Volume 41 July 1974 Number 2

PROCEEDINGS

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

Subscription $15.00 a Volume; Foreign, $15.50

CONTENTS

BIENEK, GERHARD K. AND ALBERT W. GRUNDMANN. *Catenotaenia utahensis* sp. n. (Cestoda: Catenotaeniidae) from the Merriam kangaroo rat, *Dipodomys merriami vulcani*, in Utah 134

CARNEY, W. PATRICK. Studies on the life history of *Brachylecithum stunkardi* (Pande, 1939) (Trematoda: Dicrocoeliidae) 139

COLGLAZIER, M. L., K. C. KATES, I. L. LINDAHL, AND G. SAMUELSON. Pasture trials with levamisole and thiabendazole for control of gastrointestinal helminthiasis in sheep 145

COOPER, C. LAWRENCE AND JOHN L. CRITES. Helminth parasites of the common grackle, *Quiscalus quiscula versicolor*, from South Bass Island, Ohio 233

DENTON, J. FRED AND WAYNE A. KRISSINGER. The occurrence and morphology of *Brachylecithum transversum* (Travassos, 1917) comb. n., in the eastern kingbird, *Tyrannus tyrannus* (L.), from Georgia 191

DORAN, DAVID J. *Eimeria tenella*: Merozoite production in cultured cells and attempts to obtain development of culture-produced merozoites 169

DUNAGAN, T. T. AND D. M. MILLER. Muscular anatomy of the praesoma of *Macracanthorhynchus hirudinaceus* (Acanthocephala) 199

FISCHTHAL, JACOB H. AND PIR NASIR. Some digenetic trematodes of birds and a mammal from Venezuela 178

HEINZ, MICHAEL L. AND MURRAY D. DAILEY. The trypanorhyncha (Cestoda) of elasmobranch fishes from Southern California and Northern Mexico 161

(Continued on Back Cover)

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE SOCIETY meets once a month from October through May for the presentation and discussion of papers in any and all branches of parasitology or related sciences. All interested persons are invited to attend.

Persons interested in membership in the Helminthological Society of Washington may obtain application blanks from the Corresponding Secretary-Treasurer, Dr. William R. Nickle, Nematology Laboratory, Plant Protection Institute, ARS-USDA, Beltsville, Maryland 20705. A year's subscription to the Proceedings is included in the annual dues ($8.00).

OFFICERS OF THE SOCIETY FOR 1974

President: THOMAS K. SAWYER
Vice President: ROBERT S. ISENSTEIN
Corresponding Secretary-Treasurer: WILLIAM R. NICKLE
Assistant Corresponding Secretary-Treasurer: KENDALL G. POWERS
Recording Secretary: A. MORGAN GOLDEN
Librarian: JUDITH M. SHAW (1962–)
Archivist: JUDITH M. SHAW (1970–)
Representative to the Washington Academy of Sciences: JAMES H. TURNER (1972–)
Representative to the American Society of Parasitologists:
GEORGE W. LUTTERMOSER (1969–)
Executive Committee Members-at-Large: RONALD FAYER, 1975
SHERMAN S. HENDRIX, 1975
RICHARD L. BEAUDOIN, 1974
JOHN I. BRUCE, 1974

THE PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE PROCEEDINGS are published semiannually at Lawrence, Kansas by the Helminthological Society of Washington. Papers need not be presented at a meeting to be published in the Proceedings. However, non-members may publish in the Proceedings only if they contribute the full cost of publication.

MANUSCRIPTS should be sent to the EDITOR, Harley G. Sheffield, Laboratory of Parasitic Diseases, Building 5, Room 112, National Institutes of Health, Bethesda, Maryland 20014. Manuscripts must be typewritten, double spaced, and in finished form. The original and one copy are required. Photocopies of figures and drawings may be submitted for review purposes; originals will be requested after acceptance of the manuscript. Papers are accepted with the understanding that they will be published only in the Proceedings.

REPRINTS may be ordered from the PRINTER at the same time the corrected proof is returned to the EDITOR.

BACK VOLUMES of the Proceedings are available. Inquiries concerning back volumes and current subscriptions should be directed to: Helminthological Society of Washington, c/o Allen Press, Inc., 1041 New Hampshire St., Lawrence, Kansas 66044, U.S.A.

BUSINESS OFFICE. The Society's business office is at Lawrence, Kansas. All inquiries concerning subscriptions or back issues and all payments for dues, subscriptions, and back issues should be addressed to: Helminthological Society of Washington, c/o Allen Press, Inc., 1041 New Hampshire St., Lawrence, Kansas 66044, U.S.A.

PROCEEDINGS OF THE
HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 41 JULY 1974 NUMBER 2

Acquired Immunity to Experimental Infection with *Angiostrongylus cantonensis* in Mice[1,2]

DAVID T. JOHN[3]

Department of Parasitology and Laboratory Practice, School of Public Health,
University of North Carolina at Chapel Hill

ABSTRACT: Mice were immunized by two stimulating infections with 25 *Angiostrongylus cantonensis* larvae. After a challenge infection with 25 larvae, immunized mice yielded significantly fewer and smaller worms than the nonimmunized controls. Weight loss occurred to a lesser degree in the immunized mice; they experienced a greater leukocytosis, and changes in the lymphocyte/neutrophil ratio occurred earlier than those in the nonimmunized controls.

Very little is known about the immune response of mammals to infection with *Angiostrongylus cantonensis*. Heyneman and Lim (1965); Lim, Ow Yang, and Lie (1965); and Ow Yang, Lim, and Lie (1965) have demonstrated that rats develop and acquired immunity to a normally fatal challenge infection when they are given several prior exposures to small numbers of third stage larvae. Lee (1969) was able to show a significant immunity in rats to a challenge infection with normal larvae after the animals had first received two feedings of irradiated larvae. Heyneman and Lim (1967 suggested that in Malaya man may become actively immunized against *A. cantonesis* through repeated low-level infections with larvae shed by naturally infected slugs. They were able to recover infective third-stage larvae on lettuce purchased from the public markets in Kuala Lumpur and so offer this as a possible source of human infection.

The above-mentioned laboratory studies have dealt with infections in rats, the normal definitive host for *A. cantonensis*. Recently, the course of a primary infection with *A. cantonensis* in mice, an abnormal host, has been described (John 1970). The present investigation considers the development of acquired immunity to experimental infections in mice.

Materials and Methods

EXPERIMENTAL DESIGN: A strain of Swiss albino mice maintained in this Department for many years was used in this study. Two hundred twenty female mice, 12–14 weeks old at the beginning of the experiment, were distributed randomly into four groups. The 100 mice of Group I, hereafter referred to as immunized mice, were inoculated perorally with two stimulating infections of 25 living third-stage *A. cantonensis* larvae on days 0 and 60, and then were challenged with 25 larvae on day 120. Forty-five mice (Group II) were infected once with 25 larvae on day 60 and served as nomimmunized controls for the immunized mice (Group I) receiving a second infection. A second group of 45 mice served as nonimmunized controls (Group III) for the third infection (challenge infection). Thirty mice (Group IV) were maintained as noninfected controls.

PARASITE: The strain of *A. cantonensis* used in this experiment was obtained through the

[1] A portion of a dissertation submitted to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Parasitology and Laboratory Practice, School of Public Health.

[2] This investigation was supported in part by a Predoctoral Fellowship from the National Institute of Environmental Health Sciences (5F01ES38579-03).

[3] Present address: Department of Microbiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

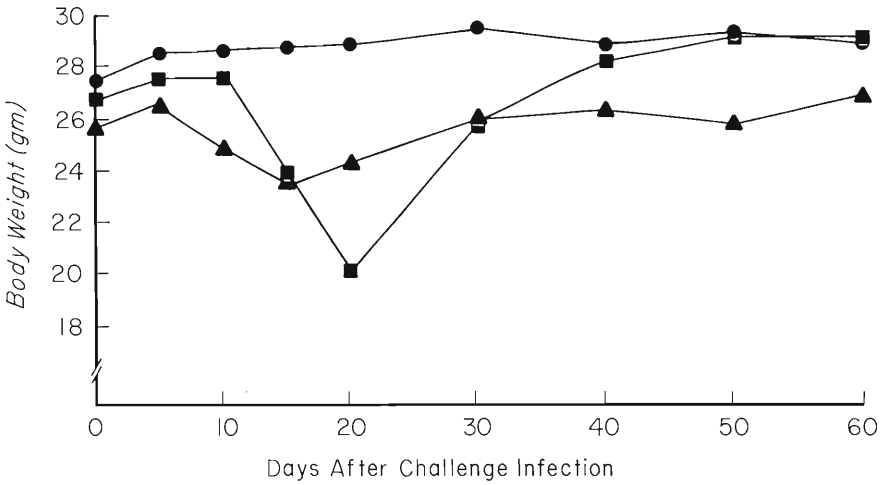


Figure 1. Average body weights of mice after a challenge infection with 25 *A. cantonensis* larvae administered 60 days after the second stimulating infection. (▲ Immunized; ■ Nonimmunized; ● Noninfected.)

courtesy of Dr. Paul P. Weinstein from the National Institutes of Health in June 1967. Since that time, the parasite has been maintained in albino rats and *Biomphalaria glabrata* snails. Infective third-stage larvae were recovered from laboratory-infected snails by peptic digestion (1% pepsin and 1% HCl) (John, 1970).

HEMATOLOGY: Blood for total and differential leukocyte counts was obtained from the clipped tails of mice. After each infection, eight mice from all groups were bled on the following days: 5, 10, 15, 20, 40, and 60. Blood counts were determined for each mouse and then the averages were calculated.

WEIGHT: Mice from all groups were weighed in lots of 10 and the average weight per mouse was determined. After each infection, animals were weighed on the following days: 5, 10, 15, 20, 30, 40, 50, and 60.

WORM COUNTS AND MEASUREMENTS: Earlier studies showed that the greatest number of worms were recovered from the mouse brain on days 5, 10, and 15 after infection. The greatest percentage recovery occurred on day 15 (John, 1970). Thus, in the present experiment, worm counts and worm measurements were determined on all three of the abovementioned days. On each day indicated, eight mice were killed from both the immunized and nonimmunized groups. The trypsin digestion technique described by Sprent (1955) was used to recover worms from brain tissue. Male and female worms were counted and measured. Measurements included total length and width at the middle of the worm.

STATISTICS: Student's *t* test was used to determine the statistical significance of the observed differences in number and size of the worms recovered from immunized and nonimmunized mice. Probability values greater than 0.05 were rejected as not being significant.

Results

VIABILITY CONTROL AND MORTALITY: After the first infection, an average of 9.9 worms per mouse (39.5%) was recovered from the eight mice used to check the viability of the larvae used for infection (viability controls). The viability controls after the second infection yielded an average of 8.9 worms per mouse (35.5%), and after the third infection, 10.4 worms per mouse (41.5%).

After the first infection, three mice died in Group I. None of the immunized mice (Group I) died after the second infection, but one of the nonimmunized controls (Group II) died. After the third infection, one immunized mouse (Group I) died and one non-

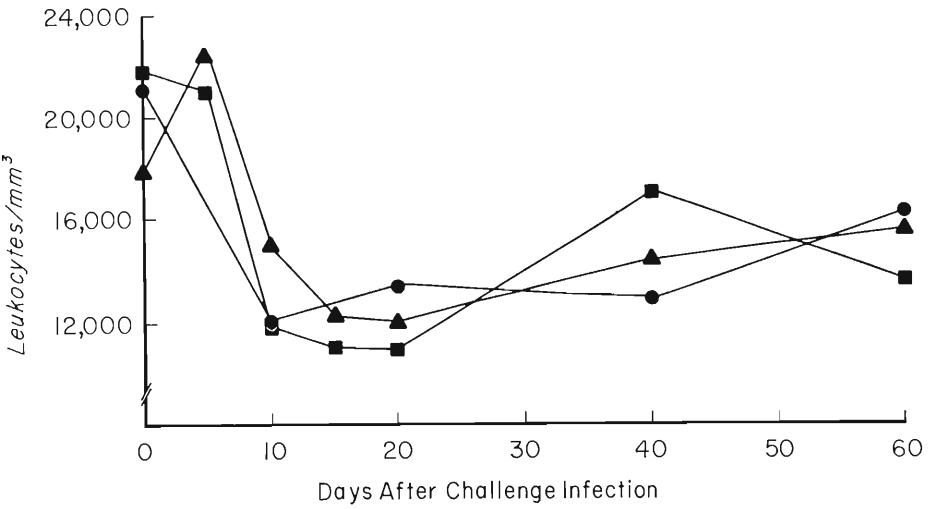


Figure 2. Average leukocyte counts from mice after a challenge infection with 25 *A. cantonensis* larvae administered 60 days after the second stimulating infection. (▲ Immunized; ■ Nonimmunized; ● Noninfected.)

immunized control mouse (Group III) died. The six deaths occurred between the 20th and 23rd days after infection.

TOTAL BODY WEIGHTS: Noninfected mice (Group IV) gained weight steadily, with only slight fluctuations, throughout the period of study. After the first infection, immunized mice (Group I) lost an average of 8.6 g per mouse (32.3% of total body weight) between days 10 and 20.

After the second infection, immunized mice (Group I) began to lose weight on day 5, and between days 5 and 15, they lost an average of 4.8 g (17.9% of total body weight). By comparison, nonimmunized mice (Group II) lost an average of 6.9 g (25.8% of total body weight) between days 10 and 20.

After the third challenge infection, weight loss for the immunized mice (Group I) was 3.0 g (11.3% of total body weight) between days 5 and 15, whereas the nonimmunized mice (Group III) lost an average of 7.6 g (27.4% of total body weight) between days 10 and 20 (Fig. 1).

TOTAL LEUKOCYTE COUNTS: A leukocytosis of 17,538 cells per mm^3 of blood occurred in the immunized mice (Group I) on day 5 after the first infection, and on the same day, the average count for the noninfected controls (Group IV) was 14,981 cells.

After the second infection, leukocytosis was present in both the immunized (Group I) and the nonimmunized (Group II) mice between days 5 and 15. A maximum of 28,781 cells for the immunized and 25,231 cells for the nonimmunized controls was recorded on day 10. The count was 19,862 cells for the noninfected controls (Group IV) on the same day.

Between days 5 and 10 after the third infection, the total leukocyte counts of immunized (Group I) and nonimmunized (Group III) were greater than the average count for the noninfected controls (Group IV). The maximum average count occurred on day 5 and was 22,412 cells for the immunized mice and 21,038 cells for the nonimmunized mice. On the same day, the cell count was about 17,000 cells for the noninfected controls. The total leukocyte counts for infected and noninfected mice after the challenge infection are illustrated in Figure 2.

DIFFERENTIAL LEUKOCYTE COUNTS: The percentage of neutrophils began to increase as the percentage of lymphocytes decreased in the immunized mice (Group I) on day 10 after the first stimulating infection. A reversal or inversion of the lymphocyte/neutrophil ratio occurred on day 20; the neutrophil count was 49.2% and the lymphocyte count was 44.0%.

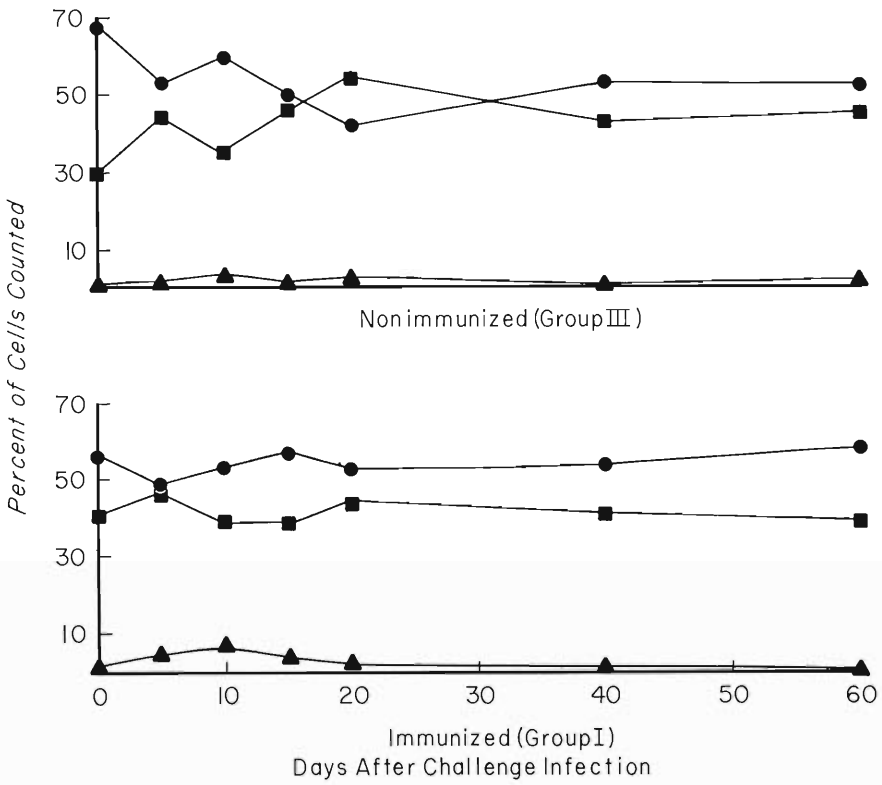


Figure 3. Average differential leukocyte counts from mice after a challenge infection with 25 *A. cantonensis* larvae administered 60 days after the second stimulating infection. (● Lymphocytes; ■ Neutrophils; ▲ Eosinophils.)

After day 20, the percentages gradually returned to normal values. Eosinophil counts reached a maximum of 6.4% on day 15.

After the second infection, the lymphocyte/neutrophil reversal occurred on day 40 for the immunized mice (Group I). On this day, the neutrophils were 52.0% and the lymphocytes were 45.4%. The reversal occurred on day 20 for the nonimmunized mice (Group II). A maximum eosinophilia of 6.2% was recorded for the immunized mice on day 10.

After the third infection, the lymphocyte/neutrophil reversal did not occur in the immunized mice (Group I). However, on day 5, the neutrophils increased to 47.0% and the lymphocytes were 48.4%. The reversal for the nonimmunized mice (Group III) occurred on day 20, when the neutrophils were 54.5% and the lymphocytes were 42.0%. A peak eosinophilia of 6.5% was recorded for the immunized mice on day 10. The average differential leukocyte counts for immunized and nonimmunized mice after challenge infection are illustrated in Figure 3.

WORM COUNTS: The average number and percentage of *A. cantonensis* recovered from immunized and nonimmunized mice are presented in Table 1. Each figure represents the average number of worms recovered from eight mice. The data show that the differences in worm numbers between *immunized* and nonimmunized control mice were significant (P 0.05 to 0.001) for all days (5, 10, 15) after both the second stimulating infection and the challenge infection.

WORM MEASUREMENTS: The most significant difference in the size of worms from im-

Table 1. The average number and percentage of *Angiostrongylus cantonensis* recovered from immunized and nonimmunized mice given infections with 25 larvae.

	Second stimulating infection			Challenge infection		
	Average number worms recovered (per cent) Day			Average number worms recovered (per cent) Day		
Group*	5	10	15	5	10	15
Immunized	9.2 (37.0)	7.5 (30.0)	1.9 (7.5)	11.5 (46.0)	5.9 (23.5)	3.0 (12.0)
Nonimmunized	15.8 (63.0)	9.6 (38.5)	8.9 (35.5)	15.0 (60.0)	10.0 (40.0)	10.4 (41.5)
t value	4.79	2.64	6.01	2.18	3.10	6.60
Level of significance	0.001	0.05	0.001	0.05	0.01	0.001

* Eight mice/group/day.

munized and nonimmunized mice was observed on day 15 after the second stimulating infection and after the challenge infection. On days 5 and 10, the differences in lengths were often significant; however, differences in widths were usually not significant. For this reason, Table 2 presents the average length and width of 10 worms each, recovered from immunized and nonimmunized mice only on day 15. On this day, the differences in both length and width of male and female worms from immunized and nonimmunized mice were statistically significant at the 0.01 or 0.001 level.

Discussion

In mice, as in man, *Angiostrongylus cantonensis* is confined to the central nervous system. Following peroral inoculation, larvae appear in the brain within 24 hr and during the next 2 weeks undergo a period of rapid growth and development within the brain tissue. Most of the larvae complete their third molt by the 5th day; by the 10th day, the fourth and last molt occurs. Shortly after the final molt, young worms migrate to the surface of the brain, where they remain in the subarachnoid space. Although live worms have been recovered from the brains of mice 180 days after infection, sexually mature adults have never been observed (John, 1970).

The results of this experiment provide evidence that mice exposed to larvae of *A. cantonensis* developed an immunity to subsequent reinfection by this parasite. After one or two stimulating infections with 25 larvae, the average number of worms recovered from the immunized mice was significantly fewer than that from the nonimmunized controls. This reduced worm burden was observed as early as day 5 after infection, but was most pronounced on the 15th day. Similarly, the size of both male and female worms recovered from the immunized mice was reduced signif-

Table 2. The average size of *Angiostrongylus cantonensis* recovered from immunized and nonimmunized mice on day 15 after infections with 25 larvae.

	Second stimulating infection Average size (mm)				Challenge infection Average size (mm)			
	Male		Female		Male		Female	
Group*	L	W	L	W	L	W	L	W
Immunized	2.92	0.07	3.75	0.08	2.58	0.07	2.93	0.07
Nonimmunized	4.69	0.09	5.70	0.12	4.04	0.08	5.04	0.11
t value	4.15	4.56	7.99	5.69	5.62	3.40	7.43	6.49
Level of significance	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001

* Eight mice/group (each figure represents the average size of 10 worms).
L = Length of worms.
W = Width of worms.

icantly, but consistently only on day 15. The worms recovered from immunized mice appear to be greatly stunted in their growth and in tissue sections are seen to be surrounded by an inflammatory cellular infiltration. Such an inflammatory reaction is not a usual feature during the early growth and development stage of the parasite following a single infection. Hence, the significant reduction in worm number and size in the immunized mice can most likely be attributed to the acute inflammatory response. These results support the findings of others [Heyneman and Lim (1965), Lim, Ow Yang, and Lie (1965), and Ow Yang, Lim, and Lie (1965)] that repeated low-level infections actively immunize animals to a challenge infection with living *A. cantonensis* larvae. However, the present is the first report of acquired immunity to experimental infection with *A. cantonensis* is an abnormal host.

Weight loss was much less after repeated infections. The immunized mice lost less than half as much weight as the nonimmunized animals. Also, weight loss in the immunized mice began and ended 5 days earlier than in the nonimmunized control mice. Concomitant with this decreased weight loss, fewer worms were present within the brain tissue of the immunized mice. Perhaps the toxic metabolic products, including exsheathing fluid, released by the worms were in some way responsible for the phenomenon of weight loss in the infected mice.

Although a leukocytosis was observed for both the immunized and nonimmunized mice on day 5 after the challenge infection, the total cell count was greater for the immunized mice. This heightened or anamnestic reaction is a characteristic response to a challenging infection (Cypess, 1972). The nonimmunized mice always exhibited a reversal of the lymphocyte/neutrophil ratio on day 20 after infection. Although the immunized mice did not show this reversal, the percentages of lymphocytes (48.4%) and neutrophils (47.0%) were nearly equal on the 5th day after the challenging infection. This near reversal occurred 15 days earlier than in the nonimmunized mice, and was associated with the host's accelerated response to reinfection. Also, eosinophilia occurred 5 days earlier for immunized mice.

Although this experiment demonstrated an acquired immunity to reinfection with *A. cantonensis*, it raises questions as to the nature of the immune response, i.e., whether the mechanism of immunity is primarily humoral or cell-mediated. It is apparent that further study is needed to shed light on this.

Acknowledgment

I am grateful to Dr. Hilton T. Goulson, Professor of Parasitology, for his counsel and direction throughout this investigation.

Literature Cited

Cypess, R. H. 1972. Blood cell count changes associated with immunity to *Nematospiroides dubius*. J. Parasit. 58: 563–566.

Heyneman, D., and B. L. Lim. 1965. Prolonged survival in rats immunized by a small number of low-level doses of *Angiostrongylus cantonensis* and challenged with lethal level of infective larvae. Med. J. Malaya, 20: 162–163.

———, **and** ———. 1967. *Angiostrongylus cantonensis:* Proof of direct transmission with its epidemiological implications. Science 158: 1057–1058.

John, D. 1970. The biology of *Angiostrongylus cantonensis* in the white mouse. Dissertation. Univ. North Carolina, Chapel Hill.

Lee, S. H. 1969. The use of irradiated third-stage larvae of *Angiostrongylus cantonensis* as antigen to immunize albino rats against homologous infection. Proc. Helminth. Soc. Wash. 36: 95–97.

Lim, B. L., C. K. Ow Yang, and K. J. Lie. 1965. Natural infection of *Angiostrongylus cantonensis* in Malaysian rodents and intermediate hosts, and preliminary observations on acquired resistance. Am. J. Trop. Med. Hyg. 14: 610–617.

Ow Yang, C. K., B. L. Lim, and K. J. Lie. 1965. Observations on the presence of immunity in rats to *Angiostrongylus cantonensis* (Chen). Med. J. Malaya 20: 70.

Sprent, J. F. A. 1955. On the invasion of the central nervous system by nematodes. II. Invasions of the nervous system in Ascariasis. Parasitology 45: 41–55.

Helminth Fauna of the Florida Scrub Jay: Host and Ecological Relationships

J. M. KINSELLA[1]
Archbold Biological Station, The American Museum of Natural History, Rt. 2, Box 380, Lake Placid, Florida 33852

ABSTRACT: Fifteen species of helminths were found in 45 Florida scrub jays, *Aphelocoma coerulescens coerulescens*, examined in south-central Florida. The normal or dominant fauna of the scrub jay appears to include one trematode, four nematodes, and one acanthocephalan, most of which appear to exhibit an ecological rather than a host specificity. A nematode, *Dispharynx nasuta*, was implicated as a possible cause of death in two fledglings. The relatively heavy parasite load of the scrub jay reflects its predominantly arthropod diet during the collection period of late winter and spring.

The Florida scrub jay, *Aphelocoma coerulescens coerulescens* (Bosc), is a relict race of the species found in the southwestern United States and northern Mexico (Pitelka, 1951). In Florida, the nonmigratory scrub jay is restricted to relatively xeric habitats composed of low dense thickets of oaks and other shrubs, with numerous open, sandy spaces. Within this habitat, breeding adults are extremely sedentary, defending a permanent territory of 20 to 30 acres (G. E. Woolfenden, pers. comm.).

Little is known about the helminth fauna of the scrub jay. Walton (1927) described two nematodes, *Diplotriaenia multituberculata* and *Diplotriaenoides hepaticus*, from single specimens collected from the Florida scrub jay, but gave no specific localities for these records. This study of the helminths of *A. c. coerulescens* in south-central Florida was undertaken to compare the fauna of a corvid with narrow habitat specificity with that of more wide-ranging corvids and to provide a base for comparison with the fauna of the scrub jay in the western part of its range.

Methods and Materials

Most birds used in this study were collected within a 2-mile radius of the Archbold Biological Station, Highlands Co., Florida. Eight birds were collected near the west shore of Lake Istokpoga, Highlands Co., and four birds in the vicinity of Roseland, Indian River Co. Adult birds were shot with an air rifle and nestlings and fledglings were caught by hand. All birds were collected between January and July 1973.

Birds were examined within a few hours after death. The gastrointestinal tract, heart, and trachea were opened and the contents washed through a 100-mesh sieve. The lungs, liver, and kidneys were teased and then washed in the sieve before examination. The body cavity and orbits also were examined for nematodes. Nematodes were fixed in glacial acetic acid and preserved in 70% alcohol with glycerine. Trematodes, cestodes, and acanthocephala were relaxed and then fixed and preserved in AFA. Trematodes and cestodes were stained with Harris' hematoxylin. Nematodes and acanthocephala were studied in temporary mounts of lactophenol.

Results and Discussion

Thirteen helminth species were recovered from 35 adult scrub jays, none of which were free of helminths. These included three species of trematodes, one species of cestode, eight species of nematodes, and one species of acanthocephala (Table 1). Ten nestlings and fledglings were examined; five birds less than 28 days old were free of helminths, while five birds 2 to 3 months of age were infected with six species of helminths. These included three species of trematodes, two species of nematodes, and one species of acanthocephala (Table 1).

Multiple infections were common in adult birds, with three or more species occurring in 21 of 35 birds. The extensity of helminth infection did not appear to decrease with age since three birds, banded as adults in 1968

[1] Mailing address: 936 Rimini Court, Missoula, Montana 59801.

Table 1. Helminth parasites of the Florida scrub jay.

	Adults			Young		
	No. infected	No. of worms		No. infected	No. of worms	
		Mean	(Range)		Mean	(Range)
Number of birds examined	35			10		
TREMATODA						
Brachylecithum nanum Denton and Byrd, 1951 (5)[1]	11	3	(1–8)	—	—	—
Brachylecithum americanum Denton, 1945 (5)	1	9	(9)	—	—	—
Echinostoma revolutum (Froelich, 1902) (3)	1	1	(1)	1	3	(3)
Stomylotrema vicarium Braun, 1901 (4)	—	—	—	1	2	(2)
Mosesia sp. (3)	—	—	—	1	1	(1)
CESTODA						
Oligorchis cyanocitti Coil, 1955 (3)	1	1	(1)	—	—	—
NEMATODA						
Microtetrameres spiculata Boyd, 1956 (1)	24	5	(1–23)	—	—	—
Cardiofilaria inornata (Anderson, 1956) (6)	23	2	(1–6)	—	—	—
Dispharynx nasuta (Rudolphi, 1819) (1)	11	8	(1–49)	1	13	(1–30)
Oxyspirura pusillae Wehr and Hwang, 1957 (7)	9	3	(1–11)	—	—	—
Acuaria quiscula Williams, 1929 (2)	8	1	(1)	1	1	(1)
Aprocta sp. (6)	1	1	(1)	—	—	—
Diplotriaenia sp. (6)	1	1	(1)	—	—	—
Strongyloides sp. (3)	1	1	(1)	—	—	—
ACANTHOCEPHALA						
Mediorhynchus robustus Van Cleave, 1916 (3)	14	3	(1–10)	1	15	(15)

[1] Location in host: (1) proventriculus, (2) under gizzard lining, (3) small intestine, (4) cloaca, (5) liver, (6) air sacs, (7) under nictitating membrane.

(Wescott, 1970), were infected with three species each. These birds, which were at least 6 years old, were occupying the same territories where they were banded, illustrating the sedentary nature of this race.

Five parasite genera (*Mosesia, Oligorchis, Strongyloides, Aprocta, Diplotriaenia*) were represented by a single specimen each. *Mosesia* sp., found in a fledging scrub jay and also a fledgling blue jay (*Cyanocitta cristata*), does not agree with the only known North American species from birds, *M. chordeilesia* McMullen, 1936, described from the common nighthawk (*Chordeiles minor*). *Oligorchis cyanocitti* was described by Coil (1955) from Steller's jay (*Cyanocitta stelleri*) in Mexico. *Oligorchis* sp., reported from one of 94 blue jays in New England by Boyd, Diminno, and Nesslinger, 1956, may be the only other record of this tapeworm, which appears to be specific to jays.

Strongyloides sp. and *Aprocta* sp. could not be further identified from the female specimens collected. A fragmented female *Diplotriaenia* differs in measurements from both *D. hepaticus* and *D. multituberculta* (synonym *Diplotriaenoides multituberculata*) described by Walton (1927) from the Florida scrub jay. These parasites are probably accidental in scrub jays.

Echinostoma revolutum, reported from many species of birds and mammals (McDonald, 1969), requires an aquatic snail in its life cycle. It was found in one of four adults collected from scrub adjoining a small lake in Indian River Co., and in a fledgling, whose feeding territory included a small artificial pond on the grounds of the Archbold Biological Station.

Stomylotrema vicarium, found in a single fledgling, has been reported in Florida from the wild turkey (*Meleagris gallopavo*), white ibis (*Eudocimus albus*), and sandhill crane (*Grus canadensis*) (Bush, 1973). *Brachylecithum americanum*, also found in a single bird, is a more common parasite of the common grackle (*Quiscalus quiscula*) and common crow (*Corvus brachyrhynchos*) (Denton, 1945). *Acuaria quiscula*, originally described from the common grackle by Williams (1929), was found in nine jays, each infected by a single worm. In five birds, only larvae were present. Since common grackles are common ecological associates of scrub jays (Woolfenden, 1969), the scrub jay may act as a secondary host for both *B. americanum* and *A. quiscula*.

The remaining six genera (*Brachylecithum, Microtetrameres, Dispharynx, Oxspirura, Cardiofilaria, Mediorhynchus*) probably constitute the "normal" or dominant parasite fauna

of the scrub jay. With the exception of *Microtetrameres spiculata*, which previously was reported only from the blue jay (Boyd et al., 1956), these parasites are known to occur in a variety of birds and probably exhibit only ecological specificity.

Brachylecithum nanum was described from the rufous-sided towhee (*Pipilo erythrophthalmus*) and the white-throated sparrow (*Zonotrichia leucophrys*) in North Carolina, Virginia, Georgia, and Texas by Denton and Byrd (1951). The rufous-sided towhee is a common associate of scrub jays in Highlands Co. (Woolfenden, 1969) and was seen in all areas where scrub jays were collected. Since 6 of 11 jays infected harbored only a single worm, the towhee may be the primary host of *B. nanum*.

Cardiofilaria inornata was reported by Anderson and Freeman (1969) from a variety of hosts including the American woodcock (*Philohela minor*), the long-eared owl (*Asio otus*), and the common raven (*Corvus corax*). Anderson and Freeman speculated that a filarid with such a wide range of hosts must be transmitted by vectors with wide feeding preferences such as mosquitoes.

Dispharynx nasuta has also been reported from a large number of hosts, but is especially common in galliforms and passeriforms (Goble and Kutz, 1945). Goble and Kutz found the incidence of this worm in juvenile birds to be significantly greater than in adults. In young ruffed grouse (*Bonasa umbellus*), infections were accompanied by a severe and often fatal proventriculitis. Two banded scrub jays, 98 and 99 days old, were found weak and extremely emaciated, and died within a few hours. These fledglings contained 28 and 30 *D. nasuta*, respectively. Although the infections could not be proven to be the cause of death, the role of this pathogenic nematode in the biology of the scrub jay deserves further attention. Cram (1931) found that isopods act as intermediate hosts for *D. nasuta*.

Pence (1973) reported *Oxyspirura pusillae* from 10 species of birds in Louisiana, including the red-bellied woodpecker (*Centurus carolinus*), brown thrasher (*Toxostoma rufum*), and common grackle. He stated that the host distribution of *O. pusillae* is probably related to an arthropod intermediate host which is restricted to a particular habitat. Its avian hosts most commonly feed on trees, removing insects from under bark, decaying wood, and leaves.

The hosts of *Mediorhynchus robustus* include the yellow-shafted flicker (*Colaptes auratus*), the brown thrasher, and the rufous-sided towhee (Van Cleave, 1947), all common associates of the scrub jay. The distribution of this parasite also seems to be related to common arthropod prey items shared by its hosts. A closely related species, *M. grandis*, uses grasshoppers as intermediate hosts (Moore, 1962).

The normal helminth fauna of the scrub jay therefore shows a complex interrelationship with the faunas of the birds with which it coexists, and the intermediate hosts of the worms. Seven helminths (*Brachylecithum* spp., *Microtetrameres*, *Dispharynx*, *Oxyspirura*, *Acuaria*, *Mediorhynchus*) almost certainly require an arthropod intermediate host, while an eighth (*Cardiofilaria*) is transmitted by a blood-sucking arthropod. The collection period of January through July represents the peak of arthropod consumption in Florida scrub jays, which feed primarily on acorns in late summer and fall (G. E. Woolfenden, pers. comm.). Since most of its parasites are arthropod-borne, it probably also represents the peak parasitic burden.

Comparative data on the helminth faunas of other North American corvids are available for the blue jay (Boyd et al., 1956), common crow (Daly, 1959; Hendricks, Harkema, and Miller, 1969), and black-billed magpie (*Pica pica*) (Todd and Worley, 1967). As presently known, the scrub jay shares only two helminth species with the magpie and crow, and four (possibly five) with the blue jay. The scrub jay differs from the other three corvids in its high rate of infection with acanthocephala. The two species of jays share a low rate of infection with cestodes, in contrast with magpies and crows which harbor four species each, and are commonly infected. The high rate of filarial infections in the blue jay and common crow reported by Robinson (1955) in Georgia parallels that found in the scrub jay in the present study. Despite its limited geographical range, relatively xeric habitat, and sedentary habits, the Florida scrub jay does not seem to have a reduced helminth fauna in comparison with other corvids,

either in terms of number of species or intensity of infection.

Acknowledgments

I wish to especially thank Glen E. Woolfenden for providing some of the birds and much valuable information on the biology of the scrub jay. I also wish to thank Fred E. Lohrer and Chet E. Winegarner for aid in collections and James N. Layne for helpful criticism of the manuscript. J. Fred Denton kindly aided in identification of liver flukes and Roy C. Anderson identified one of the filarids. I also wish to express my appreciation to Mr. Richard Archbold for financial support and use of the facilities of the Archbold Biological Station.

Literature Cited

Anderson, R. C., and R. S. Freeman. 1969. *Cardiofilaria inornata* (Anderson, 1956) from woodcock with a review of *Cardiofilaria* and related genera (Nematoda: Filarioidea). Trans. Amer. Micros. Soc. 88: 68–79.

Boyd, E. M., R. L. Diminno, and C. Nesslinger. 1956. Metazoan parasites of the blue jay, *Cyanocitta cristata* L. J. Parasit. 42: 332–346.

Bush, A. O. 1973. An ecological analysis of the helminth parasites of the white ibis in Florida. M.S. thesis, Univ. of Florida, 114 p.

Coil, W. H. 1955. *Oligorchis cyanocitti* sp. nov., a hymenolepid cestode parasitic in the Steller jay, *Cyanocitti stelleri* (Corvidae). Proc. Helm. Soc. Wash. 22: 112–114.

Cram, E. B. 1931. Developmental stages of some nematodes of the Spiruroidea parasitic in poultry and game birds. USDA Tech. Bull. 227.

Daly, E. F. 1959. A study of the intestinal helminths of the southern crow (*Corvus brachyrhynchos paulus*) in Virginia. Proc. Helm. Soc. Wash. 26: 66.

Denton, J. F. 1945. Studies on the life history of *Brachylecithum americanum* n. sp., a liver fluke of passerine birds. J. Parasit. 31: 131–141.

———, **and E. E. Byrd.** 1951. The helminth parasites of birds. III. Dicrocoelid trematodes from North American birds. Proc. U. S. Nat. Mus. 101 (3274): 157–202.

Goble, F. C., and H. L. Kutz. 1945. The genus *Dispharynx* (Nematoda: Acuariidae) in galliform and passeriform birds. J. Parasit. 31: 323–331.

Hendricks, L. D., R. Harkema, and G. C. Miller. 1969. Helminths of the crow, *Corvus brachyrhynchos* Brehm, 1822, in North Carolina. Proc. Helm. Soc. Wash. 36: 150–152.

McDonald, M. E. 1969. Catalogue of helminths of waterfowl (Anatidae). Bur. Sport Fish. Wildl. Spec. Sci. Rep. No. 126.

Moore, D. V. 1962. Morphology, life history, and development of the acanthocephalan *Mediorhynchus grandis* Van Cleave, 1916. J. Parasit. 48: 78–86.

Pence, D. B. 1972. The genus *Oxyspirura* (Nematoda: Thelaziidae) from birds in Louisiana. Proc. Helm. Soc. Wash. 39: 23–28.

Pitelka, F. A. 1951. Speciation and ecologic distribution in American jays of the genus *Aphelocoma*. Univ. Calif. Publ. Zool. 50: 195–464.

Robinson, E. J., Jr. 1955. Observations on the epizootiology of filarial infections in two species of the avian family Corvidae. J. Parasit. 41: 209–214.

Todd, K. S., Jr., and D. E. Worley. 1967. Helminth parasites of the black-billed magpie, *Pica pica hudsonia* (Sabine, 1923), from southwestern Montana. J. Parasit. 53: 364–367.

Van Cleave, H. J. 1947. The acanthocephalan genus *Mediorhynchus*, its history and a review of the species occurring the United States. J. Parasit. 33: 297–315.

Walton, A. C. 1927. A revision of the nematodes of the Leidy collection. Proc. Acad. Nat. Sci. Phila. 79: 49–163.

Wescott, P. W. 1970. Ecology and behavior of the Florida scrub jay. Ph.D. diss., Univ. of Florida, 87 p.

Williams, O. L. 1929. A critical analysis of the specific characters of the genus *Acuaria*, nematodes of birds, with descriptions of new American species. Univ. Calif. Publ. Zool. 33: 69–107.

Woolfenden, G. E. 1969. Breeding-bird censuses of five habitats at Archbold Biological Station. Audubon Field Notes 23: 732–738.

Rhigonema critesi sp. n. (Nematoda: Rhigonematidae), A Parasite of the Millipede, *Orthoporus typotopyge* (Brölemann, 1905) from Costa Rica

JORGE RAMÍREZ†
Department of Zoology, The Ohio State University, Columbus, Ohio 43210

ABSTRACT: *Rhigonema critesi* sp. n. (Nematoda: Rhigonematidae) from the millipede, *Orthoporus typotopyge* (Brölemann, 1905), is described. This is the first rhigonematid nematode to be described from Costa Rica. It differs from other *Rhigonema* species in that the female has a large, blunt, prevulvar spine and in the number and arrangement of caudal papillae in the male: Five pairs precloacal, one median, unpaired precloacal and four pairs postcloacal. Also it lacks cuticular pilosities.

The nematodes studied for this description came from the hindgut of the millipede, *Orthoporus typotopyge* (Brölemann, 1905). Twenty-three millipedes were collected in farmland just west of Juan Santamaría International Airport, Alajuela Province, Costa Rica. Twenty of them were infected with *Rhigonema critesi*. Each millipede contained four to 35 adults (41% males) and four to 30 larvae of *R. critesi*.

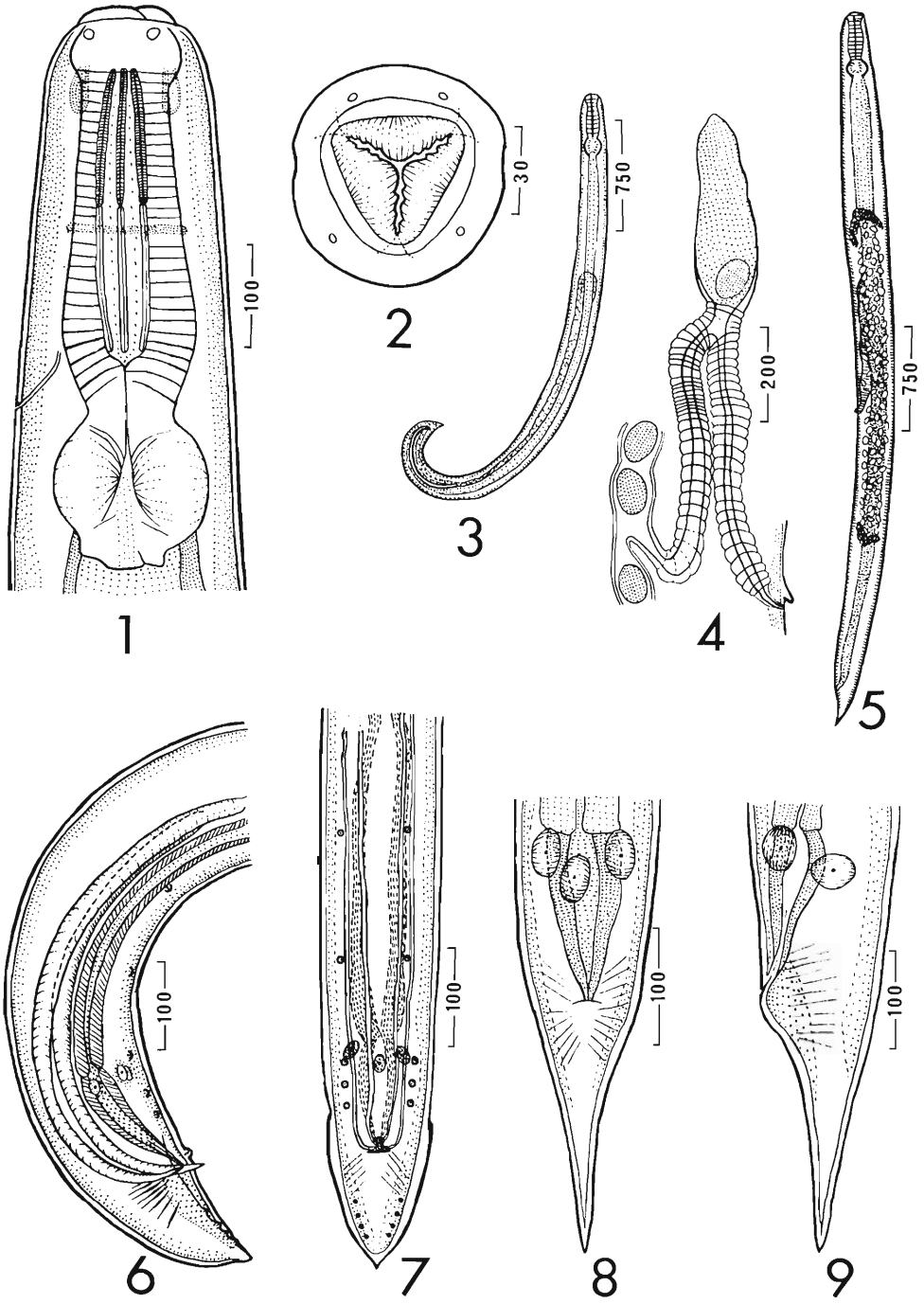
Specimens were fixed in AFA according to Crites' (1965) method. They were cleared by glycerine–alcohol dehydration and mounted in glycerine, the cover glass being supported by glass wool. Although preliminary sketches of living worms were made, all drawings were made from preserved specimens with the aid of a camera lucida and a phase microscope. Unless otherwise stated, all measurements are in microns; those in parentheses are averages of 5 males and 10 females. Measurements of curved regions of the body were made near the middle of the specimens.

Rhigonema critesi sp. n. (Figs. 1–9)

DESCRIPTION: Rhigonematidae Chitwood, 1935. *Rhigonema* Cobb, 1898. Small nematodes with short tails, particularly in males. Cuticle translucent, very faintly striated and devoid of ornamentation. Oral opening surrounded by three lips, which bear four papillae, two on dorsal lip and one on each subventral lip. Amphids not observed. Lips have very fine cuticular lines and bear denticular projections (Fig. 2). Glands (amphidial?) on each side of anterior portion of esophageal corpus. Six longitudinal rods line most of corpus lumen. Rods divided into anterior and posterior portions: anterior one weakly striated and shorter than posterior, nonstriated portion. Just posterior to end of striations on longitudinal rods, nerve ring surrounds corpus. Excretory pore opens ventrally at corpus–bulb junction or slightly anterior to it. Esophageal bulb contains corrugated valve; intestine straight. Rectal walls cuticularized and thick. Rectal glands present.

MALE (5 Specimens): Total length 2.19 to 4.46 mm (3.32); maximum width 126 to 213 (172). Distance from anterior end to end of cardia 332 to 446 (387); distance from anterior end to end of corpus 242 to 320 (279). Length of bulb (excluding cardia) 72 to 101 (89); length of cardia 17 to 25 (20); distance from anterior end to end of striated portion of rods lining corpus 120 to 170 (144). Nerve ring 122 to 177 (154) from anterior end; excretory pore (distance from anterior end) 210 to 293 (254). Cloacal opening to tip of tail 77 to 135 (110). Testis single, reflexed, and extends to 642 to 1,392 (1,091) from anterior end. Two spicules, one slightly longer than other. Longer spicule 417 to 614 (517); shorter spicule 403 to 600 (503). Spicules not reticulated and have retractor muscles attached to their anterior ends. No gubernaculum present. One medioventral precloacal papilla and nine pairs of caudal papillae present: five pairs precloacal, four pairs post cloacal. Small caudal alae extend from short distance anterior to cloacal opening to tip of tail. Rectal glands present. Tail short, tapers sharply and ends abruptly.

† Present address: Escuela de Biología, Universidad de Costa Rica. San José, Costa Rica.



Figures 1–9. Camera lucida drawings of *Rhigonema critesi* sp. n. from *Orthoporus typotopyge*. Scales in μm. 1. Female anterior end, lateral view. 2. Female, en face view. 3. Entire male, lateral view. 4. Vulvar

FEMALE (10 Specimens): Total length 2.52 to 5.85 mm (4.37); maximum width (just anterior to prevulvar spine) 163 to 259 (198). Distance from anterior end to end of cardia 308 to 570 (485); distance from anterior end to end of corpus 228 to 421 (376). Bulb length (excluding cardia) 76 to 115 (98); cardia 19 to 34 (25); distance from anterior end to end of striated portion of rods lining corpus 117 to 178 (156). Nerve ring 122 to 192 (168) from anterior end; excretory pore 201 to 313 (284) from anterior end. Vulva 1.30 to 3.12 mm (2.34) from anterior end; anus to tip of tail 155 to 242 (214). Eggs 96 to 103 (101) long by 68 wide. Strong, blunt spine just anterior to vulva, projects slightly posteriorly and outwardly (Fig. 4). Thick-walled vagina vera directed anteriorly, opening into thinner-walled vagina uterina and into large, blind sac. Vagina uterina extends posteriorly, connecting with amphidelphic uteri (Fig. 4). Ovaries reflexed and anterior ovary extends to within 521 to 1,451 (1,124) of anterior end. Oviducts become enlarged just before opening into uteri. Tail tapers to sharp point.

HOST: *Orthoporus typotopyge* (Brölemann, 1905).

LOCATION: Anterior hindgut, just posterior to valve separating mid- from hindgut.

TYPE LOCALITY: Area just west of Juan Santamaría International Airport, Alajuela Province, Costa Rica.

HOLOTYPE: Female, USNM Helm. Coll. No. 71808.

ALLOTYPE: Male, USNM Helm. Coll. No. 71809.

PARATYPES: USNM Helm. Coll. No. 71810.

Discussion

Cobb (1898) established the genus *Rhigonema* with a very brief description of *R. brevicolle* composed of a few drawings and measurements given as a formula. Christie and Cobb (1927) redescribed the genus and established *R. brevicolle* as the type species. Artigas (1930) divided *Rhigonema* into two genera: *Rhigonema* for the species in which the female has a large, blind sac or diverticulum between the vagina vera and the vagina uterina, and *Dudekemia* Artigas, 1930, for the species in which the female does not have such a diverticulum. Dollfus (1952), reviewing the genus *Rhigonema*, felt that this difference might merit a new subgenus, but not a new genus. Subsequently (1964) he recognized the two genera as valid.

Of the four characteristics listed by Travassos and Kloss (1960) as differentiating the two genera, the only one that is conclusive and without known exceptions is the presence of a diverticulum connected with the vagina in *Rhigonema* and the absence of such a sac in *Dudekemia*. The species presently assigned to the genus *Rhigonema* are: *R. infectum* (Leidy, 1849), *R. brevicolle* Cobb, 1898, *R. truncatum* Artigas, 1926, *R. nigella* Thomas, 1931, *R. longicaudatum* Dollfus, 1952, *R. alvarengai* Travassos and Kloss, 1960, *R. africana* Dollfus, 1964 (female only), *R. thysanophora* Crites, 1965, and *R. ornata* Majumdar, 1967.

R. critesi is the only species in the genus in which the female has a thick, blunt spine just anterior to the vulva (Fig. 4). *R. critesi*, *R. africana*, and *R. ornata* are the only species in the genus without cuticular pilosites. In *R. africana* only the female is known and it does not have the prevulvar spine characteristic of *R. critesi*. The rods lining the corpus in *R. africana*, *R. nigella*, and *R. thysanophora* have an anterior striated portion that is longer than the posterior nonstriated portion, while in *R. critesi* the opposite is true. A diverticulum associated with the vagina "was not observed" by Majumdar (1967) for *R. ornata*. In the absence of this diverticulum *R. ornata* cannot possibly be placed in the genus *Rhigonema*. The only males in the genus that have caudal alae are *R. critesi*, *R. infecta*, and *R. thysanophora*. All known *Rhigonema* males have a different number and arrangement of caudal papillae than *R. critesi*. The spicules of

←

opening, vagina vera, diverticulum, vagina uterina, and beginning of amphidelphic uteri. 5. Entire female, lateral view. 6. Male tail, lateral view. 7. Male tail, ventral view. 8. Female tail, ventral view. 9. Female tail, lateral view.

R. truncatum and *R. alvarengai* males have lateral extensions ("alae") which are absent in *R. critesi* spicules. Furthermore, the tail of *R. critesi* males is very different than that of *R. longicaudatum* or *R. thysanophora* males.

Acknowledgments

I would like to express my gratitude to Dr. John L. Crites, under whose direction this work was done. In appreciation for his multifaceted assistance and encouragement I am naming this species after him. I am grateful to Dr. Richard L. Hoffman who identified the millipede hosts, and to Dr. Paul C. Stromberg, Messrs. C. Lawrence Cooper and Frank F. Jaszcz, Jr., for reviewing the manscript. My thanks go to Miss Heidi E. Uotila, who drew the final figures based on my original drawings.

Literature Cited

Artigas, P. de T. 1930. Nematoideos dos generos *Rhigonema* Cobb, 1898 e *Dudekemia* n gn. (Nematoda: Rhigonemidae). Mem. Inst. Oswaldo Cruz 24: 19–30.

Christie, J. R., and N. A. Cobb. 1927. *Rhigonema* (*Isacis* Skrjabin, 1914, ?*Isacis* Baylis and Daubney, 1926; nec *Isacis* Lespés, 1856), nemas inhabiting the intestine of millipeds. J. Wash. Acad. Sci. 17: 17–19.

Cobb, N. A. 1898. Extract from M.S. report on the parasites of stock. Agric. Gaz. New South Wales 9: 296–321, 419–454.

Crites, J. L. 1965. *Rhigonema thysanophora* sp. n. (Nematoda: Rhigonematidae) from the millipede, *Euryurus erythropygus* Brandt, 1841. J. Parasitol. 51: 180–184.

Dollfus, R.-Ph. 1952. Quelques Oxyuroides de Myriapodes. Ann. de Parasitol. 27: 143–236.

———. 1964. Nematodes de myriapodes du Congo Belge. Mem. du Mus. Natl. d'Hist. Natur. Ser. A–Zool. 32: 109–169.

Majumdar, G. 1967. *Rhigonema ornata* sp. n. (Nematoda, Rhigonematidae) from the millipede, *Xenobolus carnifex* in India. Atas Soc. Biol. Rio de Janeiro 11: 109–111.

Travassos, L., and G. R. Kloss. 1960. Compendio dos nematoides parasitos intestinais de artropodos. Arq. do Mus. Nac. 50: 237–303.

Catenotaenia utahensis sp. n. (Cestoda: Catenotaeniidae) from the Merriam Kangaroo Rat, *Dipodomys merriami vulcani*, in Utah

GERHARD K. BIENEK AND ALBERT W. GRUNDMANN
University of Utah, Department of Biology, Salt Lake City, 84112

ABSTRACT: A new species of *Catenotaenia* is described and compared with other members of the genus in North America. The new species differs in having fewer and larger testes and symmetrically bilobed ovaries located in the median line toward the center of the proglottid. *Catenotaenia californica*, the species most closely allied, has an asymmetrical ovary located more anteriorly in the proglottid.

A study of the parasitism in the Merriam kangaroo rat, *Dipodomys merriami vulcani* Benson, conducted at Dixie State Park, Washington County, Utah, produced a relatively high incidence of infections with helminths, especially cestodes. Examinations of 84 hosts from trapsites in the park during 1971 produced 12 specimens of tapeworms, 10 belonging to the genera *Mathevotaenia* and 2 to *Schizorchodes*, the latter described as new (Bienek and Grundmann, 1973). During 1972, 84 cestodes were recovered from 26 of 107 hosts examined. The generic composition of the cestodes recovered varied from the previous year, and in addition to *Mathevotaenia* being present, 18 specimens of an apparently new species of *Catenotaenia* were recovered representing a 4% infection rate.

Although identical trapsites and seasonal col-

lections were involved, the new species was not recovered during 1971, probably due to changed food availability during 1972. Based on climatic data, 1971 was a quite normal year in regard to rainfall. During 1972, however, significant deviations occurred in that rainfall was much below normal during winter, spring, and early summer producing minimal growth in perennials and preventing annuals from setting seed. During August, September, and October high rainfall occurred producing springlike vegetative growth. These abnormal conditions altered the food intake of the host causing intensive use of available insects including the intermediate hosts of the new species. For further references of intestinal helminths of *D. m. vulcani* see Bienek and Grundmann (1973) and Bienek and Klikoff (1974).

The type locality is located in the transitional zone between the Great Basin Faunal Area, a cold desert habitat, the warm Mojave Desert Faunal Area to the south, and the Colorado Plateau Faunal Area to the northeast. The study site is typical Mojave desert vegetation dominated by *Larrea*.

Materials and Methods

Sherman live traps baited with rolled oats were used to collect kangaroo rats from Dixie State Park. A sample of each catch was necropsied yielding 18 specimens of *C. utahensis* which were used for the description of the new species. All specimens were collected from recently killed hosts, relaxed in distilled water, fixed in AFA, stained with Delafield's hematoxylin, cleared in Methyl salicylate, and mounted in Eukitt.

Review of Genus *Catenotaenia* in Western Utah

Because other related species such as *Catenotaenia peromysci* in the deer mouse, *Peromyscus maniculatus*, and *C. linsdalei* in the pocket mouse, *Perognathus formosus*, occur in the region, it appears that a short discussion is warranted on their morphology as compared with the new species. This is especially necessary because the specimens of *C. linsdalei* and *C. peromysci* obtained consistently deviate from the original descriptions of these species in the literature. Such differences in numbers of testes and structure of gravid proglottids are included.

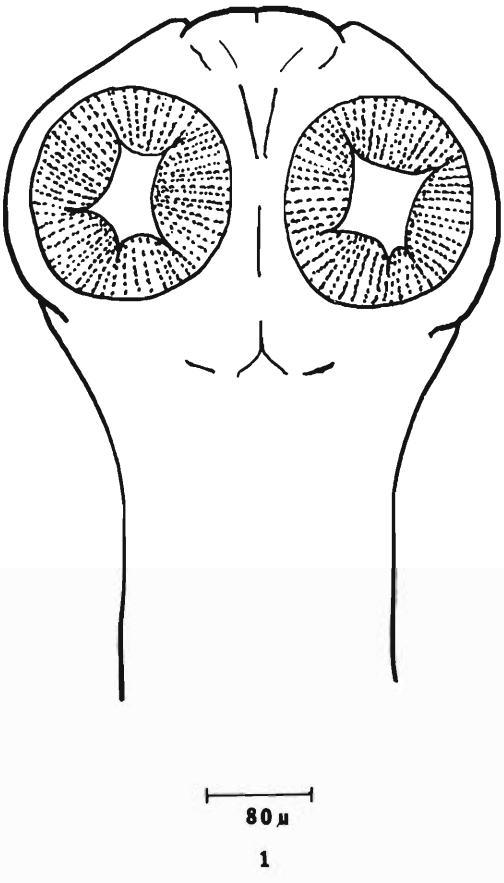
Mikhail and Fahmy (1968), in a review of the subfamily Catenotaeniinae Spasskii (1946), presented arguments for the inclusion of the family *Catenotaenia* Janicki (1904), as now constituted, as a subfamily in the Anoplocephalidae. The present authors favor retention of the family Catenotaeniidae following Spasskii (1951) and Schmidt (1970). In 1946, Akhumian split the genus *Catenotaenia* into two separate genera, namely *Catenotaenia* and *Skrjabinotaenia* Akhumian (1946). His division of the genus was based on an interpretation of the significance of the following morphology:

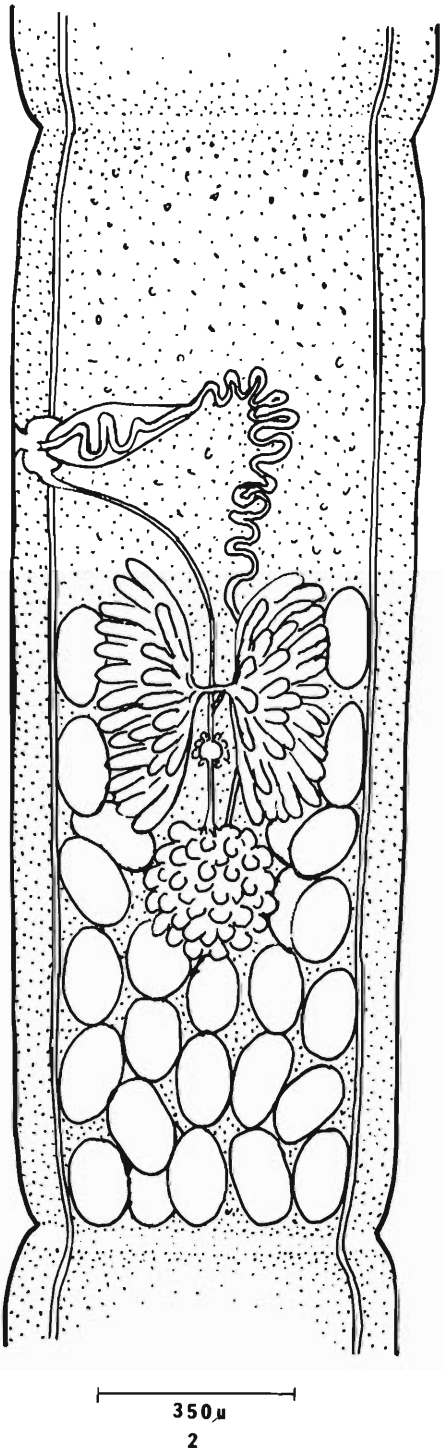
Group I: Testes always posterior to ovary; genital pore in the anterior half of segment; both mature and gravid segments longer than wide, with a long uterine stem.

Group II: Testes in two distinct lateral groups relative to the female genitalia; genital pore at anterior end of lateral margin; mature segment wider than long; uterine stem shorter than in the previous group (Mikhail and Fahmy, 1968).

Classification by Akhumian was accepted by Yamaguti (1959) and Tenora (1959). The latter author suggested a further subdivision of the genus *Catenotaenia* into three subgenera, namely *Catenotaenia*, *Spasskyela*, and *Meggitina* (Mikhail and Fahmy, 1968). In addition, Yamaguti and also Wolfgang (1956) suggested synonymizing three species, *C. laguri*, *C. peromysci*, and *C. linsdalei* with *C. dentritica*, but left *C. californica* as a distinct species. Mikhail and Fahmy also declared *C. peromysci* and *C. laguri* to be synonyms of *C. californica*.

Specimens assigned to both *Catenotaenia linsdalei* and *C. peromysci* have been recovered frequently in the Bonneville Basin with the former being present in the pocket mouse, *Perognathus formosus*, and the latter in the white-footed deer mouse, *Peromyscus maniculatus sonoriensis*. Sufficient morphological differences exist between these two, based on mature and gravid proglottids, to retain them as separate species. However, the local speci-





Figures 1, 2. *Catenotaenia utahensis*; from *Dipodomys merriami vulcani*. Scolex and mature proglottid, respectively.

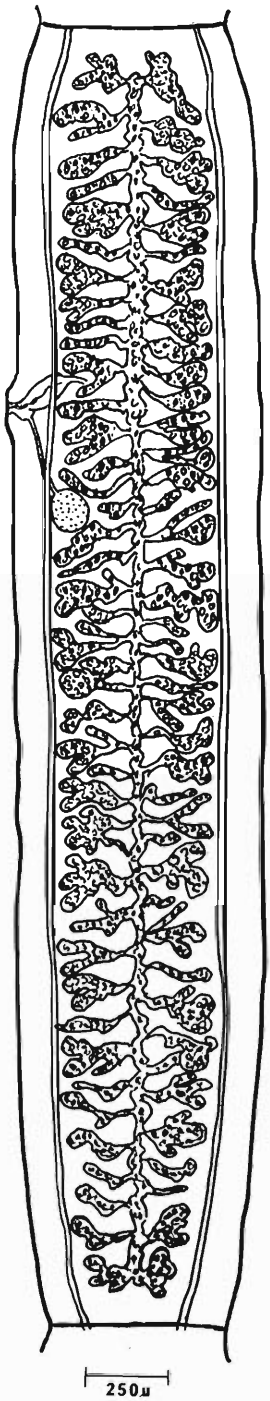
mens show some variation from original descriptions while still retaining basic morphology. Specimens referable to *C. linsdalei* have far fewer testes, averaging 77, while the type lists about 130; *C. peromysci* in western Utah also has fewer testes with an average of 50 (range 41–56) as compared with the 70–80 reported by Smith (1954) in Wyoming. Furthermore, the gravid proglottids of our *C. peromysci* have a uterus with finer and longer branches extending over a wider area of the proglottid than those of *C. linsdalei* (see Figs. 3 and 4). The specimens from the Bonneville Basin also have fewer lateral branches on the uterus, *C. peromysci* having 20–26 and *C. linsdalei* having 40–50. Depending upon the significance attached to the variation described, the two forms may be considered either as subspecies or variants of a single species where the difference may be due to genetic variability and possibly host-selected, or as separate species, each restricted to a separate host genus. The authors favor the latter position.

Catenotaenia utahensis sp. n. (Figs. 1, 2)

HOST: *Dipodomys merriami vulcani* Benson.
LOCATION: Small intestine.
TYPE LOCALITY: Dixie State Park, Washington County, Utah.
HOLOTYPE: USNM Helminth. Coll. (No. 72902).
PARATYPE: University of Utah, Department of Biology–Parasitology.

Description

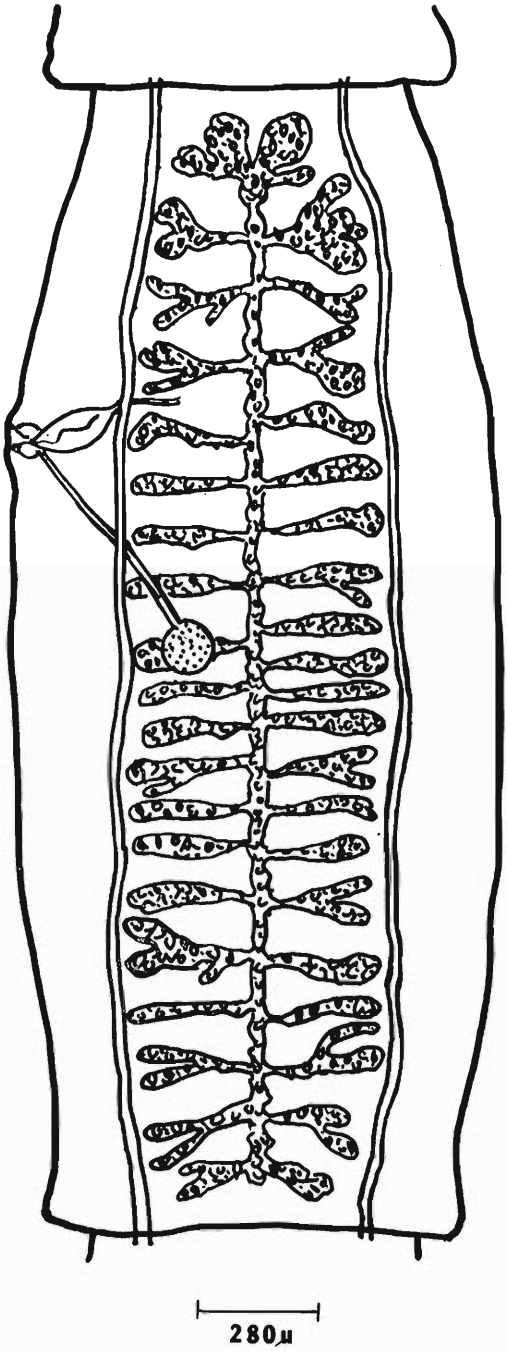
Length of strobila up to 44 mm; mature proglottids longer than wide averaging 0.74 by by 2.38 mm. Segments acraspedote, wider than long anteriorly and longer than wide posteriorly. Width of mature proglottids measuring 0.68 to 0.9 mm, and length 2.38 mm (1.5–2.46). Scolex unarmed, rostellum absent, diameter 364 μ (319–455). Four well-developed suckers present, diameter 215 μ (200–227). Neck present, long, contricted im-



3

→

Figure 3. ***C. linsdalei*; gravid proglottid from *Perognathus formosus*.**



mediately posterior to scolex. Genital pores alternating irregularly, located in anterior ⅓ of relaxed mature segment. Intersegmental membranes apparently absent. Male reproductive system positioned in posterior half of mature segment and having 24–36 oval testes ranging from 118 to 173 μ, cirrus unarmed, sac pyriform in shape measuring 137 by 100 μ. Vas deferens somewhat coiled, internal and external vesicles absent (see Fig. 2). Vagina an uncoiled tube, dorsally situated, ovary bilobed, symmetrical, highly branched, located anteriorly in midline. Median positioned vitelline gland near posterior end of ovary.

Discussion

Catenotaenia utahensis is differentiated from other members of the genus by being much smaller and by the number, size, and position of the testes. The new species possesses far fewer testes (24–36) than *C. californica* Dowell (1952) (72–90) and *C. reggiae* Rausch (1951) (up to 300 testes). In *C. utahensis* testes surround the female reproductive organs. Furthermore, *C. utahensis* possesses a larger cirrus sac (0.14 mm by 0.1 mm) than in *C. californica* (0.02 mm by 0.038 mm) or *C. peromysci* (0.05 mm by 0.07 mm), *C. laguri* (0.08 mm by 0.09 mm), and *C. dentritica* (0.05 mm by 0.07 mm). The scolex of *C. utahensis* is larger than that of other described species from this region, except that of *C. reggiae*. *C. utahensis* differs also from *C. californica* in the shape and position of the ovaries, the latter having a bilobed, asymmetrical ovary located in the anterior end of the proglottid while the former possesses a symmetrical bilobed ovary located on the median line toward the posterior half of the proglottid. Moreover, the vitelline gland in the new species is not positioned in the poral half of the proglottid as in *C. californica*, but is median and positioned posteriorly to the ovary. None of the collected specimens possessed a fully developed gravid proglottid. *C. utahensis* lacks membranes separating proglottids which are distinct in the other species.

←

Figure 4. ***C. peromysci*; gravid proglottid from *Peromyscus maniculatus*.**

Literature Cited

Akhumian, K. S. 1946. Systematics of the cestode genus *Catenotaenia* Janicki, 1904. Gelmint. Sborn. 40. Let. Deiatelnost Skrjabin 1946, p. 37–41.

Bienek, G. K., and A. W. Grundmann. 1973. A new tapeworm *Schizorchodes dipodomi* gen. et sp. n. (Cestoda: Anoplocephalidae) from the Merriam kangaroo rat *Dipodomys merriami vulcani*. Proc. Helm. Soc. Wash. 40: 192–195.

———, **and L. G. Klikoff.** 1974. Parasitological evidence of arthropods as food for *Dipodomys merriami vulcani*. Am. Midl. Nat. 91: 251–253.

Dowell, A. M. 1953. *Catenotaenia californica*, sp. nov., a cestode of the kangaroo rat, *Dipodomys panamintinus mohavensis*. Am. Midl. Nat. 49: 738–742.

Mikhail, J. W., and M. A. M. Fahmy. 1968. Study on some members of the genus *Skryabinotaenia* with a description of a new species and a review of the subfamily Catenotaeniinae Spasskii, 1946. Zool. Anz. 181: 439–450.

Rausch, R. 1951. Studies on the helminth fauna of Alaska. VII—On some helminths from arctic mormots with the description of *Catenotaenia reggiae* n. sp. (Cestoda: Anoplocephalidae). J. Parasitol. 37: 415–418.

Schmidt, G. D. 1970. How to Know the Tapeworms. W. M. C. Brown Co., Dubuque, Iowa, 266 p.

Smith, C. F. 1954. Four new species of cestodes of rodents from the high plains, central and southern Rockies and notes on *Catenotaenia dentritica*. J. Parasitol. 40: 245–254.

Spasskii, A. A. 1951. Anoplocephalate Tapeworms of Domestic and Wild Animals. The Academy of Sciences of the USSR, Moscow, 783 p.

Tenora, F. 1959. Revision of the classification of platyhelminths of the family Catenotaeniidae Spasskii, 1950. Zool. Zh. 38: 1322–1334.

Wolfgang, R. W. 1956. Helminth parasites of reptiles, birds, and mammals in Egypt: II. *Catenotaenia aegyptica* sp. nov. from myomorph rodents, with additional notes of the genus. Can. J. Zool. 34: 6–20.

Yamaguti, S. 1959. Systema Helminthum. Vol. II. Cestodes of Vertebrates. Interscience, New York, 860 p.

Studies on the Life History of *Brachylecithum stunkardi* (Pande, 1939) (Trematoda: Dicrocoeliidae)[1, 2, 3]

W. Patrick Carney

Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco 96356

ABSTRACT: *Brachylecithum stunkardi* (Pande, 1939) is reported for the first time from Clark's nutcracker, *Nucifraga columbiana*, in Montana. *Allogona ptychophora* served as an experimental first intermediate host. Fully developed cercariae contained in slimeballs were shed 498 days after the ingestion of *B. stunkardi* eggs.

Twenty (+) flukes were recovered from the bile ducts and gallbladder of a Clark's nutcracker, *Nucifraga columbiana* (Wilson), shot in Missoula County, Montana. Twelve were prepared for morphological studies and the remainder utilized in the life history experiments. These flukes were determined to be conspecific with *Brachylecithum stunkardi* (Pande, 1939). *Brachylecithum stunkardi* was originally described from specimens taken from the bile ducts of *Garrullus lanceolatus* in India by Pande (1939). It was found also in livers of *Cyanocitta cristata* (Linnaeus) from Texas and Virginia (Denton and Byrd,

[1] This study was supported by funds provided by the Bureau of Medicine and Surgery, Navy Department for Work Unit MF51.524.009-0030BF61, and the Department of Zoology, University of Montana.

[2] The opinions and assertions contained herein are those of the author and are not to be construed as official or as reflecting the views of the U. S. Navy Department.

[3] Experimental aspects of study conducted in the Department of Zoology, University of Montana, Missoula 59801. Reprint requests to Publications Office, NAMRU-2, Box 14, APO San Francisco 96263.

1951). This paper represents the first report of *B. stunkardi* from Clark's nutcracker and western North America.

The intramolluscan development has been described for five *Brachylecithum* species: *B. americanum* Denton, 1945, by Denton (1945), *B. alfortense* (Railliet in schedulis) Dollfus, 1954, by Timon-David (1956, 1957), *B. orfi* Kingston and Freeman, 1959, by Kingston (1965) and Carney (1966), *B. mosquensis* (Skrjabin and Isaitschikoff, 1927) by Carney (1967, 1970a), and *B. myadestis* Carney, 1972, by Carney (1972). Of these the complete life history is known for *B. mosquensis* and possibly *B. americanum*.

Methods

Adult flukes were teased from the bile ducts and placed in 0.85% NaCl. Those used for experimental infections were transferred to demineralized, distilled water and stored at 10C. The remaining flukes were fixed, stained, and mounted as described by Carney (1970a). Land snails collected in Missoula County, Montana, were held and bred in plastic terraria maintained at temperatures ranging from 10–15C as described by Carney (1970b). Snails exposed to eggs of *B. stunkardi* were transferred to a laboratory varying in temperature from 15–25C when infections were diagnosed. Snails were dissected in 0.3% NaCl. The sporocyst stages were preserved as the adult flukes. Slimeballs, containing fully developed cercariae, were allowed to dissolve in water before the cercariae were preserved as were other stages.

Drawings were made with the aid of a camera lucida and microprojector. Measurements are in microns unless otherwise indicated. Average measurements are followed by ranges in parentheses.

Observations

Description of *Brachylecithum stunkardi* adult from *Nucifraga columbiana* (Fig. 1)

Body elongate-cylindrical, of uniform diameter; body length 2.76 mm (1.6–4.0 mm) (estimated maximum length 9 mm or more since some fragments recovered with previtellarian length of 3 mm and others with postovarian length of more than 6 mm); body width 132 (115–192) between suckers, 150 (107–225) between testes, 130 (110–172) at posterior border of vitellaria. Tegument aspinose, without tuberculations. Oral sucker subterminal 151 (108–208) long, 124 (100–177) wide; mouth ventral; prepharynx absent; pyriform pharynx 61 (48–77) long, 55 (42–69) wide; esophagus 75 long and 5 wide, cecal bifurcation anterior to genital pores, ceca slender, course not determined posterior to ovary. Course and termination in hindbody obliterated by densely packed coils of uterus. Ventral suckers, anterior ⅙ to ⅛ body, 160 (150–210) long, 216 (190–285) wide, with distinct lateral auricles, usually folded on lateral axis and protruding slightly above the ventral surface. Separate male and female pores close together on ventral surface posterior to cecal bifurcation; male pore posterior to female. Cirrus pouch usually anterior to ventral sucker 121 (112–144) long, 55 (54–64) wide, contains unarmed cirrus and coiled bulbus seminal

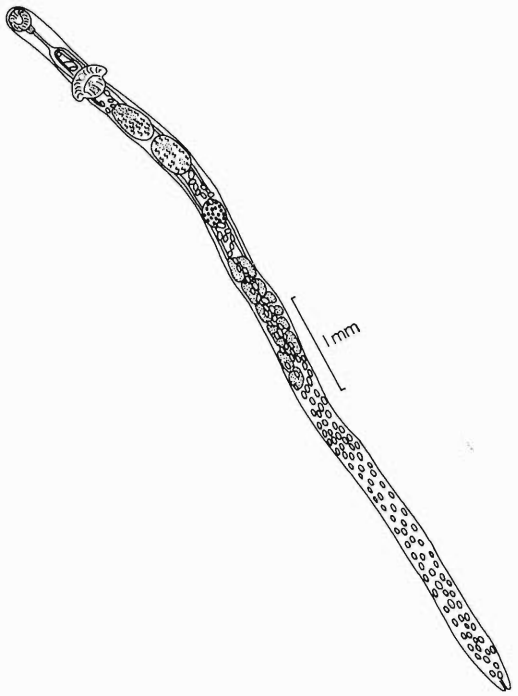


Figure 1. ***Brachylecithum stunkardi*, adult, from bile ducts of Clark's nutcracker.**

vesicle. Testes usually distinctly oval, tandem, smooth, with width often exceeding mean body width; anterior testis 305 (200–540) long, 177 (123–280) wide; posterior testis 133 (184–462) long, 176 (92–280) wide. Both testes separated from ventral sucker, ovary, and each other by loop or loops of uterus. Ovary round, posterior to testes 133 (115–192) long, 112 (100–185) wide. Seminal receptacle 50 in diameter immediately posterior and dextral to ovary. Mehlis' gland and Laurer's canal not noted. Vitellaria posterior to seminal receptacle, with eight to 10 large, overlapping follicles on each side of body. Uterus descends in loops on dorsal side of body from ovary to posterior end of body where it turns and loops anteriorly ventral to vitellaria and ovary and dorsal to testes, ventral sucker and cirrus pouch to terminate in female pore. Excretory pore terminates at end of narrow excretory duct; extent of bladder and other features of excretory system not noted.

HOST: *Nucifraga columbiana* (Wilson), Clark's nutcracker, adult female.

HABITAT: Bile ducts of liver and gallbladder.

LOCALITY: Pattee Canyon, Missoula County, Montana.

DATE: 2 October 1965.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 72327.

EXPERIMENTAL MOLLUSCAN HOST: *Allogona ptychophora* (Brown), laboratory-reared from parental specimens collected in Missoula, Montana.

Description of Larval Stages

Egg (Fig. 2)

Fully developed egg, 44.7 (41.6–51.2) in length and 26.7 (22.4–32.0) in width, oval, brown, and operculate with serrate opercular margin. Shell smooth, ca. 2–3 thick except at opercular margin were thinner. Miracidium with two granular bodies in posterior half and finely particulate gland and stylet in anterior half.

Daughter sporocyst (Figs. 3, 4)

Daughter sporocyst sacculate, 1,475 (800–2,800) in length and 175 (150–250) in width. Body wall ca. 10 thick except at poles where thickens. Undifferentiated embryos and developing cercariae in endosac. Posterior pole of sporocyst rounded, anterior pole generally attenuated. Birth canal not always distinct.

Cercaria (Figs. 5–7)

Cercarial body 376 (340–500) in length and 83 in width. Tegument cross-striated with small papillae on posterior ventral margin. Subterminal cup-shaped oral sucker 83 in length, 49 in width, and 53 in depth. Retractile stylet ca. 25 long, dorsal to apex of oral sucker, blunt at posterior with two blunt, dorsolateral extensions and ventral keel at anterior. Mouth funnels to pharynx ca. 20 in diameter. No prepharynx. Transversely oval ventral sucker 53 long, 60 wide, and 56 in depth. Flame cell pattern 2 [(2 + 2 + 2) + (2 + 2 + 2)]. Tapering tail 329 (280–400) in length, maximum width at base 63 (50–70). Cross-striations of tegument conspicuous on tail. Six giant nuclei and numerous small nuclei within tail.

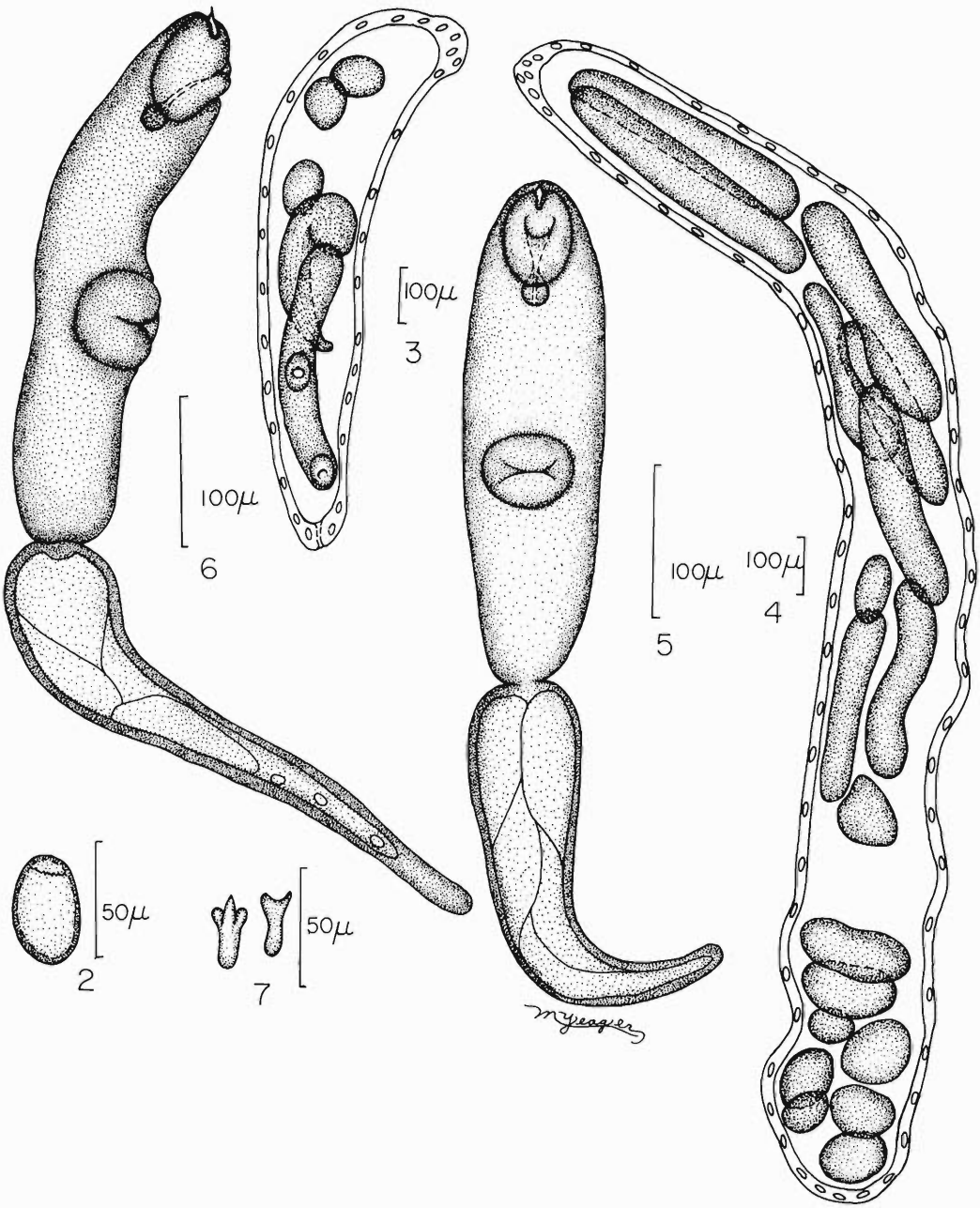
Life History Data

Eggs of *B. stunkardi* were fed to four species of land mollusks from habitats occupied by *N. columbiana*: *Allogona ptychophora* (Brown), *Triodopsis mulloni* (Bland and Cooper), *Zonitoides arboreus* (Say), and *Vallonia* sp. The *A. ptychophora* were laboratory-raised whereas the remaining molluscan species were field-collected specimens. Of the 26 *A. ptychophora* experimentally exposed to *B. stunkardi* eggs, four were positive for larval forms of the fluke. Slimeballs, first observed 498 days postingestion, were passed periodically in an amorphous mass. *Brachylecithum stunkardi* did not develop in *Z. arboreus*, *T. mulloni*, or *Vallonia* sp.

Carpenter ants, *Camponotus herculeanus* (Linnaeus), and *C. pennsylvanicus modoc* Wheeler were exposed to slimeballs of *B. stunkardi*. The ants were not observed to feed upon the slimeballs, nor were metacercariae recovered upon necropsy.

Discussion

When Denton and Byrd (1951) reported *B. stunkardi* from North America they con-



Figures 2–7. ***Brachylecithum stunkardi*****, morphology of eggs and intramolluscan stages. 2. Egg showing characteristic shape and operculum. 3. Daughter sporocyst showing fully developed cercariae and undifferentiated germinal masses. 4. Daughter sporocyst showing many developing cercariae and undifferentiated germinal masses. 5. Cercaria, ventral view. 6. Cercaria, lateral view. 7. Stylet of cercaria, ventral and lateral views, showing central keel and lateral bases.**

sidered *B. eophonae* (Yamaguti, 1941) and *Brachylecithum* sp. (Braun, 1902) conspecific with *B. stunkardi* and suggested that *B. stunkardi* might be a synonym of *B. lobatum* (Railliet, 1900). Travassos (1941) considered *B. halcyonis* (Yamaguti, 1941) identical with *B. stunkardi*. Oshmarin (1963), however, has since reported *B. eophonae* from Manchuria, and Faust (1966), although aware of Denton and Byrd's synonymy of *B. eophonae* with *B. stunkardi*, reported *B. eophonae* in the area of Peking and also questioned the synonymy of *B. halcyonis* with *B. stunkardi* by Travassos (1941).

Agrawal (1964) described as a new species a dicrocoeliid from the intestine of *Acridotheres tristis* (Myna) in the vicinity of Varanasi, India, as *Lyperosomum stunkardi*. According to Fotedar and Raina (1965) this fluke belongs to the genus *Brachylecithum*, and they suggested that it was synonymous with *B. stunkardi* which was originally included in the genus *Lyperosomum*. *Lyperosomum stunkardi* Agrawal, 1964, as described, belongs in the genus *Brachylecithum* but may not be conspecific with *B. stunkardi*. A distinguishing feature of *B. stunkardi* is the lateral auricles of the ventral sucker. These are not evident or mentioned in the description given by Agrawal. The specific name likewise is invalid since it has been used previously in the genus *Lyperosomum* to which it was originally assigned and is already used in the genus *Brachylecithum* to which it correctly belongs according to the figure and description by Agrawal (1964). Thus, *Lyperosomum stunkardi* Agrawal, 1964, is considered a *species inquirenda*.

Macko (1969) recently examined a series of *B. lobatum* from corvids in eastern Europe and concurred with Rysavy's (1960) synonymy of *B. alfortense* with *B. lobatum*. Other *Brachylecithum* spp. closely related to *B. lobatum* are *B. stunkardi, B. eophonae, B. reoi* (Jarswal, 1964), *B. chivosca* (Pratt and Cutress, 1949), *B. eugenia* (Oshmarin, 1947), *B. glandarii* (Semenov, 1927) Odening, 1964, *B. strigis* (Yamaguti, 1939), and *B. strixi* (Oshmarin, 1952) Odening, 1964. The above-mentioned species all possess distinct lateral auricles on the ventral sucker, similar morphological configuration and gonadal topography. Although closely related, the question of their synonymy with *B. lobatum*, however, will require more detailed studies of intraspecific variability of the adult as well as larval stages.

Although studies of adult structure suggest that *B. stunkardi* and *B. alfortense*, a synonym of *B. lobatum* according to Rysavy (1960) and Macko (1969), are closely related, the daughter sporocysts of both species reveal some interesting differences. When the sporocysts of *B. alfortense* are fully developed the endosac swells to an ovoid or subspherical structure in the middle while the two extremities are narrowly constricted (Timon-David, 1957). This condition resembles daughter sporocysts of some brevicercous dicrocoeliids such as *Eurytrema pancreaticum* (Janson, 1889) in which the daughter sporocysts are passed from the mollusk at maturity (Tang, 1950). The cercariae of *E. pancreaticum*, contained in the endosac, apparently mature simultaneously. Fully developed daughter sporocysts of *B. stunkardi*, on the contrary, resemble sausages with a large number of cercariae in various stages of development contained in the endosac. The latter pattern of development is common in all longicercous or magnicercous dicrocoeliids thus far studied (Carney, 1972).

Timon-David (1957) did not describe how cercariae of *B. alfortense* were released from the molluscan host. He did, however, place *B. alfortense* in Patten's (1952) longicercous group in which the cercariae are released from the molluscan host in the form of slimeballs. But from his description the daughter sporocysts appear capable of withstanding desiccation as those described for the brevicercous dicrocoeliids in which daughter sporocysts are released intact from mollusks.

In describing the cercariae of *B. alfortense*, Timon-David (1957) did not indicate the conditions under which the cercariae were studied nor did he mention the formation of slimeballs. Thus, the cercariae described may have been removed from daughter sporocysts. Carney (1970a) showed that the cercarial body of *B. mosquensis* was distended by the contents of the postacetabular glands while in the sporocyst whereas these glands were empty in the cercariae taken from slimeballs.

Contrary to expectations, life history data and larval morphology of two closely related species, such as *B. alfortense* and *B. stunkardi*,

have not solved the taxonomic interpretations of the respected adult stages. Further studies of the larval stages of members of the *B. lobatum* complex are necessary. The atypical life history at larval stages of *B. alfortense*, as described by Timon-David (1957) from field-collected snails, should be reconfirmed using laboratory-reared mollusks.

Acknowledgments

I wish to thank Dr. J. Fred Denton for his helpful comments on this manuscript. My thanks are also extended to the staffs of the Medical Illustration Department, Naval Medical Research Unit No. 2, Taipei, and the Naval Medical Research Institute, Bethesda, Maryland.

Literature Cited

Agrawal, V. 1964. On a new trematode, *Lyperosomum stunkardi* sp. nov., from the intestine of a bird, *Acridotheres tristis* (Myna). Ind. J. Helminth. 16: 1–4.

Carney, W. P. 1966. *Discus cronkhitei* (Newcomb), an experimental first intermediate host for *Brachylecithum orfi* (Kingston and Freeman, 1959) (Trematoda: Dicrocoeliidae). Can. J. Zool. 44: 768–769.

———. 1967. Notes on the life cycle of *Brachylecithum mosquensis* (Skrjabin and Isaitschikoff, 1927) from the bile ducts of the robin, *Turdus migratorius*, in Montana. Can. J. Zool. 45: 131–134.

———. 1970a. *Brachylecithum mosquensis*. Infections in the vertebrate, molluscan and arthropod hosts. Trans. Amer. Microscop. Soc. 89: 233–250.

———. 1970b. Maintenance of land mollusks in the laboratory. Nautilus 83: 136–139.

———. 1972. Studies on the life history of *Brachylecithum myadestis* sp. n. (Trematoda: Dicrocoeliidae). J. Parasitol. 58: 519–523.

Denton, J. F. 1945. Studies on the life history of *Brachylecithum americanum* n. sp., a liver fluke of passerine birds. J. Parasitol. 31: 131–141.

———, **and E. E. Byrd.** 1951. The helminth parasites of birds, III: Dicrocoeliid trematodes from North American birds. Proc. U. S. Nat. Mus. 101: 157–202.

Faust, E. C. 1966. New and previously described dicrocoeliine trematodes from Chinese birds. J. Parasitol. 52: 335–346.

Fotedar, D. N., and M. K. Raina. 1965. On a new species of the trematode genus *Lyperosomum* Looss, 1899 from *Corvus monedula soemmeringii*, common jackdaw in Kashmir. Ind. J. Helminth. 17: 54–62.

Kingston, N. 1965. On the life cycle of *Brachylecithum orfi* Kingston and Freeman, 1959 (Trematoda: Dicrocoeliidae), from the liver of the ruffed grouse, *Bonasa umbellus* L. Infections in the vertebrate and molluscan hosts. Can. J. Zool. 43: 745–766.

Macko, J. K. 1969. On the variability of the morphological features of *Brachylecithum lobatum* from the East-Slovakian Earpathian mountains. Helminthologia 10: 137–142.

Oshmarin, P. G. 1963. Parasitic worms of mammals and birds in the Primorskii Krai. Moscow. (In Russian). Akademia Nauk USSR, 323 pp.

Pande, B. P. 1939. On the trematode genus *Lyperosomum* Looss, 1899 (Dicrocoeliidae) with a description of two new species from India. Proc. Nat. Acad. Sci. India 9: 15–21.

Patten, J. A. 1952. The life history of *Conspicuum icteridorum* Denton and Byrd, 1951 (Trematoda: Dicrocoeliidae). J. Parasitol. 38: 165–182.

Pratt, I., and C. Cutress. 1949. *Olssoniella chivosca* n. sp. (Trematoda: Dicrocoeliidae) from the western evening grosbeak. J. Parasitol. 35: 361–363.

Rysavy, B. 1960. Beitrag zur Kenntnis der bei Vögeln in der Tschechoslowakei parasitär auftretenden Trematoden. Cesk. Parasitol. 7: 271–283.

Tang, C. C. 1950. Studies on the life history of *Eurytrema pancreaticum* Jansen, 1889. J. Parasitol. 36: 559–573.

Timon-David, J. 1956. Contribution a la connaissance du cycle evolutif des Dicrocoeliidae (Trematoda: Digenea) Development experimental de *Brachylecithum alfortense* (Railliet) Dollfus, 1954. Comp. Rend. Acad. Sci. 242: 1374–1376.

———. 1957. Recherches sur le development experimental de *Brachylecithum alfortense* (Railliet) R. ph. Dollfus, 1954, trematode dicrocoeliidae parasite des voies biliarires de la pie. Ann. Parasitol. Hum. Comp. 32: 353–368.

Pasture Trials with Levamisole and Thiabendazole for control of Gastrointestinal Helminthiasis in Sheep

M. L. COLGLAZIER,[1] K. C. KATES,[1] I. L. LINDAHL,[2] AND G. SAMUELSON[2]
United States Department of Agriculture

ABSTRACT: A 4-hectare pasture was contaminated with eggs of gastrointestinal helminths by grazing it from late April to early May (1971) with infected sheep; thereafter, the pasture was divided by fencing into five similar plots of 0.8 hectare each. On each plot, 11 essentially parasite-free lambs were grazed for about 18 weeks from June to November or until they died of parasitism. The lambs of one group served as unmedicated controls. At 4-week intervals beginning 20 July, lambs in the other four groups were drenched respectively with thiabendazole only (50 mg/kg), levamisole only (8 mg/kg), or the two drugs alternately and reciprocally at the same dose levels. Lamb deaths from helminthoses (mainly haemonchosis) during the test period were Group 1 (unmedicated controls), 8; Group 2 (TBZ only), 6; Group 3 (LVS only), 0; Group 4 (TBZ–LVS alternately), 2; Group 5 (LVS–TBZ alternately), 0. During the experiment, the dominant helminth species was *Haemonchus contortus*; lesser numbers of *Strongyloides papillosus, Nematodirus spathiger, Ostertagia circumcincta, Trichostrongylus* spp., *Oesophagostomum venulosum, Trichuris* spp., and *Moniezia expansa* were also involved. *H. contortus* populations and the associated clinical effects were controlled most effectively by LVS alone and least effectively by TBZ alone; an intermediate degree of control was obtained when the two drugs were used alternately. The *H. contortus* populations proved resistant to TBZ but not to LVS. All treatment regimens provided good to fair control of *S. papillosus, N. spathiger, Trichostrongylus* spp., and *O. venulosum*. Limited data indicate that none of the regimens was effective in controlling *Trichuris* spp. and *M. expansa*.

The broad-spectrum anthelmintic activity of thiabendazole (Brown et al., 1961) and of levamisole (= *l*-tetramisole, Thienpont et al., 1966; Bullock, Hand, and Waletsky, 1968) has been confirmed by many investigators (Gibson, 1965, 1969; Levine, 1968; Stone, 1969; Colglazier, Kates, and Enzie, 1969, 1971; Colglazier et al., 1971; Kates et al., 1971, 1973; and others). Drug-resistant helminth strains have been associated with thiabendazole and certain chemically related drugs, but not with levamisole (see Kates, Colglazier, and Enzie, 1973).

Populations of *Haemonchus contortus* in one of the sheep flocks at the Beltsville Agricultural Research Center are resistant to thiabendazole (Colglazier, Kates, and Enzie, 1969; Kates et al., 1971) and certain chemically related drugs (Colglazier et al., 1971; Colglazier, Kates, and Enzie, 1974; Kates, Colglazier, and Enzie, 1973) but not yet to levamisole. For these reasons, and because the alternate use of two or more antiparasitic chemicals has become common practice in control programs to prevent or minimize the evolvement of resistant parasite strains, it seemed desirable to determine whether alternate use of these drugs might improve the level of helminth control, particularly against *H. contortus*. Accordingly, experiments were designed in which these anthelmintics were administered to lambs at intervals during the grazing season, giving the drugs separately to two groups and alternately and reciprocally to two other groups.

Materials and Methods

Crossbred lambs used in these trials were purchased in Virginia and shipped to Beltsville, Maryland, in May 1971. The lambs upon arrival averaged 27.6 kg in weight and were relatively helminth-free because they had been raised on dry lot. The 4-hectare pasture used in this experiment was grazed for about 2 weeks in late April and early May by a flock of about 100 ewes lightly infected with several helminth species, including thiabendazole-resistant *H. contortus*. The pasture was then subdivided into five 0.8-hectare plots by fencing. On 21 June, five groups of 11 lambs were selected at random and confined appropriately

[1] Animal Parasitology Institute, Agricultural Research Service, Beltsville, Maryland 20705.
[2] Nutrition Institute, Agricultural Research Service, Beltsville, Maryland 20705.
Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Mean necropsy worm burdens of all lambs, including those that died. Lambs began grazing helminth-contaminated pasture plots 21 June. (TBZ = thiabendazole; LVS = levamisole.)

Helminth species	Treatment group[1]										
	Unmedicated controls			TBZ only			LVS only	TBZ–LVS alternately			LVS–TBZ alternately
	Died—8	Survived—3	Total—11	Died—6	Survived—5	Total—11	Survived—11	Died—2	Survived—9	Total—11	Survived—11
H. contortus											
4th stage	12,820	1,000	9,596	7,430	36,200	20,507	8,431	4,550	27,922	23,673	24,491
5th stage	17,710	100	12,907	10,330	3,344	7,155	145	4,000	67	782	2,827
All	30,530	1,100	22,504	17,760	39,544	27,662	8,576	8,550	27,989	24,455	27,318
O. circumcincta[2]	227	13	169	13	0	7	236	100	11	27	118
T. axei[3]	28	6	22	0	0	0	0	0	0	0	0
T. colubriformis[3]	98	340	163	0	0	0	0	0	0	0	11
S. papillosus[3]	4,080	2,453	3,636	77	8	45	536	660	282	351	15
N. spathiger[2]	695	553	656	3	4	2	247	20	40	36	58
O. venulosum[3]	5	38	14	1	0	1	0	0	0	0	0
Trichuris spp.[3]	24	12	21	7	13	9	8	2	5	4	16
M. expansa (scolices)	9	3	8	4	35	18	15	9	7	8	24

[1] Four treatments given on 20 July, 17 August, 14 September, and 13 October. Deaths occurred between 30 August and 30 September. Terminal necropsies 1–3 November.
[2] Mostly fifth stage with small numbers of fourth stage.
[3] Only fifth stage.

to the five plots. Water and salt–mineral mix were available ad lib., but no supplemental feed was given during the experiment. At the beginning of the experiment and at biweekly intervals thereafter, weight, hematocrit (packed red cell volume), and fecal egg count data were obtained from all lambs.

Group 1 lambs received no medication during the experiment; the other groups were drenched at 4-week intervals on 20 July, 17 August, 14 September, and 13 October as follows:

Group 2. Thiabendazole (TBZ) only: 50 mg/kg (Commercial Thibenzole, Merck & Co., Rahway, New Jersey).

Group 3. Levamisole (levo-tetramisole) (LVS) only: 8 mg/kg [pure chemical (hydrochloride) for experimental use; American Cyanamid Co., Princeton, New Jersey].

Group 4. TBZ 50 mg/kg and LVS 8mg/kg alternately every 4 weeks.

Group 5. LVS 8 mg/kg and TBZ 50 mg/kg alternately every 4 weeks.

Sixteen of the 55 lambs died or were killed *in extremis* between 30 August and 30 September and were necropsied immediately; the surviving lambs were necropsied at the end of the experiment, between 1 and 3 November. All residual helminths were recovered from the gastrointestinal tracts and counted by direct or standard dilution procedures (Colglazier et al., 1969).

The evaluation of the effectiveness of the drug regimens used is based upon mortality, weight gains or losses, hematocrit levels (PCV), strongyloid and *Strongyloides* fecal egg counts (EPG), and necropsy worm counts both from the lambs that died and those that survived.

The data were subjected to analysis of variance as previously reported (Colglazier et al., 1971). Necropsy worm count data were not subjected to statistical analysis because of wide variation in the time of necropsy as a result of the death of numerous control and treated lambs during the course of the experiment.

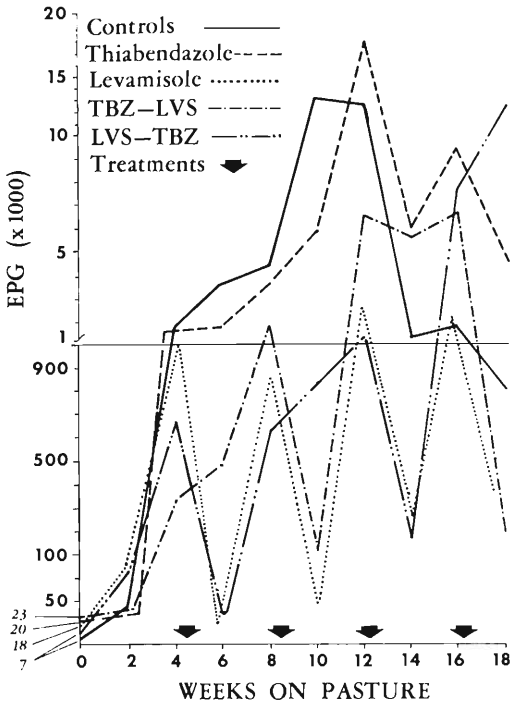


Figure 1. Mean strongyloid nematode eggs per gram of feces.

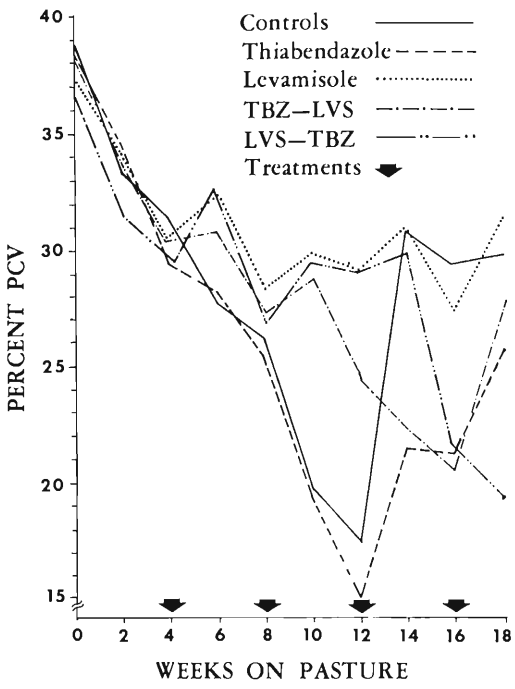


Figure 2. Mean hematocrits (packed red cell volumes).

Results and Discussion

The pertinent data are summarized in Table 1 and Figures 1–5. The helminths involved, as indicated by necropsy worm counts from the unmedicated control lambs, were *H. contortus, O. circumcincta, Trichostrongylus axei, T. colubriformis, N. spathiger, S. papillosus, O. venulosum, Trichuris* spp., and *M. expansa.*

During the 18 weeks of this experiment, the dominant helminth species in both numbers and clinical effects on the lambs was *H. contortus.*

As judged by the rapid increase in strongyloid egg counts (mainly *H. contortus*) and the sharp decline in hematocrit (Figs. 1, 2), clinical haemonchosis developed rapidly in most lambs of all groups after they had grazed for a few weeks on the contaminated pastures. Deaths, largely from acute haemonchosis, involved eight control lambs from 30 August to 24 September, six lambs treated with TBZ only from 8 to 16 September, and two lambs treated alternately with TBZ and LVS on 30 September (Table 1). No deaths occurred among lambs treated with LVS only or among those treated alternately with LVS and TBZ. The latter two regimens apparently controlled haemonchosis sufficiently to prevent deaths despite substantial exposure to *H. contortus* throughout the grazing period.

An unusual feature in the course of *H. contortus* infections in the three surviving control lambs was partial self-cure in one lamb and almost complete self-cure with subsequent resistance to reinfection in the other two lambs. The hematocrits for these lambs were much higher after the 6th week on pasture than for those lambs of the same group that died later (Fig. 3). In fact, two of the three surviving control lambs had virtually normal hematocrits at necropsy despite continuous severe exposure to *H. contortus* during the entire summer and fall grazing period. The mean strongyloid egg count for these lambs fell sharply after the 12th week on pasture (Fig. 1) in conjunction with an abrupt increase in weight gains (Fig. 4). One of the surviving control lambs had no 4th- or 5th- stage *H. contortus* at necropsy, indicating a complete self-cure of the

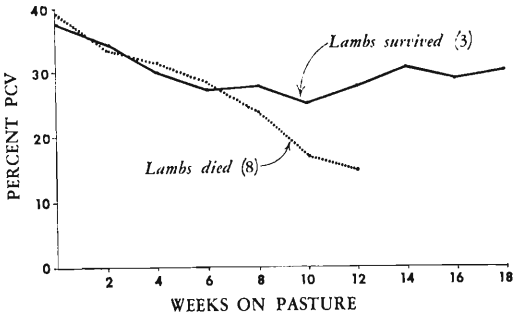


Figure 3. Mean hematocrits (packed red cell volumes) of the eight control lambs that died of parasitism and of the three that survived.

infection. A second control lamb had 20 4th-stage but no 5th-stage parasites; the third control lamb had 2,980 4th-stage and 200 5th-stage worms. When the mean numbers of *H. contortus* (4th and 5th stage) from the three surviving control lambs are compared with those from the controls that died, the differences show the startling ratio of about 1 to 30 (1,100 to 30,530, Table 1). The three surviving control lambs that self-cured all or part of their *H. contortus* also had fewer mean numbers of this species (4th and 5th stage) than any of the treated groups; and only the surviving lambs in the LVS and the TBZ–LVS groups had comparable low numbers of 5th-stage *H. contortus* at necropsy (Table 1). Only the LVS-treated lambs had relatively low numbers of 4th-stage *H. contortus* at necropsy, but even this number was eight times more than were recovered from the three surviving control lambs that self-cured.

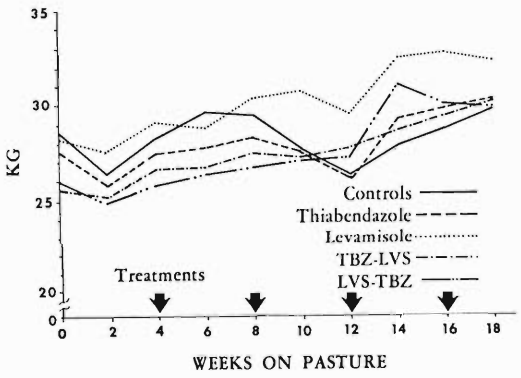


Figure 4. Mean weights of five lamb groups.

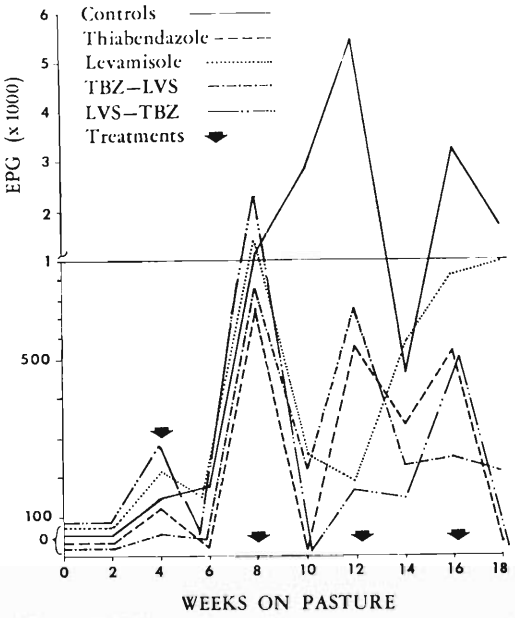


Figure 5. Mean *S. papillosus* eggs per gram of feces.

Through the 12th week, after some of the lambs in the control and TBZ groups had died, the control lambs and the TBZ-treated lambs had mean weight losses (including the terminal weights of the lambs that died) of 2.3 and 1.1 kg, respectively, whereas the lambs in the LVS, LVS–TBZ, and TBZ–LVS groups had mean weight gains of 1.3, 2.1, and 1.2 kg, respectively (Fig. 4). These group weight gains or losses that differed more than 0.25 kg were significant at $P < .05$.

Because *H. contortus* was the dominant parasite influencing the health of these lambs, those on the drug regimen that controlled this species best also showed the least overall reduction in hematocrits. After a fairly uniform reduction in mean group hematocrits during the pretreatment period, the LVS group maintained its hematocrit levels best (Fig. 2). The hematocrit levels of the control and TBZ groups at the 12th week of the experiment differed significantly ($P < 0.05$) from those of the other three treated groups. The LVS–TBZ group had mean hematocrit levels similar to those of the LVS-only group until late in the experimental period. At this time, the mean level of the LVS–TBZ group decreased

sharply, reflecting the resistance of *H. contortus* populations to the final dose of TBZ. The mean hematocrit levels of the control and TBZ-only groups were approximately equal through the 12th week; the levels of both groups increased thereafter. This trend in hematocrit levels in the TBZ group was possibly due to (a) the effects of the drug on the surviving lambs, (b) an unknown degree of immunity, and (c) the death of the most heavily infected lambs; in the control group, this trend was due to the complete or partial self-cure of the infections in the surviving lambs and to the death of the most heavily infected lambs.

The biweekly fecal egg count data, differentiated into the strongyloid type (mainly *H. contortus* in this experiment) and *S. papillosus* eggs, are summarized in Figures 1 and 5, respectively. Certain EPG trends and patterns related to the various treatment regimens are noteworthy. The strongyloid egg counts for the control and TBZ groups increased rapidly during the early weeks. Maximum levels were reached by the control group after 10 weeks (13,000 EPG) and by the TBZ group 2 weeks later (19,000 EPG), the latter despite two treatments of 50 mg/kg at the 4th and 8th weeks of the experiment. At the 12th week of the experiment, the mean strongyloid EPG of the TBZ group differed significantly ($P < 0.05$) from those of the other four groups. At this time, the other three treated groups had the lowest mean strongyloid EPG, and these differed significantly ($P < 0.05$) from those of both the control and the TBZ groups. These data provide strong evidence that the *H. contortus* populations in these lambs were resistant to TBZ at the dose level used. The strongyloid EPG counts dropped somewhat after the third and fourth treatments at 12 and 16 weeks, but by this time a number of the lambs had died. The EPG levels for the control group dropped sharply after the peak count was reached at 10 weeks, reflecting the heavy death losses and the self-cure phenomenon mentioned previously. The strongyloid EPG counts for the LVS group followed a consistent pattern throughout the experimental period. The counts reached peaks of about 1,000 EPG at 4-week intervals and for each treatment decreased to minimal levels of about 300 EPG or less at the midpoint between treatments. For the TBZ–LVS and the LVS–TBZ groups, the strongyloid egg counts declined abruptly after treatment with LVS but rose or declined only slightly after treatment with TBZ. These data also indicate that this *H. contortus* population was resistant to TBZ at the 50 mg/kg dose level. When TBZ-resistant *H. contortus* are involved, as in this test, little advantage accrues from alternating conventional therapeutic doses of the drug with other anthelmintics, even if the latter are highly effective against the TBZ-resistant population. Some measure of effective control might be obtained in this situation by substantially increasing the dose of TBZ, but it may be more economical and prudent to rely on nonbenzimidazole anthelmintics, used either separately or in a program of alternating two or more of these drugs.

The peak of *S. papillosus* egg counts of more than 5,000 EPG in the control group was in late August and early September, about the 12th week on pasture and at the time when some of the control lambs were dying of haemonchosis (Fig. 5). The *S. papillosus* egg counts were reduced by most regimens; TBZ alone had the most consistent favorable effect. The action of LVS against *S. papillosus* was less impressive. However, at the 12th week, the mean *Strongyloides* EPGs of all four treated groups were significantly lower ($P < 0.05$) than those of the controls. When the two drugs were used alternately, the first treatment with LVS was generally more effective than the second. When LVS alone was used, *S. papillosus* egg counts increased, rather than decreased, after the third and fourth doses of the drug, and the highest terminal egg counts of all the treated groups were in this group. Therefore, on the basis of egg count data, TBZ was superior to LVS against *S. papillosus* in these trials; these data are supported by terminal necropsy worm counts (Table 1).

The effect of the various treatment regimens against other helminth species was not definitive because of the small numbers of these species in the test lambs. However, necropsy worm counts indicated that some degree of control was achieved against *Trichostrongylus* spp., *N. spathiger*, and *O. venulosum*, and possibly against *O. circumcincta* in the group treated with TBZ only. None of the drug regi-

mens appeared to have any effect upon *Trichuris* spp. and *M. expansa.*

The self-cure of the *H. contortus* infections in three of the unmedicated control lambs appeared to have been the "classical" type described by Gordon (1967), but involving some degree of protection from reinfection after self-cure. In these three lambs, the hematocrits continued to rise (Fig. 3) and the strongyloid egg counts to decline (Fig. 1) from about the 12th week on pasture until the experiment was ended. Other than immunological or physiological differences, we cannot account for the self-cure phenomenon in only three of 11 lambs in the unmedicated control group because all lambs were uniform in age, weight, and breed.

None of these trials involved the administration of a combination of the two drugs in a single dose. This procedure was used successfully, however, by Romaniuk and Przeorska (1971) who gave an effective mixture to lambs that provided about 10 mg/kg of tetramisole (Nilverm) and 100 mg/kg of TBZ. Although such a formulation may have some merit in special situations, many other therapeutic alternatives are now available for control of internal helminth parasites of sheep.

Acknowledgment

The authors thank Mr. R. L. Wilson, Biometrical Services, ARS, for statistical analysis of the data.

Literature Cited

Brown, H. D., A. R. Matzuk, I. R. Ilves, L. H. Peterson, S. A. Harris, L. H. Sarett, J. R. Egerton, J. J. Yakstis, W. C. Campbell, and A. C. Cuckler. 1961. Antiparasitic drugs. IV. 2(4′-thiazolyl)-benzimidazole, a new anthelmintic. J. Am. Chem. Soc. 83: 1764–1765.

Bullock, M. W., J. J. Hand, and E. Waletsky. 1968. Resolution and racemization of *dl*-tetramisole, *dl*-6 phenyl, 2,3,5,6-tetrahydroimidazo-2,1-b thiazole. J. Med. Chem. 11: 169–171.

Colglazier, M. L., K. C. Kates, and F. D. Enzie. 1969. Anthelmintic activity of tetramisole, thiabendazole, and purified fine particle phenothiazine against experimental infections of *Haemonchus contortus* and *Trichostrongylus* species in sheep. Proc. Helm. Soc. Wash. 36: 68–74.

———, ———, **and** ———. 1971. Activity of levamisole, pyrantel tartrate, and rafoxanide against two thiabendazole-tolerant isolates of *Haemonchus contortus*, and two species of *Trichostrongylus*, in sheep. Proc. Helm. Soc. Wash. 38: 203–205.

———, ———, **and** ———. 1974. A cambendazole resistant *Haemonchus contortus* strain in sheep: Further experimental development. J. Parasit. 60: 289–292.

———, ———, ———, **I. L. Lindahl, and G. Samuelson.** 1971. Comparative activity of pyrantel tartrate, parbendazole, and levamisole at two dose levels against naturally acquired helminth infections in sheep. J. Parasit. 57: 1078–1082.

Gibson, T. E. 1965. Veterinary Anthelmintic Medication. Commonw. Agr. Bur., Bucks, England, 2nd ed., 206 p.: Addendum 1967, 5 p.

———. 1969. Advances in veterinary anthelmintic medication. Adv. Parasit. 7: 349–373.

Gordon, H. McL. 1967. Self-cure reaction. Proc. 3rd Intern. Conf. W. A. A. V. P., The Reaction of the Host of Parasitism, Vet. Med. Rev., p. 174–190.

Kates, K. C., M. L. Colglazier, and F. D. Enzie. 1973. Experimental development of a cambendazole-resistant strain of *Haemonchus contortus* in sheep. J. Parasit. 59: 169–174.

———, ———, ———, **I. L. Lindahl, and G. Samuelson.** 1971. Comparative activity of thiabendazole, levamisole, and parbendazole against natural infections of helminths in sheep. J. Parasit. 57: 356–362.

———, ———, ———, ———, **and** ———. 1973. Controlled trials with levamisole, cambendazole, and morantel tartrate against naturally acquired helminth infections in sheep. Proc. Helm. Soc. Wash. 40: 87–91.

Levine, N. D. 1968. Nematode Parasites of Domestic Animals and Man. Burgess Pub. Co., Minneapolis, Minn., 600 p.

Romaniuk, K., and B. Przeorska. 1971. [Effectiveness of some mixed anthelmintics in the course of nematode invasion in sheep.] Medyeyna Weterynaryina 27: 13–15.

Stone, O. J. (ed.). 1969. Thiabendazole Symposium. Tex. Rpt. Biol. & Med. 27 (Suppl. 2): 533–708.

Thienpont, D., O. F. J. Vanparijs, A. H. M. Raeymaekers, J. Vandenberk, P. J. A. Demoen, F. T. N. Allewijn, R. P. H. Marsboom, C. J. E. Niemegeers, K. H. L. Schellikens, and P A. J. Janssen. 1966. Tetramisole (R 8299), a new potent broad spectrum anthelmintic. Nature 209: 1084–1086.

On *Artyfechinostomum malayanum* (Leiper, 1911) Mendheim, 1943 (Trematoda: Echinostomatidae) with Synonymy of Allied Species and Genera

G. Premvati and Vibha Pande
Department of Zoology, University of Lucknow, Lucknow (U. P.), India

ABSTRACT: The genus *Artyfechinostomum* Lane, 1915, with its type species *A. sufrartyfex* Lane, 1915, has been studied from naturally infected pigs and experimentally infected white rats. Six species so far described under this genus are: *A. malayanum* (Leiper, 1911) Mendheim, 1943, from man; *A. indicum* (Bhalerao, 1931) Mendheim, 1943, from *Uromastix hardwickii*; *A. mehrai* Jain, 1960, from experimental rats; *A. paradoxuri* Baugh, 1962, from palm-civet; *A. varanum* Simha and Deshpande, 1964, from *Varanus bengalensis*; and *A. munshii* Deodhar et al., 1967, from dogs. On detailed comparison and in view of intergrading variations, all these six species have been synonymized with *A. malayanum* (regarded henceforth as type species). The three allied genera: *Reptiliotrema* Baschkirova, 1941; *Neoartyfechinostomum* Agarwal, 1963; and *Pseudoartyfechinostomum* Bhardwaj, 1963, have, on similar study, been held identical with *Artyfechinostomum*. Consequently, the species assigned to these genera, namely, *R. indicum* (Bhalerao, 1931) Baschkirova, 1941; *R. primata* Premvati, 1960; *R. tandani* Agarwal, 1963; *N. shubhrai* Agarwal, 1963; and *P. larueiformis* Bhardwaj, 1963, have been synonymized with *A. malayanum*. Generic characters of *Artyfechinostomum* have been emended. Host: reptiles and mammals.

The genus *Artyfechinostomum* Lane, 1915, with type species *A. sufrartyfex* Lane, 1915, seems to be prevalent in Southeast Asia. Its common occurrence in pigs of India and Malaya, together with its record from man, makes its study considerably important. Six other species so far described are: *A. malayanum* (Leiper, 1911) Mendheim, 1943, from man; *A. indicum* (Bhalerao, 1931) Mendheim, 1943, from *Uromastix hardwickii*; *A. mehrai* Jain, 1960, from experimental rats; *A. paradoxuri* Baugh, 1962, from palm-civet (*Paradoxurus hermaphroditus*); *A. varanum* Simha and Deshpande, 1964, from *Varanus bengalensis*; and *A. munshii* Deodhar et al., 1967, from dogs. The present study has been planned to compare morphological variations in *A. sufrartyfex* as described by earlier workers with those studied by present authors, and to ascertain the validity of all species described so far under this genus.

Besides, the question of validity of three allied genera: *Reptiliotrema* Baschkirova 1941, with its three species—*R. indicum* (Bhalerao, 1931) Baschkirova, 1941; *R. primata* Premvati, 1960; *R. tandani*, Agarwal, 1963; *Neoartyfechinostomum* Agarwal, 1963, with a single species *N. shubhrai* Agarwal, 1963; *Pseudoartyfechinostomum* Bhardwaj, 1963, with a single species, *P. larueiformis* Bhardwaj, 1963, has been examined.

These taxonomic units have mostly been erected on such morphological differences as: number and arrangement of collar spines; ratio of suckers; number of testicular lobes; posterior extent of cirrus sac with spinose or aspinose cirrus; presence or absence of seminal receptacle; anterior extent of vitellaria to anterior, middle, or posterior level of ventral sucker; size of eggs; and presence or absence of spines on excretory pore.

Metacercarial cysts, isolated from the infested renal tissue of *Rana cyanophlyctis*, were fed to albino rats. The following experiments have been conducted:

No. of cysts fed	Date of infection	Date of egg detection in feces	Date of autopsy	No. of adult flukes recovered
135	25.6.71	10.7.71	12.7.71	81
57	26.6.71	9.7.71	9.7.71	15
86	19.8.71	30.8.71	2.9.71	56

Adult flukes were also recovered from naturally infected pigs.

Encysted and excysted metacercarial stages and adults were studied alive and from stained and unstained permanent mounts. Spines were also studied after treatment with 1% KOH solution. Serial sections of adults were stained with Hematoxylin and Eosin. All measurements, unless otherwise stated, are recorded in microns.

Artyfechinostomum surfrartyfex Lane, 1915 (Plate I. Figs. 1, 2, 3, 4a, b, c; Plate II. Figs. 5a–g)

Lane (1915) described this species for the first time from an Assamese girl. Later, Bhalerao (1931b), Rai and Ahluwalia (1958), and Ahluwalia (1962) studied it from pigs; Srivastava (1964) from honey-badger; Matta and Pande (1966) from rats and piglets (experimental); Dubey et al. (1969) from cat and dog; Mohandas (1971) from white rats (experimental); Nath (1972) from lizard (experimental); and Agrawal and Pande (1972) from piglets (experimental). Present authors studied it from naturally infected pigs and from white rats infected experimentally with metacercarial cysts isolated from kidney of *Rana cyanophlyctis*.

Description of this species, as given by the above various workers, is summarized as follows.

MEASUREMENTS: Length 4.2–18.0 mm; width 1.2–6.0 mm; oral sucker 130–390 × 130–510; ventral sucker 495–1.53 mm × 448–1.8 mm; ratio of suckers 1:2.5 to 1:4; pharynx 108–450 × 120–405; anterior testis 490–2.10 mm × 530–3.21 mm; posterior testis 540–2.97 mm × 430–2.49 mm; ovary 160–800 × 120–800; eggs 56–165 × 49–90.

Collar spines: 37–39 arranged in zigzag manner (Lane, 1915); 39–42 in dorsally unbroken single row (Bhalerao, 1931b); 43 in two alternate rows (Rai and Ahluwalia, 1958; Ahluwalia, 1962; Matta and Pande, 1966; Nath, 1972 and Agrawal and Pande, 1972); 43–44 (Srivastava, 1964). Lie Kian Joe (1963) observed in *Echinostoma malayanum* Leiper, 1911 (synonym: *A. sufrartyfex*) 43 or 45 collar spines arranged in alternating dorsal rows, two lateral groups showing sometimes alternate arrangement, and two groups with five alternating corner spines each. According to Mohandas (1971), collar spines 43 in adult and 43–45 in cercariae, arranged in the pattern: 5 + 11 + 11 + 11 + 5 or 5 + 11 + 13 + 11 + 5. During present studies, the number of collar spines was mostly 41–43, except in one specimen it was 45; spines arranged uninterruptedly with 17 dorsals in two alternating series, 14 or 16 laterals, and 10 corner spines in two groups of five each. A narrow isthmus bridging ventral gap on reniform head collar, confirms the findings of Rai and Ahluwalia (1958), Ahluwalia (1962) and Lie Kian Joe (1963).

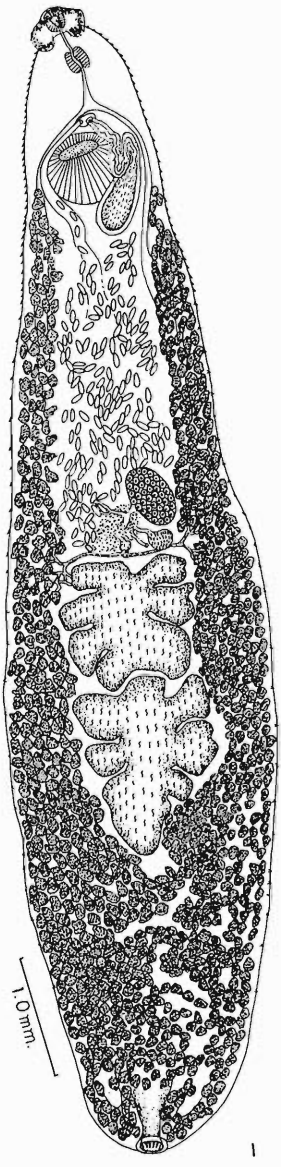
Testes deeply lobed by all, although number of lobes vary. During present study, extent of lobulation varies with degree of maturity of worm and with host (Plate II. Figs. 5 a-g). All workers reported posterior extent of cirrus sac beyond ventral sucker, though its posterior limit varies slightly. Cirrus spiny by all except Bhalerao (1931b) and Mohandas (1971).

Seminal receptacle reported to be absent by all except Faust (1930) and Craig and Faust (1943). Present authors agree with Lie Kian Joe (1965) and Mohandas (1971) in the presence of a uterine seminal receptacle. Vitellaria extend anteriorly to caudal half of cirrus sac (Lane); to middle of posterior border of ventral sucker (Bhalerao); to region of ventral sucker (Craig and Faust); to middle of ventral sucker (Rai and Ahluwalia; Ahluwalia, and Srivastava); and from middle to posterior border of ventral sucker, according to present authors. In size, eggs show slight variations—those recovered from experimentally infected rats being slightly smaller than those from pigs.

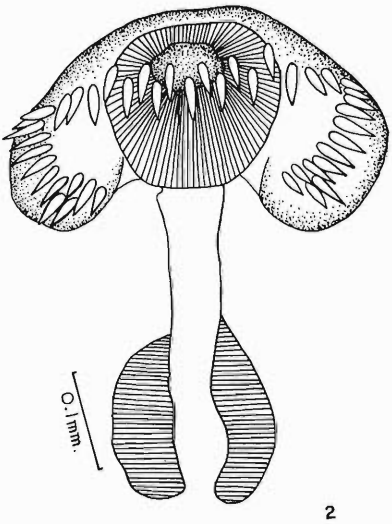
Spines on excretory pore not observed by previous workers. Present study reveals subterminal excretory pore with a crown of prominent lanceolate, eversible spines in proximal and distal groups (Plate I. Figs. 4a, b, c). Their presence has been noticed in all specimens (whole mounts and sections or following treatment with KOH), and even observed in metacercarial cysts.

→

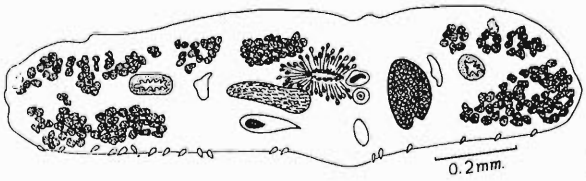
Plate I. ***Artyfechinostomum sufrartyfex*** **Lane, 1915. (Camera lucida drawings.) 1. Entire, showing 41 collar spines (from rat). 2. Head collar showing 43 collar spines (from rat). 3. Cross section, showing uterine seminal receptacle and Mehlis' glands (from rat). 4. (a) Excretory pore, end-on view (from rat). (b) Cross section of posterior end, showing spines around excretory pore (from rat). (c) Cross section of posterior end, showing spines around excretory pore (from pig).**



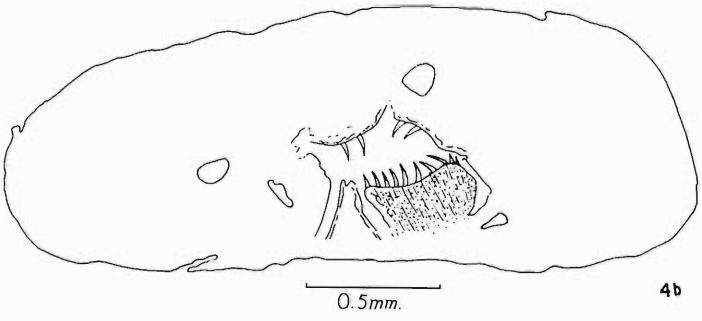
1



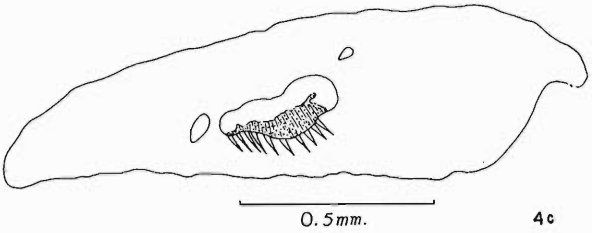
2



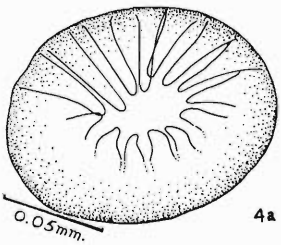
3



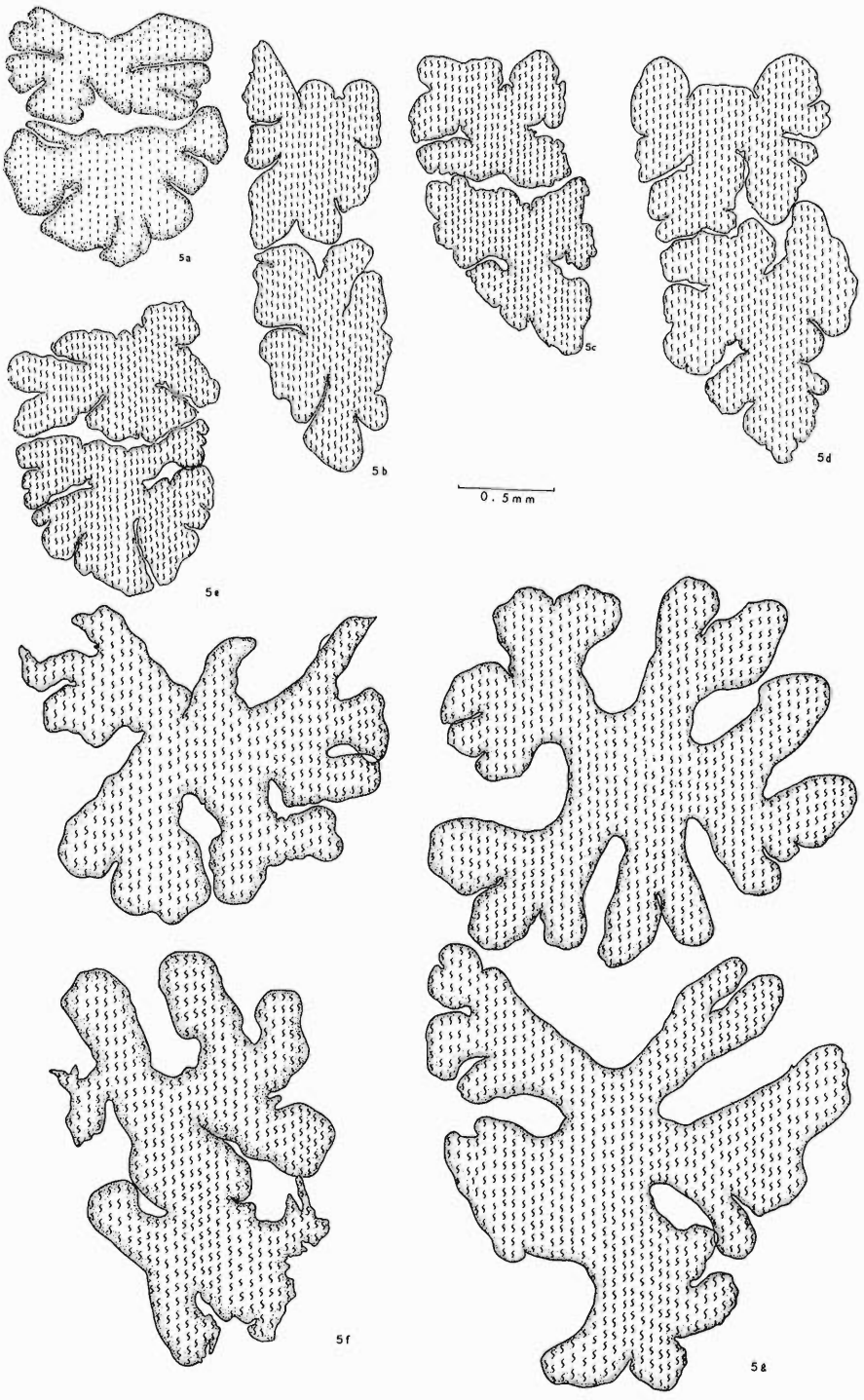
4b



4c



4a



Artyfechinostomum malayanum (Leiper, 1911) Mendheim, 1943 (Syn: *Echinostoma malayanum* Leiper, 1911; *Euparyphium malayanum* Leiper, 1911 *Euparyphium sufrartyfex* Baylis, 1929) (Plate III. Fig. 6)

Echinostoma malayanum recorded by Leiper (1911) from man in Malaya is found to infest pigs and house-shrews. It has been experimentally infected in white rats, white mice, and hamsters. Lie Kian Joe (1963), discussing the characters of *E. malayanum* and *A. sufrartyfex*, considered the latter to be a synonym of the former. He retained the name *Echinostoma* not because the worm fits in one of the definitions for this genus, but because it is most often used in medical literature. Mukherjee and Ghosh (1968) independently regarded *A. sufrartyfex* and various closely related species and allied genera as synonyms of *E. malayanum*. Mohandas (1971), after studying the life history of *A. sufrartyfex* and comparing it with that of *E. malayanum*, has stated that *A. sufrartyfex* is a synonym of *E. malayanum*.

The genus *Echinostoma* Rudolphi, 1809, according to Yamaguti (1958), is characterized by having unlobed testes, cirrus sac not extending beyond ventral sucker, and vitellaria being confluent posttesticularly or otherwise. *Artyfechinostomum*, on the other hand, has deeply lobed testes, cirrus sac extending beyond ventral sucker, and vitellaria beginning at level of ventral sucker, confluent posttesticularly. Thus, the two genera have distinct characters, and it may not be feasible to include *A. sufrartyfex* in the genus *Echinostoma*. Only one species of the genus *Echinostoma*, namely, *E. malayanum*, has all characteristics of *A. sufrartyfex*. Accordingly, *A. malayanum* (*E. malayanum* Leiper, 1911) Mendheim, 1943 and *A. sufrartyfex* Lane, 1915 are synonyms.

Artyfechinostomum indicum (Bhalerao, 1931) Mendheim, 1943 (Syn.: *Testisaculus indicum* Bhalerao, 1927; *Paryphostomum indicum* Bhalerao, 1931) (Plate III. Fig. 7 a, b)

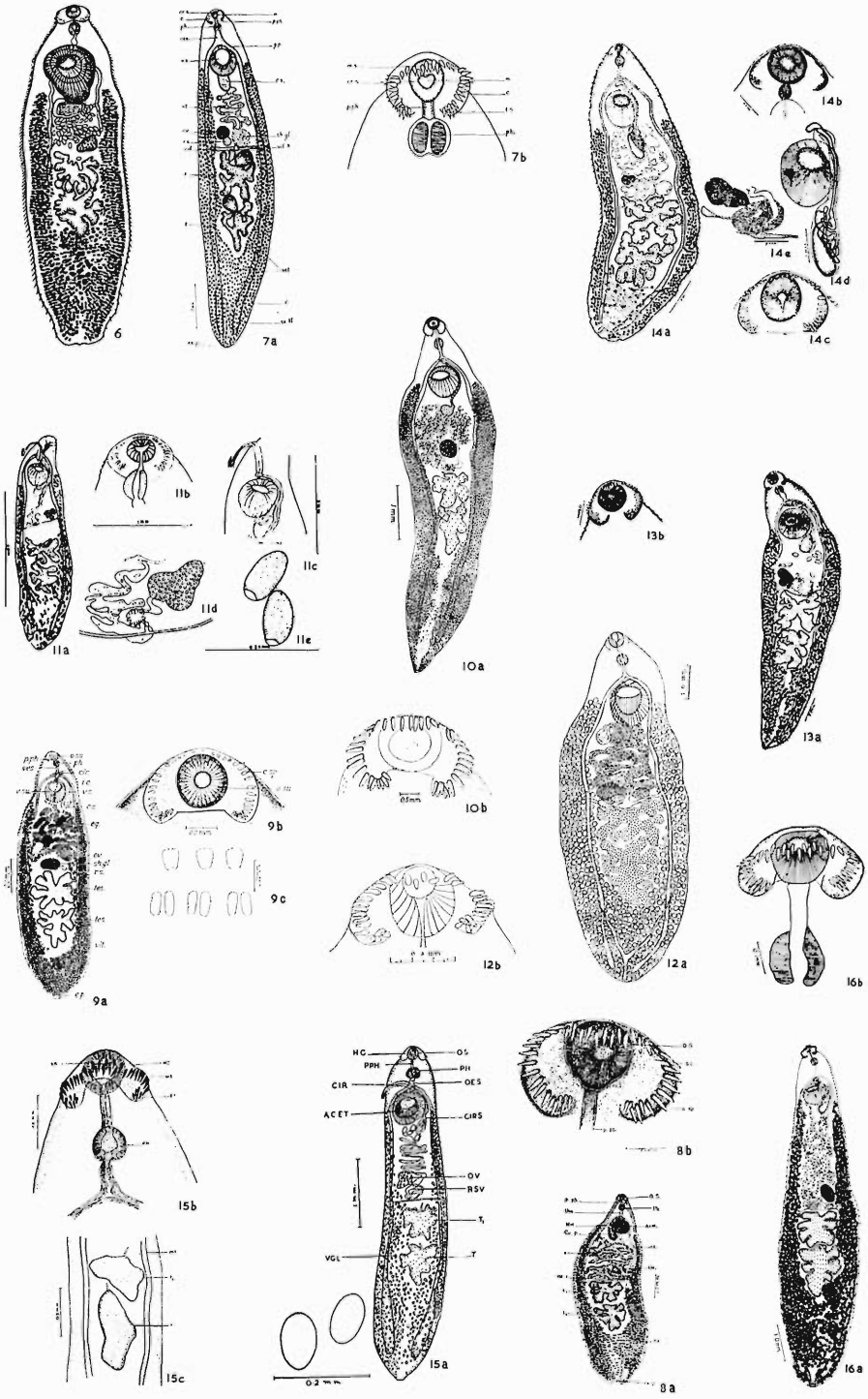
Artyfechinostomum indicum has been distinguished from *A. sufrartyfex* on account of cuticular spines extending up to ovarian zone; 42 collar spines arranged in double rows; vitellaria reaching near middle or posterior margin of ventral sucker; uterus with few coils and few eggs of smaller size. In *A. sufrartyfex*, as stated above, cuticular spines are denser in anterior half of body and sparser posteriorly; collar spines 41–45, arranged in alternating rows; vitellaria reach anteriorly from middle to posterior margin of ventral sucker; number of uterine coils and eggs depend on age of worms and on host species. Thus, distinguishing characters justifying the validity of *A. indicum* do not appear to exist. Accordingly, the latter is treated as a synonym of *A. sufrartyfex* Lane, 1915.

Artyfechinostomum mehrai Jain, 1960 (Syn.: *Paryphostomum mehrai* Jain, 1957) (Plate III. Fig. 8 a, b).

Jain (1960a) placed *A. mehrai* and *A. indicum* together, and distinguished these from *A. sufrartyfex* because collar spines in former were in double rows while, in latter, in a single row. He kept *A. mehrai* distinct from *A. indicum* as vitellaria extended to posterior margin of ventral sucker in former and to middle in latter. Further, *A. mehrai* has numerous eggs and a small seminal receptacle (Jain, 1960b). He distinguished the three species on: number and arrangement of collar spines (39 in single row in *A. sufrartyfex*, 42 in double rows in *A. indicum*, and 43 in double rows in *A. mehrai*); ratio of suckers, and num-

←

Plate II. ***Artyfechinostomum sufrartyfex*** **Lane, 1915. (Camera lucida drawings.) 5. (a) to (e). Testes showing variations in lobulations in specimens from rat. (f) to (g). Testicular lobulations in specimens from pig.**



ber of testicular lobes (Jain, 1960 a). In *A. sufrartyfex*, as mentioned above, collar spines 41–45 arranged in alternating rows dorsally; ratio of suckers 1 : 2.5 to 1 : 4, covers that found in *A. mehrai*; extent of lobulation in testes, and number of eggs depend on stage of maturity of the worm and on host; vitellaria extend anteriorly to posterior region of ventral sucker, as in *A. mehrai*; a uterine seminal receptacle, observed in *A. sufrartyfex*, is often mistaken for a seminal receptacle. Thus, the three species cannot be distinguished on these characters. Ahluwalia (1962) believed that *A. mehrai* was not distinct from *A. sufrartyfex*. Present authors also agree with Ahluwalia in treating *A. mehrai* as a synonym of *A. sufrartyfex* Lane, 1915.

Artyfechinostomum paradoxuri Baugh, 1962 (Plate III. Fig 9 a–c).

This species, based on about half a dozen specimens from palm-civet (*Paradoxurus hermaphroditus*), has been distinguished chiefly by characteristic body spines; collar spines 41–42 arranged in a single dorsally uninterrupted row; presence of seminal receptacle, and spines around excretory pore.

The body spines, in *A. sufrartyfex*, have been observed to be large and broad as in *A. paradoxuri*; arrangement of collar spines in specimens of *A. paradoxuri* has been observed to be in alternating double rows; presence of uterine seminal receptacle and spines on excretory pore have already been discussed above in *A. sufrartyfex*. Thus, *A. paradoxuri* does not differ from *A. sufrartyfex* with which it is synonymized.

Artyfechinostomum varanum Simha and Deshpande, 1964 (Plate III. Fig. 10 a, b)

This species has been differentiated only from *A. indicum*, which also parasitizes a reptilian host, on account of preacetabular portion being sharply marked off; collar spines 40; ratio of suckers 1:3; cirrus sac extending to second quarter of body, and uterus having many larger eggs.

These characters, however, come within the range of variations detected in *A. sufrartyfex*, as discussed above. *A. varanum* is, therefore, held identical with *A. sufrartyfex* Lane, 1915.

Artyfechinostomum munshii Deodhar et al., 1967 (Plate III. Fig. 11 a–e)

This species, obtained from small intestine of dog, shows affinities with *A. sufrartyfex*, *A. indicum*, and *A. mehrai* on account of general body surface; position of gonads; shape and size of cirrus sac and cirrus, according to Deodhar et al. (1967). It is stated to differ from them in having cuticular spines extending to end of anterior testis; collar spines 38; and testes with seven to nine lobes.

In *A. sufrartyfex*, body spines in posterior part of body become significantly sparser. Collar spines 41–45 are frequently lost during handling. As Deodhar et al. (1967) have probably studied preserved material, there is every possibility of spines being lost. Details of arrangement of dorsally uninterrupted collar spines have not been given. According to Yadav (1959), anterior testis of *A. sufrartyfex* has

←

Plate III. (Figures from original authors.) 6. *Euparyphium malayanum*, entire (after Skrjabin, 1956). 7. *Paryphostomum indicum*, (a) entire, (b) head collar showing arrangement of spines (Bhalerao, 1931). 8. *Paryphostomum mehrai*, (a) entire, (b) head collar (Jain, 1957). 9. *Artyfechinostomum paradoxuri*, (a) entire, (b) cephalic collar, (c) body spines (Baugh, 1962). 10. *Artyfechinostomum varanum*, (a) entire, (b) collar spines (Simha and Deshpande, 1964). 11. *Artyfechinostomum munshii*, (a) entire, (b) anterior end, (c) cirrus sac, seminal vesicle, and cirrus, (d) ovarian complex, (e) eggs (Deodhar et al., 1967). 12. *Reptiliotrema primata*, (a) entire, (b) cephalic collar (Premvati, 1960). 13. *Reptiliotrema tandani*, (a) entire, (b) head collar (Agarwal, 1963). 14. *Neoartyfechinostomum shubhrai*, (a) entire, (b) head collar, (c) arrangement of collar spines, (d) cirrus sac, (e) ootype (Agarwal, 1963). 15. *Pseudoartyfechinostomum larueiformis*, (a) entire, and two eggs, (b) anterior end showing head collar, (c) testes (Bhardwaj, 1963). 16. *Artyfechinostomum sufrartyfex*, (a) entire, with 41 collar spines, (b) head collar showing 43 collar spines (present authors).

up to 10 lobes; and in present studies, testicular lobes found to vary with age of parasite (Fig. 5 a–g of Plate II). Position of gonads, shape and size of cirrus sac and cirrus, also same as found in *A. sufrartyfex*. Besides, *A. sufrartyfex* has been reported from dog by Dubey et al. (1969) and by Nath (1969) from pup (experimental). Hence, *A. munshii* is suppressed as a synonym of *A. sufrartyfex*.

Reptiliotrema Baschkirova, 1941
R. indicum (Bhalerao, 1931) Baschkirova, 1941;
R. primata Premvati, 1960;
R. tandani Agarwal, 1963

This genus, erected to include *P. indicum* Bhalerao, 1931, has two other species: *R. primata*, from rhesus monkeys, and *R. tandani*, from *Varanus monitor*. It is already stated that *P. indicum*, synonymized with *A. indicum* by Mendheim, 1943, is held identical with *A. sufrartyfex* by present authors.

Reptiliotrema primata (Plate III. Fig. 12 a, b), described from preserved specimens, has been distinguished from *R. indicum* by cuticular spines covering whole body; number of collar spines; broader anterior testis; greater posterior extent of cirrus sac; larger size of ovary; and presence of a seminal receptacle. These features, during present study, have shown intraspecific variations which warrant suppression of *R. primata* as a synonym of *A. sufrartyfex* Lane, 1915.

According to Agarwal, *R. tandani* (Plate III. Fig. 13 a, b) differed from *R. indicum* on account of cuticular spines covering whole body; testes equal in size; ovary bilobed; and presence of an elongated seminal receptacle. In shape and size, the two testes in *R. tandani* resemble those of *A. sufrartyfex* (Plate II. Fig. 5 a–g). Jain (1960b) stated that ovary was bilobed in *A. mehrai* (a synonym of *A. sufrartyfex*. Body spines have been observed in all specimens of *A. sufrartyfex*. Thus all characters said to distinguish *R. tandani* are found in *A. sufrartyfex*. On re-examination, a uterine seminal receptacle has been observed in the genus *Reptiliotrema*. Since all the three species, under *Reptiliotrema*, have been synonymised with *A. sufrartyfex*, the genus cannot retain a status independent from *Artyfechinostomum* Lane, 1915.

Neoartyfechinostomum Agarwal, 1963
N. shubhrai Agarwal, 1963
(Plate III. Fig. 14 a–e)

This genus, with its solitary species *N. shubhrai* based on three specimens from local pig has been distinguished from closely allied genus *Artyfechinostomum* by having a pharynx larger than oral sucker; pear-shaped ovary; presence of seminal receptacle; and vitellaria extending anteriorly to hind end of ventral sucker. In *N. shubhrai* there is practically very little difference in the sizes of oral sucker (210–250 × 260–310) and pharynx (250–300 × 270–360) and these sizes are found in *A. sufrartyfex* also. The almost round ovary, when pressed, could assume a pear-shaped form and a uterine seminal receptacle could easily be mistaken for a seminal receptacle. Anterior extent of vitellaria to hind end of ventral sucker has been observed by present authors. The validity of the genus *Neoartyfechinostomum* is thus untenable. It is, therefore, regarded as a synonym of *Artyfechinostomum* Lane, 1915. The characters given for *N. shubhrai* do not support its retention even as a species distinct from *A. sufrartyfex* with which it is held identical.

Pseudoartyfechinostomum Bhardwaj, 1963
P. larueiformis Bhardwaj, 1963
(Plate III. Fig. 15 a–c)

This genus, with *P. larueiformis* as the only species, has been described from about 25 specimens collected from *Varanus*—believed to be an unreported reptilian host. It is distinguished from *Artyfechinostomum* on account of body tapering at two ends and covered with spines even below its last fourth; collar spines 39, with smaller terminal spines; prepharynx longer than esophagus; testes very irregular but never deeply lobed; and fewer eggs being largest.

Artyfechinostomum, as mentioned above, also occurs in reptiles: *U. hardwickii*, *V. bengalensis*, and *V. monitor*. In body shape, *P. larueiformis* closely resembles *A. sufrartyfex*. Large-sized prepharynx cannot be regarded as a character of generic value. Testicular lobulation and total number of eggs depend on stage of maturity. Size of eggs—0.1245 × 0.0747 to 0.10779 × 0.0664 for *P. larueiformis*

formis by Bhardwaj; 0.135 × 0.075 for *A. sufrartyfex* by Yadav (1959); 90–159 × 60–93 for *E. malayanum* by Lie Kian Joe (1963); and 0.058–0.075 × 0.09–0.113 for *P. sufrartyfex* by Bhalerao 1931b), is nearly same. *Pseudoartyfechinostomum* does not possess any distinctive characters to uphold its validity as a genus and is considered as a synonym of *Artyfechinostomum* Lane, 1915. *Pseudoartyfechinostomum larueiformis* does not reveal any distinctive character even at species level, and is, therefore, held identical with *A. sufrartyfex* Lane, 1915.

The observations have necessitated a slight emendment of the generic diagnosis given by Yamaguti:

Artyfechinostomum Lane, 1915 (Syn.: *Reptiliotrema* Baschkirova, 1941; *Neoartyfechinostomum* Agarwal, 1963; *Pseudoartyfechinostomum* Bhardwaj, 1963)

GENERIC DIAGNOSIS: Echinostomatidae; body elongate, broader posteriorly, indented near region of ventral sucker. Head collar reniform, with narrow ventral isthmus; with a crown of 39–45 collar spines arranged dorsally in uninterrupted, alternating rows, with five corner spines on each side. Oral sucker small, subterminal; prepharynx present; esophagus short; ceca terminating in front of posterior extremity. Ventral sucker prominent, in anterior fourth of body. Testes large, tandem, deeply lobed, in posterior half of body, cirrus sac long, club-shaped, extending beyond ventral sucker, enclosing prominent seminal vesicle, short prostatic duct, coiled ejaculatory duct ending in eversible cirrus carrying minute spines. Ovary pretesticular, submedian, oval; seminal receptacle present; Laurer's canal present. Uterus coiled, ending in well-developed metraterm; eggs numerous, large; vitellaria lateral, extending from region of ventral sucker to hind extremity, with follicles confluent posttesticularly. Excretory pore armed with eversible, lanceolate spines; excretory bladder Y-shaped, main stem extending to posterior testes with cornua extending near oral sucker. Intestinal parasites of reptiles and mammals.

GENOTYPE: *A. malayanum* (Leiper, 1911) Mendheim, 1943. (As *A. malayanum* has taxonomic priority over *A. sufrartyfex*, it is retained as type species.) [(Syn.: *E. malayanum* Leiper, 1911; *A. sufrartyfex* (Lane, 1915); *P. indicum* Bhalerao, 1931; *A. indicum* (Bhalerao, 1931) Mendheim, 1943; *A. mehrai* Jain, 1960; *A. paradoxuri* Baugh, 1962; *A. varanum* Simha and Deshpande, 1964; *A. munshii* Deodhar et al., 1967; *R. indicum* (Bhalerao, 1931) Baschkirova, 1941; *R. primata* Premvati, 1960; *R. tandani* Agarwal, 1963; *N. shubhrai* Agarwal, 1963; *P. larueiformis* Bhardwaj, 1963].

Acknowledgments

The authors are grateful to the State Council of Scientific and Industrial Research, Lucknow, for providing financial support.

Literature Cited

Agarwal, V. 1963. Three new trematodes of the family Echinostomatidae Poche, 1926. Indian J. Helminth. 15: 127–137.

Agrawal, R. D., and B. P. Pande. 1972. Partial life cycle of echinostomal fluke of pigs: An experimental study. Indian J. Anim. Sci. 42: 194–198.

Ahluwalia, S. S. 1962. Studies on some helminths of the domestic pig (*Sus scrofa domestica*) in western Uttar Pradesh. J. Helminth. 36: 347–364.

Baugh, S. C. 1962. Contributions to our knowledge of digenetic trematodes. IV. Z. Parasitenk. 21: 502–512.

Bhalerao, G. D. 1931a. Two new trematodes from reptiles: *Paryphostomum indicum* n. sp. and *Stunkardia dilymphosa* n. g., n. sp. Parasitology 23: 99–108.

———. 1931b. Trematode parasites of pigs in Bengal. Rec. Indian Mus. 33: 475–482.

Bhardwaj, O. N. 1963. Morphological studies and taxonomic discussion of a new trematode *Pseudoartyfechinostomum larueiformis* n. g., n. sp. (Trematoda: Echinostomatidae). Proc. Nat. Acad. Sci. India, Sect. B. 33: 303–313.

Craig, C. E., and E. C. Faust. 1943. Clinical Parasitology. Lea and Febiger, Philadelphia, 767 p.

Deodhar, M. S., V. G. Patil Kulkarni, and P. P. Karyakarte. 1967. On the occurrence of a new species of the genus *Artyfechinostomum* Lane, 1915 in a dog in Bombay. Indian J. Helminth. 19: 122–125.

Dubey, J. P., H. O. P. Srivastava, and V. K. Sahasrabudhe. 1969. *Artyfechinostomum sufrartyfex* infection in cat and dog in India. Trop. Geogr. Med. 21: 210–213.

Faust, E. C. 1930. Human Helminthology. Henry Kimpton, London, 616 p.

Jain, G. P. 1957. On the trematode *Paryphostomum mehrai* Faruqui. Proc. Nat. Acad. Sci., India, Sect. B. 27: 290–294.

———. 1960a. On the genus *Artyfechinostomum* Lane, 1915 (Echinostomatidae: Trematoda). Parasitology 50: 1–5.

———. 1960b. Further observations on *Artyfechinostomum mehrai* (Faruqui) (Echinostomatidae: Trematoda). Parasitology 50: 7–11.

Lane, C. 1915. *Artyfechinostomum sufrartyfex.* A new parasitic echinostome of man. Indian J. Med. Res. 2: 977–983.

Leiper, R. T. 1911. A new echinostome parasite in man. J. Lond. Sch. Trop. Med. 1: 27–28.

Lie Kian Joe. 1963. Studies on Echinostomatidae in Malaya. III. The adult *Echinostoma malayanum* Leiper, 1911 (Trematoda) and the probable synonymy of *Artyfechinostomum sufrartyfex* Lane, 1915. Z. Parasitenk. 23: 124–135.

———. 1965. Studies on Echinostomatidae (Trematoda) in Malaya. IX. The Mehlis' gland complex in echinostomes. J. Parasit. 51: 789–792.

Matta, S. C., and B. P. Pande. 1966. Studies on some metacercariae in local snails. Indian J. Helminth. 18: 128–141.

Mohandas, A. 1971. *Artyfechinostomum sufrartyfex* Lane, 1915, a synonym of *Echinostoma malayanum* Leiper, 1911 (Trematoda, Echinostomatidae). Acta Parasitologica Polonica 19: 361–368.

Mukherjee, R. P., and R. K. Ghosh. 1968. On the synonymy of the genus *Artyfechinostomum* Lane, 1915 (Trematoda: Echinostomatidae). Proc. Indian Acad. Sci., Sect. B. 68: 52–58.

Nath, D. 1969. *Rana cyanophlyctis* as the second intermediary of *Artyfechinostomum sufrartyfex* (Echinostomatidae: Trematoda). Curr. Sci. 38: 342–343.

———. 1972. Observations on metacercaria of *Artyfechinostomum sufrartyfex* Lane, 1915 and its experimental development in a reptile. Indian Vet. J. 49: 767–774.

Premvati. 1960. *Reptiliotrema primata* n. sp. (Echinostomatidae) from the intestine of rhesus monkeys. Annals of Zool., Agra 3: 117–121.

Rai, P., and S. S. Ahluwalia. 1958. A note on common helminths of pigs at Aligarh (Part I). Proc. Nat. Acad. Sci. India, Sect. B. 38: 195–199.

Simha, S. S., and P. Deshpande. 1964. On a new species of *Artyfechinostomum* Lane, 1915, from the intestine of the monitor lizard, *Varanus bengalensis*, in Hyderabad India. Z. Parasitenk. 24: 23–26.

Skrjabin, K. I. 1956. Trematodes of animals and man. Principles of Trematology, Vol. XII. Moscow Izdatelstvo Akad. Nauk. SSSR. 932 p. (Russian text.)

Srivastava, S. C. 1964. A new host record for *Strongyloides akbari* and *Artyfechinostomum sufrartyfex.* Indian J. Helminth. 16: 24–26.

Yadav, D. C. 1959. Further observations on *Artyfechinostomum sufrartyfex* (Lane) Bhalerao, 1931 (Trematoda: Echinostomatidae) with a note on its systematic position. Proc. Nat. Acad. Sci. India, Sect. B. 29: 157–167.

Yamaguti, S. 1958. Systema Helminthum. The Digenetic Trematodes of Vertebrates. Part I. Interscience Publishers, New York, London. Vol. I. 979 p.

The Trypanorhyncha (Cestoda) of Elasmobranch Fishes from Southern California and Northern Mexico

MICHAEL L. HEINZ AND MURRAY D. DAILEY
Department of Biology, California State University, Long Beach, California 90840

ABSTRACT: A single new genus and eight new species of Trypanorhyncha are described from elasmobranch fishes of the Pacific Coast of Southern California and Northern Mexico. These include *Sphyriocephalus pelorosoma* sp. n. from *Alopias superciliosus* (Lowe); *Mecistobothrium myliobati* gen. et sp. n. from *Myliobatis californica* Gill; *Prochristianella fragilis* sp. n. from *Rhinobatos productus* Ayres; *Prochristianella minima* sp. n. from *Urolophus halleri* Cooper; *Eutetrarhynchus litocephalus* sp. n. from *Mustelus californicus* Gill; *E. macrotrachelus* sp. n. from *Mustelus californicus*; *E. schmidti* sp. n. from *Urolophus halleri*; and *Nybelinia anthicosum* sp. n. from *Triakis semifasciata* Girard. Fourteen other species and hosts are also listed representing 13 new hosts and distribution records.

A comprehensive study of the Trypanorhyncha has been completely neglected on the west coast of the United States. Reports by workers from the Pacific Coast have remained scattered and incomplete, being inextensive in both geographical areas and types of host species. Hart (1936) recorded five species of Trypanorhyncha from the Puget Sound Region, and Pintner (1930) reported two species from Pacific Grove. Young (1954) recorded three species from Southern California and Pappas (1970) recorded four species, three of which he identified as common trypanorhynch species and the fourth he identified to the genus level.

In this paper, cestodes representing 22 species of Trypanorhyncha collected from 1966–72 are reported from 14 genera and 16 species of Pacific elasmobranchs taken in Southern California and Northern Mexico (Table 1). Eight new species and a single new genus are described and discussed.

The parasites were washed in a 1:2 dilution of sea and tap water, relaxed in tap water by refrigeration, fixed in AFA, stained in Semichon's Acetocarmine or Coelestin Blue B, and mounted in piccolyte. All measurements are in millimeters unless otherwise indicated.

Family Sphyriocephalidae Pintner, 1913
Sphyriocephalus pelorosoma sp. n.
(Figs. 1–3)

DESCRIPTION (measurements from a single specimen): Total length 118.0; maximum strobila width 15.0. Strobila craspedote, proglottids numerous, wider than long. Scolex measuring 6.16 long to posterior tip of bulbs by 3.70 wide at mid *pars bulbosa*. *Pars post bulbosa* 9.64 long. Bothridia with deep cavity, each pair encircled by thick outer margin. Bothridia 2.75 long, not overlapping bulbs. Bulbs 2.39 long by 0.36 wide; aligned in anterior–posterior position. *Pars vaginalis* length 2.94; tentacle sheath direct. Hooks hollow, arranged in longitudinal obliquely ascending rows; hooks diminishing in size at basal region. Tentacles 0.45 wide.

TYPE HOST: Bigeye thresher, *Alopias superciliosus* (Lowe).

LOCATION: Stomach.

LOCALITY: Bolsa Chica State Beach, Huntington Beach, California.

HOLOTYPE: USNM Helm. Coll. No. 72679.

REMARKS: A single specimen of *S. pelorosoma* sp. n. was recovered from *A. superciliosus* found shot through the head at Bolsa Chica State Beach. A number of morphological features distinguish *S. pelorosoma* sp. n. from *S. tergestinus* Pintner, 1913, and *S. viridis* Wagener, 1854. The tentacles (Figs. 2, 3) of this species measure 0.45 wide whereas this measurement for *S. tergestinus* and *S. viridis* is 0.15–0.22 and 0.24–0.25, respectively. The bulbs of *S. pelorosoma* sp. n. are six times longer than wide, while the bulb length is only twice the width in the other two species. The strobila (Fig. 1) measures up to 15 wide which is approximately 9.0 greater than the maximum width recorded for any other species of *Sphyriocephalus* (*S. viridis* measures 6.0).

Table 1. Trypanorhyncha of elasmobranch fishes taken from Southern California and Northern Mexico.

Parasite	Host	Locality
Family Tentaculariidae Poche, 1926		
Tentacularia coryphaena Bosc, 1802	**Carcharhinus longimanus* (Poey)	**Pacific Ocean: 17°52′ N, 103°50′ W
	**C. limbatus* (Valenciennes)	**Pacific Ocean: 16° N, 101°40′ W
Nybelinia pintneri Yamaguti, 1934	**Isurus oxyrinchus* Rafinesque	**San Diego, California
N. anthicosum sp. n.	*Triakis semifasciata*	Seal Beach, California
	Heterodontus francisci (Girard)	San Carlos Bay, Baja California, Mexico
		Playa Maria, Baja California, Mexico
Family Hepatoxylidae Dollfus, 1940		
Hepatoxylon squali Martin, 1797	**Alopias vulpinus* (Bonnaterre)	**Catalina Channel, California
Family Sphyriocephalidae Pintner, 1913		
Sphyriocephalus viridis Wagener, 1854	**A. superciliosus*	**Bolsa Chica State Beach, California
S. pelorosoma sp. n.	**A. superciliosus*	**Bolsa Chica State Beach, California
Family Dasyrhynchidae Dollfus, 1935		
Dasyrhynchus talismani Dollfus, 1935	**Carcharhinus longimanus*	**Pacific Ocean: 23° N, 112°3′ W
Callitetrarhynchus gracilis (Rudolphi, 1819)	**Prionace glauca* (Linnaeus)	**Catalina Channel, California
Floriceps saccatus Cuvier, 1817	**Notorynchus maculatus* Ayres	**Baja California, Mexico
	**Carcharhinus limbatus*	**Pacific Ocean: 17°52′ N, 103°50′ W
Family Lacistorhynchidae Guiart, 1927		
Lacistorhynchus tenuis (Van Beneden, 1858)	**Mustelus californicus*	**Mission Bay, San Diego, California
	Mustelus henlei (Gill)	Anaheim Bay, Seal Beach, California
	Rhinobatos productus	**Seal Beach, California
	Triakis semifasciata	Seal Beach, California
Grillotia smaris-gora Wagener, 1854	*Squatina californica* Ayres	**Catalina Island, California
Family Gymnorhynchidae Dollfus, 1935		
Gymnorhynchus gigas (Cuvier, 1817)	**Isurus oxyrinchus*	**San Diego, California
Molicola horridus (Goodsir, 1841)	**Isurus oxyrinchus*	San Diego, California
M. uncinatum Linton, 1924	*Alopias vulpinus*	Catalina Channel, California
		Redondo Channel, California
Family Gilquiniidae Dollfus, 1942		
Gilquinia squali (Fabricius, 1794)	*Squalus acanthias* Linnaeus	Catalina Channel, California
		San Pedro, California
Family Eutetrarhynchidae Guiart, 1927		
Eutetrarhynchus macrotrachelus sp. n.	*Mustelus californicus*	Mission Bay, San Diego, California
E. schmidti sp. n.	*Urolophus halleri*	Anaheim Bay, Seal Beach, California
	Rhinobatos productus	Seal Beach, California
E. litocephalus sp. n.	*Mustelus californicus*	Mission Bay, San Diego, California
	Triakis semifasciata	Bahia de San Quintin, Mexico
Mecistobothrium myliobati gen. et sp. n.	*Myliobatis californica*	Mission Bay, San Diego, California
	Urolophus halleri	Seal Beach, California
Prochristianella minima sp. n.	*Urolophus halleri*	Anaheim Bay, Seal Beach, California
	Platyrhinoidis triseriata (Gordon and Gilbert)	Alamitos Bay, Seal Beach, California
P. fragilis sp. n.	*Rhinobatos productus*	Mission Bay, San Diego, California
Parachristianella monomegacantha Kruse, 1959	*Rhinobatos productus*	Seal Beach, California

* New host record.
** New distribution record.

The specific name *pelorosoma* comes from the Greek meaning monstrous or gigantic body.

Family Eutetrarhynchidae Guiart, 1927 *Mecistobothrium* gen. n.

GENERIC DIAGNOSIS: Strobila craspedote; anterior proglottids wider than long; terminal proglottids longer than wide. Testes preovarian. Uterus extending to anterior end of proglottid. Neck short. Scolex acraspedote. Bothridia longer than bulbs. Armature heteromorphous with large rose thorn-shaped hooks followed by smaller hooks which terminates each row on opposite surface of tentacle. Frontal glands present.

REMARKS: The hook arrangement (Figs. 5-7) of *Mecistobothrium* gen. n. clearly places it in the family Eutetrarhynchidae. The general armature (pattern) resembles those found in the genus *Parachristianella* Dollfus, 1946 (large triangular hooks at beginning of each hook row, decreasing in size as row terminates

on opposite surface of tentacle) but differs in hook size and shape. However, *Mecistobothrium* gen. n. differs from all members of the family in having proglottids craspedote and bothridia longer than bulbs.

Family diagnosis should be amended as follows.

DIAGNOSIS: Scolex long, acraspedote. Tentacles long, cylindrical, hooks on inner surface of different size than those of outer surface. Two wide, flattened bothridia. Proglottids acraspedote or craspedote, apolytic testes numerous, crossing osmoregulatory canals laterally. No postovarian vitellaria. Parasites of elasmobranchs.

Mecistobothrium is derived from the Greek, "mecist" and "bothrium" meaning longest pit or trench, and serves as a noun to be treated as neuter in gender. The species name is taken from the type host *Myliobatis californica.*

TYPE SPECIES: *Mecistobothrium myliobati.*

Mecistobothrium myliobati gen. et sp. n. (Figs. 4–7)

DESCRIPTION (measurements from six specimens): Maximum total length 27.62; strobila craspedote; 22–55 proglottids; terminal proglottid 2.09 long by 0.75 wide. Genital pores in central third of proglottid, alternating irregularly. Testes in two horizontal rows, not extending postovarian. Cirrus pouch occupying one-third proglottid width. Ovary large, filling almost entire posterior fifth of proglottid. In gravid proglottids, uterus extending to anterior end of proglottid. Neck short. Scolex length 0.69 (0.49–0.83). Two bothridia 0.33 (0.24–0.44) long by 0.26 wide (0.24–0.27). *Pars vaginalis* 0.46 (0.34–0.55) long. Tentacle sheaths direct. *Pars bulbosa* 0.19 (0.11–0.27) long by 0.31 (0.28–0.34) wide at midbulb: individual bulb width 0.08. Armature heteroacanthous and heteromorphous, forming oblique rows beginning with large rose thorn-shaped hooks on external face and terminating with smaller hooks on the internal face of tentacle. Frontal glands in central third of scolex. Ratio of *pars bothridialis, pars vaginalis,* and *pars bulbosa* 1:1.4:0.6.

TYPE HOST: Bat ray, *Myliobatis californica* Gill; other hosts: Round stingray, *Urolophus halleri* Cooper.

LOCATION: Spiral valve.

LOCALITY: Mission Bay, San Diego, and Seal Beach, California.

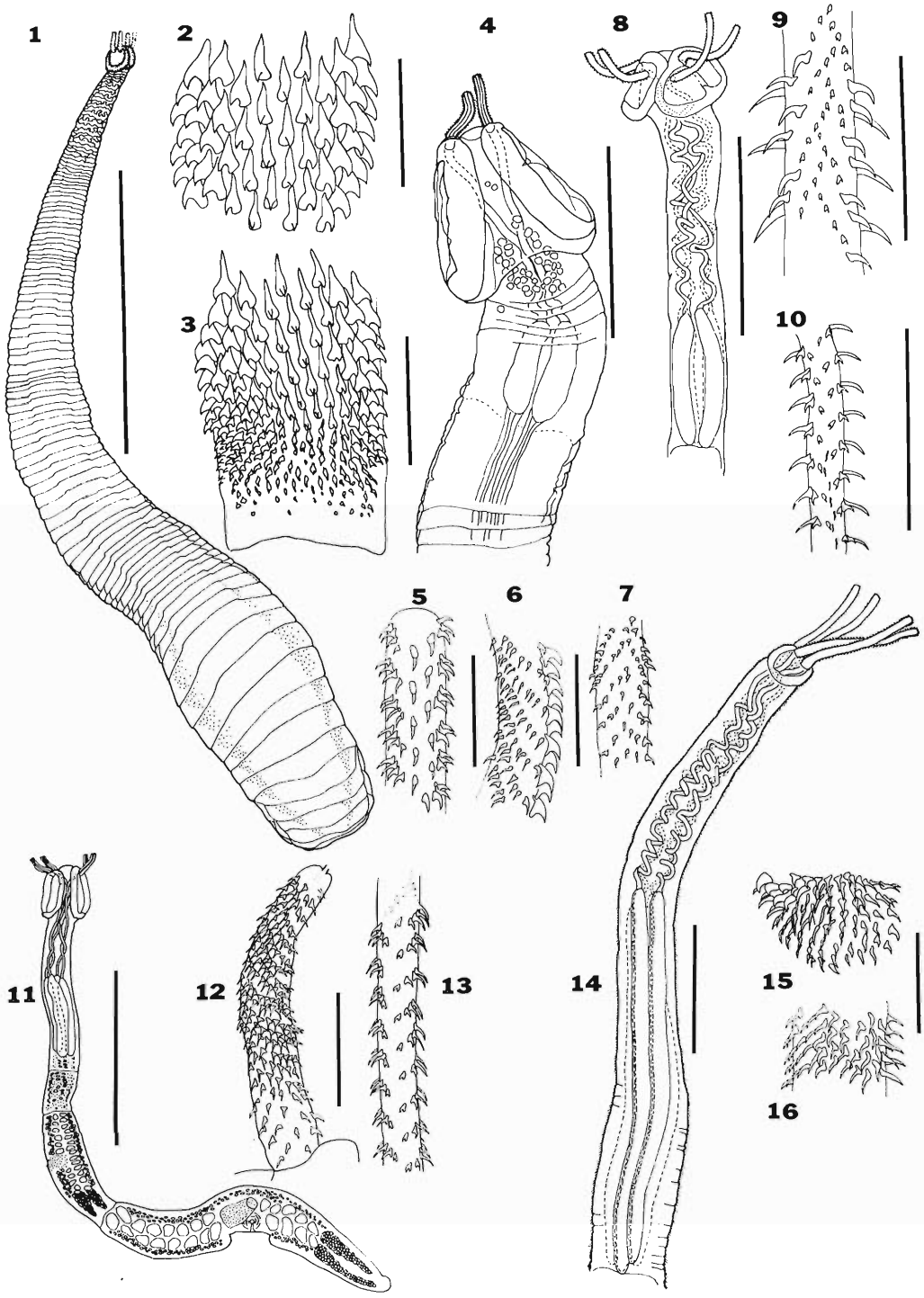
HOLOTYPE: USNM Helm. Coll. No. 72673.

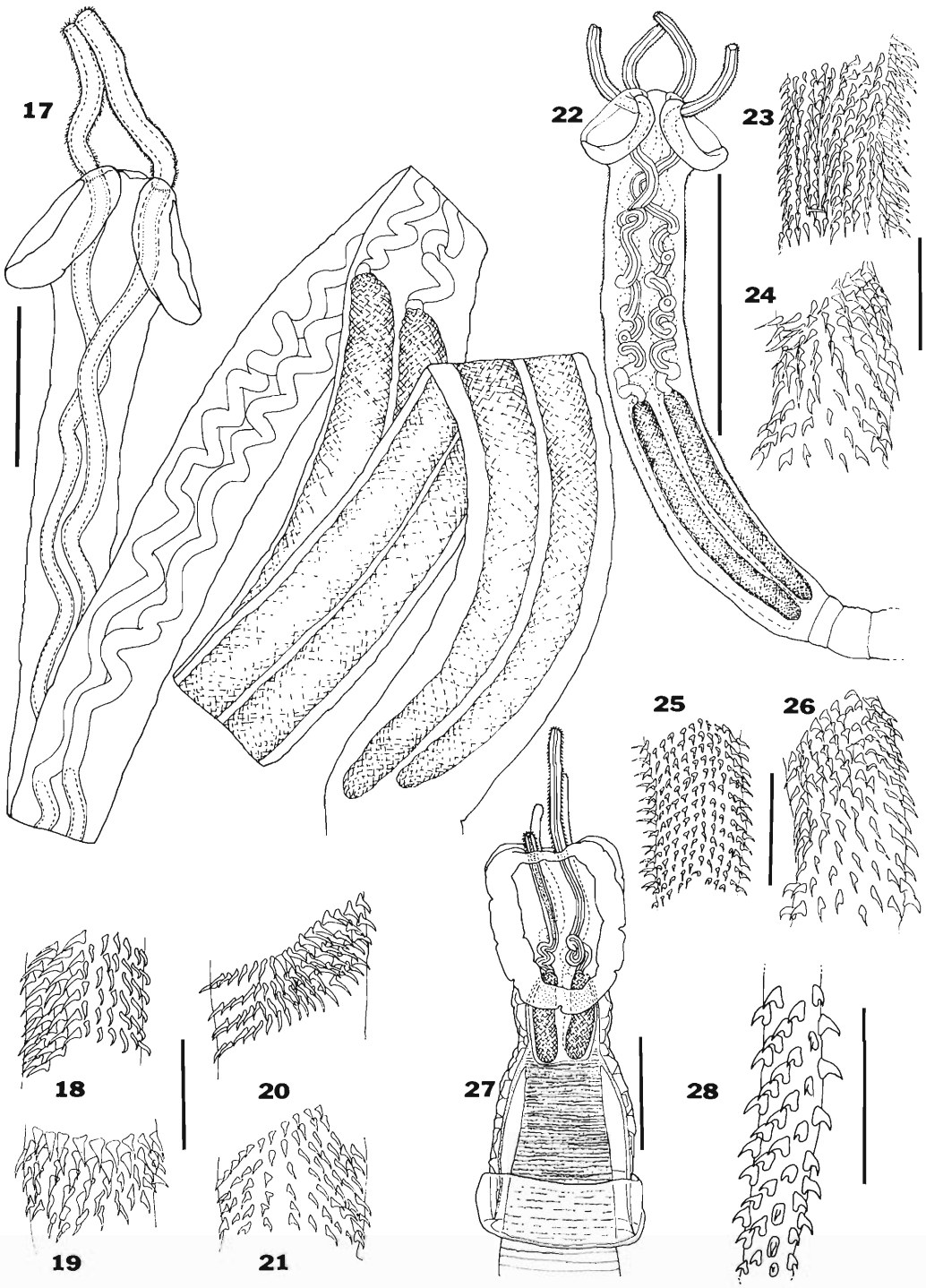
Prochristianella fragilis sp. n. (Figs. 8–10)

DESCRIPTION (measurements from five specimens): Maximum total length 7.06; width at mid *pars bulbosa* 0.15 (0.13–0.16). Acraspedote proglottids numbering to 14; anterior proglottids wider than long, latter proglottids much longer than wide. Terminal proglottid reaching 2.10 long by 0.15 wide at genital pore. Genital pore with muscular notch located slightly posterior to midproglottid. Ventral and dorsal rows of approximately 50 preovarian testes. Ovaries located at posterior end of proglottid. Vitellaria circumtesticular. Scolex length 1.00 (0.90–1.15) to posterior end of bulbs. Two slightly notched bothridia

→

Figures 1–28. Bars equal 0.05 mm unless otherwise indicated. 1. *Sphyriocephalus pelorosoma* sp. n. Entire worm (bar = 40). 2. Metabasal hooks (bar = 0.5). 3. Basal hooks (bar = 0.5). 4. *Mecistobothrium myliobati* gen. et sp. n. Scolex (bar = 0.5). 5. Hook arrangement at distal end of tentacle. 6. Metabasal hooks. 7. Basal hooks. 8. *Prochristianella fragilis* sp. n. Scolex (bar = 0.5). 9. Metabasal hooks on antibothridial surface. 10. Metabasal hooks on bothridial surface (bar = 0.10). 11. *Prochristianella minima* sp. n. Entire worm (bar = 0.5). Vitellaria illustrated in lateral bands only to express proglottid internal morphology. 12. Basal hooks (bar = 0.025). 13. Metabasal hooks (bar = 0.025). 14. *Eutetrarhynchus litocephalus* sp. n. Scolex (bar = 1.0). 15. Basal region bothridial surface. 16. Metabasal region internal surface. 17. *Eutetrarhynchus macrotrachelus* sp. n. Scolex and bulb region (bar = 0.4). 18. Metabasal region bothridial surface. 19. Basal region bothridial surface. 20. *Eutetrarhynchus macrotrachelus* sp. n. Metabasal region internal surface. 21. Basal region external surface. 22. *Eutetrarhynchus schmidti* sp. n. Scolex and bulb region (bar = 0.8). 23. Metabasal region internal surface (bar = 0.1). 24. Basal region internal surface (bar = 0.1). 25. Metabasal region bothridial surface (bar = 0.1). 26. Basal region external surface (bar = 0.1). 27. *Nybelinia anthicosum* sp. n. Anterior of worm (bar = 1.0). 28. Hooks of the basal region (bar = 0.25).





measuring 0.16 (0.15–0.18) long by 0.22 (0.19–0.25) wide. *Pars vaginalis* 0.64 (0.54–0.77) long; spiral tentacle sheath. Bulbs 0.37 (0.36–0.38) long by 0.06 (0.06–0.07) wide. Tentacles 0.02 wide at basal swelling; 0.01–0.02 wide at metabasal portion. Armature heteroacanthous with longest hooks on external and internal face of tentacle. Hook rows begin on bothridial face and terminate on antibothridial face.

TYPE HOST: Shovelnose guitarfish, *Rhinobatos productus* (Ayres).

LOCATION: Spiral valve.

LOCALITY: Mission Bay, San Diego, California.

HOLOTYPE: USNM Helm. Coll No. 72676.

REMARKS: The metabasal armature (Figs. 9, 10) of *P. fragilis* sp. n. resembles that of *P. trygonicola* Dollfus, 1946, with the largest hooks appearing in the middle of obliquely ascending half-turn spiral rows. Unlike *P. trygonicola* in which the fifth and sixth metabasal hooks are largest, the third and fourth are largest in this species. It also differs in having a scolex (to the posterior tip of *pars bulbosa*) approximately one-half the size of *P. trygonicola*. Metabasal armature distinguishes *P. fragilis* sp. n. from *P. tenuispine* Linton, 1890, and *P. penaei* Kruse, 1959. The metabasal armature of *P. penaei* is composed of a single longitudinal row of large stout hooks on the internal surface of each tentacle and a series of obliquely ascending, half-turn spiral rows of smaller hooks that start on the internal surface. The metabasal armature of *P. tenuispine* is homeomorphic. The specific name *fragilis* comes from the Latin and refers to the difficulty in keeping specimens intact.

Prochristianella minima sp. n. (Figs. 11–13)

DESCRIPTION (measurements from 19 specimens): Maximum length 3.6, consisting of two to five proglottids. Maximum terminal proglottid length 2.00; width at genital pore 0.25. Genital pores slightly posterior to midproglottid. Testes large, preovarian; numbering 18–25. Ovary bilobed, tapering posteriad. Vitellaria circumtesticular, arranged in small pockets. Strobila acraspedote; neck 0.05 (0.02–0.10). Scolex length 0.59 (0.53–0.68). *Pars vaginalis* length 0.32 (0.27–0.41); tentacle sheath lacking small red organ at posterior end; sheath sinuous. *Pars bulbosa* length 0.25 (0.18–0.35); width 0.11 (0.09–0.17); individual bulb width 0.04 (0.03–0.05); bothridia length 0.17 (0.12–0.21); width 0.13; two bothridia notched at posterior margin. Tentacles 0.012 (0.010–0.016) armed with hook rows ascending obliquely from middle line of antibothridial face; armature arranged in alternating half-circles of nine hooks terminating on bothridial face. Basal hooks sparse, becoming numerous by row 15, then decreasing to row 20 to form characteristic half-turn rows. Metabasal armature similar to row 20.

TYPE HOST: Round stingray, *Urolophus halleri* Cooper; other hosts: Thornback, *Platyrhinoidis triseriata* (Jordan and Gilbert).

LOCATION: Spiral valve.

LOCALITY: Anaheim Bay, Alamitos Bay, and Seal Beach, California.

HOLOTYPE: USNM Helm. Coll. No. 72677.

PARATYPE: USNM Helm. Coll. No. 76278.

REMARKS: The metabasal armature (Fig. 13) of *P. minima* sp. n. is arranged similar to *P. trygonicola* and *P. fragilis* sp. n. but differs in size and shape. Also, the scolex of *P. minia* sp. n. (0.59) is considerably less than *P. trygonicola* (1.93) and *P. fragilis* sp. n. (1.00). The specific name *minima* comes from the Latin and reflects the worm's small size.

Eutetrarhynchus litocephalus sp. n. (Figs. 14–16)

DESCRIPTION (Measurements from seven specimens): Maximum total length 32.97; 17–50 proglottids. Maximum size of terminal proglottid 2.21 long by 0.47 wide. Genital pore in central proglottid; testes large and rectangular extending posterior to midovary. Ovary bilobed, tapering at anterior end. Scolex covered with deciduous hairs which extend posterior to bulbs. Scolex length to posterior tip of bulb 5.19 (4.42–6.17). Two bothridia 0.36 (0.31–0.39) long by 0.29 (0.27–0.30) wide. *Pars vaginalis* 2.26 (1.82–2.77) long; tentacles long; sheaths spiral. *Pars bulbosa* 2.88 (2.40–3.40) long; individual bulb width 0.12 (0.11–0.13); small organ of unknown nature at anterior end of bulb. *Pars post bulbosa* 6.95 (4.13–10.00) long. Armature heteroacanthous. Hooks arranged in spiral half-turns; each row

starting obliquely from middle line of external face.

TYPE HOST: Gray smoothhound, *Mustelus californicus* Gill; other hosts: Leopard shark, *Triakis semifasciata* Girard.

LOCATION: Spiral valve.

LOCALITY: Mission Bay, San Diego, and Bahia de San Quintin, B. C., Mexico.

HOLOTYPE: USNM Helm. Coll. No. 72671; Paratype No. 72672.

REMARKS: The armature (Figs. 15, 16) and size of this species resembles *E. lineatus* Linton, 1909. However, *E. litocephalus* can be distinguished by (1) the *pars post bulbosa* is 6.95 compared to less than 0.5 for *E. lineatus*; (2) the *pars bulbosa* of this species is longer than the *pars vaginalis*, whereas the opposite is true for *E. lineatus;* (3) no distinct line of demarcation between *pars post bulbosa* and *pars bulbosa* in *E. litocephalus* (Fig. 14); (4) more uniformity in hook size.

The specific name *litocephalus* is derived from the Greek meaning simple or unadorned head.

Eutetrarhynchus macrotrachelus sp. n. (Figs. 17–21)

DESCRIPTION (measurements from five specimens): Strobila length 75.35 (37.30–120.33); 35 (21–51) proglottids. Terminal proglottid length 2.12; width 0.81. Genital pores alternating irregularly about midproglottid. Testes large, numerous, preovarian. Vitellaria in small packets circumtesticular. Uterus terminating near anterior end of proglottid; ovary bilobed. *Pars post bulbosa* very long 48.65 (23.11–77.33). *Pars vaginalis* length 3.88 (3.28–4.38); tentacle sheath sinuous. *Pars bulbosa* 4.10 (3.93–4.30) long; bulb width 0.16 (0.14–0.17). Small red organ at posterior end of sheath. Two patelliform bothridia, each with free posterior and lateral borders; bothridia lacking posterior notches, 0.43 (0.39–0.48) long by 0.36 wide. External hooks with toe and heel; internal hooks without toe and heel. Tentacles without special basal armature; armature heteroacanthous, forming oblique rows, beginning and ending on bothridial and antibothridial faces.

TYPE HOST: Gray smoothhound, *Mustelus californicus* Gill.

LOCATION: Spiral valve.

LOCALITY: Mission Bay, San Diego, California.

HOLOTYPE: USNM Helm. Coll. No. 72666; Paratype No. 72667.

REMARKS: The major feature that distinguishes *E. macrotrachelus* sp. n. from other members of the genus is the long *pars post bulbosa*. The tentacle armature (Figs. 18–21) most closely resembles *E. ruficollis* Pintner, 1913, in size, shape, and number, although the arrangement differs slightly. The hook rows begin and end on bothridial and antibothridial faces, unlike other species which begin and end on external and internal faces.

The specific name *Macrotrachelus* is derived from the Greek, meaning long-necked.

Eutetrarhynchus schmidti sp. n. (Figs. 22–26)

DESCRIPTION (measurements from 28 specimens): Maximum total length 8.96; mature worm with six to 10 proglottids; strobila apolytic; terminal proglottid length 2.36 (1.76–3.49); width at genital pore 0.24 (0.20–0.28); genital pore located in posterior half of proglottid; eggs with polar filament. Testes 56–70. Vitellaria circumtesticular. Ovary lobes elongate, extending forward to midway between cirrus and posterior end of proglottid. Scolex length to posterior end of bulbs 1.49 (1.32–1.64). *Pars vaginalis* 0.77 long (0.54–0.95); *pars bulbosa* length 0.70 (0.50–0.81); width at mid *pars bulbosa* 0.21 (0.16–0.26); individual bulb width 0.08 (0.07–0.10); prebulbous organ present. Bothridia measuring 0.24 long (0.17–0.27) by 0.22 wide (0.17–0.24); posterior margins free. Tentacle width 0.11; armed with heteroacanthous and homeomorphous hook arrangement. Hooks similar in size.

TYPE HOST: Round stingray, *Urolophus halleri* Cooper. Other hosts: Shovelnose guitarfish, *Rhinobatos productus* (Ayres).

LOCATION: Spiral valve.

LOCALITY: Anaheim Bay and Seal Beach, California.

HOLOTYPE: USNM Helm. Coll. No. 72668; Paratype No. 72669.

REMARKS: This species (Fig. 22) can be distinguished from all species except *E. carayoni* by its small size. It can be separated from *E. carayoni* by armature differences (*E.*

schmidti hook rows overlap) and lack of unicellular glands in the *pars vaginalis* region.

E. schmidti sp. n. is a larval parasite of ghost shrimp (*Callianassa g. gigas* Dana, 1852). Young (1954) reported *Christianella trygonisbucconis* Wagener, 1854, in a species of *Callianassa*. In this study more than 100 ghost shrimp were examined and no *C. trygonisbucconis* were revealed. However, *E. schmidti* sp. n. was found in 26% of those ghost shrimp examined. No measurements or drawings of the *Christianella* larva or adults were given by Young, thereby making it difficult to compare the worms. This species is named in honor of Dr. Gerald D. Schmidt, University of Northern Colorado, for his many contributions in the systematics of parasitic helminths.

Family Tentaculariidae
Nybelinia anthicosum sp. n.
(Figs. 27–28)

DESCRIPTION (measurements on six specimens): Strobila acraspedote; maximum total length 68.2. Proglottids wider than long, numbering to 300. Terminal end of worm tapering to a point becoming rounded. Proglottids slightly anterior to terminal end measuring 0.44 long by 1.20 wide. Testes numerous, prepostovarian. Ovary occupying nearly half proglottid width. Genital pores alternating irregularly in anterior half of proglottid. Vitellaria follicular, circumtesticular. Scolex craspedote; length to posterior end of velum 2.49 (1.86–3.00). Width at mid *pars bulbosa* 0.64 (0.54–0.74). Velum length 1.01 (0.69–1.43). Two pair of bothridia, crenulate, measuring 1.00 (0.77–1.13) long by 0.93 (0.88–0.98) wide. *Pars vaginalis* length 0.71 (0.38–0.94). Length *pars bulbosa* 0.52 (0.45–0.63); individual bulb width 0.16 (0.13–0.18); retractor muscles reaching posterior end of bulb. *Pars post bulbosa* lacking. Tentacle width 0.08. Armature homeoacanthous; hooks similar in shape, varying in size with smaller hooks at extreme distal and proximal end of tentacle.

TYPE HOST: Leopard shark, *Triagis semifasciata* Girard. Other host: Horn shark, *Heterodontus francisci* (Girard).

LOCATION: Stomach and spiral valve.

LOCALITY: Seal Beach, California; San Carlos Bay and Playa Maria, B. C., Mexico.

HOLOTYPE: USNM Helm. Coll. No. 72674; Paratype No. 72675.

REMARKS: The hooks of *Nybelinia anthicosum* sp. n. are arranged in continuous spiral rows (Fig. 28) and the armature is similar in size and shape except that basal hooks are smaller than metabasal hooks. This species is most smiliar to *N. edwinlintoni* Dollfus, 1960, differing in that the bothridia of *N. anthicosum* sp. n. are crenulate and the velum is longer. It should be noted that the fold in the velum shown in Figure 27 is an artifact of mounting and not found in most specimens.

The name *anthicosum* is from the Greek meaning flowerlike.

Discussion

Systematic problems exist in the Trypanorhyncha primarily due to the consuming task of becoming familiar with the oncotaxy. Frequently worms with a superficial resemblance belong to different families, while others in a common genus appear to be quite different. Classification, even at the family level, requires spending considerable time deciphering hook arrangements. Within a genus such as *Eutetrarhynchus* (Family Eutetrarhynchidae) the armature is very difficult to interpret due to the numerous hooks in each row and the short distances between rows. The hook rows in this genus are not as distinct as those of other genera and may appear to be continuous as stated by Schmidt (1970) and Yamaguti (1959). Actually the hook rows are arranged in spiral half-turns with only subtle difference in the armature of many species. In such cases the authors found that measurements of region ratios (*pars bothridialis*: *pars vaginalis*: *pars bulbosa*) were particularly useful characters.

The classification in all groups is not as difficult as that of *Eutetrarhynchus*. The family Sphyriocephalidae can be distinguished easily by its scolex thickness in the bothridial region and the enlarged margins of the bothridia. Yamaguti (loc. cit.) and Schmidt (loc. cit.) describe the hooks as being approximately the same size and shape. It should be pointed out that only the metabasal armature has such an arrangement. The basal armature is vastly different from the metabasal arrangement, being smaller and not at all similar in size and shape.

Most of the current workers in this order rely on the classification of Dollfus (1942), who organized and presented logical groupings based mainly on armature. Other characters such as host specificity and geographical distribution are not commonly used but are certainly considered valid by the authors. Sezen and Price (1969) proposed the new genus *Pleronybelinia* (Tentaculariidae) to contain species in which the adult form is unknown and the original description was based on the plerocercoid. Because only one name is valid for a taxon, the genus proposed by Sezen and Price (loc. cit.) would create unnecessary instability, therefore, the authors maintain that *Pleronybelinia* should be suppressed in favor of *Nybelinia*.

Acknowledgments

The authors would like to thank Mr. David Powell, curator of fishes, and his staff at Sea World, San Diego, for their help and cooperation during this study. We also express our deepest appreciation to Lorraine Peterson for her outstanding technical services.

Literature Cited

Dollfus, R. Ph. 1942. Etudes critiques sur les Tetrarhynques du Museum de Paris. Arch. Mus. Hist. Natur. Paris 19: 1–466.

Hart, J. F. 1936. Cestoda from fishes of Puget Sound. II. Tetrarhynchoidea. Trans. Am. Microscop. Soc. 55: 369–387.

Pappas, P. W. 1970. The trypanorhynchid cestodes from Humboldt Bay and Pacific Ocean sharks. J. Parasit. 56: 1034.

Pintner, T. 1930. Tetrarhynchen aus Pacific Grove, California, U.S.A. Anz. Akad. Wissensch. Wien, Mat.-Naturw. Kl. 67: 26–28.

Schmidt, G. D. 1970. How to Know the Tapeworms. Wm. C. Brown Co., Dubuque, Iowa, 266 p.

Sezen, Y., and C. E. Price. 1969. The parasites of Turkish fishes. Part II. Proposal of a new genus to contain the plerocercoids of *Nybelinia* Poche, 1926 (Cestoda: Trypanorhyncha). Riv. Parassit. 30: 35–38. (Abstr.)

Yamaguti, S. 1959. Systema Helminthum. Vol. II. The Cestodes of Vertebrates. Interscience, New York, 860 p.

Young, R. T. 1954. Cestodes of sharks and rays in Southern California. Proc. Helminth. Soc. Wash. 21: 106–112.

Eimeria tenella: Merozoite Production in Cultured Cells and Attempts to Obtain Development of Culture-produced Merozoites

DAVID J. DORAN[1]

ABSTRACT: Primary Leighton tube cultures of chicken kidney cells were inoculated with 0.85×10^5 *Eimeria tenella* sporozoites. Extracellular first-generation merozoites were most prevalent 55 hr after inoculation. The total number per tube found through 82 hr ranged from 1.3×10^5 to 4.7×10^5. Extracellular second-generation merozoites were most prevalent 120 hr after inoculation. The total number per tube found at 95 hr and at various intervals through 156 hr ranged from 12.9×10^5 to 18.2×10^5.

Culture-produced first-generation merozoites harvested 2 and 3 days after sporozoite inoculation and second-generation merozoites harvested 4 and 5 days after sporozoite inoculation were inoculated into primary cultures of chicken kidney cells. Merozoites entered cells, but none were found in which nuclear division had taken place. In addition, the merozoites did not survive well. Less than 1% of the inoculum could be accounted for 24 hr after inoculation.

Hammond, Fayer, and Miner (1969) inoculated bovine tracheal cell cultures with *Eimeria bovis* sporozoites and counted the first-generation merozoites found free in the culture medium at daily intervals for 20 days. The average number per tube in a series inoculated with 2.7×10^5 sporozoites was ap-

[1] Animal Parasitology Institute, Agricultural Research Service, Beltsville, Maryland 20705.

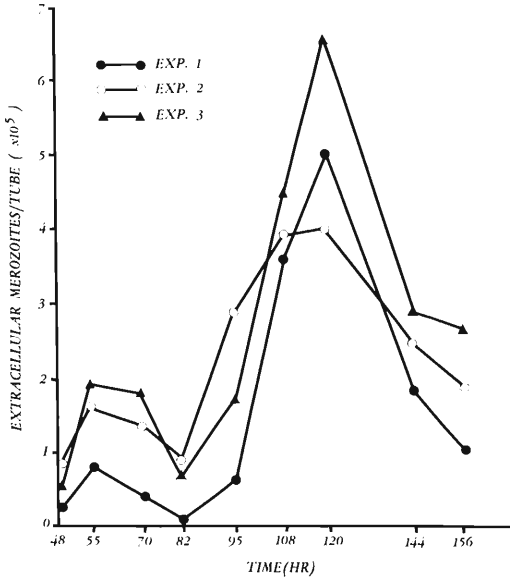


Figure 1. Numbers of extracellular merozoites 48 to 156 hr after inoculation.

proximately 150 × 10^5; the average was 20 × 10^5 in a series inoculated with 3.9 × 10^5 sporozoites. This appears to be the only report concerning the quantity of merozoites produced by a species of *Eimeria* in cell culture.

Although there are several reports (Doran, 1973) concerning the development of an eimerian after merozoites obtained from the natural host were introduced into cell culture, there are only two concerning development after merozoites produced in culture were placed into other cultures. Hammond et al. (1969) placed first-generation *E. bovis* merozoites into a variety of cultured cell types and found that merozoites either did not enter cells or entered and did not develop. Bedrnik (1969) inoculated cell cultures with *E. tenella* second-generation merozoites only and with second-generation merozoites plus trypsinized cells. With merozoites only, there was cell penetration, but no development; with merozoites plus cells, either mature third-generation merozoites or oocysts developed. In work on development of merozoites obtained from the chicken, Bedrnik (1969) found better development in cell culture with merozoites obtained on the 6th day after oocysts were fed to the host than on the 5th day.

This report concerns the production of first- and second-generation *E. tenella* merozoites in primary cultures of chicken kidney cells and attempts to obtain development in cell culture by using merozoites of the first and second generations harvested at different times after sporozoite inoculation.

Materials and Methods

SPOROZOITES: Excysted sporozoites, separated from debris (oocyst and sporocyst walls) by the method of Wagenbach (1969) as modified by Doran (1970a) and frozen and stored as previously described (Doran, 1969), were used. They were 6 weeks old when excysted and frozen and were stored 5–6 months before use.

CELL CULTURES: Kidneys were obtained from 2- to 3-week-old chicks and processed as previously described (Doran, 1971). In Exps. 1–3, 15 Leighton tube cultures (11- by 40-mm window; 10.5- by 35-mm cover slip) were prepared; in Exps. 4–6, 60 Leighton tube cultures with cover slips and five 4-oz prescription bottle cultures were prepared.

MEDIA: The medium for growing cells before inoculation was 80% Hanks' balanced salt solution (HBSS), 10% lactalbumin hydrolysate (LAH, 2.5% solution in HBSS), and 10% fetal calf serum. The media for inoculating sporozoites into culture and maintaining cultures after inoculation were the same as those used before inoculation, but with 5% LAH and 5% serum. Growth and maintenance media contained dihydrostreptomycin (100 μg/ml) and penicillin (100 units/ml). All media contained phenol red indicator (100 μg/ml) and were adjusted to pH 7.0–7.2 before use.

INOCULATION AND MAINTENANCE OF CELL CULTURES: Sporozoite suspensions were quickly thawed and placed in enough inoculation medium to dilute the dimethyl sulfoxide (freezing protectant) concentration to less than 1%. The inoculum was adjusted so that 1 ml contained 0.85 × 10^5 sporozoites. In Exps. 1–3, 1 ml was pipetted into each Leighton tube; in Exps. 4–6, 7 ml was placed in the bottle cultures. Three hr after inoculation, an additional 9 ml of medium was added to each tube and 23 ml was added to each bottle.

Merozoites were harvested from bottle cultures on days 2, 3, 4, and 5 after sporozoite

inoculation. Decanted medium was pooled and replaced with 30 ml of fresh medium. The number of merozoites/ml in the pooled medium was determined. After pH was readjusted to 7.0–7.2, 1 ml was pipetted into each of 15 Leighton tube cultures. Cultures were kept at 41.5 C.

COUNTS: In Exps. 1–3, cover slips were removed from three cultures 4 and 48 hr after inoculation. At intervals shown in Fig. 1, the medium was decanted from each of the other nine tubes and replaced with 10 ml of fresh medium. The volume of pooled, decanted medium was recorded, counts were made, and the number of extracellular merozoites per tube was determined.

In Exps. 4–6, cover slips were romoved from 3–5 Leighton tubes 2, 5, and 24 hr after inoculation. At each interval, the medium was pooled and the number of extracellular merozoites/tube was determined.

Cells on cover slips removed from tubes were fixed in 10% neutral buffered formalin and stained as previously described (Doran and Vetterling, 1967). Counts were made of the parasites in 240 microscopic fields. The location of these fields was the same as that previously reported (Doran, 1971).

Results

MEROZOITE PRODUCTION: The numbers of sporozoites and various developmental stages of the first generation found within cells fixed 4 and 48 hr after inoculation are shown in Table 1. Many of the merozoites free of schizonts were already rounded and showed evidence of starting the second generation.

Table 1. Numbers of intracellular sporozoites and developmental stages 4 and 48 hr after inoculation.

Time (hr)	Parasite	Exp. 1	Exp. 2	Exp. 3
4	Sporozoite	780(14)	810(11)	825(19)
48	Sporozoite	104(11)	74(15)	89(10)
	Trophozoite and immature schizont	284(17)	286(12)	310(14)
	Mature schizont	88(14)	40(10)	66(9)
	Merozoite free of schizont	214(19)	178(22)	195(21)

Numbers in parentheses represent percentage of deviation (average) between cover slips.

The numbers of merozoites found free in the maintenance medium from 48 to 156 hr after inoculation are shown in Figure 1. Extracellular merozoites of the first generation were most prevalent at 55 hr. The total number per tube found up to and including 82 hr ranged from 1.3×10^5 (Exp. 1) to 4.7×10^5 (Exp. 2). Merozoites of the second generation were most prevalent at 120 hr. The total number per tube found at 95 hr and thereafter ranged from 13.9×10^5 (Exp. 1) to 18.2×10^5 (Exp. 3).

DEVELOPMENT OF MEROZOITES: First-generation merozoites harvested 2 and 3 days after sporozoite inoculation and second-generation merozoites harvested 4 and 5 days after sporozoite inoculation entered cells in all three experiments (Table 2). Intracellular first-generation merozoites observed at 2, 5, and 24 hr

Table 2. Results of inoculating cultures with merozoites obtained from other cultures at different times after inoculation with sporozoites.

Time after sporozoite inoculation (days)	Experiment number	Size of merozoite inoculum ($\times 10^3$)	2 hr			5 hr			24 hr		
			A	B	C	A	B	C	A	B	C
2	4	250	65	9	26	11	9	4	2	0	<1
	5	195	42	19	21	19	5	10	1	0	<1
	6	95	12	27	14	4	10	4	0	0	0
3	4	470	50	13	10	19	10	4	0	5	<1
	5	210	19	5	9	5	7	3	0	2	<1
	6	175	21	11	12	11	5	6	2	3	1
4	4	195	7	9	3	10	3	5	1	0	<1
	5	210	14	19	7	12	17	6	0	2	<1
	6	175	19	27	11	9	21	6	0	5	<1
5	4	175	27	11	15	19	2	10	0	5	<1
	5	257	42	21	16	27	11	10	0	13	<1
	6	195	27	19	14	5	19	3	0	12	<1

A = Number of merozoites/tube remaining in medium ($\times 10^3$).
B = Number of intracellular merozoites counted (avg of 3–5 cover slips).
C = Percentage of Survival.

were all elongate. Most second-generation merozoites were elongate, but a few at 2 and 5 hr in cultures inoculated with merozoites harvested 5 days after sporozoite inoculation were rounded and contained an enlarged nucleus. However, none were found in which nuclear division had taken place. In addition to failing to undergo division, merozoites did not survive well in culture. At 2 hr, 25% or less of the merozoites were accounted for; at 24 hr, 1% or less. At 48 hr, no intracellular or extracellular merozoites could be found.

Discussion

The yield of free merozoites of both generations was extremely low. For every sporozoite inoculated into culture, only 1.5 (Exp. 1) to 5.5 (Exp. 2) first-generation merozoites and 13.9 (Exp. 1) to 21.4 (Exp. 3) second-generation merozoites were found free in the medium. Doran (1970b) found that first-generation schizonts of *E. tenella* in cell culture contained 200–250 merozoites. Based on the 200 figure, there was a potential for 170×10^5 free first-generation merozoites in the present study. However, the largest number obtained was only 4.7×10^5 (Exp. 2). The low yield may be attributed to four factors. (1) Many of the sporozoites in the inoculum did not enter cells. In Exp. 2 at 4 hr, 810 sporozoites were found intracellular. This quantity multiplied by 25.9 (240 microscopic fields is 17 mm^2, 3.86% of the culture tube window) yields 0.21×10^5 as the probable number of intracellular sporozoites in the tube. This is only 24.7% of the inoculum. (2) Many of the sporozoites and the developmental stages died between 4 and 48 hr. Disregarding merozoites free of schizonts, in Exp. 2 there was a loss of nearly 50% of the intracellular parasites. (3) Some merozoites either remained intracellular or reentered cells to continue the life cycle. In Exp. 2 at 48 hr, there were probably only about 0.05×10^5 of these per tube (178×25.9). (4) Many extracellular merozites, and perhaps intracellular developmental stages, died after 48 hr. In Exp. 2, there was a potential of 2.0×10^5 merozoites from the mature first-generation schizonts ($40 \times 200 \times 25.9$). Assuming that all sporozoites, trophozoites, and immature schizonts developed, there would be the potential for 18.6×10^5 more merozoites. Some merozoites from schizonts produced after 48 hr undoubtedly also remained intracellular or reentered cells, but the number that did so most likely would not account for the difference between 20.7 ($18.6 + 2.0 + 0.05$) $\times 10^5$ and 4.7×10^5.

In his work on development of merozoites obtained from the host, Bedrnik (1969) found that there was better development with merozoites harvested on the 6th than on the 5th day after inoculation. Although there was no development of either first- or second-generation merozoites in the present study, there was some evidence that merozoites harvested on the 5th day after sporozoite inoculation were better than those harvested on the 4th day. Some of the fifth-day merozoites were rounded and contained an enlarged nucleus. The reason for nondevelopment of culture-produced merozoites introduced into fresh cultures is obscure. Certainly, because *E. tenella* will complete its cycle in culture, culture-produced merozoites are able to develop without an activating factor or some chemical stimulus carried over from the host. Perhaps development in cell culture takes place only if merozoites leaving a schizont remain intracellular or reinvade cells immediately after leaving a cell in which they develop. As indicated by the results of Exps. 1–3, merozoites die quite readily in tissue culture medium. Bedrnik (1969) also found this to be true. Those that enter cells may be so harmed that they cannot develop. Bedrnik (1969) reported finding multinucleate schizonts 2 hr after cultures were inoculated with suspensions containing culture-produced merozoites and trypsinized cells. This appears rather rapid for multinucleate schizonts to have developed from free merozites. They probably developed from trophozoites or binucleate schizonts within the trypsinized cells.

Literature Cited

Bedrnik, P. 1969. Cultivation of *Eimeria tenella* in tissue cultures. 1. Further development of second generation merozoites in tissue culture. Acta Protozool., Warszawa 7: 87–98.

Doran, D. J. 1969. Freezing excysted coccidial sporozoites. J. Parasitol. 55: 1229–1233.

———. 1970a. Survival and development of *Eimeria adenoeides* in cell cultures inoculated with sporozoites from cleaned and un-

cleaned suspensions. Proc. Helm. Soc. Wash. 37: 45–82.

———. 1970b. *Eimeria tenella*: From sporozoites to oocysts in cell culture. Proc. Helm. Soc. Wash. 37: 84–92.

———. 1971. Increasing the yield of *Eimeria tenella* oocysts in cell culture. J. Parasitol. 57: 891–900.

———. 1973. Cultivation of coccidia in avian embryos and cell culture. *In* D. M. Hammond and P. L. Long, The Coccidia, *Eimeria*, *Isospora*, *Toxoplasma*, and Related Genera. University Park Press, Baltimore and London.

———, **and J. M. Vetterling.** 1967. Cultivation of the turkey coccidium, *Eimeria meleagrimitis* Tyzzer, 1929, in mammalian kidney cell cultures. Proc. Helm. Soc. Wash. 34: 59–65.

Hammond, D. M., R. Fayer, and M. L. Miner. 1969. Further studies on *in vitro* development of *Eimeria bovis* and attempts to obtain second generation schizonts. J. Protozool. 16: 298–302.

Wagenbach, G. E. 1969. Purification of *Eimeria tenella* sporozoites with glass bead columns. J. Parasitol. 55: 833–838.

Scanning Electron Microscopy of the Mosquito Parasite, *Reesimermis nielseni* (Nematoda: Mermithidae)[1]

W. R. Nickle[2] and Ch. H. Höegger[3]

ABSTRACT: The external anatomy of parts of the male and infective stage of the nematode parasite of mosquitoes, *Reesimermis nielseni*, is described using the scanning electron microscope. Noteworthy findings include: a fine annulation of the cuticle, the large earlike amphid openings in the male, three volcanolike pits probably containing the nerve endings in each cephalic papilla, bifurcation and structure of caudal papillae, the presence of two spicules in the male, and a tooth and a large amphid opening in the preparasitic or infective-stage juvenile.

Reesimermis nielseni Tsai and Grundmann is an important mermithid nematode parasite which causes the death of over 30 species of mosquitoes (Petersen and Willis, 1971). Field tests are currently under way in many parts of the world utilizing this parasite as a biological control agent of pest mosquitoes. Tsai and Grundmann (1969) and Nickle (1972) have studied this nematode with the light microscope. The current authors have studied the male and preparasitic infective stage of this nematode using the scanning electron microscope (SEM). A review of the literature shows that no mermithids have been viewed using SEM.

[1] The authors express their sincere appreciation to W. J. Humphries and Ben Spurlock (University of Georgia Electron Microscope Laboratory) for their suggestions, and the use of equipment and facilities.

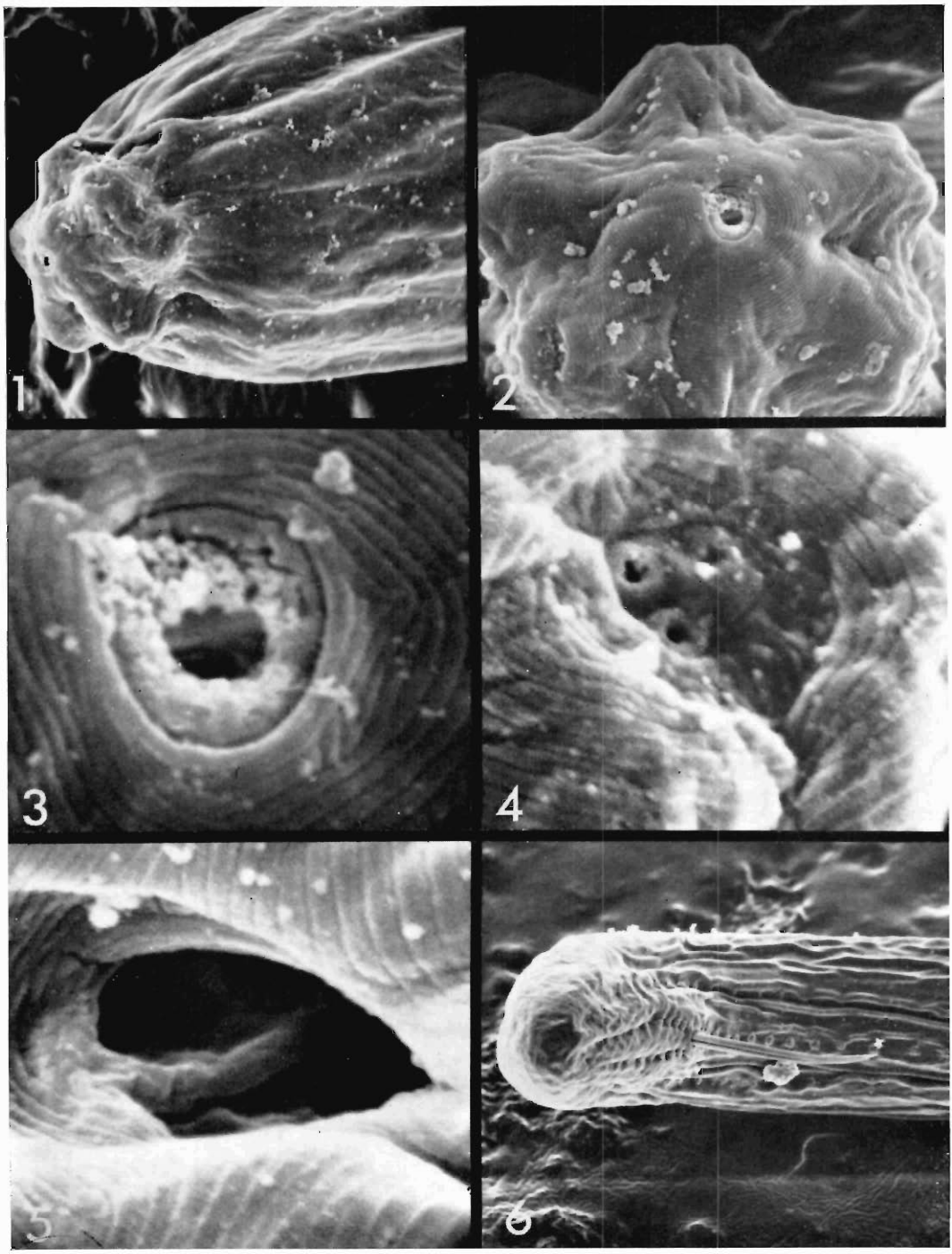
[2] Nematologist, Nematology Laboratory, Plant Protection Institute, USDA, ARS, Beltsville, Maryland 20705.

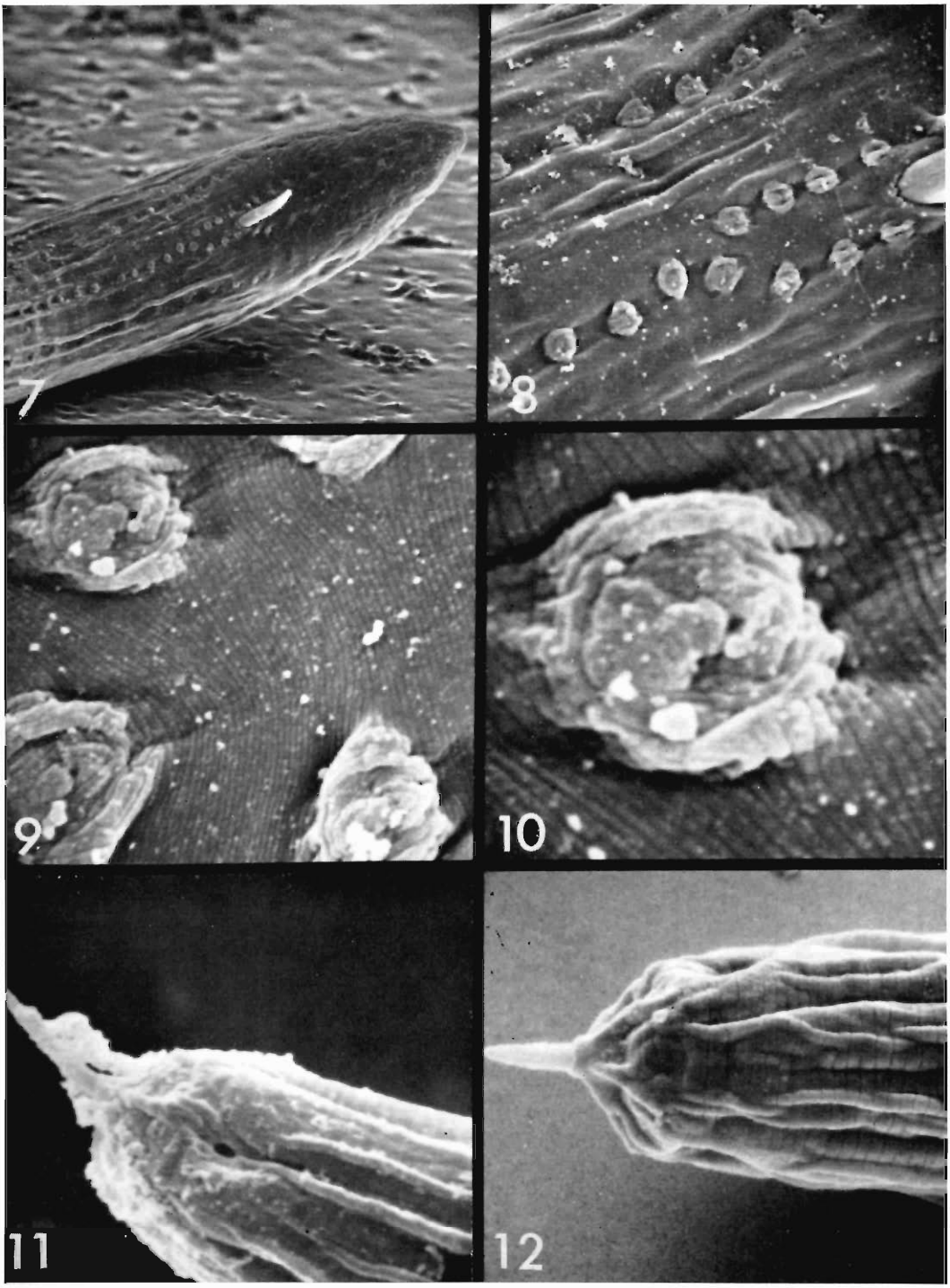
[3] Department of Plant Pathology and Plant Genetics, University of Georgia, Athens, Georgia 30602.

→

Figures 1–6. 1. Anterior end of male *Reesimermis nielseni* (× 1,650). 2. Face view showing oral opening, cuticular pattern, six lips and cephalic papillae (× 3,900). 3. Close-up of the symmetrically circular oral opening of the male (× 18,400). 4. Cephalic papilla in male with three volcanolike pits, probably containing the nerve endings (× 18,000). 5. Large earlike amphid opening in male (× 1,800). 6. Male tail showing two spicules (× 450).

Figures 7–12. 7. Male tail showing bifurcation of caudal papillae (× 430). 8. Bifurcation of caudal papillae (× 1,800). 9. Caudal papillae (× 9,000). 10. A single caudal papilla (× 18,000). 11. Anterior end of preparasitic infective-stage juvenile showing protruding tooth and amphid opening (× 8,700). 12. Dorsal/ventral view of preparasitic infective-stage juvenile showing tooth (× 8,700). [This specimen was dehydrated in acetone, air-dried, and coated with gold (Stone and Green, 1971)].





Materials and Methods

The nematode specimens were obtained from the bottom sand in an aquarium about 2 weeks after the postparasitic larval mermithids emerged from the host mosquito, *Culex pipiens quinquefasciatus* Say.

The specimens were prepared for scanning electron microscopy by a modified critical point-drying method (Hayatt and Zirkin, 1973; Högger and Bird, 1974). In this procedure biological specimens are dehydrated in ethanol or acetone; the ethanol or acetone respectively is replaced by a transitional fluid (here liquid CO_2), which upon heating to its critical point (31C for CO_2) transforms without interface from the liquid to the gaseous state leaving the specimens dry with little or no distortion. Nematodes were relaxed by gentle heat and were fixed in 2.5% formaldehyde, 1% glycerine. An incision was made in the large males about in the middle of the body to allow better penetration of dehydration fluids. Specimens were washed by shaking them in a 0.5% Kodak Photo Flo® solution for 1 min. Dehydration was initiated by placing the nematodes in 20% ethanol in a dish standing above absolute ethanol in a sealed container at 42 C overnight, similar to Stone and Green's (1971) acetone technique. On the following day absolute ethanol was added slowly to the solution containing the nematodes. Then two changes of absolute ethanol were made. In preliminary preparations *R. nielseni* males collapsed irreversibly during more rapid changes of ethanol concentrations. Also for this reason amyl acetate was omitted, in contrast to earlier investigations (Högger and Bird, 1972). The specimens, immersed in absolute ethanol in a brass well covered with 25-μm and 1-mm screens, were transferred to a pressure chamber which was subsequently filled with liquid CO_2. After a 10-min equilibration period, the chamber was flushed with liquid CO_2 for 10 mins to remove the ethanol. The chamber was heated to 42 C at 180 atm. Then the CO_2 was released slowly and dry specimens were obtained. These were hand-picked and stuck to an SEM specimen carrier. The glue was made by washing the adhesive from adhesive tape with ethyl acetate, similarly to the Stone and Green (1971) procedure. The thin sticky film on the carrier prevented the specimens from being blown off in subsequent operations. The whole adhesive layer of tape is too thick and nematodes become submerged in it. At the end of the dehydration period the juveniles often stuck irretrievably to the bottom of the small Syracuse watch glass in which they had been processed. Therefore, a small round cover glass was used as a false bottom on which the specimens then settled. This allowed the majority of specimens to be transferred at once to the brass well. After drying, the cover glass was glued to an SEM specimen carrier with electrically conductive silver paint. The specimens on the carrier were coated with gold under vacuum and observed with a Mark II Cambridge scanning electron microscope at 30kv.

Acetone permitted faster dehydration. Collapse resulting from too steep a gradient was reversible in a lower concentration. This was not the case when ethanol was used.

Juvenile specimens dehydrated in acetone and then air-dried according to Stone and Green (1971) shrank more than those which were critical point-dried after acetone or ethanol dehydration. Compare Figures 11 and 12.

The main problem in all techniques was foreign matter, probably from the habitat. Dirt may have in part become attached to the specimens during the routine formalin fixation for light microscopy. Therefore, it appears to be advantegeous to wash the specimens before fixation (Högger and Bird, 1974).

Results and Discussion

Most SEM studies concentrated on the male of *R. nielseni* and some pictures were taken of the much smaller preparasitic juvenile. Figures 1 and 2 show the male front end with the six lips, cuticle finely striated on the surface (not crisscross), the symmetrically circular oral opening Figure 3, a large amphid, and the six cephalic papillae. Figure 5 shows a close-up of the large earlike amphid and Figure 4 under high magnification shows three volcanolike pits probably containing the nerve endings in a single cephalic papilla. The original description of *R. nielseni* stated that there was only one spicule; however, Nickle (1972) emended this description to show the

presence of two spicules as shown in Figure 6. The caudal papillae form a double row around the cloacal opening as shown in Figures 7 and 8. Individual caudal papillae are seen in Figures 9 and 10.

Figures 11 and 12 show the anterior end of the preparasitic juvenile infective stage of *R. nielseni*. This stage is very slender and difficult to view under the light microscope. The tooth used to penetrate the mosquito wriggler and the large amphid opening which has not been reported before, are easily seen. The cuticle is superficially finely annulated as in the adult male (Figs. 9, 10).

Literature Cited

Hayatt, M. A., and B. R. Zirkin. 1973. Critical point-drying method. *In* M. A. Hayatt, Principles and Techniques of Electron Microscopy. Vol. 3, Van Nostrand Reinhold Co., New York, p. 297–313.

Högger, Ch. H., and G. W. Bird. 1974. Secondary male sex characteristics of *Hoplolaimus galeatus*. J. Nematology 6: 12–16.

Nickle, W. R. 1972. A contribution to our knowledge of the Mermithidae (Nematoda). J. Nematology 4: 113–146.

Petersen, J. J., and O. R. Willis. 1971. A two-year survey to determine the incidence of a mermithid nematode in mosquitoes in Louisiana. Mosquito News 31: 558–566.

Stone, A. R., and C. D. Green. 1971. A simple method of preparing nematodes for scanning electron microscopy. Nematologica 17: 490–491.

Tsai, Y. H., and A. W. Grundmann. 1969. *Reesimermis nielseni* gen. et sp. n. (Nematoda: Mermithidae) parasitizing mosquitoes in Wyoming. Proc. Helm. Soc. Wash. 36: 61–67.

Some Digenetic Trematodes of Birds and a Mammal from Venezuela[1]

JACOB H. FISCHTHAL AND PIR NASIR[2]

ABSTRACT: Ten species of digenetic trematodes from birds and one from a mammal are reported from Venezuela. New species from birds are: *Pholeter anterouterus* (Opisthorchiidae) from *Phalacrocorax olivaceus*; *Levinseniella* (*Levinseniella*) *venezuelensis* (Microphallidae) from *Ereunetes pusillus*; *Apharyngostrigea papillistomum* (Strigeidae) from *Tringa melanoleuca*. *Neodiplostomum* (*Neodiplostomum*) *obesum* (Lutz, 1928) Dubois, 1938 (Diplostomatidae) from *Phalacrocorax olivaceus* is described in detail and illustrated for the first time from its original host species and geographical locality. Other species from birds are: *Pulchrosoma pulchrosoma* Travassos, 1916 (Cathaemasiidae) from *Ceryle torquata*; *Cyclocoelum brasilianum* Stossich, 1902 (Cyclocoeliidae) from *Actitis macularia; Concinnum ellipticum* (Travassos, 1941) Travassos, 1944 (Dicrocoeliidae) from *Thraupis sayaca glaucocolpa*; *Caiguiria anterouteria* Nasir and Díaz, 1971 (Heterophyidae) from *Tringa melanoleuca*; *Odhneria odhneri* Travassos, 1921 (Microphallidae) from *Ereunetes pusillus*; *Prosthogonimus cuneatus* (Rudolphi, 1809) Braun, 1901 (Prosthogonimidae) from *Larus atricilla*. The species from a mammal is *Rhopalius coronatus* (Rudolphi, 1819) Stiles and Hassall, 1898 (Rhopaliidae) from *Didelphis marsupialis*.

The trematodes of this paper were collected by the junior author while supported by a grant (DCC/02/67/DB–02) from the Comisión de Desarrollo y Coordinación Científicas, Universidad de Oriente. Host names preceded by an asterisk (*) represent new host records. Specimens have been deposited in the U. S. National Museum Helminthological Collection. All measurements are in microns.

Neodiplostomum (Neodiplostomum) obesum (Lutz, 1928) Dubois, 1938 (Fig. 1)

HOST: *Phalacrocorax olivaceus* (Humboldt) (syn. *Carbo brasiliensis*) (Pelecaniformes: Phalacrocoracidae).

HABITAT: Small intestine.

LOCALITY: Laguna de Los Patos, near Universidad de Oriente.

SPECIMENS DEPOSITED: No. 72783.

DESCRIPTION (based on two adult worms): Diplostomatidae. Body elongate, 955–1,110 long; two segments distinctly delimited from one another; anterior segment cupuliform, larger than posterior one, 525–625 long by 450–495 wide, rounded anteriorly, truncated posterodorsally, spined to tribocytic organ level laterally and to proteolytic gland level on dorsal and ventral surfaces; posterior segment 465–530 by 340–345, inverted cone-shaped, truncated anterodorsally, rounded posteriorly, commencing posterodorsal to anterior segment, unspined; length ratio of anterior to posterior segment 1:0.85–0.89, width ratio 1:0.70–0.76. Oral sucker subterminal ventral, 49–51 by 44–49. Acetabulum muscular, 53–55 by 61–65, lying 305–347 from anterior extremity, latter distances 54–58% of anterior segment length. Sucker length ratio 1:1.04–1.12, width ratio 1:1.33–1.39. Tribocytic organ spined, 105–119 by 165–208, ratio of length to width 1:1.57–1.75, ratio of its length to anterior segment length 1:5.0–5.3, aperture transverse, contiguous with acetabulum, lying 360–400 from anterior extremity, distances 64–66% of anterior segment length. Proteolytic gland 74–96 by 215–250, dumbbell-shaped, lobes large, contiguous with tribocytic organ and anterior–posterior segment junction dorsally. Pharynx longitudinally elongate, 36–42 by 24–38; esophagus 24–55 long; ceca narrow, posterior extent not discernible. Excretory pore just subterminal dorsal.

Testes two, surfaces smooth, contiguous to slightly separated; anterior testis asymmetrical, dextral or sinistral, 103–136 by 150–177; posterior testis somewhat dumbbell-shaped in dorsal view, with lateral lobes extending ventrally, filling most of body width at its level, 117–167 by 252–280; posttesticular space 175–225 long, distances 38–42% of posterior seg-

[1] Contribution from the Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York 13901 (J. H. Fischthal).

[2] Address of Pir Nasir: Departamento de Biologia, Escuela de Ciencias, Universidad de Oriente, Apartado postal 245, Cumaná, Venezuela.

ment length. Seminal vesicle posttesticular, on same side of body as anterior testis, large, somewhat coiled. Ejaculatory duct passing through part of male papilla. Latter 83–88 by 106–110, projecting into hermaphroditic duct. Bursal pore large, dorsal, 46–53 from posterior extremity. Genital bursa not delimited from remainder of body.

Ovary smooth, 73–88 by 134–157, median to submedian, lying just posterior to anterior–posterior segment junction, anteromedian to and contiguous with anterior testis. Vitellaria commencing 177–245 from anterior extremity (distances 33–39% of anterior segment length) and 97–126 preacetabular, extending to near posterior extremity, follicles filling much of anterior segment at its level, within outermost parts of tribocytic organ, lying ventral and lateral in posterior segment. Uterus short, descending ventral to posterior testis and genital cone on side opposite seminal vesicle, entering hermaphroditic duct between male papilla and ventral wall of duct. One worm without eggs, other with two collapsed eggs measuring 72–73 by 46–54.

DISCUSSION: The original description, without illustration, of this species by Lutz (1928) as *Conchogaster obesus* from *Carbo* (*Phalacrocorax*) *brasiliensis* from Venezuela was most inadequate. Dubois (1953) listed it as a species delineata and later (1970) only mentioned it with a question mark when indicating that *Conchogaster* Lutz, 1928, was a synonym of *Neodiplostomum* Railliet, 1919; Yamaguti (1971) noted it as a species inquirenda. Our supplemental description of this species from specimens from the same host species and country adds much detail. *N.* (*N.*) *obesum* is closest to *N.* (*N.*) *biovatum* Dubois, 1937, from a falconiform (Falconidae) bird from Brazil. The latter differs in body shape, and in having the anterior segment delimited from the posterior one by a very feeble construction, the oral sucker the same size as the pharynx, a circular acetabulum, and the tribocytic organ circular and separated from the acetabulum.

Pholeter anterouterus sp. n.
(Figs. 2, 3)

HOST: *Phalacrocorax olivaceus.*

HABITAT: Small intestine.

LOCALITY: Laguna de Los Patos.

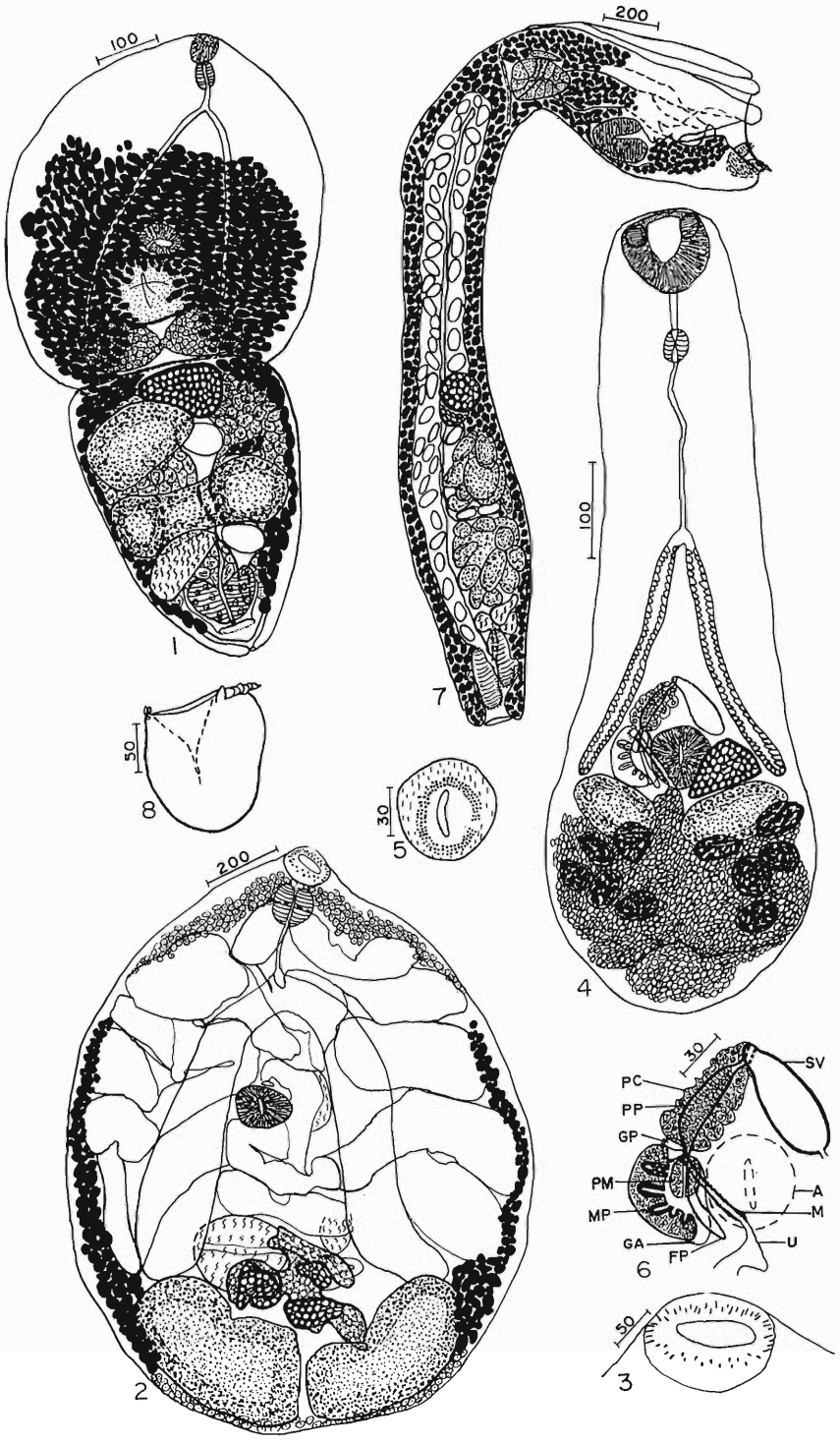
SPECIMAN DEPOSITED: No. 72785 (holotype).

DESCRIPTION (based on one adult worm): Opisthorchiidae. Body ovoid, entirely spined, 1,500 long by 1,170 wide. Forebody 620 long, gland cells laterally and dorsal to pharynx in form of inverted U; hindbody 755 long, gland cells at posterior extremity, commencing at ends of vitelline fields, overlapping testes posteriorly; forebody–hindbody length ratio 1:1.2. Oral sucker subterminal, ventral, 110 by 138; aperture transverse. About 50 circumoral spines in two alternating, uninterrupted rows; ventral spines 10–11 by 4–5; lateral oral ones 11–14 by 3–5; adoral 16–18 by 4; dorsal oral ones 8–10 by 3; adoral 8–9 by 4–5. Acetabulum 125 by 153, lip mostly insunk in parenchyma. Sucker length ratio 1:1.11. Prepharynx short; pharynx diameter 120; esophagus 120 long; cecal bifurcation preacetabular; ceca wide, extending to testes. Excretory pore subterminal dorsal.

Testes two, notched near middle of anterior margin, symmetrical at posterior end of body, nearly contiguous; right testis 265 by 450, left testis 235 by 450. Seminal vesicle large, commencing sinistrodorsal to anterior part of ovary, winding, sinistral to acetabulum. Genital pore median, just preacetabular.

Ovary median, pretesticular but overlapping level of latter, composed of three much convoluted, tubular lobes coming from central area, overall dimensions 222 by 380, lying 210 postacetabular. Seminal receptacle in front of right testis, overlapping right cecum and ovary, 220 by 245. Vitellaria in lateral fields from short distance posterior to cecal bifurcation to middle of testes, follicles intruding slightly into intercecal space dorsally only from acetabular level posteriorly. Uterus voluminous, much coiled between ovary and testes posteriorly and pharynx anteriorly, extending to lateral margins of body. Eggs numerous, brown, operculate, 10 measuring 15–20 (17.2) by 10–11 (10.7).

DISCUSSION: The genus contains only a single species, *P. gastrophilus* (Kossack, 1911) Odhner, 1914, from a cetacean (Phocaenidae) mammal from Europe. This species differs from ours in possessing only 18 circumoral spines in a single row, the testes lying a distance from the posterior extremity, and the



uterus extending anteriorly only to the cecal bifurcation level. Courtney and Forrester (1974) reported, without description or illustration, *Pholeter* sp. as a probable new species from a pelicaniform (Pelicanidae) bird from Florida.

Levinseniella (Levinseniella) venezuelensis sp. n. (Figs. 4–6)

HOST: *Ereunetes pusillus* (L.) (*Charadriiformes*: Scolopacidae).

HABITAT: Small intestine.

LOCALITY: Laguna de Los Patos.

SPECIMENS DEPOSITED: No. 72786 (holotype); No. 72787 (paratype).

DESCRIPTION (based on three adult worms, two measured): Microphallidae. Body elongate, widest posteriorly, 836–890 long, truncated anteriorly, rounded posteriorly, spined to posttesticular region; spines becoming sparser and smaller posteriorly, completely embedded in tegument from short distance postacetabular posteriorly. Forebody 545–590 long by 177–187 wide at cecal bifurcation level; hindbody 227–247 by 262–283 at posttesticular level; forebody–hindbody length ratio 1:0.4. Oral sucker subterminal ventral, broadly truncated and weakly muscular anteriorly, with pair of ventrolateral auricles, 71–82 by 85–90. Postoral circular muscle ring thick. Acetabulum 63–64 by 56–61, aperture longitudinally elongate, partly spined, with thin, short spines from outer margin inward (continuous with those of ventral body surface) but with prominent ring of coarse, blunt spines central to others, more or less interrupted posterodextrally; ring usually with three rows of spines in its width, occasionally with only two. Sucker length ratio 1:0.78–0.89, width ratio 1:0.62–0.72. Prepharynx narrow, 37–56 long; pharynx 32–38 by 37–38; esophagus narrow, 172–205 long; cecal bifurcation 192–213 preacetabular; ceca narrow, in form of inverted V, 270–285 long, extending posteriorly to anterolateral sides of testes; cecal bifurcation and ceca entirely cell-lined in one worm, devoid of cell lining at bifurcation and beginning of ceca in two worms.

Testes two, smooth, symmetrical, widely separated, overlapping level of posteriormost part of acetabulum; right testis 53–68 by 90–119, left testis 57–65 by 85–93. Seminal vesicle elongate saccular, thick-walled (up to 4), muscular, 87–88 by 35–43, commencing anterodextral to acetabulum at anterior margin of ovary, extending anteromedianly, short muscular duct connecting to pars prostatica. Latter extending posterosinistrally to side of acetabulum, thick-walled (up to 3), muscular, 57–69 by 15–16, surrounded by membrane-bound, thick glandular mass, membrane continuing onto seminal vesicle. Ejaculatory duct short, muscular. Male papilla ovoid, robust, 25 by 21 (holotype), projecting into genital atrium from anterior wall of latter. Genital atrium 52 in diameter (holotype), sinistral to acetabulum; sinistral and posterior walls very thick (up to 23–26), glandular, containing six thick-walled male pockets lacking sclerotized structures; one pair of pockets anteriorly, smaller one dorsal and larger ventral, sharing same opening into atrium; other pockets single with anterior two larger than posterior two, anterior two directed dorsolaterally, posterior two dorsoposteriorly. Female pouch simple, thick-walled (up to 6), lacking sclerotized parts, contiguous with posterosinistral margin of acetabulum, opening into genital atrium ventral to male papilla. Genital pore round (holotype), ventral to anteriormost part of sinistral wall of genital atrium and base of male papilla.

Ovary 56–62 by 74–85, wedge-shaped with

←

Neodiplostomum (Neodiplostomum) obesum (Lutz, 1928) Dubois, 1938. Fig. 1. Whole mount, dorsal view. _Pholeter anterouterus_ sp. n. Fig. 2. Whole mount, holotype, ventral view. Fig. 3. Oral sucker with spines, holotype. _Levinseniella (Levinseniella) venezuelensis_ sp. n. Fig. 4. Whole mount, holotype, dorsal view. Fig. 5. Acetabulum with spines, paratype, ventral view. Fig. 6. Terminal genitalia, holotype. _Apharyngostrigea papillistomum_ sp. n. Fig. 7. Whole mount, holotype, sinistrolateral view. Fig. 8. Oral sucker with papillae, holotype. A, acetabulum; FP, female pocket; GA, genital atrium; GP, genital pore; M, metraterm; MP, male pocket; PC, prostate cells; PM, male papilla; PP, pars prostatica; SV, seminal vesicle; U, uterus.

apex between acetabulum and right testis, overlapping both structures. Ootype complex postacetabular, intertesticular. Vitellaria in two lateral fields from testicular level to short distance from posterior extremity, follicles almost totally obscured by eggs so that number not ascertainable. Uterus filling all of posttesticular space, ascending intertesticularly. Metraterm thick-walled, dorsal to posterosinistral part of acetabulum and to genital atrium, opening into latter posterodextral to genital pore. Eggs numerous, yellow-brown, operculate, 15 measuring 15–17 (15.5) by 10–11 (10.5).

DISCUSSION: Our species could not be keyed in the keys given by Deblock (1971). The first step in the key to the subgenus *Levinseniella* Deblock and Pearson, 1970, separates the species on the basis of more or less than six male pockets in the wall of the genital atrium; our species has six. The arrangement of the male pockets differs further from all other members of the subgenus. Also, the ring of acetabular spines appears unique for our species. *L. indica* Lal, 1936, also from a scolopacid bird, from India is closest to our species, differing further in having a much larger pharynx and male papilla, a much longer pars prostatica, and four or five male pockets in the wall of the genital atrium.

Aparyngostrigea papillistomum sp. n. (Figs. 7, 8)

HOST: *Tringa melanoleuca* (Gmelin) (Charadriiformes: Scolopacidae).

HABITAT: Small intestine.

LOCALITY: Laguna de Los Patos.

SPECIMENS DEPOSITED: No. 72790 (holotype); No. 72791 (paratypes).

DESCRIPTION (based on two adult and one immature worms, adults measured): Strigeidae. Body elongate, arched dorsally, anteriorly with large, terminal opening, 2,700–3,285 long. Anterior segment cupuliform, attached somewhat eccentrically to posterior segment, finely spined from anterior extremity to about halfway to acetabulum, 940–1,025 long by 438–505 deep at acetabular level, overlapping posterior segment; latter elongate, with long narrow necklike region preceding gonads, 1,760–2,260 long by 375–440 deep at testicular level; length ratio of anterior to posterior segment 1:1.9–2.2. Oral sucker 162–165 by 90–125, subterminal ventral, muscular, papillate; holotype with nine papillae (15–20 by 10–14) on anterior lip and five (7–9 by 7–10) on posterior lip. Acetabulum muscular, 158–180 by 110–155, lying 370–460 from anterior extremity, distances 39–45% of anterior segment length. Sucker length ratio 1:0.96–1.11, depth ratio 1:1.22–1.24. Tribocytic organ finely spined anteriorly; dorsal lip bilobed; ventral lip grooved and somewhat semicircular. Proteolytic gland longitudinally elongate, multilobed; lobes uniformly granular and staining, 180–205 by 115–136, ratio of anterior segment to gland length 1:0.2, lying at posterior end of anterior segment. Small, elongate, solid mass, 92–123 by 44–61, lying ventral to and contiguous with proteolytic gland. Excretory pore ventral, 20–50 from posterior extremity.

Testes two, tandem, contiguous, multilobed, near posterior extremity. Anterior testis 255–260 by 190–280, posterior testis 265–380 by 230–295; posttesticular space 245–345 long, distances 14–15% of posterior segment length. Seminal vesicle sinuous. Genital cone 136–218 by 145–158, covered with coarse spines 2–5 long. Genital atrium opening to outside by large, wide, terminal genital pore. Copulatory bursa not delimited from remainder of body.

Ovary 133–160 by 120–143, bilobed, pretesticular, in tandem with testes, slightly separated from anterior testis, lying 835–1,070 posterior to anterior–posterior segment junction, distances 47% of posterior segment length. Laurer's canal opening on dorsal surface between ovary and anterior testis. Vitellaria extending from just posterior to oral sucker to posterior end of body, uninterrupted opposite proteolytic gland, follicles entering tribocytic organ, absent ventrally in anterior segment from acetabular level anteriorly, concentrated uniformly throughout posterior segment; vitelline reservoir intertesticular. Uterus undulating slightly, first descending dorsally to intertesticular level, then ascending dorsally to within 92–148 of anterior–posterior segment junction, finally descending ventrally, joining ejaculatory duct to form hermaphroditic duct lying within genital cone. Eggs numbering eight in one worm, 53 in other, operculate, three normally shaped ones measur-

ing 102–108 by 60–65, four slightly collapsed ones 90–100 by 56–58.

DISCUSSION: Our species is closest to *A. multiovata* (Vigueras, 1944) Dubois and Vigueras, 1949, from ciconiiform (Ardeidae) birds from Cuba and Puerto Rico, *A. ramai* (Verma, 1936) Vidyarthi, 1937, from ardeid and pelecaniform (Pelecanidae) birds from India, China, Ghana, Zambia, and Rhodesia, and *A. simplex* (S. J. Johnston, 1904) Szidat, 1929, from ardeid birds from Australia. Those species differ from it in lacking papillae on the oral sucker which in our specimens are large and prominent. *A. multiovata* differs further in being larger, in the anterior segment not being eccentrically attached to the posterior one, and in lacking spines on the genital cone. *A. ramai* differs further in the anterior segment not being eccentrically attached to the posterior one, in lacking a small, solid mass ventral to the proteolytic gland, the latter consisting of a mass posteriorly and lobules anteriorly, and in having the vitellaria interrupted at the proteolytic gland level. *A. simplex* differs further in lacking spines on the anterior end of the body, the vitellaria being interrupted at the proteolytic gland level, and the latter occupying more of the anterior segment length and having its posteriormost lobe more coarsely granular and chromophilic than the other lobes.

Previously Described Species

1. *Pulchrosoma pulchrosoma* Travassos, 1916 (Cathaemasiidae): two adult worms from the abdominal cavity of *Ceryle torquata* (L.) (Coraciiformes: Alcedinidae) collected at Laguna de Los Patos. Specimens deposited: No. 72780.

2. *Cyclocoelum brasilianum* Stossich, 1902 (Cyclocoeliidae): three adult worms from the abdominal cavity of **Actitis macularia* (L.) (Charadriiformes: Scolopacidae) collected at Laguna de Los Patos. Specimens deposited: No. 72781.

3. *Concinnum ellipticum* (Travassos, 1941) Travassos, 1944 (Dicrocoeliidae): one adult worm measuring 6,585 by 2,490 from the gall bladder of *Thraupis sayaca glaucocolpa* Cabaris (Passeriformes: Tanagridae) collected at Cantarrana, Sucre State. Specimen deposited: No. 72782.

4. *Caiguiria anterouteria* Nasir and Díaz, 1971 (Heterophyidae): five adult worms from the small intestine of **Tringa melanoleuca* collected at Laguna de Los Patos. Specimens deposited: No. 72784. Pigment granules of the disintegrated eyespots are scattered anteriorly; the testes and ovary are slightly lobed in one worm; and the vitelline follicles in the more rounded worms may extend to the lateral margin of the testes or slightly beyond, overlapping the later.

5. *Odhneria odhneria* Travassos, 1921 (Microphallidae): one adult worm from the small intestine of *Ereunetes pusillus collected* at Laguna de Los Patos. Specimen deposited: No. 72788.

6. *Prosthogonimus cuneatus* (Rudolphi, 1809) Braun, 1901 (Prosthogonimidae): one adult worm from the oviduct of **Larus atricilla* (L.) (Lariformes: Laridae) collected at Laguna de Los Patos. Specimen deposited: No. 72789.

7. *Rhopalias coronatus* (Rudolphi, 1819) Stiles and Hassall, 1898 (Rhopaliidae): two adult worms from the small intestine of *Didelphis marsupialis* L. (Marsupialia: Didelphiidae) collected at El Tacal, en route to Puerto la Cruz, Sucre State. Specimens deposited: No. 72792.

Literature Cited

Courtney, C. H., and D. J. Forrester. 1974. Helminth parasites of the brown pelican in Florida and Louisiana. Proc. Helm. Soc. Wash. 41: 89–93.

Deblock, S. 1971. Contribution à l'étude des Microphallidae Travassos, 1920. XXIV. Tentative de phylogénie et de taxonomie. Bull. Mus. Natl. Hist. Nat., Paris, 3 sér., Zool. 7: 353–468.

Dubois, G. 1953. Systématique des Strigeidae. Complément de la Monographie. Mém. Soc. Neuchatel. Sci. Nat. 8: 1–141.

———. 1970. Synopsis des Strigeidae et de Diplostomatidae (Trematoda). Fasc. 2: Familia Diplostomatidae Poirier, 1886. Ibidem 10: 259–727.

Lutz, A. 1928. Estudios sobre trematodes observados en Venezuela. *In* Estudios de Zoología y Parasitología Venezolanas. Rio de Janeiro, p. 101–125.

Yamaguti, S. 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vol. I. 1074 p. Keigaku Publ., Tokyo.

The Genus *Caryophyllaeus* Gmelin (Cestoidea: Caryophyllidea) in the Nearctic[1]

JOHN S. MACKIEWICZ
Biological Sciences, State University of New York at Albany, 12222

ABSTRACT: Review of literature records and study of specimens of *Caryophyllaeus terebrans* (Linton, 1893) from collections as well as new material from *Catostomus ardens* in Wyoming and Idaho has led to the following conclusions: there are no authentic records of *Caryophyllaeus* Gmelin in the Nearctic; *C. terebrans* (Linton, 1893) Woodland, 1923, is referable to the genus *Glaridacris* Cooper, 1920; and Hunter's (1930) description of "*C.*" *terebrans* from *Ictiobus bubalus* is based largely on an undescribed genus. *Glaridacris terebrans* comb. n. is redescribed and discussed with respect to the following species: *Hunterella nodulosa* Mackiewicz and McCrae, 1967, *G. catostomi* Cooper, 1920, and *C. laticeps* (Pallas, 1781).

The beginning of caryophyllid systematics in the Nearctic came in 1893 with Linton's description of *Monobothrium terebrans* from a catostomid fish in Yellowstone National Park, Wyoming. Transferred to the genus *Caryophyllaeus* Gmelin by Woodland (1923), Linton's species is the only one in that genus in the Nearctic and Neotropical zoogeographical regions. The presence of *C. terebrans* in North American catostomid fishes presents interesting host specificity and distributional problems because most of the other species of *Caryophyllaeus* are from cyprinid fishes in the Palearctic. Resolution of these problems forms the subject of this paper.

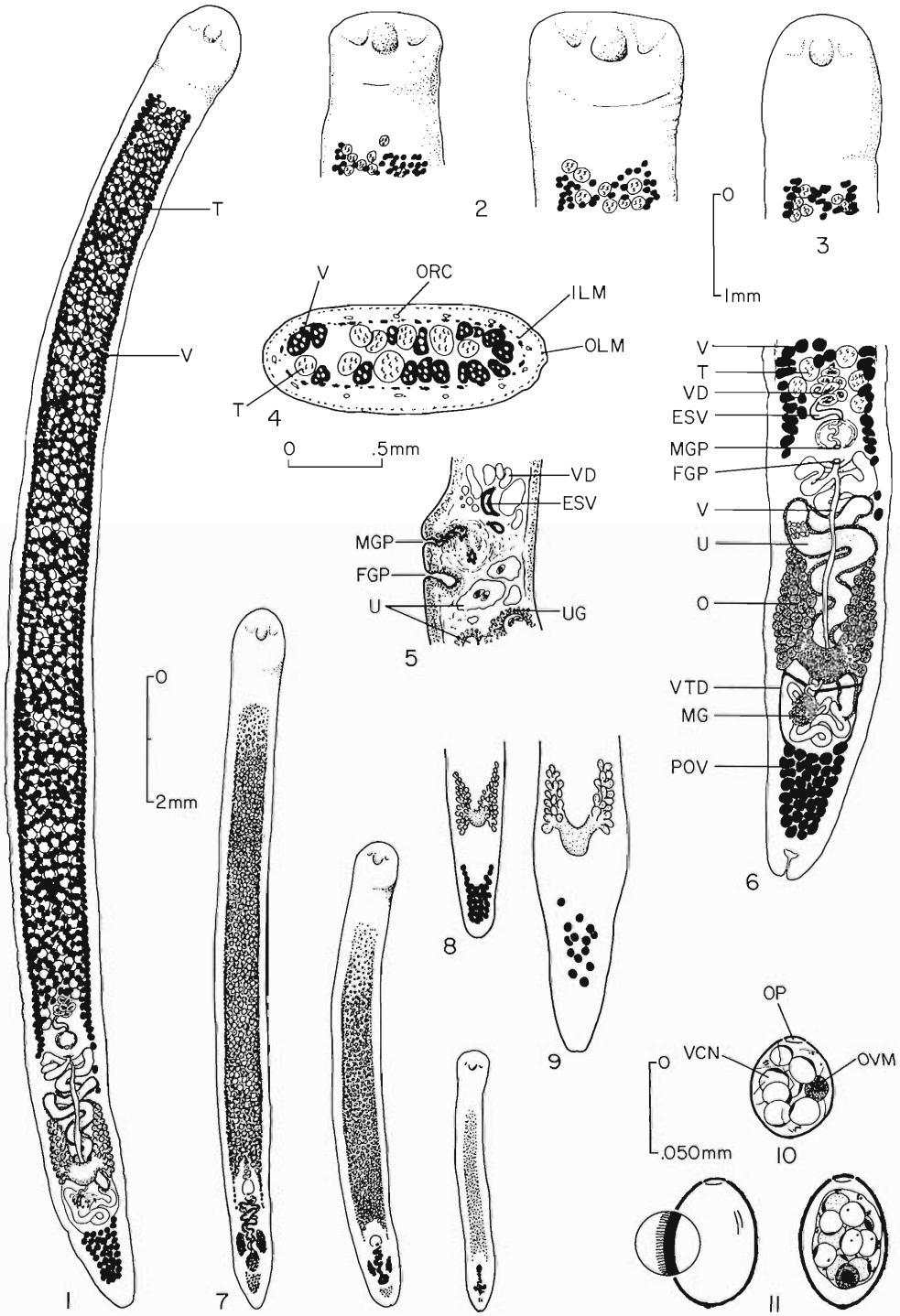
That Linton (1893) was dealing with a mixed infection, involving the small species *Hunterella nodulosa* Mackiewicz and McCrae, 1962 (Figs. 13, 14, 21) and larger *C. terebrans* (Figs. 1, 24), has been established by Mackiewicz and McCrae (1962) who designated USNM Helm. Coll. No. 51074 (35.51b) as the lectotype of *C. terebrans* (Fig. 12). Comparison of Figures 12 and 13 leaves little doubt that Linton was dealing with two separate genera. My specimens from the type host near the type locality in Wyoming and from Idaho are identical to Linton's larger worm (Fig. 12), thus providing an opportunity to clarify the morphology and systematic status of *C. terebrans*.

The morphology and taxonomic status of this species was greatly confused by Hunter (1927, 1930), who redescribed *C. terebrans* utilizing Linton's (1893) description and original slides that comprised two species (compare Fig. 13 with Fig. 31 of Hunter, 1930), and a third, different species from *Ictiobus bubalus* in Mississippi (Hunter, 1930: 30). From Dr. Hunter's collection, now in my possession, I record the following 51 slides determined as *C. terebrans*: 641.1, 645.5–6, 657.1, 659.1 as whole mounts and 645.7–.15, comprising 46 slides of sections. Two species are present in this series: a single specimen (No. 659.1) of *Biacetabulum giganteum* Hunter and another (from *I. bubalus*) of uncertain generic status

[1] Presented in part at the Wildlife Diseases Conference of The Wildlife Association, 22–25 August 1973, Storrs, Connecticut.

→

Figures 1–11. ***Glaridacris terebrans*, from Wyoming unless otherwise indicated. 1. Mature worm. 2. Scolex variations. 3. Scolex. 4. Cross section through middle of body. 5. Midsagittal section through gonopores. 6. Posterior end. 7. Immature worms. 8. Papilioniform ovary (Idaho). 9. Few postovarian follicles. 10. Ova, *G. catostomi* (N. Y.). 11. Ova, illustrating fine hairs on shell. Abbreviations (Figs. 1–20): CS, cirrus; ESV, external seminal vesicle; FGP, female gonopore; ILM, inner longitudinal muscles; ISV, internal seminal vesicle; MG, Mehlis' gland; MGP, male gonopore; O, ovary; OLM, outer longitudinal muscles; OP, operculum; ORC, osmoregulatory canal; OV, ovum; POV, postovarian vitellaria; SR, seminal receptacle; T, testis; U, uterus; UG, uterine gland; V, vitellarium; VA, vagina; VCN, vitelline cell nucleus; VD, vas deferens; and VTD, vitelline duct.**



that forms the basis for most of the redescription. This latter species cannot be *Caryophyllaeus* because it lacks a flabelliform scolex (compare Fig. 20 with 16, 17), seminal receptacle, and internal seminal vesicle (Fig. 18). On the other hand, it has a well-developed external seminal vesicle, readily visible on sections but apparently not observed by Hunter (Fig. 19a). Nor is it similar to Linton's large worm (*C. terebrans*) or those described below from Wyoming and Idaho, differing in the following ways: noncuneiform scolex and long neck (Figs. 19b, 20, 22), vitellaria and testes beginning at two different levels, and an ovarian commissure that is almost equatorial (Fig. 19a). Host differences help to reinforce these morphological ones: *I. bubalus* is a warmwater fish of large rivers and eutrophic lakes of lower elevations, feeding primarily on plankton; *C. ardens* occurs in cold rivers and oligotrophic lakes at higher elevations and is a benthic feeder. The species are allopatric.

The identity of Hunter's "*C. terebrans*" is not known. Superficially it is closest to *Promonobothrium* Mackiewicz, 1968, having a similar scolex and neck swelling (Fig. 20); it differs, however, in having postovarian vitellaria (Fig. 22). With only one suitable whole mount (No. 657.1), the identity of Hunter's "*C. terebrans*" must await new material.

Not until newly collected material was studied (Mackiewicz, 1968) did it become apparent that Linton's *C. terebrans* more closely resembled *Glaridacris* than *Caryophyllaeus* (compare Figs. 23–25). Subsequent study of these, as well as "*C. terebrans*" of literature reports, revealed conclusively that it should be transferred to the genus *Glaridacris* Cooper. Because of the absence of an accurate description of *C. terebrans*, the formation of the new combination is accompanied by a redescription. Specimens were fixed in 10% formalin and stained in Semichon's carmine. Measurements are in microns unless otherwise indicated; drawings were made with the aid of a microprojector.

Redescription

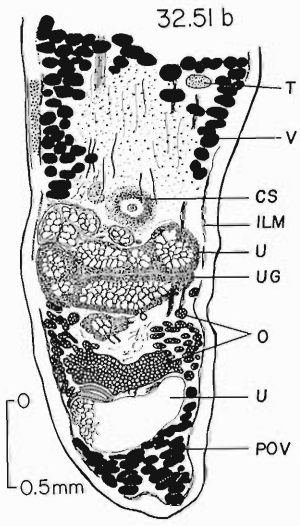
Glaridacris terebrans comb. n. (Figs. 1–7, 11, 24)

SYNONYMY: *Monobothrium terebrans* Linton, 1893 [partim]. *Caryophyllaeus terebrans* (Linton, 1893) Woodland, 1923 [partim] of Hunter (1927).

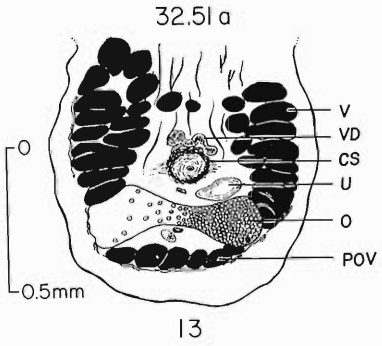
SPECIFIC DIAGNOSIS (unless otherwise specified means are based on 36 gravid individuals, 20 from three fish from Wyoming, 16 from one in Idaho, USA; ranges in parentheses): Worms 20.5 (9.8–41.5) mm long by 1.04 (0.75–1.37) mm wide at male gonopore. Body generally of uniform width throughout. Scolex well developed, slightly narrower than body, cuneiform with three shallow loculi on each side; median loculus most prominent (Figs. 1–3). Neck slightly constricted, short. Inner longitudinal muscles (ILM) widely spaced large fascicles (Fig. 4). Outer longitudinal muscles (OLM) prominent, numerous, evenly spaced small fascicles one-third distance from tegument to ILM (Fig. 4). Testes round to oval, begin 1.6 (1.2–2.9) mm from scolex apex, extend to cirrus, number 677 (502–970; $N = 30$), larger than vitellaria. External seminal vesicle long, tubular, occasionally convoluted (Figs. 5, 6). Cirrus sac 425 (280–650) in diameter ($N = 15$), from 2 to 3 times its width from ovary and 1.9–3 times into body width at male gonopore. Preovarian vitellaria oval to irregular in shape, annular arrangement; begin at same level or slightly anterior of testes, 1.5 (1.1–2.9) mm from scolex apex, extend to uterus, occasionally ovary; not continuous with postovarian vitellaria. Previtelline distance (i.e., from apex of scolex to anteriormost vitellarium) contained in length of worm 13.3 (8.3–7.9) times, represents 7.8 (5.7–13.2) per cent of worm length. Postgonopore distance (i.e.,

→

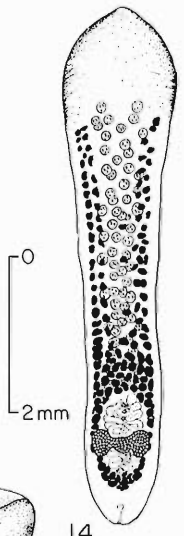
Figures 12–20. 12. Linton's *Monobothrium terebrans* (USNM Helm. Coll. No. 51074, 35.51b) = *G. terebrans*. 13. Linton's *M. terebrans* (USNM Helm. Coll. No. 51074, 35.51a) = *Hunterella nodulosa*. 14. *H. nodulosa* (after Mackiewicz, 1962: fig. 3). 15. *G. catostomi*, three scolex variations. 16. *Caryophyllaeus laticeps*, typical flabelliform scolex. 17. *C. laticeps*, scolex variations. 18. *C. laticeps*, posterior end. 19. *C. terebrans*, a. posterior end. b. scolex (after Hunter, 1930; figs. 30, 1). 20. "*C. terebrans*," scolex, slide No. 657.1, Hunter collection.



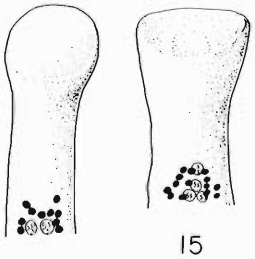
12



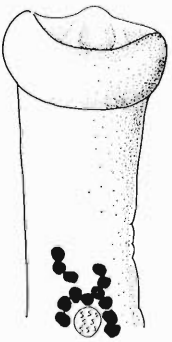
13

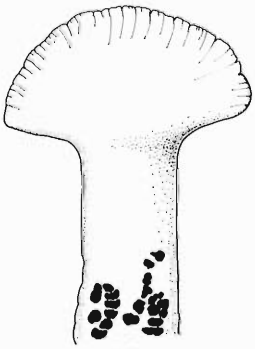


14

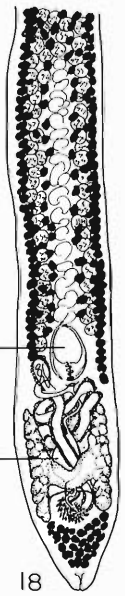


15





16

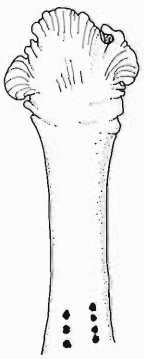


18

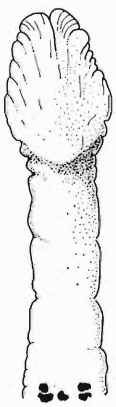
b

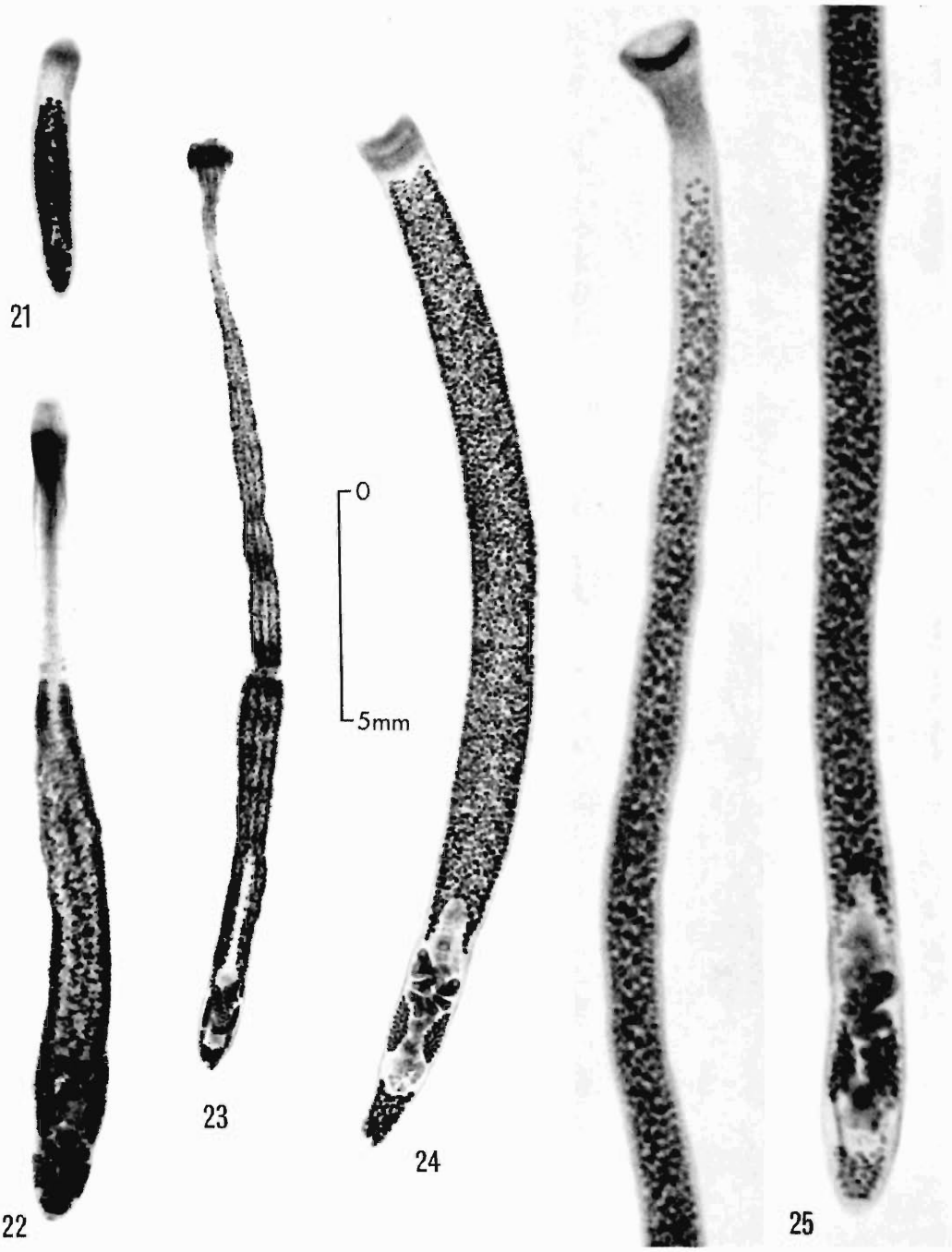
19 a

20



17





Figures 21–25. 21. *H. nodulosa*, Wyoming. 22. "*C. terebrans*" slide No. 645.5, Hunter collection. 23. *C. laticeps*, from *Abramis brama*, Switzerland. 24. *G. terebrans*, Wyoming. 25. *G. catostomi*, from *C. commersoni*, Alberta, Canada.

from male gonopore to posterior apex) 3.6 (1.9–6.7) mm, contained in length of worm 5.7 (4.7–6.5) times, represents 17.8 (13.5–21.4) per cent of worm length. Postovarian vitellaria present; rarely reaching ovary, 50 to 150 follicles. Ovary shaped like the letter U or papilioniform, follicular, 0.9 (0.5–1.7) mm long (Figs. 6, 8, 9). Seminal receptacle absent. From 14 to 16 osmoregulatory canals at midbody (Fig. 4). Eggs elliptical, large, 73 (69–80) long by 48 (46–51) wide (N = 40, 20 dissected from distal uterus of each of two Wyoming worms, measured in water); operculum 15 (14–17) wide (N = 10), shell covered with fine "hairs" (Fig. 11).

SPECIMENS STUDIED: 82 mature, 19 immature.

HOSTS: Utah sucker, *Catostomus ardens* Jordan and Gilbert (Cypriniformes: Catostomidae).

There have been numerous records of *C. terebrans* but only those of Bangham (1951) and LaBar (1969) from the upper Snake River appear to be accurate, based on examination of original material. Although *C. terebrans* is present in the Bangham (1951) collection, other caryophyllids so determined are redetermined as *H. nodulosa*, *Monobothrium* sp., and a member of the *Glaridacris laruei–oligorchis* complex. Bangham (1951) reported his records from the webug sucker, *Catostomus fecundus* Cope and Yarrow; however, according to Dr. G. T. Baxter of the University of Wyoming, the *Catostomus* of the Jackson Hole area should be *C. ardens* (pers. comm., September 1965). Furthermore, according to Moore (1968), *C. fecundus* is of uncertain status and found in Utah Lake, Utah.

The following records, originally reported as *C. terebrans* from a variety of hosts, are redetermined as follows: Linton (1941), from *Notropis rubifrons*: generic allocation uncertain by Mackiewicz (1970). Tonn (1955), from *C. commersoni*: *G. catostomi* and *H. nodulosa* by Mackiewicz and McCrae (1962). Bangham and Adams (1954), from *Mylocheilus caurinus* (Richardson): *Edlintonia ptychocheila* by Mackiewicz (1970); from *C. catostomus*: *G. catostomi* by Mackiewicz (1965) and *Isoglaridacris calentinei* Mackiewicz, 1973, from this host in the Okanagon River (several specimens from *C. catostomus* from Surveyors Lake appeared to be *G. terebrans* but poor condition of material makes positive identification impossible); from *Cyprinus carpio*: *Atractolytocestus huronensis* by Mackiewicz (1970).

LOCATION: Anterior half of intestine; occasionally in intestinal swelling in heavy infections.

LOCALITIES: *C. ardens*, Wyoming, USA: Teton Co. Fish Creek at Wilson, Snake River near Moran, Two Ocean Lake in Grand Teton National Park. Idaho: Bingham Co., Snake R.; Fremont Co., Snake R. near Ashton; Bannock Co., Portneuf R. near Inkom, Ross Fork Creek, tributary of Portneuf R. Snake and Columbia River drainage.

SPECIMENS DEPOSITED: (10) USNM Helm. Coll. Nos. 72940–2; (4) British Museum (Natural History) Helm. Coll. Nos. 1974.3.25.1–4.

Remarks

Of the six loculi, the median one is usually visible as a translucent area. The external seminal vesicle, readily seen on sections (Fig. 5), is difficult to detect on mounted specimens where it usually appears as a large thickened cylindrical tube passing horizontally near the cirrus sac. Ovarian morphology is much like that of *Glaridacris catostomi*, previously described as H-shaped (Hunter, 1930; Mackiewicz, 1965); a more accurate description would be "from papilioniform (butterfly shaped) to U-shaped" because the commissure is neither equatorial nor are there posterior arms, only follicles (Figs. 6, 9). As the commissure is bent forward the posterior follicles become more elongate thus giving the ovary a papilioniform shape (Fig. 8). However, because the U-shaped ovary occurs in relaxed specimens it is considered the basic morphological type for this species.

Anomalies are few, the most prominent being a single abortive postovarian testis in one worm and another with a much reduced number of postovarian vitelline follicles (Fig. 9).

Thirteen of 34 Wyoming fish, ranging in size from 21 to 47.5 cm, were infected (August) with from one to an estimated 300 individuals, the greatest number from the largest fish from Two Ocean Lake; most infections involved less than 15 worms. Concurrent infections with *H. nodulosa* occurred in 10 cases.

Of seven infected fish in Idaho, four had

concurrent infections with *H. nodulosa*, one with the acanthocephalan *Neoechinorhynchus venustus*, and two with *N. venustus* and *N. crassus*.

The presence of a cuneiformiloculate scolex, two gonopores, annular vitellaria, postovarian vitellaria, external seminal vesicle, and ovary that varies from papilioniform to U-shaped clearly places this species in the genus *Glaridacris* Cooper. With its scolex type, long body form (Fig. 1), annular vitellaria (Fig. 4), and numerous testes (i.e., more than 150) *Glaridacris terebrans* (Linton) most closely resembles *G. catostomi* Cooper (Fig. 25). The other three species, *G. laruei* (Lamont, 1921), *G. confusus* Hunter, 1929, and *G. oligorchis* Haderlie, 1953, have a bothrioloculodiscate scolex, short body form, lateral vitellaria, and testes numbering less than 100. It differs from *G. catostomi* in the following features: many more testes, 502–970; eggs larger and clothed with fine "hairs" (Fig. 11); more robust scolex on a less well-defined neck (Figs. 2, 3); and generally smaller size (9.8–41.5 mm). *G. catostomi*, in contrast, has from 171–347 testes, smooth eggs (Fig. 10), well-differentiated scolex and neck (Figs. 15, 25), and is from 11.5 to 55 mm long.

Discussion

There are no authenticated records of *Caryophyllaeus* in the Nearctic. In addition to those corrected in this paper, others include that of Lamont (1921), corrected by Hunter (1927), and those of Wilson (1957) and Rehder (1959), corrected by Mackiewicz (1970). As a result of these corrections and the generic reallocation made in this paper the distributional maps or analyses by Mackiewicz (1972) and Bauer and Gusev (1969) must be revised. All current evidence indicates that the genus *Caryophyllaeus* is apparently limited to the Palearctic region, except where translocated to other regions.

As more is learned through careful restudy of other species from type hosts near the type locality the explanation for the unusual host and regional distribution of several other genera may be resolved as in the case of the Nearctic *Caryophyllaeus*; these genera include *Monobothrium* Diesing, 1930, *Biacetabulum* Hunter, 1927, and *Pseudolytocestus* Hunter, 1929. At present it appears that *Archigetes* Leuckart, 1878, and *Khawia* Hsu, 1935, are the only two caryophyllid genera that definitely appear to be in the Nearctic and Palearctic regions; both occur in *C. carpio*, a Palearctic cyprinid that has become widely established in the Nearctic. Except for such introductions it appears that each zoogeographical region has its own characteristic caryophyllid fauna.

Acknowledgments

The author gratefully acknowledges the cooperation of Dr. L. Floyd Clark, Director of the Jackson Hole Research Station, and Mr. Max Rollefson and his staff of the Wyoming Game and Fish Commission at Jackson Hole for generous assistance in collecting hosts during the summer of 1965; and the following colleagues for loans or gifts of specimens: the late Dr. R. Bangham, J. Fischthal (State University of New York at Binghamton), G. LaBar (Idaho State University), and J. Adams (University of British Columbia).

This investigation was supported in part by a Faculty Research Fellowship (0922-01-020-69-0) and grants 20-160-A and 20-A022-A from the Research Foundation, State University of New York.

Literature Cited

Bangham, R. V. 1951. Parasites of fish in the upper Snake River drainage and in Yellowstone Lake, Wyoming. Zoologica 36: 213–217.

———, **and J. R. Adams.** 1954. A survey of the parasites of freshwater fishes from the mainland of British Columbia. J. Fish. Res. Bd. Canada 11: 673–708.

Bauer, O. N., and A. V. Gusev. 1969. (Parasitofauna of fish from Palearctic and Nearctic, similarities and differences.) (In Russian, English summary.) Parazit. Sbor. 24: 30–48.

Hunter, G. W. III. 1927. Notes on the Caryophyllaeidae of North America. J. Parasit. 14: 16–26; pls. I–II.

———. 1930. Studies on the Caryophyllaeidae of North America. Ill. Biol. Monog. 11 (1927), 182 p.

LaBar, G. W. 1969. *Catostomus ardens* Jordan and Gilbert, 1881, a new host record for *Neoechinorhynchus venustus* Lynch, 1936, and *N. crassus* Van Cleave, 1919, with notes on caryophyllaeids. J. Parasit. 55: 497.

Lamont, M. E. 1921. Two new parasitic flatworms. Occ. Papers Mus. Zool. Univ. Mich. No. 93, p. 1–3; pl. 1.

Linton, E. 1893. On fish entozoa from Yellowstone National Park. Rep. U. S. Comm. Fish and Fisheries, 1889 to 1891: p. 545–564.

———. 1941: Cestode parasites of teleost fishes of the Woods Hole region, Massachusetts. Proc. U. S. Nat. Mus. 90: 417–446.

Mackiewicz, J. S. 1965. Redescription and distribution of *Glaridacris catostomi* Cooper, 1920. (Cestoidea; Caryophyllaeidae). J. Parasit. 51: 554–560.

———. 1968. *Isoglaridacris hexacotyle* comb. n. (Cestoidea: Caryophyllidea) from catostomid fishes in southwestern North America. Proc. Helm. Soc. Wash. 35: 193–196.

———. 1970. *Edlintonia ptychocheila* gen. n., sp. n. (Cestoidea: Capingentidae) and other caryophyllid tapeworms from cyprinid fishes of North America. Proc. Helm. Soc. Wash. 37: 110–118.

———. 1972. Caryophyllidea (Cestoidea): A review. Exp. Parasit. 31: 417–512.

———, **and R. C. McCrae.** 1962. *Hunterella nodulosa* gen. n., sp. n. (Cestoidea: Caryophyllaeidae) from *Catostomus commersoni* (Lacépède) (Pisces: Catostomidae) in North America. J. Parasit. 48: 798–806.

Moore, G. A. 1968. Fishes, p. 22–165. *In* W. F. Blair et al., Vertebrates of the United States. Second Ed., McGraw-Hill, New York, 616 p.

Rehder, D. D. 1959. Some aspects of the life history of the carp, *Cyprinus carpio*, in the Des Moines River, Boone County, Iowa. Iowa State J. Sci. 34: 11–26.

Tonn, R. J. 1955. The white sucker, *Catostomus commersonii* (Lacepede), a new host of *Caryophyllaeus terebans* (Linton, 1893). J. Parasit. 41: 219.

Wilson, W. D. 1957. Parasites of fishes from Leavenworth County State Lake, Kansas. Kansas Acad. Sci. 60: 393–399.

Woodland, W. N. F. 1923. On some remarkable new forms of Caryophyllaeidae from the families of the Cestodaria. Quart. J. Microscop. Soc. (n.s.) 67: 435–472; pls. 24–25.

The Occurrence and Morphology of *Brachylecithum transversum* (Travassos, 1917) comb. n., in the Eastern Kingbird, *Tyrannus tyrannus* (L.), from Georgia[1]

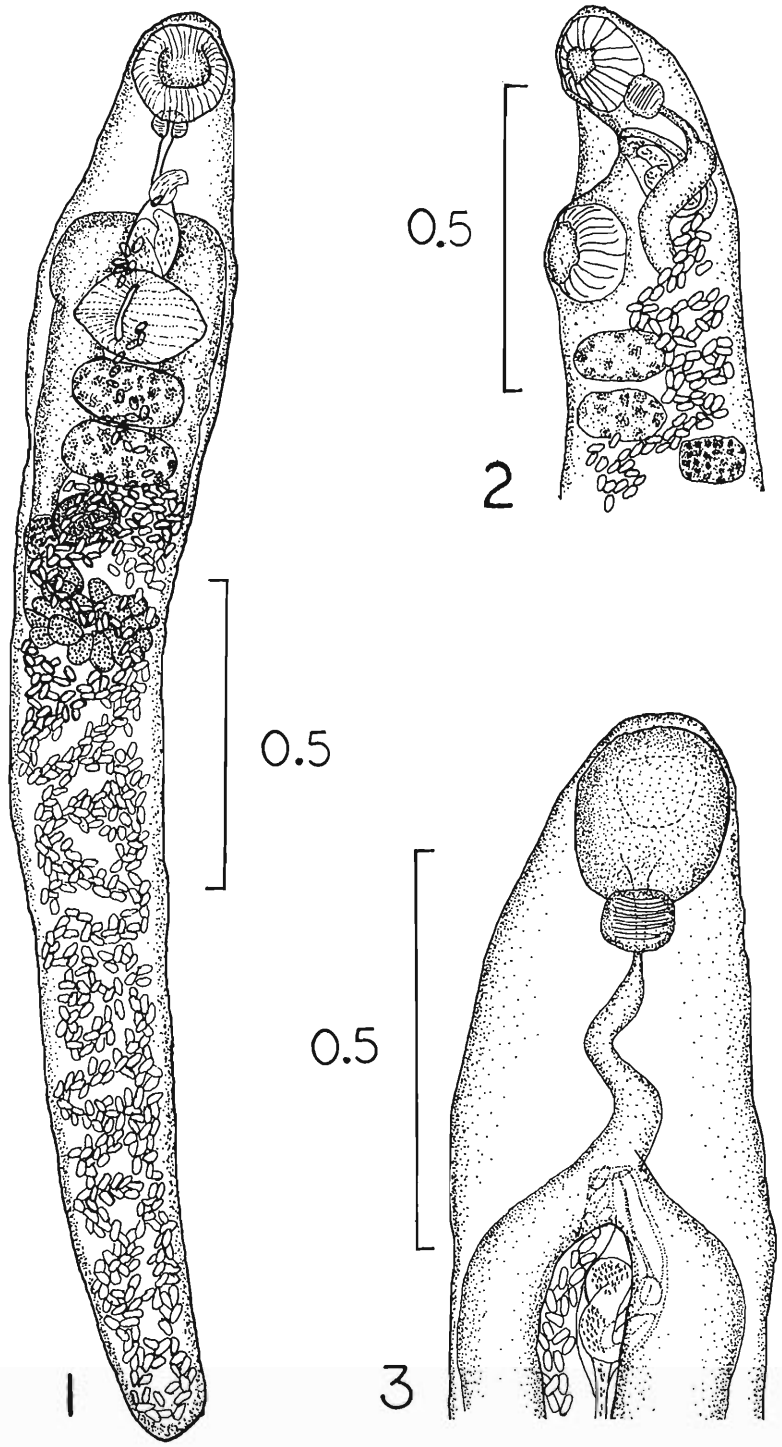
J. Fred Denton and Wayne A. Krissinger
Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30902

ABSTRACT: A dicrocoeliid trematode obtained from the livers and gall bladders of six of 20 eastern kingbirds collected in Georgia is redescribed and transferred to *Brachylecithum* as *B. transversum* (Travassos, 1917) comb. n. [Syns. *Lyperosomum transversum* Travassos, 1917; *Lutztrema transversum* (Travassos, 1917) Travassos, 1941]. This species has two ceca rather than a single cecum as previously decribed.

Ishii (1942) reported without descriptive details the occurrence of *Lutztrema momenteron* (Price and McIntosh, 1935) in the livers of two kingbirds, *Tyrannus tyrannus*, from Minnesota. Denton and Byrd (1951) examined Ishii's slides and found the specimens not identifiable even to genus and concluded they must remain *species inquirenda* pending study of more favorable material. To determine the species of dicrocoeliid occurring in the eastern kingbird in North America, 20 birds collected in the vicinity of Augusta, Georgia, were examined for helminths. Six (30%) of the birds harbored in their gall bladders and bile ducts from two to 75 specimens of a single species of the genus *Brachylecithum* Strom, 1940.

Comparison of our material with the 15 species of *Brachylecithum* previously reported from North America and with other species and subspecies recognized by Yamaguti (1971) as belonging to this and related genera has led to the conclusion that our specimens repre-

[1] Supported in part by NIH General Research Support Grant FR-5365 to the Medical College of Georgia.



sent the species previously known as *Lutztrema transversum* (Travassos, 1917). Our form agrees very closely with this species in every respect except for the digestive system which we believe was erroneously described by Travassos (1917, 1941, 1944). Therefore, we are redescribing the species and transferring it to the genus *Brachylecithum* as *B. transversum* (Travassos, 1917) comb. n.

Specimens were killed and fixed in warm 5% formalin solution under slight coverglass pressure and stained with either Harris' hematoxylin or carmine borax. Some worms were counterstained with fast green. The drawings were made with the aid of a camera lucida. All measurements are in microns.

Brachylecithum transversum (Travassos, 1917) comb. n. (Figs. 1–3)

DESCRIPTION (based on adult specimens; 15 measured): Body small, elongated, cylindrical to slightly flattened dorsoventrally, with rounded anterior and tapering posterior end, measuring 1,800–3,260 long by 224–421 wide in region of acetabulum. Tegument thin, aspinose, finely wrinkled transversely. Oral sucker elongated oval, 145–217 long by 130–239 wide, subterminal to a short preoral lip. Acetabulum located in anterior body fourth, weakly muscular and somewhat protrusible, oval to slightly lemon-shaped depending on degree of eversion, 140–213 long by 155–231 wide. Ratio of width of oral sucker to acetabulum 1:1.1 to 1:1.4. Prepharynx absent. Pharynx muscular, globular, slightly wider than long, 50–73 wide by 40–57 long, overlapping oral sucker dorsally. Esophagus slender, wavy, bifurcating from ½ to ⅔ of distance from oral sucker to acetabulum. Ceca varying from narrow to wide and voluminous in some specimens, straight to slightly sinuous, passing dorsal to acetabulum, dorsolateral to margins of gonads, dorsal to vitellaria and terminating unevenly at posterior level of vitellaria or slightly beyond at a point about ⅛ the distance from vitellaria to posterior end of body. Excretory pore terminal; excretory bladder not observed. Genital pore median and ventral to or slightly anterior to intestinal bifurcation. Testes transversely oval, margins smooth, approximately equal in size, 83–180 long by 111–232 wide, situated medially one directly behind the other; anterior testis separated from acetabulum by one to four uterine loops in some specimens; posterior testis contiguous to anterior. Vasa efferentia originating from anterolateral margins of the testes and uniting just anterior to anterior testis to form vas deferens which passes dorsal to acetabulum. Cirrus sac elongated-pyriform, 90–161 long by 47–90 wide, with posterior ⅓ to ¼ lying dorsal to acetabulum and anteriormost part lying in arch formed by cecal bifurcation, containing thick-walled coiled seminal vesicle, pars prostatica, prostate cells, and eversible cirrus. Ovary transversely oval, 70–100 long by 80–130 wide, situated slightly to right or left of body and separated from posterior testis by two or three uterine loops. Seminal receptable globular, thin-walled, situated posterodorsal to ovary, 60–75 in diameter. Mehlis' gland located posterior to ovary and medial to seminal vesicle, with ootype receiving a vitelline duct from each side of body. Laurer's canal not observed. Vitellaria consisting of a compact group of 14 to 16 large oval to irregular follicles or possibly two groups of seven to eight follicles each, occupying most of body width immediately posterior to seminal receptacle. Uterus greatly convoluted, extending posteriorly from ovarian region, ventral to vitellaria, then looping transversely from ventral to dorsal surface to near posterior end of body where it turns anteriorly and passes in a like looping pattern ventral to vitellaria and medial margin of ovary, then turning dorsally between ovary and posterior testis to pass in center of body over both testes and acetabulum to genital pore. Mature eggs operculated, with very thick opaque shells, dark brown to black in color, measuring 35–39 long by 23–24

←

Figures 1–3. ***Brachylecithum transversum*** **(scale values are in millimeters). 1. Whole worm, ventral view. 2. Anterior body segment, lateral view. 3. Anterior segment showing relative position of cirrus pouch and ceca, dorsal view.**

wide in living specimens, fully embryonated when oviposited. Miracidium ciliated and possessing a stylet and two large posteriorly situated vesicles containing refractile granules.

Two specimens have been deposited in the USNM Helm. Coll. No. 72869.

Discussion

Many investigators have had difficulty visualizing and interpreting the digestive system in delicate dicrocoeliids belonging to the genera *Brachylecithum* and *Lutztrema* Travassos, 1941. Travassos, in our opinion, was no exception as he apparently confused the termination of the cecum with the excretory bladder in *L. obliquum* (Travassos, 1917). Jimenez-Quiros and Arroyo (1960) redescribed this species but failed to clarify this point as they too were unable to see the end of the cecum. In another species, *L. marinholutzi* Travassos, 1941, he described the esophagus as dividing into two ceca, one of which is very short and terminates in the acetabular zone and the other long and sinuous, extending into the middle of the posterior body region. In no other of the more than 80 species assigned to either the genus *Brachylecithum* or *Lutztrema* has the combination of one normal and one rudimentary cecum been described. It would appear that Travassos was just unable to see one of the ceca after it passed dorsal to the acetabulum in this species which is now accepted as belonging to the genus *Brachylecithum* (Yamaguti, 1971). Travassos in describing *L. transversum* states that the esophagus continues as a single cecum but the cecum is entirely obscured by the gonads and uterus; terminating at beginning of posterior third of body. In only two of his seven figures (Plate 67, fig. 2; Plate 70, figs. 1–6) does Travassos (1944) show a cecum at all and then extending only into the acetabular zone. We believe that he failed to see the other cecum because it was obscured by the cirrus pouch, acetabulum, and gonads, a situation we have encountered in some of our specimens, and reexamination of his material would probably reveal two normal ceca.

The eastern kingbird normally winters in northern South America spending approximately 6 months there. Kingbirds returning to the United States in spring arrive with immature worms, some estimated to be no more than 3 or 4 weeks old, indicating that infection was acquired on their wintering grounds with a species endemic to South America. *B. transversum*, which has been described from worms from the tropical kingbird, *Tyrannus melancholicus*, the boat-billed flycatcher, *Megarhynchus petangua*, the crowned slaty flycatcher, *Epidonomus auratiaatrocristatus*, and the house sparrow, *Passer domesticus*, is such a species. Eastern and tropical kingbirds intermingle and share feeding grounds during the winter in South America.

Several prominent features of *B. transversum*, the combination of which easily distinguishes it from other North American species of *Brachylecithum*, are: (1) the shape of the body with a distinct bulge in the acetabular zone, (2) the location of the genital pore in a concave depression in the anterior body segment, (3) the thin-walled and usually voluminous ceca, (4) the close tandem arrangement of the transversely oval gonads, (5) the characteristic clustering of the large vitelline follicles in the middle of the body just behind the ovary, and (6) the very dark opaque mature ova.

Literature Cited

Denton, J. F., and E. E. Byrd. 1951. The helminth parasites of birds. III: Dicrocoeliid trematodes from North American birds. Proc. U. S. Natl. Mus. 101: 157–202.

Ishii, N. 1942. New parasite records from the ruffed grouse. J. Parasit. 28: 92.

Jimenez-Quiros, O., and G. Arroyo. 1960. Helmintos de la Republica de Costa Rico. XIV. Redescripcion de *Lutztrema obliquum* Travassos, 1941 y descripcion de *Zonorchis microovaricus* n. sp. Rev. Biol. Trop. 8: 53–61.

Travassos, L. 1917. Especies brazileiras do genero *Lyperosomum*. First Conf. Soc. Sud-Amer. Hig., 1916, p. 737–745.

———. 1941. *Lutztrema* n. g. (Trematoda: Dicrocoeliidae). Mem. Inst. Osw. Cruz 36: 335–343.

———. 1944. Rivisao da familia Dicrocoeliidae Odhner, 1910. Monogr. Inst. Osw. Cruz 2: 1–357.

Yamaguti, S. 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vol. 1. 1074 p. Keigaku Publ., Tokyo.

Tapeworms from Philippine Reptiles, with Two New Species of Proteocephalata[1]

GERALD D. SCHMIDT[2] AND ROBERT E. KUNTZ[3]

ABSTRACT: Tapeworms from Philippine reptiles are reported, mainly from Palawan Island. *Kapsulotaenia frezei* sp. n., from *Varanus salvator*, has 45–60 testes in two lateral fields, and a cirrus pouch one-fourth the width of the proglottid. Its egg capsules are 680–690 μ long, 65–70 μ wide, and contain 90–100 eggs each. *Acanthotaenia daileyi* sp. n., from *V. salvator*, has 46–82 testes in a single field, and a cirrus pouch one-third the width of the proglottid. *Duthiersia expansa* Perrier, 1873, and *Scyphocephalus bisulcatus* Riggenbach, 1898, are also reported from *V. salvator*. Four species of *Oochoristica* appear to be undescribed but are in too poor condition to classify.

This paper reports cestodes collected from reptiles by the second author and his colleagues of NAMRU-2 during the Silliman University–Bishop Museum Expedition to Palawan, Republic of the Philippines, in May and June 1962. Hosts were examined in the field, and all cestodes collected were relaxed in water and fixed in AFA. The first author stained the worms with Semichon's carmine for study. All measurements are in microns unless otherwise stated.

Kapsulotaenia frezei sp. n. (Figs. 1–4)

Two water monitors, *Varanus salvator* (Hosts PP-493, PP-500), were infected with a new species of *Kapsulotaenia* Freze, 1963. One had a mixed infection with a species of *Acanthotaenia*, described below, and with *Duthiersia expansa* Perrier, 1873. All specimens are fragmented. The new species is named in honor of Dr. V. I. Freze, who founded the genus and did much to stabilize the classification of the order Proteocephalata.

Description

Scolex (Fig. 1) rounded, with conical apex, 360 to 400 long (including apical cone), 360 to 465 wide. Suckers round, 145 to 165 in diameter. Apical organ 50 to 60 long, 40 to 50 wide. Neck about 800 long, slightly narrower than scolex. Entire head, neck, and strobila densely covered with small spines.

Strobila about 40 mm long, 700 greatest width. Genital pores slightly preequatorial, irregularly alternating. Genital ducts (Fig. 2) pass between osmoregulatory canals. Reproductive systems (Fig. 3) protandrous. Ventral osmoregulatory canals without transverse commissures, about 15 wide; dorsal canals 5 to 10 wide. Genital atrium simple, about 10 deep, 40 long.

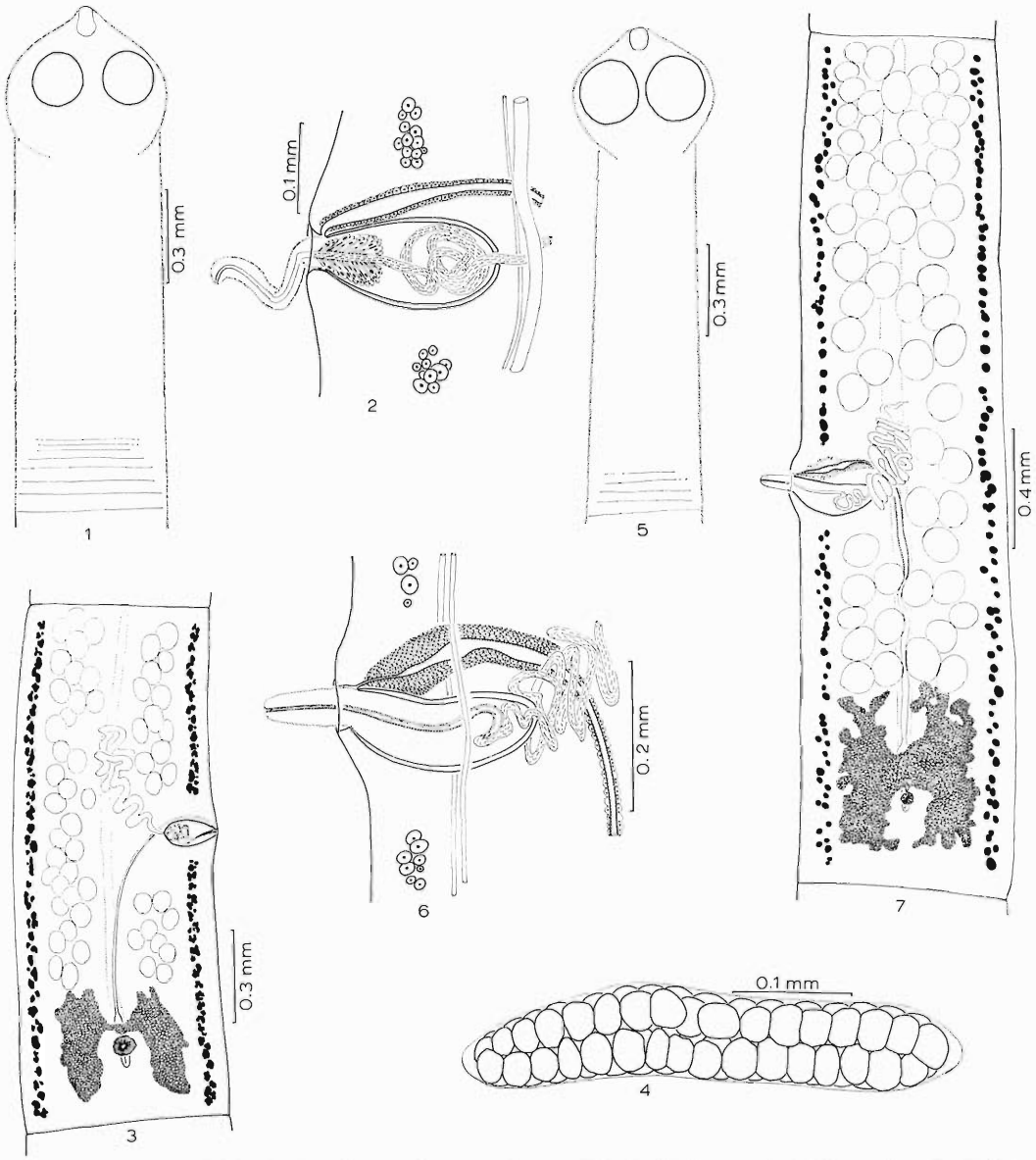
MALE GENITALIA: Forty-five to 60 testes in two lateral fields, poral field interrupted by cirrus pouch. Eight to 12 testes between cirrus pouch and ovary on poral side. Testes each 50 to 90 wide in mature segments. Seminal vesicles absent; vas deferens convoluted in anterior, median field. Ejaculatory duct convoluted inside cirrus pouch. Cirrus about 100 long, covered with small spines. Cirrus pouch elongate-ovoid, transverse, not reaching osmoregulatory canals; maximum size 130 to 200 long, 70 to 90 wide. Numerous conspicuous prostatic cells present inside cirrus pouch, each with duct opening into base of cirrus.

FEMALE GENITALIA: Ovary median, near posterior end of proglottid, with two large, lateral lobes; maximum size 400 long, 400 to 520 wide. Vitellaria in lateral, cortical margins. Distal end of vagina usually anterior, sometimes lateral or posterior to cirrus pouch. Vagina unspined, surrounded by unicellular glands along its entire length. Seminal receptacle very small. Mehlis' gland conspicuous. Uterus appears as a simple, medial, longitudinal

[1] Initial work for this study was supported by funding under Public Law 480, Section 104 (C), by funds provided by the Bureau of Medicine and Surgery, Navy Department Work Unit MR 005.20-0098, by contract NR 103-690/N0014-66-C0094, between the Office of Naval Research, Department of the Navy, and the Southwest Foundation for Research and Education. Final efforts were sponsored by a grant from the University of Northern Colorado Research and Publications Committee.

[2] Department of Biology, University of Northern Colorado, Greeley, Colorado 80639.

[3] Departments of Parasitology, Southwest Foundation for Research and Education, San Antonio, Texas 78284, and U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan.



Figures 1–4. ***Kapsulotaenia frezei*** **sp. n. from a water monitor in Palawan. 1. Scolex and neck. 2. Terminalia. 3. Mature proglottid. 4. Egg capsule.**

Figures 5–7. ***Acanthotaenia daileyi*** **sp. n. from a water monitor in Palawan. 5. Scolex and neck. 6. Terminalia. 7. Mature proglottid.**

tube, remaining so when filled with eggs; lateral diverticulae absent. Eggs 34 to 36 in diameter, contained in elongated egg capsules, each capsule surrounded by delicate membrane (Fig. 4). About 90 to 100 eggs per capsule, which is 680 to 690 long, 65 to 70 wide. Egg capsules leave uterus through ventral, median split in body wall.

TYPE HOST: Water monitor, *Varanus salvator* (Gray) (Varanidae).

LOCATION: Small intestine.

TYPE LOCALITY: Terabanon Concepcion, 73 km N of Puerto Princesa, Palawan Island, Republic of the Philippines.

TYPE SPECIMENS: USNM Helm. Coll. holotype No. 72929, paratype No. 72930.

Remarks

Freze (1963) proposed *Kapsulotaenia* for those species in *Acanthotaenia* that develop a peculiar kind of egg capsule. In this genus he placed *K. sandgroundi* (Carter, 1943) as type species, and *K. saccifera* (Ratz, 1900), *K. tidswelli* (Johnston, 1909), and *K. varia* (Beddard, 1903). Of these, *K. tidswelli* may be misplaced, for Johnston (1909) stated only that the eggs lie in small clusters in the parenchyma. This may well refer to lateral branches of the uterus, which are not always easily visible.

Kapsulotaenia frezei differs from *K. sandgroundi*, from *Varanus komodensis* in Indonesia, in having 45–60 testes rather than 98–115. Similarly, *K. varia* from Australian varanids has 80–158 testes. *Kapsulotaenia tidswelli* from Australian varanids has suckers only 80 wide, while *K. frezei* has suckers 190–200 wide. *Kapsulotaenia frezei* is most similar to *K. saccifera*, from *Varanus* sp. in New Guinea, in overall measurements, but that species has only 30–38 testes and its cirrus pouch is spheroid (150 by 138) rather than elongate-oval.

Acanthotaenia daileyi sp. n. (Figs. 5–7)

One water monitor (PP-500) was infected with several specimens of a new species of *Acanthotaenia* Linstow, 1903, in addition to *Kapsulotaenia freezi*, described above. It is named in honor of Dr. Murray D. Dailey, in recognition of his many contributions to helminthology of the Pacific area.

Description

Scolex (Fig. 5) rounded, with conical apex, 440 to 450 long (including apical cone), 440 to 450 wide. Suckers round, about 190 in diameter, apical organ 65 long, 65 wide. Neck about 960 long, slightly narrower than scolex. Entire head, neck, and strobila densely covered with small spines.

Strobila about 100 mm long, 965 greatest width. Genital pores about equatorial, irregularly alternating. Genital ducts (Fig. 6) pass between osmoregulatory canals. Reproductive systems (Fig. 7) protandrous. Ventral osmoregulatory canals 8 to 10 wide, lacking transverse commissures; dorsal canals about 6 wide. Genital atrium simple, about 20 deep, 60 long.

MALE GENITALIA: Forty-six to 82 testes in one field, poral side interrupted by cirrus pouch. Seven to 13 testes between ovary and cirrus pouch on poral side. Testes each 65 to 150 wide in mature segments. Seminal vesicle absent; vas deferens convoluted in median field at level of cirrus pouch. Ejaculatory duct convoluted inside cirrus pouch. Cirrus about 400 long, covered with small spines. Cirrus pouch elongate, transverse, exceeding osmoregulatory canals; maximum size 260 to 320 long, 90 to 105 wide, reaching about one-third width of segment. Conspicuous prostatic cells absent.

FEMALE GENITALIA: Ovary median, near posterior end of proglottid, with two large, lateral lobes. Vitellaria in lateral, cortical margins. Distal end of vagina usually anterior, sometimes lateral or posterior to cirrus pouch. Vagina lined with minute spines and surrounded by a thick mass of unicellular glands at distal end, with fewer along rest of length. Seminal receptacle and Mehlis' gland very small. Uterus appears as a simple, median tube, expanding into lateral diverticulae when gravid. Eggs single, never in capsules, 24 to 28 in diameter. Eggs leave uterus through median, longitudinal split in body wall.

TYPE HOST: Water monitor, *Varanus salvator* (Gray) (Varanidae).

LOCATION: Small intestine.

TYPE LOCALITY: Terebanon Concepcion, 73 km N of Puerto Princesa, Palawan Island, Republic of the Philippines.

TYPE SPECIMENS: USNM Helm. Coll. holotype No. 72931, paratype no. 72932.

Remarks

Freze (1965) summarized the known species of *Acanthotaenia*, including some which he placed in his genus *Rostellotaenia*. We do not accept the latter genus for it is based on vague, nongeneric characters such as weak development of Mehlis' gland, and a "piercing organ" with weak musculature. We therefore recognize the following species, all from varanid

lizards (see Freze for synonymics): *A. shipleyi* Linstow, 1903, from Malay Archipelago; *A. biroi* (Ratz, 1900) Johnston, 1909, from New Guinea; *A. tidswelli* Johnston, 1909, from Australia; *A. nilotica* Beddard, 1913, from North Africa; *A. beddardi* (Woodland, 1925) comb. n., from India; and *A. woodlandi* (Moghe, 1926) comb. n., from India. None of these has a cirrus pouch as long as one-third the width of the proglottid, as does *A. daileyi*.

The species most similar to *A. daileyi* is *A. nilotica*. It differs in the following ways: (1) its apical cone is large and massive, compared with that of *A. daileyi* which is short and conical; (2) its cirrus pouch is 190–222 long and is one-sixth to one-fourth the width of the proglottid, while that of *A. daileyi* is 260–320 long, and at least one-third the width of the proglottid; and (3) mature proglottids of *A. nilotica* are said to be ovoid in shape, while they are rectangular in *A. daileyi*.

Duthiersia expansa Perrier, 1873

This species was found in *Varanus salvator* (PP-500) from Terabanon Concepcion. It apparently is identical to the specimens reported by Tubangui (1938) as *D. fimbriata* (Diesing, 1854). However, according to Woodland (1938), *D. fimbriata* occurs in Africa and has no posterior bothridial apertures, while *D. expansa* occurs in Asia and the East Indies and has posterior bothridial apertures. These can be seen in our specimens. Specimens deposited: USNM Helm. Coll. No. 72939.

Scyphocephalus bisulcatus Riggenbach, 1898

Four specimens were found in a *Varanus salvator* (PP-213) from Terabanon Concepcion. Their measurements overlap both those of *S. bisulcatus* and *S. secundus* Tubangui, 1938. We therefore consider Tubangui's species to be a junior synonym of *S. bisulcatus*, and validate the latter in spite of the misgivings of Wardle and McLeod (1952). Specimens deposited: USNM Helm. Coll. No. 72938.

Oochoristica spp.

Four species of *Oochoristica* Lühe, 1898, were found in a variety of reptiles. All appear to be undescribed, but unfortunately all are in too poor condition to allow adequate descriptions. We have deposited them in the National Museum in hopes they will be useful for future workers who may rediscover the species. None of them is similar to *O. excelsa* Tubangui et Masiluñgan, 1936, the only species in the genus reported from the Philippines.

Species No. 1 is from house geckos, *Hemidactylus frenatus* Dumeril et Bibron (PP-712) and *H. platyurus* (Schneider) (PP-15) (Gekkonidae). Specimens deposited: USNM Helm. Coll. No. 72933.

Species No. 2 is from a sun lizard, *Mabuya multifasciata* (Kuhl) (PP-732) (Scincidae). USNM Helm. Coll. No. 72934.

Species No. 3 is from *Mabuya multifasciata* (PP-156). Specimens deposited: USNM Helm. Coll. No. 72935.

Species No. 4. is from a tree snake, *Ahaetulla ahaetulla* (Linn.) (PP-743) (Colubridae). Specimens deposited: USNM Helm. Coll. No. 72936.

Acknowledgments

We are indebted to the NAMRU-2 field group for their assistance in collecting the parasites under less than ideal conditions. The hosts were identified by Dr. W. E. Duellman, University of Kansas, Drs. R. F. Inger and D. F. Cochran (deceased), U. S. National Museum, Washington, D. C.

Literature Cited

Freze, V. I. 1963. On the convergent formation of the rostellum in two branches of Proteocephalata and the revision of the subfamilies Gangesiinae Mola, 1929 and Acanthotaeniinae Freze, 1963 (Cestoda, Proteocephalata). *In* Helminths of Man, Animals and Plants and Their Control: Papers on Helminthology Presented to Academician K. I. Skrjabin on His 85th Birthday. Izdat. Akad. Nauk, Moscow, p. 150–156. (In Russian.)

———. 1965. Essentials of Cestodology. Vol. V. Proteocephalata in Fish, Amphibians, and Reptiles. Izdat. Akad. Nauk, Moscow. (Israel Program for Scientific Translations, Jerusalem.) 597 p.

Johnston, T. H. 1909. On a new reptilian cestode. J. Proc. Roy. Soc. N. S. Wales 43: 103–116.

Tubangui, M. A. 1938. Pseudophyllidean cestodes occurring in the Philippines. Livro Jubilar do Prof. Lauro Travassos. Editado para Commemoar o 25° Anniversario de Suas

Actividades Scientificas, 1913–1938. Rio de Janeiro, p. 489–494.

———, **and V. A. Masiluñgan.** 1936. *Oochoristica excelsa*, a new reptilian cestode. Philippine J. Sci. 61: 75–77.

Wardle, R. A., and J. A. McLeod. 1952. The Zoology of Tapeworms. Univ. Minn. Press, Minneapolis, 780 p.

Woodland, W. H. F. 1938. On the species of the genus *Duthiersia* Perrier, 1873 (Cestoda). Proc. Zool. Soc. London. 108: 207–219.

Muscular Anatomy of the Praesoma of *Macracanthorhynchus hirudinaceus* (Acanthocephala)[1]

T. T. Dunagan and D. M. Miller
Department of Physiology, Southern Illinois University, Carbondale, Illinois 62901

ABSTRACT: Study of the praesoma of *Macracanthorhynchus hirudinaceus* (Acanthocephala) by light and scanning electron microscopy has clarified much of the internal musculature. The origin and insertion of all muscles including those attached to the cone of the apical sensory organ, hooks, proboscis receptacle, and proboscis neck have been mapped. The proboscis retractor muscles of previous studies are composed of six different muscle groups, two of which function only in the inversion of the apical sensory device. The longitudinal receptacle muscle is renamed the midventral longitudinal receptacle muscle and its description expanded. The interrelationships of these muscles from anterior to posterior are diagramed. Some of the nomenclature previously given these muscles by 19th-century investigators has been clarified.

A study of the muscles located in the praesoma of *Macracanthorhynchus hirudinaceus* was made by Kaiser (1893a). However, considerable work had already been performed on this and other species by Hamann (1891), Greeff (1864), and others. More recent reviews (i.e., Rauther, 1930) add to the previous information by including original drawings of sections from this parasite while others (Hyman, 1951; Yamaguti, 1963; Nicholas, 1967) review this group of parasites but do not give a detailed description of the muscles of the praesomal area. Kilian (1932) presents a detailed study on *Hamanniella* but only a single page to the "Russelapparata" of *M. hirundinaceus*. Even the best of these works fail to diagram the progressive changes that occur in the musculature or to illustrate the relationship of these muscles. Additional difficulties are encountered by nomenclature shifts that occur with the same muscle. Rauther (1930) refers to a muscle as "the proboscis sheath which consists of a one layered, very thick muscle laminus" and in a diagram labels this same muscles as contractile "Rinde" of receptacle. Problems in translation may account for some of these difficulties, but the use of such terms as retractor, invertor, or invaginator for the same muscle have compounded it further. The term which best describes the muscle and its function is not always evident. We have tried to use those terms first proposed for a muscle and hope that the illustrations will give added clarity.

Materials and Methods

Acanthocephala, along with intestinal contents, were collected from swine at Hunter Packing Company, East St. Louis, Illinois, and transported to the laboratory in Dewar flasks. Preparation of the worms for scanning electron microscopy has been explained by Miller and Dunagan (1971). The internal musculature of the praesoma was observed by cutting a small longitudinal slit in the body wall an inch or more below the praesoma. The praesoma was then inverted through this hole by adding gentle pressure to a blunt nontapered 20-gauge

[1] This work was supported by the National Institutes of Health Research Grant NB 08583 and the Graduate School of Southern Illinois University.
The authors are indebted to Mr. Ruu Tong Wang for assistance in sectioning and preparation of SEM material.

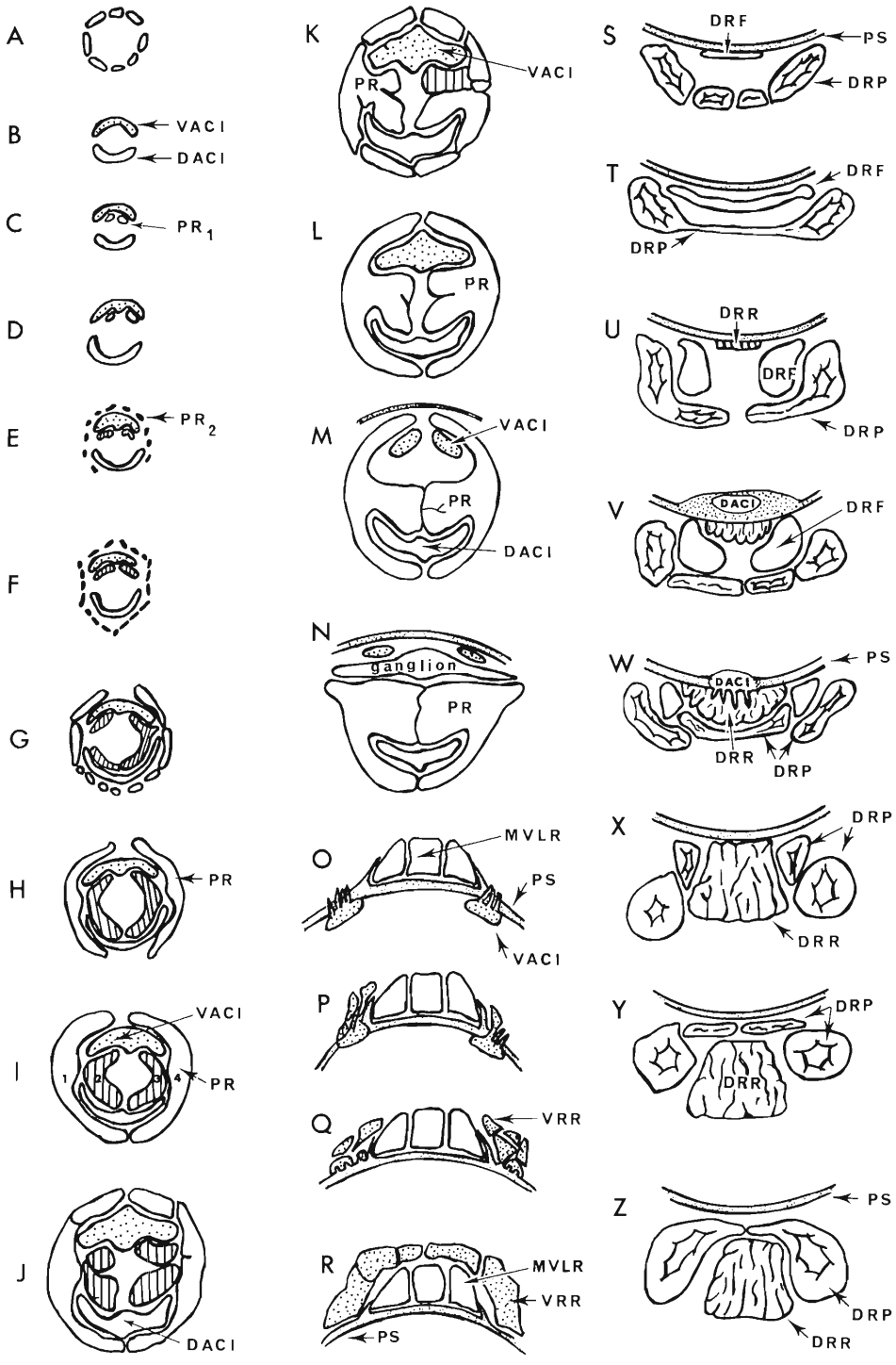


Table 1.

Abbr.	Muscle
DACI	Dorsal apical cone invertor
VACI	Ventral apical cone invertor
PR-1	Proboscis retractor—part one
PR-2	Proboscis retractor—part two
PR	Proboscis retractor—composite
MVLR	Midventral longitudinal receptacle muscle
VRR	Ventral receptacle retractor
DRP	Dorsal receptacle protrusor
DRF	Dorsal receptacle flexor
DRR	Dorsal receptacle retractor
HR	Hook retractor
PC	Proboscis circular
RW	Receptacle wall muscle
VLR	Ventral longitudinal retractor
VRP	Ventral receptacle protrusor
LRP	Lateral receptacle protrusor
R	Retinacular muscle
M	Medullary or proboscis fluid

needle which had been applied to the apex of the proboscis. As the proboscis inverted, the pseudocoel contents were lost and the inside of the body wall became the outermost layer. This process exposed the musculature as well as the surface features of the proboscis receptacle and body wall.

For light microscopy the worms were fixed for 12 hr in a solution of 0.15% formalin containing 0.03 M bromoacetate and 0.3 M sucrose, and then were moved into a glycerol–water series up to 50% glycerol. After overnight storage or longer in 50% glycerol, the specimens were dehydrated, embedded in 56 C wax, and sectioned in the usual manner. Sections were stained with either hematoxylin–eosin, toluidine blue, or PAS–Alcian blue.

Results

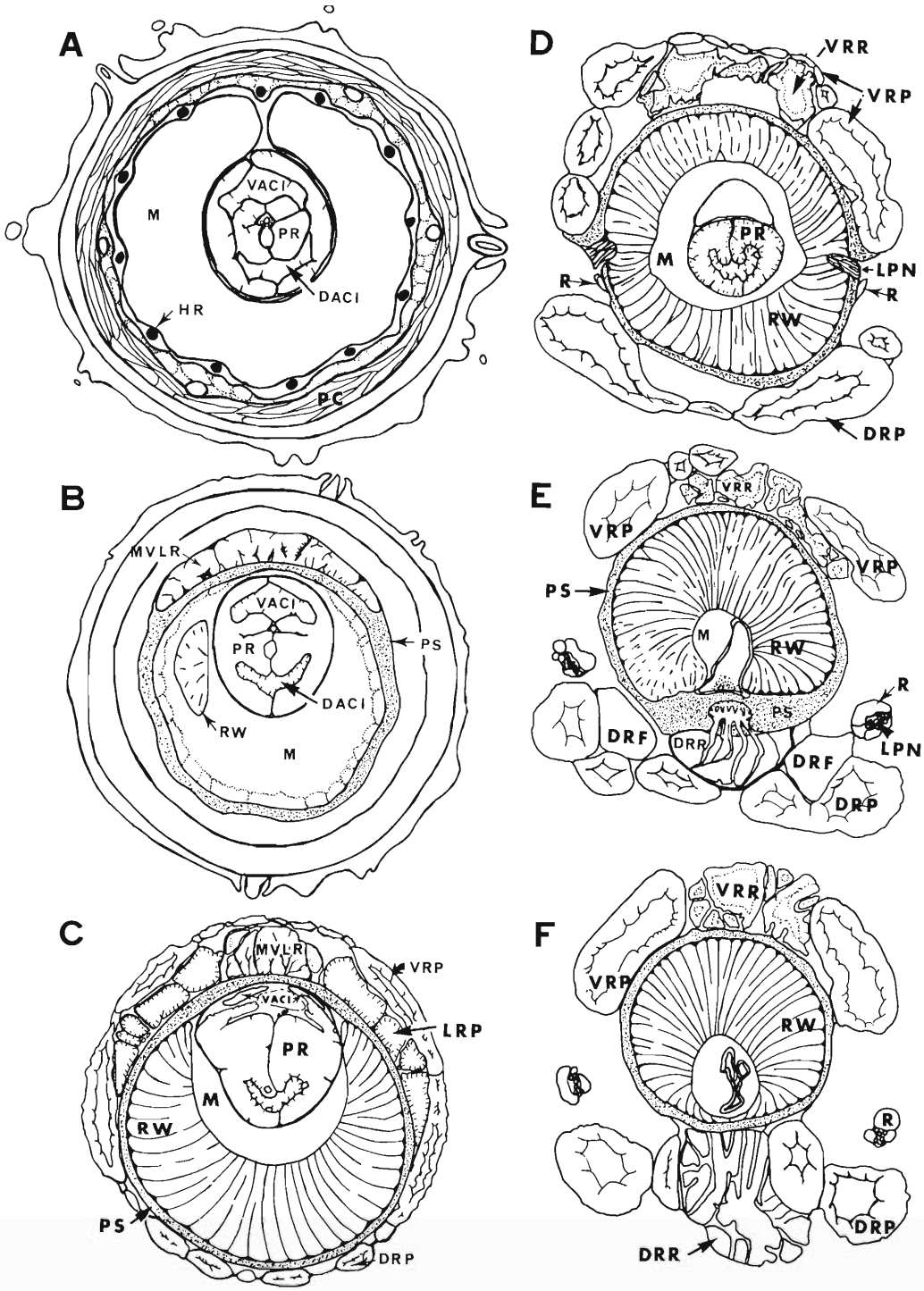
A complete listing of the names and abbreviations adopted for the muscles discussed in this study is contained in Table 1.

The summit of the inverted globose proboscis is crowned with the apical sensory device, a cone-shaped structure consisting of sensory receptors and associated nerves. Along the entire outer margin of the cone, longitudinal muscles arise and quickly divide into a dorsal and ventral group. These are the first muscles to appear in a serial section of an everted proboscis. Because of their attachment to the apical cone, we propose the names dorsal (DACI) and ventral apical cone invertors (VACI) for these muscles. Previously they have been included with the proboscis retractors. While other muscles function in the inversion of the proboscis, these appear to control the position of the apical cone. Also, the proboscis retractors insert on the receptacle wall, whereas both DACI and VACI continue into the receptacle retractors (Fig. 1).

About 60 μ from the apex and medial to the dorsal and ventral apical cone invertors another set of longitudinal muscles (PR_1) attaches on the surface of the apical cone (Fig. 1, C–H). Slightly posterior, another group of longitudinal muscle fibers (PR_2) is attached to the apical ring and encloses VACI, DACI, and PR_1 (Fig. 1–1). Because each of these two new sets of muscles eventually fuse and become indistinguishable (Fig. 1–L) and because of their origin and insertion, we call these the proboscis retractors (PR), a term previously used for these muscles but also applied to the DACI and VACI. They consist of four muscle bundles (Fig. 1–I) occupying lateral positions. The four groups merge into two bands, laterally (Fig. 1, J–L) thicken and completely enclose the apical cone invertors (Fig. 1–L). The proboscis retractors then remain as two separate lateral muscle bundles throughout their remaining length. They disappear on the inside surface of the thick receptacle wall muscle in the posterior part of the recep-

←

Figure 1. A schematic drawing of the praesoma of *M. hirudinaceus* showing some of the muscles associated with the apical cone of the proboscis and the level of appearance of each. No initial distinction is possible (A) between the dorsal apical cone invertors (DACI) and the ventral apical cone invertors (VACI). However, each can be clearly identified in more posterior sections (B). Notice how the VACI divides into two muscles (M) then moves ventrally through the proboscis sheath (PS) and finally becomes (R) a part of the ventral receptacle retractors (VRR). In a similar fashion the DACI pass through (W) the PS but rather than joining an existing muscle bundle, the DACI become the DRR. The proboscis retractors (PR) initially appear as two separate muscle groups (PR-1 and PR-2) which merge (L) into two groups. The appearance of the DRR (S) and the dorsal receptacle flexor (DRF) and their relationship to the dorsal receptacle protrusors (DRP) is illustrated in 1-S to 1-Z.



tacle. Their function is to invert the proboscis into the fluid-filled space, frequently called the Markbeutel (M), which surrounds these muscles (Fig. 2, A–B).

The concentrically arranged hooks occur in four rows of six hooks each. The base of the hooks is laterally connected by a set of dense ring muscles. At each point where a hook occurs, a single longitudinal muscle is also attached to the posterior margin of the hook base and its associated ring muscle. These fibers, called the hook retractor muscles (HR), maintain their separate identity (Fig. 2–A) until they disappear on the proboscis receptacle sheath (also called the proboscis sheath). All insertions are complete by the time of the appearance of the midventral longitudinal receptacle muscle. The function of the hook ring muscle is to give stability to the hooks in an everted proboscis, whereas the hook retractor muscles aid in the withdrawal of the hook from an attachment. The posterior row of hooks does not have longitudinal hook retractors, but apparently is operated by the invagination of the proboscis and perhaps by the proboscis circular muscles which are not very thick at this point.

At the posteriormost level of the hooks, the ventral portion of the proboscis sheath thickens and the midventral longitudinal receptacle muscle (MVLR) originates. At its origin this muscle appears to be enclosed by the proboscis sheath, but 20μ posteriorly (Fig. 2-B) the MVLR is external to the PS and considerably enlarged compared with more anterior sections. It gradually increases in size reaching its greatest diameter at the level of the cephalic ganglion where it also becomes enclosed along the outer surface (Fig. 1-R) by the ventral receptacle retractor muscle (VRR). This muscle has no counterpart along the dorsal surface on the outside of the receptacle. The MVLR disappears (Fig. 2-O) immediately posterior to the ganglion at about the same level where the receptacle wall muscle encircles the proboscis retractors and the apical cone invertors. Hyman (1951, p. 12) speaks of a longitudinal muscle attached to the midventral wall of the receptacle which acts to curve the receptacle ventrally. Rauther (1930) clearly illustrated this muscle which he terms the "aussere Langsmuskeln des Receptaculum." Kaiser (1893a) calls this the ventral catch muscle (Schliebmuskel).

The receptacle wall (RW) muscle appears (Fig. 2-B) at about the level of the lateral sensory devices which are located laterally on the neck posterior to the hooks. The anteriormost part of this muscle is bathed by the proboscis fluid (M) which apparently communicates throughout the length of the proboscis receptacle and fills most of the core of the proboscis to the base of the apical cone. The receptacle wall muscle begins (Fig. 2-B) as a very large muscle occupying the identical space of the proboscis fluid. Initially, this muscle has a crescent shape (Fig. 2-C) thickest along the dorsal surface. The "hole" in the "C" is occupied by the apical cone invertors and the proboscis retractors. We speak of these two muscles collectively as the core muscles; they

←

Figure 2. Diagrams of sections of the praesoma of *M. hirudinaceus*. Section "A" from the hook area of the proboscis illustrates the symmetry of position of the hook retractors located external to a fluid-filled area called the "Markbeutel" by early German authors and internal to the proboscis circular muscles. Notice that the proboscis retractor muscles (PR) have not completely merged at this level.

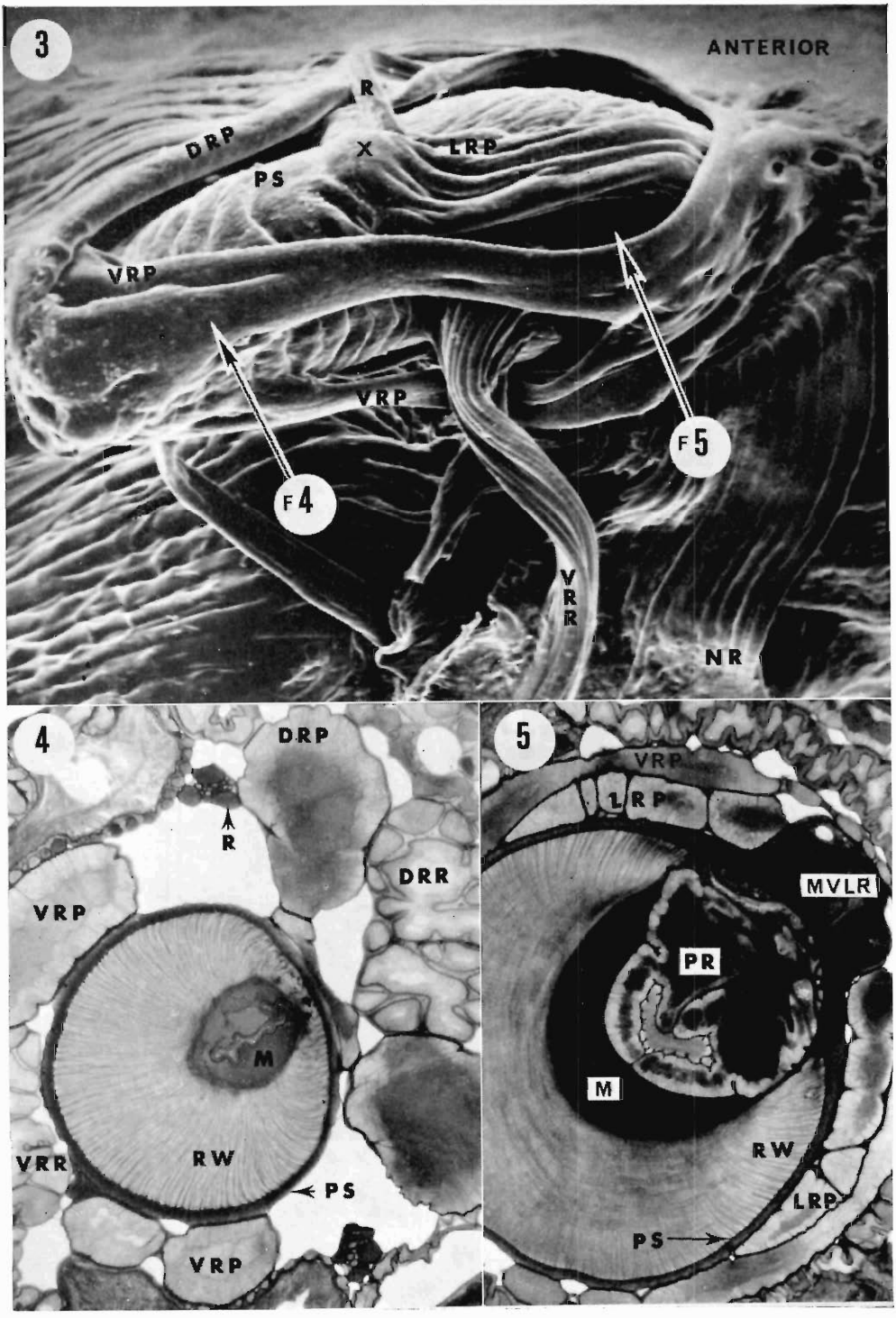
Section "B" taken from the beginning of the neck region shows the beginning of the receptacle wall muscle (RW) in the medullary fluid or Markbeutel. Notice the eccentric position of the core muscles adjacent to the proboscis sheath (PS) on the ventral surface. The ventral longitudinal receptacle muscle (VLR) caps the external surface of the PS.

Section "C" is similar in position to Fig. 5. The RW has almost replaced the medullary fluid. The receptacle retractors have not yet divided into dorsal and ventral groups and the longitudinal receptacle protrusors (LRP) are not only lateral but also largely ventral in position.

Section "D" shows the lateral posterior nerve (LPN) as it leaves the RW. The latter now encloses the core muscles. The retinaculum appears as a single muscle attached to the PS.

Section "E" shows the DACI moving through the PS into the DRR. The LPN is now enclosed by the R.

Section "F" is similar in position to Fig. 4. The protrusors and retractors are most evident.



lie adjacent to the proboscis sheath throughout most of their length. The proboscis fluid is also in contact with the medial surface of this muscle throughout its length (Fig. 2, A-E). Posterior to the ganglion, the receptacle wall muscle moves ventrally and completely encompasses the core muscles (Fig. 2-D).

The lateral posterior nerves (LPN) angle posteriorly through the receptacle wall muscle. Before they reach the outer surface of the proboscis sheath (Fig. 2-D) the proboscis retractor muscles insert on the medial surface of the receptacle wall muscle. The single remaining muscle, the dorsal apical cone invertor, gradually moves dorsally and, as previously mentioned, also goes through the dorsal proboscis sheath (Figs. 1, V-W; 2-E) and merges with the dorsal receptacle retractor muscle (DRR). The space formerly occupied by the core muscles is now filled by a membrane-enclosed fluid that frequently is granular in appearance and which occasionally reaches to the posterior level of the cerebral ganglion.

The histology of the receptacle wall muscle is unique in that it is perforated throughout by a tubular system which radiates from the ventral longitudinal axis of this muscle. Kilian (1932) discusses some of the unique features of this muscle and illustrates the organization of some of its components. Electron micrographs of this muscle show these as patent tubes that are filled with fluid (unpublished observations). PAS stains this fluid a deep red color similar to the color of the fluid in the anterior proboscis indicating that the material is rich in carbohydrates with 1, 2 glycol structure. By contracting, this muscle could cause eversion of the proboscis by exerting pressure on the fluid contents of the receptaculum.

The proboscis circular muscles (PC) appear at the posterior level of the root of the second row of hooks and immediately become four or five fibers wide (Fig. 2-A). These muscle fibers seem to be woven together in a thick pad anteriorly and a thin pad posteriorly and are associated with the epidermis of the proboscis. They disappear posterior to the last row of hooks at the beginning of the neck.

The lateral receptacle protrusors (LRP) originate in a narrow band on the medial surface of the ventral wall posterior to the lateral sensory organs and at the junction of the neck and trunk (Fig. 3-O). They consist of two muscle groups which are separated by the MVLR (Fig. 5). Each group is medial (Figs. 2-C, 5) to the ventral receptacle protrusors (VRP) and inserts on each lateral surface of the proboscis sheath (PS) approximately halfway along the length of the receptacle (Fig. 3-X). Initially, they consist of several muscles which cover the ventral and lateral surface of the receptacle. These anastomose throughout their length and finally form two separate muscles on each lateral surface of the PS.

The dorsal (DRP) and ventral receptacle protrusors (VRP) originate in the same vicinity

←

Figure 3. A scanning electron micrograph of an inverted proboscis receptacle and associated muscles. The following muscles are evident: (1) lateral receptacle protrusors (LRP). Note that they insert on the proboscis sheath at "X"; (2) retinacula (R); (3) neck retractors (NR); (4) ventral receptacle retractor (VRR); (5) dorsal receptacle retractor (DRR); (6) ventral receptacle protrusor (VRP). Note that the ligament sac is missing from its attachment to the posterior part of the protrusor muscles. The reader should be cautioned that some muscle positions are distorted because of the inversion of the proboscis. An example of this is the separation of the DRP and the VRP from F-5 anteriorly. The DRP and VRP normally separate at F-5. Light microscopy sections of the receptacle taken from areas F-4 and F-5 are depicted in Fig. 4 and 5 respectively.

Figure 4. A cross section of the proboscis receptacle at level F-4 in Fig. 3. This section is similar in position to that depicted in Fig. 2-F. The tubular elements are very evident in the receptacle wall muscle (RW) which is completely enclosed by the proboscis sheath (PS). Notice that each lateral posterior nerve and its accompanying retinacular muscle (R) is some distance from the receptacle wall at this level of sectioning.

Figure 5. A cross section of the proboscis receptacle at level F-5 in Fig. 3. This section is similar in position to that depicted in Fig. 2-C. Notice the different staining characteristics between the different muscles. This is particularly clear between the midventral longitudinal receptacle muscle (MVLR) and the lateral receptacle protrusors (LRP).

as the lateral receptacle protrusors but external to them. They completely enclose the proboscis receptacle almost to the level of the cerebral ganglion (Fig. 2-C). This enclosure by these muscles is also seen (Fig. 3) at the anterior end of the receptacle. It would normally extend to about ⅓ the length of the receptacle but the DRP and VRP have been separated by the SEM preparation and are depicted by this figure as only enclosing the entire receptacle for a short distance (anterior ⅕). The receptacle protrusors are clearly divided into two broad sheets of anastomosing muscle which cover the dorsal and ventral surface of the receptacle. Posterior to the appearance of the dorsal and ventral receptacle retractors this sheet further divides into two dorsal and two ventral groups of muscles (Fig. 2-E, F). As the ventral and dorsal receptacle retractors move posteriorly, they gradually move away from the receptacle. The protrusors then move medial to the retractors and at the posterior extremity of the receptacle they again enclose the receptacle and insert along its posterior margin forming a cap in the process (Fig. 3). It is on this cap that the ligament sac originates.

The ventral receptacle retractor (VRR) originates anterior (Fig. 2-D) to the dorsal receptacle retractor (DRR) (Fig. 2-E) (compare Fig. 1-Q with 1-U) and is the ventral apical cone invertor which has passed through the proboscis sheath (Fig. 1, O–R). Therefore, when the MVLR disappears (Fig. 2-D), the VRR occupies its space (Fig. 2-E). The VRR inserts on the trunk wall some distance below the posterior margin of the receptacle. On the other hand the DRR is not the posterior extension of the dorsal apical cone invertor in the same way as VRR is the extension of VACI. The DACI passes through the proboscis sheath (Fig. 1-W) at the level of the insertion of the lateral receptacle protrusors (Fig. 3-X). Thus the DACI joins an existing muscle (DRR) and in so doing loses its identity therein (Fig. 1, W–Z). The DRR moves through an opening formed by the separation of the dorsal receptacle protrusors. This opening is similar to that formed by the VRP for the VRR (Fig. 3). Before attaching to the trunk wall, both VRR and DRR divide again into a right and left muscle group. During isolation, this division is frequently extended almost to the muscle's origin. Goeze (1782), according to Kaiser (1893a), called the two muscle bands which stretch between the "rounded end of the receptacle" and the body wall the "hetractores receptaculi."

Another muscle, the dorsal receptacle flexor (DRF), originates on the middorsal surface of the proboscis sheath. The internal structure of the DRF stains with PAS in a manner similar to that of the muscles of the retinaculum. DRF appears at about the same level as the exit of the lateral posterior nerves from the receptacle and is largest at the level where the DACI joins the DRR (Fig. 1-W). This muscle initially occupies the dorsal surface of the proboscis sheath medial to the DRP. The DRF divides into two separate muscles at the level of the origin of the DRR (Fig. 1–4). Throughout its existence it is in close proximity to the DRR and eventually inserts on the medial surface of this muscle prior to the formation of the receptacle cap.

The two retinacular muscles (R) originate on the dorsolateral surfaces of the proboscis sheath just anterior to the exit of the two lateral posterior nerves from the receptacle (Fig. 2-D). Each encloses the neurons from the LPN and accompanies them (Fig. 4) through the pseudocoel from receptacle to the trunk. During this passage, each retinacular muscle will consist of from two to eight muscle bundles (Fig. 2, E–F) which anastomose regularly. This enclosure of a nerve by a special group of muscles is one of the unique features of *M. hirudinaceus*.

The neck retractors (NR) originate along the body wall of the anterior neck (Fig. 3). They consist of two lateral muscles which enclose the receptacle protrusors and terminate in the dorsoventral plane of the body wall. These prominent longitudinal muscles press against the lemnisci at their origin and for a small distance into the pseudocoel. Kaiser (1893a) refers to them as the "retractor colli."

Discussion

As early as 1808, Rudolphi reviewed the body wall musculature of certain acanthocephala and pointed out that it was not a homogeneous fibrous tissue as previously suggested by Goeze (1782) but constructed of longitudinal and circular muscles. Westrumb (1821), ac-

cording to Kaiser (1893a), added greater detail to these earlier descriptions and indicated that these muscles consisted of a continuous layer of strongly branching and anastomosing fibers forming a muscle cylinder. This large network of muscle fibers in the praesomal area of the trunk wall is readily observed (Fig. 5).

Descriptions of the musculature of *M. hirudinaceus*, then called *Echinorhynchus gigas*, differed from those of other species. Cloquet (1824), according to Kaiser (1893a), clearly pointed out that the circular muscles of the trunk wall did not form a coherent sheath but existed in girdles or belts. He, as well as the previously mentioned authors, also discussed the movement of the proboscis. They listed five pairs of muscles including the receptacle protrusors and receptacle retractors. Later authors (Schneider, 1868; Hamann, 1891; etc.) refined the earlier descriptions and through the use of histological preparations added new dimensions to our understanding of the proboscis apparatus. Kaiser in particular is recognized for his lengthy but excellent monograph on the Acanthocephala which appeared in two parts: the first in 1891 and the second in 1893 although both are dated 1893 in the literature. It is in his 1891 (see Kaiser, 1893a) publication that he discussed muscles using a number of illustrations of histological material.

Terminology for these muscles varied with each of the early investigators. This use of several different names for the same muscle has resulted in some difficulty in deciding on the proper term to select. Rauther (1830) is particularly difficult to understand. Perhaps it is because he has written a review article and must be sufficiently general to include all species in question. Nevertheless, he indicates proboscis sheath in Gigantorhynchidae consists of a very thick muscle laminus and equates "Die Russelscheide (Receptaculum)" or proboscis sheath with the receptacle. Yet in his illustration (Fig. 493) he labels this muscle as "kontraktile Rinde des Receptaculum" and speaks of the covering of this muscle as "Bindegewebs hulle des Receptaculum." Kaiser (1893a, Fig. 1–1) labels this same material "Fibrillenplatten des Receptaculum" and calls the covering "Sarkolemma-hullmembran des Receptaculum." Kilian (1932, Figs. 10, 16, 23) labels this identical muscle in *H. microcephala* as "Receptaculum proboscidis" and Hyman (1951, Fig. 9) uses Kilian's illustration but labels the muscle as "Dorsal Receptacle Wall." We think the receptacle wall muscle is a meaningful term and have chosen to use it throughout this article although it should be clear to the reader that many other choices were possible. We label the covering of this muscle the proboscis sheath. It is composed of dense connective tissue and has no contractile role. As viewed in whole mount of fresh unfixed material, this sheath appears as a silver-colored mantel with recognizable oblique striations. It forms an excellent protective covering and is very difficult to penetrate even with microelectrodes.

Kaiser (1893a) did not consider the proboscis retractors to be mere extensions of the receptacle retractors and cautions the reader that the separateness of these muscles might be overlooked "at first glance." Regarding the termination of these two muscles, Kaiser also states p. 98): "these (receptacle retractors) insert into the protruding end of the proboscis retractors." Figure 2-E shows one such insertion. We believe that the proboscis sheath does provide the mechanical basis of an origin and insertion for these two muscles. However, we disagree with Kaiser that there is no continuation of fibers through the sheath. To the contrary we agree with some of the later German investigators (i.e., Rauther, 1930) who stated that these muscle fibers penetrate the proboscis sheath. However, this feature is sufficiently evident that few authors who have studied these muscles fail to mention its occurrence.

Finally, some comment is in order concerning the fluid which bathes the core muscles and extends from the apical cone to the posterior part of the receptacle. The early German investigators have consistently called this substance the "markbeutel," a term which has also been applied to parts of the body wall. For example, Kaiser (1893a, p. 67) states: "Markbeutels are a peculiarity of the integumentary muscle fibers of all Echinorhychen and correspond completely with the cuticular appendages which distinguish the Colomyaren Nematodes. Evidently these arrangements have the purpose of resorbing the chyme surrounding the fi-

ber. . . ." Thus we have a term which has been applied to both a space and the contents of a space. This area is also occasionally labeled "Markraum des Russelcheide" which seems to be an appropriate term and is incorporated into this article. That still leaves the question of the fluid contents of this space unresolved in the older literature. We propose simply to call this carbohydrate-rich fluid the medullary fluid. To one degree or another this fluid communicates from the apical sensory cone to the posterior part of the receptacle. Histological sections of several of the muscles associated with the receptacle also stain the same as the medullary fluid and have about the same degree of cellular organization.

Literature Cited

*Cloquet, J. G. 1824. Anatomie des vers intestinaux ascaride lumbricoide et echinorhynque giant. Memoire couronne par l'Academie royale des sciences qui en avait mis le sujet au concours pour l'annee 1818. Paris, p. 1–130.

*Goeze, J. A. E. 1782. Versuch einer Naturgeschichte der Eingewidewurmer thierischer Korper, Blankenburg, p. 1–471.

Greeff, R. 1864. Untersuchungen uber den Bau und die Naturgeschichte von *Echinorhynchus miliarius* Zenker (*E. polymorphus*). Arch. Naturg., Berlin, 30, Bd. J. 1, p. 98–140.

Hamann, O. 1891. Monographie der Acanthocephalan (Echinorhynchen). Ihre Entwickelung, Histogenie, Anatomie, nebst Beitragen zur Systematik und Biologie. Jenaische Ztschr. Naturwiss. 25: 113–231.

* Original publication not seen.

Hyman, L. H. 1951. The Invertebrates. Vol. III: Acanthocephala, Aschelminthes, and Entoprocta. McGraw-Hill, New York.

Kaiser, J. E. 1893a. Die Acanthocephalen und ihre Entwickelung. 1. Theil, 3, Cassel. (Leuckart, K. G. F. R., and Chun, C., Biblioth. Zool. Heft 7.)

———. 1893b. Die Acanthocephalen und ihre Entwickelung. 2. Theil. Cassel. (Leuckart, K. G. F. R., and Chun, C., Biblioth. Zool. Heft 7.)

Kilian, R. 1932. Zur Morphologie und Systematik der Giganthorhynchidae (Acanthocephala). Z. Wiss. Zool. 141: 246–345.

Miller, D. M. and T. T. Dunagan. 1971. Studies on the rostellar hooks of *Macracanthorhynchus hirudinaceus* (Acanthocephala) from swine. Trans. Amer. Microscop. Soc. 90: 329–335.

Nicholas, W. L. 1967. The Biology of Acanthocephala. Advances in Parasitology, p. 209. Academic Press, New York.

Rauther, M. 1930. Sechste Klasse des Cladus Nemathelminthes Acanthocephala-Kratzwurmer. Handbuch der Zoologie. 2 Band, 1 Halfte, p. 449–482. Ed. by Willy Kukenthal and T. Krumbach.

Rudolphi, C. A. 1808. Entozoorum siue vermium intestinalium historia naturalis. Amstelaedami 1: 221.

Schneider, A. F. 1868. Ueber den Bau der Acanthocephalen. Arch. Anat., Physiol. u. Wissensch. Med. 5: 584–597.

*Westrumb, A. H. L. 1821. De helminthibus acanthocephalis. Commentatio historicao-anatomica adnexo recensu animalium, in Museo Vindobonensi circa helminthes dissectorum et singularum specierum harum in illis repertarum. Hanoverae, p. 1–85.

Yamaguti, S. 1963. Systema Helminthum. Vol. 5. Acanthocephala. Interscience Publ. 1–423.

Fine Structure of the Larval Stage of *Paragordius varius* (Leidy, 1851) (Gordiodea: Paragordidae). I. The Preseptum

JOHN E. ZAPOTOSKY[1]
Department of Zoology, Ohio State University, Columbus, Ohio

ABSTRACT: The structure of the larval stage of *Paragordius varius* has been examined by means of electron microscopy. The larval body is divided into two main areas, a preseptum (anterior) and a postseptum (posterior). Internally, these areas are separated by a septal complex consisting of a conical basal lamella enclosing six septal cells. The body wall consists of a definite cuticle and underlying hypodermis. The most salient features of the preseptum include an armature or "boring organ" (spines and proboscis), and three sets of muscles employed in the operation of this organ.

Using conventional light microscopy, the bulk of the material published on gordioid larvae is concerned with external morphology. Much of this early work is plagued by misidentification and conflicting interpretation.

The first description of a gordiod larva is attributed to Grube in 1849. One year later, Leidy (1850) noted the larval form of *Paragordius varius* (mistakenly identified as *Gordius aquaticus*). The same larval stage was redescribed in more detail by Leidy (1870), again under the name *G. aquaticus*. The embryology and a more accurate anatomical study was done by Montgomery (1904), but with errors in the description of both the armature and internal organs. More authoritative works were later published by Muldorf (1914) and Dorier (1930) for the larva of *G. aquaticus*, and by Inoue (1958) for the embryology and larval structure of *Chordodes japonensis*.

In this study the electron microscope was employed to obtain a more accurate concept of the larval anatomy of *P. varius*. This will serve as a basis for later studies of the developmental cycle and systematics of this parasite and give an insight into the functional morphology of the larval form.

Materials and Methods

Larval stages of *Paragordius varius* were collected by incubating eggstrings collected from two ovipositing females. The mating adults were fixed and permanent slides were prepared, so as to accurately identify the species. Eggstrings with fully developed larvae (i.e., armature completely developed and cuticularized, two refringent granules in pseudointestine) were fixed in ice-cold phosphate-buffered 1% osmium tetroxide (pH 7.2) for 4 hr. The specimens were dehydrated in a graded series of ethanol. Following two changes of 100% ethanol, the larvae were placed in a graded series of epoxy resin (Spurr, 1969) concentrations: 3 parts 100% ethanol–1 part resin (30 min); 1–1 (30 min); 1–3 (30 min); and full-strength resin (20 hr). Specimens in embedding medium were placed in molds and kept at 75 C for 2 days to affect polymerization. Blocks were cut with glass knives on a Sorvall Porter–Blum MT-2 ultramicrotome. Sections showing silver (0.06–0.9 μ thick) interference colors were collected on 200-mesh, noncoated copper grids. Tissue sections were counterstained with uranyl acetate and lead citrate, then examined with an RCA EMU 3G electron microscope.

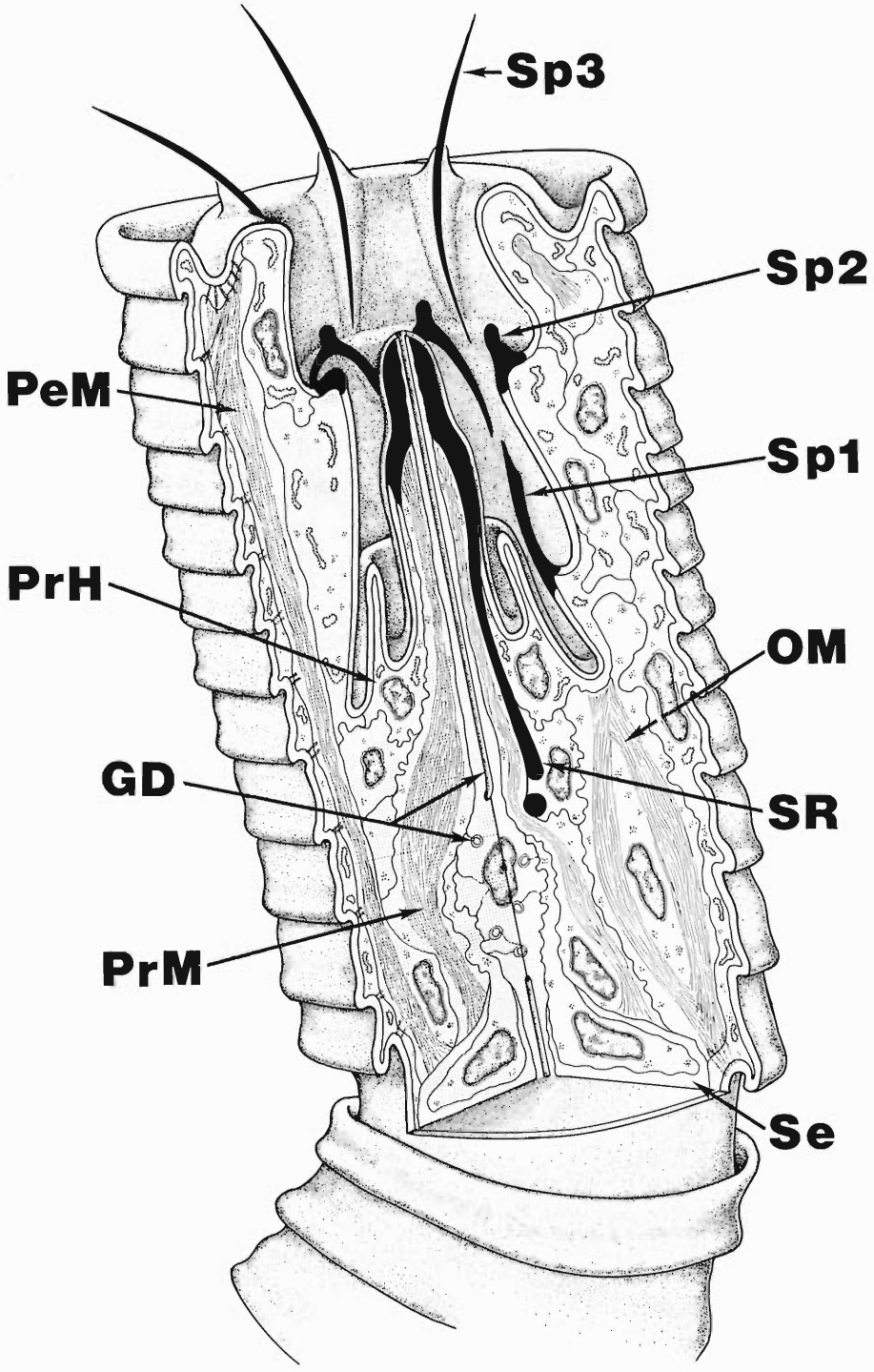
Observations

General features

The larval body of *Paragordius varius* is divided into two major areas, a preseptum and a postseptum (terminology of Dorier, 1930). Externally the body is constricted between the two areas (Fig. 2) while internally there is a conical septal complex separating these divisions (Fig. 1).

The preseptum can be further subdivided (adapted from Muldorf, 1914) into two regions, the postacanthal region and the "boring organ" or perforating apparatus. The postacanthal region (16–23 μ long and 12–15 μ diameter, when the "boring organ" is retracted) is the annulated portion of the preseptum. The

[1] Present address: F. T. Stone Laboratory, Ohio State University, Put-In-Bay, Ohio 43456.



nonannulate perforating apparatus can be retracted into the preseptum, thus forming a median, invagination pocket. The perforating apparatus consists of an acanthal region (8–10 μ long and slightly larger in diameter than the postacanthal region when everted) and a proboscis (10–11 μ long) (Fig. 3).

Body wall

There is a definite cuticular covering over the entire larval body of *P. varius* and it is composed of three distinct layers (Fig. 12). The internal layer contains fibrils. This layer varies in thickness with the number of fibrils present; in the postacanthal region (4–5 fibrils, total thickness 0.18 μ) and in the "boring organ" (1–3 fibrils, total thickness 0.07 μ). Exteriad to the fibrillar layer of the cuticle is an osmiophilic middle layer (100 Å) enveloped by an indistinct "outer" layer (200 Å). The latter two areas appear to be consistant in form and thickness over the entire surface of the cuticle, including the spines (Fig. 13).

Hypodermal cells lie beneath the cuticle, except on the most anterior portion of the proboscis. In general, the hypodermis follows the contours of the cuticle and is separated from it by a distinct plasma membrane. Along the postacanthal region of the preseptum, the nuclei of the hypodermal cells are located interiad to the parietal muscles (Fig. 8). The nucleated portions of the hypodermis are connected to the thin outer layer by narrow connection passing between the muscle cells. In the acanthal region the hypodermal cells and their nuclei are located immediately beneath the cuticle. Six hypodermal cells surround the proboscis from a point halfway up the structure to the end of the acanthal area (Figs. 1, 9). When the proboscis is withdrawn this ring of cells and its overlying cuticle is thrown into a cylindrical-like fold around the proboscis.

Septal complex

The septal complex consists of an osmiophobic intercellular matrix or basal lamella enclosing six septal cells. The septal matrix is generally in the form of a truncated squat cone (when the proboscis is inverted) with the basal portion lying perpendicular to the larva's longitudinal axis and in apposition to the cuticle along its margins (Fig. 1). This matrix is continuous with adjacent preseptal and postseptal intercellular matrices. The six septal cells enclose a median core which consists of a duct and its enveloping cell. The apical portion of the cells is in the form of a projection. Each projection ends in the vicinity of the proximal portion of a proboscial support rod.

All of the major muscle cells of the preseptum impinge on the septal complex. In addition to the proboscial muscles, which sit entirely on the septal complex, portions of the parietal and oblique muscles are found in association with the septum.

Spines

The spines are apparently formed as modifications of the cuticle. All the spines possess two upper layers similar and continuous with the rest of the cuticle (Fig. 13). The lower portions or bulk of the spine appear as a homogenous osmiophilic area, but with some indications of fibrils. As viewed with the light microscope the spines, when fully formed, are brown in color.

The acanthal region of the preseptum carries three crowns or rings of spines on its surface (Figs. 1, 6). The spines in the first ring (Sp 1) (this designation follows the work of previous authors) are adjacent to the acanthal–proboscial interface, while the spines of the third crown (Sp 3) are located near the acanthal–postacanthal interface. When the proboscis is inverted the spines of the first crown

←

Abbreviations (all figures): A, acanthal region of preseptum; C, cuticle; GD, gland duct; H, hypodermis; IP, invagination pocket; OM, oblique muscle; Pa, postacanthal region of the preseptum; Pe, preseptum; PeM, preseptal parietal muscle; Po, postseptum; Pr, proboscis; PrH, proboscial hypoderm; PrM, proboscial muscle; PrS, proboscial sheath; Sc, septum; Sp 1, spine in crown 1; Sp 2, spine in crown 2; Sp 3, spine in crown 3; SR, support rod.

Figure 1. A three-dimensional diagram of the preseptum of the larva of *Paragordius varius*. A section has been removed to show internal structures.

are near the bottom of the invagination pocket and medial to the other crown, while the spines of the third crown are highest in the pocket and external to the other crowns (Fig. 1). The second row of spines (Sp 2) are always intermediate in position to the first and third crowns.

The six spines of the first crown are somewhat sword-shaped. They appear triangular in cross section and are about 7 μ long. Proximally they possess a small core of cytoplasm, while the distal end is free and heavily cuticularized. When the acanthal region is everted the spines of the first crown are located directly over the spines of the second crown.

The spines of the second crown are leaflike structures with a "normal" cuticular covering over the outer surface. The lateral margins are cuticularized edges (Fig. 8) that converge to form a bluntly pointed tip (1 μ long and 0.4 μ in diameter). These spines are estimated to be about 7 μ long, 2.25 μ wide at their base, and 0.85 μ thick.

The third ring of spines alternate in the spaces between the common planes of the first and second crown of spines (Fig. 6). There is a double or Y-shaped spine on the ventral aspect of this crown. The other five spines (6–8 μ long) taper both proximally and distally. There is a protuberance located on the outer midportion area of the spines, giving each spine a free end of about 2 μ (Fig. 13). In cross sections these spines appear club-shaped (enlarged rounded portion on a narrower stalk) and are flanked by two other cuticular projections.

Proboscis

The proboscis is a shuttling organ that can be withdrawn into the preseptum somewhat independently of the acanthal region. It is covered by a nonannulated cuticle and possesses three cuticular support rods which are located around the proboscial periphery. These rods appear somewhat contorted along the length (13–14 μ) of the shaft. The basal end of each rod is widened into a spoon shape with a central teardroplike aperture (Fig. 5). Associated with each aperture is a cell whose nucleus lies within and partially exteriad to the aperture. The basal portion and the shaft of the support rods are externally enveloped by the cells of the proboscial sheath and hypodermis (Figs. 1, 10). Internally the rods lie adjacent to the proboscial musculature, dividing the musculature into three fields (Fig. 9). The muscles in turn envelop a central duct and its associated cell. The distal end of the rods are also expanded and these ends lie in close lateral proximity forming a subcuticular jointed ring (Fig. 4). The cuticle apparently fuses with the rods above the proboscial hypodermis.

Muscles

There are three major groupings of preseptal muscles: the parietal, oblique, and proboscial muscles (Figs. 1, 10). In addition there are apparently small muscles located in the anterior of the postacanthal region. These muscles seem to be in association with the spines of the third crown.

All of the preseptal muscles possess fibrils ranging from 200–700 Å in diameter (most of the fibrils were usually found in the lower part of this range, 220–280 Å in a diameter). No distinct smaller filaments were found adjacent to or surrounding the larger fibrils. As with the postseptal muscles, the oblique and parietal muscles show a direct attachment to the cuticle.

→

Figure 2. Light micrograph of a living larva escaping from egg membrane. × 800.

Figure 3. Phase contrast micrograph of a cleared larva, lateral view, showing the divisions of the preseptum. × 1,000.

Figure 4. Phase contrast micrograph of a living larva, ventral view. × 1,000.

Figure 5. Phase contrast micrograph of a living larva, ventral view. × 1,000.

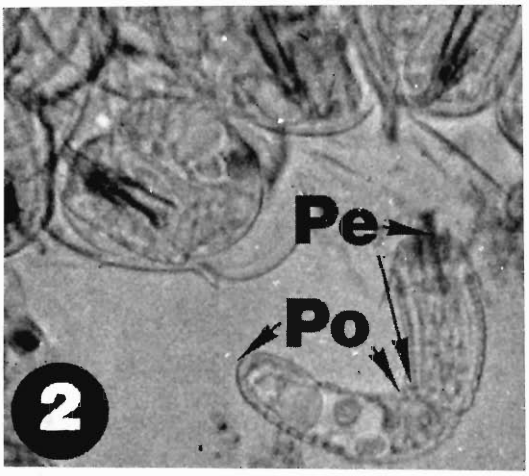
Figure 6. Phase contrast micrograph of a cleared larva, oblique lateral view of the acanthal region. × 1,000.

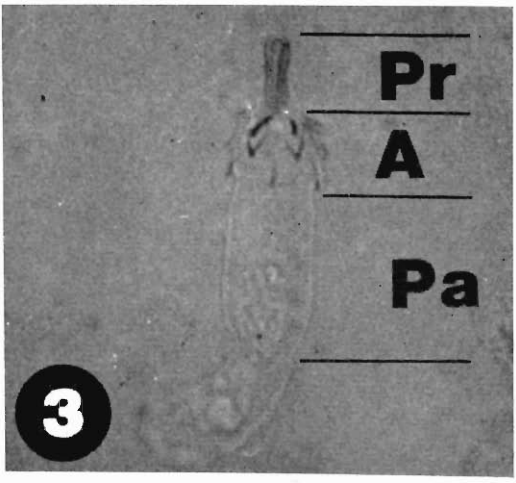
Figure 7. Phase contrast micrograph of a cleared larva, en face view. × 1,000.

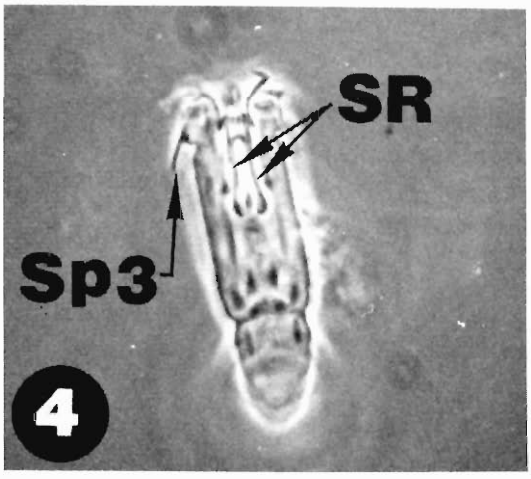
Figure 8. Slightly oblique cross section through the upper portion of preseptum. × 18,000.

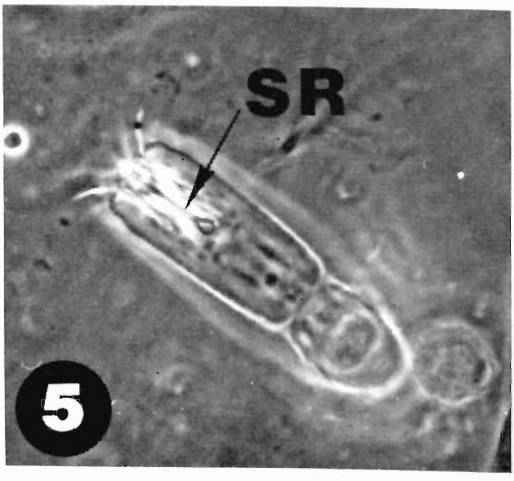
Figure 9. Cross section through the preseptum near proboscial–acanthal region interface. × 14,500.

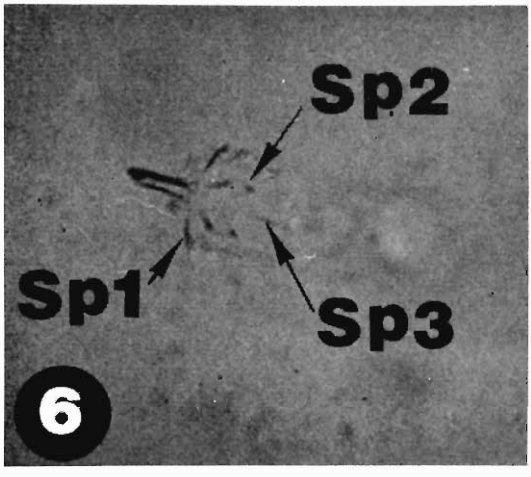
Figure 10. Cross section through the preseptum slightly lower than Figure 9. × 24,000.

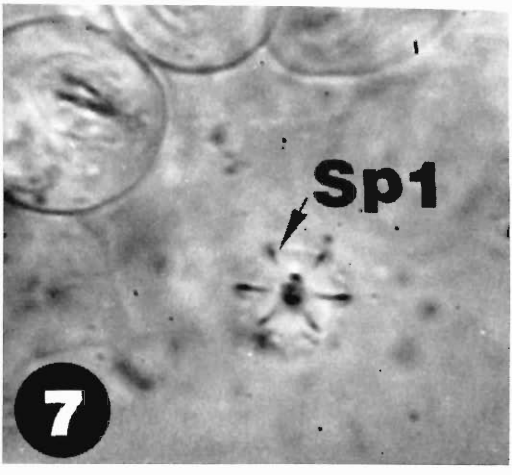


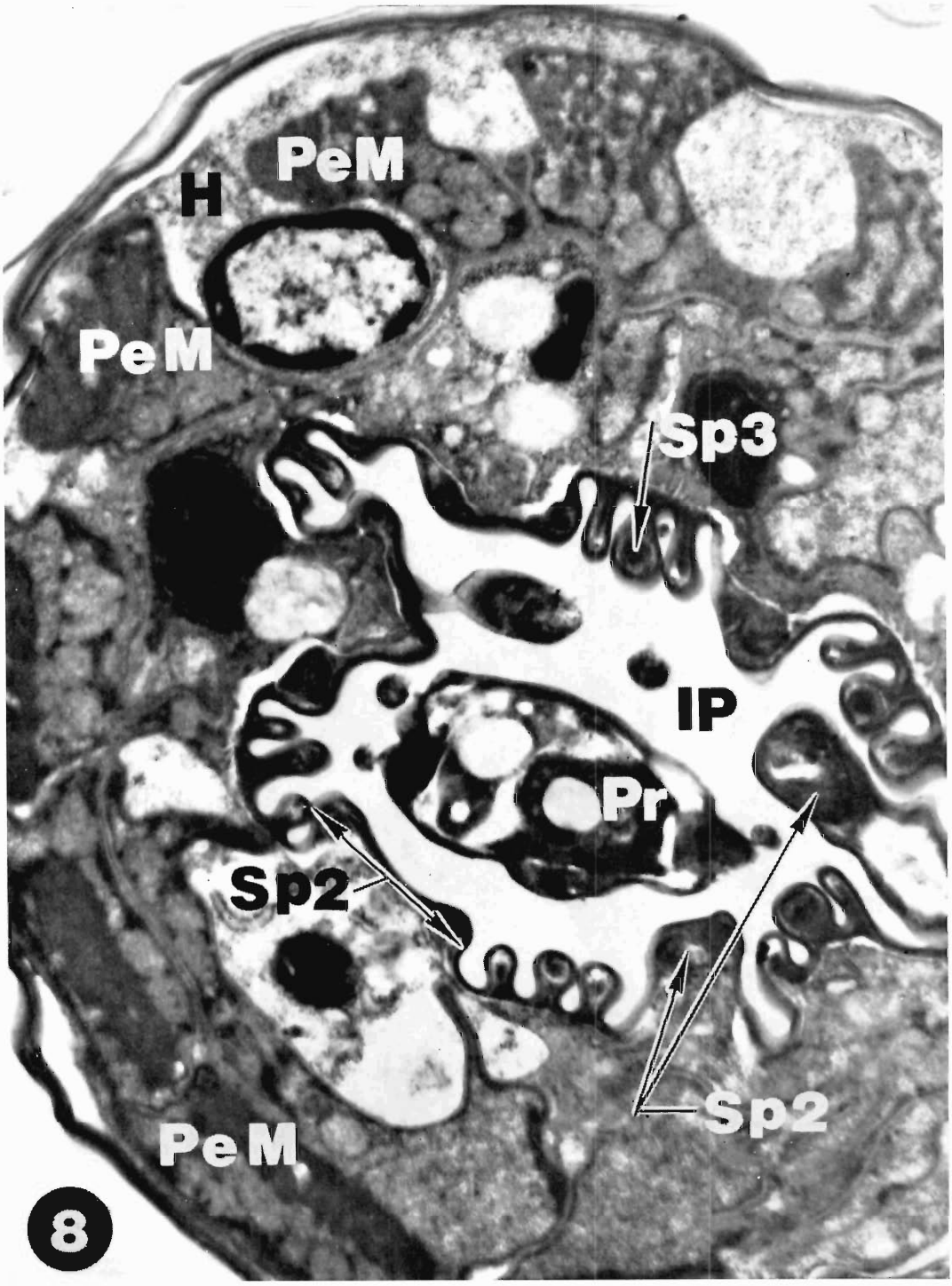


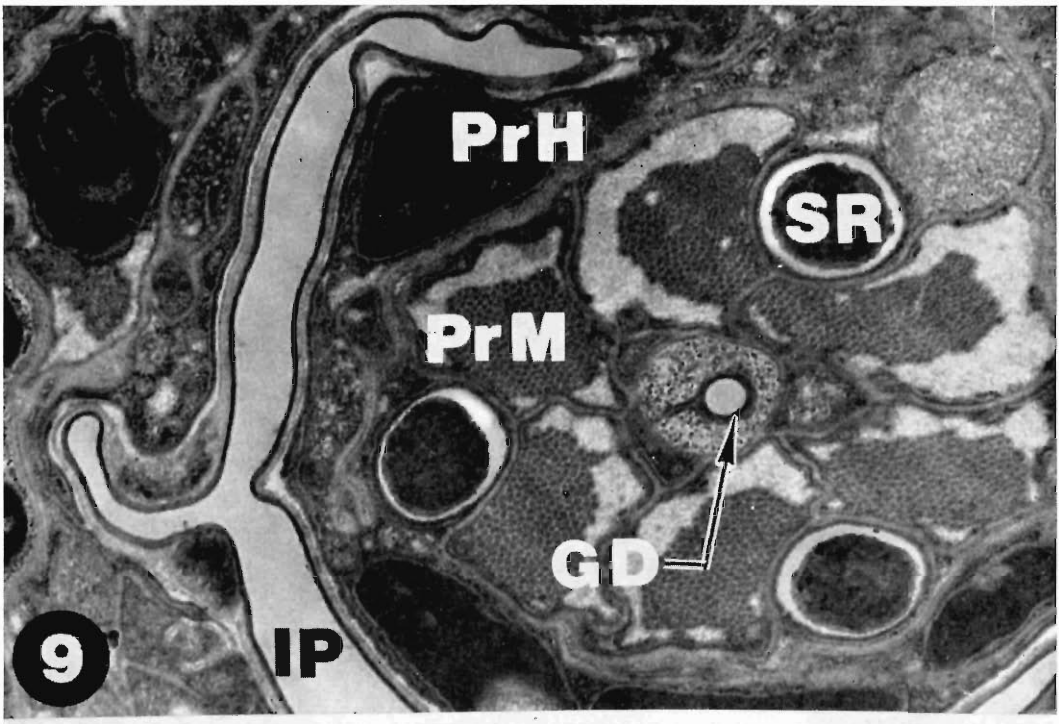


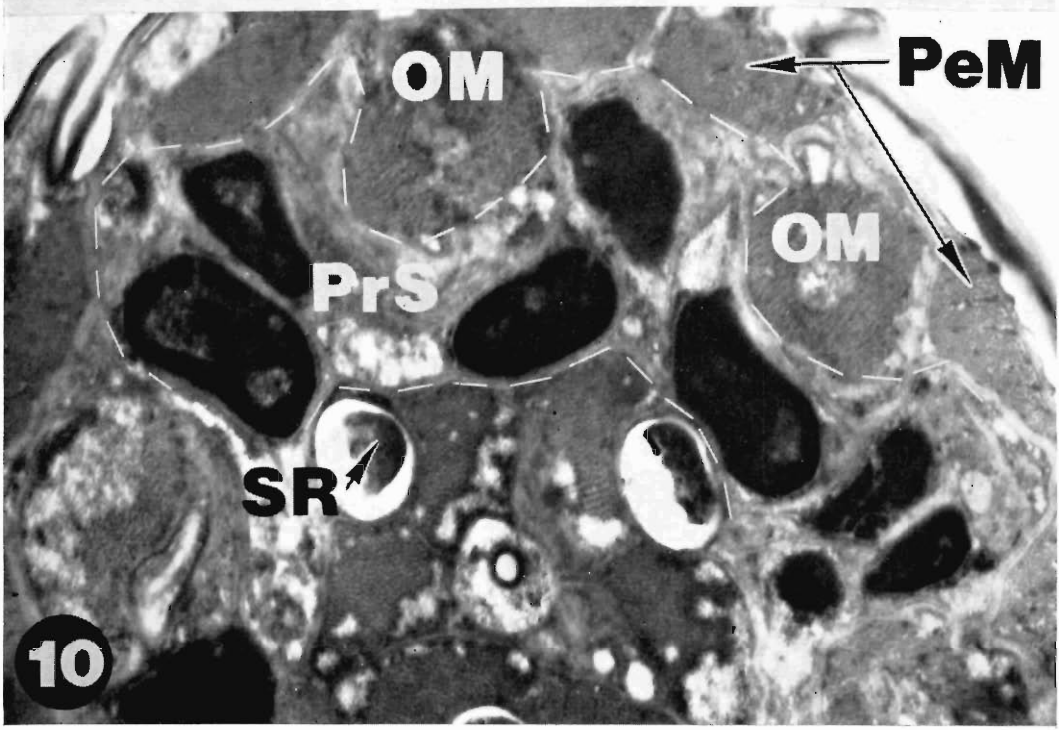


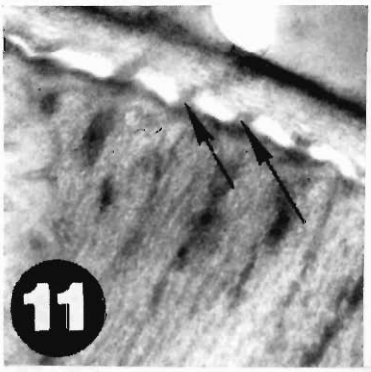


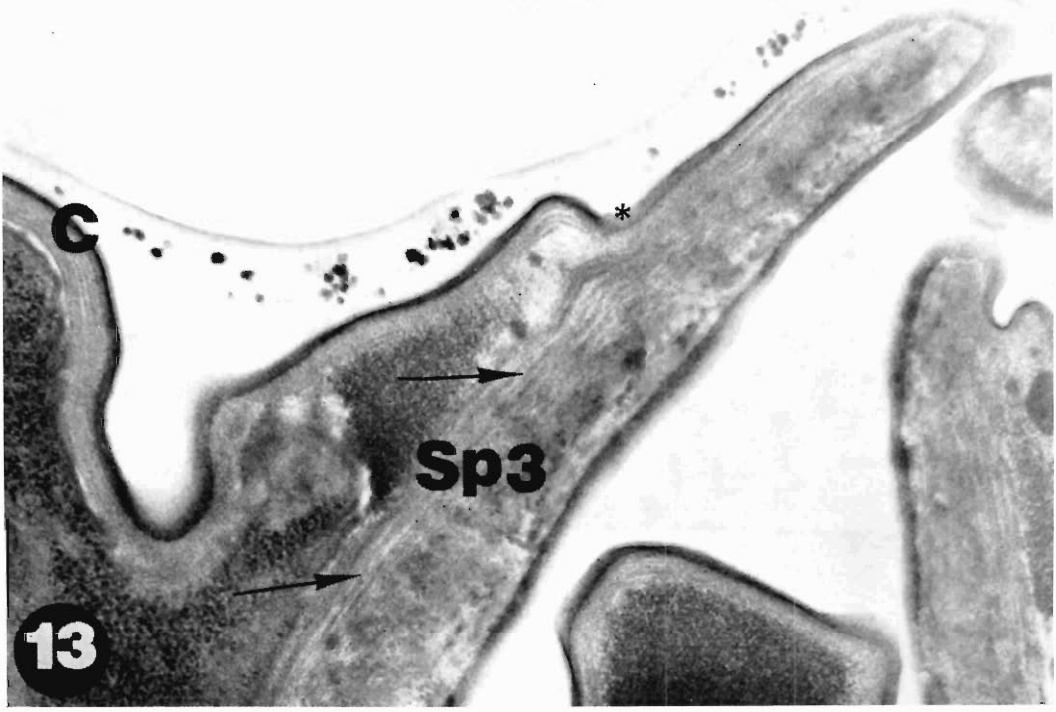












These attachments are most evident with the parietal muscle in sections oblique to the longitudinal axis, and appear as dark, osmiophilic, peglike structures (Fig. 11). The attachments run from the muscle cell through the overlying hypodermis and attach into the lower fibrils of the cuticle. The muscle attachments appear to be most evident at the inner folds of cuticle and may also serve to help anchor the hypodermis.

The 12 parietal or body wall muscles are longitudinal muscles arranged around the periphery of the postacanthal region beneath the hypodermis (Figs. 8, 10). The cells are separated from each other by a clear osmiophobic intracellular space or by a narrow extension of the hypodermis. In the basal portion of the preseptum they are also partially separated by the origins of the oblique muscles. The parietal muscles arise on the lateral portion of the septal complex and posterior portion of the preseptum, inserting in the anterior area of the postacanthal region.

The parietal muscle cells consist of a nucleated, somewhat swollen posterior portion and are filled with numerous granules and fibrils. The majority of the fibrils run in an anteroposterior direction. There are some short fibrils in the anterior portion of the cell which run obliquely from the anterior face of the preseptum (when the proboscis is inverted) to the lateral body wall.

The six oblique or acanthal muscles originate on the posterolateral margins of the postseptal body wall (and perhaps the septum), and insert into the area of the proboscial and acanthal interface (Fig. 1). The oblique muscle cell is round in cross section (Fig. 10) and appears somewhat spindle-shaped with an irregular surface in longitudinal sections (with the boring apparatus retracted). The fibrils are arranged peripherally in the middle portion of the cell but converge at the ends. Thus, a spindle-shaped, fibril-free area is located in the central portion of the cell. The nucleus is located in the fibril-free area. At both the origin and insertion the fibrils converge on a dense osmiophilic zone which is apparently the point of attachment to the cuticle.

The six proboscial muscles make up the core the proboscis (Fig. 9). These muscles originate on the slanted portion of the septal complex (Fig. 1). No micrographs were obtained showing the insertion of the muscles; still, from cross sections of the proboscis it was found these muscles continue far up into the proboscis and probably insert on the expanded distal portions of the support rods. In the basal area the proboscial musculature surrounds the projections of the septal cells and ultimately envelops the central gland duct cells. As the muscles pass up into the proboscis they also partially envelop the support rods and are in turn enclosed at their base by the proboscial sheath and proboscial hypodermis. There also arise along the anterior length of the proboscis three cells which come to separate the proboscial musculature from the gland duct cell. Since no nuclei were observed in light or electron microscopical examination of this distal area, it is assumed these are branches from three of the muscle cells.

Discussion

The division of the larval body into two major areas has been noted by all the earlier authors from Grube in 1849 to this present study. Less consistent has been the naming of the areas. The entire anterior division was called the proboscis by both Montgomery (1904) and Inoue (1958); they referred to the eversible median projection as a component of the proboscial armature. Muldorf (1914) used a variety of terms for this area "prasoma," "praesomatisher Teil," and "pracephalon." He went on to subdivide the "pracephalon" into

←

Figure 11. Cross section through the parietal muscles. Arrows indicate possible muscle attachments. × 36,250.

Figure 12. Longitudinal sections of the preseptal body wall. Arrows indicate fibrils in the lower portion of the cuticle. × 156,200.

Figure 13. Longitudinal section through a spine in the third crown. Arrows indicate fibrils in the lower portion of the spine. The star denotes the continuation of the upper cuticular layer over the surface of the spine. × 36,250.

four regions (named anteriad) "postakanthale Region," "akanthale Region," "Russellbasis," and "Russell" (the eversible projection). Dorier (1930, 1932, 1935, 1965) called this area the "preseptum" dubbing the eversible portion the "trompe."

The terminology used by Dorier is employed here because of his extensive work and the existence of an inner wall or septum separating these two areas. In addition, there is an anatomical basis for accepting three of Muldorf's (1914) subdivisions of the preseptum. The postacanthal region of the preseptum has a folded cuticle which is thicker than that found on the other regions of the preseptum. The parietal muscles are located in this area only. Secondly, the acanthal region bears the spines of the armature and has no underlying muscles. Lastly, the proboscis or "trompe" has a muscular core and is supported by three cuticularized rods. There is no clear internal or external delimitation for the "Russellbasis." The swollen posterior or proximal end of the support rods might be considered but their location is variable depending on the degree of contraction of the musculature. Therefore, here the body is divided into a preseptum and postseptum, the former is further divided into three regions: postacanthal region, acanthal region, and proboscis.

The internal wall separating the larval body into two major divisions has been interpreted in different but similar ways. Both Muldorf (1914) and Dorier (1930) interpreted the septum of *Gordius aquaticus* as a thin transverse partition onto which insert the muscles of the proboscis and those muscles associated with the acanthal region (oblique muscles). They also noted changes in the configuration of the septum with associated movements of the proboscis. In a different manner, Inoue (1958) referred to this body division in *Chordodes japonensis* as a diaphragm consisting of a layer of four cells located in the central area of the larva. Montgomery (1904) cited the presence of a wall with several cells on its anterior surface in *Paragordius varius*. He labeled the entire complex as a diaphragm. Both Inoue (1958) and Montgomery (1904) also cited the apparent attachment of the proboscial and oblique muscle cells in this area. All of the above authors appear to be partially correct in their observations. This study shows the septal complex to consist of six median septal cells surrounded by a "thick" conical intercellular area. The base of this cone apparently corresponds to the septum observed by the earlier authors.

The cuticle covers the entire surface of the larvae and serves as a flexible exoskeleton. It does not, however, prevent the desiccation of larvae as shown by Thorne (1940) for *G. robustus*. Over the postacanthal region of the preseptum and over the postseptum the cuticle is thrown into folds or annulae. These annulae are apparently of great importance in the movements of the body allowing flexions and longitudinal elongations and contractions. The cuticle on the acanthal region and the proboscis is without annulae but is pliable as indicated by the movements of these areas.

The histology of the cuticle and hypodermis of the larvae previously studied have received an understandable, meager treatment by early authors. The body wall of gordioids is presented as consisting of a thin hypodermal lamella covered by a similarly thin outer wall of cuticle. The cuticle of *P. varius* is here shown to consist of three layers. All of the spines appear to be a modification of the cuticle, apparently by the secretion of an osmiophilic substance into the lowest layer of the cuticle. The cuticularizing substance probably gives the spine its distinctive brown color.

The hypodermis arises from the primary ectoderm and the matrix of the cuticle is in turn derived from the hypodermis. In early development the ectoderm is thick but with continuing development of the cuticle is becomes thinner (Muldorf, 1914). In *P. varius* the hypodermis from the septum to the acanthal region is very thin as observed by Montgomery (1904), but the nuclei are more numerous than the few noted by him. In the acanthal region the hypodermis is thicker with a hypodermal cell and nucleus in association with each spine. This association was noted earlier for *P. varius* by Montgomery (1904) and *G. aquaticus* by Muldorf (1914). Inoue (1958) found no nuclei in the hypodermis of *C. japonensis* except in the proboscis sheath and the posterior half of the postseptum.

The armature of gordioid larvae consist of three circular rows of spines and a proboscis

supported by three "stylets" or support rods (Dorier, 1930, 1932, 1935; Muldorf, 1904; May, 1919). The first and second crown of spines are composed of six distinct spines, while the third crown contains "seven" spines. The arrangement of the spines in the third ring has been reported as follows: one dorsal, "two" ventral, two ventrolateral, and two dorsolateral (Dorier, 1930, 1932, 1935; Inoue, 1948; Montgomery, 1904). In *P. varius* the "two" ventral spines are a single wishbone-shaped spine (i.e., one spine with two free tips and a common proximal base).

Dorier (1932, 1935) describes the spines in the first crown of *P. gemmatus, P. alpestris,* and *P. violaceus* as being triangular and possessing thickened superior edge, while in *G. aquaticus* and *P. varius* they are described by Dorier (1930) and Montgomery (1904) as being flattened vertical plates terminating in a point. Inoue (1958) cites what corresponds to this row in *C. japonensis* as being rods. In *P. varius* this spine is triangular in cross section with a long free cuticularized end.

The spines of the second row are reported as being flat triangular plates with thickened edges (Dorier, 1930, 1932, 1935; Muldorf, 1914; Montgomery, 1904). The spines in the second crown in the larvae of *P. varius* fit this description. It would appear, in addition, that only a small lateral portion and the terminus of the spine is free. Montgomery (1904) reports only four spines in the second crown of *P. varius.* Observations made on material here indicate six large leaflike spines in the second crown.

The spines of the third crown in *P. gemmatus, P. alpestris, P. violaceus, G. aquaticus* and *P. varius* are described as being pointed or spurs deeply implanted in a tubercle. Inoue (1958) notes the spines of this crown in *C. janonensis* as being flat and triangular. In *P. varius* these spines are relatively long and narrow. They are not embedded in the body wall but run along the surface of the acanthal region for a distance. Externally there is a raised portion of the body wall (between the spine and postacanthal region) in association with the spine. In addition there is apparently a muscle in association with the basal portion of this spine, this giving it some mobility of its own.

In the larvae of *P. gemmatus, P. alpestris,* and *G. aquaticus* the three "stylets" of the proboscis are located around the periphery of the eversible proboscis. One "stylet" is usually located dorsally and the other two ventrolaterally (Dorier, 1930, 1932, 1935). In *C. japonensis* there is one lateral, one dorsolateral, and one ventrolateral "stylet" (Inoue, 1958). The arrangement of these rods in *P. varius* is not given by Montgomery (1904) and could not be accurately determined here. In *P. varius* the structures are not pointed protrusions and apparently function in the support of the proboscis. Thus, they have been named support rods. In *P. varius* the rods appear amber in color in light microscopic examination suggesting a cuticularization similar to that of the rest of the armature.

The support rods of the proboscis of gordioids are slender rods with either expanded distal and proximal ends or with just expanded distal ends. The expanded proximal ends, when present, are spoon-shaped with either an eyelet or just a depressed area as reported for *P. gemmatus, P. alpestris,* and *P. violaceus* (Dorier, 1932, 1935). The expanded end is absent in *C. japonensis* and *G. aquaticus* (Inoue, 1958; Dorier, 1930). Montgomery (1904) described the proximal portion of the support rods in *P. varius* as possessing a depression on each side, but it is shown here to be a true aperture. In gordioids the distal portion of the rods possess external transverse ridges and several small spines as in *G. aquaticus, P. gemmatus,* and *P. violaceus* (Dorier, 1930, 1932, 1935) or are without spines and have internal transverse ridges as in *P. varius* and *C. japonensis* (Inoue, 1958; Montgomery, 1904).

The function of the armature and proboscis is primarily as organs of penetration and secondarily used in locomotion. In penetration, the postseptum pushes the preseptum against the tissues to be penetrated stabbing the spines of the third crown into the tissue. They start at a central point and exert tensions in a radial direction thus tearing the tissues into sections. The spines of the other two crowns come out and provide footage for the thrusting proboscis. The proboscis is thrust out violently giving a blow which helps to open the wall further (Muldorf, 1914; Dorier, 1930). Dorier (1930) describes larval locomotion as a type of creep-

ing. The third crown of spicules catches to the substrate and with each evagination progresses a distance equal to the space between the first and third crown spines.

The most accurate presentation of the preseptal musculature was provided by the observations of Dorier (1930) and Muldorf (1914) for the larvae of *G. aquaticus* and by Inoue's (1958) observation of *C. japonensis*. All of these authors report (under various names) four major groups of preseptal muscles: a set of muscles lying next to the body wall, muscles running from the tip of proboscis to the septum, muscles running from base of the proboscis to the lateral edges of the septum, and a set of muscles running from the proboscial base to the base of the acanthal region. Montgomery's (1904) study of *P. varius* and May's (1919) observations on *G. robustus* noted the presence of longitudinal muscles in the preseptum but they were unable to discern their grouping or arrangement. In this study of *P. varius* only three major groupings of the preseptal musculature were observed. No muscle elements, proboscis retractor (Inoue, 1958), "muscles protracteurs do la trompe" (Dorier, 1930), or "Russelprotractoren" (Muldorf, 1914) between the proboscial base and acanthal region were found.

The parietal or body wall muscles of the acanthal region (muscles parietaux, Dorier, 1930; stylet protractor, Inoue, 1958) are not named by Muldorf (1914) and are described as a few smooth muscle elements covering the hypodermis. The number and disposition could not be determined by either Dorier (1930) or Muldorf (1914). Inoue (1958) describes these muscles as consisting of six or more cells and running from the septum to a point way up the preseptum. In *P. varius* these muscles arise on the lower body wall of the postcanthal area and insert into the cuticle slightly behind the beginning of the acanthal region. By their position these cells can serve only to shorten the preseptum.

The six oblique muscles here correspond to the proboscis retractors (Inoue, 1958), "muscles retracteurs de couronnes des spicules" (Dorier, 1930), and "rectractoren des Bohrorgans" (Muldorf, 1914) of the earlier workers. They insert on the cuticle between the proboscial base and the first crown of spines. These oblique muscles do not possess another point of insertion at the base of the third crown of spines as reported for *G. aquaticus* by Dorier (1930). The muscles originate on the body wall at the base of the septum and perhaps at the lateral margins of the septal complex. The oblique muscles apparently function as a retractor for the acanthal region of the preseptum.

The proboscis musculature, stylet retractors (Inoue, 1958), "muscles retracteurs de la trompe" (Dorier, 1930), or the "Russelretractoren" (Muldorf, 1914) consist of a ring of six muscle cells, not three as reported for *C. japonensis* by Inoue (1958). These enclose the central gland duct and its cells. As shown in this study these muscles form the internal bulk of the proboscis inserting somewhere in the anterior portion of the proboscis, not at the base of the stylets as reported by Inoue (1958) and Dorier (1930). The proboscial musculature is in turn enclosed by a sheath composed of three rings of cells. The most anterior ring, when the proboscis is inverted, consists of six folded hypodermal cells extending up along the proboscis and two lower rings of folded cells that correspond roughly to the "Russelscheede" or proboscial sheath reported for *G. aquaticus* by Muldorf (1914). Basally the muscle cells have their origin on the slanted portion of the septal complex and serve as a retractor for the proboscis.

Muldorf (1914) felt that for a precise understanding of the musculature of the preseptum, it was not only necessary to know the position but also the functioning of the muscles. Therefore, he (and Dorier 1930, 1932) studied the mode of evagination of the preseptum. These same observations were not included in Montgomery's (1904) study of *P. varius*. This author has had occasion to observe the motions of the preseptum in *P. varius* which seem to generally agree with that outlined by Montgomery and Dorier. Initially the parietal muscles contract causing a compression of the postacanthal or annulate region of the septum. The annulae in the posterior part of the postseptum become strongly compressed. The anterior face of the preseptum appears to flow slightly forward causing the acanthal region to roll out (Dorier, 1930, 1932; Muldorf, 1914.) Since there are no protractor muscles for the proboscis or acanthal regions, it would appear that the internal pressure and tension on the cuticle, caused by

the contraction of the parietal muscles, is responsible for the eversion of the perforating apparatus, while the oblique and proboscial muscles are responsible for returning the armature to the inverted position.

Literature Cited

Dorier, A. 1930. Researches biologiques et systematiques sur les gordiaces. Trav. Lab. Hydrobiol. Piscicult. Grenoble 22: 1–183.

———. 1932. Observations sur deux larves de gordiaces appartemant au genre *Parachordodes*. Trav. Lab. Hydrobiol. Piscicult. Grenoble, 24: 47–61.

———. 1935. Étude biologique et morphologique do la larva de *Parachordodes gemmatus* (Villot). Trav. Lab. Hydrobiol. Piscicult. Grenoble 25: 147–161.

———. 1965. Classe des Gordiaces, p. 1202–1222. *In* P. P. Grasse (ed.), Traité de Zoologie. Anatomie, Systematique, Biologie, Tome IV, Troisième Fascicule. Mason et Cie Editeurs, Paris.

Inoue, Il. 1958. Studies on the life history of *Chordodoes japonensis* a species of Godiacea. I. The development and structure of the larva. Jap. J. Zool. 12: 203–218.

Leidy, J. 1850. Notes on the development of *Gordius aquaticus*. Proc. Acad. Nat. Sci. Phil. 5: 98–100.

———. 1870. The gordius, or hair-worm. Amer. Entomol. Bot. 2: 193–197.

Montgomery, T. H. 1904. The development and structure of the larva of *Paragordius*. Proc. Acad. Nat. Sci. Phil. 56: 738–755.

Muldorf, A. 1914. Beitrage zur Entvicklungsgeschichte und zu dem phylogenetischen Beziehungen der Gordiuslarvae. Z. Wiss. Zool. 111: 1–75.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultra. Res. 26: 31–43.

Thorne, G. 1940. The hairworm, *Gordius robustus* Leidy, as a parasite of the Mormon cricket. J. Wash. Acad. Sci. 30: 219–231.

Parasitological Aspects of *Schistosoma intercalatum* Fisher, 1934 (Cameroon) Infection in the American Opossum (*Didelphis marsupialis* L.)[1]

ROBERT E. KUNTZ, BETTY JUNE MYERS, TAO-CHENG HUANG, AND JERRY A. MOORE
Parasitology, Southwest Foundation for Research and Education, San Antonio, Texas 78284

ABSTRACT: A study of the parasitological aspects of *Schistosoma intercalatum* infection in opossums (*Didelphis marsupialis*) showed that there was a broad range in host–parasite relationships. Adult schistosomes and their eggs occurred in the major viscera, but in contrast to infection by the closely related *S. haematobium* there was no significant pathology and no involvement of the urogenital system.

Although a number of schistosomes infect man, three species have been incriminated as the principal cause of disease. However, in some areas of Africa, *Schistosoma intercalatum*, a member of the *S. haematobium* or terminal spine egg complex, is a parasite of increasing concern to human populations. To date, laboratory investigations with this schistosome have been minimal. The present paper is concerned with parasitological aspects of *S. intercalatum* infection in the American opossum, *Didelphis marsupialis*.

[1] This investigation was supported by the United States–Japan Cooperative Medical Science Program, administered by the National Institute of Allergy and Infectious Diseases, under grant AI 08207, and by grant CA 13208, National Cancer Institute, National Institutes of Health, Education and Welfare.

Materials and Methods

S. intercalatum has been maintained in hamsters and in *Bulinus wrighti*. Three hundred cercariae were pooled from a number of snails and counted in drops of water on cover slips, then applied to shaved and cleansed skin of opossums. The latter were maintained on dry Purina dog chow. At necropsy, viscera were removed from the body and examined separately. Parasites were recovered by perfusion and/or manual extraction. Refrigerated tissues

Table 1. Parasitological aspects of *Schistosoma intercalatum* (Cameroon) in 18 American opossums (*Didelphis marsupialis*) exposed to 300 cercariae each.

a. Parasite recoveries from principal organs and total parasite recovery

Duration		Liver & Hep. Port.			Stomach	S. Int.		L. Int.		Pancreas	Spleen		Total recovery			
													Actual			
(weeks)	Lungs	Prs*	♀♀*	♂♂*	Prs	Prs	♂♂	Prs	♂♂	Prs	Prs	♂♂	Prs	♀♀	♂♂	Percentage
26–31	0	0–12	0–92	0–72	0–2	0–21	0–6	0–12	0–1	0–2	0–6	0–1	0–44	0–92	0–78	2–39

b. Total body egg count, total egg count for principal organs, and eggs/worm pair

Total body egg count†	Lungs	Liver	Stomach	S. Int.	L. Int.	Pancreas	Spleen	Mesentery	Eggs/Worm Pr
0–228.3	0–2.8	0–62.4	0–1.0	0–86.5	0–61.6	0–17.8	0–1.9	0–6.9	0–7.8

* Range in numbers.
† Counts expressed as 1,000's.

were digested in 2.5% potassium hydroxide for 12–24 hr (Cheever, 1968).

Results

Eighteen hosts exposed to 300 cercariae each were sacrificed at 26–31 weeks. Pertinent parasitological aspects of infection are indicated in Table 1. There is a broad range in host–parasite relationships as indicated by the worm returns, i.e., 2–39%, by the distribution of schistosomes in their hosts and by the values for total organ as well as total body egg counts. Most of the worms were associated with the liver and different levels of the intestine. A few were embedded in the pancreas and a few were associated with the spleen. Egg deposits were moderate, with greater numbers occurring in the liver and the small and large intestine. No schistosomes were recorded from the urogenital system even though a few eggs were detected in tissue digests. The number of eggs per worm pair is considered low.

Minimal pathology was observed, in spite of moderate numbers of eggs in digests of some organs. Lungs showed no pathology attributable to schistosome infection other than small numbers of eggs, some with circumoval granulomas. The liver demonstrated occasional small fibrotic granulomas as well as large active granulomas, some of which were exudative.

There was slight to moderate hyaline periportal material. This appeared to be amyloid and has been noted in noninfected opossums.

Discussion

As indicated by a recent review on different aspects of the biology of *S. intercalatum* and on the significance of this species (Wright et al., 1972), it is obvious that this neglected schistosome is likely to arouse more interest in the future. Laboratory investigations on *S. intercalatum*, however, are limited and the interrelationships of this parasite to the morphologically identical *S. haematobium* are unknown. Since bladder tumors have been found in association with *S. haematobium* infection in opossums (Kuntz et al., 1971), and there is a need for lower mammalian-parasite systems which could be employed in parasite carcinogenesis research, the present studies were conducted to determine whether *S. intercalatum* possessed a comparable potential for pathology leading to tumor.

The present investigation has shown that the opossum may be used as a host for S. *intercalatum*, but there is no involvement of the urogenital system. Only minor pathology occurs even though worms and eggs may be deposited in the principal viscera.

Acknowledgments

The authors are indebted to Dr. C. A. Wright, British Museum (Natural History), for providing working stocks of *Bulinus* and S. *intercalatum*. Dr. Allen W. Cheever, Laboratory of Parasitic Diseases, NIH, Bethesda, Md., has provided consultation and information on pathology observed in infected opossums, and Mr. Dennis Mynarcik and Mrs. Lydia Merrill, Parasitology, SFRE, have contributed significant technical assistance.

Literature Cited

Cheever, A. W. 1968. Conditions affecting the accuracy of potassium hydroxide digestion technique for counting *Schistosoma mansoni* eggs in tissues. Bull. World Health Org. 39: 328–331.

Kuntz, R. E., B. J. Myers, and A. W. Cheever. 1971. *Schistosoma haematobium* infection in the opossum (*Didelphis marsupialis*): Involvement of the urogenital system. Bull. World Health Org. 45: 21–25.

Wright, C. A., V. R. Southgate, and R. J. Knowles. 1972. What is *Schistosoma intercalatum* Fisher, 1934? Trans. Roy. Soc. Trop. Med. Hyg. 66: 28–64.

The Life Cycle and Infectivity to Man of *Apophallus donicus* (Skrjabin and Lindtrop, 1919) (Trematoda: Heterophyidae) in Oregon

DELBERT R. NIEMI AND RALPH W. MACY
Department of Biology, Portland State University, Portland, Oregon 97207

ABSTRACT: Metacercariae encysted in and beneath the skin of fish, including blackside dace, suckers, squawfish, redside shiners, rainbow trout, and coho salmon, collected from streams in northwestern Oregon, and when fed to gerbils, white rats, golden hamsters, cats, and chickens, produced mature trematodes identified as *Apophallus donicus* (Skrjabin and Lindtrop). Eggs typical of this species were found in the feces of one of the authors 8 days after the ingestion of encysted metacercariae. Rediae and cercariae were found in the stream snail, *Flumenicola virens* (Lea). Hatchery-reared young coho salmon were experimentally infected by exposure to cercariae from a single snail. Such salmon were killed when attacked simultaneously by as few as 35 cercariae. The taxonomy and biology of the trematode is discussed.

All blackside dace, *Rhinichthys osculus nubilus* (Girard), collected in Crystal Springs Creek at Portland, Oregon, and in Mill Creek at Salem and Turner, Oregon, were found to have blackspot disease caused by encysted metacercariae. Nearly all redside shiners, *Richardsonius balteatus* (Richardson); suckers, *Catostomus macrocheilus* Girard; squawfish, *Ptychocheilus oregonensis* Richardson; rainbow trout, *Salmo gairdneri* Richardson; and coho salmon, *Oncorhynchus kisutch* (Walbaum) from these localities were also infected with the same type of cysts. When fed to white rats, gerbils, golden hamsters, kittens, and chicks of *Gallus gallus domesticus*, these developed in the small intestine of each host into mature trematodes identified as *Apophallus donicus* (Skrjabin and Lindtrop, 1919), Price, 1931, a species previously reported by Shaw (1947) from an Oregon gull.

Heterophyid cercariae shed by a single snail, *Flumenicola virens* (Lea), were found to encyst in young, hatchery-reared coho salmon from 6 to 9 cm long, producing encysted metacercariae identical to those found in naturally infected fish previously mentioned. These metacercariae produced adult *Apophallus donicus*, when fed to white rats.

Eggs typical of this species were found in the feces of one of the authors 8 days after

ingestion of encysted metacercariae from experimentally infected coho salmon.

Materials and Methods

Fish were collected by scraping overhanging, submerged vegetation with a Needham rake, by sweeping deeper water with a 15-foot net, or by stunning them with a gasoline-powered generator provided with electrodes.

Snails were collected mainly with a Needham scraper net. For transport to the laboratory snails and small fish were placed in covered 10-inch plastic cake pans to which were added "O"-tabs (Pemble Laboratories, River Falls, Wisconsin) as a source of oxygen. The pans were then placed on ice in large styrofoam boxes. Large fish were placed directly in such boxes half-filled with water to were added "O"-tabs and ice water.

In the laboratory fish were placed in aquaria partially submerged in soft-drink coolers kept at 12 C and aerated with pumps. Snails were kept without aerators in covered plastic pans in a cold room at 6 C. When uncrowded, *Flumenicola* snails lived for 2 or more months.

For experimental infection, 50 coho salmon parr from 6 to 9 cm long were obtained from the Bonneville Fish Hatchery and were maintained in a 50-gal tank of dechlorinated tap water placed in a soft-drink cooler maintained at a constant 12 C and aerated with a pump. The fish were fed a special mixture of fish and vegetable meal used by the hatchery.

Fish to be used for infection experiments were gradually brought to 17 C to avoid shock and placed in cake pans half-filled with water and put in an environmental chamber at this temperature. No oxygenation was necessary when no more than two salmon were isolated in each 10-inch pan.

Half of the coho salmon from the Bonneville Hatchery were fed whole to experimental animals or digested in acid pepsin solution to prove that they were uninfected.

To obtain encysted metacercariae, the fish were pithed, weighted, and ground with a meat grinder. Ground fish were digested in an acid solution of 0.5 pepsin at 37 C and gently agitated on a magnetic stirer. The digest was strained, diluted with 0.85% saline, placed in a separatory funnel, and the residue removed after 15 min. After rinsing several times in saline, the residue was collected in watch glasses and the cysts were separated, counted, and used for infection of definitive hosts.

Metacercariae were excysted either mechanically or by enzymes as described by Macy et al. (1967). Those excysted were studied alive in 0.85% saline of were flattened, fixed with Gilson's fluid, stained in Ehrlich's acid hematoxylin, dehydrated, cleared, and mounted in resin.

Smaller mammals were slightly anesthetized before force-feeding with concentrated cysts using a hypodermic syringe with a fine, plastic tube on the end of the needle. Chicks were thus given cysts without anesthetic.

Human infection was brought about by swallowing cysts in a gelatin capsule washed down with water. Fecal examination was facilitated by the use of a series of sieves followed by centrifugation of the sediment which was then examined with dissecting and compound microscopes.

The principal method of obtaining heterophyid cercariae was to place groups of *Flumenicola* snails in petri dishes in an environmental chamber at 17 C with 14 hr of light. Those shedding heterophyid cercariae were individually separated and the cercariae from each snail were studied for behavior and structure. Cercariae were studied alive unstained or lightly stained with neutral red and Nile blue sulfate. Measurements of cercariae were made from specimens killed in hot 10% formalin and stained in neutral red.

Rediae from crushed snails were studied alive and then killed in AFA or Gilson's fluid from which stained permanent slides were prepared.

To determine shedding periodicity of cercariae from snails, infected snails were isolated in small dishes of water in an environmental chamber at 17 C with light and dark set to simulate spring days and nights. Each hour for a 24-hr period, each dish was examined with a dissecting microscope for cercariae, and each snail was then placed in a new dish of water. Cercariae in each dish were killed with 95% ethanol. Counting was facilitated by placing each dish over a grid.

Adult trematodes were fixed in Gilson's fluid under slight coverglass pressure, stained

in Ehrlich's acid hematoxylin, further processed and mounted for permanent preparations. Adults and pieces of salmon tailfin with metacercariae for sections were fixed in Bouin's fluid, stained in bulk in Ehrlich's acid hematoxylin, cut at 6 μ, and mounted for permanent preparations.

All measurements are in microns.

Life Cycle Stages of *Apophallus donicus*

Adult (Figs. 1, 2, 5)

Body oval, pyriform or somewhat linguiform, 311 (298–554) long by 262 (186–303) wide when slightly flattened under a cover glass. Anterior two-thirds of body covered with small scalelike spines, 1 long by 2 wide. Oral sucker subterminal, 60 (53–69) wide by 52 (45–62) long. Acetabulum 42 (36–45) long by 40 (32–56) wide, usually a short distance preequatorial. Pharynx oval or oblong, 29 (27–34) long by 30 (26–33) wide, prepharynx very short, 4 to 7 or seemingly absent. Esophagus moderately long, 40 (21–60), slender.

Intestinal ceca extending nearly to posterior end of body. Testes large, oval, and oblique in posterior third of relaxed specimens; left testis 78 (60–92) long by 92 (75–104) wide, right testis 80 (67–113) long by 95 (85–121) wide. Cirrus sac bipartate, transverse, posterior and adjacent to acetabulum, containing seminal vesicle. Gonotyl (Fig. 2) with two papillae protruding over genital pore, immediately anterior to acetabulum. Ovary usually oval, 52 (35–72) long by 66 (50–86) wide. Seminal receptacle at posterior margin of ovary. Vitelline follicles variable in size and shape, most closely packed in areas lateral to and overlapping ceca; vitelline fields extending to level of intestinal fork or just anterior to it and extending across body anteriorly. Vitelline ducts extending across body just anterior to testes. Laurer's canal not seen. Uterus confined to midbody region. Egg flask-shaped, gold-brown, 32 (21–33) long by 18 (17–20) wide, surface reticulated, containing a fully developed miracidium.

Redia (Figs. 4, 8, 9)

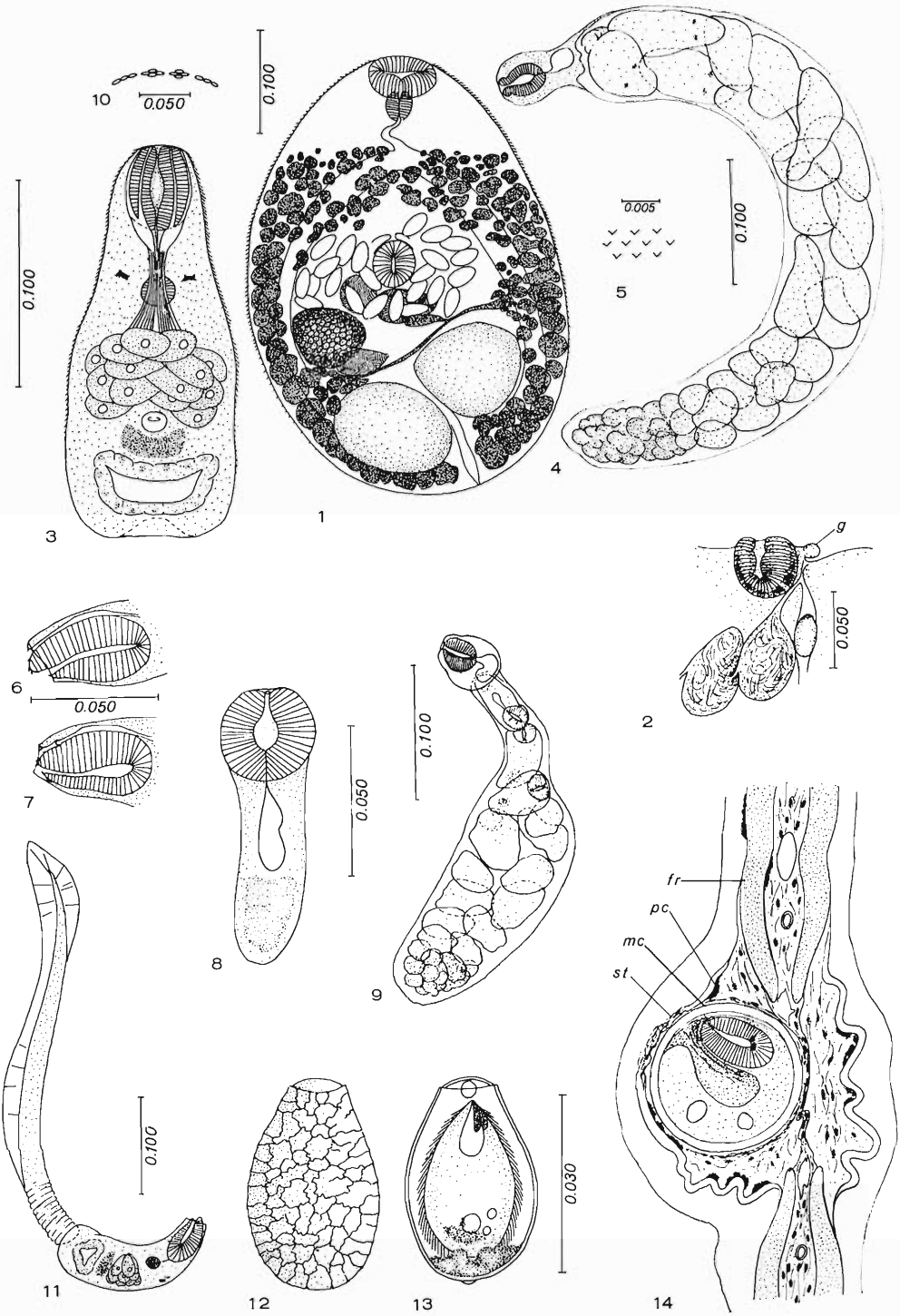
Mother redia 355 (292–396) long by 146 (130–161) wide, anterior end bulbous and only slightly broader than the pharynx which is 27 (24–32) long by 25 (23–31) wide. Body sausage-shaped, always curved in younger specimens. Cecum very small and short. Larger mother rediae contain an average of about 15 daughter rediae with the most mature ones toward the anterior region. Birth pore not seen. Daughter rediae leave the mother rediae when between 80 and 100 in length. Pharynx of daughter redia 30 (29–33) by 31 (28–36) wide, remaining this size even for large specimens, which attain a length of 485 (358–982) and a width of 140 (108–166); as in the mother redia, the anterior end of body is bulbous and the cecum is very short. Body filled with mature and developing cercariae with numerous germ balls in the posterior region.

Natural infections of mother rediae in the digestive glands of snails were found only in the spring and early summer, always with some free daughter rediae. The latter were found in up to 43% of *Flumenicola* in mid- to late summer at Crystal Springs in Portland and never less than 5% at other seasons.

Cercaria (Figs. 3, 6, 7, 10, 11)

Of pleurolophocercous type, body when moderately relaxed about 190 long by 80 wide, with surface scalelike spines extending to the posterior third. Oral sucker subterminal with several rows of minute curved spines at the anterior end, 43 (34–48) long by 30 (25–44) wide. Pharynx spherical, small, located a short distance from oral sucker, and just posterior to level of eyespots. The two eyespots 8 (7–10) long by 7 (6–9) wide, roughly rectangular, located 60 (50–82) from the anterior tips of body and 10 (9–14) from the body margins. Acetabulum located just posterior to penetration glands with average diameter of 10. The 14 unicellular penetration glands form a compact mass occupying much of the central part of the body, with ducts arranged in two dorsal bundles of four ducts, whereas the two dorsolateral bundles have three each with openings at the anterior body margin (Fig. 10). The V-shaped excretory vesicle is thick-walled and near the posterior part of the body. The flame cell pattern was not ascertained since it was obscured by orange pigment granules scattered in groups throughout the body.

Tail slender 258 (332–370) long by 26 (23–



35) wide with a continuous dorsal and ventral fin which begins 51 (46–58) from the body on dorsal surface of tail. It widens toward tip (Fig. 11) and is supported by several slender filaments near middle of tail and several more near tip.

The cercariae swim vigorously for short periods followed by rest. When swimming, the tail beats clockwise in a figure-8 pattern. When at rest the cercaria assumes the shape of the letter J.

In an environmental chamber set for 12 hr of light at 17 C, individual snails shed up to 2,380 cercariae in 24 hr. Numbers of cercariae shed reached peaks between 7 and 9 AM and 7 and 9 PM. During the middle of the day and for most of the dark period little or no shedding occurred. In dechlorinated tap water at 17 C they remained alive as long as 8 days. In a petri dish of water the oral sucker of the cercaria attached to a 6-cm salmon in as brief a period as 10 sec and tails were shed in 25 sec. After penetration of the integument of the fish in 8 to 20 min, the body migrated a short distance and began encystment. When attacked simultaneously by as few as 35 cercariae, 6-cm salmon were killed. However, in a 5-gal aquarium of water one heavily shedding snail did not appear to damage fish. A fish could tolerate the penetration of large numbers of cercariae if exposed to a few at a time over a considerable period.

The cercariae readily penetrated *Gambusia* producing large swellings at the sites of entry but there was no encystment. However, previosuly exposed *Gambusia* were refractory to penetration by this cercaria.

Metacercaria (Fig. 14)

Free, encysted metacercariae spherical, 103 (93–108) in diameter or slightly oval, with outer fibrous wall 4 (3–6) thick and a thin inner hyaline membrane. In older infections cysts surrounded by dark pigment. Excysted metacercariae oval, 305 (290–361) long by 139 (128–162) wide, anterior three-fourths of body covered with fine spines; oral sucker 36 (33–40) long by 41 (38–50) wide. Pharynx about 24 in diameter; esophagus bifurcating near body center; ceca extending along sides of body to near posterior tip. Excretory vesicle V-shaped with bifurcation anterior to testes. According to Hsu (1936), flame cell pattern for the metacercaria of *Apophallus donicus* is $2\,[(2+3)+(3+2+3)]$ but this could not be verified.

Infection of Definitive Hosts

After they were fed cysts of *Apophallus donicus*, all mammals and birds, including white rats, gerbils, golden hamsters, cats, man, and chickens, became infected with mature trematodes; eggs appeared in the feces in 7 to 8 days postinfection. In kittens eggs were seen in the feces in 7 days and continued until the 13th day. No flukes were found when the intestine was opened on the 14th day. In the human infection, eggs first appeared on the 8th day and were last found on the 23rd day.

Discussion

Skrjabin and Lindtrop (1919) erected the genus *Rossicotrema* for *R. donicus* but the genus was synonymized by Price (1931) who placed the species in *Apophallus* Luhe, 1909. Species of *Apophallus* have been treated especially by Ransom (1920), Price (1931), Ciurea (1933), Cameron (1937), Lyster (1940), Skrjabin (1952), and Yamaguti (1971). *Apophallus venustus* (Ransom, 1920), *A. similis* Ransom, 1920, and *A. brevis* Ran-

←

Figures 1–14. ***Apophallus donicus*** **Skrjabin and Lindtrop. 1. Adult raised in rat from laboratory infection of coho salmon, ventral view. 2. Adult, longitudinal section through acetabulum and genital sinus. 3. Cercaria, dorsal view of body. 4. Daughter redia with cercariae. 5. Arrangement of spines of cuticular surface of cercaria. 6. Oral sucker of cercaria showing spines and extruded lips. 7. Oral sucker of cercaria with lips in normal position. 8. Daughter redia. 9. Mother redia with daughter redia. 10. Arrangement of duct openings of penetration glands, around oral sucker of cercaria. 11. Free cercaria in resting position. 12. Egg, external surface. 13. Egg showing miracidium. 14. Metacercaria, encysted in tail of coho salmon, cross section. Figures 3, 4, 6–13 from live material. Fin ray, fr; gonotyl, g; metacercarial cyst, mc; pigment cell, pc; scar tissue, st.**

som, 1920, are considered to be synonyms of *A. donicus*. Life cycle studies of *Apophallus* include those of Lyster (1940) for *A. imperator*, Cameron (1937) for *A. venustus*, Timon-David (1936) for *A. bacalloti*, and Odening (1970) for *A. muehlingi*.

In spite of certain differences between the original description of *Apophallus donicus* and our specimens, considerable variation in the latter indicates probable identity with this species. The pharynx is slightly smaller in our material and the acetabulum is somewhat variable in position depending upon the amount of flattening or extension of specimens. Sometimes the acetabulum is nearer the intestinal fork than shown in Figure 1. Also, the anterior level of the vitellaria may extend a short distance anterior to the intestinal fork.

Cameron (1937) described the redia, cercaria, and metacercaria of *Apophallus venustus* (Ransom, 1920) in Canada and found these in the snail, *Goniobasis livescens*. Encysted metacercariae in the catfish, *Ameriurus nebulosus*, and other fish when fed to laboratory-reared cats produced mature *A. venustus*. He indicated that the entire cycle had not been observed in an experimental series. *A. venustus* is now considered to be synonymous with *A. donicus*, but there are significant differences between Cameron's findings and ours; his redia had a much longer gut and contained fewer cercariae. Further, the cercaria had 16 instead of 14 penetration glands. The snail host was *Goniobasis* instead of *Flumenicola*.

Lyster (1940) recorded the life cycle of *Apophallus imperator*, which he described as a new species, differing from *A. donicus* in that the acetabulum is slightly larger than the oral sucker, the gonotyl papillae are much larger, and the vitelline follicles are less numerous and do not extend anterior to the acetabulum. He fed encysted metacercariae from the trout, *Salvelinus fontinalis*, in eastern Canada, to cats and pigeons and later recovered from them the adult trematodes.

Miller (1941, 1946), maintaining that *Apophallus imperator* was synonymous with *A. brevis*, found the snail host to be *Amnicola limosa*, and the natural definitive host to be the loon, *Gavia immer*. The cercaria was similar to ours except that the body had numerous hairlike extensions on the surface. It is our belief that Miller's material should be referred to *A. imperator* Lyster.

Timon-David (1963), in France, fed metacercarial cysts from *Gasterosteus aculeatus* and *Gambusia affinis holbrooki* to ducklings and a pigeon and later recovered from them adult *Apophallus bacalloti* Morosov, but a cat was refractory to infection.

Odening (1970) described the life cycle of *Apophallus muehlingi* (Jagerskiold) in which the rediae were found at Berlin in the snail, *Lithoglyphus naticoides*, of the family Hydrobiidae. The cercariae encysted in several species of cyprinid fishes. This species matured in cats, dogs, and the gull, *Larus ridibundus*. The redia and cercaria stages are similar to those of *A. donicus* except that the body of the cercaria of *A. muehlingi* bears many hairlike projections; in both there are 14 pairs of penetration glands. Mature *A. muehlingi* differs from *A. donicus* in that the vitellaria of the former do not reach the intestinal bifurcation and the testes are nearly tandem.

Many heterophyid trematodes have a low host specificity for definitive hosts, and in localities where man eats fish raw, human infection with a number of species is common. Eggs resembling those of *Apophallus venustus* were reported by Cameron (1937) from a patient in a military hospital in eastern Canada. We have shown experimentally that man can be infected with *A. donicus*. Since the metacercariae can infect salmon and other fish in the Pacific Northwest, metacercarial cysts in these fishes are potential sources of human infection. It also demonstrated that under certain circumstances, encystment of this trematode kills small salmon.

Acknowledgments

Gratitude is expressed to Dr. Charlie R. Powell for help in collecting and identifying fish, to Ray G. Sheldon of the Bonneville Fish Hatchery for supplying salmon parr, and to Robin English for technical assistance.

This work was supported by National Science Foundation Grant GB-18645.

Literature Cited

Cameron, T. W. M. 1937a. Studies on the heterophyid trematode *Apophallus venustus*

(Ransom, 1920) in Canada. Part II. Life history and bionomics. Can. J. Research 15: 28–51.

———. 1937b. Studies on the heterophyid trematode *Apophallus venustus* (Ransom, 1920) in Canada. Part III. Further hosts. Can. J. Research. 15: 275.

Cuirea, I. 1933. Les vers parasites de l'homme, des mammifères et des oiseaux provenant des poissons du Danube et de la Mer Noire. Arch. Roumaines Path. Exper. Microbiol. 6: 5–135.

Lyster, L. L. 1940. *Apophallus imperator* sp. nov., a heterophyid encysted in trout, with a contribution to its life history. Can. J. Research 18: 106–121.

Macy, R. W., A. K. Berntzen, and Mary Benz. 1967. In vitro encystation of *Sphaeridiotrema globulus* metacercariae, structure of cyst, and the relationship to host specificity. J. Parasitol. 54: 28–38.

Miller, M. J. 1941. The life history of *Apophallus brevis* Ransom, 1920. J. Parasitol. 27 (Suppl.): 7.

———. 1946. The cercaria of *Apophallus brevis*. Can. J. Research 24: 27–29.

Odening, K. 1970. Der Entwicklungszyklus von *Apophallus mühlingi* (Trematoda: Opisthorchiida: Heterophyidae) in Berlin. Z. Parasitenk. 33: 194–210.

Price, E. W. 1931. A new species of trematode of the family Heterophyidae, with a note on the genus *Apophallus* and related genera. Proc. U. S. Nat. Mus. 79: 1–6.

Ransom, B. H. 1920. Synopsis of the trematode family Heterophyidae with descriptions of a new genus and new species. Proc. U. S. Nat. Mus. 57: 527–573.

Shaw, J. N. 1947. Some parasites of Oregon wildlife. Station Techn. Bull. (11) Oregon Agric. Exper. Station, 11 p.

Skrjabin, K., and G. Lindtrop. 1919. Trematodes intestinales des chiens du Don. Isvest. Donsk. Vet. Inst. 1: 1–17.

Timon-David, J. 1963. Développement expérimental d'un trématode du genre *Apophallus* Luhe (Digenea, Heterophyidae). Bull. Soc. Hist. Nat. Toulouse 98: 452–458.

Yamaguti, S. 1971. Synopsis of Digenetic Trematodes of Vertebrates, Vols. I and II. Keigoku Publishing Co., Tokyo, Japan.

A Redescription of *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae) from Primates of Panama

LARRY D. HENDRICKS

U. S. Army Medical Research Unit, Panama (Walter Reed Army Institute of Research), Box 2011, Balboa Heights, Canal Zone

ABSTRACT: Natural infections with *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae) were found in one capuchin and four marmosets from Panama. To our knowledge, this is the first report of this parasite from animals in their natural geographic range and the first report of a nonhuman primate coccidian in Central America. This report also represents two new host records and greatly enlarges the known geographic distribution of the parasite. Material from these and two experimental infections prompted this redescription of the species.

The occurrence of nonhuman primate *Isospora* infections is rare if the incidence of reports in the literature is representative. To our knowledge there are only six reports representing four isosporan species. Most of these reports are from animals that were thousands of miles from their natural environments. Recent findings that *Toxoplasma gondii* is an isosporan parasite (Overdulve 1970; Frenkel et al., 1970; Hutchison et al., 1970; Sheffield and Melton, 1970), and that this group can have both paratenic (Wallace, 1972) and vector hosts (Frenkel and Dubey, 1972), makes the *Isospora* of special interest. Natural infections with *Isospora arctopitheci* Rodhain, 1933, were found in one capuchin monkey, *Cebus capucinus*, and in four of eight marmosets, *Saguinus geoffroyi*. Material collected from these and two experimentally infected animals prompted the redescription of the species.

Materials and Methods

All animals, with the exception of the original capuchin, were purchased from local animal vendors who trap animals in the Republic of Panama. All came from the vicinity of La Chorrera, Panama Province. The original capuchin was an adult female that was apparently wild and had been baited into a large cage in Cardenas Village, Canal Zone, less than 1 week prior to its examination. This animal was probably not in its natural environment, as rarely is this species found in or near a suburban area. It is possible that it had been captive and escaped.

At the laboratory,* each animal was placed in a separate cage and feces collected daily. Fecal specimens were routinely examined by formalin–ether technique (Ritchie, 1948) and sugar flotation, specific gravity 1.275. After an animal was found positive for coccidia, the fecal samples were sieved, washed with water, suspended in 2.5% $K_2Cr_2O_7$, poured into 2-liter flasks, and aerated with a submerged air stone and a standard aquarium pump. These oocysts were allowed to sporulate at room temperature (24 C) for 5 to 7 days. Sporulation time on samples removed daily from this system was visually determined with the aid of a microscope. After sporulation the oocysts were collected via sugar flotation and then stored at 4 C until used.

All coccidia measurements were made on an American Optical Microstar microscope at 450× magnification with the aid of an ocular micrometer. All measurements are expressed in microns. The range is followed by the mean in parentheses.

Experimental infection

The oocysts of *I. arctopitheci* used for infecting two primates were obtained from the first animal discovered positive, the white-faced capuchin. These oocysts were collected as previously described.

Two experimental animals, a juvenile male, *S. geoffroyi*, and an adult male, *C. capucinus*, were housed in the laboratory in individual, well-separated cages from the time of their purchase. Fecal samples on the two experimental animals were collected and examined daily for coccidia. The marmoset and the capuchin were negative for 31 and 16 days, respectively, prior to administration by gavage of 100 sporulated oocysts suspended in 2 ml of sterile physiological saline. The number of viable sporulated oocysts were determined by counts made with the aid of a hemocytometer.

The cages housing these animals were washed daily. The diet consisted of fresh vegetables and fruits that were shipped from the United States.

Results

Isospora arctopitheci Rodhain, 1933

Description

Oocysts subspherical to ellipsoidal. Oocyst wall smooth. Wall of intact oocysts 1.0 thick with colorless outer layer, light yellowish-brown inner layer. Micropyle, polar granule, and oocyst residuum absent. Fifty nonsporulated oocysts (Fig. 1) 20.5–29.5 by 20.5–24.5 (26.0 by 22.9). Length-to-width ratios 1.00–1.33 (1.13). Fifty sporulated oocysts (Fig. 2) 22.7–32.7 by 20.5–27.3 (27.7 by 24.3). Length-to-width ratios 1.05–1.30 (1.14). Sporocysts ellipsoidal (Fig. 3) with a conspicuous residuum consisting of spherical granules usually visible in an equatorial location. No Stieda body was seen. Fifty sporocysts 13.1–20.5 by 9.8–15.9 (17.6 by 12.5). Length-to-width ratios 1.21–1.60 (1.41). Sporozoites with one large refractile body at narrow end. Nucleus not discernible. Sporulation time was 4 days.

Experimental results

Both experimentally infected animals began passing oocysts on day 8 postinoculation. The capuchin passed oocysts for 23 days and then remained negative for 45 consecutive days. The marmoset remained oocyst-positive for 49 days at which time it died from undetermined causes.

Discussion

The oocysts of *Isospora arctopitheci* Rodhain, 1933, were originally described from a

* In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

short-tusked marmoset, *Hapale jacchus penicillatus*, in captivity in Belgium. According to Walker (1968) this host is now known as *Callithrix jacchus*. The original brief description of the parasite included little detail regarding morphological characteristics. Only the size range of the oocyst and sporocyst were included, with no indication as to the number examined.

There appears to be only one other report of this parasite. Poelma (1966) stated that an "*Isospora* resembling *I. arctopitheci*" was seen. This report consisted of a brief description and measurements of three isosporan oocysts recovered from an African *Galago sengalensis* which had died the day following its arrival in the Amsterdam Zoo.

Arcay-de-Peraza (1967), in a review and comparison of the *Isospora* found in monkeys and man, described *I. scorzai* as a new species from a Uakari monkey, *Cacajao rubicundus*, in captivity in the London Zoo. Exeprimental infections of *Cebus nigrivittatus* from Venezuela were successful. The author's criteria for describing this new coccidian, the second from nonhuman primates, as different from *I. arctopitheci* were stated to be the differences in morophological characters and measurements. However, the morphological characteristics of the sporulated oocysts, usually considered the basis to separate coccidia species, do not differ in any significant way from those of *I. arctopitheci*. Both have a subspherical to ellipsoidal oocyst and lack of micropyle, polar granule, and oocyst residuum. The sporocysts of both are ellipsodial, contain sporocyst residuum, and lack Stieda bodies.

The measurements provided in Arcay-de-Peraza's description of the new species consist of averages with no indication as to the number of organisms or measurements made to derive these figures (oocysts 23.4 by 19.5; sporocysts 14.3 by 9.1). These are not markedly different from the oocyst averages Arcay-de-Peraza provided for *I. arctopitheci* (oocysts 28 to 24.4) which were apparently derived by taking the midpoint of the ranges provided by Rodhain. This liberty with Rodhain's figures does not provide a valid comparison, since his article did not provide an average or indicate the number of organisms measured to establish the size ranges of *I. arctopitheci* (oocysts 25.5–30.5 by 23.5–25.5; sporocysts 15.3 by 10.2). This liberty was further extended by mathemathical manipulation of these dubious averages to provide an oocyst index of 1.1 for *I. arctopitheci*, while that provided for *I. scorzai* is 1.2. However, a simple division of one "mean" of *I. arctopitheci* by the other results in 1.15. If this number is rounded off to the next one-tenth, as was done by Arcay-de-Peraza for her own material, then both "species" have identical oocyst indices of 1.2.

The reported sporulation time for *I. scorzai* was 4 days and the prepatent period was 8 days. These respective periods are identical for the same events to occur with *I. arctopitheci* as herein reported.

Because of similarities in the measurements of the oocysts and sporocysts, and the morphological and biological characteristics of *I. scorzai* and *I. arctopitheci*, there seems to be little justification for separation of these forms into distinct species. Final judgment of species relationships should best be reserved until information regarding other stages of the life cycle become available.

Very few additional reports are found in the literature concerning *Isospora* of nonhuman primates. Rijpstra (1967) found sporocysts which he described as resembling *I. hominis* and *I. bigemina* in the feces of a pet chimpanzee, *Pan troglodytes*, in Holland. Two other isosporan species from nonhuman primates have recently been described. Marinkelle (1969) described *I. cebi* from the monkey, *Cebus albifrons*, in Colombia. This species is easily distinguished as it is the only primate *Isopora* described that has a Stieda body. *I. papionis* McConnell, DeVos, Basson, and DeVos, 1971, was described from Chacma baboons, *Papio ursinus*, captured in Kruger National Park, Republic of South Africa. The oocysts and sporocysts of this species are smaller (Table 1) than any other *Isospora* oocyst described from primates, including those species from man (McConnell et al., 1971).

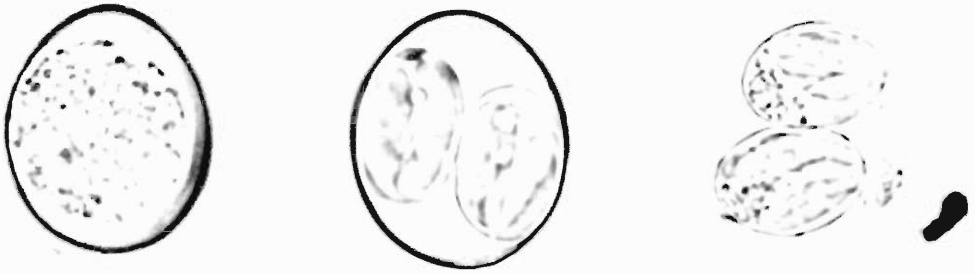
A comparison of the original author's measurements for each species of nonhuman primate *Isospora* described to date is presented in Table 1.

The finding of *I. arctopitheci* in Panama has extended the geographic range of this parasite northward. This report adds two new hosts

Table 1. *Isospora* from nonhuman primates.

Species/author	Host	Location: Captivity/ natural	Oocyst measurements (μ)	Structures	Sporocyst Measurements (μ)	Sporulation time (days)
I. arctopitheci Rodhain, 1933	*Callithrix jacchus*	Belgium/Brazil	25.5–30.5 by 23.2–25.5	Ellipsoidal 1,2,3,4, ---- absent 5 ---------- present	15.3 by 10.2	< 2
I. arctopitheci Poelma, 1966	*Galago senegalensis*	Holland/Senegal	27–33 by 21–24: x = 3	Ellipsoidal 1,3, ---------- absent 5 ---------- present	14–19 by 11–13	2
I. scorzai Arcay-de-Peraza, 1967	*Cacajao rubicundus*	England/Brazil				
	Cebus nigrivittatus (experimental)	England/Venezuela	23.4 by 19.5	Subspherical 1,2,3,4, ---- absent 5 ---------- present	14.3 by 9.1	4
I. arctopitheci This report	*Cebus capucinus*	/Canal Zone	Unsporulated: 20.5–29.5 (26.0) by 20.5–24.5 (22.9): x = 50, L–W ratio 1.00–1.33 (1.13)	Subspherical to ellipsoidal	—	—
	Saguinus geoffroyi	Canal Zone/Panama	Sporulated: 22.7–32.7 (27.7) by 20.5–27.3 (24.3): x = 50, L–W ratio 1.05–1.30 (1.14)	1,2,3,4, ---- absent 5 ---------- present	13.1–20.5 (17.6) by 9.8–15.9 (12.5): x = 50 L–W ratio 1.21–1.60 (1.41)	4
I. cebi Marinkelle, 1969	*Cebus albifrons*	/Colombia	Unsporulated: 18.0–22.0 (20.1) by 16.1–20.0 (18.8): x = 100 Sporulated: 19.1–22.9 (20.9) by 16.5–21.1 (19.8): x = 100	Subspherical 1,3,4, ---------- absent 2,5 ---------- present	12.1–16.8 (14.9) by 9.0–13.9 (11.2): x = 100	3
I. papionis McConnell et al., 1971	*Papio ursinus*	/Rep. of S. Africa	Sporulated: 15–19 (17) by 10–13 (11): x = 50, L–W ratio 1.3–1.8 (1.45)	Broadly ellipsoidal 1,2,3,4, ---- absent 5 ---------- present	10–13 (11) by 7–9 (8.5): x = 100, L–W ratio 1.2–1.7 (1.38)	Passed Sporulated
I. sp. Rijpstra, 1967	*Pan troglodytes*	Holland/Sierra Leone	Not seen	Not given 2 ---------- absent 5 ---------- present	15 by 10	—

1. Micropyle 2. Stieda body 3. Oocyst residuum 4. Polar granule 5. Sporocyst residuum



Figures 1–3. 1. Unsporulated oocyst of *I. arctopitheci*; 2. Sporulated oocyst; 3. Two sporocysts with sporozoites. All figures 1200 ×.

representing two new genera to the list of primates susceptible to this parasite.

This report is the first description of an *Isospora* from a nonhuman primate in Central America. It is the second report of a New World nonhuman primate *Isospora* from a host in its natural range.

Acknowledgments

The author wishes to thank Josesph Butera, SP/4, Army Medical Department, U. S. Army, for his aid in collecting the oocysts used in this study and Mr. John Williams for his assistance with the accompanying microphotographs.

Literature Cited

Arcay-de-Peraza, L. 1967. Coccidiosis en monos y su comparacion con la isosporosis humana, con descripcion de una nueva especie de *Isospora* en *Cacajao rubicundus* (Uakari monkey o mono chucuto). Acta Biol. Venez. 5: 203–222.

Frenkel, J. K., and J. P. Dubey. 1972. Rodents as vector hosts for feline coccidia, *Isospora felis* and *I. rivolta*. J. Inf. Dis. 125: 69–72.

———, ———, **and N. L. Miller.** 1970. *Toxoplasma gondii* in cats: Fecal stages identified as coccidian oocysts. Science 167: 893–986.

Hutchison, W. M., J. R. Dunachie, J. C. Siim, and K. Work. 1970. Coccidian-like nature of *Toxoplasma gondii*. Brit. Med. J. 1: 142–144.

Marinkelle, C. J. 1969. *Isospora cebi* sp. n. aislada de un mico de Colombia (*Cebus albifrons*). Rev. Brasil. Biol. 29: 35–40.

McConnell, E. E., A. J. DeVos, P. A. Basson, and V. DeVos. 1971. *Isospora papionis* n. sp. (Eimeriidae) of the Chacma baboon *Papio ursinus* (Kerr, 1792). J. Protozool. 18: 28–32.

Overdulve, J. P. 1970. The identity of *Toxoplasma* Nicolle and Manceaux, 1909 with *Isospora* Schneider, 1881. Proc. K. Ned. Akad, Wet. (Biol. Med.) 73: 129–141.

Poelma, F. G. 1966. *Eimeria lemuris* n. sp., *E. galago* n. sp. and *E. otolicni* n. sp. from a galago *Galago senegalensis*. J. Protozool. 13: 547–549.

Rijpstra, A. C. 1967. Sporocysts of *Isospora* sp. in a chimpanzee (*Pan troglodytes*, L.). Proc. K. Ned. Akad. Wet. Sect. C 70: 395–401.

Ritchie, L. S. 1948. An ether sedimentation technique for routine stool examinations. Bull. U. S. Army Med. Dept. 8: 326.

Rodhain, J. 1933. Sur une coccidie de l'intestine de l'Ouistiti: *Hapale jacchus penicillatus* (Geoffroy). C. R. Soc. Biol. 114: 1357–1358.

Sheffield, H. G., and M. L. Melton. 1970. *Toxoplasma gondii*: The oocyst, sporozoite, and infection of cultured cells. Science 167: 892–893.

Walker, E. P. 1968. Mammals of the World, Vol. I. 2nd Ed. The Johns Hopkins University Press, Baltimore, 644 p.

Wallace, G. D. 1972. Experimental transmission of *Toxoplasma gondii* by cockroaches. J. Inf. Dis. 126: 545–547.

Helminth Parasites of the Common Grackle, *Quiscalus quiscula versicolor*, from South Bass Island, Ohio

C. Lawrence Cooper and John L. Crites
Department of Zoology, The Ohio State University, Columbus, Ohio 43210

ABSTRACT: Fifteen species of helminths were removed from 48 of 50 common grackles, *Quiscalus quiscula versicolor*, collected on South Bass Island, Ottawa County, Ohio. These included four species of trematodes, four species of cestodes, six species of nematodes, and one species of acanthocephalan. Nine of these are new host records. Previous records of helminth parasites of the common grackle are tabulated.

The common grackle (*Quiscalus quiscula versicolor*) is present in large numbers during the summer months in the western basin region of Lake Erie. Large numbers of these birds are concentrated on South Bass Island, Ottawa County, Ohio, by the presence of a common

Table 1. Helminths of 50 common grackles (*Quiscalus quiscula versicolor*) from South Bass Island, Ohio.

Parasite	Site of infection	Prevalence (%)	Number of parasites per bird		Number of birds infected	
			Average	(Range)	Adults ($N = 13$)	Juveniles ($N = 37$)
Trematoda						
Conspicuum icteridorum Denton and Byrd, 1951	Gall bladder	46	4.5	(1–13)	10	13
Ornithodendrium imanensis Oshmarin and Dotsenko, 1951	Cloaca	4	2	(1–3)	0	2
Plagiorchis noblei Park, 1936	Cloaca	4	2	(2)	0	2
Prosthogonimus macrorchis Macy, 1934	Intestine	2	2	(1)	0	1
Cestoda						
Anonchotaenia globata (von Linstow, 1879)	Intestine	8	7.8	(1–15)	1	3
Choanotaenia musculosa (Fuhrmann, 1896)	Intestine	10	2.2	(1–3)	3	3
Hymenolepis farciminosa Goeze, 1782	Intestine	8	2	(1–3)	1	3
Orthoskrjabinia rostellata (Rodgers, 1941)	Intestine	4	2	(1–3)	0	2
Nematoda						
Capillaria exilis (Dujardin, 1845)	Intestine	6	6	(1–14)	0	3
Capillaria ovopunctatum (von Linstow, 1873)	Intestine	44	8.4	(1–25)	4	18
Chlandlerella quiscali (von Linstow, 1904)	Brain	12	2	(1–4)	5	1
Dispharynx nasuta (Rudolphi, 1819)	Proventriculus	28	6.7	(1–13)	1	13
Porrocaecum ensicaudatum (Zeder, 1800)	Intestine	46	2.3	(1–10)	3	20
Syngamus trachea (Montagu, 1811)	Trachea	72	3.2 pair	(1–8 pair)	5	31
Acanthocephala						
Plagiorhynchus formosus Van Cleave, 1918	Intestine	56	3.2	(1–10)	4	24

roosting site in the center of the island. An association of avian species including brown-headed cowbirds (*Molothrus a. ater*), grackles, red-winged blackbirds (*Agelaius p. phoeniceus*), robins (*Turdus m. migratorius*), and starlings (*Sturnus v. vulgaris*) utilize the roosting site. The feeding activities of this association of birds on ripening fruit and grain is of considerable concern to the agricultural community of the region. This study was undertaken to determine the prevalence and intensity of helminth parasitism in common grackles on South Bass Island. Fifty birds were examined between 1 July and 15 August 1968.

Materials and Methods

All of the birds examined were collected alive from a decoy trap operated by the Franz Theodore Stone Laboratory research staff. The trap is located near the roost site in the center of the island. Birds were sacrificed by exposing them to chloroform vapors for no more than 30 sec in a 2-gal jar.

Immediately upon sacrifice, the birds were autopsied. The body, brain, and nasal cavities were examined, all organs were separated, teased apart, and examined sequentially under a dissecting microscope. Helminths were fixed and preserved by standard techniques. Trematodes, cestodes, and acanthocephalans were stained with Semichon's carmine. Nematodes were cleared in glycerin–alcohol solution and studied in glycerin.

Results and Discussion

Fifteen species of helminths were removed from 48 of the 50 birds examined. Two birds were free of helminth infection. These included four species of trematodes, four species of cestodes, six species of nematodes, and one

Table 2. Published and unpublished records of helminth parasites taken from the common grackle, *Quiscalus quiscula*.

Parasite	Record	Locality
Trematoda		
Brachylecithum americanum	Welker	Indiana
Collyriclum faba	Riley, *in* Beaudette, 1940	Minnesota
Conspicuum icteridorum	Patten, 1951	New York
	Welker, 1962	Indiana
	Ellis, 1963	Iowa
	Hodasi, 1963	Manitoba, Canada
	Stanley and Rabalais, 1971	Ohio
Diplostomum crassum	Chandler and Rausch, 1948	Manitoba, Canada
Echinostoma revolutum	Welker, 1962	Indiana
	Stanley and Rabalais, 1971	Ohio
Echinostoma sp.	Ellis, 1963	Iowa
Plagiorchis sp.	Blankenspoor, 1970	Iowa
Posthodiplostomum minimum	Campbell, 1972	Experimental infection
Tanaisia bragai	Byrd and Denton, 1950	Georgia, Viriginia, Texas
Cestoda		
Anonchotaenia quiscali	Rausch and Morgan, 1947	Ohio
	Welker, 1962	Indiana
Paricterotaenia parina	Welker, 1962	Indiana
Nematoda		
Capillaria ovopunctatum	Read, 1949	Wisconsin
	Mawson, 1956b	Quebec, Canada
	Welker, 1962	Indiana
	Hodasi, 1963	Manitoba, Canada
	Stanley and Rabalais, 1971	Ohio
Chandlerella quiscali	von Linstow, 1904	USA
	Odetoyinbo and Ulmer, 1959, 1960	Iowa
	Welker, 1962	Indiana
	Robinson, 1971	Minnesota, Ohio
Diplotriaena bargusinica	Anderson, 1959, 1961	Ontario, Canada
Diplotriaena sialiae	Hodasi, 1963	Manitoba, Canada
Diplotriaena sp.	Anderson, 1957	Ontario, Canada
Dispharynx pipilonis	Stanley and Rabalais, 1971	Ohio
Oxyspirura petrowi	Pence, 1972	Louisiana
Oxyspirura pusillae	Pence, 1972	Louisiana
Porrocaecum ensicaudatum	Mawson, 1956a	Quebec, Canada
	Levin, 1957	Illinois
	Stanley and Rabalais, 1971	Ohio
Syngamus trachea	Goble and Kutz, 1945	New York
	Stanley and Rabalais, 1971	Ohio
Tetrameres sp.	Wehr, 1934	Florida
Acanthocephala		
Mediorhynchus grandis	Van Cleave, 1916	Maryland
	Van Cleave, 1947	Illinois, Kansas, Kentucky, New Jersey, Ohio
	Welker, 1962	Indiana
	Hodasi, 1963	Manitoba, Canada
Plagiorhynchus formosus	Van Cleave, 1942	Kentucky
	Stanley and Rabalais, 1971	Ohio

species of acanthocephalan. The trematodes *Ornithodendrium imanensis*, *Plagiorchis noblei*, and *Prosthogonimus macrorchis*, the cestodes *Anonchotaenia globata*, *Choanotaenia musculosa*, *Hymenolepis farciminosa*, and *Orthoskrjabinia rostellata*, and the nematodes *Capillaria exilis* and *Dispharynx nasuta* have not been previously reported from the common grackle. The results of this study are presented in Table 1.

The only extensive study of the helminth parasites of the common grackle was that by Welker (1962) in Indiana. Welker reported 10 species of helminths were removed from a total of 409 birds examined. The other records of helminth parasitism in this host are limited to reports surveying bird parasites in general or reports surveying for specific parasites. These records are presented in Table 2.

Study of previous records provides limited

information for gaining insight into the relationship which link host and parasite in a biological community. *Conspicuum icteridorum, Capillaria ovopunctatum, Dispharynx nasuta, Porrocaecum ensicaudatum, Syngamus trachea,* and *Plagiorhynchus formosus* have been previously recorded from the common grackle. The presence of these six species in relatively high percentages in both adult and recently fledged birds indicates that this host plays an important role in the maintenance and dispersal of these helminths in the avian community on South Bass Island.

Acknowledgments

We wish to express our appreciation to Dr. L. S. Putnam, Director, Franz Theodore Stone Laboratory, The Ohio State University, for providing the laboratory facilities utilized, and Dr. M. Miskimen, Director of Blackbird Migration Studies, Franz Theodore Stone Laboratory, for providing the birds examined during this study.

Literature Cited

Anderson, R. C. 1957. Observations on the life cycles on *Diplotriaenoides translucidus* Anderson and members of the genus *Diplotriaena.* Can. J. Zool. 35: 15–24.

———. 1959. Preliminary revision of the genus *Diplotriaena* Henry and Ozoux (Diplotriaenidae: Diplotroaceninae). Parassitologia 1: 195–307.

———. 1961. Further observations on *Diplotriaena* in birds. Can. J. Zool. 39: 377.

Beaudette, F. R. 1940. A case of *Collyriclum faba* infestation finch. J. Am. Vet. Med. Assoc. 96: 413–414.

Blankenspoor, H. D. 1970. Host-parasite relationships of an avian trematode, *Plagiorchis noblei* Park 1936. Ph.D. dissertation, Iowa State University, Ames, Iowa, 180 p.

Byrd, E. E., and J. F. Denton. 1950. The helminth parasites of birds. I. A review of the trematode genus *Tanaisia* Skrjabin, 1924. Am. Midl. Nat. 43: 32–57.

Campbell, R. A. 1972. New experimental hosts for *Posthodiplostomum minimum* (Trematoda: Diplostomatidae). J. Parasit. 58: 1051.

Chandler, A. C., and R. Rausch. 1948. A contribution to the study of certain avian strigeids (Trematoda). J. Parasit. 34: 207–210.

Ellis, C. J. 1963. Trematodes of passerine birds from Chickasaw County, Iowa. Iowa Acad. Sci. 70: 486–492.

Goble, F. C., and H. L. Kutz. 1945. Notes on the gapeworms (Nematoda: Syngamidae) of galliform and passeriform birds in New York State. J. Parasit. 31: 394–400.

Hodasi, J. K. M. 1964. Helminths from Manitoba birds. Can. J. Zool. 41: 1227–1231.

Levin, N. L. 1957. Life history studies on *Porrocaecum ensicaudatum,* an avian nematode. J. Parasit. 43 (Suppl.): 47–48.

Mawson, P. M. 1956a. Ascaroid nematodes from Canadian birds. Can. J. Zool. 34: 35–47.

———. 1956b. Capillarid worms from Canadian birds. Can. J. Zool. 34: 163–164.

Odetoyinbo, J. A., and M. J. Ulmer. 1959. Studies on avian filarial worms of the Subfamily Splendidofilariinae (Nematoda: Dipetalonematidae). J. Parasit. 45 (Suppl.): 58.

———. 1960. Microfilarial periodicity of *Splendidofilaria quiscali* (von Linstow, 1904) n. comb. (Nematoda: Onchocercidae). J. Parasit. 46 (Suppl.): 18–19.

Patten, J. A. 1951. The life cycle of *Conspicuum icteridorum* Denton and Byrd, 1951 (Trematoda: Dicrocoeliidae). J. Parasit. 37: 165–182.

Pence, D. B. 1972. The genus *Oxyspirura* (Nematoda: Thelaziidae) from birds in Louisiana. Proc. Helm. Soc. Wash. 39: 23–28.

Rausch, R. L., and B. B. Morgan. 1947. The genus *Anonchotaenia* (Cestoda: Dilepididae) from North American birds, with the description of a new species. Trans. Amer. Microscop. Soc. 66: 203–211.

Read, C. P. 1949. Studies on North American helminths of the genus *Capillaria* Zeder, 1800 (Nematoda): III. Capillarids from the lower digestive tract of North American birds. J. Parasit. 35: 240–249.

Stanley, J. G., and F. C. Rabalais. 1971. Helminth parasites of the red-winged blackbird, *Agelaius phoeniceus,* and common grackle, *Quiscalus quiscula,* in northwestern Ohio. Ohio J. Sci. 71: 302–303.

Van Cleave, H. J. 1916. Acanthocephala of the genera *Centrorhynchus* and *Mediorhynchus* (new genus) from North American birds. Trans. Amer. Microscop. Soc. 35: 221–232.

———. 1947. The acanthocephalan genus *Mediorhynchus,* its history and a review of the species occurring in the United States. J. Parasit. 33: 297–315.

von Linstow, O. F. B. 1904. Beobachtungen an Nematoden und Cestoden. Arch. Naturg. Berlin 70: 297–309.

Wehr, E. E. 1934. Coexistence of adult male

and female *Tetrameres* (Nematoda: Spiruridae) in proventriculus of the Florida grackle. Proc. Helm. Soc. Wash. 1: 50.

Welker, G. W. 1962. Helminth parasites of the common grackle, *Quiscalus quiscula versicolor* Vieillot in Indiana. Ph.D. dissertation, The Ohio State University, Columbus, Ohio, 103 p.

The Influence of *Nippostrongylus brasiliensis* on the Establishment of *Angiostrongylus cantonensis* in the Laboratory Rat[1]

A. Alan Kocan[2]

Department of Parasitology and Laboratory Practice, School of Public Health, University of North Carolina at Chapel Hill, 27514.

ABSTRACT: Concurrent infections with *Nippostrongylus brasiliensis* and *Angiostrongylus cantonensis* were administered to determine the effect of a *N. brasiliensis* infection on the establishment and maintenance of an *A. cantonensis* infection in the laboratory rat. Reductions in the number of adult *A. cantonensis* were noted when: (1) the initial *N. brasiliensis* infection was 1,000 or 10,000 larvae and the challenge infection of *A. cantonensis* was administered by mouth 8 days later; (2) when the *A. cantonensis* infection was administered 20 days after the initial *N. brasiliensis* infection; and (3) when the *A. cantonensis* challenge was administered by intrameningeal inoculation 8 days after the *N. brasiliensis* infection. No differences in adult *A. cantonensis* recoveries were noted in infections when adult *N. brasiliensis* were transplanted into the small intestines of clean rats and they were challenged 8 days later with *A. cantonensis* larvae. The role of the intestine and lungs is discussed in relation to the recoveries observed.

Many laboratory studies have dealt with the problems of concurrent infections with helminths in mammalian hosts. These studies have focused on two main areas of interest: (1) interspecific competition among the helminths for food, space, or both; and (2) the responses of the host resulting from a challenge infection with one species in the presence of an existing infection with another species. Nonspecific inflammation, cross-immunity, and space limitations are all well-documented conditions that explain reductions in recovery numbers in both heterologous and homologous concurrent infections (Cox, 1952; Goulson, 1958; Weinmann, 1964; Louch, 1962; Lang, 1967; Holmes, 1961, 1962). The main organ of interest in these studies has been the intestine, since this is the usual definitive attachment site or area of larval penetration. Since many larvae and adult helminths utilize the lungs either as a definitive attachment site or during migration, a study of the role of this organ, as well as the intestine, seemed appropriate.

The present study was undertaken in an attempt to determine the effect of *Nippostrongylus brasiliensis* on the establishment and maintenance of *Angiostrongylus cantonensis* in the laboratory rat. By altering the method of introduction, the timing of infections, and the infecting dosage, it was hoped that some insight would be gained into the role of various organs in the establishment and maintenance of the *A. cantonensis* as well as the degree of interaction between these two nematodes.

Materials and Methods

Adult male rats, 75 to 100 days old, were utilized in all experiments. The procedures for the maintenance of *A. cantonensis*, the oral infection of the rats, and the maintenance

[1] A portion of a dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Parasitology and Laboratory Practice.

[2] Present address: Department of Veterinary Parasitology and Public Health, Oklahoma State University, College of Veterinary Medicine, Stillwater, Oklahoma 74074.

of the snail host have been previously described (Kocan, 1972). The procedures for the maintenance and infection of the rats with *N. brasiliensis* followed that described by Thorson (1954). The procedure for the intrameningeal inoculation of rats was that of Kocan and Whitley (1972).

Experimental Procedures and Results

Experiment I

To determine the effect of an existing infection of 1,000 or 10,000 larvae of *N. brasiliensis*, at 8 days after infection, on the establishment of a 100-larvae dose infection of *A. cantonensis*, 50 rats were divided into five equal groups. Group I animals received 1,000 *N. brasiliensis* larvae subcutaneously and, 8 days later, 100 *A. cantonensis* larvae by mouth. Group II control animals received 1,000 *N. brasiliensis* larvae and were killed 8 days later and the number of adult *N. brasiliensis* present in the intestine was determined. Group III animals received 10,000 *N. brasiliensis* larvae subcutaneously and 100 *A. cantonensis* larvae 8 days later. Group IV controls received 10,000 *N. brasiliensis* larvae and were killed 8 days later and the number of adult *N. brasiliensis* present in the intestine was determined. Group V control animals received 100 *A. cantonensis* larvae by mouth. After 50 days from the time of the *A. cantonensis* infection, all animals in Groups I, III, and V were killed and the numbers of adult *A. cantonensis* present in the lungs of each were counted. The average *A. cantonensis* adult recovery from experimental Group I was 48.0 (SD = 8.8). The average *A. cantonensis* adult recovery from experimental Group III was 47.6 (SD = 5.7). The *A. cantonensis* recovery from control group V was 60.6 (SD = 4.0). The difference in individual recoveries between experimental Group I (48.0) and control Group V (60.6) was highly significant (Student's t test value = 9.72; $P < 0.01$). Also, the difference in individual recoveries between experimental Group III (47.6) and control Group V (60.6) was highly significant (Student's t test value = 8.75; $P < 0.01$). However, the comparison between experimental Group I (48.0) and experimental Group III (47.6) individual recoveries was not statistically different (Student's t test value = 0.085; $P < 0.05$). The average adult *N. brasiliensis* recovery was 658 (SD = 17.2) from Group II and 3,512 (SD = 20.1) from Group IV.

Experiment II

To determine the effect of a 20-day-old infection with 1,000 larvae of *N. brasiliensis* on the establishment of a 100-larvae dose infection of *A. cantonensis*, 30 rats were divided into 3 equal groups. Group I animals were infected subcutaneously with 1,000 larvae of *N. brasiliensis* and 20 days later were infected with 100 larvae of *A. cantonensis* by mouth. Group II control animals were infected with 1,000 *N. brasiliensis* larvae and killed 8 days later. Group III control animals were infected with 100 larvae of *A. cantonensis*. Animals in Groups I and III were killed 50 days from the time of the *A. cantonensis* infection and the numbers of adult *A. cantonensis* present in the lungs of each were determined. The average number of *A. cantonensis* adults recovered from control Group III was 40.4 (SD = 6.3) and the average number of adult *A. cantonensis* recovered from Group I was 31.9 (SD = 5.5). The difference in individual recoveries between Group I (31.9) and Group III (40.4) was highly significant (Student's t test value = 3.22; $P < 0.01$). The average adult *N. brasiliensis* recovery from Group II was 561.2 (SD = 19.8).

Experiment III

The objective of this experiment was to determine the effect of transplanting adult *N. brasiliensis* into the small intestine of normal rats, at 8 days after infection of the donors, on the ability of a 100-larvae dose infection of *A. cantonensis*, administered by mouth, to establish as adults. To accomplish this, five animals in Group I were infected with 8,000 *N. brasiliensis* larvae each. These donor animals were killed at 8 days after infection and their intestines removed. The adult worms were then separated from the intestines, washed, and transplanted surgically into recipient rats after the procedure described by Ogilvie and Hockley (1968). Ten animals in both Groups II and III received 1,000 transplanted adults each. Group III animals were

killed 8 days from the time of transplantation and the numbers of adult *N. brasiliensis* present in their intestines was determined. Animals in Group II received 100 larvae of *A. cantonensis* at 8 days from the time of the transplantation of the adult *N. brasiliensis*. Ten Group IV animals served as *A. cantonensis* controls receiving 100 larvae by mouth. Ten Group V animals served as sham controls and received inoculations of saline without larvae. Animals in Groups II, IV, and V were killed 50 days from the time of the *A. cantonensis* infection. The average adult *A. cantonensis* recovery was 39.3 (SD = 5.9) from Group II animals and 38.8 (SD = 5.9) from Group IV. The difference between individual recoveries from these two groups was not statistically significant (Student's t test value = 0.188; $P > 0.05$. The average adult *A. cantonensis* recovery from the sham control Group V was 39.2 (SD = 2.2). The difference between Group II (39.3) and Group V (39.2) was, again, not statistically significant (Student's t test value = 0.036; $P > 0.05$). The difference between individual recoveries from animals in Group IV (38.8) and Group V (39.2) was not significantly different (Student's t test value = 0.199; $P > 0.05$). The average adult *N. brasiliensis* recovery from Group III was 651 (SD = 35.8).

Experiment IV

The objective of this experiment was to determine the effect of an infection with 1,000 larvae of *N. brasiliensis* on the ability of an infection with 100 larvae of *A. cantonensis*, introduced intrameningeally, to establish as adults. Forty rats were divided into four equal groups. Group I and II animals were infected with 1,000 larvae of *N. brasiliensis*. Group II animals were killed 8 days after infection and the numbers of adult *N. brasiliensis* present in the intestines of each were counted. Animals in Groups I and IV were infected with 100 larvae of *A. cantonensis* by intrameningeal inoculation at 8 days after the *N. brasiliensis* infection. Group III animals were infected with 100 larvae of *A. cantonensis* by mouth. Animals in Groups I, III, and IV were killed 50 days from the time of the *A. cantonensis* infection. The average adult *A. cantonensis* recovery from Group I animals was 64.2 (SD = 7.0) and the average adult *A. cantonensis* recovery from Group III was 57.8 (SD = 5.5). The difference between individual recoveries from Group I (64.2) and Group III (57.8) was significant (Student's t test value = 2.386; $P < 0.05$). The average adult *A. cantonensis* recovery from Group IV controls was 67.8 (SD = 6.8). The difference between individual recoveries from Group I (64.2) and Group IV (67.8) was not significantly different (Student's t test value = 1.637; $P > 0.05$.) The average number of adult *N. brasiliensis* recovered from Group II controls was 697.9 (SD = 26.5).

Discussion

These experiments have shown a reduction in the number of adult *A. cantonensis* recovered from concurrent infections when: (1) the initial *N. brasiliensis* infection was 1,000 or 10,000 larvae and the challenge infection of 100 larvae of *A. cantonensis* was administered by mouth 8 days later; (2) when the *A. cantonensis* infection was administered 20 days after the initial 1,000-larvae infection of *N. brasiliensis*; and (3) when the *A. cantonensis* infection was administered by intrameningeal inoculation 8 days after the *N. brasiliensis* infection. No differences were noted in adult *A. cantonensis* recovery numbers when the *N. brasiliensis* infection was induced by surgical introduction of adults into the intestines of clean rats challenged 8 days later with 100 larvae of *A. cantonensis*.

Since the only two organs common to these two nematodes during their migration and development are the intestine and the lungs, their role in the reductions noted should be examined. In Experiment I, both the groups that received 1,000 and 10,000 larvae of *N. brasiliensis* and that were challenged with 100 larvae of *A. cantonensis* harbored significantly fewer adult *A. cantonensis* at 50 days after infection. The differences between the number of adult *A. cantonensis* recovered from the group receiving 1,000 *N. brasiliensis* larvae and the group receiving 10,000 *N. brasiliensis* larvae were not, however, significantly different. Based on these findings, it does not appear that the heavier infection or its resultant increased pathology altered the degree of reduction in the number of adult *A.*

cantonensis recovered. In Experiment II, the *A. cantonensis* challenge was given 20 days after the initial 1,000-larvae infection with *N. brasiliensis* and, again, the recoveries of adult *A. cantonensis* were significantly lower than those of the controls. At 20 days in the life cycle of *N. brasiliensis*, few adult worms are present in the intestine, although the pathology produced in both the lungs and intestine are still present and similar in degree to those observed at 8 days (Taliaferro and Sarles, 1939). The reductions noted in this experiment seem to eliminate the possibility of physical interference by adult *N. brasiliensis* with the migration of *A. cantonensis* larvae in the intestine and points to a tissue or inflammatory reaction in other organs as an explanation for the *A. cantonensis* reductions noted. In Experiment IV, reductions in *A. cantonensis* adult numbers were again noted when the challenge infection was administered intrameningeally 8 days after the initial *N. brasiliensis* infection. This route of administration of the *A. cantonensis* larvae allowed for the normal systemic migration by the larvae, but eliminated the intestinal and gastric penetration. In this experiment, the role of the lungs in the reductions noted becomes most apparent. By comparison, the surgical introduction of the adult *N. brasiliensis* in Experiment III allowed for the intestinal damage by the adult worms, but not for the systemic migration or migration and molting in the lungs by the larvae. In this instance, reductions in adult *A. cantonensis* numbers were not noted. With these findings, the role of the intestine must be considered minimal in the reductions noted in the other experiments.

The hypothesis that the lungs are the important organ in the reductions noted in the present study correspond to the findings of Vogel and Minning (1953), Lin and Sadun (1959), Hsü et al. (1962), and Hsü et al. (1963) that the lungs play an important role in the retention and destruction of schistosomules during repeated infections with *Schistosoma japonicum*.

Acknowledgments

The author would like to thank Dr. James R. Hendricks for his encouragement and assistance throughout the course of this study and Dr. John E. Larsh for his review of the manuscript.

Literature Cited

Cox, H. W. 1952. The effect of concurrent infection with the dog hookworm, *Ancylostoma caninum*, on the natural and acquired resistance to *Trichinella spiralis*. J. Elisha Mitchell Sci. Soc. 68: 222–235.

Goulson, H. T. 1958. Studies on the influence of a prior infection with *Ancylostoma caninum* on the establishment and maintenance of *Trichinella spiralis* in mice. J. Elisha Mitchell Sci. Soc. 74: 14–23.

Holmes, J. C. 1961. Effects of concurrent infections on *Hymenolepis diminuta* (cestode) and *Moniliformis dubius* (acanthocephala) I. General effects and comparison of crowding. J. Parasit. 47: 209–216.

———. 1962. Effects of concurrent infections on *Hymenolepis diminuta* (cestode) and *Moniliformis dubius* (acanthocephala) II. Effects on growth. J. Parasit. 48: 87–96.

Hsü, S. Y. Li, J. R. Davis, and H. F. Hsü. 1962. Pathology in rhesus monkeys infected with the Formosan strain of *Schistosoma japonicum*. Z. Tropenmed. Parasit. 13: 341–356.

Hsü, H. F., J. R. Davis, S. Y. Li Hsü, and J. W. Osborne. 1963. Histopathology in albino mice and rhesus monkeys infected with irradiated cercariae of *Schistosoma japonicum*. Z. Tropenmed. Parasit. 14: 240–261.

Kocan, A. A. 1972. Some common North American aquatic snails as experimental hosts for *Angiostrongylus cantonensis*—with special reference to *Lymnaea palustris*. J. Parasit. 58: 186–187.

———, **and L. S. Whitley.** 1972. Experimental infection of *Angiostrongylus cantonensis* by intrameningeal inoculation of third-stage larvae in laboratory rats. J. Parasit. 58: 1217–1218.

Lang, B. Z. 1967. *Fasciola hepatica* and *Hymenolepis microstoma* in the laboratory mouse. J. Parasit. 53: 213–214.

Lin, S. S., and E. H. Sadun. 1959. Studies on the host relationships to *Schistosoma japonicum* V. Reactions in the skin, lungs, and liver of normal and immune animals following infections with *Schistosoma japonicum*. J. Parasit. 45: 549–556.

Louch, C. C. 1962. Resistance to *Trichinella spiralis* in the laboratory rat following infections with *Nippostrongylus muris*. J. Parasit. 48: 24–31.

Ogilvie, B. M., and D. J. Hockley. 1968. Ef-

fects of immunity on *Nippostrongylus brasiliensis* adult worms: reversible and irreversible changes in infectivity, reproduction, and morphology. J. Parasit. 54: 1073–1084.

Taliaferro, W. H., and M. P. Sarles. 1939. The cellular reactions in the skin, lungs, and intestine of normal and immune rats after infection with *Nippostrongylus muris*. J. Infectious Dis. 64: 157–192.

Thorson, R. E. 1954. Effect of immune serum from rats on infective larvae of *Nippostrongylus muris*. Exp. Parasit. 3: 9–15.

Vogel, H., and W. Minning. 1953. Uber die erwerbene resistenz non macacus rhesus gegenuber *Schistosoma japonicum*. (English abstract.) Z. Tropenmed. Parasit. 4: 418–505.

Weinmann, C. J. 1964. Host resistance to *Hymenolepis nana*. II. Specificity of resistance to reinfection in the direct cycle. Exp. Parasit. 15: 514–526.

Helminths of Kangaroo Rats (*Dipodomys* spp.) in Nevada with Reports of Other Worm Parasites from These Hosts

SAUNDRA R. KING AND BERT B. BABERO
Department of Biological Sciences, University of Nevada, Las Vegas

Kangaroo rats, *Dipodomys*, in Nevada have been studied from the viewpoints of physiology (Howell and Gersh, 1935; Beatley, 1969; Yousef and Dill, 1970, general ecology (Deacon et al., 1964; Bradley and Deacon, 1967), and reproduction and food habits (Bradley and Mauer, 1971). Except for the investigation of deer mice, *Peromyscus*, by Babero and Matthias (1967), apparently no other helminth studies on rodents have been undertaken in the state, despite the importance of lower animals in the transmission of zoonotic diseases.

Although there have been several miscellaneous reports of helminths from kangaroo rats, including those of Hannum (1943), Tiner (1948), Voge (1948, 1958), Dowell (1953), Read (1956), Grundmann (1957, 1958), Kruidenier and Peebles (1958), Guay and Senger (1962), and Bienek and Grundmann (1973), there have been only two previous comprehensive reports on the helminth fauna of these hosts. From southern California, Read and Millemann (1953) reported the helminths from "*D. panimintinus mohavensis, D. merriami merriami, D. m. arenivagus,* and *D. morrensis.*" From Utah, Grundmann (1957, 1958) and Grundman and Warnock (1964) studied the helminths of *D. ordii* and *D. microps*. A list of helminths collected from kangaroo rats, based upon a literature survey, is presented in Table 1.

In the present study, initiated in 1971, 235 animals comprising four species (*D. merriami, D. microps, D. deserti,* and *D. panimintinus*) from southern Nevada and an adjacent area of Califoria were investigated. These animals were collected in the Mohave Desert at elevations ranging between 500–6,000 feet. *D. deserti* and *D. merriami* were collected principally in the creosote bush (*Larrea–Franseria*) community, while *D. microps* and *D. panimintinus* were taken in the blackbrush (*Coleogyne*) community. The habitat of *D. merriami* overlaps that of the latter species. The animals collected were chiefly from seven areas within three Nevada counties [Clark, Lincoln, and Nye (Table 2)].

Collections were made with snap-traps and Sherman live traps. Animals captured alive were kept in holding-cages until sacrificed. The animals killed by snap-trapping were either examined the day of capture or were frozen and examined at a later date. Postmortem examination was accomplished by routine parasitological procedures. Alcohol-formalin–acetic acid (AFA) was the fixative and Semichon's acid carmine was the stain employed for cestodes. Nematodes were cleared in lactophenol and studied using temporary mounts. No Trematoda were collected. Collection data on the parasites collected are given

Table 1. Summary of some other helminths reported from *Dipodomys* spp.*

Helminth	Hosts	Reported by	Date
	CESTODA		
Hymenolepis citelli	*D. ingens* *D. heermanni* *D. nitratoides*	Simpson and Harmon	1968
Catenotaenia linsdalei	*D. panimintinus*	Dowell	1953
	D. heermanni	Voge	1949, 1955
C. californica	*D. morroensis* *D.p. mohavensis*	Dowell	1953
	D. mohavensis *D. panimintinus*	Voge	1955
Catenotaenia sp.	*D.m. merriami* *D.p. mohavensis*	Read and Millemann	1953
Oochoristica sp.	*D.m. merriami*	Read and Millemann	1953
O. deserti	*D.p. mohavensis*	Millemann	1955
Schizorchodes dipodomis	*D.m. vulcani*	Bienek and Grundmann	1973
Raillietina retractilis	*Dipodomys* sp.	Grundmann	1958
Cysticercus sp.	*D. phillipsii*	Leiper	1935
	NEMATODA		
Trichuris minuta	*D. merriami* *D. ordii*	Hannum	1943
Wellcomia longejector	*D. merriami*	Hannum	1943
T. dipodomis	*D. ordii*	Read	1956 1957
Heligmosomum sp.	*D. ordii*	Grundmann	1957
	D. microps	Grundmann and Warnock	1964
Protospirura numidica	*D. ordii*	Grundmann	1957
	D.o. utahensis	Grundmann and Frandsen	1960
Gongylonema dipodomysis	*D.m. merriami* *D.p. mohavensis*	Kruidenier and Peebles	1958
G. neoplasticum	*D.p. mohavensis* *D.m. arenvagus*	Read and Millemann	1953
	D. merriami	Voge	1956
Mastophorus dipodomis	*D.m. mohavensis*	Read and Millemann	1953
	D. deserti	Voge	1956
Rictularia dipodomis	*D.m. merriami* *D.p. mohavensis* *Dipodomys* sp.	Tiner	1948
	D. morroensis	Read and Millemann	1953
	D. merriami	Voge	1956
Trypanoxyuris deserti	*D.m. merriami* *D.m. mohavensis* *D. morroensis*	Read and Millemann	1953
	D. merriami	Voge	1956
Capillaria americana (Syn. *C. bonnevillei*)	*D. ordii marshalli*	Grundmann and Frandsen	1960

* According to Bienek and Klikoff, University of Utah (pers. comm.), four genera of intestinal helminths known to require insect intermediate hosts were recovered from dissection of 191 Merriam kangaroo rats, namely Cestoda: *Mathevotaenia* and *Catenotaenia*; Nematoda: *Rictularia* sp. and *Mastophorus numidica*; and Grundmann, also of the University of Utah, recovered from 43 of 76 *D. microps* 460 specimens of *Trichuris dipodomys* and five of *Capillaria americana*.

in Table 3. Brief discussions of the helminths collected are presented below:

Nematoda

Genus *Abbreviata* Travassos, 1920

Abbreviata sp.

Two female specimens of *Abbreviata* sp. were recovered from the stomachs of two *D. merriami*. The lengths of the worms were 37.5 and 38.0 mm and their widths were 1.5 mm. No scar tissue or other pathological signs due to infection were discernible, although the stomachs were distended due to the presence of the worms. The occurrence of this genus in kangaroo rats constitutes a new host record. Species of *Abbreviata* are parasites principally of reptiles. The life cycle, like other spirurids, involves an arthropod intermediate host. Some of the common arthropods that may serve as intermediate hosts for the genus are: German cockroaches (*Blatella germanica*), flour beetles (*Tribolium confusum*), field crickets (*Gryllus assimilus*), ground beetles (*Harpulus* spp.), and ants (*Pogomonyremex barabatus*) (Olsen, 1967).

Table 2. Number of specimens of *Dipodomys* spp. from each collecting site.

	D. merriami	*D. deserti*	*D. microps*	*D. panimintinus*
Providence Mt., California	0	0	0	1
Pahrump Valley	110	0	0	0
Beatty–Rhyolite Road	5	0	11	0
Boulder City*	45	16	0	0
Lincoln County (Highway 93)	5	3	0	0
Potosi Mt. (Goodsprings Road)	34	0	1	0
Lee Canyon	0	0	4	0

* Boulder City represents two sites: McCaller and Searchlight roads.

Genus *Gongylonema* Molin, 1857
Gongylonema dipodomysis
Kruidenier and Peebles, 1953

This genus was first reported from *D. merriami* by Read and Millemann, who tentatively identified their specimens as *G. neoplasticum* (Fibiger and Ditlevsen, 1914). *Gongylonema dipodomysis* differs from *G. neoplasticum* by the number of genital papillae, length of gubernaculum and buccal cavity, the size of ova, and the number and distribution of the cuticular bosses. Measurements taken of two males and 13 female specimens collected in this study fitted the description of *G. dipodomysis* as presented by Kruidenier and Peebles (1958).

Members of the genus *Gongylonema* require an arthropod intermediate host to complete their life cycle. Ransom and Hall (1915) and Thomas (1952) found that various dung beetles and cockroaches serve as intermediate hosts. The life cycle of *G. pulchrum* is discussed in detail by Belding (1965). *G. dipodomysis* probably follows the same basic pattern as *G. pulchrum*; however, the precise migratory pathway of the parasite from the site of infection to embedding in the wall of the esophagus is not known (Olsen, 1967).

Genus *Mastophorus* Diesing 1853
Mastophorus dipodomis
Read and Millemann, 1953

This parasite was collected only from *D. merriami* taken from a single area (Boulder City). Three of 45 animals of this species examined harbored the parasite, with a single worm taken from each host.

Chitwood (1938) discussed the controversial status of the genera *Mastophorus* and *Protospirura* Seurat, 1914, characterized each, and assigned certain species to each genus. However, if Chitwood's mode of classification is used, *M. dipodomis* could not be placed in either *Mastophorus* or *Protospirura*. For this reason, Read and Millemann reevaluated Chitwood's separation and suppressed *Protospirura* in favor of *Mastophorus*. On the other hand, Yamaguti (1961) placed all members of these two genera under *Protospirura* and synonymized *Mastophorus*. The characters of both genera seem to be distinct enough to warrant the retension of each and possibly the establish-

Table 3. Data on helminth infections.

	D. merriami (199)		*D. deserti* (19)		*D. microps* (6)	
Helminth	No. infected	Range infection	No. infected	Range infection	No. infected	Range infection
Abbreviata sp.	2	0–1	—	—	—	—
Gongylonema dipodomysis	6	1–4	—	—	—	—
Mastophorus dipodomis	3	0–1	—	—	—	—
Pterygodermatites dipodomis	8	1–11	6	1–12	1	0–1
Heteromoxyuris deserti	7	1–26	1	0–3	—	—
Catenotaenia linsdalei	5	1–6	1	0–1	6	2–82
Oochoristica sp.	1	0–1	—	—	—	—

ment of a third genus to include *M. dipodomis, P. anodon* Hannum, 1943, and *P. tetradon* Hannum, 1943. However, at this time, the writers feel justified in retaining the name *M. dipodomis* for the spirurids of kangaroo rats.

Members of the genus *Mastophorus* employ arthropod hosts (species of *Coleoptera, Orthoptera, Siphonaptera,* and *Lepidoptera*) for development. A discussion on certain of these intermediate hosts, as well as on development and morphology of *M. numidica,* is presented by Dyer and Olsen (1967).

Genus *Pterygodermatites* Wedl, 1891
Pterygodermatites dipodomis (Tiner, 1948)

Rictularid nematodes have been reevaluated by Quentin (1969) on the basis of the position of the oral opening, the number of esophageal teeth, and the number of prevulvar spines. He defined *Rictularia* Frölich, 1802, as having an oral opening which is dorsal and transverse and with one esophageal tooth; the male has one to four pairs or eight laterally positioned cloacal papillae and the female has 34 prevulvar spines. *Pterygodermatites* differs from *Rictularia* by having an oral opening that is not totally dorsal and tranverse, three esophageal teeth, and 29–56 prevulvar spines. Using these definitions several species of *Rictularia,* including *R. dipodomis,* were removed and placed in the genus *Pterygodermatites.* Several specimens of *P. dipodomis* were found embedded in the mucosa of the small intestine of 15 kangaroo rats (*D. merriami, D. deserti,* and *D. microps*), although the majority were found within the lumen. The range of infection was from one to 12 specimens per host. At the sites of attachment, some enteritis was noticed. Groups of small translucent nodules were observed on the serosal surface. Since these nodules were not observed in other nematode infections, they were considered to be a pathological reaction to parasitism by *P. dipodomis.*

Quentin (1970) discussed the life cycle of members of the family Rictulariidae Railliet, 1916. He noted that the first three larval stages occur in the ileum wall of grasshoppers. The fourth larval stage and the adult worm were found to occur in rodents.

Genus *Heteromoxyuris* Quentin, 1973
Heteromoxyuris deserti (Read and Millemann, 1953)

This nematode was collected from *D. merriami* and *D. deserti.* The maximum number of specimens collected from a single host was 26. The parasites were usually found in the cecum, although some were recovered near the junction of the cecum and small intestine. Except for distention of the cecum, no other pathological signs were present which may have been attributed to parasitism.

The morphology of the oxyurids collected fitted the description of *"Trypanoxyuris deserti,"* as originally described by Read and Millemann. Quentin (1973) redescribed the oxyurids of rodents and subdivided the species into four genera: *"Eyphrista* Quentin, 1970, *Hilgertia* n. gen., *Heteromoxyuris* n. gen. and *Evanginuri* Skrabin and Schikhobalova, 1951." *T. deserti* was transferred to the new genus *Heteromoxyuris,* which Quentin proposed because of evolutionary differences in the formation of the cephalic structures and the morphology and number of cloacal papillae. In employing the name, *H. deserti,* the writers concur with the nomenclature as presented by Quentin.

The life cycle of *H. deserti* is direct, closely paralleling that of the oxyurid of man, *Enterobius vermicularis* (Linn., 1758). Autoinfection probably also occurs in *H. deserti,* as in the latter species. *H. deserti* was not collected from *D. microps* and *D. panimintinus*; however, additional autopsies of these hosts may reveal the presence of this helminth.

Cestoda

Genus *Catenotaenia* Janicki, 1904
Catenotaenia linsdalei McIntosh, 1941

Six *D. microps,* five *D. merriami,* and one *D. deserti* harbored specimens of *Catenotaenia.* Dowell (1953) separated this genus into three groups on the basis of the number of testes: (1) 70–150; *C. linsdalei, C. californica* Dowell, 1953, and *C. pusilla* Goeze, 1781; (2) 140–250; *C. dendritica* Goeze, 1782, *C. goesciuri* Ortlepp, 1938, and *C. oranensis* Joyeux and Foley, 1930; and (3) over 400; *C. rhombomides* Schulz and Landa, 1935. The testes number in the tapeworms found in this investi-

gation ranged from 99 to 131, thus fitting into Dowell's group 1. *C. linsdalei* differs from both *C. californica* and *C. pusilla* in the number of uterine branches (*C. californica*, 25–30; *C. linsdalei*, 40–50; *C. pusilla*, 16–20). The uterine branches from specimens collected from *D. microps* and *D. merriami* ranged in number from 40 to 49. The size of the ova from *Catenotaenia* in this study ranged from 12–17 μ in length corresponding closely with that of *C. californica* (14–15 μ). However, Dowell described *C. californica* as having from 72 to 90 oval testes arranged in two distinct lateral bands. The testes of the cestodes examined in this study resemble those described as *C. linsdalei* by McIntosh (1941). Other measurements and general arrangement of organs are basically the same for both species, except that in the case of *C. linsdalei*, the ovary may be found anterior to the vitelline gland. The ovaries of specimens in this study were posterior to the vitelline gland. Cestodes collected in this investigation corresponded closely with *C. linsdalei* and *C. californica*, although appearing closer to *C. linsdalei*.

Cestodes of the genus *Catenotaenia* were first described by Voge (1948) from two species of kangaroo rats (*D. venustus* and *D. heermanni*). Since this time *C. linsdalei* has been described from *D. merriami* and *D. panimintinus* by Dowell and from *D. spectabilis* Merriam, 1890, by Guay and Senger (1962). Adult species of *Catenotaenia* are cosmopolitan in rodents.

The life cycle of *C. linsdalei* is unknown; however, a discussion of the life cycle of *C. pusilla* is given by Wardle and McLeod (1952). The adult and deutonymph stages of the mite, *Glyciphagus domesticus*, are used as intermediate hosts. Fifteen days after the mite ingests eggs of the tapeworm, the cysticercoid larva (with solid body, formed holdfast, and terminal osmoregulatory gut) becomes infective to rodents.

Genus *Oochoristica* Luhe, 1898
Oochoristica sp.

Two specimens of adult cestodes, identified as members of the genus *Oochoristica* (parasites principally found in reptiles) were found in a *D. merriami*. They were badly damaged, rendering specific identification difficult. Read and Millemann collected a single adult cestode from *D. merriami*. Their measurements corresponded approximately with those of *O. symmetrica* Baylis, 1927 (= *O. rattis* Yamaguti and Miyata, 1937). Baylis described *O. symmetrica* from *Rattus rattus* in India and *O. rattus* from *R. r. rattus* and *R. r. alexandrinus* from animals around Japanese harbors (Yamaguti and Miyata, 1937).

Oochoristica symmetrica and *O. rattus* are parasites of murid rodents and it seems unlikely that this parasite is the same as that found in desert rodents. Read and Millemann have suggested that their *Oochoristica* may be a new species since its description is not specifically identical to that of *O. symmetrica*. Additional studies on the life cycle may determine this. Species of *Oochoristica* have been found to develop in certain tenebrionid beetles and other insects (Wardle and McLeod, 1952).

Summary and Discussion

Four species of kangaroo rats, *Dipodomys merriami, D. deserti, D. microps,* and *D. panimintinus,* were collected from seven sites (Table 2). One hundred and two *D. merriami* were collected from Clark and Nye counties in 1968. Ninety-seven *D. merriami,* 19 *D. deserti,* 16 *D. microps,* and 1 *D. panimintinus* were collected in 1971 and 1972 from Clark, Nye, and Lincoln counties. Five genera and four species of nematodes and two genera and one species of cestode were recovered and morphologically identified. The number of infections and percentage of occurrence of each helminth collected from kangaroo rats are given in Table 3. *D. merriami* harbored the greatest diversity of helminths collected in this study. This may be due to its wide distribution; its range overlaps those of *D. microps, D. panimintinus,* and *D. deserti. D. merriami* was collected in all but two of seven trapping sites (Table 2).

The percentages of parasites recovered from the species of kangaroo rats investigated are given in Table 3. *Dipodomys microps* had the heaviest worm burden, with 82 specimens of *Catenotaenia linsdalei* being recovered from a single animal. The number of parasites collected decreased sharply in the remaining hosts. Only one specimen of *C. linsdalei* was re-

covered from *D. deserti,* however, this parasite occupied most of the small intestines. *Heteromoxyuris deserti* was the next most abundant species collected (Table 3) and was restricted to *D. merriami* and *D. deserti.* This restriction may have been due to the sharing of sand dune habitats and similar foods by these two hosts. *Pterygodermatites dipodomis* was the most prevalent parasite; however, the number of parasites infecting hosts was low, with a range of infection of from one to 12. All hosts, with the exception of *D. panimintinus* from which no helminths were recovered, were infected by this nematode.* All parasites reported in this study were recovered from *D. merriami.*

Kangaroo rats from Pahrump Valley, Potosi Mountain (Goodspring Road), and Boulder City (McCaller and Searchlight Roads) were most heavily parasitized by *P. dipodomis* (Table 3). Vegetation and ecological similarity could account for the similarity in the helminth fauna of hosts collected. Pahrump Valley and parts of the Boulder City area are characterized by a creosote bush plant community and sand dunes, while other portions of the Boulder City area compare with Potosi Mountain (Goodsprings Road) in being a blackbrush community. Cestodes were collected only at higher elevations in the blackbrush creosote transition zone or in the blackbrush vegetation community (Beatty–Rhyolite Road and Potosi Mountain–Goodsprings Road). Helminths that use arthropods as an intermediate host were most abundant at higher elevations. Bradley and Mauer observed that insects comprised a portion of the diet and were taken most often during August and September when green vegetation was not readily available. The variety of arthropods also would be enhanced with an increase in elevation because of increase in environmental heterogeneity. The greastest variety and the most abundant helminth infections were from these higher elevations.

All parasites collected, with the exception of *H. deserti,* require arthropod intermediate hosts (beetles, ants, mites, and grasshoppers). These arthropods were extremely abundant on the trapping sites with some variation in abundance according to season.

Although Bradley and Mauer (1971) reported some arthropod fragments in the stomachs of their study animals throughout the year, especially August and September, none were found in this study. For this reason, 12 burrow systems were excavated in three areas: Beatty–Rhyolite Road (5), Boulder City–Searchlight Road (4), and Red Rock–Blue Diamond area (3). These sites were chosen because of previous trapping success for *D. merriami, D. deserti,* and *D. microps.* Numerous observations were made of insects entering and/or leaving these burrow systems. The burrows excavated contained tenebrionid beetles and arachnids, with some minute beetles and insect parts located in the food storage areas of these burrows. Ingestion of arthropods as reported by Reynolds (1950) and Bradley and Mauer (1971) are thus indicated by the observations of our investigations.

* Bienek and Klikoff (pers. comm.—paper in press) suggested that *D. microps* in the cold desert of the Bonneville Basin carries only parasites that have a direct life cycle; however, this is not the case in the hotter and drier Mohave Desert, which has a distinctly different vegetation and in which food appears to be selected on the basis of seasonal abundance.

Acknowledgments

The writers extend their sincere appreciation to J. Ralph Lichtenfels, USDA, Animal Parasite Institute, Beltsville, Maryland, for assistance in compiling data relative to the parasites of *Dipodomys* and to W. Glen Bradley, Department of Biological Sciences, University of Nevada, Las Vegas, for proofreading the manuscript.

Literature Cited

Babero, B. B., and D. Matthias. 1967. *Protospirura peromysci* n. sp. (Nematoda: Spiruroidea) and other helminths from *Peromyscus* spp. in Nevada. Proc. Helm. Soc. Wash. 34: 255–261.

Beatley, J. C. 1969. Dependence of desert rodents on winter annuals and precipitation. Ecology 50: 721–724.

Belding, D. L. 1965. Textbook of Parasitology. Appleton-Century-Crofts, New York, 3rd Ed., 735 p.

Bienek, G. K., and A. W. Grundmann. 1973. A new tapeworm, *Schizochodes dipodomis* gen. et sp. n. (Cestoda: Anoplocephalidae), from the Merriam kangaroo rat, *Dipodomys merriami vulcani.* Proc. Helm. Soc. Wash. 40: 192–195.

Bradley, W. G., and J. E. Deacon. 1967. Bi-

otic communities of southern Nevada. Nev. State Mus. Anthro. Papers 13: 201–295.

———, **and R. A. Mauer.** 1971. Reproduction and food habits of Merriam's kangaroo rat, *Dipodomys merriami*. J. Mamm. 52: 497–507.

Chitwood, B. B. 1938. The status of *Protospirus* vs *Mastophorus* with consideration of the species of these genera. Livr. Jub. Prof. Travassos, p. 115–119.

Deacon, J. E., W. G. Bradley, and K. M. Larsen. 1964. Ecological distribution of the mammals of Clark Canyon, Charleston Mountains, Nevada. J. Mamm. 45: 397–409.

Dowell, A. M. 1953. *Catenotaenia californica* sp. nov., a cestode of the kangaroo rat, *Dipodomys panimintinus mohavensis*. Amer. Midl. Nat. 49: 738–742.

Dyer, A. W., and O. W. Olsen. 1967. Biology of *Mastophorus numidica* (Seurat, 1914) Read and Millemann, 1953 (Nematoda: Spiruridae) with a description of the juvenile stages. Proc. Helm. Soc. Wash. 34: 98–103.

Grundmann, A. 1957. Nematode parasites of mammals of the Great Salt Lake Desert of Utah. J. Parasit. 43: 105–112.

———. 1958. Cestodes of mammals from the Great Salt Lake Desert region of Utah. J. Parasit. 44: 425–429.

———, **and J. C. Frandsen.** 1960. *Capillaria bonnevillei* n. sp. (Nematoda: Trichuridae) from the Ord kangaroo rat in Utah. Proc. Helm. Soc. Wash. 27: 206–208.

———, **and R. G. Warnock.** 1964. A population study of eight parasitic nematode species from mammals of the Bonneville Basin of Utah. Proc. Utah Acad. Sci., Arts and Letters 41: 228–233.

Guay, J., and C. M. Senger. 1962. The occurrence of *Catenotaenia* sp. in *Dipodomys spectabilis* in Arizona. J. Parasit. 48: 451.

Hannum, C. A. 1943. Nematode parasites of Arizona vertebrates. Wash. State Univ. Abstr. Theses 7, p. 229–231.

Howell, A. B., and I. Gersh. 1935. Conservation of water by the rodent *Dipodomys*. J. Mamm. 16: 1–9.

Kruidenier, F. J., and C. R. Peebles. 1958. *Gongylonema* of rodents; *G. neoplasticum* (redefinition); *G. dipodomysis* n. sp. and *G. peromysci* n. sp. Trans. Amer. Microscop. Soc. 67: 307–315.

Leidy, P. D., W. P. Carney, and C. E. Woods. 1970. Studies on sylvatic echinococcosis. III. Host occurrence and geographic distribution of *Echinococcus multilocularis* in north central states. J. Parasit. 56: 1141–1150.

Leiper, R. T. 1936. Deaths caused by animal parasites. *In* A. E. Hammerton, Report on the deaths occurring in the society's gardens during the year 1935. Proc. Zool. Soc. London, pt. 3, p. 682–684.

McIntosh, A. 1941. A new dilepidid cestode, *Catenotaenia linsdalei*, from a pocket gopher in California. Proc. Helm. Soc. Wash. 8: 60–62.

Millemann, R. 1955. Studies on the life history and biology of *Oochoristica deserti* n. sp. (Cestoda: Linstowiidae) from desert rodents. J. Parasit. 3: 424–440.

Olsen, O. W. 1967. Animal Parasites: Their Biology and Life Cycles. Burgess Publ. Co., Minneapolis, Minn., 431 p.

Quentin, J. C. 1969. Essai de classification des Nematodes Rictulaires. Mem. Mus. Nat. Hist. Nat. Nle ser., ser. A 54: 56–115.

———. 1970. Cycle biologique de *Pterygodermatites* (Mesopectines) *taterilli* (Baylis, 1928) Nematoda, Rictulariidae. Ann. Parasit. Humaine et Comparee 45: 629–635.

———. 1974. Les Oxyurinae de Rongeurs. Bull. Mus. Nat. Hist. Nat. Ser. 3e, (167)112: 1–1096.

Ransom, B. H., and M. C. Hall. 1915. The life history of *Gongylonema scutatum*. J. Parasit. 2: 80–86.

Read, C. P. 1956. *Trichuris dipodomis*, n. sp., from Ord's kangaroo rat. Proc. Helm. Soc. Wash. 23: 119.

———, **and R. E. Millemann.** 1953. Helminth parasites in kangaroo rats. Univ. Cal. Publ. Zool. 59: 61–80.

Reynolds, H. G. 1950. Relation of Merriam kangaroo rats to range vegetation in southern Arizona. Ecology 31: 456–463.

Simpson, R. L., and W. Harmon. 1968. Occurrence of *Hymenolepis citelli* McLeod, 1933 in the rodent genus *Dipodomys* Gray, 1841. J. Parasit. 54: 769.

Thomas, L. J. 1952. *Gongylonema pulchrum*, a spirurid nematode infecting man in Illinois, U.S.A. Proc. Helm. Soc. Wash. 19: 124–126.

Tiner, J. D. 1948. *Rictularia dipodomis* n. sp. (Nematoda: Thelaziidae) from the kangaroo rat, *Dipodomys* spp. J. Parasit. 34: 332–334.

Voge, M. 1948. New rodent host for *Catenotaenia linsdalei* with an addition of this cestode. Trans. Amer. Microscop. Soc. 67: 266–267.

———. 1949. New rodent hosts for *Catenotaenia linsdalei*, with an additional description of this cestode. Trans. Amer. Microscop. Soc. 67: 266–267.

———. 1955. A list of cestode parasites from California mammals. Am. Midl. Nat. 54: 413–417.

———. 1956. A list of nematode parasites from California mammals. Am. Midl. Nat. 56: 423–429.

———. 1958. A list of cestode parasites from California mammals. Am. Midl. Nat. 54: 413–417.

Wardle, R. A., and J. A. McLeod. 1952. The Zoology of Tapeworms. Univ. Minn. Press, Minneapolis, Minn., 780 p.

Yamaguti, S. 1961. The Nematoda of Vertebrates. Systema Helminthum III (Parts 1 and 2). Intersci. Publ. Inc., New York, 1261 p.

———, **and I. Miyata.** 1937. A new tapeworm (*Oochoristica rattus*) of the family Anoplocephalidae from *Rattus rattus* and *Rattus r. alexandrinus*. Japan J. Zool. 7: 501–503.

Yousef, M. K., and D. B. Dill. 1970. Physiological adjustments to low temperature in the kangaroo rat (*Dipodomys merriami*). Physiol. Zool. 43: 132–138.

Research Note

Incidence of Intestinal Parasites in Children from Scotlandville Area of Baton Rouge in Louisiana

Human intestinal parasitism is still one of the most important health hazards in the rural areas of the southern United States especially among the lower urban and rural communities of Louisiana. The earlier works of many investigators published and unpublished attest to this fact. Jung and Beaver (1952, Pediatrics, 8: 548–557) reported a remarkably high incidence of association of *Trichuris trichiura* and *Endamoeba histolytica* infections in children from the New Orleans area, and also reported cases of prolapsed rectum due to trichuriasis among some of these children. Corkum (1966, J. Parasit. 52:444–448) reported sparganosis in some vertebrates of Louisiana and made observations on a human infection. According to a report filed with the East Baton Rouge Parish Health Unit in 1970 (unpublished) by a team of investigators from the Tulane University School of Public Health and Tropical Medicine, many persons from East Baton Rouge Parish, both old and young, males and females, were found to harbor different types of intestinal helminths and protozoa.

The present survey was undertaken as the result of an outbreak of helminthiasis and amoebiasis among the school children in Scotlandville area. The East Baton Rouge Parish Health Unit was called to the scene and I (F.A.C.) was invited along with the chief (J.T.C.) to participate in the survey. Some of the children were actually passing out worms with their feces and some with bloody stool. As a result of this outbreak, many other parents in the community voluntarily brought their children's fecal samples to our laboratory for examination. Fecal samples from 452 children representing both sexes and ranging in age from 3 to 7 years old were examined. The fecal samples were examined by direct smear and centrifugal sedimentation techniques. Direct microscopic examination of the living worms picked out of the feces was also done, and in many cases trophic stages of protozoa were readily observable since the fecal samples examined were fresh in most of the cases.

Routine microscopic examination of the stool specimens were made first under glass-cover preparation containing about 2 mg of fecal sample thoroughly mixed with one drop of physiological saline solution. This was followed by addition of iodine stain made up of 1% aqueous solution of potassium iodide, saturated with iodine crystals. A drop of this stain was then placed at the edge of the cover slip and allowed to flow into the film on the slide preparation.

To further confirm our findings, other direct method of fecal smears was made by using the stock solution of MIF (merthiolate–iodine–formaldehyde) fixative stain of Sapero and Lawless (1953, Am. J. Trop. Med. Hyg. 2: 613–619). One drop of distilled water and a drop of MIF fixative stain were placed on the glass slide and adding about 2 mg of fecal sample, mixed thoroughly, and covered with a glass cover slip and examined under phase contrast microscope.

The above methods were supplemented by the formalin–ether centrifugal sedimentation technique as modified and proposed by Ritchie (1948, Bull. U. S. Army Med. Dept. 8: 326). This procedure was more of confirmation because most of the helminth eggs and protozoan cysts when present were readily observable under the microscope.

RESULTS: Following per cent infections were observed. Protozoa: about 55% of the children harbored *Endamoeba histolytica*; 54% harbored *Entamoeba coli*; 5.5% harbored *Endolimax nana*; 3.3% harbored *Giardia*. Platyhelminthes: 1% harbored *Hymenolepsis nana*; 1% *Diphyllobothrium latum*; 1% harbored undetermined tapeworm. Nematoda: 22% of the children harbored *Trichuris trichiura*; 5.5% harbored *Enterobius vermicularis*; 27.6% harbored *Ascaris*; 4% harbored hookworms.

In the above study, (1) the infections by these parasites are found in equal proportions

in both male and female children; (2) only 12 of the 452 children examined were negative for any of the above intestinal parasites; (3) there is a remarkably high incidence of multiple infections of two to four intestinal parasites in most of the fecal samples; (4) there is a remarkable association of (a) trichuriasis and amoebiasis (b) ascariasis and amoebiasis, respectively.

The results of this study have been submitted to the East Baton Rouge Parish Health Department for proper action on the treatment of the individual child infected.

We gratefully acknowledge the technical assistance of the E.B.R.P. Health Nurses and Mrs. Neola Clarke of the Biology Department, Southern University.

Supported by USDA–CSRS–La. Grant No. 8-1507.

FRED A. CHRISTIAN
Department of Biological Sciences,
Southern University,
Baton Rouge, Louisiana 70813

JAMES T. PERRET, M.D.
Chief,
East Baton Rouge Parish Health Unit,
Baton Rouge, Louisiana 70813

Research Note

New Host Record for *Setaria yehi* Disset, 1966, and Range Extension Records for *Dictyocaulus viviparus* (Bloch, 1782) and *Ostertagia mossi* Dikmans, 1931, in Fallow Deer (*Dama dama* L.)

During a parasitological survey of 15 fallow deer from Land Between the Lakes recreation area in western Kentucky, 9% harbored 16 *Setaria yehi* in the mesenteries, 21% harbored 11 *Ostertagia mossi* in the abomasum, and 28% harbored 21 *Dictyocaulus viviparus* in the lungs. Methods of diagnosis were by autopsy plus eggs or larvae in the feces.

To the author's knowledge, this is the first report of *S. yehi* from fallow deer and thus constitutes a new host record. Specimens conform to descriptions given by Yeh (1959, J. Helm. 33: 1–98) and Becklund and Walker (1969, J. Parasit. 55: 359–368).

This is also the first report of *D. viviparus* and *O. mossi* from fallow deer in North America. However, *O. mossi* has been reported from fallow deer in the Netherlands by Swierstra, Jansen, and van den Broek (1959, Tijdschr. Diergeneesk 84: 1301–1305 and Jansen and van den Broek (1966, K. Zool. Genootsch. Natura Artis Magistra Amsterdam 36: 65–68). *D. viviparus* has been reported from fallow deer in Czechoslovakia and Poland by Kotrly (1958, Cesk. Parasitol. 5: 101–110) and Drozdz (1966, Acta Parasitol. Polon. 14: 1–13), respectively. Specimens of *D. viviparus* and *O. mossi* conform to descriptions given by Olsen and Fenstermacher (1943, Univ. Minn. Tech. Bulletin 159).

All three species of nematodes have been reported many times from white-tailed deer in North America (Index-Catalogue of Med. Vet. Zool., 1970, Spec. Pub. No. 1, U. S. Govt. Printing Office, p. 18–19).

We would like to express our appreciation to Dr. Annie K. Prestwood, Southeastern Cooperative Wildlife Disease Study, Athens, Georgia, for her continued help in our deer parasite studies. This work was supported in part by an EKU faculty research grant No. 42–80.

J. H. PHILLIPS, J. P. HARLEY, AND
W. J. RUDERSDORF,
Department of Biological Sciences,
Eastern Kentucky University,
Richmond, Kentucky 40475

Research Note

Pseudosonsinotrema chabaudi (Caballero y C. and Caballero R., 1969) comb. n., a Senior Synonym of *P. echinophallus* Sullivan, 1971, from Costa Rican Frogs

Caballero y C. and Caballero R. (1969, Ann. Parasitol. 44: 539–546) established the genus *Brenesia* for *B. chabaudi* described from the intestine of *Rana pipiens* in Costa Rica. These authors assigned *Brenesia* to the Lecithodendriidae Odhner, 1911, on the basis of a V-shaped excretory bladder, the anterolateral position of the vitellaria, the short ceca, and the form and position of the reproductive organs. Although noting the apparent relationship between *Brenesia* and both *Pleurogenoides* Travassos, 1921, and *Pseudosonsinotrema* Dollfus, 1951, Caballero y C. and Caballero R. distinguished their new genus from both of these genera by the form and position of the terminal genitalia and the gonads.

Sullivan (1971, Proc. Helm. Soc. Wash. 38: 34–37) described *Pseudosonsinotrema echinophallus* from the intestine of *R. pipiens* in Costa Rica and assigned the species to the Pleurogenidae Odening, 1959. Comparison of the descriptions of *P. echinophallus* and *B. chabaudi* indicates that the two forms are conspecific. However, the establishment of the genus *Brenesia* for this form appears unwarranted.

Although the criteria used by Caballero y C. and Caballero R. (loc. cit.) preclude placement of *B. chabaudi* in *Pleurogenoides*, these same criteria do not support exclusion from *Pseudosonsinotrema*. A strongly developed metraterm is the primary generic character of *Pseudosonsinotrema*. All of the described species [*P. chamaeleonis* Dollfus, 1951; *P. megametrum* Manter and Pritchard, 1964; *P. japonicum* (Yamaguti, 1936); *P. echinophallus*; and *P. catesbeianae* Christian, 1971] show such strongly developed metraterms. However, this organ in *P. japonicum* is glandular rather than muscular (Manter and Pritchard, 1964, Ann. Mus. Roy. Afr. Centr., in-8°, Zool. 132: 75–101). Caballero y C. and Caballero R. (loc. cit.) noted a correspondence between *P. japonicum* and *B. chabaudi* in the morphology of the metraterm but indicated that the glandular metraterm was sufficient to distinguish *Brenesia* from *Pseudosonsinotrema*. Reexamination of paratypes of *P. echinophallus*, which is considered conspecific with *B. chabaudi*, demonstrates that the muscular metraterm is surrounded by glandular cells, but the presence of these cells, in the present author's judgment, is of no generic significance, when the metraterm is so well developed muscularly. Accordingly, *Brenesia* is a synonym of *Pseudosonsinotrema*, and since no characters are evident to separate *B. chabaudi* from *P. echinophallus*, the latter species is a synonym of *Pseudosonsinotrema chabaudi* n. comb.

The excretory bladder in *P. chabaudi* is distinctly Y-shaped with long arms and a short stem. Although Caballero y C. and Caballero R. (loc. cit.) interpreted the form of the bladder as V-shaped, the Y-shape of the bladder is evident in their Figure 1. Therefore, *Pseudosonsinotrema* should be retained in the Pleurogenidae, as proposed by Sullivan (loc. cit.), rather than in the Lecithodendriidae, members of which are characterized by a V-shaped bladder.

JAMES J. SULLIVAN
Institute for Medical Research
University of California ICMR
Kuala Lumpur, Malaysia

Research Note

Larval *Contracaecum* (Nematoda, Anisakidae) in the Pericardium of Fishes from East African Lakes[1]

Large, thick, unsheathed, and unencysted worms, 32–43 mm long, 1.30–1.88 mm in diameter, were found in the pericardial cavity of *Tilapia nilotica* (in 35% of 78 checked fish) and rarely they were also found in *Haplochromis* spp. from Lake George (in 0.4% of 487 checked fish) and Northern Lake Victoria (in 1% of 184 checked fish), and in *Bagrus docmac* from Lake George (in 2% of 47 checked fish). The identity of these worms as larval forms of the genus *Contracaecum* was confirmed by Dr. L. F. Khalil of the Commonwealth Institute of Helminthology. Specimens were deposited in Musée Royal Afique Central, Tervuren, Belgium.

Only a few anatomical characteristics could be observed in these larval worms. Around the mouth, one papilla was observed on each lip. Posterior appendix of the ventriculum was one-third the length of the intestinal cecum; the latter is three-thirds the length of the esophagus. The anus terminal, the caudal end is reduced to a small process. Usually, only one worm was found per infected fish; however, in rather small (60–90 mm) specimens of *Haplochromis,* two worms were occasionally found. Nematodes from *Haplochromis* spp. and *Bagrus* were longer (43.0 ± 5.8* mm) and narrower (1.44 ± 0.46) than those from *T. nilotica* (length: 37.5 ± 4.67; diam 1.54 ± 0.26). Additionally, worms from *Haplochromis* spp. were heavier than those recorded from *T. nilotica* of similar size (37.5 to 44.0 mg versus 18.2 ± 11.9). However, the worms from large *T. nilotica* were heavier (62.5 ± 21.1 mg). In the double infections noted in *Haplochromis,* the combined weight of the worms was more than twice the mean weight of single worms found in fish of similar size (53.0 and 24.0 mg against 37.5–44.0 mg). None of the infected *T. nilotica* showed signs of emaciation according to plotted length–weight relationships. On the other hand, some of the *Haplochromis,* including one of the two fish with a double infection, were emaciated, with a weight loss of up to 20%. After the death of the host, the worms were observed to leave the pericardium and migrate through the tissue, into the visceral cavity.

From the three size groups of *T. nilotica* studied (total length of 35–70 mm; 80–110 mm, and 270–350 mm), infection prevalence is the lowest in the youngest group (4% of 25 checked fish); however, the prevalence of infection in the fully grown fish (30% of 30 checked fish) unexpectedly remains much the same as in the fish of the medium size group (35% of 23 checked fish). This may be due to the fact that the infected fish suffer increased mortality or that adult fish are less likely to become infected because of their different habitat selection or feeding pattern.

I wish to acknowledge with thanks Dr. L. F. Khalil's help in the identification of the nematode larvae.

I. Paperna
Virginia Institute of Marine Science
Gloucester Point, Virginia
Present address:
The H. Steinitz Marine Biology
Laboratory at Elat, Israel

[1] Contribution No. 642 from the Virginia Institute of Marine Science, Gloucester Point, Virginia 23062.
* Standard deviation.

Research Note

Larval *Eustrongylides* sp. (Nematoda:Dioctophymatoidea) from Otter, *Lutra canadensis*, in Maryland

Eustrongylides spp. are parasitic as adults in the mucosa of the esophagus or proventriculus or in the intestine of water birds and as larvae in the connective tissue or body cavity of freshwater fish or, occasionally, amphibians and reptiles [Karmanova, 1968, Osnovy Nematodology, Vol. 20 (in Russia), Akad. Nauk SSSR, Moscow]. Reports of nematodes of this genus in mammals are extremely rare.

Three larval *Eustrongylides* sp. were found in the intestine of an otter, *Lutra canadensis*, which was trapped in Anne Arundel County, Maryland, in the winter of 1972. The nematodes are in a premolting stage of development with the cuticle of the preceding stage separated near the extremities. One entire male is 87 mm long with an esophagus 17.5 mm long. One fragment has an esophagus 19.4 mm long and another has an esophagus 18.2 mm long. The cephalic papillae are prominent in two well-separated circles of six each. The specimens have been deposited in the National Parasite Collection as USNM Helminthological Collection Number 72833.

This report appears to be only the third record of *Eustrongylides* spp. in mammals. Recently, Gibson and McKiel (1972, Can. J. Zool. 50: 897–901) found larvae in connective tissue cysts in the muscles of muskrats, *Ondatra zibethica*, in Ontario, Canada. Previously, Morishita [1923, Dobuts. Zasshi, Tokyo (415) 35: 209–210] reported *Eustrongylides* from the intestine of a raccoon. Von Brand and Cullinan (1943, Proc. Helm Soc. Wash. 10: 29–33) were unsuccessful in infecting rats or rabbits per os, but successfully infected ducks, chickens, and rats by intraperitoneal and subcutaneous implantations, which were followed by considerable parasite migration and high rates of mortality of most host species.

A search of the records of the National Parasite Collection for unpublished records of *Eustrongylides* from mammals revealed a single specimen that had been coughed up by a man. It is similiar in size to those from the otter and is a larval female. Possibly the nematodes reported herein had recently been ingested by the otter because similar specimens occur in fish and frogs. According to Levine (1968, Nematode Parasites of Domestic Animals and of Man, Burgess Pub. Co., Minneapolis), muskrats are known to serve as transport hosts for the related nematode of mammals. *Dioctophyma renale*. Any role of mammals as transport hosts in the life cycle of *Eustrongylides* spp. should not be overlooked but it is highly unlikely because of the structure of the food chains involved. However, because of the potential for high mortality from infection (Von Brand and Cullinan, loc. cit.), information on the occurrence of *Eustrongylides* in mammals should be monitored. Perhaps the larvae were found in the intestine of the otter because they were in a molt, a relatively inactive stage when ingested by the otter. Previous attempts to infect mammals per os used active third-stage larvae. Additional studies are needed to determine whether later stages of development might be infective to mammals.

This study was supported, in part, by funds from the Hampton Institute Faculty Research Fund and the Minority Schools Biomedical Research Program, National Institutes of Health. Grant No. RR08074.

James B. Abram
Department of Biology,
Hampton Institute,
Hampton, Virginia 23368

J. Ralph Lichtenfels
Animal Parasitology Institute,
Agricultural Research Service, USDA,
Beltsville, Maryland 20705

Research Note

Observations on in vitro Survival and Development of *Meloidogyne*

The life cycle of root knot nematodes, *Meloidogyne* spp., includes infective second-stage larvae, parasitic juvenile stages within plant tissue, males that migrate from the host into soil after reaching maturity, and parasitic female adults. The female root-knot nematode increases in volume about 575 times as it develops from an infective second-stage larva to the mature adult, and secretes an egg sac approximately equal to its own volume (Dropkin and King, 1956, Exp. Parasit. 5: 469–480). The female adult *Meloidogyne* has been described in the literature as a sedentary endoparasite spending its life within plant tissues. The pear-shaped contour and bulk of the adult female's body prevents migration. This research note reports on survival of female adults of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, in the absence of host tissues and observations on in vitro egg production and development.

Fifty specimens of 30-day-old *M. incognita* bearing egg sacs were dissected from infected begonia leaves. Egg sacs were teased away from the worms. Each nematode was placed in a hanging drop of soil–water medium on a depression slide, kept in moist chambers to prevent desiccation, and incubated at 27 C. The soil–water medium was prepared by steaming 1 liter of distilled water and 100 g of organic garden soil for 2 hr on 2 consecutive days.

Slides were examined periodically for vital signs: (1) pulsating of esophageal bulb, (2) thrust of stylet, (3) egg secretion and body color (the bodies of dead females were brownish instead of white).

Motile bacteria accumulated around the heads of about 75% of the worms during the first few minutes and remained there in a motile state for 24 hr. On the 2nd day of the experiment, the bacteria appeared as an inert mass, approximately 100–160 μ in diameter, around the worm's head. This mass of material remained until death of the worm. The stylet was seen thrusting back and forth; the esophageal bulb pulsated and the nematodes appeared to be feeding from the bacterial mass of dead organisms.

Data collected on survival time indicated that 60% of the female worms showed vital signs up to 3 weeks and 4% lived for 30 days. Fifty per cent of the worms secreted new egg masses within the 1st week of the study but the gelatinous matrix was sparse. Viable eggs were laid in the one-cell stage and hatching was first observed 8 days later. Hatched larvae were infective for begonia leaves.

Although the worms probably survived mainly on food reserves and secreted eggs that were already in the uterus, these observations show that the adult female of *M. incognita* can survive and produce viable eggs in the absence of host plant tissues. Observations suggest that bacterial attractants and substances that immobilize and kill bacteria are secreted at the anterior end of the adult female. It appears that a bacteriocidal agent is secreted by the female, but other factors might be responsible for death of the bacteria.

Studies on the bacteria involved and the nature of the bacterial mass are presently underway.

JACQUELINE R. SHEPPERSON AND
WILLARD C. JORDAN
Department of Natural Sciences
Winston-Salem State University
Winston-Salem, North Carolina 27101

Research Note

First Report of *Molineus* sp. Recovered from a Dog in the United States

Members of the genus, *Molineus* (Nematoda: Trichostrongylidae) have been reported from a variety of hosts which includes primates, insectivores, rodents, and carnivores (Balasingam, 1963, Can. J. Zool. 41: 599–602; Schmidt, 1965, J. Parasit. 51: 164–168). While a majority of the *Molineus* sp. has been reported from carnivore hosts, none has been reported from the Canidae of North America. However, Kozlov (1963, Trudy Gel'mint. Lab. 13: 56–74) has reported *M. patens* from domestic and wild Canidae of the far eastern U.S.S.R. Also, Balasingam (ibid.) has artificially exposed cats and dogs with infective larvae, both orally and subcutaneously, and established patent infections with *M. barbatus* obtained from the North American raccoon (*Procyon lotor*). With these positive results, Balasingam suggested the possibility of raccoons and skunks serving as reservoir hosts and establishing the potential of *M. barbatus* infections in domestic dogs and cats of North America. Natural infections of *Molineus* sp. in the dog and cat have not been reported to date and, therefore, it is of interest that we have recovered such from a dog and report this host–parasite relationship for the first time in North America.

The host was a dog of "hound-type" breeding, mature (estimated age of 2 to 5 years), and had been obtained from a dog pound in west central Ohio. This dog was moderately parasitized with *Ancylostoma*, *Uncinaria*, and *Trichuris* and had been imported for a special anthelmintic study. Pretreatment fecal egg counts indicated the presence of the aforementioned parasitic nematodes, but the presence of numerous hookworm eggs would have masked the *Molineus* eggs should they have been present. The dog was treated with TASK® TABS (Shell) and the feces collected over a 4-day period prior to necropsy. At necropsy, five small trichostrongylid-like, gravid female worms were recovered from the small intestine washings. Because of their distinct morphological differences, these unknown nematodes were submitted to a more formal identification. They were identified by one of the authors (M. B. Chitwood) as *Molineus* species, probably as *M. barbatus*, Chandler, 1942. The length of the five female worms ranged from 5.4 to 6.7 mm by 66 to 70 μ diameter. In general, their morphological identity was the same as described by A. C. Chandler (1942, J. Parasit. 28: 255–268) for *M. barbatus* from a raccoon in Texas. In the absence of any male worms, positive identification of these *Molineus* sp. remains incomplete. However, it is concluded from these findings that *M. barbatus* of the raccoon can be transmitted in nature to the domestic dog as predicted by Balasingam approximately one decade prior to our observations.

D. K. Hass
Biological Sciences Research Center,
Shell Development Company,
P. O. Box 4248,
Modesto, California 95352

M. B. Chitwood
Primate Parasite Registry,
California Primate Research Center,
University of California,
Davis, California 95616

Research Note

Psammonyx nobilis (Amphipoda: Lysianassidae), a New Host for *Bothrimonus sturionis* (Cestoda: Pseudophyllidae)

Bothrimonus sturionis has been reported from a wide variety of geographical areas and from numerous gammaridean amphipod intermediate hosts. The systematics and ecology of this tapeworm has been extensively reviewed by Burt and Sandeman (1969, J. Fish. Res. Bd. Canada 26: 975–966) and Sandeman and Burt (1972, J. Fish. Res. Bd. Canada 29: 1381–1395). All intermediate hosts reported to date have been members of the family Gammaridae, with the exception of the record of Ouspenskaia (1960, Ann. Parasitol. Hum. Comp. 35: 221–242), who found *B. sturionis* in *Anonyx nugax* (Lysianassidae) off the coast of Murmansk. We have recently found this cestode in another lysianassid, *Psammonyx nobilis,* an infaunal amphipod inhabiting intertidal and shallow subtidal sands from Long Island Sound to Newfoundland.

Collections of *Bothrimonus* were made from *P. nobilis* from three locations in northern New England: Foss Beach, adajacent to Rye Harbor, N. H.; Gerrish Island, Maine, at the mouth of the Piscataqua River; and Goose Rocks Beach, Maine, in Goosefare Bay. At each location, material from November, January, April, and July 1972 was examined. In addition, at Foss Beach, the rates of infection for August through October 1972 and November 1973 were determined. A total of 1,464 amphipods were examined.

Our data show that the rate of infection in *Psammonyx* varies not only in degree but in the season of peak infection at the three locations. At Goose Rocks, the infection rate was low and never got above 2% at any time throughout the year, while at Foss Beach and Gerrish Island, the parasite occurred from November to April but not in July. The peak infection at Gerrish Island was in the spring (9.1%) while at Foss Beach it was during the fall (1972—32.4%, 1973—34.2%). These results confirm the views of Sandeman and Burt (1972) that no infection occurs in the intermediate host during the summer months and that peak infection, at least at Gerrish Island, is in the spring. However, Foss Beach provides a striking contrast, for here there is a rapid increase in infestation from August to November and a sharp decline through the winter and spring months, to the normal noninfected state in the summer.

Sandeman and Burt, as well as Stark (1965, Parasitology 55: 415–420), observed few early developmental stages in *Gammarus* and *Marinogammarus.* They suggested that "growth and maturation of the larval worm is extremely rapid and perhaps coincides with the short spring burst of activity and growth of the gammarid host." In *Psammonyx,* numerous developmental stages, including some less than 1 mm, were found during and preceding the peak of infection in the fall at Foss Beach. Larger parasites, up to 65 mm, were found in April at both Foss Beach and Gerrish Island. However, by this time the high rate of infection at Foss Beach had declined from over 30% to about 5%. Such a decline would indicate the likelihood of a markedly higher mortality of infected as compared with uninfected amphipods. One of us (KJS) has determined that *Psammonyx* differs from *Gammarus* in that it breeds in late winter and spring, without an overwintering stage. Therefore, its period of most rapid growth and maturation takes place in late summer and fall. Presumably, infection takes place just before this. In *Gammarus,* on the other hand, the time of infection appears to be followed by a period of little growth until the period of rapid growth of the parasite in the spring.

It appears then, that the eggs are eaten by *Psammonyx* during the summer and, after hatching, the larvae continue to grow throughout the fall and complete their life cycle, after the amphipod has been ingested by a bottom fish. There is, so far, no evidence that egg production can take place in *Psammonyx* as it does in *Gammarus* and *Marinogammarus* (Sandeman and Burt, 1972).

Records of examinations of numerous marine fish by one of us (WLB) indicate that the

winter flounder (*Pseudopleuronectes americanus*) is the usual host for this cestode. All such positive records for *B. sturionis* are from flounders collected in May and June as previously indicated by Burt and Sandeman. How this is related to the developmental cycle in *Psammonyx* is not clear at this time.

Observations of live *P. nobilis* indicate that there are no noticeable changes in the coloration and the swimming or burrowing behavior of infected individuals. Also there was no significant difference in size between parasitized and nonparasitized amphipods. The males of infected *Psammonyx* have normally developed gonads but infected females have none present and this is probably the most important influence of the parasite on this species. If a one-to-one sex ratio prior to breeding is assumed, then 30% of the females at Foss Beach would be nonbreeding and the reproductive capacity of this population considerably lowered. The exact degree of the reduction will become evident as more is learned of the ecology of the intermediate host.

This research was partially funded by NSF GA–33743 to Dr. R. A. Croker, University of New Hampshire. This work is contribution No. 10 from Jackson Estuarine Laboratory, Durham, New Hampshire 03824.

K. John Scott and Wilbur L. Bullock
Jackson Estuarine Laboratory and Zoology Department
University of New Hampshire
Durham, New Hampshire 03824

Research Note

Immunosuppressive Effect of Betamethasone and the Distribution of Adult *Trichinella spiralis* in the Intestine of the Jird (*Meriones unguiculatus*)

The distribution of adult *Trichinella spiralis* in the small intestine of rodents is generally agreed to be nonuniform. It is generally accepted that a majority of the worms live in the anterior part of the small intestine in mature rats (Gursch, 1949, J. Parasit. 35: 19–26; Larsh and Hendricks, 1949, J. Parasit. 35: 101–106), in mature mice (Larsh and Hendricks, 1949), in young mice (Campbell, 1967, J. Parasit. 53: 395–397), and mice of unspecified age (Podhajecky, 1962, Wiadomosci Parazytologiczne 8: 633–636; Marty, 1966, J. Parasit. 52: 903–907). However, a number of investigators have reported that a majority of adult worms inhabit the posterior part of the small intestine in young rats and mice (Larsh and Hendricks, 1949, loc. cit.) and in rats, mice, and guinea pigs of unspecified age (Tyzzer and Honeij, 1916, J. Parasit. 3: 43–56; Roth, 1939, Am. J. Hyg. 29: 89–104; Denham, 1965, Parasitology 55: 10-11). This report presents observations on the distribution of *Trichinella spiralis* in the intestine of the jird (*Meriones unguiculatus*) and the effect of betamethasone on this distribution and "spontaneous cure" is documented.

Jirds raised in our laboratory were housed in groups of three to five and fed a commercial pelleted laboratory chow and allowed drinking water ad libitum.

Each jird was inoculated orally with approximately 600 *T. spiralis* larvae collected by standard pepsin–HCL digestion techniques. On days 7, 14, and 21 after inoculation, groups of jirds were killed and the intestines removed in their entirety. The intestines were divided into five equal portions, slit lengthwise, and placed in saline at 37C for 2 hr. Sufficient 0.4% NaOH was added to give a final concentration of 0.04% and the samples refrigerated overnight. The remaining gut portions were removed and the number of worms remaining were estimated from a 5-ml

Table 1. Effect of betamethasone phosphate on the expulsion of *Trichinella spiralis* from the jird small intestine.

Number of Animals	Days after inoculation	Average number of worms/jird (range)	Treatment
7	7	253.0 (144–352)	None
7	14	328.8 (129–428)	None
5	21	98.2 (13–152)	None
4	21	389.0 (216–535)	Betamethasone

sample (approximately 10-12%). The separation of the intestine in five equal parts results in the following divisions:

A. Anterior third of small intestine;
B. Middle third of small intestine;
C. Posterior third of small intestine;
D. Occasional small intestinal portion posterior to C, cecum, and anterior half of large intestine;
E. Posterior half of large intestine and rectum.

No attempt was made to differentiate the jirds by sex. Three of the jirds in the 7- and 14-day groups were under 3 months at inoculation (young), the remaining jirds were over 5 months at inoculation.

Cortisone, prednisone, and dexamethasone have a demonstrated inhibitory effect on the expulsion of *T. spiralis* from the host intestine between days 14 and 21 after infection. This study documents the expected inhibitory effect of the steroid betamethasone on the expulsion of *T. spiralis* from the jird small intestine.

Betamethasone was used as an experimental solution of the phosphate salt at a concentration of 2 mg/ml. Each treated jird received 0.5 mg intramuscularly on alternate days starting day 7 after inoculation and terminating at autopsy.

Treatment with betamethasone appears to prevent the expulsion of the adult worms (Table 1). Although histamine, serotonin, and inflammation may play a role in expulsion of worms, the effect of steroids is generally accepted as due to immunosuppression rather than to antiinflammatory properties (Campbell 1968, J. Parasit. 54: 452-454). A greater percentage of adults were recovered from the posterior end of the small intestine of betamethasone-treated jirds than from the controls (Table 2).

If all the worms in segment D can be attributed to worms from the small intestinal portion of this segment, a uniform distribution of worms is obtained in 7-day-old jirds. Considering only the adult (5 months +) jirds, a marked concentration of worms toward the anterior small intestine was observed. The per cent of worms in the adult jirds was recorded as 48, 29, 15.8, 7.2, and 0 for each segment. The reverse was observed with the younger jirds (3 months old). In the

Table 2. Distribution of adult *Trichinella spiralis* in the intestine of the jird (*Meriones unguiculatus*).

Days after inoculation	No. of animals	Segment of intestine	Average number of worms/segment (range)	% of total worms/segment
7	7	A	84.1 (40–120)	33.1
		B	89.6 (0–148)	33.7
		C	61.3 (19–142)	24.1
		D	22.6 (0–95)	8.9
		E	0	0
14	7	A	61.4 (20–102)	18.6
		B	91.4 (20–163)	27.8
		C	136.0 (59–177)	41.3
		D	40.0 (0–59)	12.1
		E	0	0
21	5	A	31.6 (13–45)	31.1
		B	36.0 (0–57)	29.5
		C	35.2 (0–67)	29.5
		D	4.6 (0–17)	4.5
		E	0	0
21t	4	A	22.5 (0–56)	6.1
		B	69.3 (23–114)	23.2
		C	184.3 (91–288)	45.5
		D	112.8 (0–201)	25.3
		E	0	0

t = Betamethasone phosphate-treated.

jirds examined 14 days after infection, the majority of the adult trichinae were in the posterior small intestine. No age differential was noted. In 21-day infections, an increased number of unexpelled worms in the posterior end of the intestine was observed, but the distribution was relatively uniform in untreated jirds.

The small number of animals used in this study only suggests an age effect in the distribution of the worms 7 days after infection. It also suggests a retention of worms in the posterior small intestine as a result of corticosteroid treatment.

Dr. N. F. Weatherly, University of North Carolina, kindly supplied the *T. spiralis* strain used in this study. Ms. Carol Nichols provided technical assistance.

ERIC PANITZ
Schering Corporation
Animal Health Research Center
P.O. Box 608,
Allentown, New Jersey 08501

Research Note

Formation of a Carbon Dioxide–Cysteine Complex in the Incubation Fluid Used for Excysting *Eimeria* Species in vitro

Carbon dioxide is either essential or beneficial to in vitro excystation of many coccidia (Jackson, 1962, Nature 194: 847–849; Nyberg and Hammond, 1964, J. Protozool. 11: 474–480; Nyberg, Bauer, and Knapp, 1968, J. Protozool. 15: 144–148), and is frequently used with a reducing agent to alter oocyst wall permeability prior to sporozoite activation with trypsin and/or bile (Ikeda, 1960, Jap. J. Vet. Sci. 22: 27–41; Speer, Hammond, and Kelley, 1970, J. Parasit. 56: 927–929). Although a reducing agent is commonly used and recognized as a "helping" agent in laboratory excystation, the CO_2 is generally considered to be the active agent in the process (Ryley, 1973, In The Coccidia. Hammond and Long, eds., U. Park Press, Baltimore, p. 162–166). Little direct evidence exists to indicate the mode of action of the CO_2 in mediating excystation, although some investigators have suggested the involvement of an oocystic enzyme (Jackson, 1962; Hibbert and Hammond, 1968, Exp. Parasit. 23: 161–170). The present study was designed to test the possibility of the activation of an excystation enzyme by CO_2 fixation to such an enzyme. *Eimeria tenella* and *E. stiedai* were used in all studies.

Carbon-14 dioxide ($NaH_2{}^{41}CO_2$; New England Nuclear Co.) was substituted for CO_2 in a modified excystation procedure similar to that used by Bunch and Nyberg (1970, J. Protozool. 17: 364–369). After 8 to 24 hr incubation in $^{14}CO_2$-saturated 0.2M cysteine hydrochloride (Matheson, Coleman and Bell Co.), the $^{14}CO_2$ was air-flushed from the sealed incubation flasks into a series of two traps containing 4.0M NaOH. The oocysts were washed from the incubation fluid, homogenized, and filtered through 0.45-μm Millipore filters to separate oocystic fluid from solid debris. A protein fraction within the intraoocystic fluid was concentrated by further filtering the fluid through a G-100 Sephadex column-ultraviolet (ISCO) monitor and fraction collector apparatus. All fractions were checked for radioactivity with a Nuclear Chicago scintillation counter. Results indicate that C-14 was not fixed to any specific oocystic component but did form a complex with the reducing agent in the incubation fluid (Table 1). The nature of such a complex is not known, but may be a key factor in the mechanism by which the permeability of the oocyst wall is altered in the in vitro excystation process. The possibility that CO_2 and the reducing agent cooperate to make the oocyst shell permeable

Table 1. Radioactivity (disintegrations per minute) and carbon-14 concentration resulting from incubating *Eimeria stiedai* or sodium hypochlorite-treated *Eimeria tenella* oocysts in $^{14}CO_2$ and cys-HCl.

Fraction	Radioactivity (DPM × 10^5)			Carbon-14 content (micromoles)		
	E. stiedai	*E. tenella*	Control	*E. stiedai*	*E. tenella*	Control
Oocyst debris	0.0006	0.0008	*	0.0014	0.0017	*
Prefracture wash	2.4096	2.4450	2.5200	4.9713	5.0439	5.1983
Intraoocyst fluid	0.0108	0.0011	*	0.0222	0.0223	*
I-O fluid protein[1]	0.0000	0.0000	*	0.0000	0.0000	*
Trap I	1.1280	1.3910	0.7160	2.3277	2.8705	1.4755
Trap II	0.1050	0.1166	0.0642	0.2166	0.2406	0.1317
Totals	3.6540	3.9545	3.3002	7.5392	8.1590	6.8055

* No oocysts present.
[1] Isolated from intraoocyst fluid by filtration through G-100 Sephadex and concentrated with an ISCO ultraviolet monitor and fraction collector.

is supported by other studies in which we found an increase in sulfhydryl groups to occur on the oocysts during CO_2-reducing agent incubation. The results of the latter studies will be reported elsewhere. When considered with the findings herein reported, they suggest to us that CO_2 is an allosteric effector which acts directly on a component of the oocyst wall. The CO_2 action apparently exposes wall-stabilizing disulfide bonds which can then be cleaved by the reducing agent.

Although CO_2 was not fixed to any oocystic component it is possible that another, less conspicuous reaction occurs by which an enzyme precursor is activated by the gas, e.g., pH of the intraoocyst fluid may be altered by entrance of the CO_2. We attempted to detect such an enzyme by activating oocysts, removing the incubation mixture, collecting the concentrated oocyst fluid, and incubating fresh, viable oocysts in the fluid concentrate. Oocysts so treated were not activated, suggesting that an enzyme, if present, either remains unactivated under these conditions, or is not located in the soluble portion of the oocyst.

This study was partially supported by National Science Foundation Grants GB-8295 and GB27425.

WILLIAM R. JOLLEY[1] AND PETER A. NYBERG[2]
Department of Zoology
Brigham Young University
Provo, Utah 84601

Present addresses: [1] College of Veterinary Medicine, University of Illinois, Urbana, Illinois 61801, and [2] Dixie College, St. George, Utah 84770.

Report on the Brayton H. Ransom Memorial Trust Fund

Balance on hand, 1 January, 1973	$3320.53
Receipts: Interest received in 1973	185.22
	$3505.75
Disbursements: Grant to Helminthological Society of Washington	10.00
On hand, 31 December, 1973	$3495.75

LLOYD E. ROZEBOOM
Secretary-Treasurer

MINUTES

Four Hundred Seventy-Seventh Through Four Hundred Eighty-Fourth Meetings

477th Meeting: University of Maryland, Zoology Department, College Park, Maryland, October 19, 1973. President Herlich informed the Society of the results of a poll taken during the summer to determine meeting day preference and the decision to continue to meet on Friday in consideration of the poll results. Slate of officers for 1974 presented: T. K. Sawyer (President); R. S. Isenstein (Vice-President); W. R. Nickle (Corresponding Secretary-Treasurer); A. M. Golden (Recording Secretary). Papers presented: "Canine filariasis in Maryland," W. E. Buckley and J. Mallack; "Contributions of the USDA Parasitologists," A. O. Foster; "Highlights of the LSU trip, Group 61," S. Hendrix.

478th Meeting: Conference House, Animal Parasitology Institute, Beltsville, Maryland, November 16, 1973. Dr. G. Pacheco reviewed some of the outstanding accomplishments of Dr. Elvio Sadun and presented him with the Society's Anniversary Award. The slate of officers presented at the previous meeting was elected by acclamation. Papers presented: "Life cycle and pathogenicity of *Sarcocystis fusiformis* in calves," Ronald Fayer; "Trichinellosis in swine: Another look," Robert S. Isenstein.

479th Meeting: Conference Room, Bioscience Building, Beltsville, Maryland, December 14, 1973. Papers presented: "Control of a nematode disease complex on turf," Julius Feldmesser and A. M. Golden; "Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by the reniform nematode, *Rotylenchulus reniformis*," R. V. Rebois, Philip A. Madden and B. Joe Eldridge; "A new mermithid parasite of the boll weevil and comments on black fly parasites," W. R. Nickle; "Ultrastructure of the amphidial region of a root-knot nematode, *Meloidogyne incognita*," William P. Wergin and Burton Y. Endo. Newly elected officers were installed.

480th Meeting: National Institutes of Health, Bethesda, Maryland, January 18, 1974. Papers presented: "Activity and localization of transhydrogenase in axenized *Entamoeba histolytica*," D. R. Harlow. E. C. Weinbach, and L. S. Diamond; "Sensory organelles of *Caenorhabditis elegans*: Chemo- or mechanical receptors?" R. Pertel, C. F. T. Mattern, and Ann Paran; "Bladder calcification and bladder cancer in experimental *Schistosoma heamatobium* infection," A. W. Cheever, R. E. Kuntz, B. J. Meyers, and J. A. Moore; "Invasion of erythrocytes by malaria merozoites," J. A. Dvorak, L. H. Miller, W. C. Whitehouse, and T. Shiroishi.

481st Meeting: Walter Reed Army Institute of Research, Washington, D. C., February 22, 1974. Papers presented: "Interesting spontaneous parasitological lesions of animals," LTC P. K. Hildebrandi; "A system for screening potential anti-trypanosomiasis agents," LTC K. E. Kinnamon; "Surveillance and control programs for trypanosomiasis in the Republic of Zaire," CPT W. A. Reid, Jr.

482nd Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, March 22, 1974. In view of the improved gas situation, a final decision was made to have the May meeting in Kennett Square, Pennsylvania as in recent years. Papers presented: "Further studies on the life history of *Leucocytozoon simondi* in the black fly (*Cnephia ornithophilia*)," I. B. Tarshis; "Some recent highlights in fish virology," Ken Wolf; "Environmental and experimental studies on pathogenic free-living amoebae," Joe Griffin; "Age and sex differences in malaria infection rate in the canvasback duck," R. M. Kocan.

483rd Meeting: Naval Medical Research Institute, Bethesda, Maryland, April 19, 1974. Papers presented: "Proteolytic activity of secretions from the preacetabular glands of *Schistosoma mansoni* cercariae," M. A. Stirewalt, D. Campbell, and P. Frappaolo; "*Schistosoma mansoni* circulating antigen: properties and origin," M. Bawden; "Identification and characterization of allergens from *Schistosoma mansoni*," R. Hussain. Members and guests

visited the new Experimental Parasitology Division Laboratories in Building 142.

484th Meeting: Alumni House, New Bolton Center, Kennett Square, Pennsylvania, May 11, 1974. Dr. G. F. Otto reported on the recent formation of the American Heart Worm Association in response to the increase and spread of the heart worm disease, and invited interested persons on a world-wide basis to join ($10 dues.) Papers presented: "Fate of *Litomosoides carini* adults transplanted into plural or peritoneal cavities of jirds (*Meriones unguiculatus*) or multimammate rats (*Mastomys natalensis*," D. J. Weiner; "Immunoglobulin augmentation and reagin activity during granuloma formation to *Capillaria hepatica* eggs in mice," R. B. Raybourne and G. B. Solomon; "Role of the lymphoid cells of the draining lymph nodes in the immune response to *Ascaris suum* infections," P. B. Khoury; "Quantitation of *Leishmania* infected macrophages by the uses of radio-isotopes," G. M. Groocock; "Production of metastic lesions in *Leishmania enriettii* infection in the guinea pig," D. M. H. Kadivar. Following the scientific presentations a very congenial hospitality session and dinner were enjoyed by the members and guests.

The following 25 persons were elected to membership at the meetings indicated: *477th*: Edwin J. Keppner; John S. Laurie; Stephen A. Lewis; Patrick M. Muzzall; Glenn E. White. *478th*: Omar M. Amin; Carl H. Ernst; Anne D. Frame; Gary L. Hendrickson; Parviz Jatala; Thomas R. Klei. *479th*: H. M. Johnson; Jorge Ramirez. *480th*: J. P. Dubey; Donald W. Duszynski; Mercedes Robinson. *481st*: Louis M. Chong L.; Kenneth C. Corkum; William F. Font, Jr.; Lyford K. Greene; J. W. Kimbell. *482nd*: Robert E. Croft; Robert B. Grieve; Larry D. Hendricks. *483rd*: David C. Ashley.

A. MORGAN GOLDEN
Recording Secretary

In Memoriam

Datus M. Hammond
May 20, 1911—March 17, 1974
Member since 1970
Editorial Board 1974

Guillermo H. Pacheco
June 24, 1930—April 24, 1974
Member since 1966
Executive Committee 1972
Assistant Editor 1972–1974

Elvio H. Sadun
December 9, 1918—April 23, 1974
Member since 1959
Anniversary Award—1973

INDEX TO VOLUME 41

ABRAM, JAMES B. and J. RALPH LICHTENFELS. Larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) from otter, *Lutra canadensis*, in Maryland 253
Acanthocephalus dirus, new host record 81
AMIN, OMAR M. Intestinal helminths of the white sucker, *Catostomus commersoni* (Lacépède), in SE Wisconsin 81
Angiostrongylus cantonensis, acquired immunity to 121
Angiostrongylus cantonensis, influence by *Nippostrongylus brasiliensis* 237
Announcements
In Memorian 104, 262
Minutes of the four hundred seventy-seventh through four hundred eighty-fourth meetings 261
New book translation 119
Presentation—1973 Anniversary Award of the Helminthological Society of Washington 117
Report on the Brayton H. Ransom Memorial Trust Fund 260
Apophallus donicus, life cycle and infectivity to man 223
Artyfechinostomum malayanum, synonymy of allied species and genera 151
Athesmia heterolecithodes, from birds of North Borneo 94
AUGUSTINE, PATRICIA C. (see Doran) 77
Austrobilharzia sp., new host record 89
BABERO, BERT B. (see King) 241
BARRETT, RICHARD E. (see Worley) 19
Betamethasone, immunosuppressive effect on *Trichinella spiralis* 257
BIENEK, GERHARD K. and ALBERT W. GRUNDMANN. *Catenotaenia utahensis* sp. n. (Cestoda: Catenotaeniidae) from the Merriam kangaroo rat, *Dipodomys merriami vulcani*, in Utah 134
BISHOP, CLAUDE A. and WILLIAM THRELFALL. Helminth parasites of the common eider duck, *Somateria mollissima* (L.), in Newfoundland and Labrador 25
Bothrimonus sturionis, new host record 256
Brachylecithum attenuatum, description of 94
Brachylecithum stunkardi, life history of 139
Brachylecithum transversum, occurrence and morphology of 191
BULLOCK, WILBUR L. (see Scott) 256
BUSH, ALBERT O. (see Forrester) 55
Caiguiria anterouteria from Venezuela 178
CAIN, GEORGE D. and EUGENE H. STUDIER. Parasitic helminths of bats from southwestern United States and Mexico 113
Carneophallus turgidus, new host record 89
CARNEY, W. PATRICK. Studies on the life history of *Brachylecithum stunkardi* (Pande, 1939) (Trematoda: Dicrocoeliidae) 139
Caryophyllaeus from the Nearctic 184
Catostomus commersoni, helminths of 81
CHITWOOD, M. B. (see Hass) 255
CHRISTIAN, FRED A. and JAMES T. PERRET. Incidence of intestinal parasites of children from Scotlandville area of Baton Rouge in Louisiana 249
CHRISTIANSEN, JAMES L. (see Wacha) 35
CHUTE, ANNE M. (see Lund) 73
COLGLAZIER, M. L., K. C. KATES, I. L. LINDAHL, and G. SAMUELSON. Pasture trials with levamisole and thiabendazole for control of gastrointestinal helminthiasis in sheep 145

Common grackle, *Quiscalus quiscula versicolor*, helminths of 233
Concinnum ellipticum from Venezuela 178
Contracaecum larvae in fishes of East African lakes 252
COOPER, C. LAWRENCE and JOHN L. CRITES. Helminth parasites of the common grackle, *Quiscalus quiscula versicolor*, South Bass Island, Ohio 233
Corynosoma sp., new host record 89
Cosmocephalus obvelatus, new host record 89
COURTNEY, CHARLES H. and DONALD J. FORRESTER. Helminth parasites of the brown pelican in Florida and Louisiana 89
CRITES, JOHN L. (see Cooper) 233
Cyathostoma phenisci, new host records 89
Cyclocoelum brasilianum from Venezuela 178
DAILEY, MURRAY D. (see Heinz) 161
DENTON, J. FRED and WAYNE KRISSINGER. The occurrence and morphology of *Brachylecithum transversum* (Travassos, 1917) comb. n., in the eastern kingbird, *Tyrannus tyrannus* (L.) from Georgia 191
Dictyocaulus viviparus, range extension record of 250
DORAN, DAVID J. *Eimeria tenella*: Merozoite production in cultured cells and attempts to obtain development of culture-produced merozoites 169
DORAN, DAVID J., JOHN M. VETTERLING, and PATRICIA C. AUGUSTINE. *Eimeria tenella*: An in vivo and in vitro comparison of the Wisconsin, Weybridge, and Beltsville strains 77
DUNAGAN, T. T. and D. M. MILLER. Muscular anatomy of the praesoma of *Macracanthorhynchus hirudinaceus* (Acanthocephala) 199
Duthiersia expansa from Philippine reptiles 195
Echinuria borealis, new host record 25
Eimeria, incubation fluid for excysting of 259
Eimeria tenella, comparison of three strains of 77
Eimeria tenella, cultivation of 169
ESSER, R. P. Two permanent mounting methods compared after six years 10
Eustrongylides sp. from otter in Maryland 253
FAYER, RONALD and A. J. JOHNSON. *Sarcocystis fusiformis*: Development of cysts in calves infected with sporocysts from dogs 105
FISCHTHAL, JACOB H. and ROBERT E. KUNTZ. Brachylaimid and dicrocoeliid trematodes of birds from North Borneo (Malaysia) 94
FISCHTHAL, JACOB H. and PIR NASIR. Some digenetic trematodes of birds and a mammal from Venezuela 178
Florida scrub jay, helminth parasites of 127
FORRESTER, DONALD J., ALBERT O. BUSH, LOVETT E. WILLIAMS, JR., and DAVID J. WEINER. Parasites of greater sandhill cranes (*Grus canadensis tabida*) on their wintering grounds in Florida 55
FORRESTER, DONALD J. (see Courtney) 89
GOLDBERG, AARON. Management to control helminth parasitism: Infectiousness of pastures that have been rested or grazed by resistant cattle 109
GRICE, M. J. (see Moncol) 1
GRUNDMANN, ALBERT W. (see Healey) 59
GRUNDMANN, ALBERT W. (see Bienek) 134
Grus canadensis tabida, greater sandhill crane, helminths of 55
HARLEY, J. P. (see Phillips) 250
HASS, D. K. and M. B. Chitwood. First report of *Molineus* sp. recovered from a dog in the United States 255

HEALEY, MARK C. and ALBERT W. GRUNDMANN. The influence of intermediate hosts on the infection pattern of *Protospirura numidica criceticola* Quentin, Karimi, and Rodriguez De Almeida, 1968 (Nematoda: Spiruridae) in the Bonneville Basin, Utah 59
HEINZ, MICHAEL L. and MURRAY D. DAILEY. The Trypanorhyncha (Cestoda) of elasmobranch fishes from southern California and northern Mexico 161
HENDRICKS, LARRY D. A redescription of *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae) from primates of Panama 229
HERLICH, H. Infection dynamics of the cattle parasite, *Ostertagia ostertagi*, in sheep 52
Heterakis gallinarum larvae, reciprocal transfer of 73
Himasthla compacta, new host record 25
HÖGGER, CH. H. (see Nickle) 173
HUANG, TAO-CHENG (see Kuntz) 221
IBRAHIM, I. K. A. and SAMIA I. MASSOUD. Development and pathogenesis of a root-knot nematode, *Meloidogyne javanica* 68
Ictalurus catus, white catfish, leech infestation of 64
Intestinal parasites of children in Louisiana 249
Isospora arctopitheci, redescription of 229
JACOBSON, RICHARD H. (see Worley) 19
JOHN, DAVID T. Acquired immunity to experimental infection with *Angiostrongylus cantonensis* in mice 121
JOHNSON, A. J. (see Fayer) 105
JOLLEY, WILLIAM R. and PETER A. NYBERG. Formation of a carbon dioxide-cysteine complex in the incubation fluid used for excysting *Eimeria* species in vitro 259
JORDAN, WILLARD C. (see Shepperson) 254
Kangaroo rats, helminths of 241
KATES, K. C. (see Colglazier) 145
Key to single-gonopored Caryophyllaeidae 42
Key to the genus *Pharyngodon* 46
KING, SAUNDRA R. and BERT B. BABERO. Helminths of kangaroo rats (*Dipodomys* spp.) in Nevada with reports of other worm parasites from these hosts 241
KINSELLA, J. M. Helminth fauna of the Florida scrub jay: Host and ecological relationships 127
KNIGHT, ROBERT A. and DELOYCE RODGERS. Age resistance of lambs to single inoculation with *Haemonchus contortus* 116
KOCAN, A. ALAN. The influence of *Nippostrongylus brasiliensis* on the establishment of *Angiostrongylus cantonensis* in the laboratory rat 237
KRISSINGER, WAYNE A. (see Denton) 191
KUNTZ, ROBERT E., BETTY JUNE MYERS, TAO-CHENG HUANG, and JERRY A. MOORE. Parasitological aspects of *Schistosoma intercalatum* Fisher, 1934 (Cameroon) infection in the American opossum (*Didelphis marsupialis* L.) 221
KUNTZ, ROBERT E. (see Fischthal) 94
KUNTZ, ROBERT E. (see Schmidt) 195
Levamisole, for control of gastrointestinal helminthiasis in sheep 145
LICHTENFELS, J. RALPH. Larval nematode, *Contracaecum* sp., in the hydromedusa, *Polyorchis penicillatus* (Eschscholtz) 115
LICHTENFELS, J. RALPH (see Abram) 253
LINDAHL, I. L. (see Colglazier) 145
LUND, EVERETT E. and ANNE M. CHUTE. Reciprocal transfer of *Heterakis gallinarum* larvae between ring-necked pheasants and Japanese quail: Effects on *H. gallinarum*, *Histomonas meleagridis*, and *Parahistomonas wenrichi* 73
Lutztrema bhattacharyai, description of 94

MACKIEWICZ, JOHN S. *Calentinella etnieri* gen. et sp. n. (Cestoidea: Caryophyllaeidae) from *Erimyzon oblongus* (Mitchill) (Cypriniformes: Catostomidae) in North America 42
MACKIEWICZ, JOHN S. The genus *Caryophyllaeus* Gmelin (Cestoidea: Caryophyllidea) in the Nearctic 184
Macracanthorhynchus hirudinaceus, muscular anatomy of praesoma of 199
MACY, RALPH W. (see Niemi) 223
MAHAJAN, R. *Tylenchorhynchus kashmirensis* sp. n. and *Quinisulcius himalayae* sp. n. (Nematoda: Tylenchorhynchinae) from India 13
MARTIN, W. E. *Paracardicoloides yamagutii* gen. et sp. n. from an Australian eel (Trematoda: Sanguinicolidae) 22
MARTIN, W. E. *Paralecithobotrys brisbanensis* sp. n. from an Australian mullet (Trematoda: Haploporidae) 16
MASSOUD, SAMIA (see Ibrahim) 68
Meloidogyne incognita, in vitro survival and development of 254
Meloidogyne javanica, development and pathogenesis of 68
Microparyphium facetum, new host record 89
MILLER, D. M. (see Dunagan) 199
Molineus sp. from dog, first report of 255
MONCOL, DAVID J. and M. J. GRICE. Transmammary passage of *Strongyloides papillosus* in the goat and sheep 1
Moniezia expansa, chronology of acquisition by sheep 19
MOORE, JERRY A. (see Kuntz) 221
MYERS, BETTY JUNE (see Kuntz) 221
NASIR, PIR (see Fischthal) 178
Neodiplostomum (*Neodiplostomum*) *obesum* from Venezuela 178
New combination
Brachylecithum transversum (Travassos, 1917) Denton and Krissinger, 1974 191
Glaridacris terebrans Mackiewicz, 1974 184
Pseudosonsinotrema chabaudi (Caballero y C. and Caballero R., 1969) Sullivan, 1974 251
New species (new genus indicated by *)
Acanthotaenia daileyi Schmidt and Kuntz, 1974 195
Aparyngostrigea papillistomum Fischthal and Kuntz, 1974 178
Brachydistomum api Fischthal and Kuntz, 1974 94
Brachylaima (*Brachylaima*) *sabahense* Fischthal and Kuntz, 1974 94
Brachylecithum pycnonoti Fischthal and Kuntz, 1974 94
Brachylecithum sabahense Fischthal and Kuntz, 1974 94
Brachylecithum vitellobum Fischthal and Kuntz, 1974 94
**Calentinella etnieri* Mackiewicz, 1974 42
Catenotaenia utahensis Bienek and Grundmann, 1974 134
Eimeria megalostiedai Wacha and Christiansen, 1974 35
Eutetrarhynchus litocephalus Heinz and Dailey, 1974 161
Eutetrarhynchus macrotrachelus Heinz and Dailey, 1974 161
Eutetrarhynchus schmidti Heinz and Dailey, 1974 161
Kapsulotaenia frezei Schmidt and Kuntz, 1974 195
Levinseniella (*Levinseniella*) *venezuelensis* Fischthal and Kuntz, 1974 178
Lyperosomum malaysiae Fischthal and Kuntz, 1974 94
**Mecistobothrium myliobati* Heinz and Dailey, 1974 161
Nybelinia anthicosum Heinz and Dailey, 1974 161
Octomyomermis troglodytis Poinar and Sanders, 1974 37
Paracardicoloides yamagutii Martin, 1974 22
Paralecithobotrys brisbanensis Martin, 1974 16
Pharyngodon kirbii Specian and Ubelaker, 1974 46

Pharyngodon midgi Specian and Ubelaker, 1974 46
Pholeter anterouterus Fischthal and Kuntz, 1974 178
Prochristianella fragilis Heinz and Dailey, 1974 161
Prochristianella minima Heinz and Dailey, 1974 161
Rhigonema critesi Ramirez, 1974 131
Sphyriocephalus pelorosoma Heinz and Dailey, 1974 161
Tylenchorhynchus kashmirensis Mahajan, 1974 13
NICKLE, W. R. and CH. H. HÖGGER. Scanning electron microscopy of the mosquito parasite, *Reesimermis nielseni* (Nematoda: Mermithidae) 173
NIEMI, DELBERT R. and RALPH W. MACY. The life cycle and infectivity to man of *Apophallus donicus* (Skrjabin and Lindtrop, 1919) (Trematoda: Heterophyidae) in Oregon 223
Nippostrongylus brasiliensis, influence on *Angiostrongylus cantonensis* 237
NYBERG, PETER A. (see Jolley) 259
Odhneria odhneria from Venezuela 178
Oochoristica spp. from Philippine reptiles 195
Ostertagia mossi, range extension record of 250
Ostertagia ostertagi in sheep, infection dynamics of 52
PANDE, VIBHA (see Premvati) 151
PANITZ, ERIC. Anticoccidial activity of the megalomicin complex 111
PANITZ, ERIC. Immunosuppressive effect of betamethasone and the distribution of adult *Trichinella spiralis* in the intestine of the jird (*Meriones unguiculatus*) 257
PAPERNA, I. Larval *Contracaecum* (Nematoda: Anisakidae) in the pericardium of fishes from East African lakes 252
PAPERNA, I. and D. E. ZWERNER. Massive leech infestation on a white catfish (*Ictalurus catus*): A histopathological consideration 64
Paracuaria tridentata, new host record 25, 89
Paragordium varius, fine structure of 209
Parvitaenia ibisae, new host record of 89
Pelecanus occidentalis, brown pelican, helminths of 89
Pelodera strongyloides, reaction to hydroxyl ion 4
PERRET, JAMES T. (see Christian) 249
PHILLIPS, J. H., J. P. HARLEY, and W. J. RUDERSDORF. New host record for *Setaria yehi* Disset, 1966, and range extension records for *Dictyocaulus viviparus* (Bloch, 1782) and *Ostertagia mossi* Dikmans, 1931, in fallow deer (*Dama dama* L.) 250
POINAR, GEORGE O., JR. and R. D. SANDERS. Description and bionomics of *Octomyomermis troglodytis* sp. n. (Nematoda: Mermithidae) parasitizing the western treehole mosquito *Aedes sierrensis* (Ludlow) (Diptera: Culicidae) 37
PREMVATI, G. and VIBHA PANDE. On *Artyfechinostomum malayanum* (Leiper, 1911) Mendheim, 1943 (Trematoda: Echinostomatidae) with synonymy of allied species and genera 151
Proacetabulorchis dogieli, description of 94
Proacetabulorchis prashadi, from birds of North Borneo 94
Prosthogonimus cuneatus from Venezuela 178
Protospirura numidica criceticola, infection pattern of 59
Pulchrosoma pulchrosoma from Venezuela 178
RAMIREZ, JORGE. *Rhigonema critesi* sp. n. (Nematoda: Rhigonematidae) a parasite of the millipede, *Orthoporus typotopyge* (Brölemann, 1905) from Costa Rica 131
Reesimermis nielseni, scanning electron microscopy of 173
Rhopalias coronatus from Venezuela 178
RODGERS, DELOYCE (see Knight) 116
RUDERSDORF, W. J. (see Phillips) 250

SAMSON, K. S. and G. I. WILSON. Passage through Rouen ducks of the metacercaria of *Fasciola hepatica* 112
SAMUELSON, C. (see Colglazier) 145
SANDERS, R. D. (see Poinar) 37
Sarcocystis fusiformis, development of sporocysts from dogs in calves 105
Schistosoma intercalatum, infection in *Didelphis marsupialis* 221
SCHMIDT, GERALD D. and ROBERT E. KUNTZ. Tapeworms from Philippine reptiles, with two new species of Proteocephalata 195
SCOTT, E. JOHN and WILBUR L. BULLOCK. *Psammonyx nobilis* (Amphipoda: Lysianassidae), a new host for *Bothrimonus sturionis* (Cestoda: Pseudophyllidae) 256
Scyphocephalus bisulcatus from Philippine reptiles 195
Setaria yehi, new host record of 250
SHEPPERSON, JACQUELINE R. and WILLARD C. JORDAN. Observations on in vitro survival and development of *Meloidogyne* 254
Somateria mollissima, helminths of 25
Southwellina hispida, new host record 89
SPECIAN, ROBERT D. and JOHN E. UBELAKER. Two new species of *Pharyngodon* Diesing, 1861 (Nematoda: Oxyuridae) from lizards in west Texas 46
Stephanoprora denticulata, new host record of 89
STRINGFELLOW, FRANK. Hydroxyl ion, an attractant to the male of *Pelodera strongyloides* 4
Stongyloides papillosus, transmammary passage of 1
STUDIER, EUGENE H. (see Cain) 113
SULLIVAN, JAMES J. *Pseudosonsinotrema chabaudi* (Caballero y C. and Caballero R., 1969) comb. n. a senior synonym of *P. echinophallus* Sullivan, 1971, from Costa Rican frogs 251
Synhimantus invaginatus, new host record 89
Thiabendazole, for control of gastrointestinal helminthiasis in sheep 145
THRELFALL, WILLIAM (see Bishop) 25
Trypanorhyncha of elasmobranch fishes 161
UBELAKER, JOHN E. (see Specian) 46
VETTERLING, JOHN M. (see Doran) 77
WACHA, RICHARD S. and JAMES L. CHRISTIANSEN. *Eimeria megalostiedai* sp. n. (Protozoa: Sporozoa) from the wood turtle, *Clemmys insculpta*, in Iowa 35
WEBSTER, W. A. *Diplotriaena obtusa* (Rud., 1802) and *Plagiorchis maculosus* (Rud., 1802) collected from a purple martin, *Progne subis*, in Canada 109
WEINER, DAVID J. (see Forrester) 55
WILLIAMS, LOVETT E., JR. (see Forrester) 55
WILSON, G. I. (see Samson) 112
WORLEY, DAVID E., RICHARD H. JACOBSON, and RICHARD BARRETT. The chronology of tapeworm (*Moniezia expansa*) acquisition by sheep on summer ranges in Montana and Idaho 19
Xiphinema macrostylum, preservation of 10
ZAPOTOSKY, JOHN E. Fine structure of the larval stage of *Paragordius varius* (Leidy, 1851) (Gordiodea: Paragordidae). I. The preseptum 209
ZWERNER, D. E. (see Paperna) 64

MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

(Montana through Venezuela; first half in January issue)

Montana
Kinsella, J. M.
Sogandares-Bernal, F.
Worley, D. E.

Nebraska
Kerr, E. D.
Nickol, B. B.
Pritchard, Mary H.

Nevada
Babero, B. B.
Phillips, A. W.

New Hampshire
Bullock, W. L.
Harrises, A. E.
Porter, C. A.

New Jersey
Berger, H.
Cuckler, A. C.
Doscher, Mary Ehlers
Eni, E. U.
Goble, F. C.
Green, D. F.
Hayes, T. J.
Ingalls, J. W., Jr.
Jenkins, W. R.
Kantor, Sidney
Katz, F. F.
Panitz, E.
Pankavich, J. A.
Stoll, N. R.

New Mexico
Allen, R. W.
Hopper, F. A., Jr.
Riffle, J. W.
Samson, K. S.
Wilson, G. I.

New York
Acholonu, A. D.
Braun, A. J.
Fischthal, J. H.
Granek, I.
Greene, R. E.
Krupa, P. L.
Lacey, R. J.
Levin, Norman L.
Mackiewicz, J. S.
Mai, W. F.
Mueller, J. F.
Philips, P. J.
Pike, Eileen H.
Stunkard, H. W.
Tiner, J. D.

New York (APO)
Walton, Bryce C.

North Carolina
Barker, K. R.
Bergner, J. F., Jr.
Grimes, L. R.
Harkema, R.
Johnson, C. A. III
Khan, Sekender A.
Lapp, N. A.
Laurie, J. S.
McDaniel, J. S.
Miller, G. C.
Moncol, D. J.
Reid, W. A., Jr.
Shepperson, Jacqueline R.
Tilton, Beverly E.
Triantaphyllou, Hedwig H.

North Dakota
Holloway, H. L., Jr.
Kritsky, D. C.
Larson, O. R.
Leiby, P. D.
Schneider, C. M.

Ohio
Burkhart, D. M.
Carter, O. S.
Cooper, C. L.
Crites, J. L.
Etges, F. J.
Harwood, P. D.
Martin, D. R.
Muzzall, Patrick M.
Rabalais, F. C.
Riedel, Richard M.
Stromberg, P. C.
Wharton, G. W.
Zapotosky, J. E.

Oklahoma
Kocan, A. Alan
Rogers, S. H.
Self, J. T.
Smith, P. E.

Oregon
Crook, J. R.
Jensen, H. J.
Knapp, S. E.
Lucker, J. T.
Macy, R. W.
Martin, G. W.
McCauley, J. E.
Millemann, R. E.
Pratt, I.

Pennsylvania
Bergquist, E. J.
Cheng, T. C.
Davis, G. M.
Fried, B.
Hendrix, S. S.
Klei, T. R.
Langer, B. W., Jr.
Leventhal, Ruth
Martin, H. M.
Meinkoth, N. A.
Muncey, D. W.
Ogren, R. E.
Schad, G. A.
Solomon, G. B.
Soulsby, E. J. L.
Stromberg, B. E.
Theodorides, V. J.
Uricchio, W. A.

Puerto Rico
Ayala, A.
Frame, Anne D.
Roman, J.

South Carolina
Lewis, Stephen A.

South Dakota
Greichus, A.
Hugghins, E. J.
McDaniel, B., Jr.

Tennessee
Cosgrove, G. E.
Dillon, W. A.
Jones, A. W.

Texas
Canaris, A. G.
Hopkins, S. H.
Kuntz, R. E.
Meade, T. G.
Moore, D. V.
Morrison, E. O.
Myers, Betty June
Pence, D. B.
Smith, W. N.
Specian, R. D.
Speer, C.
Thames, W. H., Jr.
Ubelaker, J. E.

Utah
Grundmann, A. W.
Havertz, D. S.

Virginia
Abram, J. B.
†Ernst, Carl H.
Ernst, E. M.
Fisher, J. E.
Hargis, W. J., Jr.
Hill, C. H.
Limber, D. P.
Miller, L. I.
Segal, Dorothy B.
Seidenberg, A. J.
Sinclair, N. R.
Sonenshine, D.
Snydam, E. L.
Wiest, L. M., Jr.

Washington
Becker, C. Dale
Seesee, Floyd M.
Senger, C. M.

West Virginia
Hall, J. E.
Hoffman, G. L.

Wisconsin
Amin, Omar M.
Taft, S. J.
Thacher, J. E.
*Thorne, G.
Todd, A. C.

Wyoming
Bergstrom, R. C.
Hendrickson, Gary L.
Honess, R. F.
Kingston, N.

Argentina
Gonzalez, Stella M.
Schmunis, G. A.

Australia
Rhode, K.
Sprent, J. F. A.

Belgium
Coomans, A.

Brazil
Franco, E.
Lordello, L. G. E.

Canada
Anderson, R. C.
Anderson, R. V.
Arthur, J. R.
Bosher, J. E.
Ching, Hilda Lei
Hopper, B. E.
Mettrick, D.
Mountain, W. B.
Mudry, D. R.
Platt, T. R.
Samuel, W. M.
Threlfall, W.
Wanson, W.
Webster, W. A.

Canal Zone
Rossan, R. N.

Chile
Tagle, Isaias V.

Colombia
Thatcher, V. E.

Costa Rica
Dunn, R. A.
Ramirez, Jorge
Salas, F., L. A.

Dakar
Taylor, D. P.

Egypt
Ibrahim, I. K. A.
Oteifa, B. A.

France
Dollfus, R. Ph.
Vitiello, P.

Gt. Brit. & N. Ireland
Franklin, Mary T.
Lee, D. L.
Mathews, H. J.
Siddiqi, M. R.
Williams, I. C.
Winslow, R. D.

Greece
Himonas, G. A.

India
Antony, N. M.
Mahajan, R.
Murty, A. S.
Premvati, Dr. G.
Saxena, S. K.

Iran
Kheiri, A.

Israel
Paperna, I.
Wertheim, Guta

Italy
Lamberti, F.

Japan
Ichinohe, M.
Inatomi, S.
Mamiya, Y.
Yamaguti, S.
Yokoo, T.

Mexico
Briseño, C. H.
Caballero y C., E.
Vázquez, José T.

Netherlands
Dorsman, W.

New Zealand
Yeates, G. W.

Nigeria
Amosu, Olakunle
Ajayi, J. A.
Caveness, F. E.
Ilemobade, A. A.
Simaren, J. O.

Pakistan
Timm, R. W.

Peru
Guerrero, C. A.
Jatala, Parviz

Philippines
DeJesus, Z.
Duncan, Bryan
Velasquez, Carmen C.

Portugal
N. de A. Santos, Maria Susana

South Africa
Heyns, J.

Switzerland
Baer, J. G.
Dubois, G.
Kreis, H.

Taiwan
Chiu, Jui-Kuang

U.S.S.R.
**Ershov, V. S.

Venezuela
Dao Dao, F.
Nasir, P.

* Life Member
** Honorary Member
† New Spouse Member

CONTENTS

(*Continued from Front Cover*)

HENDRICKS, LARRY D. A redescription of *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae) from primates of Panama 229
JOHN, DAVID T. Acquired immunity to experimental infection with *Angiostrongylus cantonensis* in mice 121
KING, SAUNDRA R. AND BERT B. BABERO. Helminths of kangaroo rats (*Dipodomys* spp.) in Nevada with reports of other worm parasites from these hosts 241
KINSELLA, J. M. Helminth fauna of the Florida scrub jay: Host and ecological relationships 127
KOCAN, A. ALAN. The influence of *Nippostrongylus brasiliensis* on the establishment of *Angiostrongylus cantonensis* in the laboratory rat 237
KUNTZ, ROBERT E., BETTY JUNE MYERS, TAO-CHENG HUANG, AND JERRY A. MOORE. Parasitological aspects of *Schistosoma intercalatum* Fisher, 1934 (Cameroon) infection in the American opossum (*Didelphis marsupialis* L.) 221
MACKIEWICZ, JOHN S. The genus *Caryophyllaeus* Gmelin (Cestoidea: Caryophyllidea) in the Nearctic 184
NICKLE, W. R. AND CH. H. HÖGGER. Scanning electron microscopy of the mosquito parasite, *Reesimermis nielseni* (Nematoda: Mermithidae) 173
NIEMI, DELBERT R. AND RALPH W. MACY. The life cycle and infectivity to man of *Apophallus donicus* (Skrjabin and Lindtrop, 1919) (Trematoda: Heterophyidae) in Oregon 223
PREMVATI, G. AND VIBHA PANDE. On *Artyfechinostomum malayanum* (Leiper, 1911) Mendheim, 1943 (Trematoda: Echinostomatidae) with synonymy of allied species and genera 151
RAMÍREZ, JORGE. *Rhigonema critesi* sp. n. (Nematoda: Rhigonematidae) a parasite of the millipede, *Orthoporus typotopyge* (Brölemann, 1905) from Costa Rica 131
SCHMIDT, GERALD D. AND ROBERT E. KUNTZ. Tapeworms from Philippine reptiles, with two new species of Proteocephalata 195
ZAPOTOSKY, JOHN E. Fine structure of the larval stage of *Paragordius varius* (Leidy, 1851) (Gordiodea: Paragordidae). I. The preseptum 209

RESEARCH NOTES

ABRAM, JAMES B. AND J. RALPH LICHTENFELS. Larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) from otter, *Lutra canadensis*, in Maryland 253
CHRISTIAN, FRED A. AND JAMES T. PERRET. Incidence of intestinal parasites in children from Scotlandville area of Baton Rouge in Louisiana 249
HASS, D. K. AND M. B. CHITWOOD. First report of *Molineus* sp. recovered from a dog in the United States 255
JOLLEY, WILLIAM R. AND PETER A. NYBERG. Formation of a carbon dioxide–cysteine complex in the incubation fluid used for excysting *Eimeria* species in vitro 259
PANITZ, ERIC. Immunosuppressive effect of betamethasone and the distribution of adult *Trichinella spiralis* in the intestine of the jird (*Meriones unguiculatus*) 257
PAPERNA, I. Larval *Contracaecum* (Nematoda: Anisakidae) in the pericardium of fishes from East African lakes 252
PHILLIPS, J. H., J. P. HARLEY, AND W. J. RUDERSDORF. New host record for *Setaria yehi* Disset, 1966 and range extension records for *Dictyocaulus viviparus* (Bloch, 1782) and *Ostertagia mossi* Dikmans, 1931, in fallow deer (*Dama dama* L.) 250
SCOTT, K. JOHN AND WILBUR L. BULLOCK. *Psammonyx nobilis* (Amphipoda: Lysianassidae), a new host for *Bothrimonus sturionis* (Cestoda: Pseudophyllidae) 256
SHEPPERSON, JACQUELINE R. AND WILLARD C. JORDAN. Observations on in vitro survival and development of *Meloidogyne* 254
SULLIVAN, JAMES J. *Pseudosonsinotrema chabaudi* (Caballero y C. and Caballero R., 1969) comb. n., a senior synonym of *P. echinophallus* Sullivan, 1971, from Costa Rican frogs 251

ANNOUNCEMENTS

In Memoriam 262
Index to Volume 41 263
Minutes—Four Hundred Seventy-Seventh Through Four Hundred Eighty-Fourth Meetings 261
Report on the Brayton H. Ransom Memorial Trust Fund 260

* * *

Date of publication, 17 October 1974

ALLEN PRESS, INC. PRINTED IN U.S.A. LAWRENCE, KANSAS