# On the ultrastructure of the cuticle of Trichodoridae Thorne, 1935 (Nematoda: Enoplia)

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**Summary** – The fine structure of the body cuticle was studied in *Trichodorus primitivus, T. similis, T. hooperi, T. viruliferus, Paratrichodorus pachydermus,* and *P. nanus.* The ultrastructure of the cuticle was mostly similar to recent observations on four species from western Africa. Some intraspecific differences were seen in the thickness of layers 4 and 6. The ultrastructure of the body cuticle in the region of the retracted spicules in *P. pachydermus* is clearly different from that in *T. primitivus.* This result confirms the diagnostic value of presence *vs* absence of caudal alae for differentiating the didelphic genera. © Orstom/Elsevier, Paris

**Résumé – *Ultrastructure de la cuticule des Trichodoridae Thorne, 1935 (Nematoda: Enoplia)*** - L'ultrastructure de la cuticule est décrite chez *Trichodorus primitivus, T. similis, T. hooperi, T. viruliferus, Paratrichodorus pachydermus* et *P. nanus.* La cuticule présente une structure largement semblable à celle décrite récemment chez quatre espèces d'Afrique occidentale mais, selon les espèces, une différence dans l'épaisseur des couches 4 et 6 est observée. La structure de la région des spicules est nettement différente chez *P. pachydermus* et chez *T. primitivus,* confirmant ainsi la valeur diagnostique du caractère présence ou absence d'ailes caudales pour différencier les genres didelphes. © Orstom/Elsevier, Paris

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The family Trichodoridae Thorne, 1935 is a small family with two didelphic genera (*Trichodorus* Cobb, 1893 and *Paratrichodorus* Siddiqi, 1974) and two monodelphic genera (*Allotrichodorus* Rodriguez-Montessoro, Sher & Siddiqi, 1978 and *Monotrichodorus* Andrássy, 1976). Although the most important diagnostic generic features are well described, overlap between the genera may occur, which makes identification to genus level difficult, especially with female specimens (Decraemer & Baujard, 1998). In males, presence *vs* absence of caudal alae differentiates *Paratrichodorus* from *Trichodorus* and *Allotrichodorus* from *Monotrichodorus,* but, here again, exceptions are known. The present study is part of a project that aims at substantiating a phylogenetic approach to the higher classification of the Trichodoridae. Currently, the families Trichodoridae and Diphtherophoridae constitute the order Triplonchida (see Siddiqi, 1983*a*), which is classified under Enoplia and is related to the order Tripylida (see Siddiqi, 1983*b*).

For a better understanding of the trichodorid genera and their phylogenetic relationships, the most important diagnostic features at genus level were reviewed from ultrastructure observations of the body cuticle. Until recently, only one illustrated description of the ultrastructure of the body cuticle in the family Trichodoridae had been published (*Paratrichodorus allius* in Raski *et al.*, 1969), plus a non-illustrated description of the fine structure of *P. minor* (Hirumi *et al.*, 1969). A study on the ultrastructure of the somatic musculature of *P. porosus* (Allen, 1957) Siddiqi, 1974 by Bird (1970) included a TEM picture of the somatic musculature showing part of the body cuticle. Within the scope of the present project, the ultrastructure of the body cuticle was studied in males of six species belonging to the two didelphic genera: *Paratrichodorus pachydermus* (Seinhorst, 1954) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974, *Trichodorus primitivus* (de Man, 1880) Micoletzky, 1922, *T. similis* Seinhorst, 1963, *T. viruliferus* Hooper, 1963, and *T. hooperi* Loof, 1973. Apart from the last one, all of these species are known to be natural vectors of economically important virus diseases of crops in Europe, and several are of world-wide significance.

In parallel with our research, Mounport *et al.* (1997) studied the ultrastructure of the body cuticle at mid-body level of females of *P. minor* (Colbran, 1956) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974, *P. rhodesiensis* (Siddiqi & Brown, 1965) Siddiqi, 1974, and *T. eburneus* De Waele & Carbonell, 1983. These species all occur in the semi-arid region of western Africa and the specimens studied were reared under laboratory conditions.

## Material and methods

For TEM observations, specimens were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 h, washed three times in buffer, then postfixed in

1% osmium tetroxide in phosphate buffer for 30 min. Nematodes were rinsed in buffer then embedded in 1% distilled water agar. Agar blocks containing single nematodes were dehydrated in a graded ethanol series and the ethanol replaced by propylene oxide. Specimens were infiltrated for 3 h in Emix epoxy resin at 37°C then placed in flat embedding moulds to be polymerised at 70°C. Specimens were sectioned at a Reichert Ultracut microtome and sections collected on plastic coated grids. These were stained with uranyl acetate and lead citrate before being examined in a JEOL 1200 electron microscope.

Transverse and/or longitudinal sections were made at the levels of the pharyngeal region and the copulatory apparatus and tail.

The fine structure of the body cuticle was studied at the level of the caudal alae in *P. pachydermus* and compared to the ultrastructure of the body cuticle at the same level in *T. primitivus* and *T. hooperi*. In addition, light-microscopic studies of series of transverse sections from the posterior body region of *T. similis* and *P. pachydermus* were compared.

## Results

All species showed the same basic structure characterised by *i*) a trilaminate external layer, *ii*) a fine granular layer, and *iii*) an electron-dense or electron-lucent layer with granulations; all three layers are also present in other taxa. Below are three inner layers (4 to 6). Layer 6 consists of three multilaminate units with the outer unit showing a marked banding. This layer of the body cuticle seems to be characteristic of trichodorids, and, according to all known data, is quite different from the structure of the body cuticle in Enoplia and Chromadoria.

### Fine structure of the body cuticle

The fine structure of the body cuticle in the species studied largely agreed with the descriptions by Mounport *et al.* (1997) and consisted of six layers; however, layer 5 was not always clearly differentiated. Some intraspecific differences were observed in the thickness of layers 4 and 6. *T. hooperi* (Fig. 2A), *P. nanus* (not illustrated), and *P. pachydermus* each possess a thick layer 4, representing more than 50% of the cuticle thickness, and a multilaminate layer 6, representing 30% of the total cuticle thickness. These data are comparable with the recent observation by Mounport *et al.* (1997). In *P. pachydermus*, the banding of layer 6 in both the anterior and the posterior body regions was not always visible (Fig. 2C, D). Conversely, the pharyngeal (Fig. 1B) and spicule (Fig. 1D) regions of *T. similis* and the spicule region of *T. primitivus* (Fig. 1A) and *T. viruliferus* (Fig. 1C) all possess a thin and amorphous layer 4 and a multilaminate basal layer 6 representing up to 75% of the cuticle thickness. No differences were observed between pharyngeal region and spicule region. The peculiar type (different from hemidesmosomes) of junctions between cuticle and somatic muscle cells that was described by Mounport *et al.* (1997) was observed (Fig. 1A), as well as intracuticular canals mainly in layers 4 and 6.

### Caudal alae

One of the most important diagnostic features in males of the didelphic genera *Paratrichodorus/Trichodorus* and the monodelphic genera *Allotrichodorus/Monotrichodorus* is presence *vs* absence of caudal alae, respectively. Caudal alae are present in all species of *Paratrichodorus*, although narrow and non-functional (*P. pachydermus*: Fig. 3A, B); in some species they are weakly developed and hardly recognisable on SEM pictures, for example *P. grandis* Rodriguez-Montessoro & Bell, 1978 (Fig. 3D, courtesy Dr. J. Baldwin, TEM Micrograph Collection Riverside) and *P. rhodesiensis* (Fig. 3C, courtesy P. Baujard, SEM Micrograph Collection, ORSTOM). In *Allotrichodorus*, caudal alae are present in various degrees of development, they are obvious but narrow in *A. brasiliensis* (Fig. 4E), and they are hardly developed in *A. longispiculis* Rashid, De Waele & Coomans, 1986. In *Trichodorus*, caudal alae are generally absent (*T. similis*: Fig. 4A; *T. primitivus*: Fig. 4B), but they are distinctly present in two species (*T. cylindricus* Hooper, 1962 and *T. paracedarus* Xu & Decraemer, 1995: Fig. 4D). Several *Trichodorus* species have 'rudimentary caudal alae', for example *T. sparsus* Szczygiel, 1968 (see Loof, 1973), *T. elefjohnsoni* Bernard, 1992, *T. nanjingensis* Liu & Cheng, 1990 (in Decraemer & Cheng, 1994), and *T. vandenbergae* De Waele & Kilian, 1992 (Fig. 4C); these were also described in *Monotrichodorus sacchari* Baujard & Germani, 1985 (see Decraemer, 1986). The 'rudimentary caudal alae' are possibly induced by temporary muscular activity in *T. elefjohnsoni* (Bernard, 1992). The fine structure of caudal alae in *Paratrichodorus* was compared with the fine structure of the body cuticle in *Trichodorus* species. Also, a comparison was made using light-microscopy observations of transverse sections of the body cuticle in the region of retracted spicules and tail in representative species of the two didelphic genera.

#### *P. pachydermus*

Light-microscopy observations of male specimens of *P. pachydermus* revealed well developed caudal alae which protrude lateroventrally from the body (Fig. 5A-C). A transverse section through the cloacal opening showed two well developed flaps of the anterior cloacal lip (Fig. 5B). Just posterior to the posterior cloacal lip (Fig. 5C), two fine subventral canals

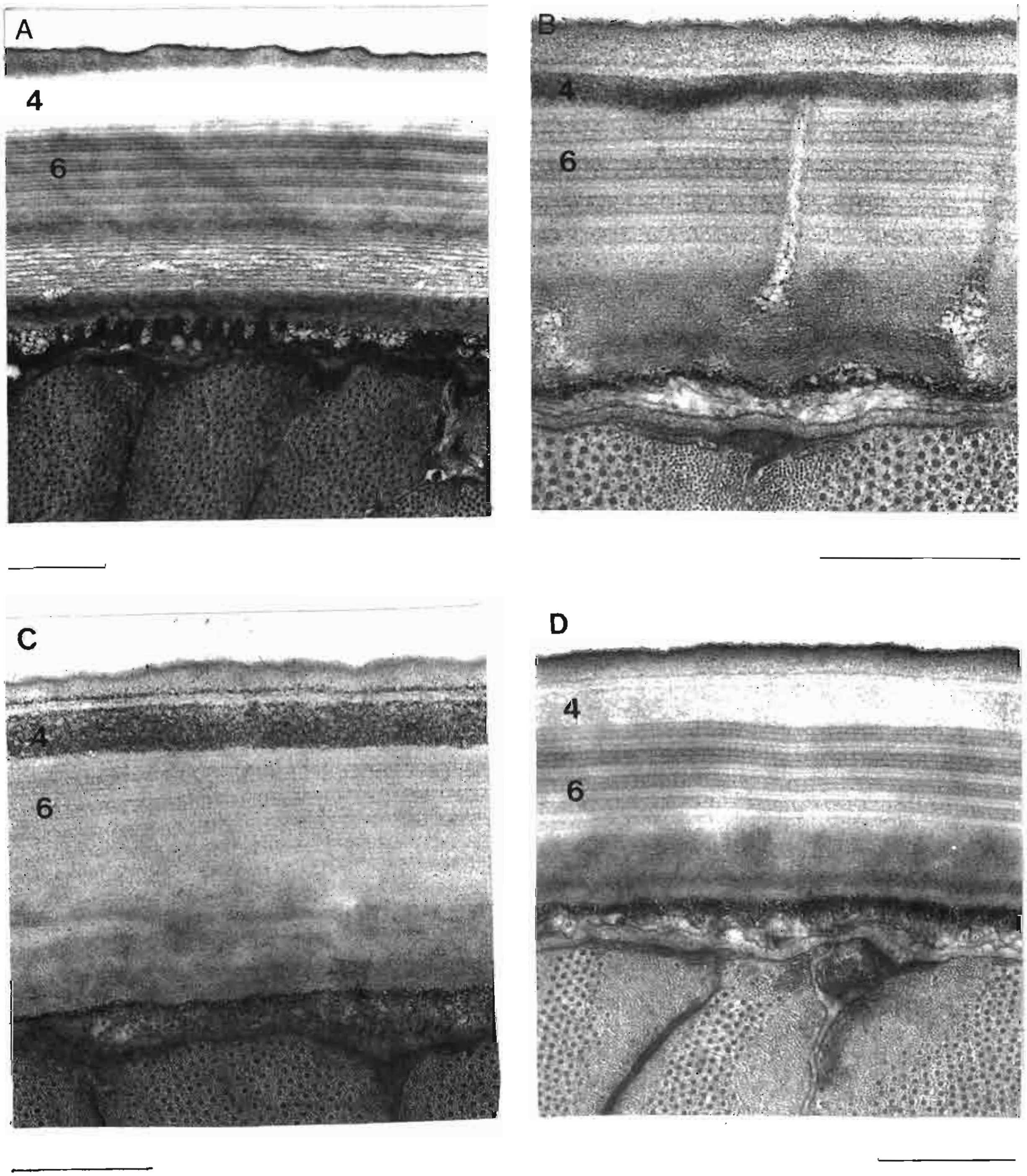


***Fig. 1.*** *Ultrastructure of the body cuticle in males; dorsal wall in transverse sections, with indication of layers 4 and 6, at level of retracted spicules (A, C, D) and in the pharyngeal region (B). A:* Trichodorus primitivus*; B:* T. similis*; C:* T. viruliferus*; D:* T. similis. *(Scale bar = 0.5 μm).*

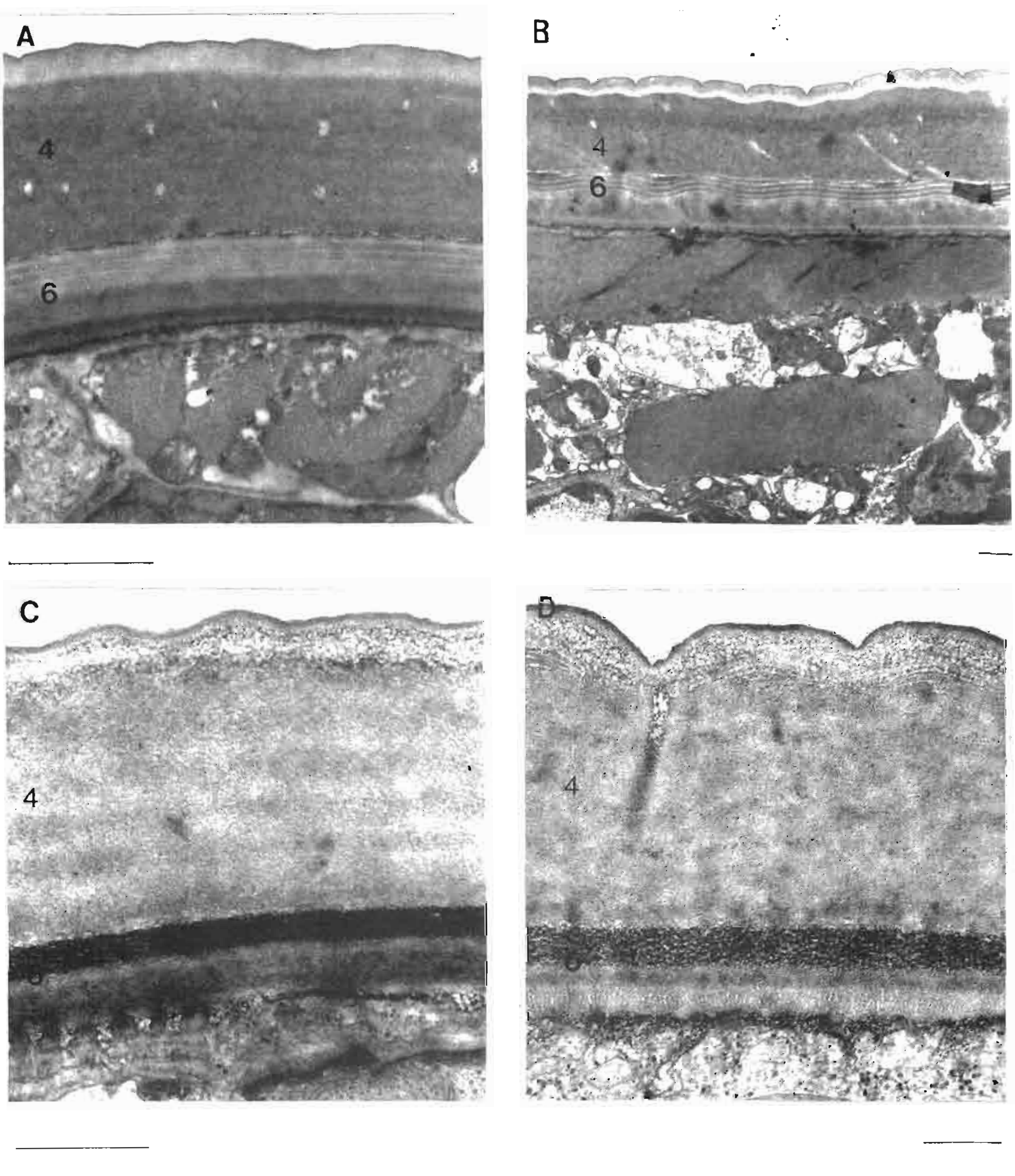


*Fig. 2. Ultrastructure of the body cuticle in males with indication of layers 4 and 6. A, B:* Trichodorus hooperi. *spicule region, dorsal wall in transverse and longitudinal section, respectively; C, D:* Paratrichodorus pachydermus, *dorsal wall in transverse section in the spicule region and longitudinal section in the pharyngeal region (Scale bar = 0.5 μm).*

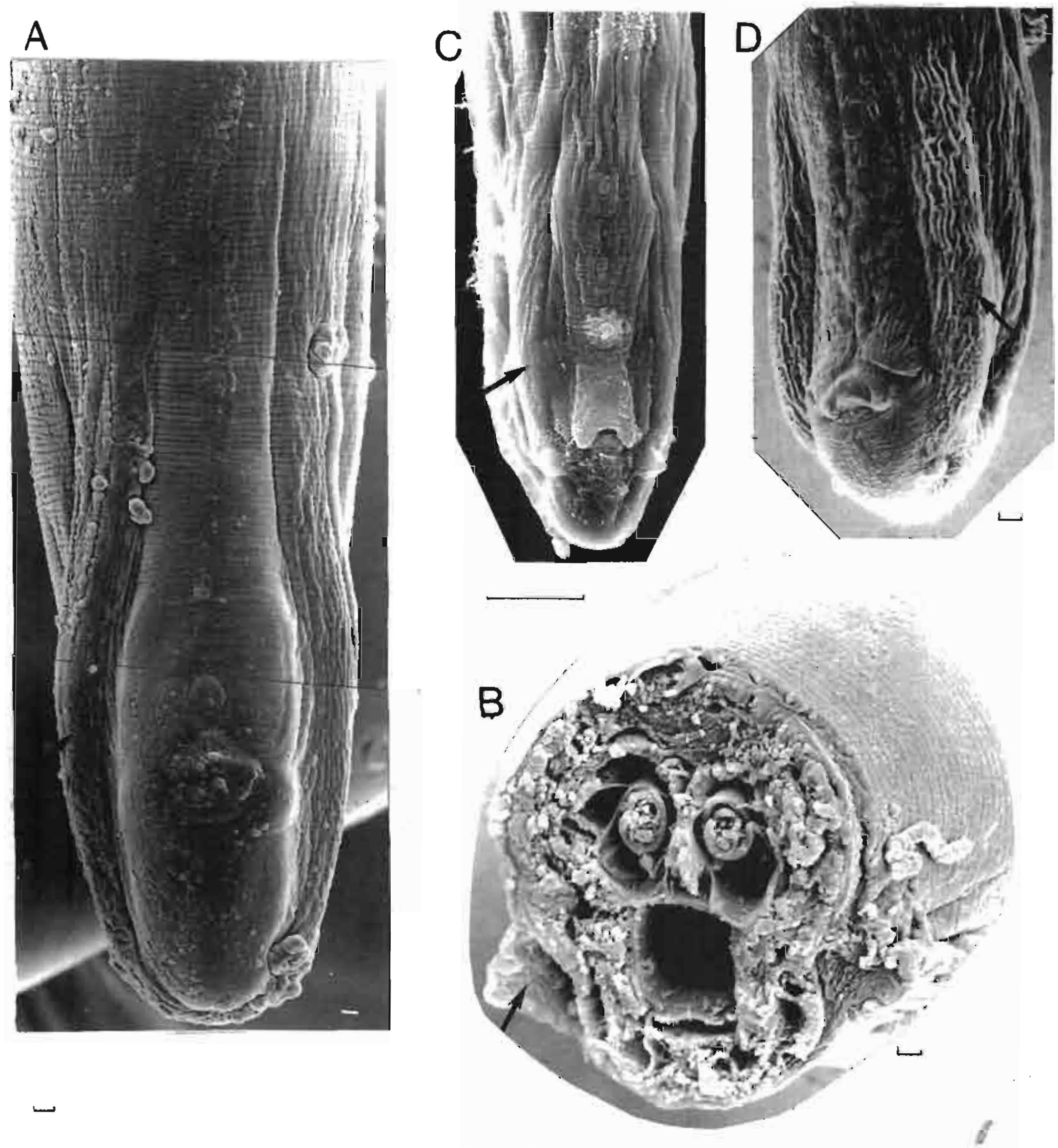


*Fig. 3. SEM observations of caudal alae region in* Paratrichodorus *males, ventral view. A, B:* P. pachydermus, *ventral view and transverse section at level of retracted spicules, respectively; C:* P. rhodesiensis *(courtesy P. Baujard); D:* P. grandis *(courtesy J. Baldwin) (Arrow indicates caudal alae. Scale bars = 1 µm in A-B, D; 10 µm in C).*

each leading to a pore were observed in the transverse optical section. This agrees with the observation by Sturhan (1985) who rejected earlier descriptions of these structures as a pair of minute postcloacal papillae (Seinhorst, 1954; Allen, 1957; Kuiper & Loof, 1962; Baujard, 1980). Transverse sections of this posterior body region show a clear demarcation of the ventral field. Baujard and Germani (1985) described the demarcation of the lateral fields by two incisures in *Monotrichodorus sacchari*. A differentiation of the lateral fields could not be confirmed by light-microscopy nor by TEM in any of the species discussed in present paper. A study of their fine structure, based on a series of transverse sections from the level of the retracted spicules to a subterminal point, showed that the caudal alae were formed lateroventrally by an additional layer of dense tissue within the inner unit of layer 6. This layer appears as a clearly distinguished

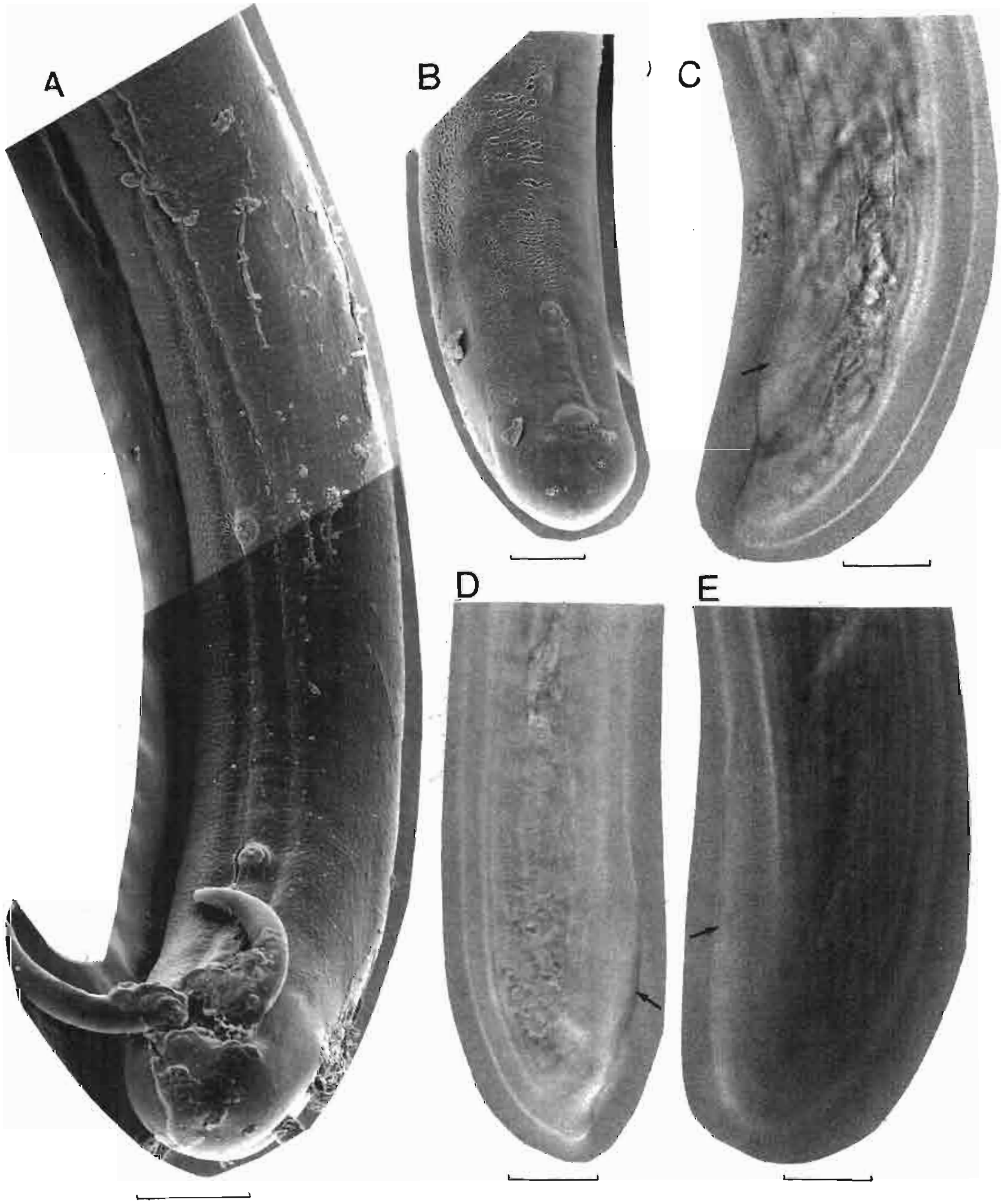


***Fig. 4.*** *SEM (A, B) and LM observations (C-E) of posterior body region in* Trichodorus *males. A:* T. similis*; B:* T. primitivus*; C:* T. vandenbergae *with rudimentary caudal alae (arrow); D:* T. paracedarus *with caudal alae (arrow); E:* Allotrichodorus brasiliensis *with caudal alae (arrow) (Scale bar = 10 µm).*

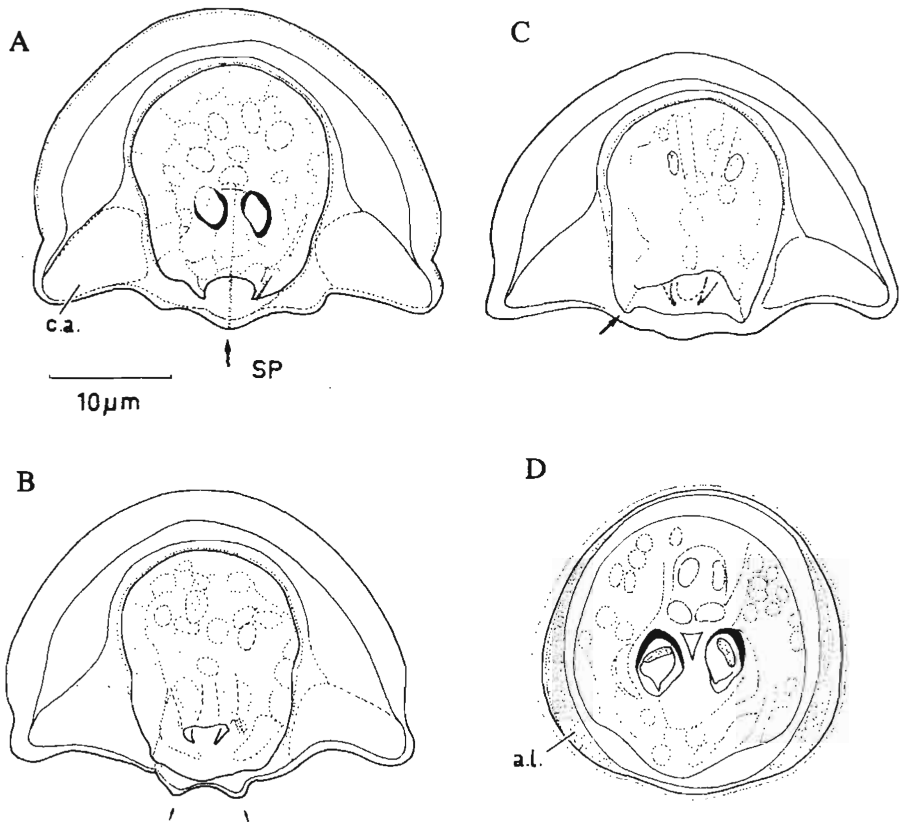


***Fig. 5.*** *Light-microscopic observations of transverse sections of* Paratrichodorus pachydermus *(A-C) and* Trichodorus similis *(D). A: At level of posteriormost precloacal supplement; B: At level of anterior cloacal lip (arrow points to bifid anterior cloacal lip); C: At level of subventral pores (see arrow) just posterior to cloacal opening; D: At level of distal part of retracted spicules (a.l. = additional layer; c.a. = caudal alae; SP = precloacal supplement).*

oval portion which fills the protruding caudal alae. Mid-ventrally, at the level of the ventral field, layer 6 with a wide outer unit forms 75% of the body cuticle (Fig. 7B).

*T. similis, T. primitivus* and *T. hooperi*

Light-microscopy observations of series of transverse sections of several male specimens of *T. similis* revealed the presence of a largely lateral to lateroventral thickening of the body wall together with a more or less pronounced dorsoventrally flattening of the body, probably resulting from muscular activity (Fig. 5D).

TEM microphotographs of transverse sections of male specimens of *T. primitivus* (Fig. 6) in the region of retracted spicules and in the tail revealed a narrow electron-dense band at the inner base of the outer unit of the multilaminate cuticle layer 6 and its connection with the mid-unit of layer 6. This electron-dense band, or layer, gradually enlarges from laterodorsal to a maximum width lateroventrally on the body, then narrows to a thin band subventrally and ventrally. At the level of the ventral field, the body cuticle is similar to that in *P. pachydermus*, with the outer unit (= with obvious banding) of layer 6 as main component.

In the fine structure of *T. hooperi*, there was some difference in the position of the cuticular thickening between layers 4 and 5 in the spicule and tail region. Midventrally, layer 6 forms 50% of the body cuticle (Fig. 7B, C).

## Discussion

The present study of the fine structure of the body cuticle of five species belonging to the two didelphic genera in Trichodoridae supports and extends the recent observations by Mounport *et al.* (1997). Our results confirmed the unique character of the cuticle structure in Trichodoridae, a feature that separates this family from the Dorylaimina.

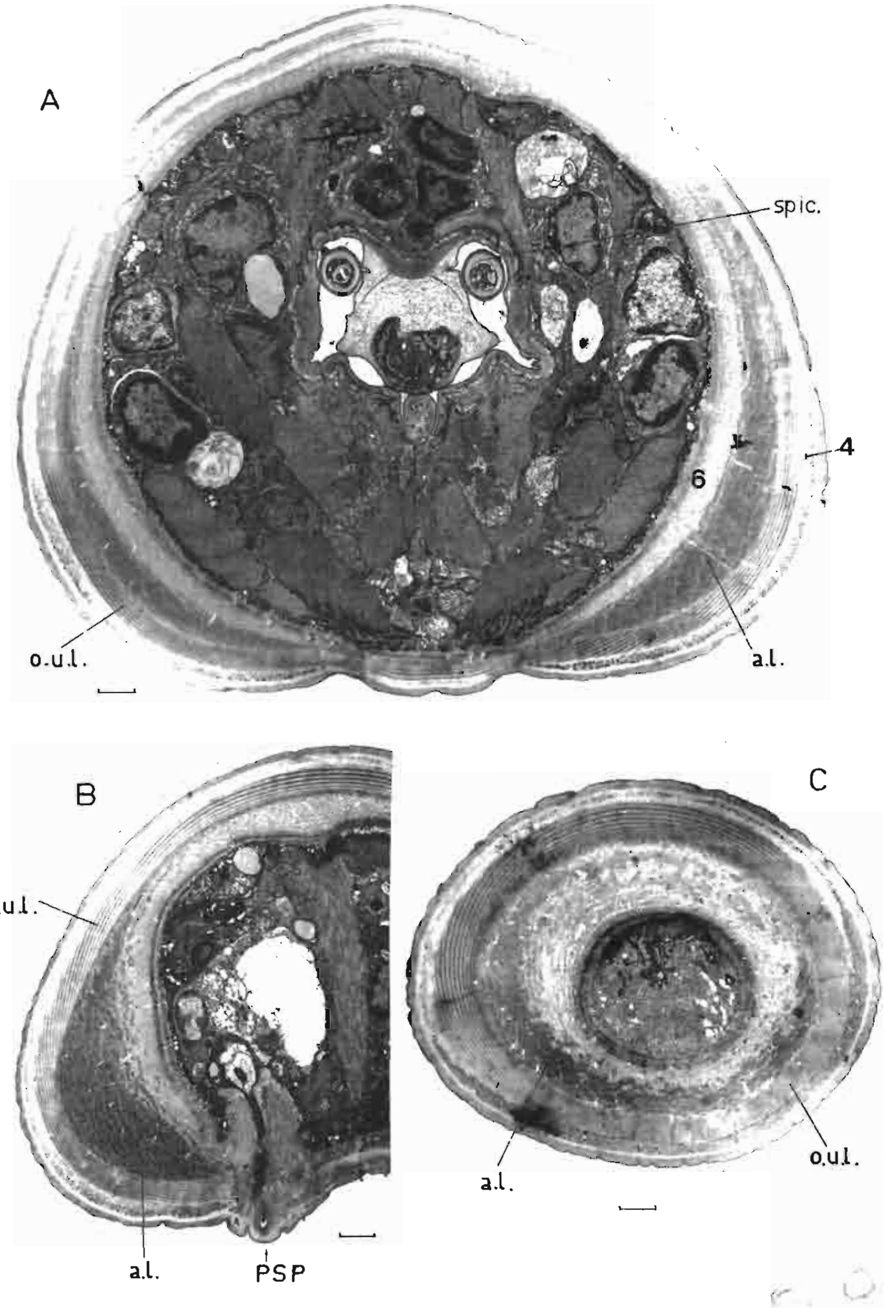


***Fig. 6.*** *Ultrastructure of body cuticle in posterior body region of* Trichodorus primitivus *males. A: Spicule region; B: At level of a post-cloacal papilla; C: Subterminal on tail (Scale bar = 1 μm; with indication of layers 4 and 6, a.l. = additional layer; o.u.l. = outer unit of layer 6; PSP = postcloacal supplement; spic. = spicule).*

There are some marked differences in the fine structure of the body cuticle in the region of retracted spicules of specimens belonging to either *Paratrichodorus* or *Trichodorus*. This result confirms the diagnostic value of the feature presence *vs* absence of caudal alae, for differentiating the didelphic genera.

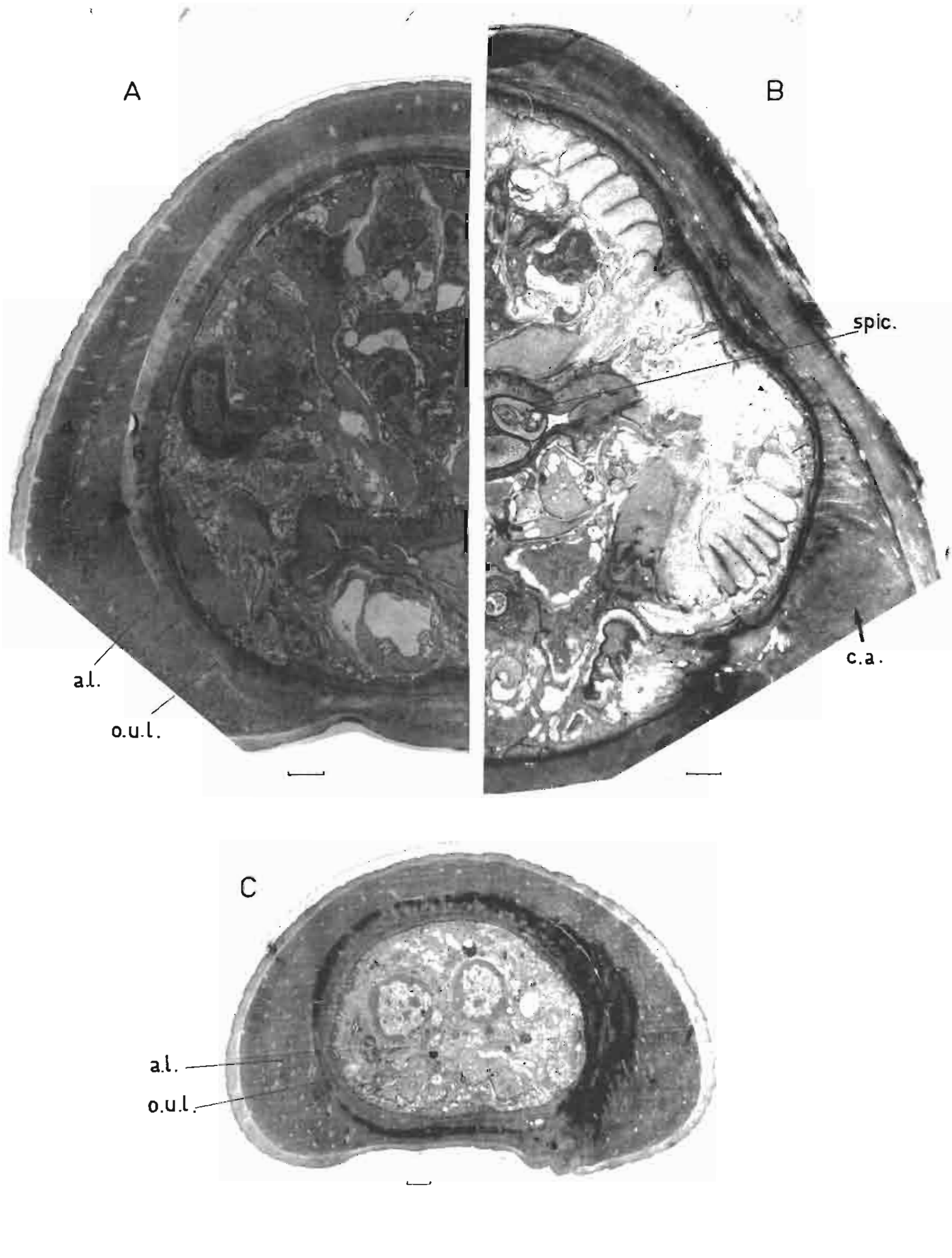


***Fig.** 7. Ultrastructure of body cuticle in males at level of caudal alae and tail of* Trichodorus hooperi *(A, C) and* Paratrichodorus pachydermus *(B). A, B: Spicule region; C: At level of a postcloacal papilla (Scale bar = 1 μm; with indication of layers 4 and 6; a.l. = additional layer; c.a. = caudal alae; o.u.l. = outer unit of layer 6; spic. = spicule).*

In the *Trichodorus* species studied, the thickening of the body cuticle is restricted to the region of the retracted spicules and tail, *i.e.*, the posterior body region which shows a more or less pronounced dorso-ventral flattening of the body. Information was not obtained on the nature of the substance forming the 'rudimentary caudal alae'. The enlargement of the body wall is possibly induced by muscular activity; however, the amount of additional tissue seems rather large as compared with a hypothetical reconstruction of a non-flattened transverse section from the more anterior body regions.

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