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# Ultrastructural observations on the cuticle of *Radopholus similis* (Nematoda: Pratylenchidae)

Catherine VALETTE*, Pierre BAUJARD**, Michel NICOLE***, Jean-Louis SARAH*, and Danamou MOUNPORT****

**CIRAD-FLHOR, B.P. 5035, 34032 Montpellier, France,*
*** Muséum National d'Histoire Naturelle, Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, 61 rue Buffon, 75005 Paris, France,*
**** ORSTOM, Laboratoire de Phytopathologie, B.P. 5045, 34032 Montpellier cedex, France, and*
*****UCAD, Faculté des Sciences, Département de Biologie Animale, Dakar, Sénégal.*

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**Summary** - The fine structure of the cuticle of males and females of *Radopholus similis* is described. In latero-dorsal and latero-ventral fields, the cuticle consisted of: *i*) a cortical zone with an external trilaminate layer and an internal granular one; *ii*) a median zone represented by an electron-dense vacuolar layer, and *iii*) a basal zone with a striated layer that averaged half of the total cuticle thickness. Beneath the lateral fields, the fine structure of the cortical zone remained inchanged; the median zone consisted of four layers, two vacuolar layers separated by two granular layers; and the basal zone was composed of two thick fibrous layers instead of the striated layer. These results agree with previous observations on *Hirschmanniella oryzae* and *H. spinicaudata*; the ultrastructural differences between these species and taxa belonging to the genus *Pratylenchus* or other phytoparasitic nematodes in Tylenchina are discussed.

**Résumé** - ***Observations ultrastructurales de la cuticule de* Radopholus similis *(Nematoda : Pratylenchidae)*** - L'ultrastructure de la cuticule des mâles et des femelles de *Radopholus similis* est décrite. Dans les champs latéro-dorsaux et latéro-ventraux, trois zones ont été observées : *i*) une zone corticale constituée d'une couche externe trilamellaire et d'une couche interne d'aspect granuleux, *ii*) une zone médiane représentée par une couche vacuolaire plus ou moins opaque aux électrons, et *iii*) une zone basale apparaissant sous la forme d'une couche striée dont l'épaisseur atteint la moitié de celle de l'ensemble de la cuticule. Dans les champs latéraux, l'ultrastructure de la zone corticale reste inchangée ; la couche médiane vacuolaire est remplacée par quatre couches, dont deux vacuolaires séparées par deux couches d'aspect granuleux; la couche basale striée est remplacée par deux couches épaisses d'aspect fibreux. Ces observations sont identiques à celles décrites chez *Hirschmanniella oryzae* et *H. spinicaudata*; elles sont, par contre, différentes des résultats obtenus chez diverses espèces du genre *Pratylenchus*. L'ultrastructure des champs latéraux chez ces genres appartenant aux Pratylenchidae est comparée à celles des nématodes phytoparasites d'autres familles du sous-ordre des Tylenchina.

**Key-words:** cuticle, nematode, Pratylenchidae, *Radopholus similis*, systematics, ultrastructure.

Light microscopy observations of the cuticle of plant parasitic nematodes including length of body annules at mid body, depth of incisures between body annules, layering of the cuticle, and ornamentation of the lateral fields are used for taxonomy (Siddiqi, 1986). Scanning electron microscopy (SEM) observations have shown that head face view, number and morphology of cephalic annules, as well as stylet and spicule morphology are also useful taxonomic criteria (Eisenback *et al.*,1980; Corbett & Clark, 1983; Baujard *et al.*, 1991). Transmission electron microscopy (TEM) has provided additional insight into the nematode organisation, including the organization of cuticle, digestive system, and the genital tract. Cuticle ultrastructure which has been studied in numerous taxa of plant-parasitic nematodes, has been useful for determing taxonomic relationships, and has contributed to new hypothesis of phylogeny as a basis for classification (Cliff & Baldwin, 1985; Baldwin & Schouest, 1990; Mounport *et al.*, 1993*a*). In the family Pratylenchidae, cuticle ultrastructure has been investigated so far only in two genera which are both characterized by weak sexual dimorphism, *Pratylenchus* (Kisiel *et al.*, 1972 ; Mounport *et al.*, 1990) and *Hirschmanniella* (Johnson *et al.*, 1970 ; Mounport *et al.*, 1995). In contrast, the genus *Radopholus* is characterized by strong sexual dimorphism but its cuticle is poorly known. The present study compares *Radopholus similis* with other taxa in the family Pratylenchidae, and in other families within the suborder Tylenchina.

## Materials and methods

Two populations of *R. similis* (Cobb, 1893) Thorne, 1949 were studied: *i*) a population collected in 1990

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at Bula (Guinea-Bissau) was reared in sterilised soil on cowpea (*Vigna unguiculata* [L.] Walp.) in Dakar (Senegal); nematodes extracted from soil and roots were prepared for TEM observations as previously described (Mounport *et al.*, 1990) ; *ii*) a second population was collected in April 1991 at Anguédédou (Ivory Coast) and reared on carrot disks (Sarah *et al.*, 1993) and vitro plants of banana (cvs Poyo and Yangambi); nematodes extracted from carrot disks were prepared according to Valette *et al.* (1997). Ultrathin sections were obtained with a Reichert OM2 ultramicrotome and observations were made with a Jeol 100EX microscope using 80 kV accelerating voltage.

## Results

Ultrathin cross sections of *R. similis* (Fig.1 A, B) show sexual dimorphism in the ratio of cuticle thickness to body diameter, 1/28 and 1/30 for males and females, respectively, and in the lateral fields which are prominent in females and non prominent in males (Fig.1 A, B). Additional incisures occur at the bottom of the external bands of the lateral fields in females (Fig.1 A, G).

The cuticle thickness is 0.55 and 0.40 µm in females and males, respectively. It is divided into three zones (cortical, median, and basal) in longitudinal, cross and tangential sections. Major differences are visible in the cuticle ultrastructure between dorso-ventral and lateral fields.

### Cuticle ultrastructure in dorso-ventral fields

The cortical zone is composed of two layers, an external trilamellate layer (not visible in overstaining sections), and an internal granular layer, which appears as two electron-dense sublayers separated by an electron-light layer, 0.18 µm thick (Fig. 1D). The median zone consists of a 0.06 µm thick, electron-dense, vacuolar layer. Slight thickenings of the vacuolar layer occur between annules. The vacuolar layer of specimens extracted from banana roots (Fig.1 C) is constantly thinner than that of other specimens (Fig.1 D). The basal zone consist of a 0.27 µm thick striated layer representing 50% of the total cuticle thickness; the periodicity of the striations is greater when viewed on cross sections than on longitudinal sections; the basal striated layer is attached to somatic muscles by hemidesmosomes (Fig.1 E).

### Cuticle ultrastructure in the lateral fields

The cuticle thickness averages 1.60 and 0.95 µm in females and males, respectively. The organisation of the cortical layer is similar to that observed outside the lateral fields, with two layers: an external trilamellate layer (Fig.1 F) bordered by a limiting membrane and an internal granular cortical layer (Fig.1 F, G). The median zone consists of four layers, *i.e.*, two vacuolar layers separated by two granular layers (Figs 1 F, G; 2 A-D). The vacuolar layers may appear electron-opaque (Fig. 1G) or electron-light (Figs 1 F; 2 A, D). The external vacuolar layer is usually thin and continuous (Figs 1 F, G; 2 A-D), but it may be discontinuous in some cases (Fig. 2 B). The internal vacuolar layer is thicker under the external bands of the lateral fields (Figs 1 F, G; 2 A-D) and very thin under the central band (Fig. 2 B, D). The basal zone is 0.65 µm thick and it consists of two fibrous layers that originate from the striated layer under the external incisures of the lateral fields (Fig. 2 B).

## Discussion

### The cuticle in the dorso-ventral fields

The ultrastructure of the cuticle in *R. similis* is identical to the cuticle ultrastructure described for various species in Pratylenchidae, including *Pratylenchus penetrans* (Kisiel *et al.*, 1972), *P. brachyurus, P. loosi* and *P. pseudopratensis* (Mounport *et al.*, 1990), *Hirschmanniella belli* and *H. gracilis* (Johnson *et al.*, 1970), and *H. oryzae* and *H. spinicaudata* (Mounport *et al.*, 1995). Similar observations were made for eleven species of the genus *Tylenchorhynchus* in the family Belonolaimidae (Ibrahim, 1967; Byers & Anderson, 1972; Mounport *et al.*, 1993*a*). This organisation of the cuticle of *R. similis* into three different zones differs from that observed in Tylenchidae (Mounport *et al.*, 1993*c*) in which the median zone is absent. It also differs from that of Hoplolaimidae which includes two or three supplementary layers in the basal zone (Mounport *et al.*, 1991, 1993*b*).

### The cuticle in the lateral fields

Observations of lateral fields with TEM showed strong modifications in the cuticle ultrastructure between the external incisures in Tylenchidae, Pratylenchidae, Belonolaimidae and Hoplolaimidae (Mounport *et al.*, 1990, 1991, 1993*a, b, c*). These modifications are quite variable. For example, in Tylenchidae, no median zone exists and the basal striated layer is replaced by two fibrous layers (Mounport *et al.*, 1993*c*) whereas in Pratylenchidae, especially in the genus *Pratylenchus*, the vacuolar layer of the median zone is replaced by three layers, *i.e.*, two granular external layers and one vacuolar internal layer. In the present study, cross sections of the lateral fields show a median zone with four layers. This structure is similar to that observed in *H. oryzae* and *H. spinicaudata* (Mounport *et al.*, 1995). The structure of the lateral fields in *H. belli* and *H. gracilis* (Johnson *et al.*,1970) has not been described. In some species of the genus *Tylenchorhynchus* (*T. indicus, T. ventralis,*

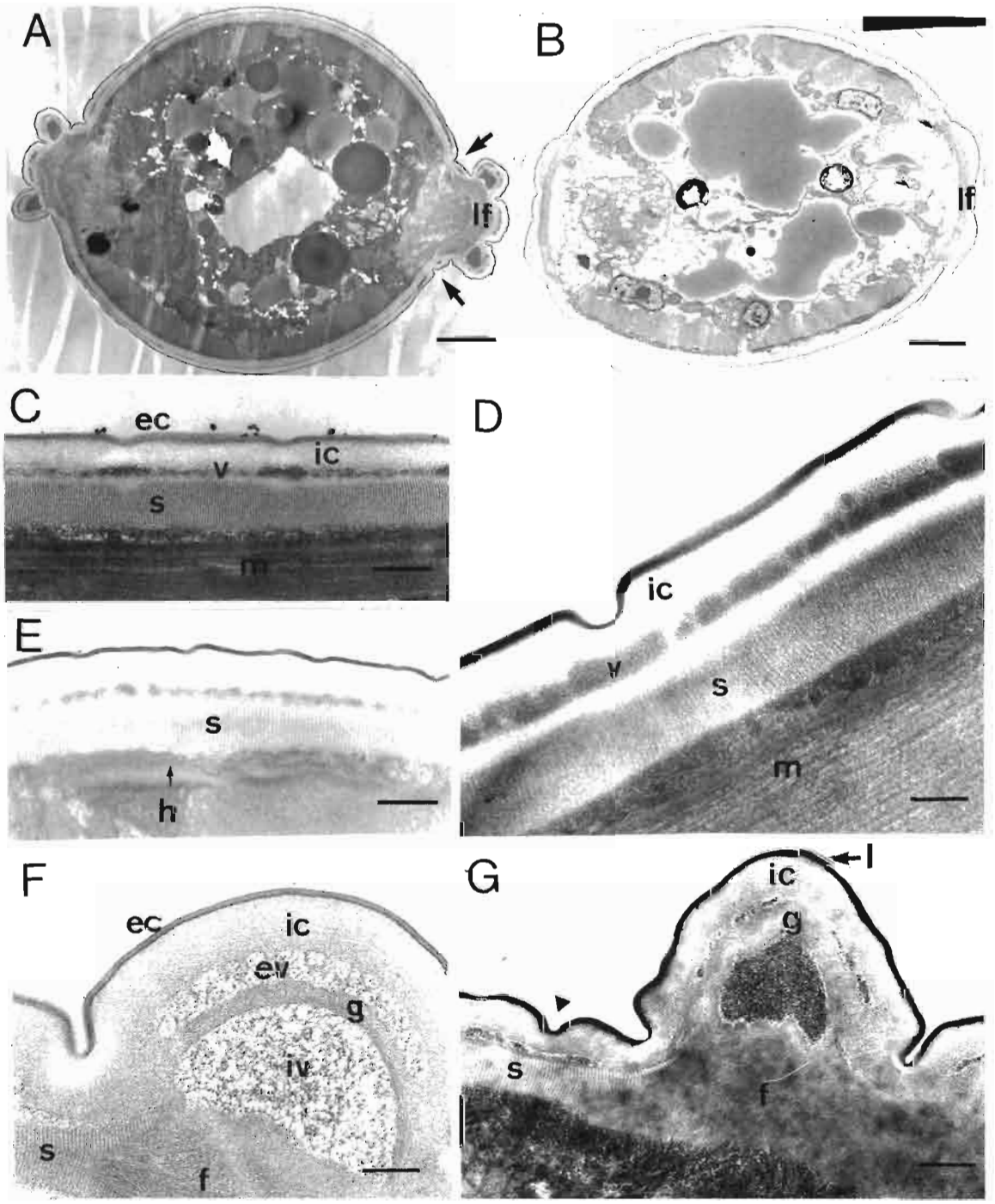


***Fig. 1.*** *Ultrastructure of the cuticle of* Radopholus similis *in cross (A, B, E, F, G) and longitudinal (C, D) sections. A: Female* in toto *(arrows show supplementary incisures); B: Male in toto; C, D, E: Female, cuticle outside of lateral fields (arrow in E shows an hemidesmosome); F, G: Female, external band of the lateral field (arrow and arrowhead show the limiting membrane and a supplementary incisure, respectively). (ec: external cortex; ev: external vacuolar layer; f: fibrillar layer; g: granular layer; h: hemidesmosomes; hy: hypodermis; ic: internal cortex; iv: internal vacuolar layer; l: limiting membrane recovering the external cortex; m: somatic muscles; s: striated layer; v: vacuolar layer. Scale bars: A = 2.2 µm ; B = 2 µm ; C = 0.36 µm ; D = 0.17 µm ; E = 0.3 µm ; F = 0.26 µm ; G = 0.36 µm).*

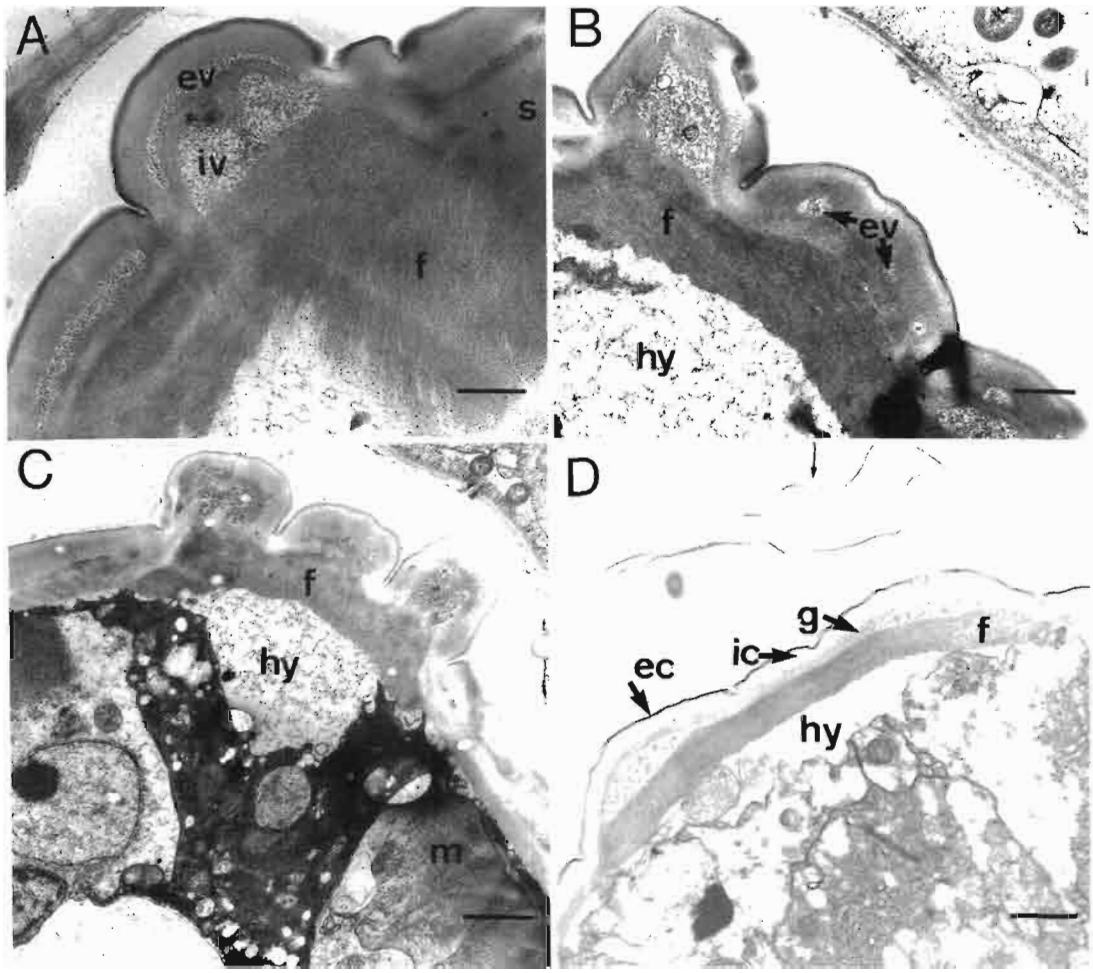


***Fig 2.*** *Ultrastructure of lateral fields of* Radopholus similis *in cross sections. A, B, C: Sections on female lateral fields; D: Section in male lateral fields (For abbreviations, see Fig. 1. Scale bars: A = 0.5 µm ; B = 0.5 µm ; C = 0.86 µm ; D = 1.5 µm).*

*T. germanii)*, supplementary granular and vacuolar layers were found in the lateral fields; however, these layers were located in the basal zone of the cuticle (Mounport *et al.*, 1993*a*).

Our observations of *R. similis* also revealed the presence of six incisures in the lateral fields, as previously observed in the genus *Pratylenchus* (Mounport *et al.*, 1990) and *Hirschmanniella* (Mounport *et al.*, 1995). Light and scanning microscopy of females belonging to the family Pratylenchidae indicated that, in lateral view, the fifth and sixth incisures are generally hidden by the outer bands of the lateral fields and are not visible. However, six incisures may be seen in specimens with distortions caused by processing techniques and/or as a specific structure of the external bands.

Based on previous and present observations, two groups of cuticular organizations can be defined: *i*) the *Pratylenchus* group, and *ii*) the *Radopholus-Hirschmanniella* group. Ultrastructural investigations are in progress to better understand the variations of this characteristic genera that have been classified in the subfamily Pratylenchinae (Luc, 1987) and/or Radopholinae (Siddiqi, 1986).

**References**

BALDWIN, J.G. & SCHOUEST, L.P., JR. (1990). Comparative detailed morphology of the Heteroderinae Filip'ev & Schuurmans Stekhoven, 1941, *sensu* Luc *et al.* (1988): phylogenetic systematics and revised classification. *Syst. Parasitol.*, 15: 81-106.

BAUJARD, P., CASTILLO, P., DOUCET, M., MARTINY, B., MOUNPORT, D. & NDIAYE, A. (1991). Variabilité intra- et interspécifique des structures cuticulaires externes dans le genre *Aorolaimus* Sher, 1963 (Nemata: Hoplolaimidae). *Syst. Parasit.*, 19: 195-213.

BYERS, J.R. & ANDERSON, R.V. (1972). Ultrastructural morphology of the body wall, stoma and stomatostyle of the nematode *Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936. *Can. J. Zool.*, 50: 457-465.

CLIFF, G.M. & BALDWIN, J.G. (1985). Fine structure of the body wall cuticle of females of eight genera of Heteroderidae. *J. Nematol.*, 17: 286-296.

CORBETT, D.C.M. & CLARK, S.A. (1983). Surface features in the taxonomy of *Pratylenchus* species. *Revue Nématol.*, 6: 85-98.

EISENBACK, J.D., HIRSCHMANN, H. & TRIANTAPHYLLOU, A.C. (1980). Morphological comparison of *Meloidogyne* female head structures, perineal patterns and stylets. *J. Nematol.*, 12: 300-313.

IBRAHIM, I.K.A. (1967). Morphological differences between the cuticle of swarming and nonswarming *Tylenchorhynchus martini. Proc. helminth. Soc. Wash.,* 34: 18-20.

JOHNSON, P.W., VAN GUNDY, S.D. & THOMSON, W.W. (1970). Cuticle ultrastructure of *Hemicycliophora arenaria, Aphelenchus avenae, Hirschmanniella gracilis* and *H. belli. J. Nematol.,* 2: 42-58.

KISIEL, M.S., HIMMELHOCH, S. & ZUCKERMANN, B.M. (1972). Fine structure of the body wall and vulva area of *Pratylenchus penetrans. Nematologica,* 18: 234-238.

LUC, M. (1987). A reappraisal of Tylenchina (Nemata). 7. The family Pratylenchidae Thorne, 1949. *Revue Nématol.,* 10: 203-218.

MOUNPORT, D., BAUJARD, P. & MARTINY, B. (1990). Etude ultrastructurale de la cuticule de *Pratylenchus brachyurus, P. loosi* et *P. sefaensis* (Nemata: Pratylenchidae). *Revue Nématol.,* 13: 249-254.

MOUNPORT, D., BAUJARD, P. & MARTINY, B. (1991). Etude ultrastructurale de la cuticule et de la région vaginale de *Scutellonema bradys, S. cavenessi* et *S. clathricaudatum* (Nemata : Hoplolaimidae). *Revue Nématol.*, 14: 261-275.

MOUNPORT, D., BAUJARD, P. & MARTINY, B. (1993*a*). Cuticle fine structure of nine species in the genus *Tylenchorhynchus* Cobb, 1913 (Nemata: Belonolaimidae). *Fundam. appl. Nematol.*, 16: 137-149.

MOUNPORT, D., BAUJARD, P. & MARTINY, B. (1993*b*). Observations on the cuticle ultrastructure in the Hoplolaiminae (Nemata: Hoplolaimidae). *Nematologica*, 39: 240-249.

MOUNPORT, D., BAUJARD, P. & MARTINY, B. (1993*c*). Ultrastructural observations on the body cuticle of four species of Tylenchidae Oerley, 1880 (Nemata: Tylenchida). *Nematol. medit.*, 21: 155-159.

MOUNPORT, D., BAUJARD, P. & MARTINY, B. (1995). Body wall ultrastructure of *Hirschmanniella oryzae* and *Hirschmanniella spinicaudata* (Nemata: Pratylenchidae). *Nematologica,* 41: 323. [Abstr.]

SARAH, J.-L., SABATINI, C. & BOISSEAU, M. (1993). Differences in pathogenicity to banana (*Musa* sp., cv. Poyo) among isolates of *Radopholus similis* from different production areas of the world. *Nematropica*, 23: 75-79.

SIDDIQI, M.R. (1986). *Tylenchida parasites of plants and insects.* Slough, U.K., Commonwealth Agricultural Bureaux, ix + 645 p.

VALETTE, C., NICOLE, M., SARAH, J.-L., BOISSEAU, M., BOHER, B., FARGETTE, M. & GEIGER, J.-P. (1997). Ultrastructure and cytochemistry of interactions between banana and the nematode *Radopholus similis. Fundam. appl. Nematol.*, 20 : 65-77.