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# PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 36

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NUMBER 1

## Osmoregulation and the Uptake of Ions in a Marine Nematode

N. A. CROLL<sup>1</sup> AND D. R. VIGLIERCHIO  
Department of Nematology, University of California, Davis, California

Past studies have demonstrated that if certain animal-parasitic, plant-parasitic and free-living nematodes are placed in hypertonic solutions they lose water causing a reduction in volume (Stephenson, 1942; Osche, 1952; Lee, 1960; Wallace and Greet, 1964; Anya, 1966; and Myers, 1966). In most cases osmoregulation has then occurred enabling the nematodes to regain their original size. Similarly when placed in hypotonic media there is an increase in volume and sometimes a return to normal body size (Stephenson, 1942; Anya, 1966; and Myers, 1966). This communication complements these observations by characterizing osmoregulation and uptake of ions in the marine nematode—*Deontostoma* (= *Thoracostoma*) *californicum*.

Reviewing the data on osmoregulatory mechanisms in invertebrates, Beadle (1957) was able to say that "There is no convincing evidence as yet that osmotic gradients between body fluid and external medium are due in any degree to the active transport of water." It is, therefore, with the movements of ions that osmoregulation is achieved and the ability to osmoregulate will depend on the ability to take up and lose ions.

### Experimental Methods and Materials

*Deontostoma californicum* was collected from the holdfasts of *Laminaria digitata* and *Egretta laevigata* at Dillon Beach, California, at extreme low water spring tide. *D. californicum* was removed from the holdfasts and stored in seawater on gauze at 5 C as this was found to reduce "clumping." The seawater was changed at weekly intervals, and under these

conditions the worms remained in an apparently good state for up to 6 weeks. All experiments were conducted on adult worms from this supply at 25 C.

Through water transfer nematodes become greatly reduced in size and distended in hypertonic and hypotonic solutions respectively. The nematode response to osmotic solutions and osmoregulation was measurable by changes in its physical dimensions. The distance from the oesophago-intestinal junction to the anterior tip was found to be a convenient sensitive parameter. In preliminary experimentation it was found that this measurement was in direct proportion to changes in width and overall length.

Each worm was placed in a perspex groove containing seawater, held by a coverglass and measured with an ocular micrometer. The nematodes then were placed in test solution in 2 cm diameter glass cells in covered Petri dishes. A little tap water was placed in each Petri dish to ensure a high humidity while allowing free gaseous exchange. The nematodes became inactive or less active during the experiments.

Frequently, exosmosis proceeded until contortion and crumpling of the worms occurred and at these extremities of water loss, volume changes were not truly reflected by length measurements. These excessive losses were accommodated within 15 min and the usual body shape was recovered. The first reliable measurement of water loss (ME) was on the recovery curve R which approximated to linearity in all but the two cases described below. T was the time taken to regain original (seawater) size (Fig. 1). The rate of recovery was expressed as ME/T or  $\tan \alpha^\circ$ .

<sup>1</sup>Fulbright Scholar, permanent address: Department of Zoology and Applied Entomology, Imperial College, University of London.

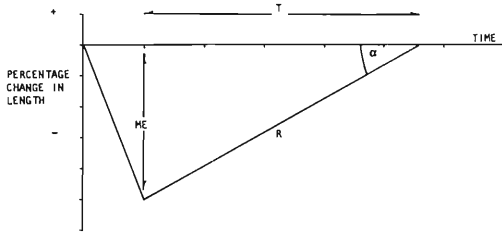


Figure 1. Schematic diagram of the measurements made of the osmoregulatory process in *Deontostoma californicum*, following immersion in hypertonic media. ME, maximum recorded exosmosis; T, recovery time; R, recovery line; tan  $\alpha$ , rate of recovery.

**Osmoregulation in hypotonic and hypertonic solutions of NaCl**

Using distilled water, 0.4, 0.6, 0.8, and 1.0 M solutions of NaCl, the length changes of *D. californicum* were measured with respect to time. The isotonicity of the worm was thus approximated as well as its ability to recover its seawater dimensions.

RESULTS: In both hypertonic and hypotonic solutions there were immediate changes in length (Fig. 2). *D. californicum* was unable to osmoregulate in hypotonic solutions (Fig. 2) even after 24 hr, but recovered its normal length and mobility in seawater. In hypertonic solutions recovery occurred at an essentially constant rate until the original length had been reached; R was treated as being linear. There frequently occurred an “over-osmoregulation” giving a length larger (usually 5–10%) than the original seawater length (Figs. 2, 4). Hav-

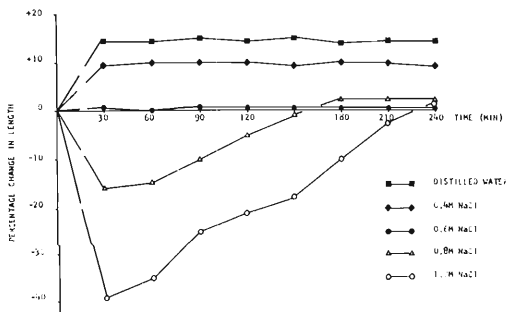


Figure 2. Percentage length changes of *D. californicum* with respect to time in NaCl solutions of varying molarity.

Table 1. Osmotic regulation of *Deontostoma californicum* following immersion in various hypertonic media.

Compound	Regulation†	Compound	Regulation†
NaCl	+	MgCl <sub>2</sub>	+
NaBr*	+	CaCl <sub>2</sub>	+
NaI	+	KMnO <sub>4</sub>	○
NaNO <sub>2</sub> *	+	Oxamic acid	○
	(partial)	(COOH·CO·NH <sub>2</sub> )	
NaNO <sub>3</sub>	+	Oxamide	○
		NH <sub>2</sub> ·CO·CO·NH <sub>2</sub> )	
Na <sub>2</sub> SO <sub>4</sub>	—	Formamide	○
		(CHO·NH <sub>2</sub> )	
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	—	Dimethyl oxalate	○
		(CH <sub>3</sub> ·CO·CO·CH <sub>3</sub> )	
Na <sub>2</sub> MoO <sub>4</sub>	—	Ribose	—
NaWO <sub>4</sub>	—	Xylose	—
KCl	+	Sorbitol	—
KBr	+	Inositol	—
KI	+	Galactose	+
			(slight)
KIO <sub>4</sub>	○	Glucose	—
K <sub>3</sub> Fe(CN) <sub>6</sub>	—	Fructose	—
K <sub>4</sub> Fe(CN) <sub>6</sub>	—	Trehalose	—
NH <sub>4</sub> Cl	+	Lactose	—
LiCl	+	Maltose	—
LiNO <sub>3</sub>	+	Sucrose	+
			(slight)
SrCl <sub>2</sub>	+		
SrBr <sub>2</sub>	+		
SrI <sub>2</sub>	+		

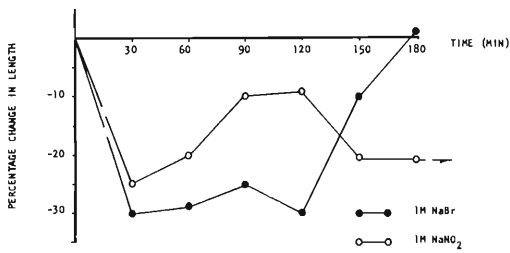
\* Nonlinear recovery.  
 † +, Recovery to original length; —, No recovery but normal on return to seawater; ○, No recovery, dead on return to seawater.

ing overcompensated, a subsequent return to normal size was never observed. Following osmoregulation in a hypertonic solution and transfer to distilled water, there was an immediate and gross increase in size which usually led to bursting of the worm. This degree of increase did not occur in changes from seawater to distilled water.

**Osmoregulation in other solutions**

A comparison was made of the ability of *D. californicum* to return to original seawater length following immersion in hypertonic solutions of selected electrolytes and non-electrolytes.

RESULTS: The ability or inability of the nematodes to recover normal length in the hypertonic media, together with the return to



**Figure 3.** Percentage length changes of *D. californicum* with respect to time to hypertonic solutions of NaBr and NaNO<sub>2</sub>, showing nonlinear recovery.

normal activity in seawater following the treatment, is tabulated (Table I). *D. californicum* returned to its original size, or increased slightly in most electrolytes. The recovery rates ( $\tan \alpha$ ) differed, while the recovery line R approximated to linearity except in the cases of NaBr and NaNO<sub>2</sub> (Fig. 3).

Very slight recovery occurred in sucrose and galactose and none was observed in any of the other sugars.

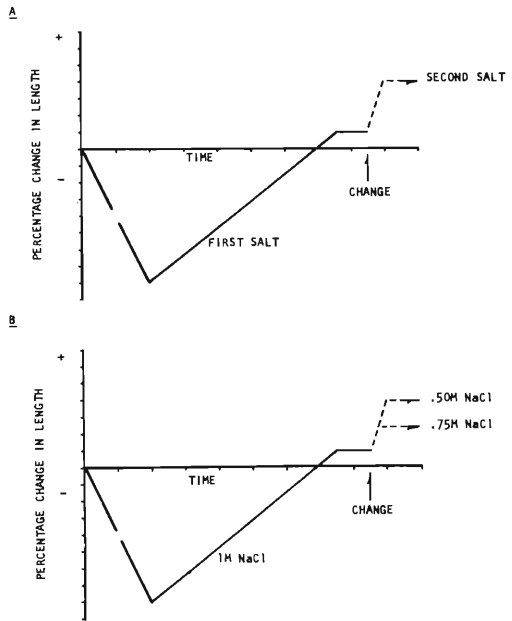
Oxamic acid, oxamide, formamide, dimethyl oxalate, and potassium periodate were used to investigate the significance of spatial configuration and charge in the regulatory mechanism. Their low solubility, together with their apparently high toxicity, made reliable conclusions impossible.

In seawater those individuals that had osmoregulated, expanded in size and frequently burst; all of the others (except the oxalic acid derivatives, formamide and potassium periodate) regained their normal size and mobility. Vigorous activity occurred for up to 30 min following transfer to sodium thiosulphate.

**The influence of successive immersions in different hypertonic solutions on osmoregulation**

After *D. californicum* had regulated in a 1 molar solution of an electrolyte, it was immersed in another molar solution of a second salt. Salts were selected from those known to allow regulation (Table 1) in order to show any cationic or anionic specificity of the regulatory mechanism.

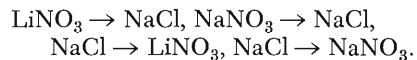
Nematodes that had osmoregulated in 1 M NaCl were also transferred to 0.5 M NaCl and



**Figure 4.** Schematic diagram showing length changes resulting from immersion in a second solution following regulation in the first. A. Transfer from 1 M solution of first electrolyte to 1 M solution of a second electrolyte. B. Transfer from 1 M NaCl to 0.5 M or 0.75 M NaCl. (Arrow indicates the point of transfer.)

0.75 M NaCl and any changes in length were measured.

**RESULTS:** Every change to a molar solution of the second salt, following regulation in the first, resulted in an additional increase in size (Fig. 4A), though the increase was subject to some individual variation. The following pairs of 1 molar successive changes were made:



*D. californicum* that had osmoregulated in 1 M NaCl also increased further when placed in 0.5 M or 0.75 M NaCl, the increase being greater with the more dilute solution (Fig. 4B).

**The influence of sulphate on the regulatory mechanism**

Since *D. californicum* was unable to regulate in sulphate (Fig. 5), thiosulphate, molybdate, and tungstate (Table 1), recovery was estimated in various volume to volume hyper-

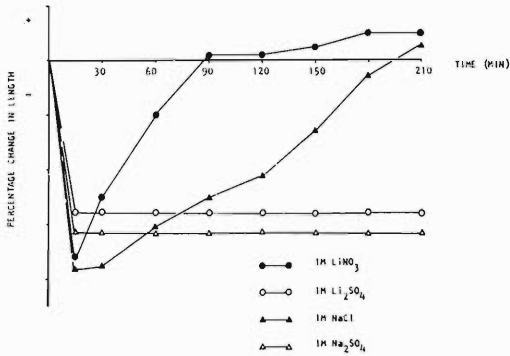


Figure 5. Percentage length changes of *D. californicum* with respect to time in hypertonic solutions of the sulphate and nitrate salts of lithium and sodium.

tonic mixtures of 1 M  $\text{Na}_2\text{SO}_4$  and 1 M NaCl.

The rate of regulation varied with different v/v mixtures of  $\text{Cl}^-$  and  $\text{SO}_4^{=}$  (Fig. 6). In 100% and 75% sulphate solutions there was no recovery from ME ( $\alpha = 0^\circ$ ). At 50:50 mixtures there was slight osmoregulation, and the rate of osmoregulation increased with greater dilutions of sulphate. From 1% to 50%  $\text{SO}_4^{=}$  the rate of recovery against dilution plot followed closely an exponential relation.

The inhibitory effect of high  $\text{SO}_4^{=}$  concentrations was temporary and the worms recovered normal size and motility on being transferred to seawater.

#### Flame photometric determination of ions

To establish the uptake of ions during osmoregulation, groups of 10–12 *D. californicum* were weighed (wet wt), dried to constant weight for 3 hr at 100 C and ashed at 600 C for 8 hr (ash wt) following treatment. In addition to controls taken directly from seawater, other groups were exosmosed (X) for 3 min in 1 M solutions of NaCl, KCl, and  $\text{MgCl}_2$ , respectively. The other treatment (R) was exosmosis and recovery in 1 M NaCl, KCl, and  $\text{MgCl}_2$  prior to weighing and ashing. The concentration of Na, K, and Mg were then estimated with a flame photometer.

RESULTS: Flame photometric measurements of ionic concentrations of  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Mg}^{++}$  following regulation (R) in 1 M KCl, NaCl, and  $\text{MgCl}_2$ , respectively, showed increases in all cases. Increases in ionic concentrations also

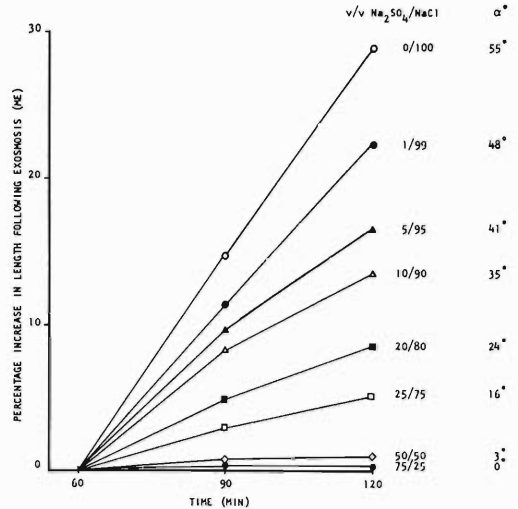


Figure 6. Percentage length changes of *D. californicum* with respect to time in various volume/volume mixtures of 1 M  $\text{Na}_2\text{SO}_4$  and NaCl; showing the dependence of angle  $\alpha$  (Fig. 1) or the  $\text{Na}_2\text{SO}_4/\text{NaCl}$  ratio.

were found for worms exosmosed only (X), this apparently being due to adsorption or some other form of binding or trapping by the integument. The increases in total cation following regulation in 1 M NaCl and  $\text{MgCl}_2$  approximated to the calculated value for a volume of 1 M solution equivalent to the volume of solution taken up in the nematodes (Table 2, volume of free water R-X). Insufficient  $\text{K}^+$  ions entered to account for the total required increment in osmotic pressure needed to attain isotonicity in 1 M KCl.

If the ionic concentration of the water lost on drying of *D. californicum* (wet wt–dry wt 100 C) is assumed equivalent to seawater for purposes of comparison (it is not, of course, because of the hydration of tissues and organic solutes, etc.), it appears that  $\text{Mg}^{++}$  and  $\text{Na}^+$  are present within the animal in concentrations of the same order present in seawater. Potassium ions, however, are present in much higher concentrations.

#### Localization of the regulatory mechanism

Following regulation in hypertonic solutions, *D. californicum* increased greatly in size and often burst when transferred to distilled water.

**Table 2. Distribution of ions in *Deontostoma californicum*, exosmosed only (X), osmoregulated (R), and from seawater controls (C).**

Ion	Ash wt (mg) 12 <i>D. californicum</i>	Dry wt (mg)	Total ash cation ( $\mu\text{M/g}$ dry wt)	Water loss ( $\mu\text{l/g}$ dry wt)	Cation in seawater $\mu\text{g/ml}$	Cation uptake in regulation (R-X) $\mu\text{g}$	Cation increase (R-X) $\mu\text{g}$	Cation in tissue water increase equivalent of salt solution $\mu\text{g}$	Cation tissue water increase equivalent of seawater $\mu\text{g}$
Potassium					9.72	1.3	4.0	50.80	0.495
K <sup>+</sup> <sub>C</sub>	22.9	37.5	2.73	18.9					
K <sup>+</sup> <sub>X</sub>	23.4	38.6	8.62	18.9					
K <sup>+</sup> <sub>R</sub>	23.6	38.5	11.30	22.3					
Sodium					457.00	0.4	17.0	9.20	4.200
Na <sup>+</sup> <sub>C</sub>	22.9	37.5	44.10	18.9					
Na <sup>+</sup> <sub>X</sub>	23.1	37.9	60.70	14.0					
Na <sup>+</sup> <sub>R</sub>	23.8	38.9	78.30	14.7					
Magnesium					53.40	1.5	22.2	36.48	1.950
Mg <sup>++</sup> <sub>C</sub>	22.9	37.5	6.37	18.9					
Mg <sup>++</sup> <sub>X</sub>	22.8	38.0	16.50	11.0					
Mg <sup>++</sup> <sub>R</sub>	23.8	38.2	40.40	14.9					

Ligaturing experiments were conducted to isolate those regions of the body important in the osmoregulatory mechanism and in the uptake of ions. Using hypertonic 1 M NaCl, ligatures were tied with 7.0 Ethicon braided silk.

**RESULTS:** When ligatured in the middle of the body while still in seawater and placed in 1 M NaCl, both anterior and posterior halves of the body decreased in size. Both halves became grossly distended on being returned to distilled water. When ligatured behind the stoma and anterior to the anus, the nematodes became smaller in hypertonic media and following osmoregulation burst when returned to distilled water.

#### Localization of the area of water uptake

The ligaturing experiments indicated that both osmoregulation and the uptake of water occurred through the body wall. After osmoregulation for 30 min in 1 M NaCl (incomplete recovery), *D. californicum* was placed in dilute vital stains to determine whether the entry of water (and with it the stain) was in any pores or canals. Cotton blue, eosin, congo red, janus green B, crystal violet, and methylene blue were all used. The reverse process was also examined where stained worms were exosmosed to see any pores or canals from which the dyes diffused out.

**RESULTS:** Crystal violet (Gentian violet), eosin, and methylene blue entered best and colored the entire body contents. No canals were found and no pores were visible for water passage in or out of living worms.

#### The longitudinal movement of water in *D. californicum*

Worms from seawater were placed across a vaseline barrier separating distilled water and 1 M NaCl. This design contrasted with ligaturing as the environment was separated but the movement of ions and water in the worm was free and continuous through its whole length.

The two halves of the worm behaved independently with respect to osmotic change. Although the stains entered the portion of the worm in distilled water, there was no indication of the dye moving longitudinally toward that part of the worm in 1 M NaCl. This may have been due to adsorption of the dyes onto membranes preventing its free passage. Nevertheless, an appreciable longitudinal movement of water could not be detected in our techniques.

#### Discussion

The environment of all the animal parasitic nematodes examined to date are hypertonic to

the body fluids of the nematodes (Pannikar and Sproston, 1941; Lee, 1960; Rogers, 1962; Anya, 1966). It has been consistently demonstrated that these forms are able to osmoregulate well in hypertonic media. They are, however, less able or unable to regulate in hypotonic media.

In contrast the free-living *Rhabditis terrestris* in culture (Stephenson, 1942) and other *Rhabditis* species (Osche, 1952) and *Panagrellus redivivus* in water (Myers, 1966) inhabit hypotonic habitats and regulate well in hypotonic media. Isotonicity for these is about 0.15 M NaCl. Because of the apparent adaptation of osmoregulation to habitat, it has been suggested that marine nematodes would be unable to regulate their osmotic pressure (Rogers, 1962; von Brand, 1960; Lee, 1965). Isotonicity of *D. californicum* is, however, 0.6 M NaCl, or about four times greater than for any nematode known. Many marine invertebrates have poor powers of osmoregulation but *D. californicum* maintains an osmotic equilibrium in hypertonic media—a feature it shares with some animal parasitic forms.

These observations suggest that *D. californicum* is partially adapted to intertidal life and can tolerate exposure and higher salinity. This nematode may be poikilosmotic as suggested by Osche (1952) for free-living soil forms and Rogers (1962) for some animal parasitic nematodes.

Beadle (1957) emphasized the importance of ionic exchange and uptake in the regulation of osmotic pressure and water content. Hobson et al. (1952a, 1952b) showed that the body fluids of *Ascaris lumbricoides* changed in ionic composition following incubation in diluted seawater. Myers (1966) reported evidence of ionic absorption in the osmoregulation of *Panagrellus redivivus* and *Aphelenchus avenae*. Regulation of *D. californicum* relates to these findings in other nematodes, taking up ions in hypertonic solutions until osmotic equilibrium is reached with the environment. Bursting of the nematode upon re-entry in distilled water, together with the flame photometric demonstration of an increased concentration of ions following regulation, supported the theory of ionic uptake. There was no suggestion of an outward movement of ions either in hypotonic media or following over-osmoregulation in hypertonic solutions (Figs. 2, 4B).

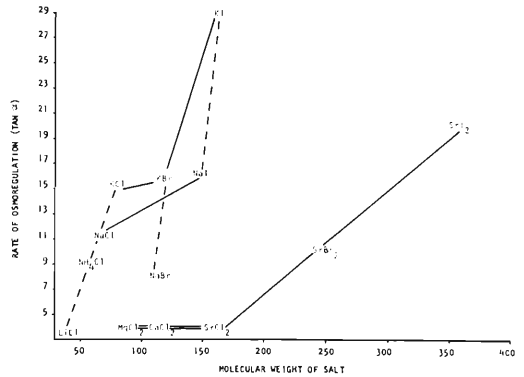
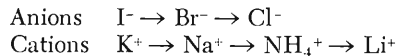


Figure 7. The relationship between molecular weight and the rate of recovery ( $\tan \alpha$ ); — joins common cations, ---- joins common anions, = joins the chlorides of divalent cations.

It may be seen from Figure 7 that the distribution of salts in a molecular weight against  $\tan \alpha$  (rate of osmoregulation) plot is not random. When using the chloride, bromide, and iodide salts of  $\text{Sr}^{++}$ ,  $\text{Na}^+$ , and  $\text{K}^+$ , iodides allowed the fastest rates of recovery, chlorides the slowest, and both  $\text{SrBr}_2$  and  $\text{KBr}$  were intermediary. The position of  $\text{NaBr}$  (Figs. 3, 7) cannot be explained, but von Brand (1943) showed it to be toxic to larval *Eustrongylides ignotus*. In a M wt  $\times$   $\tan \alpha$  plot (Fig. 7) the halogen salts of each cation had slopes of the same order. Approximately parallel lines join respectively:  $\text{NaI}$  and  $\text{KI}$ ,  $\text{NaBr}$  and  $\text{KBr}$ , and the chlorides of  $\text{Li}^+$ ,  $\text{NH}_4^+$ ,  $\text{Na}^+$ , and  $\text{K}^+$ .

The anionic and cationic sequence in decreasing order of preference in the regulatory mechanism is:



The Stoke's Law radii and ionic mobilities of  $\text{Sr}^{++}$  and  $\text{Ca}^{++}$  are practically identical and  $\text{Mg}^{++}$  is very similar (Table 3), corresponding to the rates of regulation in the chlorides of these ions (Fig. 7). The ionic mobilities and Stoke's Law radii of  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$  also form a series reflecting the rates of regulation of *D. californicum* (Fig. 7). Having the lowest M wt,  $\text{Li}^+$  has the highest charge density, greatest hydration, lowest molecular mobility and, therefore the highest Stoke's Law radius.

Of the anionic halogens the increasing order of preference for regulation is  $\text{Cl}^- \rightarrow \text{Br}^- \rightarrow \text{I}^-$



**Table 3. Ionic mobilities ( $\lambda^\circ$ ) Stoke's Law Radii, and energy of hydration ( $-\Delta_{HK}$  Kcal mole<sup>-1</sup>) of ions at 25 C.**

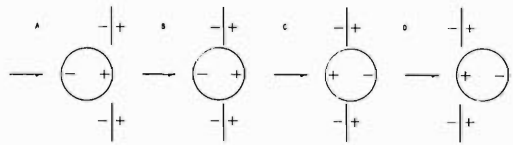
Ion	$\lambda^\circ$	Stokes' r	$-\Delta_{HK}$ Kcal mole <sup>-1</sup>
K <sup>+</sup>	73.50	1.25	- 83.37
Na <sup>+</sup>	50.10	1.84	-103.55
Li <sup>+</sup>	38.60	2.39	-129.67
Mg <sup>++</sup>	53.0	3.47	-473.29
Ca <sup>++</sup>	59.5	3.10	-394.50
Sr <sup>++</sup>	59.4	3.10	-359.22
I <sup>-</sup>	76.8	1.20	- 61.8
Br <sup>-</sup>	78.14	1.18	- 72.2
Cl <sup>-</sup>	76.35	1.21	- 80.3
SO <sub>4</sub> <sup>=</sup>		2.30	
Fe(CN) <sub>6</sub> <sup>3-</sup>		2.76	
Fe(CN) <sub>6</sub> <sup>4-</sup>		3.35	

Data after Noyes (1962) and Robinson and Stokes (1959).

(Fig. 7). These anions have low hydration, and Stoke's Law radii and their ionic mobility are essentially the same (Table 3). The series Cl<sup>-</sup> → Br<sup>-</sup> → I<sup>-</sup> does, however, manifest decreasing charge density and increasing polarizability. This significance of this polarizability appears to be correlated with increasing ease of recovery. A model scheme for a possible mechanism by which an anion could pass through a "pore" in the membrane is illustrated (Fig. 8).

The results suggest that in hypertonic solutions of single salts, ions pass in by selective diffusion until an equilibrium is reached for that salt. The "distention" over normal size in a regulated animal may be explained in part by the osmotic effect of normal residual soluble components, over and above that due to the electrolyte of hypertonicity. This is consistent with the two-step distention of an animal treated successively in two different salts. In a second hypertonic salt, following equilibrium in the first, there is a second equilibrium giving a greater resultant osmotic pressure, and further increase in size.

The recovery line R (Fig. 1) is approximately linear; the explanation for this may be found by the interaction of two opposed factors. The entry of ions by physical means would tend to be greatest at the onset of ionic entry ME (Fig. 1) and gradually become reduced. As ions enter and the worms dimensions increase, its surface area increases as a



**Figure 8. Schematic model illustrating a possible mechanism whereby polarizability of an anion facilitates its passage through a charged membrane of the body integument. A, anion approaching charged membrane; B and C show polar reversibility on passage through the "pore"; D, completion of entry.**

function of the square; there is a nonlinear increase, therefore, in the area for ionic uptake. The resultant of the decreasing rate of diffusion and increasing the surface area tends to give a linear recovery line.

It is suggested that the entry of the cation may be essentially dependent on differential ionic mobility, reflecting hydrated diameters and modified by the anion depending on its polarizability. Their entry depends on a living system possibly charged and has the characteristics of a one-way selective diffusion, there being no evidence for the outward movement of ions.

In attempting to explain the inability of SO<sub>4</sub><sup>=</sup> and other Group VI anions to enter, it was not possible to distinguish between the molecular structure or the multiple charge. Monovalent anions of like spatial configuration; ClO<sub>4</sub><sup>-</sup>, IO<sub>4</sub><sup>-</sup>, MnO<sub>4</sub><sup>-</sup> and unlike configuration, oxalic acid derivatives, as well as polyvalent anions Fe(CN)<sub>6</sub><sup>4-</sup>, Fe(CN)<sub>6</sub><sup>3-</sup> were either toxic or insufficiently soluble for testing.

The penetration of extremely dilute solutions of eosin, methylene blue, and gentian violet, would suggest that there is transfer of diverse ions and molecules through the integument, but that it is of an order several times removed from that involved in osmotic effects.

The inability to osmoregulate in hypertonic sugar solutions may rest on the very limited ability of nonelectrolytes to cross the integument (Mueller, 1929). The inability of Group VI anions to enter is consistent with the results of Tweedie and Segel (1967) and Yamamoto and Segel (1966) on *Penicillium chrysogenum* and *Aspergillus* sp. Krogh (1939) found that SO<sub>4</sub><sup>=</sup> did not enter marine invertebrates well; and Robertson (1957) found that SO<sub>4</sub><sup>=</sup> in

many marine invertebrates was usually below the equilibrium value for seawater. The inhibitory effect is probably of little importance in the ocean, where  $\text{SO}_4^-$  ion in the Dillon Beach locality are present in 2.64 parts per thousand at a salinity of 3.48% (Hedgpeth, 1964).

Nematode recovery can be visualized as involving several phases: (a) Passage of anions and cations through a selectively permeable membrane. The effect between the activity of the monovalent cation series and the monovalent anion series may be in part a reflection of manner of hydration. Since the water molecules around a cation extend their protons outward so that ions having a wide range of sizes may assume configurations more adaptable to the surrounding water structure while anions require the hydrogen of water molecules to be directed inward and as such, the configurations of the hydration spheres may be more dependent on the central ion size. (b) The formation of an osmotically active body solution, made up in part by imbibition of salts, increased solubilization of organic substances and an increase in bound water from increased tissue hydration such as may be induced by or reflected in cation mobility.

In view of experimental observation it is tempting to think that monovalent anions, depending upon their polarizabilities, control the passage of salts across the semipermeable membrane. The cations, however, are primarily involved in solubilization of organic components and enhanced hydration of tissues manifested in increased rates of recovery of body lengths, according to the series of increased ionic mobilities of monovalent cations.

The accumulation of  $\text{K}^+$  and possibly the other salts suggested in the flame photometric analyses, may be explained by the observed unidirectional movement inward of salts at high tide and the partial loss of water through drying at low tide over long periods.

If this were the case then it would be necessary to postulate an equally slow elimination of other cations  $\text{Na}^+$ ,  $\text{Mg}^{++}$  by metabolic means eg. excretion, loss through the gut, so that the balance with seawater was maintained.

The ligaturing experiments suggested that the uptake of ions occurred through the integument just under the cuticle. It was also found that the process did not depend on localized receptors, amphids or caudal glands all of

which have at some time been implicated in nematode osmoregulation. The gut was shown not to play an essential role in ionic regulation, although it probably contributes in the natural habitat.

There is good evidence that in hookworm larvae osmoregulation is restricted to the so-called excretory system as it is in flatworms (Weinstein, 1952 and 1960). This difference is consistent with the broad postulate in biology that marine organisms, which are usually isotonic with their surroundings, undergo ionic exchange and water loss over their entire surface. While with migration onto land or freshwater or another nonisotonic environment, comes a reduction in the surface area of such membranes and a localization of osmotically active surfaces.

### Summary

*Deontostoma californicum* was able to recover its osmotic pressure in hypertonic NaCl but not in hypotonic solutions. Osmoregulation was not possible in hypertonic nonelectrolytes and in  $\text{SO}_4^-$ , and other Group VI anions. In the other electrolytes used, the rate of recovery varied with ionic mobility and energy of hydration in cationic species with the degree of polarizability in anionic species. A hypothesis is presented that relates the chemical and physical characteristics of ions and the properties of the integumental membrane to the mechanism of ionic and osmotic regulation.

Using ligaturing experiments and aqueous dyes it was concluded that the uptake of ions and entry of water occurred over the entire body surface. Finally, our results have been compared to observations on other free-living and parasitic nematodes.

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## Fine Structure of the Somatic Muscles of the Free-Living Marine Nematode *Deontostoma californicum* Steiner and Albin, 1933 (Leptosomatidae)

W. DUANE HOPE<sup>1</sup>

Department of Invertebrate Zoology, Museum of Natural History, Washington, D. C.

The somatic musculature of nematodes is, in general, comprised of a single layer of longitudinally oriented, spindle-shaped muscle fibers. Each fiber has a noncontractile region, which gives rise to a "neuromuscular" process. The contractile region of the fiber is applied to the hypodermis of the body-wall and encloses an array of numerous ribbonlike bands or "fibers" (Chitwood and Chitwood, 1950). Schneider (1860) observed that these ribbons are perpendicular only to that portion of cell membrane adjacent to the hypodermis in some nematodes, whereas in others they may be perpendicular to the membrane at the base and sides of the cell as well. The former he designated as platymyarian and the latter coelomyarian muscle. Chitwood and Chitwood (1950) have since described cylindrical muscle cells in which the ribbonlike bands are perpendicular to the cell-membrane all around the cell, enclosing a central core of sarcoplasm, and which they termed circomyarian.

As others have indicated (Hanson and Lowy, 1960), our understanding of the structure of the contractile bands and other features of nematode muscle has not been advanced significantly in recent years by light microscope observations. But, recent electron microscope studies have contributed substantially to an understanding of the fine structure of nematode muscle. In a study of the coelomyarian muscles of *Ascaris lumbricoides* by Kawaguti and Ikemoto (1958c) and of *Parascaris equo-*

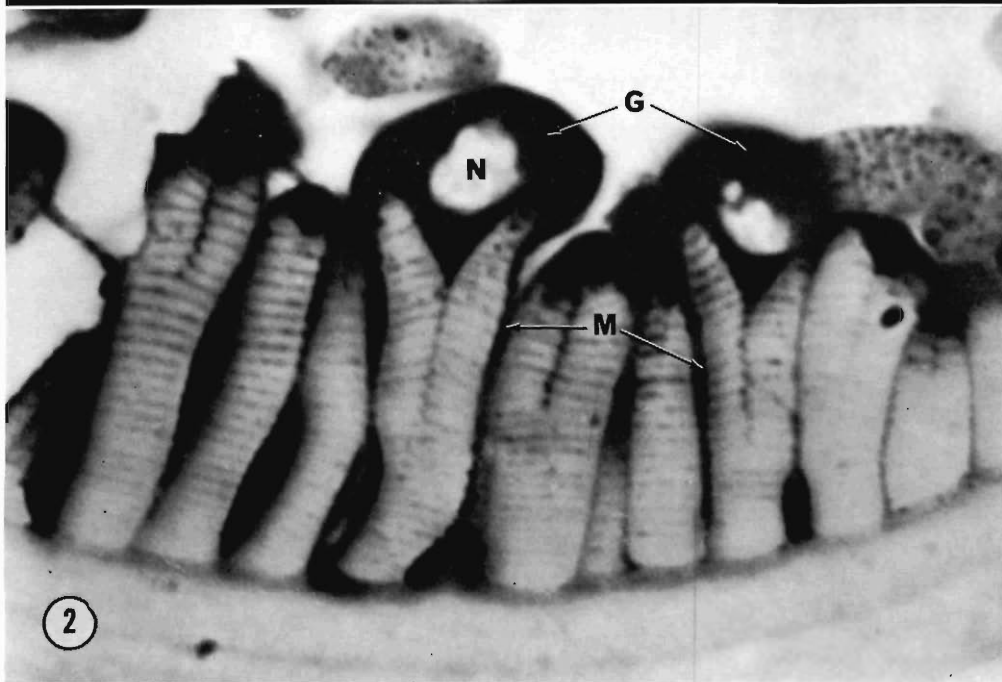
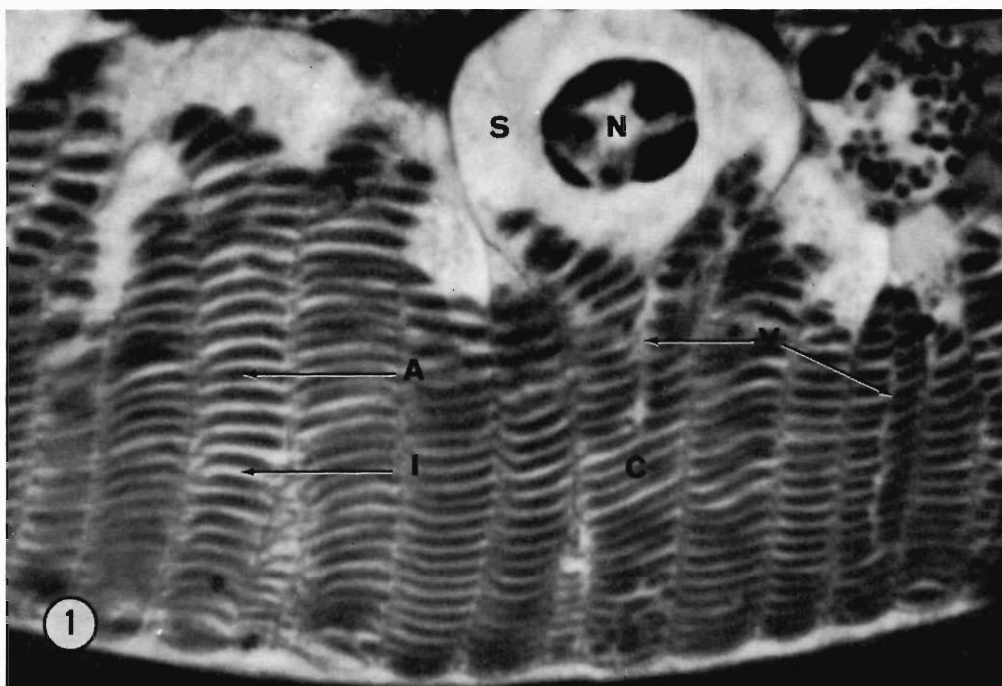
*rum* by Hinz (1963) it was demonstrated for the first time that the "contractile fibers" or "contractile ridges" of light microscope observations, are comprised of myofilaments. Hinz also mentioned the presence of invaginations of the sarcolemma which we would now recognize to be "T-tubules." But a "T-system," which is now a well-known structure of vertebrate muscle, was first clearly demonstrated in nematode muscles by Rosenbluth (1963; 1965a) and Reger (1964) in studies of *A. lumbricoides*. They also showed that cisternae of the sarcoplasmic reticulum are closely associated with the T-tubules forming both dyads and triads, and that both thick and thin myofilaments are present.

The arrangement of myofilaments in the type of muscle that occurs in nematodes differs from that in vertebrate cross-striated muscle. Our understanding of this arrangement began with early studies by Kawaguti and Ikemoto (1958c) on the muscle of *Ascaris* and on similar muscle occurring in annelids (1957b; 1958a, d), molluscs (1957a; 1961) and a sea squirt (1958b). Their studies revealed that these muscles have in common an oblique longitudinal striation and a repeating pattern of transverse bands of thick and thin filaments. Rosenbluth (1963; 1965a) and Ikemoto (1963) showed that these features were due to a uniform arrangement of longitudinally staggered myofilaments in muscle of *Ascaris* and an oligochaete, respectively. While these and other studies (Auber-Thomay, 1964; Jamuar, 1966; Lee and Miller, 1967; Watson, 1965a; Wright, 1964) have revealed that the

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Figure 1. Transverse section of muscle fibers showing noncontractile region (S) bearing the nucleus (N), and contractile regions (C) with horizontal basophilic (A) and nonstained zones (I) and vertical nonstained zones (V). Polyethylene glycol. Haematoxylin. ×2,150.

Figure 2. Transverse section. Dark stained material around the nucleus (N) is glycogen (G); that between cells is the glyco-protein of the sarcolemma (M). Paraffin. PAS. ×2,150.



arrangement of myofilaments is basically the same in most nematode muscle, they have also disclosed deviations in the extent of development and form of the "electron dense material" and sarcoplasmic reticulum, and in the presence or absence of a T-system.

The above studies constitute a significant advance in our understanding of nematode muscle, but further studies are desirable to understand not only the structural relationship between platymyarian, coelomyarian, and circomyarian muscle of nematodes, and between nematode muscle and obliquely striated and double obliquely striated muscle of other invertebrates, but the significance of variations of the sarcoplasmic reticulum and "electron dense material." In view of this, light and electron microscopy have been employed in a study of coelomyarian muscle of the free-living marine nematode, *Deontostoma californicum* Steiner and Albin, 1933, and the observations are compared with those from previous studies on nematodes and higher metazoans.

### Materials and Methods

Specimens were collected from sediment removed from holdfasts of the alga *Egregia* sp. from intertidal rocks at Dillon Beach, Marin County, California. All nematodes were retained in sea water at 15 C until fixed; some were fixed within 24 hours while others were kept in sea water at 15 C for up to one month.

**LIGHT MICROSCOPY:** Specimens used for general histological examination were fixed whole for 24 hours in 10% formalin in sea water. To avoid distortions that usually occur during paraffin embedding, the specimens were embedded in a 39 to 1 mixture of polyethylene glycol 1540 and 4000, respectively, by the following modification of the method of Riopel and Spurr (1962). Fixed specimens were washed for approximately 30 minutes in tap

water and then placed in a Bureau of Plant Industry Dish filled with a 2.5% aqueous solution of polyethylene glycol 400. The dish was covered with a cover glass to extend evaporation of the water over a period of about one week, in much the same way that nematodes are conventionally dehydrated in glycerine. This procedure, in lieu of passing the nematodes through a graded polyethylene glycol 400 series, eliminates collapsing of the body and minimizes distortion of tissues. After completing the embedding as recommended by Riopel and Spurr, the nematodes were sectioned at 5  $\mu$  on a rotary microtome and stained for 5 minutes in 0.1% aqueous hematoxylin at pH 2.4 by the method of Craig and Wilson (1937).

Fasted and nonfasted specimens to be examined for the presence of glycogen were fixed in Rossman's picric acid-alcohol-formalin fixative (Gray, 1954), embedded in paraffin, sectioned at 10  $\mu$ , and stained by the periodic acid-Schiff method. Control sections were treated with ptyalin.

**ELECTRON MICROSCOPY:** Whole specimens were fixed at room temperature for 20 minutes in a mixture of 1 volume of Acrolein and 9 volumes of M/10 solution of Sorensen's phosphate buffer adjusted to pH 7.4.

They were then rinsed in the buffer and cut into pieces approximately 2 mm long in 1% OsO<sub>4</sub> in veronal acetate buffer (pH 7.4) containing sucrose (Palades sucrose fixative) and post-fixed in the same solution for 1 hour.

After dehydration in cold ethanol, the tissues were embedded by the method of Wright and Jones (1965) in a mixture of 9 volumes of Maraglas 655, 1 volume of Cardolite Nc 513, and 0.25 volumes of DMP-30 (Freeman and Spurlock, 1962).

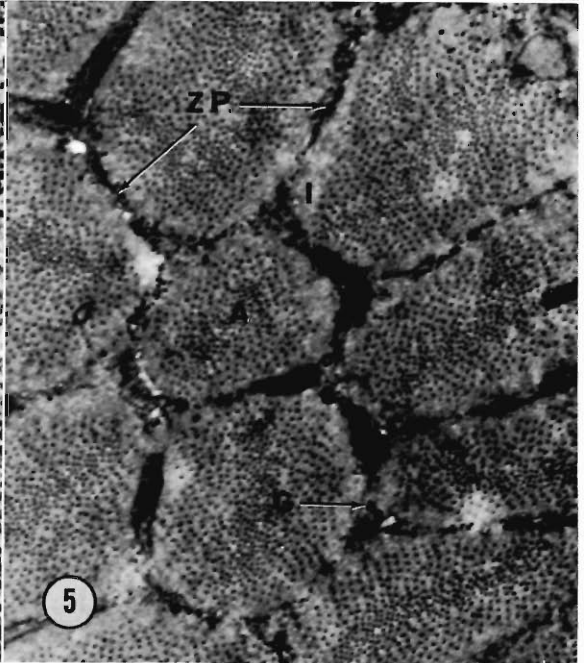
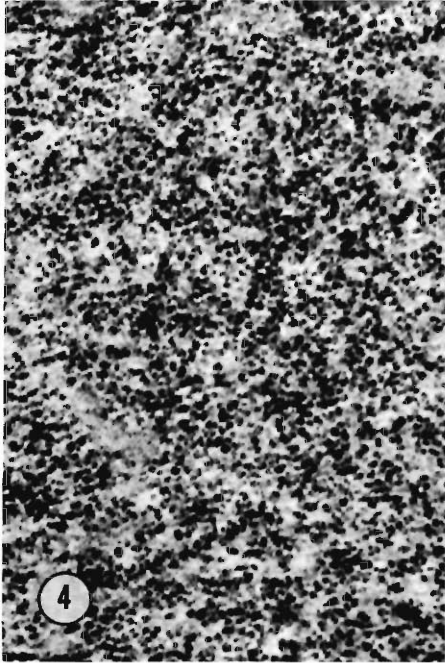
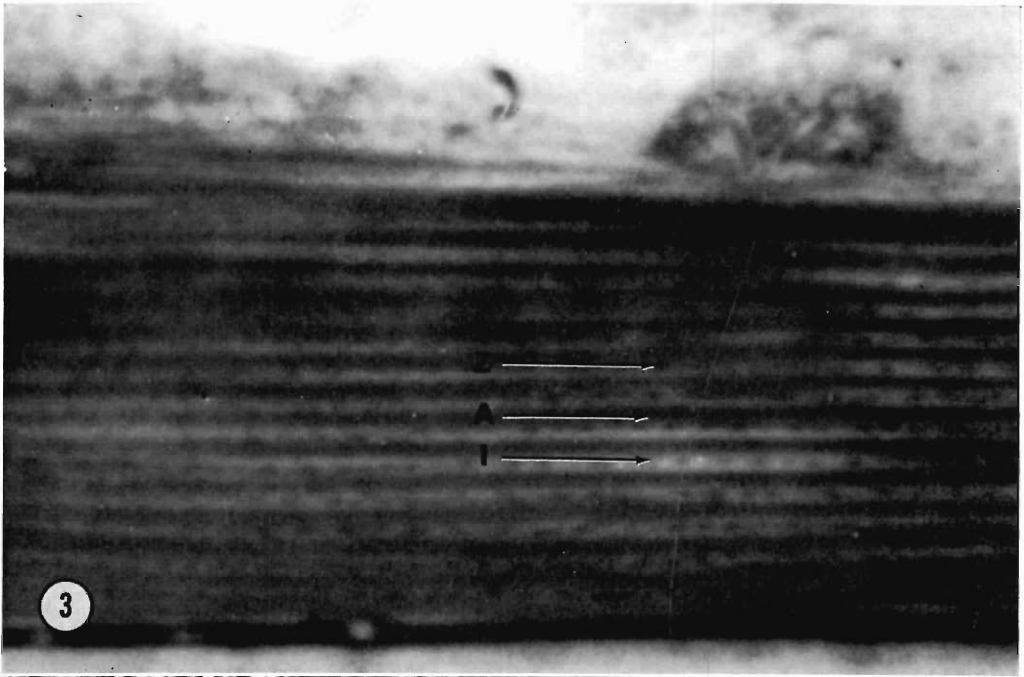
Sections were cut on LKB and Porter Blum ultramicrotomes, mounted on Formvar or

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**Figure 3.** Parasagittal section of a muscle fiber. Basophilic zones (A). Nonstained zones (I). Spindle shaped profiles (Z). Polyethylene glycol. Haematoxylin.  $\times 2,800$ .

**Figure 4.** Electron micrograph of glycogen particles in perinuclear sarcoplasm of nonfasted specimen. Lead hydroxide.  $\times 91,000$ .

**Figure 5.** Electron micrograph of cross-sectioned contractile region showing various profiles of thick zone (A and I Bands) material. Note the presence of glycogen particles (G) in the Z planes (ZP). Lead hydroxide.  $\times 33,800$ .



Formvar and carbon coated grids, stained with Watson's (1958) saturated lead hydroxide or with permanganate and lead citrate (B. L. Soloff, personal communication), and examined with a RCA EMU-3E (operated at 50 kv) or Zeiss EM 9 electron microscopes.

### Observations

**LIGHT MICROSCOPY:** The body wall musculature of *D. californicum* consists of a single layer of longitudinally oriented, spindle-shaped muscle fibers. In cross-section, an average of 24 fibers appear in each midbody quadrant. The more obvious features of each fiber include the enlarged, noncontractile region, which extends into the pseudocoelom, and a relatively slender contractile region, the base of which is adjacent to the hypodermis (Fig. 1).

The noncontractile region contains a nucleus and substantial quantities of PAS positive material (Fig. 2), which is removed by ptyalin digestion. From these observations, and those made with the electron microscope (see below), it is concluded that this material is glycogen.

The contractile portion of the fiber, when viewed in cross-section, appears to be comprised of basophilic zones which usually pass uninterrupted from one side of the fiber to the other. These zones are separated from one another by nonstained zones that also pass from one side of the fiber to the other (Fig. 1). Sometimes, however, nonstained zones occur in or near the sagittal<sup>2</sup> plane of the muscle fibers as well, extending varying distances from the perinuclear sarcoplasm toward the base of the fiber and separating the stained zones of this region into right and left halves (Fig. 1). The vertically oriented nonstained zones resemble the sarcoplasmic core of *Ascaris* muscle, but differ in being narrower, not extending as far toward the base of the fiber, and in appearing less frequently.

It is evident from parasagittal sections of the fiber, that the basophilic and nonstained zones described above are ribbonlike and extend over much of the length of the fiber (Fig. 3). In the same sections, small spindle-shaped profiles are spaced at rather regular intervals in

the nonstained zones, thus resembling similar structures appearing in longitudinal and oblique sections of *Ascaris* (see Rosenbluth, 1965a).

The entire fiber is enclosed in a PAS positive sarcolemma (Fig. 2) which remains reactive after ptyalin digestion.

**ELECTRON MICROSCOPY:** The sarcoplasm of the noncontractile region of muscles in nonfasted specimens contains a high density of lead-stained particles 100–230 Å in diameter (Fig. 4). These are surmised to be particles of the beta form of glycogen because of their size, form, and staining properties, and also their distribution is coincident with the PAS positive, ptyalin-digestible substance observed in light microscope preparations. Glycogen particles may also occur in the contractile portion of fibers (Fig. 5). Alpha particles occurred infrequently in all sections examined, and some specimens maintained in sea water for as little as one week without food, contained little glycogen in either the noncontractile or contractile regions (Fig. 6).

Mitochondria occur in the noncontractile region and occasionally were observed subjacent to the cell membrane in the contractile region as well. Slender strands of presumably noncontractile filaments and vesicles bound by smooth membranes are also evident in noncontractile regions and more readily observed in specimens depleted of glycogen. Golgi zones were not observed.

Low magnification electron micrographs of cross-sectioned muscle fibers reveal, in the contractile region, an arrangement of wide transverse zones containing small circular profiles of cross-sectioned filaments and, alternating with the above zones, much narrower zones containing bars of electron dense material (Fig. 6). This alternating pattern superficially resembles the stained and nonstained zones of light microscope observations (Fig. 1), but the difference between the widths of the stained and nonstained zones is not nearly so great as that between the wide and narrow zones of electron microscope observations. Commonly, the wide zones extend uninterrupted from one side of the fiber to the other, each appearing in cross-section as a broad rectangle with its longer sides parallel to the base of the fiber. Occasionally, in the apical region of the fiber, each wide zone may be intercepted by vertical

<sup>2</sup> Sagittal (para-) is used here to indicate longitudinal sections whose plane extends from the base of the fiber to the apex; frontal sections are also longitudinal with the plane extending between the right and left sides of the fiber.





Figure 6. Electron micrograph of cross-sectioned muscle showing wide zones (W) and narrow zones (ZP) with bars of electron dense material (Z). Note absence of glycogen in sarcoplasm at upper left and vertical Z line at right (V). Permanganate and lead citrate.  $\times 25,500$ .

narrow zones located near the sagittal plane of the fiber (Fig. 6) resembling the vertical non-stained zones from light microscope observations. When separated in this manner, the wide zones on one side of the fiber are usually at the same level as wide zones of the opposite side; less frequently are wide zones opposite narrow zones. Sometimes, several irregularly oriented narrow zones divide wide zones into profiles of various shapes, including circles (Fig. 5) and triangles, immediately basal to the nuclear region and at the base of the cell as well.

Examination of cross-sections at higher magnifications shows that the wide zones are composed of two sizes of myofilaments, the thicker with a maximum diameter of about 200 Å, and the thin about 60 Å. These filaments are distributed so as to form five transverse bands in each thick zone. That is, there are two outer bands of thin filaments (Fig. 7, i); medial to these are two comprised of both thick and thin filaments (Fig. 7, a); and in the median plane of each thick zone, a single band of thick filaments only (Fig. 7, h). This pattern is characteristic of obliquely striated muscle (Ikemoto, 1963; Rosenbluth, 1963; 1965a) and the bands are comparable to the I, A, and H bands, respectively, of vertebrate striated muscle. I bands occur on both sides of thin zone material regardless of the orientation of the latter. The filaments of the I bands do not stain with hematoxylin as employed in this study, and so the nonstained zones described above correspond to the I bands and thin zones as well, which they flank. Thus, the nonstained zones are not wholly comparable to the thin zones of electron microscope observations.

Thick filaments closest to the Z bands are slightly smaller in diameter than those of the H band and, therefore, the thick filaments are assumed to be slightly tapered as in *Ascaris* (see Rosenbluth, 1965a). Cross-linking between thick and thin filaments in the A bands

was not observed, nor did there appear to be a regular arrangement of thin filaments around the thick. Thin filaments extend to the margin of the narrow zones and here they frequently appear to be segregated in small clusters (Fig. 7) much as has been found in the case of *Ascaris lumbricoides* (see Rosenbluth, 1965a).

From parasagittal sections it is evident that the wide zones extend uninterrupted for considerable distances through the fibers in a longitudinal direction, slightly oblique to the longitudinal axis of the fiber. The bands of wide zones are not nearly as distinct in these sections as in those that are transverse, but it is at least possible to identify the A bands (Fig. 8).

The narrow zones are characterized at low magnifications by bars of electron dense material, the extent of which varies from almost none in some narrow zones to uninterrupted bars passing from one side of a fiber to the other. Most frequently, however, the dense material appears one to several times for short distances in cross-sections of narrow zones and often appears closely applied to, or in some way fused with, the plasma membrane at the sides of the fiber (Fig. 6).

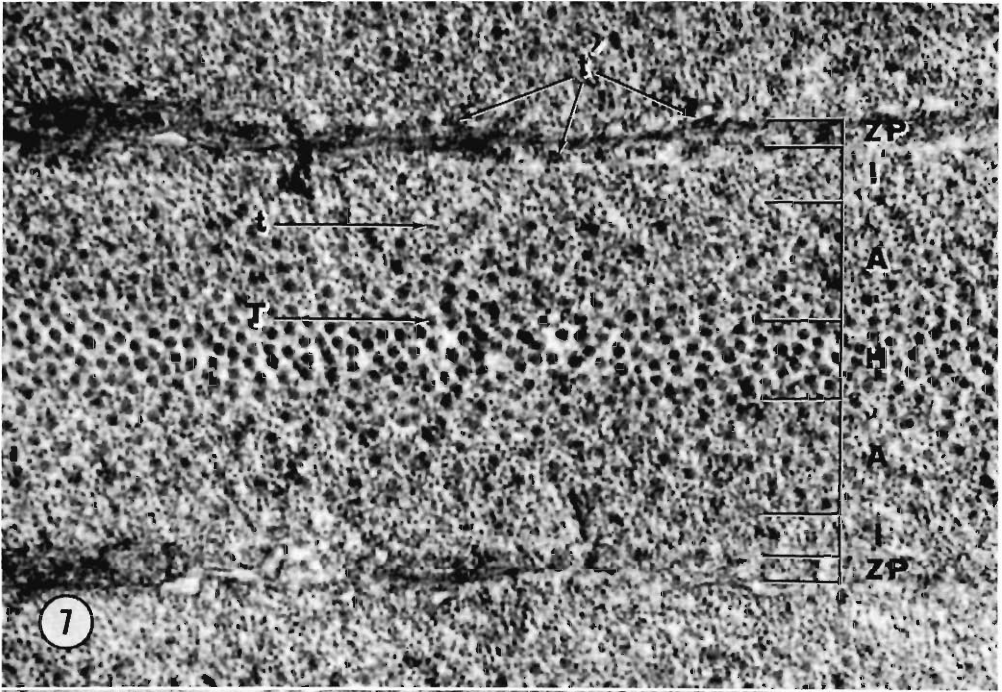
In longitudinal sections, the electron dense material is apparent as a series of spindle-shaped profiles regularly spaced in a longitudinal plane that is slightly oblique to the longitudinal axis of the fiber. Their periodicity is about 2.0  $\mu$  which agrees very closely with comparable measurements made from photomicrographs. A narrow strand, presumably a bundle of thin filaments, appears to extend from each end of each spindle, and merge with the filaments of the I band (Fig. 8), as has been observed in the case of *Nippostrongylus* muscle (Jamuar, 1966). Consequently, the filaments from one end of a spindle pass into the I band below, and those from the opposite end pass into the I band above the spindle. This apparent continuity between electron

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Figure 7. Cross-sectioned muscle showing thick (T) and thin (t) filaments arranged into I, A, and H bands. Note clusters of thin filaments (t') at the margin of the Z plane (ZP). Permanganate and lead citrate.  $\times 81,900$ .

Figure 8. Electron micrograph of a parasagittal section showing the thick filaments (T) of the wide zones (A bands) and what are interpreted to be clusters of thin filaments (t') continuous with cross-sectioned profiles of Z bars (Z). Permanganate and lead citrate.  $\times 34,400$ .



dense material and I band filaments suggests that at least a portion of the electron dense material is a Z component comparable to the Z band material of vertebrate striated muscle. For this reason, and for reasons given in the discussion, the bars of electron dense material will be referred to here as Z bars, and the oblique plane bearing them, the Z plane. Frontal sections of muscle fibers disclose that the Z planes also bear fibrils oriented at right angles to the myofilaments. In some instances it appears that the fibrils are adjacent to, but not enmeshed with the Z bars (Fig. 9), while in other cases these components do appear to be joined with each other (Fig. 10). It is also possible, however, that the latter may be an illusion attributable to superposition.

Sarcoplasmic reticulum in the form of narrow tubules and dilated vesicles also occurs consistently in Z planes. The tubules are usually closely applied to the surface of the Z bars so as to separate the latter from the I bands; in fact, wherever a Z bar is present in cross-sections, at least one and usually both of its surfaces bear sarcoplasmic reticulum (Figs. 11 and 12). Tubules of sarcoplasmic reticulum do not alternate with Z bars in the Z plane as they do in *Glycera* (see Rosenbluth, 1968).

Seldom is the lumen of the tubules more than 400 Å wide and often it is so narrow that a lumen is hardly perceptible (Figs. 11, 12). Vesicles, on the other hand, commonly have a diameter approximately equal to the width of the narrow zone (Figs. 12, 13). In the Z plane they occur most frequently in those areas devoid of Z bars. Continuity between the tubules and vesicles is clearly evident in numerous instances (Fig. 12), and tubules from both surfaces of the dense material may have continuity with a common vesicle (Fig. 12). Although it was not possible to resolve the unit membrane structure of the sarcoplasmic reticulum, or to measure accurately its membrane width, membranes enclosing vesicles do not appear to differ from those enclosing tubules.

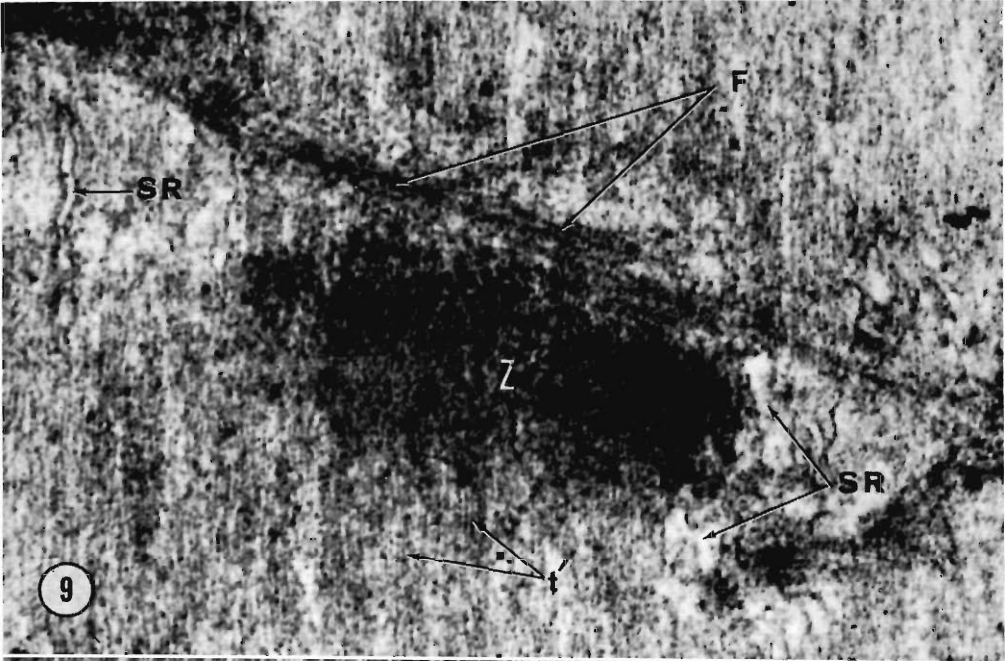
Furthermore, tubules and vesicles associated with the Z plane proper have a similar electron translucent medium within them.

Fingerlike projections of the vesicles may extend into the I band (Figs. 9, 10) where, in cross-section, they appear as small circular profiles (Fig. 12). Therefore, the sarcoplasmic reticulum is not entirely restricted to the Z plane. In no instance was it possible to identify dyads or triads within the Z plane, although vesicles (diads) occur subjacent to the lateral sarcolemma (Figs. 13, 14). The long axis of the vesicles, measured from cross-sections of the fiber, has an average length of 0.34  $\mu$  and the short axis, 0.12  $\mu$ . The average distance separating the plasma membrane from the membrane of the vesicle is 120 Å. In some instances the lumen of the vesicle is completely filled with a rather electron dense, granular material (Fig. 14), while in others it may have relatively little (Fig. 13). The association between the vesicles and the plasmalemma is here regarded as a diad and is in accord with similar views stated by Rosenbluth (1968) regarding a comparable structural arrangement in *Glycera* muscle.

A T-system was not observed in this muscle, but two types of shallow invaginations of the sarcolemma do occur and both have asymmetrical unit membranes. The first type of invagination (Fig. 15) more closely resembles the T-tubules of *Ascaris* (Reger, 1964; Rosenbluth, 1965a; and Watson, 1965b) in that they have been observed only at the level of the Z planes and have a diameter comparable to that of *Ascaris* T-tubules. They differ, however, in being very infrequent, shallow, and sarcoplasmic reticulum has never been observed near them as it occurs in diad and triad formations of *Ascaris*. The second occurs in both the noncontractile and contractile regions of the fibers. It is characteristically a very narrow infolding, the opposing membranes separated by a uniform distance of 80 Å. The infoldings may be rather extensive, occasionally

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Figures 9 and 10. Electron micrographs of frontal sections demonstrating portions of Z bars (Z) and thin filaments (t) which become continuous (t') with them. Filaments (F) presumed to be of the cyto-skeletal system, traverse the Z plane at nearly right angles to the thin actin filaments. Note tubules and vesicles of sarcoplasmic reticulum (SR) extending into the I bands. Permanganate and lead citrate. Figure 9,  $\times 80,800$ . Figure 10,  $\times 81,700$ .



appear to ramify, and always remain close to the cell surface (Fig. 16). They have never been observed to extend between bands of filaments, but may pass between filaments and subsarcolemmal cisternae, although in this instance, the distance between the membranes of the subsarcolemmal cisternae and the infoldings may be less than that between the former and the cell surface.

The sarcolemma of *Deontostoma* muscle consists of a cell membrane 100 Å wide and an extracellular coating 1.5 μ wide. It is likely that this coating is responsible for the PAS positive staining both before and after ptyalin digestion, and is a form of glycocalyx which invests cells of many types (Fawcett, 1966). Unlike *Ascaris*, however, it is not comprised of bands or lamellae.

Wright (1966) reported the occurrence of cytoplasmic bridges between muscles of *Deontostoma californicum* which were again observed in this study.

### Discussion

Light and electron microscope studies of *D. californicum* reveal that its muscles have the usual noncontractile portion which is located along the pseudocoelomic side of the fibers and a more slender contractile region adjacent to the hypodermis.

**NONCONTRACTILE REGION:** No attempt has been made to study this region of the muscle in detail, but it is worth noting that it contains the nucleus, mitochondria, noncontractile fibrils, sarcoplasmic reticulum, and glycogen particles as has been reported for other species (Wright, 1964; Reger, 1964; Rosenbluth, 1965b; Lee and Miller, 1967). The abundance of glycogen is particularly impressive as the intensity of the PAS reaction was much greater in somatic muscle than in any of the

other tissues including the gut which gave the next most intense reaction. Large deposits of glycogen have been found in *A. lumbricoides* and *Diriofilaria immitis* (Rosenbluth, 1965b; Lee and Miller, 1967). Rosenbluth has pointed out that the noncontractile region of the muscle may serve as a glycogen storage depot which may sustain the parasite during periods in which its host is fasting. This is very likely the case, but because of comparable deposits of glycogen occurring in *D. californicum*, a free-living species, it is apparent that storage of glycogen in the muscle belly is not strictly an adaptation to meet the vicissitudes of parasitism.

**CONTRACTILE REGION:** Differences between *Deontostoma* muscle and the muscle of other nematodes are to be found among components of the Z plane, namely the electron dense material or Z bars and the sarcoplasmic reticulum.

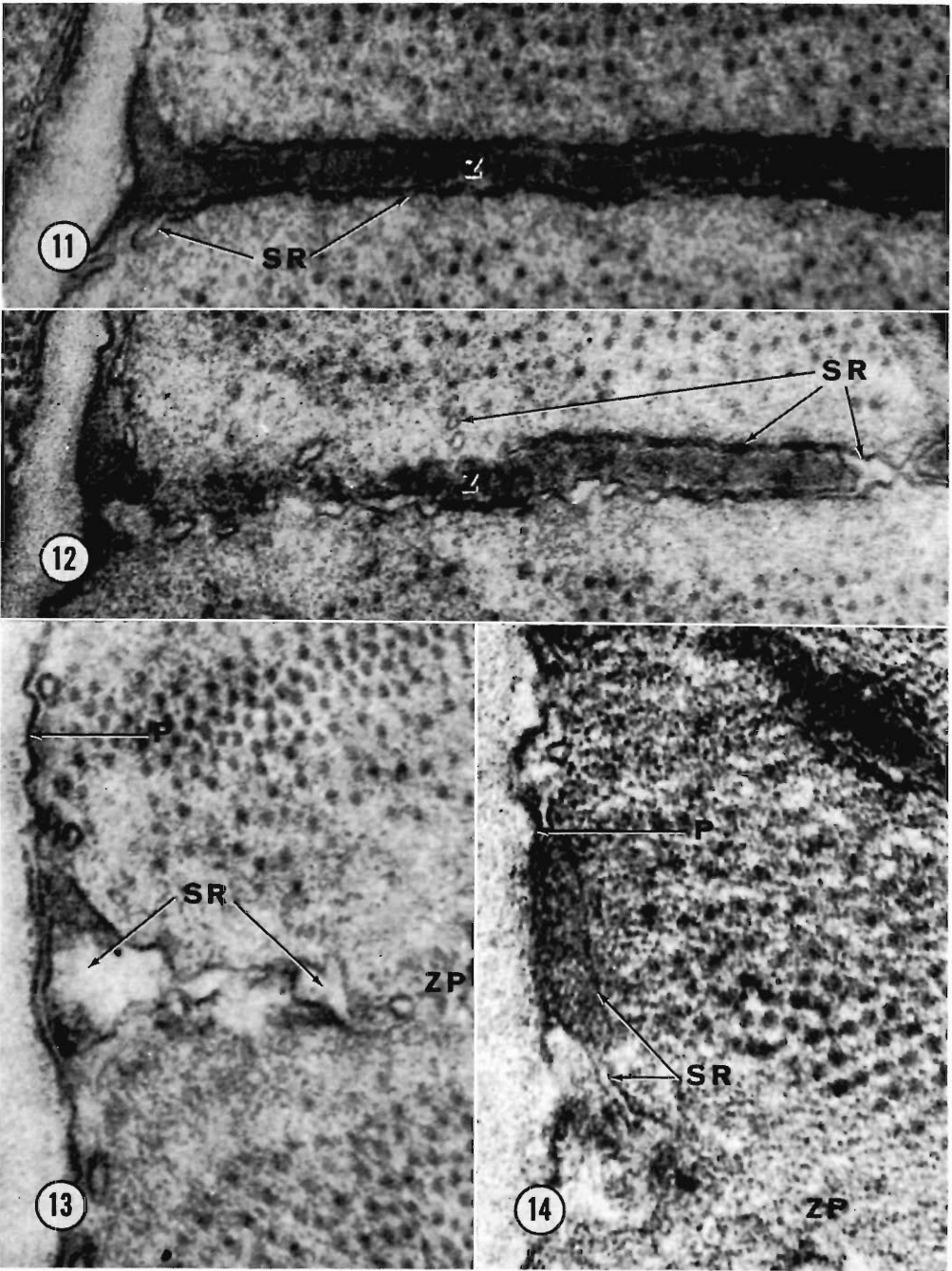
This electron dense material may extend uninterrupted for considerable distance across the width of a fiber or may appear intermittently in the same Z plane. In photomicrographs and electron micrographs of parasagittal sections of *Deontostoma* muscle, it can be seen that the obliquely regimented profiles of the dense material occur with regularity that suggests they may be an integral part of the staggered bands of myofilaments. Indeed, it can be seen at higher magnifications of the same sections (Fig. 8, Z) that each profile is tapered and appears to be continuous with thin filaments of adjacent I bands. For these reasons it is suggested that at least a part of the electron dense material of this muscle is Z material; comparable to the constituents of Z bands in vertebrate muscle. A second component in the Z bars is evidenced by frontal sections in which filaments (Figs. 9 and 10, F), presumably of the cyto-skeletal

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Figures 11 and 12. Electron micrographs of cross-sectioned muscle cell showing Z bars (Z) and tubules and vesicles of sarcoplasmic reticulum (SR). Note continuity between tubules and vesicles. Lead hydroxide. × 76,340.

Figure 13. Electron micrograph of cross-sectioned muscle fibers demonstrating an enlarged vesicle of sarcoplasmic reticulum (SR) closely applied to the plasma membrane (P) forming a diad at the level of the Z plane (ZP). The vesicle is partially filled with granular material. Lead hydroxide. × 97,400.

Figure 14. Same as Figure 13, but the diad is at the level of an A band with a tubule directed into the Z plane. The vesicle is filled with granular material. Permanganate and lead citrate. × 156,000.



system, are observed traversing the Z plane at right angles to the longitudinal axis of the fiber. These filaments were observed infrequently which suggests that they either occur infrequently or that, if they are a common component of Z bars, they are fused or enmeshed with the Z component and, therefore, difficult to identify.

Z bars, or their counterpart have not been described in detail for nematodes except in the case of *Ascaris*. According to Rosenbluth (1965a) the dense bands (Z planes) in *Ascaris* contain "bundles of fibrils" (presumably of the cyto-skeletal system) which appear in cross-section as tightly packed ovoid "dense bodies" and a second component, which he designates as "Z bundles," comprised of "small, sheaflike aggregates" of the thin filaments in which the latter "appear to be linked together." These bundles occur not only at the edges of the I zones but within it as well. He further states that "thin filaments sometimes seem to join the dense bodies" and later (1967), without further comment, states that the "thin filaments insert into opposite ends of the dense bodies." From these comments it is not entirely clear whether in *Ascaris* muscle there are Z bundles in addition to dense bodies, or whether the dense bodies themselves are the Z junctures between "sarcomeres."

What are interpreted to be aggregates of thin filaments occur in the Z plane of *Deontostoma* muscle (Fig. 7,  $\tau$ ), as in *Ascaris*. However, it is suggested that these are not Z bundles, but are aggregates of thin filaments grouped together as they converge upon (or emerge from) the Z bars. A similar situation may exist in *Nippostrongylus* muscle, since it

appears that there are aggregates of thin filaments in the Z plane (Lee, 1966) and that clearly these are bundles of thin filaments continuous with the Z component (Jamuar, 1966). By this interpretation, the structure in *Ascaris*, designated by Rosenbluth as "Z bundles" and "ovoid dense bodies," may in reality be aggregates of converging or diverging thin filaments and ovoid Z bodies, respectively.

From their study on *Dirofilaria immitis* muscle, Lee and Miller (1967) concluded that what had been termed "supporting fibers" in the myofibrillar and amyofibrillar area of nematode muscle, are identical with the Z bundles of Rosenbluth (1965a). But evidence of fibrils traversing the Z plane at right angles to the myofilaments in *Deontostoma* muscle, supports earlier concepts of a system of cytoskeletal fibrils separate and distinct from the contractile filaments.

Whereas in *Ascaris* the Z junctures are in the form of an ovoid dense body, and barlike in *Deontostoma*, they apparently have the form of dense thickenings subjacent to the plasma membrane in *Capillaria hepatica* muscle studied by Wright (1964). The distribution of these dense areas along the base and sides of the fibers suggests that it is coelomyarian, but highly specialized, and possibly degenerate. The fact that the Z juncture is plaquelike and limited to the periphery of the cell, not extending across the fiber as a series of dense bodies or a bar, may explain the absence of distinct transverse bands of thick and thin filaments.<sup>3</sup>

The Z junctures in *Nippostrongylus brasil-*

<sup>3</sup>Wright (1964) reported myofilaments of but one diameter, but disclosed in personal communication that he has since found thin filaments as well.

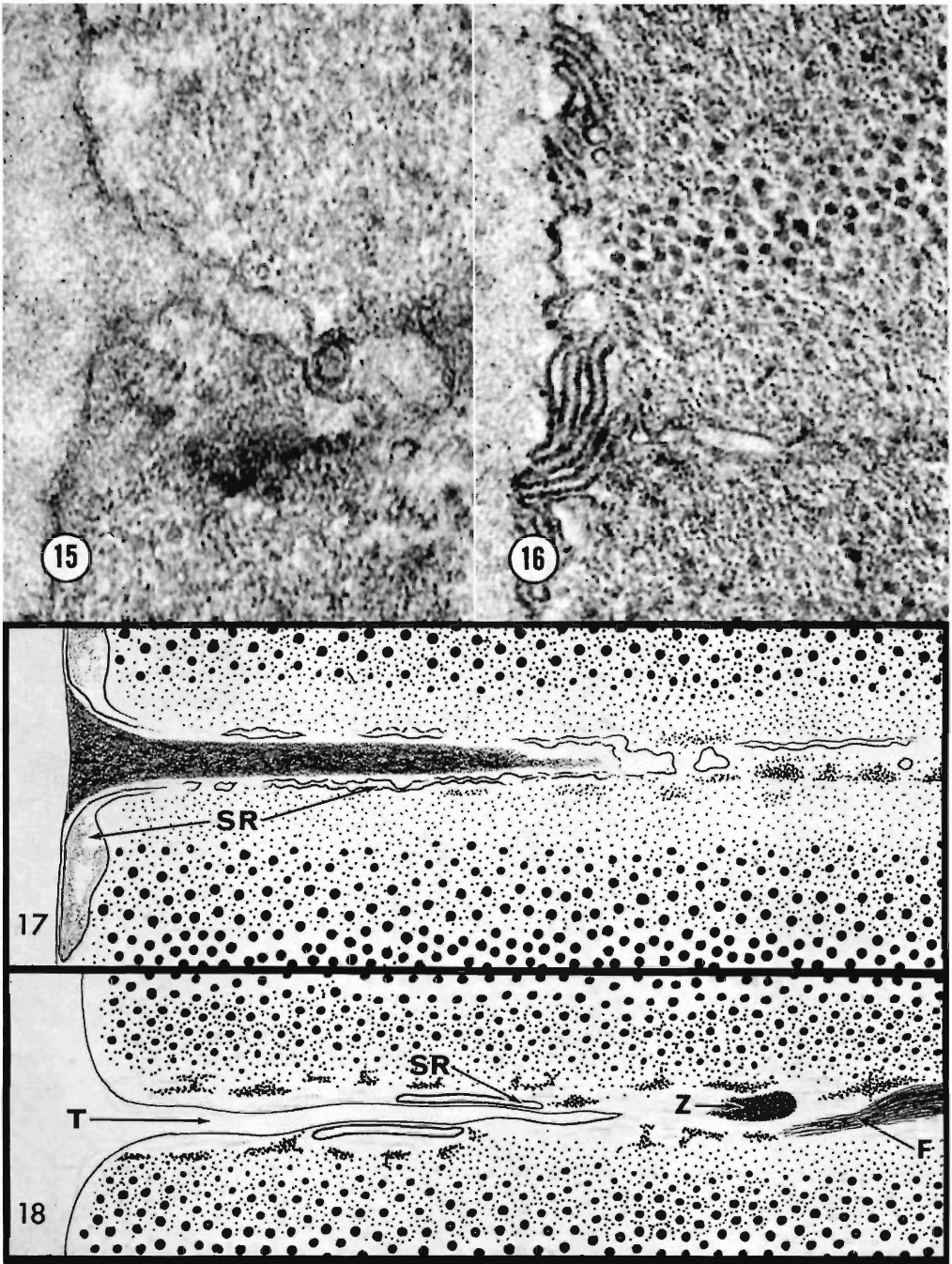
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Figure 15. Cross-section of a fiber demonstrating one of the few observed examples of relatively broad invaginations of the sarcolemma located only at the level of the Z plane. Permanganate and lead citrate.  $\times 131,200$ .

Figure 16. Cross-section of a fiber with examples of the numerous narrow invaginations of the sarcolemma that occurred in contractile and noncontractile regions of the fiber. Permanganate and lead citrate.  $\times 98,200$ .

Figure 17. Composite illustration of the Z plane of *Deontostoma* muscle. Electron dense Z material presumably with incorporated filaments of cytoskeletal system (Z); vesicles and tubules of sarcoplasmic reticulum (SR).

Figure 18. Composite illustration of the Z plane of *Ascaris* muscle based on the authors interpretation of electron micrographs published by Rosenbluth (1965A; 1967). T-tubule (T); electron dense Z material with fibrils of cytoskeletal system (Z); sarcoplasmic reticulum (SR); and filaments of the cytoskeletal system (F).





*iensis* muscle are also of interest, not because of the shape of the juncture itself, which appears to be rodlike, but in that they are oriented at right angles to the plasma membrane at the base of the fiber instead of at the sides and are, therefore, platymyarian. The significance of this is discussed below with regard to oblique striation.

The above variations in the Z component of nematode muscle, with the possible exception of *C. hepatica*, can also be found among the obliquely striated and double obliquely striated muscle of annelids and molluscs. The Z component appears to have the general shape of an ovoid structure in the heart muscle (J-granules; Kawaguti, 1963), and mantle muscle of cuttlefish (J-particles; Kawaguti and Ikemoto, 1957a), and is more rodlike in leeches (J-rods, compact sarcotubules and cross filaments; Kawaguti and Ikemoto, 1958a; Pucci and Afzelius, 1962; Rohlich, 1962), polychaetes (J-rods; Z lines; Kawaguti and Ikemoto, 1958d; Rosenbluth, 1968) and oligochaetes (J-rods; Ikemoto, 1963). But in all instances these structures are located in what is comparable to the Z plane of *Deontostoma* muscle, and, in many instances, there is evidence that thin filaments are continuous with them (Kawaguti and Ikemoto, 1957a, b; 1958a, b, c; Rohlich, 1962; Rosenbluth, 1968; Ikemoto, 1963).

At present it seems that the greatest difference between Z structures of nematode muscle, on the one hand, and those of the obliquely striated muscles of higher metazoans on the

other, is that the former appears to have a component of cytoskeletal fibrils which have not been observed in the latter. Otherwise, the Z structures seem to be quite comparable in obliquely and double obliquely striated muscle, and it seems they are also comparable to the Z bands of vertebrate cross-striated muscle.

**SARCOPLASMIC RETICULUM:** The sarcoplasmic reticulum of the Z plane of *Deontostoma* muscle is extensively developed while a T-system is lacking or poorly developed. In *Ascaris*, on the other hand, the sarcoplasmic reticulum is not as extensive and a T-system is well developed. These differences are manifest in the basic arrangement of Z plane components. Transverse sections of *Deontostoma* muscle fiber disclose that, when all components of the Z plane co-exist, the electron dense material is medial in the Z plane and the narrow membrane bound cisternae are peripheral to it. Diad or triadlike formations were never observed in the Z plane (Fig. 17). In *Ascaris*, by contrast, the medial region of the plane is occupied by a relatively broad membrane-bound structure (T-tubule) with the dense material and narrow cisternae of sarcoplasmic reticulum (which are the lateral elements of the diads and triads) displaced to the periphery of the Z plane (Fig. 18). Commonly, relatively broad vesicles limited by membranes (which are sarcoplasmic reticulum) may occupy the entire width of the Z plane in *Deontostoma* muscle, superficially resembling the T-tubules of *Ascaris*. However, when this

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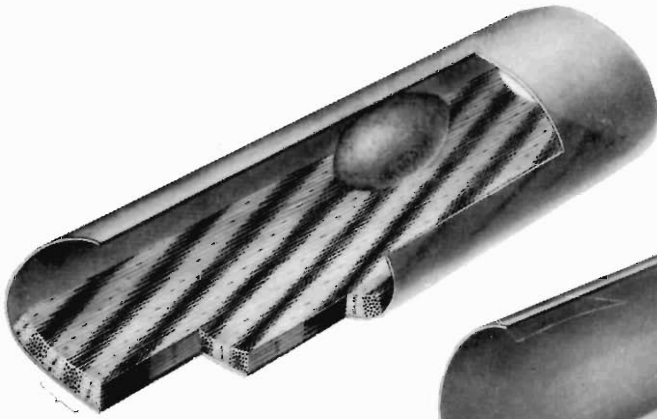
Figure 19. Cut-away diagram of platymyarian muscle fiber depicting the patterns of oblique I and A bands (H bands not shown) and Z planes. Based on the authors interpretation of electron micrographs published by Lee (1966) and Jamuar (1966).

Figure 20. Cut-away diagram of a coelomyarian muscle cell showing the pattern of double oblique striation (partial helix) that would result if the platymyarian arrangement of oblique bands were extended onto opposing sides of the fiber. Based on the authors interpretation of electron micrographs published by Rosenbluth (1965A; 1967).

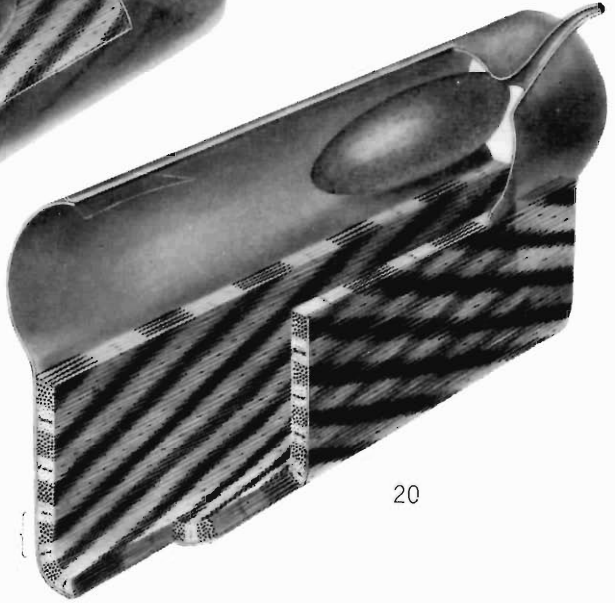
Figure 21. Cut-away diagram of circomyarian muscle fiber showing double oblique striation that would result if the platymyarian arrangement of oblique striations were extended around the entire periphery of the fiber forming complete helices. Inferred from authors interpretation of data by Chitwood (1951), Lee (1966), Jamuar (1966), and Rosenbluth (1965A; 1967).

Figure 22. Enlarged portion of comparable contractile region bracketed on each of the cut-away diagrams. The same sequence of H, A, and I band and Z planes occurs in all obliquely and double obliquely striated muscle.

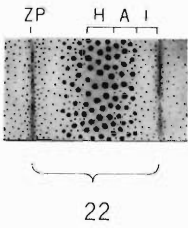
Figure 23. Cut-away diagram of *Deontostoma* muscle showing the probable arrangement of bands and Z planes inclined in the same direction and at the same angle in both right and left sides of the fiber.



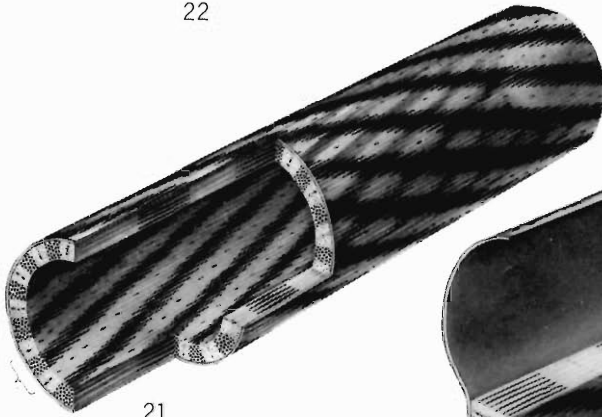
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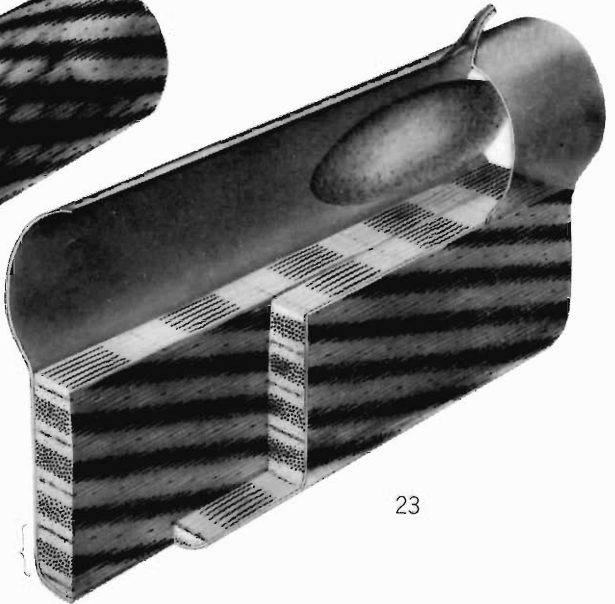
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situation exists, electron dense material and membrane-bound vesicles are not peripheral to it. That these dilated vesicles are components of the sarcoplasmic reticulum is further evidenced by their continuity with the much narrower cisternae. Continuity between the sarcolemma and either the vesicles or cisternae was never observed and, since the plasma membrane of the fiber has asymmetrical unit membrane structure, while unit membrane structure of the sarcoplasmic reticulum could not be resolved, it seems unlikely that any of the membrane-bound vesicles within the Z plane are derived from the sarcolemma. Rather, it is interpreted that the membrane-bound structures within the Z plane and subjacent to the sarcolemma are components of the sarcoplasmic reticulum and the vesicles lying adjacent to the sarcolemma, situated either above or below Z planes, are associated with the sarcolemma so as to form diads.

Sarcoplasmic reticulum in the form described for *Deontostoma* has not been previously described for other nematodes, but it appears that the "membrane units" described for *Capillaria* by Wright (1964) may also be subsarcolemmal cisternae or diads without inwardly directed tubules. *Nippostrongylus brasiliensis* muscle may also be similar to that of *Deontostoma* with respect to the absence of a T-system and greater development of sarcoplasmic reticulum, while *Euchromadora vulgaris* and *Diriofilaria immitis* seem to more closely resemble *Ascaris* muscle in this regard.

The function(s) of sarcoplasmic reticulum is, as yet, not well understood, but one postulate, which has been reviewed by Porter (1961), states that it may be involved in muscle relaxation, by uptake of calcium, by production of a "relaxing factor," or both. With regard to this theory, it is presumed that hypertrophy of the sarcoplasmic reticulum is directly related to rapid cycles of contraction and relaxation. If this theory proves to be correct, it may explain the relatively greater abundance of sarcoplasmic reticulum in *Deontostoma*, which is a free-living nematode that presumably moves about in search of food by means of frequent cycles of muscle contraction and relaxation, and less sarcoplasmic reticulum in adult *Ascaris*, which retains itself in the lumen of the gut of its host by bracing against the gut wall (Makidono, 1956), and may,

therefore, move rather infrequently with slow cycles of contraction and relaxation.

A system of sarcoplasmic reticulum similar to that in *Deontostoma* muscle is more common in annelid muscle, having been reported in the case of *Glycera muscle* (Rosenbluth, 1968), *Eisenia muscle* (Ikemoto, 1963), *Lumbricus muscle* (Heumann and Zebe, 1967), and leech muscle (Pucci and Afzelius, 1962). However, in these annelids the tubules of sarcoplasmic reticulum are not in direct association with the Z bar as they are in *Deontostoma*, but occur in the Z plane midway between each pair of Z bars. This difference may have some relevance to speed of contraction and relaxation.

**OBLIQUE AND DOUBLE OBLIQUE STRIATION:** All muscles with longitudinally staggered bundles of myofilaments (obliquely striated) appear to have similar organization and structure whether from representatives of the Nematoda, Annelida, or Mollusca. However, there are representatives of the latter two taxa for which double oblique striation has been clearly demonstrated, namely the clamworm (Kawaguti and Ikemoto, 1958d), tellinid clam (Kawaguti and Ikemoto, 1961) and the annelid *Myxicola* sp. (Hanson and Lowy, 1960). In these examples, bands of myofilaments occur on opposing sides of the cell and the bands on one side are oblique in a direction opposite to those of the other side.

Patterns of double oblique striation have not been reported for nematode muscle, but in considering the following information from light and electron microscope studies, it seems quite possible that patterns of double oblique striation may occur in at least certain types of nematode muscle. From light microscopy it is known that "contractile ridges" (bands of myofilaments) are only at the base in platymyarian (Fig. 19), at the base and sides in coelomyarian (Fig. 20) or around the entire periphery enclosing the medial sarcoplasm in circomyarian muscle cells (Fig. 21). Further, platymyarian muscle cells tend to be flattened, that is as wide, if not wider, than tall (Fig. 19); those that are coelomyarian are usually taller than wide with an enlarged perinuclear region at the apex and a narrower base containing the myofilaments (Fig. 20); and circomyarian fibers are cylindrical (Fig. 21). Many nematode muscle cells tend to have an organization and shape intermediate to those

assigned to these categories. From the illustrations in Chitwood and Chitwood (1950) it appears that the somatic muscles of *Ethmolaimus revaliensis*, are very low coelomyarian to platymyarian while those of *Theristus setosus* are higher coelomyarian tending toward circomyarian. Some muscle fibers, such as those of *Ascaris*, are coelomyarian in the midregion and circomyarian toward their ends. Furthermore, according to Chitwood and Chitwood (1950), Martini has shown that some nematodes are platymyarian as first stage juveniles, and later develop coelomyarian muscle. The above observations suggest a similar structural relationship between the muscles of these categories.

From electron microscope observations of cross-sectioned fibers it is known that the same sequence of H, A, and I bands and Z planes occur in coelomyarian muscle (Rosenbluth, 1963, 1965a; Reger, 1964; Watson, 1965a; and Lee and Miller, 1967) as well as platymyarian muscle of *Nippostrongylus brasiliensis* (see Jamuar, 1966) and that all bands and Z planes are at right angles to the cell membrane (Fig. 22). Therefore, the longitudinal striations of platymyarian muscle, if viewed from the base or other appropriate angle, would appear to have slightly oblique orientation from the right side of the fiber to the left or vice-versa (Fig. 19). If exactly the same pattern of striations were formed adjacent to a cell membrane that was not broad at its base, but curved or folded so as to appear U-shaped in cross section with continuous bands occurring on both sides of the fiber as well as at the narrower base (coelomyarian), then the same pattern of striations would pass from near the apex to the base on one side, continue across the base, and upward toward the apex of the opposite side (Fig. 20). If these assumptions are correct, then I, A, and H bands and Z planes would form partial helices in coelomyarian muscle and if the same pattern were to occur in circomyarian muscle, complete helices would be formed (Fig. 21). If fibers of either of these two types were observed in longitudinal optical section with sufficient depth of focus to simultaneously observe the bands and Z plane on both sides of the fiber superimposed on one another, it would have the appearance of double oblique striation (Figs. 20, 21) comparable to that of annelids and molluscs.

It is of interest to note further, however, that because the striations are inclined in opposite directions on opposite sides of the fiber in the above-suggested situations, each of the bands on one side, in cross section, cannot always be on the same level with a comparable band of the opposite side. In other words the bands of one side will be out of register with those of the other. An example of this, and in which double oblique striation has been demonstrated as well, is to be found in the adductor muscle of the tellinid clam (Kawaguti and Ikemoto, 1961).

From electron micrographs of cross sections of *Ascaris* muscle (Rosenbluth, 1965a) it appears that the bands of one side are out of register with those of the other which suggests the possibility of double oblique striation in the muscles of this species. However, this is contrary to the situation in *Deontostoma*, where in cross section the majority of the bands on one side of the fiber are precisely on the same level with a comparable band of the other side giving the appearance of continuity the full width of the fiber (Figs. 1, 2, 23). A similar arrangement exists in *Glycera* muscle (Rosenbluth, 1968). This "in register" condition is here interpreted to indicate that the bands are not only inclined at the same angle, but in the same direction and each myofibril band would give the appearance of a lamella that spans the full width of the fiber and extends obliquely from the apex to the base of the fiber (Fig. 23). Even where separated by a sagittal Z plane, the bands on one side are usually "in register" and parallel with those of the opposite side. Therefore, even under favorable conditions, double oblique striation most likely could not be demonstrated in *Deontostoma* muscle.

What significance these considerations have cannot be fully appreciated at this time, but differences may be relevant to the function of nematode muscle and the relationships of nematode taxa. Perhaps functional implications will not be as readily apparent and may be appreciated only as more is learned regarding the mechanics of nematode muscle contraction. But, on the other hand, if subsequent investigations disclose that somatic muscles now collectively designated as coelomyarian prove to be a composite of two types, one helical or double obliquely striated and the

other bilaterally parallel, and if all members of the order Enoplida (to which *Deontostoma* belongs) have muscle of the former type and those of *Ascarida* (to which *Ascaris* belongs) the latter, then these differences will give further justification for the wide separation between these two taxa in the taxonomic hierarchy of nematodes and may provide further clues to phylogenetic relationship within the Nemata.

### Summary

The structure of somatic muscles of *Deontostoma californicum* is described from light and electron microscope observations. The non-contractile region bears essentially the same organelles as described for previously studied nematodes and contains considerable quantities of glycogen.

The basic organization of the contractile region is that of obliquely striated muscle with characteristic repeated patterns of H, A, and I bands and Z components and resembles the obliquely striated muscles of other nematodes, annelids, and molluscs. *Deontostoma* muscle differs from other nematode muscle in that its Z components appear barlike, and a well-developed T-system is absent. Furthermore, it has a well-developed system of sarcoplasmic reticulum consisting of tubules and vesicles in the Z plane which are continuous with diads. Functional implications of the differences in the extent of the development of sarcoplasmic reticulum are briefly reviewed.

In *Deontostoma* muscle the continuity of Z components and myofilament bands from one side of the fiber to the other suggests that each band forms lamellae which span the full width of the fiber and extend obliquely from the apex to the base of the fiber. Therefore, the organization of *Deontostoma* muscle is such that it could not display double oblique striations possibly present in circomyarian and some coelomyarian nematode muscles. It is suggested that these differences may have functional and/or phylogenetic implications.

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## Infectivity of Two Species of Poultry Coccidia After Freezing and Storage in Liquid Nitrogen Vapor

DAVID J. DORAN AND JOHN M. VETTERLING

Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

Kouwenhoven (1967) reported that sporozoites of *Eimeria tenella* (Railliet and Lucet, 1891) Fantham, 1909, frozen within sporocysts released from oocysts, produced infection in chickens 24 hours after storage at  $-79^{\circ}\text{C}$ . He also found that sporozoites within intact oocysts were noninfective after the same interval at  $-79$  and  $-196^{\circ}\text{C}$ . Doran and Vetterling (1968) stored *E. tenella* and *E. meleagridis* Tyzzer, 1929 sporozoites, excysted and within released sporocysts, in liquid nitrogen vapor for one month. After either manner in which sporozoites were stored, they found that mature schizonts developed in cell culture. They also found that frozen excysted sporozoites inoculated into birds produced oocysts and lesions similar to those produced in birds inoculated with freshly excysted sporozoites, whereas sporozoites within frozen sporocysts produced less intense infections than in birds fed freshly released sporocysts.

This report pertains to the infectivity of excysted sporozoites, sporozoites within released sporocysts, and sporozoites within intact oocysts of *E. meleagridis* and *E. tenella* after storage in liquid nitrogen vapor for intervals up to 4 months.

### Materials and Methods

Oocysts were collected from droppings of chicks or turkey poults during the first 72 hr of the patent period. The methods for recovery and sporulation were the same as previously described (Vetterling, 1969). All oocysts used were from single suspensions that had been made bacteria-free by treatment with undiluted Clorox (Jackson, 1964). They were stored in Ringer's solution at 3 to 6 C and were either 35 days or 2.5 months old when ground oocysts (sporocysts) and sporozoites or oocysts, respectively, were frozen.

Sporocysts were released from oocysts by grinding with a tissue grinder (Doran and Vetterling, 1969). They were placed in

sterile distilled water for 24 hours before freezing or excysting sporozoites. Sporocysts were treated with excystation fluid as previously described (Doran and Vetterling, 1967), until more than 85% of the sporozoites had excysted.

Oocysts, sporocysts, and sporozoites of *E. tenella* were placed in media consisting of 86% Earle's (Earle, 1943) balanced salt solution, 5% lactalbumin hydrolysate (2.5% solution in Earle's B.S.S.), 2% calf serum, and 7% dimethyl sulfoxide (DMSO). Those of *E. meleagridis* were placed in media containing 88% Medium 199 (Morgan et al., 1950) in Hank's B.S.S. (Hanks and Wallace, 1949), 5% chicken serum, and 7% DMSO. The media were at 37–43 C when parasites were added.

Suspensions were quickly dispensed in 5-ml amounts into 10-ml round-bottom screw-top tubes using a Cornwall automatic pipette. The tubes were then immediately placed in a liquid nitrogen freezer (Canal Industrial Corp., Bethesda, Md.; Model 2) and frozen to  $-80^{\circ}\text{C}$  at the rate of 1 degree/min. After the temperature had dropped to that of the vapor, the tubes were placed in a cylindrical storage tank (Lansdell Cryogenics, Hyattsville, Md.; Type LR-40) about 4 to 5 inches above liquid nitrogen. Each tube contained 2.5 to 3.5 million excysted sporozoites, released sporocysts, or intact oocysts.

One tube of oocysts, sporocysts, and sporozoites of each species was removed from storage at 1 week, 1 month, 3 months, and 4 months, respectively. After their contents were quickly thawed by vigorous agitation in warm water at 43 C, the number of oocysts, sporocysts, or sporozoites per respective tube was determined. Groups of 3-week-old chicks and turkey poults, arranged by weight into lots, were inoculated with either the frozen parasites or fresh oocysts. Uninoculated groups were included to compare weight gains. Sporozoites (200,000) were inoculated by syringe into either the duodenum (*E. meleagridis*)



or cecum (*E. tenella*); oocysts (25,000) and sporocysts (100,000) were fed by gavage.

Chicks receiving *E. tenella* were maintained until 8 days after inoculation. The birds were then weighed and necropsied. After the cecal lesions were evaluated, the ceca and contents were mixed with potassium dichromate and homogenized in a Waring blender. Droppings from turkeys that received *E. meleagridis* were collected between 116 and 192 hr after inoculation. They were placed in potassium dichromate and stirred for several hours. At 192 hr the surviving poultts were weighed, necropsied, and their duodenal lesions evaluated. After the cecal homogenate and fecal suspension had been diluted with tap water, oocyst counts were made from samples with the aid of a Levy counting chamber with Fuchs-Rosenthal ruling. The total number of oocysts recovered from each group was calculated, and this was divided by the number of surviving birds in the group to give an estimate of the relative oocyst production per surviving bird.

The anticoccidial index (AI), as proposed by the Merck technical staff (Merck and Co., 1960), was calculated for each group. This appeared to be the best available method for comparing the results obtained with fresh and frozen parasites. With this method, the growth and survival of the uninfected control (UC) birds are the standards for comparison, and this group is assigned an index of 200. Lesions of the infected birds are scored on the basis of 0 = normal; 1 = detectable; 2 = moderate; and 4 = maximal. A lesion value of 2.5 is arbitrarily assigned to each bird that dies of coccidiosis during the experiment (McLoughlin and Chute, 1966). Oocyst production is scored by the following schedule:

Oocysts recovered/surviving bird $\times 10^6$	Oocyst index score
< 0.1	0
0.1-1.0	1
2-5	10
6-10	20
> 11	40

The AI is calculated according to the equation  $AI = (\% \text{ survival} + \% \text{ UC weight gain}) - (\text{mean lesion score} \times 10 + \text{oocyst index score})$ .

## Results

Oocysts of both species appeared in excellent condition when examined immediately after thawing. They were similar to fresh oocysts in all respects. However, they did not produce infection when fed to their natural host.

The membranes of many frozen sporocysts were either slightly indented or collapsed on one side. A few empty sporocysts were also found. It was impossible to determine whether sporozoites within the sporocysts were alive. In their dormant condition, they appeared morphologically similar to those in fresh oocysts. When fed to their hosts, both species produced infection but less severe than that of their controls as judged by the AI (Table 1).

Excysted sporozoites of both species appeared morphologically similar to freshly excysted sporozoites; however, because of their typically sluggish motility, it was impossible to tell whether they were alive. Counts indicated a loss of less than 5% per vial. When sporozoites of both species were injected into their natural host, infections equal to or greater than those of the controls were produced. The AI's calculated for *E. tenella* infections were nearly identical, while those of the frozen *E. meleagridis* infected principals were lower than those for the control groups.

## Discussion

The infections produced in birds with frozen sporocysts were entirely due to sporozoites within the sporocysts and not to free sporozoites released by grinding. A small number (~3%) were released, but these were killed during the 24-hour storage before freezing. We previously found that excysted sporozoites will not survive 1 hour in water only. Success with sporocysts and not oocysts was probably due to differences in permeability of the oocyst and sporocyst membranes to the freezing protectant.

After 4 months storage, frozen sporozoites of both species produced infections of equal or greater intensity than those produced by a similar number of sporozoites in the dosages of fresh oocysts. However, it is doubtful whether this really means that the 200,000 frozen sporozoites inoculated into the duo-

**Table 1. Infection of turkey poults and chicks with frozen and nonfrozen *Eimeria meleagrimitis* and *E. tenella*.**

	Birds surviving/ birds started	Mean wt grain/ surviving bird		Mean lesion score	Oocysts recovered/ surviving bird × 10 <sup>6</sup>	Anticoccidial index
		Grams	% UC*			
<i>Eimeria meleagrimitis</i>						
Uninoculated control	10/10	128	100	0	0	200
Fresh oocysts	4/10	85	66	2.0	31.0	46
Frozen sporocysts	9/10	125	98	1.0	31.0	138
Uninoculated control	9/9	121	100	0	0	200
Fresh oocysts	5/9	90	74	1.5	24.5	75
Frozen sporozoites	3/10	41	34	2.1	38.5	4
<i>Eimeria tenella</i>						
Uninoculated control	10/10	173	100	0	0	200
Fresh oocysts	10/10	93	54	2.2	4.5	122
Frozen sporocysts	10/10	173	100	0.1	0.2	198
Uninoculated control	10/10	130	100	0	0	200
Fresh oocysts	10/10	133	100	1.4	9.9	165
Frozen sporozoites	10/10	153	100	1.5	7.5	167

\* Uninoculated control.

denum or cecum were all alive and just as potentially infective or viable as 200,000 within 50,000 oocysts given orally. When a dosage of oocysts is given orally, a lot of the sporozoites are lost—some because of oocysts failing to rupture in the gizzard, some due to failure to excyst, and others, after excysting, passing out in the feces. Perhaps similar results were obtained with fresh and frozen sporozoites because a loss from the control equalized a loss in the countable frozen inoculum.

There should be no further deterioration or loss of viability during storage for years at the temperature of liquid nitrogen vapor (Levine and Andersen, 1966). Freeze-storage of coccidia (excysted sporozoites or released sporocysts) by the method described herein should save workers a considerable amount of the time, effort, and expense involved in maintaining cultures by frequent serial passage through the host. It also makes possible (1) the retention of pathogenic and drug-resistant strains without genetic alteration and (2) the establishment of a "bank" from which workers could obtain coccidia for experimental purposes. Since the morphology of intact oocysts was not altered by freezing (Kouwenhoven, 1967; present study), it is possible that a type specimen collection could also be established.

### Summary

Oocysts, sporocysts released from oocysts, and excysted sporozoites of *Eimeria meleagrimitis* and *E. tenella* were placed in media containing 7% dimethyl sulfoxide, frozen to -80 C at the rate of 1 degree/min, and stored above liquid nitrogen for intervals up to 4 months.

Oocysts produced no infection when fed to turkey poults or chicks. After 3 months, sporocysts of both species produced infections, but less than those produced by fresh oocysts. However, inoculation of sporozoites that had been stored for 4 months into the duodenum or cecum of their natural host resulted in infections comparable to those caused by fresh oocysts.

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## Influence of Storage Period on Excystation and Development in Cell Culture of Sporozoites of *Eimeria meleagridis* Tyzzer, 1929

DAVID J. DORAN AND JOHN M. VETTERLING

Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705

Prolonged storage of oocysts results in loss of infectivity. There are no reports available that indicate whether the decrease in infectivity is due to one or more of the following: (1) death of sporozoites within sporocysts during storage; (2) inability of the sporozoite to excyst because of nonremoval of the sporocyst plug or Stieda body; or (3) inability to survive and develop through the first asexual generation.

In previous work on cultivation (Doran and Vetterling, 1967a, b, 1969), oocysts less than 18 weeks old were used. In order to have such cultures available, it was necessary to propagate oocysts several times. Consequently, many bacteria-free cultures of different ages were accumulated. In an attempt to determine some of the reasons for loss of infectivity or decreased oocyst production in the host, we excysted sporozoites from these cultures and inoculated them into cell cultures. This report concerns *Eimeria meleagridis* cultures that were between 5 and 60 weeks old.

### Materials and Methods

**OOCYSTS:** All oocysts were of a single strain isolated from a single oocyst. They were collected on the third day of the patent period, and the method for their recovery and sporulation was as previously described (Vetterling, 1969). After they had been rendered free of bacteria by treatment with undiluted Clorox (Jackson, 1964), they were stored in Ringer's solution at 3 to 6 C until used.

**CELL CULTURES:** Cell cultures were of bovine embryonic kidney (cell line; 20th passage). They were on 10- by 35-mm cover glasses in 16- by 150-mm Leighton tubes. Only cultures that were confluent and did not contain cell aggregates were used. The growth and maintenance media were the same as before (Doran and Vetterling, in press).

**EXCYSTATION:** Five hundred thousand sporocysts, released from oocysts of each age by grinding (Doran and Vetterling, 1969), were treated with excystation fluid (Doran

Table 1. Survival and development of *Eimeria meleagridis*.

Exp. no.	Oocysts		5 hr	48 hr			72 hr			Per cent decrease* between 5 hr and			
	Cult. no.	Age (weeks)		Sporozoites	Schizonts†			Sporozoites	Schizonts†			48 hr	72 hr
			I		M	%	I		M	%			
1	4	5	3,242	2,525	100	26	4.8	1,735	22	32	3.0	18.2	44.8
	2	27	2,900	2,101	64	45	4.9	1,803	19	59	4.1	23.8	35.1
	1	53	920	192	4	1	2.5	151	1	0	0.7	78.6	83.5
2	4	12	2,911	2,411	89	49	5.4	1,721	19	39	3.3	12.4	38.9
	3	29	2,875	2,120	76	72	6.5	1,523	29	78	6.6	21.1	43.3
	2	34	3,146	2,477	92	18	4.2	2,311	27	82	4.5	17.8	23.0
	1	60	751	201	2	0	1.0	82	0	2	2.4	73.0	88.8

\* Total number of parasites.

† I, immature; M, mature (with merozoites).

and Vetterling, 1967a) for 20 minutes. Counts were then made of the number of empty sporocysts, sporocysts containing one and two sporozoites, and free sporozoites. Percentage excystation (PE) was calculated by two methods:

$$\text{Method A PE} = \frac{\frac{\text{number of empty sporocysts} \times 2}{\text{total number of sporocysts} \times 2} + \frac{\text{number of sporocysts with one sporozoite}}{\text{total number of sporocysts} \times 2}}{\text{total number of sporocysts} \times 2} \times 100.$$

$$\text{Method B PE} = \frac{\text{number of free sporozoites}}{\text{total number of sporozoites (free and within sporocysts)}} \times 100.$$

**INOCULATION AND MAINTENANCE OF CELL CULTURES:** Sporozoites were concentrated and resuspended in 20 ml of inoculation medium consisting of Medium 199 in Hank's (Hanks and Wallace, 1949) balanced salt solution + 3% calf serum. After adjusting the pH to 7.0–7.2 and thoroughly agitating the suspension, 1.2 ml was immediately pipetted into each of 10 cell cultures.

At 3.5 hr the sporozoite-containing medium was removed. The cells were washed once with warm inoculation medium and 5 ml of maintenance medium (pH 7.2–7.4) was added. Changes of medium were made when necessary in order to maintain the pH at 7.0–7.4. Cultures were kept in an incubator adjusted to alternating intervals of approximately 12 hr at 40.6 and 43 C.

**FIXING, STAINING, AND COUNTING:** At 5, 48, and 72 hr, cover glasses were removed from four tubes in each set of inoculated cultures. They were fixed and stained as previously mentioned (Doran and Vetterling, 1969). Counts were made at 645 $\times$ . They represent

the total number of organisms found by examining every 4th "row" across the length of each of four glasses. Variation among the four cultures ranged from 11 to 31 per cent.

## Results

Similar results were obtained in cell culture with sporozoites from oocysts 5 to 34 weeks old (Table 1). There was little difference in: (1) numbers of sporozoites found within cells at 5 hr; (2) percentage of developmental stages at 48 hrs; and (3) percentage decrease in the total numbers of parasites after 5 hr. However, the results with oocysts 53 and 60 weeks old were decidedly different.

The smaller number of sporozoites from oocysts 53 and 60 weeks old in cells at 5 hr is mostly due to death of sporozoites before inoculation of cell cultures. There was little difference between oocysts of all ages when the percentage excystation was calculated by Method A (Fig. 1). When Method B was used, however, only about 40% excystation was obtained for the 53- and 60-week oocysts. This finding indicates that many sporozoites either died before or shortly after excystation. At least half of the sporozoites inoculated into these cultures were dead at the time of inoculation. Many sporozoites from the 60-week cultures were observed to break up while emerging from the sporocyst.

## Discussion

Data indicate that decreased infectivity of older oocyst cultures is not due to the inability of sporozoites to excyst because of nonremoval

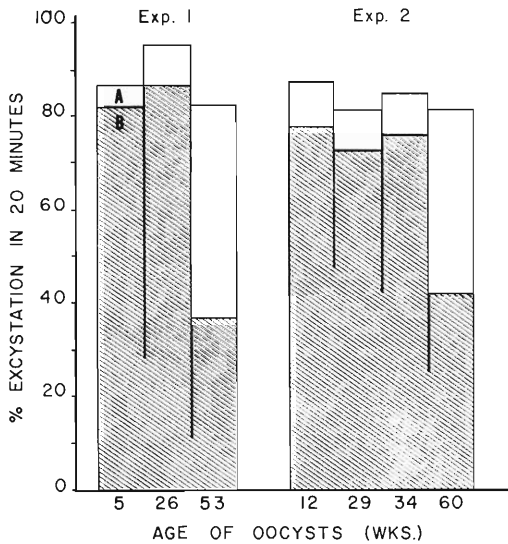


Figure 1. Excystation of sporozoites from oocysts of different ages. A = Method A; B = Method B.

of the sporocyst plug. When calculated on the basis of empty sporozoites (Method A), the percentage excystation of sporozoites from all cultures was similar.

The low percentage excystation from the older cultures when calculated on the basis of surviving sporozoites (Method B) and the presence of degenerating sporozoites emerging from sporozoites indicate that many sporozoites in older oocysts are either dead within the sporocyst or die shortly after excysting. The breaking up of sporozoites while emerging from sporocysts suggests that excystation of sporozoites depends on external factors (trypsin, bile) and is probably not due to the secretion of any enzyme by the sporozoite as previously thought (Doran, 1966).

The lower percentages of development and percentages of survival after 5 hr suggest that

many sporozoites are also unable to survive within cells and develop through the first asexual generation.

### Summary

Sporozoites, excysted from sporocysts released from *Eimeria meleagridis* oocysts of different ages, were inoculated into cell cultures of bovine embryonic kidney (cell line; 20th passage). Data obtained indicate that decreased infectivity of older oocyst cultures is due to: (1) death of sporozoites within sporozoites during storage; and (2) inability of sporozoites to survive and develop after entering cells.

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## *Megodontolaimus* New Genus (Nematoda: Chromadoridae), with a Description of Two New Species

R. W. TIMM

Visiting Professor, Department of Nematology, University of California, Davis

Two new species of a new genus of marine nematodes of the family Chromadoridae, subfamily Hypodontolaiminae, were found in the Bay of Bengal. The smaller species has six longitudinal alae and a single esophageal bulb, while the larger species has two lateral alae and a double esophageal bulb. Although the two species differ in these prominent characters, they are alike in stomatal structure and so have been kept in the same genus.

### *Megodontolaimus* new genus

**DIAGNOSIS:** Chromadoridae, Hypodontolaiminae. Four long and six short cephalic setae. Cuticle with prominent punctation; two or six alae present, with fine cross bars. Prominent pharyngeal bulb surrounding stoma, asymmetrical, much larger on dorsal side. Long ventral tooth, turning to left side, with tip hooked inwardly and dorsally. Dorsal tooth two-pronged, with crescentic thickening extending along dorsal wall of pharyngeal bulb. Transverse elliptical amphids. Ovaries amphidelphic, with flexures; single outstretched testis. Spicules cephalate; gubernaculum large, parallel, with lateral sleeves. Preanal midventral setae present. Tails conical, with pointed tip.

**TYPE SPECIES:** *Megodontolaimus coxbazari* sp. n.

**REMARKS:** *Megodontolaimus* is most closely related morphologically to that group of *Hypodontolaimus* with a prominent pharyngeal bulb in which the somatic setae are abundant and at least as long as the body diameter. The most obvious distinction between the two genera is the long thin crescentic apophysis on the dorsal side of the pharyngeal bulb in *Megodontolaimus*, together with the two-pronged dorsal tooth. In all *Hypodontolaimus* species the large tooth is said to be dorsal (cf. Wieser, 1954, p. 75), whereas in *Megodontolaimus* it is basically ventral (Fig. 1A, B; Fig. 2A). The pronounced dorsal swelling of the pharyngeal bulb may or may not be present in *Hypodontolaimus*; if present, it is usually tapering

toward the posterior (e.g. *H. steineri* Wieser, 1954), but may occasionally be as prominent as in *Megodontolaimus* (e.g. *H. inaequalis* and *H. buetschlii* in the illustrations of Bresslau and Schuurmans Stekhoven, 1940, plate 9, Figs. 44 and 45). *Hypodontolaimus* has four cephalic setae (may have six setose labial papillae), whereas *Megodontolaimus* has ten; in the latter the punctation is larger and more distinct, while the body is paler in color and the excretory cell is much less distinct.

### *Megodontolaimus coxbazari* sp. n. (Fig. 1A; Fig. 2 A-B)

**FEMALE** (n = 20): Length = 0.58–0.78 mm; maximum body diameter = 22–30  $\mu$ ; esophagus = 99–125  $\mu$ ; esophagus-vulva = 0.21–0.30 mm; vulva-anus = 0.19–0.29 mm; tail = 69–80  $\mu$ .

**MALE** (n = 13): L = 0.61–0.74 mm; mbd = 18–22  $\mu$ ; e = 99–118  $\mu$ ; e-a = 0.44–0.56 mm; tail = 58–77  $\mu$ .

**HOLOTYPE MALE:** L = 0.69 mm; mbd = 19  $\mu$ ; e = 109  $\mu$ ; e-a = 0.51 mm; t = 67  $\mu$ .

**ALLOTYPE FEMALE:** L = 0.75 mm; mbd = 26  $\mu$ ; e = 115  $\mu$ ; e-v = 0.28 mm; ov<sub>1</sub> = 0.19 mm; ov<sub>2</sub> = 0.13 mm; v-a = 0.28 mm; t = 80  $\mu$ .

**DESCRIPTION:** Body outstretched in death, with tail loosely curved to ventral side. Cuticular punctations elliptical, larger at anterior of body and bordering the alae. Annules inclined forward in cervical region and backward from opposite tip of anterior ovary. Lateral alae extending full length of body from amphids to tail tip, crossed by thin transverse bands. Head broadly rounded; head diameter 16–19  $\mu$ ; 12 fine cheilorhabdions surrounding mouth opening. Ten fine cephalic setae, the four submedian longer setae 15–18  $\mu$ , the six shorter setae 6–7  $\mu$  long. Six setiform labial papillae. Somatic setae along full length of body, but not grouped in sets at same body level; somatic setae up to 20  $\mu$  long. Amphids at extreme anterior, transversely elliptical (appear to be flattened-spiral in one specimen). Large pha-

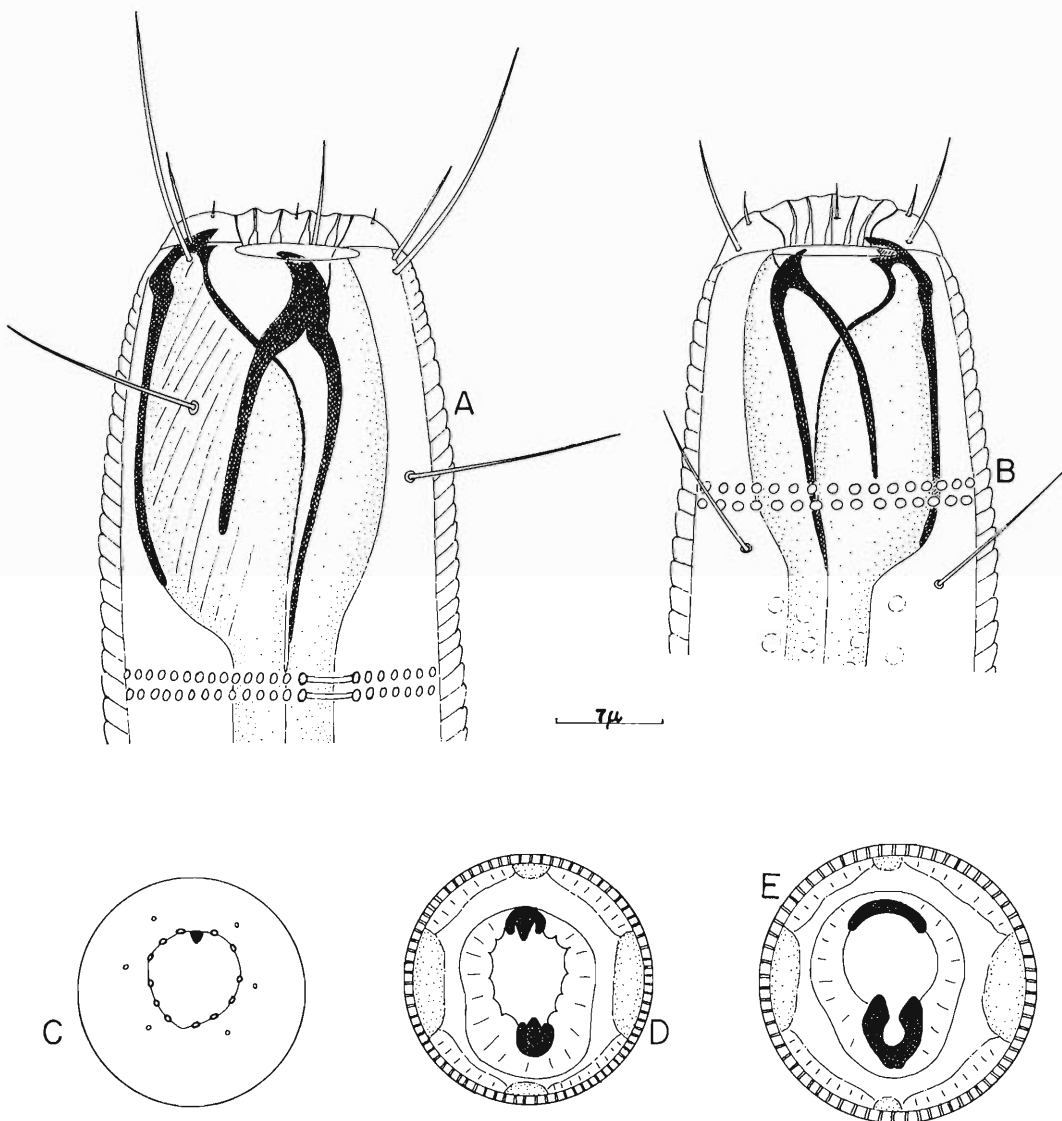


Figure 1. A. *Megodontolaimus coxbazari*, female head. *Megodontolaimus sonadiae*: B. Male head. C. En face view. D. Optical section at level of ventral tooth tip. E. Optical section just behind ventral tooth tip.

ryngeal bulb, 24–29  $\mu$  long in female, 24–26  $\mu$  in male, much more expanded dorsally; conspicuous oblique pharyngeal muscles. Large ventral tooth, appearing obliquely lateral when viewed from side; tip turned in toward center of stoma. Dorsal tooth two-pronged at tip, pro-

truding into cheilostome; long thin crescentic dorsal apophysis along outer margin of pharyngeal bulb. Large double esophageal bulb, 32–42  $\mu$  long in female, 21–32  $\mu$  in male, with external indentation into two parts and internal division. Cardia inconspicuous. Intestine

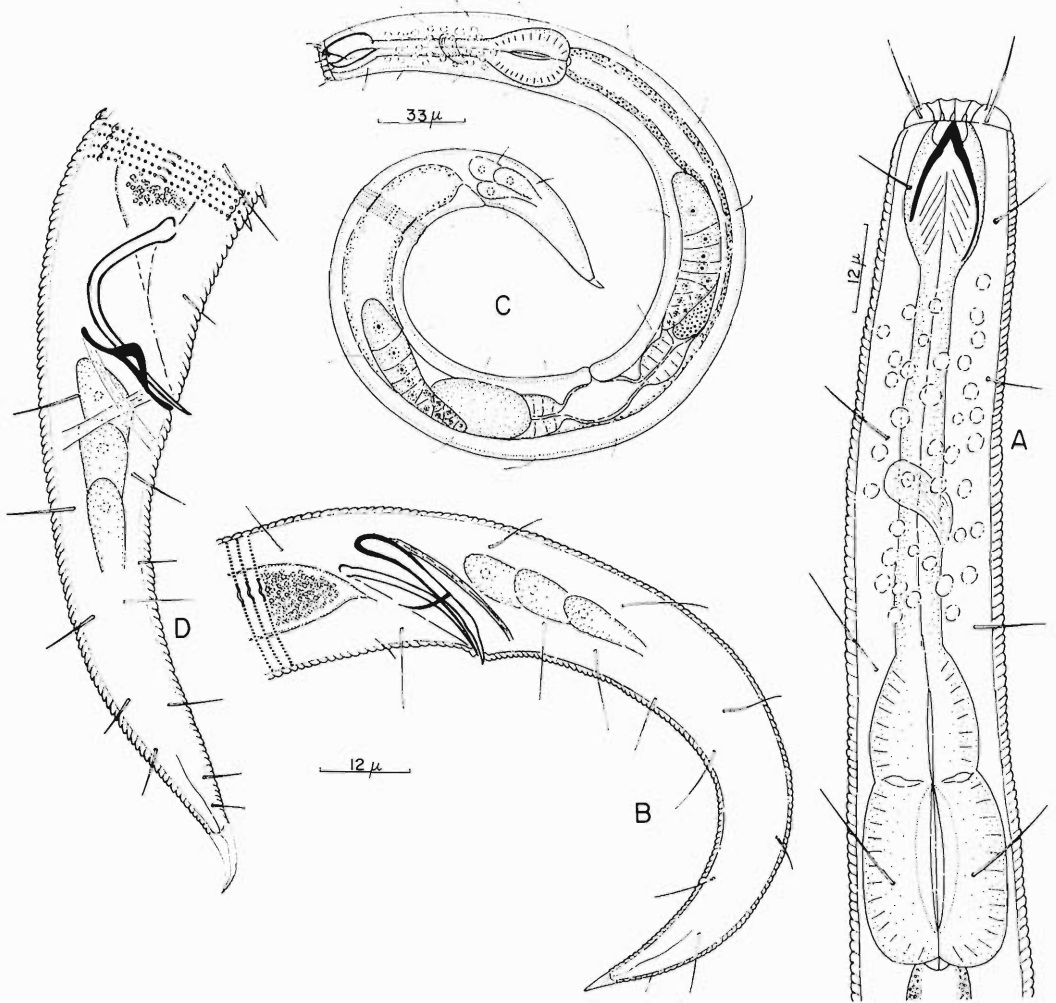


Figure 2. *Megodontolaimus coxbazari*. A. Female esophageal region, ventral view. B. Male tail. *Megodontolaimus sonadiae*. C. Entire female. D. Male tail.

thin-walled, with very scattered pale yellowish globules. Amphidelphic ovaries, with flexure up to one-half the distance to vulva; vulva at 51–56% of body length; spherical sperm in uterus,  $2\ \mu$  in diameter, with dense refringent matter in form of a crescent. Testis conspicuous, one-half body diameter in width for most of its length. Spicules slightly arcuate,  $21\text{--}22\ \mu$  long across arc, with narrow pointed tips. Gubernaculum broad, thickly cuticularized,  $20\text{--}24\ \mu$  long, with thin lateral sleeve around

spicules. Three fine midventral setae anterior to anus, equally spaced. Tails in both sexes conical, tapering to pointed tip; tail  $4\text{--}4.6$  anal body diameters long in female,  $3.4\text{--}4.8$  in male; three caudal glands opening by a simple slit.

**TYPE HABITAT AND LOCALITY:** Fine slit above sand, tidal pool, Cox's Bazar, East Pakistan.

**HOLOTYPE MALE:** Collected by R. W. Timm, 22 December 1964; Slide 1080, University of California Nematode Collection, Davis.



**ALLOTYPE FEMALE:** Same data as holotype; Slide 1081, UCNC, Davis.

**PARATYPES:** Slide CB 53 sent to B. E. Hopper; Slides CB 54-61 in collection at Notre Dame College, Dacca, East Pakistan.

**DIAGNOSIS:** This species is distinguished from the following species by its greater length, double esophageal bulb and the presence of only two alae.

***Megodontolaimus sonadiae* sp. n.**  
(Fig. 1 B; Fig. 2 C-D)

**FEMALE** (n = 20): L = 0.42-0.65 mm; mbd = 22-35  $\mu$ ; e = 80-118  $\mu$ ; e-v = 0.13-0.23 mm; v-a = 0.14-0.23 mm; t = 48-75  $\mu$ .

**MALE** (n = 20): L = 0.41-0.53 mm; mbd = 22-35  $\mu$ ; e = 73-102  $\mu$ ; e-a = 0.27-0.37 mm; t = 54-70  $\mu$ .

**HOLOTYPE MALE:** L = 0.5 mm; mbd = 29  $\mu$ ; e = 93  $\mu$ ; e-a = 0.34 mm; t = 68  $\mu$ .

**ALLOTYPE FEMALE:** L = 0.5 mm; mbd = 29  $\mu$ ; e = 96  $\mu$ ; e-v = 0.17 mm;  $ov_1$  = 112  $\mu$ ;  $ov_2$  = 128  $\mu$ ; v-a = 0.18 mm; t = 51  $\mu$ .

**DESCRIPTION:** Body assuming a spiral form or loose curve in death. Cuticle bearing conspicuous rounded punctations, larger at anterior and posterior of body and wider apart than in previous species. Annules inclined forward in cervical region and backward from opposite anterior of ovary. One pair lateral alae extending from mesesophagus to midtail; two pairs of submedian alae in region from esophagus to anus; all alae with fine cross bars. Head broadly rounded; head diameter 16-19  $\mu$ ; 12 fine cheilorhabdions surrounding mouth opening (Fig. 1C). Ten fine cephalic setae, the longer 8-13  $\mu$  long, the shorter 3  $\mu$  (may be setiform labial papillae). Somatic setae extending full length of body, up to 13  $\mu$  long. Amphids at extreme anterior, transversely

elliptical. Pharyngeal bulb 23-32  $\mu$  long in female, 19-22  $\mu$  in male, expanded dorsally. Teeth as in previous species. Single esophageal bulb, 27-33  $\mu$  long in female, 16-22  $\mu$  in male. Inconspicuous excretory cell a short distance posterior to esophageal bulb; inconspicuous excretory pore between bulb and nerve ring. Yellowish refringent circular to elliptical intestinal inclusions. Amphidelphic ovaries, with flexure up to one-half or more of distance to vulva; ova 42 by 22  $\mu$ ; vulva at 52-56% of body length. Spicules highly arcuate, 28-31  $\mu$  long across arc. Gubernaculum 18-19  $\mu$  long, with lateral sleeve around spicules. Eight mid-ventral short, thick, flabby setae anterior to anus, not equally spaced. Tails in both sexes conical, tapering to acute tip; tail 2.2-4.4 anal body diameters long in female, 2.3-3.5 in male; three caudal glands opening by a simple slit.

**TYPE HABITAT AND LOCALITY:** Mud of intertidal zone, north side of Sonadia Island, Bay of Bengal, East Pakistan. Other locality: St. Martin's Island, Bay of Bengal, East Pakistan.

**HOLOTYPE MALE:** Collected by R. W. Timm, 22 December 1964; Slide 1082, UCNC, Davis.

**ALLOTYPE FEMALE:** Same data as holotype; Slide 1083, UCNC, Davis.

**PARATYPES:** Slides 1084 and 1085, UCNC, Davis; Slide So 51 sent to B. E. Hopper; Slides So 3, 20, 33, 35, 40, 52, 53 in collection at Notre Dame College, Dacca, East Pakistan.

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## Galvanotaxis of *Pelodera strongyloides* (Nematoda: Rhabditidae)

FRED H. WHITTAKER<sup>1</sup>

Department of Biology, University of Louisville, Louisville, Kentucky

Numerous experiments concerning the responses of various organisms in an electrical field have been carried out within the past sixty years. Some examples listed by Warden et al. (1940) of the many different organisms that have exhibited a galvanotactic response are certain protozoans, bacteria, slime molds, flatworms, annelids, molluscs, insects, crustaceans, and echinoderms.

However, as far as it could be determined from the literature, there are only two reports of previous work of this nature with nematodes (Caveness and Panzer, 1960; Ronald, 1963); hence, the following experiment was undertaken to determine whether a galvanotactic response could be elicited from *Pelodera strongyloides*.

### Materials and Methods

All the stages of *P. strongyloides* used in these experiments were cultured in Petri dishes on a blood fibrin nutrient agar medium that had been inoculated with the bacterium *Pseudomonas fluorescens*.

The dauer larvae were tested as one group, and the other larval stages with the adults constituted the other group. For each series of the experiment, approximately 200 washed worms were placed centrally by means of a pipette on the bottom of a glass dish measuring 12.2 by 3.8 by 3.3 cm that had been filled with Ringer's solution.

Two calomel electrodes were constructed for use in the experiments. Each electrode consisted of a 10 by 4 cm shell vial in which were placed about 15 ml mercury and 50 ml of a saturated solution of HgCl and KCl. Each vial was fitted with a two-hole cork stopper. Through one hole was inserted a piece of glass tubing in which was fused a piece of platinum wire, one end of which passed into the mercury and the other end extended a short distance above the cork. To conduct the current

from the electrode to the Ringer's one arm of a U-shaped piece of glass tubing filled with KCl agar was inserted through the other hole into the HgCl, KCl solution, and the other arm of the agar bridge was immersed in the Ringer's solution in the dish. The agar bridge also functioned in preventing excessive polarization.

Direct current was produced by a DC power supply equipped with filters to reduce the ripple. An ammeter was placed in the circuit so that total amperage could be read at any time and current densities calculated. Decade resistance boxes were also used when small amperages were desired.

The orientation of the worms was observed with a dissecting microscope.

Since it was impossible to determine the exact values for the current passing through the worms, the responses are thus described in terms of the total amperage and/or current density at a particular moment.

For controls, worms were placed in the glass dish filled with Ringer's solution and observed for 2 hours in the absence of an electrical current.

### Results

**DAUER LARVAE:** It was found that at a total amperage of 3  $\mu\text{a}$  (current density of 0.00064  $\mu\text{a}/\text{mm}^2$ ) with a resistance of about 2 megohms, all the dauer larvae showed a definite threshold response by moving toward the anode. In all the experiments the worms first moved like one integrated mass toward the observer (perpendicular to the lines of force) for about 10 minutes and then in mass toward the anode. Gradual increase of the total amperage to 100  $\mu\text{a}$  had no effect upon the rate of movement of worms. With undulating and thrashing movements, the dauer larvae traveled the 6 cm in approximately 2 hours eventually aggregating near the anode. The experiment was repeated several times with similar results.

The presence of the electrical field appeared to affect all the worms simultaneously and with equal intensity. This showed that galvanotaxis was not due to electrolytic products,

<sup>1</sup> This work represents a portion of a thesis submitted to the Graduate School, University of Illinois, in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

in which case those worms nearest one or the other electrode would exhibit galvanotaxis first and then the other worms in succession.

Passing current through the solution before introducing the worms had no effect upon orientation or movement. There was no difference in the amperage level at which the threshold response occurred or in the rate of migration of the worms toward the anode when the direction of current flow was reversed.

The control worms which were not subjected to any current showed random movements.

**ADULTS AND OTHER LARVAL STAGES:** The adult worms showed no galvanotactic response within a total amperage range of from 1  $\mu$ a to 10 ma. Throughout the experiments these worms remained within the visual field of the dissecting microscope and distinctly showed very slow random movements, there being no indication of movement toward either of the electrodes.

In regard to the nondauer larvae, there was no change in the random movements until a total amperage of 32  $\mu$ a was reached, at which level the young larvae appeared to orientate very weakly toward the anode and extremely slow, undulating movements toward this electrode then ensued. A slow constant rate of movement continued until a total amperage of 70  $\mu$ a was reached at which point no further movement was evident. A current increase to 200  $\mu$ a did not induce any further movement; however, after decreasing the amperage below 70  $\mu$ a, a resumption of movement occurred, and the worms eventually aggregated near the anode.

The controls for these stages showed random movements.

### Discussion

The fact that the larval nematodes showed a constant rate of movement throughout the amperage ranges indicates that the anodal response should not be attributed to electrophoresis. Had the response been electrophoretic, a greater rate of movement would have resulted with an increase in current.

It cannot be stated with certainty why the dauer larvae first moved perpendicular to the lines of force before orienting and migrating toward the anode. Such perpendicular move-

ment was noticeably absent in the response of the nondauer larvae.

The cessation of movement toward the anode at 70  $\mu$ a by the nondauer larvae may indicate some inhibitory effect at this amperage level.

The results of this work differ from those of previous investigators. Caveness and Panzer (1960) working with adult *Panagrellus redivivus* and a number of other nematode genera in a field of direct current, reported a galvanotactic movement of these worms to the cathode on an agar substrate. These investigators recorded for *P. redivivus* a threshold response at 0.02 ma below which movement was not significantly different from the random movement of the controls. On a soil substrate, *P. redivivus* was reported to have migrated also to the cathode, but in numbers less significant than those which migrated to this electrode on agar.

Using distilled water and dilute saline solutions as media, Ronald (1963) was unable to obtain any directional movement with larvae of *Terranova decipiens* in continuous direct current, in intermittent direct current or by reversing the polarity. He did record, however, a condition of stasis with both polarity reversal and alternating current.

Insufficient knowledge of the physiology and biochemistry of *P. strongyloides*, especially under these experimental conditions, precludes any accurate interpretation of the responses obtained in these experiments. However, one broad explanation for such responses as proposed by Hyman and Bellamy (1922) endeavors to relate metabolic gradients to electrical gradients. These investigators suggested that differences of potential in organisms, particularly those permanent differences that occur along the main axis of animals, are due to differences in metabolic rate at different regions, the region of highest metabolic rate being the most negative in the external circuit and most positive in the internal circuit. They further suggested that internal potential differences may account for the galvanotaxis of many animals so that in a direct current, animals which are electro-positive internally become oriented toward the cathode, while those which are electro-negative, become directed toward the anode. These workers contend that such bioelectric currents are chemical in origin

and probably arise through differences in the rates of oxidation-reduction processes in different regions of the animal.

As a matter of speculation based on the aforementioned considerations, the tactic response of the larvae of *P. strongyloides* may be attributed in part to a rate of metabolism greater than that which occurs in the adult. Oxidation-reduction processes, occurring at a greater rate in the larvae, could be associated with, among other things, growth of the organism, tissue differentiation and development of the reproductive system and would produce larger amounts of organic salts and other substances. In an electrical field, the cations of these organic salts would be attracted to the cathode, and by nature of their structural simplicity and small size, would be able to pass through membrane barriers fairly readily. On the other hand, most of the corresponding organic anions, primarily because of their larger size, would be unable to pass through the organelle and cellular membranes to the anode. Such a condition may therefore result in the tactic response of the entire organism toward the anode as occurred in the case of the larvae of *P. strongyloides*.

Further investigations in the area of galvanotaxis are obviously necessary and may eventually reveal that in some cases such responses are an active behavioral process, while in other cases, the responses are of a passive nature.

### Summary

The galvanotactic response of the larval stages of the nematode *Pelodera strongyloides* is reported. Ringer's solution was employed as the medium in which the larvae migrated to the anode. A threshold response of 3  $\mu$ a was recorded for the dauer larvae and one of 32  $\mu$ a for the nondauer larvae. The adult worms showed no galvanotactic response within a total amperage range of from 1  $\mu$ a to 10 ma.

The results of two previous experiments concerning the galvanotaxis of nematodes are briefly reviewed, and an explanation, entirely speculative, is offered as to why the nematodes in the present experiments migrated to the anode.

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## IN MEMORIAM

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## New Species of Tylenchs Associated with Bark Beetles in New Mexico and Colorado

CALVIN L. MASSEY<sup>1</sup>

Members of the superfamily Tylenchoidea are among the most important nematode parasites and associates of bark beetles and other insects throughout the United States. In the opinion of the writer and other workers in the field, many of the free-living or saprophagous forms associated with insects evolve into true parasites. At times, the sexual free-living stages of nematode parasites of insects exhibit striking resemblances to soil-inhabiting or associated forms. Numerous species of internal parasites at some period in their taxonomical evolution have been placed in genera which are known to have only a free-living existence. Ruhm 1956 placed a parasite of *Pissodes pini* in the genus *Stictylus* because of the resemblance of the sexual forms of the parasite to the sexual forms of that genus. The parasite had been previously included in the genera *Tylenchus*, *Allantonema*, and *Parasitylenchus*. Recently the same parasite was placed in the genus *Sphaerulariopsis* Nickle 1963. The genus *Stictylus* Thorne 1941 includes species that are known only to be free-living, although their habitat lends credence to Ruhm's designation. The writer has been unable to associate known forms of *Stictylus* with a parasitic counterpart, but closely related genera have been collected from insect habitats.

More recently, Bedding (1967) in his studies on nematode parasites of the horntail genus *Sirex* noted the remarkable similarity of the sexual forms of the parasite to the genus *Deladenus*. The writer, working with the same parasite at Rotorua, New Zealand, confirmed the resemblance. The genus *Deladenus* Thorne 1941, at the time of erection, included two species, *Deladenus durus* and *Deladenus obesus*. *Deladenus durus* was originally described in the genus *Tylenchus* and was col-

lected from the galls of chestnut oak, decaying fungus (*Pleurota* sp.), and under the dead bark of cottonwood. Original specimens of *Deladenus obesus* were taken from the frass of bark beetles under the bark of dead white fir *Abies concolor* Lindl. It appears that both species were collected from normal insect habitats.

As research intensifies on the relation of nematodes to insects and their ecology, it can be reasonably expected that more and more examples of the similarity of parasites to free-living nematodes will arise, and the role of the associated forms will assume increasing importance.

Six new species of tylenchs were collected from trees infested with bark beetles; they were either associated with the various stages of the insect or were residing in a habitat that had been abandoned by the beetles. All type specimens are in the nematode collection of the Rocky Mountain Forest and Range Experiment Station at Albuquerque, New Mexico.

### *Neoditylenchus puniwopus* sp. n. (Fig. 1 A-C)

FEMALE (N = 3): L = 0.93–1.17 mm; a = 34.0–44.0; b = 6.2–7.4; c = 31.1–35.2; V = 90%.

MALE (N = 3): L = 0.59–0.71 mm; a = 39.2–51.3; b = 4.5–5.3; c = 23.5–30.8.

FEMALE: Figure 1B. Body slender. Cuticle nearly smooth, transverse striae faintly visible in younger specimens. Head continuous with body contour. Lip region twice as wide as deep. Stylet relatively slender with prominent basal knobs over one-third longer than width of head. Esophagus as in Figure 1B, metacarpus spindle-shaped with large central valvular plates and ending in a well-developed terminal bulb. Nerve ring a body width posterior to metacarpus. Excretory pore adjacent to nerve ring. Hemizonid posterior to excretory pore. Ovary single, outstretched, quadricolumnella two body widths in length. Postuterine branch a body width in length. Lips

<sup>1</sup> Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, with central headquarters maintained at Fort Collins in cooperation with Colorado State University; author is located at Albuquerque in cooperation with the University of New Mexico.

The writer wishes to thank Mr. Gerald Thorne for his suggestions on the preparation of the manuscript.

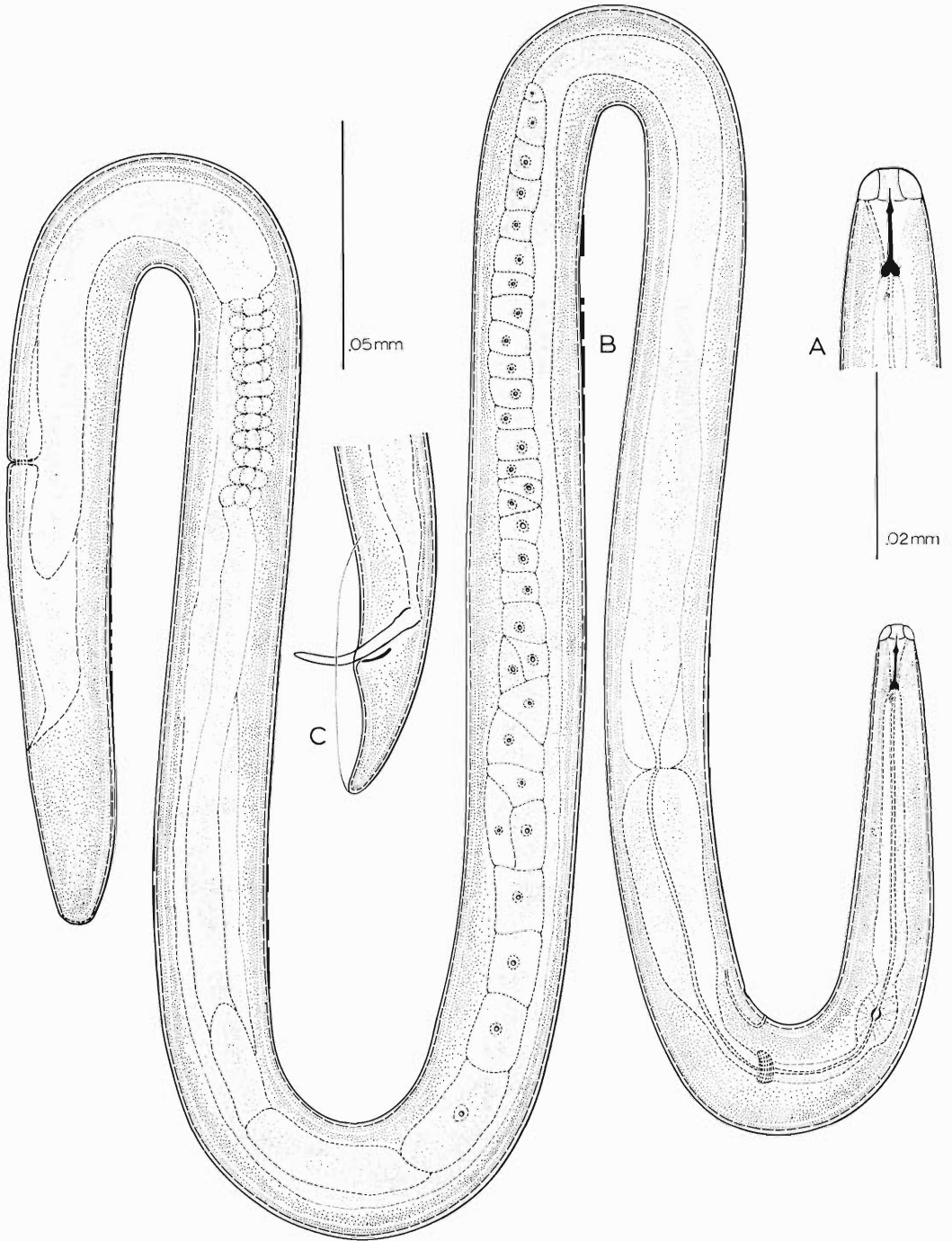


Figure 1. *Neoditylenchus puniwopus* sp. n. A, Head; B, Female; C, Male tail.

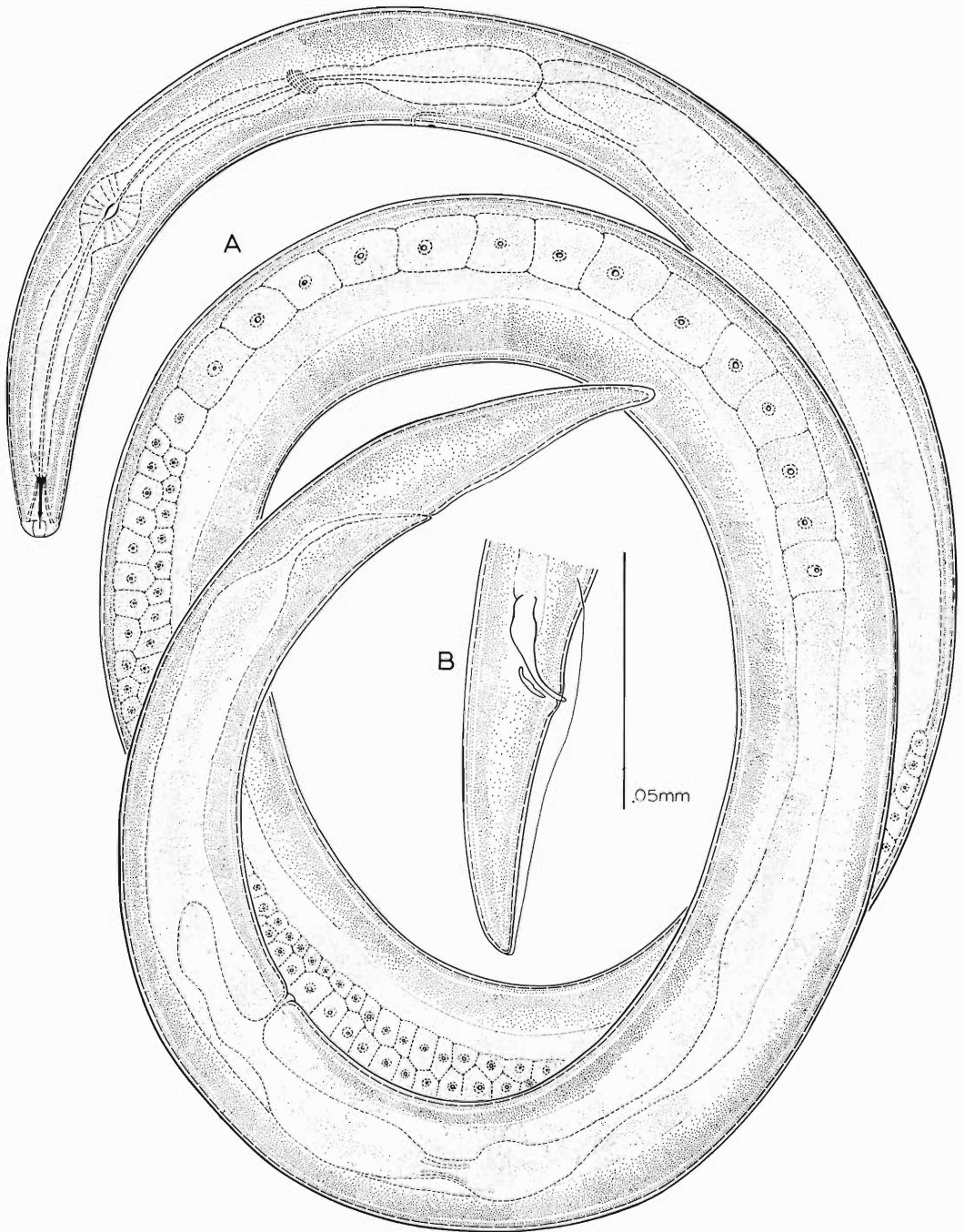


Figure 2. *Neoditylenchus yasinski* sp. n. A, Female; B, Male tail.

of vulva slightly protruding in some specimens, transverse. Anal opening barely discernible under oil immersion objective. Terminus broadly rounded.

**MALE:** With anterior body characters of female. Testes single, outstretched. Spicules paired tylenchoid, gubernaculum as in Figure 1C, one-fourth the length of the spicules. Bursa enveloping terminus which is subacute.

**DIAGNOSIS:** Closely related to *Neoditylenchus abieticolus* (Ruhm, 1956) Goodey 1963, differs in body length and width, in its longer and more slender stylet and in the nearly smooth cuticle.

**TYPE LOCALITY:** Santa Fe National Forest near Pecos, New Mexico.

**TYPE HABITAT:** Associated with the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopkins, in Douglas-fir. *Pseudotsugae menziesii* var. *glauca* (Mirb.) Sudw.

*Neoditylenchus yasinskii* sp. n.  
(Fig. 2 A–B)

**FEMALE** (N = 3): L = 1.25–1.28 mm; a = 32.60–38.60; b = 8.54–8.62; c = 20.77–22.82; V = 89%.

**MALE** (N = 1): L = 0.94 mm; a = 37.16; b = 6.49; c = 18.8.

**FEMALE:** Figure 2A. Body nearly straight when relaxed. Narrow at anterior end, widening rapidly near the vulva. Cuticle with moderately fine transverse striations. Lip region twice as wide as deep. Head continuous with contour of body. Stylet with distinct basal knobs, one-third longer than the width of the head. Esophagus typical of genus. Nerve ring two body widths posterior to metacarpus. Excretory pore three-fourths of a body width posterior to nerve ring. Hemizonid immediately posterior to excretory pore. Ovary single, outstretched on some specimens, reaching nearly to basal bulb of esophagus. Postuterine branch one body width in length. Lips of the vulva protuberant. Vulva transverse. Anal opening discernible only under oil immersion. Terminus subacute.

**MALE:** Spicules tylenchoid. Gubernaculum as in Figure 2B. Bursa enveloping tail, terminus narrowly rounded.

**DIAGNOSIS:** Closely related to *Neoditylenchus pinophilus* (Thorne) Goodey 1963. Differs from that species in shorter length, shape

of tail, and gubernaculum. Stylet knobs considerably more prominent than *pinophilus*. Also related to *Neoditylenchus panurgus* (Ruhm, 1956) Meyl 1961—differing from that species in absence of a lateral field.

**TYPE LOCALITY:** Associated with *Dendroctonus obesus* (Mannerheim) in Engelmann spruce *Picea engelmanni* Parry.

*Sychnotylenchus scolyti* sp. n.  
(Fig. 3 A–D)

**FEMALE** (N = 3): L = 0.71–0.87 mm; a = 30.6–33.8; b = 5.5–6.2; c = ?; V = 89–90%.

**MALE** (N = 3): L = 0.70–0.72 mm; a = 52–54; b = 5.2–5.4; c = 31–35.

**FEMALE:** Figure 3A. Body narrowest at anterior end, widest immediately anterior to vulva. Cuticle almost smooth, transverse striations very faint. Head slightly set off. Lip region more than twice as wide as deep, lateral lips narrower than other four and protruding beyond body contour. Figure 3B. Stylet stout without basal knobs, slightly longer than width of head, Figure 3C. Metacarpus of esophagus with prominent valvular apparatus, spindle-shaped, muscular. Isthmus slender, ending in a prominent elongate terminal bulb. Nerve ring one-half body width posterior to median bulb. Excretory pore one body width anterior to median bulb. Hemizonid about opposite anterior end of basal bulb. Ovary outstretched, relatively short. Quadricolumella prominent, approximately one-seventh ovary length. Posterior uterine branch  $1\frac{1}{3}$  body widths long. Vulva transverse, lips protuberant. Anal opening not discernible. Terminus broadly rounded.

**MALE:** With head and esophageal characters of female. Testes outstretched. Spicules paired, tylenchoid. Gubernaculum one-third length of spicules, Figure 3D. Bursa enveloping tail. Terminus subacute.

**DIAGNOSIS:** Differs from *Sychnotylenchus ulmi* Ruhm 1956 in stylet length, absence of basal knobs, form of metacarpus, and absence of a discernible anal opening. *Sychnotylenchus scolyti* is in general a smaller species than *S. ulmi*.

**TYPE LOCALITY:** Ft. Collins, Colorado.

**TYPE HABITAT:** Associated with *Scolytus multistriatus* Marsh. in American elm, *Ulmus americana* L.



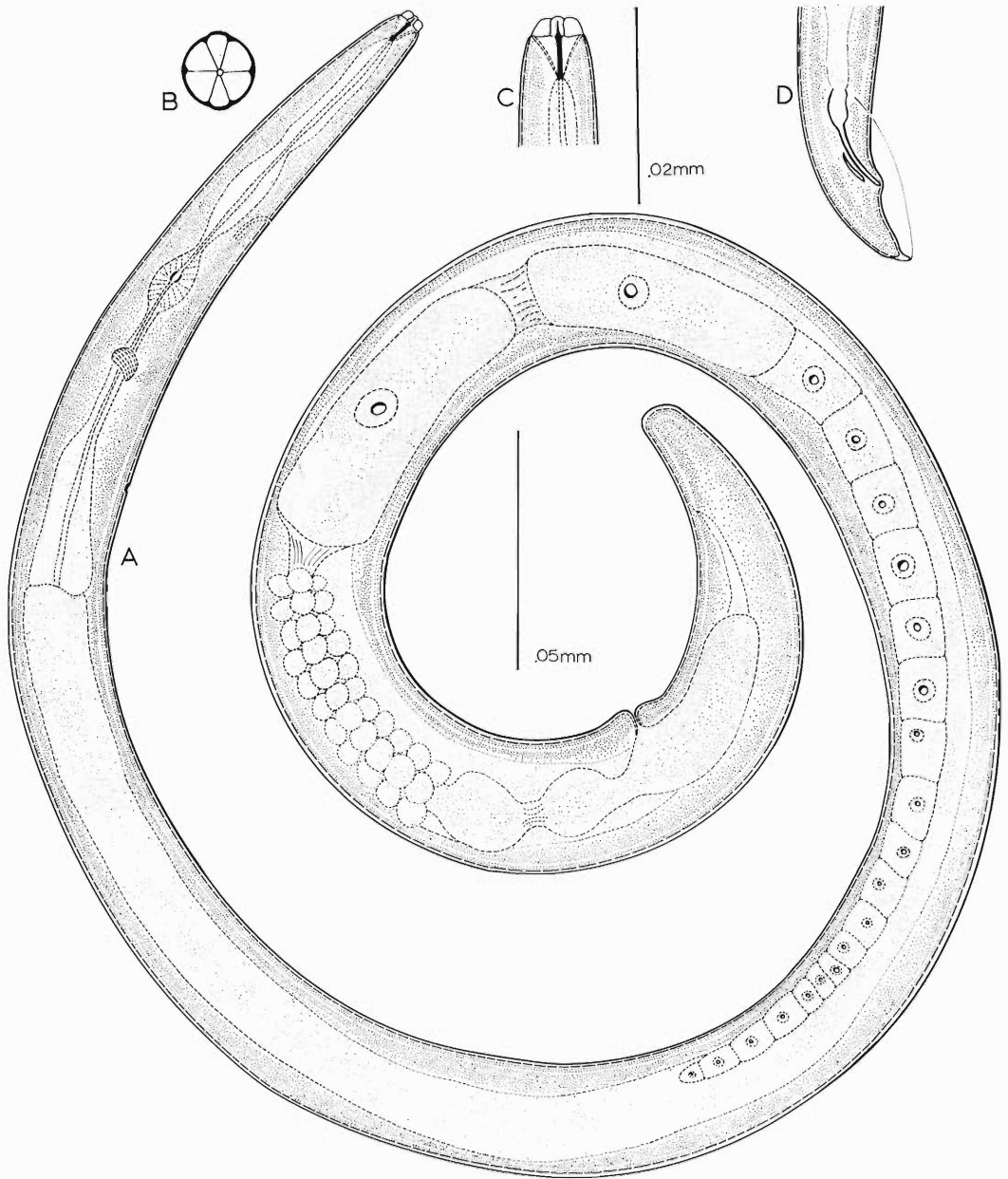


Figure 3. *Sychnotylenchus scolyti* sp. n. A, Female; B, Face view; C, Head; D, Male tail.

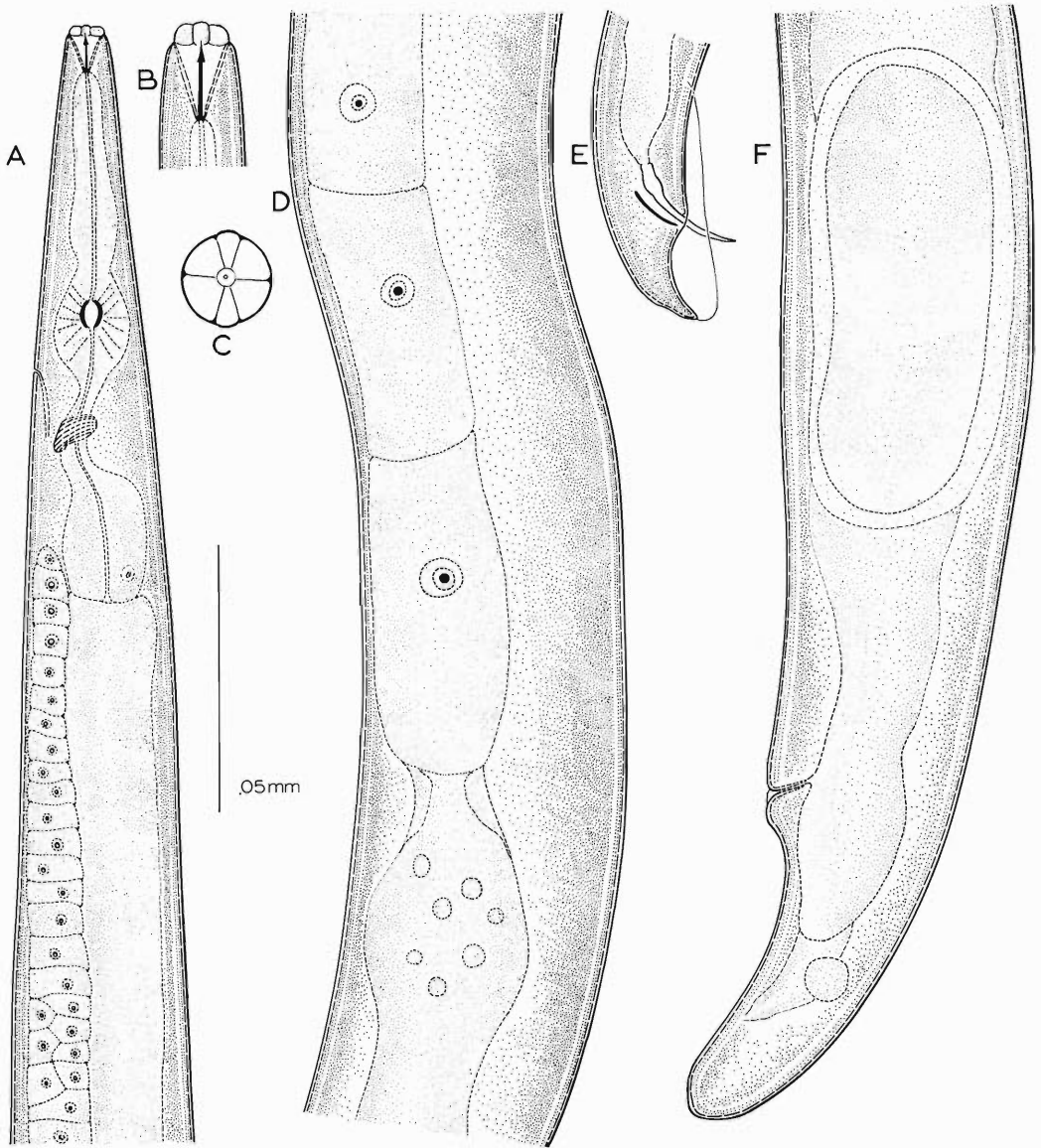


Figure 4. *Sychnotylenchus phloeosini* sp. n. A, Head and neck; B, Head; C, Face view; D, Female mid-body; E, Male tail; F, Female tail.

*Sychnotylenchus phloeosini* sp. n.  
(Fig. 4 A-F)

FEMALE (N = 3): L = 0.96–1.05 mm; a = 21–24; b = 9.3–9.5; c = ?; V = 92.6–93.6%.

MALE (N = 3): L = 0.66–0.89 mm; a = 29–38; b = 5.5–7.8; c = 38–39.

FEMALE: Body stout, narrow at anterior end, becoming widest immediately preceding vulva. Cuticle with faint longitudinal and transverse striations, the striations most discernible in neck region, becoming very faint at midbody in mature specimens. Head slightly

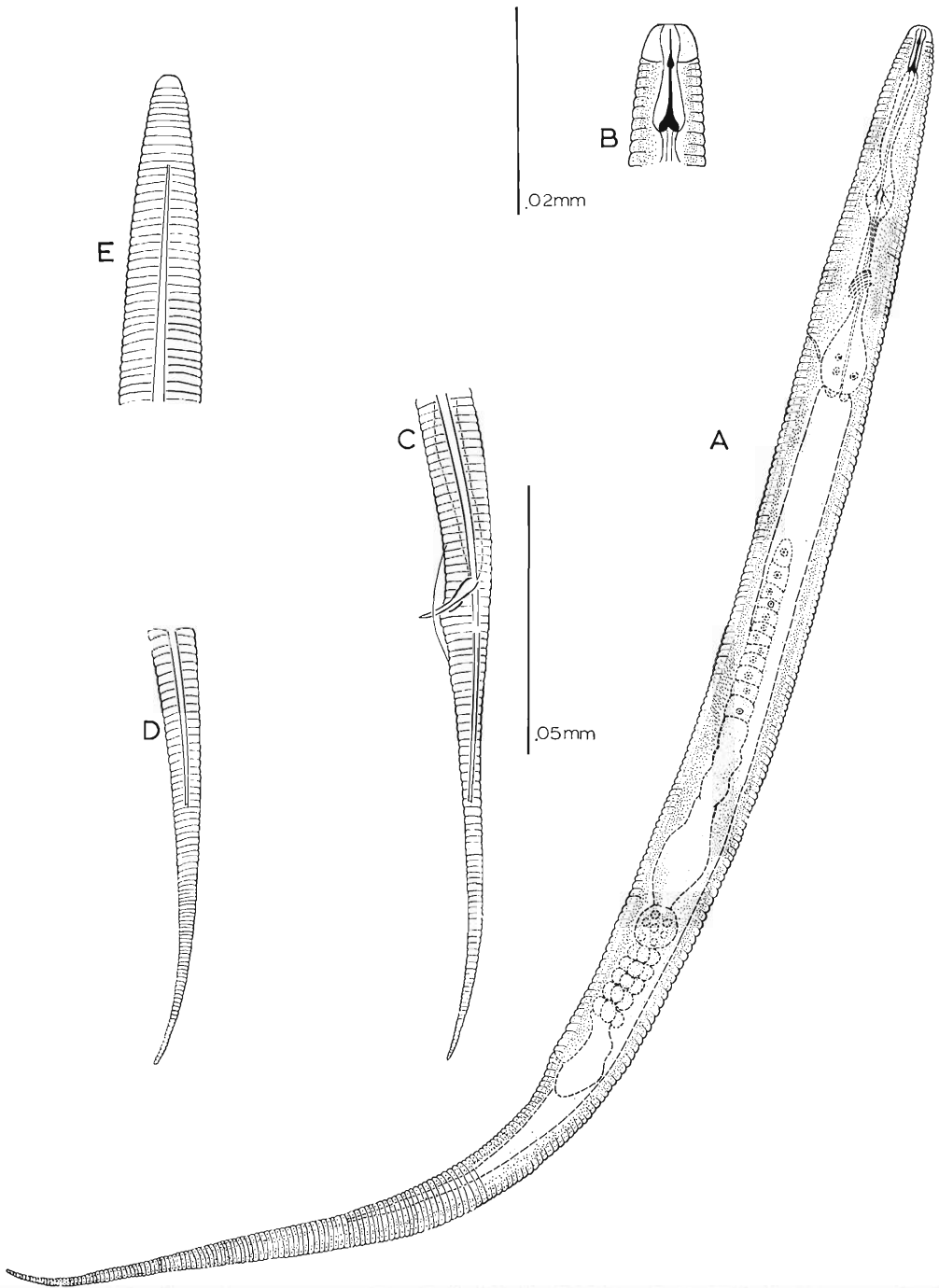


Figure 5. *Aglenchus exiguus* sp. n. A, Female; B, Head; C, Male tail; D, Tail showing lateral field; E, Head and neck showing lateral field.

set off, flattened, lip region twice as wide as deep, the lateral lips much narrower than other four, protruding beyond body contour in face view, Figure 4C. Stylet moderately slender with basal thickenings, Figure 4B. Procorpus of esophagus stout, narrowing into a prominent ovular median bulb in which the valvular apparatus is quite distinct. Terminal bulb as in Figure 4A. Nerve ring at the middle of the isthmus. Excretory pore slightly posterior to median bulb. Hemizonid not discernible in specimens examined. Amphidelphic, the ovary reaching to the median bulb in mature specimens, posterior uterine branch as in Figure 4F. Lips of the vulva protuberant. Vulva nearly transverse, anal opening not discernible. Terminus broadly rounded as in Figure 4F.

MALE: Body relatively slender. Head and lips as in female. Testes outstretched, reaching nearly to terminal bulb. Spicules paired, tylenchoid. Gubernaculum one-third to one-half length of spicules. Figure 4E. Bursa enveloping tail. Terminus subacute. Figure 4E.

DIAGNOSIS: Closely related to *Sychnotylenchus intricati* Ruhm 1956. Varies from that species in placement of excretory pore and nerve ring, absence of a lateral field, and discernible anal opening.

TYPE LOCALITY: Bandelier National Monument, New Mexico.

TYPE HABITAT: Associated with *Phloeosinus* sp. in *Juniperus utahensis* (Engelm.) Lemm.

*Aglenchus exiguus* sp. n.  
(Fig. 5 A-E)

FEMALE (N = 2): L = 0.31 mm; a = 19.7; b = 4.6-4.9; c = ?; V = 64%.

MALE (N = 2): L = 0.31-0.34 mm; a = 24.3-25; b = 4.6; c = 3.7.

FEMALE: Figure 5. Body slender ending in an elongate tail. Cuticle coarsely annulated, the annules widest at midbody and interrupted by very prominent lateral fields formed by two bright lines, Figure 5C, D, E. Lateral fields extending from two body widths posterior to anterior end to eight body widths anterior to terminus. Head slightly offset, Figure 5B. Lips only faintly discernible in lateral view. Stylet slender with prominent basal knobs, Figure 5B. Esophagus with valvate metacarpus, the procorpus and metacarpus equal in length to isthmus and terminal bulb. Excretory pore prominent, adjacent to center of

terminal bulb of esophagus. Nerve ring at middle of isthmus. Ovary single, outstretched, very short in mature specimens; postuterine branch short, one-half body width in length. Vulva transverse. Lips covered by vulval flap. Anal opening not discernible. Tail elongate, terminus acute.

MALE: With general body characters of female. Testis single, outstretched. Spicules typically tylenchoid. Gubernaculum and bursa as in Figure 5C. Tail elongate, terminus acute.

DIAGNOSIS: Closely related to *Aglenchus bryophilus* (Steiner, 1914) Meyl 1961, varies in the shape of the spicules and gubernaculum. Female without discernible anal opening.

TYPE LOCALITY: Mt. Taylor, New Mexico.

TYPE HABITAT: Taken from base of Engelmann spruce *Picea engelmanni* Parry infested with the Engelmann spruce beetle *Dendroctonus obesus* (Mannerheim).

*Neotylenchus nitidus* sp. n.<sup>2</sup>  
(Fig. 6)

FEMALE (N = 4): L = 0.79-0.86 mm; a = 31.6-37.3; b = 5.7-6.8; c = 11.1-13.3; V = 83%.

MALE: Unknown.

FEMALE: Figure 6. Body extended when relaxed. Cuticle with moderately fine transverse striations. Lip region hardly set off, continuous with body contour, twice as wide as deep. Stylet moderately coarse with prominent basal knobs. Corpus of the esophagus cylindrical, somewhat widened at the center of its length, then narrowing as it passes through the nerve ring, ending in a prominent basal bulb. Excretory pore immediately posterior to hemizonid, both approximately one-half body width anterior to the junction of the esophagus and gut. Ovary single, outstretched, quadricolumella occupying one-eighth of its total length. Posterior uterine branch well-developed, approximately one body width in length, vulva transverse. Anal opening only faintly discernible in some specimens. Terminus acute.

DIAGNOSIS: Closely related to *Neotylenchus acutus* Thorne 1941, varies from that species in the prominence of the knobs on the stylet,

<sup>2</sup> Nickle (1968) has made *Neotylenchus* a synonym of *Hexatylenchus*. It is the opinion of the writer that *Neotylenchus* is a valid genus. The character of the junction of the esophagus and intestine in members of the group is of sufficient variation to justify generic rank.

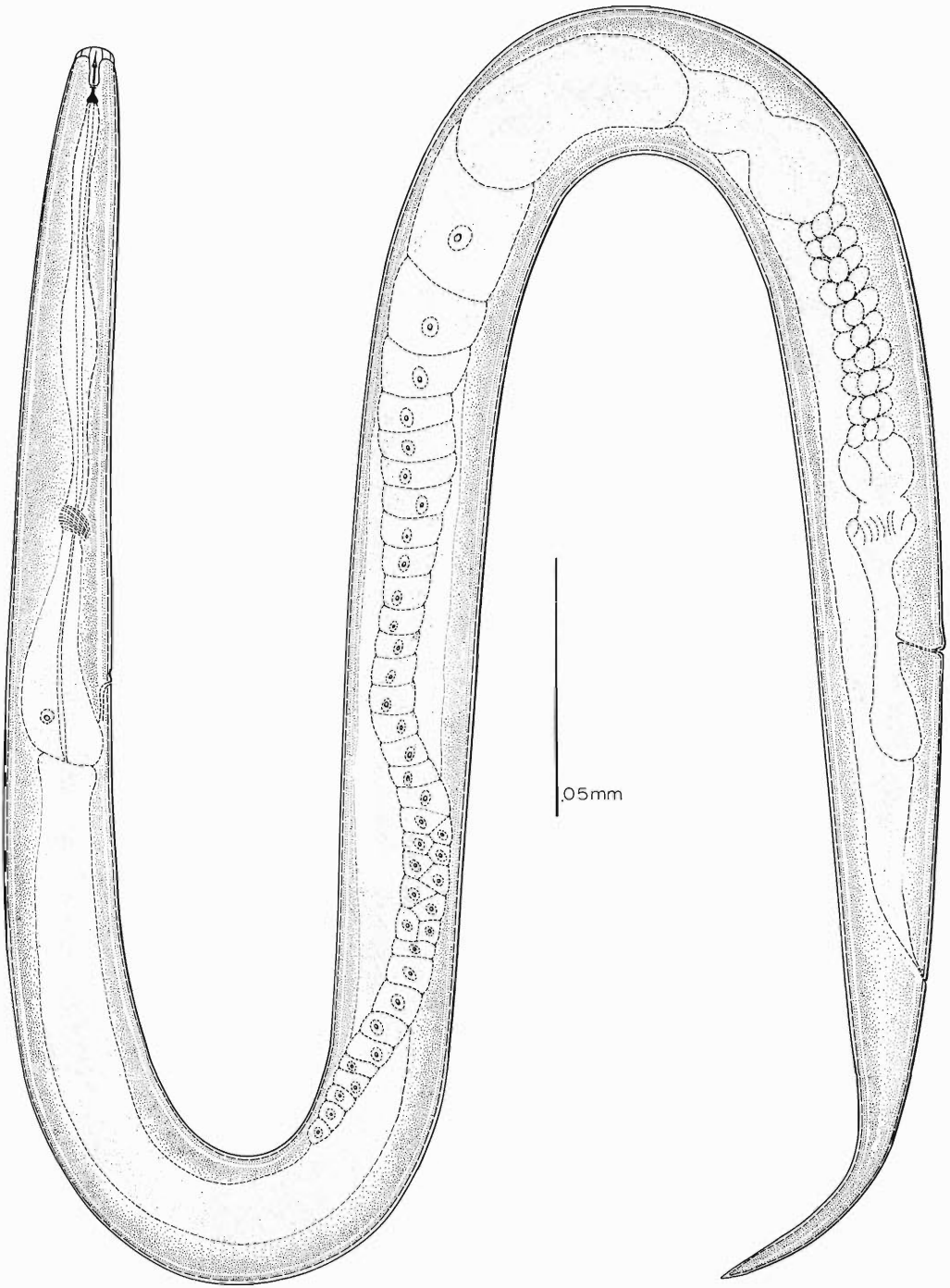


Figure 6. *Neotylenchus nitidus* sp. n. Female.

in the character of the junction of the esophagus and gut, and in the presence of a posterior uterine branch. It also varies in the absence of a lateral field.

TYPE LOCALITY: Red Feather Lakes Colorado, Roosevelt N.F.

TYPE HABITAT: Abandoned galleries of *Dendroctonus obesus* (Mannerheim) in Engelmann spruce, *Picea engelmanni* Parry.

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## The Proposed Synonymy of the Monogenean Genera *Cleidodiscus* Mueller, 1934 and *Urocleidus* Mueller, 1934, with the Proposal of *Cleidodiscus bychowskyi* sp. n.<sup>1</sup>

C. E. PRICE<sup>2</sup> AND A. MURA<sup>3</sup>

A total of 43 reports of monogenetic trematodes parasitizing North American catfishes (family Ictaluridae) has been recorded. In all cases, the parasite recovered was either *Cleidodiscus floridanus* Mueller, 1936 or *C. pricei* Mueller, 1936. In light of the numerous reports of these two monogeneans from the gills of several species of Ictaluridae, it appears that close parasite-host relationships exist between *Cleidodiscus* parasites and their ictalurid hosts. The present study includes a description of *Cleidodiscus bychowskyi*, the third species of Monogenea to be recovered from a member of this catfish family.

In a comparative study of *Cleidodiscus* and *Urocleidus*, Price (1967) expressed the opinion that these genera were very nearly identical in morphology. They could be separated from

each other by a single character: the accessory piece is basally articulated to the cirrus in *Cleidodiscus* whereas these structures exist separately in *Urocleidus*. Price (op. cit.) declined to amalgamate the two genera. After additional consideration, the authors feel that this single trait is not of generic magnitude. We consider these genera identical and recommend that species presently included under *Urocleidus* be transferred to *Cleidodiscus*.

### Materials and Methods

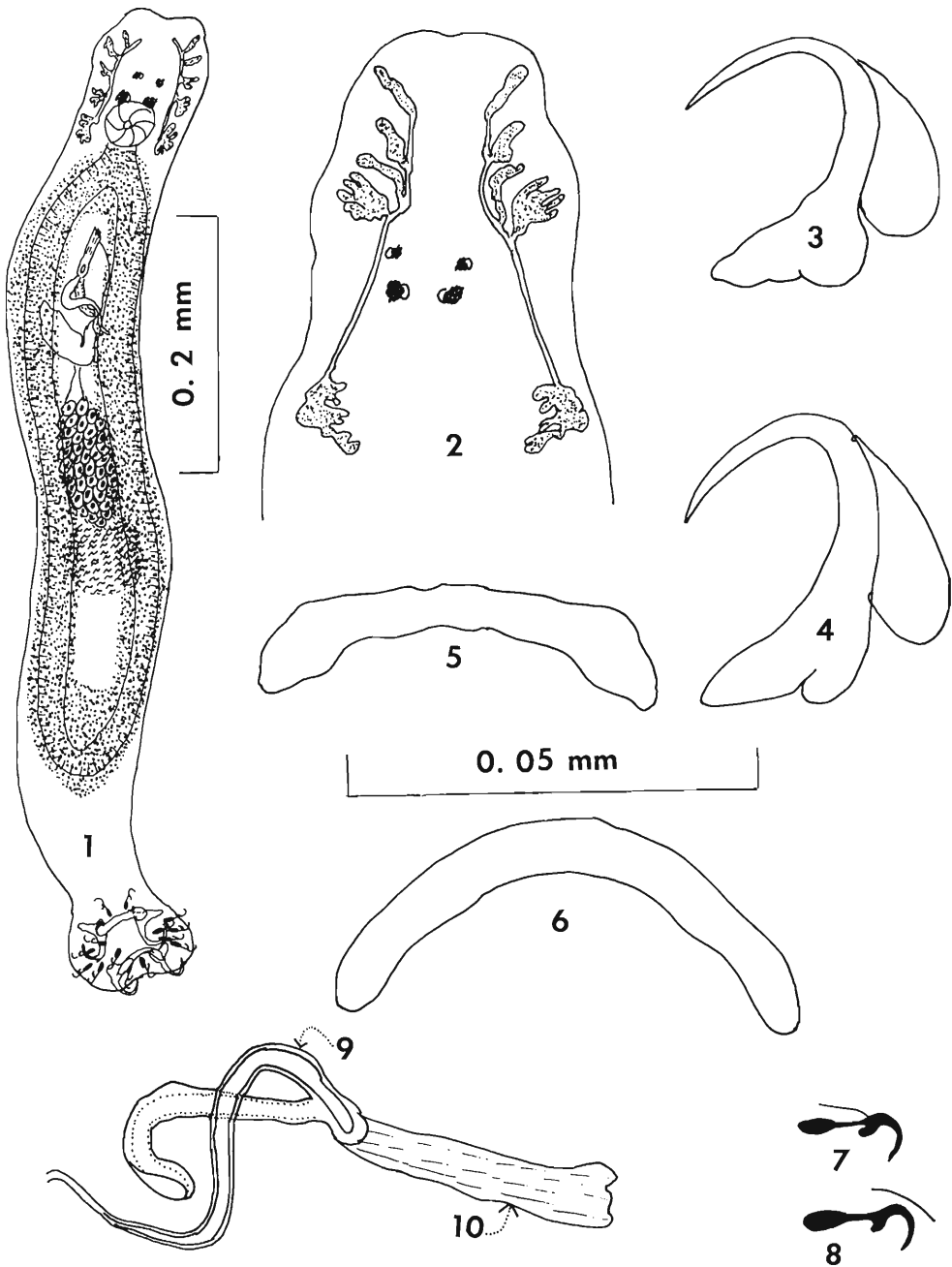
Host specimens utilized in this study were donated by Mr. W. L. McCann of Alexandria, Louisiana, to whom the authors wish to express their thanks.

Hosts were received frozen. After thawing, gills were removed and preserved in 85% isopropyl alcohol. From this point, gills and recovered parasites were treated as prescribed by Price (1966). Anatomical terminology employed is that recommended by Price and Arai (1967) for monopisthocotylean Mono-

<sup>1</sup> Study supported jointly by the Departments of Biology of Augusta College and Millersville State College and by a Grant-in-Aid of Research from the Society of Sigma Xi.

<sup>2</sup> Department of Biology, Millersville State College, Millersville, Pennsylvania 17551.

<sup>3</sup> Department of Biology, Augusta College, Augusta, Georgia 30904.



Figures 1-10. Camera lucida illustrations of *Cleidodiscus bychowskyi* sp. n. 1, whole mount (ventral view); 2, detail of anterior region, showing nature of head organs, cephalic glands, and eyespots with associated lenses; 3, ventral anchor; 4, dorsal anchor; 5, ventral bar; 6, dorsal bar; 7, 8, hooks, showing two of the many possible relative positions of the associated domus; 9, cirrus; 10, accessory piece. All figures except Figure 1 drawn to same scale.

genera and by Hargis (1958). Measurements were made according to the recommendations of Mizelle and Klucka (1953).

Appropriate measurements and illustrations were made with the aid of a calibrated filar micrometer ocular and a camera lucida, respectively. Average measurements are given first, followed by minimal and maximal values enclosed in parentheses. All measurements are expressed in microns.

We found that a tincture of Poinceau S served as an excellent stain for differentiating head organs and cephalic glands.

### *Cleidodiscus bychowskyi* sp. n.

HOST AND LOCALITY: *Ictalurus punctatus* (Rafinesque), the channel catfish; Calcasieu River, near Lake Charles, Louisiana (Calcasieu Parish).

NUMBER OF SPECIMENS STUDIED: Ten.

TYPES: Holotype USNM Helm. Coll., No. 71238. Paratypes in authors' collections.

DESCRIPTION: A dactylogyrid of moderate size, provided with a thin cuticle. Anterior two-thirds of body proper fusiform, tapering uniformly toward peduncle; length 793 (705–878), greatest width of body 162 (144 to 209). Cephalic lobes moderately developed. Peduncle and haptor variable in shape, depending upon state of contraction, haptor usually well set off from body proper (whole mount: Fig. 1). Two pairs of eyespots, members of posterior pair larger (Figs. 1, 2). Pharynx prominent, very muscular, and sub-spherical in ventral view. Four pairs of glandular head organs, connected to the multilobed cephalic glands by a common duct (Fig. 2).

Two pairs of anchors, one pair in ventral portion of haptor, other pair in dorsal. All anchors similar in both shape and size (Figs. 3, 4). Each composed of: (1) a solid base provided with a prominent superficial and a blunt deep root, (2) a solid shaft, and (3) a solid point which meets shaft at a definite angle. Anchor wings present. Ventral anchor length 45 (41–49), width of base 23 (21–25); dorsal anchor length 47 (45–51), width of base 22 (18–25). Bases of each anchor joined by a simple haptor bar, the bars similar in size but dissimilar in shape (Figs. 5, 6).

Length of ventral bar 56 (48–63); length of dorsal bar 60 (53–68).

Hooks 14 in number (7 pairs), all similar in shape and size; hook shape somewhat atypical for *Cleidodiscus* (Figs. 7, 8). Five pairs of hooks located on ventral aspect of haptor, remaining two pairs on dorsal aspect. Each hook composed of: (1) a solid elongate base joined to (2) a much narrower shaft, and (3) a sickle-shaped termination provided with a decurved opposable piece. Each hook provided with a domus. All hooks between 14 and 17 in length.

Copulatory complex composed of a cirrus and a basally articulated accessory piece (Figs. 9, 10). Cirrus equipped with an elongate, heavily sclerotized base. Cirrus tube tapers gradually to a point; length of cirrus *in situ* 80 (73–88), estimated total length 110 (95–129). Accessory piece in form of a slightly curved sclerotized bar with a flared termination and provided with a deep groove on its inner surface; length 34 (28–37). Prostatic reservoir single, filled with a yellowish granulated material and emptying into cirrus base by a fine duct. Vagina, when visible, opens ventrally near right body margin; vaginal duct opens into a thin-walled seminal receptacle. Ovary somewhat elongate longitudinally, larger than the subspherical, post-ovarian testis. Vitellaria composed of dark granules of essentially uniform size, the vitellaria forming two broad bands laterally. Intestinal limbs confluent posteriorly.

### Discussion

*C. bychowskyi* does not appear to closely resemble any known form presently ascribed to either *Cleidodiscus* or *Urocleidus*. It would serve no apparent useful purpose to designate species possessing bars and/or anchors resembling those of the present new species, as a great degree of homogeneity of these structures exists among most of the included forms. The accessory piece of *C. bychowskyi* is unique among members of either genus. The hooks of the present form are atypical for both *Cleidodiscus* and *Urocleidus*.

DERIVATION OF SPECIES NAME: This parasite is named in honor of Dr. Boris E. Bychowsky, Director of the Zoological Institute of the



USSR and author of the most complete account of the Monogenea thus far published (Bychowsky, 1957). Dr. Bychowsky (personal communication) has been a great source of both information and encouragement to the senior author.

### Summary

The very similar morphological features of the genera *Cleidodiscus* Mueller, 1934 and *Urocleidus* Mueller, 1934 have been comparatively studied. It is proposed that these genera are similar to the extent that they are considered to compose the single genus *Cleidodiscus*. It is recommended that species now included under *Urocleidus* be transferred to *Cleidodiscus*.

A new species of the North American Monogenea, *Cleidodiscus bychowskyi*, recovered from the gills of channel catfishes [*Ictalurus punctatus* (Rafinesque)] captured in Louisiana, is described.

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## Studies on *Haplobothrium bistrobilae* sp. nov. (Cestoda: Pseudophyllidea) from *Amia calva* L.

G. PREMVATI<sup>1</sup>

Department of Biology, Florida Agricultural and Mechanical University, Tallahassee, Florida

The genus *Haplobothrium* Cooper, 1914 of the family Haplobothriidae Meggitt, 1924 has so far only one species, *H. globuliforme* Cooper, 1914, described from only one host, *Amia calva* L., 1766. An examination of the same fish from Florida fresh waters revealed the presence of a new species of *Haplobothrium*. Ten bowfins were examined and all were found to be infected with this cestode. Four were male fish and had very light infection: each fish harboring not more than three to four immature worms: one to two with primary scolices and two to three with pseudoscolices. In the remaining six female fish, the number of worms varied from 10–18 with primary scolices and 55 to 65 with pseudoscolices.

The cestodes were flattened in hot water and fixed in 10% formalin. The worms were stained for whole mounts with either cochineal or Semichon's carmine or aceto alum carmine or hematoxylin. For sections, worms were fixed in hot Bouin's fluid and stained in hematoxylin and eosin. Serial frontal and cross sections were prepared from the region of primary scolex, pseudoscolex, immature strobila, and mature strobila. Osmoregulatory system was studied and photographed in living specimens.

All measurements are in microns unless otherwise indicated.

### *Haplobothrium bistrobilae* sp. nov. (Figs. 1–15)

HOST: *Amia calva* L., 1766.

LOCALITY: Lake Munson, Leon County, Florida.

<sup>1</sup> Present address: Department of Zoology, University of Lucknow, Lucknow, India.

LOCATION: First half of intestine.

HOLOTYPE and nine paratypes in USNM Helm. Coll. Nos. 71209 and 71210.

DESCRIPTION: Specimens very active and show considerable relaxation and contraction, occurring in two phases: primary strobila and secondary strobila. Primary strobila with true scolex, followed by a growth zone (neck region), 10–25 mm in length with only external segmentation, followed by a long chain of narrow segments. Each segment subdivides anteriorly into three to eight segments of secondary strobila. Anterior segments of secondary strobila, when complete, develop four auricular lobes: one dorsal, one ventral, and two lateral. Original segments with a number of anterior proglottids break off from primary strobila and develop into secondary strobila. Thus primary strobila is simply a dividing strobila which gives rise to secondary strobila. Genital organs never seen to differentiate in proglottids of primary strobila.

Primary strobila has a typical trypanorhynch scolex, with four protrusible and eversible proboscides. Each proboscis has a proximal spiny base or stump which can also be protruded or retracted, and a distal nonspiny elongated tentacle. Proboscides enclosed in invaginated saclike structures. Cross sections show each proboscis enclosed within a muscular sac, and surrounded by a cuticular layer, circular layer and longitudinal layer.

Secondary strobila between 85–100 mm in length. Anteriormost segment becomes modified into a pseudoscolex, with spines developing on its anterior margin. Segmentation complete and begins immediately posterior to pseudoscolex. Anterior segments broader than long, with distinct four auricular lobes, and then gradually become longer than broad, and appear beaded to naked eye. Reproductive organs appear after about 20–45 segments. With growth of genitalia, internal segmentation starts to disappear first, and ultimately external segmentation also not visible in gravid seg-

ments. Terminal proglottid conical in shape.

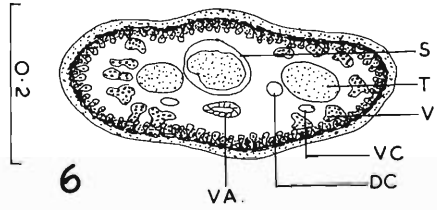
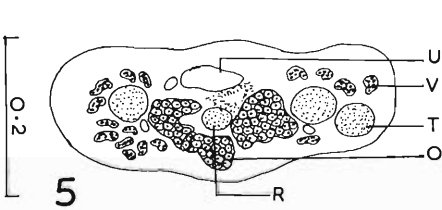
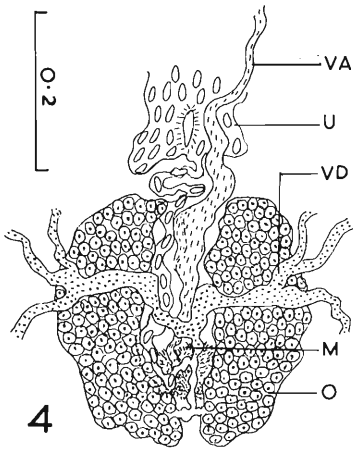
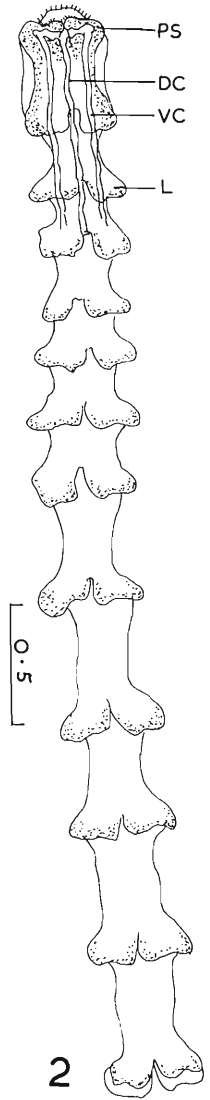
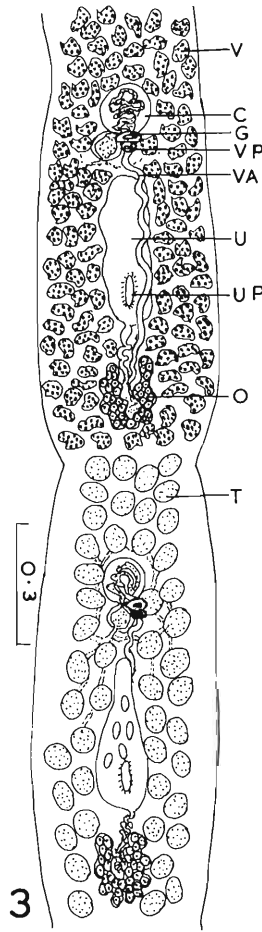
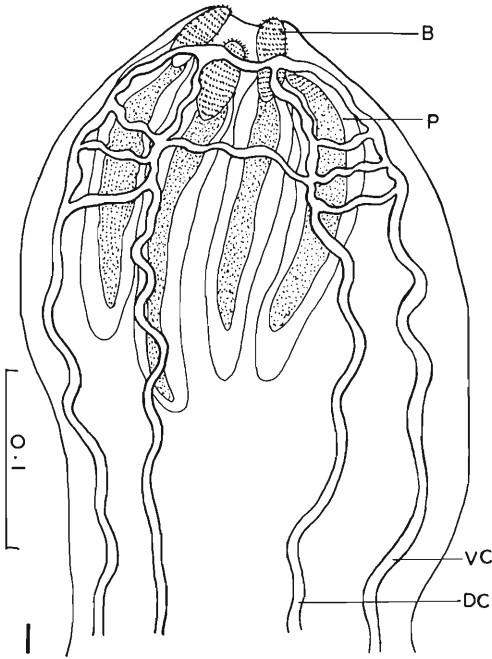
Testes rounded, medullary in position and measure 80–100 by 45–50; number of testes in immature proglottids varies from 12–14 on each side, arranged in two rows, i.e., total number 24–28. This number becomes double after about five or six proglottids, and 24–28 testes lie in double alternating rows on each side. Total number of testes not seen to exceed 56 in any proglottid. Vasa efferentia on either side join and form vas deferens, submedian in position and leads to a globular external seminal vesicle, followed by a cirrus sac enclosing internal seminal vesicle, ejaculatory duct and cirrus. Cirrus sac measures 250 in diameter; cirrus short, spiny, cylindrical, muscular and eversible, opening at level of external seminal vesicle.

Ovary measures 200–250 by 240, median, and lies at posterior portion of proglottid. Lobes of ovary connected by isthmus; oviduct arises from isthmus, runs anteriorly for a short distance, and joins common vitelline duct. Vitellaria follicular, medullary, and extend from anterior margin to posterior margin of proglottid on two lateral sides. Follicles measure 40–60 by 40. Two anterior and one posterior vitelline ducts join in region of ovary and form a thick short vitelline duct, running transversely on either side. Two transverse vitelline ducts join in space between two limbs of ovary and form a very short vitelline reservoir. From this, the common vitelline duct runs posteriorly to join oviduct just before ootype, which is surrounded by Mehlis' gland. Uterus runs anteriorly as a coiled tube for a short distance, and then enlarges to form a uterine sac, with a permanent uterine pore. Vagina opens on ventral aspect by a circular pore just posterior to cirrus opening. Vagina enters female genital space and enlarges to form seminal receptacle. Eggs measure 59–60 by 25.

Osmoregulatory system may be studied in three parts: (1) in primary scolex and neck

→

Figures 1–6. *Haplobothrium bistrobilae* sp. nov. 1. Camera lucida sketch of primary scolex of living specimen to show the proboscides and osmoregulatory system. 2. Pseudoscolex of secondary strobila with immature proglottids. 3. Two mature proglottids: the first showing vitellaria and the other position of testes. 4. Enlarged view of female genitalia. 5. Cross section from the region of ovary of a mature proglottid. 6. Cross section from the region of external seminal vesicle of a mature proglottid.



region; (2) in immature strobila; and (3) in mature and gravid strobila.

Osmoregulatory canals in primary scolex with two pairs, a ventral and a dorsal canal on either side. Ventral canals with sinuous course, run on outside and on reaching basal spiny portion of proboscides, curve inwardly; two dorsal canals also sinuous and run inner to ventral canals, and as they reach anterior half of proboscides, they are connected with a transverse canal. Three small commissures join ventral canal to dorsal canal on either side of anterior half of scolex. Thereafter dorsal canal runs anteriorly and seen to be confluent with ventral canal (Fig. 1). In neck region, two ventral canals run as two separate canals, one on either side. Dorsal canal single and median, and bifurcates into two canals after forming one or few islets (Figs. 10, 15).

In immature strobila, only three longitudinal canals run along vertical axis of body, one ventral on either side of body, and a single median dorsal canal (Fig. 13). Dorsal canal slightly thicker at places, otherwise all three canals practically of uniform width and have sinuous course.

In mature strobila, two ventral canals run longitudinally in a sinuous course, one on either side of body. Course of median canal rather unique. It runs both as a single canal and as two bifurcated canals. When present as two dorsal canals, they run on either side of genital organs, and may unite either in same proglottid or may run as two separate canals for a couple of proglottids and then join (Fig. 14), thus showing no regularity in bifurcation or union of dorsal canals in mature strobila.

In last proglottid, two ventral canals seen to open separately by excretory pores a small distance from posterior end. Two dorsal canals form two excretory vesicles at posteriormost portion of proglottid (Fig. 11). No connection between ventral canal and dorsal canal of either side in any proglottid. But when proglottids break up and anterior end forms a pseudoscolex of secondary strobila, two ventral canals connect with single median dorsal canal without forming any ring canal (Fig. 2). Flame cells numerous in each proglottid, in groups of four and as many as twenty could be counted on either side of one proglottid.

### Discussion

Genus *Haplobothrium* Cooper, 1914, family Haplobothriidae Meggitt, 1924, seems to be confined to only one host, *Amia calva* L. which is now found only in North America. *H. globuliforme* Cooper, 1914, the only species of this genus, is so far reported mostly from northern parts of the United States and from Mississippi in the South. This is the first record of this genus from Florida.

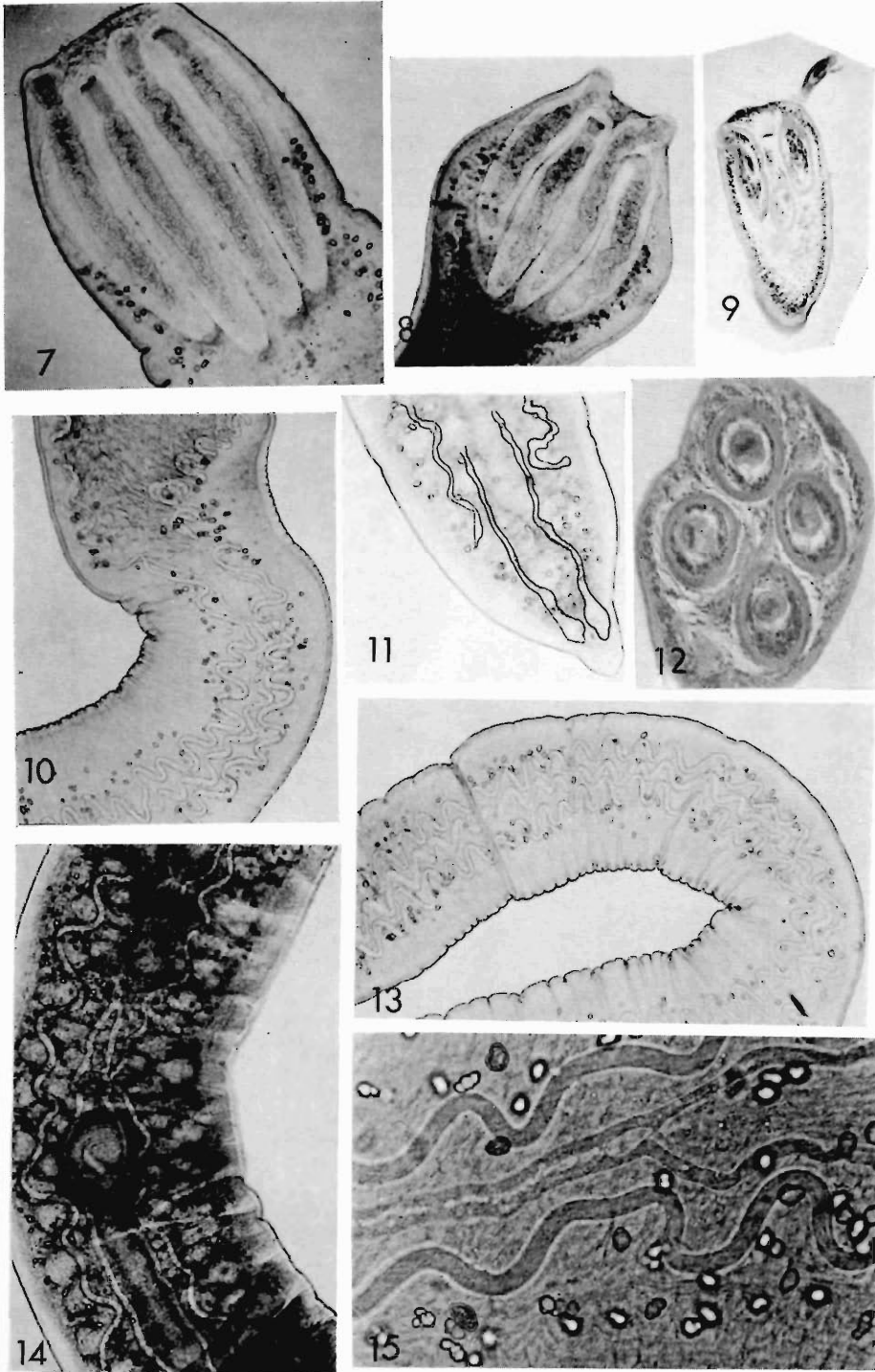
The morphology and systematic position of *H. globuliforme* was described in detail by Cooper (1914, 1919). Essex (1929), Thomas (1930), and Meinkoth (1947) described its life cycle and showed that it is similar to that of pseudophyllideans, involving the development of proceroid and pleuroceroid, and that the characteristic trypanorhynchan scolex develops later in the pleuroceroid stage.

*Haplobothrium bistrobilae* sp. nov. differs from *H. globuliforme* in the following: (1) The total number of testes is only 48-56:24-28

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Figures 7-15. *Haplobothrium bistrobilae* sp. nov. (Photomicrographs). 7. Living primary scolex showing completely retracted proboscides. 8. Living primary scolex showing protruded basal spiny portion and retracted tentacles of the proboscides. 9. Frontal section of primary scolex. 10. Osmoregulatory system in neck region of living specimen. Primary strobila, showing bifurcation of median dorsal canal. 11. Posteriormost proglottid in living specimen showing excretory pores of ventral canals and two excretory vesicles of dorsal canals. (Note: Figure retouched.) 12. Cross section of primary scolex showing proboscides. 13. Osmoregulatory system in living immature proglottids, showing two ventral canals and single median dorsal canal. 14. Osmoregulatory system in living mature proglottids, showing two ventral canals and union and bifurcation of dorsal canal. 15. A portion of dorsal canal of neck region of living primary strobila to show islets before bifurcation into two dorsal canals.

Abbreviations used: B, basal spiny portion of proboscis; C, cirrus sac; DC, dorsal canal; G, male genital pore; L, auricular lobe; M, Mehlis' gland; O, ovary; P, proboscis; PS, pseudoscolex; R, seminal receptacle; S, external seminal vesicle; T, testis; U, uterus; UP, uterine pore; V, vitellaria; VA, vagina; VC, ventral canal; VD, vitelline duct; VP, vaginal pore.



on each side of proglottid arranged in two alternate rows. In *H. globuliforme*, the number of testes is 80:40 on each side. (2) Uterine pore persists even in gravid proglottids, but ceases to exist in those of *H. globuliforme*. (3) The proboscides are completely retractable, while in *H. globuliforme* the stump or the base remains permanently protruded. (4) The disposition of vitelline ducts differs from that of *H. globuliforme*. (5) The eggs are smaller as compared to those of *H. globuliforme*. (6) The course of dorsal canal ("median vessel" by Cooper) does not remain single as in *H. globuliforme* but becomes double at places in mature and gravid proglottids. The terminal proglottid also shows two distinct vesicles of dorsal canals at posteriormost end. The two openings of ventral canals are seen to lie separately on the lateral sides of the last proglottid.

### Summary

*Haplobothrium bistrobilae* sp. nov., the second species of the genus *Haplobothrium* Cooper, 1914, family *Haplobothriidae* Meggitt, 1924, is described from *Amia calva* L. from Florida fresh waters. It is characterized by having 48-56 testes (24-28 on each side); permanent uterine pore; completely retractable proboscides with spiny base and nonspiny distal tentacles, and a unique course of dorsal osmoregulatory canal. Photomicrographs of the osmoregulatory system studied in living worms show that the two ventral canals have a sinuous course throughout the primary and secondary strobila. The dorsal canal is single in immature proglottids but bifurcates at places in mature and gravid proglottids of secondary strobila. The terminal conical proglottid shows two distinct vesicles of dorsal canals and two excretory pores of ventral canals.

*Haplobothrium bistrobilae* sp. nov. differs from the only other species, *H. globuliforme* Cooper, 1914, in the number of testes, presence of permanent uterine pore, size of eggs, in having completely retractable proboscides, and in not having a single continuous dorsal canal.

### Acknowledgments

The author wishes to thank Professor Robert B. Short of the Florida State University for the use of his personal library; Dr. N. A. Meinkoth, Department of Biology, Swarthmore College, Swarthmore, Pennsylvania, for the generous supply of slides and fixed material of *Haplobothrium globuliforme* for purposes of comparison, and Dr. W. W. Becklund of the Beltsville Parasitological Laboratory for providing type and paratype slides of *H. globuliforme* from USNM collection.

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## *Reesimermis nielseni* gen. et sp. n. (Nematoda: Mermithidae) Parasitizing Mosquitoes in Wyoming

YUAN-HWANG TSAI<sup>1</sup> AND ALBERT W. GRUNDMANN

Tropical Disease Research Center, St. Clare's Hospital, New York City, New York, and  
Department of Environmental Biology, University of Utah, Salt Lake City, Utah

There has been a renewed interest in the parasites of mosquitoes as the search for organisms that could prove valuable in biological control is pursued. As a result of such a study in northern Utah and southern Wyoming regions, a species of mermithid nematode was found inhabiting six mosquito species at a site near Lone Tree, Uinta County, Wyoming. Following an extensive study of the life history, pathogenesis in mosquitoes, and morphology of this organism, a new genus and species name is proposed.

Mermithid nematodes have been reported frequently from both larvae and adults of mosquitoes in North America and elsewhere. Jenkins (1964) listed four defined species, *Limnomermis aquatilis* Dujardin from larval Anophelines in France, *Agamomermis culicis* Stiles from adult *Aedes sollicitans* (Walker) from the United States, *Paramermis canadensis* Steiner from *Aedes vexans* (Meigen) and *A. strictus* in British Columbia, Canada, and a *Mermis* sp. from larval *Aedes aegypti* in Africa (Gendre 1909). Other records of *Mermis* sp. are: Muspratt (1945) in larval and adult Anopheles, Iyengar (1927) in India and Walandouw (1934) in Sumatra. At least fifteen additional occurrences in mosquitoes have been reported in North American literature.

Other mosquito-inhabiting species that have been described are *Hydromermis churchillensis* Welch 1960 from larval *Aedes communis* (De Geer) from Manitoba, Canada. This species was also reported from Canada by Jenkins and West (1954) from *A. communis*, *A. impiger* (Walker), *A. nigripes* (Zetterstedt), and *A. pionips* Dyar. Smith (1961) reported what is assumed to be this same species from larval *A. pullatus* in Colorado. Welch (1964) described *Romanomermis iyengari* from *Anopheles subpictus* Grassi in India. A species of

*Gastromermis* was reported by Cox (1966) from *Anopheles funestus* Giles in west Africa.

Welch (1960) reviewed the status of reported mosquito-inhabiting mermithids from North America and proposed referring *Paramermis canadensis* to species *inquirendae* because it was indicated that the description had been based on immature specimens. He further stated that a definite status for *Agamomermis culicis* would be unwise at this time since the female was unknown and thus the generic diagnosis insufficient. Reviewing the world status of mermithids inhabiting mosquitoes, three species belonging to three genera currently are determined valid, while four may be determined only to genus.

The systematics of the Family Mermithidae Braun 1883 is difficult due to the scattered literature and observations on immature forms. Best references are Filipjev (1934), Filipjev and Schuurmans (1941), and the work of a number of Russian workers that were nicely reviewed by Welch (1965).

In North America, Johnson (1963) erected the genus *Octomyomermis* from Chironomids. Poinar (1964) established *Orthomermis*, also from Chironomids. Welch (1960a, 1962b, 1963a, 1964, 1965) has described species from six genera in Simulid larvae.

### General Description Genus *Reesimermis*, gen. n.

*Reesimermis*: Mature adult worms elongate, cylindrical, with obtuse ends in both males and females. Cephalic papillae consisting of a crown of six in a hexagonal position, which may represent two lateral and four submedial cephalic nerve endings. Eight longitudinal hypodermal chords in dorsal, ventral, lateral, and submedial positions (Fig. 10). Spicules paired, slightly curved, medium-sized, fused posteriorly for more than half of their length (Fig. 8). Vagina straight, of medium length, muscular and pear-shaped. Cuticle smooth, no

<sup>1</sup> Present address: Microbiological Department, Bristol Laboratories, Syracuse, New York 13201.

criss-cross striae on the middle cuticle layer. Mouth opening terminal. Postparasitic juveniles with tail appendage. Aquatic habitat.

### Diagnosis

*Reesimermis* is the second genus to be described in the family Mermithidae possessing two spicules that are fused posteriorly for more than half their length. Four established genera are found that possess eight longitudinal chords, and *Reesimermis* differs from each of these in several distinct anatomical features. The genus *Hydromermis*, which contains *H. churchillensis* and *H. contorta*, differs from *Reesimermis* in the shape of the vagina which is S-shaped in *Hydromermis* but straight and pear-shaped in *Reesimermis*, and in the morphology of the spicules of which there are two fused for half of their length in the new genus and described as single in *Hydromermis*. However, *H. churchillensis* and *H. contorta* possess two spicules fused for more than half their length in contradiction to the generic characters but have the S-shaped vaginal characteristics of *Hydromermis*. These two species may be considered as intermediate evolutionary forms occurring between the separate, paired spicules of *Isomeris* Coman 1953 and the single spicule of *Hydromermis*. Both genera are similar in other characteristics.

The genus most closely related to the new genus is *Romanomermis* which differs primarily in having two completely separate spicules. Both genera inhabit mosquitoes. Another genus appearing in the literature that should also be considered is *Octomyomermis*, but when the published description was compared with that of *Romanomermis*, the two seemed to be almost identical. Since no specimens of either group were examined by the

authors, it is not possible to accurately state that *Octomyomermis* should be synonymized with *Romanomermis*. Other genera possessing paired spicules, pear-shaped vagina, and eight longitudinal chords are *Allomermis* and *Orthomermis*, but these differ from each other and from *Hydromermis* in the arrangement of cephalic papillae. The following key separates these genera from *Reesimermis*:

1. Four cephalic papillae in one plane; two spicules completely separated; criss-cross striae on the cuticle; mouth opening ventral ..... *Allomermis*  
Different arrangement and number of cephalic papillae; two spicules separated or fused posteriorly for more than half of their length; no criss-cross striae on the cuticle; mouth opening terminal ..... 2
2. Two lateral cepalic papillae in one plane with six papillae in ring underneath; two spicules separated ..... *Orthomermis*  
Six cephalic papillae in one plane; two spicules separated or fused more than half of their length ..... 3
3. Paired spicules separated ..... *Romanomermis* (*Octomyomermis*)  
Paired spicules fused for more than half of their length ..... *Reesimermis*

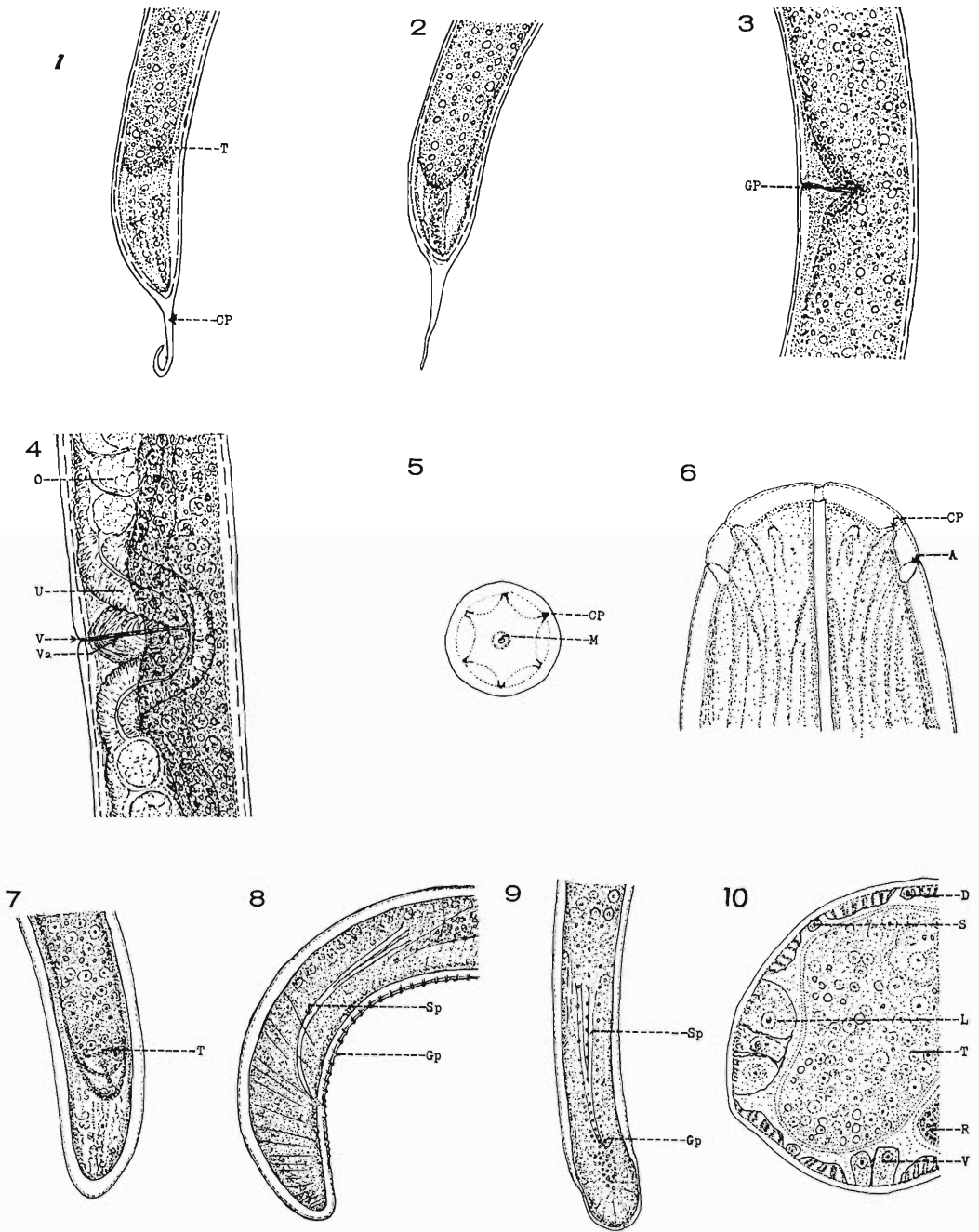
### *Reesimermis nielsenii* sp. nov.

GENERAL: All following measurements are in millimeters unless otherwise noted. Worm long and slender with smooth cuticle. Criss-cross fibers in middle cuticular layers absent. Cuticle thin, 5.5–7.7  $\mu$  in adults, and 2–3  $\mu$  in postparasitic larvae. Head convex, rounded with six cephalic papillae consisting of four submedian and two lateral papillae arranged

→

Figures 1–10. 1. Lateral view of postparasitic male juvenile tail. T, trophosome; CP, caudal appendage ( $\times 100$ ). 2. Lateral view of postparasitic female juvenile tail ( $\times 100$ ). 3. Lateral view of female postparasitic juvenile vagina. GP, genital primordium ( $\times 150$ ). 4. Lateral view of vagina (mature female worm). O, ova; U, uterus; V, vulva; Va, vagina ( $\times 200$ ). 5. Face view of mature worm head. CP, cephalic papillae; M, mouth opening ( $\times 400$ ). 6. Dorsal view of mature female head. CP, cephalic papillae; A, amphid ( $\times 500$ ). 7. Lateral view of mature female tail. T, trophosome ( $\times 100$ ). 8. Lateral view of mature male tail. Sp, spicule; Gp, genital papillae ( $\times 100$ ). 9. Ventral view of mature male tail. Sp, spicule; Gp, genital papillae ( $\times 100$ ). 10. Cross section in anterior region of female. D, dorsal hypodermal chord; L, lateral hypodermal chord; R, reproductive organ; S, submedian hypodermal chord; T, trophosome; V, ventral hypodermal chord ( $\times 400$ ).





hexagonally in one plane and in a slight depression of cuticle (Fig. 5). Mouth terminal in slight depression of cuticle. Small neck constriction visible behind papillar depression. Amphids located behind cephalic papillae pouch-shaped, pores small, situated about 2–4  $\mu$  behind lateral papillae. Ligament connecting amphids absent. Oesophagus folded, diameter 4.5–5.5  $\mu$  (average 5  $\mu$ ) near mouth opening, and 4  $\mu$  along main tract; extends over three-fourths of body length. Nerve ring 265–290  $\mu$  from mouth. Excretorylike glands 0.66–0.85 mm from mouth opening. Sticho-cytes beside oesophagus numerous and glandular-shaped. Lateral hypodermal chords with three cells (a few with paired cells). Male and female worms have obtuse terminus and are slightly curved ventrally. Free-living infective juveniles, postparasitic and parasitic juveniles retaining needle-shaped caudal appendage.

#### i. Female

Seventeen specimens. Body length 15.10 (11.0–21.2), stout, blunt at posterior end, tapering at anterior end. Greatest width of head at level of papillae 0.060 (0.055–0.065); at vulva 0.188 (0.160–0.225); and at tail measured at the terminus of trophosome 0.141 (0.125–0.195). Amphid 13 by 5  $\mu$ . Distance from anterior ovary to mouth opening 0.84 (0.32–1.33), that of posterior ovary to base of tail 0.57 (0.43–0.69).

Vulva, 45.7% (41.1–53.1%) of body length, a transverse opening slit (0.049) in ventral view, straightly located in lateral view, leading to muscular pear-shaped vagina that is straight thick-walled tunnel, 0.112 (0.095–0.130) in length, connected to opposed amphidelphic muscled uteri, 0.070 in width and 0.190 (0.180–0.198) in length.

Rate of anterior ovary to body length 41.8% (37.4–46.2%) and that of posterior ovary to length is 48.7% (42.3–53.9%), so  $v = 1.845.7^{48.3}$ . Length of posterior end of trophosome to base of tail 0.150 (0.100–0.170), that of anterior end of trophosome to degenerate mouth opening 0.420; no anal opening (Figs. 4, 6, and 7).

#### ii. Male

Eleven specimens. Body length 10.2 (6.2–14.10). Head width measured at level of

papillae 0.050 (0.048–0.053); width at middle body 0.144 (0.120–0.160); and width at anus 0.116 (0.096–0.130). Tail length 0.157 (0.140–0.175). Amphids same as in female. Paired spicules, fused posteriorly for 63–68% of their length, 250 (205–310)  $\mu$  in length. Individual bases of the separated proximal ends 22–26  $\mu$ . Width of single separate spicule 4.4  $\mu$  at midpoint, and that of the middle fused part 6.5  $\mu$ ; gently curved, tapering to sharp pin-point. Testes two parallel tubes running to middle of body possessing some flagellated sperm. Three rows of pregenital papillae. Median row with 19–22 papillae, lateral row with 16–19 papillae, postgenital papillae irregular, clustered and tiny. The two lateral papillar rows converge close to median row at anal area (Figs. 8, 9).

#### iii. Eggs

Flattened ellipsoid shaped, 84 by 74 (80–90 by 70–80)  $\mu$ , with cross-section 74 by 68 (70–80 by 63–70)  $\mu$ . Egg shell thin, transparent and sticky outside, with very thin vitelline membrane beneath the shell (visible only in preserved specimens). Eggs unembryonated with one egg cell when deposited. Size of eggs in ovaries smaller and irregular in shape, crowded side by side.

#### iv. Preparasitic juveniles

Body length 0.56–1.20; width at head region 6–7  $\mu$ , width at the middle of body trunk 15–17  $\mu$ . Onchostylet sharp and thin, about 12–14  $\mu$  in length. Numerous granular cells in body. Head rounded, tail slender, long, whiplike.

#### v. Parasitic juveniles

Body length and width vary with state of development. In 28 specimens measured, length 0.88–13.4; greatest width 0.034–0.125. Head rounded, tail long, slender.

#### vi. Postparasitic juveniles

Body length 7.4–25.5; greatest width 0.103–0.190. Ratio of head to body width 0.31–0.44. Anterior trophosome arising 0.370 (0.310–0.420) from head end, posterior trophosome arising 0.085–0.200 from base of caudal appendage. Caudal appendages 0.150–0.190 long. Sexes are distinguishable (Figs. 1, 2).

**SPECIMENS DEPOSITED:** Holotype—10 female adults and 10 male adults in the University of Utah Parasitological Collection.

**Table 1. Seasonal fluctuation in female-male ratio (FMR) for postparasitic juvenile populations of *Reesimermis nielsenii*.**

Date collected	No. of worms	Female		Male		FMR*
		No.	Per cent	No.	Per cent	
21 May 1966	142	32	22.53	110	77.47	0.29
12 June 1966	611	327	53.50	284	46.50	1.15
19 June 1966	277	105	37.90	172	62.10	0.61
28 June 1966	61	29	47.54	32	52.46	0.91
Total worms examined	1,091	493	45.20	598	55.80	0.824

\* FMR is obtained by dividing number of females by number of males.

TYPE HOST: *Aedes communis* (De Geer).

ADDITIONAL HOSTS: *Aedes cinereus* Meigen, *A. fitchii* (Felt and Young), *A. increpitus* Dyar, *A. pullatus* (Coquillet), and *Culiseta impatiens* (Walker).

LOCATION: Haemocoel of host larvae, occasionally in the pupae and adults of *Aedes increpitus* and *A. pullatus*.

LOCALITY: Lone Tree, Uinta County, Wyoming. Elevation 8,000 feet. Collected May-June 1965-66.

### Morphological differences between the male and female postparasitic juveniles

The chief morphological differences between premale and prefemale postparasitic juveniles obtained from mosquito larvae have been briefly described by Welch (1960) as being the larger size of the female and presence of the genital primordium of the vagina in the middle of the female body. Knowledge concerning the differential diagnosis of the male and female free-living postparasitic stage is inadequate because in cases of superinfection, male and female worms are often hard to separate on the basis of size, and in cases of single or double infections, the males are quite large. The presence of the genital primordium of the vagina is difficult to observe in preserved material and may be very difficult to detect in live specimens. Therefore, an attempt was made to distinguish the sexes of the postparasitic juveniles obtained following emergence from the bodies of mosquito larvae by means of the morphology of the caudal appendages and primordium of the vagina.

The caudal end of the postparasitic larvae was found to be characteristically different in males and females, with that of the premale being thin and short (150-160  $\mu$ ) and that of

the prefemale stouter at the base and longer (170-190  $\mu$ ). Also the distance from the end of the terminal trophosome to base of the caudal appendage in the premale is much greater (170-200  $\mu$ , Fig. 7) than in the prefemale (85-130  $\mu$ , Fig. 8). The morphology of the caudal end is thus a useful character that can be observed under the low power of a dissecting microscope.

The primordium of the vagina, located near the middle of the body (Fig. 3) has been previously used to separate premale and prefemale worms. It is recognizable as a small slit appearing beneath the juvenile cuticle that does not open to the outside at 46.5% of the distance from the anterior end. This feature is difficult to recognize in active specimens.

Size differences between premale and prefemale worms can be used for rapid identifications under some conditions, but should be applied in conjunction with the previous methods. Postparasitic juvenile females are longer and stouter, averaging 25 mm in length while juvenile males are shorter and thinner (average 15 mm in length). Sometimes two prominent incisions holding big excretorylike gland cells are visible on the ventral side, just under the stichosome about one-tenth to one-twelfth distance from the anterior end. These are far more prominent in premales.

### Female-male ratio (FMR) in postparasitic juveniles

Wide fluctuation appeared in sex ratios when postparasitic worms were sexed at different times during the season (Table 1). Males made up 77.4% of the population on 21 May 1966, dropped to 46.5% on 12 June, increased to 62.1% 19 June, and 52.46% on 28 June. The fluctuations correlated well with the size of worm burdens per host with the

worms being predominately males when the worm burden was greater than four and almost exclusively female when less than four were present. This phenomenon has been described previously by a number of workers (Christie, 1929; Johnson, 1955; Couturier, 1963; and Parenti, 1965) who pointed out that sex determination in mermithids may be environmentally controlled with crowding being the primary factor. Another factor was introduced by Strelkov (1964) who showed that sex of the parasite corresponds to that of the host in *Filipjevimermis singularis*. No attempt was made to resolve the latter in this study.

Female-male ratios were obtained both by examination of free-living postparasitic juveniles that had emerged spontaneously from hosts in the laboratory and through dissection of infected hosts. FMR were similar in both phases done on the same days, indicating that it would be possible to predict population changes through examination of free-living individuals in soil samples from the bottom of ponds, and thus to provide data of value to potential biological control of mosquitoes. FMR ratios are calculated by dividing the number of females by the number of males, with a low FMR being less than one (Table 1).

### Life history

The life history of *R. nielseni* is similar to that described by Welch (1960a) for *Hydromermis churchillensis*, a parasite of *Aedes communis* in Canada.

Unembryonated eggs are deposited in the bottom soil of shallow pools. Observations showed embryonation to be evident in 5–10 days and the eggs contained fully developed larvae seven days later. The larvae do not hatch upon completion of development under cold dry field conditions\* but remain dormant until favorable conditions are present. The eggs are vulnerable to desiccation so that drying is immediately fatal if the duration is more than a few seconds, but eggs appear to be resistant to freezing winter temperatures in soil.

In the type locality, snow is present from October to April when melting snow water forms the pools in which the mosquito hosts

develop. Approximately 2 weeks later the larvae emerge from the eggs and use their long, slender tails for swimming in search of a host. The larvae can exist for about 4 days under April and May temperatures and readily attach by their onchostylets to the mosquito larvae that appear at this time. Penetration into the host body cavity takes several minutes after which the larvae gradually become motionless.

The parasitic juveniles grow rapidly and are late stage juveniles in 10–15 days. Emergence from the host occurs soon after this stage of development is reached. The worms emerge by pushing the anterior end through the conjunctivum between the segments of the thorax, abdomen and neck. Complete emergence takes about 1 minute and is fatal to the host. The free-living postparasitic juveniles burrow into the bottom soil where they become motionless for 2–4 weeks. Molting occurs at the end of this period with the males preceding the females. Development and sexual differentiation are slow and it is usually 1–2 months before copulation occurs. Another 10–30 days elapse before egg production occurs and the number of eggs produced depends upon the female size and the size of the trophosome which was determined by the conditions of parasitism depending mainly on the number of larvae that inhabit a single host. A large female can produce as many as a thousand eggs. After oviposition, the female dies in 5–6 days, having exhausted the food stored in the trophosome. Males survive longer than the females since they require little stored material for sperm production.

*Reesimermis nielseni* has one generation per year, a condition well adapted to the life cycles of its hosts that are "single brooded" mountain *Aedes* species confined to the higher altitudes.

### Summary

All life stages of *Reesimermis nielseni*, gen. et sp. n., a parasite of mosquitoes from Lone Tree, Wyoming, are described. A key to the closely allied genera of Mermithidae is included. The type host is the larva of *Aedes communis* De Geer but the species was also found parasitizing *Aedes cinereus* Meigen, *A. fitchii* (Felt and Young), *A. increpitus* Dyar, *A. pullatus* (Coquillett), and *Culiseta im-*

\* The eggs, upon completion of development, could hatch after 1.5–2 months in soil water at 18–20 C in the laboratory.

*patiens* (Walker). A method of sexing post-parasitic juveniles is described as well as the seasonal fluctuation obtained in the female-male sex ratios during May and June of 1966. The life history is included and shows one generation per year.

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## Anthelmintic Activity of Tetramisole, Thiabendazole, and Purified Fine Particle Phenothiazine Against Experimental Infections of *Haemonchus contortus* and *Trichostrongylus* Species in Sheep

M. L. COLGLAZIER, K. C. KATES, AND F. D. ENZIE

Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, ARS, USDA, Beltsville, Maryland 20705

The efficacy of tetramisole (2,3,5,6-tetrahydro-6-phenyl-imidazo [2,1-b] thiazole hydrochloride), a broad spectrum anthelmintic for man and animals, was first reported by Thienpont et al. (1966). Rohrbacher et al. (1967) and Bullock et al. (1968) reported that the original optically inactive *dl*-tetramisole had been separated into optically active *d* and *l* compounds and that anthelmintic activity was ascribable to *l*-tetramisole. In preliminary trials, *l*-tetramisole had about the same anthelmintic activity as the *dl* compound when given at about half the dose rate. Published data on the activity of tetramisole in sheep and goats pertain to *dl*-tetramisole; see Cornwell et al. (1967), Fitzsimmons (1966), Forsyth (1966), Gibson (1966), Guilhon (1966), Lyons et al. (1968), Pankhurst and Sutton (1966), Reinecke (1966), Ross (1966), Shone and Philip (1967), Thomas and Bainbridge (1967), and Walley (1966). Because of the marked promise shown by tetramisole as an anthelmintic for sheep, a trial was conducted to compare the activity of the *dl* and *l* compounds with that of thiabendazole and phenothiazine against experimental infections of *Haemonchus contortus*, *Trichostrongylus axei*, and *Trichostrongylus colubriformis* in sheep.

### Materials and Methods

**EXPERIMENTAL ANIMALS:** Polled Dorset lambs (18 wethers and 2 females) used in this trial were 10–10.5 months old, weighed 21–32 kg (avg 27.7 kg), and were raised parasite-free except for insignificant infections of *Strongyloides papillosus* and coccidia. The lambs were allocated to five groups of four lambs, each group having comparable mean weights (Table 1). Each group was held in separate, concrete-floored pens which were thoroughly cleaned every 2 days. The lambs

were fed a balanced, pelleted ration in adequate quantity to maintain moderate but not maximum growth.

**ORIGIN AND ENUMERATION OF INFECTIVE LARVAE:** An isolate of *H. contortus*, designated AH-2, was established in 1966 from the sheep flock at the Animal Husbandry Division, Agricultural Research Service, Beltsville, Maryland. Infective larvae used in this trial were obtained from standard cultures of feces from a stock lamb infected with this isolate.

Infective larvae of *Trichostrongylus* spp. were of the KH isolate (Kates and Thompson, 1967), which contained, on the basis of previous sheep necropsies, about 28 per cent *T. axei* and 72 per cent *T. colubriformis*.

Prior to larval inoculation of the lambs, infective larvae in the two suspensions (one of *H. contortus* and one of *Trichostrongylus* spp.) were quantitated as follows: After appropriate dilution, the larvae in ten 1-ml samples from each suspension were counted in Scott counting chambers; undiluted formalin was added to immobilize the larvae. The per cent standard error (SE) of the larval counts of the *H. contortus* suspension was 1.87 and of the *Trichostrongylus* spp. was 1.55.

**LARVAL DOSE PREPARATION AND INOCULATION OF LAMBS:** Each larval dose per lamb contained  $5,000 \pm 93$  SE *H. contortus* and  $44,400 \pm 688$  SE *Trichostrongylus* spp. (95% confidence interval = SE  $\times$  2.26). Each dose was concentrated by centrifugation and pipetted onto filter paper. The latter was then inserted into a gelatin capsule and administered by balling gun.

**ANTHELMINTICS EMPLOYED, THEIR ADMINISTRATION, AND DOSAGES:** (1) Tetramisole: chemically pure *l* and *dl* compounds; American Cyanamid Co., Princeton, N. J. (2) Thiabendazole: Thiabendazole; Merck & Co., Rahway,

**Table 1.** Activity of anthelmintics against *H. contortus* based upon necropsy worm counts.

Drug	No. of lambs <sup>1</sup> (Avg wt, kg)	Dose rate mg/kg	Adult Avg (range)	4th-stage larvae Avg (range)	Total worms Avg (range)	Per cent efficacy	
						5th-stage	4th-stage <sup>2</sup>
<i>l</i> -Tetramisole	4 (28.8)	8	0	5 (0-20)	5 (0-20)	100	99
<i>dl</i> -Tetramisole	4 (26.8)	15	0	0	0	100	100
Thiabendazole	4 (27.5)	50	77 (0-140)	1,470 (640-2,380)	1,547 (740-2,449)	65	3
Phenothiazine purified, 2-3 $\mu$	4 (27.6)	550	<1 (0-3)	1,390 (160-3,120)	1,391 (163-3,120)	99	8
Controls	4 (27.7)	-	226 (143-393)	1,520 (920-2,040)	1,746 (1,063-2,293)		

<sup>1</sup> All males (wethers), except one female each in *dl*-tetramisole and thiabendazole groups.

<sup>2</sup> See text.

N. J. (3) Phenothiazine: 2-3  $\mu$  average particle diameter; purified grade (approximate purity 99.9%); wetting agent 1% lecithin; Atomic Basic Chemical Co., Pittsburgh, Pa.

All the anthelmintics were prepared as aqueous drenches and administered with a dose syringe on the 28th day postinfection. The two commercial products were given at commonly used dose levels, thiabendazole at 50 mg/kg and phenothiazine at 550 mg/kg. *dl*-Tetramisole and *l*-tetramisole were given at 15 and 8 mg/kg, respectively.

**EXPERIMENTAL PROCEDURES:** Data on weight gains, packed red-cell volume (micro-hematocrit), and worm eggs per gram of feces were obtained weekly on all lambs.

All lambs were killed 1 week after treatment. The parasites remaining in the treated and control lambs were recovered and counted by the following procedures: The contents of each abomasum and small intestine were recovered and stored in a large container to which undiluted formalin was added. Each organ was then separately subjected to artificial peptic digestion overnight at 37 C. The next day the contents and digests of each organ from each lamb were combined, and the final preparations made up to 2,000 ml for sampling. From each preparation, five 20-ml samples were taken for calculating the numbers of *Trichostrongylus* spp. and larval parasites in each organ. After the samples were removed from the total abomasal material, the remainder was washed through screens and the adult *H. contortus* recovered. All adult *H. contortus* were counted, but total numbers of the small nematodes were calculated from the sample counts.

Efficacy calculations were made by the standard method for the controlled anthelmintic test.

**SUPPLEMENTAL LAMB INFECTIONS:** Because substantial numbers of apparently inhibited 4th-stage *H. contortus* were recovered 35 days postinfection from lambs in the control and two treatment groups (Table 1), four more lambs were employed to determine the inhibitory influence, if any, of simultaneous infection of *Trichostrongylus* spp. on the development of *H. contortus*. Two lambs were inoculated with similar numbers of larvae of *H. contortus* and *Trichostrongylus* spp. (5,000 and 44,200, respectively) of the same isolates that were used in the anthelmintic trial, and each of two lambs was inoculated with 5,000 *H. contortus* larvae only (Table 4). Subsequently, these lambs were penned, fed, necropsied, and their parasites recovered and counted in the same manner as for the lambs in the anthelmintic trial.

## Results

This trial was planned to obtain comparative data on the activity of certain anthelmintics against adult populations of three important nematode parasites of sheep. Consequently, the lambs were treated 28 days postinfection when practically all worms ordinarily should have become adults. Surprisingly, however, the majority of the *H. contortus* recovered at necropsy from the control lambs and from two of the four treatment groups (thiabendazole and phenothiazine) were 4th-stage larvae (Table 1). Therefore, for this and other reasons discussed later, we believe that considerable numbers of inhibited 4th-

Table 2. Activity of anthelmintics against *Trichostrongylus* spp. based upon necropsy worm counts.

Drug	No. of lambs <sup>1</sup>	Dose rate mg/kg	<i>T. axei</i> <sup>2</sup>	<i>T. colubriformis</i> <sup>2</sup>	Both species Avg (range)	Per cent efficacy	
			Avg (range)	Avg (range)		<i>T. axei</i>	<i>T. colubriformis</i>
<i>l</i> -Tetramisole	4	8	65 (0-180)	0	65 (0-180)	98	100
<i>dl</i> -Tetramisole	4	15	0	0	0	100	100
Thiabendazole	4	50	0	0	0	100	100
Phenothiazine	4	550	30 (0-60)	655 (140-1,260)	685 (200-1,300)	99	94
purified, 2-3 $\mu$							
Controls	4	-	3,675 (2,660-4,400)	10,975 (7,060-13,900)	14,470 (9,720-18,300)		

<sup>1</sup> Same lambs inoculated simultaneously with *H. contortus* (Table 1).

<sup>2</sup> All *Trichostrongylus* spp. recovered were adults.

stage *H. contortus* were present in lambs of all 5 groups when the anthelmintics were administered. If this assumption is correct, data were obtained on the activity of the drugs against 4th-stage *H. contortus* as well as adults.

The data on the activity of the anthelmintics against adult and 4th-stage *H. contortus* are summarized in Table 1. Both *dl*- and *l*-tetramisole were 100% effective against adult *H. contortus* and almost as effective against 4th-stage larvae; only one 4th-stage larva was found in 20 abomasal samples from the four lambs given *l*-tetramisole. Phenothiazine was 99% effective against adult *H. contortus*, but was ineffective against 4th-stage larvae. Thiabendazole was only 65% effective against adult *H. contortus* and almost totally ineffective against 4th-stage larvae.

Data on activity of the anthelmintics against *T. axei* and *T. colubriformis* are summarized in Table 2. All were very effective (94-100%) against adults of both species.

All anthelmintics reduced the parasite egg counts 92-100% (Table 3). Thiabendazole caused the least reduction because of the re-

tention of adult *H. contortus* after treatment. No eggs were seen in the posttreatment fecal samples from lambs treated with tetramisole. The drugs were well tolerated in all trials.

Average pretreatment egg counts in the five groups of lambs were quite uniform, varying only from 2,445 EPG in the group given phenothiazine to 3,563 EPG in the control group. This uniformity in the pretreatment egg counts indicated that reasonably uniform adult infections were established initially in the lambs. However, an average of 226 adult *H. contortus* (55% females) were present in the control lambs at necropsy, whereas the same lambs had an average of 14,470 adult *Trichostrongylus* spp. (54% females) (Tables 1, 2). Therefore, substantial numbers of the eggs counted pretreatment were probably those of *Trichostrongylus* spp. No attempt was made to differentiate the eggs of *H. contortus* and *Trichostrongylus* spp. because quantitative differentiation of *T. axei* and *H. contortus* eggs in mixed infections is unreliable.

The reduction in average hematocrit values over the 4 weeks from infection to treatment

Table 3. Activity of anthelmintics against *H. contortus* and *Trichostrongylus* spp. based upon combined species fecal egg counts.

Drug	No. of lambs <sup>1</sup>	Dose rate mg/kg	Avg EPG		EPG reduction per cent
			Pretreatment 4th week	Post-treatment at necropsy	
<i>l</i> -Tetramisole	4	8	3,550	0	100
<i>dl</i> -Tetramisole	4	15	2,508	0	100
Thiabendazole	4	50	2,825	205	92
Phenothiazine purified, 2-3 $\mu$	4	550	2,445	30	98
Controls	4	-	3,563	2,645	25

<sup>1</sup> Same lambs for which necropsy worm counts are summarized in Tables 1 and 2.



**Table 4. Supplemental lamb infections. Necropsy worm counts 35 days postinfection of nonmedicated lambs infected (a) simultaneously with *H. contortus* and *Trichostrongylus* spp. and (b) with *H. contortus* only.**

No. of lambs <sup>1</sup>	Age mo.	Larvae inoculated per lamb	<i>H. contortus</i> Avg (range)		<i>T. axei</i> <sup>2</sup> Avg (range)	<i>T. colubriformis</i> <sup>2</sup> Avg (range)	<i>T. a.</i> + <i>T. c.</i> Avg (range)
			Adults	4th-stage larvae			
(a) 2	3.5	5,000 <i>H. contortus</i>	1,664	20	4,530	19,850	24,380
		44,200 <i>Trichostrongylus</i>	(806–2,522)	(0–40)	(4,300–4,760)	(16,500–23,200)	(21,260–27,500)
(b) 2	11	5,000 <i>H. contortus</i>	1,240	0	—	—	—
			(998–1,482)				

<sup>1</sup> All males (wethers) except for one female in (a); weight range 17–25 kg.

<sup>2</sup> All in the adult stage.

varied only from 5–6% per group, which supports our conclusion that average infections per group at the time of treatment were comparable.

Our conclusion that the 4th-stage *H. contortus* larvae recovered at necropsy were derived from the original inocula is supported by the fact that similar percentages of *H. contortus* (34.9%—larvae + adults) and *Trichostrongylus* spp. (32.6%—adults) of the original inocula were recovered at necropsy from the control lambs.

There appears to be no obvious explanation for the presence of large numbers of 4th-stage *H. contortus* in the control and some treatment groups at the termination of this trial. The results of the small scale test (Table 4) conducted to determine if simultaneous *Trichostrongylus* spp. infections had a reproducible inhibitory effect on the development of *H. contortus* were essentially negative. Substantial numbers of adult *H. contortus* (avg 1,664), and very small numbers of 4th-stage larvae (avg 20), were recovered at necropsy from the two lambs inoculated with all three species of nematodes. Also, substantial numbers of adult worms (avg 1,240) and no 4th-stage larvae were recovered from the two lambs inoculated with comparable numbers of *H. contortus* only. It is also noteworthy that the two lambs with mixed infections harbored larger numbers of adult *Trichostrongylus* spp. (avg 24,380 = 55% of inocula) than the control lambs in the anthelmintic trial (avg 14,470 = 32% of inocula). The results of this supplementary test indicate, therefore, that the concurrent *Trichostrongylus* spp. infection was not a major cause of the inhibited development of *H. contortus* in the anthelmintic trial.

## Discussion

Our results confirm those of previously cited authors that *dl*-tetramisole given orally at 15 mg/kg is very effective in removing adult *H. contortus*, *T. axei*, and *T. colubriformis* from lambs, and that *l*-tetramisole at about half the dose rate (8 mg/kg) of the *dl* compound is equally effective. If it is assumed that at the time of treatment the two groups of lambs treated with tetramisole compounds as well as the control group had comparable numbers of inhibited 4th-stage *H. contortus*, which seems likely, then both tetramisoles were highly effective against these larvae. Gibson (1966) reported 100% efficacy of 15 mg/kg of *dl*-tetramisole against all larval stages of *H. contortus* in lambs, and Lyons et al. (1968) reported that the *dl* compound at the same dose rate was 97% effective against 4th and 87% against early 5th-stages of *H. contortus*. Also, Shone and Phillip (1967) reported that at 15 mg/kg *dl*-tetramisole was more than 99% effective against 4th-stage *H. contortus*, but was only 72.5% effective against parasitic 3rd-stage. Furthermore, Gibson (1966) reported that, at 15 mg/kg, *dl*-tetramisole was highly effective against immature *T. colubriformis* in sheep. Data are rapidly accumulating to show, therefore, that the two tetramisoles at appropriate dose levels are highly effective against both adult and larval stages of *H. contortus* and *Trichostrongylus* spp. in sheep.

We obtained relatively poor action with thiabendazole against adult *H. contortus* (AH-2). These findings were not especially surprising, however, because certain unpublished observations involving the source flock also suggested that the sheep may harbor *H. contortus* that is somewhat resistant to thiabenda-

zole. The fact that we obtained with the AH-2 isolate an efficacy of only 65% against adult *H. contortus*, and apparently no activity against inhibited 4th-stage larvae, tends to support the earlier observations, and indicates that the AH-2 isolate may be resistant to thiabendazole at the 50 mg/kg level. Further studies are in progress to compare more precisely the efficacy of thiabendazole against this and other isolates of *H. contortus*. Most reports (see Drudge et al., 1964; Gibson, 1965) indicate that thiabendazole at 50 mg/kg is at least 90% effective against adult *H. contortus* in sheep. Indeed, Gibson (1965) recommends this regimen as the treatment of choice. Conway (1964), on the other hand, reported poor results at this dose level, but improved efficacy at 80 mg/kg. At the dose rate of 44 mg/kg, Drudge et al. (1964) administered six successive drenches at 4-week intervals to naturally infected sheep on pasture with notable lack of success in controlling *H. contortus*. Although fewer *H. contortus* were recovered at necropsy from the principals than from the controls, the residual worm burdens in the treated animals were almost pure infections of *H. contortus*. They concluded that this treatment program apparently selected, from the original population of *H. contortus*, a strain resistant to the drug at this dose level. Attention should be called to the reports of Gibson (1964), Gordon (1964), Reinecke et al. (1962), and Southcott (1963) that thiabendazole at 50 mg/kg body weight is effective against immature *H. contortus*. However, only Reinecke et al., examined digests for the immature stages.

Purified, 2–3  $\mu$  phenothiazine given at 550 mg/kg to a group of four lambs removed 99% of adult *H. contortus* (AH-2), but apparently had no effect on inhibited 4th-stage larvae. These results may be compared with those reported by Colglazier et al. (1967) for three other phenothiazine products given at the same dose rate to lambs experimentally infected with another isolate (AH) of *H. contortus* from the same sheep flock. They reported efficacies against adult parasites of 12% for N.F. 10  $\mu$  phenothiazine, 40% for N.F. 7  $\mu$  phenothiazine, and 75% for purified 6.9  $\mu$  phenothiazine. There is a lack of data on the effect of phenothiazine against immature *H. contortus*. However, Gordon (1940) reported that phenothiazine in doses of 600 mg/kg of body weight

destroyed immature *H. contortus* 10 and 15 days old. Later, Southcott (1963) reported that phenothiazine in similar doses was less effective against *H. contortus* 12 hr to 7 days old than against parasites 10–28 days old.

A unique finding in this trial was the recovery of substantial numbers of 4th-stage *H. contortus* from lambs of the control, phenothiazine, and thiabendazole groups. These 4th-stage larvae were 1.5–2.5 mm long, and were equivalent in size and development to 4th-stage larvae 4–6 days old, as described by Veglia (1915). For reasons given below, we believe these larvae developed from the original inocula, were inhibited in their development by some factor(s) not now apparent, and were present in all groups of lambs at the time of treatment 28 days postinfection. Therefore, efficacy calculations are given in Table 1 also for activity of the anthelmintics against 4th-stage *H. contortus*. On this basis, only the two tetramisoles showed action against these inhibited larvae.

We cannot be certain the 4th-stage *H. contortus* recovered from the trial lambs came from the original inocula and were inhibited in their development, but the following reasons support this thesis: (1) The animal pens were cleaned regularly. (2) The experiment was conducted in midwinter and the animal quarters were too cold for normal development of infective larvae in the pens. (3) No parasitic larvae of *Trichostrongylus* spp. were recovered from any of the 20 lambs on trial, which strongly supports our contention that reinfection did not occur from pen contamination. (4) The numbers of adult *H. contortus* were low and the numbers of adult *Trichostrongylus* spp. were high in the lambs; therefore, if reinfection had occurred in the pens, substantial numbers of immature *Trichostrongylus* spp. would have been recovered along with immature *H. contortus*, which was not the case. (5) The total numbers of *H. contortus* and *Trichostrongylus* spp. recovered at necropsy from the controls in both instances were about one-third of the original inocula.

### Summary

In a controlled test with experimental infections of *Haemonchus contortus*, *Trichostrongylus axei*, and *Trichostrongylus colubriformis* in lambs, *l*-tetramisole (8 mg/kg), *dl*-tetrami-

sole (15 mg/kg), thiabendazole (50 mg/kg), and purified, 2-3  $\mu$  phenothiazine (550 mg/kg) were all highly effective (94-100%) against adult parasites of all species, except for thiabendazole which was only 65% effective against adult *H. contortus*. The poor results obtained with thiabendazole suggest that the *H. contortus* isolate (AH-2) used in this work may be resistant to the drug at the 50 mg/kg level. Substantial numbers of apparently inhibited 4th-stage *H. contortus* were recovered from all lambs except those treated with tetramisole.

### Acknowledgment

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## A New Trematode *Cephalogonimus sireni* sp. nov. (Digenea: Cephalogonimidae) from Florida Mud Eel, *Siren lacertina*

G. PREMVAATI<sup>1</sup>

Department of Biology, Florida Agricultural and Mechanical University, Tallahassee, Florida 32307

In amphibians, a single genus *Cephalogonimus* Poirier, 1886, of the family Cephalogonimidae, has been recorded mostly from frogs of the genus *Rana*. From urodeles, only one species, *C. amphiuma* Chandler, 1923, has been reported from *Amphiuma means* in Louisiana and subsequently Manter (1938) reported the same species from *Siren* of Florida. Two mud eels, *Siren lacertina*, were examined for helminth infection, and one was found to be infected with a new trematode of the genus *Cephalogonimus*. The worms were fixed under slight coverglass pressure in hot AFA and stained with Semichon's carmine.

All measurements are in microns, unless otherwise indicated.

### *Cephalogonimus sireni* sp. nov. (Fig. 1)

HOST: *Siren lacertina* Linnaeus, 1766.

LOCALITY: Lake Munson, Leon County, Florida.

LOCATION: Latter half of intestine.

NUMBER OF WORMS: Five from one host.

SPECIMENS: Holotype and one paratype in USNM Helm. Coll. Nos. 71236, 71237.

<sup>1</sup> Present address: Department of Zoology, University of Lucknow, Lucknow (U.P.), India.

### Description

Body oval, spinous, small, 1.04-1.44 mm long, and 760-870 wide. Oral sucker sub-terminal, 170 in diameter; ventral sucker very large, 340 in diameter; both suckers have three to six rows of spines on their margins. Prepharynx thick and muscular; pharynx 125-130 by 110, very muscular, globular, resembling a sucker; esophagus absent; intestinal ceca terminate almost at posterior end of body. Distance between ceca and posterior end of body 140-150.

Genital pore median and terminal. Testes 230-270 by 200-230, ovoid to spherical, symmetrically opposite, immediately posterior to ventral sucker. Vasa efferentia join in acetabular region to form the vas deferens. Cirrus sac preacetabular, elongated; enclosing elongated seminal vesicle, ejaculatory duct, cirrus and prostatic cells; runs toward left of pharynx and then joins uterus to form hermaphroditic duct that runs dorsal to oral sucker. Ovary submedian, ovoid to lobed, dorsal and to right of ventral sucker, measures 160-170 by 100-110. Laurer's canal and seminal receptacle present. Vitellaria follicular, extends

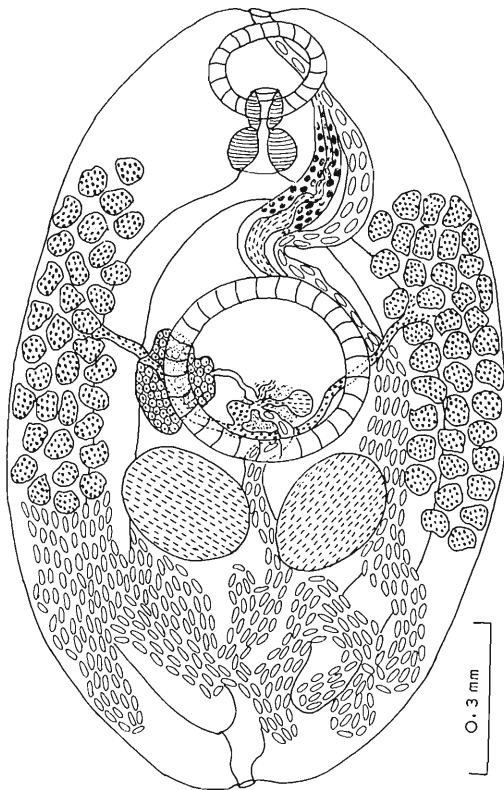


Figure 1. *Cephalogonimus sireni* sp. nov. from Florida mud eel, *Siren lacertina*. Holotype: ventral view.

from intestinal bifurcation to testicular level. Two transverse vitelline ducts dorsal to ventral sucker and join to form an elongated vitelline reservoir. Whole female genital complex lies in acetabular region. Uterus extends posteriorly and then runs anteriorly on left of ventral sucker. Excretory bladder Y-shaped, stem of Y being small. Eggs small, numerous, and measure 23–25 by 14–15 (30 measured).

### Discussion

The subfamily Cephalogoniminae Looss (1899), subsequently raised to family Cephalogonimidae Nicoll (1914), includes four genera: *Cephalogonimus* Poirier, 1886, *Emoleptalea* Looss, 1900 (Syn.: *Leptalea* Looss, 1899), *Paracephalogonimus* Skrjabin, 1950 and *Oudhia* Dayal and Gupta, 1954.

Oral sucker is larger than ventral sucker in the three genera, *Emoleptalea*, *Paracephalogonimus*, and *Oudhia*, and in all species except five, of the genus *Cephalogonimus*. Two of these five species, namely *C. compactus* Stunkard, 1924 and *C. japonicus* Ogata, 1934, have oral sucker equal to ventral sucker. In the remaining three species, the oral sucker is only slightly smaller than the ventral sucker, the sizes being: 240 and 290 in *C. lenoiri* Poirier, 1886; 220 and 270 in *C. vesicaudus* Nickerson, 1912; 220–270 and 310–330 in *C. manchuricus* Oguro, 1941. But in *C. sireni*, the oral sucker is half the size of ventral sucker and they measure 170 and 340. This ratio of 1:2 in the sizes of the two suckers is not seen in any species of the genus *Cephalogonimus*.

The position of testes in all species of the genus *Cephalogonimus* is tandem or diagonal. But in *C. sireni*, the testes are symmetrically opposite.

In all species of the genus *Cephalogonimus*, the shape of the ovary is entire. But ovary is lobed in *C. sireni*.

The position of testes and shape of ovary are two of the major characteristics used to distinguish the two families Prosthogonimidae and Cephalogonimidae: the former has symmetrically opposite testes and lobed ovary, while the latter has tandem or diagonal testes and entire ovary. *Cephalogonimus sireni* shows prosthogonimid characters in having symmetrically opposite testes and lobed ovary; but it has typical cephalogonimid shape of the body, elongated cirrus sac, genital pore at anterior extremity on the dorsal side, and an amphibian host.

Thus *C. sireni* differs from all the present species of the genus *Cephalogonimus* in the following: (1) ventral sucker very large, twice the size of oral sucker; (2) symmetrically opposite testes, and (3) lobed ovary. It is, accordingly, regarded as a new species.

### Summary

*Cephalogonimus sireni* sp. nov. (Digenea: Cephalogonimidae) is described from Florida mud eel, *Siren lacertina*. It is characterized by: (1) very large ventral sucker, twice the

size of oral sucker; (2) symmetrically opposite testes, immediately posterior to ventral sucker, and (3) lobed ovary. In the position of testes and shape of ovary, *C. sireni* shows characters of the family Prosthogonimidae; but it has typical cephalogonimid shape of the body, elongated cirrus sac, genital pore at the anterior extremity on the dorsal side, and an amphibian host.

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## Intestinal Helminths of the Bullfinch, *Pyrrhula pyrrhula* (L.), in Southern England

I. C. WILLIAMS<sup>1</sup> AND I. NEWTON<sup>2</sup>

The bullfinch, *Pyrrhula pyrrhula* (L.), is a small passerine bird which is widely distributed in Europe and northern Asia. Over most of its range it favors coniferous or mixed forests, and in Britain thick deciduous woods and hedgerows. It lives chiefly on seeds and buds, but feeds its young on a mixture of seeds and small invertebrates. By eating the buds of fruit trees it has become a serious pest in fruit growing areas of southern England, where it is resident throughout the year. The ecology and general biology of the bullfinch have received considerable attention (Wright and Summers 1960; Newton 1964, 1966, 1967a, b); on the other hand, knowledge of the helminth parasites of this bird is confined to a few scattered records and the brief report on *Urotocus rossittensis* (Mühling, 1898) by Newton and Williams (1967). The large numbers of bullfinches killed annually in pest-control programs in fruit growing areas of southern England provided an opportunity to investigate their intestinal helminths.

Through the kindness of fruit growers, dead bullfinches were received regularly between January 1965 and January 1966 from 37 localities in 10 English counties. A total of 1,265 birds was examined. The gut was removed from each bird soon after arrival and that part

from the gizzard forward was used for an analysis of the food (Newton 1967a), while the rest was stored in 5% formaldehyde and examined later for helminths. The results reported herein thus relate to the duodenum, small and large intestines, rectum, cloaca, and bursa Fabricii. Birds are classed as juvenile from the time they left the nest to the end of the postjuvenile moult (3-4 months), first-year from then until the first complete moult a year later (age 15-16 months) and adult thereafter. No nestling bullfinches were examined.

### Results

#### (a) Incidence of infection

Intestinal helminths were found in 71 (5.6%) of the bullfinches examined; they comprised three species of trematodes and two species of cyclophyllidean cestodes (Table 1). No nematodes or Acanthocephala were found. The trematodes were members of the family Brachylaimidae, namely *Brachylaima arcuata* Dujardin, 1845, *Leucochloridium macrostomum* (Rudolphi, 1803) and *Urotocus rossittensis* (Mühling, 1898), and the cestodes were *Anomotaenia borealis* (Krabbe, 1869) and *Hymenolepis passeris* (Gmelin, 1790).

Not more than one species of helminth was found in any infected host and, combining all the data, there was no significant difference in incidence of infection between male (42/

<sup>1</sup> Department of Zoology, The University, Hull, England.

<sup>2</sup> Edward Grey Institute for Field Ornithology, Botanic Garden, Oxford, England.

**Table 1.** The occurrence of five helminth species in Bullfinches, *Pyrrhula pyrrhula* (L.), from different English counties.

County	No. of localities in county	No. of bullfinches examined	<i>Brachylaima arcuata</i> <sup>1, 2</sup>		<i>Leucochloridium macrostomum</i>		<i>Urotocus rossittensis</i>		<i>Anomotaenia borealis</i> <sup>2</sup>		<i>Hymenolepis passeris</i>		Total	
			No. inf.	% inf.	No. inf.	% inf.	No. inf.	% inf.	No. inf.	% inf.	No. inf.	% inf.	No. inf.	% inf.
Bedfordshire	1	23	0	0	0	0	1	4	0	0	0	0	1	4
Berkshire	3	131	2	1.5	1	0.8	23	17.6	0	0	0	0	26	20
Buckinghamshire	5	119	3	2.5	0	0	3	2.5	0	0	1	0.8	7	6
Essex	5	207	1	0.5	0	0	2	1	1	0.5	0	0	4	2
Hampshire	1	62	1	1.6	0	0	10	16	0	0	0	0	11	18
Herefordshire	1	75	0	0	0	0	5	7	0	0	2	2.7	7	9
Kent	12	329	4	1.2	0	0	1	0.3	0	0	1	0.3	6	2
Suffolk	1	92	2	2	0	0	4	4	0	0	0	0	6	7
Sussex	6	214	0	0	1	0.5	2	1	0	0	0	0	3	1
Warwickshire	2	13	0	0	0	0	0	0	0	0	0	0	0	0
Total	37	1,265	13	1	2	0.2	51	4	1	0.1	4	0.3	71	5.6

<sup>1</sup> New record in the British Isles.<sup>2</sup> New host record in the bullfinch.

698, 6.0%) and female (29/567, 5.1%) birds. The most common parasite found was *U. rossittensis* in 51 birds from 13 of the 37 localities involved and within these areas the proportion of infected birds varied greatly. Berkshire and Hampshire provided the greatest proportion of infected birds (33/193, 17%) and the southeastern counties of Kent, Essex, and Sussex the smallest, with under 1% (5/750) infected. The second most frequent helminth found was *Brachylaima arcuata* in 13 birds, six of which were from the central-southern counties (Berkshire, Buckinghamshire, and Hampshire, 6/312, 2%) and five from the southeastern counties (Essex, Kent, 5/536, 1%); the remaining two infected birds were from one locality in Suffolk from which 92 birds were examined. The distribution of bullfinches infected with *L. macrostomum* and the two species of cestodes was sporadic but it was noticeable that no tapeworms were found in any birds from Berkshire and Hampshire, although these areas contained bullfinches with the highest incidence of trematodes.

Among birds from Berkshire and Hampshire, there was an apparent decline in incidence of infection with *U. rossittensis* with increasing age (Table 2). Thus, 25% of juveniles from these two counties contained this species compared with 18% of first-year birds and 14% adults, but these differences were not significant statistically. If the incidence of *L. macrostomum* and *Brachylaima*

*arcuata* in birds from Berkshire and Hampshire are added to the figures for *U. rossittensis* the percentage incidence of infection with age becomes 25, 19, and 16, respectively. Only one group of juveniles (hatched 1965) was examined, but two groups of first-year birds (hatched 1964 and 1965) and two groups of adults (hatched pre-1964 and pre-1965) were received. The incidence of trematodes among these five groups from Berkshire and Hampshire was: adult, hatched pre-1964, 3/20 (15%); first-year, hatched 1964, 7/53 (13%); adult, hatched pre-1965, 3/17 (18%); juvenile, hatched 1965, 6/24 (25%); first-year, hatched 1965, 18/79 (23%). Comparison of the two groups of first-year birds suggests that, within these localities, there may be differences in incidence of infection from year to year.

Among the two species of cestode it was noticeable that no infection of adult bullfinches was found.

### (b) Intensity of infection

The number of individual parasites per infected host varied considerably (Table 2), and there was a tendency for the intensity to be greater in first-year and adult birds than in juveniles, with the exception of *U. rossittensis* in which the reverse was seen. With *U. rossittensis* there was a marked difference in the mean intensity of infected male (9.4) and female (2.9) birds; but this was chiefly due to two of four juvenile males and three of 25

Table 2. The occurrence of five helminth species in Bullfinches, *Pyrrhula pyrrhula* (L.), of different age.

Age class of host		Juvenile	First winter	Adult	Total and means
Number examined		301	770	194	1,265
<i>Urotocus rossittensis</i>					
a. All localities <sup>1</sup>	Number infected	9	37	5	51
	Per cent infected	3	5	3	4
	Mean intensity <sup>2</sup>	10	7	5	7
	Range of intensity	1-42	1-73	3-7	1-73
b. Berks. and Hamps.	Number infected	6/24	22/132	5/37	33/193
	Per cent infected	25	18	14	17
<i>Leucochloridium macrostomum</i>	Number infected	0	0	2	2
	Per cent infected	0	0	1	0.2
	Mean intensity	-	-	15	15
	Range of intensity	-	-	1-29	1-29
<i>Brachylaima arcuata</i>	Number infected	1	10	2	13
	Per cent infected	0.3	1.3	1	1
	Mean intensity	1	1	1.5	1.3
	Range of intensity	1	1-2	1-3	1-3
<i>Anomotaenia borealis</i>	Number infected	1	0	0	1
	Per cent infected	0.3	0	0	0.1
	Intensity	2	-	-	2
<i>Hymenolepis passeris</i>	Number infected	1	3	0	4
	Per cent infected	0.3	0.4	0	0.3
	Mean intensity	1	6.3	-	5
	Range of intensity	1	1-16	-	1-16
All species	Number infected	12	50	9	71
	Per cent infected	4	7	5	5.6

<sup>1</sup> If data for all areas are combined, as in a, a possible decline in incidence with increase in age of host is masked by the fact that relatively more juveniles than older birds were received from areas of low infection. The data for Berkshire and Hampshire are, therefore, given separately.

<sup>2</sup> Intensity, being the number of parasites per infected host.

first-year males containing exceptionally large numbers of parasites (42, 28, 39, 73, and 31 respectively). If these birds are excluded, the mean intensity of infected males becomes 3.2 compared with the females 2.9. Corresponding adjusted figures for juveniles, first-year birds and adults then become 2.9, 2.9, and 5.2 respectively, with an overall mean intensity of 3.1 instead of 7 (Table 2). The average number of *U. rossittensis* in infected hosts from localities with few birds infected was 1.8, compared with 9.6 for Berkshire and Hampshire.

### (c) Pathology of infection

Of the five helminth species found only *U. rossittensis* was seen to have an appreciable affect on its host. This species is fusiform in shape and may attain 7 mm in length by 0.4 mm in diameter; it lives in the ovoid, saclike, bursa Fabricii where it presses upon the finger-like projections which line this organ and which

are especially rich in lymphoid follicles. The effect is to distort and, particularly in a heavy infection, destroy the functional division of lymphoid follicles into medulla, which produces lymphocytes, and cortex, where these mature (Figs. 1, 2). At the same time the extensive vascular and connective tissue network, which surrounds and supports the follicles, is compressed and ultimately destroyed. Within the bursa *U. rossittensis* feeds on blood, lymphoid cells and connective tissue. The breakdown product of hemoglobin, hematin, is especially noticeable in histological sections and can be recognized microspectroscopically. For the most part the worms lie with their mouth toward the periphery of the bursa as though browsing on its inner surface. No instance of cannibalism by *Urotocus* was seen. In addition to damage through pressure and feeding, the cuticular spines of the worm abrade the surface of the lymphoid tissue.



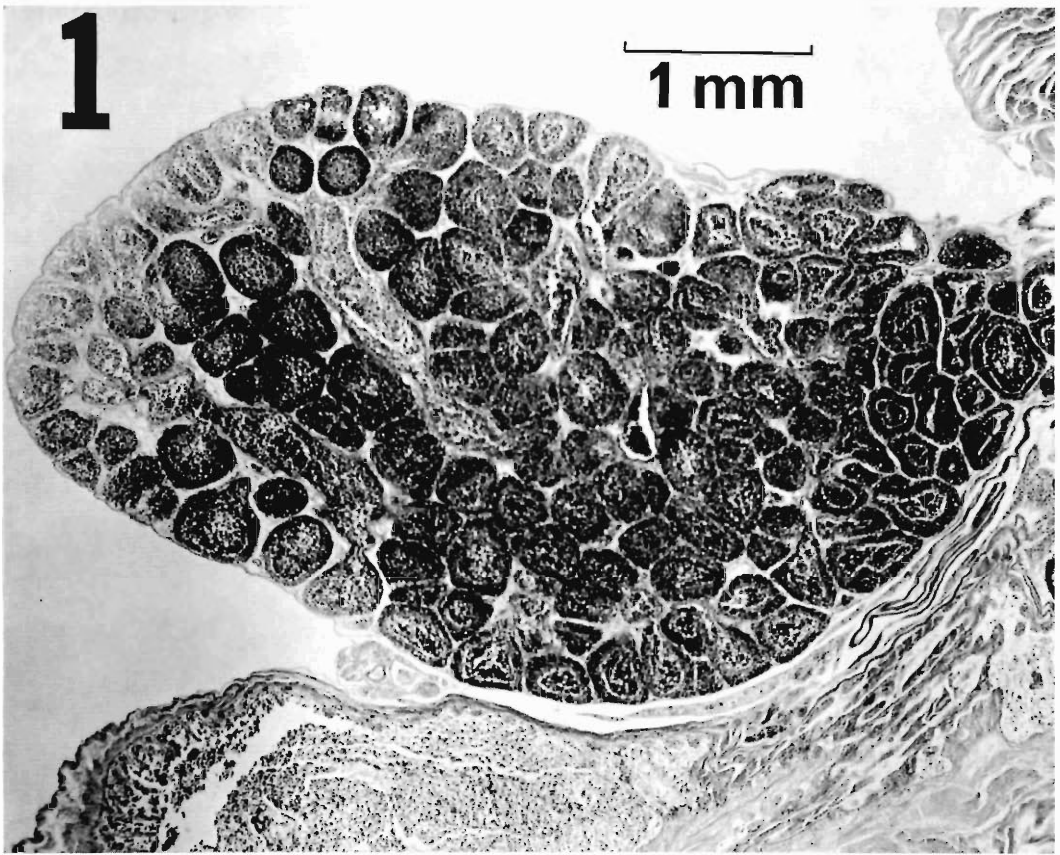


Figure 1. Sagittal section of the uninfected bursa Fabricii of a juvenile male bullfinch showing numerous lymphoid follicles.

As well as causing damage within the bursa Fabricii, heavy infections with *U. rossittensis* greatly distend the bursa producing a "cyst" which may attain 1 cm in diameter. As the bursa lies on the dorsal side of the cloaca, it is possible that a heavily infected and distended bursa might, through its presence, interfere with the normal functioning of the organs of the pelvic region.

#### Discussion

Bullfinches acquire the various helminth parasites reported above by ingesting the respective larval stages, present in certain invertebrates, with their food. As animal material is normally eaten by bullfinches only during the first 10 days or so of life, presumably

infection occurs during this early period. Although all the nestling's food, consisting mainly of caterpillars, flies, spiders, slugs, and snails, is collected by their parents the adults, apparently, do not eat invertebrates at this time, nor do they predigest it for their young (Newton 1967a); they are, therefore, unlikely to become infected. Yet many helminths were found in birds of postnestling age. This situation thus provides an unusual opportunity to examine the longevity of these various parasites in natural infections, and results show that *Brachylaima arcuata* can live in bullfinches for at least 18 months, *L. macrostomum* 25 months, *U. rossittensis* 30 months, *Anomotaenia borealis* 2 months and *Hymenolepis passeris* 4 months. As many bullfinches older than

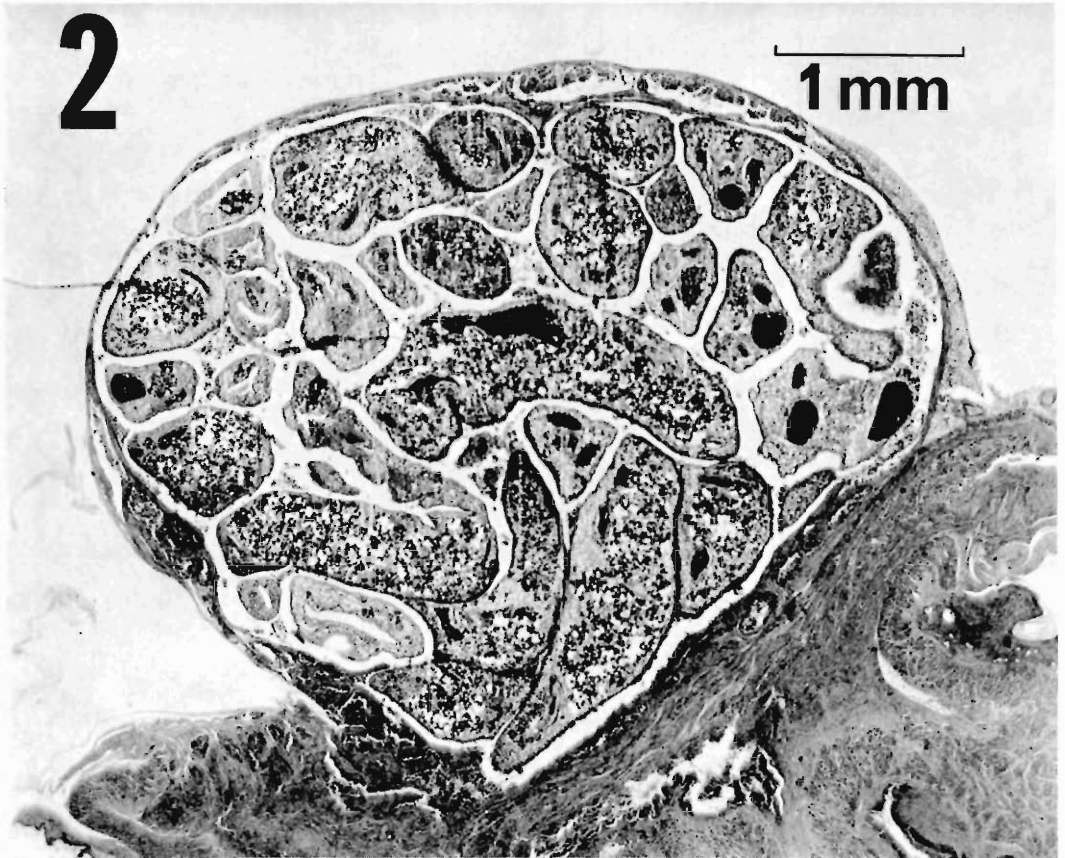


Figure 2. Sagittal section of the bursa Fabricii of a juvenile male bullfinch heavily infected with *Urotocus rossittensis*; the peripheral region of the bursa only remains.

those containing the two cestode species were examined, it is possible that these particular parasites can live only a few months in this host. This agrees with Kintner's (1938) observations on the parasites of 197 house sparrows, *Passer domesticus* (L.), in Indiana, in which cestodes were found only in birds obtained before November, with none from November to March. Kintner suggested that the cestodes concerned, which included *H. passeris*, either were short-lived, or that the intermediate hosts were not available or did not transmit them in winter. Our own observations on *H. passeris* in the bullfinch suggest that Kintner's first alternative, that the cestodes were short-lived, is very likely, although there is the possibility, in the present study at

least, that infected intermediate hosts were present but not eaten.

An interesting comparative study is that of Sokolova (1959) in Russia on the parasites of the chaffinch, *Fringilla coelebs* L., which is closely related to the bullfinch. Unlike the latter, however, invertebrates predominate in the diet of the adult chaffinch during the summer and the young are reared entirely on animal material (Newton 1967b). From 143 birds Sokolova obtained four species of trematodes; two of these, *Prosthogonimus ovatus* and *P. cuneatus*, inhabited the bursa Fabricii, and the other two, *Plagiorchis maculosus* and *Leucochloridium* sp., the intestine. The cestodes found included "*Taenia exigua* Dujardin 1845" and some unidentified specimens. None

of these parasites was found in bullfinches. Sokolova found that trematodes infected mainly nestlings but, in contrast to our results, were rare or absent in fully grown birds.

The low mean intensity of infection of bullfinches with *Brachylaima arcuata* (1.3) is in agreement with that reported for the related species *B. fuscata* in the starling, *Sturnus vulgaris* L., in northern England (3.4) by Owen and Pemberton (1962), and south Wales (4.12) by James and Llewellyn (1967). The latter authors also reported that the mean numbers of *B. fuscata* from infected mistle-thrushes, *Turdus viscivorus* L., at 5, from a song-thrush, *T. ericetorum* Turton, at 9 and from 57 infected redwings, *T. musicus* L., at 9.8. It is, perhaps, surprising that the intensities should be fairly low whereas the corresponding incidences of infection varied considerably (1% of 1,265 bullfinches; 24 of 358 (6.7%) starlings (Owen and Pemberton 1962); 25/122 (21%) starlings (James and Llewellyn 1967); 2/5 (40%) mistle-thrushes, 1/11 (9%) song-thrushes and 57/171 (33%) redwings (James and Llewellyn 1962)).

It is surprising that neither Owen and Pemberton (1962) nor James and Llewellyn (1967) found *L. macrostomum* in the 470 starlings and 235 passerines of six other species they examined, as these birds have previously been recorded as hosts for this parasite. In Poland, however, Joszt (1962) found *L. macrostomum* in 3 of 190 (1.6%) house sparrows.

*Urotocus rossittensis* presents an unusual case owing to the temporary nature of its habitat which atrophies with the approach of sexual maturity and is not normally found in the adult. Yet we found 2.6% of all adult bullfinches infected with this species, while the proportion of infected adults from Berkshire and Hampshire was much higher at 14%. Undoubtedly infection with *U. rossittensis* must take place at an age when animal food is eaten, that is as a nestling. Thus the proportion of animal material in the diet and, also, the incidence of infective larvae in intermediate hosts will influence the incidence and intensity of infection in the bird. These two factors may operate to produce the differences described above, in infection of bullfinches from different localities. The observations of Newton (1967a), that bullfinch nestlings reared in

woodland areas receive appreciably greater proportions of animal food in their diet than broods raised in farmland areas, may offer a possible explanation of the present observations but we have no direct evidence to this effect. Furthermore, infection must occur when the canal between the cloaca and the bursa is large enough to admit the young parasite, which when infective measures 0.2–0.6 mm in length (Timon-David 1957). As we were unable to measure the width of the lumen of the canal we do not know at what age it becomes too narrow to permit entry of the infective larva. Only then is the bird free from the risk of further infection with this species of parasite. Of course, some time earlier the canal will have become too narrow to permit the adult *U. rossittensis* from leaving the bursa. Thus in adult birds the worms are effectively encapsulated within the fibromuscular wall of the atrophied bursa.

As stated above, exceptionally heavy infections were found only in comparatively young birds and there was also an apparent decline in incidence with increasing age of the bird. Both these effects might be due either to the parasite dying, and presumably being eaten by their neighbors although no instances of cannibalism were seen in our sections, or to infected birds dying in greater proportion than uninfected. The role of the bursa appears to be particularly important during the first few days of life and in the fowl it is known to produce both lymphocytes (Ackerman and Knouff 1964) and circulating antibodies (Glick 1964) during this period. Thus, impairment of the host's defence mechanisms very early in life may lead to death, if not directly from worm infections, perhaps from secondary bacterial infections. In addition, it is possible that a swollen, heavily infected, bursa may press upon adjacent organs and thereby interfere with their function.

In conclusion, we believe that the acquisition of helminth parasites by bullfinches is almost certainly restricted to the nestling stage, but some species at least remain alive in the host for many months and beyond the stage at which the bird reaches sexual maturity. Of the five helminth species found only one, *Urotocus rossittensis* which inhabits the bursa Fabricii, does any appreciable damage to the

host and might influence nestling and juvenile mortality.

### Summary

Seventy-one of 1,265 (5.6%) bullfinches, *Pyrrhula pyrrhula* (L.), received from 37 localities in 10 counties in southern England between January 1965 and January 1966 were infected with helminth parasites. These comprised five species, three being trematodes and two cestodes, namely *Brachylaima arcuata* Dujardin, 1845, *Leucochloridium macrostomum* (Rudolphi, 1803), *Urotocus rossittensis* (Mühling, 1898), *Hymenolepis passeris* (Gmelin, 1790) and *Anomotaenia borealis* (Krabbe, 1869). *Urotocus rossittensis* was the most common parasite with an incidence of 4%, although in birds received from Berkshire and Hampshire the incidence was locally as high as 17%; the other four species were comparatively uncommon. *Brachylaima arcuata* has not previously been reported from the British Isles, and neither this species nor *Anomotaenia borealis* have been recorded before from the bullfinch.

The incidence and intensity of infection of the five species with respect to age, sex, and locality is considered, and the pathology of infection of the bursa Fabricii of the bullfinch with *U. rossittensis* is described. It is believed that infection of the bullfinch with helminth parasites takes place during the nestling stage because only then is animal food included in the bird's diet.

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## *Euparadistomum cercopithec* sp. n. (Dicrocoeliidae), a Digenetic Trematode from the Talapoin Monkey from Rio Muni<sup>1</sup>

JACOB H. FISCHTHAL

The specimens of this report were collected by Robert W. Cooper, D.V.M.,<sup>2</sup> Director, NIH Primate Colony, Institute of Comparative Biology, Zoological Society of San Diego. They were brought to the author's attention by G. E. Cosgrove, M.D., Biology Division, Oak Ridge National Laboratory. Dr. Cooper (personal communication) noted that "The talapoin monkey from which the five trematodes were collected was received in our colony from Bata, Rio Muni (West Africa) on 26 April 1968. This animal was an adult female. . . . Talapoin monkeys are extremely common in Rio Muni and it is unlikely that the specimen was captured more than 25 miles from Bata. . . . In a total of 20 examinations performed on talapoins in Rio Muni, in Cameroun, and here in San Diego, I have found this particular trematode on only one other occasion. In this case a single immature specimen was recovered from the gall bladder of a freshly captured advanced pregnant female which was purchased in Bata from a native on 3 January 1968." Specimens were fixed, without pressure, in Roundabush solution, stained in Semichon's acetocarmine or Mayer's carmalum, and mounted in permount. Measurements are in microns.

### *Euparadistomum cercopithec* sp. n. (Figs. 1-4)

HOST: *Cercopithecus (Miopithecus) talapoin* Schreber (Cercopithecidae).

HABITAT: Gall bladder.

LOCALITY: Vicinity of Bata, Rio Muni; Equatorial Guinea.

SPECIMENS: USNM Helm. Coll. No. 71288 (holotype); No. 71289 (paratypes).

DIAGNOSIS (based on five mature specimens from one host): Body flat, slightly longer than

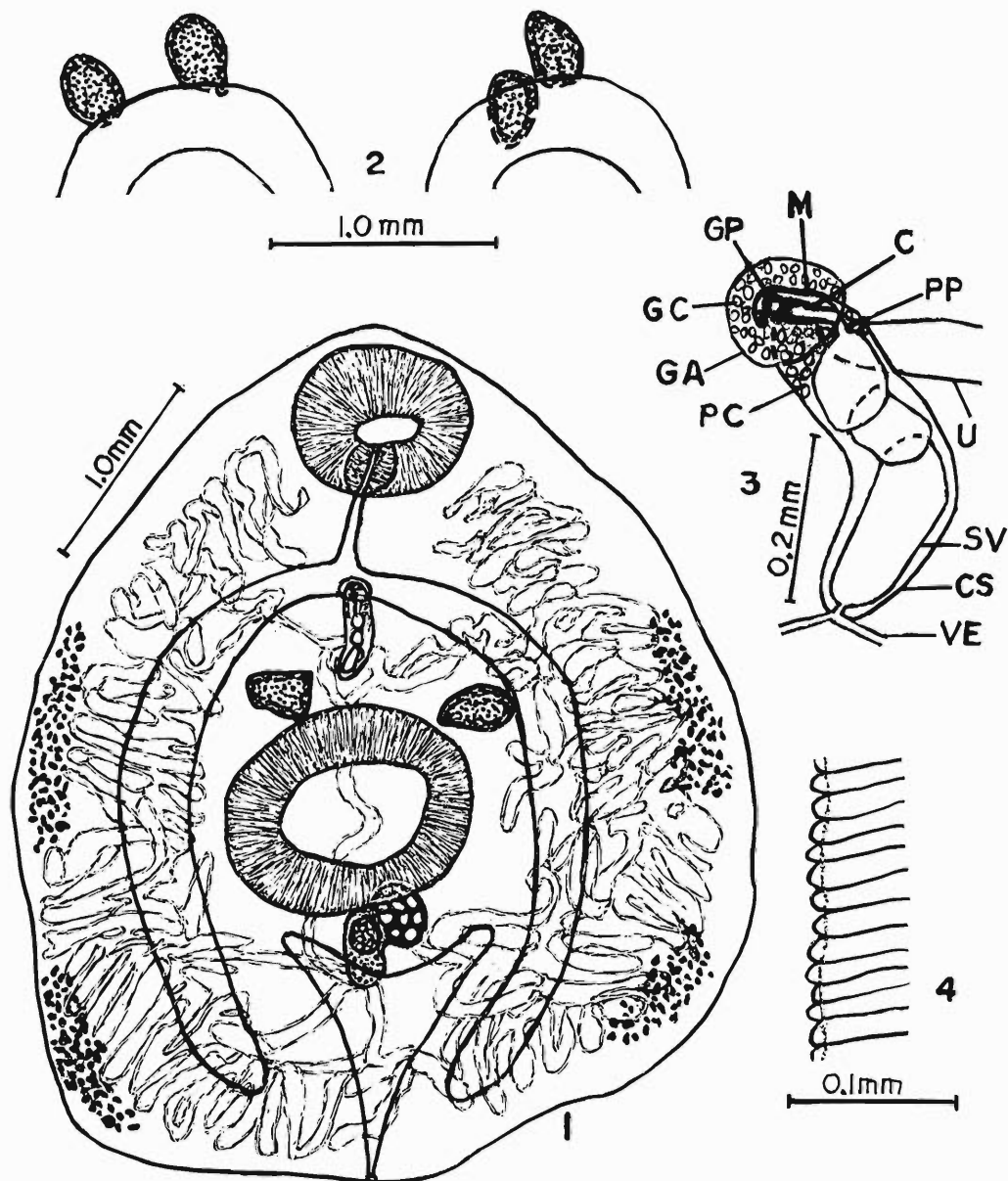
wide, anterior extremity round, posterior nearly truncate, tending toward trigonal shape, 3,180-4,385 by 2,630-3,920. Tegument thick, annulated; papillae and spines absent. Forebody 1,300-1,815 long, hindbody 997-1,450 long; forebody-hindbody length ratio 1:0.70-0.87; preoral space 60-115 long. Suckers transversely elongate; oral sucker subterminal ventral, 675-835 by 760-974; acetabulum 860-1,166 by 997-1,304, center at level of anterior 52-57 per cent of body length; sucker length ratio 1:1.22-1.39, sucker width ratio 1:1.29-1.45. Prepharynx absent; pharynx usually longitudinally elongate, 205-290 by 200-230, entirely dorsal to or considerably overlapping oral sucker; esophagus 70-450 long; cecal bifurcation 335-510 preacetabular; ceca wide, terminating 220-560 from posterior extremity.

Testes two, situated symmetrically at anterolateral margins of acetabulum in three specimens, oblique with left testis anteromedian to acetabulum in two others, usually overlapping acetabulum, margins smooth to slightly lobed, usually transversely elongate but sometimes longitudinally elongate, right testis 225-335 by 225-360; left testis 245-300 by 260-365. Vasa efferentia uniting at cirrus sac. Latter median to submedian, straight to curving slightly, 250-440 by 125-160, lying 75-160 preacetabular, containing saccular, undulating seminal vesicle, short pars prostatica surrounded by prostate cells, and muscular cirrus. Genital atrium round, wide, shallow. Genital pore median, bifurcal to postbifurcal, situated 205-520 postpharyngeal, 205-475 preacetabular.

Ovary smooth to slightly lobed, median to submedian (left), overlapping posterior part of acetabulum, longitudinally or transversely elongate, 265-375 by 290-335. Mehlis' gland well developed, very compact, round to longitudinally elongate, situated dextral to ovary in three specimens, sinistral in two others, usually overlapping ovary dorsally, 140-205 by 120-185. Seminal receptacle situated as for Mehlis' gland, large, saccular, longitudinally

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Figures 1-4. *Euparadistomum cercopitheci*. 1. Whole mount, holotype, ventral view. 2. Position of testes in two paratypes, ventral view. 3. Terminal genitalia, paratype, ventral view. 4. Annulated tegument, holotype, anterolateral part of body at level of cecal bifurcation.

Abbreviations: C, cirrus; CS, cirrus sac; GA, genital atrium; GC, gland cells; GP, genital pore; M, metraterm; PC, prostate cells; PP, pars prostatica; SV, seminal vesicle; U, uterus; VE, vas efferens.

elongate, 300–385 by 195–220. Laurer's canal extending to dorsal surface near seminal receptacle. Vitelline follicles in lateral extracecal fields, extending from pretesticular, testicular or acetabular level to level anterior or posterior to or at cecal ends, anterior and/or posterior limits of fields in individual specimen may be subequal, one or both fields may be interrupted at ovarian level or both may be uninterrupted. Uterine coils extending to posterior extremity, overlapping vitellaria ventrally, anteriorly extending to sides of oral sucker and lateral body margins; coiling essentially as schematically presented by Buckley and Yeh (1958) in figure 3 of their new species *Euparadistomum heischii*. Metraterm shorter than cirrus sac, muscular, anteriormost extent anterior to cirrus sac and genital pore, surrounded by gland cells. Eggs numerous, operculate; shells of younger eggs yellow in color, becoming yellow-brown, and finally brown as they progress from proximal to distal uterine coils; 20 older eggs measuring 34–41 by 22–26.

Excretory bladder Y-shaped, stem post-ovarian, arms extending to ovarian level; pore terminal.

### Discussion

Species of *Euparadistomum* Tubangui, 1931, have been reported from lizards, birds, and mammals from the Philippine Islands, North Borneo, British Solomon Islands, Malaya, Burma, India, Madagascar, Belgian Congo, Kenya, and Brazil. African species are: *E. varani* var. *madagascariensis* Capron, Deblock and Brygoo, 1961, from chamaeleonid lizards from Madagascar; *E. pipistrelli* (Sandground, 1937) Travassos, 1944, from a vespertilionid bat from the Belgian Congo; *E. heischii* Buckley and Yeh, 1958, from a domestic cat (Felidae) from Kenya. Species from mammalian hosts, in addition to the latter two listed above, are: *E. cerivoulae* Gogate, 1939, from a vespertilionid bat from Burma; *E.*

*paraense* (Jansen, 1941) Travassos, 1944, from a didelphid marsupial from Brazil; *E. buckleyi* S. N. Singh, 1958, from a fox (Canidae) from India. *E. cercopitheci* sp. n. appears closest to *E. paraense*, *E. buckleyi*, and *E. francolini* R. Gupta, 1959 (from a phasianid bird from India). It differs from them in body shape, and in possessing an annulated tegument. It differs further from *E. paraense* in lacking tegumental papillae, and in having smaller eggs and sucker ratios; from *E. buckleyi* in possessing a well developed Mehlis' gland; and from *E. francolini* in having smaller eggs.

The differences cited above for declaring *E. cercopitheci* a new species appear to be relatively small in view of experimental studies on host influenced intraspecific morphological variations reported for *Opisthioglyphe ranae* (Frölich, 1791) Looss, 1907 (Plagiorchiidae) by Grabda-Kazubska (1967), and for *Telorchis bonnerensis* Waitz, 1960 (Telorchidae) by Watertor (1967). However, experimental data on morphological variability and on life cycles are not known for any species of *Euparadistomum*. Additionally, *E. cercopitheci* is from Africa, whereas the most closely related species are either from Brazil or India. Therefore, the author feels that the new species designation for the present specimens is justified.

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## Survival and Egg Laying of Turtle Blood Flukes (Trematoda: Spirorchiidae) on the Chick Chorioallantois<sup>1</sup>

BERNARD FRIED AND RONALD E. TORNWALL

Biology Department, Lafayette College, Easton, Pennsylvania 18042

Turtle blood flukes, tentatively identified as *Spirorchis elegans* Stunkard, 1923 from the esophagus, and *Spirorchis scripta* Stunkard, 1923 from the head of naturally infected mid-western painted turtles, *Chrysemys picta bellei* (Gray), have been transplanted to the chick chorioallantois (Fried and Tornwall, 1965), to determine if this site would be suitable for handling spirorchiid flukes outside of the host. The details are presented herein.

### Materials and Methods

*Chrysemys picta bellei* obtained from a commercial dealer (Fried, 1965) were decapitated and the heads cut midsagittally to expose the brain and cranial cavity. *Spirorchis scripta* were removed from the surface of the brain and cranial cavity and transferred through several changes of sterile Ringer's solution (Paul, 1960). A turtle's plastron was severed with bone cutters and the esophagus was removed and placed in Ringer's solution. *Spirorchis elegans* dissected from the submucosa and periconnective tissue of the esophagus were transferred through several changes of sterile Ringer's.

Fertile white leghorn hens' eggs were incubated at 37–38 C for 9–13 days. Sterilization procedures, methods used to operate on eggs and to implant spirorchiids on chorioallantoic membranes were as previously described for eye flukes (Fried, 1962). Spirorchiids were maintained on chorioallantoic membranes for 1–3 days postimplantation at  $30 \pm 1$  C and eggs were examined as previously described (Fried, 1962).

One hundred and nineteen spirorchiids were placed on the chorioallantoic membranes of 109 eggs. One to three flukes were used per egg and the membranes were examined 1–5 days later. Fifty-nine *S. scripta* were placed

on the chorioallantoic membranes of 56 eggs, and 60 *S. elegans* were placed on the membranes of 53 eggs.

### Results

The results of the transplant experiments are presented in Table 1.

Twenty-eight of the 119 chorioallantois-spirorchiids, including the 11 flukes recovered live, laid from 1–125 eggs on chorioallantoic membranes. One live fluke recovered 2 days after transplantation laid about 70 eggs on the chorioallantois, and a second worm recovered 3 days postimplantation laid about 125 eggs on the chorioallantois. Fifty chorioallantois-spirorchiid eggs incubated in pond water at room temperature for 10 days failed to embryonate.

*S. elegans* and *S. scripta* removed from turtles and maintained at room temperature in Ringer's survived no longer than 6 hr and did not lay eggs.

Live parasites were sluggish on the chorioallantois and did not attach firmly to its surface. However, when removed to Ringer's 1–3 days after transplantation, chorioallantois-flukes were as active as in vivo spirorchiids placed in Ringer's, and displayed characteristic turtle blood fluke undulations. Dead chorioallantoic-flukes were either disintegrated and easily lifted from the chorioallantois or were walled off by the chorioallantois and firmly attached to its surface.

### Discussion

The fact that 11 (15%) of the 74 spirorchiids recovered on the chorioallantois survived for 1–3 days postimplantation at  $30 \pm 1$  C (Table 1) indicates that chick eggs can provide adequate physicochemical conditions for turtle blood flukes for at least 3 days. Since 28 spirorchiids laid eggs on the chorioallantois, including two specimens that laid an average of 35–40 eggs per day for 2–3 days, turtle

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Table 1. Survival of spirorchiids on chorioallantoic membranes at  $30 \pm 1$  C.

Exper. species	No. of eggs used <sup>1</sup>	No. of worms used <sup>2</sup>	No. of days on chorioallantois	No. of live worms recovered <sup>3</sup>	No. of dead worms recovered <sup>4</sup>	No. of contaminated or dead eggs
<i>S. scripta</i>	56	59	1-5	5	31	12
<i>S. elegans</i>	53	60	1-5	6	32	10
Total	109	119	1-5	11	63	22

<sup>1</sup> Nine to 13 days old.<sup>2</sup> One to 2 worms per egg.<sup>3</sup> One to 3 days postimplantation.<sup>4</sup> On shell or chorioallantois.

blood flukes can produce and lay eggs in this site. Spirorchiid eggs from the chorioallantois failed to embryonate, possibly because intraspecific mating is necessary for normal egg production.

A survival rate of 15% indicates that conditions on the chorioallantois although adequate are suboptimal. Physicochemical conditions on the chorioallantois differ from those in normal sites, esophageal submucosal and periconnective tissue, and cranial cavity and surface of the brain. Spirorchiids in their normal sites are usually surrounded on all surfaces by host tissue and fluid, whereas the chorioallantois makes contact only with the ventral surface of the worm. Moreover, spirorchiids have a weak oral sucker, lack an acetabulum and pharynx and might not be as capable of obtaining blood and tissue from the chorioallantois as are trematodes that have a pharynx and well-developed suckers and have been maintained on the chorioallantoic membrane for periods in excess of 3 days, i.e., *Philophthalmus hegeneri* and *Polystomoides* sp. (Fried, 1962; 1965).

Specimens of *Spirorchis scripta* obtained from the head and *Spirorchis elegans* from the esophagus of *Chrysemys picta bellei* are referable to the descriptions of *S. scripta* and *S. elegans* by Stunkard (1923), Byrd (1939), and Schroeder and Ulmer (1959). Goodchild and Kirk (1960) described the life history of *Spirorchis elegans* from the heart of *Chrysemys picta picta* in Georgia. It is questionable whether their *S. elegans* is the same as the *S. elegans* used in this study. The possibility also exists that *S. scripta* and *S. elegans* from *Chrysemys picta bellei* are morphological variants of the same species.

## Summary

Turtle blood flukes, tentatively identified as *Spirorchis elegans* from the esophagus, and *S. scripta* from the head of *Chrysemys picta bellei*, have survived on the chick chorioallantois at  $30 \pm 1$  C for up to 3 days. Spirorchiids maintained in Ringer's at room temperature survived no longer than 6 hours and did not lay eggs.

Eleven of 119 spirorchiids transplanted to the chorioallantoic membranes of 109 9- to 13-day-old chick embryos maintained at  $30 \pm 1$  C, were recovered live and laid eggs up to 3 days after transplantation. One chorioallantoic-fluke recovered 2 days after transplantation laid approximately 70 eggs, and a second worm recovered 3 days postimplantation laid about 125 eggs on the chorioallantois. Attempts to embryonate these eggs in pond water were unsuccessful.

## Acknowledgments

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## Pathogenicity of the Northern Root-knot Nematode (*Meloidogyne hapla*) to Potato<sup>1</sup>

G. D. GRIFFIN AND E. C. JORGENSON<sup>2</sup>

The northern root-knot nematode, *Meloidogyne hapla*, is considered a serious pest of potatoes in the Western United States. We have seen fields where approximately all of the tubers are either infected or galled by this nematode, making them commercially undesirable. We have also observed the occurrence of a deep-seated tuber infection (nematodes near the center of the potato tuber). We suspected that age of plant, soil temperature, and soil moisture determine the extent of invasion and severity of galling of potato roots and tubers. Gross (1940) found galling of potato tubers by *Heterodera marioni* (*Meloidogyne* sp.) less in soil irrigated every 3-5 days at a soil temperature of 17-18 C, than under less frequent irrigations of 7-9 days where soil temperature went as high as 26.5 C. Godfrey (1926) reported an increase in root-knot galling of potato plants up to 27 C. Parris (1948) reported that the higher the soil moisture, the greater tuber galling by *Heterodera marioni* (*Meloidogyne* sp.). He found larvae near the cambium below swollen lenticels and concluded that nematodes entered through lenticels of tubers grown in soil with a high moisture content. Godfrey (1926) found that optimum root-knot galling of tomato roots occurred between 50 and 80% field capacity, and Peacock (1957) obtained similar data. Wallace (1956) observed a

decline in the mobility of *Heterodera schachtii* as soil moisture decreased, which may directly affect root infection.

The role of host age on root infection by root-knot nematodes was observed by Christie (1936), who stated that second stage root-knot larvae usually enter roots near the tip. This accounts for the congregating of nematodes at or near the meristematic root tissue up to 24 hr until the root is penetrated (Godfrey and Oliveira, 1932; Peacock, 1959; Loewenberg, Sullivan, and Schuster, 1960). The ability of root-knot larvae to penetrate only delicate root tissue is evidenced by the galling of only the tip of large tap roots, in some instances in fumigated soil.

This study was made to determine (1) the relationship between soil temperature, soil moisture, and the pathogenicity of *Meloidogyne hapla* on potato under controlled conditions, and (2) if size or age of roots and tubers affect penetration and galling by root-knot larvae.

### Materials and Methods

To study the effect of temperature on pathogenicity of *M. hapla* Chitwood on potato (*Solanum tuberosum* L.), Russet Burbank potato seed pieces were surface sterilized with 1:600 solution of methylmercury dicyandiamide for 60 sec, suberized for 24 hr and planted in flats of fumigated soil. After 15 days, plants, minus seed pieces, were transplanted in 3.8 liter polyethylene containers of Provo sand (M.H.C. = 17%) and inoculated with 1,000 *M. hapla* larvae. Plants were grown

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<sup>2</sup> Research Nematologists, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Crops Research Laboratory, Utah State University, Logan, Utah.

**Table 1. Effect of temperature on pathogenicity of *Meloidogyne hapla* to Russet Burbank potatoes.<sup>1</sup>**

Temperature °C	Roots		Tubers		
	Avg wt.	Gall rating <sup>2</sup>	No. per plant	Avg wt.	Gall rating <sup>2</sup>
20	4.16b	2.14d	2.00f	6.24g	0
25	3.48b	4.00e	3.00f	2.85g	0
30	4.51b	3.71e	0	—	—
35	0.25a	0.67c	0	—	—

Numbers with same letter do not differ significantly at 5% level.

<sup>1</sup> 1,000 *M. hapla* larvae per plant.

<sup>2</sup> Gall rating: 0, none; 1, very few; 2, slight; 3, moderate; 4, severe; 5, very severe.

at temperatures of 20, 25, 30, and 35 C. Each treatment (temperature) was replicated four times, and grown under a 16 hr day. The experiment was concluded after 60 days, and plant growth and nematode pathogenicity determined.

The relationship between galling and plant age was determined by inoculating Russet Burbank seed pieces, and 3- and 6-week-old plants (minus seed pieces and rhizomes) with 1,000 larvae of *M. hapla*. Plants were grown on a greenhouse bench in 15.2 cm clay pots at  $22 \pm 4$  C; treatments consisted of six inoculated and six control plants. The experiment was ended after 140 days, and tuber and root weights, and nematode pathogenicity determined.

The effect of tuber size on nematode infection was studied by inoculating plants from which all but one potato bud or tuber were removed. Twenty-five 12-week-old Russet Burbank plants, each labeled as to size of bud or tuber, were inoculated as previously stated and transplanted into a 15.2 cm clay pot of Provo sand. Potato buds and tubers ranged from 5–57 mm in diameter. These plants were grown for 60 days at  $22 \pm 4$  C, and tuber infection was determined.

Two methods were used to study the effect of soil moisture on infection and galling. The "percent soil moisture method" involved transplanting 15-day-old plants into 3.8 liter polyethylene containers of Provo sand and inoculating each plant with 1,000 larvae of *M. hapla*. Containers of soil with the moisture levels of 25, 50, 75, 100, and 125% of field capacity (F.C.) were replicated 6 times per treatment in a chamber with relative humidity of 80–100%, and a soil temperature of  $22 \pm 2$  C. Each container was weighed daily, and distilled water added to maintain moisture at the

desired level. To be sure the soil moisture was maintained at the desired level, control plants of comparable size were weighed weekly and compensation was made for any increase in plant weight. The study was terminated after 130 days; root and tuber growth and infection and galling of roots and tubers by *M. hapla* was determined.

The other method, the "field capacity method," was similar except that the soil moisture of all containers was initially adjusted to field capacity. Water was not added to the containers until soil moisture had dropped to or below previously determined soil moisture levels of 25, 50, 75, and 100% F.C.; then enough water was added to restore F.C. (Couch, Purdy, and Henderson, 1967). Replicates consisted of six inoculated and six control plants at each soil moisture level. Plants were grown for 80 days on a greenhouse bench at  $22 \pm 4$  C, and root growth, and root infection by *M. hapla* was recorded.

## Results and Discussion

Potato plant growth was affected as much by temperature as by the pathogenicity of *M. hapla*. The least root growth occurred at 35 C, but there were no significant differences in root growth of plants from 20–30 C (Table 1). However, tubers were formed only at 20 and 25 C, and root galling was most severe at 25 and 30 C; root galling, like root growth, was adversely affected at 35 C. It appears that a temperature of approximately 25 C is optimum for maximum expression of root galling on Russet Burbank potato. The slight amount of galling at 20 C agrees with Gross (1940) who found galling of tubers to be less at 17–18 C than at temperatures up to 26.5 C. This undoubtedly affects the degree of severity of tuber galling from year to year when differ-

**Table 2.** Effect of age of plant at time of inoculation on root and tuber galling of Russet Burbank potatoes by *Meloidogyne hapla*.

Age of inoculated plant	Avg root weight (g)	Root gall rating <sup>1</sup>	Avg tuber weight	Per cent infected	Per cent tubers galled	Tuber gall rating <sup>1</sup>
6 weeks — Inoculated	0.78a	2.00c	10.50d	85	85	3.00f
Control	5.10b		21.20e			
3 weeks — Inoculated	2.25a	2.22c	8.60d	92	92	3.33f
Control	5.56b		20.74e			
Seed pieces — Inoculated	4.35b	2.29c	6.86d	83	35	3.57f
Control	5.33b		7.26d			

Numbers with same letter do not differ significantly at 5% level.

<sup>1</sup>1,000 *M. hapla* larvae per plant.

Tuber and root gall rating: 0, none; 1, very few; 2, slight; 3, moderate; 4, severe; 5, very severe; temperature, 20–25 C.

ences in soil temperatures are noted. However, we have no data on soil temperatures in potato fields, and can only speculate.

Tuber infection was not affected by differences in plant age at time of inoculation. Tubers were readily infected on plants grown from inoculated seed pieces, and from 3- and 6-week-old plants (Table 2). However, fewer tubers were galled on plants from inoculated seed pieces, which apparently was due to the late tuber set as shown by the small-sized tubers when compared with those grown from 3- and 6-week-old plants. There were significant differences between root growth of control and inoculated 3- and 6-week-old plants, which showed the pathogenicity of *M. hapla* to potato plants. However, there were no significant differences between inoculated and control plants from seed pieces, because more than one root system developed from a single seed piece.

Infection was not greatly affected by tuber size. Larvae were able to penetrate large and small tubers (Table 3). There were, however, a greater number of larvae infecting tubers

from 41–57 mm in diameter, which may be explained by the greater surface area exposed to larval invasion.

The pathogenicity of *M. hapla* to Russet Burbank potato roots and tubers was affected by soil moisture. Roots and tubers were galled at all moisture levels from 25–100% F.C. with the “percent soil moisture method” (Table 4). Tubers were not galled at 125% F.C., but 74% of the tubers were infected. Root galling was significantly less at 125% of F.C. than at other moisture levels. This was apparently explained by Shepherd and Wallace (1959), who found that the infection of hosts by *Heterodera schachtii* and *H. gottingiana* was greatest when most of the soil pores are free of water. This may also be attributable to low oxygen and gaseous exchange in near saturated soil (Van Gundy, Stolzy, Szuszkiewicz, and Rackman, 1962).

Maximum root growth occurred at 50, 75, and 100% F.C., and minimum root growth was at 25 and 125% F.C.

The “field capacity method” gave results similar to those obtained with the “percent soil moisture method,” and resulted in root and tuber infection at 25, 50, 75, and 100% F.C. (Table 5). However, tubers were not galled, because of the short duration of the study. The greatest root growth occurred at 100%, while 25% of F.C. resulted in the smallest root system. There were no differences in root weights between control and inoculated plants at 25% F.C.; roots of these plants were reduced as much or more by low soil moisture as by nematode pathogenicity. However, at 50, 75, and 100% F.C. roots of inoculated plants were significantly smaller than control plants, although

**Table 3.** Influence of bud and tuber size of Russet Burbank potato on infection by *Meloidogyne hapla*.

Bud and tuber diameter (mm) <sup>1</sup>	Per cent of buds and tubers infected	Degree of infection <sup>2</sup>
5–15	100	2.16 ± 0.42
16–25	100	2.42 ± 0.28
26–41	100	2.65 ± 0.36
41–57	100	3.17 ± 0.51

<sup>1</sup> Size at time of inoculation.

<sup>2</sup> Degree of infection: 0, none; 1, light; 2, moderate; 3, heavy; 4, very heavy.

**Table 4.** Effect of soil moisture on root and tuber galling of Russet Burbank potatoes by *Meloidogyne hapla* in controlled moisture chamber of 80–100% relative humidity at 22 ± 2 C (“Percent soil moisture method”).

Soil moisture (per cent field capacity)	Avg root wt. (g)	Root gall rating <sup>1</sup>	Per cent tubers infected	Per cent tubers galled	Tuber gall rating
25	15.80a	4.38e	95	95	4.25g
50	26.32b	4.60e	96	96	4.60gh
75	23.66b	5.00f	100	100	4.67gh
100	29.66c	5.00f	100	100	5.00h
125	18.34a	3.18d	74	0	0

Numbers with same letter do not differ significantly at 5% level.

<sup>1</sup> 15-day-old transplants inoculated with 1,000 *M. hapla* larvae per plant, and grown for 130 days. Root and tuber gall rating: 0, none; 1, very few; 2, slight; 3, moderate; 4, severe; 5, very severe.

galling was as severe at 25 as at 50% F.C.

The point of tuber penetration was apparently through the lenticels, because most of the nematodes were found immediately below or near the lenticels at 125% F.C. However, there was no affinity for the area below or near the lenticels at the other soil moisture levels, and an epidermal penetration remains a possibility. Parris (1948) stated that root-knot larvae enter potato tubers through swollen lenticels. We found no significant differences between tubers infected at 50 and 75% F.C., although tuber lenticels were swollen only at 75, 100, and 125% F.C. The low soil moistures of 25 and 50% F.C. apparently inhibited lenticel swelling, but did not prevent infection and galling of tubers by *M. hapla*. Tubers with swollen lenticels at 100% F.C. were generally the most heavily galled, which suggests that tubers may be penetrated more readily through the swollen lenticels. However, the very severe tuber galling at 100% F.C. may be partly due to the effect of soil moisture on the nematode

mobility (Wallace 1956) and migration of the nematode to the site of infection. Nematodes were found in the tuber just outside the cambial layer at all moisture levels, and less than 5% of the nematodes were found beneath the cambium. There was no deep seated infection observed in any of the infected tubers. It is, therefore, apparent that deep-seated infection of tubers by *M. hapla* is fortuitous or caused by factor(s) not considered in this study.

Comparing the two methods used for controlling soil moisture, the “field capacity method” is considered the better method since it more closely compares to field conditions, and is more easily controlled in the greenhouse.

Although there were significant differences in root and tuber infection and galling between the different soil moisture levels, it appears that soil moisture is not of major importance in the host–parasite relationship. Over 70% of all tubers were infected at all soil moisture levels; root and tuber galling was severe or very severe at all moisture levels but 125%

**Table 5.** Effect of soil moisture on root galling of Russet Burbank potatoes by *Meloidogyne hapla* on greenhouse bench at 22 ± 4 C (“Field capacity method”).

Soil moisture (per cent field capacity)	Avg root wt. (g)	Root gall rating <sup>1</sup>	Per cent tuber infected	Per cent tubers galled
25 Inoculated	4.42a	4.24e	71	0
25 Control	4.86a	—	—	—
50 Inoculated	7.86b	4.49e	100	0
50 Control	10.71c	—	—	—
75 Inoculated	8.09b	5.00f	100	0
75 Control	12.29c	—	—	—
100 Inoculated	8.31b	5.00f	100	0
100 Control	16.30d	—	—	—

Numbers with same letter do not differ significantly at 5%.

<sup>1</sup> 15-day-old transplants inoculated with 1,000 *M. hapla* larvae per plant and grown for 80 days. Root gall rating: 0, none; 1, very few; 2, slight; 3, moderate; 4, severe; 5, very severe.

F.C.; nematode infection occurred outside the cambial layer, and very few (less than 5%) were found immediately below the cambial layer. However, root weights of infected plants at all soil moisture levels but 25% F.C. were significantly less than those of uninfected plants. Therefore, we conclude that variation in soil moisture is important only in the degree of pathogenicity, and while it may contribute to difference in severity of galling, the presence or absence of root and tuber galling, and deep-seated potato tuber infection are conditioned by other factor(s).

### Summary

Root galling of Russet Burbank potatoes by the northern root-knot nematode, *Meloidogyne hapla*, increased as temperature increased from 20–30 C. Roots were slightly galled at 20 C, and severely galled at 25 and 30 C. There was a very light galling at 35 C, which was associated with very little root growth. There were no differences in root growth from 20–30 C, but tubers were formed only at 20 and 25 C.

Tuber infection was not affected by plant age or tuber size at time of inoculation.

*M. hapla* infected potato tubers at moisture levels from 25–125% F.C. in Provo sand. The greater number of larvae infected tubers at 100% F.C., and the least at 125%. Infection was mainly through the lenticels.

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## Some Gastrointestinal Helminths of *Ondatra zibethicus* Linnaeus, the Muskrat in Maryland<sup>1</sup>

JAMES B. ABRAM

Biology Department, Maryland State College, Princess Anne, Maryland

The muskrat, *Ondatra zibethicus*, has been an important wildlife entity in Maryland, both from the standpoint of adding to the economy and in providing opportunity for scientific investigations. The State has long been recognized for its large production of pelts, and muskrat meat is a table delicacy in the area.

Several muskrat studies have been conducted in the Dorchester County marshes on the Eastern Shore. The biology and habitats of the species have been written of extensively, but the aspect of parasitism has been neglected. Not since the study of helminths of Maryland muskrats by Price (1931), who described four new trematode species, has a similar study been reported.

The muskrats of the present study were trapped near Thurmont in Frederick County and in the Dames Quarter marshes in Somerset County, from November 1966 through November 1967.

The collecting areas were ecologically different in several respects. The Thurmont study area consisted of five freshwater ponds, located within a 10-mile radius of one another, near the Catoclin range of the Blue Ridge Mountains. The Dames Quarter marshes, influenced by tides from Tangier Sound and the Manokin River, are a preferred muskrat habitat. Their salinity averaged around 11.91‰ during the study.

### Materials and Methods

The muskrats were captured in a Conibear trap which kills the animals by drowning. All taken in the Thurmont area were *Ondatra zibethicus zibethicus*; all captured in the Dames Quarter area were *O. z. macrodon*. Organs of the gastrointestinal tract, stomach through large intestine, of each muskrat were separately examined for helminths. An aliquot technique or a sieving method was employed

for helminth recovery. The aliquot technique involved the use of a dissecting microscope in examining around 10% of the total volume of the contents of each organ. The sieving method entailed placing a wide strip of fine gauze material over the top of a metallic test tube rack and slowly sieving the organ contents through the gauze. The helminths were retained on the gauze and were removed by a dissecting needle. Routine methods for the preparation of the helminths followed. This included: staining trematodes with alum cochineal, borax carmine, Semichon's acetocarmine, and Delafield's hematoxylin. The nematodes were not stained. After dehydration in ethyl alcohol, the helminths were cleared in beechwood creosote or methyl salicylate and mounted with Canada balsam.

### Results and Discussion

The gastrointestinal tracts of the muskrats examined yielded five species of trematodes and one nematode species, as shown in Table I.

Approximately 71% of the Thurmont area muskrats and about 51% of those of the Dames Quarter area were infected with one or more of the helminths listed. None of the three trematode species recovered from the Thurmont group was found in the Dames Quarter group; neither trematode collected from the latter group occurred in the Thurmont group. The nematode, *Trichuris opaca* Barker and Noyes, 1915, occurred in both localities. The Dames Quarter group had a higher incidence of infection with it.

A study of the literature indicated that all of the helminths recovered in this study are widely distributed in muskrats in this country. All prior reports hereinafter cited pertain to occurrence in this host.

*T. opaca* reportedly occurs in Michigan (Ameel, 1944), Ohio (Rausch, 1946; Beckett and Gallicchio, 1967), New York (Edwards, 1949), Wisconsin (Tiner, 1950), Colorado (Ball, 1952), Illinois (Gilford, 1954), Oregon

<sup>1</sup> A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Oklahoma State University, Stillwater, Oklahoma.

Table 1. Results of survey of the gastrointestinal helminths of 154 Maryland muskrats.

Study area and helminth	Location in host	No. animals examined	No. animals infected	Per cent infected
Thurmont				
<i>Echinostoma revolutum</i>	Small intestine	79	42	53.2
<i>Quinqueserialis quinqueserialis</i>	Caecum	79	19	24.0
<i>Wardius zibethicus</i>	Caecum	79	26	32.9
<i>Trichuris opaca</i>	Caecum	79	7	8.9
Dames Quarter				
<i>Nudacotyle novica</i>	Small intestine	75	27	36.0
<i>Echinochasmus schwartzi</i>	Small intestine	75	3	4.0
<i>Trichuris opaca</i>	Caecum	75	18	24.0

(Senger and Neiland, 1955), Alaska (Dunagan, 1957), and Pennsylvania (Anderson and Beaudoin, 1966).

*Echinostoma revolutum* (Frolich, 1802), which was recovered only from the Thurmont group, had the highest helminth infection rate (53.1%). This helminth also occurs in Massachusetts (Rankin, 1946), New York (Edwards, 1949), Illinois (Gilford, 1954), Oregon (Senger and Neiland, 1955; Rider and Macy, 1947), Pennsylvania (Anderson and Beaudoin, 1966), and Ohio (Beckett and Gallicchio, 1967).

*Wardius zibethicus* (Barker and East, 1915), the second most frequently occurring helminth in the Thurmont muskrats, has been reported in Ohio (Rausch, 1946; Beckett and Gallicchio, 1967), Illinois (Gilford, 1954), and Pennsylvania (Anderson and Beaudoin, 1966).

*Quinqueserialis quinqueserialis* Barker and Laughlin, 1911, which also occurred only in Thurmont muskrats, occurs in Tennessee (Harwood, 1939), New York (Edwards, 1949), Maine (Meyer and Reilly, 1950), Illinois (Gilford, 1954), Oregon (Senger and Neiland, 1955), Alaska (Dunagan, 1957), Utah (Senger and Bates, 1957), Pennsylvania (Anderson and Beaudoin, 1966), and Ohio (Beckett and Gallicchio, 1967).

*Nudacotyle novica* Barker, 1916, the most frequently occurring helminth in the muskrats of the Dames Quarter marshes, occurs in fresh- and brackish-water habitats, and in Texas (Chandler, 1941), Louisiana (Penn, 1942), Michigan (Ameel, 1944), Ohio (Rausch, 1946; Beckett and Gallicchio, 1967), New York (Edwards, 1949), Maine (Meyer and Reilly, 1950), and Illinois (Gilford, 1954).

*Echinochasmus schwartzi* Price, 1931, although originally described from muskrats of

the Dorchester County marshes, occurred in only 4% of the Dames Quarter sample. It also occurs in Texas (Chandler, 1941), Louisiana (Penn, 1942), and Tennessee (Byrd and Reiber, 1942).

On the basis of the limited studies reported, the helminth fauna of muskrats in Maryland appears to vary from one locality to another. In this particular study, the chief environmental factor that appears to be the most influential on these parasite differences is the consistent salinity of the coastal marshes. This factor limits the ability of the freshwater gastropods, preferred intermediate hosts for certain trematodes, to persist in the Dames Quarter marshes.

While this article does not dwell on the influence that ecology plays in controlling the range of helminths, it is clear that differences in the biological and physical environment play a key role in parasite distribution.

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## The Use of Irradiated Third-stage Larvae of *Angiostrongylus cantonensis* as Antigen to Immunize Albino Rats Against Homologous Infection<sup>1</sup>

SHERIDAN H. LEE

Parasitology Laboratory, Department of Animal Sciences, University of Hawaii, Honolulu, Hawaii 96822

Lim, Ow-Yang and Lie (1965) and Heyneman and Lim (1965) reported that rats which received one to three sublethal feedings of normal third-stage larvae of *Angiostrongylus cantonensis* were able to develop some degree of resistance to reinfection as manifested by the reduced worm burden. Like many other helminthic infections, no sterile immunity was obtained (Sprent, 1963).

Jarret et al. (1959) reported success in obtaining sterile immunity to *Dictyocaulus viviparus* by using irradiated larvae. This suggested that a similar result may also be obtained with *A. cantonensis* since the latter

has a long brain phase 28-day plus, in its life cycle (Mackerras and Sandars, 1955).

### Materials and Methods

Third-stage larvae of *A. cantonensis* were harvested from laboratory infected giant African snails, *Achatina fulica*, by digesting minced snail tissue in 1% pepsin and 1% concentrated hydrochloric acid solution. Digested snail tissue was sieved through a 40-mesh wire screen into an Imhoff flask and allowed to settle for 40 min. After the supernatant liquid was siphoned off, the sediment was baermannized in saline through two 16-ply gauze pads supported by a 40-mesh copperwire sieve. The larvae were harvested 30 and 50 min later. Harvested larvae were rebaermannized and

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Table 1. Number of worms recovered from the lungs of immunized and control rats of Experiment I, II, and III following a challenge with 400 normal larvae.

Rat no.	Experiment I		Experiment II		Experiment III	
	Immunized	Control	Immunized	Control	Immunized <sup>1</sup>	Control
1	94	152	6	145	16 (2)	254
2	81	234	10	178	14 (0)	238
3	39	198	2	122	34 (6)	181
4	61	260	0	72	14 (12)	134
5	46	173	0	124	23 (8)	156
6	49	234	9	132	19 (0)	223

<sup>1</sup> Number of young adults on brain surface in parenthesis.

then washed three times in distilled water. About 10,000 washed larvae were placed in 2 cc of distilled water in small screw capped vials and irradiated with the desired dosage.

The radiation source was a 30,000-curie cobalt-60 irradiator located in the department of Food Science and Technology of the University of Hawaii. When this study began, the output was 4.7 Kr/min at 5 inches. All larvae irradiated were drawn from a common pool.

EXPERIMENT I. Twelve rats were used, each being given one feeding of 200, 40 Kr treated larvae. Six of these rats were killed on the 30th day after feeding; the other six were challenged with 400 normal larvae each, 30 days after feeding, and killed 42 days after challenge.

EXPERIMENT II. Six rats were used, each was given two feedings of 200, 40 Kr treated larvae with a 30-day interval between each feeding. Thirty days after the last feeding, each was challenged with 400 larvae and killed 42 days later.

EXPERIMENT III. In this experiment, each of six rats was given three feedings of 200, 20 Kr treated larvae with a 15-day interval between each feeding. Thirty days after the last feeding, each rat was challenged with 400 normal larvae and killed 42 days later.

An equal number of control rats were given 400 normal larvae to accompany all the above experiments. All the control rats were of the same age as the experimental rats.

EXPERIMENT IV. Two rats were given 200, 20 Kr treated larvae each. One of these rats was killed 40 days later and the other was killed 62 days later.

### Results

EXPERIMENT I. One adult female worm was recovered from one of the six rats which

received 200, 40 Kr treated larvae each. The following numbers of worms were recovered from the lungs of the experimental rats which were challenged with 400 normal larvae: 94, 81, 39, 61, 46, and 49. The number of worms found in each control rat was 152, 234, 198, 260, 173, and 234 (Table I).

EXPERIMENT II. The number of worms recovered from the lungs of each experimental rat, which had received two feedings of 200, 40 Kr treated larvae before challenge, was 6, 10, 2, 0, 0, and 9; whereas, the number of worms found in each control rat was 145, 178, 122, 72, 124, and 132 (Table I). All the worms recovered in both experiments were fertile and none of the rats had any worms in the brain. None of the rats appeared to have suffered any ill effects during the entire period of this experiment.

EXPERIMENT III. The following results were obtained from rats each of which received three feedings of 200, 20 Kr treated larvae before challenge: Rat 1 had two dead young adults on its brain surface and in its lungs were 16 adult worms, 7 males and 9 females, four of the latter being sterile. Rat 2 had in its lungs 14 adult worms, 5 males and 9 females, five of the latter being sterile. A few first-stage larvae were found in the lung tissue. Rat 3 had four live young adults and two dead young adults on its brain surface, 7 males and 27 females, in its lungs. Five of the 27 females were dead with only their cuticle and degenerate digestive tract remaining. First-stage larvae were present in the lung tissue. Rat 4 had three live young adults on its brain surface plus the remains of nine dead young adults which appeared as yellowish green lines. In its lungs were 3 sterile males and 11 females, four with eggs and

two without eggs. Five females were in the process of being absorbed. Rat 5 had seven partially absorbed young adults plus one live young adult on its brain surface. In its lungs were 13 females, three without eggs, and 10 males, seven of which were sterile. A few first-stage larvae were present in the lung parenchyma. Rat 6 had no worm on its brain surface. There were 8 males and 11 females in its lungs, seven of which were sterile. No first-stage larvae were found in the lung parenchyma. The number of worms found in the control rats for Experiment III was 254, 238, 181, 134, 156, and 223 (Table I).

EXPERIMENT IV. Eighteen sterile worms were found in the lungs of the rat killed on the 40th day after being fed 200, 20 Kr treated larvae. There were no worms in its brain. The second rat which was killed on the 62nd day after being fed 200, 20 Kr treated larvae had six sterile females in its lungs and six young adults on the surface of its brain. Two of the six young adults were still alive whereas the other four were dead.

### Discussion

Experiment I indicated that a radiation dosage of 40 Kr was quite detrimental to the further development of the third-stage larvae of *A. cantonensis*. Yet the antigenicity of these 40 Kr treated larvae was not lost as indicated by the reduced worm burden of the immunized rats when challenged. This experiment also indicated that immunity acquired by the experimental rats did not depend on the existence of live worms within the rats.

In Experiment II, the complete absence of worms in two of the experimental rats and the relative low number of worms in other rats of this group suggests that two feedings of 200, 40 Kr treated larvae offers the best immunizing procedure.

Experiment IV indicated that most likely, all the sterile worms recovered from the experimental rats of Experiment III were developed from the 20 Kr treated larvae used for immunization. It is interesting to note that Experiment IV not only demonstrated the detrimental effect of the dosage of 20 Kr to the germ cells of *A. cantonensis* but also revealed that this dosage will prolong the brain phase of this worm.

It would seem that the protracted stay of young adults in the subarachnoid space should cause the rat to have a stronger immune response. However, the results of Experiments II and III do not support this idea, at least when the quality of immunity is measured in terms of worm burden after reinfection. Why the dosage of 40 Kr makes the third-stage larvae of *A. cantonensis* serve as a better antigen as compared with 20 Kr against homologous infection remains an unsolved but interesting question.

### Summary

Of six rats which received two feedings of 200, 40 Kr irradiated third-stage larvae of *Angiostrongylus cantonensis*, two became completely refractive and four showed significant resistance when challenged with normal larvae. Twenty Kr radiation not only destroyed the germ cells of *A. cantonensis*, but also caused most of the young adults to lose their ability to migrate to the lungs of their definitive host.

### Acknowledgment

The author wishes to acknowledge the technical assistance of Dr. M. D. Upadhyia in irradiating all the larvae used in this paper.

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## *Hypsoperine ottersoni* sp. n. (Nemata, Heteroderidae) Infesting Canary Grass, *Phalaris arundinacea* (L.) Reed in Wisconsin

GERALD THORNE<sup>1</sup>

Unusual types of nematode-incited galls were observed on canary grass in the Wind Lake area near Kenosha, Wisconsin by Dr. Henry Otterson, Agricultural Chemist and Consultant, who submitted specimens for identification in 1958, which later were recognized as belonging to the genus *Hypsoperine* Sledge and Golden, 1964. Detailed studies were not begun until 1963 when Arthur Weber undertook the project as a thesis problem in graduate work under the direction of Dr. K. R. Barker, University of Wisconsin, Madison.

Validity of the genus *Hypsoperine* has been questioned by Dr. M. W. Allen (personal communication). It is true that the elevated perineal region sometimes is not evident and the cuticle is not thickened on this species, as reported by Sledge and Golden (1964) for *H. graminis*. However, the physiological effect on the host, causing an unusual type of gall formation with larvae always lying in the root with heads toward the apex, is unlike that of usual *Meloidogyne* infestations. The hemizonid of *H. graminis* is reported anterior to the excretory pore, while that of *H. spartinae* is posterior, according to Rau and Fassuliotis (1965).

Infestation always takes place just behind the apical cells and does not stop growth of the root which generally forms an arcuate, elongated gall and then proceeds to develop a series of similar galls, each containing from 1–6 females and in rare instances 15 or 20 may occur. Branch roots are produced on the outer periphery of each gall and these in turn are infested until a complicated, chainlike mass of roots results (Fig. 1A). Occasionally a progressive multiple infestation occurs in a terminal gall with mature egg-producing females at the gall base, while elongated young females are located near the terminus, resulting in a series of various-aged individuals (Fig. 1B).

In all instances observed, the heads of the

nemas were directed toward the root terminus. Eggs usually are deposited in a gelatinous matrix within the gall tissues, but in some instances they and the matrix are extruded outside the root. Feeding takes place along the cells between the xylem tubes which frequently are multiplied and broken apart (Fig. 1B). Even in extreme instances like this, the root cap and terminal cells remain intact, enabling continued growth.

### *Hypsoperine ottersoni* sp. n.<sup>2</sup> Morphology

FEMALE (10): 0.27 by 0.45 mm (0.18–0.32 by 0.39–0.52). Variable in form as indicated by these measurements (Fig. 2N–S). Spheroid to elongate pyriform with neck projecting at different angles (Fig. 2N–S). Vulva and anus usually on a slight elevation, but sometimes this is not present and the posterior end is similar to that of *Meloidogyne*. Cuticle 3–6  $\mu$  thick on main part of body, leathery with extremely fine striae except on head and neck where they are easily visible and form annules variable in width. Lip region unstriated, set off by constriction. A face view (Fig. 2L) shows six well-developed lips, the lateral ones being somewhat larger than the submedian. Amphid apertures slightly oval, located at apices of lateral lips. Vestibule slightly sclerotized, forming a spear-guiding apparatus. Spear 10–12  $\mu$  long with small rounded knobs. Dorsal esophageal gland orifice almost adjacent to spear base. Anterior portion of esophagus set off from median bulb by constriction. Bulb spheroid with refractive valve and strong radial muscles. Basal bulb a flattened, irregular shaped lobe extending back over intestine, containing the usual three nuclei which generally are difficult to observe. Isthmus very short. Nerve ring obscure. Excretory pore far forward, almost opposite spear base (Fig. 2K). Intestine and body contents densely granular, making observations very

<sup>1</sup> Professor Emeritus, Departments of Plant Pathology and Zoology, University of Wisconsin.

<sup>2</sup> First used as a *nomen nudum* by Weber and Barker (1967).

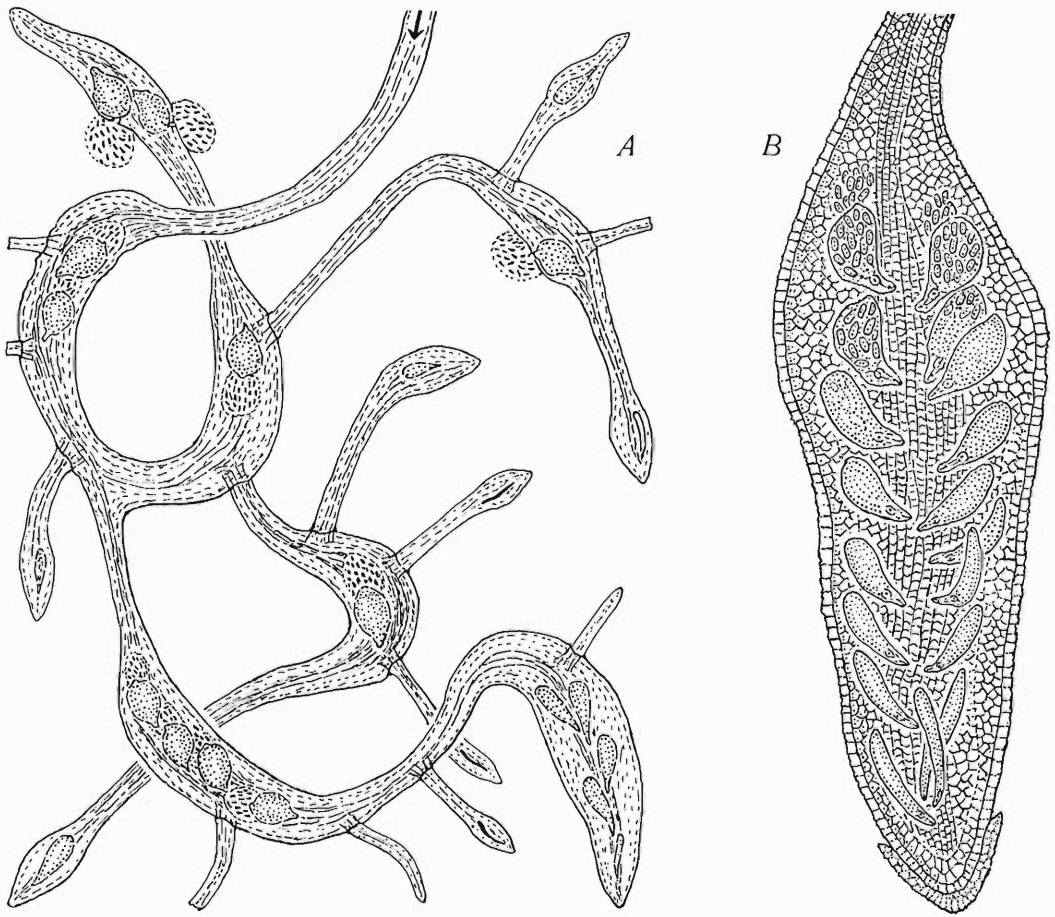


Figure 1. A, Root of *Phalaris arundinacea* infested with *Hypsoperine ottersoni*. Note how secondary roots spring from outer contours of the sickle-shaped galls. Upper left and right are females ejecting eggs from the galls as occasionally occurs. B, Unusual terminal gall with progressive infestation. The first larvae entering it are now adult females producing eggs, while those recently entering near the terminus are still in early stages of development.

difficult. Ovaries two, elaborately convoluted (Fig. 2N), at maturity completely filling body cavity. Vulva transverse. Vagina cylindroid, muscular. Perineal pattern with simple rounded striae, usually on a slight terminal elevation (Fig. 2T). Anal opening visible from a lateral view and rarely from ventral (Fig. 3C). Neither spermatheca nor spermatozoa observed.

Eggs (15) 45 by 105  $\mu$  (40–50 by 95–115), deposited before segmentation by young females. Senile individuals may retain from 5–25 eggs which segment and develop into lar-

vae which probably are not ejected. A gelatinous matrix surrounds eggs deposited either within the roots or into the soil.

MALE: (7) 0.9–1.0 mm long, rarely 0.7 mm;  $a = 34$ , rarely 40. Body twisted until a true lateral view of head and tail can be seen only by cutting specimens after fixation. Striae about 2  $\mu$  apart at midbody, narrower near head and sometimes absent on tail. Lateral fields about one-fifth body width, marked by four incisures, the two median ones sometimes very obscure. Lip region unstriated; labial disc visible only from a dorsal or ventral view when

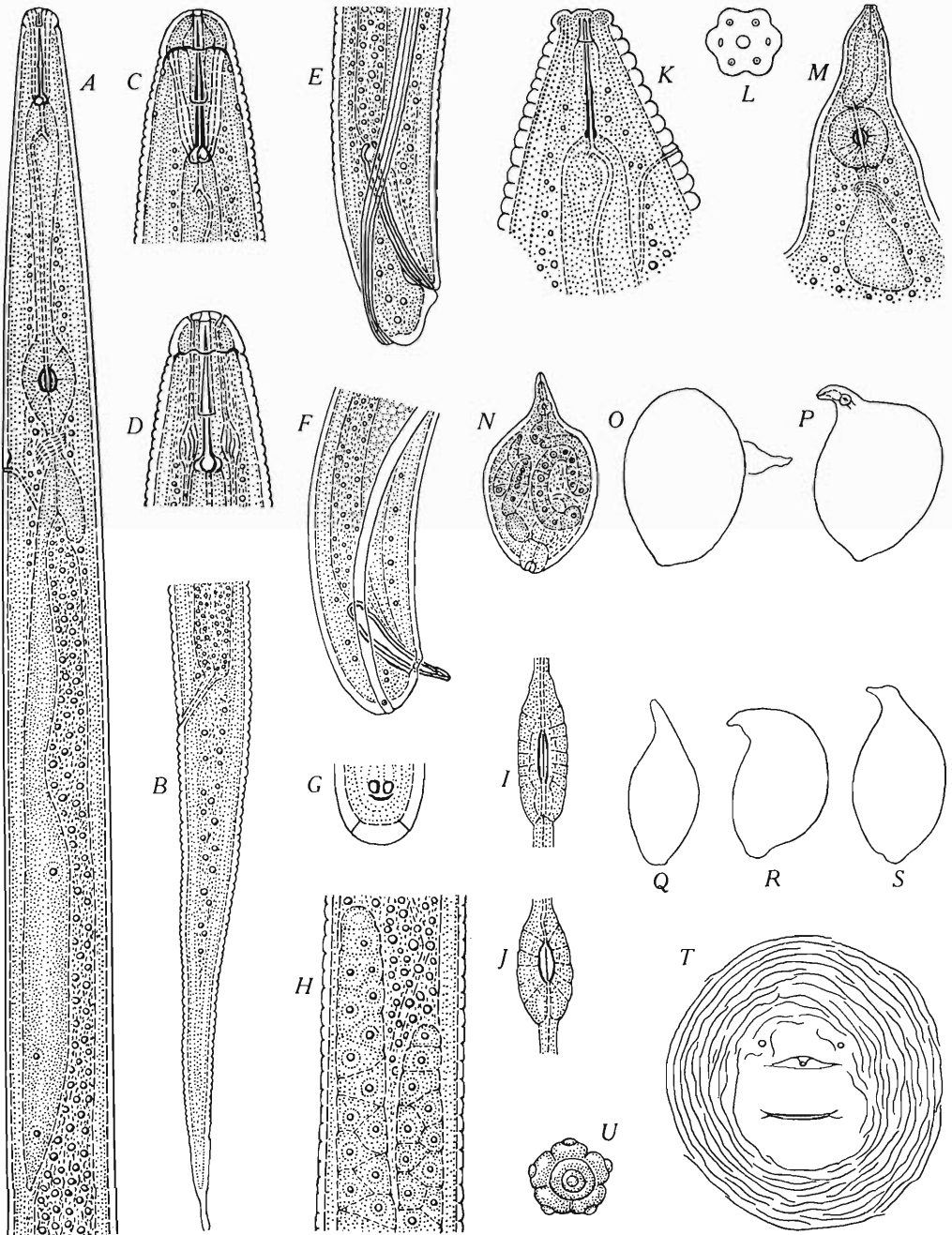


Figure 2. A, B. Anterior and posterior portions of larva  $\times 1,000$ . C, D. Male head lateral and dorsal views. E, F, G. Lateral and dorsal views of posterior portion of males. H. Specimen with two testes. I, J. Variations in median esophageal bulbs. K, L, M. Head, face view and anterior end of female. N-S. Variations in form of females. T. Perineal pattern. U. *Duboscquia* sp. parasites.

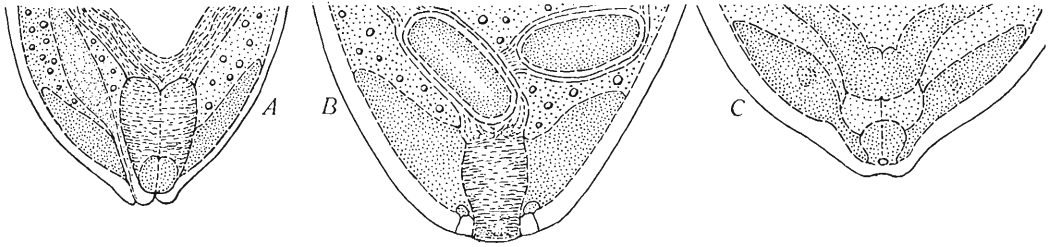


Figure 3. A, B. Lateral and dorsal views of posterior portion of female. C. Ventral view showing anus, vagina, uterus, and attachment of the two ovaries and saccate rectal glands.

the slitlike amphid apertures set it off from the lip contour (Fig. 2D). Cephalids obscure, visible only from a lateral view, located well forward of the spear base as in *Meloidogyne* (Fig. 2C). Spear 14–16  $\mu$  long with strong, backward-sloping knobs. Nerve ring one body width, and excretory pore twice that distance behind median bulb. Lumen of esophagus joining intestine about one body width posterior to bulb, the junction being rather abrupt as illustrated for the larvae (Fig. 2A). Hemizonid about two annules anterior to excretory pore. Basal esophageal lobes average about three times as long as body width. Testes 53–65% of body length, usually single, outstretched, rarely reflexed. Males rare; among 14, two were observed with double testes (Fig. 2H). Spicula slightly arcuate 19–23  $\mu$  long but difficult to measure because of the twisted bodies. Gubernaculum 3 or 4  $\mu$  long, well-thickened, troughlike. Phasmids almost terminal (Fig. 2G). Tail variable in form and length (Fig. 2E, F). Sperm ducts packed with spheroid spermatozoa indicating that males may be functional although sperms were not observed in the uteri.

LARVAE (10): 0.43–0.50 mm; a = 23–30, b and c measurements not made because the esophagus base and anal opening could rarely be accurately determined. Lateral field marked by four fine incisures visible only on favorable specimens. Framework of the rounded lip region practically undeveloped. Vestibule forming a minute sclerotized spear guide. Spear 13–15  $\mu$  long with well-developed knobs. Dorsal gland orifice about 4  $\mu$  behind spear. Nerve ring adjacent to bulb, practically enveloping the unusually short isthmus. Esophageal lumen joining intestine about one body

width behind bulb. Dorsally the esophagus base is short and rounded while ventrally it is extended in a long lobe 5–7 times the body width. Excretory pore slightly behind nerve ring. Anal opening very obscure, often unidentifiable. Tail ending in an irregularly clavate or knobbed terminus.

Holotype female, allotype male and other specimens as indexed under *Hypsoperine* 1, University of Wisconsin nematode collection.

TYPE HOST AND LOCALITY: Canary grass, *Phalaris arundinacea* (L.) Reed, Homer Ranch, Wind Lake area, Wisconsin. Found wherever canary grass is growing in Racine, Milwaukee, and Waukesha counties and near Waupun in Green Lake County. Many observations by Dr. Otterson and others have failed to reveal it attacks any crop plants.

*Hypsoperine ottersoni* is distinguished from *H. graminis*, Sledge and Golden, 1964 by the more simple annulation and absence of lateral lines in the perineal pattern, smaller males 0.9–1.5 mm, and irregular-clavate terminus of larvae. From *H. spartinae* Rau and Fassuliotis, 1965, it is recognized by absence of labial annules (two in *H. spartinae*), simple lines of perineal pattern compared to elaborate arrangement, and presence of male hemizonid anterior to excretory pore which is posterior in *H. spartinae*.

Perineal sections should be mounted in a drop of hard glycerin jelly to support the cover glass, otherwise the weight will produce folds and wrinkles which destroy the actual pattern of the striae.

Many larvae and some males were attacked with sporozoans, *Duboscquia* sp.; in some instances these were observed in clusters of

eight, unlike any previously recorded (Fig. 2U).

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## Studies on Freshwater Larval Trematodes. XXI. Two New Species of Macrocerous Cercariae<sup>1</sup>

PIR NASIR, MARCOS TULIO DÍAZ, LUIS J. HAMANA SALAZAR, AND S. GUEVARA  
Laboratorio de Parasitología, Depto. de Biología, Escuela de Ciencias,  
Universidad de Oriente, Cumaná, Venezuela

Until now, only bivalve mollusks have been reported as the first intermediate hosts of the known macrocerous cercariae. This is the first record for the involvement of univalves as well. The cercariae treated in this paper, as a result of comparative studies discussed later, proved to be new species and have been named, respectively, *Cercaria latigazica* and *C. yacalicola*. The latter is readily distinguished by the presence of a cercarial chamber, in the proximal part of its tail, in which the cercarial body is characteristically enclosed. The former lacks this structure and its tail is frequently thrust out in a peculiar whiplike fashion. It is evident from the works of other authors that various species of tadpoles and naiads of insects serve as the second intermediate hosts for macrocerous cercariae while urinary bladders of frogs, salamanders, and fishes are the usual sites of adult infections. In certain cases like *Phyllodistomum simile*, the precociously encysted cercariae while still inside sporocysts when experimentally fed to trout resulted in the development of corresponding adults (Thomas, 1958). Of course, we are not sure whether these "precocious metacercariae" were formed by the penetration of cercariae from without. Due partly to the dearth of material, we have been unable

to elucidate life cycles. Even if infected snails had been more numerous, only a few cercariae emerged at a given time; however, efforts are in progress. It may be mentioned here that on one occasion *C. yacalicola* was escaping from the same specimen of the snail, *Pomacea glauca*, which was also discharging two additional cercariae, *C. cumanensis* Nasir, 1965 and *C. allomacarapanensis* Nasir and Diaz, 1968.

The recovery of sporocysts presented an intriguing situation. Although several snails were dissected immediately after the appearance of cercariae, sporocysts were never encountered in the usual sites like gills and hepatopancreas. A careful examination revealed the presence of a few, anteroposteriorly elongated, considerably thick-walled, pigmented sporocysts in the lumen of intestine.

All observations have been made on freshly emerged cercariae, except measurements which are based on specimens killed by irrigating with hot 10% formalin.

All measurements are in millimeters.

### 1. *Cercaria latigazica* sp. n. (Figs. 1, 2, 7)

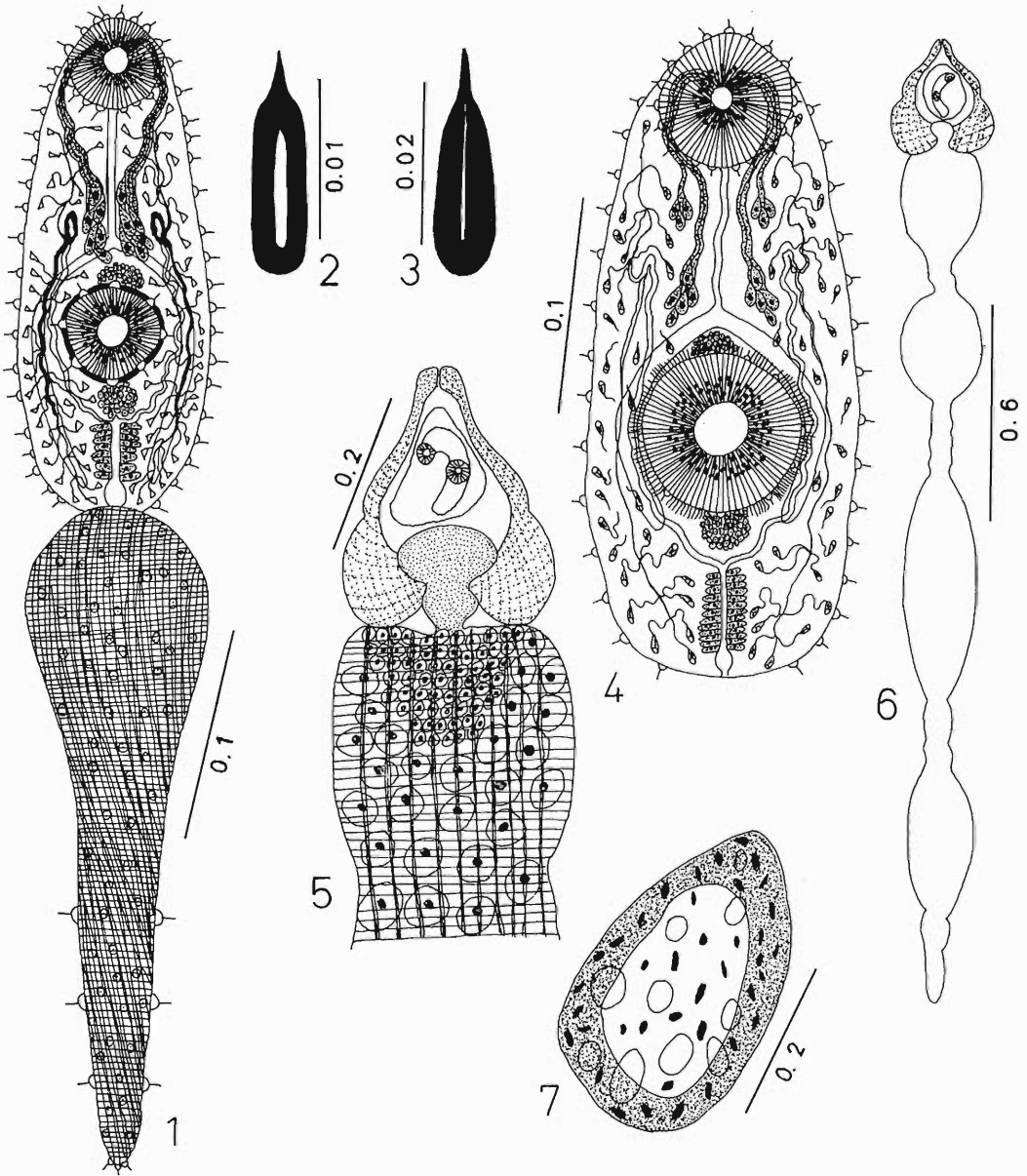
HOST: *Pomacea glauca* (L.).

LOCALITY: La Chorrera, near Caripito; Los Yacales, La Llanada de San Juan, near Universidad de Oriente, Cumaná, Venezuela.

DESCRIPTION: Body without spines, beset

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Figures 1, 2, 7. *Cercaria latigazica* sp. n. 1. Cercaria, note peculiar structure of tail, stylet not shown. 2. Stylet. 7. Sporocyst, note considerably thick wall and pigmented specks.

Figures 3-7. *Cercaria yacalicola* sp. n. 3. Stylet. 4. Body only, note two groups of penetration glands on each side. 5. Cercarial chamber and part of tail. 6. Cercarial chamber along with distal, elongated swollen region. 7. Sporocyst.

with 22 rows of setate papillae. Stylet without a basal bulb (Fig. 2). Central parts of suckers occupied by papillae without setae and irregularly distributed bristles. Periphery of both suckers with a row of setate papillae. Ventral sucker surrounded by a muscular fold. Pharynx absent. Esophagus long, not extending to ventral sucker. Intestinal ceca extending to posterior end of body. Penetration glands small, six on each side along posterior part of esophagus, not extending to ventral sucker. Genital rudiments represented by two cellular masses, one anterior and the other posterior to ventral sucker. Excretory vesicle tubular, with a swollen posterior region; eight transversely elongated glands with granular contents on either side of vesicle. Main excretory ducts extending as far anteriorly as about halfway in preacetabular region and after forming a small loop in same region dividing into anterior lateral and posterior lateral collecting excretory tubules. Flame cell formula:  $2[(3+3+3) + (3+3+3+3+3+3+3+3)] = 66$ . Tail without spines, without cercarial chamber, capable of extending in a peculiar whiplike fashion. Measurements of 10 specimens: body 0.210–0.267 by 0.057–0.090; tail 0.300–0.375 by 0.078–0.114, when extended more than twice as long as its normal length; oral sucker 0.040–0.044 in diam.; esophagus 0.060–0.065 long; ventral sucker 0.043–0.045 in diam.; stylet 0.013–0.016 by 0.003–0.005. Sporocysts 0.300–0.817 by 0.206–0.404.

## 2. *Cercaria yacalicola* sp. n. (Figs. 3–7)

HOST: *Pomacea glauca* (L.).

LOCALITY: Los Yacales, La Llanada de San Juan, near Universidad de Oriente, Cumaná, Venezuela.

DESCRIPTION: Body without spines, covered with setate papillae. Stylet without a basal bulb (Fig. 3). Central parts of suckers with papillae without setae and irregularly distributed bristles. Periphery of suckers bordered with rows of setate papillae. Pharynx absent. Esophagus long, extending to ventral sucker. Intestinal ceca extending only slightly posterior to ventral sucker. Penetration glands with uniformly granular contents, in two groups on each side of body: one group of three posterior to oral sucker and another

group of five anterior to ventral sucker; this latter group may extend laterally around ventral sucker. Genital rudiments composed of two cellular masses, one anterior and other posterior to ventral sucker. Excretory vesicle tubular, with a swollen posterior region. Twelve to 14 transversely elongated glands with coarsely granular material on each side of vesicle. Main excretory tubes dividing into anterior lateral and posterior lateral collecting tubes at about equatorial level of preacetabular region. Flame cell formula:  $2[(3+3+3) + (3+3+3+3+3+3+3+3)] = 60$ . Tail without spines or papillae, divided into two regions: proximal swollen region or cercarial chamber and a distal, narrow, elongated region which often presents irregular swellings. Measurements of 10 specimens: body of cercaria expressed from cercarial chamber 0.235–0.370 by 0.112–0.138; oral sucker 0.060–0.066 in diam.; esophagus 0.060–0.067 long; ventral sucker 0.072–0.085 in diam.; stylet 0.027–0.033 by 0.007–0.009; cercarial chamber 0.243–0.379 by 0.159–0.300; distal part of tail without cercarial chamber 2.302–2.570 by 0.159–0.300. Sporocysts similar to that of *C. latigazica*.

## Discussion

For the specific identification only those characters which are fairly constant or vary little like the shape and size of stylet, esophageal length, number and arrangement of penetration glands, number of "cystogenous glands" around excretory vesicle, type of excretory system mesostomate or stenostomate, number and arrangement of flame cells and finally presence or absence of cercarial chamber have been considered. Unless the relative differences in size are of a considerable magnitude, too much reliance on this character alone could be misleading. Some "new species" erected on the basis of minor discrepancies should be suppressed. *Cercaria eriensis* Coil, 1953 and *C. lampsilae* Coil, 1954 are indistinguishable in having approximately the same size suckers, esophageal length, intestinal length, shape, and extent of excretory vesicle, similar number of glands around excretory vesicle and finally both of the species abound in the same locality, Lake Erie, Ottawa County, USA. Furthermore, the molluscan intermediate hosts are the very closely related species,

*Lampsilis ventricosa* and *L. siliquoidea*. According to Coil, 1954, "*C. lampsilae* differs from *C. eriensis*, its closest relative, by possessing (1) a stylet which is slightly shorter and of a different shape, (2) a different disposition of sensory papillae on the oral sucker, (3) a body length which is shorter." In our experience none of these characters is of substantial taxonomic importance. The "differences" between the stylets of *C. eriensis* and *C. lampsilae*, as depicted in diagrams (Coil, 1954; fig. 5-6), are hardly differences at all, because a slight pressure of coverglass will distort the shape of one into that of the other. We have often encountered this type of distortion while studying xiphidiocercariae. Insofar as stylet

size is concerned there is only a difference of 2  $\mu$ ; the stylet of *C. eriensis* is 14-17  $\mu$  in contrast with 16-19 of *C. lampsilae*. The arrangement of papillae on oral sucker is also a matter of interpretation. Finally, the criterion of body size is also questionable. Cercaria of *Gorgoderina amplivava* Looss, 1899, as described by Krull, 1934, is 4.4-7.5 mm long whereas the same cercaria reported by Goodchild, 1945, is 12.0-17.4 mm long. It is therefore suggested that *C. lampsilae* should be synonymized with *C. eriensis*.

Only those styleted macrocercous cercariae about which sufficient structural details are known are compared and these are separated by the following key:

- I. A. Tail with a cercarial chamber ..... II
  - B. Tail without a cercarial chamber ..... III
  - II. Tail with cercarial chamber
    - A. Penetration glands arranged in two groups on each side of body
      - 1. Groups of 5 and 3 on each side ..... *C. yacalicola* sp. n.
      - 2. Groups of 6 and 4 on each side ..... *C. raiacauda*
      - 3. Groups of 8 and 2 on each side ..... *C. steelmani*
    - B. Penetration glands in only one group on each side of body
      - 1. Constant number present
        - a. eleven on each side ..... *Phyllodistomum staffordi*
        - b. four on each side ..... *C. coelocerca*
        - c. six on each side
          - 1a. Number of flame cells known
            - aa. 18 each side ..... *C. macrocerca*
            - bb. 16 each side ..... *C. donecerca*
            - cc. 24 each side ..... *Phyllodistomum simile*
          - 1b. Number of flame cells unknown
            - a'. 10 sensory tubercles around oral sucker
              - aa'. stylet 33-37  $\mu$  long ..... *Gorgoderina amplivava*
            - b'. 2 rows of 10 each
              - bb'. stylet 23-25  $\mu$  long ..... *Phyllodistomum solidum* = *C. conica*
            - c'. about 40
              - cc'. stylet 35  $\mu$  long ..... *Gorgoderina attenuata*
      - 2. Number not constant
        - a. 3 to 4 pairs on each side ..... *C. papillostoma*
        - b. not arranged in 3 to 4 pairs
          - 1a. Excretory system stenostomate ..... *C. wabashensis*
          - 2a. Excretory system not stenostomate ..... *C. sphaerocerca*
- III. Tail without cercarial chamber
  - A. Six penetration glands each side ..... *C. latigazica* sp. n.
  - B. Four penetration glands each side ..... *C. eriensis*

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## On the Morphology and Ultrastructure of the Esophageal Region of *Trichodorus allius* Jensen

D. J. RASKI, N. O. JONES, AND D. R. ROGGEN

University of California, Davis and Laboratoria voor Morfologie en Systematiek, Gent

The search by electron microscopy for plant-pathogenic viruses in nematode vectors began with *Xiphinema index*, the vector of the fanleaf strain of arabis mosaic virus. No evidence of the virus was found in *X. index* but the study did yield valuable information on the ultrastructure of the nematode vector (Wright, 1966; Roggen, Raski and Jones, 1966, 1967).

More recently, the search has been directed to *Trichodorus allius*, a vector of tobacco rattle virus, in a similar study of nematode-virus relationships. One advantage of this virus is the rod-shape of its particles which might be easier to detect in animal tissue than the polyhedral particles of fanleaf virus.

A study of ultrathin sections of *T. allius* has not been successful in locating virus particles but has been fruitful in developing a better understanding of the odontostyle and mechanism of feeding and of the ultrastructure of the esophagus.

### Materials and Methods

Nematodes used in these studies were collected originally from soil about the roots of sugarbeet (*Beta vulgaris*) and alfalfa (*Medicago sativa*) near Salinas, California. Populations were reared at Davis, California, under greenhouse conditions feeding on the roots of alfalfa, tobacco (*Nicotiana tabacum*), but principally on sweetpea (*Lathyrus odoratus*). No males of *T. allius* were seen in any of these populations.

Female and larval nematodes were wet-sieved from the soil then hand-picked into tap water. The nematodes then were transferred to 0.9% NaCl and frozen by dry ice in a fume-chamber with 2% OsO<sub>4</sub> in distilled water. After being allowed to return slowly to room temperature they were fumed overnight. This treatment was followed by 2 minutes immersion in 15% DMSO (dimethylsulfoxide) in distilled water and 20 minutes in 2% OsO<sub>4</sub> in distilled water. This procedure was used unless otherwise indicated in plate text. The nematodes then were handled according to Wright and Jones (1965). Embedded worms were cut by diamond knives on an LKB Ultratome and examined on an RCA EMU-3G or modified EMU-3E electron microscope each having a 50  $\mu$  objective aperture and operating mostly at 50 kv, occasionally at 100 kv.

### Observations

#### Cuticle

The cuticle of the adult female of *T. allius* (Plate 1, A-D; Fig. 1, A) appears to be composed of eight layers as follows:

L<sup>1</sup>. A triple-layered, thin (0.025  $\mu$ ) osmiophilic membrane.

L<sup>2</sup>. A thick (0.16  $\mu$ ) layer, uniform in texture. There may be two sub-layers making up L<sup>2</sup> because in one section (OsO<sub>4</sub> fixative plus Pb(OH)<sub>2</sub> postsectioning stain for 15 minutes) a fine, outer, whitish band and dark inner band

were observed. These were not consistently or certainly set apart in other sections.

Fine cross-striations (cs) (Plate I, A–C), approximately  $3 \mu$  apart, were present on most of the body and extended almost to the terminus. The striations were formed by an infolding of  $L^1$  and  $L^2$  and at most created a slight indent in the outer surface of  $L^3$ .

$L^3$ . A layer, varying in thickness, from  $0.48$ – $1.50 \mu$  with an average of about  $0.64 \mu$ . This layer also appeared to have two components, different in texture and somewhat in color, but there was no sharp demarcation between the two. The outer portion ( $L^{3a}$ ) comprised 74–82% of the total and was coarser-grained in appearance than the inner portion. In cross-section there was only a vague suggestion of structure as concentric lines but without regularity. In longitudinal section (Plate I, C) there were many lines running longitudinally which appeared to set off sublayers, 18–20 in number, whitish with dark borders. The inner 14 or 15 were broader, narrowing (or doubling) near  $L^2$ . The inner portion ( $L^{3b}$ ) comprised 18–25% of the total of  $L^3$ , was darker in color and of finer grain than  $L^{3a}$ . In longitudinal section there were 11 or 12 sublayers similar to those in  $L^{3a}$  but dark bands with light borders.

$L^4$ . A layer variable in thickness from minimal or nonexistent to  $0.68 \mu$ , spongy or amorphous in texture.

$L^{5-7}$ . The next three layers were granular with no apparent structure and about equal in thickness ( $0.09 \mu$  each). The innermost ( $L^7$ ) had an irregular inner surface projecting in rough, lumpy protuberances.

$L^8$ . Underlying  $L^7$  was an irregular and light gray layer the nature of which is unknown. It was intimately connected with the epidermis which, on the outer margin, formed an irregular edge with polyp like protuberances intermingled with  $L^8$ .

### Oral Aperture

The most anterior section shows the oral opening to be triradiate (each arm about  $0.5$ – $0.7 \mu$  long). These arms are almost straight but slightly posteriorly they become irregular with several short side branches, the distal branches on two of the arms give an almost forked appearance (Plate II, A). Lining the oral opening is the thin, dark osmiophilic layer

( $L^1$ ) which is continuous with the outermost layer of the body cuticle.

The opening is set in  $L^2$  of the cuticle which forms a roughly hexagonal shape, each side about  $0.7$ – $0.9 \mu$  long. The six angles of the hexagon are directed each between two inner labial papillae (IL) and anteriorly these angles extend outward joining a similar layer of tissue outside the papillae. The triradiate oral opening also is oriented with each arm directed between two inner labial papillae. Each papilla is surrounded by more coarsely granular  $L^3$ .

About  $0.6$ – $0.8 \mu$  posteriad from the anterior surface (Plate I, D) is a crease or folding of the oral aperture extending parallel with the anterior surface. This fold is about  $0.6 \mu$  long in both directions perpendicular to the longitudinal aperture. The osmiophilic layer lines this fold and is continuous with the lining of the oral aperture.

### Stoma

The first evidence of the stomatal wall (Plate II, B) appears just below the thick anterior cuticle and is composed of:

$L^1$ . The osmiophilic layer forms a very irregular outline of the stomatal lumen and is grossly convoluted or jagged. This lasts only a short distance and soon becomes more simple, triradiate with a single bulge at the midpoint of each side (Plate II, C). Where the lining is convoluted (Plate II, B) there are some membranelike folds inside the lumen of the stoma.

$L^2$ . This layer is relatively thick ( $0.1$ – $0.2 \mu$ ), at first uniform in texture, and definitely set off from the adjacent  $L^3$  by a dark line. Later a well-defined membrane appears around  $L^2$  (Plate II, C) and soon afterwards tissue appears with the membrane. This tissue probably is the most anterior projection of pharyngeal tissue (ph).

At the same level where the above membrane first appears there is also the first evidence of the lining of the pharyngeal lumen (*pll*) inside  $L^2$ . The innermost lining of the *pll* seems to be similar to, if not identical with,  $L^1$  and continuous with it. As the *pll* expands the  $L^2$  diminishes to a thin concentric band around it, finally becoming an incomplete band (Plate II, D) and later disappears.

The double-layered  $L^3$  surrounds  $L^2$ . Outside of  $L^3$  is a membrane which broadens slightly to a thin line of epidermal tissue. Sur-

rounding the epidermal layer is another which appears to be  $L^2$  and it in turn is surrounded by another layer of epidermis.

$L^3$  retains its double-layered appearance (Plate II, C) through level 1 (Fig. 1, A–B) where the pharyngeal tissue appears between it and  $L^2$ . The pharyngeal tissue continues to expand through succeeding levels further separating off  $L^3$ . As this happens  $L^3$  becomes homogeneous in color and evenly granular. Posteriorly  $L^3$  disappears (Plate II, E). This seems to be the inner limit of the external cuticle and probably marks the posterior end of the stoma. This is supported by the fact that molted cuticles of larvae show only a very short stomatal cuticular lining which is cast off.

### Pharynx

Coincident with the very first appearance of the *pll* there are light colored areas (Plate II, C; level 1, Fig. 1, A–B) which seem to be strengthening rods or bands (Pr) running longitudinally. These may serve as muscle attachments and are located centrally near the osmiophilic layer. Similar refractive bands soon appear (Plate II, D) at the ends of the tri-radiate lumen. The 3 inner bands are shorter than the distal ones and disappear just before the stylet tip is seen (Plate II, E). The three distal bands divide into three each—one at the

tip of the angles of the lumen and one slightly larger on each side.

The next change in these bands is their disappearance in the apical area on all three rays of the lumen accompanied by a lengthening of the dorsal wall which is gently arched (Plate IV, A). On the ventral ray of the lumen the refractive bands divide to form two pairs. These refractive longitudinal bands persist some distance until finally the two pairs on the ventral ray disappear (Plate II, F) and soon afterwards the pairs on the two subdorsal rays disappear.

Posteriorly the next development is a smooth, grayish thickening of  $L^1$  lining the two sides of the ventral ray of the lumen (Plate III, G). These thickenings extend posteriorly through the folds of the guide ring then disappear. The rest of the *pll* outside the  $L^1$  becomes restricted to small narrow areas on both walls of the ventral ray on each side of the swelling and finally the entire lumen wall becomes a uniform, gray texture.

### Odontostyle

The odontostyle is first seen (Plate II, E) as a simple circular body about  $0.2 \mu$  across, free in the lumen. It has a uniform gray color with a small central darker dot and a fine, slightly darker, outer border. Posteriorly it en-

→

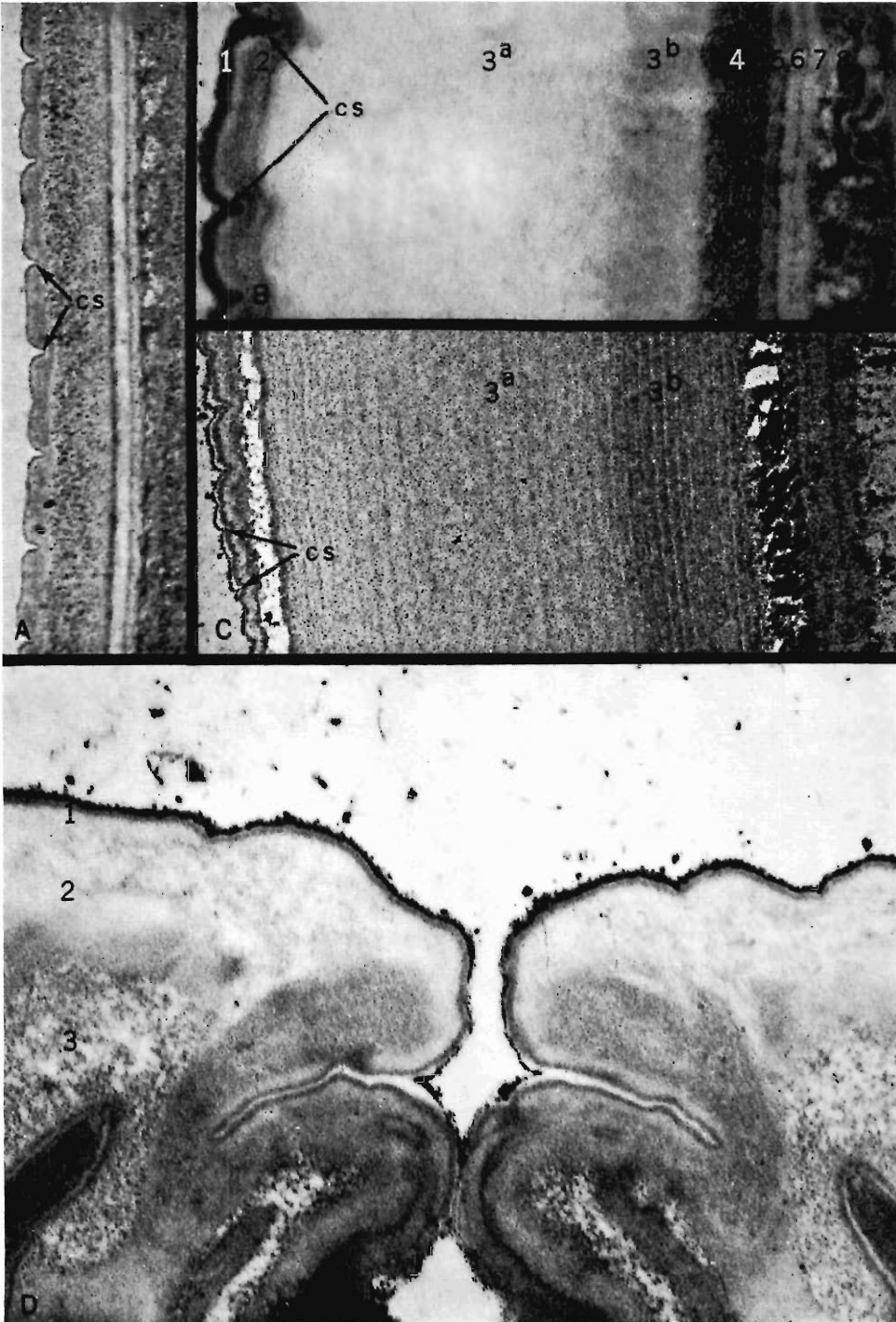
**Plate I.** Cuticular layers and markings. A, Longitudinal section, 12,000 ×; B, Longitudinal section, 38,000 ×; C, Cross section, 20,800 ×; D, Longitudinal section in stomatal region, (IL) innervations to interno-labial papillae, 43,700 ×.

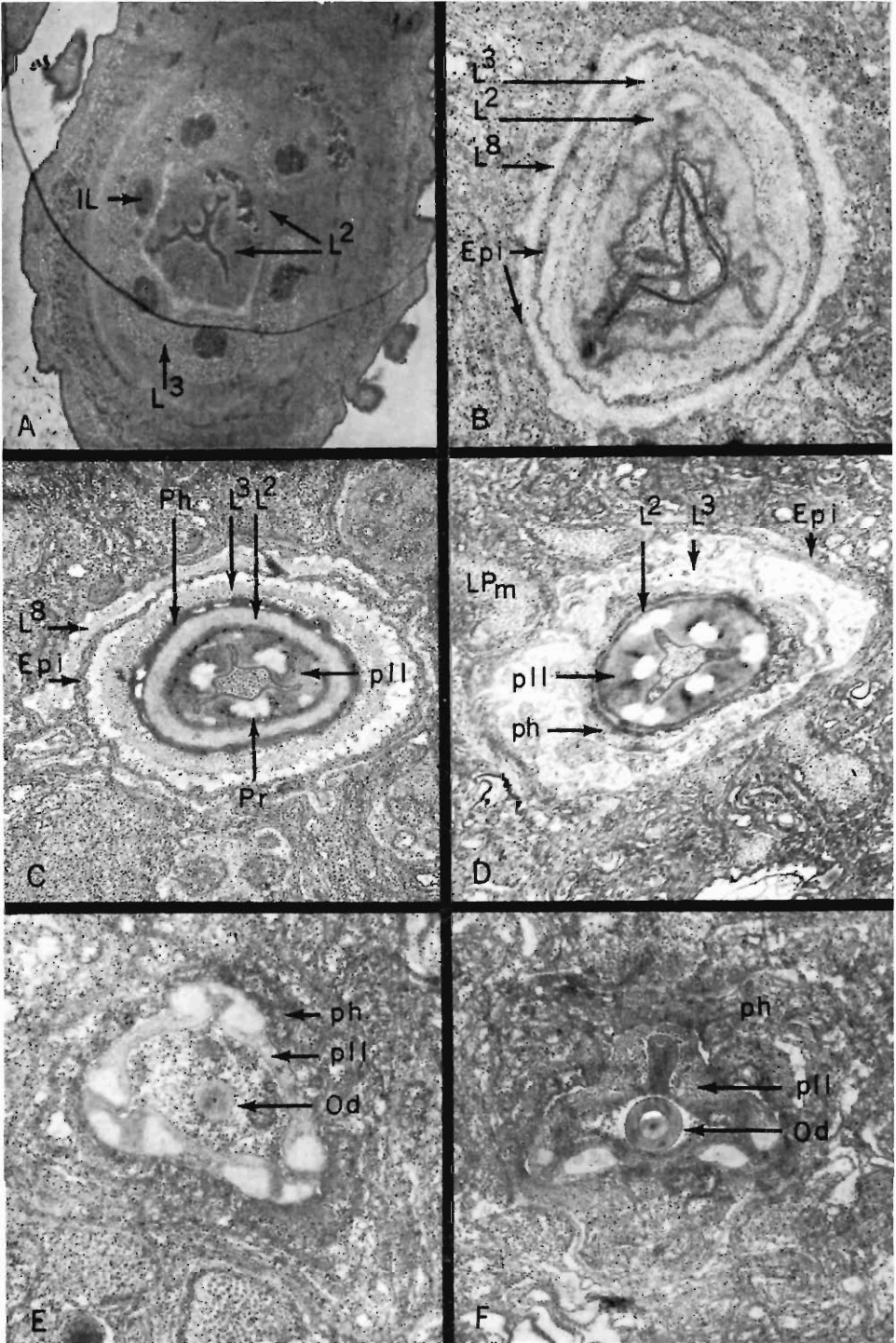
**Plate II.** Cross sections of stoma and pharynx including the odontostyle and its extension. A, Near oral aperture, 14,000 ×; B, Stoma, 23,100 ×; C, Transition from stoma to pharynx and first evidence of pharyngeal lumen lining (*pll*), 19,900 ×; D, Transition slightly posterior to D, 18,200 ×; E, Pharynx with first section of odontostyle (Od), 30,400 ×; F, Pharynx posterior to E, 20,000 ×.

**Plate III.** Cross sections of stoma and pharynx (cont'd). G, Odontostyle near attachment to dorsal wall, 19,400 ×; H, Fusion of odontostyle with dorsal wall and guide ring, 13,400 ×; I, Stylet extension posterior to H, 12,500 ×; J, Stylet extension posterior to I, 13,800 ×; K, Attachment of odontostyle protractor muscles to stylet extension, 22,000 ×; L, Transition of stylet extension with wall of pharyngeal lumen, 12,500 ×.

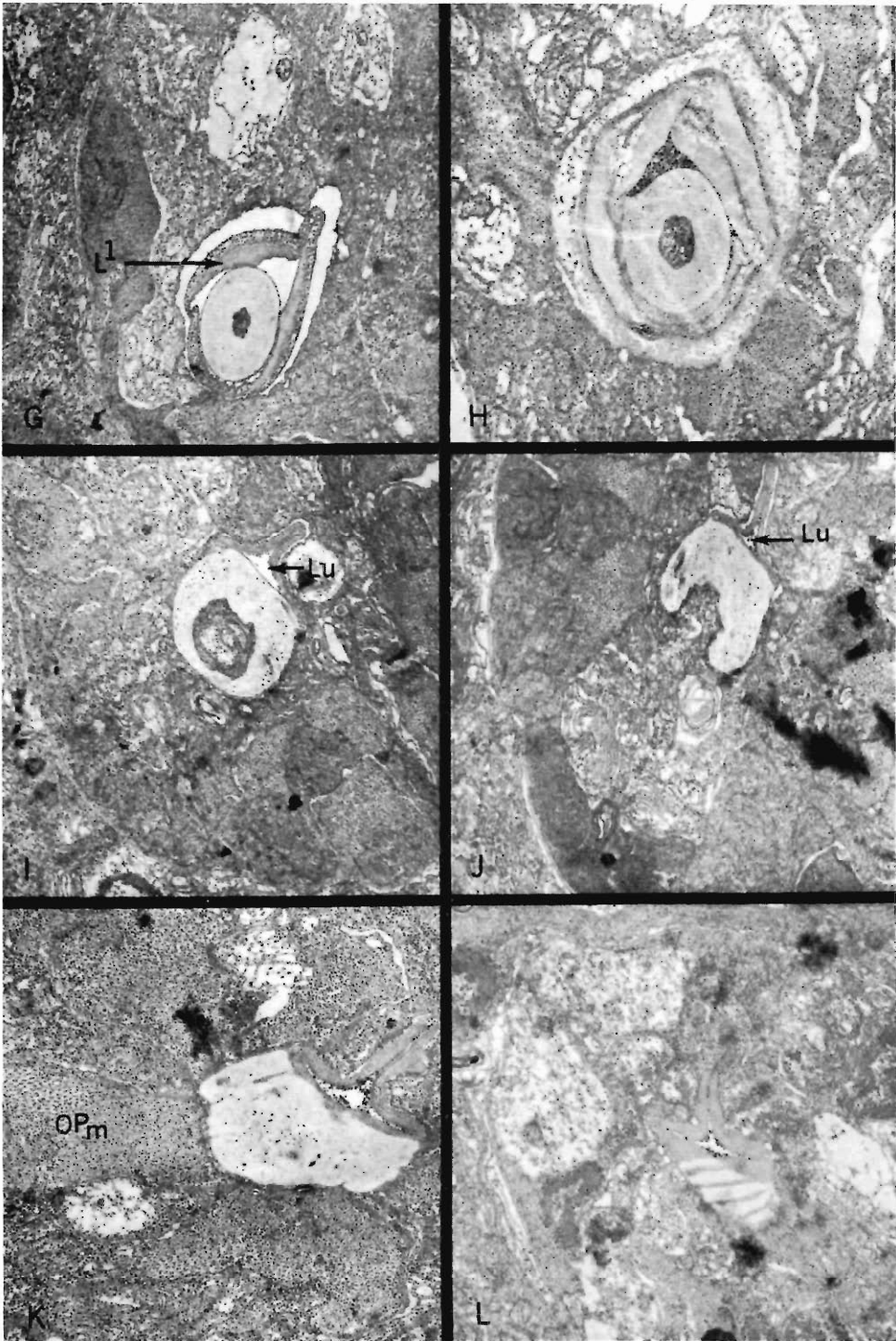
**Plate IV.** Esophageal muscles. A, Cross section at collar of (Ap) amphid, (PPm) perpendicular pharyngeal muscles, (LPm) longitudinal pharyngeal muscles, 19,800 ×; B, Cross section near base of stylet extension, (OPm) odontostyle protractor muscles, 23,100 ×; C, Cross section of esophagus in narrow region posterior to stylet, (ELm) esophageal lumen muscles, 20,200 ×.

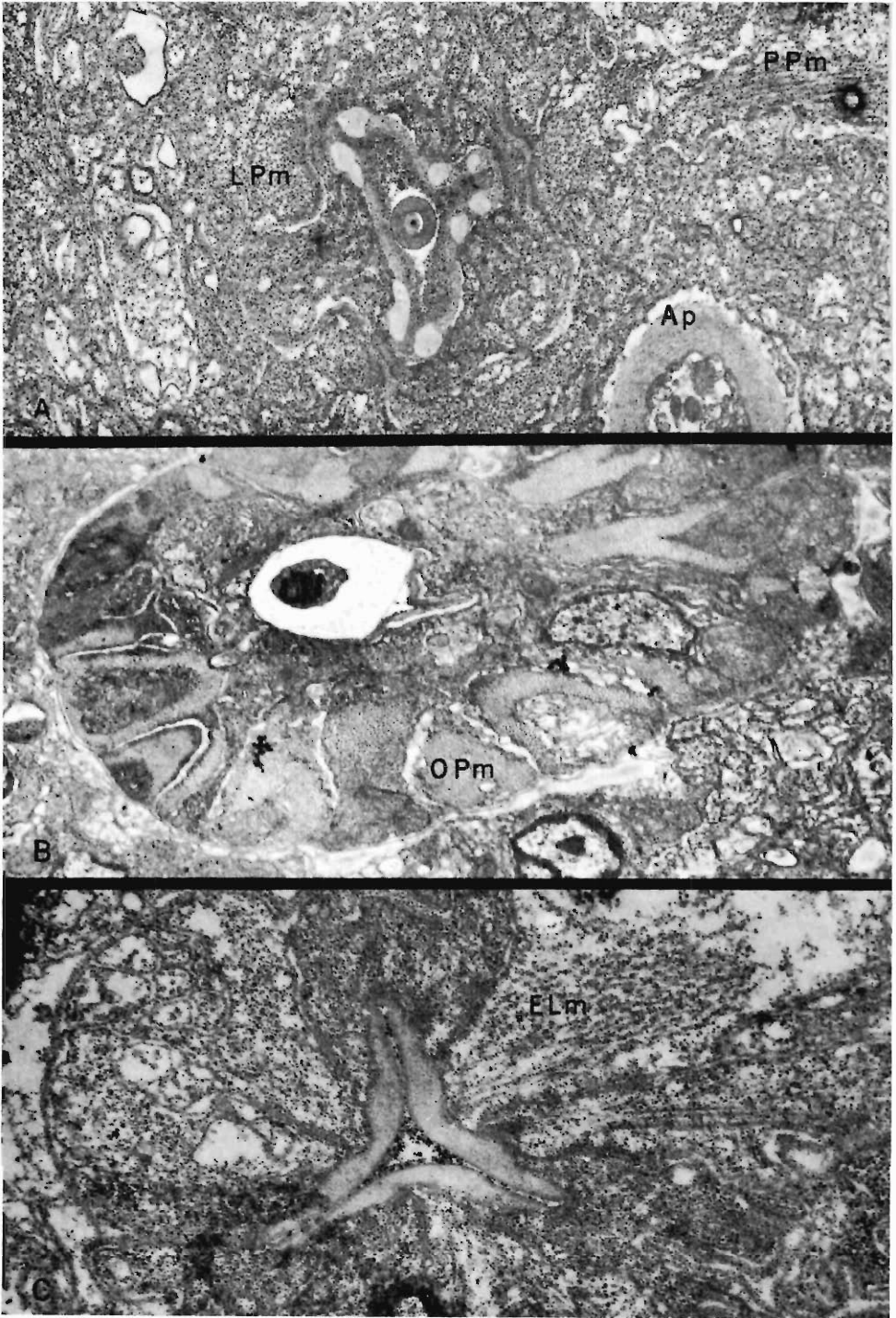
**Plate V.** A–B. Longitudinal sections of amphid (P = amphidial pouch; C = collar; → indicates basal plates of cilia). A, Near base of amphidial pouch, 9,500 ×; B, Junction (S) of cilia and nerve (N), 15,300 ×; C, Longitudinal section of esophageal gland orifice into lumen of esophagus cut across two rays of lumen (Lu), 30,400 ×; D, Longitudinal section between two rays of lumen (Lu), 31,200 ×.

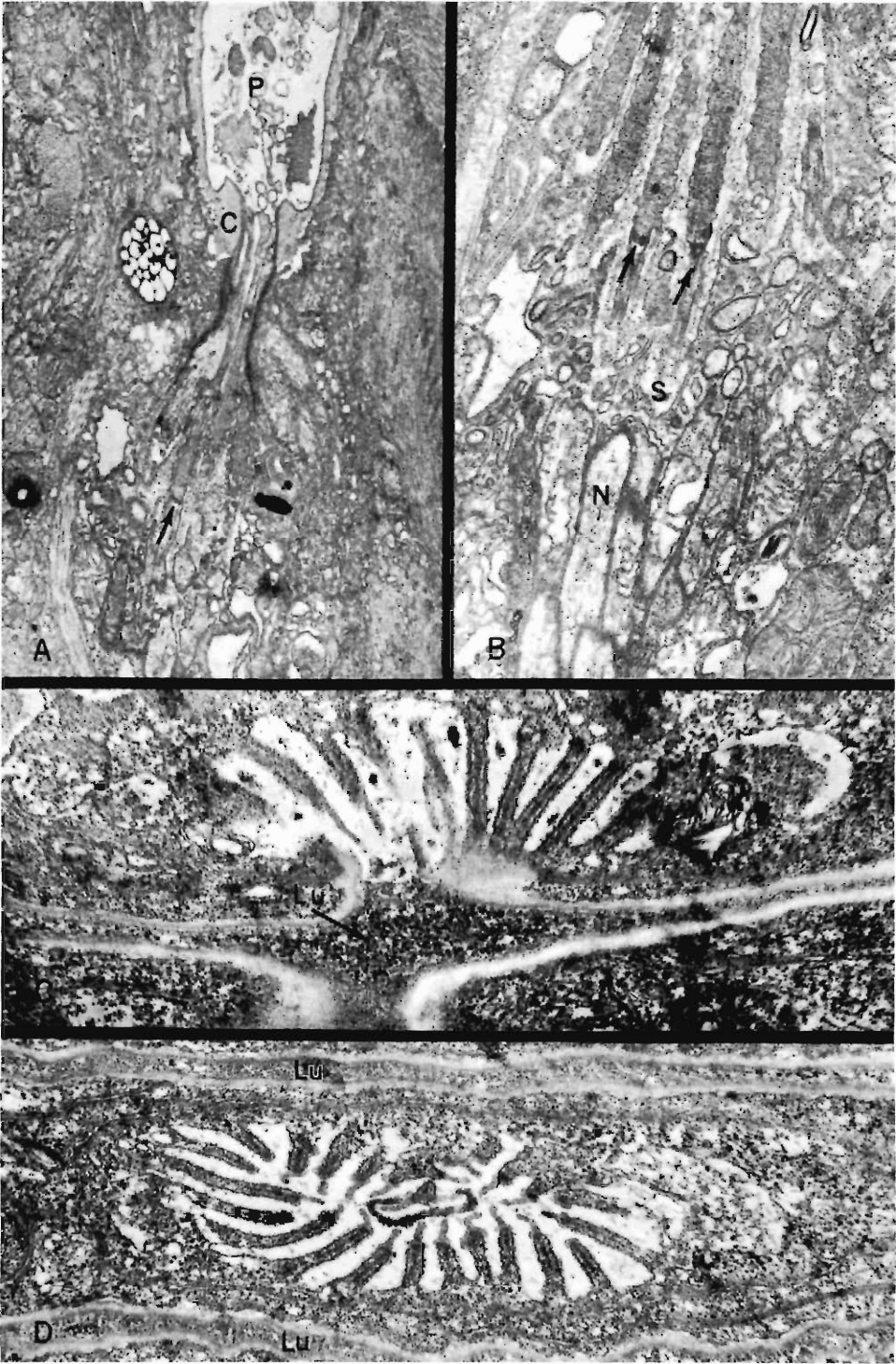












larges to about  $0.3 \mu$  across and shows two layers, a darker band (about  $0.05 \mu$  wide) as a concentric ring surrounding the solid inner core with a central black dot (Plate II, F). Later the inner core and the outer band expand and the central dot enlarges, becoming more complex and eventually developing an outer membrane surrounding tissue which includes endoplasmic reticulum and mitochondria.

The outer portion of the odontostyle reaches a maximum thickness of about  $0.1 \mu$  when the total diameter is  $0.5 \mu$ . Posteriad this outer portion becomes more narrow as the intermediate whitish layer expands to  $0.8-1.0 \mu$  in diameter. At the same time the central core enlarges with a membrane surrounding its tissue. When the outer band has disappeared (Plate III, G) the odontostyle consists of a circular, uniformly light, cuticular material surrounded by a thin, dark line with a maximum diameter about  $1.1 \mu$  and with an inner tissue core about  $0.3 \mu$  in diameter. The cuticular lining of the odontostyle is similar in texture to the odontostyle extension and continuous with it.

Posteriad the odontostyle begins its attachment to the dorsal wall and the "guide ring" appears (level 5, Fig. 1, A). The attachment begins first toward the left side of the dorsal wall (Plate III, H) and is completed in a short distance. Where the odontostyle joins the dorsal wall (Plate III, H) the *p11* folds anteriorly then again posteriorly, still showing the thickenings of the  $L^1$ . In the region of the odontostyle extension posterior to the "guide ring" the lumen of the esophagus (Lu) is small, narrow and immediately ventrad to the extension (Plate III, I, J).

In the odontostyle extension the inner core of tissue with membrane moves dorsad (Plate III, I) and finally merges (Plate III, J) with the nearby tissue (epidermal?) of the pharynx and the odontostyle extension thus forms a U-shape in cross-section with the open end to the dorsal side. By the time the core of tissue is no longer part of the extension, the latter, in cross-section, is flattened ventrally where it is part of the lumen wall and rounded dorsally. As the extension begins to diminish it becomes irregular in dorsal outline and mottled in texture (Plate III, K). Finally as the last of the whitish extension tissue disappears it becomes

banded diagonally across the dorsal wall (Plate III, L). The lumen, as it courses through the narrow esophagus to the glandular bulb, has the familiar osmiophilic membrane lining it with simple rounded ends on the three radiating arms of the lumen.

### Musculature

The somatic muscles possess two kinds of elements (myofilaments), fine and large, similar to those in *Xiphinema index*. These form identifiable areas in the cells where only large ones occur (near the midline of the cell), or where both occur (these areas surround the former). Finally the fine elements seem to be free of the larger elements in the outer margins of each cell. Neither striae nor any regular repeating markings were seen in longitudinal sections.

In the esophageal region there appear to be six or seven sets of muscles.

1. LABIAL MUSCLES (LM) (Fig. 1, A). For want of a better term these are referred to as labial because they are the first to appear at the anterior end. There seem to be four (two each subventral and sublateral), quite large, extending from the outer stomatal cuticular lining to the body wall and are almost as broad as long at their maximum development. They each occur laterad to their respective dorsal or ventral inner labial papillary innervation processes.

It is not certain just where they attach. These are not long muscles and seem to disappear before the stylet tip is found in the buccal cavity. It is possible these represent the anterior extremity of the pharyngeal muscles (PPM).

2. PHARYNGEAL MUSCLES (LPM) LONGITUDINAL. Four smaller muscles (LPM) appear at about the same level at which the labial muscles disappear. These four are closer in near the stomatal region with two each between the subventral and subdorsal inner labial papilla innervations (Plates II, D; IV, A; Fig. 1, A). The muscles are narrow and longitudinally parallel with but outside the *pharyngeal tissue*. The muscles seem to diverge and change location, one continuing on the left lateral side, the others as a single subdorsal and two subventral in location. The left lateral muscle persists longest but not quite to the end of the odontostyle proper.

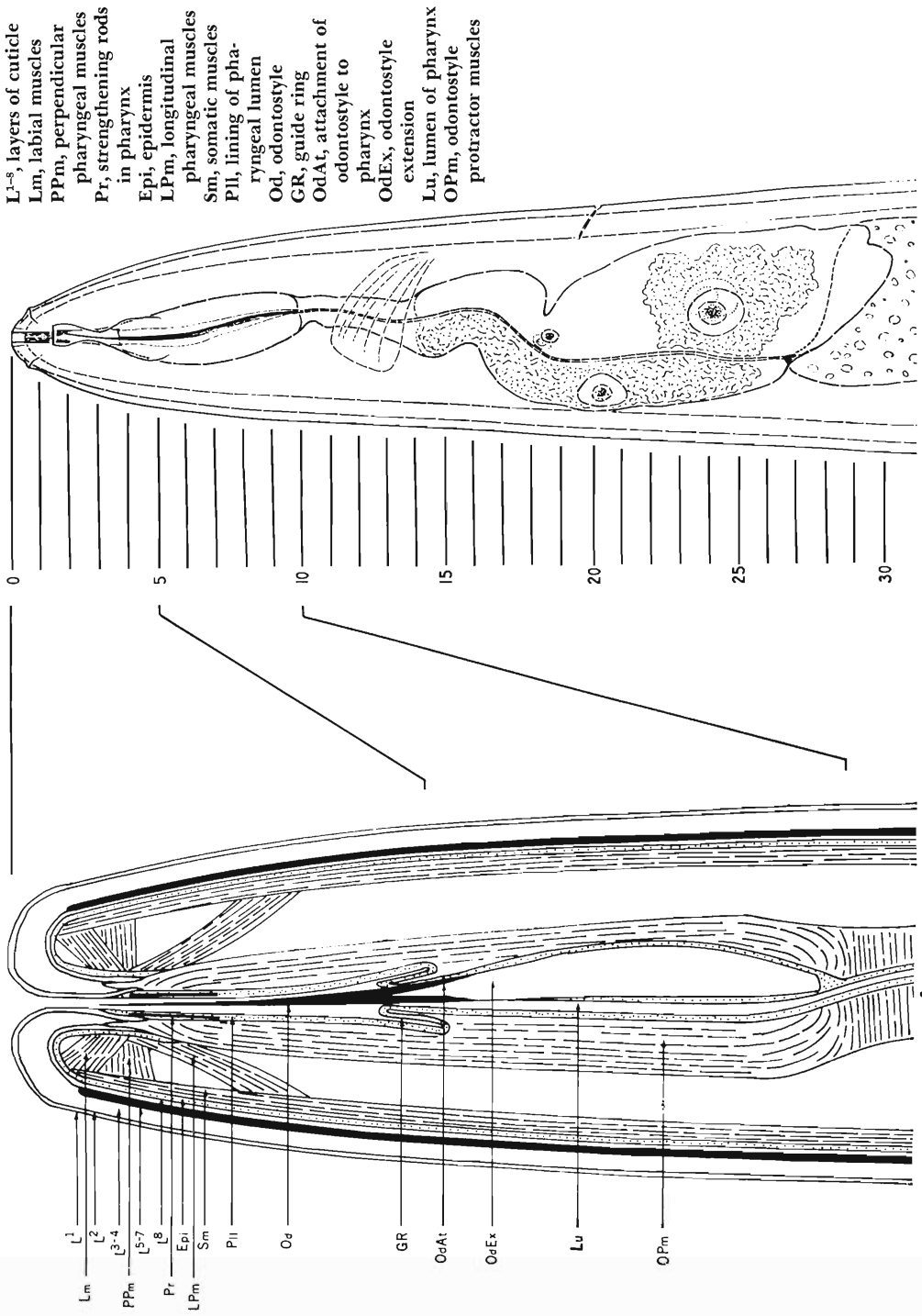


Figure 1. Schematic drawing at head region and esophagus of *T. altius*.

3. PHARYNGEAL MUSCLES (PPM) PERPENDICULAR TO LONGITUDINALS (LPM). A set of three muscles are present in the area near the anterior tip of the odontostyle when in retracted position. One is about midventral in position attaching to the ventral apex of the triradiate stoma (Plate IV, A; Fig. 1, A). The other two are subdorsal attaching to the two subdorsal apices of the stoma. The muscles extend distally and proceed between somatic muscles to the body wall, possibly to the innermost layer of the epidermis. Internally the muscles end at a perpendicular line darkened in color and adjacent to the basement membrane of the epidermal tissue surrounding the pharynx.

4. ODONTOSTYLE PROTRACTORS (OPM). These are strongly developed muscles which begin at a level anterior to the base of the odontostyle. Here, there seem to be three, one dorsal and two subventral, near the cuticular lining of the pharyngeal lumen. Nearer the end of the odontostyle these move to the outer edge of this pharyngeal tissue and appear as four or five large, irregularly shaped muscles. At the beginning of the odontostyle extension the muscles divide into elements (Plate IV, B), 15 or more in number almost lining the entire outer limit of the pharyngeal tissue. Some are dark staining while others are similar to the muscles anterior to this level. These muscles attach ventrally, two being slightly subventral leaving a corridor of epidermal tissue directly ventrad to that ray of the triradiate lumen. Each of these two becomes bifurcate as they approach the outer edge of the pharyngeal tissue. Where the core of the extension emerges, the muscles shift toward the center of the pharynx. Here mitochondria are large and numerous. Muscle attachments to the extension occur where the sclerotized wall begins to reduce in size and then almost cover the outer dorsal wall of that organ (Plate II, K).

5. SOMATIC MUSCULATURE (SM) (Fig. 1, A). These muscles begin at about the level where the amphidial pouches enter the body, appearing first dorsad and ventrad to the pouches. As the pouches move inward leaving a space laterad to them, the muscles appear between the pouches and the outer wall. Compared to *Xiphinema index*, the somatic musculature is not very high in cross-section.

6. ESOPHAGEAL LUMEN MUSCLES (ELM). Some sections of the narrow part of the esopha-

gus posterior to the odontostyle and also of the enlarged glandular region, show some muscles radiating outward from the angles of the lumen. These appear to attach at the angles of the lumen or on the swellings on the arms of the lumen (Plate IV, C). Some sections show evidence of attachments of muscles at the ends of the rays of the lumen.

### Sensory organs

1. INNER LABIAL PAPILLAE. The six inner papillae when first seen (Plate II, A) are arranged symmetrically about  $1\ \mu$  from the center of the oral aperture. The papillae show a scattered arrangement of microtubular elements. Posteriorly these arrange into four ciliary elements in each papilla (Plate II, B-C).

2. OUTER LABIAL AND CEPHALIC PAPILLAE. Six outer labial and four (two subdorsal and two subventral) cephalic papillae are present with two or three ciliary elements in each.

3. AMPHIDS. These are quite similar to those found in *X. index* and are formed by invaginations of the cuticle. The inner lining is a continuation of the osmiophilic layer. Adjacent to the osmiophilic layer and surrounding it was cuticular material apparently similar to  $L^3$  of the outer cuticle but also perhaps some remnants of  $L^2$ .

The sensillar (amphidial) pouch is evenly granular in texture near the aperture but immediately posteriad is provided with some elements. At first the pouch (P) is irregular in outline then assumes a shape roughly rectangular in outline. The shape then narrows into a circular form with a thick ring of cuticle (C) surrounding it (Plate V, A). In the longitudinal section the amphid has a goblet-shape with a concentration of ciliary elements in the narrow collar (Plate V, A). Posteriorly the amphid widens again as numerous ciliary elements with basal plates (arrows) connect (?) with nerve cells (N) (Plate V, B). Ciliary structures are similar to those in *X. index*, differing only in number of elements in individual sensory organs. The cuticular structures of the amphids are molted by larvae and persist as prominent remnants in the shed cuticles.

4. NERVE RING. First seen at the level near the posterior end of the odontostyle extension the nerve ring forms a U-shaped arc of nerves on the ventral side but not entirely encircling the esophagus. In the region of the nerve ring

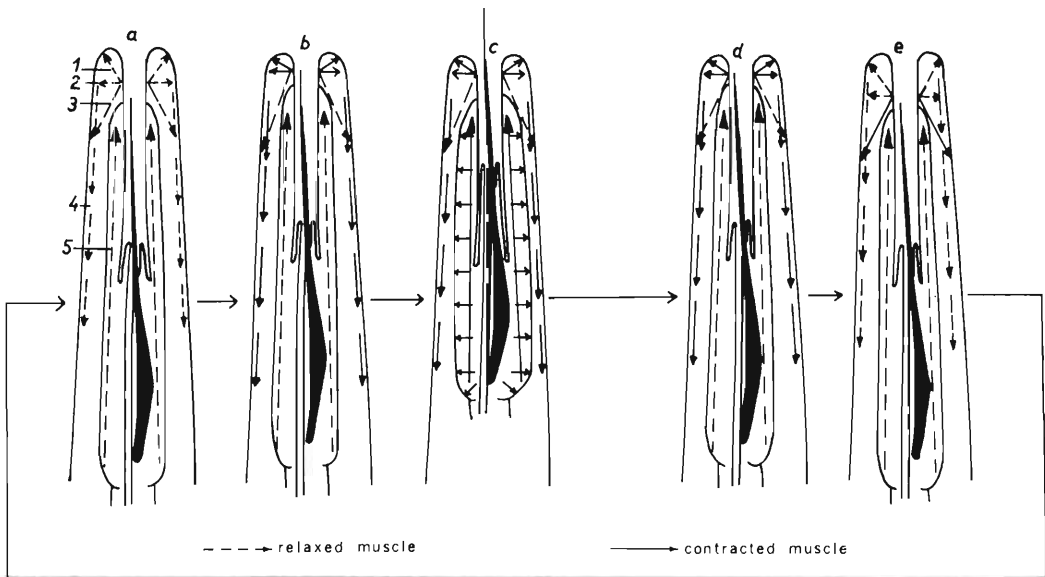


Figure 2. Schematic drawings illustrating feeding mechanism of *T. allius*. a-c, stylet protraction; d-e, stylet retraction; 1, labial muscles (Lm); 2, perpendicular pharyngeal muscles (PPm); 3, longitudinal pharyngeal muscles (LPm); 4, somatic muscles (Sm); 5, odontostyle protractor muscles (OPm).

and posteriorly around the esophagus are many nuclei with dense chromatin arranged around the periphery of and also scattered throughout the nucleus.

**ESOPHAGEAL GLANDS.** There are five nuclei visible in the esophagus of *T. allius* (Fig. 1, B). One is the large dorsal gland nucleus located in the dorsal sector of the esophagus about midway in the posterior bulb or glandular region. Two large subventral gland nuclei are more posterior in the glandular region. Slightly anterior to the dorsal gland nucleus are two more subventral nuclei, smaller in size and less noticeable.

The dorsal gland extends almost the full length of the dorsal sector but has its orifice into the lumen of the esophagus in the anterior end of the glandular region. In many specimens the narrow part of the esophagus between the stylet and the posterior bulb forms a distinct loop or S shape.

The two posterior subventral glands connect with the lumen of the esophagus in the posterior bulbar region not far from the nuclei themselves. However, the orifices of the two smaller anterior subventral glands were not certainly established.

The orifices of the dorsal and two larger subventral glands are provided with minute, complex structures which appear to serve as collection mechanisms. These measure about  $2.5 \mu$  long,  $1 \mu$  in width and height and are located next to the lumen and each is between two rays of the lumen. The structure seems to be a sinus with radiating and branching arms numbering 20 or more. It is completely surrounded by a triple-layered membrane. A sagittal section crossing both rays of the lumen shows a kidney- or oval-shaped outline (Plate V, C), becoming a common sinus in the center. Sagittal sections bisecting two rays shows three or four separated arms of the sinus or 5-10 or more fingerlike radiating arms becoming fan-shaped and converging to the orifice entering the esophageal lumen (Plate V, D).

### Discussion

**CUTICLE.** The report on *X. index* (Roggen et al., 1967) concluded that the structure of the cuticle of nematodes is too variable and too few species have been studied to permit generalizations. It was not possible to homologize the layers of the cuticle of *X. index* with those of other nematodes.

Here the same appears to be true and aside from the outer osmiophilic layer the cuticular components of *T. allius* show no certain homology with those of *X. index*. The second layer in *T. allius* may be the cortical layer but it does not appear to be at all similar to the second layer of *X. index*.

Layer four is interesting in that it varies so much in thickness and is very spongy in nature. The cuticle of most *Trichodorus* species is known to be very loose and tends to fold or wrinkle during movement. In some fixatives it swells, sometimes ballooning out and forward beyond the anterior end of the body. The sections seen here indicate most of this change takes place in L<sup>4</sup>. This suggests osmotic regulation may be active in this layer.

**FEEDING PROCESS.** The feeding process of *T. allius* is accomplished by protraction of the stylet to puncture plant cells and to ingest contents of the cells through the triradiate lumen of the pharynx and esophagus. The structure of the odontostyle being toothlike with a solid tip (Plate II, E) precludes the ingestion of food through the odontostyle even though it appears to be hollow in sections just prior to the tip. Anterior to the "guide ring" the stylet is located inside the triradiate lumen of the esophagus. Posteriorly the stylet lumen is ventrad to the extension. The muscle structure of the pharynx, as determined by their sections, suggests its function in the protraction and retraction of the stylet to be as follows (Fig. 2): (a) rest position with all muscles relaxed; (b) by contraction of the somatic muscles, labial muscles and perpendicular pharyngeal muscles, the stoma is everted with a slight protraction of the stylet; (c) contraction of the odontostyle protractor muscles results in protraction of the stylet and increased turgor pressure in the pharyngeal wall; (d) upon relaxation of the protractor muscles, turgor pressure effects stylet retraction; (e) relaxation of somatic, labial

and perpendicular pharyngeal muscles results in reinvasion of the stoma.

The functioning of the pharyngeal lumen appears to be by the classical mechanism found in most nematodes: opening of the lumen by muscular action, closing by turgor pressure.

Eversion of the stoma during feeding may possibly account for the fine folds in the inner stomatal lining or osmiophilic layer (Plate II, B). If eversion does take place the cuticular lining would be stretched and the layers closest to the lumen would be stretched most. A reasonable adaptation would be to provide folds in the cuticle which would disappear on stretching. The folds would have to be most pronounced in the innermost layers and less in the outer layers of the stoma wall. Furthermore, it would be expected that thinner membranes could not withstand as much stress as the thicker ones and should be more folded. The folded osmiophilic layer fits this concept.

### Summary

Observations are reported here on the ultrastructure of the cuticle, pharynx, musculature, sensory organs and esophagus of females of *Trichodorus allius*. The feeding process is also discussed.

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*Penarchigetes oklensis* gen. et sp. n. and *Biacetabulum carpiodi* sp. n. (Cestoidea: Caryophyllaeidae) from Catostomid Fish in North America

JOHN S. MACKIEWICZ

University of Oklahoma Biological Station, and the State University of New York at Albany

During June and July of 1963–1966 approximately 400 catostomid fish (Cypriniformes: Catostomidae), comprising five genera and nine species from southern and eastern Oklahoma, were examined for caryophyllid cestodes. From these collections three new species have been described (Calentine and Mackiewicz 1966; Mackiewicz 1968b); two other new species are described in this paper. Reference to the new species in the genus *Biacetabulum* Hunter 1927, described below was made in an earlier abstract (Mackiewicz 1964).

Cestodes were removed from freshly killed fish, fixed usually by shaking them vigorously in a vial of 5% formalin, stained in Semichon's carmine or Mayer's paracarmine and mounted in permount (Fisher) or Kleermount (Carolina Biological). Hematoxylin and eosin sections were prepared in the usual manner. Drawings were made with the aid of a microprojector; measurements are in millimeters unless otherwise indicated.

Comparative material included: *Biacetabulum meridianum* Hunter 1929, holotype USNM Helm. Coll. No. 51059 (serial longitudinal sections), and 11 of Hunter's original slides of this species (his numbers 687.2 to .6; all are serial cross or longitudinal sections) now in the author's collection; wholemounts or sections of *Archigetes sieboldi* Leuckart 1878, *A. brachyurus* Mrázek 1908, *A. cryptobothrius* Wisniewski 1928, *A. limnodrili* (Yamaguti 1934), and *A. iowensis* Calentine 1962; and a single wholemount of *Paracaryophyllaeus dubininae* Kulakovskaya 1961.

Since all available examples of *B. meridianum* consisted of serial sections, attempts were made in August 1966 to recollect this species from the type locality in North Carolina. Regrettably, only *B. infrequens* Hunter 1927 was found in two of 14 *Moxostoma* sp. (total length 225–425) and four of five *Erimyzon sucetta* (Lacépède) (total length 150–225) from the Eno River near Hillsboro, North Carolina

(Orange Co.) and University Creek, a tributary of the Eno.

Order Caryophyllidea Van Beneden  
(in Olsson 1893)

Family Caryophyllaeidae Leuckart, 1878

*Penarchigetes* gen. n.

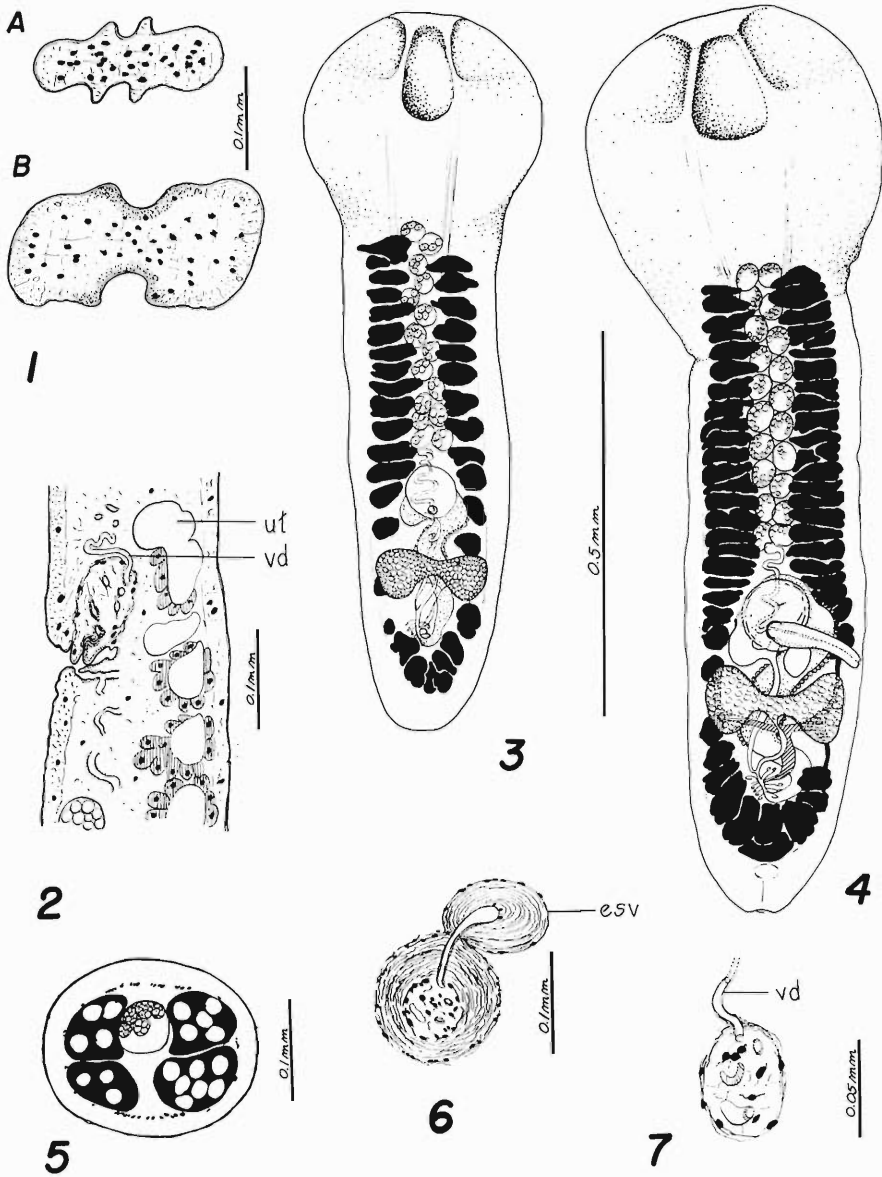
(Figs. 1–7)

GENERIC DIAGNOSIS: Caryophyllaeidae. Scolex with a pair of median bothria, two pairs of lateral loculi, and a small terminal disc. Cirrus joining uterovaginal canal. Ovary H or dumbbell shaped. Coils of uterus extending to anterior level of cirrus. Preovarian vitellaria lateral, sometimes continuous with the postovarian vitellaria. External seminal vesicle absent. Type species: *Penarchigetes oklensis*.

Remarks

In having a single gonopore, lateral vitellaria, small terminal disc, and uterus not extending anteriorly beyond the cirrus in non-gravid individuals, *Penarchigetes* most closely resembles the caryophyllaeid genus *Archigetes* Leuckart 1878. Unlike *Archigetes*, however, the new genus lacks all trace of an external seminal vesicle (Figs. 6–7) which in *Archigetes* is a prominent muscularized structure often approximating the size of the cirrus sac.

*Penarchigetes* superficially resembles *Paracaryophyllaeus* Kulakovskaya 1961; however, since the placement of the inner longitudinal muscles is not described nor the presence or absence of the external seminal vesicle established, or the number of gonopores mentioned, adequate comparisons can not be made between the two genera. These important features could not be discerned with certainty on the single specimen of *Paracaryophyllaeus* observed. Nevertheless, on the basis of an unspecialized scolex and anterior position of the uterus of *Paracaryophyllaeus* as well as a great difference in the distribution and hosts of the two genera—palearctic Cobitidae for *Paracary-*



Figures 1-7. *Penarchigetes oklensis* gen. et sp. n. from the spotted sucker, unless otherwise specified. 1A. Cross section through anterior part of bothria. 1B. Cross section through middle part of bothria illustrating the absence of acetabular musculature. 2. Midsagittal section through gonopore. 3. Immature individual. 4. Gravid individual with cirrus everted. 5. Cross section through midpart of body. 6. Frontal section through cirrus and external seminal vesicle of *Archigetes iowensis* Calentine, 1962. 7. Frontal section through cirrus and vas deferens of *P. oklensis* illustrating the absence of the external seminal vesicle. Abbreviations: esv, external seminal vesicle; ut, uterus; vd, vas deferens.

*ophyllaeus* and nearctic Catostomidae for *Penarchigetes*, the two genera are regarded as distinct from each other.

Clearly it is necessary to erect a new genus to receive this form for which the name *Pen* (pene, L. near) *archigetes* is proposed. Other genera in the family Caryophyllaeidae first described from North American Catostomidae (suckers) are: *Glaridacris* Cooper 1920, *Biacetabulum* Hunter 1927, *Hypocaryophyllaeus* Hunter 1927, *Hunterella* Mackiewicz and McCrae 1962, *Isoglaridacris* Mackiewicz 1965, and *Promonobothrium* Mackiewicz 1968.

### *Penarchigetes oklensis* sp. n.

**SPECIFIC DIAGNOSIS** (measurements based on 11 gravid and three immature specimens, five sectioned): Gravid individuals 1.2 (0.9–1.7) long and 0.2 (0.26–0.44) wide at gonopore. Immature individuals from fish 0.9 (0.8–1.0) long. Length 3.3–5.0 times combined length of neck and scolex. Scolex 0.34 (0.28–0.39) wide. Neck indistinct. Outer longitudinal muscles poorly developed. Inner longitudinal muscles consisting of small fascicles, chiefly in a dorsal and ventral group that form four large fascicles at base of scolex. Testes number 15 (11–21) in two irregular rows beginning 0.29 (0.25–0.41) from tip of scolex and extending to a short distance anterior of cirrus sac. Cirrus sac round; cirrus eversible. Preovarian vitellaria beginning 0.33 (0.26–0.41) from tip of scolex, usually continuous with postovarian vitellaria over dorsal part of ovary. Postovarian vitelline field longer than ovary. Ovary dumbbell-shaped, compact follicular wings 0.10 (0.06–0.15) long. Well developed seminal receptacle absent. Osmoregulatory canals diffuse, no specific number in midpart of body. Egg with smooth shell, 56 by 40  $\mu$  (one measured in utero); presence or absence of operculum could not be definitely established.

The specific name refers to the state of Oklahoma.

**DEFINITIVE HOST:** Spotted sucker, *Minytrema melanops* (Raf.) (Cypriniformes, Catostomidae). USA, Oklahoma, Cherokee Co., Northeastern Outing Club Lake, 22 km NE of Tahlequah on Okla. rt. 10. This lake is an ox-bow lake of the nearby Illinois River; it is approximately 10 acres in size, has a mean depth of 1 m and is periodically confluent with the river.

**HABITAT:** Intestinal swelling and fore part of intestine; weakly attached between mucosal folds.

**TYPE SPECIMENS:** Holotype No. 71262 (Coll. 14 July 1965) and Paratypes (4) No. 71263 of the USNM Helm. Collection; Paratypes (2) Nos. 1968.7.23.1 and 2 of the British Museum (Natural History) Helm. Collection.

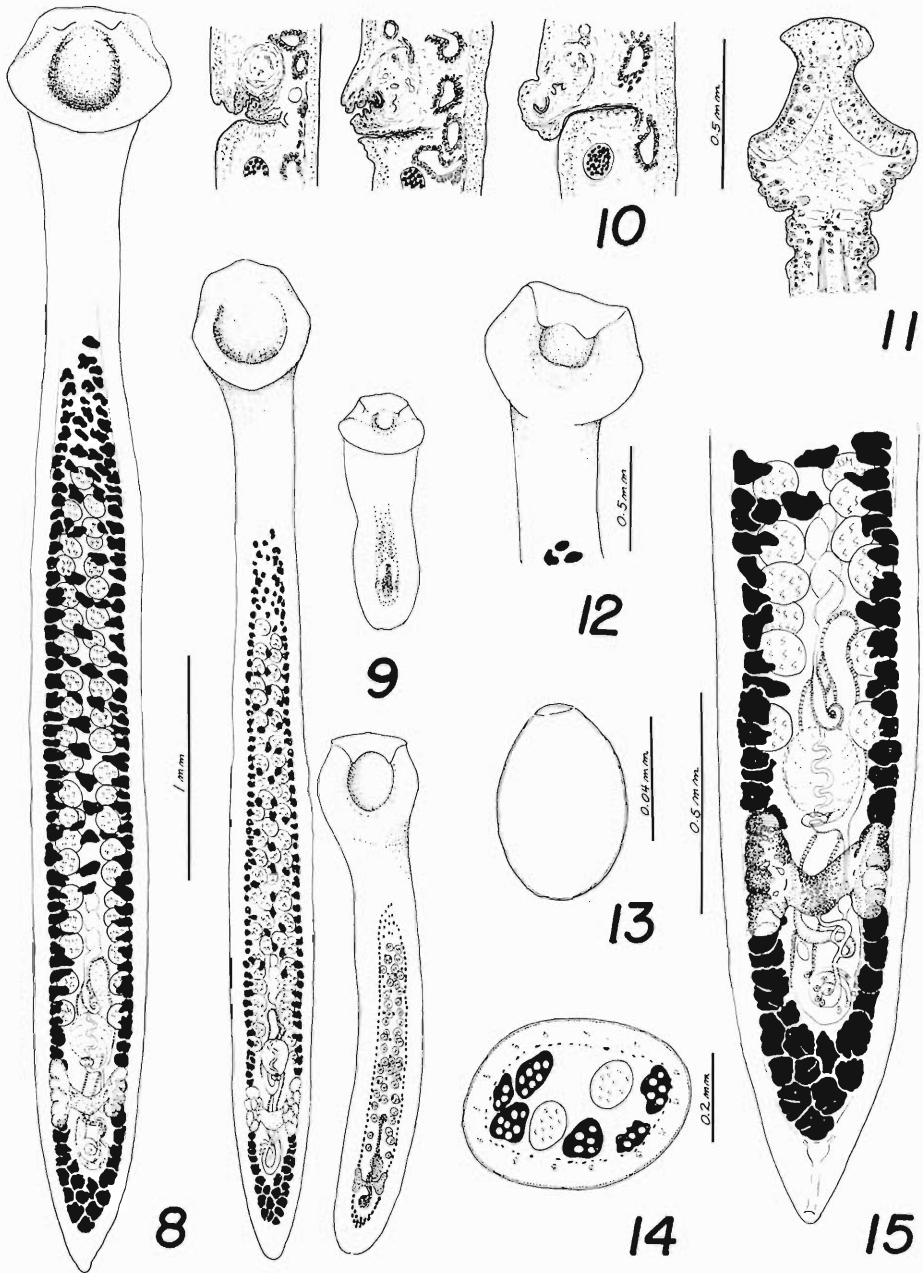
Supplementary material (six slides) consisting of immature worms and sections include: USNM Helm. Coll. No. 71263 and British Museum (Natural History) Helm. Coll. Nos. 1968.7.23.3 and 4.

### Remarks

This species, one of the smallest yet described, offers some interesting morphological characteristics. For example, the scolex is expanded and appears to be intermediate between the cuneiform-type of *Glaridacris catostomi* Cooper 1920 and the disc-type of *Glaridacris laruei* (Lamont 1921) or *Archigetes iowensis* (Figs. 3, 4). In all gravid specimens the testes contained only a few spermatogonial cells along the testis membrane; this condition made it difficult to count the testes, particularly the most anterior ones. In immature individuals, or ones with just a few ova in the uterus, the uterus does not extend anterior to the cirrus sac; in gravid individuals it does (Figs. 2–4). With some vitelline follicles dorsal to the ovary (Fig. 3) *Penarchigetes* appears to exhibit an intermediate condition between a continuous and discontinuous vitelline distribution. These vitelline cells have the vacuolated nucleus as recently described by Mackiewicz (1968a).

Of eight *M. melanops* ranging in total length 277 (143–331), only two were infected with 13 and 14 cestodes, respectively. Some cestodes were unquestionably overlooked because of their small size. *Biacetabulum banghami* Mackiewicz 1968 was the only other helminth associated with *P. oklensis*. Two *Ictiobus bubalus* (Raf.) and one *I. cyprinellus* (Val.) were not infected with caryophyllids but one of three *Cyprinus carpio* L. contained *Atractolytocoestus huronensis* Anthony 1958 and *Khawia iowensis* Calentine and Ulmer 1961.

*Penarchigetes oklensis* is the fourth caryophyllaeid to be reported from the monotypic spotted sucker, the others are: *Monobothrium ulmeri* Calentine and Mackiewicz 1966, *Bi-*



Figures 8–15. *Biacetabulum carpiodi* sp. n. from the river carpsucker. 8. Gravid individual. 9. Three immature individuals; note that the acetabular sucker is visible even on the smallest specimen (scale the same as in Fig. 8). 10. Midsagittal sections through the gonopore illustrating an expanding cirrus. 11. Midsagittal section through the scolex showing the basal membrane of the acetabular sucker. 12. Scolex with the acetabular sucker contracted. 13. Outline of an operculate egg. 14. Cross section through mid-part of body. 15. Detail of the posterior part of body, ventral view.

*acetabulum banghami* Mackiewicz 1968, *Pro-monobothrium minytrema* Mackiewicz 1968 (Mackiewicz 1968b.) Two others, still under study appear to be *Biacetabulum infrequens* Hunter 1927 and *Glaridacris* sp.

***Biacetabulum carpiodi* sp. n.**  
(Figs. 8–15)

**SPECIFIC DIAGNOSIS** (measurements based on 23 gravid and 11 immature individuals; 11 sectioned): Gravid individuals 5.5 (4–7.5) long, 0.47 (0.32–0.70) wide at gonopore. Immature cestodes from fish 3.1 (0.9–5) long. Length 3.9 to 7.2 times combined length of neck and scolex. Scolex with a pair of well-developed acetabular suckers; no accessory loculi. Neck distinct 0.31 (0.24–0.46) wide. Outer longitudinal muscles poorly developed. Inner longitudinal muscles consisting of large fascicles irregularly arranged about the cortical parenchyma. Testes begin 1.7 (1.2–2.2) from tip of scolex and extend posteriorly to the cirrus sac. Testes number 69 (59–84) and average 130 (90–210)  $\mu$  in diameter (5 from each of 5 worms). Vas deferens extends anteriorly between two rows of testes. Cirrus sac oval; male gonoduct joins uterovaginal canal very close to ventral surface forming an atrium with a large gonopore 50–100  $\mu$  in diameter. Preovarian vitellaria in median as well as lateral position, extending 1.2 (0.8–1.4) from tip of scolex to ovary or sometimes continuous with postovarian vitellaria. Postovarian vitelline field longer than ovary. Ovary shaped like letters H or M; compact follicular wings 0.3 (0.2–0.5) long. Seminal receptacle present. Fourteen osmoregulatory canals at mid-region of body. Eggs operculate, shell smooth, 53 (51–56)  $\mu$  long by 32 (28–34)  $\mu$  wide (5 measured in utero); undeveloped when shed.

The specific name refers to the host.

**DEFINITIVE HOSTS:** River carpsucker, *Carpiodes carpio* (Raf.) (Cypriniformes: Cotto-stomidae). IOWA: Des Moines River (coll. D. Williams). OKLAHOMA: Marshall Co., Lake Texoma; Payne Co., Lake Carl Blackwell (coll. R. D. Spall). TENNESSEE: Lake Co., Mississippi River at Powell's Ferry opposite Caruthersville, Missouri, New Madrid Co. TEXAS: Grayson Co., Red River below Denison Dam of Lake Texoma (Type locality). Quillback carpsucker,

*C. cyprinus* (LeSueur). TENNESSEE: Anderson Co., Oak Ridge, Clinch River at the Bullrun Steam Plant. *Carpiodes* sp. IOWA: Iowa River, South Fork Iowa River (coll. R. Calentine).

**HABITAT:** Usually in the intestinal swelling "stomach," occasionally in the anterior part of the intestine. Securely attached with scolex completely imbedded in the mucosa.

**TYPE SPECIMENS:** Holotype No. 71264 (coll. 10 July 1965) and Paratypes (5) No. 71265 of the USNM Helm. Collection; Paratypes (2) Nos. 1968.7.23.5 and 6 of the British Museum (Natural History) Helm. Collection.

Supplementary material (8 slides) consisting of immature worms and sections include: USNM Helm. Coll. No. 71265 and British Museum (Natural History) Helm. Coll. Nos. 1968.7.23.7 to 10.

**Remarks**

There are several morphological features which distinguish this species from all others in the genus. The pre- and postovarian vitellarian fields are sometimes continuous dorsally over the ovary (Fig. 15) similar to that described for *Penarchigetes*. The testes extend in two rows on each side of the vas deferens (Figs. 8, 15). And lastly, the cirrus intercepts the uterovaginal canal very close to the ventral surface and with it forms a common atrium with a large, single gonopore (Fig. 10). Because of this large gonopore, an expanded cirrus and female gonopore may give the appearance of two gonopores. By its small size and reduced number of testes, *B. carpiodi* most closely resembles *B. meridianum* and *B. macrocephalum* McCrae 1962 (Table I). However, both of these species have few postovarian vitellaria and those present do not reach the ovary as in *B. carpiodi*. In addition to the gross morphological features mentioned above, *B. carpiodi* also lacks the well-developed outer longitudinal muscles of *B. meridianum*.

Incidence and worm burden varied with host and locality. Five of 70 river carpsuckers (total length 232–475) from Lake Texoma, Oklahoma, contained 1–3 worms per fish whereas four of 16 fish (total length 232–414) from the Red River, just below Denison Dam of Lake Texoma contained 3–37 parasites. From the Clinch River, Oak Ridge, Tennessee, five of 38 quillback carpsuckers (total length 212–375) contained 1–2 parasites. In all cases,

which represented 91 cestodes, there were no concurrent infections with other caryophyllids although *Spartoides wardi* Hunter 1929 and another species of undetermined generic status (from Tenn.) occurred in the same host. Furthermore, this species appeared to exhibit a marked host-specificity for *Carpiodes* sp. not being found in approximately 275 other catostomid fish representing six genera and 11 species.

The only other *Biacetabulum* reported from carpsuckers is that of *B. meridianum* from 201 *C. carpio* from Lake Texoma (Timmons, 1951; Self and Timmons, 1955); most infected fish were collected in May. Specimens of these *Biacetabulum* were lost in the mail before they could be re-examined. Except for having testes numbering 45–53 (N = 6). Self and Timmons reported that all other characters closely complied with Hunter's description of *B. meridianum*. In the absence of the original slides it is difficult to appraise the validity of this record; however, all *Biacetabulum* from Lake Texoma *Carpiodes* were first called *B. meridianum* until critical study indicated that a new species was involved.

Self and Timmons (1955) also reported *G. confusa* from *C. carpio*; specimens that could be re-examined were redetermined as *Spartoides wardi* Hunter 1929.

### Discussion

Both of these species offer variations in certain generic characters. For example, the position of the uterus with respect to the cirrus sac has long been an important generic criterion in the order Caryophyllidea. In some genera, e.g. *Biacetabulum*, the uterus clearly twists and forms loops anterior to the cirrus sac with the uterine primordia assuming this position before gametogenesis commences (Fig. 9). In others, such as *Penarchigetes*, *Archigetes*, or *Glaridacris* (especially *G. laruei*), in which the uterus normally reaches the anterior level of the cirrus sac, its position may vary with the state of maturity of the cestode. That ova can extend the uterus beyond its normal position has recently been forcefully illustrated by Kennedy (1965) in the genus *Archigetes*. While this uterus-cirrus sac relationship may be a continuously variable character, whatever its nature it should be judged in immature and

maturing individuals before ova have greatly distended the uterus.

Whether or not the pre- and postovarian vitelline fields are continuous may be also an important systematic character although at what level is not clear at present. In some genera, for example in *Archigetes* (except for *A. sieboldi*), *Atractolytocestus* Anthony 1958 (? = *Markevitschia* Kulakovskaya 1965), *Breviscolex* Kulakovskaya 1962, *Capingens* Hunter 1927, or *Wenyonia* Woodland 1923, all of the vitellaria clearly appear as one continuous unit. In others, such as *Caryophyllaeus* Gmelin 1790, *Glaridacris*, and *Isoglaridacris*, to mention a few, the vitellaria are definitely confined to separate pre- and postovarian regions. *Penarchigetes* and *B. carpiodi* as well as *Caryophyllaeides fennica* (Schneider 1902) and *Khawia baltica* Szidat 1942 appear to exhibit an intermediate condition. In the absence of a distinct continuity it would appear that this intermediate condition is a variation of the discontinuous distribution. More precise details of the vitelline duct system might aid in the interpretation of this character.

The genus *Biacetabulum* has been subject to various interpretations since described by Hunter (1927). Szidat (1937) was the first to consider some palearctic *Archigetes* as congeneric with *Biacetabulum*. Calentine (1965), reviewing the complex *Archigetes-Biacetabulum* question, clearly demonstrated the validity of the two genera on the basis of life-history and morphological characters. A further analysis of this same problem by Kennedy (1965) resulted in the synonymy of all palearctic *Biacetabulum* under the genus *Archigetes*. Except for the Australian species *B. tandani* Johnston and Muirhead 1950, incompletely described from a single specimen and therefore of questionable status (it has been omitted in Table I), all other species (six) of *Biacetabulum* are from the nearctic.

All of these species, including *B. carpiodi*, can be arranged into distinct groups on the basis of scolex morphology and preovarian vitellaria distribution (Table I). There can be little doubt that Group I constitutes a good species group, although some of the species, *B. infrequens* for example, may represent a species complex. The relationship of Groups II and III to each other and to Group I is

Table 1. Comparison of the species of *Biacetabulum* Hunter, 1927.<sup>1</sup>

Species	Acetabular suckers	Preovarian vitellaria distribution	Posterior limit of:		Testes no.	Length (mm)	Most common host
			Testes	Preovarian vitellaria			
Group I							
<i>B. infrequens</i> Hunter, 1927	Yes	Median and lateral	Uterus	Cirrus	420-440	16-22	<i>Moxostoma anisurum</i> (Raf.)
<i>B. meridianum</i> Hunter, 1929	Yes	Median and lateral	Vas deferens	External seminal vesicle	64-95	5-15	<i>Erimyzon sucetta</i> (Lacépède)
<i>B. giganteum</i> Hunter, 1929	Yes	Median and lateral	Vas deferens	External seminal vesicle	165-215	7-16	<i>Ictiobus bubalus</i> (Raf.)
<i>B. macrocephalum</i> McCrae, 1962	Yes	Median and lateral	Uterus	Cirrus	75-95	4-6.5	<i>Catostomus commersoni</i> (Lacépède)
<i>B. carpiodi</i> Mackiewicz (this paper)	Yes	Median and lateral	Cirrus	Ovary or continuous with POV	59-84	4-7.5	<i>Carpiodes carpio</i> (Raf.)
Group II							
<i>B. biloculoides</i> Mackiewicz and McCrae, 1965	No	Median and lateral	Uterus	Cirrus	50-90	3.7-11	<i>C. commersoni</i> (Lacépède)
Group III							
<i>B. banghami</i> Mackiewicz, 1968	No	Lateral mostly, continuous anteriorly	Uterus	Ovary	63-91	3.5-13	<i>Minytrema melanops</i> (Raf.)

<sup>1</sup> Data for described species from original descriptions, illustrations, or type specimens.

much more complex, perhaps involving the introduction of subgenera or possibly of separate genera, depending upon the systematic value given the scolex and the vitelline distribution.

Similar problems of generic definition are illustrated by the palearctic and nearctic *Caryophyllaeus*, *Monobothrium*, and *Khawia* in which different scolex types and/or vitellaria distribution patterns occur within the same genus. Even in *Archigetes* there are median vitellaria in *A. sieboldi* but not in the other species; while in *Glaridacris* the vitellaria are annularly arranged in *G. catostomi* but in lateral rows in the other species. These few examples illustrate the need for a critical reappraisal of generic criteria in an order in which 60% of the approximately 35 genera are monotypic. In recent collections from catostomid fish in Tennessee, Alabama, and North Carolina, numerous new species were found that had different generic characters in various combinations with each other, thus posing serious problems of generic redefinition. In-

deed, it is becoming increasingly clear from these southern species as well as others from different parts of North America, and supported by examples from other parts of the world, that reliable generic criteria in the Caryophyllidea have not been, and can not be, established until there is a more comprehensive view of morphological types and variation in the order.

#### Acknowledgments

For assistance in collecting hosts, the cooperation of the Oklahoma Department of Game and Fish is gratefully acknowledged. Appreciation is extended to Dr. J. Teague Self and Dr. C. Riggs, Director of the University of Oklahoma Biological Station, for their generous cooperation; to Dr. O. Kulakovskaya of the Lvov Museum of Scientific Nature Study, Ukrainian Academy of Sciences, for loan of material; and to Drs. R. Calentine and C. Kennedy for examining preparations of *Penarchigetes*.

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## The Chromosomes of *Hunterella nodulosa* Mackiewicz and McCrae, 1962 (Cestoidea: Caryophyllidea)

JOHN S. MACKIEWICZ<sup>1</sup> AND ARTHUR W. JONES

Department of Zoology and Entomology, University of Tennessee, Knoxville, Tennessee

Cytological studies on tapeworms have been almost entirely confined to Cyclophyllidean cestodes (for reviews see Jones 1945, Walton 1959). Exceptions have been the studies of four species of the Pseudophyllidea by Wolcott (1959), and Wikgren and Gustafsson (1965), and of *Archigetes* sp. (order Caryophyllidea) by Motomura (1929). Where comparison between species in the same genus was made, especially of *Hymenolepis* by Jones (1945) and *Diphyllobothrium* by Wikgren and Gustafsson (1965), the chromosome numbers and/or karyotypes showed little variation and therefore could be used little as an aid in the taxonomic analyses of the polyzoic cestodes studied.

From the single investigation of Motomura (1929), who was primarily interested in the embryology rather than the cytology of *Archi-*

*getes*, it is impossible to judge the worth of similar cytotoxic studies in the monozoic tapeworms of the order Caryophyllidea. One purpose of the present study, one of a projected series, is to learn if cytological information on the Caryophyllidea will aid in the practical problems of taxonomy at the family, genus and species levels; another is to learn if such studies can help in appraising the relationship of these unusual tapeworms to the more common strobilate groups, from which they differ greatly in morphology and general biology.

*Hunterella nodulosa* Mackiewicz and McCrae, 1962 (Cestoidea: Caryophyllidea) of the family Caryophyllaeidae was chosen for detailed cytological studies for two reasons. First, in a preliminary survey of 17 species of caryophyllids studied by the squashing technique, squashes of *H. nodulosa* had large numbers of good metaphase plates with relatively large,

<sup>1</sup> On academic leave from State University of New York at Albany, New York.



well stained chromosomes in the testes and vitellaria. Second, *H. nodulosa* is easily identified, widely distributed in North America, and relatively easy to obtain, occurring as a common intestinal parasite of the white sucker, *Catostomus commersoni* (Lacépède). It is among the best known of North American Caryophyllidea with recent studies on systematics, morphology, and distribution (Mackiewicz, 1960; McCrae, 1960; Mackiewicz and McCrae, 1962) periodicity (Calentine and Fredrickson, 1965) and larval development (Calentine, 1965).

### Materials and Methods

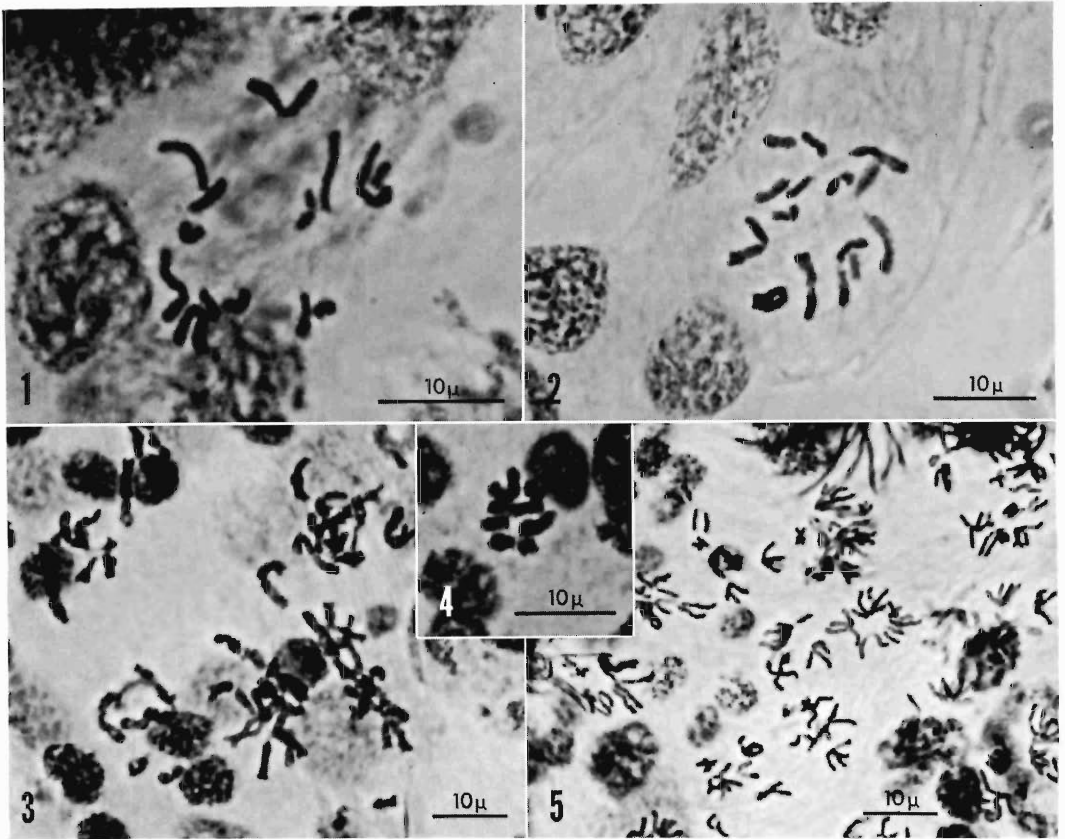
Parasites were obtained on 27 November 1967 from the white sucker, *Catostomus commersoni* (Lacépède) (Cypriniformes; Cato-stomidae) from Jacks Creek, a tributary of the Smith River (Roanoke drainage), Patrick Co., Virginia and from the same host from the Little River, Walland, Blount Co., Tennessee on 29 March 1968. Living cestodes were dropped into a vial of Carnoy's fixative (6:1:1, absolute ethyl alcohol, chloroform, glacial acetic acid) that was placed in ice water and later stored in a refrigerator until squashes were prepared in May, 1968. No differences in staining quality were detected in material stored for different periods. Carnoy fixation gave good spreads and preservation of the chromosomes, unlike the results of Wikgren (1964) who worked with plerocercoids of *D. latum*.

Squashes were prepared using the leucobasic fuchsin (Feulgen) stain prepared according to Darlington and La Cour (1960). Cestodes were removed from the vial of fixative, hydrated with successive changes (2 hrs each) of 95%, 50% (ethyl alcohol), and tap water. The whole worm was hydrolyzed in 1 N HCl at 55 C to 60 C for 9–11 min (10 min was later found to be a good average time) and removed to the Feulgen stain for one hr. Usually the testes and ovary began to show a staining reaction within the first minute. When these organs became deeply stained, (usually within one-half hour), the whole worm was placed in 45% acetic acid for 5–10 min in order to soften and swell the tissues. A small part of the body, approximately one-half mm long, containing testes and vitellaria was placed on a slide and teased apart in a drop of 45%

acetic acid. A large proportion of the testes and vitelline follicles were teased out and, along with the remaining tissues, dissected into small fragments; only large pieces of the integument were discarded. A 22 mm square coverslip (No. 1 or 0) was applied and, after excess acetic acid had evaporated, the preparation was squashed by placing the slide within one fold of paper towel and applying with the thumb the full weight of the body. The presence of Newton rings under the coverslip indicated a satisfactory squash. The preparation was made permanent by using the dry ice method of Conger and Fairchild (1953), dehydrating in 95% or 100% ethyl alcohol for 1 min and mounting with diaphane.

Dividing cells were most common in the testes but excellent mitotic figures also occurred in the vitelline follicles, particularly in cestodes in which spermatogenesis was just beginning. Mitoses were rare in the parenchymal cells.

Eighteen squash preparations representing a large number of testes and vitelline follicles from eight cestodes were studied. All illustrations and most of the observations were made on squashes from immature worms collected in Virginia; the Tennessee collections consisted of mostly gravid worms and were used to supplement and verify the other observations. Camera lucida drawings of 16 metaphase plates studied under oil immersion (900 and 1,800 powers) were prepared at an enlargement of 2,600 $\times$ . From 11 of these drawings of metaphase plates (three from testes and eight from vitelline cells of two worms) an idiogram was constructed by measuring the chromosomes to the nearest 0.5 mm and expressing each chromosome or arm as a percent of the total chromosome length in the complement. In this way there was an adjustment for the differences in chromosomes due to squashing or other factors. In those cases where either the centromere position could not be determined or the chromosome was folded, no measurements were attempted; the sample size for each chromosome measured therefore varied. The position of the centromere of the small rods could not be accurately determined but from indirect evidence, as discussed under "observations," these chromosomes appeared to be acrocentric and are so indicated on the idiogram. Karyotype analysis was based on the above eleven plates plus five



Figures 1-5. Chromosomes of *Hunterella nodulosa*. Feulgen stained squashes. 1, 2. Metaphase plates from vitelline cells. 3. Diakinesis. Note that more than one chiasma is readily visible on most bivalents. 4. Metaphase I, showing seven bivalents. 5. Metaphase II, Anaphase II, both dyads and monads are visible.

others, only parts of which were suitable for purposes of an idiogram, that included two from testes and three from vitelline cells of three worms (two from Virginia, one from Tennessee).

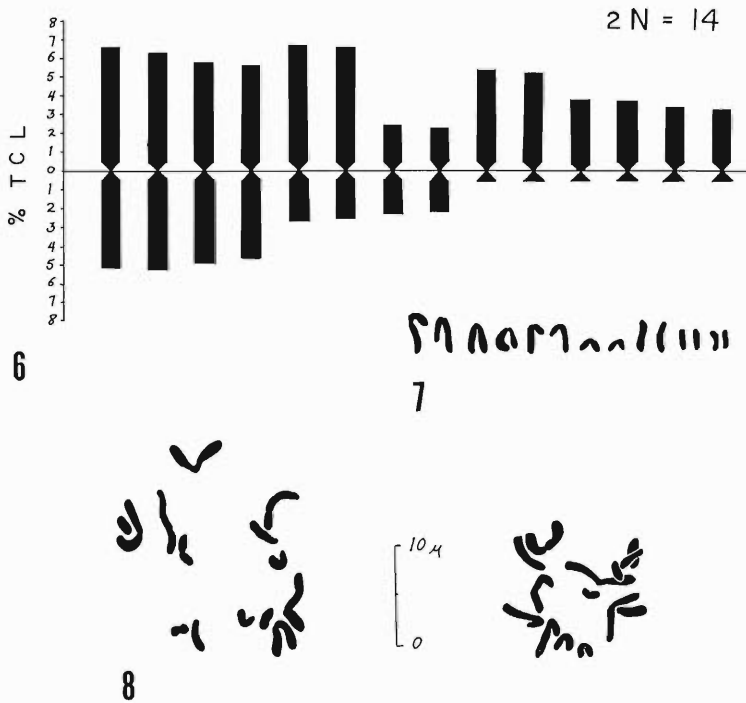
The photographs were made with a Zeiss Photomicroscope using phase contrast optics, including an apochromatic oil immersion lens and a green filter.

#### Observations

*Hunterella nodulosa* has 14 chromosomes, diploid (Figs. 1, 2, 7, 8). These include three pairs of metacentrics, ("V's", two long and one short pair), three pairs of short acrocentrics ("rods"), and one pair of long submetacentrics

("J's") (Figs. 6, 7). At mitotic metaphase in the spermatogonia and vitelline cells the longest chromosomes are 6-8  $\mu$ , the shortest 2.5-3  $\mu$ , long. As shown by the idiogram (Fig. 6) there are four large "V's," two small "V's," two large "J's," and three pairs of small rods. The largest chromosome represents approximately 12% of the total chromosome complement (TCL). No satellites or fragments were observed. The location of the centromere of the mitotic metaphase chromosomes could be determined for all but the six rods, but from observations of metaphase I of spermatogenesis it appears that the centromeres of these rods are terminal or nearly so.

Meiosis was studied only in spermatogenesis



Figures 6-8. Chromosomes of *Hunterella nodulosa* (cont'd). 6. Idiogram; TCL, total chromosome length. 7. Karyotype from a single vitelline cell nucleus. 8. Two metaphase plates from vitelline cells.

since no meiotic activity was observed in intrauterine eggs. Spermatogenesis proceeds as usual with normal sperm being formed after two meiotic divisions. Two meiotic or maturation divisions have also been reported for *Trianophorus lucii* (Müll., 1776) (Pseudophyllidea) by Rybicka (1962). At diakinesis structure of the chiasmata was readily observable in most bivalents (Fig. 3). Chiasma frequency was not worked out but it appears to be 5.0 or 6.0 per bivalent, at least in the case of the longest chromosomes. No attempt was made to ascertain the number of primary spermatocytes resulting from a single spermatogonium, details best studied in sections. A single sperm could easily be seen adjacent to or wrapped around the ovum in intrauterine eggs.

**Discussion**

Motomura (1929) was the first to report the chromosome number of a caryophyllidean; however, there appears to be some question regarding the identity of the cestode studied.

Motomura designated it as *Archigetes appendiculatus* Ratzel. Unfortunately there have been two cestodes with this same binomial, *A. appendiculatus* Ratzel 1868 and *A. appendiculatus* Mrázek, 1897. It has long been recognized, however, that while Ratzel's species was in fact the larval stage of *Caryophyllaeus laticeps* Leuckart, *A. appendiculatus* Mrázek represented a distinct species (Nybelin, 1922). Since Mrázek's name has been in more common usage with the binomial it is difficult to understand why Motomura did not use it. The complex case involving these two binomials has been thoroughly discussed by Wisniewski (1930). Kennedy (1965b) in his recent revision of *Archigetes* does not list Ratzel's species (since it refers to *Caryophyllaeus*); he places *A. appendiculatus* Mrázek in synonymy under *A. sieboldi* Leuckart, 1878. A further complicating factor has been the discovery of another species of *Archigetes*, *A. limnodridi* (Yamaguti, 1934), in *Limnodrilus* sp. and a cabbitid fish in Japan (Yamaguti, 1934; Ken-

nedy, 1965a). Motomura was most certainly dealing with *Archigetes*, because gravid specimens came from aquatic oligochetes (*Limnodrilus* and *Tubifex*) yet he did not further describe or illustrate his species. It is therefore impossible to know whether he had *A. sieboldi* (which is not reported from Japan), *A. limnodrili* (originally described from that country), or another, perhaps undescribed, species of *Archigetes*.

Adequate comparisons between the present work and that of Motomura (1929) are not possible since cytological observations were but a minor part of his study. Motomura observed a diploid number of 18 and that the chromosomes were of unequal size. From his Figure 2 of the first maturation division in the ovum, we measure the largest chromosome to be 3.3  $\mu$  long. *H. nodulosa*, on the other hand, has a diploid number of 14 and chromosomes of unequal and larger size (Fig. 8). According to Walton (1959) the only other cestode with 14 diploid chromosomes is *Liga brasiliensis* Ransom, 1909 [= *Liga punctata* (Weinl. 1856) Weinl. 1857 of Yamaguti (1959) and *L. brasiliensis* Parona, 1901 of Wardle and McLeod (1952)] a cyclophyllidean of birds (Jones, 1945) but distantly related to caryophyllideans.

More closely related are the Pseudophyllidea, an order under which caryophyllideans have often been placed as a family (Hunter, 1930; Joyeux and Baer, 1961). Working with colchicine-treated plerocerooids Wikgren and Gustafsson (1965) observed that *Diphyllobothrium latum*, *D. osmeri* and *D. dendriticum* have diploid numbers of 18 and that the karyotypes of somatic cells of all species are apparently identical. The largest chromosome of *D. latum* is 3.5  $\mu$  long with seven pairs of metacentrics and two of submetacentrics making up the complement. In contrast, the longest chromosomes of *H. nodulosa* are 8  $\mu$  long with only three pairs of metacentrics in the complement. On cytological grounds the relationship of *Diphyllobothrium* and *Hunterella* does not appear to be close.

Unlike the experience of Jones (1945) or Wikgren and Gustafsson (1965) in which little variation in chromosome number or karyotype was found among the species studied, preliminary studies on the cytology of cestodes of the order Caryophyllidea indicate that chromo-

some numbers may have systematic value. It is too early to judge if karyotypes show similar significance. Whether differences in chromosome numbers between *Archigetes* sp. and *H. nodulosa* have systematic value can not be determined until there are data from more species. Studies now in progress indicate that there is more variation in the chromosome numbers of caryophyllidean species than any other group of cestodes yet studied, that the chromosomes are the largest yet described for any group of cestodes, and that the chromosomes can be demonstrated with few technical difficulties.

#### Acknowledgments

Thanks are extended to the Departments of Fish and Game of Virginia and Tennessee for permission to collect hosts, to Dr. Etnier for assistance in collecting and to Dr. B. Hochman for photographic assistance.

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## A Protandrous Haploporid Cercaria, Probably the Larva of *Saccocoelioides sogandaresi* Lumsden, 1963

R. M. CABLE AND HADAR ISSEROFF

Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907, and  
Department of Biology, Rice University, Houston, Texas 77005

During the summer of 1967, an unusual cercaria was found among larval trematodes developing in *Amnicola comalensis* collected from a drainage canal near Galveston Bay, Texas. It not only is the first freshwater cercaria reported for the Haploporidae, a predominantly marine family of trematodes, but also is sexually precocious with a degree of protandry apparently unique among larval trematodes. Many cercariae have well developed genital primordia and a few azygid larvae even contain eggs on escape from the mollusk. The Texas species lacks eggs but the body is otherwise equivalent to an adult

trematode. It has a large seminal vesicle filled with sperms but the testis consists of a largely empty sac when the larva is shed by the snail. That gonad becomes conspicuously developed in the cercarial embryo, produces sperms, and retrogresses before development in the snail is complete. In respects other than sexual development, the cercaria agrees with the only other reported haploporid larva, a marine species (Cable, 1962). Given a testis like that of the cercarial embryo, the body would have features of the genus *Saccocoelioides* which Szidat (1954) erected for freshwater species in Argentina.

Infected snails were collected near the brackish ponds where Lumsden (1963) found sailfin mollies, *Mollienesia latipinna*, infected with a new species of *Saccocoelioides*, *S. sogandaresi*. Several dimensions of the haploporid larva fall within the ranges Lumsden gave for his species. Although the two differ in some other respects, they probably are stages of the same species as Sogandares has suggested in a personal communication. Evidence to that effect will be presented after describing the stages provided by infected snails.

The entire cercaria was drawn to scale from measurements of a representative specimen among spontaneously emerging larvae killed in near-boiling 2% formalin and measured immediately under a freely floating coverglass. Details were added freehand from the study of living specimens and wholmounts stained with Semichon's carmine and counterstained with indulin. The metacercaria was drawn from a living specimen in the same manner, and the cercarial embryo by microprojection of a wholmount. All measurements are given in microns; lengths and widths of certain cercarial structures were measured parallel to those dimensions of the entire larva.

### Description of Stages

#### Cercaria (Figs. 2-4)

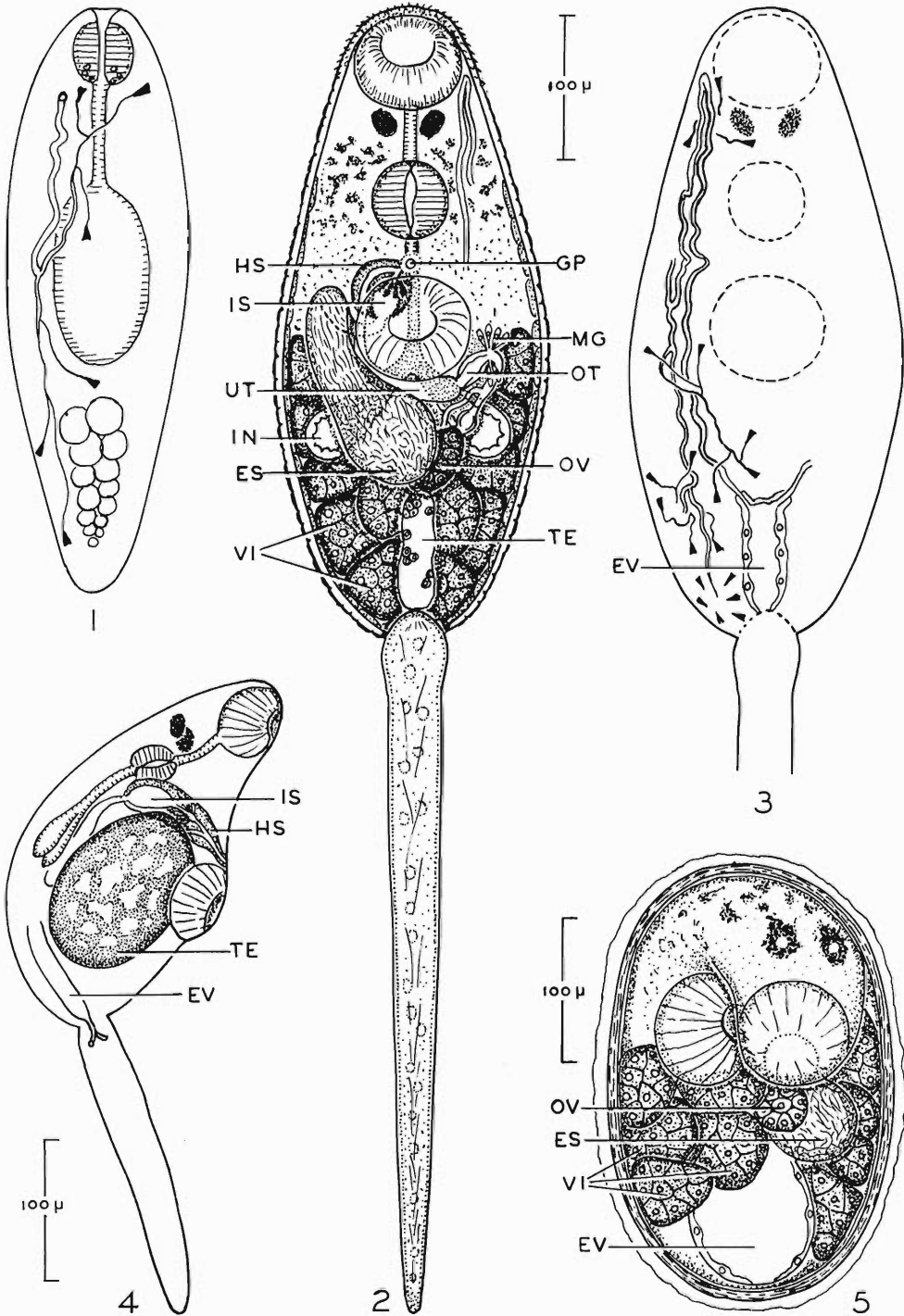
Biocellate, distomate; tail slender, unadorned, attached subventrally. Larva strongly photopositive, swimming smoothly and almost continuously before encysting in the open. Body linguiform to pyriform, 410-436 long; maximum width 175-190, at or somewhat posterior to midlevel. Tail 485-510 long, 41-45 wide at slight swelling near base. Tegument of body thick, colorless, uneven; with fine spines from anterior end almost to eyespot level; parenchyma yellowish, with much brown pigment posterior to eyespots, first clumped, then dispersed except for concentration near caudal attachment; eyespots 23 by 20. Oral sucker 61-67 long, 70-76 wide; ventral sucker equatorial, 69-76 in diameter. Mouth subterminal;

pharynx about midway between suckers, 41-58 long, 53-62 wide. Prepharynx almost as long as pharynx, esophagus longer; bifurcation near posterior edge of ventral sucker; ceca short, divergent, inflated. Testis in emerging larva an elongate, largely empty sac superimposed on excretory bladder; well developed in embryo, smooth at first but becoming slightly indented as sperms form. External seminal vesicle filled with sperms, most conspicuous feature of hindbody; beginning as a wide sac near midlevel of that region, then tapering as it extends anterolaterally and loops dorsomedially at right of ventral sucker to turn anteriorly and enter hermaphroditic sac. That sac spherical, overlapping ventral sucker anterolaterally; containing internal seminal vesicle, prostatic complex and metraterm. Ovary small, ovoid, with few cells; slightly to left, overlapping external seminal vesicle posterolaterally. From ovary, oviduct extends anterolaterally to left, joined first by Laurer's canal, then by common vitelline duct before bending medially to join oötype posterolateral to ventral sucker. Uterus short, curving to right from oötype, around ventral sucker to cross neck of external seminal vesicle, enter hermaphroditic sac and join male duct anterior to pars prostatica. Common gonoduct short; genital pore median, near anterior edge of ventral sucker. Seminal receptacle absent, amphitypy not observed. Vitelline cells large, in massive follicles not in two lateral fields but filling parenchyma of hindbody not otherwise occupied and compressing inconspicuous cystogenous glands against subtegumental muscles. Excretory system stenostomate; primary pores at sides of tail, near base. Bladder elongate sacciform, extending almost to ovary; with distinctly thickened wall containing nuclei. From bladder, main tubules extend to sides of oral sucker, then posteriorly to ventral sucker where each receives an anterior and posterior secondary tubule. Flame cells numerous, apparently three groups served by each secondary tubule; five or six flame cells per group posterior to ventral sucker. Development

→

Figures 1-5. 1. Young redia drawn freehand to show excretory system of one side. 2. Cercaria, ventral view. 3. Same, showing excretory system on one side of body. 4. Lateral view of cercarial embryo showing precociously developed testis. 5. Metacercaria.

Abbreviations: ES, external seminal vesicle; EV, excretory vesicle; GP, genital pore; HS, hermaphroditic sac; IN, intestine; IS, internal seminal vesicle; MG, Mehlis' gland; OT, oötype; OV, ovary; TE, testis; UT, uterus; VI, vitelline follicles.



completed in digestive gland of snail after escape from redia.

HOST: *Ammicola comalensis* Pilsbry and Ferriss, 1906.

HABITAT AND LOCALITY: Drainage canal near Galveston Bay, Texas.

DEPOSITED SPECIMENS: Nos. 71321–71323 USNM Helm. Coll.

### Redia (Fig. 1)

Elongate, without collar or "feet"; gut short and wide in older rediae. Pharynx of young redia 32 in diameter. Excretory pores lateral, just posterior to pharyngeal level. Excretory formula  $2[(3) + (3)] = 12$  flame cells.

### Metacercaria (Fig. 5)

Cyst symmetrically ovoid, not flattened on one side or adhering to surfaces; with indistinct outer layer and well defined inner one, fibrous, 8–11 thick when formed but becoming somewhat thicker with age and appearance of space between worm and cyst wall. Cavity of cyst 260–293 long, 200–230 wide. Eyespots begin to dissociate soon after encystment.

### Discussion

Although the bladder wall of the cercaria is appreciably thickened and contains nuclei, it does not appear to be of the epithelial type. Instead, the nuclei seem to be those of the primary excretory tubules and retained after fusion of the tubules to form the bladder, giving it the appearance of being epithelial. A similar bladder has been seen in bucephalid cercariae (unpublished studies).

Features which at first made the cercaria seem unlikely to be the larva of *Saccocoelioides sogandaresi* were the limited body spination, long prepharynx, extent of the vitellaria, large external seminal vesicle, and reduction of the testis after its conspicuous development in the cercarial embryo. The last feature suggested that the adult would be short-lived, grow little if any in the vertebrate host, and have available only the sperms provided by the cercaria. In contrast, the adult of *S. sogandaresi* is described as having a large testis. However, examination of the paratype of that trematode and several additional specimens provided by Dr. R. D. Lumsden showed that worms comparable to the cercarial body in size contained eggs but not what could be recognized as a testis. That gonad was distinct only in larger

and presumably older specimens; even then, it usually had the appearance of a young testis before the various stages of spermatogenesis are evident as cell clusters. The cercaria in question would account for that situation by providing sperms for early egg production before the testis redevelops and becomes functional. Larger adults are about one-fourth longer and considerably wider than the cercarial body but dimensions of the suckers and pharynx are essentially the same in both. During growth in the vertebrate, utilization of sperms and vitelline cells provided by the cercaria could account for the less extensive seminal vesicle and vitellaria in the adult. Meanwhile spination limited to the anterior end of the cercaria could develop over the entire body.

Morphological differences exclude as the adult of the present cercaria two other haploporids known to occur in the region. None was found in a variety of fishes which we seined from the canal where infected snails occurred, but *Mollienesis latipinna*, the definitive host of *Saccocoelioides sogandaresi* was not included. Lumsden (personal communication) found in a different snail species a haploporid cercaria which encysted in the open. He fed metacercariae to *M. latipinna* but with negative results which he considered inconclusive because he had found another haploporid adult, *Saccocoelium beauforti*, in mullet from the same region. It now seems that his cercaria may well be that species and the present one the larva of *S. sogandaresi*.

Although haploporids occur mostly in marine fishes, Szidat (1954) reported several species in Argentine freshwater hosts. He regarded the parasites as relicts of the ancient Tethys Sea which extended from the Mediterranean region to eastern South America and covered the lower watershed of rivers now in that area. In a similar manner, former inundations of central United States could account for the far inland occurrence of a few trematodes in such predominantly marine families as the Microphallidae, Lepocreadiidae, Microscaphiidae, Cryptogonimidae, and Didymozoidae. The life history has been elucidated for at least one freshwater species in each of those families except the Microscaphiidae and Didymozoidae, and recent unpublished studies have revealed the cercariae of *Microphallus opacus* and two



leporadiids, *Homalometron* sp. and *Microcreadium* sp., developing in *Ammicola limosa* Say in northwestern Indiana.

It seems significant to the zoogeography of digenetic trematodes that all known central and eastern North American freshwater cercariae in the above families as well as the Haploporidae develop in species of *Ammicola*, and brackish-water larvae in species of *Hydrobia*. The fact that malacologists disagree as to whether those genera and *Truncatella*, a genus of marine snails, belong in the same family indicates their close relationship. Formerly, all were placed in the Rissoidae which now also includes the marine host of the only other described haploporid cercaria (Cable, 1962). Knowledge of the life histories of Argentine freshwater haploporids might reveal such a close affinity of their molluscan hosts to marine gastropods. Manter (1957) pointed out that their vertebrate hosts are distantly related to those of Mediterranean haploporids, but he suggested that such euryhaline fishes as mugilids may have served as ecological "bridges" for trematodes between marine and freshwater environments.

Present knowledge of life histories strongly indicates that digenetic trematodes as a group are less specific for their vertebrate than molluscan hosts. Although both are essential to those parasites in extending their range, in doing so they would seem as a rule more adaptable in acquiring new vertebrate than molluscan hosts. Such adaptability within the marine environment is demonstrated by the cryptogonimid, *Siphodera vinalwardii*, which parasitizes shallow-water fishes from Cape Cod to South America. Throughout that range, the cercaria develops in snails of a single genus whereas the parasite changes its vertebrate host to fishes of different orders. Similarly, adults in the Bivesiculidae parasitize a variety of fishes in world-wide tropical and subtropical seas, but all of several known cercariae in that

family develop in snails of the genus *Cerithium*. It thus is evident that discussions of host specificity and zoogeography of digenetic trematodes have unduly neglected molluscan hosts, and not entirely for lack of information concerning life histories.

### Summary

A cercaria developing in *Ammicola comalensis* from a freshwater canal near Galveston Bay, Texas, is essentially an adult with a tail except for lacking eggs. A conspicuous testis develops in the embryo, fills a large seminal vesicle with sperms, and retrogresses to a largely empty sac before the cercaria emerges from the snail. Encystment is in the open. The morphology and ecology of the cercaria indicate it to be the larva of *Saccocoelioides sogandaresi*. The redia, cercaria and metacercaria are described. Representation of the Haploporidae and certain other predominantly marine families by freshwater species is discussed, suggesting that molluscan hosts have been neglected as factors in the spread of trematodes both within and between marine and freshwater environments.

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## *Lissorchis heterorchis* sp. n. (Trematoda: Lissorchiidae) from *Catostomus macrocheilus* Girard in Oregon

B. BRUCE KRYGIER AND RALPH W. MACY

Department of Biology, Portland State College, Portland, Oregon 97207

The only previous record of a species of *Lissorchis* in the Pacific Northwest appears to be that of *L. attenuatum* which was found by Bangham and Adams (1954) in *Catostomus catostomus* and *C. macrocheilus* in British Columbia. Magath (1917) established the genus *Lissorchis* which, with the new species described herein, at present includes eleven species.

### Materials and Methods

Specimens were removed from the small intestine of the host, fixed in Gilson's fluid at room temperature, under slight cover glass pressure, stained in Ehrlich's acid hematoxylin, and counterstained with 0.02% fast green in 95% alcohol. Drawings were made with the aid of a microprojector and camera lucida. The description is based upon the six largest specimens. Measurements are in microns unless otherwise stated and the average of each is followed by the minimum and maximum in parentheses.

### *Lissorchis heterorchis* sp. n. (Figs. 1-3)

#### Diagnosis

Body elongate, anterior and posterior ends rounded, 3.1 (2.4-4.0) mm long by 0.87 (0.65-1.10) mm wide; cuticular spines extending along body margins from anterior end to a short distance posterior to posterior testis, with few spines on ventral surface. Oral sucker subterminal, aspinose, 322 (283-389) long by 365 (313-454) wide, acetabulum in anterior third of body, 374 (307-460) long by 401 (319-513) wide. No prepharynx, esophagus short, ceca of moderate width, extending to near posterior end of body. Testes tandem, lobed, posterior to midbody. Anterior testis smaller, 408 (342-472) long by 330 (260-413) wide; posterior testis 605 (460-779) long by 314 (248-389) wide. Cirrus sac oblique, proximal end just posterior to acetabulum and distal end at midlevel of acetabulum

and reaching left body margin, 547 (354-708) long by 121 (83-165) wide; seminal vesicle bipartate with anterior part smaller; prostate gland cells present. Genital pore marginal at midlevel of acetabulum; cirrus protrusible, spined (Fig. 2). Ovary multilobed with variable number of lobes immediately anterior to anterior testis, 269 (212-342) long by 324 (260-401) wide. Vitelline follicles broadly interconnected, fields overlapping ceca and uterine arms, extending from midlevel of acetabulum to midlevel of posterior testis. Transverse vitelline ducts meeting at posterior margin of ovary at vitelline reservoir; common duct then proceeds anteriorly over ovarian surface where it is joined by short oviduct leading to oötype and beginning of uterus. Laurer's canal and seminal receptacle not seen. Uterus proceeds posteriorly along right side almost to end of body then returns on same side to the level of ovary, then crosses to the left side where it duplicates the same course on the left side of the body, terminating in the metraterm which lies under the cirrus sac, and reaching the common genital pore. Mature uterine eggs, brownish yellow, averaging 26 long by 16 wide.

TYPE HOST: *Catostomus macrocheilus* Girard.

HABITAT: Small intestine.

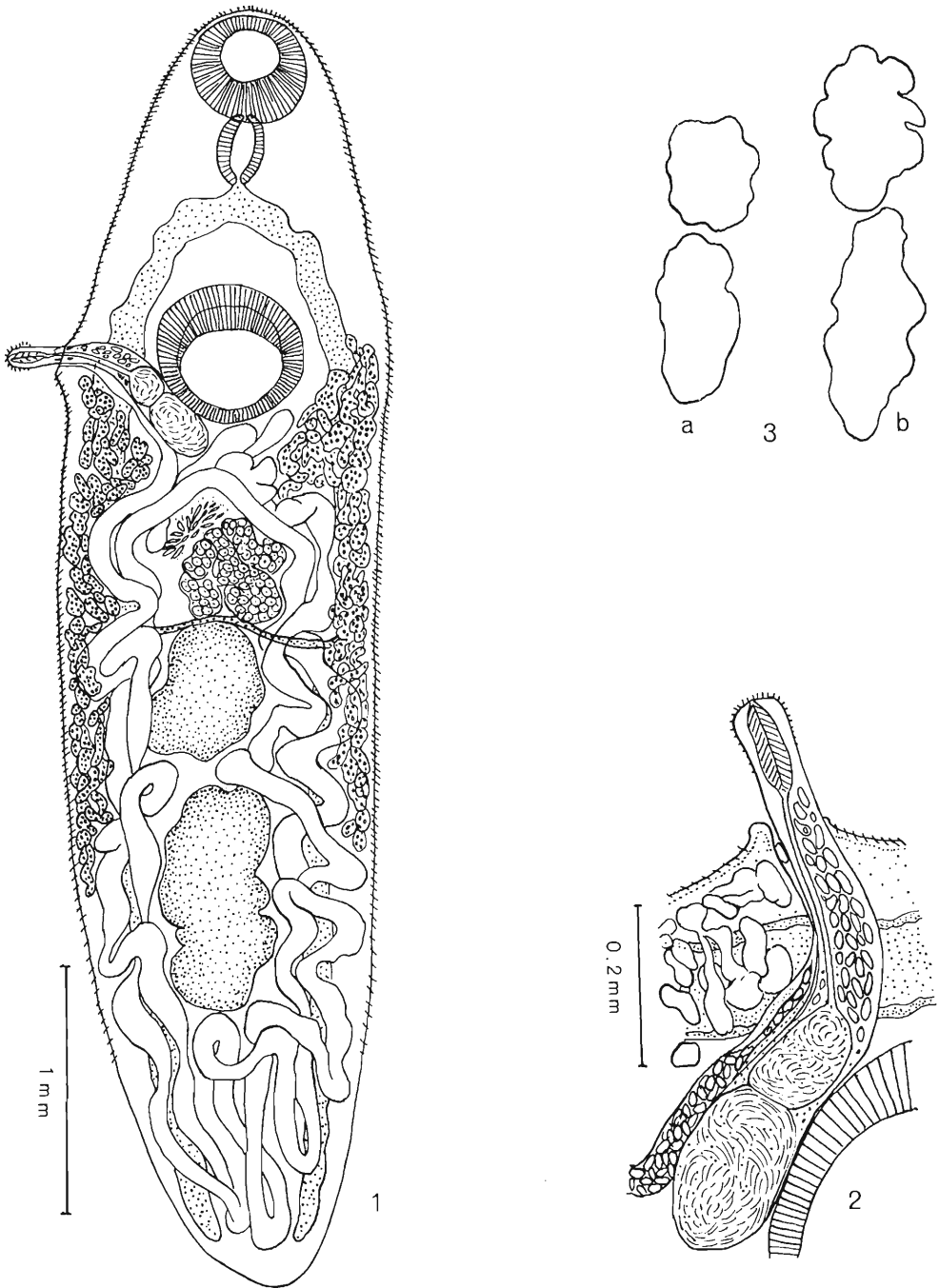
TYPE LOCALITY: Multnomah Creek, Multnomah County, Oregon.

INCIDENCE: Seven specimens found in one sucker.

SPECIMENS DEPOSITED: USNM Helm. Coll. Holotype No. 71344 Paratype No. 71345.

### Key to the species of *Lissorchis* Magath, 1917

1. Vitelline follicles 7-12 on each side ... 2  
    Vitelline follicles 16 or more on each  
    side ..... 3
2. Ovary deeply trilobed, lobes completely separated; length of body posterior to hind testis approximately equal to combined length of



Figures 1-3. *Lissorthis heterovchis* sp. n. 1. Holotype, dorsal view. 2. Detail of genital region. 3. a, b. Outline of testes of two paratypes to show variation.

- the three gonads ..... *L. simeri* (Van Cleave and Mueller, 1932) ..... *L. attenuatum* (Van Cleave and Mueller, 1932)
- Ovary compact, moderately lobed but not appearing as three separate structures; length of body posterior to hind testis less than length of the three gonads ..... *L. mutabile* (Cort, 1918)
3. Cuticular spines extending to posterior end of body; postacetabular region much narrowed and triangular ..... 4
- Cuticular spines extending no farther than the middle of the posterior testis; postacetabular region not narrowed, posterior end more broadly rounded ..... 5
4. Cirrus armed ..... *L. gullaris* Self and Campbell, 1956
- Cirrus not armed ..... *L. fairporti* Magath, 1917
5. Ovary overlapping both testes, testes wider than long, body flask-shaped ..... *L. garricki* (Simer, 1929)
- Ovary not overlapping both testes, testes longer than wide, body more elongate ..... 6
6. Anterior limits of vitelline fields much posterior to acetabulum ..... *L. hypentelii* (Fishtal, 1942)
- Vitelline fields reaching level of acetabulum ..... 7
7. Acetabulum about twice the size of the oral sucker ..... *L. translucens* (Simer, 1929)
- Acetabulum less than twice the size of the oral sucker ..... 8
8. Cuticular spines only on the ventral body surface and not on body margins; combined width of ceca equal to one-third of body width ..... *L. crassicrurum* (Haderlie, 1953)
- Cuticular spines on body margins, not confined to ventral surface; ceca narrower ..... 9
9. Testes distinctly lobed; posterior testis about one-third longer than anterior testes ..... *L. heterorchis* n. sp.
- Testes entire; posterior testis not one-third longer than anterior testis ..... 10
10. Vitelline fields largely confined to anterior half of body; testes not confined to posterior half of body .....

### Results and Discussion

The new species is relatively large with a broad posterior region. It is the only species of the genus with lobed testes. The posterior testis is much longer than the anterior one and the ovary is deeply lobed. The cirrus sac is relatively short and its proximal end does not extend much posterior to the acetabulum. The cirrus is spined.

Following Smith (1968), we consider *Triganodistomum* to be synonymous with *Lissorchis*. Simer (1929) erected a new genus *Alloplagiorchis* for *A. garricki* but this genus also is a synonym of *Lissorchis*.

An examination of paratypes of *Lissorchis crassicrurum* Haderlie and *L. polylobatum* Haderlie revealed that the vitelline follicles are more numerous than shown in the author's drawings; in the former species the vitelline fields extend nearly to the inner margins of the ceca, in the latter species the vitelline follicles are overlapping and closely packed.

Magath (1917) found that the first and second intermediate hosts of *Lissorchis fairporti* were the snail *Helisoma trivolvis* and chironomid larvae, respectively. The cercaria possessed both stylet and tail in contrast to the cercaria of *L. mutabile* which, according to Wallace (1941), lacked both stylet and tail. Cercariae of the latter encyst in the commensal annelid *Chaetogaster limnaei* in the respiratory chambers of *Helisoma trivolvis* and *H. campanulata*. The tailless cercariae of *L. mutabile* encyst after being eaten by the annelid. Thus, there is a correlation between the habitat of the second intermediate host and presence or absence of a tail on the cercariae. Smith (1968) found that *L. mutabile* also utilizes freshwater limpets and planarians as intermediate hosts.

No *Helisoma* snails are known to occur in the area inhabited by the sucker host of *Lissorchis heterorchis*. More probable snail hosts are *Flumenicola* or *Oxytrema*, common stream snails of the region.

### Summary

Seven specimens of a trematode found in the intestine of a sucker, *Catostomus macrocheilus* Girard, collected in Multnomah Creek below Multnomah Falls, Multnomah County, Oregon, were determined to be an undescribed species of *Lissorchis* Magath (1917). The new species herein described is characterized especially by lobed testes unequal in size, a spined cirrus, and numerous, broadly joined vitelline follicles. The genus *Triganodistomum* is considered to be a synonym of *Lissorchis*. A key to the species of *Lissorchis* is presented.

### Acknowledgments

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## Survival of Eggs and Larvae in Cysts of the Soybean Cyst Nematode, *Heterodera glycines*, Ingested by Swine

GROVER C. SMART, JR., AND H. R. THOMAS<sup>1</sup>

In Virginia, fields infested with the soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, are planted to peanut, *Arachis hypogaea*, in the rotation system. Although peanut is not a host of the nematode, cysts do adhere to the pods and hay when this crop is grown in infested soil (Miller, 1960; Smart and Wright, 1962). After peanuts are harvested, swine are often allowed to roam the fenced fields to eat the waste and cull peanuts and peanut vine hay. On infested land, they undoubtedly ingest nematode cysts. Swine then may be moved to noninfested fields, and the possibility exists of spreading the nematode to new locations through the fecal droppings.

Triffit (1929) reported that eggs and larvae in cysts and larvae not in cysts of *H. rosto-*

*chiensis* Wollenweber, 1923 did not survive passage through the digestive tract of the 6-week-old pig. Goffart (1934) fed 3,000 cysts of *H. rostochiensis* to a rabbit and mixed the excrement in sterilized soil planted to potato. Upon examining the potato roots, he found one larva. Ellenby (1944, 1946) reported that eggs in cysts of *H. rostochiensis* survived passage through the gut of earthworms, and yielded a greater number of larvae in larval emergence tests than cysts which did not pass through earthworms.

Girard (1887) reported that cysts of *H. schachtii* Schmidt, 1871, which were not damaged by the teeth of sheep eating infected sugarbeets, survived passage through the digestive tract of the sheep. He observed larvae emerging from such cysts and was able to obtain reproduction by adding infested sheep excrement to sand taken from a quarry and seeding sugarbeet. Chatin (1890) was unable to recover viable *H. schachtii* from sheep fed infected sugarbeet, some of which were arti-

<sup>1</sup> Respectively, Associate Professor of Nematology, Department of Entomology and Nematology, University of Florida, Gainesville, and Associate Professor of Animal Science, Virginia Polytechnic Institute Division of Research, Holland, Virginia. The research was conducted in 1962 while the senior author was located at Holland, Va. Florida Agricultural Experiment Stations Journal Series No. 3086.

ficially infested with large numbers of larvae, males, and females. He did recover eggs and larvae of parasites of sheep and believed that earlier workers had misidentified those as *H. schachtii*. Willot's (1890) results were identical to those of Chatin.

Kofoid and White (1919) reported that eggs of a nematode were recovered from the feces of military troops. Sandground (1923) showed that the eggs undoubtedly were those of a root-knot nematode, *Meloidogyne*, and conducted experiments showing that root-knot nematode eggs passed through the human digestive system and stated, "They were slightly brown in color and were in all stages of development, with the metameris so sharply defined as to indicate their viability." He further stated that the eggs were "... apparently uninjured." Martin (1968) showed that *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 survived passage through the digestive tract of a cow and subsequently infested tomato roots.

According to Leukel (1924, p. 944), Marciniowski (1909) showed that *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936 in wheat galls survived passage through the intestines of the sparrow, goldfinch, pigeon, and mouse, but did not survive passage through the chicken, gopher, or marmot. However, Davaine (1857) found that *A. tritici* in wheat galls did not survive passage through the intestines of the chicken, pigeon, or sparrow, but did survive passage through the frog, triton, salamander, and goldfish. Leukel (1924) found that *A. tritici* did not survive passage through the digestive tract of the horse, cow, hog, sheep, or chicken. Wilson (1930) found that *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 in diseased Phlox did not survive passage through the intestine of a rabbit.

Research reported in this paper was conducted to determine whether eggs and larvae in cysts of *Heterodera glycines* would survive passage through the digestive tract of swine. An abstract has been published (Smart, 1963).

### Materials and Methods

Six pigs weighing an average of 63 lb (28.6 kg) each (60–65 lb = 27.5–29.5 kg) and with an average age of 13 weeks (80–96 days) were used in this study. Three of the pigs were females and three were males. At a later date,

rectal temperatures were recorded in six other 13-week-old pigs (three of each sex) on three consecutive days. Temperatures were recorded with the pigs unrestrained and in a standing position. The average rectal temperature of the six pigs was 104.2 F (40.1 C) ranging from a low of 103.0 F (39.4 C) in a female to a high of 105.1 F (40.6 C) also in a female.

Six concrete-floored pens were washed thoroughly using a high pressure hose (110 psi pressure at the pump). One animal was placed in each pen and feed was withheld for 40 hr prior to initiating the study; however, drinking water was supplied ad lib. Once each 24 hr for 3 consecutive days each pig ingested 1,000 cysts of *H. glycines* which were mixed with the daily ration of 2 lb (0.9 kg) of a 15.6% crude protein feed. One of the pigs (pig 1 in Table 1) ingested cysts from the Holland, Virginia, area, and the other five ingested cysts from the Wilmington, North Carolina, area. Control cysts were kept at 4 C. The feed was the standard growing ration used at the Holland, Va. Station and was of the following mixture: 1,598 lb (726.3 kg) of corn, 252 lb (114.5 kg) of soybean oil meal (50% crude protein), 100 lb (45.4 kg) of tankage, 20 lb (9.1 kg) of limestone, 10 lb (4.5 kg) of salt (NaCl), 5 lb (2.3 kg) of Vitamin Pre-Mix (Charles Pfizer), 0.6 lb (272 g) of Terramycin TM50, 100 g of zinc sulphate (ZnSO<sub>4</sub>), and 12 g of copper sulphate (CuSO<sub>4</sub>).

Feces were collected initially from each pen just before the second feeding of cysts. Thereafter for 4 additional days, feces were collected 3 times a day and composited. After the third collection the feces were examined by washing through a 60-mesh and a 325-mesh screen. Residues from the 60-mesh screen were examined directly for cysts, and residues from the 325-mesh screen were placed in Baermann funnels for recovery of any larvae that might have been liberated within the alimentary canals of the pigs.

On the fourth collection day, 50 cysts recovered from the feces of each pig were placed in 4-inch (10.2 cm) pots of autoclaved soil and soybean seed, *Glycine max* Merr. var. "Lee," planted. All other cysts on all collection days were isolated and placed in sterilized distilled water in 60 mm Petri dishes at the rate

Table 1. Number and percentage of cysts of *Heterodera glycines* recovered from the feces of each of 6 pigs on 5 consecutive collection days and the number of larvae emerged during incubation of the cysts for 21 days at 22 C. Each pig ingested 3,000 cysts (1,000 on 3 consecutive days).

Day feces collected	Pig number and sex, number of cysts recovered, and number of larvae emerged													
	1-Female Cysts Larvae		2-Female Cysts Larvae		3-Female Cysts Larvae		4-Male Cysts Larvae		5-Male Cysts Larvae		6-Male Cysts Larvae		Control Cysts Larvae	
1	105	3	15	0	8	0	142	1	53	0	87	0	200	3,596
2	80	0	39	0	47	0	68	0	68	0	133	0	200	3,776
3	133	1	198	0	278	0	190	0	300	0	134	0	200	1,767
4 <sup>2</sup>	185	0	215	0	150	0	127	0	189	0	180	0	136	65
5	28	0	46	0	27	0	78	0	104	1	90	0	100	59
Total	531	4	513	0	510	0	605	1	714	1	624	0	836	9,263
Percentage of cysts recovered per pig	17.7		17.1		17.0		20.2		23.8		20.8			

<sup>1</sup> Pig 1 ingested cysts from the Holland, Va. area, and all other pigs ingested cysts from the Wilmington, N. C. area.  
<sup>2</sup> Fifty cysts from each pig collected this day were placed immediately in 4 inch clay pots of soil seeded with "Lee" soybean.

of 10 cysts per dish and incubated at 22 C. After incubation for 10 days, emerged larvae were counted and the cysts were transferred to fresh dishes of sterilized distilled water. After 11 more days the emerged larvae were again counted. The cysts which had been incubated and all emerged larvae were placed in 4-inch (10.2 cm) pots of autoclaved soil planted to "Lee" soybean. After 60 days, the soybean plants were removed from the soil. The roots were placed on a 60-mesh screen and washed with a spray of water using enough pressure to remove cysts from the roots. The soil was roiled in a container of water and washed through a 60-mesh screen with the process repeated twice. The residue was distributed in counting trays and any cysts present were counted with the aid of a dissecting microscope at 15 ×.

### Results

A total of 3,497 or 19.4% of the 18,000 cysts fed to the six pigs were recovered (Table 1). Only six larvae emerged from the 3,197 cysts subjected to larval emergence tests. This was an average emergence of 0.002 larvae per cyst. The control cysts yielded 9,263 larvae for an average emergence of 11 larvae per cyst. The six larvae which emerged from cysts recovered from the pigs were alive as determined by movement both autonomously and from tactile stimulation. The intestines of the larvae, however, were highly vacuolated. The intestines of larvae manually released from eggs in the cysts recovered from pigs also

were vacuolated. These larvae were dead; they remained in a bent position and did not move either autonomously or from tactile stimulation.

No larvae were recovered from the Baermann funnels containing the debris caught on the 325-mesh screen.

No soybean cyst nematodes developed on "Lee" soybean during a 60-day period from the cysts and six emerged larvae used as inoculum. From the control cysts, an average of 19 cysts or white females were recovered for each cyst used as inoculum.

### Discussion

Although a few larvae survived passage through the digestive tract of the 13-week-old pig, they did not appear as "normal," based on the vacuolated appearance of the intestine, as larvae not similarly treated. Balmer and Cairns (1963) stated that infectivity dropped sharply in root-knot nematode larvae containing five or more large-sized vacuoles in the intestinal region. The larvae of *H. glycines* which survived passage through the pig, as well as those which did not survive, had highly vacuolated intestines. (The number of vacuoles were not counted since this work was done prior to the publication of the work by Balmer and Cairns.)

Triffit (1929), in seeking an explanation for the death of *H. rostochiensis* ingested by the 6-week-old pig, pointed out that the temperature in young pigs is higher than in older ones, but she did not believe body heat to be the

decisive factor in death of the nematode. We recorded the average rectal temperature of pigs 5 weeks old, 13 weeks old, and 24 weeks old with the following average temperatures, respectively: 104.2 F (40.1 C), 104.2 F (40.1 C), and 102.4 F (39.1 C). Since Endo (1962) showed that larvae of *H. glycines* in cysts lived more than 8 hr at 110 F (43.3 C), we do not believe that body heat of the pigs alone was responsible for the death of most of the larvae of *H. glycines*.

Sex of the pigs apparently had no effect on survival or death of the larvae, since larvae of *H. glycines* emerged from cysts recovered from the feces of both males and females.

Since a very few larvae in cysts of *H. glycines* did survive passage through the digestive tract of swine, it is possible that the nematode could be disseminated through swine feces. However, based upon the vacuolated appearance of the intestinal region of those larvae, and upon the lack of reproduction on a susceptible host plant, we believe that dissemination of the nematode through the feces is unlikely.

### Summary

Of 18,000 cysts of *Heterodera glycines* ingested by six pigs, 19.4% were recovered from the feces. Six larvae emerged from the cysts incubated for larval emergence. The larvae were alive, but had highly vacuolated intestines. When these larvae and all cysts recovered from the feces were placed in pots of soil containing "Lee" soybean, a known host, no cyst nematodes developed.

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## The Intrapulmonary Localization of *Angiostrongylus cantonensis* in the Rat

THOMAS M. SODEMAN,<sup>1</sup> WILLIAM A. SODEMAN, JR.,<sup>2</sup> AND CHARLES S. RICHARDS<sup>3</sup>

*Angiostrongylus cantonensis* was described by Chen (1935). Mackerras and Sandars (1955) in a detailed description of its life cycle showed that the adult worm migrates from the rat's subarachnoid space to its lungs between the 28th and 31st day of infection. Fertilization takes place in the pulmonary arteries and oviposition begins about the 35th day.

The purpose of this investigation was to study the localization of adult worms, eggs, and larvae in the lungs of experimentally infected rats. Because of the difficulty in maintaining spatial relationships after removal of organs from an animal, particularly when making histologic sections from small blocks of the whole organ, it was elected to section the whole lung. The Gough-Wentworth (1960) technique for making thick sections (200  $\mu$ ) of whole, inflated fixed lungs was used. These sections permitted the localization of lesions within the matrix of the pulmonary parenchyma with little loss of the spatial relationships between pathologic changes and normal tissue.

### Methods and Materials

Weanling, Sprague-Dawley rats were infected with *A. cantonensis* by feeding of one-half of a *Biomphalaria glabrata* (= *Australorbis glabratus*) snail harboring infective third stage larvae. The rats were divided into six groups. The first group was sacrificed on the 28th day of infection and thereafter a new group was sacrificed on the 34th, 37th, 39th, 43rd, and 65th day of infection. The heart and lungs were removed *en block*, avoiding injury to the visceral pleural surfaces. The lungs were inflated with a formalin acetate mixture through the trachea by a cannula connected to a reservoir 4 feet above. The formalin acetate mixture consisted of 500 ml of liquid

formaldehyde (40%) and 200 gm of sodium acetate, brought to 5,000 ml with distilled water. The inflated lungs were fixed in the formalin acetate mixture for a minimum of 2 days.

The lungs were separated from the trachea and heart. Each lung was washed for 72 hr in running water into which 5% nitric acid was dripped. The entire lung was embedded in a solution of 250 mg 80–100 bloom gelatin, 40 ml ethylene glycol mono-ethyl ether, 5 ml carpyl alcohol, 5 ml 1% ethylmercurithio-salicylate and 850 ml distilled water. The specimen was allowed to stand for 1 hr and was then transferred to an incubator and kept for 48 hr at 35 C. The entire block was frozen and kept at -20 C until sectioning. Sections were cut at 200  $\mu$  on a Reichert microtome as thawing occurred and they were stored in tap water. The sections were floated onto glass slides for examination with a dissecting microscope. Several sections were stained with hematoxylin and eosin.

Lungs from each group were also embedded in paraffin and sectioned at 5  $\mu$ . These sections were stained with Giemsa for comparison with the thick sections.

### Results

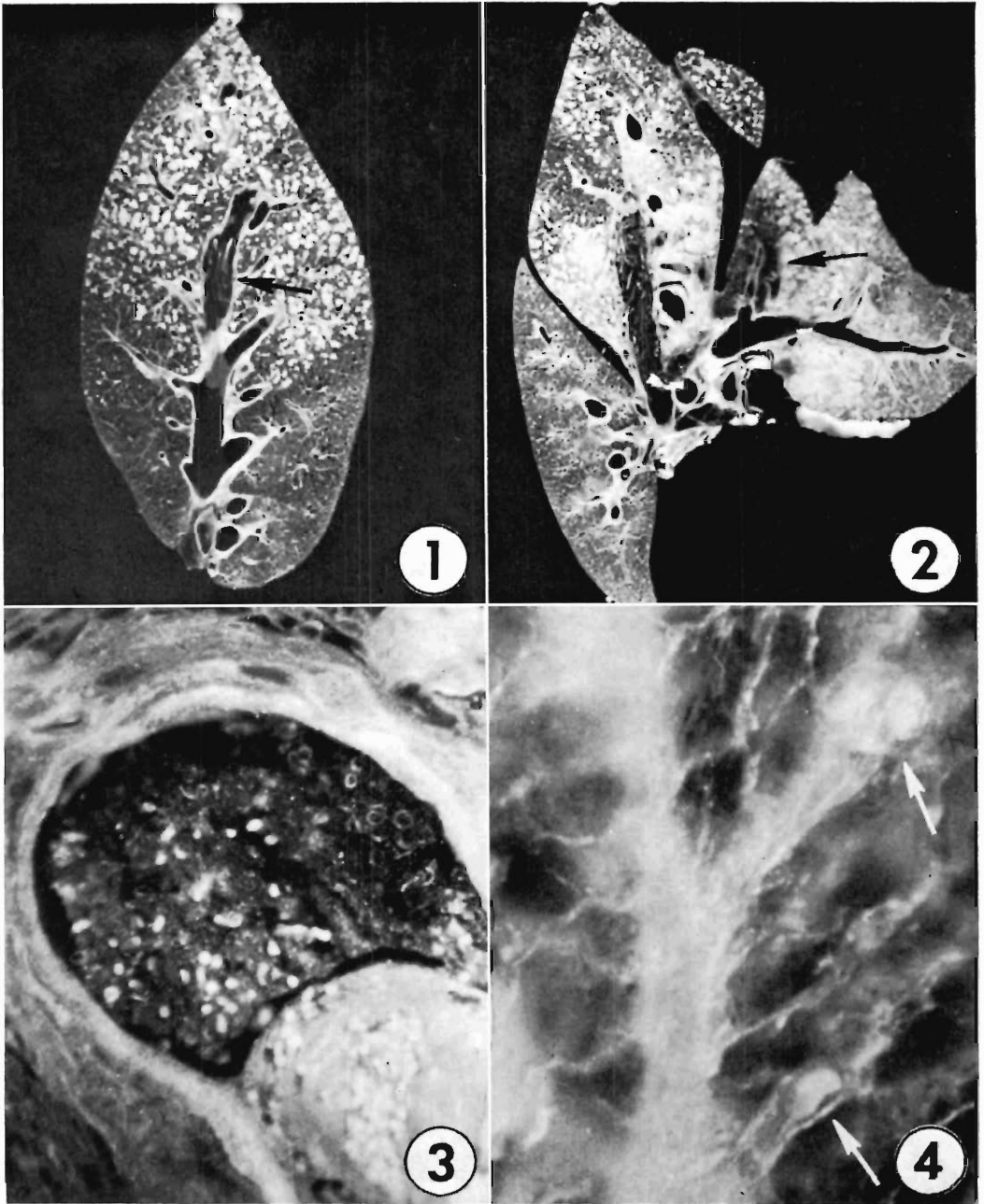
The lungs of a rat infected with *A. cantonensis* 65 days prior to sacrifice revealed mature worms only within the vascular system. They were restricted to the larger pulmonary arteries. The thick sections permitted visualization of large portions of the adult worms, most with ingested blood outlining their digestive tracts, within the mainstem pulmonary arteries and peripherally in the larger branches (Fig. 1). There was no evidence that the mature worms penetrated into the pulmonary parenchyma or the bronchial system at 65 days.

Eggs surrounded by fluffy white consolidation were scattered throughout the lung fields. From serial sections it was apparent that egg lesions were associated with blood vessels. A portion of the left middle lobe was infarcted (Fig. 2). Serial sections of the lung revealed

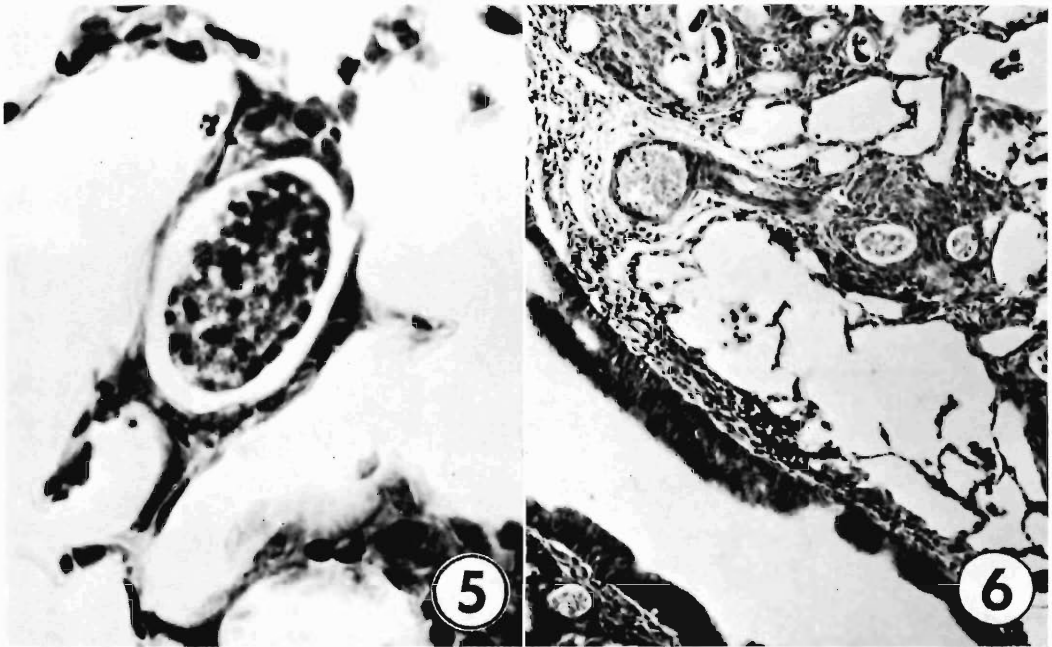
<sup>1</sup> Section on Primate Malaria, Laboratory of Parasite Chemotherapy, NIAID, P.O. Box 80190, Chamblee, Georgia 30341.

<sup>2</sup> Liberian Institute of Tropical Medicine, Harbel, Liberia.

<sup>3</sup> Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, Maryland 20014.



Figures 1-4. Thick sections of the left lung from a rat sacrificed 65 days after infection. 1. *Angiostrongylus cantonensis* fill the main pulmonary artery. Egg lesions are seen as patchy white areas ( $\times 2.5$ ). 2. Infarction of a portion of the middle lobe ( $\times 2.5$ ). 3. Thrombosis of the artery leading to the infarcted lobe in Figure 2. The proximal portion contains eggs, the distal contains developing larvae ( $\times 200$ ). 4. A thick section of the lung demonstrating eggs embolized to the terminal arteries ( $\times 200$ ).



Figures 5-6. 5. A thin section of the lung from a rat sacrificed at 39 days with an egg in a capillary of an alveolar wall ( $\times 735$ ). 6. A thin section of the lung from a rat sacrificed at 65 days with eggs in advanced stages of development surrounded by organizing tissue. A small artery can be traced to an egg lesion. The proximity of the eggs to alveoli and a bronchus is evident ( $\times 580$ ).

thrombosis of the artery to that lobe (Fig. 3). The thrombus was composed of two distinct sections; the older, more distal portion contained hatched larvae, the proximal portion, developing eggs. Where eggs were seen outside of the arterial system, the integrity of the vessel was in question because of surrounding hemorrhage or an intense inflammation with disruption of normal structures.

In many cases, the plane of the section and its thickness permitted the tracing of arterial vessels leading to egg lesions (Fig. 4). In this figure an egg can be seen occluding a branch of a small artery just distal to its bifurcation from the main blood-filled lumen. The pattern of egg lesions and their association with arteries suggested embolization. The bronchi contained no eggs.

Lungs from rats sacrificed on the 28th, 34th, and 37th days were normal. Those sacrificed on the 39th, 43rd, and 65th days postinfection and processed as  $5 \mu$  paraffin sections showed histologic changes similar to the thick gelatin sections. Adult worms were localized in the

pulmonary arteries. In the early stages of infection, eggs were clearly found only in pulmonary vessels (Fig. 5). With advancing age an extensive reaction formed about the eggs with replacement of blood vessel structure by active fibroblastic proliferation and marked leukocytic and monocytic infiltration. As the larvae developed, there was an increased eosinophilic reaction. In many places eggs lying in their connective tissue masses were separated from alveoli or bronchi only by a thin wall of tissue (Fig. 6). After 65 days of infection larvae were seen in the alveoli and surrounded by eosinophils. The patchy white consolidation in the thick sections was seen to consist of organized tissue, collections of macrophages and polymorphonuclear leukocytes in the thin sections. Alveolar septa associated with intracapillary eggs were thickened and infiltrated with polymorphonuclear leukocytes, lymphocytes, and macrophages. In the more advanced stages, the large pulmonary arteries were dilated and contained masses of eggs.

### Discussion

The results of the thick, as well as thin, sections indicated that the adult stages were restricted to the pulmonary arteries. Their size limited them to the larger arteries. No evidence in the stages studied, either in the thick gelatin or thin paraffin sections, indicated that the adult worm penetrated into the pulmonary parenchyma. The findings suggested that oviposition occurs in the blood stream and that the wide distribution of pulmonary involvement resulted from embolization of eggs. This embolization and impaction in blood vessels resulted in thrombosis and infarction. That development may progress within the thrombus was evident in Figure 3 where hatched larvae were seen in the distal portion of a thrombus, while in the recently formed proximal portion, eggs were found. There was nothing to suggest a preferential distribution of egg lesions in the lungs. However, because of the close association of the bronchial and vascular systems, many of the egg lesions were in peribronchial locations.

When eggs were seen in the pulmonary parenchyma, they were surrounded by inflammation and organization that obscured their original intravascular position. Furthermore, it was evident that eggs outside of vessels may have resulted from disruption of vessel walls with hemorrhage or from infarction. Both processes were evident in the sections. The extensive organization around the eggs in the arteries extended into the perivascular and frequently into the peribronchial tissue. The materials examined suggest that the primary site of development is within the arterial system and that

the pulmonary parenchyma acts as a secondary site for development when eggs escape from the vascular system. It is likely that the larvae migrate from the arteries through the organized tissues directly into the bronchial system or out into the alveolar sacs.

The use of the thick section technique permitted better visualization of eggs within the lung substance without loss of orientation. The authors feel this technique deserves consideration as an experimental method in studying pulmonary parasitic lesions.

### Summary

The intrapulmonary localization of *Angiostrongylus cantonensis* in experimentally infected rats was studied in lungs embedded in gelatin and sectioned at 200  $\mu$ . Adult worms were found only intravascularly. Egg deposition and larval development within lungs were primarily in blood vessels. Eggs were distributed by embolization. It is suggested that the preferred site of larval development is intravascular rather than in lung parenchyma.

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## *Tylenchus vesiculosus*, sp. n. (Nematoda: Tylenchidae) From Soil in Michigan

NATALIE A. KNOBLOCH<sup>1</sup> AND JOHN A. KNIERIM<sup>2</sup>

*Tylenchus vesiculosus* sp. n. was recovered from soil samples collected 16 October 1967 from a potato field near East Lansing, Michigan. The habitat, a field in which potatoes had been under cultivation for several years, was organic soil (muck) with two outcropping knolls of sandy mineral soil. *T. vesiculosus*, while not widely distributed throughout the field, was found in large numbers around the root systems of potato plants growing in the sandy soil. Only one male was recovered from the original soil samples.

A ratio of one male for about every 50 females was recovered from samples processed in April 1968 but these males were inadvertently destroyed in the laboratory. Since the original infestation was to be fumigated prior to spring planting, soil was transferred from the original habitat to a microplot at the Entomology Research Farm, Michigan State University in an attempt to preserve the species for future biological investigations.

The following description of the species is based on preserved specimens. Measurements were made on specimens which had been heat relaxed, fixed in F.A. 4:10, preserved and mounted according to the glycerol-ethanol method of Seinhorst (1959).

### *Tylenchus vesiculosus* sp. n. (Figs. 1-7)

HOLOTYPE FEMALE: Length = 0.55 mm; a = 29; b = 6.2; c = 4.7; V = <sup>32</sup>66; spear = 9.75  $\mu$ .

PARATYPE FEMALES (12): Length = 0.52 (0.51-0.55) mm; a = 31.3 (28-34); b = 6.1 (6.0-6.6); c = 4.8 (4.7-4.9); V = 66 (66-68); spear = 9-11  $\mu$ .

ALLOTYPE MALE: Length = 0.48 mm; a = 30; b = 5.5; c = 4.5; T = 46; spear = 9.7  $\mu$ .

FEMALE (HOLOTYPE): Body filiform slightly arcuate ventrally, tapering more towards posterior than anterior end. Cuticle of body coarsely striated; annules 1.9  $\mu$  wide at middle of body. Lateral field a plain band bordered by two bright lines one-fourth to one-fifth greatest body width (Fig. 3). Phasmids not observed. Lip region conoid, rounded, continuous with neck contour; no lip annules observed. Spear delicate, 9.75  $\mu$  long, with well-developed knobs (Fig. 2). Median bulb of oesophagus ovate with crescentic valve plates; the metacarpus obscure in many of the glycerine specimens. Isthmus slender, surrounded by the nerve ring slightly anterior to where the isthmus joins the basal oesophageal bulb. Basal bulb elongate-pyriform; cardia conical, well defined. Excretory pore opens at about the middle of the basal bulb (Fig. 1). Vulva a depressed transverse slit; vagina thick-walled, occupying about three-fifths body width; length of posterior uterine sac about four-fifths vulval body width. Ovary single, outstretched anteriorly, with a round to oval shaped spermatheca with an extended pouch-like extension (Fig. 5). Spermatheca and the extension contained many spermatozoa. Ovary with oocytes arranged in a single file and extending two-thirds of the distance to oesophagus (Fig. 4). Anus distinct; tail length about 9 times anal body width, ending in a rounded tip.

MALE (ALLOTYPE): Length = 0.48 mm; a = 30; b = 5.5; c = 4.5; T = 46; spear = 9.7  $\mu$ .

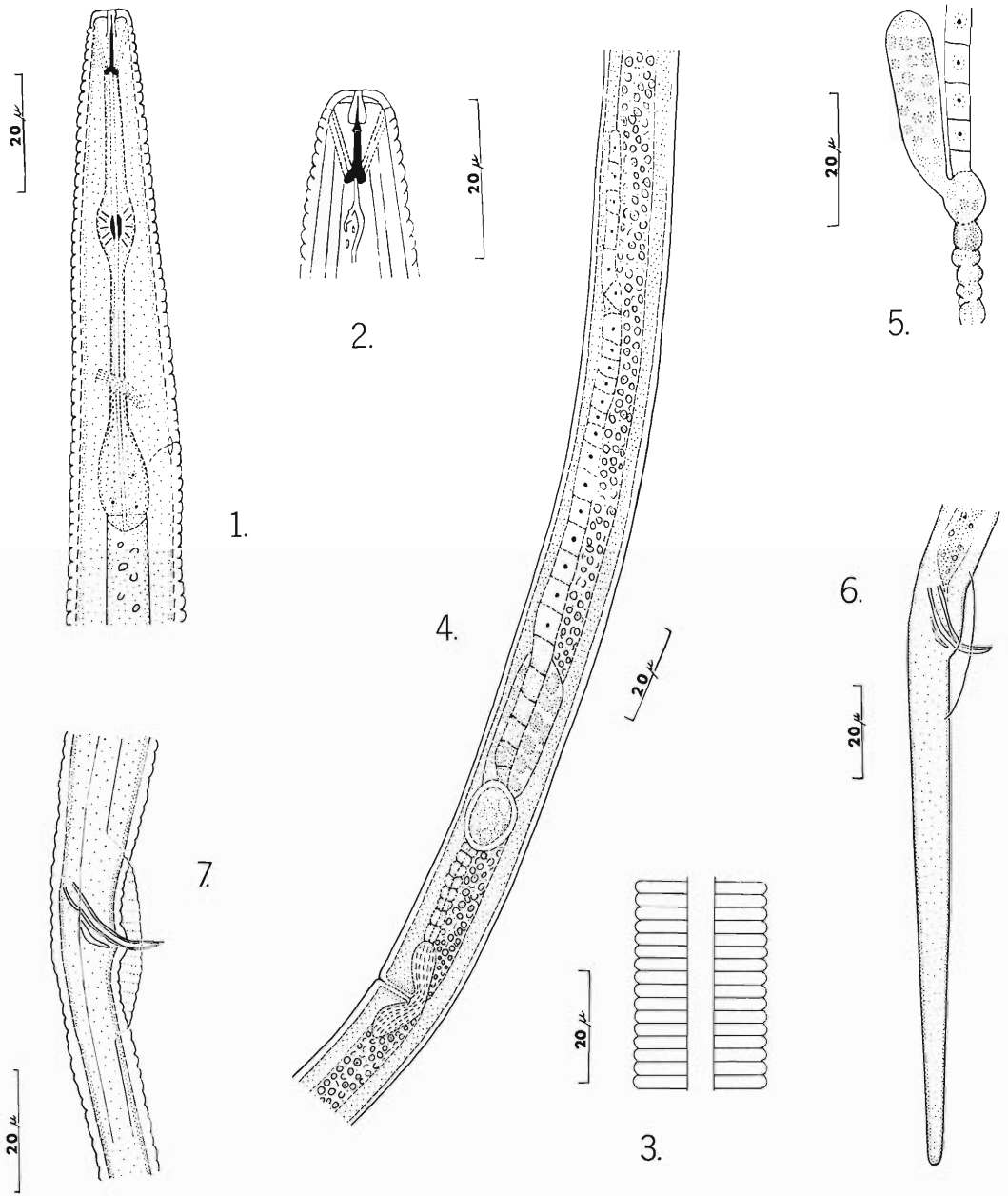
Body shape, head and oesophagus same as in female (Figs. 1, 2).

Testis single, outstretched anteriorly; spermatoocytes arranged in single file. Spicules paired, curved, of usual tylenchoid type. Gubernaculum thickened, troughlike; bursa faintly crenate, rising at a point a little above proximal ends of spicula and extending past anus a distance equal to about 1½ anal body diameter (Figs. 6, 7). Tail same shape as in female; about 10 times anal body width.

<sup>1</sup> Research Technician, Entomology Department, Michigan State University, East Lansing, Michigan.

<sup>2</sup> Assistant Professor, Entomology Department, Michigan State University, East Lansing, Michigan.

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Figures 1-7. *Tylenchus vesiculosus*. 1. Anterior portion of female. 2. Female head region. 3. Lateral field of female. 4. Female reproductive tract showing position of spermatheca. 5. Sketch of spermatheca showing ventral view of attached pouch. 6. Male tail. 7. Posterior portion of male with lateral lines.

DIAGNOSIS: *Tylenchus vesiculosus* sp. n. can be differentiated from all known species of the genus by the spermatheca with an extended pouch. It is small (0.54 mm) with thick, heavily annulated cuticle, plain bandlike lateral field and tail with a distinctly rounded terminus. It differs from *Tylenchus davainei* Bastian, 1865, in the straight tail, bandlike lateral field, unstriated lip region and the spermatheca with the pouch. Distinguished from *Tylenchus ditissimus* Brzeski (1963) by the coarser body striation, no annules in the lip region, bandlike lateral field, position of hemizonid, longer length, possession of post uterine sac and a spermatheca with a pouch. The extended pouch of the spermatheca is known for no other species of the genus *Tylenchus*. However, a similar pouch is described for *Dactyloctenychus crassacuticulus* Wu (1968).

HOLOTYPE, FEMALE: Collected 16 October 1967 from a potato field in East Lansing, Michigan. Slide T-118t deposited with the USDA Nematology Collection at Beltsville, Maryland.

ALLOTYPE, MALE: Same data as holotype, slide T-117t.

PARATYPES, FEMALES: Same data as holotype, 12 slides Tv3-Tv14 deposited in Depart-

ment of Entomology Collection, Michigan State University, East Lansing, Michigan.

TYPE HABITAT: Organic and mineral soil around roots of potato, *Solanum tuberosum* L.

TYPE LOCALITY: A cultivated farm field of potatoes grown in organic and mineral soil near East Lansing (elevation 845 feet), Michigan, USA.

The authors are grateful to Professor Gerald Thorne, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin for his advice during this work and to Richard J. Snider for his patient counseling in drawing techniques.

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## Helminths of the Crow, *Corvus brachyrhynchos* Brehm, 1822, in North Carolina<sup>1</sup>

LARRY D. HENDRICKS,<sup>2</sup> REINARD HARKEMA, AND GROVER C. MILLER  
Department of Zoology, North Carolina State University at Raleigh

There are few comprehensive reports on the helminths of crows in North America. Ward (1935) conducted the first extensive survey of crows. This work included data on the helminths of 50 crows collected in Oklahoma. Morgan and Waller (1941) examined 112 crows from Wisconsin and Iowa. Daly (1957a, 1957b) examined 103 southern crows (*Corvus brachyrhynchos paulus* Howell) from Virginia. Most references on parasites from the crow are for particular species or on surveys of hosts which included crows as part of the study. The object of our investigation was to determine the parasitic fauna and the incidence of parasitism of both external and internal parasites of the crow in North Carolina. The external parasites are reported elsewhere (Hendricks and Axtell, 1968).

### Materials and Methods

One hundred and fifty-eight crows, 76 females and 82 males, were collected and examined for parasites between 5 October 1965 and 22 April 1967. The majority of these birds was taken in the spring of 1966 and 1967 in conjunction with the annual hunt of the Southeastern Crow Shooters Association in the vicinity of Henderson, North Carolina. All hosts were data-tagged and sealed in plastic collecting bags. Whenever possible hosts were autopsied within a few hours after collection; otherwise they were frozen and stored.

Trematodes were killed and fixed in hot A.F.A.; acanthocephalans and tapeworms were killed in boiling water and then fixed and stored in A.F.A.; nematodes were killed and fixed in hot 70% ethanol and stored in 10% glycerine in 70% ethanol.

For microscopic study, trematodes, cestodes, and acanthocephalans were stained with Harris'

hematoxylin, Semichon's carmine, or fast green, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were cleared in lacto-phenol.

### Results and Discussion

Twelve species of helminths were recovered from 158 crows. These included two trematodes, two cestodes, three acanthocephalans, and five nematodes (Table 1).

*Brachylecithum americanum* Denton, 1945 is a common parasite in the liver of birds of the families Corvidae and Icteridae and was described from specimens obtained from meadowlarks, grackles, crows, and blue jays from Georgia, Tennessee, and Texas. It was found in the liver of 122 of the 158 crows examined. It was reported from the yellow throat, *Geothlypis trichas* at Durham, N. C. (Wells and Hunter, 1960).

*Echinostoma revolutum* Froelich, 1802 was found in the small intestine of seven crows. Daly (1959) reported this form from the southern crow in Virginia. This is the first report from crows in North Carolina.

Only one specimen of *Anomotaenia* sp. was found in the small intestine. It possibly is *A. constricta* (Molin, 1858) which was reported from the southern crow in Virginia (Daly, 1959). Ransom (1909) listed the fish crow (*C. ossifragus* Wilson), the raven (*C. corax* L.) and the crow as hosts for *A. constricta*.

*Variolepis variabilis* (Mayhew, 1925) was found in the small intestine of 34 of the hosts examined and North Carolina is a new locality record. It has been reported from the crow in Illinois (Mayhew, 1925), Wisconsin (Morgan and Waller, 1941) and Virginia (Daly, 1959).

*Mediorhynchus grandis* Van Cleave, 1916 was found in the small intestine of two crows. The two specimens recovered were immature males. This species has been reported from the grackle, eastern meadowlark (Van Cleave,

<sup>1</sup> From a thesis submitted to the Graduate Faculty of North Carolina State University at Raleigh in partial fulfillment of the requirements for the degree of Master of Science. This project was supported in part by Grant No. AI-05927, National Institutes of Health.

<sup>2</sup> Address: 9th Medical Laboratory, APO San Francisco 96307.



**Table 1. Helminths recovered from 158 crows, *Corvus brachyrhynchos* Brehm.**

Parasite	Number infected	Average number of helminths per infected host
Trematodes		
<i>Brachylecithum americanum</i>	122	14.5
<i>Echinostoma revolutum</i>	7	2.8
Cestodes		
<i>Anomotaenia</i> sp.*	1	1.0
<i>Variolepis variabilis</i> *	34	2.1
Acanthocephala		
<i>Mediorhynchus grandis</i>	2	1.0
<i>Macracanthorhynchus</i> sp.†	2	1.5
<i>Plagiorhynchus formosum</i>	1	1.0
Nematodes		
<i>Capillaria contorta</i> *	21	1.4
<i>Hystrichis</i> sp.*†	12	1.8
<i>Physaloptera</i> sp.*†	2	2.0
<i>Acuaria anthuris</i> *	35	2.4
<i>Diplotrriaena tricuspsis</i> *	1	1.0

\* New locality record.

† New host record.

1916), and the crow (Van Cleave, 1918, 1947; Daly, 1959).

The infection of two crows with *Macracanthorhynchus* sp. probably was accidental. The specimens are immature females and resemble *M. hirudinaceus*.

*Plagiorhynchus formosum* Van Cleave, 1918 was found in the small intestine of one crow. It has been reported from the crow in the District of Columbia (Jones, 1928) and the Macgillivray seaside sparrow in North Carolina (Hunter and Quay, 1953).

*Capillaria contorta* (Creplin, 1839) was removed from the mucosa or submucosa of the esophagus of 21 hosts. Canavan (1931) reported this species from a crow in the Philadelphia Zoological Garden and Morgan and Waller (1941) reported it from crows in Wisconsin and Iowa. This is the first report of this species from North Carolina.

*Hystrichis* sp. was recovered from the glands of the proventriculus of 12 of the 158 crows. The specimens constitute a new species and it will be described separately. This is the first report of a representative of this genus in North America and the crow is a new host.

Two immature female specimens of *Physaloptera* sp. were recovered from one host. They resemble *P. alata* Rudolphi, 1819.

*Acuaria anthuris* (Rud. 1819) was found in the crop and gizzard of 35 hosts. This species was reported from the crow in Nebraska

(Williams, 1929), Oklahoma (Ward, 1935), New Jersey (Beaudette and Hudson, 1936), Wisconsin and Iowa (Morgan and Waller, 1941). This is the first report of this species in North Carolina.

*Diplotrriaena tricuspsis* (Fedtschenko, 1874) was recovered from an abdominal air sac of one crow. Probably this is the only adult filarid species reported from crows in North America (Beaudette and Hudson, 1936). Wells and Hunter (1960) reported an immature *Diplotrriaena* sp. from the yellow throat in Durham, N. C.

It is interesting to compare this study with that of Daly (1959) who examined 103 southern crows taken in Albemarle County, Virginia. There is a distance of 100–150 miles from the counties in which the majority of the crows in this survey was taken, yet only three parasites were common to both studies. These were: the trematode *Echinostoma revolutum* Froelich, 1802, the cestode *Hymenolepis variabile* (*Variolepis variabilis* of this paper), and the acanthocephalan *Mediorhynchus grandis* Van Cleave, 1916.

### Summary

A total of 158 crows harbored the following helminths: Trematoda: *Brachylecithum americanum*, *Echinostoma revolutum*; Cestodes: *Anomotaenia* sp., *Variolepis variabilis*; Acanthocephala: *Mediorhynchus grandis*, *Macracanthorhynchus* sp., *Plagiorhynchus formosum*; Nematodes: *Capillaria contorta*, *Hystrichis* sp., *Physaloptera* sp., *Acuaria anthuris*, *Diplotrriaena tricuspsis*. New locality records are noted for *Anomotaenia* sp., *V. variabilis*, *Hystrichis* sp., *A. anthuris*, and *D. tricuspsis*, and new host records for *Macracanthorhynchus* sp., *Hystrichis* sp. and *Physaloptera* sp.

### Acknowledgments

Sincere appreciation is extended to Dr. Helen Ward for her assistance in the specific identification of the acanthocephalans, and to Mrs. MayBelle Chitwood for her opinions and suggestions on the new species of *Hystrichis*.

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## SECOND INTERNATIONAL CONGRESS OF PARASITOLOGY

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### September 6-12, 1970

The Second International Congress of Parasitology was organized by the American Society of Parasitologists and is co-sponsored by the Helminthological Society of Washington, National Academy of Science, Society of Nematologists, Society of Protozoologists, Wildlife Disease Association, and World Federation for the Advancement of Veterinary Parasitology. The program will include any and all aspects of human and animal parasitology and plant nematology except the description of new species. Parasitology is used in its generally accepted connotation and hence would not include bacteriology, virology, mycology, pests or pesticides.

Plans for the development of the meetings are progressing well. The ad hoc Organizing Committee and the ad hoc Program Committee have developed the keynote of informality for free and informal exchange. Every effort is being made to recapture the spontaneity, enthusiasm, and stimulation that has characterized some of the few unscheduled and impromptu discussions that have developed within some other international meetings. Accordingly it is proposed to limit formal presentations, with invited papers, to the morning sessions.

It is proposed to have a series of informal discussions in the afternoon sessions. Thus free contributions (submitted papers) would be grouped in appropriate sessions. It is furthermore proposed to prepublish these as longer than average résumés (perhaps 500 words) to serve as the basis for informal discussion. Thus the chairman or any participant might ask for elaboration, clarification, or discussion of any paper or any aspect therein. Lantern slide projectors will be available. However, it is hoped that free discussion will not be stifled by the routine reading of a series of short papers.

This is frankly an experiment in international meetings. The concept has been enthusiastically endorsed in many quarters. We invite comments and suggestions on the concept and any and all aspects of the program. The ad hoc Program Committee welcomes suggestions for presiding officers at these sessions which could number as many as 60 or more.

Plan to attend the Congress and participate.

GILBERT F. OTTO  
Secretary General  
Zoology Department  
University of Maryland  
College Park, Maryland 20742

## Exposure of Chicks to the Metacercaria of *Echinostoma revolutum* (Trematoda)<sup>1</sup>

BERNARD FRIED AND LINDA J. WEAVER

Department of Biology, Lafayette College, Easton, Pennsylvania 18042

Although Beaver (1937) has contributed an extensive monograph on the biology, systematics and life history of *Echinostoma revolutum* (Froelich, 1802) and Senger (1954) has studied aspects of growth, development and survival of this parasite, detailed studies on its infectivity in domestic chicks are lacking. During studies on the growth and development of this fluke in ectopic sites (Fried and Vonroth, 1968; Fried, Weaver, and Kramer, 1968) observations on the infectivity of *E. revolutum* metacercariae in the chick were made and are recorded herein.

### Materials and Methods

*Helisoma trivolvis* (Say) naturally infected with *E. revolutum* larvae were placed in finger bowls containing pond water with laboratory-reared *Physa* sp. snails. Cercariae emerged from the *H. trivolvis* and encysted primarily in the kidney of the *Physa*. The number of cysts varied depending upon the size of the *Physa* but ranged from 25–250. *Physa* were crushed in pond water from 1–10 days post-infection and cysts were transferred to 3-salt Ringer's (Paul, 1960) and teased into clumps of approximately 25, 50, or 100 cysts. Exact numbers of cysts were difficult to count so estimates of  $\pm 5$ , 10, 20 were usually made. Except in experiments F and G (Table II) where cysts were pretreated in Ringer's or distilled water and in the cloacal exposure study, cysts were pretreated in 3% NaHCO<sub>3</sub> for 30–60 min and then fed to chicks in 1–3 ml of the bicarbonate.

Day-old chicks were used in all experiments except the reinfection studies (Table III). Chicks were not given food or water on the day of exposure or autopsy. They were killed by decapitation and either total worm counts were made or parasites found in the rectum, ceca, and ileum were recorded.

### Results and Discussion

Twenty-nine (93.6%) of 31 chicks each exposed to  $50 \pm 10$  to  $100 \pm 20$  cysts and autopsied 22–35 days later were infected (Table I). Forty (95.2%) of 42 chicks each exposed to  $50 \pm 10$  cysts in 3% NaHCO<sub>3</sub>, 3-salt Ringer's or distilled water and autopsied 14 days later were infected (Table II). Senger (1954) previously demonstrated that the domestic chick could serve as a host of *E. revolutum* and reported that 22 (61%) of 36 chicks fed metacercariae in water became infected.

Bacha (1964) found that an increased worm burden of *Zygodontylenus autumalis* (Diesing, 1836) in white rats could be obtained if metacercariae were pretreated in NaHCO<sub>3</sub>, possibly because the bicarbonate counteracted the acidity of the stomach. However, in matched experiments using NaHCO<sub>3</sub> and distilled water similar worm burdens of *E. revolutum* were obtained (Table II). Moreover, when cysts were pretreated in Ringer's the worm burden increased approximately twofold. Many factors influence the rate of infectivity of *E. revolutum* in chicks including the strain of parasite and host, the emptying time of the gut, mechanical and chemical damage of cysts in the crop, proventriculus and gizzard and further investigations are needed to elucidate these factors.

In older infections more worms were found in the rectum than in the ileum. Thus when chicks were autopsied in 2 weeks (Table II) 272 worms were in the ileum and 241 in the rectum, whereas in hosts autopsied 3–5 weeks (Table I) postinfection 105 worms were in the ileum and 193 in the rectum. *E. revolutum* is shortlived in chicks and our records indicate that rarely do infections persist up to 8 weeks. Senger (1954) reported the life span of *E. revolutum* in chicks to be about 35 days. Many worms as they age migrate posteriorly from their original site and either reestablish or pass out of the host.

<sup>1</sup> This work was supported by NIH Grant AI-06835. The authors gratefully acknowledge the technical assistance of Mr. Jack Carty.

Table 1. Summary of exposure experiments using  $50 \pm 10$ – $100 \pm 20$  cysts pretreated in 3%  $\text{NaHCO}_3$ . Chicks were autopsied 22–35 days postinfection.

Exp.	No. of chicks exposed <sup>1</sup>	No. of infected chicks	No. of cysts per chick	Age of worm at autopsy	Number of worms recovered				Per cent recovery
					Ileum	Ceca	Rectum	Total	
A	8	8	$50 \pm 10$ – $100 \pm 20$	22	7	53	72	132	22.0
B	3	3	$50 \pm 10$	23	0	14	46	60	40.0
C	17	15	$50 \pm 10$	25	58	29	75	162	21.6
D	3	3	$100 \pm 20$	35	40	6	0	46	15.3
Total	31	29	$50 \pm 10$ – $100 \pm 20$	22–35	105	102	193	400	22.2

<sup>1</sup> All chicks day-old when exposed.

To determine the effects of storage on infectivity, cysts maintained for 7 weeks in 3-salt Ringer's at 15 C were fed to chicks. Three of four chicks fed  $100 \pm 20$  cysts and autopsied 7–14 days later were infected with 9, 10, and 23 worms. Senger (1954) reported that cysts stored in 0.4% saline at 5 C for 31 days were viable. Storage of cysts is important in *E. revolutum* studies since many *Physa* that become heavily infected with metacercariae die within 2 weeks postinfection.

To determine if chicks could become infected with cysts via the cloaca, cysts were implanted into the cloacas of four chicks that were autopsied 7–10 days later. Two chicks each received  $100 \pm 20$  cysts pretreated in bicarbonate and two chicks received the same number of cysts pretreated in Ringer's. None was infected.

Cysts occasionally formed ectopically on the mucus trails of *H. trivolvis*. To determine if these cysts were infective, three chicks were fed approximately 20, 60, and 175 mucus cysts and autopsied 7 days later. Only the one chick fed approximately 175 cysts was infected with four worms suggesting that ectopically formed cysts are not as infective as those obtained from the kidney of *Physa* sp. snails.

To determine the effects of a secondary

infection on a primary one 12, 3-day-old chicks were each exposed to  $100 \pm 20$  cysts and were reexposed to the same number of metacercariae 13 days later. Twelve additional chicks were used as controls, half of which received only the primary exposure and the other half the secondary exposure. Chicks were autopsied 9–10 days after the secondary exposure (Table III, H, I, J). To determine if chicks older than 1 month could become reinfected, 14 chicks that were 12 days old at the time of a primary exposure to  $50 \pm 10$  cysts were reexposed each to  $50 \pm 10$  cysts 31 days later. Chicks were autopsied 10–11 days after the secondary exposure (Table III, K). The reinfection experiments indicate that a primary infection of *E. revolutum* does not protect against a secondary infection. Fried (1963) found that a primary infection of *Philophthalmus hegeneri* Penner and Fried, 1963, did not protect chicks against a secondary infection and reviewed the literature on reinfection in trematodes. The reinfection experiments also indicate that chicks older than 2 weeks (Exp. J) can become infected and that chicks older than 1 month (Exp. K) can become reinfected indicating that an age resistance to this worm was not apparent, at least within the first month.

Table 2. Summary of exposure experiments using  $50 \pm 10$  cysts and different pretreatment solutions. Chicks were autopsied 14 days postinfection.

Exp.	No. of chicks exposed <sup>1</sup>	No. of infected chicks	Pretreatment solution	Number of worms recovered				Per cent recovery
				Ileum	Ceca	Rectum	Total	
E	18	16	3% $\text{NaHCO}_3$	104	55	63	222	27.7
F	12	12	3-salt Ringer's	130	73	105	308	51.3
G	12	12	Distilled $\text{H}_2\text{O}$	38	35	73	146	24.3
Total	42	40		272	163	241	676	32.2

<sup>1</sup> All chicks day-old when exposed.

Table 3. Summary of reinfection experiments.

Exp.	No. of chicks exposed	Age of chick at 1° infection (days)	Age of chick at 2° infection (days)	No. of pos. chicks w 1° infection	Age of chick at time of autopsy (days)	No. of worms recovered from 1° infection	No. of pos. chicks w 2° infection	No. of worms recovered from 2° infection
H	12	3	16	10	25-26	83	9	124
I	6	3	16	3	25-26	24	—	—
J	6	3	16	—	25-26	—	5	174
K	14	12	43	6	53-54	15	7	132

Chicks in Exp. H received 100 ± cysts in 1° and 2° infections.  
 Chicks in Exp. I received 100 ± cysts in 1° infection only.  
 Chicks in Exp. J received 100 ± cysts in 2° infection only.  
 Chicks in Exp. K received 50 ± cysts in 1° and 2° infections.

### Summary

Sixty-nine (94.5%) of 73 day-old chicks exposed to 50-100 *Echinostoma revolutum* metacercariae and autopsied 2-5 weeks post-infection yielded worm burdens of 15.3-51.3%. Forty (95.2%) of 42 day-old chicks exposed to metacercariae pretreated in distilled water, 3% NaHCO<sub>3</sub>, 3-salt Ringer's and autopsied 2 weeks later yielded worm recoveries of 24.3%, 27.7%, and 51.3%, respectively. Cysts stored in Ringer's at 15 C for 7 weeks were viable. Cysts formed ectopically on the mucus trails of *Helisoma trivolvis* snails were not as infective as cysts formed within the kidney of *Physa* sp. snails. Worms were not recovered from cysts implanted into the chick cloaca. A primary infection of *E. revolutum* did not protect chicks against a secondary infection. Chicks older than 2 weeks became infected and chicks older than 1 month became re-infected indicating that an age resistance to this worm was not apparent, at least within the first month.

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## Academician K. I. Skrjabin — Ninetieth Birthday

On December 7, 1968, Academician K. I. Skrjabin celebrated his ninetieth birthday and the completion of more than six decades of scientific work. Regarded as the outstanding helminthologist of the Soviet Union, he has gained worldwide recognition for his researches in this field.

Graduating as a veterinary surgeon in 1905 from the Juriev Veterinary Institute, Dr. Skrjabin first became interested in the study of helminths during an assignment in Turkestan. He made extensive collections from both domestic and wild animals and later (1912–1914), studied them under the guidance of Braun, Fuhrmann, Luhe, Railliet, and Henry. In his master's thesis (1916), based on the Turkestan collections, he stressed the importance of establishing helminthology as a science in Russia. In the following year, he was named to the first chair of parasitology in Russia, established at the Novocherkassk Veterinary Institute.

From this vantage point, Dr. Skrjabin gained increasing recognition for his concepts of the significance of helminthic disease in agriculture and public health. In the period 1920–1922 he organized or was instrumental in founding three centers of helminthological research: The Helminthological Department of the State Experimental Veterinary Institute which was later named the All-Union K. I. Skrjabin Institute of Helminthology in honor of its founder; the Helminthological Department of the Moscow Tropical Institute; and a Department of Helminthology at the Moscow State University. In addition to the organization of helminthological institutions in Moscow, he was the moving force in establishing the study of helminthology in research institutions and universities throughout the Soviet Union.

Despite his administrative activities, Dr. Skrjabin found time to continue his morphological and taxonomic studies. Beginning in 1919, he organized and directed surveys of the helminth fauna of man and animals in the Soviet Union. Publication of these studies

together with his lectures on helminthology soon attracted increasing numbers of students. Over 100 candidates have received doctorates under his guidance, and many more can be counted as students of his students. Among the former are a number of corresponding members of the Academies of Sciences of the USSR.

Dr. Skrjabin's investigations in the field of morphology, biology, phylogeny, and systematics of helminths, on ecology, epidemiology, and epizootiology of helminthic diseases and their control have been reported in over 600 publications. Now internationally known through these publications, his contributions to his country's scientific development as an educator, mentor, and administrator have been even more remarkable. To list only some of the honors accorded him in recognition of his accomplishments, he has received honorary degrees from Humboldt University (Berlin), and the Institute of Agriculture (Czechoslovakia). He is a member of the Veterinary Academy of France, a member of the Academies of Science of Bulgaria, Hungary, Czechoslovakia, and Poland, and an honorary member of the American Society of Parasitologists (1932), the Indian Society of Helminthologists, Parasitological Society of Poland, and the Rumanian Parasitological Society. He is a member of three academies in the Soviet Union; Academy of Sciences of the USSR, Academy of Medical Sciences, and the All-Union Academy of Agricultural Sciences. He is president of the All-Union Society of Helminthologists and founder and Chairman of the Editorial Board of *Helminthologia*. Among other honors bestowed by his government are five Orders of Lenin, and on the occasion of his eightieth birthday the name of Hero of Socialist Labor.

The Helminthological Society of Washington congratulates Academician Skrjabin on his ninetieth birthday, a milestone in a career that has exerted a profound influence on all phases of helminthology in the Soviet Union.

—FRANCIS G. TROMBA

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