STUDIES ON SOME SPECIES OF THE *XIPHINEMA AMERICANUM* GROUP (NEMATODA, DORYLAIMIDA) OCCURRING IN FLORIDA

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Summary. The study of the morphometrics of putative *Xiphinema americanum* group populations collected in various *habitats* in Florida, United States of America, revealed the presence of four species: *X. citricolum* Lamberti *et* Bleve-Zacheo, 1979; *X. floridae* Lamberti *et* Bleve -Zacheo, 1979; *X. georgianum* Lamberti *et* Bleve-Zacheo, 1979 and *X. laevistriatum* Lamberti *et* Bleve-Zacheo, 1979. The relationships of these species with *X. americanum sensu stricto* and similar species are discussed. It is shown for the first time that *X. citricolum* and *X. laevistriatum* have three juvenile stages. The SOD isozyme iso-electrofocusing patterns also separated the studied populations into four groups; however, it confirmed only partially the morphotypes. The analysis of the ribosomal DNA of seven populations characterized three groups identifying *X. floridae*, *X. citricolum* and *X. laevistriatum*. However, one population morphometrically identified as *X. laevistriatum* presented a pattern as *X. citricolum*.

Xiphinema americanum sensu lato is wide-spread in Florida (Tarjan, 1974) and several putative species of the group were originally described from the State (Lamberti and Bleve-Zacheo, 1979). They are *X. citricolum* Lamberti *et* Bleve-Zacheo, 1979; *X. floridae* Lamberti *et* Bleve-Zacheo, 1979; *X. intermedium* Lamberti *et* Bleve-Zacheo, 1979; *X. laevistriatum* Lamberti *et* Bleve-Zacheo, 1979 and *X. tarjanense* Lamberti *et* Bleve-Zacheo, 1979. Populations collected in Florida were also used, although not as type populations, in the original description of *X. diffusum* Lamberti *et* Bleve-Zacheo, 1979 and of *X. georgianum* Lamberti *et* Bleve-Zacheo, 1979. Later, also *X. luci* Lamberti *et* Bleve-Zacheo, 1979 and *X. sheri* Lamberti *et* Bleve-Zacheo, 1979 have been reported in Florida (Lamberti *et al.*, 2000). However, some identifications require confirmation (Robbins, 1993) and the validity of various species attributed to the *X. americanum* group is questioned (Luc *et al.*, 1998).

Several populations belonging to the *X. americanum* group were collected during a nematode survey carried out in Florida during October - November 1996. They were studied biometrically and, for some population, the superoxide dismutase (SOD) activity and the DNA of the ITS region were characterized. When available, the juvenile stages were determined and illustrated.

MATERIALS AND METHODS

Soil samples were collected from the rhizosphere of cultivated plants and in natural *habitats*. Nematodes were extracted by a wet sieving technique. Specimens for biometric studies were fixed in 5% boiling formalin and mounted in anhydrous glycerin. Measurements were taken with the aid of a camera lucida.

SOD isozymes were separated by iso-electric-focusing on lots of 15-20 specimens (Molinari *et al.*, 1997).

Genomic DNA was amplified with the ITS primers (Molinari *et al.*, 1997) and the PCR amplification products were digested with the restriction enzymes *Alu* I, *Bam* HI, *Dde* I, *Hinf* I, *Rsa* I and *Xba* I (Lamberti *et al.*, 1999). DNA of four specimens from each population was analyzed to confirm the reproducibility of the fragment profiles.

RESULTS AND DISCUSSION

The 13 populations studied (Table I) were identified as four species.

XIPHINEMA CITRICOLUM Lamberti *et* Bleve-Zacheo, 1979
(Tables I, II and VI; Figs 1-3, 9 and 10)

Xiphinema citricolum was the most commonly encountered species (Table I). Its morphometrics differ from the original description (Lamberti and Bleve-Zacheo, 1979) in its shorter odontostyle and thinner body profile. However, the old specimens of the original description were partially collapsed.

Juveniles occurred in the populations collected at Eustis and Quincy and three stages were identifiable in both populations (Fig. 3).

Two males, occurred in each of the populations from Yancey and Merrit Island. They are similar to females and the adanal pair of supplements is preceded by a row of nine or eleven ventromedian supplements.

Xiphinema citricolum differs from *X. americanum* Cobb, 1913 (Lamberti and Golden, 1984) in its more set off and frontally flattenet lip region (only slightly

Table I. Populations of *Xiphinema* from Florida studied (+).

Population number Fl. n	Locality	Host	Studies performed Biometrics	Isozymes	DNA	Species identification
124	Lake Alfred	Live oak, *Quercus virginiana* Mill.	+			*X. laevistriatum*
124 bis	Lake Alfred	Live oak	+	+		*X. georgianum*
156	Labelle	Long needle pine, *Pinus palustris* Mill.	+	+	+	*X. laevistriatum*
158	Labelle	*Citrus* sp.	+	+		*X. laevistriatum*
183	Moore Haven	Australian pine, *Casuarina* sp.	+	+	+	*X. laevistriatum*
192	Oklawaha	Swingle citrumelo *Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.	+	+		*X. citricolum*
201	Altoona	Sour orange, *Citrus aurantium* L.	+	+	+	*X. floridae*
201 bis	Altoona	Sour orange	+			*X. citricolum*
205	Altoona	Sour orange	+	+	+	*X. citricolum*
218	Eustis	Cleopatra mandarin, *Citrus reticulata* Blanco	+	+	+	*X. citricolum*
227	Quincy	Bermuda grass, *Cynodon dactylon* (L.) Pers.	+	+	+	*X. citricolum*
275	Merrit Island	Sea grape, *Coccoloba uvifera* (L.) Jacq	+			*X. citricolum*
284	Merrit Island	*Casuarina* sp.	+	+	+	X. laevistriatum

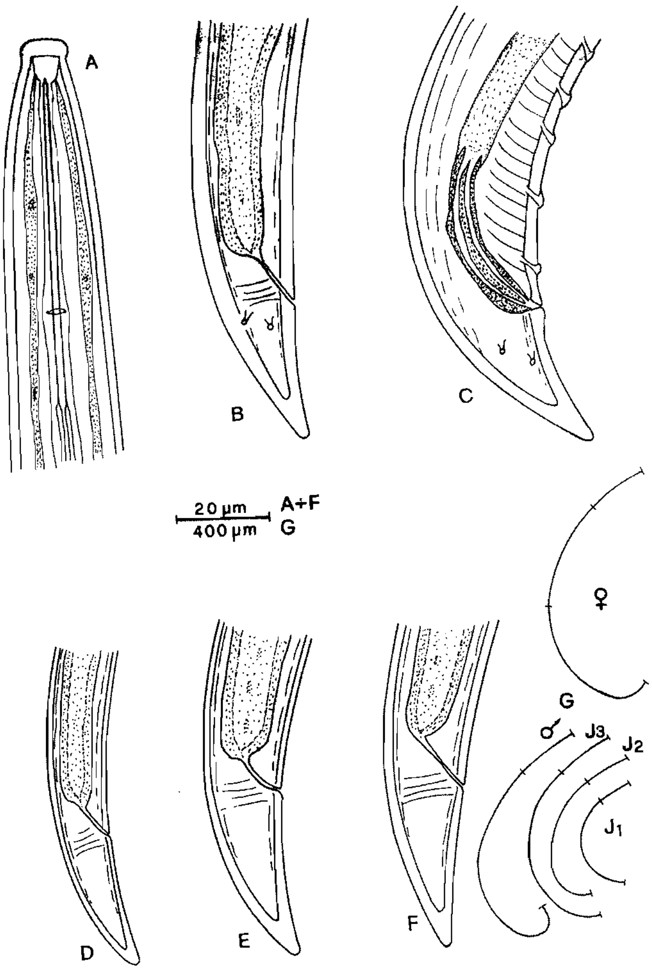


Fig. 1. *Xiphinema citricolum*: A, female anterior region; B, female posterior region; C, male posterior region; D-F, posterior region of first, second and third juvenile stage respectively; G, *habitus*.

separated from the rest of the body and frontally rounded in *X. americanum)*, straight, symmetrical, acute tail (dorsally curved and subacute terminus in *X. americanum)* and longer hyaline portion of the tail (J= 7 µm in *X. americanum)*

Xiphinema citricolum is similar to *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951; *X. lambertii* Bajaj *et* Jairajpuri, 1977; *X. intermedium* Lamberti *et* Bleve Zacheo, 1979; *X. oxycaudatum* Lamberti *et* Bleve Zacheo, 1979; *X. peruvianum* Lamberti *et* Bleve Zacheo,1979; *X. tarjanense* Lamberti *et* Bleve Zacheo, 1979; *X. tenuicutis* Lamberti *et* Bleve Zacheo, 1979; *X. bricolense* Ebsary, Vrain *et* Graham, 1989 and *X. pakistanense* Nasira *et* Maqbool, 1998.

However, compared to *X. pachtaicum* (Lamberti and Bleve-Zacheo, 1979), *Xiphinema citricolum* has lower c value (always more than 55 in *X. pachtaicum*), anterior vulva (V more than 55% in *X. pachtaicum)*, more expanded lip region (diam. at lip region ca. 8 µm in *X. pachtaicum)* and straight tail (ventrally slightly bent and concave in *X. pachtaicum* according to Lamberti and Martelli, 1971); compared to *X. lambertii* (Bajaj and Jarajpuri, 1977) *X. citricolum* has longer body (L= 1.3-1.4 in *X. lambertii*), longer odontostyle (55-64 µm in *X. lambertii*), posterior basal guide ring (48-54 µm from the anterior extremity in *X. lambertii*) and straight tail (ventrally bent and concave in *X. lambertii*); compared to *X. intermedium* (Lamberti and Bleve-Zacheo, 1979), *X. citricolum* has higher a and c' values (a= 43 and c'= 1.5 in *X. intermedium*) and straight pointed tail (ventrally bent and concave in *X. intermedium)*; compared to *X. oxycaudatum* (Lamberti and Bleve Zacheo, 1979), *X. citricolum* has more expanded lip region (slightly sett of in *X. oxycaudatum)*, longer hyaline portion of the tail (J= 9

Table II. Morphometrics (mean ± standard deviation; range) of *X. citricolum*.

Locality (populations)	Eustis (Fl. 218)				Quincy (Fl. 227)				Altoona (Fl. 205)		Merrit Island (Fl. 275)	
n	10 ♀	3 J_1	10 J_2	15 J_3	10 ♀	5 J_1	20 J_2	20 J_3	10 ♀	1 ♂	10 ♀	1 ♂
L (mm)	1.6±0.12 1.5-1.8	0.72±1.67 0.71-0.74	0.88±4.04 0.82-0.94	1.1±0.06 1.1-1.3	1.5±0.06 1.4-1.6	0.68±1.30 0.67-0.70	0.87±4.61 0.76-0.94	1.2±0.09 1.1-1.3	1.5±0.05 1.5-1.6	1.5	1.4±0.05 1.4-1.5	1.4
a	51±2.95 46.4-54.7	37.4±1.65 35.7-39	40±1.59 37.2-41.6	43.7±1.92 39.4-46.4	48.6±1.99 45.7-51	36.8±2.71 32.8-40	40±1.58 37.2-42.8	44.2±2.18 40.5-49	49±3.05 45.3-54.4	47.2	47.7±2.60 44-51.7	45.7
b	6±0.46 5.4-7	4.2±0.25 3.9-4.4	4.4±0.37 4-5	4.7±0.41 4.1-5.8	5.3±0.29 5-5.7	4±0.33 3.8-4.6	4.3±0.37 3.7-5.6	4.9±0.41 4.2-5.8	6±0.47 5.5-7	5.7	5.1±0.31 4.7-5.8	5.0
c	48.3±4.01 42.5-56.6	22±1.60 20.3-23.5	25.4±1.44 23-28.2	33±1.81 28-35.6	44.8±1.38 43.2-46.7	23.5±0.42 23-24	26.8±1.18 25-29	33.8±3.12 29.7-38.8	47±3.62 40-52.3	44.8	43±2.40 38.3-46.4	41
c'	1.8±0.09 1.7-2	2.7±0.06 2.6-2.7	2.5±0.11 2.4-2.7	2.2±0.11 2-2.3	1.8±0.09 1.6-1.9	2.5±0.07 2.4-2.6	2.4±0.08 2.3-2.5	2.1±0.09 1.9-2.2	1.7±0.09 1.6-1.8	1.6	1.8±0.10 1.7-2	1.5
V	52±1.26 50-53	---	---	---	52±1.07 50-53	---	---	---	51.7±1.58 50-53	---	52±0.92 51-53	---
Odontostyle µm	78.6±1.53 76.5-81.8	39.2±1.73 38.2-41.2	48±1.14 46-49.4	60.5±1.39 57.6-63.5	74±2.58 70-79.4	38.7±1.98 36.5-41.2	45.8±1.50 43.5-48.2	59.3±2.36 55.3-63	79.2±2.53 75.3-83	82.3	78.2±2.24 74.7-80.6	81.2
Odontophore µm	47±1.58 44-48.8	27.6±0.98 26.5-28.2	33.8±1.60 31.2-36.5	41±1.75 38.2-43.5	45.2±1.54 41.8-47.6	28.4±1.00 27-29.4	34.5±1.27 32.3-36.5	41±1.51 38-43.5	46.8±1.54 44-48.8	48.8	45.4±1.49 44-47.6	42.3
Replacement odontostyle µm	---	47.2±0.35 47-47.6	60.7±2.71 56-66	79.9±2.25 74.7-82.3	---	46.2±0.63 45.3-47	60.4±2.27 56-64	76.4±3.08 68.8-82	---	---	---	---
Oral aperture to basal guide ring µm	65.8±1.56 62.3-68.8	31.4±0.69 30.6-31.8	40±1.07 38.2-42.3	51.5±1.77 48.2-55.3	61.8±2.11 57-64.7	29.5±0.27 29.4-30	38.5±1.00 37-41.2	50±1.76 47.6-53	65.5±2.30 62.3-68.8	68.8	63.4±1.66 60.6-66	64.7
Tail µm	33.2±1.94 29.4-35.3	34±0.69 33.5-34.7	35±1.69 32.3-37	35±1.70 32.3-38.8	33±1.70 30-35.3	29.3±0.89 28.2-30.6	32.7±1.27 30.6-34.7	34.7±1.50 31.8-37	32.2±1.76 29.4-34.7	33.5	33.6±1.69 31.8-36.5	34.1
J (hyaline portion of tail) µm	11.2±1.08 10-13.5	3.5±0.55 3-4.1	5.9±0.73 4.7-7	7.8±0.85 6-8.8	9.6±1.15 8.8-11.8	3.6±0.27 3.5-4.1	5.1±0.44 4.1-6	6.8±0.73 6-8.2	10.6±0.87 9.5-12	10.6	10.5±0.60 10-11.2	10
Body diam. at lip region µm	10.8±0.31 10.6-11.2	7.6±0.00 7.6-7.6	8.7±0.25 8.2-8.8	9.5±0.26 9.4-10	10.9±0.42 10.6-11.8	7.7±0.27 7.6-8.2	8.4±0.27 8.2-8.8	9.2±0.28 8.8-9.4	10.8±0.42 10.6-11.8	11.8	10.9±0.32 10.6-11.2	10.6
Body diam. at guide ring µm	24.4±0.80 23.5-26	13.7±0.58 13-14	16.8±0.45 16-17.6	20.3±0.99 19.4-22.3	22.4±0.64 21.8-23.5	13.3±0.84 11.8-14	16.6±0.41 16-17.6	19.8±0.71 18.2-20.6	24.6±0.89 23-26	24.7	23.6±0.85 22.3-24.7	23.5
Body diam. at base of oesophagus µm	29.4±1.39 27.6-31.8	17.8±1.39 17-19.4	20.5±1.41 18.8-23	25±1.83 23-29.4	27.7±0.87 26-29.4	16.8±1.27 15.3-18.8	20.4±0.74 19.4-21.8	24.7±1.42 22.3-26.6	29±1.54 26.5-31.8	28.8	28±1.28 26-30	26
Body diam. at mid-body or vulva µm	31.4±1.05 29.4-33	19.4±1.04 18.8-20.6	22±1.79 20.6-25.3	26.6±1.88 24.7-30.6	30.5±1.39 28.2-32.3	18.6±1.24 17.6-20.6	21.8±0.97 20.6-23.5	26.5±1.44 24-28.8	31.6±2.03 29.4-35.3	31.8	30.2±1.56 27-32.3	30.6
Body diam. at anus µm	18±0.93 17-19.4	12.8±0.40 12.3-13	13.8±0.73 13-15.3	16.3±1.05 15.3-18.8	18.5±1.14 17.6-21.2	11.6±0.33 11.2-11.8	13.7±0.48 13-14.7	16.7±0.81 14.7-17.6	18.8±1.27 17-21.2	20.6	18.5±1.01 16.5-19.4	22.3
Body diam. at beginning of J µm	7.4±0.42 7-8.2	4.1±0.00 4.1-4.1	4.4±0.51 4.1-5.3	5.6±0.47 5.3-6.5	7.4±0.58 7-8.8	3.9±0.33 3.5-4.1	4.5±0.38 4.1-5.3	5.8±0.34 5.5-6.5	7.6±0.52 7-8.2	7	7.2±0.48 6.5-8.2	8.2
Spicules µm	---	---	---	---	---	---	---	---	---	44	---	41.2

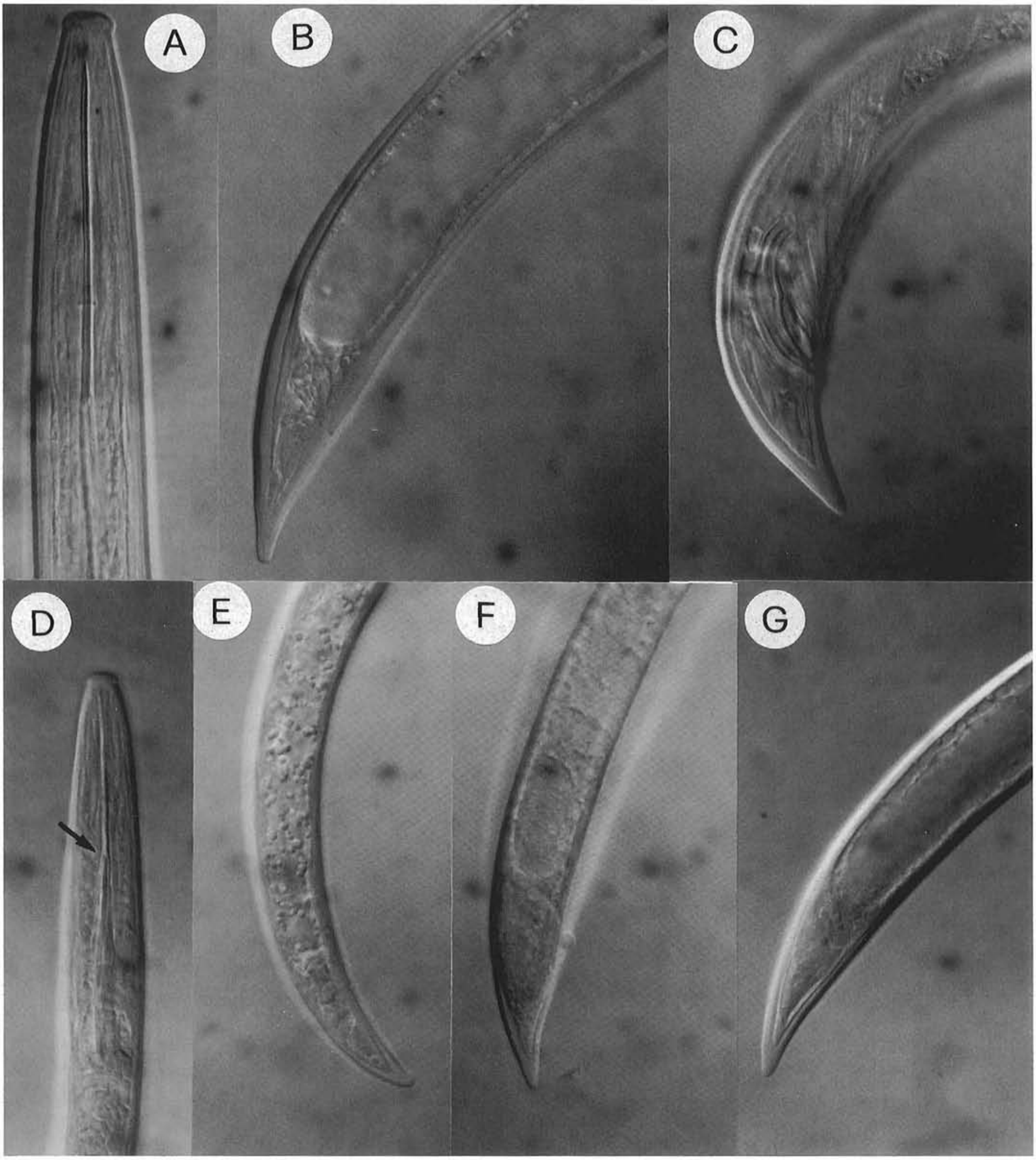
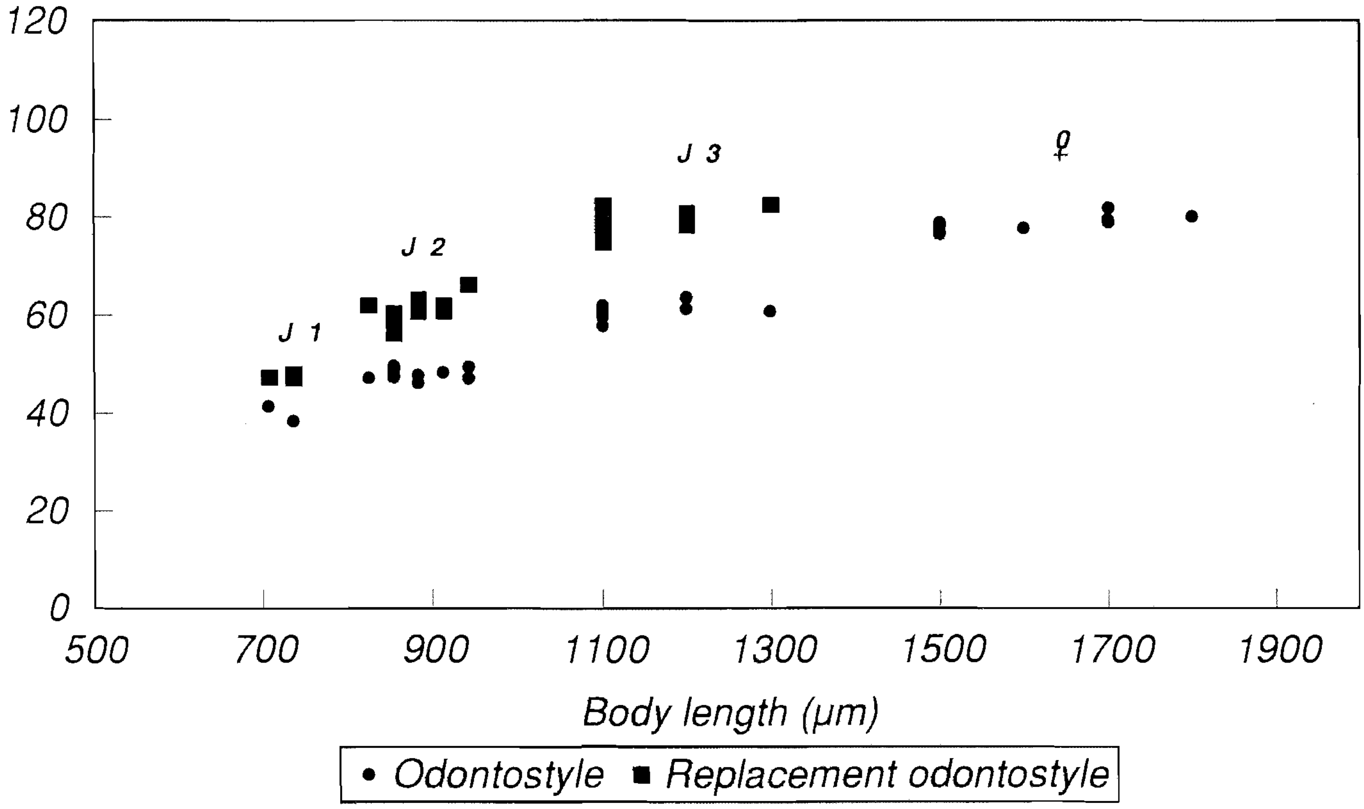


Fig. 2. Photomicrographs of *X. citricolum*: A, female anterior region; B, female posterior region; C, male posterior region; D, first stage juvenile anterior region (arrow indicates the tip of the replacement odontostyle inserted in the odontophore); E-G, posterior region of first, second and third juvenile stage respectively.

µm in *X. oxycaudatum*) and straight more symmetrical and more gradually tapering tail (dorsally convex, asymmetrical in *X. oxycaudatum*); compared to *X. peruvianum* (Lamberti and Bleve Zacheo, 1979), *X. citricolum* has lower c value (56 in *X. peruvianum*), higher c' value (1.4 in *X. peruvianum*), longer hyaline portion of the tail (J= 8 in *X. peruvianum*) and straight symmetrical tail (dorsally convex, asymmetrical in *X. peruvianum*); compared to *X. tarjanense* (Lamberti and Bleve Zacheo, 1979), *X. citricolum* has longer body (L= 1.3 mm in *X. tarjanense*) and anterior vulva (V= 54% in *X. tarjanense*); compared to *X. tenuicutis* (Lamberti and



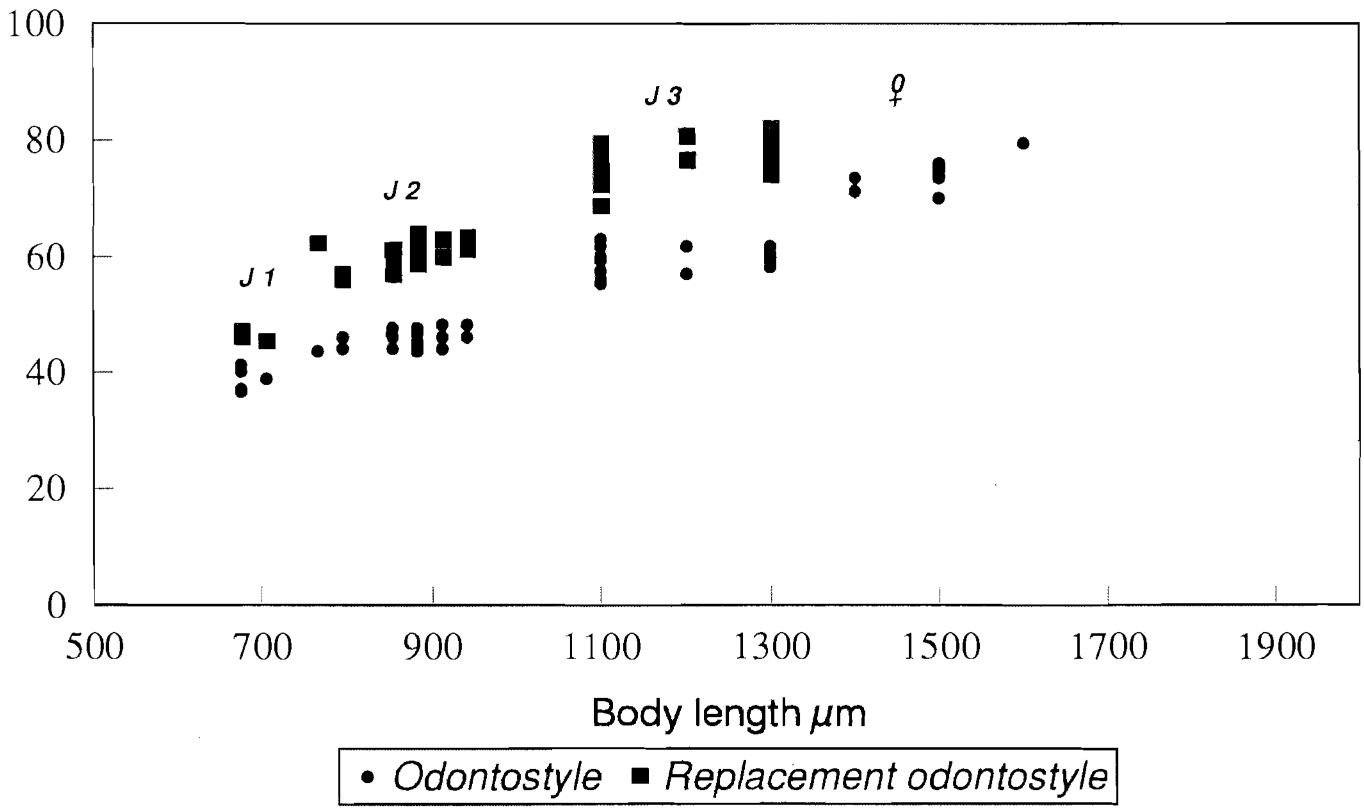


Fig. 3. Scatter diagrams plotting body and odontostyle length of individual juveniles and females of *X. citricolum* (top pop. Fl. 218, bottom pop. Fl. 227).

Bleve Zacheo, 1979), *X. citricolum* has lower c value (61 in *X. tenuicutis*) and longer tail (29 µm in *X. tenuicutis*) and hyaline portion of the tail (J= 8 in *X. tenuicutis*); compared to *X. bricolense* (Ebsary *et al.*, 1989), *X. citricolum* has shorter body (L= 1.9 in *X. bricolense*); more expanded lip region (only moderately set off in *X.*

bricolense) and more gradually tapering tail; finally, compared to *X. pakistanense* (Nasir and Maqbool, 1998), *X. citricolum* has higher c' value (1.2 in *X. pakistanense*), longer odontostyle (66 µm in *X. pakistanense*), posterior basal guide ring (56 µm from the anterior extremity in *X. pakistanense*), more clearly set off lip region (moderately in *X. pakistanense*) and longer tail (29 µm in *X. pakistanense*).

Leone *et al.*, (1997) consider *X. intermedium* the casual agent of a decline of Bermuda grass in Florida. This population should now be regarded as *X. citricolum*.

XIPHINEMA FLORIDAE
Lamberti *et* Bleve-Zacheo, 1979
(Tables I, III and VI; Figs 4, 5, 9 and 10)

A population of *X. floridae* occurred in the rhizosphere of sour orange near Altoona. Its morphometrics correspond to the original description with the only exception of being slightly longer (Lamberti and Bleve Zacheo, 1979).

Males and juveniles were not found.

X. floridae differs from *X. americanum* Cobb, 1913 (Lamberti and Golden, 1984) in its expanded lip region (separated by a shallow depression in *X. americanum*), longer body (L= 1.6 ca. in *X. americanum*), longer odontostyle (80 µm ca. in *X. americanum*), conical straight tail (ventrally bent in X. americanum) and thicker body profile.

X. floridae is similar to *X. californicum* Lamberti *et* Bleve-Zacheo, 1979; *X. citricolum* Lamberti *et* Bleve-Zacheo, 1979; *X. oxycaudatum* Lamberti *et* Bleve-Zacheo, 1979; *X. peruvianum* Lamberti *et* Bleve-Zacheo, 1979; and *X. franci* Heyns *et* Coomans, 1994.

However, compared to *X. californicum* (Lamberti *et* Bleve-Zacheo, 1979), *X. floridae* has lower a value (60 in *X. californicum*), lower c' value (1.6 in *X. californicum*), wider tail and fatter body profile; compared to *X. citricolum* (Lamberti *et* Bleve-Zacheo, 1979), *X. floridae* has higher c value (48-50 in *X. citricolum*) and lower c' value (1.6-1.7 in *X. citricolum*); compared to *X. oxycaudatum* (Lamberti *et* Bleve-Zacheo, 1979), *X. floridae* has

Table III. Morphometrics of *X. floridae*.

Locality	Altoona (pop. Fl. 201)
n	7 ♀
L (mm)	2±0.05 1.9-2
a	49.8±1.65 47.3-52.3
b	6.4±0.63 5.6-7.5
c	59.2±5.09 50.5-64.6
c'	1.4±0.05 1.3-1.4
V	52±1.21 50-54
Odontostyle µm	94.4±1.59 92.3-96.5
Odontophore µm	59.2±1.28 57.6-61.2
Oral aperture to basal guide ring µm	79±1.81 76.5-82.3
Tail µm	33±2.02 30-35.3
J (hyaline portion of tail) µm	7±0.32 6.5-7.6
Body diam. at lip region µm	13.2±0.27 13-13.5
Body diam. at guide ring µm	31±0.47 30.6-31.8
Body diam. at base of oesophagus µm	36±0.50 35.3-37
Body diam. at vulva µm	39.6±1.68 37.6-42.3
Body diam. at anus µm	24±0.64 23-24.7
Body diam. at beginning of J µm	9±0.32 8.8-9.4

Table IV. Morphometrics of *X. georgianum*.

Locality	Lake Alfred (pop. Fl. 124 bis)
n	10 ♀
L (mm)	2±0.11 1.8-2.1
a	47±1.64 44-49.6
b	5.9±0.46 5.4-6.8
c	73.3±4.26 66-79.2
c'	1.2±0.09 1.1-1.4
V	53±0.92 51-54
Odontostyle µm	118.7±3.00 113.5-125.3
Odontophore µm	59.5±2.54 54-61.8
Oral aperture to basal guide ring µm	96.5±3.38 88.8-100
Tail µm	27.3±1.66 24-30
J (hyaline portion of tail) µm	12.2±0.81 10.6-13.5
Body diam. at lip region µm	12.3±0.31 11.8-13
Body diam. at guide ring µm	32±1.44 30-34
Body diam. at base of oesophagus µm	38±2.01 35.3-41.2
Body diam. at vulva µm	42.6±2.58 38.8-47.6
Body diam. at anus µm	22.2±1.60 20.6-24.7
Body diam. at beginning of J µm	11.8±0.90 10.6-13.5

Table V. Morphometrics of *X. laevistriatum.*

Locality (populations)	Lake Alfred (pop. Fl. 124)	Labelle (pop. Fl.156)	Labelle (pop. Fl. 158)	Moore Haven (pop.Fl. 183)	Merrit Island (pop. Fl.284)			
n	10 ♀	10 ♀	10 ♀	10 ♀	10 ♀	11 J_1	16 J_2	15 J_3
L (mm)	1.7±0.07 1.7-1.9	1.8±0.07 1.7-1.9	1.7±0.12 1.6-1.9	1.7±0.07 1.6-1.8	1.7±0.07 1.6-1.8	0.70±0.22 0.68-0.73	0.96±0.59 0.88-1.1	1.3±0.05 1.2-1.4
a	50.7±1.71 49-53.8	45.9±1.62 42.5-48	48±2.12 43.7-51.7	48±2.10 45.3-52.6	45±1.58 43-47	37.4±1.59 34.8-40.3	39.8±1.30 37.5-41.6	41.7±1.73 38.8-45
b	6±0.64 5.3-7.2	6.3±0.36 5.8-7	6.4±0.55 5.7-7.5	5.8±0.24 5.4-6	6.1±0.35 5.6-6.8	3.6±0.25 3.3-4.2	4.4±0.37 3.9-5	5.2±0.36 4.5-5.8
c	55.4±2.77 52.6-61.6	51.3±3.42 45.2-55.7	46±4.04 40-52	52.3±3.37 49-57.8	50.2±3.34 45.3-55.6	22.7±1.11 20.9-24.5	28±1.73 25-36	36.5±2.18 34-41.7
c'	1.7±0.08 1.5-1.8	1.6±0.09 1.5-1.8	1.7±0.13 1.6-2	1.5±0.11 1.3-1.6	1.5±0.03 1.4-1.5	2.6±0.10 2.5-2.8	2.2±0.09 2-2.3	1.8±0.11 1.6-2
V	52±1.32 49-53	51±1.16 50-53	51±1.20 50-53	53±1.85 48-55	51±1.15 50-53	---	---	---
Odontostyle µm	90±3.15 84.7-95.3	75.8±2.02 73.5-80.6	76.4±1.83 73-79.4	78.5±4.28 74.7-88.2	82±3.51 76.5-86.5	45±0.86 43.5-46	52±1.30 50-54	63.2±2.56 60-68.2
Odontophore µm	49.7±1.92 47-53	47.8±1.42 45.3-50	47.2±1.81 44-49.4	49.4±2.07 46-53	49.3±2.18 44.7-52.3	32±1.25 30-34	36.3±1.20 34.7-38.2	42.4±2.55 36.5-46.5
Replacement odontostyle µm	---	---	---	---	---	53±1.51 50.6-56	63±2.32 58.8-66.5	80.6±2.99 75.3-86.5
Oral aperture to basal guide ring µm	75±1.77 72.3-78.2	62±2.17 59-65.3	62.7±1.21 60.6-64.7	65.8±3.43 60-71.8	67.6±1.52 65.3-70.6	35±1.16 33-36.5	42.8±1.08 41.2-44	54±1.80 50-57
Tail µm	31±2.05 27.6-35.3	34.2±2.30 30.6-37.6	37.8±2.39 34-42.3	32±2.41 29.4-36.5	34±1.53 30.6-35.3	31±1.47 28.8-33.5	34.7±2.33 30.6-40	35.7±2.46 31.2-39.4
J (hyaline portion of tail) µm	8.1±0.68 7-8.8	12±0.99 10.6-13	11.3±1.23 9.4-13	7±0.66 6-8.2	9±0.67 8-10	5.2±0.74 4-6	5.4±0.72 4.7-7	6.3±0.49 5-7
Body diam. at lip region µm	8.9±0.25 8.8-9.4	11.4±0.29 11.2-11.8	11±0.42 10.6-11.8	11.2±0.40 10.6-11.8	11.4±0.31 11.2-11.8	8±0.31 7.6-8.2	9±0.31 8.8-9.4	10.4±0.37 10-11.2
Body diam. at guide ring µm	23.7±0.79 23-25.3	25.7±0.75 24.7-27	25.2±0.88 24-27	25.3±1.07 23.5-26.5	27±0.79 26-28.8	15.5±0.57 14.7-16.5	18.7±0.57 17.6-19.4	22.8±0.62 21.8-23.5
Body diam. at base of oesophagus µm	29±0.75 28.2-30.6	34±0.90 33-36	32.8±1.68 30.6-35.3	32.2±1.63 29.4-34.7	33.4±1.23 31.2-35.3	17.4±0.30 17-17.6	22.7±1.58 20-26	28.6±1.32 26.5-31.2
Body diam. at mid-body or vulva µm	34±1.00 31.8-35.3	38.2±1.66 36.5-41.8	36.3±2.78 33-41.2	35±1.65 32.3-37.6	37.8±1.74 35.3-41.3	19±0.32 18.2-19.4	24.3±1.62 21.2-27	31.2±1.74 27.6-33.5
Body diam. at anus µm	18.8±1.00 17.6-20.6	21.3±1.11 19.4-23	21.7±0.67 20.6-23	21.6±1.12 19.4-23.5	22.8±1.14 20-23.5	11.7±0.47 10.6-12.3	15.8±1.00 14-17	20.2±1.42 18.2-23.5
Body diam. at beginning of J µm	8.3±0.46 7.5-8.8	9.6±0.81 8.8-10.6	9±0.47 8.2-9.4	8.5±0.51 7.6-9.4	9.4±0.89 8.2-10.6	4.9±0.30 4.7-5.3	5.4±0.61 4.7-6.5	6.9±0.56 6-8.2

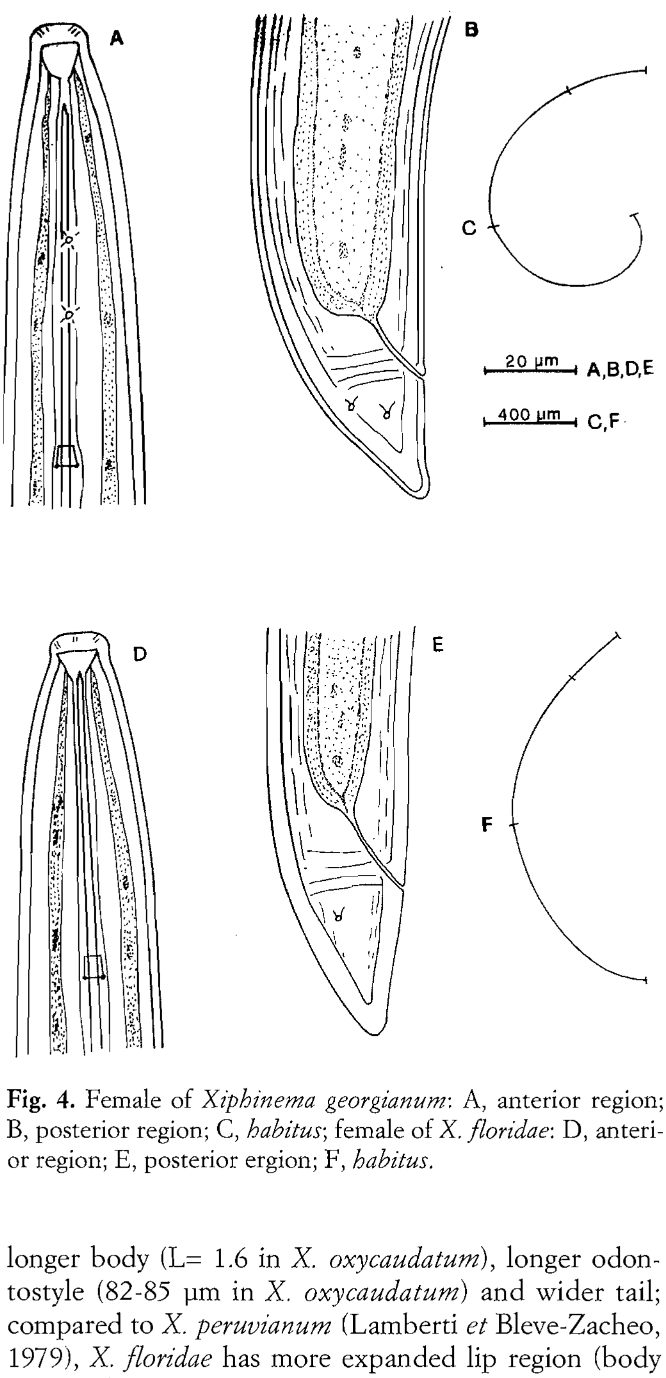


Fig. 4. Female of *Xiphinema georgianum*: A, anterior region; B, posterior region; C, *habitus*; female of *X. floridae*: D, anterior region; E, posterior ergion; F, *habitus*.

longer body (L= 1.6 in *X. oxycaudatum*), longer odontostyle (82-85 µm in *X. oxycaudatum*) and wider tail; compared to *X. peruvianum* (Lamberti *et* Bleve-Zacheo, 1979), *X. floridae* has more expanded lip region (body diam at lip region 10 µm in *X. peruvianum*) and wider more symmetrical tail (subdigitate in *X. peruvianum*); finally, compared to *X. franci* (Heyns and Coomans, 1994), *X. floridae* has expanded lip region (almost continuous with the rest of the body in *X. franci*), longer body (L= 1.4 mm in *X. franci*) and wider straight tail (slightly bent and concave ventrally in *X. franci*).

XIPHINEMA GEORGIANUM
Lamberti *et* Bleve-Zacheo, 1979
(Tables I and IV; Figs 4, 5, 9 and 10)

A population of *X. georgianum* occurred in the rhizosphere of live oak at Lake Alfred. Its morphometrics fit the original description (Lamberti *et* Bleve-Zacheo, 1979) with the only exception of having slightly longer odontostyle and odontophore.

Males and juveniles were not found.

X. georgianum differs from *X. americanum* Cobb, 1913 (Lamberti and Golden, 1984) in its longer body (L= 1.6 mm ca. in *X. americanum*), longer odontostyle (80 µm ca. in *X. americanum*) lower c' value (1.8 in *X. americanum*) and shorter tail (35 µm ca. in *X. americanum).*

X. georgianum is similar to *X. paramonovi* Romanenko, 1981, *X. longistilum* Lamberti, Bravo, Agostinelli *et* Lemos, 1994 and *X. mesostilum* Lamberti, Bravo Agostinelli *et* Lemos, 1994.

However, compared to *X. paramonovi* (Romanenko, 1981), *X. georgianum* has longer odontostyle (103 µm ca. in *X. paramonovi*), shorter tail (36 µm ca. in *X. paramonovi)* and more tapering and pointed tail (rounded terminus in *X. paramonovi*); compared to *X. longistilum* (Lamberti *et al.*, 1994), *X. georgianum* has shorter body (L= 2.8 mm in *X. longistilum),* anterior vulva (V= 56 in *X. longistilum)* and shorter tail (35 µm in *X. longistilum*); finally, compared to *X. mesostilum* (Lamberti *et al.*, 1994), *X. georgianum* possesses shorter body (L=2.5 mm in *X. mesostilum*), anterior vulva (V=57 in *X. mesostilum*) and much lower a and c values (91 and 98 respectively in *X. mesostilum*).

XIPHINEMA LAEVISTRIATUM
Lamberti *et* Bleve-Zacheo, 1979
(Tables I and VI; Figs 6-10)

Five populations were identified as *X. laevistriatum*. Their morphometrics are in the range of the original description (Lamberti *et* Bleve Zacheo, 1979); the only remarkable differences were observed in the population collected at Lake Alfred which, compared to the others, has longer odontostyle and posterior basal guide ring.

Juveniles occurred in the population from Merrit Island; males were not found.

X. laevistriatum differs from *X. americanum* Cobb, 1913 (Lamberti and Golden, 1994) in its slightly posterior vulva (V= 50% in *X. americanum),* lower value of c' (1.9 in *X. americanum*) and longer hyaline portion of the tail (J= 7µm in *X. americanum*).

X. laevistriatum is similar to *X. inaequale* (Khan et Ahmad, 1975) Khan *et* Ahmad, 1977; *X. luci* Lamberti *et* Bleve-Zacheo, 1979 and *X. minor* Ahmad, Lamberti, Rowat, Agostinelli *et* Srivastava, 1998.

However, compared to *X. inaequale* (Khan and Ahmad, 1975; Khan and Ahmad, 1977), *X. laevistriatum* has shorter body (L= 2 mm in *X. inaequale*), higher c' value (1.2 in *X. inaequale*) and pointed terminus of the tail (rounded in *X. inaequale*); compared to *X. luci* (Lamberti and Bleve-Zacheo, 1979), *X. laevistriatum* has lower c value (65 in *X. luci*), higher c' value (1.2 in *X. luci*), shorter odontostyle (95µm in *X. luci*) and pointed tail terminus (rounded in *X. luci*); finally, compared to *X. minor* (Ahmad *et al.*, 1998), *X. laevistriatum* has longer body (L= 1.5 mm in *X. minor*), longer odontostyle (69 µm in *X. minor*) and longer tail (30 µm *X. minor*).

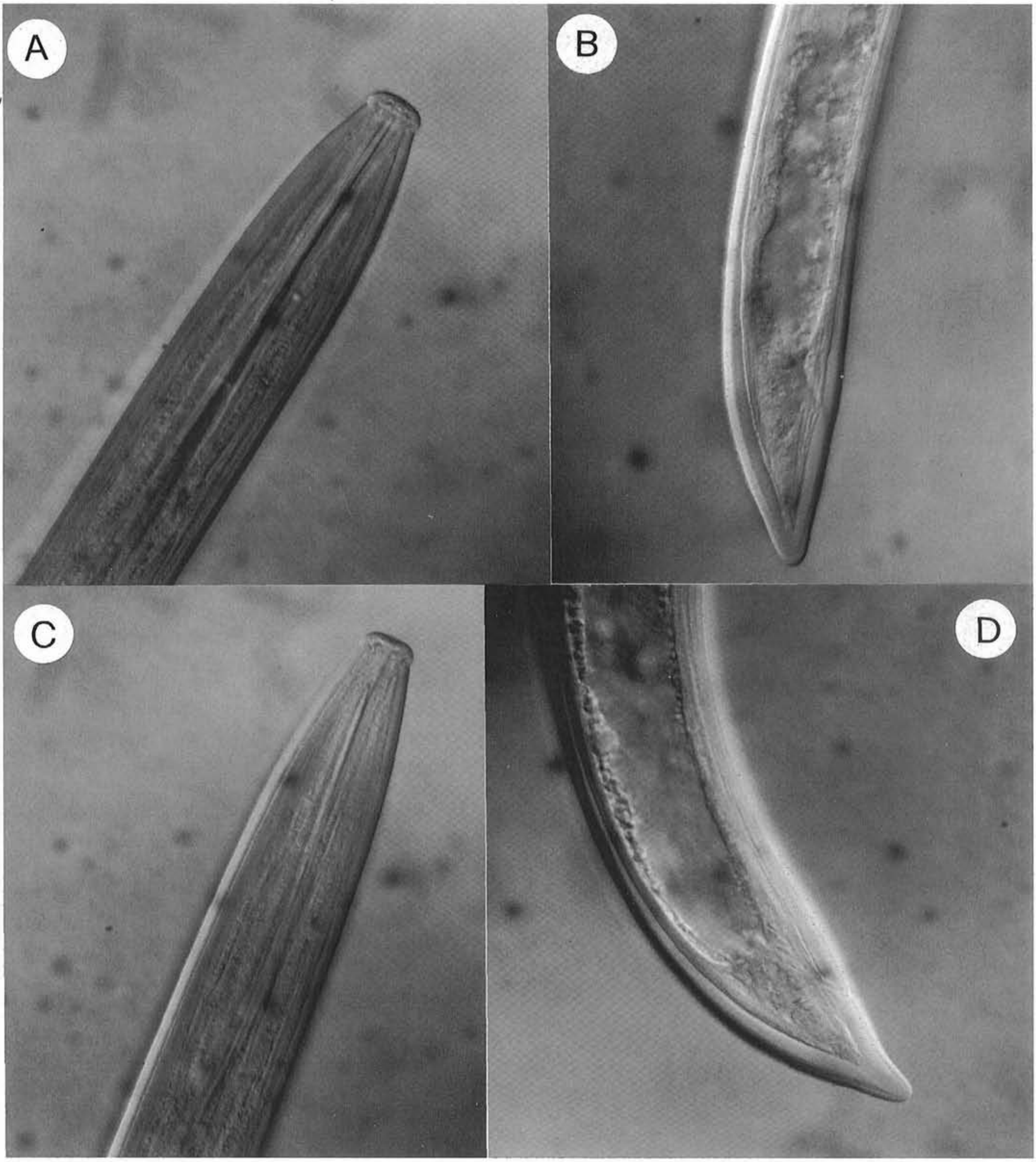


Fig. 5. Photomicrographs of females of *X. floridae*: A, anterior and B, posterior regions and *X. georgianum*: C, anterior and D, posterior regions.

Iso-electrofocusing of SOD isoforms (Fig. 9) grouped the populations into four phenotypes (Fig. 10): (1) population Fl. n. 192 from Oklawaha, identified as *X. citricolum*, which is characterized by a band of pH 7.1; (2) population Fl. n. 201 from Altoona, identified as *X. floridae* which shows only one band of SOD activity at basic pH; (3) a group comprehending populations Fl. n. 124 bis, from Lake Alfred, identified as *X. georgianum*; Fl. n. 158 from Labelle, identified as *X. laevistriatum* and Fl. n. 205 from Altoona, identified as *X. citricolum*, characterized by three main bands at pH 8.5, 6.1 and 4.8; (4) a group of five populations, including Fl. n. 156 from Labelle (*X. laevistriatum)* Fl. n. 183 from Moore Haven (*X. laevistriatum)*, Fl. n. 218 from Eustis (*X. citricolum*), Fl. n. 227 from Quincy (*X. citricolum*) and Fl. n. 284 from Merrit Island (*X. laevistria-*

Table VI. Estimated restriction fragment sizes (bp) of PCR amplified ITS of populations within the *Xiphinema americanum* group.

Enzymes	A				B	C	
	Pop. Fl. n. 156	Pop. Fl. n. 205	Pop. Fl. n. 218	Pop. Fl. n. 227	Pop. Fl. n. 201	Pop. Fl. n. 183	Pop. Fl. n. 284
ND	1500	1500	1500	1500	1500	1500	1500
Alu I	1100	(1200), (900), 750, 390, 140	730, 380	(1100, (850), 750, 380, 150	750, 380, 250, 130, 120	750, 390, 150	(1100), (850), 750, 380, 150
Bam HI	780, 650	780, 650	800, 650	780, 650	uncut	780, 650	780, 650
Dde I	430, 390, 380	420, 390, 370, 160	430, 380, 350, 150	430, 390, 380, 180	780, 360, 290, 150, 70	430, 390, 380, 180, 80	430, 390, 370, 180
HinfP I	560, 380, 300, 280, 200, 150	550, 390, 300, 250, 190, 150	550, 380, 320, 250, 200	(560), 380, 320, (290), (280), 200, 150	480, 320, 280, 200, 180, 120 110, 80	560, 380, 320, 290, 280, 200 150	560, 380, 320, 280, 200, 150
Rsa I	900, 550	900, 550	900, 550	900, 550	900, 550	900, 550, **280, 250**	900, 550, **280, 250**
Xba I	1350, 150	1350, 150	1350, 150	1350, 150	1350, 150	1350, 150	1350, 150

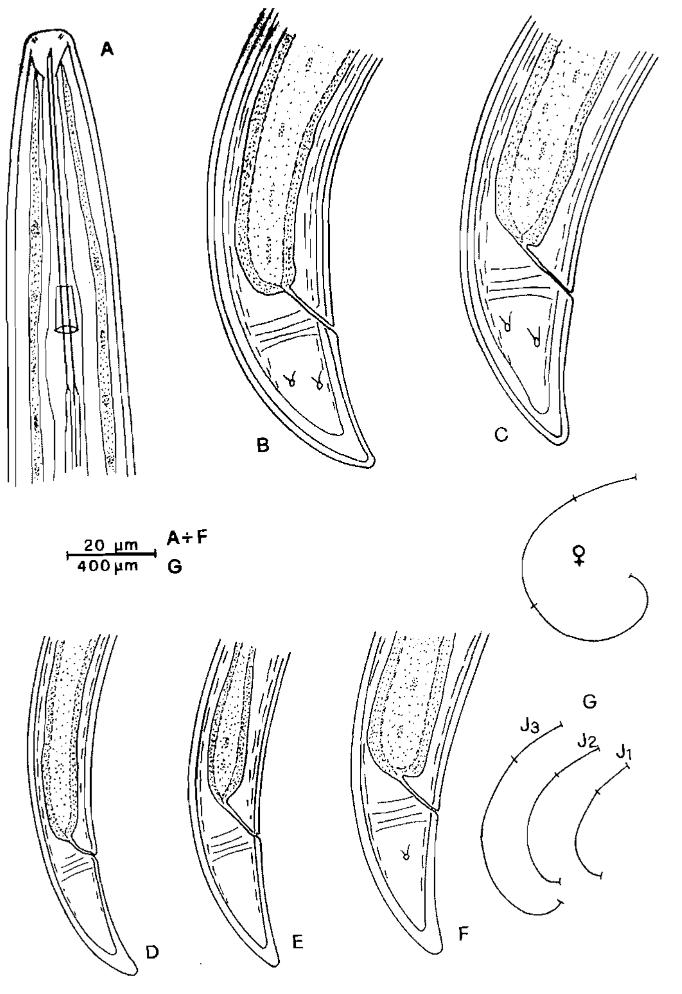


Fig. 6. *Xiphinema laevistriatum*: A, female anterior region; B and C, female posterior region; D-F, posterior region of first, second and third juvenile stage respectvively; G, *habitus*.

tum), varied from the group (3) for having an additional band at pH 5.6.

The DNA analysis, performed on seven populations (Table VI; Fig. 11) indicated that the PCR product of the ITS region of three species within the *X. americanum*-group is 1.5 kb. The population of *X. georgianum* was not analyzed; populations Fl. n. 205, 218 and 227, morphometrically identified as *X. citricolum* (Table VI, A; Fig. 11A) showed the same restriction pattern, as population Fl. n. 156 which morphometrically was identified as *X. laevistriatum*; populations Fl. n. 183 and 284, identified as X. laevistriatum, were similar to *X. citricolum*, however, showed a peculiar pattern with the enzyme *Rsa* I (Table VI, C; Fig. 11C) which yielded two fragments of 280 and 250 bp, not present in *X. citricolum*; population Fl. n. 201 identified as *X. floridae*, was not digested by the enzyme *Bam* HI and showed a peculiar restriction profile with enzymes *Dde* I and *Hinf* I (Table VI, B; Fig. 11B).

CONCLUSIONS

This work does not solve the complex and debated problem of the valid species belonging to the *X. americanum*-group. However, it permits to characterize four species which clearly posses discriminant differences and for which the following diagnoses are proposed.

X. citricolum, body length of ca. 1.6 mm, lip region set off from body profile, odontostyle length of ca. 80 μm; V= 52-54%, value of c' 1.6-1.8, tail straight, symmetrically conoid, with pointed terminus;

X. floridae, body length 1.8.2.0 mm, lip region set off from body profile, odontostyle length of ca. 90-95 μm; V= 50-52%, value of c' 1.1-1.4, tail straight, symmetrically conoid, with pointed terminus;

X. georgianum, body length of ca. 2 mm, lip region set off from body profile, odontostyle length of 110-120 μm, V ca. 53%, value of c' 1.2-1.3, tail straight, symmetrically conoid, with pointed terminus;

X. laevistriatum body length 1.7-1.8 mm, lip region continuous with body profile, odontostyle length of 75-

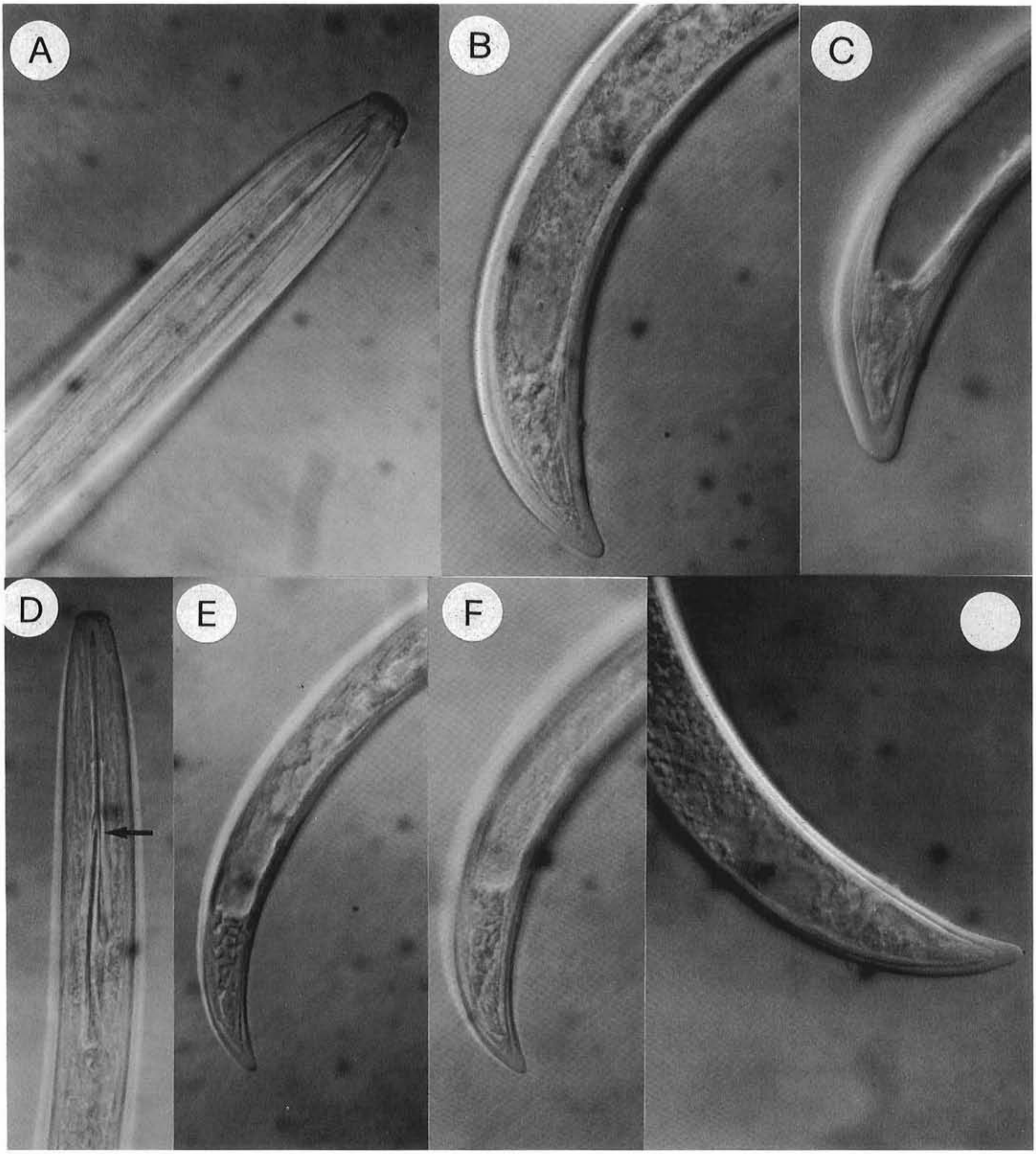


Fig. 7. Photomicrographs of *X. laevistriatum*: A, female anterior region; B and C, female posterior region; D, first stage juvenile anterior region (arrow indicates the tip of the replacement odontostyle inserted in the odontophore); E-G, posterior region of first, second and third juvenile stage respectively.

90 µm, V= 51-53%, value of c' 1.5-1.7, tail ventrally bent and slightly concave, with pointed terminus.

On the basis of our present knowledge SOD isoforms, isoelectrofocusing patterns did not discriminate between *X. americanum* populations identified by morphometric characters. It may be that small morphological differences among *X. americanum*-group species are not likely to be resolved in a system based on enzyme polymorphism.

The DNA analysis revealed that more restriction enzymes are needed to characterize populations within the *X. americanum*-group.

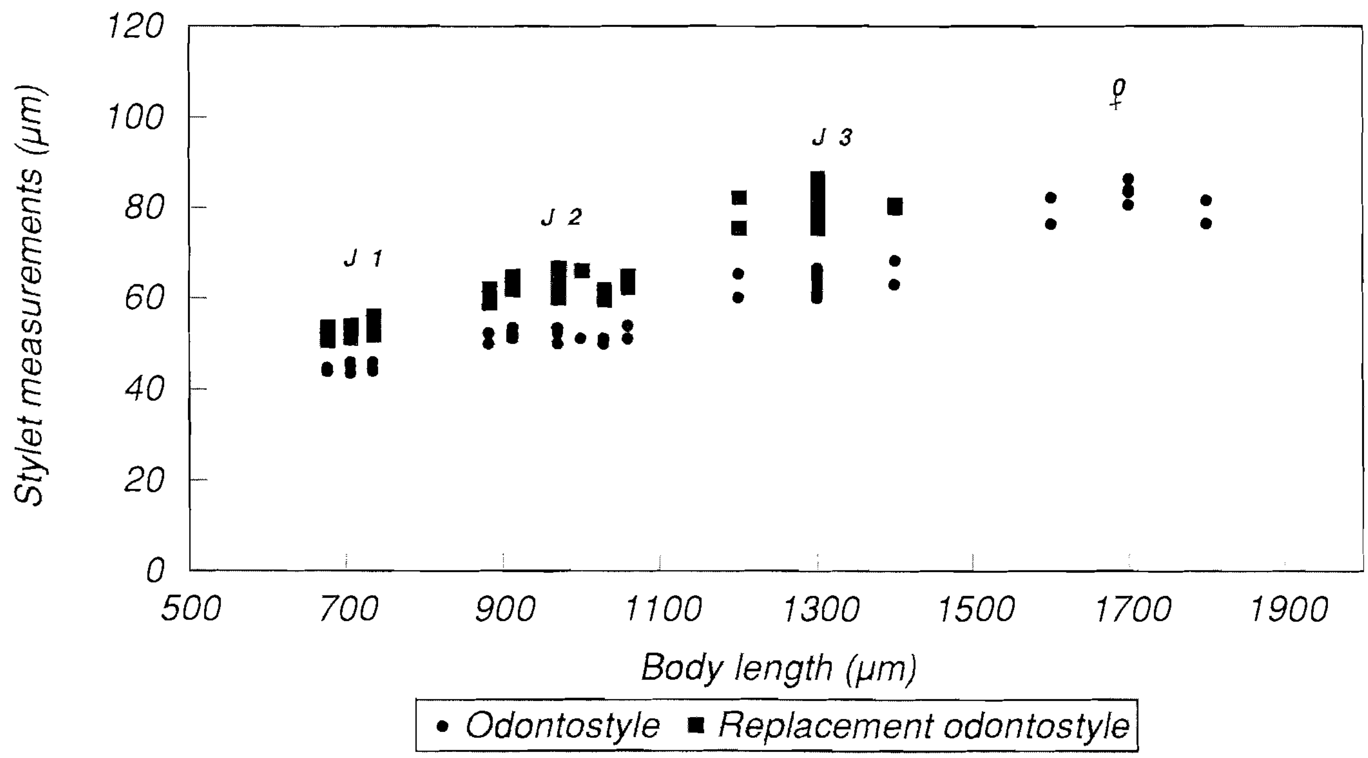


Fig. 8. Scatter diagram plotting body and odontostyle length of individual juveniles and females of *X. laevistriatum*.

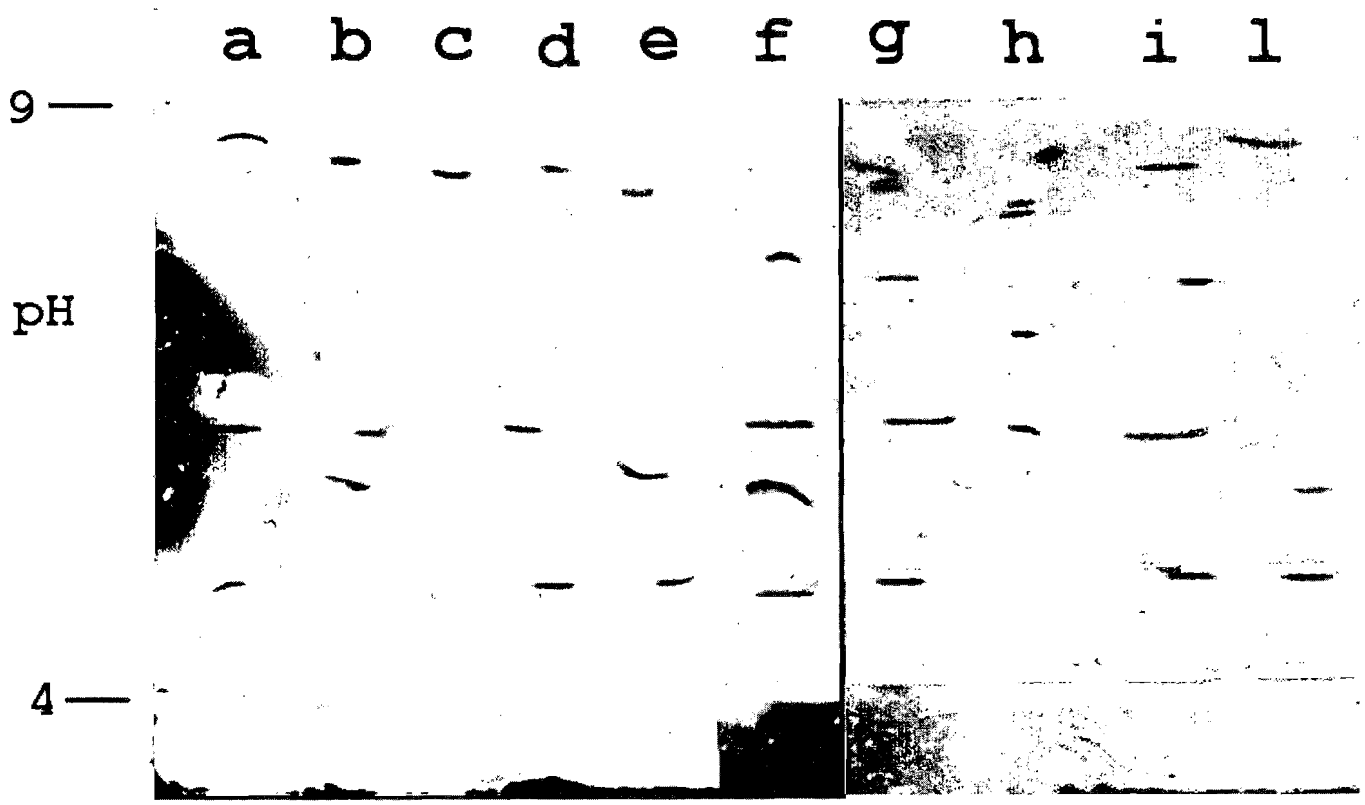


Fig. 9. Isoelectrofocusing of SOD isozymes from extracts of populations belonging to the *Xiphinema americanum*-group. Minigels were stained for SOD, dried, scanned into computer images, turned into negatives and printed on photo quality paper. Bands of SOD activity appear black over a white background. Nematode populations are shown as follows: a) *Fl 227*; b) *Fl 284*; c) *Fl 201*; d) *Fl 124 bis*; e) *Fl 183*; f) *Fl 218*; g) *Fl 205*; h) *Fl 192*; i) *Fl 158*; l) *Fl 156*.

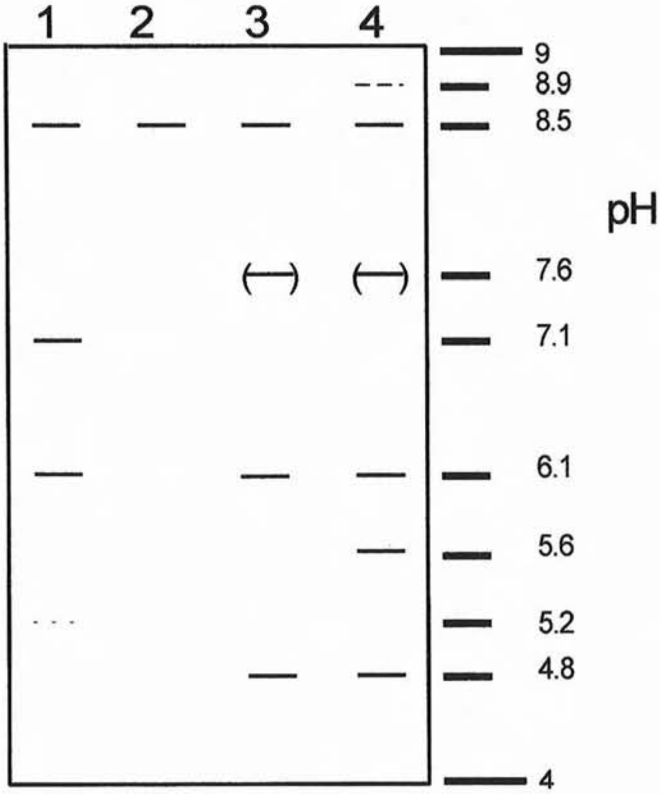


Fig 10. Diagram of the main 4 types of SOD isozyme patterns shown by the *X. americanum*-group populations. Type (1) includes Fl 192; type (2) Fl 201; type (3) Fl 124 bis, Fl 158, Fl 205; type (4) Fl 156, Fl 183, Fl 218, Fl 227, Fl 284.

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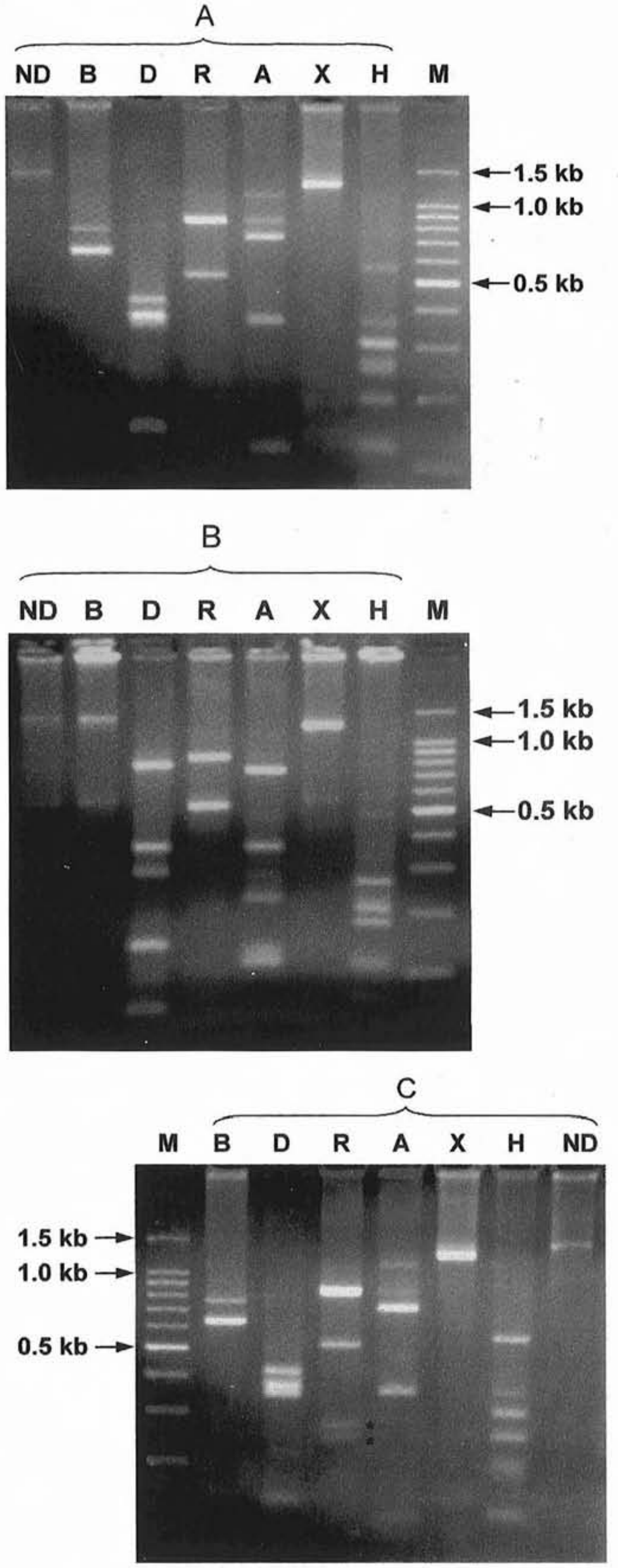

Fig. 11. Restriction digestions of the PCR amplification product of the ITS region of *X. americanum* - group populations: A, *X. citricolum* type; B, *X. floridae* type; C, *X. laevistriatum* type, separated on a 2% agarose gel and stained with ethidium bromide (M= 100bp DNA ladder, ND= not digested, B= *Bam* HI, D= *Dde* I, R= *Rsa* I, A= *Alu* I, H= *Hinf* I, X= *Xba* I.

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