Fundam. appl. Nematol., 1997, **20** (6), 587-591

Studies on the body wall ultrastructure of *Hirschmanniella oryzae* and *H. spinicaudata* (Nematoda: Pratylenchidae)

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Accepted for publication 21 February 1997.

Summary – The body wall ultrastructure of *H. oryzae* and *H. spinicaudata* is described. The cuticle consists of three major zones: a cortical zone with two layers (an outer trilaminate layer and an inner granular layer), a median zone represented by a vacuolar layer, and a basal zone consisting of a striated layer. Beneath the lateral fields, the fine structure of the cortical zone is unchanged, whereas the median zone is composed of four layers (two vacuolar layers separated by two granular layers) instead of a single vacuolar layer, and the basal zone is made of two thick fibrillar layers instead of a single striated layer. Examination of the muscle fields shows that the so-called "Thornean cells" of Sher (1968) correspond to an inflated sarcoplasmic portion of muscle cells with large amounts of glycogen, as revealed by cytochemical studies. This unusual accumulation of glycogen is discussed.

Résumé – ***Etudes ultrastructurales de la cuticule et de la musculature chez* Hirschmanniella oryzae *et* H. spinicaudata *(Nematoda: Pratylenchidae)*** – L'ultrastructure de la cuticule et de la musculature somatique chez *Hirschmanniella oryzae* et *H. spinicaudata* est décrite. La cuticule est constituée de trois zones: une zone corticale avec deux couches (une couche trilamellaire externe et une couche interne d'aspect granuleux), une zone médiane formée d'une couche vacuolaire et une zone basale représentée par une couche striée. Sous les champs latéraux, la structure de la zone corticale demeure inchangée ; par contre, la couche vacuolaire est remplacée par quatre couches (deux couches vacuolaires séparées par deux autres d'aspect granuleux) ; la couche basale striée s'interrompt sous les incisures externes du champ latéral ; elle est remplacée par deux couches épaisses d'aspect fibreux. L'observation des champs musculaires montre que les "Thorneian cells" de Sher (1968) correspondent à une hypertrophie de la partie sarcoplasmique des cellules musculaires, chargées d'importantes réserves de glycogène. Ces observations sur l'accumulation inhabituelle de glycogène sont discutées.

Key words : cuticle, *Hirschmanniella oryzae*, *Hirschmanniella spinicaudata*, glycogen, musculature, nematode, ultrastructure.

The cuticle ultrastructure has been studied in many taxa in Tylenchina but only a few studies have assessed the intraspecific (Mounport *et al.*, 1993 *a*) and intergeneric (Mounport *et al.*, 1993 *b*) variability of stratification. The fine structure of the cuticle in Pratylenchidae was studied in *Hirschmanniella belli* and *H. gracilis* (Johnson *et al.*, 1970), *Pratylenchus* spp. (Kisiel *et al.*, 1972; Mounport *et al.* 1990), and *Radopholus similis* (Valette *et al.*, 1997). Johnson *et al.* (1970) did not describe the modifications of the cuticle ultrastructure beneath the lateral fields in *Hirschmanniella* species.

The present article describes the fine structure of the body wall of two *Hirschmanniella* species, *H. oryzae* (Van Breda de Haan, 1902) Luc & Goodey, 1964 and *H. spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964. The purpose of the study was to elucidate: *i*) the ultrastructure of the cuticle in the lateral fields, and *ii*) the peculiar structures (called "Thornean cells" by Sher, 1968), that are seen under the light microscope between the somatic muscles and the intestine.

Materials and methods

Females and males of *H. oryzae* and *H. spinicaudata,* originating from Kandion Mangana, Casamance, Senegal, were cultured on rice (*Oryza sativa* L.) cv. Moroberekan under permanent water-saturated soil conditions. Nematodes extracted from roots in a mist chamber (Seinhorst, 1950) and from soil by elutriation (Seinhorst, 1962), were fixed overnight at 4 °C in glutaraldehyde 2.5 % in 0.1 M cacodylate buffer at pH 7.2. They were processed for infiltration and polymerisation in Spurr's (1969) medium as previously described (Mounport *et al.*, 1990). Ultrathin sections were cut with a diamond knife on a Sorvall Porter Blum MT1 ultramicrotome.

Two types of treatments were used for ultrastructural studies:

"Classical" treatment : ultrathin sections were collected on copper grids and stained, using uranyl acetate followed by lead citrate (Reynolds, 1963).

Cytochemical treatment: The method of Thiery and Rambourg (1974) for detecting carbohydrates was applied: it consists of three steps, separated by rinsing in distilled water (three changes of 5 min each). During the first step, gold grids with ultrathin sections were incubated in periodic acid-Schiff 1 % for 20 min at room temperature. During the second step, the grids were incubated in thiocarbazide (1 % in 20 % acetic acid) for 2 h, followed by rinsing in acetic acid in decreasing concentrations (20, 10, 5, 2, 1 %; 5 min each). During the last step, the grids were incubated in silver proteinate 1 % in distilled water for 30 min, then rinsed in three changes of distilled water (5 min each); they were not stained with uranyle acetate and lead citrate.

Protein digestion using pronase: gold grids with ultrathin sections were incubated (20 min) in a solution of periodic acid (10 %) at room temperature. They were rinsed in three changes of distilled water (5 min each), then incubated in pronase (0.5 % in phosphate buffer at pH 7.4) for periods of 30 min, 1 h, 4 h, 12 h, and 24 h. Grids were rinsed in distilled water and stained (Reynolds, 1963).

All grids were examined and photographed in a Siemens Elmiskop 101 or a Jeol 100CXII electron microscope operating at 80 kV.

Results

The cuticle

In *H. oryzae,* the thickness of the cuticle averages 0.6 µm in laterodorsal and lateroventral sectors, and 1.4 µm in the lateral fields; in *H. spinicaudata*, it averages 2 and 5 µm, respectively.

Longitudinal and cross sections showed that the cuticle consists of three major zones as defined by Bird and Bird (1991): *i*) a cortical zone consisting of an outer trilaminate layer and an inner granular layer; the trilaminate structure was not apparent in overstained sections (Fig. 1 A, B); *ii*) a median zone represented by a more or less electron-lucent vacuolar layer (Fig. 1 A, B); *iii*) a basal zone consisting of a striated layer that has a greater periodicity of striations in longitudinal than in cross sections (Fig. 1 A, B). The basal striated layer is attached to the somatic muscles by hemidesmosomes (Fig. 1 A).

These three zones were also observed in the lateral fields but with striking modifications in the median and basal zones, while the fine structure of the cortical zone is unchanged. The median zone consists of four layers: two vacuolar layers (an outer one and an inner one) separated by two granular layers (Fig. 1 C, D). The outer vacuolar layer is thin and the inner layer is thick beneath the two external bands of the lateral fields (Fig. 1 C, D) where it is electron-dense (Fig. 1 D) or electron-lucent (Fig. 1 C). The basal zone consists of two fibrillar layers instead of a single basal striated layer (Fig. 1 C, D).

The musculature

Cross sections showed that the somatic muscles of both species are of the platymyarian type (Figs 1 E, F; 2 A). Muscle cells consist of thick and thin myofilaments (not shown). The basement membrane of the muscle cells is attached to the cuticle by hemidesmosomes (Fig. 1 A). In cross sections of females and males (not shown) of both species, electron-lucent areas were visible between the myofilaments and the intestine (Fig. 1 E, F). In *H. spinicaudata* (Fig. 1 F), a cross section at the level of the anterior portion of the intestine showed these areas to occur in each laterodorsal and latero-ventral sectors and in the lateral field; in *H. oryzae* (Fig. 1 E), similar areas were visible in a cross section at the uterus level. Slightly electron-opaque ovoid granules (Fig. 1 E, F) occurred in these areas. In *H. spinicaudata* (Fig. 2 A, B), it was observed that these areas constitute the sarcoplasmic portion of muscle cells because they are surrounded by the lining of the basement membrane (Fig. 2 B). They obviously correspond to the so-called "Thornean cells" that lie between the muscles and the intestine (Sher, 1968; Luc, 1987). Ultrahistochemical studies consisting of pronase incubation of *H. oryzae* sections showed only slight points of digestion after 12 h (Fig. 2 C), which demonstrates that most of the content of the sarcoplasmic portion of muscle cells is not proteinaceous (control not shown).

The detection for carbohydrates in sections was positive for both *H. spinicaudata* (Fig. 2 D, E) and *H. oryzae*. In addition to the sarcoplasm, stained patches were seen in intestinal cells and lateral chords (Fig. 2 D, E). Detailed examination of the stained areas showed that they correspond to an accumulation of glycogen with typical aggregations of α particles (Fig. 2 E) consisting of β sub-units (not shown).

The ovoid granules observed in the sarcoplasm and in the intestinal cells were neither digested by pronase nor stained as carbohydrates. They appeared to be lipid droplets (Fig. 2 A, E).

Discussion

The structure of the cuticle outside of the lateral fields agrees with previous studies of other members of the Pratylenchidae such as *Pratylenchus* spp. (Kisiel *et al.*, 1972; Mounport *et al.*, 1990), *H. belli* and *H. gracilis* (Johnson *et al.*, 1970), and *Radopholus similis* (Valette *et al.*, 1997). The same pattern also occurs in the family Belonolaimidae, in some species of the

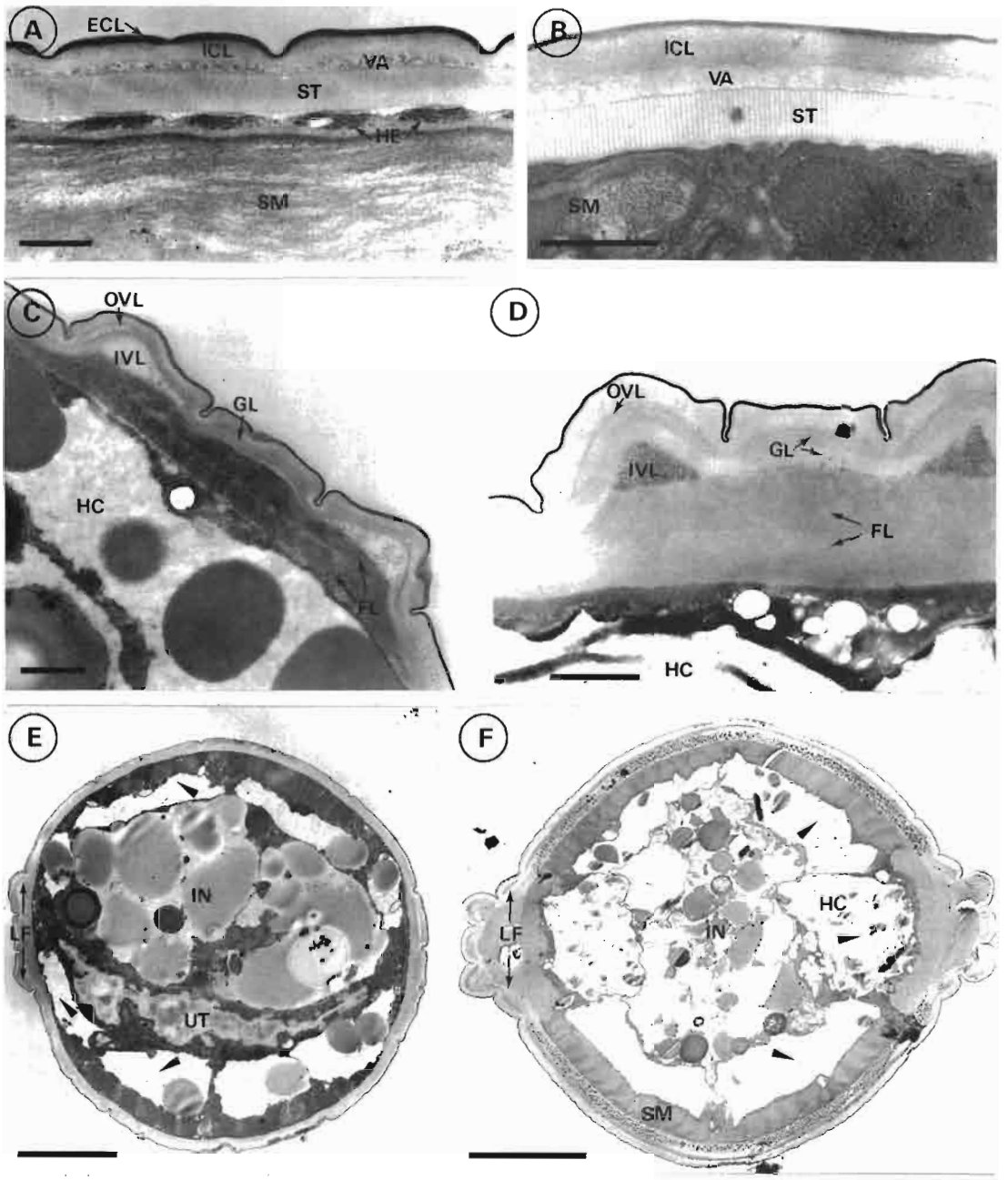

Fig. 1. *Ultrastructure of the body wall of* Hirschmanniella oryzae *(A-C, E) and* H. spinicaudata *females (D, F) in longitudinal (LS) and cross (CS) sections. A: Laterodorsal sector (LS); B: Laterodorsal sector (CS); C: CS of body at uterus level; D: CS of body in anterior intestine region showing granular layers in the median zone of the cuticle; E : CS of body showing electron-lucent areas (arrowhead) in the body wall; F: Electron-lucent areas (arrowheads) in the latero-dorsal and latero-ventral sectors, and the lateral fields. (Scale bars: A, B = 0.5 µm; C = 1 µm; D = 2 µm; E, F = 10 µm. Abbreviations. BM: basement membrane; CU: cuticle; ECL: external cortical layer; FL: fibrillar layers; FSM: fibrillar portion of somatic muscles; GL: granular layers; GP: glycogen particles; HC: hypodermal chord; HE: Hemidesmosomes; ICL: internal cortical layer; IN: intestine; IVL: inner vacuolar layer; LD: lipid droplets; LF: lateral field; OVL: outer vacuolar layer; SM: somatic muscles; SSM: sarcoplasmic portion of somatic muscles; ST: striated layer in basal zone; UT: uterus; VA: vacuolar layer in median zone).*

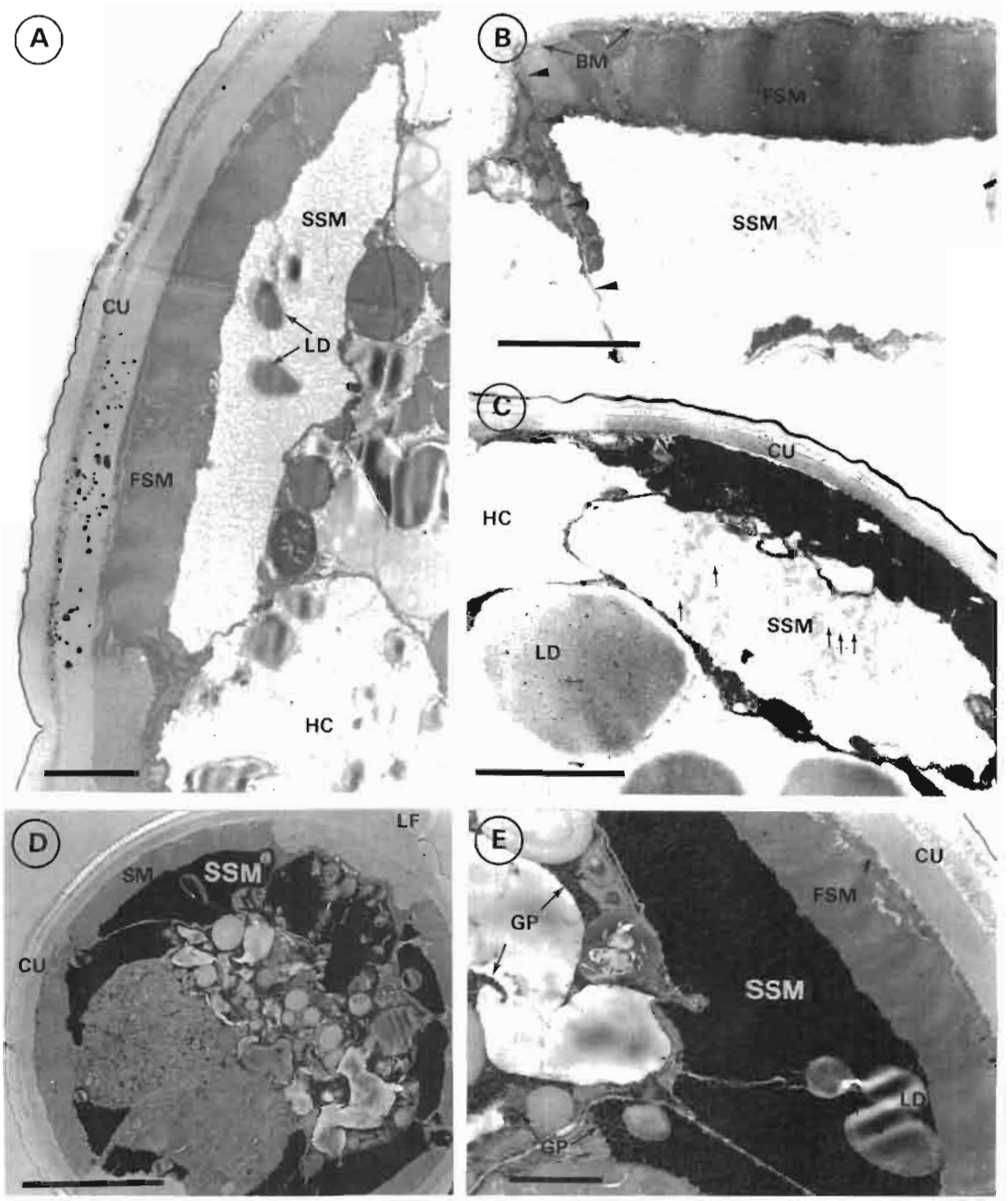

Fig. 2. *Ultrastructure of the body wall of* Hirschmanniella oryzae *(C) and* H. spinicaudata *(A, B, D, E) females in cross sections. A: Lateroventral sector showing the electron-lucent sarcoplasmic part of a muscle field; B: Magnification of a lateral portion of a muscle cell showing its basement membrane (arrowheads); C: Laterodorsal sector after 12 h pronase incubation showing slight digestion points (arrows); D: Stained section showing accumulation of polysaccharides; E: Magnification of a latero-ventral sector showing typical aggregation of glycogen particles. (Scale bars: A, B, C, E = 2 µm; D = 10 µm. For abbreviations, see Fig. 1).*

genus *Tylenchorhynchus* (Ibrahim, 1967; Byers & Anderson, 1972; Mounport *et al.*, 1993 *a*). In the family Tylenchidae, the median zone was lacking in the species examined (Mounport *et al.*, 1993 *c*). Observations of several genera in the Hoplolaimidae revealed a more complex ultrastructure with additional fibrillar layers in the basal zone of the cuticle (Durnez *et al.*, 1973; Mounport *et al.*, 1991, 1993 *b*).

The ultrastructure of the cuticle beneath the lateral fields shows, however, some peculiar features: in the present study, four layers exist in the median zone, as in *R. similis* (Valette *et al.*, 1997), whereas there are only three layers in *Pratylenchus* spp. (Mounport *et al.*, 1990). In the genus *Tylenchorhynchus*, supplementary layers of granular and vacuolar appearance occur, but only in the basal zone of the cuticle beneath the lateral fields (Mounport *et al.*, 1993 *a*).

The hypertrophy of the sarcoplasm of muscles cells with large amounts of glycogen is striking. Similar observations have not been made so far in any other phytoparasitic nematodes, even those living in the same biotope as that occupied by *Hirschmanniella* spp. Luc (1987) pointed out that, in the genus *Hirschmanniella,* "all species are found in marshy places or aquatic habitats"; we suggest that the unusual accumulation of glycogen in muscle cells might result from an adaptation of the nematodes to oxygen-limiting conditions in marshy soils. However, the lack of these accumulation of glycogen in other phytoparasitic nematodes that live in the same biotopes does not corroborate our hypothesis. Considering that, *i*) in *Hirschmanniella* spp., "three [species] are marine which is a rare case among Tylenchina" (Luc, 1987), and *ii*) important glycogen accumulation in muscle cells is known to be an adaptation of marine invertebrates (Hochachka, 1985), it may be deduced that *Hirschmanniella* spp. were originally mainland phytoparasitic nematodes that began colonising marshy places and then marine-influenced areas such as estuaries or mangrove swamps.

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