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DATA ON THE MORPHOLOGY OF THE NEMATODE SPINITECTUS MINOR (STEWART, 1914) (NEMATODA: CYSTIDICOLIDAE)

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Abstract. Morphology of the nematode *Spinitectus minor* harboured by *Mastacembelus armatus* from Burdwan, West Bengal, India was studied by light and scanning electron microscopes. The worms were described, illustrated and discussed taking into consideration of the information available relative to this species. Alate spicules, polar caps on the eggs and predelphic uteri are clearly seen in the present LM studies and SEM photomicrographs provided detailed information about the microtopography of the worms.

Spinitectus minor (Stewart, 1914) frequently occurs in the gut of different fresh water fishes of India. Since the first record this species has been reported under various specific names probably due to insufficient knowledge of its morphology as also erroneous descriptions in the literature. Soota (1984), while elaborately dealing with various Indian species of the genus *Spinitectus* Fourment, 1883 suggested the conspecificity of many nominal species with *S. minor*. Details about some significant taxonomic features, like the structures of the mouth elements, spicules and eggs, as also the number of spines in the anterior rings on the body surface, the position of excretory pore in relation to the anterior rings of spines, number and disposition of caudal papillae in male and the courses followed by the uterine branches are still either inadequate or confusing. The present light microscope (LM) and scanning electron microscope (SEM) studies aim to add some valuable information regarding the morphology of *S. minor*.

MATERIALS AND METHODS

A number of specimens (26 males and 32 females) of *S. minor* were obtained from the stomach of six out of sixteen *Mastacembelus armatus* (Lacép.) collected from the fish market at Burdwan, West Bengal, India and autopsied during the period from April to December 1983. The worms were washed by shaking thoroughly in physiological saline and transferred to 0.1 M phosphate buffer (pH 7.4) to which a few grains of the enzyme pronase had been added. They were cleared in that solution for two minutes before being fixed in Berland's fluid (Gibson 1979). Most of the specimens were transferred to 4% formaldehyde for their use in LM study.

For SEM, after primary fixation, the anterior and posterior ends of three specimens of each sex were cut off by using a sharp razor blade to promote better fixation and minimize shrinkage. The detached heads and tails were then exposed to 3% gluteraldehyde in the same buffer for 24 h before being osmicated (in 1% OsO_4). Rinsing in the buffer, however, was done twice (10 min every time) between each step and the next. The cut ends were then dehydrated in ascending series of ethanol, transferred to 100 % iso-amyl acetate through the mixtures of ethanol and iso-amyl acetate. The specimens were then dried at room temperature (26 °C), mounted on the copper stubs and finally coated with gold. Observations were carried out with a Philips 500 scanning electron microscope (PSEM 500) with a resolution of 100 Å and operating at an accelerating voltage of 24 KV.

RESULTS

Morphologically the present worms agree in general with the description of *S. minor*. Metrically also they come closer taking into consideration Soleim's (1976) view that nearly 20% increase in the measurements occurs due to the use of GAA (a major component of Berland's fluid) as fixative. However, SEM study of the present worms shows that the body cuticle is provided with distinct transverse striations (Pl. II, Figs. G and J) and anteriorly the body surface becomes elevated in the form of transverse rings, interrupted at both sides by lateral lines (Pl. I, Fig. A; Pl. II, Figs. F and G). These rings are beset with rows of backwardly directed spines. The first two rings are more raised, remain close to each other and bear larger spines with broad bases (Pl. II, Figs. G and H). First and second rings bear 33 and 31 spines in females and 24 and 20—22 in males, respectively. Thereafter, the thickened rings gradually become less pronounced, their spines also diminish in size and decrease in number. Rings, posterior to third, are again interrupted both dorsally and ventrally and each thus gives comb-like bodies (Pl. II, Figs. G and K). These comblike bodies, however, do not always lie at the same level. In the posterior region of the body the cuticular elevations are absent but circularly arranged tiny spines extend up to posterior end in female (Pl. III, Figs. N and O) and up to proximal end of caudal alae in male. A few scattered, thin sharp spines are seen (Pl. IV, Fig. R) on the postanal ventral body wall of male. Head end, anterior to the first ring, is conical and with truncated anterior margin (Pl. II, Fig. G). Dorsoventrally oval mouth opening bears two subdorsal and two subventral, elongated sclerotized plates (= cheilorhabdions) at its inner margin (Pl. II, Fig. F; Pl. III, Fig. L). Two relatively large lateral pseudolabia with indistinct dorsal and ventral lobes (Pl. II, Fig. I) at the anterior end cover greater part of the oral opening. Two submedian cephalic papillae and an indistinct lateral amphid are situated at the base of each pseudolabium (Pl. II, Fig. F). Each of the pseudolabia continues posteriorly into a thickened pulp. The vestibule is straight, thin walled, with anterior end distended to form the funnal-shaped prostom (Pl. I, Fig. A). The nerve ring encircles the oesophagus between second and third ring of cuticular spines (Pl. I, Fig. A). Crescent-shaped excretory pore is situated on a small ventral round prominence at the level of 4th or 5th ring of spines (Pl. II, Figs. G and J).

Posterior male end is coiled ventrally and bears moderately developed subventral caudal alae. The caudal alae end slightly anterior to the tail tip thus leaving a short terminal spike (Pl. I, Fig. B; Pl. IV, Fig. T). Six longitudinal rows of rectangular cuticular ridges present on the precloacal ventral body wall (Pl. I, Fig. B; Pl. IV, Fig. Q). They extend anteriorly almost to the proximal end of the larger spicule. Each of these ridges bears broad base but thin free edge (Pl. IV, Fig. S). Eleven pairs of subventral caudal papillae are present of which 4 pairs are preanal, 1 pair adanal and 6 pairs postanal in position (Pl. I, Fig. B). All, except the last pair of postanal papillae are large and pedunculated, the last pair being small and sessile (Pl. I, Fig. B). Regarding the disposition, it is observed that the preanal papillae are almost equally spaced and the second and fourth pairs lie more ventrally than the first and third ones (Pl. IV, Fig. Q); the first four pairs of postanal papillae are almost equidistantly placed from each other, the fifth pair lies at the same level as also the inner ventral side of the fourth (Pl. I, Fig. B; Pl. IV, Fig. T). The sixth pair is located in grooves formed by small petaloid cuticular formations behind the fourth and fifth pair (Pl. IV, Fig. T). Distinct transverse cuticular bands are present anterior and posterior to small cresent-shaped cloacal opening (Pl. IV, Fig. R). Spicules are unequal and dissimilar. Long and slender larger spicule is slightly bent distally, initially becomes inflated and then gradually narrows to end in a pointed tip. Two asymmetrical spicular flanges are present at the distal part, which do not continue to the tip but stop short a little before it (Pl. I, Fig. C_2). Boat-shaped smaller spicule is broad proximally and narrows down to end in a knobbed distal tip; two wide lateral flanges extend from the proximal to the distal third of the spicule where they coalesce (Pl. I, Fig. C_1).

Female tail is conical and bears small bosses at its tip (Pl. III, Fig. O). Two small phasmids are located at the posterior quarter of the tail (Pl. III, Fig. O). The vulva in the form of distinct circular marking lies near and anterior to anus (Pl. III, Fig. M). The lower lip, however, covers greater part from inner side and leaves only a narrow opening anteriorly (Pl. III, Fig. P). Anteriorly directed short muscular vagina leads to a well developed ovejector which bears huge sac-like vestibule. Shortly after its emergence the uterus bifurcates and the two branches run in parallel and anteriad (Pl. I, Fig. D). Mature eggs are oval in shape, thick-walled and embryonated and provided with fine, transparent polar formations; low polar caps (Pl. I, Fig. E) in some while irregular polar flocks in others and even in few wide ribbon-like appendage or filament on one pole.

DISCUSSION

The genus *Spinitectus* Fourment, 1883 was established with *S. oviflagellis* as the type species and based on female specimens only. Stewart (1914) described *Spiroptera denticulata* var. *minor* on two male specimens from *Walago attu* from India which was later renamed by Baylis (1939) as *Spinitectus minor*. The redescription of this species by Ali (1957) was based on a single male specimen from identical host. Both males and females were described by Soota (1984). Though descriptions of some new species from India have been added to the literature, many of them were challenged (Sood 1968, Soota 1984). Soota (loc. cit.) listed ten such species, namely *S. corti* Moorthy, 1939, *S. mastacembeli* Karve et Naik, 1951, *S. major* Khera, 1956, *S. thapari* Ali, 1957, *S. armatus* Ali, 1957, *S. singhi* Ali, 1957, *S. bengalensis* Chakravarty, Sain et Majumdar, 1961, *S. fossili* Lal, 1966, *S. batrachi* Lal, 1966 and *S. alii* Kalyankar, 1970 as junior synonyms of *S. minor*.

The present study clearly shows that these nematodes are characterized by some features which were given inadequately or erroneously in the existing literature. Moravec (1979) pointed out that the structures of the mature eggs, the number of spines in the anterior rings of the body, the situation of the excretory pore in relation with the location of the anterior ring of cuticular spines, number and disposition of the caudal papillae and the structure of the spicules were important features of the nematodes of this genus. Cephalic structures are also important in this group. Very little is known about the cephalic structures of *S. minor* and their lips are recorded as either inconspicuous or absent. Present SEM photomicrographs (Pl. II, Fig. F; Pl. III, Fig. L) not only indicate the presence of two large lateral pseudolabia with indistinct dorsal and ventral lobes but also the existence of two subdorsal and subventral elongated sclerotized plates (= cheilorhabdions) at the inner margin of dorsoventrally oval mouth opening. Similar sclerotized plates on the inner side of the oral margin were also recorded by De and Moravec (1979) in *Cystidicoloides ephemeridarum* (Linstow, 1872) and by De (1987) in *Pseudoproleptus notopteri* (Karve et Naik, 1951), members of the family Cystidicolidae Chabaud, 1975.

The number of spines in the anterior rings is reported to be variable. Baylis (loc. cit.) recorded the existence of 26 spines in the first ring of male, while Ali (1957) reported 24 spines in each of the first two rings. Number of spines in the first ring ranges from 20 to 30 and 22 to 35 in male and female, respectively, of various forms

which are now treated as synonymous with *S. minor*. In the present females, 33 spines are found in the first ring (Pl. II, Fig. F) and in the males, there are 24 spines. The location of excretory pore in relation to the anterior rings of cuticular spines seems to be species constant and, therefore, can be considered a significant feature of discrimination. Present SEM photomicrograph (Pl. II, Fig. G) clearly shows that crescent-shaped excretory pore is situated on a small round prominence at the level of the 4th or 5th rings. As to the caudal papillae, Baylis (1939) recorded 4—6 preanal and 5—6 postanal papillae on each side of *S. minor* and Soota (loc. cit.) recorded the ranges of preanal, adanal and postanal papillae as 3—4, 0—1 and 3—8, respectively. All male worms studied in the present case were found to possess 4 pairs of preanal, 1 pair of adanal and 6 pairs of postanal papillae. Most of the authors described the left spicule of this species as long, slender body with a "foot-shaped" distal portion and the right one as short, broad and boat-shaped. The present account, though confirms these observations, gives details about the "foot-shaped" formation of the left and right spicules. Baylis (loc. cit.) mentioned the presence of opposed uterine branches as a diagnostic generic feature. The synonymous species *S. mastacembeli* and *S. major* are known to possess opposed uteri, whereas the descriptions of *S. corti* and *S. thapari* clearly indicate the presence of prodelphic uteri. The present nematodes also clearly show prodelphic uterine disposition. The existence of polar caps in the eggs of *S. minor* has not so far been reported. Karve and Naik (1951) suspected the presence of polar filaments in the eggs of *S. mastacembeli*. Moravec (1979) for the first time recorded the existence of gelatinous polar caps on the eggs of *S. inermis* (Zeder, 1800) and reported it to be unique among the members of the genus *Spinitectus*. He also suggested that the type of eggs is apparently associated with the ecology of the individual species. It follows from the study of the present materials that in *S. minor* the mature eggs have gelatinous polar caps and other polar formations and thus resemble those of *S. inermis*.

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МОРФОЛОГИЯ НЕМАТОДЫ SPINITECTUS MINOR (STEWART, 1914) (NEMATODA: CYSTIDICOLIDAE)

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Резюме. С помощью световой и сканирующей электронной микроскопий изучали морфологию нематоды *Spinitectus minor* от *Mastacembelus armatus* из Бордвана (Западная Бенгалия, Индия). Паразиты описываются, изображаются и обсуждаются, учитывая все информации, касающиеся этого вида. В световом микроскопе четко выражены крылатые спикулы, полярные верхушки яиц и параллельные, вперед направленные матки. С помощью сканирующего электронного микроскопа получились подробные информации о микротопографии этой нематоды.

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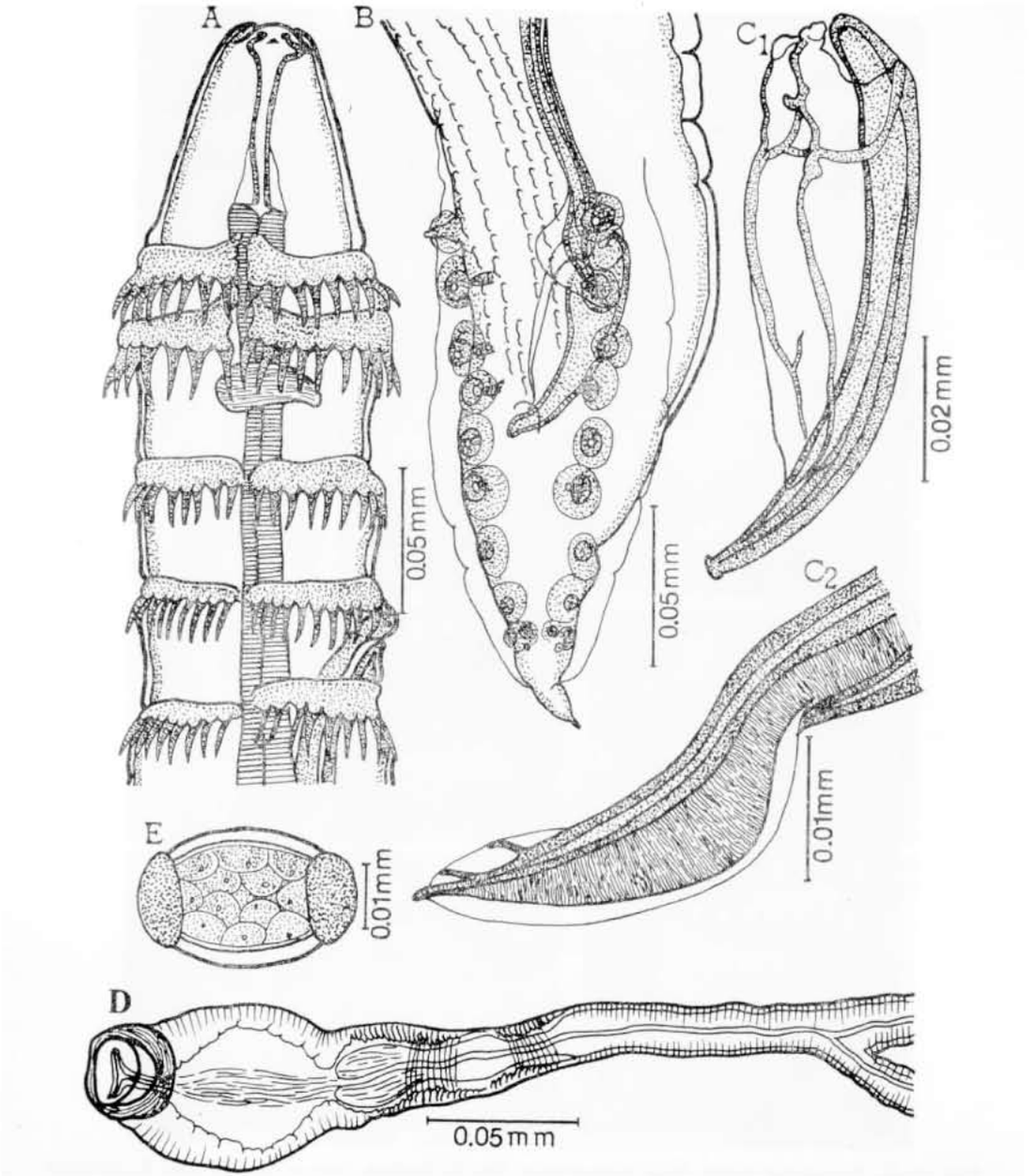
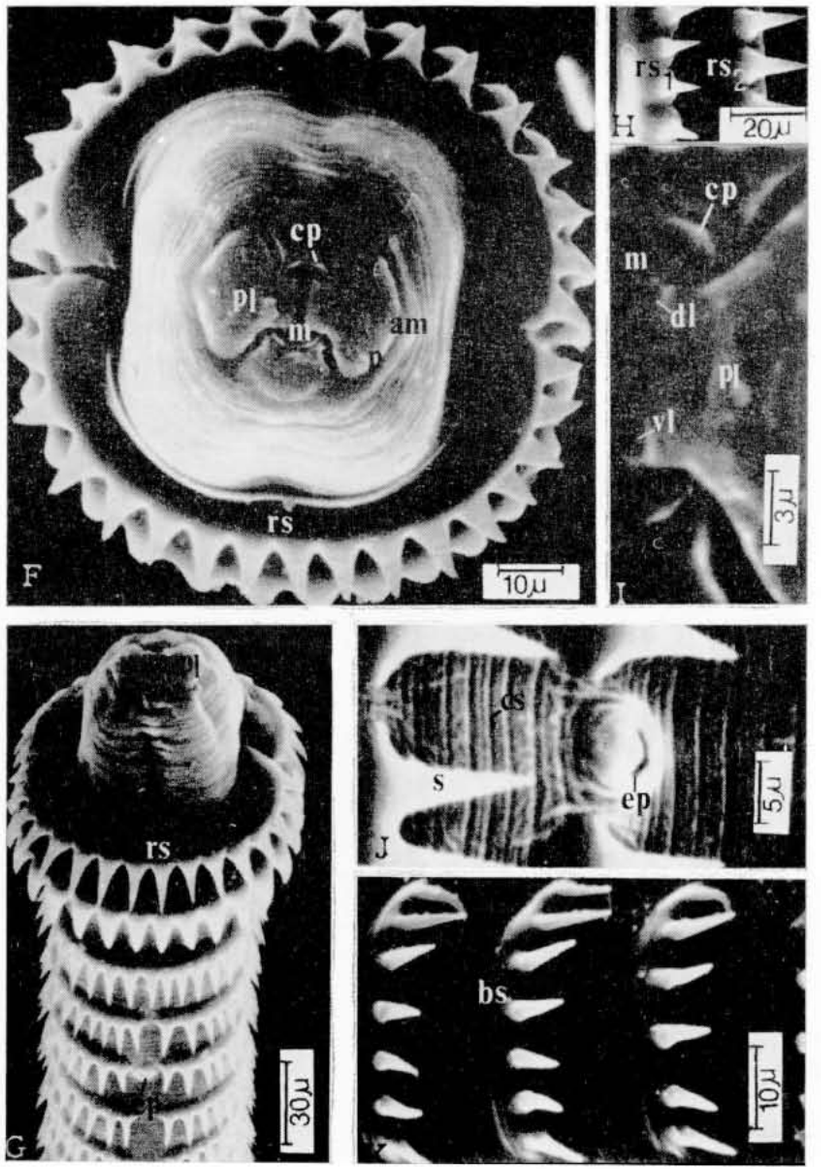
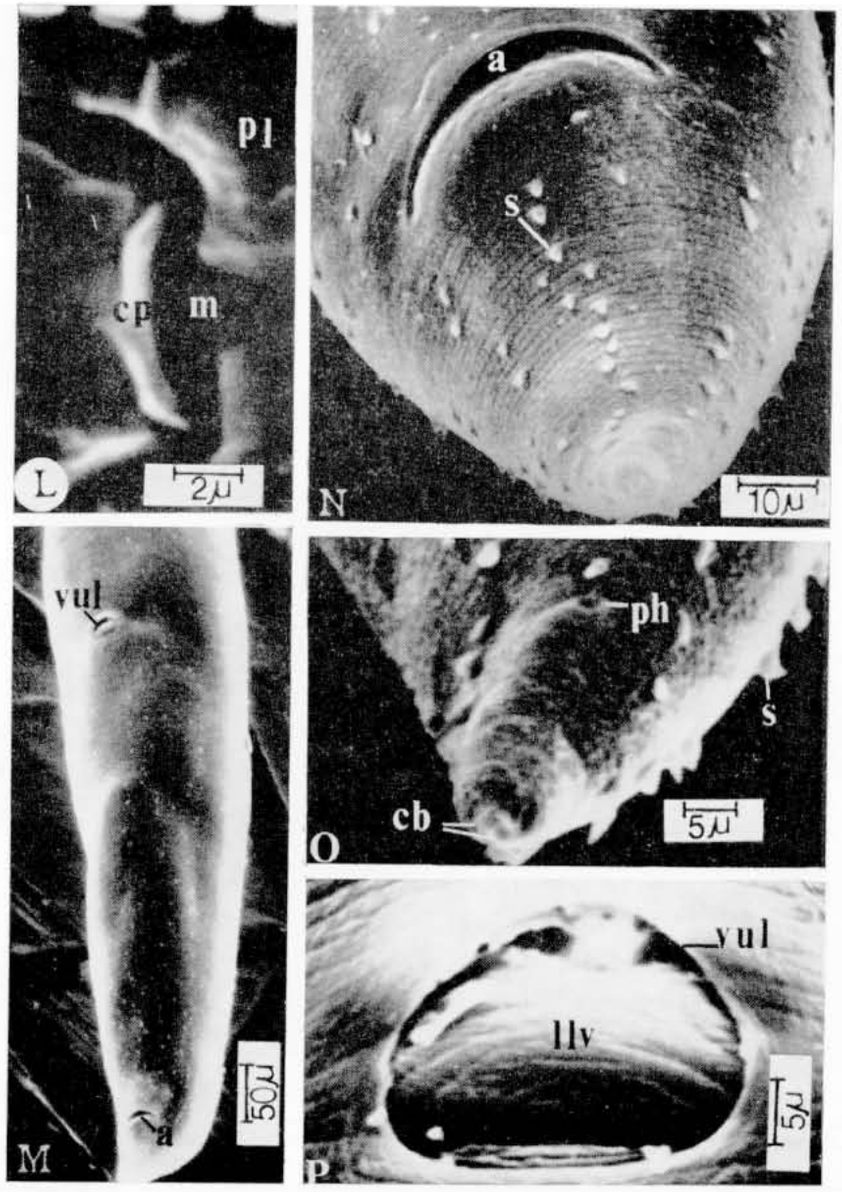


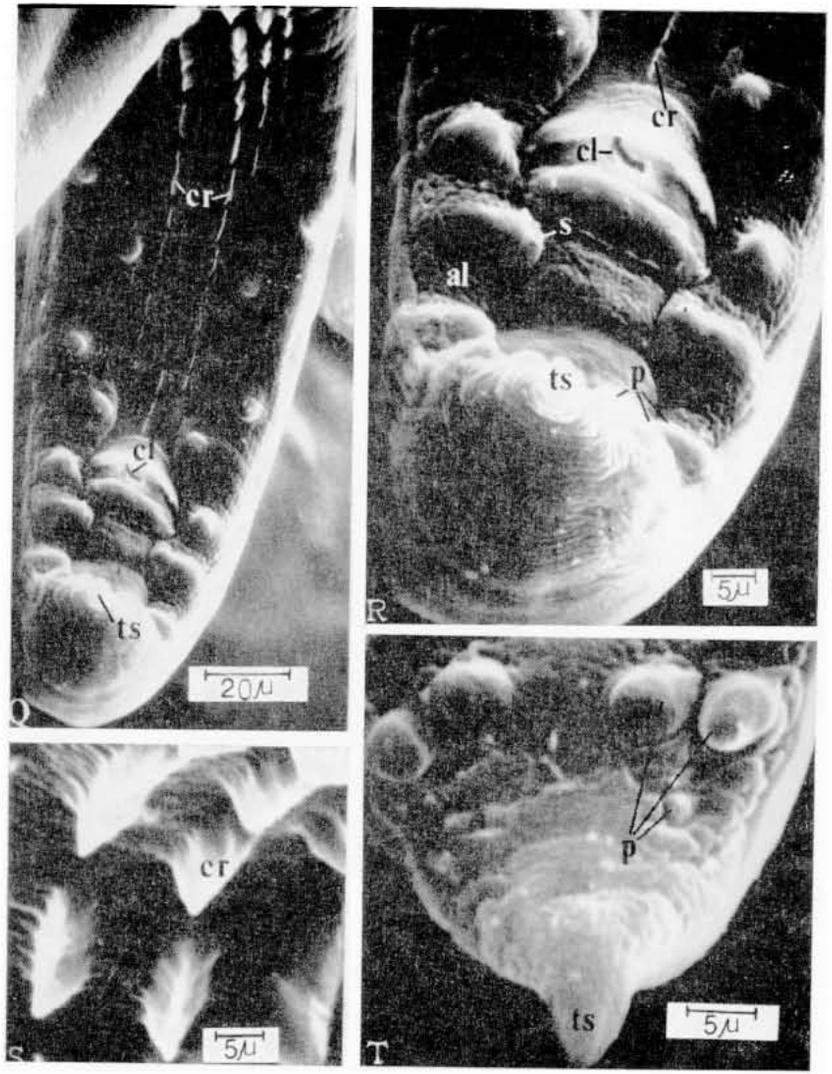
Figs. A—E. *Spinitectus minor*. **Fig. A.** Anterior end of female, lateral view; **Fig. B.** Posterior end of male, ventro-lateral view; **Fig. C_1.** Smaller spicule, lateral view; **Fig. C_2.** Distal end of larger spicule, lateral view; **Fig. G.** Vulvar region, ventral view; **Fig. E.** Egg.



Figs. F—K. *Spinitectus minor*, SEM micrographs. **Fig. E.** En-face view of female; **Fig. G.** Anterior end of female, ventral view; **Fig. H.** Cuticular spines of first and second rings; **Fig. I.** Lateral half of the apex of the head; **Fig. J.** A portion of the body wall showing excretory pore, ventral view; **Fig. K.** Cuticular spines posterior to the level of excretory pore.



Figs. L—P. *Spinitectus minor*, SEM micrographs. **Fig. L.** Dorsal half of the apex of the head; **Fig. M.** Posterior end of female, ventrolateral view; **Fig. N.** Tail of female, ventral view; **Fig. O.** Tail tip of female, enlarged lateral view; **Fig. P.** Vulva, ventral view.



Figs. Q—T. *Spinitectus minor*, SEM micrographs. **Fig. Q.** Posterior end of male, ventral view; **Fig. R.** Male tail, enlarged ventral view; **Fig. S.** precloacal cuticular ridges, ventral view; **Fig. T.** Male tail end, enlarged ventral view.

Abbreviations: a, anus; al, ala; am, amphid; bs, bar bearing spines; cb, cuticular boss(es); cl, cloaca; cp, cheilorhabdion plate; cr, cuticular ridge; cs, cuticular striation; dl, dorsal lobe of pseudolabium; ep, excretory pore; llv, lower lip of vulva; m, mouth; p, papilla(e); ph, phasmid; pl, pseudolabium; rs, ring of spines; s, spine; ts, tail spike; vl, ventral lobe of pseudolabium; vul, vulva.