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Proceedings of the Helminthological Society of Washington

VOLUME 33 JANUARY 1966 NUMBER 1

# Aerobic and Anaerobic Metabolism of Larval and Adult *Taenia taeniaeformis*. III. Influence of Some Cations on Glucose Uptake, Glucose Leakage, and Tissue Glucose

THEODOR VON BRAND AND EVALINE GIBBS[1]

It has been shown (von Brand *et al.*, 1964) that the glucose uptake of adult *Taenia taeniaeformis* is dependent on the presence of environmental $Na^+$, and that in the absence of $Na^+$ the glucose leakage previously observed in sugar-free media is considerably increased. It seemed desirable to study what influence various sodium concentrations have on these processes, and whether the absence or increased concentration of other cations would also change the rates of glucose influx and efflux under either aerobic or anacrobic conditions. The results of relevant experiments, including some determinations on the larval stage, are discussed below.

## MATERIALS AND METHODS

Only paired worms, either adults or larvae, were used. One member of the pair was kept as control in our regular Tyrode's solution, while the other was incubated in one of the experimental salines. The following salines were used; their constituents are given in gm/l.

(1) Control solution (normal Tyrode's solution): NaCl 8.0; KCl 0.2; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(2) Sodium-free Tyrode's solution: KCl 10.4; $KHCO_3$ 1.2; $KH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(3) Low sodium Tyrode's solution A: KCl 10.4; $NaHCO_3$ 1.0; $KH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(4) Low sodium Tyrode's solution B: NaCl 2.0; KCl 7.8; $NaHCO_3$ 1.0; $KH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(5) Low sodium Tyrode's solution C: NaCl 6.0; KCl 2.6; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(6) Potassium-free Tyrode's solution: NaCl 8.15; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(7) Calcium-free Tyrode's solution: NaCl 8.15; KCl 0.2; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2.

(8) Magnesium-free Tyrode's solution: NaCl 8.15; KCl 0.2; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $CaCl_2 \cdot 2H_2O$ 0.3.

(9) Phosphate-free Tyrode's solution: NaCl 8.0; KCl 0.2; $NaHCO_3$ 1.0; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

(10) High calcium Tyrode's solution: NaCl 7.3; KCl 0.2; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 3.0.

(11) High magnesium Tyrode's solution: NaCl 7.5; KCl 0.2; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.06; $MgCl_2 \cdot 6H_2O$ 2.0; $CaCl_2 \cdot 2H_2O$ 0.3.

(12) High phosphate Tyrode's solution: NaCl 7.5; KCl 0.2; $NaHCO_3$ 1.0; $NaH_2PO_4$ 0.6; $Na_2HPO_4$ 1.6; $MgCl_2 \cdot 6H_2O$ 0.2; $CaCl_2 \cdot 2H_2O$ 0.3.

In preparing the high P-Tyrode's solution, care had to be taken to avoid precipitation of calcium phosphate. To this end the phosphate was dissolved in water separately from the other constituents and added to the latter only immediately before use. Nevertheless, upon mixing a turbidity regularly developed which, however, completely disappeared when one of the $CO_2$-containing gases mentioned below was bubbled through the solution for a few minutes before dispensing it into the experimental ves-

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TABLE 1. Influence of various sodium concentrations on glucose absorption, glucose leakage, and tissue glucose of larval and adult *Taenia taeniaeformis*.

A plus sign before a figure indicates glucose leakage, rather than consumption, an * sign indicates that the difference between control and experimental animals is significant. The figures following the ± sign are the standard error of the means. The figures in parentheses are the number of determinations.

| Stage | Condition | Tyrode's solution | | Glucose-containing solutions | | | |
|---|---|---|---|---|---|---|---|
| | | | | Glucose consumed µg/100 mg/1 hour | | Tissue glucose µg/100 mg | |
| | | No. | Characteristic | Control | Experimental | Control | Experimental |
| Larval | Anaerobic | 2 | Na-free | 119 ± 24 (8) | +45 ± 4 (8) * | 176 ± 19 (8) | 250 ± 22 (7) * |
| Larval | Aerobic | 2 | Na-free | 85 ± 17 (8) | +57 ± 10 (8) * | 168 ± 9 (8) | 226 ± 10 (8) * |
| Larval | Anaerobic | 4 | Na-poor B | 40 ± 37 (4) | 5 ± 18 (5) | 155 ± 26 (5) | 234 ± 41 (5) |
| Larval | Aerobic | 4 | Na-poor B | 22 ± 20 (7) | +16 ± 9 (7) | 150 ± 11 (7) | 228 ± 21 (7) * |
| Larval | Anaerobic | 5 | Na-poor C | 111 ± 19 (7) | 122 ± 26 (7) | 171 ± 5 (7) | 183 ± 17 (7) |
| Larval | Aerobic | 5 | Na-poor C | 170 ± 24 (3) | 161 ± 10 (4) | 223 ± 28 (3) | 202 ± 21 (3) |
| Adult | Anaerobic | 2 | Na-free | 322 ± 56 (7) | 25 ± 39 (7) * | 177 ± 25 (9) | 99 ± 15 (9) |
| Adult | Aerobic | 2 | Na-free | 95 ± 8 (7) | +70 ± 34 (7) * | 205 ± 21 (7) | 116 ± 10 (7) * |
| Adult | Anaerobic | 3 | Na-poor A | 269 ± 65 (7) | 83 ± 18 (7) * | 201 ± 56 (7) | 130 ± 12 (7) |
| Adult | Aerobic | 3 | Na-poor A | 485 ± 66 (6) | 135 ± 14 (6) * | 254 ± 25 (6) | 141 ± 17 (6) * |
| Adult | Anaerobic | 4 | Na-poor B | 228 ± 60 (7) | 174 ± 40 (7) | 211 ± 26 (7) | 144 ± 10 (7) |
| Adult | Aerobic | 4 | Na-poor B | 279 ± 34 (6) | 74 ± 21 (6) * | 282 ± 35 (6) | 210 ± 15 (6) |
| Adult | Anaerobic | 5 | Na-poor C | 473 ± 63 (7) | 518 ± 99 (7) | 267 ± 39 (7) | 154 ± 19 (7) |
| Adult | Aerobic | 5 | Na-poor C | 308 ± 67 (8) | 237 ± 76 (8) | 245 ± 33 (8) | 184 ± 35 (8) |

TABLE 1. *Continued.*

| Stage | Condition | Tyrode's solution | | Glucose-free solutions | | | |
|---|---|---|---|---|---|---|---|
| | | | | Glucose leaked µg/100 mg/1 hour | | Tissue glucose µg/100 mg | |
| | | No. | Characteristic | Control | Experimental | Control | Experimental |
| Larval | Anaerobic | 2 | Na-free | +44 ± 23 (7) | +128 ± 22 (7) * | 114 ± 9 (7) | 230 ± 30 (7) * |
| Larval | Aerobic | 2 | Na-free | +34 ± 6 (5) | +127 ± 12 (5) * | 138 ± 16 (5) | 201 ± 44 (5) |
| Larval | Anaerobic | 4 | Na-poor B | +13 ± 5 (5) | +48 ± 6 (5) * | 143 ± 7 (5) | 305 ± 12 (5) * |
| Larval | Aerobic | 4 | Na-poor B | +71 ± 14 (13) | +94 ± 7 (13) | 131 ± 7 (12) | 298 ± 23 (12) * |
| Larval | Anaerobic | 5 | Na-poor C | +5 ± 2 (5) | +19 ± 9 (5) | 168 ± 16 (5) | 186 ± 9 (5) |
| Larval | Aerobic | 5 | Na-poor C | +9 ± 4 (5) | +5 ± 1 (5) | 163 ± 15 (5) | 222 ± 25 (5) |
| Adult | Anaerobic | 2 | Na-free | +21 ± 8 (8) | +139 ± 22 (8) * | 170 ± 57 (8) | 88 ± 12 (8) |
| Adult | Aerobic | 2 | Na-free | +18 ± 11 (9) | +143 ± 9 (9) * | 204 ± 21 (7) | 116 ± 10 (7) * |
| Adult | Anaerobic | 3 | Na-poor A | +11 ± 4 (8) | +63 ± 12 (8) * | 125 ± 27 (8) | 125 ± 11 (8) |
| Adult | Aerobic | 3 | Na-poor A | +17 ± 8 (9) | +67 ± 14 (9) * | 209 ± 32 (8) | 120 ± 12 (8) |
| Adult | Anaerobic | 4 | Na-poor B | +8 ± 4 (7) | +18 ± 6 (7) | 149 ± 25 (7) | 145 ± 18 (7) |
| Adult | Aerobic | 4 | Na-poor B | +7 ± 5 (5) | +29 ± 13 (5) | 143 ± 30 (5) | 259 ± 50 (5) |
| Adult | Anaerobic | 5 | Na-poor C | +1 ± 1 (6) | +1 ± 1 (6) | 157 ± 37 (6) | 123 ± 28 (6) |
| Adult | Aerobic | 5 | Na-poor C | +11 ± 27 (8) | +37 ± 20 (8) | 180 ± 22 (8) | 205 ± 28 (8) |

sels. No precipitation of calcium phosphate occurred then during the duration of the experiments.

Worms kept in the above solutions were used for the study of glucose leakage; the rates of glucose uptake were determined in salines of corresponding composition, but supplemented with 200 mg percent of glucose. All experiments lasted for 2 hours and were conducted at 37 C either aerobically in an atmosphere of 95 percent air + 5 percent $CO_2$, or anaerobically in an atmosphere of 95 percent nitrogen + 5 percent $CO_2$.

All glucose determinations were done by means of the glucose oxidase method of Saifer and Gerstenfeld (1958). All other details of the experimental procedures corresponded to those described previously (von Brand and Bowman, 1962; von Brand *et al.*, 1964).

The resulting data were analyzed statistically using the formula $S = \frac{M_1 - M_2}{\sqrt{E_1^2 + E_2^2}}$. If the resulting figure exceeded 2.0, the difference between the means was considered to be statistically significant.

## RESULTS AND DISCUSSIONS

The results of our experiments dealing with the influence of sodium on sugar consumption and leakage are presented in Table 1. It is evident that neither adult nor larval worms could withdraw glucose from a $Na^+$-free solu-

TABLE 2. Influence of lack or increased concentration of some cations on glucose absorption, glucose leakage, and tissue glucose of adult *Taenia taeniaeformis*. The same codes given in Table 1 apply.

| Condition | Tyrode's solution | | Glucose-containing solutions | | | |
|---|---|---|---|---|---|---|
| | | | Glucose consumed µg/100 mg/1 hour | | Tissue glucose µg/100 mg | |
| | No. | Characteristic | Control | Experimental | Control | Experimental |
| Anaerobic | 6 | K-free | 436 ± 63 (6) | 317 ± 66 (6) | 131 ± 21 (6) | 132 ± 30 (6) |
| Aerobic | 6 | K-free | 256 ± 71 (6) | 213 ± 72 (6) | 248 ± 40 (6) | 189 ± 13 (6) |
| Anaerobic | 7 | Ca-free | 190 ± 72 (5) | 140 ± 45 (5) | 189 ± 29 (5) | 117 ± 21 (5) |
| Aerobic | 7 | Ca-free | 104 ± 41 (5) | 101 ± 38 (5) | 240 ± 34 (5) | 170 ± 19 (5) |
| Anaerobic | 8 | Mg-free | 295 ± 41 (6) | 200 ± 46 (6) | 142 ± 41 (5) | 207 ± 46 (5) |
| Aerobic | 8 | Mg-free | 357 ± 71 (6) | 266 ± 38 (6) | 300 ± 20 (6) | 268 ± 31 (6) |
| Anaerobic | 9 | P-free | 433 ± 61 (4) | 423 ± 70 (3) | 238 ± 70 (3) | 202 ± 30 (3) |
| Aerobic | 9 | P-free | 306 ± 63 (7) | 263 ± 63 (7) | 277 ± 31 (7) | 252 ± 16 (6) |
| Anaerobic | 10 | High-Ca | 301 ± 81 (5) | 304 ± 96 (5) | 234 ± 39 (5) | 227 ± 51 (5) |
| Aerobic | 10 | High-Ca | 250 ± 97 (8) | 277 ± 76 (8) | 342 ± 31 (8) | 306 ± 24 (8) |
| Anaerobic | 11 | High-Mg | 378 ± 92 (7) | 342 ± 102 (7) | 242 ± 23 (15) | 244 ± 40 (14) |
| Aerobic | 11 | High-Mg | 128 ± 43 (5) | 200 ± 67 (5) | 276 ± 27 (5) | 354 ± 58 (5) |
| Anaerobic | 12 | High-P | 332 ± 37 (7) | 360 ± 53 (7) | 209 ± 58 (7) | 180 ± 36 (7) |
| Aerobic | 12 | High-P | 233 ± 31 (11) | 230 ± 26 (11) | 333 ± 41 (11) | 303 ± 28 (11) |

TABLE 2. *Continued.*

| Condition | Tyrode's solution | | Glucose-free solutions | | | |
|---|---|---|---|---|---|---|
| | | | Glucose leaked µg/100 mg/1 hour | | Tissue glucose µg/100 mg | |
| | No. | Characteristic | Control | Experimental | Control | Experimental |
| Anaerobic | 6 | K-free | +10 ± 7 (6) | +15 ± 6 (6) | 70 ± 12 (6) | 45 ± 13 (6) |
| Aerobic | 6 | K-free | +28 ± 15 (5) | +25 ± 19 (5) | 136 ± 37 (5) | 95 ± 24 (5) |
| Anaerobic | 7 | Ca-free | +1 ± 1 (6) | +6 ± 4 (6) | 66 ± 20 (6) | 78 ± 11 (6) |
| Aerobic | 7 | Ca-free | +1 ± 1 (6) | +2 ± 1 (6) | 127 ± 24 (6) | 93 ± 16 (6) |
| Anaerobic | 8 | Mg-free | +3 ± 2 (7) | +5 ± 4 (7) | 123 ± 21 (7) | 111 ± 23 (7) |
| Aerobic | 8 | Mg-free | +4 ± 4 (5) | +18 ± 12 (5) | 160 ± 50 (5) | 148 ± 14 (5) |
| Anaerobic | 9 | P-free | +3 ± 3 (6) | +3 ± 20 (6) | 140 ± 27 (5) | 182 ± 40 (5) |
| Aerobic | 9 | P-free | +1 ± 1 (5) | +1 ± 1 (5) | 75 ± 17 (5) | 77 ± 16 (5) |
| Anaerobic | 10 | High-Ca | +3 ± 1 (6) | +1 ± 1 (6) | 87 ± 10 (6) | 107 ± 19 (4) |
| Aerobic | 10 | High-Ca | 0 ± 0 (6) | 0 ± 0 (6) | 138 ± 13 (6) | 178 ± 24 (6) |
| Anaerobic | 11 | High-Mg | +5 ± 4 (6) | +31 ± 17 (6) | 111 ± 12 (6) | 199 ± 27 (6) |
| Aerobic | 11 | High-Mg | +52 ± 21 (8) | +40 ± 19 (8) | 202 ± 27 (8) | 207 ± 29 (8) |
| Anaerobic | 12 | High-P | +5 ± 3 (6) | +5 ± 2 (6) | 118 ± 29 (6) | 143 ± 33 (6) |
| Aerobic | 12 | High-P | +1 ± 1 (7) | +4 ± 4 (7) | 165 ± 36 (7) | 159 ± 20 (7) |

tion, a fact already known for adults (von Brand *et al.*, 1964). The larvae appear to require a higher sodium concentration in the environment than the adults to permit sugar absorption. Adult worms could consume reduced, but significant, amounts of glucose from Tyrode's solutions containing 12 and 46 mmoles Na/l (Tyrode's solutions A and B, respectively), while the larvae did not absorb glucose from Tyrode's solution B. Both stages, however, showed normal rates of glucose consumption when kept in a solution containing 115 mmoles Na/l (Tyrode's solution C).

Another definite difference existed between larval and adult worms in respect to tissue glucose. Incubation in sodium-free or sodium-poor solutions, from which, as mentioned above, no sugar was absorbed, led in the larvae to an increased glucose level of the tissues, presumably due to a breakdown of glycogen. In adult worms, on the contrary, incubation in sodium-free or -poor solutions led to a decreased glucose level in the tissues. It should be noted that the glucose levels of the larval tissues after incubation reached values in excess of 200 mg percent; that is, they surpassed the glucose concentration of the medium. As the figures of Table 1 indicate, the larvae excreted in most of these solutions some glucose, rather than withdrawing it from the medium. In view of the concentration differences mentioned, it would be unjustified to assume excretion against a concentration gradient and to postulate active transport mechanisms as involved in the glucose leakage.

In glucose-free and sodium-free, *or -poor* solutions (Tyrode's B) the sugar leakage of the larvae was increased, but the increase in leakage was far more pronounced in the total

absence of $Na^+$ than in its partial absence. Adult worms reacted in this respect essentially similarly. The tissue glucose level of the larval tissues was again increased, reaching levels comparable to those found in the corresponding glucose-containing solutions mentioned above. In adult worms, with the exception of those maintained aerobically in Tyrode's solution B, the tissue glucose levels of the experimental animals equalled or were lower than those found in the controls. We have not established the exact mechanism of glucose leakage. However our experiments seem to indicate that at least two factors are involved, a change in permeability of the cuticle induced by lack of $Na^+$, and also an increased rate of breakdown of intracellular glycogen.

Solutions from which $Ca^{++}$, $Mg^{++}$, $K^+$, or $P^{+++}$ had been omitted did not alter significantly glucose uptake, leakage, or glucose tissue levels of adult worms (Table 2). Similarly a tenfold increase in $Ca^{++}$, $Mg^{++}$ (Table 2), a thirteenfold increase in $K^+$ (Tyrode's solution C of Table 1), or a thirty-threefold increase in $P^{+++}$ did not change these parameters significantly. However, two points should be kept in mind. First, the variability was pronounced in all experiments and may have prevented us from recognizing some small-scale change as significant. Secondly, we emphasize that we consider our findings to apply only to short-term experiments. It can be assumed that in long-lasting experiments tapeworms, like most other organisms, would also require those environmental cations that could be omitted without inducing changes in glucose absorption in the present experiments.

The reactions to omission, or increased concentration of the cations mentioned, were essentially similar whether the experiments were conducted aerobically or anaerobically. This is not surprising, since it is well known that the aerobic metabolism of tapeworms is characterized by the persistence of aerobic fermentations (von Brand and Bowman, 1961).

## SUMMARY

(1) Both larval and adult *Taenia taeniaeformis* failed to absorb environmental glucose in the complete absence of $Na^+$. When kept in very $Na^+$-poor solutions, adult worms consumed some glucose while larval ones did not.

(2) In a solution containing 115 mmoles Na/l, both stages consumed normal amounts of glucose.

(3) In $Na^+$-free or -poor solutions the tissue glucose level of larval worms was increased, while that of adults had a tendency to decrease.

(4) Total lack of $Na^+$ in sugar-free solution increased considerably the glucose leakage of both larval and adult worms. Even small amounts of environmental sodium decreased this leakage and in an environment containing 115 mmoles Na/l the leakage was identical with that found in normal Tyrode's solution.

(5) Absence of $Ca^{++}$, $K^+$, $Mg^{++}$, or $P^{+++}$, or a ten to thirty-threefold increase in concentration of these cations failed to alter noticeably glucose uptake, -leakage, or tissue glucose level of adult worms.

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# Three New Species of *Criconemoides* (Nematoda : Criconematidae)

K. A. DIAB AND W. R. JENKINS[1]

During a study of the genus *Criconemoides* Taylor, 1936, collections were obtained from nematologists in various locations. Among these collections were specimens of unidentified species from coconut in the Philippine Islands, cranberry in New Jersey, and laurel in California.

The specimens from the Philippine Islands and California had been previously mounted in glycerin; those from New Jersey were living and subsequently heat relaxed at 45 C, fixed in FAA (Formalin 6 ml: acetic acid 1 ml: 95% ethyl alcohol 15 ml), and mounted in glycerin according to Seinhorst (1959). Measurements were made with the the aid of an ocular micrometer and with camera lucida tracings.

This paper includes the description of these three species.

*Criconemoides discolabium* n. sp. (Fig. 1)

MEASUREMENTS: FEMALES (3): L = 0.25 mm (0.24–0.26 mm); a = 12.8 (11.5–13.6); b = 3.8 (3.6–4.0); c = 18.6 (18.0–19.4); V = 90% (88–92%); stylet = 38 $\mu$ (37–40 $\mu$); annules = 167 (162–174).

MALE: Unknown.

HOLOTYPE (FEMALE): L = 0.26 mm; a = 13.6; b = 3.7; c = 18.5; V = 91%; stylet = 38 $\mu$; annules = 164.

DESCRIPTION: Female (Fig. 1, A–D). Body annules fine, 1.5 to 2 $\mu$; without lateral field. Annules retrorse, rounded on posterior edges. Lip region wider than body bearing a single annule, disclike in shape. Six lips present, sublateral lobes absent. Amphid slit-like, located on lateral lips. Labial disc not visible. Stylet straight with anterior surface of knobs concave. Dorsal gland orifice 2 $\mu$ posterior to stylet knobs. Cardia small, projecting into intestine about two annules in length. Excretory pore 71–80 $\mu$ from anterior end, located on 50th body annule. Vulva located on 15th to 17th annule from terminus. Lips of vulva without distinctive characters. Gonad 74–86 $\mu$ long. Spermatheca absent. Anus located on 12th to 14th annule from tail tip. Tail broadly conoid, ventrally convex, terminus pointed.

DIAGNOSIS AND RELATIONSHIPS: This species differs from all other *Criconemoides* in its unique disclike lip. It most closely resembles *C. parvulum* Siddiqi and *C. parvum* Raski but has a pointed tail terminus instead of a rounded one; its anus is more anteriorly located (12th to 14th annule) than either *C. parvum* (3rd annule) or *C. parvulum* (6th annule), and its

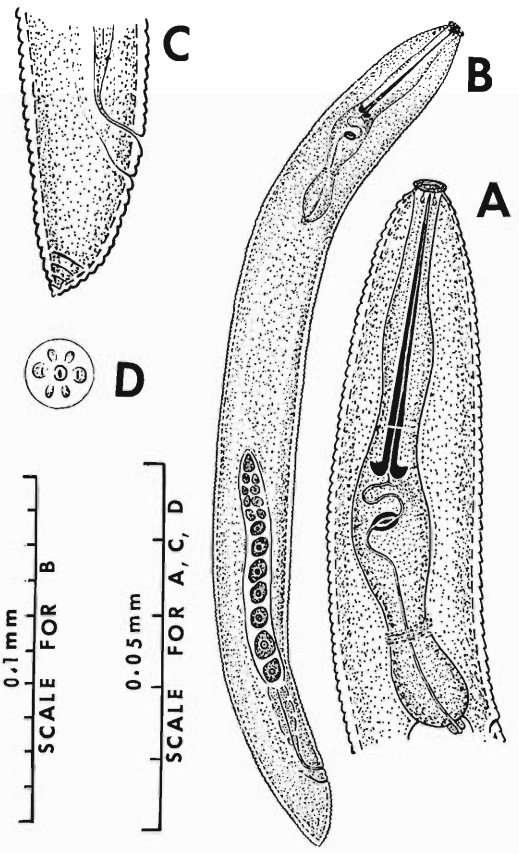


Fig. 1, A–D. Drawings of *Criconemoides discolabium* n. sp. A, esophageal region of female; B, lateral view of female; C, lateral view of tail region; D, *en face* view.

[1] Paper of the Journal Series, Department of Entomology and Economic Zoology, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

vulva is more anteriorly located (90%) than *C. parvum* (92.5–95.9%) or *C. parvulum* (93.8–95.2%). Also, it has a longer stylet (37–40 μ) than *C. parvulum* (30–34 μ), and it has a larger number of annules (162–174) than *C. parvum* (156).

TYPE LOCALITY: Soil from about coconut tree, Manila, P.I.

TYPE SLIDES: Holotype, slide no. D-176, Rutgers University collection, and two paratypes, slides 83/8/1 and 83/8/2, Department of Nematology, Rothamsted Experimental Station, Harpenden, Herts, England.

This species was kindly loaned by Mr. David J. Hooper, Rothamsted Experimental Station.

*Criconemoides reedi* n. sp. (Fig. 2)

MEASUREMENTS: FEMALES (25): L = 0.40 mm (0.36–0.46 mm); a = 8.4 (7.8–9.5); b = 3.8 (3.6–4.2); c = 24.7 (23.0–29.0); V = 91% (90–92%); stylet = 59 μ (51–62 μ); annules = 107 (104–112).

MALE: Unknown.

HOLOTYPE (FEMALE): L = 0.46 mm; a = 9.2; b = 4.2; c = 23.0; V = 91%; stylet = 62 μ; annules = 112.

DESCRIPTION: Female (Fig. 2, A–D). Body annules coarse, 3 to 4 μ; without lateral field. Annules retrorse with rounded posterior edges. Lip region continuous. Annules progressively more narrow from base of stylet to lip region. Sublateral lobes enlarged, located equidistant around labial disc. Labial disc not elevated. Amphids slit-like, occurring on lateral margins of labial disc. Stylet strongly developed with anteriorly concave knobs. Esophagus criconematoid. Dorsal gland orifice 12 μ posterior to stylet knobs. No cardia observed. Excretory pore 90 to 91 μ from anterior on 26th to 28th body annule. Vulva on 9th to 10th annule from terminus. Gonad outstretched, sometimes extending to base of esophagus. Spermatheca absent. Anus on 5th to 7th body annules from terminus. Tail acute conoid with pointed terminus.

DIAGNOSIS AND RELATIONSHIPS: This species differs from *C. rusticum* (Micoletzky), *C. lobatum* Raski, *C. curvatum* Raski, and *C. xenoplax* Raski in its pointed tail. It is most closely related to *C. morgense* (Hofmanner & Menzel), but differs in its shorter stylet (51–62 μ compared to 85 μ in *C. morgense*), also its shorter body length (0.39–0.43 mm compared to 0.55–0.59 mm in the latter).

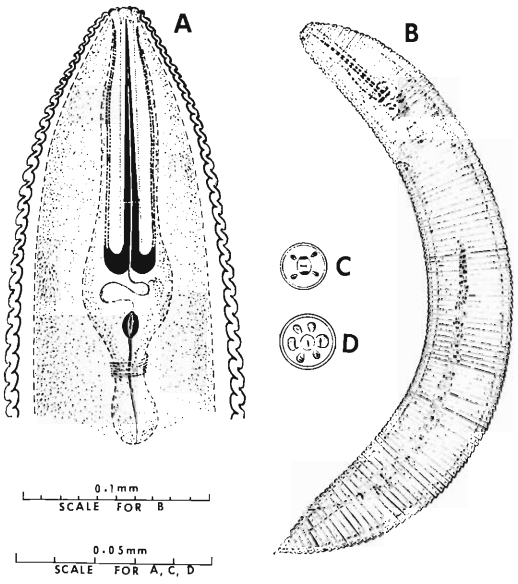


Fig. 2, A–D. Drawings of *Criconemoides reedi* n. sp. A, esophageal region; B, lateral view of female; C, *en face* view showing sublateral lobes; D, cephalic framework.

TYPE LOCALITY: Soil from about roots of cranberry, *Vaccinium macrocarpum*, near New Lisbon, Burlington County, New Jersey, USA.

TYPE SLIDES: Holotype, slide no. D-177 and 24 paratypes, slides nos. D-178 to D-192, Rutgers University collection.

This species was collected by Mr. G. W. Bird, Rutgers University, and is named in honor of Dr. J. P. Reed, Department of Entomology and Economic Zoology, Rutgers University.

*Criconemoides californicum* n. sp. (Fig. 3)

MEASUREMENTS: FEMALES (7): L = 0.33 mm (0.31–0.35 mm); a = 11.1 (10.8–11.6); b = 3.6 (3.3–4.2); c = 21.5 (20.8–23.2); V = 89% (88–90%); stylet = 64 μ (63–65 μ); annules = 105 (104–107).

MALE: Unknown.

HOLOTYPE (FEMALE): L = 0.33 mm; a = 11.6; b = 3.5; c = 21.6; V = 88%; stylet = 65 μ; annules = 104.

DESCRIPTION: Females (Fig. 3, A–C). Body annules coarse, 3 to 4 μ; without a lateral line.

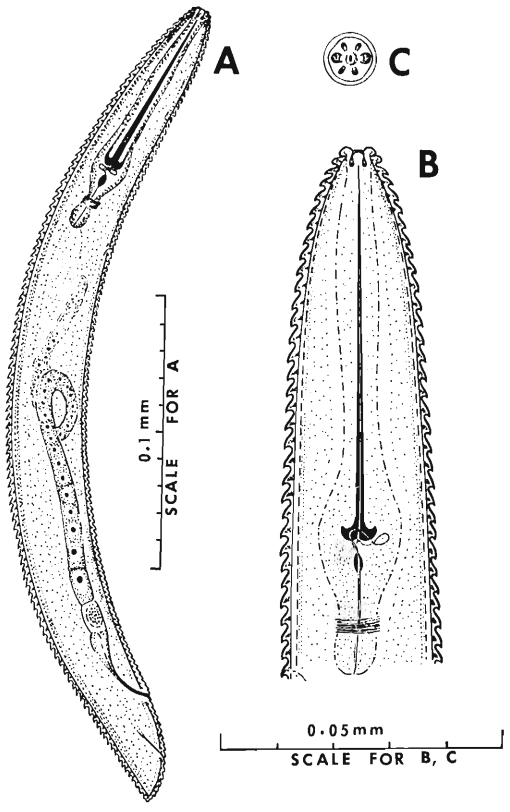


Fig. 3, A–C. Drawings of *Criconemoides californicum* n. sp. A, lateral view of female; B, esophageal region; C, *en face* view.

Annules sharply retrorse. Lip region with one annule, wider than the first body annule. Sublateral lobes absent. Six lips, amphids slit-like, occurring on lateral lips. Stylet slender with anteriorly concave knobs. Dorsal gland orifice 1 to 2 $\mu$ behind stylet knobs. No cardia observed. Excretory pore about 40 annules from anterior end. Vulva 13 to 14 annules from tail tip. Gonad with double flexure. Spermatheca a rounded portion at anterior end of uterus, not set off. Anus 7 to 8 annules from tail tip. Tail broadly conoid, ventrally convex, terminus knob-like.

DIAGNOSIS AND RELATIONSHIPS: This species is most closely related to *C. mutabile* Taylor and *C. kovacsi* Andrassy. From the former, it differs in that its tail is ventrally convex–conoid and ends in a knob-like terminus rather than being bluntly rounded; its longer stylet (63–65 $\mu$); and its more anteriorly located vulva. From *C. kovacsi* it differs in its shorter body (0.51 mm for *kovacsi*); smaller number of body annules (117 in *kovacsi*); and its longer stylet (63–65 $\mu$) as compared to *kovacsi* (59 $\mu$).

TYPE LOCALITY: Soil from about roots of laurel tree, San Francisco, California, USA.

TYPE SLIDES: Holotype, slide no. D-193, and two paratypes, D-194 and D-195, Rutgers University collection. Two paratypes in the Departmet of Nematology, University of California, Riverside, California, USA.

This species was loaned by Dr. S. A. Sher, University of California, Riverside.

## SUMMARY

Three new species of *Criconemoides; C. discolabium* from coconut in the Philippine Islands, *C. reedi* from cranberry in New Jersey, and *C. californicum* from laurel in California, were described and illustrated. The presence of a disclike lip distinguishes *C. discolabium* from all other species of this genus; *C. reedi* resembles *C. morgense* but differs in its shorter stylet and body length; *C. californicum* resembles *C. mutabile* and *C. kovacsi* but differs in its ventrally convex–conoid tail with knob-like terminus, its longer stylet, and its more anteriorly located vulva.

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# The Genus *Acrostichus* Rahm 1928, Synonym *Diplogasteritus* Paramonov 1952 (Nematoda)

CALVIN L. MASSEY[1]

In January of 1962, the writer published a paper entitled "New Species of Diplogasteridae (Nematoda) Associated with Barkbeetles in the United States." A portion of the paper was devoted to the genus *Acrostichus*. At the time the paper was authored, the writer was unaware of several publications directly related to a number of the species discussed. *Diplogaster nudicapitatus* Steiner, 1914, which is the type species of the genus *Diplogasteritus* Paramonov, 1952, was placed in the genus *Acrostichus* and, in effect, made *Diplogasteritus* a synonym of *Acrostichus*. At that time, only three of the species contained in the genus *Diplogasteritus* were moved to the genus *Acrostichus*. Dr. A. D. Baker, Research Associate Emeritus, Nematology Section, Ottawa, Canada, brought the errors to my attention, and in this paper it is hoped that much of the confusion will be rectified.

A thorough study of the literature and a review of original descriptions and illustrations confirmed the synonymous relationship between the genus *Acrostichus* Rahm, 1928, and *Diplogasteritus* Paramonov, 1952. Research on the diagnostic characteristics of the two genera revealed the following salient facts.

The genus *Acrostichus* was erected by Rahm, 1928, with *A. toledoi* Rahm, 1928, as its type species. It was treated as a valid genus (e.g., Filipjev, 1934; Filipjev and Sch. Stekhoven, 1941; Chitwood and Chitwood, 1950) until Goodey, 1951, placed it as a synonym of *Diplogaster*. Rühm, 1956, apparently agreed with Goodey and the genus remained in this position (so far as I am aware) until Massey, 1962, reestablished it as a valid genus and emended the diagnosis. Goodey, 1963, listed it among his genera and species inquirendae.

Of interest is the action of Chitwood and Chitwood, 1950. These authors placed *Acrostichus* in the subfamily Cephalobiinae together with *Cephalobium* Cobb, 1920, *Loxolaimus* (Rahm, 1928) Chitwood and Chitwood, 1950, *Butlerius* Goodey, 1929, and *Odontopharynx* de Man, 1912. Filipjev, 1934, erected the subfamily and designated *Cephalobium* as the type genus. He placed the subfamily in the family Anguillulidae. The subfamily was characterized by the Chitwoods as having a stoma consisting of two well-developed, distinctly sclerotized tandem parts, teeth if present in posterior part of stoma, labial rugae weak or absent, esophagus elongate.

In apposition, other authorities have placed the aforementioned genera in various other subfamilies of Diplogasteridae, Cephalobidae, and Odontopharyngidae. Skrjabin *et al.*, 1954, placed *Cephalobium* in Cephalobiinae of Cephalobidae. Micoletzky, 1922, put *Odontopharynx* in Diplogasterinae n. subfamily of Odontopharyngidae n. fam. The same genus was placed in Odontopharyngidae of Diplogasterata by Paramonov in 1956 and in Odontopharynginae of Diplogasteridae by Andrassy in 1958. Baker concurred in this last placement. *Butlerius* was put in Diplogasterinae of Diplogasteridae by Goodey, 1951, Paramonov, 1956, Andrassy, 1958, Baker, 1962, and Goodey, 1963. *Loxolaimus* is considered a synonym of *Diplogaster*.

In comparing the two genera, by examination of illustrations and descriptions of the type species, it is difficult to reconcile the placement of *Cephalobium microbivorum* Cobb, 1920, and *Acrostichus toledoi* Rahm, 1928, within the same subfamily. Morphologically the complicated structures at the base of the stoma, and the structure of the esophagus in *Cephalobium*, preclude its placement in the same subfamily with *Acrostichus*. Biologically the habitat of the two genera are considerably different. Members of the genus *Cephalobium* are internal parasites of arthropods, while *Acrostichus* is saprophytic and free-living. In the opinion of the writer, the genus *Acrostichus* is a valid member of the subfamily Diplogasterinae.

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Rahm's original description and the writer's emended description of *Acrostichus* together with Paramonov's description of the genus *Diplogasteritus* follows.

Genus *Acrostichus* Rahm, 1928

"Cuticle with very prominent longitudinal striations. Head rounded without cephalic papillae. The stoma with a large tooth. The genital apophyses of the female unequal. The male without a bursa. The gubernaculum massive." Rahm's illustrations of the genus reveal a tail which is also typical of *Diplogasteritus*.

Genus *Acrostichus* Rahm, 1928, Massey, 1962, diagnosis emended

Cuticle with very prominent longitudinal and moderately fine transverse striations. Head usually narrowed forward from anterior end of neck in lateral view. Stoma much deeper than wide, 10–15 microns in depth, 2.5–4 microns in width, consisting of a cheilostom with distinct cheilorhabdions, protostom with distinct prorhabdions. Meso-, meta-, and telorhabdions at times fused, forming a glottoid apparatus armed with dorsal and subventral teeth, varying from two to four in number. Esophagus typically diplogasteroid. Females amphidelphic, the ovaries usually strongly reflexed and either meeting or crossing in the region of the vulva. Females with a large reniform spermatheca, in all species closely examined. Spicules paired, ventrally arcuate, cephalated. Gubernaculum massive, variable in shape. Preanal and caudal papillae variable in number. Tails in both sexes filiform.

Genus *Diplogasteritus* Paramonov, 1952 (Translated from Russian)

The cuticulum is annular, has longitudinal stripes. The cephalic tactile–sensory organs are usually papilla-like. There are amphids on the labia. The stoma is deep; its length is greater than its width. The cheilostoma has no longitudinal folds. The cheilorhabdions are, optically, distinctly separated from the prorhabdions. The dorsal tooth is the largest, is outstanding because of its dimensions, sets in the protostoma or near the telostoma along side which subventral teeth occur. But the middle bulb is in the middle of the total length of the esophagus, is well separated. There are two ovaries. The spiculae are thin, curved; the carina is usually curved, adjoins the spiculae, less frequently, not lying close to them, aberrant. The cauda of the males is always typical, it is adorned with pre-, ad-, and postanal papillae and short setae; on the line between the proximal conical and distal threadlike parts of the cauda are the "terminal" setae.

Meyl, 1961, recognized the following generic characters for *Diplogasteritus*: the large gubernaculum; the length, shape, and armature of the pharynx; and the shape of the tail.

Goodey, 1963, mentions a rudimentary bursa as characteristic of the genus *Diplogasteritus*. Neither Paramonov nor Meyl uses this as a diagnostic character, although Meyl illustrates a rudimentary bursa on *D. nudicapitatus*.

Figure 1 (A, B, C, D) illustrates the typical characters of the genus *Acrostichus*.

*Acrostichus* Rahm, 1928

Synonym *Diplogasteritus* Paramonov, 1952

*Acrostichus toledoi* Rahm, 1928 (Type)

Synonym:

*Diplogaster toledoi* (Rahm, 1928) Goodey, 1951

*Acrostichus angustilaimus* (Sch. Stekhoven and Teun, 1938) n. comb.

Synonyms:

*Diplogaster angustilaimus* Sch. Stekhoven and Teun, 1938

*Diplogaster angustilaimus* (Sch. Stekhoven and Teun, 1938) Goodey, 1963

*Acrostichus arcuatus* Massey, 1962

*Acrostichus austriacus* (Fuchs, 1938) n. comb.

Synonyms:

*Diplogaster consobrinus* var. *austriacus* Fuchs, 1938

*Diplogaster austriacus* Fuchs, 1938 (Steiner, 1914)

*Diplogaster* (*Diplogaster*) *austriacus* Fuchs, 1938 (Körner, 1954)

*Diplogasteritus austriacus* (Fuchs, 1938) Paramonov, 1952

*Acrostichus consobrinus* var. *austriacus* (Fuchs 1938) Massey, 1962

*Acrostichus concolor* Massey, 1962

*Acrostichus consobrinus* (de Man, 1920) Massey, 1962

Synonyms:

*Diplogaster consobrinus* de Man, 1920

*Diplogaster* (*Diplogaster*) *consobrinus* de Man, 1920 (Körner, 1954)

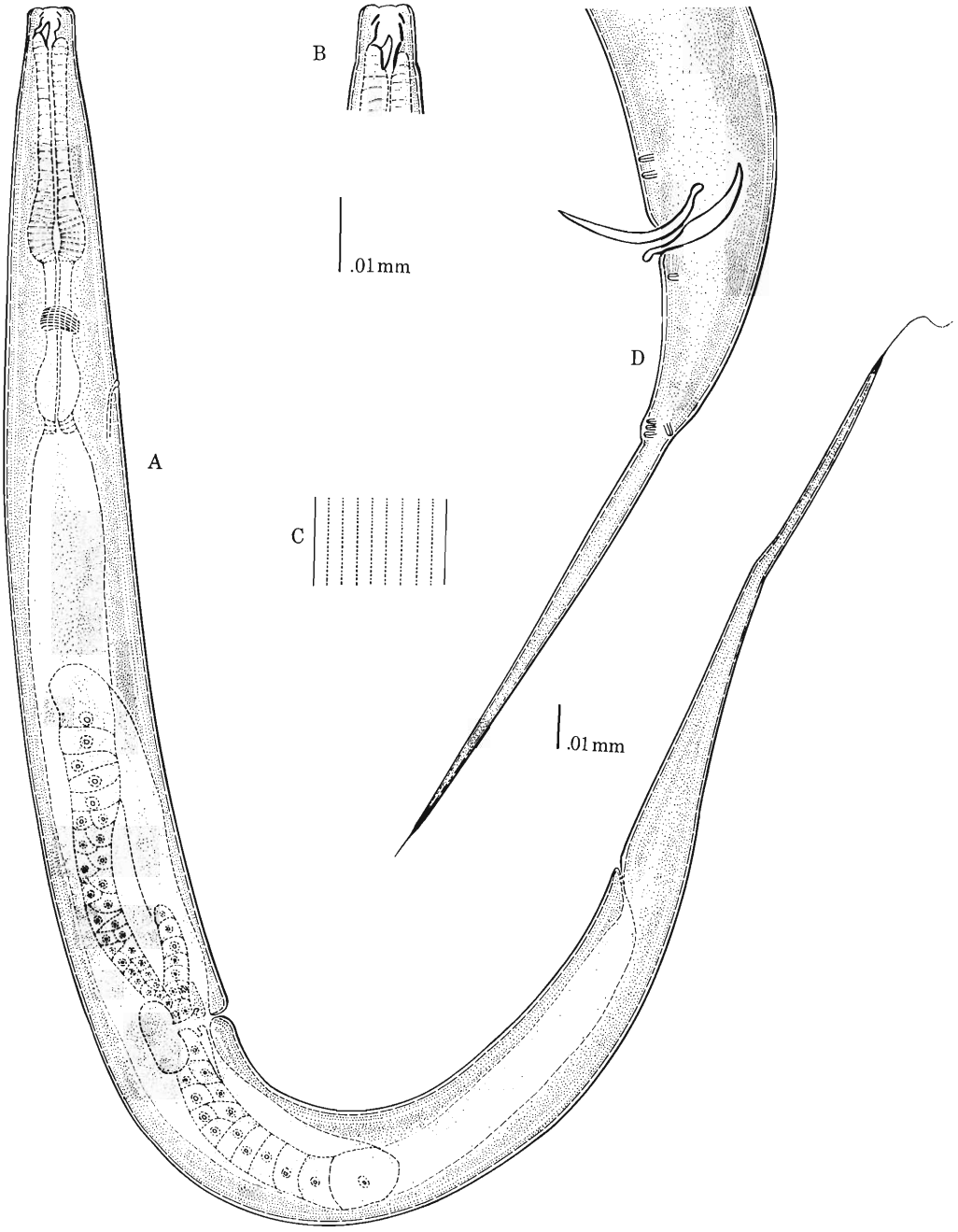


Fig. 1. *Acrostichus ponderosus*, Massey, 1962. A, female; B, head; C, cuticle pattern; D, male tail.

*Diplogasteritus consobrinus* (de Man, 1920) Paramonov, 1952
*Diplogastrellus consobrinus* (de Man, 1920) Meyl, 1961

*Acrostichus demani* (W. Schneider, 1923) n. comb.
Synonyms:
*Diplogaster demani* W. Schneider, 1923
*Diplogasteritus demani* (W. Schneider, 1923) Paramonov, 1952
*Diplogasteriana demani* (W. Schneider, 1923) Goodey, 1963

*Acrostichus dendrophilus* (Weingärtner in Körner 1954) n. comb.
Synonyms:
*Diplogaster dendrophilus* Weingärtner in Körner, 1954
*Diplogaster* (*Diplogaster*) *dendrophilus* Weingärtner in Körner, 1954 (Weingärtner, 1955)
*Diplogastrellus dendrophilus* (Weingärtner in Körner, 1954) Meyl, 1961
*Diplogasteritus dendrophilus* (Weingärtner in Körner, 1954) Goodey, 1963

*Acrostichus lineatus* (Fuchs, 1915) Massey, 1962
Synonyms:
*Diplogaster lineatus* Fuchs, 1915
*Diplogaster* (*Fuchsia*) *lineatus* Fuchs, 1915 (Micoletzky, 1922)
*Diplogaster* (*Diplogaster*) *lineatus* Fuchs, 1915 (Körner, 1954)
*Fuchsia lineata* (Fuchs, 1915) Paramonov, 1952
*Diplogasteritus lineatus* (Fuchs, 1915) Meyl, 1961
*Mikoletzkya lineata* (Fuchs, 1915) Baker, 1962

*Acrostichus microstoma* (Goodey, 1929) n. comb.
Synonyms:
*Diplogaster microstoma* Goodey, 1929
*Diplogasteritus microstoma* (Goodey, 1929) Meyl, 1961

*Acrostichus minutus* (Kreis, 1930) n. comb.
Synonyms:
*Diplogaster minutus* Kreis, 1930
*Diplogaster minor* of Kreis, 1929 (nec Cobb, 1893)
*Diplogasteritus minutus* (Kreis, 1930) Paramonov, 1952

*Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962
Synonyms:
*Diplogaster nudicapitatus* Steiner, 1914 (Type of *Diplogasteritus*)
*Diplogaster* (*Diplogaster*) *nudicapitatus* Steiner, 1914 (Hirschmann, 1952)
*Diplogasteritus nudicapitatus* (Steiner, 1914) Paramonov, 1952 (Type of *Diplogasteritus*)

*Acrostichus occidentalis* (Steiner, 1932) Massey, 1962
Synonyms:
*Diplogaster occidentalis* Steiner, 1932
*Diplogasteritus occidentalis* (Steiner, 1932) Paramonov, 1952

*Acrostichus ponderosus* Massey, 1962

*Acrostichus pterygatus* (Timm, 1961) n. comb.
Synonyms:
*Diplogaster* (*Diplogaster*) *pterygatus* Timm, 1961
*Diplogasteritus pterygatus* (Timm, 1961) Timm, 1961

*Acrostichus rhodani* (Stefanski, 1914) n. comb.
Synonyms:
*Diplogaster rhodani* Stefanski, 1914
*Diplogasteritus rhodani* (Stefanski, 1914) Paramonov, 1952
(Stefanski, 1916, placed *Diplogaster rhodani*, Stefanski, 1914, as a synonym of *Diplogaster nudicapitatus*, Steiner, 1914. The writer, however, does not feel qualified to judge the merit of the change without examining the specimens for specific variation.)

*Acrostichus stoeckherti* (Völk, 1950) n. comb.
Synonyms:
*Diplogaster stoeckherti* Völk, 1950
*Diplogaster* (*Diplogaster*) *stoeckherti* Völk, 1950 (Weingärtner, 1955)
*Diplogasteritus stoeckherti* (Völk, 1950) Paramonov and Sobolev, in Skrjabin *et al.*, 1954

*Acrostichus superbus* (Paesler, 1946) n. comb.
Synonyms:
*Diplogaster superbus* Paesler, 1946
*Diplogaster* (*Diplogaster*) *superbus* Paesler, 1946 (Hirschmann, 1952)
*Diplogasteritus superbus* (Paesler, 1946) Paramonov, 1952

*Acrostichus taedus* Massey, 1962

*Diplogaster subterraneus* Hnatewytsch, 1929, was moved to *Diplogasterium* by Paramonov, 1952, to *Anchidiplogaster* by Meyl, 1961, and to *Diplogasteritus* by Goodey, 1963. In the writer's opinion it is not a valid member of *Acrostichus*. Goodey, 1963, included several other species as possibly belonging to *Diplogasteritus*. These are: *Diplogaster zurstrasseni* Sachs, 1950 (type species of *Sachsia* Meyl, 1961); *D. eurycephalus* Völk, 1950 (to *Anchidiplogaster* by Meyl, 1961); *D. brevicaudatus* Schuurmans Stekhoven and Teunissen, 1938; and *D. labiata* Cobb in Merrill and Ford, 1916 (to *Pristionchus* by Paramonov, 1952). In the opinion of the writer, none of these are valid members of the genus *Acrostichus*.

The writer was unable to examine illustrations or specimens of *Diplogaster liratus* (A. Schneider, 1866) Orley, 1886, or of *Diplogaster filicaudatus* Butschli, 1874, and offers no opinion on their systematic position.

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## The Genus *Mikoletzkya* (Nematoda) in the United States

CALVIN L. MASSEY[1]

The genus *Mikoletzkya* was erected by Rühm in 1960. Weingärtner, 1955, had previously used the name to designate a subgenus of the genus *Diplogaster*.

Of the 10 species listed by Baker, 1962, as belonging to the genus, only two are from North America: *Mikoletzkya aerivora* (Cobb in Merrill and Ford, 1916) Baker, 1962, and *Mikoletzkya pinicola* (Thorne, 1935) Baker, 1962; the others are European in origin. All are insect associates and, for the most part, specifically associates of bark beetles.

Four new species were collected by the author in the course of studies on nematode parasites and associates of bark beetles. In addition, one other species, *Mikoletzkya cerviculata* Massey, 1966, was recovered and described in a recent paper on "Nematode Parasites and Associates of *Dendroctonus adjunctus* Blandford in New Mexico."

Members of the genus are characterized by a broadly rounded head. Cephalic papillae are weak to prominent. Stoma is usually deeper than wide with prominent cheilo and prorhabdions. Dorsal metarhabdion bears a large clawlike tooth that extends well into the pharynx. Subventral metarhabdion also bears a large tooth, which in some species extends into the lumen of the protostom and appears to bisect the dorsal tooth in lateral view. Cuticle usually bears a series of longitudinal ridges. Esophagus is typically diplogasteroid. Ovaries are paired and usually reflexed. The testis is single and

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sometimes reflexed spicules are paired. The gubernaculum is unique in appearance. It is thick proximally with a thin trough-like distal extension. There are usually eight pairs of caudal papillae although these may vary in number. Three pair are preanal, five pair are postanal with a group of three always appearing immediately anterior to the terminus or its extension. Tail may be quite elongate or short and blunt.

KEY TO THE SPECIES OF *Mikoletzkya* IN THE UNITED STATES

1. Stoma with a large dorsal and a large subventral tooth which cross at or near its center ..... 2
   Stoma with a large dorsal claw-like tooth and a smaller subventral tooth. The teeth do not cross ..... 3
2. Female tail elongate ..... *tomea* n. sp.
   Female tail short blunt ..... *bandelieri* Massey, 1960
3. Isthmus and terminal bulb of the esophagus longer than corpus and median bulb. Proximal end of gubernaculum does not appear hooked in lateral view 4
   Isthmus and terminal bulb approximately equal in length to corpus and median bulb. Proximal end of gubernaculum appears hooked in lateral view 5
4. Cheilo-, pro-, and mesorhabdions strongly sclerotized, the gubernaculum less than one-third the length of the spicules ..... *pinicola* (Thorne, 1935) Baker, 1962
   Gubernaculum over one-third the length of the spicules ..... *inedia* n. sp.
   Cheilo-, pro-, and mesorhabdions weakly sclerotized, the gubernaculum less than one-third the length of the spicules, cephalic papillae very weakly developed ..... *diluta* n. sp.
5. Main body of the gubernaculum broadened proximally ..... *cervicula* Massey, 1966
   Proximal end of gubernaculum short, broad, the distal end with a ventral prajection ..... *ruminis* n. sp.

*Mikoletzkya tomea* n. sp. (Fig. 1A, B)

FEMALES: 0.85–1.51 mm; a = 18–24; b = 4.3–5; c = 4.3–6; V = 48–50%.

MALES: Unknown.

DESCRIPTION: Cuticle with fine longitudinal striations extending from the head to the tail. Head broadly rounded with six cephalic papillae, cheilo- and prorhabdions distinct arranged as in Figure 1A with the prorhabdions overlapping the cheilorhabdions. Meso-, meta-, and telorhabdions fused, the metarhabdions armed with a large dorsal claw-like tooth and with a large subventral claw-like tooth, the teeth crossing near the center of the pharynx. Amphids pore-like, minute openings at base of lateral lips. Stoma 10 microns in width, 12 microns in depth. Corpus and median bulb of the esophagus very muscular, their length exceeding the length of the isthmus and terminal bulb. Nerve ring a third of a body width posterior to the median bulb. Excretory pore two-thirds of a body width posterior to nerve ring. Amphidelphic, the ovaries reflexed nearly to vulva. Lips of vulva slightly protuberant. Terminus long, filiform.

DIAGNOSIS: Differs from other species of the genus in the shape and length of the tail, and in the coarseness of the pharyngeal armature.

HABITAT: Associated with *Dendroctonus terebrans* (Olivier) in loblolly pine, *Pinus taeda* L.

TYPE LOCALITY: Lake City, Florida.

*Mikoletzkya bandelieri* (Massey, 1960) n. comb. (Emended) (Fig. 1C, D, E)

FEMALES: 0.77–1.2 mm; a = 21–26; b = 4.4–5.4; c = 13–17; V = 53–56%.

MALES: 0.64–1.0 mm; a = 20–24; b = 4.4–5.9; c = 16–20.

FEMALE: Cuticle with prominent longitudinal striations. Head broadly rounded with moderately prominent apical papillae. Pharynx 13 microns wide, 10 microns deep. Cheilo- and prorhabdions very distinct. Amphids at base of lateral papillae. Dorsal metarhabdion developed into a large claw-like tooth, the ventral metarhabdion developed into a large subventral tooth extending past the subdorsal tooth at the middle of pharynx (Fig. 1C). There is a small button-like tooth at the base of the pharynx that appears to be developed at the junction of the telorhabdion, which is fused with the metarhabdion. Corpus of the esophagus very muscular. Corpus and median

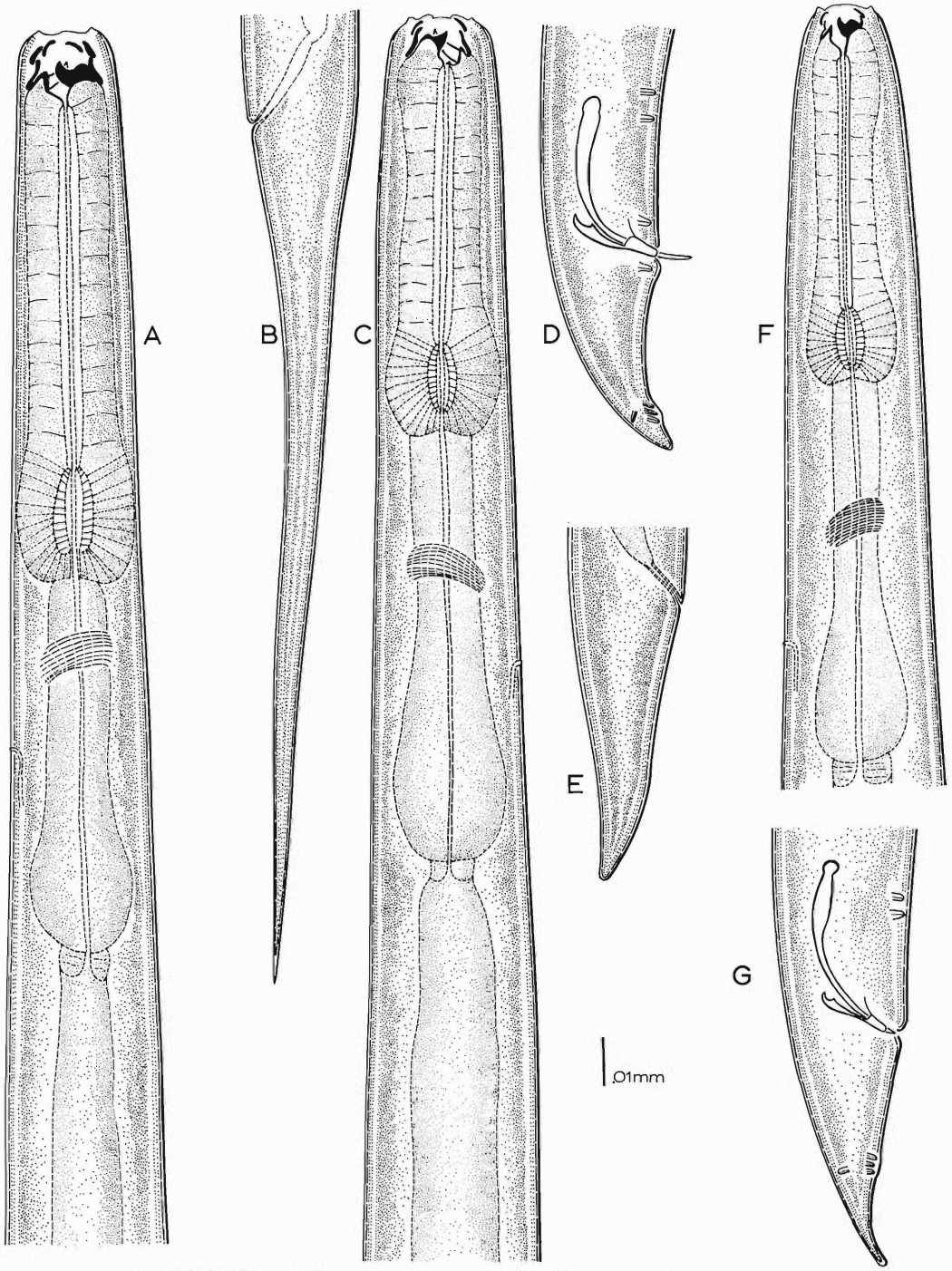


Fig. 1. A, B, *Mikoletzkya tomea* n. sp.; A, head and neck; B, female tail; C, D, E, *Mikoletzkya bandelieri* (Massey, 1960) n. comb.; C, head and neck; D, male tail; E, female tail; F, G, *Mikoletzkya cervicula* Massey, 1966; F, head and neck; G, male tail.

bulb shorter than length of isthmus and terminal bulb combined. Nerve ring at middle of isthmus. Excretory pore midway between nerve ring and terminal bulb. Hemizonid not apparent. Vulva transverse, lips protuberant. Amphidelphic, the ovaries reflexed. Spermatozoa present immediately preceding reflex of the ovary. Tail narrows to a conical terminus (Fig. 1E).

MALE: Testis single, reflexed. Spicules paired, 42–47 microns in length, gubernaculum as in Figure 1D, 16–18 microns in length. There are eight pairs of preanal and caudal papillae arranged as in Figure 1D. Terminus bluntly conoid.

DIAGNOSIS: Differs from other species in the genus in the shape and length of the tail and in the length of the corpus and median bulb of the esophagus.

HABITAT: *M. bandelieri* has been found associated with *Ips confusus* (Lec.) in piñon, with *Dendroctonus frontalis* Zimm. and *Ips avulsus* Lec. in loblolly pine, and with *Ips ponderosae* Sw. in ponderosa pine, *Pinus ponderosa* Laws.

DISTRIBUTION: Collected from Talladega National Forest in Alabama and from Bandelier National Monument and Santa Fe National Forest in New Mexico.

*Mikoletzkya pinicola* (Thorne, 1935) Baker, 1962 (Fig. 2G, H, I)

FEMALE: 1.3 mm; a = 25; b = 7.1; c = 15.1; V = 51%.

MALE: 1.1 mm; a = 31; b = 6.2; c = 15.1.

DESCRIPTION: The following is Thorne's original description: Body moderately slender, tapering anteriorly until width near lip region is about one-half that at base of neck. Female tail convex–conoid to acute terminus, its length about 2½ times anal body diameter. Male tail ventrally arcuate, convex–conoid with spicate terminus. Cuticle marked by fine transverse and longitudinal striae. Longitudinal striae low, obscure, about 44 at midbody, decreasing in number toward the extremities. Viewed laterally, these longitudinal striae present double rows of refractive, dot-like markings where they cross the transverse striae. Lip region rounded, with six forward-pointing, conical papillae. Amphids appear as minute oval markings close to the lateral papillae. Pharynx obscurely hexagonal from a face view; viewed laterally it presents two distinct chambers bearing a central, massive, arcuate, dorsal tooth. Anterior portion of esophagus four-fifths as long as posterior but broader and more muscular. Excretory pore a short distance posterior to nerve ring. Intestine densely granular, its lumen sinuous. Ovaries reflexed past vulva. Vulva a transverse slit with protuberant labia. Testis single, reflexed. Spicula yellow, arcuate, slightly cephalated. Gubernaculum thick proximally, with a thin trough-like distal extension in which the spicula glide. Eight pairs of male caudal papillae.

DIAGNOSIS: *Diplogaster* with the above measurements. Longitudinal striae 44 at midbody, low, obscure, their presence indicated by double rows of refractive dots. Tails of both sexes less than 7% of body length. Six labial papillae, forward-pointing, conical. Pharynx divided into two chambers, armed with single, massive, arcuate dorsal onchium. Female amphidelphic, ovaries reflexed past vulva. Spicula arcuate, cephalated. Gubernaculum thick proximally with thin trough-like distal extension. Eight pairs of male caudal papillae (Fig. 2H).

HABITAT: Associated with *Dendroctonus ponderosae* Hopk. in lodgepole pine, *Pinus contorta* Dougl.

*Mikoletzkya inedia* n. sp. (Fig. 2D, E, F)

FEMALES: 0.76–0.94 mm; a = 23–30; b = 5–6.2; c = 12–14; V = 55%.

MALES: 0.66–0.75 mm; a = 17–23; b = 4.5–5.3; c = 12.5–14.

DESCRIPTION: Cuticle with fine longitudinal ridges extending the entire body length. Head with six small papillae. Cheilorhabdions and prorhabdions distinct, the meso-, meta-, and telorhabdions fused, the metarhabdions bearing a large dorsal claw-like tooth and a subventral tooth as in Figure 2D. Stoma much deeper than wide. Amphids pore-like, opening at base of lateral papillae. Esophagus typically diplogasteroid. Isthmus and the terminal bulb longer than corpus and median bulb combined. Nerve ring near middle of isthmus. Excretory pore immediately anterior to initial swelling of terminal bulb, hemizonid immediately anterior to excretory pore. Ovaries paired, at times reflexed to within a body width of vulva.

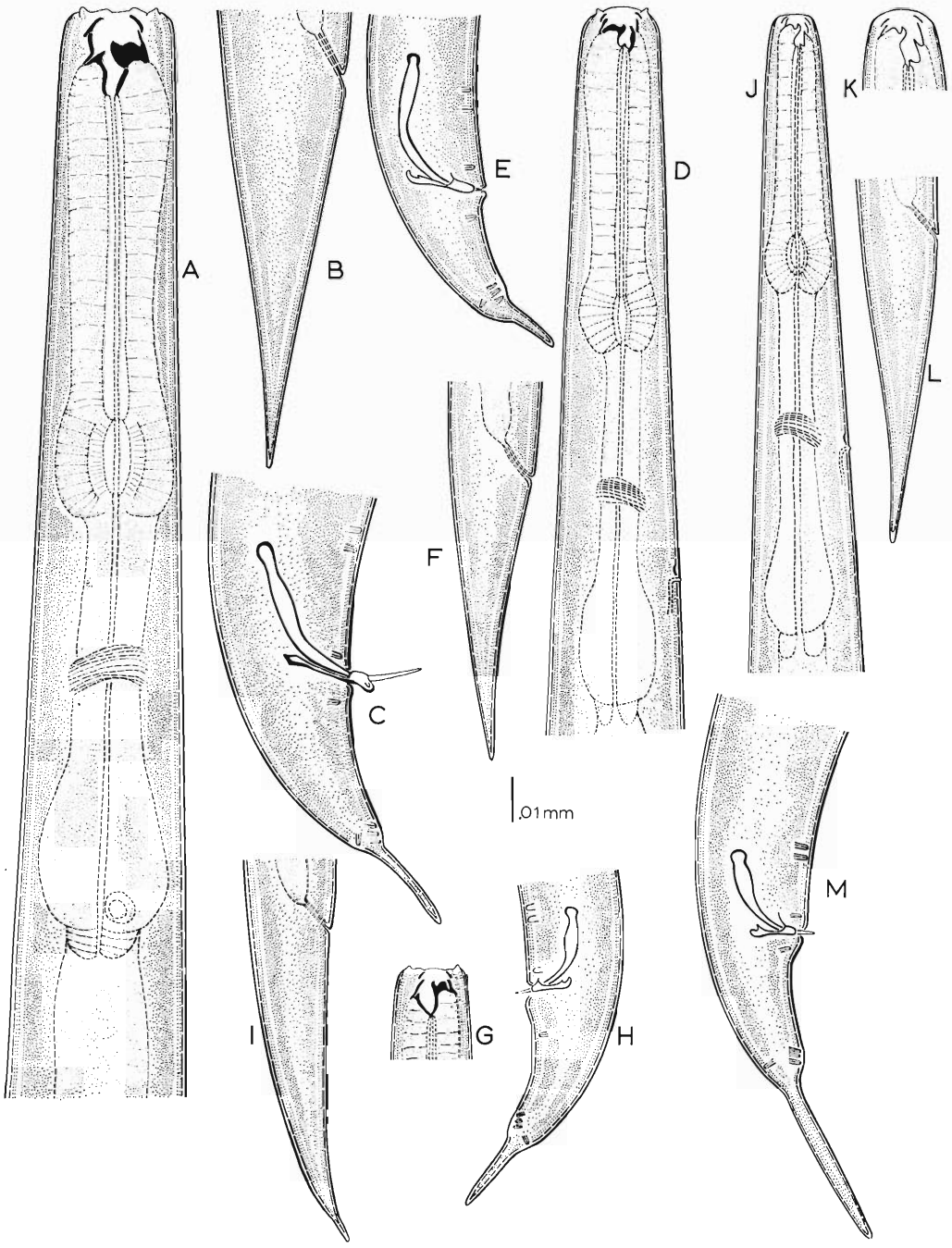


Fig. 2. A, B, C, *Mikoletzkya ruminis* n. sp.; A, head and neck; B, female tail; C, male tail; D, E, F, *Mikoletzkya inedia* n. sp.; D, head and neck; E, male tail; F, female tail; G, H, I, *Mikoletzkya pinicola* (Thorne, 1935) Baker, 1962; I, female tail; G, head; H, male tail; J, K, L, M, *Mikoletzkya diluta* n. sp.; J, head and neck; K, head; L, female tail; M, male tail.

Vulva a transverse slit, equidistant between terminal bulb and anal opening, lips protuberant. Tail conical, ending in a narrowly rounded terminus (Fig. 2F).

MALE: Testis single, sometimes reflexed; spicules paired ventrally arcuate, cephalated 38–45 microns in length. Gubernaculum from 15–18 microns long as in Figure 2E. Eight pairs of preanal and caudal papillae (Fig. 2E). Tail conoid to a spicate terminus.

DIAGNOSIS: Closely related to *M. pinicola* from which it differs in size, shape, and structure of gubernaculum.

TYPE HABITAT: Associated with *Ips* sp. and *Dendroctonus ponderosae* Hopk. in ponderosa pine.

TYPE LOCALITY: Larimer County, Colorado.

*Mikoletzkya diluta* n. sp. (Fig. 2J, K, L, M)

FEMALES: 0.80 mm; a = 20; b = 6; c = 10; V = 52%.

MALES: 0.67–0.83 mm; a = 20–23.6; b = 6–6.5; c = 11.8–12.4.

DESCRIPTION: Body tapers rapidly from midbody to head. Cuticle with fine longitudinal ridges from head to tail. Head narrowly rounded. Cephalic papillae not observed. Cheilo- and prorhabdions distinct; meso-, meta-, and telorhabdions fused, all weakly sclerotized. The metarhabdions bear a dorsal and subventral tooth. Dorsal tooth typical of genus, subventral variable in size and shape. A small tooth present at bottom of stoma, which is deeper than wide. Esophagus typically diplogasteroid, with isthmus and terminal bulb longer than corpus and median bulb combined. Nerve ring 1½ body widths behind median bulb. Hemizonid opposite nerve ring, excretory pore posterior to hemizonid. Ovaries paired, reflexed approximately their entire length. Vulva a transverse slit with protuberant lips. Tail conoid to a sharp terminus.

MALE: Testis single, reflexed, at midbody filling entire body cavity. Spicules paired, ventrally arcuate, cephalated, relatively short and stout, 23–31 microns in length. Gubernaculum 8–11 microns in length (Fig. 2M). There are eight pairs of preanal and caudal papillae. Tail conoid to a spicate terminus.

DIAGNOSIS: Closely allied to *M. pinicola*; differs in the sclerotization of the pharynx, length of tail, and in the absence of discernible cephalic papillae.

TYPE HABITAT: Associated with *Scolytus ventralis* in white fir *Abies concolor* (Gord. & Glend.) Lindl. and *Dendroctonus pseudotsugae* Hopk. in Douglas fir, *Pseudotsuga menziesii* (Mirb) Franco.

TYPE LOCALITY: Sandia Mountains, Cibola N.F., New Mexico.

*Mikoletzyka cervicula* Massey, 1965 (Fig. 1F, G)

FEMALES: Unknown.

MALES: 0.79–0.90 mm; a = 22; b = 4.7–5.6; c = 15.

DESCRIPTION: Cuticle with fine longitudinal and transverse striations. Head broadly rounded, grooves in lip ring not apparent. Six lips each with a moderately prominent apical papilla. Amphids open on outer contour of lateral lips at contour of head. Cheilorhabdions and prorhabdions distinct, about equal in length, the cheilorhabdions overlapping approximately one-third of the prorhabdions. Meso-, meta-, and telorhabdions fused, a large subdorsal claw-like tooth located on what appears to be the dorsal mesorhabdion; a large subventral tooth located on the same structure. Two large denticles at the base of pharynx. Esophagus typically diplogasteroid. Nerve ring midway of isthmus. Excretory pore a little less than one body width behind nerve ring. Hemizonid not observed. Testis single, reflexed approximately one body width. Spicules paired, ventrally arcuate, cephalated, 50 microns in length. Gubernaculum 17 microns in length, with a thin trough-like distal extension. Seven pairs of caudal papillae: three preanal ventrosubmedian, three postanal ventrosubmedian, and one subdorsal (Fig. 1G). Phasmids plainly visible. Terminus finely rounded.

DIAGNOSIS: Closely related to *M. thalenhorsti* (Rühm, 1956) Baker, 1962, and *M. pinicola* (Thorne, 1935) Baker, 1962. It differs from the former in dentation of the stoma and length and shape of tail, and from the latter in dentation of stoma, shape and size of gubernaculum, and in the shorter tail and its shape.

TYPE HABITAT: Associated with *D. adjunctus* in *P. ponderosae*.

TYPE LOCALITY: Ruidoso, New Mexico.

*Mikoletzkya ruminis* n. sp. (Fig. 2A, B, C)

FEMALE: 1.13–1.18 mm; a = 22; b = 5.4–6; c = 11; V = 55%.

MALE: 0.88–0.97 mm; a = 20–22; b = 5.0; c = 12–14.

DESCRIPTION: Cuticle with coarse longitudinal ridges extending from head to tail. In lateral view there are 11 striations at midbody, each striation appearing as a row of dots. Head broadly rounded with six prominent apical papillae. Amphids pore-like, opening at the base of the lateral papillae. Cheilo and prorhabdions distinct, coarse, the meso-, meta-, and telorhabdions fused. There is a large dorsal claw-like tooth developed by the metarhabdion and a large subventral tooth produced by the same structure. Stoma 17 microns deep, 10 microns wide. Corpus and median bulb of esophagus very muscular, somewhat longer than isthmus and terminal bulb. Nerve ring more than one body width behind median bulb. Excretory pore not discernible. Amphidelphic, the ovaries reflexed more than one-half their length. Vulva transverse with slightly protuberant lips. Tail conoid to a pointed terminus.

MALES: Testis single, outstretched. Spicules paired, ventrally arcuate, cephalated, 47–58 microns in length. Gubernaculum expanded both distally and proximally as in Figure 2C, the distal end serving as a guide for the spicules which are 18–22 microns in length.

DIAGNOSIS: Differs from other species in the genus in the shape and size of the gubernaculum.

TYPE HABITAT: Associated with *Dendroctonus obesus* Mann. in Engelmann spruce, *Picea engelmanni* Parry.

TYPE LOCALITY: Type specimen collected on Rabbit Ears Pass in Routt County, Colorado. Other specimens have been taken from the galleries of *D. obesus* in Engelmann spruce near Libby, Montana.

DISCUSSION: In the writer's opinion, there is some doubt as to the proper placement of *M. aerivora*. The figures included in Merrill and Ford's publication do not lend themselves to diagnosis. For this reason this species is not included in the keys or in the discussion.

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# Studies on Freshwater Larval Trematodes. Part XI. A Redescription of *Cercaria pygocytophora* Brown (1931)

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Brown (1931) first described *Cercaria pygocytophora* from *Planorbis carinatus*, Cheshire, England. Iles (1959) found a cercaria parasitic in *Lymnaea pereger* from Roath Park Lake, Cardiff, Wales, and later (1960) experimentally connected it with the already known adult, *Apatemon gracilis minor* (Yamaguti, 1933). At the same time she relegated *Cercaria hamburgensis* Komiya (1938), a parasite of *Lymnaea ovata* and *L. palustris* in Germany, to the synonymy of the cercaria of *A. g. minor* and also alluded to its possible synonymy with *C. pygocytophora*. The only reason advanced for the probable synonym with *C. pygocytophora* was the possible presence in the latter of posterior excretory commissure which might be found if the material were reexamined. The present author, while working as a Caroline Harrold Post-Doctoral Research Fellow in 1959 at the University of Birmingham, England, examined numerous specimens of *C. pygocytophora* extricated from the hepatopancreas of *Planorbis carinatus* as well as those which emerged naturally, but the posterior excretory commissure was not present. If it had been present Brown certainly would have noticed it, as he did observe the blind-ending anterior excretory ducts in place of a complete anterior excretory commissure. For reasons to be discussed later, it appears that not only is *C. pygocytophora* a distinct species from the cercaria of *A. g. minor*, but also the same holds true for *C. hamburgensis*. Owing to the controversy about its independent entity, *C. pygocytophora* has been redescribed in this paper. My observations agree with those given by Brown, excepting the minor details such as cuticular and acetabular spination.

Nine of the 179 specimens of *Planorbis carinatus* collected from Edgbaston Pool near the University of Birmingham were emitting *Cercaria pygocytophora*. For the relative study, measurements in mm were made of both living and fixed specimens. Fixation was accomplished by squirting naturally emerged cercariae in 10% hot formalin.

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## *Cercaria pygocytophora* Brown (1931) (Fig. 1)

DESCRIPTION: Body uniformly spinose. No special rows of spines in region of anterior organ. No forward-pointing spines. Tail-stem and furcae aspinose. Circumoral spineless area absent. Acetabular spines in three rows. A pair of unpigmented eyespots in preacetabular region. A pair of hairlike projections in postacetabular region. Six rows of these projections "flagellets" on tail-stem. Furcae laterally compressed, without a finfold. Tail-stem terminally attached. Eight pairs of caudal bodies, last pair extending in furcae. Anterior organ not thickened posteriorly. A small prepharynx and pharynx present. Esophagus tubular, bifurcating immediately anterior to ventral sucker. Ceca extending only slightly posterior to ventral sucker, not constricted. Four pairs of transversely elongated penetration glands, with finely granular contents, occupy most of space between ventral sucker and excretory vesicle. One pair of penetration gland ducts on each side of body. Anterior excretory commissure replaced by two blind-ending excretory tubes. Posterior excretory commissure absent. Division of main excretory tubes takes place at about equatorial level of ventral sucker. No ciliations in convoluted part of main excretory tubes. Caudal excretory duct dividing into two furcal branches opening on external sides of furcae. Island of Cort present. Flame cell formula: $2[(2) + (2 + 2 + (1))] = 14$. Genital rudiments represented by a group of cells anterior to excretory vesicle. Cercariae swimming, apparently restlessly, with intermittent resting periods. While resting, body and tail-stem are directed vertically downwards while furcae are stretched apart. Negatively phototactic. Measurements of ten, naturally

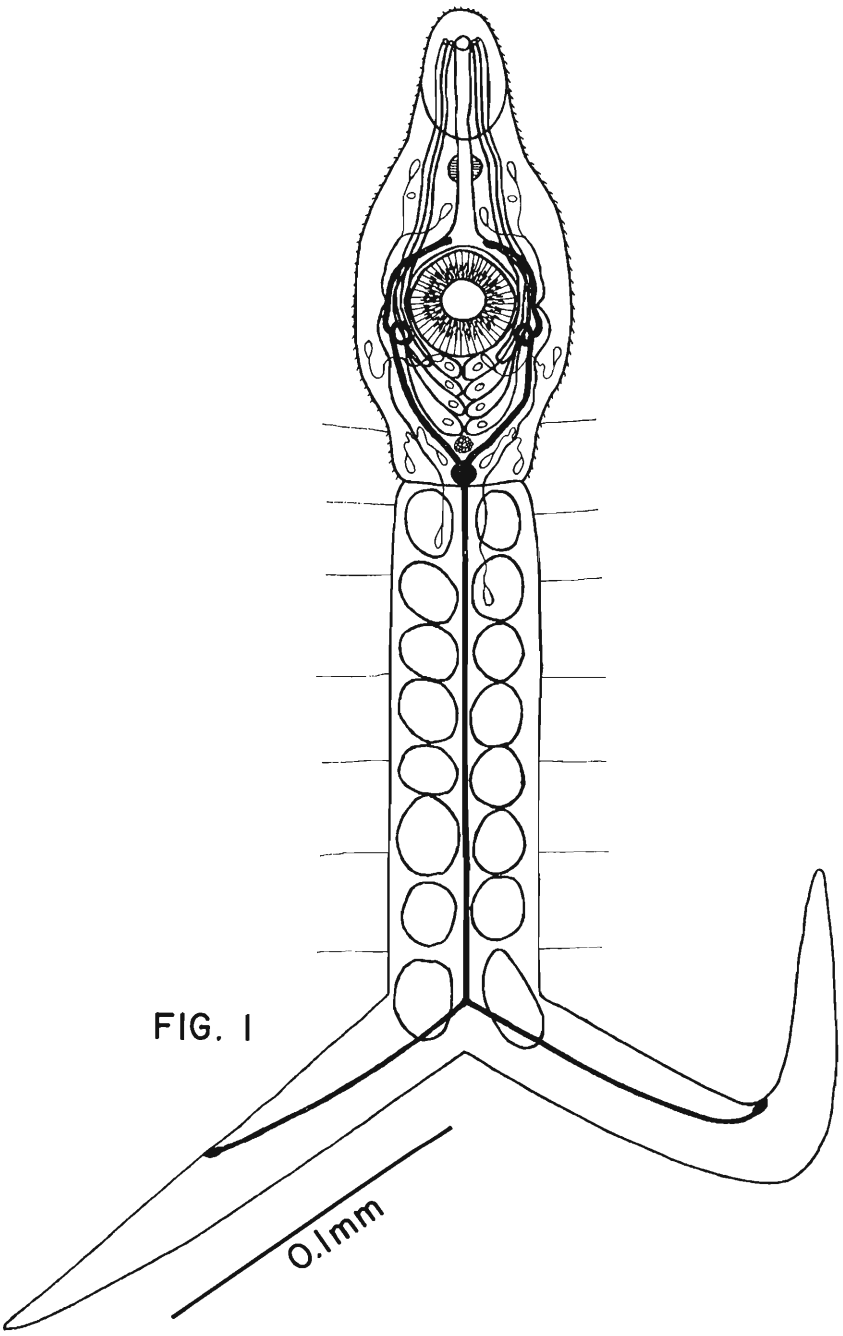


Fig. 1. *Cercaria pygocytophora* Brown (1931), ventral view.

emerged, living specimens: body, 0.084–0.142 by 0.037–0.064; tail-stem, 0.146–0.159 by 0.043–0.054; furcae, 0.147–0.168 long; anterior organ, 0.034–0.039 by 0.024–0.028; ventral sucker, 0.027–0.035 in diameter; prepharynx, 0.003 long; pharynx, 0.007–0.01 in diameter; esophagus, 0.012–0.015 long. Measurements of ten fixed specimens: body, 0.097–0.131 by 0.035–0.042; tail-stem, 0.139–0.170 by 0.038–0.047; furcae, 0.143–0.166 long; anterior organ, 0.037–0.039 by 0.020–0.023; ventral sucker, 0.025–0.027 in diameter. Development in long threadlike sporocysts with alternately constricted and swollen portions.

## DISCUSSION

The cercaria of *Apatemon gracilis minor* as described by Iles (1959) is very much like *Cercaria pygocytophora* Brown (1931) insofar as the following characters are concerned: absence of forward-pointing spines, presence of spines on body and ventral sucker, absence of spines on tail-stem; presence of unpigmented eyespots; eight pairs of caudal bodies; pattern of digestive tract; number of penetration glands and their ducts; number and arrangement of flame cells; presence of a blindly ending excretory duct on either side of body anterior to ventral sucker; resting position; and, finally, in having a negatively phototactic behavior. *Cercaria pygocytophora* differs from the cercaria of *A. g. minor* in that its furcae are aspinose, there are no special rows of spines in the region of the anterior organ, in having three rows of acetabular spines; the penetration glands are entirely postacetabular and transversely elongated; the main excretory tubes divide at equatorial level of ventral sucker and in lacking a posterior excretory commissure. The first intermediate host of *A. g. minor* is *Lymnaea pereger* while *Planorbis carinatus* is the host of *C. pygocytophora.* Thus, *C. pygocytophora* and the cercaria of *A. g. minor* are two distinct species and there is no justification for regarding them as synonymous as advocated by Iles.

*Cercaria hamburgensis* Komiya (1938) differs from the cercaria of *Apatemon gracilis minor* in the presence of an Island of Cort in excretory vesicle, in having two or three rows of acetabular spines and the larger size of its body and sucker. Furthermore, as shown by Komiya (1938, fig. 8*a*), the intestinal ceca of *C. hamburgensis* do not extend beyond the ventral sucker, whereas in the cercaria of *A. g. minor* "ceca terminate a short distance behind the posterior margin of ventral sucker." In my experience the extent of the intestinal ceca, in the case of strigeid cercariae, is a potent character for the separation of the otherwise inseparable species.

The other longifurcate pharyngeate distome furcocercariae with four pairs of postacetabular penetration glands, 14 flame cells and caudal bodies are: *Cercaria burti* Miller (1923, 1926) = larva of *Apatemon gracilis* as described by Stunkard, Willey, and Rabinowitz (1941), *C. burti* Miller (1923) variety *incusae* Giovanola (1937), *C. helvetica XXXI* Dubois (1929) Wesenberg-Lund (1934), *C. hamburgensis* Komiya (1938), *C. pseudoburti* Rankin (1939), *C. okobojensis* Brooks (1943), *C. lessoni* Johnston and Beckwith (1947) and *C. wansoni* Fain (1953). All of these species can be readily separated from *C. pygocytophora* by differing in one or more of the following characters: size of body and suckers, pattern of cuticular spination, number of the rows of acetabular spines, number of caudal bodies, esophageal length, extent of intestinal ceca and their contour, arrangement of penetration glands, state of excretory commissure, and phototactic response.

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# *Halichoanolaimus raritanensis* n. sp. (Chromadoroidea : Cyatholaimidae) from New Jersey

EDWARD R. HASBROUCK[1]

A new species of *Halichoanolaimus* was encountered during ecological investigations of the psammolittoral fauna of the Raritan estuary. These nematodes comprise from 1 to 5% of the nematode population in the sandy intertidal beaches at Port Monmouth, New Jersey. It has also been found in the Shark River estuary on similar substrates.

The specimens used in this description were fixed in 5% formalin solution buffered with $CaCl_2$. Impregnation with glycerin was accomplished by the method of Seinhorst (1959). All measurements were made from permanent glycerin mounts. Type material is being retained by the author. The measurements are presented as follows: all numbers above the line are cumulative distances in microns from the anterior end; all numbers below the line are body diameters in microns at the corresponding distance; measurements were made at the base of the buccal cavity, excretory pore, base of the esophagus, vulva of female (no corresponding measurement made in the male), and at the level of the anus. The a, b, c, and V values of de Man's formula are also included. This procedure is equivalent to that used by Hopper (1963).

*Halichoanolaimus raritanensis* n. sp.
(Fig. 1, a–d)

MEASUREMENTS:

HOLOTYPE (♂):

Slide #IV-25 $\frac{51\ 200\ 460 - 3{,}145}{68\ \ 90\ \ 90 - \ \ 65}$ 3,295; a = 36.6; b = 7.2; c = 21.9.

ALLOTYPE (♀):

Slide #IV-26 $\frac{84\ 109\ 580\ 3{,}000\ 5{,}160}{60\ 100\ 100\ \ 115\ \ \ 54}$ 5,420; a = 47.1; b = 9.3; c = 20.8; V = 55.3%

PARATYPES (♂):

Slide #II-47 $\frac{46\ 175\ 496 - 3{,}250}{84\ 105\ 109 - \ \ 70}$ 3,478; a = 31.9; b = 7.0; c = 15.3.

Slide #II-47 $\frac{53\ 179\ 584 - 3{,}370}{81\ \ 98\ \ 107 - \ \ 70}$ 3,590; a = 33.6; b = 6.2; c = 16.3.

Slide #I-16 $\frac{53\ 172\ 638 - 2{,}660}{74\ \ 98\ \ 109 - \ \ 70}$ 2,890; a = 26.5; b = 4.5; c = 12.6.

Slide #I-55 $\frac{53\ 161\ 563 - 2{,}466}{63\ \ 70\ \ 70 - \ \ 56}$ 2,981; a = 42.4; b = 5.3; c = 13.8.

Slide #III-22 $\frac{49\ 214\ 588 - 3{,}419}{74\ \ 91\ \ 98 - \ \ 74}$ 3,684; a = 37.6; b = 6.3; c = 13.9.

PARATYPES (♀):

Slide #III-41 $\frac{56\ 204\ 552\ 2{,}203\ 3{,}750}{95\ 122\ 123\ \ 126\ \ \ 56}$ 4,104; a = 33.4; b = 7.4; c = 16.2; V = 49.3%.

Slide #II-35 $\frac{60\ 171\ 580\ 1{,}786\ 3{,}364}{77\ \ 88\ \ 105\ \ 119\ \ \ 56}$ 3,627; a = 30.5; b = 6.3; c = 13.8; V = 49.4%.

Slide #II-7 $\frac{42\ 189\ 544\ 1{,}997\ 3{,}810}{81\ \ 98\ \ 91\ \ 119\ \ \ 56}$ 4,050; a = 34.1; b = 7.5; c = 16.9; V = 49.4%.

Slide #II-95 $\frac{53\ 165\ 590\ 1{,}902\ 3{,}602}{77\ \ 88\ \ 102\ \ 112\ \ \ 56}$ 3,872; a = 34.6; b = 6.6; c = 14.7; V = 49.2%.

DESCRIPTION: Body of the male stout with a perceptible posterior taper. Cuticle uniformly incised with fine circumcorporal striae 3 $\mu$ apart. Each ridge appears to be evenly punctated; however, close examination reveals these punctae to be subcuticular (Fig. 1c). The crests of the ridges are smooth. The distance between the punctae is slightly greater in the vicinity of the lateral lines. The punctae are irregularly distributed in the vicinity of the anus and are apparently larger in size.

The cephalic structures consist of six deeply incised outer lips, each subtended by two inner lips (Fig. 1b, d). Each lip is surmounted by a single papilla (Fig. 1d). The cephalic setae consist of two subdorsal pairs, two subventral pairs, and two lateral individuals. Amphids are spiral two and one-half to three turns, the

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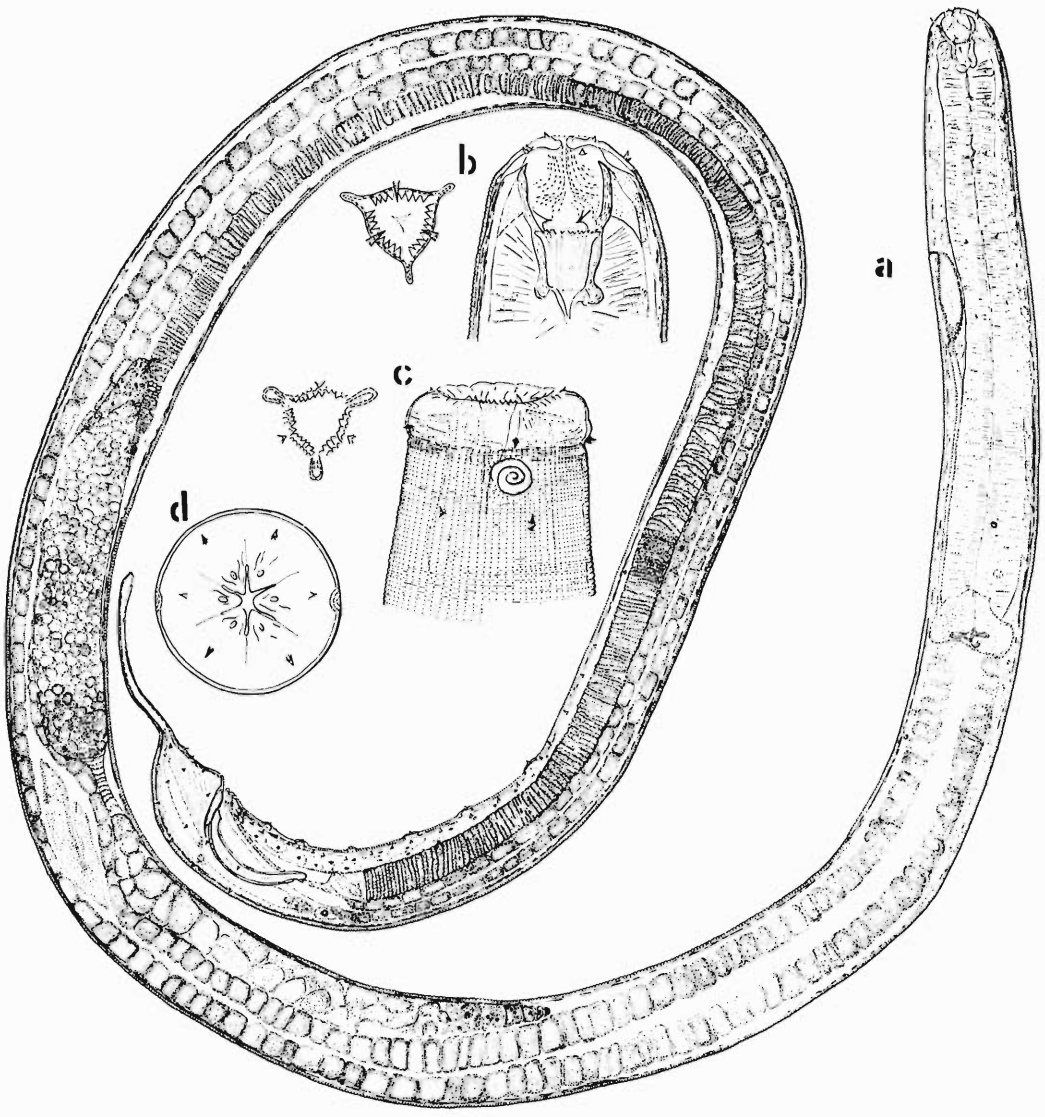


Fig. 1, a–d. *Halichoanolaimus raritanensis* n. sp. a. Male, showing position of superficial papillae; b. Head end of the male, lateral view, sagittal section, showing cephalic setae and the position of inner and outer lips when the buccal structures are abducted. The *en face* view of the teeth and denticles are shown to the left of the letter b. c. Head end, external lateral view with labial structures adducted, teeth and denticles everted. The *en face* view of the teeth and denticles is to the left of the letter c. d. *En face* view of the head, labial structures closed, showing labial papillae and cephalic setae.

outer turn being 14 to 17 microns in diameter (Fig. 1c).

The buccal cavity consists of an anterior and posterior chamber. The anterior chamber is lined with three heavily sclerotized ribs, two subdorsal and one ventral. Opposite each rib is an inwardly projecting posteriorly sloping tooth. Basal to the ribs is a ring of approxi-

mately 24 posteriorly sloping denticles which project from a sclerotized circumoral band (Fig. 1b). When the anterior ribs are adducted causing eversion of the anterior chamber, both teeth and denticles rotate anteriorly (Fig. 1c). The posterior chamber is lined with three heavily sclerotized ribs which articulate with the anterior ribs. The knob-like swellings at the base of the posterior ribs appear to be attachment points for the heavy musculature surrounding the buccal cavity (Fig. 1b). Observation of a large number of specimens indicates that both portions of the buccal cavity are capable of a considerable amount of displacement.

The cylindrical esophagus is terminated by a short anterior-posteriorly flattened cardia (Fig. 1a). The excretory system consists of a large acellular mass surrounding the esophageal intestinal junction and an easily traceable ventral duct terminating in an ampulla immediately posterior to the nonsclerotized excretory pore. The excretory pore is about three head diameters posterior to the end. Large cuboidal cells make up the anterior two-thirds of the intestine. The posterior third consists of smaller flattened cells and terminates in an elongated duct leading to the cloaca.

The two testes extend anterior and posterior to the central junction with the sperm duct. The gametes of the anterior testis are consistently larger than those found in the posterior testis (Fig. 1a). The spicular apparatus consists of a pair of smoothly curving spicules without distinct cephalization, closely applied to a short, slightly curved gubernaculum. The gubernaculum has a short distal bifurcation not readily visible in lateral view. Spicules are 110–132 $\mu$ long. The gubernaculum varies from 44–48 $\mu$ long. Anterior to the cloacal opening are six to eight papilloid supplements spaced 30 to 40 microns apart. Subventrally and parallel to the supplements are two rows of 20 to 24 papillae. There are also two converging subventral rows of three papillae each posterior to the cloaca.

The tail slopes ventrally to the filiform portion, which makes up 80–90% of its length. The terminal portion is not swollen, although a caudal gland opening is evident. As on the rest of the body, a few scattered papillae are visible on the anterior portion of the tail.

The female, except for a greater overall size and the median vulva, resembles the male. However, the arrangement of the cuticular punctations is more regular in the tail region. Also, the female lacks the subventral papillae.

DIAGNOSIS: *Halichoanolaimus raritanensis* closely resembles *H. quatrodecimpapillatus* Chitwood, 1951, except for the lesser number of preanal supplements, greater length, more slender shape of the spicules, and bifurcate gubernaculum. *H. dolichurus* Ssaweljev, 1912, differs from this species with respect to the spicular apparatus, shape of the tail, and the shape of the supplements, also, based on Gerlach's (1964) redescription, the greater number of denticles. *H. raritanensis* differs from the remaining species of the genus by greater relative length of the filiform portion of the tail.

## SUMMARY

*Halichoanolaimus raritanensis* n. sp. was discovered in the littoral substrates of Raritan Bay, New Jersey. This species is differentiated from the rest of the genus on the basis of the number of supplements (6–8), relative length of the filiform portion of the tail (80–90%), slender structure of the spicular apparatus, and bifurcate tip of the gubernaculum.

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# *Chitwoodia transvaalensis* n. gen., n. sp., and *Dorylaimoides longidens* n. sp., Two New Nematodes from South Africa[1]

J. P. FURSTENBERG AND JUAN HEYNS[2]

ABSTRACT

Two new nematodes are described and figured. *Chitwoodia transvaalensis* n. gen., n. sp., is distinguished by a prominent cap-like region offset by a deep constriction. *Dorylaimoides longidens* n. sp., is related to *D. stenodorus* Altherr, 1953, from which it differs in spear length, measurements, and number of supplements.

Measurements and drawings were made from specimens killed by the gradual application of heat, preserved in FAA, and mounted in glycerin. Slide numbers refer to the collection of the Plant Protection Research Institute, Pretoria.

GENUS *Chitwoodia* n. gen.[3] (Figs. 1–6)

Dorylaimidae. Cuticle with prominent radial dots. Lip region offset by a deep constriction; prominent cap-like; lips rounded. Papillae slightly elevated. Spear acicular, long, with a very short aperture. Spear extension long, rod-like. Guiding ring single. Esophagus with a large, enlarged basal part. Gonads paired, opposed and reflexed. Vulva longitudinal. Vagina with a thick-walled muscular tube, the anterior end having a fringed appearance. Males not found.

TYPE SPECIES: *Chitwoodia transvaalensis* n. sp.

DIAGNOSIS: This genus is characterized by the well-offset, cap-like head, the very long acicular spear, the spear extension without flanges, the large enlarged basal part of the esophagus, and the fringy appearance of the anterior part of the vagina.

DISCUSSION: *Chitwoodia* n. gen. is reminiscent of some *Enchodelus* spp. in that it possesses a long slender spear without flanges. However, it differs from these *Enchodelus* spp. in having prominent radial dots, a lip region which is offset by a deep constriction, a single guiding ring, and a longer enlarged basal part of the esophagus. Also, the ventrosubmedian gland nuclei are not located far forward as in *Enchodelus* spp.

*Chitwoodia transvaalensis* n. sp.

MEASUREMENTS: HOLOTYPE (female): L = 1.40 mm; a = 24; b = 3.6; c = 49; V = 55.4.

PARATYPES (female, n = 4: L = 1.1–1.7 (1.42) mm; a = 25–29 (27); b = 3.1–4.2 (3.7); c = 43–53 (48); V = 55.3–56.6 (55.6).

DESCRIPTION: Body slightly ventrally curved when relaxed, tapering only in the anterior part of neck. Cuticle with microscopical transverse striae and with distinct radial elements arranged in rows and forming dots on surface of the cuticle. These dots vary in size and density. Lip region prominent, cap-like; lips rounded, one-third as wide as body at base of esophagus; one and a quarter as wide as head constriction. Lips with the usual complement of 16 papillae. Spear length 28 $\mu$ to 29 $\mu$; acicular; one and a third to one and four-fifths lip region width; spear width one-fifteenth spear length; the aperture one-ninth to one-seventh spear in length. Spear extension 25 $\mu$ to 26 $\mu$ or about eight-ninths spear length. Guiding ring appearing single; 15 $\mu$ to 16 $\mu$ from the anterior end. Amphid stirrup-shaped, the aperture five-eighths as wide as head. Esophagus with two swellings anterior to nerve ring, and expanding to about half the corresponding body diameter in its basal half. Dorsal gland nucleus one-half body diameter behind esophageal expansion. Cardia hemispherical; less than one-half as wide as body;

[1] Adapted from a thesis presented by the senior author to the Institute for Zoological Research, Department of Zoology, Potchefstroom University for C.H.E., Potchefstroom, South Africa, in partial fulfillment of the requirements for the degree of Master of Science.

[2] Plant Protection Research Institute, Pretoria, South Africa.

[3] This genus is named in honor of Dr. B. G. Chitwood, in recognition of his contributions to Nematology.

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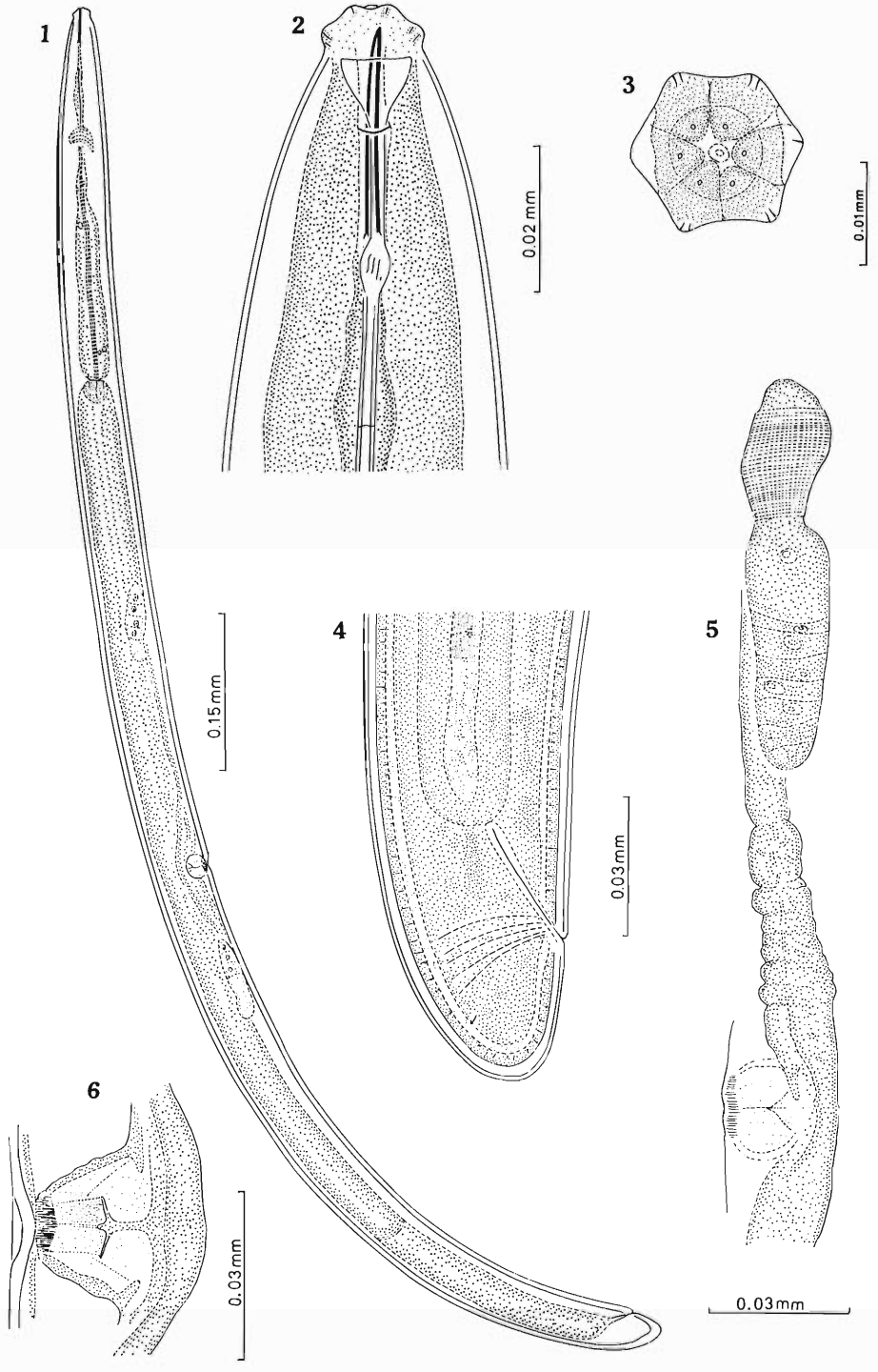
Figs. 1–6. *Chitwoodia transvaalensis* n. gen., n. sp. 1. Female. Natural position when relaxed; 2. head; 3. face view; 4. posterior portion of female; 5. female reproductive organ, anterior branch; 6. vulva.



more distinct in some specimens; 19 $\mu$ in length, 24 $\mu$ in width. Hemizonid next to nerve ring; two and a third to two and three-quarters body diameters from lip region. Intestinal cells obscure and without granules. Prerectum four to five and a half times anal body diameter in length. Rectum almost equal to anal body diameter. Tail blunt, dorsally convex; slightly shorter than anal body diameter. No caudal papillae observed. Lateral field between one-third and one-quarter body diameter in width.

Didelphic. Vulva longitudinal; vagina extending about three-sevenths the way across the body. In most of the specimens the ovaries are undeveloped; ovaries reflexed about one and a half body diameters to the vulva. Spincter about one body diameter from vulva. In one specimen a poach can be distinguished in the uterus, just anterior to the spincter.

MALE: Not found.

HOLOTYPE: Slide 1784, collected from soil around the roots of *Trifoliata* trees, Buffelspoort, Transvaal.

PARATYPES: Slide 3613, collected from soil around the roots of tea plants, Tzaneen, Transvaal, and slide 1623, collected from soil from sweet potato land, Rustenburg, Transvaal.

*Dorylaimoides longidens* n. sp. (Figs. 7–16)

MEASUREMENTS: HOLOTYPE (female): L = 7.30 mm; a = 100; b = 11.5; c = 30; V = 44.0.

ALLOTYPE (male): L = 5.79 mm; a = 107; b = 9.1; c = 24.

PARATYPES (females, n = 9): L = 5.24–7.14 (6.49) mm; a = 76–117 (98); b = 8.6–11.5 (9.9); c = 22–37 (27); V = 41.0–47.7 (44.8). (Males, n = 3): L = 5.36–7.08 (6.49) mm; a = 110–131 (120); b = 11.0–11.6 (11.3); c = 28–33 (30).

DESCRIPTION: Body ventrally curved when relaxed, especially in posterior half. Body tapering anteriorly and posteriorly. Cuticle with microscopical transverse striae and with prominent radial dots; the number of dots per square micron range from 0 to 40. Some dots are arranged in rows while others are without any definite pattern. Lips amalgamated. Lip region about one-fourth as wide as the body at the base of the esophagus. Lips with the usual complement of 16 papillae. Spear length 26 $\mu$ to 30 $\mu$, twice the width of the lip region; the aperture minute, and about one-twentieth spear length. Spear extension equals spear in length. Guiding ring double, although in most of the specimens only a single ring is displayed; when in the single state, it occupies a position 15 $\mu$ to 18 $\mu$ from anterior end, when double, the anterior and smaller ring is 8 $\mu$ to 11 $\mu$ from anterior end and the posterior one 12 $\mu$ to 15 $\mu$. Amphid stirrup-shaped, with an anterior transverse support (Fig. 9), three-quarters as wide as head. Esophagus commencing with a slight swelling in region around base of spear extension, then constricting, widening again slightly, and gradually expanding in its basal half. Enlarged basal portion one-third to one-half as wide as the corresponding body diameter. Dorsal gland nucleus less than one-third body diameter behind the esophageal expansion. Cardia small, hemispherical; one-quarter as wide as body. Nerve ring obscure in most of the specimens; three to three and a half body widths from head region. Hemizonid in some specimens distinct, opposite nerve ring. Intestine typical, two cells in circumference and with numerous, brownish granules. Prerectum and rectum obscure because tail twisted; rectum approximately equal to anal body diameter in length. Tail five and a half to seven and a half times anal body diameter; filiform; tail tips of all specimens broken off, thus a variation in tail length to be expected. Caudal papillae obscure. Lateral field one-sixth to one-quarter body width in region of cardia.

FEMALE: Vulva a short longitudinal slit; vagina extending across five-eighths of the body width. Ovaries obscure, reflexed for a distance of approximately six to ten body widths. Eggs one and three-quarters to two and a half body diameters in length; one specimen with two pairs of eggs in each uterus; others with only one or two eggs. Spincter seven to eight body widths from vulva. Uteri in all specimens packed with spermatozoa.

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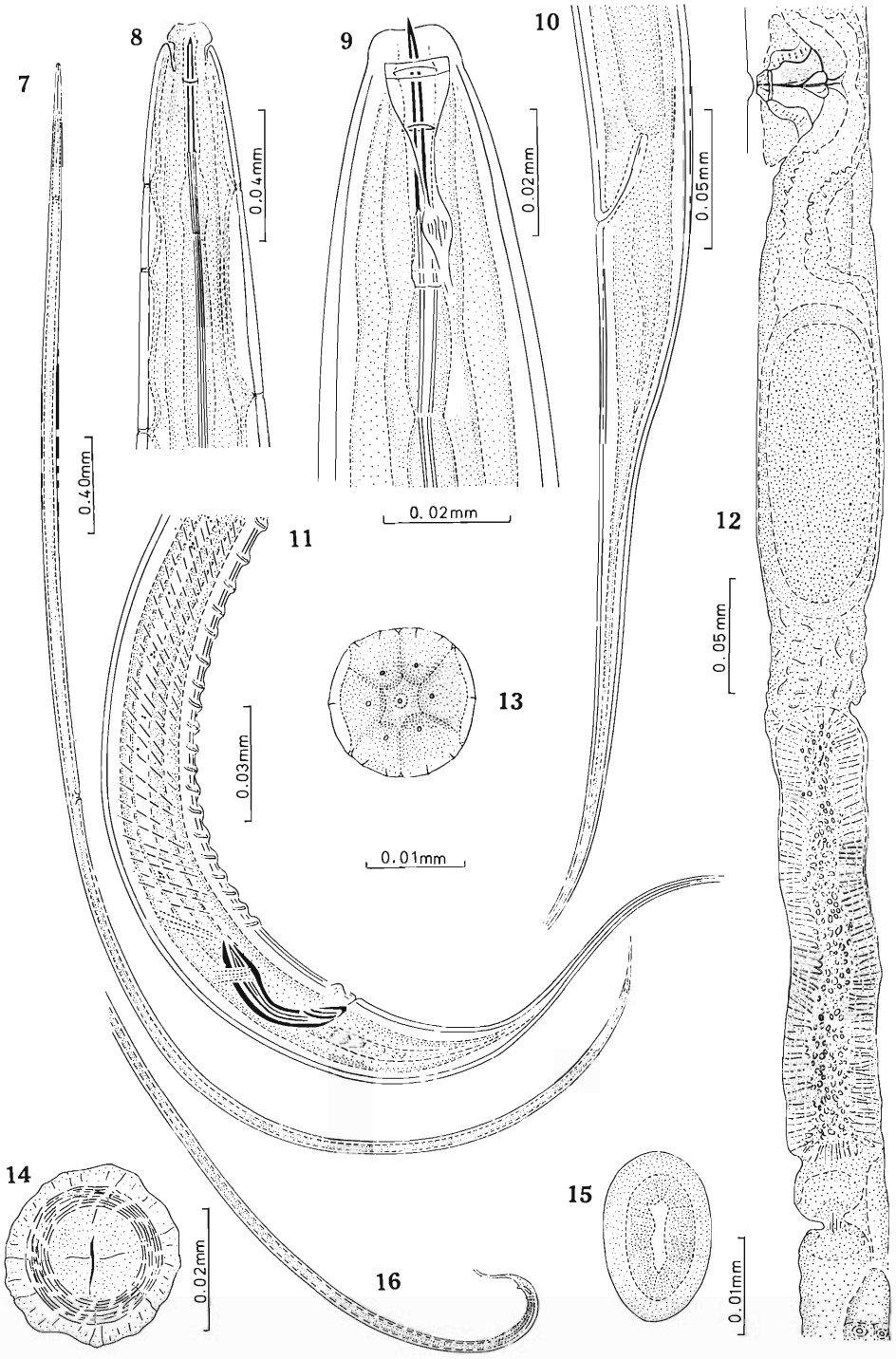
Figs. 7–16. *Dorylaimoides longidens* n. sp. 7. Female. Natural position when relaxed; 8. dorsoventral view of head; 9. head; 10. female tail; 11. male tail; 12. female reproductive organs, posterior branch; 13. face view; 14. cross section through vagina; 15. cross section through vulva; 16. male posterior part. Natural position when relaxed.



MALE: Spiculae and lateral guiding pieces typically dorylaimoid; spiculae 82 $\mu$ to 97 $\mu$; measured along the median curved line; approximately one-half length of tail. Lateral guiding pieces about one-sixth spiculae length. Supplements consisting of a preanal pair, followed by 19 to 25 ventromedian ones in continuous series; the latter beginning one anal body diameter from the preanal pair.

DIAGNOSIS: *Dorylaimoides longidens* n. sp. differs from the general description of this genus in possessing a longitudinal vulva and in the absence of an angular spear extension. It is further distinguished by the large number of supplements and the very long, thin, slightly arched spear.

DISCUSSION: *Dorylaimoides longidens* n. sp. differs from *D. stenodorus* Altherr, 1953, mainly in the spear length which is twice lip region width; the number of supplements (19 to 25 compared with 5 for *stenodorus*); the length (5.24 to 7.30 mm compared with 1.3 mm for *stenodorus*); and width (a = 76–117 compared with 43 in *stenodorus*).

TYPE LOCALITY AND HABITAT: Type specimens from water in a mudhole on bank of the Blyde River, Hoedspruit, Transvaal.

HOLOTYPE: Slide 3228.

ALLOTYPE: Slide 3230.

PARATYPES: Slides 3229 and 3231.

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## On *Basirotyleptus caudatus* n. sp., and a Redescription of *Thornenema thienemanni* (Schneider, 1937) Andrássy, 1959 (Nematoda : Dorylaimoidea)

M. SHAMIM JAIRAJPURI[1]

A new species of the genus *Basirotyleptus* Jairajpuri, 1964, obtained from soil around roots of cashew nut plants at Waltair, Visakhapatanam (A.P.) is described and its affinities discussed. A large number of specimens of *Thornenema thienemanni* (Schneider, 1937) Andrássy, 1959, were found in soil samples from around roots of grasses from Kurnool (A.P.) and Andamans. The description of *T. thienemanni* as provided by Schneider (1937) is very meager. He has given only the diagrams of tail and gonad and the details of important characters like spear, spear aperture, spear extension, and amphids are not known. It is therefore felt necessary to provide a redescription based on the present specimens.

FAMILY BELONENCHIDAE THORNE, 1964
GENUS *Basirotyleptus* JAIRAJPURI, 1964

*Basirotyleptus caudatus* n. sp. (Fig. 1, A–F)

FEMALES (ten): L = 0.4–0.5 mm; a = 19–28; b = 4.1–4.9; c = 24–30; V = 40–48.

HOLOTYPE (female): L = 0.47 mm; a = 26; b = 4.2; c = 29; V = 46.

DESCRIPTION: Body cylindroid, ventrally arcuate when relaxed. Cuticle and subcuticle distinctly striated. Radial elements present, especially abundant near tail. Lateral chords faint, about one-third of body width; lateral body pores not visible. Lips somewhat conoid, the region marked off from the body contour by a distinct depression. Six small liplets surrounding the oral aperture. Amphids cuplike, their slit-like apertures slightly more than half the head width. Sensillae pouches below the stoma. Spear 12 $\mu$ long, slender, needle like, without lumen. Spear extension about half as long as spear. Stoma inverted funnel-shaped, strongly sclerotized. Esophagus a slender tube until it expands to pyriform basal bulb. Lumen of basal bulb in two sections, the posterior one forming a triquetrous, valvular chamber. Dorsal and two pairs of subventral esophageal gland nuclei distinctly visible. Cardia hemispheroid. Nerve ring mid-

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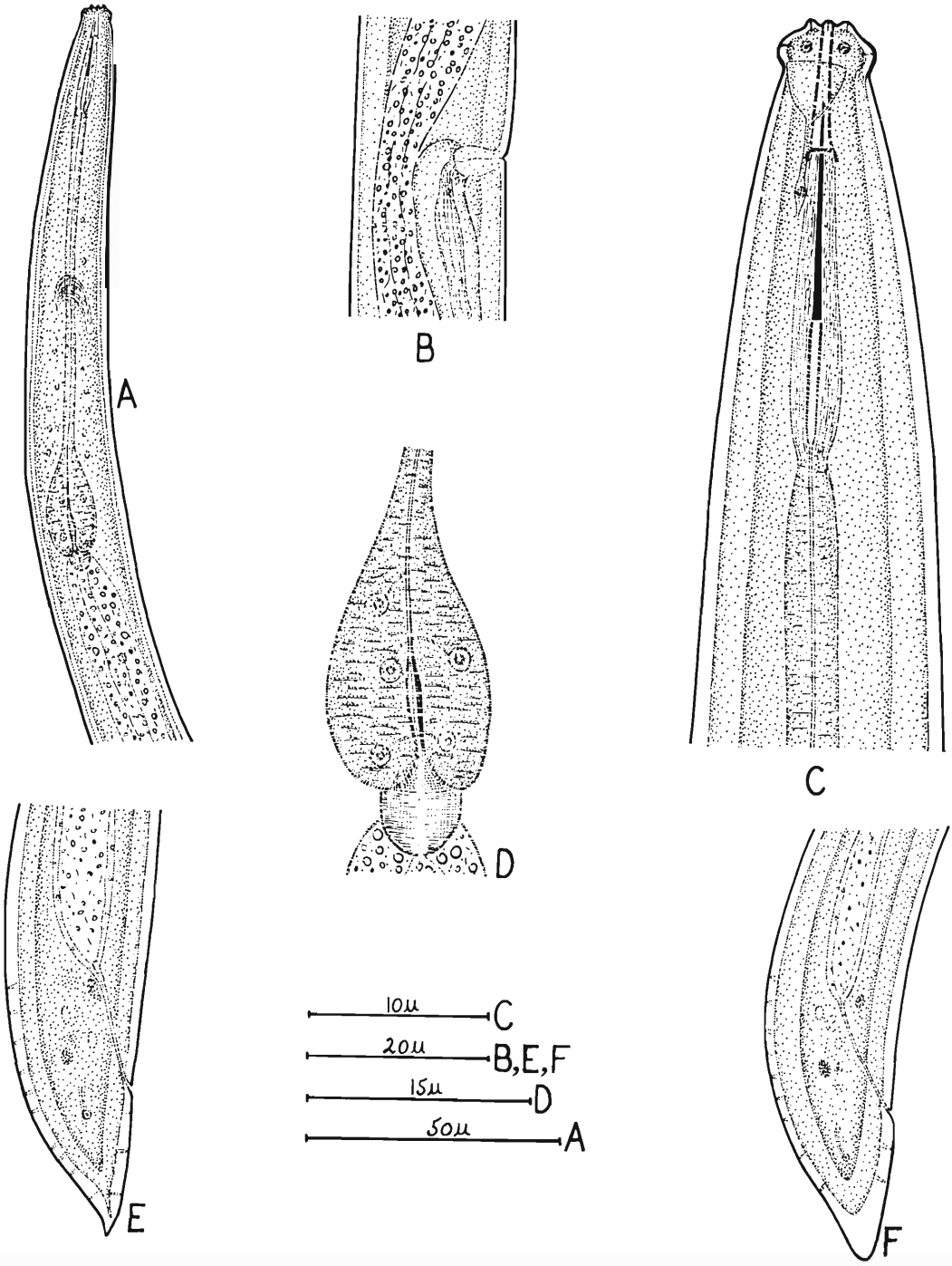


Fig. 1, A–F. *Basirotyleptus caudatus* n. sp. A, Esophageal region; B, vulval region; C, head end; D, basal bulb of esophagus; E and F, tail.

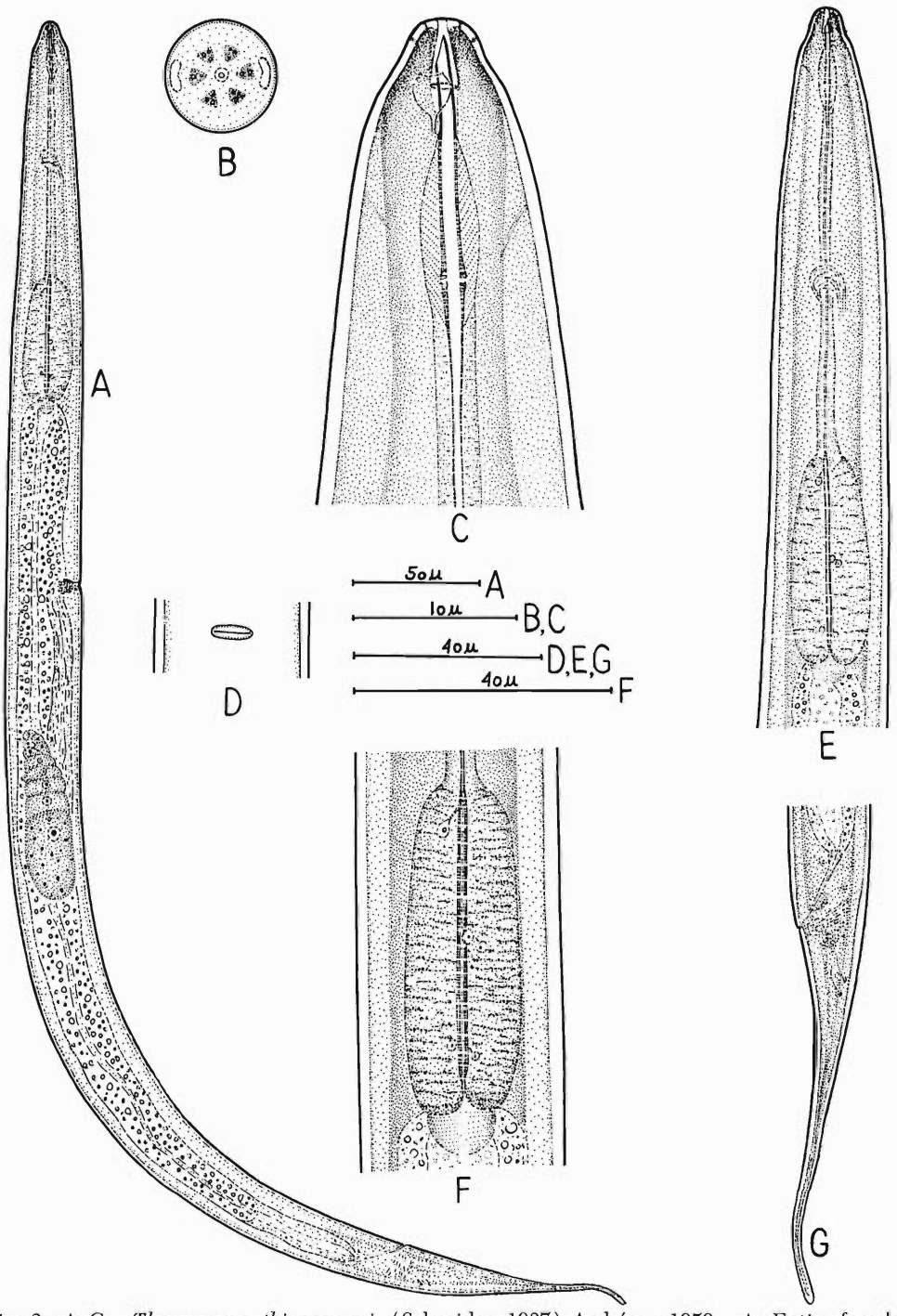


Fig. 2, A–G. *Thornenema thienemanni* (Schneider, 1937) Andrássy, 1959. A, Entire female; B. *en face* view; C, head end; D, vulva; E, esophageal region; F, basal esophageal bulb; G, tail.

way along esophagus. Vulva transverse, preequatorial. Vagina thick-walled, about one-third body width long. Ovary opisthodelphic and reflexed. Anterior uterine sac almost absent. Sperms not present in the uterus. Rectum more than one anal body width long. Prerectum about four anal body widths long. Tail conoid, somewhat digitate, slightly longer than the anal body width. A single caudal pore visible.

MALE: Not found.

HOLOTYPE AND PARATYPES: Collected by Mr. Hafeezullah on 21 October 1964, deposited with the Zoology Museum of Aligarh Muslim University.

DIFFERENTIAL DIAGNOSIS: In having opisthodelphic reproductive organs *Basirotyleptus caudatus* n. sp. comes closest to *B. basiri* Jairajpuri, 1964, *B. coronatus* Siddiqi and E. Khan, 1965, and *B. pini* Siddiqi and E. Khan, 1965, but differs considerably in having a comparatively longer conoid, somewhat digitate tail (tail hemispheroid in other species), and in having a slightly smaller and robust body and posterior position of vulva. The tail shape of *B. caudatus* is somewhat similar to *B. eximius* (Siddiqi and S. H. Khan, 1964) Siddiqi and E. Khan, 1965, but the latter has prodelphic reproductive organs.

## FAMILY DORYLAIMIDAE DE MAN, 1876
## GENUS *Thornenema* ANDRÁSSY, 1959

### *Thornenema thienemanni* (Schneider, 1937) Andrássy, 1959 (Fig. 2, A–G)

FEMALES (20): L = 0.6–0.7 mm; a = 20–26; b = 4.0–4.6; c = 6–8; V = 32–38.

DESCRIPTION: Body short, tapering towards both extremities. Cuticle and subcuticle finely striated. Lateral chords about one-third of body width at base of esophagus; lateral body pores not seen. A number of prominent radial elements present on the body except near head and tail ends. Lip region considerably narrow, about one-fifth of body width at base of esophagus and appearing yellow due to massive cuticularization. Amphids somewhat stirrup-shaped, very small, about a quarter of head width; laterally their apertures appear as obscure slits distinctly narrower than the amphidial pouches. *En face* view showing six lips and 16 cephalic papillae distributed as follows: An inner circlet of six and an outer circlet of ten of which one on each lateral and two on each submedian lip. Spear 9 $\mu$ long, slightly longer than head width, aperture about a quarter of its length. Spear extension simple, not cuticularized, slightly longer than spear. Guiding ring single. Esophagus beginning as an ellipsoidal swelling enclosing the junction of spear extension and esophageal lumen, then slightly narrowing until it suddenly expands in posterior third of its length to form the basal expanded portion. One dorsal and two pairs of subventral esophageal gland nuclei visible; their locations as illustrated. Cardia hemispheroid. Vulva transverse. Vagina with thick, spherical walls about one-fifth of body width. Ovary opisthodelphic and reflexed about one-third way back to vulva. Oocytes arranged in a single row except near tip of ovary. Anterior uterine sac absent. Posterior uterus, a short, thin-walled tube, about one and a half body widths long. Oviduct as long as uterus; the junction between oviduct and uterus not discernible. Prerectum twice anal body width long. Rectum one and a half anal body widths long. Tail at first slightly convex–conoid, then filiform to the terminus, about six anal body widths long. Caudal papillae a pair.


## SUMMARY

*Basirotyleptus caudatus* n. sp. (Dorylaimoidea:Belonenchidae) collected from soil samples from around roots of cashew nut plants at Waltair, Visakhapatanam (A.P.) is described and its affinities discussed. A redescription of *Thornenema thienemanni* (Schneider, 1937) Andrássy, 1959, based on a large number of specimens obtained from soil around roots of grasses from Kurnool (A.P.) and Andamans is also provided.


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# Significance of Males in Reproduction of the Soybean Cyst Nematode (*Heterodera glycines*)[1]

JAMES M. EPPS AND A. MORGAN GOLDEN[2]

Males of *Heterodera glycines* Ichinohe, 1952, are infrequently found in soil around the roots of soybean plants that are heavily infested with white females. Triantaphyllou and Hirschmann (1962) concluded that *H. glycines* reproduces by cross-fertilization in their study of oogensis and mode of reproduction. However, Mulvery (1958) found that the clover cyst nematode, *Heterodera trifolii* Goffart, 1932, reproduces in the absence of males. Ellenby (1957) and Fassuliotis (1957) reported that *H. rostochiensis* Wollenweber, 1923, did not develop normally or reproduce viable eggs in the absence of males. Males of *H. cyperi* Golden, Rau, and Cobb, 1962 (1965) are required for the reproduction cycle of this species. Golden (1959) found no reproduction of the sugar beet nematode (*H. schachtii* Schmidt, 1871) after inoculation of sugar beet seedlings with single larva, and concluded that the sugar beet nematode does not reproduce parthenogenetically.

We devised two experiments to determine whether males are necessary for reproduction of *H. glycines*.

*Experiment No. 1*: Four hundred 4-inch clay pots were filled with autoclaved soil and Lee soybean was seeded in the pots. When the plants were 2 weeks old a freshly hatched larva was placed on a rootlet in each of 200 pots; two larvae were placed on a rootlet in each of the remaining 200 pots. After 3 months the soil in each pot was washed from the roots, then sieved to collect cysts and white females.

*Experiment No. 2*: The only difference in the two tests was the number of pots used. In the second test 100 pots were inoculated with two larvae per pot, and 100 pots were inoculated with a single larva.

In experiment no. 1 numerous brown cysts and white females were found in 18 of the pots that had been inoculated with two larvae per pot. None was recovered from the pots inoculated with a single larva. In the second test, three white females were found in three pots that had been inoculated with single larva. These females were apparently sterile because eggs were not found in the gelatinous egg sacs. Fertilized females are known to mature, can produce eggs in approximately 25 days, and may continue producing eggs until their bodies are transformed into cysts.

In the pots inoculated with two larvae, the chances of a male and female in the same pot would be one out of three, assuming that all larvae entered the roots and developed. Because reproduction occurred on plants inoculated with two larvae and no reproduction occurred on those with one larva, we concluded that males are necessary for reproduction. Although females can develop to normal size in the absence of males, they are incapable of producing viable eggs. Our results confirm the work of Triantaphyllou and Hirschmann (1962).

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# Changes in the Redia and Metacercaria of *Metagonimoides oregonensis* Price, 1931, Transplanted from Infected to Uninfected Snails[1]

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## INTRODUCTION

This investigation was undertaken to determine the changes occurring in a trematode parasite after transplanting a larval stage from an older castrated snail to a younger uninfected snail with gonads. Szidat (1959) has given the most complete discussion to date on the effects of host hormones on trematode parasites. Particularly well suited for the present study was *Metagonimoides oregonensis* Price, 1931. Burns and Pratt (1953) described the life cycle of *M. oregonensis* and amended the description of the adult as given by Price (1931). Their study showed that both cercariae and metacercariae are produced within the redia and no daughter rediae are present. Cercariae which are shed by the stream snail, *Oxytrema silicula* (Gould), penetrate and form metacercariae in the Oregon red-legged frog, *Rana aurora aurora* (Baird and Girard). The definitive host, the raccoon, *Procyon lotor pacifica* Merriam, obtains the infection by eating the frog, and in those cases where metacercariae form in rediae, by ingesting the metacercaria with the soft parts of the snail.

## MATERIALS AND METHODS

Fifteen hundred and fifty stream snails of the genus *Oxytrema silicula* were collected during the winter, spring, and summer of 1965 from Shot Pouch Creek, Benton County, Oregon. Cracking and examination showed 4% of those over 4 cm long to be infected. Snails measuring 2 cm long possessed no infections.

Eight to 10 rediae, obtained from natural infections and concentrated in a minimum of 0.7% physiological saline, were placed in the body tissue of each of 261 young uninfected snails, 1.5 to 2.0 cm long, by use of a 1-cc syringe with an 18-gauge needle. To make the larval implants a small hole was bored midway in the length of the snail shell with a carbide-tipped burr attached to a dental-type drilling apparatus. Following transplantation, the shell opening was closed by applying melted paraffin to a thoroughly dry surface. Snails were maintained individually in finger bowls for 1 to 6 weeks at a cold room temperature which simulated Shot Pouch Creek (12 C). Lettuce and leaves, boiled to remove tannins, served as food for all snails.

Experimentally infected snails examined at weekly intervals showed either free-moving metacercariae and/or rediae. Fifty to 75 metacercariae obtained from both naturally and experimentally infected snails were fed to each of seven golden hamsters. Larval forms obtained from the transplanted infections were fed to four hamsters at the end of the first, second, third, and fourth weeks, respectively. Larval forms obtained from fifth- and sixth-week infections were fed to one hamster. Two hamsters were fed metacercariae from natural infections. After 7 days, hamsters fed larval trematodes from both natural and experimental infections were sacrificed and the intestinal contents examined.

Adult and larval trematodes were fixed in AFA, stained in Semichon's acetocarmine, cleared in oil of cloves, and mounted in balsam.

## RESULTS AND DISCUSSION

The initial mortality in snails infected experimentally with larval *M. oregonensis* exceeded 20%. Those which could be maintained for 1 day usually survived for 4 weeks. Within 4 to 6 weeks 75% mortality occurred; by 6 weeks all had succumbed.

Larvae obtained from transplanted infections produced no adult trematodes as determined by examination of the intestinal contents of five hamsters, each of which had been fed 50 to 75 living metacercariae. Two hamsters given larvae taken from natural infections were both infected. One which died had 62 egg-bearing trematodes in its intestine. Forty trematodes

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were present in the intestine of the second hamster. Similar results were obtained by Burns and Pratt (1953) when natural infections of rediae and metacercariae were fed to hamsters. Complete descriptions of all stages of *M. oregonensis* with illustrations are also given by these workers.

Rediae implanted in young uninfected *Oxytrema* often broke, releasing their metacercariae into the body cavity. However, 40% of rediae could be expected to remain intact as determined by cracking snails and examining their contents 1 day after implantation of the rediae. Of that number most failed to undergo further change and apparently died. Some metacercariae released from rediae remained active for six weeks. Average measurements in millimeters of five metacercariae recovered from 4-week infections are as follows with minima and maxima in parentheses: body length 0.23 (0.17–0.27); body width 0.20 (0.17–0.22); oral sucker diameter 0.49 (0.46–0.50). These sizes are almost identical with those from natural infections and correspond closely with the ones reported by Burns and Pratt (1953). However, metacercariae obtained from snails infected experimentally usually displayed greater activity than those obtained from natural infections. The eyespots disappeared, and the prominent Y-shaped excretory bladder which normally appears black when observed with transmitted light was often enlarged and possessed fewer granules at the end of 2 weeks.

Most notable changes were observed in transplanted infections of rediae maintained for three weeks. During that time, enlargement of the redia sometimes occurred. Mucus and debris were present in the larval cecum and the enclosed metacercariae were no longer distinguishable. Snails maintained for 4 to 5 weeks possessed rediae which were no different in appearance from those recovered at 3 weeks. In the majority of snails all transplanted rediae died even though the snails were fairly active. Enlarged and living rediae were present in only eight snails examined. Average sizes and minima and maxima of five rediae maintained in experimentally infected snails for 3 weeks are shown in Table 1. Average sizes and minima and maxima for rediae taken from the same natural infection, but not transplanted, are also given.

TABLE 1. Comparison of sizes of rediae taken from experimental and natural infections. All measurements in millimeters.

| | Experimental infections | | | Natural infections | | |
|---|---|---|---|---|---|---|
| | Average | Minima | Maxima | Average | Minima | Maxima |
| Body length | 2.56 | 2.45 | 2.80 | 1.07 | 0.95 | 1.12 |
| Body width | 0.418 | 0.325 | 0.480 | 0.182 | 0.150 | 0.210 |
| Cecal length | 0.720 | 0.600 | 0.800 | 0.119 | 0.100 | 0.125 |
| Cecal width | 0.88 | 0.060 | 0.100 | 0.017 | 0.013 | 0.020 |
| Pharynx width | 0.450 | 0.060 | 0.150 | 0.026 | 0.025 | 0.029 |

From midsummer to early autumn naturally infected *Oxytrema* shed cercariae spontaneously. Examination of rediae showed about equal numbers of cercariae and metacercariae. During the remainder of the year, only metacercariae were present in rediae and cracking was necessary to distinguish infected from uninfected snails. Daughter rediae were not present at any time of the year. Production of cercariae is possibly stimulated by slight increases in water temperature. Shot Pouch Creek is one of many streams supplied by melting mountain snow. By midsummer the majority of the winter snowfall has melted, the water level in the streams is lower and flows less rapidly, with resulting increases in water temperature.

It was expected that transplanted rediae when subjected to a hormone supply in snails which were uninfected, and therefore not castrated, would initiate production of daughter rediae and possibly maturation of their metacercariae. As shown in Table 1, the sizes of transplanted rediae were more than twice those of rediae not transplanted. Material was present in the ceca of all living rediae recovered from snails infected experimentally. In all natural infections of *Metagonimoides oregonensis* observed, only larval stages were present. The apparent stimulus given to rediae in the new host was to increase in size and ingest food material rather than to initiate alteration of development within the redia. The high mortality in transplanted rediae is not explained. The fact that metacercariae were no longer distinguishable in the redia indicates that the transplanting of rediae halted the usual sequence of development as well as the capacity to produce larval stages. The rediae were not infective to hamsters.

Metacercariae which were released into the snail by breaking of rediae during or soon after

transplantation lost one prominent larval feature, eyespots, and showed a reduction in granules of the urinary bladder. The fact that none developed into adults even when ample numbers were fed to hamsters is not explained.

SUMMARY

Larval trematodes of *Metagonimoides oregonensis* Price, 1931, were transplanted from castrated naturally infected snails of the genus *Oxytrema silicula* (Gould) to young uninfected snails with gonads. The effects of host hormones on the development of rediae and metacercariae were observed.

Snails, numbering 261 and measuring 1.5 to 2.0 cm, were infected experimentally. Initial snail mortality of 20% resulted, and mortality of rediae in all remaining snails was high. Eight snails maintained living rediae which more than doubled in size, ingested debris into their ceca, and broke down their enclosed metacercariae. These rediae were not infective to hamsters.

Metacercariae were often released into the body cavities of snails infected experimentally. Many survived for up to 6 weeks. Five golden hamsters fed 50 to 75 metacercariae each were not infected. Two hamsters fed equivalent numbers of metacercariae obtained from natural infections yielded 62 and 40 egg-bearing trematodes, respectively.

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## Note on the Ovary, Rachis, and Spermatheca of an Insect Parasitic Nematode, *Contortylenchus elongatus* (Massey, 1960) Nickle, 1963

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The morphology and development of the ovary of members of the insect parasitic nematode family Allantonematidae are unique, though little information is available on them. The spermatozoa are small. The free-living females (.60 mm in length) are fertilized in the frass of a bark beetle gallery or in the substrate where larval stages of host insects are present. Koriogamy is the normal type of fertilization. The copious sperm are packed tightly in the uterus. A parasitic sojourn in the body cavity of a suitable insect is required to complete the life cycle of the nematode. After the nematode gains entrance into an insect larva or pupa, it grows to 6–10 times its original length and 10 times in diameter. The ovary begins as a small (20 microns), fingerlike projection and grows to 100 times that length, so that eventually about 80% of the volume of a full-grown adult parasitic female is taken up by the gonad at the expense of other internal organs. Morphologically and physiologically the success of this egg-producing apparatus is centered about the rachis and the free flow of usable nutritive substances from the haemolymph of the host insect directly to the pseudocoelom of the nematode and to the developing oocytes.

Chitwood and Chitwood (1950) reported a rachis in the ovary of oxyurids, ascarids, strongylids, spirurids, thelastomids, and some tylenchs. Thorne (1949) showed the rachis of *Anguina tritici* to be a large, pulpy, cellular region encircled by a thin layer of oocytes. The rachis of *Ascaris lumbricoides* is a definite cyl-

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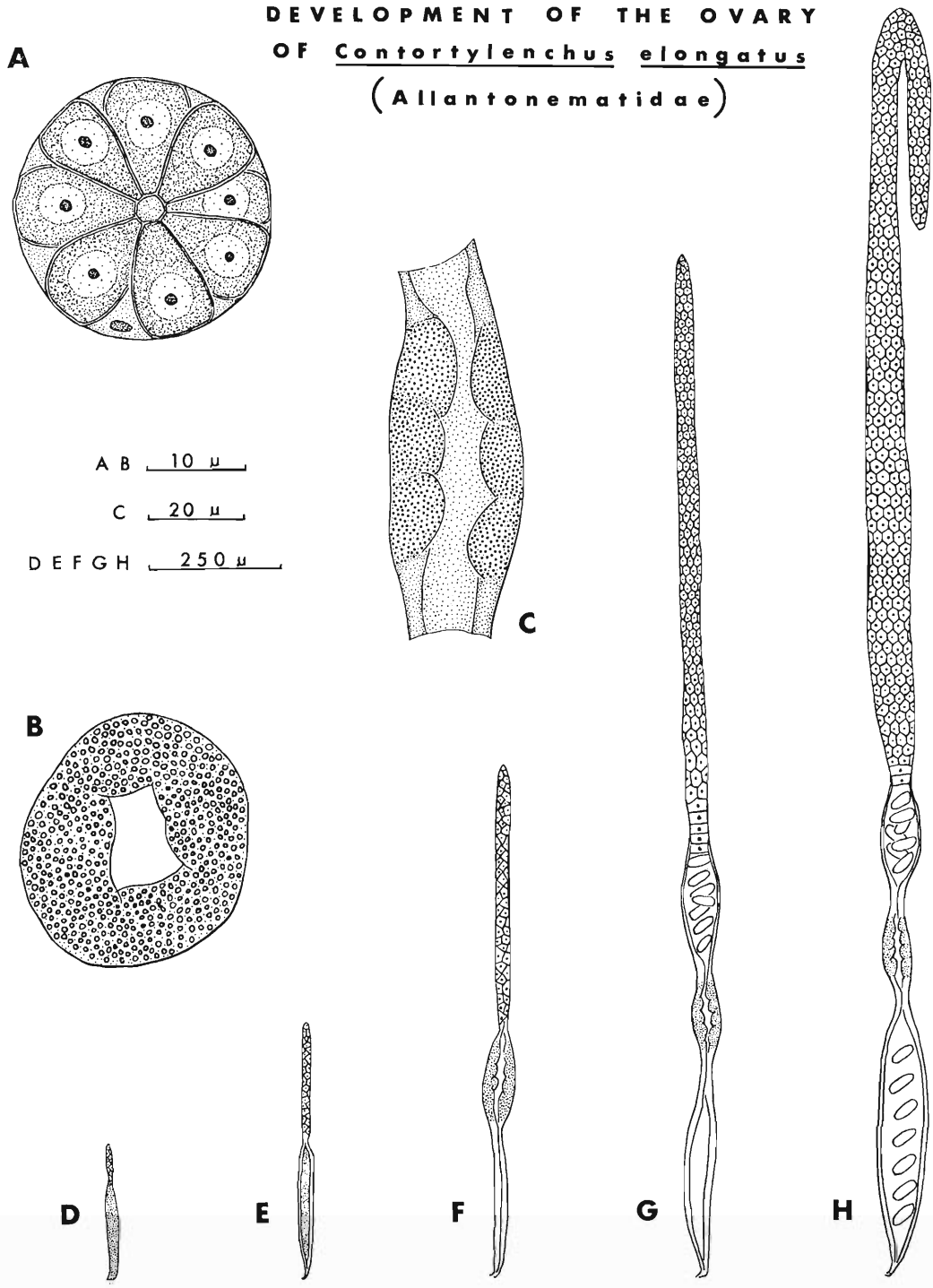

inder apparently composed of a bundle of nutritive chords (Hirschmann, 1960). Three genera of allantonematids are known to possess a rachis. Zur Strassen (1892) illustrated it in *Bradynema*, and Bovien (1937) reported one in *Heterotylenchus*. Warren (1941) described the rachis in *Howardula* as consisting of vacuolated cytoplasm without nuclei.

As a result of a study undertaken to gain information which might be of taxonomic value at the specific level, new information on the development of the ovary after penetration of the host and also on the rachis and spermatheca of *Contortylenchus elongatus* (Massey, 1960) Nickle, 1963, was uncovered in a search for structures of taxonomic value and is presented here.

A rachis is visible in serial sections of the ovary of *C. elongatus* (Fig. 1A). The rachis begins at the cap cell and extends with an increasing diameter to the region where the oocytes are largest and are arranged in pairs or singly. This rachis is similar to that illustrated by Chitwood and Chitwood (1950) for the oxyuroid, *Spironoura affine* Leidy, 1856. This central longitudinal core of the ovary has an unknown function. Of the two possible functions that will be considered, the first has more merit. If the oocytes derive their nourishment osmotically from the pseudocoelom of the nematode or indirectly by way of the haemolymph of the host, then the rachis may function morphologically solely as a central point of attachment for the oocytes and not nutritionally. In this case, as the wall of the ovary contains large nuclei, these cells may function by allowing nutrients to pass to the oocytes from the body cavity. A second possibility exists that the rachis may contain a bundle of fibrils or nutritive chords that are fed by the germarium or cap cell and extend to each oocyte. This situation is true in many insects and is indicated in *Ascaris*. Thousands of strands would be necessary in this case and their presence could not be confirmed in the sections examined. The cap cell is not very well developed and it is unlikely that it is capable of feeding as many as 8,000 oogonia. Nematodes that possess a rachis normally have a prolific reproductive potential and are often parasitic. Probably all genera in the Allantonematidae have a rachis.

The spermathecae of *C. elongatus* (Figs. 1B, C) appear as a series of pockets in the wall of the oviduct. In all probability the spermatozoa are stored inside the swollen cells. The mode of entrance could not be determined. The location of this region is constant, which indicates that a definite structure is present. The spermatozoa in the infective stage female (Fig. 1D) are transported anteriorly by undulations of the oviduct during the enormous expansion of the ovary and of the nematode after her entrance into the host insect (Fig. 1E, F). The sperms are stored in or along the wall of the oviduct (Fig. 1F) and are available for fertilization of the ova as they pass this region. The time required for the gonad to develop from that of the infective stage female (Fig. 1D) to the much enlarged gonad of the mature female (Fig. 1H) is from 2 to 3 weeks. The life history of the parasite is essentially synchronized with its host.

## SUMMARY

The development of the ovary including the rachis and spermatheca of an insect parasitic, allantonematid, *Contortylenchus elongatus* (Massey, 1960) Nickle, 1963, is discussed, and the unique ovarial development and the formation of the spermathecae of this nematode are described and illustrated. It is believed that all members of the family Allantonematidae have a rachis which facilitates morphologically the massive reproduction of these parasites.

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Fig. 1. A–C. Sections of the ovary of *Contortylenchus elongatus*. A, Oocytes arranged about a central rachis; B, transverse, through spermathecae; C, longitudinal, through spermathecae. D–H. Periodic development of nematode ovary in host. D, ovary of infective stage female; E, shortly after entrance into host; F, sperm moved into spermathecae; G, ova before fertilization; H, mature ovary.

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# The Systemic Action of Methyridine Against Helminths, Especially Whipworms, in Dogs

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Few canine anthelmintics given by mouth are uniformly effective against the whipworm, *Trichuris vulpis*. This is ascribable, in part, to factors associated with the location of the parasite in the cecum (Enzie and Colglazier, 1953). Also, because dogs vomit readily, they often expel a large part of any anthelmintic dose given *per os*.

To circumvent these barriers to reliable trichuricidal action, a variety of alternative approaches have been employed, including cecectomy, prolonged medication with anthelmintics in small daily doses, medicinal enemas, and the use of drugs that act systemically, such as phthalofyne (Eshenour *et al.*, 1957). This drug, given by intravenous injection, exhibits anthelmintic action against only *Trichuris*. Walley (1961) first reported the systemic action of methyridine in trials against a variety of helminths in ruminants. The optimum dosage, given subcutaneously, was 200 mg/kg of body weight. A few months later, Guilhon (1961) reported, on the basis of egg counts, that a similar regimen was effective against whipworms in trials with five dogs. The treatment was reasonably well tolerated although there was evidence of acute irritation at the site of injection.

Because only limited information is available on the use of systemic anthelmintics, it seemed desirable to obtain critical data on the action of methyridine against a variety of helminth parasites in animals and poultry. The present report summarizes data obtained in trials against several intestinal worm parasites of dogs.

## MATERIALS AND METHODS

The tests were made with 27 purebred beagles that ranged from 4 months to 2 years of age. The dogs had naturally acquired or experimentally established infections of one or more helminth species, namely, *Trichuris vulpis*, *Toxocara canis*, *Ancylostoma caninum*, *Dipylidium caninum*, and *Taenia hydatigena*.

One or 2 days before treatment the dogs were isolated in individual cages and the feces screened daily to detect natural elimination of parasites. A solution of methyridine[2] was given by subcutaneous injection, usually near the right shoulder, in dosages ranging from 100 to 200 mg/kg of body weight. The feces of each animal were collected daily and screened for parasites in the usual manner. When elimination of parasites ceased, usually within 3 or 4 days, the dogs were necropsied and the gastrointestinal tract examined for parasites and lesions. Five dogs were not necropsied; in these trials, the efficacy was judged on the basis of periodic fecal examinations during a posttreatment observation period of several months.

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[2] The methyridine used in these trials was supplied as the proprietary Promintic through the courtesy of Fort Dodge Laboratories, Fort Dodge, Iowa.

TABLE 1. Data on the anthelmintic action of methyridine when given to dogs by subcutaneous injection.

| Number of dogs | Dosage (mg/kg) | Parasites | Removed | Left | Efficacy (percent) | Number of tests |
|---|---|---|---|---|---|---|
| 7 | 100 | *Toxocara* | 10 | 42 | 19 | 2 |
| | | *Ancylostoma* | 46 | 126 | 27 | 4 |
| | | *Trichuris* | 1152 | 25 | 98 | 5 |
| 4 | 125 | *Toxocara* | 0 | 53 | 0 | 4 |
| | | *Ancylostoma* | 2 | 20 | 9 | 1 |
| | | *Trichuris* | 435 | 116 | 79 | 4 |
| 4 | 150 | *Toxocara* | 0 | 18 | 0 | 2 |
| | | *Trichuris* | 348 | 0 | 100 | 4 |
| 7 | 200 | *Toxocara* | 9 | 5 | 64 | 1 |
| | | *Ancylostoma* | 151 | 49 | 76 | 4 |
| | | *Trichuris* | 385 | 0 | 100 | 6 |
| | | *Dipylidium* | — | 2 | — | 1 |
| 5 | 200 | *Trichuris* | 902 | — | —[1] | 5 |
| | | *Taenia* | 1 | 0 | 100 | 1 |

[1] Animals not necropsied, but posttreatment fecal examinations indicated that all worm parasites were eliminated.

## RESULTS

The results are given in Table 1. Methyridine removed all of 348 *Trichuris* from four dogs when given at a dose rate of 150 mg/kg of body weight; and at 200 mg/kg, the drug was wholly effective against 385 *Trichuris* in six dogs. Neither dosage was markedly effective against other nematode species, and the larger dosage showed no action against *Dipylidium* in a single trial.

Additional data were obtained at the 200-mg level with dogs that were not necropsied. A total of 902 *Trichuris* were recovered from five dogs, and no whipworm eggs were found in periodic fecal examinations during a posttreatment observation period of several months. One complete *Taenia hydatigena* was recovered from one of the dogs that had been given a single cysticercus several months before treatment.

Methyridine exhibited significant trichuricidal action also when given in dosages of 100 and 125 mg/kg of body weight. The drug was not uniformly effective, however, at these levels. There was no significant action against *Toxocara* and *Ancylostoma* in these trials.

Excessive salivation, emesis, ataxia, and diarrheal feces were observed to some degree at all dosage levels. These reactions, however, were temporary, and all dogs recovered fully within 24 hours. There was evidence of acute irritation at the site of injection at all dosage levels.

## SUMMARY

Limited tests with subcutaneous injections of methyridine, a systemic anthelmintic, indicate that this chemical may compare favorably with other trichuricides for dogs. A dosage of 150 mg/kg of body weight was completely effective against 348 *Trichuris* in four dogs; at 200 mg/kg, the drug removed all of 385 *Trichuris* from six dogs. The larger dosage was presumably fully effective also in five additional dogs that were not necropsied.

Moderate activity was exhibited against *Ancylostoma* in limited trials at the 200-mg level. Anthelmintic action against *Toxocara*, *Taenia*, and *Dipylidium* was either negligible or too limited to permit even provisional interpretations of efficacy.

Emesis, ataxia, soft feces, and acute irritation at the site of injection were evidenced at all dosage levels. These reactions, however, were transitory.

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# Pancreatic Enzymes Initiating Excystation of *Eimeria acervulina* Sporozoites

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In previous studies (Doran and Farr, 1962; Farr and Doran, 1962), it was found that commercial preparations of pancreatic enzymes [trypsin l-300, lipase (steapsin), and trypsin 2× (crystalline, salt free)][2] in the presence of 5% (v/v) chicken bile or 1% (w/v) sodium taurocholate induced excystation *in vitro* of *Eimeria acervulina* sporozoites from sporocysts mechanically released from oocysts. Under optimal conditions, trypsin 2× induced only 60–65% excystation in 1 hour, whereas trypsin l-300 and the lipase preparation (each found upon assay to exhibit lipolytic and tryptic activity) induced 82–95% excystation during the same interval. The action of trypsin 2× was completely inhibited by soybean trypsin inhibitor, whereas the actions of trypsin l-300 and lipase were only partially inhibited. On the basis of these findings, it was suggested that trypsin and one or more other pancreatic enzymes, perhaps lipase, were involved in the excystation process.

The data reported herein concerns the effects of lipase, trypsin, carboxypeptidase, and chymotrypsin on excystation *in vitro*.

## MATERIALS AND METHODS

The methods for (1) collecting and treating oocysts prior to use, (2) mechanically releasing sporocysts from their oocysts, (3) sampling, counting, and obtaining percentages, and (4) maintaining hydrogen ion concentration were the same as previously described (Doran and Farr, 1962). Oocysts were between 2 and 5 months old when used.

Trypsin was obtained from Nutritional Biochemicals Corporation; lipase and the chymotrypsins from General Biochemicals; and carboxypeptidases from Worthington Biochemicals. Bile was obtained from chickens belonging to this laboratory. It was diluted to 5% (v/v) with Ringer's solution and stored at –40 C. Concentration of enzymes is expressed as gms/100 ml of 5% chicken bile.

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[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

## RESULTS

Alpha chymotrypsin and its three intermediates induced similarly high percentages of excystation (Table 1). The percentages were comparable to those previously obtained with trypsin l-300 and about 30% higher than those obtained with trypsin 2× (Table 1 and Doran and Farr, 1962). In the absence of bile, each of the chymotrypsins induced less than 15% excystation.

In either the presence or absence of bile, lipase and the two carboxypeptidases were without effect after five hours.

## DISCUSSION

The high percentages of excystation (82–90%) previously obtained with two impure preparations [trypsin l-300 and lipase (steapsin)] were most probably due to the action of chymotrypsin or the combined actions of

TABLE 1. Effect of lipase and proteolytic enzymes on excystation of sporozoites from sporocysts. (Numbers in parentheses indicate range of 3–4 determinations.)

| Enzyme | Concentration (gms/100 µl of 5% chicken bile) | pH | Percent excystation in 1 hour |
|---|---|---|---|
| Trypsin[1] | 0.00015* | 7.4 | 60 (51–72) |
| Carboxypeptidase A[2] | 0.15 | 7.4 | 0 |
| " B | 0.15 | 7.4 | 0 |
| Alpha chymotrypsin[3] | 0.0014* | 7.5 | 93 (81–97) |
| Beta " | 0.0014 | 7.5 | 90 (82–93) |
| Gamma " | 0.0014 | 7.5 | 95 (90–97) |
| Delta " | 0.0014 | 7.5 | 91 (80–95) |
| Lipase[4] | 0.25 | 6.5 | 0 |
| Trypsin | 0.00015 | 7.4 | 55 (47–68) |
| Lipase | 0.25 | | |
| Trypsin | " | 6.5 | 37 (20–50) |
| Lipase | | | |

[1] 2× crystalline (salt-free).

[2] All 2× crystalline (treated by Worthington Biochemicals to eliminate trypsin and chymotrypsin activity).

[3] All 3× crystalline (salt-free from ethanol). Assay, 11,000 µ/mg (General Biochemicals).

[4] Assay, 50 µl $CO_2$/mg/30 min (General Biochemicals).

* Lowest concentration inducing maximum excystation.

trypsin and chymotrypsin rather than to either of these proteolytic enzymes and lipase. Lipase was found to be ineffective by itself and to induce only low percentages of excystation (generally lower than with only trypsin 2×) when tested in combination with trypsin.

It is doubtful whether trypsin and chymotrypsin are solely responsible for the enzymatic digestion of the sporocystic plug. They certainly initiate the process and probably activate the sporozoites. However, it is believed that the sporozoite also secretes an enzyme that acts on the inner surface of the sporocystic plug.

SUMMARY

Trypsin and chymotrypsin in the presence of bile induced excystation of *Eimeria acervulina* sporozoites from liberated sporocysts. Carboxypeptidase and lipase were without effect.

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FARR, M. M., AND D. J. DORAN. 1962. Comparative excystation of four species of poultry coccidia. J. Protozool. 9(4): 403–407.

## Location and Time of Penetration of Duodenal Epithelial Cells by *Eimeria acervulina* Sporozoites

DAVID J. DORAN[1]

When studying the path of migration of *Eimeria acervulina* sporozoites, Doran (in press) found that the greatest number of sporozoites were present 3 hr after inoculation and that 75% of these were in the villar epithelium. It was also found that after 3 hr the total number of sporozoites declined and that 11% or less were in the villar epithelium. The others were in the lamia propria either by themselves or engulfed by macrophages. The earliest time used in the above work was 3 hr.

The present study concerns time intervals less than 3 hr. Since use of villar epithelial cells parasited with sporozoites might possibly prove to be a fruitful approach to the cultivation of the early schizogonic stages of this species, it was thought advantageous to determine (1) the area of the duodenum most penetrated by sporozoites, (2) the earliest time of penetration, and (3) the time of greatest penetration.

### MATERIALS AND METHODS

The source of *E. acervulina* oocysts and the methods for their recovery, sporulation, storage, and treatment prior to administration were the same as previously described (Doran and Farr, 1962; Farr and Doran, 1962). Oocysts were between 4 and 9 weeks old when fed to the chickens.

New Hampshire (USDA strain) chickens 2 to 3 weeks old were used. Sporulated oocysts were administered as far down into the esophagus as possible by means of a small pipette. Feed and water were available to the chickens at all times.

At desired time intervals, chickens were killed with ether and the duodenum removed. Pieces 2 to 3 mm in width from various areas (Fig. 1) were fixed in Bouin's fluid, processed, and embedded in paraffin. Ribbons from the middle and both ends of the sample were mounted and stained with Mallory's hematoxylin and eosin. The sporozoites in each of three sections, one from each of the ribbons, were counted. Counting was greatly facilitated by the fact previously found (Doran, in press) that sporozoites were present in only the tips and down to about one-half the length of the villi at 6 hr.

The crop, proventriculus, and gizzard were also removed when it was necessary to know the amount of a given dosage reaching the duodenum. Their contents were thoroughly washed into a beaker and then into a volumetric flask. The numbers of oocysts and liberated sporocysts were determined with the aid

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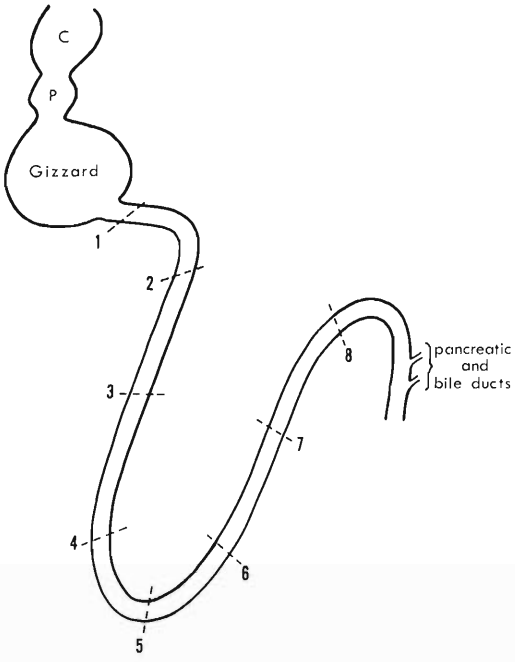


Fig. 1. Location of areas in the duodenum from which tissue was taken for study.

of a hemocytometer. The percentage of the dosage reaching the intestine was calculated using the number of sporozoites in the dosage of oocysts given and the number of sporozoites remaining above the intestine.

## RESULTS

AREA OF GREATEST PENETRATION: Sporozoites did not penetrate all areas equally. They penetrated areas 1 and 2 least; only a few were present after the heavier dosages and none after the lighter ones (Table 1). Sporozoites penetrated areas 4 through 8 most. In this length of duodenum, which extends from a point just before the curvature of the loop begins to about 2.5 cm from the pancreatic duct, the sporozoites were found in about equal amounts.

TIME OF EARLIEST AND GREATEST PENETRATION: Since there was no significant difference between the numbers of sporozoites that penetrated areas 4 through 8 at 1 hr, three of these areas were used in determining the time of greatest penetration. Table 2 shows the numbers of sporozoites found at different time intervals in areas 4, 6, and 8. The time of greatest penetration is most probably 1 hr after exposure. The number of sporozoites in the villar epithelium was highest at that time and declined thereafter, whereas the total number in all areas remained about the same. The validity of this time is increased since it was found that there was no significant difference in the dosage reaching the intestine between 30 min and 3 hr after exposure (Table 3).

TABLE 1. Numbers of sporozoites found in different areas of the duodenum 1 hr after inoculation.

| Area | Experiment number | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| 1 | 3 (1, 0, 2) | 0 | 18 (5, 7, 6) |
| 2 | 22 (7, 7, 8) | 0 | 18 (10, 7, 1) |
| 3 | 95 (21, 39, 35) | 28 (14, 7, 7) | 115 (32, 49, 34) |
| 4 | 175 (70, 61, 44) | 51 (20, 14, 7) | 195 (60, 62, 73) |
| 5 | 157 (45, 51, 61) | 52 (20, 17, 15) | 178 (51, 47, 80) |
| 6 | 176 (51, 71, 54) | 71 (20, 20, 31) | 192 (51, 72, 69) |
| 7 | 160 (70, 60, 30) | 65 (20, 20, 25) | 180 (57, 59, 64) |
| 8 | 196 (50, 72, 64) | 60 (17, 17, 26) | 190 (52, 69, 69) |

Dosage: Experiments 1 and 3, 11 million oocysts; experiment 2, 7 million.
Numbers in parentheses represent individual counts of sporozoites found in both the villar epithelium and lamina propria.

In two of the experiments, a small number of sporozoites were found in the villar epithelium as early as 10 min; in the other, 30 min.

## DISCUSSION

Tyzzer, Theiler, and Jones (1932) stated that, within 1 hr after chickens had been given heavy dosages of *E. necatrix*, sporozoites were found in the intestinal lumen and in the gland fundi. Ten minutes after feeding might appear to be an extremely short time for sporozoites of *E. acervulina* to be found in the villar epithelium. However, it does not appear unreasonable since passage of oocysts to the gizzard, liberation and passage of sporocysts out of the gizzard, and excystation of sporozoites can take place with extreme rapidity. Henry *et al.* (1933) observed that the crop in chickens can contract every 50–60 sec. They also found that feeding causes cessation of contraction for one-half hour or more, after which it resumes at regular intervals. In the present study, the chickens killed at 10 min had no food in their

TABLE 2. Numbers of sporozoites found at different times after inoculation.

| Experiment number | Area | Time (hrs ± 5 min) ⅙ A | ⅙ B | ½ A | ½ B | 1 A | 1 B | 2 A | 2 B | 3 A | 3 B |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 15 | 0 | 105 | 18 | 351 | 12 | 310 | 45 | 256 | 94 |
| | 6 | 0 | 0 | 180 | 6 | 310 | 18 | 253 | 93 | 240 | 90 |
| | 8 | 24 | 0 | 90 | 21 | 260 | 27 | 250 | 89 | 210 | 125 |
| Totals | | 39 | 0 | 375 | 45 | 921 | 57 | 813 | 227 | 706 | 309 |
| Grand total | | 39 | | 420 | | 978 | | 1,040 | | 1,015 | |
| 2 | 4 | 0 | 0 | 54 | 6 | 251 | 18 | 210 | 47 | 153 | 89 |
| | 6 | 0 | 0 | 90 | 18 | 276 | 30 | 280 | 73 | 138 | 90 |
| | 8 | 0 | 0 | 45 | 12 | 280 | 21 | 210 | 59 | 210 | 139 |
| Totals | | 0 | 0 | 189 | 36 | 807 | 69 | 700 | 179 | 501 | 318 |
| Grand total | | 0 | | 225 | | 876 | | 879 | | 819 | |
| 3 | 4 | 3 | 0 | 153 | 0 | 390 | 21 | 237 | 51 | 147 | 122 |
| | 6 | 12 | 0 | 141 | 6 | 240 | 33 | 273 | 63 | 230 | 101 |
| | 8 | 6 | 0 | 201 | 9 | 243 | 51 | 240 | 67 | 210 | 87 |
| Totals | | 21 | 0 | 495 | 15 | 873 | 105 | 750 | 181 | 587 | 310 |
| Grand total | | 21 | | 510 | | 978 | | 931 | | 897 | |
| Totals (three experiments) | | 60 | 0 | 1,059 | 96 | 2,601 | 231 | 2,263 | 587 | 1,794 | 937 |
| Percent of grand total (three experiments) | | 100 | 0 | 92 | 8 | 92 | 8 | 79 | 21 | 66 | 34 |
| Grand total (three experiments) | | 60 | | 1,155 | | 2,832 | | 2,850 | | 2,721 | |

A = In epithelium of villus.
B = In lamina propria.
Dosage—15 million oocysts/chicken.

crops. Marshall (1960) states that there are one to four contractions of the gizzard every minute and that food may reach the intestine in 10 min. Sturkee (1954) says that fluids pass through the gizzard even faster than solid food. Excystation of *E. acervulina* is rapid. Doran and Farr (1962) found that 5–10 min *in vitro* was sufficient for a few sporozoites to emerge. It was later found (unpublished) that excystation is almost immediate under the right conditions.

Doran (in press) found that, after 3 hr, many sporozoites are either destroyed by macrophages or ejected by the macrophages into the gland lumen. In the present study the percentage of sporozoites within the villar epithelium dropped gradually to 66% at 3 hr. The others were in the lamina propria—either alone or engulfed by microphages in similar amounts. Since the total numbers of sporozoites found at 1, 2, and 3 hr were about the same, it is unlikely that macrophages destroyed any of the sporozoites en route to the glandular epithelium at these earlier time intervals.

TABLE 3. Percentage of dosage reaching the duodenum.

| Experiment number | Time (hr) ⅙ | ½ | 1 | 2 | 3 |
|---|---|---|---|---|---|
| 1 | 81 | 89 | 92 | 95 | 95 |
| 2 | 69 | 91 | 96 | 98 | 92 |
| 3 | 84 | 93 | 92 | 90 | 94 |

When conditions are optimal *in vitro*, Farr and Doran (1962) found that most of the sporozoites that are going to excyst do so within one hr. In the present study, the time when most sporozoites were found in the villar epithelium was also 1 hr. They probably penetrate the villar epithelium shortly after excysting. If this were not true, the number of sporozoites found in the villar epithelium at 1, 2, and 3 hr probably would have been similar or increasingly greater after 1 hr.

## SUMMARY

*Eimeria acervulina* sporozoites penetrated the villar epithelium as early as 10 min after the chickens had been fed oocysts. The time of greatest penetration was at 1 hr.

Most sporozoites penetrated a length of the duodenum extending from a point just before the curvature of the loop begins to about 2.5 cm from where the pancreatic duct enters.

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# *Urocleidus flieri* n. sp. (Trematoda : Monogenea) from the Flier Sunfish

ROBERT E. PUTZ AND GLENN L. HOFFMAN[1]

## INTRODUCTION

Twelve flier sunfish, *Centrarchus macropterus*, were submitted 29 October 1963 for parasitological examination by Dr. Frank Roberts, a 1963 Fellow at this laboratory. The fish were collected from White Marsh Swamp, Columbus County, North Carolina.

Upon examination of the gills, two to four *Urocleidus* sp. were found per gill arch.

After comparison with known species of *Urocleidus* found on North American fish, it is here described as a new species, *Urocleidus flieri*.

## MATERIAL AND METHODS

To obtain the parasites for microscopy, gills were dissected and placed in Petri dishes containing a solution of 1:4,000 formalin (modification of Parker and Haley, 1960). After a few minutes the parasites dropped from the infected gill filaments and were pipetted to slides for study or fixed in 10% formalin for permanent preparations. Methyl green (0.25% in 1.0% acetic acid) was employed for temporary progressive staining. Harris' hematoxylin and Semichon's carmine stains were used for permanent mounts.

Gross measurements were made from parasites removed from the 1 : 4,000 formalin with ample liquid beneath the cover slip to minimize cover slip pressure distortion.

For studying the hard parts in detail, the soft body parts were allowed to decompose under a vaseline-sealed cover slip (Ikezaki and Hoffman, 1957).

All measurements are in millimeters and based on ten or more specimens. The average is given with the range following in parentheses.

## DESCRIPTION

*Urocleidus flieri* n. sp. (Figs. 1–6)

Relatively large Tetraonchinae with smooth cuticula devoid of scales and spicules; length 0.741 (0.650–0.850), area of greatest width 0.170 (0.150–0.180). Cephalic lobes, four, two on each side of midline. Eyespots, two pair, anterior pair smaller and closer together and made up of definite egg-shaped pigment granules. Pharynx, dorsal view circular in outline, diameter 0.050 (0.037–0.055). Vitellaria distributed laterally as two bands joining posteriorly. Haptor 0.100 (0.075–0.112) by 0.057 (0.037–0.065). Anchors slightly variable in size; bases bifurcate with superficial roots slightly larger than deep roots. Wings present. Ventral anchors longer than dorsal ones; length 0.025 (0.023–0.026), base 0.015 wide (Fig. 1). Dorsal anchor length 0.022 (0.021–0.023), base 0.011 (Fig. 2). Dorsal and ventral bars slightly variable in morphology. Ventral bar slightly longer, 0.023 (0.022–0.025) (Fig. 3). Dorsal bar length 0.022 (0.021–0.023) (Fig. 4). Hooks 14 in number, each composed of a base, solid shaft, and sickle-shaped termination

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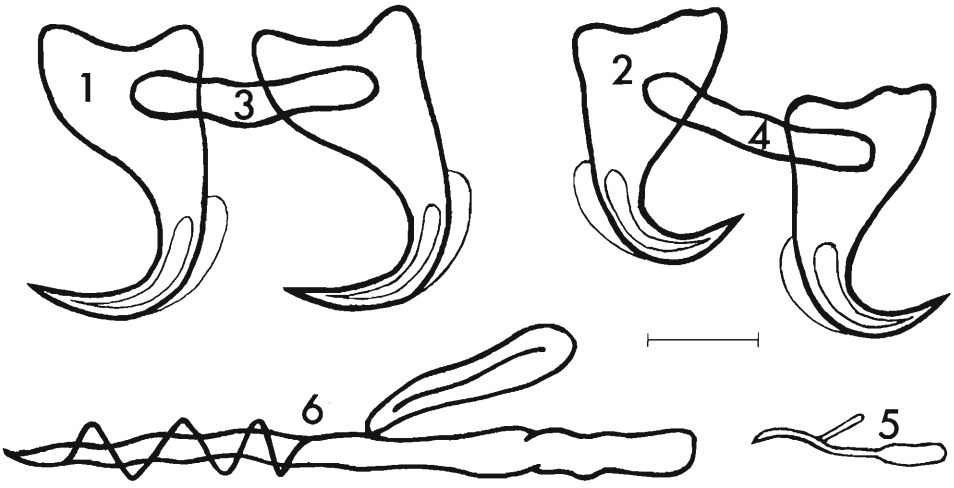


Figs. 1–6. Freehand drawing of *Urocleidus flieri* n. sp. hard parts drawn to scale. Scale represents 0.010 mm. Fig. 1. Ventral anchor. Fig. 2. Dorsal anchor. Fig. 3. Ventral bar. Fig. 4. Dorsal bar. Fig. 5. Hook. Fig. 6. Copulatory complex.

(Fig. 5), length 0.017 (0.016–0.019). Cirrus a tapering undulating chitinous tube with the terminal one-third having a cirral thread with four turns giving it an auger-like appearance, length 0.062 (0.059–0.063). Accessory piece a chitinous rod gently curved and terminally articulating with the posterior portion of the cirral threaded area of the cirrus, length 0.026 (0.025–0.029) and 0.006 wide at free end (Fig. 6). Bases of cirrus and accessory piece nonarticulate. Prostate gland, seminal vesicle, and vagina not recognized.

HOST: *Centrarchus macropterus.*

HOST LOCALITY: White Marsh Swamp, Columbus County, North Carolina.

LOCATION: Gill filaments.

TYPE: U.S.N.M. Helm. Coll. No. 60711.

PARATYPE: Eastern Fish Disease Laboratory, Kearneysville, W. Va.

## DISCUSSION AND COMPARISON

In their revision of the North American freshwater Tetraonchinae, Mizelle and Hughes (1938) stated that "sexual characters have more taxonomic value than the sporadic occurrence of spines or spurs on, or differences in size or shape of, certain haptor parts." Also in this revision a key to the North American species of *Urocleidus* is given in which the presence or absence of spiral threads and other copulatory structures are used as criteria for species differentiation. Hargis (1953) notes that a fairly high degree of host specificity exists among the monogenetic trematodes studied, stating that "even those that occur on more than one host species are, with the single exception of *Dactylogyrus aureus,* confined to a subfamily or, at most, a family." He states that "the occurrence of *D. aureus* on a fish which is not a species of its normal host family, Cyprinidae, was probably accidental."

Therefore, comparison of *Urocleidus flieri* n. sp. with other North American *Urocleidus* is limited to species having cirral threads and parasitizing the family Centrarchidae of which the flier sunfish is a member.

*U. flieri* n. sp. differs from the following *Urocleidus* species (listed below) having cirral threads and parasitizing the fish family Centrarchidae in the following ways:

*U. chaenobryttus,* Mizelle and Seamster (1937): cirral thread not uniform and lacks an accessory piece.

*U. doloresae,* Hargis (1952): cirrus half as long and accessory piece forked distally.

*U. procax,* Mizelle and Donahue (1944): cirrus half as long and nonundulating; accessory piece of variable morphology.

*U. torquatus,* Mizelle and Cronin (1943): attenuate cirrus and a sleevelike accessory

piece which is open on one side; cirrus comparatively straight with two cirral threads around its shaft; cirrus half as long; accessory piece in reality a chitinized portion of the vestibule (cirrus sac).

*U. variabilis*, Mizelle and Cronin (1943): attenuate cirrus and a sleevelike accessory piece which is open on one side; cirrus straight with a comparatively small base, half as long; accessory piece in reality a chitinized portion of the vestibule (cirrus sac).

*U. wadei*, Seamster (1948) (Harrises, 1962): cirrus a long tube whose shaft forms a distinct loop along the distal portion; cirral thread coils around proximal portion of cirrus shaft with a posteriorly projecting structure on distal curvature; accessory piece solid, partially enclosing or articulating at bifurcate distal end with cirrus.

## SUMMARY

*Urocleidus flieri* n. sp. from the flier sunfish, *Centrarchus macropterus* Lacépède, is described and compared with other *Urocleidus* species of North America.

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# *Diplophallus taglei* n. sp. (Cestoda : Cyclophyllidea) from the Viccacha, *Lagidium peruanum* Meyer, 1832 (Chinchillidae) from the Chilean Andes[1]

O. WILFORD OLSEN[2]

Two species of *Diplophallus* Fuhrmann, 1900 have been described from charadriiform birds. They are *D. polymorphus* (Rudolphi, 1819) from *Recurvirostra avosetta* and *Himantopus* sp. in Europe, and *D. andinus* Voge and Read, 1953 from *Recurvirostra andina* at 14,500 feet elevation in the Peruvian Andes. A third species of *Diplophallus* parasitizes mammals. It occurs in the small intestine of the viccacha, or mountain chinchilla, of the Andes.

The specimens, provided through the courtesy of Dr. Isaias Tagle, School of Veterinary Medicine, University of Chile, Santiago, Chile, were preserved in formalin. They were studied as whole mounts stained with Mayer's acid carmine and as sections prepared with Ehrlich's acid haematoxylin and eosin. Whole mounts were cleared in beechwood creosote. Due to the thickness of the worms, it was necessary to dissect away the cuticle, cortical parenchyma, and stout longitudinal muscles to reveal clearly the internal anatomy.

DIAGNOSIS: Strobila up to 9.5 cm long by 8 mm wide and about 800 $\mu$ or more thick; each proglottid with two complete sets of male reproductive organs and one of female organs from which vaginae are lacking; cirrus partially protrudes and is visible without magnification. Scolex 783–843 $\mu$ wide by 492–513 $\mu$ long; suckers 375–428 $\mu$ in diameter; rostellum (retracted) 246–267 $\mu$ long by 171–181 $\mu$ wide, armed with 12 hooks each 21 $\mu$ long. Neck extremely short and as wide as proglottids immediately following it. Both dorsal and ventral excretory canals with transverse connections that soon anastomose with each other, forming several simple plexus between points of fusion. Inner longitudinal muscles in two layers of prominent bundles, the inner being larger; each bundle consists of several large fibers; circular muscles sparse, encircle outer layer of longitudinal muscles. Subcuticular muscles too small to differentiate from other tissues or absent.

Cirrus pouches ventral to longitudinal excretory canals, elongated, 642–802 $\mu$ long by 117–139 $\mu$ in diameter, being more or less equal in diameter throughout length, extend about one-third their length mesad from longitudinal excretory canals, surrounded by many bands of strong circular muscles; cirrus covered with numerous small hooked spines 9–10 $\mu$ long arranged in steep spirals. Internal seminal vesicles oval to pyriform in shape, external seminal vesicles present. Genital pores open near anterior margin of proglottids, often on a prominent genital cone. Testes occur in two clumps of 25–36 each per proglottid, located near inner end of each cirrus pouch; diameter of testes averages about 29 $\mu$. Ovary elongated, with numerous finger-like lobules, approximately 500 $\mu$ long and traversing space between clumps of testes. Uterus of mature proglottid tubular, extends across space between ends of cirrus pouches. Seminal receptacle elongate, posterior to uterus and about two-thirds as long. Vitelline gland most posterior of female reproductive organs, oval to reniform in shape, about 250 $\mu$ long by 40–50 $\mu$ wide. Eggs oval, 31–39 $\mu$ wide by 73–78 $\mu$ long (average 36.6 by 75.3), with three membranes surrounding embryophore; embryophore 9.7–12.1 $\mu$ wide by 19.4–24.3 $\mu$ long (average 10.2 by 21.8), tail of embryophore broad, exceeding one-half of width of oncosphere, 29–40 $\mu$ long (average 34).

TYPE HOST: *Lagidium peruanum.*

HABITAT: Small intestine.

TYPE LOCALITY: Coquimbo Province, Chile, near Choapo River between parallels 31 and 32° S. L.

TYPE: U.S.N.M. Helm. Coll. No. 61095.

*Diplophallus taglei* has 12 rostellar hooks, each 21 $\mu$ long (Figs. 1, 2) and *D. polymorphus* has 10 that are 88 $\mu$ long (Cohn, 1900). The specimens of *D. andinus* available to Voge and Read (1953) have a well-developed rostellum

[1] Paper read at First International Congress of Parasitology, Rome, September 21–26, 1964.

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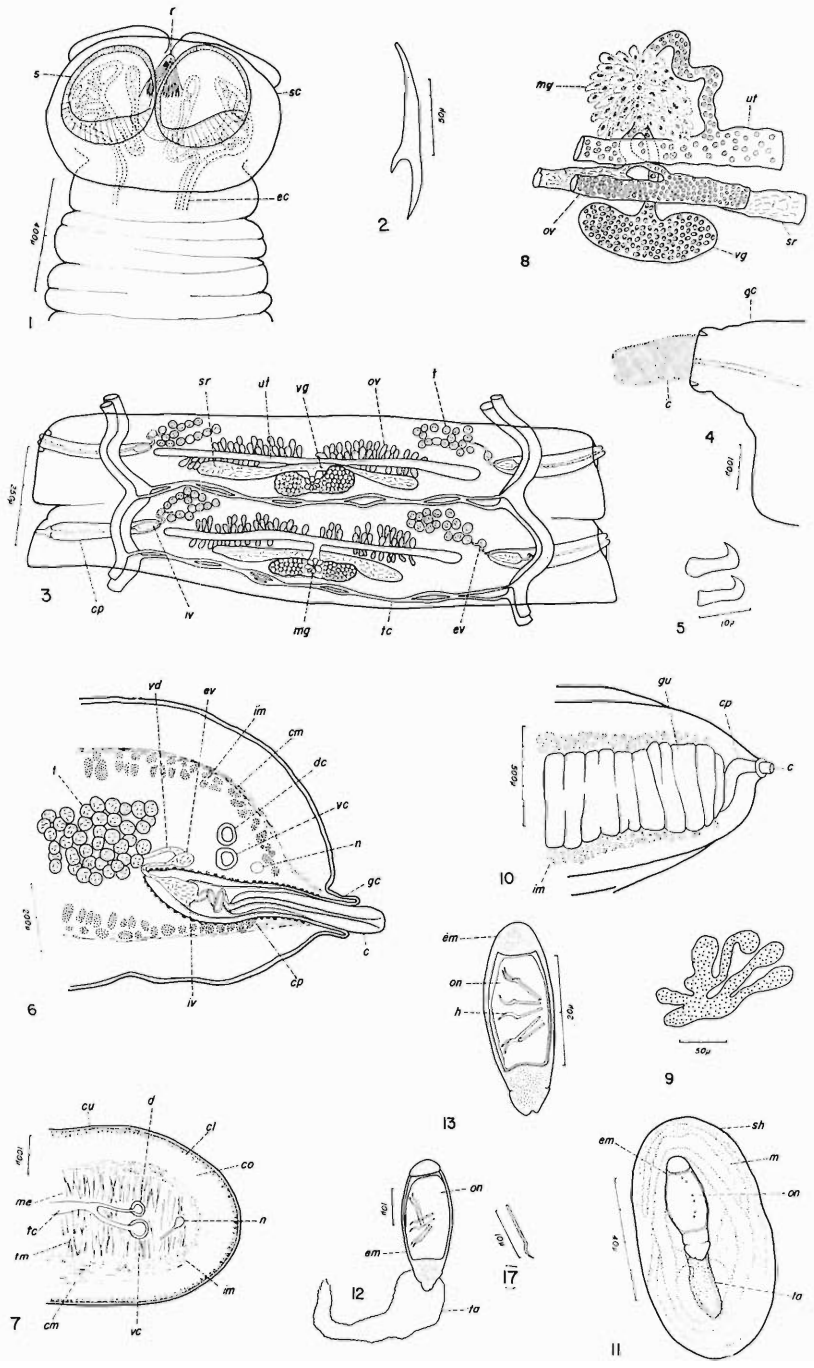

but are devoid of hooks. Some of the specimens of *D. taglei* are without hooks, indicating that they drop off readily. Further collections of *D. andinus* should show some with hooks, as it seems unlikely that they are naturally absent.

The internal longitudinal muscles of *D. taglei* are well developed and arranged in two layers, the inner one being the larger. Each muscle bundle is composed of several large fibers. The subcuticular longitudinal muscles are either absent or so small that they cannot be distinguished from other tissue. The dorsoventral muscle fibers are numerous and well developed. The circular muscles lie outside the longitudinal muscles, being in close contact with outer layer (Figs. 6, 7). In *D. andinus*, the circular muscles occur in two layers, one outside the outer and one inside the inner layer of longitudinal fibers.

MALE REPRODUCTIVE SYSTEM: The genital pores are near the anterior margin of the proglottids and commonly appear at the end of a pronounced genital cone (Figs. 4, 6). The cirrus is completely covered with minute hooked spines arranged in tightly packed steep spirals (Figs. 4, 5). There is no evidence of a spiny cap on the tip of the cirrus, such as described for *D. andinus*. All of the cirri observed were protruding freely from the genital pore and one exceeded the cirrus pouch in length. None were seen penetrating the cuticle such as reported for *D. polymorphus* by Wolffhügel (1900) and for *D. andinus* by Voge and Read (1953).

←

Drawings made with aid of camera lucida except as stated otherwise.

Fig. 1. Scolex with inverted hook-bearing rostellum.

Fig. 2. Rostellar hook.

Fig. 3. Mature proglottids.

Fig. 4. Margin of proglottid, showing genital cone and partially extended cirrus densely covered with minute spines.

Fig. 5. Detail of spine of cirrus.

Fig. 6. Cross section of lateral portion of mature proglottid.

Fig. 7. Cross section of proglottid, showing musculature and fusion of transverse canals originating from dorsal and ventral excretory canals.

Fig. 8. Freehand reconstruction of female reproductive system made from sections and dissections.

Fig. 9. Cross section of ovary, showing lobules.

Fig. 10. Anterior view of gravid proglottid, showing folds of egg-filled uterus.

Fig. 11. Entire egg removed from uterus.

Fig. 12. Embryophore with attached tail enveloping oncosphere removed from egg by mechanical pressure.

Fig. 13. Embryophore containing oncosphere, tail detached.

ABBREVIATIONS

c—cirrus
cl—cuticular layer
cm—circular muscles
co—cortical parenchyma
cp—cirrus pouch
cu—cuticle
dc—dorsal excretory canal
ec—excretory canals
em—embryophore
ev—external seminal vesicle
gu—gravid uterus
h—oncospheral hook
im—internal longitudinal muscles
iv—internal seminal vesicle
me—medullary parenchyma
mg—Mehlis' gland
n—lateral nerve cord with branch
on—oncosphere
ov—ovary
s—sucker
sc—scolex
sh—shell of egg
sr—seminal receptacle
t—testes
ta—tail
tc—transverse excretory canal
tm—transverse muscles
ut—uterus
vc—ventral longitudinal excretory canal
vd—vas deferens
vg—vitelline gland

The cirrus pouch is only slightly greater in diameter at the base than elsewhere. It is surrounded by large encircling bands of closely set muscles (Figs. 3, 6). The internal seminal vesicle varies from pear-shaped to oval. The external seminal vesicle is well developed and is located about midway between the extremities of the vas deferens (Figs. 3, 6).

FEMALE REPRODUCTIVE SYSTEM: The ovary, like that in *D. andinus*, extends across the space between the two groups of testes (Fig. 3). Numerous small lobes extend outward in all directions from the main stem of the ovary (Figs. 3, 5). The uterus of the mature proglottid is tubular before beginning to fill with eggs and commonly reaches from the inner end of one cirrus pouch to that of the other. With growth and the appearance of eggs in it, the ends begin to expand and then to lobulate. In gravid proglottids, the uterus is intricately convoluted, filling the entire space of the terminal proglottids and pushing the cirrus pouches to one side (Fig. 10). The seminal receptacle in mature proglottids is slightly shorter than the ovary and lies between it and the vitelline gland (Fig. 3). The vitelline gland is the most posterior of the female organs, and is one-third to one-half the length of the seminal receptacle in mature proglottids. It varies in shape from oval to reniform (Fig. 3).

Figure 8 is a freehand reconstruction from serial sections and dissections of the female reproductive system. The short duct from the seminal receptacle and the one from the ovary unite to form a single tube that continues anteriorly for a short distance where it is joined by the one from the vitelline gland. At this point, the common duct formed from those from each of the three organs enters the posterior side of the well-developed Mehlis' gland, following a sinuous course through it. Upon emerging from the anterior side of the Mehlis' gland, the duct, now filled with eggs, extends anterolaterally a short distance, bends posteriorly somewhat abruptly, and follows a sinuous course to the uterus.

The well-developed Mehlis' gland (Figs. 3, 8, mg) is in contrast to the condition reported for *D. andinus* by Voge and Read (1953) who could not demonstrate one with certainty.

Vaginae could not be seen in sections or dissections of the proglottids.

EGGS: Eggs of *D. taglei* are oval and contain a tailed embryophore enclosed in three broad membranes inside the shell (Figs. 11, 12, 13). The innermost membrane is almost complete, being only slightly open at the caudal end of the embryophore. At the level of the union of the embryophore and its tail, a broad extension from the inner membrane reaches inwards, tapering to a narrow point which attaches to the embryophore (Fig. 11). The middle membrane surrounds only the anterior half (opposite from the tailed end) of the embryophore and has an oval thickened area at the pole of the egg. One end of the membrane is free, whereas the other appears to be fused to the inner membrane on the opposite side. The outer membrane lines almost the entire inner surface of the eggshell; one end appears to be detached from the shell and fused laterally to the inner membrane. There is a clear spot in the outer membrane opposite the tip of the tail of the embryophore (Fig. 11).

The embryophore proper containing the oncosphere is oval with thickened ends. The end to which the broad, blunt tail is attached is the larger and is somewhat pyramidal in shape with a median transverse constriction, whereas the one at the opposite pole is oval (Figs. 11, 12, 13). The tail is about one-half the length of the embryophore (Fig. 11). The oncosphere is a truncated oval about twice as long as wide. The hooks are relatively large, being almost as long as the oncosphere is wide. The eggs of *D. andinus* also are oval in shape but very different internally. The spherical oncosphere is surrounded by three complete membranes. The two outer ones are very thin; the outermost one has at least one kink or fold in it and the intermediate one bears a more or less spherical, cell-like pole at each end. The innermost membrane is thicker than the other two. In some eggs, two slender filaments connect the inner and intermediate membranes. No detailed description is available for the eggs of *D. polymorphus*.

Comparative sizes of the eggs are: *D. polymorphus* 46 by 91 $\mu$, *D. andinus* 33–36 by 43–50 $\mu$, and *D. taglei* 31–36 by 73–78 $\mu$ (average 36.6 by 75.3).

*D. polymorphus* with 10 rostellar hooks, each 88 $\mu$ long, may be readily separated from *D. taglei* with 12 hooks, each 21 $\mu$ long. *D. taglei* with cirrus pouches that extend mesad about

one-third their total length from the longitudinal canals differs from the other two species whose cirrus pouches barely reach to the excretory canals. The tailed embryophores of *D. taglei* distinguish it from *D. andinus*. The occurrence of *D. taglei* in a rodent would appear to represent a valid specific difference from the other two species which have been found only in charadriiform birds.

TAXONOMIC POSITION: The taxonomic position of *Diplophallus* has been discussed by a number of helminthologists. Fuhrmann (1907) and Ransom (1909) considered that it belonged to the family Acoleidae. Voge and Read (1953) pointed out that the absence of vaginae and the nature of the musculature related it more closely to *Acoleus* than any other genus. They recommended that it be placed in the Acoleidae. Southwell and Hilmy (1929) considered that it rightly belonged in the family Diploposthidae Poche (1926) as did Wardle and McCleod (1952) and Yamaguti (1959).

Voge and Read (1953) reasoned that genera with double and single reproductive systems are accepted in the Anoplocephalidae and Dilepididae; hence, by implication, there should be no great difficulty in accepting *Acoleus* with single and *Diplophallus* with double male reproductive organs and both without vaginae in the family Acoleidae, an arrangement they recommend. But it might be argued with equal validity that genera with double male reproductive organs and vaginae could be included in the same family with those having single or double male reproductive organs and no vaginae, as was done by Wardle and McCleod (1953) and Yamaguti (1959) with Diplospothidae. From the same evidence, these two groups of eminent authorities have arrived at two different conclusions. Such reasoning, however, does not clarify the problems of true relationships among the tapeworms. The question of relationship remains. Is the presence or absence of vaginae in adult worms of greater significance in designating familial relationship than double or single sets of male or male and female reproductive systems? The answer does not lie in the realm of arbitrary decision but must be clarified through careful observations on the embryological development of the organ systems and detailed life history studies. While these are difficult problems, especially for rare species, they are present and must be solved before sound assessments on relationships can be made.

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# *Levinseniella carteretensis* sp. nov., a Microphallid Trematode from the Wilson Plover, *Charadrius wilsonia*[1]

WILLIAM H. COIL[2] AND RICHARD HEARD, III[3]

## INTRODUCTION

Both resident and migratory specimens of the Wilson plover were found to harbor a small microphallid in their ceca. Further study revealed that these forms belonged to the genus *Levinseniella* Stiles and Hassall, 1901, representatives of which are commonly found in the ceca of shorebirds. We found that this host generally carries a small infection of these worms, but that, on occasion, as many as 70 worms were recovered from a single host. All specimens were mature.

The genus *Levinseniella* has attracted the best efforts of helminthologists for many years and the attention accorded this fascinating group is completely out of proportion to its small size. The genus has proved vexsome, as a group, due to the small size of most species and the attendant difficulty of determining the exact nature of the complex terminal genitalia and the degree of variation present. The future appears promising, however; the study of living specimens and/or the use of phase microscopy will help solve many of the knotty problems associated with their morphology.

Rankin (1939) in his review of the genus gave as a character the presence of "four muscular, thimble-like pockets" in the male part of the terminal genitalia. Notwithstanding those worms which appear to lack the pockets (and are in an indefinite taxonomic status), this unique feature has stood for some time as the hallmark of this genus. Recently this concept has been broadened by the descriptions of worms with pockets numbering three (*L. brachysoma*), twelve (*L. polydactyla*), and seven to ten (the species described here).

## MATERIALS AND METHODS

Specimens were removed from the digestive tract by slitting the gut with scissors and shaking it in saline. Individual worms were placed in either 0.85% NaCl or Hank's BSS until either fixed or studied. Living worms were studied in saline with a small amount of neutral red added. The cover glass was ringed with vaseline to prevent drying. Worms were relaxed with heat or distilled water and then fixed in either AFA or Carnoy's fluid. Excellent whole mounts resulted from both procedures. Whole mounts were stained with Harris' hematoxylin, Semichon's carmine, or Malachite Green (for vitelline glands), and mounted in piccolyte. All measurements are in millimeters taken from worms which were relaxed by heat with very light cover-glass pressure. Phase microscopy was used on both live and stained materials.

We are indebted to Dr. W. W. Becklund, Animal Disease and Parasite Research Division, Beltsville, for the loan of the museum specimens used in this study.

## *Levinseniella carteretensis* sp. nov. (Figs. 1–2)

DIAGNOSIS: With the characters of the genus. Relatively small linguiform distomes with spinose cuticle reaching about midbody, largest spines anterior. Body 0.578–0.713 long and 0.216–0.292 wide at the broadest point. Oral sucker 0.066–0.084 by 0.057–0.080. Prepharynx 0.041–0.084 long. Pharynx 0.029–0.035 wide and 0.041–0.046 long. Ceca short with heavy, irregular epithelium, reaching to posterior quarter of body. Testes 0.031–0.053 by 0.067–0.084 symmetrically placed close to lateral margins, just postacetabular. No cirrus sac present. Seminal vesicle about 0.030 by 0.057, situated just anterior to ovary. Pars prostatica well developed, surrounded with a large mass of gland cells. Ductus ejaculatorius heavy, entering the male atrium or pocket anterior to the system of pockets. Male papilla poorly developed. Male atrium or pocket with 7–10 pockets each with a sclerotized structure at its distal end (Fig. 2), and with surrounding gland cells. Genital pore slack, slit-like with

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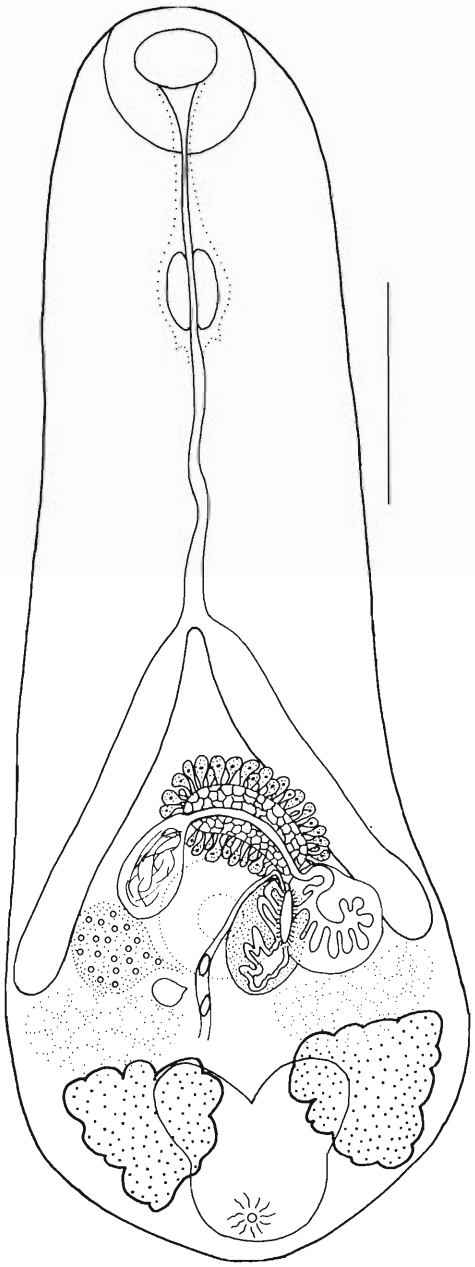

Fig. 1. Ventral aspect of *Levinseniella carteretensis* drawn with the aid of a microprojector, but details added freehand. Scale equals 0.1 mm.

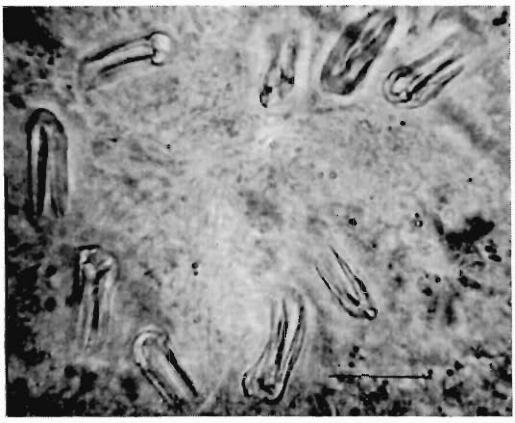

Fig. 2. Photograph of hook or toothlike structures found in each male pocket. Scale equals 0.015 mm.

little musculature; located sinistral to acetabulum. Female atrium or pocket with highly folded, heavy lining, gland cells present. Vagina thin walled, enters female pocket at anterior end. Ovary 0.044–0.062, located just to right of acetabulum and anterior to testis. Number of vitelline follicles obscured by eggs, probably 5–7 on each side, situated laterally in posterior sixth of body. Excretory bladder Y-shaped, sometimes with ragged diverticula. Excretory pore subterminal, surrounded by radiating muscle fibers. Uterus confined to posterior third of body. Eggs (living) 0.016 by 0.027–0.028.

HOST: *Charadrius wilsonia.*

LOCALITY: Carteret Co., North Carolina, U.S.A.

SITE OF INFECTION: Ceca.

TYPE SPECIMEN: Holotype in Helminthological Collection of the U.S. National Museum, No. 60770.

*L. carteretensis* is most similar to *L. polydactyla* Deblock and Rosé, 1962, the only other known member of the genus with a large number of male pockets. However, there are significant differences between the two species; *L. polydactyla* lacks, or has a reduced, female pocket, the male pouches lack the sclerotized parts in their distal ends, and the ductus ejaculatorius does not open into the male pocket. The possession of 7–10 male pouches, with sclerotized parts, serves to set this species apart from all other species in the genus *Levinseniella.*

The Wilson plover has been reported as the host for *Levinseniella leptophallus* Coil, 1956 collected in Puerto Rico by Cable *et al.* (1960). A study of the specimen submitted to the U.S. National Museum by Cable (No. 38228, submitted as *L. caribbea*, holotype) revealed that it is essentially the same as *L. leptophallus*, but that there are features in the male pocket which are different from Cable's original description. Since the present taxonomy of this group is based on the precise knowledge of the male and female pockets, it is deemed advisable to describe these features here.

Cable's specimen has a light, thin-walled ductus ejaculatorius which is highly convoluted giving the impression (in the museum specimen) that the duct enters the male atrium through a pocket. However, it is a case of the duct bending back on itself which gives this impression. One can corroborate this idea by noting the difference between the individual pockets and the terminus of the ductus ejaculatorius. Cable described three pockets (exclusive of the one associated with the ductus ejaculatorius); the specimen has, however, a fourth, smaller pocket mediad to the terminus of the ductus ejaculatorius. Another difference is the paucity of gland cells surrounding the pars prostatica. *L. leptophallus* has a relatively heavy ductus ejaculatorius (as depicted, Coil, 1956) which is almost papilla-like at its terminus. It also possesses a male atrium which has prominent muscle fibers in it.

It is felt that these differences are not sufficient to warrant the separation of these species at this time.

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## Study of Two Syngamid Nematodes from the Eastern Belted Kingfisher, *Megaceryle a. alcyon* and a New Host Record for *Aproctella stoddardi* Cram 1931

ELIZABETH M. BOYD[1]

Through an examination of 50 kingfishers from Eastern North America for parasites, 8 birds, all from Massachusetts, yielded new nematode records. Two hosts harbored *Syngamus alcyone* n. sp. (Syngamidae) in their tracheae. Detergent washings of the body revealed the presence of a second syngamid, *Cyathostoma* sp. from one bird, and *Aproctella stoddardi* (Dipetalonematidae) from the other 5 kingfishers. No species of the Syngamidae nor *Aproctella* have been previously reported for this host.

*Syngamus alcyone* n. sp. (Fig. 1, A–G)

FEMALE (Fig. 1, A–C): 19.50 mm long; 0.86 mm wide. Bucchal capsule cup-shaped, depth 990 $\mu$, diameter outside 900 $\mu$, inside 660 $\mu$; teeth 8, 100 $\mu$ by 45 $\mu$ and 144 $\mu$ by 32 $\mu$. Esophagus 1.0 mm long. Vulva 1.96 mm from anterior end, 0.16 mm posterior to end of esophagus; tail 300 $\mu$. Eggs operculate, 96 $\mu$ by 46 $\mu$.

MALE (Fig. 1, D–G): 4.05 mm long; 0.40

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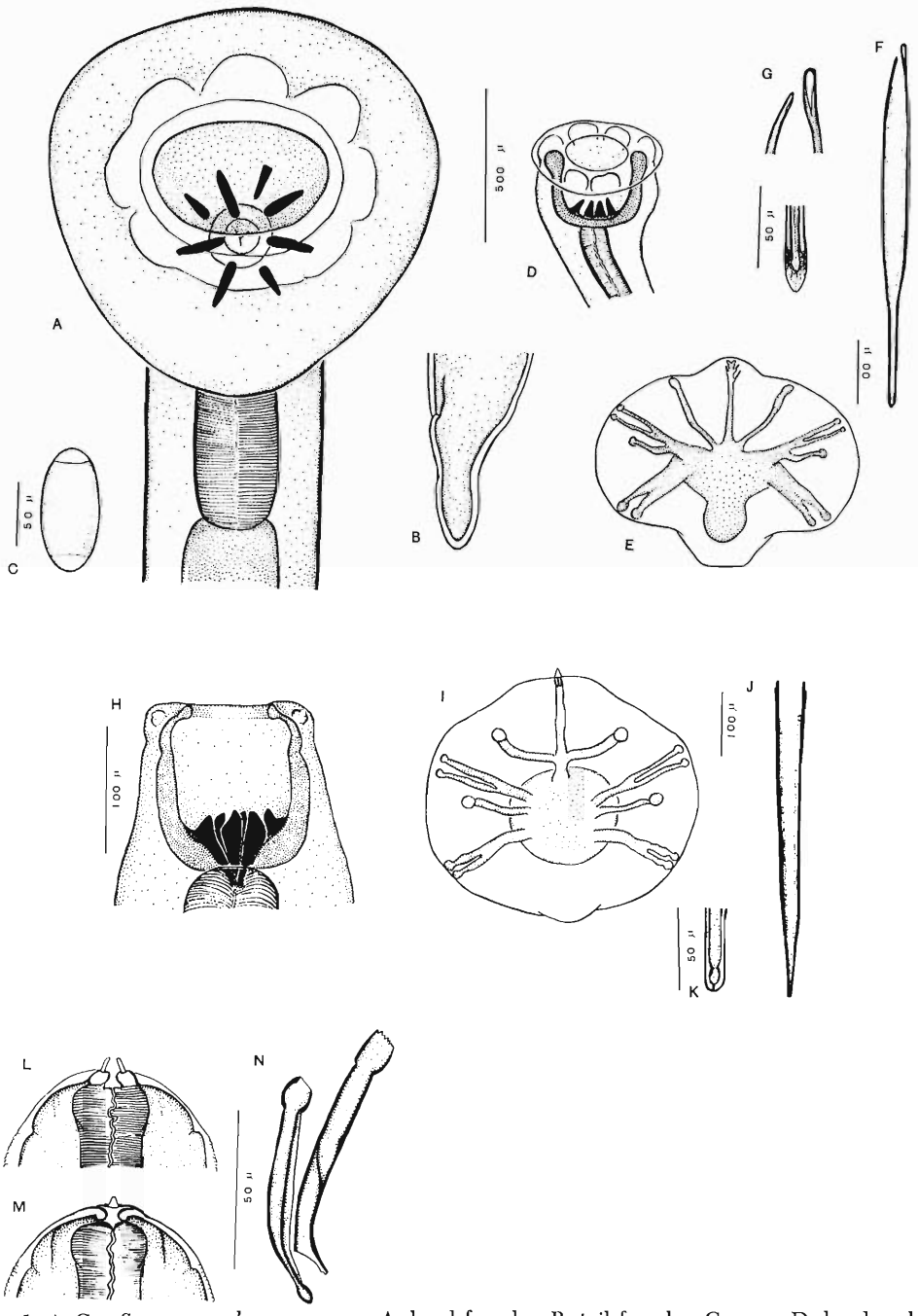


Fig. 1, A–G. *Syngamus alcyone* n. sp. A, head female; B, tail female; C, egg; D, head male; E, bursa; F, spicules; G, both ends of spicules. H–K. *Cyathostoma* sp. male. H, head; I, bursa; J, spicules; K, distal end of spicules. L–N. *Aproctella stoddardi.* L, anterior extremity, dorsal view; M, anterior extremity, lateral view; N, spicules.

mm wide. Buccal capsule cup-shaped, depth 250 $\mu$, diameter outside 350 $\mu$, inside 220 $\mu$. Esophagus 670 $\mu$ long, nerve ring 339 $\mu$, and excretory pore 720 $\mu$ from anterior end. Bursal rays as in Figure 1E, with dorsal ray terminating as five minute asymmetrical processes. Spicule lengths 557 $\mu$ and 562 $\mu$, fringed on inner surface, fused together for 150 $\mu$ distally forming a single pointed end, 15 $\mu$ in length.

HOST: *Megaceryle a. alcyon.*

LOCATION: Trachea.

LOCALITY: Massachusetts.

HOLOTYPE: U.S.N.M. Helm. Coll. No. 60526.

REMARKS: This nematode has been assigned to *Syngamus* because the sexes are fused in copula and the teeth number 8. However, unlike other species of *Syngamus* in which the spicules are up to 150 $\mu$ in length, its spicules are long. In this respect it resembles the genus *Cyathostoma*, in which the spicules are 400 $\mu$ or longer. Burt and Eadie (1958) redefined the genus *Syngamus* based on the following characters: (1) long, slender spicules, (2) 6–12 teeth, (3) externodorsal ray arising near the root of median dorsal ray. Lewis (1928), in *Syngamus* specimens collected from starlings, rooks, and domesticated birds, observed great variation in the dorsal ray of the bursa and in tail shape of the female. He concluded that, except for S. *merulae* Baylis 1926 from the blackbird, all belonged to the same species, S. *trachea* Montagu 1811. The dimensions of *Syngamus* from the kingfisher fall within the wide range listed for S. *trachea*, except in the greater depth of the buccal capsule of the female and in the markedly long spicules of the male. *Syngamus alcyone* is close to S. *merulae*, which is considered by Madsen (1950) to be synonymous with S. *trachea*. The new species from the kingfisher may be readily distinguished from other species of the genus by the length of its spicules.

*Cyathostoma* sp. (Fig. 1, H–K)

MALE: Body reddish, 9.67 mm long; 3.20 mm wide, attenuated at both ends especially anteriorly. Mouth opening 61 $\mu$ bordered by prominent knoblike swellings, each with a papilla; posteriorly a second circle of 6 rounded papillae. Cuticle smooth. Buccal capsule approximately as wide as deep, 132 $\mu$ and 134 $\mu$ respectively; chitinous portion 19 $\mu$ thick; constricted distally as a rim to mouth. Teeth 6, averaging 45 $\mu$ in length. Esophagus 693 $\mu$ long, 70 $\mu$ in width anteriorly, 96 $\mu$ posteriorly. Bursa 400 $\mu$ diameter; rays as in Figure 1I, with dorsal ray ending in 3 short branches and dorsally a spinelike process to bursal membrane. Spicules brown, 480 $\mu$, fused distally and winged; gubernaculum absent.

REMARKS: Only one specimen, a male, was found; this was obtained from the detergent washings of the head and skin of one kingfisher. Of the species of *Cyathostoma* listed in Cram (1927), only three have short spicules (less than 600 $\mu$): *C. tadornae* Chatin 1874 from the trachea of sheld duck, *C. americanum* Chapin (1925) from the post-thoracic air sacs of the hawk, *Buteo borealis*, and *C. lari* Blanchard 1894 from the nasal cavities and orbits of gulls.

According to the key of Skrjabin and Ryzhikov (1959) the kingfisher specimen is closest to *C. tadornae*, but it differs from this species in its attenuated body, smooth cuticle, and spicules less than 500 $\mu$. Some of its features are similar to those of *C. americanum* and *C. lari* which both possess a gubernaculum. In addition the kingfisher specimen differs from the former in that it has a shorter body, buccal capsule, esophagus, and bursa, and spinelike bursal extension. Burt and Eadie (1958) have described *C. lari* and reported its presence also in several Corvidae and the redshank, *Tringa totanus*. The kingfisher specimen is similar to *C. lari* in the cuticular rim of the mouth, the shape and size of the spicules, the bursa, and spinelike bursal extension. However, this specimen differs from *C. lari* not only in absence of gubernaculum but also in possessing only 6 teeth, a longer esophagus, and a smooth cuticle.

*Aproctella stoddardi* Cram 1931 (Fig. 1, L–N)

FEMALE: 9.28 mm average length (range 7.5–14.6 mm). Liplike structures as in Figure 1, L and M. Nerve ring 143–175 $\mu$ from anterior end. Esophagus 329–420 $\mu$ long. Tail length 80–157 $\mu$. Vulva 1.05–1.35 mm from anterior end.

MALE: 5.47 mm average length (range 4.73–6.76 mm). Nerve ring 80–150 $\mu$ from anterior end. Esophagus 262–430 $\mu$ long. Tail length 49–59 $\mu$. Spicules (Fig. 1N), right 53–68 $\mu$, left 70–80 $\mu$ in length.

REMARKS: This species was found in 5 of 50 kingfishers, either clinging to the digestive tract, including the liver, or in detergent washings of the body cavity. These five hosts were among the 23 birds in which detergent washings of the carcasses had been included in the parasitic examination. Had this been adopted for all 50 kingfishers, the incidence might have been much higher. One to 6 worms were collected with each host. Cram (1939) obtained an 11% incidence in 64 bobwhite quail, *Colinus virginianus*, the type host, with an average of 13 per bird.

Specimens of *A. stoddardi* from the kingfisher have been deposited in the U.S.N.M. Helm. Coll. as Nos. 41681 and 65581. *Aproctella* from the kingfisher corresponds with the redescription of *A. stoddardi* (Anderson, 1957, 1961), except for its smaller size—9.3 mm in females, 5.5 mm in males. Its dimensions approximate those for the species collected from the rosebreasted grosbeak, *Pheucticus ludovicianus* (Anderson, 1961). The range in length of females overlaps that of *A. stoddardi* (14–16.5 mm) and for the specimens from *P. ludo vicianus* (9.5–10.7 mm). The range in male lengths from the kingfisher is shorter than the figure of 7.6 mm listed for the species collected from various passerines by Anderson (1957), but overlaps the length of those obtained from *C. virginianus* (6 mm) and *P. ludovicianus* (5–6 mm) (Anderson, 1961). The present finding represents not only a new host record for the kingfisher but it is the first report of the parasite in the order Coraciiformes; previous hosts are members of the Galliformes and Passeriformes (Anderson, 1957, 1961). Thus *A. stoddardi* shows a wide range in body size as well as a wide range in host distribution.

SUMMARY

*Syngamus alcyone* n. sp. and *Cyathostoma* sp. are described from the eastern belted kingfisher, *Megaceryle a. alcyon*, in Massachusetts, marking the first report of the Syngamidae for the order Coraciiformes. The finding of *Aproctella stoddardi* Cram (Dipetalonematidae) in this bird is also presented and represents a new host record.

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# Revision of the Family Atylenchidae Skarbilovich, 1959 (Nematoda : Tylenchoidea)

S. A. SHER, D. C. M. CORBETT, AND R. C. COLBRAN[1]

Nematodes of the little-known family Atylenchidae Skarbilovich, 1959, occur in widely distributed areas of the world. There are two monotypic genera and recent classifications (Thorne, 1961 and Goodey, 1963) consider them as members of the family Tylenchidae.

Specimens of these genera have been reported in the literature on four occasions. *Eutylenchus setiferus* (Cobb, 1893) Cobb, 1913 based on a few male specimens has not been recorded since its original description over 70 years ago. Recently specimens of *Eutylenchus* have been collected from the type locality, Australia, and in four different areas in Africa. *Atylenchus decalineatus* Cobb, 1913 described from New Jersey and Florida, U.S.A., had been reported from Europe (Hirschmann, 1954) and again from the type localities by Chitwood and Tarjan (1957).

Cobb (1913), in proposing the genera *Atylenchus* and *Eutylenchus*, did not indicate their relationship to other taxa. Filipjev (1934) considered *Eutylenchus* in the subfamily Tylenchinae and *Atylenchus* in the Hoplolaiminae. This was probably done as the original illustration and description of *Eutylenchus* did not indicate the presence of the peculiar deep annulation of the cuticle due to the longitudinal ridges which was illustrated for *Atylenchus*. He again considered *Eutylenchus* in the Tylenchinae in his classification of this subfamily (Filipjev, 1936).

Filipjev and Schuurmans Stekhoven (1941) placed these two genera in separate subfamilies of Tylenchidae, *Eutylenchus* in the Tylenchinae and *Atylenchus* in Criconematinae. Skarbilovich (1947) accepted the Hoplolaiminae classification of Filipjev (1934) but also included *Eutylenchus* in this subfamily.

The classifications of Thorne (1949) and T. Goodey (1951) have considered these genera of uncertain position in the Tylenchidae. Skarbilovich (1959) proposed the family Atylenchidae and subfamily Atylenchinae for *Atylenchus* and *Eutylenchus*. This has not been accepted by Thorne (1961) or by J. B. Goodey (1963).

The present paper redescribes the nominal genera and species from type material, proposes a new species in the genus *Eutylenchus*, and considers this closely related, unusual group of nematodes as a valid family of the Tylenchoidea.

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## FAMILY ATYLENCHIDAE

DIAGNOSIS EMENDED: Tylenchoidea. Lip region with four well-developed setae, cephalic framework weakly developed. Cuticle coarsely annulated with prominent protuberances arranged as 10 to 12 longitudinal ridges on the cuticle. Esophagus with median bulb with valve, esophageal glands enclosed in terminal bulb. One ovary and postuterine sac in female. Vulva in posterior part of body. Tail elongate, filiform.

TYPE GENUS: *Atylenchus* Cobb, 1913.

### Genus *Atylenchus* Cobb, 1913

*Atylenchus* Cobb, 1913, p. 437; Filipjev, 1934, p. 33; Filipjev and Schuurmans Stekhoven, 1941, p. 160; Skarbilovich, 1947, p. 308; Chitwood and Tarjan, 1957, p. 48–52; Skarbilovich, 1959, p. 130.

*Anguillulina*: Baylis and Daubney, 1926, p. 65 (in part).

DIAGNOSIS EMENDED: Atylenchidae. Lip region not set off from body, annulated. Cuticle bearing 10 longitudinal ridges in adult, lateral ridges higher than other ridges. Male without caudal alae.

TYPE SPECIES: *Atylenchus decalineatus* Cobb, 1913.

### *Atylenchus decalineatus* Cobb, 1913 (Fig. 1)

*Atylenchus decalineatus* Cobb, 1913, p. 437; Hirschmann, 1954, p. 352; Chitwood and Tarjan, 1957, p. 49–52.

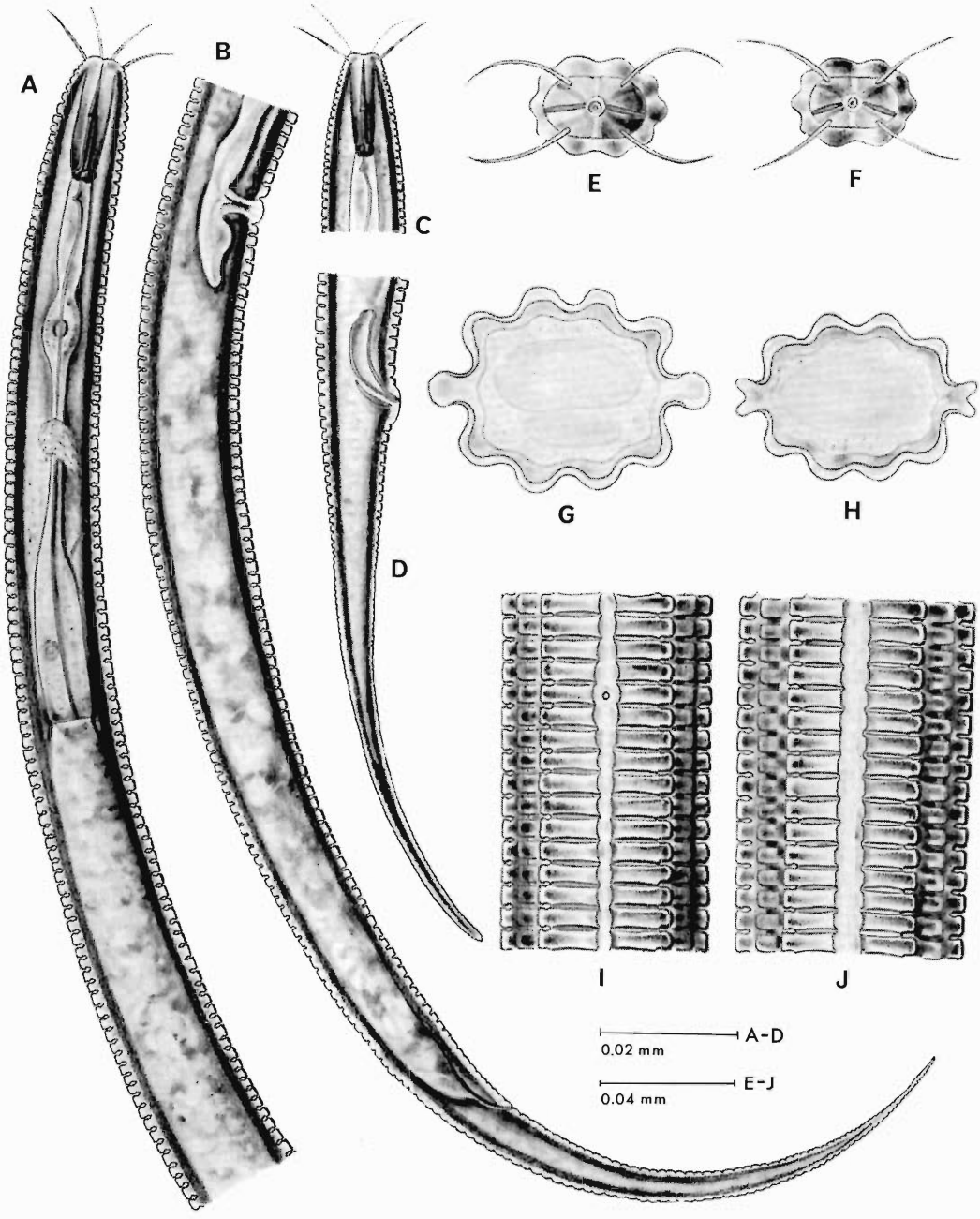


Fig. 1. *Atylenchus decalineatus*. A. Female, anterior region. B. Female, posterior region. C. Male, anterior end. D. Male, posterior end. E. Female, face view. F. Juvenile, face view. G. Female, cross section near center of body. H. Juvenile, cross section near center of body. I. Female, surface view in region of deirid. J. Female, surface view near center of body.

*Eutylenchus decalineatus*: Micoletzky 1922, p. 576.

MEASUREMENTS (10 ♀ topotypes): L = 0.53–0.66 mm; a = 37–48; b = 5.3–6.6; c = 6.5–9.4; V = 62–69; spear = 17–19 μ.

(1 ♂ syntype): L = 0.62 mm; a = 42; b = 6.4; c = 10.4; spear = 17 μ; gubernaculum = ?; spicules = 20 μ.

NEOTYPE (♀, after Chitwood and Tarjan, 1957): L = 0.836 mm; a = 33; b = 6.5; c = 10; V = 66; spear = 16 μ.

FEMALE: Body slightly flattened dorsoventrally; lateral longitudinal ridge of cuticle higher than other ridges. Lip region flattened, rounded edges, 4 or 5 annules, not set off from body; setae tapering to fine points, 6 to 9 μ long. Anterior portion of spear slightly shorter than posterior portion. Spear knobs rounded. Excretory pore at level of posterior portion of isthmus of esophagus, inconspicuous. Hemizonid not seen. Deirids just above level of excretory pore, inconspicuous. Spermatheca oval, usually with sperms. Postuterine sac about as long as body width at vulva. Phasmids not seen.

MALE: Similar in general body form to female. Testis single, outstretched. Spicules arcuate; gubernaculum slightly curved. Caudal alae absent.

JUVENILE: Similar to female in general body form except for absence of sexual organs and the lateral longitudinal ridges of the cuticle which are bifurcated (6 juvenile cross sections, Fig. 1H).

TYPE HABITAT AND LOCALITY: On roots of cranberries (*Oxycoccus macrocarpus* Pers.), cranberry bog, New Lisbon, New Jersey, U.S.A. Cobb in his original description of *A. decalineatus* also lists Atwood Grove, Florida, U.S.A. as the type locality. His first listed locality, New Lisbon, New Jersey, is considered the type locality under the rules of priority.

The description and illustrations of *A. decalineatus* are based on syntypes supplied by A. M. Golden and deposited in the USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland; topotypes collected by G. W. Bird; and the male (Fig. 1C–D) and females described by Chitwood and Tarjan (1957) and supplied by A. C. Tarjan. Males appear to be rare in this species as only 1 specimen was found in the original 37 syntypes. No males were present in a collection from the type locality containing 40 females and 10 juveniles.

## Genus *Eutylenchus* Cobb, 1913

*Eutylenchus* Cobb, 1913, p. 437; Filipjev, 1934, p. 32; Filipjev and Schuurmans Stekhoven, 1941, p. 274; Skarbilovich, 1947, p. 308; Skarbilovich, 1959, p. 130.

*Anguillulina*: Baylis and Daubney, 1926, p. 65 (in part).

DIAGNOSIS EMENDED: Atylenchidae. Body round in cross section. Lip region set off from body, not annulated. Cuticle bearing 12 similar longitudinal ridges on most of body, divided into blocks by transverse striae. Males with conspicuous caudal alae.

TYPE SPECIES: *Eutylenchus setiferus* (Cobb, 1893) Cobb, 1913.

### *Eutylenchus setiferus* (Cobb, 1893) Cobb, 1913 (Fig. 2)

*Tylenchus setiferus* Cobb, 1893, p. 813.

*Eutylenchus setiferus*: Cobb, 1913, p. 437.

MEASUREMENTS (10 ♀ topotypes): L = 0.56–0.70 mm; a = 36–46; b = 5.2–6.3; c = 5.9–7.9; V = 68–72; spear = 18–21 μ.

(10 ♂ topotypes): L = 0.50–0.65 mm; a = 40–57; b = 5.1–6.1; c = 5.3–6.3; spear = 18–20 μ; gubernaculum = 6–7 μ; spicules = 15–18 μ.

FEMALE (topotypes): Lip region flattened, deeply set off; setae with inward-pointing projection near base, tapering to fine points, 9–12 μ long. Face view with 6 lips, lateral lips much reduced. Anterior portion of spear shorter than posterior portion. Spear knobs flattened anteriorly. Excretory pore at level of isthmus of esophagus, anterior to the hemizonid. Deirids not seen. Spermatheca oval, usually with sperms. Postuterine sac one or less body width at vulva in length. Longitudinal flap on each side of vulva. Phasmids not seen.

MALE (topotypes): Similar to female in general body form except for shorter rod-shaped setae (4–6 μ) without projections and body protruding ventrally in region of cloaca opening. Testis single, outstretched. Spicules curved; gubernaculum simple, slightly curved. Caudal alae prominent approximately one body

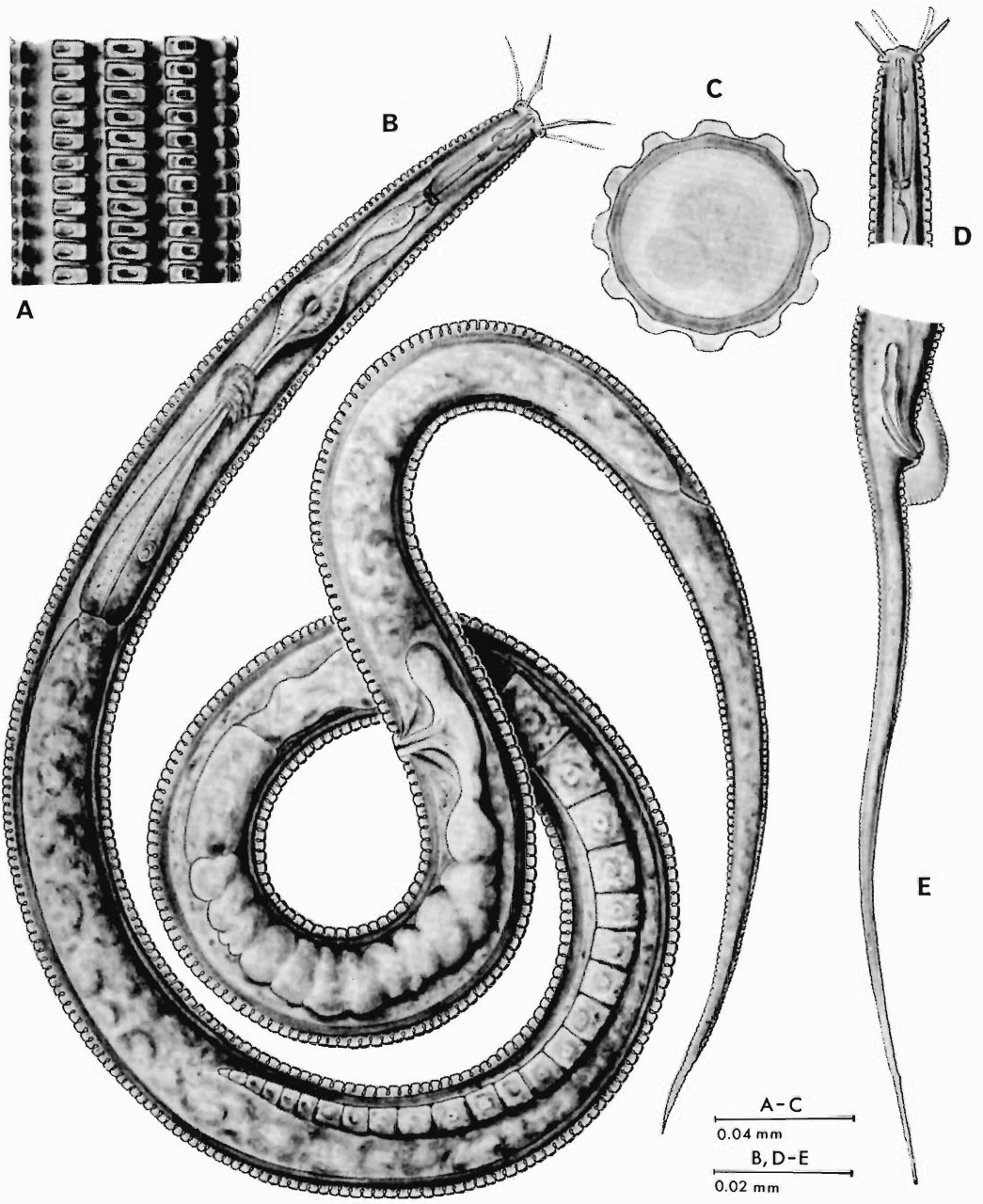


Fig. 2. *Eutylenchus setiferus.* A. Surface view of female. B. Female, entire body. C. Female, cross section near center of body. D. Male, anterior end. E. Male, posterior end.

width in front and one body width behind the cloaca opening.

JUVENILES: Similar to females in general body form except for absence of sexual organs.

TYPE HABITAT AND LOCALITY: Soil from hills opposite Harwood, Clarence River, New South Wales, Australia.

TOPOTYPES: 35 ♀ ♀, 18 ♂ ♂, 27 juveniles collected August 1963 and 25 January 1965 by R. C. Colbran at the lookout at Maclean opposite Harwood, Clarence River, N.S.W., Australia; distributed to the following institutions: Department of Nematology, Riverside, California, U.S.A.; Canadian National Collection, Ottawa, Canada; Department of Nematology, Rothamsted Experimental Station, Harpenden, England; Queensland Department of Primary Industries Nematology Collection, Australia; and Plantenziektenkundige Dienst, Wageningen, The Netherlands.

*Eutylenchus africanus* n. sp. (Fig. 3)

MEASUREMENTS (15 ♀ paratypes): L = 0.75–1.03 mm; a = 40–65; b = 5.1–6.9; c = 5.2–7.8; V = 67–75; spear = 20–24 μ; setae = 7–10 μ.

(15 ♂ paratypes): L = 0.71–1.01 mm; a = 42–64; b = 5.2–6.4; c = 5.5–8.1; spear = 20–23 μ; setae = 4–7 μ; gubernaculum = 7–12 μ; spicules = 21–27 μ.

FEMALE (allotype): L = 0.84 mm; a = 51; b = 6.5; c = 5.8; V = 69; spear = 22 μ. Lip region flattened, deeply set off, setae with inward-pointing projection near base, tapering to fine points, 9 μ long. Anterior part of spear shorter than posterior part. Spear knobs flattened anteriorly. Excretory pore at level of isthmus of esophagus, just posterior to hemizonid. Deirids not seen. Postuterine sac longer than body width at vulva. Longitudinal flap on each side of vulva. Phasmids not seen.

MALE (holotype): L = 0.86 mm; a = 55; b = 6.4; c = 5.4; spear = 22 μ; gubernaculum = 7 μ; spicules = 23 μ. Lip region flattened, deeply set off, setae rod shaped without projection, 4 μ long. Anterior portion of spear shorter than posterior portion. Spear knobs flattened anteriorly. Excretory pore at level of posterior portion of isthmus of esophagus just posterior to hemizonid. Deirids not seen. Ventral portion of body in region of cloacal opening protruding. Caudal alae prominent, extending one body width in front and less than one body width behind cloaca opening. Spicules slightly curved; gubernaculum simple, slightly curved.

JUVENILES (paratypes): Similar to females in general body form except for absence of sexual organs.

HOLOTYPE: Male, collected by F. E. Caveness, 15 August 1961, catalog number 632, University of California Survey Collection, Davis, U.S.A.

ALLOTYPE: Female, same data as holotype, catalog number 633, University of California Survey Collection, Davis, U.S.A.

PARATYPES: 30 ♀ ♀, 21 ♂ ♂, 25 juveniles, same data as holotype, distributed as follows: 2 ♀ ♀, 1 ♂, Department of Nematology, University of California, Davis; 21 ♀ ♀, 14 ♂ ♂, 16 juveniles, University of California, Riverside; 1 ♀, 1 ♂, 6 juveniles, USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland; 2 ♀ ♀, 1 ♂, 1 juvenile, Department of Nematology, Rothamsted Experimental Station, Harpenden, England; 2 ♀ ♀, 1 ♂, Plantenziektenkundige Dienst, Wageningen, The Netherlands; and 2 ♀ ♀, 3 ♂ ♂, 2 juveniles, Queensland Department of Primary Industries, Nematology Department, Australia.

TYPE HABITAT AND LOCALITY: Cocoa (*Theobroma cacao* L.) soil, W.A.C.R.I. Plantation, Gambari, western Nigeria.

DIAGNOSIS: *Eutylenchus africanus* can be distinguished from the type species *E. setiferus* by the larger body, lower position of the excretory pore posterior to the hemizonid, longer postuterine sac, longer spicules and larger size and more trapezoid form of the caudal alae.

The face views of *E. africanus* (4 ♀ ♀, 2 ♂ ♂) appear similar to *E. setiferus*. There are ten longitudinal ridges immediately behind the lip region.

Specimens identified as *E. africanus* have been examined from the following habitats and localities in Africa: soil, Moor Plantation, Ibadan, Nigeria (collected by F. E. Caveness); rain forest, Victoria Falls, Northern Rhodesia (collected by G. C. Martin); soil, Lamto, Ivory Coast (collected by M. Luc); and *Fimbristylis* sp., soil, Zomba Mountain, Malawi (collected by D. C. M. Corbett).

## DISCUSSION

The genera *Atylenchus* and *Eutylenchus* possess two unique morphological character-

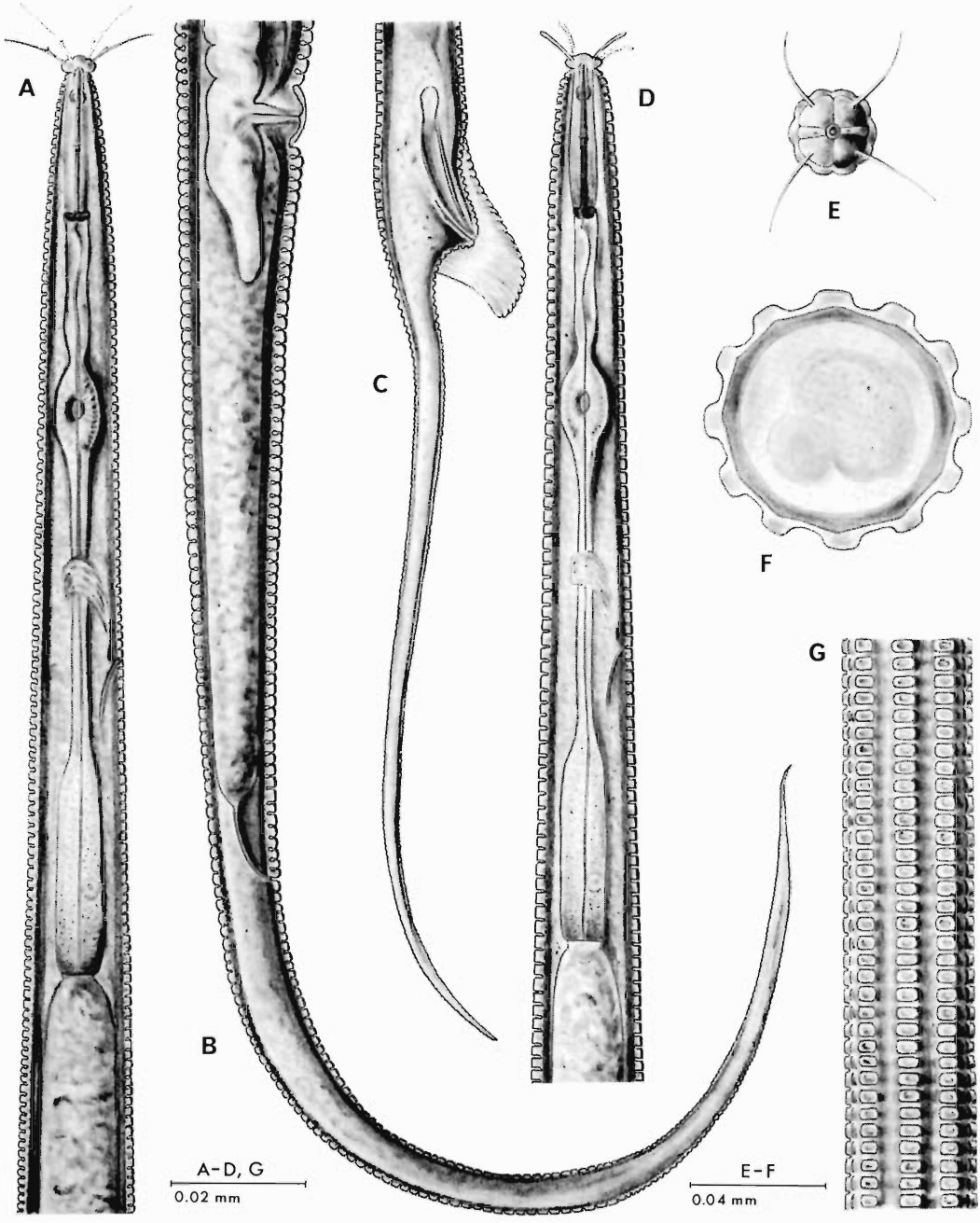


Fig. 3. *Eutylenchus africanus* n. sp. A. Female, anterior region. B. Female, posterior region. C. Male, posterior end. D. Male, anterior region. E. Female, face view. F. Female, cross section near center of body. G. Female, surface view near center of body.

istics for Tylenchida—lip region setae and prominent longitudinal ridges of the cuticle. These are considered sufficiently distinctive to consider these genera a family of Tylenchoidea most closely related to the family Tylenchidae.

No justification can be seen at present for the subfamily Atylenchinae Skarbilovich, 1959 and it is therefore rejected.

*Eutylenchus* can be distinguished from *Atylenchus* by the set off lip region without annulation and the prominent, distinctively shaped caudal alae. This latter character is useful in identifying *Eutylenchus*, even at low magnifications but of much less value in *Atylenchus* where the caudal alae are absent and males apparently rare.

*Atylenchus decalineatus* is also distinctive from *Eutylenchus* species by the dorsoventral flattening of the body with the lateral longitudinal ridges being higher than the other eight ridges. This lateral ridge is bifurcate in juvenile specimens—a condition not seen in juvenile specimens of *Eutylenchus*.

Although *Atylenchus* appears to have deirids and *Eutylenchus* not to have them, this structure is seen with difficulty because of the peculiar cuticle and it is therefore not considered as a generic character.

In face view, these two genera also appear different, *Eutylenchus* being round with four distinct lips bearing setae and two less distinct much smaller lateral lips with obscure amphid apertures (Fig. 3E). *Atylenchus* has an oval appearance with what appears to be four large lips. Elongated darker structures appear where the lateral lips would be expected and it cannot be ascertained whether these are amphid apertures or the lateral lips (Fig. 1E–F).

The following assisted in the preparation of nematode slides, measurements, and illustrations: A. H. Bell, K. B. Brown, and C. S. Papp.

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# Nematode Parasites of the Coelomic Cavity of Earthworms. VI. *Macramphida* and *Sucamphida*, Two New Genera with Unusual Amphids

R. W. TIMM[1]

Two new genera of earthworm parasites contained in the Gates–U.S.D.A. Collection possess unusual amphids. In *Macramphida* n. g. the amphids are extremely large and in *Sucamphida* n. g. they are distinctly sucker-like. All specimens were remounted in glycerin and given their original slide numbers. The collection dates given on the slides are the dates on which Dr. Gates sent letters to Beltsville listing the hosts for each collection. Dr. Gates has been able to furnish some of the actual dates for his collections.

## *Macramphida* new genus

DIAGNOSIS: Drilonematidae. Nonungellate. Female head swollen. Amphids circular, very large, slightly sucker-like. Esophagus short and narrow, slightly clavate. Symmetrical circular to elliptical caudal suckers in both sexes. Long spermatheca in female; shell of ova punctate. Copulatory apparatus in male.

TYPE SPECIES: *Macramphida sinense* n. sp.

Based on the nature of the esophagus, male and female reproductive systems, caudal suckers, and subventral excretory gland cells, this genus belongs in the Drilonematidae. In the larger size of the body and in the size and shape of the esophagus it resembles *Drilonema* Pierantoni, 1916, but neither amphids nor phasmids were described for that genus and the male is much smaller and lacking a copulatory apparatus. It perhaps most closely resembles *Dicelis* Dujardin, 1845, but the esophagus is less developed and the caudal suckers are much larger. The relative size of the suckers is reminiscent of *Siconema* Timm (in press), which belongs to the Ungellidae. The amphids are by far the largest in any of the Drilonematidae and, in fact, are as large as those reported for any nematode.

## *Macramphida sinense* new species (Fig. 1, A–F)

FEMALE (5): Length = 6.06 mm (5.62–7.34); esophagus = 173 $\mu$ (163–176); esophagus to vulva = 3.55 mm (3.11–4.19); vulva to anus = 2 mm (1.62–2.57); tail = 357 $\mu$ (270–416); maximum body diameter = 168 $\mu$ (138–216).

MALE (5): Length = 4.41 mm (3.14–6.18); esophagus = 142 $\mu$ (128–154); esophagus to anus = 4.08 mm (2.84–5.81); tail = 194 $\mu$ (150–220); maximum body diameter = 63 $\mu$ (57–67).

HOLOTYPE FEMALE: L = 7.34 mm; esophagus = 176 $\mu$; esophagus–vulva = 4.19 mm; vulva–anus = 2.57 mm; tail = 400 $\mu$; maximum body diameter = 216 $\mu$.

ALLOTYPE MALE: L = 6.18 mm; esophagus = 150 $\mu$; esophagus–anus = 5.81 mm; tail = 220 $\mu$; maximum body diameter = 57 $\mu$.

DESCRIPTION: Female body stout; male body long and narrow. Cuticle striated; striae 2.5 $\mu$ apart, composed of rough punctations; subcuticle granulated; hypodermis sometimes vacuolate at anterior. Head swollen in female, rounded at anterior, 106–112 $\mu$ in diameter at amphids; head not swollen in male, truncate at anterior, 48 $\mu$ in diameter at amphids. Cephalic hooks absent. Large circular amphids, about 80% of head diameter wide, with thin sucker-like rim; located 1.2 head diameters behind the anterior in male. Amphidial pouch with sensilla leading from central innervation. Esophagus short and narrow, slightly narrower at isthmus, slightly clavate at base, curved to dorsal side in head region; cardia flat to conical. Excretory pore obscure, possibly opposite midesophagus; sclerotized terminal excretory duct lacking, but polynucleate subventral excretory gland cells present. Nerve ring surrounding esophagus at isthmus. Anus obscure in female; three prominent rectal glands in male. Ovary single, anterior; begins in tail dorsal to caudal suckers, loops once or twice behind vulva, and extends anteriorly to connect with very long spermatheca, 0.6–0.73 mm long, containing small spherical sperm; spermatheca distinctly set off, located two to four body diameters behind esophageal base. Uterus not convoluted; vulva inclined slightly ante-

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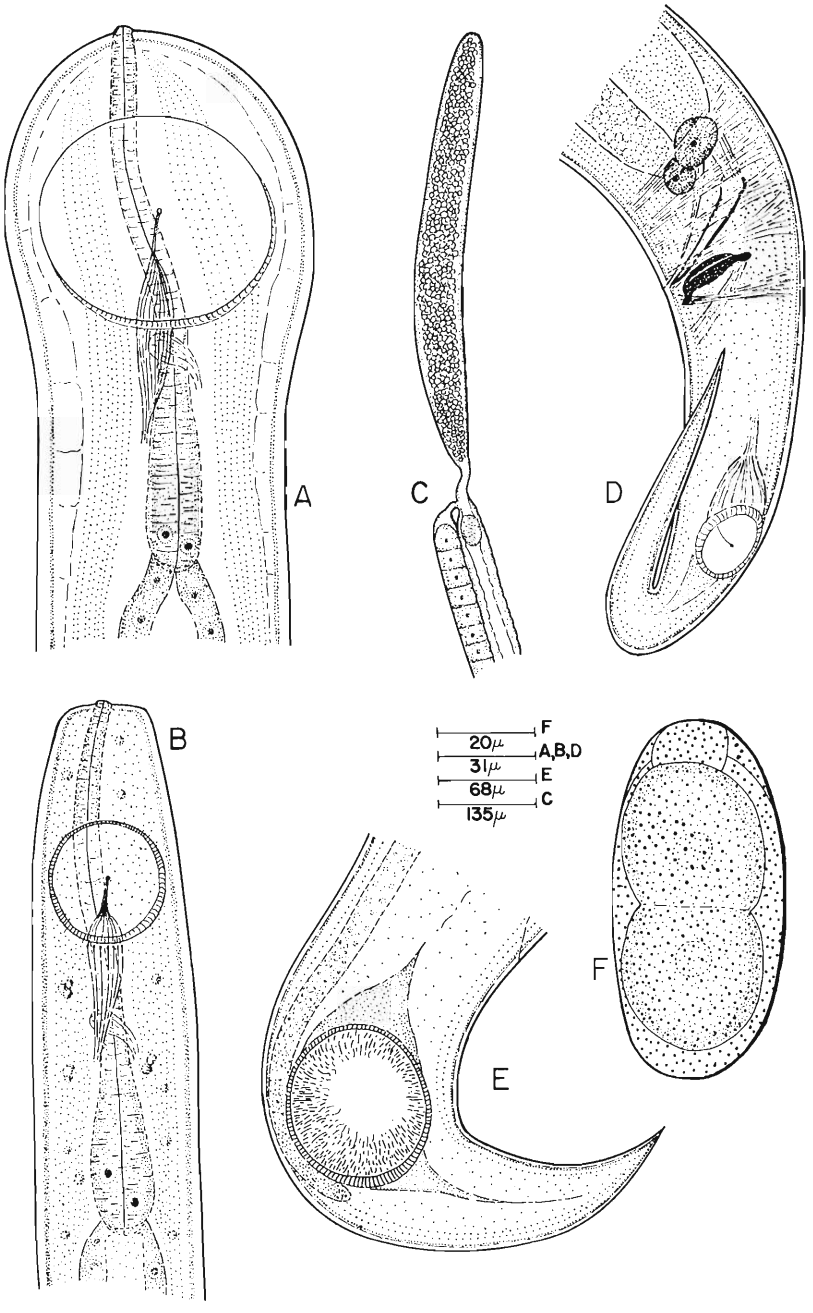


Fig. 1. *Macramphida sinense* n. gen., n. sp. A, Female esophagus; B, Male esophagus; C, Anterior of female reproductive system; D, Male tail; E, Female tail; F, Ovum.

riorly; 71–106 ova in uterus, in two to four rows, 61–67 × 32 $\mu$; shell thick, with large punctations on surface. Single testis extending to within three to four body diameters from esophageal base, reflexed ventrolaterally 0.34–0.48 mm. Spicules almost straight, thin, distinctly cephalated, 35–45 $\mu$ long. Gubernaculum boat shaped, dark colored, with dorsal and ventral posterior projections, 23–28 $\mu$ long. Fine copulatory muscles near anal region. Female tail short, ventrally curved, tapering to acute tip, 2.5–3.7 anal body diameters long. Male tail long and narrow, ventrally curved, tapering to acute tip, 3.1–5 anal body diameters long. Symmetrical caudal suckers located at about midtail, 86–112 $\mu$ in internal diameter in female, with muscular rim and delicate funnel-like membrane extending beneath surface; 23 $\mu$ in diameter in male, with central innervation and sensilla extending anterior to sucker.

TYPE HOST: *Pheretima szechuanensis* Chen.

TYPE LOCALITY: Hai Tang, Szechuan, China.

TYPE HABITAT: Coelom.

HOLOTYPE FEMALE: Sent by Dr. G. E. Gates on 20 September 1933; Slide No. $4P_1$.

ALLOTYPE MALE: Same data as holotype; Slide No. $4P_2$.

PARATYPES: Males and females on Slide Nos. $4P_2$–$4P_6$.

### *Sucamphida* new genus

DIAGNOSIS: Ungellidae. Ungellate; head bearing two sturdy dorsal hooks with thick ventral apophyses. Amphids elliptical, with distinct sucker-like rims. Caudal suckers large, symmetrical, broadly elliptical. Terminal excretory duct heavily sclerotized. Esophagus short and thick, swollen at posterior. Ova ornamented with polygonal pattern; thin outer membrane of ova supported by ribs; punctate at one end.

TYPE SPECIES: *Sucamphida robustum* n. sp.

This genus represents an advanced form of the genus *Siconema* Timm (in press); the cephalic hooks are more sturdily constructed, the amphids prominent and sucker-like, the esophageal base not set off as a dorsally displaced bulb, a copulatory swelling lacking in the male, and the female tail not swollen in the region of the suckers.

### *Sucamphida robustum* new species (Fig. 2, A–H)

FEMALE (3): Length = 1.69 mm (1.61–1.80); esophagus = 124 $\mu$ (118–128); esophagus–vulva = 1.07 mm (1.05–1.09); vulva–anus = 0.34 mm (0.22–0.42); tail = 197 $\mu$ (192–208); maximum body diameter = 151 $\mu$ (144–163).

MALE (allotype): Length = 1.13 mm; esophagus = 137 $\mu$; esophagus to anus = 0.85 mm; tail = 131 $\mu$ (tip broken); maximum body diameter = 51 $\mu$.

HOLOTYPE FEMALE: L = 1.67 mm; esophagus = 128 $\mu$; esophagus–vulva = 1.09 mm; vulva–anus = 0.38 mm; tail = 192 $\mu$; maximum body diameter = 144 $\mu$.

DESCRIPTION: Body of female stout; body of male small and narrow. Fine cuticular striation. Head bearing one pair of stout spreading dorsal hooks, 16 $\mu$ long from tip to top of dorsal apophysis, 19 $\mu$ long from top to bottom of sclerotized part in lateral view in male, 22–25 $\mu$ in female; hooks connected by a broad transverse bar in form of an H, with thick ventroposterior apophyses spreading outwards. Amphids just behind hooks, transversely elliptical, with broad sensilla and protruding muscular rim, best seen in ventral view of head. Esophagus one-half the neck diameter wide, with swollen base; cardia conical. Nerve ring surrounding isthmus. Heavily sclerotized terminal excretory duct, located opposite esophageal base in male, and one body diameter posterior in female. Intestinal cells containing yellowish globules. Three prominent rectal glands in both sexes. Several prominent nuclei posterior to anus, located within the large, finely granular subventral excretory gland cells. Cloacal aperture of male inconspicuous, without swollen copulatory papilla; copulatory apparatus lacking. Ovary single, anterior, beginning between vulva and anus and looped two to four times in this region; spermatheca short, not set off; uterus convoluted, containing 32–57 ova, 45–51 × 20–23 $\mu$; shell ornamented with irregular polygonal pattern, punctate at one end; ribs from shell support a delicate outer egg membrane. Lips of vulva protruding; vulva located near anus. Tail of both sexes swollen in anterior sucker-bearing region, then bluntly tapering to narrowly rounded tip in female. Caudal suckers symmetrical, 36–58 × 26–30 $\mu$ in female, with covering membrane bearing

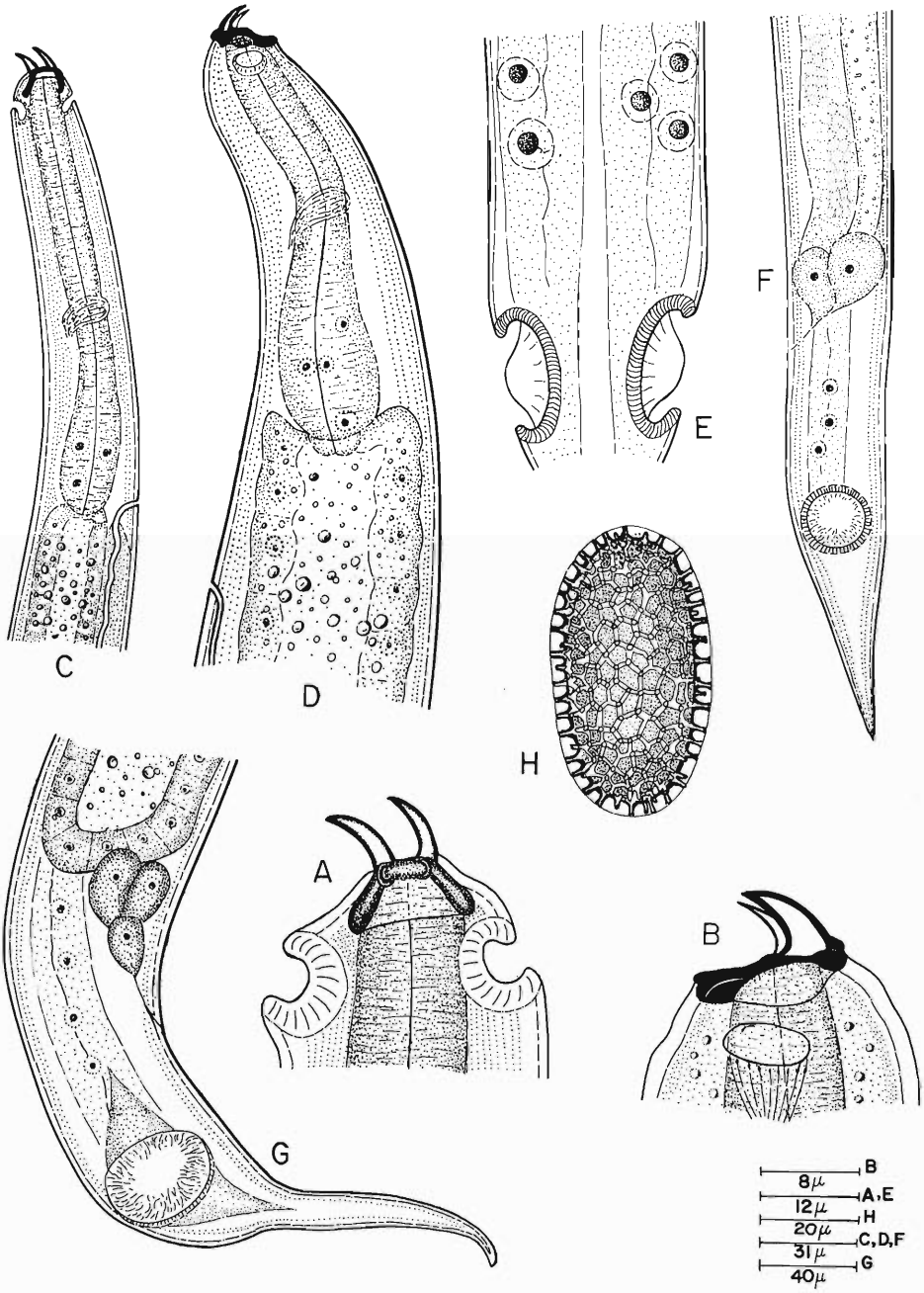


*Fig. 2. Sucamphida robustum* n. g., n. sp. A, Female head, ventral view; B, Female head, lateral view; C, Male esophagus; D, Female esophagus; E, Anterior region of male tail; F, Male tail; G, Female tail; H, Ovum.

threadlike markings. Suckers in male 22 $\mu$ in diameter, with central raised portion.

TYPE HOST: *Pheretima doliaria* Gates, 1931.

TYPE LOCALITY: Nam Hpen Noi, Yunnan, China, across the border from northeastern Burma (corrected locality).

TYPE HABITAT: Coelom, segments xi–xiv.

HOLOTYPE FEMALE: Sent by Dr. G. E. Gates on 2 April 1932; Slide No. $3A_2$.

ALLOTYPE MALE: Same data as holotype; Slide No. $3A_2$.

PARATYPES: Females on Slide Nos. $3A_1$ and $3A_4$.

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# The Life Histories of *Seinura celeris*, *S. oliveirae*, *S. oxura*, and *S. steineri* (Nematoda : Aphelenchoididae)[1]

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The predaceous habit of members of the genus *Seinura* Fuchs, 1931, has been known since 1937 when their feeding habits were described by Linford (1937) and Linford and Oliveira (1937). However, the life history of only one species, *Seinura tenuicaudata* (de Man, 1895) J. B. Goodey, 1960, has been investigated to date (Hechler, 1963). The life histories of four more species, *S. celeris* Hechler, 1965, *S. oliveirae* (Christie, 1939) J. B. Goodey, 1960, *S. oxura* (Paesler, 1957) J. B. Goodey, 1960, and *S. steineri* Hechler, 1965, are described in this paper.

## MATERIALS AND METHODS

The sources of the species of *Seinura* used in this investigation were listed by Hechler and Taylor (1965). Maintenance of these in culture, as well as cultures of *Aphelenchus avenae* Bastian, 1865, used as a source of food for the *Seinura* spp., was also described.

Time of development of immature stages and the total life cycle were determined on nematodes reared in small cells made by attaching 18-mm glass rings to microslides with a film of petroleum jelly. Prior to the addition of nematodes, 1 ml of 2% water agar was added to each cell. *A. avenae* were concentrated in a small drop of distilled water by centrifugation and the suspension was placed on the agar. The *Seinura* were added with a nylon pick, and after the small amount of water was absorbed the nematodes moved into the agar. The cells were stored in Petri dishes at 28 C. Observations were made with a stereoscopic microscope at 90× magnification.

To determine the time from laying to hatching of eggs, single gravid females were placed in sterilized distilled water in sterilized Bureau of Plant Industry watch glasses. Immediately after an egg was laid, the female was removed and the time was recorded. The dishes, each containing one egg, were stored at 28 C. When eggs were about to hatch, as shown by preliminary tests, they were examined at half-hour intervals until hatching occurred.

Hanging drop preparations were used to investigate details of development within the egg. A gravid female was placed in a small drop of distilled water on a cover slip, and the female was removed after the egg was laid. The cover slip was inverted over a depression slide, its edges were sealed with petroleum jelly, and observations were made with a 97× oil-immersion objective.

The need for males for reproduction was determined as described by Hechler (1963).

Measurements of immature stages and stud-

[1] Portion of a Ph.D. thesis submitted by the senior author to the Graduate College, University of Illinois, Urbana.

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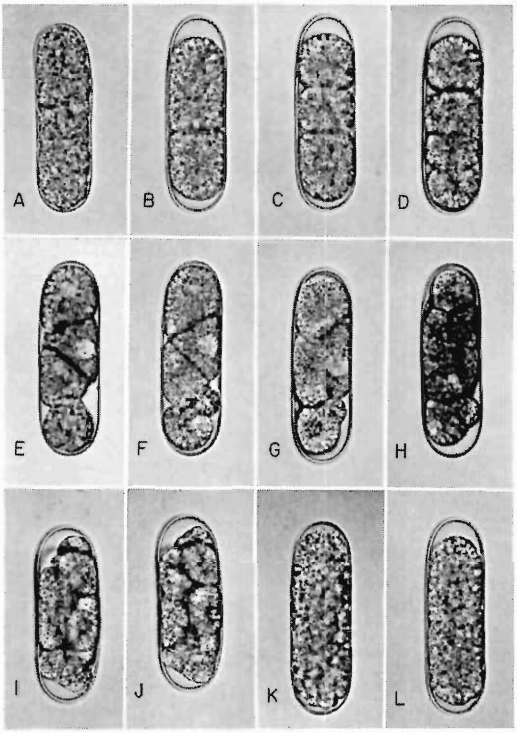


Fig. 1. *Seinura steineri*, embryogeny. (A) newly laid; (B) age ½ hour; (C) age 1 hour; (D) age 3½ hours; (E) age 3 hours 45 minutes; (F) age 4½ hours; (G) age 5 hours; (H) age 8 hours; (I) age 10 hours; (J) age 12 hours; (K) age 14 hours; (L) age 24 hours.

ies of the morphology of developing gonads were made on heat-relaxed specimens mounted in 5% formalin containing a small amount of acid fuchsin. Satisfactory staining was achieved 24 hours or longer after the nematodes were placed in the dye.

Chromosomes were stained as described by Hechler (1963).

## LIFE HISTORIES

### *Seinura steineri*

DEVELOPMENT WITHIN THE EGG: Eggs measure 60 to 78 $\mu$ × 19 to 23 $\mu$ when laid. The shell is finely rugose, and both ends are the same width. The shell is entirely filled with protoplasm (Fig. 1A). The protoplast contracts and cleavage begins in ¾ to 1½ hours resulting in two cells of unequal size (Fig. 1B),

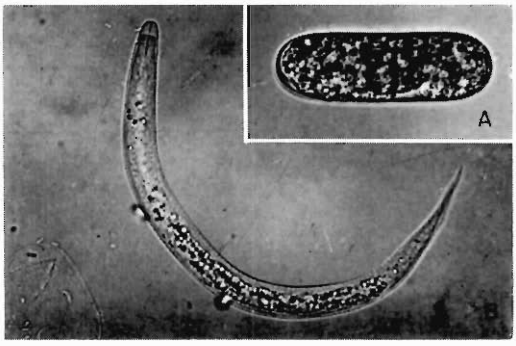


Fig. 2. *Seinura steineri*. (A) embryogeny, age 30 to 33 hours; (B) second molt.

the larger in the anterior end. After[3] 3½ hours the larger cell divides (Fig. 1C), then the smaller 10 to 15 minutes later, resulting in four cells in a row (Fig. 1D). After 4½ hours the second cell from the anterior end begins to move back dorsal to the third cell and both cells become triangular in outline (Fig. 1E). After 5 hours the posterior cell divides obliquely, with the much smaller daughter cell formed anteriorly (Fig. 1F). The second cell continues to move posteriad and after 8 hours it is beside the third cell (Fig. 1G). After 10 hours all three anterior cells have divided and the small daughter cell from the posterior cell has moved posteriad (Fig. 1H). After 12 hours the anterior and posterior cells have divided again (Fig. 1I), and after 14 hours there are too many cells to follow further divisions easily (Fig. 1J). After 18 hours the embryo is composed of many small cells, the inner ones somewhat larger than those around the periphery (Fig. 1K). After 24 hours the embryo is well formed, with the anterior end more hyaline (Fig. 1L); the tail begins to show after 30 to 33 hours (Fig. 2A), and motion begins about 35 hours after the egg was laid. The first stage has conical lips, no stylet or valve plates in the metacorpus, and an elongate conoid tail. Dimensions are: L =150–170 $\mu$; a = 11.0; b = 5.0; c = ? (anus not seen). A molt similar to that seen by Hechler (1963) in *S. tenuicaudata* occurs after 38 to 40 hours. The second stage has a stylet and valve plates. Dimensions are: L = 200 $\mu$; a = 13.5; b = 5.4; c = ? (anus not

[3] In this and subsequent usage the term "after" refers to the elapsed time between egg laying and the observation.

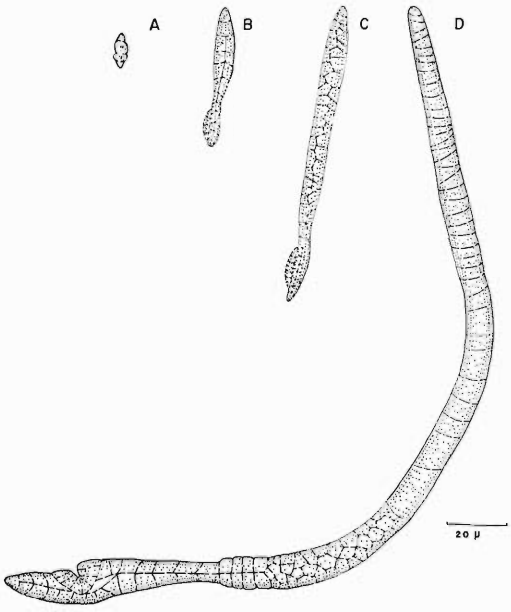


Fig. 3. *Seinura steineri*, gonad development in hermaphrodite. (A) hatching stage; (B) early third stage; (C) early preadult; (D) final molt.

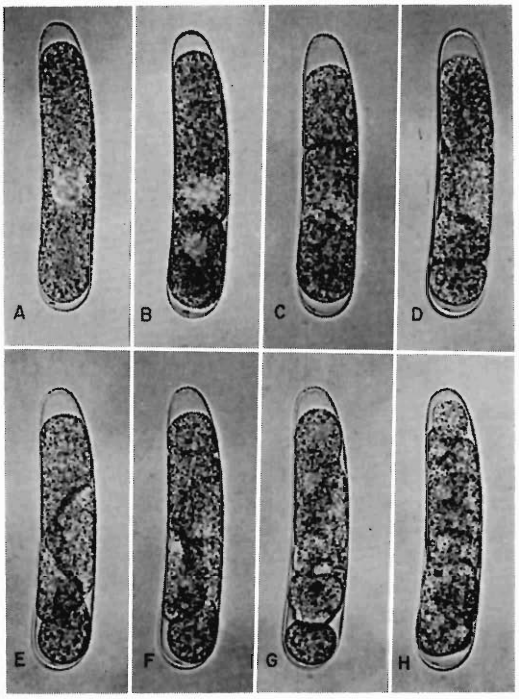


Fig. 4. *Seinura oxura*, embryogeny. (A) age 15 minutes; (B) age 45 minutes; (C) age 65 minutes; (D) age 68 minutes; (E) age 1 hour 20 minutes; (F) age 1 hour 30 minutes; (G) age 1 hour 40 minutes; (H) age 2 hours 15 minutes.

seen). Periodic pulsation of the median bulb begins immediately after the molt, and the nematode moves around within the eggshell. The shell gradually softens and stretches to correspond to the shape of the moving nematode. Finally, after 44 to 48 hours, the head breaks through the shell by pressing against it at one end of the egg. No probing of the stylet was observed during hatching.

HATCHING STAGE: L = 210–260 $\mu$; a = 18.0–21.8; b = 4.9–5.7; c = 6.3–7.7. Stylet 11 to 12 $\mu$ long, gland lobe 45 $\mu$ long. The genital primordium consists of four cells in a row, two large cells in the middle, two smaller ones at either end (Fig. 3A). It is 7 to 10 $\mu$ long and located at 56 to 64% of body length. Cuticular striations are more prominent than in later stages, and the body is curved, as described by Hechler (1963) for *S. tenuicaudata*. No feeding by this stage has been seen. Molting occurs 18 to 24 hours after hatching (Fig. 2B).

THIRD STAGE: L =250–450 $\mu$; a = 23.5–30.1; b = 5.1–7.1; c = 5.0–6.6. Stylet 13.0 to 13.5 $\mu$, gland 55 to 65 $\mu$ long. Genital primordium, at 68 to 70% of body length, increases in length to about 45 $\mu$. The germinal section is 30 $\mu$ long with 6 to 10 large cells arranged in two rows (Fig. 3B). A smaller fusiform group of cells about 15 $\mu$ long forms the gonoduct primordium. Four large ventral chord cells can be seen opposite the gonoduct primordium in favorably stained specimens. The third molt occurs about 24 hours after the second, and the nematode feeds before the molt. Nematodes removed from a food source after the second molt do not develop further.

PREADULT: L = 460–710 $\mu$; a = 24.0–27.7; b = 6.4–8.9; c = 5.8–6.6. Stylet 16.5–17 $\mu$, gland 110 to 130 $\mu$ long. The genital primordium increases in length to 180 to 240 $\mu$. Early in the stage spermatogonia arranged in two rows occupy the anterior part of the gonad (Fig. 3C). Late in the fourth stage spermatocytes begin to mature near the posterior part of the gonad and oogonia arranged in one row form in the anterior part (Fig. 3D). The

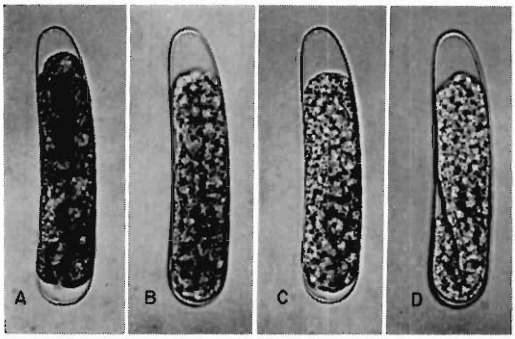


Fig. 5. *Seinura oxura*, embryogeny. (A) age 3 hours; (B) age 5 hours; (C) age 8 hours; (D) age 10 hours.

spermatheca and uterus are formed by division of the epithelial cells in the posterior part, and during the final molt large cells from the ventral chord move in to line the vagina. Spermatogenesis continues throughout the molt. The final molt occurs 24 to 30 hours after the third. Preadults must feed in order to continue their development.

ADULT: Males are extremely rare, varying from 20 to less than one male per 10,000 females. Sperm formation takes place in the gonad of the hermaphrodites during and immediately after the final molt. About 100 sperm cells are produced. The sperm cells remain in the posterior part of the gonad where they were formed until the passage of the first egg forces them back into the gonoduct and the postvulvar sac. Egg laying begins 24 to 28 hours after the nematode emerges from the final molt, and well-fed nematodes continue to lay an egg every 2 hours. When removed from a food source, females lay only four, rarely five, more eggs.

When the supply of sperm cells is exhausted, eggs continue to enlarge and move into the uterus, but they are not laid. Such eggs are usually smaller than eggs produced when sperm cells are abundant. A few of these females have been seen with an egg pushed into the postvulvar sac by the next egg to develop.

The total generation time is 6 to 6½ days at 28 C.

*Seinura oxura*

DEVELOPMENT WITHIN THE EGG: *Embryogeny*: Eggs measure 90 to 114 $\mu \times 18$ to

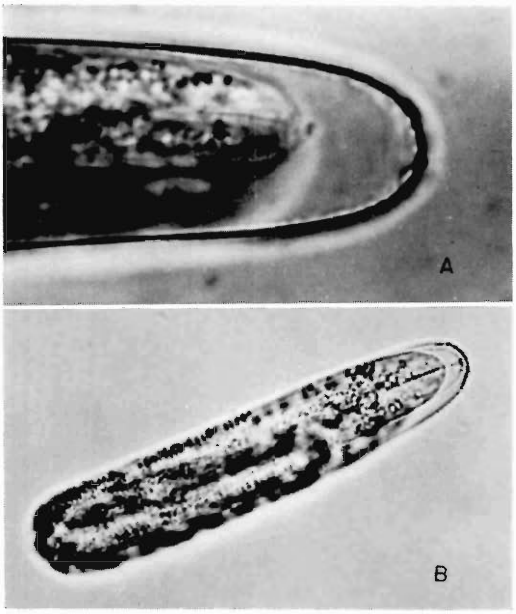


Fig. 6. *Seinura oxura*. (A) first molt; (B) second molt.

23 $\mu$ when laid. They are rugose, straight to slightly curved, with the anterior end, which emerges from the female first, more narrow. The egg is unsegmented when laid, the protoplast contracts 10 to 15 minutes later (Fig. 4A), and the first division occurs after 45 minutes, with the anterior blastomere larger (Fig. 4B). Twenty minutes later the large cell divides (Fig. 4C), then 2 or 3 minutes later, the smaller one, resulting in four cells in a row (Fig. 4D). Then the second cell from the anterior end moves dorsad to the anterior and third cells, and all three cells become generally triangular in outline (Fig. 4E). Within the next 20 minutes all three cells divide (Fig. 4F), and after 1 hour 40 minutes the posterior cell divides (Fig. 4G). The cells continue to divide and shift in position. After 2 hours 15 minutes over 12 cells are present, with one prominent cell in the anterior end of the egg (Fig. 4H), and an hour later most of the cells have divided again and the anterior cell is no longer conspicuous (Fig. 5A). After 5 hours the embryo is composed of many small cells and is more hyaline at the anterior end (Fig. 5B) and after 8 hours the head has

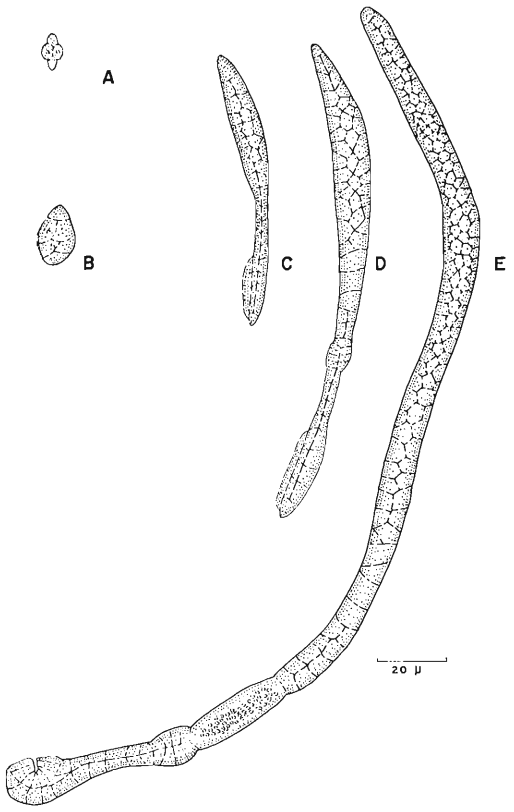


Fig. 7. *Seinura oxura*, gonad development in hermaphrodite. (A) second stage; (B) early hatching stage; (C) late hatching stage; (D) early preadult; (E) final molt.

become flattened (Fig. 5C). The tail begins to form after nine hours, and the oral opening shows as a large cone-shaped or rounded depression (Fig. 5D). Motion begins after 10 to 12 hours. The nematode is 1½ times as long as the egg, and the head is nearly as broad as the egg, with six conical lips. No stylet or valve in the metacorpus is present. Motion consists of both a back-and-forth movement and rotation in the longitudinal plane.

*First Molt*: Development through the first molt requires 14 hours and proceeds as follows: The stylet, faintly visible at first, develops in the hyaline V-shaped pharynx, and later the lumen of the esophagus and intestine appear. Meanwhile the outline of the metacorpus begins to show, later the crescentic valve plates, and finally the muscular structure. Active motion ceases for about one-half hour except for contractile twitching throughout the body, especially in the cephalic region, and gradually the new cuticle loosens from the old. When back-and-forth motion resumes, the cuticles separate. The cast cuticle is visible around the head and tail for only 3 to 5 minutes (Fig. 6A). Periods of pulsation of the metacorpus begin, the nematode rapidly lengthens to twice the egg length, and the old cuticle is no longer visible.

*Second Stage*: L = 125–135 μ; a = 8.1–10.5; b = 3.6–4.9; c = 8.9–10.4. Stylet 11 μ long, very delicate compared to that of subsequent stages. Tail very short with rounded terminus. Genital primordium 8 μ long at about 55% of body length, with four cells arranged in a diamond pattern, the two larger side by side and the two smaller at the anterior and posterior ends (Fig. 7A). The second stage lasts about four hours. Then active motion ceases, the stylet and valve plates disappear, and a new stylet and valve are formed. These structures are much more prominent in the third stage than in the second, and the lips are larger and more offset from the body. Twitching movements separate the cuticles, and the molt is complete at age 20 to 24 hours, with the cast cuticle and the anterior part of the second-stage stylet clearly visible (Fig. 6B). The valve in the bulb begins to pulsate periodically and the nematode rapidly increases in size until it fills the eggshell tightly. Meanwhile the nematode moves back and forth within the shell with the head and tail always in the narrow end. The shell softens and stretches, and finally the head breaks through the shell at the narrow end.

Hatching of the third stage occurs at 24 to 26 hours.

HATCHING STAGE: L = 200–240 μ; a = 17.6–21.8; b = 4.7–5.7; c = 9.2–11.8. Stylet 12 to 14 μ long, gland lobe 45 to 65 μ long. The genital primordium is 10 to 20 μ long at 65 to 73% of body length. There are usually four cells in one row, two large germinal cells in the center, and one smaller cell at each end. Occasionally there are three to four cells arranged in two rows (Fig. 7B). Tail short with rounded or pointed terminus. Three incisures are visible in the lateral field.

The nematodes grow to 370 μ in length.

The genital primordium lengthens to about 80 $\mu$, with two elongate fusiform groups of cells. The anterior group, twice as long as the posterior, consists of about 20 large germinal cells arranged in two rows. The posterior group, composing the gonoduct primordium, consists of two rows of 16 smaller cells (Fig. 7C).

The nematodes begin to feed as soon after hatching as suitable prey is located, and feeding is necessary for further development. The third stage takes about 16 hours, and the nematodes kill six to eight prey during this time.

PREADULT: L = 390–560 $\mu$; a = 22.5–26.0; b = 7.0–9.3; c = 10.7–14.0. Stylet 12.5 to 15.5 $\mu$ long, gland lobe 60 to 100 $\mu$ long. Genital primordium increases from 70 to 160 $\mu$, with the posterior end at 77 to 84% of body length. Early in the stage, the uterus and spermatheca are visible as separate sections of the gonoduct (Fig. 7D). Cells from the ventral body wall move inward to form the lining of the vagina at the final molt. By the time the stylet of the adult is formed, spermatozoa are mature in the gonad (Fig. 7E). The sperm remain in the posterior end of the gonoduct until the first eggs push them back into the spermatheca and uterus. The fourth molt occurs about 24 hours after the third.

ADULT: During the molt the gonad lengthens to about 300 $\mu$. Sperm formation continues until after the adult nematode emerges from the cast cuticle. There are more than 115 sperm cells in the gonoduct when egg production begins. The first egg is laid 6 to 8 hours after the molt and production continues at the rate of one egg every two hours. Egg production stops when the sperm supply is exhausted. Females removed from food lay up to six eggs before body reserves are exhausted.

Males are not necessary for reproduction and their numbers are extremely variable. In one isolate there were 12.2 to 1.3 females per male, and in another isolate 332 females per male. One culture had all males! Copulation has not been seen.

The total generation time is 3 to 3½ days at 28 C.

*Seinura celeris*

DEVELOPMENT WITHIN THE EGG: Eggs measure 20 to 22 $\mu$ × 80 to 85 $\mu$ when laid. They are unsegmented, with a finely rugose shell, straight to slightly curved, with the end which emerges first from the female more narrow. One polar body is visible near the midpoint of the egg length. Cell division begins about 15 minutes after the egg is laid, with the larger anterior blastomere in the narrow end of the egg. The cleavage pattern is similar to that of *S. oxura*, with the larger cell dividing after 50 minutes, and the smaller one 5 to 10 minutes later. The central anterior cell moves dorsal to the central posterior cell, both become triangular in outline and divide longitudinally. After 1½ hours there are many cells present, with one conspicuous cell in the anterior end of the egg. After 4½ hours the anterior end of the embryo has become flattened and more hyaline than the posterior end, and the oral depression shows after 5½ hours. The tail begins to curve along the ventral side after 8 hours, the embryo is 1½ times as long as the egg after 9 hours, and motion begins. After 11 hours the nematode is twice the egg length and a faint stylet and valve in the metacorpus appear. The head is broad with small conical lips. A hyaline space forms around the stylet, and the cuticle over the lips begins to loosen (Fig. 9A). No period of quiescence or shedding of cuticle corresponding to the first molt in *S. oxura* has been seen at this stage of development. After 12 to 13 hours the nematode is 2½ times as long as the egg. Dimensions are: L = 150–190 $\mu$; a = 10.0–13.5; b = 5.3–5.8; c = ? (anus not visible). The genital primordium is 12 $\mu$ long and consists of two cells.

After 14 to 16 hours a heavier stylet and valve are formed and a molt takes place. The lips are larger than in the first stage and more offset from the body. After the old cuticle is loosened, the valve in the metacorpus pulsates periodically. The nematode increases in length to three times as long as the egg. Dimensions are: L = 210–220 $\mu$; a = 14.0–14.5; b = 4.7–4.9; c = ? (anus not visible). Two small cells are visible at either end of the two larger cells in the genital primordium.

The egg hatches about 18 hours after it is laid.

HATCHING STAGE: Dimensions: L = 210–260 $\mu$; a = 17.7–21.6; b = 5.9–6.9; c = 5.9–7.6. Stylet 10 to 13 $\mu$ long. Gland lobe 55 to 60 $\mu$ long. Genital primordium 10 to 12 $\mu$ long,

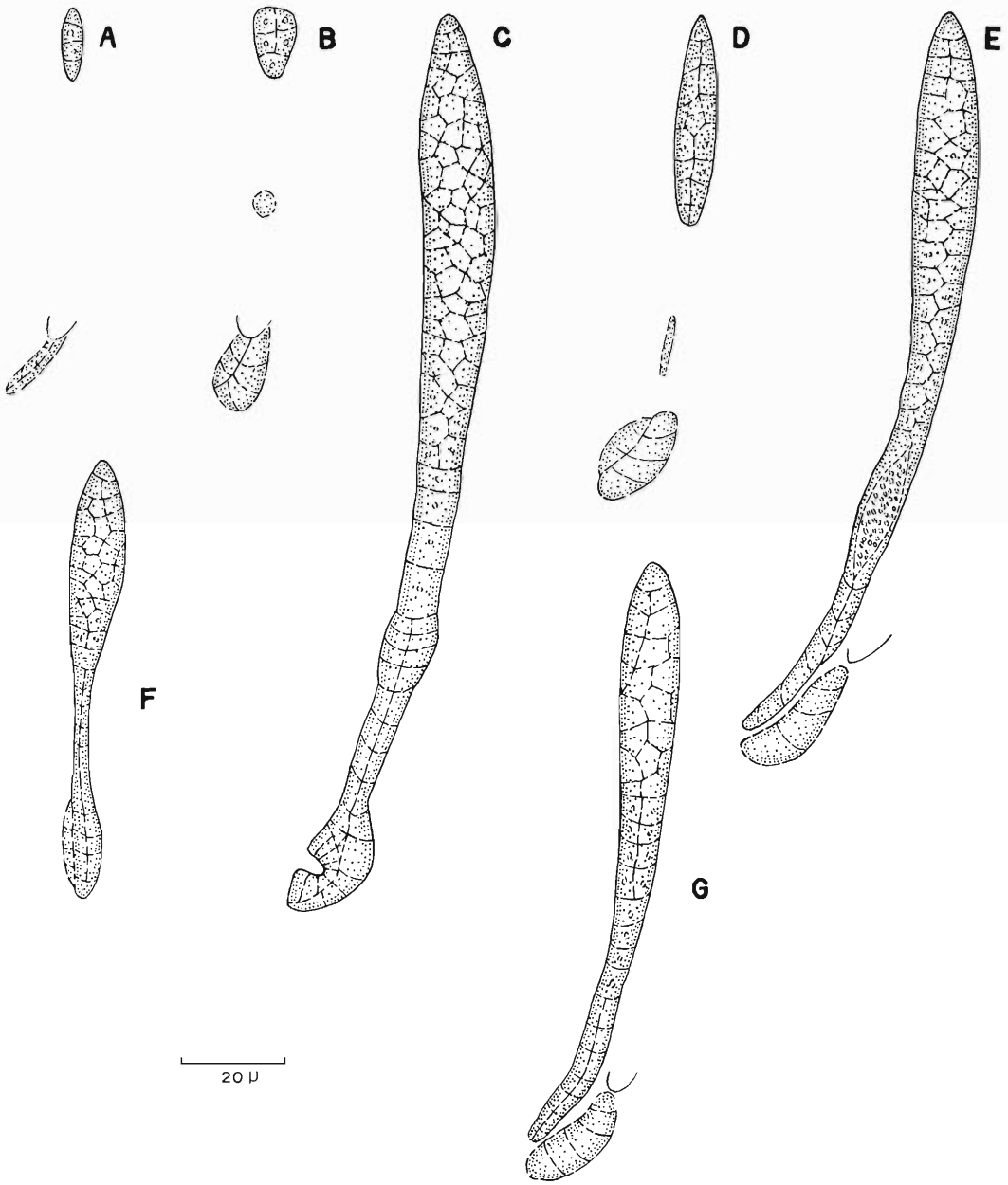


Fig. 8. *Seinura celeris*, gonad development. (A) hatching stage, female; (B) hatching stage, male; (C) late preadult, female; (D) second molt, male; (E) final molt, male; (F) early preadult, female; (G) early preadult, male.

located at about 60% of body length from anterior end, consisting of a row of four cells in females (Fig. 8A). In males it consists of a cluster of five to seven cells, the rectum is more prominent than in females, with large cloacal primordium cells present, and at about half the distance between anus and gonad there is often a pair of small dark-staining cells, one on either side of the ventral chord (Fig. 8B). The nematodes begin to feed as soon as they locate suitable prey. Females grow to about 420 $\mu$, males to about 330 $\mu$, before the next molt. The genital primordia increase to about 60 $\mu$. The nematodes molt about 12 hours after hatching, and they do not develop further if removed from a source of food.

PREADULT: Dimensions: Females: L = 400–560 $\mu$; a = 22.1–24.6; b = 6.5–8.3; c = 6.6–7.3. Stylet 13.5 $\mu$. Males: L = 330–400 $\mu$; a = 18.2–24.6; b = 6.5–7.0; c = 7.3–10.3. Stylet 12 $\mu$. Early in the stage the female genital primordium is 90 to 100 $\mu$ long. The ovary is fusiform, with three or four oocytes at its widest part (Fig. 8F). Posteriorly it tapers to two rows of cells, then widens at the vagina primordium. It increases in length to about 240 $\mu$ and the vagina and spermatheca differentiate during the final molt. The vagina is lined during the molt with cells from the ventral chord (Fig. 8C). In males the genital primordium increases from 85 to 120 $\mu$ in length. The cells located along the ventral chord divide and may form part of the vas deferens (Fig. 8D). Early in the stage the gonad is joined to the cloacal primordium (Fig. 8G). Many sperm cells are mature before the final molt is complete (Fig. 8E). Development of the preadult stage takes about 12 hours and the nematodes must feed to continue development.

ADULT: Copulation occurs as soon as the adult nematodes emerge from the final molt, and the first egg is laid about 4 hours later. Egg laying continues at the rate of one egg every 1½ hours. Females removed from a source of food lay up to five eggs before nutrient reserves are exhausted. More than 275 sperm cells were counted in the genital tract of females. Virgin females have not been seen to lay eggs. Sex ratio varied from 1.78 to 7.4 females per male. The total generation time is 2¼ to 2½ days at 28 C.

*Seinura oliveirae*

DEVELOPMENT WITHIN THE EGG: Eggs measure 72 to 83 $\mu$ × 17 to 19 $\mu$ when laid. They are straight, rugose, with the end which comes from the female first more narrow. They are evenly filled with protoplasm at first, but within 15 minutes after laying the protoplast contracts, and the vitelline membrane can be seen loose in the smaller end. One or two polar bodies are present at one-half the egg length. Cleavage is similar to that of *S. oxura*, with a large blastomere in the narrow anterior end of the egg and a smaller one in the posterior end after the first division. There are two cells after 40 minutes, four cells after 1 hour, six cells after 1½ hours, and eight cells after 2 hours. After 5 hours the embryo is well formed with the head end flattened and more hyaline than the posterior end. The oral depression is visible after 6 hours, the tail is visible after 8 to 10 hours, and after 12 hours motion begins. At the time the nematode is twice as long as the egg the first stage is mature, with a faintly visible stylet and valve in the metacorpus. L = 180 $\mu$; a = 14.5; b = 8.5; c = ? (anus not visible). Stylet 7 to 8 $\mu$ long. The head is broad, and the tail is elongate conoid. A molt similar to that in *S. celeris* takes place after 18 to 20 hours (Fig. 9B). After the molt the nematode is about 2½ times longer than the egg; the lips, stylet, and valve are more prominent; and the tail is longer, occasionally with a short filiform portion. Hatching occurs 22 to 24 hours after the egg is laid.

HATCHING STAGE: L = 220–260 $\mu$; a = 20.0–26.0; b = 4.7–6.2; c = 4.9–7.1. Stylet 10 to 12 $\mu$ long, gland lobe 45 to 55 $\mu$ long. Genital primordium about 15 $\mu$ long at 61 to 64% of body length. In females it consists of a single row of four cells, two large central cells, and two smaller ones at either end (Fig. 10A). In males there are five cells in a cluster, and the cells around the rectum are larger than those in females, indicating the presence of the cloacal primordium (Fig. 10B). A pair of cells, one on either side of the ventral chord, is present at half the distance between the gonad and the anus. Females grow to about 400 $\mu$ in length and the genital primordium increases to 40 to 52 $\mu$. The anterior germinal portion consists of 10 to 12 cells, with three rows of oocytes at the widest part (Fig. 10G). The posterior

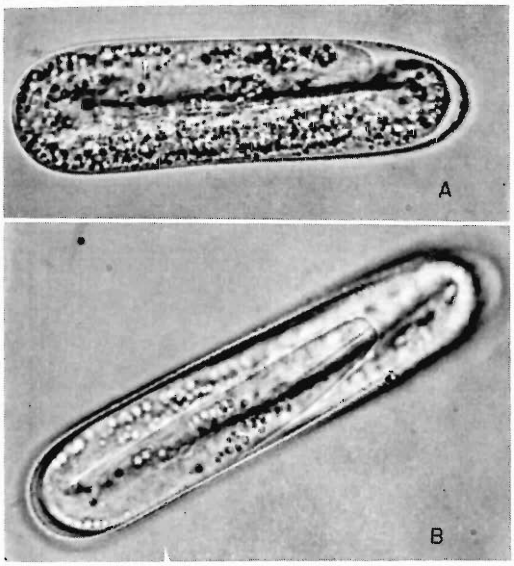


Fig. 9. (A) *Seinura celeris*, early first molt; (B) S. *oliveirae*, late first molt.

gonoduct is much smaller. Four prominent cells in the ventral chord increase in size. Males grow to about 340 $\mu$ in length, the gonad increases to 30 to 40 $\mu$, and the ventral cells divide (Fig. 10C). Late in this stage and during the second molt the anterior part of the genital primordium begins to grow posteriad, and the cloacal primordium becomes increasingly conspicuous (Fig. 10D). Feeding begins as soon as the nematodes hatch, and molting occurs about 16 hours later.

PREADULT: Females: L = 380–550 $\mu$; a = 22.1–30.0; b = 6.7–9.1; c = 5.5–6.0. Males: L = 300–410 $\mu$; a = 24.1–27.1; b = 6.0–7.7; c = 5.4–6.8. Stylet 15 to 16 $\mu$, gland lobe 80 to 100 $\mu$ long. In males the genital primordium continues to grow posteriad, and apparently the ventral cells contribute to the formation of the vas deferens. The entire gonad, about 95 $\mu$ long, joins the cloaca before the final molt (Fig. 10E). At the time of the molt it may be straight or the anterior end may be curved back. Spermatogenesis begins before the molt. In females the genital primordium increases to about 240 $\mu$ with five germinal cells at the widest part. The spermatheca appears as a somewhat wider section of the anterior part of the uterus, and the four cells in the ventral chord divide to eight. During the final molt they move in to line the vagina (Fig. 10F). The time for development of the preadult is about 24 hours.

ADULT: Copulation occurs immediately after the nematodes emerge from the final molt. There is one male for every four to 20 females. Egg laying begins in about 8 hours, and an egg is laid once every 1½ hours as long as there is an adequate food supply. Females removed from food produce up to three eggs before food stored in the body is exhausted. Virgin females have not been seen to lay eggs.

The total time for the life cycle is 2½ to 3 days.

## CHROMOSOMES

Chromosome number was determined by examining oocytes and spermatocytes in meiosis. For each species there was a constant number of tetrads at the first metaphase, a constant number of dyads after the first division, and mature sperm cells contained a constant number of chromosomes. The number of these groups was considered to be the haploid chromosome number. There are six pairs of chromosomes in S. *oxura* and S. *steineri*, and three pairs in S. *oliveirae* and S. *celeris*.

Individual chromosomes at the second metaphase of spermatogenesis are spherical to extremely short rods, about 0.5 $\mu$ or less long. No morphological differences between individual chromosomes were seen within or between species.

## SPERMATOGENESIS

In males rapid mitotic division of spermatogonia begins late in the preadult stage and continues for several days after the final molt. Chromocenters, dark-staining bodies in a circle surrounding the nucleus, can be seen in the spermatogonia and appear identical to those in oogonia (Fig. 11A). By the beginning of the final molt in all species studied a few primary spermatocytes are mature at the posterior end of the testis and meiosis begins. Both divisions of meiosis take place throughout the length of the testis, beginning at the posterior end. The sperm move back toward the cloaca as they mature. At the first metaphase tetrads can be seen in favorable material (Fig. 11B), and dyads are seen at the second metaphase. In

mature sperm cells the chromatin remains condensed and dark-staining (Fig. 11C, D).

In hermaphrodites the primary spermatocytes are mature by the beginning of the final molt. Meiosis begins in the posterior part of the gonad, and continues until the end of the molt in S. *oxura*, and for several hours after the molt in S. *steineri* (Fig. 11E, F). The sperm remain in the posterior part of the gonad until they are pushed into the gonoduct by the first developing egg.

### OOGENESIS

Mitotic divisions were seen in the anterior half of the ovary in all species (Fig. 11G). The chromosomes were very small and close together during these divisions. Chromocenters were seen in older oocytes (Fig. 11A). The first metaphase is visible when the egg begins to separate from the younger oocytes and the posterior end enters the oviduct. The haploid number of tetrads are present at the first metaphase (Fig. 11I), and the haploid number of dyads in each half of the nucleus during the first anaphase (Fig. 11H). At this time a sperm cell can be seen in the end of each egg in S. *steineri* (Fig. 11K), but they were seen in only a few eggs of the other species.

At the beginning of meiosis the first division spindle is centrally located and parallel with the long axis of the egg, but later it moves to the egg membrane and becomes perpendicular to it. One polar body is formed at half the egg length. The polar body and the secondary oocyte have the haploid number of dyads.

In S. *steineri*, stained chromosomes in the second division were seen but the second polar body was not detected in the egg (Fig. 11J). The second division was not seen in the other species, although occasionally two polar bodies were seen in developing eggs of S. *oliveirae*. The sperm cell remains unchanged in the end of the egg until the egg is laid. Further activity of the sperm cells was not studied.

### DISCUSSION

Of the five species of *Seinura* studied to date, four patterns of development have been found. S. *tenuicaudata* reproduces bisexually, with one molt in the egg, one after hatching but before feeding, and it must feed before each of the next two molts (Hechler, 1963). S. *steineri* is hermaphroditic with the molting pattern like that of S. *tenuicaudata*. In both species the hatching stage has smaller lips, more prominent cuticular striations than in subsequent stages, and the nematodes are curved ventrally. S. *oxura* is hermaphroditic with two molts in the egg, the hatching stage feeds, and two molts occur after hatching. In all the species mentioned above the first stage has no stylet or median bulb, and in the second stage, which does not feed, these structures are more delicate than in later stages.

In both S. *celeris* and S. *oliveirae* the molt within the egg between the stage with no stylet and the stage with a delicate stylet, which would correspond to the first molt in S. *oxura*, has not been seen. This can be explained in three ways: (1) it does not occur; (2) it was not seen although it does occur; (3) it occurs, but the two cuticles are superimposed, as suggested by Linford and Oliveira (1940) for the second molt in *Rotylenchulus reniformis* Linford and Oliveira, 1940. Five eggs of S. *oliveirae* and 11 eggs of S. *celeris* were observed carefully throughout the period when the molt could occur, and several eggs of both species were crushed under the cover slip for careful examination of the cast cuticles. No evidence of either a molt or presence of a superimposed cuticle was seen. Furthermore, only one period of quiescence occurred during the development of the eggs after motion had begun. Therefore, the authors believe that these species pass through only one molt in the egg.

The first stage in both S. *celeris* and S. *oliveirae* has a delicate stylet. The hatching stage has a well-developed stylet and valve in the metacorpus, and it feeds. There are two more molts, making a total of only three for these species. They also have shorter life cycles than the other species, only 2½ and 3 days, whereas the life cycle is as long as 6½ days for S. *tenuicaudata* and S. *steineri*, and 3½ days for S. *oxura*.

It is interesting that it is in the species with one molt deleted, or in S. *oxura* with two molts in the egg, that the total development time is drastically reduced. With the deletion of one molt, and therefore one stage, it would be reasonable to expect a reduction of one-fifth to one-fourth in the development time. How-

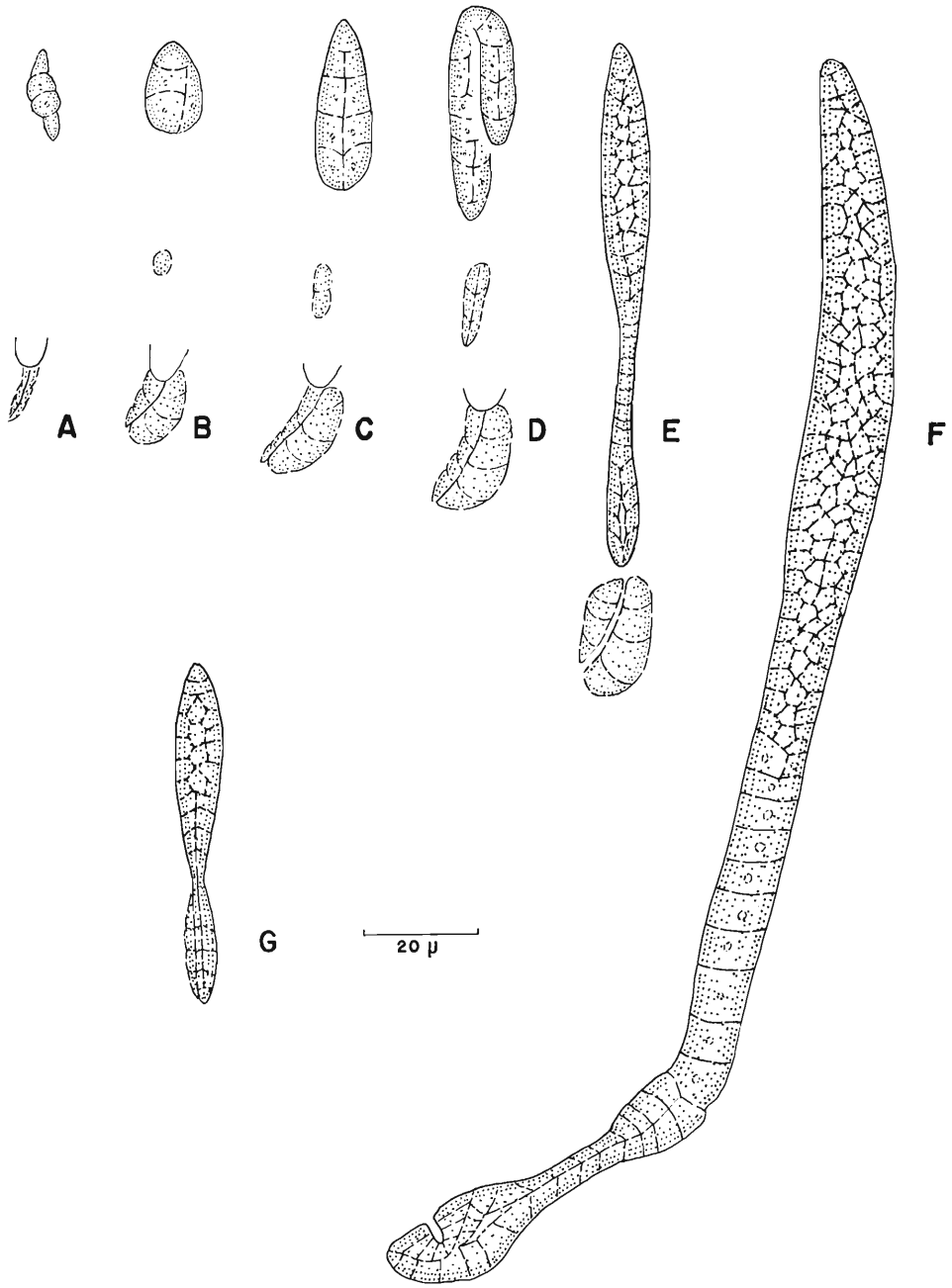


Fig. 10. *Seinura oliveirae*, gonad development. (A) hatching stage, female; (B) hatching stage, male; (C) late hatching stage, male; (D) second molt, male; (E) late preadult, male; (F) late preadult, female; (G) late hatching stage, female.

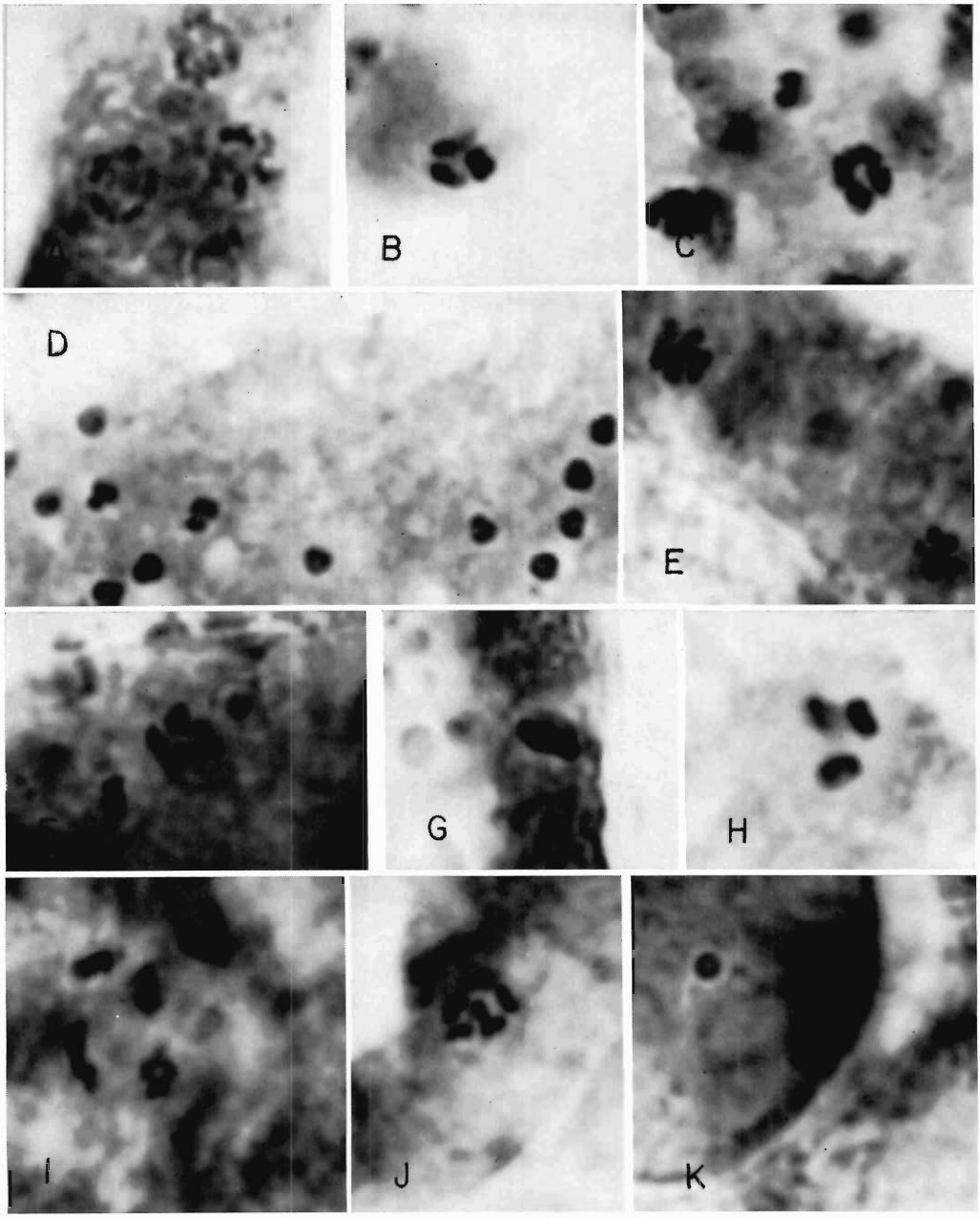


Fig. 11. (A) *Seinura celeris*, chromocenters in growth zone of ovary; (B) *S. celeris*, first metaphase, spermatogenesis; (C) *S. celeris*, four mature sperm cells from a single primary spermatocyte; (D) *S. oliveirae*, sperm cells in uterus of female; (E) *S. steineri*, spermatogenesis in hermaphrodite; (F) *S. oxura*, spermatogenesis in hermaphrodite; (G) *S. celeris*, mitosis in anterior part of ovary; (H) *S. celeris*, first anaphase of oogenesis; (I) *S. steineri*, first metaphase of oogenesis, spread nucleus in crushed egg; (J) *S. steineri*, second metaphase of oogenesis, nucleus in crushed egg; (K) *S. steineri*, sperm within egg.

ever, *S. celeris* develops in less than half the time of *S. steineri*.

There are six pairs of chromosomes in *S. oxura*, *S. steineri*, and *S. tenuicaudata*, and three pairs in *S. oliveirae* and *S. celeris*. This suggests that tetraploidy is present in the former species. However, *S. celeris* and *S. oliveirae* show an unusual molting pattern, with only three molts during the life cycle. This suggests that these species evolved more recently, and if this is true, they could hardly have been ancestors of the species with six pairs of chromosomes. This situation could be explained by the possible existence of a common ancestor with three pairs of chromosomes from which both the tetraploid species and those with three molts evolved. The discovery of a species with three pairs of chromosomes and four molts in the life cycle, which may have evolved without change in these characters, would give this suggestion more significance.

## SUMMARY

The development of four species of *Seinura* feeding on *Aphelenchus avenae* in Petri dish culture at 28 C was studied. *S. steineri* reproduces by protandric hermaphroditism, with about 100 sperm cells produced during the final molt. After the molt egg production begins, with one egg produced every 2 hours when food is abundant. Egg production stops when the supply of sperm cells is exhausted. The first stage has no stylet or valve plates in the metacorpus. There is one molt in the egg, and the second stage has a delicate stylet. The nematode hatches, but does not feed, before the second molt. The third and fourth stages and the adult have heavier stylets and feed. Males are extremely rare, less than one per 10,000 adults. Development within the egg takes 44 to 48 hours and the complete life cycle takes 6 to 6½ days. There are six pairs of chromosomes.

*S. oxura* reproduces by protandric hermaphroditism, with about 115 sperm cells produced during the final molt. The number of males in cultures varies from none to 45% of the adults. Copulation has not been seen. An egg is laid every two hours when food is abundant. Egg production ceases when the sperm supply is exhausted. The first stage has no stylet or valve. During the first molt within the egg the cast cuticle is visible for only 3 to 5 minutes. The second stage has a delicate stylet. The second molt, also within the egg, is more prominent and the cast cuticle is clearly visible. The hatching stage, with a heavier stylet, as well as the preadult and adult, feeds. Development of the egg takes 24 to 26 hours, and the total life cycle takes 3 to 3½ days. There are six pairs of chromosomes.

*S. celeris* reproduces bisexually. The number of males varies from 15 to 40% of the adults. Females may have over 250 sperm cells in the reproductive tract at one time. They lay an egg every 1½ hours when food is abundant. The first stage has a delicate stylet and valve. The first molt occurs within the egg. The hatching stage has a heavier stylet and valve and feeds, as does the preadult and adult. Only three molts were seen. Egg development takes 18 hours and the total life cycle takes 2½ days. There are three pairs of chromosomes.

*S. oliveirae* reproduces bisexually. Males number 5 to 20% of the adults. The life history is similar to that of *S. celeris*. Females lay an egg every 1½ hours when food is abundant. Development within the egg takes 22 to 24 hours, and the total life cycle takes 2½ to 3 days. There are three pairs of chromosomes.

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# *Tyleptus variabilis* n. sp., with a Key to the Species of *Tyleptus* (Nematoda : Leptonchidae)

M. SHAMIM JAIRAJPURI AND P. A. A. LOOF[1]

In 1964 the first author collected two populations of an undescribed species of the genus *Tyleptus* Thorne, 1939. These populations are regarded conspecific despite some small differences. The specimens were fixed in hot 4% formalin and mounted in glycerin. Dimensions from mounted specimens.

*Tyleptus variabilis* n. sp.

FEMALES (6): L = 0.85–1.26 mm; a = 29–36; b = 4.1–4.9; c = 74–104; V = $^{3-6}29$–$34^{23-30}$.

HOLOTYPE: L = 1.07 mm; a = 29; b = 4.6; c = 74; V = $^{6}33^{27}$.

MALES (3): L = 1.10–1.33 mm; a = 36–38; b = 5.0–5.6; c = 53–65.

DESCRIPTION: Body subcylindrical, female slightly curved in posterior half when killed; male more strongly curved; tapering little posteriorly, more distinctly anteriorly, the width of the lip region being about one-third of the body diameter at the base of the esophagus. Lateral field about one-third of body width; containing numerous granules, apparently without lateral pores except on the tail. Lip region offset by depression, with six well-developed, separate inner liplets, and the usual 16 papillae; in the outer circle the four papillae lie anterior to the six. Amphids cup-shaped, more than half the corresponding body width. Cuticle smooth, with radial striae less numerous than in *T. projectus*. Spear reminiscent of *Dorylaimoides*, irregular in shape, the aperture occupying about one-quarter of its length; in lateral view the extensions appear to be flanged. Cross section shows the dorsal sector to be less heavily sclerotized than the ventrosublateral ones. Length of spear 8.5 $\mu$, extensions 9.5 $\mu$. Esophagus slender, terminating in a pyriform bulb about half as wide as the body; walls of lumen thickened. Excretory pore apparently absent. The nerve ring surrounds the esophagus anterior to its middle. Glandular organs in neck as in *T. parvus*.

FEMALE: Vulva transverse. Anterior gonad rudimentary, its length equal to 1.5 (1.1–1.9) corresponding body widths or 27–63% of the distance from esophagus base to vulva. Posterior gonad normally developed. Uterus nearly three body widths long, separated from the oviduct by a well-developed globoid sphincter. The oviduct extends almost to the flexure; the ovary runs parallel to its distal part; oviduct and ovary are joined by an irregular chamber into which both open, the oviduct opening posterior to the ovary. This chamber may correspond to the "proximal part of the ovary forming a blunt sac" described for *Discolaimus* by Coomans (1965). Though males occur, most females do not contain sperm. Tail broadly rounded, distinctly shorter than the anal body diameter. Rectum slightly longer than, prerectum 3–4 times as long as, anal body width. Intestine without postrectal blind sac, but a conspicuous thickening of the dorsal wall of the rectum (the postanal pulvillus? See Chitwood and Chitwood, 1950) may suggest the presence of one. Core leaving an irregular chamber as in other species. On each lateral side of the tail there is a conspicuous lateral papilla.

MALE: Testes two. Spicules dorylaimid, 34–35 $\mu$ long. Lateral guiding pieces present. Apart from the adanal pair there are three preanal supplements, located 2, 3, and 4 body diameters from the anus. Tail slightly longer and more conoid than in the female. Two caudal papillae.

HOLOTYPE: Female on slide WT-550.

PARATYPES: Five females, three males, and two juveniles with end-on view of head, on slides WT 551–557. Types in the Nematode Collection of the Plantenziektenkundige Dienst, Wageningen, Netherlands; paratypes also in the Zoology Museum, Aligarh Muslim University, Aligarh, India.

TYPE HABITAT AND LOCALITY: Soil near

[1] Aligarh Muslim University, Aligarh, India, and Landbouwhogeschool, Wageningen, The Netherlands, respectively.

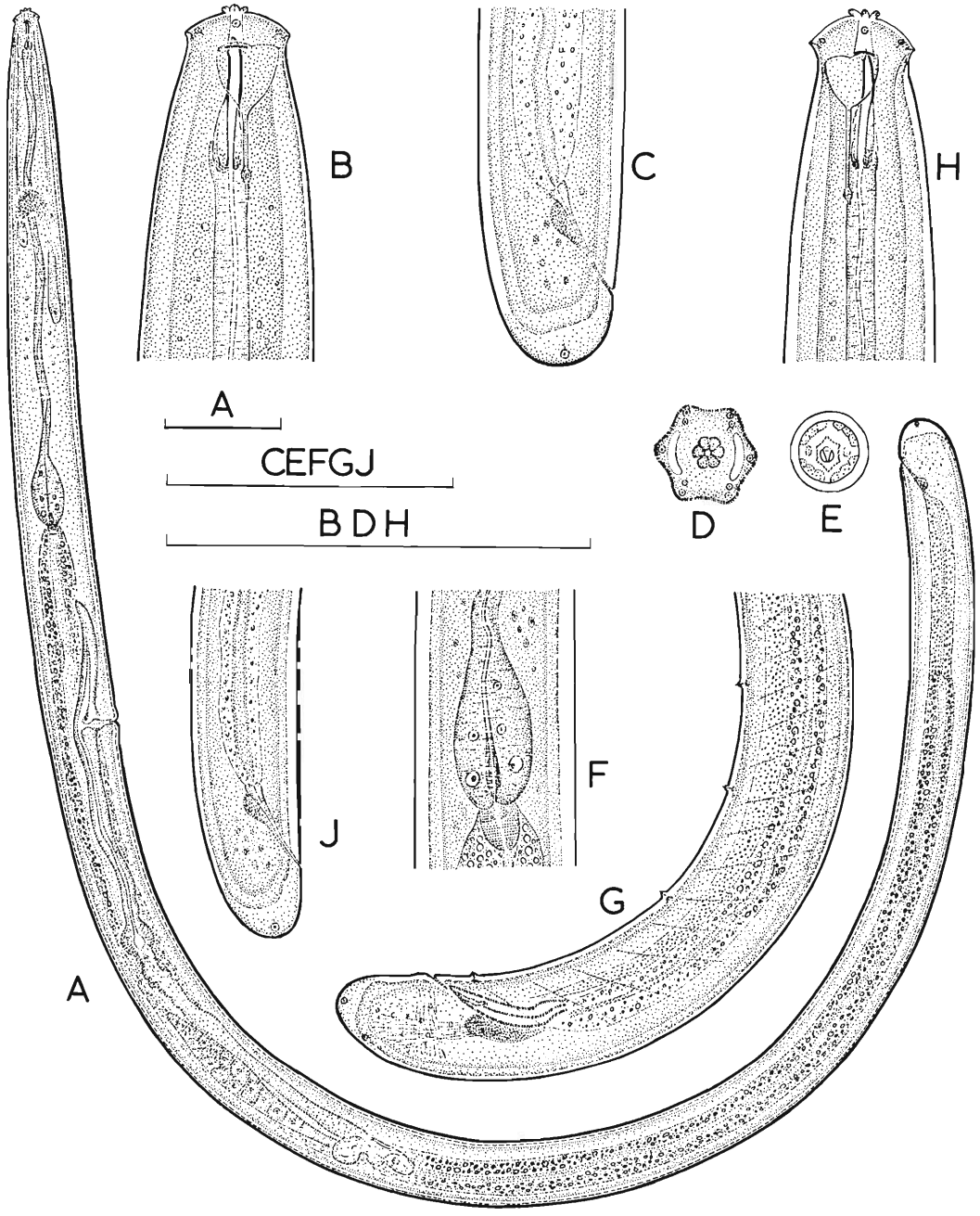


Fig. 1. *Tyleptus variabilis* n. sp. A–G, Bombay population. A, Female; B, Female, head end; C, Female, tail; D, Juvenile, *en face* view; E, Juvenile, cross section at level of spear extensions; F, Female, base of esophagus; G, Male, posterior portion. H–J, Coonoor population. H, Female, head end; J, Female, tail. The scale lines indicate 50 $\mu$.

roots of guava (*Psidium guajava* L.), Bombay, India.

DIAGNOSIS: In possessing distinct inner liplets and refractive radial cuticular striae, *Tyleptus variabilis* comes closest to *T. projectus* Thorne, 1939 and *T. amalgans* Thorne, 1964. From the former it differs by the irregular shape of the spear and the short, flanged extensions; from the latter also by the separate inner liplets.

A second population was obtained from soil at Coonoor, Madras, India.

FEMALES (13): L = 0.95–1.25 mm; a = 35–41; b = 3.9–4.7; c = 74–101; V = $^{5-7}31-35^{19-28}$.

MALES: Not found.

This population differs from the Bombay one in that males may be absent, females are more slender (average value of a = 38 against 32), the body tapers more strongly posteriorly, the lateral field contains only scattered granules, the tail is less broadly rounded, and the gonads are much more prominent. Also there appears to be a slight difference in the shape of the spear tip and the radial striae in the cuticle are more numerous. These differences are for the moment not considered important enough to justify regarding the Coonoor population specifically distinct from the Bombay one.

### KEY TO THE SPECIES OF *Tyleptus*

1. Inner liplets absent, lip region rounded ..... *gymnochilus* Loof, 1964
   Inner liplets present ..... 2
2. Cuticle with refractive radial striae ..... 3
   Cuticle without refractive radial striae ..... 5
3. Liplets amalgamated ..... *amalgans* Thorne, 1964
   Liplets separate ..... 4
4. Spear extensions flanged, short; spear somewhat irregular in shape ..... *variabilis* n. sp.
   Spear extensions long, linear; spear regular in shape ..... *projectus* Thorne, 1939
5. Base of spear extensions widened; vulva not close to esophagus base ..... *striatus* Heyns, 1963
   Base of spear extensions not widened; vulva close to esophagus base ..... *parvus* Jairajpuri, 1965

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# Studies on Freshwater Larval Trematodes. Part XIV. A New Species of a Strigeid Cercaria, *Cercaria allotropicalis*, from Venezuela with a Key to the Related Species

PIR NASIR[1] AND JOSÉ V. SCORZA[2]

In two previous papers Nasir (1964a, 1964b) described two new species of strigeid cercariae, *Cercaria manzanaresensis* and *Cercaria cumanacoensis*, parasites of *Pomacea glauca* (L.), from Rio Manzanares and its various tributaries. The present paper deals with the third new strigeid cercaria, *Cercaria allotropicalis*, found in *Australorbis glabratus* (Say) in the lagoon of Carrizales, Los Teques, Edo. Miranda, Venezuela.

*Cercaria allotropicalis* n. sp. (Figs. 1–3)

DESCRIPTION: Pharyngeate longifurcate distomate furcoccercaria without furcal finfolds. A pair of unpigmented eyespots present. Oral cap with five to seven rows of hooklike spines. Preoral region with a group of seven simple spines arranged in a staggered row. Circumoral spineless area present. No special forward-pointing spines. Behind oral cap, body uniformly spinose. Acetabular spines in a single row. Tailstem and furcae aspinose. A pair of flagellets slightly anterior to excretory vesicle. Tailstem subterminally attached, with 14–17 rows of flagellets. In freshly emerged cercariae, in tailstem, five to six pairs of caudal bodies. Caudal bodies of each pair not exactly opposite to each other and often not extending to extreme posterior region of tailstem. Two pairs of longitudinal muscle bands in tailstem, one dorsal and one ventral. Furcae laterally compressed. Anterior organ not thickened posteriorly. Ventral sucker protrusible. A small prepharynx present. Pharynx weakly muscled. Esophagus not septate or bulbous, narrower than intestinal ceca, dividing anterior to central pair of penetration glands. Intestinal ceca not extending to genital rudiments, in postacetabular region, characteristically divided into three septa; in preacetabular region, ceca with slight undulations. Penetration glands in two pairs, anterolateral to ventral sucker, with uniformly granular contents. Two of these glands central, always transversely elongate, mostly preacetabular, rarely overlying preequatorial region of ventral sucker. Other two glands anterolateral to ventral sucker, distinctly separated from central pair. Two penetration ducts on each side of body. Genital rudiments represented by a cellular mass lying anterior to excretory vesicle. Excretory vesicle tripartite. Main excretory tubes dividing into anterolateral and posterolateral collecting tubules at equatorial level of ventral sucker. Each of main excretory tubes, just before dividing, lined with a ciliated patch. Anterior excretory commissure frequently lying dorsally over preequatorial region of ventral sucker, rarely anterior. No posterior excretory commissure. Caudal excretory duct, in posterior region of tailstem, dividing into two furcal branches which open on lateral aspects of corresponding furcae. Island of Cort present. Flame cell formula: $2[((2+2)+(2+2+(2))] = 20$. Caudal flame cells highly variable in position. Cercariae swimming, apparently aimlessly, with intermittent resting periods. While resting, body and tailstem held vertically downwards, furcae stretched apart and directed upwards. Development in long filiform sporocysts as characteristic of other strigeid cercariae. Measurements (in mm) of freshly emerged cercariae killed by squirting into hot 10% formalin: body, 0.142–0.189 × 0.30–0.049; tailstem, 0.204–0.218 × 0.030–0.038; furcae, 0.196–0.207 long; anterior organ, 0.030–0.040 × 0.019–0.025; ventral sucker, 0.016–0.025 × 0.019–0.030; prepharynx, 0.001–0.003 long; pharynx, 0.008–0.011 in diameter; esophagus, 0.015–0.018 long; postintestinal extent, 0.016–0.026; preacetabular extent, 0.084–0.103.

HOST: *Australorbis glabratus* (Say).

LOCALITY: Laguna de Carrizales, Los Teques, Edo. Miranda, Venezuela.

## DISCUSSION

The other species of longifurcate pharyngeate distomate furcocercous cercariae like *Cercaria*

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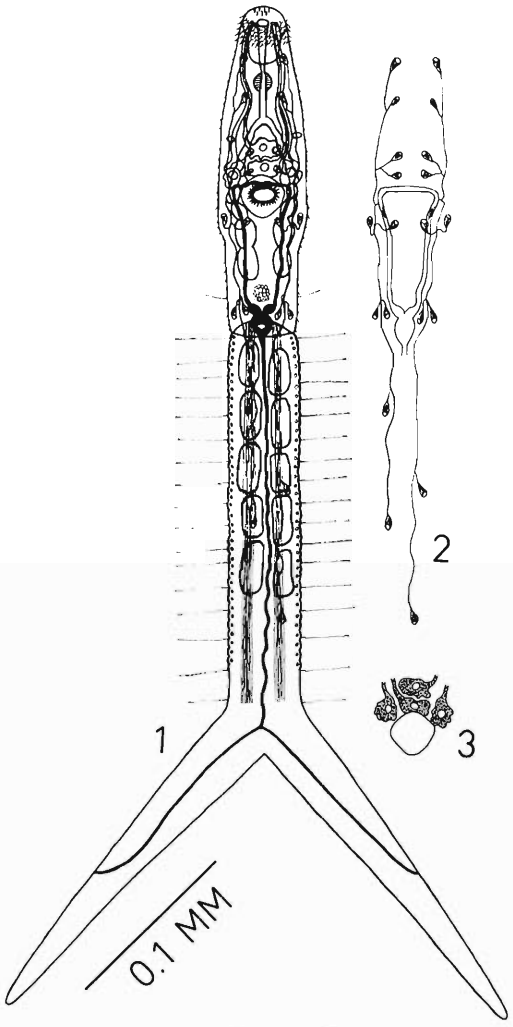


Fig. 1. *Cercaria allotropicalis* n. sp., ventral view.

Fig. 2. *Cercaria allotropicalis*, excretory system.

Fig. 3. Arrangement of penetration glands of *C. allotropicalis* in relation to ventral sucker.

*allotropicalis* characterized with two pairs of penetration glands anterolateral to ventral sucker, an anterior excretory commissure, 20 flame cells in all and without furcal finfolds are: cercaria of *Cotylurus flabelliformis* (Faust, 1917), Cort and Brooks (1928), Olivier and Cort (1941); *Cercaria A* Szidat (1924), Dubois (1929), Wesenberg-Lund (1934) = larva of *Cotylurus cornutus* as described by Szidat (1928, 1929); *C. sanjuanensis* Miller (1927); *C. berghei* Fain (1953); *C. tetraglandis* Iles (1959); and cercaria of *Cotylurus brevis* Dubois and Rausch (1950) as described by Nasir (1960, 1962), syn. *Cercaria helvetica XXXIV* Dubois (1934). To determine the specific identity of *Cercaria allotropicalis* characters like relative size of body and tail, forward-pointing spines, spines on furcae, number of rows of acetabular spines, length of esophagus, point of esophageal bifurcation, straight or tortuous condition of digestive tract, arrangement of penetration glands, and position of the bifurcation of main excretory tubes have proven to be of diagnostic importance. Since the caudal bodies in older specimens of the same species of a cercaria vary not only in numbers, as a result of gradual disintegration, but also disappear in still older specimens, the number and arrangement of these structures is not a worthwhile diagnostic criterion unless freshly emerged larvae have been studied. On several occasions while making observations on *C. allotropicalis*, the senior author was beguiled as to the exact number and arrangement of caudal bodies; sometimes even their presence was overlooked. It was only with freshly emerged specimens that the real pattern of caudal bodies was ascertained. In general, *C. allotropicalis* is readily distinguished from the aforementioned species by the characteristic arrangement of its penetration glands, i.e., the central and transversely elongated position of two glands, by the absence of forward-pointing spines, and by the possession of a single row of acetabular spines. In addition, other points of disagreement with related cercariae are discussed below.

*C. allotropicalis* differs from *C. douglasi* in having a tailstem longer than its body while the tailstem of *C. douglasi* is shorter than its body. The esophagus of the cercaria of *Cotylurus flabelliformis* bifurcates slightly in front of the ventral sucker and the intestinal ceca are straight. In *C. allotropicalis* the esophagus divides a considerable distance anterior to the ventral sucker and the intestinal ceca, in the postacetabular region, are invariably triseptate with slight undulations in the preacetabular region. The point of esophageal bifurcation in *Cercaria A* lies slightly anterior to the ventral sucker, in contrast with *C. allotropicalis*, where the esophagus divides about midway

between the pharynx and the ventral sucker. The "alimentary canal" of *C. sanjuanensis* "is of narrow calibre throughout its extent" in contradistinction to *C. allotropicalis*, the intestinal ceca of which are markedly dilated. Furthermore, in *C. sanjuanensis*, Miller (1927) states there are "four penetration glands between the origin of the ceca and the ventral sucker, three ventrally located and one wedged dorsally between the ceca," an arrangement which is quite distinct from the pattern of the penetration glands encountered in *C. allotropicalis*. The digestive tract of *C. berghei* is sinuous only in the esophageal region which has two characteristic dilatations. The esophagus of *C. allotropicalis* is straight and the intestinal ceca are sinuous. The division of the main excretory tubes in *C. berghei* takes place posterior to the ventral sucker but a similar division in *C. allotropicalis* occurs at the equatorial level of the ventral sucker. In *C. tetraglandis* and cercaria of *Cotylurus brevis* the main excretory tubes also divide posterior to their ventral suckers. Moreover, *C. tetraglandis* possesses a long esophagus, straight intestinal ceca, and its pharynx occupies a position about midway between the anterior organ and the ventral sucker. The esophagus of *C. allotropicalis* is short, its intestinal ceca are tortuous, and the pharynx is almost immediately posterior to the anterior organ.

Marín (1928) described a new cercaria, *Cercaria II*, from *Planorbis guadeloupensis* in Puerto Rico, but the description is too inadequate to render a suitable comparison possible. Faust and Hoffman (1934) reduced *Cercaria II* Marín to the synonymy of a new species named *Cercaria neotropicalis* found in *Australorbis glabratus* in Puerto Rico. *Cercaria neotropicalis* differs from *C. allotropicalis* in having three to four rows of acetabular spines, a long esophagus, straight ceca, differently arranged penetration glands, a different flame cell formula, $2[((2) + (2 + (2))] = 12$, and there is no anterior excretory commissure.

### KEY TO THE SPECIES RELATED TO *C. allotropicalis*

1.(2) Two of penetration glands central and transversely elongated ..... *Cercaria allotropicalis*

2.(1) Two of penetration glands not transversely elongated ..... 3

3.(4) Pharynx about midway between anterior organ and ventral sucker ..... *Cercaria tetraglandis*

4.(3) Pharynx not midway between anterior organ and ventral sucker .... 5

5.(6) Esophagus with two dilatations .. ..... *Cercaria berghei*

6.(5) Esophagus without two dilatations 7

7.(8) Esophagus dividing at level of penetration glands ..... *Cercaria sanjuanensis*

8.(7) Esophagus not dividing at level of penetration glands ..... 9

9.(12) Esophagus short, not extending more than halfway between pharynx and ventral sucker ..... 10

10.(11) Tailstem longer than body ..... Cercaria of *Cotylurus brevis*

11.(10) Tailstem shorter than body ..... *Cercaria douglasi*

12.(9) Esophagus long, extending more than halfway between pharynx and ventral sucker ..... 13

13.(14) Three rows of acetabular spines ..... *Cercaria A*

14.(13) Four to five rows of acetabular spines ..... Cercaria of *Cotylurus flabelliformis*

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## The Molting Process in Species of *Seinura* (Nematoda : Aphelenchoididae)[1]

HELEN CAROL HECHLER AND D. P. TAYLOR[2]

The investigation on molting reported here was done primarily on *Seinura oxura* (Paesler, 1957) J. B. Goodey, 1960, and all the photographs are of this species. Supplementary observations on *S. celeris* Hechler in Hechler and Taylor, 1965, *S. oliveirae* (Christie, 1939) J. B. Goodey, 1960, *S. steineri* Hechler in Hechler and Taylor, 1965, and *S. tenuicaudata* (de Man, 1895) J. B. Goodey, 1960 show that these species molt in the same manner. Preliminary results have been published (Hechler, 1966).

[1] Portion of a Ph.D. thesis submitted by the senior author to the Graduate College, University of Illinois, Urbana.

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### MATERIALS AND METHODS

Nematodes were extracted from stock cultures maintained as described by Hechler and Taylor (1965). Nematodes in the final molt were selected and placed in a small drop of water on a microslide, and a drop of cooled molten 2% water agar was added. A cover slip was applied and pressed down gently to align the nematodes in a horizontal plane throughout their length. Observations were made using 40× and 90× oil-immersion objectives. It was often possible to observe a single individual throughout the entire molting process before the preparation dried. Photographs were made with a Zeiss photomicro-

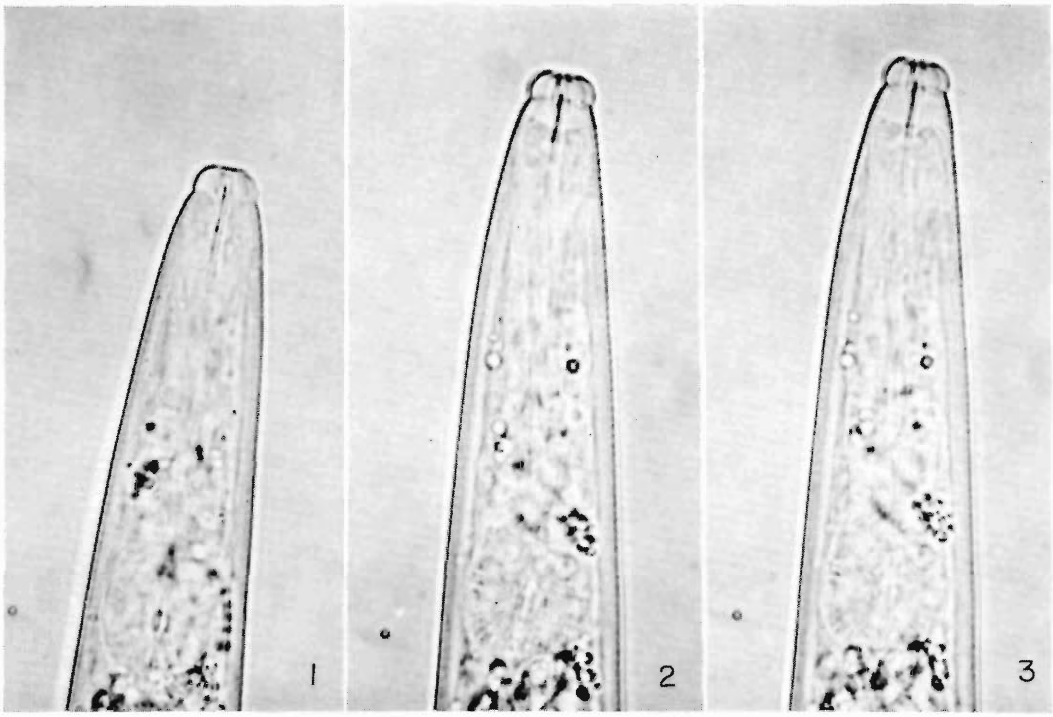



Fig. 1. Early in molt. Lips just forming, hyaline cavity visible.

Fig. 2. After 1 hour. Lips retracted from head; posterior stylet, esophageal lumen, and valves much less sclerotized.

Fig. 3. After 2 hours. Sclerotization of new stylet just beginning.

scope, and when nematodes were moving flash illumination was used. Time-lapse motion pictures were also made.

The time intervals involved are reported here only to give an idea of the proportion of time each phase of the molting process required, since all observations were made with the nematodes on the stage of the microscope rather than under controlled temperature conditions.

## MOLTING

At the beginning of each molt after hatching active motion ceases and the nematode lies straight, with only occasional back-and-forth movements and twitching of the head. Gradually even these movements cease. A flask-shaped hyaline cavity appears surrounding the anterior section of the stylet, with its base a short distance behind the junction of the conical and cylindrical sections, and the newly developing lips begin to differentiate just behind the junction of the lip region and body (Fig. 1). First the lining of the esophageal lumen, and then the valve plates in the metacorpus and the posterior part of the stylet, gradually become less refractive. The head begins to retract from both the old cuticle and the anterior part of the stylet, and occasionally strands close to the stylet connecting the head with the old cuticle are visible. By the time one half of the anterior part of the stylet has emerged from the newly developing oral aperture, the posterior part of the cylindrical section of the stylet has disappeared except for a short piece just posterior to the conical section which finally emerges from the lips attached to the conical section, and a short portion anterior to the guiding rings which appears as two faint lines. Occasionally even this section entirely

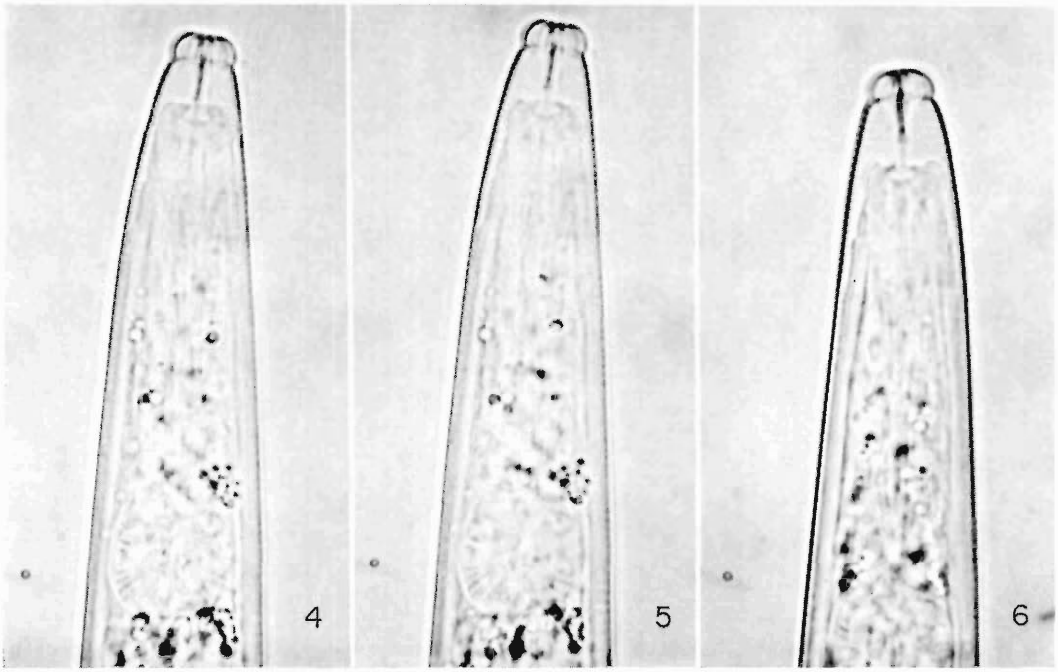



Fig. 4. After 2½ hours. Further sclerotization of stylet anterior to guiding rings.

Fig. 5. After 2¾ hours. Further sclerotization of stylet.

Fig. 6. After 3 hours. Conical section and portion of cylindrical section of old stylet nearly clear of oral aperture. Sclerotization posterior to guiding rings.

disappears. At this time the guiding rings are also hardly visible (Fig. 2). Sclerotization of the new stylet begins immediately. By the time the conical section of the old stylet has emerged 80% of its length, the new guiding rings are faintly visible and new sclerotization is evident just anterior to them (Fig. 3). As the head continues to retract sclerotization of the stylet proceeds anteriad (Figs. 4, 5, 6). The short portion of the stylet shaft which is pulled out with the conical section becomes pliable and less refractive by the time it is completely free of the oral aperture (Fig. 6). A short portion of the lining of the conical section is also pulled out. The guiding rings become more and more prominent and sclerotization of the new stylet begins to advance posterior to them (Fig. 7). Meanwhile, as the new lips continue to develop, the hyaline cavity becomes smaller and smaller and finally disappears (Figs. 6, 7, 8). At this time the conical section of the new stylet is visible to its apex. Sclerotization of the new stylet continues posteriad and the valve in the metacorpus and the esophageal lumen become more noticeable (Fig. 8). In *S. oliveirae*, a species having a knobbed stylet, the knobs are the last part of the stylet to become visible. All sclerotized structures continue to become more and more refractive until active back-and-forth motion of the nematode begins, 5 to 6 hours after the beginning of the molt in *S. oxura* (Fig. 9).

Sclerotization of the spicules in the male tail begins about the time the head is completely separated from the stylet, starting with the ventral limb (Figs. 10, 11), then the posterior dorsal limb (Fig. 12), then the transverse bar connecting the apex and rostrum, and finally the anterior dorsal limb and the apex (Fig. 13). A hyaline cavity anterior and ventral to the spicule primordium becomes progressively more narrow and finally disappears as the spicules develop.

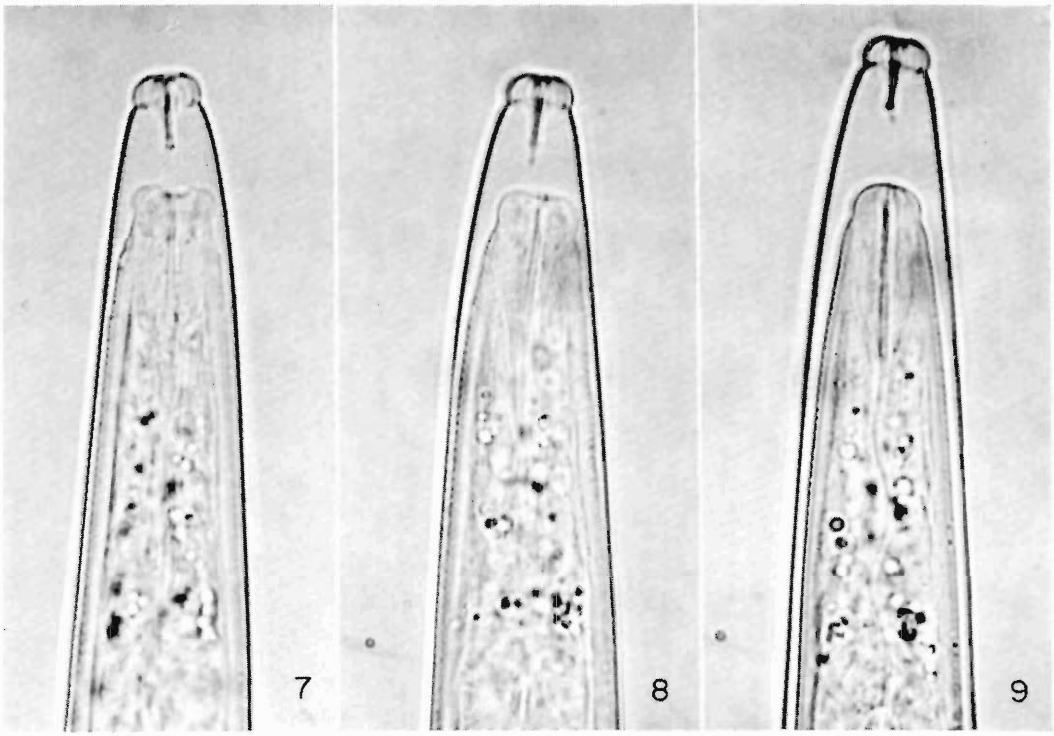
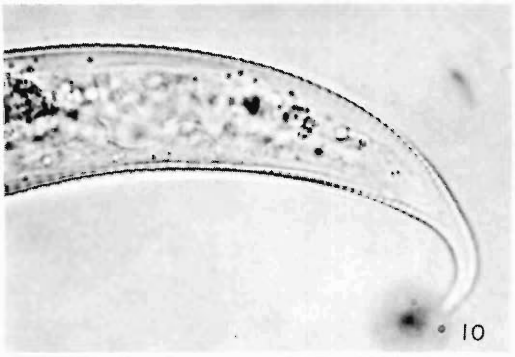



Fig. 7. After 3½ hours. Apex of stylet visible. Hyaline cavity much smaller.
Fig. 8. After 4 hours. Anterior section of stylet well developed, lips completely developed.
Fig. 9. After 5 hours. Sclerotization of stylet, lumen, and valve nearly complete.

During the time that sclerotization of the stylet is proceeding and the head is retracting from the old lips, occasional contractile twitching movements can be seen in the head region. By the time the stylet has cleared the oral aperture the movement has become less jerky, and finally the head begins to swing from side to side. At this time contractile twitching movements begin throughout the remainder of the body, which separate the two cuticles. After they are separated the nematode begins to move actively back and forth within the old cuticle and occasionally to rotate in the longitudinal plane. During periods when the nematode head is farthest from the lips of the old cuticle the metacorpus pulsates as in feeding, and often the stylet is protruded far enough for its aperture to be exposed (Fig. 14). Although no flow of material through the stylet, esophagus, and intestine has been detected, material already present in the intestine moves posteriad as though it were being displaced by fluid entering the intestine. The periods of pulsation last about 30 seconds. When pulsation stops the nematode begins to move forward within the old cuticle, and a copious flow of granular material moves from the esophageal gland lobe into the anterior part of the metacorpus. It has never been seen to flow forward through the esophageal lumen and stylet and out into the cavity between the cuticles. When the nematode is in the anterior part of the cuticle it often pushes the old stylet out of the oral aperture (Fig. 15), or it may merely push it to one side. The stylet has never been seen to protrude when the nematode is in the anterior position. The nematode lengthens until it completely fills the old cuticle, but it continues flexing movements which withdraw the head from the anterior end of the cuticle. The



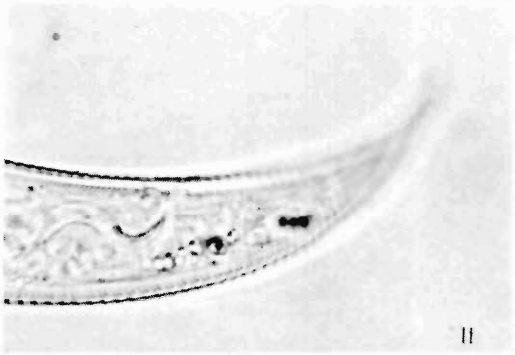
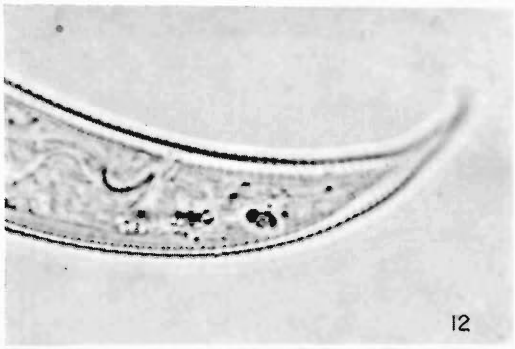




Fig. 10. Early spicule development showing spicule primordium, hyaline cavity anterior to it.
Fig. 11. Sclerotization of ventral limb.



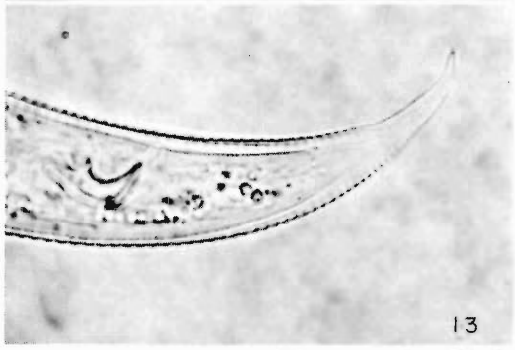




Fig. 12. Sclerotization of posterior dorsal limb.
Fig. 13. Spicule well developed.

old cuticle remains rigid until, after 4 or 5 hours of back-and-forth activity, it finally becomes pliable and often loosens around the body. The nematode head continues to move back in the old cuticle occasionally, but most of the time the head is now turned to one side, pressing against the old cuticle just behind the lips (Fig. 16). The head rotates in this position for 15 to 30 minutes and eventually breaks through the old cuticle just behind the lips and the nematode moves out (Fig. 17). The stylet has never been seen to protrude at this time or function in breaking through the old cuticle.

## DISCUSSION

In *Seinura* spp. sclerotization of the stylet apparently begins just behind the junction of the anterior conical and posterior cylindrical sections, advances anteriad to the conical section, and before this part is entirely formed proceeds posteriad also. The point of separation between the two sections is never clearly seen until the whole stylet is well developed. This is in contrast to stylet development in *Criconemoides xenoplax* Raski, 1952, where at the first molt the anterior section of the stylet develops well ahead of the posterior section (Seshadri, 1964), and also in *Ditylenchus destructor* Thorne, 1945, where sclerotization of the conical section is well advanced before it begins in the cylindrical section and the two sections are easily seen as separate parts of the stylet (Anderson and Darling, 1964). Also the three cutinized rings reported by the latter authors were not seen in *Seinura* spp.

In a species of *Xiphinema* the entire lining of the basal portion of the stylet is pulled out during the molt (Coomans and *de Coninck*, 1963). In *Caenorhabditis briggsae* (Dougherty and Nigon, 1949) Dougherty, 1953 the linings of both the buccal capsule and the esophagus are shed (Jantunen, 1964). In *Seinura* spp. only a very short strand of the stylet shaft lining is shed. However, apparently a short portion

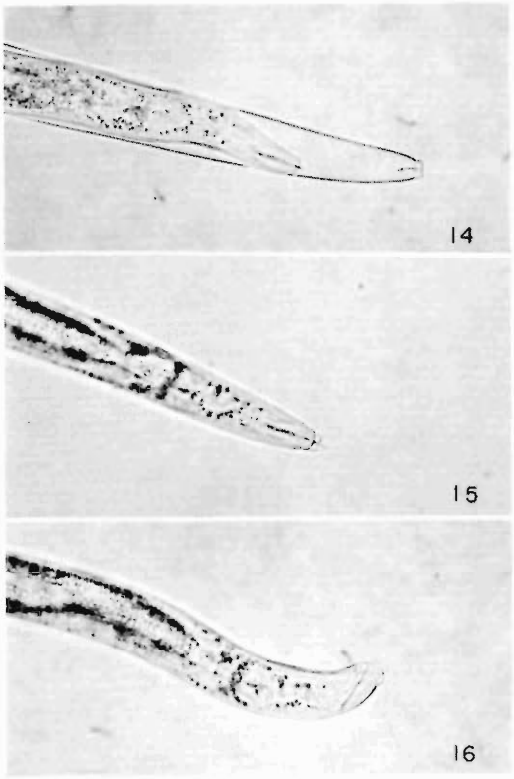



Fig. 14. Nematode moved back within cuticle, metacorpus pulsating, stylet protruded.

Fig. 15. Nematode moved forward within cuticle, cuticle rigid.

Fig. 16. Cuticle pliable, nematode pressing against it with lips.

of the cylindrical section of the stylet is shed along with the conical section.

In *Ditylenchus destructor* the amphids and sensillae pouches are very conspicuous during the molt, and strands leading from the amphids connect the head to the old cuticle (Anderson and Darling, 1964). Connecting strands near the stylet are also visible occasionally in molting *Seinura* specimens but they are so inconspicuous that it is difficult to be certain whether they are from the amphids, the cephalic papillae, or the lining of the stoma.

Pulsation of the bulb as in feeding, and accompanying backward movement of intestinal content, suggests that fluid is entering the intestine and displacing material already present in

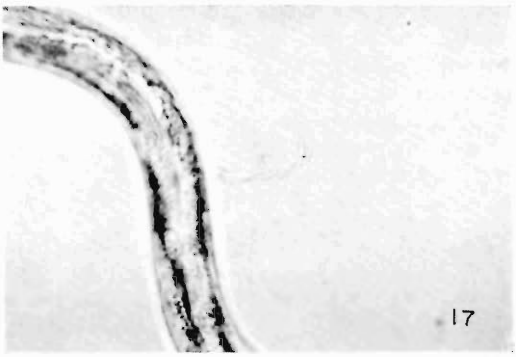



Fig. 17. Nematode emerging from cuticle. Head section of old cuticle with cast stylet section at side.

the lumen. This suggestion is further supported by the elongation of the nematode at the same time, a process which would presumably be aided by fluid creating an internal pressure.

In *Haemonchus contortus* (Rudolphi, 1803) and other nematodes parasitic in animals, Sommerville (1957) showed that loosening of the cast cuticle was initiated by a fluid produced at a point less than 130 $\mu$ from the anterior end of the animal. This caused formation of a refractive ring in the cuticle 19 $\mu$ from the anterior end. Eventually this 19 $\mu$ tip of the old cuticle was lost and the nematode escaped. The cap-like tip of the old cuticle is also lost at each molt in *Caenorhabditis briggsae* (Jantunen, 1964). No such refractive ring was seen in molting *Seinura* specimens. However, copius flow of material from the gland was seen, and this was followed by softening of the old cuticle. It is possible that the material produced in the gland escapes through the stylet, even though the flow was not detected, and that this material acts to soften the old cuticle.

Hooper (1961) suggested that *Longidorus* spp. emerge from the old cuticle by piercing it with the stylet. Puncture of the cuticle by the stylet was not seen in molting *Seinura*; instead the old cuticle seemed to be torn, often at one of the striae, by pressure of the lips against it. Wallace (1963) suggested that movement of nematodes against abrasive soil particles may help to shed the cuticle, and this theory is supported by the observations of van Gundy (1958) on *Tylenchulus semipenetrans*, Cobb, 1913, and Linford and Oliveira (1940) on

*Rotylenchus reniformis* Linford and Oliveira, 1940, who reported that nematodes in water tend to retain the cast cuticle. *Seinura* spp. also retain the cuticle longer in water than in agar, although they eventually emerge. Soil particles or agar may speed the process of emergence, but they are not essential.

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## Studies on Freshwater Larval Trematodes. Part XIII. Some New Species of Cercariae from Venezuela

PIR NASIR AND AMADO ACUÑA CEDEÑO[1]

During August 1964, while on a scientific expedition to Territorio Delta Amacuro, the senior author collected three species of freshwater snails: *Pomacea urceus* (Müller), 417 specimens; *P. glauca* (Linné), 379 specimens; and *Neritina* sp. (?), 62 specimens. Three individuals of *P. urceus* were infected with a polyadenous xiphidiocercaria while six others were discharging a microcotylous xiphidiocercaria. After comparison with the known related species, these proved to be new and are named, respectively, *Cercaria urceus* and *Cercaria homocotylea*.

1. *Cercaria urceus* n. sp. (Figs. 1–2)

DESCRIPTION: Polyadenous xiphidiocercaria. Body spinose, beset with 12 rows of flagellets. Tail aspinose, subterminally attached. Caudal pockets lined with needlelike spines. Suckers without special spines. Oral sucker larger than ventral sucker. Stylet without a basal bulb, shape of stylet as in Fig. 2. Prepharynx, pharynx, and esophagus present. Esophagus dividing about halfway between two suckers. Intestinal ceca not extending beyond anterolateral aspects of ventral sucker. Penetration glands in eight pairs, with uniformly granular contents. Shape of excretory vesicle as shown in Fig. 1. Main excretory tubes arising subterminally. Division of main excretory tubes at postequatorial level of ventral sucker. No caudal excretory duct. Flame cell formula: $2[(3 + 3 + 3) + (3 + 3 + 3)] = 36$. Development in sausage-shaped sporocysts. Measurements (in mm) of freshly emerged cercariae killed by squirting in hot 10% formalin: body, 0.386–0.469 × 0.153–0.201; tail, 0.273–0.364 × 0.039–0.086;

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The authors are greatly indebted to Dr. J. P. E. Morrison, Smithsonian Institution, Washington, D.C., USA, for the identification of the snails involved in this study.

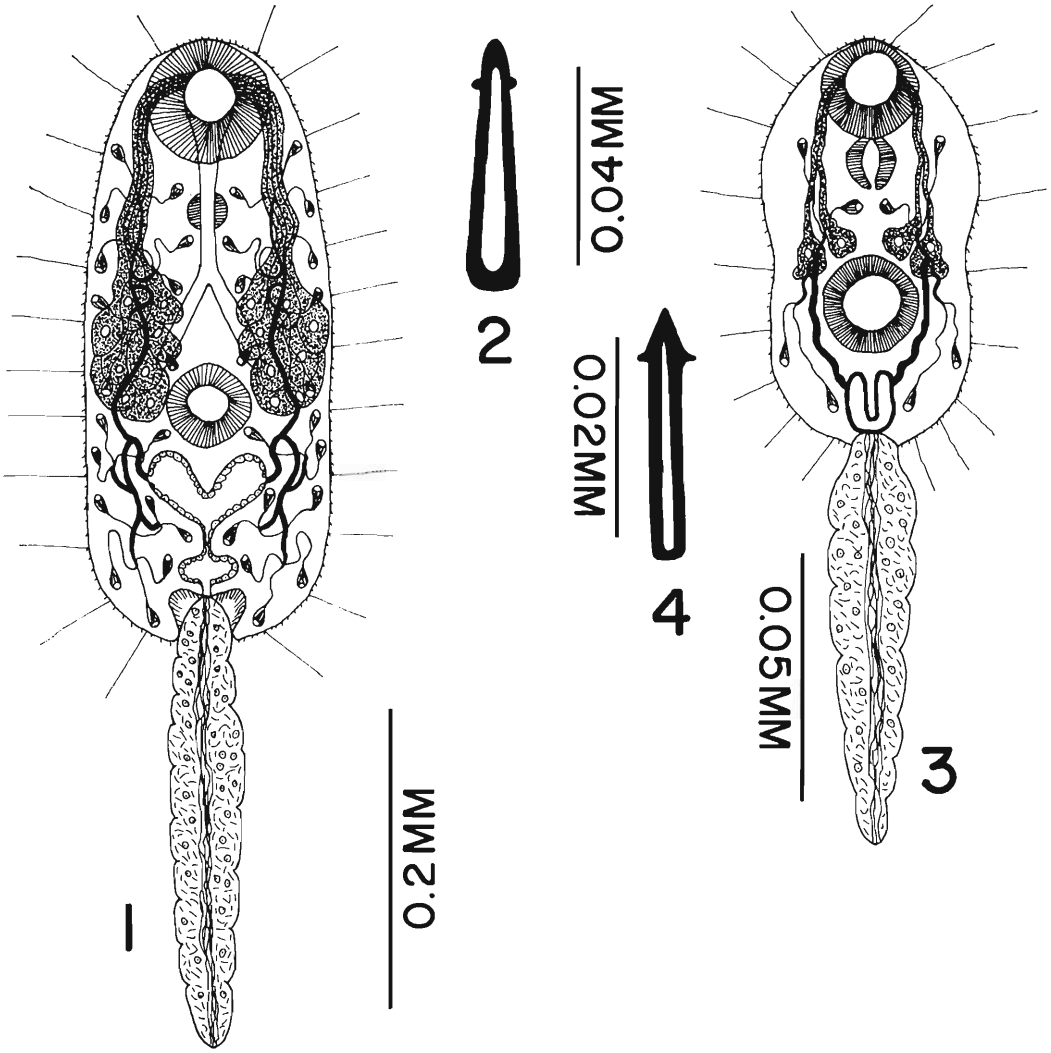


Fig. 1. *Cercaria urceus* n. sp., ventral view.
Fig. 2. *Cercaria urceus*, ventral view of stylet.
Fig. 3. *Cercaria homocotylea* n. sp., ventral view.
Fig. 4. *Cercaria homocotylea*, ventral view of stylet.

oral sucker, 0.080–0.093 in diameter; ventral sucker, 0.051–0.058 in diameter; prepharynx, 0.013–0.020 long; pharynx, 0.019–0.028 in diameter; esophagus, 0.016–0.026 long; stylet, 0.048–0.054 long; width of shoulder, 0.008–0.010; width at base of stylet, 0.010–0.011.

2. *Cercaria homocotylea* n. sp. (Figs. 3–4)

DESCRIPTION: Microcotylous xiphidiocercaria. Body spinose, with nine rows of flagellets. Tail aspinose, subterminally attached. No caudal pockets. Suckers almost isodiametric. Stylet without a basal bulb, shape of stylet

shown in Fig. 4. Prepharynx and esophagus absent. Pharynx present. Two penetration glands, with uniformly granular contents on each side of body, anterolateral to ventral sucker. Two penetration ducts on each side of body. Excretory vesicle U-shaped. Main excretory tubes arising subterminally. Division of main excretory tubes anterior to anterior border of ventral sucker. No caudal excretory duct. Flame cell formula: 2[(2) + (2)] = 8. Development in anteroposteriorly elongated sporocysts. Measurements (in mm) of freshly emerged cercarie killed by squirting in hot 10% formalin: body, 0.080–0.088 ×0.043–0.047; tail, 0.072–0.088 × 0.016–0.019; oral sucker, 0.020–0.027 in diameter; ventral sucker, 0.018–0.025 in diameter; pharynx, 0.010–0.012 in diameter; stylet, 0.020–0.025 long; width of shoulder, 0.006; width of shaft, 0.003.

## DISCUSSION

*Cercaria isocotylea* Cort (1914); *C. polyadena* Cort, 1914, Cort (1919) = larva of *Plagiorchis proximus* as described by McMullen (1937); *C. micropharynx* Faust (1917); *C. indicae XVII* Sewell (1922); *C. reptans* Uribe (1925); cercaria of *Dasymetra conferta* as described by McCoy (1928); cercaria of *Pneumatophilus variabilis* as described by McCoy (1928); *C. helvetica XXX* Dubois (1929); *C. helvetica V (= VII)* Dubois (1929); *C. helvetica IV* Dubois (1929); *C. helvetica XXVII* Dubois (1929); *C. sudanensis No. 2* Archibald and Marshall (1931); cercaria of *Zeugorchis syntomentera* as described by Ingles (1933); *C. acanthocoela* Miller (1935); *C. cystonchnoides* Miller (1935); *C. concavocorpa* Sizemore (1936) = larva of *Tetrapapillotrema concavocorpa* as described by Ralph (1938); cercaria of *Plagiorchis muris* as described by McMullen (1937); cercaria of *P. micracanthos* as described by McMullen (1937); *C. holthauseni* Rankin (1939); *C. macrostyla* Byrd (1940); *C. brevicauda* Byrd and Reiber (1940); *C. nolfi* Brooks (1943); *C. aalbui* Brooks (1943); *C. eta* Brooks (1948); *C. goodmani* Najarian (1952) = larva of *Plagiorchis goodmani* Najarian (1961); cercaria of *P. (M.) megalorchis* Rees (1952); *C. blukwa* Fain (1953); *C. ramanujami* Peter (1955); cercaria of *Opisthoglyphe locellus* as described by Macy and Moore (1958); *C. edgbastonensis* Nasir (1960); *C. baldai* Nasir (1964); and cercaria of *Plagiorchis dilimanensis* Velasquez (1964) are the other polyadenous xiphidiocercariae in which the contents of penetration glands like *C. urceus*, apparently, are not differentiated into finely and coarsely granular cytoplasmic inclusions and which possess a total number of eight or almost eight pairs of penetration glands. Of these, *C. polyadena*, *C. indicae XVII*, cercaria of *Dasymetra conferta*, *C. helvetica V (= VII)*, *C. helvetica IV*, *C. helvetica XXX*, *C. helvetica XXVII*, *C. acanthocoela*, *C. cystonchnoides*, *C. concavocorpa*, *C. holthauseni*, *C. macrostyla*, *C. eta*, *C. goodmani*, cercaria of *Plagiorchis (M.) megalorchis*, *C. blukwa*, cercaria of *Opisthoglyphe locellus*, *C. edgbastonensis*, *C. baldai*, and cercaria of *Plagiorchis dilimanensis* all have 36 flame cells. In addition, *C. helvetica IV*, *C. helvetica XXX*, *C. holthauseni*, *C. macrostyla*, *C. eta*, *C. goodmani*, cercaria of *P. (M.) megalorchis*, *C. blukwa*, cercaria of *O. locellus*, *C. edgbastonensis*, *C. baldai*, and cercaria of *P. dilimanensis* are characterized with exactly eight pairs of penetration glands and, therefore, stand a close chance of comparison with *C. urceus*.

Among other characters to be discussed below, *C. urceus* differs from all these species in having a considerably larger stylet, the shape of which is very different.

*C. helvetica IV*, *C. helvetica XXX*, *C. eta*, cercaria of *P. (M.) megalorchis*, *C. blukwa*, and cercaria of *P. dilimanensis* have intestinal ceca extending to the posterior ends of their bodies whereas in *C. urceus* the intestinal ceca are limited to the anterolateral aspects of its ventral sucker. *Cercaria helvetica IV* and *C. helvetica XXX* both possess a long esophagus in contrast with the short esophagus of *C. urceus*. Furthermore, *C. helvetica IV* contains characteristic refractile granules which are lacking altogether in *C. urceus*. There are also refractile granules in *C. eta*. In cercaria of *P. (M.) megalorchis* and *C. blukwa* the main excretory tubes arise terminally from the corresponding arms of their excretory vesicles and both have caudal excretory ducts in comparison with *C. urceus*, where the main excretory tubes arise subterminally and there is no caudal excretory duct. The main excretory tubes of cercaria of *P. dilimanensis* divide at the anterior border of its ventral sucker but the corresponding division in *C.*

*urceus* occurs at post equatorial level of the ventral sucker.

The stylet of *C. holthauseni* as well as that of *C. macrostyla* is furnished with a basal bulb which is absent in that of *C. urceus*. Moreover, in *C. holthauseni* the main excretory tubes arise terminally and in *C. macrostyla* the arms of the excretory vesicle encompass its ventral sucker in contradistinction to a condition met with in *C. urceus*, i.e., its main excretory tubes originate subterminally and the arms of the excretory vesicle do not even extend to its ventral sucker.

The intestinal ceca of *C. goodmani* are two short appendages, not extending to the ventral sucker, and its tail is spinose. The intestinal ceca of *C. urceus* extend to the ventral sucker and its tail is aspinose.

The cercaria of *Opisthoglyphe locellus* differs from *C. urceus* by the fact that its esophagus is long and the main excretory tubes arise terminally.

*C. baldai*, found in *Marisa cornuarietis* from Quebrada Seca, a freshwater stream near Güiria, has the following features in common with *C. urceus*: body furnished with spines, presence of flagellets on body, aspinose tail, subterminal attachment of tail, presence of spines in caudal pockets, pattern of digestive system, position of penetration glands, number of penetration ducts, shape and extent of excretory vesicle, subterminal origin of main excretory tubes and the point of bifurcation of main excretory tubes. However, *C. urceus* is considerably larger, has a larger and differently shaped stylet, and lacks the refractile globular bodies present in *C. baldai*. Although these two species have been described from the same country, they occur in distant localities and infect different intermediate hosts.

As far as the identification of *Cercaria homocotylea* is concerned there are only six species of microcotylous xiphidiocercariae with two pairs of penetration glands, about which sufficient structural details are known. These are: *Cercaria cellulosa* as described by Wesenberg-Lund (1934), *C. indicae LVII* Sewell (1922), *C. paucadena* Faust (1926), *C. cystorhysa* Miller (1935), *C. meniscadena* Miller (1935), and *C. cumanensis* Nasir (1965). *Cercaria homocotylea* differs from these in having almost isodiametric suckers, in the shape of its stylet, and, where the size of stylet is known, in having a larger stylet.

The contents of the penetration glands in *Cercaria cystorhysa* and *C. meniscadena* are differentiated into fine and coarse granules, and, therefore, these two species are readily separated from *C. homocotylea* where the granules are uniform. *Cercaria cellulosa* is a larger species and its excretory vesicle is faintly bicornuate in contrast with *C. homocotylea*, which is smaller and has a U-shaped excretory vesicle. *Cercaria paucadena* is also larger and has prepharynx and esophagus, *C. homocotylea* lacks both prepharynx and esophagus. *Cercaria indicae LVII* and *C. cumanensis* are the only other related species with known flame cell formula and this arrangement, i.e., $2[(2) + (2)] = 8$, is similar to that of *C. homocotylea*. However, in *C. indicae LVII* the main excretory tubes, which arise terminally, divide at equatorial level of the ventral sucker and there is a caudal excretory duct. The main excretory tubes of *C. homocotylea* arise subterminally, divide anterior to the anterior level of the ventral sucker, and there is no caudal excretory duct. *Cercaria cumanensis* found in *Marisa cornuarietis*, San Juan de Marcarapana, near the Universidad de Oriente, differs from *C. homocotylea* in that it possesses an esophagus, the main excretory tubes arise terminally, and the excretory vesicle is Y-shaped.

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# Effects of Cold Storage on the Infectivity of Third-Stage Larvae of the Intestinal Worm, *Trichostrongylus colubriformis* (Giles, 1892) Ransom, 1911[1]

HARRY HERLICH[2]

Maintenance of cultures of parasitic nematodes of ruminants requires repeated passages of the organisms through successive numbers of parasite-free animals. This is both costly and time consuming. Storage of infective larvae is feasible; such techniques as keeping larvae on sphagnum moss at room or refrigerator temperature or in water at refrigerator temperature are used. For some years, I have routinely stored the infective larvae of various species of trichostrongyles on moistened filter paper in jars in a refrigerator kept at 4 C. Such larvae have been used to maintain isolates of the several species of nematodes, but uncertainty concerning the effects of this storage on larval infectivity and potency to produce disease has prevented their use for experimental purposes. This study was conducted, therefore, to determine the effects of cold storage on the infectivity of the intestinal worm, *Trichostrongylus colubriformis*, a common parasite of sheep and cattle.

## MATERIALS AND METHODS

Infective larvae were isolated from cultures made up of sphagnum moss and feces collected from a lamb experimentally infected with *T. colubriformis*. One hundred thousand larvae were inoculated into each of nine vials containing pieces of filter paper. Sufficient water (1 ml) was used to moisten the paper completely but to preclude water standing on the bottom of the vial. The vials were stored in a refrigerator kept at 4 C. At the same time, 50,000 freshly isolated larvae were used to establish an index of infectivity. Each of 10 male guinea pigs, 2 months old, was inoculated orally with 5,000 larvae. All guinea pigs were killed 12 days later, and worms were recovered by cutting the small intestine into one-inch long strips and exposing them to pepsin-HCl acid solution overnight. Numbers of worms were determined by counting those present in a 10-percent sample; where less than 100 were indicated, total counts were made.

## RESULTS AND DISCUSSION

Ninety to 100% of the larvae isolated from cultures stored from 0 to 12 months were motile within 1 hr of being exposed to room temperature (20–23 C), whereas only 60 and 8% of those stored for 15 and 18 months, respectively, were motile. As each vial had originally been seeded with 100,000 larvae and only 50,000 were required to infect each group of guinea pigs, the inocula for all animals receiving larvae stored from 0 to 15 months consisted of 5,000 motile larvae plus from 0 to 3,333 nonmotile larvae. On the other hand, the inocula for the guinea pigs receiving larvae stored for 18 months contained only 800 motile larvae.

The numbers of worms recovered from the seven groups of guinea pigs are shown in Table 1. The differences between the mean number of worms recovered from guinea pigs inoculated with freshly isolated larvae and those inoculated with larvae stored for 3, 6, 9, and 12 months, respectively, are not significant. The mean numbers of worms recovered from all of these groups differed very significantly from the mean numbers recovered from the guinea pigs inoculated with larvae stored for 15 and 18 months.

No deaths occurred in any groups of guinea pigs, probably because the animals were killed too soon for fatal infections to develop. However, at least half of each group inoculated with larvae stored no longer than 12 months developed diarrhea and became emaciated, indicating that the larvae were still capable of causing enteritis. None of the guinea pigs receiving larvae stored for 15 and 18 months showed any signs of disease.

Besch (1964) found that the infective larvae of *Cooperia punctata*, another nematode para-

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TABLE 1. Numbers of *Trichostrongylus colubriformis* recovered from 7 groups of 10 guinea pigs, each inoculated with 5,000 infective larvae stored for different lengths of time at 4 C.

| Guinea pig | Length of storage time in months | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| 1 | 960 | 380 | 510 | 1,090 | 840 | 130 | 1 |
| 2 | 480 | 1,420 | 450 | 890 | 390 | 190 | 0 |
| 3 | 1,680 | 760 | 1,730 | 610 | 660 | 280 | 0 |
| 4 | 1,620 | 910 | 1,020 | 240 | 930 | 430 | 1 |
| 5 | 630 | 480 | 1,410 | 1,200 | 1,040 | 330 | 0 |
| 6 | 840 | 1,600 | 870 | 1,370 | 1,770 | 160 | 0 |
| 7 | 1,200 | 1,100 | 1,620 | 650 | 900 | 240 | 0 |
| 8 | 720 | 740 | 880 | 930 | 1,620 | 300 | 1 |
| 9 | 810 | 690 | 1,110 | 430 | 960 | 260 | 0 |
| 10 | 630 | 570 | 390 | 1,230 | 1,260 | 350 | 0 |
| Average | 957 | 865 | 999 | 864 | 1,037 | 267 | 0.3 |

site of cattle, retained their infectivity for 15 months when stored on moistened sphagnum moss at 6 to 8 C; however, he concluded this from the fact that the prepatent period of infection was unaffected and that patent infections were established. Based on these criteria, *T. colubriformis* larvae may remain infective for as long as 18 months in cold storage, but the low numbers recovered indicated that viability and infectivity were significantly reduced after 15 and 18 months of storage.

## SUMMARY

When guinea pigs were used as experimental hosts to test the effects of cold storage on the infectivity of third-stage larvae of *Trichostrongylus colubriformis*, there was no apparent effect on larvae stored as long as 12 months at 4 C; whereas, the viability and infectivity of larvae stored for 15 and 18 months were affected detrimentally.

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# Amino Acid Composition of Some Strongyle Parasites of Cattle[1]

HARRY HERLICH[2]

As part of a broad study on the relationship of the three species of *Cooperia* that commonly parasitize cattle in the United States, previous reports have dealt with the comparative aspects of their life history, pathogenicity, and immunogenicity (Herlich, 1965a, b, c). This is a report on the amino acid composition of these nematodes and of other strongyles parasitic in cattle.

## MATERIALS AND METHODS

Infective larvae of *Cooperia punctata*, *C. pectinata*, *C. oncophora*, *Ostertagia ostertagi*, *Trichostrongylus axei*, *T. colubriformis*, and *Oesophagostomum radiatum* were isolated from cultures of sphagnum moss and feces collected from bovines with experimentally produced monospecific infections. The larvae were then freed of debris and sterilized by the method of Cryan (1963).

Adults of the three species of *Cooperia* were harvested by the technique of Herlich (1964). They were freed of debris and washed in six successive baths in sterile distilled water, three baths in water containing streptomycin and penicillin, and three more baths in sterile water. The worms were then blotted lightly on pieces of filter paper until no further water was absorbed by the paper.

Acid hydrolysates of the larvae and worms were prepared by exposing 2 million larvae and 3 gm of adult worms to 15 and 30 ml, respectively, of 6N HCl acid for 24 hours. Hydrolysates were diluted with distilled water, decolorized with Norit-A, filtered through Whatman No. 2 paper, and concentrated by evaporation.

Descending unidimensional and two-dimensional chromatograms were made. Whatman No. 3MM paper was cut into $2.5 \times 30$ cm strips and used for the unidimensional chromatograms. Ten lambda of hydrolysate was applied, and separation of the amino acids was effected by a phenol-water solvent (4 : 1 v/v) over a 12-hour period. Two-dimensional chromatograms were made on sheets of Whatman No. 3MM, $46 \times 57$ cm. Twenty lambda of hydrolysate was applied. A mixture of butanol : acetic acid : water (4 : 1 : 5 v/v) was used as the solvent for one direction over a 16-hour period, and the phenol : water mixture was used as solvent for the second direction over a 10-hour period.

Standard solutions were prepared (Block, Durrum, and Zweig, 1958) and run in parallel with all unknowns.

Strips and sheets of paper were dried thoroughly in a hood and then sprayed with a 0.25 percent ninhydrin-in-acetone solution to locate the amino acids. Rf values were calculated for the unidimensional chromatograms.

## RESULTS AND DISCUSSION

In the unidimensional chromatograms, 11 ninhydrin-positive spots were detected on the control strip and 10 were detected on each of the strips to which worm hydrolysate had been applied. Based on Rf values, the amino acids thus revealed were identified presumptively and they are listed in Table 1. As a mixture of 16 amino acids constituted the standard solution used as a control, it was evident that some of the ninhydrin-positive spots represented more than one amino acid. Levy and Chung (1953) found that it was impossible to separate glutamic-threonine, methionine-valine, and glycine-serine by ascending unidimensional chromatography, and in addition to these amino acid complexes, it was not possible to separate arginine-histidine and leucine-isoleucine by the unidimensional descending chromatographic technique of this study.

Separation of the above-listed amino acid complexes was effected by two-dimensional chromatography, as 15 ninhydrin-positive spots appeared on the chromatograms. The amino acids so revealed were tentatively identified as: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, iso-

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TABLE 1. Rf values of ninhydrin positive spots on chromatograms of acid hydrolysates of infective larvae and adults of *Cooperia punctata*, *C. pectinata*, and *C. oncophora* and of the infective larvae of some other strongyle parasites of cattle.

| Spot no. | Amino acid | Standard solutions | Infective larvae | | | | | | | Adults | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | C.p. | C.pe. | C.o. | O.o. | T.a. | T.c. | O.r. | C.p. | C.pe. | C.o. |
| I | Cystine | .08 | .07 | .08 | .08 | .07 | .08 | .07 | .07 | .07 | .08 | .07 |
| II | Lysine | .12 | .12 | .12 | .12 | .10 | .11 | .11 | .11 | .10 | .11 | .11 |
| III | Arginine–histidine | .15 | .14 | .15 | .15 | .13 | .13 | .13 | .13 | .14 | .15 | .15 |
| IV | Aspartic acid | .21 | .19 | .20 | .20 | .18 | .19 | .19 | .19 | .19 | .19 | .20 |
| V | Glycine–serine | .24 | .23 | .24 | .24 | .21 | .23 | .22 | .24 | .24 | .25 | .25 |
| VI | Glutamic–threonine | .27 | .28 | .28 | .27 | .24 | .26 | .26 | .28 | .27 | .28 | .27 |
| VII | Alanine | .35 | .34 | .34 | .32 | .32 | .33 | .33 | .34 | .33 | .34 | .32 |
| VIII | Proline | .38 | .37 | .37 | .38 | .36 | .37 | .36 | .38 | .37 | .39 | .38 |
| IX | Tyrosine | .45 | — | — | — | — | — | — | — | — | — | — |
| X | Methionine–valine | .53 | .52 | .52 | .53 | .50 | .53 | .52 | .52 | .52 | .54 | .53 |
| XI | Leucine–isoleucine | .65 | .62 | .66 | .66 | .65 | .66 | .66 | .66 | .66 | .67 | .66 |

C.p. = *Cooperia punctata*; C.pe. = *C. pectinata*; C.o. = *C. oncophora*; *O.o.* = *Ostertagia ostertagi*; T.a. = *Trichostrongylus axei*; T.c. = *T. colubriformis*; O.r. = *Oesophagostomum radiatum*.

leucine, lysine, methionine, proline, serine, threonine, and valine. Since no basic hydrolysates were prepared, the status of tryptophan was not determined; however, it has been reported as present in the excretions of *Nematodirus* spp. (Rogers, 1955), in the eggs and larvae of *Ascaris suum* (Jaskoski, 1962) and its presence was suspected in the larvae of *Nippostrongylus brasiliensis* (Friedman and Kagan, 1958). Only tyrosine and phenylalanine were absent from the hydrolysates of all of the species of worms used in this study. Friedman and Kagan (1958) also were unable to find tyrosine in the larvae of *N. brasiliensis*, whereas, Haskins and Weinstein (1957) identified it positively in excretions of the larvae of *Trichinella spiralis*. Failure to demonstrate phenylalanine is surprising in view of its importance to most living organisms. It is present in *A. suum* (Jaskoski, 1962) and in excretions of *T. spiralis* larvae (Haskins and Weinstein, 1957).

There were no qualitative differences in amino acid composition between the larvae and adults of the three species of *Cooperia* nor between the larvae of *Cooperia* spp. and those of the other species of strongyles. However, quantitative differences that might exist could not be determined by the methods employed in this study.

## SUMMARY

In unidimensional chromatograms of acid hydrolysates of the infective larvae and adults of *Cooperia punctata*, *C. pectinata*, and *C. oncophora*, and of the infective larvae of *Ostertagia ostertagi*, *Trichostrongylus axei*, *T. colubriformis*, and *Oesophagostomum radiatum*, 10 ninhydrin-positive spots were detected. When two-dimensional chromatography was used, these spots were resolved into 15 that were tentatively identified as the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, methionine, proline, serine, theonine, and valine. Tyrosine and phenylalanine were not found in any of the hydrolysates.

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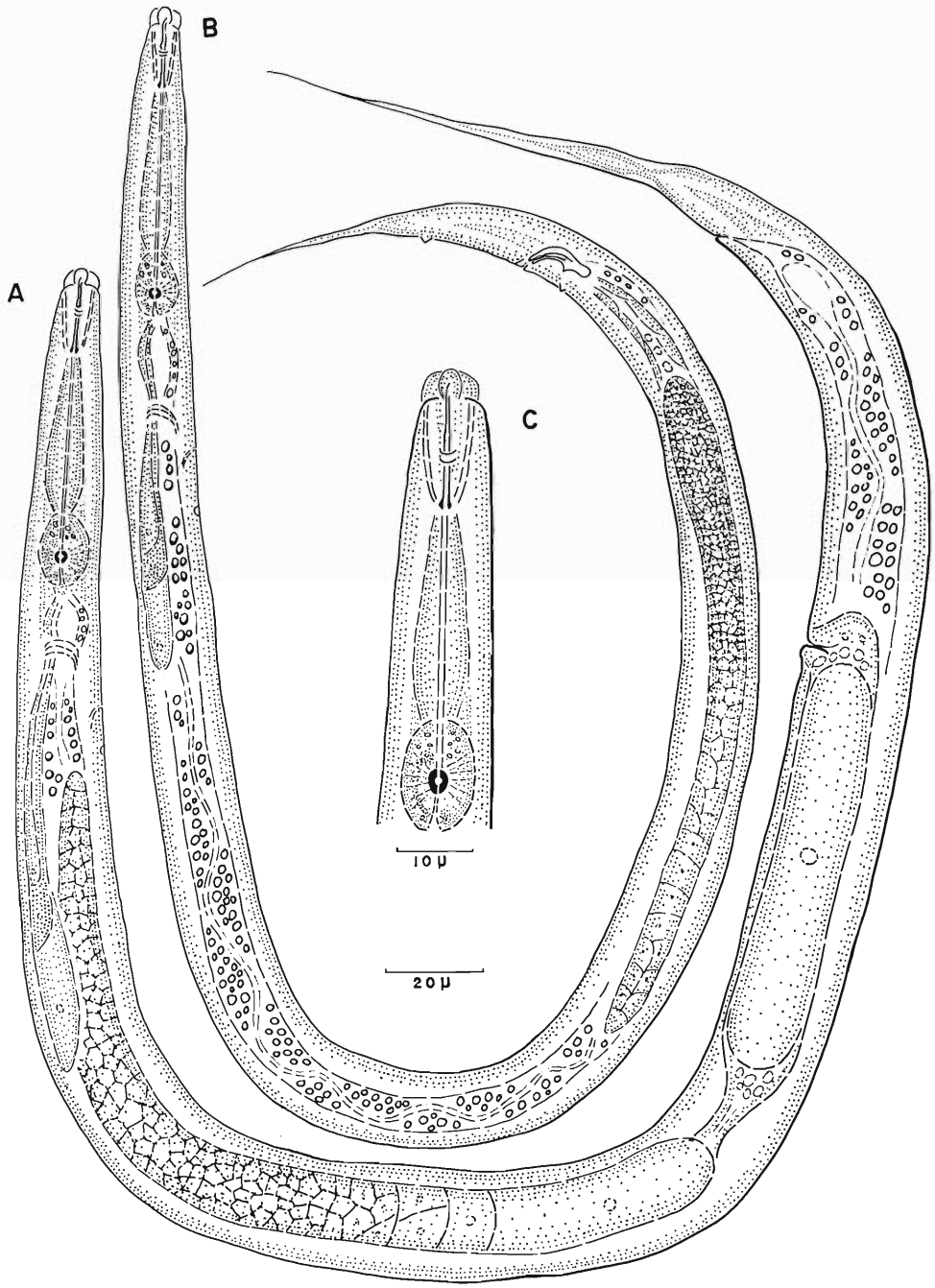
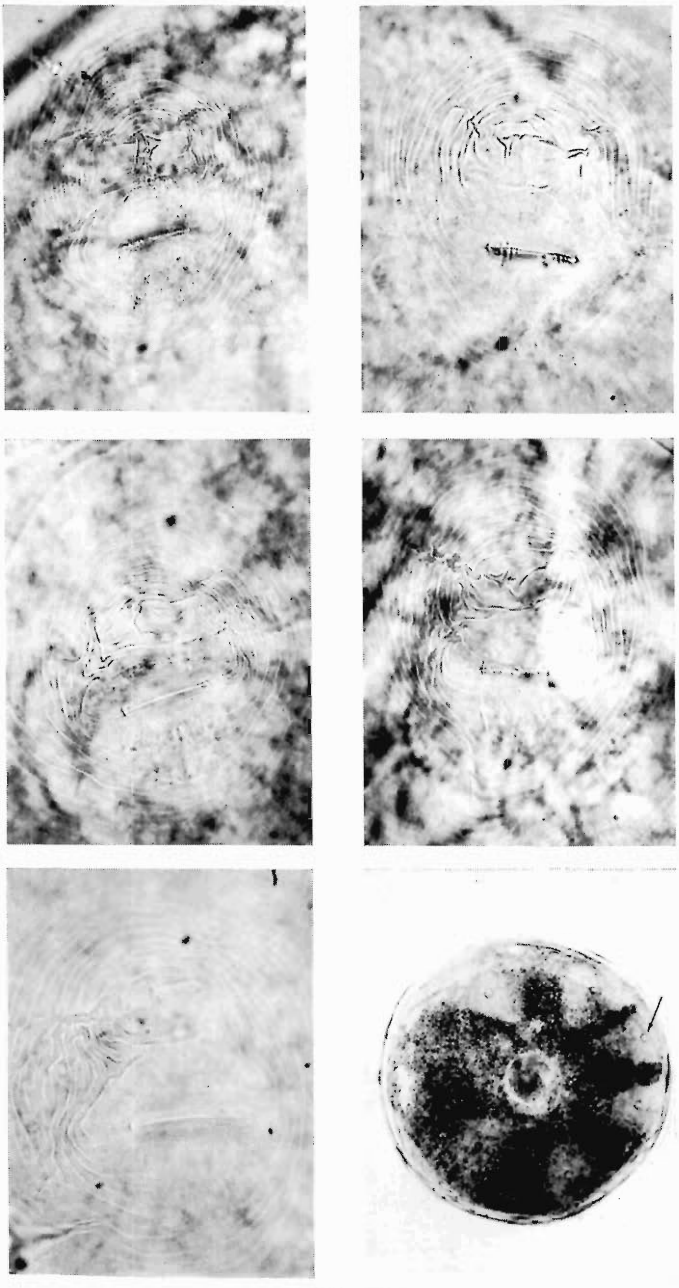



## ERRATA

In the article by Hechler and Taylor (Proc. Helminthol. Soc. Wash. 32: 205–219) Fig. 4, p. 213 is a duplicate of Fig. 6, p. 218. The correct Fig. 4, *Seinura oliveirae* A. Female; B. Male; C. Female head, appears above.



## ERRATA

In the article by Golden and Birchfield (Proc. Helminthol. Soc. Wash. 32: 228–231) Fig. 3, p. 230 was inadvertently reduced. The correct Fig. 3, Photomicrographs of *M. graminicola*, n. sp. Five perineal patterns and, lower right, posterior portion showing rectal glands (note large nuclei, one of which is at arrow), appears above.

(Alabama through Maryland; remainder of list will appear in July issue)

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