Nematol. medit. (1999), 27: 83-93

[1] *Istituto di Nematologia Agraria, C.N.R., 70126 Bari, Italy*
[2] *Laboratorio de Nematología Agrícola, Instituto de Zoología Agrícola, Universidad Central de Venezuela, Apdo. 4579, Maracay - 2101-A, Venezuela*

TWO SPECIES OF *XIPHIDORUS* MONTEIRO (NEMATODA: DORYLAIMIDA): NEW RECORDS FOR VENEZUELA

by

F. LAMBERTI [1], R. CROZZOLI [2], S. MOLINARI [1], F. DE LUCA [1], A. AGOSTINELLI [1] and N. GRECO [1]

Summary. *Xiphidorus amazonensis* Uesugi, Huang *et* Cares and *X. minor* Rashid, Coomans *et* Sharma constitute new records for Venezuela. *X. amazonensis* is reported for the first time since its description from Brazil. It is characterized by a body length of 5 mm, odontostyle length 97 µm, mid-body vulva, offset lip region and conoid tail. *X. minor*, which also occurs in Argentina, Brazil and Uruguay, is characterized by a body length of 2 mm, odontostyle length 68 µm, slightly anterior vulva, continuous lip region and conoid tail. *Xiphidorus minor* is also characterized by two acidic bands in its superoxide dismutase isozyme profile. PCR-RFLP analysis are reported for *X. minor*.

The genus *Xiphidorus* Monteiro, 1976 seems to be restricted to South America in its geographic distribution (Doucet *et al.*, 1998). According to Decramer *et al.* (1996) species of *Xiphidorus* have been recorded only in Argentina, Brazil and Uruguay, and Hunt (1993) states of having seen an undescribed species from Bolivia.

During a nematode survey in Venezuela two species of *Xiphidorus* were identified; they constitute new records for the country, although the occurrence of the genus in Venezuela has been reported by Renaud and Briceño in the XV Congreso Venezolano de Fitopatologia held in Maracaibo from the 23th to the 27th of November 1997 (Compendio p. 106).

The two populations identified here as *X. amazonensis* Uesugi, Huang *et* Cares, 1985 and *X. minor* Rashid, Coomans *et* Sharma, 1986 are briefly described.

Nematodes were extracted from soil by the wet sieving technique and mounted in anhydrous glycerine after fixation in hot 5% formalin.

Descriptions

XIPHIDORUS AMAZONENSIS

(Table I; Figs 1-3)

A population composed by females and juveniles, males did not occur, was found at San Fernando in the Santa Luisa Farm, Apure State, in the rhizosphere of an old tamarind tree, *Tamarindus indica* L.

Female of medium size (approx 5 mm long), assuming a more or less closed C *habitus* when dead; body tapering gradually towards anterior. Lip region cylindrical laterally rounded, separated from the rest of the body by a depression; amphidial pouch as a wine glass, with barely visible slit-like aperture. Odontostyle long, forked at the base; odontophore strongly flanged, guide ring single. Oesophagus dorylaimoid with three nuclei in the basal bulb, which measures 55-72 µm long and 15-17 µm wide and occupies about 1/5 of the oesophagus total

TABLE I - *Morfometrics of* Xiphidorus amazonensis *from Venezuela.*

Locality:	Santa Luisa, San Fernando				
Host:	Tamarind				
n:	15 ♀	9 J1	7 J2	8 J3	6 J4
L (mm)	5.1±0.30 (4.5-5.6)	1.1±0.04 (1-1.1)	1.7±0.10 (1.6-1.8)	2.3±0.21 (2-2.5)	3.8±0.23 (3.5-4.1)
a	146±8.17 (132.6-158.8)	58±1.48 (55.9-59.5)	69.4±2.09 (65.8-72.6)	89.9±4.99 (82.3-96)	125.5±8.44 (110-132.2)
b	16.6±1.30 (15.1-18.6)	5.1±0.24 (4.8-5.4)	7.4±0.43 (6.9-7.9)	10.2±0.73 (9.3-11.2)	14.9±1.00 (14.0-16.9)
c	184.9±9.52 (172.9-203.8)	35.2±3.54 (30.4-40.4)	49.1±4.20 (43.9-54.7)	68.9±4.64 (63.4-75.5)	118.6±9.08 (106.4-128.9)
c'	1.2±0.07 (1-1.3)	2.7±0.09 (2.5-2.8)	2.3±0.15 (2.1-2.5)	1.9±0.05 (1.8-2.0)	1.5±0.04 (1.4-1.5)
V	51±1.40 (48-53)	–	–	–	–
Odontostyle µm	98.1±2.53 (95.4-102.3)	50.8±2.59 (47.9-54.3)	62.5±1.83 (60.1-65.9)	72.3±2.52 (69.4-76.9)	85.8±2.61 (82.7-89.6)
Odontophore µm	43.4±1.76 (40.5-45.7)	27.9±1.73 (26-30.6)	32.2±0.63 (31.2-32.9)	35.9±1.50 (33.5-37.6)	39.5±1.21 (37.6-40.5)
Replacement odontostyle µm	–	62.6±2.40 (58.4-66.5)	74.5±1.86 (72.2-78)	84.1±1.69 (80.9-86.7)	97.5±1.42 (95.9-100)
Oral aperture to guide ring µm	83.5±1.68 (80-86.7)	38.8±1.06 (37.6-41)	47.2±2.57 (43.5-50.9)	59.5±3.95 (54.9-64.7)	67.1±3.86 (61.8-71.7)
Tail µm	27.3±1.32 (26-29.5)	30.4±2.29 (27.2-32.9)	34.3±2.13 (30.6-36.4)	33.9±2.13 (30.6-37)	31.5±1.63 (28.3-32.9)
J (hyaline portion of tail) µm	7.9±0.76 (6.4-8.7)	4.2±0.34 (3.7-4.6)	5.3±0.32 (5-5.8)	5.9±0.66 (5.2-6.9)	6.7±0.68 (5.8-7.5)
Body diam. at lip region µm	11.7±0.34 (10.9-12.1)	7.2±0.32 (6.9-7.5)	8.4±0.32 (8.1-8.7)	9.1±0.18 (8.7-9.2)	10.8±0.48 (10.4-11.6)
Body diam. at guide ring µm	26.3±0.82 (24.8-27.7)	15.2±0.52 (14.4-16.2)	18.3±1.11 (17.3-20.2)	20.8±0.62 (20.2-21.9)	23.5±1.09 (21.9-24.8)
Body diam. at base of oesophagus µm	30.5±1.11 (28.9-31.8)	17±0.56 (16.2-17.9)	21.9±0.68 (21.4-23.1)	24.5±1.19 (23.1-26.6)	27.6±1.13 (26-28.9)
Body diam. at mid-body or vulva µm	34.8±1.31 (32.9-36.9)	18.6±0.47 (17.9-19.1)	24.3±0.80 (23.1-25.4)	25.9±1.16 (24.3-27.7)	30±1.39 (28.8-31.8)
Body diam. at anus µm	23.7±1.00 (21.9-24.8)	11.4±0.65 (10.4-12.1)	14.9±0.61 (14-15.6)	17.6±0.86 (16.2-19)	21.8±0.41 (21.4-22.5)
Body diam. at beginning of J µm	11.8±0.75 (10.4-13)	4.8±0.44 (4.5-5.8)	5.8±0.00 (5.8-5.8)	6.8±0.59 (5.8-7.5)	9.3±0.54 (8.7-9.8)

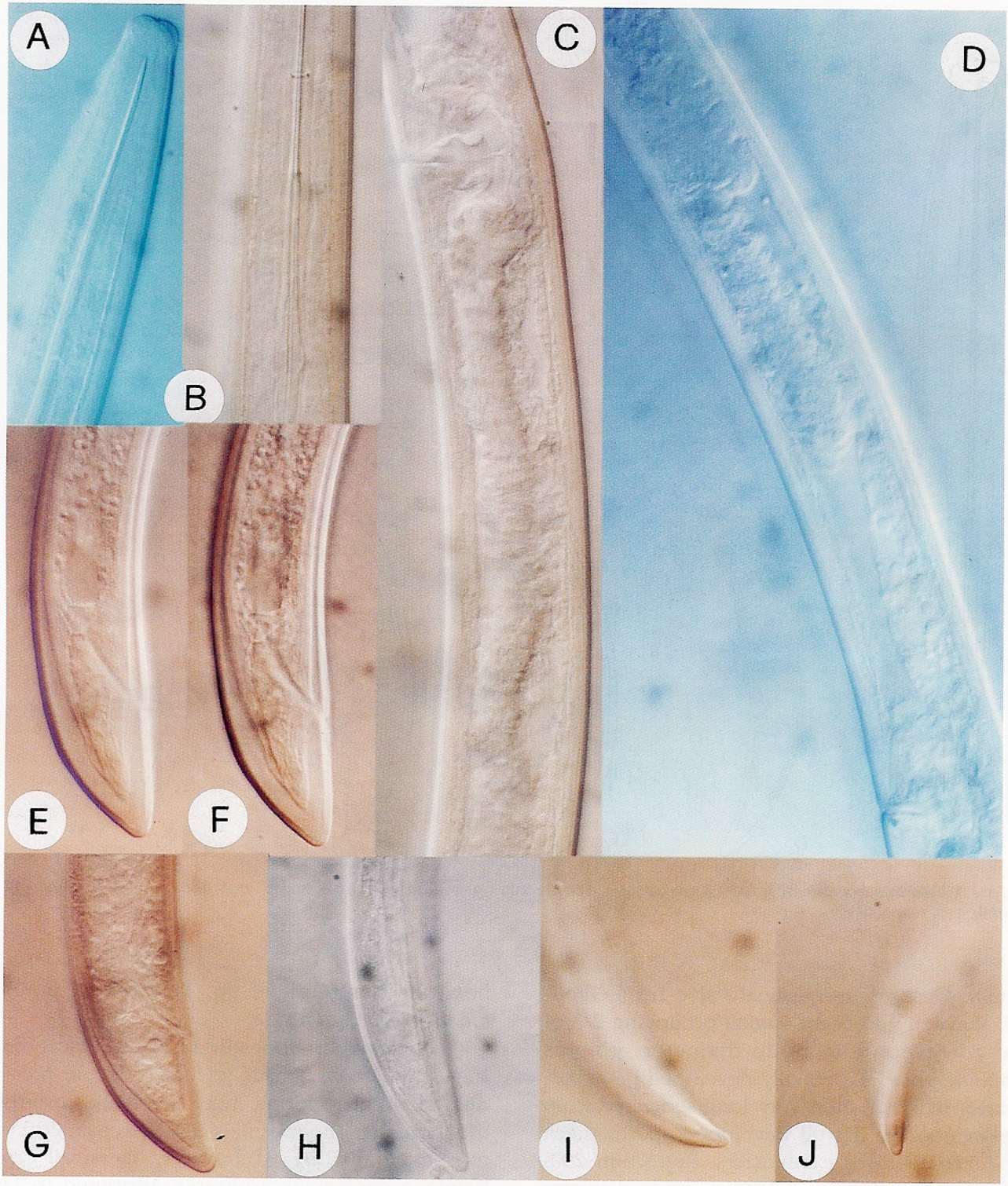

Fig. 2 - Photomicrographs of *X. amazonensis* from Venezuela: A, female anterior region; B, guide ring, forked base of the odontostyle and odontophore; C, vulva region and posterior uterus; D, spermatheca; E and F, female tail; G-J, J4, J3, J2, J1 juvenile tail, respectively.

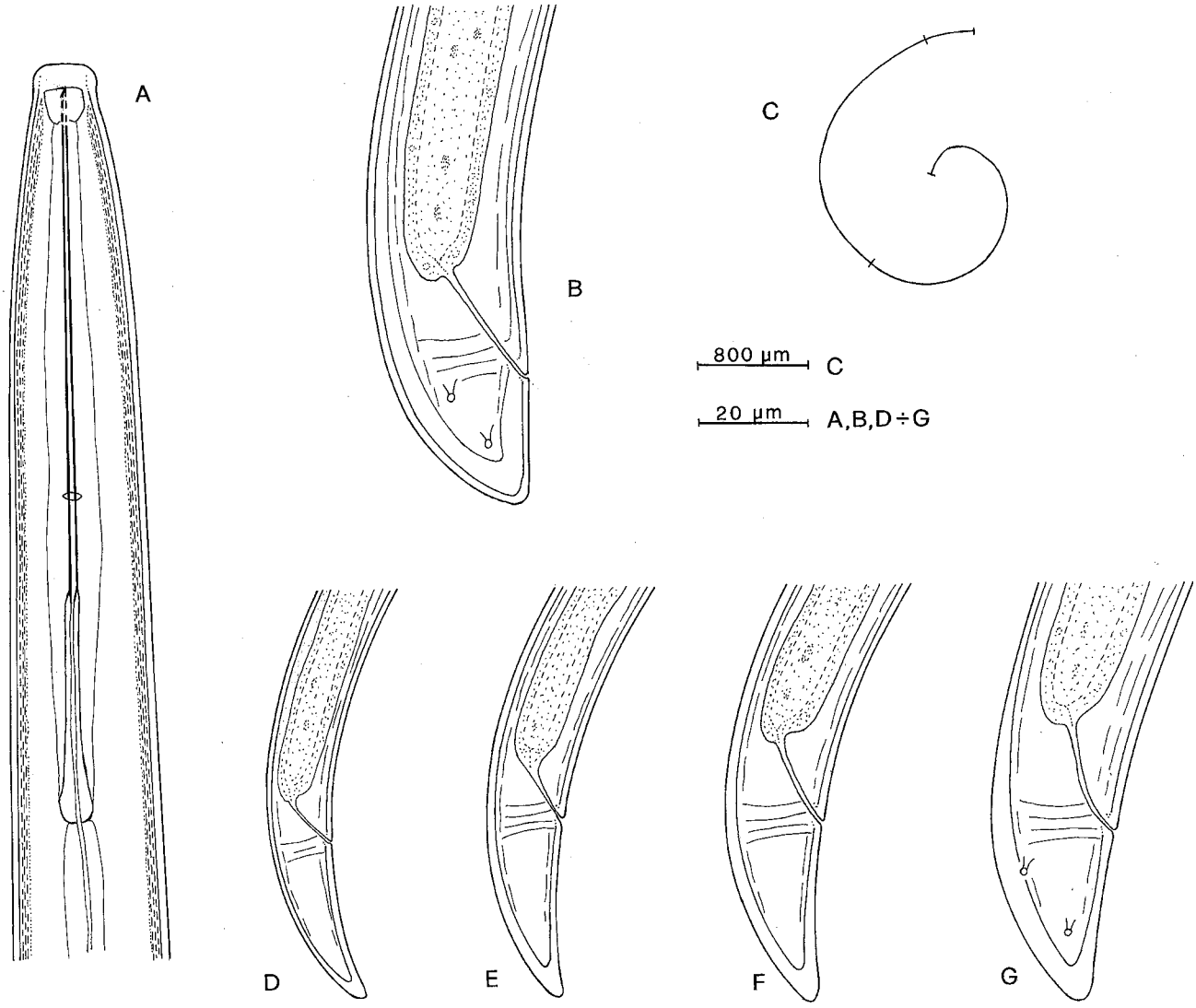

Fig. 1 - *Xiphidorus amazonensis* from Venezuela: A, female anterior region; B, female tail; C, *habitus*; D-G, J1, J2, J3 and J4 juvenile tail, respectively.

length. Oesophago-intestinal valve hemi-elliptical. Vulva at mid-body; vagina occupying 2/3 of the corresponding body diameter; gonads amphidelphic with equally developed branches; ovaries reflexed; uteri very long separated from the oviduct by a well developed spermatheca delimited by strong sphincters. Prerectum 290-340 µm long; rectum as long as body diameter at anus. Tail short, conoid with wide base, tapering abruptly towards the terminus, which, however, has rounded tip. Two caudal pores are visible on each side.

Juveniles with elongate tail in the first three stages; tail more similar to female in the preadult, although tapering more gradually towards the terminus. The juveniles can be separated into four groups (Fig. 3), however, the small gap between specimens arbitrarily attributed to J2 and J3 might be an indication that only three juvenile stages occur.

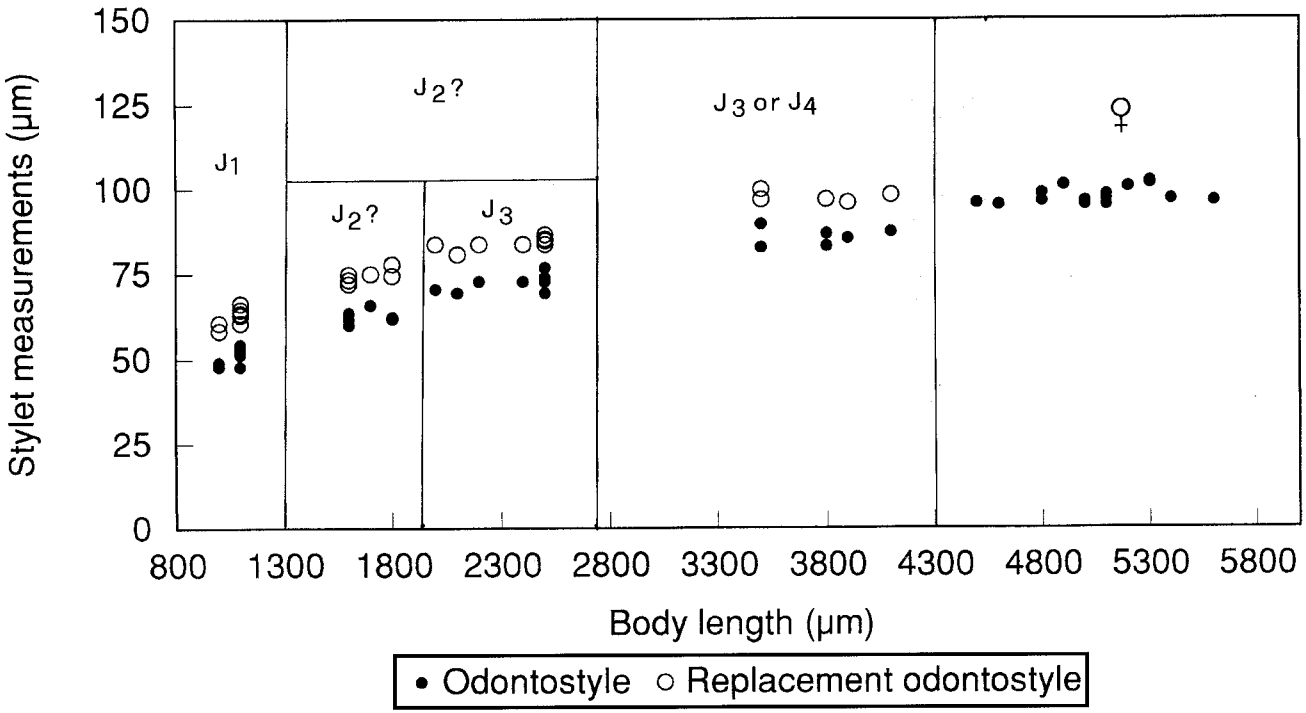

Fig. 3 - Scatter diagram plotting body and odontostyle lengths of individual juveniles and females of *X. amazonensis* from Venezuela.

Compared to the type population (Uesugi *et al.*, 1985), *X. amazonensis* from Venezuela has a slightly shorter body (5.35 mm in type population) and slightly anterior vulva (V around 50% in the type population).

This is the first record of *X. amazonensis* outside Brazil.

XIPHIDORUS MINOR

(Table II; Figs 4-8)

The population studied was found in the rhizosphere of ground orchids, *Oeceoclades maculata* (Lindl.) Lindl., on the Campus of the Universidad Central de Venezuela at Maracay, Estado Aragua. Females and juveniles were present; males were not present.

Female of small size (approx 2 mm long), assuming a closed C to spiral habitus, when dead; body tapering gradually towards both extremities. Lip region frontally rounded, almost continuous with the rest of the body, distinguished only by a very faint constriction. Amphidial pouch as a wine glass, with indistinct aperture. Odontostyle forked at its base; odontophore moderately flanged, guide ring single. Oesophagus dorylaimoid; the basal bulb contains three nuclei and measures 60-65 µm long and 13-15 µm wide, occupying 1/5 of the oesophagus total length. Oesophago-intestinal valve amorphus. Vulva slightly anterior to mid-body; vagina occupying 1/2 of the corresponding body diameter; gonads amphidelphic, and reflexed ovaries with equally developed branches; very short uteri, separated from the oviduct by a sphincter. Prerectum 230-260 µm long; rectum as long as body diameter at anus. Tail conoid, dorsally convex and ventrally flattened, with rounded terminus, bearing two caudal pores on each side.

Juveniles with more elongate tail, compared with females; in particular the tail of the first

TABLE II - *Morfometrics of* Xiphidorus minor *from Venezuela.*

Locality:	Maracay U.C.V. Campus				
Host:	Ground orchid				
n:	20 ♀	3 J1	1 J2	3 J3	13 J4
L (mm)	1.8±0.17 (1.5-2.1)	0.75±0.05 (0.7-0.8)	1.0	1.3±0.06 (1.2-1.3)	1.45±0.05 (1.4-1.5)
a	64.7±4.83 (58-75.2)	40.1±2.17 (37.8-42.1)	46.7	54.7±1.70 (52.9-56.3)	54.9±2.20 (49.5-57.7)
b	7.6±0.78 (6.5-9)	4.4±0.17 (4.3-4.6)	5.3	7.2±1.58 (5.8-8.9)	7±0.63 (6-8.1)
c	75.6±7.04 (63.3-90.9)	27.4±0.98 (26.3-28.2)	39.4	46.5±3.29 (43.5-50)	53.1±3.48 (48.4-57.7)
c'	1.3±0.06 (1.2-1.4)	2.6±0.06 (2.5-2.6)	1.8	1.8±0.17 (1.7-2)	1.6±0.10 (1.4-1.7)
V	46.1±1.41 (44-48)	–	–	–	–
Odontostyle µm	69.4±2.32 (65.9-73.4)	42±2.33 (39.9-44.5)	50.5	57.2±3.00 (53.7-58.9)	60.7±1.30 (57.8-63)
Odontophore µm	33.8±1.88 (30.6-38.1)	23.9±2.33 (21.4-26)	27.8	27.8±0.57 (27.2-28.3)	30.1±0.97 (28.9-31.8)
Replacement odontostyle µm	–	50.5±0.86 (49.7-51.4)	55.5	65.5±3.32 (61.8-68.2)	70±2.48 (65.5-73.9)
Oral aperture to guide ring µm	60.5±1.93 (56.6-64.7)	34.1±1.04 (33.5-35.3)	40.5	46.4±0.87 (45.7-47.4)	50.9±2.61 (46.2-54.9)
Tail µm	23.8±1.16 (21.4-25.4)	27.4±1.33 (26.6-28.9)	25.4	27.5±1.33 (26-28.3)	27.5±1.77 (24.3-30.1)
J (hyaline portion of tail) µm	5.5±0.67 (4.6-6.9)	3.7±0.29 (3.5-4)	3.5	4.2±0.35 (4-4.6)	4.5±0.49 (4-5.8)
Body diam. at lip region µm	7.9±0.39 (7.5-8.7)	6±0.29 (5.8-6.3)	6.9	7±0.00 (7-7)	7.1±0.30 (7-7.5)
Body diam. at guide ring µm	19.4±0.86 (17.9-20.8)	13.5±0.92 (12.7-14.5)	15.6	15.8±0.35 (15.6-16.2)	17.5±0.57 (16.8-19.1)
Body diam. at base of oesophagus µm	24.9±1.51 (22.5-28.3)	16.8±0.55 (16.2-17.3)	20.2	20.6±0.92 (19.6-21.4)	23.6±0.92 (22.5-25.4)
Body diam. at mid-body or vulva µm	27.8±1.95 (24.3-32.9)	18.7±0.29 (18.5-19)	21.4	22.5±1.53 (20.8-23.7)	26.4±1.06 (25.4-28.3)
Body diam. at anus µm	17.7±1.04 (15.6-19.6)	10.8±0.69 (10.4-11.6)	14.5	15.4±1.46 (13.9-16.8)	17.2±0.52 (16.2-18.5)
Body diam. at beginning of J µm	9.1±0.62 (8-9.8)	4±0.55 (3.5-4.6)	5.8	6.4±0.55 (5.8-6.9)	7.5±0.78 (6.3-8.7)

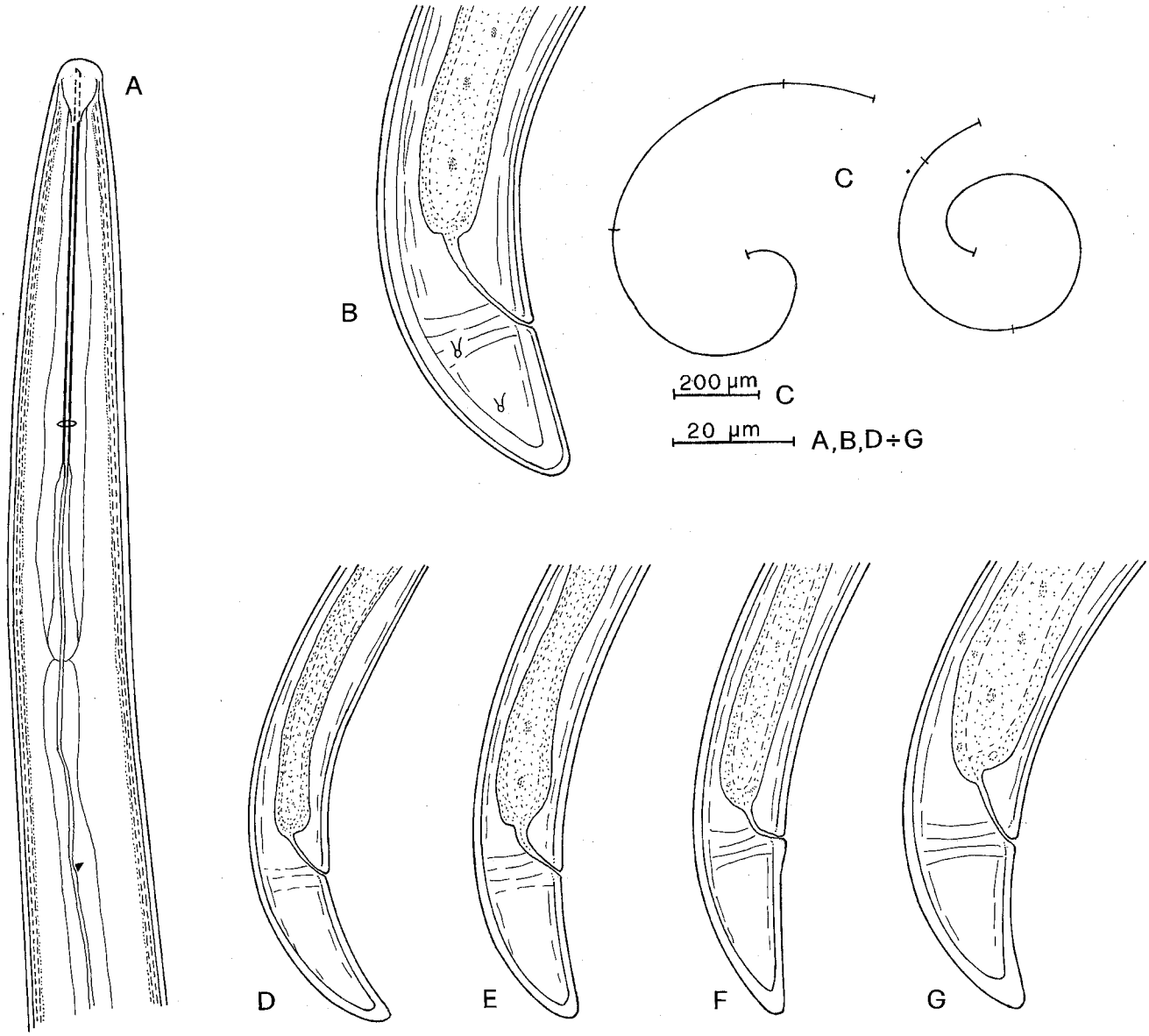

Fig. 4 - *X. minor* from Venezuela: A, female anterior region; B, female tail; C, *habitus*; D-G, J1, J2, J3, and J4 juvenile tail, respectively.

stage is ventrally slightly concave and has a more pointed tail. Juveniles can be separated into four groups (Fig. 6), however, it seems that there is an overlap between the J3 and J4 stages, perhaps indicating the presence only of three juvenile stages.

Compared to the type population (Rashid *et al.*, 1986), *X. minor* from Venezuela has a shorter odontostyle (73-79 µm in type population) and an anterior guide ring (68 µm in type population). With respect to the specimens from Uruguay (Coomans *et al.*, 1996), it has a shorter

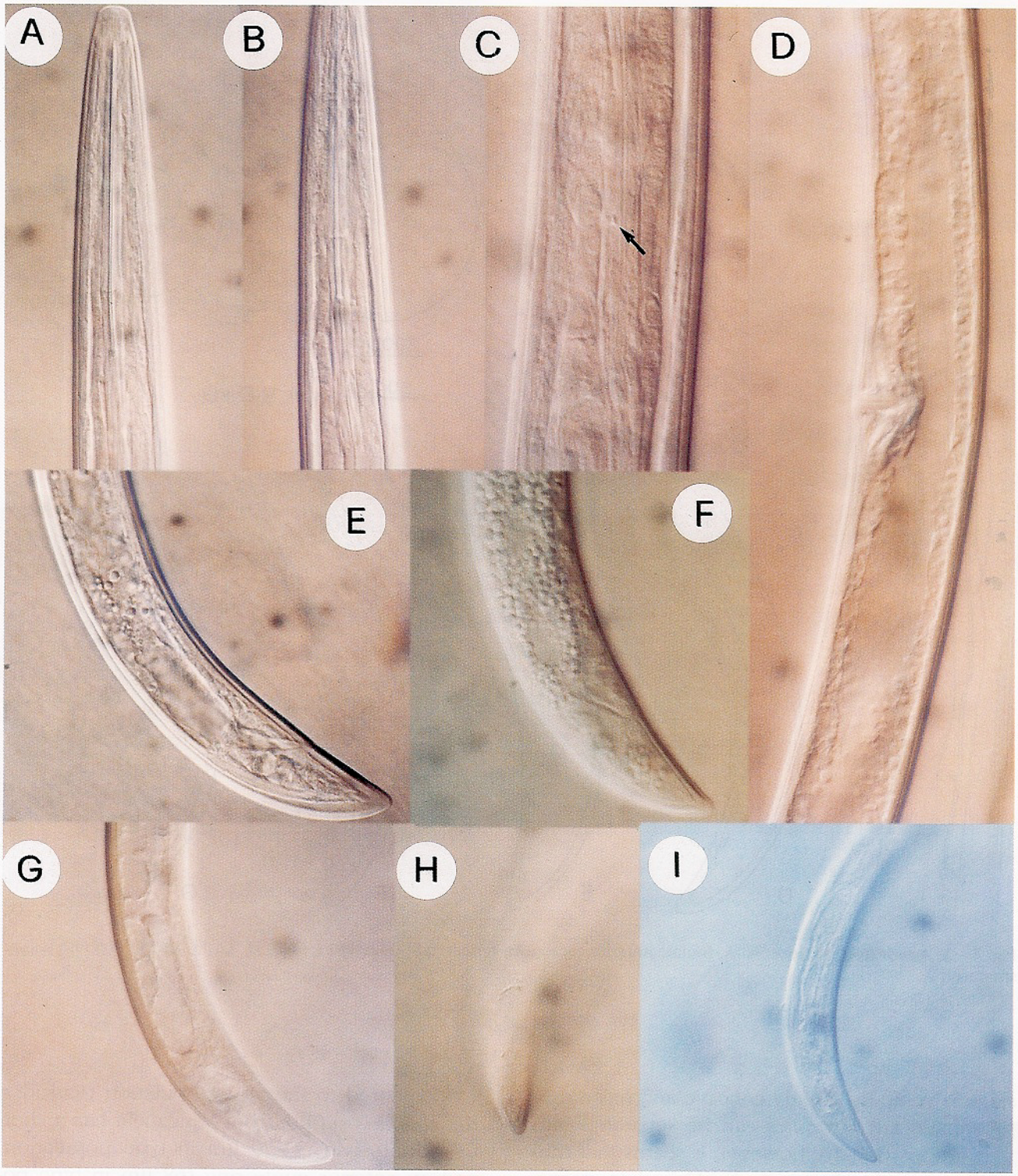

Fig. 5 - Photomicrograph of *X. minor* from Venezuela: A, female anterior region; B, guide ring, forked base of the odontostyle and odontophore; C, mucro in the tubular portion of the oesophagus; D, vulva region with uteri; E, female tail; F-I, J4, J3, J2, and J1 juvenile tail, respectively.

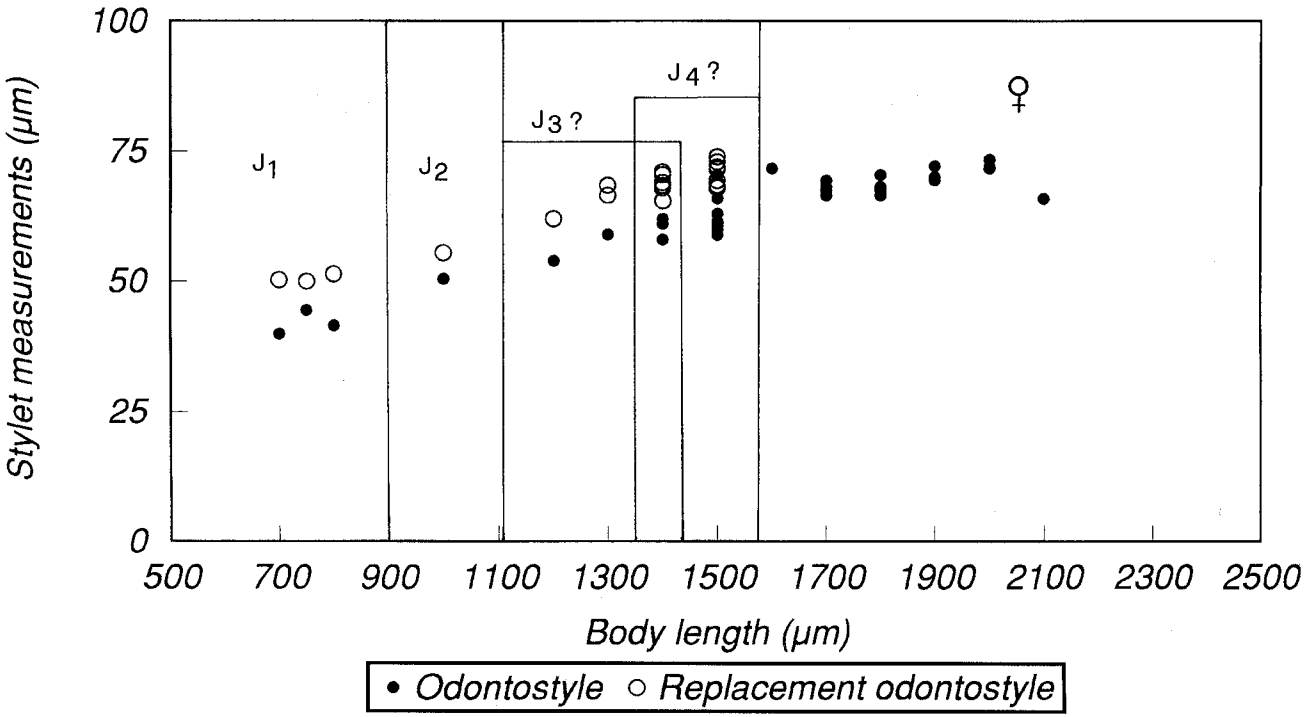

Fig. 6 - Scatter diagram plotting body and odontostyle lengths of individual juveniles and females of *X. minor* from Venezuela.

body and odontostyle (L=2.47 mm and odontostyle = 84 µm in the Uruguay population), and anterior vulva (V=49 in the Uruguay population). *Xiphidorus minor* from Venezuela also differs from an Argentinian population (Decramer *et al.*, 1998) in having a shorter body (L=2.15 in the Argentinian population), shorter odontostyle (79 µm in the Argentinian population) and slightly shorter tail (25.5 µm in the Argentinian population).

Xiphidorus minor, characterized by super oxide dismutase (SOD) isozyme profile (Fig. 7), carried out as described in Molinari *et al.*, (1997), shows two acidic bands which differentiate it from most species of *Xiphinema*.

Polymerase chain reaction (PCR) was used to amplify a fragment of ribosomal DNA (rDNA) containing the ITS (Internal Transcribed Sequences) region in *X. minor* with heterologous primers which hybridize with conserved regions. Single nematodes were ruptured mechanically and the DNA extraction was carried out as described in Molinari *et al.* (1997). The amplification of the ITS region produced only one band of approximately 1.5 Kb. The amplified product was digested with six restriction enzymes: Bam HI, Dde, Rsa I, Alu I, Hinf I and Xba I (Fig. 8). The PCR-RFLP profiles may be used to specifically identify *X. minor*.

Venezuela, after Brazil, Uruguay and Argentina is the fourth South American country where *X. minor* is reported to occur.

Comments on the number of juvenile development stages occurring within the genera Xiphidorus *and* Paraxiphidorus *Coomans* et *Chaves, 1995.*

Among the species belonging to the genera *Xiphidorus* and *Paraxiphidorus* the information concerning juvenile developmental stages is as follows:

– *X. achalae* Luc *et* Doucet, 1984 was described with only third and fourth juvenile stages (Luc and Doucet, 1984); however, it is not known

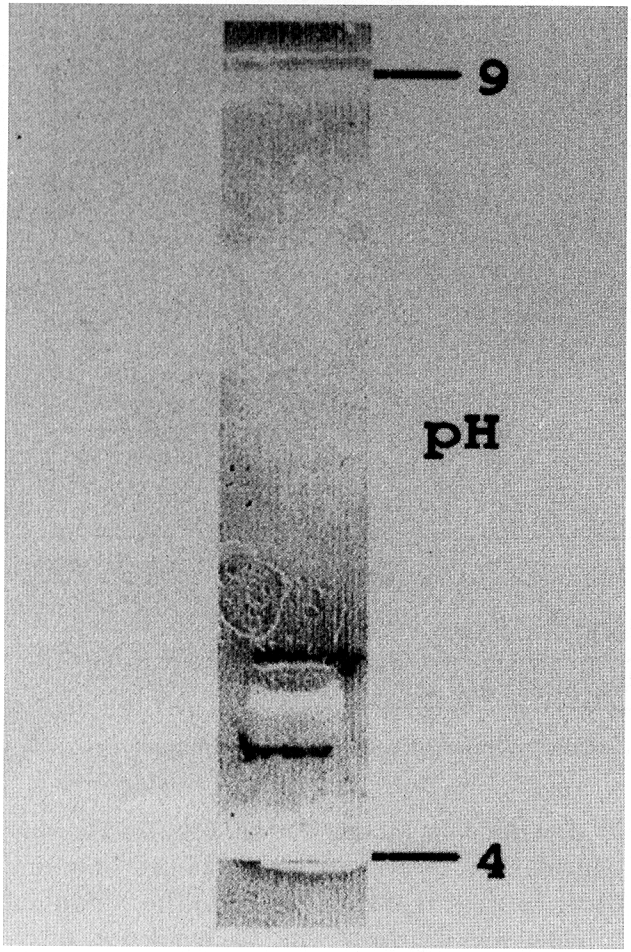

Fig. 7 - SOD isozyme pattern of *X. minor* from Venezuela.

if four stages occur, therefore, the third and fourth stages reported may in fact represent the second and third if only three such stages occur;

– *X. amazonensis*, the 13 juveniles reported in its description were not separated in different stages (Uesugi *et al.*, 1985);

– *X. balcarceanus* Chaves *et* Coomans, 1984 is the only species within the two genera described with four juvenile stages (Chaves and Coomans, 1984). However, the same authors, in describing *X. tucumanensis* (Chaves *et* Coomans, 1984), which is considered a junior synonym of *X. balcarceanus* (Decraemer *et al.*, 1996), report juveniles only, identified as J1, J2 and J4 and a further description of *X. balcarceanus* (Decraemer *et al.*, 1996), reports J2, J3 and J4 only;

– *X. minor*, in its original description J2, J3 and J4 only are reported (Rashid *et al.*, 1986);

– *X. parthenus* Monteiro, Lordello *et* Nakasomo, 1981 does not include juveniles in its original description (Monteiro *et al.*, 1981); however, in a further description, *X. yepesara* s.sp. *parthenus*, measurement of J2, J3 and J4 are given (Decraemer *et al.*, 1996);

– *X. saladillensis* Chaves *et* Coomans, 1984 was found in its type population with only third and fourth stage juveniles (Chaves and Coomans, 1984); a further description of this species includes J2, J3 and J4 (Decraemer *et al.*, 1996);

– *X. uruguayensis* Coomans, Chaves *et* De Leon, 1996 is described with one J1, one J2, three J3, one J4 and a moulting specimen considered a J2 turning to J3 (Coomans *et al.*, 1996);

– *X. yepesara* Monteiro, 1976 is originally described with J3 and J4 and a further description reports J2, J3 and J4 (Decraemer *et al.*, 1996);

– *P. brevistylus* Decraemer, Doucet *et* Coomans, 1998 is described with only J2 and J3 (Decraemer *et al.*, 1998);

– *P. heynsi* Coomans, Chaves *et* De Leon, 1996 is described with six preadult (J4) juveniles plus two juveniles moulting from J3 to J4 (Coomans *et al.*, 1996);

– *P. michelluci* Coomans *et* Chaves, 1995 was found only with a preadult juvenile (Coomans and Chaves, 1995).

None of the described species can be clearly attributed as having four juvenile developmental stages, except *X. balcarceanus* for which only two J2 are reported. Of the two populations from Venezuela, *X. amazonensis* does not show a clear separation between J2 and J3 and in *X. minor* there is an overlap between J3 and J4. However, it should be recognized that population densities of *Xiphidorus* and *Paraxiphidorus* are generally very low, therefore, in most cases the population structure cannot be clearly defined. Moreover, it is likely that the first stage juveniles, those most frequently missing in the descriptions, are lost in the extraction procedures because of their small size. Nevertheless, the available information

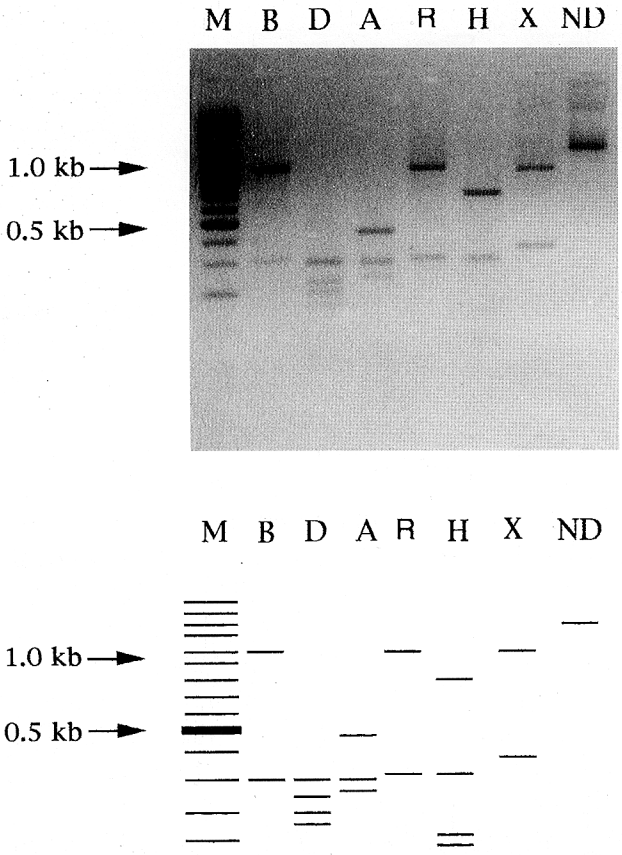

Fig. 8 - PCR-RFLP patterns of amplified ITS region of *X. minor* from Venezuela (schematic representation of restriction patterns below the gel).

provides some evidence that only three juvenile stages might occur in such genera, typical of South America. If this is true, it might be assumed that *Xiphidorus* and *Paraxiphidorus* are closely related to the American species of *Xiphinema* determined to have only three juvenile stages (Robbins *et al.*, 1996). Alternatively, and perhaps most likely, the evolutionary process resulting in the development of three, instead of, or from, four such stages, has occurred indipendently, in at least two, if not three, genera in the Longidoridae occurring in the Americas.

Aknowledgment. Work carried out in the frame-work of the scientific cooperation between Consiglio Nazionale delle Ricerche (C.N.R.), Italy and Consejo Nacional de Investigaciones Cientifica y Tecnologica (C.O.N.I.C.I.T.), Venezuela.

The authors are grateful to Mr. V. Radicci for preparing the illustrations and to Mr. F. Zacheo, for mounting the nematodes on slides.

Literature cited

CHAVES E. and COOMANS A., 1984. Three new species of *Xiphidorus* from Argentina, with comments on *Xiphinema sandellum* Heyns, 1966. *Révue Nématol.*, 7: 3-12.

COOMANS A. and CHAVES E., 1995. *Paraxiphidorus michelluci* n.g., n. sp. from Argentina (Nematoda: Longidoridae). *Fundam. appl. Nematol.*, *18*: 303-306.

COOMANS A., CHAVES E. and DE LEON L., 1996. Xiphidorini (Nematoda: Longidoridae) from Uruguay. *Bull. Inst. Royal Scie Natur Belgique/Biol.*, *66*: 5-15.

DECRAMER W., LUC M., DOUCET A. and COOMANS A., 1996. Study of the genus *Xiphidorus* Monteiro, 1976 (Nematoda: Longidoridae). *Fundam. appl. Nematol.*, 19: 207-225.

DECRAMER W., LUC M., DOUCET M. and COOMANS A., 1998. Longidoridae from Argentina with the description of *Paraxiphidorus brevistylus* sp.n. (Nematoda: Longidoridae). *Fundam. appl. Nematol.*, *21*: 371-388.

DOUCET M. E., FERRAZ C. B. L. C., MAGUNACELAYA J. C. and BROWN D. J. F., 1998. The occurrence and distribution of longidorid nematodes in Latin America. *Russian J. Nematol.*, *6*: 111-128.

HUNT D. J., 1993. *Aphelenchida, longidoridae and trichodoridae: their Systematics and Bionomics.* CAB International, Wallingford, U. K., 332 pp.

LUC M. and DOUCET M. E., 1984. Description of *Xiphidorus achalae* n. sp. and proposal for a classification of longidorids (Nematoda: Dorylaimida). *Révue Nématol.*, 7: 103-112.

MOLINARI S., DE LUCA F., LAMBERTI F. and DE GIORGI C., 1997. Molecular methods for the identification of Longidorid nematodes. *Nematol. medit.*, *25*: 55-61.

MONTEIRO A. R., 1976. *Xiphidorus yepesara* n. gen., n. sp. (Nematoda: Longidoridae) from Brazil. *Nematol. medit.*, *4*: 1-6.

MONTEIRO A. R., LORDELLO L. G. E. and NAKASOMO K., 1981. *Xiphidorus parthenus* n. sp. (Nematoda: Longidoridae) from Brazil. *Revista Agricultura*, *56*: 93-97.

RASHID F., COOMANS A. and SHARMA R. D., 1986. Longidoridae (Nematoda: Dorylaimida) from Bahia State, Brazil. *Nematol. medit.*, *14*: 235-250.

ROBBINS R. T., BROWN D. J. F., HALBRENDT J. M. and VRAIN T. C., 1996. Compendium of juvenile stages of *Xiphinema* species (Nematoda: Longidoridae). *Russian J. Nematol.*, *4*: 163-171.

UESUGI C. H., HUANG C. S. and CARES J. E., 1985. *Xiphidorus amazonensis* n. sp. (Nematoda: Longidoridae) from the Brazilian Amazon Basin. *J. Nematol.*, 7: 310-313.

Accepted for publication on 23 December 1998.