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SCANNING ELECTRON MICROSCOPY OF ADULT DIPLOTRIAENA TRIDENS (MOLIN, 1858) BOULENGER, 1928 (NEMATODA: DIPLOTRIAENOIDEA)

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Abstract. Several males and females *Diplotriaena tridens* were examined by scanning electron microscopy. The entire cuticle of the worms presents a wrinkled aspect. A regular striation is found in whole region of the body with the exception of the head, which is separated by a circular constriction, and shows a rectangular promontory. The morphology of the mouth, "apex" of tridents, the trident pouch openings, oral papillae, the amphidial openings, the vulva and the anus, is presented. In the male, the variability of the caudal papillae regarding their number and distribution is presented. The shape of the two spicules and of the duct of the left one is illustrated.

The difficulties of identifying species of the genus *Diplotriaena* because of their classic and incomplete descriptions, notoriously variable sizes, proportions, and details of spicules and caudal papillae are well known.

Anderson (1959) points out these difficulties and is sure that *Diplotriaeninae* is the most widespread and diverse group of the Filarioidea, but it is also one of the most distinctive by the existence of chitinoid structures (the tridents) in the head.

However, when the species have been defined more rationally it is evident that they are much less variable than it would be expected. This evidence for the general morphology can be extended to other features, and Sonin (1968) points out that the number of caudal papillae in the male tail is constant for the genus.

In recent years the SEM has proven to be an important aid in observing the microtopographic features of several nematode species (Gibbons 1984, 1986, Snyder 1985, etc.). The purpose of this paper is to present more detailed information on the surface morphology and other features of adult *Diplotriaena tridens* (Molin, 1858) Boulenger, 1928 with the aid of the SEM.

MATERIALS AND METHODS

The nematodes studied were obtained from the air sacs of *Sylvia attricapilla* L. (Sylvidae). They were cleaned in saline solution (0.9%) and subsequently separated in two groups: a) for light microscopy studies; b) for scanning electron microscopy.

In the group a) the nematodes were fixed in 10% formalin, cleared in 70% alcohol—100% glycerine (95 : 5), and brought into 100% glycerine; in the group b) they were fixed in 2.5% glutaraldehyde-Milloning's solution during 5 min, cut in 0.5 cm pieces and brought again in 2.5% glutaraldehyde-Milloning's solution. The procedure lasted 2 hours. The specimens were post-fixed in 2% osmiun tetroxide (1 h), dehydrated in a graded series of ethanol, and finally placed in 100% acetone before critical-point drying from CO_2. After drying, they were coated with gold, examined and photographed in an ISI mod. SX-25 scanning electron microscope at 25 kV.

RESULTS

Anterior end

The "en face" view of the anterior end in both sexes showed a rectangular cephalic elevation (CE, Pl. I, Figs. 1 and 3) which was less marked in the male specimens. Sometimes the dorsal and ventral sides of this elevation were slightly depressed.

The mouth (M, Pl. I, Figs. 1 and 3) was dorsoventrally elongated and had no cuticular ring in any specimens. Two well-marked openings of trident cavities (t, Pi. I, Figs. 1, 4 and 5) were on either side of the oral opening. A consistency in the pattern of oral papillae, which were well visible in all specimens, was observed. The mouth, pores of trident cavities and the four papillae of the internal circle were located on the cephalic elevation already mentioned.

Outside the cephalic elevation, there were four big papillae of the external circle (P, Pl. I) and two amphids (a, Pl. I, Figs. 3 and 4) with small opening. The head was constantly marked in all specimens by a circular constriction (C, Pl. I) behind the external papillae circle. The cuticle in the head region showed a wrinkled aspect but no other ornamentation.

Midbody region

All specimen of *D. tridens* have a fine and transverse cuticular striation (→, Pl. I, Fig. 2) extending to the posterior end and maintaining an uniform distance between the striae (3. 11 μm). In this region the cuticle also shows its wrinkled aspect (Pl. I, Fig. 2). Other marks visible in Fig. 2 may be due to fixation artifact. No details of the tipe described can be seen at light microscopy.

The vulva is situated on the ventral side, aproximately 600 μm behind the anterior end. The opening appears as a transverse (and archaete) fissure which presents a prominent and thick anterior lip (V, Pl. II, Fig. 1). No other features can be seen by SEM in the male and female in this region.

Posterior end

The anus (A, Pl. II, Fig. 2) of the female specimens, which is not functional in this species (Chabaud 1955), is in subterminal position and has a very small opening. In the males, it is transverse, with prominent lips, the rear one of which is larger (Pl. II, Fig. 4). These lips have an ornamentation in the form of creases. The tail length (from the anus to the tip of the tail) is 55 μm in the males.

The pattern of caudal papillae presents an evident variability. Practically a variable number and disposition of these can be seen in each of several caudal extremities studied. In the most complete one (Pl. II, Fig. 4) this pattern is:

Two symmetrical rows (with four papillae each) in lateral and terminal position (PCL).

Two innermost rows (also symmetrical) with five papillae each (PCI), the first pair of which being in a slightly preanal position.

In addition to these papillae, two other rows can be seen with a most irregular disposition and no constant among specimens. Another variation is the irregular presence of an odd terminal papilla (PCT).

In our specimens, the outer and inner rows are constant, although the number of papillae is variable.

The spicules, which are well visible at light microscopy, show their typical morphology and other details in SEM. The right one (RS, Pl. II, Figs. 3A and 5) is shorter (463 μm on average at light microscopy) and exhibits a spiral shape, twice twisted. Neither alae nor other structures on this spicule can be seen. The left spicule (LS, Pl. II, Fig. 3A), sword-shaped, is longer (693 μm on average at light microscopy), slightly arched on the ventral side of the distal end, and presents a longitudinal fissure. Such fissure (→ Pl. II, Fig. 3A) is the result of a fold of wall that penetrates inside the spicule originating an internal channel which can be seen in cross sections (→, Pl. II, Fig. 3B).

DISCUSSION

The microtopographic features of adult *Diplotriaena tridens* as revealed by SEM confirm to a great extent the previous findings under the light microscope, although SEM observation allows for a more accurate definition of the features.

The most exhaustive description of this species was given by Anderson (1959), apart from proving the two denominations of *D. monticelliana* and *D. tridens* to be synonyms. Sonin (1968) does little more than compile Anderson's (1959) findings. Further relevant data about the species were provided by Chabaud (1955). Anderson (1968) in a study of the cephalic end of Filarioidea includes *Diplotriaena* in a general sense.

In all the specimens examined under SEM the head always appears as distinctly differentiated due to a circular constriction, which is difficult to visualize under the light microscope and hence has not been reported in the literature (Chabaud 1955, Anderson 1959, 1968, Sonin 1968).

The four large papillae of the outer circle are located exactly above the constriction and present the typical configuration which coincides with Anderson's (1959) illustrations, SEM, however, permitting more accurate morphological assessment.

The remaining cephalic structures are situated on the top of a rectangular cephalic elevation, not described to date, whilst the laterally positioned amphids with their small openings are located outside this area.

The four papillae forming the inner circle, smaller in size, occupy the edge of the cephalic elevation. The laterally positioned tridents are equidistant from the buccal opening. The opening itself is of diffuse outline and oval in a dorso-ventral direction. SEM confirms the trident apex as a tri-lobulate structure (Anderson 1959). The pouch opening of the tridents shows a salient, circular and clearly marked rim, which has not been described to date (cf. Gibbons 1986 for *D. ozouxi*). The cuticle in this body region has a wrinkled aspect but no regular striation.

Caudally to the neck structure, which limits the head, there appears a tenuous striation, which so far has been missed in the literature (Chabaud 1955, Anderson 1959), extending down to the caudal region, the cuticle preserving its wrinkled aspect. Neither deirids nor excretory pore were observed, (nor were they found in histological sections, according to forthcoming data).

Caudal papillae variability in the males is notorious, as has been described in the literature. Due to their small size, however, these papillae proved difficult to describe and characterize. The images obtained in this research allowed a convenient observation and differentiation of caudal papillae. In addition, Sonin's (1968) hypothesis regarding the consistent presence of 11 caudal papillae pairs proved to be untenable.

Finally, the copulatory spicules of the males can be examined by SEM when they are outside the body. In all specimens whose right spicule could be studied it consistently appeared as a double coil. In spite of the fact that Anderson (1959) describes it as an alated structure, SEM observation did not reveal any alae or other structures.

For the left spicule a new feature not yet described in the literature has to be stressed:

the existence of a longitudinal fissure, originating laterally and ending ventrally at the distal tip. This fissure corresponds to the internal duct, as has been confirmed by cross sections of the spicule. Franz (1980) points out a similar structure for the same spicule of *Onchocerca volvulus* males.

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СКАНИРУЮЩАЯ ЭЛЕКТРОННАЯ МИКРОСКОПИЯ ПОЛОВОЗРЕЛЫХ ЭКЗЕМПЛЯРОВ НЕМАТОДЫ *DIPLOTRIAENA TRIDENS* (MOLIN, 1858) BOULENGER, 1928 (NEMATODA: DIPLOTRIAENOIDEA)

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Резюме. С помощью сканирующей электронной микроскопии изучали несколько самцов и самок нематоды *Diplotriaena tridens*. Целая кутикула червей морщинистая. Правильная исчерченность находится во всех частях тела, кроме головы, которая отделена круговой перетяжкой и имеет прямоугольный выступ. Показаны морфология рота, апекс трехзубчатых полостей, их отверстия, ротовые сосочки, амфидиальные отверстия, вульва и анус. Показана вариабильность хвостовых сосочков, что касается их количества и распределения. Изображена форма обеих спикул и протока левой спикулы.

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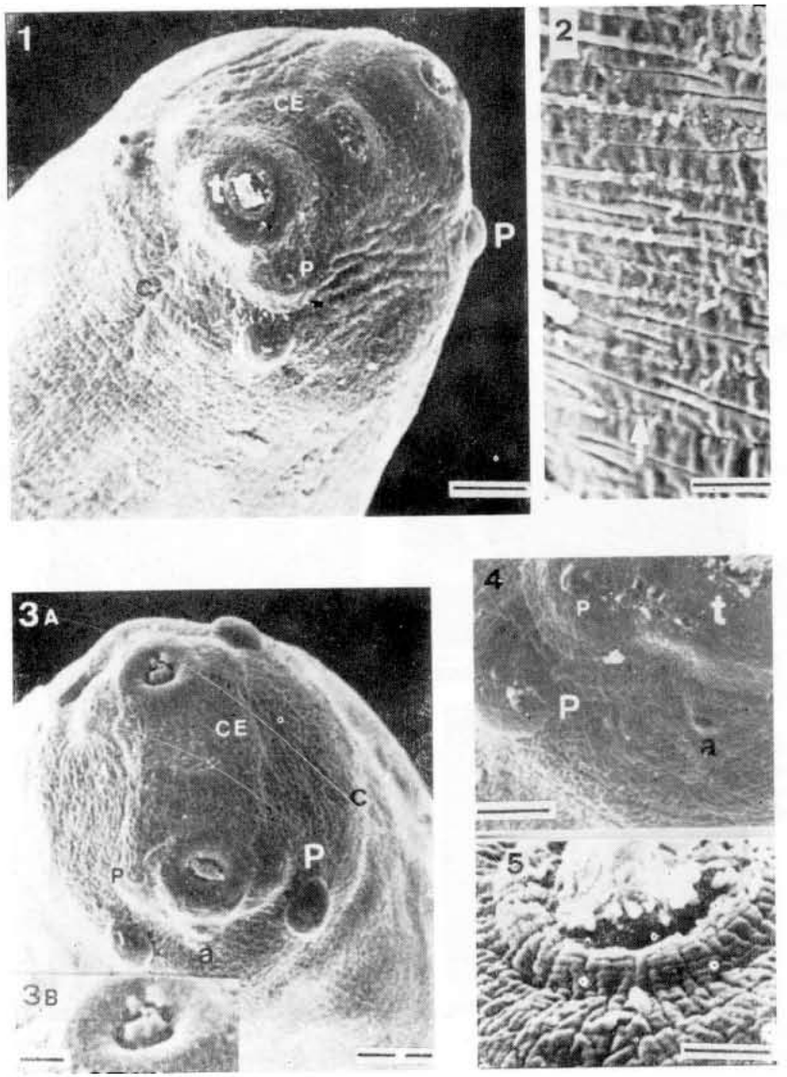
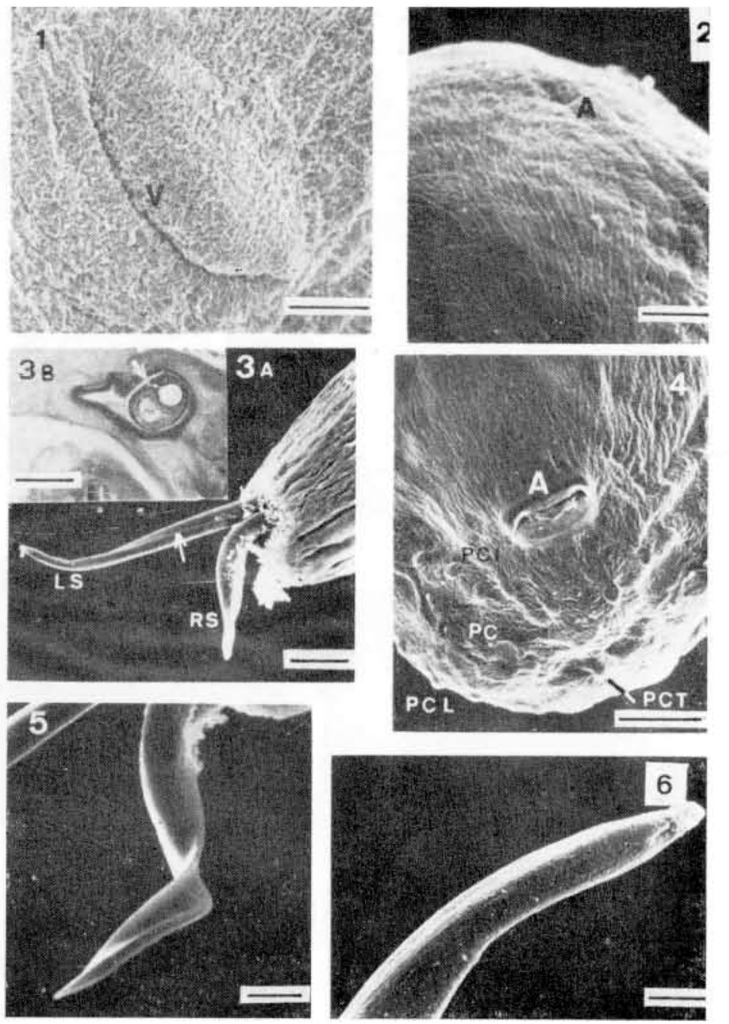


Figs. 1—5. *D. tridens*, anterior end of male and female. **Fig. 1.** Lateral view of head in male (scale bar 20 μm). **Fig. 2.** Detail of the wrinkled aspect of cuticle (→ striation) (scale bar 5 μm). **Fig. 3A.** "*En face*" view of the head in female (scale bar 20 μm). **Fig. 3B.** Detail of the apex of trident (scale bar 5 μm). **Fig. 4.** A detail of the head (scale bar 10 μm). **Fig. 5.** Opening of trident cavity (scale bar 5 μm).
a — amphidial opening, CE — oval cephalic elevation, C — circular constriction that marks the head, p — inner papillae, P — outer papillae, t — opening of trident cavity.



Figs. 1—6. *D. tridens*, vulva and posterior end of female and male. **Fig. 1.** View of the vulva (scale bar 20 μm). **Fig. 2.** Anus on the female specimen (scale bar 10 μm). **Fig. 3A.** Posterior end of male with the spicules (→ the longitudinal fissure on left one) (scale bar 100 μm). **Fig. 3B.** Histological section through left spicule (→ fissure) (scale bar 20 μm). **Fig. 4.** Ventral view of the tail of a male (scale bar 40 μm). **Fig. 5.** Detail of right spicule (scale bar 20 μm). **Fig. 6.** Distal end of left spicule (scale bar 20 μm).
A — anus, LS — left spicule, PC — additional row of smaller papillae, PCI — caudal papillae of the inner row, PCL — caudal papillae of the lateral and terminal row, PCT — terminal ventral papilla, R S — right spicule, V — vulva.