Tern Key, Porjoe Key, South Park Key, North Park Key, Pigeon Key, Central Jimmy Key, Cowpens Key, Crane Key, East Buchanan Key, Grassy Key, Key Largo), and 1 in western Florida Bay (Sandy Key) (Fig. 1). Nestlings were assigned to 1 of 2 categories based on bill length measured from the base of the bill to the tip of the maxilla and into juvenile or adult categories based on plumage (Spalding and Forrester, 1993). The nestling categories included small nestlings, less than 60% of adult bill length, and large nestlings, greater than 60% of adult bill length. The sample included a total of 19 small nestling males, 48 small nestling females, 11 large nestling males, 11 large nestling females, 39 nestlings of unknown sex, 1 juvenile female, 2 adult males, 1 adult female, and 1 bird of unknown age. In addition, the carcasses of 1 adult male found at Lake Okeechobee in July 1971, of 1 juvenile female from Tamiami Trail collected in February 1990, and 1 juvenile male from Key West collected in July 1991 were included in the study (Fig. 1). Body fat reserves were assessed as: abundant, moderate, slight, or none.

Complete necropsy examinations were performed on fresh or frozen carcasses and techniques for recovering, fixing, and staining helminths were similar to those described by Kinsella and Forrester (1972). The terms

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prevalence, mean intensity, and abundance follow the definitions given by Margolis et al. (1982).

Chi-square and Wilcoxon tests were used to evaluate differences in prevalence and mean intensities of infection respectively, between years, colonies, ages, sexes, and amount of body fat on the birds (SAS Institute Inc., 1988). Prevalence and mean intensities for birds collected during 1986–1987 and 1990–1991, and of juveniles and adults were excluded from the statistical analysis by year and by age, respectively, because of the small sample sizes. Helminth fauna comparison was performed using the index of similarity from Holmes and Podesta (1968).

Voucher specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM 82336 and 83028–83053).

Results and Discussion

Twenty-eight species of helminths with a mean of 4.3 (range, 1–13) per host were identified from 121 of 136 (89%) roseate spoonbills. Of the birds that were not infected, 13 were small nestlings, and 2 were large nestlings. Helminths identified comprised 15 trematodes, 7 nematodes, 3 cestodes, and 3 acanthocephalans. The prevalence, number of helminths per infected bird, abundance, and location in the host of each of the species are presented in Table 1. Twenty species are new host records, while 7 are recorded from Florida for the first time. In Table 2 the prevalence and mean intensity of helminths collected from nestling, juvenile, and adult roseate spoonbills are listed.

Trematoda

Eighty-three birds (61%) harbored at least 1 species of digenetic trematode. The family Heterophyidae was represented by four species: *Ascocotyle chandleri* (Lumsden 1963), *Ascocotyle mcintoshi* (Price, 1936), *Phagicola longa* (Ransom, 1920), and *Pygidiopsis pindoramensis* (Travassos, 1929). *Ascocotyle chandleri*, the most frequently occurring trematode in this study, was usually found buried inside small nodules in the small intestine, alone or in groups of up to 3 individuals. Roseate spoonbills are the only known definitive hosts for this species (Dronen, 1985). *Phagicola longa* occurred in high intensities (on 1 occasion, more than 47,000 specimens were found in a bird). *Ascocotyle mcintoshi* and *P. longa* have been reported in Florida from ciconiiforms and pelecaniforms (Price, 1936; Hutton and Sogandares-Bernal, 1960; Hutton, 1964; Courtney and Forrester, 1974; Bush and Forrester, 1976; Threlfall, 1982). *Pygidiopsis pindoramensis* has been described from herons

from Brazil and Argentina (Yamaguti, 1971; Ostrowski de Nuñez, 1976). The present study is the first report of this parasite in the United States.

Three species of echinostomes were found: *Echinochasmus dietzevi* (Issaitschikoff, 1927), *Stephanoprora denticulata* (Rudolphi, 1802), and *Microparyphium facetum* (Dietz, 1909). Even though *E. dietzevi* was the second most abundant trematode collected during the study, this is the first report for the parasite in Florida. A single specimen of *M. facetum* was collected from the cloaca of an adult male collected in Lake Okeechobee. *Stephanoprora denticulata* and *M. facetum* have been reported in Florida from herring gulls (*Larus argentatus*) (Hutton and Sogandares-Bernal, 1960; Hutton, 1964), black skimmers (*Rynchops nigra*) (Sogandares-Bernal, 1959; Kinsella, 1972), brown pelicans (*Pelecanus occidentalis*) (Courtney and Forrester, 1974), and white ibises (*Eudocimus albus*) (Bush and Forrester, 1976).

Two species of microphallids were found: *Microphallus turgidus* (Leigh, 1958) and *Levinseniella* sp. Leigh (1958) originally described *M. turgidus* from racoons (*Procyon lotor*) collected in the Everglades, and since then it has been found in clapper rails (*Rallus longirostris*), white ibises, and brown pelicans from Florida (Heard, 1967; Courtney and Forrester, 1974; Bush and Forrester, 1976). Dronen (1985) described this microphallid from roseate spoonbills in Texas as *Carneophallus choanophallus* (=*M. turgidus*, Deblock, 1971). *Levinseniella* sp. was found in a single adult bird from Lake Okeechobee.

Renicola ralli (Byrd and Heard, 1970) was the only member of the family Renicolidae found during this study. This species was first described from the clapper rail-inhabiting marshes and mangrove areas of Florida (Byrd and Heard, 1970). The black skimmer is also a host for this species in Florida (Kinsella, 1972). Members of the genus *Renicola* are found commonly in kidneys of birds. However, in the present study specimens of *R. ralli* were collected not only from kidneys (29%) but also from cloaca (39%), large intestine (14%), and body cavity (13%). The latter 3 locations are possibly accidental resulting from the migration of the worms after the death of the host.

The family Clinostomidae was represented by the species *Clinostomum complanatum* (Rudolphi, 1819). In Florida, this fluke has been collected from the oral cavity of the white ibis and from the trachea and lungs of the double-crested
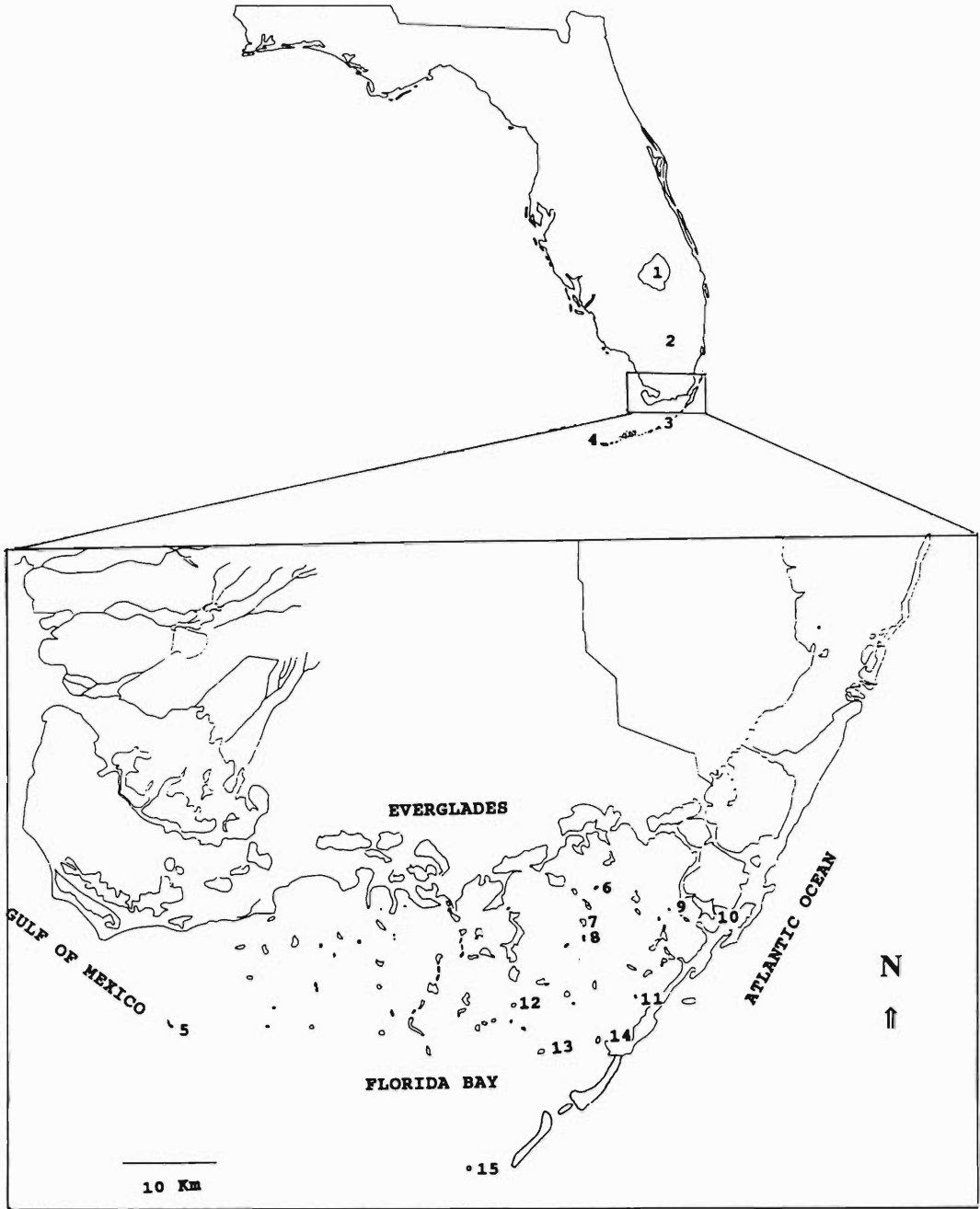
Figure 1.   Collection sites of 136 roseate spoonbills in southern Florida, with numbers of birds collected in parentheses. 1. Lake Okeechobee (1); 2. Tamiami Trail (1); 3. Grassy Key (1); 4. Key West (1); 5. Sandy Key (34); 6. Tern Key (48); 7. North Park Key (8); 8. South Park Key (5); 9. Porjoe Key (24); 10. Key Largo (1); 11. Pigeon Key (2); 12. Central Jimmy Key (3); 13. Crane Key (3); 14. Cowpens Key (3); 15. Buchanan Key (1).

Table 1.   Helminths of 136 roseate spoonbills from southern Florida.

Helminth*	Prevalence %	Number of worms per infected bird		Abundance
		Mean	Range	
Trematoda				
Ascocotyle chandleri† (3)				
(USNM 83028)	45	385	1–3,986	173
Echinochasmus dietzevi†‡ (3)				
(USNM 83029)	43	518	1–3,140	223
Phagicola longa‡ (3)				
(USNM 83030)	38	1,942	1–47,449	738
Microphallus turgidus (3, 4)				
(USNM 83031)	33	112	1–1,386	37
Ascocotyle mcintoshi‡ (3)				
(USNM 83032)	29	84	1–963	24
Renicola ralli‡ (4, 5, 7, 8)				
(USNM 83033)	12	22	1–140	3
Clinostomum complanatum‡ (1)				
(USNM 83034)	11	2	1–3	<1
Stephanoprora denticulata‡ (3)				
(USNM 83035)	10	54	2–248	5
Posthodiplostomum minimum‡ (3, 4)				
(USNM 83036)	10	34	1–202	3
Pygidiopsis pindoramensis†‡ (3, 4)				
(USNM 83037)	7	104	1–782	7
Apharyngostrigea multiovata (3, 4)				
(USNM 83038)	4	6	1–10	<1
Mesoophorodiplostomum anterovarium† (3)				
(USNM 83039)	3	46	1–140	1
Dendritobilharzia pulverulenta‡§ (6)				
(USNM 83040)	<1	1	—	<1
Microparyphium facetum‡§ (5)				
(USNM 83041)	<1	1	—	<1
Levinseniella sp.‡§ (3, 4)				
(not deposited)	<1	6	—	4
Nematoda				
Contracaecum multipapillatum‡ (1–3)				
(USNM 83042)	63	13	1–152	8
Cosmocephalus obvelatus‖ (1, 2)				
(USNM 83043)	40	8	1–68	3
Capillaria mergi†‡ (2–4)				
(USNM 83044)	8	5	1–9	<1
Syncuaria diacantha† (2, 4)				
(USNM 83045)	6	2	1–3	<1
Tetrameres micropenis‡ (2)				
(USNM 83046)	2	2	—	<1
Eustrongylides sp.¶ (2)				
(USNM 82336)	<1	1	—	<1
Synhimantus sp.‡# (2)				
(USNM 83047)	<1	107	—	79
Cestoda				
Cyclustera capito† (3)				
(USNM 83048)	43	39	1–240	17
Parvitaenia ibisae‡ (3)				
(USNM 83049)	17	26	1–307	4
Microsomacanthus sp.‡ (3)				
(USNM 83050)	<1	25	—	18
Acanthocephala				
Southwellina hispida‡ (3)				
(USNM 83051)	24	11	1–135	3

Table 1.   Continued.

| Helminth* | Prevalence % | Number of worms per infected bird | | Abundance |
		Mean	Range	
Arhythmorhynchus sp.‡ (3)				
(USNM 83052)	<1	1	—	<1
Leptorhynchoides sp.‡ (2)				
(USNM 83053)	<1	1	—	<1

* Numbers in parentheses indicate most frequent location in host: (1) oral cavity/esophagus, (2) proventriculus/ventriculus, (3) small intestine, (4) large intestine, (5) cloaca, (6) heart, (7) kidneys, and (8) body cavity.
† New record for Florida.
‡ New host record.
§ Found in an adult male, Lake Okeechobee, 1971.
‖ A complex of adults and larvae.
¶ Immature specimen probably of *Eustrongylides ignotus.*
# Probably represents an undescribed species.

cormorant, *Phalacrocorax auritus* (Bush and Forrester, 1976; Threfall, 1982).

Three species of strigeids were found: *Posthodiplostomum minimum* (MacCallum, 1921), *Apharyngostrigea multiovata* (Perez Vigueras, 1944), and *Mesoophorodiplostomum anterovarium* (Dronen, 1985). *Posthodiplostomum minimum* has been reported from the small intestine of white ibises in Florida (Bush and Forrester, 1976). Dubois and Macko (1972) reported *A. multiovata* from roseate spoonbills from Cuba, and Conti et al. (1986) found this species in reddish egrets, *Egretta rufescens,* collected at Tern Key, Florida Bay. Specimens from roseate spoonbills in Florida were found in very low prevalence and were always immature or nongravid, suggesting that this bird is probably not the normal definitive host. *Mesoophorodiplostomum anterovarium* was described originally by Dronen (1985) from specimens obtained from roseate spoonbills in Texas.

A single female of the schistosome *Dendritobilharzia pulverulenta* (Braun, 1901) was found in the heart of an adult male collected in Lake Okeechobee. The low prevalence and intensity of infection observed during this study is not surprising since this fluke has been described mainly from anseriforms (Vande Vusse, 1979). However, the actual prevalence of this trematode may be higher because blood vessels and hearts were not commonly examined.

Nematoda

A total of 107 birds (79%) was infected with 1 or more species of nematode belonging to the orders Ascarididea, Spiruridea, Trichuridea, and Dioctophymidea.

Contracaecum multipapillatum (Drasche, 1882; family Heterocheilidae) was the most common nematode encountered during this study. Barus (1966) reported larvae of *Contracaecum* sp. from roseate spoonbills from Cuba. In Florida, this nematode has been reported from the water turkey (*Anhinga anhinga*) (Huizinga, 1971), the brown pelican (Courtney and Forrester, 1974), the double-crested cormorant (*P. auritus*) (Threlfall, 1982), and the reddish egret (Conti et al., 1986).

The family Acuariidae was represented by *Cosmocephalus obvelatus* (Creplin, 1825), *Syncuaria diacantha* (Petter, 1961), and *Synhimantus* sp. *Cosmocephalus obvelatus* has been reported from roseate spoonbills from Cuba and Texas (Barus, 1966; Huey and Dronen, 1981) and from black skimmers and brown pelicans from Florida (Kinsella, 1972; Courtney and Forrester, 1974). *Syncuaria diacantha* was described originally by Petter (1961) from a roseate spoonbill held in captivity in Paris, France. Barus (1966) reported this species from roseate spoonbills from Cuba. The present report represents the first record of *S. diacantha* in the United States. One small nestling collected at South Park Key was infected with what appears to be a new species of *Synhimantus.* The same acuariid has been found in a laughing gull (*Larus atricilla*) from Cedar Key, Florida (Kinsella, unpubl. data).

A single species of the family Tetrameridae was found: *Tetrameres micropenis* (Travassos, 1915). This nematode was identified from 3 nest-

Table 2.   Prevalence and mean intensity of helminths of nestlings, juveniles, and adults of roseate spoonbills from Florida.

Helminth	Nestlings (N = 128)		Juveniles and adults (N = 7)	
	Prevalence %	Mean intensity	Prevalence %	Mean intensity
Trematoda				
A. chandleri	45	332	43	1,444
E. dietzevi	45	545	43	129
P. longa	36	2,068	57	595
M. turgidus	32	114	43	87
A. mcintoshi	30	86	14	1
R. ralli	13	22	0	—
C. complanatum	11	2	14	1
S. denticulata	11	54	0	—
P. minimum	9	36	14	5
P. pindoramensis	8	104	0	—
A. multiovata	4	6	0	—
M. anterovarium	3	46	0	—
D. pulverulenta	0	—	14	1
M. facetum	0	—	14	1
Levinseniella sp.	0	—	14	6
Nematoda				
C. multipapillatum	64	13	43	23
C. obvelatus	39	8	71	10
C. mergi	5	3	71	9
S. diacantha	5	2	29	3
T. micropenis	2	2	0	—
Eustrongylides sp.	<1	1	0	—
Synhimantus sp.	<1	107	0	—
Cestoda				
C. capito	42	33	57	115
P. ibisae	16	12	29	169
Microsomacanthus sp.	0	—	14	25
Acanthocephala				
S. hispida	21	5	57	53
Arhythmorhynchus sp.	<1	1	0	—
Leptorhynchoides sp.	<1	1	0	—

lings, and only males were collected from the proventriculus of each bird.

The family Trichuridae was represented by a single species, *Capillaria mergi* (Madsen, 1945). Specimens of *Capillaria* similar to *C. mergi* have been reported from the brown pelican and the white ibis in Florida (Courtney and Forrester, 1974; Bush and Forrester, 1976).

One immature specimen of *Eustrongylides* sp. (family Dioctophymidae) was found in a nestling collected on Porjoe Key. This specimen probably belongs to the species *E. ignotus* (Jägersk, 1909), since it has been described from other species of wading birds in southern Florida (Spalding et al., 1993).

Cestoda

Cyclophyllidean cestodes were found in 63 (46%) of the birds examined. Two species belonging to the family Dilepididae were identified: *Cyclustera capito* (Rudolphi, 1819) and *Parvitaenia ibisae* (Schmidt and Bush, 1972). *Cyclustera capito* was the most abundant cestode occurring during this study. This species was originally described from a roseate spoonbill from Brazil (Yamaguti, 1959), and more recently it has been reported from the same host in Mexico and Texas (Coil, 1955; Huey and Dronen, 1981). Mature specimens of *P. ibisae* were found frequently in birds of all ages, and unhatched cysticercoids of this cestode were found in the stomachs of 3 nestlings. In Florida, immature specimens have been reported from brown pelicans (Courtney and Forrester, 1974) and black skimmers (Kinsella, 1972), while mature cestodes have been collected only from white ibises (Schmidt and Bush, 1972).

A single species belonging to the family Hymenolepididae was found. *Microsomacanthus* sp. was found in a single adult bird collected on Lake Okeechobee. The specimen had 10 hooks that were 25 μm long and may be the same species reported by Bush (1973) in white ibises from Florida.

Acanthocephala

A total of 35 birds (26%) was infected with acanthocephalans belonging to the order Echinorhynchidea. The family Polymorphidae was represented by *Southwellina hispida* (Van Cleave, 1925) and *Arhythmorhynchus* sp. *Southwellina hispida,* the most abundant acanthocephalan encountered during this study, has been reported from Florida brown pelicans (Courtney and Forrester, 1974). One nestling collected in Tern Key was infected with an immature specimen of *Arhythmorhynchus* sp. Unfortunately, it could not be identified further since the proboscis was retracted.

A single immature specimen of an unidentified species of *Leptorhynchoides* (family Echinorhynchidae) was found in the proventriculus of a nestling collected in Porjoe Key. The specimen closely resembles *L. thecatus* (Linton, 1891), a common parasite of fishes in southern Florida (Bangham, 1940), but differs in having 3 more longitudinal rows of longer hooks. This parasite is probably accidental in roseate spoonbills.

Table 3.   Significant differences of prevalence and mean intensity of helminth infections, by year.

Helminth	Prevalence %					Intensity				
	1987–1988 ($N = 38$)	1988–1989 ($N = 33$)	1989–1990 ($N = 31$)	1991–1992 ($N = 25$)	$P*$	1987–1988 ($N = 38$)	1988–1989 ($N = 33$)	1989–1990 ($N = 31$)	1991–1992 ($N = 25$)	$P*$
Trematoda										
A. chandleri	32	52	61	40	NS†	237	82	832	269	0.03
P. longa	16	42	55	44	0.002	383	4,614	167	2,018	0.03
M. turgidus	18	52	29	44	0.006	74	218	23	49	NS
A. mcintoshi	13	39	48	20	0.04	12	109	105	23	NS
Nematoda										
C. multipapillatum	50	27	26	48	0.04‡	22	5	9	16	0.0009

* P = level of significance for comparisons of prevalence (χ^2) and mean intensity (Wilcoxon test).

† NS = not significant ($P > 0.05$).

‡ Significant differences were found for adult parasites only.

Effect of host sex on helminth infections

The mean intensity of *Echinochasmus dietzevi* infection was significantly higher in nestling females ($\bar{x} = 548$) than nestling males ($\bar{x} = 245$) ($P = 0.04$). Factors responsible for this difference are difficult to explain, particularly because these birds were being fed by their parents. Thul et al. (1985) described a similar phenomenon in immature Florida wood ducks (*Aix sponsa*) infected with the trematode *Prosthogonimus ovatus* and attributed it to anatomical or immunological differences between sexes.

Effect of host body fat on helminth infections

The prevalence of *Cosmocephalus obvelatus* larvae was significantly greater in nestlings with no fat than in nestlings with abundant fat (55 vs. 28%, $\chi^2 = 7.58$, $P = 0.006$). This suggests that *C. obvelatus* infection may lead to poor nutritional condition, or that birds in poor nutritional condition are more susceptible to infection by this particular species.

Effect of year on helminth infections

Prevalence and intensity analyses by year are summarized in Table 3. In general, the prevalences of the trematodes *P. longa, M. turgidus,* and *A. mcintoshi* followed a similar trend over the years, with significant differences explained mainly by the small number of birds infected during 1987–1988 and the high prevalences in 1988–1989 to 1989–1990. *Ascocotyle chandleri,* the most prevalent trematode found during this study, followed the same trend; however, the differences were not significant.

In contrast, the prevalence of the nematode *C. multipapillatum* followed an opposite trend and in 1987–1988, 50% of the birds examined were found infected. During the 1991–1992 breeding season, a similar high prevalence of infection (48%) was observed. In addition, significantly higher intensities of infection with this nematode were reported during those years. Prevalence and intensity of infection with this nematode were significantly lower during 1988–1989 and 1989–1990.

Data available on the life cycles of these helminths indicate that, in general, immature stages of *P. longa, M. turgidus,* and *A. mcintoshi* develop in salt or brackish water intermediate hosts, while larval stages of *C. multipapillatum* are found in freshwater copepods and fishes. Hutton and Sogandares-Bernal (1959) found no metacercariae of *P. longa* in mullet (*Mugil cephalus*) from a freshwater lake in Florida. However, they did find the metacercariae in mullet (*M. cephalus, M. curema,* and *M. trichodon*) from brackish waters in southern Florida and believed that the first intermediate host for this trematode was a salt or brackish water mollusk. Leigh (1956) found mosquitofish (*Gambusia affinis*) from the Everglades infected with metacercariae of *A. mcintoshi* and speculated that the cercariae occurred in small brackish water snails. He later described the prosobranch snail, *Littoridinops monroensis,* as the first intermediate host and added the brackish-water fish *Poecillia latipinna* to the list of second intermediate hosts for this trematode (Leigh, 1974). The microphallid *M. turgidus* utilizes the snails *L. monroensis* and *L. tenuipes* as first intermediate hosts in southern Florida, and

shrimp of the genus *Palaemonetes* sp. as second intermediate hosts (Heard and Overstreet, 1983). Even though *G. affinis* and *Palaemonetes* sp. are found commonly in freshwater habitats, Bush and Forrester (1976) found no specimens of *A. mcintoshi* and *M. turgidus* in white ibises from freshwater areas in Florida.

Larval stages of *C. multipapillatum* have been found in freshwater fishes in Florida (*Micropterus salmoides, G. affinis, Lepomis* sp.) and experimentally this nematode has been found to be highly infective to freshwater copepods (Huizinga, 1965).

The lower prevalences of trematodes observed during 1987–1988 are likely to be a reflection of a decrease in the availability of infected brackish water intermediate hosts. On the other hand, the high prevalence and intensity by *C. multipapillatum* in 1987–1988 and 1991–1992 can be attributable to an increase in the presence of infected freshwater copepods and fishes. Even though other factors probably contributed to the observed differences in prevalence and intensity of infection, it is possible that they were the result of hydropattern changes on the roseate spoonbills' feeding grounds through the years. In fact, during the dry seasons of 1988–1989 and 1989–1990, the drying out of brackish water wetlands concentrated fishes and invertebrates and thus made them more available as prey for roseate spoonbills (Bjork and Powell, 1993). In contrast, during 1987–1988 and 1991–1992, the surrounding wetlands were flooded by rainfall and/or canal discharges (Bjork and Powell, 1993). The effect of the decrease in salinity during the wet years on populations of marine and brackish water fishes is unknown. However, during those years, eggs of *C. multipapillatum,* which are thin-shelled and nonresistant to drying (Huizinga, 1965), probably found excellent conditions for their development.

The absence of a major decrease in the prevalence of trematodes during 1991–1992 is difficult to understand. However, the significant differences in prevalence and intensity of *C. multipapillatum* between wet and dry years might mean that this nematode is more susceptible to drought conditions than the trematodes.

Of particular interest were the differences in intensities of *A. chandleri* and *P. longa* infections between years. Generally, in years of high intensity of *A. chandleri* there was a low intensity of *P. longa* and vice versa. In addition, for both parasites, years of high intensity of infection were generally followed by years of low intensity of infection.

Effect of locality on helminth infections

Prevalence and intensity analyses by locality are summarized in Table 4. Generally, prevalence and intensity of helminths were greater in roseate spoonbills from the east colonies than in birds from the west colony. *Clinostomum complanatum, C. multipapillatum, C. obvelatus,* and *S. hispida* were found in significantly higher prevalences and/or intensities in nestlings from the east colonies, while *S. diacantha* and *P. ibisae* were found in significantly greater prevalences and/or intensities in nestlings collected in the west colony (Table 4).

Differences in roseate spoonbill food habits and/or in prevalence and intensity of infection of intermediate hosts between east and west Florida Bay are some factors that may have caused the differences observed in prevalence and intensity of infection between the 2 areas. Because of the scarce information available on the food habits of roseate spoonbills and on the degree of infection of invertebrates and fishes from the area, few conclusions can be drawn from the present study.

Immature stages of *Parvitaenia* sp. have been reported from *Fundulus confluentus, F. grandis, Cyprinodon variegatus,* and *G. affinis* (Bush, 1973). Lorenz and Powell (1992) found that *G. affinis* was a very common species in west Florida Bay, and that *F. confluentus* and *F. grandis* occurred very rarely in that area. The fact that Powell and Bjork (1990) found a higher percentage of *C. variegatus* in regurgitations of nestlings collected in the eastern colonies indicates that the difference in prevalence of *Parvitaenia ibisae* between birds from west and east Florida Bay may be the result of differences in the availability of infected mosquitofish (*G. affinis*) between the 2 areas.

Effect of host age on helminth infections

Prevalence and intensity analyses by age of the host are summarized in Table 4. In general, prevalence and intensity of helminths were greater in larger nestlings than in smaller nestlings. *Ascocotyle chandleri, R. ralli, C. obvelatus, C. mergi,* and *C. capito* were found in significantly greater prevalences and/or intensities in larger nestlings, while *E. dietzevi* was the only helminth that showed a significantly higher intensity of infection in smaller nestlings (Table 4).

Table 4. Significant differences of prevalence and mean intensity of helminth infections, by locality and age.

Helminth	Prevalence %						Intensity					
	Locality			Age*			Locality			Age		
	West (34)	East (99)	P†	I (105)	II (23)	P	West (34)	East (99)	P	I (105)	II (23)	P
Trematoda												
A. chandleri	50	44	NS	45	48	NS	108	492	NS	136	1,152	0.02
E. dietzevi	47	46	NS	46	48	NS	643	474	NS	648	88	0.002
R. ralli	12	12	NS	10	26	0.02	49	13	NS	27	14	NS
C. complanatum	0	15	0.01	13	4	NS	—	2	NS	1	3	NS
Nematoda												
C. multipapillatum	24	45	0.01‡	67	61	NS	6	15	0.002	14	9	NS
C. obvelatus	53	35	NS	10	35	0.007‡	5	10	0.03	7	11	0.04
C. mergi	3	9	NS	1	26	0.0002	2	3	NS	1	3	NS
S. diacantha	15	2	0.02	3	13	NS	1	2	NS	2	2	NS
Cestoda												
C. capito	53	41	NS	42	48	NS	4	36	NS	29	51	0.03
P. ibisae	32	11	0.02	18	13	NS	20	6	0.01	14	3	NS
Acanthocephala												
S. hispida	12	28	0.02	23	17	NS	3	13	NS	5	6	NS

* I = small nestlings; II = large nestlings.

† P = level of significance.

‡ Significant differences were found for adult parasites only.

The higher prevalences and intensities of helminths observed in older nestlings can be attributed to the fact that these birds had a greater opportunity for successive infections than younger ones, and that the time required for helminths to mature had probably occurred in the majority of the older birds. Additionally, this difference could be a reflection of changes in the availability of infected prey for parent birds and/or changes in the intensity of infection of these food items as the breeding season progresses.

The present study points out some discrepancies regarding the effect of age on the intensities of infection of members of the genus *Ascocotyle*. Sogandares-Bernal and Bridgman (1960) and Sogandares-Bernal and Lumsden (1964) found that nestling ardeids (1–4 days old) had higher intensities of infection with *Ascocotyle* spp. than their parents and attributed this decrease to an age immunity factor. Due to the fact that only nestling roseate spoonbills were included in the *A. chandleri* intensity statistical analysis, a decrease in intensity as birds grow older cannot be disproven. However, juveniles and adults were found harboring apparent higher numbers of this parasite than nestlings (Table 2).

The increase in prevalence and intensity of *C. obvelatus* with age has been reported previously in ring-billed gulls (*Larus delawarensis*) from Lake Ontario, Canada (Wong and Anderson, 1982). Examining young-of-the-year, those authors found a general increase in prevalence (reaching 100% in 2 wk) and in intensity (increased for approximately 28 days and then again at 35–42 days of age) as birds grew older.

The decline in intensity of *E. dietzevi* in large nestlings may be attributable to changes in intermediate host availability during the nesting season, development of immunity, physiological and anatomical changes associated with age, and/or competition with other helminths inhabiting the small intestine.

The significance of helminth infections to roseate spoonbills

The occurrence of a relatively high prevalence and/or high intensity of *P. longa*, *A. chandleri*, *E. dietzevi*, *C. multipapillatum*, *R. ralli*, and *S. hispida* leads us to believe that these helminths might act as potential pathogens to roseate spoonbills. Heterophyids, members of the genus *Renicola*, and *C. multipapillatum* have been implicated in severe pathological changes and even death of avian hosts (Sogandares-Bernal and Lumsden, 1964; Huizinga, 1965; Riley and Owen, 1972).

The similarity between the helminthofaunas of roseate spoonbills and white ibises in Florida, both members of the family Threskiornithidae, was high (index of similarity for all helminths was 41%). This is a strong indication of the similarity of food habits and habitat use by both species. In contrast, roseate spoonbills in Florida appear to share only a few helminth species with Texas roseate spoonbills (Huey and Dronen, 1981; Dronen, 1985), which may mean that there are differences in diets between populations. However, because only 2 Texas spoonbills have been examined and the age of these birds was not reported, further work is needed for a better understanding of these differences.

Acknowledgments

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Life Cycle of *Oligogonotylus manteri* (Digenea: Cryptogonimidae), a Parasite of Cichlid Fishes in Southern Mexico

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ABSTRACT: The life cycle of the cryptogonimid, *Oligogonotylus manteri* Watson, 1976, was studied under natural and experimental conditions. Field study showed that aquatic snails, *Benthonella gaza* (Prosobranchiata: Rissoidae), were the first intermediate host and cichlid fish, *Cichlasoma urophthalmus,* either the second intermediate or definitive hosts. Laboratory-reared cichlids, *Cichlasoma synspilum,* were exposed to *O. manteri* cercariae from naturally infected snails by placing them into water or force-feeding with remnants of snails harboring *O. manteri* cercariae. The development of metacercariae in experimentally infected *C. synspilum* was completed 6 days postexposure (DPE) at 22–24°C. Metacercariae from the gills, fins, body surface, and intestinal walls of naturally infected *C. urophthalmus* and experimentally infected *C. synspilum* were used to expose *C. synspilum,* *Oreochromis niloticus* (Cichlidae), and *Poecilia reticulata* (Poecilidae). Adult worms were detected in *C. synspilum* 16 DPE at 22–24°C and juveniles only in *O. niloticus* and *P. reticulata.* Results of both feeding experiments and examination of naturally infected cichlid fish from the Yucatan Peninsula revealed that metacercariae previously reported as *Echinochasmus zubedakhaname* were *O. manteri.*

KEY WORDS: *Oligogonotylus manteri,* Digenea, experimental infection, life cycle, *Benthonella gaza, Cichlasoma* spp., Yucatan, Mexico, developmental stages.

The cryptogonimid *Oligogonotylus manteri* was originally described from cichlid fishes in Nicaragua (Watson, 1976). Since that time, it has been reported as a common intestinal parasite of cichlids in Mexico as well (Osorio-Sarabia et al., 1987). Despite the wide distribution and frequent occurrence of this digenean, little is documented about its biology. Consequently, the purpose of this study was to provide information on the life cycle of this parasite.

Another aim of this investigation was to test the assumption that metacercariae, frequently found encysted in the intestinal wall, gills, and fins of *Cichlasoma urophthalmus* (Günther) in southeastern Mexico and hitherto apparently misidentified as *Echinochasmus zubedakhaname* Nasir et Díaz, 1968 (Lamothe-Argumedo & Aguirre-Macedo, 1991a), represent a developmental stage of *O. manteri.*

Materials and Methods

Study areas

The first was the coastal lagoon of Celestun, situated NW of Merida (20°45′–20°58′N, 90°15′–90°25′W), Yu-

catan, Mexico. The lagoon ranges in depth from 0.4 to 3.5 m. It is 28 km long and from 0.4 to 2.3 km wide, with a total area of 31 km², and opens southward into the Gulf of Mexico (Valdés et al., 1988). Salinity values in the lagoon fluctuate from almost fresh water in its northern part, where numerous springs are present, to 22.0–36.8 ppt in the southern part where the lagoon opens into the sea.

The second study area was a flooded quarry in the Mitza limestone factory, 25 km north of Merida (21°15′N, 89°40′W). The total area of the quarry is 9.3 ha and average depth of the water is 5.2 m, with a maximum depth of 8.5 m (Flores-Nava, 1990).

Samples of *C. urophthalmus* were also collected from other localities in the Yucatan Peninsula: the coastal lagoon at Rio Lagartos, Yucatan (21°34′–21°36′N, 87°51′–88°13′W); permanent lakes at Noh-Bek (19°04′N, 88°49′W) and Guerrero (18°42′N, 88°15′W), both in the state of Quintana Roo; the Champoton River (19°21′N, 90°40′W), Laguna El Vapor and Estero Pargo, a tidal channel, both in the Laguna Terminos complex (18°20′–19°00′N, 91°10′–92°00′W), all in the state of Campeche.

Examination of naturally infected hosts

During April, May, and August 1993, 589 snails, *Benthonella gaza* Dall (Prosobranchiata: Rissoidae), were sampled from Celestun and 290 from Mitza. In the laboratory, they were placed individually in glass tubes containing 10 ml of pond water and exposed to the light for several hours. The water was then examined for the presence of released cercariae by visual inspection. Thereafter, all snails were dissected and their rediae and cercariae studied as temporary mounts.

A total of 88 fishes included 30 *C. urophthalmus,* 26

Table 1. Records of fish with *Oligogonotylus manteri* metacercariae.

Fish species	Locality	No. fishes Examined	No. fishes Infected	Prevalence (%)	Total no. worms	Intensity of infection Mean	Intensity of infection Range
Cichlasoma urophthalmus	Celestun*	30	28	93	506,374	18,033	1–178,871
C. urophthalmus	Celestun†	30	30	100	11,734‡	391‡	3–1,615
Bairdiella ronchus	Celestun	11	11	100	513	47	6–132
Lutjanus griseus	Celestun	26	15	60	373	25	1–127
Strongylura timucu	Celestun	3	1	33	6	6	6
Sphoeroides testudineus	Celestun	8	6	75	647‡	108‡	2–570
C. urophthalmus	Rio Lagartos	27	24	89	130,017	5,417	1–23,363
C. urophthalmus	Noh-Bek	30	2	7	45	23	4–41
C. urophthalmus	Guerrero	4	2	50	157	79	58–99
C. urophthalmus	Champoton	30	15	50	9,266	618	1–4,993
C. urophthalmus	El Vapor	30	7	23	33	5	1–19

* May 1988.
† April, May, and August 1993.
‡ Metacercariae from the intestinal wall are not included.

Lutjanus griseus (L.), 2 *Lutjanus synagris* (L.) (Lutjanidae), 7 *Lagodon rhomboides* (L.) (Sparidae), 11 *Bairdiella ronchus* (Cuvier), 1 *Cynoscion nebulosus* (Cuvier) (Sciaenidae), 3 *Strongylura timucu* (Walbaum) (Belonidae), and 8 *Sphoeroides testudineus* (L.) (Tetraodontidae) were collected by angling from the lagoon of Celestun. An additional 163 *C. urophthalmus* from the study localities mentioned above, including Celestun (see Table 1), were collected between May and July 1988.

All fish were examined externally and internally by routine helminthological procedure, as outlined by Bykhovskaya-Pavlovskaya (1969). Metacercariae were studied alive, either while encysted or after removal from cysts. Adult worms were observed and measured either as temporary or permanent mounts, fixed either with ammonium-picrate (Ergens, 1969) or with 4% formalin under slight coverslip pressure, stained with Schuberg's (acid) carmine, dehydrated in alcohol, and mounted in Canada balsam. All measurements are in μm, unless otherwise stated. The mean with standard deviation (SD) as well as minimum and maximum values (in parentheses) are included in the descriptions of developmental stages. Drawings were made using a Leitz drawing attachment.

Experimental animals

Five species of experimental animals were used: laboratory-reared, parasite-free cichlid fishes, *Cichlasoma synspilum* (Hubbs) and *Oreochromis niloticus* (L.); guppies, *Poecilia reticulata* (L.) (reared in an ornamental fish farm in Merida); laboratory mice (2 wk old, from laboratory stocks in the Laboratory of Parasitology, Faculty of Veterinary Sciences, University of Yucatan, Merida); and chicks (5 days old, from a chicken farm in Merida).

Experimental design

SECOND INTERMEDIATE HOST: Eleven hatchery-reared *C. synspilum* housed in individual small aquaria were exposed to 200–300 cercariae for 3–12 hr. Exposed fish were maintained in 10-liter aquaria on a diet of pelletized food and examined for metacercariae 1, 3, 4, 5, 6, 10, 15, and 20 days postexposure (DPE).

Tissues containing cercariae of *O. manteri* from naturally infected *B. gaza* snails were injected directly into the stomachs (i.e., force-fed) of 3 additional *C. synspilum*, using a Pasteur pipette. Regurgitated material was collected and readministered to the fish. Fish exposed in this manner were maintained as described above and examined 5, 7, and 16 DPE.

DEFINITIVE HOST: Pieces of intestine (approximately 1–3 mm long) and gills of naturally infected *C. urophthalmus* were force-fed to 19 *C. synspilum*, 9 *O. niloticus*, and 12 *P. reticulata*, which were dissected and examined 1, 2, 3, 4, 5, 7, 10, 13, 16, 19, 22, 25, and 28 DPE. An additional 7 *C. synspilum* were each force-fed between 8 and 50 metacercariae (6–7 days old) from the fins, body surface, and gills of experimentally infected *C. synspilum*. The exposed fish were held in 10-liter aquaria and examined 2, 3, 7, and 16 DPE.

Oral infections of *O. manteri* metacercariae from naturally infected *C. urophthalmus* were administered to 3 groups of chicks and laboratory mice (each group consisting of 5 chicks and 2 mice) as follows:

Group 1: Each host was fed pieces of the anterior third of the intestine of *C. urophthalmus* (from a swamp in Mitza) infected with several hundred *O. manteri* metacercariae.

Group 2: Each host was fed pieces of gills of *C. urophthalmus* (from Celestun) infected with 100–200 *O. manteri* metacercariae.

Group 3: Each host was fed pieces of the anterior third of the intestine of *C. urophthalmus* (from Celestun) infected with at least 1,000 *O. manteri* metacercariae.

Ten chicks and 4 mice served as controls. After infection, all animals, experimentals and controls, were fed pelletized food, and examined 1, 3, 8, 13, and 15 DPE (chicks) and 4 and 11 DPE (mice).

Reference specimens, including metacercariae from naturally infected *C. urophthalmus* and adults from experimentally infected *C. synspilum*, are deposited in

the National Parasite Collection, U.S. National Museum, Beltsville, Maryland, Coll. No. 83754-55, and helminthological collection of the Laboratory of Parasitology, CINVESTAV-IPN, Merida.

Results

Natural infections

FIRST INTERMEDIATE HOST: A total of 136 *B. gaza* from Celestun (23.1%) and 32 from Mitza (11.0%) were found to be infected with larval stages (rediae, cercariae; Figs. 1–7) of *O. manteri* in their hepatopancreas.

SECOND INTERMEDIATE HOST: Metacercariae of *O. manteri* were found in 5 fish species from the lagoon of Celestun, with the highest infection level in *C. urophthalmus* (see Table 1); *L. synagris, L. rhomboides,* and *C. nebulosus* were negative. Whereas a majority of *O. manteri* larvae found in *C. urophthalmus* (Fig. 8) and *B. ronchus* were alive without signs of degeneration, larvae recorded in *S. testudineus, L. griseus,* and *S. timucu* were dead and partially decomposed.

Internal infections (metacercariae encysted in the wall of the anterior intestine) were found only in *C. urophthalmus* and 2 specimens of *S. testudineus*; other fishes were infected with *O. manteri* larvae located only superficially (in the gills, fins, or on the body surface).

Out of 11,734 metacercariae found in *C. urophthalmus* (those encysted in the intestinal wall not counted), 7,457 larvae were encysted in the gills and 4,262 in the fins; other encysting sites (body surface, heart, spleen) were quite exceptional. In other fish species, *O. manteri* larvae predominated in the fins, where from 53% of larvae in *L. griseus* to 100% in *S. timucu* were found.

DEFINITIVE HOST: Adult worms were found only in *C. urophthalmus* and they were recorded from all 7 sampling sites (Table 2). Worms were most common in the posterior third of the intestine.

Experimental infections

SECOND INTERMEDIATE HOST: All 11 *C. synspilum* subjected to free-swimming cercariae were found to harbor metacercariae, independent of the time of postexposure (1–20 days). A total of 353 *O. manteri* metacercariae were found in experimental fish. Encysting sites included the pectoral fins (56.7% of larvae found), caudal fin (18.4%), body surface (10.8%), gills (9.1%), pelvic fins (2.3%), dorsal fin (1.4%), anal fin (0.8%), and ventral fin (0.6%), respectively.

All 3 *C. synspilum,* force-fed with snails harboring *O. manteri* cercariae were found to be infected with a total of 171 *O. manteri* metacercariae, independent of the time postexposure. The sites of infection were the intestinal wall (62.0% of larvae), dorsal fin (16.4%), caudal fin (8.2%), body surface (5.3%), anal fin (2.9%), gills and pelvic fins (1.8%), ventral fin (1.2%), and pectoral fins (0.6%). External infections with metacercariae, i.e., those in gills, fins, and body surface, were apparently caused by penetration of free-swimming cercariae released from snail tissues vomited by fishes, because no migration of larvae from the intestine to these sites was observed.

Development of *O. manteri* metacercariae in experimentally infected *C. synspilum* at 22–24°C was as follows: 1 DPE: cercariae encysted, enclosed by a thin, hyaline membrane; pharynx faintly visible, ventral sucker partially formed; 2 DPE: ventral sucker still incomplete, anterior end still provided with spination typical of cercariae, including preoral spines; 3 DPE: preoral spines still present; pharynx clearly visible; 4 DPE: ventral sucker almost completely formed; preoral spines not observed; 5 DPE: metacercariae fully formed, with completely developed ventral sucker and digestive system; 6 and 7 DPE: metacercariae proved to be infective for the definitive host. In the following days (10–20 DPE), no changes were recorded in the morphology of metacercariae.

DEFINITIVE HOST: Since there were no differences between infections of fish challenged either with *O. manteri* metacercariae from the intestinal wall or those encysted in the gills of *C. urophthalmus,* results of experimental infections are summarized together (Table 3). Complete development of the trematode was only recorded in *C. synspilum* and it was as follows (Figs. 9–13; Table 4):

One and 2 DPE: worms show well-developed eyes and a large excretory bladder, filled with numerous dark granules; 3 and 4 DPE: remnants of eyes present in the form of diffused, dark granules; cephalic glands present; spherical, small anlagen of testes lying oblique to each other, near posterior extremity; 5 DPE: testes small but discernable; excretory bladder distinguishable; 7 DPE: remnants of eyes still present; excretory bladder difficult to distinguish; 10 DPE: testes large and seminal receptacle containing live spermatozooa; first gonotyls (2 in most specimens) present, situated anterior to ventral sucker; 13 DPE: eggs in uterus, but with not fully formed
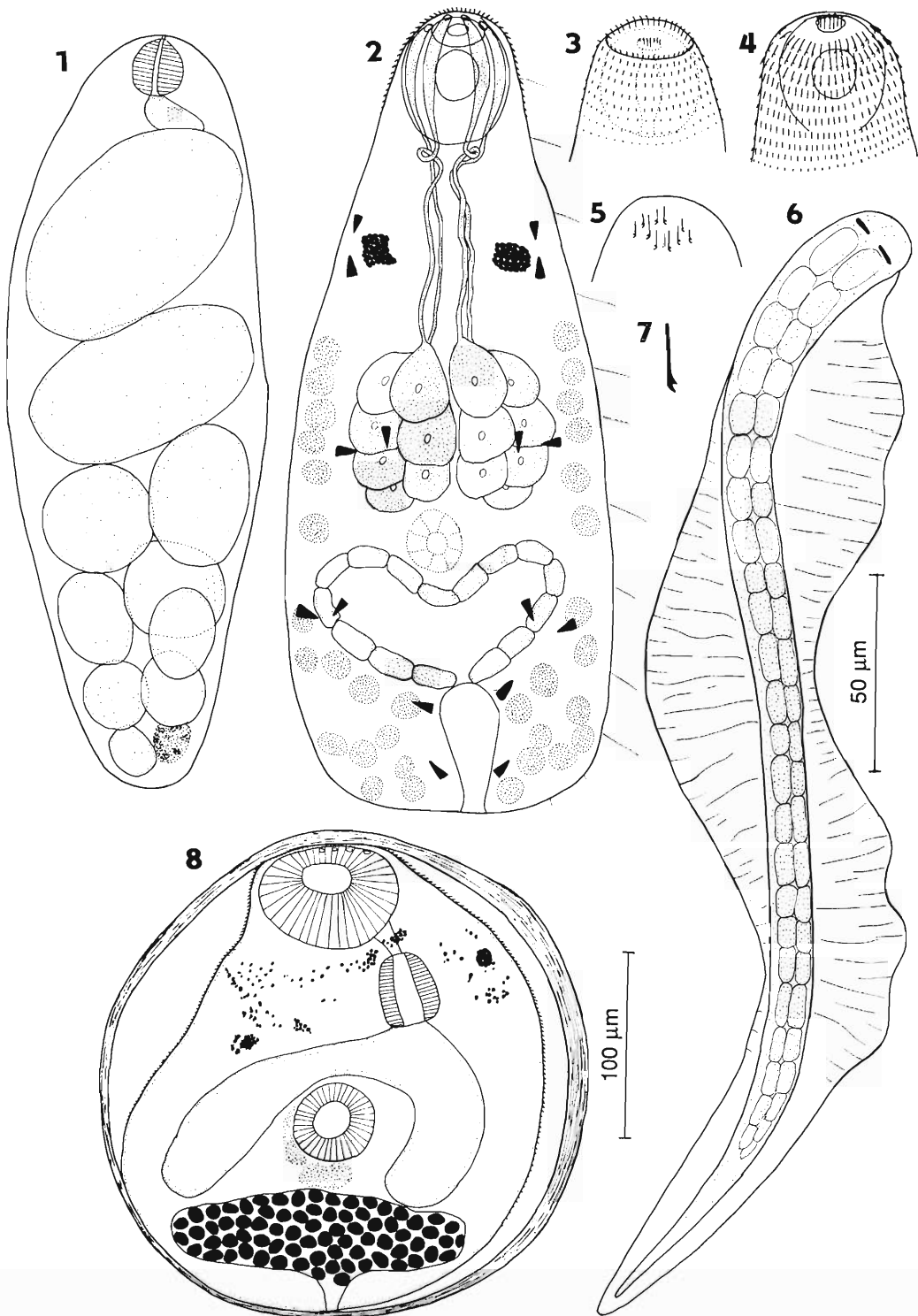
Figures 1-8. Larval stages of *Oligogonotylus manteri* from *Benthonella gaza* (1-7) and *Cichlasoma urophthalmus* (8). 1, Daughter redia with scale bar; 2-7, cercaria (2, body; 3, 4, anterior end with tegumental spines; 5, distribution of circumoral spines; 6, tail with scale bar; 7, circumoral spine, enlarged; length 5 μm); 8, metacercaria from the pectoral fins.

Table 2. Occurrence of adult *Oligogonotylus manteri* in *Cichlasoma urophthalmus.*

Locality	Date	Number of fish		Prevalence (%)	Total no. worms	Infection intensity	
		Examined	Infected			Mean	Range
Celestun	V.88	30	30	100	1,252	37.0	2–115
Celestun	IV–VIII.93	24*	19	79	329	17.3	1–53
Rio Lagartos	VI.88	27	16	59	186	11.6	1–69
Noh-Bek	VI.88	30	17	56	215	2.6	1–47
Guerrero	VI.88	4	3	75	42	14.0	2–31
Champoton	VII.88	30	22	73	74	3.3	1–7
El Vapor	V.88	30	2	7	11	5.5	5–6
Estero Pargo	V.88	12	3	25	13	4.3	1–10

* Of 30 fish sampled, only 24 were examined for the presence of intestinal helminths.

contents and thin-walled capsules; 16 DPE: ripe, fully developed eggs in uterus.

In the following days (19–28 DPE), measurements of worms (Table 4), as well as the proportion of gravid worms in the samples, gradually increased.

No mature or gravid worms were found in either *O. niloticus* or *P. reticulata*. Bodies of juvenile trematodes found in the intestinal lumen of *P. reticulata* 2 and 5 DPE were filled with numerous granules and vacuoles, which indicated that they were in the process of disintegration. All trematodes recorded in *C. synspilum* were located in the posterior (distal) third of the intestine.

Out of a total of 7 *C. synspilum* infected with

O. manteri metacercariae from experimentally infected *C. synspilum,* serving as second intermediate hosts, only the 2 examined 3 and 7 DPE, harbored 2 and 8 trematodes, respectively. Trematodes were not found in either experimental or control chicks and mice.

DESCRIPTIONS OF DEVELOPMENTAL STAGES:

Redia from naturally infected *B. gaza* (*N* = 20; Fig. 1): Daughter rediae elongate, sacciform, without locomotory appendages; body 125 ± 41 (66–197) long, 43 ± 14 (20–75) wide. Oral opening terminal, pharynx strongly muscular, oval, 11 ± 3 (8–16) long, 11 ± 3 (8–16) wide. Cecum very short, sacculate. Birth pore located just posterior to pharynx. Several developing cercariae (up to 10) in rediae, with larger and more de-
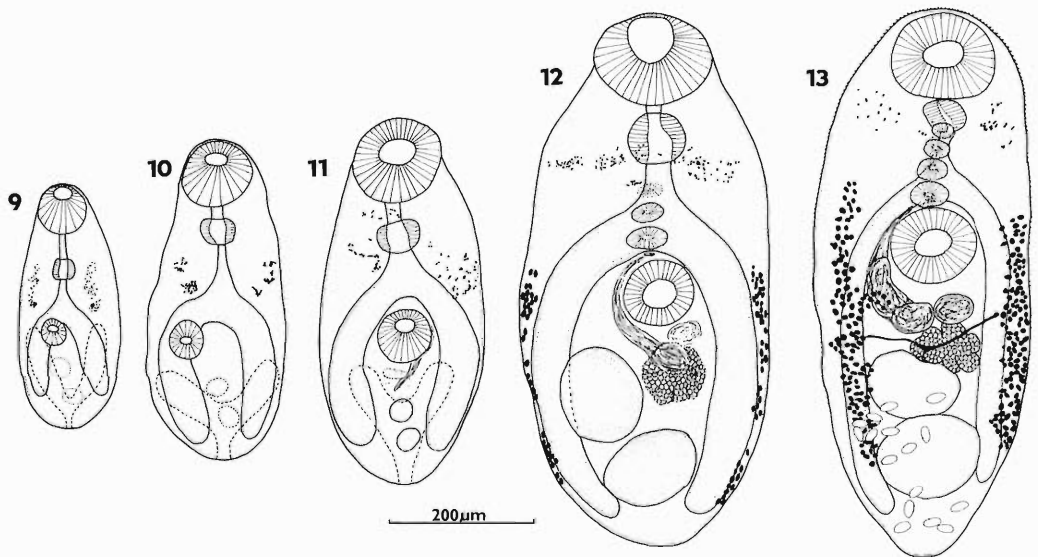
Figures 9-13. Development of *Oligogonotylus manteri* adults in experimentally infected *Cichlasoma synspilum* at 22-24°C (9, 1 DPE; 10, 3 DPE; 11, 7 DPE; 12, 10 DPE; 13, 19 DPE).

Table 3. Results of experimental infection of fish with *Oligogonotylus manteri* metacercariae from naturally infected *Cichlasoma urophthalmus.*

Fish	No. fish		Σ worms	Intensity mean (range)	Days post-exposure*	State of maturation
	Examined	Infected				
Cichlasoma synspilum	19	19	1,781	94 (4–620)	1, 3, 4, 7	Juvenile
					10, 13	Mature
					16, 19, 22, 25, 28	Gravid
Oreochromis niloticus	9	4	87	22 (17–41)	1, 2, 5	Juvenile
Poecilia reticulata	12	3	7	2 (1–4)	2, 4	Juvenile

* Only positive fish are mentioned; those examined 5, 10, 16 days postexposure (DPE) (*O. niloticus*), and 7 DPE (*P. reticulata*), respectively, were free of infection.

veloped cercariae in anterior part of redial body; measurements of largest cercariae up to 113 by 45. Mother rediae not observed.

Cercaria from naturally infected *B. gaza* (*N* = 16; Figs. 2–7): Oculate, pleurolophocercous cercaria. Body bell-shaped, 198 ± 28 (153–258) long by 108 ± 19 (68–130) wide; tail length 356 ± 56 (311–385), width 31 ± 3 (23–35); oral sucker 33 ± 6 (26–38) long by 31 ± 4 (20–35) wide.

Body spinous from anterior extremity to level of eye spots (Figs. 3, 4). Three or four anterior rows formed by hooklike spines, posterior spines (about 12–15 rows) simple and smaller. Hooklike preoral spines (Figs. 5, 7), usually 11 in number, located on dorsal lip of oral sucker; length of hooks 4–5. Body margins with long, hairlike sensory structures, distributed irregularly from anterior extremity to posterior end of body. Tail slightly curved dorsoventrally, provided with dorsoventral, hyaline fin-fold, beginning dorsally at first sixth and narrowing at last third of tail, beginning ventrally immediately behind proximal end of tail stem, with narrower part near middle of tail.

Oral sucker subterminal, pharynx poorly developed, almost indiscernible, esophagus and ceca not developed. Eye spots located anterior to penetration (cephalic) glands. Penetration glands, occupying central part of body, each formed by 7 pairs of cells; diameter of gland cells 15–17. Ducts of glands forming 2 bundles of 2 tubules each, coursing over and opening around anterior margin of oral sucker as four large orifices. Cystogenous glands in 2 groups; anterior group lying lateral to penetration glands, formed usually by 6 glands; posterior group filling posterolateral part of body, consisting of approximately 13 glands (Fig. 2). Ventral sucker weakly developed, spherical, located between penetration glands and excretory bladder. Excretory vesicle thick-walled,

Y-shaped. Flame cell formula 2 (2 + 2 + 2 + 2) = 16.

Metacercaria from naturally infected *C. urophthalmus* (*N* = 20; Fig. 8): Metacercariae found in experimentally infected *C. synspilum* were biometrically and morphologically identical to those found in naturally infected *C. urophthalmus.* The following description is based on larvae from the fins of *C. urophthalmus* from Celestun (the morphology, as well as measurements, of worms from the wall of the anterior intestine were identical to those of larvae from the gills, body surface, and fins).

Encysted metacercariae enclosed by thin, hyaline membrane of parasite origin and thick-layered cyst of variable thickness; size of cysts 178 ± 17 (149–207) by 164 ± 19 (132–201), thickness of outer wall 4 ± 1 (3–6). Body surface of metacercariae covered with numerous, simple, single-pointed tegumental spines, posteriorly smaller and less dense. Oral sucker large, subterminal, 48 ± 6 (35–59) long by 59 ± 7 (47–76) wide. Ventral sucker slightly postequatorial, its diameter 32 ± 4 (24–36) by 33 (33); sucker considerably smaller than oral sucker. Suckers' ratio (*N* = 7) 1.40:1 ± 0.34 (0.93–2.08) for sucker length and 1.64:1 ± 0.04 (1.60–1.69) for sucker width. Prepharynx short, 14 ± 4 (6–21) long; pharynx strongly muscular and large, 30 ± 4 (24–41) long by 33 ± 5 (27–50) wide. Esophagus relatively short, ceca wide, reaching far posterior to ventral sucker. Remnants of eye spots located lateral to oral sucker and pharynx. Two pairs of penetration glands, each consisting of 7 cells, located on lateral side of body between pharynx and ventral sucker. Gland openings clearly visible, as 4 orifices on anterior rim of oral sucker. Genital primordium poorly visible, located posterior to ventral sucker. Excretory bladder large, Y-shaped, with large lateral branches reaching

Table 4. Measurements of *Oligogonotylus manteri* in experimentally infected *Cichlasoma synspilum*.

	Days postexposure									
	1	3	7	10	13	16	19	22	25	28
Number of worms	4	14	20	20	20	15	18	13	15	20
Body length*	258 ± 62	333 ± 81	362 ± 61	752 ± 155	764 ± 102	825 ± 233	844 ± 247	874 ± 51	891 ± 208	1,130 ± 204
	170–329	217–477	277–490	417–947	602–999	397–1,169	721–1,220	767–993	529–1,128	516–1,367
Body width	138 ± 19	146 ± 27	162 ± 23	342 ± 106	383 ± 61	431 ± 113	405 ± 100	474 ± 49	329 ± 41	585 ± 130
	106–161	103–199	126–206	153–520	300–488	220–593	198–573	370–553	273–418	388–861
Oral sucker										
Length	59 ± 9	72 ± 14	84 ± 141	131 ± 25	133 ± 19	136 ± 38	148 ± 20	156 ± 12	118 ± 12	174 ± 24
	44–68	50–103	38–108	94–153	106–173	103–179	94–173	143–91	100–141	141–244
Width	61 ± 64	79 ± 18	93 ± 13	140 ± 23	158 ± 59	158 ± 28	160 ± 22	163 ± 10	152 ± 22	196 ± 20
	47–66	53–108	70–126	73–167	120–409	123–176	133–176	92–103	132–176	162–235
Prepharynx	16 ± 4	21 ± 10	34 ± 12	28 ± 11	28 ± 11	27 ± 13	79 ± 84	22 ± 7	86 ± 18	36 ± 18
	47–66	9–53	14–59	11–62	11–53	9–49	68–138	12–41	9–58	47–74
Pharynx										
Length	45 ± 1	40 ± 6	42 ± 7	64 ± 10	68 ± 12	68 ± 11	46 ± 61	72 ± 5	64 ± 9	82 ± 19
	44–47	33–53	29–59	44–79	49–103	50–88	59–85	62–79	47–74	59–132
Width	42 ± 19	43 ± 11	48 ± 8	78 ± 11	84 ± 13	88 ± 26	79 ± 22	107 ± 7	80 ± 19	118 ± 19
	29–56	23–64	26–51	59–94	62–103	14–118	59–103	91–118	36–121	82–147
Acetabulum										
Length	34 ± 14	45 ± 9	54 ± 7	99 ± 21	107 ± 23	116 ± 31	112 ± 13	176 ± 30	104 ± 16	171 ± 20
	26–41	29–59	38–70	59–153	59–161	62–153	103–129	134–193	76–126	135–176
Width	30 ± 4	44 ± 9	52 ± 6	100 ± 19	104 ± 12	120 ± 35	99 ± 28	127 ± 16	147 ± 25	157 ± 20
	26–35	32–59	35–61	59–123	82–123	68–158	94–138	94–147	132–182	132–185
Position†	174 ± 77	191 ± 46	233 ± 92	375 ± 86	379 ± 64	361 ± 143	373 ± 146	423 ± 53	465 ± 156	522 ± 114
	153–196	123–279	161–300	106–493	291–488	64–599	59–92	332–526	252–861	356–873
Sucker's ratio										
Length	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	1.3 ± 0.1	1.2 ± 0.3	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
	1.6–2.0	1.4–2.0	1.3–1.8	0.9–1.7	1.1–1.6	0.7–1.8	1.1–1.7	1.1–1.7	1.0–1.4	1.0–1.6
Width	2.0 ± 0.5	1.7 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.1
	1.3–2.5	1.4–1.9	1.3–1.7	1.2–1.8	1.2–1.6	1.0–1.7	1.2–1.6	1.1–1.5	1.0–1.4	1.1–1.5
Anterior testis										
Length	n.m.‡	n.m.	n.m.	n.m.	86 ± 25	69 ± 18	63 ± 32	112 ± 19	129 ± 12	122 ± 32
					50–133	44–81	33–118	79–141	118–15	65–168
Width	n.m.	n.m.	n.m.	n.m.	111 ± 28	61 ± 25	94 ± 83	123 ± 19	140 ± 14	143 ± 34
					53–153	44–91	53–156	85–144	123–171	65–190

Table 4. Continued.

	Days postexposure									
	1	3	7	10	13	16	19	22	25	28
Posterior testis										
Length	n.m.	n.m.	n.m.	n.m.	103 ± 21 59–144	58 ± 11 26–74	70 ± 35 50–129	98 ± 30 49–162	132 ± 11 118–147	127 ± 32 74–185
Width	n.m.	n.m.	n.m.	n.m.	113 ± 43 41–173	64 ± 10 47–74	93 ± 36 47–144	145 ± 29 129–162	137 ± 10 118–153	138 ± 30 82–179

* Mean ± SD and range.
† Distance of the middle of the acetabulum from anterior extremity.
‡ Not measured.

anteriorly to level of acetabulum, and with short posterior stem (Fig. 8).

Adult (Figs. 9–13): The morphology of adult worms recovered from naturally infected *C. urophthalmus* as well as their measurements (Table 4; Fig. 13) were identical to those of worms described by Watson (1976) and Osorio-Sarabia et al. (1987).

Discussion

The present study demonstrated that the developmental cycle of *O. manteri,* involving the aquatic snail *B. gaza* as the first intermediate, and cichlid fish, both as second intermediate or as definitive hosts, is similar to those of other trematodes of the family Cryptogonimidae, which have been studied (Yamaguti, 1971, 1975; Greer and Corkum, 1979, 1980; Font, 1987). A high level of infection in *B. gaza* snails from Celestun (prevalence 23.1%), together with the fact that they are abundant in this lagoon and that *C. urophthalmus* eats these snails in large quantities (Salgado-Maldonado, unpubl. obs.), may explain extremely high worm burdens in fish from this locality.

Morphology of larval stages from naturally infected snails was similar to that of rediae and cercariae of other cryptogonimid trematodes (Yamaguti, 1971, 1975; Greer and Corkum, 1979). Daughter rediae of *O. manteri* were typified by the absence of any locomotory appendages, presence of cercariae in different degrees of development and a very short, sacculate intestine. *Oligogonotylus manteri* cercariae were characterized by the presence of a tail with a hyaline fin-fold, 7 pairs of large penetration glands filling the middle region of the body, small cystogenous glands situated posterolaterally, and a Y-shaped, thick-walled excretory bladder, typical of the Cryptogonomidae (Yamaguti, 1971, 1975).

Experiments with *O. manteri* cercariae showed their high infectivity for *C. synspilum,* because all the fish were found to be infected with metacercariae. Free-swimming cercariae resulted in a high proportion (57%) of metacercariae encysted in pectoral fins, which tallies with data from natural conditions. On the other hand, cysts in gills of experimental hosts were remarkably few (9%); this contrasts to the findings in *C. urophthalmus* in nature.

Successful experimental infections of fish with *O. manteri* cercariae clearly demonstrated 2 modes of infection of the second intermediate

host and explained 2, quite different types of en-cysting sites in their fish host—internal, i.e., in the wall of the intestine, and external, notably in the gills, fins, and on the body surface. In the former case, the fish acquired the infection by ingesting snails containing cercariae of the trem-atode, and in the latter case, they became infected after free-swimming cercariae penetrated their body surface, gills, and fins.

Development of *O. manteri* metacercariae (metamorphosis of cercariae) in experimental hosts was relatively quick, and as early as 5 DPE metacercariae were fully formed; experiments confirmed that 6-day-old worms were infective for the definitive host. Greer and Corkum (1979), however, reported at least 14 days as the mini-mum time for cercariae of other cryptogonimids to develop into infective metacercariae.

With the exception of *C. urophthalmus,* which is freshwater, with rather high salinity tolerance, all other fish species studied are euryhaline. The infection of 4 of these fishes, *L. griseus, B. ron-chus, S. timucu,* and *S. testudineus,* showed that cercariae of *O. manteri* were able to penetrate through the surface of these fishes when they enter into this lagoon. However, almost all these larvae were dead, which indicated that these spe-cies did not represent suitable second interme-diate hosts. Only *B. ronchus* harbored a larger proportion of live larvae. However, experimen-tal infection of the cichlid *C. synspilum* with metacercariae encysted in the fins of *B. ronchus* was not successful (unpubl. data).

Experimental infections of laboratory-reared *C. synspilum* with metacercariae from naturally infected *C. urophthalmus* confirmed the as-sumption that these larvae, hitherto misidenti-fied as those of the echinostomatid *E. zubeda-khaname,* belong to the species *O. manteri* (compare Figs. 1 and 2 in Lamothe-Argumedo and Aguirre-Macedo [1991a] with Fig. 8 in the present study, as well as the descriptions of these larvae in the 2 papers). The identification of these metacercariae by Lamothe-Argumedo and Aguirre-Macedo (1991a) was based on experi-mental infections of chicks and laboratory mice fed the intestines and gills of *C. urophthalmus* containing metacercariae. These yielded mature echinostome trematodes, morphologically iden-tical to those described by Nasir and Díaz (1968) as *E. zubedakhaname* (Lamothe-Argumedo and Aguirre-Macedo, 1991a, b). However, the mor-phology of these larvae, as described by La-mothe-Argumedo and Aguirre-Macedo (1991a),

did not resemble that typical of an echinosto-matid: a collar was absent, collar spines, well visible in echinostome metacercariae, were also lacking, the oral sucker was much larger than the relatively rather small acetabulum, being of the same size as the pharynx, and the excretory blad-der was Y-shaped. Spines described by the above authors were most probably openings of cephalic glands, which are well developed both in the cer-caria and metacercaria of *O. manteri* (Fig. 8 in the present paper). Lamothe-Argumedo and Aguirre-Macedo (1991a) evidently used at least 2 species of metacercariae for their experimental infections. Examination of *C. urophthalmus* from different localities in the Yucatan Peninsula (un-publ. data) revealed the presence of *Echino-chasmus* metacercariae. These occurred exclu-sively in gills and can easily be differentiated from those of *O. manteri* by their internal mor-phology, size, and shape of cyst as well as their location in gill filaments. Our suspicion that La-mothe-Argumedo and Aguirre-Macedo (1991a) carried out mixed experimental infections was confirmed by our recovering *Echinochasmus* adults in mice and chicks experimentally infected with gills containing echinostome metacercariae from a swamp in Mitza.

The trematode *O. manteri* completed its de-velopment in experimentally infected *C. syn-spilum* within 16 DPE at 22–24°C, when the first embryonated eggs were recorded. In contrast to the relatively rapid developmental times for metacercariae of this species, maturation took about twice as long as in related trematodes (Greer and Corkum, 1979; Font, 1987).

The occurrence of *O. manteri* adults exclu-sively in *C. urophthalmus* showed limited host specificity at the level of the definitive host. Re-sults of experimental infections of tilapias (*O. niloticus*) and guppies (*P. reticulata*) with *O. manteri* metacercariae, in which no adult worms were found, further indicate a narrow host spec-ificity of this cryptogonimid. It seems that *O. manteri* is a specific parasite of cichlid fishes from the genus *Cichlasoma* and the related species *Pe-tenia splendida* in southern Mexico and Central America (Watson, 1976; Osorio-Sarabia et al., 1987).

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Fourth Edition of the International Code of Zoological Nomenclature

The International Commission on Zoological Nomenclature proposes to publish a new edition of the code, taking into account the large number of possible amendments that have been received. It is planned that the Fourth Edition will be published during 1995 and that on 1 January 1996 its provisions will supersede those in the current (1985) edition.

The Commission's Editorial Committee met in Hamburg from 12–16 October 1993 to prepare a discussion draft for the new edition of the Code. Copies of this draft will be sent without charge to all subscribers to the *Bulletin of Zoological Nomenclature* and to members of the American and European Associations for Zoological Nomenclature. Any other institution or individual may order a copy from the Executive Secretary, I.C.Z.N., % The Natural History Museum, Cromwell Road, London, SW7 5BD, England. Bank charges on currency exchanges make it uneconomic to charge the cost of printing and postage (£3 or US$5) except for payment in sterling or US dollars. The draft will therefore be sent free of charge, but those able to pay in sterling or US dollars are asked to enclose a check for £3 or US$5 to cover the cost.

Before completing the definitive text of the Fourth Edition, the Commission will (in accordance with Article 16 of its Constitution) carefully consider all comments and suggestions on the draft. Zoologists and others are asked to send these to the Executive Secretary of the Commission at the above address as soon as convenient, and in any event not later than February 1995.

A Redescription of *Telorchis auridistomi* (Digenea: Telorchiidae) with Comments on the Oral Sucker Papillae

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ABSTRACT: *Telorchis auridistomi* (Byrd, 1937) Wharton, 1940 is redescribed from type material, voucher specimens collected by Brooks (1979) from *Farancia abacura* (subspecies unspecified) in Florida, and newly collected voucher specimens from the small intestine of the western mudsnake, *F. a. reinwardti* in Louisiana. The redescription includes extension of the ranges of the measurements of all of the morphometric features of the species. A detailed illustration is presented to show the arrangement of the tegumental spines. The oral sucker of *T. auridistomi* was investigated with histological sections and scanning electron microscopy to resolve the discrepancies among descriptions of the number of oral sucker papillae in this species. Both techniques revealed the presence of only a single ventrolateral pair of papillae on the oral sucker of *T. auridistomi*. Examination of the holotype and 3 paratypes of *Telorchis dollfusi* (Stunkard and Franz, 1977) Wharton, 1940 confirmed the presence of only a single pair of papillae in this species as well. The key to species of *Telorchis* given by MacDonald and Brooks (1989) is emended to reflect this discovery.
KEY WORDS: *Farancia abacura*, *Telorchis auridistomi*, Telorchiidae.

Byrd (1937) originally described *Cercorchis auridistomi* from 1 complete and 2 partial specimens collected from *Farancia abacura* (Holbrook) from a canal in Harvey, Louisiana. Although the taxonomy of this species has been discussed subsequently in the literature (see Wharton, 1940; Stunkard and Franz, 1977; Stunkard, 1979) to our knowledge the only data added to this description were those of MacDonald and Brooks (1989) from 5 voucher specimens of the species. The discovery of numerous specimens of a digenean in the small intestine of the western mud snake, *Farancia abacura reinwardti* (Schlegel), provided us with the opportunity to redescribe this still poorly known telorchiid species. Particular attention was paid to the papillae associated with the oral sucker as there exists disagreement between Byrd's original description of this feature in this species (Byrd, 1937) and most of the subsequent references to the species (e.g., Stunkard and Franz, 1977; Stunkard, 1979; MacDonald and Brooks, 1989). In addition, the deposition of the type specimens of *C. auridistomi*, previously lost among Byrd's personal possessions and therefore unavailable to workers subsequent to Byrd, allowed us to confirm all details of the redescription with the original material.

Materials and Methods

Twenty-five specimens of the western mud snake, *Farancia abacura reinwardti*, were collected from St. John the Baptist Parish, Louisiana, in June of 1987. Digeneans were recovered from the small intestine of 22 of the snakes, fixed in alcohol/formalin/acetic acid (AFA) for 12 hr, and stored in 70% ethanol. Twenty specimens were stained with Gill's hematoxylin, cleared in xylene, and mounted in Canada balsam as whole mounts according to conventional techniques. Five specimens were embedded in paraplast (Sherwood Medical Industries, St. Louis, Missouri), sectioned at 10 μm, stained in hematoxylin and eosin, and mounted in Canada balsam according to conventional techniques. Three specimens were dried for scanning electron microscopy as follows: specimens were hydrated, transferred to 1% osmium tetroxide overnight, dehydrated in an ethanol series, transferred to a 1:1 mixture of Peldri II (Ted Pella, Inc., Redding, CA) and absolute alcohol at 25°C on a slide warmer for 1 hr, transferred to 100% Peldri II at 25°C on a slide warmer for 1 hr, and transferred to a well slide filled with 100% Peldri II on a petri dish that had been cooled in the freezer and left overnight in a fume hood to sublimate. Specimens were mounted on stubs with silver paint, sputter coated with 100 Å of gold, and examined with a Coates and Welter Field Emission scanning electron microscope. The line drawing was done with the aid of a drawing tube. Sections were photographed with an Olympus PM-10AD camera system. Measurements are given in micrometers in the text as range followed in parentheses by the mean, the standard deviation, the number of worms examined (n), and the number of measurements taken (*n*) when more than 1 measurement was made per worm. In addition to the material of *Telorchis auridistomi* listed below, for comparative purposes, 1 slide containing both the holotype (AMNH 872) and 3 paratypes (AMNH 873) of *Paratelorchis dollfusi* Stunkard & Franz, 1977 (=*Telorchis dollfusi* (Stunkard & Franz, 1977) MacDonald and Brooks, 1989) was borrowed from the American Museum of Natural History.

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Telorchis auridistomi (Byrd, 1937)
Wharton, 1940
(Figs. 1-5)

SYNONYMS: *Cercorchis auridistomi* Byrd, 1937; *Paratelorchis auridistomi* (Byrd, 1937) Stunkard & Franz, 1977; *Auritelorchis auridistomi* (Byrd, 1937) Stunkard, 1979.

MATERIAL EXAMINED: Holotype of *C. auridistomi* (U.S. National Museum Helminthology Collection, Beltsville, Maryland, USNM 80696); 1 paratype of *C. auridistomi* (The Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, HWML 31103); 10 voucher specimens of *P. auridistomi* (HWML 20896); 20 voucher specimens *T. auridistomi* (HWML 37545); sections of *T. auridistomi* (HWML 37545).

HOSTS AND LOCALITIES: *Farancia abacura,* Harvey, Louisiana (type host and locality); *F. abacura,* Payne's Prairie, Alachua County, Florida; *F. abacura,* Willowood Pond, Jefferson Parish, Louisiana; *F. a. reinwardti,* St. John the Baptist Parish, Louisiana (new locality).

SITE OF INFECTION: Small intestine.

REDESCRIPTION (based on holotype, 1 paratype, and 26 voucher specimens): Body elongate, tapering slightly posteriorly, 1,199–3,188 (2,114 ± 497; n = 27) long by 123–520 (328 ± 79; n = 28) wide, greatest width at level of acetabulum. Spines present on anterior half of body excluding ventral surface of oral sucker (Figs. 1, 2), densely arranged on tegument from anterior extremity of body to anterior margin of acetabulum (Figs. 3, 4), more sparsely arranged from anterior margin of acetabulum to anterior margin of ovary. Oral sucker subterminal, with oral aperture and 1 pair of lateral muscular papillae; oral sucker measurements excluding papillae: 153–268 (199 ± 33; n = 27) long by 170–280 (216 ± 28; n = 27) wide. Papillae rounded distally, 28–73 (49 ± 11; n = 27; *n* = 54) long by 43–110 (75 ± 15; n = 27; *n* = 54) wide. Acetabulum 103–188 (144 ± 25; n = 27) long by 113–199 (151 ± 24; n = 27) wide. Oral sucker to acetabulum width ratio 1.1:1–1.8:1 (1.5:1 ± 0.18; n = 26). Forebody 430–990 (588 ± 143; n = 26) long; hindbody 769–2,198 (1,518 ± 407; n = 26) long; forebody to hindbody length ratio 0.28:1–0.64:1 (0.4:1 ± 0.09; n = 26).

Short prepharynx present. Pharynx 50–100 (74 ± 12; n = 27) long by 55–110 (84 ± 17; n = 27) wide; often surrounded by numerous, large gland
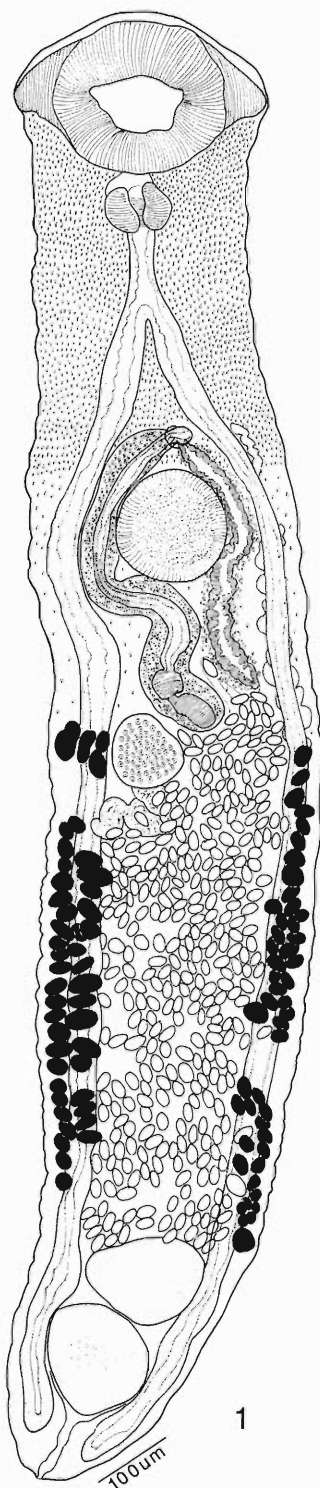
Figure 1. *Telorchis auridistomi* from *Farancia abacura reinwardti* in Louisiana, ventral view.

cells. Esophagus 38–188 (85 ± 35; n = 24) long, often surrounded by few gland cells; cecal bifurcation midway between oral sucker and acetabulum; ceca extending almost to posterior end of body. Testes contiguous, tandem, round to somewhat irregular in shape, near posterior end of body. Anterior testis 68–255 (131 ± 39; n = 28) long by 88–260 (132 ± 35; n = 28) wide; posterior testes 83–268 (152 ± 35; n = 28) long by 75–235 (126 ± 33; n = 28) wide. Genital pore ventral, median, at anterior margin of acetabulum. Cirrus sac elongate, somewhat convoluted, extending posteriorly to level of ovary, 288–813 (560 ± 147; n = 27) long by 38–150 (73 ± 25; n = 27) wide; containing bipartite seminal vesicle and well-developed cirrus, each surrounded by numerous cells. Ovary round to transversely oval, usually dextral, immediately posterior to or overlapping posterior margin of cirrus sac, 40–160 (87 ± 24; n = 28) long by 50–235 (102 ± 39; n = 28) wide; seminal receptacle elongate, posterior to ovary; Mehlis' gland present; Laurer's canal not seen. Metraterm sinistral, approximately two-thirds as long as cirrus sac, surrounded by many, large gland cells. Vitelline follicles generally lateral, extending from anterior margin of ovary posterior to anterior margin of anterior testis. Eggs 50–1,190 (292 ± 249; n = 20) in number, 25–35 (30 ± 5; n = 28; n = 132) long by 15–20 (17 ± 2; n = 28; n = 132) wide, operculate. Uterus with coiled descending and ascending loops. Excretory pore terminal; excretory bladder median, long, giving rise to cornua at about level of ovary; cornua ending posterior to acetabulum.

Discussion

Observation of a large number of newly collected voucher specimens of *T. auridistomi* as well as the type material allowed us to extend the ranges of the measurements for all of the morphometric features of this species as described by Byrd (1937) and MacDonald and Brooks (1989). In addition, the tegumental spines require some comment. Byrd (1937, p. 359) described *C. auridistomi* to possess "cuticula beset with fine spines anteriorly to about the level of the ovary." The tegumental spines of *T. auridistomi* were not described by MacDonald and Brooks (1989), but minute lateral spines were shown extending to a point midway between the ovary and the testes of the specimens they figured. Our Figure 1 illustrates the typical tegu-

mental spine condition in *T. auridistomi*. The spines are conspicuously more dense in the region between the oral sucker and the anterior margin of the acetabulum. We did not find tegumental spines posterior to the ovary in any of the specimens we examined.

There is current disagreement in the literature regarding the number of papillae associated with the oral sucker of *T. auridistomi*. In the original description of this species Byrd (1937) described and figured 2 anterolateral liplike appendages on the oral sucker, but this is inconsistent with the diagnosis of *Auritelorchis,* a genus erected by Stunkard (1979) to contain this and 2 other papillose telorchid species, in which the oral sucker is described as having 2 pairs of papillae (1 dorsal and 1 ventral). It is also inconsistent with the most recent treatment of this species (see MacDonald and Brooks, 1989) in which the oral sucker is described as having both a pair of ventrolateral and a pair of dorsolateral lappets. This species has undergone several generic transfers throughout its taxonomic history and the discrepancy seems to have arisen as a result of 1 of these transfers. Wharton (1940) transferred this species to the genus *Telorchis* and mentioned that the oral sucker had lappets but did not discuss the number. Stunkard and Franz (1977) later proposed that the homogeneity of the unwieldy family Telorchiidae Stunkard, 1924 could be improved by removing the species with ear-like expansions of the oral sucker from the genus *Telorchis*. They therefore erected the genus *Paratelorchis* for 2 species previously assigned to *Telorchis* (*Telorchis auridistomi* and *Telorchis bifurcus* (Braun, 1900) Braun, 1901) and their new species, *P. dollfusi* Stunkard and Franz, 1977, all of which they said (Stunkard and Franz, 1977, p. 383) possessed "dorsal and ventral ear-like lobes on the anterolateral faces of the sucker." Thus it would appear that Stunkard and Franz believed that these species possessed a total of 4 papillae on the oral sucker (1 pair on the dorsal anterolateral faces of the sucker and 1 pair on the ventral anterolateral faces of the sucker). There is no indication that Stunkard and Franz (1977) examined specimens of either *P. bifurcus* or *P. auridistomi* to confirm the presence of 4, rather than 2, oral sucker papillae in these species when they proposed the genus *Paratelorchis*. It now seems that they erred about the configuration of the papillae in these species when they included them under their proposed generic description for *Paratelorchis*. This error was sub-
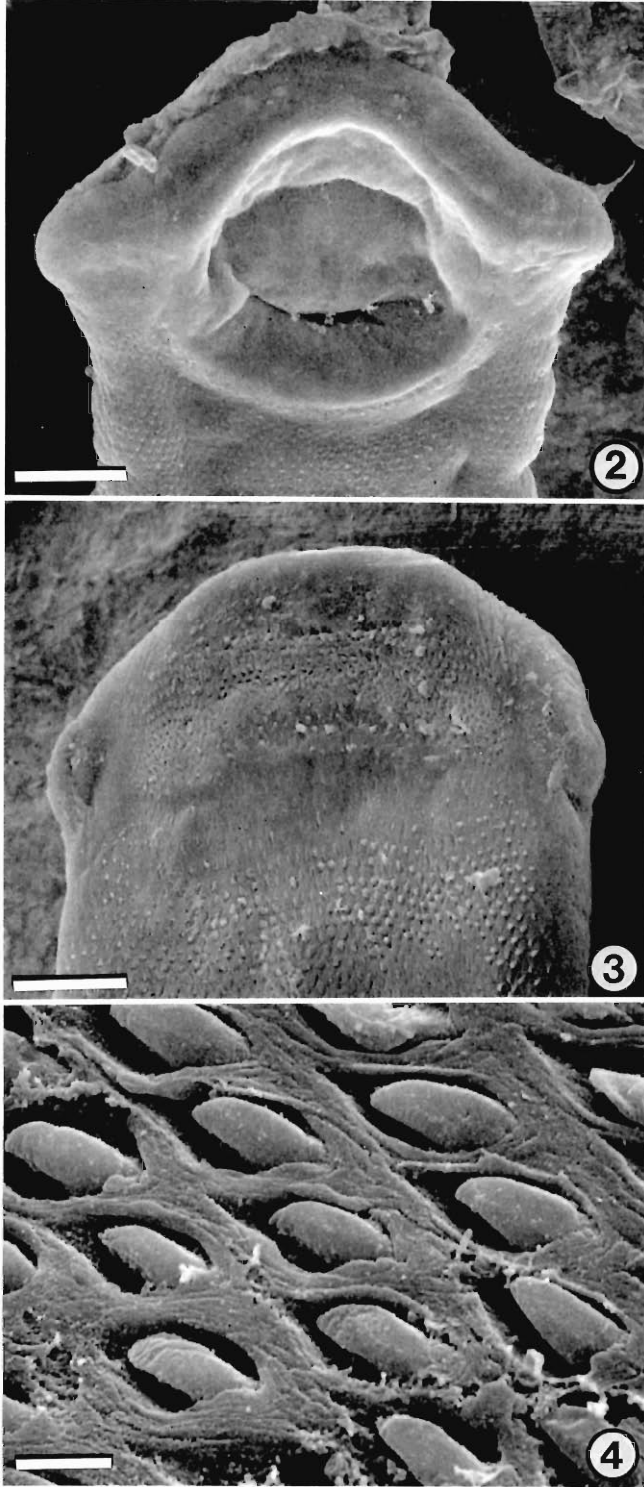
Figures 2-4.   Scanning electron micrographs of *Telorchis auridistomi*. 2. Ventral view of oral sucker. Note presence of lateral papillae. Scale bar = 50 μm. 3. Dorsal view of anterior extremity. Note absence of dorsal papillae. Scale bar = 50 μm. 4. Enlarged view of tegumental spines. Scale bar = 4 μm.
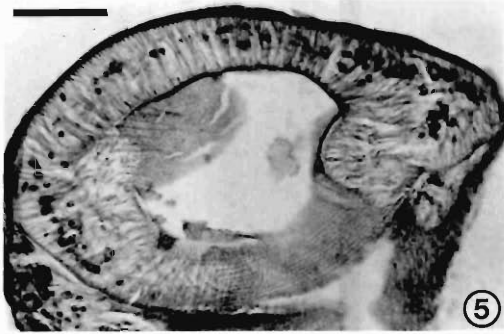
Figure 5. Frontal section through oral sucker of *Telorchis auridistomi* **showing musculature of 1 papilla. Scale bar = 50 μm.**

sequently repeated by Stunkard (1979) and Mac-Donald and Brooks (1989).

To resolve the issue of number of oral sucker papillae in *Telorchis auridistomi,* we investigated the oral sucker papillae of this species in detail. Examination of the holotype and the paratype revealed only a single pair of papillae, 1 papilla on each of the ventrolateral surfaces of the oral sucker. Examination of 3 voucher specimens with scanning electron microscopy confirmed the presence of a single pair of papillae on the ventrolateral margins of the oral sucker (Fig. 2), and verified the total absence of papillae on the dorsal surfaces of the sucker (Fig. 3). Sections through the oral sucker showed that these papillae are extensions of the musculature of the oral sucker proper (Fig. 5) similar to those of the papillose allocreadiids (see Caira, 1989). We have therefore chosen to use the term papillae rather than lappet. Three of the 28 specimens we examined (2 of which were from material examined by MacDonald and Brooks [1989]) had enlargements of the oral sucker musculature that might be mistaken for a second pair of papillae; however, we believe these are artifacts associated with contraction of the oral sucker.

Our work requires a change in the key to the species of North American Telorchiidae presented by MacDonald and Brooks (1989). In their key, the feature used to distinguish *T. auridistomi* from *T. dollfusi* is the number of pairs of oral sucker papillae (1 versus 2). As it now seems clear that both species possess only a single pair of papillae on the oral sucker, some other feature must be used to distinguish between the two species. We suggest that the following couplet re-

place couplet 6 in the key of MacDonald and Brooks (1989, p. 2314):

"6a. Vitellaria extending posteriorly to level of testes; acetabulum distinctly smaller than oral sucker . . . . *Telorchis auridistomi*

b.   Vitellaria extending posteriorly ½ to ¾ distance from ovary to testes; acetabulum equal to or only slightly smaller than oral sucker . . . . *Telorchis dollfusi.*"

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Drepanidotaenia teshekpukensis sp. n. (Cestoda: Hymenolepididae) from Black Brant, *Branta bernicla nigricans,* from the Teshekpuk Lake area of Alaska

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ABSTRACT: Specimens of *Drepanidotaenia teshekpukensis* sp. n. (Eucestoda: Hymenolepididae) were recovered from 68 black brant, *Branta bernicla nigricans* at Teshekpuk Lake, Alaska. Of the species of *Drepanidotaenia* with 10 rostellar hooks *D. teshekpukensis* sp. n. is most similar to *D. barrowensis* and *D. bilateralis,* which also occur in black brant. It differs from these 2 species in shape and size of rostellar hooks, position of the reproductive systems relative to the lateral excretory canals, and shape and size of the cirrus spines. The new species can be distinguished from all other species of the genus in having the centers of the ovary and vitellarium immediately poral of the center of the most antiporal testis.

KEY WORDS: *Drepanidotaenia teshekpukensis* sp. n., Cestoda, Hymenolepididae, *Branta bernicla,* Alaska.

Black brant, *Branta bernicla nigricans* Linnaeus, 1758 are commonly found along the northern Pacific coastlines of Asia and North America. Eleven genera of tapeworms, including 2 species of the genus *Drepanidotaenia* Railliet, 1892, *D. barrowensis* (Schiller, 1952) Yamaguti, 1959, and *D. lanceolata* (Block, 1782) Railliet, 1892 have been reported previously from this subspecies of brant in North America (Neraasen and Holmes, 1975). A third species, *D. bilateralis* (Linstow, 1905) Railliet, 1892 has been found in *B. bernicla* in Russia (Linstow, 1905; Spasskaya, 1966). The purpose of this study was to provide additional information on the cestode fauna of black brant.

Materials and Methods

Cestodes removed from intestines of 68 black brant collected by netting and shooting from the Teshekpuk Lake area of Alaska from June through August from 1987 to 1989 were fixed without coverslip pressure in 10% formalin, stained in Semichon's carmine and mounted in Kleermount or Canada balsam. Some specimens were sectioned by conventional paraffin technique. Measurements were from whole mounts of adults and are given in μm, with the mean followed by the range in parentheses, unless otherwise stated. Type materials of *D. barrowensis* (no. 37341, USNM Helminthological Collection) were examined. No specimens of *D. bilateralis* were available for comparison.

Results

All black brant examined were infected with an undescribed species of hymenolepidid cestode of the genus *Drepanidotaenia.*

Drepanidotaenia teshekpukensis sp. n.
(Figs. 1-6)

DESCRIPTION (based on 20 specimens): With the characteristics of the genus. Strobila craspedote, total length of worms 16 mm (6–19), composed of 70–200 proglottids. Scolex 180 (140–220) long by 140 (105–200) wide. Suckers well developed, 70 (45–95) in diameter. Rostellum 65 (45–85) long with 10 hooks, arranged in a circle, each 33 (29–35) long with blade 7 (4–8) long, rostellar sac 109 (96–122) long by 49 (46–52) wide. Immature proglottids wider than long, mature proglottids 150 (100–280) long by 900 (400–1,500) wide. Genital atria unilateral opening in the anterior fourth of proglottids. Three testes 86 (62–114) long by 88 (70–110) wide arranged in a straight line. Cirrus sac 360 (285–500) long by 82 (45–115) wide. Cirrus 230 (145–300) long, armed with symmetrical, similarly shaped spines, largest situated near the base and middle of cirrus, 23 (18–27) long, and smallest at the tip, 5 (4–8) long. External seminal vesicle 150 (72–235) long by 88 (27–140) wide. Ovary antiporal, deeply lobed 90 (57–140) long by 130 (70–215) wide with its center situated ventrally, immediately poral of the center of the most antiporal testis. Vitellarium lobed, situated directly under the ovary, 47 (27–70) wide. Genital ducts passing between ventral and dorsal excretory ducts, excretory ducts outside reproductive organs, ventral canals 33 (30–41) wide, dorsal excretory canals 11 (10–13) wide. Vagina posterior and ventral to cirrus sac. Seminal receptacle 67 (56–78) wide. Gravid proglottids wider than long,
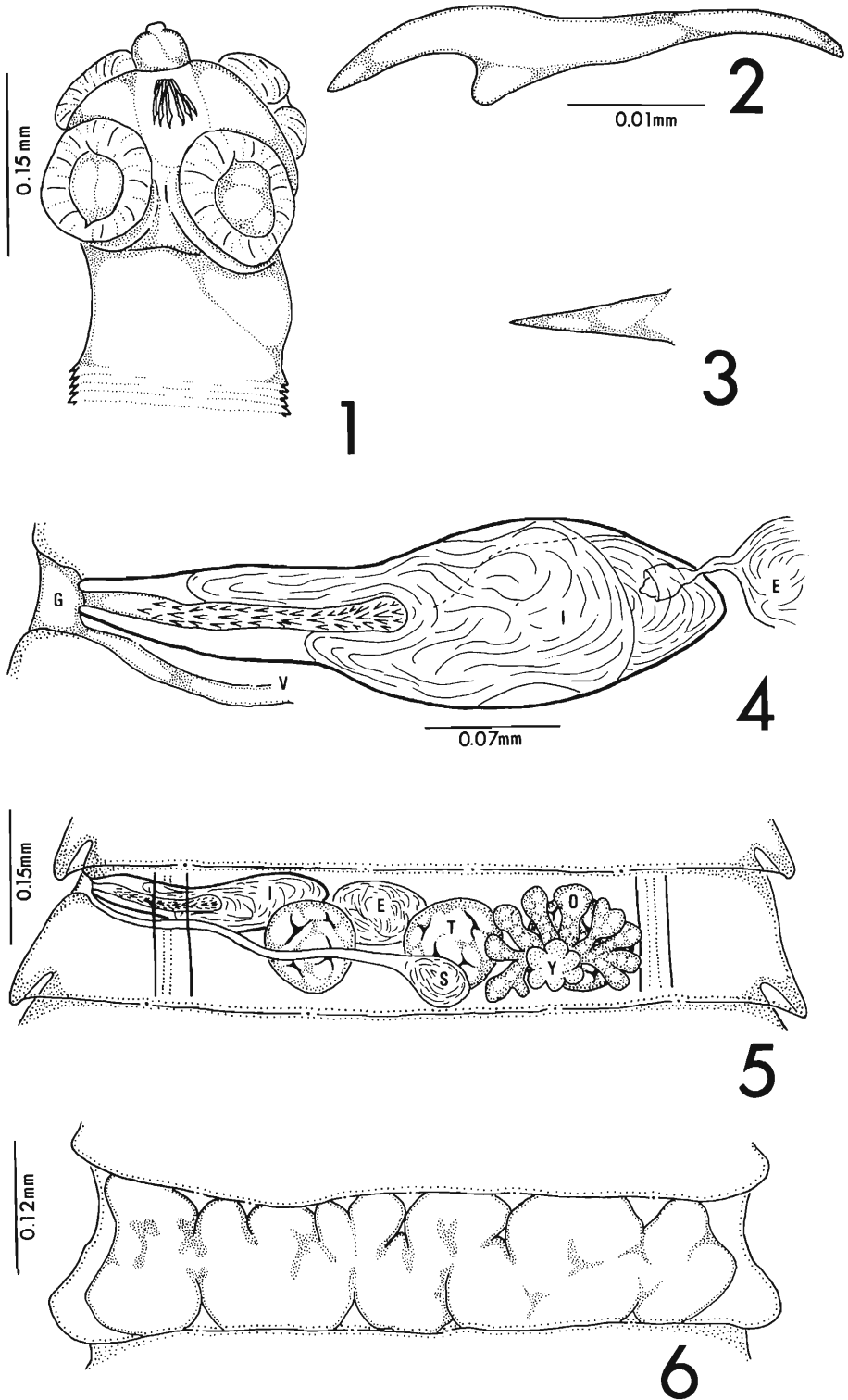
Figures 1-6. *Drepanidotaenia teshekpukensis* sp. n. from *Branta bernicla nigricans.* 1. Scolex. 2. Hook from rostellum. 3. Cirrus spine. 4. Enlarged view of genital atrium and seminal vesicle region showing the genital atrium (G), vagina (V), internal seminal vesicle (I), and external seminal vesicle (E). 5. Mature proglottid showing the internal seminal vesicle (I), external seminal vesicle (E), testis (T), seminal receptacle (S), vitellarium (Y), and ovary (O). 6. Gravid proglottid showing uterus.

150 (90–300) long by 1,300 (730–1,900) wide. Gravid uterus a transverse sac. Eggs ovoid, 28 (27–30) long by 34 (35–38) wide, oncospheres 19 (17–25) long by 23 (22–24) wide.

HOST: *Branta bernicla nigricans.*

SITE OF INFECTION: Small intestine.

LOCALITY: Teshekpuk Lake, Alaska (70°49′N, 153°15′W).

HOLOTYPE: USNM Helm. Coll. No. 82531.

PARATYPES: USNM Helm. Coll. No. 82533; Texas A&M Cooperative Wildlife Coll. No. 84-89AL, Department of Wildlife and Fisheries Sciences, Texas A&M University and The University of Nebraska State Museum No. 35446, The Harold W. Manter Laboratory, University of Nebraska.

ETYMOLOGY: The species name refers to the largest lake in the area where specimens were collected.

Discussion

Of the species of *Drepanidotaenia* with 10 rostellar hooks, *D. teshekpukensis* sp. n. resembles both *D. barrowensis* and *D. bilateralis,* which have been reported previously from black brant (Neraasen and Holmes, 1975; Spasskaya, 1966). In both *D. barrowensis* and *D. bilateralis* the ovary can be substantially overlapped by the antiporal testis, however, in both of these species the center of the ovary is always situated antiporal of the most antiporal testis (Schiller, 1952; Spasskaya, 1966). The new species can be distinguished from all described species of *Drepanidotaenia* because the center of its ovary is consistently situated immediately poral of the center of the most antiporal testis.

The new species differs from *D. barrowensis* in that it has a shorter total length, 16 mm, as compared to 70 mm reported by Schiller (1952) and 90 mm reported by Spasskaya (1966); its scolex is larger, 180 long by 140 wide, as compared to 72 long by 96 wide (Schiller, 1952) and 105–115 wide (Spasskaya, 1966); its rostellar hooks are longer, 33 (29–35) long, as compared to 22 long (Schiller, 1952) and 21–23 long (Spasskaya, 1966); it has a longer cirrus, 230 long as compared to 140 long (measured from type material from USNM Helm. Coll.); it has larger and more symmetrical cirrus spines, largest spines 23 (18–27) long, as compared to 8–10 long (Schiller, 1952) and 2–13 long (Spasskaya, 1966); it has smaller testes, 88 (80–91) wide, as compared to 129–144 wide (Schiller, 1952) and 150–220 wide

(Spasskaya, 1966); its external seminal receptacle is more spherical; its ovary is smaller, 130 (70–215) wide as compared to 330–440 (Spasskaya, 1966) and does not extend laterally past the excretory canals; it has a smaller vitellarium, 47 (27–70) wide, as compared to 72 wide (Schiller, 1952) and 111–140 (Spasskaya, 1966); and its uterus is a uniform transverse sac lacking the antiporal expansion described by Schiller (1952).

The new species differs from *D. bilateralis* in that it has a shorter total length, 16 mm, as compared to 68 mm (Linstow, 1905; Spasskaya, 1966); its scolex is smaller, 180 long, as compared to 220 long (Linstow, 1905; Spasskaya, 1966); its rostellar hooks are shaped differently, having a proportionally smaller blade that is approximately 20% of the total length of the hook, as compared to approximately 38% (Spasskaya, 1966); its ovary is smaller, approximately ½ of proglottid width, as compared to ⅓ of the proglottid width (Spasskaya, 1966); its excretory canals are outside of the region occupied by the reproductive organs, rather than being more medially situated (Spasskaya, 1966) and its cirrus sac and vagina pass between the ventral and dorsal excretory ducts, rather than their being positioned ventral of the cirrus sac (Spasskaya, 1966).

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Odilia mallomyos sp. n. (Nematoda: Heligmonellidae) from Mallomys rothschildi weylandi (Rodentia: Muridae) of Irian Jaya, Indonesia

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ABSTRACT: Odilia mallomyos sp. n. (Nematoda: Heligmonellidae: Nippostrongylinae) is described based on adults, fourth- and third-stage larvae from the small intestine of Mallomys rothschildi weylandi (Rodentia: Murinae) captured around Wamena, Irian Jaya, Indonesia. Adults of this species are distinguished from other members of the genus Odilia by the following features: synlophe with continuous ridges (16 in number at midbody of both sexes and the hypertrophied left lateral ridge located about midway between the adjacent ventral and dorsal ridges), spicules with slender pointed tips, bursa copulatrix with a right lobe larger than the left, and absence of gubernaculum. This is the first record of Odilia species outside of Australia. Presence of Odilia species in Irian Jaya is not unexpected because New Guinea and Australia belong to the same zoogeographical region. The synlophe of adult and fourth-stage larva suggests that Odilia is of archaic origin in the subfamily Nippostrongylinae.

KEY WORDS: Odilia mallomyos sp. n., Nematoda, Mallomys rothschildi weylandi, Murinae, Irian Jaya, Indonesia.

Mallomys rothschildi weylandi Flannery et al. is a giant rat inhabiting montane forests of West New Guinea (Flannery et al., 1989). This murid is hunted for food by the local people. During a survey of murine helminth fauna in 1993, 2 individuals of M. rothschildi weylandi were examined, and a nematode belonging to the genus Odilia was collected from the small intestine. Close examination has revealed that this species is new to science and is described herein.

Materials and Methods

Two individuals of M. rothschildi weylandi were purchased at the central market of Wamena, Irian Jaya, Indonesia. They were dissected and their viscera were fixed and preserved in 10% formalin solution and then transported to the laboratory. The alimentary canals were cut open and examined under a stereomicroscope for nematodes. Collected nematodes were rinsed in 70% ethanol and then cleared with a glycerol–alcohol solution for microscopic examination. In order to study male genital organs, some worms were mounted in chloral-gum. Freehand cross sections were made for observation of the synlophe (Durette-Desset, 1985). Figures were made with the aid of a drawing tube. Measurements (in micrometers unless otherwise stated) are given for the holotype male and the allotype female, followed in parentheses by the range of paratype males and females. Ranges of measurements are given for larval stages. The terminology of the synlophe and female genital organs follows Durette-Desset (1983). Specimens are deposited in the Museum Zoologi Bogor (MZB), Bogor, Indonesia, and the United States National Museum Helminthological Collection (USNM Helm. Coll.), Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A.

Results

Numerous individuals of a heligmonellid species were found in the upper small intestine of both of the 2 M. rothschildi weylandi we examined.

Odilia mallomyos sp. n.
(Nematoda: Heligmonellidae: Nippostrongylinae)

Adult (Figs. 1-14)

GENERAL: Small worm bent ventrally forming sinistral coils. Cephalic vesicle widening posteriorly, with distinct annulation (Figs. 1, 2). Mouth triangular with rounded corners, and buccal cavity small (Figs. 1, 2). Six minute labial papillae, amphidial pores and 4 double cephalic papillae present (Fig. 1). Cuticle finely striated. Synlophe well developed with pointed ridges, commencing just posterior to cephalic vesicle and ending anterior to level of proximal ends of spicules in male and anterior to level of infundibulum in female (Figs. 2–5, 7, 10–14). Axis of orientation of ridges passing through ventral-right and dorsal-left sides, inclined about 30–40°, 45°, and 70° from sagittal axis in anterior, middle, and posterior body, respectively (Figs. 3, 4, 10–12). In midbody left lateral ridge strongly hypertrophied, located slightly laterodorsally, forming prominent carene of type A; right lateral 2 ridges hypertrophied; dorsal ridges and left ventral ridges moderately developed; ridge just

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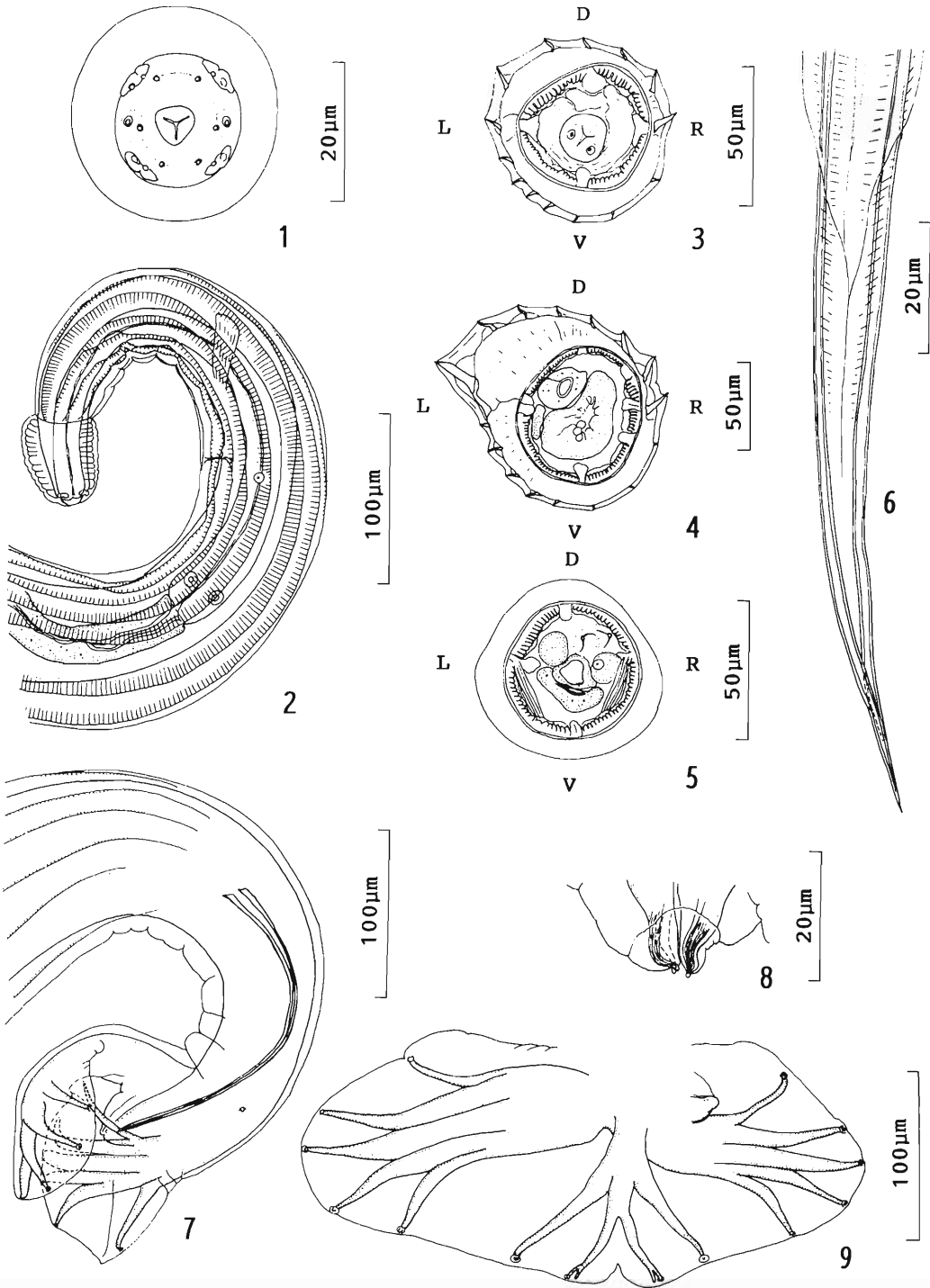
Figures 1–9. Male adult of *Odilia mallomyos* sp. n. from *Mallomys rothschildi weylandi* of Irian Jaya, Indonesia. 1. Cephalic extremity, apical view. 2. Anterior part of holotype, left lateral view. 3–5. Cross sections through nerve ring (3), midbody (4) and proximal portion of spicules (5). 6. Distal ends of spicules, ventral view. 7. Posterior part of holotype, left lateral view. 8. Genital cone, right lateral view. 9. Bursa copulatrix, ventral view. Abbreviations: D, dorsal; L, left; R, right; V, ventral.
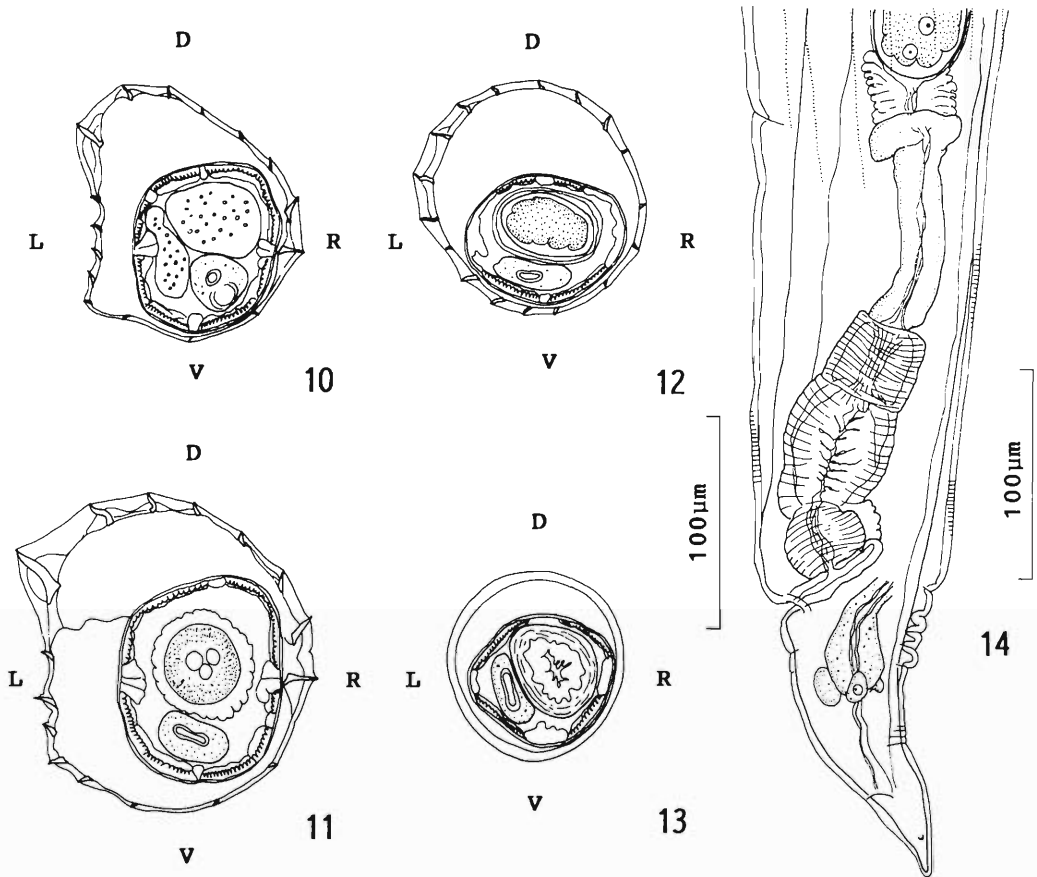
Figures 10-14. Female adult of *Odilia mallomyos* sp. n. from *Mallomys rothschildi weylandi* of Irian Jaya, Indonesia. 10-13. Cross sections through anteriormost portion of intestine (10), midbody (11), uterus (12), and vestibule (13). 14. Posterior part of allotype, left lateral view. Abbreviations: D, dorsal; L, left; R, right; V, ventral.

ventral to hypertrophied left lateral ridge slightly smaller than next ventral ridge; right ventral ridges minute (Figs. 4, 11). In both sexes, 16 ridges present in midbody (Figs. 4, 11); in esophageal region, left lateral 2 ridges and right lateral 2 ridges hypertrophied (Fig. 3); and ridges becoming smaller in posterior body (Fig. 12). Esophagus club-shaped. Nerve ring slightly anterior to middle of esophagus, excretory pore located posterior to middle of esophagus; deirids prominent, at same level with excretory pore (Fig. 2).

MALE (holotype and 10 paratypes): Length 3.7 (3.3–3.9) mm, width at midbody 152 (117–155). Cephalic vesicle 56 (37–53) long by 45 (40–46) wide. Nerve ring 183 (135–190), excretory pore 258 (194–276) and deirids 264 (204–280) from cephalic end. Esophagus 350 (273–376) long and 35 (24–34) wide near posterior end. Bursa

copulatrix markedly asymmetrical with larger right lobe and dorsal incision present (Figs. 7, 9). Prebursal papillae minute (Fig. 7). All bursal rays terminating near bursal rim: ventroventral ray much shorter than lateroventral ray, divergent from each other widely; externolateral and mediolateral rays almost same length, divergent distally; posterolateral ray thinner and shorter than other laterals and divergent from slightly distal to middle of mediolateral ray; dorsal ray and externodorsal rays with common, thick trunk. Dorsal ray divided into 2 divergent branches at middle, and each branch divided again into 2 unequal offshoots near tip; externodorsal ray arising from basal ⅓ of dorsal ray (Fig. 9). Genital cone moderately protruded, with 1 pair of sessile papillae; anterior lip of cloaca with unpaired papilla (Fig. 8). Spicules relatively short, equal, alate, distal ends fused and pointed, 207 (200–238) long

(occupying 5.5–7.1% of worm length) (Figs. 6, 7). Gubernaculum not observed.

FEMALE (allotype and 10 paratypes):   Length 5.2 (4.6–5.2) mm, width at midbody 156 (128–187). Cephalic vesicle 45 (45–50) long by 46 (43–51) wide. Nerve ring 155 (128–158), excretory pore 235 (193–257), and deirids 230 (208–253) from cephalic end. Esophagus 328 (315–360) long and 27 (26–34) wide near posterior end. Vulva 140 (120–158) and anus 44 (38–53) from caudal end (Fig. 11). Postvulval body slightly torsioned to left (Fig. 14). Vagina vera with thick wall forming diverticulum dorsally, 62 (45–64) long, vestibule with compact light-refractive distal portion, 84 (64–84) long, sphincter 35 (26–37) long, and infundibulum usually straight, 120 (104–140) long (Fig. 14). Tail conical, with phasmidial pores subapically (Fig. 14). Eggs ellipsoidal, thin-shelled, containing early cleavage-stage embryos, and 69–77 by 34–45 (Fig. 14).

Fourth-stage larva (Figs. 15-22)

GENERAL: Minute transparent worm bent ventrally forming irregular coils. Cephalic vesicle short, with distinct annulation (Figs. 15, 16). Cuticle posterior to cephalic vesicle with relatively rough annulation and rest of body cuticle with fine transverse striations (Fig. 16). Mouth almost round, and buccal cavity with thick wall (Figs. 15, 16). Six minute labial papillae, amphidial pores and 4 double cephalic papillae present (Fig. 15). Synlophe commencing slightly posterior to cephalic vesicle and ending about 200 from posterior extremity (Figs. 16, 17–19, 22). Axis of orientation of ridges passing through ventral-right and dorsal-left sides, inclined about 25–30° in midbody (Figs. 20, 21). In midbody 10 ridges present in both sexes: 3 dorsal, 5 ventral, and 1 in each lateral side; 1 of dorsal ridges located slightly right to middorsal line less developed lacking clear intracuticular skeleton; lateral and laterodorsal ridges relatively developed; ventral ridges minute (Fig. 20). In midbody of worm with formation of adult cuticle, left ventral 3 ridges becoming hypertrophied with development of some faint additional ridges ventrally (Fig. 21). Esophagus club-shaped (Fig. 16). Nerve ring slightly anterior to middle of esophagus, excretory pore located posterior to middle of esophagus; deirids at same level with excretory pore (Fig. 16).

MALE (10 worms):   Posterior end swollen and with terminal process (Figs. 17–19). Length 2.00–2.46 mm, width at midbody 74–93. Cephalic vesicle 19–40 long by 26–34 wide. Nerve ring 102–128, excretory pore 123–163 and deirids 130–164 from cephalic end. Esophagus 240–320 long and 22–32 wide near posterior end. Various stage of development of bursa copulatrix and spicules seen under cuticle of posterior region (Figs. 17–19). Asymmetry of bursa copulatrix observed from early stage (Fig. 17). Anus hardly discernible in late stage (Fig. 19). Spicules gradually elongated anteriorly (Figs. 17–19). Gubernaculum formation not observed. Tail including terminal process 36–53 long.

FEMALE (8 worms):   Posterior end tapering (Fig. 22). Length 1.86–2.69 mm, width at midbody 42–99. Cephalic vesicle 19–32 long by 32–36 wide. Nerve ring 106–117, excretory pore 123–163 and deirids 130–170 from cephalic end. Esophagus 255–303 long and 18–27 wide near posterior end. In premolt larvae, vulva, vagina and ovejector observed under cuticle (Fig. 22). Postvulval body slightly torsioned to left (Fig. 22). Primordial vulva 121–138 from posterior extremity (6 worms). Tail conical, 48–56 long (Fig. 12).

Third-stage larva (Figs. 23-26)

GENERAL (3 worms):   Slender transparent larva tapering to both extremities (Figs. 23, 25, 26). Length 0.99–1.23 mm, width at midbody 34–45. Cephalic end round, with submedian papillae and amphidial pores. Cuticle with wide longitudinal elevation in each lateral field having groove with side elevations on lateral line (Figs. 23, 24). Esophagus club-shaped, 265–365 long by 21–22 wide near posterior end; nerve ring just anterior to midesophagus and excretory pore slightly posterior to nerve ring, 110–146 and 135–183, respectively, from anterior extremity (Fig. 23). Deirids not seen. Genital primordium oval, 14–21 long, located ventrally at 380–568 from anterior extremity. Tail with 1 pair of small processes subterminally, 58–62 long (Fig. 25). In premolt worm subterminal processes becoming less prominent (Fig. 26).

TYPE HOST: *Mallomys rothschildi weylandi* (Muridae: Murinae).

SITE IN HOST: Small intestine (duodenum and upper jejunum).

TYPE LOCALITY: Highland forest near Wamena (4°11′S, 138°58′E; 1,500 m elevation), Irian Jaya, Indonesia.

DATE OF COLLECTION: 4 August 1993.

ETYMOLOGY: Species name is derived from the generic name of the type host.
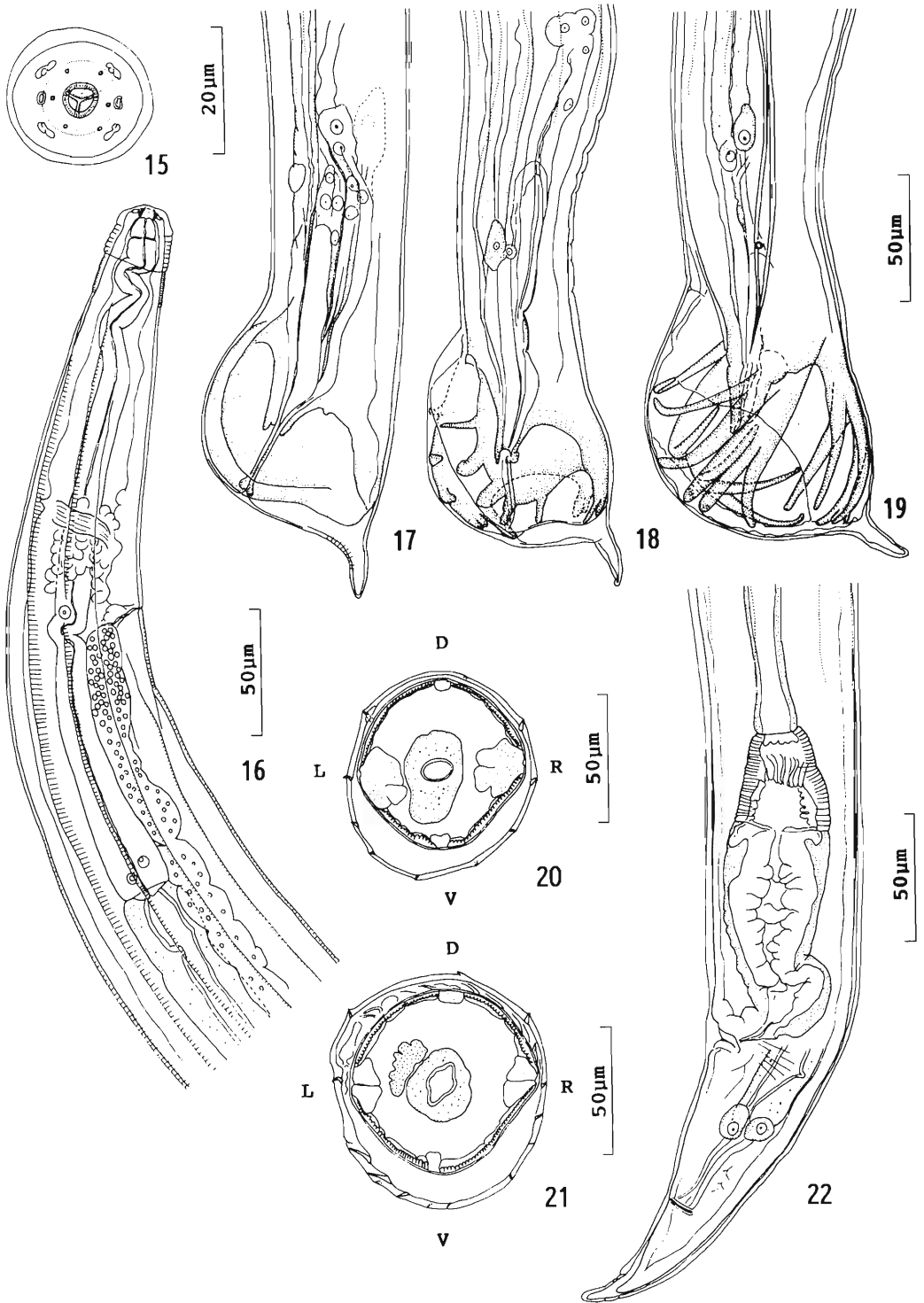
Figures 15–22.   Fourth-stage larva of *Odilia mallomyos* sp. n. from *Mallomys rothschildi weylandi* of Irian Jaya, Indonesia. 15. Cephalic extremity of male, apical view. 16. Anterior part of late fourth-stage male, right lateral view. 17–19. Posterior extremities of males of various developmental stages, left lateral view. 20, 21. Cross sections through midbody of early fourth-stage male (20) and late fourth-stage male (21). 22. Posterior part of female, left lateral view. Abbreviations: D, dorsal; L, left; R, right; V, ventral.

TYPE SPECIMENS: MZB Na-274 (holotype and allotype); MZB Na-275 (5 male and 5 female paratypes), USNM Helm. Coll. 83732 (5 male and 5 female paratypes, 10 male and 8 female fourth-stage larvae, and 3 third-stage larvae).

REMARKS: The present species belongs to the genus *Odilia* Durette-Desset, 1973 (syn. *Austrostrongylus* sensu Durette-Desset, 1971 nec Chandler 1927) because the left lateral ridge of synlophe in midbody of adult is hypertrophied, supporting carene of type A, the bursa copulatrix is asymmetrical, and the externodorsal rays are of the same size (Durette-Desset, 1971, 1983). *Odilia mallomyos* is unique because the gubernaculum is apparently lacking. All other representatives of this genus have readily discernible gubernaculum (Mawson, 1961; Durette-Desset, 1969).

Odilia mallomyos is easily distinguished from *Odilia polyrhabdote* (Mawson, 1961), and *Odilia mawsonae* (Durette-Desset, 1969) because in the latter 2 species there are more than 20 synlophe ridges in the midbody and the left lateral ridge is not hypertrophied (Durette-Desset, 1969). *Odilia mallomyos* is also easily distinguished from *Odilia uromyos* (Mawson, 1961) because the latter species has numerous (more than 40) ridges at the widest portion of its body (Mawson, 1961). The present species resembles *Odilia brachybursa* (Mawson, 1961), *Odilia mackerrasae* (Mawson, 1961), *Odilia emanuelae* (Mawson, 1961), and *Odilia melomyos* (Mawson, 1961). However, *O. brachybursa* has spicules that are expanded near the tips into 2 alate branches, *O. mackerrasae* has intermittent ridges in the ventral field, and *O. emanuelae* has a larger left lobe in the bursa copulatrix, all being readily distinguished from the present species (Mawson, 1961; Durette-Desset, 1969).

The synlophe of *O. melomyos* is similar to that of the present species although it has 15 ridges in the anterior body and midbody (Durette-Desset, 1969). Nevertheless, *O. melomyos* is distinguishable from *O. mallomyos* because the left lateral hypertrophied ridge is much closer to the adjacent ventral ridge than to the adjacent dorsal ridge and in having much longer spicules (over 350 long in the males with body length of 3.4–3.5 mm) (Mawson, 1961; Durette-Desset, 1969).

Discussion

The nematodes of the genus *Odilia* have been known only from Australia (Durette-Desset,

24

23

50 μm
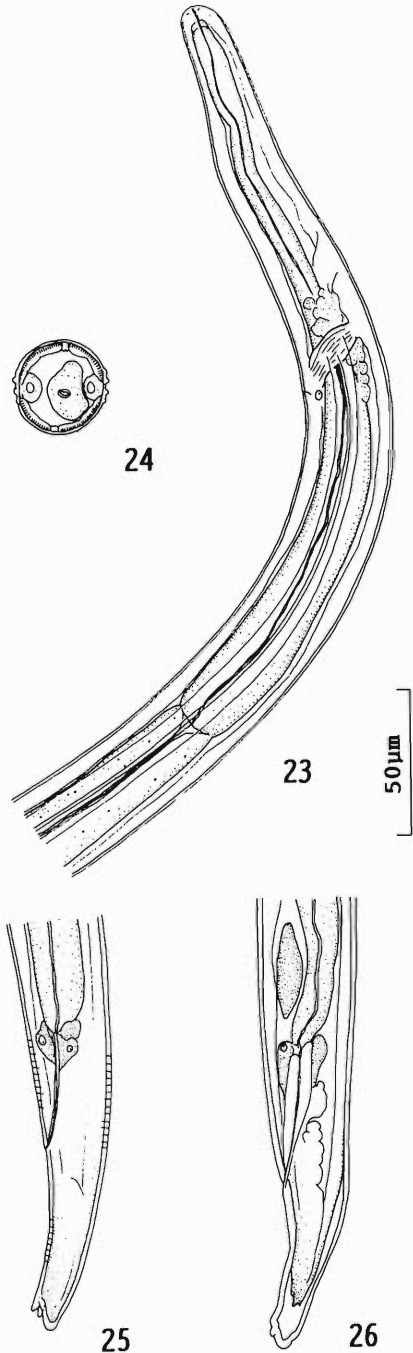
25   26

Figures 23–26. Third-stage larva of *Odilia mallomyos* sp. n. from *Mallomys rothschildi weylandi* of Irian Jaya, Indonesia. 23. Anterior part, left lateral view. 24. Cross section of midbody. 25, 26. Posterior extremity of early (25) and late (26) third-stage larvae, left lateral view.

1971, 1973, 1985; Obendorf, 1979; Smales, 1992). Thus this report is the first of an *Odilia* species outside of Australia. No description has been made of *Odilia* species larval stages. Durette-Desset (1985) presented a hypothesis that the ancestral *Odilia* was introduced to Australia from Southeast Asia by *Melomys*-group murines during lower and middle Pliocene from which they subsequently speciated. The presence of an *Odilia* species in New Guinea is not unexpected because this large island zoogeographically belongs to the Australian Region and many murines including *Melomys* and *Uromys* are shared by both Australia and New Guinea (cf. Musser and Carleton, 1993).

Durette-Desset (1985) recognized 2 groups in the synlophe of the fourth-stage larva of Nippostrongylinae. It is apparent that *O. mallomyos* belongs to the second group because it has a ridge directly adjacent to the left lateral chord (Durette-Desset, 1985). Its synlophe resembles those of *Neoheligmonella* and *Heligmonoides* in having a total of 10 ridges of which 3 are in the dorsal side (Durette-Desset, 1985). However, *O. mallomyos* is closer to *Nippostrongylus* than to *Neoheligmonella* or *Heligmonoides* in the degree of inclination of the axis of synlophe orientation with respect to the sagittal axis. It inclines more than 50° from the sagittal axis in the fourth-stage larva of *Neoheligmonella* and *Heligmonoides,* while in *Nippostrongylus* it inclines less than 50° (Durette-Desset, 1985, Fig. 7).

It has been considered that the axis of orientation has rotated from sagittal to frontal during the course of Nippostrongylinae evolution (Durette-Desset, 1985). The synlophe of the fourth-stage larva and adult of *O. mallomyos* suggests that the genus *Odilia* is an early derivative from a common evolutionary stem with *Neoheligmonella* and *Heligmonoides*. The fact that only *Odilia* and *Nippostrongylus* have been known as intestinal nippostrongylines in Australian region may indicate that these genera have archaic origins in this subfamily.

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Andrya apodemi sp. n. (Cestoda: Anoplocephalidae), a Parasite of *Apodemus argenteus* (Rodentia: Muridae) from Hokkaido, Japan

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ABSTRACT: *Andrya apodemi* sp. n. from the small intestine of small Japanese field mice, *Apodemus argenteus,* from Hokkaido, Japan is described. This is the first species of the genus *Andrya* Railliet, 1893 that has been found in murid rodents in Asia. This new species can be distinguished from related species by total length of strobila, genital pores situated unilaterally, number of testes (48–76), distribution of testes, which are mostly situated in the antiporal half of proglottids and do not extend beyond the antiporal excretory canal, and the size of eggs. In addition, upon review of specimens of *Paranoplocephala omphalodes* (Hermann, 1883) Lühe, 1910 of Ishimoto (1974) in Hokkaido, we identified them as *A. apodemi* because of the network-like structure of the uterus in postmature proglottids.

KEY WORDS: *Andrya apodemi* sp. n., Cestoda, Anoplocephalidae, *Apodemus argenteus,* Muridae, rodents, Japan.

During a survey of the helminths from the small Japanese field mouse, *Apodemus argenteus* Temminck, from Hokkaido, Japan, an undescribed species of *Andrya* Railliet, 1893 was found. This is the first time that a cestode of the genus *Andrya* was found from murid rodents in Asia. Thus, we describe the specimens as *Andrya apodemi* sp. n.

Materials and Methods

Animals were collected at Nopporo National Forest in June 1967–May 1968, at Tohbetsu in July 1985, and at Iwamizawa in May 1993. After removal from the host, worms were lightly pressed and fixed in 70% ethanol, stained by Semichon's acetocarmine or Delafield's hematoxylin and mounted in Canada balsam or MGK® (Matsunami Glass Ind., Ltd., Japan). All measurements are of fixed specimens, are in micrometers unless otherwise indicated, and are given as a range with the mean in parentheses.

Description

Andrya apodemi sp. n.
(Figs. 1-5)

The following description is based on 3 complete specimens having gravid proglottids and 4 incomplete strobila from 7 *A. argenteus.*

Total body length 48.0–86.5 (65.3) mm. Maximum width 5–9 (6.7) mm at the posterior end of strobila. Total number of proglottids 128–175 (155), including 30–38 (34) immature, 14–20 (17) mature, 60–101 (74) postmature, and 20–50 (30) gravid proglottids. Scolex 680–1,296 (953) wide. Four suckers 264–396 (332). Neck 614–886 (790)

wide. All proglottids craspedote, wider than long, 213–440 (293) long by 1,018–4,100 (2,903) wide in the last mature proglottids. Proglottids' margin serrate. Genital pores unilateral, opening in second third on margin of proglottids. Cirrus sac elongate 231–277 (254) long by 61–125 (78) wide in the last mature proglottids, extending slightly across poral excretory canal. Cirrus lacking spines. External vesicula seminalis 79–145 (110) long by 26–79 (48) wide in mature proglottids. Testes 48–76 (60) in number, 46–99 (70) in diameter in the last mature proglottids, mostly distributed between antiporal ventral excretory canal and antiporal end of ovary, but do not overlap the antiporal canal. Occasionally some testes can be found dorsal to the antiporal part of the ovary. Vagina situated posterior to cirrus sac. Receptaculum seminis beginning to fill in the last mature proglottid, 528–792 (655) long by 198–323 (246) in anterior postmature proglottids. Ovary 726–1,082 (980) wide, occupying almost one-third of the width in the poral half of the proglottid. Vitellarium 231–310 (278) wide, situated at posterior margin of the proglottid, near middle part of ovary. Uterus initially situated on both sides of proglottid, progressively spreading laterally with development, overlapping the testes, forming a network-like structure in fully gravid proglottids; testes never displaced posteriorly. When the receptaculum seminis becomes fully developed in postmature proglottids, the ovary degenerates. Eggs spherical, 43–56 (51), with pyriform apparatus present. Oncosphere 9–16 (13).
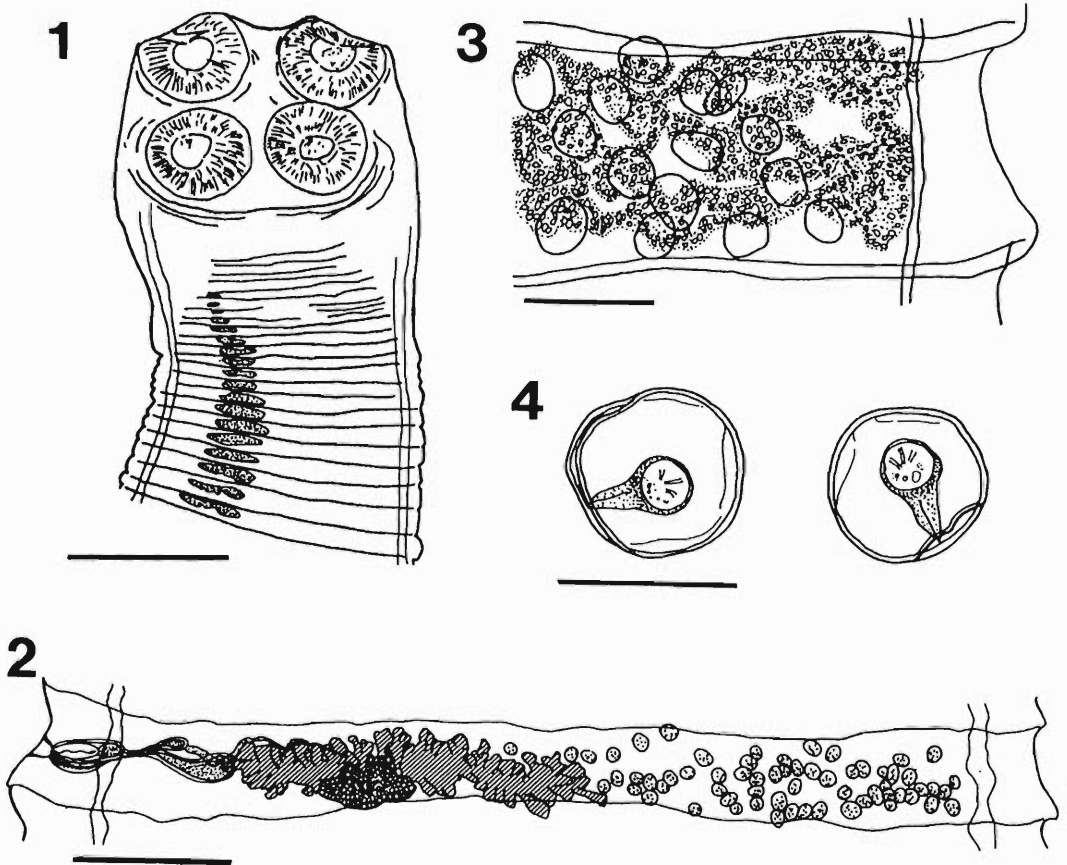
Figures 1-4. *Andrya apodemi* sp. n. 1. Scolex. Scale = 0.5 mm. 2. Mature segment. Scale = 0.5 mm. 3. Development of reticulate uterus forming network-like structure among testes. Scale = 0.2 mm. 4. Eggs with pyriform apparatus in gravid proglottid. Scale = 50 μm.

HOST: *Apodemus argenteus.*

SITE: Small intestine.

TYPE SPECIMENS: Holotype: Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, No. P-722; paratypes: No. P-723 and P-731.

TYPE LOCALITY (holotype):   Tohbetsu, Hokkaido, Japan. Other localities (paratypes): Nopporo National Forest (No. P-723), and Iwamizawa (No. P-731), Hokkaido, Japan.

ETYMOLOGY: This is the first species of the genus *Andrya,* which is found from murid rodents of the genus *Apodemus,* so it is named *A. apodemi.*

Discussion

Generic-level identification of *Andrya apodemi* sp. n. was based on the structure of the uterus as determined in postmature proglottids. The most important feature of the genus *Andrya,* by which the genus is separated from related genera, such as *Paranoplocephala* Lühe, 1910, and *Anoplocephaloides* Baer, 1923, is the pattern of development of the uterus in postmature proglottids (Tenora et al., 1981–1982, 1986). According to the identification key of Tenora et al. (1986, p. 44), the uterus of *Paranoplocephala* is "situated ventrally, positioned transversally, gradually pushed back the testes and forms an organ with network structure," while the uterus is "not positioned transversally, suddenly proliferated among the testes forming the network uterus" in species of *Andrya.* These features of the postmature proglottids are possessed by several species, although the structure of the uterus is observed only in gravid proglottids of other species in some related genera.

The following 20 species have been described

as belonging to the genus *Andrya*: (A) Parasites of Lagomorpha: *Andrya rhopalocephala* (Riehm, 1881) Railliet, 1893; *A. cuniculi* (Blanchard, 1891), Railliet, 1893. (B) Parasites of Rodentia: *A. africana* Baer, 1933; *A. arctica* Rausch, 1952; *A. bairdi* Schad, 1953; *A. bialowiezensis* Soltys, 1949; *A. caucasica* Kirschenblat, 1938; *A. communis* Douthitt, 1915; *A. dasymidis* Hunkeler, 1972; *A. kalelai* Tenora, Haukisalmi and Henttonen, 1985; *A. macrocephala* Douthitt, 1915; *A. microti* Hansen, 1947; *A. monodi* Joyeux and Baer, 1930; *A. montana* Kirschenblat, 1941; *A. neotomae* Voge, 1946; *A. ondatrae* Rausch, 1948; *A. primordialis* Douthitt, 1915; *A. primordialis* var. *gundii* Joyeux, 1923; *A. sciuri* Rausch, 1947; *A. translucida* Douthitt, 1915. One additional species, *Paranoplocephala petauristae* (Sawada and Kugi, 1979) Schmidt, 1986, found in Japan, is also similar to the new species morphologically.

Compared with descriptions of the above-mentioned species by Douthitt (1915), Soltys (1949), Spasskii (1951), Rausch (1952), Schad (1954), Hunkeler (1972), Sawada and Kugi (1979), Tenora et al. (1985, 1986), and Schmidt (1986), the new species can be distinguished by total length of strobila from *A. africana, A. bairdi, A. cuniculi, A. monodi,* and *A. rhopalocephala*; distinguished by unilateral arrangement of genital pores from *A. arctica, A. cuniculi, A. kalelai, A. microti, A. neotomae, A. ondatrae, A. sciuri,* and *P. petauristae*; distinguished by number of testes from *A. africana, A. bairdi, A. bialowiezensis, A. caucasica, A. communis, A. dasymidis, A. kalelai, A. microti, A. monodi, A. montana, A. ondatrae, A. rhopalocephala, A. primordialis, A. primordialis* var. *gundii,* and *A. sciuri*; distinguished by distribution of testes, which are mostly situated in the antiporal half of proglottids and do not extend beyond the antiporal ventral excretory canal, from *A. caucasica, A. communis, A. dasymidis, A. macrocephala, A. microti, A. ondatrae,* and *A. translucida*; distinguished by having smaller eggs from *A. macrocephala, A. primordialis,* and *A. primordialis* var. *gundii.*

Andrya apodemi is the first species of the genus *Andrya* found from murid rodents in Asia. None of the above-mentioned species have been found from murid rodents, except *A. dasymidis,* which was found in the African marsh rat, *Dasymidis incomtus rufulus* Miller, in Africa. Anoplocephalid cestodes have been reported in wild rodents in Japan, by Ishimoto (1974), Rausch (1976), Asakawa et al. (1983), and Sato et al. (1993).
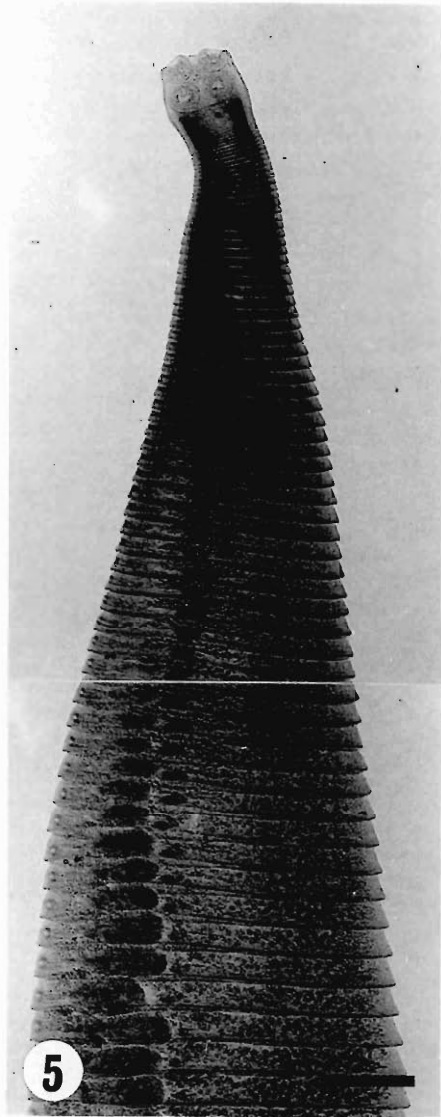
Figure 5. View of scolex and part of strobila. Scale = 1 mm.

However, until now there have been no reports of the genus *Andrya* found in Japan. Ishimoto (1974) described *Paranoplocephala omphalodes* (Hermann, 1783) Lühe, 1910 from *Apodemus speciosus* Thomas and *A. argenteus* Temminck. Although he presented a figure of a tubelike uterus in the anterior part of mature proglottid of his *P. omphalodes,* such a structure could not be observed in his specimens, which displayed only reticulate uteri. Thus, we identified his specimens as *A. apodemi,* not *P. omphalodes.* Rausch (1976, p. 538) wrote: "The cestodes identified as

P. omphalodes by Ishimoto (1974) from *Apodemus* spp. in Hokkaido appear to be distinct from *Anoplocephaloides blanchardi* and may represent an undescribed species that is host-specific for these murine rodents." Our results are in agreement with this contention.

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Meeting Schedule
1994-1995

Dactylogyrus boopsi sp. n. (Monogenea: Dactylogyridae) from the Bigeye Shiner, Notropis boops Gilbert (Pisces: Cyprinidae)

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ABSTRACT: *Dactylogyrus boopsi* sp. n. is described from the bigeye shiner, *Notropis boops* Gilbert, from Arkansas. *Dactylogyrus boopsi* closely resembles *D. perlus* Mueller, 1938, but the attenuated portion of the cirrus is shorter, and the basal process of the cirrus is narrower in *D. boopsi* than in *D. perlus*.

KEY WORDS: *Dactylogyrus boopsi* sp. n., *Notropis boops,* Monogenea, Dactylogyridae, morphology, taxonomy.

A new species of *Dactylogyrus* Diesing, 1850, is described from the bigeye shiner, *Notropis boops* Gilbert. This is the first report of any parasite from the bigeye shiner.

Materials and Methods

Immediately after capture, bigeye shiners were placed in jars containing a 1:4,000 formalin solution; after approximately 1 hr, enough formalin was added to make a 10% solution (Putz and Hoffman, 1963). The parasites, collected from the gills of their hosts, were mounted in glycerin jelly, and observations were made with a Zeiss phase-contrast microscope. Drawings were made with the aid of a Zeiss drawing tube. Measurements, in micrometers, were made as presented by Mizelle and Klucka (1953); means are followed by ranges in parentheses. Numbering of haptoral hooks is after Mueller (1936).

Type specimens were deposited in the helminthological collections of the National Museum of Natural History (USNM) and of the Harold W. Manter Laboratory, University of Nebraska State Museum (HWML). Nontype specimens are in the author's (DGC) and Wilmer A. Rogers' (WAR) collections. For comparative purposes, all original descriptions and redescriptions of North American *Dactylogyrus* species, and the following specimens of *D. perlus* Mueller, 1938 (synonym = *D. banghami* Mizelle and Donahue, 1944) (see Cloutman, 1988), were examined: USNM 73552 (1 syntype of *D. banghami*); HWML 21545 (2 syntypes of *D. banghami*); USNM 71454, 71457 (3 syntypes of *D. perlus*); HWML 21305 (1 voucher specimen).

Results and Discussion

Dactylogyrus boopsi sp. n.
(Fig. 1)

TYPE LOCALITY: Arkansas: Franklin Co., Mulberry River at Redding Access, 3 km E of Cass.

TYPE SPECIMENS: Holotype, USNM 83280; 9 paratypes, USNM 83281 (6 specimens) and HWML 36962 (3 specimens).

OTHER LOCALITIES: Arkansas: Fulton Co., Spring River near Mammoth Spring (WAR); Newton Co., Buffalo River near Hasty (DGC); Polk Co., Ouachita River, 3 km S of Cherry Hill (DGC); Washington Co., Clear Creek at Hwy 112 bridge (DGC).

DESCRIPTION: With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; length 244 (180–288), greatest width 60 (29–72). Two pairs of eyes, anterior pair usually smaller and farther apart than posterior pair. Peduncle 23 (7–39) long, 30 (19–42) wide. Haptor 36 (28–49) long, 40 (28–49) wide.

Dorsal anchor composed of solid base with short deep root, elongate superficial root, solid shaft, and sharp recurved point; length 21 (19–23); greatest width of base 13 (10–15). Ventral 4A (see Kritsky and Kulo, 1992) length 4. Dorsal bar length 21 (18–22). Vestigial ventral bar length 14 (13–15). Fourteen hooks (7 pairs), similar in shape, and normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece. Hook lengths: no. 1, 12 (11–13); 2, 15 (14–15); 3, 15 (14–15); 4, 13 (11–14); 5, 15 (14–15); 6, 12 (10–13); 7, 14 (13–15).

Copulatory complex composed of cirrus, articulated accessory piece. Cirrus with enlarged base bearing a straight, tapering process and curved tubular shaft attenuated to a point. Cirral length 37 (33–41). Accessory piece bifurcate; distal ramus curved, attenuated to a point; mesial ramus recurved, attenuated to a point. Accessory piece length 20 (18–21). Vagina sclerotized, irregular in shape, opening dextroventrally posterior to cirrus, length 14 (12–16). Vitellaria light to moderate, usually distributed from pharynx to haptor.

REMARKS: *Dactylogyrus boopsi* closely resembles *D. perlus,* but comparisons revealed 2 distinct differences: (1) the attenuated portion of the cirrus shaft of *D. boopsi* is shorter (goes ca. 4 times into shaft length) than that of *D. perlus* (goes ca. 2.5 times into shaft length), and (2) the
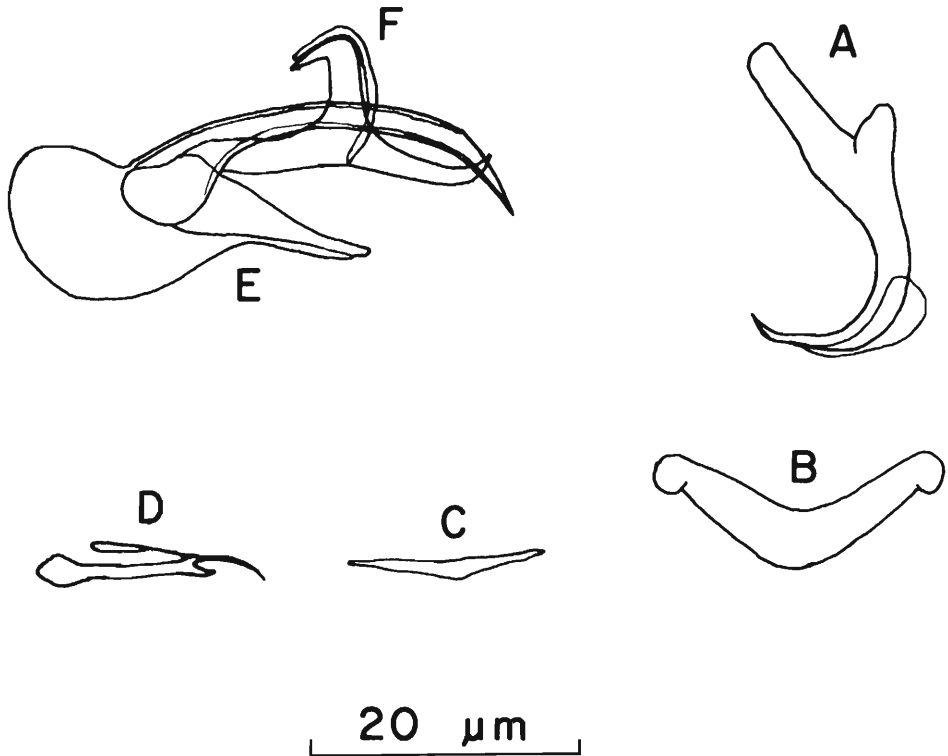
Figure 1. *Dactylogyrus boopsi* **sp. n., holotype. A. Anchor. B. Dorsal bar. C. Ventral bar. D. Hook. E. Cirrus. F. Accessory piece.**

basal process of *D. boopsi* is not as robust as that of *D. perlus*.

ETYMOLOGY: *Dactylogyrus boopsi* is named after its host.

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Research Note

New Host and Distribution Records for Coccidia (Apicomplexa: Eimeriidae) from North American Lizards (Reptilia: Sauria)

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ABSTRACT: Four hundred twenty-three lizards, representing 33 species within 7 families (Anguidae, Crotaphytidae, Gekkonidae, Phrynosomatidae, Polychridae, Scincidae, Teiidae) were examined for coccidia. Eighty-two (19%) lizards harbored coccidia. Eighty-four percent of the infected lizards were gekkonids (69/139 or 50%) while 6 non-gekkonid families (13/283 or 5%) accounted for 16% of infected lizards. New host records are reported for *Isospora scinci* Upton, McAllister, and Trauth, 1991, from the broadhead skink, *Eumeces laticeps,* and *Eimeria sceloporis* Bovee and Telford, 1965, from the crevice spiny lizard, *Sceloporus poinsettii.* In addition, this represents the first report of *E. sceloporis* from Texas.

KEY WORDS: Apicomplexa, Coccidia, Eimeriidae, *Isospora scinci, Eimeria sceloporis,* lizards, Gekkonidae, survey, prevalence, Reptilia, Sauria.

Between March 1986 and December 1993, we conducted surveys on protozoan parasites of select herpetofauna of the southcentral and southwestern United States and Mexico and found several species of lizards harboring coccidia. Further examination revealed 6 previously undescribed coccidia, which we have since described as new (McAllister et al., 1988, 1990, 1991a; Upton et al., 1988, 1991), or either redescribed (McAllister and Upton, 1989) or provided new host records (McAllister et al., 1991b). Herein, we add a new locality record and 2 additional host records and summarize our data on the coccidia of 7 families of North American lizards.

Four hundred twenty-three saurians (see Table 1) were collected by hand, noose, or shot with .22 caliber rat shot from 16 counties or parishes in Arkansas, 24 in Texas, 2 in Louisiana, and 1 each in New Mexico and Oklahoma, and from the state of Veracruz, Mexico. Specimens were returned to the laboratory and intestinal contents and feces were examined for coccidia following previously published methods (Upton et al., 1988). Except for lizards collected in Mexico, which were released, host voucher specimens are deposited in the Arkansas State University Museum of Zoology (ASUMZ) and the Carnegie Museum of Natural History (CM).

Of the 423 individual lizards, representing 33 species within 7 families, 82 (19%) were found to harbor 9 species of coccidians (Table 1). Eighty-four percent of these were gekkonids as 69 of 139 (50%) had 4 species of eimerians. The remaining 16% of the infected lizards were in 4 families as 3 of 131 (2%) of the phrynosomatids had a single eimerian, 2 of 14 (14%) of the polychrids had a caryosporan, 7 of 59 (12%) of the scincids had an eimerian and an isosporan, and 1 of 58 (2%) of the teiids had an eimerian. Three anguids and 18 crotaphytids were negative. There is a significant difference in prevalence of coccidia among gekkonids and non-gekkonids ($\chi^2 = 118.0$, 1 df, $P < 0.00001$). If gekkonids are excluded from the data set, only 13 of 283 (5%) of the lizards were infected.

A new host record was documented for *Isospora scinci* Upton, McAllister, and Trauth, 1991, in the broadhead skink, *Eumeces laticeps* (Schneider, 1801). The infected juvenile male skink (ASUMZ 19148, snout–vent length = 70 mm) was collected on 18 August 1993 in Independence County, Arkansas, 12.1 km NW Possum Grape at Gold Mine Springs. Three additional Arkansas *E. laticeps,* 2 from Desha County and 1 from Searcy County were negative. *Isospora scinci* was originally described from five-lined skinks, *Eumeces fasciatus* (Linnaeus, 1758) from Van Buren and Woodruff counties, Arkansas (Upton et al., 1991). These sites are 111 km west and 54 km southeast of the new locale, respectively. This finding was not surprising given that these skinks are broadly sympatric throughout their range (Conant and Collins, 1991).

Sporulated oocysts of an eimerian matching the description of *Eimeria sceloporis* Bovee and

Table 1. Lizards surveyed and the coccidian species collected.

Lizard family/species*	Locality	Prevalence†	Coccidian
Anguidae			
Ophisaurus attenuatus	Arkansas	0/2 (0%)	—
	Texas	0/1 (0%)	—
Crotaphytidae			
Crotaphytus collaris	Texas	0/17 (0%)	—
C. reticulatus	Texas	0/1 (0%)	—
Gekkonidae			
Cyrtopodion scabrum	Texas	8/20 (40%)	*Eimeria lineri*
Hemidactylus frenatus	Texas	7/12 (58%)	*E. dixoni*
H. mabouia	Mexico	1/1 (100%)	*E. boveroi*
H. turcicus	Louisiana	3/7 (43%)	*E. lineri*
	Texas	36/99 (36%)	*E. lineri*
	Texas	14/99 (14%)	*E. turcicus*
Phrynosomatidae			
Cophosaurus texanus	Texas	0/65 (0%)	—
Holbrookia lacerata	Texas	0/1 (0%)	—
H. propinqua	Texas	0/1 (0%)	—
Phrynosoma cornutum	Texas	0/6 (0%)	—
Sceloporus cyanogenys	Texas	0/1 (0%)	—
S. olivaceus	Texas	0/24 (0%)	—
S. poinsettii	Texas	1/4 (0%)	*E. sceloporis*
S. undulatus	Arkansas	0/9 (0%)	—
	New Mexico	0/1 (0%)	—
	Texas	0/1 (0%)	—
S. variabilis	Mexico	1/1 (100%)	*E. sceloporis*
	Texas	0/1 (0%)	—
Urosaurus ornatus	Texas	0/7 (0%)	—
Uta stansburiana	Texas	0/8 (0%)	—
Polychridae			
Anolis carolinensis	Arkansas	0/4 (0%)	—
	Louisiana	2/10 (20%)	*Caryospora ernsti*
Scincidae			
Eumeces anthracinus	Arkansas	0/2 (0%)	—
E. fasciatus	Arkansas	3/14 (21%)	*Eimeria fasciatus*
	Arkansas	3/14 (21%)	*Isospora scinci*
E. laticeps	Arkansas	1/4 (25%)	*I. scinci*
E. obsoletus	Texas	0/2 (0%)	—
E. septentrionalis	Texas	0/3 (0%)	—
E. tetragrammus	Texas	0/8 (0%)	—
Scincella laterale	Arkansas	0/15 (0%)	—
	Oklahoma	0/5 (0%)	—
	Texas	0/6 (0%)	—
Teiidae			
Cnemidophorus gularis	Texas	0/22 (0%)	—
C. inornatus	Texas	0/2 (0%)	—
C. laredoensis	Texas	0/2 (0%)	—
C. septemvittatus	Texas	0/1 (0%)	—
C. sexlineatus	Arkansas	1/28 (4%)	*E. sexlineatus*
	Texas	0/2 (0%)	—
C. tesselatus	New Mexico	0/1 (0%)	—

* Family nomenclature for iguanian lizards follows Frost and Etheridge (1989) and current scientific names follow Collins (1990).

† Number infected/number examined (percent).

Table 2. Summary of hosts, localities, and prevalence of *Eimeria sceloporis*.

Host	Locality	Prevalence		Reference
Sceloporus clarkii	Mexico	4/4	(100%)	Bovee and Telford, 1965a
S. jarrovii	Arizona	11/38	(31%)	Mitschler et al., 1993
S. magister	California	1/1	(100%)	Bovee and Telford, 1965a
		1/52	(2%)	Telford, 1970
S. poinsettii	Texas	1/4	(25%)	This paper
S. occidentalis	California	?		Bovee and Telford, 1965a
	Washington	149/178	(84%)	Clark and Colwell, 1973
S. variabilis	Mexico	2/2	(100%)	McAllister and Upton, 1989

Telford, 1965, were found in the feces of a gravid adult female (ASUMZ 19021, SVL = 119 mm) crevice spiny lizard, *Sceloporus poinsettii* Baird and Girard, 1852. The host was collected on 28 May 1993 from Llano County, Texas, 3.2 km N Enchanted Rock State Park, off FM 965. *Eimeria sceloporis* was originally described from Clark's spiny lizards, *Sceloporus clarkii* Baird and Girard, 1852, western fence lizards, *Sceloporus occidentalis* Baird and Girard, 1852, and desert spiny lizards, *Sceloporus magister* Hallowell, 1854 (Bovee and Telford, 1965a). This coccidian is apparently genus specific for it was reported from 6 species of sceloporiine lizards of the southwestern and Pacific northwestern United States and Mexico (Table 2). In addition, Mitschler et al. (1993) recently provided a redescription of *E. sceloporis* from Yarrow's spiny lizard, *Sceloporus jarrovii* Cope, 1875.

It is somewhat difficult to explain the disparity in prevalence of coccidia among the lizard families. However, the number of previous reports of coccidia from saurian hosts (other than gekkonids) in North America suggests a low prevalence of infection. Indeed, Matuschka and Bannert (1987) and Matuschka (1989) collectively list 30 species of *Eimeria* and 19 species of *Isospora* from lizards, excluding gekkonids. Of the species listed, only 20% of the eimerians and 11% of the isosporans are known from North America (6 from phrynosomatids, 2 from xantusids, and 1 from a scincid). In addition, all of the lizards that have been reported to serve as hosts for coccidia were collected from humid regions of the extreme western or Gulf Coastal area of the United States, in California, Louisiana, and Washington (Bovee and Telford, 1965a, 1965b; Bovee, 1966, 1969; Clark, 1970; Pellérdy, 1974; Atkinson and Ayala, 1987).

It is possible that many of the lizards from study sites did not serve as suitable hosts for coccidia because of defecation habits. Most of

the lizards were observed to bask upon limestone outcroppings, logs, and miscellaneous debris. Feces deposited on the substrate were desiccated within minutes. During periods of increased lizard activity, typical substrate temperatures can range from 35 to 45°C. If these conditions predominate over the period of lizard activity, the upper high temperatures would not represent ideal conditions for coccidia to develop or remain viable. The process of oocyst sporulation is temperature dependent (Long and Joyner, 1984). For example, Lindsey et al. (1982) reported *Isospora suis* oocysts were unable to sporulate at temperatures above 37°C. Furthermore, the majority of non-gekkonid lizards were observed to be most active during the warmer periods of the day. If lizards defecated at these times, it would serve to limit viability of oocysts that have exogenous sporulation.

We believe the reason that gekkonids have a 10-fold prevalence of infection, when compared to other lizards in this study, is also related to their ecology. Most gekkonids are diverse nocturnal lizards that are distributed throughout the tropics and subtropics of the Old and New Worlds. All 4 species of geckos in this study (see Table 1) are Old World natives that have been introduced into North America and have established themselves in buildings, docks in tropical seaports, in cargoes of fruit, and other humid areas. Feces deposited in these microhabitats would tend to favor increased viability of coccidian oocysts when compared to lizards inhabiting more hostile and dryer sites. Interestingly, these data showing a lower prevalence of infection for coccidia in non-gekkonid lizards are similar to those reported for western rodents inhabiting arid environments with a low prevalence of coccidia (Ford et al., 1990; McAllister et al., 1993).

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Research Note

Gastrointestinal Helminths of the Japanese Treefrog, *Hyla japonica* (Anura: Hylidae), from Japan

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ABSTRACT: Of 104 *Hyla japonica* examined, 38 (37% prevalence) harbored helminths. The cestode *Cylindrotaenia japonica* (prevalence 11%, mean intensity 2.5) and the nematodes *Cosmocerca japonica* (prevalence 20%, mean intensity 2.1) and *Oswaldocruzia insulae* (8% prevalence, 1.9 mean intensity) were found. *Oswaldocruzia insulae* in *Hyla japonica* represents a new host record.

KEY WORDS: Cestoda, *Cylindrotaenia japonica*, Nematoda, *Cosmocerca japonica*, *Oswaldocruzia insulae*, Hylidae, *Hyla japonica*.

The Japanese treefrog, *Hyla japonica* Günther, 1859, is a hylid frog occurring on Goto, Hokkaido, Honshu, Iki, Kyushu, Osumi, Shikoku, Tsushima, and Sado islands, Japan (Nakamura and Uéno, 1970) as well as in Korea, central Mongolia, northeastern China, and eastern Russia (Frost, 1985). Uchida (1975) listed, without comment, the cestode *Cylindrotaenia* (=*Baerietta*) *japonica* (Yamaguti, 1938) Jones, 1987, and the nematodes *Rhabdias* (=*Angiostoma*) *bufonis* (Schrank, 1788) Stiles and Hassall, 1905, and *Cosmocerca japonica* Yamaguti, 1938 as parasites of *H. japonica*. The purpose of this note is to report the results of a survey of gastrointestinal helminths of *H. japonica*.

One hundred four adult (80 male, 24 female) *Hyla japonica* (mean snout–vent length 29.8 ± 3.1 mm SD, range 20–37) were examined. Sixty-nine (55 male, 14 female) were collected at Beppu, Oita Prefecture, Kyushu Island, Japan (33°17′N, 131°26′E ca. 300 m elevation) 7 June 1992, 22 (17 male, 5 female) were collected at Gotemba, Shizuoka Prefecture, Honshu Island, Japan (35°18′N, 138°56′E ca. 476 m elevation) 27 May and 2 June 1993 and 13 (8 male, 5 female) were collected at Odawara, Kanagawa Prefecture, Honshu Island, Japan (35°15′N, 139°10′E ca. 152 m elevation) 20 October 1993.

The body cavity was opened ventrally and the esophagus, stomach, small intestine, and large intestine were slit longitudinally and examined under a dissecting microscope. The liver and body cavity were also examined for helminths. All helminths were identified utilizing a glycerol wet mount. Selected cestodes were stained with hematoxylin and mounted whole in Canada balsam.

Thirty-one of 80 males and 7 of 24 females were parasitized, 38/104 (37% prevalence). *Cylindrotaenia japonica* (11% prevalence, 2.5 mean intensity, range 1–5) and *Oswaldocruzia insulae* Morishita, 1926 (8% prevalence, 1.9 mean intensity, range 1–4) were found in the small intestines while *Cosmocerca japonica* (20% prevalence, 2.1 mean intensity, range 1–5) was found in both the small and large intestines. There was no significant difference in prevalence of infection between male and female frogs (chi-square = 0.35, 1 df, $P > 0.05$).

The Beppu population harbored *Cylindrotaenia japonica* (4 of 55 male frogs, 0 of 14 female frogs), *O. insulae* (6 males, 2 females), and *Cosmocerca japonica* (5 males). Two males harbored co-parasites: 1 *Cylindrotaenia japonica* and 2 *O. insulae* in one and 1 *Cylindrotaenia japonica* and 1 *Cosmocerca japonica* in the other. The Gotemba population harbored only *Cosmocerca japonica*; 16 of 22 (13 of 17 males, 3 of 5 females) were infected as compared to 5 of 69 from Beppu. The Odawara population harbored only *Cylindrotaenia japonica* 7 of 13 (5 of 8 males, 2 of 5 females) as compared to 4 of 69 from Beppu. The prevalence of infection for the 3 populations was statistically different (chi-square = 8.65, 2 df, $P < 0.05$).

Selected helminth specimens were placed in 70% alcohol in glass vials and deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705); *Cylindrotaenia japonica* Beppu (83291), Odawara (83395); *Cosmocerca japonica* Beppu (83292), Gotemba (83338); *O. insulae* Beppu (83293). All frog specimens were deposited in the herpetology collection of the Natural History Museum of Los Angeles County

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Table 1. Amphibian hosts from Japan for the helminths recovered from *Hyla japonica*.

Helminth	Host	N	Prevalence (%)	Reference
Cylindrotaenia japonica	*Hyla japonica*	—	—	Uchida, 1975
		—	—	Jones, 1987
		104	11	This note
	Rana japonica	—	—	Uchida, 1975
		—	—	Jones, 1987
	Rana ornativentris	—	—	Jones, 1987
	Rhacophorus schlegelii	—	—	Uchida, 1975
	Rhacophorus viridis	— a*	—	Jones, 1987
Cosmocerca japonica	*Buergeria japonica*	23 a	13	Hasegawa, 1989
	Bufo japonicus	—	—	Uchida, 1975
	Bufo melanostictus	—	—	Uchida, 1975
	Cynops ensicauda	94 a	3	Hasegawa, 1989
	Hyla japonica	—	—	Yamaguti, 1938
		104	20	This note
	Microhyla ornata	12 a	92	Hasegawa, 1989
	Polypedates leucomystax	9 a	11	Hasegawa, 1989
	Rana ishikawae	4 a	25	Hasegawa, 1989
	Rana japonica	—	—	Yamaguti, 1938
	Rana limnocharis	135 a	41	Hasegawa, 1989
		6 b	33	Hasegawa, 1989
		7 c	57	Hasegawa, 1989
		4 d	100	Hasegawa, 1989
	Rana narina	40 a	30	Hasegawa, 1989
	Rana nigromaculata	—	—	Yamaguti, 1938
	Rana ornativentris	—	—	Uchida, 1975
	Rana rugosa	—	—	Yamaguti, 1938
Oswaldocruzia insulae	*Bufo japonicus*	—	—	Uchida, 1975
	Bufo gargarizans miyakonis	20 b	95	Hasegawa, 1989
	Bufo japonicus formosus	—	60	Morishita, 1926
	Hyla japonica	104	8	This note
	Rana rugosa	—	—	Morishita, 1926

* a = From Okinawa; b = from Miyako; c = from Iriomote; d = from Yonaguni islands.

(Beppu LACM 140421–140489; Gotemba 140784–140805; Odawara 140888–140900).

None of the parasites found in this study are unique to *H. japonica* (Table 1). *Cylindrotaenia japonica,* a parasite of the small intestine, has a limited geographical distribution and has been reported only from anurans. Although nothing is known of its life cycle, Joyeux (1924) considers the life cycle of *Cylindrotaenia americana* Jewell, 1916 to be direct with infection occurring when a contaminated fecal pellet is swallowed by a frog.

Cosmocerca japonica, a parasite primarily of the rectum, has a similar geographical distribution pattern to *Cylindrotaenia japonica* but has been reported from a greater number of anurans. Hasegawa (1989) suggested a synonymy of *Cosmocerca japonica* with *C. ornata* (Dujardin, 1861) Diesing, 1861, which has been found in all biogeographic realms except the Nearctic and Australian (Baker, 1987); but further review is necessary before this synonymy can be accepted. The life cycle of *C. japonica* is not known. However, the life cycle of *Cosmocera commutata* (Diesing, 1851) Diesing, 1861 (=*Cosmocerca kashmirensis* Fotedar, 1959 sensu Baker, 1987) was studied by Fotedar and Tikoo (1968). Eggs hatched in 2–4 hr. Larvae penetrated the skin of *Bufo viridis* and migrated through the viscera reaching the lungs 3 days postinfection and the rectum 10–14 days postinfection.

Oswaldocruzia insulae, a parasite of the small intestine, is more limited in geographical distribution than the other 2 species reported here and is restricted to Japan (Baker, 1987). It should be noted that Travassos (1937) synonymized *O. insulae* and *O. socialis* Morishita, 1926 with *O. filiformis* (Goeze, 1782) Travassos, 1917 which has a wide distribution in Europe; but this synonymy requires confirmation (Baker, 1987). The life cycle of *O. insulae* is not known, however Baker (1978) reported that in *Oswaldocruzia pip-*

iens Walton, 1929, development to infective larvae occurred in fecal pellets with transmission to new hosts by skin penetration. *Hyla japonica* is a new host record for *O. insulae.*

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Research Note

Glypthelmins pennsylvaniensis (Trematoda: Digenea) in the Spring Peeper, *Pseudacris c. crucifer* (Anura: Hylidae), from Southwestern West Virginia

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ABSTRACT: Fifty-one of 120 northern spring peepers, *Pseudacris c. crucifer* (Wied-Neuwied 1838), collected from 3 different habitats in Wayne County, West Virginia were infected by *Glypthelmins pennsylvaniensis* Cheng, 1961. The lowest mean intensity (2.5) of infection was recorded from hosts in a temporary ditch habitat, while the highest mean intensity (8.9) was recorded for hosts collected in 1 of 2 marsh sites. There was no significant difference between the size (as weight) of infected versus uninfected hosts. There was a slight negative correlation between host weight and the number of *G. pennsylvaniensis* individuals present (i.e., larger hosts had fewer trematodes), but the regression coefficient was not significant (i.e., b = 0).

KEY WORDS: *Glypthelmins pennsylvaniensis, Pseudacris c. crucifer,* spring peeper, West Virginia.

The northern spring peeper, *Pseudacris c. crucifer,* is a small anuran that ranges from Ontario, Quebec, and southeastern Manitoba, south to northern Florida and eastern Texas (Green and Pauley, 1987). This springtime breeder is abundant in areas of brushy growth near small temporary or semipermanent ponds or swamps. There are no reports of parasites from this host species in West Virginia. After recovering specimens of *Glypthelmins pennsylvaniensis* Cheng 1961, from a small sample population of spring peepers in Wayne County, West Virginia, we set out to broaden our study to determine preva-

Table 1. Prevalence and mean intensity of *Glypthelmins pennsylvaniensis* infections in spring peepers from Wayne Co., West Virginia.

Collection site	No. hosts examined	% prevalence/ no. hosts infected	Mean intensity (SE)
BFD	24	8.3/2	2.5 (NC†)
BFM	46	52.2/24*	8.9‡ (1.28)
SHM	50	50.0/25*	2.6‡ (0.41)

* $\bar{x} = 0.0007$, 1 df, $P > 0.05$.

† NC = not calculated because only 2 hosts, both gravid females, were infected.

‡ Means are significantly different ($P < 0.05$).

Figure 1. Scatter plot of infected host weight with numbers of *Glypthelmins pennsylvaniensis* present. Each symbol on the plot represents a single infected host. Closed circles indicate BFM and BFD males; closed squares indicate BFM and BFD females; closed triangles indicate SHM males. X̄ represents mean host weight (in grams). Ȳ represents intensity (as mean number of trematode individuals per infected host). Curved dashed lines represent 95% confidence limits around the regression line.

lence rates, intensity of infections, and the relationship between host size and numbers of this trematode species at 3 different collection sites (i.e., habitats) in the county mentioned above. All 3 western West Virginia collection sites can be found on the Lavalette, West Virginia quadrangle, USGS topographic map, photorevised 1989. Site #1 was a grass-lined drainage ditch located approximately 0.4 km downstream from the Beech Fork Lake Dam (BFD). Water accumulation in this shallow, 50 m long by 1 m wide ditch, was temporary (approximately 3 wk duration in late March to mid-April 1993). The entire BFD host sample population of 24 individuals was collected on the evening of 27 March 1993. Site #2 was a ≅0.5-ha marsh located at the headwaters of Stower's Branch of Beech Fork Lake (BFM), a U.S. Army Corps of Engineers flood control/recreation reservoir. This site is flooded from April through October (a summer pool). Dominant vegetation at BFM is soft rush (*Juncus effuses*) and cattails (*Typha latifolia*). The sample population of 48 BFM hosts was collected on the evenings of 21 and 25 April 1992. Site #3 located at Shoals, West Virginia (SHM), approximately 6 km north northwest of Site #2, is a permanently flooded marsh covering an area of ≅0.8 ha. A dense cover of buttonbush (*Cephalanthus occidentalis*) lined the perimeter of SHM, with bladderwort (*Litricularia gibba*) located extensively throughout the open water. A host sample population of 50 individuals was collected from SHM on the evenings of 17 and 19 April 1993.

Spring peepers from all 3 sites were collected by hand, placed in 4-liter screw-cap jars (no more than 10 animals per jar) containing moist paper toweling, and returned to the laboratory within 1 hr of capture. Hosts were maintained in a refrigerator at 4°C, no longer than 24 hr. Upon removal from the refrigerated jar, each host was weighed to the nearest tenth of a gram on a Mettler BB300 balance, then killed by pithing. At necropsy, hosts were sexed, and all trematodes found in the small intestine of each host were removed, placed on slides, then killed and fixed with 10% buffered formalin under light coverslip pressure. Selected trematode specimens were stained with Semichon's acetocarmine, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted in Permount®. Two voucher specimens of *G. pennsylvaniensis* are deposited in the U.S. National Museum Parasite Collection (Beltsville, Maryland 20705) under Helminth Collection Number 83429.

A total of 283 *G. pennsylvaniensis* individuals were recovered from 51 of 120 spring peepers, *P. c. crucifer,* examined from the 3 Wayne Co., West Virginia sites described previously. Prevalence of infection for hosts collected in the ditch (i.e., BFD habitat) was obviously lower than prevalences for hosts in either semipermanent (BFM) or permanent (SHM) marsh conditions (Table 1). While differences in prevalences between hosts in the 2 marsh habitats were insignificant ($\chi^2 = 0.0007$), the mean intensity of infection in BFM hosts was significantly higher (t = 4.782, 47 df, $P < 0.05$) than that found in their SHM counterparts (Table 1). Our observed prev-

Table 2.   Mean size (as weight in grams) of hosts infected with *Glypthelmins pennsylvaniensis* versus uninfected hosts. Weights of males and females were combined to calculate means and standard errors.

Collection site	Infected hosts			Uninfected hosts		
	No. of ♂♂	No. of ♀♀	Mean (SE)	No. of ♂♂	No. of ♀♀	Mean (SE)
BFD	0	2	3.74 (—)	19	3	2.14 (0.096)
BFM	22	2	1.68 (0.107)	21	1	1.75 (0.070)
SHM	25	0	1.70 (0.050)	25	0	1.85 (0.046)

alences for *G. pennsylvaniensis* in *P. c. crucifer* from West Virginia marshes are higher than the 11.4% and 38% recorded by Muzzall and Peebles (1991) from 2 marsh areas in Michigan. Mean intensities of *G. pennsylvaniensis* infection found in hosts from the ditch (BFD) and permanent marsh (SHM) were relatively low (Table 1), but the mean intensity (± 1 SD) of 8.92 (6.29) in temporary marsh (BFM) hosts is virtually identical to the 9.0 (6.1) recorded by Muzzall and Peebles (1991).

Comparisons between infected versus uninfected host weights at both marsh sites revealed no statistical differences (Table 2). Then too, there was no significant relationship ($b = 0$; $t = -1.79$, 49 df, $P > 0.05$) between size of infected hosts and the number of trematodes present (Fig. 1). Any predictive value of y relative to its corresponding value of x is obviously unreliable, as evidenced by the scatter around the regression line (Fig. 1) and the calculated *r*-value of -0.248. This lack of correlation between host size and intensity of infection was also referred to by Muzzall and Peebles (1991) who noted that, "There were also no distinct increases in infection for each helminth species ... with frog length."

Glypthelmins pennsylvaniensis was described by Cheng (1961) from spring peepers in Pennsylvania. Since then this trematode species has been reported in *Pseudacris* spp. from Georgia (Sullivan and Byrd, 1970), Wisconsin (Coggins and Sajdak, 1982), and Michigan (Muzzall and Peebles, 1991).

We thank Daniel Brooks, University of Toronto, for confirming our identification of *G. pennsylvaniensis*; Jeffrey May, Marshall University, for critically reviewing our manuscript; and Lance Fontenot, Clemson University, for his helpful suggestions.

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Obituary Notice

Paul C. Beaver

died December 23, 1993.

Elected to Life Membership, 1986

Research Note

Gastrointestinal Nematodes of the Cuban Treefrog, *Osteopilus septentrionalis* (Hylidae) from San Salvador Island, Bahamas

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ABSTRACT: The gastrointestinal tracts of 72 *Osteopilus septentrionalis* were examined for helminths. Five nematode species were present: *Oswaldocruzia lenteixeirai, Parapharyngodon osteopili, Physalopteroides bahamensis,* an unidentified female strongylid, and an unidentified larval oxyurid. *Physalopteroides bahamensis* had the greatest prevalence (83%) and highest mean intensity (8.4). This represents the first report of *O. lenteixeirai* and *P. osteopili* in the Bahamas.

KEY WORDS: Hylidae, *Osteopilus septentrionalis*, Nematoda, *Oswaldocruzia lenteixeirai, Parapharyngodon osteopili, Physalopteroides bahamensis,* Bahamas, prevalence.

The Cuban treefrog, *Osteopilus septentrionalis* Duméril and Bibron, 1841 is known from Cuba, the Bahamas, and Cayman Islands and has been introduced in Puerto Rico, St. Croix, St. Thomas, Florida Keys, and mainland Florida (Schwartz and Henderson, 1991). It is widespread but occurs primarily in mesic habitats. To our knowledge, there are 7 previous reports of nematodes from *O. septentrionalis*: Walton, 1940; Barus, 1973; Coy Otero et al., 1980; Adamson, 1981; Coy Otero and Barus, 1982; Coy Otero and Ventosa, 1984, Bursey and Goldberg, 1994. The purpose of this note is to report the gastrointestinal helminths of *O. septentrionalis* from San Salvador Island, Bahamas as part of an ongoing study of the biogeography of helminths in the Caribbean herpetofauna.

Seventy-two *O. septentrionalis* (mean snout–vent length, SVL = 48 mm ± 5.7 SD range 41–66) were hand collected and fixed in 10% formalin at the Bahamian Field Station (24°07′N, 74°28′W, 0 m elevation), San Salvador Island, Bahamas, 7–10 June 1991. The abdominal wall was slit to allow rapid penetration of fixative into the internal organs. Males were more abundant (N = 63) and smaller (mean SVL = 47 mm ± 3.1 SD, range 41–53 mm) than females (N = 9; mean SVL = 60 mm ± 5.6 SD, range 52–66 mm). The specimens were deposited in the herpetology collection of the Natural History Mu-

seum of Los Angeles County (LACM 139733–139804).

The body cavity was opened by a longitudinal incision from throat to vent, and the gastrointestinal tract was removed by cutting across the anterior esophagus and the rectum. The esophagus, stomach, small intestine, and large intestine were examined separately under a dissecting microscope. Recovered helminths were removed and identified utilizing the standard glycerol wet mount procedure.

Three species of nematodes, *Oswaldocruzia lenteixeirai* Pérez Vigueras, 1938, *Parapharyngodon osteopili* Adamson, 1981, and *Physalopteroides bahamensis* Bursey and Goldberg, 1994, were recovered and identified. In addition, 1 gravid female strongylid and 1 larval oxyurid were found but not identified. The female strongylid was 1.75 mm long × 0.046 mm wide, measured at the vulva, which was equatorial in placement. The body was alate throughout its length. The uteri were amphidelphic. The eggs formed a single row within the uterus and measured 30 μm × 40 μm; they were barrel-shaped within the uterus but became oval when released. Because no males were found, identification was not attempted. The esophagus of the larva was typical of oxyurid nematodes, containing a corpus, isthmus, and bulb; a digitiform tail was present. Again, identification was not attempted. Of the nematodes reported here, *P. bahamensis* had the highest prevalence and mean intensity (Table 1). *Physalopteroides bahamensis* was originally described from *O. septentrionalis* from the Bahamas (Bursey and Goldberg, 1994). This is the first report of *O. lenteixeirai* and *P. osteopili* in the Bahamas. Selected specimens were placed in vials of alcohol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *O. lenteixeirai* (82786); *P. osteopili* (82784); *P. bahamensis* (82785); female strongylid (82788); oxyurid larva (82787).

Table 1. Prevalence, mean intensity (range), and location of nematodes from 72 *Osteopilus septentrionalis* from San Salvador Island, Bahamas.

Parasite	Prevalence (number infected/ number examined, %)	Mean intensity (range)	Location*
Oswaldocruzia lenteixeirai	31	3.8 (1–25)	b, c
Parapharyngodon osteopili	79	5.8 (1–24)	c, d
Physalopteroides bahamensis	83	8.4 (1–31)	a, b, c, d
Unidentified oxyurid	1	1.0	d
Unidentified strongylid	1	1.0	c

* a = Esophagus, b = stomach, c = small intestine, and d = large intestine.

The 12 nematode species previously recorded for *O. septentrionalis* are listed in Table 2. *Oswaldocruzia lenteixeirai* is known from 17 species of amphibians and 22 species of reptiles from Cuba and has also been reported from Puerto Rico (see Baker, 1987). The prevalence we report here for *O. lenteixeirai* is similar to that reported previously by Coy Otero and Ventosa (1984). *Parapharyngodon osteopili* is known only from Cuba and from a single *O. septentrionalis* as reported by Adamson (1981). Our findings extend the range of these 2 species to the Bahamas. *Physalopteroides bahamensis* is known only from San Salvador Island, Bahamas. It is differentiated from the closely related *Physalopteroides valdesi,* reported only from Cuba where it occurs in 2 eleutherodactylid frogs and an anole (Baker, 1987), by the distribution of caudal papillae (*P. bahamensis* has 8 precloacal, 2 paracloacal, 8 postcloacal; *P. valdesi* has 10 precloacal, 2 paracloacal, 6 postcloacal) and spicule size and form (the spicules of *P. bahamensis* are unequal in size and form and are approximately one-half the length of the equal spicules of *P. valdesi*). In addition, *P. bahamensis* is roughly twice the length of *P. valdesi*.

The other nematodes reported from *O. septentrionalis* may occur at such low prevalences (Table 2) that our sample size might not account for them. Alternatively, there may be some aspect of the biogeography of these helminths that prevents their occurrence in *O. septentrionalis* in the Bahamas. For example, *Batracholandros bassi* is currently known only from Cuba but has been found in 15 species of frogs (Barus, 1973; Coy Otero and Ventosa, 1984). Its life cycle is apparently unknown, although we would expect a typical oxyurid life cycle with direct infection by ingestion of eggs (Anderson, 1992). We did recover a larval oxyurid; the prevalence was similar to that reported by Coy Otero and Ventosa (1984) for unidentified larval oxyurids in *O. septentrionalis* from Cuba.

Cosmocercoids are parasites of the intestine of amphibians and reptiles (Anderson, 1992). Females of *Aplectana* spp. produce larvated eggs that hatch outside the host to develop into infective third-stage larvae with the final host becoming orally infected (Anderson, 1992). With the exception of Cuba, species of *Aplectana* are unknown from Caribbean islands. *Hammerschmidtiella diesingi,* a parasite of cockroaches (Coy Otero et al., 1980), apparently represents an incidental occurrence resulting from ingestion with prey and is not typically found in *O. septentrionalis.*

The definitive hosts of species from the anisakid genus *Contracecum* are piscivorous birds and mammals (Anderson, 1992). Unembryonated eggs pass out in the feces of the host. Subsequent development and hatching as second-stage larvae occur in water. Larvae are ingested by a wide variety of invertebrate hosts especially copepods (see Norris and Overstreet, 1976), which in turn are fed upon by fish. Larvae are thought to pass from 1 fish intermediate host to another through predation and reinvasion of tissues of the new host.

Species from the ascarid genus *Porrocaecum* are widely distributed parasites of the intestine of birds (Anderson, 1992). Eggs hatch after ingestion by earthworms; a large number of earthworm species are known as intermediate hosts (see Supriaga, 1972). Shrews and other small mammals that consume earthworms act as paratenic hosts, capable of transferring *Porrocaecum* to definitive carnivorous bird hosts; species in birds that do not consume small mammals are infected directly from ingesting earthworms (Anderson, 1992).

Table 2. Previously reported nematodes of *Osteopilus septentrionalis*.

Parasite	Prevalence (number infected/ number examined, %)	Mean intensity (range)	Site*	Locality	Reference
Dioctophymatoidea					
Eustrongylides sp. (larvae)	Not given	Not given	—	Cuba	Walton, 1940
Oxyuroidea					
Batracholandros bassi	Not given	Not given	d	Cuba	Walton, 1940
	33 (4/12)	Not given (7–50)	d	Cuba	Barus and Moravec, 1967
	56 (98/175)	8.0 (not given)	d	Cuba	Coy Otero and Ventosa, 1984
Parapharyngodon osteopili	100 (1/1)	43.0	d	Cuba	Adamson, 1981
Larval oxyurids	1 (1/175)	17.0 (not given)	b	Cuba	Coy Otero and Ventosa, 1984
Cosmocercoidea					
Aplectana sp.	1 (1/175)	3.0 (not given)	d	Cuba	Coy Otero and Ventosa, 1984
Aplectana hamatospicula	2 (3/175)	3.6 (not given)	d	Cuba	Coy Otero and Ventosa, 1984
Hammerschmidtiella diesingi	100 (1/1)	19.0	b	Cuba	Coy Otero et al., 1980
Ascaridoidea					
Contracaecum sp. (larvae)	1 (1/175)	6.0 (not given)	b	Cuba	Coy Otero and Ventosa, 1984
Porrocaecum sp. (larvae)	2 (3/175)	2.3 (not given)	b	Cuba	Coy Otero and Ventosa, 1984
Physalopteroidea					
Abbreviata sp. (larvae)	2 (3/175)	1.7 (not given)	b	Cuba	Coy Otero and Ventosa, 1984
Physalopteroides valdesi	1 (2/175)	2.0 (not given)	b	Cuba	Coy Otero and Ventosa, 1984
Filarioidea					
Foleyellides brachyoptera	1 (1/175)	4.0 (not given)	e	Cuba	Coy Otero and Ventosa, 1984
Trichostrongyloidea					
Oswaldocruzia lenteixeirai	58 (7/12)	Not given (1–15)	b, c	Cuba	Barus and Moravec, 1967
	34 (59/175)	2.5 (not given)	c	Cuba	Coy Otero and Ventosa, 1984

* a = Esophagus, b = stomach, c = small intestine, d = large intestine, and e = body cavity.

Likewise, piscivorous birds (Chitwood, 1969) are the definitive hosts for the dioctophymatid genus *Eustrongylides*. Freshwater oligochaetes are thought to be the first intermediate host and fish are assumed to be the second intermediate host, although numerous paratenic hosts have been identified (see Bursey, 1986). *Osteopilus septentrionalis* apparently can serve as a paratenic host for these nematodes.

Physalopterids occur mainly in the stomach of reptiles, birds, and mammals; rarely in amphibians and fishes (Anderson, 1992). Only larval *Abbreviata* sp. are known from *O. septentrionalis* (Table 2). *Abbreviata ranae* was described from larvae recovered from amphibians (*Rana catesbeiana, Rana utricularia sphenocephala, Bufo woodhousii, Hyla cinerea*) of North America and has been reported by a number of authors, but is considered by Baker (1987) to be a species inquirenda and may, in fact, represent more than 1 physalopterid species. Larval physalopterids, but not adults, are frequently encountered in frogs, toads, salamanders, and a few species of lizards (Goldberg et al., 1993). Because insects serve as intermediate hosts, diet may be important in the initial infection; however, some internal mechanism may produce an unfavorable environment that prevents survival to adult stage (Goldberg et al., 1993). Because these physalopterids do not occur in cysts and are apparently short lived, we do not consider the herptiles in which they are found to be paratenic hosts.

Species of the genus *Folleyellides* are parasites of amphibians (Baker, 1987). *Folleyellides brachyoptera* is known only from North America (*R. utricularia sphenocephala*) and Cuba (*Peltaphryne peltacephala, O. septentrionalis*) (see Baker, 1987). Larval stages develop in mosquitos (Causey, 1939). The factors responsible for the distribution of nematodes among different is-

land/mainland amphibian populations have not been elucidated and warrant subsequent investigation.

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Editor's Acknowledgment

Research Note

Helminths of the Western Lesser Siren, *Siren intermedia nettingi* (Caudata: Sirenidae), from Arkansas

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ABSTRACT: Sixteen juvenile and adult western lesser sirens, *Siren intermedia nettingi* Goin, 1942, were collected from 2 counties in Arkansas and examined for endoparasites. Eleven (69%) sirens harbored 1 or more parasites, including 1 (6%) with *Diplostomum* sp. metacercariae, 9 (56%) with *Proteocephalus sireni,* 1 (6%) with larval *Capillaria* sp., and 2 (13%) with *Falcaustra chabaudi.* New locality records are documented for *P. sireni* and *F. chabaudi,* and new host records are reported for *Diplostomum* sp. and *Capillaria* sp. The histopathology of the larval *Capillaria* integumental infection is described in the new host. A summary is provided on helminths of *Siren* spp.

KEY WORDS: Amphibia, Arkansas, *Capillaria* sp., Caudata, *Diplostomum* sp., *Falcaustra chabaudi,* histopathology, intensity, prevalence, *Proteocephalus sireni,* Sirenidae, *Siren intermedia nettingi,* survey.

The western lesser siren, *Siren intermedia nettingi* Goin, 1942, is a large eel-like amphibian that ranges through the Mississippi River Valley east to western Alabama and west to eastern Texas (Martof, 1973; Conant and Collins, 1991). Much is known about the natural history and ecology of this salamander (Martof, 1973), including information on its helminths (Nickol, 1972; Dunagan and Miller, 1973; Dyer, 1973; Brooks and Buckner, 1976; Brooks, 1978; Buckner and Nickol, 1979). Except for Louisiana populations, these reports concern sirens from more northern parts of the range in Illinois. Herein, we report on helminths of a small sample of *S. i. nettingi* from Arkansas and provide a summary of helminths of North American *Siren* spp.

A total of 16 juvenile and adult specimens of *S. i. nettingi* (mean ± SE snout–vent length [SVL] = 180.2 ± 85, range 53–290 mm) were collected alive between October 1990 and March 1991, and again during January 1993, with minnow traps, dip nets, or by hand at wetland sites in Montgomery ($N = 13$) and Clay ($N = 3$) counties of Arkansas and examined for endoparasites. Methods for necropsy, coccidial isolation, and preparation and staining of blood films and helminths follow those used by McAllister and Upton (1987). Voucher specimens of hosts are deposited in the Arkansas State University Museum of Zoology (ASUMZ). Specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Diplostomum* sp. (USNM 82843), *Proteocephalus sireni* (USNM 82844), *Capillaria* sp. (USNM 82845), *Falcaustra chabaudi* (USNM 82842).

Eleven sirens (mean ± SE SVL = 189.7 ± 20.3, range 53–290 mm) were infected with 1 or more helminths, including *Diplostomum* sp. metacercariae, *Proteocephalus sireni,* larval *Capillaria* sp., and *Falcaustra chabaudi.* Two sirens harbored multiple infections of either larval *Capillaria* sp. and *P. sireni* or *Diplostomum* sp. metacercariae and *P. sireni.* Sirens were negative for coccidians, blood hematozoans, and myxozoans.

Numerous strigeoid metacercariae of the diplostomulum type were free in the coelomic and pericardial cavities of a single *S. i. nettingi* (SVL = 252 mm) collected in February 1991 from Montgomery County. The diplostomula appeared identical to the description of the metacercarial stage of *Diplostomum variable* (Chandler, 1932) Dubois, 1937 (=*Didelphodiplostomum variable* (Chandler, 1932) Dubois, 1944 (see Harris et al., 1967). This present finding represents a new host record for *Diplostomum* sp.

A total of 39 tapeworms fitting the description of *Proteocephalus sireni* (Brooks and Buckner,

Table 1. Summary of helminths of *Siren* spp.

Host taxon/parasite	Locality	Reference(s)
Siren intermedia nettingi		
Trematoda		
Allassostomoides louisianensis	Illinois	Brooks and Buckner, 1976
Diplostomum sp.	Arkansas	This report
Progorgodera foliata	Illinois	Brooks and Buckner, 1976
Cestoidea		
Proteocephalus sireni	Arkansas	This report
	Illinois	Brooks and Buckner, 1976
	Louisiana	Brooks, 1978
Acanthocephala		
Fessisentis fessus	Illinois	Landewe, 1963; Nickol, 1972; Dunagan and Miller, 1973
	Louisiana	Nickol, 1972; Buckner and Nickol, 1979
Neoechinorhynchus sp.	Illinois	Miller and Dunagan, 1971
Nematoda		
Capillaria sp.	Arkansas	This report
Falcaustra chabaudi	Illinois	Dyer, 1973
	Arkansas	This report
Siren intermedia texana		
Nematoda		
Contracaecum sp.	Texas	McAllister and McDaniel, 1992
Siren lacertina		
Trematoda		
Cephalaogonimoides sireni	Florida	Premvati, 1969
Diplostomum variabile	*	Harris et al., 1967
Gorgodera minima	Louisiana†	Bennett and Humes, 1938;
	Florida	Brooks and Buckner, 1976
Lechriorchis sp.	Louisiana†	Bennett and Humes, 1938
Progorgodera foliata	Florida	Brooks and Fusco, 1978
Stomatrema guberleti	Florida	Brooks and Buckner, 1976
Telorchis sireni	*	Zeliff, 1937
T. stunkardi	Florida	Brooks and Buckner, 1976
Cestoidea		
Proteocephalus sp.	Louisiana†	Bennett and Humes, 1938;
	Florida	Loftin, 1960
P. aberrans	Florida	Brooks, 1978
Nematoda		
Brevimulticaecum tenuicolle	Louisiana†	Bennett and Humes, 1938
Cosmocercoides dukae or *C. variabilis*	Florida	Walton, 1938; Baker, 1987
Falcaustra catesbeianae	Florida	Walton, 1938
Siren sp.‡		
Trematoda		
Cephalogonimus amphiumae	Florida	Manter, 1938
Clinostomum marginatum	Florida	Manter, 1938
Diplostomulum sp.	Florida	Manter, 1938

* Data on locality not provided in reference.

† Sirens collected from the campus of Louisiana State University, Baton Rouge, Louisiana, and reported originally to be *S. lacertina*; however, its range does not include Louisiana (Conant and Collins, 1991). In all probability the host is *S. intermedia*.

‡ Species of siren not reported. The ranges of *S. intermedia, S. lacertina, Pseudobranchus axanthus,* and *P. striatus* include Florida (Conant and Collins, 1991).
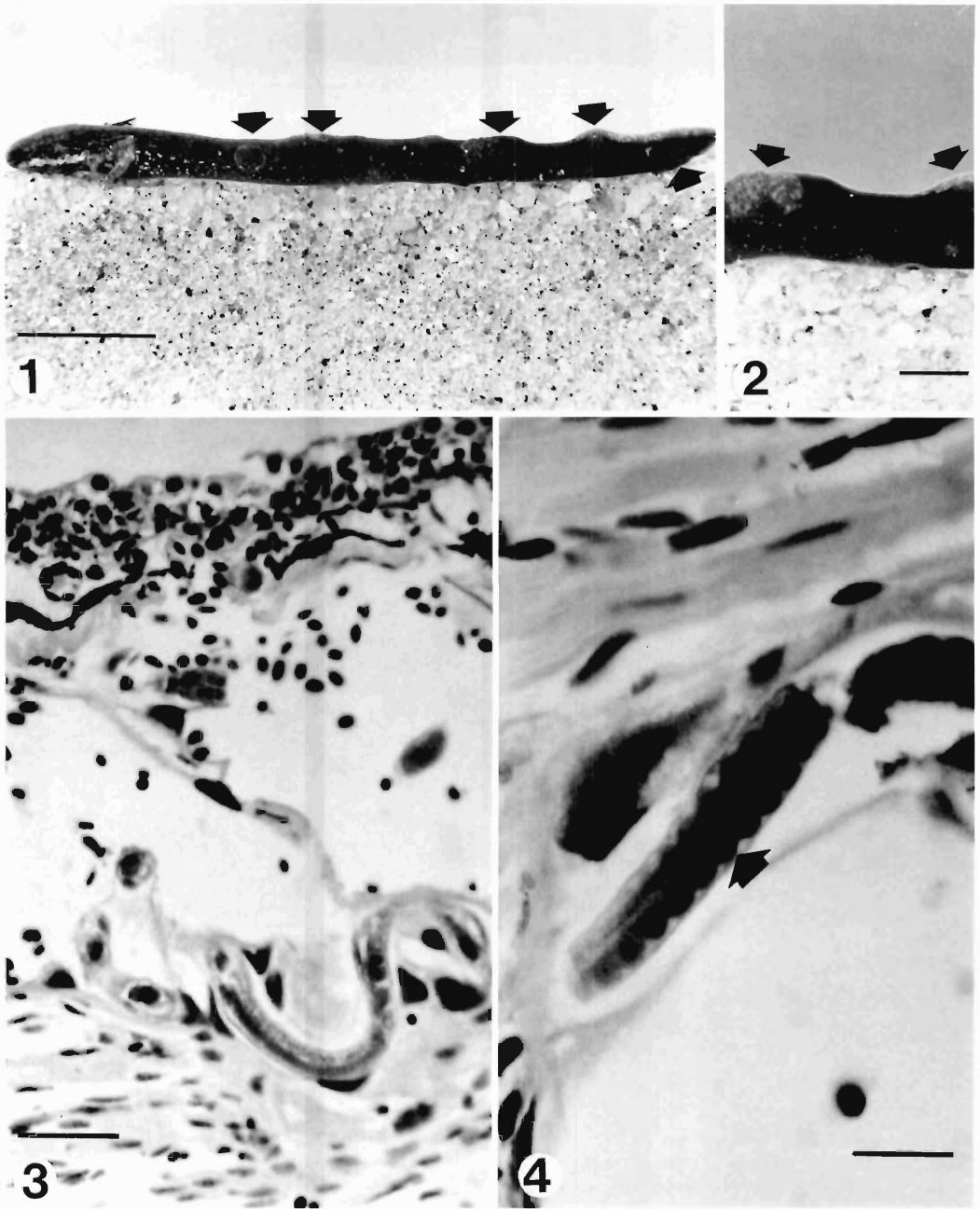
Figures 1–4.   Larval *Capillaria* sp. infecting the integument of a juvenile *Siren intermedia nettingi* (ASUMZ 16992) from Montgomery County, Arkansas. 1. View of siren showing raised and discolored areas on integument (arrows) infected with larvae. Scale bar = 15 mm. 2. Closer view showing areas of infection on integument. Scale bar = 7.5 mm. 3. Section of skin showing parasite in wall of dermal capillary with severe ectasia of superficial vasculature as well as degeneration and edema in overlying epidermis. Scale bar = 100 μm. 4. Closer view of parasite in dermal vessel. Note stichostome (arrow). Scale bar = 35 μm.

1976) Brooks, 1978, were recovered from the duodenum of 9 sirens (173.6 ± 20.7, 53–252 mm) collected only at the Montgomery County site; mean intensity was 4.3 ± 1.4 (range 1–15) worms. This cestode has been reported previously from *S. i. nettingi* in Illinois and Louisiana (Table 1).

Four kathlaniid nematodes, *Falcaustra chabaudi* Dyer, 1973, were in the rectum of 2 *S. i. nettingi* (235 and 290 mm SVL) collected in February 1991 from Clay County; mean intensity was 2.0 ± 1.0 (range 1–3) worms. This is the first report of *F. chabaudi* from Arkansas. Dyer (1973) described *F. chabaudi* from 2 *S. i. nettingi* in southern Illinois.

A single juvenile *S. i. nettingi* (SVL = 53 mm; ASUMZ 16992) collected in October 1990 from the Montgomery County site was infected with larval *Capillaria* sp. in the integument (Figs. 1, 2). The epidermis of ASUMZ 16992 showed separation of the pigment layer from the underlying basement membrane with moderate hydropic change at the dermal interface. In the dermis there was marked edema with dilation of numerous vascular channels. The larvae adhered to the walls of these dilated vessels (Fig. 3) while provoking no discernable inflammatory response. Dermal connective tissue was unaffected by the presence of this parasite. The stichostome, typical of trichuroid nematodes (Noble et al., 1989), is shown in Figure 4.

A summary of the helminths of *Siren* spp. is presented in Table 1. There appears to be some host specificity among helminths from different siren taxa, particularly among trematodes of lesser and greater sirens. Although *S. intermedia* and *S. lacertina* may be found in sympatry, they appear to be partitioned by habitat differences. The former tends to inhabit more acidic pH waters while the latter is found in aquatic sites with circumneutral pH (P. E. Moler, pers. comm.). This may help explain differences in the trematode faunas of the 2 species as intermediate hosts may also be partitioned in the same manner.

We thank the Arkansas Game and Fish Commission for Scientific Collecting Permits (issued to S.E.T. and B.G.C.), Dr. Steve J. Upton for examining samples for coccidia, and Paul E. Moler for information on sirens.

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Research Note

New Host and Distribution Record of *Raillietina (Raillietina) coreensis* (Cestoda) from *Apodemus argenteus* (Rodentia) in Japan

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ABSTRACT: *Raillietina (Raillietina) coreensis* Honda, 1939 is redescribed from the small intestine of *Apodemus argenteus* (Rodentia: Muridae) in Hokkaido, Japan. This report represents a new host and distribution record for *R. coreensis* in the host and in Japan.
KEY WORDS: *Raillietina (Raillietina) coreensis,* cestode, Davaineidae, *Apodemus argenteus,* rodent, Japan.

Cestodes of the genus *Raillietina* Fuhrmann, 1920 have been reported from various rodents in the tropical and subtropical zones. However, there are few records of *Raillietina* from *Apodemus* Kaup, 1829 (Rodentia). We obtained *Raillietina (Raillietina) coreensis* Honda, 1939 from a small Japanese field mouse, *Apodemus argenteus* (Temminck) in Hokkaido, Japan, representing a new host and distribution record for the parasite.

Cestodes were collected from the small intestine of an *A. argenteus* (female, 10–18 mo old) captured at Abuta, Hokkaido, Japan in October 1991. Worms were lightly pressed, fixed in 70% ethanol, stained with acetocarmine, dehydrated in an ethanol series, cleared in xylene, and mounted in MGK® (Matsunami Glass Ind., Ltd., Japan). All measurements in fixed specimens are in micrometers unless otherwise indicated and given as a range with the mean in parentheses.

Raillietina coreensis Honda, 1939
(Figs. 1-3)

Redescription (based on 2 specimens): Total body length 61 and 114 (88) mm, maximum width 2 mm. Scolex 363 and 554 (459) long by 264 and 528 (396) wide. Four suckers oval, 106–123 (117) long by 92–115 (102) wide, with numerous hooks 7–8 long, arranged diagonally with about 7 hooks per row on the inside of the sucker. Rostellum 86–99 (93) wide. Most of rostellar hooks lost during processing. One remaining hook hammer-shaped and 13 long. Proglottids trapezoidal. Mature proglottids 132–238 (180) long by 785–1,465 (1,033) wide. Genital pore unilateral, usually located in anterolateral position in mature proglottids; some situated near middle of margin. Testes 24–29 (27) in number lying on

1

2

3
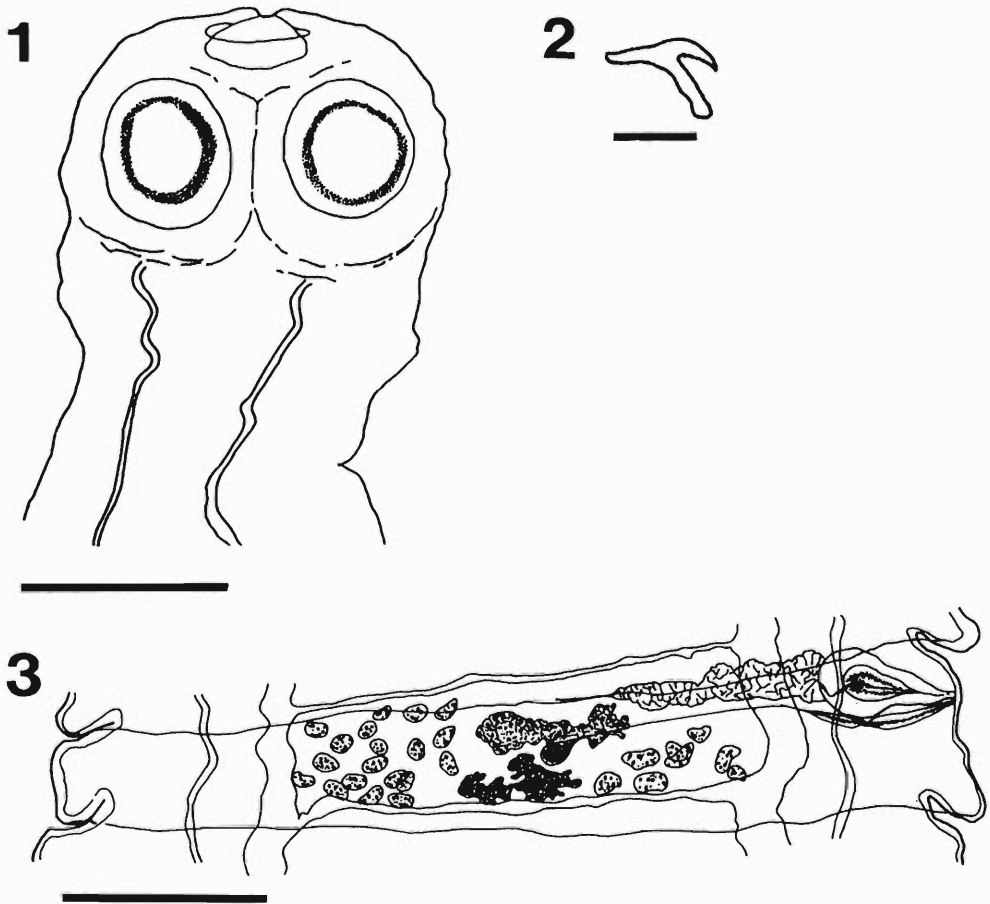
Figures 1-3.   1. Scolex. Scale = 0.2 mm. 2. Rostellar hook. Scale = 10 μm. 3. Mature proglottid. Scale = 0.2 mm.

both sides of mature proglottids. Cirrus sac 92–119 (101) long by 43–59 (52) wide. Ovary is bilobed, situated almost at center of proglottid. Vitelline gland situated posterior to ovary. Gravid proglottids wider than long, containing 102–190 (136) egg capsules. Six to 12 (8) eggs are found in each capsule. Spherical oncospheres, 11–16 (13) in diameter, lie nearly in center of eggs.

HOST: *Apodemus argenteus.*
SITE: Small intestine.
LOCALITY: Abuta, Hokkaido, Japan.

SPECIMENS DEPOSITED: Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, No. P 721.

Several species of *Raillietina* in rodents have been reported in Japan. Miyazaki (1950) reported *Raillietina madagascariensis* (Davaine,

1869) from *Rattus norvegicus* (Berkenhout) in Kagoshima. Kamiya et al. (1968) found *Raillietina celebensis* (Janicki, 1902) in *R. norvegicus* and *Rattus rattus* (Linnaeus) in southern Amami. Goto and Nishimura (1988) found *Raillietina* sp. in *Apodemus speciosus* (Temminck) in Aomori. In Korea, Honda (1939) described *R. coreensis* in *Apodemus agrarius coreae* (Thomas) at prevalence of about 3%. Seo et al. (1968) also reported this species from *A. agrarius* (Pallas) and *Microtus fortis pellceus* Thomas in southern Korea, but with no morphological description.

Our specimens were identified as *R. coreensis* based on form of mature proglottids, position of genital pore, measurements for suckers (109 μm in Honda's description), and cirrus sac (96–129 long by 40–56 wide), presence of hooks in suckers, number of testes (26–28), and number of eggs in capsule (7–13). Though we were unable to

count number of rostellar hooks in our specimens Honda (1939) noted that there were 80. *Raillietina* sp. of Goto and Nishimura (1988) shows similar measurements to Honda's (1939) and ours; thus, it may also be identified as *R. coreensis.*

Though both Miyazaki (1950) and Kamiya et al. (1968) reported *Raillietina* from *Rattus* in southern parts of Japan, specimens of ours and of Goto and Nishimura (1988) from *Apodemus* were obtained in the far north of the country. It suggests that cestodes in *Apodemus* have a different distribution and host range than those in *Rattus.* Including this study, *R. coreensis* has been reported from 2 species of *Apodemus.* We suggest that *R. coreensis* has a close host–parasite relationship with *Apodemus* in east Asia.

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Research Note

Helminth Parasites of Ringed Seal, *Phoca hispida,* from Northern Quebec, Canada

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ABSTRACT: Five ringed seals, *Phoca hispida* Schreber, 1775 collected by Inuit hunters near Salluit (Quebec) in eastern Arctic Canada were examined for helminths. Four nematodes, *Acanthocheilonema spirocauda* (Leidy, 1858), *Otostrongylus circumlitus* (Railliet, 1899), *Filaroides (Parafilaroides) krascheninnikovi* Yurakhno and Skrjabin, 1971, and *Phocascaris phocae* Höst, 1932; 2 acanthocephalans, *Corynosoma strumosum* (Rudolphi, 1802) and *C. reductum* (von Linstow, 1905); 2 cestodes, *Diplogonoporus tetrapterus* (von Siebold, 1848) and *Anophryocephalus* sp., were found. New geographic records of *A. spirocauda, P. krascheninnikovi, P. phocae,* and *D. tetrapterus* are reported.

KEY WORDS: parasitic helminths, ringed seal, *Phoca hispida,* Arctic Canada, *Acanthocheilonema spirocauda, Otostrongylus circumlitus, Filaroides (Parafilaroides) krascheninnikovi, Phocascaris phocae, Corynosoma strumosum, Cornyosoma reductum, Diplogonoporus tetrapterus, Anophryocephalus* sp.

The ringed seal, *Phoca hispida* Schreber, is still an important "country food" in some Inuit communities in Arctic Canada. It is the most common and most widely distributed Arctic seal and has a circumpolar distribution. Its helminth fauna in eastern Arctic Canada has been poorly studied (Cooper, 1921; Lyster, 1940; Myers 1957a, b). Recently, Kennedy (1986) and Onderka (1989) studied lungworms of ringed seals in western Arctic Canada. During some preliminary work with ringed seals in northern Quebec various helminths were collected and the present study reports our findings.

Five ringed seals were collected by Inuit hunters in inshore waters of Hudson Strait near Salluit (Quebec), Canada (62°13′N, 75°39′W) 25–28 August 1992. From each animal standard length, axial girth, maximum girth, blubber thickness, body weight, and sculp weight were measured (American Society of Mammalogists, 1967). Sculp weight is the weight of the skin with

attached blubber dissected from the body begin-
ning at the proximal end of the hind flippers to
a point midway between the eyes and external
ear openings. The lower jaw was collected for
determination of age. Thin transverse sections
of canine teeth were cut and growth layers count-
ed with transmitted light (McLaren, 1958). Four
of five ringed seals examined were young-of-the-
year (YOY). They were probably 4–5 mo old as
ringed seals give birth in March or April in east-
ern Arctic Canada (McLaren, 1958; Smith, 1973).
The stomachs of all ringed seals were full and
contained crustaceans identified as *Mysis oculata*
(O. Fabricius, 1780). The lungs, trachea, heart,
stomach, and intestine were examined for hel-
minths. The muscle and other organs and tissues
were kept by the hunters. Nematodes were fixed
in hot 10% glycerin–alcohol (9 parts 70% alco-
hol : 1 part glycerin) and cleared by evaporation.
Acanthocephalans and cestodes were fixed in 10%
buffered formalin and stained with Semichon's
acetocarmine (Pritchard and Kruse, 1982). Thick
transverse sections of some cestode proglottids
were made by hand using a razorblade. Mea-
surements and morphological study of helminths
were made using a Leitz Diaplan microscope
equipped with a drawing tube interfaced with a
digitizer tablet and computer. Nomenclature of
pinnipeds follows Honacki et al. (1982).

The following ringed seals were examined: Seal
1 was a YOY male weighing 14 kg with a total
length of 72 cm; Seal 2 was a YOY female, 18
kg, 79 cm; Seal 3 was a YOY male, 28 kg, 94
cm; Seal 4 was a YOY male, 15 kg, 81 cm; Seal
5 was an adult female, 10 yr old, 30 kg, 110 cm.
Parasites found in each of these seals are indi-
cated in Table 1.

Specimens of all helminths were deposited in
the Canadian Museum of Nature, Ottawa, On-
tario, Canada K1P 6P4 and National Parasite
Collection, Beltsville, Maryland, U.S.A. 20705-
2350 (Table 1).

We report parasites not previously reported in
ringed seals from the eastern Arctic, specifically
northern Quebec.

Acanthocheilonema spirocauda (Leidy, 1858)
(=*Dipetalonema spirocauda*) (2 males and 3 fe-
males) was found in the right ventricle of Seal 2.
No gross lesions associated with *A. spirocauda*
were observed. This heartworm has been re-
ported from most phocid seals of the Holarctic
including ringed seals (Dailey, 1975). It has also
been reported from the California sea lion (*Zal-
ophus californianus* (Lesson)) (Taylor et al., 1961).

Table 1. Helminths found in ringed seals collected from Salluit, Quebec.

Helminth	Seal no. infected	Museum specimens deposited*
Acanthocheilonema spirocauda	2	CMNP1993-0050
Otostrongylus circumlitus	1, 2, 4	CMNP1993-0051
Parafilaroides krascheninnikovi	2, 3, 4, 5	CMNP1993-0052
Phocascaris phocae	2, 3, 4	CMNP1993-0043
Corynosoma strumosum	1, 2, 3, 4, 5	CMNP1993-0044 and CMNP1993-0046
Corynosoma reductum	1, 2, 3, 4, 5	CMNP1993-0045 and CMNP1993-0047
Diplogonoporus tetrapterus	1, 2, 4, 5	CMNP1993-0048 and CMNP1993-0049
Anophryocephalus sp.	2, 3	CMNP1993-0031 to CMNP1993-0037 USNM82826–82828

* Museum numbers given are inclusive.

The allocation of specimens from the northern
fur seal (*Callorhinus ursinus* (Linnaeus)) to *A.
spirocauda* are in doubt (see Anderson, 1959;
Perry, 1967). The present study is the first report
of this parasite in Arctic Canada. The sole report
of *A. spirocauda* in Canada is from a captive
harbour seal (*Phoca vitulina* Linnaeus) in Nova
Scotia (McClelland, 1980). This parasite is known
to cause cardiovascular and pulmonary arterial
lesions and in severe infections may cause oc-
clusion of arteries (Dunn and Wolke, 1976).

Otostrongylus circumlitus (Railliet, 1899) was
present in Seals 1, 2, and 4. Mean intensity (range)
of *O. circumlitus* was 34 (7–77). Cephalic ex-
tremities of these worms, in right and left lungs,
were attached deep in the parenchyma and as-
sociated with thick white to yellow mucus. Cau-
dal extremities extended anteriorly within the
lumen of bronchioles and bronchi to the trachea.
Left and right lungs were equally infected. The
sex ratio of female to male worms was 1.8–2.0:
1.0 in Seal 4 and 1, respectively, and 1.0:1.3 in
Seal 2. All *O. circumlitus* were adults and females
were gravid. This important lungworm has been
reported in ringed seal from western Arctic Can-
ada (Onderka, 1989) and from southeastern Baf-
fin Island in eastern Arctic Canada (Smith et al.,
1979). The present study confirms the latter re-
port. *Otostrongylus circumlitus,* which has a hol-
arctic distribution, has been reported in other pho-
cids and otariids (Dailey, 1975). This parasite
induces extensive pulmonary mucus secretion as

seen in the present study, mucosal hyperplasia, verminous pneumonia, and obliterative bronchitis (Stroud and Dailey, 1978). It has been suggested that *O. circumlitus* may affect health and recruitment of seals. Young-of-the-year seals are infected predominantly (Smith et al., 1979; Onderka, 1989). In the present study only YOY ringed seals were infected. All morphometrics of infected YOY seals ($N = 3$) measured were less than that of the single uninfected YOY seal. For example, infected seals had total body weights 50, 54, and 64% of the total weight of the uninfected seal. In addition, condition indices (body weight/standard length × 100) of the former were less than that of the uninfected YOY seal. In a larger study, Onderka (1989) did not observe a difference in condition factor (axillary girth/standard length × 100) or standard length of infected compared to uninfected ringed seals. "Stunted" ringed seals may also result from pups being born in suboptimum habitat (McLaren, 1958; Finley et al., 1983) or from poor nourishment during lactation and postweaning (Smith, 1987).

Another lungworm, *Filaroides (Parafilaroides) krascheninnikovi* Yurakhno and Skrjabin, 1971, was found in the lung parenchyma of Seals 2, 3, 4, and 5. Small, white nodules observed in lung parenchyma were associated with this small nematode. Originally reported from ringed seals in the North Pacific Ocean by Yurakhno and Skrjabin (1971), *Parafilaroides krascheninnikovi* has not been reported prior to the present study in seals from eastern North America. However, *Parafilaroides hispidus* Kennedy, 1986 was reported in ringed seal from western Arctic Canada by Onderka (1989). Pathologic lesions associated with species of *Parafilaroides* have been observed in otariids as well as phocids.

Phocascaris phocae Höst, 1932 present in the first 91–122 cm of the small intestine of Seals 2, 3, and 4 had a mean intensity of 32 (3–66). In Seal 4 the intestinal mucosa was eroded in several places where the cephalic extremities of *P. phocae* had been attached. These erosions and 1 group of attached *P. phocae* were aggregated near the beginning of the small intestine. Höst (1932) described mucosal damage of the pyloric wall due to attachment of *P. phocae*. All *P. phocae* were adults except 1, which was in the fourth stage. The sex ratio of female to male worms was 2.0–2.2:1.0. This nematode has a holarctic distribution and has been reported from phocid seals including the ringed seal (see Adams, 1988). In Canada, Lyster (1940) described *P. netsiki* from

ringed seal from eastern Arctic Canada. *Phocascaris* sp. has been reported from ringed seals from eastern Arctic Canada (Myers, 1957a), harp seal (*Phoca groenlandica* Erxleben), and grey seal (*Halichoerus grypus* (Fabricius)) from the southeastern coast of Atlantic Canada (Myers, 1957b; McClelland, 1980; Brattey and Ni, 1992). Brattey (1990) reported *Phocascaris phocae* in harp seals collected off the coast of Newfoundland. The present study thus confirms the previous reports of *P. phocae* in North America and reports *P. phocae* for the first time in ringed seals from eastern Arctic Canada.

Corynosoma spp. were present in the intestine of all seals. From a random subsample of 130 *Corynosoma* collected (some from all seals), 125 *C. strumosum* (Rudolphi, 1802) and 5 *C. reductum* (von Linstow, 1905) were identified. Most female *C. strumosum* were mature with eggs. Both female *C. reductum* were immature, and no eggs were observed. *Corynosoma strumosum* was found throughout the small and large intestine anterior to the cecum. *Corynosoma reductum* was observed only in the region of the large intestine adjacent and posterior to the cecum and in the rectum. *Corynosoma strumosum* is widespread in pinnipeds including ringed seal (Dailey, 1975). It has been reported in ringed seals from eastern Arctic Canada (Lyster, 1940). *Corynosoma reductum*, however, appears to be restricted to ringed seal and has been reported in ringed seals from Baffin Island (Van Cleave, 1953).

Diplogonoporus tetrapterus (von Siebold, 1848) was found in the intestine of Seals 1, 2, 4, and 5 and *Anophryocephalus* sp. was found in the intestine of Seals 2 and 3. No gross lesions associated with acanthocephalans or cestodes were observed. The former cestode, which is holarctic in distribution, has been reported in otariids and phocids including ringed seal (Markowski, 1952). In Canada it has been reported in Steller sea lions (*Eumetopias jubatus* (Schreber)) from the Pacific coast (Margolis, 1956) and *Diplogonoporus* sp. has been reported in bearded seals and harp seals from the Atlantic coast (Margolis and Arai, 1989). Thus the present study reports *D. tetrapterus* in eastern Canada for the first time.

An examination of the *Anophryocephalus* specimens found in ringed seals in the present study revealed a previously undescribed species, the description of which is to be published elsewhere (Hoberg, pers. comm). *Anophryocephalus anophrys* Baylis, 1922 was reported from a harp seal collected off the coast of Newfoundland

(Smith and Threlfall, 1973) and McClelland (1980) found *Anophryocephalus* sp. in a captive harbour seal in Nova Scotia. The latter report is in doubt (see Hoberg et al., 1991). The infection of the harp seal with *A. anophrys* is considered incidental (Hoberg and Adams, 1992). Species of *Anophryocephalus* presently known from ringed seals are *A. anophrys* and *A. skrjabini* (Krotov and Delyamure, 1955) with *A. anophrys* known from the Subarctic to Arctic of the Atlantic Basin (Hoberg et al., 1991; Hoberg, 1992).

Of the various species of parasites found in the present study only *A. spirocauda* and the lungworms, *O. circumlitus* and *P. krashcheninnikovi*, are likely to be important as etiological agents of disease in seal populations. The lungworms may be important in predisposing seals to secondary lung infections as seen in the bighorn sheep lungworm pneumonia complex (Bergstom and Honess, 1982; Claussen et al., 1991). Managers of ringed seal populations should be aware that these lungworms may affect survival or recruitment of young seals to local populations. Certainly more data on the importance of these lungworms to ringed seal populations are needed.

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Obituary Notice

Robert Jennings Chinnis
died April 12, 1994.

Elected Member December 12, 1984
Executive Board 1987–1988

Research Note

Effect of Differential Permeability to Plasma Proteins on Localization of *Leptorhynchoides thecatus* (Acanthocephala) in Green Sunfish, *Lepomis cyanellus* (Centrarchidae)

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ABSTRACT: An Evans blue dye injection technique was employed to compare vascular leakage into various regions of the alimentary canal of uninfected and *Leptorhynchoides thecatus*-infected green sunfish, *Lepomis cyanellus*. Results revealed no significant difference in permeability to dye among cecal, between cecal, and intestinal regions. Additionally, *L. thecatus* appeared to have neither a systemic nor local effect on mucosal permeability as there was no significant difference in leakage between infected and uninfected fish or between occupied and unoccupied ceca within infected fish. Consequently, differential permeability of mucosal regions to plasma proteins is unlikely to be a factor in the localization of *L. thecatus* in green sunfish.

KEY WORDS: *Leptorhynchoides thecatus*, parasite localization, *Lepomis cyanellus*, plasma proteins.

Leptorhynchoides thecatus (Linton, 1891) has been shown in laboratory infections of *Lepomis cyanellus* Rafinesque, 1819 (green sunfish) (Uznanski and Nickol, 1982) and *Micropterus salmoides* (Lacepède, 1802) (largemouth bass) (Leadabrand and Nickol, 1993) to localize in the pyloric ceca and the region of the alimentary canal immediately at their base (between ceca area). After initial establishment along the intestinal tract, worms that do not gain access to these favored sites by 3 wk postinfection are lost from green sunfish (Ewald and Nickol, 1989) or migrate into extraintestinal sites in largemouth bass (Leadabrand and Nickol, 1993). Attributes of the ceca and between cecal area that render them suitable for *L. thecatus* are unknown.

A histological study of the ceca and anterior intestine of green sunfish (Williams and Nickol, 1989) revealed no difference to suggest differences in function that could account for the localization of *L. thecatus*. The bile duct was found to enter through 1 of the pyloric ceca, but distribution and maturation of worms could not be shown to vary among ceca of green sunfish (Ewald and Nickol, 1989) or largemouth bass (Leadabrand and Nickol, 1993).

Szalai et al. (1988) demonstrated leakage of plasma proteins in acanthocephalan-infected fish and suggested that plasma leakage provides nutrition for the parasites. Thus, the possibility that differences in permeability to plasma proteins between the ceca and intestine could account for the localization of *L. thecatus* in the ceca of green sunfish was investigated.

Green sunfish were seined from various sites in Lancaster County, Nebraska, where *L. thecatus* is known to be absent. Nine individuals were fed 15 cystacanths each by the procedure described by Leadabrand and Nickol (1993). Infections were allowed to persist for 1 wk, a time that permitted establishment of worms but not their localization solely within ceca. Nine uninfected fish served as the control group.

A 0.1% (g/ml) solution of Evans blue dye in Ringer's solution for cold-blooded vertebrates was prepared to serve as a marker for plasma protein. The injection procedure was applied to fish one at a time. To inject dye solution, a fish was anesthetized to the point of immobilization in 3.8 liters of fresh water containing 0.8 g of tricaine methanesulfonate. The fish then was weighed, and 0.4 ml of dye solution per kilogram of body weight was injected slowly into the subcutaneous region just behind the posterior end of the spinal cord by use of a 20-cc syringe and a 26-gauge needle. The fish was returned to its tank, and every 5 min its gills were checked for the appearance of dye. When the gills appeared completely blue, the fish was killed.

The intestinal tract was removed, cut along its ventral surface, and pinned open. Any worms present were removed and their locations recorded. Color photographic slides of the intestinal tract were taken immediately following each dissection. Slides were compared and rated blindly for the intensity of blue dye in the mucosae of the ceca; between ceca area; and anterior, middle, and posterior thirds of the intestine. The rating scale ranged from 0 to 4 in one-half-unit increments. A rating of one-half corresponded to the lightest intensity of blue dye observed

Table 1. Mean ratings (±SD) for dye leakage in the alimentary tracts of *Lepomis cyanellus* uninfected or infected with *Leptorhynchoides thecatus*.*

Infection status	Ceca	Between ceca	Intestine
Uninfected fish†	1.6 ± 1.5 (0.0–3.5)	2.4 ± 1.6 (0.0–4.0)	2.5 ± 1.2 (0.5–4.0)
Infected fish†			
Occupied and unoccupied sites combined	1.1 ± 1.0 (0.0–4.0)	2.5 ± 1.1 (1.0–4.0)	1.8 ± 0.8 (0.0–3.5)
Unoccupied ceca‡	1.2 ± 1.0 (0.0–3.0)		
Occupied ceca‡	0.9 ± 1.0 (0.0–4.0)		

* Seven fish in uninfected group, 7 fish in infected group, and ranges in parentheses.

† Means not significantly different with respect to fish infection status and site using 2 × 3 factorial analysis of variance, $P \leq 0.05$.

‡ Means not significantly different within fish using paired t-test, $P \leq 0.05$.

among fish; a rating of 4 corresponded to the darkest intensity. Although applied judgmentally, ratings were assumed to reflect absolute levels of dye intensity in intervals of equal magnitude that were consistent across fish. Ceca were rated individually, then these measurements were pooled across ceca and means were calculated. Subdivisions of the intestine were treated in an identical fashion. Within infected fish, unoccupied and occupied ceca were pooled separately and compared. Means and standard deviations presented in Table 1 are pooled ratings; ranges are unpooled values. Two infected and 2 uninfected fish that failed to circulate dye were excluded from the analysis.

Because Evans blue dye binds readily to plasma proteins, particularly albumin (Rawson, 1943), permeability of the epithelium to dye is assumed to reflect leakage of plasma proteins. Although the green sunfish intestines were not tested for the presence of free dye, in rats and quillback injected with similar concentrations (Nawa, 1979; Szalai et al., 1988, respectively), all dye that entered the intestinal tract was determined to be bound to protein. Results of the study (Table 1) reveal no significant difference in mucosal permeability with respect to site. Additionally, *L. thecatus* appears to have neither a systemic nor local effect on mucosal permeability, because there was no significant difference in dye leakage between infected and uninfected fish (systemic effect) or between occupied and unoccupied ceca within infected fish (local effect). Consequently, differential permeability of mucosal regions to plasma proteins, either parasite induced or uninduced by parasites, is unlikely to be a factor in the localization of *L. thecatus* in green sunfish.

David W. T. Crompton asked the question and suggested the technique that stimulated this study. The study was supported, in part, by the Arthur William Sampson Fund.

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Research Note

Growth of *Naegleria* with Insulin

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ABSTRACT: The growth factor insulin was added to axenic cultures of pathogenic *Naegleria fowleri* and nonpathogenic *Naegleria gruberi* to determine whether it would enhance the growth of amebae. The growth of *N. fowleri* was not affected by the presence of insulin; however, *N. gruberi* was inhibited by 100 μg/ml insulin. Based on these results, *N. fowleri* could tolerate the levels of insulin in the brain and especially that of the olfactory bulbs, the tissues invaded, which have much higher concentrations of insulin than the serum.

KEY WORDS: *Naegleria fowleri, Naegleria gruberi,* insulin, growth, culture.

Naegleria fowleri Carter, 1970 is a pathogenic free-living ameboflagellate and the cause of a fatal human disease known as primary amebic meningoencephalitis (reviewed by John, 1993). *Naegleria gruberi* Schardinger, 1899 (Fulton, 1970), a nonpathogenic relative of *N. fowleri,* is a common and widely distributed ameba in freshwater and terrestrial habitats (Page, 1988). The hormone insulin has been used as a growth promoter with a variety of cell types in culture (Ellis, 1989). The purpose of this study was to determine whether insulin would enhance the growth of *N. fowleri* and *N. gruberi* in culture.

The LEE strain of *N. fowleri,* originally isolated from cerebrospinal fluid by E. Clifford Nelson in Richmond, Virginia, in 1968 (Duma et al., 1971), was obtained from Clifford Nelson. Amebae were grown axenically in Nelson's medium (Nelson and Jones, 1970; for composition see Weik and John, 1977) in 25-cm² polystyrene tissue-culture flasks (Corning Glass Works, Corning, New York). Cultures were inoculated with 1 × 10⁵ amebae and incubated at 37°C. The NEG strain of *N. gruberi,* derived from the EG strain (Fulton, 1970), originally isolated from soil by Frederick L. Schuster in Berkeley, California, in 1960 (Schuster, 1969), was kindly supplied by Chandler Fulton. Amebae were adapted to grow at 37°C by gradually increasing the incubation temperature from 30°C over a period of months and were cultivated in Mix ameba medium (John, 1993) in 25-cm² tissue-culture flasks (Corning). Mix ameba medium is an equal mixture of Bal-

amuth's (Balamuth, 1964) and Nelson's media. Cultures were inoculated with 1 × 10⁵ amebae and incubated at 37°C. Cell counts were made with a Coulter counter (model Z_{BI}; Coulter Electronics, Inc., Hialeah, Florida) using settings described elsewhere (John and John, 1989).

Insulin, purchased from Sigma Chemical Company (St. Louis, Missouri), was solubilized in 1 M acetic acid and added to Nelson's and Mix ameba media, containing reduced calf serum (1%), at concentrations of 1, 10, or 100 μg/ml. The serum was reduced from 2% in Nelson's medium and 4% in Mix ameba medium in order to make any changes in growth more marked. The control cultures contained medium, 1% calf serum, and 1% acetic acid, the concentration of acetic acid equivalent to that present in cultures containing 100 μg/ml insulin. Triplicate cultures were prepared of each concentration of insulin and of controls.

Figures 1 and 2 show the growth of *N. gruberi* and *N. fowleri,* respectively, in the presence of varying concentrations of insulin. *Naegleria gruberi,* the nonpathogen, was affected by the addition of insulin to the medium. Concentrations of 1 μg and 10 μg/ml insulin had little effect on the growth of *N. gruberi*—1 μg may have slightly increased growth and 10 μg/ml slightly decreased growth—however, 100 μg/ml inhibited growth (Fig. 1). In contrast, the growth of *N. fowleri* was not affected by the addition of insulin to the medium, even at a concentration of 100 μg/ml (Fig. 2).

The working range of insulin in cell culture media is 0.001–20 μg/ml (Sigma, 1991). For example, H-Y medium, a medium specifically designed to support hybrid cells in culture, requires 8.3 μg/ml insulin. Thus, the 2 lower concentrations used in the present study, 1 μg and 10 μg/ml, were within the range of added media supplements. However, these quantities would be considerably higher than the levels of insulin that occur in the blood, approximately 0.008 μg/ml (Dittmer, 1961), and would be considered su-

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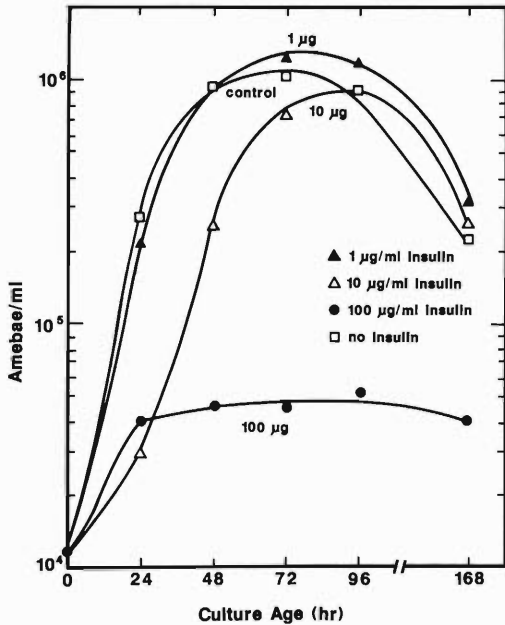
Figure 1. Growth of *Naegleria gruberi* (NEG) in Mix ameba medium with insulin at 37°C. Each point represents the average of 9 counts for triplicate cultures.

Figure 2. Growth of *Naegleria fowleri* (LEE) in Nelson's medium with insulin at 37°C. Each point represents the average of 9 counts for triplicate cultures.

praphysiological concentrations, as would concentrations greater than 0.1 µg/ml (Koontz and Iwahashi, 1981).

The results presented here show that the growth of *N. fowleri* was unaffected by the addition of insulin to the medium, even at concentrations of 100 µg/ml. Insulin is present in the mammalian central nervous system and in the cerebrospinal fluid (Plata-Salamán, 1991) and there is accumulating evidence for its de novo synthesis by neurons in the brain (Schechter et al., 1992). Experimental studies in the rat have demonstrated that insulin levels in the brain averaged 25 times higher than plasma levels, with the olfactory bulbs and hypothalamus having concentrations 75–100 times higher than plasma (Havrankova et al., 1978). Thus, for an organism such as *N. fowleri* to invade via the nasal mucosa and olfactory bulbs it would have to tolerate substantial concentrations of insulin. Although the growth of *N. fowleri* was not affected by high concentrations of insulin, nonpathogenic *N. gruberi* was inhibited by such concentrations.

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Research Note

Identification of a *Haemonchus placei*-Specific DNA Probe

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ABSTRACT: A partial DNA library was generated from *Haemonchus placei* and differentially screened to identify clones containing repetitive and species-specific sequences. A DNA sequence, which hybridized with *H. placei* genomic DNA by dot-blot analysis and did not hybridize with *Haemonchus contortus* DNA, was identified and characterized. The probe, designated pHp3, was sequenced and found to be 723 bp in length, constituting 0.34% of the *H. placei* genome. The pHp3 probe is useful in differentiating the morphologically similar parasites *H. placei* and *H. contortus.*

KEY WORDS: *Haemonchus placei, H. contortus,* DNA probe, species specific.

Haemonchus is a genus among trichostrongyle nematodes that parasitizes the abomasum of ruminants. The different *Haemonchus* species (9–10 species have been recognized [Gibbons, 1979; Lichtenfels et al., 1993]) develop in a variety of domesticated and wild ruminant hosts, with *Haemonchus placei* and *Haemonchus contortus* being species typically found in cattle and sheep,

respectively (see Lichtenfels et al., 1986). Another species, *H. similis,* occurs in cattle especially in southern North America, Central and South America, but it is easily distinguished morphologically from *H. contortus* and *H. placei.* Mixed infections with the 2 morphologically similar species, *H. placei* and *H. contortus,* occur in both domesticated hosts, leading to discussion of the validity of 2 species. Some authors (Gibbons, 1979) have synonymized *H. contortus* and *H. placei* while others (Le Jambre, 1979, 1981; Lichtenfels et al., 1986, 1993) have provided evidence for the recognition of both species. Lichtenfels et al. (1986, 1988, 1993) have described morphological characteristics in detail for identification of individual worms of the 2 species, i.e., cuticular ridge patterns and spicule lengths. These characters make it difficult to differentiate female worms without training and require the recovery of adult worms and hence killing of the host.

In the current report, we describe the development and application of an *H. placei*-specific DNA probe, which can discern *H. placei* and *H.*

pHp3   **rDNA**
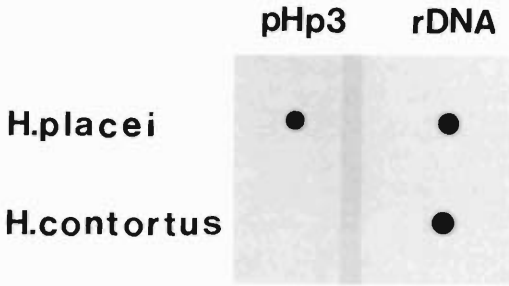
Figure 1.   Dot-blot analysis demonstrating genus specificity of cloned repetitive sequence, pHp3. Genomic DNA (~0.2 μg) of *H. placei* and *H. contortus* was heat denatured, vacuum filtered onto Nytran^R membranes, and screened with radiolabeled probes (pHp3; rDNA). The probes were hybridized overnight to the blots at 65°C in hybridization solution (0.6 M NaCl, 0.5% SDS, 1% Denhardt's solution (80×), 10 μg/ml salmon sperm DNA, 10 μg/ml heparin) then washed (2 × 30 min) in 0.1% SDS and 0.2× SSC (1× SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) at 60°C.

contortus, and which may additionally be adapted to an antemortem test to differentiate *Haemonchus* eggs excreted in the feces, thereby identifying the presence of *H. placei* in infected animals.

The pHp3 probe was developed using methods described by Christensen et al. (1994). Differential screening of a *H. placei* genomic DNA library, using radiolabeled *H. placei* and *H. contortus* genomic DNA, was performed to identify clones containing repetitive and species-specific sequences. Reactive clones were selected and rescreened with radiolabeled homologous genomic DNA to select the clones giving the strongest reactions.

To verify the specificity of pHp3 for *H. placei,* equivalent amounts (approximately 0.2 μg) of genomic DNA from *H. placei* and *H. contortus* were heat-denatured and blotted onto duplicate Nytran^R membranes. The blots were screened

pHp3

```
  1      TATGTCCTGT AGAGGAGAAA ATACCATAAA ATCGATGCTC CTGAACGAAG GTGTTTCAGA

 61      GAATGAGAAA AGCTGAGTGG AAACTAGCAC AGAAAGCGAG ATCCCGTTCC TAGCTCCAAA

121      TAGTGCATGG CTCATTCGTA GACGGTCTTG GGGAAGCCAT CGAAATGGTA CAGGGCTTCC

181      GATACCTGAA AAGTACTTGC CGATGATGGC AGCGTGGACC AGGCGGTAAA AGCCAGAGTA

241      ATCGAGGCGT GGATGAGGTG GAGGGAATTC ACCGTTATCC TCTACGACCA TAGGTGCTTA

301      GGAGTCAGAG GAAAGGGATA CCAAACAGTA GTTAGGCCCA TGCAGTGGCT GGCAGCCGAG

361      ATGGTGCTAG GAGGAGGGAG GGAGACAGAC AGGACCCTTC TACGCGGTCG CGTCGAGCCC

421      ATGTCCCTTC GAAGCTGAAA ACAAGGAGAA ACAAGATCAG AACAATTTGC CTTTGCCCTT

481      TTTCCCTTGG GCGCGACGCG ACCGCGTTGA AAGGTCCCGT CTGCCTCCTC TCCCACCCAG

541      CACTACCTCG GCGGCACGCC GGCACGCCGG TCGGCCGGAC TATGAAAGCA GCCAGTGCTT

601      TACGGCAGCG AATGTTGGCC GTTGGTAAAC ACATGAAAGA CAACTGCACT CTGCTGAAAT

661      GAGGATGTTG CGGTTGGAAG CGACTGGATG GGATCCGTGA AGTGCTACGA GAACTAATTT

721      GAG
```

Figure 2.   Nucleotide sequence of *H. placei*-specific repetitive sequence, pHp3.

with radiolabeled probes (Feinberg and Vogel-stein, 1983) of either pHp3 DNA or ribosomal DNA (rDNA) (Zarlenga et al., 1994). The blots were washed under high stringency conditions (60°C, 0.2× SSC, 0.1% SDS) to reduce nonspecific hybridizations. The pHp3 probe reacted only with *H. placei* genomic DNA, whereas the rDNA probe hybridized equally to genomic DNA from both *H. placei* and *H. contortus,* thus verifying the presence of equivalent amounts of genomic DNA on the filter (Fig. 1). The specificity of the pHp3 probe was tested and confirmed using *H. placei* and *H. contortus* laboratory strains maintained at Beltsville, Maryland, and 3 different *H. contortus* strains provided by G. Conder at The Upjohn Company. Previous results (Zarlenga et al., unpubl. obs.) demonstrated that these parasite species (wild type and domestic type) from various geographic locations are genetically similar. Thus, we suggest that this probe will have unilateral application in differentiating *H. placei* and *H. contortus.*

The nucleotide sequence of pHp3 was determined by dideoxy sequencing (Sanger et al., 1977) using the Sequenase™ kit (US Biochemical Corp., Cleveland, Ohio) in both directions employing both forward and reverse *pUC* primers as well as synthetic internal primers. The cloned sequence was 723 bp in length, containing 47% AT, with no notable internal repeats (Fig. 2). The clone constitutes approximately 0.34% of the total genome (data not shown), determined as previously described (Zarlenga et al., 1991).

Techniques similar to those cited above are commonly applied to identify sequences repeated within the parasite genome (Zarlenga et al., 1991; Egwang et al., 1992; Christensen et al., 1994). Such repeats are believed to represent noncoding regions of the genome, which tend to undergo evolutionary change at a faster rate than coding regions. This makes these regions excellent targets for the identification of species-specific sequences (Flavell, 1982; Hammond and Bianco, 1992).

The application of the described probe requires only small amounts of DNA. In addition, radiolabeled pHp3 can be used to identify DNA extracted from *H. placei* eggs (extracted as described by Christensen et al. [1994]). With this probe the presence of *H. placei* in animals can be determined. As such this probe will be very useful in defining the distribution of *H. placei* in cattle herds without killing of selected individuals, and as a research tool to verify the purity of *H. contortus* strains. Complete definition of the distribution of *H. placei* and *H. contortus* will require the identification of a probe specific for both *H. contortus* and *H. placei* as the use of the present probe cannot rule out the presence of *H. contortus* in infected animals.

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Special Offer for Students

The Executive Committee has voted to offer a special one-year $6.00 student subscription to the *Journal*. To be eligible for this special rate, the student's major professor must nominate the student on departmental letterhead and include the letter with the application and $6.00. Mail the completed application to: Dr. Joan E. Jackson, Department of Parasitology, Division of Experimental Therapeutics, WRAIR, Washington, D.C. 20307-5100. An application form may be found at the end of recent numbers of the *Journal*.

Report on the Brayton H. Ransom
Memorial Trust Fund

The Brayton H. Ransom Memorial Trust Fund was established in 1936 to "Encourage and promote the study and advance the Science of Parasitology and related sciences." Income from the Trust currently provides token support of the *Journal of the Helminthological Society of Washington* and limited support for publication of meritorious manuscripts by authors lacking institutional or other backing. Donations or memorial contributions may be directed to the Secretary-Treasurer. Information about the Trust may be found in the following articles: *Proceedings of the Helminthological Society of Washington* (1936) 3:84–87; (1983) 50:200–204; and (1993) 60:144–150.

Financial Report for 1993

Balance on hand, January 1, 1993	$13,143.48
Receipts:	
Interest received in 1993	$884.64
Donations, including a memorial for Frank D. Enzie	$1,025.00
Total	$1,909.64
Disbursements:	
Grant to the Helminthological Society of Washington for 1993	($50.00)
Membership in the American Association for Zoological Nomenclature for 1993	($50.00)
Page Chart Support	($180.00)
Total	($280.00)
Balance on hand, December 31, 1993	$14,773.12

J. Ralph Lichtenfels, Secretary-Treasurer
USDA, ARS, BARC-East, No. 1180
Beltsville, MD 20705-2350

Presentation of the 1993 Anniversary Award to
Dr. Ralph P. Eckerlin

Ralph P. Eckerlin
at the Anniversary Dinner Meeting, October 6, 1993.

Often many of us are asked to serve on committees during our professional society careers, and even though being a committee member is an easy task, the work begins when it is your turn to be chair. I must say that as "my turn" came this year for the Anniversary Awards committee chair, I had an especially easy time. This was due to the suggestions from the committee members of worthy candidates for this prestigious society honor. When a very special nomination is presented for consideration, not much discussion has to take place for a unanimous vote to be quickly reached. Unanimous is the word for this year's Anniversary Award Recipient, and it gives me great pleasure to present Dr. Ralph Eckerlin with that honor.

Dr. Eckerlin is being recognized for this award because of his tireless contributions to the science of parasitology through research and teaching and for his support of the Helminthological Society of Washington and the other many societies to which he also devotes his time.

Ralph was born in New York City and crossed the border into New Jersey to receive his Bachelor of Arts degree from Rutgers University. He then migrated to the University of Miami for his Master's degree. His Master's research was on a trematode and his thesis was titled "Life cycle studies on *Platynosomum fastosum* in South Florida." This is a very special cat liver fluke that has been immortalized in embroidery on the back of a shirt by his wife, Mary. After receiving his M.S., he was the recipient of a National Institutes of Health training grant to study the epidemiology of human parasitism, concentrating on Chagas' Disease. This was his first trip to Costa Rica; he has returned there often.

After Costa Rica, he returned to New York as a Research Biologist with American Cyanamid Co. He developed in vitro and in vivo screening tests for chemotherapy for parasites. After several years in New York, he was awarded an American Cyanamid Co. educational fellowship to work on a Ph.D. at the University of Connecticut. He completed his dissertation on "Studies of the life cycle of *Strongyloides robutus*." He then slightly shifted his interests and worked with the Division of Marine Sciences, University of Connecticut, on mapping coastal wetlands as determined by plant distribution. At the same time, he became an instructor at the Waterbury Branch, University of Connecticut, where he taught zoology.

In the early 1970's, he moved to northern Virginia were he has been a Professor of Biology at Northern Virginia Community College ever since. He has lectured and taught laboratory courses since 1971 in many areas of biology, including general biology, health sciences, human anatomy and physiology, body structure and function, and invertebrate zoology. Of course, Ralph has done

more than just teach. He developed the courses in body structure and function and invertebrate zoology, which are now part of the Virginia state curriculum. He coauthored the biology laboratory manual in use at the multicampus college. To keep himself busy, each fall he teaches parasitology at George Washington University. This has been valuable to the Helminthological Society, as he has brought numerous students to the meetings. He has managed, in his spare time, to sponsor wonderful learning trips for students to Costa Rica to study the local flora and fauna of the country.

The most amazing of his contributions is to professional societies. He is a member of the American Society of Parasitologists, American Society of Tropical Medicine and Hygiene, Helminthological Society of Washington, Association of Tropical Biology, Entomological Society of America, Entomological Society of Washington, International Society for Medical and Applied Malacology, Southeastern Society of Parasitology, Tropical Medicine Society of Washington, Virginia Association for Biological Education, and Wildlife Disease Association. Furthermore, he is a member of the Virginia Academy of Science. As most of us know, however, he is not just a member of these societies but attends meetings regularly and holds or has held offices in most of them.

But tonight, the Helminthological Society of Washington is honoring him, for not only all of the above, but for his tireless participation and service to the society. He has served in about every known capacity of the society from Council Member at Large to President. The greatest service of all, however, has been as Editor of the *Journal of the Helminthological Society of Washington*. He has served as Editor starting in 1988 and completed his term in 1993. Even though Dr. Eckerlin has worn many hats in the society, we know he will always continue his support for "Helm Soc."

On behalf of the Helminthological of Washington and the Anniversary Awards Committee, it gives me great pleasure to present the 1993 Anniversary Award to Dr. Ralph Peter Eckerlin.

Robin N. Huettel
Chair, Anniversary Awards Committee

Honorary Membership Recipients, 1992 and 1993

Louis Euzet (right) receiving the 1992 Honorary Membership certificate from Sherman Hendrix at the Second International Symposium on Monogenea, Sète, France.

John C. Holmes (right) receiving the 1993 Honorary Membership certificate from Sherman Hendrix at the Anniversary Dinner meeting of the Helminthological Society of Washington, October 6, 1993.

MINUTES

Six Hundred Thirty-Seventh Through Six Hundred Fortieth Meeting

637th Meeting: Uniformed Services University of the Health Sciences, Bethesda, MD, 6 October 1993. The Anniversary Dinner Meeting and program was presided over by President Ruth Kulstad. Recognizing the achievements in parasitology of Dr. John C. Holmes of the University of Alberta, Sherman Hendrix, Chairman of the 1993 Honorary and Life Membership Committee, presented Dr. Holmes with a Certificate of Honorary Membership on behalf of the Society. Dr. Robin Huettel, Chairwoman of the Awards Committee, presented the 1993 Anniversary Award to Dr. Ralph P. Eckerlin. The Keynote Speaker for the evening was John Holmes who spoke on "Peregrinations of a Parasitologist at Play." The slate of officers for 1994 was presented: Mark C. Jenkins, President; Eileen D. Franke, Vice President; Joan E. Jackson, Corresponding Secretary-Treasurer; Ellen M. Andersen, Recording Secretary.

638th Meeting: National Institutes of Health, Bethesda, MD, 10 November 1993. Ruth Kulstad presided over the business meeting and Allen Cheever presided over the scientific session. The following papers were presented: Molecular epidemiology of pyrimethamine and proquanil-resistant *Plasmodium falciparum* in Mali, by Chris B. Plow; and T helper cell responses to schistosomiasis, by Edward J. Pearce. The new officers were elected and installed.

639th Meeting: Johns Hopkins University, Montgomery County Campus, Rockville, MD, 6 April 1994. Mark Jenkins presided over the business meeting and Clive Shiff presided over the scientific session. Fifteen individuals were elected to membership. The Executive Committee recommended, and the membership elected, Dr. Louis Diamond and Mary Lou Pritchard to Life Membership. Purnomo, from Indonesia, was elected to Honorary Membership. John Cross gave a talk on Strategies for control of parasitic diseases in modern China, and Ellen Boudreau and Gary Posner gave a two-part presentation on the History and recent advances in antimalarial chemotherapy using Qinghaosu.

640th Meeting: New Bolton Center, University of Pennsylvania, Kennett Square, PA, with the New Jersey Society of Parasitologists, 7 May 1994. Dr. Jay Farrell presided over the scientific program, which consisted of three presentations on aspects of the immune response: Joseph Urban spoke on The role of IgE and immediate hypersensitivity responses to gastrointestinal nematode infections; Thomas Nutman presented Human filarial infection: a model to study IgE regulation; and Phillip Scott reported on The role of IL-12 in Th1 cell development following *Leishmania major* infection. Support for the meeting was provided by SmithKline Beecham Animal Health and the Laboratory of Parasitology, University of Pennsylvania.

The Helminthological Society of Washington welcomed 15 new members at the 639th meeting: Michael Bangs, Jackie Bird, Janet Bjordahl, Wendy Rae Cooper, Rillardo Fiorillo, Maxine Kellman, Marc Labeau, Sandra Marin, Rhonda Pinckney, Carla Siefker, Scott Snyder, Belal Soliman, Reginald Valdez, A. Lee Willingham III, and Jyh-Herng Yen.

Respectfully submitted,

Ellen M. Andersen
Recording Secretary

AUTHOR INDEX FOR VOLUME 61

KEY WORD AND SUBJECT INDEX FOR VOLUME 61

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Journal is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues of $20.00 domestic or $22.00 foreign are payable on notification of election. Send this completed form to:

> Dr. Joan E. Jackson
> Dept. of Parasitology
> Division of Experimental Therapeutics
> WRAIR
> Washington, D.C. 20307-5100

Print name: _____ Date of birth: _____

Mailing address: _____

_____

_____

Degree and year received: _____

Present position: _____

Field of interest: _____

_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
  (a member)

ANNIVERSARY AWARD RECIPIENTS

*Edna M. Buhrer	1960	*Horace W. Stunkard	1977
*Mildred A. Doss	1961	Kenneth C. Kates	1978
*Allen McIntosh	1962	*Everett E. Wehr	1979
*Jesse R. Christie	1964	*O. Wilford Olsen	1980
*Gilbert F. Otto	1965	*Frank D. Enzie	1981
*George R. LaRue	1966	Lloyd E. Rozeboom	1982
*William W. Cort	1966	Leon Jacobs	1983
*Gerard Dikmans	1967	Harley G. Sheffield	1984
*Benjamin Schwartz	1969	A. Morgan Golden	1985
*Willard H. Wright	1969	Louis S. Diamond	1986
Aurel O. Foster	1970	Everett L. Schiller	1987
Carlton M. Herman	1971	Milford N. Lunde	1988
May Belle Chitwood	1972	J. Ralph Lichtenfels	1989
*Elvio H. Sadun	1973	A. James Haley	1990
E. J. Lawson Soulsby	1974	Francis G. Tromba	1991
David R. Lincicome	1975	Thomas K. Sawyer	1992
Margaret A. Stirewalt	1975	Ralph P. Eckerlin	1993
*Leo A. Jachowski, Jr.	1976		

HONORARY MEMBERS

*George R. LaRue	1959	Bernard Bezubik	1980
*Vladimir S. Ershov	1962	Hugh M. Gordon	1981
*Norman R. Stoll	1976	E. J. Lawson Soulsby	1990
*Horace W. Stunkard	1977	Roy C. Anderson	1991
*Justus F. Mueller	1978	Louis Euzet	1992
John F. A. Sprent	1979	John C. Holmes	1993

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	*Benjamin Schwartz	1976
*Albert Hassall	1931	*Mildred A. Doss	1977
*Charles W. Stiles	1931	*Everett E. Wehr	1977
*Paul Bartsch	1937	Marion M. Farr	1979
*Henry E. Ewing	1945	John T. Lucker, Jr.	1979
*William W. Cort	1952	George W. Luttermoser	1979
*Gerard Dikmans	1953	*John S. Andrews	1980
*Jesse R. Christie	1956	*Leo A. Jachowski, Jr.	1981
*Gotthold Steiner	1956	Kenneth C. Kates	1981
*Emmett W. Price	1956	Francis G. Tromba	1983
*Eloise B. Cram	1956	A. James Haley	1984
*Gerald Thorne	1961	Leon Jacobs	1985
*Allen McIntosh	1963	*Paul C. Beaver	1986
*Edna M. Buhrer	1963	Raymond M. Cable	1986
*Benjamin G. Chitwood	1968	Harry Herlich	1987
Aurel O. Foster	1972	Glenn L. Hoffman	1988
*Gilbert F. Otto	1972	Robert E. Kuntz	1988
*Theodor von Brand	1975	Raymond V. Rebois	1988
May Belle Chitwood	1975	Frank W. Douvres	1989
Carlton M. Herman	1975	Thomas K. Sawyer	1989
Lloyd E. Rozeboom	1975	*J. Allen Scott	1990
*Albert L. Taylor	1975	Judith H. Shaw	1990
David R. Lincicome	1976	Milford N. Lunde	1991
Margaret A. Stirewalt	1976	Everett L. Schiller	1991
*Willard H. Wright	1976	Harley G. Sheffield	1991

*Deceased.

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Date of publication, 15 July 1994

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