Fundam. appl. Nematol., 1994, **17** (5), 479-483

Ultrastructure of the body cuticle of *Atalodera gibbosa* Souza & Huang, 1994 (Tylenchida : Heteroderinae) [1]

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Accepted for publication 6 December 1993.

Summary – The ultrastructure of the body cuticle of *Atalodera gibbosa* Souza & Huang, 1994 is described. The cuticle consists of A, B, C and D layers, which may be subdivided into additional layers designated by subscripts. A_1, A_2 and A_3 layers are similar to those of other heteroderids. A new layer, A_4, is reported in second-stage juveniles (J2). Besides an usual B layer in J2 and males, a unique arrangement of fibres parallel and radial (relative to the cuticle surface) characterizes this layer in females. Third and fourth stage juveniles present a structured C layer and a new-formed D layer. Young females present one common D layer, and mature females show an additional layer D_2 (parabolic-formed fibres in two lamellae), which is reported for the first time.

Résumé – ***Ultrastructure de la cuticule d'*Atalodera gibbosa *Souza & Huang, 1994 (Tylenchida : Heteroderinae)*** – L'ultrastructure de la cuticule d'*Atalodera gibbosa* Souza & Huang, 1994 est décrite. La cuticule est composée des couches A, B, C et D qui peuvent être subdivisées en couches supplémentaires désignées par des sous-exposants. Les couches A_1, A_2 et A_3 sont semblables à celles des autres Heteroderidae. Une nouvelle couche – A_4 – est signalée chez les juvéniles de deuxième stade (J2). La couche B est normale chez les J2 et les mâles, alors que chez les femelles, elle est caractérisée par une disposition particulière de fibres parallèles – par rapport à la surface de la cuticule – et de fibres radiales. Les troisième et quatrième stades juvéniles présentent une couche C structurée et une couche D néo-formée. Les jeunes femelles possèdent une couche D normale, mais chez les femelles matures existe une couche supplémentaire, D_2, rapportée ici pour la première fois, et formée de fibres paraboliques disposées en deux strates minces.

Key-words : *Atalodera*, cuticle, ultrastructure.

Detected in an extensive survey of natural *cerrado* (savanna) in central Brazil (Cares & Huang, 1991), *Atalodera gibbosa* Souza & Huang, 1994 was first described and subsequently studied in its seasonal fluctuations (Souza & Huang, 1994). These authors also proposed *Thecavermiculatus* Robbins, 1978 as a junior synonym of *Atalodera* Wouts & Sher, 1971. This article reports the ultrastructure of the cuticle of second (J2), third (J3), and fourth (J4) juvenile stages as well as young and mature females, and males of *A. gibbosa*.

Materials and methods

The nematodes were fixed at 4 °C in formaldehyde 2 % – glutaraldehyde 3 % in sodium cacodylate buffer 0.05 M, pH 7.3 for at least 24 h, and osmium tetroxide 1 % in sodium cacodylate buffer 0.1 M for 1-2 h, dehydrated with acetone at room temperature, embedded in Spurr's resin, and sectioned in ultramicrotome with glass knives. Sections with 50-60 nm thickness were mounted in formvar covered grids and stained with potassium permanganate 0.9 %, uranyl acetate 2 % and lead citrate (Reynolds, 1963; Knight, 1977). Ten J2, six J3-J4 and three males were observed in transverse sections through median region of body. One young female and ten mature females were observed in transverse sections through median, median-posterior, and vulval regions. One young female and seventeen mature females were examined in longitudinal sections. All TEM was with a JEOL JEM 100 C transmission electron microscope.

Results

J2 : (Fig. 1 A, B). The A_1 layer (40 nm) is divided into three zones. Layers A_2 and A_4 (0.2 μm each) consist of fibrils embedded in an electron-lucent matrix. Layer A_3 (0.3 μm) consists of electron-dense deposits in

(1) A part of the MS thesis of the first author.

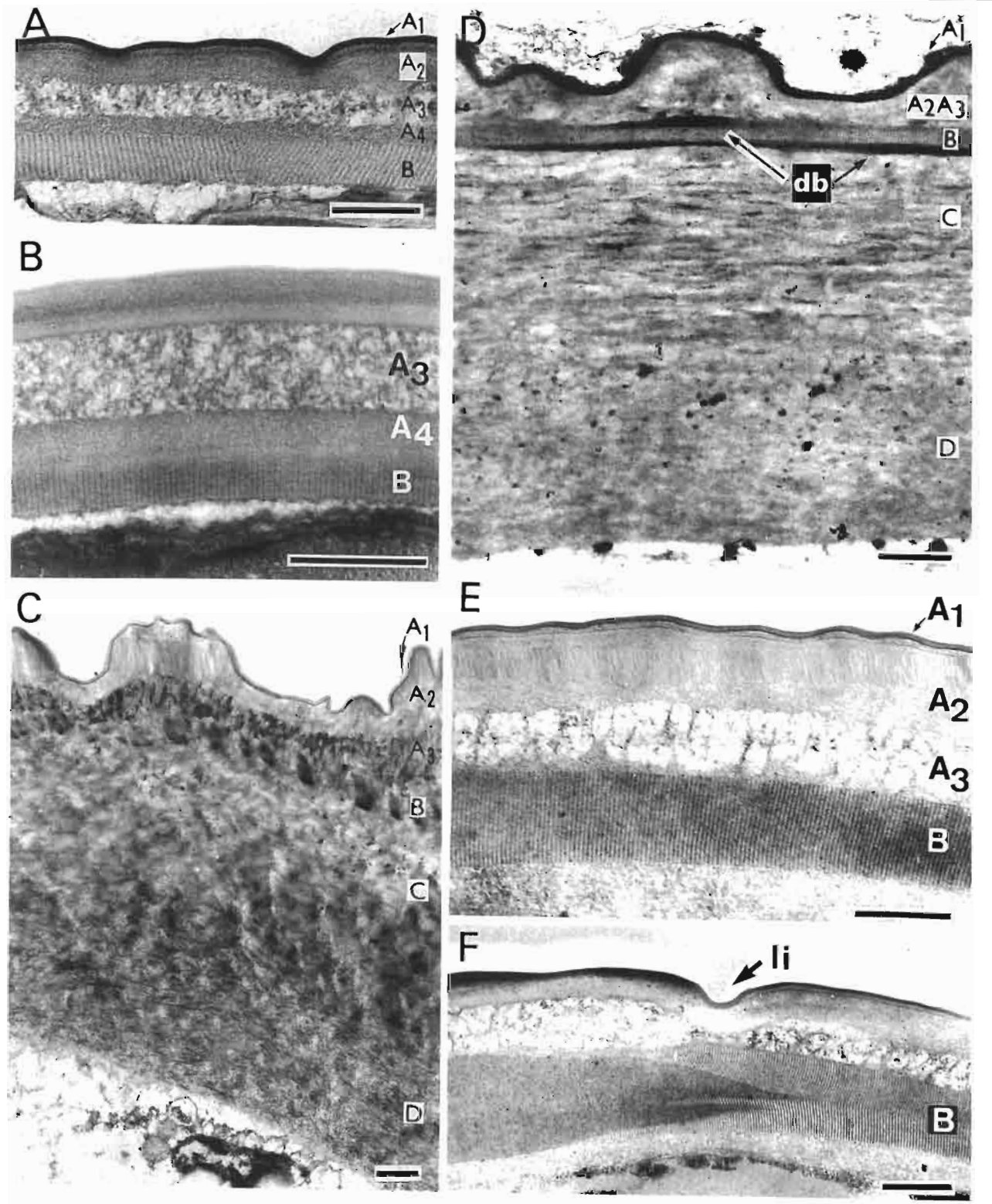

Fig. 1. *Ultrastructure of the body cuticle of* Atalodera gibbosa. *A : J2, longitudinal section; B : J2, transverse section; C : Young female, longitudinal section; D : J3-J4, transverse section, E, F : Male, transverse section (db = horizontal fibres seen as dense bands; li = lateral incisure).* Bar equivalents : A, B, E, F = 0.5 μm; C, D = 1.0 μm.

an electron-lucent matrix. Layer B (0.3 μm) is composed of striated fibres in electron-lucent matrix. These fibres are oriented radially and obliquely in transverse and oblique sections, respectively. In the region of the lateral field in J2 and in males (Fig. 1 F), The B layer is forked by one or two electron-dense layers.

Male : (Fig. 1 E, F). Layers A_1 (50 nm), A_2 (0.4 μm), A_3 (0.4 μm) and B (0.5 μm) are shown as in J2.

J3-J4 : (Fig. 1 D). The layer A_1 (80 nm) was viewed as a high electron-dense band. Layers A_2 and A_3 (0.7 μm) consist of electron-dense spots immersed in an electron-lucent matrix. Layer B (0.3 μm) is organized as in J2, but also presents horizontal fibres (electron-dense bands in figure). Layer C (2.5 μm) consists of horizontal filaments in an electron-lucent matrix. Layer D (2.5 μm) has a felt-work texture.

Young female : (Fig. 1 C). Layer A_1 (90 nm) is divided into two zones : electron-lucent and electron-dense. Layer A_2 (0.8 μm) and layer A_3 (0.4 μm) are similar to those of J2. Layer B (0.4 μm) consists of groups of horizontal fibres separated by radial ones (as in mature females, Fig. 2 C, D), whereas layer C (3.0 μm) is organized as in J3-J4. Layer D (4.0 μm) consists of fibers embedded in an electron-lucent matrix, presenting circular and irregular patterns at longitudinal and transverse sections, respectively.

Mature female : (Fig. 2). Layers A_1 (0.1 μm) and B (0.5 μm) are similar to those of young females, and so are layers A_2 and A_3 (0.8 μm each) to J2, layer C (3.0 μm) to J3-J4, and layer D_1 (3.5 μm) to D layer of young females. There were electron-dense deposits in layer D_1 in some mature females (Fig. 2 A). The layer D_2 (0.9 μm) consists of parabolic-form fibres in two lamellae in longitudinal and an irregular pattern in transverse section (Fig. 2 A, B and E). There were " islands " of horizontal fibres disposed in different directions to longitudinal axis in layers A_2 and C (Fig. 2 C).

Discussion

Layers A_1, A_2 and A_3 of *A. gibbosa* are similar to those of J2, females and males of other heteroderids (Shepherd *et al.,* 1972; Baldwin & Hirschmann, 1975; Johnson & Graham, 1976; Johnson, 1981; Baldwin, 1983; Cliff & Baldwin, 1985). Layer A_4 in J2 of *A. gibbosa* has not been reported previously. Layer B in J2 is also similar to those of other heteroderids, but in females it is characterized by groups of horizontal fibres separated by radial ones. This pattern has not been reported in other species. The D layer in young females (as D_1 in mature ones) of *A. gibbosa* is similar to those of *A. lonicerae, A. ucri, A. gracililancea* and *Cactodera* sp. (Baldwin, 1983; Cliff & Baldwin, 1985). In *A. gibbosa* layer D_1 is distinguished from D_2 by the parabolic pattern with two lamellae in the latter, and by larger diameter of fibres (50 nm *vs* 30 nm). The layer D_2 is different from the layer E in *A. ucri* and layers E_1-E_2 of *Heterodera schachtii* and *Bellodera utahensis* (Cliff & Baldwin, 1985; Cordero & Baldwin, 1990; Baldwin & Eddleman, 1992).

Cuticular modifications of female with age have been described in *Heterodera* spp., *Globodera* spp., and *B. utahensis* (Shepherd *et al.,* 1972; Cordero & Baldwin, 1990; Baldwin & Eddleman, 1992). In *A. gibbosa* the changes in layer D from J3-J4 to mature female may be attributed to collagen crystallization, as mentioned by Shepherd *et al.* (1972). Furthermore, layer D_2, the electron-dense deposits in layer D_1, and the " islands " of fibres in A_2 and C were observed only in mature females.

The structure of the body wall cuticle has been used in studies on taxonomy and phylogeny of Heteroderinae (Baldwin, 1983; Cliff & Baldwin, 1985; Baldwin & Bell, 1985, Luc *et al.,* 1988; Baldwin & Schouest, 1990; Baldwin & Eddleman, 1992). However, five of nine non-cyst forming genera are monospecific, suggesting fewer intrageneric variations. Similarly, only two of seven *Meloidodera* species and one of two *Verutus* species were studied (Baldwin, 1983; Cliff & Baldwin, 1985). In *Cryphodera* with four species, only limited optical microscope studies were done (Baldwin & Schouest, 1990). *Atalodera* sensu Souza and Huang (1994), with nine species, is the most widely studied genus. Thus, *A. gracililancea* has C and D layers, *A. lonicerae* presents C_1, C_2 and D, *A. gibbosa* has C, D_1 and D_2, and *A. ucri* shows C_1, C_2, D and E (Baldwin, 1983; Cliff & Baldwin, 1985; this article). Because of this great intrageneric variability in C and D layers, and the absence of diagnostic characters in A_1, A_2, A_3 and B ones, only the presence *vs* absence of D-layer in mature females, as used by Luc *et al.* (1988), is suggested as differentiated character.

Acknowledgements

The authors are thankful to Miss Maria Isabel Lima, an MS graduate student at Departamento de Fitopatologia, Universidade de Brasília, for teaching the ultrastructure procedures.

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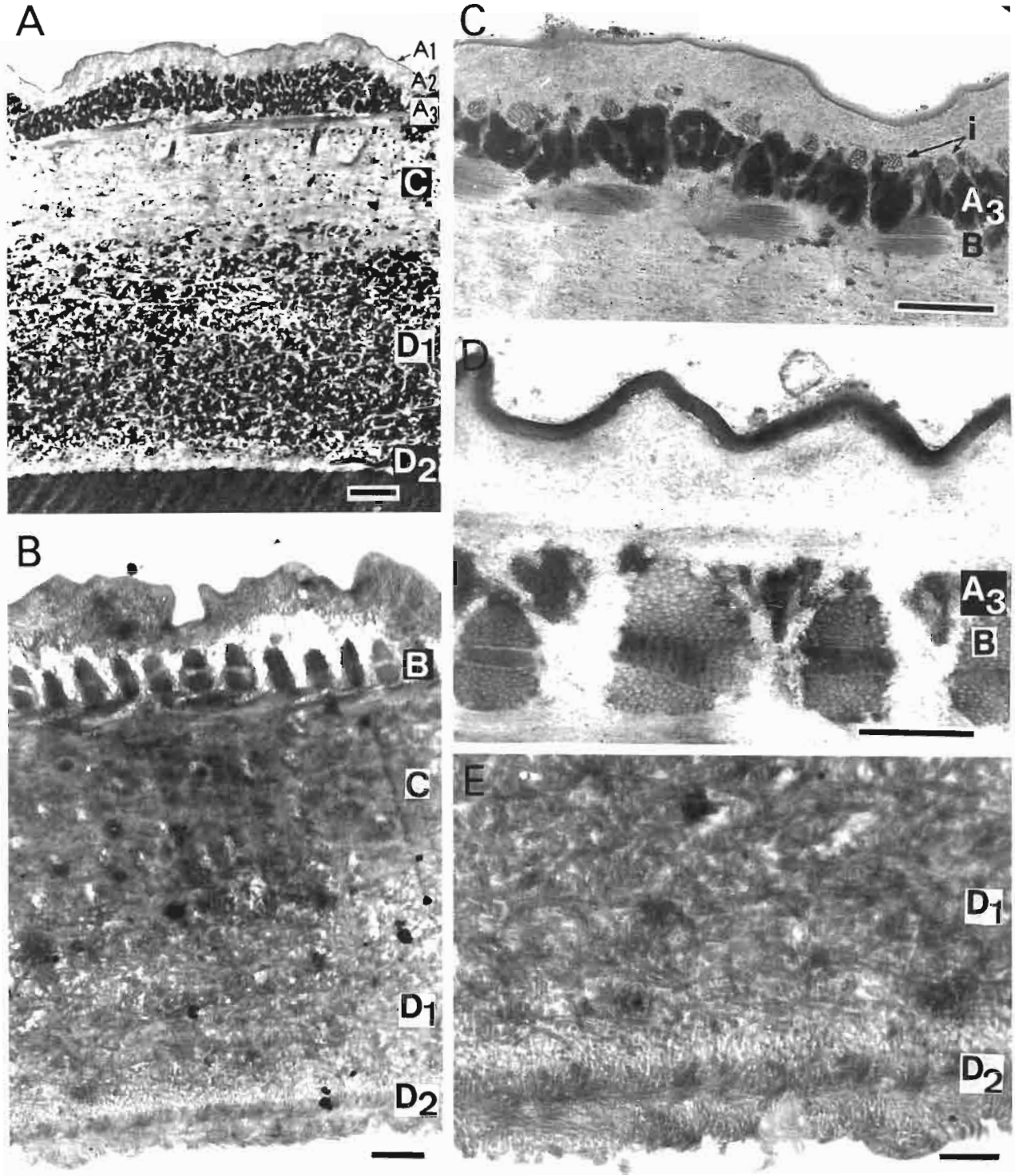

Fig. 2. *Ultrastructure of the body cuticle of mature female of* Atalodera gibbosa. *A, C : Transverse section; B, D, E : Longitudinal section. (Fig. E enlarged from B; i = " islands " of fibres).* Bar equivalents : A-C = 1.0 µm; D, E = 0.5 µm.

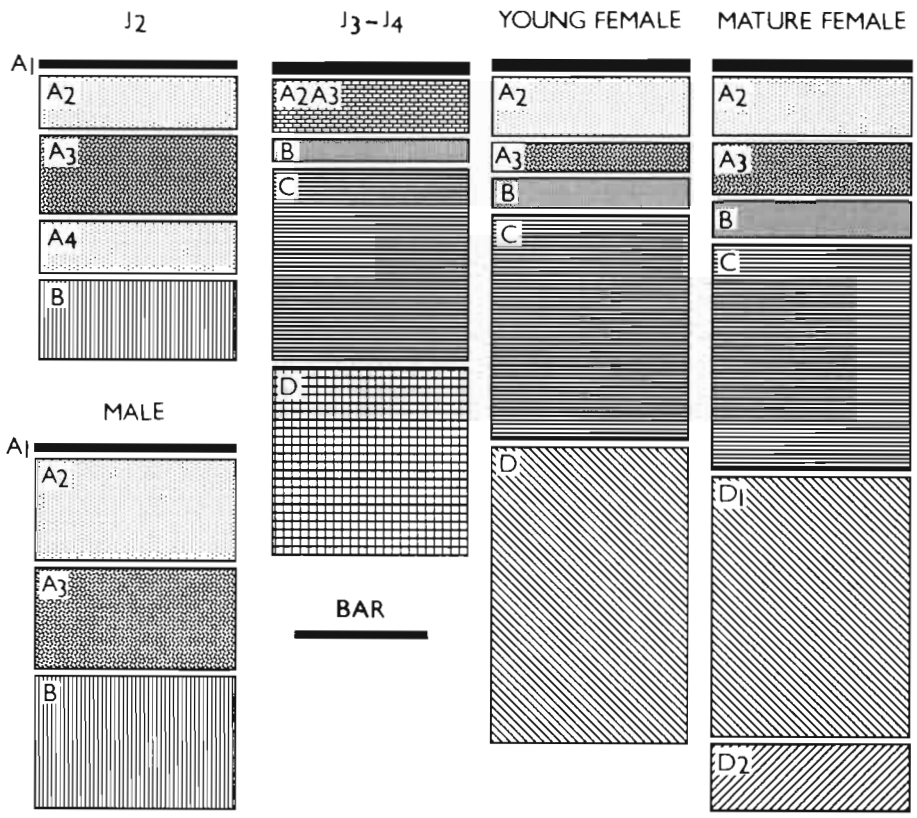

Fig. 3. *Schematic drawing of the body cuticle of J2, J3-J4, young females, mature females and males of* Atalodera gibbosa. *Similar patterns correspond to same organization of layers.* (Bars equivalents : J2 and male = 1 µm; J3-J4 and females = 1.8 µm.

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