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Morphology, homogonic development, and lack of a free-living generation in *Strongyloides robustus* (Nematoda, Rhabditoidea), a parasite of North American sciurids

C. M. Bartlett

Biology, University College of Cape Breton, Sydney, Nova Scotia, Canada B1P 6L2

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Abstract. Adult females of *Strongyloides robustus* Chandler, 1942, a parasite of sciurids in North America, were found in the duodenal mucosa of 30 of 32 red squirrels (*Tamiasciurus hudsonicus* (Erxleben)) collected in Cape Breton Island, Nova Scotia, Canada. The parasitic female is illustrated and redescribed; characteristics include: body 3.8–8.0 mm long, cephalic extremity with X-shaped mouth and 8 circumoral lobes, ovaries spiralling around intestine, and tail bluntly rounded. Eggs in fresh feces contained tadpole-stage larvae. In fecal cultures, eggs hatched and larvae invariably developed to the filariform infective third stage; i.e. a free-living generation did not occur and is probably absent in *S. robustus* in Cape Breton and possibly other parts of North America. It is hypothesized that homogonically developing *S. robustus* might be more fecund or more efficiently transmitted than species of *Strongyloides* that exhibit both homogonic and heterogonic development. Larvae of *S. robustus* in fecal cultures, i.e. homogonic larvae, are described in detail. Intestinal walls of second- and third-stage larvae, as well as the lateral chords of young third-stage larvae, contained numerous round bodies, likely nutrient stores. Third-stage larvae were present within 2 days in cultures maintained at 30°C, 4 days at 20°C, and 7 days at 15°C. They lived for at least 33 and 30 days at 15° and 20°C, respectively. Third-stage larvae probably die when their nutrient stores are exhausted.

Nematodes of the rhabditoid genus *Strongyloides* Grassi, 1879 parasitize the mucosa of the small intestine of numerous and diverse vertebrates; approximately 50 species are recognized (Speare 1989). They have an unusual biology, generally assumed to include only parthenogenetic females as parasites and one or more free-living generations with both females and males. Heterogonic (indirect, by way of a free-living generation) and homogonic (direct) routes of development give rise to the skin-penetrating, infective third-stage larvae (Schad 1989, Anderson 1992).

Strongyloides robustus Chandler, 1942 occurs in sciurids in North America and is reported herein from red squirrels (*Tamiasciurus hudsonicus* (Erxleben)) in Cape Breton Island, Nova Scotia, Canada. The species has been reported only once before in Canada but frequently in the United States (Patrick 1991a); the present report is the furthest northeast on the continent that it has been found. Its discovery offered an opportunity to redescribe it, bearing in mind recent taxonomic comments by Speare (1989) and Viney et al. (1991) on species of *Strongyloides*. As part of this endeavour, development in fecal cultures was studied to obtain free-living adults. A free-living generation, however, did not develop. This enabled detailed morphologic observations to be made on larvae known to be of the homogonic route. Earlier authors who have reported in detail on the development of other species of *Strongyloides* have had to contend with larvae of both routes in cultures (Alicata 1935, Lucker 1942, Basir 1950, Little 1966a) or they did not have homogonic larvae (Griffiths 1940, Premvati 1958, Mackerras 1959).

MATERIALS AND METHODS

Red squirrels (*Tamiasciurus hudsonicus*) were live-trapped near Sydney (46°09'N; 60°11'W), on Cape Breton Island, Nova Scotia, Canada, in May and June; 10 were trapped in 1991, 12 in 1992, and 10 in 1993. They were killed with an overdose of inhaled chloroform, their abdominal cavity opened, and the small intestine removed and examined for nematodes. Intensity of infection was not determined. Representative adult nematodes were fixed either in hot 5% glycerin-70% alcohol (and studied in pure glycerin) or in steaming 10% formalin (then transferred to glycerin-alcohol and studied in pure glycerin). Specimens of parasitic females of *Strongyloides robustus* have been deposited in the parasite collection of the US National Museum in Beltsville, Maryland, USA (accession numbers 84840 and 84841).

In a few freshly-killed squirrels, the mucosa of the intestinal region containing *S. robustus* was gently scraped and the scrapings transferred to 0.85% physiological saline in a small Petri dish. Dishes were left for a specific period of time at

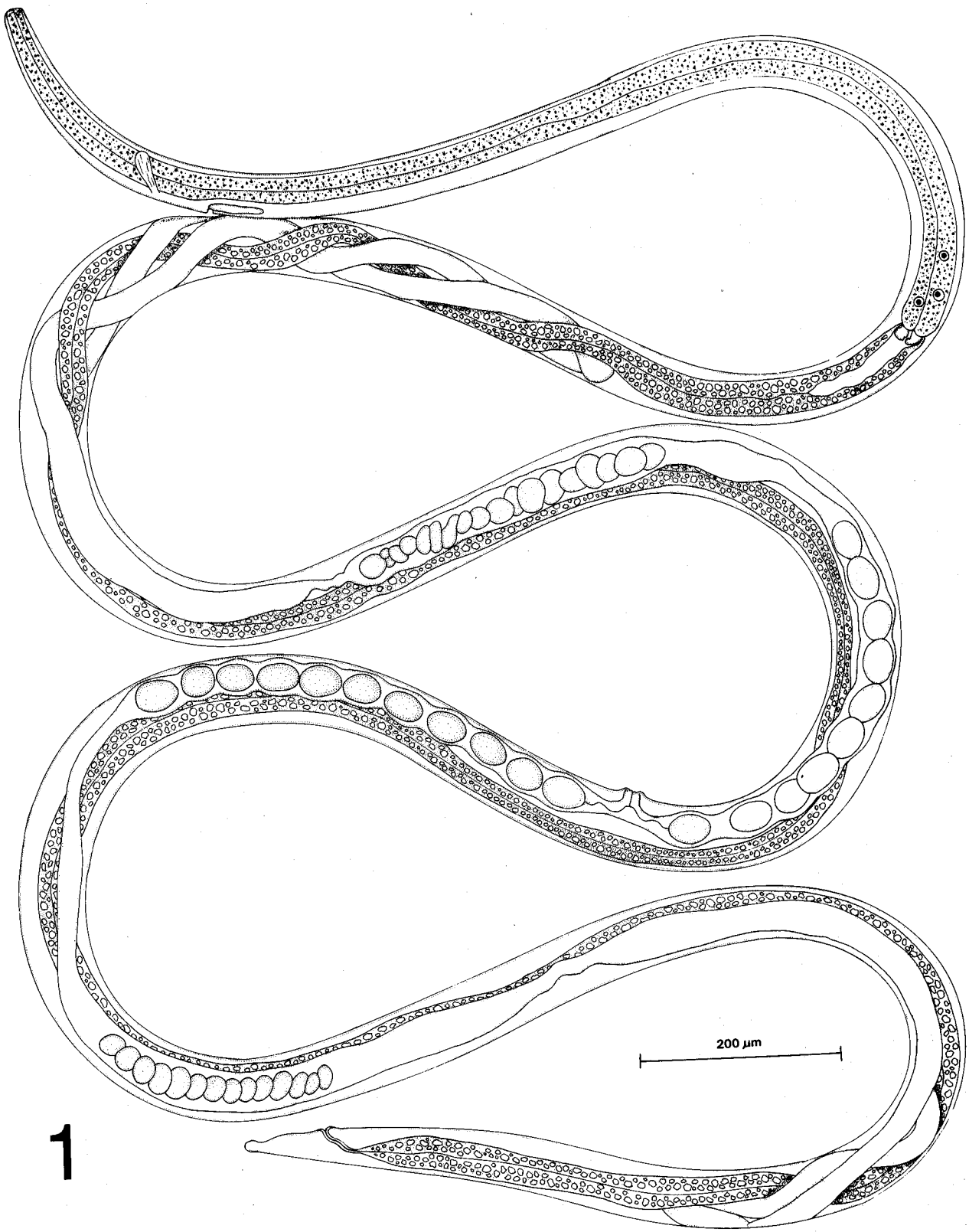
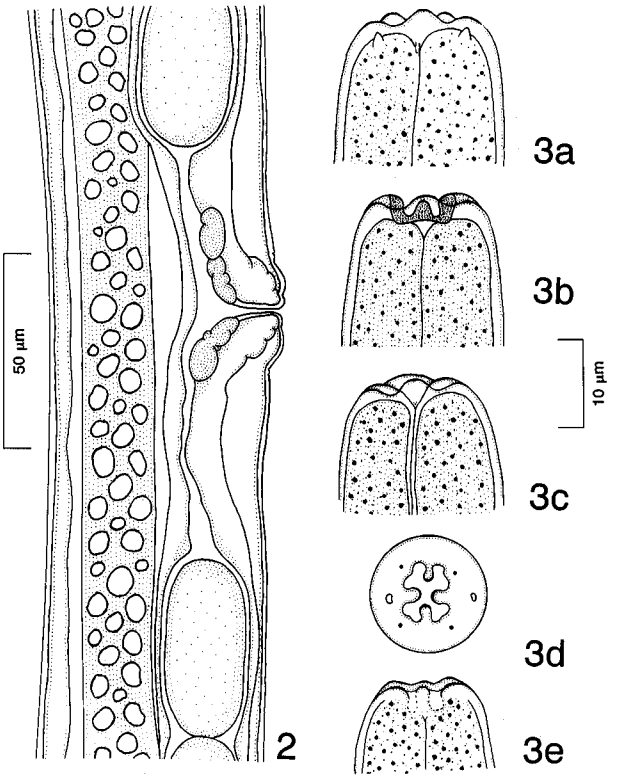


Fig. 1. Parasitic adult female of *Strongyloides robustus* Chandler, 1942.

room temperature (22°C). Some of the scraped mucosal material was then removed with a pipette, placed on a microscope slide, and an equal volume of 10% formalin added. The mixture was covered with a petroleum jelly ringed coverglass and examined with a compound microscope.

Feces of some squirrels were collected for culture purposes. Three to six fecal pellets were placed in plastic Petri dishes (100 mm × 15 mm) containing a 1 mm depth of distilled water; dishes were covered (with their normal lids) and transferred to incubators at 15°, 20°, and 30°C. At specific times after cultures had been prepared, 2–3 drops of medium containing larvae were removed using a pipette and placed on a glass microscope slide. Two to three drops of 10% formalin were added and the mixture covered with a petroleum jelly ringed coverglass. Preparations were examined with a compound microscope. Third-stage filariform larvae were studied in *en face* view following the technique of Anderson (1958) except the entire larva was substituted for the amputated head.

When a free-living generation did not develop in cultures prepared as described above, culture techniques were modified slightly, as follows (one modification per dish): (1) use of tap water rather than distilled water; (2) addition of sterile soil such that the medium was damp rather than watery; (3) addition of an inclined microscope slide covered with filter paper (following Little 1966a); and (4) initial incubation at 10°C for 3 d before transfer to temperatures previously specified.



Figs. 2–3. Parasitic adult female of *Strongyloides robustus* Chandler, 1942. **Fig. 2.** Vulva, lateral view. **Fig. 3.** Cephalic extremity; **a–c** – lateral views at decreasing depths of focus; **d** – en face; **e** – dorsal view.

RESULTS

Gravid adult females of *Strongyloides robustus* were found in the anterior-most 2–3 cm of the duodenum of 9 of 10 squirrels collected in 1991, 11 of 12 in 1992, and 10 of 10 in 1993. In general, part of the body of each nematode was threaded into the mucosa. Rarely, nematodes were free in the lumen.

Description of the parasitic female (Figs. 1–3; Table 1) ***Strongyloides robustus*** Chandler, 1942 (Rhabditoidea, Strongyloididae): N = 40 (20 fixed in 5% glycerin-70% alcohol, 20 fixed in 10% formalin), measurements in Table 1. Cephalic extremity with complex X-shaped mouth, 4 papillae, and 8 lobes on circumoral elevation (1 dorsal, 1 ventral, 2 subdorsal, 2 lateral, 2 subventral). Esophagus 17.8–28.9% of body length in specimens fixed in glycerin-alcohol and 18.1–25.0% in specimens in formalin. Vulva located 57.8–65.2% along body length from anterior end in specimens fixed in glycerin-alcohol and 57.3–64.8% in specimens in formalin. Lips of vulva protuberant but prominence variable. Uteri containing eggs in single row, 1–19 in each uterus but generally 8–13. Branches of ovary spiralled around intestine; anterior branch spiralling 2–2.5 times, posterior branch spiralling 1–1.5 times. Tail digitiform and bluntly rounded; extremity frequently (but not always) slightly swollen and thus knob-like in appearance.

Eggs

Strings of eggs in the 1–2 cell stage were found in the duodenal mucosa adjacent to females. Individual eggs only were found in freshly-passed fecal pellets; they (N = 15) were 45–72 (average 57.9) µm long and 33–42 (35.7) µm wide and contained tadpole-stage embryos.

In mucosal scrapings in saline at 22°C for 1 hr, most eggs contained multi-celled or tadpole stage embryos while a few contained recognizable larvae folded in half. The oolemma was visible as a slightly detached membrane. At 6 hr, a few eggs contained the same developmental stages as at 1 hr, but most contained coiled ready-to-hatch larvae and a few had hatched. Eggs vacated by larvae contained wrinkled remnants of the oolemma.

Development in fecal cultures

General: The higher the temperature at which fecal cultures were maintained, the earlier the eggs hatched (Table 2). Larvae invariably developed to the filariform third stage, i.e. a free-living generation did not develop, regardless of temperature (Table 2) or culture technique. Development was asynchronous, regardless of temperature. Thus, the earliest cultures containing second-stage rhabditiform larvae also contained first-stage rhabditiform larvae. Similarly, those containing molting

Table 1. Measurements, as mean followed by range (in µm unless otherwise stated), of gravid parasitic females of *Strongyloides robustus* obtained from four different red squirrels *(Tamiasciurus hudsonicus)* near Sydney, Nova Scotia, Canada. Specimens were fixed live in hot 5% glycerin-70% alcohol or steaming 10% formalin.

	Squirrel # 92–5		Squirrel # 93–4		Squirrel # 93–7		Squirrel # 93–13	
Fixative	glycerin-alcohol		glycerin-alcohol		formalin		formalin	
N	9		11		11		9	
Body length (mm)	4.3	3.6–4.9	4.1	3.8–4.7	6.0	5.5–6.5	7.3	6.4–8.0
Body width at end of esophagus	42	36–50	46	40–52	56	52–58	58	56 –62
Body width at vulva	44	42–50	50	46–56	61	56–64	62	56–65
Esophagus, length (mm)	0.90	0.80–1.10	1.01	0.90–1.10	1.29	1.14–1.40	1.56	1.45–1.70
Esophagus, % of total length of body	20.9	17.8–25.6	24.6	21.3–28.9	21.5	18.1–23.3	21.4	18.8–25.0
Vulva, from anterior (mm)	2.7	2.3–3.0	2.55	2.3–3.0	3.6	3.3–3..9	4.5	3.7–5.0
Vulva, % of body length from anterior	62.7	61.2–65.2	61.7	57.8–64.8	60.0	58.3–63.6	61.6	57.8–64.8
Extent of anterior branch of ovary*	153	140–200	114	30–240	238	168–348	244	132–348
Extent of posterior branch of ovary**	172	120–220	166	96–228	279	160–428	246	160–384
Anus from posterior extremity	77	64–96	71	52–84	101	90–116	96	88–110

* distance behind posterior end of esophagus
** distance anterior to anus

second-stage larvae also contained rhabditiform first and second stages and those containing filariform third-stage larvae also contained rhabditiform larvae and molting second-stage larvae.

Filariform third-stage larvae were first observed within 2–7 days, depending on temperature (Table 2). Live filariform larvae were still present when cultures at 20°C were last examined at 30 days and cultures at 15°C at 33 days; motility of larvae in older cultures was markedly reduced over that at earlier times (< 15 days), however. At 30°C, all larvae died by 10 days.

Newly hatched rhabditiform L_1 in 6 hr culture at 20°C (Fig. 4; Table 3): Cephalic cuticle not closely adherent to hypodermis; cuticle over remainder of body and tail closely adherent to hypodermis. Four delicate cephalic papillae present. Mouth opening into cylindrical buccal cavity; walls of cavity lined anteriorly by thin cuticle and posteriorly by thicker hyaline cuticle.

Table 2. Time of first appearance of different larval stages of *Strongyloides robustus* in fecal cultures of red squirrels *(Tamiasciurus hudsonicus)* at different temperatures.

	15°C	20°C	30°C
Newly hatched larva	30 hr	6-8 hr	3 hr
Molting L_1	-	30 hr	17 hr
Rhaditiform L_2	6 d	2.5 d	23 hr
Molting L_2	-	3.5 d	30 hr
Filariform L_3	7 d	4 d	2 d

Esophagus rhabditiform, i.e. consisting of: (1) corpus with anterior, short vestibule (slightly constricted at posterior end) and longer, broader posterior portion; (2) long, narrow isthmus; and (3) valved bulb. Muscular striations within esophagus strongly marked in corpus and bulb but delicately marked in isthmus. Intestine well-developed with thick, translucent walls containing prominent pairs of large nuclei; lumen narrow and empty. Anus readily apparent. Tail attenuated with tip delicately teardrop shaped. Excretory pore near anterior of isthmus. Nerve ring difficult to distinguish. Genital primordium near mid-body, consisting of few cells.

Rhabditiform L_1 in 18 hr culture at 20°C (Fig. 5, Table 3): Morphologically similar to larva at 6 hr except: Body longer. Hyaline cuticle of buccal cavity with short elongate or squat anterior portion and longer elongate posterior portion. Intestinal walls as previously; uppermost 20–30 µm of intestinal lumen dilated and containing brownish particulate material; remainder of intestinal lumen narrow. Cuticle detached at tip of tail.

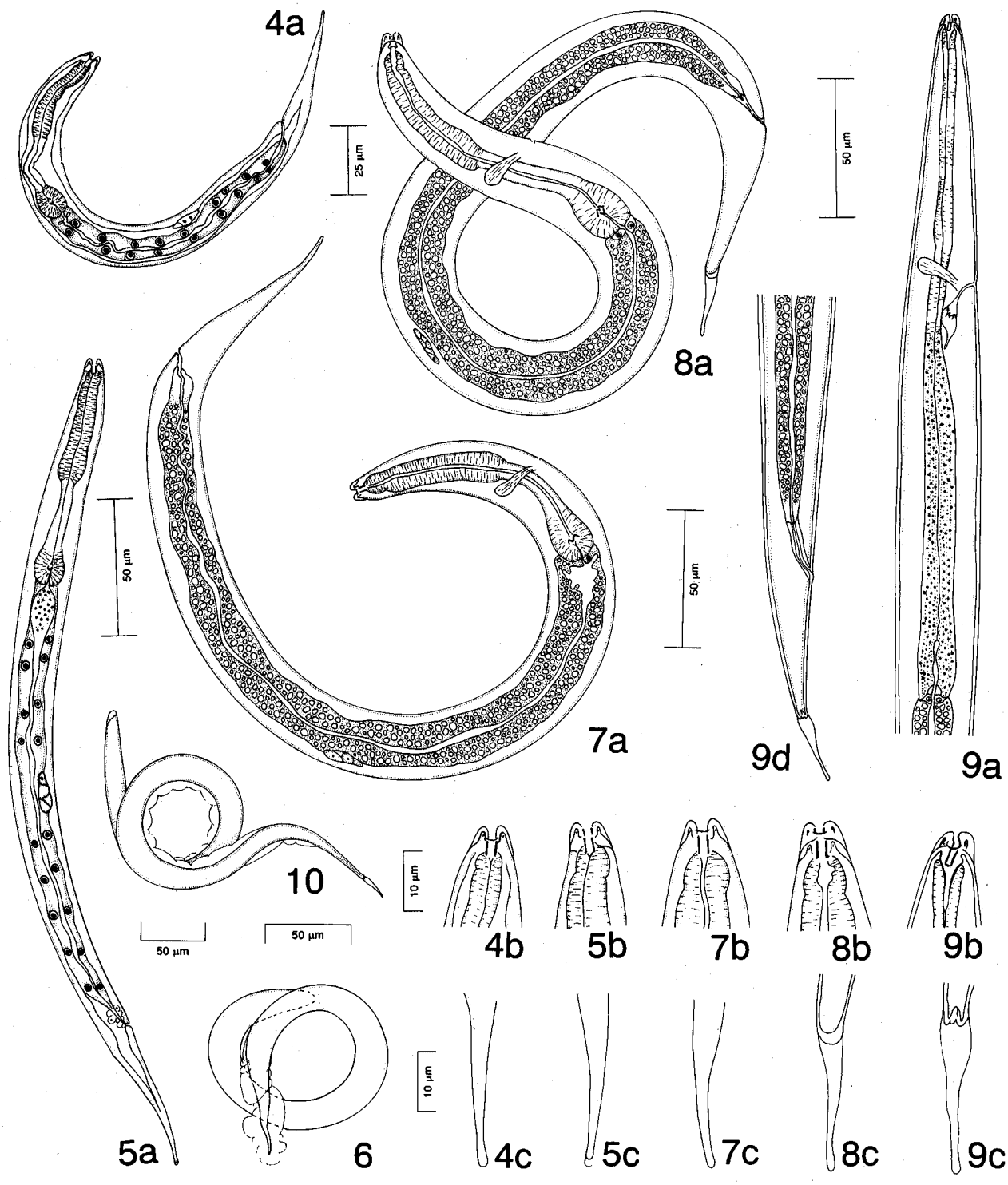
Late rhabditiform L_1 in 23 hr culture at 20°C (not illustrated, not measured): Morphologically similar to larva at 18 hr except: Cuticle of cephalic extremity doubled; outermost layer lifted and containing faint outlines of papillae; innermost layer with prominent papillae. Cuticle of buccal cavity doubled; outermost portion elongate or squat and clearly separated from innermost elongate portion. Intestinal walls with inconspicuous nuclei. Cuticle detached at tip of tail.

First molt: Once the cuticle over the entire body loosened (in addition to that at the cephalic and tail extremities that had detached earlier, as previously

Table 3. Measurements (mean, followed by range in parentheses) in μm unless otherwise specified of different larval of stages of *Strongyloides robustus* in fecal cultures of red squirrels (*Tamiasciurus hudsonicus*).

	Rhabditiform L_1, 6 hr, 20°C	Rhabditiform L_1, 18 hr, 20°C	Early rhabditiform L_2, 23 hr, 30°C	Late rhabditiform L_2, 23 hr, 30°C	Filariform L_2 30 hr, 30°C	Filariform L_3 48 hr, 30°C
N	5	5	5	5	5	10
Body length	229.0 (224–236)	262.6 (254–274)	481.6 (431–553)	565.4 (503–655)	636.6 (572–694)	670 (613–702)
Buccal cavity, depth	–	–	–	–	7.4 (7–8)	8.6 (7–10)
thin cuticle	2.4 (2–3)	4.0 (4)	2.2 (2–3)	4.0 (4)	–	–
elongate, hyaline cuticle	3.6 (3–4)	4.0 (4)	3.4 (3–4)	4.0 (4)	–	–
Esophagus length, total	76.8 (70–79)	75.4 (72–79)	104.0 (97–117)	153.2 (135–190)	274.4 (260–286)	282.6 (266–294)
vestibule	4.8 (4–6)	5.6 (5–7)	7 (6–8)	125.2 (100–160)	–	–
remainder of corpus	31.6 (30–32)	33.4 (30–36)	48.4 (45–56)		–	–
isthmus	19.8 (18–21)	22.0 (20–24)	127.0 (22–32)		–	–
bulb	20.6 (15–24)	14.1 (13–15)	23.2 (20–27)	26 (20–30)	–	–
Intestine length	106.8 (97–118)	131.2 (127–137)	294.8 (250–354)	317.2 (274–412)	265.6 (214–314)	312.8 (280–330)
Body with, at junction of esophagus and intestine	15 (15)	15.6 (15–16)	25.2 (24–28)	29.2 (26–30)	19.6 (18–20)	22.2 (20–24)
Body width, at anus	8.2 (6–9)	9.2 (8–10)	17.4 (17–18)	17.6 (16–18)	14.6 (13–16)	15.1 (13–16)
Anus from tip of tail	39.4 (30–45)	48.0 (46–50)	75.2 (70–90)	59.2 (54–70)*	63.6 (60–70)*	66.0 (60–72)
Length of detached tail cuticle	–	–	–	28.2 (20–30)	25.0 (20–30)	–

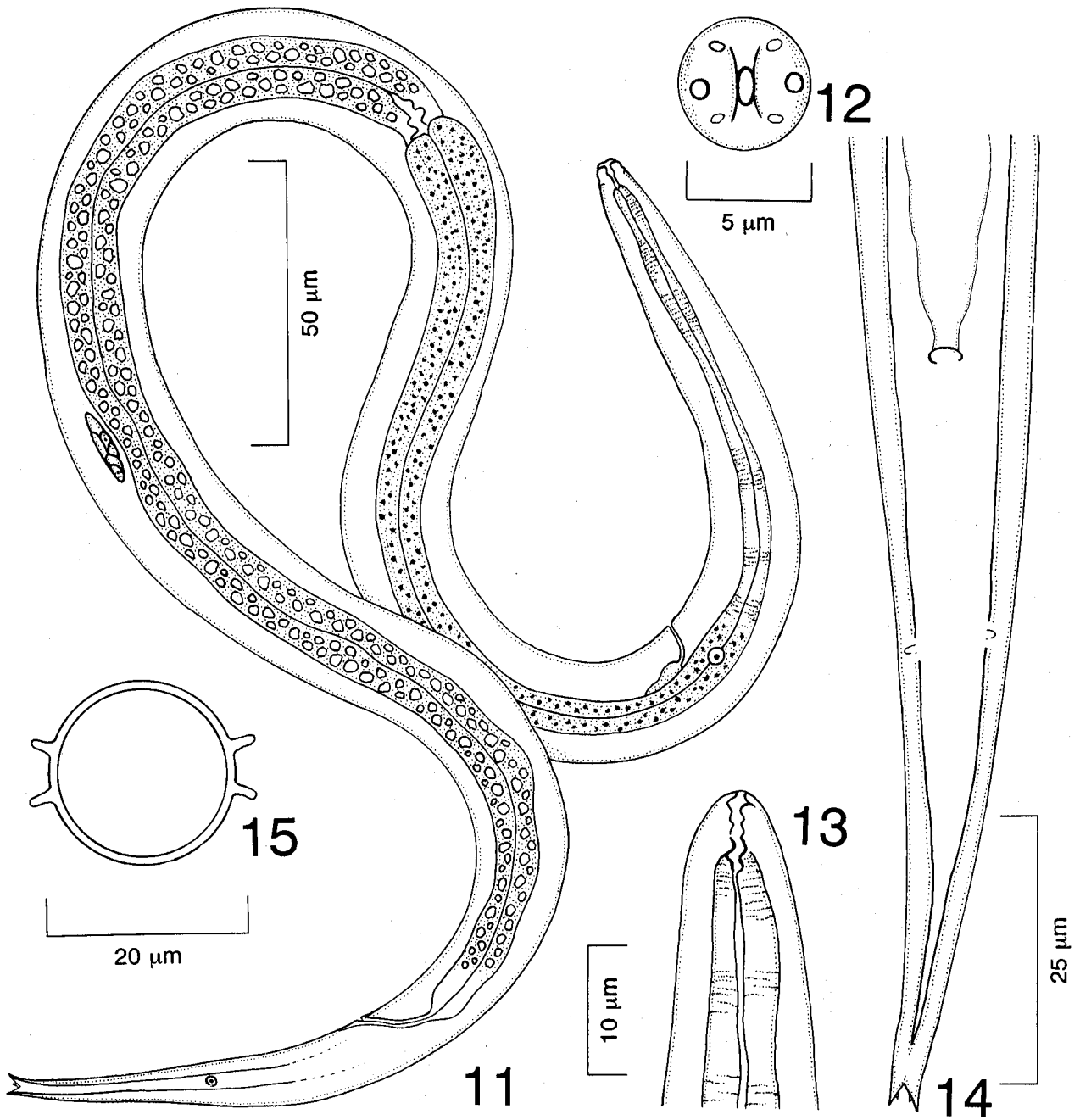
* distance to posterior-most extent of hypodermis



Figs. 4–10. First- and second-stage homogonic larvae of *Strongyloides robustus* Chandler, 1942. **Fig. 4.** Newly hatched rhabditiform first stage in 6 hr culture at 20°C; **a**. whole specimen; **b**. anterior extremity showing buccal cavity and anterior corpus including vestibule; **c**. posterior extremity. **Fig. 5.** Rhabditiform first stage in 18 hr culture at 20°C; **a**. whole specimen; **b**. anterior extremity; **c**. posterior extremity. **Fig. 6.** Molting first stage. **Fig. 7.** Early rhabditiform second stage in 23 hr culture at 30°C; **a**. whole specimen; **b**. anterior extremity; **c**. posterior extremity. **Fig. 8.** Late rhabditiform second stage in 23 hr culture at 30°C; **a**. whole specimen; **b**. anterior extremity; **c**. posterior extremity. **Fig. 9.** Filariform second stage in 30 hr culture at 30°C; **a**. anterior end; **b**. anterior extremity; **c**. posterior extremity; **d**. posterior end. **Fig. 10.** Molting second stage.

mentioned), it was shed exceedingly rapidly. Thus, few larvae were seen in the actual process of molting, regardless of temperature; cuticle was shed as a wrinkled, rolled sheath from the tail region (Fig. 6). Shed cuticles were not seen in fecal cultures. (Note: In comparison, and with respect to larvae developing on mucosal scrapings rather than in fecal cultures, shed cuticle was commonly seen on mucosal material adjacent to second-stage larvae.) Larvae molted earlier in cultures maintained at higher temperatures (Table 2).

Early rhabditiform L_2 in 23 hr culture at 30°C (Fig. 7; Table 3): Morphologically similar to late L_1



Figs. 11–15. Filariform third-stage larva of *Strongyloides robustus* Chandler, 1942. **Fig. 11.** Whole specimen, lateral view; note slight constriction in diameter of body 4–5 µm from tip of anterior extremity and, also, deirid located near excretory pore. **Fig. 12.** Anterior extremity, en face view. **Fig. 13.** Anterior extremity, dorsal view. **Fig. 14.** Posterior extremity, ventral view; note post deirids located midway between anus and tip of tail. **Fig. 15.** Transverse section at midbody showing doubled form of lateral alae (diagrammatic).

except: Cephalic cuticle not closely adherent to hypodermis, i.e. similar to that of newly hatched L_1. Intestinal walls generally packed with round bodies that impart dark brown appearance to whole of intestine when viewed with light microscope; lumen empty. Cuticle adherent to hypodermis at tip of tail.

Late rhabditiform L_2 in 23 hr culture at 30°C (Fig. 8, Table 3): Morphologically similar to early rhabditiform L_2 except: Cuticle detached at both extremities but closely adherent to hypodermis over remainder of body. Cuticle of cephalic extremity and buccal cavity doubled. Intestine always packed with round bodies and dark brown in appearance. Anus with slightly protuberant posterior lip. Nerve ring and excretory pore both readily apparent near anterior third of isthmus. Cuticle of tail detached over posterior third of extent; tip of detached cuticle teardrop shaped.

Filariform L_2 in 30 hr culture at 30°C (Fig. 9; Table 3): Morphologically similar to late rhabditiform L_2 except: Body longer and narrower. Buccal cavity consisting of detached, outermost, squat cuticle and inner, elongate cuticle. Esophagus filariform i.e., club-shaped with anterior muscular and posterior glandular portions; muscular striations delicate. Nerve ring and excretory pore readily apparent near posterior end of muscular portion of esophagus. Detached cuticle of tail similar to late L_2 or slightly swollen in region immediately behind hypodermis; in some larvae, one or more small hyaline bodies present within area bounded by detached cuticle.

Second molt: Once the cuticle over the entire body loosened (in addition to that at the cephalic and tail extremities that had detached earlier, as previously mentioned), it was retained for some time before being shed completely. Thus, many larvae retaining a loose second-stage cuticle were observed (Fig. 10). Shed cuticles were rarely observed in the culture medium. The intestinal walls of molting larvae contained numerous round bodies but were generally lighter brown than in younger larvae. In many larvae, round bodies were also present anterior to the esophageal-intestinal junction, particularly in the lateral chords of third-stage larvae; these bodies were much less dense than those in the intestine.

Filariform L_3 in 48 hr culture at 30°C (Figs. 11–15, Table 3): Larva slender; in lateral view diameter of anterior body slightly constricted 4-5 μm from tip. Cuticle delicately striated, closely adherent to hypodermis over whole of body. Cephalic extremity with four papillae, two amphids, two lateral lobes, and oval oral opening. Buccal cavity narrow, lined anteriorly by thin cuticle and posteriorly by hyaline cuticle, each 3–4 μm long. Esophagus filariform; anterior portion narrow, with few overt muscular striations; posterior portion glandular and increasing in width posteriorly. Esophageal gland nuclei not observed. Intestinal wall with round bodies (note: numbers and size of bodies decreased as larvae aged); lumen empty. Rectum lined with hyaline cuticle. Anus readily apparent, posterior lip slightly protuberant. Nerve ring and excretory pore near posterior end of anterior portion of esophagus. Genital primordium near mid-body, consisting of few cells. Lateral alae present, doubled, readily apparent from approximately 50 μm behind anterior extremity of body to tip of tail and culminating in four terminal points. Tail attenuated, tip quadripartite due to alae (note: twisting of body frequently causes tip to appear tripartite, rather than quadripartite). Many larvae with round bodies in lateral chords; density varying considerably among larvae. Deirids present, one within each ala near excretory pore. Phasmids present, one within each ala approximately halfway between anus and tip of tail.

DISCUSSION

Parasitic females in red squirrels in the present study were morphologically similar to those described as *Strongyloides robustus* by Chandler (1942) and Eckerlin (1974). The female is characterised by her length (see below) and the presence of an X-shaped mouth, 8 circumoral lobes, ovaries that spiral around the intestine, and a bluntly rounded tail (traits recommended by Speare 1989 as taxonomically important). There are numerous reports of *S. robustus* in various other sciurids in many parts of North America (Table 4) but few of these reports contain morphological or mensural information. The parasite was first reported in red squirrels by Lichtenfels (1966) although Patrick (1991a) felt the unidentified species reported earlier in red squirrels by Rausch and Tiner (1948) was *S. robustus*.

Speare (1989) attemptedd to facilitate identification of species of *Strongyloides* by using body length of parasitic females to separate 49 species into three groups; he placed *S. robustus* in the "large" (> 5 mm) group. Some females in the present study were shorter (range 3.8–8.0 mm) than 5 mm, however, as were some of Chandler's (1942) (4.5–6.8), Parker's (1971) (3.90–7.84), Eckerlin's (1974) (4.72–7.36) and Patrick's (1991a) (3.18–7.97). Lichtenfels (1966) reported females 5.14–6.71 mm long; he also determined that Dozier and Hall's (1944) specimens averaged 6.06 mm in length. Speare (1989) recognized the problems in using body length as a taxonomic character, as did Viney et al. (1991) and Little (1966a, b), and thought it would be most useful when combined with other taxonomic characters. Among authors who have studied *S. robustus*, only Chandler (1942) and Eckerlin (1974) provided both measurements and morphologic details.

If body length is used as a taxonomic character, a standardised fixation technique is required. The present

Table 4. Reports of *Strongyloides robustus* Chandler, 1942 (or *Strongyloides* sp., as indicated) in sciurids in North America.

		Sciurid species*							
	location	fox	gray	red	northern	southern	Richardson's	13-lined	chipmunk
Chitwooda Graham 1940**	United States		+						
Chandler 1942	Texas	+	+						
Reiber and Byrd 1942***	Tennessee		+						
Dozier and Hall 1944	Maryland	+							
Rausch and Tiner 1948[+]	Michigan, Wisconsin	+	+	+					
Heck 1951	Kansas	+	+						
Packard 1956	Kansas	+	+						
Lichtenfels 1966	Maryland			+					
Lichtenfels and Haley 1968	Maryland			+					
Olexik et al. 1969**	Tennessee	+	+						
Parker 1971	Virginia		+						
Parker and Holliman 1971	North Carolina		+						
Parker et al. 1972	Florida		+						
Eckerlin 1974	Connecticut		+	+	+	+			
	Florida		+			+			
	New Jersey		+						
Davidson 1976	10 states[++]		+						
McGee 1980	Saskatchewan			+			+	+	
Conti et al. 1984	Florida		+						
Parker 1984	Georgia		+						
Eckerlin 1985	Virginia		+						
Pagels et al. 1990	Virginia				+				
Patrick 1991a	Pennsylvania		+	+		+			+
Patrick 1991b	Pennsylvania					+			
Eckerlin 1993	Maryland, Virginia	+							
Wetzel and Weigl 1994	North Carolina					+			
present study	Nova Scotia			+					

* Sciurid species are: fox squirrel *(Sciurus niger)* (type), gray squirrel (*Sciurus carolinensis),* red squirrel *(Tamiasciurus hudsonicus*), northern flying squirrel *(Glaucomys sabrinus*), southern flying squirrel (*Glaucomys volans*), Richardson's ground squirrel (*Spermophilus richardsonii),* thirteen-lined ground squirrel *(Spermophilus tridecemlineatus*), and eastern chipmunk (*Tamias striatus).*

** reported as *"Strongyloides"* sp.

*** described as *"S. papillosis"* (Wedl, 1856)"; description considered by Patrick (1991a) as "comparable to that of *S. robustus.*"

[+] reported as *"Strongyloides* sp."; considered by Parker (1971) and Patrick (1991a) as likely to be *S. robustus.*

[++] Alabama, Georgia, Kentucky, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, West Virginia

study used steaming 10% formalin, as recommended by Speare (1989) and Viney et al. (1991), and hot 5% glycerin-70% alcohol, a standard fixative for parasitic nematodes. All specimens fixed in formalin easily qualified for Speare's (1989) "large" group, as did some fixed in glycerin-alcohol. Others fixed in glycerin-alcohol fell into the "medium" group (2–5 mm). Speare (1989) noted that 70% alcohol sometimes causes shrinkage of specimens of *Strongyloides*. Only Patrick (1991a) has reported specimens of *S. robustus* shorter than those in the present study; his specimens were also fixed in hot glycerin-70% alcohol.

Little (1966a, b) demonstrated the utility of including the free-living adults in descriptions of species of *Strongyloides*. *Strongyloides robustus* might, however, lack a free-living generation as development in cultures proceeded directly and invariably to the filariform third stage (the stage infective to the host and the only type of third-stage larva in the homogonic developmental route – see Anderson 1992). Eckerlin (1974) and Wetzel and Weigl (1994) also studied development of *S. robustus* and, although not stated *per se*, apparently did not observe a free-living generation. Wetzel and Weigl (1994) simply proposed that the "life cycle pattern of *S. robustus* is dominated by a homogonic strategy" in cooler habitats or northern ranges of some sciurids, including their study in North Carolina. Eckerlin (1974), on the other hand, mentioned first-stage larvae "probably not of the direct cycle." His failure, therefore, to mention free-living adults is curious because: (1) he would have been aware of their taxonomic importance (as established by Little 1966a,b) since his study involved a redescription of *S. robustus* and (2) they should have appeared in cultures soon after those larvae supposedly of the indirect (heterogonic) developmental route were observed. (Note: In contrast to the above, R. P. Eckerlin [personal communication, February 1995] states that he has observed a free-living generation in fecal cultures from some populations of gray squirrels, *Sciurus carolinensis*, in Virginia, New Jersey, and Connecticut, United States; this requires additional study.) In species that have a free-living generation, adults usually appear within 2–5 days at 25°C and the ease in culturing them, regardless of the species, is reflected in Little's (1966a) statement "The free-living adults have been more thoroughly studied in the past than have the parasitic females, the probable reason being that these stages are more easily obtained in the living condition." Dawkins (1989, p. 291), however, noted that "some larvae from 'pure' homogonic strains of *S. ratti* have been observed to go through a free-living cycle in cultures that have remained moist and been left for long periods of time (Grove and Northern, personal communication)."

The absence of a free-living generation is rare among species of *Strongyloides*, having been noted in some strains of *S. stercoralis* (Bavay, 1876), *S. ratti* Sandground, 1925, and *S. venezuelensis* Brumpt, 1934 (see Wertheim and Lengy 1964, Dawkins 1989, Speare 1989). Numerous workers (e.g. Moncol and Triantaphyllou 1978, Yamada et al. 1991, Viney et al. 1992) have sought to determine factors that influence development disproportionately along the homogonic or heterogonic route. Genetic factors and conditions in the host and culture media are considered important.

Eggs of *S. robustus* passed in the feces contained larvae at the tadpole stage of development (Eckerlin 1974, present study). The stage in fresh feces is considered taxonomically important (Little 1966a); other possibilities include first-stage larvae, a combination of larvae and eggs, or strings of eggs (Ashford and Barnish 1989). Strings were observed in the present study, but only in the intestinal mucosa.

First-stage larvae of species of *Strongyloides* in fecal cultures frequently must be differentiated from those of other parasites or free-living contaminants (e.g. *Rhabditis* species). Schad (1989) states that rhabditiform larvae of *S. stercoralis* have a "buccal capsule [that] is a shallow, cup-shaped depression" and that this "salient differential diagnostic feature" distinguishes these larvae from those of hookworms where the buccal capsule is a "chitinoid buccal tube." Little (1966a), however, states that the typical first-stage larva of various *Strongyloides* species (including *S. stercoralis*) has a cylindrical "stoma 5–8 µm long." Observations in the present study agree with those of Little. Lucker (1942) emphasized the importance of the vestibule in differentiating larvae of *Strongyloides* species from those of hookworms and *Rhabditis* species. This structure was prominent in the rhabditiform esophagus of *S. robustus*.

Basir (1950) noted that the first larval molt of *S. papillosus* (Wedl, 1856) is easily overlooked since the shedding of cuticle occurs exceedingly quickly. This is also true of *S. robustus* and probably accounts for reports in the early literature (see summary in Alicata 1935) of only one molt in some species prior to the appearance of filariform third-stage larvae. Among many parasitic nematodes, detached cephalic cuticle indicates that a molt is imminent. In *S. robustus* and other species of *Strongyloides*, however, detached cephalic cuticle is apparent shortly after eggs hatch and for many hours before the first molt. The difficulty in ascertaining the first molt is further increased by the fact that tail morphology does not change between the first and early second stages. Conclusive evidence of the first molt of *S. robustus* was found among larvae developing in mucosal scrapings where shed cuticle adhered to mucosal material and was readily apparent. In contrast, larvae in fecal cultures probably quickly moved away from shed cuticles which then became lost in the medium.

Readily apparent, round bodies appeared in the

intestinal cells of second-stage larvae in the present study. This suggests, based on Bird and Bird's (1991) statement that dietary sources of proteins and lipids are often stored as round bodies in the intestinal cells of nematodes, that it is the second stage of *S. robustus* that actively stores nutrients. Reports of similar intestinal bodies (as "granules" or as a "granulated" or "granular" intestine) are rare for second-stage larvae of other species of *Strongyloides*; Schuurmans Stekhoven (1928) reported them in *S. stercoralis*. They have more commonly been reported in third-stage larvae of various species (Leuckart 1883, Alicata 1935, Lucker 1942, Eckerlin 1974) and Stepanow-Grigoriew and Hoeppli (1926) stated that the bodies ("refractile cells") "have a fat-like consistency" (see Kreis 1932). Curiously, Schad's (1989) review of the morphogenesis of *S. stercoralis* does not mention them, nor do the studies of Little (1966a) or Basir (1950), who made (after the peculiar life cycles of species of *Strongyloides* were understood) some of the most detailed observations on the morphology of larvae of any species. Indeed, Little (1966a) observed that cells of the intestinal walls divided during the second stage; in the present study, the packed nature of intestinal bodies precluded a similar observation.

Round bodies were also noted in third-stage larvae in the present study, both in the intestinal walls and lateral chords. Previous authors have not reported them (or "granules") in the latter location. Their presence in the lateral chords is likely related to the presumably high energy demands of the nearby muscles of the body wall. Continuous, rapid movement characterises young third-stage larvae of species of *Strongyloides*.

The *en face* morphology of the infective third stage of *S. robustus*, as determined herein, agrees with the cephalic morphology reported by Little (1966a) for various species. Schad (1989) commented, however, that the *en face* proper of *S. stercoralis* had not been studied and that Little's observations should be viewed with caution. Schad suggested a lateral pair of papillae should be present, in addition to the reported subventral and subdorsal pairs, and that electron microscopy may be required to see them. Schuurmans Stekhoven (1928) thought three lips were present; Little (1966a) reported two lateral lobes.

Larvae took longer in the present study to develop to the third stage than those of *S. robustus* studied by Eckerlin (1974) and Wetzel and Weigl (1994). At 15°C, 7 versus 4 days were required and at 20°C, 4 versus 2 days. Larvae in the present study survived longer, however. Motile third-stage larvae were still present when cultures at 15°C and 20°C were examined in the present study at 30 days, whereas Wetzel and Weigl (1994) reported that all larvae died by 12 days at these same temperatures. Thus, Wetzel's and Weigl's (1994) conclusion that "the opportunity [for *S. robustus*] to infect a host decreases as environmental temperature decreases because of the declining numbers of juveniles [=strongyliform larvae] available to infect a host ..." is less applicable to the strain of *S. robustus* in the cooler climate of Cape Breton than theirs in the warmer climate of North Carolina. Filariform larvae probably do not feed and thus, die once their nutrient stores are exhausted. In the present study, the presumed nutrient stores (i.e. round bodies) were abundant in young filariform larvae but not those 30 or more days old. Depletion of food storage bodies has been noted in other nematodes as they age (Bird and Bird 1991).

In species of *Strongyloides* with one or more free-living generations, the skin-penetrating, infective filariform larvae arise through both homogonic and heterogonic developmental routes. The latter route presumably augments the numbers of infective larvae available in the environment and thus, presumably enhances fitness. The possibilities that *S. robustus* (having eliminated the heterogonic route) exhibits higher fecundity in the parasitic phase than other species or that transmission is comparatively more efficient (possibly taking place in the microenvironment of the host's nest) merit additional study.

The high prevalence of *S. robustus* in Cape Breton (94% of 32 red squirrels) tends to conflict with the conclusion of Chandler and Read (1961, p. 467) who stated "The larvae of *Strongyloides* are easily destroyed by cold, desiccation, or direct sunlight, and are rather short-lived even under the most favourable conditions. This probably accounts for the infrequencies of *Strongyloides* infections outside warm, moist climates." The possibility mentioned above, namely that the nest might be a site of transmission of *S. robustus*, could also be important as it is undoubtedly a warm, as well as confined, environment. Wetzel and Weigl (1994) thought nests of the southern flying squirrel (*Glaucomys volans*) may be important in the transmission of *S. robustus* as they sometimes contain fecal material and because filariform larvae were found to move toward temperatures approximating those of the host in its nest.

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