PROCEEDINGS

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The Helminthological Society

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

CONTENTS

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The Sensitivity and Responses of *Rhabditis* sp. to Peripheral Mechanical Stimulation*

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ABSTRACT: The locomotory response of *Rhabditis* sp. to mechanical stimulation has been described. A technique for quantitating the intensity of the stimulus is outlined and the results of varying the stimulation are given. All stages of *Rhabditis* respond to mechanical stimulation, the direction of the response varying with the location of the stimulus. The whole sense shows adaptation, probably not at the sensory level, and the response is superimposed on the endogenous pacemaker system of the nematode. We interpret the reaction as an escape mechanism from predation, the mechanical threshold of the response approximating to that of contact with mites and predatory nematodes.

Tactile or mechanical stimuli must provide a significant part of the sensory information required by nematodes in their movement, hatching, feeding, mating and for many forms, their penetration into and migrations within hosts. The behavioral or physiological basis of the mechano-sensitivity in nematodes has, however, never been investigated (Lee, 1965). The setae and bristles of marine nematodes have been described as possible mechanoreceptors (Maggenti, 1964) and a peripheral nerve net has been demonstrated in *Deontostoma californicum* connecting the somatic setae (Croll and Maggenti, 1968). Inglis (1963), using the light microscope, observed 'campaniform-type receptors' in marine Cyatholaimidae, but this has been questioned by Wright and Hope (1968), the latter authors using electron micrographs.

Doubtless some of the tactile responses in the biological events listed above are the result of a complicated integration of senses, applying only to certain stages of nematodes. For these reasons we selected a simple, recognizable response: the short-lived locomotory response of *Rhabditis*, elicited by local mechanical stimulation.

* This work was supported in part by a Royal Society grant providing a 16 mm cine camera.

Materials and Methods

Rhabditis sp. were originally isolated from leaf litter, in Ascot, Berkshire, U.K. They were cultured on 3–5% water agar; at lower concentrations excessive migration into the agar occurred, a hazard for the experiments. The nematodes carried sufficient bacteria when subcultured to support their growth and reproduction. All stages responded to mechanical stimulation, nevertheless adults gave the most reliable results, because of their greater size, and so were preferentially selected.

Mechanical stimulation of the nematodes by a consistent and measurable force was achieved by using the apparatus figured (Fig. 1). Stimulation was observed through a binocular microscope, and effected by dropping a pin, of known weight (5.0 mg), down a directed glass capillary at 85° to the horizontal. The capillary was marked in millimetres, and an electromagnetic coil was moved up and down the capillary, permitting the pin to be released from a series of measured heights. The coil was wired to a 6 volt dry cell, and included resistances (totalling 8.9 ohms) to prevent overheating of the coil. A switch was included to release the pin by breaking the current, and for drawing the pin back. This design allowed immobile individuals to be found on the agar
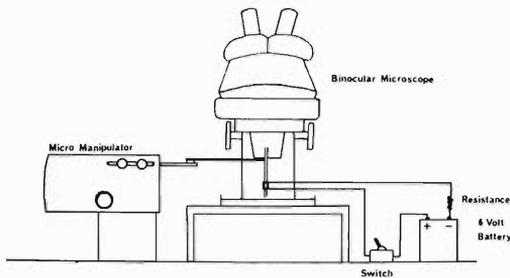
1

Figure 1. The design for stimulating nematodes, in a known region with a known kinetic force. A marked capillary was moved using the micromanipulator, and the electromagnetic coil was moved along the capillary, the switch released the pin (see text for further details).

Figure 2. The relative response, forwards (+) or backwards (−), when different parts of *Rhabditis* were given a uniform stimulus. Each worm was immobile, having not been previously stimulated.

cultures, the capillary to be aimed, and the pin released. Only stationary nematodes were used, and when the stimulated worms were activated to move continuously they were disregarded, this occurred in 3% of cases. The distance fallen was used to calculate the velocity in cm/sec., and expressed as kinetic energy in dynes/cm or ergs.

The nematode body was considered as being made up of 14 arbitrarily distinguished regions, each of equal length, from the anterior to the posterior tip. Sometimes it was difficult to be sure that the pin gave a 'square knock,' but we felt that any possible error was constant, and was compensated for in replication. Light elicited a slight photoresponse, when the nematodes were illuminated following a period of darkness, or after storage in dimmer light. A "cold light" of constant intensity was used, and the nematodes were always exposed to light for at least 10 min prior to experimentation.

Regional Sensitivity and Response to a Single Stimulus

Using a fresh individual for each record, a pin was dropped 3.0 cm (with a force of 14.5 ergs) onto each of the 14 regions of the body. At least 25 replications were made for each region. After stimulation, the number and direction of waves of locomotory movement was counted, to the nearest 0.5 of a wave. The waves were sufficiently slow to permit accurate counting through the binocular microscope, and cine films were analysed by frame analysis.

Results

All the nematodes responded when stimulated, irrespective of the regions of stimulation, but the extent and direction of the response varied (Fig. 2). If stimulated on the anterior half, the nematodes moved backwards, and they moved forwards if stimulated posteriorly. The direction of the wave was assumed simultaneously by all parts of the nematode, and it would be incorrect to assume that the wave was initiated posteriorly or anteriorly. Around the mid point, movement was forwards or backwards, but almost always unidirectional. Very rarely (3 individuals only) a hit half way down the body caused the waves in each half to move simultaneously in opposite directions. The extent of the response for the anterior regions ranged from 1.0 to 2.5 undulations (mean 2.19), and for all the posterior regions it was 1.0 to 2.2 undulations (mean 2.19). It was therefore treated as being of the same order in both halves. The first and fourteenth regions of the body, as well as the median region showed somewhat lower responses than did the remainder of the body.

Response to Stimulation of Variable Intensity

By dropping the pin from varying heights onto motionless individuals the response in undulations was measured. It was established

Figure 3. The variation in response, using an increasing intensity of stimulation. All individuals were stimulated at the oesophago-intestinal junction.

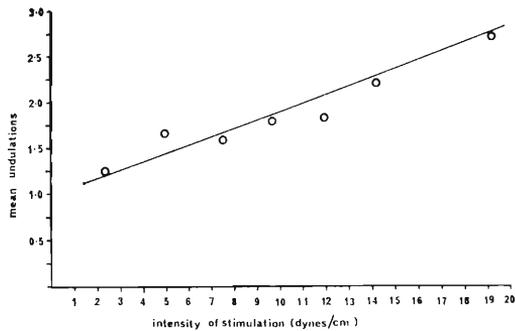Figure 4. A hypothetical model to explain the relationship between mechanical sensitivity and responses. Mechanical stimulation, from a number of different mechanical sensory receptors, elicits a co-ordinated response, superimposed on the pacemaker. Adaptation occurs in the whole sense, not at sensory level, and does not interfere with other senses. The dotted line represents an alternate system which connects the MSOs directly to motor nerves. (MSO = mechanical sensory receptor)

above that the response varied with the region of stimulation, all nematodes were therefore stimulated at the oesophago-intestinal junction. Each kinetic force was applied to at least 25 individuals.

Results

While individual responses varied quite widely, there was a consistently higher (and faster) response with an increasing intensity of stimulation. The relationship between ergs and undulations approximated to linearity up to 4 cm or 19.2 ergs (Fig. 3), this being the upper limit of our system.

Adaptation to Successive Stimulation

Individuals were repeatedly stimulated (using a drop of 0.5 cm, or 2.30 ergs) at the oesophago-intestinal junction, each successive stimulus being applied imediately following the response to the previous stimulation. The direction and extent of successive responses to stimulation were observed. Other individuals were stimulated at the tail end, following inactivity through repeated stimulation at the oesophago-intestinal junction. When no longer responsive to successive stimulation at the same point, some individuals were immediately immersed in a drop of hot (approximately 35 C) water, to expose them to a second source of stimulation.

Results

Each anterior stimulus consistently evoked a backwardly directed locomotion. After re-peated stimulation the nematodes became unresponsive. The mean number of stimuli required before there was no further response was 12, but varied from 4 to 21. The unresponsiveness lasted for about 15 sec, after which a locomotory response again resulted from further mechanical stimulation. Although the number of waves occasionally increased over the number resulting from the initial stimulus, the response always becomes weaker prior to unresponsiveness.

Nematodes that were unresponsive through successive stimulation at the oesophago-intestinal junction, were not responsive when stimulated posteriorly, the whole tactile sense becoming equally unresponsive. Individuals that were continuously moving forwards, could be stimulated to reverse when hit anteriorly. When adapted to successive stimuli, no further response could be observed and the active worms still moved forwards normally.

When unresponsive to mechanical stimulation, the worms responded vigorously to sudden immersion in water, 15° C above the ambient temperature.

Biological Interactions and direct observations

Mites and predatory nematodes in the Mononchidae were added to agar cultures of *Rhabditis sp.* and the interactions between *Rhabditis* and these predators observed.

Results

We found no evidence to support predatory mechanisms in prey locations, all the contacts appeared to be the result of random encounters. Some *Rhabditis* sp. were seen to draw away, both anteriorly and posteriorly, when suddenly contacting mites or predatory Dorylaimoidea, the reaction being more marked with the mites.

Responses of Isolated Pieces

In attempting to investigate the general neural pathways of the mechano-sensitivity, some individuals were cut using a fine pair of scissors and the resultant pieces were mechanically stimulated.

Results

Most pieces of the worm did not respond to mechanical stimulation, the pieces often collapsed, everting all the contents, although there were individuals which did respond after being cut. The anterior pieces largely remained normal in their sensitivity and responses, but most posterior halves collapsed. There were a few definite examples where posterior pieces, severed behind the nerve ring, showed distinct responses to mechanical stimulation.

Discussion

Rhabditid nematodes occur freely in the soil, feeding microbivorously, and are found in large numbers in areas of organic decay. Here they undoubtedly fall prey to other nematodes, mites, dipterous larvae and other small carnivores (Esser, 1963). It is tempting to think that the mechanical response described in this paper enables *Rhabditis* sp. to avoid these predators. Bilaterally symmetrical vermiform organisms tend toward cephalization, but in the sense described here, both extremities evoke a lower response to the same stimulation than the general body, and the sense does not show cephalization (Fig. 2). It is also possible that the fewer undulations resulting from stimulation of the head and tail, which are the narrowest fourteenths, may be due to more near misses or glancing blows, than at the wider parts of the nematode. Such blows would yield a stimulus of somewhat lower intensity, and would result in fewer undulations, in accordance with the data in Figure 3. If the biological basis of mechanoreception is in predator avoidance these observations may relate to

escape from lateral attack, and the probing head would have a higher threshold for its response, as it is continuously exposed to mechanical sensory stimulation. The threshold for the response is very low, being elicited by contact with predatory nematodes and arthropods. The mechanical stimulation required is very small, and we have observed the response being elicited when mites and nematodes touch *Rhabditis* sp.

From our observations we postulate a peripheral nerve system, of connected mechano-receptors, although we were unable to demonstrate a nerve net using the silver technique of Croll and Maggenti (1968). The peripheral sense shows adaptation but not at the receptor level, as an individual adapted in one area shows total adaptation to further stimulation in other regions. Adapted worms do however respond to other stimuli (e.g. heat), or revert to their original state of activity or inactivity. We therefore believe that the tactile sensitivity evokes a response which is superimposed on the inate endogenous rhythm, a conclusion which could be applied to the data for *Ditylenchus dipsaci*, reported by Doncaster and Webster (1968).

The presence of peripheral sensory cells has been established many times, in nematodes (reviewed by Bullock and Horridge, 1965), and the evidence here is that local excitation can propagate for some distance. The response may be a reflex, but the interpretation of direct responses from isolated pieces of nematode, must at best be tenuous, as there must be a gross sensory stimulation. The area of stimulation in each worm was constant, using a pin of constant size, although the extent of the response varied depending on the region of stimulation.

Rhabditids, often largely inactive in agar, are highly mobile in water. It is possible that a thigmo-orthokinesis occurs, when there is a low substrate feedback, leading to a maximum activity when a small area of the body is in contact with a solid substrate.

Neurophysiological work on many nematodes other than *Ascaris* (Jarman, 1959) is lacking, and is often complicated by small size and the nematode hydrostatic skeleton. The morphological and physiological basis of this response will await confirmation by electrophysiologists and electron microscopists.

A hypothetical system for the neural connections of the sense is presented (Fig. 4).

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Two New Species of Strigeid Metacercariae from an Indian Fresh Water Fish, *Xenentodon cancilla* (Ham.)

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ABSTRACT: Two new species of strigeid metacercariae from the Indian fresh water fish, *Xenentodon cancilla*, are described. They were found as concurrent infections in four of 81 fish from the reservoir, Nishatganj Bundha, and the local fish market. Encysted *Neascus hepatica* sp. n. were found in the liver, and encysted *Tetracotyle xenentodoni* sp. n. attached to the mesentery.

Sixty-one specimens of *Xenentodon cancilla* (Ham.) were examined by the writer from September 1966 to January 1968. Most of the fish were obtained from the local fish market, but eight were collected from "Nishatganj Bundha," a reservoir of the Gomti river at Lucknow; of the latter, only two specimens (12 August 1967) were found to have double infection with cysts of strigeid metacercariae. The metacercariae belonged to two separate larval strigeid groups, viz., *Neascus* Hughes, 1927 and *Tetracotyle* Fillipi, 1859. Twenty more specimens of *X. cancilla* were examined from the local fish market and "Nishatganj Bundha"; of these, two from fish market and one from "Nishatganj Bundha" were infected with both species of strigeid metacercariae. This apparently shows the occurrence of double infection in *Xenentodon cancilla* not to be accidental, but a usual phenomenon in nature. The metacercariae are described here as new species. All measurements are in millimeters.

Neascus hepatica sp. n.
(Figs. 1–6)

Cyst

Oval and transparent cysts (Fig. 1) containing the metacercariae were found embedded only in the liver of the fish. They numbered 15 to 25 in individual hosts. Cysts single layered, devoid of pigment, taking light stain with Mallory triple stain, measuring 0.72–0.87 by 0.44–0.52. Metacercarial body much smaller than cyst, consequently metacercaria performs free movements in the spacious accommodation available. Cyst also contains transparent fluid laden with excretory products. When
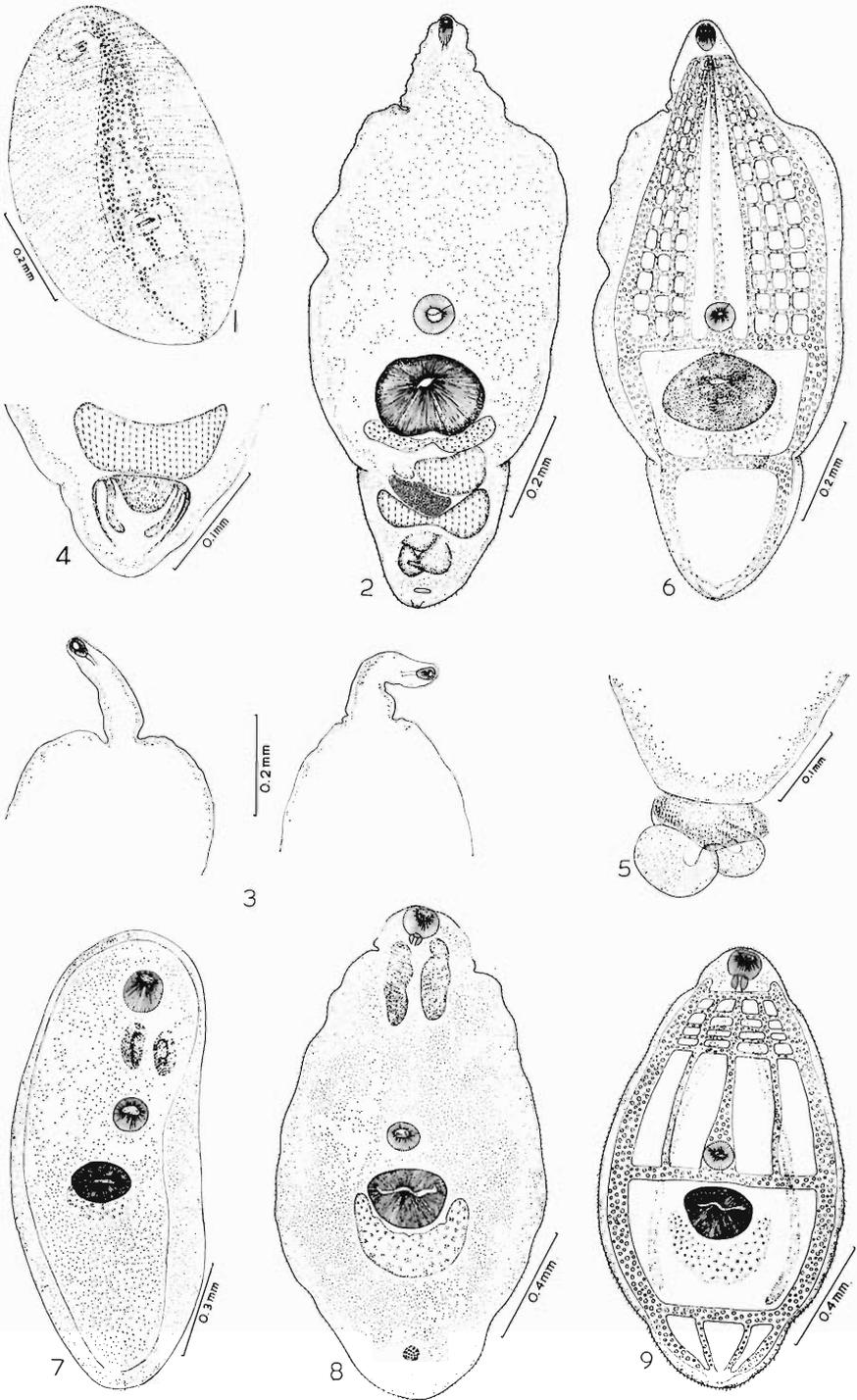
teased out in saline, the metacercaria performs active movements, particularly with its agile anterior part.

Metacercaria

Body (Fig. 2) spinose, foliaceous, well-marked into forebody and hindbody. Forebody broad and much larger than hindbody, its anterior end slender and much attenuated in some specimens (Fig. 3) measuring 0.46–0.75 in length and 0.23–0.39 in maximum width in region of ventral sucker. Hindbody truncated, measuring 0.18–0.26 in length and 0.15–0.24 in maximum width in region of posterior testis.

Suckers fairly well developed; oral sucker terminal, longitudinally elongated, measuring 0.04–0.07 by 0.03–0.05, ventral sucker slightly larger than oral sucker, circular in outline, located in postequatorial region of the forebody, measuring 0.05–0.09 in diameter. Holdfast organ strongly developed, situated behind ventral sucker in hind part of forebody. Holdfast gland a bilobed mass of dark staining cells, located close to posterior border of holdfast organ.

Esophagus short easily visible in live specimens (Fig. 6) but not in fixed and stained ones. Pharynx absent. Intestinal ceca in live specimens extending to level of anterior testis.

Fundaments of gonads in hindbody in form of three densely staining masses, two large and one comparatively small. Two larger masses, situated one behind the other represent testes; anterior mass smaller, subglobular, pear-shaped, or even oval in outline, deflected to the left of median line, measuring 0.10–0.15 by 0.06–0.10; posterior mass more variable in shape, being band-like, crescent-shaped, V-shape, or dumbbell shape transversely placed in median field, measuring 0.16–0.22 by 0.02–0.07. Smaller mass of cells, constituting pri-

mordium of ovary, elongated in outline, obliquely placed between the testicular anlagen, deflected slightly to right of median line, measuring 0.08–0.12 by 0.04–0.08. Mehlis' gland complex prefigured by small mass of dark staining cells located close to anterior side of ovary which often masks it.

Genital sinus (Fig. 4) containing copulatory bursa opening to outside by subterminal genital pore. Copulatory bursa when protruded through genital pore (Fig. 5) clearly reveals one median and two lateral pieces. In retracted condition, median piece conical and flanked (Fig. 4) by lateral pieces. When everted, three pieces of bursa capable of great extension, lateral pieces being more expansible and extending beyond median piece. Moreover, lateral pieces showing active twisting movements and often rolling at sides of median piece, forming infundibular structure resembling that of *Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936, figured by Ulmer (1961).

Reserve excretory system (Fig. 6) is of usual type found in *Neascus* group of strigeid larvae. V-shaped excretory bladder located at end of hindbody, opening to outside by terminal excretory pore.

Excretory canals of reserve system are as follows: Two main reserve excretory canals arising, one on each side, from cornua of excretory bladder, gradually widening as they approach region of ventral sucker thereafter, gradually becoming narrow as they proceed anteriorly towards the oral sucker where they interconnect by slender transverse canal, the anterior transverse canal. One median longitudinal canal and, on each side, three lateral longitudinal canals arising from anterior transverse canal. Median longitudinal canal running posteriorly along median line of body, widen-

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Figures 1–6. *Neascus hepatica* sp. n. 1. Encysted metacercaria. Drawn from a live specimen. 2. Ventral view. Drawn from a mounted specimen (hindbody slightly contracted) showing reproductive organs. 3. Ventral view. Drawn from mounted specimens showing the attenuated anterior end. 4. Ventral view. Drawn from a mounted specimen showing copulatory bursa in retracted condition. 5. Ventral view. Drawn from a mounted specimen showing copulatory bursa in everted condition. 6. Ventral view. Drawn from a live specimen showing reserve excretory system.

Figures 7–9. *Tetracotyle xenentodoni* sp. n. 7. Encysted metacercaria. Drawn from a live specimen. 8. Ventral view. Drawn from a mounted specimen showing reproductive organs. 9. Ventral view. Drawn from a live specimen showing reserve excretory system.

ing posteriorly from region of ventral sucker. Further behind ventral sucker, median longitudinal canal connected by transverse canal (median transverse canal) with main lateral reserve excretory canals. Median longitudinal canal continuing its course posteriad, greatly widening in zone of holdfast organ, eventually joining posterior transverse canal connecting directly the two main reserve excretory canals at level of junction of forebody and hindbody. Thus, in reserve excretory system of this metacercaria, three transverse excretory canals connect median longitudinal canal with main excretory canals.

The three lateral longitudinal canals, viz., internal, median, and external, of each side are equidistant, running parallel to one another and to median longitudinal excretory canal, eventually joining median transverse canal behind ventral sucker. Inner lateral longitudinal canal slightly more apart from median longitudinal canal than lateral longitudinal canals are from one another. Fine cross-connections, numbering about fifteen, existing between adjacent lateral longitudinal canals and main reserve excretory canal of each side, presenting lattice pattern with quadrangular or oblong meshes.

A colorless fluid with round corpuscles of different sizes present in all canals of reserve system. Fluid and corpuscles observed moving hither and thither within canals and also passing out of excretory pore with contractions of body.

Excretory tubules of the primary system could not be traced.

Discussion

Several species of strigeid metacercariae have been described under the larval group *Neascus* Hughes, 1927. Kaw (1950), for the first time in India, described a metacercaria of the larval genus *Neascus*, viz., *N. vetastai*. Khera (1958) described the second species, *N. chelai*. Subsequently, Thapar (1967) added two more forms, viz., *N. indicus* and *N. cirrhinus*. Recently, Pandey (1967) studied two apparently new species from freshwater fishes of Lucknow. To date, only seven Indian species of *Neascus* are known, viz., *N. vetastai*, *N. chelai*, *N. elongatus**, *N. indicus*, *N. cirrhi-*

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* Chakarbarti (1968) transfers *Diplostomulum elongatus* Singh, 1957 to *Neascus*.

nus and Pandey's species; of these, the present form closely resembles one of Pandey's forms from which it can be distinguished by its smaller body size and larger cysts. It also differs from it in having a spinose body and in the relative size of the suckers. In Pandey's form the ventral sucker is double the size of oral sucker, while in the present form the ventral sucker is slightly larger than oral sucker. Lastley, they differ in the pattern of the reserve excretory system.

Among the species found outside India, the present form resembles mostly *Neascus grandis* Mueller and Van Cleave, 1932 and *Neascus* of *Posthodiplostomum minimum* from which it differs in being apharyngeal. The present form further differs from *N. grandis* in having a holdfast gland, and from *Neascus* of *P. minimum* in the extension of the intestinal ceca to the anterior testis rather than to the posterior of the hindbody, and in details of the reserve excretory system.

Tetracotyle xenentodoni sp. n.
(Figs. 7–9)

Cyst

Cysts numbering four or five per host, found attached to the mesentery of visceral organs of *Xenentodon cancilla*. Cyst oval, elongated (Fig. 7) under cover-glass pressure measuring 1.14–1.64 by 0.54–0.78. Cyst wall tough, devoid of pigment, single-layered, fibrous in nature, staining faintly with Mallory triple stain, appearing partly parasitic in origin. Besides metacercaria, cysts contain fluid laden with chalky sediments. Metacercaria, when teased out of cyst in saline or in Tyrode's solution, showing active contraction and expansion as well as twisting movements of forepart of body.

Metacercaria

Body oval (Fig. 8), spinose, measuring 1.58–1.92 in length and 0.68–1.14 in maximum width in middle region. Suckers well developed. Oral sucker terminal, slightly larger than ventral sucker, measuring 0.09–0.13 by 0.12–0.17. Ventral sucker slightly preequatorial or at most equatorial, measuring 0.08–0.12 in diameter. Pseudosuckers large, fairly developed, located behind pharyngeal region. Holdfast organ strongly developed, bowl-shaped, located posterior to ventral sucker in

postequatorial field of the body, measuring 0.21–0.26 by 0.26–0.30. Large crescentic mass of dark staining cells, representing holdfast gland, located close behind holdfast organ, measuring 0.11–0.19 by 0.24–0.42.

Prepharynx absent. Pharynx subglobular, feebly muscular, measuring 0.05–0.07 in diameter. Esophagus (Fig. 9) short, distinct. Intestinal ceca extending up to posterior end of forebody as observed in live specimens, hardly traceable in stained and mounted specimens.

Fundaments of gonads represented by small mass of dark staining cells located mesially at posterior end of body.

Excretory bladder V-shaped (Fig. 9) but may present triangular appearance during contraction of body, located at hind end of body, opening to outside by subterminal excretory pore.

Details of the reserve excretory system: two main excretory canals of reserve system arise, one on each side, from ends of cornua of excretory bladder, appearing to be continuations of these; extending anteriorly along lateral margins of body, in their course are interconnected by the posterior transverse canal about mid-way between ventral sucker and hind end of body; continuing anteriad to pharyngeal region are again interconnected by anterior transverse canal. Median longitudinal canal and, on each side, one lateral longitudinal canal arising from the anterior transverse canal, former much thicker than latter. Median longitudinal canal gradually widening as it runs posteriorly, behind ventral sucker connecting with median transverse canal which unites with main lateral excretory canals. Smaller lateral longitudinal canals running backward through lateral fields of body, also joining median transverse canal behind ventral sucker. Each lateral longitudinal canal connected, in the anterior part of the body, with median longitudinal canal on one side and with main reserve excretory canal on other side by four thin cross-connections. Of three transverse canals found in reserve excretory system, anterior and median ones connect median longitudinal canal with lateral longitudinal canals and latter with main reserve excretory canals, while posterior one connects directly the two main reserve excretory canals. Further, two slender canals, arising from an-

terior aspect of excretory bladder, run forward to join posterior transverse canal.

All canals of reserve system containing transparent fluid laden with rounded corpuscles of different sizes. Fluid and corpuscles voided through the excretory pore when the body contracts.

Details of primary system of excretory tubules could not be traced due to extensive development of reserve system.

Discussion

The larval genus *Tetracotyle* Fillipi, 1859, includes only four Indian species, viz., *T. ranae* Kaw, 1950, *T. indicus* Singh, 1956, *T. sophoriensis* Singh, 1956, and *T. lucknowensis* Pandey, 1967; a description of a fifth species, viz., *T. muscularis*, is soon to be published (Chakrabarti, in press). Of these, the present form closely resembles *T. indicus*, but differs from it in the presence of spines, in the pattern of the reserve excretory system in the preequatorial position of the ventral sucker, and in the ratio of suckers. In *T. indicus* the ventral sucker is larger than oral sucker, whereas in the present form it is smaller.

Among the species of *Tetracotyle* found outside India the present form closely resembles *T. communis* Hughes, 1928, *T. diminuta* Hughes, 1928 and *T. biwaensis* Goto and Ozaki, 1930, but differs from them in the presence rather than absence of a holdfast gland, and in the relative size of suckers. In *T. communis* and *T. biwaensis* the ventral sucker is larger than the oral sucker, in *T. diminuta* the suckers are equal in size, while in the present form the ventral sucker is smaller than the oral sucker. Further, the present form differs from *T. communis* and *T. biwaensis* in the position of the ventral sucker: in the former the ventral sucker is roughly equatorial, whereas in the latter two species it is definitely postequatorial. The present form also differs from both *T. communis* and *T. diminuta* in the larger size of its body, and from the latter in the smaller size of its holdfast organ. From *T. biwaensis*, the present form differs further in having a spinose body.

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Population Study of Nematodes From Drying Beds

John L. Murad[1]

ABSTRACT: Moisture, temperature, biological oxygen demand and depth were parameters used to determine their effects on sludge drying bed nematodes either as single influencing factors or in combination. A multiple regression analysis of environmental factors showed that nematode numbers were moisture dependent in a positive correlation while the other single parameters varied between positive and negative relationships to nematode numbers during the 20 weeks of sampling. The results indicated that no one environmental factor affected nematode numbers as significantly as did their combinations. Rhabditid nematodes were the predominant population, peaked highest in June and were always present. Diplogasterids occurred in considerably fewer numbers, peaked in May and October, then ostensibly were absent at other times.

Cobb (1918) identified and described several new species of nematodes from slow sand filter beds. He noted that of the 35 species identified, one species made up 96% of the total population during certain periods of the year. Peters (1930) studied nemic populations in trickling filters and reported population fluctuations throughout the year.

Natural enemies of nematodes are known to produce harmful effects on nematodes (Dreschler, 1946; Dollfus, 1946; Doncaster, 1956; Duddington, 1957). Pipes (1965) found that predatory fungi caused a decline in the populations of rotifers and nematodes.

The cuticular remains of these dead organisms created a mechanical distribution problem in treatment plants due to bulking of the sludge. Metabolic by-products of other organisms in the sludge may also affect nematode populations (Murad, 1966).

This work was concerned with the influence that selected factors—moisture, temperature, biological oxygen demand and depth—might have on sludge bed nematodes either as single influencing factors or in combination.

Materials and Methods

One of six sludge beds at the Texas A & M disposal plant was used throughout this study. Experimental areas were enclosed by three wooden frames 30 cm² placed randomly

within the sludge bed. Samples were taken from within the frames at weekly intervals from April through October. Each sample was divided into subsamples from the 1-, 2-, 3- and 4-inch layers.

Temperature was read directly using a centigrade thermometer calibrated at 1-inch intervals. Reading were made at the same hour on each day of sampling. Moisture content was determined by placing a constant volume of sludge into a BPI watch glass which was then heated to 103 C for 24 hr. The biological oxygen demand (BOD) was determined by the Rideal-Stewart modification of Winkler's method (Theroux, 1943). The sludge remaining from each subsample was processed by the technique of Christie and Perry (1951) for nematode recovery. All samples were processed within three hours after initial collection.

A multiple regression analysis was used to determine relationships between the environmental factors and the variation in nematode number in the sludge (Snedecor, 1956). The variables used in this analysis included nematode number, moisture content, temperature, BOD and depth. For purpose of the equation, nematode numbers were considered dependent on all other variables of the equation; therefore, the term *dependent variable* was applied to the total number of nematodes of any sample. All other variables used in this analysis were assumed to be independent of any variation of nematode densities and therefore the term *independent variable* was applied to them. All independent variables were interacted to determine combination effects on the total number of nematodes. Variables with nonsignificant *t*-values were eliminated in successive order until only values for independent variables significant at the 5% level remained. The anaylsis for each sampling period ended when all remaining variables had *P*-values ≤ 0.05.

Results and Discussion

Cobb (1918) found *Mononchus longicaudatus, Ironus ignavus, Tripyla monohystera,* and *Ironus longicaudatus* to be the most common species from slow sand filter beds. From London, England, Peters (1930) reported *Diplogaster striatus, D. liratus, D. nudicapitatus, Diploscapter coronata, Dorylaimus saprophilus* and *Rhabditis* sp. to be most abundant in trick-ling filters. Freeliving nematodes from municipal water supplies in the U. S. were studied by Chang et al. (1960) and these nematodes were identified: *Aphelenchus, Cephalobus, Cheilobus, Pelodera chitwoodi, Diplogaster, Dorylaimus, Monohystera, Rhabditis* and *Turbatrix aceti.* By comparison, the genera most commonly found in this study were: *Rhabditis, Pelodera, Mesorhabditis, Plectus, Diplogaster* and *Dorylaimus.* Generally, four nematode families are represented in sewage throughout the world: Rhabditidae, Diplogasteridae, Mononchidae and Dorylaimidae. The nematode population variance for two nematode families in the Texas A & M drying beds is shown in Figure 1.

Of the nematodes identified in this study rhabditid and diplogasterid genera were the most common and abundant. Diplogasterids were first noted in April, reached their highest peak in May then declined sharply until early October when they again peaked followed by a rapid decline. Attempts to recover them from the sand layer (23 inches deep) during their absence from the top 4 inches of sludge were unsuccessful. The rhabditids were the predominant population and reached their highest peak in early June although lesser peaks occurred regularly during the 20-week period. May, June, and August showed only three weeks of sampling because the drying beds were routinely cleared and refilled during these months.

Comparisons of the mean number of nematodes in the separate layers showed interesting relationships to the total population curve in Figure 1. Nematode mean number from the 1-inch layer peaked in June and declined until September when it peaked again but to a lesser magnitude. In the 2-inch layer, the populations peaked highest in June and declined considerably for the remainder of the study. The 3-inch layer was similar to the 2-inch layer. The sand (4 inches) layer nematodes peaked in early and late July and again in mid-September. In general, the 1- and 4-inch layers showed less abundance of nematodes than did the other layers.

The moisture content varied most in the top layer and the sand layer. Exposure of the top layer reduced moisture through evaporation while the 4-inch layer retained less than 10% average moisture because of its sand structure.
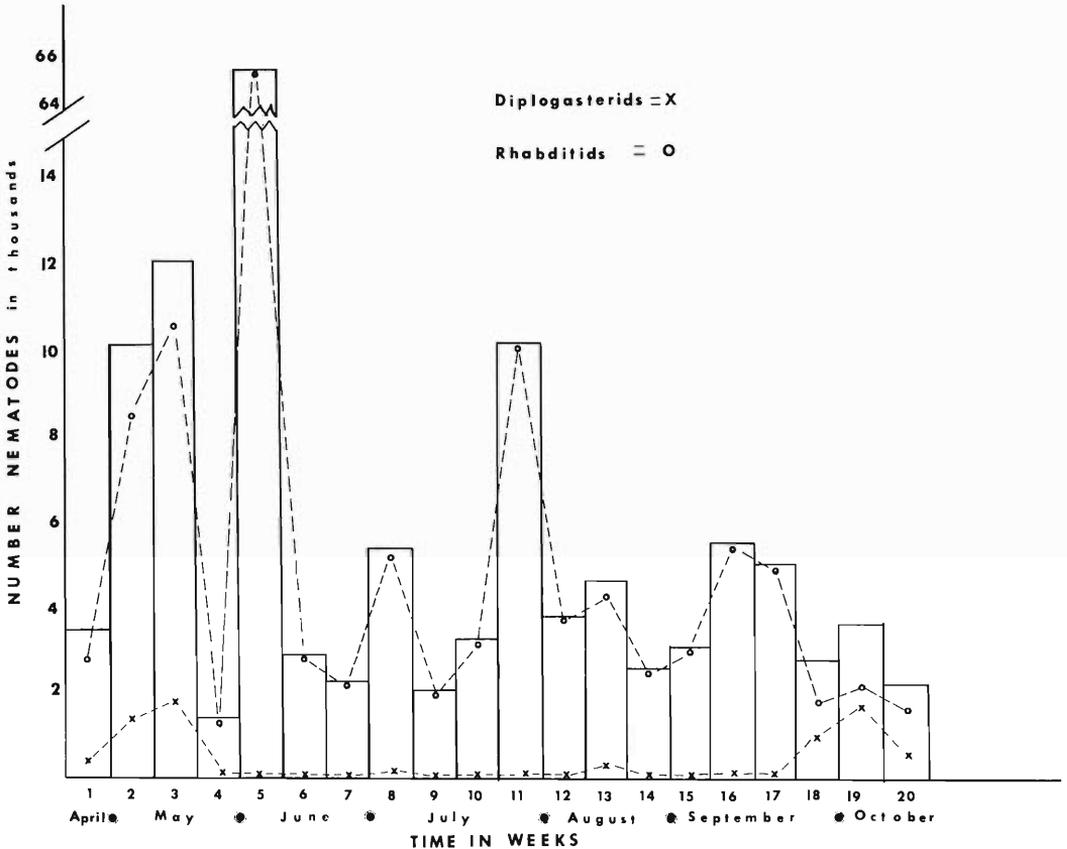
Figure 1. Weekly averages showing fluctuations in rhabditid and diplogasterid populations over a 20-week period.

Correlated with low moisture content were fewer nematodes. Layers 2 and 3 did not vary greatly from each other in moisture; likewise their nematode counts were similar.

Temperature of the sludge decreased as the sampling depth increased except in late September and early October. The BOD did not appear to be correlated with temperature, nematode numbers or moisture content.

The multiple regression analysis of environmental factors showed that nematode numbers were moisture dependent in a positive correlation while the other single parameters varied between positive and negative relationships to nematode abundance during different periods of sampling. However, when all the parameters were interacted, positive correla-

tions existed in all combinations except when temperature was involved. The greatest occurrence of significant values for all parameters was in May and June. The indication from these results is that no single environmental factor influenced nematode numbers as significantly as did combinations of those factors.

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A Histochemical Study of the Cyst Wall of the Metacercaria of *Nanophyetus salmincola* (Chapin)*

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ABSTRACT: A histochemical study of the metacercarial cyst of *Nanophyetus salmincola* was undertaken in order to ascertain the chemical nature of the structure. The cyst wall was examined for carbohydrates, lipids, proteins and enzymes and was found to consist of a carbohydrate-protein complex.

The general apearance of the metacercaria of *Nanophyetus salmincola* has been described by Bennington and Pratt (1960) and Gebhardt, et al. (1966). They describe the cyst wall as a thin, glistening, transparent structure that thickens as the metacercaria ages. Bennington and Pratt (1960) state that the cyst wall of the parasite is formed by the parasite and consists of only one layer. Wood and Yasutake (1956) studied the histopathology associated with the metacercaria on *N. salmincola* and found little tissue reaction or inflammation around the parasite. To this date only histological techniques have been used to study the metacercarial cyst of *N. salmincola* even though various investigators have demonstrated the use of histochemical techniques in ascertaining the chemical nature of cyst walls (Singh and Lewert, 1959; Bogitsh, 1962;

Dixon, 1965; Erasmus, 1967; Rees, 1967; and Thakur and Cheng, 1968). The present study was undertaken to explore more fully the histochemical nature of the cyst of *N. salmincola*.

Materials and Methods

The kidneys of several species of salmon were collected from state fish hatcheries and the metacercaria recovered by using the homogenation-sedimentation technique devised by Nyberg (in Gebhardt, et. al. 1966). Approximately 50 harvested metacercaria were inserted into a slit cut in a small piece of salmon kidney, and sectioned at 8 μ in a cryostat. The sections were air dried or fixed in 10% phosphate buffered formalin depending on the test to be carried out. The histochemical tests carried out were mainly those suggested by Barka and Anderson (1965). Tests were selected to investigate the presence of various categories of proteins, carbohydrates, lipids, connective tissue, and enzymes.
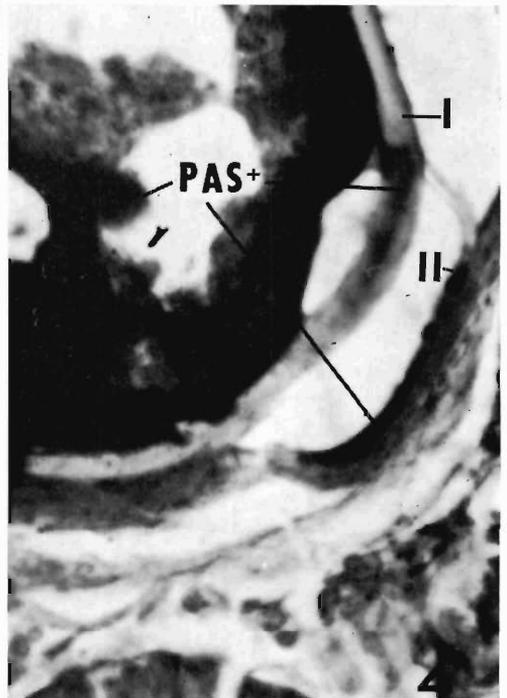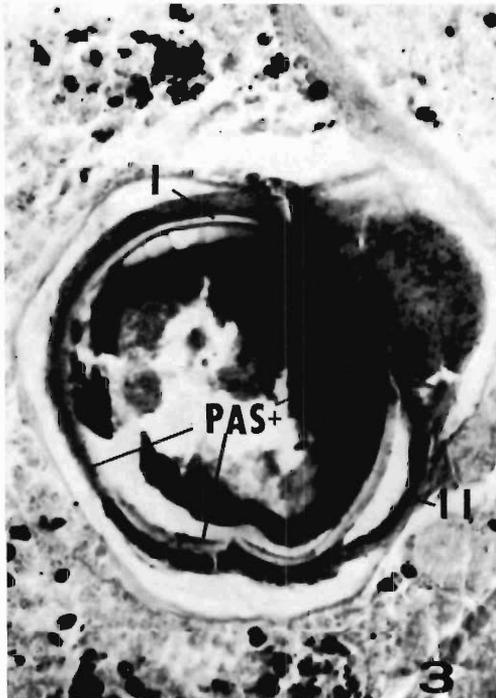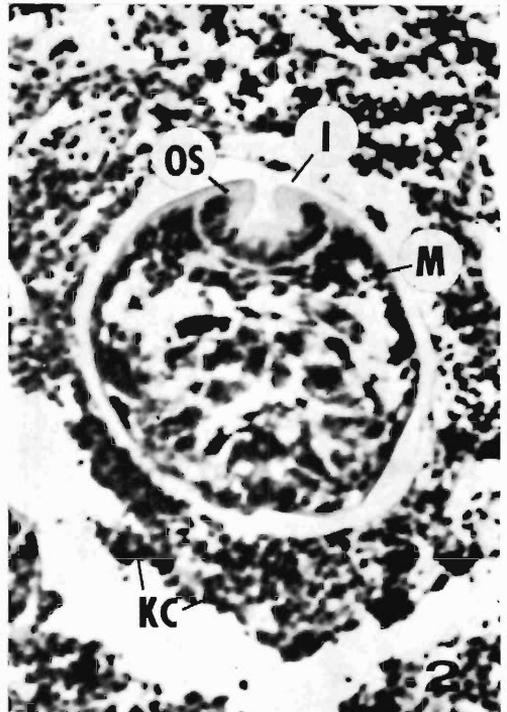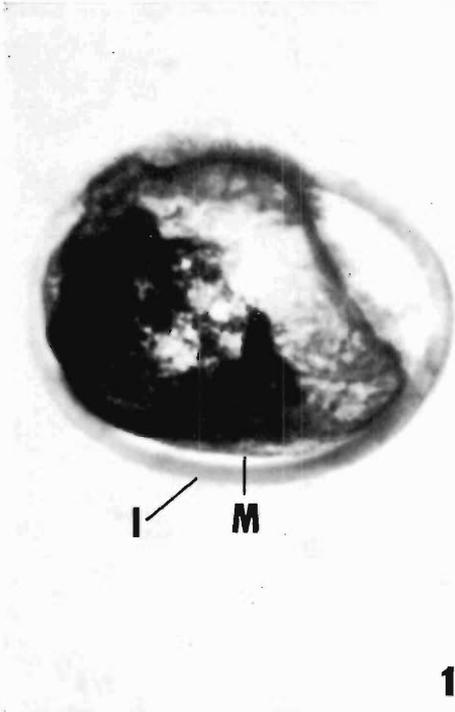
Tests for carbohydrates utilized the periodic acid-schiff (PAS) technique. In an attempt to differentiate among the various PAS-positive materials, control sections were treated in several ways. One group of control slides was subjected to the PAS stain without the benefit of periodic acid oxidation in order to determine the presence of free aldehyde groupings. Other control sections were treated with methyl alcohol-chloroform at 60° C for 6 hr in order to determine the relative amount of glycolipid in the tissue. A third group of slides was subjected to digestion by diastase, pectinase, and hyaluronidase. A positive reaction with PAS indicates such complexes as neutral muco-polysaccharides, muco- and glycoproteins. Alcian blue and toluidine blue were used for the determination of acid mucopolysaccharides.

Mercuric bromphenol blue (HgBPB), alloxan and ninhydrin were used for general proteins. Millon's and the Morel-Sisley technique, for tyrosine, Liebman's method for arginine, and the ferric ferricyanide and the dihydroxy dinaphthyl disulfide (DDD) methods for SH groups. As suggested by Dixon (1965) and Rees (1967) carbohydrate-protein complexes are determined by PAS and protein reactions. Muco-proteins can be identified by a PAS-postive reaction and a positive reaction with HgBPB; and neutral muco-polysaccharides which contain less protein by a PAS-positive reaction and a negative reaction with HgBPB.

Alternate frozen sections were treated with oil red O and nile blue A for the study of fats and fatty acids.

The Gomori methods used in conjunction with simultaneous azocoupling techniques were used for the study of acid and alkaline phosphatases. The alpha-napthyl acetate method was utilized for esterases. Known positive tissue of snail hepatopancreas was used as controls to determine the reliability of the specific substrates.

Mallory's triple stain was used to stain for

Table 1. Histochemical characteristics of the structures comprising the cyst wall of *N. salmincola* metacercaria.

Histochemical test	Inner layer	Outer layer
P.A.S.	+	+
P.A.S. without oxidation	–	–
P.A.S. with lipid extraction	+	+
P.A.S. with diastase digestion	–	–
P.A.S. with pectinase	–	faint
Toluidine blue	–	–
Toluidine blue with hyaluronidase	–	–
Alcian blue	+	–
Mercuric bromphenol blue (HgBPB)	+	+
Alloxan	–	+
Ninhydrin	–	+
Morel-Sisley	+	+
Millons	–	+
Liebman's arginine	+	+
Ferric-ferricyanide (SH groups)	–	–
DDD (SH groups)	–	–
Nile blue A	–	–
Oil red O	–	–
Mallory's triple	blue	red
Acid phosphatase	–	–
Alkaline phosphatase	–	–
Esterase	–	–
Diastase	resistant	resistant
Hyaluronidase	resistant	resistant

connective tissue in both fresh frozen sections and infected fish kidney fixed in 10% buffered formalin and embedded in paraffin (56–58° C melting point). Paraffin embedded material was also stained for carbohydrates and proteins.

Results

The appearance and general structure of the cyst wall is that of a two-layered structure consisting of an inner (layer I) and an outer (layer II) layer. The inner cyst wall is in accord with the report of Bennington and Pratt (1960), noncellular and hyaline (Figs. 1–4). The outer layer is cellular and thinner than the inner layer (Figs. 2, 4), and the cells appear elongated and flattened. The metacercaria is generally ovid in shape and has a large fluid-filled excretory bladder (Fig. 1).

The results of the histochemical tests are summarized in Table 1. It is apparent from the results that the inner hyaline layer is composed of a material which gives the reaction

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Figures 1–4. Photomicrographs of sectioned metacercaria of *Nanophyetus salmincola;* 1–3 (110×), 4 (430×). 1. Cyst wall (layer I), PAS. 2. Young metacercariae in salmon kidney, H & E. 3. and 4. Cyst wall showing PAS positive material and the relationship between layers I and II.

Key to figures: I = layer I; II = layer II; M = enclosed larva; PAS+ = PAS positive; KC = kidney cells; OS = oral sucker.

for acid mucopolysaccharides. The structure is resistant to enzyme digestion and gives a positive reaction with mercuric bromphenol blue and the Morel-Sisley technique indicating the presence of proteins. The layer is rich in tyrosine and arginine as indicated by the Morel-Sisley and the Liebman's technique.

The outer cellular layer was positive with PAS and HgBPB. Diastase digestion destroyed the reaction with PAS leading to the conclusion that the positivity was probably due to the presence of glycogen. Layer I was negative for lipids but layer II exhibited evidence of containing fat.

Neither layer gave a positive reaction for acid and alkaline phosphatase nor for esterase.

Discussion

Bennington and Pratt (1960) indicated that the metacercaria of *N. salmincola* is enclosed within a thin, glistening, transparent structure that is easily ruptured. Wood and Yasutake (1956) recognized a hyperplasia of epithelial cells or fibrous walls around the parasite in Coho salmon, but failed to recognize the surrounding cells as a distinct layer of the cyst wall. The author suggests that epithelial cells do form a distinct layer of the metacercarial cyst wall (Fig. 4), and that the tissue response becomes more apparent with the age of the infection as observed by Hunter and Hunter (1940), with *Posthodiplostomum minimum* in some rock fish. Both Bennington and Pratt (1960), and Wood and Yasutake (1956) worked with experimental infections and observed only early encystations.

The present study indicates that the cyst wall of *N. salmincola* is of a complex nature. The structure is made up of two layers: a thick, hyaline, noncellular inner layer that surrounds the metacercaria, and an outer cellular layer comprised of fish kidney epithelial cells.

The cyst wall of *N. salmincola* resembles that of *Posthodiplostomum minimum* as observed and reported by Hunter and Hunter (1940) and Bogitsh (1962) in consisting of two layers. In both species the inner layer is more complex than the outer and appears to to composed of a carbohydrate-protein complex. Bogitsh (1962) found that layer I of *P. minimum* was positive for both arginine and tyrosine, and the results of this study indicate

the presence of those same amino acids in layer I of *N. salmincola*.

Layer II is cellular in appearance and look like modified kidney cells that have become oriented around the metacercaria constituting a well-defined layer. The capsulated appearance created in the formation of the outer layer suggests a tissue reaction of the fish host to the presence of the parasite.

Both layers are positive for carbohydrates with the PAS test (Figs. 3, 4). Layer I was positive for acid mucopolysaccharides with alcian blue, whereas layer II was negative. PAS with diastase removed the stain from both layers indicating that some glycogen was involved. Both layers were resistant to diastase digestion. The absence of color when stained with PAS without prior oxidation eliminates the presence of free aldehyde groups.

Both layers I and II were positive for mercuric bromphenol blue and the Morel-Sisley technique indicating the presence of protein. Both layers were found to contain both tyrosine and arginine.

The intimacy of the cellular layer with the hyaline layer and its regular appearance accompanying the parasite in infected salmon kidney leads the author to conclude that although it indicates a tissue reaction to the fluke it also forms a part of the cyst wall (Fig. 4).

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Monogenetic Trematodes of Some Alabama Freshwater Fishes with Descriptions of Four New Species and Redescriptions of Two Species[1]

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abstract: The following new species of Dactylogyridae are described from Alabama fishes: *Actinocleidus bennetti* from *Lepomis auritus*, *Urocleidus circumcirrus* from *Elassoma zonatum*, *Urocleidus tuberculatus* from *Lepomis auritus*, and *Urocleidus udicola* from *Elassoma evergladei* and *Elassoma zonatum*. *Dactylogyrus minutus* Kulwiec, 1927, is redescribed for the first time from North America and *Actinocleidus georgiensis* Price, 1966, is redescribed. In all 43 described species involving six genera and two subfamilies of Dactylogyridae are reported. New host records are reported for 11 of these.

Four new species of the genera *Actinocleidus* and *Urocleidus* are described and one species of *Actinocleidus* and one of *Dactylogyrus* is redescribed. Forty-three described species involving six genera and two subfamilies of Dactylogyridae are reported.

Branchial material was collected from a total of 370 individual hosts. Twenty-two host species, representing three familes of fish were examined. Preliminary field work began at Auburn University during the summer of 1957 and was continued through 1960. Collections were taken from 30 localities within Alabama.

Host fish were collected with chemicals, seines, and by angling. Hosts were not separated to species immediately after capture in every case but were often held in a common container.

Technical procedures used in the preparation of specimens for microscopic study are essentially those of Mizelle (1936, 1938) and Mizelle and Cronin (1943). All measurements are given in microns and were made with a compound microscope and a calibrated Bausch and Lomb Filar micrometer. Descriptions presented in this work are based upon living specimens and unstained glycerine jelly mounts studied with a phase-contrast microscope. Figures were made with the aid of a camera lucida.

Actinocelidus georgiensis Price, 1966
(Figs. 1–10)

HOST AND LOCALITIES: Lepomis auritus (Linn.)—Uchee Creek, Crawford, Alabama; Loblockee Creek, (Macon's Hill) Loachapoka, Alabama; Chattahoochee River (Bartlett's Ferry) Lanett, Alabama.

SPECIMENS STUDIED AND MEASURED: 16.

TYPE SPECIMENS: Four hypotypes: U. S. National Museum No. 70455.

REDESCRIPTION: Length 371 (279–495) by 77 (62–71) wide. Haptor subcircular, 53 (45–62) long by 72 (66–100) wide; peduncle usually elongate depending upon state of contraction. Haptoral bars dissimilar in shape, articulated. Ventral bar (Fig. 2) length 41 (32–46), dorsal bar (Fig. 3) length 27 (23–32). Anchors similar in size and shape; anterior (Fig. 4) slightly bifurcate with superficial root much larger than deep root, length 32 (30–35); posterior (Fig. 5) with deep root vestigal or wanting, 32 (30–33) in length. Hooks (Fig. 1) 14 in number, characteristic in arrangement (Mizelle, 1938). Seventh pair, numbered according to Mizelle (1938), conspicuously longer, 17 (16–18 in length. Remaining hooks subequal in length, averaging 14. Pharynx circular to ovate, 20 (18–24) in transverse diameter. Cirrus basally articulated with accessory piece; (Figs. 7, 9) short, 26 (21–30). Accessory piece (Figs. 8, 10) comparatively short, 12–13 long, bifurcate basally. Vagina sinistral, situated on conical projection (Fig. 6) of body wall near mid-portion of body, 17 (16–21) in length. Small seminal receptacle present. Internal structures obscured by well-developed vitellaria which extend to peduncle. Seminal vesicle or prostates not observed.

REMARKS: *Actinocleidus georgiensis* was first collected during summer of 1959 and determined to be undescribed. Price (1966) subsequently described the species from 2 specimens. The present redescription extends the knowledge of the species.

All 22 specimens of *Lepomis auritus* taken from three Alabama locations were infested with *A. georgiensis*. This species occurred in mixed infestations with *Urocleidus dispar, Actinocleidus bennetti* sp. n., *Urocleidus tuberculatus* sp. n., *Cleidodiscus venardi*.

Actinocleidus bennetti sp. n.
(Figs. 11–17)

HOST AND LOCALITIES: *Lepomis auritus* (Linn.)—Uchee Creek, Crawford, Alabama; Loblockee Creek (Macon's Mill), Loachapoka, Alabama; Chattahoochee River (Bartlett's Ferry), Lanett, Alabama.

SPECIMENS STUDIED AND MEASURED: 13.

TYPE SPECIMENS: Holotype and 3 paratypes: U. S. National Museum Nos. 70447 and 70448.

DESCRIPTION: Length 409 (328–453) by 68 (49–79) wide. Haptor subcircular, disc-like, 57 (44–63) long by 88 (77–98) wide. Haptoral bars dissimilar in shape; articulated. Ventral bar (Fig. 12) 45 (42–48). Dorsal bar (Fig. 13), 27 (25–31) in length. Ventral and dorsal anchors similar in size and shape. The former (Fig. 14) 35 (33–38) and the latter (Fig. 15), 36 (33–38) in length. Hooks (Fig. 11) normal. Cirrus and accessory piece articulated basally. Cirrus 35 (32–39) in length (Fig. 16). Accessory piece (Fig. 17) 24 (22–27) long, somewhat sigmoid. Vagina sinistral, opens on body wall near mid-portion of body. Vaginal tube short, heavily chitinized. Internal structures mostly obscured by well-

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Figures 1–10. *Actinocleidus georgiensis* Price, 1966. 1. Hook; 2. ventral bar; 3. dorsal bar; 4. ventral anchor; 5. dorsal anchor; 6. vagina; 7. and 9. cirri; 8. and 10. accessory pieces. Scale applies to all figures.

Figures 11–17. *Actinocleidus bennetti* sp. n. 11. hook; 12. ventral bar; 13. dorsal bar; 14. ventral anchor; 15. dorsal anchor; 16. cirrus; 17. accessory piece.

Figures 18–24. *Urocleidus circumcirrus* sp. n. 18. ventral anchor; 19. dorsal anchor; 20. hook; 21. cirrus; 22. accessory piece; 23. dorsal bar; 24. ventral bar.

Figures 25–31. *Urocleidus udicola* sp. n. 25. ventral anchor; 26. dorsal anchor; 27. hook; 28. accessory piece; 29. cirrus; 30. dorsal bar; 31. ventral bar.

Figures 32–39. *Urocleidus tuberculatus* sp. n. 32. dorsal anchor; 33. dorsal bar; 34. ventral bar; 35. hook; 36. small pair number five hooks; 37. accessory piece; 38. cirrus; 39. ventral anchor.

Figures 40–46. *Dactylogyrus minutus* Kulwiec, 1927. 40. accessory piece; 41. cirrus; 42. 4-a hooks; 43. hook; 44. bar; 45. anchor; 46. wings.

50μ
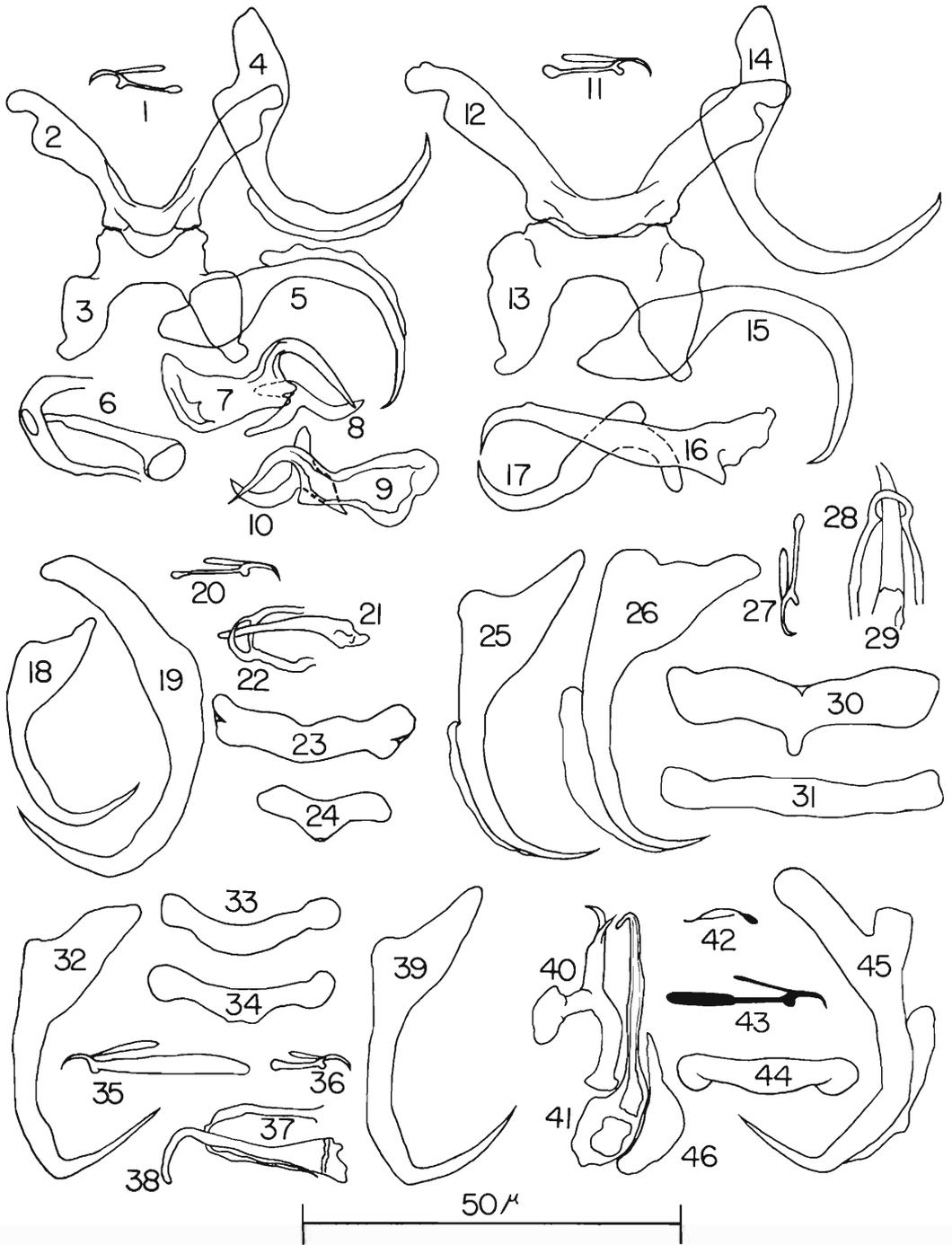
developed vitellaria. Seminal vesicle and prostates not observed.

REMARKS: All 22 specimens of *Lepomis auritus* taken from three Alabama locations were infected with *Actinocleidus bennetti*. This species occurred in mixed infestations with *Urocleidus dispar*, *Actinocleidus georgiensis*, *Urocleidus tuberculatus* sp. n., and *Cleidodiscus venardi*. The haptoral armament of this species resembles that of *A. articularis*, but can be readily differentiated from the latter by the characteristic copulatory complex and vaginal tube. This species is named in honor of Dr. Harry J. Bennett.

Urocleidus circumcirrus sp. n.
(Figs. 18–24)

HOST AND LOCALITIES: *Elassoma evergladei* Jordan (type host)—Roadside marshes, Gulf Shores, Alabama; *Elassoma zonatum*, Uchee Creek, Crawford, Alabama.

SPECIMENS STUDIED AND MEASURED: 10.

TYPE SPECIMENS: Holotype and 3 paratypes: U. S. National Museum Nos. 70449 and 70450.

DESCRIPTION: Length 472 (201–669), 138 (81–190) in greatest body width. Haptor poorly defined because of large dorsal anchors. Bars dissimilar in size and shape. Ventral bar 12–13 in length (Fig. 24). Dorsal and ventral anchors dissimilar in size and shape. The former (Fig. 19) 50 (42–65); latter (Fig. 18) 27 (23–32) in length. Fourteen pairs of hooks (Fig. 20) subequal in length; normal in arrangement. Gonads in inter-intestinal space anterior to loop of intestine. Vagina not observed. Cirrus short, straight (Fig. 21). Accessory piece (Fig. 22) appears to be chitinized wall of cirrus sac and forms ring around cirral aperture through which cirrus is extruded. Cirrus 20 (16–27); accessory piece 16 (13–21) in length. Vitellaria moderately developed, extending from pharynx to base of peduncle.

REMARKS: This species resembles others of the genus by having dorsal anchors of much greater length than ventral anchors. It can be readily distinguished by the characteristic cirrus and accessory piece.

This species was found in association with *Urocleidus udicola* sp. n. (which follows) on the gills of the Everglades pigmy sunfish and the banded pigmy sunfish. This record constitutes the first report of ancyrocephalid

parasites from these hosts. The name refers to the accessory piece forming a ring around the cirrus.

Urocleidus udicola sp. n.
(Figs. 25–31)

HOST AND LOCALITIES: *Elassoma evergladei* Jordan (type host)—Roadside marshes, Gulf Shores, Alabama. *Elassoma zonatum* Jordan—Uchee Creek, Crawford, Alabama.

SPECIMENS STUDIED AND MEASURED: 15.

TYPE SPECIMENS: Holotype and 3 paratypes: U. S. National Museum Nos. 70453 and 70454.

DESCRIPTION: Length 308 (227–462) by 95 (57–150) wide. Dorsal and ventral anchors similar in shape, the latter slightly longer. Anchor bases slightly bifurcate, deep roots vestigal or wanting. Ventral anchors (Fig. 25) 41 (35–44); dorsal (Fig. 26) 39 (36–42). Dorsal bar 38 (36–42) long, (Fig. 31). Ventral bar 30 (23–34) in length (Fig. 30). Fourteen hooks subequal (Fig. 27) with exception of pair 5; normal in arrangement. Pair 5 smaller than remaining pairs. Accessory piece (Fig. 28) appears as chitinized wall of the cirrus vestibule and forms ring around cirral aperture through which cirrus is extruded; length 16 (10–20) by 20 (17–22) wide. Gonads and associated ducts not observed.

REMARKS: This species was found on the Everglades pigmy sunfish and the banded pigmy sunfish in association with *Urocleidus circumcirrus*. The name is from Latin and refers to the habitat of the host—(Swamp dweller).

Urocleidus tuberculatus sp. n.
(Figs. 32–39)

HOST AND LOCALITIES: *Lepomis auritus* (Linn.)—Loblockee Creek (Macon's Mill), Loachapoka, Alabama; Chattahoochee River (Bartlett's Ferry), Lanett, Alabama.

SPECIMENS STUDIED AND MEASURED: 5.

TYPE SPECIMENS: Holotype and 2 paratypes: U. S. National Museum Nos. 70451 and 70452.

DESCRIPTION: Length 345 (315–369), width 72 (60–84). Diameter of pharynx 20 (18–23). Dorsal and ventral anchors similar in shape, ventrals averaging slightly longer. Dorsal anchor (Fig. 32) 36 (30–42), ventral (Fig. 39) 38 (31–42) in length. Dorsal and ventral bars similar in size; former more robust with rounded ends. Dorsal bar (Fig. 33) 24

(20–27); ventral (Fig. 34) 23 (17–27) in length.

Hooks 14 in number and normal in arrangement. Pair number 5 (Fig. 36) comparatively small, situated between ventral anchor shafts. Remaining hooks subequal (Fig. 35); pairs 2 and 3 somewhat smaller than others.

Posterior eyespots larger and closer together. Cephalic lobes vestigal or wanting. Vitellaria moderately developed, extended from pharynx to peduncle. Haptor well differentiated, 93 long by 84 wide. Gonads and seminal vesicle not observed. Cirrus (Fig. 38) 22 (17–25) in length, curved at distal end, without cirral thread. Accessory piece broad, sleeve-like, appears to be chitinized portion of cirrus sac (Fig. 37); length, 15 (7–17). Vagina not observed.

REMARKS: The anchors of this species resemble those of *Urocleidus torquatus*, but it can easily be distinguished from the latter by its characteristic cirrus and accessory piece.

This species was found in association with *Actinocleidus bennetti* sp. n., *Actinocleidus georgiensis* and *Urocleidus dispar* on the gills of *Lepomis auritus*. The name is from Latin and refers to the swellings on the anchors.

Dactylogyrus minutus Kulwiec, 1927
(Figs. 40–46)

HOST AND LOCALITY: *Cyprinus carpio* Linn. —Auburn University Agricultural Experiment Station Ponds, Auburn, Alabama.

PREVIOUSLY REPORTED HOST AND LOCALITIES: *Cyprinus carpio* Linn.—Poland (Kulweic, 1927); Sweden (Nybelin, 1937); Ukraine (Markievicz, 1961); Russia (Gussev, 1955), Israel (Paperna, 1959).

SPECIMENS STUDIED AND MEASURED: 25.

REDESCRIPTION: Dactylogyrid collected from *Cyprinus carpio* Linn. imported from Haiti. In general, measurements (Table 1) agree with those of Kulweic (1927) and Paperna (1959).

Cuticle thin, vitellaria only moderately developed. The latter extend from pharynx to posterior loop of intestine. Ovary in interintestinal space anterior to intestinal loop. Thin-walled uterus extends from ovary to copulatory apparatus. Uterine aperture ventral, surrounded by "Chitin Plate" of Kulweic (1927). Vagina distinct, on right body margin. Short vaginal canal leads to bag-like

Table 1. Measurements of *Dactylogyrus minutus* from Different Localities.

Structure	This Study (Alabama)	Kulwiec 1927 (Poland)	Paperna 1959 (Israel)
Total length	366 (266–477)	(320–440)	(257–532)
Greatest width	65 (45–88)	(48–72)	
Pharynx (Length)	19 (18–20)	16	
Pharynx (Width)	17 (15–18)	(21–23)	
Haptor (Length)	45 (40–60)	(38–45)	
Haptor (Width)	63 (45–90)	(64–77)	
Cirrus	33 (29–37)	(35–40)	(25–36)
Accessory Piece	24 (20–25)	(25–28)	
Anchor	41 (37–44)	46 (43–49)	(33–48)
Dorsal root	14 (11–16)	(9–14)	(15–18)
Ventral root	3 (3–4)	(2–5)	(4–7)
Dorsal bar	24 (20–25)	(25–30)	(22–26)
1st hook	19 (15–25)	(21–25)	(18–26)
2nd hook	21 (18–24)	(22–25)	
3rd hook	22 (18–27)	(23–28)	
4th hook	18 (17–20)	(23–25)	
5th hook	18 (18–22)	(22–25)	
6th hook	12 (11–22)	(20–23)	
7th hook	16 (14–22)	(22–26)	

seminal receptacle; joins distal margin of ovary.

Single median testis located in interintestinal space ventral to ovary, partially covered by the latter. Vas deferens thin-walled, fibrous, reaching anteriorly to bifurcation of intestine; expands to form sac-like, seminal vesicle. Two prostate glands with individual ducts forming common duct opening at base of cirrus. Cirrus a hollow sclerotized tube, tapers distally (Fig. 41). Accessory piece, forked distally, bears median curved process (Fig. 40), articulates basally with side of cirrus. Separate wing-like outgrowth (Fig. 46) at base of cirrus.

Haptor distinct, discoidal, with 16 marginal hooks (Fig. 43), 14 on margin and two between anchors. Hook No. 4a (Fig. 42) as described by Mizelle and Price (1964). Anchors (Fig. 45) connected with bar (Fig. 44).

REMARKS: This species of *Dactylogyrus* was probably introduced with a variety of *Cyprinus carpio* which this laboratory imported from Haiti in 1954.

Host and Distribution Records

The following gill parasites were collected. Since there were no reports in the literature of Ancyrocephalinae from Alabama freshwater fish prior to this study, each represents a new distribution record. Rogers (1966, 1967a, 1967b) reports new ancyrocephalids collected after the present study. New host records are indicated by an asterisk. The number of hosts examined is given following each host.

Host and Parasite

Cyprinus carpio Linnaeus—28
  Dycylogyrus anchoratus Wagener, 1857
  Dactylogyrus minutus Kulwiec, 1927
Ictalurus nebulosus (Le Sueur)—10
  Cleidodiscus pricei Mueller, 1936
Ictalurus punctatus (Rafinesque)—50
  Cleidodiscus pricei Mueller, 1936
  Cleidodiscus floridanus Mueller, 1936
Ictalurus catus (Linnaeus)—10
  Cleidodiscus pricei Mueller, 1936
Ictalurus furcatus (Le Sueur)—15
  Cleidodiscus pricei Mueller, 1936
  Cleidodiscus floridanus Mueller, 1936

Pylodictus olivaris (Rafinesque)—10
  Cleidodiscus pricei Mueller, 1936
  Cleidodiscus floridanus Mueller, 1936

Lepomis machrochirus (Rafinesque)—81
  Actinocleidus fergusoni Mizelle, 1938
  Actinocleidus oculatus (Mueller, 1934) Mueller, 1937
  Anchoradiscus sp.
  Clavunculus bifurcatus (Mizelle, 1941) Mizelle et al, 1956
  Clavunculus bursatus (Mueller, 1936) Mizelle et al, 1956
  Cleidodiscus nematocirrus Mueller, 1937
  Cleidodiscus robustus Mueller, 1934
  Urocleidus acer (Mueller, 1936) Mizelle and Hughes, 1938
  Urocleidus acuminatus (Mizelle, 1936) Mizelle and Hughes, 1938
  Urocleidus antennuatus Mizelle, 1941
  Urocleidus chaenobryttus Mizelle and Seamster, 1939
  Urocleidus dispar (Mueller, 1936) Mizelle and Hughes, 1938
  Urocleidus ferox Mueller, 1934
  Urocleidus perdix (Mueller, 1937) Mizelle and Hughes, 1938

Lepomis cyanellus Rafinesque—18
  Actinocelidus longus Mizelle, 1938
  Clavunculus bursatus (Mueller, 1936) Mizelle et al, 1956
  Cleidodiscus diversus Mizelle, 1938
  Cleidodiscus pricei Mueller, 1936
  Urocleidus cyanellus (Mizelle, 1938) Mizelle and Hughes, 1938
  Urocleidus furcatus (Mueller, 1937) Mizelle and Hughes, 1938

Lepomis megalotis (Rafinesque)—19
  Actinocleidus articulatus (Mizelle, 1936) Mueller, 1937
  Clavunculus bifurcatus (Mizelle, 1941) Mizelle et al, 1956
  Cleidodiscus bedardi Mizelle, 1936
  Urocleidus acuminatus (Mizelle, 1936) Mizelle and Hughes, 1938
  Urocleidus furcatus (Mueller, 1937) Mizelle and Hughes, 1938

Lepomis punctatus (Valenciennes)—2
  Urocleidus affinis (Mueller, 1937) Mizelle and Hughes, 1938
  Urocleidus biramosus (Mueller, 1937) Mizelle and Hughes, 1938
  Urocleidus miniatus Mizelle and Jaskoski, 1942

Lepomis humilis (Giarard)—10
  Actinocleidus fergusoni Mizelle, 1938
  Cleidodiscus nematocirrus Mueller, 1937
  Urocleidus dispar (Mueller, 1936) Mizelle and Hughes, 1938

Lepomis microlophus (Gunther)—22
  Actinocleidus bifidus Mizelle and Cronin, 1943
  Actinocleidus harquebus Mizelle and Cronin, 1943
  Clavunculus bifurcatus (Mizelle, 1941) Mizelle et al, 1956
  Urocleidus furcatus (Mueller, 1937) Mizelle and Hughes, 1938
  Urocleidus acer (Mueller, 1936) Mizelle and Hughes, 1938
  Urocleidus parvacirrus Mizelle and Jaskoski, 1942
  Urocleidus torquatus Mizelle and Cronin, 1943
  Urocleidus variabilis Mizelle and Cronin, 1943

Lepomis auritus (Linnaeus)—22
  Actinocleidus georgiensis Price, 1966
  Actinocleidus bennetti sp. n.
  Cleidodiscus venardi Mizelle and Jaskoski, 1942
  Urocleidus tuberculatus sp. n.
  Urocleidus dispar (Mueller, 1936) Mizelle and Hughes, 1938

Centrarchus macropterus (Lacepede)—3
  Urocleidus macropterus Harrises, 1962
  Urocleidus wadei Seamster, 1948

Elassoma evergladei Jordan—10
  *Urocleidus circumcirrus sp. n.
  *Urocleidus udicola sp. n.

Elassoma zonatum Jordan—11
  *Urocleidus circumcirrus sp. n.
  *Urocleidus udicola sp. n.

Chaenobryttus coronarius (Bartram)—13
  Actinocleidus flagellatus Mizelle and Seamster, 1939
  Urocleidus chaenobryttus Mizelle and Seamster
  Urocleidus doloresae Hargis, 1952
  Urocleidus grandis Mizelle and Seamster, 1939

Micropterus salmoides (Lacepede)—22
  Actinocleidus fergusoni Mizelle, 1938
  Actinocleidus fusiformis (Mueller, 1934) Mueller, 1937
  *Clavunulus unguis (Mizelle and Cronin, 1943) Mizelle et al, 1956
  Urocleidus dispar (Mueller, 1936) Mizelle and Hughes, 1938
  Urocleidus furcatus (Mueller, 1936) Mizelle and Hughes, 1938
  Urocleidus helicis (Mueller, 1936) Mizelle and Hughes, 1938
  Urocleidus principalis (Mizelle, 1936) Mizelle and Hughes, 1938

Micropterus punctatus (Rafinesque)—1
  Clavunculus bursatus (Mueller, 1936) Mizelle et al, 1956

Micropterus coosae Hubbs and Bailey—5
  *Cleidodiscus banghami (Mueller, 1936) Mizelle et al, 1940

Promoxis nigromaculatus (Le Sueur)—4
  Cleidodiscus vancleavei Mizelle, 1936
Pomoxis annularis Rafinesque—4
  Cleidodiscus vancleavei Mizelle, 1936
  Cleidodiscus uniformis Mizelle, 1936

Remarks

Cleidodiscus pricei reported from *Lepomis cyanellus* is an unusual situation since *C. pricei* normally only infests catfishes of the family Ictaluridae. In this case the green sunfish were in a channel catfish culture pond with a very high stocking density (2,000 per acre) and the catfish had suffered mortality due to an extremely heavy infestation of *C. pricei*. Examinations of green sunfish subsequent to the kill in catfish ponds did not show infestation with *C. pricei* so this is undoubtedly a case of accidental parasitism.

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Immunity of Sheep to *Dictyocaulus filaria* Following Vaccination with *Dictyocaulus viviparus*[1]

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ABSTRACT: Sheep vaccinated with *Dictyocaulus viviparus* larvae appeared to develop a definite but variable resistance to later infection by *Dictyocaulus filaria*. On the average, eight vaccinated sheep had fewer and smaller worms, lower larval production, higher gamma globulin and eosinophil percentages, and less lung pathology than eight control sheep similarly infected. *Dictyocaulus viviparus* larvae were able to develop and molt in sheep but did not mature and produced no evidence of infection other than increased gamma globulin and eosinophil percentages. Gamma globulin and eosinophil increases did not appear to be directly related to the strength of the immunity.

Attempts to elicit an immune response against one species of parasite by prior infection of the host with a related species have met with varied success in a variety of host-parasite systems. Such experiments are based on the assumption that the immunizing parasite is antigenically similar to the native parasite but is relatively nonpathogenic to the abnormal host. This report concerns two attempts to immunize sheep against *Dictyocaulus filaria*, the thread lungworm of sheep, by prior vaccination with *Dictyocaulus viviparus*, the cattle lungworm. Experiments to produce immunity to *D. viviparus*, in cattle by vaccination with *D. filaria* have been reported by Hildebrandt (1962), Lucker et al. (1964), and by Parfitt and Sinclair (1967). Previous work (Wilson, 1970) showed that sheep have high resistance to reinfection after recovery from *D. filaria* infection. It was postulated that a lower but substantial resistance might be produced by *D. viviparus* vaccination.

Materials and Methods

D. viviparus Viability Test

To determine if *D. viviparus* would survive long enough in sheep to stimulate the immune mechanisms of the host, three lambs 3–5 months old were given 5,000, 9,000, or 4,000 larvae and killed after 5, 6, and 12 days, respectively.

TEST NO. 1: Four parasite-free lambs 3–4 months old were given 2,500 *D. viviparus*

larvae each, followed by a similar dose 3 weeks later (Table 1). Three weeks after the second vaccination, the vaccinated lambs and four comparable control lambs were given 5,000 *D. filaria* larvae each. Three vaccinated and three control lambs were killed 6 weeks after the challenge infection and the recovered lungworms were counted and measured. The lamb with the lowest fecal larval count in each group was not killed at this time to permit development of any inhibited worms which might mature later.

TEST NO. 2: Four parasite-free yearling sheep were each given three immunizing doses of *D. viviparus* larvae at 9-week intervals over a period of 18 weeks (Table 2). The vaccination schedule was not thought to be ideal but was based on the availability of larvae. The four vaccinated sheep and four comparable controls each received 10,000 *D. filaria* larvae 8 weeks after the last vaccination. One vaccinated and one control sheep were killed 2 weeks after the challenge infection and one sheep from each group was killed at 2-week intervals thereafter.

Recovered worms from both tests were counted and measured under a dissecting microscope. All worms were measured if less than 100 were found, otherwise a representative sample was removed and 50 worms of each sex were measured. Fecal examinations, serum protein determinations, leukocyte counts, and hematocrit levels were recorded weekly. Serum was collected and serum protein analyses were made as described in Turner and Wilson (1962). Lung fluid was aspirated into a pipette by means of a rubber bulb and processed in the same manner as

Table 1. Data from lambs receiving two vaccinations of 2,500 *D. viviparus* larvae each, 6 and 3 weeks prior to challenge with 5,000 *D. filaria* larvae. Lambs killed 6 weeks after challenge.

Group	Lamb No.	Maximum LPG[1]	Worms Recovered	Size Range Males	Avg Size Males	Size Range Females	Avg Size Females	Lung Pathology[2]
Vaccinated	1	0	not killed	—	—	—	—	—
	2	1	70	15.0–30.0	23.2	11.0–32.0	23.3	Slight
	3	2	75	15.0–31.0	22.6	11.0–35.0	23.6	Slight
	4	84	273	28.0–37.0	31.7	35.0–46.0	39.3	Slight
Controls	5	12	not killed	—	—	—	—	—
	6	100	364	25.0–36.0	31.9	33.0–55.0	42.8	Slight
	7	550	864	33.0–51.0	42.6	51.0–61.0	50.7	Moderate
	8	850	743	33.0–45.0	37.2	42.0–60.0	48.3	Moderate

[1] LPG = Larvae per gram of feces.
[2] Slight = 0 to ⅛ of lung surface affected; Moderate = ⅛ to ¼ of lung surface affected.

blood serum. Total leukocyte counts were made using an AO Bright-line hemocytometer and differential cells counts were made from thin blood smears stained with Giemsa. Fecal examinations were made by placing approximately 3 g of feces in warm water for 24 hr and counting the larvae recovered. Smears of lung fluid and pus were made at necropsy and stained with Giemsa. All data were analyzed using the analysis of variance test for significance.

Results

D. viviparus Viability Test

No lungworms were found in the lambs killed 5 and 12 days after *D. viviparus* infection but 60 larvae were recovered from the lamb killed 6 days after dosing. Slight pathological changes were present in the lungs of all three lambs. All 3rd stage worms recovered had undergone considerable development, and some appeared to be molting. Others had reached the 4th stage and could be distinguished as to sex. Average length of the larvae was 566 μ, which is approximately the length of larvae of the same age from cattle.

Fecal and postmortem examinations

TEST NO. 1: The four vaccinated lambs passed no larvae and developed no clinical signs as a result of the vaccinations, but passed an average maximum of 22 LPG (larvae per gram of feces) 5–6 weeks after the *D. filaria* challenge (Table 1). The controls passed an average maximum of 369 LPG, a ratio of 1 to 16. At necropsy a total of 418 worms were recovered from three vaccinated lambs and 1,971 worms from the three controls, an estimated reduction of 79%. The difference between

groups was significant ($P < 0.05$). The lambs with the lowest larval count in each group were not killed to permit development of any inhibited worms that might be present, but counts did not increase. The consolidation (hepatization) in the lungs of lambs was approximately proportionate to the number of worms recovered.

TEST NO. 2: Since two vaccinated and two control sheep were killed before patency, larval counts were obtained on only two animals in each group. One vaccinated sheep had passed no larvae at necropsy 6 weeks after challenge and the other started passing larvae on day 36 and reached a maximum of 92 LPG 48 days after challenge (Table 2). The two control sheep began passing larvae 32 and 35 days after challenge but the average count never exceeded 6 LPG.

Worm counts from the vaccinated sheep were considerably less than those from comparable controls except in the pair killed at 8 weeks. This latter vaccinated sheep had many more worms than its control. Thus, a total of 2,201 worms were recovered from the vaccinated sheep and 4,794 from the controls. Since sheep within the groups were killed at 2-week intervals the data were not amenable to statistical analysis, but the apparent reduction of 54% may not have been significant.

Lung consolidation was negligible in all four sheep killed 2 and 4 weeks after challenge exposure and slight to moderate in the vaccinated sheep killed at 6 and 8 weeks. Consolidation was moderate to heavy in the controls killed 6 and 8 weeks after challenge. In the latter two, damage was much greater than would be expected from the number of worms recovered.

Table 2. Data from yearling sheep receiving three vaccinations of 2,000, 1,500, and 2,500 *D. viviparus* larvae each 26, 17, and 8 weeks prior to challenge with 10,000 *D. filaria* larvae. Sheep killed at 2-week intervals after challenge.

	Lamb No.	Week Killed	Maximum LPG[1]	Worms Recovered	Size Range Males	Avg Size Males	Size Range Females	Avg Size Females	Lung Pathology[2]
Vaccinated	9	2	0	1,461	0.44– 2.65	1.8	1.6– 3.8	2.4	None
	10	4	0	359	3.0 –19.5	10.6	6.5–16.5	10.4	Slight
	11	6	0	37	4.0 – 8.0	6.4	5.0–15.0	7.7	Slight
	12	8	92	354	18.0 –41.0	27.8	13.0–54.0	34.7	Moderate
Controls	13	2	0	3,142	1.2 – 4.2	2.4	1.0– 6.5	2.7	None
	14	4	0	1,527	7.5 –39.0	25.0	5.0–47.0	27.6	Slight
	15	6	7	66	17.0 –33.0	23.8	7.0–40.0	23.5	Heavy
	16	8	5	59	16.5 –29.0	21.4	9.5–30.0	19.6	Moderate

[1] LPG = Larvae per gram of feces.
[2] Slight = 0 to $\frac{1}{8}$ of lung surface affected; Moderate = $\frac{1}{8}$ to $\frac{1}{4}$ of lung surface affected; Heavy = more than $\frac{1}{4}$ of lung surface affected.

Worm size comparisons

TEST No. 1: On the average, male worms from the vaccinated lambs were 26% smaller than those from the controls ($P < 0.005$), and female worms 38% smaller ($P < 0.005$), (Table 1). In every case, the average size was related to the number of worms present, the smaller sized worms being in lambs with the smaller numbers of worms.

TEST No. 2: Female worms from the vaccinated sheep killed 2 weeks after challenge exposure were not significantly smaller than those from its control. However, male worms from the lamb killed at 2 weeks and both male and female worms from lambs killed at 4 and

Figure 1. Average gamma globulin percentages (A) and average eosinophil percentages (B) from vaccinated and control lambs of Test 1.

6 weeks after challenge were somewhat smaller ($P < 0.005$) than the worms from the controls, showing increased inhibition as the age of the infection increased (Table 2). Worms from the vaccinated sheep killed 8 weeks after challenge were significantly larger ($P < 0.01$) than those from its control but were smaller than the average of the worms from any of the control lambs at 6 weeks in Test No. 1.

Serum protein studies

TEST No. 1: Serum protein percentages were not noticeably affected by the two *D. viviparus* vaccinations. After *D. filaria* challenge, gamma globulin percentages increased an average of 6.7% in the vaccinated lambs and 7.2% in the controls. These differences were not statistically significant. Although the increase was about equal in the two groups, the increase began 2 weeks earlier in the vaccinated group (Fig. 1A). The A/G ratios (albumin to globulin) generally decreased in both groups as gamma and beta globulins increased.

TEST No. 2: The percentage of gamma globulin increased in the vaccinated sheep after the third *D. viviparus* vaccination (Fig. 2A). Four weeks after the *D. filaria* challenge the vaccinated sheep averaged 27.8% gamma globulin as compared to 28.1% for the controls. These increases averaged 10% and 14% respectively as the vaccinated sheep had a higher average at the time of challenge, but these differences were not significant. A/G ratios in the two groups were not markedly different from the second through the sixth week after challenge.
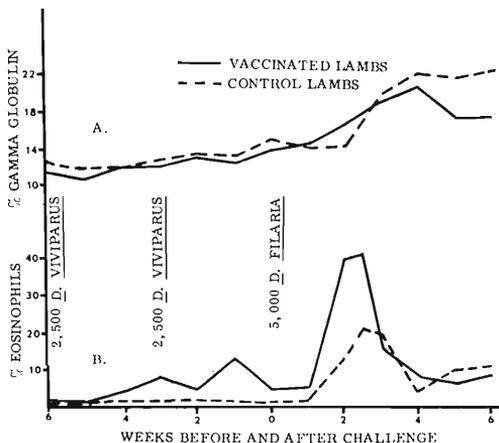
Figure 2. **Average gamma globulin percentages (A) and average eosinophil percentages (B) from vaccinated and control lambs of Test 2.**
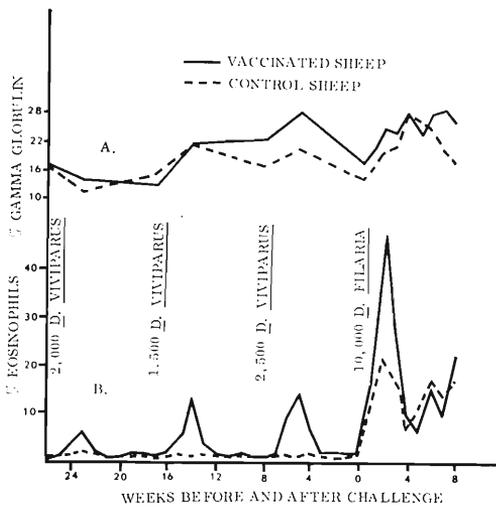
Lung fluid analysis

TEST No. 2: The lungs of the pair of sheep killed 2 weeks after challenge exposure did not contain enough fluid for analysis. Analysis of fluid from the lungs of the other animals in each group showed a considerable reduction in gamma globulin and albumin and an increase in beta globulin in comparison to the blood sera from the same animals (Table 3).

Differential leukocyte counts

TEST No. 1: Eosinophils increased in only two lambs after the first vaccination, but an increase was evident in all four after the second, rising to an average of 13% (Fig. 1B).

During the second and third weeks after challenge inoculation, eosinophils averaged 40% in the vaccinated lambs and 21% in the controls ($P < 0.01$). Increases in total leukocyte counts corresponded to increases in eosinophils as other leukocyte components were not noticeably affected.

TEST No. 2: Average eosinophil counts increased after each vaccination with *D. viviparus* but returned to normal between exposures (Fig. 2B). Two weeks after the challenge infections the vaccinated sheep averaged 46% eosinophils compared to an average of 22% in the controls ($P < 0.005$). No important changes were noted in other leukocyte components. Large numbers of cells were often present in the lung fluid with up to 90% being eosinophils.

Hematocrit readings

TEST No. 1: No detectable changes in hematocrit values were noted as a result of either the vaccinations or the challenge infection.

TEST No. 2: No important changes in hematocrit levels occurred as a result of the vaccinations or the challenge infections. Averages in Test 2 appeared to vary abnormally as sheep were being killed at 2-week intervals.

Discussion

The viability test demonstrated that *D. viviparus* larvae could develop in sheep and persist long enough to complete one molt. However, the absence of worms in the lambs at 5 and 12 days and the recovery of only 60 worms at 6 days indicated that infectivity was low and that any immunity produced might also be low grade.

Table 3. **Electrophoretic analysis of lung fluid from vaccinated and control sheep of Test 2. Principals received three *D. viviparus* vaccinations prior to administration of 10,000 *D. filaria* larvae to principals and controls.**

| Group | Sheep No. | Week Killed | Serum Fractions | | | | | |
			Albumin	Alpha	Beta	Beta₂	Gamma	A/G Ratio[1]
Vaccinated	10	4	33.1	3.4	47.6	15.9	0.0	0.49
	11	6	31.8	6.8	12.5	29.6	19.3	0.47
	12	8	23.5	9.8	44.4	20.2	2.1	0.31
Controls	14	4	36.5	3.1	42.4	16.5	1.5	0.54
	15	6	46.1	7.9	10.1	15.7	20.2	0.85
	16	8	16.4	2.0	74.8	5.8	1.0	0.20

[1] A/G = Albumin to globulin ratio.

In the two subsequent tests, three of the four sheep in each test appeared to develop a substantial immunity to *D. filaria* infection as a result of *D. viviparus* vaccinations. The other two sheep may have developed a slight immunity. However, one control in the first test and two in the second test appeared to be relatively resistant to *D. filaria* infection without prior contact with the parasite. Worms from the yearling controls killed 6 and 8 weeks after challenge were not only few in number but were smaller than the average of the worms from both the vaccinated and control lambs killed at 6 weeks in Test 1 and also smaller than those from the control yearling killed at 4 weeks in Test 2. The extensive lung pathology in these two yearlings indicated that many more worms may have reached the lungs but were eliminated before patency. It has been noted by Kauzal (1934) and others that lungworm infectivity and worm sizes are somewhat variable, particularly in older sheep. This natural resistance in control animals made it difficult to determine to what extent the observed immunity was due to the vaccinations.

Despite evidence of natural resistance, six factors indicated that the immunity of the vaccinated lambs was enhanced by the *D. viviparus* exposures: (1) larval counts were lower in comparison to comparable controls in all but one instance; (2) individual worm counts were lower in all but one instance and average worm counts were 2 to 5 times lower; (3) worm sizes were substantially inhibited in all but one vaccinated sheep in which development may have been slightly inhibited; (4) gamma globulin increased in yearling sheep as a result of exposure to *D. viviparus* indicating that foreign protein had stimulated some immunological mechanisms, even though the gamma globulin itself might not have been protective; (5) increased eosinophils following the *D. viviparus* vaccinations demonstrated that the invading worms were penetrating the host and persisting long enough to produce an allergic response, and the greater increases which followed *D. filaria* challenge gave evidence of an immunological relationship between the two lungworm species; (6) the lungs of the vaccinated sheep showed less pathology than those of the controls, suggesting that fewer worms had reached the lungs and developed there.

Djafar et al. (1960) stated that rapid increases in gamma globulin and eosinophils indicated recovery from lungworm disease, whereas calves that did not produce these increases within 10 days had an unfavorable prognosis. No activity was attributed to either the gamma globulin or the eosinophils and was not necessarily implied. Whether these factors are important in themselves or are merely indicators of general immunologic responsiveness remains to be demonstrated. Although early increases occurred in both gamma globulin and eosinophils in the vaccinated lambs in Test 1 and in some of the vaccinated and control sheep in Test 2, there was no direct evidence that these increases were related to the degree of immunity in these sheep. The early increases in vaccinated sheep presumably occurred because of the sensitization produced by the *D. viviparus* vaccinations. Eosinophilia as evidence of prior sensitization to *D. filaria* by *D. viviparus* was also shown by MacKenzie and Michel (1964) in calves. Antigenic similarities between the two species of worms would seem to be responsible for this sensitization. Further evidence that antigenic relationships exist between the two species was shown by Parfitt and Sinclair (1967). They found that sera from calves infected with *D. filaria* reacted with antigens from both *D. filaria* and *D. viviparus* in agar gel-diffusion tests. Even though eosinophilia and gamma globulin increases may not be directly related to immunity, they served as reliable indications of vaccination and challenge lungworm infections in the absence of other symptoms. The unusual electrophoretic pattern of lung fluid may indicate that the immune activity of the globulin fractions of this substance should be investigated further.

The *D. viviparus* vaccinations produced little or no disease in lambs or yearling sheep and none of the worms developed to patency. *D. filaria*, however, can produce disease in calves. Parfitt (1963) reported three deaths in six calves exposed to a total of 30,000 *D. filaria* larvae and some worms matured. Hildebrandt (1962) and Lucker et al. (1964) also found that *D. filaria* infection in cattle caused considerable respiratory distress even though the worms did not reach maturity. The failure of *D. viviparus* to persist very long in sheep may be responsible for the variability of the immune

response seen in the present tests. Incomplete development and low infectivity would reduce the variety as well as the amount of antigen produced by the vaccinations. Much greater development, however, might increase the pathogenicity of the vaccine beyond practicality. As expected the immunity produced by *D. viviparus* vaccination was considerably less than that produced by *D. filaria* in sheep as reported by Wilson (1970).

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Scanning Electron Microscopy of *Eimeria tenella* Oocysts[1]

Peter A. Nyberg[2] and Stuart E. Knapp

ABSTRACT: Scanning electron micrographs of *Eimeria tenella* oocysts were taken before and after pretreatment with gaseous carbon dioxide. Oocysts appeared broadly ovoid with size measurements similar to those reported by authors using light microscopy. The micropylar area was not evident on oocysts prior to CO_2-pretreatment, however, it showed up as an alteration on oocysts that had been exposed to this gas. The alteration occurred at the narrow end of oocysts and consisted of a circular depression surrounding a "pin-point" opening in a centrally elevated portion. The outer layer of the oocyst wall appeared roughened on some oocysts, apparently resulting from the presence of finely granular debris particles not removed during the cleaning process.

Carbon dioxide is apparently an important stimulant in initiating the release of larval parasites during infection of the definitive host. It is required for exsheathment of nematode larvae (Rogers and Sommerville, 1960), markedly stimulates activation of acanthocephalan cystacanths (Graff and Kitzman, 1965), is involved in activation of trematode metacercariae prior to excystment (Dixon, 1966), and alters the micropyle area of oocysts of ovine, bovine, rodent and chicken coccidia (Jackson, 1962; Nyberg and Hammond, 1964; Landers and Colley, 1964; Nyberg, et al., 1968; Hibbert and Hammond, 1968). The involvement of this gas in similar processes of at least four phyla suggests that its biochemical action is a general and important phenomenon.
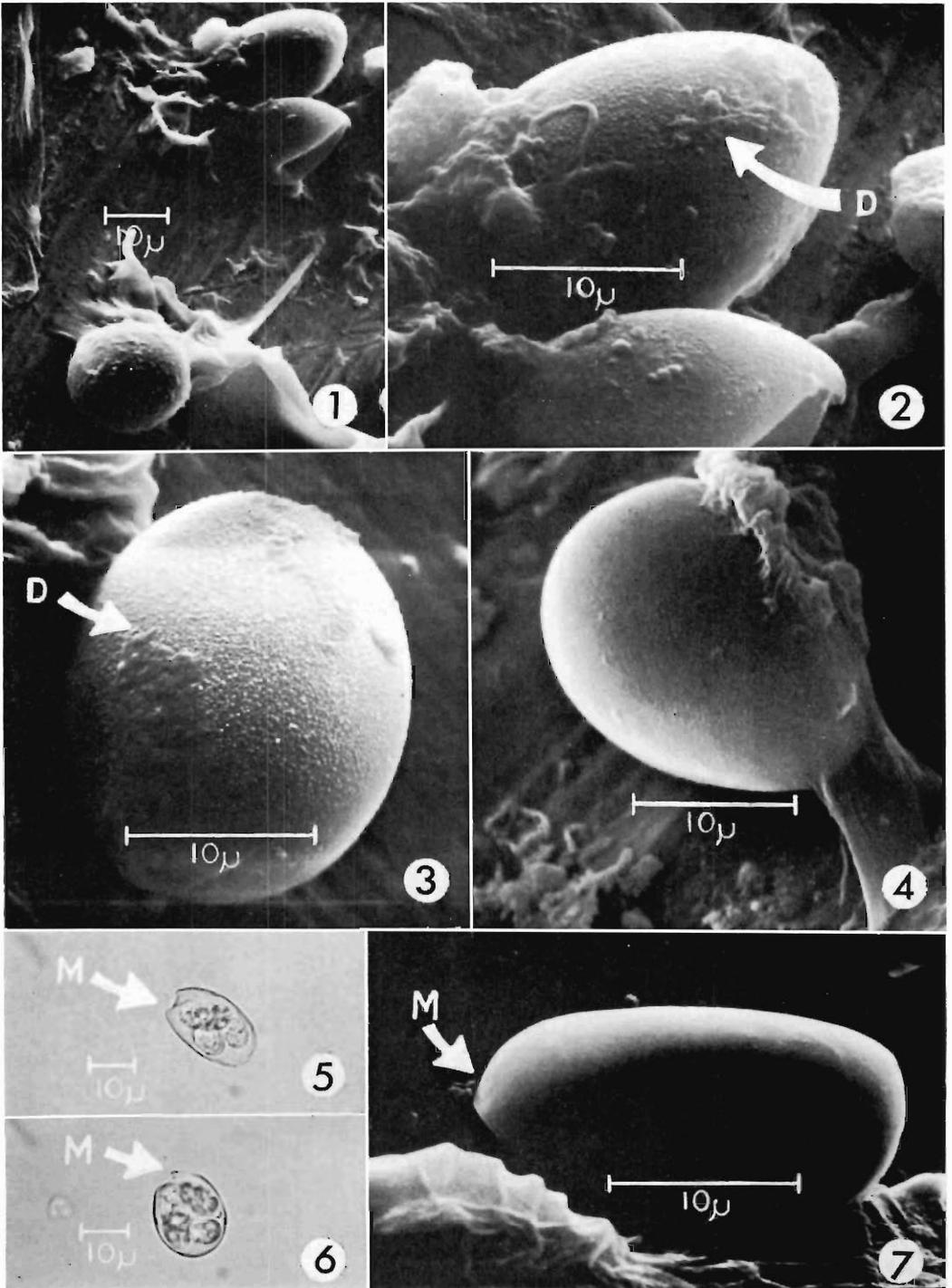
During our in vitro studies of the action of carbon dioxide on excystation of *Eimeria tenella* oocysts, we observed with the light

microscope, a micropylar thinning and indentation as shown in Figures 5 and 6. The intent of the present study was to use scanning electron microscopy to obtain photographs of normal sporulated *E. tenella* oocysts, and if possible to amplify detection of observed micropylar changes caused by CO_2.

Materials and Methods

Oocysts were collected, sporulated, and pretreated with gaseous CO_2 according to techniques outlined by Nyberg, et al. (1968). They were separated from debris using a glycerol-water gradient (50–50). Oocysts were fixed in phosphate buffered 2% glutaraldehyde for 2 hr at 4 C, and stored in this solution until time to use. A drop of the oocyst-glutaraldehyde suspension was pipetted onto a specimen plug for the scanning electron microscope, and allowed to air dry. Mounted specimens were placed in a vacuum evaporator and coated with a layer of gold, 100 Å thick. The plug containing coated oocysts was placed in a Japanese Scanning Microscope (JSM-2) and examined at several different magnifications. Photographs were made with a polaroid camera.

Results and Discussion

Observations of normal sporulated oocysts

Eimeria tenella oocysts have been reported by Levine (1961) to be broadly ovoid in shape with size measurements of 14–31 by 9–25 μ (mean 22.9 by 19.1 μ). The outer layer of the oocyst wall is reportedly smooth and a visible micropyle is absent, although a micropylar area is presumed to be located terminally at the narrow end of the oocyst (Levine, 1961).

In the present study, some oocysts became distorted when they were placed in the high intensity electron beam of the scanning microscope (Fig. 1). The problem was corrected, however, by decreasing the time used to adjust the microscope for higher magnification. After the microscope was adjusted to prevent distortion, light microscope descriptions of the size and shape of oocysts were confirmed, as well as most other features within the observable capabilities of the instrument. One difference existed, however, in that quite often the outer layer of the oocyst wall appeared roughened (Fig. 3). This was in direct contrast to the smooth appearance normally seen with the light microscope. The nature of this roughened surface is not clear, but presumably is an abnormal condition. Since light microscopy reveals a smooth outer surface (Figs. 5, 6), as does scanning microscopy on most similarly handled oocysts (Fig. 4), it is suggested that this roughened appearance is due to finely-granular debris particles not removed during the cleaning process or to artifacts resulting from fixation. Nyberg and Knapp (1970) presented supportive evidence for this when they saw tiny particles on the outer surface of the oocyst wall in electron micrographs of sectioned *E. tenella* oocysts. Particulate material observed by them appeared to be extraneous debris rather than an actual component of the oocyst wall.

Observations of CO_2-pretreated oocysts

In light microscope studies of excystation, a thinning and indentation of the micropylar area is often observed on CO_2-pretreated oocysts of *E. tenella* (Figs. 5, 6). A similar alteration has been observed with ovine species by Jackson (1962, 1964) and with bovine species by Nyberg and Hammond (1964), and Hibbert and Hammond (1968). Detection of a thinning of the micropylar membrane is beyond the capabilities of scanning microscopy, and therefore, that alteration was not observed in the present study. The micropylar indentation, however, was observed on some oocysts and appeared as shown in Figure 7. It consisted of a small circular depression with a "pin-point" opening located in a centrally elevated portion.

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Figures 1–7. *Eimeria tenella* oocysts. 1, 2, 3, 4. Scanning electron micrographs before CO_2-pretreatment, showing general shape and size of oocysts. 5, 6. Light microscope photographs after CO_2-pretreatment, showing alteration of the micropyle. 7. Scanning electron micrograph after CO_2-pretreatment. Note the micropylar alteration as a circular depression surrounding a small opening in a centrally elevated portion. Abbreviations: D, debris particles; M, micropylar area. Magnifications: Fig. 1, × 675; Figs. 2, 3, × 2,000; Fig. 4, × 1,750; Figs. 5, 6, × 500; Fig. 7, × 2,000.

This alteration appeared similar to that observed by light microscopy (Figs. 5, 6).

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Effect of Sodium Hypochlorite on the Oocyst Wall of *Eimeria tenella* as Shown by Electron Microscopy[1]

PETER A. NYBERG[2] AND STUART E. KNAPP

ABSTRACT: Electron micrographs are presented showing the effect of sodium hypochlorite on the oocyst wall of *Eimeria tenella*. Before treatment, the oocyst wall showed considerable opacity, was nonporous and consisted of two primary layers. Each layer appeared to have a different consistency. The sporocyst membrane appeared to consist of a single layer. Limiting membranes of sporozoites were apparently ruptured during fixation and thus not observed. Polysaccharide granules and other sporozoite organelles were distributed randomly throughout the inner contents of the sporocysts. After treatment, the oocyst wall consisted of a single layer. The outer primary layer had been removed during treatment with sodium hypochlorite. Other structures in treated oocysts appeared similar to those in untreated oocysts.

Sodium hypochlorite has been used successfully to clean fecal debris from oocysts of several species of coccidia (Monné and Hönig, 1954; Jackson, 1964; Wagenbach, et al., 1966; Nyberg, et al., 1968). It has been reported by

Monné and Hönig (1954) and Jackson (1964) to remove the outer layer of the oocyst wall in chicken and sheep species, respectively, without hindering the ability of oocysts to excyst. During our studies of the action of carbon dioxide on excystation of *Eimeria tenella* oocysts, we observed with the light microscope, a visible separation between the inner and outer layers of the oocyst wall, as shown in Figures 3 and 4. The intent of the present study was to compare, by electron microscopy, sections of sporulated oocysts before and after treatment with sodium hypochlorite.

Materials and Methods

Collection and cleaning of oocysts

Oocysts were collected and sporulated as reported by Nyberg et al. (1968). Separation of oocysts from debris was accomplished using a glycerol-water gradient (50–50) or by a modification of the technique reported by Wagenbach et al. (1966). In this modified technique, oocysts were combined in a proportion of 1 : 10 (v/v) with a 2.6% sodium hypochlorite (Clorox)-water solution (1 : 1) and this mixture was held for 15 min in an ice bath with frequent stirring. After repeated washing with water to remove traces of clorox, the oocysts were suspended in a small volume of distilled water, and held at 4 C until used.

Fixation and embedding of oocysts

Cleaned oocysts were centrifuged into a pellet, and then washed by centrifugation in phosphate buffer (0.125 M Na_2HPO_4 – NaH_2PO_4, pH 7.1). The supernatant buffer was removed and oocysts were combined with fixative (3% glutaraldehyde in phosphate buffer, plus 1 drop dimethylsulfoxide [DMSO] per 2 ml of fixative) and allowed to stand 1 hr at room temperature. The fixative was then removed and the oocysts washed twice with phosphate buffer. Following the second washing and centrifugation, supernatant buffer was removed, and an osmium tetroxide mixture (1% osmium tetroxide in phosphate buffer, plus 1 drop DMSO per 2 ml osmium solution) added. This mixture was allowed to stand 1 hr at room temperature. The fixative was removed by centrifugation, after which oocysts were washed twice in phosphate buffer. Following the second washing, oocysts were combined with 1% nonnutritive agar and placed in an ice bath until the agar hardened. Agar chunks containing the oocysts were dehydrated in ethanol and washed twice in a propylene oxide-plastic mixture (Dodecenyl Succinic Anhydride [DDSA] 40 ml; Araldite 502, 28 ml; Epon 812, 8 ml; Benzyldimethylamine [BDMA] 3–4 drops/ml) in a ratio of 1 : 1. At each washing, the mixture was shaken thoroughly and allowed to stand at room temperature for 2 hr. Agar chunks were then put into a final plastic mixture and incubated for 12 hr at 40 C, followed by 24 hr at 65 C. Ultrathin sections of oocysts were cut with a glass knife on a Porter-Blum microtome. Observations were made using a Phillips EM-300 electron microscope.

Results

General observations of untreated oocysts

Figures 1 and 2 are electron micrographs of sectioned oocysts cleaned on a glycerol-water gradient and not treated with sodium hypochlorite. The wall shows considerable opacity, is nonporous and consists of two primary layers. The composition of each primary layer is apparently different. The inner layer appears slightly narrower than the outer layer. Also a narrow dense band about one-fifth the width of the entire inner layer is evident on the extreme outer periphery. The external surface of the outer primary layer of oocysts shown here has a roughened appearance, resulting from the presence of numerous finely granular projections 5–10 mμ high.

Sporocysts and sporozoites underwent variable amounts of distortion during the fixation process (Figs. 1 and 2). Abnormal protrusions of the sporocyst membrane were present and contents of the sporozoites, including polysaccharide granules (Ryley, et al., 1969), were distributed randomly throughout the sporocysts. Individual sporozoites could not be seen, since their limiting membranes had apparently been ruptured during fixation. The sporocyst membrane appeared to consist of a single layer. A row of unidentified granules was seen adjacent to the sporocyst membrane within the oocyst cavity (Figs. 1 and 2). These granules appeared different from the finely granular projections on the outer surface of the oocyst wall, and could be artifacts of fixation.

Effect of sodium hypochlorite on the oocyst wall

Light microscopy indicated that the outer layer of the oocyst wall was removed during treatment with sodium hypochlorite since a ballooned outer layer was often observed (Figs. 3, 4). Figure 5 is an electron micrograph of a sectioned oocyst that was treated with sodium hypochlorite. It shows that after treatment only the inner layer remains, including the dense narrow band located at the outer periphery.
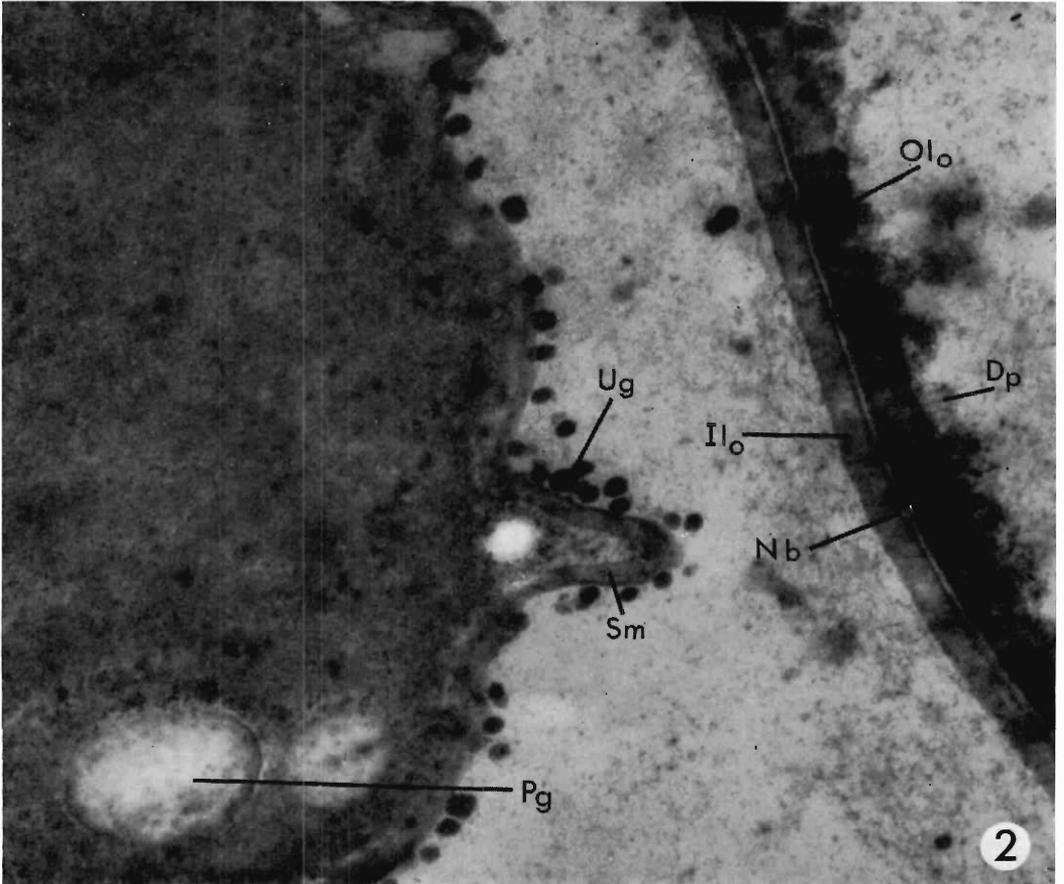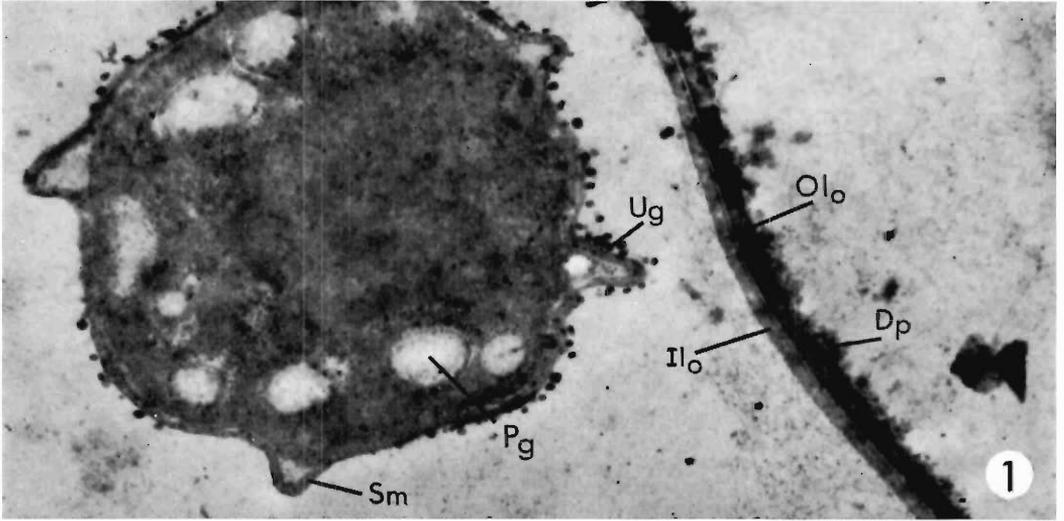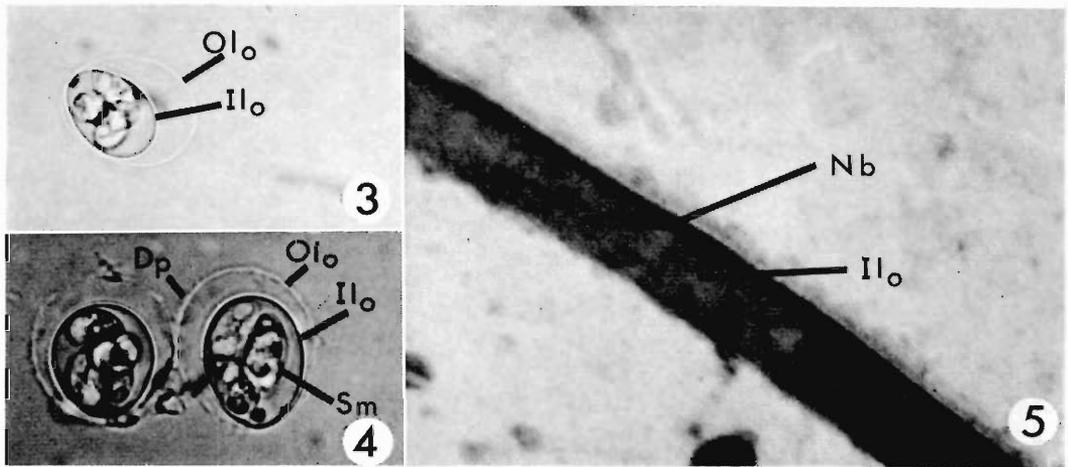
Figures 3–5. *Eimeria tenella* oocysts. 3, 4. Light microscope photographs after treatment with sodium hypochlorite. 5. Electron micrograph of the inner layer of the oocyst wall after sodium hypochlorite treatment. Compare the narrow peripheral band to that identified in Fig. 2. Abbreviations: Sm, sporocyst membrane; Ilo, inner layer oocyst wall; Olo, outer layer oocyst wall; Dp, finely-granular debris particles; Nb, narrow band on periphery of inner layer. Magnifications: Figs. 3 and 4, × 500; Fig. 5, × 99,750.

Discussion

Monné and Hönig (1954), using light microscopy, reported the oocyst wall was composed of two primary layers. The outer layer was reportedly made up of a quinone-tanned protein and the inner layer of a protein lamella firmly associated with a lipid. The lipid portion was situated internal to the lamella. Scholtyseck and Weissenfels (1956), using the electron microscope, also reported two primary layers of the oocyst wall, but did not distinguish parts of the inner layer as Monné and Hönig (1954) had done. In addition to the two primary layers, Scholtyseck and Weissenfels (1956) reported a thin lamella covering the external surface of the outer primary layer. We noted that the external part of the inner layer was much narrower than that reported by Monné and Hönig (1954), and we did not observe the thin lamella covering the outer primary layer as reported by Scholtyseck and Weissenfels (1956). However, we observed, numerous finely-granular projections 5 to 10 mμ high, which covered the entire external surface of the outer primary layer. We presume that these projections are either debris not removed during the cleaning process or artifact resulting from fixation. In the present study these particles may have obscured the fine covering lamella as described by Scholtyseck and Weissenfels (1956). Using scanning electron microscopy, Nyberg and Knapp (1970) reported a roughened outer surface of the oocyst wall of some *E. tenella* oocysts similar to that described herein, while others appeared to be smooth. Therefore, we suggest that this particulate material is extraneous debris rather than an actual component of the oocyst wall. Similar particulate material appeared on oocysts in photographs taken by Scholtyseck

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Figures 1–2. Electron micrographs of sectioned *Eimeria tenella* oocysts not treated with sodium hypochlorite. Abbreviations: Sm, sporocyst membrane, Pg, polysaccharide granule; Ilo, inner layer oocyst wall; Olo, outer layer oocyst wall; Dp, finely-granular debris particles; Ug, unidentified granules; Nb, narrow band on periphery of inner layer. Magnifications: Fig. 1, × 20,235; Fig. 2, × 51,300.

and Weissenfels (1956), however, they did not mention it.

Monné and Hönig (1954) reported that the outer layer of the oocyst wall was rapidly dissolved when oocysts were combined with sodium hypochlorite. They reported that in oocysts treated with sulfuric acid prior to sodium hypochlorite, the outer layer of the oocyst wall became wrinkled, then elevated away from the inner layer, apparently the result of hydrotropic properties of the acid. We obtained similar results in the present study; however, we also observed an elevation and ballooning of the outer layer following treatment with sodium hypochlorite without sulfuric acid. Results similar to ours have been reported by Jackson (1964) for *E. arloingi* from sheep. He described the process as an initial separation and rapid ballooning of the outer layer from the inner layer of the wall, and noted that the two layers remain attached only at the micropyle. The ballooned wall appears to become progressively thinner, and eventually collapses and disappears.

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Helminths of the Opossum (*Didelphis virginiana*) in North Carolina[1]

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ABSTRACT: Thirteen species of helminths were recovered from 54 opossums in North Carolina. These included: Trematoda—*Brachylaima virginianum, Rhopalias macracanthus, Diplostomum variabile, Maritreminoides nettae*; Cestoda—*Mesocestoides variabilis*; Nematoda—*Cruzia americana, Physaloptera turgida, Viannaia hamata, Longistriata didelphis, Capillaria aerophila, Trichuris* sp., Acanthocephala—Two species of immature and probably accidental parasites were recovered. Thirteen of the 18 specimens appear to be *Centrorhynchus* sp. The helminths reported herein are similar to those listed by other investigators of opossum parasites.

During recent years our studies have provided additional knowledge on the helminthic fauna in our native wild mammals. These pertain to helminths of the raccoon (Harkema and Miller, 1964), mink (Miller and Harkema, 1964), and the otter, bobcat, grey fox and red fox (Miller and Harkema, 1968). Information is presented here on helminths of 54 opossums collected from 13 counties in eastern North Carolina.

Most of the animals were trapped. A few were road kills or brought to the laboratory by local hunters. All were examined soon after death. Trematodes, cestodes, and acantho-

Table 1.   Helminths of 54 Opossums from eastern North Carolina.

Species of Parasite	Number infected
Trematoda	
Brachylaima virginianum (small intestine)	48
Rhopalias macracanthus (small intestine)	26
Diplostomum variabile (small intestine)	22
Maritreminoides nettae (small intestine)	1
Cestoda	
Mesocestoides variabilis (small intestine)	2
Nematoda	
Cruzia americana (cecum and large intestine)	48
Physaloptera turgida (stomach)	48
Viannaia hamata (small intestine)	39
Longistriata didelphis (small intestine)	37
Capillaria aerophila (lungs)	10
Trichuris sp. (large intestine)	1
Acanthocephala	
Centrorhynchus sp. (immature, small intestine)	1
unidentified acanthocephalan (immature, small intestine)	2

cephalans were killed and fixed in AFA, stained, and mounted for study. Nematodes were killed in hot 70% ethanol, stored in glycerin-alcohol, cleared in lacto-phenol or glycerin and studied in temporary mounts.

Results and Discussion

Thirteen species of helminths were recovered from the 54 opossums, none of which was helminth free. The helminths included four species of trematodes, one cestode, six nematodes, and two acanthocephalans (Table 1).

Trematoda

Brachylaima virginianum (Dickerson, 1930) was the most common trematode recovered. Infections varied from two to 357 per host. Its absence from six young animals might be attributable to their age, or in some instances to locality. *B. virginianum* is nearly ubiquitous throughout the range of the opossum. Babero (1957, 1960), Byrd, Reiber, and Parker (1942), Ulmer (1952), Kaplan (1964) and others have reported it from diverse localities.

Rhopalias macracanthus Chandler, 1932 reportedly is also fairly common and host specific for the opossum. However, less than half of our animals harbored it, and rarely more than two to five worms. One animal from a swampy area in Hertford County contained 303 specimens. As far as we are aware the life cycle of this species is unknown. We have obtained adults by feeding naturally infected cricket frogs (*Pseudacris sp.*) to white mice. Eggs were incubated and hatched but various species of snails exposed to a limited number of miracidia did not become infected. Originally reported from Texas, (Chandler 1932) it has been reported from other states (Babero, 1957, 1960); (Byrd et al., 1942); (Leigh, 1940); (Self and McKnight, 1950). Apparently its range also approximates that of the opossum.

Diplostomum variabile (Chandler, 1932) is the common strigeoid reported from the opossum. About 40% of our animals were infected with one to 307 worms per host. Since the second intermediate hosts are salamanders (Harris et al., 1967) it follows that opossums collected from swampy or wet areas where salamanders are abundant are more apt to be infected. Our data support this conclusion.

Maritreminoides nettae (Gower, 1936) was recovered from one opossum from Hertford County. Though described from ducks in Michigan and presumably a bird parasite, it has been reported from the raccoon, (Harkema and Miller, 1964). Our specimens from the opossum were in good shape and not difficult to identify but it is possible that their presence was due to recent consumption of a bird. This is the first report of this parasite from the opossum.

Cestoda

Mesocestoides variabilis Mueller, 1927 was restricted to an animal from Edgecombe County and another from Hertford County. Four specimens were found in one and a single specimen in the other which indicates the relative scarcity of tapeworms in opossums in North Carolina. Our studies on raccoon parasites indicate that this tapeworm is more common in the coastal areas than elsewhere. Voge (1955) considers *M. variabilis* to be typical of carnivores and *M. latus* of the opossum. Babero (1957, 1960) reported *M. variabilis* from the opossum in Illinois and Georgia. According to Voge (1955) the specimens recovered from the opossum in Tennessee by Byrd and Ward (1943), identified as *M. variabilis*, were probably *M. latus*. Webster (1949) made observations on the life history of *M. latus* from the opossum in Texas.

Nematoda

Cruzia americana Maplestone, 1930 was one of the two most common roundworms recovered. It generally occurred in large numbers (2060 in one host) in the cecum and large intestine. Even in such numbers no noticeable pathology was observed. Crites (1956) found opossums from Ohio infected and redescribed this nematode. Reports from other localities in North America indicate its range coincides with that of the opossum.

Physaloptera turgida (Rudolphi, 1819) was equally common. Infections ranged from two to 170 worms. In apparent contrast to *C. americana*, *P. turgida* does considerable harm, in some instances by penetrating the stomach wall. Much of the information on *Physaloptera* spp. was summarized by Morgan and Hawkins (1949) and Krupp (1962) reported a treatment of opossums with physalopteran infections.

Viannaia hamata Travassos, 1914 and *Longistriata didelphis* (Travassos, 1914) occurred in about 70% of the animals and in nearly equal numbers in each infected host. Both species have been reported from South, Central, and North America throughout the range of the opossum.

Capillaria aerophila (Creplin, 1839) occurred in the lungs of about 20% of the opossums. Commonly called the fox lungworm it has been reported many times from European and North American foxes. The detection of this form is facilitated by palpating the lung tissue for the cyst-like nodules. Dissection of these nodules reveals the tightly coiled worms. Some may be free in the respiratory tract of the host. Christenson (1935, 1938) studied the morphology and life history of *C. aerophila* and Read (1949) presented a key to the species of *Capillaria* spp. in North American mammals.

Trichuris sp. was found only in one animal from Durham County with but three specimens present. Species identification is difficult even with abundant material. Babero (1960) presented a detailed discussion of five species of trichurids from marsupials, one of which, *T. didelphis*, he described as new. Only this species and *T. minuta* Rud., 1819 have been reported from *Didelphis virginiana*.

Two species of immature acanthocephalans were recovered from three hosts. Thirteen of the eighteen appear to be *Centrorhynchus* sp., the other five are unidentified. It is presumed that both are accidental forms.

Summary

Fifty-four opossums from North Carolina were examined for parasitic helminths. Thirteen species were recovered. For the most part, the helminths reported herein appear to parallel those listed by other investigators of helminths of the opossum.

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The Effects of Parasitism by the Trematode *Plagioporus virens* on the Digestive Gland of its Snail Host, *Flumenicola virens*[1]

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ABSTRACT: The effects of parasitism by the trematode *Plagioporus virens* on the digestive gland of the snail *Flumenicola virens* has been studied. The parasite causes damage to the organ by increased pressure, active ingestion of the digestive gland cells, and by the increased burden placed upon the snail in disposing of the waste of the parasite. Glycogen and lipid uptake from the digestive gland by the parasite was found to occur via two routes: active ingestion of gland cells by the parasite and absorption across the body membrane of the parasite. Aminopeptidase was found associated with the body wall of mature rediae and developing cercariae and is thought to cause some lysis of the host cells. Alkaline phosphatase was found in greater quantities in snails infected with *P. virens*. This enzyme is thought to function in the metabolism of glycogen.

The literature on the cytological and metabolic effects of parasitism by larval digenea on their molluscan host has been reviewed by Cheng and Snyder (1962a, b) and James (1965). The host species studied to date include two freshwater lamellibranchs, one freshwater prosobranch, two marine prosobranchs, and ten freshwater pulmonates.

In the present investigation the cytological effects by *Plagioporus virens* on the structure and histochemistry of the digestive gland of *Flumenicola virens*, along with the disposition of glycogen, glucose, lipids, phosphomonoesterase, and aminopeptidase is compared in the hepatopancreatic cells of uninfected and infected snails.

Materials and Methods

Specimens of *Flumenicola virens* were collected from Gales Creek, located 35 miles west of Portland, Oregon. During a 6-month period, between April, 1968, and September, 1968, approximately 250 snails were brought into the
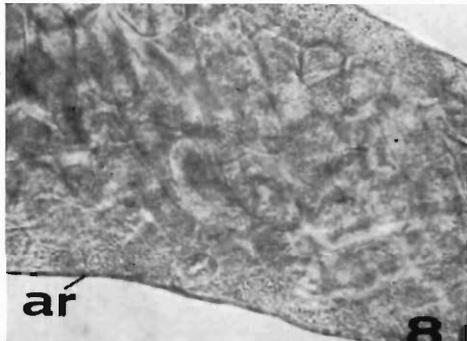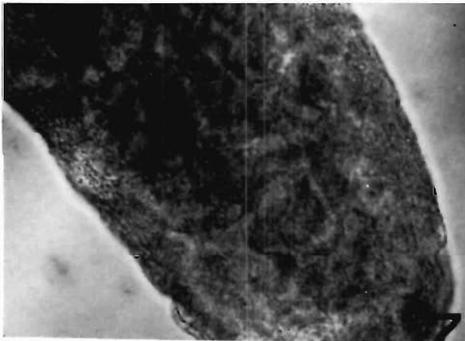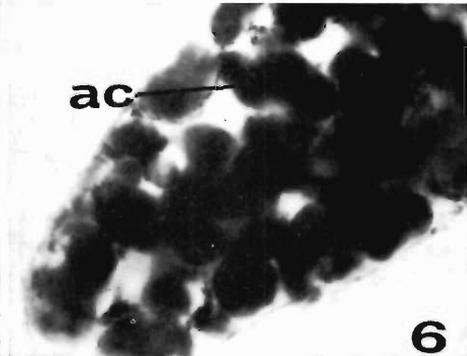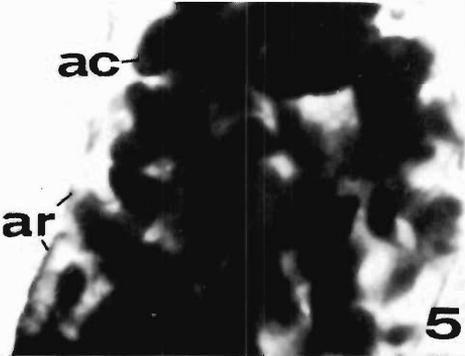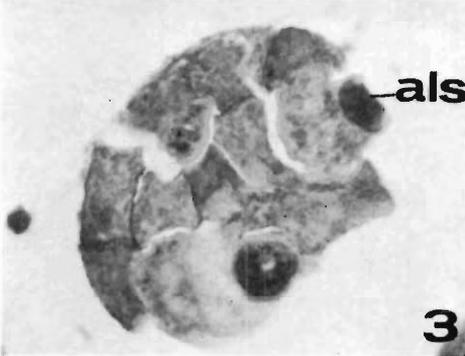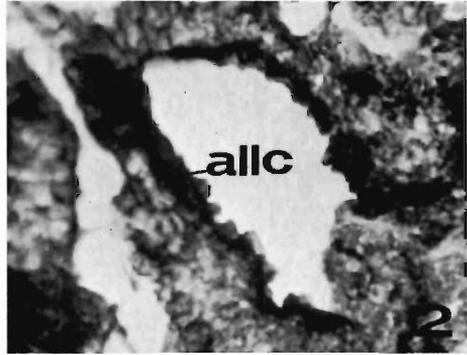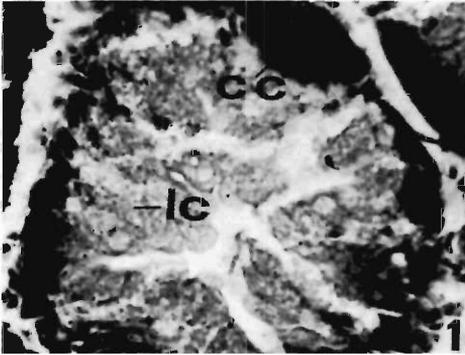
laboratory, and isolated individually in glass dishes containing distilled water at 20 C for detection of infection. Two sets of 25 specimens each were used for specific tests, one set consisting of uninfected snails and the other of infected snails. Members of both sets were removed from their shells, and the digestive gland was removed and prepared for histological and histochemical evaluations.

Routine sections were prepared by the paraffin method after fixation in 10% neutral formalin, and stained with Harris hematoxylin and eosin.

For more detailed histochemical examination, tissues were fixed in formol calcium or in absolute methanol saturated with barium hydroxide and sectioned at 8 μ. The periodic acid schiff (PAS) reaction coupled with diastase digestion for glycogen, Okamoto's method for glucose, the nile blue A and oil red O techniques for lipids, simultaneous Azodye coupling techniques and Gomori's techniques for acid and alkaline phosphatase, and the alpha naphthylacetate method for esterases were performed according to the procedures outlined by Barka and Anderson (1965). Appropriate controls were used with each test.

A modification of Nachals, Crawford and Seligman's L-Leucyl-β-Naphthylamine (LBN) method described by Cheng and Yie (1968) was used for aminopeptidase examinations. As controls two ions known to inhibit aminopeptidase activity were added individually to the incubation media. The inhibitors used were 0.01 M solutions of $CuCl_2 \cdot 2H_2O$, and $MnSO_4 \cdot 7H_2O$.

Observations and Results

Normal Digestive Gland

In *Flumenicola virens* the digestive gland occupies the center of the visceral mass. It rests on the dorsal side of the snail beneath the spire and is intimately associated with the gonads along its dorsal surface. It consists of a series of blindly ending racemose glandular tubules bound together by a meshwork of interstitial cells, between which are found branches of the hepatic artery, blood lacunae, and numerous nerve endings. The entire gland is enclosed by a tunica propria.

The digestive gland is composed of liver cells and calcium cells (Fig. 1). The elongate liver cells possess a basal nucleus which contains deeply staining chromatin granules, and a single, small almost central nucleolus. The cytoplasm contains widely scattered basophilic granules and a number of small vacuoles devoid of pigments. Droplets of fatty material are distributed in the area of the nucleus. Calcium cells (Fig. 1) are less numerous and occur in groups of two to six interspersed among the liver cells. The cell is triangular with a broad base and a rather narrow, elongated apex at the lumen of the tubule. The cell is intensely basophilic with a large spherical nucleus inside of which is a single, variably-disposed nucle-

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Figure 1. Normal digestive gland tubule of *Flumenicola virens* showing two cell types. Liver cells (lc). Calcium cells (cc). H. & E. stain. × 430.

Figure 2. Cross section of uninfected digestive gland tubule of *Flumenicola virens* showing site (s) of alkaline phosphatase. Gomori's calcium-cobalt method. Allc (alkaline phosphatase in liver cell × 100).

Figure 3. Rediae infecting the digestive gland of *F. virens* showing sites of alkaline phosphatase activity. Calcium-cobalt method. Als (alkaline phosphatase in sucker. × 100).

Figure 4. Anterior portion of adult rediae of *Plagioporus virens* showing aminopeptidase activity in wall. (L-Leucyl-β-naphthylamide medium. × 200). Ar (aminopeptidase in rediae).

Figure 5. Middle portion of adult rediae of *P. virens* showing aminopeptidase activity in wall and in the developing cercariae. (L-Leucyl-β-naphthylamide medium. × 200). Ac (aminopeptidase in cercaria).

Figure 6. Posterior portion of adult rediae of *P. virens* showing aminopeptidase activity in the developing cercariae. (L-Leucyl-β-naphthylamide medium. × 200).

Figure 7. Portion of adult rediae of *P. virens* showing absence of aminopeptidase in wall. (L-Leucyl-β-naphthylamide medium with $CuCl_2 \cdot 2H_2O$, × 200.)

Figure 8. Portion of adult rediae of *P. virens* showing traces of aminopeptidases in wall, but absence of aminopeptidase in developing cercariae. (L-Leucyl-β-naphthylamide medium with $MnSo_4 \cdot 7H_2O$, × 200).

olus and diffuse chromatin granules. Deposits of calcium are scattered throughout the cytoplasm of these cells.

The Parasitized Digestive Gland

The parasitized digestive gland of *Flumenicola virens* appears darker than the normal structure. The rediae permeate the structure and are readily apparent to the naked eye in heavy infections.

The parasites invade the digestive gland after having completely devoured the gonads and take up positions between the tubules in the connective tissues. During the rapid growth of the rediae, the tubule cells become cuboidal. This decrease in height appears to be due to pressure and crowding associated with the great increase in size of the parasite as well as of its numbers.

Glycogen Utilization

The digestive gland of *F. virens* is normally rich in glycogen. Uniformly distributed clumps of fine PAS-positive granules are present throughout the cytoplasm of the liver cells. Occasional glucose droplets are present in the proximal halves of the digestive gland.

Two distinct means of transfer appear to be involved in the movement of host glycogen into the parasite. The most direct involves the ingestion of host cells, while the other is operative on the physiological level of transfer of the glycogen from the liver cells to the parasite without destruction of the former. This depletion is seen as a decreased PAS reaction in the host cells contiguous with the rediae.

The ingestion of the host cells is evidenced by the destruction of tubules and the appearance of large amorphous aggregates of PAS-positive materials in the sucker, pharynx and digestive tract of the developing rediae. The initial loss of host cells occurs at the periphery of the digestive gland, but spreads in heavy infections to include the destruction of nearly all of the digestive gland tubules.

Lipid Utilization

In healthy digestive glands little or no neutral fat was seen with nile blue A and oil red O. There appeared to be an increase in the amount of neutral fats present in the parasitized digestive gland. The fat was scattered throughout most of the cells of the tubules and increased with the number of parasites present. In addition to neutral fat globules, others of fatty acid were found within the liver cells. Both neutral fats and fatty acids were observed around the redial wall and within the somatic cells of the developing cercariae.

Acid and Alkaline Phosphatases

Acid Phosphatase, indicated by finely granular brownish-black precipitates with Gomori's method and by the red-brown Azo dye with the simultaneous Azo coupling technique, was found in the nuclei and sparsely scattered throughout the cytoplasm of the digestive cells. No acid phosphatase was associated with the luminal surface.

Similarly, alkaline phosphatase was scattered throughout the cytoplasm but was primarily concentrated around the lumen of the tubules (Fig. 2). A heavy concentration of the enzyme was also observed in the calcium cells around the calcium spherules.

Alkaline phosphatase was observed to have increased in the parasitized digestive gland, and was very prominently associated with the redial body wall and suckers of the developing cercariae (Fig. 3). No increase in acid phosphatase activity was observed.

Esterases

No reactions for esterases were observed in either the uninfected or the infected digestive glands using the alpha-napthyl acetate technique.

Aminopeptidase

The digestive gland cells of *Flumenicola virens* were negative for aminopeptidase activity when stained with LBN.

Mature rediae, enclosing well developed cercariae, revealed aminopeptidase activity associated with their body walls (Figs. 4–6). The activity was uniformly distributed over the entire body surface. The enclosed cercariae also revealed sites of aminopeptidase activity along their body proper and the tail (Figs. 5, 6).

That the characteristic purplish-blue color did indicate aminopeptidase activity was verified in the specimens treated with enzyme-inhibiting ions. Larvae incubated in the copper-containing medium were completely

devoid of any color reaction (Fig. 7). Those larvae incubated in manganese-containing medium revealed a definite reduction of the color reaction (Fig. 8).

Discussion

There are numerous reports of the destruction of snail digestive glands by larval trematodes (Cheng and James, 1960; Cheng and Snyder, 1962a; B. L. James, 1965; James and Bowers, 1967; and Porter et al., 1967).

The impairment of digestive gland function by rediae of *Plagioporus virens* manifests varying degrees of severity as demonstrated by changes in cell shape and decreases in glycogen and fat content.

Mechanical pressure caused by the infiltration of rediae and the movement of cercariae leads to shrinkage of cells, and eventually to a decrease in their number and of the liver tubules. Cheng and Snyder (1962a) reported the destruction of the digestive gland of *Helisoma trivolvis* (Say) by the extrinsic mechanical pressure caused by *Glypthelmins pennsylvaniensis* Cheng, and Porter et al. (1967) reported a similar situation in *Oxytrema siliqua* infected with *Nanophyetus salmincola*. Their suggestion that the presence of cercarial excreta supplemented this activity is in keeping with the interpretation of Faust (1920) and Hurst (1927), both of whom attributed digestive gland destruction to the accumulation of waste products.

The presence of aminopeptidase along the body wall of mature rediae and the enclosed developing cercariae suggests that some lysis of the digestive gland cells in the immediate proximity of those larvae might be due, at least in part, to digestion by this enzyme. The possible digestion of host cells by larval aminopeptidase was first proposed by Cheng and Yie (1968) who also suggested that amino acids resulting from this enzymatic hydrolysis can be utilized by the rediae and cercariae in addition to amino acids occurring in the snail's hemolymph.

Although aminopeptidase activity can account for some lysis of the surrounding host cells, the findings of this study suggest that damage to the organ is also brought about by the ingestion of tubule cells by the rediae. The histochemical demonstration of glycogen inside the sucker and pharynx of the rediae

is interpreted as evidence of active feeding by the parasite.

The concurrent decrease of glycogen from the digestive gland and the increase in PAS positive material in the rediae and the developing cercariae suggest that two distinct means of transfer are involved in the movement of host glycogen into the parasite. one involves the ingestion of host cells, while the other involves the physiological transfer of the glycogen from the gland cells to the parasite without destruction of the former. The depletion of glycogen from cells immediately adjacent to the larvae plus the localization of glucose along the redial body suggest the possibility of glycogen breakdown, and absorption across the body surface of the parasite as a smaller molecule. It appears as if the mechanism postulated by Cheng and Snyder (1962a, 1963) for the conversion and resynthesis of glycogen after passage across the larval body wall as free glucose is operative in *F. virens*.

Additional support for this interpretation comes from the distribution of alkaline phosphatase in the redial body wall and the cercarial suckers. Several investigators (Von Brand, 1952; Erasmus, 1957a, b; Rogers, 1957; Robinson, 1961; Cheng, 1964, and Porter et al., 1967) have all suggested that alkaline phosphatase probably plays a role in the carbohydrate metabolism of the digestive gland infected by larval trematodes by hydrolysing the sugar into simpler compounds. It is suggested that *Flumenicola virens* can also hydrolyse some substrate in its environment and that this hydrolytic activity is correlated with glycogen degradation.

The evidence in this study confirms the presence of neutral fat in the digestive gland of the snail *F. virens*. It was not determined in this study whether the transfer of the fat of the host to the larvae was made possible by a lipase secreted by the host cells, or by the parasite. However, the localization of neutral fats and fatty acids along the body wall of the rediae suggest that an enzyme elaborated by the larvae plays a role in fat utilization.

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Survival and Development of *Eimeria adenoeides* in Cell Cultures Inoculated with Sporozoites from Cleaned and Uncleaned Suspensions

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ABSTRACT: Bovine embryonic kidney cell cultures were inoculated with 100, 250, and 500 thousand and 1 million *E. adenoeides* sporozoites from suspensions cleaned of debris (oocyst and sporocyst shells, oocysts, and sporocysts) and from suspensions that contained debris. At 5 hr, the numbers of intracellular sporozoites from both suspensions were directly proportional to the size of the inoculum, but, at all inocula levels, the number was less within cultures inoculated with sporozoites from the cleaned suspensions. At 48 hr, the number of schizonts in cultures inoculated with sporozoites from cleaned suspensions was directly proportional to inocula size; in those inoculated from uncleaned suspensions, the number increased, but not in proportion to the size of the inoculum. Between 5 and 48 hr following inoculation with 500,000 and 1 million sporozoites there was a greater loss of parasites in cultures inoculated with sporozoites from uncleaned suspensions. The lack of proportionality and loss of parasites is attributed to debris in the inoculum.

After coccidial sporozoites are excysted *in vitro*, the suspension also contains oocyst and sporocyst shells, intact oocysts, and sporocysts containing sporozoites. If the oocyst suspension is not cleaned well before the release of sporocysts and excystation, the resultant sporozoite suspension also contains fecal debris. In the initial work on cultivation of coccidia, Patton (1965) reported that debris and shells of oocysts and sporocysts carried in a suspension of *Eimeria tenella* sporozoites were toxic to cell cultures. Fayer and Hammond (1967) stated that oocyst and sporocyst debris of *E. bovis* obscured observation of parasites, but did not have an appreciable toxic effect on cells.

The present report compares survival and development in cell culture of *E. adenoeides* sporozoites from suspensions cleaned of debris with those from suspensions that were not cleaned.

Materials and Methods

Oocysts were collected, freed of fecal debris, and sporulated as previously described (Vetterling, 1969). They were made bacteria-free by the method of Jackson (1964) and stored in Ringer's solution at 3 to 6 C. All were less than 3.5 months old when used.

Sporocysts were released from oocysts by grinding (Doran and Vetterling, 1969). They were then washed twice with 15 ml of Ringer's solution and treated with trypsin-bile solution

(Doran and Vetterling, 1967) at 43 C until 85–90% of the sporozoites within released sporocysts had excysted.

Bovine embryonic kidney cells were cultured on 10- by 35-mm cover glasses in Leighton tubes and were prepared from a frozen supply of cells that had been serially passed 22 times before freezing. Only cultures that were confluent and did not contain cell aggregates were used. The growth and maintenance media were the same as previously used (Doran and Vetterling, 1969).

Sporozoite suspensions were cleaned of all debris by passage through two columns (240 by 15 mm) of packed glass beads (100 μ). Sporozoites in both cleaned and uncleaned suspensions were concentrated by centrifugation. Those in the uncleaned suspension were washed twice with 15 ml of Ringer's solution. Both were resuspended in a medium consisting of 95% Medium 199 + 5% chicken serum at pH 7.0–7.2. In each of three replications, the concentration in both suspensions was adjusted so that 1 ml contained 1 million sporozoites. Only those showing refractileness were counted. As previously mentioned (Doran, 1969), these did not stain with 1% nigrosin and showed motility when examined at 37–43 C. Sufficient amounts of each suspension were then placed in three other tubes so that, when diluted to 10 ml, 1 ml contained 100, 250, and 500 thousand sporozoites. After thorough agitation, 1 ml of each of the dilutions was
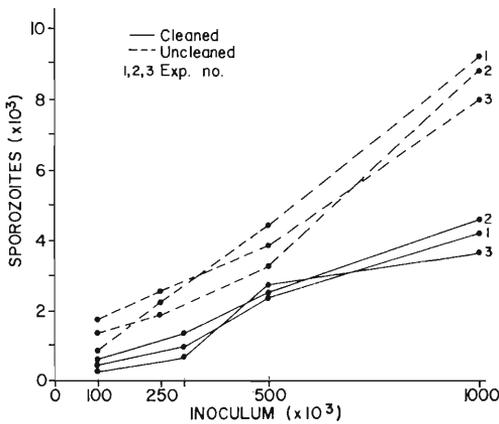
Figure 1. Number of intracellular sporozoites at 5 hr. In this Figure and in Figure 5, quantities represent total of counts from 3 cover glasses. There was less than 17% variation between counts.

pipetted into six cultures. At 4 hr, the medium in each tube was replaced with 5 ml of maintenance medium. Cultures were kept in a walk-in incubator with a regulated cycle as previously described (Doran and Vetterling, 1969).

At 5 and 48 hr after inoculation, cover glasses were removed from three tubes in each set of inoculated cultures. After fixing and staining as before (Doran and Vetterling, 1969), counts were made of the number of sporozoites, trophozoites, and schizonts in a strip 285 μ wide across the middle of each of the three cover glasses. Counts included only those parasites that were within cells of the monolayer attached to the cover glass.

Results

In cultures inoculated with sporozoites from either cleaned or uncleaned suspensions, the number of intracellular sporozoites at 5 hr was directly proportional to the size of the inoculum (Fig. 1). However, at all inocula levels there were fewer intracellular sporozoites from cleaned suspensions than from the uncleaned suspensions. Figure 2 shows a monolayer at 5 hr after inoculation with 1 million sporozoites from a cleaned suspension; Figure 3 shows one at the same time after receiving the same number from an uncleaned suspension.
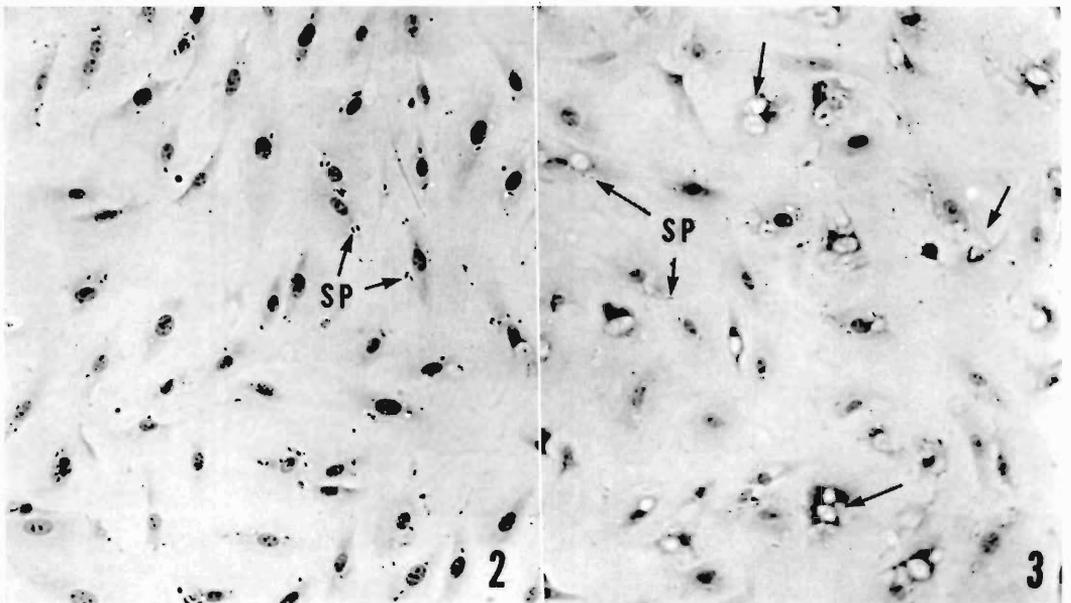
Figures 2 and 3. Cell cultures 5 hr after inoculation with sporozoites from cleaned and uncleaned suspensions, respectively. Stained preparations, phase contrast. Magnification, 625 ×. Arrows indicate some of the debris. SP = sporozoite.
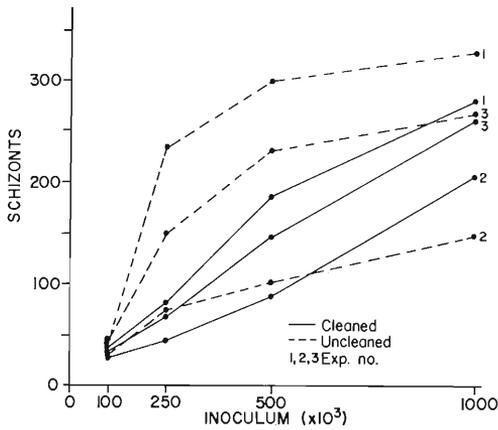
Figure 4. Number of schizonts at 48 hr.

Figure 5. Percentage of loss of parasites between 5 and 48 hr.

At 48 hr, the number of schizonts in cultures inoculated with cleaned suspensions was also directly proportional to the size of the inoculum (Fig. 4). In cultures inoculated with uncleaned sporozoite suspensions, this proportionality did not occur. With inocula of 500,000 and 1 million, the number of schizonts increased, but not in proportion to the inocula size. At this time, cultures that received 500,000 and 1 million sporozoites from uncleaned suspensions contained more dead or dying cells that were detached from the cover glass. Many of these contained parasites as previously illustrated for *E. meleagrimitis* (Doran and Vetterling, 1969; Fig. 24). Schizonts in cultures receiving the two larger inocula from cleaned suspensions were predominantly rounded; in cultures from uncleaned suspensions they were mostly irregular in shape, caused by growth around oocyst or sporocyst debris.

Between 5 and 48 hr, there was a greater loss of parasites in cultures inoculated with 500,000 and 1 million sporozoites from the uncleaned suspensions than there was in those inoculated with similar numbers of sporozoites from cleaned suspensions (Fig. 5).

Discussion

Sharma and Foster (1964) reported that extracts of *E. tenella* oocysts (fluid with oocysts) produced a toxic effect when inoculated into rabbits. Fayer and Hammond (1967)
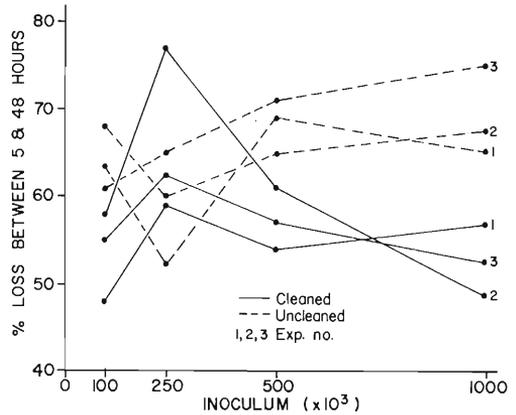
found that fluid obtained after mechanical release of sporocysts from *E. bovis* oocysts killed cells within hours when inoculated into cell cultures. It is very unlikely that such material, or residual excystation fluid, was responsible in this study for lack of proportionality and greater loss of parasites in cultures inoculated with 500,000 and 1 million sporozoites from uncleaned suspensions. Released sporocysts and excysted sporozoites were both washed twice before resuspending in at least 50 ml of media necessary to obtain the 1 million/1 ml concentration. It seems more probable that debris (oocyst and sporocyst shells, oocysts, and sporocysts) was the cause. Development of schizonts could have been suppressed, but it is more likely that debris disrupted the cell layer and caused death of cells containing parasites.

It is most desirable to use sporozoites in cleaned suspensions for inoculating cell cultures. Because of the absence of debris, counts are much easier to make, schizonts are more uniform in shape, and larger dosages can be used without diminished schizont development. The smaller numbers of sporozoites found within cells at 5 hr was probably due to the method of cleaning. It is probable that some of those in the inoculum, counted because of their refractileness, were either dead or altered so that they could not enter cells. This situation has also been observed with sporozoites that had been frozen (Doran, 1970).

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Aphasmatylenchus straturatus sp. n. (Nematoda : Hoplolaimidae) from West Africa

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ABSTRACT: *Aphasmatylenchus straturatus* sp. n., found around roots of *Arachis hypogaea* L. from Haute-Volta (West Africa), is described and figured. The new species is distinguished by the longitudinal striation of the cuticle, and by the measurements of the male.

Sher (1965) described a new genus, *Aphasmatylenchus*, with the type and only species *A. nigeriensis*. *Aphasmatylenchus straturatus* sp. n. was recovered from soil around the roots of the peanut (*Arachis hypogaea* L.) in Haute-Volta. The description of this species is based on specimens fixed and mounted in glycerin by Seinhorst's method (1962).

Measurements

FEMALES (36): L = 1.43 (1.18–1.75) mm; a = 31.1 (26–35.6); b = 7.8 (6–9.1); c = 22.4 (18–27.5); V = 52.9 $(15.3–24.3 \quad _{50–56} \quad 13.8–26)$; spear = 33 (30–36) μ.

MALES (6): L = 1.13 (0.99–1.26) mm; a = 34.8 (29.1–38); b = 6.8 (6.1–7.2); c = 16.4 (14.6–17.4); T = 28.7 (28–29.4); spear = 29 (28–30) μ.

HOLOTYPE (FEMALE): L = 1.47 mm; a = 33.4; b = 7.7; c = 23.7; V = 51.9, spear = 32 μ.

ALLOTYPE (MALE): L = 1.18 mm; a = 37; b = 7.24; c = 16.5; spear = 28 μ.

Description

FEMALES: Body curved ventrally to C-shape sometimes forming a closed curve. Annules 1.4–1.8 μ wide in center of body. Cuticle

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Figure 1. *Aphasmatylenchus straturatus* sp. n. Female: A; entire body, lateral view. B, C, D; anterior region. E; face view. F; cross section through basal annule of lip region. G; ovary. H; cross section at center of body. I; areolation of lateral field in middle of body. J, K, L; posterior end, lateral view showing cuticular ornamentation diagrammatically. M; posterior end, ventral view.
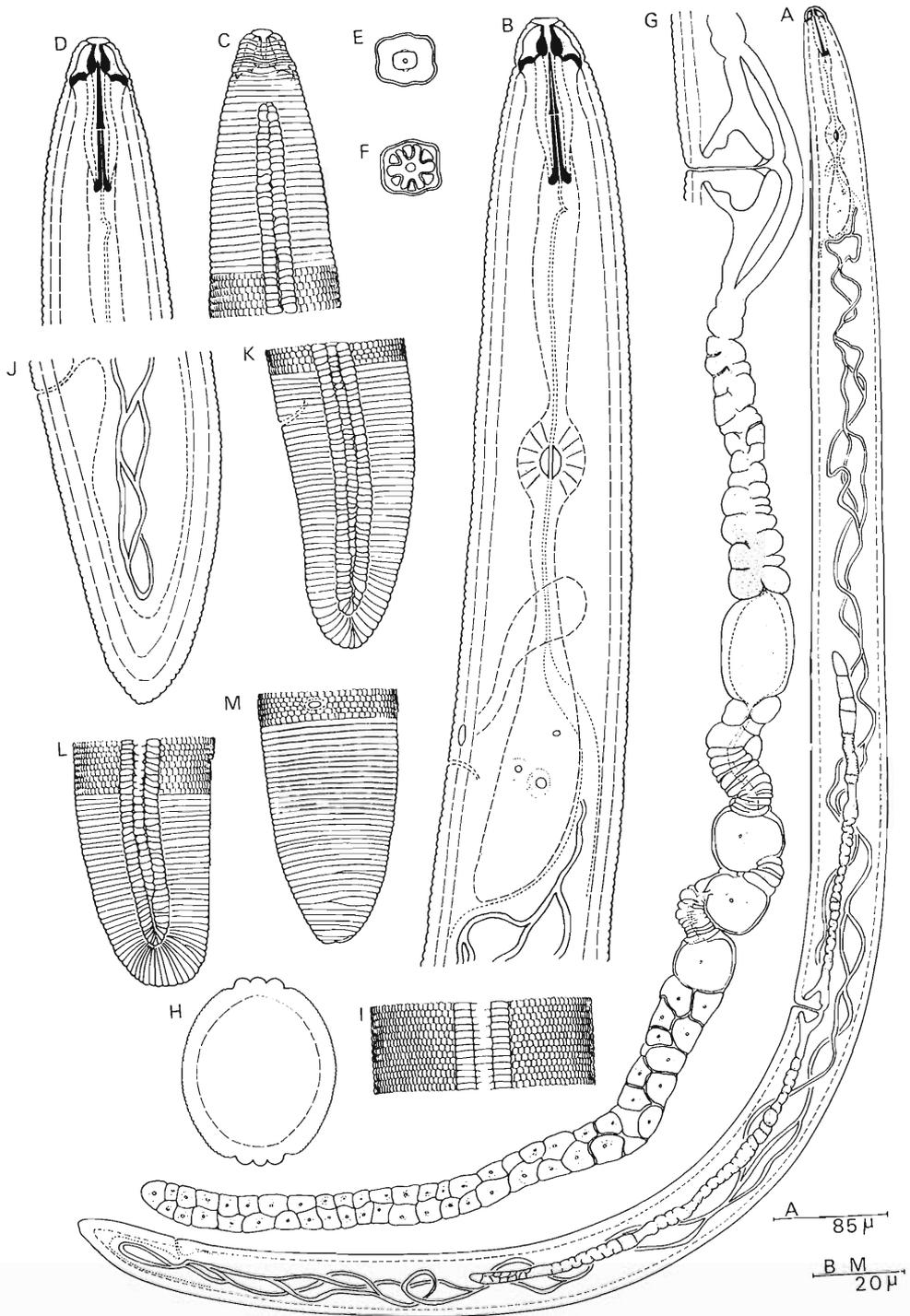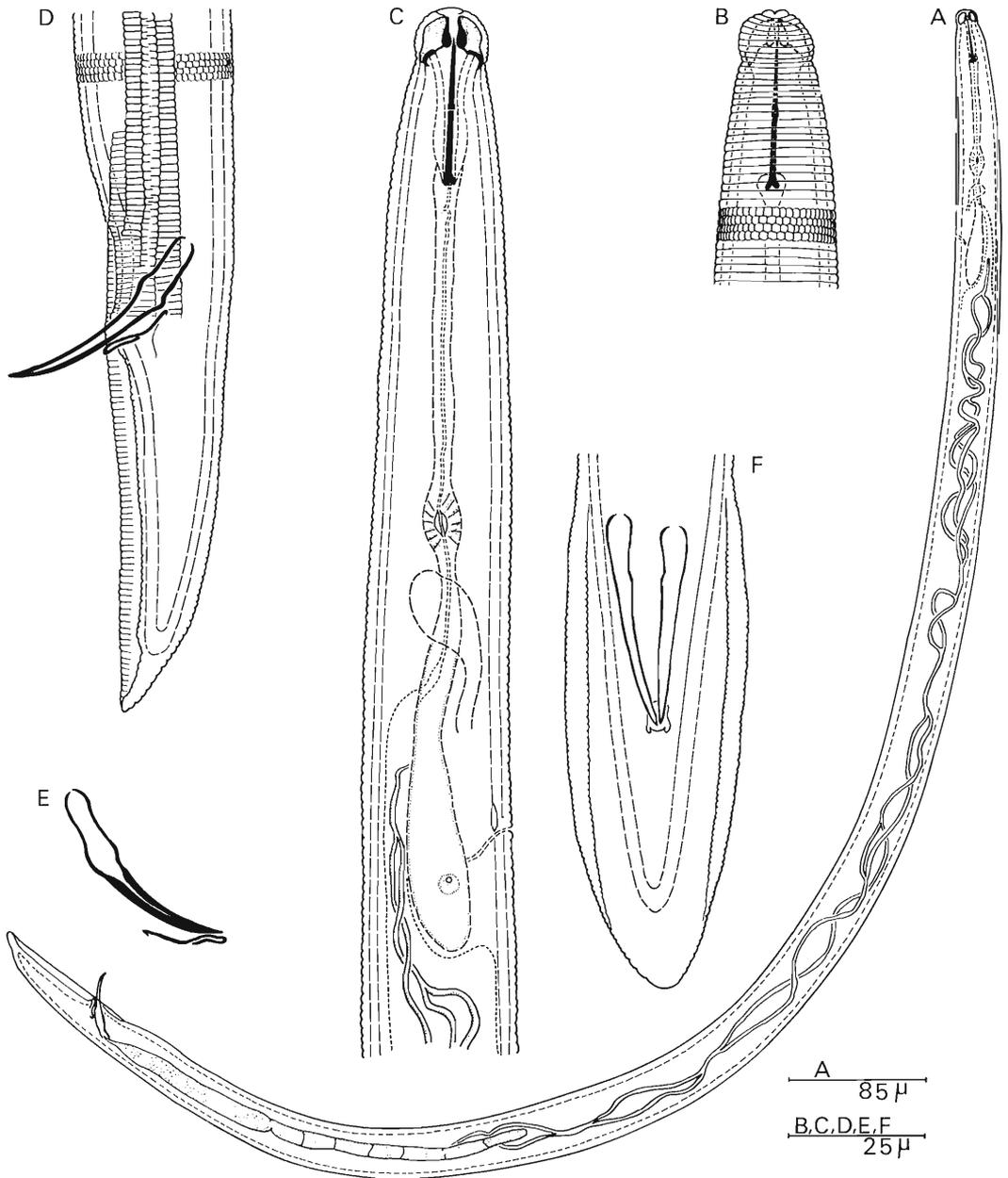
Figure 2. *Aphasmatylenchus straturatus* sp. n. Male: A; entire body, lateral view. B; anterior end, ventral view, showing cuticular ornamentation diagrammatically. C; anterior region, lateral view. D; posterior end, lateral view showing articular ornamentation diagrammatically. E; spicules and gubernaculum. F; posterior end, ventral view.

exhibits an irregular corn-cob pattern, each body annule being divided (with the exception of the lateral field) by fine cross-striae. Lateral field occupying about ¼ of body width, with four incisures (three longitudinal bands); outer bands striated transversally areolation of central band inconspicuous and incomplete.

Lip region a truncated cone, subhexagonal and flattened laterally in face view, set off from body contour, with 8–11 annules, the anterior one being conspicuously larger. Length of stylet 30–36 μ (anterior part: 13–17 μ).

Esophagus: procorpus sometimes slightly narrowing at posterior end; dorsal esophageal gland opening 8–9 μ behind spear base; esophageal glands with three distinct nuclei.

Excretory pore situated 142–196 μ from anterior end, 1–3 annules below hemizonid, the latter being 2–3 annules wide. Spermatheca almost rectangular, with sperm-like bodies.

Tail cylindro-conical, tapering to round or bluntly-round, 44–82 μ or 1.4–2.5 anal-body width long.

MALES: Lip region almost hemispherical, distinctly set off, with 9–10 annules. Stylet and esophagus not as well developed as in female. Only one esophageal nucleus could be observed in the small number of male specimens available. Cuticular ornamentation and areolation as in female. Excretory pore situated 150–164 μ from anterior end. Testis single, outstretched. Spicules curved, 42–44 μ long; gubernaculum 14–16 μ long, with titillae. Caudal alae enveloping tail, edges of the bursa annulated. Tail 3 to 3.3 body widths long at cloaca.

A tube-like or "serpentine" resembling that described by de Guiran and Germani, 1968 in Brachydorus tenuis can be seen within the intestinal walls winding its way over the entire length of the intestine, the post-anal sac included. A "serpentine" has also been observed by us in type specimens of A. nigeriensis Sher, 1965.

TYPE HABITAT AND LOCALITY: Soil around roots of peanut (Arachis hypogaea L.). I.R.H.O. Station, Niangoloko, Haute-Volta.

HOLOTYPE: Female, slide n° 5872—Laboratoire de Nematologie, O.R.S.T.O.M., Abidjan, Ivory Coast.

ALLOTYPE: Male, slide n° 3878, same collection.

PARATYPES: 17 females, 4 males in same collection; 2 females: Nematology Department, Rothamsted Experiment Station, Harpenden, Herts., England; 3 females: Nematology Department, Plantenziektenkundige Dienst, Wageningen, the Netherlands; 3 females: Nematology Department, Instituut voor Plantenziektekundig Onderzoek, Wageningen, the Netherlands; 2 females: Nematology Department, University of California, Davis USA; 5 females, 1 male: USDA Nematode collection, Beltsville, Maryland, USA; 3 females: Department of Nematology, University of California, Riverside, California, USA.

Diagnosis

Aphasmatylenchus straturatus sp. n. can be differentiated from the single species of the genus, A. nigeriensis Sher, 1965 by the ornamentation of the cuticle.

The male of A. straturatus sp. n. differs from the male of A. nigeriensis Sher by its greater length (L = 0.99–1.26 mm against 0.82–0.96 mm) and its longer spear (stylet = 28–30 μ against 24–27 μ) and spicules (42–44 μ against 31–36 μ).

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Surface Ultrastructure of the Acanthocephalan Lemnisci[1]

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ABSTRACT: The pseudocoelomic lemniscal surface of *Moniliformis dubius* Meyer, 1933 exhibits ultrastructural features similar to the basal surface of the metasomal tegument. These include a 100 Å thick plasmalemma associated externally with an amorphous basement lamina, and internally with half desmosome-like structures. The trilaminate membranes are extensively infolded into channels spacially continuous with the body cavity. Glycogen-like particles and deposits of lipoid material are prominent cytoplasmic inclusions. This morphology is consistent with concepts of lemniscal development, and suggestive of a similar role in physiological processes.

The lemnisci of acanthocephalans are outgrowths of the subcuticula of the neck, which extend back into the pseudocoel. Crompton (1963) and King (1965) felt they are concerned with the movement of fluid when the proboscis is active. Bullock (1949) and Pflugfelder (1949) suggested a possible role of the lemnisci in lipid metabolism. Crompton and Lee (1965) thought they excreted absorbed lipids into the body cavity, whereas Hammond (1967, 1968) alternately suggested the reverse process occurs with lipids being discharged as a waste product at the surface of the proboscis. In conjunction with current studies of the body wall structure and function (Wright and Lumsden, 1968, 1970), the lemnisci of *Moniliformis dubius* Meyer, 1933 were examined by electron microscopy. Surface features of these organs are described in the present paper.

Materials and Methods

Adult female *Moniliformis dubius* were recovered from albino rats and transferred to ice cold 6% glutaraldehyde in Millonig's (1961) 0.12M phosphate buffer (pH 7.4) containing 2 mM calcium chloride and 3% sucrose. The lemnisci were immediately excised and left intact during subsequent tissue preparation. The anterior portion of the metasoma was cut into small pieces, and both tissues fixed for two hours. After washing overnight in 0.12M phosphate buffer containing 2mM calcium chloride and 5% sucrose, both tissues were post-fixed 90 min using ice cold phosphate-buffered 1% osmium tetroxide, dehydrated in ascending concentrations of cold ethanol and embedded in epon. Ultrathin sections were cut on diamond knives using a Sorvall MT-2 ultramicrotome, collected on uncoated grids, and stained with aqueous uranyl acetate (Watson, 1958), and lead citrate (Reynolds, 1963). Electron microscopy was carried out with a Siemens Elmiskop 1A operated at 80 kv.

Observations and Discussion

The basal surface of the metasomal tegument is illustrated in Figure 1. As described by Wright and Lumsden (1970), it is limited by a 100 Å thick plasmalemma intimately associated with an externally positioned amorphous basement lamina approximately 0.2 μ thick. Invaginations of the membrane form channels penetrating the hypodermis to a depth of approximately 1 μ. Half desmosome-like structures appear to line the cytoplasmic side between the inpocketings. The lemnisci exhibit similar morphology (Fig. 2). The tri-

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Figure 1. Electron micrograph illustrating the basal surface of the metasomal hypodermis. The basal plasmalemma is characterized by numerous invaginations (i) between the half desmosome-like structures (hd) lining the cytoplasmic side of the membrane. The cytoplasm contains extensive deposits of glycogen (g) and lipoid material (L). Externally positioned to the membrane is the basal lamina (bl) and associated connective tissue (ct). × 50,000.
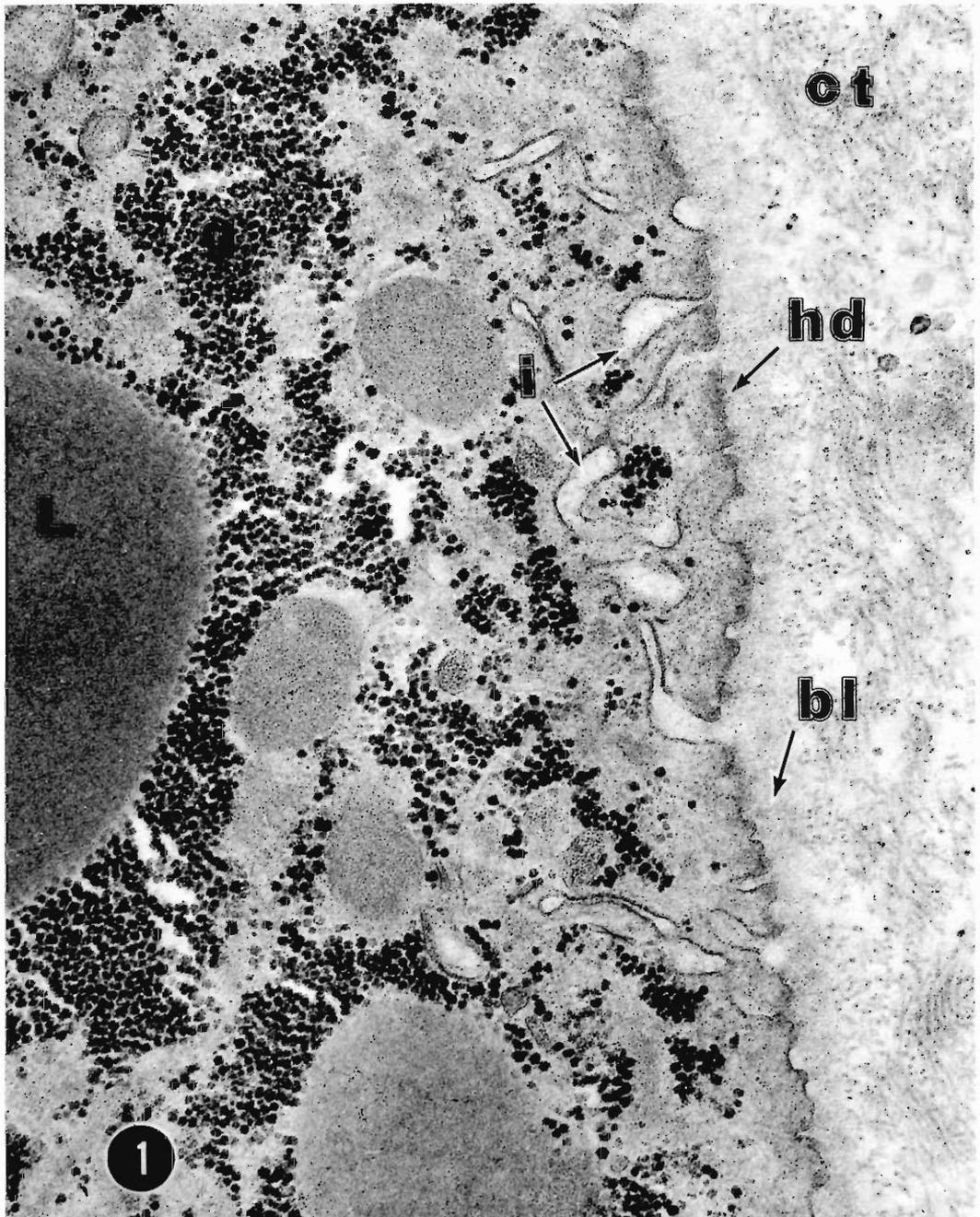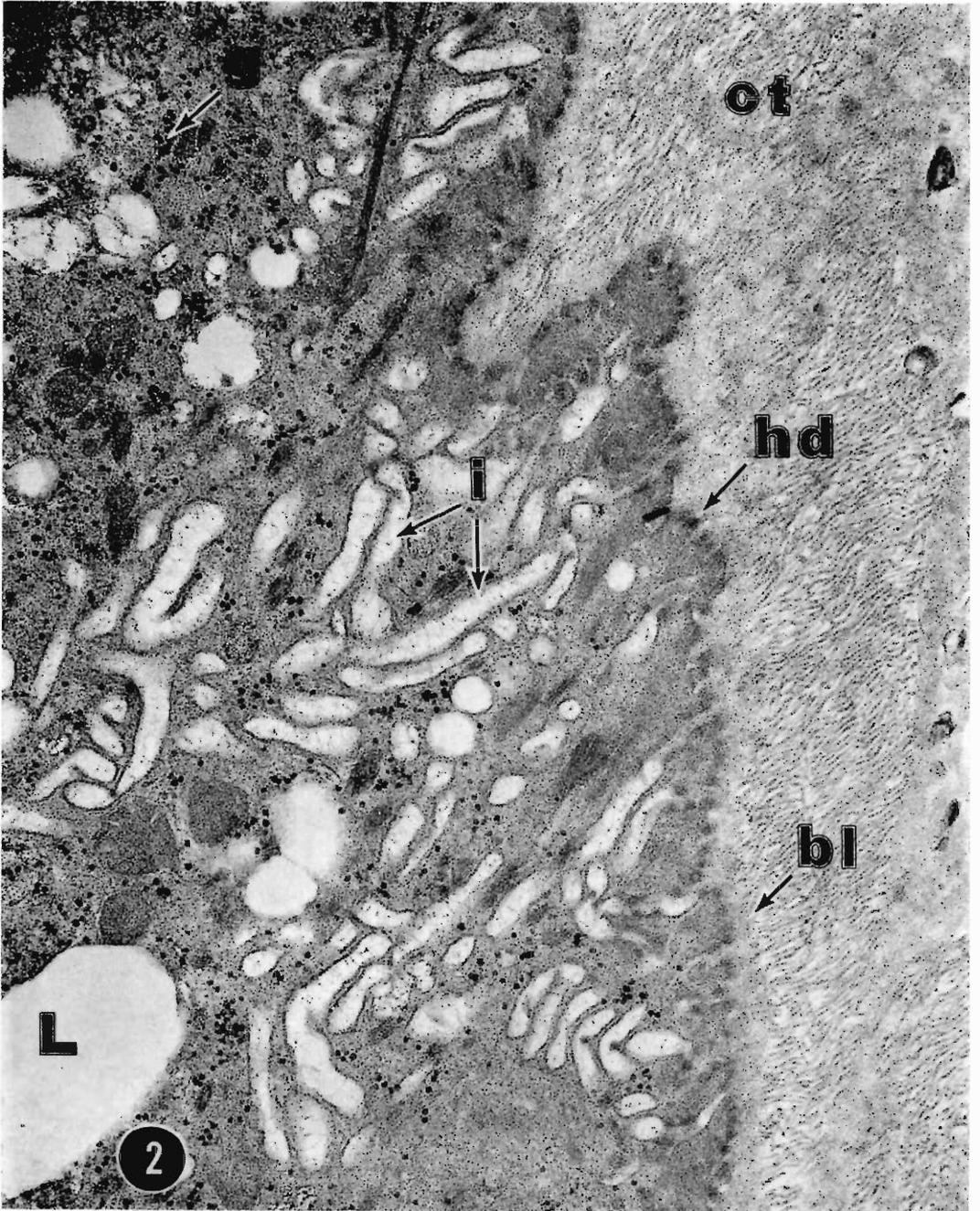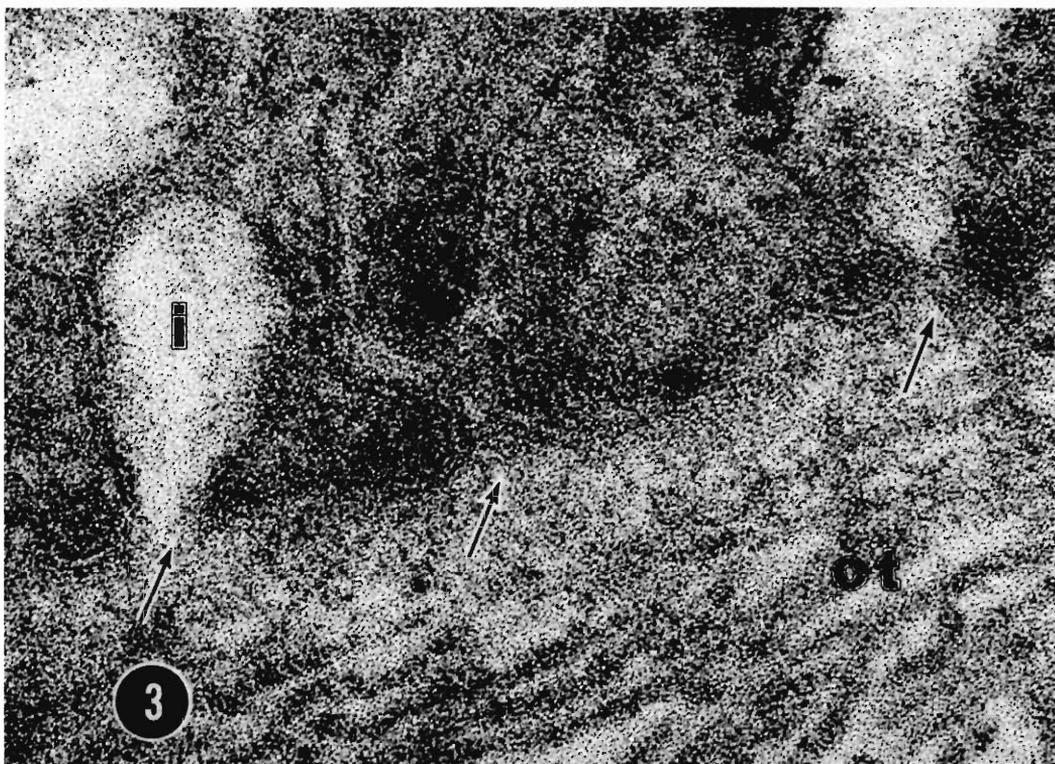
Figures 2 and 3. Surface morphology of the lemniscus. 2. Surface infolding of the plasmalemma forming invaginations (i) comparable to those seen at the basal surface of the body wall (Fig. 1) is a cardinal morphological feature of the lemniscus. Also note the half desmosome-like structures (hb), glycogen particles (g), lipoid material (L), adjacent basement lamina (bl) and connective tissue (ct). × 40,000. 3. High magnification image illustrating the continuity of the plasmalemma with that lining the invaginations (i) at the regions of membrane infolding (arrows). Conective tissue (ct). × 253,000.

laminate plasmalemma measures approximately 100 Å in thickness and is extensively infolded. Half desmosome-like structures are spaced between the invaginations along the cytoplasmic side of this membrane. A "basal" lamina is associated with fibrillar connective tissue on the coelomic side of the plasmalemma. Abundant glycogen-like particles are scattered throughout the cytoplasm of the lemniscus and body wall, and extensive deposits of lipoid material are a prominent feature of both tissues.

Moore (1946) observed that the lemnisci develope as evaginations of the hypodermal layer. The lemniscal nuclei, before migrating into the developing buds, constitute the lemniscal ring, formed from nuclei of the hypodermal

primordia. The similar ultrastructural morphology of the lemnisci and hypodermis is consistent with the idea that both tissues develop from the same anlage.

The prominent invaginations of the lemniscal plasmalemma greatly amplify the free surface area exposed to the pseudocoel. Spacial conflux between these invaginations and the body cavity is indicated in images such as Figure 3 where the limiting plasmalemma is seen to be continuous with that lining the infoldings. This surface amplification would be expected to enhance the efficiency of molecular interchange involving any diffusion process across this membraneous interface. It might therefore be suggested that, in view of their common ultrastructure, the pseudocoelomic surfaces of the

body wall and lemnisci both have an important physiological role in transporting material relative to the metasomal cavity.

Acknowledgment

The author wishes to express sincere gratitude to Dr. Richard D. Lumsden, Tulane University, for his continued support and interest during this investigation.

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An Evaluation of the Baermann Technic using Infective Larvae of *Haemonchus contortus*[1]

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abstract: An evaluation of the modified Baermann technic, using infective *Haemonchus contortus* larvae, was made to determine the effects of time, type of filter, temperature, vehicle (solution), illumination, size of funnels, amount and length of grass, and weight and type of soil on the numbers of larvae recovered. A higher percentage of larvae was retrieved from funnels in which cheesecloth was used as a filter than from those containing cellulose tissue. Greater percentages of larvae were recovered at 4, 10, 20 and 25 C than at 30–50 C. Tap water, 0.9% NaCl solution, mammalian Ringer's solution and 0.2% HCl were equally effective as vehicles for baermannization, but a non-ionic and two anionic detergents were less satisfactory and at some concentrations were toxic to the larvae. There was no significant difference in larval recovery in the light or dark. The greater the diameter of funnel used for baermannization, the fewer larvae were recovered. The greater the weight of grass placed in the funnels, the lower was the recovery rate. Retrieval of larvae was better from sand than from silty clay loam soil, and better from silty clay loam than from clay loam.

A method to extract hookworm larvae from soil was described by Baermann (1917a). A modified Baermann apparatus was described by Cort et al. (1922) and is widely used by parasitologists to recover nematodes from soil, grass and feces. Dinaburg (1942) studied the efficiency of this technic and the variation in results that are obtained when using it. Because of the routine use of the modified Baermann technic in our laboratory to recover infective larvae of *Haemonchus contortus* and other trichostrongylids from soil, grass and feces, a detailed evaluation of this method was needed.

Materials and Methods

Fecal pellets containing 5,000–25,000 *Haemonchus contortus* eggs per gram were collected from monospecifically infected sheep. The pellets were incubated at 30 C for 5–7 days, at which time infective third stage larvae were present.

To determine the factors which affect the efficiency of the Baermann technic, the following variables were evaluated: (1) time of baermannization, (2) type of filter, (3) temperature, (4) vehicle (solution) used, (5) illumination, (6) size of funnel, (7) amount and length of grass, and (8) soil type and amount of soil.

The baermannization apparatus that was used as a standard for comparison consisted of a glass funnel 10 cm in diameter with a capacity of approximately 175 ml. A piece of 6 mm mesh galvanized wire screen was placed in the funnel approximately 4 cm from its top. A single layer of cheesecloth was placed over the wire screen, and the funnels were filled with warm tap water (about 25 C) to a level about 2 cm above the wire mesh. The fecal pellets were then placed in the water, which was of sufficient depth to cover them. As samples were withdrawn from the funnels water was added to restore the approximate initial level.

To determine the total number of larvae remaining in the Baermann apparatus after the samples had been withdrawn, the remaining fluid was removed, the funnel was washed with water, the tissue or cheesecloth was shaken in tap water to remove larvae, and the pellets were crushed and mixed in water. Aliquots of all these solutions were examined and the live (moving) larvae present were counted.

Experiment I, which was to determine the effects of time and the type of filter through which the larvae had to pass, was divided into six parts. Ten funnels, each containing 10 g of feces were used for each part. Five ml samples were withdrawn hourly for 8 hr and then at 12 hr; 24 hr after beginning the ex-

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periment, a final 15 ml sample was taken. In part one of the experiment, whole pellets and a single layer of cheesecloth were used; in part two, whole pellets and a single layer of cellulose tissue ("Kimwipes") were used. In part three, pellets were crushed and a single layer of cheesecloth was used. Part four was the same as part three except that a single layer of tissue was placed in the funnels. In part five crushed pellets were autoclaved, allowed to cool and a known number of larvae in 5 ml of tap water were mixed with the feces. The samples were covered and placed in the dark for 16 hr to allow uniform mixing of the larvae, after which they were baermannized using a single layer of cheesecloth. In part six, the Baermann apparatus, containing a single layer of cheesecloth and whole pellets, was shaken at 1 oscillation/sec throughout the experiment.

Experiment II was designed to determine the effect of temperature on baermannization. Five 10 cm funnels, each containing 10 g of feces, were placed in incubators at the following temperatures: 4, 10, 20, 25, 30, 35, 40, 45, and 50 C. Five ml samples were taken from each funnel at 2 and 4 hr and a 15 ml sample was removed 6 hr after beginning the experiment.

Experiment III was identical to part one of experiment I, i.e., whole pellets and a single layer of cheesecloth were used; however, samples were withdrawn at 2, 4 and 6 hr, and no wire screens were used in the funnels. The following solutions, in addition to tap water, were tested: 0.9% NaCl, mammalian Ringer's solution, 2.0% HCl, 0.2% Tergitol Wetting Agent No. 7, 0.2% Tergitol Penetrant 4, and 0.2% Tween-80.

Experiment IV was designed to determine the effect of illumination on baermannization. Five funnels were placed in the dark and five funnels were placed in a well lighted room at about 25 C. Whole pellets and a single layer of cheesecloth were used, and samples were withdrawn at 2, 4 and 6 hr.

Experiment V was similar to experiment I, part one except that different sizes of funnels were used. Five funnels of the following diameters and capacities were used for each part of the experiment; 7 cm, 80 ml; 10 cm, 175 ml; 13 cm, 350 ml; 15 cm, 650 ml; 25 cm, 3,700 ml; and 30 cm, 8,000 ml. All of the funnels

were made of glass, except that the 25 cm funnels were plastic, and the 30 cm funnels were galvanized metal. Screens were of sufficient size so that they were situated approximately 4–6 cm from the top of the funnel. The mean total number of larvae remaining in the 10 cm funnels were used in computing the percentage recovery from the larger funnels. Samples were taken at 2, 4, 6 and 24 hr.

Experiment VI was conducted to determine the effects of the amount and length of grass on the efficiency of the technic. Because the grass samples we examine from pastures often contain dehydrated larvae, larvae were desiccated on grass for this experiment. Grass was cut from a Kentucky bluegrass pasture, washed in tap water and excess water was evaporated at room temperature. In part one of the experiment, grass approximately 2.5 cm long was used. Five samples each of grass weighing 5, 10, 20, 50, 100 and 150 g were mixed with known numbers of larvae. Each sample was then desiccated at 30 C and 70% relative humidity for 24 hr. After desiccation the containers in which drying took place were examined to determine the number of larvae that did not remain on the grass. At the same time that the grass samples were dried, larvae were desiccated in a Petri dish. This sample was rehydrated to determine the number of larvae that survived desiccation. The 5, 10 and 20 g samples were baermannized in 15 cm funnels, and the 50, 100, and 150 g samples in 30 cm funnels. Part two of the experiment was identical to part one, except that grass approximately 8.0 cm long was used. Samples were taken at 2, 4, 6 and 24 hr. All samples were placed in 2% HCl before counting to kill free-living nematodes that might have been on the grass.

In experiment VII the types and amounts of soil used for baermannization were evaluated. Three soil types (sandy, silty clay loam and clay loam) were used. Soil textural composition followed the particle class limits and basic soil textural classes defined by the Soil Survey Staff (1951). The content of clay and silt size particles was determined by the hydrometer method of Bouyoucos (1951). Particle dispersion was obtained by using sodium hexametaphosphate (Calgon) as a dispersing agent and a reciprocating shaker at 180 strokes/min. Soil pH was determined with a Leeds and Northrup glass electrode using a

Table 1.   Characteristics of soil samples used for baermannization experiments.

Textural Class Name	% Sand 2.0–0.05 mm	% Silt 0.05–0.002 mm	% Clay < 0.002 mm	pH
Sand	93.6	1.2	5.2	7.3
Clay Loam	29.7	42.1	28.2	7.5
Silty Clay Loam	10.9	55.4	33.7	5.9

Figure 1.   Percentage of larval recovery when cheesecloth or tissue was used with whole or crushed pellets: 1. Whole pellets, cheesecloth. 2. Whole pellets, cellulose tissue. 3. Crushed pellets, cheesecloth. 4. Crushed pellets, cellulose tissue.

1 : 1 soil-water solution. All determinations were made in duplicate. Mean values are given in Table 1. Known numbers of larvae were mixed with 10 samples of each soil type of the following weights: 20, 40, 60, 80 and 100 g and placed in 10 cm funnels. A single layer of cellulose tissue was used for all parts of the experiment and samples were withdrawn at 2, 4, 6 and 24 hr.

In addition to the baermannization experiments, the effects of diluting larval suspensions on the accuracy of the counts were determined. When more than 200 larvae/ml were present, accurate counting was difficult because of the close proximity and activity of the larvae; it was also time-consuming. Samples containing approximately 100,000, 50,000, 25,000, 10,000 and 5,000 larvae were diluted to 500, 250, 125, 50 and 25 times their original volume, respectively, and 50 one ml samples were withdrawn with a pipette from each after bubbling air through the solution to mix the larvae.

Results

Experiment I:

Effects of time and type of filter

Figure 1 illustrates the effects of the use of cheesecloth and cellulose tissue on the recovery of larvae from crushed and whole pellets. Because a higher percentage of larvae was obtained using cheesecloth, this material was used for the other experiments. The following mean percentages (of the total number originally in the pellets) of larvae were recovered after 24 hr: whole pellets and cheesecloth, 87%; whole pellets and cellulose tissue, 67%; crushed pellets and cheesecloth, 85%; crushed pellets and tissue, 73%; larvae added to crushed pellets, 93%; and whole pellets and cheesecloth, sample shaken, 90%. The results

of this experiment revealed that relatively few larvae were recovered after 6 hr. For this reason, in the other experiments samples were taken at 2 hr intervals up to 6 hr and in some experiments a final sample was taken 24 hr after the experiment began.

Experiment II: Effect of temperature

The effects of different temperatures on the percentages of larvae recovered are given in Figure 2. After 6 hr the following mean percentages of larvae were recovered: 4 C, 59%; 10 C, 64%; 20 C, 75%; 25 C, 63%; 30 C, 38%; 35 C, 39%; 40 C, 13%; 45 C, 5%; and 50 C,
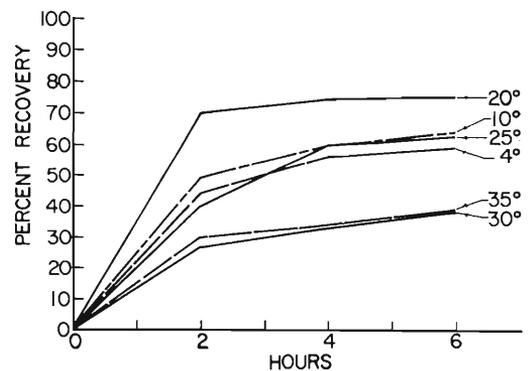
Figure 2.   Effect of temperature on percentage of larval recovery.

Figure 3. Effect of funnel diameter on percentage of larval recovery.

5%. At temperatures above 40 C most of the larvae were dead after 2 hr.

Experiment III: Effect of vehicle used

There were no significant differences in percentages of larvae recovered when water, salt or acid solutions were used for baermannization, but lower numbers were obtained when detergents were used. After 6 hr the following mean percentages of larvae were recovered: tap water, 83%; 0.9% NaCl, 76%; Ringer's solution, 96%; 2.0% HCl, 88%; 0.2% Tergitol Wetting Agent No. 7, 57%; 0.2% Tergitol Penetrant 4, 59%; and 0.2% Tween-80, 49%.

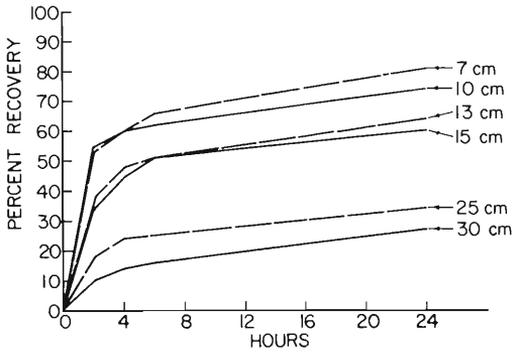## Experiment IV: Effect of illumination

This experiment revealed that there was no significant difference in percentages of larvae recovered when the funnels were placed in a well lighted room or in the dark. After 6 hr the mean percentages of recovery from the funnels placed in the dark and light were 84% and 86%, respectively.

Table 2. Percentages of recovery after 24 hr of baermannization of larvae desiccated on grass.

Weight of Sample (g)	% Recovery	
	2.5 cm Grass	8.0 cm Grass
5	36	28
10	28	32
20	19	33
50	19	7
100	19	7
150	4	4

Experiment V: Effect of size of funnel

The size of the funnel proved to be an important factor in determining the percentage of larvae recovered. The percentage decreased progressively as the funnel diameter increased (Fig. 3). After 24 hr the following mean percentages of larvae were recovered: 7 cm funnel, 83%; 10 cm, 76%; 13 cm, 68%; 15 cm, 65%; 25 cm, 47%; and 30 cm, 27%. The correlation coefficient for the percentage of larvae recovered from the above series after 24 hr was −0.99, which indicates a nearly perfect linear correlation.

Experiment VI:
Effect of amount and length of grass

The results of parts one and two of experiment VI are given in Table 2. The values given are calculated as the mean percentages of larvae surviving desiccation in the controls placed in petri dishes.

Experiment VII:
Effect of soil type and amount of soil

The results for this experiment are given in Table 3 and indicate that larger percentages of larvae were recovered from sandy and clay loam soils than from silty clay loam soil. A

Table 3. Percentages of larvae recovered from various weights of different soil types after 2, 4, 6 and 24 hr of baermannization.

Hours	Sample Weight in Grams														
	Sand					Clay Loam					Silty Clay Loam				
	20 g	40 g	60 g	80 g	100 g	20 g	40 g	60 g	80 g	100 g	20 g	40 g	60 g	80 g	100 g
2	42	34	21	22	15	33	21	14	14	10	8	9	8	7	7
4	47	53	31	27	19	39	26	22	18	13	14	13	9	9	10
6	52	58	41	29	22	43	29	26	20	16	18	16	10	11	11
24	66	63	51	37	26	45	32	29	23	17	21	18	11	11	11

higher mean percentage of recovery was obtained when small soil samples were used. Recovery from 20 or 40 g soil samples was generally two to three times greater than from 80 or 100 g samples.

In comparing the accuracy of the aliquot method of counting larvae, we found that when approximately 100,000, 50,000, 25,000, 10,000 and 5,000 larvae were diluted to 500, 250, 125, 50 and 25 times their original volume, respectively, the standard deviations for the numbers present were similar. The standard deviations for the above diluted volumes were 20.6, 24.0, 17.0, 18.9 and 19.6, respectively. However, the standard deviations for the calculated numbers of larvae present were: 10,312 for samples containing approximately 100,000 larvae, 6,015 for 50,000, 2,131 for 25,000, 950 for 10,000 and 495 for samples with about 5,000 larvae.

Discussion

Although higher percentages of larvae were recovered from funnels containing cheesecloth than from those containing cellulose tissue, large particles of debris passed through the cheesecloth and settled to the bottom of the funnel. Such debris did not pass through the cellulose tissues. Cort et al. (1926) used one or two layers of cloth in funnels and obtained slightly better results when only one layer was used. Tobar Jiménez (1963) evaluated five methods of recovering three genera of eelworms from soil samples. The modified cottonwool method (Tobar Jiménez, 1962) and the Oosterbrink method, which used a double layer of milk filter pads through which the nematodes had to pass, gave significantly lower returns of the small nematode *Paratylenchus* spp. than the Seinhorst 2-flask method (Seinhorst, 1956) and the Seinhorst mistifier (Seinhorst, 1950), which did not have filters. When recovery of larger nematodes (*Helicotylenchus* and *Tylenchus*) was attempted, better results were obtained from the apparatuses which had filters. Kauzal (1940) used the Baermann apparatus; with a sieve only he recovered 58% of *H. contortus* and *Trichostrongylus* spp. larvae which had been placed in soil plots, but with gauze in the funnel, he recovered only 37% of the larvae.

Crushing the pellets did not increase the numbers of larvae recovered in our study and baermannized samples from crushed pellets were so turbid that the samples had to be centrifuged and washed before the larvae could be easily counted.

The relationship between the length of time samples are baermannized and the recovery of nematodes has not been adequately studied. Cort et al. (1922) reported that the moisture content of the soil affected the time period during which most of the larvae were recovered. The "great majority" of larvae was recovered from moist soil within 6 hr; however, a larger percentage of larvae was recovered from saturated soil between 6 and 24 hr. Most reports state that samples were baermannized for 24 or 48 hr. We found that putrefaction killed many of the larvae when grass and fecal samples were baermannized for more than about 24 hr. In our study most of the larvae were recovered by 6 hr, and relatively few were recovered between 6 and 24 hr.

Our work substantiated the fact that temperature influences the numbers of larvae recovered from the Baermann apparatus. Figure 2 illustrates that a temperature about that of most laboratories is the most efficient for recovering *H. contortus* larvae. Baermannization at lower temperatures did not give a significantly higher yield, and temperatures of 30 C and higher greatly decreased the numbers of larvae recovered. The optimum temperature evidently varies for different species of nematodes. Cort et al. (1922) found that the water used must be at least 10 F higher than the soil in order to obtain good recovery of *Necator americanus* larvae. Cort et al. (1926) reported that water temperatures between 35 and 45 C gave the best recovery for *Ancylostoma* and *Necator* larvae. Kauzal (1940) found that higher numbers of *H. contortus* and *Trichostrongylus* spp. larvae were recovered at temperatures between 5 and 22 C than at 37 C. Slightly higher numbers of *H. contortus* larvae were recovered at room temperatures of 22–26 C than in an incubator at 24–28 C. He found that recovery of *Trichostrongylus* spp. was about the same at these two temperature ranges. Adams (1965) found that the optimum temperature for recovery of soil nematodes was between 15 and 25 C. Higher or lower temperatures yielded fewer nematodes. His data indicated that temperature could affect the species of nematodes that were recovered in highest numbers.

We found that there was no advantage in using solutions other than water for baermannization. Rohrbacher (1957) reported that adding 0.5 ml of a nonionic detergent (Triton X-100) per liter of water increased the recovery of *Trichostrongylus axei* and *Ostertagia ostertagi* from Bermuda grass, orchard grass and crimson clover. We found that 0.2% solutions of the anionic detergents, Tergitol Wetting Agent No. 7, Tergitol Penetrant 4 and the nonionic detergent Tween-80, gave lower returns than tap water. Concentrations above 0.5% of these solutions were toxic to the larvae, and most were dead within 24 hr after having been placed in the solutions.

Placing the Baermann apparatus in the light or dark did not alter the numbers of larvae recovered. Kauzal (1940) attempted to attract *H. contortus* and *Trichostrongylus* spp. larvae to the base of funnels by blackening all but the lower stem of the funnels and by applying both light and heat to the neck of the funnels. Neither method gave better results than when the usual technic was used.

The results of the present study indicate that the size of the funnel is an important factor in determining the percentage of larvae recovered. According to Cort et al. (1922), Baermann (1917a) first used a small funnel and later (1917b) used a larger one which gave uniformly satisfactory results. Kauzal (1940) found that when 50 g of soil were placed in 6 inch funnels the mean recovery of *H. contortus* and *Trichostrongylus* spp. was 33.6%. When 100 g of soil were placed in 6- and 9-inch funnels, the mean recoveries were 17 and 43%, respectively. Mönnig (1930) reported that the best recovery of *Trichostrongylus* spp., *H. contortus* and *Oesophagostomum columbianum* larvae from fecal and soil samples was from 6 inch funnels and stated that if a large amount of material was to be examined it was better to use a number of funnels rather than a large funnel.

The effects of the amount and length of grass on baermannization have evidently not been reported in the literature. Our results indicate that when more than 50 g of longer grass was used there was a significant decrease in recovery. However, these data are misleading because the 50, 100 and 150 g samples were baermannized in 30 cm funnels, while the 5, 10 and 20 g samples were in 15 cm funnels.

In experiment V 38% larvae were recovered from 30 cm funnels than from 15 cm funnels.

The influence of soil type on the results of baermannization has been studied by several authors. Our experiments confirm the previous reports that larger numbers of larvae are recovered from loose sandy soil than from clay soil. Baermann (1917b) reported that *Ancylostoma* larvae move through loose soil faster than through compact soil. Cort et al. (1922) obtained low recoveries of *Necator americanus* larvae from clay, but obtained increasingly better results with clay loam and sand, respectively. The closely packed particles of clay evidently hinder movement of the larvae between the spaces in the soil. Cort et al. (1922) found that mixing coarse gravel with clay loam greatly increased the percentage of larvae recovered. Stoll (1923) mixed known numbers of *N. americanus* larvae with humus, sand and loam, and clay, and recovered about 90, 80, and 30–50% of the larvae, respectively. Mönnig (1930) obtained "best results" with coarse soil and 6 inch funnels when he tested the efficiency of the Baermann technic with *Trichostrongylus* spp., *H. contortus* and *O. columbianum.*

Many technics other than or modifications of the Baermann apparatus have been described for recovery of nematodes from feces, grass and soil. Some authors have concluded that the Baermann technic should be used for qualitative rather than quantitative purposes. However, many of the other methods for determining the number of larvae present in soil, grass and fecal samples are time consuming and require elaborate equipment. We believe that the Baermann technic is a useful tool in determining the relative number of larvae present, if the investigator realizes its limitations.

Acknowledgments

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Monogenetic Trematodes from Costa Rica with the Proposal of *Anacanthocotyle* gen. n. (Gyrodactylidae: Isancistrinae)

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ABSTRACT: Four species of Gyrodactylidae are comparatively or originally described from Costa Rican fishes as follows: *Anacanthocotyle anacanthocotyle* sp. n. and *Gyrodactylus neotropicalis* sp. n. both from *Astyanax fasciatus* (Cuvier); and *G. costaricensis* sp. n. and *G. bullatarudis* Turnbull, 1956, both from *Poecilia sphenops* Valenciennes. *Anacanthocotyle* gen. n. is proposed. This genus differs from the related *Isancistrum* de Beauchamp, 1912, principally by possessing cephalic lobes which contain a spicule and portions of the head organs, 16 instead of 15 adult haptoral hooks, and a tapered peduncle on which a cup-shaped haptor occurs. *Anacanthocotyle* gen. n. lacks anchors and bars and possesses posteriorly confluent intestinal crura.

The description of *Cleidodiscus travassosi* and *C. chavarriai* from *Rhamdia rogersi* (Regan) was the first report of freshwater Monogenea from Costa Rica (Price, 1938). Recently, Price and Bussing (1967, 1968) described *C. costaricensis*, *C. strombicirrus* and *Palombitrema heteroancistrium* from *Astyanax fasciatus* (Cuvier) from Costa Rica. The present study includes four additional species from Costa Rican fishes and represents the first report of members of the Gyrodactylidae from the Neotropical Region.
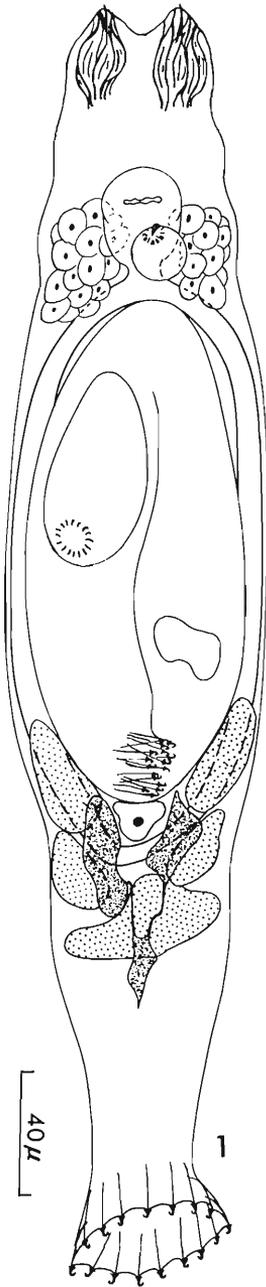
A. ANACANTHOCOTYLE
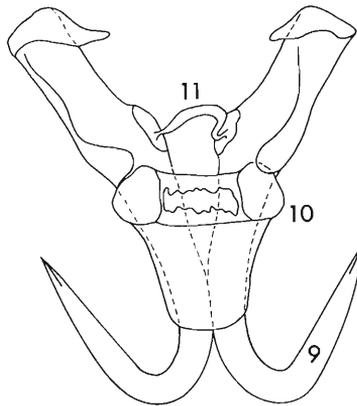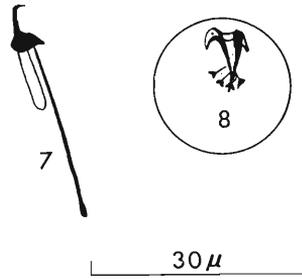
G. BULLATARUDIS

G. NEOTROPICALIS

G. COSTARICENSIS

Fish hosts were seined from a marshy area approximately 3 km SW of Rincón, Puntarenas Province, Costa Rica, during July, 1968. Methods of host preparation and prep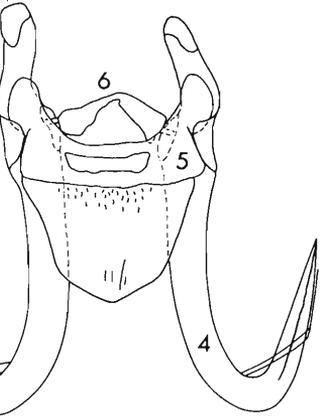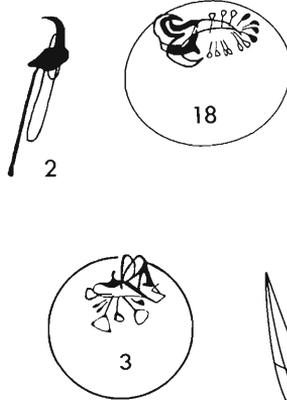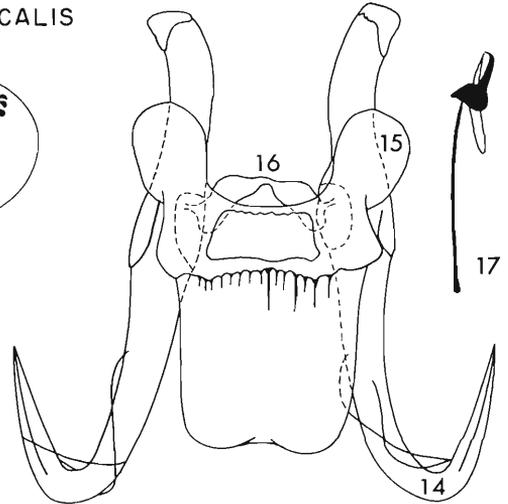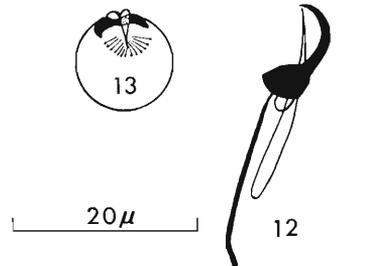aration, observation, measurement, and illustration of gyrodactylid specimens were those described by Mizelle and Kritsky (1967a). In addition, several parasites were stained with Delafield's hematoxylin or Gomori's trichrome for elucidation of internal anatomy and haptoral bars. Terminology is as proposed by Kritsky and Mizelle (1968) and Mizelle and Kritsky (1967a). Measurements are in microns. Paratypes are in the authors' collections.

Isancistrinae Fuhrmann, 1928

Emended subfamily diagnosis: Gyrodactylidae: Cephalic area bilobed or truncate, head organs present. Haptor with 15 or 16 marginal hooks; anchors and bars absent.

Anacanthocotyle anacanthocotyle gen. n., sp. n.
(Figs. 1–3)

HOST: *Astyanax fasciatus* (Cuvier), Characidae.
LOCATION ON HOST: External surface.
SPECIMENS STUDIED: 30.
HOLOTYPE: USNM Helm. Coll. No. 70542; two paratypes, USNM Helm. Coll. No. 70543.

Description

Length 329 (236–378), greatest width 79 (59–104) near midlength. Cephalic lobes moderate to incipient, spicule large. Head organs well developed, longitudinally striated; cephalic glands moderate to well developed. Anterior pharyngeal bulb width 27 (22–32), posterior bulb width 25 (20–30); intestinal crura in dorsal half of trunk. Peduncle moderate to broad. Haptor opening posteriorly or posteroventrally, width 52 (41–57), length 37 (30–53); hooks arranged radially (holocentric) in haptor. Hook shank with slight proximal

enlargement; hooklet point open or slightly recurved, heel diagonally truncate, toe with shelf and pointed tip; hook length 18 (17–20), hooklet length 6. Filamentous hooklet (FH) loop extends 0.6–0.7 shank length; secondary filamentous hooklet (SFH) loop indistinct. Cirrus ventral to posterior pharyngeal bulb, present in 11 specimens, with 2–8 spinelets usually arranged in two rows; cirrus diameter 11 (10–12).

Remarks

Sequential development of sclerotized haptoral structures in *A. anacanthocotyle* sp. n. follows that described for hooks of *Gyrodactylus* spp. (Mizelle and Kritsky, 1967a). Initially the hooklet points and shafts appear simultaneously in a concentric ring with points directed inward. The base and shank form next, after which the FH loop becomes visible in near-term embryos. Portions of anchors or bars are not present at any stage in development. The cirrus apparently forms after parturition. The first trace of sclerotized parts of a second embryo appears after complete formation of hooks of the older embryo. Third generation embryos were not observed.

This is the only species described for the genus. Genus and species names are from Greek and refer to the absence of haptoral anchors.

Anacanthocotyle gen. n.

GENERIC DIAGNOSIS: Gyrodactylidae, Isancistrinae: Elongate body divisible into a cephalic region, trunk, peduncle and haptor. Cuticle thin, smooth. Cephalic lobes (two) terminal, each containing a spicule and portions of the head organs. Cephalic glands in two bilateral groups in anterolateral trunk. Eyes absent. Pharynx composed of two bulbs; esophagus short, intestinal crura confluent posteriorly. Gonads uncertain; several variably shaped bodies of undetermined function posterior to uterus (Fig. 1). Cirrus ventral, with

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Figures 1–3. *A. anacanthocotyle* sp. n. 1, Whole mount (ventral view). 2, Hook. 3, Cirrus. Figures 4–8. *Gyrodactylus bullatarudis* Turnbull, 1956. 4, Anchor. 5, Superficial bar. 6, Deep bar. 7, Hook. 8, Cirrus. Figures 9–13. *G. neotropicalis* sp. n. 9, Anchor. 10, Superficial bar. 11, Deep bar. 12, Hook. 13, Cirrus. Figures 14–18. *G. costaricensis* sp. n. 14, Anchor. 15, Superficial bar. 16, Deep bar. 17, Hook. 18, Cirrus. Figures are drawn to the following scales: Fig. 1 (40 micron), anchor and bar complexes (30 micron), hooks and cirri (20 micron).

spine and several spinelets. Sequential development of embryonic generations typical of family (e.g. Braun, 1966); embryo in shape of inverted U. Haptor cup shaped, with 16 hooks; anchors and bars absent. Parasitic on external surface of freshwater fishes.

TYPE SPECIES: *A. anacanthocotyle* sp. n.

TYPE HOST: *Astyanax fasciatus* (Cuvier).

Anacanthocotyle gen. n. is similar to the monotypic genus *Isancistrum* de Beauchamp, 1912, in that members of both lack anchors and bars. *Anacanthocotyle* gen. n. differs from the latter by possessing a tapered peduncle, a cup-shaped haptor and cephalic lobes which contain a spicule and portions of the head organs. Further, the original figure of *I. loliginis* de Beauchamp, 1912, shows 15 adult haptoral hooks (possibly erroneously, *cf.* Bychowsky, 1957) whereas *A. anacanthocotyle* sp. n. possesses 16.

Although Yamaguti's (1963) diagnoses of the subfamily Isancistrinae Fuhrmann, and the genus *Isancistrum* de Beauchamp, state that members are "parasitic on Cephalopoda, occasionally on gills of marine fishes," he fails to cite a fish host. A review of the literature similarly failed to disclose previous reports of a fish host for these taxa. Therefore, it appears that the different host preferences of *Isancistrum* spp. (cephalopods) and *Anacanthocotyle* spp. (fishes) may further serve to separate the genera.

Gyrodactylus bullatarudis Turnbull, 1956
(Figs. 4–8)

HOST: *Poecilia sphenops* Valenciennes, Poeciliidae.

LOCATION OF HOST: External surface.

SPECIMENS STUDIED: 21, and USNM Helm. Coll. No. 38154 (holotype).

Comparative description

With characters of the genus as emended by Mizelle and Kritsky (1967a). Cuticle thin, smooth. Length 391 (325–518), greatest width 83 (66–108) near midlength. Cephalic lobes prominent, conspicuous spicule in each. Head organs longitudinally striated; cephalic glands large. Anterior pharyngeal bulb width 26 (19–33), posterior bulb width 22 (18–24); gut normal. Peduncle moderate; haptor subovate, length 60 (51–72), greatest width 67

(51–82); hook distribution extrahamular. Superficial anchor root variably bent; anchor length 54 (51–59), base width 10 (7–20); anchor filament double. Ends of superficial bar developed anteriorly; bar length 27 (24–29); shield short, with chromophilic areas anteriorly. Deep bar with terminal and medial constrictions; length 18 (15–23). Hook shank with small proximal enlargement; hooklet point short, shaft straight, base with short shelf; hook length 25 (24–26), hooklet length 6. FH loop extends 0.4 shank length. Hemispherical ovary submedian, immediately posterior to uterus. Two large bilateral testes posterior to ovary. Large lobulate body ventral to each testis. Cirrus post-pharyngeal and slightly sinistral, present in 13 specimens, with 4 or 5 spinelets; diameter 13 (10–15). Sequential development of haptoral parts normal; temporal development late; uterus with a maximum of two embryos.

Remarks

This is the first report of *G. bullatarudis* from a natural habitat. Previous reports are from the guppy, *Poecilia (Lebistes) reticulata* Peters, in aquaria (Turnbull, 1956; Rogers and Wellborn, 1965).

Gyrodactylus costaricensis sp. n.
(Figs. 14–18)

HOST: *Poecilia sphenops* Valenciennes, Poeciliidae.

LOCATION ON HOST: External surface.

SPECIMENS STUDIED: 15.

HOLOTYPE: USNM Helm. Coll. No. 70545.

Description

With characters of the genus as emended by Mizelle and Kritsky (1967a). Cuticle thin, smooth. Length 438 (325–607), greatest width 74 (55–89) near midlength. Cephalic lobes prominent, with two large spicules each. Head organs well developed; cephalic glands conspicuous. Anterior pharyngeal bulb width 29 (25–33), posterior bulb width 26 (22–30); gut normal. Peduncle broad; haptor semicircular, length 83 (76–91), greatest width 91 (68–106); hook distribution extrahamular. Superficial anchor root elongate, bent ventrally; anchor length 72 (69–76), base width 17 (13–21). Anchor filament inconspicuous. Ends of superficial bar extended anteriorly; bar

length 40 (37–44); shield longitudinally striated anteriorly, posterior margin indented. Deep bar with lateral and medial constrictions, length 20 (18–23). Hook shank with small proximal enlargement; hooklet point short, shaft straight, base with sloping shelf and globose heel; hook length 28 (27–29), hooklet length 7 or 8. FH loop extends 0.4 shank length. Subovate ovary situated medially and post-uterine. Two bilateral testes post-ovarian. Lobulate body situated anteroventral to each testis. Cirrus posterior to pharynx and slightly sinistral, present in nine specimens, with 8–13 spinelets; cirrus diameter 14 (10–17). Available embryonic stages inadequate to determine sequential development of haptoral parts; each specimen with one embryo.

Remarks

This species closely resembles *Gyrodactylus lacustris* Mizelle and Kritsky, 1967b, and *G. prolongis* Hargis, 1955. *G. costaricensis* sp. n. is distinguished from the former by the morphology of the deep bar and from the latter by the absence of a peduncular skirt (Figs. 14–18; Mizelle and Kritsky, 1967b, figs. 18–22; and Hargis, 1955, figs. 4–7). The species name indicates the locality.

Gyrodactylus neotropicalis sp. n.
(Figs. 9–13)

HOST: *Astyanax fasciatus* (Cuvier), Characidae.
LOCATION ON HOST: External surface.
SPECIMENS STUDIED: 5.
HOLOTYPE: USNM Helm. Coll. No. 70544.

Description

With characters of the genus as emended by Mizelle and Kritsky (1967a). Cuticle thin, smooth. Length 352 (303–392), greatest width 97 (74–126) near midlength. Cephalic lobes moderate to well developed, one or two large spicules in each. Head organs and cephalic glands inconspicuous. Pharyngeal bulbs subequal, width 28 (25–30); gut normal. Peduncle broad; haptor subovate, width 78 (61–99), length 74 (66–80); hook distribution extrahamular. Tip of superficial anchor root folded mesially, anchor folds extensive, filament not observed; anchor length 59 (54–63), base width 20 (19–21). Superficial bar

with enlarged ends, posterior margin of shield rounded or truncate, bar length 28 (25–32). Rod-shaped deep bar variably bent; length 15 (12–18). Proximal hook shanks uniform; hooklet shaft and point evenly recurved; base with rounded proximal margin, shelf absent; hook length 31 (30–33), hooklet length 11 (10–12). FH loop extends 0.6 shank length; SFH loop short, conspicuous. Two ovate testes bilateral, post-uterine. Cirrus post-pharyngeal and slightly sinistral, present in three specimens, with numerous small spinelets; cirrus diameter 11 (10–12). Ovary not observed. Available embryonic stages inadequate to determine sequential development of haptoral parts; each specimen with one embryo.

Remarks

This species most closely resembles *Gyrodactylus cyprini* Diarova, 1964. They are easily distinguished by the morphology of haptoral armaments (Figs. 9–13; and Rogers, 1968, fig. 3). The species name indicates the biogeographical region.

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On the Plant-parasitic Nematode Genera *Merlinius* gen. n. and *Tylenchorhynchus* Cobb and the Classification of the Families Dolichodoridae and Belonolaimidae n. rank

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ABSTRACT: *Merlinius* gen. n. is proposed for 32 species of *Tylenchorhynchus, sensu lato*, which have six incisures in the lateral fields, rather cylindroid spicules with prominently notched distal end, a nonprotruding gubernaculum and a moderately developed bursa. *Tylenchorhynchus* Cobb is redefined and *T. uliginosus* sp. n. and *T. papyrus* sp. n. are described from swampy areas in Uganda. The diagnoses, composition and relationships of Dolichodoridae and Belonolaimidae n. rank are detailed. *Tetylenchus* Filipjev is placed in a new subfamily Tetylenchinae under Tylenchidae.

In his review of the genus *Tylenchorhynchus* Cobb, 1913, Allen (1955) pointed out the existing diversity in the morphological characters exhibited by various species and prophesied the creation of new genera within this group; two related genera, *Nagelus* and *Geocenamus*, have since been proposed by Thorne and Malek (1968). Tarjan (1964) and de Guiran (1967) gave differential keys for 68 and 71 valid species of *Tylenchorhynchus* respectively and 25 more new species have since been described. I had the opportunity of studying the specimens of over fifty valid species in this genus including the type species, *T. cylindricus* Cobb (made available by Dr.

Allen) and *T. dubius* (Bütschli) from Holland, Belgium and England which was regarded as type by Filipjev (1934) for his new subgenus *Bitylenchus*, later (1936) synonymized by him with *Tylenchorhynchus*.

This study shows that whereas *T. dubius* and *T. cylindricus* are congeneric, a number of other species show considerable differences in their morphology which are considered of generic status. Consequently, *Merlinius* gen. n. is here proposed for those species of *Tylenchorhynchus, sensu lato*, which have six incisures in the lateral fields, deirids frequently present, a small trough-shaped nonprotrusible gubernaculum and characteristic spicules which

markedly differ from those of *Tylenchorhynchus*. Both the genera are described below.

Merlinius[1] gen. n.

DIAGNOSIS: Tylenchorhynchinae: Dolichodoridae. Lateral fields with six incisures. Deirids frequently present. Lip region symmetrical, with four or more annules but without a perioral disc. Amphids prominent pores or oblique slits close to oral opening. Labial framework lightly to heavily sclerotized. A single papilla on outer margins of each submedian lip. Protrudor muscles of spear attached to the base of labial framework. Basal bulb large, with well developed cardia. Vulva usually with double epiptygma and lateral membranes; cloaca with two pedunculate papilla-like protuberances ventro-laterally, here named as hypoptygma. Ovaries symmetrical. Female tail cylindroid to subcylindroid; male tail enveloped by a moderately developed bursa. Spicules stout, rather cylindroid; distal end broadly rounded, notched and devoid of large ventral flanges. Gubernaculum small, trough-shaped in lateral view, not protrusible.

Type species

Merlinius brevidens (Allen, 1955) comb. n.
  syn. *Tylenchorhynchus brevidens* Allen, 1955

Other species

Merlinius affinis (Allen, 1955) comb. n.
  syn. *Tylenchorhynchus affinis* Allen, 1955
M. alpinus (Allen, 1955) comb. n.
  syn. *T. alpinus* Allen, 1955
M. bavaricus (Sturhan, 1966) comb. n.
  syn. *T. bavaricus* Sturhan, 1966
M. berberides (Sethi and Swarup, 1968) comb. n.
  syn. *T. berberides* Sethi and Swarup, 1968
M. bogdanovikatjkovi (Kirjanova, 1941) comb. n.
  syn. *T. bogdanovikatjkovi* (Kirjanova, 1941) Loof, 1959
    Anguillulina bogdanovikatjkovi Kirjanova, 1941
M. conicus (Allen, 1955) comb. n.
  syn. *T. conicus* Allen, 1955
M. cylindricaudatus (Ivanova, 1968) comb. n.
  syn. *T. cylindricaudatus* Ivanova, 1968

M. dubius (Steiner, 1914) comb. n.
  syn. *Aphelenchus dubius* Steiner, 1914
    Anguillulina macrura Goodey, 1932
    Tylenchorhynchus macrurus (Goodey, 1932) Filipjev, 1936
    ? *T. graminicola* Kirjanova, 1951
M. galeatus (Litvinova, 1946) comb. n.
  syn. *T. galeatus* Litvinova, 1946
M. grandis (Allen, 1955) comb. n.
  syn. *T. grandis* Allen, 1955
M. hexagrammus (Sturhan, 1966) comb. n.
  syn. *T. hexagrammus* Sturhan, 1966
M. hexincisus (Jairajpuri and Baqri, 1968) comb. n.
  syn. *T. hexincisus* Jairajpuri and Baqri, 1968
M. icarus (Wallace and Greet, 1964) comb. n.
  syn. *T. icarus* Wallace and Greet, 1964
M. laminatus (Wu, 1969) comb. n.
  syn. *T. laminatus* Wu, 1969
M. lenorus (Brown, 1956) comb. n.
  syn. *T. lenorus* Brown, 1956
M. leptus (Allen, 1955) comb. n.
  syn. *T. leptus* Allen, 1955
M. lineatus (Allen, 1955) comb. n.
  syn. *T. lineatus* Allen, 1955
M. macrodens (Allen, 1955) comb. n.
  syn. *T. macrodens* Allen, 1955
M. microdorus (Geraert, 1966) comb. n.
  syn. *T. microdorus* Geraert, 1966
M. nothus (Allen, 1955) comb. n.
  syn. *T. nothus* Allen, 1955
M. obscurisulcatus (Andrássy, 1959) comb. n.
  syn. *T. obscurisulcatus* Andrássy, 1959
M. obscurus (Allen, 1955) comb. n.
  syn. *T. obscurus* Allen, 1955
M. quadrifer (Andrássy, 1954) comb. n.
  syn. *T. quadrifer* Andrássy, 1954
    T. ornatus Allen, 1955
M. rugosus (Siddiqi, 1962) comb. n.
  syn. *T. rugosus* Siddiqi, 1962
M. socialis (Andrássy, 1962) comb. n.
  syn. *T. socialis* Andrássy, 1962
M. stegus (Thorne and Malek, 1968) comb. n.
  syn. *T. stegus* Thorne and Malek, 1968
M. superbus (Allen, 1955) comb. n.
  syn. *T. superbus* Allen, 1955
M. tartuensis (Krall, 1959) comb. n.
  syn. *T. tartuensis* Krall, 1959
M. tessellatus (Goodey, 1952) comb. n.
  syn. *T. tessellatus* Goodey, 1952
M. undyferrus (Haque, 1967) comb. n.
  syn. *T. undyferrus* Haque, 1967

[1] Named in honor of Dr. Merlin W. Allen, University of California, U. S. A.
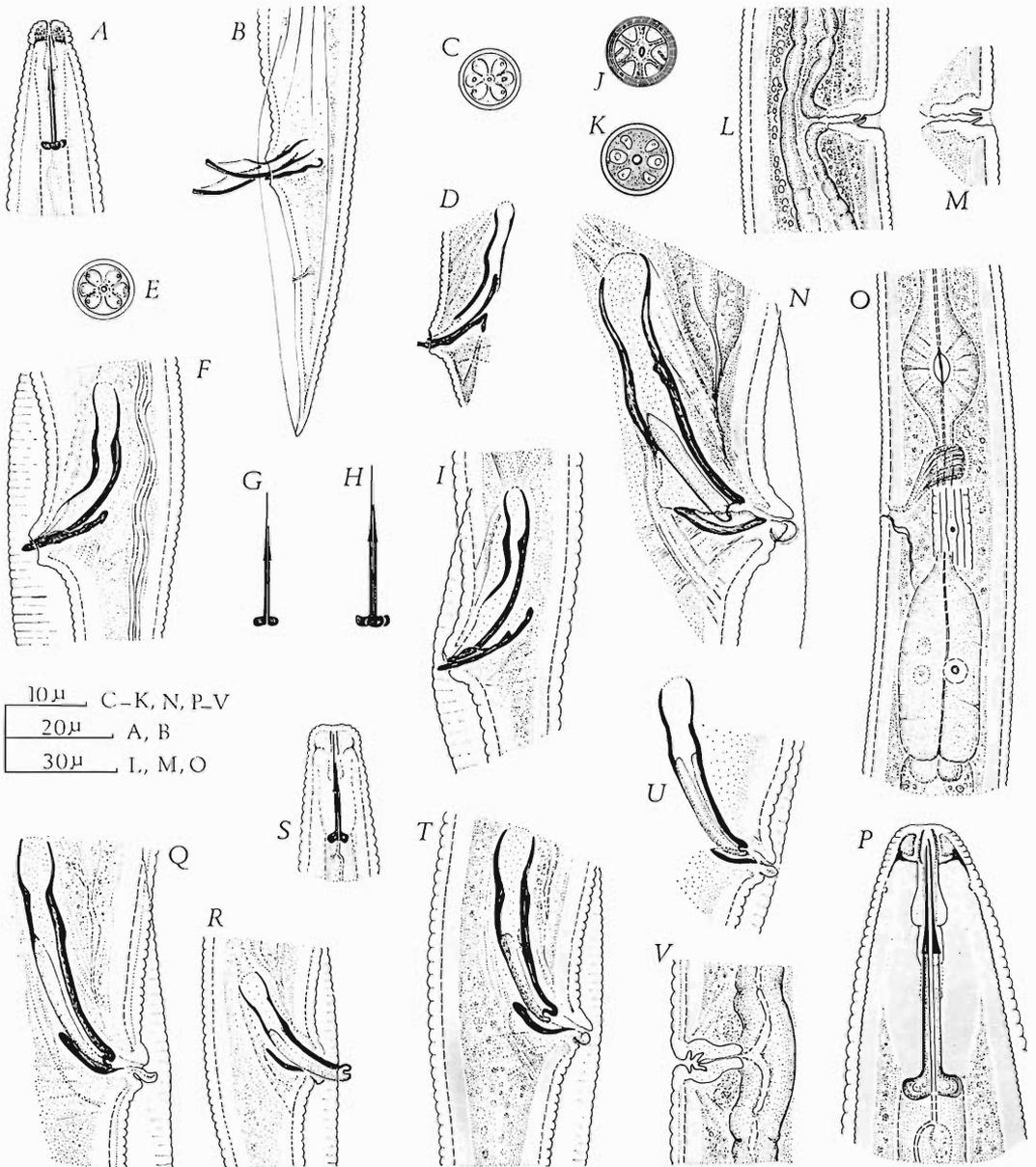
Figure 1. A–I. *Tylenchorhynchus*, J–V. *Merlinius*. A and B. *T. cylindricus*. C and D. *T. capitatus*. E–G. *T. dubius*. H and I. *T. nudus* from blue grass, Wisconsin, U.S.A. J–P. *M. icarus* from grasses, St. Albans, England. Q. *M. alpinus*, paratype. R. *M. nanus* from Belgium. S. *M. microdorus* from onion soil, Rampur City, India. T. *M. grandis*, paratype. U and V. *M. quadrifer* from Belgium. C, E and J. *En face* of female. K. Transverse section through basal plate. A and P. Head end of female. S. Head end of male. L, M and V. Vulval region showing epiptygma. B, D, F, I, N, Q, R, T and U. Spicular regions. J. Part of female esophagus showing deirid in lateral field.

M. varians (Thorne and Malek, 1968) comb. n.
syn. *T. varians* Thorne and Malek, 1968

Tylenchorhynchus brachycephalus Litvinova, 1946 is a problematical species. The female measuring 0.70–0.77 mm long has head-end and tail-end like those of *Helicotylenchus* whereas the male measuring 0.93–1.20 mm is apparently a *Merlinius*. However the female is reported to have six incisures in the lateral field which is contrary to the definition of *Helicotylenchus*.

RELATIONSHIP: *Merlinius* differs from *Tylenchorhynchus* in having six incisures in the lateral field, usual presence of deirids, the males having characteristic spicules lacking large ventral flanges and small, nonprotruding gubernaculum.

Nagelus Thorne and Malek, 1968 lacks a labial framework, has asymmetrical lip region, angular spear knobs and protractor muscles of the spear attached to the cuticularized inner walls of the labial cavity.

Geocenamus Thorne and Malek, 1968 is characterized by having a refractive labial disc from which a slender spear guide extends back almost one-third length of the exceedingly slender spear. According to Thorne and Malek (1968) the head and spear of *Geocenamus* are reminiscent of those of *Dolichodorus* rather than *Tylenchorhynchus*.

I have seen deirids in the following species: *Merlinius affinis, M. alpinus, M. brevidens, M. conicus, M. dubius, M. grandis, M. icarus, M. microdorus* and *M. nanus*. Figure 1 (J–V) gives further information on certain species of this genus.

Genus *Tylenchorhynchus* Cobb, 1913
syn. *Bitylenchus* Filipjev, 1934

DIAGNOSIS (emended): Tylenchorhynchinae : Dolichodoridae. Lip region symmetrical, offset or continuous with body; labial framework lightly to heavily sclerotized; labial disc absent. Lateral fields with 3–5 incisures. Deirids rarely present. Spear usually well developed with prominent basal knobs and anteriorly tapering portion appearing nontubular and needle-like distally; protractor muscles attached to the base of the labial framework. Median and basal bulbs of esophagus well developed. Convoluted tubules running along entire intestinal region present in many species. Spicules cephalated, ventrally arcuate, with distal portion pointed and prominently flanged ventrally. Gubernaculum large, rod-like in lateral view, sometimes with proximal portion dorsally bent, capable of protruding through anus. Bursa terminal, well developed.

TYPE SPECIES: *Tylenchorhynchus cylindricus* Cobb, 1913.

Two new species of this genus, *T. uliginosus* and *T. papyrus*, collected from swampy areas in Uganda are described below. These come close to *T. rhopalocercus* Seinhorst, 1963, which was transferred by Seinhorst (1968) to the genus *Trichotylenchus* Whitehead, 1959. I have collected specimens of *T. rhopalocercus* from sugarcane soil samples originating in Jebba, Nigeria and these fit well the original description. The species has a definite basal esophageal bulb and is, therefore, retained in *Tylenchorhynchus*.

Tylenchorhynchus uliginosus sp. n.
(Fig. 2, A–I)

Measurements

FEMALES (25): L = 0.40–0.64 mm; a = 30–37; b = 4.4–5.6; c = 10.5–13.0; V = 52–58; spear = 14–16 μ.

FEMALE (HOLOTYPE): L = 0.6 mm; a = 34; b = 4.8; c = 12; V = ^{26}54^{25}; spear = 15.5 μ.

MALES (10): L = 0.48–0.54 mm; a = 31–37; b = 4.4–5.5; c = 12–14; T = 55–62; spear = 14–15 μ; spicules (measured along dorsal line) = 19–21 μ; gubernaculum = 9.5–10.5 μ.

Description

FEMALE: Body in an open "C" form when relaxed by gentle heat; transverse striae rather coarse, 1.25 μ apart near middle. Lateral fields aerolated, about one-fourth body width; three incisures; outer ones crenate. Phasmids distinct, variable in position from a little anterior to slightly posterior to middle of tail. Lip region conoid-rounded, with 6–7 annules; framework lightly sclerotized, with outer margins extending 2–3 body annules from basal plate. *En face* of female head shows six papillae in inner and eight in outer circlet of which four nearer to amphids are most prominent and large, and circular openings of the amphids (Fig. 2, B). Three-fourths of anterior part of spear needle-like, not tubular; orifice of dorsal esophageal
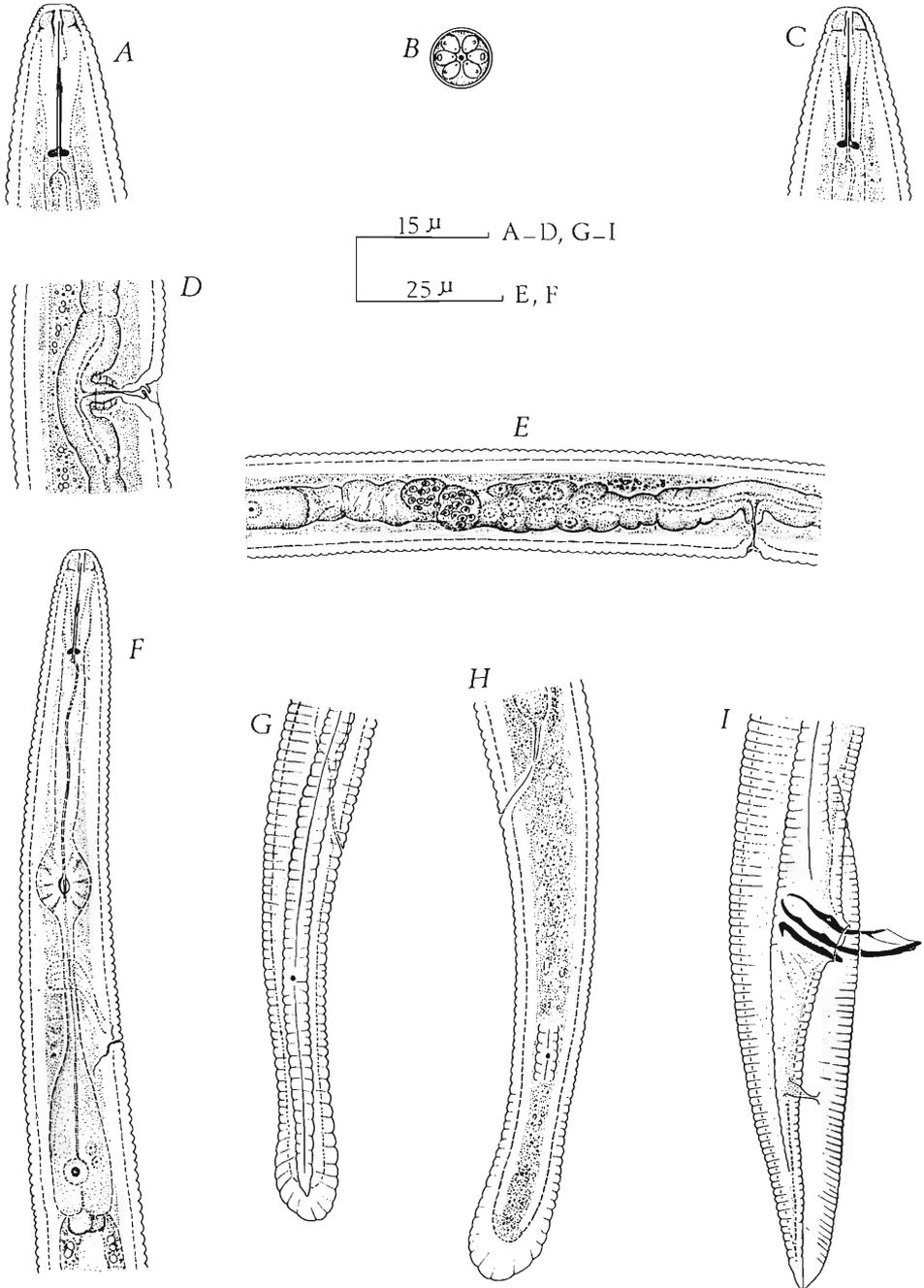
Figure 2, A–I. *Tylenchorhynchus uliginosus* n. sp. A, B and D–H. Female. C and I. Male. A and C. Head ends. B. *En face* view. D. Vulval region. E. Part of anterior reproductive branch. F. Esophageal region. G–I. Tail ends.

gland 2 μ behind spear base. Esophagus typical (Fig. 2, F), cardia apparently made up of two large cells. Excretory pore near anterior end of basal esophageal bulb. Hemizonid two body annules long, just anterior to excretory pore. Vulva with double epiptygma; vagina extending half-way across body (Fig. 2, D); spermatheca packed with round sperms 2 μ in diameter; ovaries with 1–2 rows of oocytes. Tail elongate-clavate, 4.6–5.0 × anal body-width long; tail terminus with thick cuticle, coarsely striated.

MALE: Essentially similar to female. Bursa large, finely crenate arising at about 1½ spicular lengths in front of anus. Phasmids near middle of tail. Lateral field, spicule and gubernaculum as shown in Figure 2, I.

RELATIONSHIP: This species is close to *T. rhopalocercus* Seinhorst, 1963, *T. bifasciatus* Andrássy, 1961 and *T. palustris* Merny and Germani, 1968. *T. rhopalocercus* has females with 0.62–0.81 mm long body, spear measuring 17–19 μ long and tail almost six anal body-widths long. *T. bifasciatus* has 0.65–0.73 mm long body, 19–20 μ long spear and female tail only 2.6–2.7 anal body-widths long. *T. palustris* has fewer labial annules and female tail measuring 2.7–3.9 anal body-widths long and having unstriated terminus.

TYPE HABITAT AND LOCALITY: Collected by Prof. W. B. Banage from swampy soil near a fish pond in Kabanyolo, near Kampala, Uganda.

TYPE MATERIAL: Holotype female, four paratype females and two paratype males at Nematology Department, Rothamsted Experimental Station, Harpenden, England; two paratype females and two paratype males at each of the following centers: Nematology Department, University of California, Davis, California, USA; Nematology Department, Landbouwhogeschool, Wageningen, The Netherlands; Department of Zoology, Aligarh Muslim University, Aligarh, India; the remainder at the Commonwealth Bureau of Helminthology, St. Albans, England.

Tylenchorhynchus papyrus sp. n.
(Fig. 3, A–F)
Measurements

FEMALES (5): L = 0.80–0.94 mm; a = 37–43; b = 5.5–6.6; c = 12.5–16.0; V = 52–55.

FEMALE (HOLOTYPE): L = 0.93 mm; a = 40; b = 6.6; c = 14.3; V = ^{28}–52–^{30}; spear = 23.5 μ.

MALES (4): L = 0.69–0.75 mm; a = 35–40; b = 5.1–5.7; c = 14–15; T = 55–65.

Description

FEMALE: Body ventrally arcuate, transverse striae 1.2–1.8 μ apart near mid-body. Lateral fields with three incisures. Lip region conoid-rounded, with 7–8 annules; framework lightly sclerotized. Spear very thin 23–24 μ long, with minute, backwardly directed basal knobs, its anterior part longer than the posterior. Dorsal esophageal gland opening 2.5–3.0 μ behind base of spear. Hemizonid three body annules long, a little behind nerve ring. Excretory pore in the region of the hemizonid or just behind. Basal esophageal bulb rather elongate, its base applied to anterior face of intestine. Cardia large, rounded to slightly discoidal. Large spherical granules present in the intestinal cells. A post-intestinal sac absent but coiled tubules possibly associated with excretory system extend into tail cavity a little behind anal region. Tail elongate-subclavate with striated terminus, 3.7–4.2 times anal body-width long; phasmids at middle of tail or further anterior (Fig. 3, E). Vulva depressed. Spermatheca irregularly rounded. Ovaries with single row of oocytes.

MALE: General characters as for female. Spear averaging 23.5 μ long. Spicules 26–29 μ long as measured along their dorsal side, with large ventral flanges; gubernaculum 13 μ long, with a large dorsal spine at proximal end; lateral fields aerolated, disappear before the phasmids; bursa distinctly crenate (Fig. 3, C). Phasmids prominent, anterior to middle of tail.

RELATIONSHIP: *T. papyrus* sp. n. differs from *T. bifasciatus* in having a longer spear, a subclavate female tail measuring over three anal body-widths long and in the more anterior position of the phasmids (in latter species spear = 19–20 μ long, tail = 2.6–2.7 times anal body-widths and phasmids in female behind the middle of the tail). *T. rhopalocercus* has spear 17–19 μ, spicules 21 μ and gubernaculum 8 μ long and the female tail is almost six anal body-widths in length.

TYPE HABITAT AND LOCALITY: Collected by Professor W. B. Banage from soil in Papyrus swamp, Namulonge, Uganda.
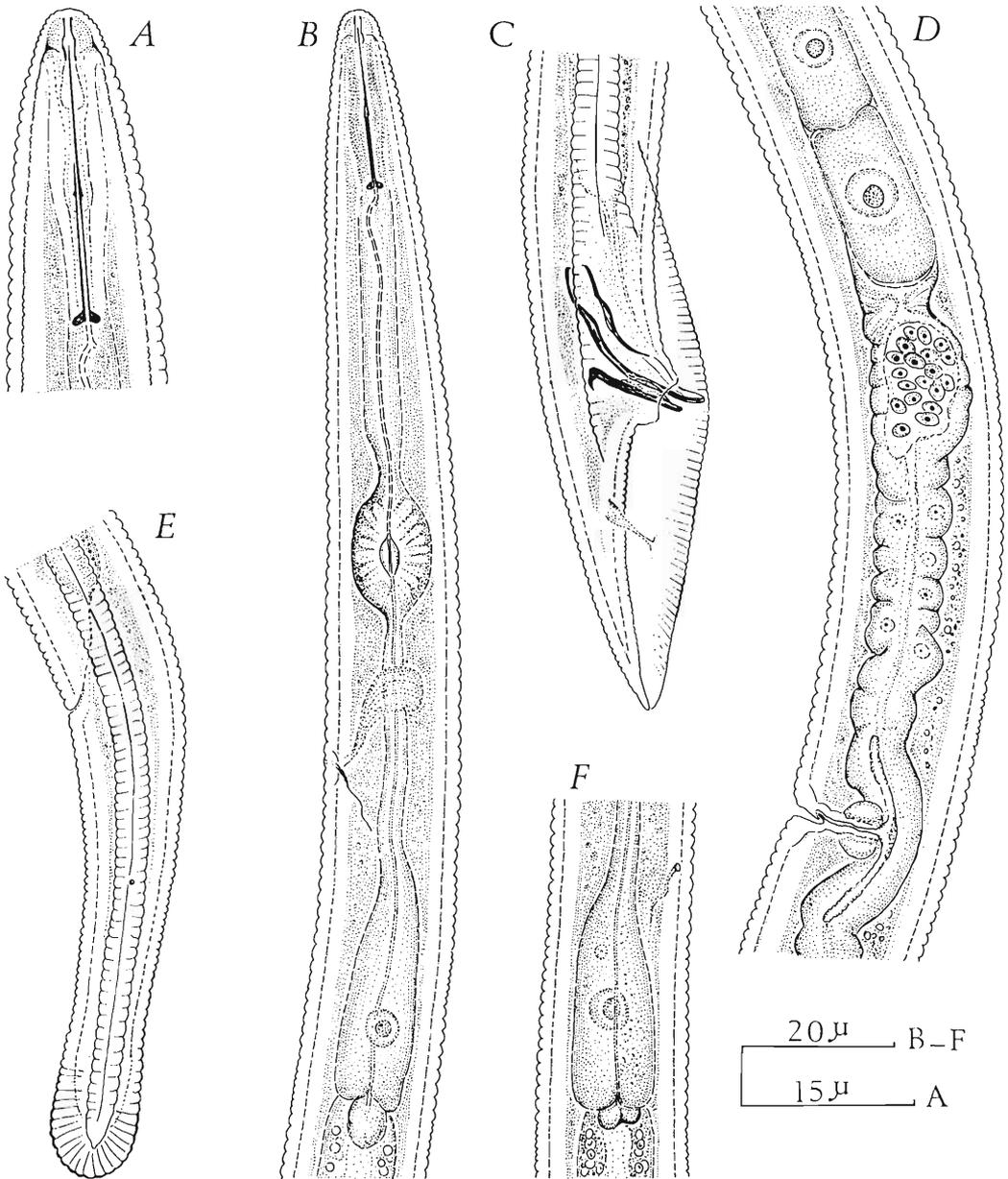
Figure 3, A–F. *Tylenchorhynchus papyrus* n. sp. C. Male, rest female. A. Head end. B. Esophageal region. C and E. Tail ends. D. Part of anterior reproductive branch. F. Basal Esophageal bulb and cardia.

TYPE MATERIAL: Holotype female and a pair of paratypes (1 ♂, 1 ♀) at Nematology Department, Rothamsted Experimental Station, Harpenden, Hertfordshire, England; a pair of paratypes (1 ♂, 1 ♀) at Nematology Department, University of California, Davis, California, USA and the remainder at Commonwealth Bureau of Helminthology, St. Albans, England.

The systematic position of Tylenchorhynchinae Eliava, 1964 is not clear at the moment. Allen and Sher (1967) assigned this subfamily along with Dolichodorinae, Telotylenchinae, Belonolaiminae, Hoplolaiminae, Pratylenchinae etc., to the family Tylenchidae. Paramonov (1967) considered it under Hoplolaimidae along with Hoplolaiminae, Rotylenchoidinae, Belonolaiminae, Dolichodorinae and Trophurinae. Husain and Khan (1967) defined it as a subfamily of Tylenchidae considering under it the genus *Telotylenchus* Siddiqi, 1960. Thorne and Malek (1968) questioned this latter action and gave an emended diagnosis of the subfamily to exclude *Telotylenchus*.

As elaborately discussed by Paramonov (1967, 1968) there are sufficient reasons, both morphological and ecological, to support the view that the subfamilies Dolichodorinae, Tylenchorhynchinae, Trophurinae, Tylodorinae, Belonolaiminae, Telotylenchinae, and Aphasmatylenchinae do not belong in the family Tylenchidae but are closer to Hoplolaimidae in being sufficiently advanced ectoparasites of plant roots. However, the first four of these have a fundamentally different type of esophagus than the rest, in that the esophageal glands do not lie free in the body cavity but form a compact basal esophageal bulb joined to the intestine through a prominent cellular cardia. The former group of subfamilies is regarded to constitute the family Dolichodoridae (Chitwood and Chitwood, 1950) Skarbilovich, 1959 and the latter is here proposed to form a separate family Belonolaimidae (Whitehead, 1959) n. rank. Diagnoses, composition and relationships of these two families are given below.

Dolichodoridae (Chitwood and Chitwood, 1950) Skarbilovich, 1959

DIAGNOSIS (EMENDED): Tylenchoidea. Lateral fields with six or less incisures. Labial framework lightly to heavily sclerotized, absent in *Nagelus*. Spear usually well developed and over 15 μ in length, with prominent basal knobs; its protractor muscles almost parallel to body axis. Amphids labial; deirids sometimes present; phasmids pore-like, near middle of tail. Median esophageal bulb strongly muscular. Esophageal glands enclosed in and forming basal esophageal bulb. A cellular cardia present, projecting into lumen of intestine. Ovaries usually paired; spermatheca a small pouch; sperms small, rounded, with little cytoplasm. Bursa completely enveloping tail except in Tylodorinae, with phasmid extending as a false rib. Tail in female elongate (very rarely under twice anal body-width), conoid, cylindrical or filiform. Ectoparasitic on roots of higher plants.

Dolichodoridae is related to Tylenchidae in having a basal esophageal bulb enclosing the esophageal glands but is differentiated by its sclerotized labial framework, well developed spear with tubular arrangement of the protractor muscles, a strongly muscular median esophageal bulb and large bursa which is usually terminal. Members of this family represent a higher stage over those of Tylenchidae in their adaptation to the parasitism of the roots of the spermatophytes (see Paramonov, 1967 and 1968).

Key to subfamilies and genera of Dolichodoridae

1. Tail in both sexes filiform, bursa adanal .... Tylodorinae Paramonov, 1967; _____ *Tylodorus* Meagher, 1964
   Tail in both sexes not filiform, bursa terminal ------------------------------ 2

2. Bursa trilobed .... Dolichodorinae Chitwood and Chitwood, 1950 3
   Bursa simple ------------------------------ 4

3. Spear abnormally long, lip region four-lobed .... *Dolichodorus* Cobb, 1914
   Spear not as long, lip region smoothly rounded .... *Brachydorus* de Guiran and Germani, 1968

4. Body cuticle abnormally thick especially on tail, indistinctly striated .... Trophurinae Paramonov, 1967 5
   Body cuticle not abnormally thick, distinctly striated .... Tylenchorhynchinae Eliava, 1964 6

5. Ovaries paired, spear abnormally long
   .... *Macrotrophurus* Loof, 1958
   Ovary single, spear not as long ....
   .... *Trophurus* Loof, 1956
   (syn. *Clavaurotylenchus* Caveness, 1958)

6. Lateral fields with 6 incisures 7
   Lateral fields with 3–5 incisures ....
   .... *Tylenchorhynchus* Cobb, 1913

7. Protractors of spear attached to the
   cuticularized inner walls of the
   labial cavity, labial framework ab-
   sent .... *Nagelus* Thorne and Malek, 1968
   Protractors of spear attached to the
   base of the labial framework 8

8. Labial disc very conspicuous ....
   .... *Geocenamus* Thorne and Malek, 1968
   Labial disc absent .. *Merlinius* gen. n.

Belonolaimidae (Whitehead, 1959) n. rank

DIAGNOSIS: Tylenchoidea. Body with promi-
nent transverse striae; lateral fields with four
incisures or less. Female tail elongate-conoid
or cylindroid, very rarely under twice anal
body-widths in length, with phasmids located
near middle; male tail completely enveloped
by bursa. Lip region offset or continuous, not
low or flattened, with light to heavy sclerotiza-
tion. Amphids labial, a single papilla on outer
margins of each submedian lip. Spear well
developed, with tubular protractor muscles.
Median esophageal bulb strongly muscular;
basal bulb absent. Esophageal glands free,
forming a long overlap over anterior end of
intestine; dorsal gland enormously enlarged,
extending past subventrals which are often
greatly reduced. Cardia reduced or absent.
Ovaries paired, opposed, outstretched. Spicules
and gubernaculum well developed. Ectopara-
sitic on roots of higher plants.

Belonolaimidae differs from Hoplolaimidae
(Filipjev, 1934) Wieser, 1953 in having the
dorsal esophageal gland prominently larger
and extending well past the subventrals which
are considerably reduced and the elongate tails
in both sexes with phasmids located near the
middle. From Pratylenchidae (Thorne, 1949)
Siddiqi, 1963 this family differs in having a
lip region which is neither low nor flattened, a
large dorsal esophageal gland extending past
the subventrals and in not being endoparasites
of roots.

Key to subfamilies and genera of Belonolaimidae

1. Phasmids absent, esophageal glands
   mostly on ventral side of intestine
   .... Aphasmatylenchinae Sher, 1965;
   Aphasmatylenchus Sher, 1965
   Phasmids present, esophageal glands
   mostly on dorsal and lateral sides of
   intestine 2

2. Lip region four-lobed with lateral lips
   considerably reduced ....
   Belonolaiminae Whitehead, 1959 3
   Lip region not so ....
   Telotylenchinae Siddiqi, 1960 4

3. Labial disc circular ...... *Belonolaimus*
   Steiner, 1949
   Labial disc lemon-shaped due to
   lateral extensions ....
   .... *Morulaimus* Sauer, 1966

4. Labial disc present, prominent ....
   .... *Carphodorus* Colbran, 1965
   Labial disc indistinct or absent 5

5. Spear well developed, lateral fields
   with 4 incisures ....
   Telotylenchus Siddiqi, 1960
   Spear greatly attenuated, lateral fields
   with 3 incisures ....
   Trichotylenchus Whitehead, 1959

Thorne and Malek (1968) considered the
genus *Tetylenchus* Filipjev, 1936 under Tylen-
chorhynchinae. However, it appears to belong
to Tylenchidae because the protrudor muscles
of the spear are oblique to body axis being
attached to the cuticular band at the base of
the lip region (see Thorne and Malek, 1968);
the labial-framework, spear and median
esophageal bulb are weakly developed and
the bursa is not terminal. The genus does not
fit any of the existing subfamilies of Tylenchi-
dae i.e. Tylenchinae, Psilenchinae Paramonov,
1967 or Anguininae Paramonov, 1962, and
therefore a new subfamily, Tetylenchinae, is
proposed to receive it.

Tetylenchinae subfam. n.

DIAGNOSIS: Tylenchidae. Deirids and phas-
mids usually distinct. Amphids pore-like,
labial, close to oral opening. Four prominent
submedian papillae on the outer contour of lip
region. Spear elongate-slender, with or without
basal knobs. Median esophageal bulb oval;

cardia usually discoidal. Vulva median; ovaries paired, outstretched. Tail in both sexes elongate-conoid, not filiform; in male enveloped by a subterminal bursa.

TYPE AND ONLY GENUS: *Tetylenchus* Filipjev, 1936.

Tetylenchinae differs from Psilenchinae in having pore-like amphids which are labial in position, conoid tail and large, subterminal bursa. From Tylenchinae it is differentiated in having conoid tail, paired ovaries and large, subterminal bursa.

Acknowledgments

The author wishes to thank Doctors M. W. Allen, E. Geraert, P. A. A. Loof and J. W. Seinhorst for providing him with specimen slides of several species of *Tylenchorhynchus* and *Merlinius* and Professor W. B. Banage for the specimens of the new species described here.

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Aphelenchoides cibolensis (Nematoda: Aphelenchoididae), a New Mycophagous Nematode Species

Jerry W. Riffle[1]

ABSTRACT: *Aphelenchoides cibolensis* sp. n., a mycophagous nematode that feeds on many root-inhabiting fungi under laboratory conditions, is described and differentiated from similar species of the genus.

An undescribed *Aphelenchoides* species was recovered from rootlets of southwestern tree species during an investigation of plant-parasitic nematodes in marginal *Pinus ponderosa* Laws. stands in central New Mexico. Subsequent laboratory studies revealed that this nematode was mycophagous, and fed on 50 of 53 known or suspected mycorrhizal fungi, and on 3 of 5 root-pathogenic fungi (Riffle, 1970).

Aphelenchoides cibolensis sp. n.
(Fig. 1)

FEMALE (N = 10): 0.41 (0.36–0.46) mm; a = 26 (23–29); b = 8.0 (7.3–8.9); c = 15 (13–16); V = $^{45(38-54)}70(69-71)^{3(2-3)}$; stylet 10 (9–11) μ.

HOLOTYPE: 0.44 mm; a = 25; b = 8.3; c = 15; V = $^{41}70^3$; stylet 10 μ.

MALE: Unknown.

FEMALE: Cuticle marked by fine transverse striae, 0.7–1.0 micron wide. Lateral fields with three incisures (Fig. 1D), extending from median bulb nearly to tail terminus, occupying about one-fifth body width near middle. Lip region set off by constriction (Fig. 1B), divided into six equal sectors (Fig. 1C). Cephalic framework lightly sclerotized with refractive cheilorhabdions at oral opening (Fig. 1B, 1C). Spear 9 to 11 microns long, slender, with distinct basal swellings (Fig. 1B). Median bulb spheroid, occupying about three-fourths of body width. Nerve ring about one-half median bulb length posterior to median bulb. Excretory pore at level of nerve ring, hemizonid two body widths posterior to excretory pore (Fig. 1A). Esophageal gland extends dorsally over intestine 3.5–4 body widths. Ovary outstretched, extending nearly to base of esophageal gland. Oocytes 12–22, arranged in single file. Posterior uterine branch rudimentary, less than one body width in length (Fig. 1F). Vulva with slightly protuberant lips, vagina obliquely transverse for one-third body width (Fig. 1F). Egg about 2.5 times long as wide, eight eggs measured 13 × 35 (12–15 × 32–38) microns. Tail dorsally almost angular as it forms a single sharply pointed ventral mucro (Fig. 1E). Phasmid not observed.

HOLOTYPE: Female on slide number *Aphelenchoides* 1, collected by author on 17 June 1965 and deposited in the Rocky Mountain Forest and Range Experiment Station Nematode Slide Collection, Albuquerque, New Mexico.

PARATYPES: Twenty-two females, same data as holotype; deposited: 20 females, slide numbers *Aphelenchoides* 1a–1h, Rocky Mountain Forest and Range Experiment Station Nematode Slide Collection, Albuquerque, New Mexico; two females, slide numbers 1209–1210, University of California Nematode Survey Collection, Davis.

TYPE HOST: Recovered from rootlets of *Juniperus deppeana* Steud. at 7100 feet elevation.

TYPE LOCALITY: Riley Loop Road one mile north of its junction with Tajique-Torreon Loop Road in the Cibola National Forest near Tajique, New Mexico.

DIAGNOSIS: *Aphelenchoides cibolensis* belongs to the "parientinus" species group and is similar to *A. dactylocercus* Hooper, 1958, *A. parientinus* (Bastian, 1865) Steiner, 1932, *A. sacchari* Hooper, 1958, *A. trivialis* Franklin and Siddiqi, 1963, *A. spinosus* Paesler, 1957, *A. cyrtus* Paesler, 1957, and *A. clarus* Thorne in Thorne and Malek, 1968.

Aphelenchoides cibolensis can be distinguished from *A. dactylocercus* by its shorter tail length, in the shape of the tail terminus
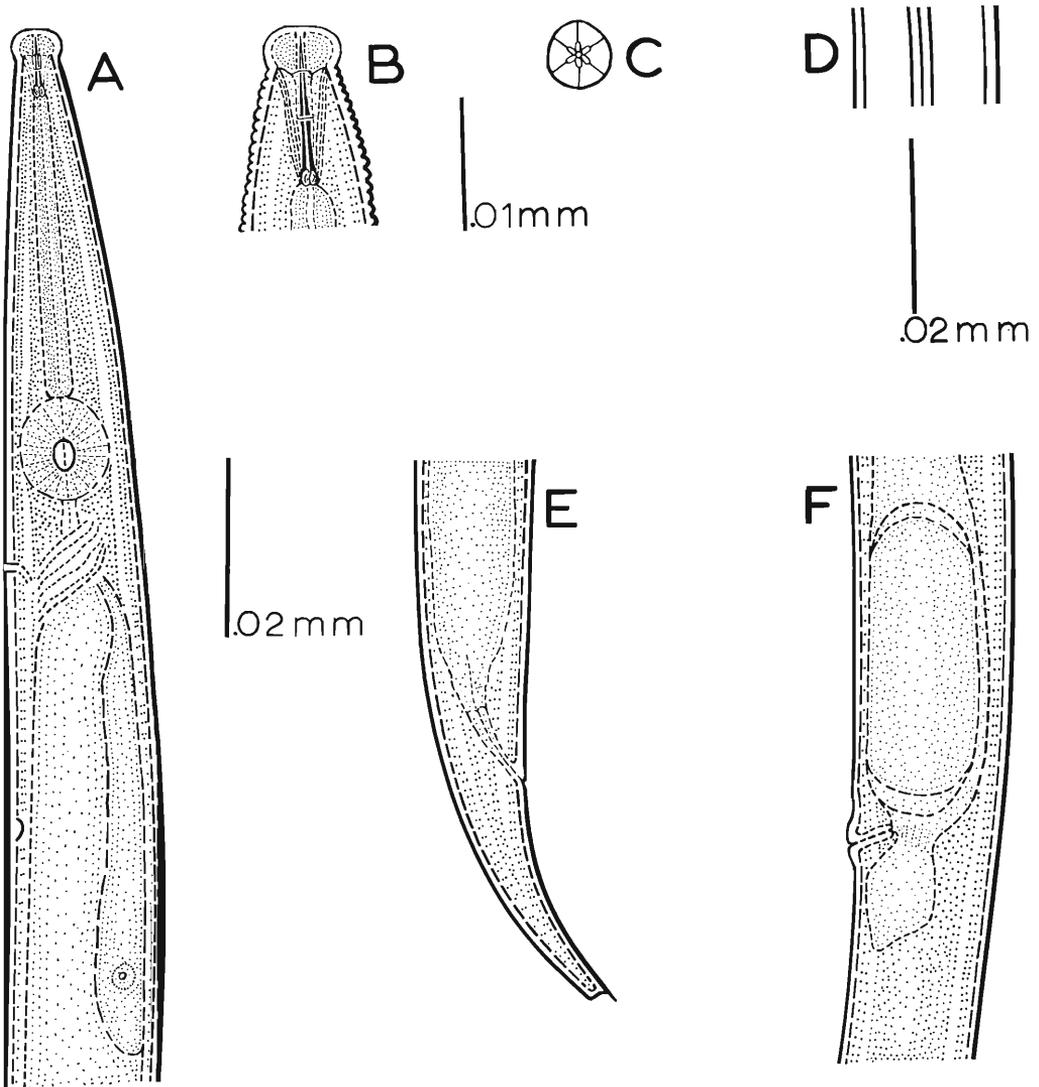
Figure 1. *Aphelenchoides cibolensis* sp. n. A, Lateral view of head and neck region; B, Head; C, Face view; D, Lateral field; E, Tail region; F, Vulval region.

and mucro, in the number of oocytes in the ovary, and in the position of the excretory pore in relation to the nerve ring and median bulb. It differs from *A. parientinus* in having only three incisures in the lateral field, a shorter posterior uterine sac, and in the angularly shaped tail terminus bearing the sharply pointed mucro. The new species can be dis-

tinguished from *A. sacchari* by a shorter body length, a longer neck, a much shorter posterior uterine sac, and an angular tail terminus with a spine-like mucro. It can be separated from *A. trivialis* by a longer neck and tail, and a more anterior vulva position. From *A. cyrtus* and *A. spinosus*, the new species can be distinguished by a shorter body length, a shorter

posterior uterine sac, a longer tail, and a more anteriorly positioned vulva. In addition, the shape of the tail terminus of *A. cibolensis* is quite different from that of *A. spinosus*. It can be separated from *A. clarus* by a longer tail, a more posteriorly located hemizonid in relation to the excretory pore, a shorter posterior

uterine sac, and a mucro that is more ventrally located on the tail terminus.

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Differential Morphology of Adult *Ascaridia galli* (Schrank, 1788) and *Ascaridia dissimilis* Perez Vigueras, 1931

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ABSTRACT: The confusing literature on the morphological differentiation of *A. galli* of chickens and *A. dissimilis* of turkeys was reviewed, and the differential characters of the adults were restudied. These species are highly host specific. They are approximately the same size and are similar in appearance. The females cannot be easily separated on the basis of morphological characters, but the males can be identified readily by differences in (1) position of the first and fourth pairs of ventral caudal papillae, and (2) spicular morphology.

The common *Ascaridia* species of chickens (*Gallus gallus*) and of turkeys (*Meleagris gallopavo*) are respectively *A. galli* and *A. dissimilis*. These species show a high degree of host specificity. Recently, we (Kates et al., 1969) had occasion to review the literature on the differential characters of *Ascaridia* species of chickens and turkeys to identify large numbers of specimens recovered from turkeys. We noted some inaccuracies and omissions in the literature which were repeated in recent books and monographs. Consequently, we restudied numerous adult specimens of both species from several lots; only *A. galli* was identified from chickens and only *A. dissimilis* from turkeys. Because of the economic importance and common occurrence of these species, a brief account of the significant literature is given, as well as the results of our study of adult specimens.

Literature Review

Ackert (1931) studied the life history and morphology of *A. galli* and accurately described the adults. He provided descriptions and figures of the caudal papillae and spicules of the

male, but did not mention any caudal papillae of the female as did some later authors. The same year, Perez Vigueras (1931) published a description of a new species, *A. dissimilis*, from turkeys, but this paper was not widely available. He described the caudal papillae of the male crudely but accurately, figured two pairs of small caudal papillae in the female but did not figure the spicules, mentioning only that they were subequal, 2.016 and 2.080 mm long, respectively. Wehr (1940), in a paper describing a new species, *Ascaridia bonasae*, from the ruffed grouse, refigured the caudal ends of the males of *A. galli* and *A. dissimilis* and keyed out the three species on the basis of differences in position of the first and fourth pairs of caudal papillae of the males. No mention was made of differences in spicular morphology or of caudal papillae of females. Horton-Smith and Long (1957) refigured the caudal ends and papillae of males of *A. galli* and *A. dissimilis*, and pointed out, as did Wehr (1940), the diagnostic value of the position of the fourth pair of ventral papillae; again no mention was made of spicular differences.

Table 1. Lengths in mm of adult *A. galli* and *A. dissimilis* recorded by various authors.

| Authors | A. galli | | | | A. dissimilis | | | |
| | Male | | Female | | Male | | Female | |
	Range	Avg	Range	Avg	Range	Avg	Range	Avg
Ackert, '31	51–76	63	72–116	88	(Not studied)			
Perez Vigueras, '31	(Not studied)				40–65	52	50–85	67
Wehr, '42	(*A. dissimilis* slightly smaller than *A. galli*)							
Mozgovoi, '53	26–70	46	65–100	82	(Not given)			
Horton-Smith & Long, '57	(Not given)				37–45	41	53–70	61
Vasilev, '62	(Not given)				(Not given)			
Baruš, '66	(Not given)				38–52	45	54–72	63
Present authors[1]	60–65	62	80–100	90	50–58	55	70–105	87

[1] Twenty largest specimens measured of each sex of each species.

Kerr (1958) reported that *A. galli* and *A. dissimilis* males were easily separated by differences in the caudal papillae and in length and shape of the spicules, stating that "The spicules of *A. galli* are almost twice as long and the angle of the funnel portion is less acute than those of *A. dissimilis*." We found it impossible to differentiate the males on the basis of these spicular characters. Vasilev (1962) described correctly for the first time the spicules of *A. dissimilis*, and added some minor details to the description of *A. galli* spicules by Ackert (1931). Baruš (1966) redescribed *A. dissimilis*, and also published figures of *A. galli* without accompanying description and measurements. However, Baruš' sketch of the distal spicule tips of *A. galli* is not typical. Both Vasilev and Baruš figured three pairs of small caudal papillae for female *A. dissimilis*, and Vasilev stated that female *A. galli* have one pair of caudal papillae, Baruš did not mention or figure such papillae. Perez Vigueras (1931) originally reported "dos pares de papilas caudales" for female *A. dissimilis*.

Materials and Methods

Several dozen adult specimens of both sexes of *A. galli* and *A. dissimilis* were used in this study; all the former species came from chickens and the latter from turkeys necropsied at this Laboratory. All specimens were fixed and preserved in 70% ethyl alcohol. Although spicules were dissected from many adult males of various sizes, only the largest specimens were selected for measurement of body and spicule length (Tables 1, 2). After the body length of specimens was measured, the caudal ends were severed and cleared in lacto-phenol-glycerine solution. These were studied intact from all aspects, and the male caudal ends were later dissected and the spicules removed intact and mounted in the above-mentioned clearing agent for study and photography.

Results and Discussion

Since the identity of *A. dissimilis* became known in 1931, there is a paucity of data in the literature on the comparative lengths of this species and *A. galli*. Some worm length measurements from the literature and our own are summarized in Table 1. Ackert's (1931) measurements for *A. galli* are similar to ours. Although all specimens measured by the several authors may have been sexually mature, it is possible that the shorter worms had not reached their potential maximum size. Our measurements of large *A. dissimilis* do not differ much from those of *A. galli*. Wehr (1942) stated that *A. galli* adults are slightly larger than *A. dissimilis*, but gave no measurements. Our observations indicate that the two species do not differ much in size when full grown, and that size is not a significant differential character.

Table 2. Lengths in mm of the spicules in male *A. galli* and *A. dissimilis* recorded by various authors.

Authors	A. galli		A. dissimilis	
	Range	Avg	Range	Avg
Ackert, '31	1.0–2.4	1.94	(Not studied)	
Perez Vigueras, '31	(Not studied)		2.016 & 2.080 (Subequal)	2.048 (?)
Mozgovoi, '53	0.65–1.95	1.30	(Not given)	
Kerr, '58	(*A. galli* spicules almost twice as long as *A. dissimilis*)			
Vasilev, '62	(No measurements given)			
Baruš, '66	(Not given)		1.32–1.79	1.55
Present authors[1]	1.5–2.4	1.9	1.6–2.16	1.92

[1] Spicules about equal size. Ten sets of spicules of each species measured after dissection from largest available specimens.

The most useful diagnostic characters of *A. galli* and *A. dissimilis* are found only in the males, i.e., (1) the arrangement of the caudal papillae, and (2) the morphology of the spicules. The differences in the caudal papillae of males have been described by Wehr (1940) and others, and need no further comment. However, the length and morphology of the spicules need some clarification. The number of caudal papillae in females is often difficult to determine and is probably not of much diagnostic value, but will be discussed briefly.

Spicule lengths reported in the literature for both species show considerable variation (Table 2). From our study, spicule length appears to be directly related to size or length of the male specimens. The largest males from each collection had the longest spicules. Our measurements of spicule length of *A. galli* averaged close to those given by Ackert (1931), but Ackert's range of lengths was greater. Our measurements of spicule lengths of *A. dissimilis* averaged close to those given by Perez Vigueras (1931). The latter author, however, may have measured spiclues of only one male specimen, as he reported only a single measurement for each spicule. Whether the spicules of either species are equal or subequal in length appears to be moot, and not of diagnostic significance.

Regardless of the length of the males and the spicules of both species, the males can be separated easily on the basis of spicular morphology (Figs. 1–4). The spicules of *A. dissimilis* look like small drumsticks (Fig. 1),

are broad and truncate proximally, 90–100 μ wide, taper gradually distally, and terminate in rounded clear tips 11–12 μ wide (Fig. 2). Baruš (1966) gave the following measurements: proximal ends 79–92, distal ends 11–18, μ wide. The spicules of *A. galli*, when observed at low magnification, superficially resemble those of *A. dissimilis*, and are quite similar for the proximal third of their length. Over the distal two-thirds, however, the heavily sclerotized part of the spicules gradually narrows to a thin strand almost to the distal end, and is accompanied by a membranous structure about twice as wide as the sclerotized strand (Fig. 3). The distal tips of the spicules are more than twice as wide (24–28 μ) as those of *A. dissimilis*, and their typical terminal outline is blunt with a slight indentation at the center (Fig. 4). Variations in the shape of the distal tips of *A. galli* spicules may occur as illustrated by *Ackert* (1931); in some cases these may be real, or due to variations in orientation, or distortion, of the spicules on slide preparations.

Although Perez Vigueras, Vasilev, and Baruš reported that female *A. dissimilis* have two or three pairs of small caudal papillae, and Vasilev reported that *A. galli* females have one pair, we have been unable to confirm these observations fully. On some cleared specimens of female *A. dissimilis* we have seen what appeared to be one or two pairs of such papillae, but have not seen caudal papillae on adult female *A. galli*. If such papillae do occur in a uniform manner on females of these species,
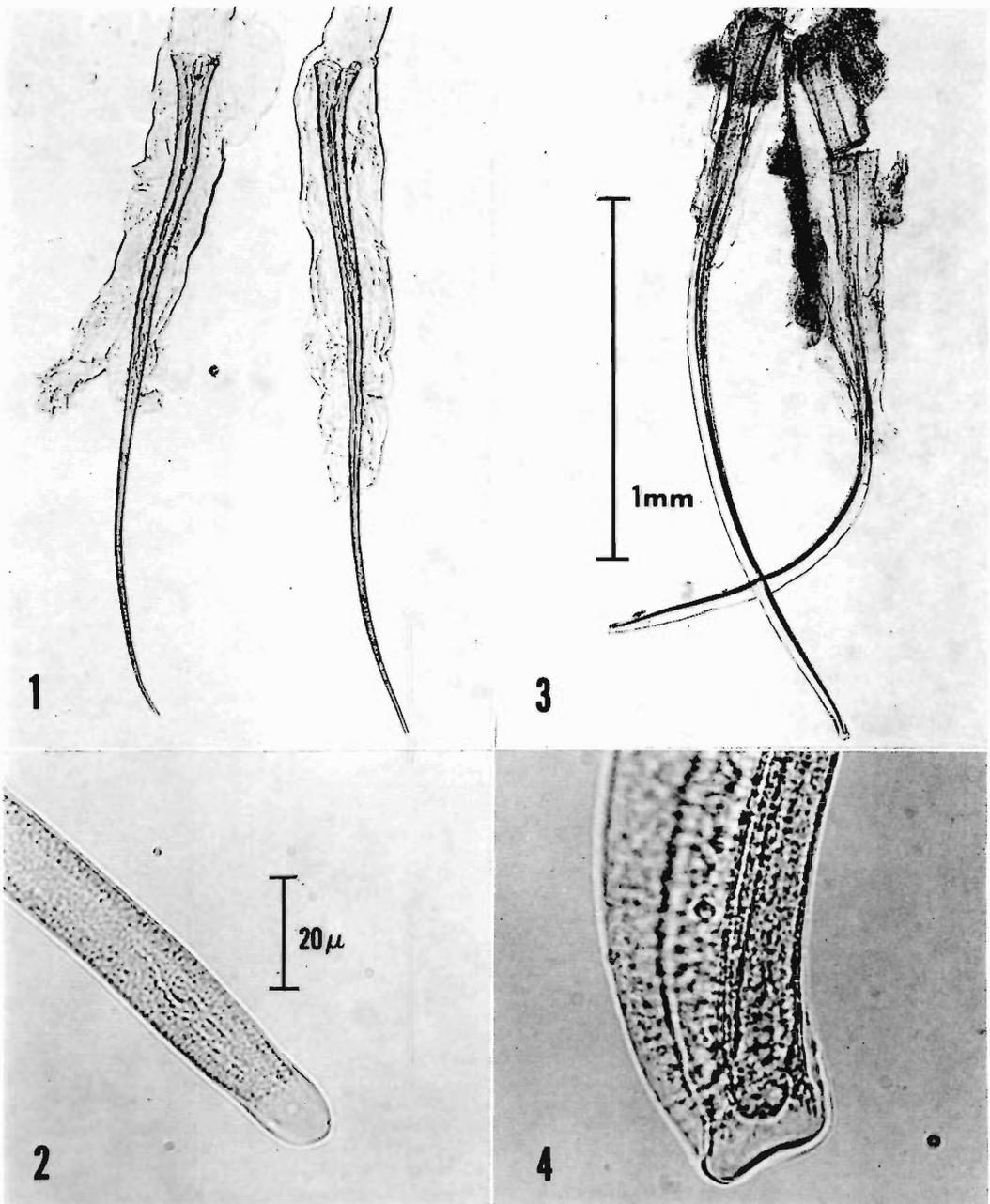
Figures 1–4. Photographs of dissected spicules. 1. Complete spicules of *A. dissimilis*. 2. Distal end of spicule of *A. dissimilis*. 3. Complete spicules of *A. galli*. 4. Distal end of spicule of *A. galli*. Figures 1 and 3 same magnification; 2 and 4 same magnification.

they are not always readily discernible, and thus have little practical value.

Summary of differential character of males

A. galli: First pair of ventral caudal papillae anterior to precloacal sucker; fourth pair of ventral papillae widely separated just posterior to second pair of lateral papillae. Spicules about equal; average length 1.9 mm, with marked membranous structure over about the distal half; distal ends typically blunt with slight indentation, 24–28 μ wide.

A. dissimilis: First pair of ventral caudal papillae opposite precloacal sucker; fourth pair of ventral papillae slightly separated just posterior to cloaca. Spicules about equal; average length 1.92 mm, without marked membranous structure; distal ends clear and rounded about 11–12 μ wide.

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Eimeria tenella: From Sporozoites to Oocysts in Cell Culture

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ABSTRACT: Excysted sporozoites of *Eimeria tenella* were inoculated into monolayer cell cultures of embryonic chick kidney (ECK) and nonembryonic chick kidney (CK). Only two asexual generations were found in cultures prepared from cell suspensions containing few cell aggregates; gametocytes and oocysts were found in cultures prepared from suspensions where cell aggregates were abundant. Gametocytes and oocysts developed faster and in greater quantity in CK cultures. In CK, 175(120–193) oocysts per coverslip were found at 7 days; in ECK, only a few were found at 8 to 9 days. Oocysts that developed in culture sporulated and produced infection when fed to the natural host. During the first 5 days of the patent period, 15,000,000 oocysts were recovered.

Although *Eimeria tenella* has undergone schizogony in all cell types tested, only one complete asexual generation has been obtained in cell cultures established from other than chick tissues. Using sporozoites as the inoculum, mature first generation schizonts were found in cell line Japanese quail fibroblasts and cell line bovine kidney (Patton, 1965), in

cell line bovine embryonic kidney (Doran and Vetterling, 1968; Doran 1970), and in cell line bovine embryonic trachea (Matsuoka et al., 1969). Bedrnik (1967a) found mature third generation schizonts in Hela cells inoculated with second generation merozoites obtained from the chicken.

Better growth of *E. tenella* has been obtained with cells from the chicken. Although Patton (1965) and Bedrnik (1969) found only the first asexual generation when primary cultures of chick embryo fibroblasts were inoculated with sporozoites, Bedrnik (1967b) found gametocytes and oocysts when the same cell type was inoculated with second generation merozoites. Strout and Ouellette (1968) inoculated primary embryonic chick kidney cells with sporozoites and obtained gametocytes. The latter work was especially significant because it showed the possibility of obtaining the complete endogenous cycle in cell culture.

The present paper compares the development of excysted *E. tenella* sporozoites in embryonic and nonembryonic chick kidney cells and reports for the first time the production of oocysts which, when sporulated, contained infective sporozoites.

Materials and Methods

SPOROZOITES: Excysted sporozoites, frozen and stored as previously described (Doran, 1969), were used in all experiments. They were 3–8 weeks old when excysted and frozen, and they had been stored 3–5 months when thawed and used.

CELL CULTURES: Kidneys were obtained from 14- to 17-day-old chick embryos and from 2- to 3-week-old chicks. For cultures in Experiments one and two, the tissue was trypsinized using four 15-min periods; for those in Experiments three and four, the third and fourth trypsinization periods were 3 min each. Only cells from the 4th period were used. In Experiments one and two, the cell suspension contained very few cell aggregates; in Experiments three and four, small cell aggregates were abundant. Approximately 500,000 cells were placed in Leighton tubes containing 10- by 35-mm coverslips. In the first three experiments, 24 cultures of each cell type were prepared; in the fourth, 24 of embryonic chick kidney (ECK) and 32 of nonembryonic chick kidney (CK) were prepared. Cultures were

incubated at 40–41 C until approximately 25% of the coverslip was covered with cells. This period was 1–2 days for ECK and 2–3 days for CK. The growth medium was 80% Hanks' balanced salt solution (B.S.S.), 10% lactalbumin hydrolysate (2.5% solution in Hanks' B.S.S.), and 10% fetal calf serum. It also contained phenol red indicator (10 mg/ml), dihydrostreptomycin (100 μg/ml), and penicillin (100 units/ml).

INOCULATION OF CELL CULTURES: Sporozoites, which were in Medium 199 + 5% chicken serum when frozen, were quickly thawed and placed in enough additional medium to dilute the dimethyl sulfoxide (freezing protectant) concentration to less than 1%. Sporozoites were counted and more medium was added so that 1 ml contained 300,000 sporozoites. When adjusted to pH 7.0–7.2, 1.0 ml was pipetted into each of the culture tubes.

After 3–4 hours, the sporozoite-containing medium was removed and replaced with 2 ml of a maintenance medium. This was the same as the growth medium except that the concentrations of both hydrolysate and serum were 5 rather than 10%. From 3 days after inoculation the medium was changed daily, to maintain the pH at 7.0–7.2.

FIXING, STAINING, AND EVALUATION OF DEVELOPMENT: At 24-hour intervals from 2 through 9 days after inoculation, coverslips were removed from three tubes, fixed, and stained as previously described (Doran and Vetterling, 1969). The entire coverslip was examined, and counts were made only of oocysts. Oocysts that developed in culture were easily distinguishable from the few unbroken oocysts that were carried over in the inoculum because they were unsporulated and were surrounded by an intracytoplasmic space.

SPORULATION OF CULTURE-GROWN OOCYSTS: At 7 days, 8 CK cultures were removed from incubation (40–41 C) to room temperature (21–23 C). One culture was examined for the presence of oocysts and sporulation progress using an inverted microscope. After 3 days, the medium in each of the tubes was replaced with 2 ml of 2.5% potassium dichromate. One day later, all culture material was removed from the tubes and concentrated to 2 ml.

INOCULATION AND EXAMINATION OF CHICKS: Three 3-week-old chicks, whose droppings
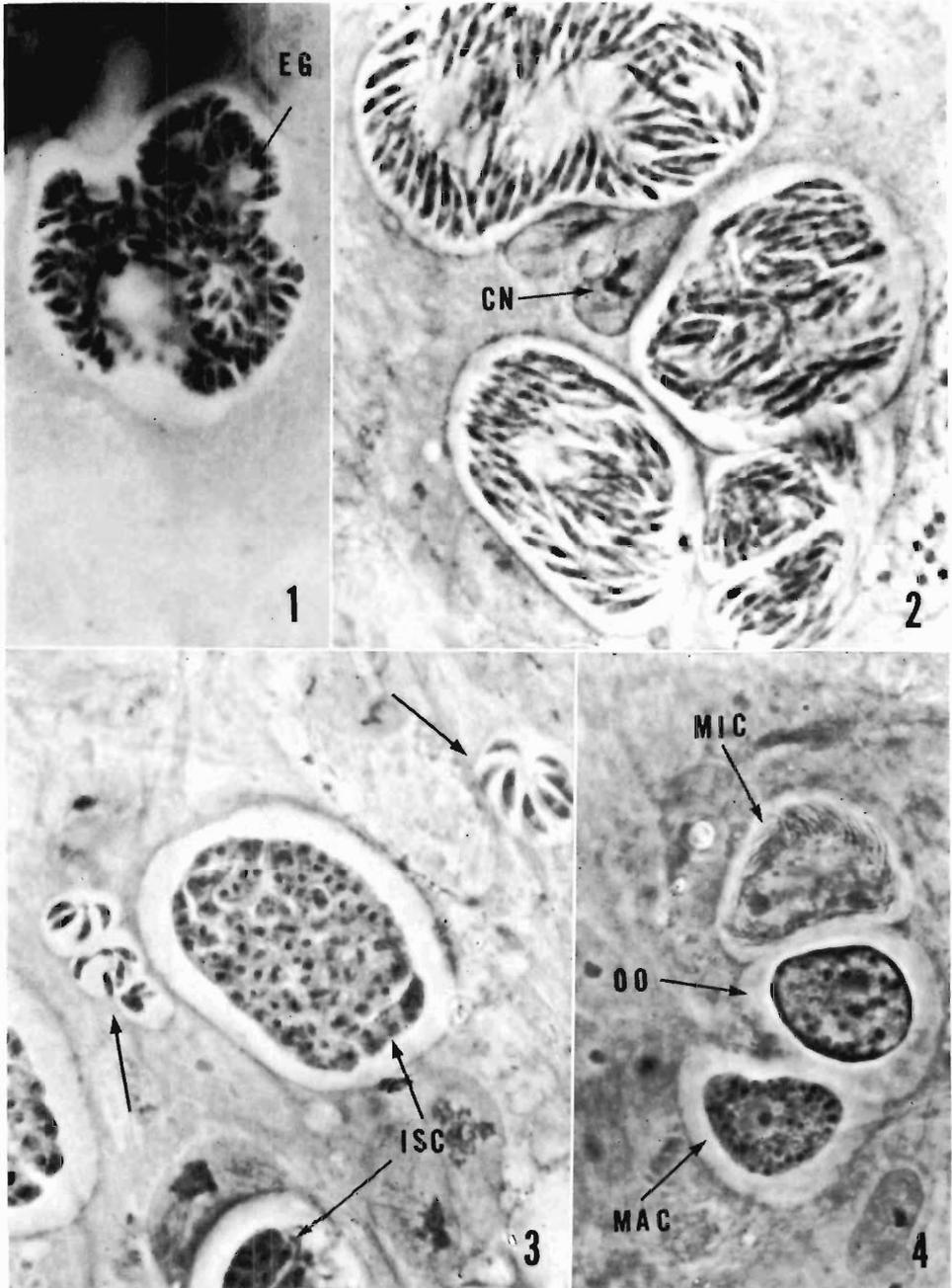
Figures 1–10. Photomicrographs of stages of *Eimeria tenella* in cell culture. Magnification × 1000, except Figs. 5–8 (× 430). Abbreviations: EG = eosinophilic globule; CN = host cell nucleus; ISC = immature schizont; ME = merozoite; MIC = microgametocyte; MAC = macrogamete; OO = oocyst. 1. First generation mature schizont. 2. Second generation mature schizonts. 3. Two small, mature schizonts

Table 1. The occurrence of *Eimeria tenella* first and second generation mature schizonts, gametocytes, and oocysts in chick kidney (CK) and embryonic chick kidney (ECK) cell cultures.

Developmental stage	Cell type	Exp. no.	Time (days)							
			2	3	4	5	6	7	8	9
Mature schizont (generation 1)	ECK	1	−	+	+	+	−	−	−	−
		2	−	+	+	+	−	−	−	−
		3	−	+	+	−	−	−	−	−
		4	+	+	+	−	−	−	−	−
	CK	1	+	+	+	−	−	−	−	−
		2	−	+	+	+	−	−	−	−
		3	+	+	+	−	−	−	−	−
		4	+	+	+	+	−	−	−	−
Mature schizont (generation 2)	ECK	1	−	−	+	+	+	+	+	+
		2	−	−	−	+	+	+	+	+
		3	−	−	+	+	+	+	+	+
		4	−	−	+	+	+	+	+	+
	CK	1	−	−	+	+	+	+	+	+
		2	−	−	+	+	+	+	+	+
		3	−	−	+	+	+	+	+	+
		4	−	−	+	+	+	+	+	+
Gametocyte	ECK	1	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−
		3	−	−	−	−	+	+	+	+
		4	−	−	−	−	+	+	+	+
	CK	1	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−
		3	−	−	−	−	+	+	+	+
		4	−	−	−	−	+	+	+	+
Oocyst	ECK	1	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−
		3	−	−	−	−	−	−	−	+
		4	−	−	−	−	−	−	+	+
	CK	1	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−
		3	−	−	−	−	+	+	+	+
		4	−	−	−	−	−	+	+	+

were free of oocysts, were placed in separate cages. One was given the concentrated composite of the 8 CK cultures. Another was given 25,000 sporulated oocysts from a stock culture that had been incubated at 41 C for 7 days. The latter was necessary to determine whether the infection produced in the chick given the culture concentrate could have been due to oocysts carried over in the inoculum. The third chick was an uninoculated control.

Droppings were examined for oocysts by sugar-flotation on the 5th, 7th, 8th, and 12th days. Counts were made of the number of oocysts passed between days 7 and 12 in the droppings of the bird fed the culture concentrate.

←

of probably the third generation within a cluster of large, immature second generation schizonts. 4. Microgametocyte with gametes (4–5 μ long), early macrogamete, and an oocyst. 5. Sexual stages and oocysts. The merozoites were not intracellular and were probably dead. 6. Young microgametocytes. 7. Oocysts similar in size to those obtained from the natural host. The small body just below the intracytoplasmic space is a trophozoite of a generation other than the first. 8. An early oocyst with an incompletely formed wall (arrow). 9. A large oocyst 36 × 22 μ) and a greatly elongated macrogamete. The folding at the edge of the oocyst is probably due to fixation. 10. A greatly elongated oocyst (65 × 15 μ) in which nuclear division appears to be taking place.

Results

Only the first and second asexual generations were found when cultures were established from cell suspensions containing only a few cell aggregates (Exps. one and two). However, when cell aggregates were abundant (Exps. three and four), development proceeded to the formation of oocysts. Gametocytes were found in both cell types at 6 days, but oocysts were found 2 days sooner in CK than in ECK (Table 1).

Mature first generation schizonts (Fig. 1) generally occurred singly within either fibroblast-like or epithelial-like cells. They were most prevalent in both cell types one day after they were first found. They were not found after 5 days, although many sporozoites and a few 2–4 nucleated immature schizonts were present. At 2 days, 15 mature schizonts measured 37 (30–45) × 30 (17–45) μ in CK; at 3 days, 10 measured 32 (28–41) × 25 (17–37) μ in ECK. All schizonts contained approximately 200–250 merozoites of which 30 in 8 different schizonts measured 7 (6–8) × 1.0–1.5 μ. An eosinophilic (= refractile) globule was observed only in a few schizonts.

Mature second generation schizonts (Fig. 2) were present at 4 days in both cell types. They were most prevalent at 5 days and were nearly always situated in clusters mostly within epithelial-like cells at 4–5 days and within the dense areas of growth containing mostly fibroblast-like cells after 5 days. At 4 days, 25 in both cell types were similar in size. They measured 42 (30–65) × 19 (14–21) μ. Most schizonts contained approximately the same number of merozoites as those of the first generation; a few contained only 37–45. Thirty-five merozoites in 6 schizonts measured 12 (10–15) × 1.4 (1.2–1.8) μ.

In the CK of Exp. four at 6 and 7 days only, schizonts that were probably third generation (Fig. 3) were found within the denser areas of cell growth. The 15 found at 6 days measured 15 (12–20) μ and contained only 5–17 merozoites. These schizonts were all close to a cluster of immature or mature second generation schizonts. Merozoites were similar in size to those of the second generation.

Only a few microgametocytes and macrogametes (Fig. 4) were in ECK, but in CK, especially at 7 days, they were very numerous.

In ECK they were usually situated singly; in CK there were many in clusters (Fig. 5). All were confined to the denser areas of growth containing mostly fibroblast-like cells. In CK, the sexual stages were usually together, but occasionally each sex was by itself in clusters of 5 to 18 (Fig. 6).

Comparatively few oocysts were in ECK cultures. Three coverslips in Experiment 4 at 8 and 9 days contained an average of 7 oocysts. However, those of CK at 6 days contained 175 (120–193). All were found in the denser growth areas that contained mostly fibroblast-like cells. Most of the oocysts (Fig. 7) were similar in size to those obtained from infected chickens, but some were larger (Figs. 8–10). Some were the same size and shape as macrogametes (Fig. 10).

In one culture held at room temperature and examined for oocysts after 7 days, 7 oocysts, similar in size to those from the host, were in one cluster. After 8 hr, the protoplasmic mass had contracted in three of them; after 12 hr, it had divided into 4 sporoblasts. Because of poor optical quality of the culture tube, it was impossible to follow sporulation further or tell whether sporozoites had formed.

Oocysts appeared in the droppings 7 days after the material from 8 cultures was fed to a chick. The oocyst yield between days 7 and 12 was 15.0 ± 0.32 × 10⁶. No oocysts were recovered from the chick given 25,000 heat-treated oocysts or from the uninfected control chick.

Discussion

The degree to which kidney tissue was trypsinized was apparently an important factor for *E. tenella* development. Gametocytes and oocysts were not found in cultures when the cell suspension from which the cultures were established contained only a few cell aggregates. At 2 days, very few patches of epithelial-like cells were seen; most cells were widely dispersed and growing in a fibroblast-like manner. However, gametocytes and oocysts were found when the suspension was composed mostly of cell aggregates. At 2 days, epithelial-like patches of cells were abundant and most of them contained parasites. After 5 days, however, there were only a few epithelial-like patches. Instead, there were areas of dense growth containing mostly fibroblast-like cells

6
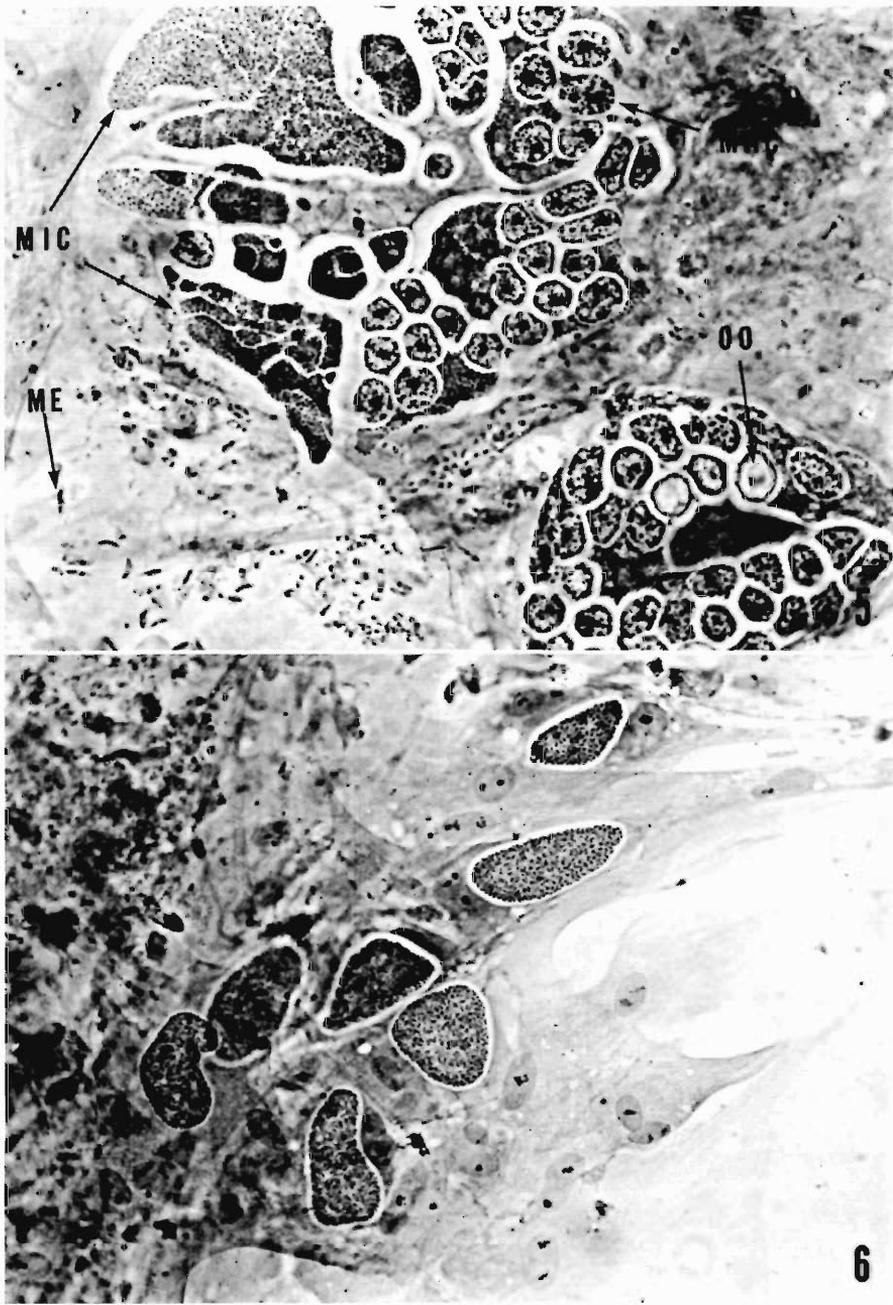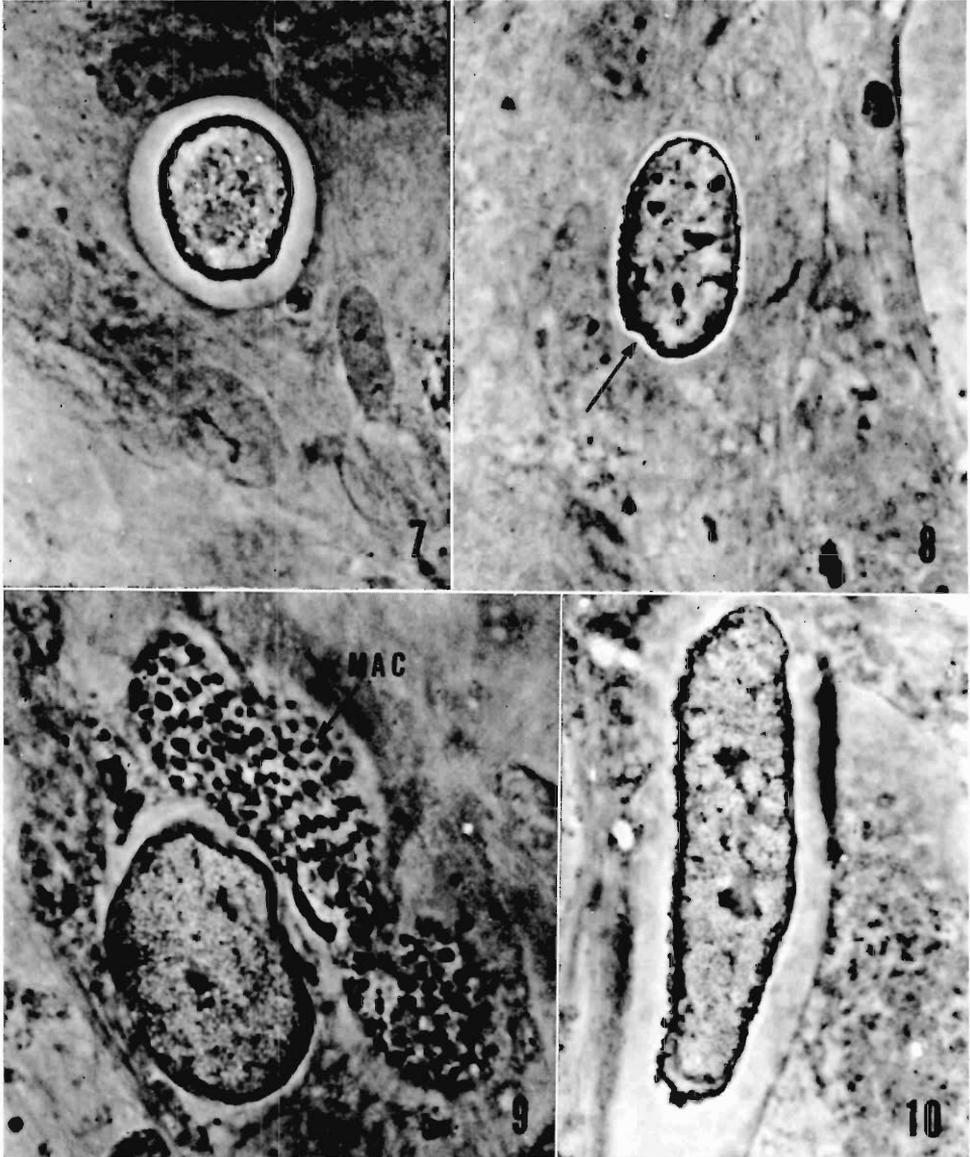
and these contained the parasites. Epithelial-like cells apparently changed to fibroblast-like. Bedrnik (1967b) reported that sexual stages were not in fibroblasts, but were strictly limited to the islands of epithelial-like cells. He used second generation merozoites as the inoculum and the interval between inoculation and appearance of sexual stages was only 1–2 days.

I believe that sexual stages and oocysts develop only from sporozoites that enter patches of cells, epithelial-like at first, that are produced from cell aggregates. Since development from the first generation on took place primarily in clusters, merozoites probably did not migrate very far before entering cells. It appeared that those that underwent further development en-

tered cells adjacent to the cell in which the schizont developed.

Development in nonembryonic cells was better than in embryonic cells in two ways: First, there was greater development. Only a few gametocytes and oocysts were in ECK; in CK there were 175 (120–193) oocysts per coverslip. Strout and Ouellette (1968) also found only a few gametocytes in ECK. Second, development was more rapid. First generation schizonts were generally more prevalent one day earlier and oocysts were found at 6–7 days rather than 8–9 days. Present findings concerning the time when mature schizonts first appeared agree with those of Strout and Ouellette (1968), but differ slightly from the time intervals in the host. In the host, Tyzzer (1929) found that the first generation was completed in 2.5–3 days and that the second generation was completed in 4.5–5 days. In culture, first and second generation mature schizonts were found at 2 and 4 days, respectively. The slight differences are probably due to the time required for the organism to reach the intestine, excyst, and migrate to the site of development in the host; in culture, cells in which development can occur are available upon inoculation. The length of merozoites in culture also differed somewhat from those in the host. In culture, first generation merozoites were 6–8 μ long and second generation merozoites were 10 to 15 μ long; in the host, they were 2–4 μ and 16 μ, respectively. The small schizonts in CK at 6–7 days, which were thought to be those of the third generation, also differed from the third generation in the host. Mature schizonts in the host measure 5.6–13.2 μ and contain 4–30 merozoites that are 6–8 μ long. As previously reported (Doran and Vetterling, 1967), one should expect some variation in a highly artificial system such as monolayer cell culture. The important point upon which there was no difference between CK cultures and the host is that oocysts appeared at 6–7 days.

Strout and Ouellette (1968) used Minimum Essential Medium of Eagle + 10% newborn calf serum for starting cultures of ECK cells, inoculating sporozoites, and maintaining the cells after inoculation. In several pilot tests, I compared growth and maintenance in this medium with that in Hanks' B.S.S., lactalbumin

hydrolysate, and fetal calf serum. The latter gave much healthier looking cells. In the present study, the concentrations of hydrolysate and serum were lowered to 5% in the maintenance medium to reduce the rate of cell multiplication. When the growth medium was used for maintenance, cells grew too fast and peeled off the coverslip 5–7 days after inoculation. Neither the growth nor maintenance media were used to inoculate sporozoites into culture because unpublished work showed that *E. tenella* sporozoites survive better in Medium 199 and chicken serum. The inoculation medium was not used for maintenance because of the poor condition of cells encountered in another pilot test.

Fifteen million oocysts were recovered when the material from 8 cell cultures was fed to a chick. Brackett and Bliznick (1950) reported that, in light infections, over 100,000 oocysts are produced from each infective oocyst. This number would indicate that about 150 oocysts, which developed in culture, sporulated and were infective. It is unlikely that infection was due to viable sporozoites or merozoites or to oocysts carried into culture with the inoculum. Sporozoites and merozoites, whether intra- or extracellular, will not survive in potassium dichromate; Wagenbach and Burns (1969) and the present study show that oocysts subjected to 41 C for 6–7 days do not produce infection.

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Helminths of the Striped Skunk, *Mephitis mephitis* Schreber, in North Dakota

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ABSTRACT: Ten species of helminths were recovered from 42 striped skunks, *Mephitis mephitis*, in North Dakota. The 31 infected skunks harbored one or more of the following species: *Alaria taxideae*, *Plagiorchis muris*, *Mesocestoides corti*, ? *Oschmarenia* (*Morosovella*) *mephitis*, *Ascaris columnaris*, *Filaroides mephitis*, *Molineus patens*, *Physaloptera maxillaris*, *Trichinella spiralis*. As skunk parasites, all represent new locality records and *P. muris* a new host record.

The first extensive review of the literature on the parasites of North American carnivores was published by Stiles and Baker (1935) and includes a list of the helminths reported from mustelids. Erickson (1946) provided lists of the hosts of, and a key to, the North American species of helminths of mustelids and reported on the incidence of these helminths in Minnesota. Rare prior and subsequent reports pertain in part or wholly to helminths of *Mephitis mephitis* Schreber, the host investigated by the writer, and include data on their distribution in this country and Canada. However, few of them are based on comprehensive surveys. Others are restricted to one or a few of the helminths of this host. Some of these reports are cited hereinafter.

The present study was undertaken to determine the incidence of helminths in a sample of *M. mephitis* from three ecologically similar counties (Ward, McHenry, McLean) in North

Dakota; collections were made between 26 April 1968 and 1 April 1969. The only helminth of this host heretofore reported from North Dakota (Shumard and Bolin, 1958) is *Physaloptera* sp.

Materials and Methods

The majority of the 42 skunks collected were either trapped or shot; a few were road-killed. Whenever possible, they were autopsied within a few hours after collection; otherwise they were frozen and stored. Organs of the gastrointestinal tract, esophagus through large intestine, of each skunk were separately examined for helminths. The liver, lungs, kidneys, spleen and skeletal muscles also were examined.

Results and Discussion

Ten species of helminths were recovered (Table 1). Two were trematodes, two cestodes and six nematodes. This is the first report of these parasites from *M. mephitis* in North Dakota.

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Table 1. Prevalence of helminth infection in 42 striped skunks from North Dakota.

Species of parasite	Location in host	No. animals infected	Per cent infected
Trematoda			
Alaria taxideae	Small intestine	3	7.1
Plagiorchis muris	Small intestine	2	4.8
Cestoda			
Mesocestoides corti	Small intestine	6	14.3
? *Oschmarenia* (*Morosovella*) *mephitis*	Small intestine	2	4.8
Nematoda			
Ascaris columnaris	Small intestine	8	19.0
Crenosoma canadensis	Bronchi	1	2.4
Filaroides mephitis	Lungs	1	2.4
Molineus patens	Small intestine	3	7.1
Physaloptera maxillaris	Stomach	21	50.0
Trichinella spiralis	Skeletal muscle	2	4.8

Alaria taxideae Swanson and Erickson, 1946 has been reported from *M. mephitis* or related hosts from Minnesota, Michigan, Maryland and Louisiana.

Plagiorchis muris Tanabe, 1922 has not previously been reported from skunks to my knowledge.

Mesocestoides corti Hoeppli, 1925 [*M. variabilis* Muller, 1928 and *M. manteri* Chandler, 1942 are synonyms of *M. corti*, according to Voge (1955)] has been reported from striped skunks in California, Minnesota, Wisconsin, and Louisiana and from several other mammalian hosts. Of the various carnivores examined in this laboratory, *M. mephitis* appears to be the important definitive host in North Dakota.

Immature specimens of a species of a cyclophyllidean cestode were recovered from two skunks. The scolices and proglottids resemble those of *Oschmarenia* (*Morosovella*) *mephitis* (Skinker, 1935) Spassky, 1951 which was described from *M. mephitis* and has since been reported from it (Leigh, 1940; Self and McKnight, 1950; Loftin, 1961). However, my material was inadequate for positive identification.

Ascaris columnaris Leidy, 1856 has been reported from *M. mephitis* in Quebec, Maryland, Louisiana and California.

Crenosoma canadensis Webster, 1964 has previously been reported from *M. mephitis* in Quebec only.

Filaroides mephitis Webster, 1967 has been previously reported from *M. mephitis* in Quebec only.

Molineus patens (Dujardin, 1845) Petrov,

1928 has been reported from *M. mephitis* in Maryland and Louisiana and from related hosts.

Physaloptera maxillaris Molin, 1860, the most common helminth collected has been reported from skunks in Quebec, Maryland, Louisiana and California.

Trichinella spiralis (Owen, 1835) has been reported from *M. mephitis* in Louisiana and Maryland.

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Two New Species of *Pterygodermatites* (*Paucipectines*) Quentin, 1969 (Nematoda : Rictulariidae) with a Key to the Species from North American Rodents

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ABSTRACT: *Pterygodermatites coloradensis* (Hall, 1916) Quentin, 1969 (= *Rictularia coloradensis*) and a species formerly confused with it—*Rictularia coloradensis, sensu* Tiner (1948)—are separated. The former is redescribed; the latter is defined and named *Pterygodermatites peromysci* sp. n. A third species, previously reported from tree squirrels and a wood rat as *Rictularia* sp., is described and named *Pterygodermatites parkeri* sp. n. The three species have apical mouths; three equal buccal teeth; 61–67 cuticular processes, of which 29–31 are prevulvar; and 20 or fewer perioral denticals. They can be separated from each other by differences in the morphology of the buccal teeth, size of the buccal teeth relative to the size of the buccal cavity, size of cuticular processes, and ratio of the lengths of left and right spicules.

In the course of identifying *Rictularia* sp. from *Peromyscus leucopus* it became clear that specimens commonly identified as *R. coloradensis* Hall, 1916 exhibited sufficient differences from the syntypes of Hall's species to warrant recognition as a separate species. The original description of *R. coloradensis* from a Colorado chipmunk was based on one male with nearly equal spicules and one incomplete female with its tail missing. However, most workers accept the "redescription" given by Tiner (1948) as the definition of *R. coloradensis*. Tiner's (1948) specimens were from *Peromyscus leucopus* and *P. maniculatus* from Wisconsin and more Eastern localities and the males had markedly unequal spicules. Tiner recognized that his males differed sufficiently to be regarded as a separate species, but he apparently found no distinguishing characters between the females. He decided to follow earlier workers and identify his specimens as *R. coloradensis* until more males could be studied. Unfortunately, he "redescribed *R. coloradensis*" solely from the specimens from *Peromyscus*.

My study of the syntypes of *R. coloradensis* and the species Tiner (1948) "redescribed" revealed differences between both males and females. In addition, study of *Rictularia* sp. from other North American rodents has led to the recognition of another new species. *R. coloradensis* is redescribed and two new species are described below.

The genus *Rictularia* Froelich, 1802 was recently divided by Quentin (1969) into two genera: *Rictularia* Froelich, 1802 *s. str.*, containing the species that have a completely dorsal transverse mouth with a single buccal tooth (= esophageal teeth *sensu* Quentin, 1969); and *Pterygodermatites* Wedl, 1861, *s. str.*, containing the species that have mouths ranging from apical to dorsal, but never completely dorsal and transverse, with three buccal teeth. He further subdivided *Pterygodermatites* into five subgenera.

Five species from North American rodents—*Pterygodermatites coloradensis* (Hall, 1916); *P. onychomis* (Cuckler, 1939); *P. ondatrae* (Chandler, 1941); *P. dipodomis* (Tiner, 1948), *sensu* Read and Millemann (1953); and *P. microti* (McPherson and Tiner, 1952)—are among 15 species with apical mouths assigned by Quentin (1969) to the subgenus *Paucipectines* (Type: *P. coloradensis*). All species described or discussed herein are members of this subgenus.

Materials and Methods

Nematodes of the subgenus *Paucipectines* from nine species of rodents were studied. In addition, types of all five previously described North American species of the subgenus *Paucipectines* were examined.

The nematodes were studied as temporary whole mounts cleared in phenol-alcohol, except for the syntypes of *P. coloradensis* which are permanent whole mounts. *En face* preparations were made of females only because of the shortage of males. The buccal teeth of a

few females were studied by rolling severed heads under cover glasses in temporary mountings. All drawings were made with the aid of a camera lucida. Measurements are given in microns unless otherwise indicated. They were made with an ocular micrometer or a projection microscope and a measuring wheel. In the descriptions of females only measurements of mature worms containing embryonated eggs are given. Observations on smaller immature fifth stage females are given in the "Remarks," the "Discussion," and the figures.

The numbering system of Chabaud and Petter (1961) is followed for the genital papillae. The arrangement for the subgenus *Paucipectines* from anterior to posterior is: 1, 2, 4, 3, 5, 6, 8, 7, 9, phasmids, 10.

Results

The morphology of the buccal teeth and buccal capsule are basically the same in both sexes of all species that were studied or examined. Although there is a great difference between the sexes in size, and in size and proportion of cuticular combs, comparative comb size was useful in matching males and females of a species. With these characters, and because all males were found only with females of their own species, it was possible to determine conspecificity of males and females with confidence. Thus, characters of both sexes can be used in species determinations.

Pterygodermatites Wedl, 1861 *sensu* Quentin, 1969

Rictulariidae. Mouth apical or inclined dorsally, but never totally dorsal and transverse; three buccal teeth; 29–56 cuticular processes prevulvar.

Subgenus *Paucipectines* Quentin, 1969

Pterygodermatites. Mouth apical; genital papillae prs. no. 1, 2, 4, and 8 placed lateral to line of other papillae; 29–39 prs. of cuticular processes prevulvar.

Pterygodermatites coloradensis (Hall, 1916) (Figs. 1–5)

HOSTS: (Type) *Eutamias quadrivittatus* (Say), Colorado chipmunk; (Other) *Peromyscus maniculatus* (Wagner), deer mouse.

LOCATION: Small intestine.

LOCALITIES: (Type) Pagosa Springs, Colorado; (Other) Utah.

SPECIMENS: USDA Par. Coll. Nos. 16569 (lectotype: female and paralectotype: male); 66238 (fragment of immature female from deer mouse).

Redescription

Buccal cavity contains three equally large buccal teeth extending anteriorly from its floor about one-half its depth. Center portion of each buccal tooth longer than lateral portions giving anterior edge of each tooth convex appearance. (The shape of the anterior edges of the buccal teeth can best be determined as follows: the dorsal tooth in a dorsal or ventral view; the subventrals in a subventral view. Subsequent references to tooth shape refer to the appearance in these aspects.)

FEMALE (LECTOTYPE): Length of incomplete specimen 8.46 mm. Estimated length of specimen when complete 9.0 mm. Diameter: maximum 360; at vulva 270; at base of buccal capsule 120. Buccal cavity 54 deep by 78 wide (dorso-ventrally). Mouth bordered by about 17 perioral denticles up to 9 long. Esophagus 1.5 mm long; anterior muscular portion 470 long. Nerve ring 250 from anterior end. Cervical papillae 400 from anterior end between 6th and 7th prs. of combs. (Excretory pore not seen.) Vulva at level of posterior end of esophagus between comb prs. nos. 31 and 32. (Cuticle and esophagus wrinkled and contracted so that normal relationship of vulva and esophagus cannot be determined.) Eggs embryonated 38–40 by 22–24. Transition from combs to spines not distinct; 32–34 pairs of combs plus spines totaling 65 pairs of cuticular processes. Largest comb 105 long by 42 high. Longest spine 81. (Tail missing.)

MALE (PARALECTOTYPE): Length 3.3 mm. Maximum diameter 250; at base of buccal capsule 70. Buccal cavity 27 deep by 49 wide (latero-dorsally). Mouth bordered by perioral denticles (could not count or measure). Esophagus 875 long. (Nerve ring, cervical papillae and excretory pore not seen; according to original description, nerve ring 100 from anterior end.) Forty-two pairs of combs, begin slightly posterior to base of buccal capsule, end 481 from tail end. Largest comb 85 long by 45 high. Ventral cuticular fans absent. Vent
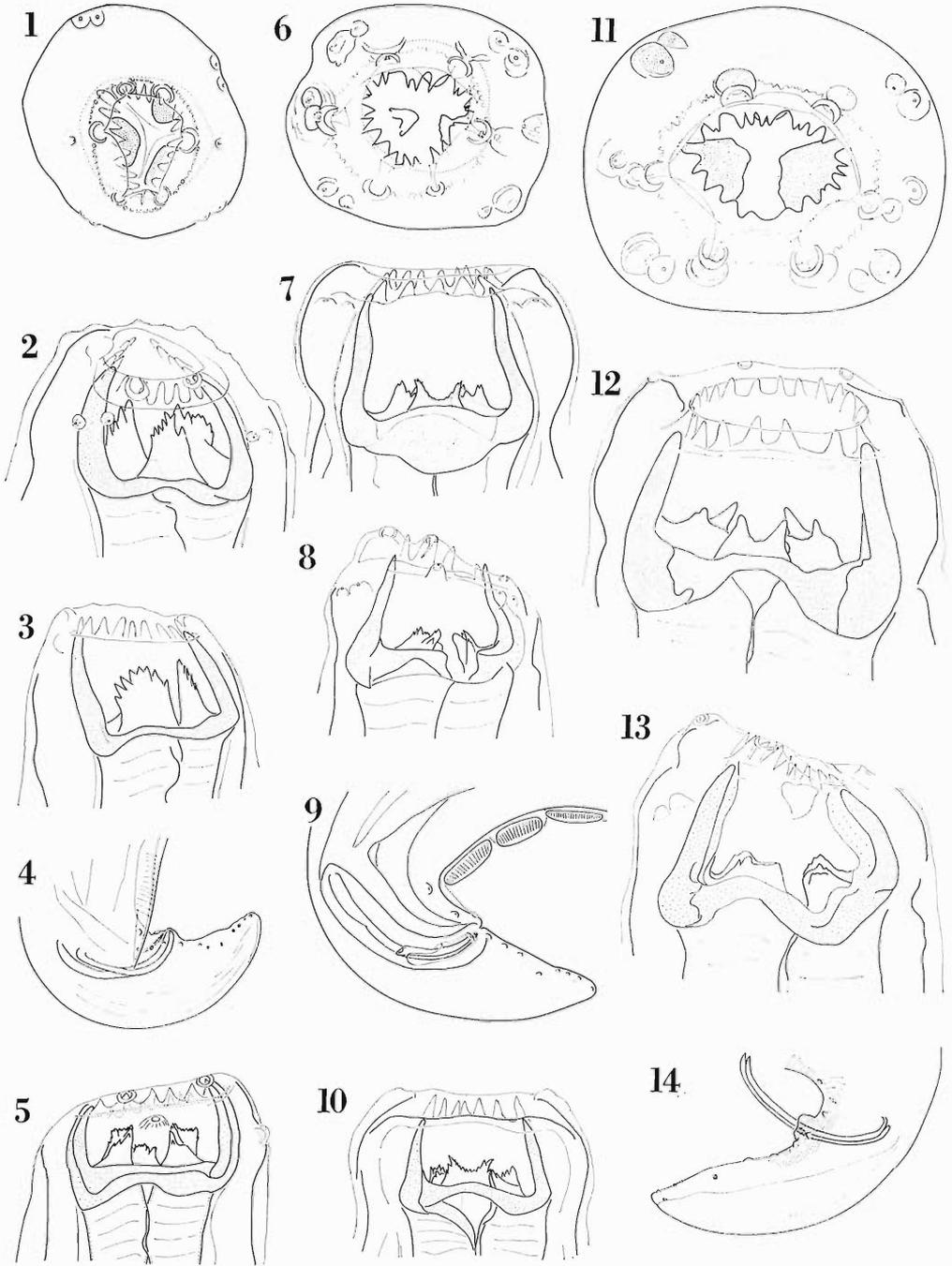
.1 mm
Figs. 5-9, 11-13

.05 mm
Figs. 1-3, 10

.2 mm
Figs. 4, 14

on elevation 130 from end of blunt conical tail. Spicules nearly equal, ventrally curved; left 240, right 215, long. Gubernaculum not discernible. A delicate accessory piece located ventrally near spicule tips, 57 long. Ventral surface of tail and pericloacal region with 10 paired and one unpaired genital papillae and a finely rugose papillation in the pericloacal region (Fig. 4).

Remarks

This redescription agrees with the description by Hall (1916) except in details of the buccal teeth, lengths of the spicules, and genital papillae of the male. Hall's (1916) measurements of the spicules were made in a straight line from tip to tip. They are, therefore, shorter than mine which followed the curvature.

The female syntype is designated the lectotype, although the posterior one-tenth is missing, for two reasons: (1) most species of the genus are represented by female holotypes; and (2) males are rarely collected.

It was possible to estimate the length of the incomplete female lectotype fairly accurately because, although the tail and anus are missing, the intestino-rectal valve is present.

An additional specimen that I believe to be *P. coloradensis* collected in Utah from *Peromyscus maniculatus* by Dr. A. W. Grundmann was found in the National Parasite Collection (USDA Par. Coll. No. 66238). It is a fragment of a young adult female containing no eggs, that probably was 5–10 mm long. Its mouth is elongated dorso-ventrally and the teeth are large and markedly convex on the anterior edge. The buccal cavity is round in *en face* and is not elongated like the mouth (Figs. 1–3). I regard this specimen, the lectotype female and the paralectotype male to be the only known specimens of *P. coloradensis*.

Pterygodermatites peromysci, sp. n.
(Figs. 6–10)

Host: (Type) *Peromyscus leucopus* (Rafinesque), white-footed mouse; (Others) *Peromyscus maniculatus* (Wagner), deer mouse; *P. floridanus* (Chapman), Florida mouse; *P. polionotus* (Wagner), oldfield mouse; *Tamias striatus* (Linnaeus), Eastern chipmunk.

Location: Small intestine.

Localities: (Type) Montgomery County, Maryland; (Others) Virginia, Wisconsin, Georgia, Florida.

Specimens: USNM Helm. Coll. Nos. 63204 (holotype: female) and 63205 (allotype: male); USDA Par. Coll. Nos. 65598, 65599, 41768, 46320, 40571, and 19390 (paratypes). Nos. 46320 and 19390 include males.

Description

Buccal cavity contains three equally large buccal teeth that extend anteriorly from its floor about one-third its depth. Buccal teeth consist of three main parts with middle portion shorter than lateral portions, giving anterior edge of each tooth a concave appearance.

Female (Based on 30 specimens): Length 16.7–29.3 mm. Diameter: maximum 562–784; at vulva 315–422; at base of buccal capsule 134–200. Buccal cavity round in *en face*; 68–115 deep by 65–97 wide. Mouth roughly oval in shape, elongated laterally very slightly; bordered by 16–19 perioral denticles, up to 13–24 long. Esophagus length 2.65–4.05 mm; anterior muscular portion 650–910 long. Nerve ring 288–389, and excretory pore 496–629, from anterior end. Cervical papillae 448–623 from anterior end at level of 6th or 7th pair of combs. Vulva anterior to posterior end of esophagus, between pairs of cuticular processes numbers 29 and 32, 2.00–3.38 mm from anterior end. Vagina vera 174–208 long. Uterus bifurcates 348–380 posterior to vulva. Eggs

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Figures 1–14. Three species of *Pterygodermatites* (*Paucipectines*) from North American rodents. 1–3. Immature female *Pterygodermatites coloradensis* (Hall, 1916) from *Peromyscus maniculatus*: 1. En face. 2. Dorsal view of head. 3. Left lateral view of head. 4. Male tail (paralectotype), *P. coloradensis*. 5. Right lateral view of head, female (lectotype), *P. coloradensis*. 6–10. *Pterygodermatites peromysci* sp. n. from *Peromyscus* spp: 6. En face, female. 7. Dorsal view of head, female (holotype). 8. Left lateral view of head, female. 9. Male tail. 10. Dorsal view of head, immature female. 11–14. *Pterygodermatites parkeri* sp. n. from *Sciurus carolinensis*: 11. En face, female. 12. Dorsal view of head, female. 13. Left lateral view of head, female. 14. Male tail.

embryonated, 43–46 by 30–32. Total number pairs of cuticular processes 61–64, including 28–30 pairs of combs. Largest combs 94–127 long by 40–56 high. Longest spine 70–103. Tail length 355–470.

MALE (Based on 3 specimens): Length 1.92–2.88 mm. Maximum diameter 118–138. Buccal cavity 18–20 deep by 15–17 wide; circle of 12 perioral denticles 3–4 long. Esophagus length 562–640; anterior muscular portion 150 long. Nerve ring 111 from anterior end. Cervical papillae 230 from anterior end at level of 6th or 7th pair of combs. (Excretory pore not seen.) Forty-one pairs of combs; longest comb 50–65; last comb 216–420 from tail end. Three ventral cuticular fans anterior to vent; posterior fan 41–59 long by 19–20 high, others smaller. Tail length 67–70. Spicules markedly unequal, curved ventrally; left 92–98, right 43–50, long. Gubernaculum 40 long, portion extends ventrally around spicules (difficult to discern in two of three specimens). Ventral surface of tail and pericloacal region with 10 paired and one unpaired genital papillae and a very finely rugose papillation in the pericloacal region (Fig. 9).

Remarks

This is the species described by Tiner (1948) in his "redescription" of *P. coloradensis.* Specimens examined by both Tiner and me include USDA Par. Coll. Nos. 40571, 46320, and 19390.

Measurements given above include only those that I made, although the range includes, almost without exception, the measurements given by Tiner (1948). Females as short as 5.4 mm were studied and will be referred to in the discussion.

Both male and female *P. peromysci* differ from *P. coloradensis* in the morphology of the buccal teeth and the size of the teeth relative to the size of the buccal cavity. The males can also be separated by the ratios of the lengths of the left and right spicules.

Pterygodermatites parkeri, sp. n.
(Figs. 11–14)

HOSTS: (Type) *Sciurus carolinensis* Gmelin, Eastern gray squirrel; (Others) *Neotoma magister* Baird, Pennsylvania packrat; *Napeo-*

zapus insignis (Miller), woodland jumping mouse.

LOCATION: Small intestine.

LOCALITIES: (Type) Montgomery County, Virginia. (Others) Ohio, Maryland and Connecticut.

SPECIMENS: USNM Helm. Coll. Nos. 63207 (holotype: female) and 63208 (allotype: male); USDA Par. Coll. Nos. 42690, 24977, 66286, and 57254 (paratypes: female).

Description

Buccal cavity contains three equally large buccal teeth that extend anteriorly from its floor about one-third its depth. Buccal teeth consist of three main portions with middle portion of each tooth shorter than lateral portions, giving anterior edge of each tooth a concave appearance.

FEMALE (Based on 12 complete specimens and some fragments): Length 19.9–34.4 mm. Diameter at vulva 315–536. Buccal cavity round in *en face* 65–100 deep by 65–105 wide. Mouth roughly oval, slightly elongated laterally, bordered by 14–19 perioral denticles up to 13–16 long. Esophagus length 2.70–4.14 mm; anterior muscular portion 610–810 long. Nerve ring 315–449 and excretory pore 518–555 from anterior end. Cervical papillae 415–700 from anterior end at level of 6th or 7th pr. of combs. Vulva posterior to end of esophagus (except specimens from *Napeozapus* in which it is anterior), between pairs of cuticular processes numbers 30 and 32, 2.25–4.27 mm from anterior end. Eggs embryonated, length 40–50; width 30–33. Total number pairs cuticular processes 61–67 including 32–34 pairs of combs. Largest combs 147–240 by 60–120 high. Maximum spine length 97–167. Tail length 255–442.

MALE (allotype): Length 6.18 mm. Diameter: maximum 268; at base of buccal capsule 100. Buccal cavity 43 by 43. Mouth bordered by 13 perioral denticles up to 11 long. Esophagus length 1.40 mm; anterior muscular portion 400. (Excretory pore not seen.) Nerve ring 175 from anterior end between 3rd and 4th combs. Cervical papillae between 5th and 8th pairs of combs 300 from anterior end. Total of 42 pairs of combs; largest 162 long by 59 high; last one 500 from tail end. Ventral cuticular fans absent. Tail

length 243. Spicules nearly equal, curved ventrally, left 270, right 256, long. (Neither gubernaculum nor accessory piece discernible.) Ventral surface of tail and pericloacal region with 10 paired and one unpaired genital papillae, and a finely rugose papillation in the pericloacal region (Fig. 14).

Remarks

The description of the female refers to specimens from all three hosts listed above unless noted otherwise. This species includes, at least in part, those referred to as the "long-combed group" by Tiner (1948) and McPherson and Tiner (1952). Some of the females from the gray squirrel and those from the wood rat were examined by both Tiner and me. According to Tiner (1948) similar specimens have been collected in Ohio by Rausch and Tiner (1948) from the fox squirrel, *Sciurus niger*; the red squirrel *Tamiasciurus hudsonicus*; and the Eastern gray squirrel.

P. parkeri differs from both *P. coloradensis* and *P. peromysci* in the length of its combs. It further differs from *P. coloradensis* in the morphology of its buccal teeth and the size of the teeth relative to that of the buccal cavity; and from *P. peromysci* in the ratio of the lengths of the spicules. *P. parkeri* is named in honor of James C. Parker who provided me with the only known male of the species and several females from an Eastern gray squirrel (Parker, 1968).

Discussion

The possibility was considered that differences in morphology of buccal teeth were due to wearing, with the older specimens having teeth like *P. peromysci*, since the only specimens of *P. coloradensis* are smaller and perhaps younger than the others. However, this possibility was eliminated by finding that immature specimens of *P. peromysci* (Fig. 10), smaller than the *P. coloradensis* lectotype, have buccal teeth like the adult *P. peromysci* and that mature adult *P. microti* from Alaskan voles have teeth like *P. coloradensis*.

Separate keys are given herein to males and females of the seven North American species of the subgenus *Paucipectines*. It appears that only three exotic species are sufficiently similar to those described herein to require discussion: (1) *P. baicalensis* (Spasskii, Ryzhikov and Sudarikov, 1952) is similar to *P. coloradensis*, but its buccal teeth are bicuspid and smaller in relation to the size of the buccal cavity. (2) *P. sibiricensis* (Morozov, 1959) has buccal teeth similar to *P. coloradensis* and *P. microti*, but differs by having 33 pairs of prevulvar cuticular processes in the female and 44 pairs of combs and markedly unequal spicules in the male. (3) *P. zygodontomis* (Quentin, 1967) is similar to *P. peromysci*, but differs from it in the position of the nerve ring and length of esophagus of males and females and in the number of cuticular processes of the females.

McPherson and Tiner (1952) reported finding two kinds of males in a single white-footed mouse in Illinois. One had unequal spicules and conformed with the description of *P. peromysci*; the other had equal spicules 238 long. Similarly, Oswald (1958a, 1958b) reported both kinds of males in experimental infections derived from naturally infected white-footed mice collected in Ohio. Since the known geographical distribution of *P. coloradensis* now includes only Colorado and Utah, it is probable that the males with equal spicules reported from the white-footed mice were *P. parkeri* which occurs in Ohio.

The results of the present study emphasize the importance of a valid species determination of organisms used in experimental work. The identity of the nematodes used in the life cycle work of Oswald (1958a, 1958b) and Swartz (1959) on "*P. coloradensis*" is questionable since it is uncertain whether *P. coloradensis*, *P. peromysci*, *P. parkeri*, or some mixture of them was used. Questions of taxonomy may be avoided by depositing samples of organisms in museums such as the National Parasite Collection.

Acknowledgments

Key to Male *Pterygodermatites* (*Paucipectines*) Quentin, 1969 from North American Rodents*

I. Spicules equal or nearly equal in length

    A. 52 pairs of cuticular processes; left spicule 110, right 98 long ___ *P. ondatrae*

    B. 45 pairs of cuticular processes; both spicules 132 long _____ *P. microti*

    C. 42 pairs of cuticular processes; spicules 215 to 270 long

        1. top edge of buccal teeth convex; longest comb 85 _____ *P. coloradensis*

        2. top edge of buccal teeth concave; longest comb 162 _____ *P. parkeri*

II. Spicules markedly unequal, right about ½ as long as left

    A. 38 to 40 pairs of cuticular processes; knob on proximal end of left spicule twice diameter of shaft ___ *P. dipodomis*

    B. 41 pairs of cuticular processes; proximal end of left spicule only slightly wider than shaft __ *P. peromysci*

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* The male *P. onychomis* is unknown.

Key to Female *Pterygodermatites* (*Paucipectines*) Quentin, 1969 from North American Rodents

I. More than 70 pairs of cuticular processes

    A. 40 of 71 to 74 pairs of processes prevulvar; buccal cavity small, 26 deep by 40 wide _____ *P. dipodomis*

    B. 32 of 73 to 75 pairs of processes prevulvar; buccal cavity large, 90 deep by 85 wide _____ *P. ondatrae*

II. 61 to 67 pairs of cuticular processes

    A. 24 to 26 perioral denticles; 32 to 33 pairs of processes prevulvar

        1. maximum comb length 131 to 145; maximum spine length 150 to 160; ratio, comb length/spine length .87 to .91 _____ *P. microti*

        2. maximum comb length 270; maximum spine length 113; ratio, comb length/spine length 2.4 _____ *P. onychomis*

    B. 14 to 20 perioral denticles; 29 to 31 pairs of processes prevulvar; ratio, comb length/spine length 1.1 to 1.6

        1. top edge of buccal teeth convex; buccal teeth extended anteriorly about ½ depth of buccal cavity _____ *P. coloradensis*

        2. top edge of buccal teeth concave; buccal teeth extend anteriorly about ⅓ depth of buccal cavity

            a. maximum comb length 94 to 127 _____ *P. peromysci*

            b. maximum comb length 147 to 240 _____ *P. parkeri*

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Ascocotyle sexidigita sp. n. (Trematoda: Heterophyidae) with Notes on its Life Cycle*

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ABSTRACT: The new species *Ascocotyle sexidigita* is described from Southern California. Naturally infected killifish, *Fundulus parvipinnis parvipinnis* (Girard), harbored the metacercariae in the stomach and intestinal wall. Metacercariae were fed to hatchery-raised chicks and all of them served as hosts to the adult worms. The worms developed in the ceca of the chicks which they reached within twenty-four hours after feeding. They required about six days to become egg bearing. *A. sexidigita* differs from all species in the genus in the nature of the gonotyl which bears six digits supported by what appear to be calcareous deposits.

The killifish, *Fundulus parvipinnis parvipinnis* (Girard), collected in an estuary at the Bolsa Chica Gun Club near Huntington Beach, California had metacercariae embedded in the wall of the intestine. These metacercariae possessed characters of the genus *Ascocotyle* Looss, 1899. They were fed to hatchery-raised chicks and adult worms were recovered from the ceca and large intestine. Adults are described as a new species.

Living, fixed (Heidenhain's "Susa") whole mounts stained with Mayer's paracarmine, and sections stained with Mallory's Triple were studied. Measurements are expressed in millimeters unless noted otherwise.

Diagnosis

Ascocotyle sexidigita sp. n.
(Figs. 1, 4)

Body pyriform to cylindrical depending on state of contraction. Body length 0.35–0.81, av. 0.57 (measurements based on 16 egg-bearing specimens); maximum body width 0.14–0.29, av. 0.24. Integument covered with small spines arranged in quincuncial pattern.

Prepharynx about 0.05 long, opens into ventral side of oral appendage. Pharynx oval, 0.034–0.05, av. 0.045 long, 0.02–0.047, av. 0.033 wide. Esophagus length varies with degree of contraction but when extended is about 0.05 long. Prepharynx and esophagus walls contain fine longitudinal and transverse muscle fibers. Ceca relatively short, not reaching acetabular level in extended specimens. Mouth surrounded by coronet of two rows of spines of nearly equal size, each row having 29–30 spines. Spines of anterior row average about 10 μ in length, while those of the posterior row average about 9μ. Oral appendage extends to pharynx and turns back on itself a short distance, or if nearly straight, extends posterior to pharynx, has muscular sheath and cellular core, when contracted it produces an expansion of spine-bearing region, averages about 0.08 in transverse diameter. Dorsal to mouth is a protrusible lip upon whose anterior margin are openings of 14 cephalic glands retained from the metacercarial stage plus those of about 12 short tubular glands. Ventral sucker 0.038–0.056, av. 0.048 in length, 0.037–0.06, av. 0.05 in width, enclosed in ventro-genital sack. Vitellaria consist of numerous small follicles laterally

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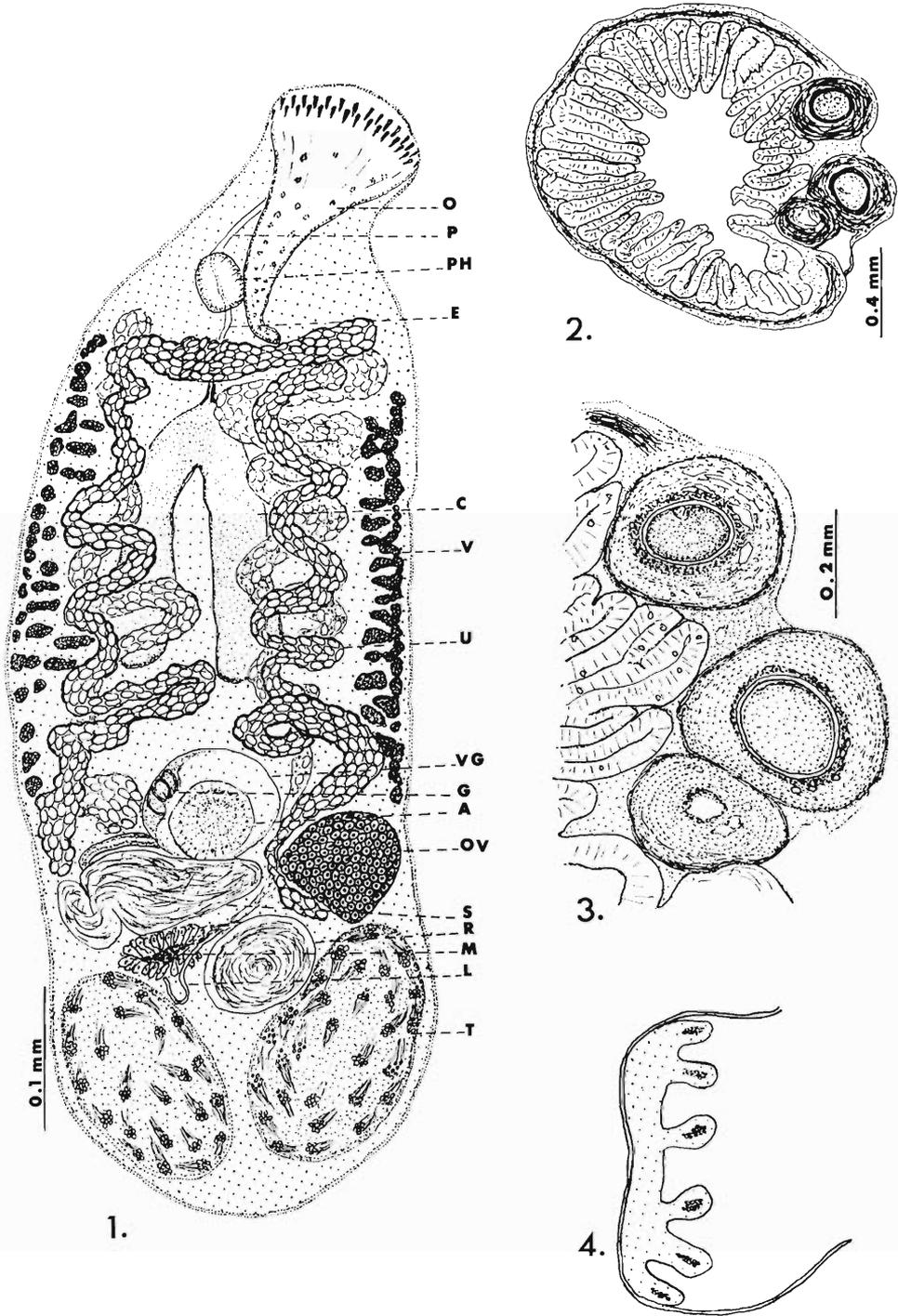
placed, extending between the ovarian level to near level of pharynx. Right and left vitelline ducts empty into slightly expanded common duct just anterior to testes. Ovary usually on right side in posterior part of body between testes and acetabulum, 0.042–0.07, av. 0.059 in length, 0.042–0.08, av. 0.066 in width. Laurer's canal present; Mehlis' gland at the anterior testes level. Close to Mehlis' gland, but more median, is a seminal receptacle usually distended with sperm. Uterus ascends nearly to pharyngeal level, passes posteriorly to near acetabular level, then anteriorly again to pharyngeal level, all on the right side of body, then passes transversely to left side of body where similar pattern is followed and terminates at ventro-genital sack, beginning loops of uterus contain many free yolk granules as well as eggs. Eggs operculate, colorless when newly formed, but turn brown with age, 15–19, av. 16 μ long, 6–9, av. 8 μ wide. Two spherical to oval testes located in posterior part of body. In living specimens, testicular surface is weakly lobed but smooths out under fixation pressure. Right testis averages 0.122 long and 0.059 wide, while left is 0.116 long and 0.06 wide. Vasa efferentia extended from testes to unite before emptying into seminal vesicle. Seminal vesicle a large sac immediately posterior to acetabulum, empties by way of muscularly-walled duct into ventro-genital sack. Ventro-genital sack encloses acetabulum and a gonotyl, the latter having six short, finger-like projections each enclosing what appears to be a calcareous deposit too poorly organized to be called a spine. Excretory pore terminal. Excretory bladder has scalloped margins and two arms that extend to ovarian level. Excretory pattern of flame cells is $2[(2+2)+(2+2)] = 16$.

Type specimen deposited in the Hancock Foundation Parasitology collection No. 691. Paratypes in W. E. Martin's collection.

Host (experimental): *Gallus domesticus.*

Habitat: Ceca and large intestine.
Locality: Southern California.

Metacercaria
(Figs. 2, 3)

The metacercariae were found in and on the stomach and intestinal walls of the killifish, *Fundulus parvipinnis parvipinnis.* Apparently the cercariae penetrate the wall to the submucosa where they encyst. The submucosa increases in thickness and a rich supply of vascular passages is provided to assure the growing encysted worm a good supply of nutrients. As the cyst enlarges, the muscularis layer is disrupted. The cysts bulge from the wall of the gut and may even produce pedunculate bodies. Cysts, including modified host tissues, are 0.154–0.35, av. 0.26 in diameter. Diameters of metacercarial cysts without host tissues are 0.084–0.252, av. 0.147 in diameter. The cyst wall, presumably secreted by the trematode, is 6–12, av. 10 μ in thickness. The worm in the cyst is bent upon itself. The excretory bladder is greatly distended with fluid that contains globules of various sizes. Small, fairly uniform in size, globules adhere to the wall of the bladder. The ceca are filled with circular, flattened concretions of fairly uniform size. The gonads are evident. Excretory pattern is similar to that of the adult.

Different sizes of metacercariae were found along the *Fundulus* digestive tract. Younger worms lacked the thick cyst wall characteristic of fully grown metacercariae. This wall stained yellow with Mallory's triple stain suggesting a possible lipid-protein material. Lenhoff, Schroeder, and Leigh (1960) examined the metacercarial cysts of *Ascocotyle* obtained from the Southern Sheepshead killifish, *Cyprinodon variegatus* Lacépède, and found them to contain hydroxyproline and be collagen-like, apparently the first report of such material in the phylum Platyhelminthes. Lumsden (1968)

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Figures 1–4. All drawings made with the aid of a camera lucida except fig. 4 which was drawn free hand from living material. 1. Adult, seven days old, dorsal view. 2. Cross section of fish intestine showing three metacercarial cysts. 3. Cyst area enlarged. 4. Gonotyl with six digits and calcareous (?) supports. Abbreviations: A, acetabulum; C, cecum; E, esophagus; G, gonotyl; L, Laurer's canal; M, Mehlis' gland; O, oral appendage; OV, ovary; P, prepharynx; PH, pharynx; R, seminal receptacle; S, seminal vesicle; T, testis; U, uterus; V, vitellaria; VG, ventrogenital sack.

described the ultrastructure of *Ascocotyle chandleri* cysts from the liver of *Cyprinodon variegatus* and stated that the cyst wall did not contain collagen though collagen fibers of host origin may be adjacent to the cyst.

Development

A series of seven hatchery-raised chicks were fed metacercariae and examined 1, 2, 3, 6, 7, 8 and 9 days after feeding. The worms reached the ceca of the host within the first day. They were firmly attached and had host cecal material in their ceca. No eggs were produced. At two days after feeding there was some growth; there were sperm in the seminal vesicle, but no eggs were produced. The same held true for three-day old worms. Eggs were present at six days and thereafter. At nine days some of the worms were found in the large intestine, possibly the beginning of elimination of the worms from host. Schroeder and Leigh (1965) reported that *Ascocotyle pachycystis* matured in the ceca of chicks, but that they were eliminated after a few days.

Discussion

The genus *Ascocotyle* was proposed by Looss (1899) with the type species *A. coelostoma* (Looss, 1896) which he had found in the ceca and large intestine of a pelican collected in Egypt. Most of the additional species of this genus are listed by Hutton and Sogandares-Bernal (1958). Species of the genus described since 1958 include *A. ampullacea* Miller and Harkema, 1962; *A. chandleri* Lumsden, 1963; and *A. pachycystis* Schroeder and Leigh, 1965. The gonotyl of *A. sexidigita* with six digits supported by deposits distinguishes this species from all others in the genus. In circumoral spine count *A. sexidigita* is closest to *A. leighi* Burton, 1956 which has 48–52 spines. However, in *A. sexidigita* the vitellaria are mainly preacetabular, while in *A. leighi* they are mainly postacetabular. Also, the metacercariae of *A. sexidigita* are found in the intestinal wall and not in the heart of the fish host, while *A. leighi* metacercariae are found only in the heart. *A. pachycystis* has 44–58 circumoral spines, but the vitellaria are distributed as in *A. leighi*.

The life cycle of *A. pachycystis* was worked out by Schroeder and Leigh (1965). They reported the raccoon as the definitive host in southern Florida, the fish, *Poecilia* (*Molliensia*) *latipinna* (Le Sueur, 1821) Rosen, 1963 as the second intermediate host, and the snail, *Littoradinops tenuipes* (Couper) as an experimental first intermediate host. They described the cercaria as ocellate and parapleurolophocercous with the fins extending the full length of the tail. To date we have not found such a cercaria in the region where the fish are infected. They further stated that there were four penetration glands on each side of the body, but we suspect that only one bundle of gland ducts was counted on each side. We found seven on each side in the metacercariae and adults of *A. sexidigita*. It has been demonstrated that many related heterophyid cercariae have a 3-4-4-3 arrangement of cephalic or penetration gland ducts (Martin, 1950, 1958, Martin and Kuntz, 1955).

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Description of Entaphelenchidae fam. n., *Roveaphelenchus jonesi* gen. n., sp. n., and *Sheraphelenchus entomophagus* gen. n., sp. n. (Nematoda : Aphelenchoidea)

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ABSTRACT: Two new nematode genera and one new family are established in the Aphelenchoidea with descriptions of two new species. *Roveaphelenchus jonesi* gen. n., sp. n., a parasite of the rove beetle, *Aleochara tristis*, is placed, together with the genera *Entaphelenchus, Peraphelenchus,* and *Praecocilenchus*, into the Entaphelenchidae fam. n. *Sheraphelenchus entomophagus* gen. n., sp. n., is described from two nematode populations, one from decaying oranges and the other associated with nitidulid beetles. This genus is placed temporarily in the Aphelenchoididae.

The nematode superfamily Aphelenchoidea contains four ecological groupings, which may be classified as plant parasites, fungus feeders, predators, and obligate parasites of insects. Phenetically, the superfamily is recognized by the dorsal esophageal gland emptying into the lumen of the esophagus within the median muscular bulb anterior to the crescentic valve plates. Also, though rudimentary in the genus *Aphelenchus*, the spicule is rosethorn shaped. Recently I have studied two interesting populations of aphelenchs which are described herein. They are of particular interest because of their unique morphology and value in understanding the phylogeny of this major group of nematodes. A collection of nematode specimens, parasitic in the body cavity of the rove beetle, *Aleochara tristis* Gravenhorst, was received from Dr. Calvin M. Jones, Lincoln, Nebraska. This predaceous rove beetle was imported from Europe to control the face fly, *Musca autumnalis* De Geer.

The literature dealing with the endoparasitic aphelenchoid parasites of insects is rather brief, and began when Wachek (1955) described two insect-parasitic genera, *Entaphelenchus* and *Peraphelenchus*. Nickle (1967) moved the sphaerulariids out of the Aphelenchoidea and Poinar (1969) described *Praecocilenchus*, an aphelenchoid genus, in which the young reach sexual maturity within the adult parasitic female. After studying specimens of these four genera, a new family category is proposed here to include all the described aphelenchoid insect parasites.

Entaphelenchidae fam. n.

DIAGNOSIS: Aphelenchoidea. With at least three distinct adult forms, including a vermiform male and female, and a swollen endoparasitic female. Stylet present, with or without small basal flanges. Esophagus with a large median bulb and overlapping glands. Male without caudal alae. Spicules rosethorn shaped. Gubernaculum absent. Obligate insect parasites.

TYPE GENUS: *Entaphelenchus* Wachek, 1955.

OTHER GENERA: *Peraphelenchus* Wachek, 1955, *Praecocilenchus* Poinar, 1969, *Roveaphelenchus* gen. n.

This family Entaphelenchidae can be separated from the Aphelenchidae because it is parasitic in insects and lacks a gubernaculum, caudal alae, and caudal rays. It can be distinguished from the Aphelenchoididae on the basis that the Entaphelenchidae is parasitic in insects, and the female is swollen and found in the body cavity of insects. The Entaphelenchidae of the Aphelenchoidea has a status similar to that of the Sphaerulariidae of the Tylenchoidea; both are ecologically and morphologically distinct from other families.

Genus: *Roveaphelenchus* gen. n.

DEFINITION: Entaphelenchidae. With three adult forms found in body cavity of the adult rove beetle. Stylet without knobs. Males die with only tail in tight coil, not corkscrew shaped. Spicules not fused. Caudal alae and gubernaculum absent. Median esophageal bulb may be constricted, violin-like. Vermiform female with only a single larva or single embryo
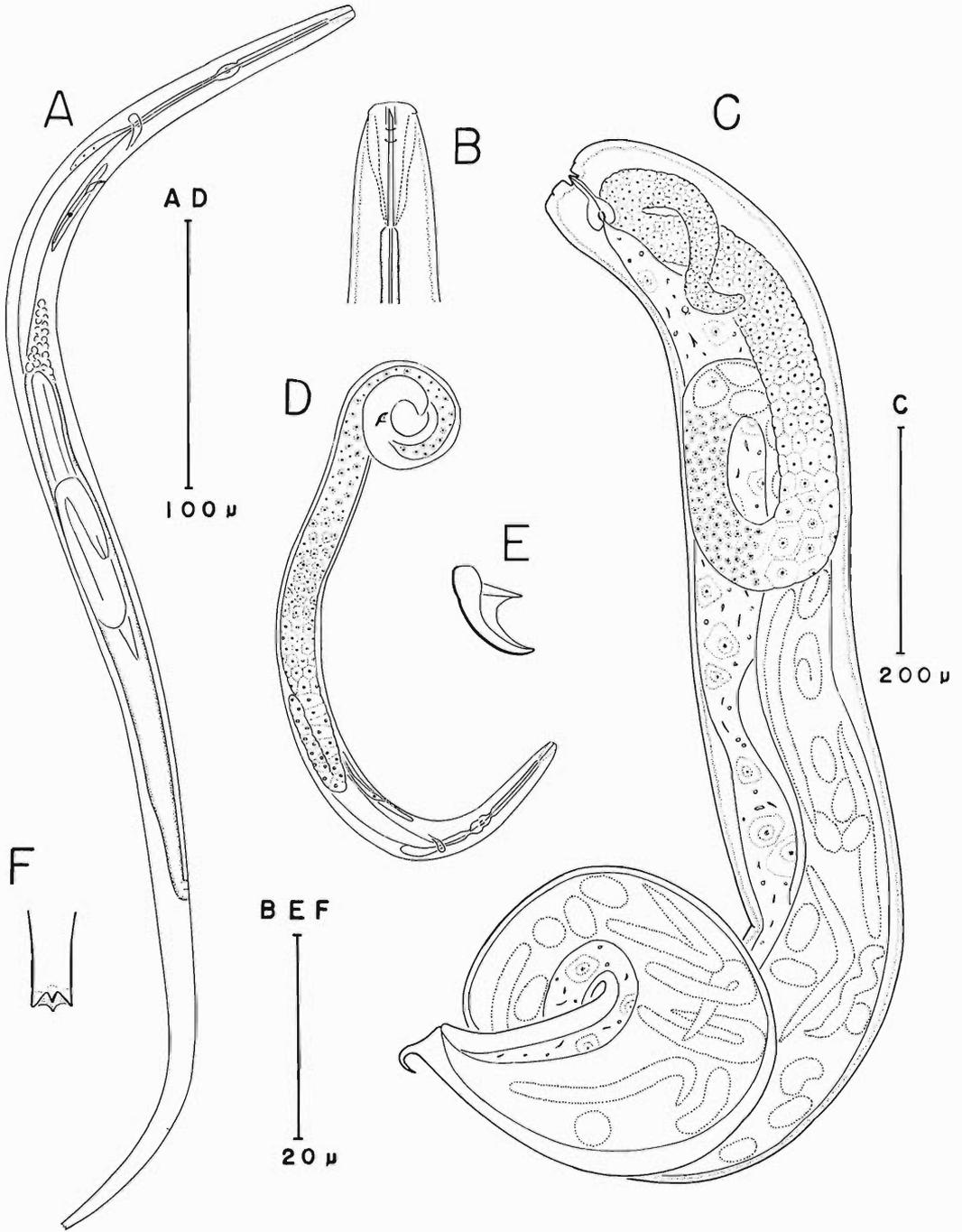
in uterus, postuterine sac absent. Large adult parasitic female ovoviviparous.

TYPE SPECIES: *Roveaphelenchus jonesi* sp. n.

Roveaphelenchus jonesi gen. n., sp. n.
(Fig. 1, A–F)

FEMALES (5): L = 0.542 mm (0.508–0.583); W = 19.7 μ (19.0–20.6); a = 27.5 (26.0–27.9); b = 5.7 (5.4–5.9); c = 9.9 (8.6–11.1); V = 71.6% (67.7–72.5); stylet = 12.6 μ (11.7–13.4).

HOLOTYPE ♀: L = 0.540 mm; W = 19.3 μ; a = 27.9; b = 5.7; c = 10.3; V = 67.7%; stylet = 13.4 μ.

MALES (5): L = 0.354 mm (0.341–0.364); W = 12.8 μ (10.9–14.7); a = 28.1 (24.2–32.5); b = 5.1 (4.7–5.2); c = 13.2 (10.7–17.2); spicule L = 11.6 μ (10.9–12.6); stylet = 10.8 μ (10.1–11.8).

ALLOTYPE MALE: L = 0.354 mm; W = 14.3 μ; a = 25.0; b = 5.1; c = 13.1; spicule L = 11.3 μ; stylet = 11.1 μ.

ADULT PARASITIC FEMALES (5): L = 1.76 mm (1.54–2.22); W = 0.156 mm (0.130–0.182); a = 11.2 (9.9–11.8); V = 96.4% (95.9–96.8); stylet = 12.7 μ (11.3–14.7).

Description

Three adult forms occur in the body cavity of the adult beetle along with numerous larvae.

MALE: Cuticle with fine annulation. Cephalic framework faint. Lips not set off. Excretory canal well developed. Testis single, flexed, extending almost to end of esophageal glands. Spicule with prominent pointed rostrum, apex continuing less strongly along the curve of the shaft (Fig. 1E). Tail bluntly rounded.

FEMALE: Body tends to relax in an arcuate or S-shape. Cuticle with fine annulation. Cephalic framework and stylet (Fig. 1B) better developed than in male. Excretory canal well developed. Gonad with small degenerate ovary and with single larva in uterus (Fig. 1A). Vulva not protruding. Tail attenuated, with 4 points on tip.

ADULT PARASITIC FEMALE: Swollen, milky white. Median esophageal bulb large, overlapping glands degenerate. Gonad almost fills body, often doubly flexed. Vulva far posterior. Tail tip acute, conical, often bent dorsad.

HOLOTYPE: Female, collected by C. M. Jones, 25 August 1968. Slide T-147t. USDA Nematode Collection, Beltsville, Maryland.

ALLOTYPE: Male, same data as holotype. Slide T-148t. USDA Nematode Collection, Beltsville, Maryland.

PARATYPES: Several males, females, and adult parasitic females deposited in the USDA Nematode Collection, Beltsville, Maryland, and the University of California Nematode Survey Collection, Davis, California, U.S.A.

TYPE HOST: *Aleochara tristis* Gravenhorst.

TYPE LOCALITY: Lincoln, Nebraska, U.S.A.

This species is named after Dr. Calvin M. Jones, Lincoln, Nebraska, who first found this nematode.

Genus *Sheraphelenchus* gen. n.

DEFINITION: Aphelenchoididae. Stylet without knobs. Esophageal valve in center of median bulb. Anterior part of gonad in both sexes usually with three cells across. Tail without mucrons. Male tail characteristically narrows abruptly just posterior to spicule, then attenuates conically to rounded terminus. Spicules partially fused, with curved narrow rostrum, apex continuing less strongly along the curve of the shaft (Fig. 2E). One anal pair and two postanal pairs of papillae. Caudal alae and gubernaculum absent. Female tail attenuates conically posterior to vulva. Uterus often contains two or more eggs with embryos. Postuterine sac absent.

TYPE SPECIES: *Sheraphelenchus entomophagus* sp. n.

The genus has no known close relatives, but is near *Parasitaphelenchus* because of the fused spicules. It differs from *Parasitaphelenchus* in the shape of the spicules, the length of postuterine sac, and the shape of the tail in both sexes.

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Figure 1 A–F. *Roveaphelenchus jonesi* gen. n., sp. n., parasitic in the rove beetle, *Aleochara tristis*. A. Female, full body, lateral view. B. Female, anterior end, lateral view. C. Adult Parasitic Female, full body, lateral view. D. Male, full body, lateral view, showing coiled tail. E. Spicule, lateral view. F. Tail tip of sexual female.
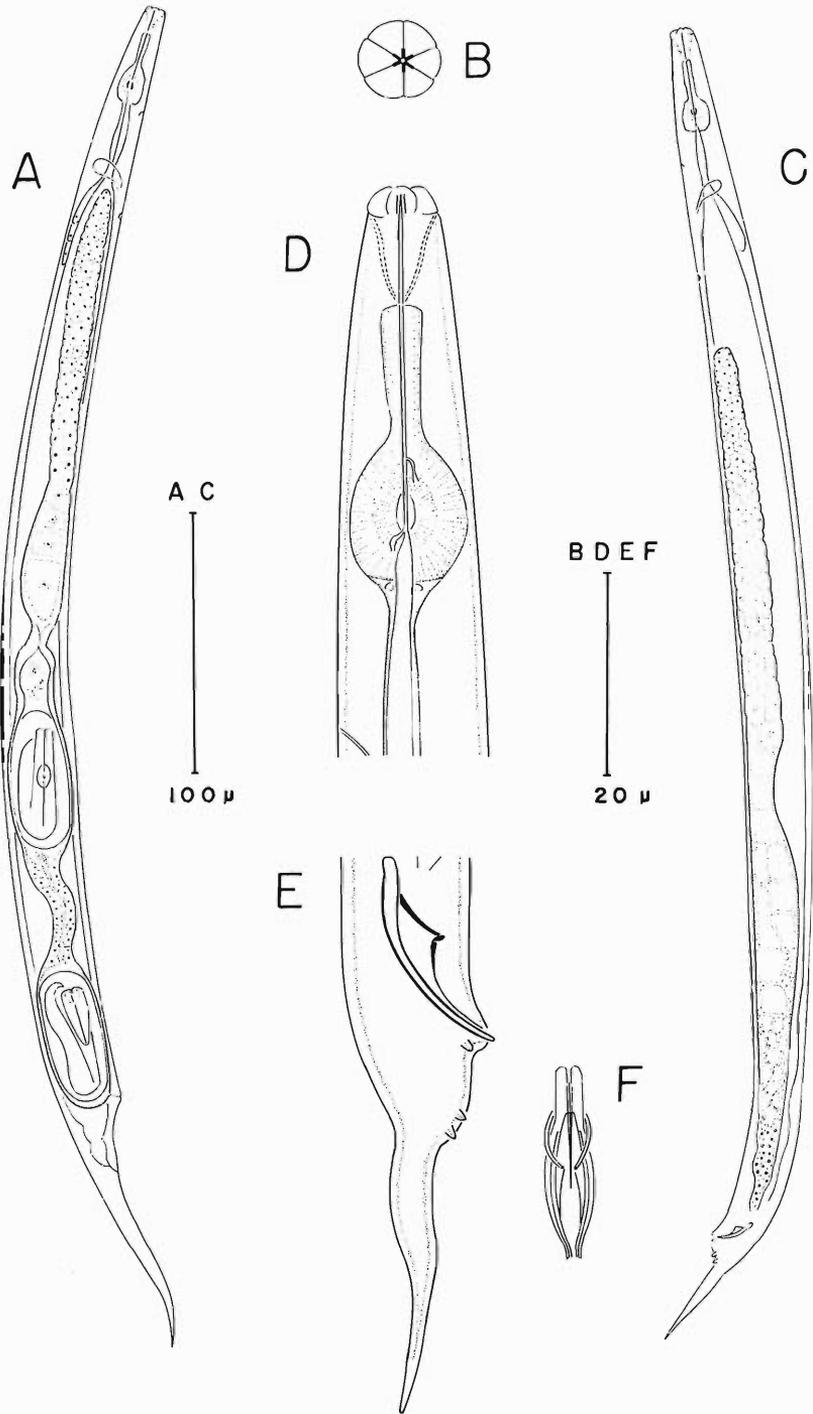
Professor S. A. Sher, Riverside, California, collected a population of this nematode in 1952 from decaying oranges in Pahoa, Hawaii. In addition, the author has received this nematode, though smaller in size, from a fig culture of the nitidulid beetle, *Haptonchus luteolus* (Erichson) from Dr. W. R. Kellen, Fresno, California. The nematode has a phoretic relationship with nitidulid beetles and is probably mycophagous. Originally the Kellen nematode population came from Brazo County, Texas, from the nitidulids *Carpophilus* (*Urophorus*) *humeralis* (Fabricius) and *Carpophilus mutilatus* Erichson.

This genus is named after Dr. S. A. Sher, Riverside, California, who was first to collect this nematode and also in recognition of his outstanding contributions to the systematics of the Tylenchoidea.

Sheraphelenchus entomophagus
gen. n., sp. n.
(Fig. 2, A–F)

MALES (12): L = 0.682 mm (0.478–1.186); W = 28.4 μ (21.4–53.3); a = 24.1 (20.5–27.3); b = 8.6 (7.0–10.3); c = 14.4 (10.7–22.6); spicule L = 21.9 μ (18.9–25.2); stylet = 12.3 μ (11.3–13.0); excretory pore = 59.2 μ (39.9–71.4); hemizonid = 104.4 μ (80.2–130.2).

HOLOTYPE MALE: L = 0.706 mm; W = 27.3 μ; a = 25.9; b = 8.8; c = 16.8; spicule L = 21.5 μ; stylet = 12.6 μ; excretory pore = 65.9 μ; hemizonid = 101.6 μ.

FEMALES (12): L = 0.697 mm (0.532–0.975); W = 29.9 μ (22.8–45.4); a = 23.3 (19.7–26.3); b = 9.6 (7.8–12.2); c = 8.7 (7.5–10.2); V = 82.9% (77.8–88.1); stylet = 12.0 μ (11.3–13.0); excretory pore = 57.2 μ (39.9–72.7); hemizonid = 98.9 μ (86.1–113.4).

ALLOTYPE FEMALE: L = 0.706 mm; W = 29.4 μ; a = 24.0; b = 9.9; c = 9.3; V = 84.0%; stylet = 13.0 μ; excretory pore = 64.7 μ; hemizonid = 99.5 μ.

Description

Both sexes slightly arcuate when relaxed. Excretory pore position variable but always posterior to median esophageal bulb and about equidistant between hemizonid and front end of nematode.

MALE: Cuticle with fine annulation. Six lips, not set off. Stylet present, without basal flanges. Testis single, outstretched, not flexed.

FEMALE: Cuticle with fine annulation. Stylet well developed, without basal flanges or knobs. Gonad single, outstretched, not flexed, usually with two or more ova in uterus. Vulva lips protruding. Postvulval sac absent.

HOLOTYPE: Male, collected by S. A. Sher, 29 November 1952. Slide T-149t. USDA Nematode Collection, Beltsville, Maryland.

ALLOTYPE: Female, same data as holotype. Slide T-150t. USDA Nematode Collection, Beltsville, Maryland.

PARATYPES: Several males and females deposited in USDA Nematode Collection, Beltsville, Maryland, University of California Nematode Survey Collection, Davis, California, University of California Survey Collection, Riverside, California, USA, and Canadian National Collection, Ottawa, Canada.

TYPE HOST: Found associated with decaying oranges.

TYPE LOCALITY: Pahoa, Hawaii, USA.

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Figure 2 A–F. *Sheraphelenchus entomophagus* **gen. n., sp. n. Drawing of the Kellen fig cultured population, phoretic on *Haptonchus luteolus*. A. Female, full body, lateral view. B. An *en face* view of female. C. Male, full body, lateral view. D. Female, anterior end, lateral view. E. Male tail, lateral view, showing spicule. F. Spicule, ventral view.**

Edlintonia ptychocheila gen. n., sp. n. (Cestoidea : Capingentidae) and Other Caryophyllid Tapeworms from Cyprinid Fishes of North America

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ABSTRACT: Schell's caryophyllid is described from the intestine of the freshwater fish *Ptychocheilus oregonense* (Richardson) and *Mylocheilus caurinus* (Richardson) from British Columbia, Canada and Idaho, United States. *Edlintonia* is morphologically close to *Adenoscolex* Fotedar, of Kashmir, but has an external seminal vesicle absent in the latter genus. A comparative table of diagnostic features and a simplified key to genera of the Capingentidae are presented. All other records of caryophyllids from minnows (Cyprinidas) in North America are tabulated. Other North American Capingentidae are illustrated.

The family Capingentidae Wardle and McLeod 1952 (= Pseudolytocestinae Hunter, 1929; Capingentidae Hunter, 1930) (Cestoidea: Caryophyllidea) contains seven genera and eight species: four from the oriental, three from the nearctic, and one from the palearctic region. In these regions the definitive hosts are primary-division freshwater ostariophysan fishes of the families Cyprinidae (oriental, palearctic), Catostomidae (nearctic) and Clariidae (oriental). This report presents a description of a new genus and species of capingentid cestode, the first from a cyprinid fish in the nearctic.

Comparative material in the family Capingentidae included many examples of *Capingens singularis* Hunter, 1927 (Fig. 8) and *Spartoides wardi* Hunter, 1929 (Fig. 10); the type of *Pseudolytocestus differtus* Hunter, 1929 (Fig. 9) and *Adenoscolex oreini* Fotedar, 1958; and a single specimen of *Breviscolex orientalis* Kulakovskaja, 1962. Examples of *Pseudocaryophyllaeus* Gupta, 1961 and *Capingentoides* Gupta, 1961, could not be obtained.

The following description is based on 19 gravid specimens collected by Dr. Stewart C. Schell, University of Idaho, and on 18 additional specimens from the slide collection of Dr. J. Adams, University of British Columbia. Whole mounts were stained with paracarmine; paraffin sections were stained with hematoxylin and eosin. Measurements are in microns unless

otherwise indicated; drawings were made with the aid of a microprojector.

Order Caryophyllidea Van Beneden (in Carus, 1863) Family Capingentidae Wardle and McLeod, 1952
Edlintonia gen. n.

GENERIC DIAGNOSIS: Capingentidae. Scolex cuneiform, lacking loculi or bothria. Cirrus opening separately from uterovaginal canal. Ovary H-shaped. Uterus not extending anteriorly beyond cirrus sac. Preovarian vitellaria median and lateral. Postovarian vitellaria present. External seminal vesicle present. Type species: *Edlintonia ptychocheila.*

Remarks

Edlintonia can easily be separated from the other genera of nearctic Capingentidae on the basis of scolex morphology, cirrus sac-uterus relationships, and the presence of postovarian vitellaria (Table 1, Figs. 1–3). *Edlintonia* most closely resembles *Adenoscolex* Fotedar, 1958, both having the same number of gonopores, and similar scolexes, vitelline gland distribution, and cirrus sac-uterus relationships. It differs from *Adenoscolex* and *Breviscolex* Kulakovskaja, 1962, in having an external seminal vesicle. The relationship of the new genus to others in the family Capingentidae is expressed in the key below.

The name *Edlintonia* is proposed in honor of Dr. Edwin Linton, American helminthologist, who described the first caryophyllid

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[1] Preliminary work was done while the author was on sabbatical leave at the University of Tennessee, Knoxville.

from North America; the generic name is feminine in gender.

Edlintonia ptychocheila sp. n.
Schell's caryophyllid
(Figs. 1–7)

SPECIFIC DIAGNOSIS (37 observed: 10 gravid individuals measured except where otherwise indicated; four sectioned): Gravid worms 21.3 (15–29) mm long, 1.6 (1–2) mm wide at male gonopore (N = 11). Total length 8.5–16.3 times length of neck and scolex. Scolex slightly wider than body, 1.9 (1.3–3) mm wide. Constricted neck absent. Outer longitudinal muscles poorly developed; inner longitudinal muscles in small fascicles, irregularly spaced. Testes begin 1.4–2.2 mm from tip of scolex, extend to cirrus pouch, number 422 (263–568), average 225 (100–350) in diameter (N = 60, 10 from each of six worms). Vas deferens short, in small area between testes and cirrus pouch. External seminal vesicle small, narrow, twisted. Cirrus pouch anterior to ovary, 570 (440–690) in diameter. Preovarian vitellaria begin 1.5–2.2 mm from tip of scolex, extend to cirrus or uterus, in lateral and median regions, average 168 (75–225) in diameter (N = 60, 10 from each of six worms). Postovarian cluster of vitelline follicles well developed, not touching ovary. Ovary H-shaped, with narrow commissure; follicular arms 1.2 (0.64–1.62) mm long. No definite number of osmoregulatory canals at midregion of body. Seminal receptacle poorly developed. Eggs operculate, shell smooth, 66 (63–67.5) long by 47.6 (45–49.5) wide (five measured in water); operculum 14.8 (13.5–15.7) wide; 7–9 vitelline cells per egg. Eggs undeveloped when shed.

DEFINITIVE HOSTS: (Cyprinidae): Columbia River chub, *Mylocheilus caurinus* (Richardson). BRITISH COLUMBIA: Kathlyn Lake near Smithers, on highway 16 (Skeena River drainage). This record was originally reported as *Caryophyllaeus terebrans* (Linton) by Bangham and Adams (1954). Squawfish, *Ptychocheilus oregonense* (Richardson), type host. IDAHO: Bonner Co., Clark Fork River (Columbia River drainage), type locality, collected by Stewart C. Schell.

HABITAT: Intestinal swelling (stomach; Squawfish); attached.

TYPE SPECIMENS: Holotype, USNM Helm. Coll. No. 70513. Paratypes (2), USNM Helm. Coll. No. 70514; Paratypes (1) British Museum (Natural History) Helm. Coll. No. 1969.6.27.1.

Supplementary material (7 slides) consisting of sections include: USNM Helm. Coll. Nos. 70515, 70516, and British Museum (Natural History) Helm. Coll. Nos. 1969.6.27.2 and 3.

Remarks

The external seminal vesicle, though readily visible on sections can not be easily seen on whole mounts. The loosely coiled, thin walled vas deferens constricts to 20–25 μ and bends sharply before expanding abruptly to 45–50 μ to form the thicker walled external seminal vesicle that twists through a half turn before entering through the dorsal surface of the cirrus sac. Unlike the external seminal vesicle of *Pseudolylocestus differtus*, it is not greatly expanded but rather narrow and twisted, distinguished from the vas deferens by its thicker walls and circular layer of muscles (Fig. 5).

Near the ovary of some specimens there was an additional transverse duct connecting the two lateral vitelline ducts (Fig. 3); normally there is only one transverse duct.

The great variation in testes number on gravid specimens (263–568) may be more a reflection of testes loss (degeneration?) through senescence rather than normal variation. Testes normally extend to the cirrus sac in a continuous and homogeneous group (Fig. 1); however, in a single individual (15 mm long) having 263 testes (Fig. 7a) they became progressively less numerous in the posterior one-half of the testicular field, extending only to a point well anterior to the cirrus sac. In another individual, approximately the same size (15.5 mm) and having the normal testes distribution, there were 469 testes. Another specimen, slightly smaller (Fig. 7b) and having only 48 testes (confined to the anterior one-half of the testicular field), had few preovarian vitellaria and no postovarian vitelline follicles or ovarian structures could be found on these whole mounts, even under oil immersion.

Little is known of the growth pattern of caryophyllideans, however, the above observations on testes distribution and number suggest that in *E. ptychocheila* senescense is characterized by a degeneration of the testes, beginning in the posterior part of the testicular field,
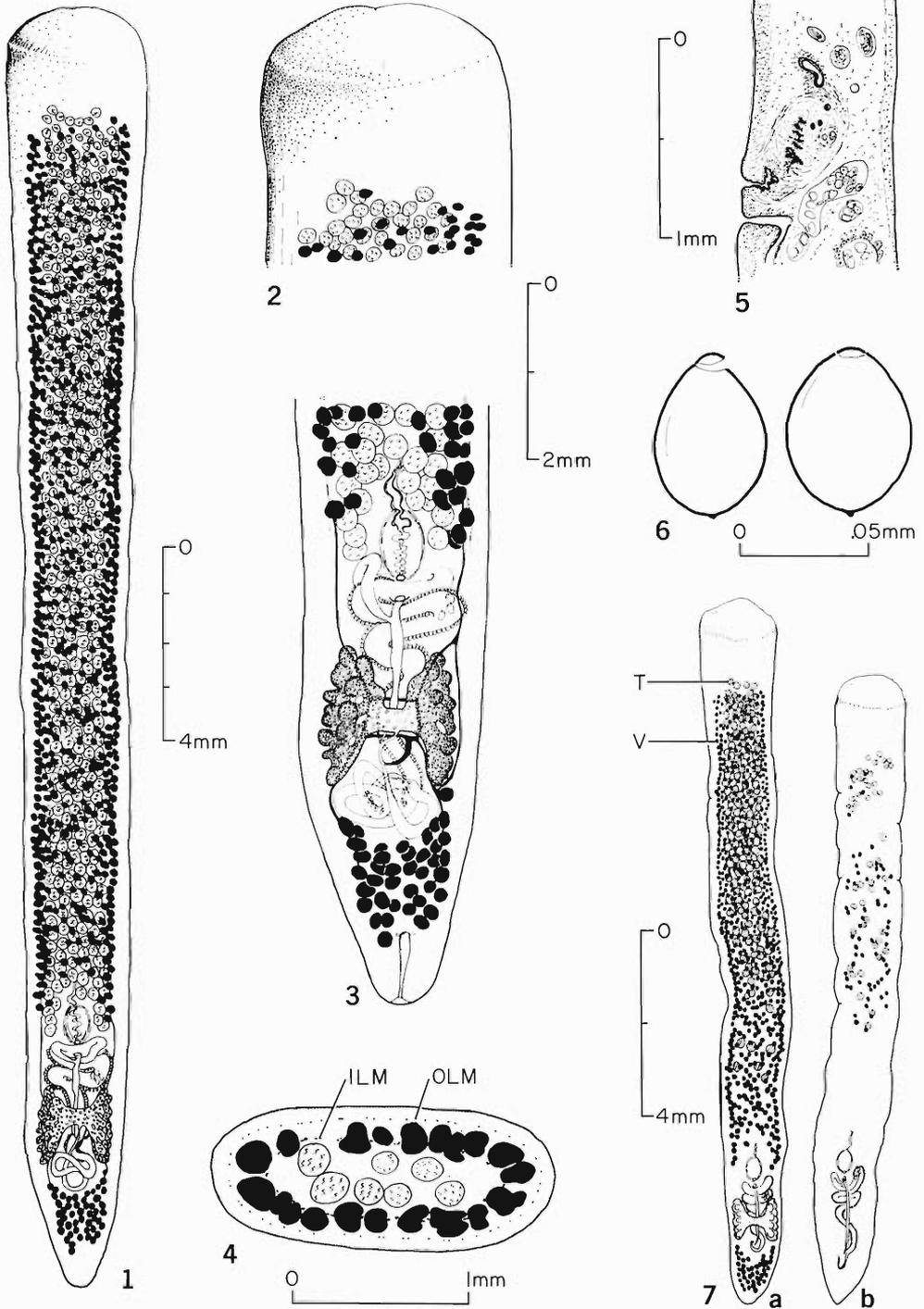
followed by degeneration of the pre- and post-ovarian vitelline follicles and the ovary. In the absence of a larger sample an alternate interpretation of the above observations may be that the two specimens, the smallest gravid worms observed, are simply extreme examples of intraspecific variation.

According to Hoffman (1967) the only other cestodes recorded from *P. oregonense* are *Eubothrium salvelini* (Schrank, 1790), the larva of *Ligula intestinalis* (Linn., 1758) (Pseudophyllidea), and *Proteocephalus ptychocheilus* Faust, 1920 (Proteocephalidea). The first two are also recorded for *M. caurinus*.

Discussion

From Table I it is clear that the Capingentidae are widely distributed in the world. In North America there are three genera, each with a single species (Figs. 8–10). These genera are remarkably distinct from each other, having few characteristics in common save the relationship of the inner longitudinal musculature (ILM) to the vitellaria. A fourth, but undescribed species (from California) is the "*Glaridacris* sp." of Haderlie (1953), described from a single whole mount (USNM Helm. Coll. No. 59511); the ILM-vitellaria relationships of this species is similar to that of another having only lateral vitellaria, *Spartoides wardi*. Haderlie's "*Glaridacris* sp." differs so much from other North American Capingentidae in morphology and host relationships (being found in a *Catostomus* sucker rather than *Ictiobus* or *Carpiodes*) that additional material should be sought in the Sacramento sucker, *Catostomus occidentalis* Ayres, its original host.

The host-parasite relationships of *E. ptychocheila* are different from any other member of the Capingentidae in North America. Not only is it found in minnows (Cyprinidae), while all other nearctic capingentid tapeworms are from suckers (Catostomidae), but one of the hosts, the squawfish, is distinctly predaceous in its food habits (Clemens and Munro, 1934;

Schultz, 1941; Thompson, 1959; Weisel, 1962). Generally, caryophyllidean definitive hosts are planktonic, phytophagous, or insectivorous feeders, usually feeding near or on the bottom. According to Thompson (1959) squawfish less than 8 inches in total length feed predominantly on insects; from 8–11 inches they feed mostly on insects and fish; over 11 inches, mostly on fish and crayfish. In the present study *E. ptychocheila* was found only in the larger fish over 10 inches long. If the life cycle of this tapeworm involves a benthic oligochaete, as is the case for the known cycles, then it is difficult to explain its occurrence in a fish described as ". . . pikelike in general habits, feeding voraciously on other aquatic animals, mostly fish . . ." (Schultz, 1941:31). Either the life cycle of *E. ptychocheila* does not involve an oligochaete intermediate host, or the squawfish feeds on benthic organisms, including tubificids, to a greater degree than hitherto suspected. Tubificids, because of their small size and soft body parts, are rapidly digested, remaining in the intestinal swelling for a short period (Kennedy, 1969); thus they are generally not reported in food studies. Another, though remote possibility, is that a paratenic host may be involved, although none has been reported in any caryophyllidean cycle.

All of the cestodes from *P. oregonense* were found in the intestinal swelling ("stomach"); a true stomach, with gastric glands and a pyloric spincter, is absent, as with other minnows and the suckers (Weisel, 1962). Most caryophyllideans are found posterior to the intestinal swelling; a notable exception is *Capingens singularis* which appears restricted to the intestinal swelling of *Carpiodes* and *Ictiobus* (Catostomidae). All other capingentid cestodes inhabit the intestine posterior to the intestinal swelling.

The food habits and biology of the second cyprinid host, the peamouth chub, *Mylocheilus caurinus*, are poorly known. One significant aspect of its biology is that this minnow has

Figures 1–7. *Edlintonia ptychocheila* gen. n., sp. n., Schell's caryophyllid. 1. Holotype. 2. Scolex. 3. Posterior end. 4. X-section through center of body. 5. Midsagittal section through gonopores. 6. Outlines of eggs. 7a, b. Gravid specimens exhibiting abnormal testes (a, b) and vitellaria distribution (b). Abbreviations: ILM, inner longitudinal muscles: OLM, outer longitudinal muscles; T, testis; V, vitellarium.
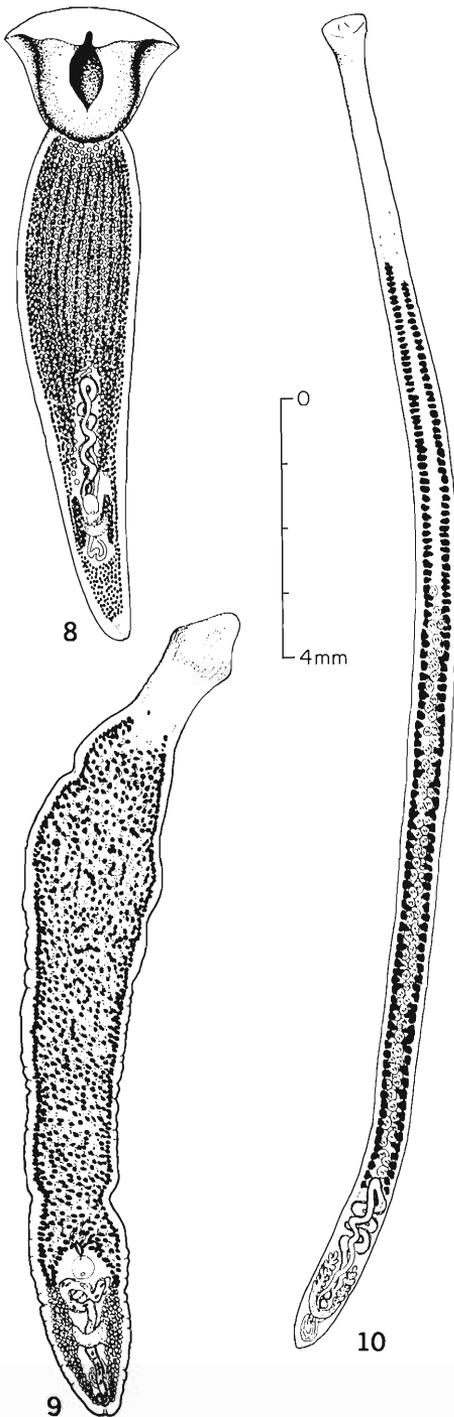
Table I. Comparison of genera of the Capingentidae.

Genus	Scolex	Gono-pore no.	Uterus anterior to cirrus	External seminal vesicle	Ovary shape	Post-ovarian vitellaria (POV)	Pre-ovarian vitellaria	Distribution and Host family(ies)
Capingens Hunter, 1927	two bothria	2	yes	present	H	present	lateral & medial, continuous with postovarian vitellaria	North America: Catostomidae
Pseudolytocestus Hunter, 1929	conical, unspecialized	2	no	present	H	absent	lateral & medial	North America: Catostomidae India : Clariidae
Spartoides Hunter, 1929	cuneiform, 3 pairs loculi	2	yes	present	U	absent	lateral	North America: Catostomidae
Adenoscolex Fotedar, 1958	cuneiform, no loculi	2	no	"apar-ently"[4] absent	H	present	lateral & medial, not continuous with postovarian vitellaria	Kashmir : Cyprinidae
Pseudocaryophyllaeus Gupta, 1961	"oval or cone shaped, truncated anteriorly:[1]	2	no	absent	H	absent	lateral & medial	India : Clariidae
Capingentoides Gupta, 1961	"oval or globular and truncated anteriorly"[1]	1	no	absent	H or transverse band	present	lateral & medial, con-tinuous (?) with postovarian vitellaria	India : Clariidae
Breviscolex Kulakovs-kaja, 1962	"short, simple . . smooth anterior margin"[2]	?	no	absent	"butter-fly"[2] "bowtie"[3]	present	lateral & medial, con-tinuous with postovarian vitellaria	Russia : Cypri-nidae
Edlintonia (this paper)	cuneiform, no loculi	2	no	present	H	present	lateral & medial, not continuous with postovarian vitellaria	North America: Cyprinidae

[1] From: Gupta, 1961.
[2] From: Dubinina, 1964.
[3] From: Kulakovskaja, 1962.
[4] From: Fotedar, 1958.

the ability to enter the sea (Jordan and Ever-man, 1896; Carl, Clemens and Lindsey, 1967). This ability may be of great zoogeographical importance in helping to explain the occur-rence of capingentid cestodes in Asian cyprin-ids (*Breviscolex* from the Amur River Basin) and *Edlintonia* from northwestern North America. *Catostomus catostomus* (Forster), longnose sucker, is the only other cary-ophyllidean host from the western part of

North America that is able to tolerate brackish conditions (Walters, 1955). *Hemibarbus maculatus* Bleeker, *Chilogobio czerskii* Berg, and *Oreinus sinuatus* (Heckel), cyprinid hosts of *Breviscolex* in the Amur basin of the USSR and *Adenoscolex* in Kashmir appear to be re-stricted to freshwater. According to Darling-ton (1957) the family Clariidae, which includes the only other capingentid host, *Clarias batrachus* (L.), is restricted to fresh-

water. This last host has recently been introduced into North America (Florida) where it is rapidly expanding its range (Idyll, 1969).

It has long been established that catostomid fish are the principal hosts of caryophyllids in North America (Hunter, 1930). In Europe, where catostomids are absent, cyprinids are the principal hosts of these tapeworms. As Table II illustrates, the cyprinids are also important hosts in the nearctic. Two significant facts are evident from this table. One, an introduced species, carp, has the richest fauna. Caryophyllids have not been reported, however, from some other introduced cyprinids such as goldfish, *Carassius auratus* (L.), tench, *Tinca tinca* (L.) or rudd, *Scardinius erythrophthalmus* (L.), normally common hosts in Europe. Except for *A. sieboldi*, none of the other carp cestode species has been reported from Europe or Asia where the carp has been established for a much larger time. Furthermore, the other cestodes in the carp exhibit a high host specificity, with only *Khawia iowensis* being reported, though rarely, from another host (*Ictiobus*; Catostomidae). These facts make it difficult to explain how the carp introduced into North America became a host for *Archigetes iowensis*, *Atractolytocestus huronensis* and *K. iowensis*, cestodes that are apparently absent in the European and Asian fauna and not normally found in native palearctic fishes. A second fact is that only one species, *Caryophyllaeus terebrans*, also commonly occurs in catostomid fish. Unfortunately, this record of Linton (1941) is clearly a misidentification, as restudy of the original material reveals. Unlike *C. terebrans*, the Linton cestodes (USNM Helm. Coll. No. 8852; two gravid individuals) are small (4 and 6 mm long), have between 25 and 30 testes, possess an unusually large number of postovarian vitelline follicles, and have an unspecialized scolex; serial sections and additional material are de-

←

Figures 8–10. North American Capingentidae. 8. *Capingens singularis* **Hunter, 1927, from** *Ictiobus bubalus* **(Raf.), Lake Texoma, Oklahoma, USA. 9.** *Pseudolytocestus differtus* **Hunter, 1929, holotype, USNM Helm. Coll. No. 51203, from** *I. bubalus*, **Tallahatchie River, Mississippi. 10.** *Spartoides wardi* **Hunter, 1929, from** *Carpiodes carpio* **(Raf.) Lake Texoma, Oklahoma, USA.**

Table II. Annotated List of the Caryophyllidea (Cestoida) Recorded from Cyprinidae in North America.

Acrocheilus alutaceum Agassiz and Pickering, chiselmouth.

"Cestodaria." CANADA, British Columbia (Bangham and Adams, 1954). Caryophyllidea.

Cyprinus carpio (L.), carp.

Archigetes iowensis Calentine. 1962. IOWA (Calentine, 1962).

Archigetes sieboldi Leuckart. 1878. WISCONSIN (Calentine and DeLong, 1966).

Atractolytocestus huronensis Anthony, 1958. CALIFORNIA: San Joaquin Co., delta of Sacramento and San Joaquin Rivers (coll. S. Edwards). CANADA: British Columbia (Bangham and Adams, 1954, det. as *Caryophyllaeus terebrans*). MICHIGAN (Anthony, 1958). NEW YORK: Tompkins Co., Cayuga Lake; Albany Co., Mohawk River (coll. J. Mackiewicz). OKLAHOMA: Payne Co., Lake Carl Blackwell (coll. R. Spall). TENNESSEE: Anderson Co., Clinch River; Knox Co., Loudon Lake; Lake Co., Mississippi River, Reelfoot Lake (coll. J. Mackiewicz, D. Etnier et al.). TEXAS (Mackiewicz, 1964) WASHINGTON (Griffith, 1953, det. as *Capingens* sp.).

Caryophyllaeidae. OHIO (Bangham, 1941).

"Cestodarian." NEW YORK (Van Cleave and Mueller, 1934). Most certainly a caryophyllidean, probably *A. huronensis* or *K. iowensis*.

Khawia iowensis Calentine and Ulmer, 1961. CALIFORNIA: San Joaquin Co., delta of Sacramento and San Joaquin Rivers (coll. S. Edwards). IOWA (Calentine and Ulmer, 1961) (Rehder, 1959 det. as *Caryophyllaeus laticeps* Pallas). KANSAS (Wilson, 1957 det. as *Caryophyllaeus* sp.). OKLAHOMA (Mackiewicz, 1964). OREGON (coll. I. Pratt). TENNESSEE: Knox Co., Loudon Lake; Lake Co., Mississippi River, Reelfoot Lake (coll. J. Mackiewicz, D. Etnier et al.). WISCONSIN (Anthony, 1963).

Gila atraria (Girard), Utah chub.

Hypocaryophyllaeus gilae Fischthal, 1953. WYOMING. (Fischthal, 1953). Based on a redescription of the "*Glaridacris laruei*" of Bangham (1951).

Hybopsis biguttata (Kirtland), hornyhead chub.

Bialovarium nocomis Fischthal, 1954. WISCONSIN (Fischthal, 1954).

Notemigonus crysoleucas (Mitchell), golden shiner.

Glaridacris sp. CONNECTICUT (Hunter, 1942).

Pliovitellaria wisconsinensis Fischthal, 1951. WISCONSIN (Fischthal, 1951). VIRGINIA (coll. W. Hargis, det. J. Fischthal).

Notropis bifrenatus (Cope), bridled shiner.

Pliovitellaria wisconsinensis Fischthal, 1951. NEW YORK: Suffolk Co., Nissequaque River at Smithtown (coll. A. Anderson, det. J. Mackiewicz).

Notropis deliciosus straminaeus (Cope), northeastern sand shiner.

Caryophyllaeidae. OHIO (Bangham, 1941).

Notropis rubellus (Agassiz), rosyface shiner.

Carophyllaeus terebrans (Linton). MASSACHUSETTS (Linton, 1941), recorded from "*Notropis rubifrons* (?)." Determination incorrect, see text.

Pimephales notatus (Raf.), bluntnose minnow.

Pliovitellaria wisconsinensis Fischthal, 1951. WISCONSIN (Fischthal, 1951, recorded from *Hyborhynchus notatus*).

Pimephales promelas Raf., fathead minnow.

Biacetabulum sp. WISCONSIN (Fischthal, 1950).

Richardsonius balteatus hydrophlox (Cope), Utah silver-side minnow.

"Cestodaria." WYOMING (Bangham, 1951). Caryophyllidea.

sirable in order to determine the generic status of these cestodes from *Notropis*. Other genera, such as *Biacetabulum*, *Glaridacris* and *Hypocaryophyllaeus* have also been found in catostomid fish, yet there is some reason to question some of these records. For example the *Biacetabulum* record is represented by a single immature specimen and thus it could be an accidental infection. Furthermore, neither the *Biacetabulum* nor *Glaridacris* were specifically identified, raising some questions as to the identity of the worms. Another species of *Hypocaryophyllaeus*, *H. paratatius*, was originally described from *Caripodes* and *Ictiobus* by Hunter (1927) but has not been reported since. Because the scolex of *H. gilae* has a terminal disc while that of *H. paratarius* is weakly cuneiform there is some reason to doubt the present systematic treatment of *H. gilae*.

From the above discussion it would appear that, with few exceptions, the caryophyllid fauna of cyprinid and catostomid hosts in North America are distinct from each other

and that the fauna of North American cyprinids, including introduced species such as the carp, is distinct from that of the cyprinids of Eurasia. The presence of these tapeworms in such diverse genera as *Acrocheilus* Agassiz, *Gila* Baird and Girard, *Notropis* Raf., *Pimephales* Raf. and *Richardsonius* Girard indicates a wide host spectrum. Indeed, helminth surveys of species in the above genera, and particularly of others in North America, such as *Phenacobius* Cope and *Hybopsis* Agassiz, should reveal additional species so necessary to better appraise the evolutionary relationships between the caryophyllidean cestode fauna of cyprinid and catostomid fishes.

Simplified Key to the genera of Capingentidae

1. Postovarian vitellaria present 2
1. Postovarian vitellaria absent 6
2. Uterus passing anterior to cirrus sac; scolex large with two deep, well-developed bothria ....
.... *Capingens* Hunter, 1927
2. Uterus not passing anterior to cirrus sac; scolex without large, deep bothria 3
3. Pre- and postovarian vitelline groups continuous with each other; ovary dumbbell-shaped 4
3. Pre- and postovarian vitelline groups not continuous with each other; ovary distinctly H-shaped, or in the form of an inverted letter A 5
4. Neck narrow, distinct; scolex expanded, oval in ventral view ....
.... *Capingentoides* Gupta, 1961
4. Neck absent; scolex not expanded, continuous with stout body ....
.... *Breviscolex* Kulakovskaja, 1962
5. Ovary in shape of inverted letter A, posterior wings not fused; postovarian vitellaria cover posterior tips of ovary. External seminal vesicle absent ....
.... *Adenoscolex* Fotedar, 1958
5. Ovary distinctly H-shaped, posterior wings widely separated; postovarian vitellaria do not cover posterior tips of ovary. External seminal vesicle present .... *Edlintonia* gen., n.
6. Uterus passing anterior to cirus; ovary U-shaped; vitellaria in lateral bands ....
.... *Spartoides* Hunter, 1929

6. Uterus not passing anterior to cirrus; ovary H-shaped; vitellaria in lateral and median positions 7
7. External seminal vesicle present ....
.... *Pseudolytocestus* Hunter, 1929
7. External seminal vesicle absent ....
.... *Pseudocaryophyllaeus* Gupta, 1969

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Neoaplectana hoptha, sp. n. (Neoaplectanidae: Nematoda), A Parasite of the Japanese Beetle, Popillia japonica Newm.[1]

C. P. Turco[2]

ABSTRACT: Neoaplectana hoptha sp. n., a nematode parasite of the Japanese beetle, Popillia japonica Newm., found in Moorestown, New Jersey in 1938 is described and illustrated. It causes death of the insect host.

The family Neoaplectanidae was instituted by Sobolev (1953) to contain all known species of the genus Neoaplectana Steiner, 1929. These nematodes have been used in many attempts at biological control of insect pests. While studying nematodes of the family Neoaplectanidae made available by the Nematology Investigations Section, USDA, Beltsville, Maryland, the author encountered an undescribed form. It is named and described here as Neoaplectana hoptha sp. n.[3] The specimens were obtained from the body cavity of the Japanese beetle, Popillia japonica Newm., recovered from soil in Moorestown, New Jersey in 1938 by L. Strong.

Beginning with the description of Neoaplectana glaseri by Steiner (1929), the presence and arrangement of the labial and cephalic papillae have been used, to a limited extent, as a taxonomic character. The Neoaplectanidae are characterized as possessing a full complement of well developed papillae consisting of six labial and six cephalic papillae. Examination of these papillae in various specimens in this study produced a wide range of results. Although they appeared inconspicuous or absent in a few, they were quite evident in most of the specimens. Nevertheless, enough detail was found to substantiate the presence and general arrangement of the papillae. Until further work is done on the number and arrangement of these papillae in all known species of the genus Neoaplectana, the taxonomic value placed on them should be reduced.

Neoaplectana hoptha sp. n.

MALES (PARATYPES) (8): L = 729 (554–837) μ; a = 20.3 (17.6–22); b = 7.2 (5.9–8.2); c = 28.9 (18.1–37.4); Spicule = 47 (43–60) μ; Gubernaculum = 28 (26–30) μ.

FEMALES (PARATYPES) (14): L = 3343 (2826–3983) μ; a = 15.2 (12.7–19.3); b = 15.9 (12.4–20.7); c = 57.7 (46.9–67.2); Vulva = 47 (43–49) %.

MALE (HOLOTYPE): L = 738 μ; a = 18.9; b = 7.5; c = 35.1; Spicule = 49 μ; Gubernaculum = 27 μ.

FEMALE (ALLOTYPE): L = 3302 μ; a = 14.7; b = 13.1; c = 66; Vulva = 48%.

DESCRIPTION OF MALE: Occurs in body cavity of host insect. Body smaller and more slender than the female. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae (Fig. 1A). Stoma reduced or vestibulate. Esophagus with simple corpus, slender isthmus, and terminal bulb with reduced musculature. Nerve ring encircling esophagus in front of basal bulb. Excretory pore opening at or near nerve ring. Testis single, large, reflexed; spicules paired large, curved slightly, ending in an enlarged proximal region, distal end unhooked; gubernaculum long and narrow (Fig. 1B). Tail convex-conoid, with rounded tip; genital papillae as follows: four pair preanal, two pair adanal, and six pair postanal.

DESCRIPTION OF FEMALE (Fig. 1C): Occurs in body cavity of host insect. Body stout, doubling in diameter rather abruptly at level of basal bulb of esophagus. Cuticle not striated. Head region similar to that of male. Stoma reduced or vestibulate. Esophagus with simple corpus, indistinct isthmus, and terminal bulb with reduced musculature (Fig. 1D). Nerve ring encircling terminal bulb region. Excretory pore opening at or near
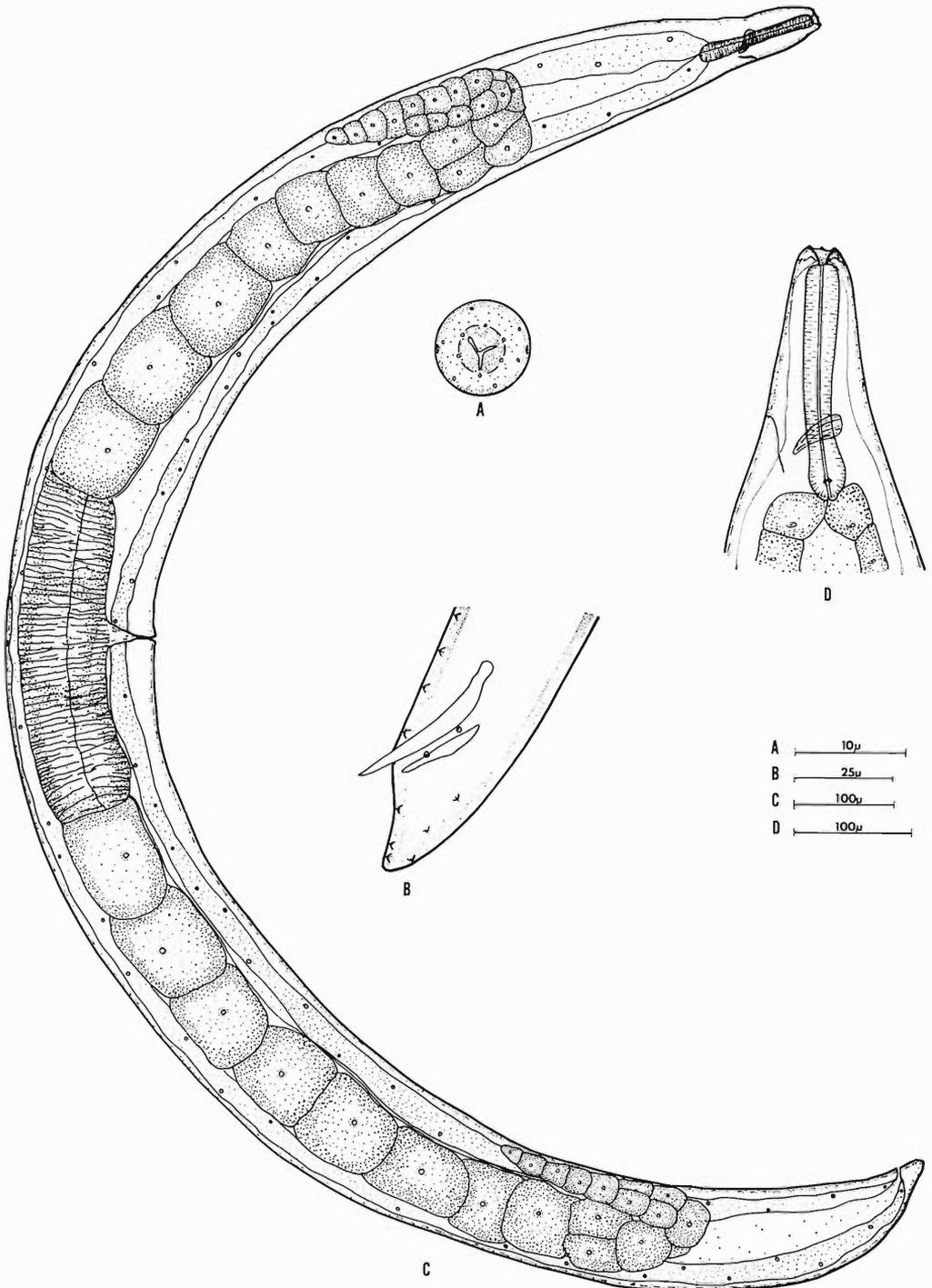
Figure 1. *Neoaplectana hoptha* sp. n. A, Face view; B, Male tail; C, Female; D, Head and neck of female.

nerve ring. Gonads amphidelphic, large, symmetrical; ovaries with flexure at terminal bulb of esophagus and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous, with large postanal lip. Tail conoid, tip pointed.

TYPE MATERIAL: Paratypes (males) Slide No. T-668p, paratypes (females) Slide No. T-668p, Holotype (male) Slide No. T-121t, and Allotype (female) Slide No. T-122t, deposited with the Nematology Investigations Collection, USDA, Beltsville, Maryland.

TYPE HABITAT AND LOCALITY: Body cavity of the Japanese beetle, *Popillia japonica* Newm., from Moorestown, New Jersey.

DIAGNOSIS AND RELATIONSHIP: *Neoaplectana hoptha* sp. n. is closely related to *Neoaplectana glaseri* Steiner, 1929. It varies from this species by the absence of distally hooked spicules and by the female body doubling in diameter rather abruptly at level of the basal bulb.

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Research Note

A New Distribution Record for the Genus *Paryphostomum* (Echinostomatidae)*

The Helminthological Collection, Division of Microbiology and Veterinary Medicine, University of Wyoming, contains, among other helminths, 15 specimens, on slides, of a 27-spined echinostomatid from a "Turkey Vulture" taken in the vicinity of Laramie, Wyoming. Mr. Ralph Honess, former Professor of Parasitology, University of Wyoming, collected these specimens (and ca. 35 more) on 20 August 1930; the bird had been shot by a Mr. Cook, then Superintendent of Wyoming State Fish Hatchery, at that time located at Red Buttes, eleven miles south of Laramie. The bird was identified by Dr. J. W. Scott and Mr. C. E. McCafferty. Examination of the specimens and a perusal of the literature dealing with echinostomatid trematodes (Skrjabin, 1956, Trematodes of Animals and Man,

Vol. 12) requires that the Wyoming specimens be placed in the genus *Paryphostomum* Dietz, 1909; a cosmopolitan genus with species recorded from Asia, Western Europe (Germany), South America (Brazil), Australia, Africa, and India and found in cormorants (*Phalacrocorax* spp.), water turkeys (*Anhinga anhinga*), ducks (*Anas platyrhynchos*, *Anser indicus*, and *Dendrocygna javanica*), snipe (*Capella gallinago*), and various vultures (*Oenops* (= *Cathartes*) *aura*, *Catharista* (= *Coragyps*) *atrata*, *Sarcorhamphus papa*, *Cathartes urubutinga*, and *Vultura* sp. (Skrjabin *loc. cit.*). The present record from *Cathartes aura téter* Friedmann, 1933 extends the distribution of this genus to the continental USA. (*C. aura* is the sole cathartid listed from Wyoming (Knight, 1902, Birds of Wyoming, Exp. Stat. Bull. 55, 1–174; and McCafferty, 1930, Unpublished Master's Thesis, An annotated and distributional list of the birds of Wyoming, 1–288),

and *C. a. téter* is considered the subspecies in the area (A.O.U. Check List of North American Birds, 5th Ed., 1957).

Measurements of sexually mature specimens from Wyoming fall within the range of measurements of *Paryphostomum segregatum* Dietz, 1909 as given by Lie and Basch (J. Parasit., 1967, 53, 280–286) and thus the Wyoming material is considered conspecific with the Brazilian species described by these authors. Lie and Basch (*loc. cit.*) using cercariae from *Biomphalaria* spp. experimentally infected fishes and tadpoles and used the metacercariae from such sources to infect an urubu vulture (*Coragyps atratus foetens*). No snails infected with 27-spined cercariae have been found in the vicinity of Laramie, Wyoming (though a 45-spined cercaria (= *Echinoparyphium* sp.) has been shed by *Lymnaea palustris*) and thus it is not known whether the infected vulture taken in Wyoming acquired the infection here or elsewhere. Migration of western Turkey Vultures apparently takes them to southwest Arizona, to Texas (Peterson, A field guide to the birds, Houghton Mifflin Co. 1958, 1–290) and south as far as Ecuador (A.O.U. Check List, *loc. cit.*). It seems unlikely that vultures from the continental USA would migrate as far as Brazil and acquire their infections there; it seems more probable that infection with *Paryphostomum segregatum* in this instance took place within the limits of the continental USA or in nearby countries to the south of the USA.

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Research Note

Histochemical Lipid Studies on *Echinostoma revolutum*

Studies on the histochemical distribution of lipids in adult echinostomes are not available. The purpose of this work was to demonstrate the presence and distribution of lipids in *Echinostoma revolutum* from the chick intestine, the chorioallantois and worms starved 24 hr in non-nutrient Tyrode's.

Seven-day-old preovigerous and 14-day-old ovigerous *E. revolutum* obtained from laboratory infected domestic chicks were separated into three groups of six or eight worms each. Group one flukes were untreated controls, while group two were starved for 24 hr in non-nutrient Tyrode's as described previously (Fried and Kramer, 1968, J. Parasit. 54: 942–944). Group three consisted of only 7-day-old flukes maintained an additional 7 days on the chick chorioallantois (Fried, et al., 1968, J. Parasit. 54: 939–941). Three or four worms from each group were frozen at −20 C, sectioned on a cryostat at 8 μ, fixed in formalin fumes, stained with Oil Red O (Lillie, 1944, Stain Tech. 19: 55–58) and counterstained with Mayer's haemalum. The remaining worms were flattened on slides with moderate coverslip pressure, fixed in 10% formalin, stained with Oil Red O and prepared as whole mounts. Some worms prepared as sections or whole mounts were treated with ether for lipid extraction prior to staining. To facilitate penetration of the stain the cuticle of worms prepared as whole mounts was pierced with insect pins. Sections and whole worms were prepared as aqueous mounts with glycerine jelly and coverslips were rimmed with paraffin to preserve the slides.

Oil Red O stained lipids bright red and the Mayer's haemalum stained nuclei blue. Cryostat sections revealed the presence of lipids in the excretory system of group one to three flukes and whole mounts confirmed these findings (Figs. 1, 2). Lipids were extracted from the excretory system with ether in sections and in whole mounts. Preliminary studies indicated that it was not necessary to pierce the whole mounts to extract the lipids. Ether extraction did not adversely effect the morphology of sections or whole mounts. Lipids were not ob-
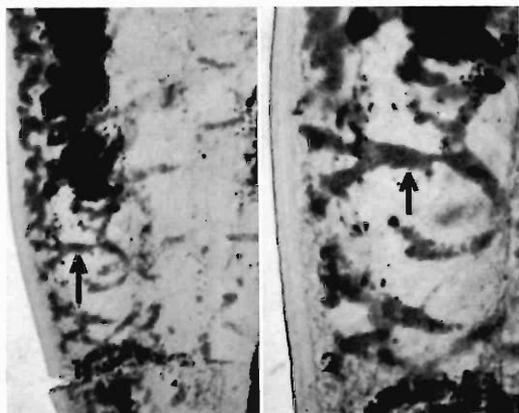
Figures 1, 2. **Histochemical lipid studies on** *Echinostoma revolutum.*

Figure 1. Excretory tubules visualized in a whole mount of a chorioallantoic-echinostome fixed in formalin and stained with Oil Red O. The black material is hematin-like pigment in the worm's intestine.

Figure 2. An enlargement of an excretory tubule (arrow) from the specimen in Figure 1.

served in the tegument, parenchyma, vitellaria or intestine. No differences in the presence or distribution of lipids were observed in pre-ovigerous and ovigerous intestinal or starved flukes or in chorioallantoic-echinostomes.

The results on *E. revolutum* are in accord with other studies on trematodes (Vogel and von Brand, 1933, Ztschr. Parasitenk. 5: 425–431; Stephenson, 1947, Parasitology 38: 140–144; Pantelouris and Threadgold, 1963, L. Cellule 64: 63–67; Pantelouris, 1965, The Common Liver Fluke, Pergamon Press, London; Threadgold and Gallagher, 1966, Parasitology 56: 299–304; Öhman, 1965, Para-

sitology 55: 481–502; Öhman, 1966, Parasitology 56: 209–226; Öhman, 1966, Parasitology 56: 481–491; Erasmus, 1967, J. Parasit. 53: 525–536 and Erasmus, 1967, J. Parasit. 53: 703–714) in that the excretory system is the primary site for localization of lipids in adult flukes and the parenchyma and tegument are generally negative. Lipids were not found in *E. revolutum* intestine but were found in the cecal wall and contents of *Apatemon gracilis minor* (Öhman, 1966, Parasitology 56: 209–226). Fat droplets observed in the tegumentary and vitelline cells of *Cyathocotyle bushiensis* by Erasmus (1967, J. Parasit. 53: 525–536) were not found in *E. revolutum.*

Whole mounts stained with Oil Red O confirmed the presence of lipids in the excretory system and also demonstrated the complex branching of the tubules. This latter finding suggests that the Oil Red O staining procedure on fixed worms may provide a simple and effective method to study the morphology of the trematode excretory system. To our knowledge this technique *per se* has not been used for this purpose. A temporary procedure for the visualization of the excretory system of adult *Fasciola hepatica* in which a vital dye is injected into the excretory bladder of live flukes has been discussed and figured by Pantelouris (1965, The Common Liver Fluke, Pergamon Press, London).

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Research Note

Helminth Parasites of Some Birds in Puerto Rico

The birds examined were collected from December 1967 through February 1969 from rural and suburban areas in Puerto Rico. Grackles (*Quiscalus niger*) were collected near

Caguas, Fajardo, Isabela, La Parguera, Mayaguez and Rio Piedras; plovers (*Charadrius semipalmatus*), gray kingbirds (*Tyrannus dominicensis*), the ground dove (*Columbigal-*

Table 1. Helminth parasites of some birds in Puerto Rico.

Bird	Number examined	Parasite	New record of parasite for	
			Bird	Puerto Rico
Quiscalus niger brachypterus Cassin	124	Acanthocephala *Lueheia inscripta* (Westrumb, 1821) Travassos, 1919 *Mediorhynchus emberizae* (Rudolphi, 1819) VanCleave, 1916	* *	* *
		Cestoda *Choanotaenia* sp. *Raillietina* sp.		
		Nematoda *Acuaria quiscula* Williams, 1929 *Diplotriaena thomasi* Seibert, 1944	*	* *
Charadrius semipalmatus Bonaparte	2	Nematoda *Stellocaronema skrjabini* Gilbert, 1931	*	*
Tyrannus dominicensis (Gmelin)	3	Nematoda *Acuaria* sp.	*	
Bubulcus ibis (L.)	5	Nematoda *Tropisurus* sp. ♀		*
Agelaius xanthomus xanthomus (Sclater)	1	Nematoda *Acuaria* sp.	*	
Columbigallina passerina (L.)	1	Cestoda *Choanotaenia* sp.	*	

lina passerina) and the yellow-shouldered blackbird (*Agelaius xanthomus*) were collected near Rio Piedras; cattle egrets (*Bubulcus ibis*) were all collected near the University of Puerto Rico Biological Station at La Parguera. To our knowledge, there is no record of previous examination of these birds for helminth parasites in Puerto Rico.

The number of birds of each species examined, the helminth parasites found and new host and locality records are listed in Table 1. It is noteworthy that only two trematodes were recovered. These were of the family Echinostomatidae and were found in the small intestine of one of the cattle egrets. Their unfortunate loss precluded further identification.

Although the *Choanotaenia* sp. and the *Raillietina* sp. from *Quiscalus niger brachypterus* appear to be new, additional material is necessary to warrant description.

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Presentations

1969 Anniversary Awards of The Helminthological Society of Washington 445th Meeting 22 October 1969
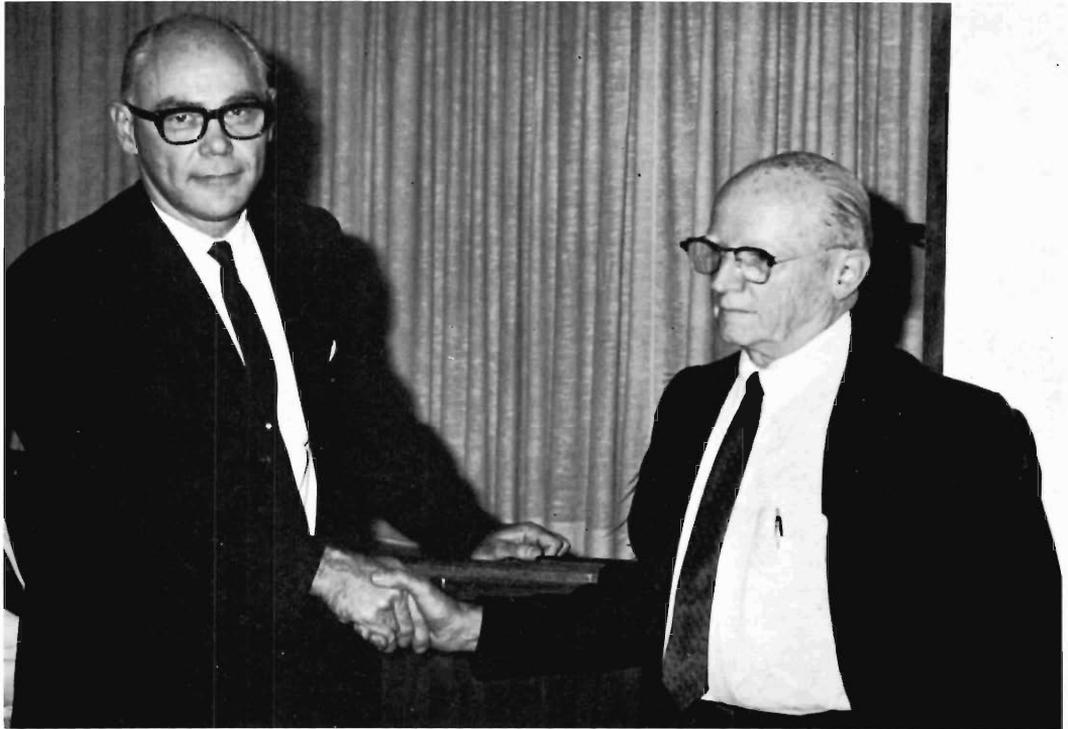
Dr. J. S. Andrews (right) accepting on behalf of Dr. Benjamin Schwartz a 1969 Anniversary Award of the Helminthological Society of Washington (presented by W. W. Becklund)

Dr. Benjamin Schwartz

Benjamin Schwartz, as a resident in the Washington area and member of this Society for over forty years, is well known to most of us here tonight. In fact, as Chief of the Zoological Division of the old Bureau of Animal Industry he was instrumental in bringing many of us to the Washington area. During recent years he has resided in New York City. His great knowledge of parasitology, total recall of events in research, tales of his association with the early members of this Society, and stimulating conversation have been missed.

Born in Austria on 25 November 1889, he came to this county in 1901 and was naturalized in 1912. He graduated from City College of New York (B.A., 1911), Columbia University (M.A., 1913), and George Washington University (Ph.D., 1920). After receiving his M.A. degree he worked briefly as a Scientist Assistant in the U. S. Bureau of Fisheries, before becoming an Instructor in Biology for two years at the University of Arkansas. In September 1915, he accepted an appointment as Junior Zoologist in the Zoological Division of the USDA. He remained in the Washington area until December 1920, at which time he traveled to the Philippines where he taught for two and one-half years at the University of the Philippines. In August 1923, he re-entered the Zoological Division, and subsequently became Assistant Zoologist of the

Division in 1932, and Chief Zoologist in 1936. The latter position he filled until 1953, when he became Consultant in Parasitology in the Animal Disease and Parasite Research Division. He retired in November 1959, after completing over 42 years service. His retirement was short lived, as he soon became Consultant in Parasitology with the Meat Inspection Division, and also was a lecturer of parasitology at the University of Wisconsin.

Dr. Schwartz has written numerous articles, reports, bulletins, and abstracts on many aspects of parasitology. The titles of his articles cover several pages in the Index-Catalogue of Medical and Veterinary Zoology. I attempted to summarize his contributions, but lost heart when I found that he was the author, or senior author, of at least 340 articles, and a junior author of many additional ones. The subjects of his papers concerned toxic products, physiology, taxonomy, life history studies, immunity, and control of parasites of livestock, as well as those transmissible to man. The name trichinosis frequently occurs in the title of his contributions and this disease was the subject of his first two papers. He was one of the first workers to test the effects of x-rays on parasites by exposing trichinae. Because of his interest, and research, on trichinae, he was frequently called upon to represent the USDA in national and international matters relating to trichinosis.

The number of committees, societies, and other organizations with which he was affiliated, and the number which he served as an officer are too many to name. Among them are the National Research Council, Washington Academy of Science, National Trichinosis Committee, Association of Land Grant Colleges and Universities, Committees on the Yearbook of Agriculture, Agricultural History Series of the USDA, and the Livestock Sanitary Association, in which he served as committee chairman of the Committee on Parasites for eight years. In an editorial capacity, he helped in publishing the Philippine Journal of Science, Bacteriological Abstracts, Journal of Parasitology, and Journal of Agricultural Research. In addition he has been a delegate to international congresses. Dr. Schwartz was on the council of the American Society of Parasitologists, its vice president in 1944, its president in 1951, and was elected an emeritus member in 1965. He served on a committee, with Drs. Cort and Stoll, to establish the Journal of Parasitology as the official organ of the American Society of Parasitologists. The Journal was established by Dr. H. B. Ward in 1914, and was maintained by him as managing editor. When the Society came into being in 1924, all records of its meetings were submitted to Dr. Ward for publication in the Journal. The committee's job was to approach Dr. Ward and persuade him to give up the Journal. In 1932, the Journal became the official organ of the Society, but not until Dr. Ward referred to Dr. Schwartz as "a pirate."

In 1924, Dr. Schwartz was the Recording Secretary of the Helminthological Society of Washington, its president in 1926, and its representative to the Washington Academy of Sciences in 1927. He is the father of the Brayton H. Ransom Memorial Award. At the 141st meeting of the Society in October 1931, he suggested that the Society arrange to use the Ransom Memorial Fund for an award to someone for outstanding work in parasitology. Although a committee was appointed at that time to select a suitable recipient, considerable confusion and inactivity followed. The first award was given in 1960, 24 years and five months after his suggestion.

In addition to his scientific accomplishments, Dr. Schwartz has many hidden talents which become apparent when one carefully goes over his records. Among these talents is his role as a poet. When called upon in 1920 to write a limerick for a place card for Dr. M. C. Hall, at the banquet to celebrate the tenth anniversary of our Society, he wrote:

"There was a young fellow named Hall,
Who went to a fancy dress ball.
He dressed up as a Taenia,
But his host got anemia
And took some male fern—That is all!"

Thus, for his achievements in research, successful administration of a large parasitological research agency, activities on committees and in societies including "Helm Soc.", and for greatly contributing to the furtherance of parasitology, through publications and talks, we honor Dr. Schwartz with the Anniversary Award.

(Committee: W. W. Becklund, L. S. Diamond, L. A. Jachowski. Presentation: W. W. Becklund).

Dr. Willard H. Wright (right) receiving a 1969 Anniversary Award of the Helminthological Society of Washington (presented by L. A. Jachowski)

Dr. Willard H. Wright

In 1928, Dr. Willard H. Wright joined the Helminthological Society of Washington. Since then he has served as Secretary (1930–32) and President (1936–37). He was a charter member of the American Society of Parasitologists in which he served as member of the Council (1942–49), Vice-President (1949) and President (1950). In the American Society of Tropical Medicine and Hygiene, he served as Vice-President (1956) and President (1960). Dr. Wright has been an active member of a number of professional societies, including our own.

What are the origins of this scientific leader? Dr. Wright was born in Findlay, Ohio on December 9, 1894. In 1913, he graduated from Findlay High School and four years later received his D.V.M. (cum laude) from the George Washington University here in Washington, D. C. With this degree, he was commissioned Second Lieutenant in the Veterinary Corps, U. S. Army. From 1917–1919, he saw front-line action in many bitter engagements— LeReine and Boucq Sector, Sector of Haute Alsace, the Battle of the Marne, Oise-Aisne Offensive, and the Mause-Argonne Offensive. He received the World-War I Victory Medal with five battle stars.

Dr. Wright began his scientific career with the Department of Agriculture. From 1919–1928 he was a veterinarian working on animal disease control in North Carolina, Texas, and Maryland. Then, from 1928–1936, he was a parasitologist in the Zoological Division, U. S. Department of Agriculture. During this tenure, he clarified the life cycle of *Toxascaris leonina* and began work with his colleagues on *Dirofilaria immitis*. Somehow, during this period, he managed to earn his M.S. from American University (1932) and his Ph.D. from the George Washington University (1935).

In 1936, Dr. Wright transferred to (what

is now) the National Institutes of Health. Initially, he was Senior Zoologist. Later, he became Chief, Division of Zoology (1939). From 1936–1945, he and his coworkers investigated many problems in medical parasitology. These included studies of intestinal helminths, trichinosis, filariasis, and schistosomiasis. Also, during this period, he served as consultant on onchocerciasis to the Pan American Sanitary Bureau.

Then came World War II and Dr. Wright was appointed Scientist Director (Colonel), U. S. Public Health Service and assigned to the Army (1945–1946). Most of this time was spent in the Western Pacific and was devoted to a series of studies on *Schistosoma japonicum*. For his war service, he received the Legion of Merit (1945), World War II Victory Medal, National Defense Service Medal, Asiatic-Pacific Campaign Medal with one battle star, Philippine Liberation Medal with one battle star, Philippine Presidential Unit Citation and the Army of Occupation Medal (Japan).

After the War, he returned to N.I.H. and his contributions and recognition increased. Initially, he was Chief, Division (later Laboratory) of Tropical Diseases (1947–1958). From 1952–1953, he was also Assistant Director, National Microbiological Institute. His research interests focused on filariasis and schistosomiasis and a long series of publications resulted. In recognition of his talents, he was appointed to many international organizations. A climax of this period in his scientific career was the awarding of the Walter Reed Medal in 1958 by the American Society of Tropical Medicine and Hygiene.

In 1959, Dr. Wright retired from Government service, but not from parasitology. He continued from 1959–1962 as Director of the Tropical Health Survey, National Academy of Sciences—National Research Council. This endeavor was climaxed with publication of "Tropical Health."

Dr. Wright had previously served the World Health Organization in a great variety of short-termed capacities. It was not surprising that he was appointed (1963) as temporary advisor. He was in Geneva in 1963, 1964, and 1966 and in Washington in 1965.

These do not terminate all of his parasitological responsibilities. There is still unfinished business. Originally appointed in 1967, Dr. Wright continues to serve as Consultant to the Gorgas Memorial Institute of Tropical and Preventive Medicine. Also, since 1952, he has been a member of the Commission on Parasitic Diseases of the Armed Forces Epidemiological Board.

This biographical sketch is far from complete. Inadequate mention has been made of the scientific papers which number over 200; of the various schools, both here and abroad in which he has lectured; and the many committees, boards and commissions on which he has served. In recognition of his many and diverse contributions to parasitology, we proudly present the Anniversary Award of the Helminthological Society of Washington to Dr. Willard H. Wright.

(Committee: W. W. Becklund, L. S. Diamond, L. A. Jachowski. Presentation: L. Ā. Jachowski).

Sixtieth Anniversary of the Society

The year 1970 will mark the sixtieth anniversary of the founding of the Helminthological Society of Washington. A special banquet meeting is planned to commemorate this event.

It will be held during the period when the Second International Congress of Parasitology meets in Washington, D. C. Particulars of time and place will be given in a later announcement.

Errata

In the Minutes of the 444th Meeting of the Helminthological Society of Washington (v 36:289) the paper "A comparative study on Dimensions of the Filariform larvae of *Strongyloides ratti* and *S. venezuelensis*" was presented by F. F. Katz. S. R. Sylk presented a paper entitled "Nuclear distribution patterns in *Ascaris*."

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