

Volume 61

January 1994

Number 1

JOURNAL

of

# The Helminthological Society of Washington

*A semiannual journal of research devoted to  
Helminthology and all branches of Parasitology*

Supported in part by the  
Brayton H. Ransom Memorial Trust Fund

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ISSN-1049-233X

∞ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

## Historical Basis of Binomials Assigned to Helminths Collected on Scott's Last Antarctic Expedition

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**ABSTRACT:** Scientific investigations were a feature of Captain R. F. Scott's ill-fated expedition to the South Pole in 1910–1912. Among them was a study of parasitic worms in the coastal wildlife of Antarctica. It was the special project of Surgeon Edward L. Atkinson, whose scientific contributions, like his passion for high adventure, have largely been forgotten. The new parasitic species that he discovered were given names that were intended to honor the Expedition and many of its members. However, it was not then usual for new species descriptions to include an explanation of the proposed new binomials, and the significance of these particular names is not obvious to modern readers. This article examines the historical connection between the names of Atkinson's worms and the individuals and exploits commemorated by those names.

**KEY WORDS:** Antarctica, helminths, history, marine parasites.

One way or another, parasites and parasitologists have been a feature of several Arctic and Antarctic expeditions, and the association between poles and parasites is particularly strong in the case of Captain Scott's famous and fatal *Terra Nova* Expedition to the South Pole. The parasitological contribution of 2 medical members of that expedition, Dr. Edward A. Wilson and Dr. E. Leicester Atkinson, has been discussed elsewhere (Nelson, 1977; Campbell, 1988, 1991). Only passing mention, however, has been made of the new helminth species discovered by Atkinson in the course of his Expedition duties. It is the purpose of this article to examine the historical significance of the names conferred on these worms, which Atkinson brought with him when he sailed back to England with the widow of Edward Wilson. (Mrs. Scott and Mrs. Wilson had traveled to New Zealand to greet their returning husbands, unaware that neither man had survived the trek to the South Pole.)

Apart from a few worms (ascaridids and heartworm) collected from Expedition dogs, the worms in Atkinson's collection were recovered from wildlife taken from the coastal waters of Antarctica or the temperate and tropical islands visited en route. The worms were collected in the period 1 July 1910 to 9 July 1912. Understandably, few dissections were carried out in the months between the demise of Scott and his Polar Party in March 1912 and the departure of the Expedition from Antarctica in January 1913, although Atkinson did undertake a 3-wk parasitological excursion during that time. The parasitological findings were recorded in 3 notebooks,

which are extant and have been described (Campbell, 1991). Parasites were examined microscopically, but apparently cursorily, in Antarctica and were preserved for later study.

Description of the parasites was subsequently carried out at the London School of Tropical Medicine, where Atkinson (who had been recently promoted to Staff Surgeon in the Royal Navy) worked under the tutelage of Robert T. Leiper. On 17 February 1914, Leiper and Atkinson gave a "lantern demonstration" of their helminth specimens at a meeting of the Zoological Society, and an account of the event was published in the same year (Leiper and Atkinson, 1914). The report consists of "diagnoses," or brief descriptions, of the new helminth species, but it does not include illustrations or incidental information. A more complete account was published in the following year (Leiper and Atkinson, 1915), but it, too, did not mention the origin or significance of the names assigned to the parasites.

Because most of the new taxa were named after members of Scott's *Terra Nova* Expedition (or their spouses), we could easily link most new binomials with the appropriate persons. These associations are discussed here on a systematic basis: the Digenea, Cestoda, Acanthocephala, and Nematoda. Atkinson's collection of worms included some species that had been described previously. Leiper and Atkinson (1915) tabulated these species together with species collected by earlier Antarctic expeditions, but these known species will not be considered here. The biographical information in the following notes has

been gleaned from many published accounts of the Expedition, but special reference should be made to Huxley (1978), Ponting (1923), Thomson (1977), and Quartermain (1981).

#### Digenea

##### *Hemiurus oatesi* (= *Elytrophalloides oatesi*) (Leiper and Atkinson, 1914)

This trematode species, from the fish *Trematomus bernacchii* (emerald notothen), also had been placed in *Parahemiurus*, *Plerurus*, and *Elytrophallus* before being established as the type species for the genus *Elytrophalloides*. It is named after Captain L. E. G. Oates of the Sixth Inniskilling Dragoons. As an Army man, he was an exception among the naval personnel of the *Terra Nova* Expedition. He had served heroically in the Boer War. He was devoted to horses and hounds and was responsible for the welfare of the ponies on the Expedition. To join the Expedition, "Titus" Oates had come from India and had contributed 1,000 pounds sterling. He is of special interest in the present context because he and Leicester Atkinson became close friends on the Expedition. On the return journey from the South Pole, Oates became incapacitated by frostbite. To enhance his companions' chances of survival, he left their encampment and walked to certain death in the surrounding snow. His heroic gesture proved futile, but Captain Oates is featured prominently in histories of the Expedition.

##### *Aponurus bowersi* (= *Genolinea bowersi*) (Leiper and Atkinson, 1914)

This trematode was collected from *Trematomus bernacchii*. *Genolinea leiperi* Byrd, 1963 (named for Robert Leiper) as well as other names are junior subjective synonyms of *G. bowersi*. The species is named after Lieutenant Henry Bowers of the Royal Indian Marine, who was 1 of the 2 companions (the other being Wilson) who died with Captain Scott at the final encampment. An intensely loyal and devoutly religious man, Bowers calmly awaited death, full of respect and admiration for Scott and confident that the party's demise was the work of divine providence. Bowers later became the subject of a biography written by Wilson's biographer, the Reverend George Seaver (Seaver, 1938).

##### *Lepodora garrardi* (= *Lepidapedon garrardi*) (Leiper and Atkinson, 1914)

This species was recovered from *Trematomus bernacchii* and is named after Mr. Apsley Cherry-

Garrard, Assistant Zoologist on the *Terra Nova* Expedition. He contributed 1,000 pounds sterling to join the Expedition and served as an unpaid member of the scientific staff. His much-lauded book, *The Worst Journey in the World*, includes an account of the "side trip" that he, Wilson, and Bowers made to Cape Crozier in the middle of winter (Cherry-Garrard, 1951). He commanded a squadron of armored cars on the Western Front in World War I until invalidated home. He subsequently lived a quiet and private life, becoming increasingly exasperated by what he perceived as a loss of moral fiber on the part of his countrymen, and being continually haunted by the realization that he could conceivably have saved the lives of the returning Polar Party (Thomson, 1977; Huxley, 1978). In February 1912, Atkinson, unable to leave base himself, had asked Cherry-Garrard to go south to One Ton Camp in the hope of meeting the returning Polar Party and escorting them back to base. Cherry-Garrard got there, with dogs and dog-driver Gerof, but even after a wait of several days no one else appeared. The supplies would have allowed them to push farther south for perhaps 20 mi, but it would have been useless. We know that 4 of the 5 members of the Polar Party were still alive at that time, but they were more than 70 mi away. Cherry-Garrard could have ventured even farther south if he had killed some of the dogs to feed the other dogs, but this would have been a violation of Scott's standing orders. An emergency might have justified the breaking of rules, but Cherry-Garrard perceived no emergency because the Polar Party would not be considered overdue for another 24 days. In any case, there was a strong possibility that Cherry-Garrard, in proceeding farther south, would miss the north-bound Polar Party and perish along with Gerof and the dogs. He lacked navigational skills, the weather was dangerously cold, the visibility was poor, and Gerof became seriously ill. Cherry-Garrard decided to return to base, and no one doubted the wisdom of that decision—except Cherry-Garrard.

##### *Podocotyle pennelli* (= *Macvicaria pennelli*) (Leiper and Atkinson, 1914)

This fluke, also, was taken from *Trematomus bernacchii*. The species was later transferred to the genus *Plagioporus* and then to *Macvicaria*. It is named after Lieutenant Harry L. L. Pennell, navigator of the *Terra Nova*. He was not a member of the Shore Party but had a crucially important position of leadership; it was his respon-

sibility to get the ship away from Antarctica before the winter freeze and to land and embark the various subsidiary parties. In World War I, during the Battle of Jutland, Pennell (who had been promoted to the rank of Commander) served on HMS *Queen Mary*, the newest and fastest battle cruiser in the Allied fleet. She was sunk by enemy shells during that famous battle, with the loss of all but 18 of her complement of 1,258 men. Pennell was not among the survivors.

***Allocreadium fowleri***  
**Leiper and Atkinson, 1914**

The immature specimens of this species were collected from *Trematomus bernacchii*. The name *Allocreadium fowleri* is considered a junior subjective synonym of *Macvicaria pennelli*. The Expedition roster did not include anyone named Fowler, and the significance of the original specific name is not known with certainty. According to Dr. David Gibson, of the Department of Zoology of the Natural History Museum in London, the name may refer to George Herbert Fowler, who was closely involved with material from the *Challenger* and *Discovery* expeditions.

**Cestoda**

***Oriana wilsoni* Leiper and Atkinson, 1914**

This tapeworm was collected by biologist D. G. Lillie from the rorqual whale *Balaenoptera borealis* (sei whale). The name is a junior synonym of *Tetrabothrius affinis*. *Oriana wilsoni* may or may not be named after Edward Wilson (after whom Shipley had named *Dibothriocephalus wilsoni* in 1907). If named after Wilson's wife and grammatically correct, the species should be *wilsonae*. The genus, however, is certainly named after Wilson's wife, Oriana Wilson née Souper. The 2 met while engaged in mission work in the London slums and were married 3 wk before Wilson sailed on Scott's first (*Discovery*) Antarctic expedition. Though severely shaken by the loss of her husband on the *Terra Nova* Expedition and by other family tragedies, she worked with the New Zealand Red Cross during World War I. She was made Commander of the British Empire in recognition of her outstanding service. She did not remarry and died in England in 1945, after a long illness.

***Dibothriocephalus lashleyi***  
**(=*Diphyllobothrium lashleyi***  
**(Leiper and Atkinson, 1914))**

This worm was recovered from a Weddell seal, *Leptonychotes weddelli*. It is named after Chief

Stoker William Lashly, who served under Scott in both the *Discovery* and *Terra Nova* expeditions. He was awarded the Albert Medal for gallantry in helping to save the life of Lieutenant Edward Evans (later Lord Mountevans) when the latter was stricken with scurvy during the polar journey. At the head of the Beardmore Glacier, Wilson (probably enquiring on behalf of Scott) asked Atkinson whom he would choose if an extra man were to be picked for the final polar assault. According to Huxley (1978), Atkinson's first choice was Lashly, followed by Thomas Crean. Scott, however, chose Bowers as the extra member of that ill-fated party. Like Atkinson, Lashly subsequently served in the Gallipoli Campaign during World War I, but he served at sea rather than on land. His ship, HMS *Irresistible*, was 1 of 3 battleships knocked out of action when the Allied fleet tried to storm the Dardanelles (a setback that had profound implications for the Allied cause). Most of the crew were taken off before the ship was sunk, and Lashly subsequently served on HMS *Amethyst*, surviving the war to become a customs officer in Wales. He died in 1940, and his diaries were published posthumously (Ellis, 1969).

***Dibothriocephalus archeri***  
**(=*Diphyllobothrium archeri***  
**(Leiper and Atkinson, 1914))**

This tapeworm was recovered from a Weddell seal, *Leptonychotes weddelli*. The name is a junior subjective synonym of *Diphyllobothrium wilsoni* (Shipley, 1907), as is *D. scotti* (Shipley, 1907). We think the parasite is named after Mr. W. W. Archer, Chief Steward on board the *Terra Nova*, who replaced Mr. Thomas Clissold as cook for the Shore Party when the *Terra Nova* returned to McMurdo Sound early in 1912. It is at first surprising that a species should have been named after someone who is not generally mentioned in accounts of the Expedition—one who had held a very "lowly" position in an expedition in which the sense of caste was deeply ingrained and one who had missed all the action. It is significant, however, that Archer had served in Antarctica under the command of Atkinson, who had done so much to break down the barriers of caste and who had gained the respect of all ranks in the dark winter days following the loss of the Polar Party. It is perhaps of special significance that Archer had accompanied Atkinson and Cherry-Gerrard (2 distinguished "upper-class" members of the Expedition) on a 3-wk parasitological excursion in December 1912. Apparently, Archer

had impressed Atkinson in some way that we shall never know but that led to Atkinson's commemoration of Archer in the name of a new species.

***Diphyllobothrium rufum*  
Leiper and Atkinson, 1914**

This tapeworm was found in a specimen of the Weddell seal, *Leptonychotes weddelli*. Its specific name refers to the brick-red pigmentation around the base of the living worm's suckers. Of the 19 taxa that the authors named in their report, it is only 1 of 2 that were not patronymics or named after the Expedition vessel.

***Tetrabothis rufus*  
Leiper and Atkinson, 1914**

This tapeworm was found in *Pygoscelis adeliae*, the Adélie, or black-throated penguin. It was named after Charles (later Sir Charles) Wright, physicist and glaciologist on the *Terra Nova* Expedition. As a member of one of the supporting sledging parties, Wright helped to haul a sledge almost to the top of the Beardmore Glacier before being ordered to return to base camp. He learned navigation on the Expedition and put this knowledge to good use when serving as a member of Atkinson's Search Party, which found Scott's last encampment in November 1912. He subsequently served as a radio officer in World War I, winning both the Military Cross and the Legion of Honour. Until his retirement at the end of World War II, Wright served in various distinguished scientific positions in the Admiralty. When he died in 1977, he left Trygve Gran as the last survivor of the Expedition.

***Anthobothrium wyatti*  
Leiper and Atkinson, 1914**

This tapeworm was recovered from *Trematodus bernacchii*. It is presently considered a junior synonym of *Oryzmatobothrium versatile*. We think it was named after Mr. George Wyatt, who was not a member of the *Terra Nova* Expedition but who was its business manager. Scott's preparations for the Expedition were handled, at least nominally, by a small business staff with offices in London. In fact, Scott delegated little responsibility to others.

***Tetrabothis creani* Leiper and Atkinson, 1914**

This parasite was found in the herald petrel, *Pterodroma arminjoniana* (reported as *Oestrelata trinitatis* and *O. arminjoniana*). It was named

after Thomas Crean, an Irishman of the "wild" variety who had served with Scott on both the *Discovery* and *Terra Nova* expeditions and indeed during the interim. A noncommissioned officer, he had to drop out of the depot-laying journey (February 1911) to take care of Surgeon-Lieutenant Atkinson, who was unable to continue because of a chafed and infected heel. He survived moments of great danger on the breaking sea-ice of McMurdo Sound and was one of those who traveled farthest with the main Southern Party. He and fellow Petty Officer Lashly reportedly shed tears of disappointment when, having struggled to within 169 statute mi of the South Pole, they were sent back to base camp. Thus, with Lashly and Lieutenant Edward Evans, he was a member of the last Supporting Party, the 3 men who were sent back on 4 January 1912 and who almost met a fate similar to that of the 5 men who continued to the Pole itself. When Lieutenant Evans collapsed with scurvy on the homeward journey, Lashly stayed with him. Crean, having already trudged 1,500 mi on foot, hauling a sledge most of the way, now walked 35 mi in 18 hr, with no camp equipment to save him in the event of a blizzard, and reached base camp half an hour before a blizzard came down. Atkinson was thereby alerted in time to go to the rescue of Evans. For this exploit, Crean was awarded the Albert Medal for gallantry. On return from the Expedition, Crean gave up his Navy pension to join Shackleton's *Endurance* Expedition, and he was a member of the small party that accomplished the incredible rescue mission that became that Expedition's chief claim to fame. He got back in time to serve in the War for 1 yr before retiring to the South Pole Inn in the village of Anascaul in Ireland.

***Tetrabothis aichesoni*  
Leiper and Atkinson, 1914**

This tapeworm was collected from the petrel *Pterodroma arminjoniana* (reported as *Oestrelata trinitatis*). Its name is a junior synonym of *T. creani*. According to Ms. Adrienne Reynolds, a British authority on Atkinson, "Aicheson" is an old variant of Atkinson's family name. It is extremely unlikely that Atkinson would have named the species after himself, but he may have been trying to honor his family. It is also conceivable that Leiper would have wished to name a species after his colleague and that Atkinson would accept only an indirect and obscure form of recognition. Atkinson was described by one

who knew him well as “almost pathologically modest” (Cherry-Garrard, 1951).

***Tetrabothrius catherinae***  
**Leiper and Atkinson, 1914**

This tapeworm, also, was recovered from the petrel *Pterodroma arminjoniana* (reported as *Oestrelata trinitatis*). The name is a junior synonym of *T. creani*. The species was probably named after Atkinson's aunt, Catherine Leycester (=Leicester) Atkinson, later Lady Nicholson of Banff, Scotland. As a schoolboy, Atkinson spent some of his vacations in her home because his parents were living in the West Indies (A. Reynolds, 1988, unpubl. letter to W. C. Campbell).

***Tetrabothrius priestleyi***  
**Leiper and Atkinson, 1914**

This tapeworm was reported from the frigate bird *Fregata aquila* or *F. ariel* and is presently considered a junior synonym of *Tetrabothrius pelecani*. It was named after Raymond (later Sir Raymond) E. Priestley, geologist on Shackleton's *Nimrod* Expedition and Scott's *Terra Nova* Expedition. He was a member of the Eastern (originally Northern) Party, which almost perished when they had to spend the winter months in an ice cave far from base camp. He served in the Signal Corps during World War I and subsequently held distinguished academic positions. Each of his 2 sisters married other members of the *Terra Nova* Expedition (Griffith Taylor and Charles Wright).

***Tetrabothrius nelsoni***  
**Leiper and Atkinson, 1914**

This parasite was collected from the light-mantled sooty albatross, *Phoebastria palpebrata*, and was named after Edward W. Nelson, biologist on the Expedition. Nelson was rich and urbane, and Scott thought him lazy and superficial. He had little impact on the Expedition, but was 1 of the dozen men who passed the second winter under Atkinson's command.

**Acanthocephala**  
***Echinorhynchus campbelli***  
**(= *Metacanthocephalus campbelli*)**  
**(Leiper and Atkinson, 1914))**

This acanthocephalan species has been transferred to *Leptorhynchoides*, then to *Metechinorhynchus*, and finally to *Metacanthocephalus*. It was found in *Trematomus bernacchi* and was

named after Lieutenant Victor L. A. Campbell, one of the Navy officers on the *Terra Nova* Expedition and Commander of the Eastern Party (originally Northern Party). The survival of this party, sheltering for 7 winter months in an ice cave and returning unaided to base camp, was largely attributed to his leadership and courage. In World War I, Campbell served in the Gallipoli Campaign and won the Distinguished Service Order.

***Echinorhynchus rennicki***  
**(*Leptorhynchoides rennicki*)**  
**(Leiper and Atkinson, 1914))**

This spiny-headed worm also infected *Trematomus bernacchii*. Its name, however, is now considered a junior synonym of *Metacanthocephalus campbelli*. The name *rennicki* was in honor of Lieutenant Henry Rennick, who was the hydrographic expert on board the *Terra Nova*. Not members of the Shore Party, he and Pennell were in charge of the ship while Captain Scott was on shore. Rennick died when his ship, *HMS Hogue*, was sunk during World War I.

***Echinorhynchus debenhami***  
**Leiper and Atkinson, 1914**

This worm, too, was found in the fish *Trematomus bernacchi*. The original name is considered more appropriate than *Leptorhynchoides debenhami*, a synonym that also occurs in the literature. The species was named in honor of Frank Debenham, an Australian-born geologist on the *Terra Nova* Expedition and a member of the Eastern Party. He served in the Infantry in World War I and later became the first professor of geography at Cambridge University. He was a co-founder and first director of the Scott Polar Research Institute.

**Nematoda**

***Kathleena scotti* (= *Contracaecum scotti*)**  
**(Leiper and Atkinson, 1914))**

This roundworm was found in *Diomedea melanophrys*, the mollymawk, or black-browed albatross. The generic name *Kathleena* is now a junior synonym of *Contracaecum*. Because of the masculine, rather than feminine or plural, Latin ending in *scotti*, we must assume that the trivial name honors R. F. Scott and not also Kathleen.

The genus is named in honor of Kathleen Bruce Scott. A talented sculptor, the young Kathleen Bruce lived for a time in Paris, where she studied,

informally at least, under Rodin. She subsequently returned to England, where she married Scott in 1908 and gave birth to their son just over a year later. (That son, the late Sir Peter Scott, was the celebrated wildlife artist, naval officer, wildfowl conservationist, Olympic yachtsman, and champion glider-pilot.) Kathleen's artistic background and high-spirited personality were in marked contrast to her husband's conventional discipline and introspection. She did not learn of his death until 11 mo after the event. As the widow of a national hero who would have been knighted, she was dubbed Lady Scott. She had a special friendship with the explorer Nansen, who tried unsuccessfully to persuade her to marry him. Through her sculpture and her social connections, she attained a fulgent but ephemeral fame. Lady Scott worked in a munitions factory in World War I and subsequently married a politician, E. H. Young (the couple later becoming Lord and Lady Kennet).

Robert Falcon Scott, leader of 2 prominent Antarctic expeditions, has been the subject of numerous books and articles and is sufficiently well known to require little comment here. His final (*Terra Nova*) expedition ended in disaster but became the most famous of all Polar expeditions. Among the 5 members who died on the return journey from the South Pole were 4 (Wilson, Oates, Bowers, Scott) after whom helminth species were named. Scott and Wilson had also been twice honored in this way, their names having previously been given to tapeworms collected on Scott's *Discovery* Expedition (*Dibothriocephalus scotti* Shipley, 1907, and *D. wilsoni* Shipley, 1907).

#### **Crassicauda Leiper and Atkinson, 1914**

Leiper and Atkinson (1914) erected the generic name *Crassicauda* for *Filaria crassicauda* Creplin, 1829, for specimens that occurred in renal tubules of the hump-backed whale. The name, in apposition to the species group name and also like the cestode *Diphyllobothrium rufum*, is descriptive in nature rather than a patronymic or named after the Expedition vessel.

#### ***Terranova antarctica* Leiper and Atkinson, 1914**

This ascaridoid nematode was collected from a shark, and its specific name refers to the Expedition's destination. The generic name refers to the commercial whaling ship *Terra Nova*, which Scott bought for his second Antarctic ex-

pedition. It was barque-rigged, renovated, and fitted out under the command of Lieutenant Edward R. G. R. Evans, whose name, together with that of Petty Officer Edgar Evans, is conspicuously absent from these helminth names (Campbell, 1991). In theory, the *Terra Nova* was a sailing ship with auxiliary steam power; in practice, she was a steamship with auxiliary sail.

#### **Other Antarctic Names**

The purpose of this article is to treat those helminths described by Leiper and Atkinson that pay recognition to people involved in many capacities in the *Terra Nova* Expedition. As already indicated, other helminths such as *Dibothriocephalus wilsoni* Shipley, 1907, and its junior synonym *D. scotti* Shipley, 1907, are also named after some of the same people. The same can be said of some fishes in the Antarctic region such as the nototheniids *Trematomus scotti* (Boulenger, 1907) (blackfin notothen) and *T. penellii* Regan, 1914 (sharp-spined notothen), the channichthyids *Chionodraco kathleenae* Regan, 1914 (a synonym of *C. hamatus*), and *Chaenodraco wilsoni* Regan, 1914 (spiny icefish), and the artedidraconid *Artedidracon orianae* Regan, 1914 (plunderfish). Scientists apparently shared with others a deep interest in Scott's ill-fated expedition and used the naming of new species as a means of honoring many of the individuals associated with it.

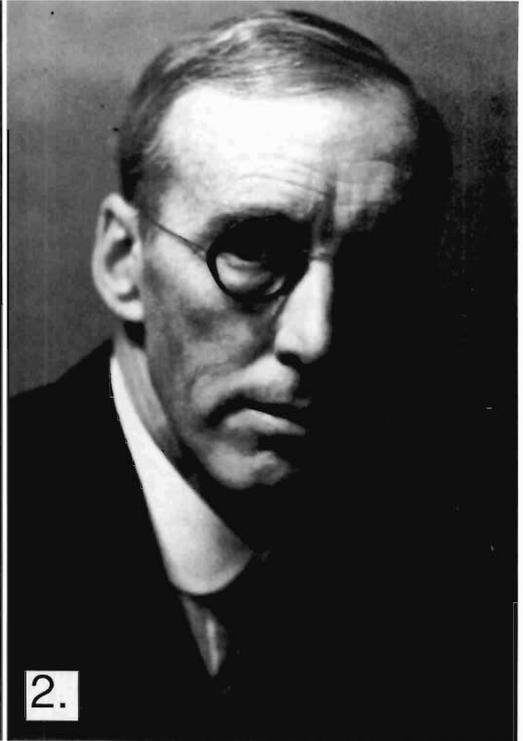
#### **Acknowledgments**

Figures 1 and 4 courtesy of the Scott Polar Research Institute. Figure 2 courtesy of Dr. D. A. Denham and the trustees of the London School of Hygiene and Tropical Medicine. Other figures from Ponting (1923) or from photographs of uncertain origin. The authors are grateful to Mr. Reid Zeigler and Mr. Ron Maturro, Merck Laboratories, for preparation of the plates.

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Figures 1-4. Historical links between helminthology and Scott's Antarctic expedition. 1. E. L. Atkinson. 2. R. T. Leiper. 3. L. E. G. Oates. 4. H. R. Bowers.



Figures 5-8. Historical links, continued. 5. A. Cherry-Garrard. 6. H. L. L. Pennell. 7. H. E. de P. Rennick. 8. W. Lashly.



Figures 9-12. Historical links, continued. 9. F. Debenham. 10. T. G. Taylor. 11. C. S. Wright. 12. T. Green.



Figures 13-16. Historical links, continued. 13. K. Scott and son. 14. The *Terra Nova*. 15. O. Wilson. 16. R. F. Scott.

## *Caryospora tremula* and *Sarcocystis* sp. from Turkey Vultures, *Cathartes aura*: Descriptions of Oocysts and Sporocysts and Attempted Transmission to Rodents

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**ABSTRACT:** Oocysts of *Caryospora tremula* (Allen, 1933) were observed in the feces of 3 turkey vultures, *Cathartes aura*, 1 from Kansas and 2 from Alabama. Oocysts from all birds were structurally similar. Oocysts were subspherical to ellipsoidal and measured  $33.4 \times 28.0 \mu\text{m}$ . Oocysts enclosed a single spherical sporocyst that measured  $20.4 \times 20.1 \mu\text{m}$ . Each sporocyst contained 8 sporozoites that measured  $16.3 \times 5.3 \mu\text{m}$ . Sporozoites contained anterior and occasionally posterior granular refractile bodies. Free *Sarcocystis* sporocysts were observed in the feces of 1 of the 2 turkey vultures from Alabama. The sporocysts were fully sporulated, contained 4 sporozoites, and measured  $11.4 \times 8.9 \mu\text{m}$ . Developmental stages of *C. tremula* or *Sarcocystis* sp. were not observed in mice or cotton rats inoculated orally with oocysts and sporocysts.

**KEY WORDS:** Apicomplexa, *Caryospora tremula*, *Sarcocystis* sp., oocyst, sporocyst, transmission studies, turkey vulture, *Cathartes aura*.

Oocysts that were structurally similar to those of *Caryospora tremula* (Allen, 1933) Hoare, 1934, were observed in the feces of 3 turkey vultures, *Cathartes aura*. One turkey vulture also was excreting sporocysts of a species of *Sarcocystis*. Because *C. tremula* has not been reported for nearly 50 yr and because the original description was incomplete, we present a redescription of the oocysts of this species. In addition, we report the structure of sporocysts of a *Sarcocystis* species from 1 turkey vulture and our attempts to transmit these coccidians to mice and cotton rats.

### Materials and Methods

The turkey vulture (vulture 1) from Kansas was submitted for treatment of a fractured wing after being struck by a car to Kansas State University, College of Veterinary Medicine, Manhattan, Kansas, in September 1991. The 2 turkey vultures (vultures 2 and 3) from Alabama were submitted for treatment to the Southeastern Raptor Rehabilitation Center (SRRC) at the College of Veterinary Medicine, Auburn University, Alabama, in June and October 1992. Raptors at the SRRC are fed food that has been frozen and then thawed. Feces from all birds were examined by flotation in Sheather's sugar solution, and feces containing oocysts or sporocysts were mixed in 2.5% (w/v) potassium dichromate solution in a thin-layer (<5 mm) in plastic Petri dishes and sporulated at room temperature (22-24°C). Fecal samples were examined from vultures 2 and 3, once or twice weekly, over a 2-mo period. Additionally, single fecal samples were examined from 2 black vultures (*Coragyps atratus*) that were

residents at the SRRC. Oocyst cultures were examined daily with Nomarski interference-contrast or bright-field microscopes to determine when sporulation had occurred. Oocysts were concentrated by flotation in Sheather's sugar solution and counted in a hemacytometer prior to experimental transmission studies. Oocysts and sporocysts were stored at 4°C and were less than 2 mo old when used. Oocysts and sporocysts were measured with a calibrated ocular micrometer. All measurements are in micrometers and expressed as means  $\pm$  standard deviation, followed in parentheses by the range and number (*N*) of stages measured.

The oocyst and sporocyst shape indices (length/width ratios) were compared using analysis of variance (ANOVA). Significant differences were considered to be present if  $P < 0.05$  was detected by ANOVA.

Seven female Hsd:ICR mice and 4 female cotton rats were used for experimental oral inoculations with *C. tremula* oocysts and *Sarcocystis* sp. sporocysts. Three of the mice intramuscularly received 4 mg of methylprednisolone acetate (MPA; The Upjohn Company, Kalamazoo, Michigan) in an attempt to enhance infectivity of the inoculum. Mice received  $3 \times 10^4$  *C. tremula* oocysts, and cotton rats received  $2 \times 10^4$  *C. tremula* oocysts. The number of *Sarcocystis* sp. sporocysts present in the inoculum was not determined. Two female Hsd:ICR mice and 2 male cotton rats were not inoculated and served as controls.

Inoculated MPA-treated mice were killed and examined at necropsy 21, 35, and 49 days postinoculation (PI); inoculated mice not treated with MPA and inoculated cotton rats were killed and examined at necropsy 7, 21, 35, and 49 days PI; and control mice and cotton rats were killed and examined 35 and 49 days PI. A portion of cerebral cortex was collected from each rodent and examined as an unstained smear with

**Table 1.** Measurements and shape indices (length/width ratios) of *Caryospora tremula* oocysts and sporocysts from turkey vultures.\*

	Vulture 1 sporulated	Vulture 2 unsporulated	Vulture 2 sporulated	Vulture 3 sporulated
Oocyst length	34.0 ± 1.18 (32.0–36.2)	33.3 ± 1.46 (30.0–36.0)	34.3 ± 1.38 (32.0–37.0)	32.1 ± 1.69 (30.0–38.0)
Oocyst width	29.0 ± 1.03 (27.6–32.0)	27.5 ± 1.45 (25.0–30.0)	28.6 ± 1.47 (25.0–32.0)	26.8 ± 0.87 (25.0–28.0)
Oocyst index	1.17 ± 0.04 (1.08–1.23)	1.21 ± 0.05 (1.10–1.31)	1.20 ± 0.04 (1.10–1.28)	1.20 ± 0.08 (1.07–1.52)
Sporocyst length	20.4 ± 0.73 (18.6–21.6)	NA† NA	20.8 ± 0.60 (19.0–22.0)	20.0 ± 0.54 (19.0–21.0)
Sporocyst width	ND‡ ND	NA NA	20.3 ± 0.61 (19.0–21.0)	19.8 ± 0.58 (19.0–21.0)
Sporocyst index	ND ND	NA NA	1.02 ± 0.03 (1.00–1.11)	1.01 ± 0.02 (1.01–1.05)

\* Measurements are expressed in micrometers as means ± standard deviation, with the ranges in parentheses. All measurements are based on 25 observations.

† NA = not applicable to unsporulated oocysts.

‡ ND = not determined for sporocysts of this isolate because sporocysts were considered to be spherical.

light microscopy. The remaining brain, eyes, and portions of tongue, heart, thigh (semitendinosus and semimembranosus), gastrocnemius, diaphragm, abdominal muscles, lung, thymus, liver, spleen, pancreas, kidney, adrenal gland, stomach, ileum, cecum, ear, and facial tissue were fixed in 10% neutral-buffered formalin solution. Tissues were processed by routine histological methods and stained with hematoxylin and eosin for light microscopic examination.

Portions of skeletal muscle were obtained from the carcass of each rodent (1–2 g for mice, 3–6 g for cotton rats) and digested in hydrochloric acid–pepsin solution (0.52 g pepsin, 0.50 g NaCl, 1.4 ml concentrated HCl, and 98.6 ml H<sub>2</sub>O) for 10 min. The suspension was washed once in Hanks' balanced salt solution (HBSS) by centrifugation, resuspended in HBSS, and examined with bright-field microscopy for parasites.

## Results

Measurements of *C. tremula* oocysts from each turkey vulture are presented in Table 1. A general composite description is presented here.

### *Caryospora tremula* (Allen, 1933) Hoare, 1934 Apicomplexa: Eimeriorina

**DESCRIPTION OF OOCYSTS** (Figs. 1–4): Oocysts subspherical to ellipsoidal, occasionally ovoidal, 33.4 ± 1.65 × 28.0 ± 1.50 (30–38 × 25–32, *N* = 100); shape index (length/width) 1.2 ± 0.06 (1.07–1.52, *N* = 100); oocyst wall bilayered and smooth; outer layer 1.0–1.4 thick; inner layer 0.6–0.8 thick; micropyle and polar granule absent; small, spherical oocyst residuum rarely present; sporocysts spherical or occasionally subspherical, 20.4 ± 0.7 × 20.1 ± 0.7 (18.6–21.6 × 18.6–21.6, *N* = 75); shape index 1.01 ± 0.02

(1.00–1.05, *N* = 75), with smooth single-layered wall 0.8–1.0 thick; sporocyst residuum present consisting of hundreds of diffuse granules on day 2 of sporulation, as a compact mass on days 3, 4, and occasionally 5 of sporulation, and usually as scattered granules on and beyond day 5 of sporulation; 8 sporozoites present in each sporocyst, 16.3 ± 0.73 × 5.2 ± 0.29 (15.2–18.4 × 4.8–5.6, *N* = 25), arranged parallel or randomly in sporocyst; anterior ends of sporozoites tapering slightly; each sporozoite usually with single, spherical to ellipsoidal posterior granular refractile body 3.0 × 2.8 and rarely spherical anterior granular refractile body 2.5; nucleus prominent, centrally or posteriorly located.

**HOST:** *Cathartes aura* Vieill “turkey vulture” (Falconiformes: Cathartidae)

**LOCALITY:** Type locality is Washington, D.C. Other localities (present study) Kansas and Alabama, U.S.A.

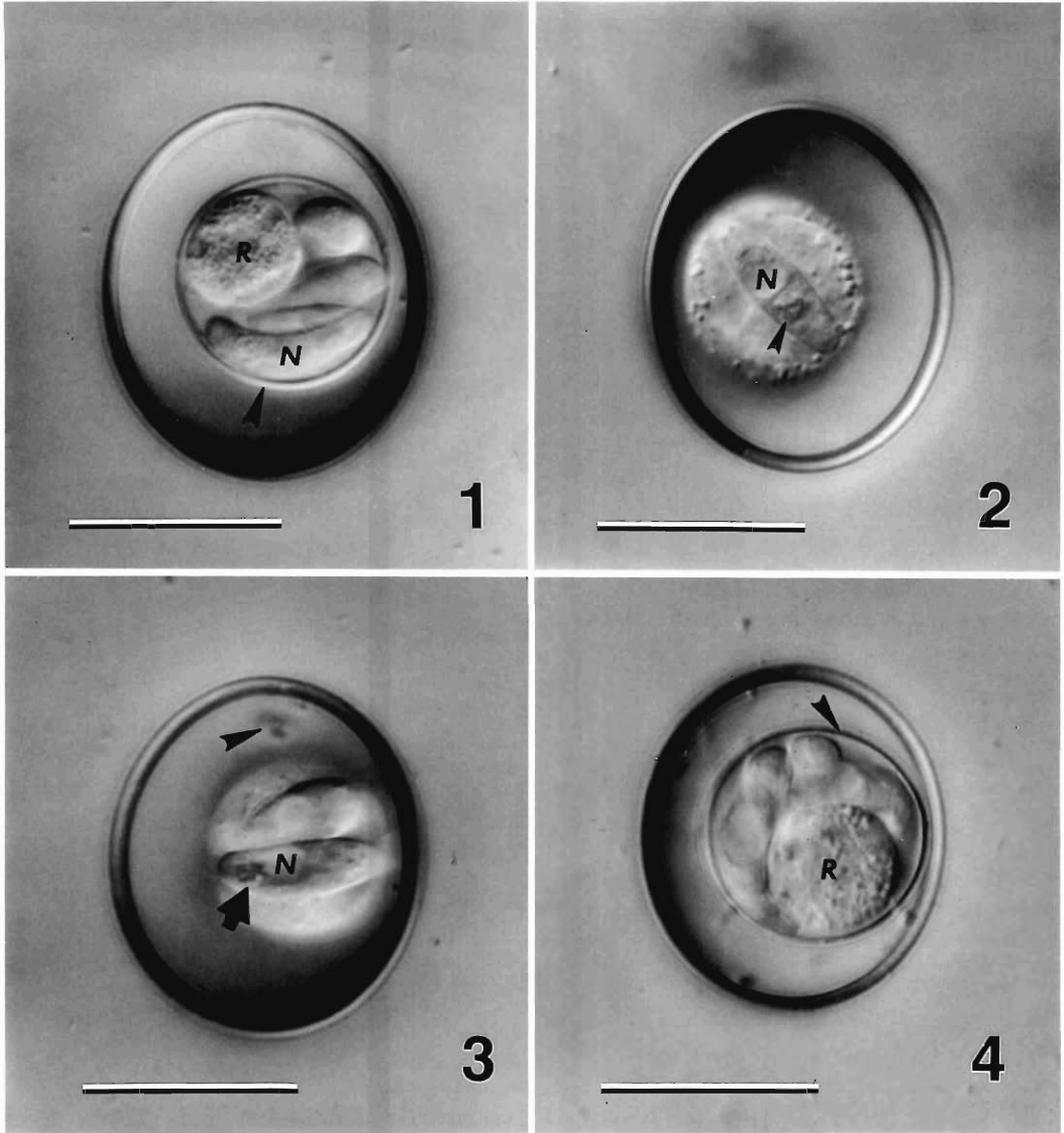
**LOCATION IN HOST:** Oocysts were originally described from the intestine of a turkey vulture. Oocysts in this study were recovered from feces.

**SPORULATION:** Some oocysts had completed sporulation within 48 hr; all had sporulated within 4 days at room temperature (22–24°C).

**TRANSMISSION TO SECONDARY HOSTS:** No stages of *C. tremula* were observed in mice or cotton rats.

**COMMENTS:** No significant differences (*P* > 0.05) were detected in the oocyst or sporocyst shape indices among the 3 *C. tremula* isolates.

Exposure of *C. tremula* oocysts to 2.5% po-



Figures 1-4. Sporulated oocysts of *Caryospora tremula* from a turkey vulture from Kansas. Bars = 20  $\mu$ m. 1. Oocyst with a sporocyst with a compact sporocyst residuum (R). Note the sporocyst wall (arrowhead) and the centrally placed sporozoite nucleus (N). 2. Oocyst with a sporocyst with a dispersed sporocyst residuum. Note the nucleus (N) of a sporozoite and the granular refractile body (arrowhead). 3. Oocyst containing a small oocyst residuum (arrowhead). The sporozoite in the field of focus contains a nucleus (N) and a small granular refractile body (arrow). 4. Oocyst with a sporocyst that contains sporozoites in cross or tangential sections. Note the sporocyst wall (arrowhead) and the compact sporocyst residuum (R).

tassium dichromate solution for periods of 1 wk or more apparently caused the oocyst wall to become fragile because oocysts with collapsed walls surrounding the sporocysts were observed after flotation in sugar solution. Free *C. tremula*

sporocysts were occasionally observed in sugar flotations and probably represent complete collapse and removal of the oocyst wall.

Oocysts of *C. tremula* were present in all fecal samples examined from vultures 2 and 3 over

the 2-mo period. Oocysts of *C. tremula* were not observed in the fecal samples from the 2 black vultures.

### *Sarcocystis* sp.

#### Apicomplexa: Eimeriorina

**DESCRIPTION OF SPOROCASTS (Fig. 5):** Sporocysts elongate with 1 side slightly flattened,  $11.4 \pm 0.65 \times 8.9 \pm 0.60$  ( $10.0\text{--}12.0 \times 8.0\text{--}10.0$ ,  $N = 25$ ); residuum composed of dispersed, discreet granules; 4 sporozoites present; no refractile bodies observed in sporozoites.

**HOST:** *Cathartes aura* Wiedl "turkey vulture" (Falconiformes: Cathartidae)

**LOCALITY:** Alabama, U.S.A.

**LOCATION IN HOST:** Unknown. Sporocysts in this study were recovered from feces.

**TRANSMISSION TO INTERMEDIATE HOSTS:** No stages of *Sarcocystis* were observed in mice or cotton rats.

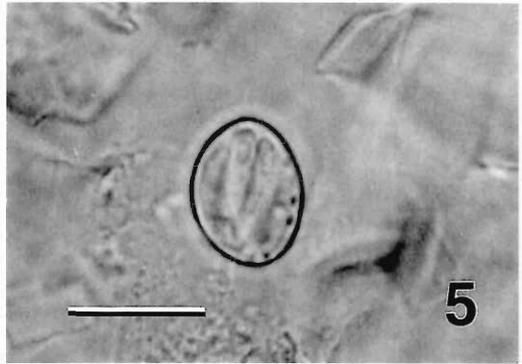
**COMMENTS:** The turkey vulture (vulture 2) was excreting low numbers of sporocysts for the 2-mo observation period. No oocysts were seen.

### Discussion

Oocysts of *C. tremula* were originally reported as ellipsoidal and  $33\text{--}35 \times 28\text{--}30$  in diameter and sporocysts as  $23.5\text{--}25$  in diameter (Allen, 1933). In the present study, oocysts were  $30\text{--}38 \times 25\text{--}32$  and sporocysts were  $18.6\text{--}21.6 \times 16.6\text{--}21.6$ . The original measurements of *C. tremula* oocysts by Allen (1933) fall within the range of oocysts in our study, but the sporocyst measurements are larger by  $2\text{--}6 \mu\text{m}$ . We observed refractile bodies in sporozoites and a small amount of oocyst residual material in *C. tremula* oocysts, which Allen (1933) did not mention in her description.

Allen (1933) originally created a new genus (*Eumonospora*) to accommodate the new coccidian, which she termed *Eumonospora tremula*. This genus was quickly synonymized with *Caryospora* by Hoare (1934), which was accepted by the original author (Allen, 1934).

Several members of the genus *Caryospora* use rodents as secondary hosts in their life cycles (Stockdale and Cawthorn, 1981; Cawthorn and Stockdale, 1982; Wacha and Christiansen, 1982; Upton et al., 1984). Severe disease and death can occur in cotton rats and mice inoculated with oocysts of *C. bigenetica* Wacha and Christiansen, 1982 (Wacha and Christiansen, 1982; Lindsay



**Figure 5.** Sporocyst of *Sarcocystis* species from a turkey vulture from Alabama. Note the sporozoites and dispersed granules that compose the sporocyst residuum. Bar =  $10 \mu\text{m}$ .

et al., 1988; Upton and Barnard, 1988). Severe disease occurs in mice infected with oocysts of *C. simplex* Leger, 1904 (Upton et al., 1984). Both *C. bigenetica* and *C. simplex* have viperiid snakes as the primary hosts. *Caryospora bubonis* Cawthorn and Stockdale, 1981, of the great horned owl, *Bubo virginianus*, is transmissible to mice (Stockdale and Cawthorn, 1981; Cawthorn and Stockdale, 1982); however, no other species of *Caryospora* from avian hosts have been shown to have secondary hosts (see Lindsay and Sundermann, 1989; Upton et al., 1990; Upton and Sundermann, 1990).

### Acknowledgments

We thank Natasha S. Rippey and Cindy Tily for technical assistance and David Kralovanic and other student members of the Southeastern Raptor Rehabilitation Center, College of Veterinary Medicine, Auburn University, for assistance with vultures.

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## Eimerian Species (Apicomplexa: Eimeriina) in Gunnison's Prairie Dogs (*Cynomys gunnisoni zuniensis*) and Rock Squirrels (*Spermophilus variegatus grammurus*) from Southeastern Utah

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**ABSTRACT:** *Eimeria callospermophili-morainensis* (prevalence = 78.9%), *E. becheyi* (62.6%), *E. spermophili* (9.8%), *E. bilamellata* (8.1%), *E. larimerensis* (3.3%), *E. cynomysis* (1.6%), and 1 unidentified eimerian species were recovered from 123 fecal samples of Gunnison's prairie dogs (*Cynomys gunnisoni zuniensis*) collected in southeastern Utah. *Eimeria spermophili* has not been reported previously in any prairie dog species, and Gunnison's prairie dog is a new host record for 7 of the preceding species. Eighty-five percent of the samples contained at least 1 eimerian species, with multispecific infections of 2-4 species in 65% of the 123 samples. Species richness was 2.0 species/infected host. *Eimeria becheyi* (100%), *E. morainensis* (85.7%), *E. callospermophili* (57.1%), and *E. bilamellata* (28.6%) also were recovered from 7 rock squirrels (*Spermophilus variegatus grammurus*). Individuals harbored from 2-5 eimerian species (species richness = 3.5). *Eimeria becheyi* and *E. morainensis* have not been reported previously infecting rock squirrels. The unidentified eimerian species was recovered from 6 of the 123 prairie dog samples (4.9%) and 5 (71.4%) rock squirrels live-trapped in southeastern Utah. The eimerian complex described for these 2 sympatric host species is similar to those described in Wyoming ground squirrels and several other sciurid species. We propose that the ability of the spermophiline species of *Eimeria* to infect related host species contributes to the stability and persistence of this eimerian guild.

**KEY WORDS:** Gunnison's prairie dog, *Cynomys*, *Eimeria*, host specificity, prevalence, rock squirrels, *Spermophilus*.

Three species of *Eimeria* (*E. cynomysis* Andrews, 1928, *E. ludoviciani* Vetterling, 1964, and *E. larimerensis* Vetterling, 1964) have been reported infecting black-tailed prairie dogs (*Cynomys ludovicianus* Ord, 1815) (Andrews, 1928; Vetterling, 1964), and 7 species (*E. cynomysis*, *E. ludoviciani*, *E. larimerensis*, *E. bilamellata* Henry, 1932, *E. becheyi* Henry, 1932, *E. callospermophili* Henry, 1932, and *E. morainensis* Torbett, Marquardt, and Carey, 1982) have been reported in white-tailed prairie dogs (*Cynomys leucurus* Merriam, 1890) (Todd and Hammond, 1968a, b; Seville and Williams, 1989; Shults et al., 1990). However, no species of *Eimeria* has been reported from Gunnison's prairie dogs (*Cynomys gunnisoni zuniensis* Baird, 1858).

Two eimerian species (*E. bilamellata* and *E. larimerensis*) have been reported in wild rock squirrels (*Spermophilus variegatus grammurus* Erxleben, 1777) (Todd and Hammond, 1968b; Todd et al., 1968). Todd and Hammond (1968a) infected rock squirrels with *E. callospermophili* experimentally, but this species has not been recovered from naturally infected rock squirrels.

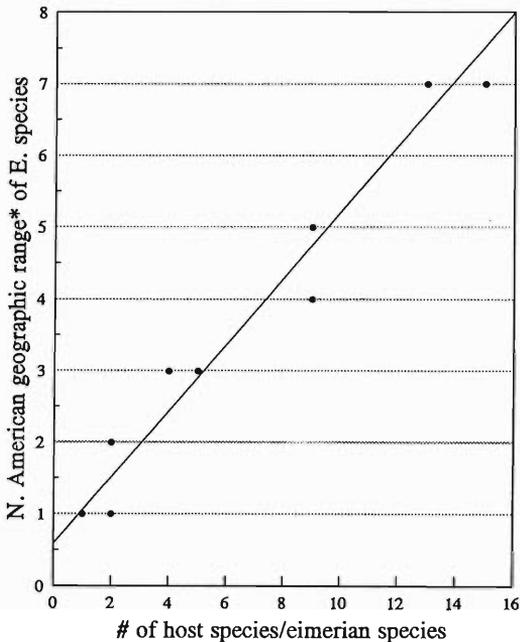
Here we report the occurrence of *Eimeria* recovered from 2 sympatric sciurid species from southeastern Utah, Gunnison's prairie dogs and rock squirrels, and compare the observed ei-

merian complex with those described in other sciurid rodents.

### Methods

Fecal samples were collected from a population of Gunnison's prairie dogs 7 km east of Monticello, Utah (38°15'N, 109°51'W), over a 4-day period in May 1991. The colony was located in a rye (*Secale cereale*) field. Fresh fecal pellets were collected from the entrances of individual prairie dog burrows between sunrise and 0800 hours each morning. Only pellets in or at the entrance of the burrows were collected to avoid duplicate samples from the same individual. Fecal samples also were collected from 7 adult rock squirrels that were live-trapped within a 12 km radius of Monticello, Utah.

Feces were soaked in 2% aqueous potassium dichromate at room temperature (26-28°C) for several weeks to allow oocyst sporulation to occur. Oocysts were isolated by floatation and centrifugation in Benbrook's sugar solution, and species were identified based on oocyst size, shape, and internal structure. Because size ranges for *E. callospermophili* and *E. morainensis* overlap, differentiation of these 2 species depends on the internal structure of the sporulated oocyst. All 7 rock squirrel samples achieved sufficient sporulation of oocysts to differentiate between these 2 species. These eimerian species were also identified in the prairie dog samples; however, incomplete sporulation in some samples made prevalence impossible to determine. Therefore, although both species were positively identified, we combined the 2 (*E. callospermophili-morainensis*) for data on prevalence in the prairie dog.



n = 18

\*Number of states (including Canada and Mexico)

Figure 1. The correlation between the size of the geographic range and the number of host species reported for 18 eimerian species of ground-dwelling sciurids in North America.

### Results

Eighty-five percent of 123 prairie dog fecal samples were positive for *Eimeria*. Sixty-five percent of the samples had more than 1 eimerian species: 48.8% had 2 species, 13.8% had 3 species, and 2.4% had 4 species. The *E. callospermophili-morainensis* complex was the most prevalent (78.9%), followed by *E. beecheyi* (62.6%), *E. spermophili* Hilton and Mahrt, 1971 (9.8%), *E. bilamellata* (8.1%), *E. larimerensis* (3.3%), and *E. cynomysis* (1.6%). An unidentified eimerian species was found in 6 (4.9%) of the prairie dog samples.

All 7 rock squirrels had multiple eimerian infections: 2 squirrels harbored 2 species, 1 had 3 species, 3 were infected with 4 species, and 1 animal harbored 5 species. *Eimeria beecheyi* (100%) was the most prevalent, followed by *E. morainensis* (86%), *E. callospermophili* (57%), and *E. bilamellata* (29%). Oocysts of an unidentified species were also recovered from 5 (71%) of the rock squirrels. Although no cross-trans-

mission studies or species-differentiating tests were performed, it appears that these oocysts are of the same species as the unidentified species recovered from the Gunnison's prairie dogs.

Oocysts of this species were ovoid to ellipsoidal, with a length-width ratio of approximately 1.2. Fifty-eight sporulated oocysts averaged  $21.2 (19.6-23.8) \times 26.0 (24.0-29.0) \mu\text{m}$ , and 53 unsporulated oocysts measured  $21.3 (20.0-23.8) \times 26.0 (24.0-28.5) \mu\text{m}$ . The oocyst wall appeared to be smooth, 1-layered, and approximately  $0.8-1.0 \mu\text{m}$  thick. Oocysts lacked distinct micropyles and no polar granules were observed; however, sporulation appeared to be incomplete and internal structures were difficult to define. Of the eimerian species previously described in sciurid rodents, only *E. spermophili* is similar to this species in size and shape, but oocysts of the unidentified species display no thinning of the anterior oocyst wall characteristic of *E. spermophili*. Therefore, it appears that this species has not been described previously.

### Discussion

Vetterling (1964) found 3 species of *Eimeria* in 86 black-tailed prairie dogs in northern Colorado, but data are insufficient to calculate species richness (the mean number of species/infected host). Seville and Williams (1989) reported 3 eimerian species in 17 white-tailed prairie dogs from Park County, Wyoming (species richness = 1.5), and Shults et al. (1990) found 3 species in 17 white-tailed prairie dogs (species richness unknown) and 6 species from 1,007 Wyoming ground squirrels from southeastern Wyoming (species richness = 1.9). Seville et al. (1992) reported 4 species of *Eimeria* in thirteen-lined ground squirrels (*Spermophilus tridecemlineatus* Mitchell, 1821) from 2 populations in Wyoming (species richness = 1.2 and 1.8). In this study, 8 eimerian species were recovered from Gunnison's prairie dogs, and the species richness was 2.0, despite the combination of *E. callospermophili* and *E. morainensis* into a single species complex. Gunnison's prairie dog is a new host record for 7 of these eimerian species.

Although only 7 rock squirrels were sampled, 3 of the 5 eimerian species recovered have not been previously recorded for this host. Species richness was high (3.5), but the sample size was small.

Gunnison's prairie dogs and rock squirrels are sympatric in southeastern Utah, and all 5 eimerian species found in rock squirrels were also

Table 1. Reported host species of 8 eimerian species of ground-dwelling sciurid.\*

Host species	Eimerian species							
	<i>E. beecheyi</i>	<i>E. callospermophili</i>	<i>E. morainensis</i>	<i>E. larimerensis</i>	<i>E. bilamellata</i>	<i>E. spermophili</i>	<i>E. cynomys</i>	UNID†‡
<i>Spermophilus elegans</i>	17, 18	17, 18	17, 18	17, 18	17, 18	17, 18	—	—
<i>S. armatus</i>	—	10	—	9	8	—	—	—
<i>S. beecheyi</i>	1	10	—	9	8	—	—	—
<i>S. variegatus</i>	21	10, 21	21	9	8, 21	—	—	21
<i>S. lateralis</i>	—	1, 10	14	4, 9	1, 8	—	—	—
<i>S. tridecemlineatus</i>	19	10, 19	19	9, 19	19	—	—	—
<i>S. columbianus</i>	—	11	—	11	11	—	—	—
<i>S. franklinii</i>	—	11	—	—	2, 7, 11	11	—	—
<i>S. richardsonii</i>	20	10, 11, 20	20	11, 20	11, 20	11, 20	—	—
<i>S. beldingii</i>	—	15	—	—	—	—	—	—
<i>S. spilosoma</i>	—	7	—	12	—	—	—	—
<i>S. townsendii</i>	23	23	23	23	23	—	—	—
<i>S. citellus</i>	—	—	—	—	3, 5	—	—	—
<i>S. maximus</i>	—	7	—	—	—	—	—	—
<i>S. relictus</i>	13	—	—	—	—	—	—	—
<i>Cynomys leucurus</i>	17	10	17	9, 16	17	—	16	—
<i>C. ludoviciani</i>	—	—	—	6	—	—	6	—
<i>C. gunnisoni</i>	21	21	21	21	21	21	21	21
<i>Marmota flaviventris</i>	22	22	22	—	—	22	—	—
Total number of host species	10	16	9	13	13	5	3	2
Total number of host genera	3	3	3	2	2	3	1	2

\* Numbers indicate references: 1 = Henry (1932), 2 = Hall and Knipling (1935), 3 = Pellérdy and Babos (1953), 4 = Levine et al. (1957), 5 = Ryšavý (1957), 6 = Vetterling (1964), 7 = Levine and Ivens (1965), 8 = Todd and Hammond (1968b), 9 = Todd et al. (1968), 10 = Todd and Hammond (1968a), 11 = Hilton and Mahrt (1971), 12 = Broda and Schmidt (1978), 13 = Abenov and Svanbaev (1982), 14 = Torbett et al. (1982), 15 = Veluvolu and Levine (1984), 16 = Seville and Williams (1989), 17 = Shults et al. (1990), 18 = Stanton et al. (1992), 19 = Seville et al. (1992), 20 = Seville and Stanton (1993), 21 = Thomas and Stanton (this study), 22 = Thomas (unpubl. data), 23 = Wilber (pers. comm.).

† Seville and Stanton (1993) suggested synonymizing *E. larimerensis* and *E. lateralis*, so data for these 2 species are combined.

‡ Unidentified species, this study.

found infecting Gunnison's prairie dogs. Additionally, *E. beecheyi*, *E. callospermophili*, and *E. morainensis* exhibited high prevalences in both hosts. The unidentified eimerian species occurred at much higher prevalences in rock squirrels than in prairie dogs, but this may be due to small sample size. However, it is possible that the rock squirrel is a primary host and that the prairie dog is a host of secondary importance.

Stanton et al. (1992) described a stable eimerian guild in Wyoming ground squirrels (*Spermophilus elegans* Kennicott, 1863), with 6 species present at consistent prevalences across populations and over time. The observed eimerian assemblage in Gunnison's prairie dogs more closely resembles that described for Wyoming ground squirrels (Shults et al., 1990; Stanton et al., 1992) than of any other sciurid host for which prevalence data are available (Vetterling, 1964; Seville and Williams, 1989; Seville et

al., 1992). All 6 species found in Wyoming ground squirrels also infect Gunnison's prairie dogs. Additionally, the 3 most prevalent species in both Wyoming ground squirrels and Gunnison's prairie dogs were *E. beecheyi*, *E. callospermophili*, and *E. morainensis*, whereas *E. larimerensis*, *E. bilamellata*, and *E. spermophili* always occurred in prevalences below 20% in both host species.

Characteristics of host populations (e.g., geographic distribution, population density, body size, population growth rate) play an important role in shaping parasite communities (Price, 1990). Positive correlations have been reported between host geographic range and the species richness of the associated parasite community (e.g., Price, 1980; Price and Clancy, 1983; Price et al., 1988; Aho, 1990), and the probability of exchange of parasites within and among host species increases with increased host geographic range (Price, 1990). There is a strong correlation

( $R^2 = 0.971$ ) between the size of the geographic range of eimerian species of ground-dwelling sciurids and the number of host species/eimerian species (Fig. 1). As parasites are exchanged between related sympatric host species, parasite host specificity declines. Additionally, the high densities and population growth rates characteristic of most sciurids allow maintenance of a diverse parasite community on a local level by increasing the incidence of transmission between hosts and maintaining a large population of new hosts available (e.g., with low immunological resistance) for parasite colonization (Price, 1990).

Duszynski (1986) challenged the paradigm of high host specificity among eimerian species (Marquardt, 1973; Joyner, 1982) and proposed that mammalian coccidia are less rigid in their host requirements, especially when hosts occur in high densities and the probability of transmission is high. The 8 eimerian species in this study inhabit a wide geographic range and have been recovered from 19 host species representing 3 genera (Table 1). The individual eimerian species have been reported infecting as many as 16 of these host species in 3 distinct genera. We propose that this ability to infect a wide range of abundant and ubiquitous host species across large geographic regions enhances the stability and persistence exhibited by this eimerian assemblage.

#### Acknowledgments

This research was supported by NSF grant #BSR-89-9887 and the University of Wyoming Office of Research. Special thanks are extended to Newton Kingston, who provided valuable comments on the manuscript.

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## Two New Species of *Dactylogyrus* (Monogenea: Dactylogyridae) from *Notropis alborus* (Pisces: Cyprinidae), with Comments on Inferred Host Relationships

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**ABSTRACT:** *Dactylogyrus albori* sp. n. and *D. onychoaccessorius* sp. n. are described from *Notropis alborus* Hubbs and Raney, 1947. *Dactylogyrus albori* differs from its closest apparent relative, *D. alabamensis* Rogers and Mizelle, 1966, most notably by possessing smaller anchors, hooks, and a copulatory complex. *Dactylogyrus onychoaccessorius* possesses a distinctive cirrus and 2-pronged accessory piece. Its relationship to other species of *Dactylogyrus* is uncertain, but it may be most closely related to *D. distinctus* Mizelle and Klucka, 1953. These parasites indicate that *N. alborus* may be more closely allied with the subgenus *Hybopsis* than with other *Notropis*.

**KEY WORDS:** *Dactylogyrus albori* sp. n., *Dactylogyrus onychoaccessorius* sp. n., *Notropis alborus*, Monogenea, Dactylogyridae, morphology, taxonomy.

Two new species of *Dactylogyrus* Diesing, 1850, are described from the whitemouth shiner, *Notropis alborus* Hubbs and Raney. Also, evolutionary relationships of *N. alborus* based on infesting *Dactylogyrus* are hypothesized in light of taxonomic revisions by Mayden (1989). This is the first report of any parasites from *N. alborus*.

### Materials and Methods

Taxonomy of hosts follows Robins et al. (1991). *Notropis alborus* were collected through use of a minnow seine on 17 August 1978 and 28 June 1989. Immediately after capture, the fish 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 haptor hooks follows the scheme of Mueller (1936). Type specimens were deposited in the helminthological collection of the National Museum of Natural History (USNM) and the Harold W. Manter Laboratory, University of Nebraska State Museum (HWML). For comparative purposes, all original descriptions and redescrptions of North American *Dactylogyrus* species and specimens of the following species were examined: *D. alabamensis* Rogers and Mizelle, 1966, holotype (USNM 60782), 2 paratypes (USNM 60783); *D. amblops* Mueller, 1938, 7 syntypes (USNM 71453); and *D. heterolepis* Hanek, Molnar, and Fernando, 1975, 1 paratype (USNM 73158).

### Results

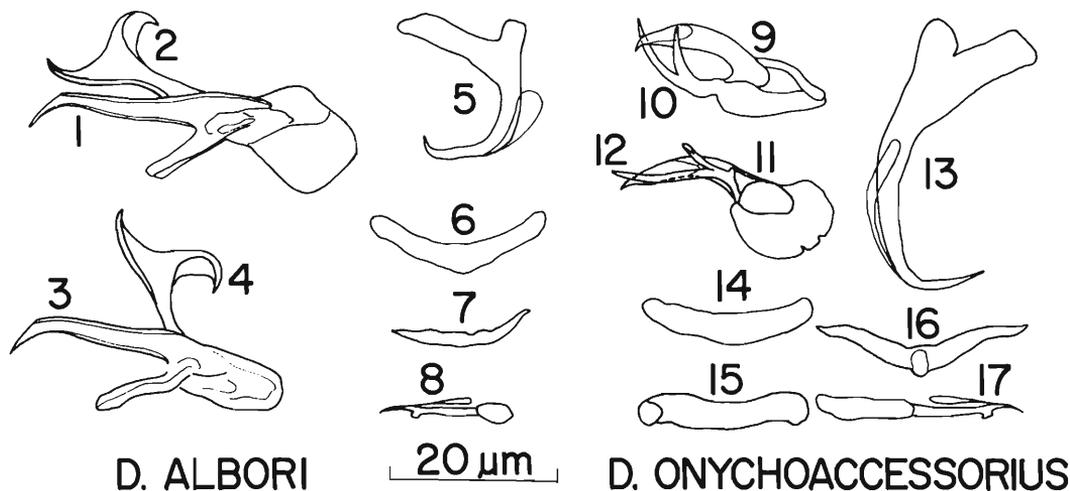
#### *Dactylogyrus albori* sp. n. (Figs. 1–8)

**TYPE LOCALITY:** North Carolina: Mecklenburg Co., Mallard Creek, U.S. Highway 29 bridge. Pee Dee River system.

**TYPE SPECIMENS** Holotype, USNM 82879; 19 paratypes, USNM 82880 (1 specimen), USNM 82881 (10 specimens), HWML 36608 (8 specimens).

**LOCATION ON HOST:** Gills.

**DESCRIPTION:** With characters of the genus *Dactylogyrus*, as emended by Mizelle and McDougal (1970). Body with thin tegument; length 283 (216–374), greatest width 74 (50–101). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, posterior pair usually farther apart than anterior pair. Pharynx circular to ovate (dorsal view), transverse diameter 21 (18–25), gut not observed. Peduncle usually present, 21 (11–35) long, 37 (28–46) wide. Haptor 39 (32–49) long, 53 (46–63) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, solid shaft, and recurved point. Anchor length 18 (17–20), greatest width of base 14 (12–16). Dorsal bar length 20 (18–22). Vestigial ventral bar length 15 (11–22). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane,



Figures 1–17. Sclerotized parts of *Dactylogyrus* species (drawings are of holotypes unless otherwise specified). 1–8. *Dactylogyrus alborig* sp. n.: 1, 3 (USNM 82880), cirrus; 2, 4 (USNM 82880), accessory piece; 5, anchor; 6, dorsal bar; 7, ventral bar; 8, hook. 9–17. *Dactylogyrus onychoaccessorius* sp. n.: 9, 11 (USNM 82883), cirrus; 10, 12 (USNM 82883), accessory piece; 13, anchor; 14, 15 (USNM 82883), dorsal bar; 16, ventral bar; 17, hook.

1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 14 (11–15); 2, 15 (13–16); 3, 15 (14–18); 4, 16 (14–18); 4A, 6 (6–7); 5, 15 (14–16); 6, 14 (13–16); 7, 14 (13–15). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus with rounded base bearing slender, usually straight process and slightly recurved tubular shaft that is attenuated to a point. Cirrus length 35 (32–39). Process length (measured from base of cirrus shaft to distal tip of process) 10 (9–12). Shaft length 28 (27–32). Accessory piece bifurcate, distal ramus curved and attenuated to a point; medial ramus a recurved blade, attenuated to a point. Accessory piece length 20 (15–23). Vagina sclerotized, irregular in shape, opening dextroventrally posterior to cirrus. Vitellaria obvious, usually distributed from pharynx to peduncle.

REMARKS: *Dactylogyrus alborig* most closely resembles *D. alabamensis* by possessing similar-shaped anchors and cirrus, but comparison of type specimens and the original description of *D. alabamensis* (Rogers and Mizelle, 1966) reveals trenchant differences. The sclerites of *D. alborig* are notably smaller. The dorsal bar of *D. alabamensis* is robust and v-shaped, whereas that of *D. alborig* is more slender and gently curved.

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

*Dactylogyrus onychoaccessorius* sp. n.  
(Figs. 10–17)

TYPE LOCALITY: North Carolina: Mecklenburg Co., South Prong Clarks Creek, Asbury Chapel Road. Pee Dee River system.

TYPE SPECIMENS: Holotype, USNM 82882; 7 paratypes, USNM 82883 (1 specimen), USNM 82884 (3 specimens), HWML 36609 (3 specimens).

OTHER LOCALITY: North Carolina: Mecklenburg Co., Mallard Creek, Highway 29 bridge. Pee Dee River system.

LOCATION ON HOST: Gills.

DESCRIPTION: With characters of the genus *Dactylogyrus*, as emended by Mizelle and McDougal (1970). Body with thin tegument; length 294 (216–374), greatest width 57 (43–72). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, anterior pair ranges from farther apart to closer together than posterior pair. Pharynx circular to ovate (dorsal view), transverse diameter 21 (18–23), gut not observed. Peduncle 34 (14–84) long, 30 (21–49) wide. Haptor 46 (28–70) long, 65 (49–77) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, and elongate solid shaft that curves to a sharp point. Anchor length 35 (33–36), greatest width of base 14

(13–15). Dorsal bar length 22 (21–22). Vestigial ventral bar with medial knob, length 23 (21–25). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 20 (18–23); 2, 22 (21–23); 3, 22 (19–25); 4, 25 (22–29); 4A, 6 (5–6); 5, 22 (21–24); 6, 21 (20–22); 7, 22 (21–24). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus with base bearing robust curving shaft that attenuates to a point, length 25 (23–28). Accessory piece composed of 2 clawlike rami that are fused near the proximal end, length 14 (13–15). Vagina not observed. Vitellaria moderate, distributed from pharynx to peduncle.

**REMARKS:** *Dactylogyrus onychoaccessorius* possesses a distinctive cirrus and accessory piece. Its relationship to other North American species of *Dactylogyrus* is uncertain, but it may be most closely related to *D. distinctus*. Type specimens of *D. distinctus* could not be located; however, comparison of the original description (Mizelle and Klucka, 1953) indicates that the cirrus base is much larger and the cirrus shaft and accessory piece rami are much more robust in *D. albori* than in *D. distinctus*.

**ETYMOLOGY:** The specific name is from Greek (*onycho* = claw), referring to the clawlike accessory piece.

### Discussion

*Notropis alborus* was originally compared with *N. atrocaudalis* Evermann, *N. bifrenatus* (Cope), *N. heterolepis* Eigenmann and Eigenmann, *N. stramineus* (Cope) (= *N. deliciosus* (Girard) and *N. ludibundus* (Girard)) (see Mayden and Gilbert, 1989), *N. procne* (Cope), and *N. volucellus* (Cope) (Hubbs and Raney, 1947). Although unable to determine precise relationships, ichthyologists have generally speculated that *N. alborus* is closely related to *N. heterolepis*, which has variously been placed in the *N. procne* species group (Snelson, 1971; Burr and Mayden, 1981; Page and Beckham, 1987), the *N. heterolepis* group as part of a "tooth reduction" group, which includes several of the preceding species plus some others (Coburn, 1982), and the *N. volucellus* group (Mayden, 1989). Coburn (1982) stated that the *N. heterolepis* group, which he thought should possibly include *N. alborus*, may share synapomorphies with the subgenus *Hybopsis* (sensu

Robins et al., 1991). Mayden (1989) concluded that *N. alborus*, but not *N. heterolepis*, shared synapomorphies with *Hybopsis* and thus transferred *N. alborus* to *Hybopsis*. Robins et al. (1991) did not include *N. alborus* in the subgenus *Hybopsis*.

Assuming that the Farenholz rule (co-speciation) is the prevailing pattern rather than homoplasy (co-accommodation) (Brooks, 1979), I hypothesize that the apparent close relationship between *Dactylogyrus albori* and *D. alabamensis* indicates that *N. alborus* is closely related to *Hybopsis* because *D. alabamensis* has been found only on species of *Hybopsis* (Mizelle and McDougal, 1970). In contrast, the apparent relationship between *D. onychoaccessorius* and *D. distinctus* indicates a possible relationship between *N. alborus* and the *N. volucellus* species group (which includes *N. heterolepis* according to Mayden [1989]) because *D. distinctus* has been reported only from *N. volucellus*. This possible relationship is further strengthened by *D. heterolepis*, found only on *N. heterolepis* (Hanek et al., 1975), being somewhat similar to *D. albori* and *D. alabamensis*. However, *D. albori* and *D. alabamensis* are apparently more closely related to each other than to *D. heterolepis*, indicating that *N. alborus* is more closely related to *Hybopsis* than to the *N. volucellus* species group, including *N. heterolepis*. These data also indicate that the *N. volucellus* species group, including *N. heterolepis*, may be fairly closely related to the subgenus *Hybopsis*. Further ichthyological and parasitological studies, including cladistic analyses of all species of hosts and parasites, are needed before the relationships between *Hybopsis* and the preceding groups of *Notropis* and their parasites will be thoroughly resolved.

### Acknowledgments

I thank Dr. Edward F. Menhinick, University of North Carolina at Charlotte, and Larry E. Miller and Sherry M. Reid, Duke Power Company, for help in collecting hosts. Drs. J. Ralph Lichtenfels and Mary Hanson Pritchard loaned type specimens of *Dactylogyrus* from the USNM and HWML, respectively.

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## Neotropical Monogenoidea. 19. Dactylogyridae of Cichlids (Perciformes) from the Yucatán Peninsula, with Descriptions of Three New Species of *Sciadicoleithrum* Kritsky, Thatcher, and Boeger, 1989

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**ABSTRACT:** Three new species of *Sciadicoleithrum* are described from the gills of cichlids from the Yucatán Peninsula in North America: *Sciadicoleithrum mexicanum* sp. n. from "*Cichlasoma*" *urophthalmus* (Günther); *Sciadicoleithrum bravohollisae* sp. n. from "*C.*" *pearsei* (Hubbs) (type host), "*C.*" *synspilum* Hubbs, and *Petenia splendida* Günther; and *Sciadicoleithrum splendidae* sp. n. from *P. splendida*. *Cichlidogyrus sclerosus* Paperna and Thurston, 1969, is reported from the gills of *Oreochromis niloticus* (Linnaeus), an introduced fish in Mexico.

**KEY WORDS:** Mexico, Monogenoidea, Dactylogyridae, *Sciadicoleithrum*, *Sciadicoleithrum mexicanum* sp. n., *Sciadicoleithrum bravohollisae* sp. n., *Sciadicoleithrum splendidae* sp. n., *Cichlidogyrus sclerosus*, "*Cichlasoma*" *urophthalmus*, "*Cichlasoma*" *pearsei*, "*Cichlasoma*" *synspilum*, *Petenia splendida*, *Oreochromis niloticus*.

Members of the Dactylogyridae (Polyonchoinea, Dactylogyridae) have been reported as parasites of cichlid fishes from nearly throughout their hosts' geographic range. *Gussevia* Kohn and Paperna, 1964, *Sciadicoleithrum* Kritsky, Thatcher, and Boeger, 1989, and *Trinidactylus* Hanek, Molnar, and Fernando, 1974, are restricted to and include the only known Dactylogyridae (Ancyrocephalinae) infesting Neotropical Cichlidae (see Kritsky et al., 1986, 1989). In continental Africa, native cichlids support species of *Cichlidogyrus* Paperna, 1960, *Onchobdella* Paperna, 1968, and *Enterogyrus* Paperna, 1963, and in Madagascar species of *Insulaeleidus* Rakotofringa and Euzet, 1983 (see Paperna, 1979; Rakotofringa and Euzet, 1983). Asian cichlids are parasitized by species of *Ancyrocephalus* Creplin, 1839 (sensu lato), *Ceylonotrema* Gussev, 1963, and *Enterogyrus* (see Gussev, 1963).

During surveys of helminth parasites of fishes from the Yucatán Peninsula, 3 undescribed species of *Sciadicoleithrum* were recovered from the gills of native North American cichlids. In addition, a species of *Cichlidogyrus*, apparently introduced with its host from Africa, was found. These geographic and host records, descriptions of the new species of *Sciadicoleithrum* and a brief discussion of origin and speciation of *Sciadicoleithrum* species in Central America are provided herein.

### Materials and Methods

Cichlid<sup>1</sup> hosts, "*Cichlasoma*" *urophthalmus*, "*C.*" *synspilum*, "*C.*" *pearsei*, *Petenia splendida*, and *Oreochromis niloticus*, were collected from several localities on the Yucatán Peninsula during 1989-1992. Methods of collection, preparation, measurement, and illustration of helminths were those described by Kritsky et al. (1986). In addition, some specimens were fixed in Bouin's, stained with Gomori's trichrome and mounted in Canada balsam; others were cleared in lactophenol and mounted unstained in glycerine jelly. Measurements are in micrometers; the mean is followed by the range and number (*n*) of specimens measured in parentheses. Type specimens are deposited in the helminthological collections of the U.S. National Museum (USNM), Beltsville, Maryland, and the University of Nebraska State Museum (HWML), Lincoln; vouchers of each species are in the Colección Helminológica del Instituto de Biología, Universidad Nacional Autónoma de México, México City, México.

### *Sciadicoleithrum mexicanum* sp. n. (Figs. 1-8)

**DESCRIPTION** (based on 50 specimens): Body 320 (245-398; *n* = 24) long, fusiform; greatest width 76 (59-117; *n* = 25) near midlength or in

<sup>1</sup> Stiasny (1991) suggested that cichlid species formerly included in *Cichlasoma* and now lacking formal generic placement as a result of Kullander's (1983) revision of the genus should be referred to by the generic name in quotes until phylogenetic analysis of the group allows formal generic assignment.

posterior trunk. Cephalic lobes moderately developed. Eyes 4; members of posterior pair with conspicuous lens, larger, closer together than members of anterior pair; eye granules variable in size, elongate ovate; accessory granules in cephalic region, uncommon in anterior trunk. Pharynx spherical, 18 (15–20;  $n = 20$ ) in diameter; esophagus moderately long. Peduncle broad; haptor subhexagonal, 75 (55–98;  $n = 25$ ) wide, 59 (50–70;  $n = 26$ ) long. Ventral anchor 33 (29–35;  $n = 22$ ) long, with short deep root, tapering superficial root, shaft and point with longitudinal lateral grooves; base width 16 (15–17;  $n = 17$ ). Dorsal anchor 39 (35–41;  $n = 19$ ) long, with slightly appressed roots, shaft and point with longitudinal lateral grooves; base width 14 (13–16;  $n = 16$ ). Ventral bar 34 (30–37;  $n = 21$ ) long, robust, with enlarged ends; dorsal bar 31 (29–33;  $n = 21$ ) long, broadly V-shaped rod with enlarged ends. Hooks similar; each 15 (14–17;  $n = 71$ ) long, with upright thumb, delicate point, shank varying in diameter along length; domus  $\frac{2}{3}$  shank length. Gonads slightly overlapping. Testis 61 (50–72;  $n = 13$ )  $\times$  27 (24–32;  $n = 11$ ), elongate ovate; apparent seminal vesicle elongate fusiform, with thick wall; prostatic reservoir saccate. Coil of male copulatory organ loose, comprising about 0.5 poorly defined ring, frequently appearing U-shaped; base of copulatory organ with sclerotized margin; copulatory organ length 62 (53–68;  $n = 17$ ). Accessory piece 45 (37–52;  $n = 12$ ) long, comprising delicate sheath enclosing copulatory organ, terminating in diagonal opening. Germarium 32 (27–43;  $n = 12$ )  $\times$  21 (14–25;  $n = 11$ ), with irregular margin; oviduct, ootype, uterus not observed; vagina dextral, distal opposing funnel-shaped sclerites guarding aperture; seminal receptacle midventral, small; vitellaria dense throughout trunk, except absent in regions of reproductive organs.

**HOST AND LOCALITIES:** Gills of "*Cichlasoma*" *urophthalmus* (Günther); Progreso (type) and Río Lagartos, Yucatán, Mexico, and Champotón, Atasta, and Laguna Silvituc, Campeche, Mexico.

**SPECIMENS STUDIED:** Holotype, USNM 82796; 49 paratypes, USNM 82797, HWML 36295–36299.

**REMARKS:** *Sciadicleithrum mexicanum* most closely resembles *S. bravohollisae* sp. n. Both species possess dorsal and ventral anchors with longitudinal lateral grooves on the shafts and points, dorsal anchors with appressed roots, gonads slightly overlapping or tandem, vaginae with

opposing "funnel-shaped" sclerites near their apertures, accessory pieces comprising delicate sheaths enclosing the distal portions of the U-shaped shafts of the male copulatory organs, and thickened walls of the apparent seminal vesicles. *Sciadicleithrum mexicanum* differs from *S. bravohollisae* by having a male copulatory organ with a thin shaft and simple base lacking a proximal lobed projection and a more delicate vaginal sclerotization. This species is named for the country from which it was collected.

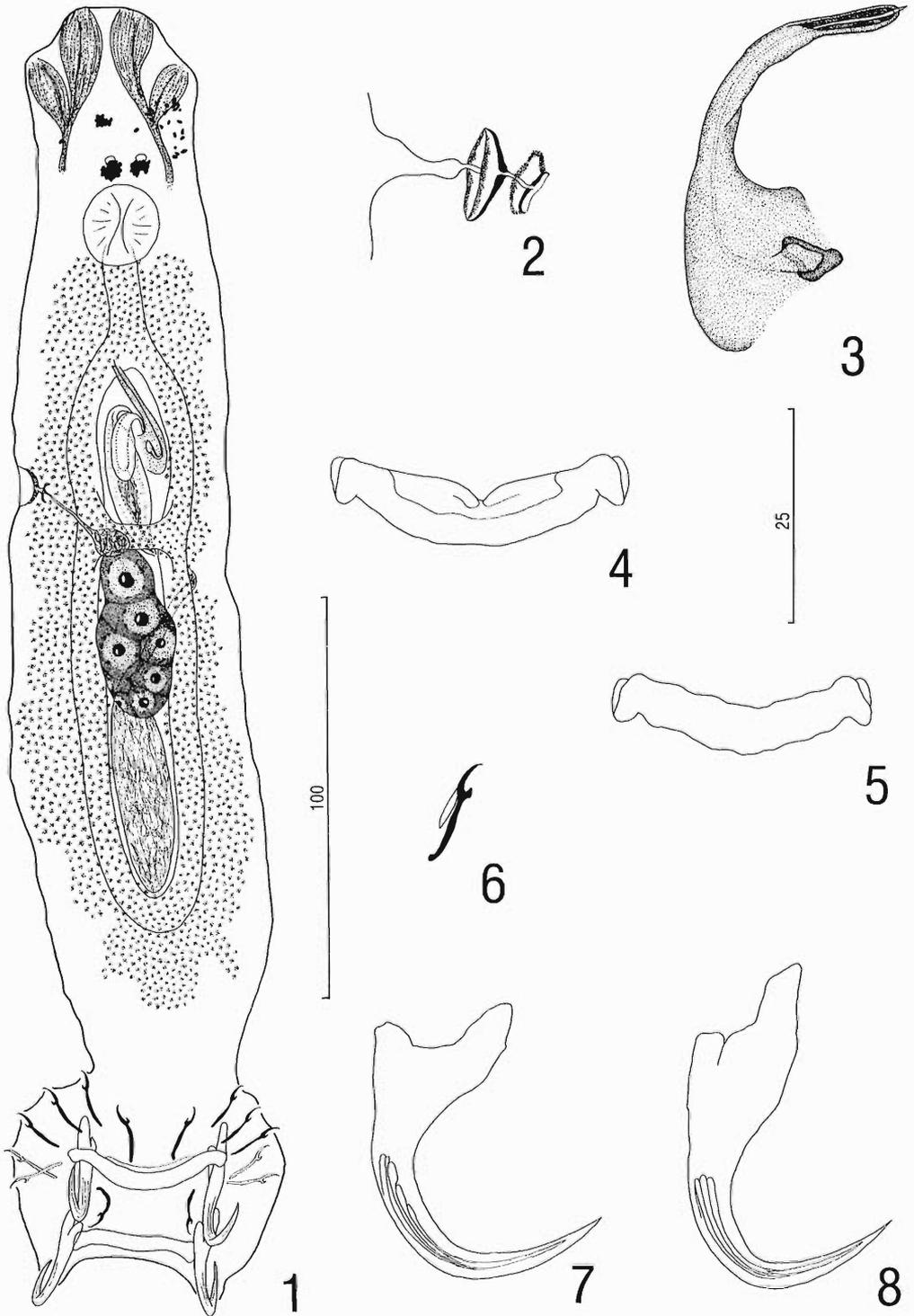
***Sciadicleithrum bravohollisae* sp. n.**

(Figs. 9–17)

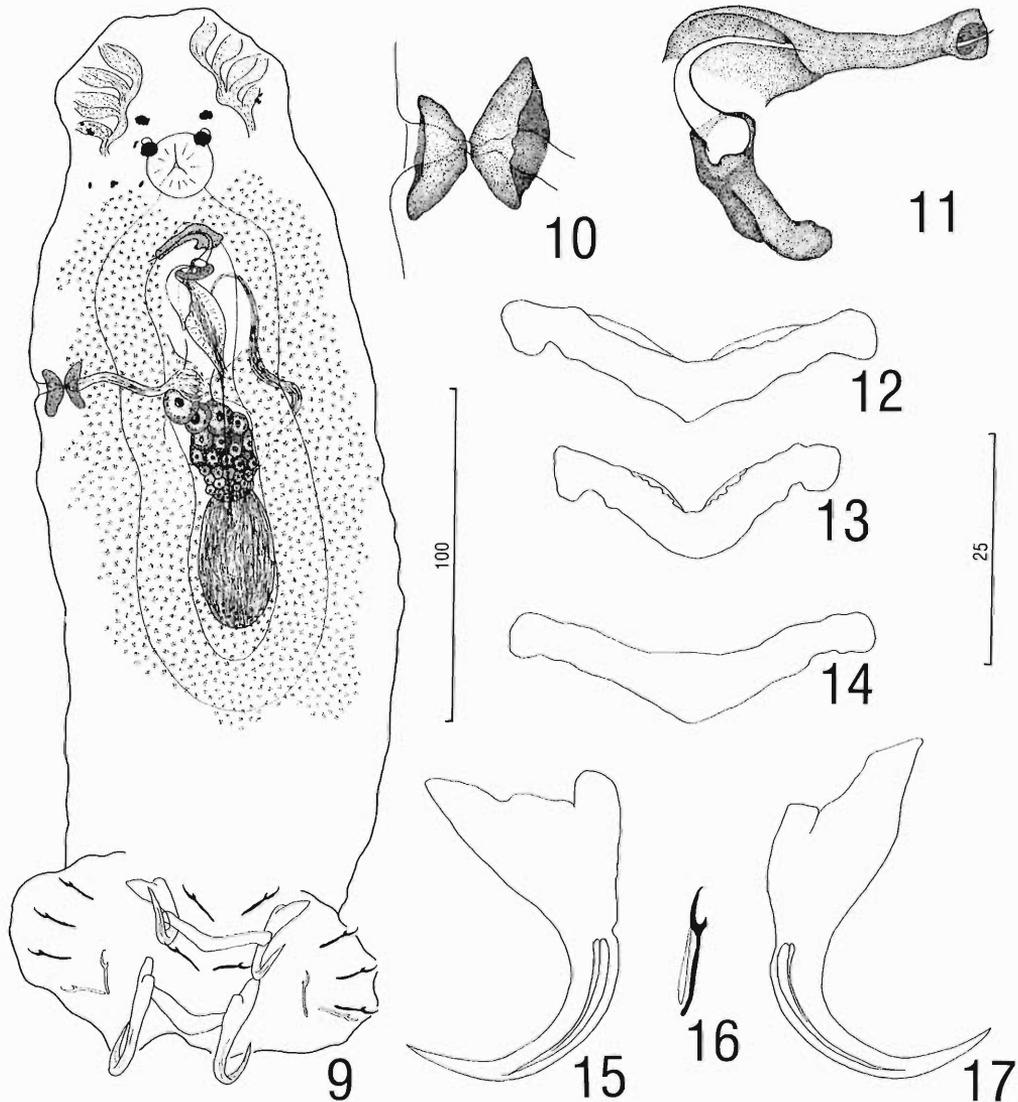
**DESCRIPTION** (based on 16 specimens from "*Cichlasoma*" *pearsei*; comparative measurements by host presented in Table 1): Body stout; greatest width near midlength. Cephalic margin broad; cephalic lobes moderately developed. Eyes 4; members of posterior pair with conspicuous lens, larger, closer together than members of anterior pair; eye granules variable in size, usually elongate ovate; accessory granules in cephalic, anterior trunk regions. Pharynx spherical; esophagus short (contracted specimens) to moderately long. Peduncle broad; haptor subovate. Ventral anchor with short roots, shaft and point with longitudinal lateral grooves. Dorsal anchor with slightly appressed roots, shaft and point with longitudinal lateral grooves. Ventral bar yoke-shaped, with delicate umbelliform membranes; dorsal bar expanded medially, with slightly enlarged ends. Hooks similar; each with upright thumb, delicate point, shank; domus  $\frac{1}{10}$  shank length. Gonads slightly overlapping. Testis ovate; apparent seminal vesicle with thick wall, fusiform; prostatic reservoir fusiform. Coil of male copulatory organ loose, comprising less than 1 poorly defined ring; shaft of copulatory organ proximally tapered; base with sclerotized margin, lobed proximal projection. Accessory piece comprising variable sheath enclosing distal shaft of copulatory organ. Germarium with irregular margin; oviduct, ootype, uterus not observed; vagina dextral, with 2 opposing funnel-shaped distal sclerites, opening into small medial seminal receptacle; vitellaria dense throughout trunk, except absent in regions of reproductive organs.

**TYPE HOST AND LOCALITY:** Gills of "*Cichlasoma*" *pearsei* (Hubbs); El Vapor Lagoon, Campeche, Mexico.

**OTHER RECORDS:** Gills of "*Cichlasoma*" *synspilum* Hubbs; El Vapor Lagoon and Atasta,



Figures 1–8. *Sciadicleithrum mexicanum* sp. n. 1. Composite illustration of entire specimen (ventral). 2. Vaginal aperture and sclerotization. 3. Copulatory complex. 4. Ventral bar. 5. Dorsal bar. 6. Hook. 7. Ventral anchor. 8. Dorsal anchor. All drawings are made to the 25- $\mu$ m scale except Figure 1 (100- $\mu$ m scale).



Figures 9–17. *Sciadicleithrum bravohollisae* sp. n. 9. Ventral view of entire specimen (composite). 10. Vaginal aperture and sclerotization. 11. Copulatory complex. 12, 13. Ventral bars. 14. Dorsal bar. 15. Ventral anchor. 16. Hook. 17. Dorsal anchor. All drawings are to the 25- $\mu$ m scale except Figure 9 (100- $\mu$ m scale).

Campeche, Mexico. Gills of *Petenia splendida* Günther; El Vapor Lagoon, Campeche, Mexico.

**SPECIMENS STUDIED:** Holotype, USNM 82794; 15 paratypes, USNM 82795, HWML 36289; 13 vouchers (from "*C.*" *synspilum*), HWML 36290, 36291; 2 vouchers (from *Petenia splendida*), HWML 36292.

**REMARKS:** *Sciadicleithrum bravohollisae* resembles *S. mexicanum* sp. n. Morphologic details separating them are presented in the remarks for *S. mexicanum*. This species is named for Dr. M. Bravo-Hollis, Universidad Nacional

Autonoma de Mexico, in appreciation and recognition of her significant contributions to present understanding of the Monogenoidea of Central and North America.

***Sciadicleithrum splendidae* sp. n.**  
(Figs. 18–24)

**DESCRIPTION** (based on 2 specimens): Body 250 ( $n = 1$ ) long, with parallel lateral margins; greatest width 83 (74–92;  $n = 2$ ). Cephalic lobes moderately developed. Eyes 4; members of posterior pair with conspicuous lens, larger, closer together

**Table 1.** Comparative measurements (in micrometers; mean with range in parentheses; *n* = number of specimens measured) of *Sciadicleithrum bravohollisae* from 3 different hosts collected from the Yucatán Peninsula, Mexico.

	" <i>Cichlasoma</i> " <i>pearsei</i>	<i>n</i>	" <i>Cichlasoma</i> " <i>synspilum</i>	<i>n</i>	<i>Petenia</i> <i>splendida</i>	<i>n</i>
Body length	391 (296–558)	8	401 (307–580)	5	515 (508–522)	2
Greatest width	103 (80–121)	9	140 (136–155)	6	101 (79–123)	2
Pharynx	22 (20–25)	7	23 (22–27)	6	21 (17–25)	2
Haptor length	72 (57–89)	9	65 (62–66)	5	70	1
Haptor width	97 (83–111)	8	103 (92–120)	5	79	1
Dorsal anchor length	41 (39–44)	7	33 (30–35)	5	30	1
Dorsal anchor width	17 (14–18)	7	17 (16–18)	3	16	1
Ventral anchor length	36 (34–39)	6	28–29	3	27	1
Ventral anchor width	22 (21–24)	6	19 (18–20)	3	17	1
Dorsal bar length	32 (30–33)	6	31 (30–32)	6	30	1
Ventral bar length	31 (28–34)	5	29 (27–32)	6	28	1
Hook length	16 (14–17)	27	15 (14–16)	21	15–16	3
Male copulatory organ length	54 (45–60)	7	53 (50–58)	4	—	0
Accessory piece length	39 (31–45)	4	31 (26–37)	5	—	0
Germarial length	38 (27–53)	7	43 (24–84)	6	62	1
Germarial width	19 (16–23)	7	30 (20–43)	6	20	1
Testis length	45 (36–57)	5	45 (31–64)	5	—	0
Testis width	27 (23–30)	5	30 (27–39)	5	—	0

than members of anterior pair; eye granules variable in size, irregular to elongate ovate; accessory granules in cephalic, trunk regions. Pharynx sub-spherical, 20 (*n* = 1) in diameter. Peduncle broad; haptor subtrapezoidal, 77 (*n* = 1) wide, 62 (*n* = 1) long. Ventral anchor 32 (*n* = 1) long, with short deep root, protruding superficial root, shaft and point with longitudinal lateral grooves; base width 17 (*n* = 1). Dorsal anchor 40 (*n* = 1) long, with slightly appressed roots, shaft and point with longitudinal lateral grooves; base width 14 (*n* = 1). Ventral bar 39 (38–40; *n* = 2) long, robust, with enlarged ends; dorsal bar 37–38 (*n* = 2) long, broadly V-shaped. Hooks similar; each 15–16 (*n* = 6) long, with upright thumb, delicate point, shank; domus  $\frac{3}{4}$  shank length. Gonads slightly overlapping. Testis 47 (*n* = 1) × 22 (*n* = 1), elongate ovate; seminal vesicle, prostatic reservoir not observed. Coil of male copulatory organ loose, comprising about 2.5 rings; base of copulatory organ with sclerotized margin, bilobed proximal branch; length of copulatory organ 230 (228–233; *n* = 2), proximal ring diameter 31 (27–35; *n* = 2). Accessory piece 22 (*n* = 1) long, comprising delicate sheath enclosing subterminal portion of shaft of copulatory organ. Germarium 30 (*n* = 1) × 21 (*n* = 1), subovate; oviduct, ootype, uterus not observed; vagina dextral, a dilated tube looping anteriorly to level of copulatory complex, with distal funnel guarding aperture; seminal receptacle dextromedial, small; vitellaria dense throughout trunk, except absent in regions of reproductive organs.

HOST AND LOCALITY: Gills of *Petenia splendida* Günther; El Vapor Lagoon, Campeche, Mexico.

SPECIMENS STUDIED: Holotype, USNM 82793; paratype, HWML 36293.

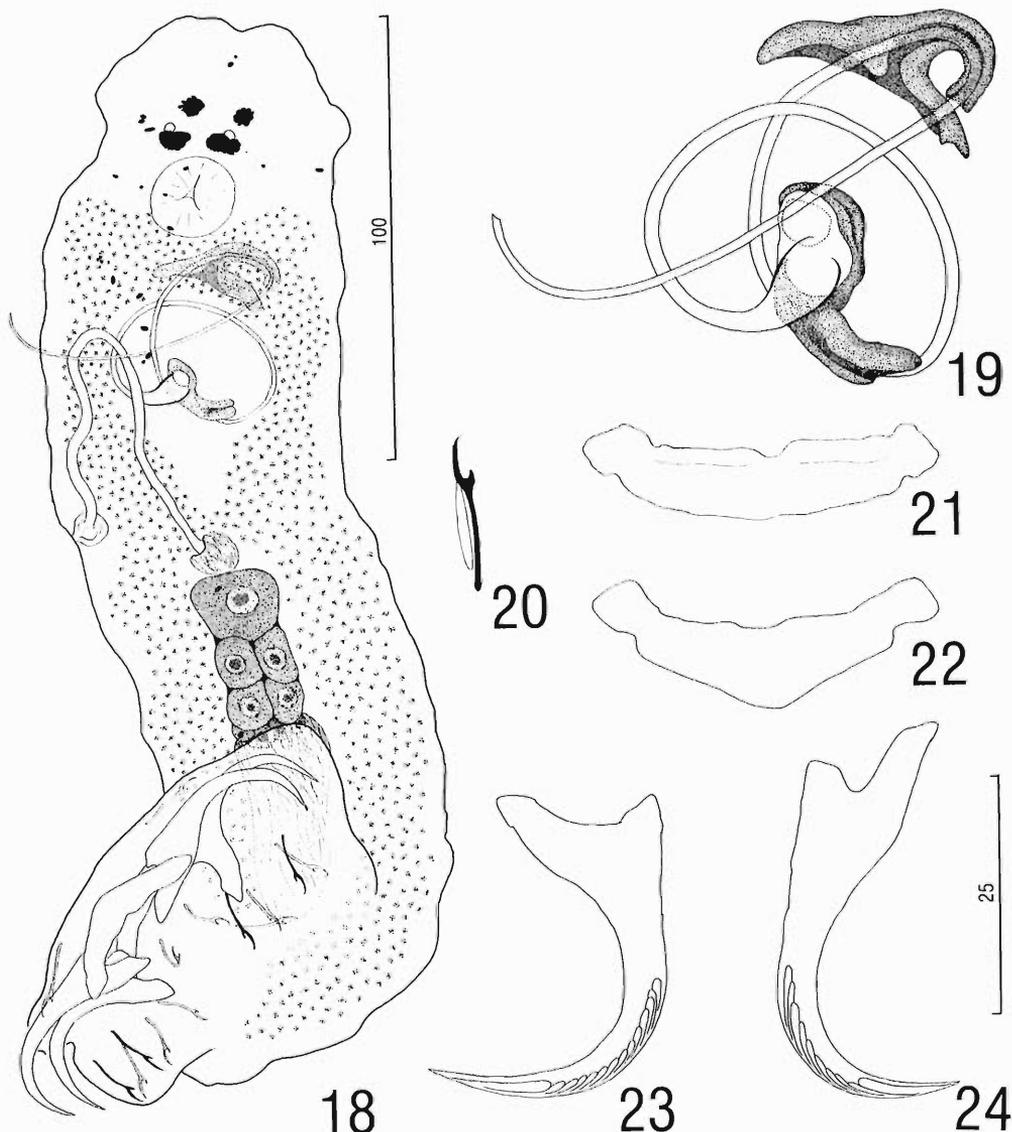
REMARKS: Only 2 specimens of this species were found on 1 specimen of *Petenia splendida*. Both specimens were mounted unstained in Gray and Wess' medium, which precluded complete study of the internal anatomy. Nonetheless, morphology of haptor and copulatory sclerites clearly indicate that these specimens comprise an undescribed species of *Sciadicleithrum* with affinities to *S. bravohollisae* and *S. mexicanum* spp. n. This species differs from *S. bravohollisae* and *S. mexicanum* by possessing an elongate vaginal canal lacking a "double funnel" sclerotization of its external aperture, dorsal anchors with comparatively elongate straight shafts, and a coiled male copulatory organ with more than 1 complete ring. The specific name is derived from that of its host.

#### *Cichlidogyrus sclerosus* Paperna and Thurston, 1969

HOST AND LOCALITY: Gills of *Oreochromis niloticus* (Linnaeus); Laguna Noh-Bek, Quintana Roo, Mexico.

SPECIMENS STUDIED: Two vouchers, HWML 36294.

MEASUREMENTS: Pharyngeal diameter 40 (35–45; *n* = 2); ventral anchor length 28 (*n* = 1), width 18 (*n* = 1); dorsal anchor length 25 (*n* = 1), width 16 (*n* = 1); ventral bar length 41 (*n* =



Figures 18–24. *Sciadicleithrum splendidae* sp. n. 18. Holotype (ventral view); haptor (dorsal view) folded ventrally. 19. Copulatory complex. 20. Hook. 21. Ventral bar. 22. Dorsal bar. 23. Ventral anchor. 24. Dorsal anchor. All drawings are to the 25- $\mu$ m scale except Figure 18 (100- $\mu$ m scale).

1); dorsal bar length 31 ( $n = 1$ ); length of male copulatory organ 55 ( $n = 1$ ); accessory piece length 43 (42–45;  $n = 2$ ); egg length 55 ( $n = 1$ ), width 39 ( $n = 1$ ).

REMARKS: *Cichlidogyrus sclerosus*, a natural parasite of cichlids in Africa, was first reported from the Western Hemisphere by Kritsky and Thatcher (1974) on *Oreochromis mossambicus* (Peters) (= *Tilapia mossambica*) from Colombia. The parasite has subsequently been reported repeatedly from Cuba on *O. aureus* by Vinjoy et

al. (reported in Prieto et al., 1985) and on *O. aureus*, *Sarotherodon hornorum*, and hybrids of *O. mossambica* [sic]  $\times$  *S. hornorum* by Prieto et al. (1985) and Prieto and Fajer (1987). Martínez (1980) reported *Cichlidogyrus* sp. on tilapia roja [sic] in Cuba (paper not seen by present authors; reported in Prieto et al., 1985); this report probably included *C. sclerosus*. All reported occurrences of *C. sclerosus* in the Western Hemisphere apparently are results of concomitant human introductions of the parasite and its hosts.

### Discussion

Although some Central American fish species clearly have their origins within North America, the fish fauna of this region is dominated by forms with evolutionary links to South America. The invasion of Central America by South American fishes probably occurred more than once (cf. Rosen, 1975), with that of the cichlids preceding the dispersal of primary freshwater fishes into the area (Myers, 1966). According to Myers (1966), evolution of "*Cichlasoma*" (and of its relatives) in Central America has been rapid since invasion, which probably occurred during the Miocene or late Oligocene. Miller (1966) listed 68 described species of Central American "*Cichlasoma*" occurring south of the Isthmus of Tehuantepec in Mexico.

*Sciadicleithrum* includes a group of 12 described species infesting gills of New World Cichlidae in the Amazon Basin and Guyana (Kritsky et al., 1989) and Central America (nobis). Although species of this genus have not previously been reported as parasites of *Cichlasoma* in South America, Central American species of *Sciadicleithrum* are clearly more closely related to each other than to any known South American species, suggesting that they have undergone speciation since dispersal of a common ancestor to the area. Among apparent synapomorphic characters supporting sister relationships of Central American *Sciadicleithrum* are (a) the longitudinal lateral grooves on the shafts and points of the ventral and dorsal anchors, (b) the sheathlike accessory piece, (c) the slightly overlapping or tandem gonads, (d) the slightly appressed roots of the dorsal anchors, and (e) the thickened walls of what appears to be the seminal vesicle.

Species of *Sciadicleithrum* apparently have experienced a parallel evolutionary history with that of their hosts in Central America. Although they may be assigned to the South American-Caribbean generalized distributional tract defined by Rosen (1975), it is not presently clear whether the *Sciadicleithrum* clade in Central America represents descendants from early or late invasions to the area because surveys for dactylogyrids infesting cichlids of the Antilles and Mexico north of the Isthmus of Tehuantepec are lacking. If *Sciadicleithrum* does not occur in the Antilles and northern Mexico, the distribution would suggest 2 possibilities: (a) that it entered Central America during late invasions predicted by Rosen's (1975) vicariance model, or (b) that

ancestral *Sciadicleithrum* reaching Central America during the early wave(s) of dispersal experienced subsequent extinction within the Antilles after relative displacement eastward had effectively separated the Antillian area from Central America. The latter scenario is not unlikely, because extinction rates are presumed to be locally high as historical geographic ranges fragment or dispersal to new areas occurs (Wilson, 1992). If, however, *Sciadicleithrum* species are found in either the Antilles or northern Mexico, the new distribution would support early dispersal of the taxon to Central America.

It is apparent that further survey of Dactylogyridae infesting cichlids in South and Central America and both the Greater and Lesser Antilles will be necessary to understand the evolutionary history of *Sciadicleithrum*. Knowing the parasites harbored by cichlids occurring north of the Isthmus of Tehuantepec might provide insight on the temporal aspects of invasion by their ancestors. Absence of a phylogenetic hypothesis for members of all dactylogyrid genera infesting cichlids in the New World also limits our ability to determine potential coevolutionary relationships of these parasites and their hosts in the region.

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## Neotropical Monogenoidea. 20. Two New Species of Oviparous Gyrodactylidea (Polyonchoinea) from Loricariid Catfishes (Siluriformes) in Brazil and the Phylogenetic Status of Ooegyrodactylidae Harris, 1983

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**ABSTRACT:** Two new species of oviparous gyrodactylideans are described from the external surface of Brazilian loricariid catfishes: *Phanerothecium spinatus* sp. n. from *Hypostomus punctatus* (Valenciennes) from Rio Guandú, State of Rio de Janeiro; and *Hyperopletes malmbergi* gen. et sp. n. from *Rhineloricaria* sp. from Igarapé Candiru, State of Amazonas. The monotypic *Hyperopletes* gen. n. is proposed for species having a seminal vesicle located within the copulatory sac, a second external seminal vesicle, and an eversible copulatory organ armed with spines. Cladistic analysis of the oviparous species, *H. malmbergi* sp. n., *Nothogyrodactylus amazonicus* Kritsky and Boeger, 1991, *N. clavatus* Kritsky and Boeger, 1991, *N. plaesiophallus* Kritsky and Boeger, 1991, *Ooegyrodactylus farlowellae* Harris, 1983, *Phanerothecium caballeri* Kritsky and Thatcher, 1977, *P. harrisi* Kritsky and Boeger, 1991, and *P. spinatus* sp. n., and the viviparous Gyrodactylidae (considered a single monophyletic taxon), indicates that Ooegyrodactylidae Harris, 1983, is paraphyletic. Ooegyrodactylidae is rejected, and the genera *Hyperopletes*, *Nothogyrodactylus*, *Ooegyrodactylus*, and *Phanerothecium* are transferred to the Gyrodactylidae Van Beneden and Hesse, 1863. Synapomorphies supporting the new composition of the Gyrodactylidae are presence of (a) a muscular copulatory organ, (b) spike sensilla in the head organs, (c) an unciliated larva (oncomiracidium absent), (d) a deep bar associated with the deep roots of the anchor pair, (e) separate genital pores, (f) a massive Mehlis' gland, (g) an amorphous cap on the egg filament, and absence of (h) a vagina and (i) eyes in the larva and adult. All oviparous genera received evolutionary support except the paraphyletic *Nothogyrodactylus*.

**KEY WORDS:** Monogenoidea, Ooegyrodactylidae, Gyrodactylidae, *Hyperopletes* gen. n., *Hyperopletes malmbergi* sp. n., *Phanerothecium spinatus* sp. n., Loricariidae, *Rhineloricaria* sp., *Hypostomus punctatus*, cladistics, phylogeny.

During a survey of ectoparasites of Neotropical freshwater fishes from the States of Amazonas and Rio de Janeiro in Brazil, 2 new species of oviparous Gyrodactylidea were recovered from armored catfishes (Loricariidae). In this article, these species are described, and *Hyperopletes* gen. n. is proposed to accommodate 1 of them.

The Ooegyrodactylidae was proposed by Harris (1983) for oviparous Monogenoidea with close relationships to viviparous Gyrodactylidae. Monophyly of Harris' family was challenged by Kritsky and Boeger (1991) because of the apparent absence of synapomorphic features, and Boeger and Kritsky (1993) tentatively considered the family a junior synonym of the Gyrodactylidae in their phylogenetic analysis of the Monogenoidea. To evaluate the evolutionary status of this family, the phylogenetic relationships of oviparous gyrodactylid-like species and the Gyrodactylidae (sensu stricto) are reconstructed us-

ing methods of phylogenetic systematics. Results of this analysis are presented herein.

### Materials and Methods

*Rhineloricaria* sp. were caught during February 1991 by E.B.J. from the "igarapé" Candiru, a small tributary of the Rio Puraquequara on Highway AM-010 near Manaus, Amazonas, Brazil. *Hypostomus punctatus* (Valenciennes) were captured during January 1991 from the Rio Guandú, Nova Iguaçu County, State of Rio de Janeiro, Brazil, by a professional fisherman. Hosts were placed individually or pooled in a container containing a 1:4,000 formalin solution for removal of helminths according to procedures of Putz and Hoffman (1963). After 1 h, the vial was shaken vigorously, and formalin was added to increase concentration to 5%. Some specimens were mounted unstained in Gray and Wess' medium for study of sclerotized structures. Other specimens were stained with Gomori's trichrome for determination of internal morphology. Illustrations were prepared with the aid of a camera lucida. Measurements, all in micrometers, were made according to procedures of Mizelle and Klucka (1953); the av-

erage is followed by the range and number ( $n$ ) of structures measured in parentheses. Type specimens were deposited in the helminthological collections of the Instituto Oswaldo Cruz (IOC), Rio de Janeiro; the University of Nebraska State Museum (HWML), Lincoln; and the U.S. National Museum (USNM), Beltsville, Maryland.

Transformation series used in the phylogenetic analysis were defined from the literature and available specimens. Homologous series in which the apomorphic state represents an autapomorphy of a single ingroup taxon were not utilized. In group taxa included all species described in *Hyperopletes* gen. n., *Nothogyrodactylus* Kritsky and Boeger, 1991, *Ooegyrodactylus* Harris, 1983, and *Phanerothecium* Kritsky and Thatcher, 1977; the group of viviparous species traditionally comprising the Gyrodactylidae (sensu stricto) was also included as a single in group taxon. For this analysis, viviparity was considered the synapomorphy supporting monophyly of the latter taxon; we assumed that viviparity developed only once in the evolutionary history of the Gyrodactylidae. The Gyrodactylidae (sensu stricto) is represented in the analysis as the viviparous ancestor; the hypothetical ancestor, developed by outgroup and functional outgroup comparison, represents the contrived ancestor of the ingroup (Table 1).

An initial hypothesis on the evolutionary relationships of in group taxa was constructed using Hennigian argumentation (Hennig, 1966; Wiley, 1981); the topology of the cladogram was then subjected to PAUP (Phylogenetic Analysis Using Parsimony, Version 2.4.1; D. L. Swofford, Illinois Natural History Survey, Campaign) to confirm that it was a most-parsimonious tree. A total of 35 character states comprising 19 transformation series was used in the analysis. Polarization of homologous series was determined by outgroup and functional outgroup analyses (Watrous and Wheeler, 1981; Maddison, et al., 1984). The Anoplodiscidae, Bothitrematidae, and Tetraonchoiidae were chosen as outgroups based on a previous reconstruction of the phylogeny of the Monogenoidea by Boeger and Kritsky (1993). The matrix is presented in Table 1.

## Results

### Taxonomic Account

#### Monogenoidea Bychowsky, 1937

#### Polyonchoinea Bychowsky, 1937

#### Gyrodactylidea Bychowsky, 1937

#### Gyrodactylidae Van Beneden and Hesse, 1863

#### *Phanerothecium spinatus* sp. n.

(Figs. 1–7)

HOST AND LOCALITY: *Hypostomus punctatus* (Valenciennes), Loricariidae; Rio Guandú, Nova Iguaçu, Rio de Janeiro, Brazil.

TYPE SPECIMENS: Holotype, IOC 33051a; 30 paratypes, IOC 33051b-k, USNM 82823, HWML 36346.

DESCRIPTION: Body fusiform, 1,124 (775–1,410;  $n = 12$ ) long; greatest width 175 (125–

215;  $n = 13$ ) near midlength; tegument smooth with 6 bilateral pairs of “sensory” pustules spaced along lateral margin from body midlength to distal peduncle. Bilateral excretory pores at level of anterior pharyngeal bulb. Cephalic lobes well developed; head organs conspicuous. Distal pharyngeal bulb 69 (60–80;  $n = 9$ ) in diameter, proximal pharyngeal bulb 69 (63–85;  $n = 9$ ) in diameter. Intestinal ceca terminating at level of posteriormost vitelline follicle. Testis subspherical, variable in size (depending on age of specimen), becoming inconspicuous in adult worms; proximal seminal vesicle with thick wall; distal seminal vesicle elongate; copulatory sac ovate; spines of copulatory organ numerous, minute. Germarium ovate, 70 (45–90;  $n = 8$ ) long, 75 (58–95;  $n = 8$ ) wide; seminal receptacle subspherical. Uterine pore a transverse slit, with sphincterlike muscle. Uterus delicate, containing a maximum of 10 eggs; egg elongate ovate, 162 (151–180;  $n = 6$ ) long, 41 (34–55;  $n = 6$ ) wide; filament moderately long; filament cap urn-shaped. Dextral pregermarial vitelline follicles absent. Peduncle elongate; haptor 87 (79–97;  $n = 9$ ) long, 95 (86–110;  $n = 9$ ) wide. Anchor 59 (52–65;  $n = 16$ ) long, with elongate superficial root, short angular deep root, slightly curved shaft, recurved point; base 25 (17–33;  $n = 14$ ) wide. Superficial bar 48 (32–60;  $n = 13$ ) long, plate like, with slightly enlarged ends; deep bar rod-shaped, variably bent. Hook with straight diagonal shaft, erect thumb, slender proximally tapered shank; hooklet 6 (4–7;  $n = 23$ ) long, shank 29 (27–34;  $n = 23$ ) long, domus  $\frac{1}{4}$  shank length.

REMARKS: *Phanerothecium spinatus* sp. n. differs from other congeneric species by having conspicuous spines on the copulatory organ and lacking a keel on the shank of the hooks. The specific name is from Latin (*spinatus* = spined) and refers to the presence of spines on the copulatory organ.

#### *Hyperopletes* gen. n.

DIAGNOSIS: Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Cephalic lobes 2, terminal; each with spike sensilla, portion of head organ. Cephalic glands unicellular, in 2 bilateral groups posterolateral, dorsal to level of pharynx. Eyes absent. Mouth ventral; pharynx comprising 2 tandem bulbs, proximal bulb glandular, distal bulb muscular with digitiform projections; intestinal ceca 2, lacking diverticula, nonconfluent. Worms oviparous, protandrous. Gonads tandem, in-

**Table 1. Character matrix used in the reconstruction of the evolutionary relationships of the Gyrodactylidae (sensu nobis).**

Taxon	Homologous series*																		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
<i>Ooegyrodactylus farlowellae</i>	1	1	0	1	1	1	1	0	1	2	1	1	1	0	0	1	9	9	9
<i>Phanerothecium caballeri</i>	1	1	0	1	1	1	1	0	1	9	1	1	9	9	1	1	9	9	9
<i>P. harrisi</i>	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	9	9	9
<i>P. spinatus</i>	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	9	9	9
<i>Nothogyrodactylus clavatus</i>	1	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1
<i>N. amazonicus</i>	1	1	0	1	1	1	0	0	0	1	1	1	1	0	0	1	0	0	1
<i>N. plaesiophallus</i>	1	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	0	1	1
<i>Hyperoletes malmbergi</i>	1	1	0	1	1	1	1	1	1	2	1	1	1	0	0	1	9	9	9
Viviparous ancestor	1	1	1	1	1	1	2	1	1	2	2	1	9	0	0	1	9	9	9
Hypothetical ancestor	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0

\* Homologous series are presented in an order corresponding to that of the character analysis. 9 = missing data.

terceal; testis postgermarial. External seminal vesicle subspherical. Copulatory sac well defined, with eversible copulatory organ, internal seminal vesicle; copulatory organ armed with spines. Germarium submedian with internal seminal receptacle; ootype surrounded by large Mehlis' gland; uterus with thin wall; uterine pore dextroventral at level of copulatory sac; vagina absent. Uterus containing a maximum of 1 egg; egg with single proximal filament; egg filament embedded in amorphous cap. Vitellaria comprising numerous large, postgermarial follicles. Haptor ventrally concave, with pair of ventral anchors, superficial bar lacking shield, deep bar connecting to deep roots, 16 gyrodactylid hooks marginal; hooks with extrahamular distribution (Kritsky and Mizelle, 1968). Parasitic on external surface of loriciariid fishes.

TYPE SPECIES: *Hyperoletes malmbergi* sp. n.

REMARKS: *Hyperoletes* gen. n. is monotypic. Characters that distinguish this genus from other oviparous gyrodactylids are the combined presence of (a) a spined copulatory organ, (b) a seminal vesicle lying within the copulatory sac, and (c) a second external seminal vesicle. Species of the host family Loriciariidae are commonly referred to as the "armored" catfishes; the name of the new genus is from Greek (*hyper* = above + *hopl/o* = armor + *-etes* = one who) and has the intended meaning of "the one who dwells on armor."

***Hyperoletes malmbergi* sp. n.**

(Figs. 8–13)

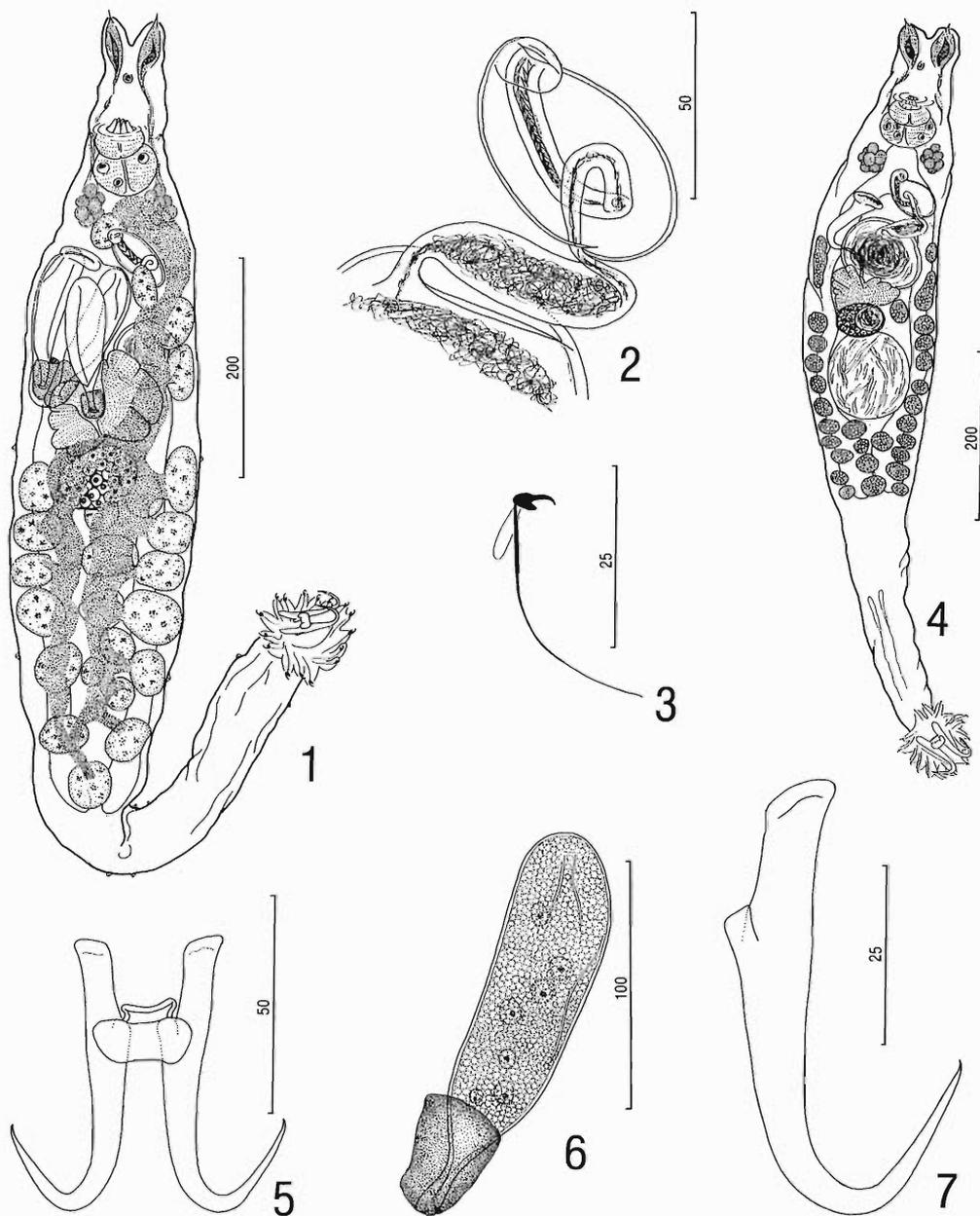
HOST AND LOCALITY: *Rhinelorica* sp., Loriciariidae; Igarapé Candiru, a tributary of Rio Pu-

raquequara, Highway AM-010, near Manaus, Amazonas, Brazil.

TYPE SPECIMENS: Holotype, IOC 33050a; 27 paratypes, IOC 33050b-1, USNM 82822, HWML 36347; 13 vouchers, HWML 36348.

DESCRIPTION: Body robust, fusiform, 682 (460–870;  $n = 13$ ) long; greatest width 143 (105–200;  $n = 15$ ) near midlength. Cephalic lobes well developed; head organs conspicuous. Distal pharyngeal bulb 66 (43–69;  $n = 10$ ) in diameter; proximal pharyngeal bulb 74 (52–95;  $n = 9$ ) in diameter. Testis subspherical, variable in size (depending on age of specimen), becoming inconspicuous in adult worms; proximal seminal vesicle subspherical, delicate; copulatory sac pyriform; copulatory organ armed with 2 regions of numerous small rectangular spines. Germarium ovate, 57 (50–67;  $n = 6$ ) long, 81 (68–102;  $n = 6$ ) wide, with internal seminal receptacle. Uterus delicate; uterine pore surrounded by weak sphincter; egg 145 (132–154;  $n = 4$ ) long, 34 (32–38;  $n = 3$ ) wide, elongate ovate; filament short, flared; filament cap subspherical to subovate. Vitelline follicles extending from level of germarium posteriorly to peduncle. Peduncle short; haptor 141 (97–172;  $n = 17$ ) long, 152 (117–183;  $n = 16$ ) wide. Anchor 81 (75–86;  $n = 21$ ) long, with elongate robust superficial root, short deep root, straight shaft, recurved point; base 14 (12–18;  $n = 16$ ) wide. Superficial bar 21 (13–25;  $n = 20$ ) long, with slightly enlarged ends; deep bar rod-shaped, variably bent. Hook with straight shaft, short erect thumb, proximally tapered shank; hooklet 6 (4–7;  $n = 29$ ) long, shank 32 (29–35;  $n = 29$ ); domus  $\frac{1}{6}$  shank length.

REMARKS: *Hyperoletes malmbergi* sp. n. is the

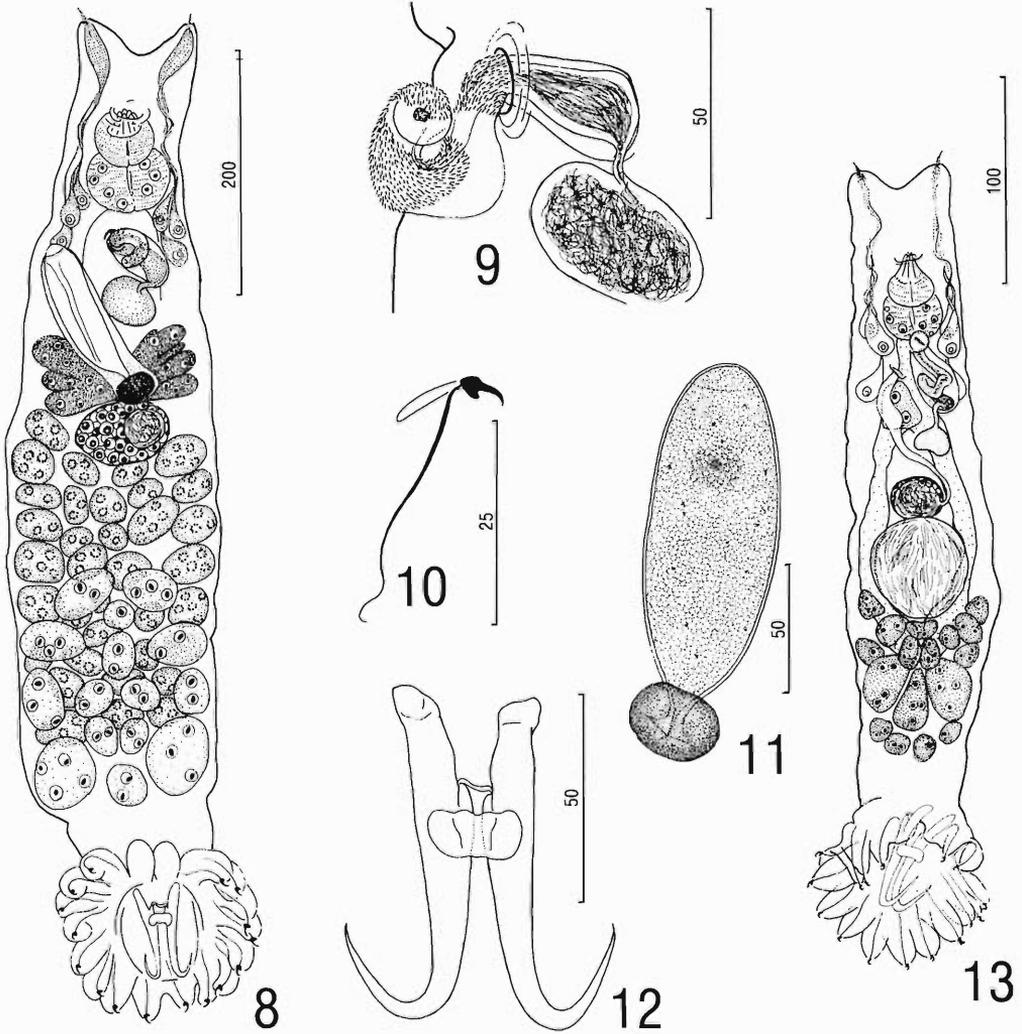


Figures 1–7. *Phanerothecium spinatus* gen. et sp. n. 1. Holotype (ventral view). 2. Terminal ducts, seminal vesicles and copulatory sac of male reproductive system. 3. Hook. 4. Ventral view of young specimen. 5. Anchor/bar complex (ventral view). 6. Egg. 7. Anchor. Figures are drawn to respective micrometer scales.

type species of the genus. It is named for Dr. Göran Malmberg, Swedish Museum of Natural History, Stockholm University, a friend, in recognition of his contributions in systematics of the Monogeneoidea.

#### Character Analysis

Homologous series used in the analysis follow with comments on character evolution. Numbers in parentheses preceding the definition of a



Figures 8–13. *Hyperopletes malmbergi* gen. et sp. n. 8. Holotype (ventral view). 9. Terminal portion of male reproductive system (copulatory organ is partially everted). 10. Hook. 11. Egg. 12. Anchor/bar complex (ventral view). 13. Young specimen (ventral view). Figures are drawn to respective scales.

character state refer to the coding that state received in the data matrix (Table 1); bold numbers in brackets following the definition refer to respective evolutionary changes depicted in the cladogram (Fig. 15).

A. *Eyes*. Plesiomorphy: (0) present. Apomorphy: (1) absent [1].

B. *Spike sensilla*. Plesiomorphy: (0) absent. Apomorphy: (1) present [2].

C. *Mode of reproduction*. Plesiomorphy: (0) oviparous. Apomorphy: (1) viviparous [24].

D. *Larva*. Plesiomorphy: (0) oncomiracidium ciliated. Apomorphy: (1) Larva (oncomiracidium?) unciliated [3]. Species of *Anoplo-discus*

have a ciliated oncomiracidium (Ogawa and Egusa, 1981). Although the nature of ciliation of the larvae/oncomiracidia of bothitrematids and tetraonchoids is unknown, ciliated larvae would be predicted for them based on the phylogenetic hypothesis for the Monogenoidea offered by Boeger and Kritsky (1993).

E. *Genital pore*. Plesiomorphy: (0) common. Apomorphy: (1) male and female pores separate [4].

F. *Copulatory organ*. Plesiomorphy: (0) sclerotized. Apomorphy: (1) muscular [5].

G. *Copulatory sac*. Plesiomorphy: (0) absent. Apomorphies: (1) present [15]; (2) modified into

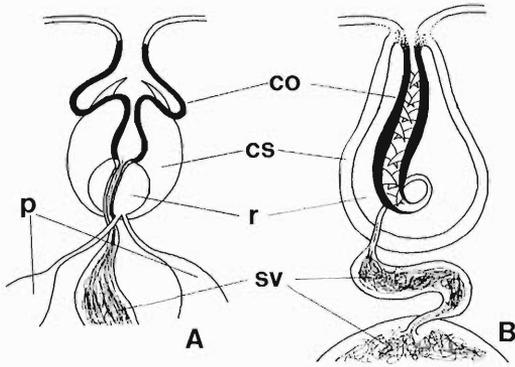


Figure 14. Schematic diagrams of terminal genitalia of viviparous (A) and oviparous (B) Gyrodactylidae showing probable homologous structures. Abbreviations: co = copulatory organ, cs = copulatory sac, p = prostate gland, r = prostatic reservoir, sv = seminal vesicles. Figure 14A is modified from Kritsky (1971).

muscular bulb [22]. In the 3 *Nothogyrodactylus* species, Kritsky and Boeger (1991) describe the copulatory sac as poorly defined, although their drawings suggest that the extrusive copulatory organ does not lie within a sac. Thus, we assigned the plesiomorphic state to *N. clavatus*, *N. plaesiohallus*, and *N. amazonicus*.

The "cirral bulb" of viviparous Gyrodactylidae apparently represents a modified copulatory sac. In these taxa, the bulb is muscular and has probably developed from the muscular wall of a plesiomorphic sac similar to that of *Ooegyrodactylus farlowellae* (see fig. 28 in Kritsky and Boeger, 1991). The prostatic reservoir in viviparous taxa is enclosed within the bulb (Kritsky, 1971) and is probably homologous to the lumen of the sac containing prostatic secretions in species of *Phanerothecium*. The copulatory organ

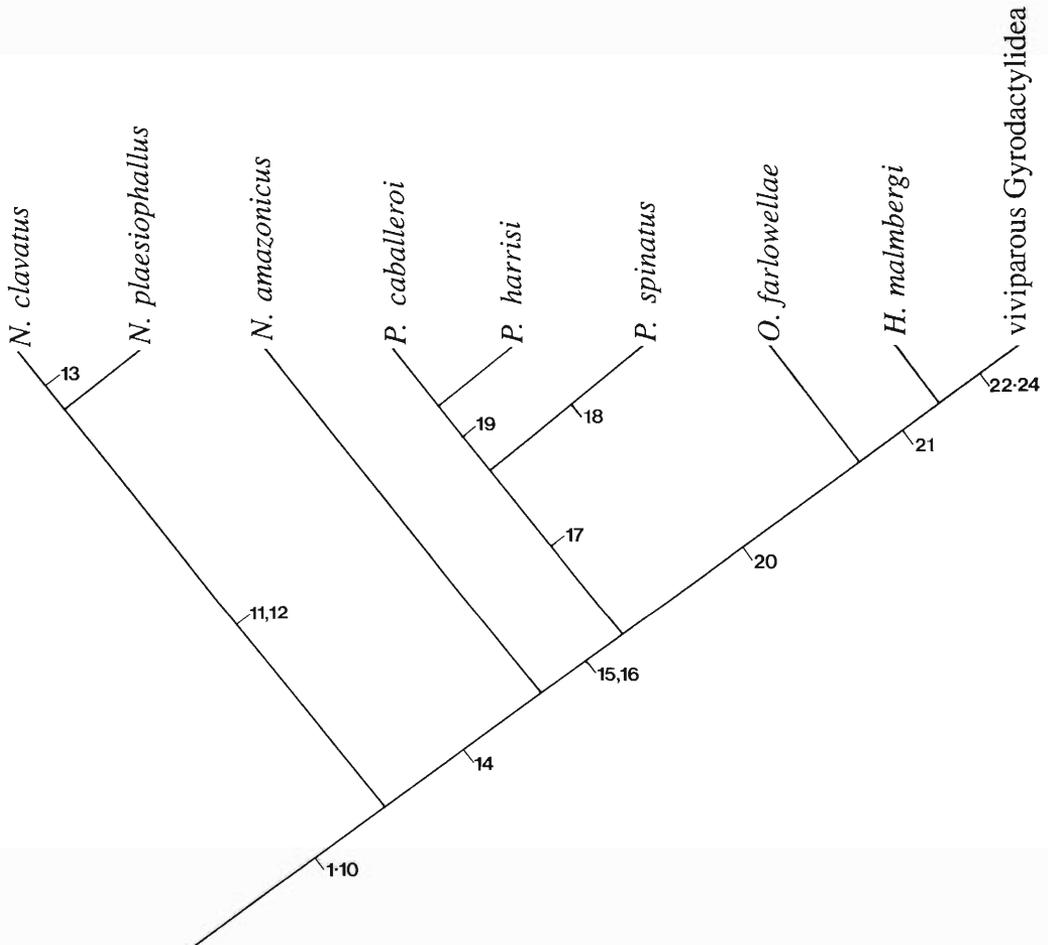


Figure 15. Cladogram depicting the evolutionary relationships within the Gyrodactylidae (sensu nobis). Slashes with numbers refer to postulated evolutionary changes in character states indicated in the text.

(the armed epithelium) of viviparous gyroductylids is eversible and lies on the superficial surface of the bulb (Fig. 14).

H. *Spines of copulatory organ*. Plesiomorphy: (0) absent. Apomorphy: (1) present [18, 21]. The analysis indicates that not all copulatory spines present in ingroup taxa are homologous. The spines of *Hyperopletes malMBERGI* appear to be homologous to those of the viviparous Gyroductylidae, whereas the larger spines of *Phanerothecium spinatus* apparently evolved independently. Although we assign a (9) to the hypothetical ancestor for this series, because presence or absence of spines on the copulatory organ may be related to the type of copulatory organ present (see Series F), the analysis does not exclude the possibility that absence of spines is symplesiomorphic for the ingroup taxa.

I. *Accessory piece*. Plesiomorphy: (0) present. Apomorphy: (1) absent [16]. Although no evidence to support homology other than location exists, the accessory sclerites associated with the copulatory organ of *Nothogyrodactylus* species are considered to be homologous to the accessory piece of other Polyonchoinea based on Hennig's (1966) auxiliary principle of presumed homology. If these structures are shown not to be homologous to the accessory piece, "absence (loss) of an accessory piece" would represent another synapomorphy for the Gyroductylidae (sensu nobis) and development of the accessory sclerites as new structures would provide a synapomorphy for *Nothogyrodactylus*.

J. *Distribution of vitellaria*. Plesiomorphy: (0) follicles post- and pregermarial. Apomorphies: (1) vitellaria primarily postgermarial, with a few sinistral pregermarial follicles present (dextral pregermarial follicles absent) [14]; (2) vitelline follicles postgermarial only [20]. The morphology of the vitelline collecting ducts corresponds to the distribution of the follicles. In ventral view, species with the plesiomorphic state depict H-shaped collecting ducts, whereas those with apomorphic states have reversed h-shaped and inverted U-shaped ducts, respectively.

K. *Mehlis' gland*. Plesiomorphy: (0) comprised of small unicellular glands. Apomorphies: (1) follicular, forming a large mass of cells [6]; (2) absent [23]. In a study of the ultrastructure of the reproductive system of *Gyrodactylus*, Kritsky (1971) reported the absence of the Mehlis' gland. This absence is apparently derived and linked to development of viviparity and/or absence of an egg shell surrounding the ovum.

L. *Vagina*. Plesiomorphy: (0) present. Apomorphy: (1) absent [7].

M. *Egg cap*. Plesiomorphy: (0) absent. Apomorphy: (1) present [8]. An amorphous cap is present on the egg filament of oviparous ingroup species (egg morphology is unknown for *Phanerothecium caballeroi*). This cap is composed of a sticky substance that allows fixation of the egg or egg cluster to surfaces when laid by the parent (Kritsky and Boeger, 1991).

N. *Maximum number of eggs (or embryos) in utero*. Plesiomorphy: (0) one. Apomorphy: (1) more than one [17]. The apomorphic state does not include the condition of multiple in utero generations of embryos that may occur in viviparous forms. The viviparous taxa did not develop multiple egg production, although in utero generational development may represent another strategy for increased reproductive potential.

O. *Keel on hook shank*. Plesiomorphy: (0) absent. Apomorphy: (1) present [12, 19].

P. *Deep bar associated with deep roots of anchors*. Plesiomorphy: (0) absent. Apomorphy: (1) present [9].

Q. *Cup-like accessory sclerite*. Plesiomorphy: (0) absent. Apomorphy: (1) present [13].

R. *Sheath-like accessory sclerite*. Plesiomorphy: (0) absent. Apomorphy: (1) present [11].

S. *Hook-like accessory sclerite*. Plesiomorphy: (0) absent. Apomorphy: (1) present [10].

Series Q, R, and S indicate the presumed homology and transformation of the various accessory sclerites associated with the copulatory organs of species of *Nothogyrodactylus*. In the matrix, we assign a (0) to the hypothetical ancestor for each series because of our presumption of homology of the accessory piece of the outgroups and other Polyonchoinea and the accessory sclerites present in members of this genus (see Series I).

We initially considered the number and position of seminal vesicles as potentially useful characters for the analysis. Two seminal vesicles appear to be symplesiomorphic for the Gyroductylidae (sensu nobis). In outgroup species, *Pavlovskioides antarcticus* Bychowsky, Gusev, and Nagibina, 1965, and *P. trematomi* Dillon and Hargis, 1968 (both Tetraonchoidea), 2 seminal vesicles are depicted by Bychowsky et al. (1965) and Dillon and Hargis (1968), respectively. Although Kritsky (1971) reported a single seminal vesicle (external) in *Gyrodactylus*, this homologous series did not provide information on phylogeny of the ingroup. Loss of the distal

seminal vesicle appears to have occurred secondarily within the Gyrodactylidae (sensu stricto), because species of *Accessorius* Jara, An, and Cone, 1991, apparently members of 1 of the first evolutionary lines to diverge within the viviparous taxon, clearly possess both proximal and distal vesicles (see fig. 1 in Jara et al., 1991). Therefore, number of seminal vesicles is a constant character that did not provide information on the evolutionary history of the ingroup taxa; consequently, this series was not used in the analysis.

*Hyperopletes malmbergi* is the only known species within the ingroup with the distal seminal vesicle within the copulatory sac. As such, this feature is an autapomorphy and is also not used in the analysis.

Lastly, a pharynx composed of 2 tandem sub-hemispherical bulbs was initially considered a synapomorphy for the Gyrodactylidae (sensu nobis). Members of the Tetraonchoididae and Bothitrematidae possess a pharynx comprised of a single bulb (Bychowsky et al., 1965; Dillon and Hargis, 1968), and previous reports on species of Anoplodiscidae indicated a single bulbed pharynx (see Ogawa and Egusa, 1981). We examined specimens of an unidentified species of *Anoplodiscus* from *Pagrus pagrus* (Linnaeus) collected off the coasts of Uruguay and Argentina; these specimens clearly show a pharynx comprised of 2 bulbs, suggesting that this feature is symplesiomorphic for the ingroup. Because subsequent evolution of this feature has not occurred in any of the ingroup taxa, this character was not utilized in the analysis.

#### Phylogenetic Analysis

The cladogram (CI = 91.7%) depicting the phylogenetic relationships of the oviparous gyrodactylids and the ancestor of the viviparous species of Gyrodactylidae is presented in Figure 15. Elimination of the homologous series that only indicate monophyly of the ingroup (Series A, B, D, E, F, L, M, P, and S) results in the same cladogram with a consistency index of 86.7%. The analysis failed to identify synapomorphies for a taxon containing just the oviparous species of the ingroup, and sister-group relationships of these taxa suggest that the Ooegyrodactylidae, as diagnosed by Harris (1983), is paraphyletic. All oviparous genera received phylogenetic support except *Nothogyrodactylus* Kritsky and Boeger, 1991, which is paraphyletic.

As a result of the analysis, the Ooegyrodac-

tylidae Harris, 1983, is rejected as a junior synonym of the Gyrodactylidae. The following genera of oviparous gyrodactylideans, and their species, are transferred to or placed within the Gyrodactylidae along with all viviparous taxa: *Hyperopletes* gen. n. (with *H. malmbergi* sp. n.); *Nothogyrodactylus* Kritsky and Boeger, 1991 (with *N. clavatus* Kritsky and Boeger, 1991, *N. plaesiophallus* Kritsky and Boeger, 1991, and *N. amazonicus* Kritsky and Boeger, 1991); *Ooegyrodactylus* Harris, 1983 (with *O. farlowellae* Harris, 1983); and *Phanerothecium* Kritsky and Thatcher, 1977 (with *P. caballeroi* Kritsky and Thatcher, 1977, *P. harrisi* Kritsky and Boeger, 1991, *P. spinatus* sp. n., and *P. sp.* [= *P. caballeroi* forma major of Kritsky and Thatcher, 1977]). Although paraphyletic, *Nothogyrodactylus* is retained for *N. amazonicus*, *N. clavatus*, and *N. plaesiophallus* until additional evidence for or against homology of the accessory piece (of outgroups) and accessory sclerites (of *Nothogyrodactylus* species) is obtained.

#### Discussion

Prior to Harris' (1983) discovery of the oviparous *Ooegyrodactylus farlowellae* on a South American catfish, the taxon containing the viviparous monogenoideans (the Gyrodactylidae sensu stricto) enjoyed a relatively stable acceptance and embodiment based on unique synapomorphic features. Among the most important of these characters was the viviparous mode of reproduction, which students have previously recognized as having developed only once in the evolutionary history of the Monogenoidea. However, these highly derived features without known intermediates in character evolution precluded consensus on origins, phylogenetic relationships, and classification of the taxon within the Monogenoidea (see Bychowsky, 1937, 1957; Price, 1937a, b; Yamaguti, 1963; Llewellyn, 1965; Lebedev, 1988; Malmberg, 1990). The recognition of oviparity in a gyrodactylid-like monogenoidean by Harris (1983) represented a milestone in determining sister-group relationships for the taxon (Boeger and Kritsky, 1993).

Apparently unwilling to challenge the longstanding diagnostic features of the Gyrodactylidae (sensu stricto), Harris (1983) proposed the Ooegyrodactylidae for the oviparous forms. Kritsky and Boeger (1991) provisionally accepted the family but indicated that it had a high probability for being paraphyletic because features used to establish the family "either repre-

sent symplesiomorphies of the Gyrodactylidae (sensu stricto) (misspelled 'Gyrodactylidea' in Kritsky and Boeger, 1991) or are primitive characters shared by the Gyrodactylidea and its sister taxon, the Dactylogyridea . . . (parentheses, ours) p. 14." Our discoveries of additional genera and species of oviparous gyrodactylid-like forms, including *Nothogyrodactylus* Kritsky and Boeger, 1991, allowed the present phylogenetic analysis to test monophyly of the Ooegyrodactylidae. This analysis shows the family to be paraphyletic and supports its rejection and the transfer of all included genera and species to the Gyrodactylidae. Thus, viviparity developed secondarily within the Gyrodactylidae and can no longer be used as a definitive diagnostic feature of the family. However, we were able to identify several synapomorphies supporting the new configuration of the Gyrodactylidae including (a) a muscular copulatory organ (see Series F), (b) spike sensilla in the head organs (Series B), (c) an unciliated larva (oncomiracidium absent) (Series D), (d) a deep bar associated with the deep roots of the anchor pair (Series P), (e) separate genital pores (Series E), (f) a massive Mehlis' gland (Series K), (g) an amorphous cap on the egg filament (Series M), and absence of (h) a vagina (Series L) and (i) eyes in the larva and adult (Series A).

The analysis also suggests that *Nothogyrodactylus* is paraphyletic. Kritsky and Boeger (1991) based the genus primarily on presence of 1 or more accessory sclerites associated with the extrusive copulatory organ. In the present analysis, the accessory sclerites are assumed to be homologous to the accessory piece of outgroup taxa grounded on Hennig's auxiliary principle on homology. As a result of this assumption, presence of accessory sclerites is symplesiomorphic and does not lend support to presumed congeneric status of *N. clavatus*, *N. amazonicus*, and *N. plae-siophallus*. Although no synapomorphies were identified for the group of 3 species, *N. amazonicus* is provisionally retained in the genus pending further study of character evolution within this homologous series.

Based on their respective positions within the cladogram, all other oviparous genera received evolutionary support through the analysis. Species of *Phanerothecium* share a single synapomorphy, presence of more than 1 egg in utero (Series N). *Hyperopletes* is the only species with the distal seminal vesicle enclosed within the copulatory sac (see the character analysis), and

*Ooegyrodactylus* has unique features associated with the morphology of the egg, egg filament, copulatory sac, and wall of the distal seminal vesicle (see Kritsky and Boeger, 1991). However, conclusive phylogenetic support for *Hyperopletes* and *Ooegyrodactylus*, both of which are monotypic, will depend on discovery of other congeneric species.

The development of viviparity in the Gyrodactylidae apparently has had profound effect on several characteristics related to structures associated with the oviparous mode of reproduction. Apparently unlike all oviparous forms, the eggs of viviparous gyrodactylids lack a shell and yolk (Kritsky, 1971). While controversy exists concerning the function of their products, absence of the Mehlis' gland and presence of apparently nonfunctional vitellaria in viviparous gyrodactylids may be related to the egg structure and/or modified mechanisms to meet embryonic nutritional needs. The phylogeny proposed herein suggests that these and similar morphological and functional characters were lost or modified in the ancestor of the viviparous Gyrodactylidae.

Some features associated with reproductive strategy, particularly those associated with parental care of offspring, appear to have begun development early in the evolutionary history of the Gyrodactylidae (sensu nobis). In general, eggs of other polyonchoineans (including the gyrodactylidean taxa used as outgroups) are maintained in utero for a short time before being released into the environment where they embryonate and eclose producing the free-swimming, ciliated oncomiracidium that actively searches for an adequate definitive host. Among oviparous Gyrodactylidae, accentuated parental care of the young has already developed. The eggs are stored in utero, where embryonation occurs (Harris, 1983; Kritsky and Boeger, 1991). The embryonated eggs are then deposited on the skin or bony plates of the host where they are secured by the sticky egg caps. Hatching occurs comparatively quickly, producing young unciliated individuals with a male reproductive system at or near maturity. Kritsky and Boeger (1991) found that eggs hatched within 6 days after expulsion in *Phanerothecium harrisi*, and Harris (1983) indicated that the male system develops rapidly and is functional about 7 days posthatching in *Ooegyrodactylus farlowellae*. Parental care of the eggs, use of the egg cap for attachment of the eggs to the skin or gills of the

host, and rapid posthatching development increase probability of survival for the young. However, the highest level of parental care for the offsprings is seen among the viviparous Gyrodactylidae, where in utero development is prolonged until the young individual approaches adult form.

#### Acknowledgments

The authors wish to thank the following individuals for support of this study: Rogério Nunes da Mota assisted during collecting efforts in Rio de Janeiro; Michel Jégu (Instituto Nacional de Pesquisas da Amazônia) and Heraldo A. Britsky (Museu de Zoologia da Universidade de São Paulo) provided host identifications; Flávio Popazoglo helped with laboratory work; and Sherm Hendrix allowed us to examine specimens of *Bothitrema* in his collection. The Fundação de Amparo a Pesquisas do Estado do Rio de Janeiro (Proc. E-29/170.033/90) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Proc. 406184/90-9 and 500711/90-9), Brazil, provided funding for this project.

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## *Hadwenius pontoporiae* sp. n. (Digenea: Campulidae) from the Intestine of Franciscana (Cetacea: Pontoporiidae) in Argentinian Waters.

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**ABSTRACT:** *Hadwenius pontoporiae* sp. n. from the intestine of franciscana (*Pontoporia blainvillei*) in Argentinian waters is described. This new species differs from the other *Hadwenius* species in body and cirrus pouch dimensions, shape, and disposition of the gonads and distribution of vitellaria. This is the first record of a campulid in the franciscana, and the genus *Hadwenius* is reported for the first time in the South Atlantic Ocean.

**KEY WORDS:** *Hadwenius pontoporiae* sp. n., Digenea, *Pontoporia blainvillei*, Cetacea, South Atlantic Ocean.

*Pontoporia blainvillei* (Gervais and d'Orbigny, 1844), known as franciscana, or La Plata dolphin, has a limited distribution along the Atlantic coast of South America (Brownell, 1989). The parasite fauna of this species is insufficiently known, although some previous surveys were carried out in Uruguayan waters (Schmidt and Dailey, 1971; Kagei et al., 1976; Brownell, 1981, 1989).

In cooperation with a research project concerning the biology and factors limiting the population of *P. blainvillei* in Argentinian waters, a parasitological survey was carried out. So far, no digeneans have been reported in this host, but in our study 2 species were found, one in the submucosa of the main stomach, *Pholeter gastrophilus* (Kossack, 1910), and another in the intestine. The latter was identified as an undescribed species of the family Campulidae and is the subject of this article.

### Materials and Methods

This study was based on 23 franciscanas incidentally caught in shark fishery nets in Necochea and Claramécó (Buenos Aires Province, Argentina) between 1988 and 1990. The examination of 16 fresh and 7 frozen intestines revealed the presence of digenetic trematodes.

The helminths were washed in saline solution, fixed, and preserved in 70% ethanol. Twenty specimens from 3 fresh intestines were selected for study. They were stained in Semichon's acetocarmine and celestine blue B, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Serial sections of additional specimens were cut at 10-15  $\mu$ m and stained in Ehrlich's acid hematoxylin and eosin.

All measurements are in micrometers unless otherwise indicated. Ranges are given with means in paren-

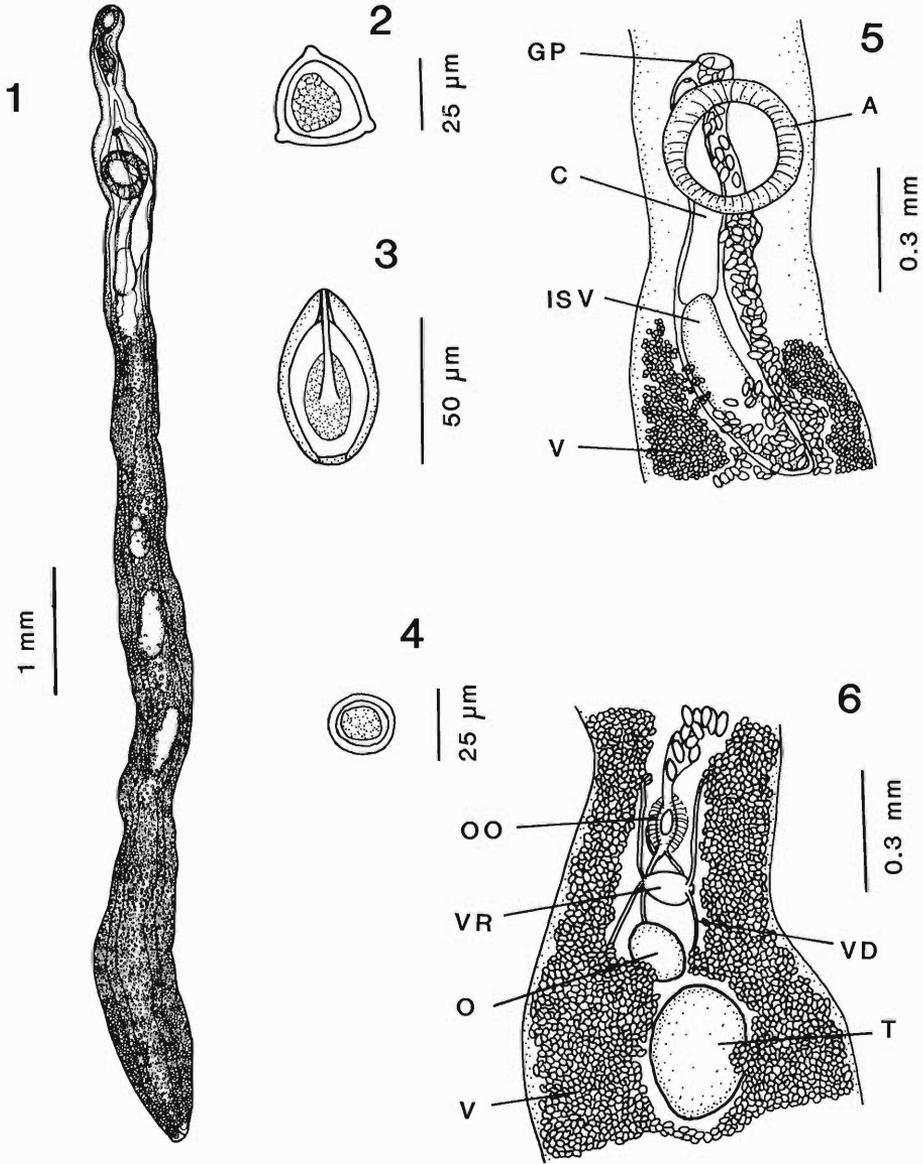
theses. Illustrations were made with the aid of a drawing tube.

### Results

#### *Hadwenius pontoporiae* sp. n. (Figs. 1-6)

**DESCRIPTION** (based on 20 specimens): With characters of the genus. Body elongate, slender, dorsoventrally flattened, 3.37-9.77 mm (6.11 mm) long, maximum width behind posterior testis 341-813 (605). Tegument spinose, spines more concentrated on anterior midbody, length of spines 11-18 (14). Oral sucker subterminal, 125-198 (160) long by 116-166 (147) wide. Acetabulum larger than oral sucker, in first quarter of body, 246-349 (297) long by 258-369 (316) wide. Distance between suckers 629-1,903 (1,021). Prepharynx, 24-317 (130) long. Pharynx pyriform, 143-227 (184) long by 88-123 (100) wide. Esophagus extremely variable in length, usually short. Intestine H-shaped, ceca terminating blindly near posterior margin of body. Anterior intestinal branches extending up to posterior margin of oral sucker. Excretory vesicle tubular, pore terminal. Ceca and anterior intestinal branches without inner or outer lateral diverticula.

Testes elongate, oval in shape, entire, tandem; situated in third quarter of body. Anterior testis 193-513 (322) long by 115-205 (164) wide. Posterior testis 205-431 (320) long by 147-267 (214) wide. Intertesticular distance 90-472 (291). Posterior testis at 1.09-3.06 mm (1.99 mm) from caudal extremity of body. Cirrus pouch 0.68-1.15 mm (0.91 mm) long, extends posteriad well



Figures 1-6. *Hadwenius pontoporiae* sp. n. 1. Whole mount. 2. Egg, cross-section at abopercular pole. 3. Egg, lateral view. 4. Egg, cross-section at opercular pole. 5. Acetabular region. 6. Proximal female genitalia. Abbreviations: A = acetabulum, C = cirrus, GP = genital pore, ISV = internal seminal vesicle, O = ovary, OO = ootype, T = testis, V = vitellarium, VD = vitelline duct, VR = vitelline reservoir.

beyond acetabulum, contains coiled seminal vesicle located at its proximal extremity. Cirrus armed. Cirrus pouch opening into medial genital pore immediately anterior to margin of acetabulum. Genital pore at 0.56-2.00 mm (1.05 mm) from anterior margin of body.

Ovary oval or subglobular, pretesticular,

slightly dextral to midline, 100-256 (177) long by 65-178 (119) wide, situated at 8-170 (124) from anterior testis and 0.80-2.09 mm (1.2 mm) from acetabulum. Ootype and Mehlis' gland situated anterior to ovary. Laurer's canal present. Seminal receptacle absent. Vitelline reservoir conspicuous, ovoid, between ootype and ovary,

sometimes ventral to ovary, 53–170 (102) long by 32–90 (71) wide. Vitellaria arranged in acinous bunches, profuse, commencing at seminal vesicle extending to posterior extremity. Uterus coiled in preovarian intercecal field, widening into unarmed metraterm before opening into genital pore. Eggs oval, truncate at the opercular pole, triangular in cross-section at abopercular pole, circular in cross-section at opercular pole, 54–59 (56,  $n = 40$ ) long by 32–37 (34,  $n = 40$ ) wide.

**DEFINITIVE HOST:** *Pontoporia blainvillei* (Gervais and d'Orbigny, 1844), franciscana, or La Plata dolphin.

**SITE:** Small intestine.

**TYPE LOCALITY:** Necochea (38°37'S, 58°50'W) (Buenos Aires Province), Argentina, South Atlantic Ocean.

**PREVALENCE:** 100%.

**INTENSITY OF INFECTION:** 8–1,023 specimens ( $\bar{x} = 255$ ). No apparent pathological effects were observed.

**SPECIMENS DEPOSITED:** Holotype USNM Helm. Coll. No. 82915 and paratypes USNM Helm. Coll. No. 82916; other paratypes British Museum (Natural History) Reg. No. 1993.5.17.1–2 and Department of Animal Biology, University of Valencia, Coll. Nos. PB.N88.1–21, PB.N89.1–16, and PB.N90.1–11.

**ETYMOLOGY:** The specific name *pontoporiae* is derived from the generic name of the host, emphasizing that this is the first occurrence of a member of the genus *Hadwenius* in a host of the family Pontoporiidae.

### Discussion

There are currently 36 species recognized in the family Campulidae Odhner, 1926. All parasitize aquatic mammals, mainly marine and freshwater cetaceans, but also pinnipeds and the sea otter (*Enhydra lutris*). Campulids are found in all oceans of the world.

The taxonomy of this family is confusing and in need of review. Yamaguti (1971) considered 6 subfamilies: Campulinae Stunkard and Alvey, 1930, Hunterotrematinae Yamaguti, 1971, Lecithodesminae Yamaguti, 1958, Odhneriellinae Yamaguti, 1958, Orthosplanchninae Yamaguti, 1958, and Synthesiinae Yamaguti, 1958. However, Adams and Rausch (1989) synonymized Odhneriellinae with Orthosplanchninae, and Skrjabin (1976) indicated that, due to the presence of anterior intestinal branches, *Synthesium*

*tursionis* (Marchi, 1873), the only member of the subfamily Synthesiinae, should be transferred to the Orthosplanchninae. Thus, pending further studies, this leaves 4 subfamilies: Campulinae, Lecithodesminae, Hunterotrematinae, and Orthosplanchninae.

The specimens studied exhibit an elongate body, the intestine shows no lateral diverticula, and the acetabulum is situated in the anterior third of the body, which are all characters of the Orthosplanchninae (Yamaguti, 1971; Adams and Rausch, 1989). According to the most recent taxonomic criteria (Adams and Rausch, 1989), this subfamily is composed of 3 genera: *Orthosplanchnus* Odhner, 1905, *Oschmarinella* Skrjabin, 1947, and *Hadwenius* Price, 1932. The present specimens should be included in the latter because they show a high length/width ratio of the body; possess a long, clavate cirrus lined with spines; possess an unarmed metraterm; and vitellaria do not extend to the acetabular level.

Adams and Rausch (1989) considered 5 species within the genus *Hadwenius*: *H. seymouri* Price, 1932, *H. nipponicus* Yamaguti, 1951, *H. mironovi* (Krotov and Delyamure, 1952), *H. elongatus* (Ozaki, 1935), and *H. subtilis* (Skrjabin, 1959), but the authors recognize 1 additional species, *H. delamurei* (Raga and Balbuena, 1988) (see Balbuena, 1991).

Four Orthosplanchninae species have an uncertain generic allocation: *Orthosplanchnus sudarikovi* Treshchev, 1966, *Odhneriella (Campula) gondo* (Yamaguti, 1942), *Leucasiella arctica* Delyamure and Kleinenberg, 1958, and *Synthesium tursionis* (see Skrjabin, 1976; Adams and Rausch, 1989). However, until further evidence is available, these species should be regarded as incertae sedis and will not be considered in the present discussion.

*Hadwenius seymouri* differs from the new species in body and egg size, situation of oral sucker and gonads, and extension of vitellaria (Price, 1932), whereas *H. elongatus* differs in body size and morphology of the testes (Ozaki, 1935) (Table 1). *Hadwenius nipponicus* shows larger body and eggs than *H. pontoporiae* and vitellaria commencing at testicular level (Yamaguti, 1951) (Table 1). *Hadwenius mironovi* can be distinguished from *H. pontoporiae* by the disposition of the gonads and vitellaria and having the cirrus pouch shorter and the eggs larger (Delyamure, 1964) (Table 1). *Hadwenius subtilis* shows a much larger body and the oral sucker is terminal (Balbuena

Table 1. Differential characters of *Hadwenius* species.\*

	<i>H. seymouri</i>	<i>H. elongatus</i>	<i>H. nipponicus</i>	<i>H. mironovi</i>	<i>H. subtilis</i>	<i>H. delamurei</i>	<i>H. pontoporiae</i> n. sp.
Body length	27–60	13–18	17.5–22	8.90–12.89	14.03–38.34 (33.35)	9.7–16.8 (12.1)	3.37–9.37 (6.11)
Maximum width	1.5–2	1–2.1	0.95–1.25	0.72–1.25	1.23–1.95 (1.52)	0.59–0.79 (0.67)	0.34–0.81 (0.60)
Oral sucker position	Terminal	Subterminal	Subterminal	Terminal	Terminal	Subterminal	Subterminal
Cirrus pouch length	1.8	0.9–1.5	1.55–2	—	2.4–6.1 (4.4)	2.0–3.4 (2.5)	0.6–1.1 (0.9)
Testis shape	Entire	Lobed	Entire	Entire	Entire	Entire	Entire
Gonads position	Anterior 1/3	Medial 1/3	Medial 1/3	Anterior 1/3	Medial 1/3	Posterior 1/3	Medial 1/3
Anterior extent of vitellaria	Anterior testis	Seminal vesicle	Anterior testis	Anterior testis	Seminal vesicle	Seminal vesicle	Seminal vesicle
Egg size ( $\mu\text{m}$ )	97 × 52	49–55 × 25–31	80–90 × 45–50	72–90 × 33–37	75–98 × 44–55 (89 × 49)	57–72 × 32–50 (67 × 40)	54–59 × 33–37 (56 × 34)
Host†	DI	Np	Pd, Pp	DI	Oo, DI, Gm	Gm	Pb
Geographical distribution	Alaska	Japan	Japan, U.S.A. (Pacific Coast)	North Pacific	North Pacific, White Sea, North Atlantic	Mediterranean, North Atlantic	South Atlantic
References	Price, 1932	Ozaki, 1935	Yamaguti, 1951; Ching and Robinson, 1959	Delyamure, 1964	Balbuena et al., 1989	Raga and Balbuena, 1988; Balbuena, 1991	Present study

\* Measurements in millimeters unless stated otherwise, followed by the mean in parentheses.

† DI = *Delphinapterus leucas*, Gm = *Globicephala melas*, Np = *Neophocoena phocoenoides*, Oo = *Orcinus orca*, Pb = *Pontoporia blainvillei*, Pd = *Phocoenoides dalli*, Pp = *Phocoena phocoena*.

et al., 1989) (Table 1). The specimens studied closely resemble *H. delamurei* in body and egg size, position of the oral sucker, shape of testes, and distribution of vitellaria. However, they clearly differ in the position of the gonads and length of the cirrus pouch (Table 1). They also exhibit a different egg shape. The eggs are circular in cross-section at the opercular pole in *H. pontoporiae* sp. n. (Fig. 4), whereas they are triangular in *H. delamurei* (Raga and Balbuena, 1988; Balbuena, 1991).

This is the first report of a campulid trematode parasitizing franciscanas, and a species of *Hadwenius* is reported for the first time in the South Atlantic Ocean and Southern Hemisphere. Given the high prevalence and mean intensity of *H. pontoporiae* sp. n. observed in this study, the absence of this species in previous reports from Uruguayan waters (situated about 500 km from the study area) is surprising.

#### Acknowledgments

The fieldwork was done in the Estación Hidrobiológica de Puerto Quequén (Necochea). We wish to thank Mr. J. Corcuera and Ms. F. Monzón (Consejo Nacional de Investigaciones Científicas y Técnicas and Museo Argentino de Ciencias Naturales) for their hospitality and valuable assistance while sampling in the field. Two anonymous reviewers provided very helpful, detailed critical comments. Funds were provided by the General Directorate of Scientific and Technological Research (DGICYT) of the Spanish Government (project No. PB87-146-C2-2). This article was completed during a visit by one of the authors (J.A.R.) to the California State University at Long Beach, made possible by a grant from the DGICYT.

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## Avian Cestodes of the Ivory Coast

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**ABSTRACT:** Between 1985 and 1988, the cestodes of 1,252 wild birds (174 species) from the Ivory Coast (West Africa) were collected and studied. Of these birds, 15.7% were parasitized by an estimated 95–100 species of tapeworms, 55 of them having been determined or described. Most of the parasites were found in new hosts, and numerous new geographical records are reported. In general, both the prevalence and intensity of infection were low. All the parasites were cyclophyllideans distributed mainly in the families Dilepididae, Hymenolepididae, and Paruterinidae. Of particular interest was the presence of 3 species of the family Metadilepididae in the collections.

**KEY WORDS:** Platyhelminthes, cestodes, birds, Ivory Coast, taxonomy, survey.

Avian cestode faunas in the Republic of the Ivory Coast (West Africa) have been poorly studied. Only 8 species of tapeworms have been reported from avian hosts in this region (Morel, 1959; Quentin, 1964; Baer, 1972), and recent investigations are lacking. The fauna of neighboring countries is not better known. With the exception of the parasites of some poultry or game birds such as *Numida* sp., virtually nothing is known on platyhelminths of wild birds in tropical Africa. The only noticeable exceptions are the works by Southwell and Lake (1939) and Mahon (1954), both in Zaire; however, these surveys were done in a country very distant from the Ivory Coast and concerned few species in common with those presented in this work. Consequently, I initiated a study of the avian tapeworm fauna of this region with the goal of developing an inventory of species diversity for these poorly known organisms in a region that has received little attention from parasitologists.

During 3 field seasons (each of 4 mo duration), in 1985, 1987, and 1988, I examined 1,252 birds, primarily representing the commonly occurring avian species of the Ivory Coast, with an emphasis on nonmigratory landbirds. Although collecting was concentrated in the southern areas of the country, fieldwork was widespread and included all biotopes existing in the region, extending from the southern rainforests and coastal zones to the northern savannahs. The systematics of some cestodes derived from these collections has been treated previously (Mariaux and Vaucher, 1988, 1989, 1990, 1991; Mariaux, 1989, 1991a, b; Mariaux and Georgiev, 1991; Mariaux et al., 1992). The present article summarizes the overall results of these collections and documents new information for host–para-

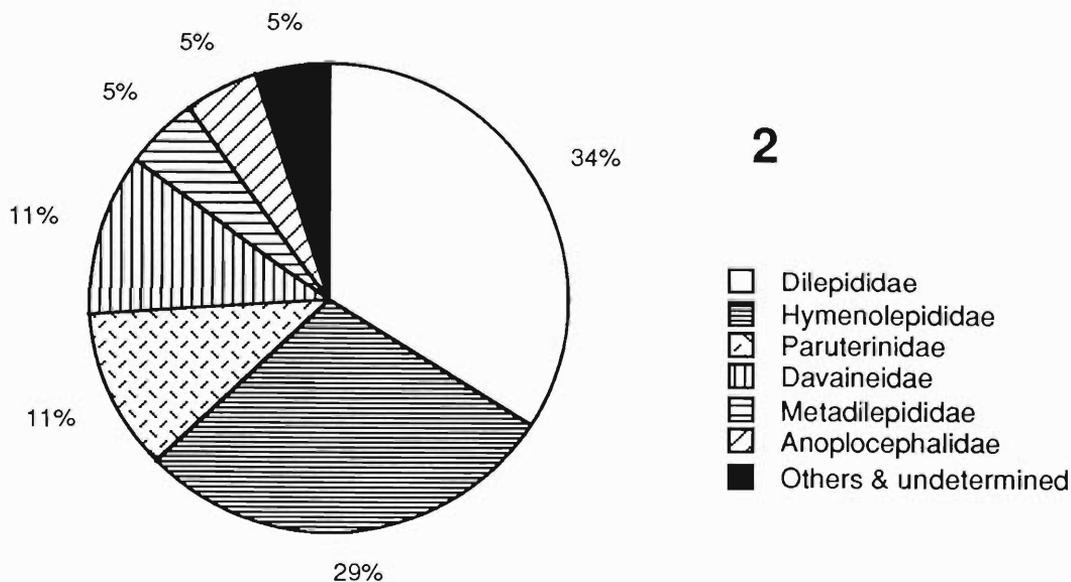
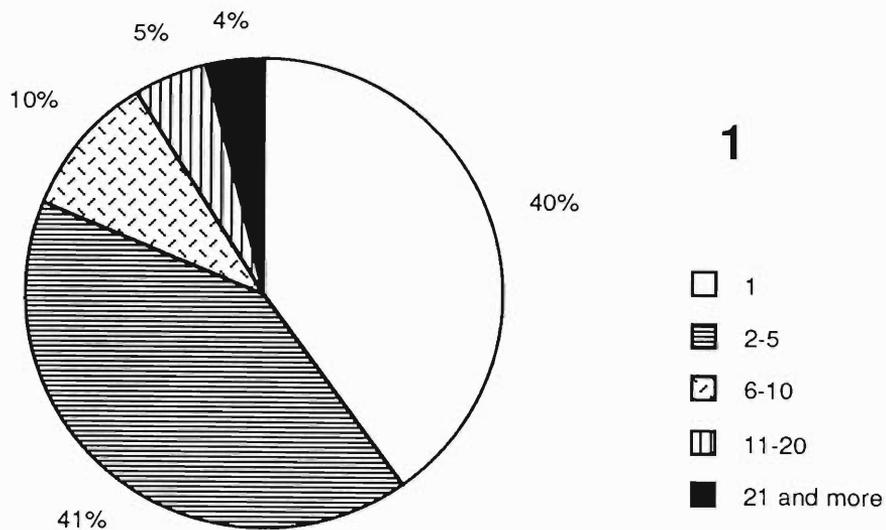
site distribution of this poorly known avian cestode fauna.

### Materials and Methods

Methods were described in previous works (Mariaux and Vaucher, 1988, 1990). In summary, birds were captured with mist nets or shot. They were necropsied and examined for parasites immediately after death. Cestodes were removed from the dissected gut with forceps and fixed with hot 5% formalin. They were then transferred into 70% ethanol for long-term storage. The worms were stained with alcoholic hydrochloric carmine and mounted in Canada balsam for light or Nomarsky microscopy. Some specimens were also prepared for scanning electron microscopy. All the parasites studied and most of the hosts (skins and skeletons) have been deposited at the Muséum d'Histoire Naturelle de Genève (MHNG) in Geneva, Switzerland.

### Results

The 1,252 birds studied belong to 174 species distributed in 104 genera and 39 families. One to 48 specimens ( $\bar{x} = 6 \pm 0.6$ ) of each species were examined. The prevalence of infection by cestodes was highly variable by host family. Overall, 197, or 16%, of the birds were parasitized by tapeworms, but this value was as low as 0%, for example, in Alcedinidae (29 specimens examined) and Indicatoridae (14), 6% in Nectariniidae (129), and 8% in Ploceidae (182) and as high as 80% in Corvidae (10) and 56% in Cuculidae (9). The intensity of infection was rather low, with a clear predominance of parasitism by 1–5 specimens and extremely rare infections by more than 20 specimens (Fig. 1). Infections were usually due to a single species of parasites (98%), whereas only 23 mixed infections (22 double and 1 triple) were noted. At the familial level, the most important groups collected were the Dilepididae and Hymenolepi-



Figures 1, 2. 1. Intensities of tapeworm infection among 197 of 1,252 birds examined from the Ivory Coast (220 values). 2. Composition of the cestode fauna of 1,252 birds examined in the Ivory Coast by family (220 occurrences).

dididae, followed by Paruterinidae and Davaineidae (Fig. 2). At this time, 55 taxa are determined to the generic or specific level, but the collection is estimated to be composed of about 95–100 distinct species.

In Table 1, a synoptic overview of these collections is presented with a complete host-parasite list based on the material that has been determined at least to the generic level. This list demonstrates how poorly known is this fauna: Among the records not previously published, there are 52 new host records. Furthermore, all records not treated in previous papers are new for the Ivory Coast, and 19 are new for West Africa or for the entire continent.

### Discussion

The overall prevalence found in the present study (16%) was low as compared to that reported in similar works (e.g., 23% by Petrova [1978]; 26–44% by Rausch [1983]; 27% by Spasskaja and Spassky [1971]; 28% by Schmidt et al. [1985]; 33% by Ryšavý [1955]; 34% by Yun [1973]; 43% by Zhuk et al. [1982]; 18–69% by various authors, as reported by Rausch [1983]). However, in the only comprehensive work in tropical Africa that includes some numerical data, Southwell and Lake (1939) reported about 20% prevalence among parasitized birds (Mahon [1954], who worked in Zaire, did not report that kind of information). This is a remarkable coincidence even if it seems probable that the low observed prevalences in the present study and that of Southwell and Lake (1939) were more probably due to the composition of the avifauna than to geographical reasons (the other works mentioned here were mainly focused on birds from aquatic biotopes).

The species composition of the fauna from the Ivory Coast is particularly diverse and interesting. Although some of the cestodes have yet to be specifically determined, the diversity and the proportion of new material is relatively great. The most notable aspect of fauna structure was the relatively high proportion of worms belonging to the family Metadilepididae (Fig. 2). This family, originally erected by Spassky (1959) and reviewed by Spassky and Spasskaja (1977) and Kornushin and Georgiev (in press), has largely been ignored by Western authors. Renewed interest in the family derived from the redescription of *Skrjabinoporus merops* by Mariaux and Vaucher (1989) and the subsequent descriptions

of new members of the group (Mariaux, 1991a; Mariaux et al., 1992).

The parasite fauna of some families of hosts warrants some additional remarks with respect to diet and the distribution of cestodes. Species of the Alcedinidae (kingfishers) and Jacanidae (jacanas) have never been found infected, despite the examination of 29 and 6 specimens, respectively. This is rather surprising given the ecology and habitat of these birds. This point has been previously discussed for the Alcedinidae, and it was suggested that cestodes could be replaced by trematodes in these hosts (Mariaux and Vaucher, 1989). A similar explanation could perhaps be applicable to jacanas, because trematodes, but not cestodes, were present in my captures; however, the small number of individuals examined does not allow unequivocal support of this contention. Another family, the Nectariniidae (sunbirds), was parasitized by 3 distinct species of cestodes (Mariaux and Vaucher, 1991). This was in contrast to the very limited list of known cestodes of these birds and tended to suggest that the minute arthropods swallowed by the sunbirds when sucking nectar from flowers could serve as intermediate hosts. The Estrildidae, with more than 1 bird out of 4 parasitized by cestodes, also are infected more heavily than their diet would indicate. These examples (of insectivorous/carnivorous birds almost unparasitized and granivorous/nectarivorous birds with a diverse array of parasites) prevent us from formulating simplistic conclusions about the relationships between diet and parasitic fauna. Even if general trends exist—for example, aquatic birds are certainly more heavily infected by cestodes than terrestrial ones—exceptions do occur.

In comparison with Rausch's (1983) survey of bird helminths, it is worth noticing some other results of the present study. In contrast to the low prevalences reported by Rausch, both in the United States and in Eurasia, I observed a high prevalence of cestodes in Cuculidae. Five of the 9 specimens (5 species) I collected were parasitized with 3 different species of tapeworms. The same observation can be made about Meropidae. I studied 45 individuals belonging to 3 species, and 8 of them (18%) harbored cestodes. This was about twice as many as reported in Rausch's summary. In contrast, only 15% of the 32 individuals (5 species) of Hirundinidae I examined had cestodes. Rausch reported prevalences varying from 40 to 100% for these birds. Finally, I

Table 1. Host-parasite list ordered alphabetically by host.

Host family	Genus and species	Parasite family	Genus and species	Prevalence	Intensity	Status <sup>a</sup>	MHNG accession #
Accipitridae	<i>Accipiter badius</i>	Davaineidae	<i>Idiogenes flagellum</i>	1/1	1	*	987.245
Ardeidae	<i>Ardea purpurea</i>	Dilepididae	<i>Dendrouterina macrosphincter</i>	1/1	2	○1, 2	988.172
			<i>Neogryporhynchus lasiopeius</i>	1/1	215	●	988.171
Capitonidae	<i>Butorides striatus</i>	Dilepididae	<i>Valipora</i> sp.	1/3	45	*○2	988.170
		Hymenolepididae	<i>Drepanidotaenia</i> sp.	2/3	1	*	985.607; 988.169
	<i>Gymnobucco calvus</i>	Davaineidae	<i>Raillietina</i> (P.) <i>bargetzii</i>	1/2	8	P	987.230
		Hymenolepididae	<i>Thaumasiolepis microarmata</i>	2/2	2-4	P	987.228-9
	<i>Lybius dubius</i>	Hymenolepididae	<i>Thaumasiolepis microarmata</i>	4/4	2-11	P	987.225-7; 988.177
Charadriidae	<i>Pogoniulus scolopaceus</i>	Paruterinidae	<i>Paruterina</i> sp.	1/17	4	*	988.174
	<i>Charadrius hiaticula</i>	Hymenolepididae	<i>Wardium hughesi</i>	2/3	1-2	P	985.603-4
Columbidae		Progynotaeniidae	<i>Progynotaenia odhneri</i>	3/3	6-27	P	985.601-2; 988.162
	<i>Streptopelia semitorquata</i>	Davaineidae	<i>Raillietina</i> sp.	1/2	5		985.609
	<i>Turtur afer</i>	Anoplocephalidae	<i>Aprina delafondi</i>	1/22	1	*○1, 2, 3	987.247
		Davaineidae	<i>Raillietina</i> sp.	1/22	1		985.608
Corvidae	<i>T. tympanistris</i>	Davaineidae	<i>Raillietina</i> sp.	1/9	2		987.248
	<i>Corvus albus</i>	Davaineidae	<i>Raillietina</i> (P.) <i>reynoldsiae</i>	2/2	1-2	○2, 3	987.244; 988.173
	<i>Malaconotus blanchoti</i>	Paruterinidae	<i>Anonchotaenia</i> (P.) <i>malaconoti</i>	1/2	2	P	988.182; 988.184
	<i>Prionops plumata</i>	Dilepididae	<i>Anomotaenia</i> sp.	3/7	1-12	*	987.302-3; 988.416
Cuculidae		Paruterinidae	<i>Anonchotaenia</i> (P.) <i>prionopos</i>	3/7	4-6	P	987.251-3
	<i>Centropus leucogaster</i>	Davaineidae	<i>Raillietina</i> (R.) <i>permista</i>	1/1	9	*○2	988.166
	<i>C. senegalensis</i>	Davaineidae	<i>Raillietina</i> (R.) <i>macrocirrosa</i>	1/2	7		987.237
			<i>Raillietina</i> (R.) <i>permista</i>	1/2	7	*○2	988.165
	<i>Ceuthmochares aereus</i>	Dilepididae	<i>Bonaia africana</i>	1/1	100	P	988.163-4
Estrildidae	<i>Chrysococcyx klaas</i>	Davaineidae	<i>Raillietina</i> (R.) <i>permista</i>	1/1	1	*○2	985.606
	<i>Estrilda melpoda</i>	Davaineidae	<i>Raillietina</i> (R.) sp.	1/15	2	*	985.633
	<i>Lonchura cucullata</i>	Hymenolepididae	<i>Echinocotyle dolosa</i>	13/32	1-12		985.622; 985.624-7, 985.964; 987.278-84
Glareolidae	<i>L. fringilloides</i>	Hymenolepididae	<i>Echinocotyle dolosa</i>	1/3	17	*	985.623
	<i>L. poensis</i>	Hymenolepididae	<i>Echinocotyle dolosa</i>	1/1	1	*	987.277
	<i>Pirenestes ostrinus</i>	Davaineidae	<i>Raillietina</i> sp.	1/7	2		987.288
	<i>Glareola pratincola</i>	Hymenolepididae	<i>Wardium</i> sp.	2/2	1-3	*	988.175-6
	Hirundinidae	<i>Hirundo rustica</i>	Hymenolepididae	<i>Passerilepis</i> sp.	1/5	1	*
		Paruterinidae	<i>Anonchotaenia</i> sp.	1/5	1		985.619
<i>H. semirufa</i>		Paruterinidae	<i>Anonchotaenia longiovata</i>	1/14	10	P	987.254
			<i>Anonchotaenia</i> sp.	1/14	1		987.255
			<i>Neyraia</i> (?) sp.	1/14	8	P	987.238 <sup>b</sup>
Incertae sedis	<i>Psadiloprocne obscura</i>	Paruterinidae	<i>Anonchotaenia globata</i>	1/8	8	P	988.183
	<i>Hylia prasina</i>	Davaineidae	<i>Raillietina</i> sp.	1/16	1	*	987.307

Table 1. Continued.

Host family	Genus and species	Parasite family	Genus and species	Prevalence	Intensity	Status*	MHNG accession #
Laniidae	<i>Tchagra senegalensis</i>	Dilepididae	<i>Anomotaenia</i> sp.	1/6	2	*	988.417
		Hymenolepididae	<i>Variolepis</i> sp.	2/6	4-5	*	988.412-3 988.414
Meropidae	<i>Merops albicollis</i>	Paruterinidae	<i>Biuterina africana</i>	1/6	10		987.224; 988.178
		Metadilepididae	<i>Skrjabinopus merops</i>	2/16	1-2	P	987.221-2; 988.179-81
Motacillidae	<i>M. gularis</i> <i>Anthus leucophrys</i>	Paruterinidae	<i>Biuterina macranciostrota</i>	5/16	2-8	P	
		Metadilepididae	<i>Skrjabinopus merops</i>	1/2	1	P	987.223
Muscicapidae	<i>Macronyx croceus</i> <i>Batis senegalensis</i>	Dilepididae	<i>Sobolevitaenia sobolevi</i>	6/19	1-25	*●	985.954-9
		Hymenolepididae	<i>Variolepis</i> sp.	4/19	1-3		985.960-1; 987.304; 988.419
	<i>Macronyx croceus</i> <i>Batis senegalensis</i>	Dilepididae	<i>Sobolevitaenia</i> sp.	1/3	9	*●	987.256
		Hymenolepididae	<i>Variolepis</i> sp.	1/3	1	*	987.257
	<i>Platysteira blisseti</i> <i>P. castanea</i> <i>Terpsiphone rufiventer</i>	Dilepididae	<i>Anomotaenia</i> sp.	4/12	2-9	*	985.620-1; 987.305; 988.420
		Paruterinidae	<i>Pseudochoanotaenia eburnea</i>	1/12	2	*	987.306
	<i>Anthreptes fraseri</i> <i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Dilepididae	<i>Sphaeruterina</i> sp.	1/3	1	*●	987.262
		Hymenolepididae	<i>Anomotaenia</i> sp.	1/11	2	*	988.185
	<i>Anthreptes fraseri</i> <i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Metadilepididae	<i>Passerilepis</i> sp.	1/11	3	*	987.261
		Dilepididae	<i>Pseudodelphoscolex eburnensis</i>	3/11	1	P	987.258-60
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Hymenolepididae	<i>Emberizotaenia</i> sp.	1/3	1	*	988.411
		Hymenolepididae	<i>Staphylepis ambilateralis</i>	1/6	1	P	985.614
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Hymenolepididae	<i>Staphylepis ambilateralis</i>	2/6	1	P	985.612; 985.615
		Hymenolepididae	<i>Staphylepis ambilateralis</i>	1/1	2	P	985.617
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Hymenolepididae	<i>Staphylepis ambilateralis</i>	1/38	2	P	985.613
		Hymenolepididae	<i>Staphylepis ambilateralis</i>	2/23	1-2	P	985.610-1; 985.616
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Davaineidae	<i>Raillietina</i> sp.	1/2	11	*	987.246
		Davaineidae	<i>Raillietina</i> sp.	1/2	1	*	988.407
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Dilepididae	<i>Paradilepis delachauxi</i>	1/1	2		988.415
		Davaineidae	<i>Raillietina</i> (S.) <i>campetherae</i>	1/1	1	P	987.233
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Davaineidae	<i>Raillietina</i> (S.) <i>campetherae</i>	1/1	4	P	987.231-2
		Davaineidae	<i>Raillietina</i> (P.) <i>vapoensis</i>	1/1	4	P	987.234-5
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Paruterinidae	<i>Paruterina</i> sp.	1/18	1	*	985.628
		Dilepididae	<i>Anomotaenia</i> sp.	2/17	2	*	987.275-6
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Paruterinidae	<i>Biuterina</i> sp.	1/17	11	*	988.186
		Paruterinidae	<i>Sphaeruterina</i> sp.	1/6	1	*●	988.187
	<i>Nectarinia chloropygia</i> <i>N. cuprea</i> <i>N. cyanolaema</i> <i>N. olivacea</i> <i>Nectarinia</i> sp. <i>Oriolus brachyrhynchus</i>	Dilepididae	<i>Anomotaenia</i> sp.	3/27	1-2	*	985.632; 985.637; 988.402
		Dilepididae	<i>Anomotaenia</i> sp.	1/16	2	*	987.290
Pycnonotidae	<i>P. nigricollis</i> <i>Andropadus latirostris</i>	Anoplocephalidae	<i>Paronia calcarutrina</i>	2/21	1	*●	985.292-3

Table 1. Continued.

Host family	Genus and species	Parasite family	Genus and species	Prevalence	Intensity	Status <sup>a</sup>	MHNG accession #
	<i>A. virens</i>	Anoplocephalidae	<i>Paronia calcaruterina</i>	3/48	1-2	*●	985.639; 987.291; 987.294
		Hymenolepididae	<i>Passerilepis passeris</i>	3/48	1-6	*	985.947-9
	<i>Bleda canicapilla</i>	Incertae sedis	<i>Spreotaenia abassena</i>	1/16	1	*○ <sup>3</sup>	985.962
	<i>Chlorocichla simplex</i>	Davaineidae	<i>Raillietina</i> sp.	1/15	1	*	985.946
	<i>Criniger barbatus</i>	Hymenolepididae	<i>Passerilepis</i> sp.	1/3	2	*	987.297
	<i>C. calurus</i>	Davaineidae	<i>Raillietina</i> sp.	1/3	5	*	987.295
		Hymenolepididae	<i>Passerilepis</i> sp.	2/3	1-7	*	987.298-9
	<i>Phyllastrephus icterinus</i>	Metadilepididae	<i>Yapolepis yapolepis</i>	5/19	1-5	P	987.239-42; 988.167-8
	<i>Pycnonotus barbatus</i>	Anoplocephalidae	<i>Paronia calcaruterina</i>	5/34	1-2	*●	985.638; 985.640; 985.943-5
		Dilepididae	<i>Emberizotaenia</i> sp.	6/34	1-5	*	985.965-70
Scolopacidae	<i>Tringa hypoleucis</i>	Dilepididae	<i>Anomotaenia hypoleucis</i>	4/12	2-20	P	985.595-9
			<i>Kowalewskiella cingulifera</i>	4/12	1-9	P	985.592-4; 987.236
		Davaineidae	<i>Raillietina permista</i>	1/12	1	P	985.600
Sylviidae	<i>Acrocephalus arundinaceus</i>	Paruterinidae	<i>Paruterina</i> sp.	2/9	2	*	987.273-4
	<i>Camaroptera brachyura</i>	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	1/21	1	*	985.963
	<i>Cisticola cantans</i>	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	1/1	1	P	985.583
	<i>C. erythropus</i>	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	1/2	2	P	985.590
	<i>C. galactotes</i>	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	2/2	3-8	P	985.581-2; 985.584
	<i>C. lateralis</i>	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	3/3	1	P	985.586-7
	<i>C. natalensis</i>	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	1/1	1	P	985.588
	<i>Cisticola</i> sp.	Dilepididae	<i>Pseudochoanotaenia eburnea</i>	10/87	1-6	P	985.585; 985.589; 985.591; 985.618; 987.264-70
		Hymenolepididae	<i>Variolepis</i> sp.	1/87	1	*	987.272
	<i>Sylvietta virens</i>	Paruterinidae	<i>Buterina</i> sp.	1/7	3	*	987.271
Timallidae	<i>Malacocincla cleaveri</i>	Hymenolepididae	<i>Passerilepis</i> sp.	1/1	1	*	987.300
	<i>Turdoides plebejus</i>	Hymenolepididae	<i>Variolepis farciminosus</i>	1/3	3	*●	988.410
Turdidae	<i>Turdus pelios</i>	Dilepididae	<i>Emberizotaenia</i> sp.	4/13	2-10	*	988.421-4
		Hymenolepididae	<i>Variolepis fernandensis</i>	3/13	2-4	*●	988.403-5
Turnicidae	<i>Turnix sylvatica</i>	Davaineidae	<i>Raillietina</i> sp.	2/6	1-65	●	987.249-50

<sup>a</sup> \* = new host record; ● = new African record; ○ = new West African record (1, already known from Northern Africa; 2, Central Africa; 3, Eastern Africa); P = published in previous works by the same author.

<sup>b</sup> Part of this material is deposited at the Central Laboratory for Helminthology in Sofia (Bulgaria) with the accession number 1989.12.27.1.

found only 4 out of 42 Columbidae with tapeworms. This is comparable to Rausch's findings in the United States and in sharp contrast to the much higher prevalences (30–46%) he reported from works done in Eurasia.

#### Acknowledgments

I am grateful to Dr. A. Aeschlimann (Neuchâtel), Dr. F. Bona (Torino), Dr. B. B. Georgiev (Sofia), Mr. O. Porgo (Abidjan), and Dr. C. Vaucher (Geneva) for their invaluable support during this work. I thank Eric Hoberg for his many helpful suggestions. I am also indebted to the Swiss Academy of Sciences, the Foundation J. de Giacomi, the Roche African Research Foundation, and the University of Neuchâtel for their financial support.

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## Diversity in the Genera *Avitellina* and *Thysaniezia* (Cestoda: Cyclophyllidea): Genetic Evidence

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**ABSTRACT:** The isoenzyme electrophoretic study of 2 species of cestodes, *Avitellina centripunctata* and *Thysaniezia ovilla*, sampled in African (Senegal) domesticated ruminants, revealed a complex of cryptic species. Four species of *Avitellina* were found in sheep and goats and 1 in cattle. Two species of *Thysaniezia*, 1 specific to cattle and the other to sheep, were also revealed. Despite a probable preponderant selfing mode of reproduction, the existence of the detected species was confirmed by high levels of genetic differentiation.

**KEY WORDS:** Cestoda, *Avitellina*, *Thysaniezia*, ruminants, isoenzyme electrophoresis, specificity.

*Avitellina centripunctata* (Rivolta, 1874) Gough, 1911, and *Thysaniezia ovilla* (Rivolta, 1878) Skrjabin, 1926, are 2 cestode species found in the small intestine of numerous herbivorous mammals (Schmidt, 1986). In domesticated ruminants, numerous other species of these two genera of Anoplocephalidae have been described by different authors, none of which is valid (Spas-skii, 1951; Troncy et al., 1981). It is difficult to explain this lack of parasite diversity considering the heterogeneity of potential hosts and their diets.

In this article, we present a population genetic study, based on isoenzyme electrophoresis, of African (Senegal) *A. centripunctata* and *T. ovilla*, sampled in sheep, goats, and cattle. This enabled us to test the genetic homogeneity and the degree of specificity within the 2 cestode species. This study revealed a broader diversity of species and a narrower range of host specificity than suspected.

### Material and Methods

#### Sampling of the worms

Two morphological species of parasite were studied, *Avitellina centripunctata* and *Thysaniezia ovilla* (Cyclophyllidea: Anoplocephalidae). Cestodes were taken

from the small intestine of cattle ( $n = 30$ ), sheep ( $n = 80$ ), and goats ( $n = 38$ ) from the Dakar (Senegal) slaughterhouse during the summer of 1992. For *A. centripunctata*, prevalences were 15, 8, and 7% in sheep, goats, and cattle, respectively. For *T. ovilla*, prevalences were 6 and 13% in sheep and cattle, respectively (goats not infected). The exact origin of each host is unknown; they may come from any region of the northern part of Senegal. Parasites were kept alive in physiological saline (0.9% w/v NaCl). After being identified under a dissecting stereoscope, the cestodes were stored in liquid nitrogen. It is known that such treatment prevents the contamination with host enzymatic material (e.g., Nadler, 1987; Johnson and Hoberg, 1989; Chilton et al., 1992). Parasites were then carried to Montpellier (France) on dry ice.

#### Preparation of the worms

In the laboratory, worms were thawed. One portion was fixed in alcoholic Bouin fixative, stained with aceto-carmine, mounted in Canada balsam (Martoja and Martoja, 1967), and observed under a light microscope. This enabled a precise diagnosis of the cestodes, using the criteria described by Schmidt (1986). At this time, no morphological heterogeneity could be found within each of the 2 species. Another portion of each parasite, corresponding to a volume of 0.5 ml, was homogenized in Eppendorf tubes filled with an equal volume of distilled water, centrifuged at 12,000 rpm for 1 min, and the homogenates were used as the protein source.

#### Electrophoresis

Starch gel electrophoresis was performed as described by Renaud and Gabrion (1988). The enzyme systems studied and their corresponding Enzyme Commission numbers were as follows: glucose phosphate isomerase (GPI, EC 5.3.1.9), hexokinase (HK, EC 2.7.1.1), malate dehydrogenase (MDH, EC 1.1.1.37),

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**Table 1. Genotypes observed for *Avitellina centripunctata*. The 4 genetic entities observed (A1, A2, A3, and A4) are shown separately. The cestodes came from 3 host species: sheep, goat, and cattle (represented by the third letter S, G, and C, respectively). Alleles were numbered according to their anodal mobility.**

	GPI	MDH	PEP-A	HK	NP	PGM	ME	N*
A1S1	2/2	1/1	2/2	3/3	4/4	2/2	2/2	9
A1S2	2/2	1/1	4/4	3/3	4/4	2/2	2/2	1
A1G1	2/2	1/1	4/4	3/3	4/4	3/3	2/2	1
A2S1	3/3	3/3	5/5	4/4	2/2	1/1	1/1	2
A2G1	3/3	3/3	5/5	4/4	2/2	1/1	1/1	2
A2S2	3/3	3/3	5/5	3/3	2/2	1/1	1/1	1
A2G2	3/3	3/3	4/4	4/4	2/2	1/1	1/1	1
A2G3	3/3	3/3	5/5	4/4	2/2	1/1	3/3	1
A3S1	3/3	2/2	3/3	2/2	3/3	3/3	3/3	7
A3G1	3/3	2/2	3/3	2/2	3/3	3/3	3/3	1
A3S2	3/3	2/2	3/3	1/1	3/3	3/3	3/3	1
A3S3	3/3	2/2	3/3	1/1	3/3	3/3	4/4	1
A4C1	1/1	2/2	1/1	5/5	1/1	4/4	1/1	10

\* Number of individuals.

malic enzyme (ME, EC 1.1.1.40), mannose phosphate isomerase (MPI, EC 5.3.1.8), nucleoside phosphorylase (NP, EC 2.4.2.1), phosphoglucomutase (PGM, EC 2.7.5.1), and peptidase A (PEP-A, EC 3.4.1.1). The number of worms analyzed and the host species from which they came are given in Tables 1 and 2.

## Results

### Species diversity

The different genotypes obtained are presented in Tables 1 and 2 for *A. centripunctata* and *T. ovilla*, respectively. It can be noted that the 2 "species" actually correspond to 2 species complexes. *Avitellina centripunctata* is subdivided into 4 genetically distant species. Out of the 7 loci studied, each species displayed a level of fixed allelic differences ranging from 71 to 100% (Table 1). Two species, discriminated with 7 (out of 8) diagnostic loci (87% of fixed differences), were observed for *T. ovilla* (Table 2).

### Parasite specificity

In the *A. centripunctata* complex (Table 1), parasite specificity isolates the small ruminants (sheep and goats) from the large ruminants (cat-

tle), with 1 species (A4) specific to cattle and the remaining 3 found in sheep and goats. For *T. ovilla*, the 2 species observed displayed a strict specificity (Table 2) for cattle and sheep.

### Within-species diversity

Within-species genetic heterogeneity could only be found in *A. centripunctata* species infecting small ruminants (Table 1). This heterogeneity is represented by rare alleles in several of the loci studied. These loci are PEP-A and PGM for species A1; PEP-A, HK, and ME for species A2; and HK and ME for species A3 (Table 1). Within outcrossing species, homozygosity is highly unlikely for rare alleles (more likely to be found at a heterozygous stage) (Hartl and Clark, 1989). No heterozygote could be found, even for rare alleles. This strongly suggests that selfing may be the preponderant mode of reproduction for these cestodes.

## Discussion

As demonstrated in similar studies, parasite species diversity is often much more complex

**Table 2. Genotypes obtained for *Thysaniezia ovilla* from sheep (TS) and cattle (TC). Alleles were numbered according to their anodal mobility.**

	MDH	PEP-A	NP	PGM	ME	HK	MPI	GPI	N
TS	1/1	2/2	2/2	2/2	1/1	1/1	1/1	1/1	14
TC	2/2	1/1	1/1	1/1	2/2	2/2	2/2	1/1	16

\* Number of individuals.

than what morphological taxonomy has previously postulated. This is true for various kinds of parasitic organisms: cestodes (Renaud et al., 1983; Renaud and Gabrion, 1984, 1988; de Chambrier et al., 1992), trematodes (Reversat et al., 1989), nematodes (Nascetti and Bullini, 1982; Andrews et al., 1989; Chilton et al., 1992), acanthocephalans (de Buron et al., 1986), and caligid copepods (Zeddami et al., 1988).

The level of biological diversity characterized within the cestodes studied appeared much higher than what has been reported (e.g., Euzéby, 1966; Soulsby, 1968; Troncy et al., 1981; Schmidt, 1986). Specificity was found to separate worms infecting small ruminants (sheep and goats) from those found in large ruminants (cattle). For certain kinds of organisms, in particular cestodes, selfing may make species characterization more difficult (Lymbery, 1992). Here, the high levels of genetic differentiation strongly validate the 6 species characterized, even for those represented by few individuals.

Species that self are likely to display high heterozygote deficiencies and, thus, low levels of polymorphism (homozygosity lowers the effective population size, i.e., accelerates drift) (Li, 1976). Accordingly, no variation was found within the 2 cryptic species of *Thysaniezia* and within 1 *Avitellina* species (cattle parasite). Some loci studied appeared polymorphic within 3 *Avitellina* species. No heterozygous individuals could be observed within these 3 species. Hosts probably came from a wide area. However, some migration must occur due to human activity (host migrations). Attributing the observed absence of heterozygotes to population structuring would require a total geographical isolation between the different units (no migration). High levels of selfing thus represents a suitable explanation.

In the small intestine of African domesticated ruminants, species of *Avitellina* and *Thysaniezia* coexist with other cestodes: *Stilesia globipunctata*, *Moniezia expansa*, and *M. benedeni* (e.g., Euzéby, 1966), some of which are themselves species complexes (unpubl. data). The ecological factors allowing such a species diversity remain unknown. However, differences in intermediate hosts and in host grazing behaviors may explain heterogeneities in host infections. The goats, for example, were rarely infected, compared to sheep. Moreover, it is probable that all these coexisting species display different ecological and transmission strategies. This remains to be studied. It is probable, as well, that the effect of anthel-

mintic treatments is different on these different parasite species. Consequently, the control of these diseases of veterinary importance may be more complicated than expected.

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## Meeting Schedule

### HELMINTHOLOGICAL SOCIETY OF WASHINGTON 1994

- |                             |  |
|-----------------------------|--|
| (Wednesday) 9 February 1994 | Animal Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD   |
| (Wednesday) 6 April 1994    | Johns Hopkins University, Baltimore, MD  |
| (Saturday) 7 May 1994       | Joint Meeting with the New Jersey Society for Parasitology, at the New Bolton Center, University of Pennsylvania, Kennett Square, PA |
| October 1994                | Site to be announced   |
| November 1994               | Site to be announced   |

## *Monoecocestus centroovarium* sp. n. (Cestoda: Anoplocephalidae) from Attwater's Pocket Gopher, *Geomys attwateri*, from the San Antonio Area of Texas

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**ABSTRACT:** Two of 6 (33%) Attwater's pocket gophers, *Geomys attwateri*, collected from Atascosa County south of San Antonio, Texas, in October and December 1986 were found to be infected with an undescribed anoplocephaline tapeworm, *Monoecocestus centroovarium* sp. n. The new species differs from existing species of *Monoecocestus* in having the ovary centrally located and no overlap between the ovary and the seminal receptacle. *Monoecocestus centroovarium* sp. n. most closely resembles *M. anoplocephaloides* from *Geomys breviceps* and *M. sigmodontis* from *Sigmodon hispidus*. It differs from both of these species by having fewer testes (36–39), smaller eggs (26  $\mu\text{m}$  in diameter), a smaller scolex (280  $\mu\text{m}$  wide), and excretory canals that do not anastomose.

**KEY WORDS:** *Monoecocestus centroovarium* sp. n., Cestoda, Anoplocephalidae, *Geomys attwateri*, Texas.

During a survey of the helminths of Attwater's pocket gopher (*Geomys attwateri* Tucker and Schmidly, 1981) from Atascosa County, Texas, an undescribed species of *Monoecocestus* Beddard, 1914, was found. Six species of *Monoecocestus* have been reported from rodents in North America: *M. americanus* Stiles, 1895, from *Erethizon dorsatum* and *Ondatra zibethicus* by Olsen, 1939; *M. anoplocephaloides* Douthitt, 1915, from *Geomys breviceps*; *M. giganticus* Buhler, 1970, from *E. dorsatum*; *M. sigmodontis* Chandler and Suttles, 1922, from *Sigmodon hispidus*; *M. thomasi* Rausch and Maser, 1977, from *Glaucomyx sabrinus*; and *M. variabilis* Douthitt, 1915, from *E. dorsatum*. *Monoecocestus anoplocephaloides* is the only species known from pocket gophers (Douthitt, 1915). The purpose of this study was to provide additional information on the helminths of the Attwater's pocket gopher.

### Materials and Methods

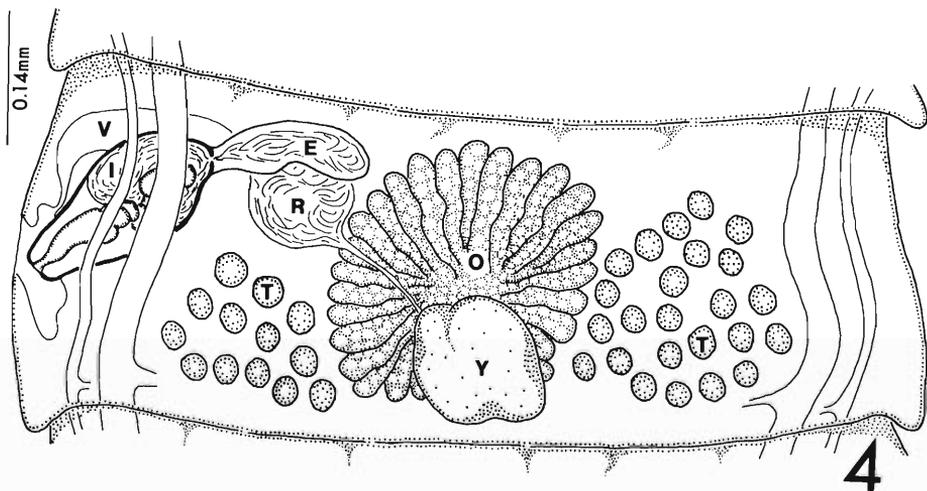
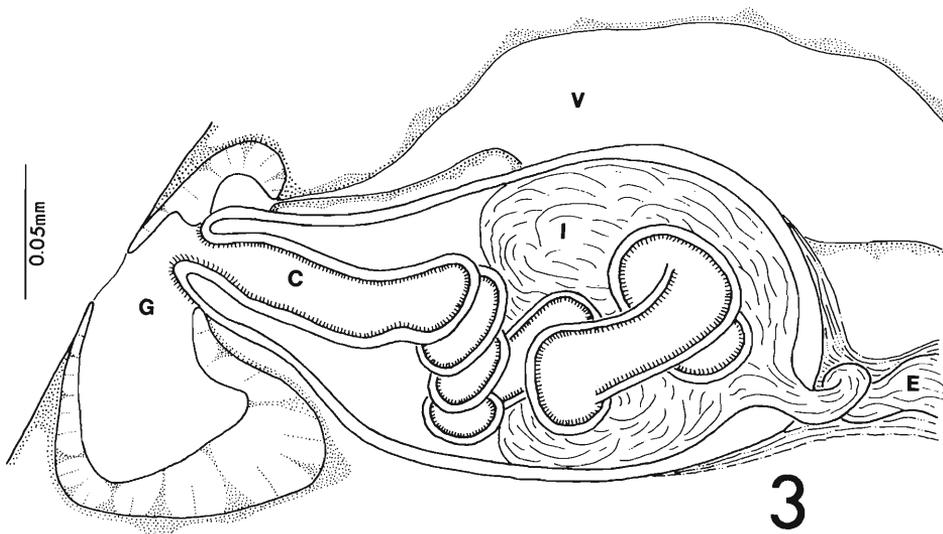
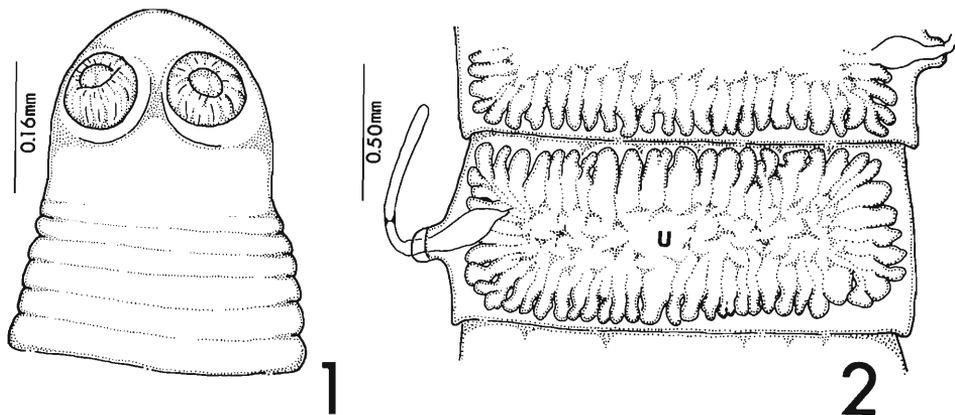
Six specimens of *Geomys attwateri* were trapped in Atascosa County, south of San Antonio, Texas, in October and December 1986 and examined for helminths. Cestodes were fixed in 10% formalin, stained in Semichon's carmine, and mounted in Canada balsam. Some specimens were sectioned by conventional paraffin technique. Measurements are in micrometers, with the mean followed by the range in parentheses, unless otherwise stated.

### Results

Two of 6 (33%) *G. attwateri* from Texas were infected with an undescribed species of *Monoecocestus*.

### *Monoecocestus centroovarium* sp. n. (Figs. 1–4)

**DESCRIPTION** (based on 15 specimens): Strobila craspedote, total length of worms 33 mm (28–39), composed of 60–80 proglottids. Scolex 210 (195–235) long by 280 (240–305) wide. Suckers well developed, 108 (100–112) in diameter. Neck short, first proglottids wider than long, approximately 500 wide. Mature proglottids 364 (360–370) long by 960 (930–1,010) wide. Genital atria regularly alternating, 47 (45–50) deep in mature proglottids, 145 (140–155) long in gravid proglottids when everted. Thirty-six to 39 testes present (12–13 poral, 24–26 antiporal), 37–44 in diameter, scattered laterally in the regions between the ovary and the excretory ducts. Cirrus sac approximately  $\frac{1}{4}$  as long as width of mature proglottids, 218 (208–221) long by 120 (114–130) wide. Extended cirrus approximately 600 long armed with numerous minute spines. External seminal vesicle 218 (200–225) long by 26 (23–32) wide. Ovary centrally located, 241 (224–262) long by 220 (211–246) wide, composed of 26–30 digitiform lobes. Vitellarium located in posterior  $\frac{1}{3}$  of mature proglottids, ventral to ovary, 93 (85–99) long by 104 (98–112) wide. Vagina opening into genital atrium immediately anterior to opening of male pore. Vagina and cirrus apparatus located dorsally to both lateral excretory canals. Excretory canals not anastomosing, ventral excretory canals 40 (35–50) wide, dorsal excretory canals 11 (9–14) wide. Seminal receptacle poral to ovary, not over-



lapped by ovary, 93 (88–97) long by 94 (88–96) wide. Gravid proglottids 750 (690–790) long by 1,975 (1,850–2,010) wide. Gravid uterus distinctly reticulate. Eggs ovoid, 26 (23–29) in diameter, oncospheres 16 (15–18) in diameter, pyriform apparatus indistinct, approximately 3–5 long.

TYPE HOST: *Geomys attwateri* Tucker and Schmidly, 1981.

TYPE LOCALITY: Atascosa County, Texas. 30 km south of San Antonio, 29°12'N, 94°45'W.

HOLOTYPE: USNM Helm. Coll. No. 83061.

PARATYPES: USNM Helm. Coll. Nos. 83062 (2 specimens); Texas Cooperative Wildlife Coll. No. 93-4593 (5 specimens), Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas.

ETYMOLOGY: The species name refers to the unique central placement of the ovary for this species of *Monoecocestus*.

#### Discussion

Of the 6 species of *Monoecocestus* known from North American rodents, *M. sigmodontis* from *Sigmodon hispidus* and *M. anoplocephaloides* from *Geomys breviceps* most closely resemble *M. centroovarivum* sp. n. in general proglottid morphology and in having the ovary nearly centrally located. The new species differs from both *M. sigmodontis* and *M. anoplocephaloides* by having fewer testes (36–39 compared to 70 and 70–110, respectively), smaller eggs (26 in diameter as compared to 47–53 and 30–40, respectively), a smaller scolex (280 wide as compared to 380–450 and 320–390, respectively), and excretory canals that do not anastomose (Chandler and Suttles, 1922; Douthitt, 1915; Spasskii, 1951).

Also, in all specimens of *M. centroovarivum* sp. n. examined, the ovary was centrally located rather than being poral, and the ovary does not overlap the seminal receptacle, as is found in all other described species of *Monoecocestus*.

#### Acknowledgments

We are indebted to Dr. J. R. Lichtenfels for the loan of type materials of *M. americanus*, *M. anoplocephaloides*, *M. giganticus*, and *M. sigmodontis* from the U.S. National Parasite Collection.

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 Figures 1–4. *Monoecocestus centroovarivum* sp. n. (Anoplocephalidae) from *Geomys attwateri*. 1. Scolex. 2. Gravid proglottid showing reticulate uterus (U). 3. Genital atrium region showing genital atrium (G), cirrus (C), internal seminal vesicle (I), external seminal vesicle (E), and vagina (V). 4. Mature proglottid showing internal seminal vesicle (I), external seminal vesicle (E), vagina (V), seminal receptacle (R), ovary (O), vitellarium (Y), and testes (T).

## New Species of *Skrjabinoclava* (Nematoda: Acuarioidea) from the Semipalmated Sandpiper (*Calidris pusilla*) (Aves: Scolopacidae)

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**ABSTRACT:** *Skrjabinoclava deltensis* from the proventriculus of a semipalmated sandpiper (*Calidris pusilla* (L.)) collected at the Delta Marsh in Manitoba, Canada, is distinguished from all other members of the genus by its long, slender right spicule divided into a shaft and a blade.

**KEY WORDS:** Nematoda, Acuarioidea, Charadriiformes, *Skrjabinoclava deltensis* sp. n.

Three species of *Skrjabinoclava* have been reported in semipalmated sandpipers (*Calidris pusilla* (L.)) in North America. *Skrjabinoclava morrisoni* Wong and Anderson, 1987, and *S. pusillae* Wong and Anderson, 1987, were found in 46-47% of semipalmated sandpipers collected in New Brunswick, Canada, and they are basically parasites of this shorebird (Wong and Anderson, 1987). *Skrjabinoclava bakeri* Wong and Anderson, 1987, has been found rarely (3%) in *C. pusilla*, but it is essentially a parasite of western sandpipers (*Calidris mauri*). Similarly, *S. tupacincal* Freitas and Ibanez, 1970, has been found in *C. pusilla* (7%), but it is mainly a parasite of sanderlings (*Calidris alba*). In the present article, a new species of *Skrjabinoclava* is described from *C. pusilla*. It is apparently an uncommon species in this host because it was recovered from only 1 of 26 birds examined.

### Materials and Methods

Semipalmated sandpipers (26) were collected by netting and were examined for acuarioid nematodes. After removal from the proventriculus, the specimens were washed in saline and fixed in hot glycerine alcohol. They were cleared and studied in glycerin. Measurements are given in micrometers, unless indicated otherwise.

### *Skrjabinoclava deltensis* sp. n. (Figs. 1-5)

**GENERAL:** Small worms with cordons as broad as long, constricted laterally. Body spines forming broad arch behind cordons and decreasing in size posteriorly (Fig. 1). Cuticle thick with regular transverse striations.

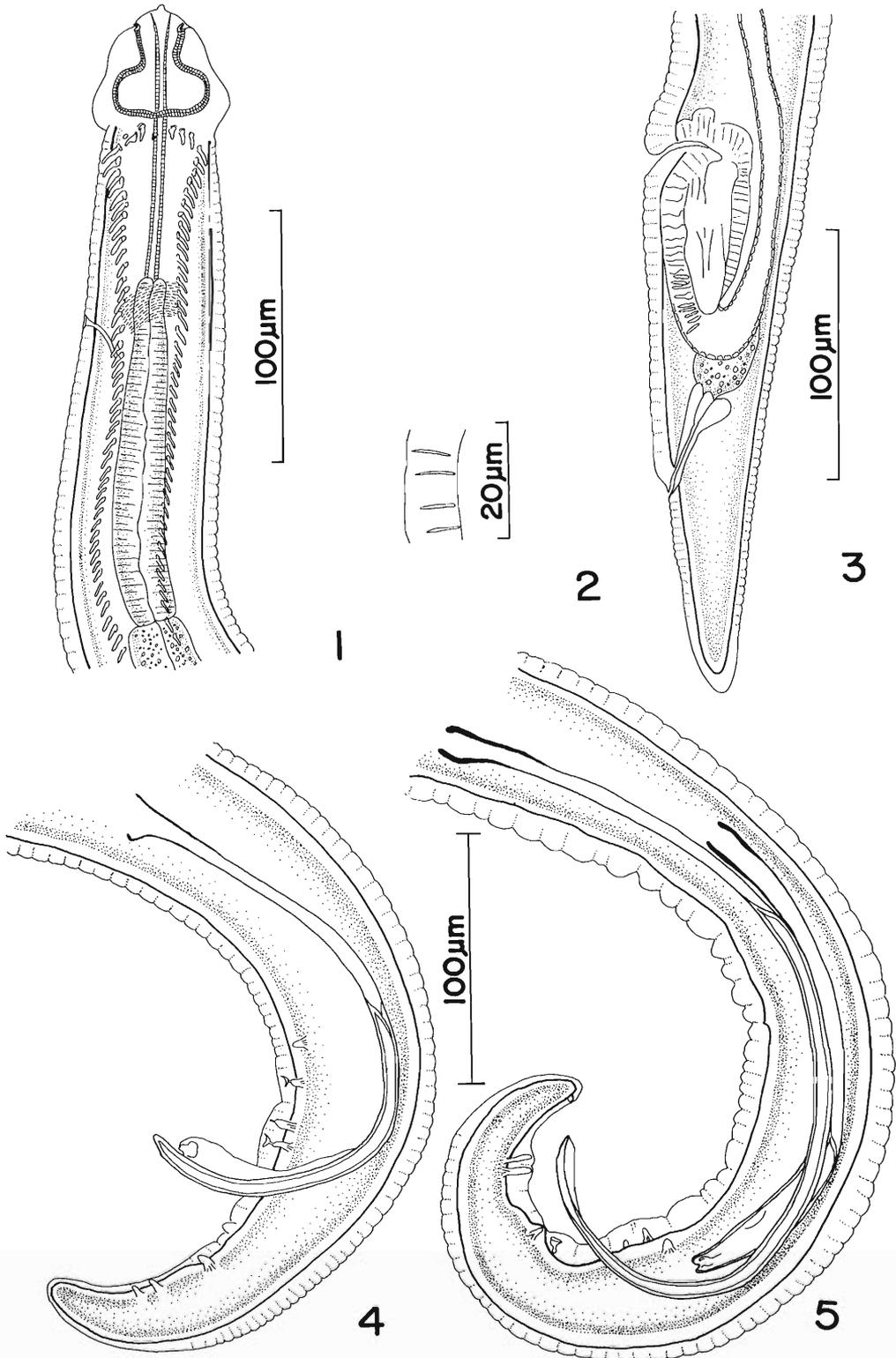
**MALE HOLOTYPE:** Length 1.8 mm. Maximum width, near middle of body, 78. Cordons 33 long by 35 wide. Buccal cavity 96 in length. Deirids 43, nerve ring 101, and excretory pore 130 from anterior extremity. Muscular esophagus 106, club-shaped, and glandular esophagus 550 in length. Total esophagus 656 in length. Left spicule 410 in length, narrow, ending in tapered point with ventral membranous wing (Fig. 5). Right spicule 210 in length (51% of the length of left spicule) with distal third expanded and distal end with cleft, divided into calamus and lamina. Caudal papillae consisting of 3 pairs preanal and 5 pairs postanal. Area rugosa (Fig. 2) consisting of 4 parallel rows of cuticular ridges. Tail 96 in length, slightly tapered.

**MALE PARATYPE:** Length 2.3 mm. Maximum width 72. Cordons 30 long by 33 wide. Buccal cavity 95 in length. Deirids 49, nerve ring 114, and excretory pore 139 from anterior extremity. Muscular esophagus 132 and glandular esophagus 660 in length. Total esophagus 792 in length. Left spicule 257 in length. Right spicule abnormal (Fig. 4). Caudal papillae consisting of 3 pairs preanal and 5 pairs postanal.

**FEMALE ALLOTYPE:** Length 2.3 mm. Maximum width 77. Cordons 36 long by 40 wide. Buccal cavity 106 in length. Deirids 102, nerve ring 116, and excretory pore 128 from anterior extremity. Muscular esophagus 135 and glandular esophagus 786 in length. Total esophagus 921 in length. Vulva 221 from caudal extremity. Vagina directed posteriorly, leading to single anteriorly directed uterus. Eggs in uterus few and undeveloped into larvae. Tail 82 in length, tapered with rounded tip (Fig. 3).

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Figures 1-5. *Skrjabinoclava deltensis* sp. n. 1. Anterior end female, lateral view (allotype). 2. Area rugosa. 3. Posterior end female, lateral view. 4. Posterior end male, lateral view (paratype with deformed right spicule). 5. Posterior end male, lateral view (holotype with normal spicules).



HOST: Adult female *Calidris pusilla* (L.) semipalmated sandpiper (Scolopacidae).

LOCATION IN HOST: Attached close together to the mucosa of the proventriculus.

LOCALITY AND DATE OF COLLECTION: Delta Marsh, Manitoba, Canada; June 1986.

PREVALENCE: The specimens were found in 1 of 26 birds examined.

SPECIMENS: United States National Museum, Helminthological Collection (Holotype ♂, paratype ♂, allotype ♀) No. 82910.

ETYMOLOGY: After the type locality.

COMMENTS: *Skrjabinoclava deltensis* is readily distinguished by its long, slender right spicule divided obviously into a calamus (shaft) and lamina (blade) and about 51% of the length of the longer left spicule. In other members of the genus, the right spicule varies in shape depending on the species (see Wong and Anderson, 1987), but it is never as long as 50% of the left spicule and never divided into a shaft and blade as in *S. deltensis*.

We include in the description the paratype male with what is obviously an abnormally developed right spicule. In all other respects, this specimen agrees with the holotype, and we believe that it belongs to the same species.

A single male and a single female of *S. pusillae* Wong and Anderson, 1987, were also found in the sandpiper containing the new species. Females of species of the genus *Skrjabinoclava* are difficult to distinguish; however, the female here-in assigned to *S. deltensis* is much smaller than the female of *S. pusillae*, and there is no overlap in the various measurements of the 2 species (see Wong and Anderson, 1987).

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Wong, P. L., and R. C. Anderson. 1987. New and described species of *Skrjabinoclava* (Nematoda: Acuarioidea) of the proventriculus of nearctic waders (Aves: Charadriiformes) with a review of the genus and a key to species. *Canadian Journal of Zoology* 65:2760-2779.

### New Editor

I am honored to be appointed as the new editor of the *Journal*. I would like to publicly thank the previous editor, Ralph Eckerlin, who has completed his 5-year term and who has worked very hard in maintaining the high quality of this publication and for making the editorial transition as smooth as possible. I am looking forward to working with the Editorial Board, Allen Press, and the authors during my term of office. Moreover, I would like to invite parasitologists working in all aspects of the discipline to consider submitting manuscripts to the *Journal*.

Sherman S. Hendrix

## Parasites of Alewives, *Alosa pseudoharengus*, from the Great Lakes

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**ABSTRACT:** A total of 302 alewives, *Alosa pseudoharengus* (Clupeidae), were collected from 2 locations in Lake Michigan and from Saginaw Bay, Lake Huron, between May 1990 and July 1992 and examined for parasites. Ten parasite species (2 Trematoda, 4 Cestoda, 1 Nematoda, 1 Acanthocephala, 1 Copepoda, and 1 Protozoa) infected alewives from Lake Michigan, with *Echinorhynchus salmonis* Müller, 1784, being most common. *Diplostomum* sp., *Contracaecum* sp., and *Ergasilus luciopercarum* Henderson, 1927, infrequently infected alewives from Saginaw Bay. Alewives from Ludington, Michigan, had the highest mean parasite species richness (0.7229). A summary and comparison of the parasites found in alewives from the Great Lakes are presented with 22 parasite species reported. This information revealed that the alewife is an important intermediate and transport host for helminths that mature in piscivorous fishes and birds in the Great Lakes area.

**KEY WORDS:** alewife, *Alosa pseudoharengus*, Clupeidae, parasites, survey, Michigan, Great Lakes.

The alewife, *Alosa pseudoharengus* (Wilson), is an anadromous fish species of eastern North America (Scott and Crossman, 1973). Its origin in the Great Lakes has not been established with certainty (R. R. Miller, 1957). The alewife was first reported in Lake Huron in 1933 and in Lake Michigan in 1949, and since then it has become well established in many parts of these lakes. The importance of the alewife as a food item for salmonids (Jude et al., 1987; M. A. Miller and Holey, 1992) and piscivorous birds (Ludwig, 1966; Fox et al., 1990) in the Great Lakes is well known.

The objectives of the present study were (a) to provide information on the occurrence and abundance of parasites in alewives from 2 locations in Lake Michigan and from the western portion of Saginaw Bay, Lake Huron; (b) to summarize and compare the known information about parasites infecting this important forage species in the Great Lakes; and (c) to examine the role of alewives as intermediate and transport hosts for parasites that mature in piscivorous fishes and birds in the Great Lakes area.

### Materials and Methods

Alewives were collected by beach seine and trawl from 2 locations in Lake Michigan and from the western portion of Saginaw Bay, Lake Huron. Fish data are given below with information on location; month and year of collection; number of fish examined; total length, with range in millimeters (followed by mean  $\pm$  SD); and 95% confidence intervals.

1. Southern Lake Michigan, Michigan City, Indiana; August 1991;  $n = 75$ ; 101-212 (151  $\pm$  29.1); 144.1-157.5.

2. Eastern Lake Michigan, Ludington, Michigan; May-August 1990, April-June 1991;  $n = 166$ ; 102-239 (160  $\pm$  28.6); 155.8-164.6.
3. Western portion of Saginaw Bay, Lake Huron, Michigan; May-July 1992;  $n = 61$ ; 62-203 (141  $\pm$  35.1); 132.1-150.1.

Ludington, Michigan, is approximately 247 km north of Michigan City, Indiana. Alewives were frozen in the field and measured and sexed at necropsy. The skin, fins, gills, eyes, kidney, gonads, spleen, liver, gall bladder, mesenteries, esophagus, gastrointestinal tract, heart, and the left or right side of the musculature were examined. Parasites were collected and processed using routine procedures. Prevalence is the percentage of fish infected, and mean intensity is the mean number of worms of a species per infected fish. Voucher specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Diplostomum* sp. (83226), *Tetracotyle* sp. (83227), *Cyathocephalus truncatus* (83228), *Diphyllobothrium* sp. (83229), *Eubothrium salvelini* (83230), *Proteocephalus* sp. (83231), *Haplonema hamulatum* (83233), and *Echinorhynchus salmonis* (83232). Specimens of *Ergasilus luciopercarum* are in the collection of L. Roberts. Specimens of other helminth species were not retained by the author and therefore were not deposited.

Information on the parasites of alewives was obtained from examining published studies performed in the Great Lakes. Species richness refers to the number of parasite species infecting alewives from each Great Lake. The Jaccard coefficient of community similarity was calculated as

$$C_j = C / (S_1 + S_2 - C),$$

where  $S_1$  and  $S_2$  are the number of parasite species in communities 1 and 2, respectively, and  $C$  is the number of species common to both communities (Brower and Zar, 1984). For calculations of the Jaccard coefficient of community similarity and species richness, *Diplostomum* (present study) and *Diplostomum* in other studies were considered to be a single genus.

## Results

Thirty (40%) alewives from Michigan City, Indiana, 91 (55%) from Ludington, Michigan, and 7 (11%) from Saginaw Bay, Michigan, were infected with 1 or more parasites. A total of 10 parasite species (4 from Michigan City and 9 from Ludington) infected alewives from both locations in Lake Michigan (Table 1). *Echinorhynchus salmonis* had the highest prevalence and mean intensity at each location. Correlation coefficients between *E. salmonis* intensity and length of infected alewife from Michigan City and Ludington were 0.172 ( $P > 0.05$ ) and 0.592 ( $P < 0.01$ ), respectively. Of the helminth species found, only *Cyathocephalus truncatus* (Pallus, 1781) and *E. salmonis* were gravid. *Diplostomum* sp. was more common in alewives from Michigan City than from Ludington, whereas cestodes were more common in alewives from Ludington than from Michigan City. Three parasite species (*Diplostomum* sp., *Contracaecum* sp., and *Ergasilus luciopercarum*) infrequently infected alewives from Saginaw Bay. There were no significant differences in prevalence (chi-square analysis,  $P > 0.05$ ) and intensity (Student's *t*-test,  $P > 0.05$ ) of parasitism between female and male alewives at each location. The alewife is a new host record for *Haplonema hamulatum* Moulton, 1931, and *E. luciopercarum*.

Jaccard's coefficients of similarity for the parasite faunas of alewives between locations are 0.30 for Michigan City–Ludington, 0.40 for Michigan City–Saginaw Bay, and 0.09 for Ludington–Saginaw Bay. When the data for uninfected and infected fish were combined, mean parasite species richness  $\pm$  SD, range, and 95% confidence intervals in alewives from Michigan City ( $0.5333 \pm 0.6830$ , 0–2, 0.3670–0.6997), from Ludington ( $0.7229 \pm 0.7991$ , 0–4, 0.6016–0.8441), and from Saginaw Bay ( $0.1148 \pm 0.3214$ , 0–1, 0.0324–0.1971) were significantly different (analysis of variance,  $F = 16.7$ ,  $P < 0.001$ ). However, when data from infected fish only were used in the analyses, mean parasite species richness  $\pm$  SD, range, and 95% confidence intervals in alewives from Michigan City ( $1.3333 \pm 0.4795$ , 1–2, 1.1543–1.5124), from Ludington ( $1.3187 \pm 0.5940$ , 1–4, 1.1949–1.4424) and from Saginaw Bay ( $\bar{x} = 1$ , range = 1) were not significantly different (analysis of variance,  $F = 1.13$ ,  $P > 0.05$ ).

## Discussion

*Echinorhynchus salmonis* was the most common parasite species found in alewives from Lake

Michigan in the present study. Alewives in this lake feed on the amphipod, *Pontoporeia affinis*, which serves as an intermediate host for this parasite (Amin, 1978). Morsell and Norden (1968) reported that as alewives in western Lake Michigan increased in length a greater proportion of *P. affinis* was found in their diet. This could explain the significant relationship between *E. salmonis* intensity and length of infected alewives from Ludington, Michigan. Webb and McComish (1974) found that the largest percentage of volume and percent frequency of occurrence of *P. affinis* in alewives from southern Lake Michigan occurred in August 1972; however, it was of negligible importance in their diet in 1971. Rhodes and McComish (1975) reported that alewives consumed the largest volume of *P. affinis* in October in southern Lake Michigan.

Amin and Burrows (1977) found *E. salmonis* infecting alewives in western Lake Michigan. A mean intensity of 1.9 *E. salmonis* per infected fish was calculated using their data in Table 1. This value and prevalence are low compared to the infection values of *E. salmonis* in the present study. Fish length and time of collection do not appear to play major roles in these infection differences, because examined fish had similar lengths and were collected during similar months. Possible explanations for these infection differences may involve the availability of the amphipod intermediate host and its importance in the diet of alewives from 1 year to the next or *E. salmonis* may be more common in 1 location than in another.

Muzzall (1989) hypothesized that the alewife serves as an important transport host for *E. salmonis* to salmonids in eastern Lake Michigan. Hnath (1969) experimentally demonstrated the transfer of *E. salmonis* between coho salmon, *Oncorhynchus kisutch*, and brook trout, *Salvelinus fontinalis*, from Lake Michigan. Jude et al. (1987) reported that the alewife made up 78% of the identifiable prey species eaten by salmonids in Lake Michigan. The high infection values of *E. salmonis* in alewives in the present study supports this suggestion that alewives serve as important transport hosts for *E. salmonis* to salmonids. Seng (1975) estimated that approximately one-eighth of *E. salmonis* flows through transport hosts in the ecosystem of Cold Lake, Alberta. Furthermore, it is known that as salmonids become older (larger) they ingest a larger proportion of alewives (Muzzall, 1989). Amin and Burrows (1977) suggested that other species of

**Table 1. Prevalence (P), mean intensity (MI), and maximum number of parasites (max.) found in *Alosa pseudoharengus* from Lake Michigan (Michigan City, Indiana, and Ludington, Michigan) and Saginaw Bay, Lake Huron, 1990-1992.\***

Parasite	Michigan City (n = 75)†		Ludington (n = 166)		Saginaw Bay (n = 61)		Site
	P	MI ± 1 SD (max.)	P	MI ± 1 SD (max.)	P	MI ± 1 SD (max.)	
<b>Digenea</b>							
<i>Diplostomum</i> sp.‡	21	1.6 ± 1.0 (4)	0.6	1	3	1	Lens
<i>Tetracotyle</i> sp.‡	—	—	1	1	—	—	Encysted in mesenteries
<b>Cestoda</b>							
<i>Cyathocephalus truncatus</i>	—	—	12	1.6 ± 0.9 (4)	—	—	Pyloric ceca
<i>Diphyllobothrium</i> sp.‡	—	—	0.6	1	—	—	Encysted around pyloric cecum
<i>Eubothrium salvelini</i> §	—	—	8	2.0 ± 1.5 (5)	—	—	Anterior intestine, pyloric ceca
<i>Proteocephalus</i> sp.§	1	1	1.8	1	—	—	Anterior intestine
<b>Nematoda</b>							
<i>Contracaecum</i> sp.‡	—	—	—	—	5	3.7 ± 1.5 (5)	Encysted in mesenteries
<i>Haplonema hamulatum</i> §	—	—	1	1	—	—	Small intestine
<b>Acanthocephala</b>							
<i>Echinorhynchus salmonis</i>	29	4.0 ± 3.4 (13)	48	8.4 ± 11.3 (72)	—	—	Intestine
<b>Copepoda</b>							
<i>Ergasilus luciopercarum</i>	1	1	—	—	3	1	Gills
<b>Protozoans</b>							
<i>Trichodina</i> sp.	—	—	0.6	—	—	—	Gills

\* Unless otherwise indicated, parasites were gravid.

† Number of fish examined.

‡ Metacercariae or larvae.

§ Immature parasites.

Table 2. Parasites reported from *Alosa pseudoharengus* in the Great Lakes.

Parasite	Lake Michigan (n = 241)*	Lake Superior (n = 12)	Lake Huron (n = 297)	Lake Erie (n = 14)	Lake Ontario (n = 61)
<b>Monogenea</b>					
<i>Octomacrum</i> sp.	—	—	—	10	—
<b>Digenea</b>					
<i>Diplostomum flexicaudum</i>	—	—	7	—	—
<i>Diplostomum spathaceum</i>	—	5	6, 8	—	12
<i>Diplostomum</i> sp.	1, 2	—	9	10	—
<i>Posthodiplostomum minimum</i>	—	—	—	—	12
<i>Tetracotyle intermedia</i>	—	—	6	—	—
<i>Tetracotyle</i> sp.	2	—	8	—	—
Metacercariae	—	—	—	10	—
<b>Cestoda</b>					
<i>Cyathocephalus truncatus</i>	2	—	—	—	—
<i>Diphyllbothrium</i> sp.	2	—	—	—	—
<i>Eubothrium salvelini</i>	2	—	—	—	—
<i>Proteocephalus</i> sp.	1, 2	—	—	—	—
<b>Nematoda</b>					
<i>Camallanus oxycephalus</i>	—	—	—	11	—
<i>Capillaria</i> sp.	—	—	—	10	—
<i>Contracaecum</i> sp.	—	—	9	—	—
<i>Haplonema hamulatum</i>	2	—	—	—	—
<b>Acanthocephala</b>					
<i>Acanthocephalus dirus</i>	3	5	6, 7, 8	—	12
<i>Echinorhynchus salmonis</i>	1, 2, 4	—	6, 7	—	—
<b>Copepoda</b>					
<i>Ergasilus luciopercarum</i>	1	—	9	—	—
<b>Fungi</b>					
<i>Saprolegnia</i> sp.	—	5	6, 7, 8	—	12
<b>Protozoa</b>					
<i>Trichodina</i> sp.	2	—	—	—	—
<b>Acarina</b>					
<i>Hydrachna</i> sp.	—	—	6	—	—

\* Total number of fish examined. Entries are abbreviations for published investigations on the parasites of alewives from the Great Lakes. 1 = present study, Michigan City, Indiana; 2 = present study, Ludington, Michigan; 3 = Amin (1977); 4 = Amin and Burrows (1977); 5 = Dechtiar and Lawrie (1988); 6 = Collins and Dechtiar (1974); 7 = Dechtiar and Berst (1978); 8 = Dechtiar et al. (1988); 9 = present study, Saginaw Bay, Michigan; 10 = Bangham (1972); 11 = Stromberg and Crites (1975); 12 = Dechtiar and Christie (1988).

forage fish in Lake Michigan serve as transport hosts for *E. salmonis*.

This is the first published study to summarize the parasites of a fish species in the Great Lakes with a total of 22 parasite species being reported from the alewife (Table 2). Taxonomically, larval digenean species are most common followed by cestode and nematode species. No parasite species has been reported from alewives in all the Great Lakes. *Diplostomum* is the only genus found in alewives from all the Great Lakes. Cestodes were reported from alewives only from Lake

Michigan. *Acanthocephalus dirus* has been found in alewives from 4 Great Lakes. Although *E. salmonis* infect fishes from Lake Huron, it was not found in alewives from Saginaw Bay. None of the parasite species infecting alewives in the Great Lakes is specific to this fish species.

Mean parasite species richness was significantly higher in alewives from Ludington than from the other locations because more parasite species were found and the high prevalence of *E. salmonis*. Parasite species richness (in parentheses) in alewives from each lake are Lake Mich-

**Table 3.** Jaccard's index of community similarity based on presence of parasite species reported from *Alosa pseudoharengus* in each lake.\*

Lake†	1	2	3	4	5
1	1.0	0.08	0.29	0.07	0.07
2		1.0	0.27	0.00	0.75
3			1.0	0.07	0.15
4				1.0	0.00
5					1.0

\* See Table 2 for the parasite species used in these calculations and specific investigations.

† 1 = Lake Michigan, 2 = Lake Superior, 3 = Lake Huron, 4 = Lake Erie, 5 = Lake Ontario.

igan (11), Lake Superior (3), Lake Huron (11), Lake Erie (5), and Lake Ontario (4). Species richness is highest in Lake Michigan and Lake Huron, where the largest numbers of alewives were examined. Jaccard's coefficients of similarity for the parasite faunas in alewives between the Great Lakes do not follow a specific pattern (Table 3). Alewives from Lake Erie had the lowest coefficients ( $\leq 0.07$ ), indicating that they shared the fewest parasite species with alewives from the other Great Lakes. Alewives from Lake Superior and Lake Ontario had the highest coefficient (0.75), indicating that they shared the most parasite species of those species found in alewives from each lake.

Several hypotheses have been proposed and discussed by Wisniewski (1958), Chubb (1963, 1964, 1970), Esch (1971), Halvorsen (1971), Kennedy (1978), Holmes and Price (1986), and Marcogliese and Cone (1991) to explain the patterns of distribution and abundance of metazoan parasites in freshwater fishes. The present study is the first to investigate the distribution of parasites in 1 fish species in the Great Lakes. None of these hypotheses, however, explains the distribution and number of helminth species in alewives from the Great Lakes. An explanation is confounded by the small number of alewives examined from Lake Superior and Lake Erie. It is believed more parasite species will be found when more alewives are examined from these 2 lakes.

Eight (7 larval digenean species and *Diphylobothrium* sp.) of the 18 helminth species reported from alewives in the Great Lakes (Table 2) are allogenic, maturing in piscivorous birds. Both Ludwig (1966) and Fox et al. (1990) have reported that the alewife and rainbow smelt, *Osmerus mordax*, accounted for at least 80% of the fish eaten by herring gulls, *Larus argentatus*, in

the Great Lakes. *Eubothrium salvelini* (Schrank, 1790), *Proteocephalus* sp., *Capillaria* sp., *Contracaecum* sp., and *Haplonema hamulatum*, listed in Table 2, mature in other fish species and not in the alewife. It is believed that alewives serve as transport hosts for these helminth species and *E. salmonis*. Therefore, it is hypothesized that the dominance of allogenic helminth species in alewives and the occurrence of helminths in alewives that mature in other fish species is attributable to the position of alewives in the food web as planktivores and macroinvertebrates that are prey species for piscivorous fishes and birds in the Great Lakes area.

### Acknowledgments

I thank Dan Brazo, Indiana Department of Natural Resources, Michigan City, Indiana, and Tom McComish, Ball State University, Muncie, Indiana; Rob Elliott and Doug Peterson, Michigan State University, East Lansing, Michigan; and Bob Haas, Jack Hodge, and Larry Shubel, Michigan Department of Natural Resources, Mount Clemens, Michigan, for providing the alewives; and Larry Roberts for confirming my identification of *Ergasilus luciopercarum*.

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## Gastrointestinal Helminths of *Sceloporus* Lizards (Phrynosomatidae) from Arizona

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**ABSTRACT:** Five species of Arizona spiny lizards were examined for gastrointestinal helminths. *Sceloporus clarkii* ( $N = 20$ ) harbored the cestodes *Mesocestoides* sp. and *Oochoristica scelopori* and the nematodes *Atractis penneri*, *Physaloptera retusa*, *Piratuba prolifica*, *Skryabinoptera phrynosoma*, and *Spauligodon giganticus*. *Sceloporus magister* ( $N = 15$ ) harbored *A. penneri*, *Ph. retusa*, and *Sk. phrynosoma*. *Sceloporus undulatus consobrinus* ( $N = 30$ ) harbored *O. scelopori* and *Ph. retusa*. *Sceloporus undulatus tristichus* ( $N = 18$ ) harbored *Mesocestoides* sp., *Ph. retusa*, and *Sp. giganticus*. *Sceloporus virgatus* ( $N = 23$ ) harbored *A. penneri* and *Ph. retusa*. No helminths were recovered from *Sceloporus graciosus* ( $N = 20$ ). *Sceloporus clarkii* is a new host record for *Mesocestoides* sp., *O. scelopori*, *Ph. retusa*, *Pi. prolifica*, and *Sk. phrynosoma*. *Sceloporus undulatus consobrinus* is a new host record for *O. scelopori*. *Sceloporus undulatus tristichus* is a new host record for *Mesocestoides* sp. *Sceloporus virgatus* is a new host record for *A. penneri* and *Ph. retusa*. The highest prevalence in the study (55%) was recorded for *Ph. retusa* in *S. clarkii*. The highest mean intensity (1,682) was recorded for *A. penneri* in *S. magister*. It appears that larger species of sceloporine lizards have more diverse helminth faunas than smaller species.

**KEY WORDS:** Cestoda, *Mesocestoides* sp., *Oochoristica scelopori*, Nematoda, *Atractis penneri*, *Physaloptera retusa*, *Piratuba prolifica*, *Skryabinoptera phrynosoma*, *Spauligodon giganticus*, Phrynosomatidae, *Sceloporus*, prevalence, intensity, survey.

Seven species of lizards in the genus *Sceloporus* occur in Arizona (Stebbins, 1985). *Sceloporus scalaris* Wiegmann, 1828, *Sceloporus virgatus* Smith, 1938, and *Sceloporus jarrovi* Cope, 1875, are mountain species restricted to elevations above 1,500 m. *Sceloporus clarkii* Baird and Girard, 1852, is found on lower mountain slopes usually below elevations of 1,500 m. *Sceloporus magister* Hallowell, 1854, is found on arid plains. *Sceloporus undulatus* (Bosc and Daudin, 1801), represented by subspecies *S. u. consobrinus* Baird and Girard, 1853, and *S. u. tristichus* Cope, 1875, is found in a variety of habitats. *Sceloporus graciosus* Baird and Girard, 1852, occurs in brushlands but is restricted to northern Arizona.

Several reports on helminths from these lizards are available: Gambino (1958), Gambino and Heyneman (1960), and Goldberg and Bursey (1992c) for *S. clarkii*; Goldberg and Bursey (1990a, 1992c) and Bursey and Goldberg (1991a, b, 1992a) for *S. jarrovi*; Walker and Matthias (1973) and Benes (1985) for *S. magister*; and Goldberg and Bursey (1992b) for *S. scalaris*. To our knowledge, there are no published reports of helminths from *S. graciosus*, *S. undulatus*, and *S. virgatus* from Arizona, although reports of helminths from *S. graciosus* in California (Stebbins and Robinson, 1946; Goldberg and Bursey, 1989b, c), in Utah (Woodbury, 1934; Pearce and

Tanner, 1973) and from *S. undulatus* from Utah (Pearce and Tanner, 1973) have been published. The purpose of this article is to present data on helminths from 5 species of sceloporine lizards from Arizona and to compare helminth infections among the various species of Arizona lizards.

### Materials and Methods

*Sceloporus clarkii* were borrowed from the Herpetology Collection, Natural History Museum of Los Angeles County (LACM) and from the Museum of Northern Arizona (MNA); *S. graciosus* were borrowed from the LACM and Monte L. Bean Life Science Museum, Brigham Young University (BYU); and *S. magister*, *S. undulatus consobrinus*, *S. undulatus tristichus*, and *S. virgatus* were borrowed from the LACM. The number of specimens of each species examined, body sizes as snout-vent length, and collection dates are given in Table 1. Museum accession numbers and collection site latitudes, longitudes, and elevations are given in the Appendix.

The body cavity was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was excised by cutting across the anterior esophagus and rectum. The esophagus, stomach, and small and large intestines were slit longitudinally and examined under a dissecting microscope. Each helminth was examined and identified using a glycerol wet mount. Selected cestodes were stained with hematoxylin and mounted in balsam. Representative specimens were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705 (see the Appendix for accession numbers).

**Table 1. Collection locality, year of collection, and mean size of the sceloporine lizards examined in this study.**

Species	County	N	Year	Mean snout-vent length (mm)	Range (mm)
<i>Sceloporus clarkii</i>	Gila	1	1992	49	—
	Mohave	1	1992	103	—
	Pima	9	1966, 1967, 1991	91	78–98
	Pinal	4	1967	94	86–117
	Yavapai	5	1967	92	85–97
<i>S. graciosus</i>	Coconino	13	1984	39	34–52
	Navajo	7	1966	48	41–52
	—*	1	1983	56	—
<i>S. magister</i>	Gila	1	1992	109	—
	Mohave	2	1992	98	87–109
	Pima	9	1966, 1967, 1969	93	68–114
	Pinal	1	1967	124	—
	Yavapai	2	1967, 1969	72	48–95
<i>S. undulatus consobrinus</i>	Pinal	30	1967, 1969	56	43–65
<i>S. u. tristichus</i>	Pima	18	1967	55	33–68
<i>S. virgatus</i>	Cochise	23	1967	46	31–64

\* The 1 infected specimen was from Washington County, Utah.

### Results and Discussion

Two species of cestode, *Mesocestoides* sp. (as tetrathyridia) and *Oochoristica scelopori* Vogt and Fox, 1950, and 5 species of nematodes, *Atractis peneri* (Gambino, 1957) Baker, 1987, *Physaloptera retusa* Rudolphi, 1819, *Pirartuba prolifica* Pelaez and Perez-Reyes, 1958, *Skrjabinoptera phrynosoma* (Ortlepp, 1922) Schulz, 1927, and *Spauligodon giganticus* (Read and Amrein, 1953) Skrjabin, Schikhobalova and Lagodovskaja, 1960, were recovered during the course of this study. Prevalences and mean intensities (Margolis et al., 1982) and infection sites for helminths occurring in Arizona lizards (including this study) are given in Table 2.

Of the 44 species of lizards in Arizona (Stebbins, 1985), 31 are now reported to harbor at least 20 species of helminths in 13 genera (Table 2). We question the validity and have therefore deleted *Alaeuris* sp. (reported by Benes, 1985), *Spauligodon* (= *Pharyngodon*) *extenuatus* (reported by Hannum, 1941), *Spauligodon* (= *Pharyngodon*) *oxkutzcabiensis* (reported by Benes, 1985), and *Macracis* sp. and *Ozolaimus* sp. (reported by Benes, 1985) from the Arizona helminth list. Ten species of *Alaeuris* have been reported from coastal environments of North America (California, Florida, and Georgia) (Baker, 1987), but there are no other reports of *Alaeuris* sp. from Arizona and no reports of another species of *Cnemidophorus* infected by a species of *Alaeuris*. We prefer to think of Benes' (1985) report of *Alaeuris*

sp. in terms of "an oxyurid species." *Spauligodon* (= *Pharyngodon*) *extenuatus* and *S.* (= *P.*) *oxkutzcabiensis* are unknown from North America (Baker, 1987), *S. extenuatus* is known only from Spain, Italy, and North Africa, and *S. oxkutzcabiensis* is known only from Central America. We prefer to think of these nematodes as *Pharyngodon* sp. The genus *Macracis* was synonymized with the genus *Ozolaimus* by Inglis et al. (1960) and *Ozolaimus* is unknown from mainland North America (Baker, 1987). Again, we refer to the report of this genus as "an oxyurid species." We believe the *Oochoristica* sp. reported by Benes (1985) in *Phrynosoma solare* to be *Diochetos phrynosomatis* and have recorded it as such.

Tetrathyridia of *Mesocestoides* sp. have previously been reported from 35 species of lizards from 9 states and Mexico (Goldberg and Bursey, 1990c; McAllister, 1991; McAllister et al., 1991a, b). Of the 44 species of lizards found in Arizona, tetrathyridia of *Mesocestoides* sp. have been reported previously from 7 species (2 teiid and 5 phrynosomatid). This report brings the total number of Arizona lizard species infected to 9, 2 teiid and 7 phrynosomatid. Tetrathyridia are most commonly seen in the body cavity, although they may invade the viscera (Goldberg, 1985) as well as skeletal muscle (Goldberg et al., 1993d). The life cycle of species in the genus *Mesocestoides* is incompletely known, but adults are intestinal parasites of carnivorous birds and mammals. The first intermediate host is an arthropod, probably an insect (Webster, 1949).

Table 2. Helminths recovered from Arizona lizards.

Host Helminth	Prevalence	Mean intensity (range)	Site	Reference
<i>Callisaurus draconoides ventralis</i>				
<i>Atractis</i> sp.	50% (1/2)	71 —	Large intestine	Benes, 1985
<i>Cnemidophorus</i> sp.				
<i>Pharyngodon papillocauda</i>	—	— —	—	Hannum, 1941
<i>Cnemidophorus burti stictogrammus</i>				
<i>Mesocestoides</i> sp.	2% (1/57)	— —	Liver, ovary, mesenteries	Goldberg, 1987
<i>Oochoristica bivitellobata</i>	2% (1/57)	1 —	Small intestine	Goldberg and Bursey, 1989a
<i>Physaloptera retusa</i>	14% (8/57)	2 (1–5)	Stomach	Goldberg and Bursey, 1989a
<i>Pharyngodon cnemidophori</i>	5% (3/57)	26 (18–35)	Large intestine	Goldberg and Bursey, 1989a
<i>Thubunaea cnemidophorus</i>	4% (2/57)	2 (1–3)	Stomach	Goldberg and Bursey, 1989a
<i>Skrjabinoptera phrynosoma</i>	2% (1/57)	1 —	Stomach	Goldberg and Bursey, 1989a
<i>Cnemidophorus inornatus arizonae</i>				
<i>Oochoristica bivitellobata</i>	13% (10/78)	2 (1–4)	Small intestine	Goldberg and Bursey, 1990b
<i>Pharyngodon warneri</i>	23% (18/78)	15 (1–73)	Large intestine	Goldberg and Bursey, 1990b
<i>Physaloptera</i> sp. (larvae)	1% (1/78)	2 —	Stomach	Goldberg and Bursey, 1990b
<i>Cnemidophorus sonora</i>				
<i>Oochoristica bivitellobata</i>	7% (1/14)	5 —	Small intestine	McAllister, 1992
<i>Physaloptera</i> sp. (larvae)	29% (4/14)	1 (1–3)	Stomach	McAllister, 1992
Acanthocephalan larva	7% (1/14)	1 —	Coelom	McAllister, 1992
<i>Cnemidophorus tigris</i>				
<i>Mesocestoides</i> sp.	2% (1/50)	2 —	Small intestine	Benes, 1985
<i>Oochoristica</i> sp.	6% (3/50)	— (1–3)	Small intestine	Benes, 1985
<i>Pharyngodon warneri</i>	67% (4/6)	— —	Large intestine	Babero and Matthias, 1967
Oxyurid nematodes	4% (2/50)	29 (7–45)	Small and large intestine	Benes, 1985
Acanthocephalan larvae	2% (1/50)	4 —	Coelom	Benes, 1985
<i>Cnemidophorus uniparens</i>				
<i>Oochoristica bivitellobata</i>	26% (8/31)	2 (1–8)	Small intestine	Goldberg and Bursey, 1990b
Acanthocephalan larva	3% (1/31)	1 —	Stomach	Goldberg and Bursey, 1990b
<i>Coleonyx variegatus</i>				
<i>Oochoristica</i> sp.	8% (4/53)	— (1–3)	Small intestine	Benes, 1985
<i>Pharyngodon</i> sp.	36% (19/53)	— (2–61)	Large intestine	Benes, 1985
	— (2/?)	total of 32	—	Hannum, 1941

Table 2. Continued.

Host Helminth	Prevalence	Mean intensity (range)	Site	Reference
<i>Thubunea</i> sp.	2% (1/53)	2 —	Stomach	Benes, 1985
Spirurid larvae	8% (4/53)	— (1–9)	Stomach and large intestine	Benes, 1985
<i>Cophosaurus texanus scitulus</i>				
<i>Oochoristica</i> sp.	2% (1/53)	1 —	Small intestine	Goldberg and Bursey, 1992a
<i>Oochoristica</i> sp.	— (?/3)	— —	—	Walker and Matthias, 1973
<i>Atractis penneri</i>	— (?/3)	— —	—	Walker and Matthias, 1973
<i>Thubunaea iguanae</i>	8% (4/53)	3 (1–8)	Stomach	Goldberg and Bursey, 1992a
Acanthocephalan larva	2% (1/53)	1 —	Small intestine	Goldberg and Bursey, 1992a
<i>Dipsosaurus dorsalis dorsalis</i>				
<i>Atractis scelopori</i>	100% (2/2)	— many	Large intestine	Benes, 1985
<i>Heloderma suspectum</i>				
<i>Oochoristica whitentoni</i>	2% (2/110)	3 (1–40)	Small intestine	Goldberg and Bursey, 1991b
<i>Oswaldocruzia pipiens</i>	5% (6/110)	20 (1–67)	Small intestine	Goldberg and Bursey, 1991b
<i>Piratuba prolifica</i> (larvae)	100% (1/1)	— —	Liver	Goldberg and Bursey, 1991b
<i>Skrjabinoptera phrynosoma</i>	5% (6/110)	3 (1–8)	Stomach	Goldberg and Bursey, 1991b
<i>Splendidofilaria corophila</i>	— (1/?)	— —	—	Hannum, 1941
<i>Holbrookia maculata</i>				
<i>Oochoristica</i> sp.	— (?/40)	— —	—	Walker and Matthias, 1973
<i>Atractis penneri</i>	65% (11/17)	— —	Large intestine	Gambino and Heyneman, 1960
	— (?/40)	— —	—	Walker and Matthias, 1973
	13% (2/15)	61 (31–91)	Large intestine	Goldberg and Bursey, 1992a
<i>Physaloptera</i> sp. (larvae)	7% (1/15)	4 —	Stomach	Goldberg and Bursey, 1992a
<i>Phrynosoma cornutum</i>				
<i>Diochetos phrynosomatis</i>	71% (5/7)	86 (22–181)	Small and large intestine	Goldberg et al., 1993a
<i>Atractis penneri</i>	14% (1/7)	137 —	Large intestine	Goldberg et al., 1993a
<i>Skrjabinoptera phrynosoma</i>	86% (6/7)	611 (9–1,579)	Stomach	Goldberg et al., 1993a
<i>Phrynosoma douglassii</i>				
<i>Diochetos phrynosomatis</i>	— (?/2)	— —	—	Walker and Matthias, 1973

Table 2. Continued.

Host Helminth	Prevalence	Mean intensity (range)	Site	Reference
<i>Atractis penneri</i>	11% (2/19)	476 (323–636)	Small and large intestine	Goldberg et al., 1993a
<i>Skrijabinoptera phrynosoma</i>	11% (2/19)	47 (34–60)	Stomach and small intestine	Goldberg et al., 1993a
<i>Phrynosoma modestum</i>				
<i>Skrijabinoptera phrynosoma</i>	80% (4/5)	5 (1–13)	Stomach, small intestine, lung	Goldberg et al., 1993a
<i>Phrynosoma platyrhinos</i>				
<i>Atractis penneri</i>	40% (2/5)	511 (396–625)	Large intestine	Goldberg et al., 1993a
<i>Skrijabinoptera phrynosoma</i>	40% (2/5)	8 (6–10)	Stomach	Goldberg et al., 1993a
<i>Phrynosoma solare</i>				
<i>Diochetos phrynosomatis</i>	100% (8/8)	30 (21–70)	Small intestine	Goldberg et al., 1993a
	29% (4/14)	— (63–250)	Small intestine	Benes, 1985
	75% (3/4)	—	Large intestine	Gambino and Heyneman, 1960
<i>Atractis penneri</i>	21% (3/14)	— (58–1,258)	Large intestine	Benes, 1985
	63% (5/8)	1,113 (2–2,364)	Large intestine	Goldberg et al., 1993a
	75% —	—	—	Hannum, 1941
<i>Skrijabinoptera phrynosoma</i>	79% (11/14)	— (1–360+)	Stomach	Benes, 1985
	100% (8/8)	524 (16–1,804)	Stomach and intestines	Goldberg et al., 1993a
<i>Sauromalus obesus obesus</i>				
<i>Atractis scelopori</i>	100% (1/1)	—	Large intestine	Gambino and Heyneman, 1960
<i>Atractis</i> sp.	100% (2/2)	— many	Large intestine	Benes, 1985
<i>Sceloporus clarkii</i>				
<i>Mesocostoides</i> sp.*	5% (1/20)	32 —	Coelom	This study
<i>Oochoristica scelopori</i> *	5% (1/20)	2 —	Small intestine	This study
<i>Atractis penneri</i>	50% (1/2)	—	Large intestine	Gambino and Heyneman, 1960
	35% (7/20)	330 (1–997)	Large intestine	This study
<i>Physaloptera retusa</i> *	55% (11/20)	14 (1–76)	Stomach	This study
<i>Piratuba prolifica</i> *	10% (2/20)	2 (2)	Coelom	This study
<i>Skrijabinoptera phrynosoma</i> *	30% (6/20)	48 (9–94)	Stomach	This study
<i>Spawligodon giganticus</i>	10% (3/20)	3 (2–4)	Large intestine	This study

Table 2. Continued.

Host Helminth	Prevalence	Mean intensity (range)	Site	Reference
<i>Sceloporus jarrovi jarrovi</i>	3% (15/489)	5 (1-22)	Coelom	Goldberg and Bursley, 1990a
<i>Mesocestoides</i> sp.	10% (47/489)	2 (1-10)	Small intestine	Goldberg and Bursley, 1990a
<i>Oochoristica scelopori</i>	3% (1/31)	7 —	Small intestine	Goldberg and Bursley, 1992c
<i>Physaloptera retusa</i>	34% (167/489)	12 (1-271)	Stomach and intestines	Goldberg and Bursley, 1990a
<i>Spauligodon giganticus</i>	94% (459/489)	20 (1-258)	Small and large intestines	Goldberg and Bursley, 1990a
<i>Thubunaea intestinalis</i>	74% (23/31)	—	Stomach and intestines	Goldberg and Bursley, 1992c
<i>Acanthocephalus</i> sp.	3% (16/489)	3 (1-8)	Small intestine	Bursley and Goldberg, 1991b
<i>Sceloporus magister</i>	1% (3/489)	1 —	Small and large intestines	Goldberg and Bursley, 1990a
<i>Mesocestoides</i> sp.	2% (1/52)	124 —	Coelom and liver	Benes, 1985
<i>Oochoristica scelopori</i>	— (0/3)	—	—	Walker and Matthias, 1973
<i>Oochoristica</i> sp.	4% (2/52)	1 —	Small intestine	Benes, 1985
<i>Atractis peneri</i>	— (0/3)	—	—	Walker and Matthias, 1973
<i>Physaloptera retusa</i>	33% (5/15)	1,682 (24-5,048)	Large intestine	This study
	— (0/3)	—	—	Walker and Matthias, 1973
<i>Physaloptera</i> sp. (larvae)	13% (2/15)	2 (1-3)	Stomach	This study
<i>Skrjabinoptera phrynosoma</i>	2% (1/52)	2 —	Stomach	Benes, 1985
	— (1/?)	—	—	Hannum, 1941
<i>Thubunaea</i> sp.	2% (1/52)	2 —	Stomach	Benes, 1985
	2% (5/15)	12 (1-33)	Stomach	This study
	2% (1/52)	2 —	Stomach	Benes, 1985
<i>Sceloporus scalaris slevini</i>	8% (3/38)	39 (8-58)	Coelom	Goldberg and Bursley, 1992b
<i>Mesocestoides</i> sp.	3% (1/38)	6 —	Stomach	Goldberg and Bursley, 1992b
<i>Physaloptera</i> sp.				
<i>Sceloporus undulatus consobrinus</i>				
<i>Oochoristica scelopori</i> *	10% (3/30)	6 (2-1)	Small intestine	This study
<i>Physaloptera retusa</i>	7% (2/30)	2 (2)	Stomach	This study
<i>Sceloporus undulatus trisitchus</i>				
<i>Mesocestoides</i> sp.*	11% (2/18)	399 (203-595)	Coelom	This study
<i>Physaloptera retusa</i>	6% (1/18)	1 —	Stomach	This study
<i>Spauligodon giganticus</i>	22% (4/18)	8 (1-21)	Large intestine	This study

Table 2. Continued.

Host Helminth	Prevalence	Mean intensity (range)	Site	Reference
<i>Sceloporus virgatus</i>				
<i>Atractis penneri</i> *	13% (3/23)	1.18 (106–125)	Large intestine	This study
<i>Physaloptera retusa</i> *	52% (12/23)	3 (1–5)	Stomach	This study
<i>Urosaurus ornatus</i>				
<i>Mesocestoides</i> sp.	1% (1/100)	3 —	Coelom	Benes, 1985
<i>Pharyngodon warneri</i>	— (0/3)	—	—	Walker and Matthias, 1973
<i>Pharyngodon</i> sp.	9% (9/100)	— (1–141)	Large intestine	Benes, 1985
<i>Spauligodon giganteus</i>	20% (3/15)	4 (1–9)	Large intestine	Goldberg et al., 1993d
<i>Uta stansburiana stejnegeri</i>				
<i>Mesocestoides</i> sp.	7% (7/100)	— (5–293+)	Coelom and liver	Benes, 1985
<i>Oochoristica</i> sp.	3% (3/100)	— (1–2)	Small intestine	Benes, 1985
<i>Thubunea</i> sp.	4% (4/100)	— (1–4)	Stomach	Benes, 1985

\* New host record.

Whether lizards are important intermediate hosts or are only paratenic hosts is yet to be determined. McAllister et al. (1992) administered tetrathyridia recovered from *Sceloporus undulatus hyacinthinus* from Arkansas to hamsters and recovered gravid adult *Mesocestoides lineatus*, a common parasite of raccoons. Such studies have not yet been performed on tetrathyridia recovered from Arizona lizards. *Sceloporus clarkii* and *S. undulatus tristichus* are new host records for tetrathyridia of *Mesocestoides* sp. (Table 2).

*Oochoristica scelopori*, 1 of 14 species of *Oochoristica* reported from North America, is known from 9 crotaphytid and phrynosomatid lizards from 5 western states (see Bursey and Goldberg, 1992b). It previously had been reported in only 2 species from Arizona, *S. jarrovi jarrovi* by Goldberg and Bursey (1990a) and *S. magister* by Walker and Matthias (1973). This report brings the total number of species infected in Arizona to 4, all sceloporine lizards. *Oochoristica scelopori* is replaced by *Oochoristica bivitellobata* in some species of *Cnemidophorus* spp. (Goldberg and Bursey, 1990b), by *Oochoristica whitentoni* in *Heloderma suspectum* (Goldberg and Bursey, 1991b), and by *Diochetos phrynosomatis* in lizards of the genus *Phrynosoma* (Goldberg et al., 1993a). The life cycle of *O. scelopori* is unknown. Cysticercoids of *O. osheroffi* were recovered from both coleopteran and orthopteran insects (Widmer and Olsen, 1967). Coleoptera have been implicated as intermediate hosts for *Oochoristica anolis* in *Anolis carolinensis* from southeastern Louisiana (Conn, 1985). Thus, all studies of life cycles of species of *Oochoristica* to date indicate an insect intermediate host. *Sceloporus clarkii* and *S. undulatus consobrinus* are new host records for *O. scelopori* (Table 2).

*Atractis penneri* has been reported from 23 species of carnivorous lizards from North America (Baker, 1987, and Table 2). It is replaced in herbivorous lizards by *A. scelopori*. We recovered *A. penneri* from 4 of the 5 species examined from Arizona. A single *S. graciosus* harboring 286 *A. penneri* in the large intestine was examined from Washington County, Utah, which is adjacent and continuous to our Arizona collection site. Thus, although we did not record *A. penneri* from *S. graciosus* in Arizona, it has been previously reported from 8 other species of lizards from Arizona and had the highest mean intensity (1,682 in *S. magister*) of all the helminths we recovered (Table 2). *Atractis penneri* has been found in *S. graciosus* from Utah (Pearce

and Tanner, 1973) and California (Gambino and Heyneman, 1960). Third-stage larval atractids are known to autoinfect the host (Anderson, 1992). Baer (1951) suggested that these nematodes, which occur in such large numbers and in all stages of development in a single host, are possibly living on the partially digested vegetable matter and should be considered as commensals rather than true parasites. *Sceloporus virgatus* represents a new host record for *A. penneri* (Table 2).

*Physaloptera retusa* has been reported from 12 species of North American lizards (Burse and Goldberg, 1991a). In Arizona, Goldberg and Bursey (1989a, 1990a) previously found *Cnemidophorus burti stictogrammus* and *S. jarrovii jarrovii* to harbor *Ph. retusa*. We recovered it from 4 of the 5 species examined in this study. The highest prevalence in our study (55%) was recorded for *Ph. retusa* in *S. clarkii*. As was the case for *A. penneri*, we found *P. retusa* in 1 *S. graciosus* from Washington County, Utah. *Physaloptera retusa* has previously been reported in *S. graciosus* from Utah and California (Woodbury, 1934; Goldberg and Bursey, 1989b). Although the life cycle has not been studied for *Ph. retusa*, the life cycles of 2 related species (*Ph. hispida* and *Ph. maxillaris*) have been examined in detail (Hobmaier, 1941; Schell, 1952; Lincoln and Anderson, 1975). Insects scavenging fecal material ingest physalopterid eggs, which hatch in their gut and then migrate into body tissue for subsequent development to third-stage larvae, infective to both definitive and paratenic hosts. *Physaloptera retusa* has been shown to cause ulcerative lesions in the stomach of sceloporine lizards (Goldberg and Bursey, 1989b). Records of *Physaloptera* sp. in Table 2 may well represent larvae of *Ph. retusa*; it is apparently incapable of reaching maturity in a number of lizard species (Goldberg et al., 1993c). *Sceloporus clarkii* and *S. virgatus* are new host records for *Ph. retusa* (Table 2).

Smith (1910) described *Filaria mitchelli* from *Heloderma suspectum* in the southwestern United States. Chabaud and Frank (1961) stated that the adult parasites from Smith's (1910) study should be placed in the genus *Piratuba* but the microfilariae in the *H. suspectum* blood belonged to another species. Sonin (1966) synonymized *F. mitchelli* with *Piratuba prolifica*, which has been reported from *Sceloporus mucronatus* from Mexico (Pelaez and Perez-Reyes, 1958) and *H. suspectum* from Arizona (Goldberg and Bursey,

1990d) under the synonym *Piratuba mitchelli*. The intermediate hosts are thought to be ticks (Smith, 1910; Pelaez and Perez-Reyes, 1958). *Sceloporus clarkii* is a new host record for *Pi. prolifica* (Table 2).

*Skrjabinoptera phrynosoma*, the only member of the genus *Skrjabinoptera* reported from North America, has been recovered from 20 lizard species in the United States and Mexico (Goldberg and Bursey, 1991a). Eight lizard species from Arizona have been reported previously to harbor *Sk. phrynosoma*; this report brings the number of infected lizards to 9. It is second only to *A. penneri* in terms of intensity (Table 2). Lee (1957) experimentally showed that the ant *Pogonomyrmex barbatus* served as an intermediate host for *Sk. phrynosoma*. Pearce and Tanner (1973) suggested that several species of ants may serve as intermediate hosts for this nematode. *Sceloporus clarkii* is a new host record for *Sk. phrynosoma* (Table 2).

*Spauligodon giganticus* has been found in 10 lizard species from western North America (Burse and Goldberg, 1992a, and Table 2). *Sceloporus jarrovii*, *S. clarkii*, and *Urosaurus ornatus* are the only Arizona species previously to have been reported to harbor *Sp. giganticus* (Table 2). *Spauligodon giganticus* is thought to have a direct life cycle with infection occurring by fecal contamination of the substrate (Burse and Goldberg, 1992a). Infection in *S. jarrovii* may occur shortly after birth (Goldberg and Bursey, 1992c). The presence of *Sp. giganticus* in lizards may be related to local climatic conditions. *Spauligodon giganticus* was present in *S. undulatus tristichus* but absent in *S. undulatus consobrinus*. The *S. undulatus tristichus* population was situated in a mesic environment on the top of the Santa Catalina Mountains (ca. 2,438 m) and was separated from the drier foothills population of *Sceloporus undulatus consobrinus* (ca. 1,280 m). Furthermore, Goldberg et al. (1993d) found higher prevalences of *Sp. giganticus* in New Mexico *Urosaurus ornatus* from a more mesic habitat as opposed to *U. ornatus* from a drier habitat. Similarly, Goldberg et al. (1993b) found *Sp. giganticus* present in New Mexico *Sceloporus poinsettii* but absent in a Texas population from a drier habitat. Finally, it is noteworthy that *Sp. giganticus* has not been found in *S. magister*, which occurs in xeric habitats. These observations suggest that moisture may be a limiting factor in *Sp. giganticus* distribution.

Nine of the 20 helminth species reported to

occur in Arizona lizards are harbored by sceloporine lizards. Two of these, *A. penneri* and *Sp. giganticus*, have direct life cycles, and infection may be gained through contact with contaminated substrate. Lizard population density may be important in determining infection intensities. The remaining 7 helminth species require an arthropod intermediate host. Diet may be most important in determining infection intensities for these species.

*Sceloporus clarkii* in Arizona harbors 7 species of helminths, *S. magister* 7, *S. jarrovi* 6, *S. undulatus* 4, *S. scalaris* 2, and *S. virgatus* 2 (Table 2). Thus, the sceloporine lizards with the largest body sizes (*S. magister*, *S. clarkii*, and *S. jarrovi*) contain the most diverse helminth faunas, whereas the smaller lizards (*S. scalaris* and *S. virgatus*) have the least. Whether or not having a larger digestive tract supports a more diverse helminth fauna is a question that warrents further investigation.

#### Acknowledgments

We thank the following for allowing us to examine specimens from their respective institutions: Robert L. Bezy and John W. Wright (LACM), Scott Cutler (MNA), and Jack W. Sites, Jr. (BYU).

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- Appendix**
- Museum Accession Numbers, Locality Data, and USNM Helminthological Collection Numbers**
- Sceloporus clarkii*: Gila County, Arizona: LACM 139987, 33°36'N, 111°12'W, 609 m elevation. Mohave County, Arizona: LACM 139986, 35°26'N, 113°38'W, 1,219 m elevation. Pima County, Arizona: LACM 140101–140102, 31°95'N, 111°59'W, 1,884 m elevation; LACM 140092–140094, 140098, 140100, 140103–140104, 32°20'N, 110°49'W, 883 m elevation. Pinal County, Arizona: LACM 140095–140097, 32°30'N, 110°43'W, 1,417 m elevation; LACM 140099, 32°51'N, 111°27'W, 1,219 m elevation. Yavapai County, Arizona: MNA 1762, 1767, 1770, 1772–1773, 34°40'N, 111°46'W, 1,169 m elevation. USNM Helm. Coll. Nos.: *Mesocoestoides* sp., 82711; *Oochoristica scelopori*, 82712; *Atractis penneri*, 82713; *Physaloptera retusa*, 82715; *Piratuba prolifica*, 82714; *Skrjabinoptera phrynosoma*, 82716; *Spauligodon giganticus*, 82717.
- S. graciosus*: Coconino County, Arizona: BYU 37876–37888, 36°06'N, 111°20'W, 1,383 m elevation. Navajo County, Arizona: LACM 95730–95735, 97551, 36°56'N, 110°26'W, 1,859 m elevation. Washington County, Utah: BYU 37388, 37°13'N, 112°57'W, 1,473 m elevation. USNM Helm. Coll. Nos.: *Atractis penneri*, 82718; *Physaloptera retusa*, 82719.
- S. magister*: Gila County, Arizona: LACM 139993, 33°36'N, 111°12'W, 609 m elevation. Mohave County, Arizona: LACM 139990, 34°32'N, 113°44'W, 827 m elevation; LACM 139992, 35°06'N, 114°06'W, 846 m elevation. Pima County, Arizona: LACM 140112–140114, 140117, 32°20'N, 110°49'W, 907 m elevation; LACM 140118, 32°02'N, 111°32'W, 944 m elevation; LACM 140110–140111, 140115–140116, 32°15'N, 110°05'W, 731 m elevation. Pinal County, Arizona: LACM 140119, 32°36'N, 110°51'W, 1,158 m elevation. Yavapai County, Arizona: LACM 139988–139989, 34°36'N, 113°08'W, 1,248 m elevation. USNM Helm. Coll. Nos.: *Atractis penneri*, 82720; *Physaloptera retusa*, 82721; *Skrjabinoptera phrynosoma*, 82722.
- S. undulatus consobrinus*: Pinal County, Arizona: LACM 140120–140149, 32°36'N, 110°51'W, 1,158 m elevation. USNM Helm. Coll. Nos.: *Oochoristica scelopori*, 82723; *Physaloptera retusa*, 82724.
- S. undulatus tristichus*: Pima County, Arizona: LACM 140150–140167, 32°26'N, 110°45'W, 2,438 m elevation. USNM Helm. Coll. Nos.: *Mesocoestoides* sp., 82725; *Physaloptera retusa*, 82726; *Spauligodon giganticus*, 82727.
- S. virgatus*: Cochise County, Arizona: LACM 140168–140190, 31°58'N, 109°22'W, 1,700 m elevation. USNM Helm. Coll. Nos.: *Atractis penneri*, 82728; *Physaloptera retusa*, 82729.

## Parasitic Helminths and Arthropods of Fulvous Whistling-Ducks (*Dendrocygna bicolor*) in Southern Florida

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**ABSTRACT:** Thirty fulvous whistling-ducks (*Dendrocygna bicolor*) collected during 1984-1985 from the Everglades Agricultural Area of southern Florida were examined for parasites. Twenty-eight species were identified and included 8 trematodes, 6 cestodes, 1 nematode, 4 chewing lice, and 9 mites. All parasites except the 4 species of lice and 1 of the mites are new host records for fulvous whistling-ducks. None of the ducks were infected with blood parasites. Every duck was infected with at least 2 species of helminths (mean 4.2; range 2-8 species). The most common helminths were the trematodes *Echinostoma trivolvis* and *Typhlocoelum cucumerinum* and 2 undescribed cestodes of the genus *Diorchis*, which occurred in prevalences of 67, 63, 50, and 50%, respectively. Only 1 duck was free of parasitic arthropods; each of the other 29 ducks was infested with at least 3 species of arthropods (mean 5.3; range 3-9 species). The most common arthropods included an undescribed feather mite (*Ingrassia* sp.) and the chewing louse *Holomenopon leucoxanthum*, both of which occurred in 97% of the ducks.

**KEY WORDS:** fulvous whistling-duck, *Dendrocygna bicolor*, trematodes, cestodes, nematodes, chewing lice, mites, survey, prevalence, southern Florida.

Fulvous whistling-ducks, *Dendrocygna bicolor* (Vieillot), occur in much of the Western Hemisphere, but they have been documented as regular winter visitors in Florida only since 1955 (Jones, 1966). The first breeding record of this species in Florida was in 1965 at Lake Okeechobee in southern Florida (Ogden and Stevenson, 1965). Turnbull et al. (1989a) reported that it was nesting regularly in 1988 and increasing in numbers in the Everglades Agricultural Area (Palm Beach County). Other than one report on pesticide residues (Turnbull et al., 1989b), there is no published information on the diseases and parasites of this newly established resident anatid in Florida. Herein we report on the parasitic helminths and arthropods collected from a sample of fulvous whistling-ducks in southern Florida.

### Materials and Methods

Fulvous whistling-ducks were obtained from the Everglades Agricultural Area in southern Florida south and east of Lake Okeechobee (Palm Beach County).

The primary crops in this area were sugarcane, row crops, sod, and, to a lesser extent, rice. Turnbull et al. (1989b) described the collection site in detail.

Nine ducklings (5 males and 4 females), which died during a banding operation, were obtained on 28 August 1984. An additional 21 ducks were collected by shotgun on 15 November 1985 and consisted of 7 hatch-year and 2 adult males, 7 hatch-year and 3 adult females, and 2 ducks of unknown age and gender. Carcasses were frozen within 4 hr of collection and later thawed and examined at necropsy. Techniques for recovering, fixing, staining, and examining parasites followed Forrester et al. (1974). Thin blood films were prepared from the 21 ducks collected in 1985, and these were fixed in absolute methanol, stained with Giemsa, and examined microscopically for blood parasites. Representative specimens have been deposited as follows: helminths in the U.S. National Parasite Collection (Beltsville, Maryland), mites in the Parasite Collection of the USDA, National Veterinary Services Laboratories (Ames, Iowa), and chewing lice in the Arthropod Collection of the University of Minnesota (St. Paul, Minnesota).

### Results and Discussion

Fifteen species of helminths were collected from the 30 ducks, none of which were free of helminths. These included 8 species of trematodes, 6 cestodes, and 1 nematode. All are new host records for fulvous whistling-ducks. Sites, prev-

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alences, and intensities of each of the species are given in Table 1. Every duck was infected with at least 2 species of helminths (mean 4.2; range 2–8 species). In the 30-duck sample, multiple infections were as follows: 3 ducks had 2 species of helminths, 5 had 3 species, 9 had 4 species, 8 had 5 species, 2 had 6 species, 2 had 7 species, and 1 had 8 species. The overall mean intensity was 20.1 (range 3–83). A total of 604 helminth specimens was collected.

Two unidentified species of *Diorchis* were found in the small intestine, including a small form with hooks 20–22  $\mu\text{m}$  long and strobila up to 36 mm in length and a larger form with hooks 38–40  $\mu\text{m}$  long and strobila up to 75 mm in length. No scoleces were found for an unidentified species of *Sobolevicanthus*, which had a cirrus sac up to 240  $\mu\text{m}$  long and a prominent accessory sac. All 3 of these species are probably undescribed (J. D. McLaughlin, pers. comm.). An undescribed species of *Sobolevicanthus* was reported from the black-bellied whistling-duck, *Dendrocygna autumnalis* (Linnaeus), in Texas by George and Bolen (1975), but no specimens could be obtained for comparison in our study.

Four of the helminths (*Apatemon gracilis*, *Typhlocoelum cucumerinum*, *Echinostoma trivolvis*, and *Cloacotaenia megalops*) were considered to be characteristic species of waterfowl by McDonald (1969) and, except for *C. megalops*, have been found in 2 other anatids that breed in Florida, the wood duck, *Aix sponsa* (Linnaeus), and the mottled duck, *Anas fulvigula* Ridgway (Kinsella and Forrester, 1972; Thul et al., 1985). In comparison to the mottled duck and the wood duck in Florida, the fulvous whistling-duck has less species richness and lower overall prevalences and intensities of infection. The most striking difference is in the nematode fauna, in which there is only 1 species in the whistling-duck versus 10 in the wood duck and 14 in the mottled duck. The explanation may be found in the habitat and diet of the whistling-duck in the rice fields of southern Florida (Turnbull et al., 1989a). Forrester et al. (1987) found that the helminth communities of round-tailed muskrats (*Neofiber alleni* True) in monocultural sugarcane fields in southern Florida were reduced significantly in comparison to muskrats from natural habitats. Similarly, the rice field monoculture may have resulted in the elimination of intermediate hosts of the heteroxenous nematodes that are common in ducks. George and Bolen (1975) reported 14 species of helminths from the black-bellied whis-

ling-duck, the only other species of the subfamily found in North America. Surprisingly, the 2 hosts have only 2 trematodes and 2 cestodes in common, assuming that the unidentified species of *Sobolevicanthus* is the same.

A study of the helminth fauna of fulvous whistling-ducks in Florida might provide an opportunity to test the hypothesis of Brown (1984) concerning the relationship of species abundance and distribution. Fedynich et al. (1986) and Radomski et al. (1991) have applied Brown's concept to helminth communities in beaver (*Castor canadensis* Kuhl) and long-nosed armadillos (*Dasyurus novemcinctus* Linnaeus), respectively, and proposed that the helminths of hosts at the periphery of their range are less diverse and abundant than those at the epicenter of the host's origin. At the present time, this hypothesis cannot be tested for fulvous whistling-ducks because there are no comparative data on the helminth fauna of this species of duck in other parts of its range. However, the almost total lack of nematodes in the fauna of fulvous whistling-ducks examined in the present study may be an indication that such a phenomenon has occurred in this duck since it invaded southern Florida in the early 1950s, but proof of this idea is not currently available.

Thirteen species of parasitic arthropods were collected, including 7 species of feather mites, 1 skin mite, 1 quill mite, and 4 chewing lice. All are new host records except 1 of the feather mites and the 4 chewing lice. Prevalences and total numbers of each species collected are presented in Table 2. Only 1 duck was free of parasitic arthropods. Each of the other 29 ducks was infested by at least 3 species of arthropod parasites (mean 5.3; range 3–9 species). In the 29-duck sample, multiple infestations were as follows: 5 ducks had 3 species of arthropods, 7 had 4 species, 6 had 5 species, 3 had 6 species, 3 had 7 species, 3 had 8 species, and 2 had 9 species. A total of 1,832 specimens of arthropods was collected and identified, but intensities could not be calculated because quantitative techniques were not used to obtain every parasitic arthropod from each host as they were for the parasitic helminths.

All of the mites collected from fulvous whistling-ducks were sarcoptiform mites and have been found previously on ducks in general, with 2 exceptions (Gaud, 1982). The 2 exceptions are 1 specimen each of *Scutomegninia* sp. and *Paralges* sp. These are probably stray contaminants

**Table 1. Parasitic helminths of 30 fulvous whistling-ducks from southern Florida, 1984-1985.**

Species of helminth	USNM Accession No.	No. ducks		No. worms/infected duck	
		Infected	%	Mean	Range
<b>Trematodes</b>					
<i>Echinostoma trivolvis</i> (Cort, 1914) (4)*	82888	20	67	4.8	1-31
<i>Typhlocoelum cucumerinum</i> (Rudolphi, 1809) (1)	82887	19	63	9.8	1-31
<i>Cotylurus gallinulae</i> (Lutz, 1928) (3)	82885	12	40	3.1	1-7
<i>Echinoparyphium</i> sp. (3)	82889	11	37	2.5	1-10
<i>Prosthogonimus ovatus</i> (Rudolphi, 1803) (6)	82892	7	23	3.4	1-10
<i>Tanaisia fedtschenkoi</i> Skrjabin, 1924 (7)	82891	3	10	8.3	1-22
<i>Philophthalmus gralli</i> Mathis and Leger, 1910 (2)	82890	3	10	2.7	1-5
<i>Apatemon gracilis</i> (Rudolphi, 1819) (3)	82886	1	3	1.0	1
<b>Cestodes</b>					
<i>Diorchis</i> sp. I (3)	82896	15	50	3.4	1-20
<i>Diorchis</i> sp. II (3)	82897	15	50	5.3	1-20
<i>Hymenolepis teresoides</i> Fuhrmann, 1906 (3)	82893	10	33	3.3	1-15
<i>Cloacotaenia megalops</i> (Nitzsch, 1829) (6)	82895	9	30	1.9	1-4
<i>Sobolevicanthus</i> sp. (3)	82898	7	23	4.4	1-15
<i>Hymenolepis hopkinsi</i> Schiller, 1951 (5)	82894	4	13	3.5	1-6
<b>Nematodes</b>					
<i>Strongyloides</i> sp. (3)	82899	3	10	2.3	1-4

\* Numbers in parentheses indicate locations in host: (1) trachea/air sacs, (2) eye, (3) small intestine, (4) large intestine, (5) caeca, (6) cloaca, and (7) kidney.

from some sympatric aquatic birds. *Scutomegninia* spp. (family Avenzoariidae) usually occur on cormorants, gannets, ibises, or petrels. *Paralges* spp. (family Dermoglyphidae) have been reported from ostriches and godwits, but information on quill mites is so meager that this could be a true but unknown mite of ducks. Dermoglyphid mites live inside the quills of their hosts. One other unusual feature of the mite assemblage taken from the fulvous whistling-ducks in Florida is the absence of any representatives of the genus *Bdellorhynchus* (family Avenzoariidae), all of which are duck parasites, or of the family Freyanidae, 7 genera of which occur on ducks (Gaud, 1982); the fulvous whistling-duck has been documented by Dubinin (1950) as a host for *Freyana dendrocygni* Dubinin, 1950.

Very little taxonomic work has been done on the sarcoptiform mite fauna of North American birds; therefore, comments on the mites found in the present survey can be made only in relation

to knowledge of extralimital taxa. The most common mite on fulvous whistling-ducks was *Ingrassia* sp. (family Xolalgidae). About 24 species in this genus are described from shorebirds, sea birds, grebes, and ducks (Gaud and Atyeo, 1981). Of the 2 duck mites, the present specimens most closely resemble *I. (Vingrassia) velata* (Megnin, 1877), found on domestic ducks, mallards, and many other species, but not previously on any species of *Dengrocygna*. Species of *Brephosceles* (family Alloptidae) are typically feather mites of aquatic birds, including some from ducks (Peterson, 1971); the 2 species on fulvous whistling-ducks have not been described. Members of the mite family Dermationidae are found on the skin of birds in at least 8 avian orders, but only species of *Dermation* (*Neodermtion*) occur on ducks (Fain, 1965). The species *Dermation* (*N.*) *anatum* Fain is known from many species of ducks, including the white-faced tree-duck, *Dendrocygna viduata* (Linnaeus), of Central Africa, but the

**Table 2. Parasitic arthropods of 30 fulvous whistling-ducks from southern Florida, 1984–1985.**

Species of arthropod	No. ducks		Total number collected
	Infected	%	
Feather mites			
<i>Ingrassia (Vingrassia) sp.</i> near <i>velata</i> *	29	97	1,288
<i>Brephosceles sp. I</i> *	12	40	74
<i>Zygochelifer edentulus</i> *			
Atyeo, 1984	11	37	31
<i>Brephosceles sp. II</i> *	6	20	11
<i>Alloptoides sp.</i> *	5	17	8
<i>Rectijanua striata</i>			
Atyeo and Peterson, 1976	4	13	8
<i>Scutomegnina sp.</i> *	1	3	1
Skin mites			
<i>Dermation (Neodermaton) sp.</i> *	13	43	43
Quill mites			
<i>Paralges sp.</i> *	1	3	1
Chewing lice			
<i>Holomenopon leucoxanthum</i> (Burmeister, 1938)	29	97	248
<i>Acidoproctus rostratus</i> (Rudow, 1866)	23	77	54
<i>Anatoecus icterodes</i> (Nitzsch, 1818)	19	63	64
<i>Trinoton aculeatum</i> Piaget, 1885	1	3	1

\* New host records.

mites from *D. bicolor* are different and not described. All known species of *Zygochelifer* (family Avenzoariidae) come from ducks (Atyeo, 1984). *Zygochelifer edentulus* has been found previously on only 2 species of ducks in Central Africa, including *D. viduata* in Burundi. The known species of *Alloptoides* (family Alloptidae) have been collected from ducks, but this genus is poorly understood, and species cannot be identified without male specimens; no males were found on *D. bicolor*, and no species of *Alloptoides* have been recorded previously from any of the species of *Dendrocygna*. *Rectijanua striata* (family Rectijanuidae) is the only mite found in the present study that has been taken previously from *D. bicolor*, and all known members of this mite family are duck parasites (Atyeo and Peterson, 1976).

Two of the 4 species of chewing lice taken from the fulvous whistling-ducks appear to be restricted in their host distribution, and 2 have a very broad distribution. *Acidoproctus rostratus* is known only from *Dendrocygna bicolor* and *D. viduata*, whereas *Trinoton aculeatum* has been

recorded from only *D. bicolor*, *D. arborea* (Linnaeus) (the West Indian tree-duck), *D. autumnalis*, and *D. viduata*. Contrasted to these, *Holomenopon leucoxanthum* has been identified from over 35 species of Anatidae, including 11 species of *Anas*, 8 of *Aythya*, and 5 of *Dendrocygna*, whereas *Anatoecus icterodes* has been recorded from even more hosts, having been identified from over 60 species of Anatidae. Price (1971) provided the majority of the records for the former and Kéler (1960) reviewed many of the records for the latter; undoubtedly both of these lice will prove to have a much broader distribution among the Anatidae than known to date.

None of the blood films were positive for hematozoan parasites. This is in keeping with previous studies on waterfowl that breed in Florida, that is, Canada geese (*Branta canadensis* Linnaeus), mottled ducks, and wood ducks (Thul et al., 1980). The appropriate vectors necessary for transmission of some of the hematozoans of waterfowl may be absent from Florida; in addition, the peak populations of vectors that are present in Florida may not occur at the same time of

year (i.e., winter) when infected migratory waterfowl are present and have parasitemias high enough to allow transmission to occur.

#### Acknowledgments

We thank F. Montalbano III and D. H. Brakhage for assistance in collecting the ducks and J. H. Bogue, S. E. Ross, and G. W. Foster for technical laboratory assistance. We are also grateful to W. T. Atyeo for confirmation of the feather mite identifications and to J. D. McLaughlin for his comments and advice on the taxonomy of several of the species of tapeworms. We also thank E. C. Greiner and M. D. Young who reviewed an early draft of the manuscript and offered some useful suggestions for its improvement. This is Florida Agricultural Experiment Stations Journal Series No. R-03242.

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## Coccidia, *Giardia* sp., and a Physalopteran Nematode Parasite from Black-footed Ferrets (*Mustela nigripes*) in Wyoming

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**ABSTRACT:** Oocysts of 3 species of *Eimeria* were found in feces and intestinal contents of free-ranging and captive black-footed ferrets (*Mustela nigripes*) from Wyoming. Oocysts, meronts, and gamonts of 2 of the intestinal coccidia; meronts and oocysts of an unidentified coccidian in the respiratory tract; and merozoites of an unidentified coccidian from the wall of the urinary bladder were seen histologically or in impression smears. Based on oocyst morphometry, 2 of the intestinal coccidia were identified as *E. ictidea* and *E. furonis*. The third intestinal coccidian and the respiratory and urinary bladder forms were not identified. Other parasites observed included *Giardia* sp. and *Physaloptera* sp. All parasites constitute new host records. The coccidia also represent new distribution records and the developmental stage descriptions are previously unreported in black-footed ferrets.

**KEY WORDS:** Black-footed ferret, *Mustela nigripes*, coccidia, *Eimeria ictidea*, *Eimeria furonis*, *Giardia* sp., *Physaloptera* sp.

The black-footed ferret (*Mustela nigripes*), once thought to be extinct, was rediscovered near Meeteetse, Wyoming in 1981. The colony occupied a white-tailed prairie dog (*Cynomys leucurus*) town and was intensively studied over the next few years (Forrest et al., 1988). In the autumn of 1985, 6 animals (2 adult and 1 juvenile males, 1 adult and 2 juvenile females) were trapped to establish a captive breeding colony (Thorne and Williams, 1988). When captured, 2 of 6 animals were incubating canine distemper, a viral disease invariably fatal in this species. Subsequently, all of these animals died of canine distemper (Williams et al., 1988). Canine distemper, later found to be widespread in the free-ranging black-footed ferret population, was a major factor in making carcasses and excretory products available for examination and prompted a trapping operation that was completed in the spring of 1987.

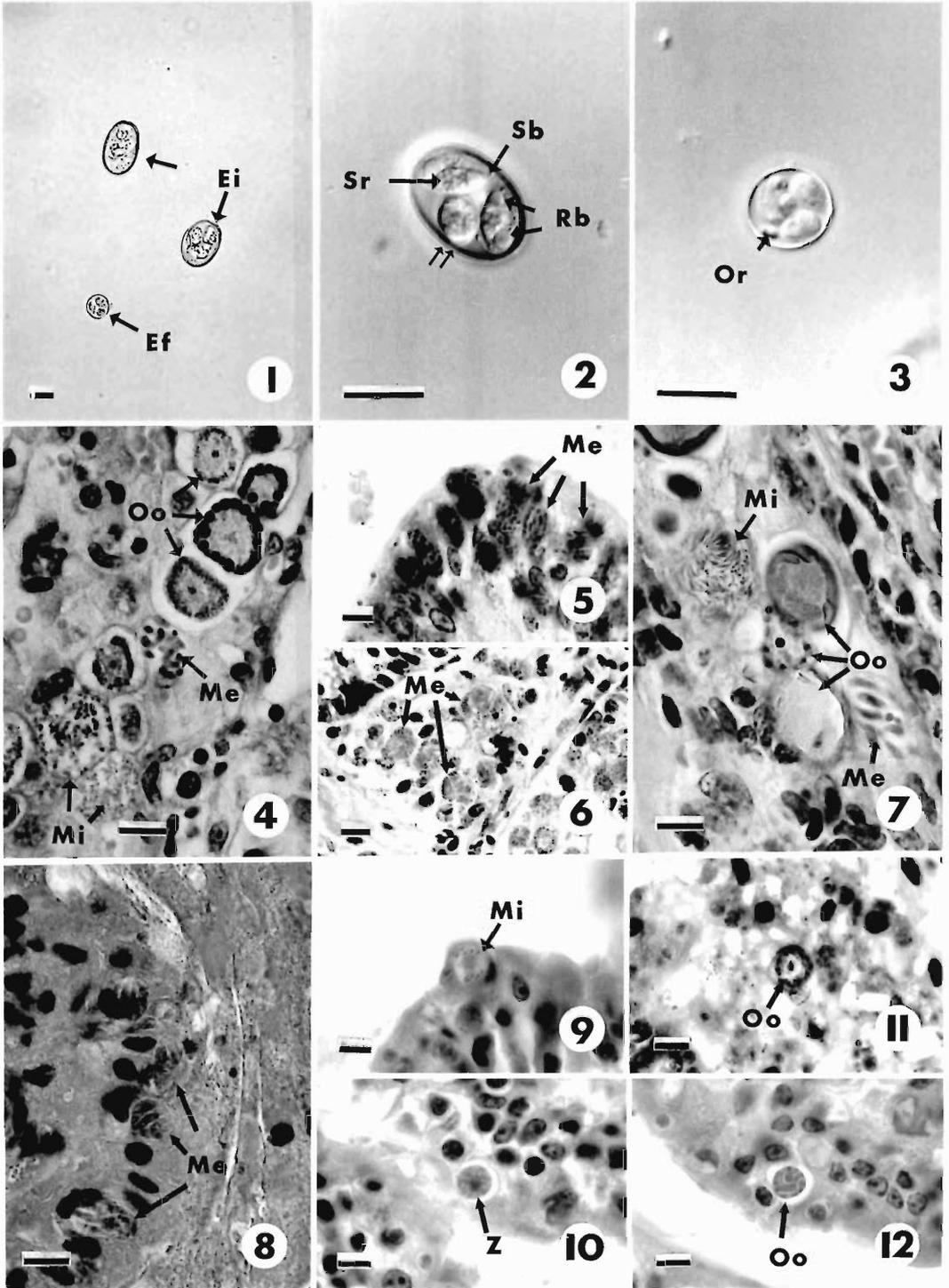
Parasites have not been studied extensively in black-footed ferrets. Most attention has been on the ectoparasites: *Ixodes kingi* Bishopp, 1911; *I. sculptus* Neumann, 1904; and *Otodectes* sp. (Boddicker, 1968; Carpenter and Hillman, 1979; Schroeder, 1983). Other ectoparasites reported include: *Oropsylla* (*Opisocrostitis*) *hirsuta* Baker, 1895 and *O. idahoensis* Baker, 1904; *Rhadinopsylla* (= *Rectofrontia*) *fraterna* Baker, 1895 and *Nearctopsylla brooksi* Rothschild, 1904; and unidentified ticks and fleas. *Molineus mustelae* Schmidt, 1965 and *Taenia* sp. have also been reported (Boddicker, 1968; Carpenter and Hill-

man, 1979; Schroeder, 1983). The cestode has since been identified through life history and structure as *T. mustelae* Gmelin, 1790 (Rockett et al., 1990). Unidentified coccidia were reported from 2 groups of captive black-footed ferrets (Carpenter and Hillman, 1979; Williams et al., 1988). Sarcocysts were found in skeletal muscle of several free-ranging black-footed ferrets (Schroeder, 1983; Williams et al., 1988).

The present report documents the occurrence of coccidia, a flagellate protozoan parasite (*Giardia* sp.) and a nematode (*Physaloptera* sp.), recovered from free-ranging and/or captive black-footed ferrets.

### Materials and Methods

Feces from free-ranging black-footed ferrets were collected from holding cages following capture in 1982, 1984, and 1985 and from captive ferrets when cages were cleaned. Feces were macerated and held in 2% potassium dichromate solution at room temperature (22°C) and aerated to promote sporulation of oocysts. Samples were examined initially on arrival and every 24 hr thereafter by brightfield and/or phase-contrast microscopy of direct wet preparations or after flotation with a saturated sucrose solution (specific gravity 1.12). Sporulation was considered complete when at least 75% of oocysts contained sporocysts and sporozoites by microscopic examination. Complete necropsies were conducted on black-footed ferrets found dead in the field or those that died in captivity. Tissues were fixed in 10% buffered formalin and processed for histologic examination. Gastrointestinal tracts of free-ranging animals were examined for metazoan parasites. Measurements of coccidia and *Giardia* cysts were made with a calibrated filar micrometer.



Figures 1-12. Photomicrographs of sporulated oocysts and endogenous developmental stages of *Eimeria ictidea* and *E. furonis* from black-footed ferrets. 1. Two oocysts of *E. ictidea* (Ei) and 1 of *E. furonis* (Ef) showing relative sizes. 2. Oocyst of *E. ictidea* with double layer wall (double arrows), sporocysts showing residuum (Sr) and Stieda body (Sb), and sporozoites with refractile bodies (Rb). Oocyst polar body is not shown. 3. *Eimeria furonis* oocyst

**Table 1. Measurements in micrometers of coccidial oocysts recovered from the feces of black-footed ferrets.**

Form	Length (range)	Width (range)	L/W ratio
Large oval (N = 5)	37.0* ± 1.3 (35.0–38.6)	22.3* ± 2.3 (21.2–23.2)	1.66:1
Small oval (N = 64)	23.2* ± 2.3 (18.2–27.4)	15.5* ± 1.0 (13.0–16.2)	1.50:1
Spherical-sub-spherical (N = 60)	12.6* ± 1.2 (10.8–15.2)	11.9* ± 0.9 (10.1–12.9)	1.06:1

\* ± 1 SD.

### Results

*Eimeria* spp. were encountered from field-collected black-footed ferret feces and from feces from all 6 captive black-footed ferrets with canine distemper. All passed medium-sized oval oocysts, 3 also passed smaller, spherical or sub-spherical oocysts, and a larger oval oocyst was occasionally seen. These large oocysts were seen again in a different black-footed ferret in 1991. Measurements of coccidial oocysts are listed for comparison in Table 1.

The medium oval form (Figs. 1, 2) sporulated in no less than 48, nor more than 72 hr, forming the 4 dizoic sporocysts typical of eimerian oocysts. Oocyst walls were bilaminar, without a micropyle; a polar body/granule was formed, but no residuum was seen. The elongate sporocysts possessed a Stieda body and a fine granular sporocyst residuum. The sporozoites lay in opposing directions, anterior-to-posterior, in the sporocysts, with a prominent refractile body near each posterior end. Often, sporocysts lay in pairs, with one at a right angle to the other pair. The morphometric and structural features are compatible with the species *Eimeria ictidea* Hoare, 1927 described from the domestic ferret (*M. putorius furo*) from England (Hoare, 1927).

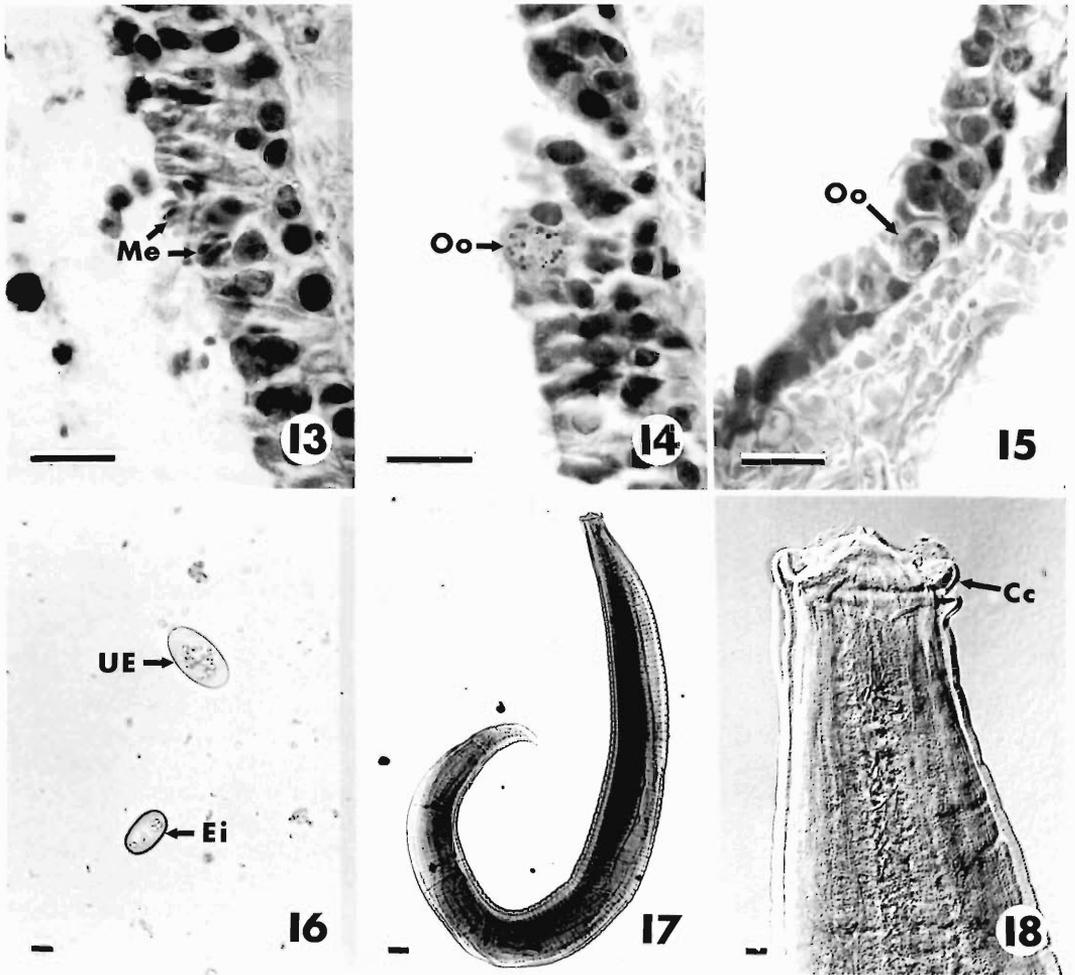
The small spherical to subspherical form (Figs. 1, 3) sporulated in no less than 48, nor more than

72 hr, producing typical eimerian oocysts, with a pink double-layered wall and a coarse, sparse granular residuum; no micropyle or polar granule were seen. Elongate sporocysts with a Stieda body contained sporozoites with refractile bodies. Dimensions and internal structure of sporulated oocysts are consistent with the description of *Eimeria furonis* Hoare, 1927 reported from the domestic ferret in England and mink (*M. vison*) in Kazakhstan (Nukerbaeva and Svanbaev, 1973, cited by Levine and Ivens, 1981).

Merogony and gamogony of *E. ictidea* and *E. furonis* were seen in villar epithelial cells throughout the small intestine, but were most prevalent in the jejunum. Two morphologic types of meronts were seen in intestinal sections infected predominantly with *E. ictidea*; one was commonly seen near the bases of the villi and rarely in the crypts, whereas the other occurred at or near the tips. Merozoites formed by ectopolygenic merogony (Levine, 1985) were visible in the meronts near the bases of the villi (Fig. 6). The meronts seen in more apical regions of the villi contained larger merozoites and lacked an undifferentiated mass (Figs. 4, 5, 7). Sequential specimens were not available to allow determination of merogonic generations.

*Eimeria ictidea* gamogony also occurred throughout the small intestine, mainly in epithelial cells in the apical half of villi. Microga-

←  
with indistinct sporocysts and oocyst residual granules (Or). Figures 4–7. Endogenous stages of *E. ictidea*. 4. Oocysts in various stages of development with wall-forming bodies (Oo), a meront with fully formed merozoites (Me), and microgamonts in development (Mi). 5. Meronts in cells at the tip of jejunal villus, with merozoites formed by fission (Me). 6. Meronts in cells at the base of jejunal villus, with merozoites formed by ectopolygeny (Me). 7. Oocysts, 2 fully formed, 1 of which shows finger-like interdigitations resulting from fusion of wall-forming bodies; central oocyst, still developing, shows wall-forming bodies (Oo). Meront (Me) shows fully formed merozoites. Microgamont (Mi) shows fully formed microgametes. Figures 8–12. Endogenous stages of *E. furonis*. 8. Meronts with fully formed merozoites (Me). 9. A microgamont with forming microgametes (Mi). 10. A macrogamont/zygote prior to formation of wall-forming bodies (Z). 11. A developing oocyst with wall-forming bodies (Oo). 12. A developing oocyst with wall-forming bodies undergoing fusion into interdigitations (Oo). Scale bars = 10 μm.



Figures 13-18. Photomicrographs of tracheal coccidia, oocysts of intestinal coccidia, and juvenile nematodes from black-footed ferrets. Figures 13-15. Endogenous stages of tracheal coccidia. 13. Meronts with fully formed merozoites (Me). 14. The surface of a developing oocyst with wall-forming bodies (Oo). 15. An oocyst with sporont and wall fully formed (Oo). 16. Oocysts of *E. ictidea* (Ei) and unidentified eimerian (UE), showing relative sizes. Scale bars Figures 13-16, 10  $\mu$ m. Figures 17-18. *Physaloptera* sp. 3rd-stage larvae. 17. Whole mount, entire worm. Scale bar = 100  $\mu$ m. 18. Anterior end showing cephalic collarette (Cc). Scale bar = 10  $\mu$ m.

monts with developing or fully formed microgametes were common near macrogametes/zygotes and oocysts in various stages of development (Figs. 4, 7). As wall-forming bodies began to fuse, interdigitations (Fig. 7) commonly formed prior to the completion of oocyst wall development.

Endogenous stages of *E. furonis* were seen commonly in the top third but not in crypts or low basal regions of villi. Meronts were small, with 16 or fewer merozoites, and ectopolygony was not seen (Fig. 8). Microgamonts and mac-

rogametes/zygotes usually were seen in clusters (Figs. 9, 10) and occurred most commonly in cells of the apical one-third of villi, as were oocysts with wall-forming bodies or the oocyst wall interdigitations (Figs. 11, 12).

Sporulated oocysts of the large oval form (Fig. 16) were rarely seen, and no endogenous stages were seen that could be attributed to it, thus precluding its description and identification. Attempts to sporulate this species were variably successful under the conditions described, often with little or no development seen after a 10-day

incubation period in the potassium dichromate solution. Oocysts taken from the intestinal lumen failed to sporulate, whereas some of those passed in feces successfully did so. Measurements are given in Table 1. No coccidial oocysts with these measurements were reported by Levine and Ivens (1981) from mustelids or other carnivores, nor were any reported by Levine and Ivens (1990) or Thomas and Stanton (1994) from prairie dogs, rock squirrels, thirteen-lined, or other ground squirrels.

Meronts and oocysts of a small unidentified coccidian were found in the cells lining the trachea, a bronchus, and in associated bronchial glands in 1 black-footed ferret with canine distemper (Figs. 13–15). Identification awaits finding sporulated oocysts. Merozoites of another unidentified coccidian species were found in an impression smear of the epithelium of the urinary bladder of the same ferret with the respiratory coccidia (Fig. 19).

Cysts and trophozoites of a *Giardia* sp. were found in feces and in intestinal luminal contents in histologic sections from a black-footed ferret with canine distemper. The species was probably *G. lamblia*, based on cyst size (mean,  $11 \times 8 \mu\text{m}$ ) and trophozoite morphological features.

Ten nematodes were recovered from the half-consumed carcass of a free-ranging black-footed ferret found in July 1983. Eight worms were found in the intact stomach and 2 in the remaining portion of the small intestine. These were identified as 3rd stage larval ( $L_3$ ) *Physaloptera* sp. by virtue of the head collar and other morphological features (Figs. 17, 18).

### Discussion

Coccidial oocysts, predominantly *E. ictidea*, were noted in relatively small numbers in feces from free-ranging black-footed ferrets. Oocyst production in ferrets with canine distemper was markedly higher, apparently due to immunosuppression associated with the viral disease (Kauffman et al., 1982). This clinical condition resembles that described by Hoare (1927) in domestic ferrets with canine distemper. In spite of the massive intestinal involvement noted by both Hoare (1927, 1935) and Williams et al. (1988), intestinal inflammation attributable to the coccidial infections was mild, though intestinal function was probably compromised. Mortality of black-footed ferrets due to coccidiosis has recently been reported (Williams et al., 1992).

Although mixed infections occurred, *E. furo-*

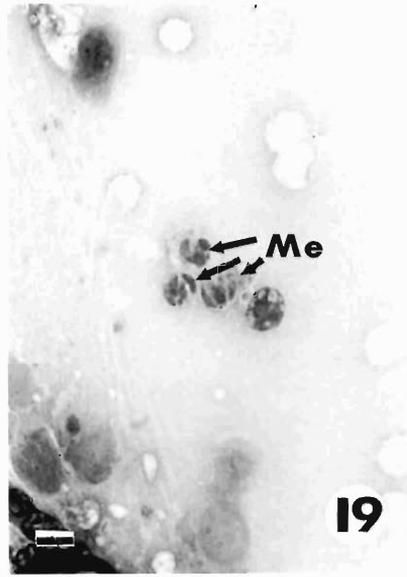


Figure 19. Photomicrograph of coccidian merozoites (Me) in impression smear from the wall of the urinary bladder from a black-footed ferret. Scale bar =  $10 \mu\text{m}$ .

*nis* was much less prevalent than *E. ictidea* both in healthy ferrets and those immunocompromised by canine distemper, and the large oval species was rare. The difficulty in getting the large oocyst to sporulate during incubation, and its scarcity in both normal and ill black-footed ferrets suggests that it may be a species poorly adapted to the black-footed ferret as a host. Those seen probably developed in the black-footed ferret rather than a prey species for several reasons; first, because it was found in feces of animals with canine distemper that were in isolation, anorexic, and fed only laboratory mice, skinned pieces of prairie dog, and nutritional supplements, and second, because none of the eimerians described from prairie dogs, ground squirrels, or mice are as large.

Neither the identity nor the clinical importance of the tracheal or the urinary bladder coccidian species is clear, as no lesions could be attributed to their presence. Identifying criteria were lacking but they are likely different species, based on morphometric and structural differences. Oocyst morphology, immunohistologic tests, and/or life cycle details would aid in their identification. The bladder form would need differentiation from *Toxoplasma*, *Neospora*, and *Hepatozoan* species, which it resembles in size,

ability to invade a variety of tissue types, and merogonic development. Unfortunately, those seen in the bladder were the only stages seen of coccidia outside of the intestinal and broncho-tracheal linings, and immunohistologic tests were not done. *Hepatozoon mustelis* from the Siberian polecat (Novilla et al., 1978) and *Hepatozoon* sp. from mink (Presidente and Karstad, 1975) also resembled the bladder form in the ferret in this report. We are not aware of any reports of *Neospora* in mustelids, although its host range capability presently includes canidae and felidae in which natural infections have been reported in dogs and experimental infections established in cats (Dubey and Lindsay, 1989a, b). The clinical significance of the *Giardia* sp. infection is unknown, but it may have contributed to fluid and electrolyte loss.

The *Physaloptera* larvae could not be identified to species because mature worms are required for such identification. Any clinical effect on the ferrets by the nematodes was probably minor due to the relatively small number of worms and their stage of development.

This report constitutes new host records for *E. ictidea*, *E. furonis*, *Giardia* sp., and *Physaloptera* sp. and new distribution records for the coccidia. The intestinal, respiratory, and urinary bladder coccidians require additional study to determine their identities and significance in the black-footed ferret.

#### Acknowledgments

We acknowledge the assistance of Drs. E. Tom Thorne and Don R. Kwiatkowski, Wyoming Game and Fish Department and Sharisse Berk, Department of Veterinary Sciences, University of Wyoming.

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## Helminth Parasites Collected from *Rattus rattus* on Lanyu, Taiwan

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**ABSTRACT:** The helminth fauna of 53 *Rattus rattus* captured on Lanyu, Taiwan, was studied and compared with that found in a survey carried out on this island in 1959. One trematode, 3 cestode, and 14 nematode species were detected. Among the nematodes, *Calodium hepaticum* (= *Capillaria hepatica*), *Strongyloides ratti*, *Strongyloides venezuelensis*, *Orientostrongylus tenorai*, *Mastophorus muris*, *Ascarops strongylina*, and *Pterygodermatites whartoni* were recorded for the first time from the rat on this island. *Gongylonema neoplasticum* and *Protospirura muricola*, which had been recorded by the survey in 1959, were not observed. Morphological and morphometric data are given for *O. tenorai*, *Globocephalus connorfilli*, *A. strongylina*, *Pterygodermatites tani*, and *P. whartoni*. *Orientostrongylus ratti* was synonymized with *O. tenorai*. *Globocephalus connorfilli* and *A. strongylina* were assumed to be shared between rat and swine on this island.

**KEY WORDS:** Nematoda, Cestoda, Trematoda, survey, *Rattus rattus*, Lanyu, Taiwan.

Lanyu (Orchid Island, Koto-sho or Botel Tobago; 22°00'–05'N, 121°29'–36'E; ca. 45 km<sup>2</sup>) is a small island located about 70 km southeast of the main island of Taiwan. This island is famous ethnologically for its aboriginal Yami tribe but has been also noticed zoogeographically because its fauna is composed of both the Philippine and Taiwan elements (cf. Kano, 1935–1936). On Lanyu, *Rattus rattus* is abundant in the fields. During a survey on tsutsugamushi disease (scrub typhus) on this island in 1990, 53 rats were trapped. This article reports the helminths collected from the rats in comparison with the results of a similar survey carried out on Lanyu in 1959 (Myers and Kuntz, 1960). Brief descriptions are made for 5 nematode species that have not been described adequately from murines and/or have special taxonomical interest.

### Materials and Methods

Rats, *Rattus rattus*, were captured with wire-cage live traps in the bushes along the southwestern coast of Lanyu during the period 5–10 August 1990. They were anesthetized to death with ether. Then, laparotomy was performed, and the liver was checked with naked eyes for the presence of *Calodium hepaticum* (= *Capillaria hepatica*) eggs and *Taenia taeniaeformis* strobilocerci. The alimentary canal and lungs were resected and fixed in 5% formalin solution. Helminths were collected from these viscera under a dissecting microscope. Trematodes and cestodes were stained with Meyer's hematoxylin or cleared in glycerin-alcohol solution. Nematodes were cleared in glycerin-alcohol or creosote for microscopical observation. Figures were made with the aid of a drawing tube equipped on a Nikon Optiphoto microscope. Measurements are in micrometers unless otherwise stated. Representative specimens are deposited in the National Science Museum, Tokyo (NSMT).

### Results

The helminths detected are shown in Table 1 and are compared with the data of Myers and Kuntz (1960). One trematode, 3 cestode, and 14 nematode species were collected. *Echinostoma* sp. was strongly shrunken, preventing species identification. *Gongylonema neoplasticum* and *Protospirura muricola*, which had been recorded by Myers and Kuntz (1960), were not observed, whereas 6 nematode species were first recorded in the present study (i.e., *C. hepaticum*, *Strongyloides ratti*, *Strongyloides venezuelensis*, *Orientostrongylus tenorai*, *Mastophorus muris*, and *Ascarops strongylina*). *Angiostrongylus cantonensis* was not recorded in Myers and Kuntz (1960) but later reported by the same authors (Kuntz and Myers, 1964) from Lanyu. Concurrent infection with *S. ratti* and *S. venezuelensis* was common; however, it was difficult to obtain all worms because they were embedded in the villi of the small intestine. Hence, the exact prevalence of each species could not be determined.

### *Orientostrongylus tenorai* Durette-Desset, 1970

Syn. *Orientostrongylus ratti*

Kamiya and Ohbayashi, 1980

(Trichostrongyloidea: Heligmonellidae:  
Nippostrongylinae)

**MALE:** Length 1.47–1.78 mm, width at mid-body 50–70. Cephalic vesicle 36–42 long by 22–28 wide. Nerve ring 103–133 and excretory pore 154–179 from anterior extremity. Esophagus 282–313 long by 20–26 wide near posterior end.

**Table 1. Prevalence of helminth parasites in *Rattus rattus* on Lanyu, Taiwan (%).**

Site in host	Year of examination: 1959*		1990
	No. of rats examined:	80	53
<b>Trematoda</b>			
<i>Echinostoma</i> sp.	Small intestine	—	1.9
<b>Cestoidea</b>			
<i>Taenia taeniaeformis</i>	Liver	—	5.7
<i>Raillietina celebensis</i>	Small intestine	—	35.8
<i>Hymenolepis diminuta</i>	Small intestine	—	3.8
<b>Nematoda</b>			
<i>Calodium hepaticum</i>	Liver	0.0	18.9
<i>Eucoleus bacillatus</i>	Stomach	0.0	9.4
<i>Capillaria</i> sp.	—	2.5	0.0
<i>Strongyloides</i> spp.†	Small intestine	0.0	71.7
<i>Angiostrongylus cantonensis</i>	Pulmonary artery	0.0	1.9
<i>Nippostrongylus brasiliensis</i>	Small intestine	38.8	69.8
<i>Orientostrongylus tenorai</i>	Small intestine	0.0	66.0
<i>Globocephalus connorfili</i>	Small intestine	6.3‡	11.3
<i>Syphacia muris</i>	Cecum	1.3‡	20.8
<i>Heterakis spumosa</i>	Large intestine	1.3	22.6
<i>Gongylonema neoplasticum</i>	Stomach	20.0	0.0
<i>Protospirura muricola</i>	Stomach	6.3	0.0
<i>Mastophorus muris</i>	Stomach	0.0	22.6
<i>Ascarops strongylina</i>	Stomach	0.0	5.7
<i>Pterygodermatites</i> spp.§	Small intestine	11.3	13.2

\* Data from Myers and Kuntz (1960).

† Mixture of *S. ratti* and *S. venezuelensis*.

‡ Species identification was not made.

§ Mixture of *P. tani* and *P. whartoni*.

Cuticular ridges number 19 or 20 at midbody. Spicules 82–94 long.

**FEMALE:** Length 1.70–2.33 mm, width at midbody 52–70. Cephalic vesicle 38–44 long by 24–32 wide. Nerve ring 105–125 and excretory pore 128–179 from anterior extremity. Esophagus 269–344 long by 26–32 wide near posterior end. Cuticular ridges number 20 at midbody. Vulva 102–132 and anus 42–60 from posterior extremity. Vagina vera 29–40 long, vestibule 30–42 long, sphincter 28–46 long, and infundibulum 80–104 long. Eggs 64–80 by 24–36.

**HOST:** *Rattus rattus*.

**SITE IN HOST:** Small intestine.

**LOCALITY:** Lanyu, Taiwan.

**SPECIMENS DEPOSITED:** NSMT As-2261.

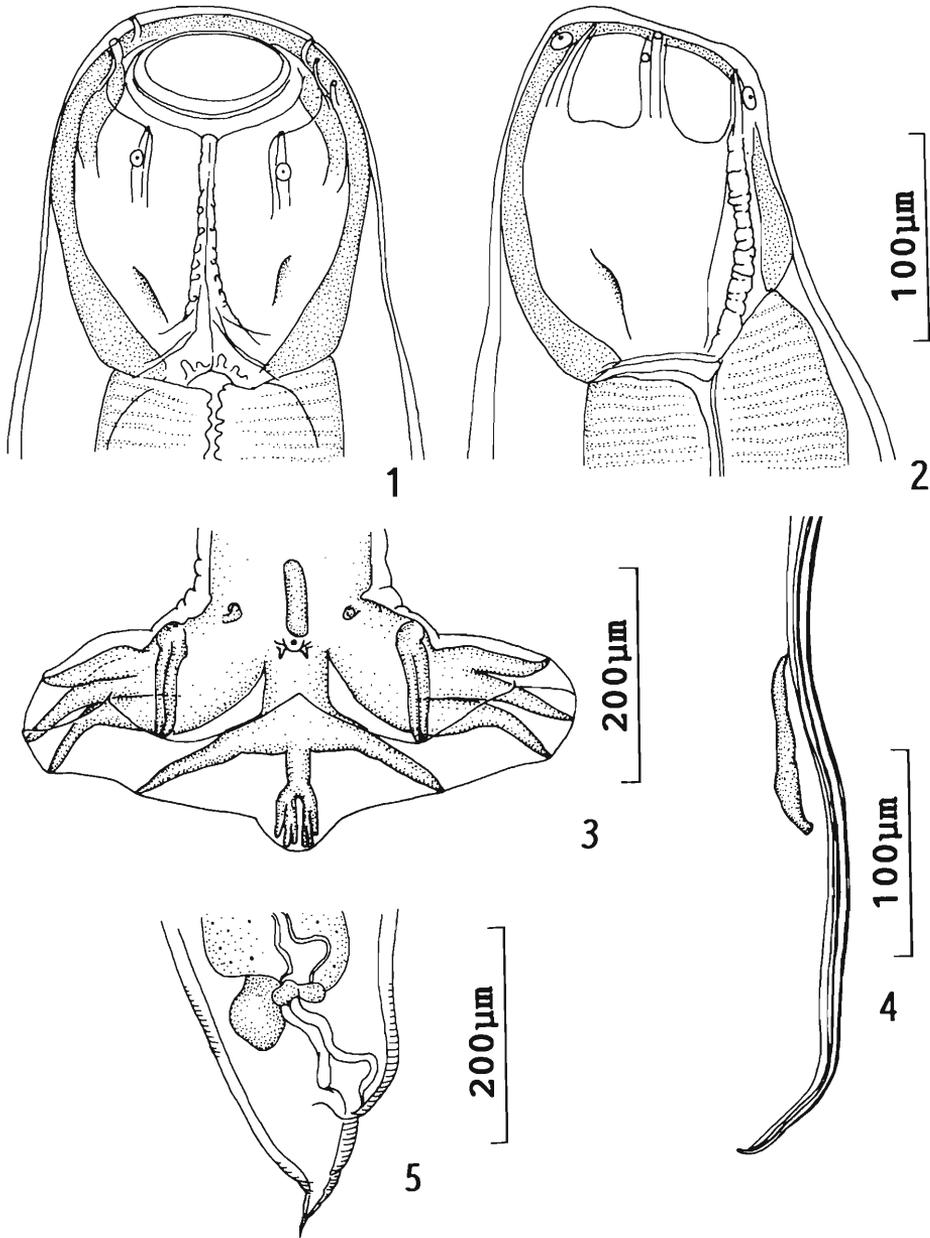
**REMARKS:** The number of cuticular ridges of the present material is identical to that described for *Orientostrongylus ratti* Kamiya and Ohbayashi, 1980, which was collected from *R. rattus* and *R. norvegicus* in Thailand (M. Kamiya and Ohbayashi, 1980). However, *O. ratti* is distinguished from *O. tenorai* only by the slight difference in number of cuticular ridges (M. Kamiya

and Ohbayashi, 1980). Fukumoto and Ohbayashi (1985) reported a considerable variation in the number of the cuticular ridges of *Orientostrongylus ezoensis* Tada, 1975, from *R. norvegicus* in Japan. It is highly probable that the difference in the number of the cuticular ridges between *O. tenorai* and *O. ratti* is an intraspecific variation. *Orientostrongylus ratti* is thus regarded as a junior synonym of *O. tenorai*.

***Globocephalus connorfili* Lane, 1922**  
(Ancylostomatoidea: Ancylostomatidae:  
Ancylostomatinae)  
(Figs. 1–5)

**GENERAL:** Small but stout worm. Buccal cavity well developed, globular, with thick wall (Figs. 1, 2). Oral opening inclined dorsally (Fig. 2). Esophageal gutter extending to level slightly posterior to oral opening (Figs. 1, 2). Subventral lancets of buccal cavity weakly developed (Figs. 1, 2).

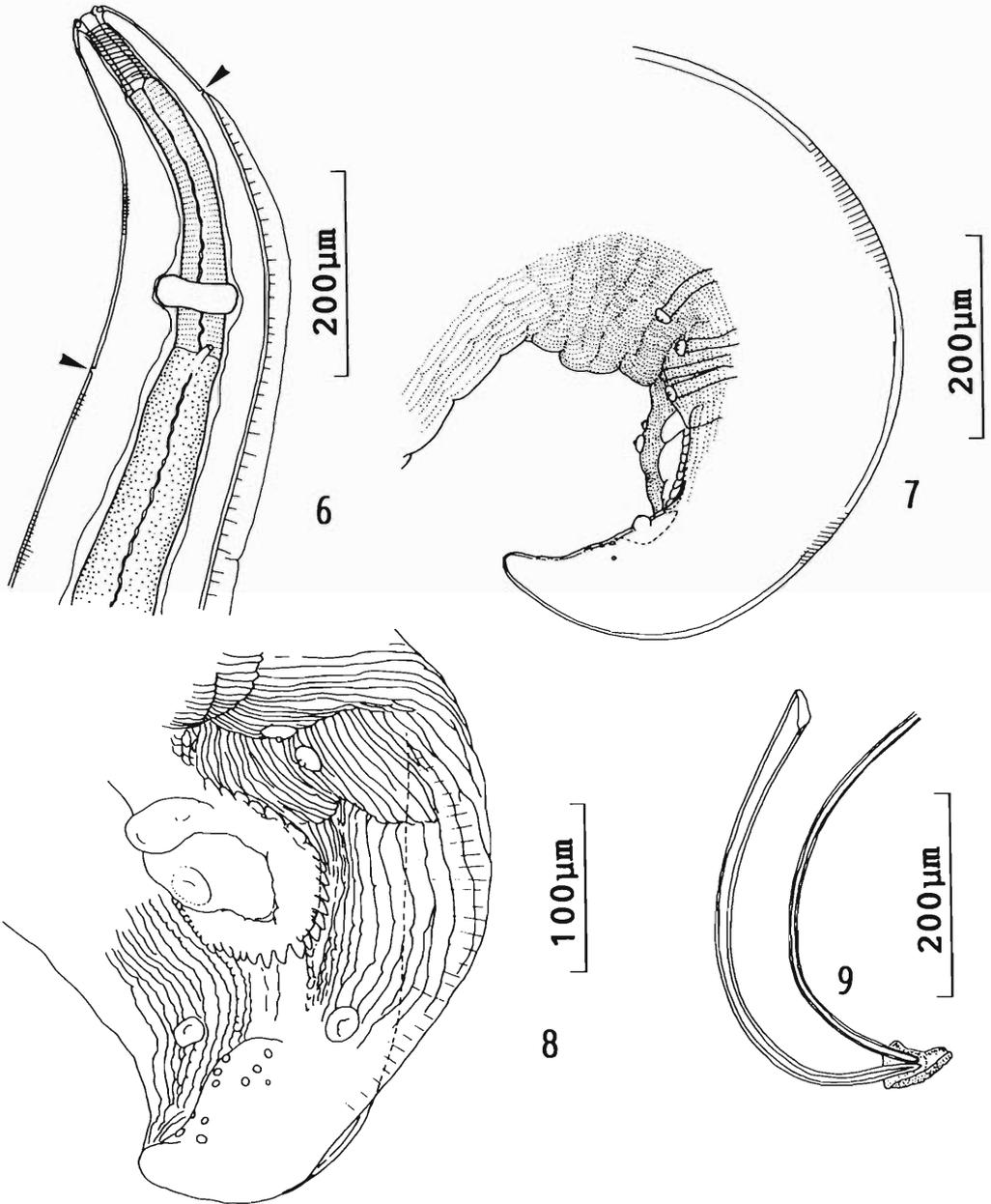
**MALE (4 worms):** Length 4.15–4.60 mm, width at midbody 246–265. Buccal cavity 140–



Figures 1–5. *Globocephalus connorfilii* collected from *Rattus rattus* on Lanyu, Taiwan. 1, 2. Cephalic extremity, dorsal (1) and left lateral (2) views. 3. Bursa copulatrix, ventral view. 4. Spicules and gubernaculum, right lateral view. 5. Tail of female, right lateral view.

160 long by 101–113 wide. Nerve ring 382–454, excretory pore 324–390, and deirids 332–474 from anterior extremity. Esophagus 632–679 long by 133–150 wide. Bursa copulatrix well developed, symmetrical: prebursal rays minute; ventral rays thin, equal, fused along total length;

lateral rays stout, divergent from each other, and externolateral ray shorter than other laterals and ending distant from bursal rim; externodorsal rays arising from middle of dorsal ray, directing posterolaterally; dorsal ray divided at distal 1/2 into 2 branches, each of which trifid distally (Fig.



Figures 6-9. *Ascarops strongylina* collected from *Rattus rattus* on Lanyu, Taiwan. 6. Anterior part of male, ventral view. Arrowheads indicate deirids. 7. Posterior part of male, left lateral view. 8. Caudal extremity of male, left subventral view. 9. Spicules and gubernaculum, right lateral view.

3). Genital cone with 1 pair of minute pedunculate papillae on posterior lip and 1 unpaired sessile papilla on anterior lip (Fig. 3). Spicules equal, filiform, distal ends fused, 450-488 long (Fig. 4). Gubernaculum boat shaped, about 80 long (Figs. 3, 4).

FEMALE (7 worms): Length 4.53-7.15 mm, width at midbody 245-435. Buccal cavity 160-183 long by 117-140 wide. Nerve ring 356-514, excretory pore 348-411, and deirids 363-425 from anterior extremity. Vulva 2.88-4.48 mm from anterior extremity, dividing body unequal-

ly, with forebody: hindbody ratio of 1.51–1.84: 1. Esophagus 600–814 long by 152–198 wide. Tail conical, distally pointed sharply, 129–176 long (Fig. 5). Eggs present in females larger than 6.5 mm in length, elliptical, thin-shelled, containing 1–2-cell-stage embryos, 54–64 by 28–36.

HOST: *Rattus rattus*.

SITE IN HOST: Small intestine.

LOCALITY: Lanyu, Taiwan.

SPECIMENS DEPOSITED: NSMT As-2262.

REMARKS: Most of the morphological characteristics of the present worms are identical to the original description of *G. connorfilii* by Lane (1922, 1923). Although it has been stated that the esophageal gutter is protruded only slightly into the buccal capsule (cf. Popova, 1955), the figure in the original description (Lane, 1923) indicates that the gutter is fairly extended as in the present material. *Globocephalus connorfilii* is a common parasite of swine of the Oriental Region and Pacific islands and has been also recorded from *Leopoldamys sabanus* (= *Rattus sabanus*) in Malaysia (Singh and Cheong, 1971).

***Ascarops strongylina* (Rudolphi, 1819)**

(Spiruroidea: Spirocercidae: Ascaropsinae)  
(Figs. 6–9)

GENERAL: Slender worm. Lips poorly developed. Buccal cavity with 6 teeth. Pharynx straight, with 15–19 spiral thickenings (Fig. 6). Deirids asymmetrical (Fig. 6). Left lateral ala well developed, arising from left deirid (Fig. 6). Right lateral ala absent.

MALE (1 worm): Posterior body coiled ventrally (Fig. 7). Length 13.5 mm, width at midbody 253. Pharynx 86 long by 32 wide. Muscular esophagus 336 long by 46 wide; glandular esophagus 2.83 mm long by 110 wide. Nerve ring 316, excretory pore 363, right deirid 386, and left deirid 120 from anterior extremity. Caudal alae thick and asymmetrical: right ala larger. Perianal surface with rugose longitudinal ridges (Figs. 7, 8). Anus surrounded by large hemicircular disc with prominently serrated margin (Fig. 8). Four pairs of pedunculate papillae situated preanally, 1 pair of pedunculate papillae at midtail, and 4 pairs of minute sessile papillae and phasmidial pores grouped on ventromedian elevation near posterior extremity (Fig. 8). Spicules markedly dissimilar: right one 520 long and left one filiform, 2.10 mm long (Fig. 9). Gubernaculum triangular in ventral view, 64 long (Fig. 9). Tail 312 long.

FEMALE (2 worms): Length 17.8–21.3 mm,

width at midbody 277–300. Pharynx 90–102 long by 32–34 wide. Muscular esophagus 312–376 long by 44–50 wide; glandular esophagus 2.85–3.59 mm long by 119–132 wide. Nerve ring 293–320, excretory pore 343–395, right deirid 339–394, left deirid 152–261, and vulva 8.3–10.9 mm from anterior extremity. Tail 250–254 long. Only unfertilized eggs present.

HOST: *Rattus rattus*.

SITE IN HOST: Small intestine.

LOCALITY: Lanyu, Taiwan.

SPECIMENS DEPOSITED: NSMT As-2263.

REMARKS: Although the present male has a somewhat shorter left spicule, other characteristics are identical to those of *Ascarops strongylina* (cf. Shmitova, 1964; Shoho and Machida, 1979). *Ascarops strongylina* is a well-known, cosmopolitan nematode of swine, but it has not been recorded from *Rattus*.

***Pterygodermatites tani* (Hoepli, 1928)**

(Rictularioidea: Rictulariidae)

MALE (2 worms): Length 1.66–6.80 mm, width at midbody 126–514. Number of comb pairs 64–68; last several pairs small and pointed. Buccal depth 28–54. Muscular esophagus 162–442 long by 28–78 wide; glandular esophagus 0.51–1.75 mm long by 48–137 wide. Nerve ring 132–328, excretory pore 188–458, and deirids 246–593 from anterior extremity. Posterior end not bent ventrally. Preanal fans 2 or 3 in number, anterior 1 or 2 rudimentary, posterior 1 moderately developed. Tail conical, 76–171 long. Caudal papillae 10 pairs: 2 pairs preanal, 1 pair adanal, and 7 pairs postanal. Spicules almost equal, simple, slightly bent ventrally: right spicule 76–81 long and left spicule 70–84 long.

HOST: *Rattus rattus*.

SITE IN HOST: Small intestine.

LOCALITY: Lanyu, Taiwan.

SPECIMENS DEPOSITED: NSMT As-2264.

REMARKS: Morphology of the present males is identical to the previous descriptions of the male *P. tani* (cf. Le-Van-Hoa, 1965; Hasegawa et al., 1993)

***Pterygodermatites whartoni***

**Tubangui, 1931**

(Rictularioidea: Rictulariidae)

GENERAL: Morphology identical to that of *P. tani* except male caudal structure.

MALE (3 worms): Length 4.58–6.15 mm, width at midbody 254–359. Number of comb

pairs 60–62. Buccal depth 46–51. Muscular esophagus 265–394 long by 54–76 wide; glandular portion of esophagus 1.13–1.61 mm long by 98–128 wide. Nerve ring 238–312, excretory pore 308–421, and deirids 419–600 from anterior extremity. Posterior end bent ventrally. Preanal fans 3–4 in number, posterior 3 well developed. Tail conical, 160–228 long. Caudal papillae 10 pairs: 2 preanal, 1 adanal, and 7 postanal. Spicules dissimilar, simple, curved ventrally: right spicule 65–83 long and left spicule 138–170 long.

HOST: *Rattus rattus*.

SITE IN HOST: Small intestine.

LOCALITY: Lanyu, Taiwan.

SPECIMENS DEPOSITED: NSMT As-2265.

REMARKS: The present males are identical morphologically to the previous descriptions of *P. whartoni* (cf. Schmidt and Kuntz, 1967; Hasegawa et al., 1993). Females of *P. whartoni* are indistinguishable from those of *P. tani* (cf. Hasegawa et al., 1993).

### Discussion

All of the present nematode species from Lanyu except *G. connorfilii* and *A. strongylina* had been apparently introduced to this island from the adjacent areas by the rats because these parasites have also been recorded from *R. rattus* and *R. norvegicus* of the Philippines, the main island of Taiwan, South China, and the Ryukyu Archipelago (cf. Yokogawa, 1920; Tubangui, 1931; Chen, 1936; M. Kamiya et al., 1968; H. Kamiya and Machida, 1977; M. Kamiya and Kanda, 1977; Hasegawa et al., 1988, 1993; Hasegawa, 1990). The rats on Lanyu are considered to have arrived relatively recently because the Yami people have an oral tradition that there was no rat before large ships called at the ports of the island (Kano, 1933). The geographical origin of *R. rattus* on Lanyu has not been adequately studied, although its morphological affinity with the Philippine population was pointed out (cf. Tokuda, 1941).

There are several discrepancies in the species composition and prevalence of the rat parasites on Lanyu between the survey by Myers and Kuntz (1960) and the present one (Table 1). It is probable that the helminth fauna of the rats on this island has changed during the past 3 decades. It is also likely, however, that some of the species were overlooked by Myers and Kuntz (1960) because minute nematodes such as *Strongyloides* spp. and male *S. muris* were not recorded in their

report. *Orientostrongylus tenorai* resembles immature *Nippostrongylus brasiliensis* in appearance, and both have been confused in many surveys until recently.

It is of special interest that 2 swine nematodes, *G. connorfilii* and *A. strongylina*, were found from *R. rattus* on Lanyu. They were apparently mature adults, indicating that the rat could be a suitable host of these parasites, although the prevalence and intensity of *A. strongylina* was low and its females had only unfertilized eggs. On Lanyu, the Yami people rear, often pasture, small short-eared black pigs, which have affinities with those of the Philippines (Tanaka et al., 1981). Although no parasitological examination of the pigs was made, it is highly probable that *G. connorfilii* and *A. strongylina* are shared by the pigs and the rodents on this island.

Rodents have been known as paratenic hosts of *A. strongylina* but have not been recorded as natural final hosts of it (cf. Dimitrova, 1966). However, Ono (1933) observed that *A. strongylina* developed to adult stage in rabbits experimentally infected with the third-stage larvae from dung beetles, demonstrating that this nematode has a wider host range. This observation was confirmed by Chowdhury and Pande (1969), who also recovered mature *A. strongylina* from the stomachs of rabbits and guinea pigs by experimental infection. Moreover, Webster and Speckmann (1977) reported many adults of *A. strongylina* from the proventriculus of a cockatoo. It is thus suggested that the presence of adult *A. strongylina* in a wild rodent is not an exceptional phenomenon.

A species of the genus *Globocephalus* has been recorded from the feral rat, *Diplothrrix legata*, on Amami and Okinawa islands, the Ryukyu Archipelago, Japan, where wild boar, *Sus scrofa riukiuanus*, is distributed (Itagaki et al., 1981; Hasegawa, 1987). Itagaki et al. (1981) identified it as *G. longemucronatus* (Molin, 1861) based on Popova (1955) and Yamaguti (1935). Hartwich (1986), however, claimed that the criteria used by Popova (1955) were incorrect and considered that *G. longemucronatus* sensu Yamaguti, 1935, was *G. connorfilii*. The species of *Globocephalus* parasitic in *D. legata* is hence considered to be *G. connorfilii*, although the males have somewhat longer spicules (0.60–0.67 mm in males with body length of 3.40–5.40 mm [Itagaki et al., 1981], 0.60–0.77 mm in males with body length of 5.4–6.4 mm [Hasegawa, unpubl. data]).

Two additional species of the genus *Globo-*

*cephalus* are known from rodents: *Globocephalus howelli* (Khalil, 1973) from *Cricetomys gambianus* of Tanzania and *Globocephalus callosciuri* Cassone and Krishnasamy, 1986, from *Callosciurus caniceps* of Malaysia (Khalil, 1973; Cassone and Krishnasamy, 1986). *Globocephalus callosciuri* resembles *G. connorfili* closely in having weakly developed subventral lancets in the buccal cavity but differs in having a longer buccal capsule (240 long in holotype male with body length of 5.89 mm and 310 long in paratype female with body length of 9.65 mm [Cassone and Krishnasamy, 1986]).

*Pterygodermatites tani* and *P. whartoni* are indistinguishable by female morphology, but their males are quite different in genital morphology (cf. Le-Van-Hoa, 1965; Hasegawa et al., 1993). The concurrent infection of both species in a rat on the small island may indicate that *P. tani* has 2 types of males, 1 of which has been regarded as *P. whartoni* (cf. Hasegawa et al., 1993).

#### Acknowledgments

Sincere thanks are rendered to Mr. H. M. Lin for his kind collaboration in collecting rats on Lanyu and to Dr. K. Tuchiya of Miyazaki Medical School, Dr. K. Tanaka of Tokyo University of Agriculture, Dr. M. Asakawa of Rakuno Gakuen University, and Dr. Y. Yokohata of Hokkaido University for their kind advice on the taxonomy of the hosts and parasites. This research was supported partly by a Grant-in-Aid for Overseas Scientific Survey No. 63041108 from the Ministry of Education, Science and Culture, Japan.

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## Genetic Variability in the M-Line Stock of *Biomphalaria glabrata* (Mollusca: Planorbidae)

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**ABSTRACT:** Genetic variation among samples of the M-line stock of *Biomphalaria glabrata* is described. Snail samples from 5 separate research laboratory facilities display differences in allozyme frequencies among 9 of 24 enzymes examined. Genetic variability of M-line snails is discussed relative to the origin and maintenance of the stock and its use as a model in studies of snail-schistosome interactions. Inquiries among investigators maintaining and using snails have indicated that multiple albino stocks of *B. glabrata* are or have been used for snail-schistosome research. Exchange of these stocks among investigators and subsequent interbreeding are the most probable explanations for the patterns of genetic divergence observed.

**KEY WORDS:** *Biomphalaria glabrata*, *Schistosoma mansoni*, genetic variability, allozymes.

The M-line stock of *Biomphalaria glabrata* is among the snail stocks most often used in studies of snail-schistosome compatibility (Richards and Merritt, 1972; Richards, 1973, 1975, 1984), molluscan immunobiology (e.g., Loker et al., 1987; Noda and Loker, 1989; see references in Bayne, 1983), and where large numbers of infected snails are needed as in vaccine research (Stirewalt et al., 1983). Newton (1955) established the M-line stock to combine albinism and a high level of susceptibility to a Puerto Rican strain of *Schistosoma mansoni*. The derivation of the M-line stock involved a cross between a pigmented Puerto Rican snail susceptible to this strain of *S. mansoni* and an albino Brazilian snail not susceptible to this parasite. Ten generations of inbreeding (selfing) and selection for susceptibility yielded an albino stock that was >90% (although usually not 100%) susceptible to a Puerto Rican *S. mansoni* strain. Additionally, there may have been strong (although inadvertent) selection for early reproduction, because the first individual to reproduce was used to found the subsequent generation. The breeding and selection scheme imposed by Newton (1955, p. 528) led to the prediction that the stock should be "genetically rather homogeneous, although it is to be expected that with random breeding and the occurrence of mutations there will be a gradual loss in this high degree of homozygosity." An assumption inherent in the use of laboratory stocks for studies of snail-schistosome compatibility and snail immunobiology is that they are more ge-

netically homogeneous and will provide a more uniform response to experimental manipulation than would field-collected specimens.

Mulvey and Vrijenhoek (1981) provided evidence that M-line snails were not genetically uniform but were unexpectedly variable. Using electrophoretically detectable genetic variation, they estimated a level of heterozygosity following the initial hybridization between the Puerto Rican and Brazilian stocks (although not the original stocks of Newton) to be approximately 0.32. Ten generations of selfing in which a single selfed snail founded each generation and subsequent selection for susceptibility would be predicted to reduce such heterozygosity to 0.0003. These estimates of initial and subsequent heterozygosity are based on several a posteriori assumptions: 1) that the Puerto Rican and Brazilian stocks used to estimate heterozygosity were representative of the original stocks, 2) that reproduction was exclusively by single self-fertilizing snails, and 3) that the M-line stock sampled is a direct descendant of the original stock and has been isolated from other snail stocks since its origin (i.e., no migration). The M-line sample described by Mulvey and Vrijenhoek was more than 100 times more heterozygous than predicted ( $H = 0.057$ ). These authors suggested the following possibilities to account for the unexpectedly high levels of heterozygosity in their sample: 1) that there was selection to maintain heterozygosity, 2) that the sample was not representative of all M-line material, or 3) that these snails had been inadvertently outcrossed to other laboratory stocks of *Biomphalaria*.

Studies of snail-schistosome compatibility often have reported variation in snail susceptibil-

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ity. Although such variation may have a number of underlying causes, genetic differences among host individuals is undoubtedly involved. The present study was undertaken to extend the investigation of electrophoretically detectable enzyme polymorphism and heterozygosity in the M-line of *B. glabrata*. If electrophoretic markers can be viewed as an indication of overall genomic variation, these studies may suggest the extent to which genetic variation is important in this stock of snails. A second impetus for this study is the observation that natural populations of *Biomphalaria* generally have levels of electrophoretically detectable polymorphism and heterozygosity comparable to or lower than those reported by Mulvey and Vrijenhoek (1981) for the M-line. This laboratory stock resembles naturally occurring snail populations with respect to genetic population structure more than previously expected.

Here we report on electrophoretically detectable genetic variation in the M-line of *B. glabrata* maintained at 5 laboratories in the U.S.A. The following points will be addressed. 1) Is the variation reported earlier limited to the original sample studied or characteristic of the M-line generally? 2) Do M-line snails maintained at different laboratories collectively represent a homogeneous stock?

### Materials and Methods

Samples of approximately 30–40 M-line snails were generously provided by the following: E. S. Loker (University of New Mexico, Albuquerque), W. Granath (University of Montana, Missoula), C. Bayne (Oregon State University, Corvallis), F. Lewis (Biomedical Research Institute, Rockville, Maryland), and T. Yoshino (University of Wisconsin, Madison). These investigators also provided information concerning the history and maintenance of each colony.

Methods for starch gel electrophoresis of *B. glabrata* have been described elsewhere (Mulvey and Vrijenhoek, 1981; Mulvey et al., 1988). For the present study, the lithium-hydroxide buffer of Selander et al. (1971) was used for resolution of esterase allozymes; additionally, the esterase-staining solution contained  $\alpha$ -naphthyl acetate and  $\beta$ -naphthyl propionate as substrates. Peptidase enzymes were detected using the following peptide substrates: leucylglycylglycine, leucylalanine, and phenylalanylproline.

Data were analyzed using BIOSYS-1 (Swofford and Selander, 1981) and SAS (SAS Institute, 1985). Genetic variation within and among samples was examined using Wright's (1978) *F*-statistics. Rogers (1972) genetic identity values were clustered using the UPGMA (Sneath and Sokal, 1973) and distance Wagner methods (Farris, 1972).

### Results

Twenty-four enzymes were examined in the M-line samples, and 15 were invariant. Allozyme frequencies for the variable loci and overall heterozygosity varied markedly among samples (Table 1). Genotypic frequencies within samples differed significantly from expectations under Hardy-Weinberg equilibrium and random mating in 9 of 37  $\chi^2$ -tests. All of these deviations reflected a deficiency of heterozygous genotypes. Contingency  $\chi^2$ -tests indicated that there was significant heterogeneity in allele frequencies among samples at 8 of the 9 polymorphic loci ( $P < 0.00002$ ). Only allele frequencies for Pep-1 were homogeneous among the 5 samples.

The  $F_{it}$  value is a measure of correlation among gametes in individuals relative to the entire population (Table 3). The observed value,  $F_{it} = 0.482$ , indicates an excess number of homozygotes in the total sample relative to that predicted if the entire sample represented a single randomly mating assemblage. The correlation among gametes within individuals in the total sample ( $F_{it}$ ) is affected by nonrandom mating (heterozygosity measured by  $F_{is}$ ) and population size (differences among populations, measured by  $F_{st}$ ). The  $F_{is}$  value indicates mean heterozygosity at the local level; again, positive values indicate an excess of homozygous genotypes relative to expectations under random mating. Among-sample variation can be expressed as a percentage, measured by the  $F_{st}$ . Therefore, 17.3% of the total genetic variation is attributable to differences among samples.

Figure 1 provides genetic identity values clustered using UPGMA. The cophenetic correlation was 0.85, indicating a good fit at the original data matrix to the diagram.

### Discussion

The M-line samples display unexpectedly high levels of genetic variability. Approximately 30% of the loci examined were polymorphic within the samples. Average individual heterozygosities ranged from 0.051 to 0.098. These levels of genetic variation are comparable to levels found in many naturally occurring populations of *Biomphalaria* (Table 2). Our findings confirm previous reports of high levels of genetic variation in laboratory stocks of *B. glabrata* (Mulvey and Vrijenhoek, 1981; Knight et al., 1991). Working with the 10-R2 stock of *B. glabrata*, an

**Table 1.** Allozyme frequencies for enzymes in colonies of the M-line stock of *Biomphalaria glabrata*. Allozymes are designated A, B, or C for each locus. Fifteen other enzymes were invariant.\*

Locus	Loker	Granath	Lewis	Bayne	Yoshino
Aconitate hydratase-2					
A	0.81	0.18	0.28	0.14	0.64
B	0.19	0.62	0.72	0.86	0.36
6-Phosphogluconate dehydrogenase					
A		0.06	0.29	0.61	0.36
B	1.00	0.94	0.71	0.39	0.64
Phosphoglucomutase-1					
A	0.92	0.72	0.84	0.57	0.54
B	0.08	0.28	0.16	0.43	0.46
Esterase-1					
A	0.99	0.53	0.86	1.00	0.51
B	0.01	0.47	0.14		0.39
C					0.10
Esterase-4					
A	0.55	0.97	0.70	0.32	0.75
B					0.03
C	0.45	0.03	0.30	0.68	0.22
Xanthine dehydrogenase					
A					0.15
B	0.92	0.87	0.82	0.67	0.74
C	0.08	0.13	0.18	0.33	0.11
Peptidase-1					
A	0.08	0.09	0.04		0.10
B	0.69	0.60	0.81	0.69	0.71
C	0.23	0.31	0.15	0.31	0.19
Peptidase-4					
A	0.35			0.24	
B	0.65	1.00	1.00	0.76	1.00
Acid phosphatase					
A				0.23	0.26
B	1.00	1.00	1.00	0.77	0.74
Mean sample size	33.0 ± 4.7	31.0 ± 1.3	30.9 ± 1.1	30.3 ± 3.1	34.1 ± 1.2
% Polymorphic loci	29	29	29	33	33
Mean heterozygosity	0.054	0.051	0.063	0.094	0.098

\* Aconitate hydratase-1, adenosine deaminase, aspartate aminotransferase-1, aspartate aminotransferase-2,  $\alpha$ -glycerophosphate dehydrogenase, hemoglobin, hydroxybutyrate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase-1, malate dehydrogenase-2, malic enzyme, nucleoside phosphorylase, phosphoglucoisomerase, and phosphoglucomutase-2.

M-line derivative, Knight et al. (1991) observed restriction fragment-length polymorphism variation among snails from various laboratories as well as among progeny of selfed snails. Mulvey and Vrijenhoek (1981) reported a high degree of intrastrain polymorphism even in some presumably inbred stocks.

In agreement with the earlier work of Mulvey and Vrijenhoek (1981), levels of genetic varia-

tion observed in laboratory populations of snails are not consistent with predictions based on the intensive regime of inbreeding and selection during the derivation of the M-line stock. Given the hybrid origin of these snails and the estimated initial heterozygosity, it might be possible to maintain these levels of heterozygosity by some kind of balancing selection. However, the occurrence of 3 alleles at the Est-1, Xdh and Pep-1

**Table 2.** Levels of genetic variability in natural populations of *Biomphalaria* compared with *B. glabrata* M-line.  $N_s$  = number of samples,  $N_l$  = number of loci,  $P$  = proportion of polymorphic,  $H$  = mean heterozygosity per sample.

	$N_s$	$N_l$	$P$	$H$	References
<i>B. glabrata</i>					
Puerto Rico	7	26	0.15	0–0.06	Mulvey and Vrijenhoek, 1982
Dominican Republic	2	21	0.19–0.24	0.022–0.027	Mulvey et al., 1988
St. Lucia	2	21	0.09–0.24	0.003–0.037	Mulvey et al., 1988
<i>B. alexandrina</i>					
Qalubia	7	26	0.16–0.19	0.044–0.092	Graven, 1984
<i>B. straminea</i>					
Hong Kong	4	19	0.26	0.056–0.097	Woodruff et al., 1985
<i>B. Pfeifferi</i>					
Kenya	12	10	0.00–0.40		Bandoni et al., 1990
<i>B. glabrata</i>					
M-line	5	24	0.29–0.33	0.051–0.098	This study

loci demands an alternative explanation. A single selfing snail could not have more than 2 alleles. There are 2 possible explanations for the presence of the third allele: mutation and migration.

The spread of a neutral allele in a population would be determined by the effective population size and reproductive rate. Although mutation cannot be entirely excluded, it seems unlikely given the relatively high frequencies observed and its necessarily recent origin. For example, the Yoshino stock has been isolated from the other M-line stocks for at most 20 generations. However, a sequence resembling the LINE-1 element of *Drosophila* has recently been described from *B. glabrata* (Knight et al., 1992). The LINE-1 element is a transposable element associated with higher levels of mutation. Migration is another probable explanation. Even with diligent care, movement of snails among aquaria

is a possibility during cleaning, feeding, or handling of the colony. All laboratories from which M-line snails were obtained also maintain other stocks of *B. glabrata*.

Among-sample differentiation accounted for approximately 17% of the observed variation. This among-sample differentiation is less than that observed between naturally occurring snail populations but considerably more than would be expected from a homogeneous, inbred laboratory stock (Table 4).

The Lewis colony is an uninterrupted descendant of the original Newton Stock (F. Lewis and C. S. Richards, pers. comm.). Snails have been shipped from this colony to initiate other colonies. The Loker colony was secondarily derived from the Bayne colony in 1983 and the Granath colony from the Yoshino colony in 1984. The Granath and Yoshino colonies were periodically supplemented with snails from J. Bruce (University of Lowell, Lowell, Massachusetts). Laboratories maintaining M-line snails identified additional factors that would be expected to reduce heterozygosity and lead to divergence in the M-line samples. Stocks have been reselected for susceptibility (Bayne, every 30–48 mo) and albinism (Richards, early to mid-1970s). Selection would be expected to reduce the number of genotypes in the population. Additionally, although all laboratories report maintaining their M-line stock in numbers greater than 50 individuals, occasional bottlenecks in population numbers have occurred. For example, Bayne (pers. comm.) reported having only 15 M-line

**Table 3.** Wright (1978)  $F$ -statistics for 5 colonies of M-line *Biomphalaria glabrata*.

Locus	$F_s$	$F_{it}$	$F_{st}$
ACON-2	0.261	0.474	0.288
PGD	0.323	0.492	0.249
PGM-1	0.384	0.449	0.106
EST-1	0.510	0.627	0.239
EST-4	0.205	0.372	0.210
XDH	0.309	0.351	0.060
PEP-1	0.425	0.437	0.022
PEP-4	0.269	0.425	0.213
ACP	0.967	0.973	0.167
$\bar{x}$	0.374	0.482	0.173

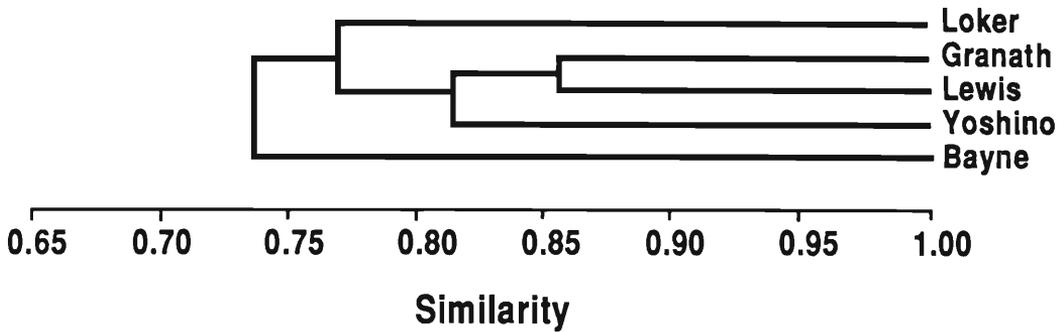


Figure 1. Genetic identity values for 5 colonies of M-line *Biomphalaria glabrata* clustered using unweighed pair group method of averaging. Cophenetic correlation = 0.85.

snails in December 1986. Again, these low numbers might be associated with genetic drift and would be expected to lead to a reduced number of genotypes and differentiation among samples. Finally, *B. glabrata* is a self-compatible hermaphrodite, and although outcrossing is the most frequent mode of reproduction, to whatever extent selfing occurs it would tend to reduce heterozygosity.

Several important points emerge from the data. M-line snails are not genetically homogeneous within populations. Additional genetic differentiation is apparent among populations. It is possible that some contamination of the stock has taken place. The M-line stock is as genetically polymorphic and heterozygous as any natural population of *Biomphalaria* examined to date.

High levels of electrophoretic variability observed for the M-line snails were most likely associated with interbreeding among stocks. Albino stocks of *B. glabrata* currently used may have separate origins and histories. The M-line was the result of laboratory selection to combine albinism and susceptibility. Another albino stock was isolated by Dr. Paul Thompson in 1953 from the field in Puerto Rico (J. Bruce, pers. comm.). This stock had been maintained by Drs. Henry

van der Schalie (deceased) and Elmer Berry (retired) at the University of Michigan and John I. Bruce at the University of Massachusetts-Lowell. Both stocks of *B. glabrata* display susceptibility to *S. mansoni* and albinism; however, because these stocks had separate origins, there is no reason to expect a single underlying genetic control for these traits. The observation of high genetic heterogeneity and polymorphism and the occurrence of 3 alleles for some loci would be consistent with mixing and interbreeding these 2 stocks.

One of the advantages of defined laboratory stocks is their potentially more uniform response to experimental manipulation. Our information suggests that it would be useful to clarify the history of *Biomphalaria* stocks used in research so that stock designations can be uniformly applied to single genetic stocks of snails and so that the genetic integrity of particular stocks can be maintained.

#### Acknowledgments

The authors are grateful to E. S. Loker, W. Granath, C. Bayne, F. Lewis, and T. Yoshino for making snails available. John I. Bruce, E. S. Lo-

Table 4. Comparison of  $F_{st}$  for studies with multiple samples of some species of *Biomphalaria*.

Species	Number and location of samples	$F_{st}$	References
<i>B. glabrata</i>	5 M-line colonies	0.173	This study
<i>B. glabrata</i>	6 West Indian samples	0.805	Mulvey et al., 1988
<i>B. glabrata</i>	7 Puerto Rican samples		Mulvey and Vrijenhoek, 1982
<i>B. straminea</i>	4 Hong Kong samples	0.098	Woodruff et al., 1985
<i>B. alexandrina</i>	11 Egyptian samples	0.200	Graven, 1984
<i>B. Pfeifferi</i>	12 Kenyan samples	0.589	Bandoni et al., 1990

ker, F. Lewis, C. S. Richards, M. Knight, and T. Yoshino provided comments on an earlier draft of the manuscript. Support came from contract DE-AC09-765SR00-819 between the U.S. Department of Energy and the University of Georgia Savannah River Ecology Laboratory and University/DOE Cooperative program for graduate research participation.

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Research Note

Biological Studies of Filamentous Bacteria Associated with Cyathostomes from a Burchell's Zebra Hindgut

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ABSTRACT: Nematode-filamentous microbe association with cyathostomes collected from the hindgut of a Burchell's zebra was quantified. Cyathostomes were examined for the presence of filamentous bacteria with the aid of light and scanning electron microscopy. Sixty-seven percent of the females and 3% of the male nematodes were affected. The presence of filamentous bacteria was noted at or near the vulvar opening in the 5 most abundant cyathostome species. These species of cyathostomes from the most abundant to the least abundant were as follows: *Cyathostomum montgomeryi*, *Cylicocycylus triramosus*, *Cylicostephanus minutus*, *Cylicostephanus calicatus*, and *Cyathostomum tetracanthum*. In *C. montgomeryi*, bacteria colonized the anal orifice. The uterine tracts of the female cyathostomes were examined for the presence of eggs as a possible indication of the reproductive capacity, but the results were inconclusive. In 1 species, *C. triramosus*, the filamentous bacteria formed a dense mass that prevented thorough examination of the bacteria's position in possible penetration of the reproductive tract.

KEY WORDS: filamentous bacteria, cyathostomes, nematodes, zebra.

Large numbers of parasitic nematodes inhabit the hindguts of free-ranging zebras, and microbial communities have been reported in association with 2 of these nematode groups, the cy-

athostomes and atractids (Mackie et al., 1989; Krecek et al., 1992). Previous reports include ultrastructural studies of the components of these communities as well as proposed life cycles of 3 of the filamentous bacteria (Krecek et al., 1987; Els et al., 1991).

Cultivation of these microbial organisms has been attempted, with varying success (Mackie et al., 1989; Krecek et al., 1992). Currently, nematode material is obtained from the hindguts of zebras during postmortem examinations in various national parks in southern Africa. Not all populations of nematodes examined harbored these microbial communities. When microbes are present, the nematodes are stored in liquid nitrogen for further cultivation studies. The present study is the first to quantify the association of the filamentous bacteria and their cyathostome host species. The prevalence of female and male cyathostomes affected and the particular species of cyathostomes associated with the filamentous bacteria and the anatomical sites of colonization on these cyathostomes are described.

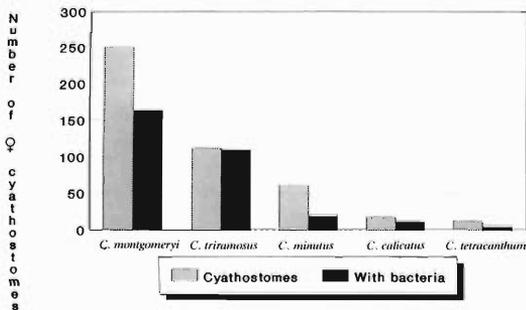


Figure 1. The number of females of the 5 most abundant cyathostome species associated with filamentous bacteria.

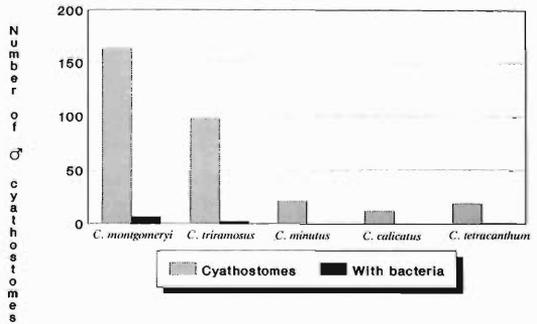


Figure 2. The number of males of the 5 most abundant cyathostome species associated with filamentous bacteria.

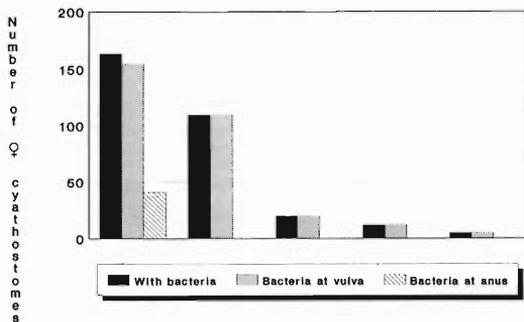
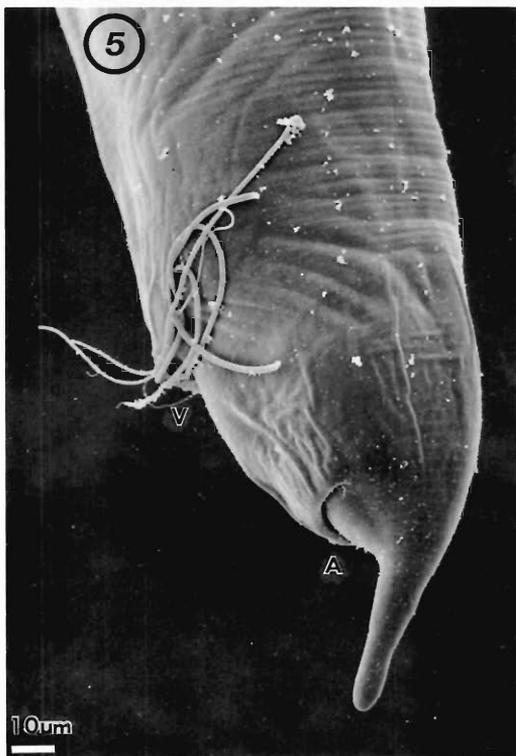


Figure 3. The number of female cyathostomes demonstrating filamentous bacteria at the vulvar and anal orifices.

A Burchell's zebra, *Equus burchelli antiquorum* H. Smith, 1841, from Etosha National Park, Namibia, was killed during August 1989 and processed at necropsy according to methods described previously (Malan et al., 1981; Els and Krecek, 1990). An aliquot of 100th of the ventral colon ingesta was examined, and the nematodes

were recovered, mounted on glass slides in lactophenol, a clearing agent, and identified to species level (Theiler, 1923; Lichtenfels, 1975; Scialdo-Krecek, 1984). The attachment sites of filamentous bacteria to the cyathostomes were noted, especially to the oral, excretory, anal, and vulvar orifices. The orifices were examined for the presence of microbes both inside and outside these openings. The vulvar orifice was examined for the presence of eggs in the reproductive tract. For each cyathostome species, the number of nematodes affected at each orifice was counted. This quantification for each species and the number of male and female cyathostomes affected were recorded.

Abundance of each nematode species was derived by dividing the separate species by the total count of the aliquot, 877. For each species, 55 females were examined and the data extrapolated to the total. If there were fewer than 55 females all were examined and all of the males were examined. The chi-square test was used to compare sexes and species with sexes. The binomial test



Figures 4, 5. *Cyathostomum montgomeryi* female with filamentous bacteria on the posterior extremity. 4. Light microscopy shows a bacterial plug in vagina (arrow) with embedded microbes. 5. Scanning electron microscopy (SEM) with anus (A) and vulva (V).

was used when comparing the association of bacteria at the vulva and anus among species.

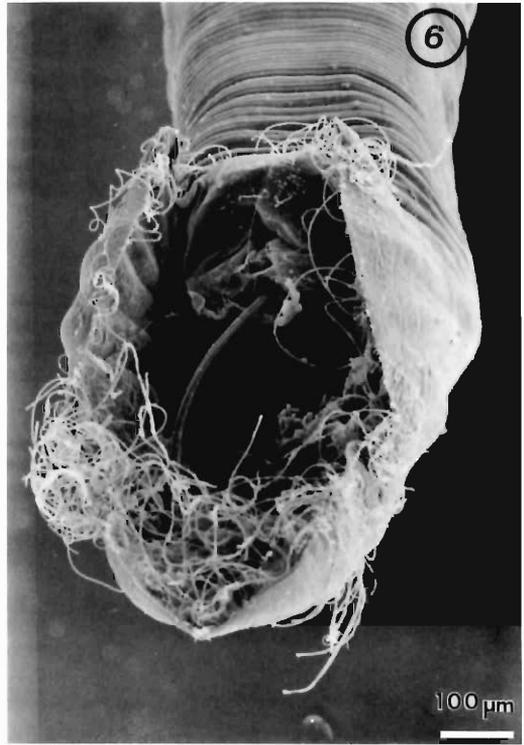
Whole cyathostomes were prepared for scanning and transmission electron microscopy by conventional techniques previously described (Els and Krecek, 1990).

The aliquot yielded 877 cyathostomes. Eight species were identified from the most to the least abundant as follows: *Cyathostomum montgomeryi* (Boulenger, 1920) K'ung, 1964, *Cylicocyclus triramosus* (Yorke and Macfie, 1920) Chaves, 1930, *Cylicostephanus minutus* (Yorke and Macfie, 1918) Cram, 1924, *Cylicostephanus calicatus* (Looss, 1900) Cram, 1924, *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861, in part, Looss, 1900, *Cylicodontophorus reineckeii* Scialdo-Krecek and Malan, 1984, *Cylicostephanus longiconus* Scialdo-Krecek, 1983, and *Cylindropharynx* sp. The presence of filamentous bacteria was not noted on the latter 2 species, and only 1 of 3 specimens of *C. reineckeii* demonstrated these bacteria. Therefore, these 3 species were not included in this study.

Among cyathostomes, 312 of 463 females examined and 11 of 414 males demonstrated an association with the microbes. The 5 most abundant cyathostome species in the population together with the proportion of females and males associated with the filamentous bacteria are given in Figures 1 and 2, respectively. Figure 3 indicates the presence of filamentous bacteria associated with the vulvar and anal orifices of the females of the 5 most abundant cyathostome species. Scanning electron and light microscopy demonstrate the presence of filamentous bacteria on females of *C. montgomeryi* and *C. triramosus* and a male cyathostome in Figures 4–8.

Various filamentous organisms inhabit mammalian guts including mice, rats, swine, and humans (Savage, 1983a, b). However, the cyathostomid nematode–filamentous bacterial association discussed here is, as far as can be determined, the first report of its kind in a mammalian host. This is the first attempt to quantify this association.

The predominance of female cyathostomes affected as compared to that of male cyathostomes is significant ( $P < 0.001$ ), and the colonization of the vulvar orifices in this population is evident in this study. The numbers of each species (Fig. 1) of female cyathostome were compared with and without bacteria and differed significantly from one another ( $P < 0.001$ ). There were, however, no significant differences among species of

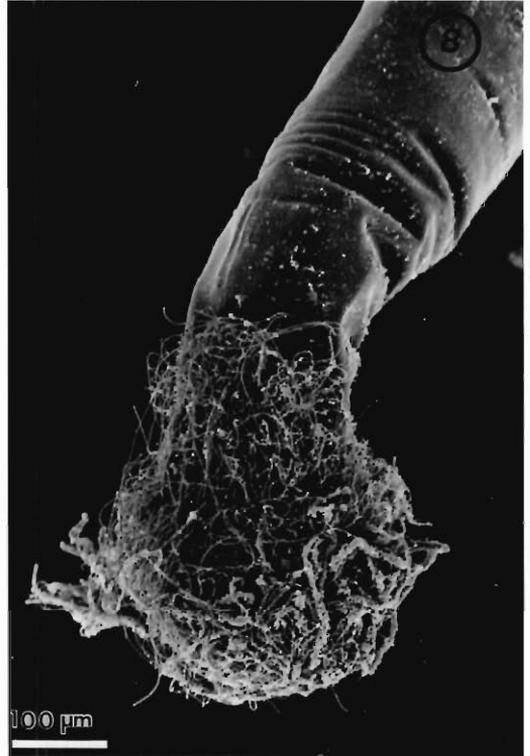


**Figure 6.** Ventral surface of dorsal ray of copulatory bursa of male cyathostome. Presence of filamentous bacteria suggests transmission during copulation.

male cyathostomes (Fig. 2) for a similar comparison. Numbers of female cyathostomes were compared for each species with the association of bacteria at the vulva and at the anus, and in all cases there was a significant difference ( $P \leq 0.031$ ).

Few adult male cyathostomes demonstrate the association, and only the ventral side of the copulatory bursa of the males is affected. Because this ventral side would clasp the female cyathostome during copulation, it is possible that the small numbers of males become affected at this time. It is possible that only reproductively successful males are colonized by the bacteria and these males infected all of the females.

When males are affected, it is always a smaller proportion than in females of the same species. In *C. triramosus*, the second most abundant species, most females (110 out of 112) were affected. Although the vulva is the orifice predominantly affected throughout, the anus of a small number of *C. montgomeryi* was sometimes colonized. Earlier studies suggested that the colonization of



Figures 7, 8. *Cylicocyclus triramosus* female with dense mass of filamentous bacteria on the posterior extremity. 7. Light microscopy shows mass of microbes near vulva (arrow). 8. Scanning electron microscopy showing mass of microbes.

the anus by filamentous bacteria was caused by the release of an excretory product that provided a suitable environment (Mackie et al., 1989). However, the present study indicates that the vulva, and not the anus, is the colonized site. Perhaps some substance in the female reproductive tract serves as a suitable substrate for colonization and, consequently, this association may influence egg production.

Uterine tracts of the cyathostomes in the present study were examined for the presence of eggs. Examination for the penetration of the microbes into the reproductive tract canal was inconclusive in some species, such as in *C. triramosus*, because the dense mass of microbes (Fig. 7) prevented thorough examination using light microscopy. In future studies, the penetration of these bacteria in the reproductive and digestive tracts could be examined with the use of differential staining and serial sectioning of the uterine tract of these females.

Observations such as those in the present study are urged by the difficulty to cultivate these fil-

amentous bacteria. Though attempts have been made (Krecek et al., 1992), this inability hampers possible studies on the dynamics of this association.

We thank Mr. T. E. Krecek and Miss R. Hartman for their technical assistance; Professor T. Britz for his helpful comments with the manuscript; and the Foundation for Research Development and the University of Pretoria for financial support. This study is part of the Wildlife Research Program at the Faculty of Veterinary Science, University of Pretoria.

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*J. Helminthol. Soc. Wash.*  
61(1), 1994, pp. 113–114

### Research Note

## *Sarcocystis felis* (Protozoa: Sarcocystidae) from the African Lion (*Panthera leo*)

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**ABSTRACT:** Sarcocysts of *Sarcocystis felis* were found in skeletal muscle of a 7-yr-old African lioness (*Panthera leo*) from Kenya, Africa. Sarcocysts were up to 1,650  $\mu\text{m}$  long and up to 150  $\mu\text{m}$  wide. The cyst wall was 1.0–1.5  $\mu\text{m}$  thick and had characteristic fingerlike villar projections.

**KEY WORDS:** *Sarcocystis felis*, lion, *Panthera leo*, Sarcocysts.

*Sarcocystis* spp. undergo a 2-host life cycle involving prey and predator animals. Infection of muscles by *Sarcocystis* spp. (sarcocysts) is common in herbivores. Until recently, infection of muscles by sarcocysts in carnivores was considered rare (Dubey et al., 1989). Sarcocysts were found in more than half of bobcats, cougars, and panthers examined in 3 studies (Greiner et al., 1989; Anderson et al., 1992; Dubey et al., 1992). Only one morphologic type of sarcocyst was found in bobcats (*Felis rufus*), domestic cats (*Felis do-*

*mesticus*), Florida panthers (*Felis concolor coryi*), and cougars (*Felis concolor floridanus*). Dubey et al. (1992) proposed the name *Sarcocystis felis* for *Sarcocystis* in muscles of Felidae. Although sarcocysts were reported previously from lions from India (Bhatavedkar and Purohit, 1963; Somvanishi et al., 1987) and Africa (Bwangamoi et al., 1990), the purpose of this article is to report species of *Sarcocystis* from the African lion, *Panthera leo* (*Felis leo*).

Specimens of heart and skeletal muscle from a 7-yr-old lioness from Nairobi National Park, Kenya, were fixed in 10% formalin solution (Bwangamoi et al., 1990). The lioness was euthanized because of rabies virus infection (Bwangamoi et al., 1990). Paraffin sections were cut at 5–6  $\mu\text{m}$  thickness and examined microscopically after staining with hematoxylin and eosin.

Sarcocysts were seen in 11 of 15 sections of

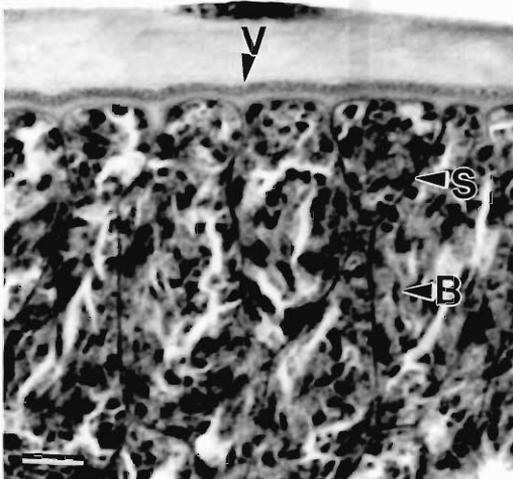


Figure 1. *Sarcocystis felis* sarcocyst in section of skeletal muscles from the African lioness. Note villar projections on the cyst wall (V), septa (S), and bradyzoites (B). Hematoxylin and eosin stain. Bar = 10  $\mu$ m.

skeletal muscle but not in 22 sections of the heart. Sarcocysts were up to 1,650  $\mu$ m long and up to 150  $\mu$ m wide, were septate, and contained mostly bradyzoites. The cyst wall was 1.0–1.5  $\mu$ m thick and had small fingerlike villar projections (Fig. 1).

One sarcocyst from the paraffin block was deparaffinized and examined ultrastructurally (Dubey et al., 1992). Although the sarcocyst was fixed poorly, the villar projections on the sarcocyst wall from the lioness were similar to those of *S. felis* from the bobcat (Dubey et al., 1992).

Structurally, the sarcocysts in the African lion were identical to *S. felis* sarcocysts in the bobcat

(Dubey et al., 1992). Sarcocysts were previously reported from lions from India (Bhatavedkar and Purohit, 1963; Somvanshi et al., 1987) and Africa (Bwangamoi et al., 1990), but it is difficult to judge the species involved from the descriptions provided by the authors. *Sarcocystis felis* sarcocysts were recently found in the musculature of 7 of 10 cheetahs (*Acinonyx jubatus*) from the U.S.A. (Briggs et al., 1993).

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## Research Note

# Prevalence and Isolation of *Toxoplasma gondii* from Wild Turkeys in Alabama

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**ABSTRACT:** Hearts from 16 adult, male wild turkeys (*Meleagris gallopavo*) from eastern central Alabama were examined for encysted *Toxoplasma gondii* by hydrochloric acid–pepsin digestion and mouse inoculation. *Toxoplasma gondii* was isolated from 8 (50%) of the 16 wild turkeys examined. Six of the 8 isolates caused fatal infections in mice following primary isolation; a seventh isolate did not cause death, although the mice had clinical signs of infection prior to necropsy. Sera and/or tissue fluids from these 16 wild turkeys and an additional wild turkey were examined for *T. gondii* antibodies in the modified direct agglutination test. Twelve (71%) of the 17 samples were positive at dilutions of 1:50 or higher.

**KEY WORDS:** *Toxoplasma gondii*, tissue cysts, bradyzoites, tachyzoites, wild turkeys, *Meleagris gallopavo*.

Little is known about the prevalence or importance of encysted *Toxoplasma gondii* Nicolle and Manceaux, 1909, in wild game birds. Howerth and Rodenroth (1985) described a case of suspected fatal systemic toxoplasmosis in a female wild turkey (*Meleagris gallopavo*) from Georgia. The carcass had been frozen prior to examination at necropsy. Gross lesions consisted of splenomegaly, focal to confluent gray areas of consolidation in the lungs, and 1–3-mm-diameter erosions in the cecum and colon. Microscopic examination of tissue sections stained with hematoxylin and eosin indicated that protozoans were present in the brain, spleen, lungs, liver, adrenal glands, kidneys, esophagus, proventriculus, and colon. Ultrastructural examination of parasites in the lungs was suggestive of *T. gondii*. A direct fluorescent antibody test performed on a section of the spleen yielded results suggestive of the presence of *T. gondii*. Howerth and Rodenroth (1985) attempted to demonstrate *T. gondii* in mice inoculated with splenic tissues from the wild turkey but failed to isolate the parasite. Interestingly, Burrige et al. (1979) examined serum samples from 20 wild turkeys in Florida using the indirect hemagglutination test but failed to detect antibodies to *T. gondii* in any of the birds.

The present study was conducted to determine the prevalence of *T. gondii* in free-ranging wild turkeys and to attempt to isolate the parasite from their tissues. Experimental studies in domestic turkeys have demonstrated that the heart is most often infected with *T. gondii* tissue cysts and that the modified direct agglutination test (MDAT) using formalin-fixed tachyzoites will detect *T. gondii* antibodies in their sera (Dubey et al., 1993).

The hearts from 16 hunter-killed and sera/tissue fluids from 17 hunter-killed male wild turkeys were examined. The turkeys were from eastern central Alabama originating in Lee, Macon, and Russell counties. Hunters eviscerated the wild turkeys, placed the heart and viscera in a plastic food storage bag, and refrigerated the samples until they were delivered to the College of Veterinary Medicine, Auburn University. Blood and contaminating tissue fluid were collected from the plastic bags and centrifuged in a microfuge, and the supernatant was collected and examined in the MDAT for antibodies against *T. gondii* at dilutions of 1:25, 1:50, and 1:500 as described by Dubey and Desmonts (1987). Nineteen to 25 g of heart tissue was collected from each bird and digested individually in hydrochloric acid–pepsin solution (0.52 g pepsin, 0.5 g NaCl, 1.4 ml concentrated HCl, and 98.6 ml distilled water) and used for subcutaneous inoculations of groups of 4–5 female, 20–24-g Hsd:ICR mice. Four weeks later, serum was collected from all surviving mice and examined for IgG antibodies to *T. gondii* at dilutions of 1:50 and 1:100 in an indirect immunofluorescent antibody assay against RH isolate *T. gondii* tachyzoites as described by Lindsay et al. (1990). After serum was collected, the mice were killed, and squashes from their cerebrums were examined unstained for *T. gondii* tissue cysts as described by Lindsay et al. (1991). Smears were made from the lungs and/or cerebrums of mice that died during the study and examined as unstained preparations for stages of *T. gondii*.

**Table 1. Serological prevalence and results of isolation of *Toxoplasma gondii* from wild turkeys from Alabama.**

Turkey	Heart*	NI/NP/NPD†	% mortality‡	MDAT§
1	NA	NA/NA/NA	NA	1:50
2	25	5/5/1	20	1:50
3	25	4/4/4	100	1:50
4	25	4/4/1	25	1:50
5	25	4/4/1	25	1:50
6	21	4/0/0	0	<25
7	19	4/0/0	0	1:50
8	25	5/0/0	0	1:50
9	25	4/0/0	0	1:50
10	25	4/4/0	0	1:50
11	25	4/0/0	0	1:50
12	25	4/4/1	25	1:50
13	25	4/4/0	0¶	<25
14	25	4/0/0	0	<25
15	22	5/0/0	0	<25
16	22	4/4/4	100	1:500
17	25	4/0/0	0	<25

\* Number of grams of heart used for digestion and mouse bioassay.

† Number of mice inoculated/number of mice positive for *T. gondii*/number of mice that died from inoculation of heart tissue.

‡ Percentage of mice inoculated with that isolate that died.

§ Titers obtained in the MDAT for *T. gondii*.

|| Not applicable.

¶ No mice inoculated with this isolate died; however, mice had clinical signs of infection prior to necropsy.

*Toxoplasma gondii* was isolated from 8 (50%) of the 16 wild turkey hearts examined (Table 1). Six (75%) of the 8 isolates caused fatal infections in mice on primary isolation (Table 1). Prior to necropsy, mice in another group had clinical signs of weight loss and rough hair coats that were suggestive of *T. gondii* infection. Results of the modified direct agglutination tests demonstrated that 12 (71%) of the 17 samples had titers  $\geq$  1:50 (Table 1). *Toxoplasma gondii* was isolated from the heart of 1 wild turkey that was negative at 1:25 in the MDAT (Table 1).

The prevalence of *T. gondii* infection in wild turkeys from Alabama in the present study is much higher than that reported by Burrige et al. (1979) for wild turkeys from Florida. Burrige et al. (1979) found no positive samples from the 20 wild turkeys they examined using an indirect hemagglutination test. However, negative serological reactions to *T. gondii* in these birds were probably a result of the serological test employed. Avian sera do not react as do mammalian sera in all serological tests (Frenkel, 1981), and tur-

keys experimentally infected with *T. gondii* do not develop titers or have low titers in the indirect hemagglutination test (Dubey et al., 1993). We used the MDAT to detect *T. gondii* antibodies in wild turkey sera because the MDAT has been validated in domestic turkeys (Dubey et al., 1993) and other wild birds (Kirkpatrick et al., 1990; Lindsay et al., 1991; Dubey et al., 1992).

In the present study, the serological prevalence of *T. gondii* was 71%, and the direct isolation prevalence was 50%. In one instance, *T. gondii* was isolated from the heart, but the serum/tissue fluid had a titer of <1:25 and was considered negative (Table 1). This may represent a recent infection in this bird, in which case it did not have sufficient time to mount a serological immune response. Alternatively, the bird may have had an antibody titer that was less than 1:25 and not detected. The lack of isolation of *T. gondii* from 4 wild turkeys that were MDAT-positive (Table 1) probably indicates that no tissue cysts were present in the heart but may have been present in other tissues. *Toxoplasma gondii* has been isolated from the brain, heart, breast muscles, and leg muscles of experimentally infected domestic turkeys (Drobeck et al., 1953; Dubey et al., 1993).

Ground-feeding birds, such as wild turkeys, probably acquire infection with *T. gondii* by ingesting sporulated oocysts from the soil. Feral cats (*Felis catus*) and bobcats (*Felis rufus*) are likely sources of oocysts of *T. gondii* for free-ranging wild turkeys.

The high prevalence of encysted *T. gondii* in wild turkeys indicates that these birds are potential sources of human infection. Freezing ( $-12.4^{\circ}\text{C}$  for <1 min) and adequate cooking ( $\geq 64^{\circ}\text{C}$  for 3.6 min) readily kill *T. gondii* tissue cysts in pork (Dubey et al., 1990; Kotula et al., 1991); therefore, common food preparation practices should kill most bradyzoites in tissue cysts in wild turkey tissue. Viscera from wild turkeys should not be fed to domestic cats. If turkeys are field-dressed, then the viscera should be buried or burned to prevent infection in wild felids or other animals.

We thank Natasha S. Rippey and Lisa C. Parsons for technical assistance. We thank Dr. J. P. Dubey, USDA, Zoonotic Diseases Laboratory, Beltsville, Maryland, for conducting the MDAT on turkey sera/tissue fluids. This study was supported in part by funds from the Department of Pathobiology, College of Veterinary Medicine, Auburn University.

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J. Helminthol. Soc. Wash.  
61(1), 1994, pp. 117-121

## Research Note

Seroprevalence of *Toxoplasma gondii* in Wild Mammals in Kansas

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**ABSTRACT:** Between 1989 and 1993, serum samples from 516 mammals in Kansas representing 17 species were examined for antibodies to *Toxoplasma gondii* using a modified direct-agglutination test. The overall prevalence was 84/516 (16%) mammals infected. When considering only animals where adequate sample sizes were available, the highest prevalences were found in raccoons (*Procyon lotor*), of which 14/20 (70%) were infected; white-tailed deer (*Odocoileus virginianus*), with 47/106 (44%) seropositive; and Virginia opossums (*Didelphis virginiana*), with a prevalence of 9/28 (32%). The seroprevalence in rodents and pronghorn antelope was <8%.

**KEY WORDS:** *Toxoplasma gondii*, coccidia, survey, deer, raccoon, Kansas.

The intermediate host range of *Toxoplasma gondii* (Nicolle and Manceaux, 1908) Nicolle and Manceaux, 1909, is unusually wide for a coccidian, currently comprising >350 known species of vertebrates (Beyer and Poljansky, 1989). Felids, the definitive hosts, are thought to become infected principally by ingesting tissues of infected mammals and birds, and most cats are seropositive by weaning time (Dubey and Beatlie, 1988).

Although numerous studies have examined the seroprevalence of antibodies to *T. gondii* in humans and wild and domestic animals, limited information exists concerning the seroprevalence of this coccidian in Kansas. Lindsay et al.

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**Table 1. Seroprevalence of *Toxoplasma gondii* in various wild mammals from Kansas.**

Host	County	No. positive/ No. tested (%)		Antibody titer			
				<1:25	1:25	1:50	≥ 1:500
<i>Antilocapra americana</i> (pronghorn antelope)	Greeley	0/02		2	0	0	0
	Logan	0/16		16	0	0	0
	Sherman	1/11	(9)	10	1	0	0
	Wallace	2/34	(6)	32	1	1	0
Subtotal		3/63	(5)				
<i>Blarina hylophaga</i> (Elliott's short-tail shrew)	Geary	0/01		1	0	0	0
<i>Castor canadensis</i> (beaver)	Riley	0/02		2	0	0	0
<i>Didelphis virginiana</i> (Virginia opossum)	Coffey	3/07	(43)	4	2	1	0
	Lyon	5/16	(31)	11	3	2	0
	Pottawatomie	0/01		1	0	0	0
	Riley	1/04	(25)	3	1	0	0
Subtotal		9/28	(32)				
<i>Dipodomys ordii</i> (Ord kangaroo rat)	Harvey	0/05		5	0	0	0
	Morton	0/10		10	0	0	0
<i>Mephitis mephitis</i> (striped skunk)	Morris	0/01		1	0	0	0
<i>Microtus ochrogaster</i> (prairie vole)	Mitchell	0/01		1	0	0	0
	Sherman	1/01	(100)	0	0	1	0
<i>Mus musculus</i> (house mouse)	Pottawatomie	0/06		6	0	0	0
	Riley	0/04		4	0	0	0
	Sherman	0/01		1	0	0	0
<i>Neotoma floridana</i> (eastern woodrat)	Geary	1/02	(50)	1	1	0	0
	Greenwood	0/01		1	0	0	0
	Lyon	0/01		1	0	0	0
	Pottawatomie	1/24	(4)	23	1	0	0
Subtotal		2/28	(7)				
<i>Odocoileus virginianus</i> (white-tailed deer)	Chautauqua	10/16	(63)	6	2	3	5
	Crawford	2/08	(25)	6	1	0	1
	Ellis	1/01	(100)	0	1	0	0
	Finney	0/02		2	0	0	0
	Jefferson	0/01		1	0	0	0
	Linn	6/15	(40)	9	0	1	5
	Lyon	4/06	(67)	2	1	1	2
	Marshall	2/05	(40)	3	0	2	0
	Mitchell	0/01		1	0	0	0
	Rawlins	2/02	(100)	0	1	1	0
	Reno	2/02	(100)	0	2	0	0
	Riley	18/46	(39)	28	5	6	7
	Thomas	0/01		1	0	0	0
Subtotal		47/106	(44)				
<i>Peromyscus leucopus</i> (white-footed mouse)	Cherokee	1/22	(5)	21	0	1	0
	Geary	1/10	(10)	9	0	1	0
	Mitchell	0/04		4	0	0	0
	Osborne	0/12		12	0	0	0
	Pottawatomie	0/41		41	0	0	0
	Riley	0/13		13	0	0	0
	Russell	0/11		11	0	0	0
	Trego	0/02		2	0	0	0
Subtotal		2/115	(2)				
<i>Peromyscus maniculatus</i> (deer mouse)	Cherokee	0/01		1	0	0	0
	Geary	0/06		6	0	0	0
	Greenwood	0/03		3	0	0	0
	Osborne	0/09		9	0	0	0
	Pottawatomie	0/03		3	0	0	0
	Riley	1/05	(20)	4	0	1	0
	Russell	2/24	(8)	22	2	0	0
	Sherman	0/05		5	0	0	0
Subtotal		3/56	(5)				

Table 1. Continued.

Host	County	No. positive/ No. tested (%)		Antibody titer			
				< 1:25	1:25	1:50	≥ 1:500
<i>Procyon lotor</i> (raccoon)	Coffey	2/04	(50)	2	0	0	2
	Lyon	11/15	(73)	4	0	5	6
	Riley	1/01	(100)	0	0	0	1
Subtotal		14/20	(70)				
<i>Reithrodontomys megalotis</i> (western harvest mouse)	Osborne	1/02	(50)	1	1	0	0
<i>Sigmodon hispidus</i> (hispid cotton rat)	Butler	0/12		12	0	0	0
	Cherokee	0/05		5	0	0	0
	Geary	0/10		10	0	0	0
	Greenwood	0/01		1	0	0	0
	Mitchell	0/03		3	0	0	0
	Osborne	0/12		12	0	0	0
	Pottawatomic	0/01		1	0	0	0
	Riley	0/10		10	0	0	0
	Russell	0/08		8	0	0	0
<i>Sylvilagus floridana</i> (eastern cottontail rabbit)	Osborne	1/02	(50)	1	0	1	0
<i>Vulpes velox</i> (swift fox)	Wallace	1/02	(50)	1	0	1	0
Overall prevalence		84/516	(16%)				

(1990) found 57/229 (25%) dogs in Kansas seropositive using the direct agglutination test, and Dubey (1973) reported 16% of cats in Kansas seropositive by the Sabin-Feldman dye test. A recently published nationwide survey of *T. gondii* in swine suggests a seroprevalence of about 21% in Kansas and Nebraska (Dubey et al., 1991). Because we are aware of no studies examining the seroprevalence of *T. gondii* in Kansas wildlife, we chose to conduct a statewide serologic survey for the parasite in various wildlife species.

Animals were collected during all seasons between December 1989 and March 1993. Rodents were captured using Sherman live-traps baited with a combination of peanut butter and oatmeal. Blood was collected from each animal by direct heart puncture following the administration of an overdose of ether. Blood from other mammals was obtained from the Kansas Department of Wildlife and Parks either at check stations during hunting season or by trapping. Blood was centrifuged at 800 g, and serum was collected and frozen at -20°C until tested. Sera were shipped frozen to the Zoonotic Diseases Laboratory, USDA, Beltsville, Maryland, where they were tested at titers of 1:25, 1:50, and 1:500 using the modified direct agglutination technique (Dubey et al., 1991). Titers at or above 1:25 were considered positive, as has been utilized previously (Dubey et al., 1991). The direct agglutination technique was chosen because of its ease

of use, reproducibility, and sensitivity (Dubey and Beattie, 1988; Dubey et al., 1993) and because we felt that complement fixation tests may not be as accurate for some of the hemolysed samples inherent in this type of survey.

Antibodies to *Toxoplasma gondii* were found in 84/516 (16%) mammals tested (Table 1). Of those animals where adequate (> 10) sample sizes were obtained, highest prevalences were found in raccoons, *Procyon lotor* (70%), white-tailed deer, *Odocoileus virginianus* (44%), and Virginia opossums, *Didelphis virginiana* (32%). Although different serologic tests can give varying results, raccoon and deer seem to have relatively high seroprevalences in the U.S.A. (Walton and Walls, 1964; Franti et al., 1976; Burrige et al., 1979; Lindsay et al., 1991; Dubey et al., 1992), and it is not surprising that opossums are commonly infected considering their omnivorous habits and that they have been reported previously as infected (Walton and Walls, 1964; McCulloch et al., 1967; Franti et al., 1976; Burrige et al., 1979; Frenkel and Sousa, 1983). We also found a rabbit and a fox to be infected, but sample sizes were too low to obtain an accurate prevalence. However, both of these hosts, as well as many additional medium-sized carnivores and omnivores, are considered common carriers of toxoplasmosis (Morris et al., 1956; Havlík and Hübner, 1958; Eyles et al., 1959; Kulasiri, 1962; Galuzo et al., 1964; Walton and Walls, 1964; McCulloch

et al., 1967; Čatár, 1972; Šíma and Rašín, 1973; Doby et al., 1974; Franti et al., 1976; Marchiondo et al., 1976; Burrige et al., 1979; Schowalter et al., 1980; Frenkel and Sousa, 1983; Beyer and Shevkunova, 1986; Dubey and Beattie, 1988; McCue and O'Farrell, 1988).

The seroprevalence in pronghorn antelopes was about 5%. Although *T. gondii* has been previously isolated from the skeletal muscle of pronghorn (Dubey, 1981), this apparently represents the largest number of animals of this species surveyed at one time. The low prevalence in pronghorns when compared to that of white-tailed deer may be due to geographic locality and climate, as all samples from antelopes were collected in the western portions of the state, where human populations are lower and where vegetation is less dense.

Our results are consistent with others and show that most species of rodents have a relatively low prevalence of toxoplasmosis (Perrin et al., 1943; Morris et al., 1956; Eyles et al., 1959; Pokorný et al., 1961; Umiński et al., 1961; Kulasiri, 1962; Galuzo et al., 1964; Smith and Munday, 1965; Stroczyńska and Umiński, 1967; Dymon et al., 1971, 1988; Čatár, 1972; Doby et al., 1974; Chinchilla, 1978; Burrige et al., 1979; Ruiz and Frenkel, 1980; Frenkel and Ruiz, 1981; Dubey, 1983; Hay et al., 1983; Jackson et al., 1986; de Diego et al., 1987). Only in a few species such as Old World rats (*Rattus* spp.), house mice (*Mus musculus*), bandicoot rats (*Thylacis obesulus*), and water rats (*Hydromys chrysogaster*) have higher prevalences sometimes been found (Pope et al., 1957; Galuzo et al., 1964; Smith and Munday, 1965; Rifaat et al., 1973; Chinchilla, 1978; Dubey et al., 1981; Zardi et al., 1983; Childs and Seegar, 1986; Dymon et al., 1988). Some of these reports appear to represent isolated epizootic foci, although *Rattus* spp. often seem to have a somewhat higher prevalence than many other rodents.

The authors thank Oliver Kwok and S. Krumin for performing serologic tests for *T. gondii*. This research was supported, in part, by Kansas Agricultural Experiment Station grant No. KAN081865 to S.J.U. This is Kansas Agricultural Experiment Station Contribution No. 93-509-J.

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**Research Note**

**Enhanced Development of *Cryptosporidium parvum* In Vitro by Removal of Oocyst Toxins from Infected Cell Monolayers**

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**ABSTRACT:** Previous studies have suggested that coccidian oocysts of the genus *Eimeria* contain toxic substances that can inhibit parasite development in cultured cells. To examine whether or not oocysts of *Cryptosporidium parvum* may also contain such toxic substances, Madin-Darby bovine kidney cells were plated into 24-well tissue culture plates in RPMI 1640 medium supplemented with 10% fetal bovine serum and inoculated with CsCl purified oocysts of *C. parvum*. Plates were sealed in candle jars at 37°C so that sporozoites could excyst. After 3 hr, candle jars were opened, and the medium containing excysted and unexcysted oocysts was removed and replaced with fresh medium. <sup>3</sup>H-uracil was then added to wells, and plates were reincubated for an additional 65 hr at 37°C in candle jars. Control cultures consisted of infected cultures sealed and incubated in candle jars but not opened, as well as infected cultures exposed to atmospheric oxygen concentrations but not washed free of old medium. Parasite growth was measured as incorporation of [<sup>3</sup>H]uracil by scintillation counting. Results revealed that washing inoculated monolayers and then adding fresh medium significantly enhanced uracil incorporation.

**KEY WORDS:** *Cryptosporidium parvum*, Apicomplexa, coccidia, in vitro, cell culture, uracil, candle jar.

Recent studies have suggested that sporozoites of *Cryptosporidium parvum* are frail and must be manipulated minimally following excystation (Woodmansee, 1987; Robertson et al., 1993). Even washing free sporozoites using centrifugation appears to reduce the numbers of viable sporozoites (Woodmansee et al., 1987). Because previous studies had shown that *C. parvum* is capable of excysting at 37°C without the use of traditional excystation conditions such as trypsin and bile salts (Fayer and Leek, 1984; Woodmansee, 1987), Upton et al. (1991) chose to inoculate cell monolayers directly with intact oocysts so that sporozoites would have immediate access to host cells upon excystation. However, inoculating intact monolayers with *C. parvum* oocysts has the potential of adversely affecting the cell monolayer. Several studies have shown the presence of toxic substances within coccidian

oocysts that might affect development (Burns, 1959; Rickimaru et al., 1961; Sharma and Foster, 1964; Patton, 1965; Fayer and Hammond, 1967; Doran, 1970). Sharma and Foster (1964) found that sporozoites and oocyst and sporocyst walls of *Eimeria tenella* were nonlethal for rabbits following intravenous inoculation whereas fluids in oocysts were highly toxic in vivo. Fayer and Hammond (1967) found that fluid extracts from *Eimeria bovis* oocysts were toxic to bovine cells in vitro. Patton (1965) suggested that oocyst and sporocyst debris were toxic to cell monolayers infected with *E. tenella*.

The preceding studies collectively suggest that although inoculating monolayers directly with oocysts of *C. parvum* may be an effective method of establishing infections in vitro, further studies are needed to determine whether or not toxic substances liberated by this parasite upon excystation have a negative impact on parasite development. Below we present results that demonstrate the adverse effect of toxic substances associated with *C. parvum* oocysts on development of this parasite in cultured cells.

*Cryptosporidium parvum* was passaged through 2-5-day-old goats (*Capra hircus*) as described previously (Tilley and Upton, 1990). Oocysts were partially purified using discontinuous sucrose gradients (Arrowood and Sterling, 1987) and then further purified using CsCl gradients (Taghi-Kilani and Sekla, 1987). Oocysts purified by CsCl gradient purification were resuspended in 10% (v/v) aqueous Clorox® bleach for 10 min on ice. Oocysts were then washed 3 times with ice-cold, sterile distilled water and 2 times with ice-cold, sterile phosphate-buffered saline (PBS) (pH 7.2). At the time when oocysts were added to the cells, a small aliquot in the experimental medium was removed, placed in a separate test tube, and incubated at 37°C for 60 min. After 1 hr, the sample was viewed under ×40, and the first 100 oocysts observed were counted to determine percentage of excystation. Aliquots wherein <50% of the oocysts were excysted after

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1 hr were deemed unsuitable for in vitro studies, and the experiments were terminated.

The cell culture medium consisted of RPMI 1640 with L-glutamine, supplemented with 0.02 g/ml sodium bicarbonate, 10 mM HEPES, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B. For routine cell passage, 5% fetal bovine serum (FBS) was used, whereas experiments with parasites employed 10% FBS.

Madin-Darby bovine kidney cells (ATCC #6071) were maintained in 75 cm<sup>2</sup> tissue culture flasks. A trypsin/ethylenediamine tetraacetic acid (EDTA) (trypsin 1:25/0.53 mM EDTA in PBS) solution was then added to lift the cells off the plate. Cells were routinely tested for contamination with species of *Mycoplasma* using 4',6-diamidino-2-phenylindole stain (Russell et al., 1975; Mitchell and Finch, 1977; Uphoff et al., 1992).

Four hours prior to inoculation with *C. parvum*, cells were plated into 24-well cluster plates at a concentration of  $1.5 \times 10^5$  viable cells in 0.5 ml medium. Preliminary experiments showed that this concentration of cells allowed near-confluent monolayers to be achieved after about 68 hr postinoculation. Cell viability was assessed using trypan blue exclusion (0.15% w/v in PBS), and numbers were quantitated using a hemacytometer. Plates were incubated in a 37°C humidified incubator supplemented with 5% CO<sub>2</sub> until inoculated with oocysts.

Oocysts were added to tissue culture wells in ½ final volume of medium with 15% FBS, bringing the FBS concentration in all wells to 10%. Various concentrations of oocysts were examined, ranging from 1.75–6.25 × 10<sup>5</sup>/well (Fig. 1). The plates were then placed in prewarmed desiccator jars (candle jars), a candle inside was lit, and the jars were sealed with stopcock grease. After 3 hr at 37°C, plates were removed from some of the candle jars, the medium removed from some of these, and fresh medium with 10% FBS added. [<sup>3</sup>H]uracil was also added to wells at this time at a concentration of 4 µCi/well (specific activity 37.7 Ci/mmol). Previous studies have shown these parasites to incorporate free uracil (Upton et al., 1991). Plates removed from candle jars were then placed back in candle jars, resealed following relighting of the candles, and incubated at 37°C for an additional 65 h. Control cultures consisted of infected and noninfected wells without [<sup>3</sup>H]uracil, cultures incubated within candle jars without breaking the seal, and cultures in-

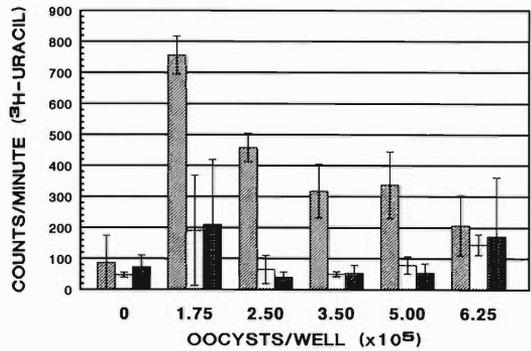


Figure 1. Effects of washing Madin-Darby bovine kidney cells 3 hr postinoculation (PI) with oocysts of *Cryptosporidium parvum*. Diagonal lines represent wells washed 3 hr PI; stippling represents those wells placed under same conditions as washed wells, removed from candle jar and incubator, placed at room temperature for 45 min, and then returned to the jar without replacing media. Cross-hatching represents those unwashed wells left in a second sealed candle jar at 37°C during the entire procedure. Experiments were performed in 24-well plates with 1 ml medium and 4 µCi [<sup>3</sup>H]uracil per well. Cells were plated 4 hr preinoculation at a concentration of  $1.5 \times 10^5$  per well. Incubation was at 37°C in a sealed candle jar for 65 hr postwashing. All data points represent the mean ± SD of 4–6 replicates.

cubated in candle jars, exposed to atmospheric conditions, and then resealed in candle jars without a change of medium. For all experiments, tissue culture plates were examined immediately upon removal from candle jars by using an inverted microscope at ×20–40. Any well detected to have either bacterial or fungal contamination, or sloughing of the monolayer greater than what appeared to be 25% of the total cells, was eliminated from further consideration. Cells were then harvested by vacuum aspiration onto 0.45-µm pore size glass fiber filters using an Inotech cell harvester (Inotech Biosystems International, Lansing, Michigan). Medium was removed by aspiration, and each well was washed 2 times with distilled water. To each well, 100 µl of 10% (w/v) trichloroacetic acid (TCA) (5 g/50 ml) was added, maintaining vacuum to prevent backwash. After 8–10 min, the TCA was then removed and wells washed 2 additional times with distilled water. To each well, 0.2 M NaOH was then added at a concentration of 400 µl/well, again maintaining vacuum. After 5 min, plates were then washed and aspirated onto filters 3 times with distilled water, followed by 2 washes with 70% EtOH. Filters were detached from the

harvester and placed in a 37°C incubator until dry. Once dried, the filter discs corresponding to each well were placed in 5-ml plastic scintillation vials with 2 ml scintillation cocktail for counting in a Beckman LS 6000SC scintillation counter.

Four to 6 wells were employed for each variable. Data are presented as the mean of 4–6 wells  $\pm$  SD of the mean. Individual data points were compared among and between groups using a 2-tailed Mann-Whitney *U*-test. In addition, all 3 treatments for each oocyst concentration were compared using the Kruskal-Wallis test. Differences were considered significant for both tests when  $P \leq 0.05$ .

Two of 5 (40%) experiments resulted in enough development beyond uninfected control cultures to be termed successful. All unlabeled control cultures had tritium counts  $< 20$  counts per minute and are not shown in Figure 1. These studies suggested that washing empty and unexcysted oocysts from the monolayers, as well as fluids released from oocysts during excystation, has a positive effect on [ $^3\text{H}$ ]uracil incorporation (Fig. 1). After comparison using both tests, it was determined that washing significantly increased uracil incorporation when all oocyst concentrations were employed except  $6.25 \times 10^5$  ( $P < 0.05$ ). Thus, it appears that debris and/or oocyst fluid from excystation does have a negative effect on *in vitro* quantitation of *C. parvum*.

Multiple concentrations of oocysts were added to wells for each experiment. Results of these and additional studies in our laboratory have suggested that an oocyst-to-host cell ratio of about 1:1 to 1:2 results consistently in highest uracil incorporation. However, these numbers are highly variable as the percentages of oocysts that excyst within culture vary considerably among experiments. It should be noted that Rasmussen et al. (1993) also found that better parasite development can be achieved with low parasite-to-host cell ratios.

Many studies have collectively demonstrated *Cryptosporidium parvum* to be capable of completing at least some development in a variety of cultured cells (Woodmansee and Pohlenz, 1983; Current and Haynes, 1984; Naciri et al., 1986; Wagner and Prabhu Das, 1986; Lumb et al., 1988; Datry et al., 1989; Bonnin et al., 1990; McDonald et al., 1990; Aji et al., 1991; Buraud et al., 1991; Flanigan et al., 1991; Gut et al., 1991; Kuhls et al., 1991; Upton et al., 1991; Marshall and Flanigan, 1992; Martinez et al., 1992; Rasmussen et al., 1993; Rosales et al.,

1993). Although these studies have helped define some parameters that enhance development of this parasite *in vitro*, additional studies are needed to provide more reliable, reproducible, and cost-effective *in vitro* systems that will allow for both studying the basic biology of the parasite and for large-scale pharmaceutical testing.

This work is a portion of a thesis entitled "Development of a  $^3\text{H}$ -Uracil Incorporation Assay to Monitor Development of *Cryptosporidium parvum*," submitted by M.T.E. in partial fulfillment of the requirement for a Master of Science degree in Biology, Kansas State University. This research was supported by NIH grant AI31774 to S.J.U. and is Kansas Agricultural Experiment Station contribution No. 93-374-J.

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## Research Note

# Hatching Dynamics of Eggs as Further Evidence for the Existence of Two Separate Species of *Sphaeridiotrema* (Digenea) in Eastern North America

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**ABSTRACT:** The hatching rate and success was determined and compared for 2 recently distinguished species of digeneans in the genus *Sphaeridiotrema*: *S. globulus* and *S. pseudoglobulus*. Hatching success did not differ between the 2 species, but the mean hatching time at 20°C for *S. pseudoglobulus* ( $18.0 \pm 0.1$  days) was almost 10 days less than that observed for *S. globulus* ( $27.9 \pm 0.2$  days). These results provide further evidence for the existence of 2 separate species.

**KEY WORDS:** *Sphaeridiotrema globulus*, *Sphaeridiotrema pseudoglobulus*, eggs, hatching.

Recent studies (McLaughlin et al., 1993) have demonstrated that 2 species of *Sphaeridiotrema* (Digenea) occur in waterfowl in eastern North America. *Sphaeridiotrema globulus* (Rudolphi, 1814) occurs in waterfowl in New England (Roscoe and Huffman, 1982, 1983); *Sphaeridiotrema pseudoglobulus* McLaughlin, Scott, and Huffman, 1993, occurs in waterfowl along the St. Lawrence River and its tributaries in southern Québec (Hoeve and Scott, 1988). Both species have been implicated in waterfowl mortality (Price, 1934; Huffman and Roscoe, 1986; Hoeve and Scott, 1988). The life history stages and intermediate hosts of both species are known from natural infections (Huffman and Fried, 1983; Ménard and Scott, 1987). However, neither life cycle has been studied experimentally and few details are available.

This study examines the comparative hatching dynamics of the eggs as part of a larger study on the life cycles of the 2 species. An unexpected consequence of this work was the observation that the egg hatching dynamics differed significantly between the 2, providing further evidence to separate *S. globulus* from *S. pseudoglobulus*.

Metacercariae of *S. pseudoglobulus* were obtained from naturally infected *Bithynia tentaculata* collected from Rivière du Sud, Québec, Canada. Metacercariae of *S. globulus*, obtained from naturally infected *Goniobasis virginica* from Lake Musconetcong, New Jersey, were kindly supplied by Dr. J. E. Huffman, East Stroudsburg

University of Pennsylvania. Three lots of about 65 *S. pseudoglobulus* and 5 lots of about 65 *S. globulus* metacercariae were fed to 3 and 5 12-day-old pekin ducklings (*Anas platyrhynchos dom.*) (Brome Lake Duck Farms, Knowlton, Québec), respectively. Eggs were harvested on days 5 and 6 pi by passing the duck droppings through a series of screens (120, 70, and 37  $\mu\text{m}$ ). The eggs were caught on the 37- $\mu\text{m}$  screen and separated from the fine debris by repeated sedimentation. Eggs collected on days 5 and 6 pi were pooled and, for experimental purposes, assumed to have been collected at the same time.

For each species, 6 24-well tissue culture plates were filled with water (3 ml/well), and a single egg was deposited in each well. The plates were then incubated simultaneously at 20°C ( $\pm 1^\circ\text{C}$ ) under constant incandescent illumination. Eggs were examined daily for 40 days and were considered hatched when the operculum was open and the miracidium was free of the egg. Eggs not hatching during this period or not exhibiting development were considered dead.

There was no significant difference in hatching success between the two species (*G*-test,  $G = 3.06$ ,  $P = 0.080$ ). Overall, 90% of the *S. pseudoglobulus* and 83% of the *S. globulus* eggs hatched. The hatching times of all viable *S. pseudoglobulus* and *S. globulus* eggs are shown in Figure 1. There was almost no overlap in hatching times observed between the 2 species, and a highly significant difference in mean hatching time was found (Mann-Whitney *U*-test,  $Z = 13.787$ ,  $P < 0.00005$ ). The mean number of days required for hatching ( $\pm \text{SE}$ ) was  $18.0 \pm 0.1$  for *S. pseudoglobulus*, whereas eggs of *S. globulus* required, on average,  $27.9 \pm 0.2$  days to hatch.

*Sphaeridiotrema globulus* and *S. pseudoglobulus* exhibit substantial overlap in morphometrics. The major difference between the 2, as determined from experimental populations, is egg size. Live *S. pseudoglobulus* eggs from feces mea-

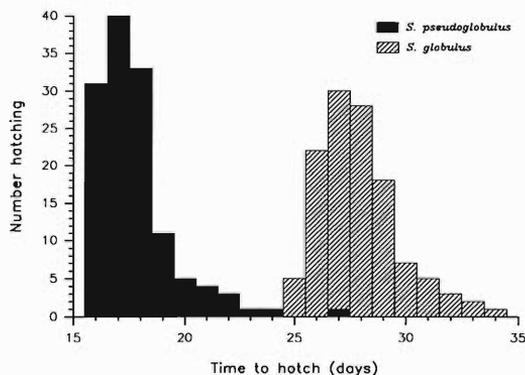


Figure 1. Hatching times of *Sphaeridiotrema pseudoglobulus* (solid bars) and *Sphaeridiotrema globulus* (diagonally striped bars) eggs maintained under identical conditions at 20°C with constant light.

sure, on average,  $126 \times 82 \mu\text{m}$ , whereas those of *S. globulus* measure  $105 \times 73 \mu\text{m}$  (McLaughlin et al., 1993). Features such as egg number (mean in utero number of 9 in *S. pseudoglobulus*, 23 in *S. globulus*) and cirrus shape are also useful in distinguishing between the 2 species (McLaughlin et al., 1993). Pathogenicity of the 2 species also differs. Pekin ducklings and blue-winged teal (*Anas discors*) do not develop enteritis comparable to that caused by *S. globulus* in mallards (*Anas platyrhynchos*), Canada geese (*Branta canadensis*), or mute swans (*Cygnus olor*) described by Huffman and Roscoe (1989) when infected experimentally with comparable numbers of *S. pseudoglobulus* (Gagnon, 1990). Nonetheless, the massive infections of *S. pseudoglobulus* present in ducks found dead compared to the numbers seen in healthy, hunter-shot ducks suggests that this helminth may well be a factor in waterfowl mortality (Hoeve and Scott, 1988), although the mechanism clearly differs from that of *S. globulus*. Differences in the egg-hatching dynamics reported here provide further evidence for the separate identity of the 2 species.

We thank Dr. Jane Huffman, East Stroudsburg University of Pennsylvania, for the *S. globulus*

metacercariae. The work was supported in part by a Concordia University Faculty Development Research Grant to J. D. M.

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**Research Note**

***Heterobilharzia americana* (Trematoda: Schistosomatidae) from White-tailed Deer (*Odocoileus virginianus*) in Southern Florida**

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**ABSTRACT:** Nongravid females of *Heterobilharzia americana* were found in mesenteric blood vessels from 3 of 40 (7.5%) white-tailed deer (*Odocoileus virginianus*) examined at necropsy during October and November 1990 in southern Florida. All infected deer were females ranging in age from 2 to 5.5 yr. This represents the first report of this schistosome from white-tailed deer in Florida and the second report from this host in North America.

**KEY WORDS:** schistosome, *Heterobilharzia americana*, prevalence, white-tailed deer, *Odocoileus virginianus*, Florida.

The parasitic helminths of white-tailed deer, *Odocoileus virginianus* (Zimmermann), have been well studied, and numerous published accounts exist on their prevalence and distribution in North America (Davidson et al., 1981). There is one report of the schistosome *Heterobilharzia americana* Price, 1929, from white-tailed deer in South Carolina (Byrd et al., 1967). Herein we report information on the prevalence of *H. americana* in white-tailed deer in southern Florida.

Deer were collected by shooting at night during October and November 1990. The sample consisted of 8 bucks and 32 does of which 9 were fawns (<13 mo of age), 4 were subadults (13-24 mo), and 27 were adults (>24 mo). Ages were determined by the patterns of toothwear and replacement in the lower jaw (Severinghaus, 1949; Harlow and DeFoor, 1962). The deer originated from 5 locations in Collier County; these locations were described in detail by Atkinson et al. (1993). Carcasses were kept under refrigeration until examined at necropsy, usually within 6-8 hr of death. At necropsy, mesenteries were removed from each deer and frozen in plastic bags for later examination, at which time they were thawed and processed. Large vessels were cut open, the entire mesentery of each deer was stretched and torn by hand in a container of water, and subsequent washings from this were passed through a 100-mesh sieve to collect blood flukes. Specimens were fixed in AFA, dehydrated in an ethanol series, cleared in methyl salicylate,

and mounted in Permount® for microscopic study and identification. The specimens were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705 (USNM Helm. Coll. Nos. 82830-82832).

Specimens of *Heterobilharzia americana* were recovered from 3 of the 40 deer. Infected deer originated from 2 of the 5 areas sampled (i.e., the Bear Island Unit in Big Cypress National Preserve [ $n = 2$ ] and the Florida Panther National Wildlife Refuge [ $n = 1$ ]). These 2 sites are adjacent and ecologically similar (Atkinson et al., 1993). Two flukes were obtained from 1 deer and 1 each from the other 2 animals; all were nongravid females. All hosts were females and were 2, 3, and 5.5 yr of age.

This is the first report of *H. americana* from white-tailed deer in Florida and the second report of this schistosome from white-tailed deer in North America. It was previously recovered from 4 of 15 white-tailed deer from Barnwell County in South Carolina, all of which were 6 mo to 1 yr of age. In Florida, *H. americana* has been found in black bears (*Ursus americanus*), bobcats (*Felis rufus*), Florida panthers (*Felis concolor coryi*), and raccoons (*Procyon lotor*) (Forrester, 1992). The latter appears to be the most common host in Florida, and deer may be an accidental host. Although lesions due to infections of *H. americana* in raccoons have been described (Bartsch and Ward, 1976; McKown et al., 1991), nothing is known about the effects of this blood fluke on white-tailed deer.

We acknowledge J. W. McCown and other personnel of the Florida Game and Fresh Water Fish Commission for collecting the deer. The technical assistance of G. A. Burrell and S. J. Tucker is appreciated. We thank Dr. J. M. Kinsella for verification of our identification of the schistosomes. This research was supported by a contract from the Florida Game and Fresh Water Fish Commission and is a contribution of Federal Aid to Wildlife Restoration, Florida Pittman-Rob-

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*J. Helminthol. Soc. Wash.*  
61(1), 1994, pp. 129–132

#### Research Note

### *Skrjabinoclava aculeata* (Acuarioidea: Acuariidae) in Dunlins (*Calidris alpina*) from Both Iceland and Louisiana, U.S.A.

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**ABSTRACT:** *Skrjabinoclava aculeata* (Creplin, 1825) was found in 11 of 24 dunlins (*Calidris alpina hudsonia*) wintering in Louisiana. This nematode was previously reported only in dunlins from Europe (Germany) and in dunlins collected in Iceland migrating from Palaearctic and Ethiopian staging and wintering areas. This is the first report of the same species of *Skrjabinoclava* in both New and Old World waders.

**KEY WORDS:** *Skrjabinoclava aculeata*, Nematoda, charadriiform birds, Old and New Worlds.

Anderson and Wong (1992) recently reported mature *Skrjabinoclava aculeata* (Creplin, 1825) in the proventriculus of 68% (15/22) of dunlins (*Calidris alpina schinzii*) collected in Iceland in the spring of 1989; intensity was 12 (1–83). Larvae were not found in the birds, and it was concluded that the latter had acquired infections either on their wintering grounds in Morocco and Mauritania (Pienkowski and Dick, 1975) or on staging areas in Morecambe Bay and the Dee Estuary, Britain (Wilson, 1973; Eades, 1974), when en route to breeding areas in southwest Iceland and southeast Greenland. *Skrjabinocla-*

*va aculeata* was regarded as exclusively a parasite of dunlins wintering in Palaearctic and Ethiopian regions because it had never been found in the numerous ( $N = 105$ ) specimens of *Calidris alpina pacifica* collected from the Pacific coast of North America or in any other shorebird species from North America (Wong and Anderson, 1987, 1990). However, *Skrjabinoclava bakeri* Wong and Anderson, 1987 (26%, 3.7 [1–11]), and *S. tupacincuae* Freitas and Ibanez, 1970 (16%, 3.2 [1–24]), were fairly common in Pacific dunlins along with a few *S. pusillae* Wong and Anderson, 1987 (4%, 5.0 [3–8]), and *S. myersi* Wong and Anderson, 1987 (1%, 168).

Dunlins (*Calidris alpina hudsonia*) were collected by shooting on their wintering grounds near Port Fourchon, Louisiana, U.S.A., on 20–25 January 1988. The birds were examined for proventricular worms of the genus *Skrjabinoclava*. Specimens were washed in saline and fixed in hot glycerine alcohol with 5% glycerine. The worms were cleared for study in pure glycerine

**Table 1. Morphometric characteristics of *Skrjabinoclava aculeata* (Creplin, 1825) in dunlins (*Calidris alpina*) from Iceland and Louisiana, U.S.A.\***

	Iceland	Louisiana
Males	N = 20	N = 10
Length	4.0 (3.6–4.3) mm	4.2 (2.8–4.4) mm
Maximum width	107 (85–120)	126 (112–146)
Buccal cavity	150 (128–183)	197 (160–215)
Deirids†	74 (60–88)	86 (79–92)
Nerve ring‡	177 (146–206)	185 (170–199)
Excretory pore†	201 (180–249)	219 (189–249)
Muscular esophagus	221 (192–280)	216 (192–240)
Glandular esophagus	1.0 (0.8–1.2) mm	1.0 (0.7–1.4) mm
Left spicule	568 (530–620)	572 (530–630)
Right spicule	140 (125–148)	137 (111–149)
Tail	147 (118–166)	145 (120–164)
Females	N = 20	N = 10
Length	5.6 (5.1–6.1) mm	5.6 (4.3–6.4) mm
Maximum width	180 (160–220)	208 (184–240)
Buccal cavity	170 (145–193)	234 (182–241)
Deirids†	102 (78–115)	107 (94–122)
Nerve ring‡	192 (165–218)	215 (201–239)
Excretory pore†	242 (210–287)	259 (220–308)
Muscular esophagus	254 (220–290)	246 (188–360)
Glandular esophagus	1.2 (1.0–1.3) mm	1.3 (1.1–1.5) mm
Vulva‡	329 (285–385)	319 (240–367)
Tail	114 (90–135)	103 (85–124)
Eggs	20–22 × 32–38	21–23 × 33–35

\* Mean and range in micrometers except where indicated otherwise.

† Distance from anterior extremity.

‡ Distance from posterior extremity.

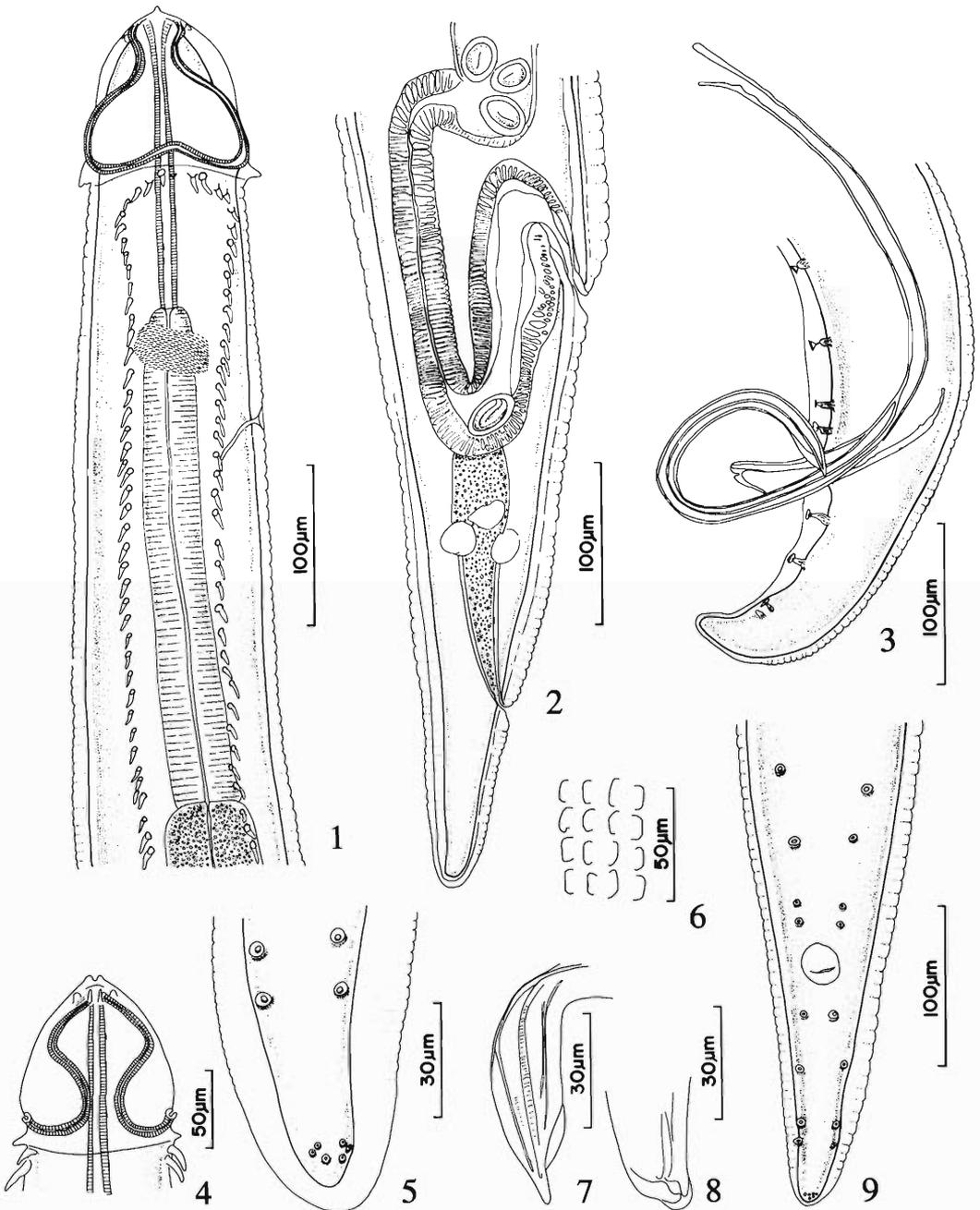
or lactophenol. The specimens have been placed in the Helminthological Collection of the United States National Museum (Nos. 82846–82847).

We were surprised to discover that the specimens found in 11 of 24 (46%) dunlins examined from Louisiana were *Skrjabinoclava aculeata*. Intensity was 3.4 (2–5), and the male female ratio was 0.7:1. *Calidris alpina hudsonia* breeds in the central Canadian arctic and winters in the Gulf coast of the U.S.A. The morphometric correspondence between the nematodes from dunlins from Iceland and those from Louisiana was remarkably close (Table 1), and all morphologic characters were indistinguishable (Figs. 1–9). This is the first time the same species of *Skrjabinoclava* has been found in shorebirds in both the New and Old Worlds.

Two hypotheses for this unusual distribution are outlined below, both of which require additional study. Various points must be kept in mind for either hypothesis. First, species of *Skrjabinoclava* are heteroxenous, utilizing crustaceans such as amphipods as intermediate hosts (Wong and Anderson, 1990). Second, transmis-

sion occurs in wintering or staging areas (i.e., not on the breeding grounds [Wong and Anderson, 1990]). Third, charadriiforms are thought to have evolved during the Tertiary and early glaciations to have been responsible for the formation of species living in the tundra (Larson, 1957). Finally, the last glaciation was responsible for the distribution of existing subspecies, which developed in tundra refuges (Larson, 1957).

*Skrjabinoclava aculeata* may be an evolutionary stable parasite that occurred in dunlins prior to the time when subspecies of the host evolved. If so, the absence of *S. aculeata* in New World *C. a. pacifica* is somewhat problematic. However, the parasite could have been retained in some populations of the host and not others. Greenwood (1984, 1986) suggested that *C. a. pacifica* (which breeds in southern Alaska) and *C. a. hudsonia* (which breeds in the central Canadian arctic) originated from a parent population occupying a refuge in central North America whereas *C. a. arctica* (which breeds in Greenland) and *C. a. schinzii* (which breeds in Iceland, Britain, and northwestern Europe) originated in



Figures 1–9. Morphology of *Skrjabinoclava aculeata* from *Calidris alpina hudsonia* from Louisiana, U.S.A.  
 1. Anterior region of male, lateral view. 2. Posterior region of female, lateral view. 3. Caudal end of male, lateral view. 4. Cephalic extremity of male, ventrolateral view. 5. Distal end of tail of male, ventral view. 6. Area rugosa. 7. Left spicule, distal end. 8. Right spicule, distal end. 9. Caudal region of male, ventral view.

French and central Siberian refuges. In contrast to Greenwood, Wenink et al. (1993) suggested that *C. a. pacifica* was derived from birds from a Beringia refuge. They also concluded that *C. a. hudsonia* was widely separated from other dunlins.

*Skrjabinoclava aculeata* may have originated as a parasite of dunlins after the time when subspecies of the host evolved and, thus, originally have been a parasite of only 1 subspecies of host. Subsequently, it spread to a second subspecies. This hypothesis requires spread of the parasite from 1 continent to another in recent times, which, in view of our knowledge of where the parasite is transmitted, is somewhat difficult to envision but cannot be ruled out.

Future studies using molecular methods might help to determine whether the parasites from Iceland and North America are the same or sibling species.

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## Research Note

# Some New Records of Marine and Freshwater Leeches from Caribbean, Southeastern U.S.A., Eastern Pacific, and Okinawan Animals

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**ABSTRACT:** Thirteen specimens of *Trachelobdella lubrica* were found attached near the eyes of 6 closely associated *Pomacentrus partitus* on a coral reef in Puerto Rico. *Myzobdella lugubris* is recorded for the first time in the West Indies and may have been introduced into eastern Puerto Rico. Varied sizes of *Ozobranchius branchiatus* found in a large tank with tilapia may demonstrate the first record of off-turtle development of this leech and of its occurrence in the insular Caribbean. *Branchellion torpedinis*, previously reported only in temperate regions, is reported in the tropics.

New host records (number in parentheses) are noted for *B. torpedinis* (1), *Myzobdella lugubris* (3), *Stibirobdella macrothela* (2), and *Trachelobdella lubrica* (12) from the West Indies; *Actinobdella inequiannulata* (1), *Myzobdella lugubris* (6), and *Piscicolaria reducta* (2) from the southeastern U.S.A.; *Trachelobdella* sp. (1) from the Pacific coast of Panama; and *Trachelobdella* sp. (1) from the southern islands of Japan.

**KEY WORDS:** Annelida, Hirudinea, leeches, fishes, crabs, new hosts.

Sawyer and Kinard (1980) listed freshwater and marine leeches from Puerto Rico and some Caribbean areas. New host and locality records have been noted for leeches from Alabama (E. H. Williams, 1979) and the Caribbean (E. H. Williams, 1982). A series of routine collections from West Indian and Japanese marine vertebrates and invertebrates (L. B. Williams and Williams, 1986) and from southeastern U.S. freshwater and brackish water fishes included 27 new host records, 3 new leech records for the Caribbean, and 2 unusual field observations (Table 1).

The positions of external leeches observed with SCUBA were drawn on dive slates prior to collection. Hosts were collected with SCUBA with elastic-band spearguns and multiprong-microbarb spears, underwater lights and dipnets, rotenone, and quinaldine; fish traps; monofilament gillnets; boat and backpack shockers; 15- and 25-m seines; hook and line; and trawls and from fish kills and mass mortalities and from strandings. The majority of the collections in the Ca-

ibbean were made in Puerto Rico, Mona Island, the U.S. Virgin Islands, the Dominican Republic, and Panama; in the southeastern U.S.A. in Alabama, Georgia, and Florida; and in Japan in Okinawa. Geographic locations for leeches are reported in Table 1.

Leeches were relaxed by refrigeration and preserved in 10% formalin. The specimen of *Actinobdella inequiannulata* was flattened between microscope slides when preserved in formalin, stained in carmine, and mounted in Permout. All specimens were deposited in the U.S. National Museum, Division of Worms (USNM).

### *Trachelobdella lubrica* (Grube, 1840)

A closely spaced group of 6 bicolor damselfishes, *Pomacentrus partitus* Poey (Pomacentridae), each infested with *T. lubrica*, was observed at Buoy #1 off Mayaguez Bay at a depth of 25 m (Table 1). Thirteen leeches were collected from the skin of 6 fishes. No other bicolor damselfishes, or other fishes observed during the dive, were infested externally with leeches. Sixty-nine percent were attached around the eyes of these hosts, and this is similar to early attachment positions that we found in extensive field studies of juvenile parasitic isopods (L. B. Williams, 1984). This may indicate a similar mechanism among externally attaching fish associates in locating and attaching to fish hosts.

After more than 3,500 hr of closely observing fishes underwater for externally visible parasites and tumors, we are certain that leeches do not commonly attach externally on Caribbean reef fishes. Specimens of *T. lubrica* usually attach on the gills. These external attachment sites may indicate that they first attach externally and then move to the preferred site on the gills and that our observation found them in this process. Monogeneans and isopods have been found to

Table 1. Caribbean, southeastern U.S.A., eastern Pacific, and Okinawan marine and freshwater leeches.

Host	N/H*	Site	I/E†	Host sizes (cm)	Locality	Date	USNM no.
<b>Family Ozobranchidae</b>							
<i>Ozobranchus branchiatus</i> (Menzies, 1791)‡							
No host	1-40	—	—	—	Magueyes, Parguera, Puerto Rico	18 Apr 1990	132423
<b>Family Glossiphoniidae</b>							
<i>Actinobdella inequiannulata</i> Moore, 1901							
<b>Class Osteichthyes—bony fishes/order Cypriniformes/family Catostomidae—suckers</b>							
<i>Minytrema melanops</i> §	1	body	1/1	—	Lee County, Alabama, U.S.A.	3 May 1969	144227
<b>Family Piscicolidae</b>							
<i>Branchellion torpedinis</i> Savigny, 1822							
<b>Class Chondrichthyes—cartilaginous fishes/order Rajiformes/family Myliobatidae—eagle rays</b>							
<i>Aetobatis narinari</i> §	5	nare	1/1	285	off Parguera, Puerto Rico	10 Aug 1989	132445
<i>Trachelobdella lubrica</i> (Grube, 1840)							
<b>Class Osteichthyes—bony fishes/order Elopiformes/family Elopidae—tarpons</b>							
<i>Elops saurus</i> §	1	gills	1/3	36.5	Cayo Santiago, Puerto Rico	10 Dec 1991	155354
<b>Order Myctophiformes/family Synodontidae—lizardfishes</b>							
<i>Synodus intermedius</i> §	1	skin	1/10	20.0	Turumote, Parguera, Puerto Rico	5 Apr 1989	132437
<b>Order Perciformes/family Centropomidae—snooks</b>							
<i>Centropomus undecimalis</i> §	1	mouth	1/2	48.0	Urban Pond, Carolina, Puerto Rico	30 Apr 1991	144225
<b>Family Serranidae—sea basses</b>							
<i>Epinephelus cruentatus</i> §	1	body	1/5	—	Freeport, Grand Bahama, Bahamas	12 Jun 1991	144224
<i>Epinephelus guttatus</i>	1	gills	2/10	22.0	Magueyes, Parguera, Puerto Rico	1 Feb 1985	132438
<i>Liopropoma rubre</i> §	1	gills	1/1	6.4	Salinas, Puerto Rico	8 May 1978	132439
<b>Family Lutjanidae—snappers</b>							
<i>Lutjanus apodus</i> §	1	gills	1/3	38.0	north of Sardinero, Mona Island	15 Apr 1975	132440
<i>Lutjanus synagris</i> §	1	gills	1/2	24.5	Punta Santiago, Humacao, Puerto Rico	5 Feb 1992	155352
<b>Family Gerreidae—mojarras</b>							
<i>Gerres cinereus</i> §	2	gills	1/1	16.5	Punta Santiago, Humacao, Puerto Rico	30 Apr 1991	144226
	1	gills	1/1	27.9	Cayo Santiago, Humacao, Puerto Rico	10 Dec 1991	155353
	2	gills	2/2	25-29	Cayo Santiago, Humacao, Puerto Rico	5 Feb 1992	155351
<b>Family Haemulidae—grunts</b>							
<i>Haemulon flavolineatum</i>	1	gills	1/10	16.7	shelf edge, Parguera, Puerto Rico	22 Jan 1977	132446
<i>Haemulon plumieri</i> §	1-2	gills	2/12	20-22	shelf edge, Parguera, Puerto Rico	10 Sep 1992	164035

Table 1. Continued.

Host	N/H*	Site	I/E†	Host sizes (cm)	Locality	Date	USNM no.
<i>Haemulon sciurus</i>	2	gills	2/5	10.2-15	Ensenada, Puerto Rico	10 Oct 1977	132441
	2	mouth	1/1	19.0	Parguera, Puerto Rico	11 Nov 1977	
<i>Abudefduf saxatilis</i> §	1	gills	1/10	11.0	shelf edge, Parguera, Puerto Rico	11 Feb 1981	132442
<i>Pomacentrus partitus</i> §	1-7	skin	6/6	2.5-9	off Mayaguez Bay, Puerto Rico	20 Oct 1988	132443
<i>Sparisoma aurofrenatum</i> §	1	gills	1/2	—	Family Scaridae—parrotfishes Salinas, Puerto Rico	20 May 1978	132436
<i>Acanthurus bahianus</i>	2	gills	1/4	15.0	Family Acanthuridae—surgeonfishes shelf edge, Parguera, Puerto Rico	14 Oct 1977	132444
<i>Sciaenops ocellatus</i> §	1	body	1/1	—	<i>Trachelobdella</i> sp. A Class Osteichthyes—bony fishes/order Perciformes/family Sciaenidae—drums Agromarina, El Dorado, Panama	4 Feb 1988	132448
<i>Oplegnathus punctatus</i> §	1	tongue	1/1	39.0	<i>Trachelobdella</i> sp. B Class Osteichthyes—bony fishes/order Perciformes/family Oplegnathidae—knifejaws Amitori Bay, Inomote Island, Japan	25 Nov 1985	144437
<i>Carcharhinus perezi</i> §	1	fin	1/1	—	<i>Stribarobdella macrothela</i> (Schmarda, 1861) Class Chondrichthyes—cartilaginous fishes/order Squaliformes/family Carcharhinidae—requiem sharks Saba, Netherland Antilles	4 Nov 1986	132433
<i>Ginglymostoma cirratum</i>	5	mouth	1/1	—	Parguera, Puerto Rico	29 Apr 1969	132450
	2	mouth	1/1	—	Parguera, Puerto Rico	22 Jun 1971	132449
	1	mouth	1/1	220	Parguera, Puerto Rico	30 Aug 1974	132451
	3	mouth	1/1	—	Parguera, Puerto Rico	—	132455
<i>Galeocerdo cuvier</i>	1	mouth	1/1	300	Parguera, Puerto Rico	21 Jan 1982	132452
<i>Negaprion brevirostris</i>	3	mouth	1/1	226	Laurel Reef, Parguera, Puerto Rico	6 Jul 1978	132454
"Shark"	1	—	1/1	—	Parguera, Puerto Rico	14 Dec 1962	132453
"Shark"	1	—	1/1	—	Parguera, Puerto Rico	1977	
<i>Aetobatis narinari</i> §	5	skin	1/1	310	Order Rajiformes/family Myliobatidae—eagle rays Salt River Canyon, St. Croix, USVI	16 Mar 1984	132434
	1-15	skin	2/2	158	Margarita, Parguera, Puerto Rico	2 Aug 1989	132435
Not on a host	1	—	—	—	Guayacan, Lajas, Puerto Rico	5 May 1955	3517†
<i>Callinectes bocourti</i> §	4-10	body	17/17	7-7.5	<i>Myzobdella lugubris</i> Leidy, 1851    Class Crustacea—crustaceans/order Decapoda/family Portunidae—swimming crabs Santa Teresa Lagoon, Puerto Rico	29 May 1992	155347
<i>Callinectes sapidus</i>	6	body	1/1	7.9	Santa Teresa Lagoon, Puerto Rico	29 May 1992	

Table 1. Continued.

Host	N/H*	Site	I/E†	Host sizes (cm)	Locality	Date	USNM no.
<b>Class Osteichthyes—bony fishes/order Elopiformes/family Elopidae—tarpons</b>							
<i>Megalops atlanticus</i> §	1	fin	1/12	48.8	Santa Teresa Lagoon, Puerto Rico	29 May 1992	155349
<b>Order Cypriniformes/family Cyprinidae—carps and minnows</b>							
<i>Notropis callistius</i> §	1	skin	1/32	7.6	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132424
<b>Family Catostomidae—suckers</b>							
<i>Hypentelium etowanum</i> §	1	skin	1/15	25.0	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132425
<b>Order Siluriformes/family Ictaluridae—bullhead catfish</b>							
<i>Ameiurus catus</i> §	3	fins	2/21	27.0	Euphapy Creek, Alabama, U.S.A.	27 Mar 1972	132426
<i>Ameiurus natalis</i> §	1	skin	1/8	15.2	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132427
<b>Order Atheriniformes/family Cyprinodontidae—killifishes</b>							
<i>Fundulus jenkinsi</i> §	1	skin	1/1	4.1	Bon Secour River mouth, Alabama, U.S.A.	22 Jan 1971	132429
<b>Order Perciformes/family Centrarchidae—sunfishes</b>							
<i>Lepomis gulosus</i>	1	skin	1/43	12.7	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132430
<i>Micropterus coosae</i> §	1	skin	1/5	13.0	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132431
<b>Family Cichlidae—cichlids</b>							
<i>Tilapia rendalli</i> §	1	gills	1/12	18.0	Santa Teresa Lagoon, Puerto Rico	29 May 1992	155350
<b>Family Mugilidae—mulletts</b>							
<i>Mugil curema</i>	1	fin	1/1	49.0	Santa Teresa Lagoon, Puerto Rico	29 May 1992	155348
<b><i>Piscicola reducta</i> Meyer, 1940</b>							
<b>Class Osteichthyes—bony fishes/order Cypriniformes/family Ictaluridae—bullhead catfish</b>							
<i>Noturus leptacanthus</i> §	2	skin	1/4	6.2	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132428
<b>Family Percidae—perches</b>							
<i>Percina palmaris</i> §	1	skin	1/1	6.0	Loblockee Creek, Lee County, Alabama, U.S.A.	15 Feb 1972	132432

\* Number of leeches per host.

† Number of hosts infected/number of hosts examined.

‡ New locality record for the insular Caribbean (Sawyer et al. [1975] reported this leech from the Caribbean coast of Costa Rica).

|| New locality record for the Caribbean.

§ New host record.

¶ Department of Marine Sciences, University of Puerto Rico, Invertebrate Collection, Acct. No.

attach initially in various locations on the body of their fish host and move to preferred sites (Kearn, 1976; Cone and Burt, 1981; L. B. Williams, 1984). More than half of the leeches attached to the largest host.

E. H. Williams (1982) did not publish the USNM numbers for his specimens of this leech on *Acanthurus bahianus* (USNM 73880), *Archosargus rhomboidalis* (73883), *Cantherhines macrocerus* (73887), *Epinephelus guttatus* (73892), *Epinephelus striatus* (73886), *Haemulon album* (73888), *Haemulon flavolineatum* (73885), *Haemulon sciurus* (73889), *Lachnolaimus maximus* (73891), and *Scorpaena plumieri* (73884) from the Caribbean.

#### *Myzobdella lugubris* Leidy, 1851

A month-long fish kill occurred in the Santa Teresa Lagoon during May 1992. The Lagoon is brackish water and is located near Humacao, Puerto Rico. Losses were largely confined to Mozambique tilapia, *Tilapia mossambica* (Peters), but a few other brackish and freshwater fishes died. Water temperatures in the Lagoon were 30–32°C, which is unusually high for that time of year. The tilapia were infected with a variety of protozoan parasites, high intensities of nematodes encysted in the skin, fins, and internal organs, and systemically infected with *Vibrio vulnificus* (identification via API 20E® System, API Products Limited, Quebec, Canada).

During the last week of the kill, blue crabs (*Callinectes bocourti* Milne-Edwards and *Callinectes sapidus* Rathbun) began to die in the Lagoon. The crabs were reported to be infested with very high numbers of leeches identified as *M. lugubris*. Those we collected (Table 1) had only 4–10 leeches per crab, which are not unusual levels (Sawyer et al., 1975). The mortalities were probably caused by poor water quality conditions.

*Myzobdella lugubris* is found on freshwater fishes in the continental U.S.A. (Table 1). We have not seen this leech during the examination of thousands of freshwater fishes from a variety of locations and habitats in Puerto Rico or during extensive examinations of brackish water fishes and crustaceans from other parts of Puerto Rico. Its absence in the past suggests that the leech may have been introduced recently into Puerto Rico. Mariculture projects in eastern Puerto Rico have brought in exotic organisms for culture including species from the U.S.A. This record is a range extension of at least 1,700 km for *M. lu-*

*gubris*. The previously known southern range for this leech was Florida, U.S.A. (Sawyer et al., 1975). Leeches are not known to infest freshwater fishes in Puerto Rico. *Myzobdella lugubris* may eventually invade all freshwater habitats in Puerto Rico.

#### *Ozobranchus branchiatus* (Menzies, 1791)

A circular plastic pool, 3.5 m in diameter and 0.9 m deep, was filled with seawater from the seawater system of Magueyes Island, La Parguera, Puerto Rico. Twenty to 30 small blue tilapia, *Tilapia aurea* (Steindacher) (Cichlidae), which had been acclimated to seawater, were added to this tank. Graduate students soon began complaining of leeches attaching to their arms while in this tank. One student standing in the pool was covered immediately with 30–40 leeches of varied sizes (5–11 mm). Samples from this student were removed with forceps, preserved in 10% formalin, and identified as *O. branchiatus* (Table 1).

The establishment of leeches in the plastic pool is difficult to explain, as is the range of sizes of leeches present in the tank. Sawyer et al. (1975) suggested that *O. branchiatus* completed its life cycle on turtle hosts. This case would suggest that the leech sometimes occurs free living off the host. No leeches or wounds were found on the blue tilapia specimens, and no evidence of leech reproduction was found when the tank was drained and disinfected.

#### *Branchellion torpedinis* Savigny, 1822

The specimens collected from the spotted eagle ray, *Aetobatis narinari* (Euphrasen) (Table 1), had 33 pairs of branchiae, typical of *B. torpedinis*. According to Sawyer et al. (1975), *B. torpedinis* is a temperate species that has not been reported south of North Carolina in the western Atlantic or south of Senegal in the eastern Atlantic. The southern, warm-water counterpart of *B. torpedinis*, *Branchellion ravenelii*, known from Florida, Alabama, and Mississippi, would be expected in Puerto Rico; however, *B. ravenelii* has only 31 pairs of branchiae. On the basis of the number of branchiae and other external characters, we consider the leeches from the spotted eagle ray to be *B. torpedinis*.

#### *Trachelobdella* sp. A

Red drum ("red fish"), *Sciaenops ocellatus* (Linnaeus), have been introduced for culture in a number of Caribbean and eastern Pacific lo-

cations. A leech on this fish taken from a culture pond on the Pacific coast of Panama (Table 1) may be *Trachelobdella lubrica*, the common gill leech of marine tropical fishes; however, the specimen was curled and contracted, making observation difficult. Large pulsatile vesicles typical of *T. lubrica* were not obvious, and the trachelosome was much shorter than typical specimens of *T. lubrica*. However, both these features may have been the result of the state of contraction of the specimen. In addition, 1 pair of punctiform eyespots was present on the oral sucker. Eyespots have not been reported on *T. lubrica* from the Atlantic, and they were not observed on specimens identified as *T. lubrica* during this study. However, eyespots may be present on *T. lubrica* from Hawaii (Epshtein, 1973), and since the leech from the red drum was collected from the Pacific, the presence of eyespots may not be a reason for ruling out *T. lubrica*. The red drum were brought into Panama as fry from hatcheries in Texas and were unlikely to have been infested with leeches. The presence of *Nerocila californica* Schiodte and Meinert, 1881 (Isopoda: Cymothoidae), also indicates that these fishes were exposed to Pacific parasites. These leeches were not a problem in the culture of red drum in Panama. They were only recorded twice from cage-cultured fishes.

#### *Trachelobdella* sp. B

The specimen from the knifejaw, *Oplegnathus punctatus* (Temminck and Schlegel), was too contracted to be identified to species (Table 1). We examined 289 fish specimens, representing 186 species in 61 families, for leeches from May 1985 through March 1986 from 9 islands in the Ryukyu Islands of Japan (Williams and Williams, 1986). This was the only leech recovered.

#### *Stibarobdella macrothela* (Schmanda, 1861)

Williams (1982) did not publish the USNM numbers for his specimens of this leech on *Ginglymostoma cirratum* (73880), *Galeocerdo cuvieri* (73882), *Negaprion brevirostris* (73881), and *Sphyrna mokarran* (73879) from the Caribbean.

We thank Humberto A. Garces, Hiroyoshi Kohno, Patrick L. Colin, Ileana E. Clavijo, Tom van't Hof, Rosa M. Steele, Ronald P. Phelps,

and Joseph R. Sullivan for collecting leeches or hosts; Donald Klemm for identifying the specimen of *Actinobdella inequianmulata*; and Hiroyuki Yokochi and Hiroyoshi Kohno for use of facilities. Support was provided by the Department of Fisheries and Allied Aquacultures, Auburn University, in part by Dingle Johnson Funds; the Department of Natural Resources, Commonwealth of Puerto Rico, and Wallop Breaux Funds, Project F-28; and the Ministry of Education, Science and Culture of the Japanese Government. This manuscript is a contribution of the Sesoko Marine Sciences Center.

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Research Note

Helminths of the Charal Prieto, *Chirostoma attenuatum*  
(Osteichthyes: Atherinidae), from Patzcuaro Lake, Michoacan, Mexico

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ABSTRACT: Eight helminth species (*Posthodiplostomum minimum*, *Allocreadium mexicanum*, *Bothriocephalus acheilognathi*, a cyclophyllidean cysticeroid, *Arhythmorhynchus brevis*, *Spinitectus carolini*, *Capillaria patzcuarensis*, and *Eustrongylides* sp.) were found in 195 fish, *Chirostoma attenuatum*, from Patzcuaro Lake from October 1989 to December 1990. *Posthodiplostomum minimum* had the highest prevalence and intensity. Our findings represent new host records.

KEY WORDS: *Chirostoma attenuatum*, charal prieto, *Posthodiplostomum minimum*, *Allocreadium mexicanum*, cyclophyllidean, *Bothriocephalus acheilognathi*, *Arhythmorhynchus brevis*, *Spinitectus carolini*, *Capillaria patzcuarensis*, *Eustrongylides* sp., helminths, Patzcuaro, Mexico.

*Chirostoma attenuatum* is an endemic atherinid fish from Patzcuaro Lake, and this and other species of the same genus have a commercial importance in fisheries of this lake. All species of *Chirostoma* are endemic to Mexico, and their taxonomy has been reviewed by Barbour (1973a, b). Osorio et al. (1986) previously recorded 8 helminth species as parasites of "white fish," *Chirostoma estor*, another endemic fish of Patzcuaro Lake. The purpose of this note is to describe the prevalence and intensity of the helminth fauna of *C. attenuatum*.

Fish were collected in Patzcuaro Lake, one of the largest lakes in Mexico, which is situated in the State of Michoacan, in southwest Mexico. The lake occupies 10% of an endorreic basin of tectovolcanic origin and has no outlet and no important water inlets. It is fed by temporary streams during the rainy season, May to October. It is in a eutrophication stage and has a total area of 130 km<sup>2</sup> and a mean depth of 4.97 m (Chacon et al., 1989).

From October 1989 to December 1990, 195 fish were collected by "spoon" and seine nets. Fish were kept frozen until their dissection 4 hr after capture. Digestive tract, viscera, mesentery, muscle, eyes, and brain were examined separately using a stereoscopic microscope. Trematodes and cestodes were fixed in Bouin's fluid with light coverglass pressure, stained in Harris

or Delafield's hematoxylin, dehydrated, cleared in methyl salicylate, and mounted in Canada balsam. Acanthocephalans were kept in distilled water 6 hr at 4°C, fixed in 70% ethanol, and then stained and mounted as already described. Nematodes were fixed in hot 70% ethanol and were studied as temporary whole mounts cleared in lactophenol. Specimens were deposited in the Colección Helmintológica del Instituto de Biología de la UNAM México (IBUNAM) and in the U.S. National Museum Helminthological Collection, Beltsville, Maryland (USNM Helm. Coll.) as follows: *Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936: IBUNAM No. 246-21; USNM Helm. Coll. No. 82105. *Allocreadium mexicanum* Osorio, Pérez, and Salgado, 1986: IBUNAM No. 248-13; USNM Helm. Coll. No. 82160. *Bothriocephalus acheilognathi* Yamaguti, 1934: IBUNAM No. II-269; USNM Helm. Coll. No. 82162. Cyclophyllidean cysticeroid: IBUNAM No. II-270; USNM Helm. Coll. No. 82161. *Arhythmorhynchus brevis* Van Cleave, 1916: IBUNAM No. II-271; USNM Helm. Coll. No. 82159. *Spinitectus carolini* Holl, 1928: IBUNAM No. 187-4; USNM Helm. Coll. No. 82163. *Eustrongylides* sp.: IBUNAM No. 187-5; USNM Helm. Coll. No. 82164. The ecological terms are used in accordance with Margolis et al. (1982).

The helminths include 8 species, 4 of them larval forms as well as 4 adults. The 4 larval forms—*P. minimum* metacercariae, cyclophyllidean cysticeroids, *A. brevis* cystacanths, and *Eustrongylides* sp. larvae—probably complete their life cycles in birds that feed on fish. We have found (unpubl. data) adults of *P. minimum* and *A. brevis* in several bird species of the family Ardeidae, but we have been unable to find adults of the cyclophyllidean cysticeroids and *Eustrongylides* sp. larvae in their natural hosts. Because of the small number of larvae of those species found here, we could not feed larval forms to an experimental definitive host to get the adult worms. The presence of cyclophyllidean cysti-

**Table 1. Helminths of the charal prieto, *Chirostoma attenuatum*, in Patzcuaro Lake, Michoacan, Mexico, N = 195.**

Helminth	Site*	Prevalence (%)	Intensity ( $\bar{x}$ )
Trematoda			
<i>Posthodiplostomum minimum</i> (Metacercariae)	L, M, Me, E, B	98.4	111.3
<i>Allocreadium mexicanum</i>	I	4.1	2.9
Cestoda			
<i>Bothriocephalus acheilognathi</i>	I	6.7	3.5
Cyclophyllidea (Cysticeroid)	I	0.5	8.0
Acanthocephala			
<i>Arhythmorhynchus brevis</i> (Cystacanth)	L, Me	3.6	2.8
Nematoda			
<i>Spintectus carolini</i>	I	14.3	2.9
<i>Capillaria patzcuarensis</i>	I	0.5	1.0
<i>Eustrongylides</i> sp. (larvae)	Me	1.5	1.3

\* B = brain, E = eyes, I = intestine, L = liver, M = muscle, Me = mesentery.

cercoids in fish has been documented by several authors; Olsen (1939) found minute cysticeroids of *Dendrouterina nycitoracis* in the gall bladder of the fish *Ameiurus melas* in Minnesota, U.S.A.

Prevalence and mean intensity for each helminth species in the host sample are given in Table 1. *Posthodiplostomum minimum* had the highest prevalence (98.4%) and mean intensity (111.3) of infection. We found a total of 21,399 larvae of this diplostomid from liver, muscle, mesentery, eyes, and brain, with highest numbers found in the liver. Other helminths, in diminishing order of prevalence, were *S. carolini*, *B. acheilognathi*, *A. mexicanum*, and *A. brevis*. The lower values of prevalence and intensity for the cyclophyllidean cysticeroids, *Eustrongylides* sp. larvae, and *Capillaria patzcuarensis* suggest that they may be sporadic or accidental infections.

None of the parasites found in this study is unique to *C. attenuatum*, but each represents a new host record. The population of *C. attenuatum* examined is sympatric with the population of *C. estor*. Thus, it is not unexpected that these populations should share some of the same helminth species; actually, they share 7 helminth

species when our results are compared to those of Osorio et al. (1986) in *C. estor*.

The high prevalences of *P. minimum* in freshwater fish of North America are well documented (Meade and Bedinger, 1967; Spall and Summerfelt, 1969; McDaniel and Bailey, 1974; Sutherland and Holloway, 1979; Ingham and Dronen, 1980; Amin, 1982; Threlfall and Watkins, 1982; Bailey, 1984) and are in agreement with our findings. This trematode may produce severe damage to its host, and this is an aspect to be evaluated in the future.

We thank J. Ralph Lichtenfels from the Bio-systematic Parasitology Laboratory, Beltsville, Maryland, for his advice on this manuscript; Rafael Lamothe for the review and comments on this manuscript; and Patricia Ramos, Rocio Hernández, and Ma. Antonieta Arizmendi for their assistance with the fieldwork. Luis García Prieto assisted in the field as well as with laboratory work and gave technical advice. Araceli Orbe permitted us to use the field facilities in CRIP-Patzcuaro. This research was supported by the Programa de Apoyo a las Divisiones de Estudios de Posgrado (PADEP-UNAM) Nos. DFC9002 and DFC9148. The comments and suggestions of 2 anonymous reviewers are greatly appreciated.

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*J. Helminthol. Soc. Wash.*  
61(1), 1994, pp. 141-145

### Research Note

## Persistence of the Component Parasite Community of Yarrow's Spiny Lizard, *Sceloporus jarrovi*, 1967-1991

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**ABSTRACT:** Persistence of the component parasite community of *Sceloporus jarrovi* was examined from samples taken 22 yr apart. Of the nematodes recovered, *Spauligodon giganticus* represents a core species; *Physaloptera retusa*, *Thubunaea intestinalis*, *Oochoristica scelopori*, and *Mesocestoides* sp. are satellite species. Species composition, prevalences, and intensities were similar after 22 yr, suggesting a persistent helminth component community.

**KEY WORDS:** *Sceloporus jarrovi*, Phrynosomatidae, *Physaloptera retusa*, *Spauligodon giganticus*, *Thubunaea intestinalis*, *Oochoristica scelopori*, *Mesocestoides* sp., helminth community.

Parasite community structure is hierarchical: a parasite infrapopulation represents all members of a single species of parasite within an individual host (Esch et al., 1975), a parasite infracommunity includes all of the infrapopulations within an individual host (Bush and Holmes, 1986), and a component parasite community represents all of the infracommunities within a given host population (Holmes and Price, 1986). A component parasite community is composed

of core species, those species that occur with relatively high frequencies (prevalences) and densities (mean intensities), and satellite species, which occur with less frequency and are relatively less numerous than core species (Hanski, 1982).

Persistence, a measure of continued presence, and stability, a measure of constancy over time (see Meffe and Minckley, 1987), of helminth infections in lizards have been infrequently reported (Telford, 1970; Goldberg and Bursley, 1990b; Bursley and Goldberg, 1991, 1992). In this note, we present data on the component helminth community in samples taken 22 yr apart from a population of Yarrow's spiny lizard, *Sceloporus jarrovi* Cope. This phrynosomatid lizard (see Frost and Etheridge, 1989, for revised taxonomy of iguanian lizards) is restricted to the mountains of southeastern Arizona (Stebbins, 1985). Goldberg and Bursley (1990a) provided a list of the helminth fauna of *Sceloporus jarrovi*.

Specimens of *Sceloporus jarrovi* were collect-

ed by hand-held noose at Kitt Peak (31°95'N, 111°59'W; elevation 1,889 m) in the Baboquivari Mountains, Pima County, Arizona. A total of 489 lizards collected from October 1967 to January 1970 from elevations 1,730–1,884 m and 50 specimens collected October 1991 at 1,889 m elevation, representing 21 monthly samples, were examined for helminths. The 1967–1970 specimens were deposited in the Department of Biology Vertebrate Collection at Whittier College, Whittier, California. The 1991 specimens were deposited in the herpetology collection of the Natural History Museum of Los Angeles County (LACM Nos. 139668–139717).

The body cavity was opened and the gastrointestinal tract was excised by cutting across the esophagus and the rectum. The digestive tract was slit longitudinally and examined under a dissecting microscope. Each helminth was removed to a glass slide and identified using a glycerol wet-mount procedure; selected nematodes were stained with iodine, and selected cestodes were stained with hematoxylin. Representative specimens were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705). Terminology use is in accordance with Margolis et al. (1982).

Three nematode species (*Spauligodon giganticus* (Read and Amrein, 1953), *Physaloptera retusa* Rudolphi, 1819, and *Thubunaea intestinalis* Bursey and Goldberg, 1991), 2 cestode species (*Mesocestoides* sp. and *Oochoristica scelopori* Voge and Fox, 1950), and a juvenile acanthocephalan were recovered (USNM Helm. Coll. Nos. 80867, 82468; 80868, 82467; 80869, 82468; 80870, 82469; 80871, 82470; 80877, respectively). Specific comparison of helminth species presence/absence and intensity was made for the October 1968, 1969 ( $N = 21, 26$ , respectively) and October 1991 ( $N = 50$ ) samples, and no significant differences were found: Jaccard coefficient = 1; Morisita's Index = 0.995; Kruskal-Wallis statistic = 2.61, 2 df,  $P > 0.05$ .

Of the 539 lizards examined, 514 (95.4%) were infected and harbored 12,710 helminths. From an infrapopulation/infracommunity perspective, 282 (52.3%) lizards were infected with a single species of parasite (mean intensity = 18.0, range 1–258): 274 with *S. giganticus*, 5 with *P. retusa*, 2 with *O. scelopori*, and 1 with *Mesocestoides* sp. One hundred ninety-one (35.4%) were infected with 2 species of parasites (mean intensity = 32.1, range 2–279): 134 were infected with *S. giganticus* and *P. retusa*, 43 with *S. giganticus* and *O.*

*scelopori*, 8 with *S. giganticus* and *T. intestinalis*, 3 with *S. giganticus* and *Mesocestoides* sp., 2 with *P. retusa* and *O. scelopori*, and 1 with *S. giganticus* and a juvenile acanthocephalan. Forty (7.4%) were infected with 3 species of parasites (mean intensity = 29.8, range 5–124): 15 with *S. giganticus*, *P. retusa*, and *O. scelopori*; 12 with *S. giganticus*, *P. retusa*, and *Mesocestoides* sp.; 7 with *S. giganticus*, *P. retusa*, and *T. intestinalis*; 4 with *S. giganticus*, *T. intestinalis*, and *O. scelopori*; 2 with *S. giganticus*, *P. retusa*, and a juvenile acanthocephalan; and 1 with *S. giganticus*, *O. scelopori*, and *Mesocestoides* sp. A single lizard was infected with 4 species (intensity = 68): *S. giganticus*, *P. retusa*, *O. scelopori*, and *Mesocestoides* sp. Helminths were not recovered from 25 (4.6%) lizards.

From a frequency of occurrence perspective, 505 (93.6%) lizards were infected with *S. giganticus*, 173 (32.1%) with *P. retusa*, 19 (3.5%) with *T. intestinalis*, 68 (12.6%) with *O. scelopori*, 18 (3.3%) with *Mesocestoides* sp., and 3 (0.5%) with juvenile acanthocephalans. In the 34 lizards not infected with *S. giganticus*, 25 were those not infected, 5 were infected with *P. retusa* only, 2 with *O. scelopori* only, and 1 with *Mesocestoides* sp. only. The remaining lizard was infected with *P. retusa* and *O. scelopori*. Monthly prevalences for *S. giganticus*, *P. retusa*, *T. intestinalis*, *Mesocestoides* sp., and *O. scelopori* are shown in Figure 1.

From an abundance perspective, of the 12,710 helminths recovered, 10,388 (81.7%) were *S. giganticus*. There were 1,948 (15.3%) *P. retusa*, 75 (0.6%) *T. intestinalis*, 130 (1.0%) *Mesocestoides* sp., and 166 (1.3%) *O. scelopori*. Acanthocephalans appeared in the collection (<0.1%) only in July 1968 ( $N = 2$ ) and September 1968 ( $N = 1$ ). A Shannon diversity index of 0.848 was calculated. The *Sceloporus jarrovi* composite helminth community is depauperate with >75% of the individuals belonging to a single species.

Because core species are defined as those species that occur with relatively high prevalence and mean intensity whereas satellite species occur with less frequency and are relatively less numerous than core species, we constructed a scatter plot of average monthly prevalence and average monthly mean intensity in order to categorize members of the component parasite community (Fig. 2). We would expect core species to appear in the upper-right quadrant of the graph and satellite species to appear in the other quadrants. *Spauligodon giganticus* was recov-

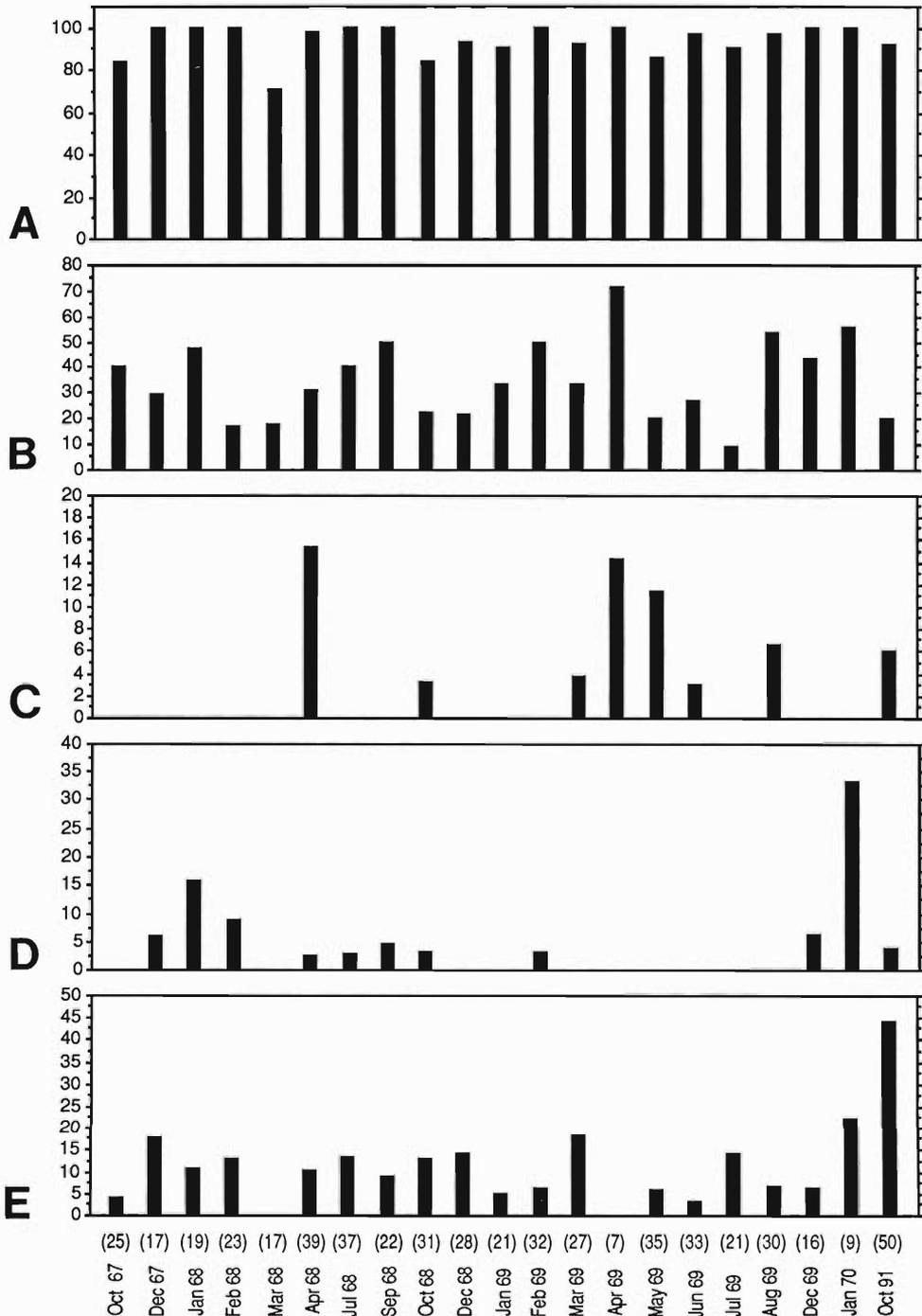


Figure 1. Monthly prevalences as percentages for *Spauligodon giganticus* (A), *Physaloptera retusa* (B), *Thubunaea intestinalis* (C), *Mesocostoides* sp. (D), and *Oochoristica scelopori* (E) recovered from 539 *Sceloporus jarrovi*. Numbers in parentheses are the number of lizards examined each month.

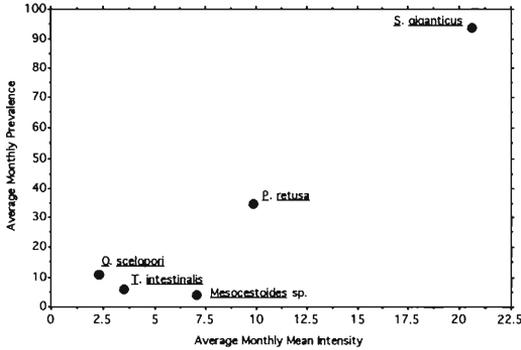


Figure 2. Scattergram of average monthly prevalence versus average monthly mean intensity of helminths from *Sceloporus jarrovi*. Core species appear in the upper-right quadrant of the graph.

ered from each of the 21 samples; average monthly prevalence was 94% (range 70.6–100), and average mean intensity was 20.6 (range 6.8–41.3). *Physaloptera retusa* was recovered from each of the 21 monthly samples; average monthly prevalence was 35% (range 9.5–71.4), and average mean intensity was 9.8 (range 1.4–47.3). *Thubunaea intestinalis* was recovered from 8 of the 21 samples; average monthly prevalence was 3% (range 0–15.4), and average mean intensity was 3.4 (range 1–10). *Oochoristica scelopori* was recovered from 19 of the 21 samples; average monthly prevalence was 11% (range 0–33), and average mean intensity was 2.3 (range 1–5). Tetrathyridia of *Mesocoestoides* sp. were recovered from 11 of the 21 samples; average monthly prevalence was 4% (range 0–33), and average mean intensity was 7.4 (range 1–26.5). Acanthocephalans appeared in the collection only in July 1968 ( $N = 2$ ) and September 1968 ( $N = 1$ ). Based on Figure 2, *Spauligodon giganticus* is a core species within the composite helminth community of *Sceloporus jarrovi*. *Physaloptera retusa*, *Oochoristica scelopori*, *Thubunaea intestinalis*, and *Mesocoestoides* sp. are satellite species. The acanthocephalans are incidental.

From an epizootiological perspective, the composite helminth community is composed of 1 core species and 4 satellite species. Persistence over a 22-yr period is demonstrated. *Spauligodon giganticus* develops directly, no intermediate host is necessary, and infection can occur from fecal contamination of the substrate (Telford, 1971). Infection of lizards can occur shortly after birth, and the life cycle of this oxyurid nematode is completed in less than 98 days (Goldberg and

Burseley, 1992). Substrate licking by *Sceloporus jarrovi*, a well-documented behavior (De Fazio et al., 1977), may be primarily responsible for early infection of juvenile lizards and the high prevalence of *Spauligodon giganticus* seen in the adult lizard population. The other 4 parasites of the component helminth community presumably involve arthropod intermediate hosts. The life cycle of *Thubunaea intestinalis* has not been determined, but most spiruroids are associated with arthropod intermediate hosts (Olsen, 1974). Arthropods infected with third-stage larvae of *Physaloptera retusa* are the source of infection for lizards (Olsen, 1974). Intermediate hosts for *Mesocoestoides* sp., although presumed to be an arthropod, have not been demonstrated (Webster, 1949). Linstowiine cestodes such as *Oochoristica scelopori* are known to develop in beetle intermediate hosts (Millemann and Read, 1953). Because *Sceloporus jarrovi* is insectivorous (Goldberg and Bursey, 1990c), the potential for repeated infection by these 4 helminth species depends primarily on the density of local insect populations.

We thank Jorge Martinez, Douglas Booth, Adrian Sales, Linda Bone, Thomas A. Bienz, and Rana Tawil for assistance in collection of parasites.

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**Research Note**

**Avian Hematozoa of Adult and Nestling Cooper's Hawks (*Accipiter cooperii*) in Wisconsin**

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**ABSTRACT:** Blood from 80 adult and nestling Cooper's hawks in 8 Wisconsin counties was examined for hematozoa. In 1991, 13 of 19 adults harbored *Haemoproteus* sp., 17 of 19 had *Leucocytozoon toddi*, and 1 of 19 exhibited one unidentified microfilaria; and 2 of 4 nestlings were infected with *L. toddi*. In 1992 16 of 28 adults had *Haemoproteus* sp., and 26 of 28 adults and 2 of 29 nestlings possessed *L. toddi*. Adults as old as 6 years were infected with *L. toddi* and *Haemoproteus* sp.

**KEY WORDS:** Cooper's hawks, *Leucocytozoon toddi*, *Haemoproteus* sp. microfilariae, hematozoa, Wisconsin.

In the early to mid 1900s, populations of Cooper's hawks (*Accipiter cooperii*) were in serious decline in the eastern U.S. (Rosenfield, 1988). In Wisconsin it was listed as a threatened species in 1979. As part of a long-term study of a stable breeding population of Cooper's hawks in Wisconsin (Rosenfield et al., 1991) blood samples were collected and analyzed to establish baseline data for future hematozoan studies.

Adult Cooper's hawks were trapped near their nests with mist nets as described by Rosenfield et al. (1992). Nestlings were collected by hand from 38 tree nests, 10–23 m above ground in 8 counties in central and southeastern Wisconsin. Blood was taken with a syringe from the brachial vein of breeding adult and nestling (ca. 10–25-day) Cooper's hawks during 23 June–11 July 1991 and 14 June–10 July 1992. Thin smears were made on microscope slides, air dried, fixed in methanol, stained in Giemsa, and mounted in balsam. Cells were examined at 400, 600, and 1,000 $\times$ . Two slides with only *L. toddi* as re-described by Greiner and Kocan (1977) from falconiforms and one slide with *L. toddi* and *Haemoproteus* sp. Kruse, 1890, were deposited in the University of Nebraska State Museum Harold W. Manter Laboratory Collection (HWML Coll.) as follows: HWML Nos. 36341 *Leucocytozoon toddi* Sambon, 1907, and *Haemoproteus* sp. Kruse, 1890, 36342 and 36342 with *L. toddi*, ex *Accipiter cooperii*.

Haematozoa were collected from hawks in 5 counties in central and southeastern Wisconsin in 1991, and 3 of the same plus 2 additional

counties in this area in 1992. No distribution pattern was discernible.

A single, small (62.5  $\mu$ m) unsheathed microfilaria was observed on one slide. Bennett et al. (1982) reported microfilariae from 8 species in the genus *Accipiter*, but none in *A. cooperii*. Members of the genus *Cardiofilaria* have been reported from raptors, but their microfilariae measure approximately 300  $\mu$ m (Anderson, 1992).

The number of Cooper's hawks in this study harboring *L. toddi* and *Haemoproteus* sp. was higher in all cases than reported by Greiner et al. (1975), Kocan et al. (1977), Stabler and Holt (1965), and Williams and Bennett (1978), perhaps because our samples were collected during the nesting season.

The differences in prevalence of infection rates of *L. toddi* and *Haemoproteus* sp. between the 1991 and 1992 samples can probably be explained in part by the ratios of adults to nestlings and sample sizes (Table 1). In 1991 we examined mainly adults, a smaller sample; in 1992 half of the birds in a larger sample were nestlings. In both years the prevalence of infection in breeding adults was high, ranging from 82 to 100% between sexes and years. According to Atkinson and Van Riper (1991), transmission of avian hematozoa in the northern hemisphere occurs mainly during the breeding season when the insect vectors are present and when immunologically naive nestlings and fledglings are exposed. Peirce and Marquiss (1983) found higher numbers of *L. toddi* gametocytes in nestlings than in

Table 1. Age and sex of Cooper's hawks and prevalence of infections with hematozoa.

Age/sex	1991					1992				
	$N_c$ *	$N_p$ (%)	$N_L$ (%)	$N_H$ (%)	$N_M$ (%)	$N_c$	$N_p$ (%)	$N_L$ (%)	$N_H$ (%)	$N_M$ (%)
Adult males	8	8 (100)	8 (100)	7 (88)	1 (12)	11	10 (90)	10 (90)	7 (64)	0 (0)
Adult females	11	9 (82)	9 (82)	6 (55)	0 (0)	17	17 (100)	16 (94)	9 (53)	0 (0)
Nestling males	2	1 (50)	1 (50)	0 (0)	0 (0)	17	2 (12)	2 (12)	0 (0)	0 (0)
Nestling females	2	1 (50.0)	1 (50)	0 (0)	0 (0)	12	0 (0)	0 (0)	0 (0)	0 (0)
Total	23	20 (87)	19 (83)	13 (57)	1 (4)	57	29 (51)	28 (49)	16 (44)	0 (0)

\*  $N_c$  = number examined;  $N_p$  = number parasitized;  $N_L$  = *Leucocytozoon*;  $N_H$  = *Haemoproteus*;  $N_M$  = microfilariae.

adults. They detected extraordinarily high parasitemias of *L. toddi* in some raptor nestlings as early as 14 days posthatching. The number of infected nestlings versus infected adults available to compare parasitemias was insufficient to show statistical differences. However, our data established that at least some *L. toddi* infections were acquired at nesting sites in Wisconsin.

Concurrent infections were not unusual. In the 1991 males, 1 had *L. toddi*, 6 were found to have both *L. toddi* and *Haemoproteus* sp., and 1 had *L. toddi*, *Haemoproteus* sp., and a microfilaria. Four females harbored *L. toddi*, 5 harbored *L. toddi* and *Haemoproteus* sp., and one harbored *Haemoproteus* sp. The nestlings were infected with only *L. toddi*. In 1992, 3 adult males harbored *L. toddi*, 7 had *L. toddi* and *Haemoproteus* sp., while 8 adult females had *L. toddi*, 8 *L. toddi* and *Haemoproteus* sp., and 1 was infected with *Haemoproteus* sp. As in 1991 infected nestlings had only *L. toddi* (Table 1).

Adult birds of relative and known age (Rosenfield et al. 1992) were analyzed for prevalence of *L. toddi* and *Haemoproteus* sp., and birds of all ages were infected. The infected 5- and 6-yr-old birds showed that either they retained *L. toddi* and/or *Haemoproteus* sp. for long periods or they were being reinfected.

Using published and unpublished data on the prevalence of avian hematozoa in North America, Greiner et al. (1975) analyzed the distribution of parasite genera by region, host family, and vertical stratification of nesting sites. In their analysis, correlation between prevalence of hematozoa showed an inverse relationship to nest height on a local geographic basis—the higher the nest the fewer the parasites. All our Cooper's hawk nest sites were in their highest stratum, 4 (8 m or more). Our study suggests, however, that nest height does not protect these birds from hematozoan vectors in Wisconsin. Bennett et al.

(1975) stated that no geographic generalizations can be made about avian hematozoa epizootiology. Continuing research on blood parasites of fall migrant raptors, including Cooper's hawks, may elucidate additional aspects of these host-parasite relationships.

We thank Dr. Pat Redig for critically reviewing the manuscript, Marc Thwaites for field assistance, and The Wisconsin Department of Natural Resources for facilitating the research. This research was supported in part by The National Raptor Rehabilitators Association.

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## IN MEMORIAM

Frank Dorr Enzie, a member of the Helminthological Society of Washington since 1942, recently passed away at Clearwater, Florida. Dr. Enzie had been suffering from Parkinson's Disease for several years and succumbed to a fatal stroke in late September, 1993.

The following brief review of his contributions to parasitology was taken from a more complete commentary by K.G. Powers on the occasion of the presentation of the 1981 Anniversary Award to Dr. Enzie (Proceedings Helminthological Society of Washington 49:165-167).

Dr. Enzie was an active supporter of the Society and published 18 papers in the Proceedings. He served as Society Recording Secretary in 1948, Vice-president in 1954 and President in 1956. When the need for format standardization between the Proceedings and other parasitological journals was brought to his attention, he organized, supported, and co-chaired the Conference of Parasitology Editors at the Animal Parasitology Institute (API) in 1978. Not the least of his contributions to the Helminthological Society was the continuing encouragement he provided for young scientists and other staff researchers to take an active role in Society affairs. His success in these efforts is evident from the contributions that have been made over the years by many members of the Beltsville parasitology group.

His considerable expertise in parasite chemotherapy is well recognized by the scientific community. Dr. Enzie had a major role in the development of sodium fluoride for the removal of large roundworms from swine and to toluene as an antiparasitic for companion animals. He and his co-workers were the first to develop effective agents against the fringed tapeworm in sheep. They were among the first to report the occurrence of phenothiazine-resistant *Haemonchus contortus*, and the first to demonstrate that certain nonbenzimidazole anthelmintics were effective against benzimidazole-resistant strains of the parasite. Although researchers had tried unsuccessfully for many years to produce drug-resistant strains of worm parasites experimentally, Dr. Enzie and his co-workers achieved this goal with cambendazole and *H. contortus* of sheep in 1973. He prepared and contributed to a number of government publications on the chemical control of parasitic diseases of domestic animals, and was consulted frequently by researchers in the Food and Drug Administration (FDA) and other federal agencies, veterinary practitioners, and representatives from industry in this country and abroad on various matters pertaining to the treatment and control of livestock and poultry parasites. In 1958, he was selected to represent parasitology as a member of the first six-man U.S. Veterinary Exchange Delegation to review veterinary education and research in the Soviet Union. From 1966 to 1968, he served as Chairman of the Panel on Anthelmintics established by the National Research Council/National Academy of Sciences Committee on Veterinary Drug Efficacy. This work served as the basis for the efficacy evaluation of anthelmintics submitted to the FDA for market approval.

Among his notable achievements was the upgrading and augmentation of facilities and staff of the Agricultural Research Service, Animal Parasitology Institute. During his tenure as Institute Chairman, he recruited several outstanding scientists for API, reassigned staff personnel to the mutual advantage of both scientist and project requirements, and provided encouragement, guidance, and appropriate fiscal support for the several Institute programs. This approach contributed to the development and recognition of award-winning scientists and to the establishment of an exceptionally productive research environment at the Animal Parasitology Institute.

K.D. Murrell, USDA, ARS, Beltsville, Maryland 20705-2350.

**In Memoriam**

MILDRED A. DOSS

born September 2, 1903  
died December 22, 1993  
Recording Secretary 1945  
Vice President 1950  
Anniversary Award 1961  
Life Member 1977

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* Willard H. Wright	1976	Harley G. Sheffield	1991

\* Deceased.

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Date of publication, 4 February 1994

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