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LONGIDORUS EDMUNDSI (NEMATODA, DORYLAIMIDA), A NEW RECORD FOR SOUTH AMERICA

by
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Summary. Soil samples collected from the rhizosphere of coconut palms and *Coccoloba uvifera* shrubs on the beach of Cuyagua on the Caribbean sea in northern Venezuela contained specimens of *Longidorus edmundsi* Hunt *et* Siddiqi, 1977. The Venezuelan population of *L. edmundsi* is biometrically smaller than the type population with the lip region slightly expanded compared with the original specimens in which it is continuous with the rest of the body. Isoelectrofocusing of SOD isoforms showed three main bands with highest SOD activity at pH 6.9. The PCR products of the ITS region was about 2.2 Kb and was digested by ten restriction enzymes but not by Mva I.

Soil samples collected in December 1998 and November 1999 from the rhizosphere of seagrape, *Coccoloba uvifera* (L.) Jacq., shrubs, and coconut palms, *Cocus nucifera* L., on the beach of Cuyaga, Aragua State, on the Caribbean sea in northern Venezuela, contained numerous specimens of *Longidorus*, which were identified as *L. edmundsi* Hunt *et* Siddiqi, 1977.

Records of *Longidorus* are rare in Latin America (Doucet *et al.*, 1998) the only specific report being *L. laevicapitatus* in Columbia (Andrade *et al.*, 1979), the identification of which is not, however, supported by morphometric characters.

Therefore, it is considered useful to illustrate and briefly describe the population of *L. edmundsi* found in Venezuela. The description is also complemented with isozyme and DNA characterization.

Materials and methods

Nematodes were extracted from sand samples by Cobb's wet sieving technique. Specimens for taxonomic studies were killed and fixed in 5% hot formalin, mounted in dehydrated glycerine, and measured with the aid of a camera lucida.

Superoxide dismutase (SOD) isozymes were separated by isoelectrofocusing on lots of 10-20 hand-picked live, active nematodes as specified by Molinari *et al.* (1997).

Nematodes for DNA studies were stored in 1M NaCl solution and frozen until used. DNA extraction was performed on single females cut into pieces under a dissecting microscope and transferred to a 200 µl eppendorf containing 10 µl worm lysis buffer (100 mM KCl, 20 mM Tris-Cl pH 8.3, 2 mM $MgCl_2$, 2 mM DTT, 4.5%

Tween 20) and 2 µl proteinase K (600 µg/ml). The total volume of the reaction was 20 µl. Eppendorfs were incubated at 65 °C for one hour and at 95 °C for ten minutes. They were then centrifuged (12,000 g) for about 30 seconds. The ITS of the ribosomal gene cluster was amplified by PCR performed in a PTC-100 thermocycler (MJ Research Inc.) with 1.5 mM Mg^{2+}, 5 µl 10X *Taq* buffer, I U *Taq* DNA polymerase, 200 µM of each dNTP (*Taq* PCR Core Kit, Qiagen, Germany), 10 µl DNA, 0.5 µM of each primer. The total reaction volume was 50 µl. Primers used in this study were: forward primer TW81 (5'-GTTTCCGTAGGTAACCTGC-3') and reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Joyce *et al.*, 1994). The thermocycling profile started with an initial denaturation of two minutes at 92 °C, followed by 35 cycles at 92 °C for 30 seconds denaturing, 55 °C for 40 seconds annealing and 72 °C for two minutes extension with an additional ten minutes extension at 72 °C. Five microliter PCR product was run on a 1% agarose gel. Eleven restriction enzymes (*Tru*9I, *Mva*I, *Kpn*I, *Hae*III, *Cfo*I, *Alu*I, *Mbo*I, *Dde*I, *Hinf*I, *Rsa*I and *Msp*I) were used to digest the PCR products obtained. RFLP patterns were obtained by running the digestion products on a 2% agarose gel for two hours.

Results

The morphometrics of the Venezuelan population of *Longidorus edmundsi* Hunt *et* Siddiqi, 1977 are listed in Table I, and illustrated in Figs 1 and 2.

Dead females have a J posture and a medium sized cylindrical body which is of uniform diameter thoughout its length. Lip region truncate, slightly expanded, less in dorso-ventral view. Amphidial pouches wide, slightly bilobed with an obscure opening. Vulva at mid-body; vagina occupying more or less 1/2 of the corresponding diameter; uteri filled with large sperms; amphidelphic, genital system with almost equally developed branches; ovaries reflected. Tail short, hemielliptical, bearing two caudal pores on each side.

Male with coiled posterior region. Testes well developed and apparently functional, containing many sperms. Spicules robust, strongly bent ventrally. The adanal pair of supplements is preceeded by a row of 8 to 13 ventromedian supplements.

Juvenile development stages separate into four groups (Fig. 3). Their body is less arcuate compared to the female, expecially in the first two stages that have a more elongated tail.

Some specimens were invaded by *Pasteuria*-like organisms (Fig. 2).

The Venezuelan population of *L. edmundsi* was compared with paratypes (Fig. 4). Morphologically it is very similar to the type population except for the lip region, which in the paratypes is continuous with the rest of the body. However, all paratypes examined were in a dorso-ventral position and as in the Venezuelan specimens the post labial slight depression is much less evident.

Biometrically the Venezuelan population of *L. edmundsi* is smaller than the original populations from the Windward Islands (Hunt and Siddiqi, 1977).

On the basis of such consideration, the identification code for *L. edmundsi* in the polytomus key of Chen *et al.* (1997) should be modified as it follows:

A3/4, B5, C2, D 3/4 (1 in Chen *et al.*, 1997), E1 (2/3 in Chen *et al.*, 1997), F 2/3, G 2/3, H1, I2.

Isoelectrofocusing of SOD isoforms of *L. edmundsi* showed three main bands (Fig. 5a, b). The highest SOD activity occurred at pH 6.9, which is a change isoform also observed in *L. elongatus*, but this species can be easily differentiated from *L. edmundsi* by the isoelectric point of its other two SOD activity bands.

The PCR product of the ITS region of *L. edmundsi* was about 2.2 Kb and it was digested by ten restriction enzymes, but not by Mva I (Table II; Fig. 6).

TABLE I - *Morphometrics of* Longidorus edmundsi *from Venezuela.*

n	12 ♀	10 ♂	1 J_1	15 J_2	16 J_3	10 J_4
L mm	4.8±0.51 4.2-5.7	4.8±0.52 4.0-5.7	1.0	1.6±0.16 1.4-1.9	2.2±0.19 1.9-2.6	3.3±0.27 3.0-3.8
a	97.0±12.77 82.3-125.3	107.2±9.36 88.4-121.3	34	43.3±3.06 39.7-49.7	53.7±3.93 49.7-63	80.3±8.16 69-92.2
b	12.4±1.84 10.6-16.0	12.4±1.52 9.7-14.7	4.2	5.7±0.70 4.6-6.8	7.3±0.86 6.2-9	10±0.62 9-11
c	170±25.62 141.5-218.6	139.7±24.00 88.4-170	63	65.4±5.51 56.7-76.6	83.6±7.03 72.5-94.2	118.9±12.96 102-137.7
c'	0.8±0.08 0.6-0.9	1.0±0.05 0.9-1.0	0.9	0.9±0.06 0.7-0.9	0.8±0.06 0.7-0.9	0.8±0.07 0.7-0.9
V	49±1.29 48-52	–	–	–	–	–
Odontostyle µm	99.2±4.55 94.0-107	99±6.38 90.6-110.6	65.3	72.6±3.49 67.6-80	83.7±3.11 80-90	89±2.98 85.3-92.3
Odontophore µm	58.3±1.86 54.7-60.6	58.3±2.33 56-61.8	38.2	43.7±1.12 41.2-45.3	47.3±1.55 44-48.8	50.2±1.00 49-53
Replacement odontostyle µm	–	–	74	85.7±2.53 80.6-89.4	93.7±4.51 86.5-99.4	97±4.28 93-104
Oral aperture to guide ring µm	23.4±2.18 21.0-27.6	23.6±1.43 22.3-27	13.5	17.9±0.84 17-20.6	19.2±0.87 17.6-20.6	20.3±0.91 19-22
Tail µm	28.3±2.65 24.7-32.9	33.4±2.50 29.4-38.2	15.9	25±1.53 23.5-28.2	26.4±1.67 23.5-28.8	27.7±1.99 23-29.4
J (hyaline portion of tail) µm	9.7±0.77 8.2-10.6	7.3±0.80 6.0-8.8	5.9	7.3±0.56 6.5-8.2	7.6±0.66 7-8.8	7.8±0.75 7-8.8
Body diam. at lip region µm	27±1.89 24.7-30.6	27.5±2.14 24.5-30.6	16	21±0.83 19.4-22.3	23±1.43 20.6-26	24.5±1.60 22-26.5
Body diam. at guide ring µm	29.3±2.08 26.5-32.3	29.6±2.57 26.0-33.5	18.2	24.3±1.07 22.9-26.6	26.2±2.25 22.3-30.6	27±1.94 23.5-29.4
Body diam. at base of oesophagus µm	42.6±3.52 36.5-48.2	41±2.86 37-44.7	27	35±2.25 31.2-38.8	37.7±2.35 33.5-41.2	38±1.98 35.3-41
Body diam. at mid-body or vulva µm	49.4±3.47 44-54.7	45±2.35 41.2-47	29.4	37.8±2.21 34-41.2	41±2.34 38.2-44	41.4±2.26 38.2-45
Body diam. at anus µm	37±2.81 31.2-40.6	35.8±2.66 33-41.2	17.6	28.9±2.48 26.5-33.5	32±2.38 27.6-35	33.8±2.68 29.4-36.5
Body diam. at beginning of J µm	25.8±2.10 20.6-29.4	19.7±1.55 17.6-22.3	11.8	18.0±1.65 16.5-20.6	20.4±2.15 16.5-22.3	22±1.91 18.8-23.5
Spicules µm	–	74.3±4.76 67.6-79	–	–	–	–

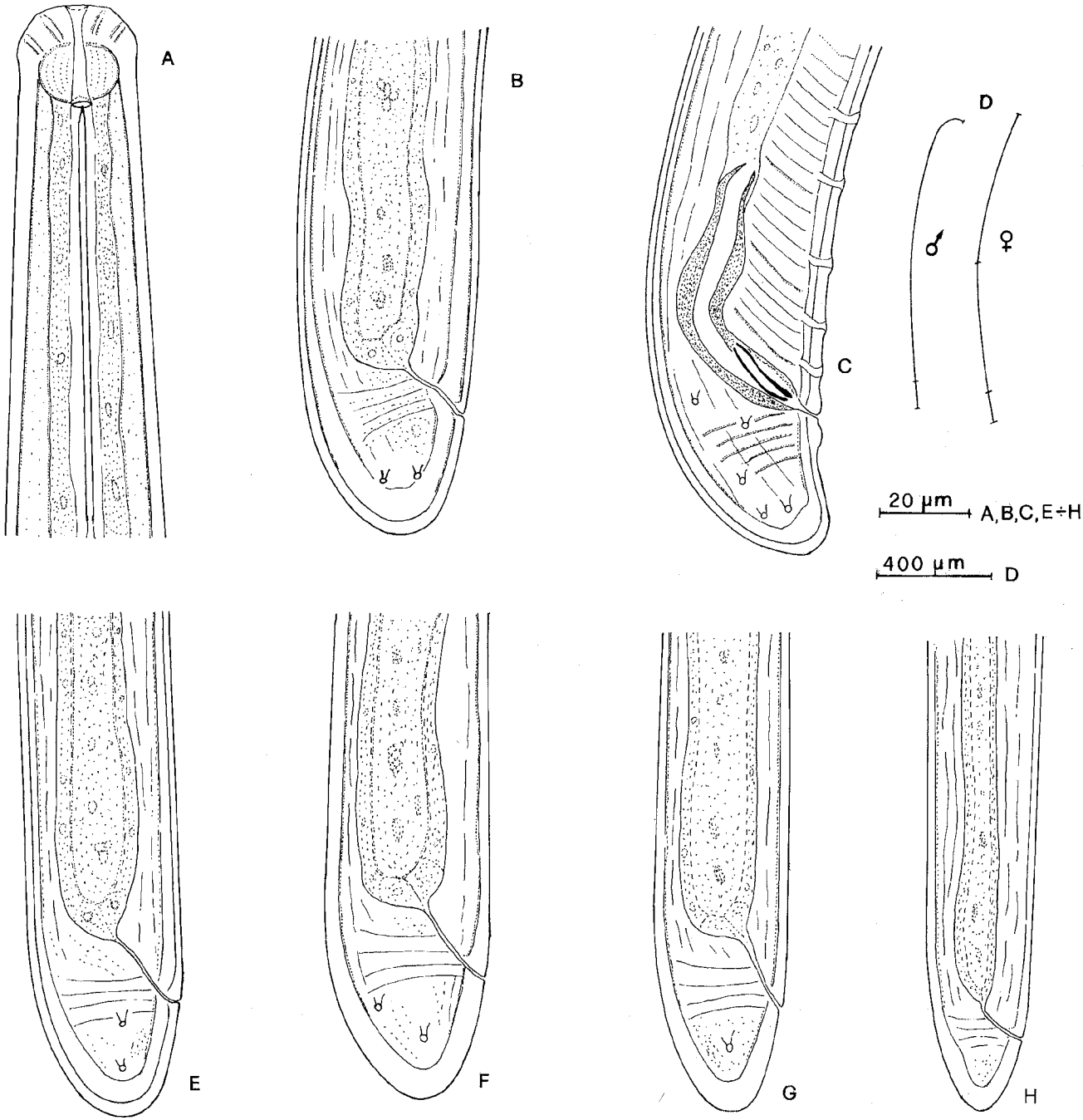

Fig. 1 - *Longidorus edmundsi* from Venezuela: A, female anterior region; B, female posterior region; C, male posterior region; D, *habitus*; E-H, posterior region of fourth, third, second and first juvenile stages, respectively.

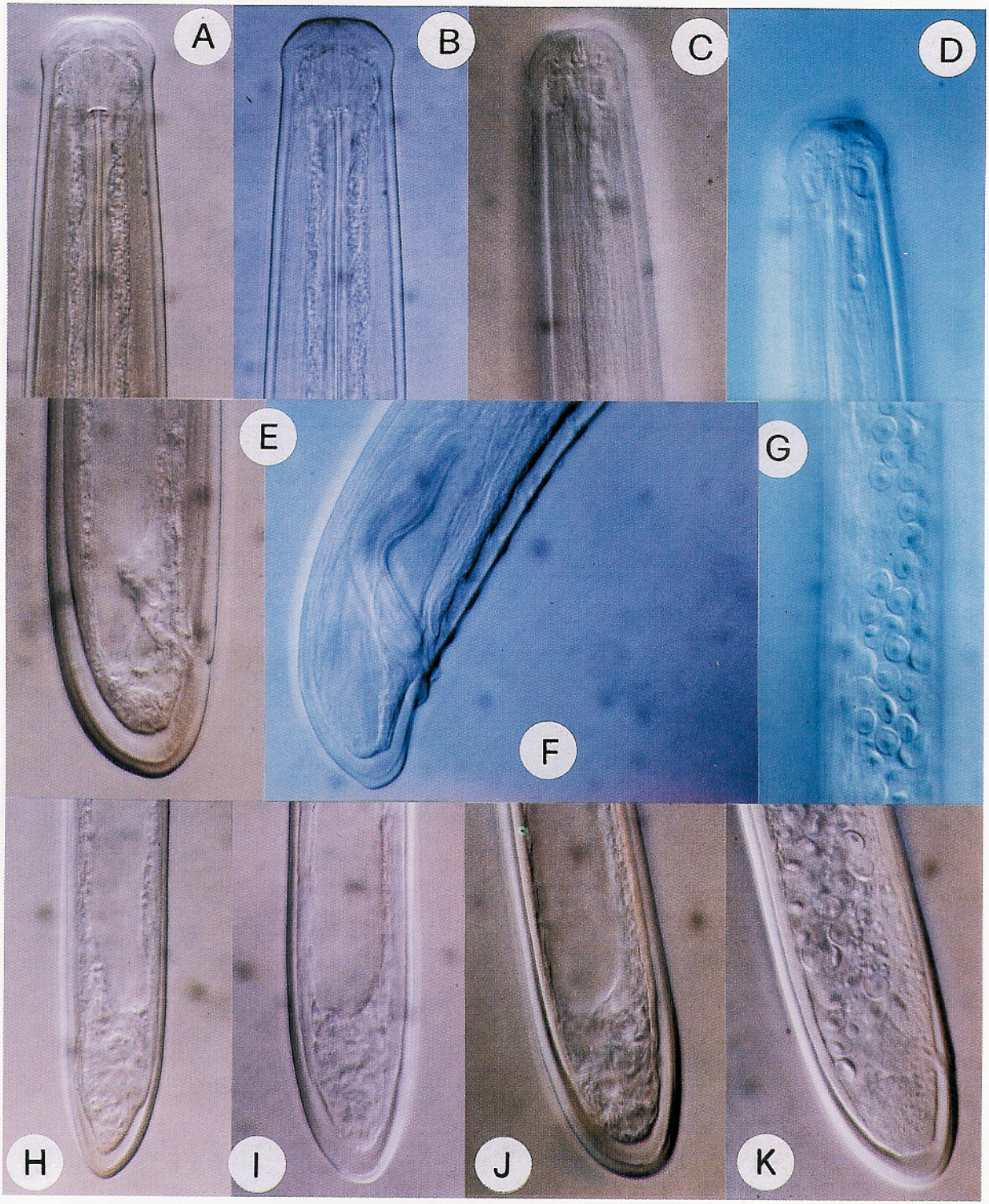

Fig. 2 - Photomicrographs of *L. edmundsi* from Venezuela: A and B, female anterior region; C and D, female anterior region in dorso-ventral view; E, female posterior region; F, male posterior region; G, specimen with *Pasteuria* - like organisms; H-K, first, second, third, and fourth juvenile stage tails, respectively.

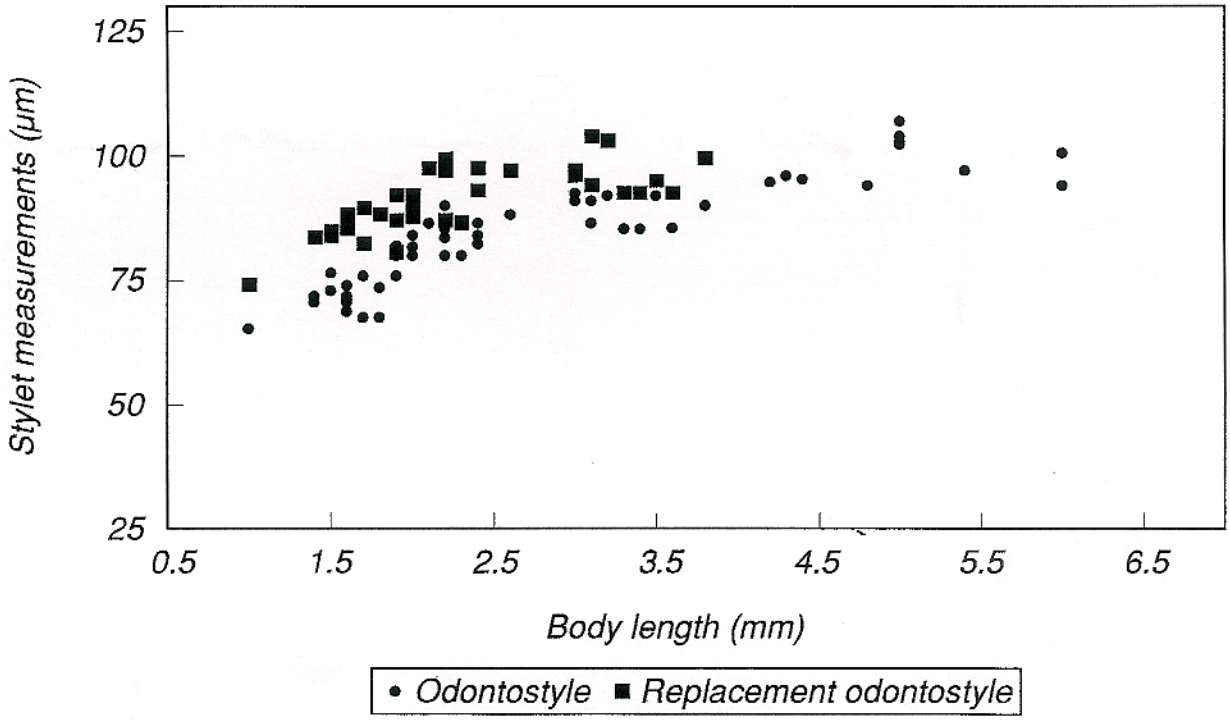

Fig. 3 - Scatter diagram plotting body and odontostyle length of individual juveniles and females of *L. edmundsi* from Venezuela.

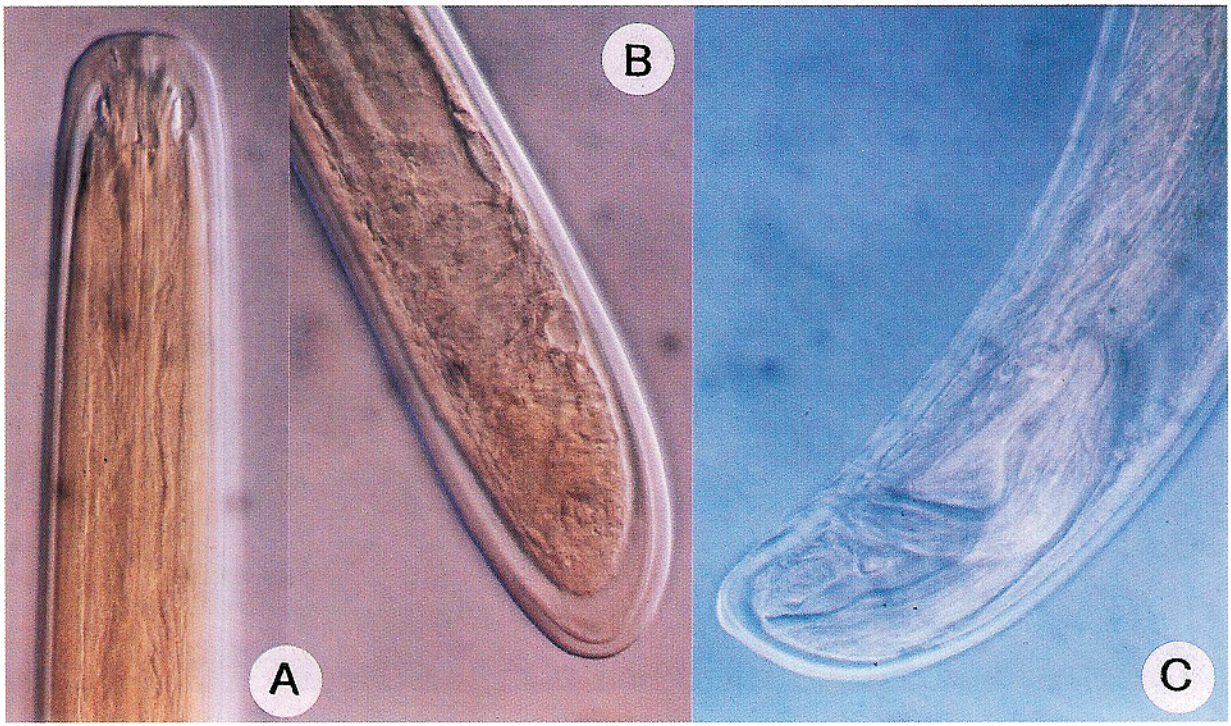

Fig. 4 - Photomicrographs of paratypes of *L. edmundsi*: A, male anterior region; B, female posterior region; C, male posterior region.

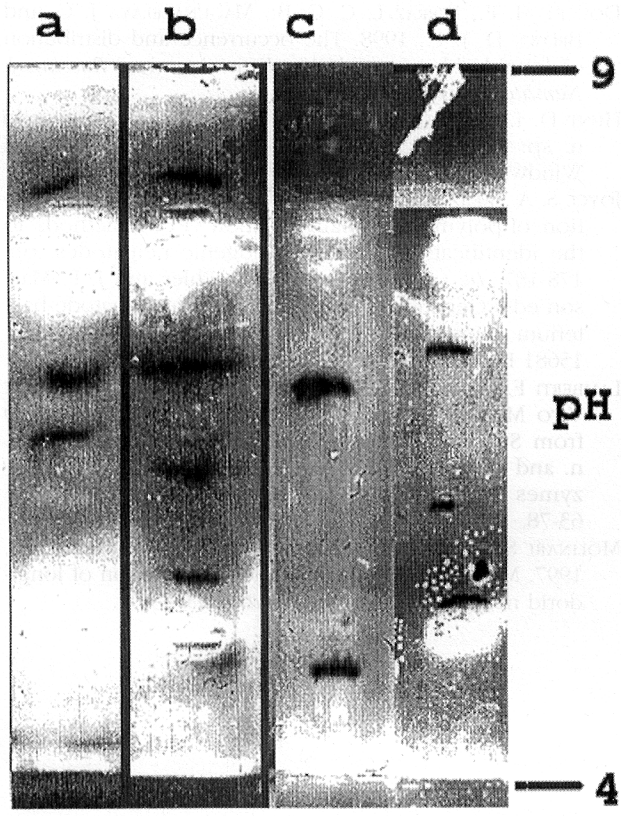

Fig. 5 - Isoelectrofocusing of nematode extracts from different *Longidorus* species. Gels were stained for SOD activity, scanned, turned into negative images and printed. SOD activity appears as black bands over a brighter background: a, *L. edmundsi* from coconut palms in Venezuela (juveniles); b, *L. edmundsi* from seagrape in Venezuela (adults); c, *L. pauli* (Lamberti *et al.*, 1999); d population of *L. elongatus* from Scotland.

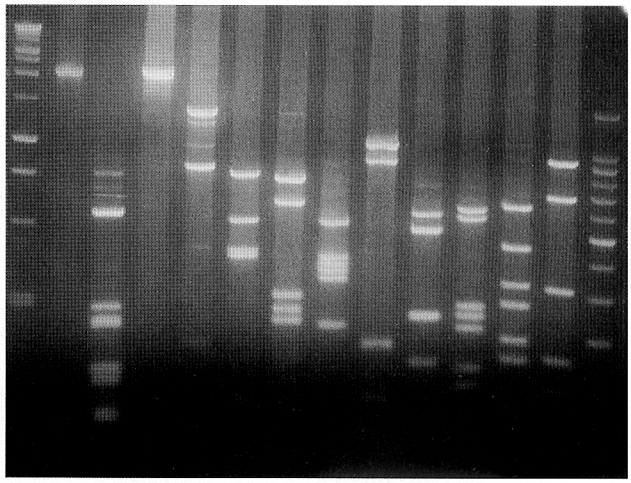

Fig. 6 - RFLP pattern of *L. edmundsi* from Venezuela: from left to right respectively, lane 1 to 14 are 1 Kb ladder, complete ITS PCR products, about 2.2 Kb, patterns for Tru 9I, MvaI, KpnI, HaeIII, CfoI, AluI, MboI, DdeI, HinfI, RsaI, MspI, and 100bp ladder.

The occurrence of *L. edmundsi* in northern Venezuela on a beach of the Caribbean sea is not considered unusual as previous records of this species refer to St. Lucia and Dominica in the West Indies (Hunt and Siddiqi, 1977).

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TABLE II - *Bands sizes (bp) for all RE digestions used in the experiment.*

Band size	1	2	3	4	5	6	7	8	9
Restriction Enzyme									
*Tru*9I	550	550	250	230	210	130	110	130	30
*Mva*I	No digestion								
*Kpn*I	1350	850							
*Hae*III	800	550	410	390	50				
*Cfo*I	770	630	290	260	220	30			
*Alu*I	550	390	380	340	320	220			
*Mbo*I	1080	900	170	50					
*Dde*I	580	520	520	250	250	80			
*Hinf*I	620	580	270	240	220	150	120		
*Rsa*I	650	450	350	280	200	150	50		
*Msp*I	950	690	310	150	100				

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