

Studies on the Nematode *Subulura suctorica*

III. Development in the Definitive Host

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Abstract. The life-history of the nematode *Subulura suctorica* has been studied in the definitive host. In addition to the morphology of the individual larval stages the length of time of the prepatent period (37–46 days) and the longevity (6–8.5 months) have been established. Histopathological examination revealed that no marked pathological changes are caused either by the larval stages or by the adults in the definitive host.

The few studies available on the development of the nematode *Subulura suctorica* (MOLIN 1860) – (CUCKLER and ALICATA 1944; ABDOU and SELIM 1963; BUŠA and LORENZO HERNÁNDEZ 1966), offer little information on this developmental phase. In view of the fact that this nematode is a frequent parasite of domestic fowl in tropical and subtropical regions we carried out a number of experiments in order to elucidate its development in the definitive host and to obtain information on the duration of the prepatent period and on the longevity of the parasite. The development of the nematode *S. suctorica* in its intermediate host has been described in an earlier paper (BARUŠ 1970).

MATERIAL AND METHODS

The definitive hosts and the mode of infection: Our experiments were carried out with Leghorn chickens obtained at the age of one day from the incubator and kept under laboratory conditions, without the possibility of a natural invasion, to the age of 4 weeks. Infected intermediate hosts (naturally invaded beetles of the species *Alphitobius diaperinus* Panzer) collected on the chicken farm J. A. Mella in the province Havana were used as the source of infection. Part of the beetle population had been examined beforehand in order to establish the average incidence of larvae of *S. suctorica*. Infection with *S. suctorica* was produced by artificially administering infected beetles to either starved or unstarved chickens (duration of starvation before infection 24 and 12 hrs). All chickens were kept in the laboratory under infection-free conditions and fed only with Cuban produced chickenfood mixture.

The development of the larvae: We fed each of the 50 chickens with 500 infected beetles (approximately 800 invasive larvae). The results of infection and development of the larvae were obtained

in postmortem examinations. On the first day we dissected chickens at intervals of 2, 4, 6, 12 and 24 hrs after infection, on the following days at intervals of 2—3 days until day 36. From day 30 onwards, the faeces of each chicken were examined with Breza's method (BREZA 1964). The experiment was completed when eggs were found and adult nematodes recovered. The morphology of the larvae has been under constant observation; the most important details have been measured and figured.

Part of the material of this experiment has been examined histopathologically (the caeca were fixed in 10 % formalin). Material from guinea fowl, Japan quail and from naturally infected chickens has also been examined.

Observation of the prepatent period and the longevity of the nematode: In this experiment, after starving 20 chickens, each was fed with a single dose of 200 naturally infected beetles (approximately 300 invasive larvae per dose). Faecal examination started on day 30. The faeces of each chicken were inspected twice a day with Breza's method. The finding of eggs of *S. suctoria* was confirmed in the postmortem of 10 chickens. The faeces of the remaining 10 chickens were examined every second day. If no eggs were present in the faeces of a chicken in three consecutive faecal examinations, the absence of the nematodes was confirmed in postmortem.

Our experiments have been carried out in the Biological Institute of the Cuban Academy of Sciences in Havana and at the experimental centre Combinado Avícola Nacional, farm "Jesús Menéndez", Havana.

RESULTS

A. DEVELOPMENT AND MORPHOLOGY OF THE LARVAL STAGES

The intermediate hosts (beetles of the species *A. diaperinus*) when ingested by the chickens, are crushed mechanically in the gizzard. The invasive larvae emerge from the cysts, straighten and shed the cuticle from the second moulting in the intermediate host (either in the gizzard or in the upper part of the small intestine). They reach their definitive site of location in the caeca by being carried along with the contents of the intestine. The length of time which the larvae need to reach the caeca depends on the degree of filling of the digestive tube. In our experiments with hosts starved for 24 hrs, larvae were present in the caeca 6 hrs after invasion, while in normally fed hosts they reached the caeca after 24 hrs. No further migration occurs in the definitive host, larval development being completed in the lumen of the caeca.

Parasitic 3rd-stage larvae (Fig. 1A, B) measure 6 hrs after invasion 0.70 to 0.94 mm in length, their maximum width is 0.086—0.110 mm. The anterior end of the body is blunt, width 0.020—0.024 mm. The mouth is terminal, surrounded by 6 small papillae. The buccal cavity is small, depth 0.006—0.008 mm, width 0.008 mm. The oesophagus is 0.205—0.221 mm long and divided into a corpus, isthmus and a bulbus with the valvular apparatus. The nerve ring is at 0.079 to 0.094 mm, the excretory pore at 0.086—0.114 mm from the anterior end. The excretory organ is visible. The intestine is straight, occupied by dark granules. The posterior end of the body attenuates ultimately into a tail with a rounded peak. The anus is at 0.090—0.114 mm from the posterior end of the tail.

At the age of 12 hrs the larvae are 0.093—1.04 mm long, maximum width 0.061—0.069 mm (distinctly more slender than the younger larvae). No great

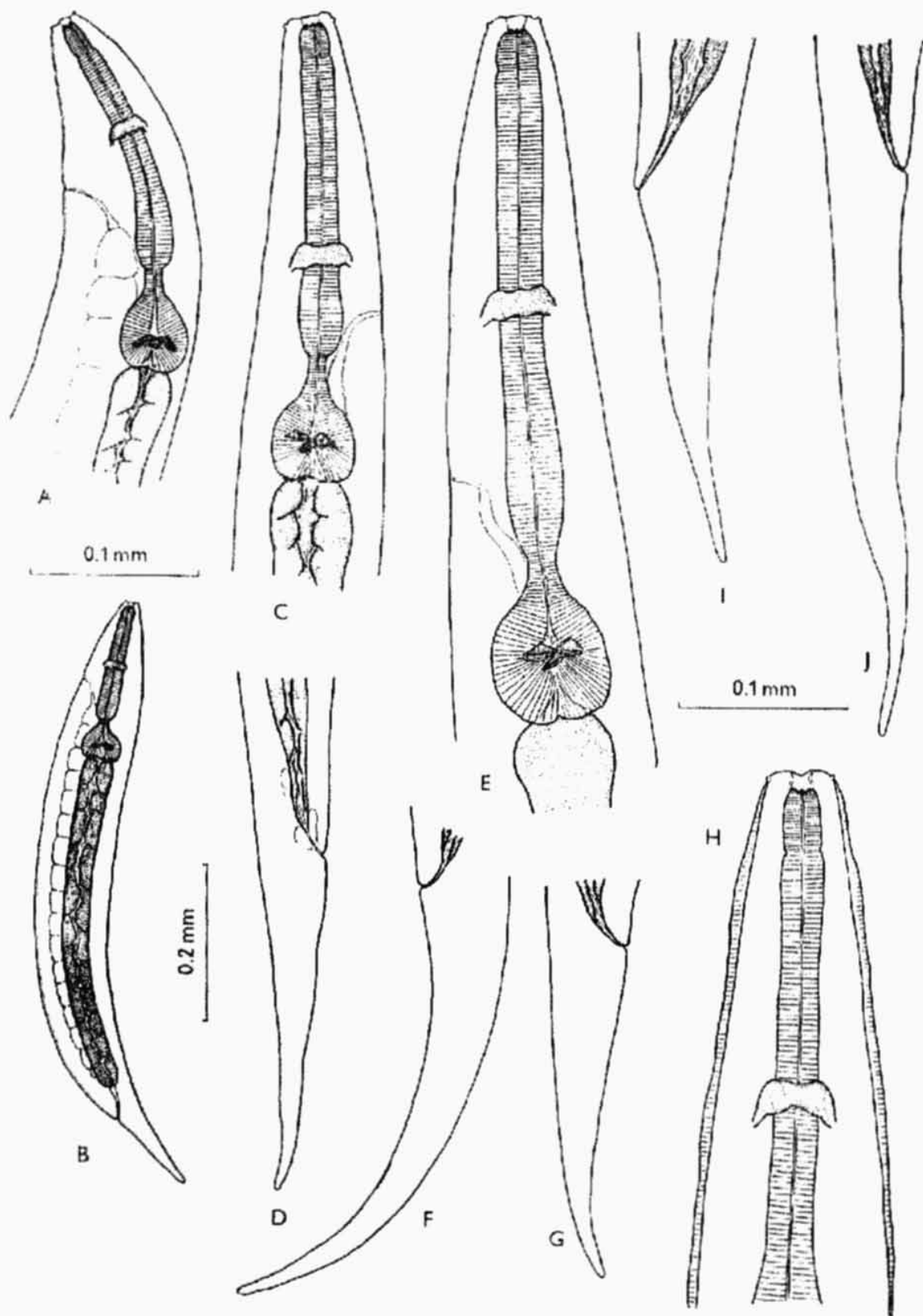


Fig. 1. Parasitic 3rd-stage larvae of the nematode *Subulura suctorica* (Molin, 1860) from the caecum of *Gallus gallus f. domestica*. A—anterior portion of larva (6 hrs p.i.); B—overall view (6 hrs p.i.); C, D—anterior and posterior portion of larva (24 hrs p.i.); E—anterior portion of larva (5 days p.i.); C, D—anterior and posterior portion of larva (24 hrs p.i.); E—anterior portion of larva (5 days p.i.); F, G—posterior portion of female and male larvae (5 days p.i.); H—anterior portion of larva (7 days p.i.); I, J—posterior portion of male and female larvae (9 days p.i.).

Table 1. Measurements of the parasitic 3rd-stage larvae of the nematode *Subulura suctorica* (Molin, 1860) from the caecum of *Gallus gallus f. dom.*

Age of larvae in days	3	5	7	9
Overall length	1.80—2.05	2.09—2.48	1.97—2.50	2.27—3.23
Maximum width	0.086—0.115	0.110—0.130	0.098—0.122	0.098—0.130
Width of anterior portion	0.028—0.032	0.029—0.036	0.032—0.041	0.036—0.045
Depth of mouth capsule	0.004—0.006	0.006—0.008	0.007—0.010	0.011—0.012
Width of mouth capsule	0.007—0.008	0.008—0.012	0.008—0.012	0.012—0.016
Oesophagus length	0.299—0.319	0.324—0.352	0.344—0.492	0.362—0.548
Bulbus width	0.045—0.057	0.053—0.057	0.049—0.074	0.057—0.086
Nerve ring	0.129—0.156	0.159—0.179	0.150—0.205	0.163—0.185
Excretory pore	0.167—0.205	0.205—0.233	0.225—0.260	0.227—0.286
Anus distance (male larvae)	0.123—0.180	0.185—0.205	0.175—0.205	0.187—0.205
Anus distance (female larvae)	0.123—0.180	0.225—0.269	0.225—0.286	0.274—0.369

changes have been observed in the other body measurements except the distance of the anus from the posterior end of the body (0.106—0.134 mm). The excretory organ which seems to be of importance for the contraction of the invasive larva in the intermediate host and the straightening of the 3rd-stage larva after liberation in the definitive host, is markedly reduced.

At the age of 24 hrs (Fig. 1C, D) the larvae are 1.18—1.43 mm long, maximum width 0.041—0.061 mm. The cuticle bears a fine transverse striation. The buccal cavity measures 0.004—0.007 mm in depth and 0.008 mm in width. The oesophagus is 0.230—0.272 mm long, its bulbus is 0.032—0.041 mm wide. The nerve ring is at 0.124—0.148 mm from the anterior end of the body, the excretory pore at 0.164 to 0.172 mm. The anus is at 0.123—0.180 mm from the posterior end. The daily rate of growth of the male worm is 0.17 mm, that of the female 0.21 mm. On day 5 after invasion (Fig. 1F, G) the males can be differentiated from the females by the length of their tail. The tail of the females is longer than that of the males. According to CUCKLER and ALICATA (1944) such differentiation is possible from the second day onwards. In 5 day-old larvae the cervical alae start to appear; these are very distinct on day 7 (Fig. 1H). The buccal cavity enlarges gradually; its walls start to sclerotize from the 7th day onwards forming a pseudochitinous buccal capsule. The measurements of the larvae of this stage are given in Table 1.

Moulting and morphology of 4th-stage larva: The larva attains this stage after 12 days, this being accompanied by the typical shedding of the cuticle (the first larval moulting in the definitive host). At first the cuticle separates in waves from the body surface (Fig. 2A, B), being torn away by vehement motion at the level of the oesophagus termination (in all larvae observed the split occurred at this place). Now the cuticle forms two pouches the shorter covering the anterior portion of the body, the longer the posterior portion. The moulting process becomes complete by the shedding of these two pouches.

The moulting larva 4 (the future male) is 2.74—3.61 mm long, maximum width 0.122—0.175 mm. Width of the anterior portion 0.036 to 0.041 mm. Depth of the

mouth capsule 0.010—0.012 mm, width 0.009 mm. Nerve ring at 0.163—0.185 mm from anterior end of body, excretory pore at 0.246 to 0.290 mm. Oesophagus length 0.495—0.533 mm, its bulbus is 0.077—0.144 mm wide. Anus at 0.193—0.205 mm from end of tail.

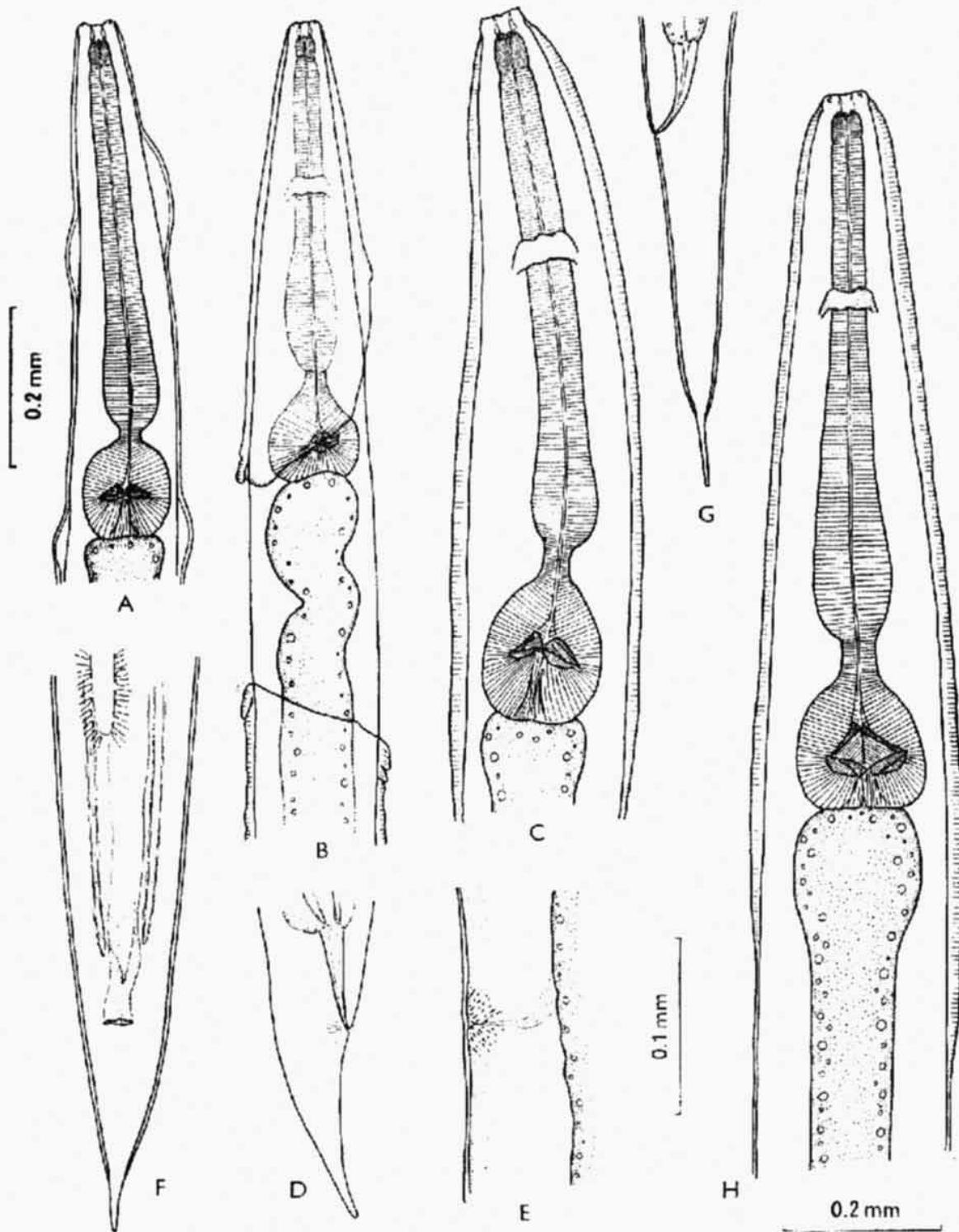


Fig. 2. Moulting process and 4th-stage larvae of the nematode *Subulura suctorica* (Molin, 1860) from the caecum of *Gallus gallus f. dom.* A, B—anterior portion of the body of exsheathing larvae (12 days p.i.); C—anterior portion of larva (15 days p.i.); D—posterior portion of male larva (15 days p.i.); E—formation of vulva (15 days p.i.); F—posterior portion of male larva (18 days p.i.); G—posterior portion of female larva (18 days p.i.); H—anterior portion of larva (18 days p.i.).

Table 2. Measurements of 4th-stage larvae of the nematode *Subulura suctorio* (Molin, 1860) from the caecum of *Gallus gallus f. dom.*

Age of larvae in days	15		18		21	
	males	females	males	females	males	females
Sex						
Overall length	4.18-4.52	4.17-4.75	4.25-5.16	5.22-6.45	4.43-5.70	5.64-6.65
Maximum width	0.174-0.201	0.193-0.213	0.154-0.214	0.205-0.254	0.170-0.229	0.230-0.262
Width of anterior portion	0.040-0.047	0.049-0.057	0.041-0.049	0.049-0.060	0.047-0.057	0.057-0.064
Depth of mouth capsule	0.014-0.016	0.014-0.016	0.014-0.016	0.016-0.018	0.016-0.020	0.016-0.020
Width of mouth capsule	0.012-0.014	0.012-0.016	0.016	0.016-0.020	0.016-0.020	0.016-0.020
Oesophagus length	0.643-0.708	0.585-0.592	0.615-0.664	0.581-0.750	0.730-0.810	0.636-0.822
Bulbus width	0.102-0.122	0.102-0.122	0.102-0.134	0.122-0.156	0.110-0.170	0.122-0.163
Nerve ring	0.185-0.205	0.179-0.213	0.142-0.217	0.191-0.229	0.205-0.240	0.205-0.246
Excretory pore	0.279-0.307	0.295-0.356	0.270-0.352	0.310-0.367	0.348-0.369	0.369-0.410
Spicule length			0.469-0.615		0.602-0.755	
Gubernaculum length			0.069-0.094		0.073-0.106	
Distance of pseudosucker from end of tail			0.163-0.208		0.574-0.615	
Distance of cloaca	0.190-0.229		0.205-0.254		0.218-0.250	
Distance of anus		0.423-0.471		0.410-0.490		0.470-0.494
Distance of vulva from anterior end		1.51-2.25		2.13-3.05		2.56-3.13

The moulting larva 4 (future female) measures 3.28—4.10 mm, maximum width 0.126—0.163 mm. Width of anterior portion 0.041—0.049 mm. Depth of mouth capsule 0.012 mm, width 0.012 mm. Length of oesophagus 0.574—0.615 mm, width of bulbus 0.090—0.102 mm. Nerve ring at 0.171—0.205 mm from anterior end of body, excretory pore at 0.277—0.315 mm. Anus at 0.307—0.377 mm from end of tail.

The rate of growth of the larva 4 is about the same as that of larva 3, the average daily increase in length being 0.22 mm (males and females). In addition to the gradual increase of all body sizes, a number of important morphological changes occur in the larva during this developmental stage. At the age of 15 days the vulva, at first appearing under the cuticle like rays of muscle fibres (Fig. 2E) starts to develop in the female; it remains closed during the development of larva 4. In males of the same age (15 days) we observed the formation of the spicules; on day 18 the spicules resemble moderately pseudochitinized tubes (Fig. 2F). The pseudosucker of these larvae is very distinct (especially its radially arranged muscles), less distinct is the gubernaculum. The tail of the male and female larvae loses its smoothly conical appearance and attains a shape typical of the imaginal stage (the peak extends into a cuticular process). A steadily progressing sclerotization leads ultimately to the complete formation of the spicules and the gubernaculum (day 21); also the two layers of the cuticle become visible. The larvae 4 complete their development at the age of 23 days and start their second moulting in the definitive host (measurements of the larva 4 are given in Table 2).

Moulting and morphology of the 5th-stage larva: The second moulting preceding stage 5 is similar to the first moulting. The cuticle separates from the larval body together with the cuticular lining of the mouth cavity, the rectum and the cloaca (Fig. 3A, B, D). The caudal papillae start to develop in the moulting male larvae. The male body measures 4.56—5.77 mm, maximum width 0.163—0.260 mm. The anterior portion is 0.045—0.057 mm wide. The mouth capsule is 0.024—0.028 mm

deep and 0.026–0.028 mm wide. The oesophagus is 0.760–0.930 mm long, the bulbus is 0.126–0.185 mm wide. The nerve ring is at 0.185–0.244 mm from the anterior end, the excretory pore at 0.296–0.369 mm. The cloaca is at 0.209 to 0.266 mm from the end of the tail, the pseudosucker at 0.492–0.570 mm. Its

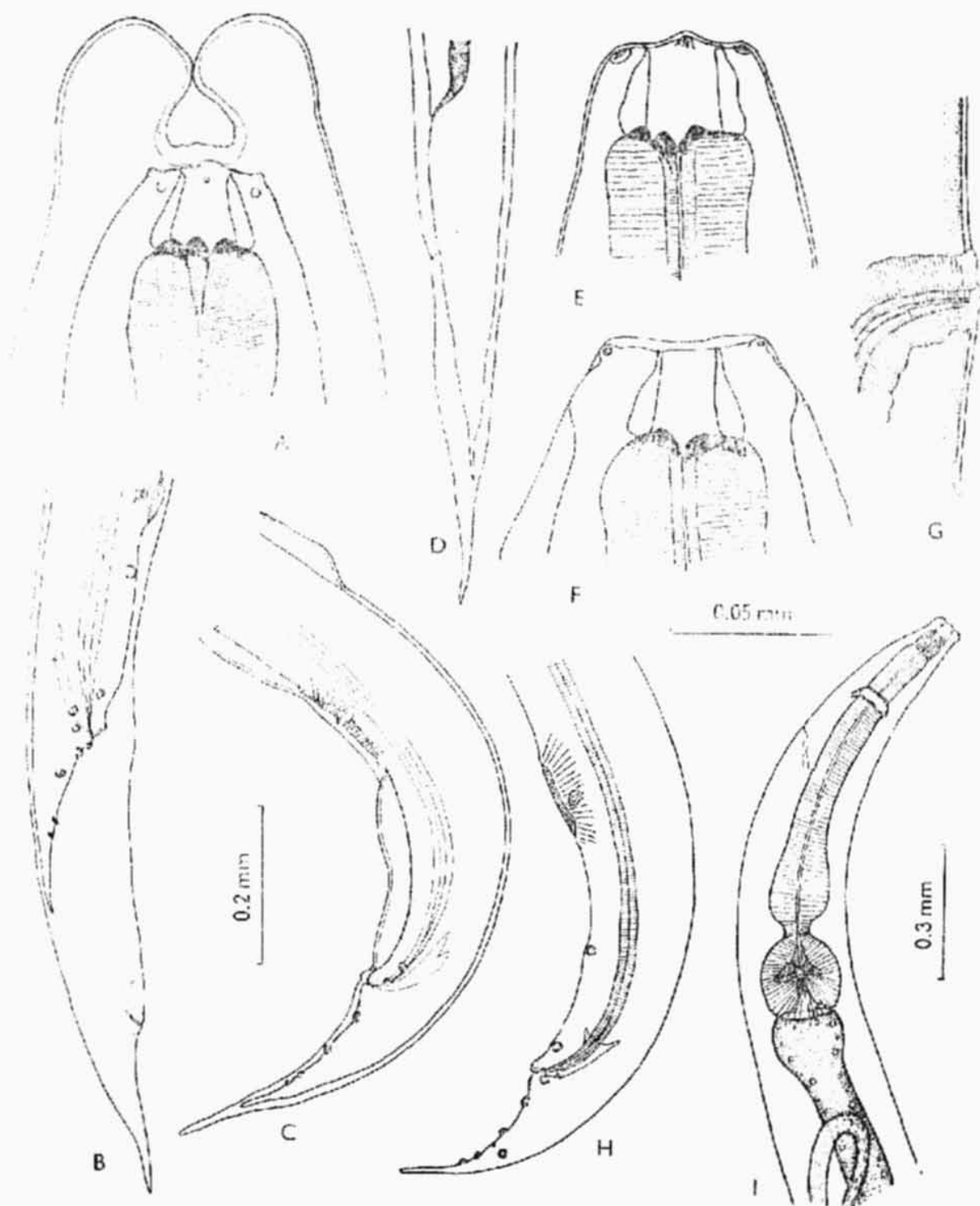


Fig. 3. Moulting process and 5th-stage larvae of the nematode *Subulura suctorica* (Molin, 1860) from the caecum of *Gallus gallus f. dom.* A—separating of the cuticle from the anterior portion of the body (23 days p.i.); B, C—shedding of the cuticle from the posterior end of the male larva (23 days p.i.); D—exsheathing of the cuticle from the posterior portion of the female larva (23 days p.i.); E, F—detail of the anterior portion of the body—lateral and dorsal view—(26 days p.i.); G—vulva (26 days p.i.); H—posterior end of male's body (26 days p.i.); I—anterior portion of body—overall view—(26 days p.i.).

Table 3. Measurements of 5th-stage larvae of the nematode *Subulura suctorica* (Molin, 1860) from the caecum of *Gallus gallus f. dom.*

Age of larvae in days	26		36	
	males	females	males	females
Sex				
Overall length	5.70—6.46	6.40—7.41	7.38—8.90	8.96—11.70
Maximum width	0.225—0.279	0.260—0.293	0.210—0.330	0.304—0.348
Width of anterior portion	0.049—0.057	0.057—0.069	0.057—0.069	0.057—0.069
Depth of mouth capsule	0.024—0.028	0.028—0.032	0.028—0.035	0.032—0.036
Width of mouth capsule	0.029—0.034	0.036—0.041	0.032—0.036	0.036—0.041
Oesophagus length	0.855—0.950	1.00—1.04	0.893—1.14	1.00—1.24
Bulbus width	0.163—0.192	0.189—0.205	0.142—0.205	0.185—0.209
Nerve ring	0.213—0.262	0.205—0.250	0.238—0.287	0.246—0.315
Excretory pore	0.340—0.400	0.269—0.402	0.307—0.410	0.307—0.410
Spicule length	0.950—1.10	—	0.980—1.16	—
Gubernaculum length	0.102—0.130	—	0.122—0.144	—
Distance of pseudosucker from end of tail	0.517—0.600	—	0.458—0.610	—
Distance of cloaca	0.190—0.250	—	0.185—0.246	—
Distance of anus	—	0.380—0.532	—	0.570—0.665
Distance of vulva from anterior end	—	3.03—3.42	—	4.65—4.97

length is 0.082—0.122 mm. The spicules are 0.902—0.973 mm long, the gubernaculum 0.082—0.118 mm.

The females of the same age are 5.57—6.84 mm long, maximum width 0.225 to 0.262 mm. The anterior end of the body is 0.053—0.064 mm wide. The mouth capsule is 0.024—0.028 mm deep and 0.024—0.030 mm wide. The oesophagus is 0.817—1.08 mm long, its bulbus is 0.154—0.193 mm wide. The nerve ring is at 0.205—0.254 mm from the anterior end of the body, the excretory pore at 0.300 to 0.402 mm. The vulva is at 2.66—3.24 mm from the anterior end of the body, the anus at 0.380—0.497 mm from the end of the tail.

The outer morphology of the larva 5 after moulting is the same as that of the adult nematode (Fig. 3E, I). At this stage of development, the larvae still grow (Table 3) and complete the development of the sexual organs (mainly the females). The daily increase in body length is 0.15 mm in the males and 0.30 mm in the females. On day 43 the larvae reach the imaginal stage.

Morphological and metrical characters of 43 day-old nematodes (Fig. 4): The body is cylindrical, white to yellowish. The cuticle bears fine transverse striations. The cervical alae terminate close behind the oesophagus. The anterior end of the body is obtuse, width 0.061—0.082 mm. The mouth with no lips is surrounded by 6 papillae. The walls of the mouth capsule are well-chitinized. Three rounded teeth arise from its bottom. The bulbus contains the valvular apparatus.

Male: Overall length 7.98—9.50 mm, maximum width 0.205—0.300 mm. Depth of mouth capsule 0.032—0.041 mm, width 0.036—0.041 mm. Length of oesophagus 0.920—1.17 mm, width of bulbus 0.185—0.209 mm. Nerve ring at 0.246—0.266 mm from anterior end of body, excretory pore at 0.307—0.410 mm. The posterior end of the body is bent mostly in ventral direction. Caudal alae very narrow. Of the

11 pairs of caudal papillae 3 are precloacal, 2 paracloacal and 6 postcloacal. Length of precloacal pseudosucker 0.163—0.225 mm, situated at 0.494 to 0.632 mm from end of tail. Spicules well-chitinized, of equal length with sharp distal ends. Length of spicule 0.984—1.14 mm. Width of proximal end 0.032—0.045 mm. Length of gubernaculum 0.122—0.158 mm.

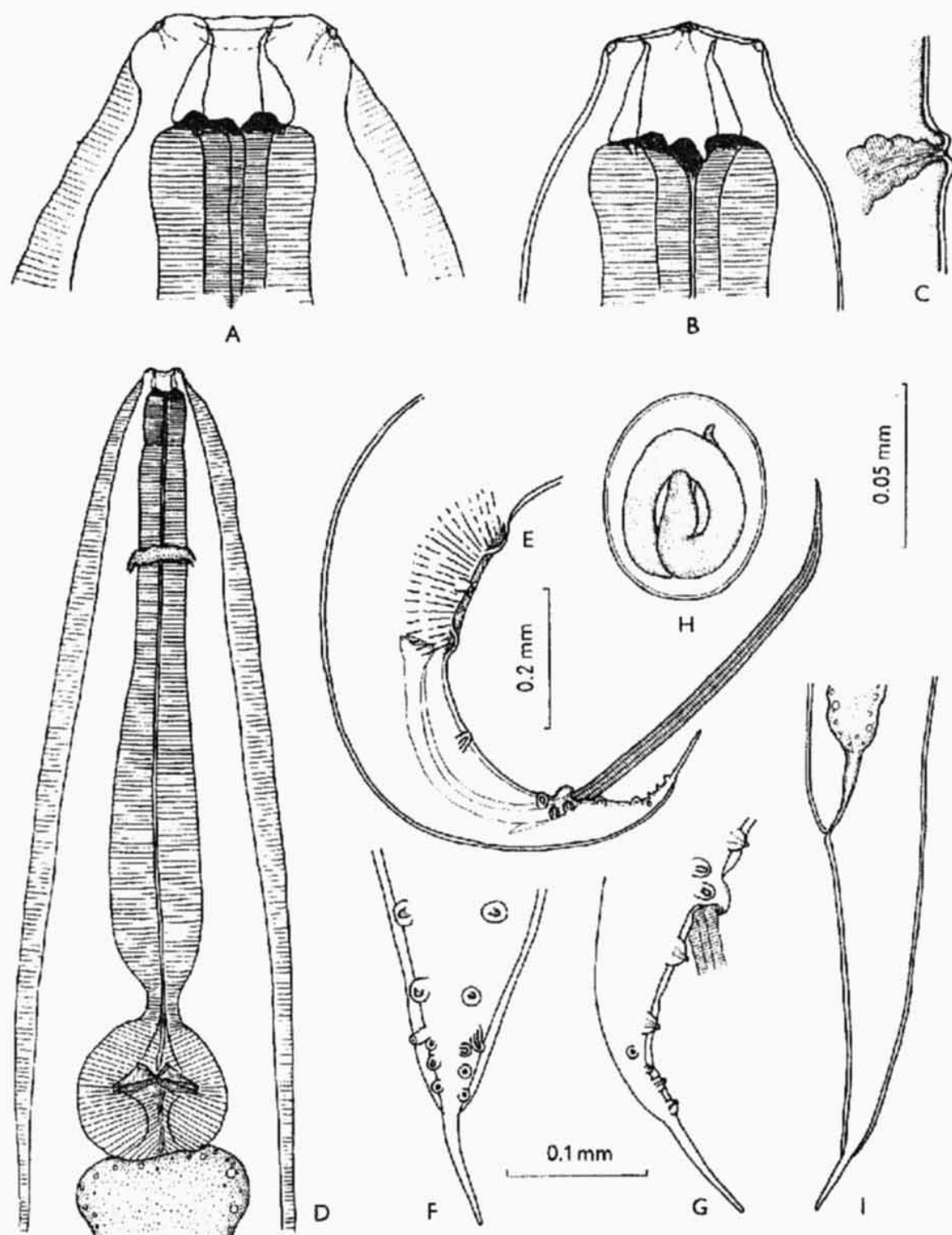


Fig. 4. Adult nematode *Subulura suctorica* (Molin, 1860) from the caecum of the *Gallus gallus f. dom.* (43 days p.i.); A, B—anterior portion of body (detail, dorsal and lateral view); C—vulva; D—anterior portion of body (overall view); E—posterior portion of male's body (lateral view); F, G—arrangement of postcloacal papillae (ventral and lateral view); H—egg, I—posterior end of female body (lateral view).

Female: Overall length 11.02—13.11 mm, maximum width 0.319—0.380 mm. Depth of mouth capsule 0.036—0.047 mm, width 0.011—0.045 mm. Nerve ring at 0.225—0.295 mm from anterior end of body, excretory pore at 0.450—0.492 mm.

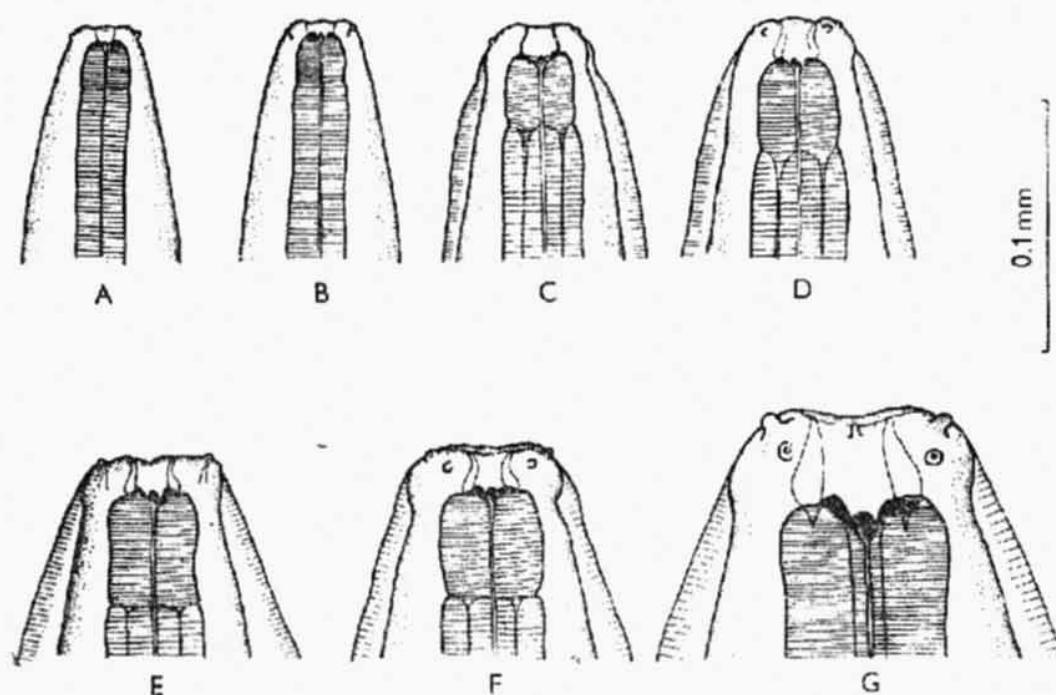


Fig. 5. Anterior end of 3rd-stage larva (A—D), 4th-stage larva (E, F) and 5th-stage larva (G) of the nematode *Subulura suctorica* (Molin, 1860) from the caecum of the host *Gallus gallus f. dom.* A—24 hrs p.i.; B—5 days p.i.; C—7 days p.i.; D—9 days p.i.; E—15 days p.i.; F—18 days p.i.; G—23 days p.i.

Length of oesophagus 1.19—1.27 mm, width of bulbous 0.213—0.225 mm. Vulva at 4.75—5.68 mm from anterior end of body. The eggs with thin translucent shells contain a coiled larva. Size of eggs 0.061—0.070 × 0.045—0.055 mm. Anus at 0.722 to 0.874 mm from end of tail.

B. PREPATENT PERIOD, LONGEVITY OF THE PARASITE AND RESULTS OF THE HISTOPATHOLOGICAL EXAMINATION

Faecal examination of a group of 20 chickens showed that the nematode *S. suctorica* matures sexually and starts egg production from day 37—46 after invasion of the host (after 43 days on the average). In 10 chickens we confirmed the finding of eggs in the faeces by postmortem examination. In all instances adult female worms with eggs containing coiled larvae were found in the caeca of the chickens. According to CUCKLER and ALICATA (1944) the prepatent period lasts 42 days. ABDOU and SELIM (1963) give 45 days. Our observations revealed a top and bottom limit of the length of the prepatent period. The length of time recorded by all these authors is within the range of this period. The longevity of *S. suctorica* is 6—8.5 months (average 7.5 months) according to the results of our coprological examination of a group of 10 chickens.

The caeca of artificially invaded chickens were examined histologically on day 33, 36, 39, 43; after 2.5—3.5 months p.i.; those of a guinea fowl on day 59 p.i. and also the caeca of naturally invaded chickens from the farm. Our examinations showed that the nematode *S. suctorius* (young and adult stages) do not cause any marked pathological changes in the mucosa of the caecum. The nematodes are located in the lumen of the caecum; only at sites where the parasite presses more intimately into the mucous fold, a compression of the epithelial layer has been observed to cause a reduction in height of the cells of the epithelium. Neither did CUCKLER and ALICATA (1944) observe any marked pathological effect of the larvae on the caeca which they examined histologically 3—4 days after invasion.

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