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CONTENTS



(*Continued on Outside Back Cover*)

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# Life History of *Rhabditis* (*Pelodera*) *orbitalis*—A Larval Parasite in the Eye Orbits of Arvicolid and Murid Rodents

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ABSTRACT: Whereas microbotrophic adults and developmental stages of *Rhabditis* (*Pelodera*) *orbitalis* live only in the nesting material of the host, its parasitic third-stage larvae have hitherto been found frequently in the conjunctival sacs of 15 species of mice and voles during the past ~30 years.

In addition to the parasitic ("infective") larvae, the third stage may exist as dauer larvae as well as normal larvae, differing in morphological as well as ecological details. Only the infective larvae can successfully locate a host by the vibrations of the nesting material and by means of a thermotactic response.

Food is taken up from the lachrymal fluid by endosmosis and is stored as fat in the intestinal cells. After 3–19 days in the conjuctival sac, larvae will leave the host but remain in the nesting material, where they molt twice to the adult stage. The phenomenon designated in this paper as "obligate parasitism" pertains only to the infective larvae.

KEY WORDS: nematodes, *Rhabditis* (*Pelodera*) *orbitalis, Rhabditis* (*Pelodera*) *strongyloides,* larval parasitism, parasites of small rodents, parasites of eye orbits, nest fauna, sibling species, life cycle, host finding, thermotactic response.

The lachrymal fluid of conjunctival sacs of voles and mice from Europe and North America (Table 1) often harbors up to 100 or more nematode larvae per eye. According to Osche (1956), Poinar (1965), and Cliff et al. (1978) these third-stage larvae are assumed to belong to the species *Rhabditis* (*Pelodera*) *strongyloides* (Schneider, 1860), which is known among nematologists for its easy cultivation and its use as a "potential research tool" in experimental studies (Scott and Whittaker, 1970; Stringfellow, 1974, 1976).

In a more recent revision, however, this well-known nematode was recognized as belonging to a sibling species complex (see Sudhaus and Schulte, 1986; Sudhaus et al., 1987), confusing the biological and ecological patterns of several different species.

1) *Rhabditis* (*P.*) *strongyloides* (Schneider, 1860) lives as a microbophage in fecal matter of stables and chicken houses. Although without phoretic or parasitic association, third-stage larvae of a separate strain (*Rhabditis* (*P.*) *strongyloides dermatitica* Sudhaus and Schulte, 1988) may cause intense dermatitis in warm-blooded animals.

2) *Rhabditis* (*P.*) *cutanea* Sudhaus, Schulte, and Hominick, 1987, third-stage larvae can be found coiled in hair follicles of the skin of wood mice (*Apodemus sylvaticus* and *A. flavicollis*).

3) *Rhabditis* (*P.*) *nidicolis* Sudhaus and Schulte, 1986, has been found only once in nesting material of a field vole (*Microtus agrestis*). Its life cycle remains to be elucidated.

4) *Rhabditis* (*P.*) *orbitalis* Sudhaus and Schulte, 1986, third-stage larvae are regularly found in the conjunctival sacs of lemmings (Cliff et al., 1978), voles (Poinar, 1965; Canning et al., 1973; Prokopič et al., 1974), and, to a lesser extent, of mice and rats (Cross and Santana, 1974). The life history of *Rhabditis orbitalis* is described in this paper.

## Results

### Cultivation and cross-mating experiments

All the species of the complex can successfully be cultivated on pure agar plates (2%) with little pieces of uncooked meat (as a substratum for bacterial growth). They are morphologically nearly identical, although they are reproductively isolated.

For interspecific cross-mating experiments, males and females of the above-mentioned species were put on the surface of an agar plate. Mating took place in all combinations, but the development of eggs or larvae soon ceased, indicating that the species are metagametically isolated (see Sudhaus and Schulte, 1986; Sudhaus et al., 1987).

Conspecificity with *R. orbitalis,* on the other hand, was confirmed by the same method for different strains recovered from the orbits or

**Table 1. Small rodent species known as hosts of *Rhabditis orbitalis* (combined data from literature and my own findings).**

| |
|---|
| Muridae |
| *Apodemus sylvaticus* (Linné) |
| *Apodemus flavicollis* (Melchior) |
| *Apodemus agrarius* (Linné) |
| *Mus musculus* (Linné) |
| *Rattus norvegicus* (Berkenhout) |
| Arvicolidae |
| *Arvicola terrestris* (Linné) |
| *Clethrionomys glareolus* Schreber |
| *Clethrionomys gapperi* (Vigors) |
| *Pitymys subterraneus* (de Sélys-Longchamps) |
| *Microtus agrestis* (Linné) |
| *Microtus arvalis* (Pallas) |
| *Microtus californicus* (Peale) |
| *Microtus longicaudus* (Merriam) |
| *Lemmus trimucronatus* (Richardson) (=*Lemmus sibiricus* (Kerr)) |
| *Dicrostonyx groenlandicus* (Traill) |

nesting material of various rodent species (*Microtus agrestis, M. arvalis, Clethrionomys glareolus,* and *Apodemus agrarius*).

## Life history of *Rhabditis orbitalis*

HABITAT AND SAMPLING: All developmental stages could be extracted easily from nesting material of *Microtus agrestis* and *M. arvalis,* respectively, by a Baermann funnel: in Berlin (West), 23 out of a total of 33 examined nests (i.e., 70%) revealed larvae and adults of this nematode living there as bacterial feeders. Another way of obtaining *R. orbitalis* for laboratory studies was to live trap infected voles and put them in cages supplied with sterilized, moist hay or soil, from which all developmental stages could be extracted a few days later (as mentioned by Poinar [1965, 1983] for "*P. strongyloides*").

Parasitic larvae were recovered even faster by killing the rodents, flooding the conjunctival sac with 0.22 M saline, and drawing the nematodes up into a pipette. However, only very few of these larvae survived and molted to the adult stage on agar plates.

LIFE CYCLE AND "LARVAL TRIPHENISM": Once established, *R. orbitalis* can be grown indefinitely in a laboratory culture, provided that an adequate amount of bacteria is offered as food. The life cycle of one generation takes between 5 and 7 days (at 20°C) with an average of 5.4 days ($N = 26$). Third-stage larvae of *R. orbitalis* appear in three morphologically different shapes, which arise simultaneously from a common pre-stage.

After 5–10 days in laboratory culture, infective larvae will occur, which are morphologically different from the "normal" third-stage larvae as well as from the dauer larvae, although belonging to the same developmental stage (Fig. 1).

This phenomenon should be referred to as a "larval triphenism," obviously induced by environmental factors (quality of bacteria available for food). While dauer larvae may tolerate lack of food and desiccation (up to 28 days), only the infective larvae are able to find the rodents' conjunctival sacs.

Once formed, the infective larvae will not continue their development, not even on fresh agar plates seeded with bacteria (as dauer larvae do). These larval nematodes will only continue their life cycle by finding the eye orbits of an adequate host.

HOST FINDING: Small mammals such as arvicolid rodents possess a short-term rhythm of activity and rest or sleep (Lehmann, 1976). At least every few hours, each rodent will return to its own nest. Before sleeping the rodent causes disturbance of the nesting material by grooming and scratching. Resulting vibrations can be perceived by the infective larvae of *R. orbitalis*: twirling the petri dish on a table for about 20 sec will cause these larvae to move intensively 3–7 min later. They climb up to the petri dish lid or similar dry areas. Infective larvae in a dish that is not moved will never show such a behavior, but remain quiescent up to 6 wk before dying without further development.

Once activated and exposed to the air, the larvae can locate their host by the heat of its body within a striking distance of about 40 mm, which was shown under experimental conditions (Fig. 2a, b). After the plexiglass triangle had vibrated for about 30 sec, hundreds of infective larvae left the agar surface, wandering directly to the heated metal point and ignoring the unheated reference.

While perception of heat stimuli from the host (Fülleborn, 1924, 1932) is well known for moist soil inhabiting infective larvae of *Ancylostoma* and *Strongyloides,* infective larvae of *R. orbitalis* can perceive radiation heat (infrared beams), a capability hitherto best known for certain snakes (Boinae and Crotalinae). Locomotion on the dry plexiglass takes place in a very strange manner: bending of the stoma against the substrate, thus pulling the rest of the body forward (sometimes even a somersault was performed by a moving larva). That mode of locomotion should be considered an adaptation to the dryness of the nesting material of small rodents, which makes the

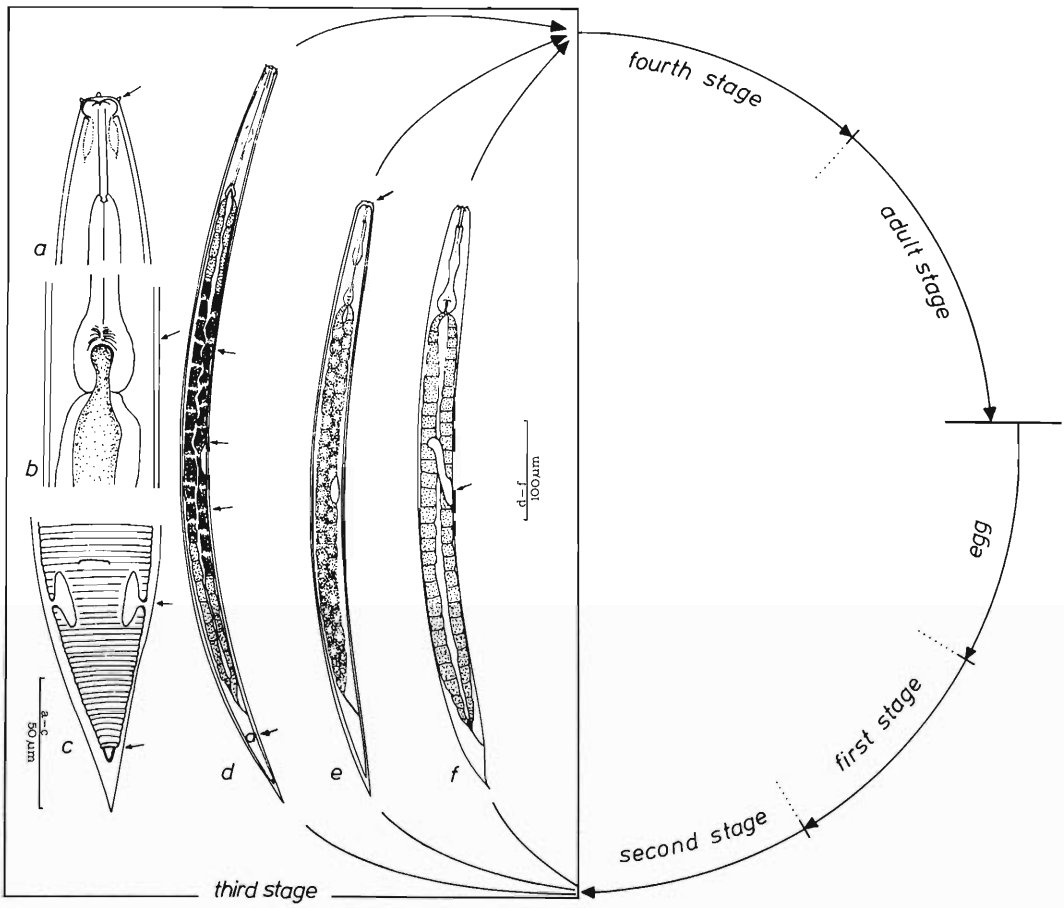


**Figure 1. Triphenism of third developmental stage in the life cycle of *Rhabditis orbitalis*. a–d. Infective larvae. a. Anterior region (ventral). b. Pharyngeal area (ventral). c. Caudal region with enlarged phasmids and a mucro on tip of the tail (ventral). d. Infective larva in toto (lateral). e. Dauer larva in toto (lateral). f. Normal third-stage larva in toto (lateral).**

normal "gliding"-type movement of nematodes on moist substrate impossible. Larvae will reach the warm metal point with a maximum speed of about 1.2 mm/min. Then the nematodes may stand on their tails and wave back and forth or even jump from the metal, ready for a new attack.

INFECTION OF LABORATORY MICE: Quest for a host by the infective larvae was tested under more natural conditions on laboratory mice (*Mus musculus*). The rodents were kept individually in a wire cage (70 × 70 × 125 mm) covered with artificial nesting material containing all developmental stages of *R. orbitalis*. After 12–16 hr, examination of the conjunctival sacs revealed between 11 and 54 larvae per eye (number of mice = 7). This seems to be roughly the "normal" number of larvae counted from a single rodent under field conditions according to Canning et al. (1973), Cross and Santana (1974), Prokopič et al. (1974), and my own findings (min. 1/max. 78 for 12 specimens of *Clethrionomys glareolus* Schreber at Berlin).

Once in contact with the fur of their host, regardless of the region, larvae will climb among the hairs searching for the rodents' eyes in a "trial and error" method; similar to their behavior described for the heated metal point (under given conditions) larvae will start jumping from the fur of the host, making a new attempt to find its eyes.

Poinar (1965, 1983) has proposed a hypothesis that the larvae might be picked up by the rodents' feet and transmitted to their eye orbits while grooming. In order to prove this hypothesis, mice were kept in a special narrow cage, completely

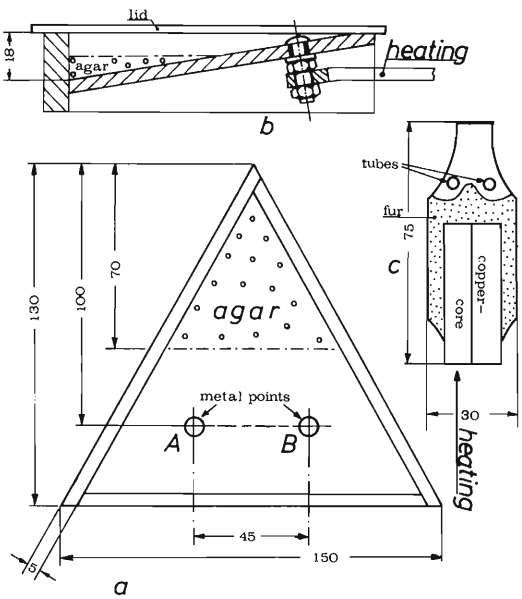


**Figure 2. a, b. Plane view of the arrangement for testing thermotactic responses of the infective larvae of *Rhabditis orbitalis*. A population was established on the agar surface. After the plexiglass triangle had vibrated for about 30 sec, hundreds of infective larvae left the agar and moved directly to the heated (up to 35°C) metal point (A or B, respectively) but ignored the unheated reference. c. "Dummy" consisting of soft plastic draped with a muskrat (*Ondatra zibethicus*(Linné)) fur. The copper core could be heated up to 35°C. The little tubes in the eye position were filled with 0.22 M NaCl solution or tap water, respectively, prior to exposition.**

preventing movements associated with grooming. As a result, the conjunctival sacs of mice contained between 120 and 184 larvae per eye (3 mice tested) after 12–16 hr. In fact, within the same time, lachrymal fluid of "fixed" mice harbored far more larvae than non-fixed mice. Thus Poinar's hypothesis cannot be confirmed.

Cliff and Anderson (1980) tried to inoculate lemmings (*Lemmus trimucronatus* = *L. sibiricus*) with *R. orbitalis* ("*P. strongyloides*") under laboratory conditions, using dauer larvae instead of infective larvae. They obviously did not know about the larval triphenism as a special character of this species. In their experiments, dauer larvae left the conjunctival sacs of the lemmings within 48 hr, thus leading Cliff and Anderson (1980) to the wrong conclusion that the association might be phoretic only.

"DUMMY" EXPERIMENT: The essential stimuli causing the larvae to search for the host's eyes (vibrations of the nesting material, radiation heat from the host's body, and finally the wetness of its eyes) were affirmed by the aid of a "dummy" (Fig. 2c). The dummy was heated up to 35°C and covered with the artificial nesting material containing *R. orbitalis* for 12 hr. Infective larvae invaded the little tubes filled with tap or saline water, respectively, in nearly the same numbers as they invaded conjunctival sacs of laboratory mice. This result was obtained only when the different stimuli (as mentioned above) were given simultaneously.

LARVAL PARASITISM IN *R. ORBITALIS*: Shortly after the infective larvae gain entrance to the orbit, they free themselves from the previous-stage cuticle and begin to move through the lachrymal fluid.

It had been unclear for a long time whether the heavy incidence of larvae of *R. orbitalis* in the conjunctival sacs of small rodents (confused with "*R. strongyloides*") is part of a phoretic or parasitic association. A considerable amount of information has been published on this problem.

**Table 2. Body dimensions of *Rhabditis orbitalis* parasitic larvae obtained from the lachrymal fluid of laboratory mice at different times (in μm). Prior to examination, larvae were heat-relaxed and stored in saline (to avoid bursting). Figures in parentheses are means.**

| | Age of larvae | | |
|---|---|---|---|
| | 1 day | 3 days | 5 days |
| Number of larvae measured | 10 | 10 | 10 |
| Body length | 646–720 (648) | 711–936 (874) | 819–936 (889) |
| Body width | 30–34 (31) | 36–58 (56) | 45–65 (58) |
| Buccal cavity (length) | 23–23 (25) | 23–29 (25) | 24–29 (25) |
| Pharynx length | 162–172 (167) | 162–188 (179) | 162–176 (172) |
| Tail length | 67–78 (77) | 72–97 (82) | 90–99 (95) |
| Gonad primordium (length) | 68–123 (96) | 86–144 (103) | 84–144 (100) |

Osche (1956, 1963), Poinar (1965, 1983), and Prokopič et al. (1974) evaluated the phenomenon involved as facultative parasitism (or phoresis), i.e., preadaptation of the larvae of microbotrophic nematodes to parasitism.

My findings indicated that the only reason why the infective larvae of *R. orbitalis* invade the conjunctival sacs is to obtain their nourishment from the lachrymal fluid. The larvae increase in size while on the host (Table 2) and the intestinal cells are densely packed with lipid droplets, giving the nematodes opaque bodies (Fig. 3). Furthermore, none of the larvae resume development without having stayed in the conjunctival sacs for at least 72 hr.

Since the stoma remains closed at its beginning and the pharynx musculature is reduced or degenerated (Fig. 3b), proteins from the lachrymal fluid are apparently taken up through the modified cuticle ("endosmosis"). That mode of nutrition is common in some parasitic nematodes from the body cavity of invertebrates.

If stored, namely in an auto-sterile saline solution (containing Na-EDTA) for several hours, injury inside the epidermis became visible all over the body surface. Immersion of older parasitic larvae recovered from the lachrymal fluid in tap or distilled water led to rapid bursting.

A special feature connected with the inevitable uptake of inorganic salts from the lachrymal fluid (mainly NaCl) is the enlargement of the phasmids (Fig. 3d). This suggests that they may serve as excretion organs. The hypertrophy is not caused by the salt, since it will occur even when the infective larvae are obtained from the culture and stored at 30°C in a tap water film on the surface of an agar plate.

Former investigators in this association were often amazed by the fact that the rodents do not show pathological effects caused by the nematodes, although the lachrymal fluid of one eye can harbor up to some 100 larvae (Stammer, 1956; Poinar, 1965). Most probably, the larvae become sterile with their exsheathment, thus becoming more tolerable to their host. Furthermore, the nematodes never cause damage of surrounding tissues, but only use the lachrymal fluid in the "mild" endosmotic manner.

Several experiments were run to determine the time period of larval parasitism: laboratory mice were infected with *R. orbitalis* for 12 hr as described above. Infected mice were kept singly in a plexiglass box (95 × 95 × 60 mm) containing

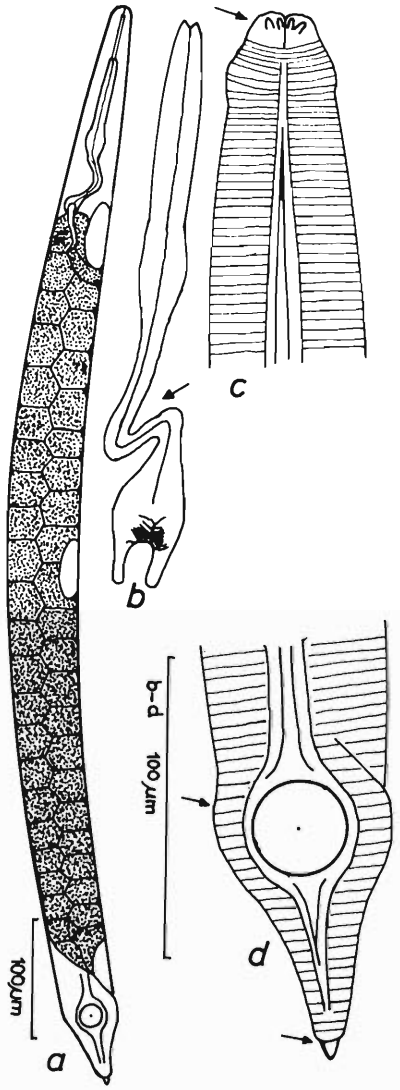


**Figure 3. Morphological characters of *Rhabditis orbitalis* parasitic larvae obtained from the lachrymal fluid of a vole (*Microtus agrestis*). a. Larva in toto (lateral). b. Pharynx with bend (indicated by arrow). c. Anterior region of the body (lateral). d. Caudal region with enlarged phasmids.**

sterilized moist hay (artificial nesting material), which was put in a glass aquarium with a layer of sawdust. Emigrated larvae were extracted from the hay daily by taking samples which were then examined by the Baermann method. Parasitic larvae remained in the conjunctival sac between a minimum of 3 and a maximum of 19 days (average $8/N = 9$) before leaving the host and resuming development (Fig. 4).

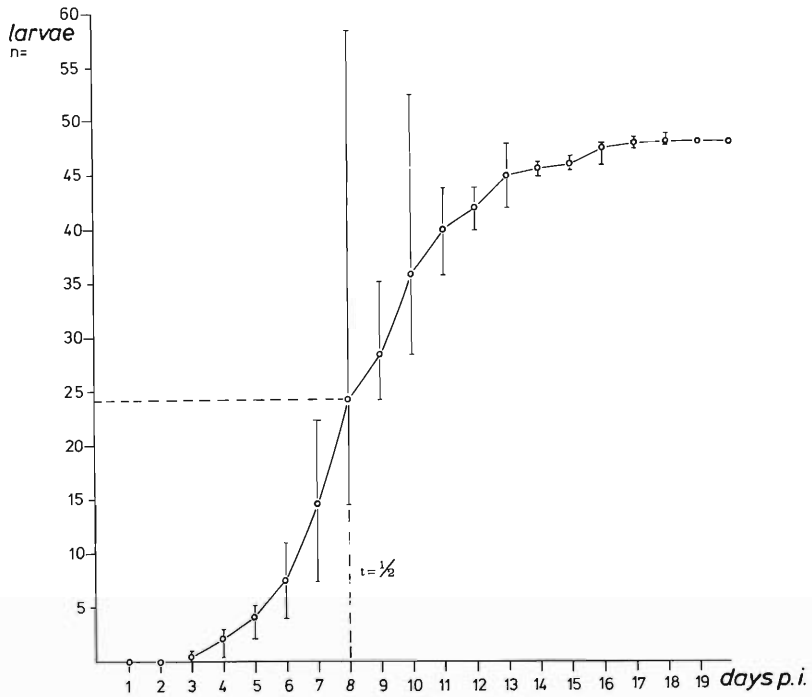


**Figure 4. Duration of stay in the conjunctival sacs of laboratory mice (*Mus musculus*) by parasitic larvae of *Rhabditis orbitalis*. Method as described in the text. Data for infection and following examination of 9 mice were summarized for every single day p.i.**

LEAVING THE HOST: Leaving the conjunctival sac as well as resuming the microbotrophic mode of life must take place obligatorily within the rodents' nest. Since *R. orbitalis* is a bisexual species, only the nesting material provides the chance to find a mate and thus continue its life cycle. While still in the lachrymal fluid, the larva has to perceive internal stimuli from the host connected with its periodic stay in the nest. In fact, emigrated larvae from laboratory mice could only be extracted from nesting material itself, and never from the surrounding substratum.

Possibly oscillation of endothermy will give this internal stimulus, but the exact mechanism remains unknown.

In contrast to the results presented by Poinar (1965), 2 molts were always observed in post-parasitic larvae before they became adults.

## Discussion

*Rhabditis orbitalis* is only found in the scanty nesting material, where its infective larvae can invade the conjunctival sacs of mice and voles. The infective larvae exhibit a number of unique, specialized features connected with their obligate parasitism in the lachrymal fluid. However, *R. orbitalis* still has the ability to undergo a complete free-living (i.e., microbotrophic) cycle similar to that of the closely related *R. strongyloides*. Periods of adversity, such as lack of food or drought, may be tolerated with the aid of the dauer larva stage. That is the reason why *R. orbitalis* can be grown indefinitely in laboratory culture.

Infective larvae of *R. orbitalis* were able to persist in the nesting material up to 6 wk, thus increasing their chance to meet a host and obtain their nourishment from the lachrymal fluid.

Since rodents' nests are like small islands in the surrounding soil, decomposing organic material of a nest will only persist for a comparatively short time. If the parasitic larvae are not distributed by their host and transmitted to a new habitat, populations of *R. orbitalis* would soon perish without reproduction.

This complicated association only would have evolved in connection with clear advantages even for the post-parasitic generation. Possibly this could mean better fitness for the offspring (depending on the food stored in the intestinal cells

of the parasitic larva) or the predictability for completing the life cycle.

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# Reconsideration of the Acanthocephalan Genus *Echinopardalis*, with a Description of Adult *E. atrata* and a Key to Genera of the Oligacanthorhynchidae

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ABSTRACT: Five of 5,360 *Canis familiaris* from Cairo, Egypt, harbored 47 specimens of *Echinopardalis atrata* Meyer, 1931. The species was originally described from juvenile specimens, and until now it has not been reported again. Study of adults revealed that males reach lengths up to 64 mm and gravid females range from 68 to 94 mm long. The elongate trunk tapers to the posterior extremity where for gravid females it terminates in an appendix-like process dorsal to the genital pore. Capsular protonephridial organs are present in both sexes, and the male reproductive system extends into the anterior third of the trunk. Eggs, removed from females, average 92 × 46 μm.

Restitution of separate generic status for *Echinopardalis* Travassos, 1918, is proposed. *Echinopardalis* is among the genera of Oligacanthorhynchidae characterized by 36 proboscis hooks. The anterior extent of the male reproductive system and general shape of the trunk distinguish it from *Oligacanthorhynchus* Travassos, 1915; and capsular protonephridial organs separate it from *Macracanthorhynchus* Travassos, 1917, and *Oncicola* Travassos, 1916.

KEY WORDS: *Echinopardalis, Echinopardalis atrata,* adult morphology, acanthocephalans of Egyptian carnivores, *Canis familiaris,* key to genera of Oligacanthorhynchidae, *Oligacanthorhynchus mariemily* (Tadros, 1969) comb. n.

As a result of a project to rid streets in Cairo, Egypt, of stray dogs, 5,360 *Canis familiaris* were collected between 30 August 1986 and 30 March 1987. Five of these dogs harbored a total of 47 acanthocephalans similar to *Echinopardalis atrata* Meyer, 1931. One dog each from the districts of Torah and Maasara harbored a single acanthocephalan. One dog from El-Basateen harbored a single worm, and each of two other dogs from that district had 22.

*Echinopardalis atrata* was described by Meyer (1931) from specimens in the Berlin Museum that had been collected from Egyptian carnivores of the families Canidae, Felidae, and Viverridae. It has not been reported since. Comparison with syntype specimens confirmed that the acanthocephalans from Cairo dogs are conspecific with *E. atrata.* Because specimens examined by Meyer were all juveniles and many features, including dimensions of the proboscis hooks, were not given, the species is herein redescribed to include adults.

Probably because of their large size and the difficulty in studying them microscopically, members of the family Oligacanthorhynchidae have a complex systematic history. The revision by Schmidt (1972) brought clarity and stability to systematics of the group, but there is little information for the less common members of the family. Generic assignment of the species from dogs in Cairo was difficult from the descriptions and key provided in Schmidt's revision. In that work, the genus *Echinopardalis* Travassos, 1918, was synonymized with *Oligacanthorhynchus* Travassos, 1915. New information regarding protonephridial systems and reconsideration of morphological features lead us to propose the resurrection of *Echinopardalis.*

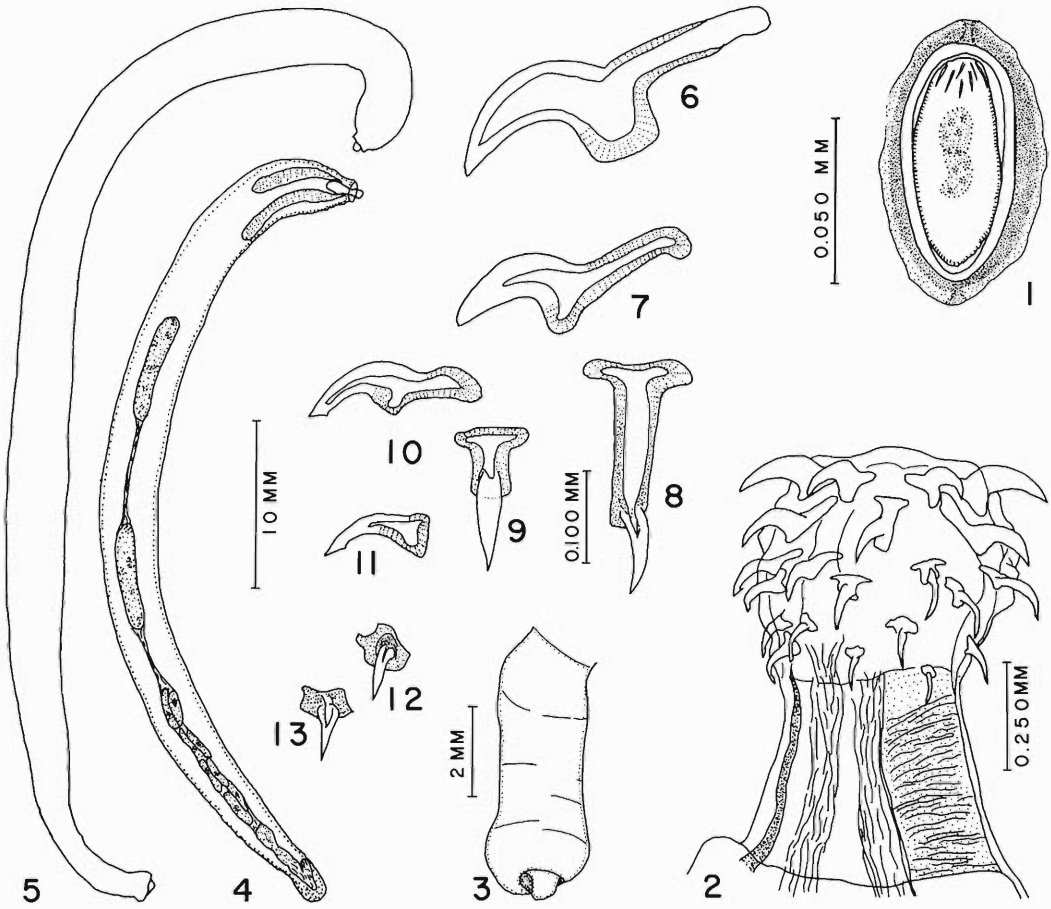
## Materials and Methods

Acanthocephalans were removed alive from the intestines of dogs and allowed to die in tap water before being fixed in AFA or 2% glutaraldehyde. After dehydration in ethanol and embedding in paraplast, protonephridia were studied from 8-μm serial sections stained by standard hematoxylin and eosin methods. Other morphological features were studied from dissected specimens or from entire worms cleared in xylene. Proboscides and eggs were removed from some specimens, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam for microscopic study. All hooks were measured in flat lateral profile. Unless otherwise noted, measurements are in micrometers with ranges followed by means in parentheses.

## Results

### Redescription

The following redescription is based on 6 juvenile syntypes from the study by Meyer (1931) (Museum für Naturkunde der Humboldt-Uni-




**Figures 1–13. Camera lucida drawings of *Echinopardalis atrata*. 1. Egg removed from pseudocoel. 2. Proboscis. 3. Posterior extremity of female. 4. Male. 5. Outline of female showing shape of trunk. 6. Hook from first (apical) circle, lateral view. 7. Hook from second circle, lateral view. 8. Hook from second circle, surface view. 9. Hook from third circle, surface view. 10. Hook from third circle, lateral view showing chisel-shaped tip. 11. Hook from sixth (proximal) circle, lateral view showing chisel-shaped tip. 12. Hook from fifth circle, surface view. 13. Hook from sixth circle, surface view. Scale beside Figure 4 applies equally to Figures 4 and 5; that between Figures 8 and 9 applies equally to Figures 6–13.**

versität zu Berlin numbers 2445, 2644, and 6031) and 7 mature male and 11 gravid female specimens of this collection.

### *Echinopardalis atrata* Meyer, 1931 (Figs. 1–13)

GENERAL: Trunk elongate and thick; black in juveniles, pale yellow to white in adults. Trunk widest anteriorly, tapering to posterior extremity. Proboscis globular; armed with 6 circles of 6 hooks each, tips of those in proximal 4 circles with chisel-shaped barbs. Length of hooks in each circle, distally to proximally: 197–221 (205), 154–178 (164), 134–154 (146), 106–130 (121), 101–115 (109), and 101–106 (105). Hooks in distal 4 circles with strong anteriorly directed roots that are expanded laterally in a T-shape. Hooks in proximal 2 circles with small, nearly trapezoidal, roots. Neck averages 460 long by 510 wide at junction with trunk; 403 long by 490 wide in juveniles. Proboscis receptacle typical of Archiacanthocephala; attached in neck at base of proboscis and 1,344–1,690 (1,524) long by 653–816 (723) wide; averaging 1,325 by 413 in juveniles. Lemnisci, approximately equal in length, 6.9–10.9 mm (8.6) long; 4.9–5.5 mm long in juveniles. Protonephridial organs of capsular type measure approximately 350 long by 200 wide.

MALE (mature specimens): Trunk, 55–64 mm (60) long, tapering gradually from greatest width,

3.0–4.0 mm (3.7), anteriorly to 1.5–2.0 mm (1.8) posteriorly. Proboscis 480–499 (490) long by 590–595 (593) wide. Reproductive tract extending into anterior third of trunk. Testes elongate and not contiguous. Anterior testis 6.1–6.4 mm (6.2) long by 0.7–1.1 mm (0.9) wide; posterior testis 6.3–7.5 mm (7.0) long by 0.8–1.0 mm (0.9) wide. Eight uninucleated rectangular cement glands in a tight row of 4 pairs begin 2.9–4.5 mm (3.9) behind posterior margin of second testis. Glands, usually smaller posteriorly, 1.7–2.6 mm (2.0) long by 326–461 (382) wide. Protonephridial organs on outer surface of dorsal ligament sac at junction with genital sheath.

FEMALE (gravid specimens): Trunk, 68–94 mm (83) long, tapering rapidly from greatest width, 3.5–6.5 mm (4.9), anteriorly to 1.5–2.8 mm (2.0) in posterior fifth; terminating in cylindrical to conical, withdrawable process. Proboscis 538–595 (572) long by 586–670 (653) wide. Anterior edge of uterine bell approximately 2.5 mm from genital pore. Uterus and vagina approximately 1,000 and 400 long, respectively. Protonephridial organs on outer, lateral surface of dorsal ligament sac at level of anterior rim of uterine bell. Fully formed eggs removed from pseudocoel 87–99 (92) long by 41–50 (46) wide.

HOSTS: *Canis familiaris* (domestic dog), *Felis catus* (domestic cat), *Herpestes ichneumon* (Egyptian mongoose), and *Vulpes vulpes aegyptiaca* (=*Canis vulpecula*) (Egyptian red fox), all in Egypt.

SPECIMEN DEPOSITION: Museum für Naturkunde der Humboldt-Universität zu Berlin: syntypes from Meyer (1931) study including numbers 2445, 2644, and 6031; vouchers from this study numbers 7179, 7180, 7181, and 7182.

### Remarks

Proboscis armature on the specimens from dogs collected in Cairo is identical in all respects to that of the specimens described by Meyer (1931) from other Egyptian carnivores. Differences are related to the fact that Meyer studied only juveniles. The proboscis size he reported is slightly smaller than that of adult females, and he noted the absence of a terminal process on females. Such a process is present on gravid worms (Fig. 3) although it may be partially withdrawn. It is unknown whether the coloration observed by Meyer resulted from fixation and storage; but, as he noted, preserved juveniles were black. Specimens freshly preserved for this study were yellow to white.

Individuals of *Echinopardalis atrata* attain substantially larger sizes than those of other species historically assigned to *Echinopardalis*. Eggs of *E. atrata* are also much larger than those of other species of the genus except *E. lerouxi* Bisseru, 1956. *Echinopardalis atrata* has larger hooks than does *E. lerouxi*. The lengths of proboscis hooks on *E. atrata* differ also from those of other species of the genus except that those in the apical circle are similar to the sizes reported in the original descriptions of *E. macrurae* Meyer, 1931, and *E. bangalorensis* Pujatti, 1951, and by Travassos (1918) for *E. pardalis* (Westrumb, 1821) Travassos, 1918. However, hooks in the second circle of the latter 2 species are longer than those of the apical circle. This is not the case for *E. atrata*. Hooks in the basal 2 circles of *E. macrurae* are much shorter than corresponding hooks of *E. atrata*.

## Discussion

### Status of *Echinopardalis* Travassos, 1918

*Echinopardalis* was proposed by Travassos (1918) to replace *Pardalis* Travassos, 1917, preoccupied by *Pardalis* Gray, 1867, for a mammal. Originally the type species, *E. pardalis* (Westrumb, 1821) Travassos, 1918, was the only known member of the group. As additional species were described, the concept of the genus was modified through several emendations until it stood as defined by Petrochenko (1958). Yamaguti (1963) offered a definition similar to that of Petrochenko, but he less accurately stated the position of the male reproductive system. Because it was felt that the only morphological character separating *Echinopardalis* from other genera of Oligacanthorhynchidae was the presence of a terminal process on the trunk of gravid females, *Echinopardalis* has been synonymized with *Oncicola* Travassos, 1916, by Golvan (1962) and with *Oligacanthorhynchus* Travassos, 1915, by Schmidt (1972). Schmidt discussed the weakness in considering a genital papilla on females as a generic-level character, and his view that species formerly assigned to *Echinopardalis* should be included in *Oligacanthorhynchus* is the prevalently accepted position.

Members of *Oligacanthorhynchus* are described as possessing (among other traits) long slender trunks that are narrower anteriorly than posteriorly and have the male reproductive system confined to the posterior half. Species formerly assigned to *Echinopardalis* do not fit this prototype. Despite the fact that some are de-

scribed with "testes at about mid-body," measurement of illustrations and examination of specimens reveal that the anterior testis is always preequatorial, decidedly so (Fig. 4) in some species. All other species of *Oligacanthorhynchus,* including those formerly assigned to *Hamanniella* Travassos, 1915, have male reproductive systems restricted to the posterior quarter of the trunk. Further, the trunk of species of *Echinopardalis* is not noticeably narrower anteriorly than posteriorly. There is a conspicuous difference in this regard when trunks of species formerly assigned to *Oligacanthorhynchus, Hamanniella,* and *Echinopardalis* are compared.

Species previously included in *Echinopardalis* more closely resemble those of *Oncicola* in shape of the trunk and location of the male reproductive system. However, the trunks of those in *Echinopardalis* are elongate and attain much greater lengths than those of *Oncicola.* Further, a review of the literature (Dunagan and Miller, 1986) indicated that protonephridial organs in species of *Oncicola* are of the dendritic type while those of *Echinopardalis* are of the capsular type. Protonephridia were restudied for *O. oncicola,* type species; *O. canis,* a well-accepted member of the genus; and *O. luehei,* a species originally assigned to *Prosthenorchis* and reassigned to *Oncicola* by Schmidt (1972). The protonephridia in each of these was confirmed as being dendritic.

Additionally, *Echinopardalis* may differ from *Oligacanthorhynchus* and *Oncicola* in the nature of the egg. Descriptions of *Echinopardalis* routinely mention that eggs lack a compact external shell. This condition was apparent in eggs removed from the body cavity of female *E. atrata* (Fig. 1). No such egg has been described for species of either *Oligacanthorhynchus* or *Oncicola.* Because of the size and shape of the trunk, position of the male reproductive system, and type of protonephridia, it is proposed that separate generic status be resurrected for *Echinopardalis.*

## Emended diagnosis of *Echinopardalis* Travassos, 1918

The following diagnosis is basically that of Petrochenko (1958) with acknowledgment of Schmidt's (1972) view that the Pachysentidae do not constitute a family distinct from Oligacanthorhynchidae. The subsequent key to genera of Oligacanthorhynchidae is essentially that of Schmidt (1972). Emendations incorporate new information about protonephridial systems and morphological interpretations made possible by the opportunity to study adult *Echinopardalis atrata.*

DIAGNOSIS: Oligacanthorhynchidae. Body elongate. Sexually mature females with a small process at the posterior end, dorsal to the genital aperture. Proboscis orbicular, with 36 hooks in 6 circles of 6 hooks (12 regularly alternating, approximately longitudinal rows of 3 hooks each). Lemnisci nearly cylindrical, and long. Lacunar system slightly developed with amebiform giant nuclei present in the region of the main canals. Testes oval to elongate, extending anteriad to midbody, tandem, but separated from one another and from cement glands. Eight cement glands in a column of pairs. Protonephridial organs capsular. Eggs oval, with concentric membranes and without compact external shell. Adults parasitic in intestines of carnivorous mammals. Type species: *E. pardalis* (Westrumb, 1821) Travassos, 1918.

### Key to the Genera of Oligacanthorhynchidae

1a. Anterior end of trunk with conspicuous, festooned collar ... *Prosthenorchis* Travassos, 1915
1b. Anterior end lacking such a collar ... 2
2a. Anterior trunk with dorsal and ventral sagittal crests ... *Tchadorhynchus* Troncy, 1970
2b. Anterior end lacking such crests ... 3
3a. Proboscis with 12 longitudinal, regularly alternating rows of 2 and 3 hooks each, total of 30 ... *Neoncicola* Schmidt, 1972
3b. Proboscis with more than 30 hooks ... 4
4a. Proboscis with 36 hooks ... 5
4b. Proboscis with more than 36 hooks ... 8
5a. Both testes conspicuously postequatorial ... *Oligacanthorhynchus* Travassos, 1915
5b. At least 1 testis preequatorial ... 6
6a. Protonephridial organs capsular type ... *Echinopardalis* Travassos, 1918
6b. Protonephridial organs dendritic type ... 7
7a. Trunk very long, females more than 100 mm long ... *Macracanthorhynchus* Travassos, 1917
7b. Trunk short to medium, females up to 50 mm long ... *Oncicola* Travassos, 1916
8a. Anterior trunk narrower than posterior trunk; proboscis hooks in 12 longitudinal, regularly alternating, approximately straight rows of 4 each, total 48 ... *Nephridiorhynchus* Meyer, 1931
8b. Anterior trunk wider than posterior trunk; proboscis hooks in 12 longitudinal, regularly or irregularly alternating rows of 3–12 each, total 42–102 ... *Pachysentis* Meyer, 1931

## Assignment of species

The protonephridial system of acanthocephalans seems to be evolutionarily conservative. There appear to be only 2 basic patterns and a rudimentary type (Dunagan and Miller, 1986),

and it seems reasonable that all species of a genus should share a common type. The nature of the protonephridial system is known for relatively few species, and conflicting accounts remain for some species of *Oligacanthorhynchus*. When more widely known, the types of protonephridia might resolve some systematic uncertainties of the Oligacanthorhynchidae. For example, as presently defined *Oncicola* includes species of 2 general forms; a group with roughly saccular or pyriform-shaped trunks that are less than 20 mm long and a group with elongate trunks that may reach lengths of nearly 50 mm. The type and all other species for which the nature of protonephridial organs is known (dendritic type) belong to the former group. Trunks of those in the latter group more closely resemble the shape of species assigned to other genera, including *Echinopardalis*. It is critical to learn the form of protonephridial organs in a wider variety of species.

Aside from the type, *Echinopardalis pardalis* (Westrumb, 1821) Travassos, 1918, 8 species have been assigned to *Echinopardalis*: *E. atrata* Meyer, 1931; *E. bangalorensis* Pujatti, 1951; *E. decrescens* Meyer, 1931; *E. lamasi* Freitas and Costa, 1964; *E. lerouxi* Bisseru, 1956; *E. macrurae* Meyer, 1931; *E. mariemily* Tadros, 1969; and *E. pachyacanthus* (Sonsino, 1889) Neveu-Lemaire, 1936.

Meyer (1931) described *Oncicola macrurae* from *Felis macrura* collected in Paraguay and *Echinopardalis macrurae* from *F. macrura* collected in "South America." Later Witenberg (1938) examined the same specimens and declared them conspecific. However, the specimens of *O. macrurae* were juveniles, and generic assignment of juvenile Oligacanthorhynchidae is virtually impossible. Lent and Freitas (1938) identified adult specimens from *F. macrura* collected in Brazil as *O. macrurae*. Their adult specimens are of the size (females 9–10 mm long) and shape typical of *Oncicola* and differ conspicuously from the long, slender adults of *E. macrurae* illustrated by Meyer (1931). Van Cleave (1953) later identified as *E. macrurae* specimens taken from a bobcat, *Lynx rufus*, that had been residing in a San Diego zoo. These specimens had elongate bodies up to 43 mm long. Holloway (1964) identified two male acanthocephalans from a spotted skunk, *Spilogale putorius*, taken in Virginia as *E. macrurae*. Subsequent collections produced females with long bodies and prominent terminal processes. Evidence suggests that *O. macrurae* and *E. macrurae* are distinct, or at least that *E. macrurae* was properly assigned by Meyer.

The protonephridial system has been described as capsular for *Echinopardalis atrata, E. decrescens*, and *E. macurae*. These species, therefore, conform fully to the present view of *Echinopardalis*. The protonephridial systems are unknown for *E. lamasi* and *E. lerouxi*, but they are assigned to *Echinopardalis* for the present because they conform in all other respects. *Echinopardalis bangalorensis* is known only from juveniles and must remain incertae sedis. *Echinopardalis mariemily* conforms fully with *Oligacanthorhynchus*, but its assignment has been previously overlooked. *Oligacanthorhynchus mariemily* (Tadros, 1969) comb. n. is proposed. *Echinopardalis pachyacanthus* is currently unrecognizable and has been previously assigned to several genera. Its host, *Canis aureus*; geographical occurrence, Egypt; and the size of its eggs, 90–100 × 50–60 μm, all suggest that it is conspecific with *E. atrata*.

**Acknowledgments**

Dr. R. M. A. Rashed, Ain Shams University, Cairo, Egypt, made the specimens of *Echinopardalis atrata* available for study and kindly provided host data. Specimens of *Oncicola oncicola* and *O. luehei* were collected by Dr. Vernon E. Thatcher. Dr. Danny B. Pence provided specimens of *O. canis*. Syntypes of *E. atrata* were borrowed from the Museum für Naturkunde der Humboldt-Universität zu Berlin through the assistance of Dr. Gerhard Hartwich, Curator Wurm-Abteilung. The cooperation of these colleagues is gratefully acknowledged.

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## Meeting Announcement

The 22nd meeting of the Association of Marine Laboratories of the Caribbean will be hosted by the Department of Marine Sciences, University of Puerto Rico, 5–8 June 1989, in La Parguera, Puerto Rico. A *Proceedings* including abstracts of contributed papers will be published prior to the meeting. For information contact Dr. Manuel L. Hernandez Avila, President AMLC, Department of Marine Sciences, Box 5000, University of Puerto Rico, Mayaguez, Puerto Rico 00709-5000; Telephone (809) 832-4040; Telex: UPR MAY 3452024; Fax: (809) 834-3031.

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# Morphology of Urogenital System in Male *Echinopardalis atrata* (Acanthocephala)

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ABSTRACT: The urogenital system of male *Echinopardalis atrata* consists of the products from: 1) 2 testes, 1 of which is located preequatorially, 2) a capsular type of protonephridial system attached on the dorsal surface at the junction of the dorsal ligament sac and the genital sheath, and 3) 8 rectangular cement glands immediately anterior to the excretory bladder and whose individual ducts are enclosed by the genital sheath. Each of the 2 vasa efferentia pass beneath the bladder and adjacent to the genital sheath. These ducts fuse near the posterior terminus of the bladder. The vas deferens so formed is medial to the excretory canal but remains in a dorsal position. The two capsular protonephridia extend anteriorly from the anterior dorso-lateral surface of the excretory bladder. Each enters the bladder through an obliquely oriented nephridial canal. A single excretory canal exits the ventral surface of the bladder. This canal is lined with cilia for most if not its entire length. Anterior to the penis it is joined by the vas deferens to form the urogenital canal into which the ducts from the cement glands empty. All 3 systems empty to the outside through the penis by way of the gonopore. Each of these systems is depicted using photographs of cross sections taken at various positions between the cement glands and the bursa.

KEY WORDS: urogenital, *Echinopardalis atrata,* Acanthocephala.

In a recent review of protonephridial excretory systems, Dunagan and Miller (1986) noted that current information suggested that there were 3 designs for this system. The 2 most common, dendritic and capsular, are the best described but still poorly understood. Indeed, the information on the capsular design is largely from Meyer (1931a) who first recognized and named this type following his studies of *Oligacanthorhynchus taenioides.* However, his studies and line drawing were based only on female specimens. Von Haffner (1942a) was the next person and the last in our opinion to make a significant contribution toward understanding the organization of this design. He studied both sexes of *Oligacanthorhynchus thumbi* and represented his results in the form of a series of line drawings of cross sections of the urogenital system. Included in this system was the dorsal ligament sac. Von Haffner (1942a, p. 285) stated that the area enclosed by the genital sheath ("Leitungsschlauch") was but the third part of the ligament sac. We do not include the ligament sac in this study and do not address the question of its relationship to the systems mentioned.

Several general accounts of acanthocephalan urogenital systems have been published since the work of Meyer (1932). These reviews (Hyman, 1951; Petrochenko, 1956; Yamaguti, 1963; Miller and Dunagan, 1985; etc.) include information on capsular protonephridia but do not expand previous studies. Likewise, taxonomic papers have typically added little, if anything, on the excretory system or its relationship to other components of the urogenital system. For example, Machado Filho (1950) reviewed and revised the genus *Prosthenorchis* members of which are presumed to have a capsular-type excretory design. Yet, most of his illustrations omit these structures. However, when illustrated, the figures are very small (3 mm) and include only the capsule. The text is equally brief, pointing out that the type of excretory system has not historically been important in taxonomic studies.

The purpose of this study in *Echinopardalis atrata* is to show the relationship of a capsular designed protonephridial system to other components of the urogenital system.

## Materials and Methods

Live worms were removed from dogs (*Canis familiaris*) exterminated in Cairo, Egypt. Infected dogs were from the districts of Torah, El-Basateen, and Maasara. Upon removal from the intestine, the worms were briefly washed in tap water and then fixed in AFA or 2% glutaraldehyde. No attempt was made to control osmotic pressure or pH during fixation. Specimens were prepared for routine paraplast embedding and sectioned at 8 $\mu$m. Staining was accomplished by standard methods for hematoxylin and eosin. Two sets of serial sections were prepared and form the basis of this report. The position of Saefftigen's pouch which Kaiser (1893) described as ventral was used as the basis for dorsal-ventral orientation. All photographs are organized with dorsal to the top. Figures 1–12 have the

same scale which is depicted in Figures 1, 2, 7, 10, and 12.

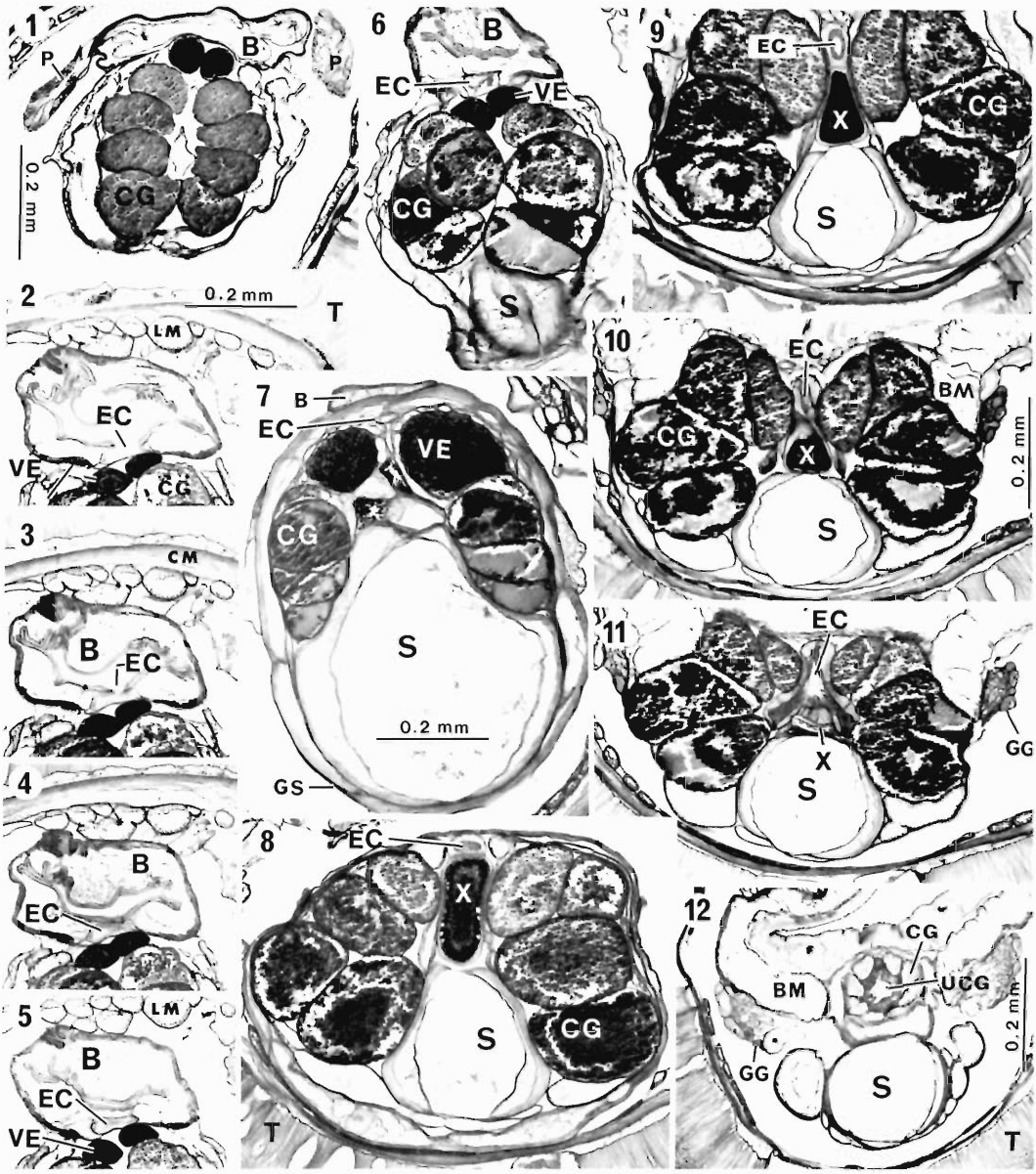
## Results

The urogenital system of male worms consists of: 1) a dorsally located capsular-type protonephridial excretory system, 2) a series of 8 rectangular, tightly packed cement glands and their associated ducts, and 3) testes and exit passages for sperm. However, in a larger sense, this system also includes Saefftigen's pouch and various muscle groups including the bursa complex. The paired capsular protonephridia extend anteriorly from the anterior fourth of the excretory bladder. The exact shape and position of these oblong capsules vary with compression of body wall musculature as well as with the degree of expansion of the excretory bladder. This variation is obvious by comparing the 2 capsules in Figure 1. Flame bulbs which cover the capsule surface are seldom oriented perpendicular to that surface but appear to enter from an oblique angle (Figs. 14, 15). A large number of cilia fill each flame bulb. The bladder wall varies considerably in thickness (Fig. 2 vs. Fig. 6), and both outer and inner surfaces may have many infoldings (Figs. 2–4). The posterior terminus of the bladder (Fig. 7) is not patent but a narrow band of solid tissue attached to the genital sheath. The anterior attachment is similarly organized. Each flame bulb appears to open individually into a capsule which has a small lumen which empties into a nonciliated canal leading into the bladder along its dorso-lateral surface. The description of this entry point varies depending on fullness of the bladder. The wall of each capsule has 3 nuclei evenly distributed but located predominately on its outer pseudocoelomate surface. The contents in the lumen of the bladder contain material which precipitates during fixation often forming a cobweb-like appearance. The bladder contains no cilia and empties via a single excretory canal (Figs. 2–11) originating in the posterior half of the bladder wall (Figs. 3–6) along its medial surface immediately dorsal to paired vasa efferentia. This origin is about the same level as the anterior margin of Saefftigen's pouch (Fig. 6). Notice that the excretory canal travels in the wall of the bladder (Figs. 4, 5) a short distance prior to penetrating the genital sheath (Fig. 6). By the time the posterior terminus of the bladder is reached (Fig. 7), this canal lies between and slightly dorsal to the vasa efferentia. It will remain in this position until the vicinity of the bursa where it merges (Fig. 11) with the vas deferens to form the urogenital canal. Cilia are observed for much of the length of the excretory canal and may be present throughout although this is not obvious in the anterior portion of this tube. Prior to the junction with the vas deferens, the excretory canal enlarges (Fig. 16) forming a "Y"-shaped structure (Fig. 11) which reverts to a cylinder-shaped urogenital canal (Fig. 12) as the cement gland ducts prepare to enter. Cilia are observed throughout this area.

Four pairs of tightly packed rectangular-shaped cement glands are located immediately anterior to the genital sheath. Each gland has a single large nucleus. Two testes are anterior to the cement glands although separated from the latter and from each other by short distances. A vas efferens from each testis and a single duct from each cement gland extend posteriorly. At the level where dorsal ligament sac and genital sheath join these 10 tubes occupy the space enclosed by the genital sheath. This area is narrowed (in comparison with that more anterior or posterior) by the yoke of the bursa protrusor muscles. Thus, Figure 1 is smaller than Figure 7. The appearance of Saefftigen's pouch rapidly displaces remaining structures in a dorsal direction (Fig. 7). This highly muscular organ occupies much of the space enclosed by the genital sheath for a distance of about 1,600 $\mu$m after which it becomes smaller (Figs. 8–12) but rather uniform in size until it enters the bursal muscles. As it becomes smaller, the cement gland ducts increase in diameter (Figs. 8–10). After formation of the urogenital canal, they become much smaller and encircle this tube (Fig. 12). After the penis musculature has encircled the urogenital canal, these ducts rapidly become smaller and empty individually into this canal beginning with the most ventral pair and concluding with the most dorsal pair (Fig. 13). All components exit the penis through a common gonopore.

## Discussion

A very small number of papers exist that include information on the excretory system of Acanthocephala or its relationship to the urogenital system. This is largely because these systems are found only in a single family, Oligacanthorhynchidae. Also, since most publications on these parasites are taxonomic in nature and taxonomists have not considered the excretory system of discriminating value, this sytem has been overlooked, ignored, and underrepresented



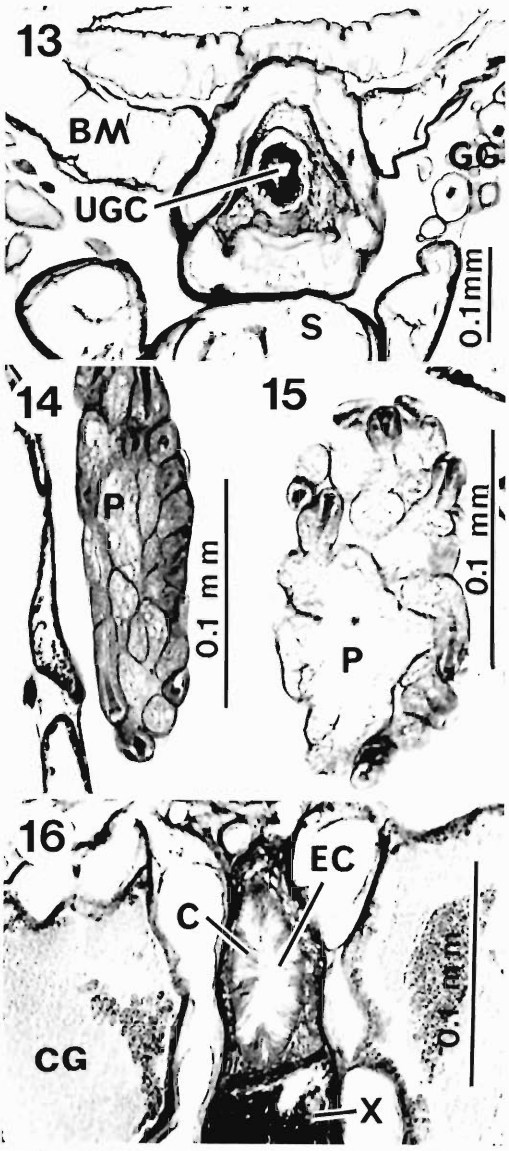

**Figures 1–12.** Cross sections of urogenital system of male *Echinopardalis atrata*. 1. Junction of dorsal ligament sac and genital sheath showing capsular protonephridium including bladder. 2. Initial formation of excretory canal along ventral surface of bladder. 3. Last stage in formation of "roof" of excretory canal. 4. Formation of excretory canal complete. 5. Bladder wall dorsal to excretory canal. Notice thickness of wall. 6. Excretory canal penetrates genital sheath. Anterior terminus of Saefftigen's pouch. 7. Posterior terminus of excretory bladder. Excretory canal ventral to genital sheath. Notice size of Saefftigen's pouch. 8. Excretory canal dorsal to vas deferens. Note enlargement and position of cement gland ducts. 9. Anterior edge of bursa complex just prior to formation of urogenital canal. 10. Organization of excretory canal before entry of vas deferens. 11. Entry of vas deferens to excretory canal forming urogenital canal. 12. Cement gland ducts prior to entry into urogenital canal. Penis musculature surrounds ducts. B, excretory bladder; BM, bursal muscle; CG, cement gland ducts; CM, circular muscle; EC, excretory canal; GG, genital ganglion; GS, genital sheath; LM, longitudinal muscle; P, capsular protonephridium; S, Saefftigen's pouch; T, tegument; UCG, urogenital canal; VE, vas efferens; X, vas deferens. Dorsal is top of photograph. All photographs to same scale organized from anterior to posterior.

in their descriptions of new species or evaluations of previous information. Apparently, Golvan (1959) spoke to this point at the 15th International Congress of Zoology. Until 1931 (Meyer, 1931a) descriptions of protonephridia and associated excretory systems were focused on *Macracanthorhynchus hirudinaceus.* This information had been reviewed and updated by Kaiser (1892, 1893) and Schepotieff (1908). Meyer (1931a) erected a new class of protonephridia (capsular) based on his observations on female *Oligacanthorhynchus taenioides.* The third and final type was proposed by von Haffner (1942b) in female *Gigantorhynchus echinodiscus.* This last type consists of a single cell with an intracellular ciliated pouch and has not been reported since its original description.

The work of 2 authors (Meyer, 1931a, b; von Haffner, 1942a) form the basis of our current information on urogenital systems with capsular designs. The work of Kilian (1932) might be thought to add to this since he gave a good description of *Hamanniella microcephala* which is currently (Amin, 1985) listed in the genus *Oligacanthorhynchus* along with *O. taenioides.* However, Kilian described a dendritic system in *O. microcephala* and Meyer (1931a) described a capsular system in *O. taenioides.* This conflict suggests that the generic placement of some species in this family may have to be reconsidered.

Meyer's (1931a) description of the capsular excretory system in female *O. taenioides* depicted a capsule with a large number of small diameter flame bulbs radiating perpendicular from its surface and whose wall contained 3 nuclei. The excretory bladder was described as thin walled. That same year Meyer (1931b) described several new species with capsular protonephridia. His textural descriptions were seldom more than single sentences, but he made 3 line drawings (figs. 27, 49, 73) one of which (fig. 49) was more detailed and most nearly compares with our observations. His figure 49 shows a capsule in *Pachysentis procumbens* covered with stubby flame bulbs that radiate from the surface in perpendicular fashion. Three large nuclei are also shown in the capsule wall. Their size is such that they cover the entire lumen of the capsule.

Von Haffner (1942a) described the urogenital system in juvenile *Oligacanthorhynchus thumbi.* His figure 15 (p. 281) showed the flame bulbs to be very long narrow projections radiating in perpendicular fashion from the capsule surface. However, his drawing of a cross section of the same area (fig. 27, p. 287) had the orientation of flame bulbs in a more random fashion which




**Figures 13–16. Cross sections of urogenital system of *Echinopardalis atrata.* 13. Urogenital canal following entry of cement gland ducts. Penis musculature surrounds canal. 14, 15. Capsular protonephridium showing cilia in flame bulbs. 16. Enlargement of excretory canal showing cilia. BM, bursal muscle; C, cilia; CG, cement gland ducts; EC, excretory canal; GG, genital ganglion; P, capsular protonephridium; S, Saefftigen's pouch; UGC, urogenital canal; X, vas deferens. Dorsal is top of photograph except Figures 14, 15.**

corresponds with information presented here. Von Haffner's figure 15 also depicted the capsule emptying into the excretory bladder in a mid-dorsal position, whereas in this species, this system empties more anteriorly and along the dorso-lateral surface. The excretory bladder was considered by von Haffner (1942a, p. 286) in a narrow sense. He apparently restricted this term to the lumen or cavity and considered its surrounding wall the "Polsterstiel" or swollen section of the "Leitungsschlauch." His drawings confirm this view. In contrast, we consider the entire structure into which the capsule empties to be the excretory bladder. Its location on the dorsal surface at the junction of dorsal ligament sac and genital sheath agrees with all previous descriptions regardless of excretory type. The variety of shapes and sizes of this structure suggests that it is an expandable organ.

The vasa efferentia form the vas deferens about halfway between excretory bladder and bursa in *O. thumbi.* Meyer (1931a) did not examine male *O. taenioides.* In *Pachysentis* the vas deferens forms at the posterior terminus of the excretory bladder which is much anterior to the location where this occurs in *O. thumbi.* However, the ventral entry of the vas deferens into the excretory canal is the same for both descriptions and occurs at the anterior edge of the penis. Moreover, Saefftigen's pouch is shown by von Haffner (1942a) as a spindle-shaped organ that never forms a uniform diameter duct prior to entry into the bursa. In this species, the enlarged spindle-shaped portion occupies less than half the length of the genital sheath. The remaining length consists of a large muscular rather uniformly shaped extension (Figs. 8–12) which occupies a prominent position adjacent to the genital sheath along its ventral surface. The distribution of cilia in the urogenital system and excretory canal is about the same. We are unable to verify the presence of cilia in the anterior part of the excretory canal because of the small size of the tube in these specimens. Considerable difference occurs between von Haffner's (1942a, p. 290) description of the entry of cement gland ducts into the urogenital canal in *O. thumbi* and our observations for *Echinopardalis atrata.* In *O. thumbi* the 4 cement gland ducts on each side unite into 2 very short "Ausführgänge (Sammelgängen)" which enter the vas deferens shortly before (i.e., anterior) it joins the excretory canal. An examination of Figures 11 and 12 clearly shows that the cement gland ducts are very much intact posterior to the formation of the urogenital canal. Our serial cross sections show the cement gland ducts entering individually in pairs beginning with the most ventral ducts. The remaining part of the urogenital system compares favorably in both species. We believe this is only the second description of a male urogenital system containing a capsular-type protonephridium. The differences observed may reflect their different generic positions. Unfortunately, as pointed out previously, we cannot be sure of their proper taxonomic position.

### Acknowledgments

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# *Cosmocercella iwatsukii* sp. n. (Nematoda: Cosmocercidae) from *Rhacophorus viridis viridis* (Anura: Rhacophoridae) on Okinawa Island, Japan

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ABSTRACT: *Cosmocercella iwatsukii* sp. n. from the intestine of the frog, *Rhacophorus viridis viridis,* collected in Kunugami-son, Okinawa Island, Japan, is described. *Cosmocercella iwatsukii* is readily distinguished from other representatives of the genus on the absence of gubernaculum, and the presence of two pairs of papillae on posterior anal lip and/or post-vulval position of posterior ovary.

KEY WORDS: *Cosmocercella iwatsukii* sp. n., new species, Nematoda, Cosmocercidae, taxonomy, frog, *Rhacophorus viridis viridis,* Anura, Rhacophoridae, Okinawa Island, Japan.

The genus *Cosmocercella* Steiner, 1924, includes a small group of nematodes parasitic in amphibians and reptiles from North and South America and Asia (Baker and Adamson, 1977; Baker and Crusz, 1980). During a survey of amphibian helminths from the Ryukyu Archipelago, Japan, an undescribed species of *Cosmocercella* was collected from a rhacophorid frog on Okinawa Island, Japan. The species is described herein.

## Materials and Methods

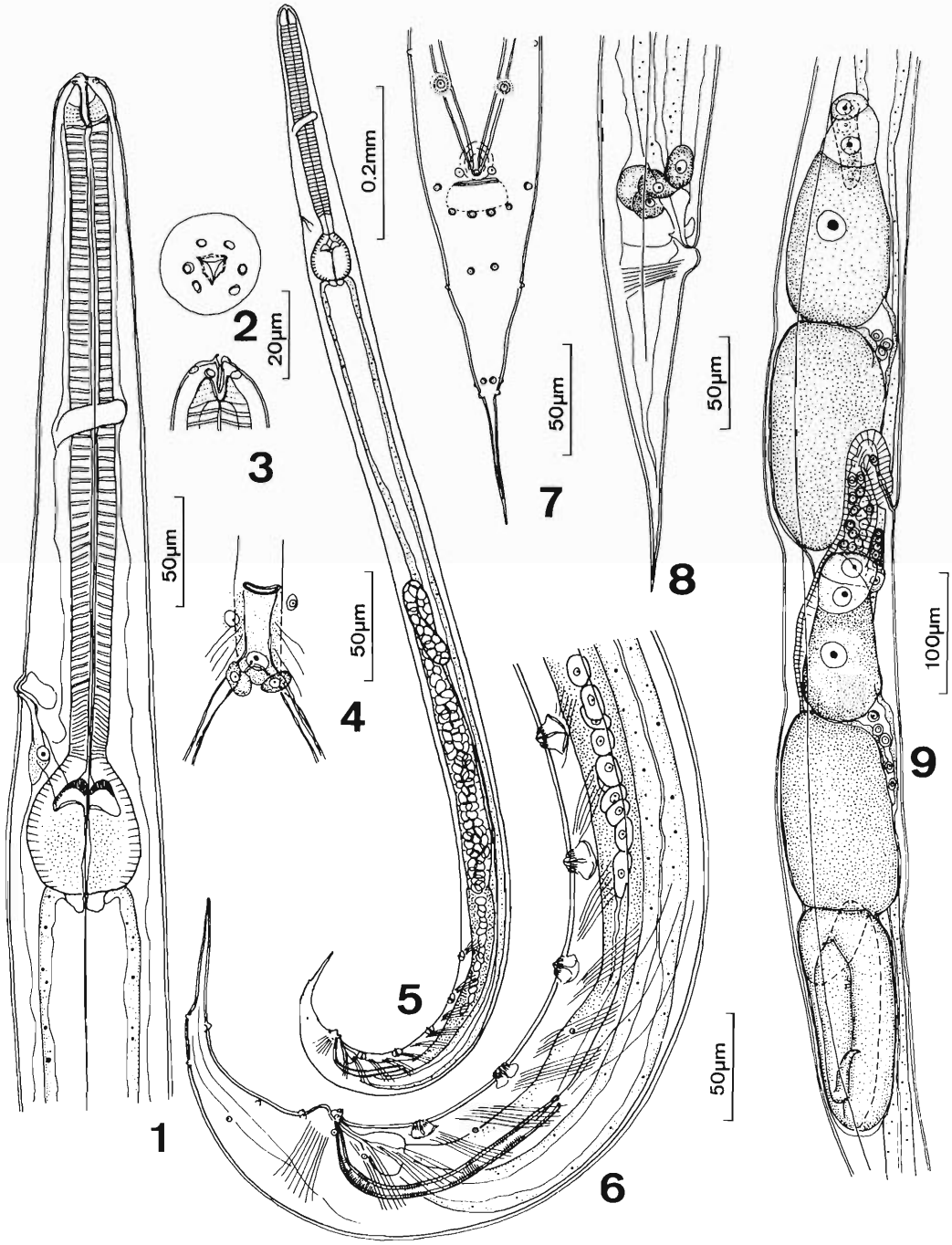
Frogs were collected by hand at Kunigami-son, Okinawa Island, Japan. They were examined after being killed with ether inhalation. Nematodes were fixed in hot 70% ethanol, cleared in glycerin-alcohol, and mounted on slides with glycerin for microscopical observation. Figures were made with the aid of a drawing apparatus, Olympus BH-DA-LB. Measurements are in micrometers unless stated otherwise; with those of the holotype or allotype being followed by those of the paratypes in parentheses.

## *Cosmocercella iwatsukii* sp. n. (Figs. 1–9)

GENERAL: Ascaridida, Cosmocercoidea, Cosmocercidae, *Cosmocercella* Steiner, 1924. Minute worms with tapered extremities (Figs. 1, 5, 7, 8). Cuticle with fine transverse striations. Lateral alae narrow, commencing from level far anterior to nerve ring (Fig. 1) and ending at precloacal region in male (Fig. 6), and anterior to midtail in female (Fig. 8). Oral opening triangular, surrounded by 3 lips (Fig. 2); each lip with cuticular flap overhanging oral opening (Fig. 3); dorsal lip with 1 pair of papillae; subventral lips each with a subventral papilla and an amphid (Fig. 2). Anterior extremity of pharynx with 3 protuberances (Fig. 3). Esophagus composed of cylindrical portion with short isthmus and bulbous portion (Fig. 1). Nerve ring at midlevel of cylindrical portion of esophagus (Fig. 1); excretory pore at level of posterior end of cylindrical esophagus (Fig. 1); excretory vesicle small, surrounded by a cellular mass with 1 large and 4 small nuclei; 2 posteriorly directed lateral canals arising from excretory vesicle (Fig. 4).

MALE (holotype and 15 paratypes): Length 1.73 (1.66–1.80) mm. Maximum width 70 (65–70). Caudal end bent ventrally (Figs. 5, 6). Pharynx 20 (15–20) long; cylindrical portion of esophagus 0.29 (0.28–0.32) mm long by 25 (23–25) wide; bulbous portion of esophagus 58 (60–63) long by 50 (48–55) wide. Nerve ring 156 (143–168) and excretory pore 0.28 (0.27–0.31) mm from cephalic extremity. Spicules subequal, nonalate, distally pointed, bent ventrally, 140 (128–145) long (Figs. 6, 7). Gubernaculum absent. Caudal alae absent. Oblique musculature present in preanal ventral portion (Fig. 6). Tail 140 (123–140) long, tapering abruptly in posterior one-third to spinelike distal portion (Figs. 6, 7). Posterior lip of anus protruded (Fig. 6). Caudal papillae as follows: 5 pairs of large rosette papillae on surface of clear vesicles and 1–3 lateral pairs of small papillae present preanally; 1 pair of small papillae and 1 unpaired papilla on anterior anal lip; 1 pair adanal; 2 pairs at posterior margin of posterior anal lip; 1 ventral and 1 lateral pair at posterior end of anterior one-third of tail; 1 ventral, 1 lateral, and 1 subdorsal pair at base of spinelike distal portion of tail (Figs. 6, 7).

FEMALE (allotype and 15 paratypes): Length 2.07 (1.97–2.15) mm. Maximum width 120 (108–130). Pharynx 20 (18–25) long; cylindrical portion of esophagus 0.33 (0.31–0.34) mm long by




**Figures 1–9.** ***Cosmocercella iwatsukii*** **sp. n. 1. Anterior part of male (holotype), lateral view. 2. Anterior end of female (paratype), apical view. 3. Anterior end of female (paratype), lateral view. 4. Excretory pore of female (paratype), ventral view. 5. Male (holotype), lateral view. 6. Posterior end of male (holotype), lateral view. 7. Posterior end of male (paratype), ventral view. 8. Posterior end of female (allotype), lateral view. 9. Reproductive organs of female (allotype), lateral view.**

29 (25–30) wide; bulbous portion of esophagus 68 (65–73) long by 58 (58–63) wide. Nerve ring 163 (153–180) and excretory pore 0.33 (0.31–0.34) mm from cephalic extremity. Vulva small, slitlike, slightly lateral to midventral line, 1.18 (1.09–1.24) mm from cephalic extremity (Fig. 9); vagina first directing anteriorly, then flexed posteriorly (Fig. 9); uteri divergent; anterior uterus directing anteriorly; posterior uterus directing posteriorly, then flexed anteriorly; each uterus with 1 or 2 large eggs; anterior ovary flexed posteriorly and ending far anterior to vulval level; posterior ovary ending at vulval level; oviducts compressed (Fig. 9). Tail long, conical, 190 (168–203) in length (Fig. 8). Eggs elliptical, thin-shelled, mean 184.6 ± 14.4 (SD) by 99.0 ± 7.8 ($N = 23$) (Fig. 9). Posterior-most egg in each uterus often containing fully developed larva (Fig. 9). Free larva sometimes observed in uterus.

HOST: *Rhacophorus viridis viridis* (Hallowell, 1860).

LOCATION: Small intestine and rectum.

PREVALENCE AND INTENSITY: One of 14 frogs examined. About 100 worms were collected.

LOCALITY: Hentona, Kunigami-son, Okinawa Island, Japan.

DATE OF COLLECTION: 31 October 1987.

SPECIMENS DEPOSITED: United States National Museum, USNM Helm. Coll. No. 80327 (holotype and allotype), 80368 (10 paratypes); National Science Museum, Tokyo, NSMT-As 1880 (20 paratypes).

ETYMOLOGY: The species name is dedicated to Mr. Nobuki Iwatsuki who kindly collected the frogs.

## Discussion

Five species of *Cosmocercella* have been recognized as valid: *C. haberi* Steiner, 1924 (type species); *C. minor* (Freitas and Dobbin, 1961) Baker and Adamson, 1977; *C. uropeltidarum* (Crusz and Ching, 1975) Baker and Crusz, 1980; *C. anothecae* Baker and Adamson, 1977; and *C. phyllomedusae* Baker and Vaucher, 1983. Among them, *C. haberi, C. minor, C. anothecae,* and *C. phyllomedusae* are parasitic in frogs of the New World, while *C. uropeltidarum* is a parasite of uropeltid snakes of Sri Lanka and South India.

The present species belongs to the genus *Cosmocercella* in having vesiculated rosette papillae and a few large eggs in the uteri (Chabaud, 1978) although it is unusual in that the gubernaculum is absent and the posterior ovary is posterior to the vulva. *Cosmocercella iwatsukii* is also clearly distinguished on other characteristics: from *C. haberi, C. minor,* and *C. anothecae* in lacking caudal alae and digitiform caudal papillae (Baker and Adamson, 1977; Baker and Vaucher, 1983); from *C. uropeltidarum* which has wide lateral alae, many somatic papillae, external appendices on vesiculated papillae, and a well-developed gubernaculum (Crusz and Ching, 1975; Baker and Crusz, 1980); and from *C. phyllomedusae* which has wide lateral alae and markedly large rosette caudal papillae (Baker and Vaucher, 1983). Baker and Adamson (1977) provisionally retained *C. neveri* Hsü and Hoeppli, 1933, in *Cosmocercella* although they suggested an affinity with *Cosmocerca. Cosmocercella iwatsukii* is easily distinguished from *C. neveri* since the latter has numerous somatic papillae, alate spicules, and its eggs are smaller and more numerous (Hsü and Hoeppli, 1933).

There are many inconstant characteristics among *Cosmocercella* species, i.e., presence or absence of caudal alae, digitiform caudal papillae, and gubernaculum in male, and pre- or post-vulval position of posterior ovary in female. Old World representatives possess caudal papillae on the margin of the posterior anal lip which are absent in New World species. Therefore, separation of *Cosmocercella* into at least two genera may be appropriate. However, more knowledge on the genus, especially that on the Old World species, should be accumulated before final conclusion on this problem.

## Acknowledgments

Thanks are tendered to Prof. Y. Sato, University of the Ryukyus, for his valuable criticism, and to Dr. A. Ichihara, the Meguro Parasitological Museum, Tokyo, for his kindness in making available the related papers.

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# Filariasis in Colombian Capybaras: Circadian and Spatial Distributions of Microfilariae in the Skin

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ABSTRACT: Three capybaras harboring concomitant natural infections with *Yatesia hydrochoerus, Mansonella longicapita, M. rotundicapita,* and *Cruorifilaria tuberocauda* were captured and transported to the United States where studies of spatial and circadian distributions of microfilariae in the skin were performed. *Mansonella longicapita, M. rotundicapita,* and *C. tuberocauda* showed a similar, distinctive pattern of distribution in the skin. Snips taken from the top of the head, the back, or the ears contained highest concentrations while snips from the venter and extremities contained few or no microfilariae. Circadian fluctuations in the concentrations of *C. tuberocauda* and *M. longicapita* microfilariae were similar. Both species demonstrated peak concentrations of microfilariae at approximately 1000 hours. Concentrations of *M. rotundicapita* did not appear to fluctuate in a periodic manner. *Yatesia hydrochoerus* microfilariae were invariably present in low concentrations in skin snips and absent from blood samples. Thus, periodicity was not evaluated for this species. These findings suggest that natural vectors of *M. longicapita, M. rotundicapita,* and *C. tuberocauda* may display peak biting activity on the head, ears, or backs of capybaras, and for *M. longicapita* and *C. tuberocauda* peak biting activity of vectors would be likely in the morning hours.

KEY WORDS: skin snips, periodicity, Colombia, *Hydrochoerus hydrochaeris, Yatesia, Mansonella, Cruorifilaria,* filarioidea, capybara, circadian and spatial distribution.

Capybaras taken from the wild in Colombia and Venezuela, South America are often found to harbor up to 4 concomitant filarial infections (Eberhard et al., 1976; Yates and Jorgenson, 1983; Eberhard et al., 1984). A high prevalence of renal infections with *Cruorifilaria tuberocauda* has been reported (Ojasti, 1973; Eberhard et al., 1976) and extensive associated pathology of the renal vasculature and adjacent tissues has been described by Morales et al. (1978). Infections of the dermis with adult *Mansonella rotundicapita* or *M. longicapita* and infection of skeletal muscle fascia with *Yatesia hydrochoerus* adults apparently cause insignificant tissue damage (Yates and Jorgenson, 1983; Eberhard et al., 1984).

*Cruorifilaria tuberocauda* microfilariae circulate in the peripheral blood vascular system while microfilariae of the other three species inhabit the superficial layers of the skin (Eberhard et al., 1976; Yates and Jorgenson, 1983). Interestingly, superficial, apparently bloodless skin snips readily detected infections with all four species (Yates and Jorgenson, 1983; Campo-Aasen et al., 1985).

There is a dearth of microfilarial periodicity studies of skin-dwelling species, although, *Onchocerca volvulus* microfilariae display a diurnal concentration peak in human skin (Duke et al., 1967). Periodicity of skin-dwelling species in hosts other than humans apparently has received little attention. An understanding of the behavior of animal microfilariae that infect the skin may provide information relevant to human infections with *O. volvulus* and *M. streptocerca.* The following report summarizes our observations on the behavior of microfilariae in capybara skin.

## Materials and Methods

The capybaras used in these studies were captured in Casanare, Colombia and transported to the Delta Regional Primate Research Center in Covington, Louisiana where they were maintained in the manner outlined by Crandall (1964). A 12-hr photoperiod was provided in the animal quarters. Small, relatively uniform skin snips were obtained with a Holth® sclerocorneal biopsy punch. Skin snips were placed individually into screw-top vials containing 1 ml of physiologic saline and allowed to incubate for approximately 6 hr at room temperature. Thereafter, skin snips were removed from the vials and 10 ml of 2% formalin were added to each vial to fix microfilariae which had emerged from the tissue. The samples were individually concentrated by centrifugation and the sediment was examined microscopically. Slides containing microfilariae were dried and stained with hematoxylin and eosin. Skin snips were blotted on filter paper and weighed on a torsion balance to enable calculation of microfilarial concentrations (i.e., number of microfilariae per mg of skin).

The numerical distributions of 3 species of microfilariae in capybara skin were determined from 100 skin snips per animal, taken from capybaras anesthetized with ketamine HCl. In this study, 2 animals which harbored moderate infections with 3 species of microfilariae and light infections with a fourth species were examined. Snips were taken with a biopsy punch from 10 different areas of the body: top of head, ears, submandibular, back, axilla, abdomen, chest, perianal,

**Table 1. The distribution of 3 species of microfilariae in the skin of 2 capybaras. Mean microfilarial counts per mg of skin were determined from 10 weighed skin snips from each of 10 parts of the body.**

| | Head | Back | Ear | Chin | Abdo-men | Foreleg | Perianal | Hindleg | Chest | Axilla |
|---|---|---|---|---|---|---|---|---|---|---|
| *C. tuberocauda* | | | | | | | | | | |
| Animal YC103 | 9.3 | 9.3 | 5.8 | 0.5 | 0.2 | 0.1 | 0.5 | 0.1 | 0.2 | 0.3 |
| Animal YC104 | 2.4 | 0.9 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *M. longicapita* | | | | | | | | | | |
| Animal YC103 | 7.8 | 4.1 | 1.9 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Animal YC104 | 19.2 | 18.4 | 8.0 | 0.6 | 0.3 | 0.1 | 0 | 0 | 0 | 0 |
| *M. rotundicapita* | | | | | | | | | | |
| Animal YC103 | 12.2 | 2.4 | 1.3 | 0.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Animal YC104 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

foreleg, and hind leg. Before collecting skin snips, each site was washed, shaved, and swabbed with ethanol. The snips were placed individually into vials containing 1 ml of Hanks' balanced salt solution and evaluated as described above. Microfilariae of the 4 species were readily distinguishable from each other in wet 2% formalin preparations at ×40 magnification.

Circadian fluctuations in the concentration of 3 species of microfilariae in skin snips were evaluated by examining snips collected at 2-hr intervals over 24 hr from the backs of 2 capybaras. At each time interval, 10 skin snips were taken and examined as described above. Capybaras submitted to the repeated skin snipping with minimal distress and thus required no anesthesia for this study.

## Results

In preliminary studies it became apparent that skin snips from the heads or ears of infected capybaras contained high concentrations of microfilariae while snips from the abdomens of corresponding animals invariably contained few or no microfilariae. In order to substantiate this observation, 2 capybaras naturally infected with all 4 species were anesthetized and systematically examined. From each animal 100 skin snips were taken; 10 snips from 10 parts of the body (Table 1). Concentrations of *C. tuberocauda, M. rotundicapita,* and *M. longicapita* displayed a similar, distinctive pattern of distribution in the skin. Snips taken from the top of the head, the back, or the ears contained highest concentrations of microfilariae while snips from the venter, thorax, and extremities contained few or no microfilariae. *Yatesia hydrochoerus* concentrations were too low to be evaluated in these animals (low microfilarial counts are typical of this species in our experience). This study was repeated after 6 mo in order to evaluate possible seasonal influences. There was no appreciable difference between the mean counts or the spatial distributions previously observed.

Circadian fluctuations in the concentrations of microfilariae in the superficial layers of the skin were evaluated by examining skin snips from the backs of 2 capybaras with multiple infections at 2-hr intervals over 24 hr. Before considering the variations in microfilarial concentrations over time, it was necessary to determine that these differences were independent of snip weight. The range of weights of the 224 snips was 0.15–5.64 mg. More than 90% of the snips weighed between 0.26 and 2.25 mg. Typical examples of frequency distributions by weight and ratios of mean concentrations found during peak hours to those found in hours of low density, for snips of different weights, are shown in Tables 2 and 3. The ratios of mean microfilarial density in hours of high density to that in hours of low density were fairly constant for *C. tuberocauda* and *M. longicapita* at all snip weights compared in this study. This finding indicated that concentrations of these 2 species of microfilariae found at different hours of the day were substantially independent of the weights of the individual snips taken. Mean microfilarial concentrations from 20 skin snips (10 from each animal) at each interval revealed a low diurnal peak in *C. tuberocauda* microfilarial concentrations (Fig. 1). Mean concentrations began to rise between 0400 and 0600 hours, peaked at 1000 hours, then declined by 1200 hours. Counts remained low but detectable throughout the evening and night. A similar but more striking diurnal peak in *M. longicapita* concentrations was observed (Fig. 2). Mean concentrations began to rise sharply between 0400 and 0600 hours, peaked at 1000 hours then declined by 1400

**Table 2. The ratios of the concentrations of *C. tuberocauda* microfilariae found during hours of peak microfilarial density to the concentrations found in hours of low density for snips of different weights from capybara YC103.**

| Weight of snip (mg) | Frequency of snips | Mean no. of mf/snip | A: Mean no. of mf/snip, peak hours (0600–1000) | B: Mean no. of mf/snip, trough hours (1800–2200) | A/B: Nearest whole number ratio |
|---|---|---|---|---|---|
| <0.26 | 1 | 1.0 | 0 (0)* | 0 (0) | — |
| 0.26–0.75 | 34 | 3.9 | 7.1 (9) | 2.1 (11) | 3:1 |
| 0.76–1.25 | 33 | 3.6 | 6.2 (10) | 1.8 (9) | 3:1 |
| 1.26–1.75 | 17 | 5.7 | 8.8 (4) | 2.6 (5) | 3:1 |
| 1.76–2.25 | 9 | 6.8 | 11.0 (3) | 6.5 (2) | 2:1 |
| 2.26–2.75 | 6 | 2.8 | 0 (0) | 1.0 (1) | — |
| 2.76–3.25 | 1 | 14.0 | 0 (0) | 0 (0) | — |
| >3.25 | 7 | 5.7 | 0 (0) | 0 (0) | — |

* Values in parentheses represent the number of snips included in the sample.

hours. Again counts remained low but detectable in both capybaras until the following morning. There was no recognizable periodic fluctuation in the levels of *M. rotundicapita* microfilariae (data not shown).

## Discussion

The pattern of microfilarial distribution in capybara skin is similar for *C. tuberocauda, M. rotundicapita,* and *M. longicapita.* Microfilariae of these 3 species all concentrated along the dorsal surface of the body. High densities of microfilariae were consistently detected in snips from the top of the head, ears, and back, while snips from the extremities and ventral parts of the body were usually free of microfilariae. Distribution studies have been conducted to evaluate microfilariae of *Onchocerca gutturosa* in cattle (Eichler and Nelson, 1971; Bain et al., 1977), *O. cervicalis* in horses (Mellor, 1973), *O. tarsicola, O. tubingensis, O. flexuosa,* and *Cutifilaria wenki* in deer (Shultz-Key, 1975), and *O. volvulus* in chimpanzees and people (Kershaw et al., 1954; Woodruff et al., 1963; DeLeon and Duke, 1966; Duke and Moore, 1974). In all of these cases where natural vectors are known, a direct correlation has been shown between sites of high microfilarial concentrations in the skin and body areas preferentially attacked by feeding arthropod hosts.

Circadian periodicity of blood-dwelling microfilariae is a well-studied phenomenon (for example, Sasa, 1976). Little attention, however, has been given to periodicity in skin-dwelling species. A diurnal peak in skin densities of *O. volvulus* microfilariae has been observed and confirmed by various workers (Duke et al., 1967; Lartigue, 1967; Duke and Moore, 1974; Anderson et al., 1975). Skin-dwelling species in hosts

**Table 3. The ratios of the concentrations of *M. longicapita* microfilariae found during hours of peak microfilarial density to the concentrations found in hours of low density for snips of different weights from capybara number YC104.**

| Weight of snip (mg) | Frequency of snips | Mean no. of mf/snip | A: Mean no. of mf/snip, peak hours (0800–1200) | B: Mean no. of mf/snip, trough hours (2200–0200) | A/B: Nearest whole number ratio |
|---|---|---|---|---|---|
| <0.26 | 1 | 41.0 | 0 (0)* | 0 (0) | — |
| 0.26–0.75 | 28 | 46.9 | 90.8 (8) | 30.8 (4) | 3:1 |
| 0.76–1.25 | 48 | 31.2 | 50.8 (13) | 14.9 (7) | 3:1 |
| 1.26–1.75 | 24 | 41.7 | 112.8 (5) | 23.8 (11) | 5:1 |
| 1.76–2.25 | 10 | 37.5 | 79.0 (1) | 31.0 (5) | 3:1 |
| 2.26–2.75 | 2 | 257.0 | 502.0 (1) | 0 (0) | — |
| 2.76–3.25 | 1 | 34.0 | 0 (0) | 34.0 (1) | — |

* Values in parentheses represent the number of snips included in the sample.

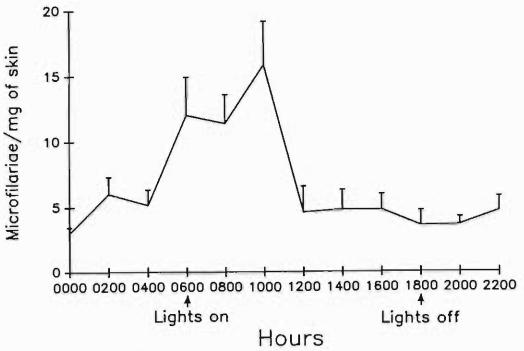


**Figure 1. Mean concentrations (with standard errors) of *Cruorifilaria tuberocauda* microfilariae in skin snips from 2 capybaras over 24 hr. Each point represents the mean of 20 snips, 10 from each animal.**

other than humans apparently have received little attention. In the present work a large number of skin snips were examined at 2-hr intervals over 24 hr from 2 capybaras. This protocol included enough samples to minimize distributional irregularities while still being tolerated by unanesthetized capybaras. Both *C. tuberocauda* and *M. longicapita* exhibited periodic fluctuations in concentrations, with peak levels observed at approximately 1000 hours. Levels of *M. rotundicapita* microfilariae did not appear to fluctuate in a periodic pattern. Future studies of vector requirements for *C. tuberocauda, M. rotundicapita,* and *M. longicapita* may be facilitated if potential hosts are experimentally fed on the body areas with highest levels of microfilariae, during the hours of peak concentrations in the skin. Hematophagous arthropods with peak biting activity on capybaras around 1000 hours

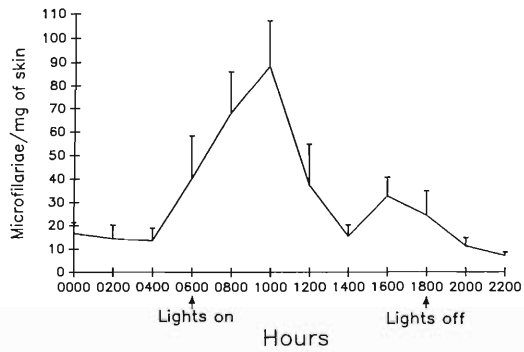


**Figure 2. Mean concentrations (with standard errors) of *Mansonella longicapita* microfilariae in skin snips from 2 capybaras over 24 hr. Each point represents the mean of 20 snips, 10 from each animal.**

would be good candidates for evaluation as natural vectors of *C. tuberocauda* and/or *M. longicapita.* Vector requirements of *Y. hydrochoerus* were previously reported (Yates and Lowrie, 1984).

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# Ultrastructural Study of Attachment of *Gyrodactylus colemanensis* (Monogenea) to Fins of Fry of *Salmo gairdneri*

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ABSTRACT: Attachment of the monogenean *Gyrodactylus colemanensis* to the fin edges of young *Salmo gairdneri* is examined in detail with transmission electron microscopy. Attachment sites become surficial patches of necrotic epidermis that serve as stable, but temporary, platforms. Tissue damage is caused both from pressure exerted by hamulus blades and by penetration of epithelial cells by blades of the 16 marginal hooks. Structural integrity of attachment sites is maintained by aggregations of microfilaments which remain inside necrotic epithelial cells and is enhanced by the inherent ability of both dead and live cells to remain bound together. Smallness of *G. colemanensis* likely allows its attachment to such a fragile site. The observed minor pathologic host response is explained by both the surficial attachment and the parasite's behavior of regularly relocating.

KEY WORDS: Monogenea, *Gyrodactylus colemanensis, Salmo gairdneri,* gyrodactyliasis, ectoparasite, TEM.

*Gyrodactylus colemanensis* Mizelle and Kritsky, 1967, parasitizes fin margins of various North American salmonid fishes. Experimental studies by Cusack (1986) established, in contrast to observed pathogenicity of species such as *Gyrodactylus salmonis* Yin and Sproston, 1948, and *Gyrodactylus salaris* Malmberg, 1957 (see Heggberget and Johnsen, 1982; Cusack and Cone, 1986), that *G. colemanensis* does not adversely affect growth or survival of young fry of *Salmo gairdneri* Richardson.

Differential susceptibility to infection by species or races of the hosts is a probable underlying factor in gyrodactyliasis (Madhavi and Anderson, 1985). However, a scanning electron microscopy study by Cone and Cusack (1988) suggested that apparent lack of gross pathologic response to infections with *G. colemanensis* may be due, in part, to a delicate mode of attachment of the parasite to the host's epithelial surface.

In the present study we investigated the nature of this attachment at the cellular level by use of transmission electron microscopy.

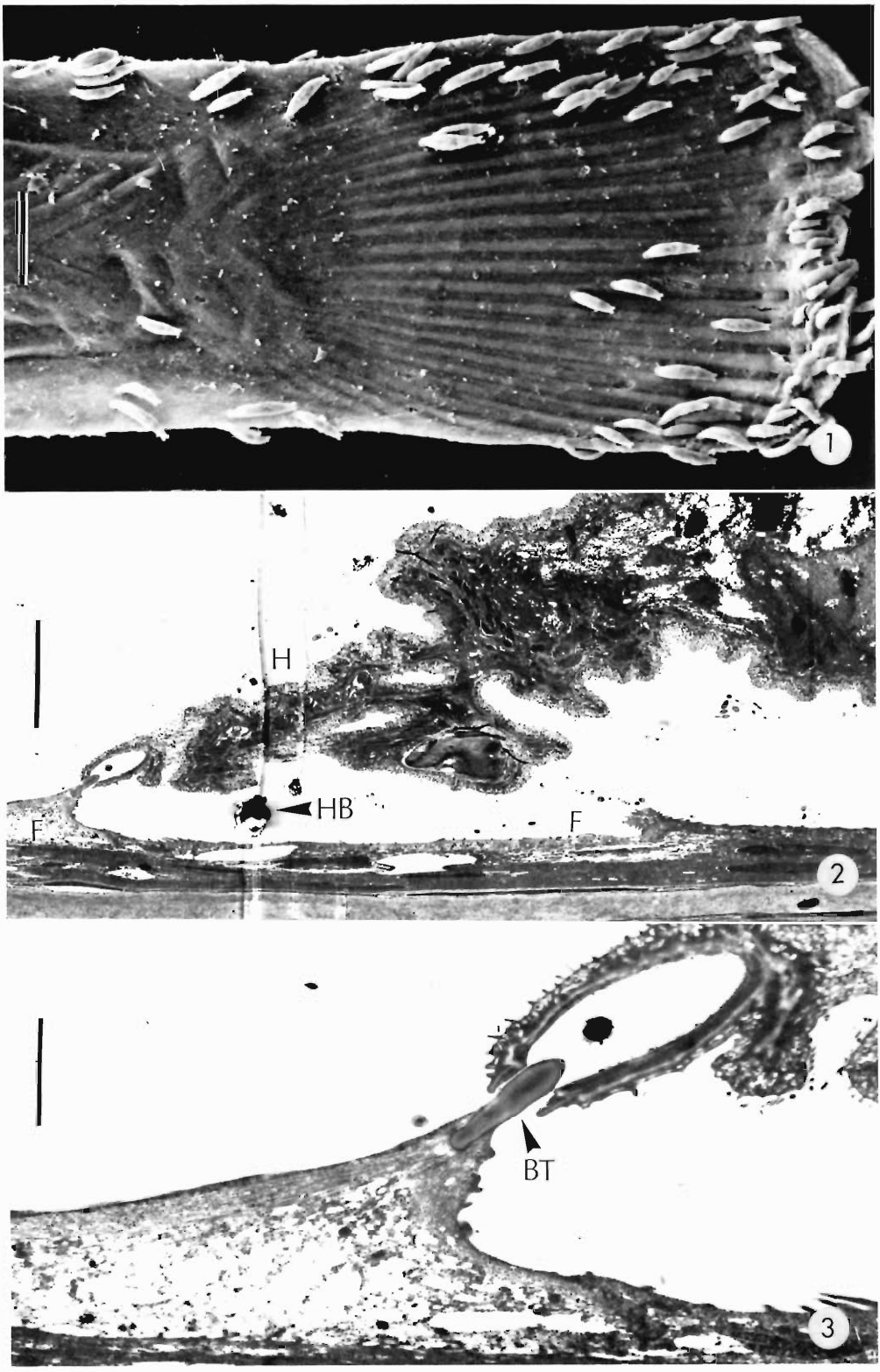
## Materials and Methods

Specimens of *G. colemanensis* were obtained from a laboratory maintained stock of infected rainbow trout, *S. gairdneri.* Two-month-old, nonparasitized fry of *S. gairdneri* (2–3 cm long; 0.2–0.3 g) were obtained in February 1985 from Fraser's Mills Fish Hatchery, Antigonish, Nova Scotia, and experimentally infected with 1–12 *G. colemanensis* using a bath exposure technique (Cusack, 1986). The fry were held in dechlorinated tap water at 15°C and fed ad libitum. On the tenth day postinfection, 11 small pieces of fin with attached parasites were fixed for 2 hr in ice-cold Karnovsky's solution. Fixed tissues were rinsed thoroughly in phosphate buffer at a pH of 7.2 and postfixed for 1 hr in 1% osmium tetroxide in the same buffer. After a second buffer rinse the specimens were embedded in eponaraldite. Thin sections were stained with uranyl acetate and examined by means of a Philips 400 electron microscope. Pieces of fins from three noninfected fry were processed as controls. Specimens obtained from our stock laboratory culture were prepared as described by Cone and Odense (1984) and examined by use of scanning electron microscopy.

## Results

The skin surface covering fins of fry of *S. gairdneri* consists of a squamous epithelium 3–4 cells thick. *Gyrodactylus colemanensis* attaches to this surface with its forebody trailing downstream in relation to water passing over the host's body (Fig. 1). The haptor's ventral face is applied to the fin surface and the blades of the pair of large, centrally-located hamuli lie parallel to the fin surface. The hamuli apparently do not penetrate the underlying host tissue. However, host cells beneath the haptor are necrotic and distinctly vacuolated (Fig. 2). The blade tips of the 16 peripherally-located marginal hooks each pierce an epithelial cell, whose plasma membrane is not readily identifiable, and pull them into distorted conical shapes (Fig. 2). The penetrating marginal hook blade is anchored to an aggregation of microfilaments near the edge of the host cell (Fig. 3). The damaged epithelial cells seem to be firmly bound both to each other and to adjacent live cells. However, no cell junctions were observed. The maximum diameter of the area containing affected host cells is about 75 μm. No inflammatory host response was evident either at the attachment sites or elsewhere on the fins. There was no ulceration.

Study of live parasites on stock fish revealed that when the parasite is not moving in an inch-



worm fashion across the body surface to occupy another site, it is sedentary during periods of 1–12 hr. Fin epithelium is presumably taken in as food during these sedentary times. The secure adherence to the epithelial surface and the extreme flexibility of the forebody of the monogenean combine to permit grazing by the parasite over an extended arc centered at the actual attachment site.

## Discussion

Intense infections of *G. colemanensis* produce no obvious clinical signs of disease in fry of *S. gairdneri* (Cusack, 1986). Clearly, the delicate and surficial nature of the attachment explains the lack of a gross pathological response by the infected fish. What tissue damage there is arises both from microscopic punctures in epithelial cells caused by the marginal hooks and by pressure exerted on adjacent underlying tissue by the hamulus blades. We saw no larger ruptures in the epithelium of infected fish that might allow tissue invasion by aquatic bacteria or fungi. Lying beneath the small patches of necrotic tissue there were normal epithelial cells that are readily available for surface repairs after the dead cells have been sloughed off.

During its attachment at a necrotic site, the haptor assumes the general shape of a tent. The marginal hooks are in the positions of securing pegs and the hamuli are situated centrally as though they were support poles. Such a configuration of the haptor would constitute a sufficiently mechanically strong platform for attachment for a monogenean that is as small as *G. colemanensis*. However, it would probably not be strong enough to hold in place large skin-dwelling monogeneans. Shear forces would likely cause tearing of the epithelium and the expected consequence of this is dislodgment. Large species of monogeneans use various attachment devices, including penetrating sclerites, water pressure actuated disks, and sticky glandular secretions (Kearn, 1976; Rand and Wiles, 1987).

With *G. colemanensis* on fins of fry of *S. gairdneri,* several intrinsic factors do augment host tissue stability at attachment sites to the extent of maintaining a continuous epidermal barrier. Firstly, necrotic epithelial cells remain bound both to one another and to neighboring living cells. Secondly, an aggregation of microfilaments in a punctured dead cell provides a solid anchoring site for the penetrating tip of a marginal hook. They likely also serve as a mechanical strengthener for the damaged cell itself which would prevent cell tearing. The assumption of taut conical shapes by punctured cells probably serves to stabilize attachment through a reduction of the elasticity of the outer surface of the cells. Stretching of epidermal cells at attachment sites has been reported in other species of *Gyrodactylus* and in *Fundulotrema* (Cone and Odense, 1984, 1988).

According to information given by Lester (1972), *Gyrodactylus alexanderi* appears to attach to the skin surface of *Gasterosteus aculeatus* in a manner similar to that of *G. colemanensis.* However, *G. alexanderi* must contend with increased production by host epidermal cells of cuticle, a glycocalyx-like material inimical to it (Whitear, 1970, 1986). There was no evidence of a similar host mechanism for removal of skin parasites in the present study.

Frequent relocating of the parasites on the body surface further explains the absence of a gross pathological response by the fish to *G. colemanensis.* It is unknown whether this species relocates when it has depleted its food resources within its feeding arc or because its rather fragile site of attachment eventually becomes damaged and thus unstable. In either case, short-term stays followed by movement away from attachment sites would not result in formation of distinct wounds, which in turn would explain the absence

←

**Figures 1–3. 1. Caudal fin of a stock fry of *Salmo gairdneri* infected with *Gyrodactylus colemanensis.* Most worms are positioned near the edge of the fin and all have the forebody directed posteriorly with respect to the path of water currents passing along the length of the host's body. Scale bar is 1 mm. 2. Transmission electron photomicrograph of the haptor (H) of *Gyrodactylus colemanensis* attached to the caudal fin (F) of fry of *Salmo gairdneri.* The 2 parallel knife marks were invariably present in the sections because of the failure of the hamulus blades (HB) to become infiltrated with embedding resin. Host tissue lying beneath the haptor is compressed and necrotic. Scale bar is 10 μm. 3. Enlarged portion of Figure 2 showing the penetration of an epithelial cell by the blade tip of a marginal hook (BT). Note that the tip is rooted in an aggregation of microfilaments near the cell's edge. Scale bar is 2.5 μm.**

of inflammation. Such behavior would ensure that there would be minimal damage as a result of grazing over a surface that represents both a food source and a substrate for attachment. It would be particularly appropriate when intensity of infection is high.

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# Activity of Fenbendazole and Oxfendazole Against Experimental Infections of *Trichostrongylus axei* in Dairy Calves

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ABSTRACT: *Trichostrongylus axei* larvae ($L_3$), cultured from a bovine isolate, maintained in rabbits for over 25 yr, were administered to 14 male Jersey calves raised worm free. Patency occurred in all the calves. Three controlled tests were undertaken to determine anthelmintic activity of 2 compounds, fenbendazole (FBZ) and oxfendazole (OFZ), administered once as paste formulations, against adult *T. axei* in the calves. Test A included 3 calves treated with FBZ (5 mg/kg) and 3 nontreated calves. The other 2 tests (B and C) were comprised of calves treated with OFZ (2.5 mg/kg); in each of these 2 tests, 2 calves were treated and 2 were nontreated. Removals of *T. axei* were 100% for fenbendazole and >99% for oxfendazole.

KEY WORDS: Nematoda, Trichostrongyloidea, *Trichostrongylus axei,* experimental infections, dairy calves, anthelmintics, fenbendazole, oxfendazole, infectivity, >25 yr passage, rabbits.

A recent publication by the present authors showed that *Trichostrongylus axei* (equid and bovid strains) were still infective to dairy calves ($N = 19$) after maintenance in rabbits for over 25 yr (Lyons et al., 1987). Data in the present paper are on 1 of these strains (bovid) of *T. axei* administered to 14 additional dairy calves to determine infectivity and also to evaluate anthelmintic activity of fenbendazole and oxfendazole. Both of these compounds are reported to have excellent activity against *T. axei* in cattle (Baker et al., 1978; Benz and Ernst, 1978; Chalmers, 1978; Craig and Bell, 1978).

## Materials and Methods

A bovid strain (O) of *T. axei,* maintained in rabbits for 26 years, was administered to 14 dairy calves which were separated into 3 controlled tests (A, B, and C).

The calves were all male Jerseys, ranging in age (at the beginning of the tests) from about 4 to 10 months. They were raised worm free. *Trichostrongylus axei* infective larvae ($L_3$) were administered with a dose syringe fitted with a Whitlock nozzle. The number of larvae ($L_3$) administered was 19,000 to test A calves ($N = 6$) on 15 August 1986, 7,440 to test B calves ($N = 4$) on 8 October 1986, and 12,800 to test C calves ($N = 4$) on 20 March 1987.

Anthelmintics used in the 3 controlled tests were paste formulations of fenbendazole (Hoechst-Roussel) at the dose rate of 5 mg/kg of body weight and oxfendazole (Syntex) at the dose rate of 2.5 mg/kg. Individual doses were weighed into separate plastic syringes and administered intraorally. Test A calves included 3 treated with fenbendazole 35 days after administration of larvae and 3 were nontreated. Oxfendazole was administered to 2 calves in test B and to 2 calves in test C at 100 days and 40 days, respectively, after larvae were given. There were 2 nontreated calves in both tests B and C. At either 7 days (tests A and B) or 9 days (test C) posttreatment, the calves were killed and their abomasums examined for *T. axei.*

*Trichostrongylus axei* eggs per gram of feces (EPG) were determined on the day of treatment and at necropsy. Specific methods have been published for culturing and administering larvae (Leland et al., 1959), determining EPG (Drudge et al., 1963; Lyons et al., 1976), and recovering *T. axei* from the abomasum (Leland and Drudge, 1957).

## Results and Discussion

Pre- and posttreatment EPG and number of worm specimens recovered at necropsy are summarized (Table 1).

Removals of *T. axei* were 100% for fenbendazole and >99% for oxfendazole. Excellent removal of *T. axei* by these 2 benzimidazoles was similar to other reports (Baker et al., 1978; Benz and Ernst, 1978; Chalmers, 1978; Craig and Bell, 1978).

All 14 test calves had positive EPG at the time of treatment and there was a range of 3,060 to 5,510 specimens of *T. axei* in the nontreated calves at necropsy. These factors re-verified the continued infectivity of this bovid strain after passage in rabbits for over 25 yr (Lyons et al., 1987).

## Acknowledgments

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**Table 1. Data on *Trichostrongylus axei* egg counts per gram of feces (EPG) and specimens recovered at necropsy of dairy calves in 3 controlled tests of activity of paste formulations of fenbendazole and oxfendazole.**

| Calf no. | *Trichostrongylus axei** Eggs per gram of feces (EPG) Pretreatment | Posttreatment | No. of specimens recovered at necropsy |
|---|---|---|---|
| | Test A—Fenbendazole at 5 mg/kg | | |
| Treated | | | |
| 271 | 50 | 0 | 0 |
| 272 | 20 | 0 | 0 |
| 278 | 10 | 0 | 0 |
| Mean | 27 | 0 (100%)† | 0 (100%)‡ |
| Nontreated | | | |
| 274 | 10 | 10 | 5,510 |
| 280 | 210 | 40 | 5,160 |
| 281 | 110 | 120 | 5,000 |
| Mean | 110 | 57 (52%) | 5,223 |
| | Test B—Oxfendazole at 2.5 mg/kg | | |
| Treated | | | |
| 286 | 10 | 0 | 20 |
| 289 | 10 | 0 | 0 |
| Mean | 10 | 0 (100%) | 10 (>99%) |
| Nontreated | | | |
| 287 | 20 | 20 | 3,720 |
| 292 | 10 | 20 | 3,060 |
| Mean | 15 | 20 (0%) | 3,390 |
| | Test C—Oxfendazole at 2.5 mg/kg | | |
| Treated | | | |
| 310 | 70 | 0 | 20 |
| 311 | 20 | 0 | 0 |
| Mean | 45 | 0 (100%) | 10 (>99%) |
| Nontreated | | | |
| 301 | 20 | 10 | 3,060 |
| 313 | 10 | 40 | 3,780 |
| Mean | 15 | 25 (0%) | 3,420 |

* The number of $L_3$ administered was 19,000, 7,440, and 12,800 to calves in tests A, B, and C, respectively.

† % reduction.

‡ % removal.

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# Comparative Studies on Two Similar Species of *Haplorchis* and *Metagonimus* (Trematoda: Heterophyidae)— Surface Ultrastructure of Adults and Eggs

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ABSTRACT: The surface ultrastructure of adults and eggs of *Haplorchis pumilio* and *H. taichui,* and of *Metagonimus yokogawai* and *M. takahashii,* are each compared by scanning electron microscopy. The adults of these species bear scalelike spines with a posterior serration. It is difficult to distinguish the similar species by their surface ultrastructure, such as the shape of the tegumental spines, the number and localization of sensory papillae, and the shape of the opening of the ventrogenital complex. The eggs of *H. pumilio* which bear surface granular tubercles are different from those of *H. taichui* which are characterized by slender coiled projections. The eggs of both *Metagonimus* species are marked by granular tubercles on the surface, and they appear difficult to separate from each other.

KEY WORDS: *Haplorchis pumilio, H. taichui, Metagonimus yokogawai, M. takahashii,* Heterophyidae, ultrastructure, scanning electron microscopy, adult worms, eggs, heterophyidiasis.

Adult trematodes in the family Heterophyidae are small intestinal parasites of birds and mammals including man. Besides species in the genus *Heterophyes* producing heterophyiasis, members of this family include other medically important species in the genera *Haplorchis* and *Metagonimus.* Two species of *Haplorchis, H. pumilio* (Looss, 1896) and *H. taichui* (Nishigori, 1924), whose life cycles and morphologies were described by Faust and Nishigori (1926) and Pearson (1964), have similar morphological features. Two species of *Metagonimus, M. yokogawai* (Katsurada, 1912) and *M. takahashii* Suzuki, 1930, are also similar to each other and remained undefined specifically, although Takahashi (1929) and Ochi (1957) showed some morphological and biological differences between them. Recent studies showed that they are separate species (Takahashi, 1967; Saito, 1972, 1973; Saito and Tsuji, 1973).

Recent investigations using scanning electron microscopy (SEM) have revealed the surface ultrastructure of several trematodes (Fujino et al., 1979) and eggs (Ishii and Miyazaki, 1971). With respect to the species of *Haplorchis* and *Metagonimus* there have so far been no reports of SEM work except for the comparative study of cercariae of *M. yokogawai* and *M. takahashii* (Fujino et al., 1976) and observations on eggs of *M. yokogawai* (Ishii and Miyazaki, 1971; Ishii, 1972).

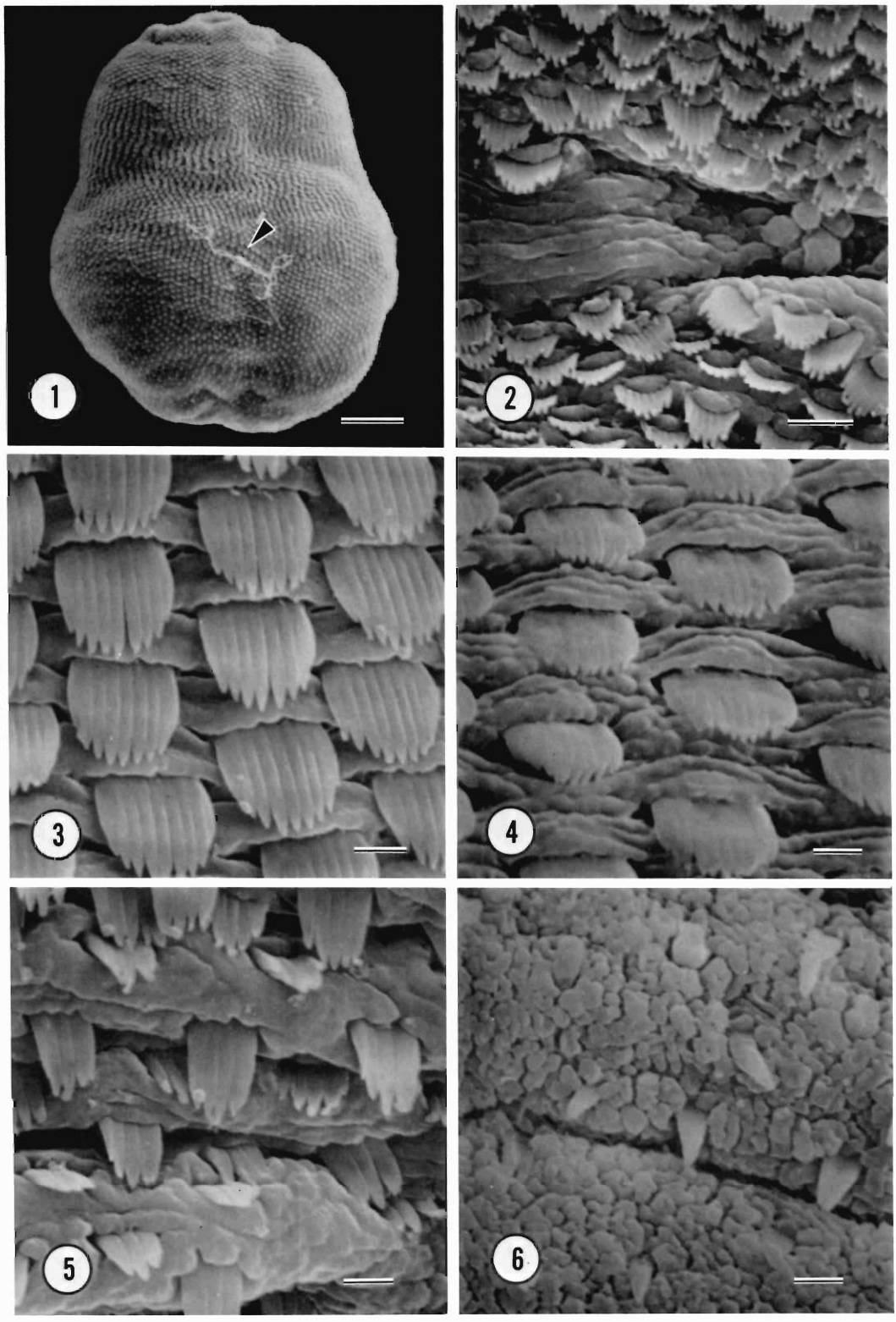
The present investigation was undertaken to compare using SEM the surface ultrastructure of the adult worms and eggs of 2 similar species of each of the genera *Haplorchis* and *Metagonimus*; *H. pumilio* and *H. taichui,* and *M. yokogawai* and *M. takahashii.*

## Materials and Methods

The metacercariae of *Haplorchis pumilio* were removed from the natural freshwater fish host, *Hemiculter leucisculus,* at Chenching Lake, Kaohsiung County, southern Taiwan, and the adults were recovered from a golden hamster 7 days after oral inoculation. The metacercariae of *H. taichui,* taken from unidentified freshwater fish in Suimen Village, Santi, Pingtung County, southern Taiwan, were inoculated into a golden hamster and the adults were recovered 14 days later. The metacercariae of *Metagonimus yokogawai,* obtained from the muscle of the freshwater fish, *Plecoglossus altivelis,* in the Ota River, Hiroshima City, central Japan, were inoculated into a golden hamster which yielded adults 14 days later. *Metagonimus takahashii* adults were taken from a golden hamster 10 days after inoculation of the metacercariae which were removed from the freshwater fish, *Carassius carassius,* taken at the Toyano Lake, Niigata City, northern Japan.

For SEM preparation adult worms and eggs which were naturally shed were rinsed well with Ringer's solution. The specimens were then fixed with phosphate buffered (pH 7.3) 4% glutaraldehyde for 2–4 hr, followed by 2 hr postfixation in 1% osmium tetroxide. They were dehydrated with an ethanol series, and then critical-point dried. After coating with gold, the specimens were examined under a JEOL-U3 scanning electron microscope at 15 kV. Ten specimens each were examined, measured, and their averages were taken.

For the examination of eggs by transmission electron microscopy (TEM), part of the uterus was dissected



and fixed for 3 hr in Karnovsky's (1965) fixative. After a wash in cacodylate buffer the material was osmicated, dehydrated, and embedded in Spurr Resin or Quetol 812 (Nisshin EM, Tokyo). Cut sections were viewed in a Hitachi HS-9 electron microscope at 75 kV.

## Results

### Adult worms

*Haplorchis pumilio* resembled *H. taichui* in the surface ultrastructure of the body. The body was oblong or slightly elongate, and encircled by rows of flat, oval, or scalelike spines with a serrated posterior edge (Figs. 1, 3–6). The spines measured approximately 2.0 μm long by 2.3 μm in *H. pumilio* (Figs. 3, 5, 6), and 2.0 μm long by 2.5 μm in *H. taichui* (Fig. 4). The surface of the spines was almost flat with 6–8 shallow furrows extending from near the basal part of the spine to the distal edge. The spines near the middle of the body were larger than the anterior and posterior ones. The posterior spines were short and narrow and simple or double- or triple-pointed (Figs. 5, 6). There were no spines around the excretory pore. No marked difference was found in shape and size between the dorsal and ventral spines. There were many sensory papillae, each with a short cilium, on the lip of and around the oral sucker. Two, 3, or 4 papillae often occurred close together. Several pairs of papillae were arranged bilaterally dorsally and ventrally. The opening of the ventrogenital complex, which was devoid of spines, appeared as a round or slitlike depression of the tegument in about the anterior third of the body (Fig. 2).

*Metagonimus yokogawai* and *M. takahashii* were similar to each other in the surface fine structure of the body. The body was flat and oval or pyriform, and was covered with flat, oblong or oval spines with a serrated posterior end (Figs. 7–12). The spines measured approximately 1.5 μm long by 1.7 μm in *M. yokogawai* and 1.5 μm long by 1.5 μm in *M. takahashii* (Figs. 11, 12). The posterior serration of the spines was more bluntly pointed in *M. takahashii* than in *M. yokogawai*. The spines were smaller and narrower and simple or double-pointed posteriorly, and no spines were present around the excretory pore. The ventrogenital complex occurred in the anterior third of the body (Fig. 7), and the opening appeared as a round swelling directed anteriorly (Fig. 8). Small knobbed protuberances and sensory papillae were scattered on or around the lip of the oral sucker (Fig. 9). Sensory papillae were arranged bilaterally in the anterior half of the body dorsally and ventrally. Each papilla had a short cilium.

### Eggs

The eggs of *Haplorchis pumilio,* measuring 22–23 μm long by approximately 10 μm, tapered toward the abopercular end which had a tubercular nodule. The operculum was prominent. The surface of the eggshell including the operculum was coarse with minute tubercles measuring 0.2–0.3 μm by 0.1 μm (Fig. 13). There were longitudinal irregular ridges of various lengths whose surfaces were also tuberculate. TEM showed irregular minute tubercles on the egg surface with occasionally raised extensions or projections whose surface was tuberculate (Fig. 14). The basal part of the operculum and the shell edge bordering the operculum were raised to form low "shoulders."

The eggs of *H. taichui,* measuring approximately 18 μm by 8 μm, were smaller than those of *H. pumilio* and the abopercular end was broader than in *H. pumilio.* The egg surface was marked by irregularly coiled ropelike ridges, each of which was approximately 0.1 μm wide (Fig. 15). The edge of the eggshell bordering the operculum and the basal part of the operculum were slightly raised, but not forming "shoulders." The narrow area along the eggshell edge was devoid of ridges. TEM showed the presence of irregularly curved slender projections covered with a filamentous coat (Fig. 16).

The eggs of *Metagonimus yokogawai,* mea-

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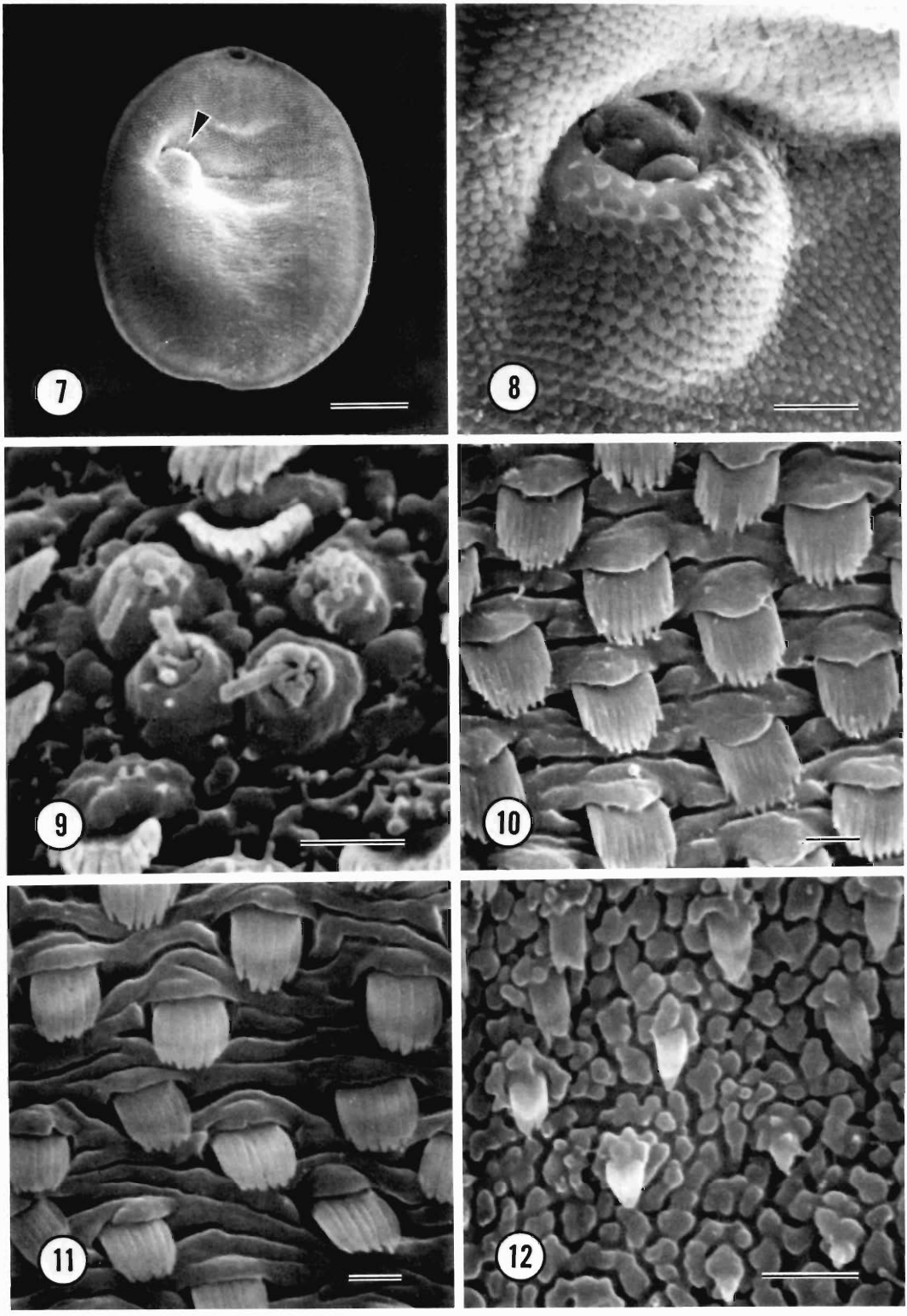
**Figures 1–6. 1. *Haplorchis pumilio.* Dorsal aspect of the body. The body is flattened, elongate, and spinous almost all over the surface. Spermatozoa from Laurer's canal opening are seen (arrowhead). Bar = 20 μm. 2. *H. pumilio.* The opening of the ventrogenital complex. No spines are present around the opening. Bar = 2.0 μm. 3. *H. pumilio.* Spines of middorsal area. The spines are scalelike with longitudinal furrows and a serrated posterior edge. Bar = 1.0 μm. 4. *H. taichui.* Spines of middorsal area. The spines are similar to those of *H. pumilio.* Bar = 1.0 μm. 5. *H. pumilio.* Spines of posteroventral area. The spines are narrower than those of middorsal area. Bar = 1.0 μm. 6. *H. taichui.* Spines of posteroventral area. The spines are simple or double-pointed. The tegumental surface is granular. Bar = 1.0 μm.**



suring about 20 μm by 12–13 μm, were ellipsoidal with a broad abopercular end which had a small knoblike projection occasionally. The egg surface was coarse with small granular tubercles measuring 0.2 μm by 0.1 μm (Fig. 17). The operculum was prominent. The basal part of the operculum and the shell edge were raised to form narrow ridges. The eggs of *M. takahashii* resembled those of *M. yokogawai,* being slightly longer and wider than *M. yokogawai.* The surface features of *M. takahashii* were very close to those of *M. yokogawai* except that the granular tubercles appeared more conspicuous than those of *M. yokogawai* (Fig. 18).

## Discussion

The adults of 2 species of *Haplorchis* and of *Metagonimus* are revealed to be very similar to each other under SEM observations. All of the species examined bear scalelike tegumental spines with a serrated posterior edge. Similar spines are reported in the heterophyid *Cryptocotyle lingua* by Køie (1977). It seems possible that species of this family commonly bear this type of spine. Heterophyid worms are known to inhabit the intestines of birds and mammals (Faust and Nishigori, 1926). These serrated spines on the body surface probably help the abrasion of host tissue for feeding and also function in attachment to the host or for easy movement among the intestinal villi (Køie, 1977).

Although it is hard to distinguish *M. pumilio* from *H. taichui* by the shape of the tegumental spines, the shape and localization of the sensory papillae, and the opening of the ventrogenital complex, eggs of the 2 species differ markedly.

The *Metagonimus* species, *M. yokogawai* and *M. takahashii,* are also very similar in the surface ultrastructures of the adults except for a slight difference in the serration of the tegumental spines. Unlike the eggs of the *Haplorchis* species, the eggs of the *Metagonimus* species are so similar that it seems difficult to distinguish them from each other. Saito (1972) compared both of the species in their developmental stages, cercaria, metacercaria, and adult, including eggs, under the light microscope. He found differences in the number of oral spines and the shape of the penetration gland cells of the cercaria, the body color of the metacercaria, and the body size of all the stages. Fujino et al. (1976) confirmed with SEM observations of the cercariae that the number and size of the oral spines are the most distinguishing characteristics and that other features are too similar to use in separating the species from each other.

Africa et al. (1935, 1936a, b, 1937) reported that some heterophyids including *H. taichui* and *H. pumilio* were recovered from human intestines and that their eggs caused cardiac, cerebral, or spinal heterophyiasis. In these cases, the eggs have been found associated with lesions in the organs. Africa et al. (1935, 1936a) mentioned that heterophyid eggs plugged capillaries and other vessels and caused rupture of these vessels. It seems possible that the eggs of these species, which are comparatively small, are easily carried by the blood stream from the intestine to the above-mentioned regions. The egg surfaces of *H. taichui* and *H. pumilio,* as observed in SEM, are coarse with surface granular tubercles and slender coiled projections, respectively. These structures of the eggs may be responsible for the embolism of eggs in the capillaries of various organs.

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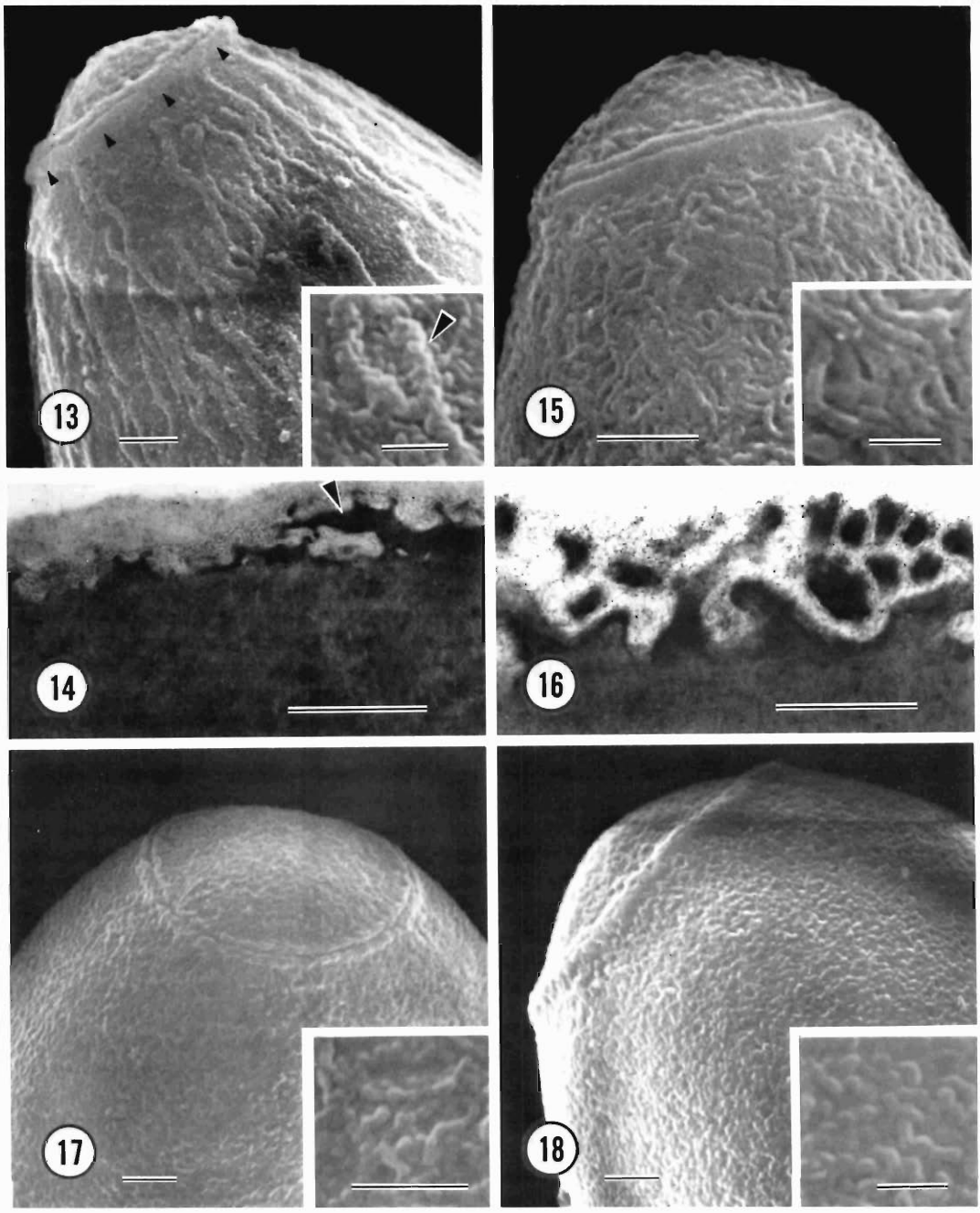
**Figures 7–12. 7. *Metagonimus yokogawai.* Ventral aspect of the body. The body is flattened, elongate, oval or pyriform, and spinous. The ventrogenital complex is marked (arrowhead). Bar = 50 μm. 8. *M. yokogawai.* Enlarged view of the opening of the ventrogenital complex. The swollen wall of the complex is covered with spines and the opening is directed anteriorly. Bar = 10 μm. 9. *M. yokogawai.* Grouped ciliated papillae near the oral sucker. Bar = 1.0 μm. 10. *M. yokogawai.* Spines of middorsal area. The scalelike spines have a finely serrated posterior edge. Bar = 1.0 μm. 11. *M. takahashii.* Spines of middorsal area. The posterior edge is serrated bluntly. Bar = 1.0 μm. 12. *M. takahashii.* Spines of posteroventral area. The spines are simple or double-pointed. Bar = 1.0 μm.**




**Figures 13–18.** 13. ***Haplorchis pumilio.*** **The operculum and the distal part of the eggshell. Note "shoulders" of the basal part of the operculum and eggshell edge (small arrowheads). The eggshell is marked by minute tubercles all over the surface. Longitudinally running irregular ridges are characteristic. Bar = 1.0 μm. Inset: Enlarged view of the shell surface. An arrowhead indicates a tubercular ridge. Bar = 0.5 μm. 14. *H. pumilio.* TEM of part of the eggshell showing irregular minute tubercular projections and longer extensions (arrowhead) on the surface. Bar = 0.5 μm. 15. *H. taichui.* The operculum and part of the eggshell. The surface is characterized by irregularly coiled ridges. "Shoulders" are inconspicuous. Bar = 1.0 μm. Inset: Enlarged view of the eggshell surface. Bar = 0.5 μm. 16. *H. taichui.* TEM of part of the eggshell showing irregularly curved projections with a filamentous cover. Bar = 0.5 μm. 17. *Metagonimus yokogawai.* The operculum and part of the eggshell. The**

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**surface is coarse with round or oval granules. Bar = 1.0 μm. Inset: Enlarged view of the eggshell. Bar = 0.5 μm. 18. *M. takahashii.* The operculum and part of the eggshell. The surface is coarse with round or oval granules. The opercular base and the edge of the eggshell are slightly raised. Bar = 1.0 μm. Inset: Enlarged view of the eggshell. Bar = 0.5 μm.**

Proc. Helminthol. Soc. Wash.
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# *Sebekia mississippiensis* (Pentastomida) from Juvenile American Alligators in North Central Florida

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ABSTRACT: The pentastome *Sebekia mississippiensis* was found in the lungs of 16 of 60 (27%) juvenile American alligators (*Alligator mississippiensis*) from Orange Lake, Alachua County, Florida. Hatchlings (<40 cm in total length) were not infected. Prevalences (and mean intensities) for alligators 41–60 cm in length and 61–80 cm in length were 20% (1.5) and 60% (2.4), respectively. It was concluded from these data that sebekiosis in wild alligator hatchlings such as reported previously for captive animals is probably rare in Florida.

KEY WORDS: pentastomes, *Sebekia mississippiensis*, survey, prevalence, intensity, American alligators, *Alligator mississippiensis*, Florida.

The American alligator frequently serves as definitive host of a pentastome, *Sebekia mississippiensis* (Cherry and Ager, 1982; Boyce, 1985; Overstreet et al., 1985). Adults of this parasite are found in the respiratory system of alligators and produce eggs which are passed in the hosts' feces (Deakins, 1971). Developmental stages have been found in various tissues of crocodilians, snakes, turtles, fishes, and mammals (Boyce, 1985).

Sebekiosis has been described as a clinical disease in captive alligator hatchlings (Boyce et al., 1984) manifesting as weight loss, anorexia, respiratory distress, and death. At necropsy pentastomes were found in the lungs at a mean intensity of 7.0 (SD = 2.3). Lesions in respiratory and hepatic tissues have been noted also from wild alligators (Shotts et al., 1972; Hazen et al., 1978). Previous surveys for *Sebekia* in northern Florida conducted by Boyce (1985) examined subadult (1.2–1.8 m, total length [TL]) and adult (>1.8 m) alligators. His sample did not include alligators <1.2 m. In order to assess the impact of sebekiosis as a disease in wild populations of alligators, we present data on prevalence and intensity of *Sebekia* in juvenile alligators (<1.2 m TL) from a lake in northern Florida.

## Materials and Methods

Sixty alligators, ranging in TL from 28.6 to 88 cm (0.5 to 1.6 kg), were collected during September 1986 from Orange Lake, Alachua County, Florida. Alligators were separated into 3 size categories of 20 animals each. These included individuals <40 cm (hatchlings), 41–60 cm (approximately 1 yr old), and 61–80 cm TL (more than 1 yr of age). Carcasses were frozen and livers and lungs subsequently examined after thawing. Techniques for recovery of parasites were those described by Forrester et al. (1974). Pentastomes were fixed in Roudabush's AFA solution, cleared, and mounted in lactophenol. Examination and measurements were completed with a light microscope and a micro-projector. Representative specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland) as USNM Helm. Coll. No. 80287. Terms such as prevalence, intensity, and abundance are used as defined by Margolis et al. (1982). Confidence intervals were determined for data on prevalence assuming a binomial distribution (Snedecor and Cochran, 1967) and for intensity assuming a Poisson distribution (Hogg and Craig, 1970).

## Results and Discussion

Prevalences and intensities are given in Table 1. Alligators <40 cm TL were free of pentastomes. For other size categories, the abundance of *Sebekia mississippiensis* increased linearly with TL of the alligators. This trend was similar to that reported by Boyce (1985) who examined larger alligators from the same lake in 1983 and found a prevalence of 90% and a mean intensity of 14.

All pentastomes recovered in this study were obtained from lungs; none was found in hepatic tissues. All except 2 specimens were adults. One alligator, a 59-cm male, was infected with 2 nymphs. Delany and Moore (unpubl. data) found that the non-insect portion of juvenile alligator diets from Orange Lake increased with the total length of the alligator. The mosquitofish (*Gambusia affinis*), an intermediate host of *S. mississippiensis* in Florida (Boyce et al., 1984), was present in the stomachs of 4 of the 60 alligators examined in the present study (Delany and Moore, unpubl. data). It is likely that the increase

**Table 1. Prevalence and intensity of the pentastome (*Sebekia mississippiensis*) in the lungs of American alligators from Orange Lake, Florida (September 1986).**

| Total length of alligators (cm) | Prevalence | | | Intensity* | | |
|---|---|---|---|---|---|---|
| | Number examined | Number positive (%) | Confidence intervals | Mean | Range | Confidence intervals |
| <40 | 20 | 0 (0) | 0–15 | 0 | — | — |
| 41–60 | 20 | 4 (20) | 7–42 | 1.5 | 1–2 | 1.0–1.3 |
| 61–80 | 20 | 12 (60) | 41–86 | 2.4 | 1–6 | 1.4–2.5 |

* Number pentastomes per infected alligator.

in prevalence and intensity of infection with TL is related to the food habits of the alligators.

Infection with *S. mississippiensis* without manifestation of disease (sebekiosis) is probably the most frequent scenerio in free-ranging juvenile alligators under normal conditions. A gradual accumulation of numbers of this particular parasite over time most likely allows a degree of resistance to the effects of these infections to develop as the alligators mature. The infections in captive hatchlings described by Boyce et al. (1984) may have been abnormal and a result of the ingestion of unusually high numbers of pentastome larvae. Mosquitofish were fed to these alligators in large numbers and at a higher frequency than would be normal for wild hatchlings (Delany and Moore, unpubl. data). Sebekiosis, therefore, in wild hatchlings is probably rare.

## Acknowledgments

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# Diagnostic Morphometry: Identification of Helminth Eggs by Discriminant Analysis of Morphometric Data

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ABSTRACT: The perimeters of strongylid eggs were digitized electronically, the resulting coordinate point data converted into 8 geometric parameters, and these parameters subjected to stepwise discriminant analysis. This procedure distinguished 92% of *Ancylostoma caninum* eggs from those of *Uncinaria stenocephala* and 100% of *U. stenocephala* eggs from those of *A. caninum*. When applied to strongylid species infecting sheep, eggs of the following species were correctly classified as follows: *Bunostomum trigonocephalum* (60%), *Chabertia ovina* (58%), *Cooperia curticei* (60%), *Haemonchus contortus* (67%), *Nematodirus battus* (98%), *N. filicollis* (100%), *N. spathiger* (96%), *Oesophagostomum columbianum* (72%), *Oe. venulosum* (100%), *Teladorsagia circumcincta* (49.5%), *Trichostrongylus axei* (76%), and *T. colubriformis* (73%). Comparable accuracy of classification was achieved for equine parasites only after pairs of species having morphometrically identical eggs were pooled before analysis and all of the cyathostomin categories except *Gyalocephalus capitatus* further pooled after analysis. The final diagnostic categories, "large strongyle," *Triodontophorus serratus, T. tenuicollis,* "small strongyle," *G. capitatus,* and *Trichostrongylus axei,* were correctly classified in 76.6, 74.3, 83.0, 79.1, 100, and 80.0% of the cases, respectively. *Haemonchus contortus* and *T. colubriformis* eggs recovered from fresh feces were significantly wider than eggs dissected from the uteri of female worms collected from the alimentary tracts of the same sheep. Thorough mixing of sheep feces and 10% formalin (1 g/30 ml) yielded fixed *H. contortus* and *T. colubriformis* eggs that were morphometrically indistinguishable from fresh ones.

KEY WORDS: diagnostic morphometry, quantitative microscopy, digitizing tablet, discriminant analysis, coprology, fecal examination, helminth eggs, hookworm eggs, strongylid eggs, Strongyloidea, Ancylostomatoidea, horse, sheep, dog.

Antemortem diagnosis of helminth infections is based primarily on identification of eggs in fecal preparations. In many cases, specific or generic diagnosis can be reached by an experienced diagnostician on the basis of qualitative characteristics alone. When qualitative differences fail, micrometry may provide sufficient additional information to allow differentiation. Unfortunately, in many very important cases, the dimensions are not sufficiently distinctive to permit differentiation by comparison with tabled values of ranges even if simple statistical analysis is brought to bear on the problem. Under such circumstances, micrometry falls short of providing sufficient information to yield a positive diagnosis.

The eggs of the important nematode superfamilies Strongyloidea, Trichostrongyloidea, and Ancylostomatoidea are thin-walled, ovoid or ellipsoid in shape, and contain an embryo in the morula stage of development when laid. With few exceptions exemplified by the genera *Nematodirus* in ruminants and *Gyalocephalus* in horses, both of which are exceptionally large, the generic or specific identity of individual strongylid eggs usually cannot be established by inspection or even by micrometry, so similar are they in appearance and size (Shorb, 1939). Most of the serious effort to differentiate helminth eggs by mathematical analysis of their dimensions has been expended on the eggs of strongylids (Tetley, 1941; Krug and Mayhew, 1949; Cunliffe and Crofton, 1953; Christie and Jackson, 1982). However, there remain many diagnostic dilemmas to be resolved beyond the order Strongylida and we hope that the technique described here will find much broader application in coprology.

Differential diagnosis based on identification of strongylid infective larvae was pioneered in sheep by Dikmans and Andrews (1933) and Andrews (1935) and later applied to cattle by Keith (1953). This approach affords reliable qualitative differentiation of strongylid larvae at the generic level. Russell (1948), for example, made brilliant use of larval identification in her study of the development of nematode parasite burdens in thoroughbred foals.

The principal disadvantages of diagnosis based

on larval culture and identification are that it requires the attention of a highly skilled diagnostician and is very time consuming and tedious to perform routinely on any considerable scale. Other drawbacks of diagnostic larval culture include a delay of at least a week before cultures can be read and the inherently non-quantitative nature of the results. The non-quantitative nature of diagnostic culture methods stems from the differential response of the several species of developing larvae to the ambient cultural conditions which are difficult to control precisely. Therefore, differential counts of the species of infective larvae present in a culture do not provide an accurate estimate of the relative abundances of species of strongylid eggs in the original sample (Cunliffe and Crofton, 1953).

Identification of preinfective larvae (Whitlock, 1959; Ogbourne, 1971) reduces the delay inherent in methods based on identification of infective larvae but the other disadvantages remain.

The morphologic and geometric characters of the egg afford the most direct approach to differential diagnosis of strongylid infections. The first detailed and comprehensive effort to identify the eggs of ruminant parasites appears to have been that of Shorb (1939) who published a key to the genera. This was a pioneer effort and included detailed investigation of the composition and dimensions of eggs of many species of parasites. Unfortunately, Shorb's key required accurate measurement of shell thickness in the range 1–2 $\mu$m, with distinctions drawn as precisely as 0.1 $\mu$m, certainly a difficult undertaking with the best of optical equipment. Shorb's key also required counts of the number of cells in the embryo of the freshly passed egg. For example, eggs with combined thickness of the second and third layers of the eggshell equal to 1.5 $\mu$m and with 24 cells or fewer were identified as *Bunostomum*, whereas like eggs with 24 or more cells were identified as *Haemonchus*. The identity of eggs with exactly 24 cells apparently remained problematical. Shorb's key also required the user to reach subjective decisions regarding nuances of shape such as "Eggs tapering toward one or both ends," etc., a process that leaves even the experienced diagnostician with an uncomfortable feeling. Although Shorb's key may have enjoyed very limited if any adoption by parasitologists working with ruminant parasites, it certainly marked the starting point of all serious efforts in the direction of differentiating strongylid nematode eggs.

Tetley (1941) showed, by bivariate graphs, how certain individual strongylid eggs belonging to a specified set of 10 species could be identified. However, eggs with length and width coordinates lying in areas of overlap between species predominated and were unidentifiable by this means. Therefore, in effect, many eggs had to be measured in order to identify a few and the procedure was generally inapplicable to mixed infections.

Cunliffe and Crofton (1953) developed a procedure by which large samples of eggs of 8 species of sheep nematodes could be differentiated on the basis of "the statistical chances of eggs of different species falling into particular size classes." "The accuracy of differentiation increases with the number of eggs classified, but the results can only be regarded as an estimate which is more accurate for the more numerous species in any sample." Again, the onerous nature of the actual microscopic work and the uncertainty of the results have led few to adopt the technique of Cunliffe and Crofton.

The most recent published account of and effort to identify strongylid eggs is that of Christie and Jackson (1982). By combining egg dimensions and information regarding the state of their embryonic development after specified conditions of incubation, these authors claimed to be able to identify more than 90% of *Ostertagia* spp. and *Trichostrongylus vitrinus* eggs but admitted that, "For other species supporting information from larval cultures may be needed but this need will vary according to the composition of the sample."

Measurements of nematode eggs are presented in almost every relevant taxonomic work, but the above 4 reports represent the major efforts that have been applied to the problem of differential diagnosis of ruminant strongylid infections. Unfortunately, none of these has provided an accurate, efficient, and practical solution.

The electronic digitizing tablet affords a convenient means of collecting measurements of nematode eggs and the digital computer and multivariate statistical analysis enable us to draw considerably more detailed conclusions from those data than we have been able to do in the past. We report here the results of our initial investigations of multivariate analysis (specifically, stepwise discriminant analysis) of the geometric parameters length, width, area, perimeter, and areas and arc lengths of specified polar areas of eggs, these having been derived from complete sets of coordinate points of the perimeters of eggs

collected by a digitizing tablet. Inasmuch as our information consists basically of the contour of the egg, we have accepted the term *morphometry,* the "measurement of external form" (Webster's New International Dictionary, 2nd ed., 1935) to denote the general process involved.

## Materials and Methods

### Morphometric procedures

Instruments used in collecting coordinate point data included a compound microscope (Zeiss Standard or Zeiss Photoscope I) fitted with a drawing tube (Zeiss Camera Lucida for Standard microscope or Zeiss Camera Lucida for Photoscope I), a digitizing tablet with cursor (IBM 5083 or Zidas), a lamp to illuminate the surface of the digitizing tablet, and a microcomputer with a 640-kilobyte random access memory and a 10–30-megabyte hard disk memory (AT&T PC6300 or IBM PCXT). With the drawing tube focused on the surface of the digitizing tablet and illumination of the latter suitably balanced with illumination of the microscopic field, the image of an egg under the objective lens of the microscope was made to appear to lie at the surface of the digitizing tablet. As the contour of this image was traced with the cursor, all coordinate points were transmitted to the microcomputer and recorded in memory as a point-capture file consisting of several hundred pairs of coordinates, the ordinal number of the measurement, and an end marker.

All measurements were conducted at ×1,000 magnification except when *Nematodirus* eggs were the subject, in which case magnification was reduced to ×640 to accommodate the image in the microscopic field. Magnification was carefully calibrated before every series of measurements by comparing the scale of an objective micrometer with that of a millimeter rule lying on the surface of the digitizing tablet. Scales were compared at both top and bottom of the microscopic field so that any distortion due to misalignment of the optical axis of the drawing tube could be detected and corrected.

### Conversion of coordinates into geometric parameters

Coordinate point data were converted into geometric parameters (length, width, area, perimeter, areas and arc lengths of poles) with a microcomputer program (PARAM) developed by Miles McCreadie. PARAM first uses the distance formula based on the Pythagorean Theorem to determine the distance between every pair of points on the contour (Fig. 1A). The greatest of these distances is the *length* or *major axis* which is inclined at the angle (theta) whose tangent is equal to the ratio of the difference in ordinates to the difference in abscissas of the end points, i.e., the 2 points of intersection of the major axis with the perimeter (Fig. 1B). The inclinations (alpha) of all line segments drawn from the end point with the smaller value of X to all points on the contour and represented by "d" of Figure 1C are also determined. PARAM then rotates the egg to the horizontal by translating each point on the perimeter by means of the trigonometric equations of Figure 1C. *Width* is then measured as the greatest difference between ordinates of all points lying on the contour of the rotated egg (Fig. 1D). *Area* is estimated as the sum of the areas of all trapezoids inscribed between the contour and the major axis and *perimeter* is estimated as the sum of all distances between adjacent points on the contour (Fig. 1E). *Areas* and *arc lengths* of each pole are calculated in the same manner, each "pole" defined to include $\frac{1}{20}$ of the major axis (Fig. 1F). PARAM is written in Fortran and is available from the senior author as the source code or as an IBM-compatible program on double-sided, double-density diskettes at the cost of reproduction and mailing. The senior author will also be happy to assist those seriously interested in developing a diagnostic morphometry work station with operational details and morphometric data.

PARAM works well for objects with convex contours but does not deal effectively with concavities because the geometric analysis used is appropriate only to shapes made up of convexities and straight lines. Even the gentle concavity of a heat-relaxed strongylid larva is not measured accurately by PARAM. Circles and triangles are measured accurately, but PARAM estimates the diagonals of squares, parallelograms, and certain trapezoids instead of the length of the sides. The reason becomes apparent when the above sequence of steps are thought out in relationship to these geometric forms.

### Stepwise discriminant analysis

In the normal operation of the discriminant analysis program (P7M, available from BMDP Statistical Software, 1964 Westwood Blvd., Suite 202, Los Angeles, California 90025), the variables that lend the greatest separation of the species groups are automatically chosen for inclusion in the discriminant function. For some comparisons, 1 variable will prove sufficient, while for other comparisons, nearly all the variables will be included in the analysis. Once the variables that are to be included have been selected, the discriminant functions are calculated as the combinations of the selected variables that best separate the species groups. Specifically, linear combinations of the selected variables are considered and the coefficients in the linear combinations are chosen to maximize the ratio of the between-group to within-group variation (Johnson and Wichern, 1988, Sec. 11.8). The discriminant functions calculated in such a manner can then be used to sort the eggs into groups representing the species under consideration.

A note on terminology is appropriate here. Statisticians refer to the process of sorting cases into groups on the basis of discriminant analysis as "classification." As long as the analysis is restricted to groups of known identity, this use of the term is entirely appropriate in a zoological sense, inasmuch as it is an inductive process similar to the classification of known taxa. However, when we present discriminant analysis with an "unknown" (statistically, any data set not included in the calculation of canonical coefficients employed to sort its cases) the process becomes deductive rather than inductive and is properly termed "identification" instead of "classification." We observe the distinction between identification and classification in both statistical and zoological senses.

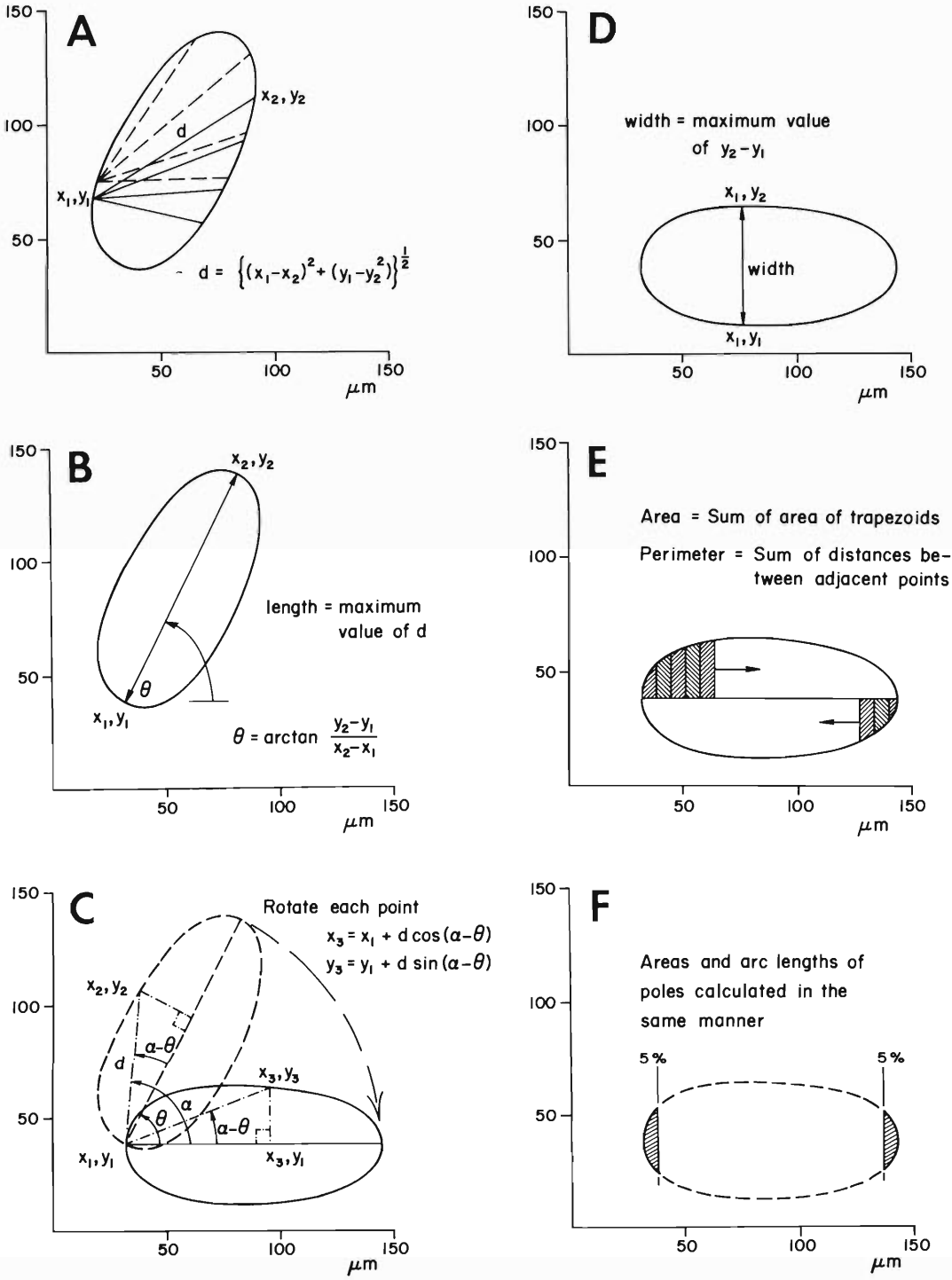


**Figure 1. Graphic representation of conversion of coordinate point data into geometric parameters by PARAM, the computer program written by Miles McCreadie. For each egg entered, the computed length, width, area, perimeter, and areas and arc lengths of each pole are stored in a computer file in form suitable for stepwise discriminant analysis.**

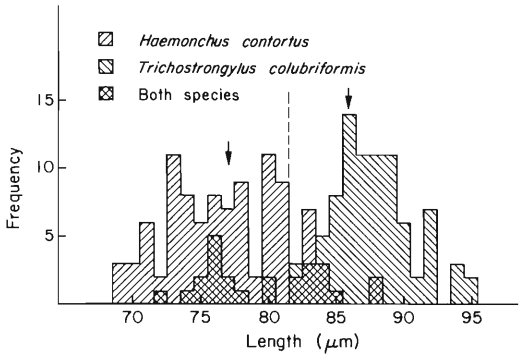


**Figure 2. Frequency distribution of lengths of 100 *Haemonchus contortus* eggs and 100 *Trichostrongylus colubriformis* eggs. The arrows indicate the mean lengths; the vertical line lies at the midpoint between means. Eight-five percent of the *H. contortus* and 86% of the *T. colubriformis* egg lenths fell on the same side of this vertical line as their respective means. Therefore, classifying each egg as *H. contortus* or *T. colubriformis* according to whether its length was closest to one mean or the other resulted in 85.5% correct classification, on the average.**

**Table 1. Jackknifed classification matrix of *Ancylostoma caninum, Uncinaria stenocephala,* and *Haemonchus contortus* based on stepwise discriminant analysis.**

| Group | Percent correct | Number of cases classified into group: ANCN | UNST | HMCT |
|---|---|---|---|---|
| ANCN | 90.0 | 90 | 7 | 3 |
| UNST | 73.0 | 0 | 73 | 27 |
| HMCT | 75.0 | 6 | 19 | 75 |

## Specimens

Strongylid eggs of known identity were obtained for morphometry from the uteri of adult female specimens and from the feces of hosts with purported single species infections of strongylid worms. Dissection of adult female worms was carried out on both fresh and fixed specimens. The usual and more satisfactory preservative was 10% formalin, but the only available specimens of *Cooperia curticei* were in 70% ethanol. Certain small clear specimens such as fresh *Trichostrongylus colubriformis* eggs were measured in situ.

ARTIFICIAL INFECTIONS: One lamb was infected with 3,000 infective larvae of *Haemonchus contortus* and another with 6,000 infective larvae of *Trichostrongylus colubriformis*; both infections became patent 21 days later. Samples of rectal feces were collected on the first day of patency and at intervals of 12, 14, 20, and 31 days thereafter. On day 31, the lambs were slaughtered, the purity of infection established by examination of their alimentary tracts, and worm specimens collected so that dimensions of uterine and fecal eggs could be compared.

FORMALIN FIXATION OF FECAL SPECIMENS: As a standard procedure, 10 g feces were suspended in 75 ml water and the suspension strained through a sieve with 1-mm apertures. The solid material retained by the sieve was compressed, washed with an additional 75 ml water, and compressed again. The resulting 150 ml

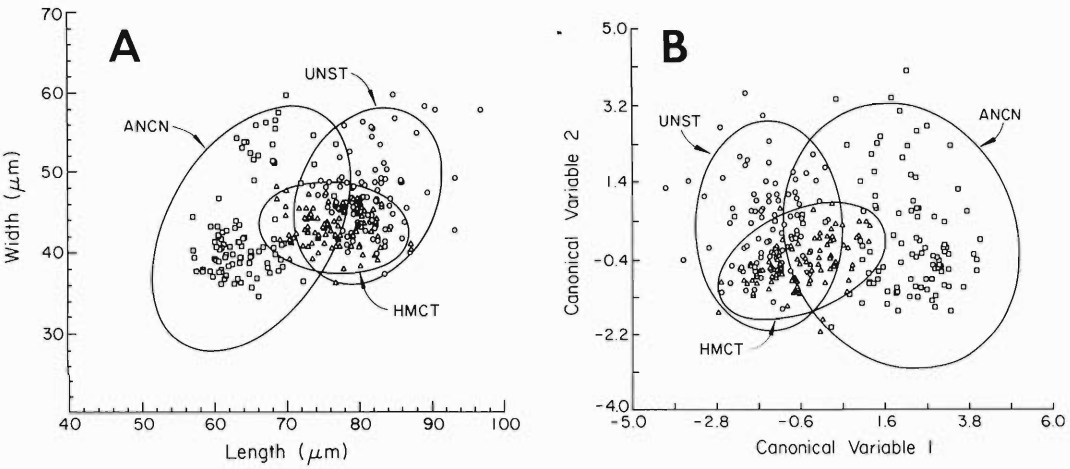


**Figure 3. A. Distribution of lengths and widths of 100 eggs each of *Ancylostoma caninum* (ANCN), *Uncinaria stenocephala* (UNST), and *Haemonchus contortus* (HMCT). Ellipses include 95% of the data points. B. Distribution of points representing the first 2 canonical variables derived by discriminant analysis of the same data set represented in Figure 3A. The canonical variables are based on length and perimeter, the parameters chosen by the discriminant analysis program. Despite the considerable degree of overlap, discriminant analysis correctly classified 90% of the *A. caninum,* 73% of the *U. stenocephala,* and 75% of the *H. contortus* eggs (Table 1). Ellipses include 95% of the data points.**

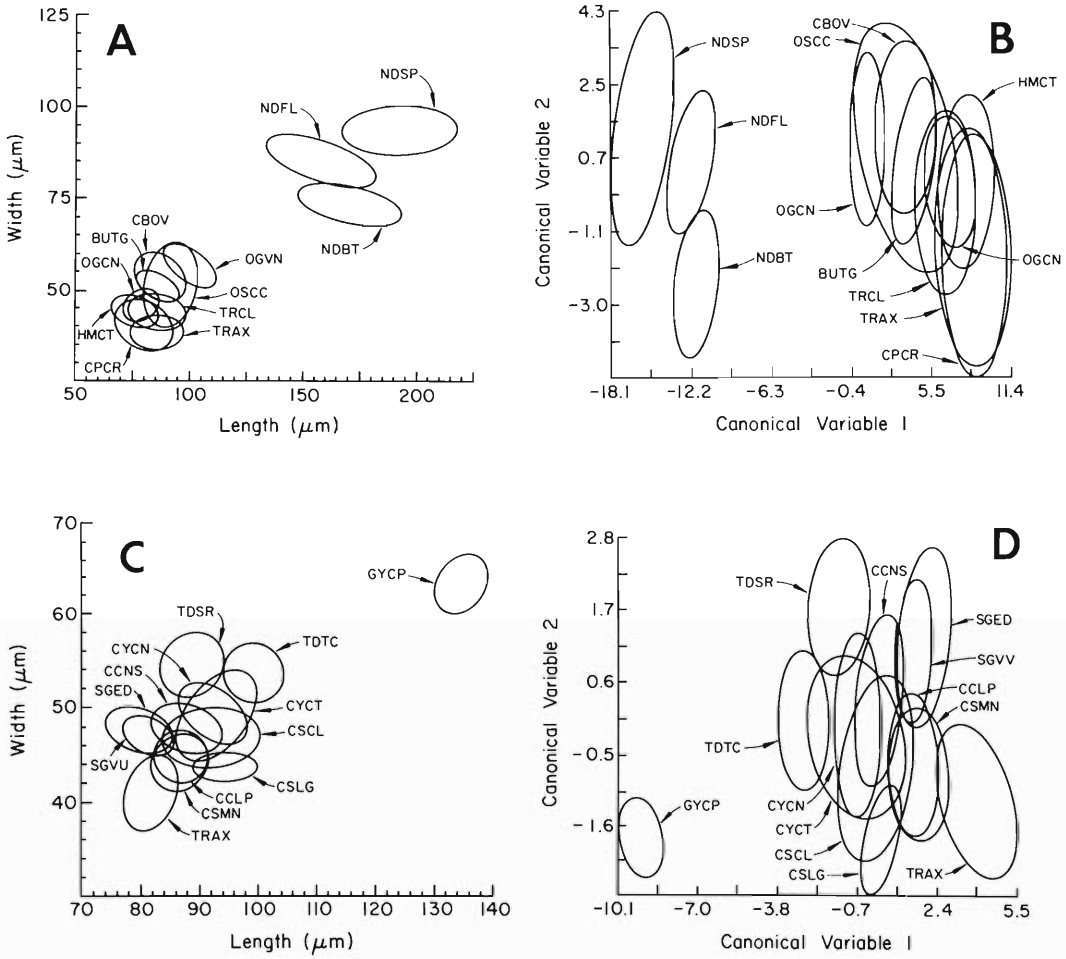


**Figure 4. A. Ellipses including 95% of the points representing width vs. length of 12 species of ovine strongylid eggs. Data points deleted for clarity. B. Ellipses including 95% of the points representing canonical variables 1 and 2 based on 7 geometric parameters (area, length, width, area pole 1, perimeter, arc length pole 1, arc length pole 2) of the same data set as that represented in Figure 4A. Stepwise discriminant analysis correctly identified 75.8% of these 12 species, on average (Table 2). Data points deleted for clarity. C. Ellipses including 50% of the points representing width vs. length of 13 species of equine strongylid eggs. Data points deleted for clarity. D. Ellipses including 50% of the points representing canonical variables 1 and 2 based on 4 geometric parameters (area, length, width, perimeter) of the same data set as that represented in Figure 4C. Discriminant analysis correctly identified only 46.8% of these 13 species, on average (Table 3). Data points deleted for clarity.**

of egg suspension free of coarse debris was mixed with 150 ml 20% formalin solution (20 ml stock 37% formaldehyde solution + 80 ml water) to yield a final concentration of 10% formalin. Fecal specimens from other laboratories usually contained intact fecal pellets and so were not preserved in the manner specified above. Eggs from fresh fecal specimens were examined when the opportunity arose.

SETS OF STRONGYLID EGG DATA: Morphometry data representing the following 12 species of ovine strongylids were analyzed as a set to evaluate discriminant analysis in multidimensional sample space; abbreviations are those used in tables, the number of eggs representing each species is indicated: *Bunostomum trigonocephalum* (BUTG, 25), *Chabertia ovina* (CBOV, 50), *Cooperia curticei* (CPCR, 50), *Haemonchus contortus* (HMCT, 100), *Nematodirus battus* (NDBT, 100), *N. filicollis* (NDFL, 20), *N. spathiger* (NDSP, 100), *Oesophagostomum columbianum* (OGCN, 25), *Oe. venulosum* (OGVN, 50), *Teladorsagia circumcincta* (OSCC, 99), *Trichostrongylus axei* (TRAX, 25), and *T. colubriformis* (TRCL, 100).

Morphometry data representing the following 13 species of equine strongylids were analyzed as a set to evaluate discriminant analysis in multidimensional sample space; abbreviations are those used in tables, the number of eggs representing each species is indicated: *Strongylus edentatus* (SGED, 55), *S. vulgaris*

**Table 2. Jackknifed classification matrix of 12 species of ovine strongylid eggs based on stepwise discriminant analysis and identification of 3 "unknown" samples from pure infections with the indicated species of strongylid.**

| Group | Percent correct | Number of cases classified into group: BUTG | CBOV | CPCR | HMCT | NDBT | NDFL | NDSP |
|---|---|---|---|---|---|---|---|---|
| | | | | Reference set | | | | |
| BUTG | 60.0 | 15 | 7 | 0 | 0 | 0 | 0 | 0 |
| CBOV | 58.0 | 9 | 29 | 0 | 0 | 0 | 0 | 0 |
| CPCR | 60.0 | 0 | 0 | 30 | 8 | 0 | 0 | 0 |
| HMCT | 67.0 | 0 | 0 | 9 | 67 | 0 | 0 | 0 |
| NDBT | 98.0 | 0 | 0 | 0 | 0 | 98 | 2 | 0 |
| NDFL | 100.0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 |
| NDSP | 96.0 | 0 | 0 | 0 | 0 | 0 | 4 | 96 |
| OGCN | 72.0 | 1 | 0 | 0 | 5 | 0 | 0 | 0 |
| OGVN | 100.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OSCC | 49.5 | 6 | 14 | 0 | 0 | 0 | 0 | 0 |
| TRAX | 76.0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| TRCL | 73.0 | 1 | 0 | 9 | 3 | 0 | 0 | 0 |
| | | | | "Unknowns" | | | | |
| HMCT | 66.0 | 0 | 0 | 3 | 66 | 0 | 0 | 0 |
| OSCC | 53.0 | 5 | 20 | 1 | 0 | 0 | 0 | 0 |
| TRCL | 80.0 | 0 | 0 | 1 | 15 | 0 | 0 | 0 |

(SGVU, 99), *Triodontophorus serratus* (TDSR, 70), *T. tenuicollis* (TDTC, 100), *Cylicostephanus calicatus* (CSCL, 37), *C. longibursatus* (CSLG, 52), *C. minutus* (CSMN, 100), *Cylicocyclus nassatus* (CCNS, 100), *C. leptostomus* (CCLP, 85), *Cyathostomum catinatum* (CYCT, 100), *C. coronatum* (CYCN, 100), *Gyalocephalus capitatus* (GYCP, 20), and *Trichostrongylus axei* (TRAX, 47).

The nomenclature of Lichtenfels (1975) is followed here with all due respect to the excellent monograph of Hartwich (1986) on the subject of cyathostomin systematics.

## Results

### Analysis in one dimension; differentiation of eggs of *Haemonchus contortus* and *Trichostrongylus colubriformis*

DISTRIBUTION OF LENGTHS: One hundred *Haemonchus contortus* eggs differed from 100 *Trichostrongylus colubriformis* in mean length ($P < 0.001$, $t_{198df} = 13.8$) but not in mean width. A frequency distribution of lengths of eggs of these 2 species is presented in Figure 2. A line drawn perpendicular to the X-axis between 81 and 82, which is midway between the mean of 77 for *H. contortus* and the mean of 86 for *T. colubriformis,* divided each population of eggs into 2 subpopulations, 1 lying on the same side as its population mean and the other lying on the side of the opposite population mean. Eighty-five percent of the *H. contortus* eggs and 86% of the *T. colubriformis* eggs lay on the same side as their respective population means. Stepwise discriminant analysis of the same data yielded an identical result, i.e., 85% correct classification of *H. contortus* eggs and 86% correct classification of *T. colubriformis* eggs.

### Analysis in two dimensions; differentiation of eggs of *Ancylostoma caninum, Uncinaria stenocephala,* and *Haemonchus contortus*

In Figure 3A, widths versus lengths of 100 eggs of each of the 3 species, *Ancylostoma caninum* (ANCN), *Uncinaria stenocephala* (UNST), and *Haemonchus contortus,* and ellipses bounding 95% of the data points are presented for each species. The populations of *A. caninum* and *U. stenocephala* are seen to overlap each other and to completely engulf *H. contortus.* A plot of the canonical variables based on the 2 geometric parameters selected by discriminant analysis (i.e., perimeter vs. length, Fig. 3B) also conveys the impression that the classification of *A. caninum* eggs must be completely confounded with the other 2 species, but in fact, discriminant analysis classified the majority of each species correctly (Table 1). In Table 1, we report the jackknifed classification matrix. A simple classification matrix tends to be overly optimistic in estimating

**Table 2. Extended.**

| Number of cases classified into group: | | | | |
|---|---|---|---|---|
| OGCN | OGVN | OSCC | TRAX | TRCL |
| | | Reference set | | |
| 1 | 0 | 2 | 0 | 0 |
| 2 | 4 | 6 | 0 | 0 |
| 5 | 0 | 0 | 4 | 3 |
| 16 | 0 | 0 | 1 | 7 |
| 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 1 |
| 0 | 50 | 0 | 0 | 0 |
| 3 | 13 | 49 | 0 | 14 |
| 0 | 0 | 0 | 19 | 3 |
| 8 | 0 | 2 | 4 | 73 |
| | | "Unknowns" | | |
| 28 | 0 | 0 | 0 | 3 |
| 0 | 20 | 53 | 0 | 1 |
| 2 | 0 | 1 | 1 | 80 |

the correct classification rate because the eggs being classified are included in the set used to determine the discriminant functions. In jackknifing, each egg in turn is left out of the calculations and classified using a discriminant function based on all the other eggs. This gives a truer estimate of the correct classification rate. Discriminant analysis estimated correct classification rates of 90% for *A. caninum,* 73% for *U. stenocephala,* and 75% for *H. contortus* (Table 1).

When *A. caninum* and *U. stenocephala* were compared with *H. contortus* absent, correct classifications based on length rose to 92% and 100%, respectively. The difference in correctness of classification was due to the presence of 8 "long" eggs in the *A. caninum* set misclassified as *U. stenocephala*; the *U. stenocephala* set contained no "short" ones to be misclassified as *A. caninum.*

### Multidimensional analysis; differentiation of 12 species of ovine strongylids and 13 species of equine strongylids

Ellipses including 95% of the points representing width vs. length of 12 species of ovine strongylid eggs are plotted in Figure 4A; the 744 data points have been deleted to avoid cluttering the figure. Ellipses including 95% of the points representing canonical variables 1 and 2 based on 7 geometric parameters (area, length, width, area pole 1, perimeter, arc length pole 1, arc length pole 2) selected by discriminant analysis are presented in Figure 4B and the corresponding jackknifed classification matrix is presented in Table 2. The overall correctness of this classification was 75.8% and varied from 49.5% for *Teladorsagia circumcincta* to 100% for *Nematodirus filicollis* and *Oesophagostomum venulosum.* The compactness of the distribution of *Oe. venulosum* data points and the relatively wide dispersion of those of *T. circumcincta* are largely responsible for the contrasting accuracy of classification of eggs of these 2 species.

Ellipses including 50% of the points representing width vs. length of 13 species of equine strongylid eggs are plotted in Figure 4C, ellipses including 50% of the points representing canonical variables 1 and 2 based on 4 geometric parameters (area, length, width, perimeter) selected by discriminant analysis are presented in Figure 4D, and the corresponding jackknifed classification matrix is presented in Table 3. The 965 data points have been deleted from Figures 4C and 4D and the domain of the ellipses reduced from 95% to 50%; otherwise, the high degree of overlap in dimensions of equine strongylid eggs would have rendered these figures illegible.

POOLING OF NEARLY IDENTICAL GROUPS: Using the data set of the previous paragraph, those species having nearly identical eggs were pooled as follows: *Strongylus edentatus* + *S. vulgaris* (EDVU), *Cylicostephanus calicatus* + *Cylicocyclus nassatus* (CLNS), *Cylicostephanus minutus* + *Cylicocyclus leptostomus* (MNLP), and *Cyathostomum catinatum* + *C. coronatum* (CTCN). These pooled categories were again subjected to discriminant analysis along with TDSR, TDTC, CSLG, CYCP, and TRAX. The resulting classification matrix is presented in Table 4. Classification remained 100% in the case of *Gyalocephalus capitatus* and was substantially improved for *Triodontophorus serratus, T. tenuicollis, Cylicostephanus longibursatus,* and *Trichostrongylus axei.* Classification of the "large strongyles" (EDVU) was raised to a level of possible practical utility (76.6%) but classification of the 3 "small strongyle" groups (CLNS, MNLP, and CTCN) remained unsatisfactory. Therefore, the number of cases classified as *C. longibursatus* (CSLG) plus these 3 groups (see boxes, Table 4) were added together to form a pooled "small strongyle" group (*Gyalocephalus capitatus* not

**Table 3. Jackknifed classification matrix of 13 species of equine strongylid eggs based on stepwise discriminant analysis.**

| Group | Percent correct | Number of cases classified into group: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SGED | SGVU | TDSR | TDTC | CSCL | CSLG | CSMN | CCNS |
| SGED | 61.8 | 34 | 10 | 0 | 0 | 1 | 0 | 3 | 0 |
| SGVU | 56.6 | 18 | 56 | 1 | 0 | 0 | 0 | 4 | 9 |
| TDSR | 72.9 | 0 | 0 | 51 | 10 | 0 | 0 | 0 | 6 |
| TDTC | 83.0 | 0 | 0 | 5 | 83 | 0 | 0 | 0 | 0 |
| CSCL | 5.4 | 3 | 2 | 0 | 2 | 2 | 8 | 2 | 5 |
| CSLG | 80.8 | 0 | 0 | 0 | 0 | 3 | 42 | 2 | 2 |
| CSMN | 38.0 | 8 | 3 | 0 | 0 | 5 | 6 | 38 | 2 |
| CCNS | 36.0 | 2 | 16 | 3 | 0 | 9 | 6 | 0 | 36 |
| CCLP | 21.2 | 6 | 7 | 0 | 0 | 8 | 5 | 28 | 2 |
| CYCT | 8.0 | 0 | 4 | 6 | 21 | 11 | 7 | 6 | 17 |
| CYCN | 28.0 | 4 | 0 | 17 | 5 | 9 | 11 | 0 | 11 |
| GYCP | 100.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TRAX | 76.6 | 3 | 1 | 0 | 0 | 0 | 0 | 5 | 0 |
| Total | 46.8 | 78 | 99 | 83 | 121 | 48 | 85 | 88 | 90 |

included) to which 454 out of a total of 574 (79%) were correctly assigned.

### Identification of "unknown" eggs

Data from a fecal specimen containing an unknown assemblage of nematodes are entered into the program for identification only, i.e., the "unknown" data do not enter into calculation of the discriminant functions but are merely identified on the basis provided by analysis of the reference set of eggs. The "unknown" eggs referred to in this section were recovered from the feces of animals with purported single species artificial infections or natural infections of known composition but were entered into the program in exactly the same manner as true unknowns would have been. The identification of these "unknown" eggs was therefore governed by the geometric information supplied by the reference set and the percentage correctly identified therefore provided an unbiased estimate of the diagnostic accuracy of the procedure.

SHEEP PARASITES: One hundred eggs each of *Haemonchus contortus* (Beltsville, Maryland), *Teladorsagia circumcincta* (Armidale, New South Wales, Australia), and *Trichostrongylus colubriformis* (West Chester, Pennsylvania) were identified 66%, 53%, and 80% correctly when subjected as "unknowns" to discriminant analysis based on the reference set of Table 2. When all diagnostic categories except the 3 species presented as "unknowns" were eliminated from the reference set, classification of the reference set and identification of the "unknowns" both improved substantially (Table 5, Fig. 5). Note that "unknown" HMCT and OSCC were identified more precisely than were the corresponding groups in the reference set were classified (Table 5). This is because more of their data values fell closer to the reference set means than did data values of the reference set itself; i.e., these 2 "unknown" sets happened to be more compact and to display less overlap of neighboring reference sets.

HORSE PARASITES: Four "unknown" samples were subjected to discriminant analysis using the pooled reference set of Table 4; they consisted of 100 eggs from a pure *Strongylus edentatus* infection (Baton Rouge, Louisiana), 100 eggs from a pure *S. vulgaris* infection (Baton Rouge, Louisiana), 100 eggs from a naturally acquired mixed cyathostome infection (*Cylicostephanus longibursatus, C. minutus, Cylicocyclus nassatus,* and *Cyathostomum catinatum,* Ithaca, New York), and 100 eggs from a naturally acquired mixed cyathosome infection (same 4 species + *C. calicatus, C. goldi,* and *C. insigne*) plus large numbers of *Trichostrongylus axei* (Ithaca, New York). The nature of the last 2 samples was determined at necropsy. Identification of these "unknowns" accorded reasonably well with the type of infection (Table 4).

### Procedural sources of morphometric variation

WORM EGGS VS. FECAL EGGS: One hundred eggs from feces of a lamb with the pure *Haemonchus contortus* infection were compared with 100 eggs dissected from the uteri of 20 *H. con-*

**Table 3. Extended.**

| Number of cases classified into group: | | | | |
|---|---|---|---|---|
| CCLP | CYCT | CYCN | GYCP | TRAX |
| 4 | 0 | 1 | 0 | 2 |
| 11 | 0 | 0 | 0 | 0 |
| 0 | 1 | 2 | 0 | 0 |
| 0 | 10 | 2 | 0 | 0 |
| 3 | 6 | 4 | 0 | 0 |
| 2 | 0 | 1 | 0 | 0 |
| 24 | 3 | 2 | 0 | 9 |
| 6 | 9 | 13 | 0 | 0 |
| 18 | 1 | 4 | 0 | 6 |
| 6 | 8 | 14 | 0 | 0 |
| 0 | 15 | 28 | 0 | 0 |
| 0 | 0 | 0 | 20 | 0 |
| 2 | 0 | 0 | 0 | 36 |
| 76 | 53 | 71 | 20 | 53 |

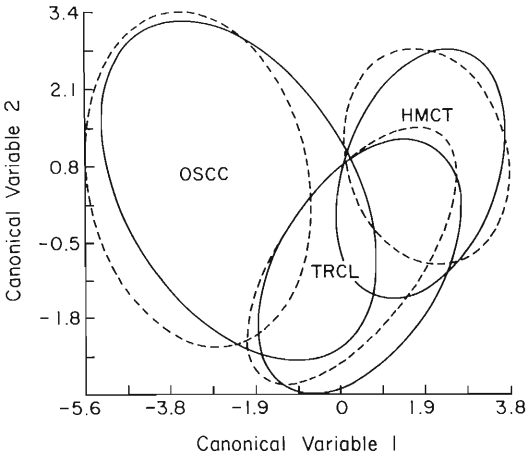


**Figure 5. Ellipses including 95% of the points representing canonical variables 1 and 2 based on length and perimeter. *Haemonchus contortus, Ostertagia circumcincta,* and *Trichostrongylus colubriformis* reference sets (unbroken contours) are compared with independent sets of data representing the same species and presented to the computer as "unknowns" (broken contours). Data points deleted for clarity.**

*tortus* worms (5 eggs from each of 10 linguiform and 10 smooth morph females) recovered from the abomasum of the same lamb. In addition, 100 eggs from the feces of a lamb with pure *Trichostrongylus colubriformis* infection were compared with 100 eggs in the uteri of *T. colubriformis* worms recovered from the small intestine of the same lamb. Fecal eggs of both *H. contortus* and *T. colubriformis* were significantly greater in width than uterine eggs of the same species. The degree of difference was greater for *T. colubriformis* (46.1 vs. 41.8 $\mu$m, $P < 0.01$, $t_{198df} = 11.7$) than for *H. contortus* (44.7 vs. 43.8 $\mu$m, $P < 0.01$, $t_{198df} = 3.19$).

FRESH EGGS VS. FORMALIN-FIXED EGGS: There was no statistically significant difference between the dimensions of fresh *Haemonchus contortus* eggs measured promptly and eggs from the same fecal sample subjected to fixation in 10% formalin according to the procedure described in Materials and Methods followed by storage at room temperature for several days. However, when 5% formalin was substituted, the formalin-treated eggs were significantly wider (49.6 vs. 44.7 $\mu$m, $P < 0.01$, $t_{198df} = 17.6$) and had advanced as far as the "tadpole" stage of development.

## Discussion

Ideally, we would like to be able to identify each egg and estimate the relative abundance of each species in a sample of feces. Unfortunately, great similarity exists in the shape and dimensions of many important species of eggs, appreciable variation may occur among different isolates of the same species, and changes in dimensions may be induced by differences in handling, fixation, and storage of specimens, all leading to greater overlap and decreased diagnostic resolution. The most realistic relative abundances are obtained when the reference set contains the same species as the unknown. This is sometimes possible as, for example, when the species composition involved in a natural or experimental epidemic is known.

### Differentiation of strongylid eggs

COMPARISON OF *HAEMONCHUS CONTORTUS* AND *TRICHOSTRONGYLUS COLUBRIFORMIS*: In the simple case provided by the distribution of lengths of *Haemonchus contortus* and *Trichostrongylus colubriformis* eggs, the differences between the length of a particular egg and the population mean for each species provided a criterion that allowed correct classification of 85 of 100 *H. contortus* and 86 of 100 *T. colubriformis* eggs. Application of stepwise discriminant analysis to this simple case yielded an identical result automatically but by essentially the same process.

HOOKWORM EGGS: The eggs of *Ancylostoma caninum* and *Uncinaria stenocephala* are sufficiently distinctive so that a qualitative diagnosis of either infection or of a mixed infection can be

**Table 4. Jackknifed classification matrix of 5 species and 4 species pairs of equine strongylid eggs (data of similar species pooled prior to analysis). Boxes enclose data pooled after analysis, forming a 7-species group of "small strongyles" to which a total of 79% of the 574 cyathostome eggs were correctly assigned. Four "unknowns" include pure infection of *Strongylus edentatus* (SGED), *S. vulgaris* (SGVU), a natural mixed cyathostome infection, and a natural mixed cyathostome infection with large numbers of *Trichostrongylus axei* (TRAX). Percentage correct values for these 4 "unknowns" are enclosed in single quotation marks because they involve composite groups instead of single species.**

| Group | Percent correct | Number of cases classified into group: EDVU | TDSR | TDTC | CLNS | CSLG | MNLP | CTCN | GYCP | TRAX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Reference set | | | | | | |
| EDVU | 76.6 | 118 | 1 | 0 | 13 | 0 | 22 | 0 | 0 | 0 |
| TDSR | 74.3 | 1 | 52 | 11 | 3 | 0 | 0 | 3 | 0 | 0 |
| TDTC | 83.0 | 0 | 9 | 83 | 0 | 0 | 0 | 8 | 0 | 0 |
| CLNS | 38.0 | 20 | 5 | 3 | 52 | 15 | 13 | 29 | 0 | 0 |
| CSLG | 86.5 | 0 | 0 | 0 | 2 | 45 | 4 | 1 | 0 | 0 |
| MNLP | 62.2 | 18 | 0 | 0 | 9 | 15 | 115 | 12 | 0 | 16 |
| CTCN | 32.5 | 6 | 24 | 28 | 36 | 27 | 14 | 65 | 0 | 0 |
| GYCP | 100.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 |
| TRAX | 80.9 | 2 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 38 |
| | | | | "Unknowns" | | | | | | |
| Pure SGED | '61.0' | 61 | 3 | 0 | 1 | 1 | 25 | 4 | 0 | 5 |
| Pure SGVU | '67.0' | 67 | 12 | 0 | 7 | 0 | 8 | 5 | 0 | 1 |
| Mixed cyathostomes | '80.0' | 1 | 2 | 17 | 15 | 18 | 5 | 42 | 0 | 0 |
| Mixed cyathostomes + TRAX | '86.0' | 10 | 1 | 3 | 0 | 4 | 35 | 6 | 0 | 41 |

based on simple length measurements of a few eggs. However, morphometry and discriminant analysis make it possible to estimate conveniently and accurately the relative abundances of eggs of these 2 species in a mixed infection and, at least in certain cases, to detect spurious parasites. In the example presented, *U. stenocephala* eggs resemble those of *Haemonchus contortus* more closely than those of *A. caninum,* yet all 3 species could be distinguished morphometrically with reasonable accuracy. Detecting spurious parasites requires a suitable hypothesis, e.g., that *Haemonchus contortus* eggs might reasonably be found contaminating the feces of a dog with access to sheep feces or offal during summer, so that a set of *H. contortus* eggs can logically be included in the reference set under such circumstances.

The classification of equine strongylids proved a formidable task. As Figures 4C and 4D and Table 3 reveal, only *Gyalocephalus capitatus* was classified 100% correctly. *Triodontophorus serratus, T. tenuicollis, Cylicostephanus longibursatus,* and *Trichostrongylus axei* were also classified reasonably well, but the classification of the other species was disappointing. Classification of *Cylicostephanus calicatus* and *Cyathostomum catinatum* was at or below the *a priori* level; i.e., one could do better by drawing numbers out of a hat. The reason for the particularly poor classification of these 2 species is once again the great dispersion and intersection of their data sets with those of several other species; too many of the data points of *C. calicatus* and *C. catinatum* lay closer to the population means of other species than to their own. Correct classification of 80.8% of *Cylicostephanus longibursatus* might not be anticipated considering its degree of overlap with neighboring species but its eggs were more uniform in size than the others and that is what accounts for the precision of classification in this case.

ADVANTAGES OF POOLING DIAGNOSTIC CATEGORIES: Stepwise discriminant analysis deals effectively with overlap in dimensions of different species but it cannot deal with identity of dimensions. It is tempting to combine species into groups that represent practical diagnostic categories. For example, "large strongyles," "small strongyles," "*Triodontophorus,*" "*Gyalocephalus,*" and "*Trichostrongylus*" would satisfy most clinical diagnostic needs. For the purpose of analysis, however, it is important to pool only those data sets that are virtually indistin-

guishable morphometrically. When this is done, a general improvement in classification is achieved as can be appreciated by comparing Tables 3 and 4. Classification of species left standing as independent data groups is also uniformly improved. On the other hand, when species with morphometrically dissimilar eggs are pooled, the dispersion of data points results in increased misclassification in all groups and a general decrease in precision.

In any case, there is no harm in combining species into desired diagnostic categories *after* analysis is completed. Adding all cases classified as *Cylicostephanus longibursatus* and the 3 pooled cyathostome groups (i.e., all data at intersections of CSLG, CLNS, MNLP, and CTCN in Table 4) and dividing by the 574 total cases representing these groups resulted in 79% correct classification as "small strongyle."

"UNKNOWNS": In general, the "unknown" samples tended to be identified with about the same degree of precision with which species (individual and pooled) of the reference set were classified (Tables 2, 4). The relative performance of reference and "unknown" sets of the same 3 species of sheep parasites (Table 5) can probably be explained on the basis of the slight differences in the degree of dispersion of their respective distributions (Fig. 5); the more compact the distribution, the greater the precision of identification.

The more categories available for classification and identification, the greater the potential for misclassification and misidentification. Optimum results are achieved when the reference set contains exactly the same species or species groups as the unknown samples to be identified. Usually, this ideal would only obtain in experimental situations in which the composition of artificial infections is under the control of the investigator, at least at the outset. In such a system, diagnostic morphometry would provide an estimate of relative abundance/fecundity of the species present with unprecedented accuracy. At the very least, knowledge of the life histories of the parasites involved should be applied where possible to delete irrelevant categories from the reference set. For example, removing *Oesophagostomum columbianum* from the reference set when analyzing data from samples collected outside the geographic range of this parasite effectively removes the most serious pitfall to the correct identification of *Haemonchus contortus* eggs (Table 2). For another example, the only advantage of including the EDVU category in analysis of a fecal sample from a foal under 6 months of age might be to assess its tendency to practice coprophagy (Russell, 1948). In principle, any species or category may be removed from the reference set for any sound reason. The result will usually be a substantial improvement in precision of classification and identification.

**Table 5. Jackknifed classification matrix of 3 species of ovine strongylid eggs and identification of 3 "unknown" samples representing purported pure infections of the same 3 species.**

| Group | Percent correct | Number of cases classified into group: HMCT | OSCC | TRCL |
|---|---|---|---|---|
| | | Reference set | | |
| HMCT | 85.0 | 85 | 0 | 15 |
| OSCC | 85.9 | 1 | 85 | 13 |
| TRCL | 84.0 | 13 | 3 | 84 |
| | | "Unknowns" | | |
| HMCT | 97.0 | 97 | 0 | 3 |
| OSCC | 99.0 | 0 | 99 | 1 |
| TRCL | 81.0 | 17 | 2 | 81 |

The pattern of classification of each species provides important clues which can be used to enhance interpretation of the identification pattern of the unknown set. For example, in Table 5, 97% HMCT and 99% OSCC in the identification patterns of "unknowns" clearly represent relatively pure infections, but we might question the purity of 81% TRCL with 17% of its eggs identified as HMCT and 2% as OSCC. However, classification of the reference set is seen to follow an almost identical pattern and reassures us as to the purity of the TRCL "unknown" set.

A high percentage value (e.g., above 80%) in a particular diagnostic category represents a condensed clustering of data values within the boundaries of that category; a situation that is very unlikely to arise through scatter from other categories. In principle, therefore, the highest percentage value in any identification matrix represents a virtual certainty that the corresponding species of strongylid is actually present in the host and that its eggs are the most numerous in the fecal specimen examined.

On the other hand, any species present in the unknown set but not represented in the reference set will be misidentified as whatever species it most resembles. If a sample of dog feces containing only spurious *H. contortus* eggs is ana-

lyzed morphometrically using only *A. caninum* and *U. stenocephala* in the reference set, an incorrect diagnosis of mixed hookworm infection with a preponderance of *U. stenocephala* will result. The reference set must be as small as possible but must include all possibilities.

FECAL VS. WORM EGGS: Tetley (1941) reported that swelling of *Haemonchus contortus* eggs manifested principally as an increase in width took place in the interval between being laid and appearing in the feces. Our observations concur with Tetley's with respect to *H. contortus* and were even more marked in the case of *Trichostrongylus colubriformis*. Species whose egg dimensions and shapes differ least require the greatest precision of measurement in establishing reference data. These should be based on fresh or properly formalized fecal eggs from single species infections of the parasite in question. Unfortunately, "pure infections" represent considerable labor and expense and a few contaminant eggs from an unwanted species ruin the resulting data set. However, if a practical need exists for the differentiation of a given set of closely similar species of eggs, the most satisfactory results will be achieved by careful work with pure infections. On the other hand, where distinctions are relatively easily drawn, it is a waste of time and resources to base reference data on any other than eggs from worm uteri; the small changes in dimensions experienced from worm uterus to host's feces do not interfere provided the interspecific differences are sufficiently large in the first place.

FRESH VS. FORMALIN-FIXED EGGS: Fecal samples carefully preserved in 10% formalin yielded *Haemonchus contortus* eggs which, after storage for a few days at room temperature, remained dimensionally indistinguishable from eggs measured promptly after being passed. Five percent formalin, on the other hand, was inadequate to prevent partial development and concomitant increase in width. Care must be exercised to obtain thorough mixing of fecal material and formalin. If fecal pellets are simply dropped into 10% formalin, a mix of developmental stages from morula to first-stage larva results, the degree of development of a particular egg depending on its distance from the surface of the fecal mass and consequent time required for the formalin to reach it. Such a sample is of limited value for morphometric analysis.

APPRAISAL AND PROSPECTS FOR IMPROVEMENT: We consider our technique in its present stage of development to be ready for the hands of competent parasitologists. The optical and electronic equipment and software are relatively inexpensive and present no serious obstacle to a modest research budget.

As it now stands, diagnostic morphometry is better suited to systems in which repeated measurements and supplementary observations permit suitable reduction of reference sets than it is to the differential diagnosis of casual specimens. However, as the process of measurement is facilitated, the amount of information gathered increased, and the analysis of that information refined, diagnostic morphometry can be expected to cope with more difficult problems and to find more general application.

Highly sophisticated videographic image analysis systems are potentially capable of accomplishing the same ends as the system described herein and perhaps demand less time and effort on the part of the observer. Image analysis systems are also capable of measuring infinitessimal differences in color and optical density and thus provide information that the digitizing tablet cannot. However, in our experience, any color observed in strongylid eggs represents spurious and inconstant optical effects. Whether or not optical density will provide a reliable diagnostic criterion remains to be determined. High cost is the principal disadvantage of videographic image analysis systems.

The value of classification patterns to the interpretation of identification patterns was pointed out above. An objective mathematical method of analyzing these patterns would be a distinct improvement over their subjective interpretation, but remains to be developed.

APPLICATIONS: Effective application of morphometric analysis in resolving diagnostic problems that have no easier solution requires detailed knowledge of taxonomy and biology of the parasitic organisms under study, skill in the operation of microscope and computer, and meticulous attention to detail at every step of the operation. The serious student of strongylid life history and epidemiology stands to gain most from adopting these procedures. In order to obtain an accurate list of the strongylid species infecting a group of hosts in a natural setting, the contemporary investigator must resort to postmortem examination of those hosts, to exposure of "sentinal" parasite-free hosts to the same environment and subsequent postmortem examination of these "sentinals," or to larval cultures

with the limitations of that technique discussed above. By contrast, diagnostic morphometry is completely non-destructive and requires only correctly identified fecal samples to determine the more abundant species or species groups present and to supply an estimate of their relative abundances. When applied in a longitudinal study with repeated sampling of the same individual hosts, the temporal pattern of growth and decline of populations of different species can be quantified with unprecedented accuracy.

We believe that diagnostic morphometry can profitably be applied to other parasite groups presenting diagnostic dilemmas (e.g., coccidia, taeniid eggs) and may eventually find application in the routine diagnostic laboratory. Of course, it would be a simple matter to develop programs capable of differentiating those taxa that are easily identified on sight by a competent parasitologist and indeed such programs might enjoy the greatest commercial potential. Diagnosis based on egg micromorphometry could then be accomplished by any intelligent and painstaking technician after a few hours' instruction and would allow the parasitologist time to study molecular biology and maybe keep his job.

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Proc. Helminthol. Soc. Wash.
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# Appendicitis or Trichuriasis? A Case History

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ABSTRACT: A 37-yr-old man, who recently emigrated from India, was diagnosed as having acute appendicitis, i.e., presented with right lower quadrant abdominal pain, an oral temperature of 39.2°C, and a white cell count of 17,100 × $10^3/\mu l$. An appendectomy was performed. Sections of the appendix revealed no definite associated inflammatory infiltrate, but *Trichuris trichiura* was noted in the lumen and subsequent stool samples revealed eggs of *T. trichiura*. The elevated temperature and neutrophilia were eventually ascribed to phlebitis in the right arm, and verminous involvement of the large bowel was the apparent reason for the abdominal discomfort.

KEY WORDS: *Trichuris trichiura,* trichiuriasis, appendicitis, pseudoappendicitis.

The human whipworm, *Trichuris trichiura,* has been incriminated in verminous appendicitis (Haines et al., 1968) and clinical disease mimicking appendicitis (Barss, 1983). The following case history of suspected appendicitis illustrates the importance of considering trichuriasis as a possible cause of right lower quadrant abdominal pain.

On 1 February 1987 a 37-yr-old male, who had emigrated from India 2 years before, presented at the emergency room with severe intestinal cramps. The abdominal pain and concurrent anorexia had persisted for 24 hr, but he denied nausea and vomiting. The patient had an oral temperature of 39.2°C, a blood pressure of 188/76 mm Hg, and a pulse rate of 100. Abdominal examination revealed bowel sounds and localized tenderness of the right lower quadrant with rebound tenderness. Prothrombin and partial thromboplastin time, amylase, lipase, urinalysis, and liver function tests were all within normal limits. A white cell count revealed 17,100 × $10^3/\mu l$, with a differential of 77 neutrophils, 4 band forms, 12 lymphocytes, 7 monocytes, 0 eosinophils, and 0 basophils. The stool was positive for eggs of *T. trichiura* and hookworm. An appendectomy was performed, and on gross examination the appendix appeared inflamed; however, tissue sections, which included representative samples throughout the length of the resected organ, showed no associated inflammatory infiltrate within the mucosa. A helminth was noted in one of the tissue sections with characteristics consistent with *T. trichiura*: a bacillary band, short muscle fibers, and a stichosome esophagus (Fig. 1). The patient was subsequently treated with a 3-day course of mebendazole. The postoperative course was significant for increased temperature, which, along with the neutrophilia, was eventually attributed to phlebitis in the right arm. The patient was discharged on the tenth day following surgery.

On the basis of the pathological findings, the presumptive diagnosis of acute appendicitis as the cause for the abdominal distress is improbable. The erythrocytes and debris within the lumen (Fig. 1) were considered a result of the surgical procedure and not a manifestation of appendicitis. Also, there is no definitive evidence to support a notion that a single worm infecting the appendix could promote exaggerated abdominal pain, but a concurrent infection in other sites of the large bowel might cause marked discomfort. The likelihood that the cecum and colon were also infected with *T. trichiura* was high, which would account for the eggs being recovered in the feces but not being observed in the tissue sections. The hookworm infection could have exacerbated the situation. In view of the clinical presentation of the patient when admitted to the hospital, surgical intervention probably was warranted. Furthermore, the possibility that *T. trichiura* could mimic appendicitis should be recognized by health professionals in the United States, especially when considering persons who lived in tropical areas of the world. The clinical importance of trichuriasis should not be underestimated.

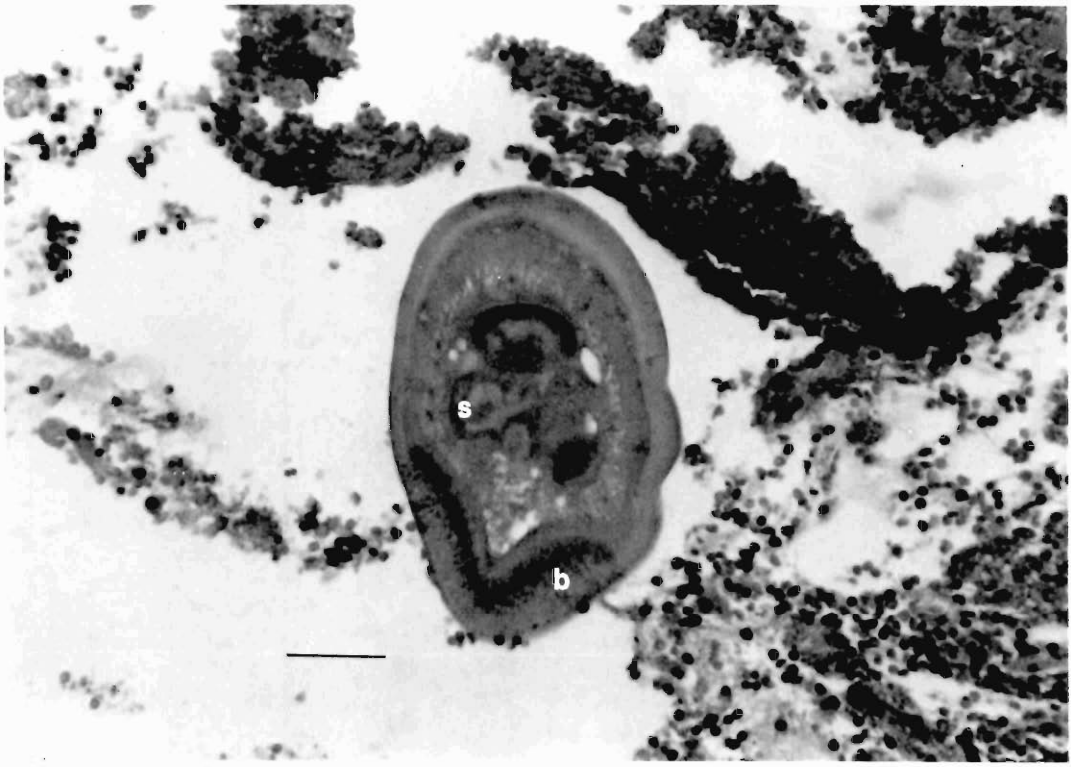


**Figure 1. Cross section of *Trichuris trichiura* in the lumen of the resected appendix: s = stichocyte, b = bacillary band. Scale = 50 μm.**

VOUCHER SPECIMEN: Tissue sections of the resected appendix, USNM Helm. Coll. No. 80392.

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Proc. Helminthol. Soc. Wash.
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# Scanning Electron Microscopy of *Dracunculus medinensis* First-Stage Larva

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ABSTRACT: Scanning electron microscopy of the first-stage larva of *Dracunculus medinensis* showed that the cuticle is marked by distinct annulations and 2 lateral cords. The cephalic end has a triangular denticle and an elongated oral opening. There are 2 large phasmids each placed across the lateral cords and each containing 2 semilunate membranous structures. There is a tiny excretory pore towards the anterior end and a larger anus towards the posterior end.

KEY WORDS: *Dracunculus medinensis*, scanning electron microscopy.

Dracunculosis is an important parasitic infection in some parts of the world and it has been estimated that approximately 120 million persons are at risk of dracunculosis in Africa and 20 million in the Indian subcontinent (Hopkins, 1987). During the life cycle of the parasite the mature female produces first-stage larvae that are expelled to the exterior. These are ingested by cyclops (vector) and continue further development. Although the morphology of the first-stage larvae has been well studied with the light microscope (Moorthy, 1938; Ivashkin et al., 1971) this is the first study using the SEM.

## Results

The larva has a rounded anterior end and a long pointed tail (Fig. 1). The whole body is covered with distinct cuticular annulations (Figs. 1, 2, 6) that become indistinct towards the tail end (Fig. 1). At the midbody area each annule is about 800 nm broad (Fig. 6). There are 2 lateral cords which appear as elevated ridges and run longitudinally from the anterior to the posterior end (Figs. 1, 2, 5). The 2 large phasmidial apertures are situated across the lateral cords and are diamond-shaped (Figs. 5, 7). At high magnification the phasmidial apertures display 2 membrane-like semilunate bodies at their openings (Fig. 7).

At the anterior end of the larva a dorsal denticle or spine is clearly visible (Figs. 1–3). The denticle is triangular in shape and measures between 250 and 500 nm in length (Fig. 3). When the head region is seen en face a laterally elongated mouth or oral opening is also visible and the denticle appears as a rounded structure lying below the mouth (Fig. 4). The excretory pore is a tiny opening near the anterior end (Fig. 1). The anus is a larger opening, lying slightly anterior to and ventral to the 2 phasmids (Figs. 5, 6).

## Discussion

An excellent description of the larval morphology using the light microscope has been given by Moorthy (1938). All the structures that he described fit in with the observations made with the SEM. The dorsal denticle, because of its shape, appears to be an organ for penetrating the vector tissues. As it is generally believed that the infection of cyclops occurs by the ingestion of the larvae (Muller, 1975), the denticle is probably used for the penetration of the gut rather than the cuticle, so as to enter the hemocoel.

Muller and Ellis (1973) have examined the phasmids of the first-stage *Dracunculus* larva with the transmission microscope and postulated a 'purse-string' type closure mechanism at the opening. The SEM observations reveal that the phasmids have a sort of closing device which is made up of a pair of semilunate membranes. This is quite consistent with the closure mechanism postulated by Muller and Ellis (1973) although it looks more likely that in the closed position there would be a slit rather than a hole. Further studies would be required to fully understand the structure of the phasmids and confirm the observations reported in this paper.

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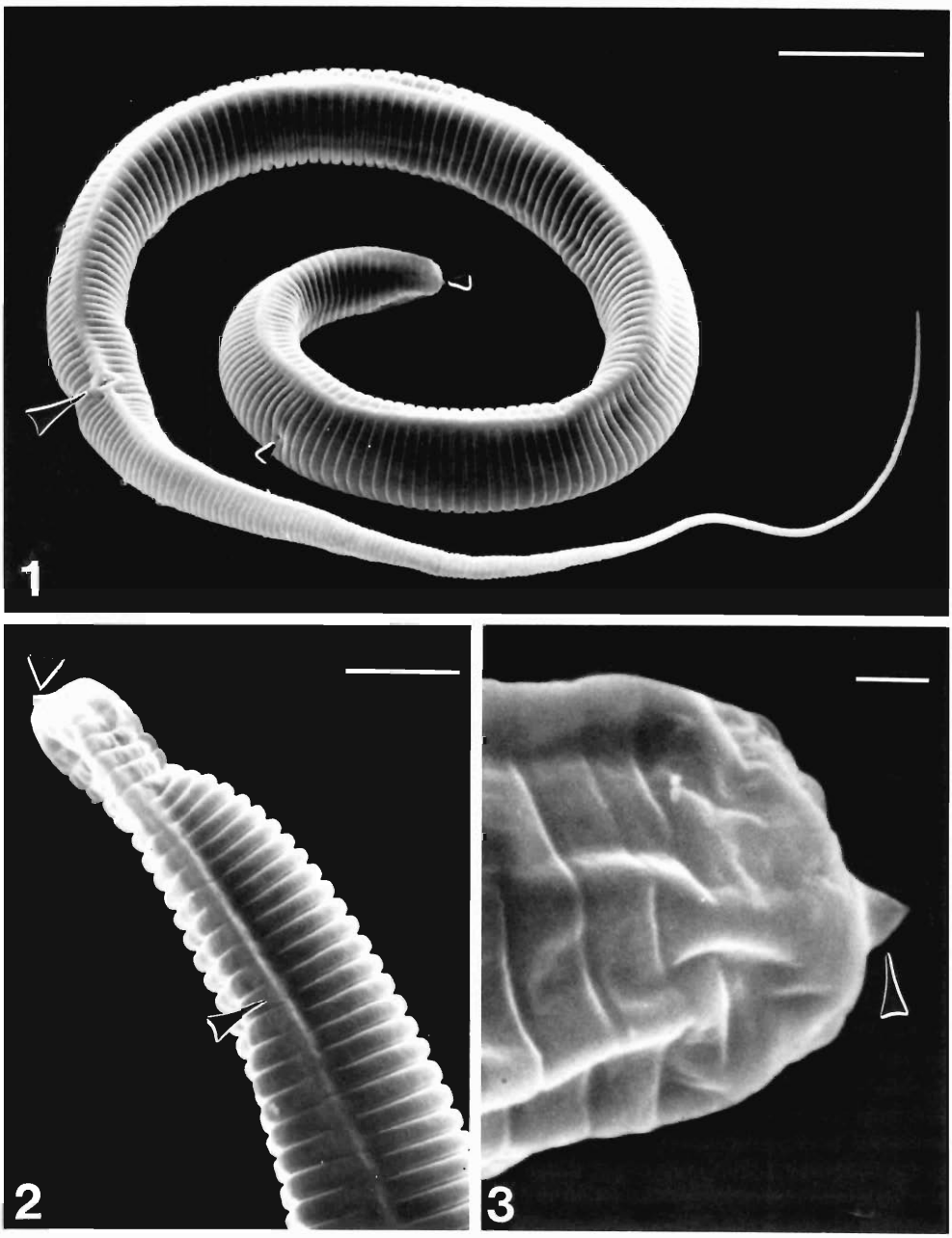
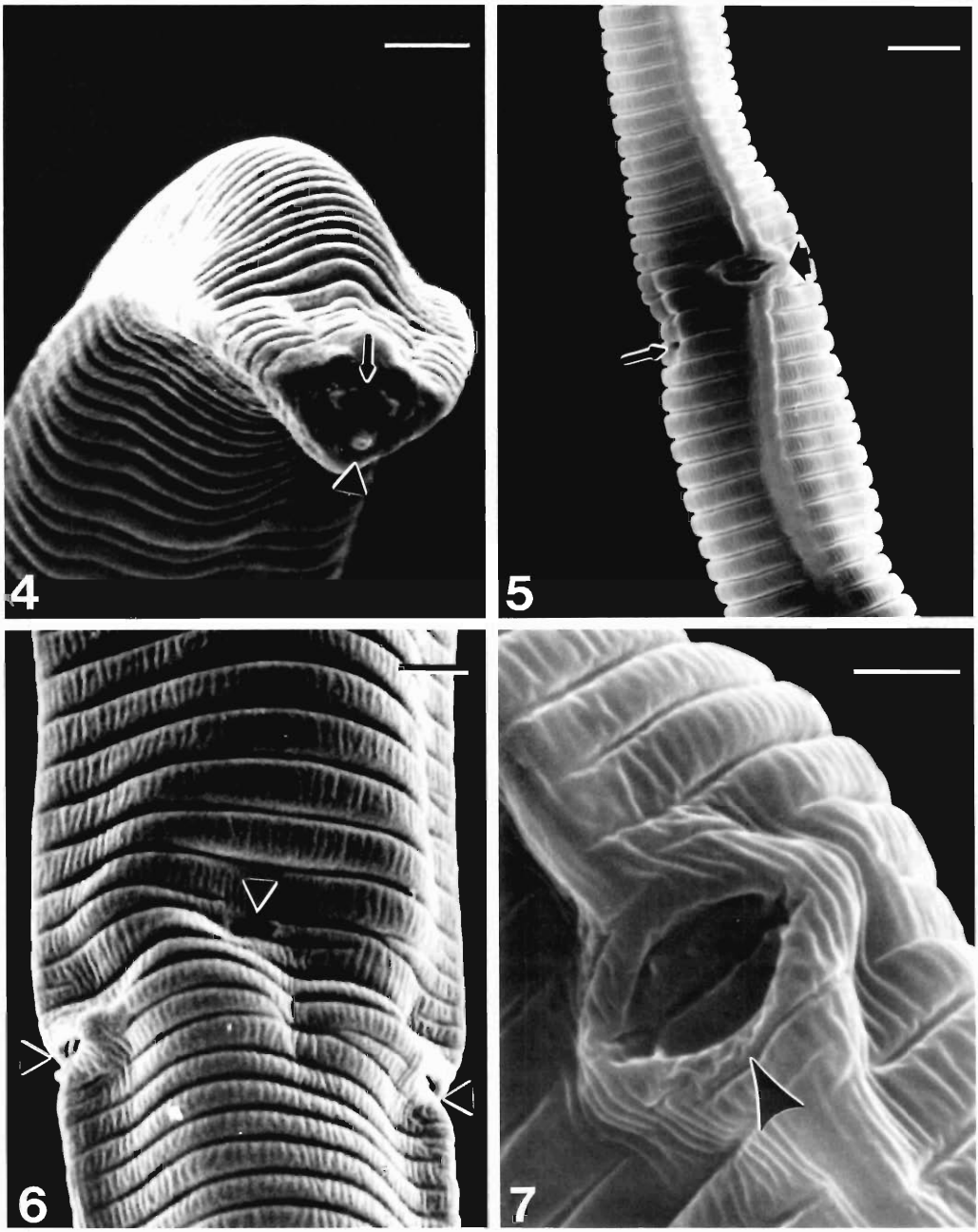



**Figures 1–3.** 1. The whole worm showing a rounded anterior end and a long pointed tail. The 2 small arrows mark the denticle and excretory pore. The long arrow marks a phasmid that lies across a lateral cord. Bar = 10 μm. 2. The anterior end in profile showing the distinct annulations and a lateral cord (long arrow). The denticle is visible at the anterior end (small arrow). Bar = 2 μm. 3. Anterior end showing the denticle (arrow) with a pointed extremity and a triangular shape. Bar = 1 μm.




**Figures 4–7. 4. En face view of the anterior end. Long arrow marks the elongated mouth and the small arrow the denticle. Bar = 1 μm. 5. A phasmid (small arrow) lying across a lateral cord and anus (long arrow) lying slightly anteriorly. Bar = 2 μm. 6. The phasmids (2 lateral arrows) and anus (central arrow). Distinct annulations of the body are also visible. Bar = 1 μm. 7. A phasmid (arrow), showing the 2 membrane-like structures at the opening. Bar = 500 nm.**

### Acknowledgment

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# Monogeneans from Marine Fishes of Okinawa, Japan

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ABSTRACT: Monogeneans were recovered from 21 of 348 fishes representing 52 families, 115 genera, and 154 species between May and September 1985 in the coastal waters of Okinawa. Three families of Monogenea representing 9 genera and 12 species were recorded, including 6 monogeneans not previously known from Okinawa. Fifteen new host records were established. Only 1 of 12 species identified transgressed family bounds in their hosts. Representatives of *Protogyrodactylus* and *Lepidotrema,* heretofore reported only from freshwater teleosts, were also detected but not described.

KEY WORDS: Monogenea, survey, fish, Okinawa Island, Japan, new host records, new localities.

While contributions to our knowledge of monogeneans from marine fishes of the Japanese Archipelago have been made by numerous workers, probably the most comprehensive studies from this region have been those reported by Yamaguti (1934, 1937, 1938, 1939, 1940, 1942a, 1958). With the exception of the report by Yamaguti (1942b) on the digeneans of marine fishes of Okinawa, information on other helminth parasites of this region of the Japanese Archipelago is lacking. Thus, the present study was initiated to examine over a 5-month period as many fishes as possible from the coastal waters of Okinawa for monogeneans.

## Materials and Methods

Between May and September 1985, monogeneans were collected from 348 fishes representing 50 families of teleosts and 2 families of elasmobranchs (Table 1). A variable mesh gill net, seine traps, spearfishing and hook and line supplemented by quinaldine were used to collect species inhabiting shallow waters. Fishes were individually placed in plastic bags containing seawater and held in styrofoam containers for transport to the laboratory. Gills and skin were examined with a dissecting microscope. Specimens were immediately fixed and stored in AFA (alcohol–formalin–acetic acid). Some specimens were mounted unstained in Gray and Wess' medium for observation of sclerotized structures. Others were stained with Harris' hematoxylin, Mayer's carmalum, or Gomori's trichrome solution according to the technique of Kritsky et al. (1978). Voucher specimens have been deposited in the United States National Museum (USNM) Helminthological Collection, Beltsville, Maryland, under the accession numbers listed in Table 1. Other specimens are in the authors' collections.

Locality coordinates are given for each host listed in Table 1 except for a specimen of *Taeniura melanospila* (Bleeker) examined after the stingray died about 1 mo after being placed in the Okinawa Expo Memorial Park Aquarium, Motobu-cho, Okinawa, Japan, and a specimen of *Acanthopagrus sivicolus* Akazaki held at the Prefectural Fish Hatchery also located in Motobu-cho Okinawa.

## Results and Discussion

Monogenea were recovered from 21 (6.0%) of 348 fishes representing 15 (28.9%) of 52 families, 17 (14.8%) of 115 genera, and 21 (13.6%) of 154 species. The 12 species of monogeneans collected represented 3 families and 9 genera (Table 1). Of the 21 species of fishes that were infested, none harbored more than 1 species of Monogenea. Fish negative for monogeneans are listed in Appendix 1.

The intensity of a given species ranged from 1 to 1,000 monogeneans per host. Each of 7 fishes yielded 1–10 specimens; 8, 15–25; 1, 50; 1, 60; 2, 200; and 2, 1,000.

Of the fish species examined, the prevalence of infestation was high. Each of 11 (91.6%) of the 12 species of Monogenea recovered occurred on only 1 host species and 1 (20.0%) on 5.

Six of the 12 species are reported for Okinawa for the first time. These include: *Entobdella squamula*; *Ancyrocephalus spinicirrus*; *Haliotrema upenei*; *Pseudohaliotrema sphincteroporus*; *Protolamellodiscus convolutus*; and *Lamellodiscus elegans.*

To our knowledge, the following are new host records: *Benedenia seriolae* on *Cantherhines pardalis*; *Entobdella squamula* on *Taeniura melanospila*; *Metabenedeniella hoplognathi* on *Plectorhynchus chaetodontoides*; *Ancyrocephalus spinicirrus* on *Variola albimarginata*; *Haliotrema alatum* on *Acanthurus bariene*; *Haliotrema japonense* on *Zanclus cornutus*; *Haliotrema upe-*

**Table 1. Monogenetic flukes of marine fishes from coastal water of Okinawa, Japan.**

| Hosts | Parasite | Locality | No. hosts examined/no. infected/ mean intensity | USNM Helm. Coll. No. |
|---|---|---|---|---|
| Chondrichthyes | | | | |
| Dasyatididae | | | | |
| *Taeniura melanospila* Bleeker | *Entobdella squamula* (Heath, 1902) | | 1/1/1,000 | 80216 |
| Osteichthyes | | | | |
| Acanthuridae | | | | |
| *Acanthurus bariene* Lesson | *Haliotrema alatum* Yamaguti, 1942 | 26°39.18′N; 127°52.32′E | 1/1/25 | 80217 |
| *Acanthurus lineatus* (Linnaeus) | *Pseudohaliotrema sphincteroporus* Yamaguti, 1953 | 26°39.71–39.96′N; 127°52.09–52.50′E | 1/1/15 | 80224 |
| *Acanthurus nigrofuscus* (Forsskål) | *Haliotrema upenei* Yamaguti, 1953 | 26°37.95′N; 127°52.00′E | 2/1/10 | 80219 |
| *Acanthurus olivaceus* Schneider | *Pseudohaliotrema sphincteroporus* | 26°37.95′N; 127°52.00′E | 2/1/4 | 80225 |
| Belonidae | | | | |
| *Tylosurus crocodilus crocodilus* (Le Sueur) | *Ancyrocephalus* sp. | 26°37.95′N; 127°52.00′E | 1/1/1,000 | 80210 |
| Chaetodontidae | | | | |
| *Heniochus chrysostomus* Cuvier | *Pseudohaliotrema sphincteroporus* | 26°39.71–39.96′N; 127°52.09–52.50′E | 3/1/20 | 80228 |
| *Heniochus singularius* Smith and Radcliffe | *Ancyrocephalus* sp. | 26°39.71–39.96′N; 127°52.09–52.50′E | 1/1/6 | 80208 |
| Dactylopteridae | | | | |
| *Dactyloptena orientalis* (Cuvier) | *Protancyrocephalus strelkowi* Bychowsky, 1957 | 26°37.95′N; 127°52.00′E | 1/1/60 | 80223 |
| Gerreidae | | | | |
| *Gerres oyena* (Forsskål) | *Pseudohaliotrema sphincteroporus* | 26°37.95′N; 127°52.00′E | 1/1/50 | 80227 |
| Lethrinidae | | | | |
| *Lethrinus harak* (Forsskål) | *Protolamellodiscus convolutus* (Yamaguti, 1953) | 26°37.95′N; 127°52.00′E | 4/1/200 | 80220 |
| Monacanthidae | | | | |
| *Cantherhines pardalis* (Rüppell) | *Benedenia seriolae* Yamaguti, 1934 | 26°39.71–39.96′N; 120°52.09–52.50′E | 1/1/2 | 80213 |
| Pomadasyidae | | | | |
| *Plectorhynchus chaetodontoides* (Lacepède) | *Metabenedeniella hoplognathi* (Yamaguti, 1942) | 26°37.95′N; 127°52.00′E | 1/1/15 | 80222 |
| Scombridae | | | | |
| *Grammatorcynus bilineatus* (Rüppell) | *Caballerocotyla* sp. | 24°20.91′N; 123°42.32′E | 1/1/1 | 80214 |
| Scorpaenidae | | | | |
| *Pterois lunulata* Temminck and Schlegel | *Ancyrocephalus* sp. | 26°40.62′N; 127°49.20′E | 1/1/25 | 80209 |

**Table 1. Continued.**

| Hosts | Parasite | Locality | No. hosts examined/no. infected/ mean intensity | USNM Helm. Coll. No. |
|---|---|---|---|---|
| Serranidae | | | | |
| *Cephalopholis urodelus* Schneider | *Pseudohaliotrema sphincteroporus* | 26°39.71–39.96′N; 127°52.09–52.50′E | 1/1/20 | 80226 |
| *Plectropomus leopardus* (Lacepède) | *Entobdella* sp. | 26°39.18′N; 127°52.32′E | 1/1/6 | 80215 |
| *Variola albimarginata* Baissac | *Ancyrocephalus spinicirrus* Yamaguti, 1953 | 26°37.37′N; 127°51.77′E | 4/1/15 | 80211 |
| Sparidae | | | | |
| *Acanthopagrus sivicolus* Akazaki | *Lamellodiscus elegans* Bychowsky, 1957 | | 1/1/20 | 80221 |
| Tetraodontidae | | | | |
| *Arothron mappa* (Lesson) | *Benedenia synagris* Yamaguti, 1953 | 26°37.95′N; 127°52.00′E | 1/1/3 | 80212 |
| Zanclidae | | | | |
| *Zanclus cornutus* (Linnaeus) | *Haliotrema japonense* Yamaguti, 1934 | 26°39.71–39.96′N; 127°52.09–52.50′E | 1/1/200 | 80218 |

*nei* on *Acanthurus nitrofuscus*; *Protancyrocephalus strelkowi* on *Dactyloptena orientalis*; *Pseudohaliotrema sphincteroporus* on *Acanthurus lineatus, Acanthurus olivaceus, Heniochus chrysostomus, Gerres oyena,* and *Cephalopholis urodelus*; *Protolamellodiscus convolutus* on *Lethrinus harak*; and *Lamellodiscus elegans* on *Acanthopagrus sivicolus.*

In addition, representatives of 2 additional genera previously reported only from freshwater teleosts were collected. One represents a species of *Protogyrodactylus* from *Parupeneus spilurus* (Bleeker), and the other represents a species of *Lepidotrema* from *Epinephelis fasciatus* (Forsskål). We prefer to withhold description of these until additional specimens are available.

As visiting fish disease specialists, we (E.H.W., L.B.W.) were requested to examine a number of fish mortalities which occurred in Okinawa during our visit. Two of these involved Monogenea.

Two to 3 "minami-kurodai," *Acanthopagrus sivicolus,* 27 cm in standard length, reared in concrete, flow-through tanks at the Prefectural Fish Hatchery, Toguchi, Montobu-cho, Okinawa, began dying each day in each culture tank in late September 1985. We examined apparently stressed specimens on site from these tanks on 24 September. These fishes were heavily infested (51 to 100 parasites) (Williams, 1972) with *Lamellodiscus elegans.* A few nauplii and copepodid stages of a parasitic copepod (to be reported elsewhere) occurred on the skin and gill filaments of the fishes, which also had inflamed areas on their ventral surfaces. A static formalin treatment to remove the Monogenea was recommended.

Very heavy infestations (100+ parasites) (Williams and Phelps, 1976) of *Entobdella squamula* had recently killed 2 specimens of "madara-ei," *Taeniura melanospila,* when we were invited to the Expo Aquarium, Montobu-Cho, Okinawa, on 20 July 1985. We examined a freshly dead 34.5-kg female ray, 174 cm in total length, disc length 96 cm, width 104 cm, that was covered with monogeneans. The ray had been collected on 17 June 1985, at Haneji, Nago, Okinawa, and had survived slightly less than a month in captivity. This parasite has been a long-term problem at the aquarium and has caused mortalities in every species of dasyatid ray held in their large display tanks (Expo Aquarium Staff, pers. comm.). Due to the size of the tank, size, number, and diversity of aquarium animals, no treatment

could be designated that was more satisfactory than periodically replacing moribund rays.

Although 154 species of fishes were examined, the number of individuals of each species was regrettably low, resulting in insufficient data to form convincing conclusions on the prevalence and intensity of their parasites. Table 1 reveals a striking degree of specificity between the Monogenea and their hosts in Okinawa. Only 1 of the 12 species identified in this study, namely *Pseudohaliotrema sphincteroporus,* has been found to transgress family bounds on its hosts. While the present study provides information on new host and locality records, more extensive sampling of marine fishes of Okinawa is needed before a list of Monogenea approaching completeness can be presented.

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## Appendix 1

### Species of Fish Negative for Monogenea

(listed alphabetically by family with the number of individuals examined in parentheses)

**Chondrichthyes**

Rhincodontidae: *Rhincodon typus* Smith (1).

**Osteichthyes**

Acanthuridae: *Acanthurus bleekeri* Günther (1), *A. glaucopareius* Cuvier (2). Apogonidae: *Apogon aroubiensis* (Hombron and Jacquinot) (4), *A. cyanosoma* Bleeker (7), *A. doederleini* Jordan and Snyder (45), *Cheilodipterus macrodon* (Lacepède) (2), *C. quinquelineatus* Cuvier (39), *Rhabdamia gracilis* (Bleeker) (1), *Siphamia versicolor* (Smith and Radcliffe) (4). Atherinidae: *Atherion elymus* Jordan and Starks (2). Aulostomidae: *Aulostomus chinensis* (Linnaeus) (1). Balistidae: *Balistoides conspicillum* (Schneider) (1), *Rhinecanthus aculeatus* (Linnaeus) (1). Blenniidae: *Ecsenius lineatus* Klausewitz (3), *E. yaeyamaensis* (Aoyagi) (2), *Istiblennius lineatus* (Valenciennes) (1), *Meiacanthus atrodorsalis atrodorsalis* (Günther) (1), *M. grammistes* (Valenciennes) (2), *Plagiotremus laudandus* Whitley (2), *P. tapeinosoma* Bleeker (2), *Salarias fasciatus* (Bloch) (3). Callionymidae: *Diplogrammus xenicus* (Jordan and Thompson) (3). Carapodidae: *Encheliophis vermicularis* Müller (2). Centriscidae: *Aeoliscus strigatus* (Günther) (2). Chaetodontidae: *Chaetodon argentatus* Smith and Radcliffe (1), *C. bennetti* Cuvier (1). Cheilodactylidae: *Goniistius zebra* (Doderlein) (2). Cirrhitidae: *Cirrhitichthys aprinus* (Cuvier) (1), *C. falco* Randall (3), *Paracirrhites forsteri* (Schneider) (1). Diodontidae: *Diodon holocanthus* Linnaeus (5). Ephippididae: *Platax pinnatus* Linnaeus (1), *P. teira* (Forsskål) (1). Fistulariidae: *Fistularia petimba* Lacepède (2). Girrellidae: *Girella melanichthys* (Richardson) (2), *G. mezina* Jordan and Starks (1). Gobiesocidae: *Diademichthys lineatus* (Sauvage) (1). Gobiidae: *Amblyeleotris fasciata* (Herre) (1), *A. japonica* Takagi (5), *Bathygobius fuscus* (Rüppell) (3), *Chasmichthys dolichognathus* (Hilgendorf) (3), *Ctenogobiops feroculus* Lubbock and Polunin (1), *Eviota smaragdus* Jordan and Seale (2), *Gantholepis scapulostigma* Herre (2), *Istigobius campbellli* (Jordan and Snyder) (2), *I. decoratus* (Herre) (2), *I. ornatus* (Rüppell) (2), *Ptereleotris evides* (Jordan and Hubbs) (1), *P. heteroptera* (Bleeker) (2), *Trimma caudomaculata* Joshima and Araga (1), *Valenciennea puellaris* Tomiyama (2), *V* sp. (1), *V. strigata* (Broussonet) (4). Grammistidae: *Diploprion bifasciatus* Cuvier (1), *Grammistes sexlineatus* (Thunberg) (1). Holocentridae: *Flammeo sammara* (Forsskål) (1), *Myripristis violaceus* Bleeker (1). Labridae: *Bodianus axillaris* (Bennett) (3), *Cheilinus bimaculatus* Valenciennes (1), *Cheilio inermis* (Forsskål) (1), *Cirrhilabrus cyanopleura* (Bleeker) (2), *Coris aygula* Lacepède (2), *Halichoeres melanurus* (Bleeker) (1), *H. trimaculatus* (Quoy and Gaimard) (1), *Hemigymnus fasciatus* (Bloch) (1), *H. melapterus* (Bloch) (2), *Hologymnosus annulatus* (Lacepède) (1), *Labroides dimidiatus* (Valenciennes) (2), *Pseudochelinus hexataenia* (Bleeker) (1), *Pteragogus flagellifera* (Valenciennes) (1),

*Xyrichtys dea* Temminck and Schlegel (1). Lethrinidae: *Lethrinus semicinctus* Valenciennes (1), *Monotaxis grandoculis* (Forsskål) (2). Lutjanidae: *Lutjanus fulviflamma* (Forsskål) (3). Malacanthidae: *Malacanthus latovittatus* (Lacepède) (1). Monacanthidae: *Parapercis cylindrica* (Bloch) (1), *C. polyophthalma* (Cuvier) (1). Mullidae: *Upeneus tragula* Richardson (1). Muraenidae: *Echidna delicatula* (Kaup) (1), *Gymnothorax flavimarginatus* (Rüppell) (1). Nemipteridae: *Pentapodus nagasakiensis* (Tanaka) (1), *Scolopsis bilineatus* (Bloch) (1), *S. cancellatus* (Valenciennes) (2), *S. dubiosua* Weber (1). Ostraciidae: *Ostracion immaculatus* Temminck and Schlegel (1). Plesiopidae: *Calloplesiops altivelis* (Steindachner) (2). Pomacanthidae: *Centropyge ferrugatus* Randall and Burgess (1), *C. heraldi* Woods and Schultz (3), *C. tibicen* (Cuvier) (2), *Chaetodontoplus mesoleucus* (Bloch) (1), *Genicanthus lamarck* (Lacepède) (1), *Heniochus singularius* Smith and Radcliffe (1). Pomacentridae: *Amblyglyphidodon cauracao* (Bloch) (1), *A. leucogaster* (Bleeker) (1), *Amphiprion clarkii* Bennett (2), *A. frenatus* Brevoort (1), *Chromis flavomaculatus* Kamohava (2), *C. margaritifer* Fowler (2), *C. weberi* Fowler and Bean (1), *Chrysiptera cyanea* (Quoy and Gaimard) (1), *C. rex* (Snyder) (1), *C. starcki* (Allen) (1), *Dascyllus aruanus* (Linnaeus) (2), *D. trimaculatus* (Rüppell) (1), *Paraglyphidodon nigroris* (Cuvier) (1), *Pomacentrus alexanderae* Everman and Seale (2), *P. philippinus* Evermann and Seale (1), Pomadasyidae: *Plectorhynchus diagrammus* (Linnaeus) (1). Priacanthidae: *Priacanthus hamrur* (Forsskål) (1). Pseudochromidae: *Dampieria cyclophthalma* (Müller and Troschel) (2), *Pseudochromis porphyreus* Lubbock and Goldman (2). Scorpaenidae: *Dendrochirus zebra* (Quoy and Gaimard) (3), *Scorpaenopsis diabolus* (Cuvier) (3). Scorpididae: *Microcanthus strigatus* (Cuvier) (2). Serranidae: *Cephalopholis sexmaculatus* (Rüppell) (1), *Cromileptes altivelis* (Valenciennes) (1), *Epinephelus merra* Bloch (7), *E. summana* (Forsskål) (9), *Franzia squamipinnis* (Peters) (1), *Mirolabrichthys pascalus* (Jordan and Tanaka) (2), *Plectropomus leapardus* (Lacepède) (1). Siganidae: *Siganus argenteus* (Quoy and Gaimard) (1), *S. spinus* (Linnaeus) (1), *Variola louti* (Forsskål) (1). Sphyraenidae: *Sphyraena barracuda* Walbaum (1). Syngnathidae: *Corythoichthys haematopterus* (Bleeker) (2), *C. schultzi* Herald (1), *Dunckerocampus dactyliophorus* (Bleeker) (1). Synodontidae: *Saurida gracilis* (Quoy and Gaimard) (1), *Synodus variegatus* (Lacepède) (1). Tetraodontidae: *Arothron meleagris* (Schneider) (1), *Canthigaster valentini* (Bleeker) (1). Tripterygiidae: *Enneapterygius etheostomus* (Jordan and Seale) (1), *Helicogramma* sp. (5).

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# The Prehatching Development of *Leidynema portentosae* (Nematoda: Oxyuroidea)

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ABSTRACT: The embryonic development of *Leidynema portentosae* starts with a pseudocleavage. The first true cleavage is unequal, forming a larger AB and smaller $P_1$ blastomere. The second turn of cleavage starts with the division of the AB and then the $P_1$ blastomere. The "lima bean" stage of embryo has an indentation at the middle of the body. The first-stage juvenile fills the full length of the eggshell with the tail tip bent. The resting, second-stage juvenile is shortened with a diminished tail tip and the prehatching development ceases when this developmental stage is reached. The opercular groove of the eggshell always corresponds with the posterior end of the juvenile. The total time for prehatching development lasts approximately 73 (69–77) hr at 25°C.

KEY WORDS: *Leidynema portentosae,* Nematoda, Oxyuroidea, *Gromphadorhina portentosa,* cockroach, embryo, prehatching development, pseudocleavage.

Nematodes have a unique combination of favorable properties for the microscopic observation of development. These include small eggs that are usually transparent, reproducible cleavage leading to a juvenile with a constant number of cells and position, and relatively simple anatomy. They have attracted much research especially in recent years. Some species, such as the parasitic horse roundworm *Parascaris equorum* (zur Strassen, 1896; Boveri, 1899; Bonfig, 1925) and the free-living nematode *Caenorhabditis elegans* (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston and White, 1980; Sulston et al., 1983), have been extensively studied and complete cell lineages have been determined. However, relatively few parasitic nematodes especially among the Oxyuroidea have been studied from the standpoint of embryonic development. Only 1 species (Spiridonov, 1983) in the family Thelastomatidae and none in the genus *Leidynema* has been documented thus far, though Dobrovolny and Ackert (1934) described very briefly the prehatching juvenile stage of *L. appendiculatum.* The present research was undertaken to determine the prehatching development of *Leidynema portentosae* Van Waerebeke, 1978, a parasitic nematode of the Malagasy cockroach *Gromphadorhina portentosa.*

## Materials and Methods

Host cockroaches, *Gromphadorhina portentosa,* were supplied from the Insect Culturing Laboratory at The Ohio State University. Gravid females of *Leidynema portentosae* were removed from the hindgut of adult *G. portentosa* and placed in Ringer's invertebrate solution. Eggs were collected immediately after being laid by the female *L. portentosae* and transferred into another dish containing tap water that had been dechlorinated by standing for 2 days. The eggs were then incubated at 25°C (±0.1°C) and observed frequently until the prehatching development ceased. Ten eggs were studied. Progress of the prehatching development was recorded by microphotography. The nomenclature used here follows Boveri's terminology (1899) which is widely accepted.

## Results

The egg of *Leidynema portentosae* is oval, elongate, and flattened slightly on one side. The size of the egg is 115 × 42 μm and the thickness of the eggshell 2–3 μm. An opercular groove is present on one end of the eggshell that always corresponds with the posterior end of the future juvenile. At the time an egg was laid, the eggshell was filled by the cytoplasm with little air space (Fig. 1). However, in most cases, the cytoplasm of the egg was already condensed to various degrees when the egg was laid. The cytoplasm appeared rich with yolk granules that were evenly distributed within the cell. During the first hour, the cytoplasm of the egg condensed greatly forming a slightly elongated ball 48 × 38 μm in the center of the eggshell leaving 2 large spaces at both ends (Fig. 2). Towards the end of the first hour, a pseudocleavage appeared. The cell ($P_0$) started to pinch off incompletely at the middle of the cell body by a temporary constriction of the cell plasma membrane (Fig. 3). The cell, however, did not divide at this time. The anterior part quickly diminished to form a protuberant-like projection, pointing to the end opposite the

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operculum (Fig. 4). In some cases, the pseudodivision was less visible and the anterior part narrowed directly to form the projection. Finally the protuberance also disappeared (Fig. 5), but sometimes the diminished protuberance was retained until the first cleavage was finished. This whole process lasted approximately 20 min. About 10 min after the pseudocleavage, a constriction of cell plasma membrane reappeared at the posteriormost one-third of the cell $P_0$. At this time a transverse cell cleavage occurred forming a large anterior blastomere AB with a diameter of about 38 μm and a small posterior blastomere $P_1$ with a diameter of approximately 30 μm (Fig. 6). These 2 blastomeres were almost completely separated from each other for a short time when the division was completed, but then the contact area between them quickly increased. About 3 hr after the egg was laid, the large AB blastomere started to elongate perpendicularly to the axis of the egg. Because of the normal orientation of the egg, it was difficult to observe this process unless the egg was turned through a right angle. Before the division of the AB blastomere, the $P_1$ blastomere also started to elongate (Fig. 7). The cleavage of AB occurred first to give 2 blastomeres, A and B. The B blastomere migrated posteriorly before the cleavage was completed so that it became gradually visible (Fig. 8). At the very beginning after the AB cell started to elongate, the embryo appeared somewhat T-shaped, but it quickly became λ-shaped as the B blastomere continued to migrate. Next, $P_1$ divided into EMSt and $P_2$ with the former anterior and the latter posterior (Fig. 9). These four blastomeres, A, B, EMSt, and $P_2$, rearranged shortly after the completion of this cleavage cycle to form a rhomboid, with A anterior, B dorsal, EMSt ventral, and $P_2$ posterior (Fig. 10).

An hour later after the rhomboid formed, the blastomeres were ready for the next cycle of cell cleavages. At this point A and B almost simultaneously elongated in the direction nearly perpendicular to the axis of the egg and to the elongation axis of AB blastomere. In 10 min A gave rise to 2 blastomeres, a and α, with α slightly ahead of a, and B gave rise to 2 blastomeres, b and β, with β slightly ahead of b (Fig. 11). The constriction of EMSt followed and it split to form MSt and E with the former anterior and the latter posterior (Fig. 12). The last cell division in this cycle was the $P_2$ blastomere that started to elongate about 15 min after the EMSt split and gave rise to c and $P_3$ in approximately 15 min, with $P_3$ anterior and c posterior (Fig. 13). Thus an 8-cell stage appeared around 5½ hr after the egg was laid.

While cleavages continued, the total volume of the embryo was kept relatively unchanged although cell number increased constantly. The embryo appeared somewhat ball-shaped (Fig. 14). Nearly 21 hr after the egg was laid, the embryo became bean-shaped with an indentation in the middle of the body (Fig. 15). A small projection formed at the apex of the posterior end of the embryo indicating the initiation of embryo elongation (Fig. 16). The projection always pointed toward the operculum of the eggshell and became the tail-tip of the future juvenile. The first muscular contraction was observed between the beginning of embryo elongation and the embryo reaching two-thirds of its first-stage juvenile length (Fig. 17). After about 8 hr elongation, the embryo reached the full length of the first-stage juvenile. It filled almost the complete length of the eggshell with the tail tip bent. At this stage, the juvenile was quite active inside the eggshell. It was observed rotating and rocking frequently. The first-stage juvenile had a very short intestine, only about one-fifth of the body length, and a long esophagus which occupied almost four-fifths of the body length (Fig. 18). The body annulation appeared approximately 12 hr after the embryo reached its full length. The first-stage juvenile lasted 41 (38–45) hr. After that, the juvenile shortened and molted (Fig. 19). When this point was reached, the nematode simply ceased development and became a resting, second-stage

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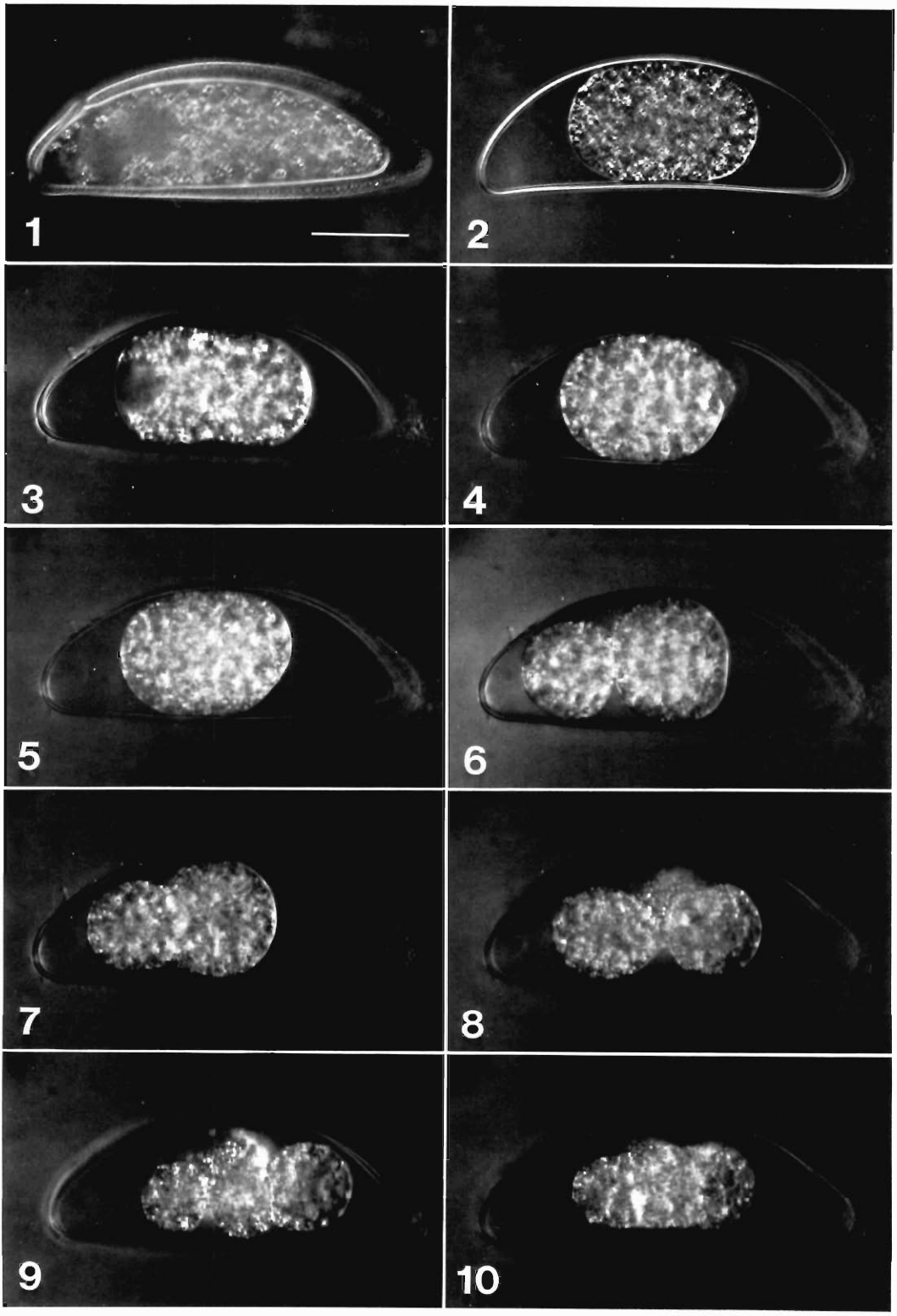
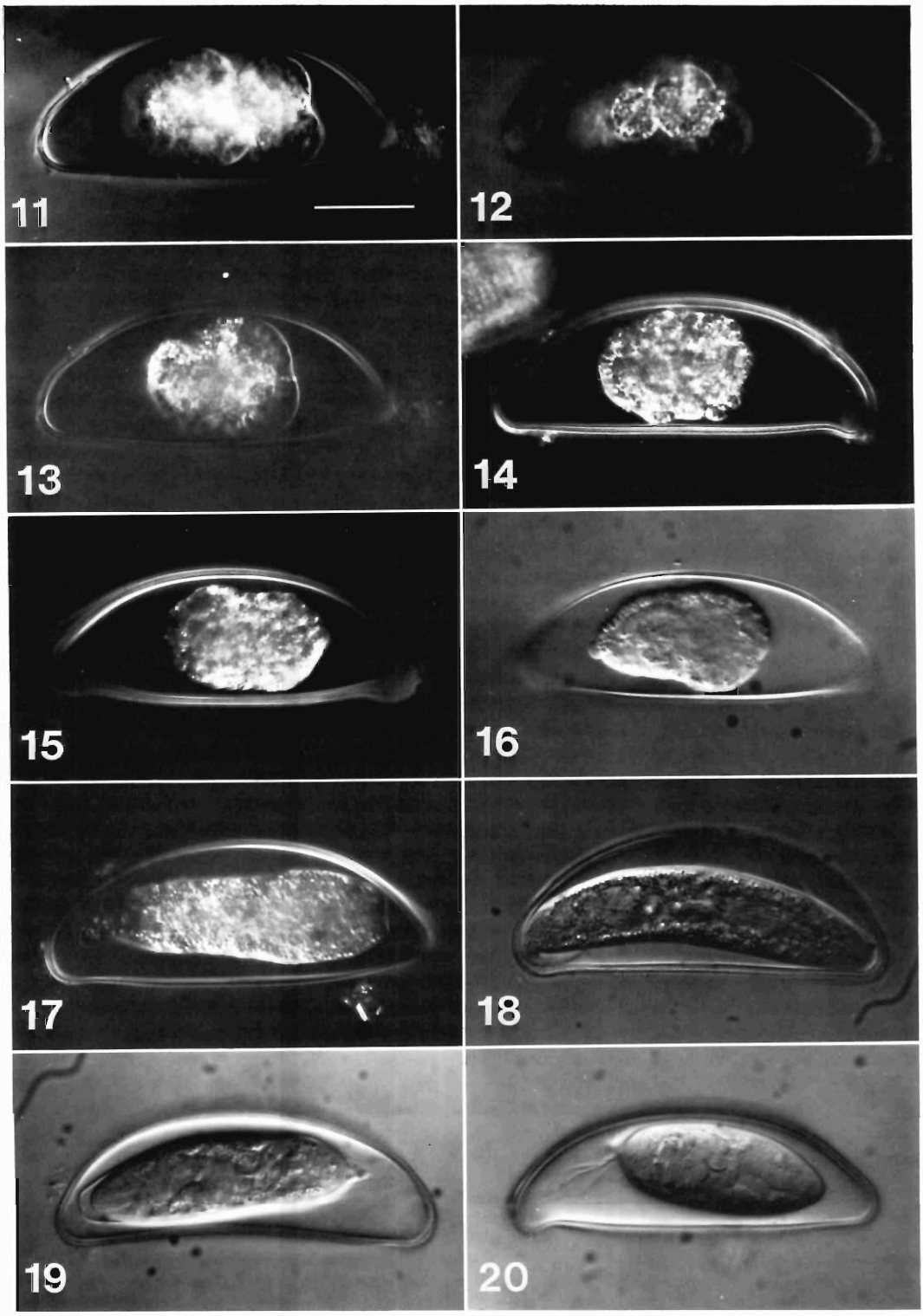
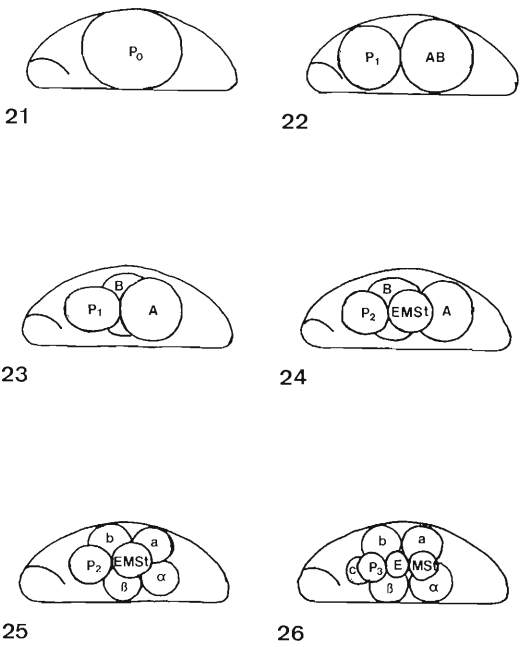
**Figures 1–10. The prehatching development of *Leidynema portentosae*. Bar = 30 μm for all photos. The time indicates the period from when the egg was laid until the photo was taken. 1. Egg completely filled with cytoplasm of $P_0$. 0 min. 2. Fully condensed $P_0$. 1 hr. 3. Constriction for pseudocleavage. 1 hr 30 min. 4. Diminishing of anterior part of $P_0$ to form protuberant-like projection. 1 hr 42 min. 5. End of pseudocleavage. 1 hr 47 min. 6. First cleavage completed forming AB and $P_1$ blastomeres. 2 hr 9 min. 7. Constriction of $P_1$ after AB. 3 hr 19 min. 8. B blastomere became visible after migration. 3 hr 27 min. 9. Four-blastomere stage (A, B, EMSt, and $P_2$). 3 hr 42 min. 10. Forming rhomboid. 4 hr 26 min.**





juvenile, which was only half the length of the first-stage juvenile, but with an increased body width (79 × 25 μm). The second-stage juvenile was oval-shaped with a relatively pointed mouth and a tiny tail tip (Fig. 20). The esophagus was two-thirds of the body length and the intestine was folded in the posterior end of the body. The juvenile stopped developing at this stage until it was ingested by another host. The total time for the prehatching development of *L. portentosae* within the eggshell was 73 (69–77) hr at 25°C.

The sequence of early blastomere cleavages of *L. portentosae* is summarized in Figures 21–26.




**Figures 21–26. Summary of early blastomere cleavage sequence of *Leidynema portentosae*. 21. Zygote stage. The operculum corresponds to posterior end of future juvenile. 22. Two-blastomere stage. 23. Three-blastomere stage. 24. Four-blastomere stage. 25. Six-blastomere stage. 26. Eight-blastomere stage.**

## Discussion

The embryonic cleavage of *Leidynema portentosae* is holoblastic, but neither radial nor spiral as is typical of most other nematodes (Nigon, 1965; Chitwood, 1974). The first cleavage of *L. portentosae* gives 2 unequal blastomeres, AB and $P_1$. The unequal first cleavage has also been reported in some other nematodes (Van Weerdt, 1960; Yuksel, 1960), although the AB and $P_1$ are about equal in *Parascaris equorum* (Boveri, 1899). The cleavage of AB in *L. portentosae* is longitudinal and oblique and the following $P_1$ cleavage is transverse so that the 4-blastomere stage is λ-shaped. This early cleavage pattern seems close to, but still differs from, that of *P. equorum,* which has a unique, T-shaped 4-blastomere stage (Boveri, 1899).

Pseudocleavage was reported in *Caenorhabditis elegans* and some other free living species (Nigon et al., 1960). The whole process in *L. portentosae* is similar to that of *C. elegans* except no posterior projection was reported in the latter species. A large gap between the outer eggshell and the anterior egg cytoplasm is formed after the pseudocleavage in *C. elegans,* which may be important to the embryonic development (von Ehrenstein and Schierenberg, 1980). In *L. portentosae,* the blastomere volume appears to be slightly smaller after the pseudocleavage, presumably due to further cytoplasmic condensation. However, increasing space between the eggshell and the cytoplasm may not have a significant meaning in *L. portentosae* since the large spaces are already present before this event. Therefore, the significance of pseudocleavage is unclear.

The embryonic development of nematodes can be divided into 2 phases, proliferation and morphogenesis (von Ehrenstein et al., 1979). In the proliferation phase, cell divisions continue until the embryo reaches a fixed cell number. The cells in this stage are rearranged considerably but all of them are essentially undifferentiated. In the morphogenesis phase, the embryo changes without additional cell division from ball-shaped to a form as a round worm with fully differentiated tissues and organs. Morphogenesis begins from

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**Figures 11–20. The prehatching development of *Leidynema portentosae* (continued). Bar = 30 μm for all photos. 11. Cleavages of A and B blastomeres to give a, α, b, and β blastomeres. 4 hr 43 min. 12. E and MSt formed from EMSt cleavage. 5 hr 3 min. 13. Cleavage of $P_2$ blastomere. 5 hr 34 min. 14. Nineteen-hour embryo. 15. "Lima-bean" stage embryo. 21 hr. 16. Tail projection formed. 23 hr. 17. Elongation of embryo. 26 hr. 18. First-stage juvenile. 54 hr. 19 Molting of first-stage juvenile. 71 hr. 20. Second-stage juvenile. 73 hr.**

the so-called "lima bean" stage in *C. elegans* (Krieg et al., 1978). An identical stage occurred in *L. portentosae* (Fig. 15). The embryo of *L. portentosae* starts elongation, and head, tail, and internal organs become differentiated soon after this stage. However, *L. portentosae* has its indentation at the middle of the body at the "lima bean" stage rather than at the posterior one-third as in *C. elegans*. Another noticeable characteristic is that the first muscular contraction can be observed between the beginning of embryo elongation (Fig. 16) and the embryo reaching two-thirds of its first-stage juvenile length (Fig. 17). A similar observation was reported in *C. elegans* between stage "comma" and "tadpole" (von Ehrenstein and Schierenberg, 1980).

The only species of Thelastomatidae for which the embryonic development has been studied is *Hammerschmidtiella diesingi* (Spiridonov, 1983). Most of the developmental stages of *H. diesingi* are similar to those of *L. portentosae* although the description documented for *H. diesingi* is less detailed. It was reported that in *H. diesingi* the second cleavage was started from the $P_1$ blastomere instead of the AB. However, since *H. diesingi* and *L. portentosae* are within the same family and since most of the developmental stages are so similar to each other, it is unlikely, though not yet proven, that *H. diesingi* has a different second cleavage pattern from that of *L. portentosae*. Constriction of the AB blastomere may occur first in *H. diesingi* as it does in *L. portentosae*, but it may have been overlooked by the observer because the elongation is perpendicular to the axis of the egg and because of the position the eggs usually lay, therefore making it difficult to see.

Another interesting characteristic of the first-stage juvenile of *L. portentosae* as well as *H. diesingi* is the very short intestine (Fig. 18). Spiridonov (1983) suggested that this resulted from the small size of the E blastomere, which is responsible for the development of the intestine.

In conclusion, the prehatching development of *L. portentosae* is essentially similar to that of most other nematodes and almost identical to that of *H. diesingi*. The major developmental stages, such as the pseudocleavage, the start of morphogenesis, and the muscle contraction, are even identical to the free-living nematode *C. elegans*, although they are quite distant systematically. Further studies of embryonic development of additional species in Thelastomatidae should provide more information on the basic developmental biology of this group of nematodes.

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## Obituary Notice

MERRITT P. SARLES

Elected Member: 20 March 1940
Vice-President: 1941–1942
President: 1954
Deceased: October 1988

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# *Trichuris elatoris* sp. n. (Nematoda: Trichuridae) from the Texas Kangaroo Rat (*Dipodomys elator*)

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ABSTRACT: This paper represents the first report of an endoparasite from the endangered Texas kangaroo rat (*Dipodomys elator*). Thirteen of 20 hosts examined harbored a previously undescribed species of trichurid. *Trichuris elatoris* is described and illustrated. SEM's of spicule and spicule sheath of both *Trichuris elatoris* and *Trichuris dipodomis* are included.

KEY WORDS: taxonomy, morphology, *Dipodomys elator, Trichuris dipodomis,* Trichuridae, nematode taxonomy, north-central Texas, southwestern Oklahoma.

The Texas kangaroo rat (*Dipodomys elator*) is limited in distribution to north-central Texas and 2 localities in southwestern Oklahoma (Baumgardner, 1987; Best, 1987; Jones et al., 1988). In a recent phenomorphic study among populations from 3 counties in Texas, Best (1987) observed considerable geographic differences in sexual dimorphism and morphometric variation. Best (1987) suggested that restricted resources, harsh peripheral environmental conditions, or perhaps even physiological dimorphism may account for the observed phenetic differences. In addition to morphological variation, significant interpopulation variation was also detected from electrophoretic samples (Hamilton et al., 1987). Because of the significant morphologic and genetic differences among populations within such a limited geographic area, we felt it would be of interest to examine hosts for possible impact of host variation on parasites.

The extent to which host isolation may have effected parasite speciation or the extent to which parasitism may have acted as a selective force on the hosts is not known. While examining *D. elator* for parasites we encountered a previously undescribed species of trichurid. The purposes of this paper are (1) to provide a description of the new species and (2) to include SEM's of the spicule and spicule sheath of both *T. elatoris* and *Trichuris dipodomis* Read, 1956. Information regarding prevalence and intensity of parasitism in *D. elator* will be presented elsewhere.

## Materials and Methods

*Dipodomys elator* were live-trapped from 7 localities in Texas on 12 and 13 March 1985. These are the same specimens examined by Hamilton et al. (1987) and are preserved as standard museum specimens and deposited at Texas Tech University. A scientific collecting permit was granted to E. Rex Wahl, and traps were provided by the Texas Parks and Wildlife Department. Partial funding was provided by The Nature Conservancy—Texas Natural Heritage Program.

We examined trichurids recovered from 13 infected hosts among 20 examined from Hardeman, Wilbarger, and Wichita counties, Texas. The material studied comprised 8 male and 6 female whipworms recovered from the large intestine of 13 infected hosts, of which 7 were infected females.

Nematodes were fixed in 36% acetic acid, gradually cleared in 70% ethanol/5% glycerin mixture followed by pure glycerin, and then stored in FGA (5 parts formalin, 5 parts glycerin, 90 parts 70% ethanol). Permanent mounts were in euparal (neutral mounting medium, Carolina Biological Supply Company). Measurements were obtained using a Graticules LTD (200 × 0.01 = 2 mm) stage micrometer. All illustrations were made using a Leitz laborlux binocular microscope with camera lucida attachment.

For scanning electron microscope (SEM) studies trichurids were subjected to a dehydration series (90%, 95%, absolute ethanol), critical point dried, coated with gold-palladium, and examined using an ISI-100B SEM at an accelerated voltage of 15 kV. All descriptive and illustrative measurements occur in millimeters unless stated otherwise. In the following description the character designation is followed by 3 measurements. The first measurement is that of the holotype or allotype, the second measurement is a range (in parentheses), and the third is a mean measurement. Where single measurements appear they were consistent for all specimens examined.

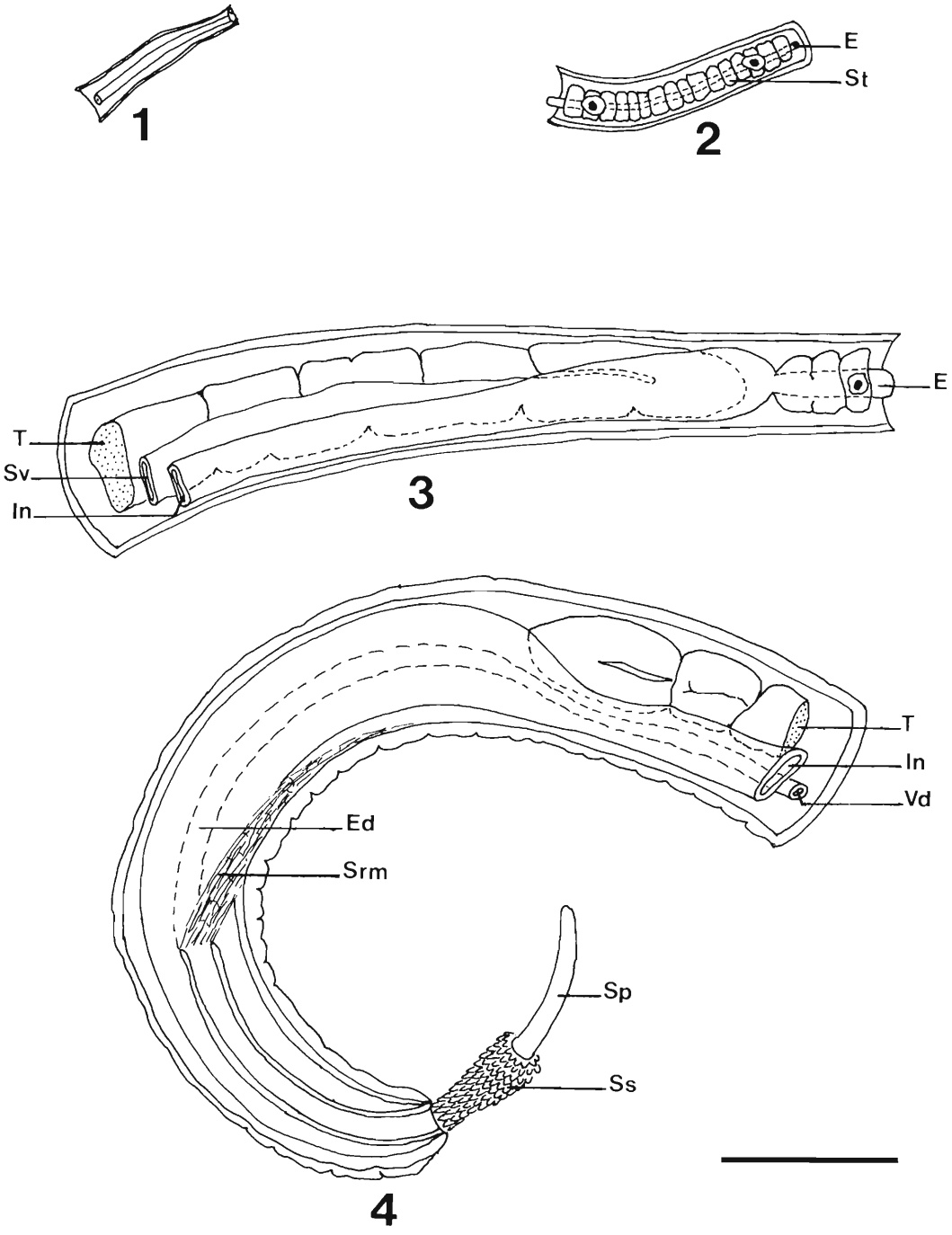
Type specimens of *Trichuris dipodomis* were obtained from the U.S. National Museum Helminthological Collection (U.S. Department of Agriculture, Beltsville, Maryland 20705) accession number 38035.

## *Trichuris elatoris* sp. n. (Figs. 1–8)

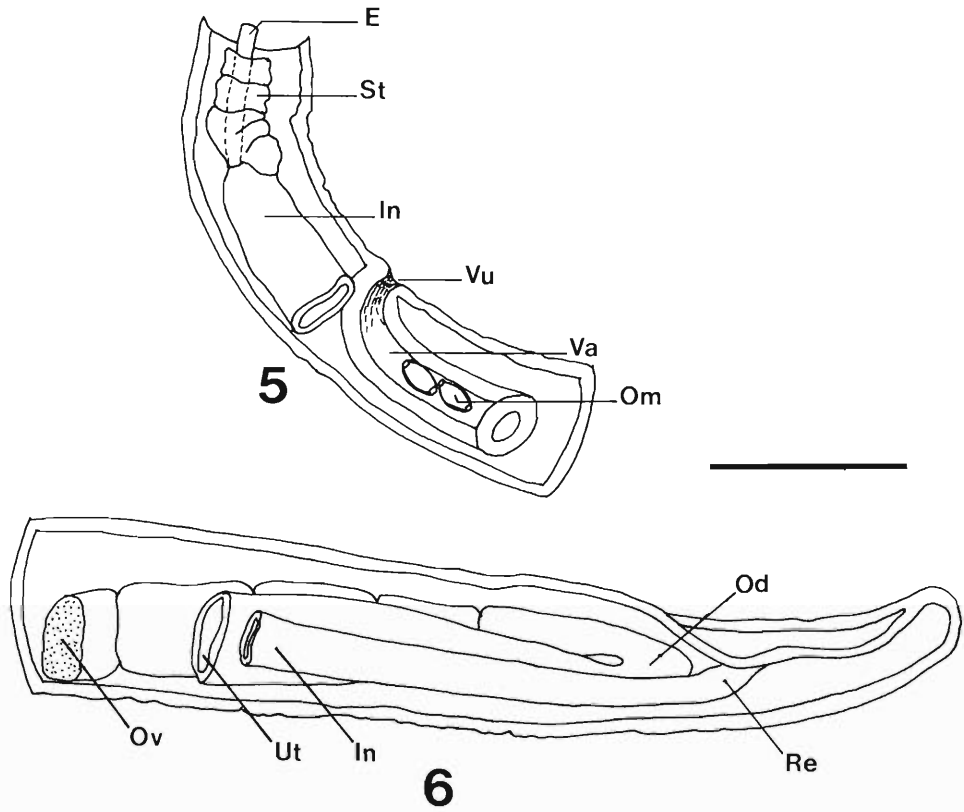
### Description

GENERAL: Trichuridae, with characteristics of the genus.

MALE (Figs. 1–4): Total length 18.75, (15.0–



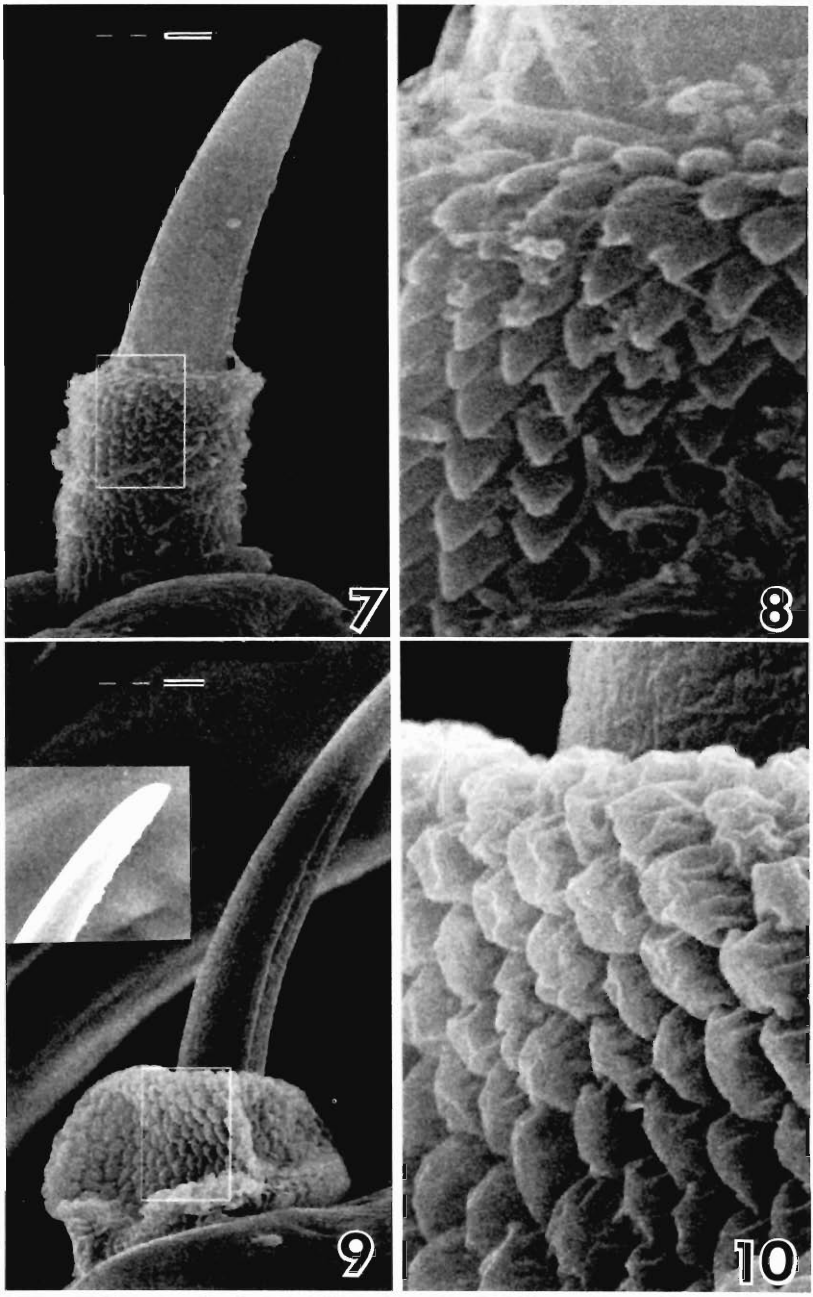

**Figures 1–4.** *Trichuris elatoris* **sp. n. 1. Anterior end of male and female. 2. Mid-stichosomal region of male and female. 3. The junction of the narrow anterior and posterior fleshy portions of the male. 4. The posterior end of the male. E = esophagus, Ed = ejaculatory duct, In = intestine, Sp = spicule, Srm = spicule retractor muscle, Ss = spicule sheath, St = stichocyte of stichosome, T = testis, Vd = vas deferens. Scale = 0.2 mm.**




**Figures 5, 6.** ***Trichuris elatoris*** **sp. n. 5. Junction of the narrow anterior and posterior fleshy portions of the female. 6. Posterior end of the female. E = esophagus, In = intestine, Od = oviduct, Om = ovum, Ov = ovary, Re = rectum, St = stichocyte of stichosome, Ut = uterus, Va = vagina, Vu = vulva. Scale = 0.2 mm.**

21.0), 18.81; narrow anterior end with collar (Fig. 1), length of narrow anterior end 9.45, (8.0–11.0), 10.1; length of fleshy posterior end 9.30, (8.0–11.0), 9.36. Stichosome (Fig. 2) begins 0.33, (0.31–0.34), 0.33 from collar. Stichocytes larger near mid-esophageal region (Fig. 2), mid-esophageal distance between stichocyte nuclei 0.20, interstichocyte nuclear distance decreasing to 0.13 near proximal and distal ends. Esophagus at anterior end 0.01 enlarging to 0.035 at junction with intestine. Testis arches into the seminal vesicle (Fig. 3) near esophageal–intestinal junction. Spicule (Figs. 4, 7, 8) without ornamentation or grooves, length 0.94, (0.92–1.03), 0.95; width of spicule at proximal end 0.05, (0.04–0.06), 0.05; at middle of shaft 0.03, (0.03–0.05), 0.035; at blunt distal end 0.014, (0.014–0.015), 0.014. Observed surface of spicule sheath (Figs. 4, 7, 8) covered with longitudinal columns of uniform-sized, pointed projections. Basal width of projections 12 μm, length to tip 18 μm. Shape of spicule sheath mostly parallels shaft of spicule (Fig. 7), may also appear slightly campanulate at apical end (Fig. 4). Spicular pouch 0.73, (0.70–0.75), 0.74 long. Spicule retractor muscle (Fig. 4) originates 0.4 from anal opening along ventral curvature of coiled tail. Cloaca 0.89, (0.85–1.07), 0.93 long. Ejaculatory duct (Fig. 4) 0.80, (0.73–1.22), 0.89 long; vas deferens (Fig. 4) 5.14, (4.22–6.80), 5.46 long. Testis lobated (Figs. 3, 4) with 47–56 sacculations, maximum width 0.10, (0.09–0.10), 0.10.

FEMALE (Figs. 5, 6): Body length 29.0, (25.0–31.0), 29.8; esophageal region (Fig. 2) 11.0, (9.0–12.0), 10.7; posterior body 18.0, (16.0–19.0), 18.3. Maximum body width 0.45, (0.33–0.50), 0.44, width at esophageal junction 0.18, (0.14–0.19), 0.18. Vulva (Fig. 5) without prominent lips, 0.23, (0.15–0.33), 0.24 from intestinal–esophageal junction. Length of vagina (Fig. 5) 0.62, (0.50–1.03), 0.62; width 0.07, (0.05–0.08), 0.067. Ovary (Fig. 6) 0.33, (0.29–0.34), 0.33 from posterior




**Figures 7–10. 7. Spicule and sheath of *Trichuris elatoris*. Scale = 10 μm. 8. Dual image from Figure 7, showing longitudinal rows of pointed projections. 9. Spicule and sheath of *T. dipodomis*. Notice ventral groove on spicule. Inset is tip of spicule. Scale = 10 μm. 10. Dual image from Figure 9, showing oblique rows of sacculelike projections.**

end. Rectum (Fig. 6) 0.07, (0.06–0.08), 0.07 long. Anus 0.2, (0.17–0.21), 0.19 from posterior end of bluntly rounded tail.

EGGS: Figure 5. Dimensions of eggs within vagina include length 0.061–0.066 (includes polar plugs), width 0.027–0.029.

TYPE HOST: *Dipodomys elator* Merriam, 1894.

LOCATION: Large intestine.

TYPE LOCALITY: 3.2 mi N Jct FM 2006 & US 287, Hardeman Co., Texas.

ETYMOLOGY: Named for host.

TYPE SPECIMENS: U.S.N.M. Helm. Coll. Nos. 78988 (holotype male), 78987 (allotype female), and 79015 (paratypes, vialed males and females). Specimens in Eastern New Mexico University Medical Zoology Collection under accession numbers 1363–1397 (slides) and 223–231 (vials).

LOCALITY OF INFECTED HOSTS: Number and sex of infected hosts collected at each of the following localities appear in parentheses following dates of collection. 12-III-85: (2 females) 2 mi W Harrold, Wilbarger Co.; (2 males) 2 mi W, 5 mi N, (1 male, 1 female) 9 mi N, Iowa Park, Wichita Co., Texas. 13-III-85: (1 female) 4.1 mi N, 3 mi W, (1 male) 3.5 mi N, 2 mi E, (1 male, 1 female) 3.8 mi N, 1.8 mi E, (1 male, 2 females) 3.2 mi N Jct FM 2006 & US 287, Hardeman Co., Texas.

## Discussion

Only 4 species of parasites, all ectoparasites, have been recovered from *D. elator* (Carter et al., 1985). Carter et al. (1985) also identified 19 different species of mammals that typically cohabit the same general areas where Texas kangaroo rats have been collected. Among those only 1 host (*Peromyscus maniculatus*) has been reported as host to a species of *Trichuris* (*Trichuris stansburyi* Frandsen and Grundmann, 1961). *Trichuris elatoris* is readily distinguished from *T. stansburyi* by the shape of the spicule. Spicular dimensions of *T. elatoris* demonstrate only a gradual taper from proximal to distal ends, whereas the proximal spicular end of *T. stansburyi* is 1.2–1.7× wider than mid-shaft which narrows abruptly toward the distal end (Frandsen and Grundmann, 1961).

Because the types of all other described *Trichuris* species were not examined during this study we encountered difficulty when we compared our description with other vague and oftentimes incomplete descriptions. From the literature we determined that *T. dipodomis* and *Trichuris perognathi* were closely related morphotypes. Although the Ord's kangaroo rat (*Dipodomys ordii* Woodhouse) has not been observed within the geographic range of *D. elator* (Carter et al., 1985), its trichurid parasite (*T. dipodomis*) appears to be most like *T. elatoris*.

*Trichuris elatoris, T. dipodomis,* and *T. perognathi* may be distinguished by combinations of spicular dimensions and body lengths. *Trichuris perognathi* is longer in body length (25.0–30.0) and possesses a spicular constriction between the broadened proximal end (0.05) and the expanded mid-portion (0.045) (Chandler, 1945). *Trichuris dipodomis* is characterized by an intermediate body length (19.7–25.1) and a much longer spicule (1.21–1.33; Read, 1956) than observed in the other 2 species. The width of the proximal spicule in *T. dipodomis* is also 1.5× greater than the 0.05 observed in the other 2 species. Finally, the body length of *T. elatoris* is somewhat shorter (15.0–21.0) as is the spicular length (0.92–1.03) when compared to *T. dipodomis*.

An SEM of the spicule and sheath of *T. dipodomis* is shown in Figures 9 and 10. By comparing Figures 7 and 8 with 9 and 10, additional differences are evident which may be of significance. The presence of sacculations on the sheath and an apparent groove along the ventral curvature of the spicule of *T. dipodomis* are enlightening. However, the relevance of these and other characters will only become meaningful when all described *Trichuris* species can be examined and compared.

## Acknowledgments

We thank E. Rex Wahl for obtaining the scientific collecting permit and assistance in the field; Derik de Bruin, Richelle Fawcett, Karen Poulos, and Sharon Willoughby for host necropsy; and Brad Kinneer for the scanning electron micrographs. We also thank 2 anonymous reviewers for their constructive comments.

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# DIAGNOSTIC PARASITOLOGY COURSE

## 31 July–11 August 1989

## Uniformed Services University of the Health Sciences

The first week of this course, 31 July–4 August 1989, will be on the diagnosis of helminthic infections and the second week, 7–11 August, will be devoted to the diagnosis of protozoan parasites. The course will consist of a series of lectures and laboratory exercises covering the diagnosis of parasitic infections of humans. In addition to the examination of specimens, participants will practice various methods used in the diagnosis of parasitic diseases. Diseases encountered worldwide will be included. The course will be held on the campus of the Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814. For further information, please contact Dr. John H. Cross or Dr. Edward Michelson at the Department of Preventive Medicine/Biometrics at the University or telephone (202) 295-3139 or 295-3138.

There will be a minimal charge for U.S. military and other U.S. government employees to cover the cost of supplies, and a cost of $400.00 to all others for either week of the course, in case a person only wants to enroll for one aspect of the course, or $600 for the entire 2-week course.

A previous background in laboratory methods is recommended.

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56(1), 1989, pp. 82–85

*Research Note*

# Occurrence of Larval *Sulcascaris sulcata* (Nematoda: Anisakidae) in the Calico Scallop, *Argopecten gibbus,* Collected Along the Eastern Coast of Florida, with Comments on Histopathology

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ABSTRACT: Data collected from a 3-yr study (1983–1985) are presented concerning the prevalence and intensity of third- and fourth-stage larvae of *Sulcascaris sulcata* larvae infecting the calico scallop, *Argopecten gibbus.* Of the 7,319 scallops examined during this 3-yr study, 1,973 (27%) were infected with 1 or more worms (range 1–6). Concerning infected scallops, 89% had single worm infections, 7% had 2 worms, 2% had 3 worms, and 1% had 4. No scallops had 5-worm infections and 1 scallop was infected with 6 nematodes. The effect that encapsulated *S. sulcata* have on the surrounding tissue is described. The response is characterized grossly by the presence of a yellowish-brown area surrounding the tightly coiled larva. Histological sections show a distinct reaction zone that included aggregates of inflammatory cells, fibroblasts, and connective tissue. The finding of necrotic nematode material in some scallops surrounded by a compact zone of dense connective tissue that included many histocytic elements suggests that the scallop possesses a defensive mechanism against infection. This study also reports metacestodes of a tetrarhynchean tapeworm encapsulated in the adductor muscle of the calico scallop. Histopathology associated with these metacestodes is described.

KEY WORDS: *Sulcascaris sulcata,* calico scallop, larval nematode, histopathology, *Argopecten gibbus,* parasite, prevalence, tetrarhynchoid plerocercoid, eastern Florida.

The presence of third- and fourth-stage anisakid larvae of *Sulcascaris sulcata* (Rudolphi, 1819) (as described by Lichtenfels et al., 1978) encapsulated in or on the adductor muscle of commercially important shellfishes has caused much concern from financial, aesthetic, and public health points of view. This larval nematode has been reported from 12 marine molluscan hosts (Lichtenfels et al., 1980) and 1 gastropod (Cannon, 1978) throughout the world. Along the eastern coast of Florida, the calico scallop, *Argopecten gibbus* (Linnaeus), represents the most commonly infected intermediate host of *S. sulcata.* These larvae mature in the esophago-gastric junction of molliscivorous marine turtles that feed on infected scallops (Berry and Cannon, 1981). Eggs, released by the mature worms into the gastrointestinal tract of the definitive host, pass out of the host with the feces and are filtered from the seawater by the scallop. This ascaridoid life cycle appears to be unique because it involves only 2 hosts. All other known marine ascaridoid life cycles involve 3 hosts.

Reports have documented prevalence and intensity data on these larvae in various hosts throughout the world (e.g., Cannon, 1978; Lichtenfels et al., 1980; Payne et al., 1980; Otwell et al., 1984; Barber et al., 1987) and survival of the helminth during commercial processing (Blake et al., 1985). The effect these encapsulated worms have on the surrounding tissue of *A. gibbus,* however, has not been studied. The present study provides additional prevalence and intensity data and describes the interactions between the calico scallop and these larval nematodes and between the scallop and a larval tetrarhynchean tapeworm.

Calico scallops were collected by commercial harvestors from beds offshore from Cape Canaveral, Florida, during 1983–1985. For histopathology studies, the scallops were immediately eviscerated and the adductor muscle was fixed in Bouin's solution and processed using standard procedures; 8-$\mu$m sections were stained with hematoxylin and eosin.

Of the 7,319 scallops examined during this study, 1,973 (27%) were infected with 1 or more worms. Concerning infected scallops, 89% had single-worm infections, 7% had 2 worms, 2% had 3 worms, and 1% had 4 worms. No scallops harbored 5-worm infections; 1 was infected with 6 nematodes (range 1–6). Table 1 summarizes prevalence and intensity data for this 3-yr study. Worm burdens fluctuated from various samples and different years. For example, in 1983, 450 (45%) of the 1,001 scallops were infected; in 1984, 512 (12%) of 4,229 were infected; and in 1985, 1,011 (48%) of 2,089 were infected. Otwell et al.

(1984) reported a similar percentage of infection (14 to 40%) during a survey in 1981–1982 of scallops from the southeast coast of Florida.

Encapsulation of a nematode in or on the muscle of a scallop was generally characterized by the presence of a yellowish-brown area surrounding the tightly coiled worm (Fig. 1). This distinct color usually alerts food inspectors and potential consumers to the presence of the parasite. Colored areas were more vivid when larvae encapsulated on the surface of the adductor muscle rather than within the muscle.

Histological sections of worms encapsulated in the adductor muscle of several scallops revealed some degree of host reaction in all specimens. Most nematode larvae appeared to be viable and were surrounded by a distinct reaction zone that included aggregates of inflammatory cells, fibroblasts, and connective tissue (Fig. 2). The reaction zone surrounding the nematodes varied in intensity. Necrotic nematodal material, found in some calico scallops, was surrounded by a compact zone of dense connective tissue with many histocytic elements.

The yellowish-brown color associated with nematodal encystment (Fig. 3) appeared to be a result of the host response to either the invasive nematode and subsequent breakdown and resorption of tissues or the excretory–secretory (ES) materials produced by the worms. Cannon (1978) reported that tissue surrounding encapsulated larval *Sulcascaris* in the Queensland sea scallop, *Amusium balloti* Bernardi, was stained orange or brown and speculated that ES products from the worms may contribute to the staining. Preliminary studies by Deardorff (1986) indicate that scallop nematodes produce bioactive materials similar to the ES materials of the third-stage larvae of *Anisakis simplex,* a closely related ascaridoid nematode (see Raybourne et al., 1983, 1986). Other encapsulated parasites, or their ES products, induce a distinct color in the surrounding tissue of their hosts. For example, a similar yellow color induced by the larval gnathostomatid nematode, *Echinocephalus pseudouncinatus* Millemann, 1963, in the foot of the pink abalone, *Haliotis corrugata* (Gray), and the metacercariae of the fellodistomatid digenean, *Proctoeces maculatus* (Looss), in the mantle of the hooked mussel, *Ischadium recurvum* (Rafinesque), were reported by Millemann (1951) and Turner (1985), respectively. The dark coloration caused by the hyperparasitic protozoa *Urosporidium spisuli* as reported by Perkins et al. (1975) was not observed in any of the scallops examined during this study.

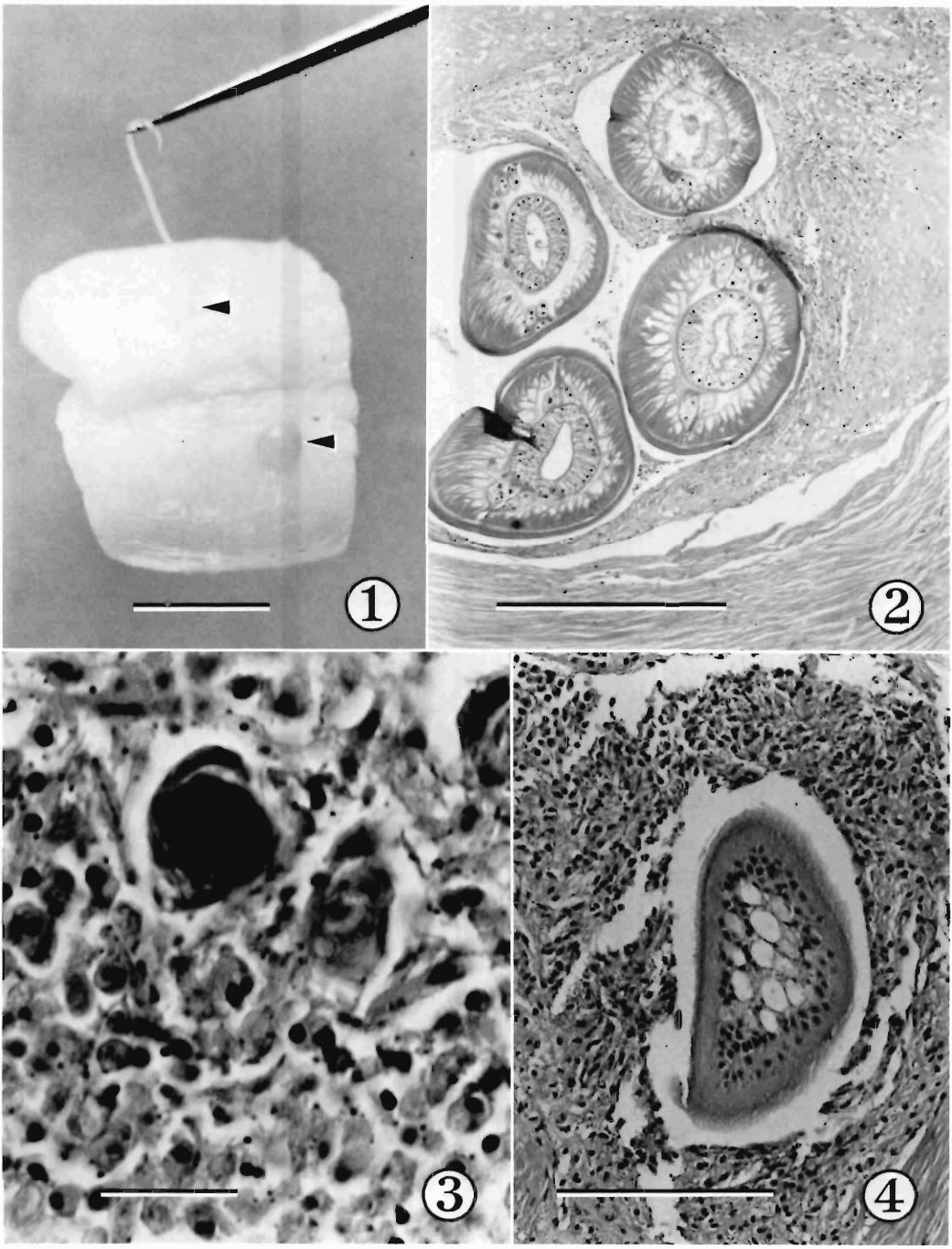
**Table 1. Prevalence and intensity of larval *Sulcascaris sulcata* in the calico scallop, *Argopecten gibbus*.**

| No. of worms/scallop | No. of hosts examined |
|---|---|
| 0 | 5,343 |
| 1 | 1,764 |
| 2 | 146 |
| 3 | 45 |
| 4 | 20 |
| 5 | 0 |
| 6 | 1 |
| | Total 7,319 |

Host response to the larvae varied in intensity, and fragments of degenerating nematodes were found within the muscles of the scallops suggesting a mechanism of host defense to infections; thus, the host may be effective in eventually ridding itself of the parasite. More research in this area is necessary.

Histological sections of the adductor muscle of 2 scallops revealed a metacestode, probably a plerocercoid tapeworm (Fig. 4), in addition to the larval nematode. In contrast to the host response to larval *S. sulcata,* the adductor muscles of scallops infected with the larval tapeworm were not yellowish brown. Gross examination gave no indication of the presence of the larval cestodes. The host response to the cestodes was less cellular and not as extensive as that of the larval nematodes.

Because of insufficient information, positive identification of the larval worm is not possible. In the only other report of a larval cestode infecting *A. gibbus,* Hutton (1964) did not identify the larval cestode from specimens collected from the Gulf of Mexico and did not list the site of infection, describe the host response, or mention the collection location of the scallops. It is possible that several species of larval tapeworms may infect *A. gibbus.* Cake (1975) found the Atlantic bay scallop, *A. irradians concentricus* (Say), collected from several Gulf coast localities, harbored the following larval and postlarval cestode species: *Acanthobothrium* sp., *Anthobothrium* sp., *Rhinebothrium* sp., *Eutetrarhynchus* sp., *Parachristianella* sp., *Polypocephalus* sp., and *Tylocephalum* sp. In some cases as many as 7 distinct species of larval and postlarval cestodes infected *A. i. concentricus.*




**Figures 1–4.** Parasites on or in the adductor muscle of calico scallop, *Argopecten gibbus*. 1. Yellowish-brown pustule (arrows) indicating an area of encystment of a fourth-stage larva of *Sulcascaris sulcata*; probe shows larval nematode being removed from pustule. Scale bar is 2 cm. 2. Extent of cellular response surrounding encapsulated nematodes. Scale bar is 300 μm. 3. Brown pigmentation surrounding encapsulated nematode. Scale bar is 20 μm. 4. Plerocercoid metacestode of a tetrarhynchean tapeworm. Scale bar is 100 μm.

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*Research Note*

# Helminths of the Giant Spotted Whiptail, *Cnemidophorus burti stictogrammus* (Sauria: Teiidae)

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ABSTRACT: Investigation of 57 specimens of *Cnemidophorus burti stictogrammus* revealed the presence of 4 nematodes (*Physaloptera retusa, Pharyngodon cnemidophori, Thubunaea cnemidophorus,* and *Skrjabinoptera phrynosoma*) and 1 cestode (*Oochoristica bivitellobata*). Prevalence of infection for helminth parasites was 14/57 (24.6%). The highest prevalence of infection (14.0%) was with *P. retusa.* Greatest mean intensity of infection (26.3) was recorded for *P. cnemidophori.*

KEY WORDS: Nematoda, cestode, prevalence, intensity, Teiidae, *Cnemidophorus burti stictogrammus.*

The giant spotted whiptail, *Cnemidophorus burti stictogrammus,* occurs in extreme southeastern Arizona, extreme southwestern New Mexico, and Sonora (Stebbins, 1985). In a previous note, Goldberg (1987a) found the tetrathyridia of a *Mesocestoides* sp. in this host. While there have been other reports on the helminths of North American *Cnemidophorus* (Read and Amrein, 1953; Babero and Matthias, 1967; Benes, 1985; Lyon, 1986; McAllister et al., 1986), this is the first helminthological survey on *C. b. stictogrammus.* The purpose of this note is to describe the prevalence and intensity of helminth infection in a population of *C. b. stictogrammus* from Tucson, Pima County, Arizona.

A total of 57 lizards collected by the senior author was examined. Fifty-six were collected from May through August 1966 while 1 was collected in April 1967. The majority of lizards were from Sabino Canyon (elev. 883 m), Santa Catalina Mountains, west of Tucson, Pima County, Arizona (32°20′N, 110°49′W). Six lizards were collected at the base of the Santa Catalina Mountains (elev. 907 m) at the north end of Campbell Avenue, north of Tucson, Pima County, Arizona. Lizards were shot with a 22 caliber pistol using dust shot and preserved in Bouin's fixative. They were later transferred to neutral buffered 10% formalin.

Of the 57 specimens examined, 14 *C. b. stictogrammus* contained helminths (including 1 with *Mesocestoides* sp.) (Goldberg, 1987a), for an infection prevalence of 24.6%. Prevalence, location, and mean intensity of infection are presented in Table 1. More lizards contained *Physaloptera retusa* than any other parasite. However, lizards had heavier infections with *Pharyngodon cnemidophori.* Representative specimens were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *Physaloptera* (80202), *Pharyngodon* (80203), and *Thubunaea* (80204).

Prevalence of infection in adult males (>89 mm snout–vent length [SVL]), adult females (>90 mm SVL) (Goldberg, 1987b), and juveniles according to month of collection is given in Table 2. Our small sample size prevents a meaningful discussion of seasonal trends in infection prevalences. However, the infection prevalence between male and female lizards was evaluated by the Kruskall-Wallis test, a nonparametric procedure where ranks are analyzed (Eckblad, 1984). The statistic calculated is approximately distributed as a chi-square. The calculated value (j = 0.08 with 1 df) indicates that infection prevalence between males and females was not significantly different ($P > 0.05$).

**Table 1. Prevalence, location, and intensity of gastrointestinal helminths in *Cnemidophorus burti stictogrammus.***

| Parasite | Prevalence (%) | Intensity mean (range) |
|---|---|---|
| Nematoda | | |
| *Physaloptera retusa** | 14.0 | 1.9 (1–5) |
| *Pharyngodon cnemidophori*† | 5.2 | 26.3 (18–35) |
| *Thubunaea cnemidophorus** | 3.5 | 2.0 (1–3) |
| *Skrajbinoptera phrynosoma** | 1.8 | 1.0 (1) |
| Cestoda | | |
| *Oochoristica bivitellobata*† | 1.8 | 1.0 (1) |

* Stomach.
† Large intestine.

**Table 2. Monthly percent prevalence of gastrointestinal nematodes in *Cnemidophorus burti stictogrammus*, April–September 1966.**

| | Adult | | | | | |
|---|---|---|---|---|---|---|
| | Male | | Female | | Juvenile | |
| Month | *N* | % infected | *N* | % infected | *N* | % infected |
| April | 0 | — | 0 | — | 1 | 0 |
| May | 3 | 0 | 2 | 0 | 0 | — |
| June | 12 | 16.7 | 5 | 20.0 | 0 | — |
| July | 8 | 37.5 | 5 | 0 | 1 | 0 |
| August | 10 | 10.0 | 4 | 25.0 | 0 | — |
| September | 2 | 100.0 | 4 | 75.0 | 0 | — |

Babero and Matthias (1967) described *Thubunaea cnemidophorus* in *Cnemidophorus tigris* from Clark County, Nevada. They also reported what they tentatively identified as *P. retusa* and the cestode *Oochoristica bivitellobata* from *C. tigris*. *Pharyngodon cnemidophori* was described by Read and Amrein (1953) from *Cnemidophorus tesselatus tesselatus*.

In conclusion, we have presented new host records and infection prevalences for 5 endoparasites infecting *C. b. stictogrammus* in southern Arizona.

We thank Linda Bone and Adrian Sales for assistance in collection of parasites.

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***Research Note***

# Attrition of Adult *Schistosoma mansoni* in A/J Mice

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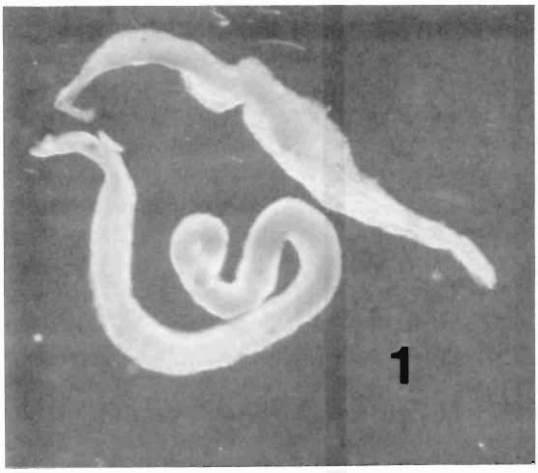
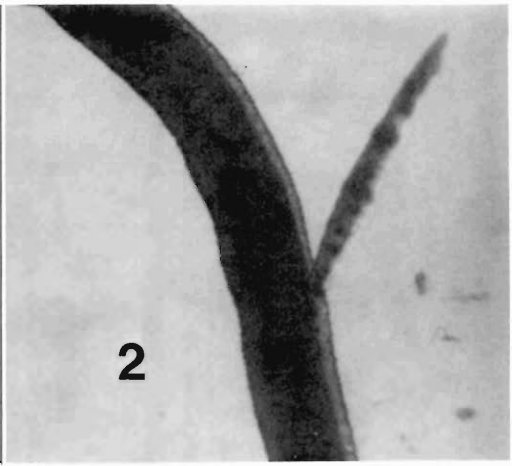
ABSTRACT: Inbred mice (A/J) were infected by tail immersion with *Schistosoma mansoni* cercariae and the adults isolated from the hepatic portal and mesenteric veins at 6, 7, 8, and 10 wk postinfection. Dead adult worms were not observed at 6 and 7 wk postinfection. At 8 and 10 wk, dead worms, predominantly females, were observed among the worm populations. The dead females were irregular in shape, very rigid, and much smaller than the normal adult females. They lacked pigment and were frequently situated within the gynecophoral canals of apparently healthy male worms. Viable eggs were observed within the tissues of one-half of the dead female worms.

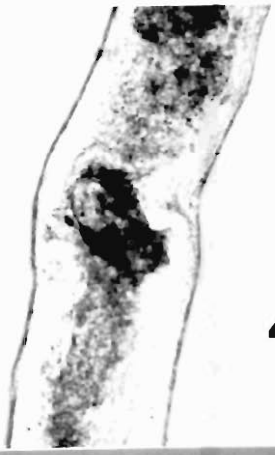
KEY WORDS: *Schistosoma mansoni*, adult worms, attrition, A/J mouse, permissive host.

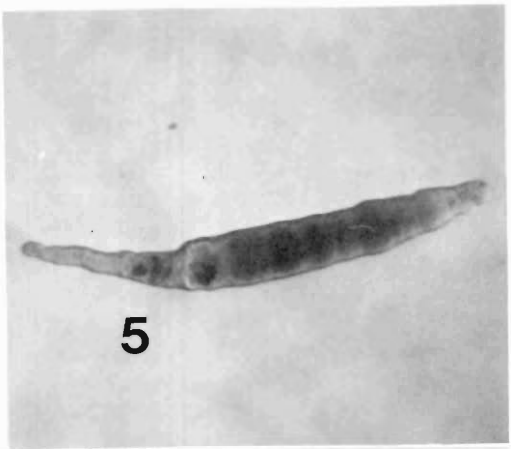
In recent years, much attention has been focused upon the elimination of larval schistosomes from permissive vertebrate hosts. In contrast, relatively little information is available on

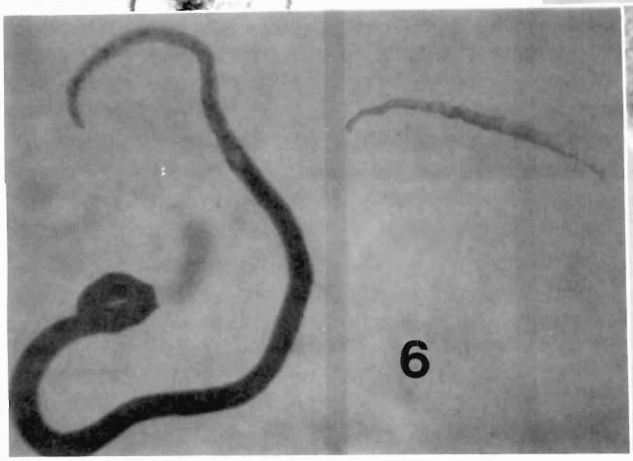


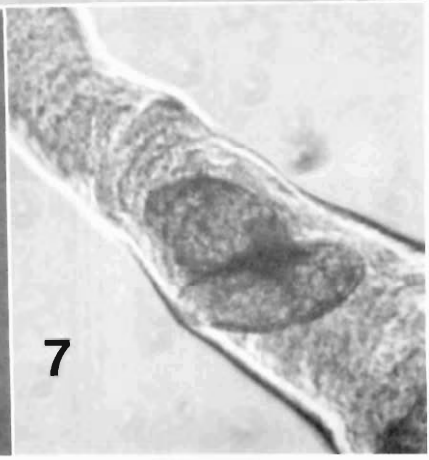







the elimination of adult schistosomes from these hosts. This reflects the widely held belief that, while larval stages are vulnerable to immune effectors during infection of permissive hosts, adults are long-lived and remain in the vertebrate host for some years. Nevertheless, reports do exist which describe attrition of adult populations in permissive hosts, thus suggesting that adult schistosomes also may be vulnerable to acquired host immune responses. These reports have relied on the numerical decrease in adult worm populations over time or the discovery of dead parasites in the livers of permissive hosts (for review see Damian, 1984). Death of adult worms in the final sites of *Schistosoma mansoni* infection, has not, however, been documented. The purpose of the work reported here, therefore, was to document attrition of adult *S. mansoni* within the mesenteric and portal veins of the A/J mouse, a permissive host for this parasite.

A Puerto Rican strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snails and laboratory mice by standard methods. A/J mice were infected with 75 cercariae per mouse by tail immersion (Bruce and Radke, 1971). Adult worms were recovered by perfusion (Duvall and DeWitt, 1967) at 6, 7, 8, and 10 wk postinfection and their viability determined by careful microscopic examination. Infections of longer than 10 wk duration were not investigated due to the high mouse mortality associated with schistosome egg deposition and its subsequent pathology.

Dead adult schistosomes were never observed in infections of less than 8 wk duration even though 500 mice were perfused and more than 15,000 viable adults were examined in the sixth and seventh wk of infection. At 8 wk postinfection, 1 dead female was observed among 1,500 live worms recovered from 47 mice. Examination of worms at 10 wk postinfection revealed 1 dead male and 5 dead female worms among the 1,800 live worms recovered from 58 mice (Figs. 1–7).

The dead female schistosomes only vaguely resembled living worms. They were irregularly shaped and very rigid (Figs. 3, 5), they possessed little pigment other than infrequent areas of pink—possibly due to undigested hemoglobin, and they were much smaller than living females (Fig. 6), ranging in size from 0.82 to 2.95 mm while living females were 5.64 to 9.74 mm in length. Three of the 6 dead females were found either entirely within, or protruding from, the gynecophoral canal of a living male. Eggs were found within 3 of the dead females, 1 worm possessing 2 eggs (Fig. 7). Only their location within the gynecophoral canals of living males and the presence of laterally-spined eggs clearly identified these worms as female *S. mansoni*. In 2 cases, miracidia were observed moving within these eggs, indicating their viability. The presence of viable eggs indicates that the reduction in size of the dead females was due to size regression rather than the stunting of females in immature stages. Size regression is also supported by the fact that no dead worms were observed at 6 or 7 wk postinfection even though the majority of worms were mature at this time.

The results presented here directly demonstrate that adult *S. mansoni* located within the mesenteric and hepatic portal veins are susceptible to elimination in a permissive host. Indeed, observation of dead worms in the perfusate may underestimate the level of this adult attrition. Dead adult worms in the venous circulation are difficult to observe because of their low numbers and the fact that dead females, due to their small size, may be entirely hidden within the gynecophoral canals of living male worms. It is also quite possible that dead worms which are not held within living males are swept away with the blood and quickly cleared from the circulatory system. This could explain the observed disparity in the numbers of dead females versus dead males observed.

The cause of the adult schistosome attrition is unknown, but it is occurring after worms have been mature for only a few weeks, long before senescence would be a factor. The fact that no dead worms were observed at 6 and 7 wk postinfection, even though most worms were mature at this time, suggests that mechanisms discrete from those acting against larval forms may be responsible. This delay in the appearance of dead

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**Figures 1–7. 1. Dead male schistosome with normal adult male, ×12. 2. Dead female within canal of living adult male, ×25. 3. Dead female schistosome, ×40. 4. Close-up of egg within dead female of Figure 3, ×100. 5. Dead female schistosome, ×40. 6. Dead female with live adult female, ×12. 7. Close-up of eggs within dead female of Figure 5, ×200.**

worms also suggests that acquired responses may be responsible for adult schistosome attrition in the mouse.

In nonpermissive hosts for *S. mansoni* infection such as the rat, adult worms are eliminated beginning at 4 wk postinfection (Smithers and Terry, 1965). While there is an immune component to this "self cure" (Phillips et al., 1975), host hormones are known to participate in the nonpermissive status of rats by preventing normal maturation and development of the worms (Knopf and Soliman, 1980). These developmentally stunted worms fail to migrate to the mesenteric veins and do not produce viable eggs (Cioli et al., 1977). Therefore, the self-cure phenomenon in nonpermissive hosts is fundamentally different from the adult attrition observed in the A/J mouse permissive host model where worms complete their maturation, producing viable eggs, before succumbing to as yet undetermined mechanisms of elimination. The elucidation of the mechanisms responsible for adult schistosome attrition in permissive laboratory hosts could be instrumental in the development of new strategies for the treatment and prevention of human schistosomiasis. If acquired immune responses are involved, then the development of an adult schistosome vaccine could complement those vaccines directed against larval stages which are currently under development.

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***Research Note***

# Morphometric Comparison of the Oocysts of *Cryptosporidium meleagridis* and *Cryptosporidium baileyi* from Birds

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ABSTRACT: The structures of oocysts of *Cryptosporidium meleagridis* from turkeys and *C. baileyi* from chickens were compared. Oocysts of *C. meleagridis* were 5.2 by 4.6 μm and contained sporozoites that were 5.2 by 1.4 μm. Oocysts of *C. baileyi* were 6.6 by 5.0 μm and contained sporozoites that were 6.1 by 1.5 μm. The length/width ratio was 1.13 for *C. meleagridis* and 1.33 for *C. baileyi*. The mean lengths, widths, and length/width ratios of oocysts were significantly different ($P < 0.05$) following analysis using the Student's *t*-test. Oocysts of both species were passed fully sporulated in the feces. Oocysts of *C. meleagridis* were infectious for young chicks.

KEY WORDS: chicken, turkey, oocyst, *Cryptosporidium meleagridis, C. baileyi.*

*Cryptosporidium* spp. are coccidian parasites that inhabit the microvillous border of a variety of epithelial surfaces of man and other vertebrates (reviewed by Fayer and Ungar, 1986). Four species have been named from birds, however, 2 of these species, *C. tyzzeri* and *C. anserinum,* were inadaquately described and their validity is questionable (Current et al., 1986). Slavin (1955) described the oocysts and endogenous life cycle of *C. meleagridis* from naturally infected turkey poults with diarrhea. He reported that oocysts were 4.5 by 4.0 μm and were passed unsporulated in the feces. These measurements were made on stained oocysts, and therefore, are smaller than the actual size of this species. No data were presented on sporulated oocysts or the structure of *C. meleagridis* sporozoites. Current et al. (1986) described the oocysts and life cycle of *C. baileyi* from experimentally infected chickens. Oocysts were 6.3 by 5.2 μm and were passed sporulated in the feces. The present study describes the structure of sporulated *C. meleagridis oocysts* and compares their structure to that of *C. baileyi* oocysts. Information reported in this study should aid in the diagnosis of species causing avian cryptosporidiosis.

Turkey feces containing *C. meleagridis* oocysts were obtained from Dr. M. G. Levy, North Carolina State University. Oocysts were then passaged in 2 groups of 5 3- to 4-day-old poults. To remove contaminating *Eimeria* species, the oocysts collected from the poults were passaged in 2 groups of 5 3- to 4-day-old chicks. Feces from poults and chicks were collected 5–10 days postinoculation. *Cryptosporidium meleagridis* oocysts were concentrated from feces by methods routinely used for *C. baileyi* (Lindsay et al., 1986a, b, 1987). Oocysts of the AU-B1 isolate of *C. baileyi* (Lindsay et al., 1986b) were used for structural comparisons. Oocysts of both species were stored in Hanks' balanced salt solution containing antibiotics (Lindsay et al., 1988) for less than 60 days prior to structural observations. Sporozoites were obtained from oocysts using methods previously described (Sundermann et al., 1987). Oocysts and sporozoites were examined with an Olympus BH-2 microscope equipped with Nomarski interference-contrast optics and a calibrated ocular micrometer. Measurements are expressed as means ± standard error of the mean followed by the ranges and number ($N$) of stages examined in parentheses. Means were evaluated using the Student's *t*-test (Huntsberger and Billingsley, 1977) to determine if significant differences ($P < 0.05$) in size were present.

Oocysts of *C. meleagridis* were passed fully sporulated in the feces of both poults and chicks. Small numbers of oocysts were passed by both poults and chicks during the 6-day collection period. Oocysts were spherical, irregularly spherical, or slightly elongate. Four sporozoites and an oocyst residuum were present. A micropyle was absent. The wall was about 0.5 μm thick. Oocysts were 5.2 ± 0.08 by 4.6 ± 0.04 μm (4.5–6.0 by 4.2–5.3 μm, $N = 40$). The length/width ratio was 1.13 ± 0.02 (1.00–1.33, $N = 40$). Sporozoites

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were released from the oocysts through a suture that formed in ⅓ to ½ of the oocyst wall. Excysted sporozoites were 5.2 ± 0.10 by 1.4 ± 0.03 μm (4.5–6.0 by 1.2–1.8 μm, $N = 40$). No refractile bodies were present in sporozoites.

Oocysts of *C. baileyi* are passed fully sporulated in the feces (Current et al., 1986; Lindsay et al., 1986b). They are usually elongate, contain an oocyst residuum, and lack a micropyle. The wall is about 0.5 μm thick. Oocysts of the AU-B1 isolate examined in this study were 6.6 ± 0.10 by 5.0 ± 0.05 μm (6.0–7.5 by 4.8–5.7 μm, $N = 40$). The length/width ratio was 1.33 ± 0.02 (1.05–1.79, $N = 40$). Excysted sporozoites were 6.1 ± 0.10 by 1.5 ± 0.03 μm (4.5–7.5 by 1.2–1.8 μm, $N = 40$). No refractile bodies were present in sporozoites.

The mean lengths, widths, and length/width ratios of the oocysts were significantly different ($P < 0.05$). The mean lengths of excysted sporozoites were significantly different ($P < 0.05$) but the widths were not ($P > 0.05$).

Upton and Current (1985) compared the structure of *C. muris* and *C. parvum* oocysts from mammals. They found that oocysts of *C. muris* were larger and more elongate than those of *C. parvum*. This is similar to the relationship that was found to exist between the size and shape of *C. baileyi* and *C. meleagridis* oocysts in the present study. Slavin (1955) also observed a similarity in oocyst structure between *C. meleagridis* and *C. parvum*.

Measurements of *C. baileyi* oocysts in the present study are similar to those previously reported (Current et al., 1986; Lindsay et al., 1986b). Measurements of *C. meleagridis* oocysts in the present study are slightly larger than those reported by Slavin (1955). However, the length/width ratios are identical (1.13). His use of fixed material probably accounts for these size differences.

*Cryptosporidium baileyi* has been shown to exhibit little host specificity for birds (Current et al., 1986; Lindsay et al., 1986a). It is not infectious for mammals. In the present study, *C. meleagridis* was transmitted to chicks. Further studies are needed to determine the susceptibility of other avian species and mammals to this parasite.

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On behalf of all the members of the Helminthological Society of Washington we thank J. Ralph Lichtenfels and Patricia A. Pilitt for the five years of outstanding service they gave to the Society as co-editors of the *Proceedings*. Our journal and our scientific discipline have grown as a result of their efforts.

The Editor

## New Books

*Monogenetic Trematodes; Their Systematics and Phylogeny,* by Boris E. Bychowsky, 1961, 627 pp. The English Version has been reprinted by the Virginia Institute of Marine Science and is available from the following address: Cashier, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062, U.S.A. Cost: $US 25.00 hardbound; $US 16.00 paperbound (includes postage and handling).

*Les Nematodes Syphaciinae, parasites de Rongeurs et de Lagomorphes. Taxonomie. Zoogeographie. Evolution,* by Jean-Pierre Hugot, 1988, Memoires du Museum National D'Histoire Naturelle, Serie A Zoologie, Tome 141, 153 pp. is available from Editions du Museum, Service de Vente, 38 Rue Geoffroy Saint-Hilaire, 75005 Paris. Cost: 208.00 Francs plus postage.

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

## HONORARY MEMBERS

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

## CHARTER MEMBERS 1910

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

## LIFE MEMBERS

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

* Deceased.

VOLUME 56 JANUARY 1989 NUMBER 1

## CONTENTS

(*Continued from Front Cover*)



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