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OBSERVATIONS ON THE CUTICLE ULTRASTRUCTURE IN THE HOPLOLAIMINAE (NEMATA: HOPLOLAIMIDAE)

BY

D. MOUNPORT[1]), P. BAUJARD[2]) and B. MARTINY[2])

[1]) Département de Biologie Animale, Faculté des Sciences, Université Cheikh Anta Diop, Dakar, Sénégal; [2]) Laboratoire de Nématologie, Centre Orstom, B.P. 1386, Dakar, Sénégal

The fine structure of the cuticle of *Aorolaimus macbethi, Aphasmatylenchus straturatus, A. variabilis, Helicotylenchus dihystera, H. multicinctus, Hoplolaimus pararobustus, H. seinhorsti* and *Pararotylenchus hopperi* is described. Six layers are identified in *Aphasmatylenchus, Helicotylenchus* and *Pararotylenchus* species *vs* seven in *Hoplolaimus* and *Aorolaimus* species. The ultrastructure of the five outer layers of the cuticle is identical in all species and consists of an external cortex, an internal cortex, a granular or fibrillar layer with electron dense ovoid structures, a striated layer and an electron dense fibrillar layer; the basal zone of the cuticle consists of a thin electron-lucent layer in *Helicotylenchus* and *Pararotylenchus* species and a thick electron-lucent layer representing half of the total thickness of the cuticle in *Aphasmatylenchus* species; in *Hoplolaimus* and *Aorolaimus* species, two layers are present: a thin electron-dense layer consisting of densely packed osmophilic corpuscules and a thick electron-lucent layer. Intracuticular canals previously described in other genera in the subfamily occur in all species studied and may be considered constant in Hoplolaiminae; observations on lateral fields in cross section reveal a variability of their shape and of the deepness of incisures. Three major groups in the subfamily may be distinguished based on ultrastructure and relative thickness of the layers of the cuticle: *i) Hoplolaimus, Scutellonema* and *Aorolaimus, ii) Pararotylenchus* and *Helicotylenchus, iii) Aphasmatylenchus* and *Rotylenchus*. Cuticle ultrastructure in Hoplolaiminae appears totally different from that observed in other taxonomic groups of the order Tylenchida.

Keywords: cuticle, ultrastructure, Hoplolaiminae, taxonomy

Cuticle ultrastructure of Hoplolaiminae has been studied in three genera: *Hoplolaimus columbus* (Lewis & Huff, 1976), *Rotylenchus robustus* (Durnez *et al.,* 1975) and *Scutellonema* species (De Grisse & Roose, 1975; Wang & Chen, 1982; Mounport *et al.,* 1991). Seven cuticular layers have been identified in *Hoplolaimus* and *Scutellonema* species *vs* six in *Rotylenchus robustus*. This paper presents observations on the ultrastructure of the cuticle in eight species belonging to four other genera of the subfamily.

MATERIALS AND METHODS

Species used in this study originated from different locations in West Africa, except *Pararotylenchus hopperi* Baldwin & Bell, 1984 paratypes which were obtained from Dr. J. G. Baldwin and M. Mundo-Ocampo (USA). They were cultured on different plants in the laboratory since the sampling date indicated in Table I.

TABLE I

Origins of the species

Species	Sampling location and date	Cultured on
Hoplolaimus pararobustus Schuurmans, Stekhoven & Teunissen, 1938	NDindy, Senegal 1982	*Pennisetum typhoides*
Hoplolaimus seinhorsti Luc, 1958	Martinique 1989	*Sorghum vulgare*
Aorolaimus macbethi Sher, 1964	Dombe Senegal 1984	*Pennisetum typhoides*
Aphasmatylenchus variabilis Germani & Luc, 1984	Sine Saloum, Senegal 1988	*Pennisetum typhoides*
Aphasmatylenchus straturatus Germani, 1970	Niangoloko, Burkina 1989	*Arachis hypogaea*
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961	NDindy, Senegal 1984	*Pennisetum typhoides*
Helicotylenchus multicinctus (Cobb, 1893) Golden, 1956	Bula, Bissau Guinea	*Vigna unguiculata*
Pararotylenchus hopperi Baldwin & Bell, 1984	—	

Nematodes were extracted from soil using Seinhorst's (1962) elutriation technique. Adult specimens were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7,2; they were then cut into at least three pieces, rinsed in buffer alone and post-fixed in 1% osmium tetroxide in the same buffer for 2 h; pieces were dehydrated in a graded ethanol series followed by two changes of propylene oxide and embedded in low viscosity epoxy resin (Spurr, 1969). Thin sections were cut with a diamond knife on a Sorvall MT1 Porter Blum or a Reichert-Jung E ultramicrotome, stretched with xylene vapour and stained on copper grids with aqueous uranyl acetate followed by lead citrate (Reynolds, 1963). Grids were examined with a Jeol 100 CXII or a Siemens Elmiskop 101 electron microscope operating at 80 Kv.

RESULTS

Longitudinal and cross sections of about ten specimens of each species revealed seven cuticular layers in *Hoplolaimus* and *Aorolaimus* species *vs* six in the other species; cuticle thickness ranges from 0.74 µm in *Helicotylenchus multicinctus* to 2.40 µm in *Aphasmatylenchus variabilis* (Table II). The ultrastructure of the five outer layers in all species is identical; in a centripetal direction can be distinguished:

— an external cortex, trilaminate appearing as two osmophilic sublayers separated by a non-osmophilic one (Figs 1F, 2F, 3E).

— an internal cortex with a granular appearance; in all species the outer edge seems to be stratified (Figs 3E, 4B); longitudinal incisures in *A. straturatus* are confined to this layer (Fig. 1C).

TABLE II

Measurements (μm) of cuticle layer thickness at mid-body in some of the hoplolaimid species studied. The percentage of the total thickness for each layer is given in brackets

Layers	Nematode species				
	Helicotylenchus multicinctus	*Pararotylenchus hopperi*	*Aphasmatylenchus variabilis*	*Aorolaimus macbethi*	*Hoplolaimus pararobustus*
External cortex	0.035 (4.8)	0.040 (4.0)	0.050 (2.1)	0.040 (3.0)	0.050 (2.0)
Internal cortex	0.150 (20.5)	0.230 (23.0)	0.100 (4.2)	0.110 (8.0)	0.120 (6.9)
Vacuolar layer	0.150 (20.5)	0.170 (17.0)	0.760 (31.7)	0.220 (16.0)	0.360 (20.8)
Striated layer	0.190 (25.7)	0.300 (30.0)	0.330 (13.8)	0.260 (19.0)	0.300 (17.3)
Fibrillar layer	0.020 (2.7)	0.060 (6.0)	0.050 (2.1)	0.050 (3.6)	0.120 (6.9)
Osmophilic corpuscules layer	absent (-)	absent (-)	absent (-)	0.050 (3.6)	0.060 (3.4)
Basal layer	0.190 (25.8)	0.190 (19.0)	1.100 (45.8)	0.640 (46.7)	0.720 (41.5)
Total thickness	0.740	1.00	2.400	1.370	1.800

— a vacuolar layer consisting of electron-dense ovoid structures in a granular matrix; in *Aphasmatylenchus* species we observed a particular pattern: the layer appears as a thick fibrillar matrix with electron-dense structures located at its base; radial projections of these structures can be observed in the upper area of the layer (Fig. 1E, F); the matrix in tangential sections appears as cylindric electron-lucent structures arranged in five rows under each annulation (Fig. 1G).

— a striated layer consisting of alternate osmophilic and non-osmophilic thin bands (Figs 1E, 2F, 3C); the frequency of the radial bands is greater in longitudinal than in cross sections.

— an electron-dense fibrillar layer whose thickness in longitudinal sections may vary particularly in *Hoplolaimus* and *Aorolaimus* species (Fig. 4B).

The ultrastructure of the basal zone of the cuticle is variable:

i) in *Aphasmatylenchus* species we observed only one electron-lucent layer consisting of thick fibres (Fig. 1C, H); the thickness of the layer is about half of the whole cuticle;

ii) in *Helicotylenchus* and *Pararotylenchus* species, an electron-lucent layer with thin fibres was observed; it represents approximately a quarter of the cuticle thickness (Figs 2E, 3C);

iii) in *Hoplolaimus* and *Aorolaimus* species two layers are present: a layer of densely packed osmophilic corpuscules (Fig. 4C, E) and a thick electron-lucent layer identical to the basal layer observed in *Aphasmatylenchus* species; in tangential sections the basal layer consists of thick crossing fibres (Fig. 4F).

The basal layer of the cuticle in all species is attached to underlying somatic muscles by desmosomoids (Figs 2E, 3D). Two sets of intracuticular canals occur in all species: the first set connects the vacuolar layer (layer 3) and the electron-dense fibrillar layer (layer 5) passing through the striated layer (Figs 1F, 2E, 3D,

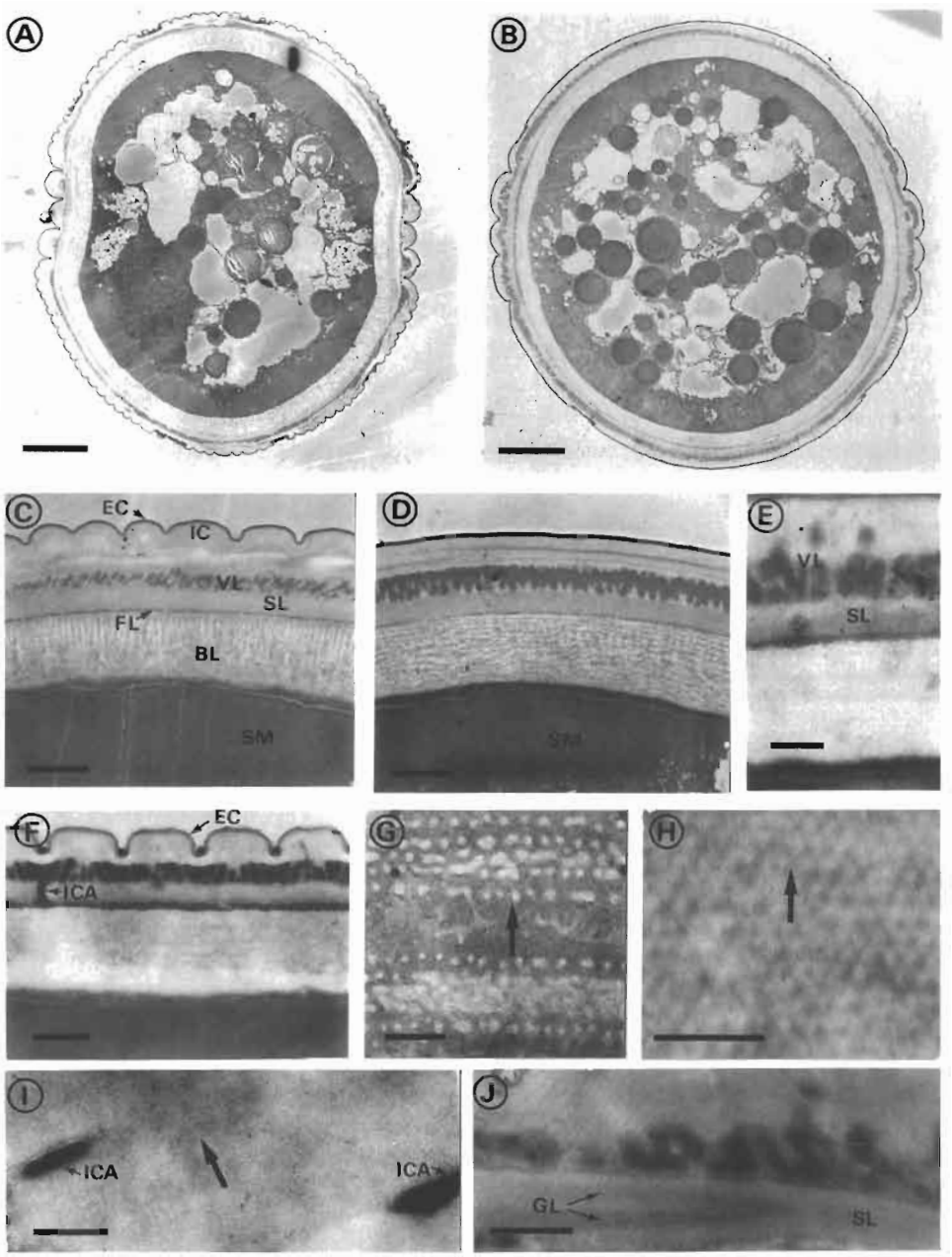

Fig. 1. Ultrastructure of the cuticle of *Aphasmatylenchus strauratus* (A, C) and *A. variabilis* (B, D-J) females in longitudinal (LS), cross (CS) and tangential (TS) sections. A, B: CS of entire female; C: CS at the level of incisures; D, E: CS; F: LS; G, H, I: respectively TS of vacuolar, basal and striated layers (arrow indicates longitudinal axis of the nematode); J: CS at the level of an external incisure of a lateral field. Bar = 5 μm (A, B), 1 μm (C, D & F), 0.5 μm (E, G-J). Abbreviations: BL, basal layer; EC, external cortex; FL, fibrillar layer; GL, granular layers; IC, internal cortex; ICA, intracuticular canals; SL, striated layer; SM, somatic muscles; VL, vacuolar layer.

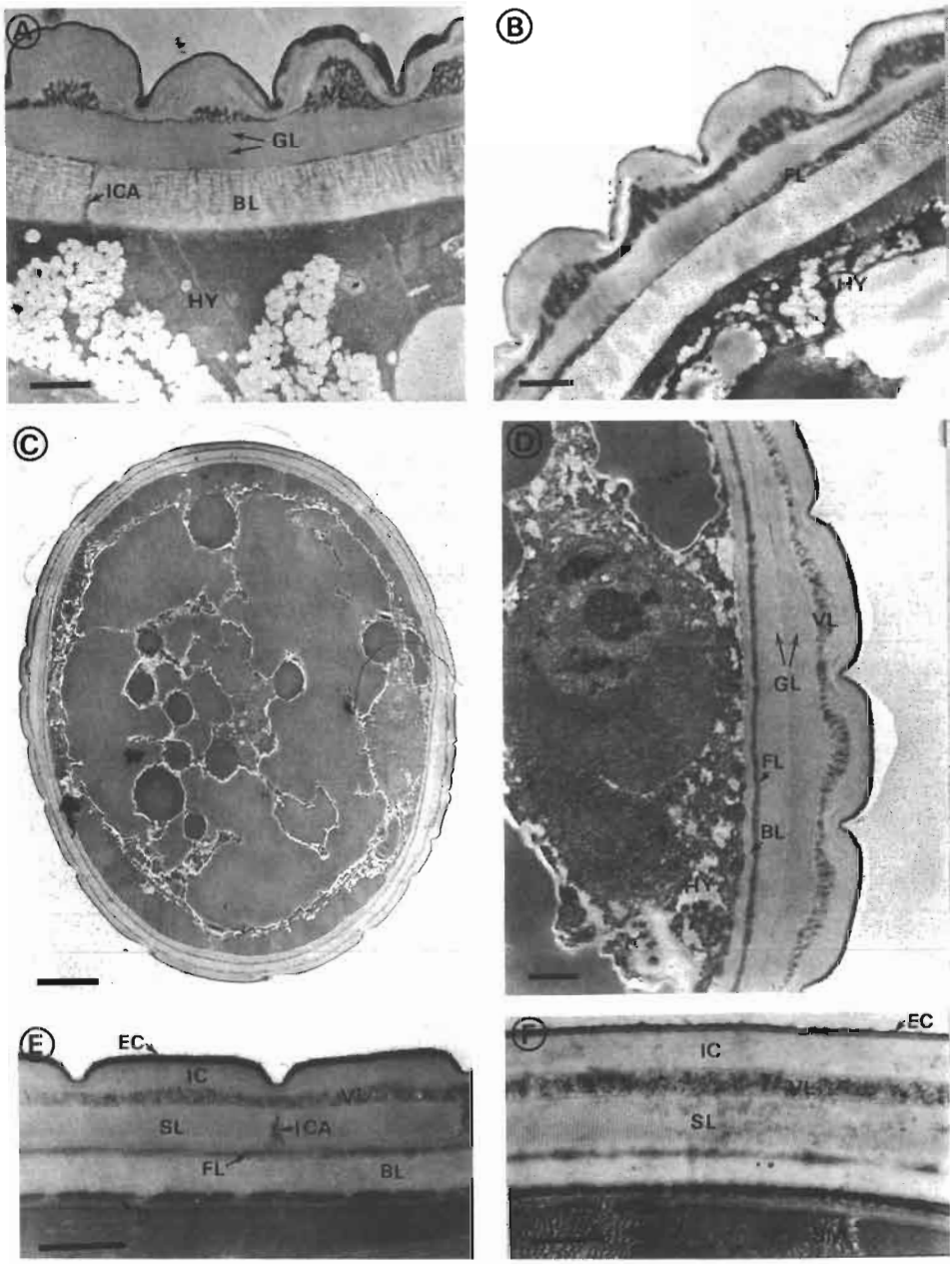

Fig. 2. Ultrastructure of the cuticle of *Aphasmatylenchus straturatus* (A), *A. variabilis* (B) and *Pararotylenchus hopperi* (C-F) females in longitudinal (LS) and cross (CS) sections. A, B, D: CS of a lateral field; C: CS of entire female; E, F: respectively LS and CS at mid-body. Bar = 5 µm (C), 1 µm (A, B, D) and 0.5 µm (E, F). Abbreviations: BL, basal layer, D, desmosomoid; EC, external cortex; FL, fibrillar layer; GL, granular layers; HY, hypodermis, IC, internal cortex; ICA, intracuticular canals; SL, striated layer; SM, somatic muscles; VL, vacuolar layer.

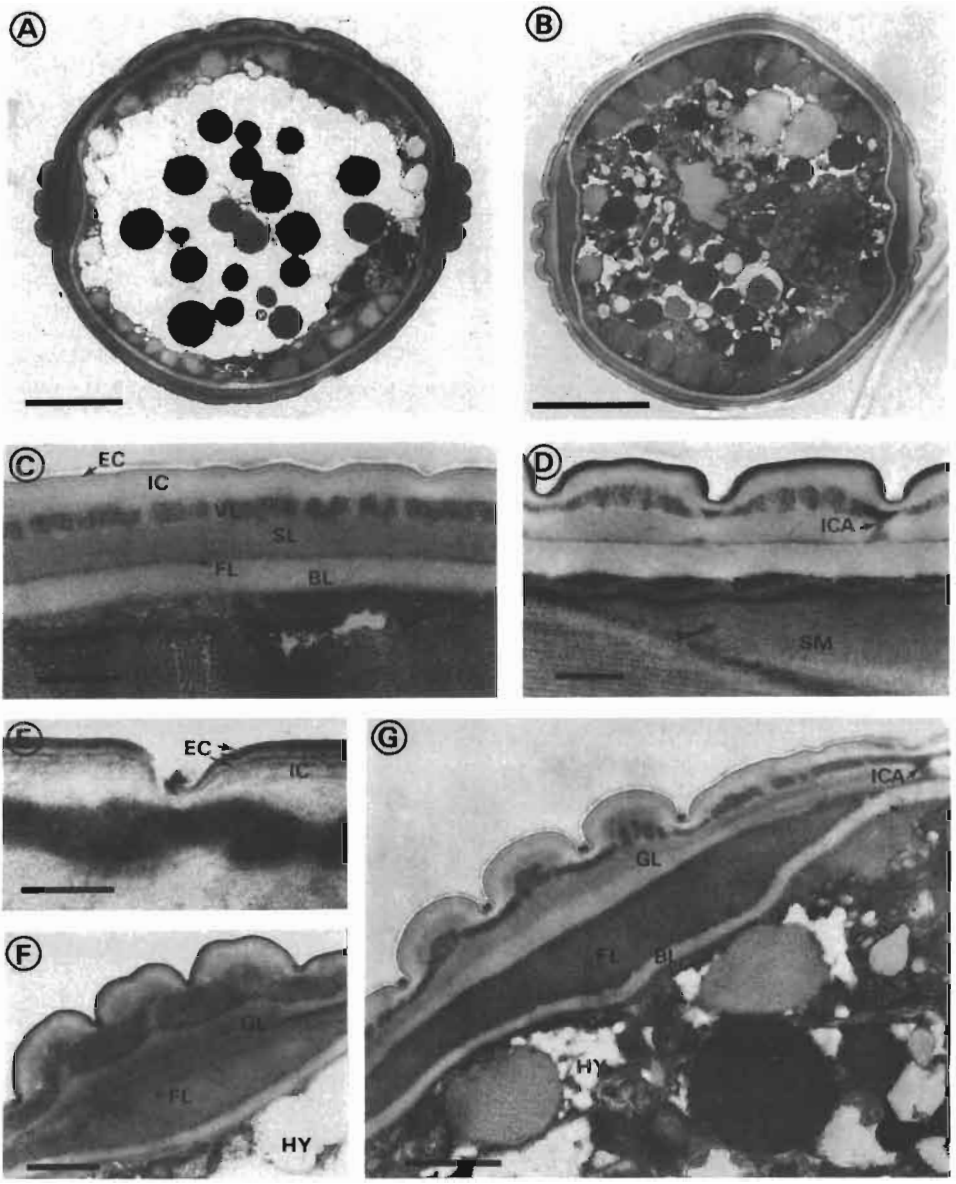

Fig. 3. Ultrastructure of the cuticle of *Helicotylenchus dihystera* (A, E, F) and *Helicotylenchus multicinctus* (B-D, G) females in longitudinal (LS) and cross (CS) sections. A, B: CS of entire female; C: CS at mid-body; D, E: LS at mid-body; F, G: CS of the lateral field. Bar = µm (A, B), 1 µm (F, G), 0.5 µm (C, D) and 0.25 µm (E). Abbreviations: BL, basal layer; D, desmosomoid; EC, external cortex; FL, fibrillar layer; GL, granular layers; HY, hypodermis; IC, internal cortex; ICA, intracuticular canals, SL, striated layer; SM, somatic muscles; VL, vacuolar layer.

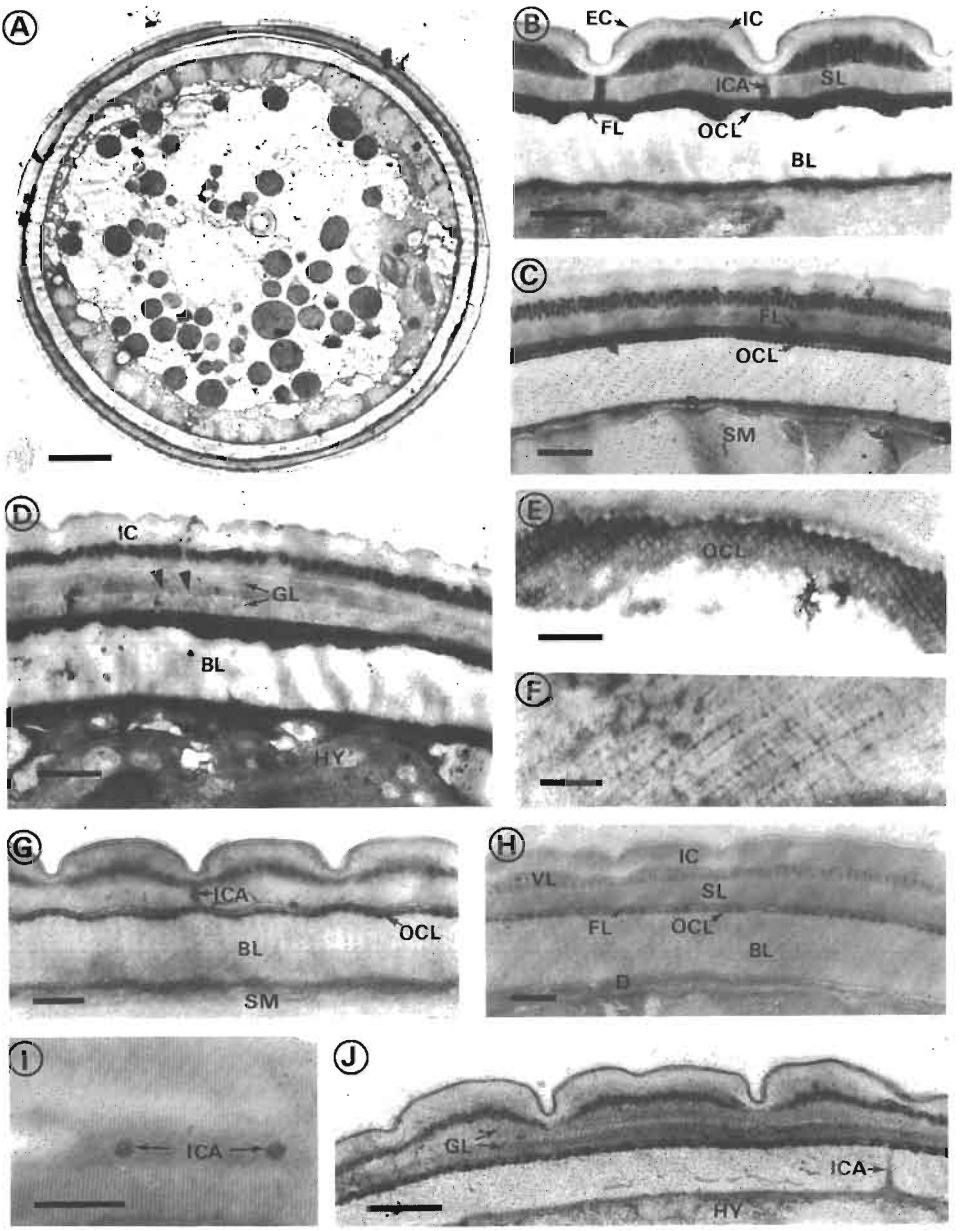

Fig. 4. Ultrastructure of the cuticle of *Hoplolaimus pararobustus* (A-F) and *Aorolaimus macbethi* (G-J) females in longitudinal (LS), cross (CS) and tangential (TS) sections. A: CS of entire female; B, C: respectively LS and CS at mid-body; D: CS of lateral field; E, F: respectively TS of osmophilic corpuscules layer and basal layer; G, H: respectively LS and CS at mid-body; I: TS of the striated layer; J: CS of a lateral field. Bar = 5 µm (A), 1 µm (B-D, J) and 0.5 µm (E-I). Abbreviations: BL, basal layer; D, desmosomoid; EC, external cortex; FL, fibrillar layer; GL, granular layers; HY, hypodermis; IC, internal cortex; ICA, intracuticular canals; OCL, osmophilic corpuscules layer; SL, striated layer; SM, somatic muscles; VL, vacuolar layer.

4B); sections of these canals appear circular (Fig. 4I) in all species except in *Aphasmatylenchus* species where sections are elliptical (Fig. 1I); the second set of canals connects the electron-dense fibrillar layer (layer 5) to the hypodermis (Figs 2A, 4J). At the level of the lateral fields, the striated layer becomes forked in cross section (Fig. 1J); it is replaced by two electron-lucent granular layers. In *Hoplolaimus* species, the two granular layers are separated by an electron-lucent fibrillar layer (Fig. 4D). An important thickening of the lateral fields cuticle occurs in *Helicotylenchus* species (Fig. 3A, B) and the electron-dense fibrillar layer (layer 5) gets thicker under lateral fields (Fig. 3F, G). Longitudinal incisures are particularly deep in *Helicotylenchus* and *Aphasmatylenchus* species (Figs 1A, 1B, 3A, 3B).

DISCUSSION

Eight genera are recognised in Hoplolaiminae (Fortuner, 1987); this paper details the fine structure of the cuticle of seven genera in the subfamily. Cuticle ultrastructure of *Hoplolaimus* and *Aorolaimus* species corresponds with what was observed previously in *Hoplolaimus columbus* (Lewis & Huff, 1976) and *Scutellonema* species (De Grisse & Roose, 1975; Wang & Chen, 1982; Mounport *et al.*, 1991). These three genera are distinct from the others by the presence of an electron-dense layer (layer 6) consisting of densely packed corpuscules. The ultrastructure of the cuticle of *Aphasmatylenchus* species is in agreement with previous observations on *Rotylenchus robustus* (Durnez *et al.*, 1973). *Helicotylenchus* and *Pararotylenchus* species are distinct from all other genera: the basal layer of the cuticle is proportionately thinner and consists of thinner fibres without any particular orientation; fibres in other genera are thick and form a crossing network in the basal part of the cuticle. Three major groups may be identified in the subfamily depending on ultrastructure and relative thickness of the basal layer of the cuticle:

— *Aorolaimus* sp., *Hoplolaimus* sp. and *Scutellonema* sp.: seven-layered cuticle; lateral field incisures not deep when present; basal layer with thick fibres representing one half of cuticle thickness.

— *Aphasmatylenchus* sp. and *Rotylenchus* sp.: six-layered cuticle; lateral fields with deep longitudinal incisures; basal layer with thick fibres representing one half of cuticle thickness.

— *Helicotylenchus* sp. and *Pararotylenchus* sp.: six-layered cuticle; lateral field incisures deep in *Helicotylenchus* sp.; basal layer with thin fibres representing less than a quarter of cuticle thickness.

Intracuticular canals previously described in *Rotylenchus robustus* (Durnez *et al.*, 1973) and *Scutellonema* species (Mounport *et al.*, 1991) occur in all species that we have studied; they suggest the possibility of exchange of material between cuticle layers and hypodermis.

These observations on cuticle ultrastructure in Hoplolaiminae reveal the following constant features: *i)* the number of layers (6-7 or 8-9 if the three

sublayers of the external cortex are counted) which distinguishes Hoplolaiminae from all families or subfamilies previously studied in Tylenchida; three layers are observed in Aphelenchinae (Johnson *et al.*, 1970), or Anguinidae (Yuen, 1967); four layers in Pratylenchidae (Mounport *et al.*, 1990) or Telotylenchinae (Ibrahim, 1967; Byers & Anderson, 1972); four or five layers in Heteroderidae (Cliff & Baldwin, 1985); *ii)* the presence of intracuticular canals; they may be considered constant in the subfamily.

At the subfamily level, cuticle ultrastructure is a criterion that allows determination at the level of groups of genera. Further studies on more species in each genus including range of cuticle and layer thickness may improve discrimination at the generic level.

RÉSUMÉ

Observations sur l'ultrastructure de la cuticule des Hoplolaiminae (Nemata: Hoplolaimidae)

L'ultrastructure de la cuticule est décrite chez *Aorolaimus macbethi, Aphasmatylenchus straturatus, A. variabilis, Helicotylenchus dihystera, H. multicinctus, Hoplolaimus pararobustus, H. seinhorsti* and *Pararotylenchus hopperi*. Six couches sont identifiées chez les espèces des genres *Aphasmatylenchus, Helicotylenchus* et *Pararotylenchus vs.* sept dans les genres *Hoplolaimus* et *Aorolaimus*. L'ultrastructure des cinq couches externes de la cuticule est identique chez toutes les espèces étudiées et consiste en une couche corticale externe, une couche corticale interne, une couche granuleuse ou fibrillaire renfermant des structures ovoïdes denses aux électrons, une couche striée et une couche fibreuse opaque aux électrons; la zone basale de la cuticule consiste en une couche fine peu dense aux électrons dans les genres *Helicotylenchus* et *Pararotylenchus* et en une couche épaisse peu dense aux électrons représentant la moitié de l'épaisseur totale de la cuticule dans le genre *Aphasmatylenchus*; chez les espèces des genres *Hoplolaimus* et *Aorolaimus*, deux couches sont présentes: une couche fine et dense aux électrons formée de corpuscules agglomérés et une couche épaisse peu dense aux électrons. Des canaux intracuticulaires précédemment décrits dans d'autres genres de la sous-famille apparaissent dans toutes les espèces étudiées et peuvent être considérés comme constants dans ce groupe; les observations faites sur les champs latéraux en coupe transversale révèlent une variabilité de leur forme et de la profondeur des incisures. Trois grands groupes peuvent être formés dans la sous-famille des Hoplolaiminae en relation avec l'ultrastructure et l'épaisseur relative des couches de la cuticule: *i) Hoplolaimus, Scutellonema* et *Aorolaimus, ii) Pararotylenchus* et *Helicotylenchus, iii) Aphasmatylenchus* et *Rotylenchus*. L'ultrastructure de la cuticule chez les Hoplolaiminae apparait totalement différente de celle observée jusqu'ici dans d'autres groupes taxonomiques des Tylenchida.

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