

DETAILED STUDIES ON THE MORPHOLOGY AND INTRA-SPECIFIC VARIATIONS OF *PARAHADRONCHUS SHAKILI* (JAIRAJPURI, 1969) MULVEY, 1978 WITH SOME OBSERVATIONS ON ITS JUVENILE STAGES

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(With 7 Text-figures)

INTRODUCTION

The nematodes belonging to the Order Mononchida Jairajpuri, 1969, generally referred to as mononchs, constitute one of the most important groups among the soil nematodes because of their predatory feeding habits. Several workers have reported and described a large number of species of mononchs from different parts of the world (Cobb, 1916 & '17 ; Clark, 1960—'63 ; Mulvey, 1961—'78 ; Mulvey & Jensen, 1967 and Jairajpuri, 1969—'75). But only a few workers have worked exclusively on the morphology of mononchs (Coomans & Lima, 1965 and S. Z. Baqri & Jairajpuri, 1972). Since the detailed morphological and anatomical observations on a group or a species help in understanding the evolutionary trend in the nematodes, the present work was initiated on *Parahadronchus shakili* (Jairajpuri, 1969) Mulvey, 1978 which is a widely distributed species of mononchs in North and North-Eastern part of India.

In the present study an attempt has been made to describe in detail the intra-specific variations, the musculature, the digestive and the reproductive organs of *Parahadronchus shakili*. Besides, observations have also been made on different juvenile stages of this species. The specimens included in the present study were collected from soil around roots of *Litchi chinensis* Sonn. at Company gardens, Bareilly, Uttar Pradesh.

MATERIAL AND METHODS

The specimens of *Parahadronchus shakili* were fixed in hot 4% formalin, dehydrated slowly in a desiccator and mounted in dehydrated glycerine. The transverse sections of body at various levels were cut with the help of a sharp razor blade in glycerine-jelly and were mounted in the same medium.

GROSS MORPHOLOGY AND INTRA-SPECIFIC VARIATIONS OF *PARAHADRONCHUS SHAKILI* (JAIRAJPURI, 1969) MULVEY, 1978

Dimensions :

Females (140) : L=2.40–3.35 mm ; a=40–52 ; b=3.8–6.0 ;
c=6.4–11.8 ; c'=5.6–9.5 ; V= $9^{-1.5}60 - 66^{9-1.5}$

Males (62) : L=2.32–3.22 mm ; a=35–53 ; b=4.2–5.2 ; c=7.3–11.8 ; c'=4.5–7.0 ; T=39–45.

Description :

Female : Lip region fairly expanded, marked off from body, 45–60 μm wide, 15–25 μm high, distinctly wider than the adjoining body. *En face* view (Text-fig. 4, A) shows the vestibulum bounded by six lips which are of same shape and size. There are two dorsolateral, two ventrolateral and two lateral lips. Each lip is provided with an outer and an inner papilla. Thus the two circlets have six papillae each. Near the middle of lips on either side of internal papillae are present prominent dots. Amphids are small cup-like, apertures 5–6 μm wide, at 18–21 μm from anterior end of body. The amphidial chamber is connected to an amphidial duct which leads to the sensillar pouch containing sensory neurons.

Stoma consists of a hexaradiate vestibulum and a barrel-shaped tri-radial buccal cavity 50–65 \times 32–39 μm . Apex of dorsal tooth at 21–30 μm from the base of stoma. Walls of buccal cavity are formed by two sets of heavily cuticularized plates, the vertical and the oblique sets. The dorsal plate of the vertical set bears a large tooth in the posterior half of buccal cavity. The subventral walls bear 2–7 teeth and the oblique wall have two foramina each.

Oesophagus is roughly cylindrical and strongly muscular, with tri-radiate lumen, slightly expanded at its anterior end, narrowing a little at nerve ring and then widening towards base. The oesophago-intestinal junction is tuberculate with non-sclerotized tri-radiate lumen. Orifices of oesophageal glands are located as follows : dorsal 281–302 μm from anterior end of body ; the first pair of subventrals 109–140 μm from the orifice of dorsal one ; the second pair 168–196 μm from the first pair. Nerve ring encircles the oesophagus at 148–197 μm from anterior end of body. Intestine is sac-like, flattened dorsally in the region of gonads with 6–7 cells in circumference. Rectum 33–54 μm or about one anal body-width long.

Vulva transverse, slit-like, post-equatorial in position with cuticularized lips. Pre-vulval papillae 0–3, post-vulval papillae 0–2. Vagina short, thick-walled. Gonads amphidelphic, ovaries reflexed. Each sexual

branch consists of an ovary with 10-25 oocytes, an oviduct with a narrow distal and an enlarged sac-like proximal part, well developed sphincter and a large flexible uterus. Uterine eggs measure $124-138 \times 51-56 \mu\text{m}$.

Tail 262-430 μm or about 6-10 anal body-widths long, elongate-conoid, tapering sharply. Caudal glands well developed, opening terminal. Caudal papillae 4 on either side.

Male : Body generally strongly curved in posterior half of its length, often 'C' shaped. Buccal cavity $49-60 \times 29-35 \mu\text{m}$. Apex of dorsal tooth $19-27 \mu\text{m}$ from base of stoma. Spermatozoa spindle-shaped measuring $6-10 \mu\text{m}$. Four ejaculatory glands generally visible from one side. Spicules $91-106 \mu\text{m}$ medially, gubernaculum $18-24 \mu\text{m}$ and lateral accessory pieces $12-18 \mu\text{m}$. Supplements 11-16. Rectum $38-56 \mu\text{m}$ or about one anal body-width long. Tail $223-354 \mu\text{m}$ or about 5-7 anal body-widths long.

ANATOMY OF *PARAHADRONCHUS SHAKILI*

MUSCULATURE

The musculature can broadly be divided into 'somatic or unspecialized musculature' which are arranged in the interchordal zones of the hypodermis and the 'specialized musculature' are those associated with a particular organ.

Somatic musculature (Text-fig. 3, I—K) :

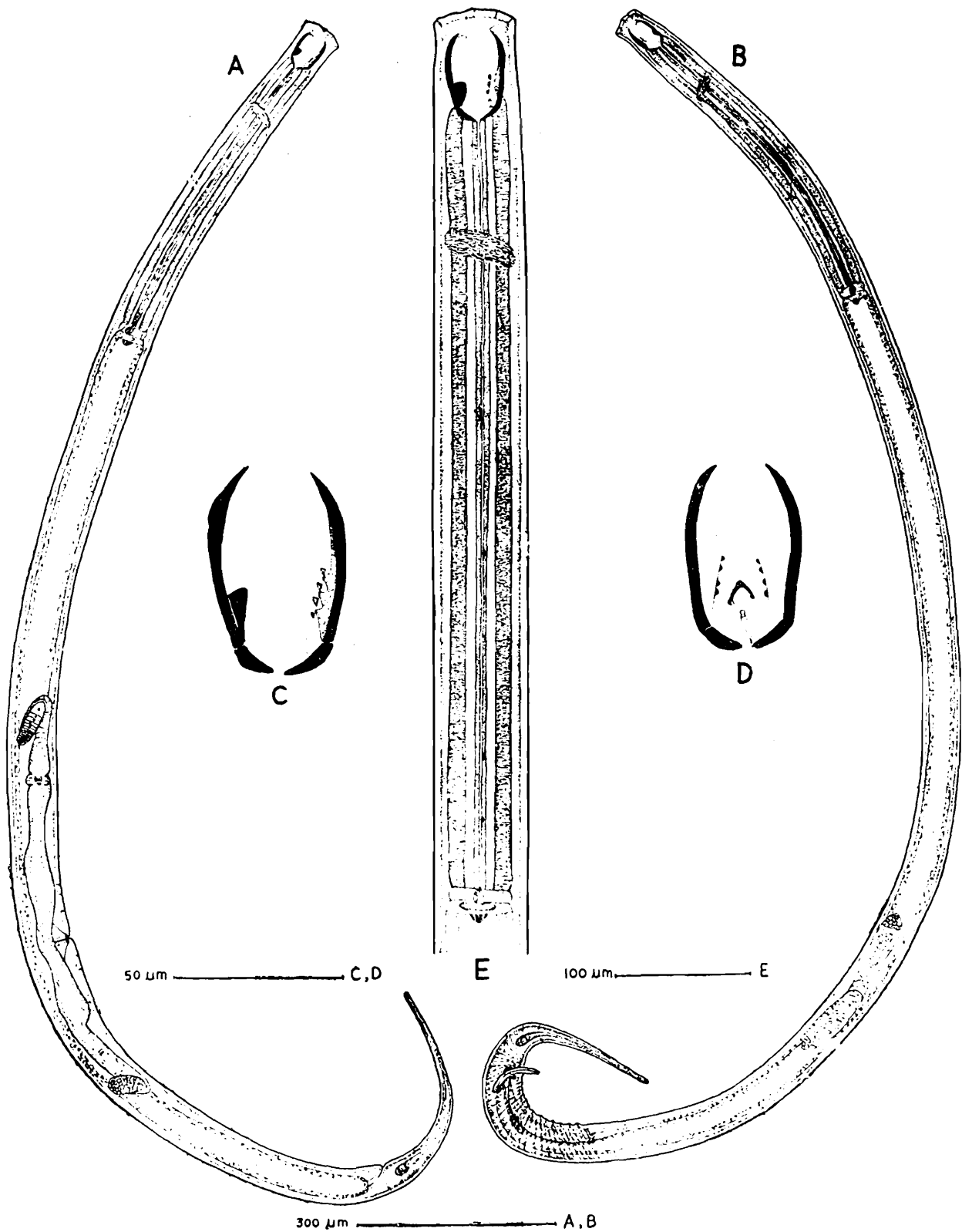
The somatic musculature consists of a single layer of cells situated beneath the hypodermis within the interchordal zones. In each quadrant four cells are present which shows that the nematodes are typically meromyarian. The cells are typically of platymyarian type. The shape and size of the muscle cells may be variable at the same or at different levels of body.

Specialized Musculature :

(i) *Cephalic muscles* : These are two sets of muscles. One set is attached to the lips and is known as labial muscles. The other set is attached to the walls of the stoma and is called stomatal muscles.

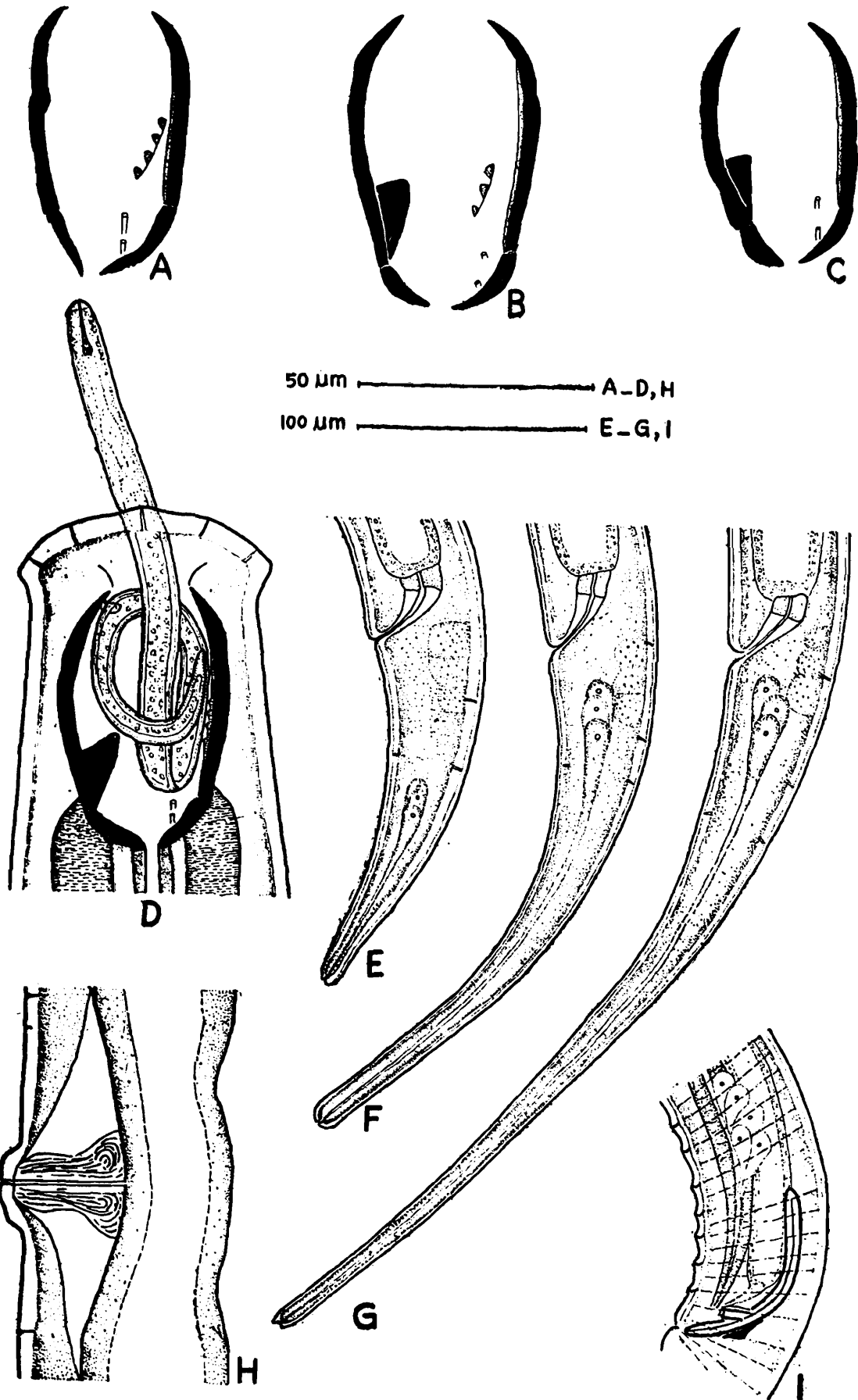
a. *Labial muscles* (Text-fig. 3, B and 4, D : 1) : These are six in number bifurcated at their anterior ends. The forked bands of labial muscles are attached to adjoining lips. Thus each lip is bounded by two different labial muscles. Most probably dots near the middle of lips on either side of the internal labial papillae are the points of attachment of these muscles.

b. *Stomatal muscles* (Text-fig. 3, B and 4, D : 2) : These are eight in number attached to the vertical walls of stoma. The point of their attachment is posterior to labial muscles. In cross section of body at level of stoma it becomes evident that two of these are subdorsal, two subventral and four sublateral in position. The contraction and relaxation of these muscles brings about the opening and closing of stoma.

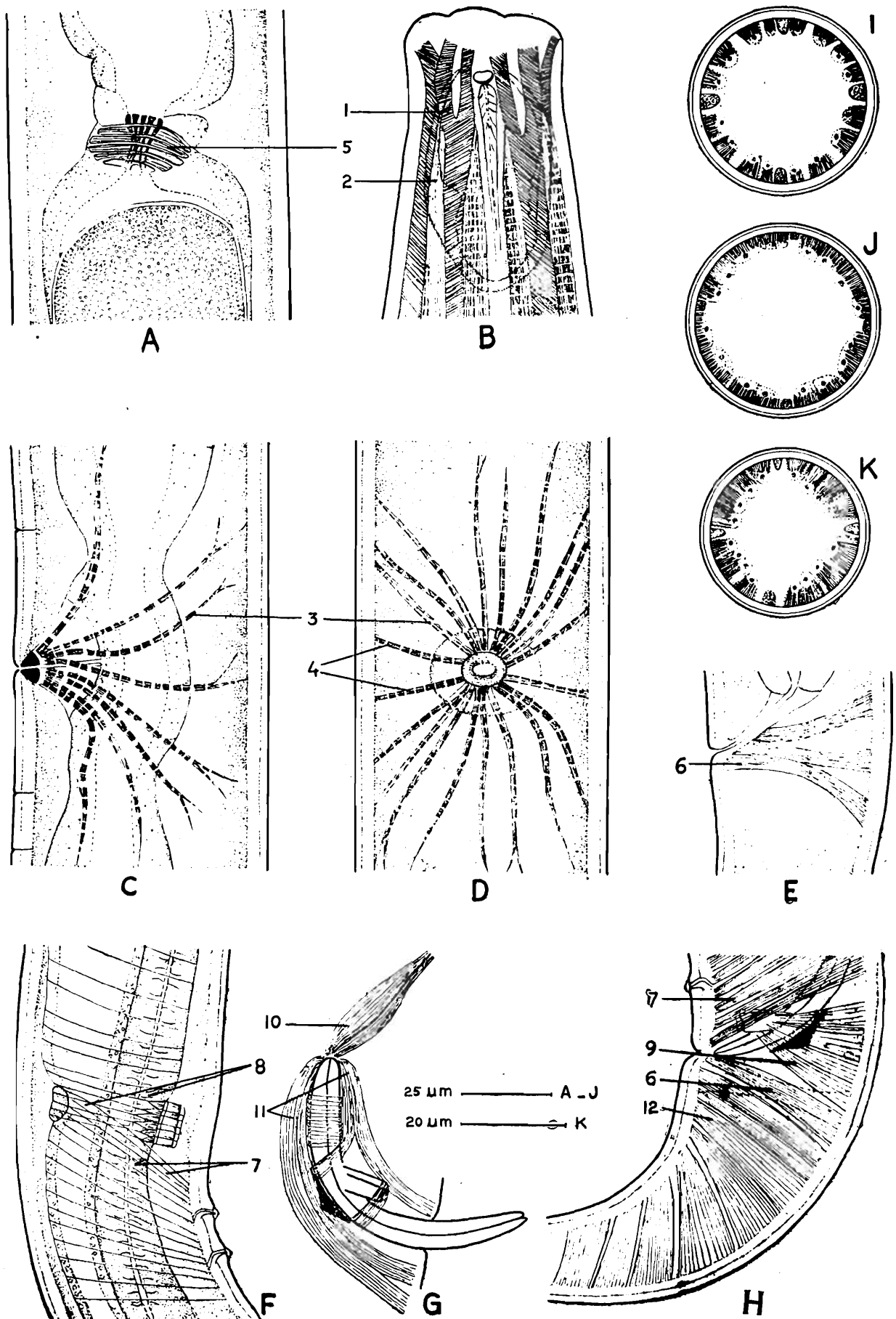


Text-fig. 1. A—Entire female; B—Entire male; C—Buccal cavity (Lateral view); D—Buccal cavity (Dorso-ventral view); E—Oesophagus.

(ii) *Vulval muscles* : The muscles associated with vulva bring about its dilation and contraction. Basically two sets of muscles are found associated with the vulva.



Text-fig. 2. A-C—Abnormal buccal cavities ; D—Anterior region of body showing a nematode (Prey) in the buccal cavity ; E-G—Abnormal tails ; H—Vagina without cuticularized pieces ; I—Posterior region of male showing abnormal number of ejaculatory glands (five).

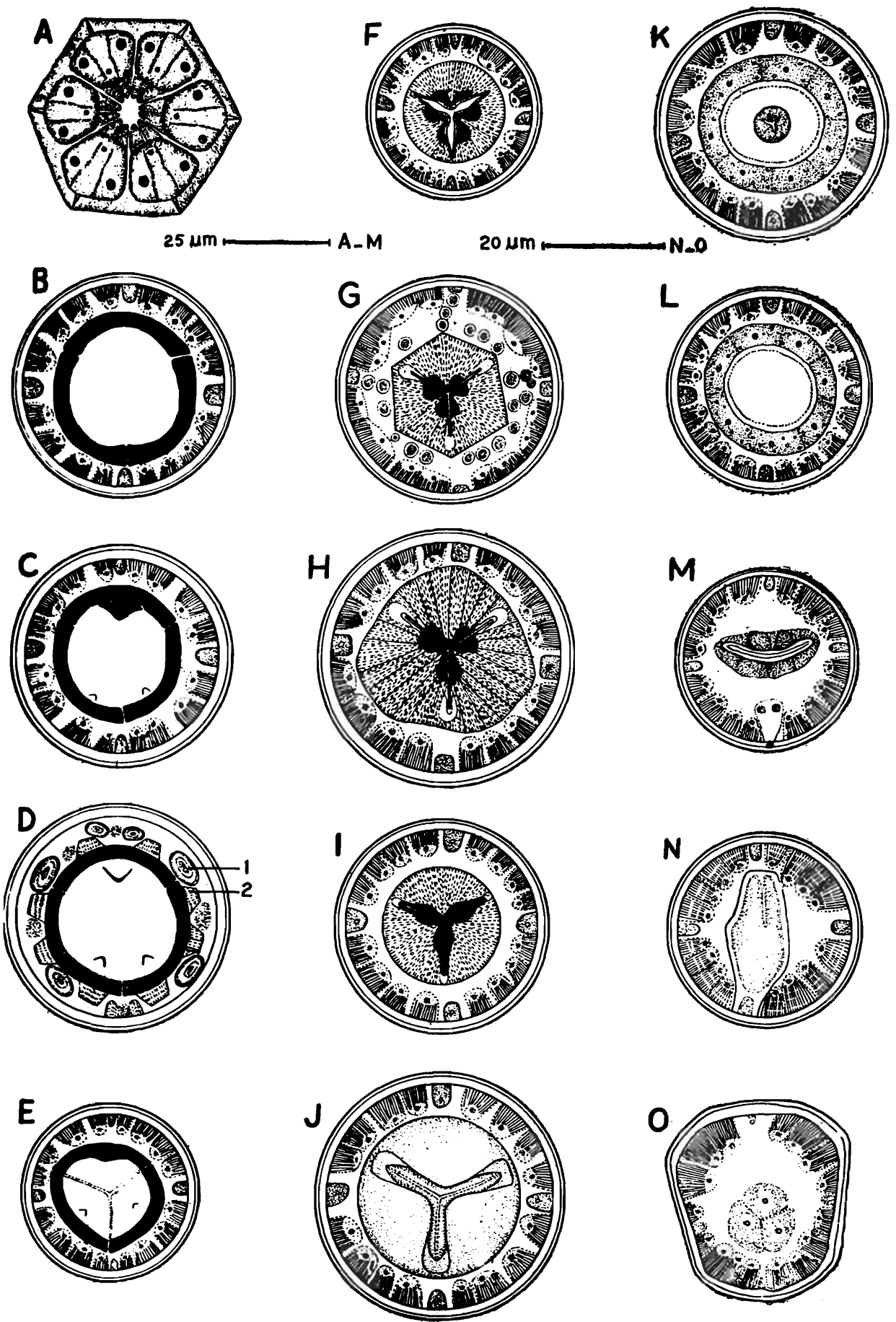


MUSCULATURE

Text-fig. 3. *Specialized muscles* A—Sphincter muscles ; B—Cephalic muscles ; C & D—Vulval muscles ; E—Anal muscles (female) ; F—Accessory copulatory muscle ; G—Spicular muscles ; H—Anal, copulatory, gubernacular and caudal copulatory muscles (male).

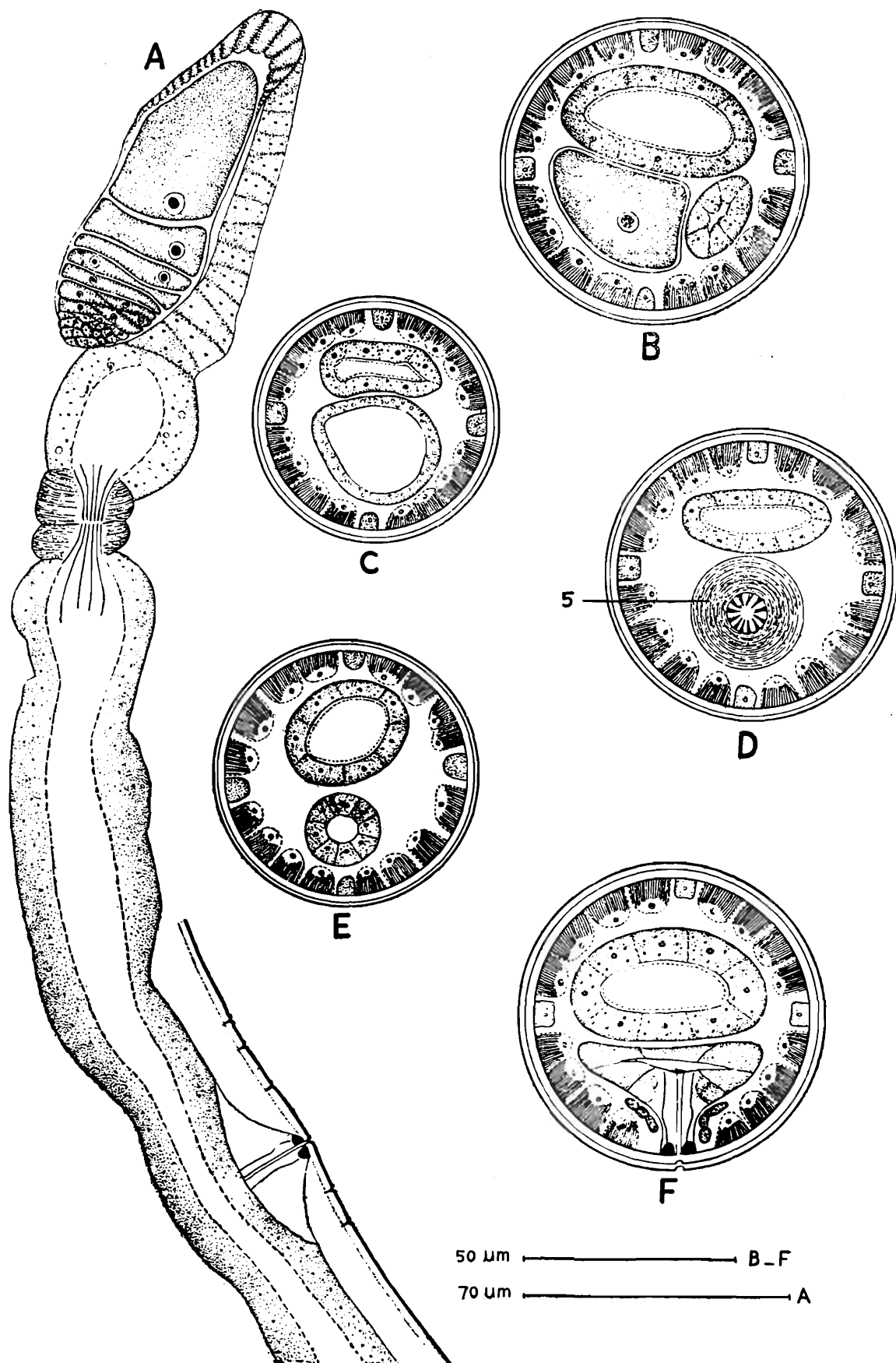
Somatic muscles (Cross-sections) I—At base of buccal cavity ; J—At level of nerve ring ; K—At level of anus.

1. Labial muscles, 2. Stomatal muscles, 3. Dilator muscles, 4. Constrictor vulvae, 5. Sphincter muscles, 6. Anal muscles, 7. Copulatory muscles, 8. Accessory copulatory muscles, 9. Retractor gubernaculi, 10. Retractor spiculi, 11. Protractor spiculi, 12. Caudal copulatory muscles.



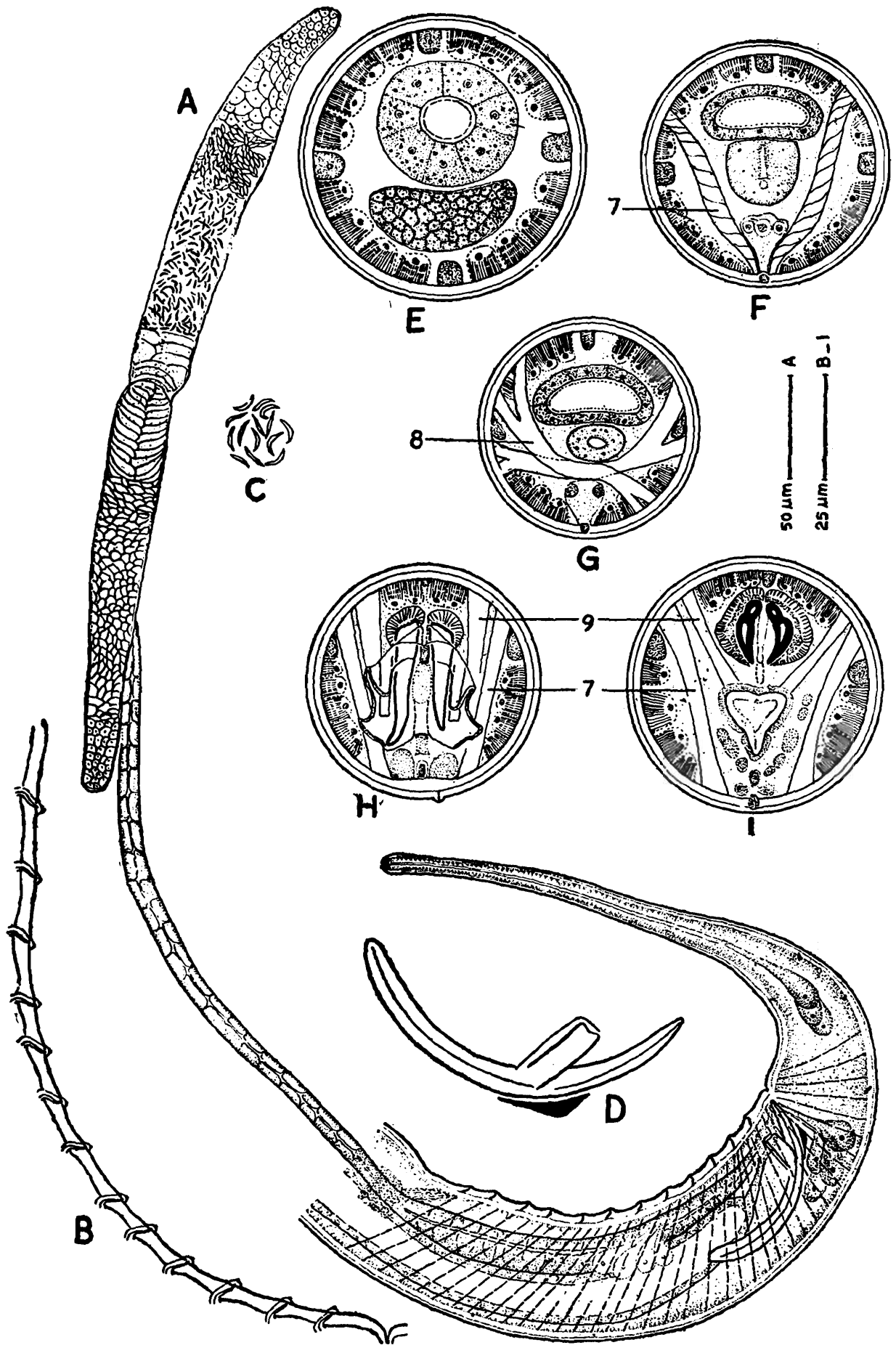
CROSS-SECTIONS OF BODY OF *P. SHAKILI*

Text-fig. 4. A—*En face* view ; B—Above dorsal tooth ; C—At level of dorsal tooth ; D—At basal level of dorsal tooth showing cephalic muscles ; E—At level of foramina ; F—At base of buccal cavity ; G—At level of nerve ring ; H—Through middle of oesophagus ; I—At base of oesophagus ; J—Through tubercles ; K—Through conical organ of oesophago-intestinal junction ; L—Through intestine ; M—Through rectum ; N—At level of anus ; O—At level of caudal glands.



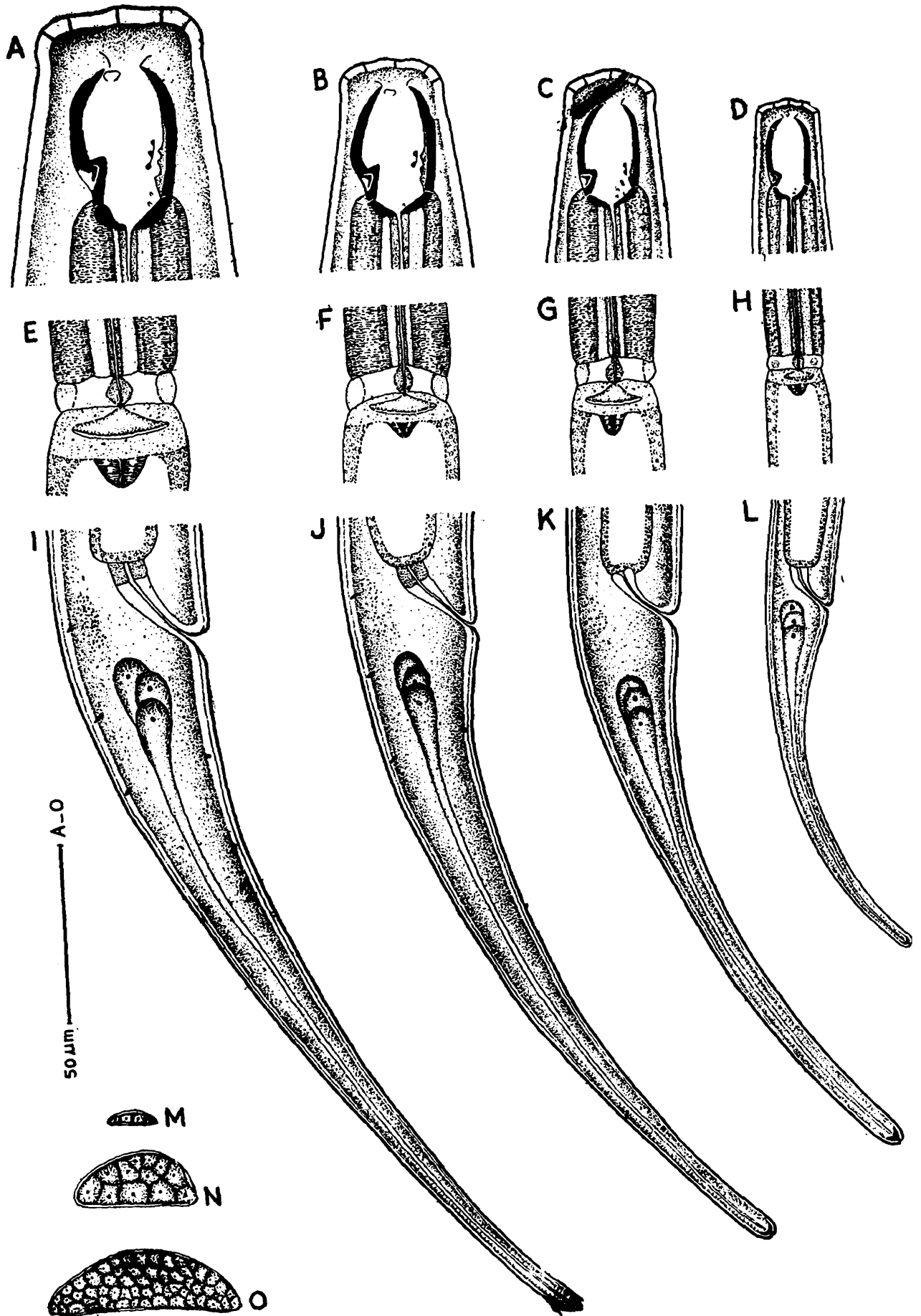
FEMALE GONAD

Text-fig. 5. A—Anterior gonad ; B—C. S. through ovary and distal part of oviduct, C—C. S. through proximal part of oviduct ; D—C. S. at level of sphincter; E—C. S. through uterus ; F. —C. S. at level of vulva.



MALE GONAD

Text-fig. 6. A—Entire gonad ; B—Supplements ; C—Sperms, D—Spicule, gubernaculum and accessory pieces ; E—C. S. through germinal zone of testis ; F—C. S. through vas deferens ; G—C. S. at level of transversely oriented accessory copulatory muscles ; H—C. S. through spicule ; I—C. S. through head of spicule.



JUVENILE STAGES

Text-fig. 7. A-D—Head ends of L_4 , L_3 , L_2 , L_1 respectively; E-H—Oesophago-intestinal junction of L_4 , L_3 , L_2 , L_1 respectively; I-L—Tails of L_4 , L_3 , L_2 , L_1 respectively; M—Genital primordium of L_2 ; N—Genital primordium of L_3 ; O—Genital primordium of L_4 .

a. *Dilator vulvae* (Text-fig. 3, C-D : 3) : These are 16 in number appearing in 8 pairs, 4 of which are anterior to vulva and 4 posterior. Originating from vulva, they run ventrolaterally and some of them are bifurcated at their ends. The contraction of these muscles opens the vulva.

b. *Constrictor vulvae* (Text-fig. 3, D : 4) : The constrictor vulvae are 4 in number and occur in pairs. Each pair originates from base of vagina and is attached to ventrolateral body wall. The muscles function in direction opposite to the dilator muscles and bring about the closure of vulva.

(iii) *Sphincter muscles* (Text-fig. 3, A and V, D : 5) : The oviduct leads to uterus through a valve called as sphincter. The lumen of the valve is controlled by a set of circular muscles which are called as the sphincter muscles.

(iv) *Anal muscles* (Text-fig. 3, E & H : 6) : These muscles are associated with the anus. They originate below the anus, run laterally and ventrodorsally. The number of the anal muscle bands is 2 to 3 in females but single in males.

(v) *Copulatory muscles* (Text-fig. 3, F & H and 6, F, H & I : 7) : The copulatory muscles extend from the region where the vas deferens and intestine appear constricted and continue up to the upper margin of cloaca. Each copulatory muscle originates at the laterodorsal body wall, runs lateroventrally and is finally attached between the somatic muscles and ventral chord. The anterior most copulatory muscles are not attached to the dorsolateral body wall but surround the intestine from its dorsal side. In this region the intestine appears transparent. The number of these muscles varies from 30 to 35 on either side of the body wall.

(vi) *Accessory copulatory muscles* (Text-fig. 3, F and 6, G : 8) : These are 14 to 16 in number, lying in front of copulatory muscles, of these 8-10 are rather faint, oblique bands followed by 6-8 transversely oriented muscles. The latter run from the dorso-lateral body wall of one side to the lateroventral wall of the other side. They cross each other ventral to vas deferens and usually bifurcate before their lateroventral attachment. These muscles along with the first copulatory muscles form a powerful constricting unit around the intestine and vas deferens.

(vii) *Gubernacular muscles* : These muscles are associated with gubernaculum and are basically of two types :

a. *Retractor gubernaculi* (Text-fig. 3, H and 6, H & I : 9) : They are 4 in number extending from the laterodorsal body wall to the gubernaculum. The anterior most originates dorsal to the copulatory muscles, curves on the inner side ventral to the spicules and is attached to the anterior end of the gubernaculum ventrally. The last muscle band runs laterodorsally and is attached to the gubernaculum on its laterodorsal side while the rest are attached laterally.

b. *Protractor gubernaculi* : The protractor gubernaculi are indistinct.

(viii) *Spicular muscles* : These are two sets of muscles attached to the spicules, one retractor spiculi and the other protractor spiculi.

a. *Retractor spiculi* (Text-fig. 3, G : 10) : A pair of muscle bands extending from the head of the spicules to the dorsolateral body wall.

b. *Protractor spiculi* (Text-fig. 3, G : 11) : A pair of muscle bands surrounding the spicules from dorsal, lateral and latero-ventral sides. These are attached to the spicular sheath which in turn envelops the spicules. One muscle band extends posteriorly from the head of the spicules beyond the gubernaculum and connects the dorsal and laterodorsal parts of the gubernaculum, then extends further posteriorly and gets bifurcated. The bifurcated bands are attached to the ventral body wall a short distance behind the anus. Another muscle band extends posteriorly from the head of the spicules runs lateroventrally and then bifurcates. A forked band extends laterally from the spicules and is attached to the laterodorsal parts of it. Another forked band passes laterally from the lateral accessory pieces and is attached with the ventral body wall near the upper lip of cloaca.

(ix) *Caudal copulatory muscles* (Text-fig. 3, H : 12) : Posterior to the anus there are about 5-9 bands of muscles arranged one behind the other. They extend from the laterodorsal body wall to the ventral body wall. The anterior most muscles band is attached to the lower lip of cloacal opening.

DIGESTIVE ORGANS

The digestive organs consist of stoma, oesophagus, oesophago-intestinal junction, intestine, rectum and anus. These organs have been described below in detail.

Stoma (Text-fig. 1, C-E and 4, A-E) : The stoma opens anteriorly through a hexaradiate oral aperture or vestibulum guarded by six lips. The vestibulum leads to a barrel-shaped buccal cavity formed by two sets : one vertical and another oblique set. Each set has three walls, one dorsal and the other two subventral in position. The dorsal wall of

vertical set bears a large dorsal tooth in the posterior half of buccal cavity pointing anteriorly. The subventral vertical walls are provided with denticles opposite the dorsal tooth. The subventral oblique walls bear two foramina each. Though the walls of both sets are almost similar in shape but those of the oblique set are smaller and less sclerotized. A cross section through vertical set shows the three walls to be similar anterior to dorsal tooth. The buccal cavity narrows posteriorly and joins the oesophageal lumen.

A few abnormal types of buccal cavities were also observed. In one specimen the buccal cavity lacked dorsal tooth (Text-fig. 2, A) in other subventral denticles were absent on one of the vertical wall (Text-fig. 2, B). In another case the subventral denticles were absent from both the walls (Text-fig. 2, C).

Oesophagus (Text-fig. 1, E and 4, F-K) : The buccal cavity is followed by a long cylindrical and highly muscular oesophagus. The oesophagus is slightly expanded at its anterior end surrounding the basal part of stoma, but slightly narrowing at level of nerve ring, then widening gradually towards the base. Various kinds of thickenings occur on the walls of the triradiate lumen. These serve as points of attachment for the radial muscles which originate from the walls of oesophagus. These muscles are strongly developed in the middle of oesophagus. The width of the lumen of oesophagus is uniform throughout its length except at the base of buccal cavity where it is wider.

Oesophago-intestinal junction (Text-fig. 1, E and 4, J-K) : The oesophago-intestinal junction or cardia connects the oesophagus with intestine and is tuberculate type. It consists of three tubercles, a filtration valve and a conical organ (Clark, 1960a). These tubercles open in the lumen of oesophagus which in turn opens into a funnel-shaped filtration valve situated in the somatic zone which lies below the transparent zone. The conical organ is heart-shaped projecting into the lumen of intestine. It also has triradiate lumen, but lacks cuticularized thickenings.

Intestine (Text-fig. 4, L) : The intestine is a long uniform tube with a wide lumen, narrowing posteriorly in the region where it joins the rectum. In a cross section it is single-layered with 6-8 cells in the circumference. The cells have distinct nucleus but are irregular in shape. The intestine on its dorsal walls where it is surrounded by anterior most copulatory muscles lacks granules and appears hyaline. The lumen of the intestine is also covered by a hyaline layer, but no cilia or rod-like structures are present.

Rectum (Text-fig. 3, E and 4, M) : The intestine is followed by the rectum which is a narrow, dorsoventrally flattened tube internally

lined with cuticle. The rectum leads to the exterior through anus. In males the rectum joins the genital duct to form the cloaca. The cloaca opens outside through the cloacal aperture.

Rectal glands (Text-fig. 6, A) : In males four rectal glands are present on each side dorsal and posterior to spicules. Their ducts run a short distance parallel to spicules turn upwards and open into the cloaca.

REPRODUCTIVE ORGANS

Female reproductive organs (Text-fig. 5, A-F) : The female reproductive system is amphidelphic. Each sexual branch consists of an ovary, oviduct, sphincter, uterus, vagina and vulva.

Ovary (Text-fig. 5, A) : The ovary is a thin-walled epithelial sac enclosing the germ cells and lying on ventral side of the body. The epithelium of the sac consists of long flattened cells which are clearly visible towards the proximal end. Typically the ovary may be divisible into two zones : the germinal zone and the growth zone (Text-fig. V, A & B). The oviduct is connected to the ovary subterminally which results in the formation of a blind sac in which the ripening oocytes or oogonia grow until they reach their full length. When the oocytes have reached maturity they leave the ovary and pass on to the oviduct.

Oviduct (Text-fig. 5, A-C) : The oviduct is at first a narrow tube and then enlarges proximally. The distal part differs from the proximal in having transverse markings (Coomans, 1964). In a cross section the distal part appears circular with a narrow lumen and made up of 6-7 high columnar cells. The proximal part also appears cellular in structure but the outlines of the individual cells are indistinguishable from one another and the whole region gives a rather granular vacuolated appearance.

Technically, the oviduct is followed by the uterus, but they are separated by a well developed structure which is called *sphincter* (Text-fig. 5, D). It is a truncated cone-like structure having longitudinal cuticularized linings of fine strands or fibers which arise from the inner walls of the uterus and converge at the centre of sphincter where they become slightly thickened and more refractive in nature. The fibers from the centre form the linings of the proximal end of the oviduct in the lumen of which narrow end of the sphincter is protruded.

Uterus (Text-fig. 5, E) : The uterus is a highly extensible tube with low columnar epithelial cells in circumference. The distal part of the uterus serves a fertilization chamber while the eggs are present in the proximal part and are provided with a visible shell. The maximum

number of eggs recorded at one time in the uterus was only 2, the sperms were also seen in the proximal part of uterus of some specimens.

Vagina (Text-fig. 5, F) : The uteri of both sides join to form a common short tube, the vagina. It is lined with cuticle and also adequately supported with muscles. It opens outside through vulva.

Vulva (Text-fig. 3, C, D and 5, F) : It is a ventral transverse slit, post-equatorial in position. It has cuticularized lips formed by the invagination of the cuticle. On either side of the vulva there are present vulval papillae which are variable in number. The prevulval vary from 0-3, and postvulval 0-2. In one female, the cuticularized pieces were absent (Text-fig. 2, H). A pair of vulval glands (Text-fig. 5, F) are situated ventrally which open into the vulva through their ducts.

Male reproductive organs (Text-fig. 6, A-I) : The male gonad consists of testes, vas deferens, ejaculatory duct and cloaca.

Testes (Text-fig. 6, A & E) : There are two testes, one directed anteriorly and the other posteriorly. Each testis is enclosed in a thin-walled sac made up of epithelial cells. Each testis is differentiated into a germinal zone and a zone of maturation. At the distal end of the maturation zone there are usually present several rows of spermatocytes. These are rectangular or hexagonal in shape and give rise to spindle-shaped spermatids which are present in clusters. The length of spermatids may be variable. These ultimately transform into spermatozoa or sperms (Aboul-Eid, 1969). The sperms (Text-fig. 6, A & C) are numerous and fill the entire proximal end of the growth zone.

Vas deferens (Text-fig. 6, F & G) : The proximal ends of the two testes join a single long thin-walled tube, the vas deferens. It runs posteriorly up to the constriction formed by the last accessory copulatory muscles and first copulatory muscles. The vas deferens at this point appears very much constricted.

Ejaculatory duct (Text-fig. 6, A) : Posterior to the horizontal accessory copulatory muscles, the vas deferens differentiates into a thin-walled tube, the ejaculatory duct. It narrows posteriorly to join the rectum to form a cloaca.

There are four ovoid or rounded ejaculatory glands lying over a part of the intestine or ejaculatory duct or both. Posteriorly each one narrows and joins a duct which opens into the lumen of the ejaculatory duct (Text-fig. 6, A). In one male the ejaculatory glands were five in number (Text-fig. 2, I).

Cloaca (Text-fig. 6, A & I) : The middorsal wall of the cloaca is first flattened, than compressed and finally folds backward where the spicules enter the cloaca. A buldging of the midventral wall also appears about halfway its length.

Spicules (Text-fig. 6, D, H & I) : These are a pair, equal in size and similar in shape. They are long and slender ventrally arcuate, sclerotized, narrowing towards their tips. Each spicule may be differentiated into two regions, a head region (capitulum) and a blade (Lamina). No region similar to shaft (calomus) was found to be present. Each spicule is covered by a spicular sheath. In a cross section at level of head of spicules, they appear flattened with two cavities and gradually inward curving flanges.

Gubernaculum & lateral accessory pieces (Text-fig. 6, D & H) : The gubernaculum lies dorsal to spicules from which a pair of lateral accessory pieces extend forward on either side of the spicules. In a cross section the gubernaculum appears trough-shaped with lateral parts first extending along the spicules and lateral accessory pieces (crura) then turned inwards in front of the crura and finally fused with the lateroventral walls of the cloaca as in *Anatonchus amiciae* by Coomans & Lima (1965) and *Cobbonchus pounamua* by Clark (1960b). The lateral accessory pieces are cuticularized and bifurcated at their distal end.

Supplements (Text-fig. 6, B) : Each supplement is an elevated structure supplied with a nerve ending.

TAIL (Text-fig. 6, A)

The tail is long filiform, tapering uniformly, with a ventral curvature and slightly clavate tip. The tail is provided with 4 prominent caudal papillae. The three caudal glands lead to a common duct which opens into an ampulla-like structure, the spinneret. The tuboid opening of the spinneret is provided with a cone-like structure which acts as a valve (Text-fig. 6, A & 4, O).

Apart from the normal type of tails, some abnormal tails were also recorded. In one specimen the tail is elongate conoid about half the normal tail length. The caudal glands are situated posteriorly and the spinneret absent (Text-fig. 2, E). In another specimen the tail is provided with a mucro (Text-fig. 2, G). Often the tail is broad especially in the hinder region (Text-fig. 2, F).

THE JUVENILE STAGES

First stage Juvenile (Text-fig. 7, D, H & L) :

Dimensions (16) : $L=0.61-0.93$ mm ; $a=22-31$; $b=2.5-3.7$;
 $c=6.0-7.9$; $c'=5.4-7.3$.

Description : Body almost straight upon fixation, tapering slightly anteriorly, but markedly posteriorly. Cuticle (inner and outer) smooth, $2\ \mu\text{m}$ thick at various places on the body. Lip region marked off, $18-20\ \mu\text{m}$ wide, $5-7\ \mu\text{m}$ high, distinctly wider than the adjoining body. Amphids small cup-like, apertures $3-4\ \mu\text{m}$ wide at $11\ \mu\text{m}$ from anterior end of body.

Buccal cavity $21-23 \times 11\ \mu\text{m}$. Apex of dorsal tooth at $5-8\ \mu\text{m}$ from base of stoma. The dorsal wall of buccal cavity $18-20\ \mu\text{m}$ in length. No tooth or teeth present on the vertical subventral walls. The oblique subventral walls have two foramina on each. Orifices of the oesophageal glands located as follows : dorsal $88-94\ \mu\text{m}$ from anterior end of body ; the first pair of subventrals $30-39\ \mu\text{m}$ from the orifices of dorsal one ; the second pair $44-60\ \mu\text{m}$ from the orifices of the first pair. Oesophago-intestinal junction tuberculate. Nerve ring at $53-60\ \mu\text{m}$ from anterior end of body. Rectum $14-16\ \mu\text{m}$ or about one anal body-width long. Genital primordium not observed. Tail $82-126\ \mu\text{m}$ or about 3-5 anal body-widths long. Caudal glands well developed, opening terminal, caudal papillae invisible.

Second Stage Juvenile (Text-fig. 7, C, G, K & M) :

Dimensions (10) : $L=1.06-1.26$ mm ; $a=30-31$; $b=3.4-4.5$;
 $c=6.3-7.2$; $c'=6.5-7.5$.

Description : Body arcuate upon fixation, tapering slightly anteriorly, but markedly posteriorly. Cuticle (inner and outer) smooth, $2\ \mu\text{m}$ thick at midbody. Lip region marked off, $24-26\ \mu\text{m}$ wide, $9-11\ \mu\text{m}$ high, distinctly wider than adjoining body. Amphids small cup-like, apertures $3-4\ \mu\text{m}$ wide at $11-13\ \mu\text{m}$ from anterior end of body.

Buccal cavity $29-31 \times 15-17\ \mu\text{m}$. Apex of dorsal tooth at $9-11\ \mu\text{m}$ from base of stoma. Dorsal wall $24-28\ \mu\text{m}$ high. The vertical subventral walls bear 1-2 teeth and the oblique walls have two foramina on each. Orifices of oesophageal glands located as follows : dorsal $102-156\ \mu\text{m}$ from anterior end of body ; the first pair of sub-ventrals $46-53\ \mu\text{m}$ from the orifices of dorsal one ; the second pair $68-95\ \mu\text{m}$ from the orifice of the first pair. Oesophago-intestinal junction tuberculate. Nerve ring at $88-96\ \mu\text{m}$ from anterior end of body. Rectum $20-24\ \mu\text{m}$ or about 2-3 anal body-widths long. The genital primordium $14\ \mu\text{m}$ long and consists of 4 serially arranged cells. Tail elongate-conoid, $156-195\ \mu\text{m}$

or about 6-8 anal body-widths long. Caudal glands well developed, opening terminal. Caudal papillae invisible.

Third Stage Juvenile (Text-fig. 7, B, F, J & N) :

Dimensions (14) : $L=1.49-1.73$ mm ; $a=32-37$; $b=3.9-4.1$;
 $c=7.5-8.6$; $c'=5.6-6.9$

Description : Body almost straight upon fixation, tapering slightly anteriorly, but markedly posteriorly. Cuticle (inner and outer) smooth, 2-4 μm thick at various places on the body. Lip region marked off, 29-33 μm wide, 11-15 μm high distinctly wider than the adjoining body. Amphids small cup-like, apertures 5-6 μm wide at 12-15 μm from the anterior end of body.

Buccal cavity 35-39 \times 18-20 μm . Apex of dorsal tooth at 13-17 μm from base of stoma. The dorsal wall of buccal cavity 29-31 μm . Vertical subventral walls bear 3-4 teeth and the oblique walls have two foramina on each. Orifices of oesophageal glands located as follows : dorsal 170-194 μm from anterior end of body ; the first pair of subventrals 56-63 μm from orifice of the dorsal one ; the second pair 94-105 μm from orifice of first pair. Oesophago-intestinal junction tuberculate. Nerve ring at 113-117 μm from anterior end of body. Rectum 27-28 μm or about 4-5 anal body-widths long. The genital primordium consists of 12 cells and measures 34 μm in length. Tail elongate-conoid, 184-230 μm or about 6-7 anal body-widths long. Caudal glands well developed, opening terminal. Caudal papillae 4.

Fourth Stage Juvenile (Text-fig. 7, A, E, I & O) :

Dimensions (22) : $L=1.81-2.31$ mm ; $a=40-42$; $b=4.0-4.5$;
 $c=6.6-8.8$; $c'=6.2-8.5$.

Description : Body almost straight upon fixation, tapering slightly anteriorly, but markedly posteriorly. Cuticle (inner and outer) smooth, 4-6 μm thick at various places on the body. Lip region marked off, 35-40 μm wide, 13-18 μm high, distinctly wider than adjoining body. Amphids small cup-like, apertures 5-6 μm wide at 16-18 μm from anterior end of body.

Buccal cavity 42-48 \times 22-26 μm . Apex of dorsal tooth at 18-20 μm from base of stoma. The dorsal wall of buccal cavity 37-40 μm high. Vertical subventral walls bear 3-6 teeth and the oblique wall have two foramina on each. Orifices of the oesophageal glands are located as follows : dorsal 205-231 μm from anterior end of body ; first pair of subventrals 65-95 μm from orifice of dorsal one ; second pair 106-141 μm from orifices of first pair. Oesophago-intestinal junction tuberculate. Nerve ring at 138-143 μm from anterior end of body. Rectum 30-33 μm

or about 4-5 anal body-widths long. The genital primordium measuring 48-123 μm and consisting of 32-60 cells. Tail elongate-conoid, 226-314 μm or about 6-7 anal body-widths long. Caudal glands well developed, opening terminal, caudal papillae 4.

DISCUSSION AND CONCLUSION

The detailed morphological studies on *Parahadronchus shakili* (Jairajpuri, 1969) Mulvey, 1978 show many intra-specific variations which are important for the taxonomic study of the genus *Parahadronchus* and the related groups.

The shape of the body and the number of the labial papillae was found to be constant. The dot-like additional structures near the middle of lips on either side of internal labial papillae were observed which are supposed to be the points of attachment of six labial muscles which have been recorded for the first time for this species.

Important variations were observed in total body length, shape and size of buccal cavity, number of denticles on the subventral walls, number of ventromedian supplements etc. The size of the buccal cavity is to some extent related with the sex and body length. Larger specimens have larger buccal cavities and also the females usually have larger buccal cavities than males. The position and shape of the dorsal tooth are constant and is therefore of diagnostic value. The orifices of the oesophageal glands are variable and thus are not much significant.

The characters for the separation of different juvenile stages confirm with those given by Coomans & Lima (1965) with slight deviations. The first stage juveniles differ from all the other stages in the absence of denticles on the vertical subventral walls. These denticles are 1-2 in the second stage, 3-4 in the third stage and 3-6 in the fourth stage. The genital primordium was not observed in the first stage, in the second stage it is small having 4 serially arranged cells, in the third stage it is oval with 12 cells, while in the fourth stage it is elongate-oval with 32-60 cells. In the last juvenile stage usually a rectangular hyaline area also makes its appearance which indicates the place of formation of the future vulva.

The presence or absence and number of denticles on the subventral walls are helpful only up to the third stage. The tail length does not overlap between first and 2nd stages, yet it can not be taken as reliable character because it overlaps in the rest of the stages. The a, b, c and c' values are not useful distinguishing characters. The other features like amphids, tubercles, spare tooth etc., are unreliable in the

separation of juvenile stages. The presence of spare tooth clearly differentiates the juveniles from the adults.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Zoology, Aligarh Muslim University for providing necessary laboratory facilities. The first author also thanks the ICAR, New Delhi for the award of a Senior Research Fellowship.

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