

# Redescription of *Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977 (Nematoda : Tylenchina)

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## SUMMARY

*Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977 which was described on a single female from Brazil, is redescribed using an abundant material from Senegal. Female, male and various juvenile stages are studied in detail, with special emphasis on the morphology (SEM) of the labial area and the structure of the labial sclerotization.

## RÉSUMÉ

Redescription de *Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977 (Nematoda : Tylenchina)

*Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977, décrit sur une seule femelle provenant du Brésil, est redécrit grâce à un abondant matériel originaire du Sénégal. Les femelles, les mâles ainsi que les juvéniles des divers stades sont étudiés; la morphologie (MEB) de la région labiale et la structure de la sclérotisation labiale font l'objet d'une étude détaillée.

*Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977 was described, as *Plesiodoris rostrulatus* Siddiqi, 1976, on a single female sampled in marine sandy soil around the roots of a diseased coconut-tree (*Cocos nucifera* L.) at Valença, Bahia State, Brazil. The creation of a new species on the basis of a single specimen was justified by the possession of two unique characters : *i*) the lip region composed of only two wide annules and *ii*) the *vagina vera*, heavily sclerotized and (in profile) shaped like "the beak of a bird of prey".

The species was found again in soil from the rhizosphere of tomato (*Lycopersicon esculentum* Mill.) cultivated in a niaye\*\* near Tiaroye, Senegal. That population of *N. rostrulatus* was composed of numerous specimens, including males and the different juvenile stages. So, a complementary description of the species was possible.

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\*\* A "niaye" is in Senegal a shallow depression near the coast which is filled with water during the rainy season and on the edges of which vegetables are grown following the descent of the water level during the dry season. The soil is generally heavily sandy.

## Methods

The specimens were fixed with FP 4:1 (Netscher & Seinhorst, 1969) and mounted in dehydrated glycerine following Seinhorst's (1959) rapid method.

Glycerine embedded nematodes were used for scanning electron microscopy, they were transferred to a drop of glycerine in a small embryo dish and stored overnight in a closed jar with ethanol 95 % at 30°. Then the nematodes were washed several times with ethanol 95 % to remove as much glycerine as possible. Subsequently distilled water was added drop by drop until the nematodes were in pure distilled water. Then the nematodes were passed two times 45 sec. under ultrasonic to remove adhering particles. The water was then suck off and replaced by a solution of osmium tetroxide in cacodylate buffer (equal amounts of OsO<sub>4</sub> and buffer) which is allowed to stand overnight. Subsequent dehydration was done with a series of ethanol (30 %, 50 %, 75 %, 95 %, 100 %); the absolute ethanol was twice renewed in one hour's time. Half of the absolute alcohol was then replaced by Spurr's low viscosity epoxyresin and then rotated (at 10 rev./min in a TAAB rotator) during one night in a cold room (4°). The resin solution was then replaced by a new one and

the rotation is continued for one day. After that, the nematodes were transferred to pure Spurr's resin and put for one night in an exsiccator under vacuum in a cold room. The nematodes are stored for two hours in an oven at 60° and then separately washed with absolute alcohol to remove the resin from the outside of the nematodes. Finally the nematodes were placed in an oven at 60° for 48 h to complete polymerisation of the infiltrated resin.

***Neodolichodoros rostrulatus***  
**(Siddiqi, 1976), Siddiqi, 1977**

(Figs 1-7)

MEASUREMENTS

See Table 1.

DESCRIPTION

*Female* : Body elongated, cylindrical, C-shaped to semicircular when heat relaxed, tapering anteriorly, but not posteriorly; marked by a shallow ventral depression in the vulval area, most often filled with copulatory plug matrix. Posterior terminus subclavate, bluntly rounded. Annules prominent, 4.2-4.6 µm\* wide in anterior body region, i.e. until excretory pore level, but only 2.3-2.6 µm\* wide behind that level. Lateral field with four lines, exterior ones indented, interior ones plain; external bands of lateral field regularly crossed by body annules, central band narrower, irregularly crossed by transverse striae which are thinner than those of the outer bands; at vulva level the lateral field often irregular, the central lines sometimes disappearing over a short distance. Labial area high and expanded, flattened anteriorly, roughly cushion-shaped, measuring 7.5-8 µm wide and 4.5-5 µm high, separated from rest of body by a deep constriction and provided with a strong sclerotization (see below for detailed description of the labial area). Anterior and posterior cephalids dot-like in lateral view, situated 5-8 µm and 15-21 µm posterior to basal plate of the labial sclerotization, respectively. Stylet long and thin, basal knobs rounded, smooth anteriorly, directed backward. Procorpus cylindrical to slightly barrel-shaped; dorsal pharyngeal gland orifice situated 4-6 µm from the base of the stylet; duct of dorsal pharyngeal gland wide, filling nearly all the procorpus, with a densely granular content; pharyngeal lumen sinuous; median bulb strong, heavily muscular, about half of the corresponding body diameter, ovate in profile, with strong valve; orifices of ventrosublateral pharyngeal glands followed by a wide ampulla; isthmus narrow and short; basal bulb ovate in profile, anterior part (dorsal gland) densely granular,

posterior part (ventrosublateral glands) more translucent; dorsal gland nucleus large, in the middle of the basal bulb; ventrosublateral nuclei smaller, situated far posteriorly; exceptionally a female presented seven nuclei in the basal bulb, six of them smaller, resulting most probably from a triplication of the ventrosublateral gland nuclei (Fig. 1L); pharyngo-intestinal valve strong, roughly conoid-truncate. Intestine overlapping rectum, filling nearly all the tail volume; canals (= fasciculi) present. Excretory pore at about the level of isthmus; anterior part of excretory duct sclerotized. Nerve ring thick. Hemizonid flat, posterior to excretory pore. Hemizonion lenticular, about at the level of the pharyngo-intestinal junction. Vulva a transverse ellipse or oval without flaps, situated in a depression of the cuticle (Fig. 1E, H). Distal part of vagina with heavy sclerotization, forming a yellowish mass, rounded in profile, often asymmetrical; in ventral view, the sclerotization appears flattened, with a sinuous slit-like lumen ( Fig. 1F, G, I & J). The vulval dilators are attached to thin sclerotization (Fig. 1 F & J); more inward the musculature comprises of the perivaginal sphincter, vaginal dilators and vaginal suspensors. Two genital branches opposite and outstretched, with well developed axial spermathecae, full of sperm. Tail rounded; tail annules about the same size as the body annules, except two or three larger terminal ones, separated by a deeper incisure; external lines of lateral field rounded and continuous on tail or interrupted far posteriorly. Phasmids dot-like, anterior to anus, in the central band of the lateral field or on the dorsal inner line. Caudalids rarely seen, small, lenticular, situated ventrally 8-15 µm in front of anus.

*Male* : Body similar to that of female, but shorter and more curved posteriorly. Labial area not so expanded, measuring 6-7 µm wide and 3.5-4.5 µm high; constriction separating it from the rest of the body not so pronounced as in female; one or two narrow basal annules and one wide anterior annule; labial disc not visible laterally; labial sclerotization strong, similar to that of female. Anterior and posterior cephalids dot-like, situated 5-9 µm and 12-18 µm from anterior end, respectively. Stylet, pharynx, intestine, excretory pore, hemizonid, hemizonion and nerve ring similar to that of female, except in dimensions and/or in distance to anterior end. Spicules slightly ventrally curved, not cephalated, devoid of a velum, distal end obtuse. Gubernaculum slightly protruding from cloaca, composed of corpus, cuneus and two titillae; corpus distally recurved, almost straight to slightly sinuous, proximally with a more or less dorsally curved apophysis which appears triangular in cross section (Fig. 2M, left); remainder of corpus thin except for the mediodorsal sclerotized part (Fig. 2M, middle and right). Cuneus wide, weakly sclerotized, occupying most of the area between the distal parts of the spicules (Fig. 2M, right).

\* 20 consecutive annules measured in five females.

Table 1

*Neodolichodoros rostrulatus*, measurements of juveniles and adults.

	<i>J</i> <sub>2</sub>	<i>J</i> <sub>3</sub>	<i>J</i> <sub>4</sub>	Females	Males
n	10	10	10	20	20
L	0.48-0.56 (0.53 ± 0.024)	0.65-0.79 (0.72 ± 3.93)	1.03-1.30 (1.20 ± 2.16)	1.57-2.01 (1.80 ± 11.74)	1.36-1.66 (1.50 ± 8.14)
a	25.5-27.9 (28.1 ± 1.39)	25.3-30.4 (28.3 ± 1.59)	29.2-35.9 (33.5 ± 2.16)	34.2-44.5 (40 ± 3.14)	32.4-46.6 (40.0 ± 2.81)
b	3.7-4.6 (4.0 ± 0.23)	4.0-4.8 (4.4 ± 0.22)	5.5-6.1 (5.8 ± 0.17)	6.6-7.8 (7.2 ± 0.37)	5.6-7.0 (6.4 ± 0.29)
Tail length	41.5-50 (46.5 ± 2.69)	35.5-40.5 (37 ± 1.7)	18-30 (24 ± 4.02)	18-32 (23 ± 2.99)	21-33 (29 ± 3.28)
c	10.5-12.7 (11.4 ± 0.76)	18.2-20.8 (19.4 ± 0.92)	40.5-69.2 (50.5 ± 8.53)	63.3-106.1 (79.4 ± 12.17)	43.6-66.7 (53.0 ± 5.88)
c'	3.1-4.2 (3.8 ± 0.34)	1.5-2.4 (2.0 ± 0.24)	0.7-1.0 (0.9 ± 0.10)	0.5-0.8 (0.6 ± 0.154)	0.9-1.4 (1.2 ± 0.12)
v	—	—	—	53.1-58.2	—
V	—	—	—	(55.9 ± 1.39)	—
Stylet	44-52 (48.5 ± 2.15)	61-67.5 (65 ± 1.92)	74-85 (81.5 ± 3.5)	91-103 (96 ± 3.97)	87-99 (94 ± 3.09)
Stylet cone	22.5-31 (29 ± 1.47)	36-40 (38 ± 1.12)	42.5-52 (48 ± 2.9)	50-61 (56.5 ± 2.85)	47-58 (55 ± 2.78)
Center median pharyng. bulb <sup>1</sup>	74-82 (78.5 ± 2.8)	88-107 (101 ± 4.9)	116-139 (132 ± 6.74)	148-175 (163 ± 6.27)	140-161 (154 ± 5.31)
Pharyngo-intestinal junction <sup>1</sup>	125-141 (134.5 ± 5.35)	159-168 (163 ± 2.9)	183-215 (204 ± 9.5)	225-263 (250 ± 8.73)	221-248 (235 ± 8.01)
Excretory pore <sup>1</sup>	96-109 (102 ± 4.36)	111-128 (123 ± 5.18)	135-163 (155 ± 9.24)	166-207 (188 ± 12.56)	152-186 (173 ± 7.41)
Hemizonide width	2-3 (2.5 ± 0.35)	3-5.5 (4 ± 0.75)	4-5.5 (5 ± 0.47)	5-7 (6 ± 0.89)	5-9 (6 ± 1.11)
Hemizonide <sup>1</sup>	96-107 (100 ± 3.53)	112-127 (123 ± 4.88)	149-171 (164 ± 7.1)	189-230 (207 ± 10.87)	173-201 (195 ± 7.27)
Hemizonion width	—	—	—	1.5-3 (2 ± 0.35)	1.5-3 (2 ± 0.40)
Hemizonion <sup>1</sup>	—	—	—	219-267 (237 ± 13.35)	198-230 (220 ± 7.89)
Max. diam.	18-22 (19 ± 1.14)	23.5-30 (23.5 ± 2.16)	32-38 (35.5 ± 2.00)	41-53 (45 ± 3.66)	34-42 (38 ± 2.15)
Lat. field width	4-5.5 (4.5 ± 0.35)	6.5-10 (8. ± 1.24)	9-11.5 (10.5 ± 0.72)	14-17 (15.5 ± 0.95)	12-15 (14 ± 0.84)
Lat. field (% corr. diam.)	21.6-29.7 (24.5 ± 2.49)	22.8-38.8 (31.8 ± 6.07)	27.7-34.4 (29.8 ± 1.9)	28.0-42.6 (34.8 ± 3.75)	31.6-41.2 (36.2 ± 2.25)
Phasmids <sup>2</sup>	(+) 0.5-(+) 12.5 (+) 9.5	(-) 3-(+) 5.5 (+) 2	(-) 12.5-(+) 1.5 (-) 16	(-) 12-(-) 28 (-) 19.5 ± 4.18	(-) 6-(-) 21 (-) 14 ± 3.14
Spicules <sup>3</sup>	—	—	—	—	52-59 (55 ± 1.65)
Gubernaculum	—	—	—	—	22-25 (23 ± 0.97)

All measurements are in µm, except L, in mm.

(1) Distance, in µm, from anterior end.

(2) Distance from anus or cloaca : (+) = posterior and (-) anterior to anus or cloaca level.

(3) Measured following median axis.

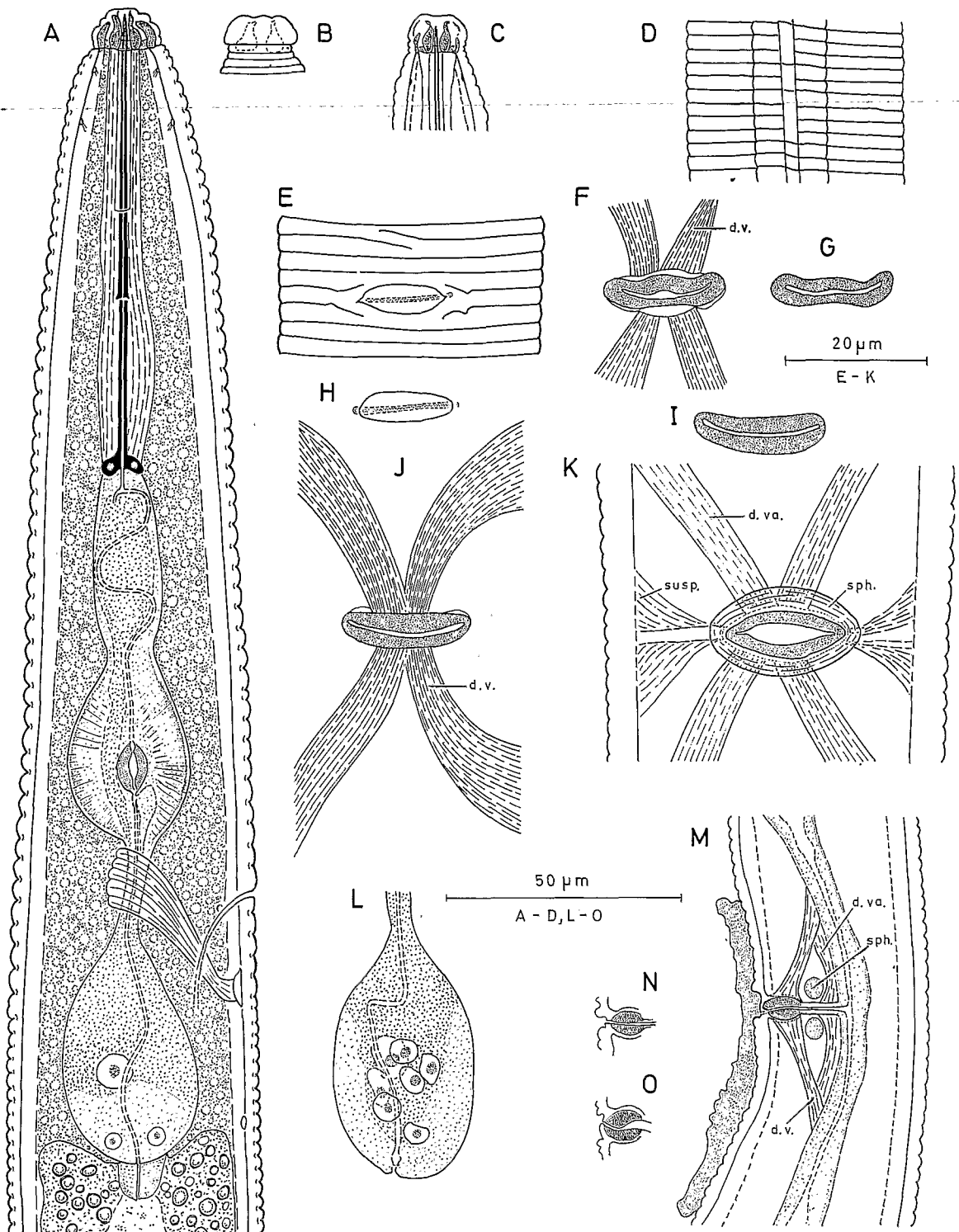


Fig. 1. *Neodolichodorus rostrulatus*. A, B, D-O : Female. A : Anterior portion; B : Labial area, surface view; D : Body annules and lateral field; E, H : Vulva, surface view (ventral view); F, G, I, J : Cuticularized anterior part of the vagina and attached muscles (ventral view); K : Vagina at level of vaginal sphincter and attached muscles (ventral view); L : Abnormal pharyngeal bulb with seven gland nuclei; M : Vulva area (lateral view) showing the copulatory plug (c.p.); N, O : Cuticularized anterior part of the vagina (lateral view). Male. C : Labial area.  
 (muscles : d.v. = dilatores vulvae; d.va. = dilatores vaginae; susp. = suspensores vaginae; sph. = vaginal sphincter).

Tail short, pointed in lateral view. Caudal alae well developed, indented posteriorly; lateral wings rounded, annulated, edges crenate; central part ending at same level as lateral wings, with truncate or bifid extremity (Fig. 2J). Phasmids punctiform, anterior to cloaca level.

*Juveniles, stage 2* : Habitus regularly curved, bracket-shaped. Labial area smaller than that of female, similar in shape, but no annulation visible. Body annules well demarcated, 1.4-1.5  $\mu\text{m}$  wide in the anterior body

region, progressively smaller posterior to excretory pore to become 1.1-1.3  $\mu\text{m}$  wide at mid-body. Lateral field with four lines, the two central ones so close to each other as to appear as a single central line; outer bands regularly areolated; central band, when present, narrow and devoid of transverse striae. Hemizonid flat, generally surrounding the excretory pore. Stylet, pharynx, intestine as in adult forms. Tail long conical; lateral field ending posterior to mid-tail; phasmids punctiform, situated on tail.

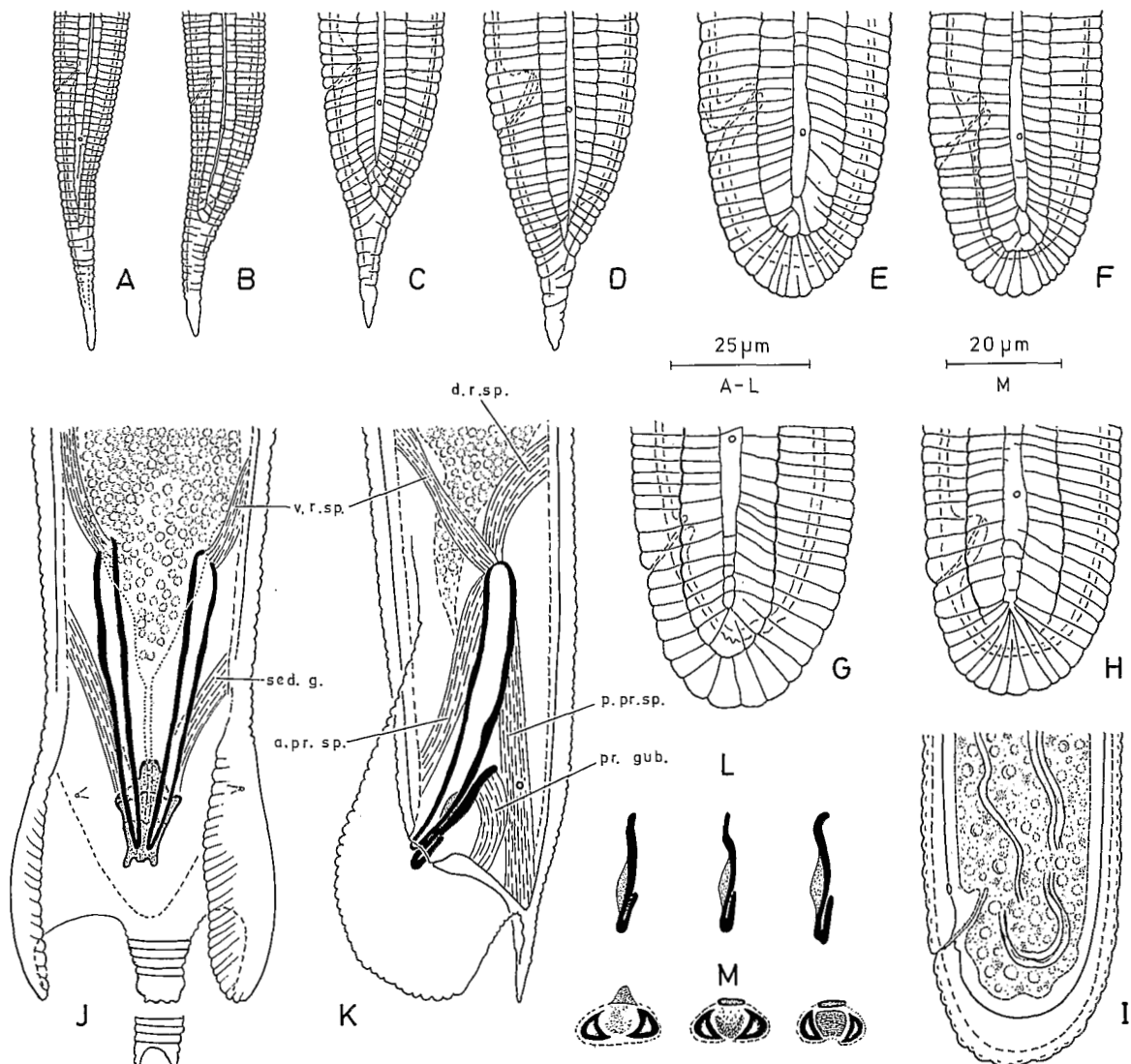


Fig. 2. *Neodolichodorus rostrulatus*. A, B : Tails, 2nd stage juveniles; C, D : Tails, 3rd stage juveniles; E, F : Tails, 4th stage juveniles; G, H, I : Tails, females; J : Male tail, copulatory organs and musculature (ventral view); K : Same as J (lateral view); L : Gubernaculum (lateral view); M : Arrangement of spicules and gubernaculum at three different levels (transverse sections). (muscles : v.r.sp = ventral retractor spiculi; d.r.sp. = dorsal retractor spiculi; a.pr.sp. = anterior protractor spiculi; p.pr.sp. = posterior protractor spiculi; sed.g. = seductor gubernaculi; pr.gub. = protractor gubernaculi).

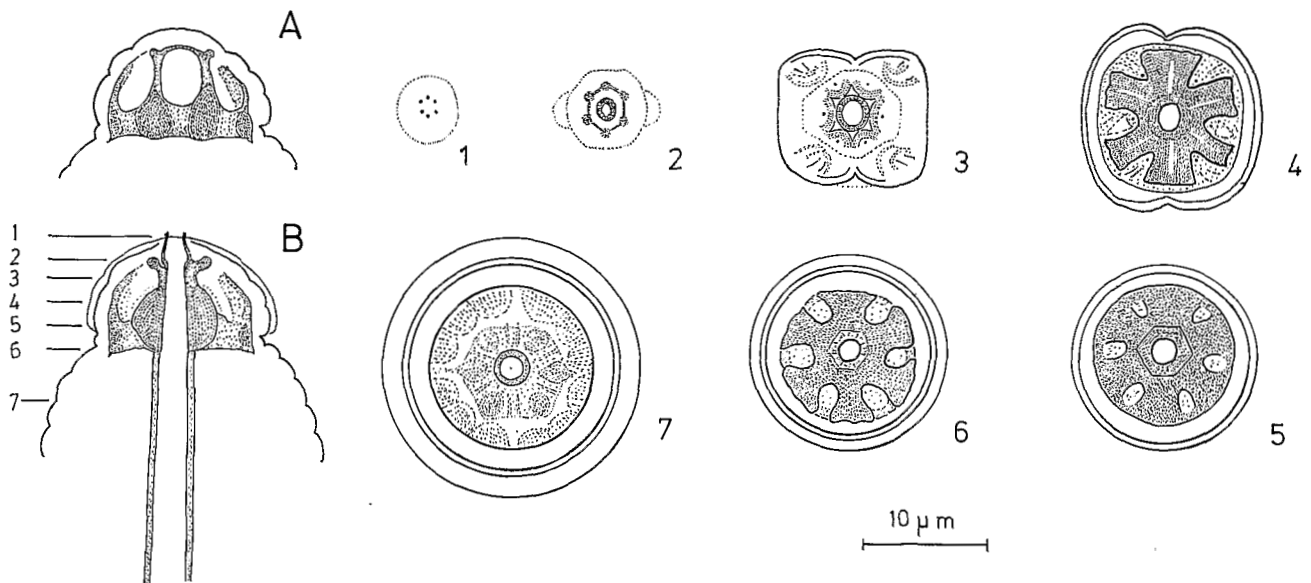


Fig. 3. *Neodolichodoros rostrulatus*. Female, labial sclerotization. A : Submedian view showing the development of the basal plate; B : Median view; 1 to 7 : transverse sections at different levels (see B).

*Juveniles, stage 3* : Habitus C-shaped. Labial area as in female, but smaller. Body annules well demarcated, slightly wider anterior to excretory pore : 2.0-2.3 µm vs 1.5-1.9 µm. Lateral field composed of three bands, the outer ones regularly areolated, the central one narrower areolated only posteriorly. Hemizonid flat, surrounding excretory pore. Stylet, pharynx and intestine as in adult forms. Tail irregularly conical; phasmid punctiform, situated slightly anterior to slightly posterior to anus level, generally located on the central band of the lateral field, but occasionally on one of the inner lines.

*Juvenile, stage 4* : Habitus bracket- to open C-shaped. Labial area as in female. Body annules well demarcated, but contrary to other juvenile stages and adults the annules have about the same width throughout the body : anterior body region : 3.0-3.2 µm; mid-body : 2.7-3.9 µm; posterior region : 3.3-3.7 µm. Lateral field similar to that of female. Hemizonid flat, posterior to excretory pore. Stylet, pharynx and intestine as in adult. Tail rounded, resembling that of female, but more elongated; phasmid generally situated in front of the anal level, rarely slightly posterior to it.

#### DETAILED DESCRIPTION OF THE LABIAL AREA

Siddiqi (1976) describes and illustrates (his figure 3) the labial area as being divided into two large annules of about the same width. Actually *i*) a narrow annule exists (which would have been the third one) at the base of the lip region, and *ii*) the separation in two wide

annules is not complete all around the head and, in fact, is only visible over a short distance, at median level, in lateral view (Fig. 1A), but not in surface view (Fig. 1B). This particular structure of the labial area was especially evident under the SEM.

SEM-face views of females (Figs 4D, E; 5) show a rounded lip region with slight indentations dorsally and ventrally; the oral disc is rounded or more rectangular, with lateral expansions and central part slightly elevated; in some cases (Fig. 4C, D) small, irregular expansions are also present. In the middle of the oral disc, the rounded oral aperture is surrounded by six small, refractive bud-like projections, which represent the anterior extremities of the labial framework (see Fig. 3B); alternating with these processes are the slit-like apertures of the six internal labial sensillae. The amphidial apertures lie close to the lateral expansions of the oral disc, are bracket-shaped and of medium size. The lateral lip sectors are only represented by a depression between the subventral and subdorsal lobes. The subdorsal and subventral lip sectors are somewhat swollen; mediodorsally and medioventrally these sectors are separated by a narrow wider, but always deep, longitudinal groove; this groove is connected with a deep, transverse groove, which separates the subdorsal or subventral sectors dorsally, resp. ventrally from the rest of the labial area. The transverse grooves may delimit half or more of the subdorsal or subventral sectors, but never extend so far as to reach the lateral sectors. In lateral view, these grooves give the impression that the lip region is divided into two annules (see above).



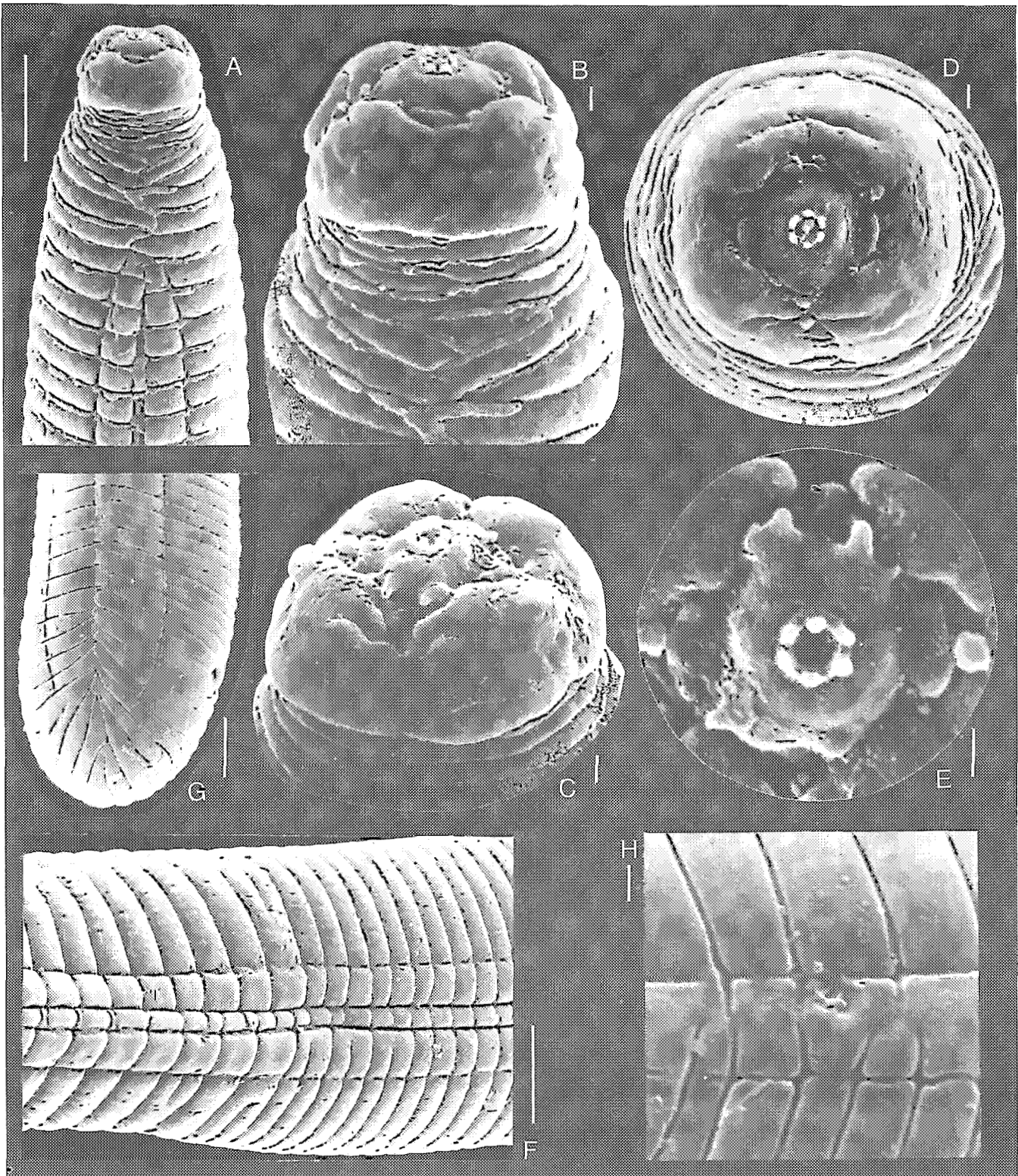


Fig. 4. *Neodolichodoros rostrulatus*. SEM pictures of females. A : Anterior end (lateral view, slightly tilted towards the observer). B : Labial area (as in A); C : Labial area (ventro-dorsal view, slightly tilted towards the observer); D : En face view of labial area; E : En face view of an oral disc with expansions; note the prominent bud-like projections; F : Lateral field and body annulation in the transition area from large (left) to narrow (right) annules; G : Tail; H : Phasmid. (Bars represent : A, F, G : 10  $\mu$ m; remaining : 1  $\mu$ m.)

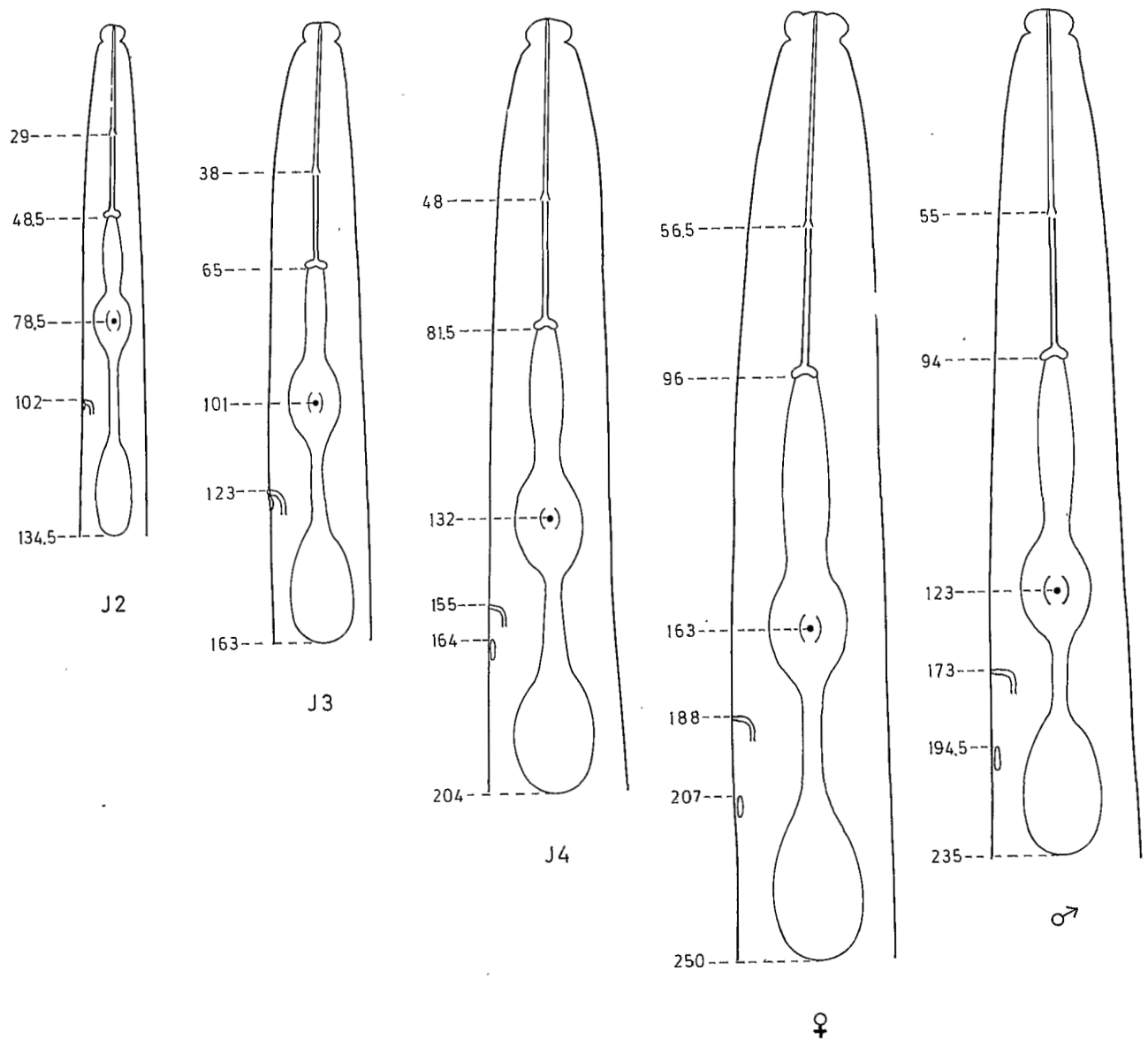


Fig. 6. *Neodolichodus rostrulatus*. Schematic illustration of differential growth of some elements of the anterior body region. Are figured : stylet (cone and basal part), median bulb, pharyngo-intestinal junction, hemizonid and excretory pore (based on mean values for ten juveniles of each stage and twenty adults of each sex; scale in  $\mu\text{m}$ ).

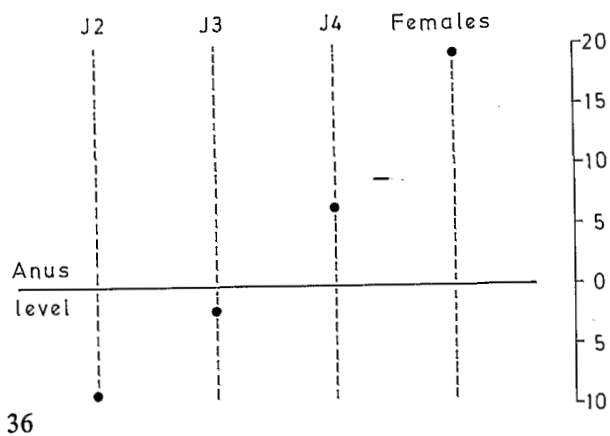


Fig. 7. *Neodolichodus rostrulatus*. Position of the phasmids in juvenile stages and females (based on mean values of ten juveniles of each stage and of twenty females; scale in  $\mu\text{m}$ ).



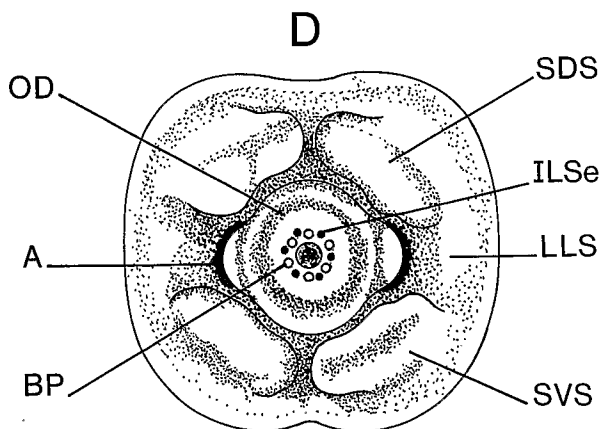


Fig. 5. *Neodolichodorus rostrulatus* : Schematic representation of the face. (OD = oral disc; SDS = subdorsal lip sector; SVS = subventral lip sector; LLS = lateral lip sector; BP = bud-like projections; ILSe = internal labial sensilla; A = amphid aperture).

Labial framework very well developed and heavily sclerotized, as usual in the family Dolichodoridae. In lateral view (Fig. 3A & B), the basal plate and the six arches appear very thick. Anteriorly the framework starts with six circumoral projections (see above and Figs 3B, 1 & 4B, C, D, E) that join posteriorly with a hexagonal structure, composed of six outwardly directed knob-like projections interconnected by fainter and thinner parts (Fig. 3B, 2); then follow the narrow and soon the wide parts of the six arches (Fig. 3B, 3 & 4). The dorsal and ventral arch are more developed than the sublateral ones. The arches first fuse around the stomatal lining and, further backward, also at their peripheries, thus forming the basal plate and enclosing six labial tissue sectors (Fig. 3B, 4 & 5); still further backward the sectors open again at their peripheries (Fig. 3B, 6). The stomatal lining is rounded at first and only loosely connected to the framework (Fig. 3B, 2 & 3), then completely incorporated in the framework (Fig. 3B, 4) and finally hexagonal with rounded lumen (Fig. 3B, 5 & 6). Behind the cephalic framework it again becomes rounded in cross section (Fig. 3B, 7) and constitutes the stylet guide.

#### MORPHOLOGICAL CHANGES DURING POSTEMBRYONIC DEVELOPMENT

Juveniles of the three stages resemble well the adults. Apart from the normal growth, the main changes during postembryonic development affect the following structures :

— *labial area* : less prominent in J2 than in other stages, transverse grooves not discernable.

— *body annules* : in J2 the annules are notably wider in front of the excretory pore than posterior to it, exactly as in the adult. In J3 this difference is not so

pronounced, whereas in J4, the annules are of about the same width all along the body.

— *lateral field* : in J2 the two inner lines are so close together that the lateral field appears as three-lined; in J3 and J4 it is as in the adult.

— *tail and phasmids* : the tail shape is the major difference and allows to recognize immediately the stage to which a juvenile belongs (Fig. 2 A-F). It is long conical in J2, shorter and conoid-spicate in J3, whereas it has a rounded terminus in J4, as in the female. Apart from the shape, also the actual length of the tail varies, the mean value being 46.5  $\mu\text{m}$  in J2, 37  $\mu\text{m}$  in J3 and only 24  $\mu\text{m}$  and 23  $\mu\text{m}$  in J4 and females, respectively. In connection with this, the phasmid position also varies (Fig. 7) : in J2 the phasmid is far posterior to the level of the anus; in J3 and J4 it is close to that level, but generally posterior to it in J3 and anterior to it in J4. In females the phasmids are always anterior to the anal level.

— *excretory pore and hemizonid* : in J2 and J3 the excretory pore is surrounded by the hemizonid, whereas in J4, it is situated anterior to the hemizonid as in the adult but at a shorter distance (see Fig. 6).

Other structures, e.g. the stylet and the pharynx (Fig. 4), show a regular increase in length across the different juvenile stages.

#### VOUCHER SPECIMENS

Deposited in the collection of the Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris, France.

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