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Scanning electron microscope study of two African populations of *Radopholus similis* (Nematoda: Pratylenchidae) and proposal of *R. citrophilus* as a junior synonym of *R. similis*

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Summary – Cuticular structures of two African populations of *Radopholus similis* from the Ivory Coast and Guinea Bissau were observed under the scanning electron microscope. These two populations have similar cuticular structures, which exhibited a range of variation overlapping that described in American population of *R. similis* and *R. citrophilus*. Consequently, *R. citrophilus* is proposed as a junior synonym of *R. similis*. Additional data are provided on female tail shape and lateral fields and male head and bursa shape. © Elsevier - ORSTOM

Résumé – ***Étude en microscopie électronique à balayage de deux populations africaines de* Radopholus similis *(Nematoda: Pratylenchidae) et proposition de* R. citrophilus *comme synonyme mineur de* R. similis - Les structures cuticulaires de *Radopholus similis* provenant de Côte d'Ivoire et de Guinée Bissau ont été étudiées en microscopie électronique à balayage. Les résultats montrent que les structures cuticulaires sont identiques dans les deux populations et exhibent une variabilité supérieure à celle observée dans les populations américaines de *R. similis* et de *R. citrophilus* qui est proposé comme un synonyme mineur de *R. similis*. Des données additionnelles sont apportées sur la forme de la queue et les champs latéraux chez la femelle, et sur la forme de la région céphalique et de la bursa chez le mâle. © Elsevier - ORSTOM

Keywords: cuticular structures, *Radopholus citrophilus*, *R. similis*, scanning electron microscopy, taxonomy, variability.

Two races have long been recognized (Ducharme & Birchfield, 1956) in *Radopholus similis* (Cobb, 1893) Thorne, 1949; one race parasitizes banana but not citrus, the other parasitizes both citrus and banana. Other differences were described between the two races, *i.e.*, karyotype with n=4 in the banana race vs. n=5 in the citrus race (Huettel & Dickson, 1981; Huettel *et al.*, 1984), differences in isoenzymes and proteins (Huettel *et al.*, 1983*a, b*), and differences in sexual behaviour (Huettel *et al.*, 1982).

Latter, Huettel *et al.* (1984) proposed the citrus race as a new species, *R. citrophilus* Huettel, Dickson & Kaplan, 1984, emphasizing the fact that it was the first sibling species described in plant parasitic nematodes and that it was not morpho-anatomically distinguishable from *R. similis*. Consequently, the diagnosis only referred to karyotypes, enzymes and proteins, and sexual behaviour. Recently Hahn *et al.* (1996) demonstrated that the chromosome number cannot be used as a diagnostic feature in *R. similis*.

Absence of morpho-anatomical differences between the two species led some taxonomists to consider *R. citrophilus* as a subspecies within *R. similis* (see Siddiqi, 1986).

Huettel and Yaegashi (1988) examined specimens of the two taxa with scanning electron microscopy (SEM) and claimed that they can be differentiated at the species level by using minute external characters, clearly visible only under SEM. These characters were the shape of oral disc and lateral lip sectors in females, the number of body annules at level of the vulva, and the number of "genital papillae" on the anterior cloacal lip in male.

The examination of specimens from two African populations of *R. similis* show that the differentiating power of these SEM characters are not as strong as claimed by Huettel and Yaegashi (1988).

Materials and methods

Two populations of *R. similis* were studied under SEM: *i*) one population collected in 1990 at Bula, Guinee-Bissau (= GB pop.) and reared on cowpea (*Vigna unguiculata* [L.]) Walp.) in the laboratory at Dakar (Senegal), and *ii*) one population collected at Anguédédou, Ivory Coast (= IC pop.) and reared in the laboratory on carrot disks culture (Fallas *et al.*, 1995).

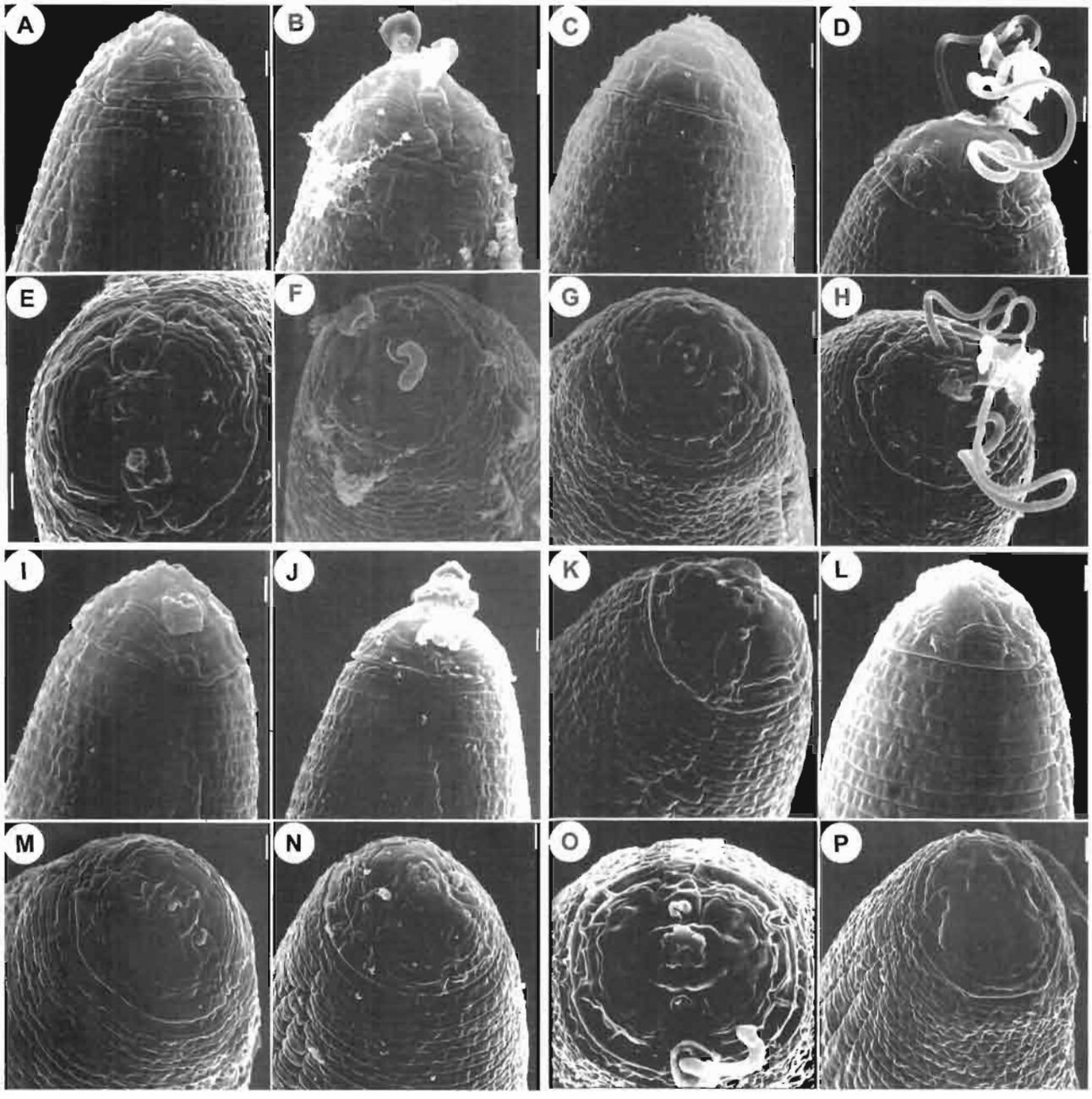

Fig. 1. *Female head morphology of* Radopholus similis, *Guinea Bissau population (A, E; B, F; C, G; D, H: Lateral and face views of the same specimen, respectively; scale bars = 1 μm).*

Nematodes extracted from soil and roots (GB pop.) or from carrot disks (IC pop.) were prepared for SEM as described by Baujard and Pariselle (1987). Thirty females and males of each population were observed.

Results

HEAD MORPHOLOGY

Females: Shape of the labial disc variable in the GB pop., depending on the degree of dorsal and ventral

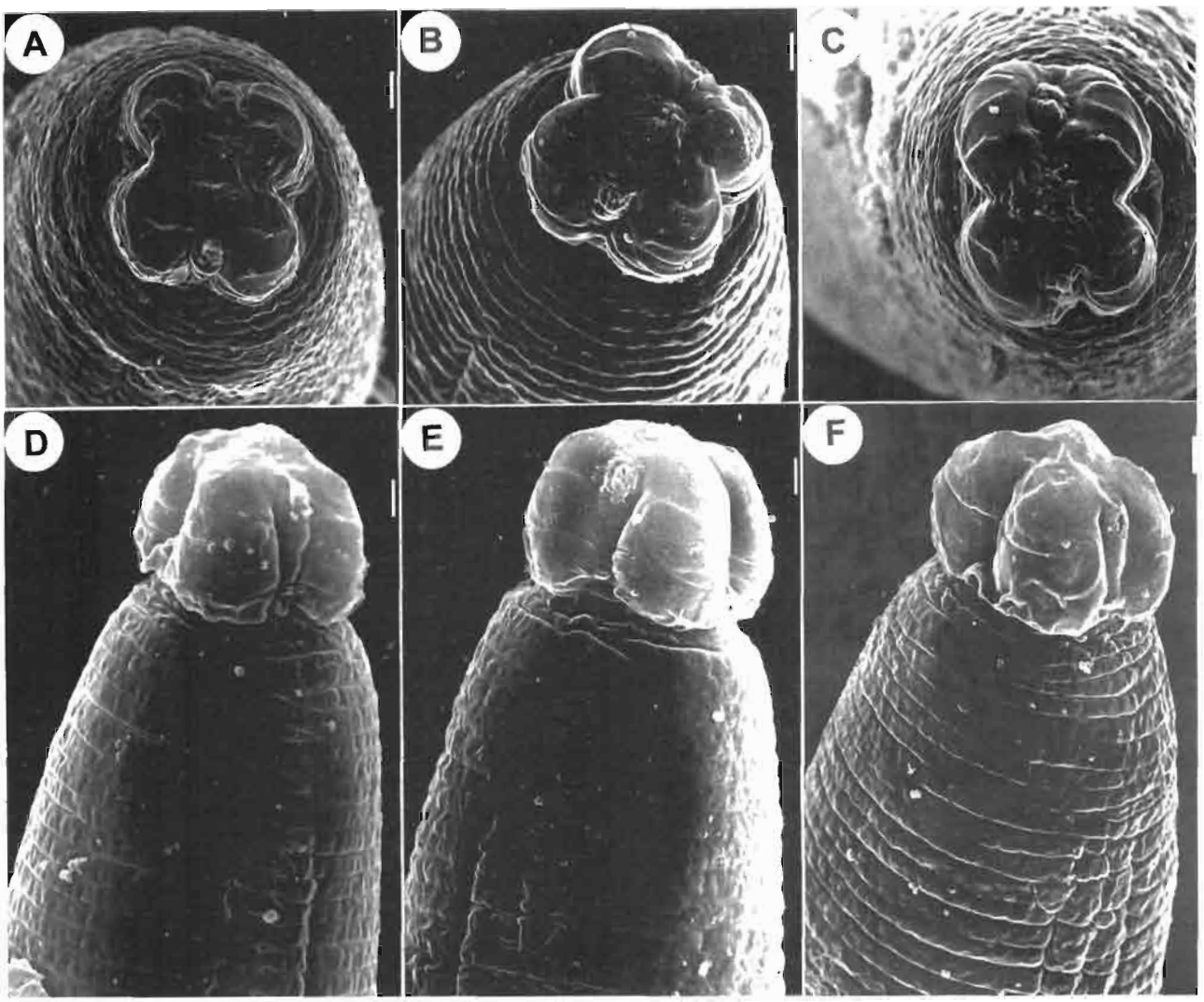

Fig. 2. *Male head morphology of* Radopholus similis, *Guinea Bissau population (A, D; B, E; C, F: Face and lateral views of the same specimen, respectively; scale bars = 1 µm).*

fusion of the labial disc with the first head annule; in the IC pop., labial disc rounded to elongate (Fig. 1). In both populations, the lateral lips extend over the whole cephalic region in 50 % of the females, and stop just before the last head annule in the other specimens. Number of head annules variable in the GB pop.: two (13 % of the specimens), three (27 %), four (27 %), or five (7 %); in 27 % of the specimens, one or two head annules are partly subdivided, which means that the number of head annules is different on each side of the head: two-five, three-four, or three-five. In the IC pop., three head annules are always present.

Males: Head quadrilobed in face view (Fig. 2), with four deep dorso-ventral and lateral grooves; laterally, these grooves partially covered by the lateral lip sectors extending longitudinally over the whole head; first head annule with a small, more or less rounded, not well marked labial disc; submedian lip sectors appearing totally fused together; head with two to four head annules.

VULVA AREA

Vulva, a transverse slit of medium length (30-40 % of the corresponding body diameter); vulval lips protruding or not; at the level of the vulva, transverse body annulation completely interrupted by vulva opening (=annules terminating at vulva in Huettel and Yaegashi, 1988) over two (15 %), three (71 %), or

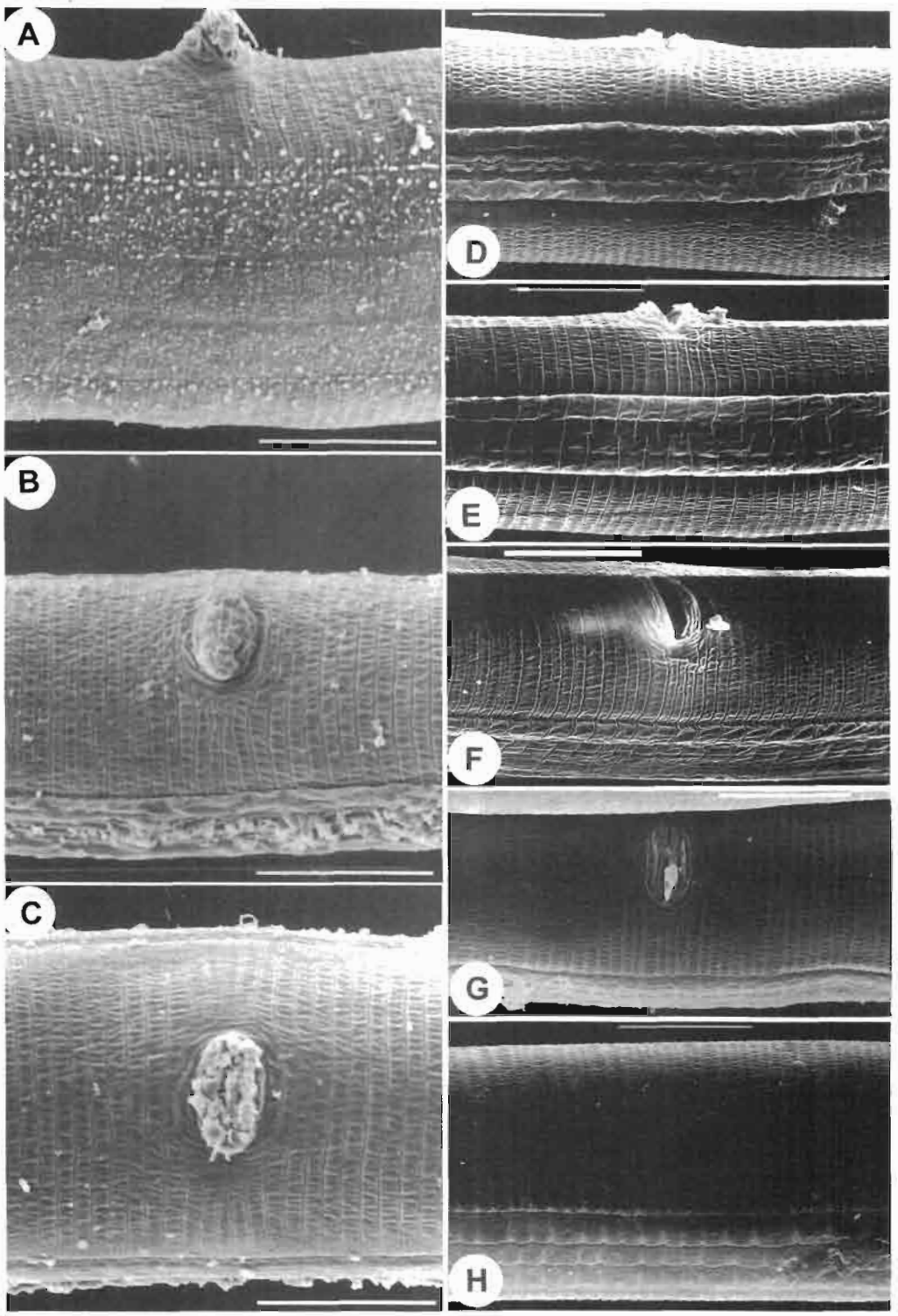

Fig. 3. *Female vulval region and lateral fields of* Radopholus similis, *Ivory Coast (left column) and Guinea Bissau (right column) populations (E, F: Lateral and subventral views of the same specimen, respectively; scale bars = 10 µm).*

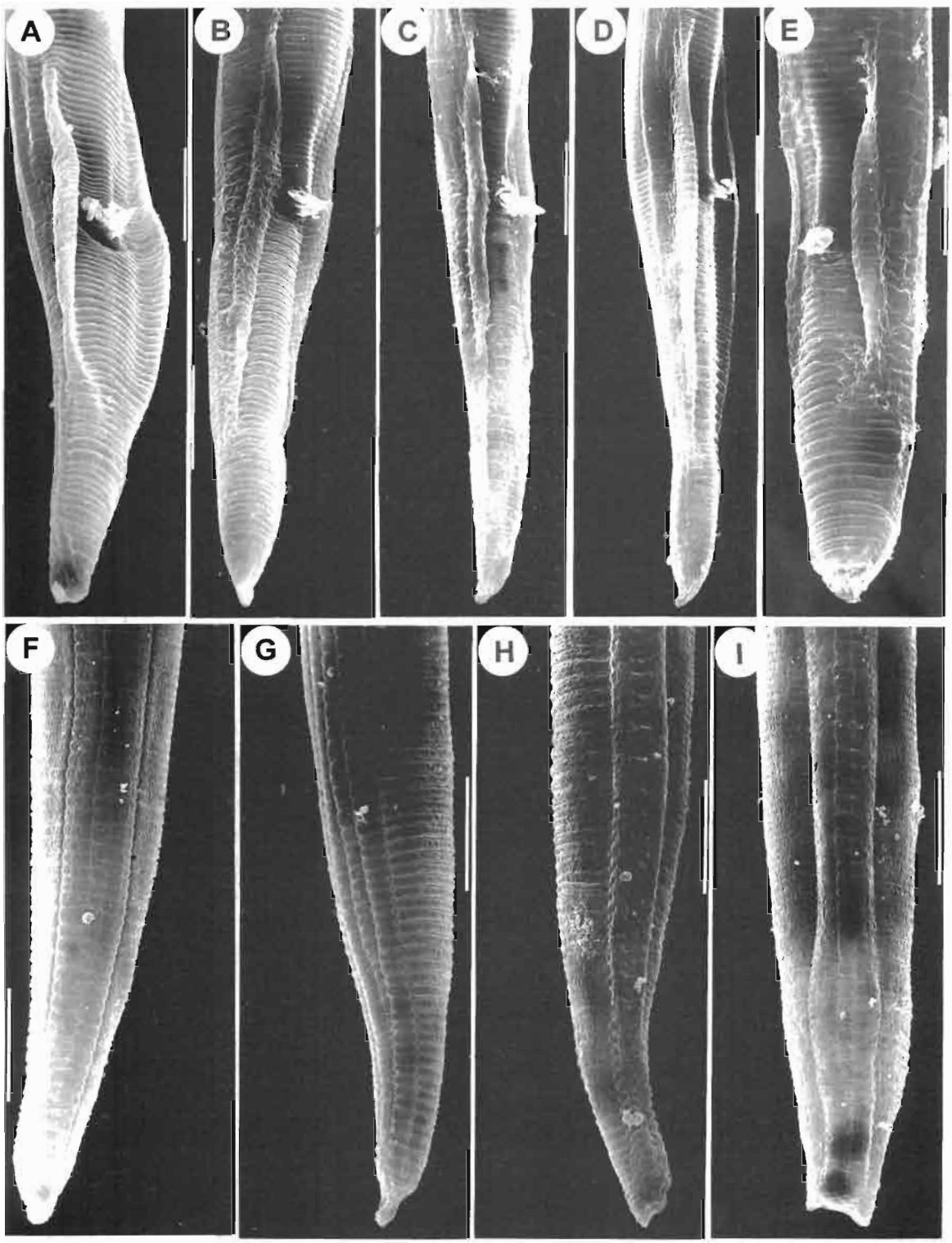

Fig. 4. *Male (A-E) and female (F-G) tail of* Radopholus similis, *Guinea Bissau population (Scale bars = 10 µm).*

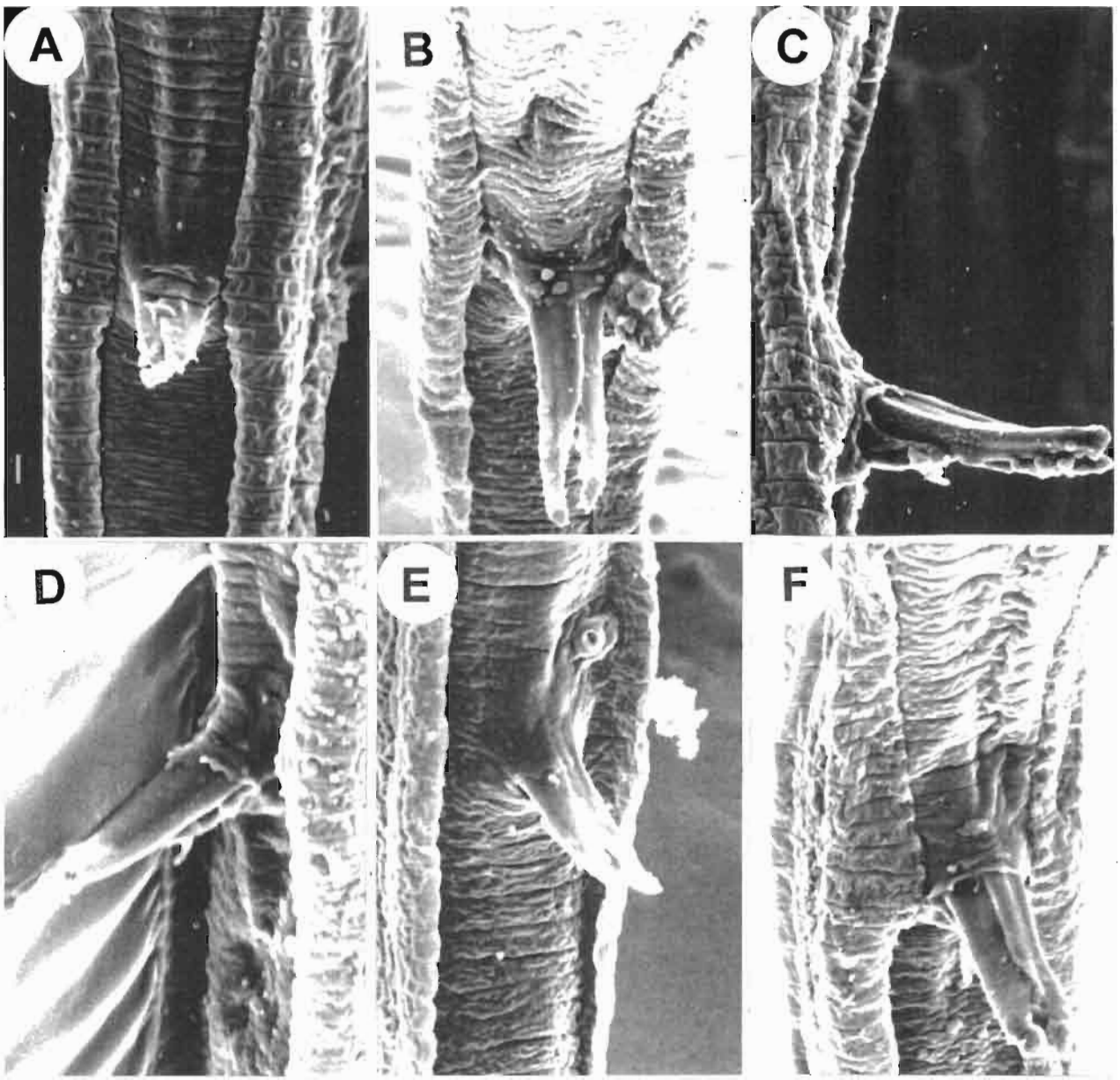

Fig. 5. *Cloacal region of* Radopholus similis, *Guinea Bissau population (Scale bars = 1 µm).*

four (15 %) body annules in the IC pop (Fig. 3, A-C) and one (15 %), two (28 %), or three (57 %) body annules in the GB pop. (Fig. 3, D-H).

FEMALE LATERAL FIELDS

Lateral fields occupying 27-53 % of the corresponding body diameter, with four incisures delimiting three bands of equal size, more or less regularly areolated (Fig. 3, A, B, D, E, H).

TAIL SHAPE

Female: Tail appearing most often regularly conoid, rarely subcylindrical; distal part of the tail variable in shape in both populations: truncate, rounded, or bearing a short (3-4 µm) and well marked mucro (Fig. 4, F-G).

Male: Tail generally regularly conoid, rarely subcylindrical; tail ending mostly in a short mucro as

Table 1. *Variability of characters used for the differentiation of* Radopholus similis *and* R. citrophilus.

	R. similis		*R. citrophilus*
	Huettel & Yaegashi (1988)	Present study	Huettel & Yaegashi (1988)
Genital papillae	0-2	0-6	3-7
Annules interrupted by vulva	2	1-5	3
Labial disc	± hexagonal	rounded/oval	± rounded
Lat. lip sectors	reaching base of 3rd lip annule	reaching penultimate lip annule (2-5)	not reaching base of 3rd annule

described for female, rarely in an abrupt truncate terminus (Fig. 4, A-E). Bursa adanal, occupying 33-63 % of the tail length. In both populations, 50 % of the specimens have anterior cloacal lip devoid of any ornamentation; in the other specimens, one to six appendices present, variable in length (from a short tubercle to a thin appendice, 0.6 µm long) and appearing as simple outgrowths of the cloacal lip and not as papillae since no pore or other type of aperture can be observed (Fig. 5).

Discussion

In the two African populations of *R. similis* studied, variations are observed in the cuticular structures greater than previously described (Orton Williams & Siddiqi, 1973): females with two-five vs. three-four head annules, lateral fields regularly areolated vs. areolated only towards extremities, tail terminus rounded to truncate, with or without terminal mucro vs. rounded or indented without any terminal mucro; males with two-four vs. three-five head annules, bursa extending over 33-63 vs. about 66 % of the tail.

The male head, observed for the first time under SEM, exhibited a four-lobed shape, very close to that observed in the genus *Belonolaimus* in the family Belonolaimidae; this shape, unusual in the suborder Tylenchina, has never been observed in the family Pratylenchidae or in other families with endoparasitic forms; Baldwin and Cap (1992) observed that "the submedial lips are separated by deep indentations" in males of *Radopholus* spp. and considered this pattern close to that of *Nacobbus* males, although SEM observations by Doucet (1989) revealed a "classical" head pattern without any longitudinal indentations in *N. aberrans*.

In the two populations studied, the SEM characters considered as diagnostic by Huettel and Yaegashi (1988) all showed variation overlapping the differences between *R. similis* and *R. citrophilus* described by these authors (Table 1).

The present observations show that the two species cannot be differentiated morphologically, even with the use of SEM.

Also karyotypes do not appear a safe way for such a differentiation (see above). Finally differences recorded in enzyme and protein patterns cannot be assumed to be characteristic at species or at race level. Moreover, it has been demonstrated that *R. similis* can reproduce by parthenogenesis (Brooks & Perry, 1962), a fact that lessens the importance of the sexual attraction.

Therefore it appears more reasonable to consider *R. citrophilus* Huettel, Dickson & Kaplan, 1984 as a junior synonym of *R. similis* (Cobb, 1893) Thorne, 1949, the citrus and banana races representing differentiated pathotypes.

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