MORPHOGENESIS OF *CONTRACAECUM RUDOLPHII* (NEMATODA: ASCARIDOIDEA), A PARASITE OF FISH-EATING BIRDS, IN ITS COPEPOD PRECURSOR AND FISH INTERMEDIATE HOSTS

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Summary :

Eggs of *Contracaecum rudolphii* obtained from female worms in double-crested cormorants (*Phalacrocorax auritus*) in Nova Scotia, Canada, hatched in 9-17 days in sea water at 15-20 °C. The newly-emerged, free-living (and presumably second-stage) larva is described in detail, as are larvae from experimentally-infected copepods (*Tigriopus* sp.), amphipods (*Gammarus* sp.), and fish (*Lebistes reticulatus*, *Fundulus heteroclitus*). Copepods are considered precursor hosts and morphogenesis of the parasite in them primarily involved body size, the ventricular appendix, and the excretory system. Infection of amphipods and fish was much more successful when invading larvae were from copepods than when they were free living; amphipods served as paratenic hosts and fish as intermediates. In fish, second-stage larvae 20 or fewer days postinfection were within the intestinal wall. At 44 or more days postinfection, larvae were considered third stage; all were in the abdominal cavity, many within a closely adhering « sleeve » of material resembling cuticle. The longest larvae (3.1-3.9 mm) in fish were associated with the oldest infection (152 d), suggesting that larvae continue to grow for a considerable period of time; growth was also found to be asynchronous, however.

KEY WORDS : nematode, parasite, *Contracaecum spiculigerum*, Ascaridoidea, morphogenesis, intermediate, paratenic, cormorant, bird.

Résumé : MORPHOGENÈSE DE *CONTRACAECUM RUDOLPHII* (NEMATODA : ASCARIDOIDEA), PARASITE DES OISEAUX DE MER, CHEZ SON HÔTE PRÉCURSEUR, LE COPÉPODE, ET SON HÔTE INTERMÉDIAIRE, LE POISSON

La larve de deuxième stade de Contracaecum rudolphii, *de connaissance récente et vivant sans hôte, est décrite en détail ainsi que les larves venant de copépodes infectés en laboratoire (*Trigriopus *sp.), d'amphipodes (*Gammarus *sp.) et de poissons (*Lebistes reticulatus, Fundulus heteroclitus*). La morphogenèse du parasite dans le copépode, considéré comme hôte précurseur, inclut la taille, l'appendice ventriculaire et le système excrétoire. L'infection des amphipodes et des poissons est beaucoup plus importante lorsque les larves proviennent des copépodes que lorsqu'elles vivent sans hôte; les amphipodes ont servi d'hôtes paraténiques et le poisson d'hôte intermédiaire. Chez le poisson, les larves ont atteint le troisième stade 44 jours ou plus après l'infection; toutes se trouvaient dans la cavité abdominale, plusieurs à l'intérieur d'un « sac » étroitement ajusté et d'un matériau évoquant une cuticule. Chez le poisson, les larves les plus longues mesuraient de 3,1 à 3,9 mm (152 d). Il est probable que les larves continuent à grandir chez le poisson pendant une longue période mais la croissance en est cependant asynchrone.*

MOTS CLÉS : *nématode, parasite,* Contracaecum spiculigerum, *Ascaridoidea, morphogenèse, intermédiaire, paraténique, cormoran, oiseau.*

INTRODUCTION

Ascaridoid nematodes, common parasites of the stomach or intestines of vertebrates, are often rather plastic with respect to transmission (Anderson 1992). *Contracaecum rudolphii* Hartwich, 1964, for example, undergoes « partial development » in copepods followed by later development in fish to the stage infective to the avian final host (e.g. cormorants, *Phalacrocorax* spp.) (Huizinga 1966) (note: Hartwich (1964) proposed *C. rudolphii* as a new name for the *Contracaecum* sp. found mainly in cormorants; Huizinga (1966) referred to his specimens as *Contracaecum spiculigerum*). However, fish can also be infected directly, i.e. without the agency of copepods (Huizinga 1966), suggesting development in copepods may be trivial. The present study sought to clarify our understanding of these events through a detailed examination of the parasite's morphology in copepods, amphipods, and fish. It worked from the premise of Huizinga (1966) that a second-stage larva emerges from the egg of *C. rudolphii* (although studies on related species [e.g. Køie and Fagerholm 1993; Measures and Hong 1995] suggest this requires re-examination using transmission electron microscopy). Morphogenesis occurred in both copepods and fish, enabling their designation as precursor and intermediate hosts, respectively.

METHODS AND MATERIALS

Live females of *C. rudolphii* were removed from the proventriculus of double-crested cormorants (*Phalacrocorax auritus*) shot on Delorier Island (45° 31′N, 61° 06′W) near Arichat or on Red Islands (45° 48′N, 60° 46′W) near Johnstown, Nova Scotia,

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Canada, in June and July, 1992 and 1993. Eggs were dissected from females and incubated at 10°, 15°, and 20 °C in Syracuse dishes (4 × 4 cm) containing sea water that had been passed through a millepore filter (0.47 µm grid size).

Once larvae hatched, various experiments (outlined below) were conducted. Copepods (*Tigriopus* sp.), amphipods (*Gammarus* sp.), and larval fish (mummichog, *Fundulus heteroclitus*, 0.75-1,25 cm long) used in the experiments were collected in a tidal marsh near Port Morien (46° 08′N, 59° 52′W), Nova Scotia (note: 50 copepods, 50 amphipods, and 50 larval mummichog were also examined as controls; nematode larvae were not present). Guppies (*Lebistes reticulatus*) were purchased from a local pet store.

Experiments A-C exposed recently-emerged larvae to copepods (A), amphipods (B), and fish (C). Hundreds of larvae were placed in filtered water in each of numerous Syracuse dishes and then dozens of copepods, four amphipods, or one fish were added to each dish. Crustaceans and mummichog were exposed in sea water; guppies in pond water. After 1 d, amphipods and fish were transferred to clean water in crystallization dishes (100 × 50 mm) and exposure dishes examined to determine if all larvae had been eaten (they had). Crustaceans were maintained at 15° C and fish at 22° C. Amphipods were fed pulverized trout chow and fish were fed tropical fish food flakes.

Experiments D and E exposed infected copepods to amphipods (D) and fish (E). Dozens of infected copepods (from Experiment A) were placed in filtered sea or pond water (as appropriate) in each of numerous Syracuse dishes and then four amphipods or one fish were added to each dish. At 1 d, amphipods and fish were transferred to clean water and maintained as previously outlined; exposure dishes were examined to determine if all copepods had been eaten (they had).

Experiment F involved exposure of fish to infected amphipods. Ten amphipods (from Experiment B, and presumed to contain larvae but not verifiable) were placed in filtered sea water in each of numerous Syracuse dishes and then one fish was added to each dish. At 1 d, fish were transferred to clean water and maintained as previously outlined; exposure dishes were examined to determine if all amphipods had been eaten (they had).

At various times postexposure, crustaceans and fish were killed (via decapitation) and dissected in saline on microscope slides. Larvae from crustaceans and fish 20 or fewer days postinfection were fixed by adding an equal volume of 10 % formalin to the saline; a petroleum-ringed coverglass was then placed over the mixture and larvae studied. Larvae from other fish were fixed in a hot solution of 5 % glycerin/70 % alcohol and studied in glycerin. A microscope equipped with differential interference contrast lighting was used to study larvae.

RESULTS

Hatching of eggs

Larvae first emerged from eggs at 9 d in water at 20 °C and at 11 or 17 d at 15 °C (eggs from worms from two cormorants). At 10 °C, eggs had not embryonated by 40 d and were discarded. Eggs stored at 5 °C for 10 months did not embryonate after being moved to 20 °C for 15 d and the experiment was terminated.

Experiments A-F

Experiment A: At 1-2 d postexposure, about half of the hundreds of copepods examined contained larvae (prevalence and intensity not precisely determined). Larvae were motile and generally free in the abdominal haemocoel where they were readily visible. Live, infected copepods were noticeably lethargic in comparison to copepods without larvae. Many infected copepods died within 2-3 d postinfection; larvae within them frequently remained motile for an additional 2 d.

Experiment B: One larva was found in one amphipod and none in 57 others (Table I).

Experiment C: Three of 6 guppies contained larvae (all motile), none of 27 mummichog did (Table I). At 20 d postinfection, larvae were within the intestinal wall. At 53 and 66 d, they were in the abdominal cavity. Some of the latter were in a delicate, closely adherent « sleeve » of indeterminate nature resembling shed cuticle with a delicate fibrous texture (Table I). In addition, numerous host cells sometimes surrounded these « sleeves » or larvae by themselves, giving the appearance of a delicate capsule.

Experiment D: Five of nine amphipods contained larvae (all motile) (Table I).

Experiment E: Four of four mummichog contained larvae, as did six of seven guppies (Table I). All larvae were motile. Those in fish examined 10 or fewer days postinfection were in the intestinal wall. At later times (44-152 d), larvae were in the abdominal cavity, some free and others within « sleeves », and some also surrounded by host cells (Table I).

Experiment F: Two of two mummichog contained larvae, as did two of seven guppies (Table I). All larvae were motile. Those in fish examined six or fewer days postinfection were in the intestinal wall and those in fish at 96 and 132 d were in the abdominal cavity. As in Experiment E, some larvae were within a « sleeve » and some free.

Description of second-stage larvae recently emerged from eggs

Larvae (Fig. 1) emerging from eggs with retained, moulted cuticle forming loose, striated sheath slightly

wider and quarter to half longer than larva within. Anterior extremity of sheath generally with several, irregularly digitiform extensions and larvae attaching, by way of anterior extremity, to bottoms of dishes and waving vigorously in medium. Posterior end of sheath attenuated and sharply pointed. At 15° C, larvae remaining highly active for approximately 10 d after which becoming increasingly less motile, dying between 30 and 40 d.

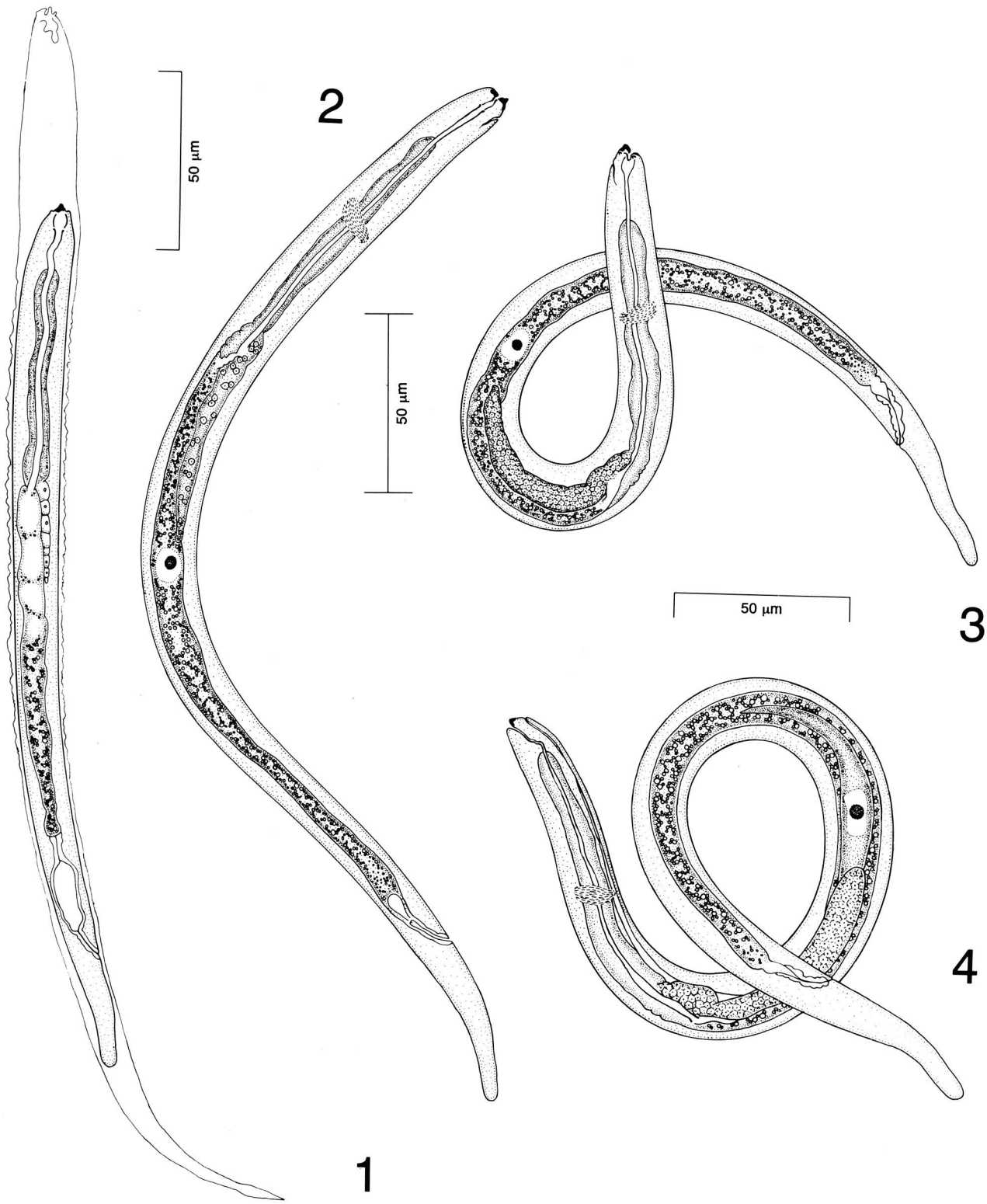
Free-living, recently-emerged (< 24 hr) and 10 d old larvae: Long, slender (Table II), with striated cuticle.

	Days post-exposure	No. infected/ No. exposed	No. larvae found	Condition of host prior to dissection and comments on location of larvae
Experiment B[a]				
amphipod	3	1/1	1	amphipod live; larva in haemocoel
amphipods	2-33	0/57	—	amphipods live
Experiment C[b]				
mummichog	1-12	0/27	—	some fish live, some dead
guppy	20	1/1	2	fish dead; larvae in intestinal wall
guppy	34	0/1	—	fish dead
guppy	37	0/1	—	fish dead
guppies	53	1/2	2	fish dead; larvae in abdominal cavity adjacent to intestine within « sleeve »[f] covered by host cells
guppy	66	1/1	1	fish live; larva in abdominal cavity with a few adherent host cells but not within a « sleeve »
Experiment D[c]				
amphipods	3	2/2	1,6	amphipod live; larvae in haemocoel
amphipods	14	1/5	1	amphipod live; larva in haemocoel
amphipod	22	1/1	5	amphipod live; larvae in haemocoel
amphipod	33	1/1	1	amphipod live; larva in haemocoel
Experiment E[d]				
mummichog	4	1/1	41	fish moribund; larvae in intestinal wall
mummichog	5	1/1	>100	fish dead; larvae in intestinal wall
mummichog	8	1/1	112	fish dead; larvae in intestinal wall
mummichog	10	1/1	70	fish dead; larvae in intestinal wall
guppy	44	1/1	10	fish dead; larvae within fat around intestine (presence or absence of « sleeve » not determined)
guppy	45	1/1	5	fish dead; larvae within abdominal cavity, one within « sleeve » and four free
guppies	87	2/2	3, 10	one fish live, one dead; larvae either covered in host cells or within « sleeve »
guppy	112	1/1	1	fish dead; larva free within fat around intestine
guppies	152	1/2	19	fish live; larvae free within fat around intestine and free in abdominal cavity
Experiment F[e]				
mummichog	3	1/1	1	fish dead; larva in intestinal wall
mummichog	6	1/1	16	fish dead; larvae in intestinal wall
guppy	56	0/1	—	fish dead
guppies	76	0/2	—	one fish live, one dead
guppy	86	0/1	—	fish dead
guppies	96	1/2	2	fish live; larvae within « sleeve » with attached host cells within fat around intestine
guppy	132	1/1	1	fish live; larva within mesentery and observed to pull itself free of « sleeve » when placed in saline

[a] Experiment B, amphipods exposed directly to recently-emerged, second-stage larvae.
[b] Experiment C, mummichog and guppies exposed directly to recently-emerged, second-stage larvae.
[c] Experiment D, amphipods exposed to copepods containing second-stage larvae.
[d] Experiment E, mummichog and guppies exposed to copepods containing second-stage larvae.
[e] Experiment F, mummichog and guppies exposed to amphipods presumed to contain second-stage larvae.
[f] « sleeve » = closely adhering, sleeve-like structure resembling cuticle and often with fibrous texture.
Note: Larvae at 20 or fewer days in second stage; larvae at 44 or more days in third stage.

Table I. — Numbers of amphipods (*Gammarus* sp.) and fish (mummichog, *Fundulus heteroclitus*; guppies, *Lebistes reticulatus*) infected with larvae of *Contracaecum rudolphii* at different times after exposure to larvae in different experiments (B-F); numbers and locations of larvae and condition of fish also indicated.

Prominent, hyalinized, cuticular tooth present on cephalic surface ventral to oral opening. Oral opening leading into tube of hyalinized cuticle; latter generally more dilated for anteriormost 5 µm than remainder of length. Cuticular tube continuing posteriorly and appearing to lack immediately adjacent tissue for



Figs. 1-4. — *Contracaecum rudolphii*, second-stage larvae (free-living, from copepods (*Tigriopus* sp.), and from amphipods (*Gammarus* sp.)). Fig. 1. Larva recently emerged from egg. Fig. 2. Larva from copepod 42 hr postexposure. Figs. 3, 4. Larvae from amphipods exposed to infected copepods 14 and 22 d previously, respectively.

approximately 15-20 µm before becoming surrounded by delicate, thin wall; together these comprising oesophagus. Presumptive ventriculus marked by slight increase in width of wall at posterior end of oesophagus. Ventricular-intestinal junction readily apparent. Presumptive ventricular appendix present as delicate column of narrow cells adjacent to ventral side of anteriormost intestine and extending posteriorly for average of 77 µm at 24 hr and 84 µm at 10 d. Presumptive intestinal caecum not distinguishable. Intestine generally containing large clear areas in anterior third and small, round, brown particles throughout remainder. Short, narrow constriction joining intestine to rectum. Rectum joined by narrow canal to anus. Nervous and excretory systems not discernable. Posterior end of larva digitiform with bluntly rounded extremity.

DESCRIPTIONS OF SECOND-STAGE LARVAE FROM CRUSTACEANS

Larvae from copepods (Fig. 2): Larvae 42 hr postexposure (Experiment A) wider and longer than recently-emerged larvae (Table II). Other characteristics: cuticle delicately striated; excretory pore visible on cephalic surface ventral to cuticularized tooth; oesophageal wall and ventriculus more apparent than in recently-emerged larvae; ventricular appendix digitiform, extending posteriorly for an average of 102 µm from ventricular-intestinal junction and containing several small, scattered cells; presumptive intestinal caecum marked by small swelling anterior to ventricular appendix and latter containing few small, scattered cells; nerve ring apparent at approximately mid length of oesophagus; prominent cell with large nucleus present in pseudocoelom slightly posterior to tip of ventricular appendix and not appearing associated with any other structure (although presumably cell of excretory gland).

Larvae from amphipods: Larva 3 d postexposure (Table I, Experiment B) similar in length and width (Table II) and morphologically to larvae from copepods. Larvae 14 and 22 d postexposure (Table I, Experiment D) similar in length and width to larvae from copepods (Table II) but differing slightly as follows: oesophagus and ventriculus more apparent; ventricular appendix and presumptive intestinal caecum slightly larger and packed with small cells with inconspicuous borders; in larva at 14 d (Fig. 3), cell with large nucleus present in pseudocoelom posterior to tip of ventricular appendix but appearing not associated with any other structure; in larva at 22 d (Fig. 4), this cell associated with excretory gland of excretory system.

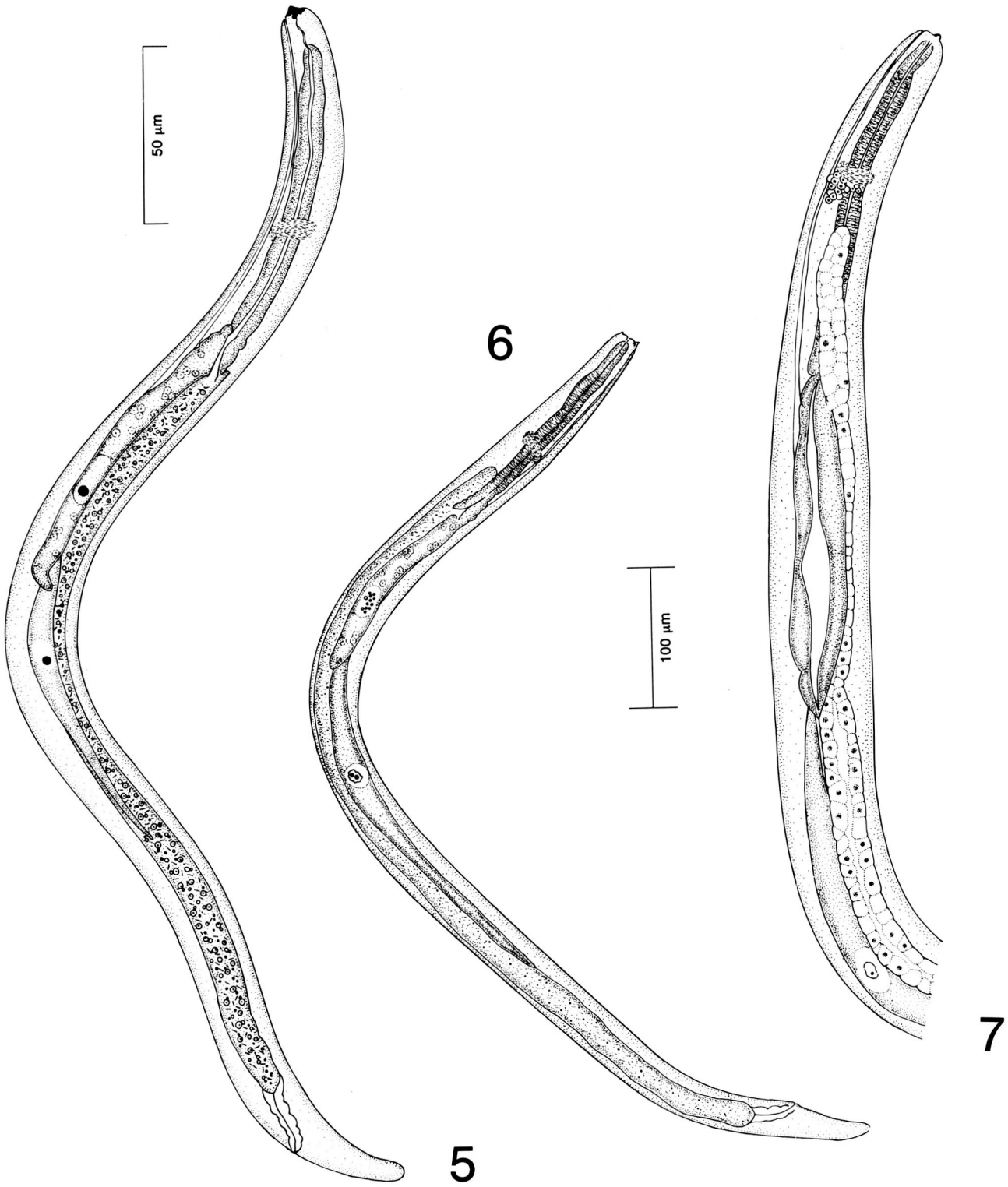
DESCRIPTIONS OF LARVAE FROM FISH

At 8 d, larvae (from mummichog; Table I, Experiment E) similar in size and morphology (Fig. 5) and slightly longer and wider than recently-emerged larvae or larvae from crustaceans (Table II). Other differences including: cuticle thin with delicate transverse striations; intestinal caecum apparent as tiny anterior projection from ventricular-intestinal junction; ventricular appendix slightly longer and containing, in posterior third, large cell with prominent nucleus; intestinal wall containing small round cells.

	Second-stage larvae[a] 24 hr after emergence from eggs	Second-stage larvae[a] 10 d after emergence from eggs	Second stage-larvae 42 hr in copepods (Experiment A)	Second stage larvae 3 d in amphipod (Experiment B)	Second-stage larvae 14 & 22 d in amphipod (Experiment D)	Second-stage larva 8 d in mummichog (Experiment E)	Third-stage larva 45 d in guppy (Experiment E)	Third-stage larva 53 d in guppy (Experiment E)	Third-stage larva 66 d in guppy (Experiment C)
N	10	10	12	1	1, 1	10	1	1	1
Total length	238 (223-255)	245 (215-285)	365 (318-417)	310	340, 385	407 (370-470)	860	1,600	2,700
Maximum width	14 (13-15)	14 (13-15)	17 (13-20)	17	17, 18	19 (18-22)	35	70	110
Nerve ring	—	—	52 (32-64)	53	—, 55	54 (45-63)	100	110	140
Length oesophagus	77 (75-85)	84 (75-90)	102 (92-118[b])	100	83, 85	98 (90-110)	142	260	310
Length ventriculus	—	—	[c]	13	15, 15	16 (12-21)	30	20	20
Length ventr. appendix	35 (25-53)	26 (20-33)	—	50	53, 60	72 (57-93)	140	240	260
Length intestinal caecum	—	—	—	—	—, —	—	40	120	140
Excretory cell	—	—	—	165	160, 180	198 (180-220)	400	680	1,150
Anus	23 (22-25)	—	49 (44-54)	25	40, 47	35 (27-45)	54	70	110

[a] free-living.
[b] includes ventriculus.
[c] included in length of oesophagus.

Table II. — Morphometrics of larvae of *Contracaecum rudolphii* at various times after emergence from eggs or postinfection in copepods (*Tigriopus* sp.), amphipods (*Gammarus* sp.), or fish (guppies, *Lebistes reticulatus*; mummichog, *Fundulus heteroclitus*).



Figs. 5-7 — *Contracaecum rudolphii*, larvae from fish (mummichog, *Fundulus heteroclitus;* guppies, *Lebistes reticulatus*). Fig; 5. Larva from mummichog 8 d postinfection. Fig. 6. Larva from guppy 45 d postinfection. Fig. 7. Larva from guppy 53 d postinfection.

At 20 d, larvae (from guppy; Table I, Experiment C) slightly contracted in appearance but otherwise similar to larvae at 8 d.

At 45 d, larva (from guppy; Table I, Experiment E; Fig. 6) slightly longer and wider than larvae at 8 d (Table II). Other differences including: cuticle with pro-

minent transverse and delicate longitudinal striations; cephalic extremity with four prominent papillae; narrow, non-striated wall of tissue surrounding tube of hyalinized cuticle immediately posterior to buccal region (an area previously lacking visible tissue wall); intestinal caecum extending halfway to nerve ring; large cell in ventricular appendix containing numerous round bodies (rather than nucleus); nucleus of cell of excretory gland with irregular margin and containing two round bodies. Larva not within « sleeve ».

At 53 d, larva (from guppy; Table I, Experiment C; Fig. 7) about twice as long and wide as larva at 45 d (Table II). Other differences including: lumen apparent within ventricular appendix; intestinal caecum and intestinal wall containing faintly defined, large cells. Larva within « sleeve ».

At 66 d, larva (from guppy; Table I, Experiment C) considerably longer and wider than larva at 53 d (Table II), but otherwise similar to it. Larva not within « sleeve ».

At 87 d, larvae (from guppy; Table I, Experiment E) varying in length and width (Table III). Genital primordium in longer larvae (≥ 2 mm) visible as single cell between ventral hypodermis and excretory gland at approximately mid length of body. Larvae otherwise similar to larva at 53 d. Some larvae within « sleeve » and some not.

At 112 d, larva (from guppy; Table I, Experiment E) 1150 µm long, 40 µm wide, and similar to larva at 53 d. Larva not within « sleeve ».

At 132 d, larva (from guppy; Table I, Experiment F) 2750 µm long, 90 µm wide, and similar to long larvae at 86 d. Larva within « sleeve ».

At 152 d, larvae (from guppy; Table I, Experiment E; Figs. 8-11) varying in length and width. Five largest larvae (Table III) longer and wider than larvae at all previous times but otherwise similar to larvae at 86 d. Larvae not within « sleeve ».

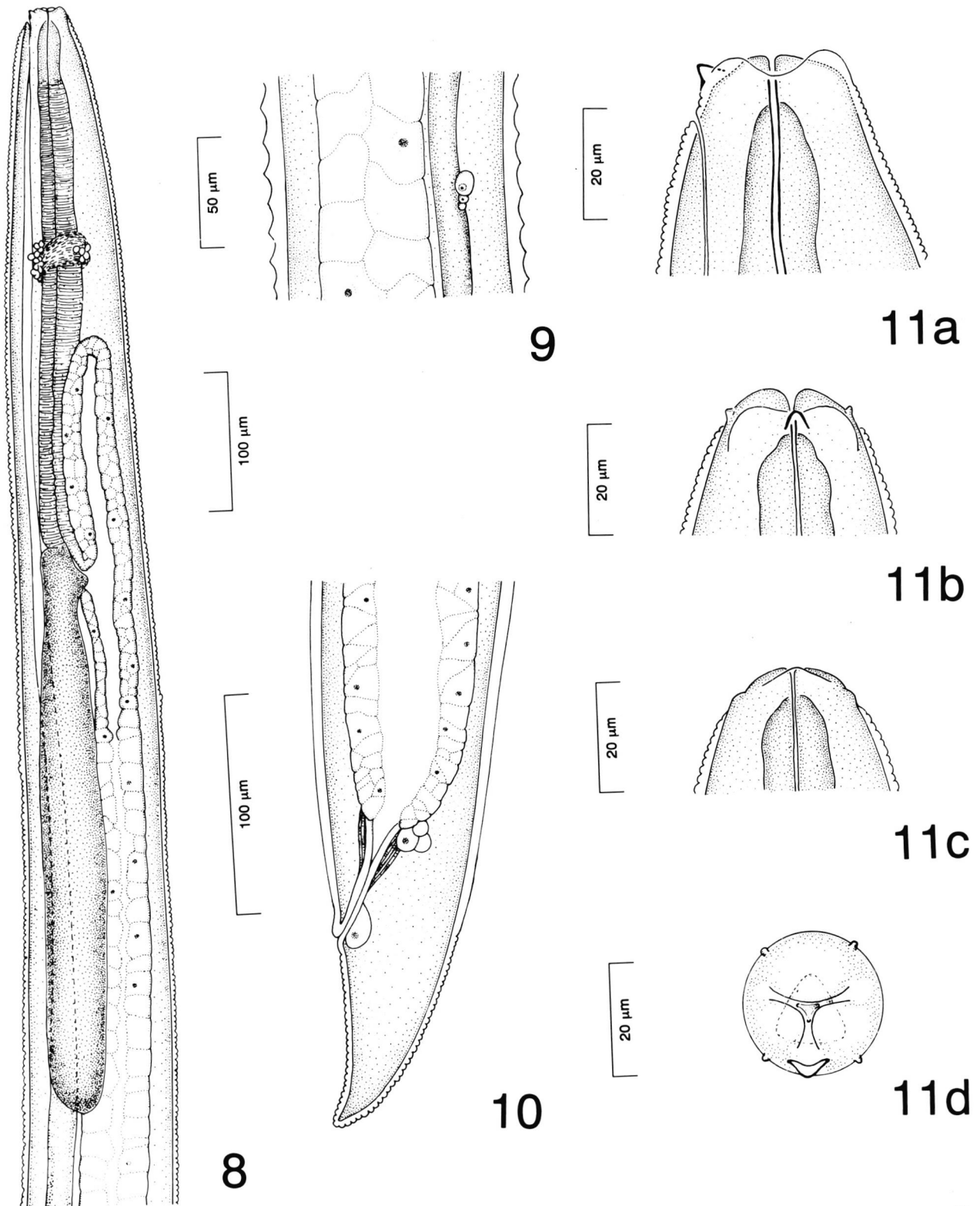
DISCUSSION

Changes in *C. rudolphii* while in the copepod (*Tigriopus* sp.) primarily involved body size and development of the ventricular appendix (from a delicate column of narrow cells to a longer, digitiform structure containing several small, scattered cells) and the excretory system (from unapparent to a prominent cell with a large nucleus). Huizinga (1966) reported a similar slight increase in size of larvae in the copepod *Tigriopus californicus* (as well as unexplained « partial development ») but « no morphological change » in *Cyclops vernalis*. The present study considers the changes that occurred in the parasite while in the copepod to be developmentally significant; they were associated with markedly increased success by the parasite in establishing in its fish host (10 of 11 fish exposed to copepods harbouring larvae became infected versus none of 27 mummichog and 3 of 6 guppies exposed directly to recently-emerged larvae). Huizinga (1966) reported no (0 of 25) to limited (4 of 25) success in infecting guppies with *T. californicus* and *C. vernalis* harbouring larvae, respectively, and considerable success (3 of 5) in infecting mummichog (= killifish) with *T. californicus* harbouring larvae.

Amphipods, in addition to copepods, were found capable of transporting larvae of *C. rudolphii* to fish, something not previously reported for the species. Successful infections in amphipods were generally established only when amphipods ate copepods containing larvae (5 of 9 became infected), however, and not when amphipods ate free-living, second-stage larvae in water (1 of 58 became infected although all larvae were consumed). This requirement of larvae to pass through copepods before being infective to amphipods is strikingly similar to that noted for infectivity of larvae to fish and supports the suggestion that the

	Larvae 87 d										**Larvae 152 d**
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	(N = 5)
Total length (mm)	0.8	1.1	1.5	1.8	1.9	2.0	2.2	2.5	2.7	2.9	3.5 (3.1-3.9)
Maximum width	30	35	50	60	65	70	85	90	95	90	114 (110-120)
Nerve ring	60	—	—	130	110	—	130	150	140	140	167 (150-200)
L. œsoph.	165	—	—	230	200	210	—	370	340	310	391 (370-415)
L. ventr.	15	—	—	20	25	20	—	30	20	30	29 (25-30)
L. ventr. app.	130	—	—	210	220	220	—	325	270	350	359 (320-380)
L. int. caecum	40	—	—	80	90	80	—	190	150	150	191 (170-225)
Excr. cell (mm)	—	—	—	0.6	0.7	—	—	0.9	0.9	1.0	1.2 (1.1-1.3)
Anus	—	50	65	60	65	60	65	75	85	85	88 (85-90)

Table III. — Morphometrics (in µm, unless otherwise specified) of third-stage larvae of *Contracaecum rudolphii* in guppies *(Lebistes reticulatus)* 87 and 152 d after ingesting copepods (*Tigriopus* sp.) containing second-stage larvae. Note: Ten larvae of considerable size range were recovered at 86 d and five larvae of more similar size at 152 d.



Figs. 8-11. — *Contracaecum rudolphii*, larva from guppy (*Lebistes reticulatus*) 152 d postinfection. Fig. 8. Anterior end, lateral view. Fig. 9. Genital primordium, lateral view. Fig. 10. Tail, lateral view. Figs. 11A-D. Anterior extremity, lateral, ventral, dorsal, and *en face* views, respectively.

copepod serves a partial developmental role in the transmission of *C. rudolphii*. Within the genus *Contracaecum*, the copepod is reported to serve different roles in transmission ranging from that suggested herein for *C. rudolphii* (i.e., a « precursor » role, see below) to being the site of a moult (e.g. *C. micropapillatum* (Stossich, 1850)) (see Anderson 1992). Extreme plasticity also apparently characterizes *Pseudoterranova decipiens* (Krabbe, 1878) where a moult has been suggested in macrocrustaceans (McClelland, 1990).

The amphipod is considered herein a paratenic host of *C. rudolphii*. Amphipods have also been reported to harbour larval *C. micropapillatum* although, in contrast to the present study, infections were said to have been acquired when amphipods ate free-living larvae (Anderson, 1992). *Pseudoterranova decipiens* can also pass in the food chain from one crustacean host to a second (« serial transmission ») and acquisition by amphipods is, as in the present study, much more efficient when a copepod is initially involved (McClelland, 1990). Copepods serving this role have previously been termed « precursor » hosts (note: other authors may use the term « metaparatenic host » (see Odening, 1976)) and consumption of them by macroinvertebrates likely explains the presence, in the latter, of larvae of various species of marine and freshwater ascaridoids (Overstreet, 1983; McClelland, 1990). It would be informative to know whether morphogenesis occurs in these parasites in the copepod, similar to that noted herein.

When copepods are involved in a life cycle, it probably is advantageous for the parasite to use a subsequent paratenic host (macroinvertebrate) or be quickly transmitted to fish. Copepods harbouring *C. rudolphii* are adversely affected by the presence of larvae, becoming lethargic or moribund or dying within a few days of infection (Huizinga 1966; present study), as do copepods harbouring other ascaridoid larvae (McClelland, 1990). Affected copepods in the present study were readily fed upon by amphipods. In nature, transmission likely involves copepods which acquire larvae from the free-living environment and are then fed upon by paratenic hosts or fish. McClelland (1990) reported altered behaviour in amphipods harbouring larvae of *P. decipiens*; the limited observations herein precluded conclusions.

Huizinga (1966) suggested that at 18 d postinfection in fish, « a moult (of *C. rudolphii*) was in progress » although « larvae did not shed the second-stage cuticle while encapsulated, but retained it as a closely adhering layer. » In the present study, larvae at 20 d did not appear to be moulting. However, observations at later times support Huizinga (1966); at 45 d and later times many larvae were within a closely adherent « sleeve » resembling cuticle. In addition, larvae were sometimes surrounded by host cells, giving the appearance of a delicate capsule. Onset of a moult may be associated with migration of larvae from the intestinal wall (their site 20 or fewer days postinfection) to the abdominal cavity (their site 44 or more days postinfection). Huizinga (1966) recovered larvae in fish at times earlier than 18 d postinfection by using a pepsin digest and thus did not know their specific locations; his larvae at 18 d were « encapsulated along the intestinal mesenteries ».

In the present study, third-stage larvae continued to grow in length, with the longest (3.9 mm) having been obtained from the fish with the oldest (152 d) infection. Different larvae in a given fish were sometimes of different lengths, however (e.g. at 87 d, Table III), suggesting that growth is asynchronous. Huizinga (1966) also noted the longest (1.4 mm) larva came the fish with the oldest (37 d) infection; Moravec (1994) stated that « advanced » third-stage larvae ranged from 15-24 mm. McClelland and Ronald (1974*b*) indicated that second-stage *C. osculatum* Rudolphi, 1802 grew continuously for 32 weeks in cultures at 15 °C but commenced to moult to the subadult (fourth) stage when the temperature was raised to 35 °C. McClelland and Ronald (1974*a*, 1974*b*) found a similar phenomenon, namely development from the second to the fourth stage with only one intervening moult, in cultured *P. decipiens*.

The detailed description of larvae of *C. rudolphii* provided herein will help facilitate identification of anisakid larvae in invertebrates and fish (also, see Moravec 1994). In addition to Huizinga (1966), the life cycle of the *Contracaecum* sp. in cormorants has previously been studied by Thomas (1937*a, b*, 1940), Dubinin (1949), and Mozgovoy et al. (1965, 1968). However, within the genus *Contracaecum* as a whole, only the development of *C. osculatum* has previously been studied in a manner as detailed as that presented herein for *C. rudolphii*. The study of *C. osculatum* was done with cultured specimens; its 450 µm long, second stage has a large nucleus in the ventricular appendix, a readily distinguishable excretory system, and a genital primordium (McClelland and Ronald, 1974*b*, Fig. 3) not seen in second-stage *C. rudolphii* from crustaceans. Cephalic structures of third stage *C. rudolphii* from fish at 152 d postinfection were basically similar to those of 6 mm long, cultivated larvae of *C. osculatum* (see McClelland and Ronald 1974*b*; Figs. 1, 2) although the lips were not as prominent and the amphids not visible. They also resembled the cephalic structures of second-stage larvae of *P. decipiens* (see McClelland and Ronald, 1974*a*; Fig. 1), *Anisakis simplex* (Rudolphi, 1809) (see Smith, 1983; Plate 1, Figs. *a, b, e*), and *Raphidascaris acus* (Bloch, 1779) (see Smith 1984; Fig. 5).

Anderson and Bartlett (1993) suggested that precocity may be widespread but unrecognized among many nematodes, especially those that exhibit plasticity in transmission. None of the four ways proposed by Anderson (1992) as manifestations of precocity was observed in *C. rudolphii*. Indeed, its single-celled genital primordium was first visible in larvae at 86 d in fish and it remained unchanged in the oldest larvae. The single-celled genital primordium in *C. osculatum* only grew after larvae moulted to the fourth stage (McClelland and Ronald, 1974 *b*). *Contracaecum rudolphii* is rather unusual, however, in that growth of some larvae apparently continues while what is presumed to be cuticle from an earlier moult is retained. McClelland and Ronald (1974 *a*, 1974 *b*) suggested somewhat similar developments in cultured *C. osculatum* and *P. decipiens* to be neoteny or artifact.

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