

SCANNING ELECTRON MICROSCOPIC STUDIES ON MALES OF TRICHINELLA SPECIES

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Abstract. Ultrastructure of the cuticle and pseudobursa of adult males of four species of *Trichinella* has been studied by SEM. *T. nativa* differs markedly from *T. spiralis*, *T. nelsoni* and *Trichinella* sp. in the form of the pseudobursa. *Trichinella* sp. differs only slightly from *T. spiralis* and *T. nelsoni*. The ultrastructure of the cuticle revealed no characters suitable for the differentiation of the taxons under study.

The pionner papers by Britov (1969, 1971 a, b, 1972) contain basic information on the reproductive isolation of various strains of nematodes of the genus *Trichinella* Railliet, 1895. After a series of experiments with these nematodes, Britov and Boev (1972) and Garkavi (1972) arrived at important taxonomic conclusions and described three new species of this genus. In addition to the type species, *Trichinella spiralis* (Owen, 1845), they described the following species: *T. nativa* Britov et Boev, 1972, *T. nelsoni* Britov et Boev, 1972 and *T. pseudospiralis* Garkavi, 1972. The taxonomic value and validity of these species are a topical problem in helminthology and has recently been evaluated by Boev (1978). This author maintains that *T. spiralis*, *T. nelsoni*, *T. nativa* and *T. pseudospiralis* are so-called sibling species and gives criteria for their validity as regards the ecology, symbiotology, zoogeography and etiology (Boev 1978).

Possible morphological differences among these sibling species have received little attention. In the original description of *T. nelsoni* and *T. nativa*, these species are reported to be morphologically identical to *T. spiralis* (Britov and Boev 1972). Boev (1978) also stressed that these species are identical in their morphology. The authors arrived at these conclusions while evaluating the morphology of *Trichinella* species by classical methods and observations under the optical microscope. However, the ultrastructural morphology of sibling species was sometimes different. In order to get some knowledge necessary for the possible morphological differentiation of *Trichinella* we used the method of scanning electron microscopy for the evaluation of two criteria, namely ultrastructure of the cuticle and pseudobursa of males. Our results supplement the morphological characteristics of four taxons of the genus *Trichinella*.

MATERIAL AND METHODS

The ultrastructure of the surface of cuticle and pseudobursa of four nematode species (adult males only) of the genus *Trichinella* has been studied by SEM. The material was obtained from a series of identification experiments described in detail in the paper by Komandarev et al. (1975). The following species were evaluated in the present paper: 1. *Trichinella spiralis* from *Sus scrofa* f. *domestica* from Canada (10 males); 2. *T. nelsoni* from *Sus scrofa* f. *domestica* and *Vulpes vulpes* L. from Bulgaria (5 males from each host); 3. *T. nativa* from *V. vulpes* L. from Mordovian A.S.S.R. (6 males); 4. *Trichinella* sp. from *Canis aureus* L. from Bulgaria (4. males).

The nematodes were fixed in 10 % formalin and specimens for SEM studies were prepared by the method described in the paper by Wiger et al. (1978). JEOL JSM-S1 microscope was used for examination of the material and photographic documentation.

RESULTS

1. *Trichinella spiralis* (Owen, 1835)

The surface of the cuticle of the whole body, except for the head part and pseudobursa, is markedly longitudinally wrinkled. Individual longitudinal ridges are rounded and adjacent to one another (Plate I, Fig. 1). Transverse striations of the cuticle are quite irregular, of various depths, length and branching and never form entire cuticular rings (Plate I, Fig. 4). Bacillary bands are situated laterally, originating slightly posterior to the head and ending just anterior to the base of the pseudobursa. Characteristic for each bacillary band are the pores of lateral hypodermal glands which are arranged in two irregular longitudinal rows (Plate I, Figs. 2–3). The pores are rounded, in some instances their margins are slightly upraised above the body surface and smooth. The pore is filled with a secretion sometimes rising above the surface of the cuticle.

The pseudobursa consists of two lateral processes (0.017–0.020 mm long) and a group of four circumcloacal papillae. The lateral processes of the pseudobursa rise from a wide base (Plate I, Fig. 5, Plate II, Fig. 1). Their greatest width occurs at one third of their length from the base, after which they become somewhat narrowed and terminate in a rounded, relatively wide tip. The cloacal aperture is situated subventrally between the lateral processes and is distinctly triangular (Plate II, Fig. 2). The circumcloacal papillae are divided into two pairs. The first pair is evidently paracloacal, situated laterally on the margins of the cloacal aperture. The second pair is situated at the level of dorsal margin of the cloacal aperture or slightly higher, and more laterally (Plate II, Fig. 3). The circumcloacal papillae are either ovoid or conical. In some instances a part of the cloacal wall extended to but did not overlap the lateral processes of pseudobursa.

2. *Trichinella nelsoni* Britov et Boev, 1972

The surface of the cuticle (Plate II, Fig. 4) is similar to that of *T. spiralis* (with conspicuous, dense longitudinal ridges and irregular transverse striations). Lateral bacillary bands are present and are characterized by two rows of pores of hypodermal glands. These pores are distributed in tandem, at irregular intervals (Plate II, Figs. 5–6).

The pseudobursa has a smooth cuticle and consists of two massive lateral processes (0.018–0.019 mm long) and a group of 4 circumcloacal papillae. As in *T. spiralis*, the lateral processes have a wide base (Plate III, Fig. 1) and their tip is broadly rounded. The cloacal aperture is slightly shifted subventrally. The first pair of circumcloacal papillae is situated on the sides of cloacal aperture. The second pair lies at the level of dorsal margin of the cloacal aperture and is slightly lateral (Plate III, Fig. 2). The circumcloacal papillae are ovoid or conical. The inner wall of the cloacal opening extends laterally to the level of base of the lateral processes of the pseudobursa.

3. *Trichinella nativa* Britov et Boev, 1972

There are longitudinal, densely distributed ridges and irregular transverse striations (Plate III, Figs. 3–4) of various depth (sometimes only feebly distinct) on the surface of the cuticle. The lateral bacillary bands are present. The pores of hypodermal glands are arranged in two longitudinal rows and are not very distinct (Plate III, Fig. 5).

The pseudobursa is characteristic with a smooth cuticle, and distinctly differing in its morphology from that of the above described species. Two lateral processes of pseudobursa (0.014–0.016 mm long) rise from a wide base. Approximately at one third of their length they are markedly narrowed and then they are fingershaped (Plate IV, Fig. 1). They are constantly narrower than the lateral processes of the pseudobursa of *T. spiralis* and *T. nativa*. The cloacal aperture is triangular (Plate III, Fig. 6) or slitlike (Plate IV, Fig. 2) and is situated slightly subventrally. A fine structural difference between the cuticle of pseudobursa and surface of inner wall of cloaca is visible in the micrograph (Plate III, Fig. 6). The circumcloacal papillae are divided into two pairs, being of different size and shape (Plate IV, Fig. 3). The first pair is situated on either side of the cloacal aperture (close to it). The papillae are low and flat, with a rounded and slightly protruding tip. Their height does not exceed half of the height of papillae of the second pair. The papillae of the second pair are located at the level of the dorsal margin of cloacal aperture and are slightly lateral to the first pair. The dorsal pair of papillae has a wide base and their tips markedly protrude in front of hemispherical or conical processes.

4. *Trichinella* sp.

The surface of the cuticle and bacillary bands are of the same structure as those of *T. spiralis*.

The pseudobursa has a smooth cuticle. Its lateral processes are massive, wide and with rounded tips (Plate IV, Figs. 4–5). Their length, 0.012–0.014 mm, is smaller than in *T. spiralis* and *T. nelsoni*. The circumcloacal papillae are separated into two pairs. The papillae in both pairs are similar in size and shape. The first pair of papillae is situated on sides of cloacal aperture, the second pair at level of the dorsal margin of cloacal aperture. In contrast to *T. spiralis* and *T. nelsoni*, the papillae of the dorsal pair are not distinctly laterally shifted, but are situated in a line with the papillae of the first pair (Plate IV, Fig. 6). The cloacal aperture is triangular, situated slightly subventrally.

DISCUSSION

The theoretical and practical significance of studying sibling species was discussed in the paper by Boev (1978) using helminth species as an example. This is still more important in case of *Trichinella* species, which are responsible for trichinellosis, a serious disease of man.

According to the present taxonomic classification by Britov and Boev (1972) and Garkavi (1972) the various strains of *Trichinella* are four independent species, *T. spiralis*, *T. nelsoni*, *T. nativa* and *T. pseudospiralis*. This classification is based on repeated experimental confirmation of their reproductive isolation (Britov 1971 a, b, 1972, 1975, Komandarev et al. 1975, Shaikenov et al. 1977 and others). The degree of reproductive isolation, however, is different in these species. According to the results of Britov (1972, 1975), Komandarev et al. (1975) and other workers, there exists a total reproductive isolation between *T. nativa* and *T. pseudospiralis* (from *T. spiralis* and *T. nelsoni*, as well as from one another). On the other hand, copulation and crossing between *T. spiralis* and *T. nelsoni* is possible as it was confirmed experimentally (e.g., Britov 1972, Komandarev et al. 1975). In our studies of these four *Trichinella* species we took into consideration that differences in the degree of reproductive isolation might also be reflected in their morphology. From this point of view the pseudobursa

of males and its ultrastructure is an important criterion (the ultrastructure of the cuticle is only a supplementary criterion).

Although in the original description of *T. nativa* and *T. nelsoni* these species were found to be morphologically identical with *T. spiralis*, some differences were observed in our material. The morphology of pseudobursa of *T. nativa* distinctly differs from that of other three species in the shape of the lateral processes of the pseudobursa and the circumcloacal papillae. These findings support the concept that complete reproductive isolation of this species from the other *Trichinella* species has a morphological basis. According to the ultrastructure of pseudobursa, *T. spiralis*, *T. nelsoni* and *Trichinella* sp. are very closely related, almost identical. More exactly, *T. spiralis* and *T. nelsoni* were found to be identical in the morphology of their pseudobursa, whereas *Trichinella* sp. was slightly different. Our observations thus corroborate the experimental results of Britov (1972) concerning the possible hybridisation between *T. spiralis* and *T. nelsoni*.

Trichinella sp., which cannot be classified more exactly, differs from *T. spiralis* and *T. nelsoni* in two details: length of lateral processes of pseudobursa and location of the second pair of circumcloacal papillae. The length of lateral processes of pseudobursa is 0.012–0.014 mm in *Trichinella* sp., 0.017–0.020 mm in *T. spiralis* and 0.018 to 0.019 mm in *T. nelsoni*. The second pair of circumcloacal papillae of *Trichinella* sp. is situated in line or almost in line behind the first pair. In *T. spiralis* and *T. nelsoni*, the second pair of papillae is shifted more laterally. These difference were found by measuring the distance between the bases of the papillae. In the three species the distance between the bases of the first and second pair of papillae respectively, are as follows: *T. spiralis* – 0.006–0.007 mm and 0.009–0.012 mm; *T. nelsoni* – 0.008 mm and 0.012–0.014 mm and in *Trichinella* sp. – 0.009 mm and 0.010 mm.

The ultrastructure of the surface of cuticle cannot be used as a criterion for species differentiation in this genus, since all species possess conspicuous longitudinal ridges and irregular, more or less distinct transverse striations. We have observed the presence of lateral bacillary bands which were studied in detail by Bruce (1970) in *T. spiralis*. The longitudinal lines of pores of lateral hypodermal gland cells marking the bacillary bands are also characteristic, to a different extent, for the species of the suborder Trichocephalata (Wright and Chan 1973), where they are very variable in distribution and shape.

It can be concluded that the reproductive isolation of *T. spiralis*, *T. nelsoni*, *T. nativa* and *Trichinella* sp. studied in the present paper is related with the morphology of their pseudobursa. In our results the ultrastructure of pseudobursa confirm the independence and validity of the species *T. nativa*, but could not be used for the differentiation of *T. spiralis* and *T. nelsoni*.

ИЗУЧЕНИЕ САМЦОВ РОДА *TRICHINELLA* ПОД СКАНИРУЮЩИМ ЭЛЕКТРОННЫМ МИКРОСКОПОМ

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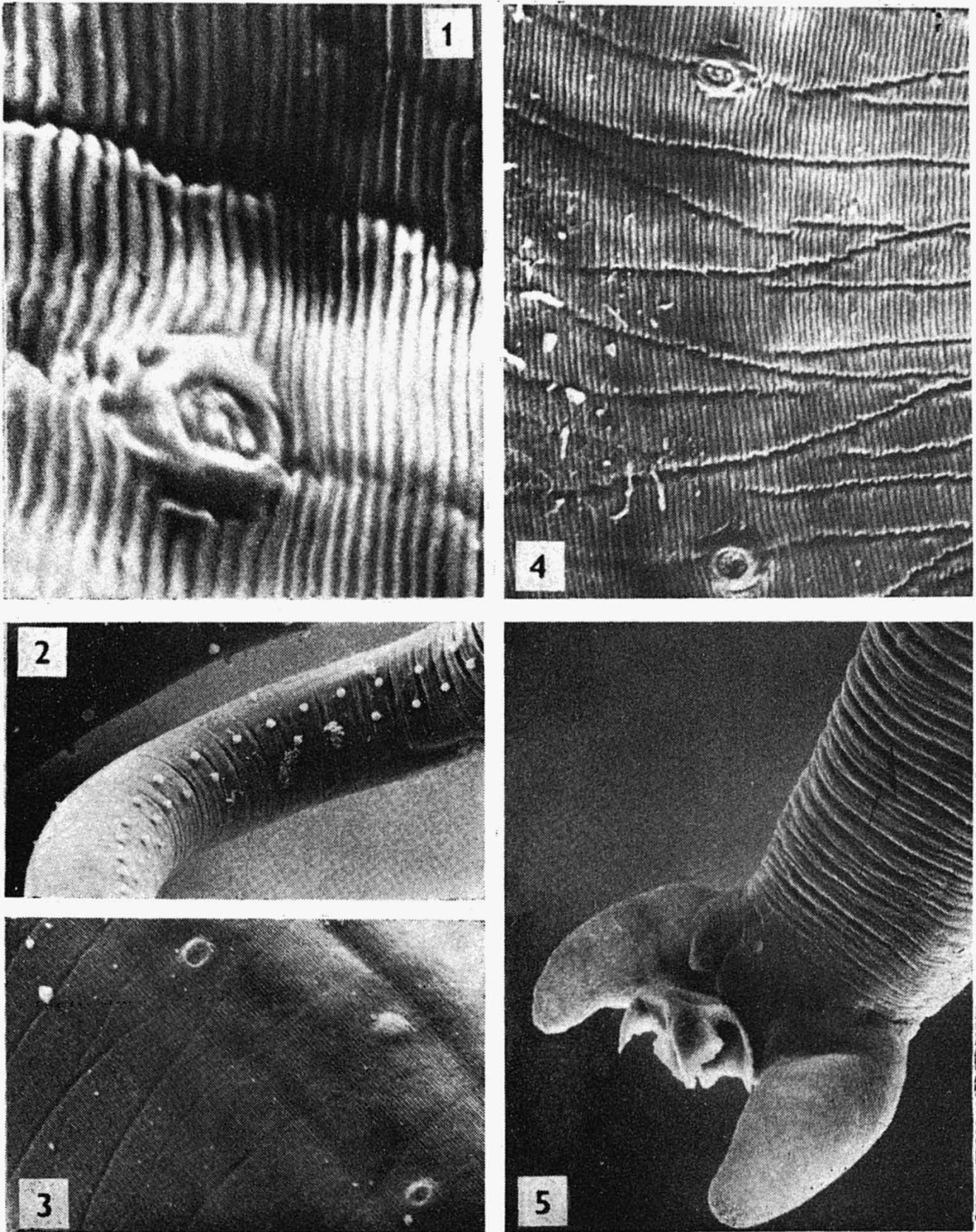
Резюме. Методом СЭМ изучали ультраструктуру кутикулы и псевдобурсы взрослых самцов 4 видов рода *Trichinella*. Вид *T. nativa* отличается от *T. spiralis*, *T. nelsoni* и *T. sp.* по форме псевдобурсы. *Trichinella* sp. отличается от *T. spiralis* и *T. nelsoni* только незначительно. Ультраструктура кутикулы не показывает признаков удобных для дифференцировки изучаемых таксонов.

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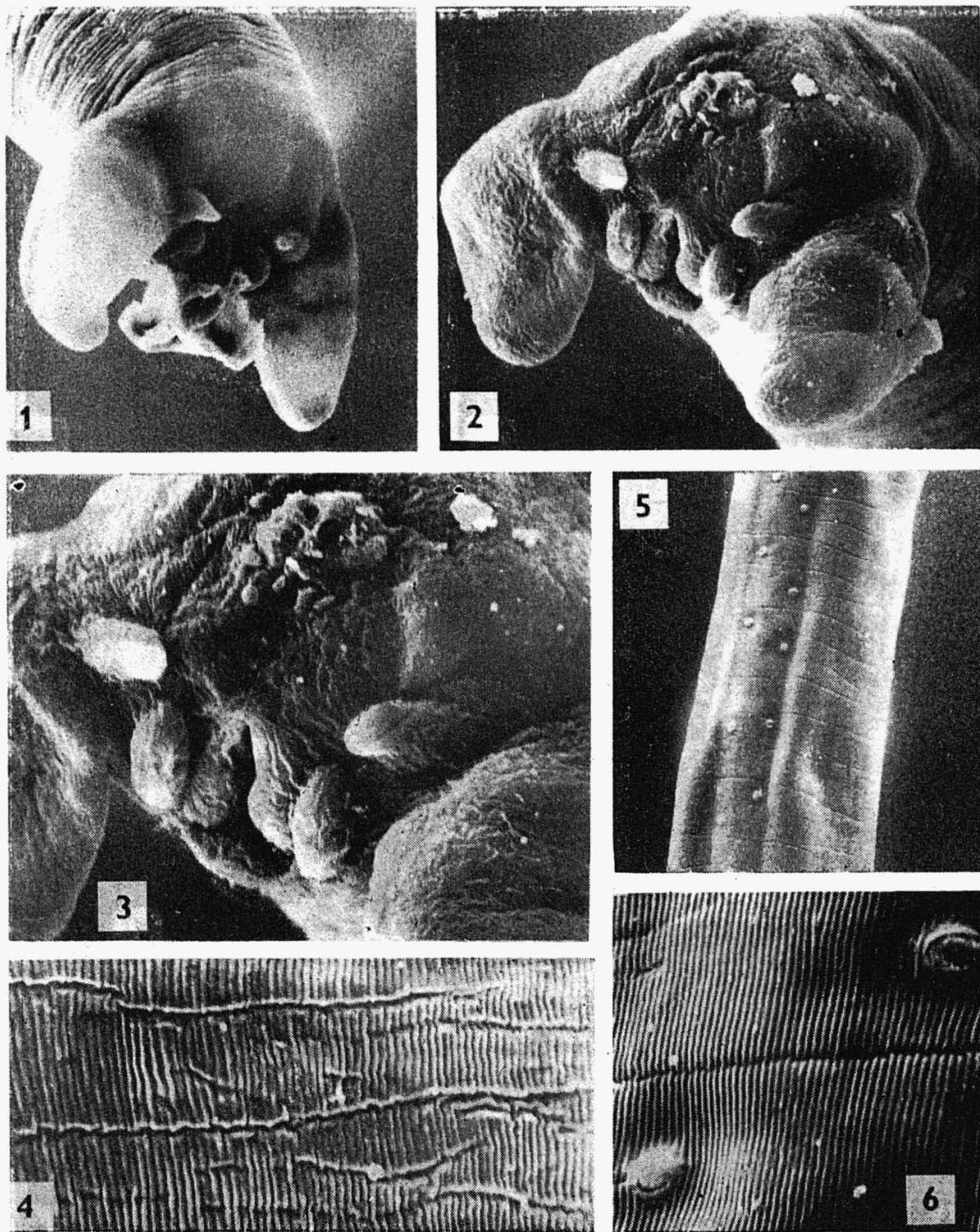
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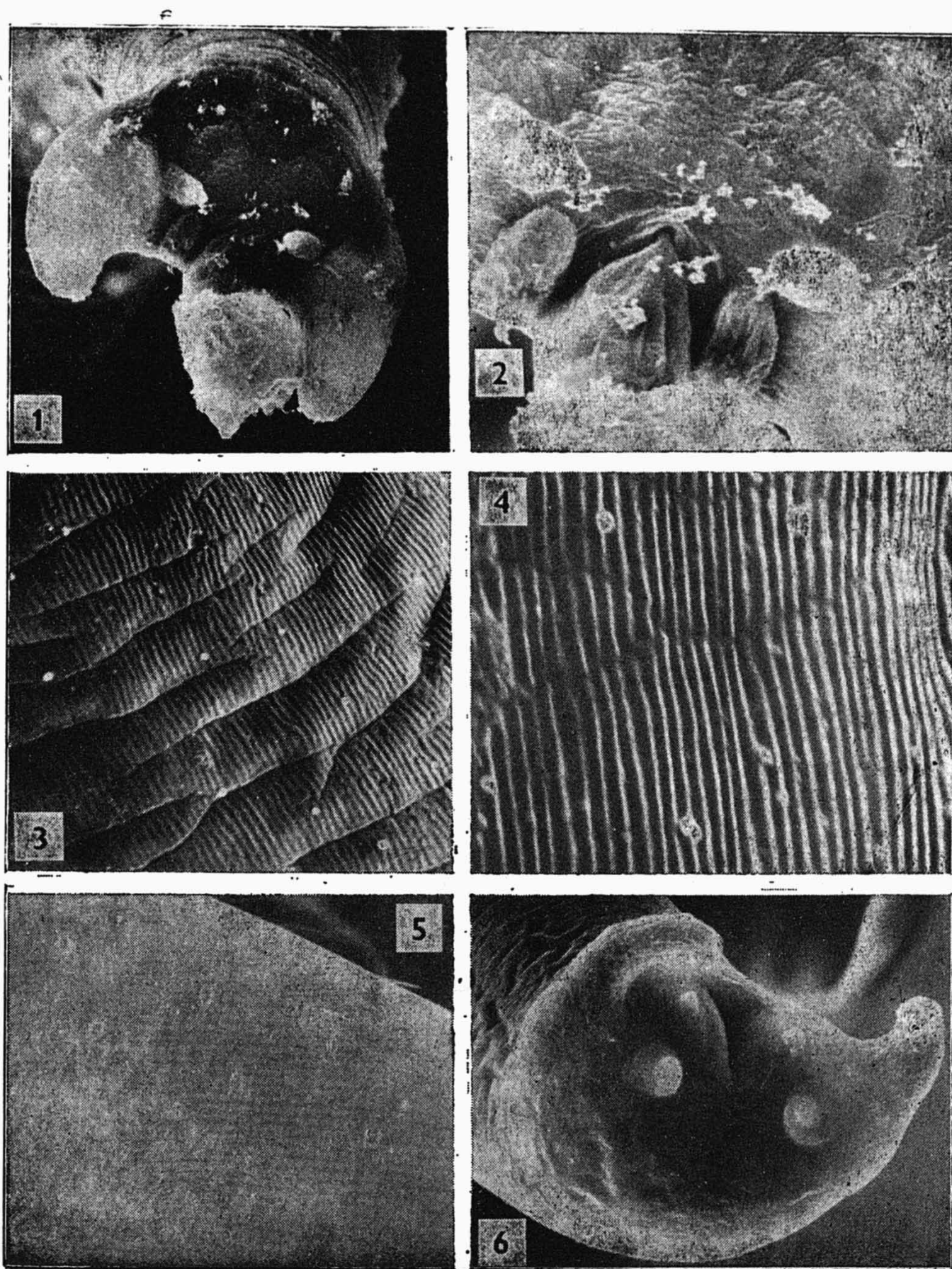
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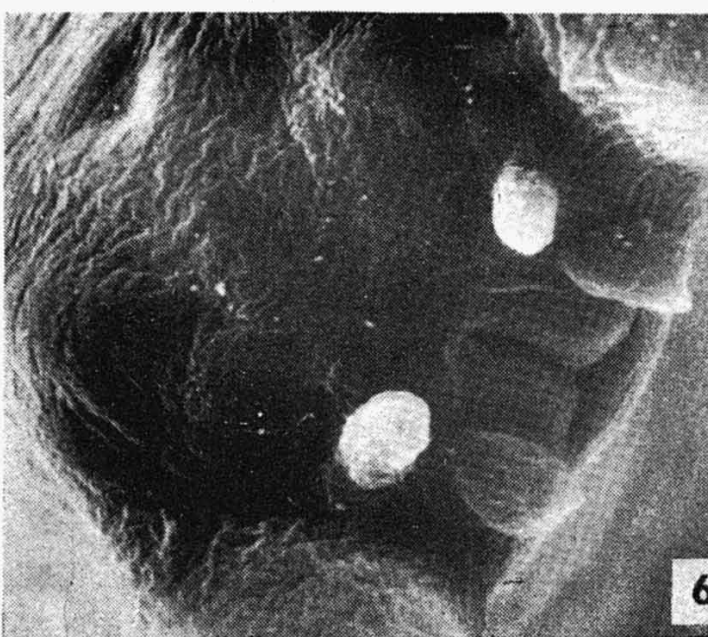
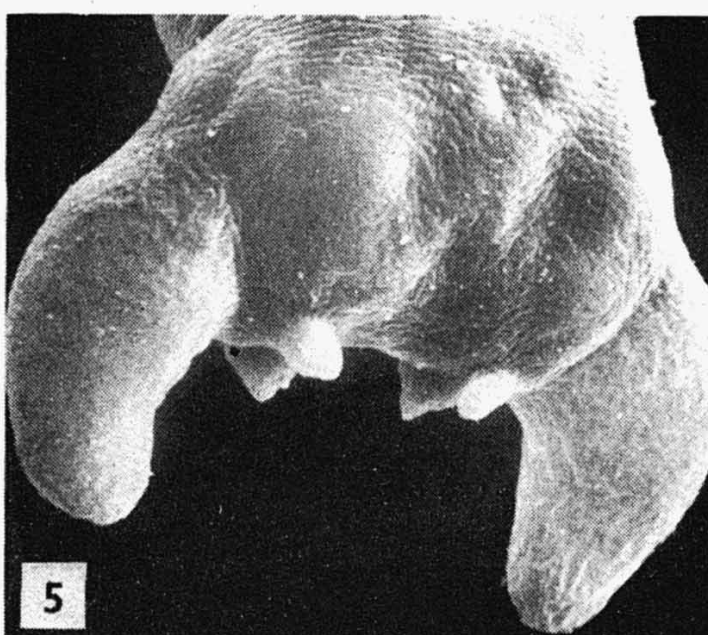
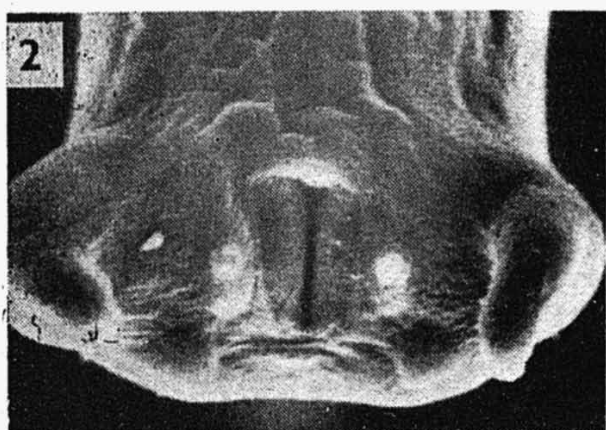
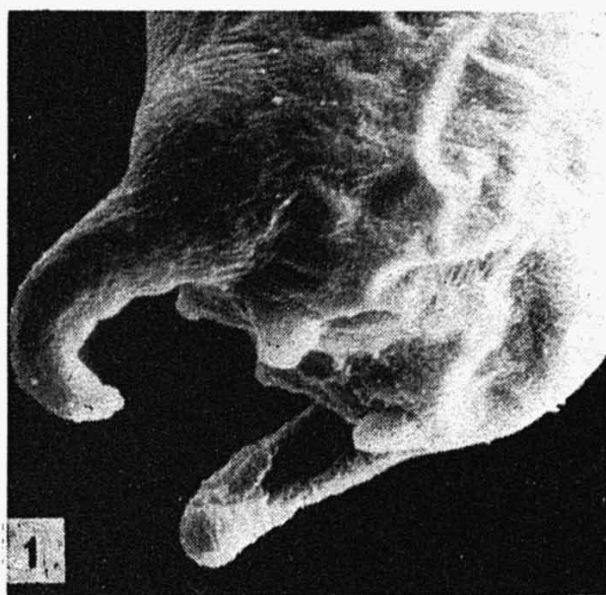
Figs. 1—5. Scanning electron micrographs of *Trichinella spiralis* (Owen, 1835). **Fig. 1.** Detail of the surface of the cuticle, with longitudinal ridges and pore of hypodermal gland ($\times 21\,600$). **Fig. 2.** Bacillary band in the middle of body length ($\times 720$). **Fig. 3.** Detail of bacillary band ($\times 3\,600$). **Fig. 4.** Cuticle in the vicinity of bacillary band (with pores of hypodermal glands) ($\times 7\,200$). **Fig. 5.** Pseudobursa with everted cloacal wall (ventral view) ($\times 1,450$).



Figs. 1—3. Scanning electron micrographs of *Trichinella spiralis* (Owen, 1835). **Figs. 4—6.** SEM of *Trichinella nelsoni* Britov et Boev, 1972. **Fig. 1.** Pseudobursa with everted cloacal wall (dorso-lateral view) ($\times 1\ 400$). **Fig. 2.** Pseudobursa (general dorsal view) ($\times 2\ 100$). **Fig. 3.** Detail of cloacal aperture and circumcloacal papillae ($\times 3\ 500$). **Fig. 4.** Longitudinal ridges of cuticle and irregular transverse striations ($\times 7\ 000$). **Fig. 5.** Bacillary band with pores of hypodermal glands ($\times 1\ 050$). **Fig. 6.** Detail of bacillary bands with pores of hypodermal glands ($\times 7\ 000$).



Figs. 1–2. Scanning electron micrographs of *Trichinella nelsoni* Britov et Boev, 1972. **Figs. 3–6.** SEM of *Trichinella nativa* Britov et Boev, 1972. **Fig. 1** Pseudobursa with everted cloacal wall (dorsal view) ($\times 1\,400$). **Fig. 2** Detail of cloacal aperture (with everted wall) and circumcloacal papillae ($\times 3\,500$). **Fig. 3** Cuticle with longitudinal ridges and transverse striations ($\times 7\,000$). **Fig. 4** Detail of longitudinal ridges of cuticle ($\times 14\,000$). **Fig. 5** Bacillary band with pores of hypodermal glands ($\times 2\,100$). **Fig. 6** Pseudobursa (general dorso-ventral view). ($\times 2\,100$).



Figs. 1–3. Scanning electron micrographs of *Trichinella nativa* Britov et Boev, 1972. **Figs. 4–6.** SEM of *Trichinella* sp. **Fig. 1.** Pseudobursa (dorso-lateral view) ($\times 2\,700$). **Fig. 2.** Pseudobursa (ventral view) ($\times 2\,050$). **Fig. 3.** Detail of cloacal aperture and circumcloacal papillae ($\times 3\,400$). **Fig. 4.** Pseudobursa (dorsal view) ($\times 2\,050$). **Fig. 5.** Pseudobursa (dorso-lateral view) ($\times 2\,700$). **Fig. 6.** Detail of cloacal aperture and circumcloacal papillae ($\times 3\,400$).