

Scanning Electron Microscopy (SEM) of Some *Setaria* Species (Filarioidea, Nematoda)*

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Summary. Some *Setaria* spp., such as *S. digitata*, *S. marshalli*, *S. marshalli pandei*, *S. equina* and *S. labiatopapillosa* were studied by scanning electron microscopy at the critical points in the anterior and posterior parts of the adult worms. The amphids, Deirids, fine transverse striations of the cuticle, and phasmidial pore, as well as the transverse bands (lugae), postdeirid and ventral papillae (only of the male) were visualized clearly at this examination. They contribute towards the identification of *Setaria* spp., in addition to the characteristics which are demonstrated by common light microscopy.

The specific distinctions among *Setaria* spp. recognizable by the common light microscope were sometimes the cause of misidentification. Inaccessibility of the original literatures, ignorance of a language, by which the original description was made, or common occurrence of a species at unexpected host (like new-born) were partly responsible for the confusing situation around identification of some *Setaria* spp. In this paper *Setaria* spp. from *Bos taurus* of the Far East and from *Bubalus bubalis* of South India, and that from *Equus caballus* of Kazakhstan and Japan are dealt with.

Materials

S. digitata (von Linstow, 1906), commonly found from the peritoneal cavity (this parasitic location applies to all other spp.) of *Bos taurus* and *Bubalus bubalis* of the area east to "Thar Desert" till down to the Amur Valley (Shoho, 1976a), freshly collected from the indigenous cattle, *Bos taurus*, of Japan at Toyonaka abattoir near Osaka.

S. marshalli Boulenger, 1920, occurring in the same area as that of *S. digitata* and usually from the new-born calves, but rarely from the adult cattle, sheep, goats and horses (see Shoho, 1965) got from a few-days-old male bovine calf (of imported breed, Holstein-Friesian) at Chitose abattoir by Mr. K. Sugawa.

S. marshalli pandei Shoho, 1965 from the indigenous water buffalo, *B. bubalis*, of Trichur, South India, by Dr. N.S. Simon (FAO-expert).

S. equina (Abildgaard, 1789) from a horse, *Equus caballus*, of Kazakhstan at Alma-Ata by Prof. N.S. Boev (supplemented by the material from a race horse of Japan by Prof. Noda). (see Shoho, 1976b).

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S. labiatopapillosa (Alessandrini, 1848) from a cow (of an imported breed) at Toyonaka abattoir (this animal was recorded as coming from Gumma Prefecture near Tokyo¹).

Method

S. digitata and *S. labiatopapillosa* were exposed in 5% glutaraldehyde, postfixed in 1% osmium tetroxyde, dehydrated with ethyl alcohol, treated with isoamyl acetate (for replacement of alcohol), then dried with liquid CO₂ in a critical point apparatus (HCP-1) and finally coated by Au-sputter in an ion bombardment apparatus (IB-3). Other specimens, prefixed in formol-saline, were washed by the distilled water, so as to be treated in the same way as described above. The preparations were examined with JEOL JSM-50A scanning electron microscope, operated at 15 kV.

Results

The Anterior Part

The en face view by SEM enables us to confirm the characteristic form of the mouth opening surrounded by the peribuccal crown for each examined species (Figs. 1, 19, 23, 26, 27 and 35 respectively for *S. digitata*, *S. marshalli*, *S. marshalli pandei*, *S. equina* and *S. labiatopapillosa*). The form of the lateral lips of the peribuccal crown is more clearly seen at the lateral view, and here it is shown only at male *S. marshalli* (Fig. 11). The position of an amphid and of 2 pairs of the submedian papillae in different levels can be seen clearly.

Figs. 1–10. *S. digitata* (Figs. 3–9 for the male and Figs. 1, 2 and 10 for the female)

1. The en face view of the head showing the roundness of the mouth opening. 1,000 ×
2. The hilly elevation of the amphid with its pore at the lower bottom. 5,000 ×
3. The side view of a deirid. 3,800 ×
4. The ventral view of the male posterior part showing clearly the papillar arrangement; 3 pairs of precloacal, a pair of adcloacal and 3 pairs of postcloacal (of which the 1st pair being not neatly arranged) papillae, plus a central papilla just in front of the cloaca. A pair of lateral appendages accompanied with 2 pairs of tiny papillae anteriorly (only visible of one side) is also seen. Besides these papillae a pair of the extremely tiny papilla is at the extreme end. 300 ×
5. This showing the position of the postdeirid (marked with *) unilaterally left and the transverse bands 150 ×
6. The postdeirid enlarged looking not exactly similar to the deirids (Fig. 3). 4,900 ×
7. The central papilla, dorsoventrally elongated. 3,600 ×
8. The ventral bands composed of the parallel longitudinally running microstriations. 2,600 ×
9. The phasmidial pore at the upper armpit of the lateral appendage. 2,400 ×
10. The tapering terminal end with a smooth knob. 700 ×

Figs. 11–22. *S. marshalli* (Figs. 11–18 for the male and Figs. 19–22 for the female)

11. The lateral view of the head showing non-indented lateral lip (usually at the male) of the peribuccal crown and 2 pairs of the submedian papillae in different levels, in the centre of which the amphid is situated. 560 ×
12. The amphid showing its cuticular mosaic with its pore at the lower bottom. 3,800 ×
13. The deirid encircled by the fine striations, running round the basement of the deirid. The dotted dark line at the upper left is for the lateral line. Its needle-like formation is thickly bifurcated and an extra low thorn is present at left. (The mosaic of the deirid body could be artefact?) 4,900 ×

¹ Finding of *S. labiatopapillosa* in a certain area near Tokyo was elucidated first in 1973 (Shoho, 1974). It may be related to the importation of foreign cattle in the postwar period.

14. The postdeirid, at the base of which cuticular vertical striations intermit, with the trifurcated needle-like formation. 4,900 ×
15. The transverse bands composed, here, of longitudinal microstriations (compare with Fig. 8). 1,300 ×
16. The central papilla. 3,200 ×
17. The ventral view of the posterior part with the characteristic arrangement of papillae; 4 pairs of the precloacal, and 3 pairs of the postcloacal (of which the first and the third pairs being not neatly arranged) papillae with 3 lateral papillae (of one side of 3 pairs). The last one of these 3 pairs is the lateral appendages (see Fig. 18). 340 ×
18. The area with 3 lateral papillae enlarged (marked with * at Fig. 17). The phasmidial pore is recognizable at the upper armpit of the most distal papilla, thus assuring the nature of its being the lateral appendage among 3 papillae of nearly the same size. 2,100 ×
19. The female mouth opening, dorsoventrally lengthy oval. Three summits of the lateral lips (twice indented) of both sides are recognizable. 1,000 ×
20. The deirid with two short thorns at its base right and left. (The position of the picture can be orientated from the running direction of the transverse striations of the cuticle.) 4,400 ×
21. The obtusely ending tail with the roughly furcated terminus. The lateral appendage with the distinct phasmidial pore is marked with *. 1,300 ×
22. The lateral appendage enlarged and the phasmidial pore distinctly visible. 3,200 ×

Figs. 23–25. *S. marshalli pandei* (female only)

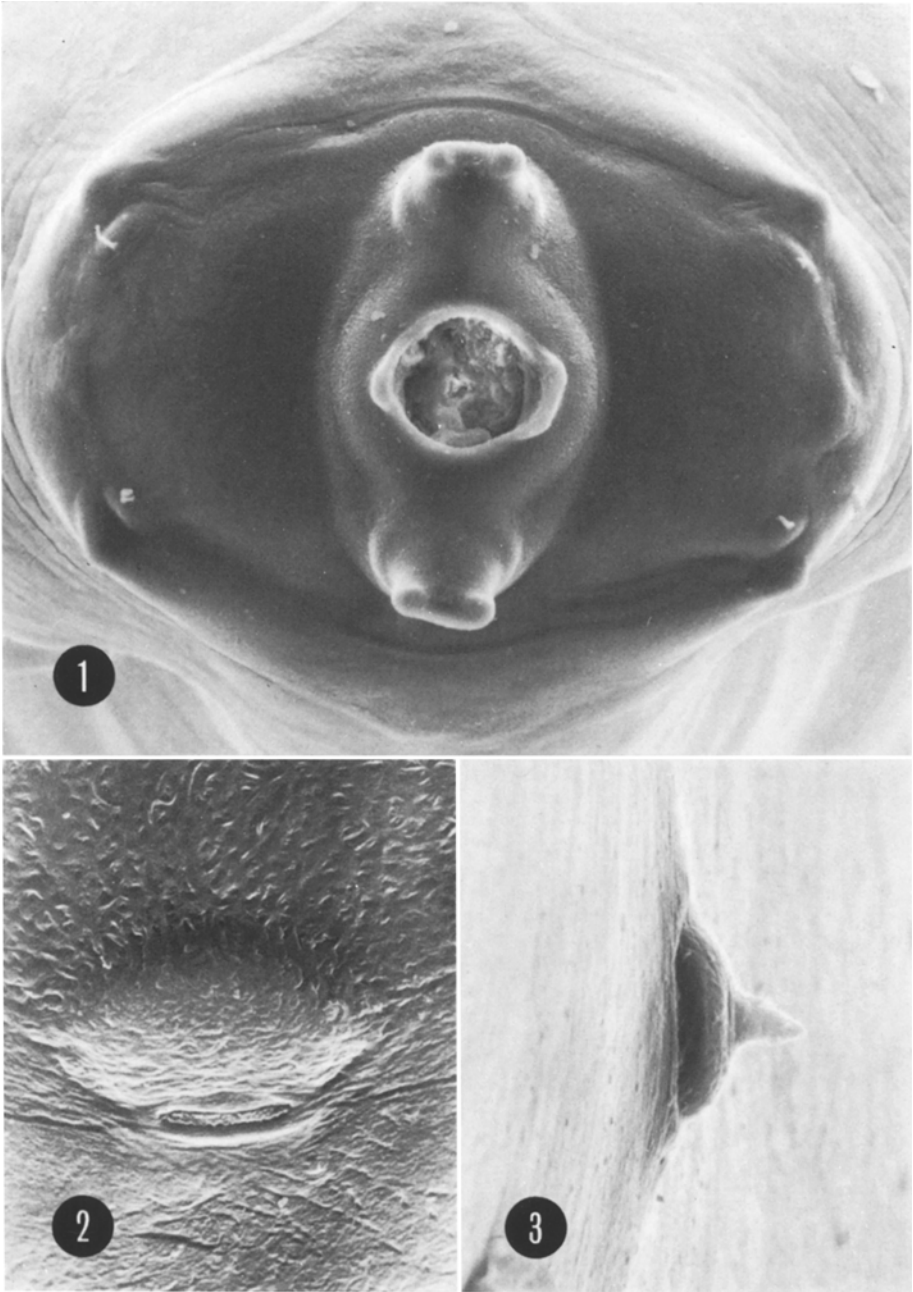
23. The en face view of the head showing the slit-like mouth opening. An amphid is marked with *. 500 ×
24. The amphid, marked with * at Fig. 23, is shown enlarged. 3,000 ×
25. The lateral deirid looks like a nipple. 1,800 ×

Figs. 26–34. *S. equina* (Figs. 26 and 34 for the female (Japan), Figs. 27 and 28 and Figs. 29–33, respectively for the female and male (Kazakhstan))

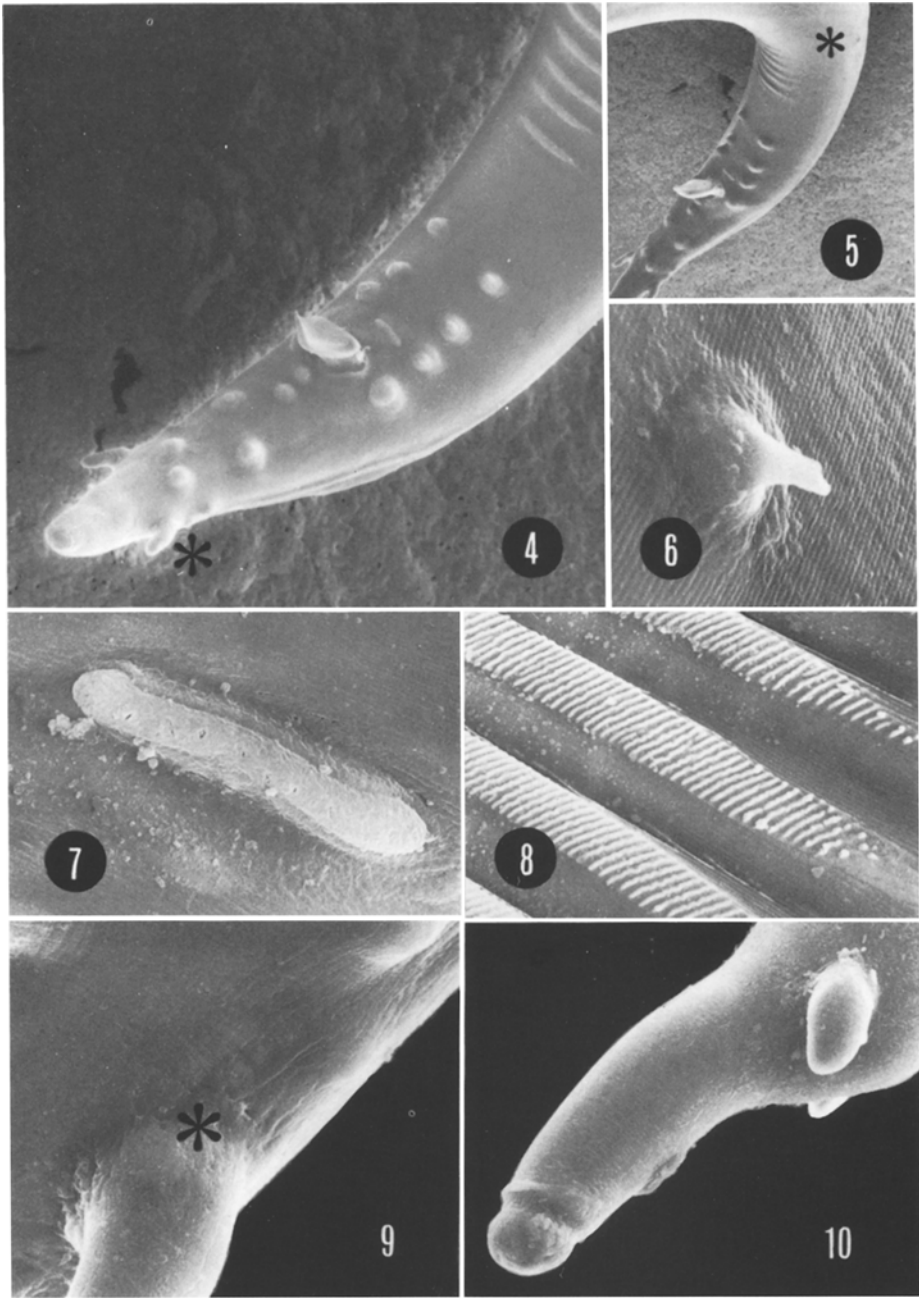
26. The en face view of the head. 210 ×
27. The same, but shrinkage is evident (perhaps of longer preservation in the formol-saline). 240 ×
28. The amphid, marked with * at Fig. 27, is shown in the same position. Note the walnut-like surface of the hilly body and the loose amphidial pore. 5,700 ×
29. The deirid lying close to the lateral line (the whitish line in the picture) on the dorsal side. 2,700 ×
30. The ventral view of the posterior part with the protruding spicula. The papillae surround the cloaca: a pair of precloacal (very near to the cloaca), each one of pre- and postcloacal central, and a pair of postcloacal papillae plus an adcloacal papilla only right (perhaps an anomaly?). Other pre- and postcloacal papillae are well recognizable. The bosses at this area are quite densely distributed on the cuticle, but, as shown in Fig. 31, they tend to become sparsely distributed at the area with the transverse bands, which are, yet, distributed with the bosses at both end parts. 400 ×
31. The area with transverse bands and fewer bosses. 320 ×
32. The postdeirid (unevenness of the cuticle may be due to long preservation in the fixative). 2,800 ×
33. The terminal end showing a pair of the flat papillae at the extreme end. (The phasmidial pore is indistinct.) 1,000 ×
34. The terminal end of the female with a lateral appendage and the mild terminal knob. 500 ×

Figs. 35–41. *S. labiatopapillosa* (Figs. 35 and 38–40 for the male and Figs. 36, 37 and 41 for the female)

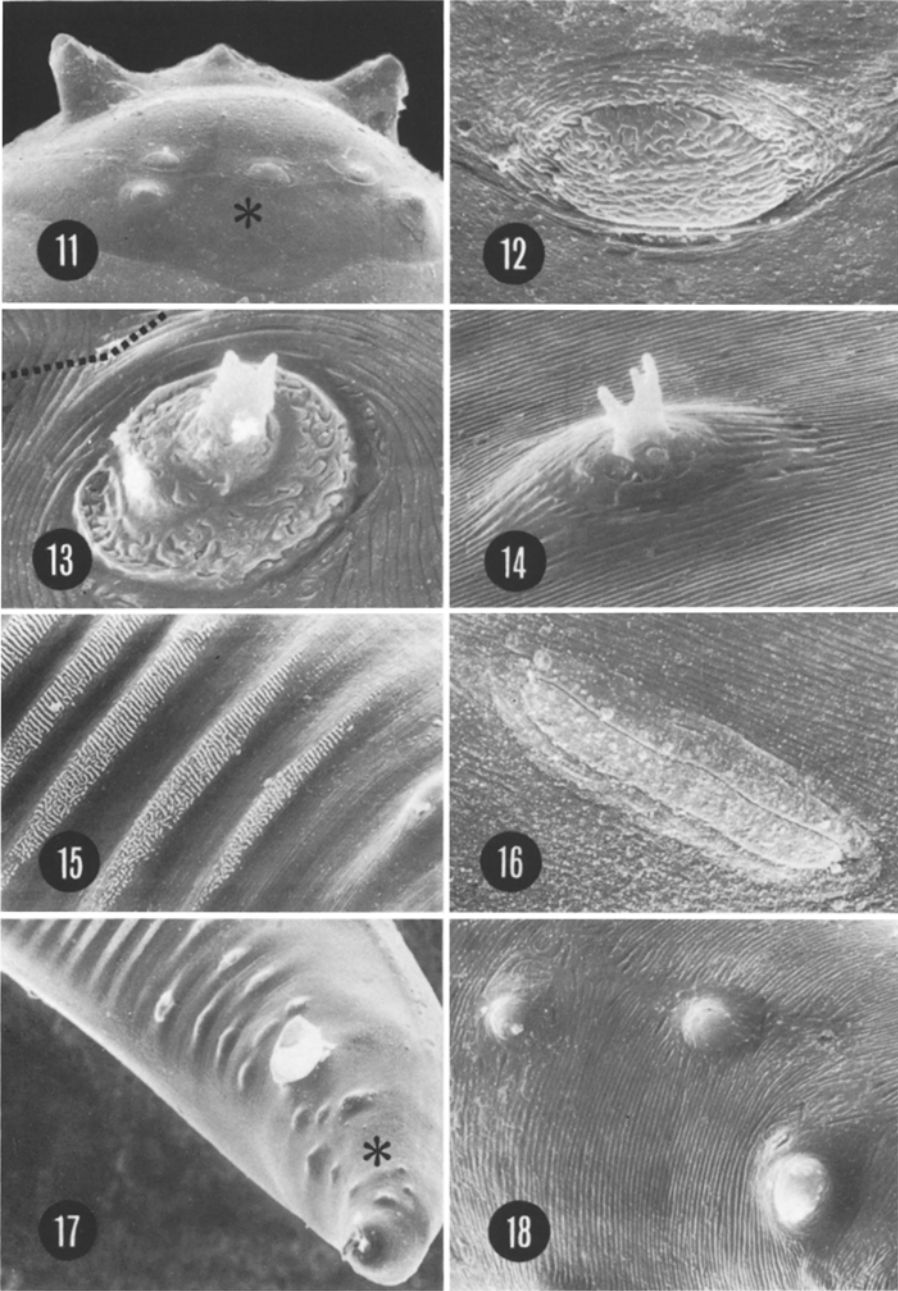
35. The slant apical view of the head. The dorsal and ventral projections of the peribuccal crown are distinctly notched. 600 ×
36. The amphid. Note the cuticular mosaic surface of its relevant body. 4,900 ×
37. The deirid looks like a cap with thorny formation, the tip of which is somewhat widened (transverse striations suggest the angle of observation). 5,800 ×
38. The transverse bands. 3,000 ×
39. The oddly bifurcated postdeirid. 4,500 ×
40. The ventral view of the posterior part shows clearly the papillar arrangement. Note 4 pairs (instead of 3 pairs at *S. digitata*, Fig. 4) of the postcloacal papillae, and only one lateral tiny papilla anterior to the lateral appendage (this being 2 at *S. digitata*). 380 ×
41. The tail end of the female with distinct blunt spines. The phasmidial pore is marked with *. 800 ×



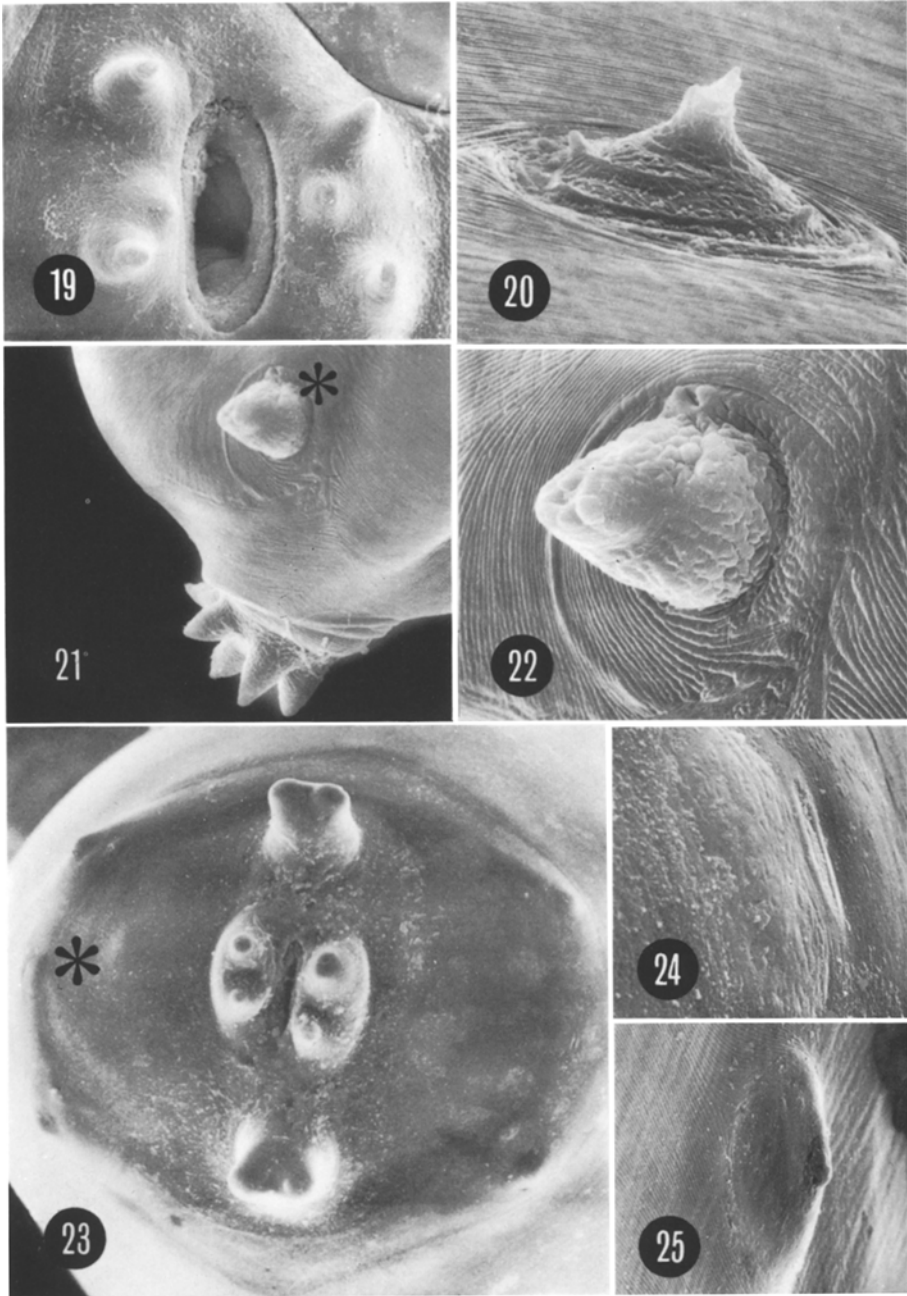
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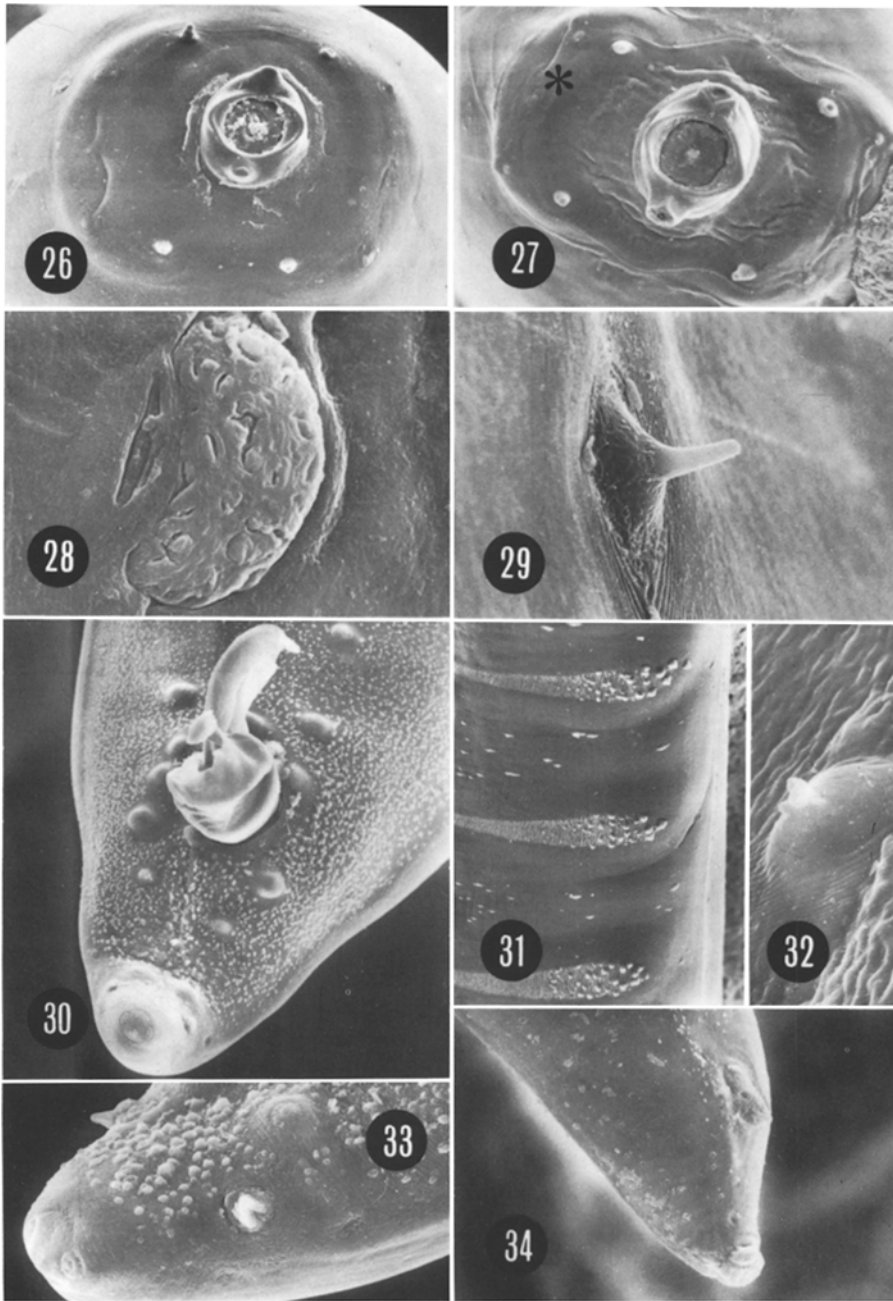
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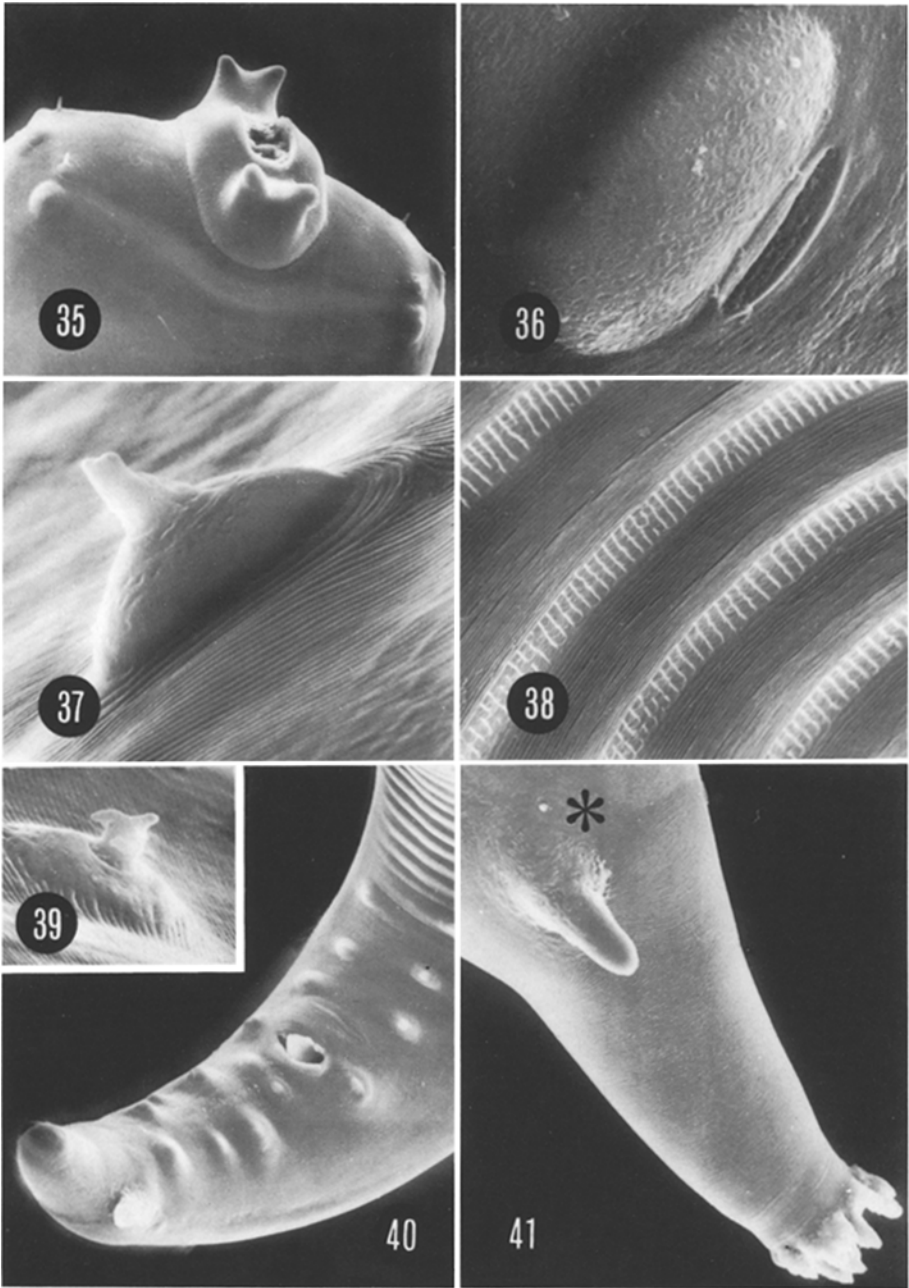
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The *amphids* situated laterally and recognizable usually as a small papillar formation in weak magnification by light microscopy at all *Setaria* spp. are illustrated more in detail by SEM; a hilly elevation covered with the cuticle of characteristic mosaic and having a dorsoventrally elongated amphidial pore at the lower bottom (Figs. 2, 12, 24, 28 and 36 respectively for *S. digitata*, *S. marshalli*, *S. marshalli pandei*, *S. equina* and *S. labiatopapillosa*).

The *deirids*, cervical papillae, situated close to the lateral line at the level between nerve ring and the end of the anterior oesophagus by light microscopy are visualized in finer detail by SEM; a small cap-like formation situated closely along the lateral line (at dorsal side) with a needle-like protrusion, furcated or non, on it are surrounded by the fine vertical striations (of the cuticle) running round its basement (Figs. 3, 13, 20, 25, 29 and 37 respectively for *S. digitata*, *S. marshalli*, *S. marshalli pandei*, *S. equina* and *S. labiatopapillosa*).

The Posterior Part

The *phasmidial pore* of the *Setaria* worm usually unrecognizable by light microscopy becomes visible first by SEM in both the male and female. It is found at the upper armpit of the lateral appendages (marked with * at Figs. 9, 21, 22, 41, and not marked but clear at Fig. 18).

Male

The general arrangement of the sexual papillae can be seen well at the ventral view.

The *postdeirid* unilaterally left at about the level posterior to the retracted position of the left spicule is recognizable as a similar formation to the deirid by light microscopy, but its fine appearance is revealed by SEM in detail (Figs. 6, 14, 32, and 39 respectively for *S. digitata*, *S. marshalli*, *S. equina* and *S. labiatopapillosa*). Its position is shown representatively at *S. digitata* (Fig. 5). It is distinct that the cuticular vertical striations intermit at the base of the postdeirid, while they are running round the deirid (see Figs. 13, 14, 37, 39).

The *central papilla* just anterior to the cloaca is dorsoventrally elongated, while this used to be recognized as a small papilla by light microscopy (Figs. 7, 16).

The *ventral bands*, lugae, are shown as their being composed of finer longitudinal microstriations (Figs. 8, 15 and 38 respectively for *S. digitata*, *S. marshalli* and *S. labiatopapillosa*). Exceptionally at *S. equina* (Fig. 31) bosses are distributed at both ends of the bands.

Female

The posterior end of the female is equipped with a knob (Fig. 10) at *S. digitata*, with roughly furcated end (Fig. 21) at *S. marshalli*, with a less prominent knob (Fig. 34) at *S. equina*, and with blunt spines (Fig. 41) at *S. labiatopapillosa*, as shown already by the workers by light microscopy.

Discussion

SEM brings all critical points of *Setaria* species into relief and there is beyond any doubts about the independency of species, here treated. The amphids which have been well known by light microscopy, are now illustrated more in detail about its hilly appearance with its pore at the posterior side of the hill-foot. The phasmidial pores have been overlooked until present by light microscopy just at the specimens *Setaria* because of the obscure location of its pore, but they are now clearly demonstrated at the upper armpit of the lateral appendages in both male and female. The deirids, which are of diagnostic value for Desset (1965), are illustrated in detail at the present SEM pictures, and their finer features suggest possible presence of certain range of deviation even within a species. Examination of more numerous specimens may be required before getting the final answer for its diagnostic evaluation. The postdeirid, which has been commonly overlooked, but described by some (for example by Anderson, 1959 at *Dirofilaria roemeri*) was first recognized at *Setaria nelsoni* (Shoho, 1976a) among all *Setaria* spp., and has been found later at all species of the genus *Setaria* examined by him. As in the case of the deirids more examination on it is necessary to decide its diagnostic value. The ventral transverse bands were first demonstrated by Helle and Blix (1973) by means of SEM at *Dipetalonema spirocauda* Leidy, 1858. The finer, longitudinal microstriations, composing those bands, are illustrated even at the specimens, prefixed with formol-saline. This indicates that the prefixation seems not to affect much for demonstrating these finer microstriations.

Finally existence of *Setaria marshalli* should be again stressed by the present presentation. This is one of the rare nematodes in which the life-cycle involves transmission by an intermediate host (mosquito) and intrauterine passage within the body of the definitive host, perhaps a unique example in this respect. Besides the slit-like narrow mouth opening and its rare, but repeated findings in other host spp. than the normal, the arrangement of three pairs of the lateral papillae, including the lateral appendages, of nearly the same size at the male tail end may provide us with a *Setaria* species, just at this *S. marshalli*, that may eventually lead us to clarify the phylogenetical clue of *Setaria* spp. of Bovinae.

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