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BIOMETRIC AND MOLECULAR CHARACTERIZATION AND JUVENILE DEVELOPMENT STAGES OF *XIPHINEMA VULGARE* TARJAN, 1964 (NEMATODA, DORYLAIMIDA)

by

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Summary. Morphometric and molecular studies were undertaken of Floridian populations of *Xiphinema vulgare* Tarjan, 1964. All populations were found to be almost morphometrically identical with the type populations and those from Sâo Tomé. Juveniles were clearly separated in four developmental stages. Isoelectricfocusing profiles of specimen extracts stained for superoxide dismutase activity showed one or two close bands at pH 7.6 and 7.6/7.3, respectively. The PCR products of the ITS region was between 1 and 1.5 kb and was digested by six restriction enzymes.

The validity of *Xiphinema vulgare* Tarjan, 1964 is still debated and its synonymy with *X. setariae* Luc, 1958 has been proposed by various authors and rejected by others (Lamberti *et al.*, 1995b). In 1995 Lamberti and coworkers processed by principal component and hierarchical cluster analysis populations attributed to each species and concluded that the two species could be considered as separate entities differing from each other in five taxonomic characters. However, such differences considered individually were estimated to be insufficient to justify the separation of the two species and *X. vulgare* Tarjan, 1964 was designed as a junior synonym of *X. setariae* Luc, 1958 (Luc and Baujard, 1996).

Both opinions are to be respected and to discuss the criteria used by the authors to arrive at their divergent conclusions is probably unproductive.

More recent techniques of characterization such as isozyme and DNA analysis may prove useful to clarify relationships between these populations (Molinari *et al.*, 1997). However, it is difficult to obtain sufficient live material from distant geographical regions where populations of one or the other species occur. Information can be perhaps more easily accumulated step by step, as populations become available. We begin this process in this paper. Several populations, all identified as *X. vulgare*, were collected in various localities in Florida, United States of America, some very close to the type locality of this species (Tarjan, 1964). Morphometric and molecular variability among the populations are characterized.

Materials and methods

Soil samples were collected during October and November 1996 from the rhizosphere of plants in cultivated and natural habitats in different localities in Florida. Nematodes were ex-

tracted by the wet sieving technique. Among the many populations tentatively identified as *X. vulgare*, under a dissecting microscope, twenty were selected to confirm the identification on the bases of the morphometric characters observed on five to ten adult females for each population.

Upon confirmation of their identity (Table I), the morphometric variability of six populations was studied on specimens fixed in boiling 5% formalin and mounted in anhydrous glycerin. Measurements were taken with the aid of a camera lucida. With two populations, the juvenile development stages also were described. Juveniles stages were not studied for *X. setariae* or *X. vulgare* from original localities and were incompletely reported from Brazil (three fourth stage specimens; Loof and Sharma, 1979) and Java (16 fourth, eight third and five second stage specimens; Brown *et al.*, 1981).

Superoxide dismutase (SOD) isozymes were separated by isoelectric focusing and processed as indicated by Molinari *et al.*, (1997) for seven populations (Table I) in lots of 10 to 20 specimens.

Genomic DNA was isolated and amplified as described by Molinari *et al.*, (1997) singularly on 7 to 10 specimens from each of three populations (Table I). Following PCR, 1/10 of each amplification product was digested with the restriction enzymes Bam Hi Dd e I, Rsa I, Alu I, Xba I and Hinf I (Lamberti *et al.*, 1999).

Results and discussion

Morphometrically the six populations of *X. vulgare* studied are almost identical (Table II); only population 179, from the sugarcane rhizosphere, being slightly larger in body size. They also coincide with the type population, as reported by Lamberti *et al.*, (1995b) from which the only noticeable differences were in the distance of the basal guide ring from the anterior

TABLE I - *Populations* of Xiphinema vulgare *from Florida studied.*

N° of sample	Locality (Country)	Host	Biometrics		SOD isozymes	DNA
			Adults	Juveniles		
1	Polk City	Swingle citrumelo [*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf]	+	+	+	
53	Bartow	Sour orange (*Citrus aurantium L.*)	+	+	+	+
65	Bartow	Long needle pine (*Pinus palustris* Mill.)			+	
145	Dade City	Sour orange	+		+	
179	Moore Haven	Sugarcane (*Saccharum officinarum* L.)	+		+	
183	Moore Haven	*Casuarina* sp.	+			
232	Lake Alfred	*Citrus* sp.				+
241	Lake Wales	*Citrus* sp.	+		+	
255	Merrit Island	Live oak (*Quercus virginiana* Mill.)			+	+

+ studied.

TABLE II - *Biometrics of females of* X. vulgare *from Florida.*

Population (sample)	N° 1	N° 53	N° 145	N° 179	N° 183	N° 241
n	10♀♀	10♀♀	10♀♀	10♀♀	10♀♀	10♀♀
L mm	2.6 ± 0.13 2.5 - 2.9	2.7 ± 0.09 2.5 - 2.8	2.7 ± 0.08 2.6 - 2.8	3 ± 0.10 2.8 - 3.1	2.7 ± 0.08 2.6 - 2.9	2.6 ± 0.08 2.5 - 2.7
a	63.1 ± 1.7 60.7 - 65.7	62.6 ± 2.62 58.5 - 67	63.6 ± 2.14 59.8 - 67	63.5 ± 2.14 60 - 67	61.4 ± 1.91 57.4 - 64.6	62.4 ± 2.06 58.9 - 65.4
b	6.6 ± 0.42 5.8 - 7.4	6.5 ± 0.38 6 - 7	6.7 ± 0.37 6 - 7	7 ± 0.36 6.5 - 7.3	6.3 ± 0.23 6 - 6.6	6.2 ± 0.42 5.5 - 6.8
c	52.2 ± 3.81 43.8 - 58	52.5 ± 3.44 47 - 56	52.8 ± 1.67 49 - 55.3	55.7 ± 3.23 50 - 60.7	53 ± 2.90 49.4 - 58	53 ± 2.10 50.6 - 57.4
c'	1.9 ± 0.10 1.7 - 2.0	1.8 ± 0.12 1.6 - 2	1.9 ± 0.12 1.6 - 2	1.8 ± 0.09 1.7 - 1.9	1.8 ± 0.09 1.6 - 1.9	1.8 ± 0.08 1.7 - 1.9
V %	39.2 ± 1.62 37 - 42	38.3 ± 1.42 37 - 41	38.4 ± 1.96 36 - 41	38.9 ± 1.20 37 - 40	37.7 ± 1.42 36 - 41	38.8 ± 1.32 37 - 40
Odontostyle µm	111.8 ± 3.91 106 - 116	114.4 ± 1.55 111.8 - 116	112.9 ± 2.98 109.4 - 118.8	114 ± 2.28 110.6 - 117.6	115.7 ± 1.24 113.5 - 117.6	113.8 ± 1.83 111.8 - 117
Odontophore µm	70.4 ± 1.79 67.6 - 72.3	71.8 - 1.24 69.4 - 73.5	70.6 ± 2.28 67.6 - 74	73.2 ± 1.26 70.6 - 75.3	71.6 ± 1.92 69.4 - 76	71.2 ± 2.27 68 - 76.5
Oral aperture to basal guide ring µm	104.4 ± 3.35 100 - 108.8	106.6 ± 2.14 103 - 110	103.3 ± 3.12 98.2 - 107	102.7 ± 2.57 100 - 106.5	102.4 ± 3.78 95 - 107	106.6 ± 2.54 103 - 110.6
Tail µm	49.8 ± 2.54 46.5 - 54.7	51.2 ± 2.41 48.2 - 55.3	50.8 ± 1.94 47 - 53	53.3 ± 3.03 49.4 - 58.8	51.2 ± 2.74 47 - 54.7	48.6 ± 1.50 47 - 51.2
J (hyaline portion of tail) µm	17.3 ± 0.68 16.5 - 18.8	17 ± 1.10 15.3 - 18.8	17.5 ± 1.20 16 - 20	18.3 ± 1.29 17 - 20.6	17 ± 1.36 14.7 - 18.8	17 ± 0.68 15.5 - 17.6
Body diam. at lip ring µm	13 ± 0.16 12.5 - 13	12.8 ± 0.39 11.8 - 13	13 ± 0.24 12.5 - 13	13 ± 0.16 13 - 13.5	13 ± 0.38 11.8 - 13	13 ± 0.24 12.5 - 13
Body diam. at guide ring µm	33.6 ± 0.89 32.3 - 35.3	33.2 ± 0.56 32.3 - 34	33.3 ± 0.89 31.2 - 34	35 ± 0.77 34 - 36	32.7 ± 2.34 28.2 - 36.5	33.8 ± 1.09 32.3 - 35.3
Body diam. at base of oesophagus µm	37.9 ± 1.50 35.3 - 40	38.6 ± 1.10 37 - 40.6	38.3 ± 0.99 36 - 39.4	41.4 ± 1.42 38.8 - 43.5	40.4 ± 1.36 37.6-41.8	38 ± 1.71 35.5 - 40
Body diam. at vulva µm	41.3 ± 1.60 38.8 - 44	43 ± 0.96 41.8 - 44.7	42.2 ± 1.37 39 - 43.5	46.7 ± 2.79 41.8 - 50.6	44.5 ± 1.93 41.8 - 47.6	41.3 ± 2.24 38.2 - 44
Body diam. at anus µm	26 ± 0.98 24.7 - 27.6	28.2 ± 0.75 27 - 29.4	26.6 ± 1.28 24.7 - 28.8	29.6 ± 0.94 28.2 - 31.2	28.5 ± 0.91 27 - 29.4	27 ± 1.17 26 - 29.4
Body diam. at beginning of J µm	10.2 ± 0.89 9.5 - 11.8	10.6 ± 0.62 9.5 - 11.2	10.6 ± 0.46 9.5 - 11.2	11.7 ± 0.84 10.6 - 13	10.2 ± 0.68 9 - 11.2	10.3 ± 0.40 9.5 - 10.6

end, being much shorter in the paratypes. The Floridian populations of *X. vulgare* are also in the range of the populations from Sâo Tomé attributed to this species (Lamberti *et al.*, 1995a).

In the two populations in which juveniles were studied, these clearly separated into four groups (Table III and Fig. 1), indicating that *X. vulgare* possesses four juvenile stages.

Morphometrically juveniles are very similar to females, except for the smaller size. However, the tails of the first and second stages are more elongated and more gradually tapering with respect to the third and fourth stages, and in the preadults identical to the female (Fig. 2).

IEF SOD patterns of all the population tested were characterized by one basic band; an additional close band may be present (Fig. 3a-g). The specific pattern did not depend on the relative host of the population and was different from that characterizing *X. index* (Fig. 3h).

The isoelectric point (pI) of the common band was 7.6 whilst the pI of the additional band was 7.3 (Fig. 3).

All the three populations of *X. vulgare* amplified *ca* 1.9 kb fragment upon PCR amplification with the ITS. The amplification products of four individuals for each population were digested by restriction enzymes and the sizes of

TABLE III - *Juvenile stages of two populations of* X. vulgare *from Florida.*

Population (sample)	N. 1				N. 53			
n	8 J_1	6 J_2	11 J_3	10 J_4	10 J_1	8 J_2	10 J_3	10 J_4
L (mm)	0.780±35.14 0.74-0.85	1.1±0.04 1-1.1	1.4±0.08 1.3-1.5	1.9±0.12 1.7-2.1	0.781±25.58 0.765-0.823	1.1±0.02 1.1-1.1	1.4±0.09 1.3-1.6	2.0±0.10 1.9-2.2
a	40±0.98 38-41	43.7±1.48 42-46	47.7±1.44 45.3-51	57.4±2.31 54.4-61	40.3±0.75 38.8-41	44±1.73 43-47.6	50±2.59 46-54.4	57.3±1.45 55-59.4
b	4.0±0.54 3.6-5.2	4.2±0.22 4-4.6	4.7±0.22 4.4-5	5.4±0.41 4.5-5.8	3.7±0.19 3.4-4	4.3±0.29 4-4.9	4.6±0.31 4-5.1	5.3±0.27 5-5.9
c	13±0.61 12.2-13.8	17±0.67 16-17.7	22.6±1.55 21-26	33.2±2.07 30.4-37.3	13±0.33 12.4-13.3	16.6±0.62 16-17.6	23±1.60 20.5-25.5	33.5±2.05 30.9-36.6
c'	5±0.14 4.8-5.9	4.1±0.18 3.9-4.4	3.3±0.18 3-3.6	2.5±0.12 2.3-2.6	5.0±0.14 4.8-5.2	4.1±0.16 3.9-4.4	3.3±0.17 3-3.5	2.4±0.12 2.2-2.6
Odontostyle µm	45±1.14 44-47.6	59±1.85 56-61.8	77±1.46 74.7-80	92.6±1.60 90-94.7	45.4±1.84 41.2-47.6	59±0.68 58.2-60	77.4±2.20 74-80	94.6±1.67 92.3-98.2
Odontophore µm	35±0.31 34.7-35.3	43.7±1.15 42.3-45.3	52.2±1.54 48.8-54	62±2.46 58.8-66.5	35.3±1.50 33-37	43.5±0.95 42.3-45.3	51.6±1.35 50-54	62±2.08 58.8-64.7
Replacement odontostyle µm	59.4±1.06 57.6-60.6	77.6±1.53 74.7-78.8	92.2±2.10 86.5-94	112.2±2.77 107.6-118.2	61.2±1.60 59.4-64	77.8±2.64 74.7-80.6	95±2.81 91.2-100	114±1.98 110.6-116.5
Oral aperture to basal guide ring µm	36.8±1.01 35.3-38.2	52.3±2.62 48.8-55.3	69.3±1.65 67.6-72.3	84±2.79 80.6-89.4	39.5±2.37 37-54.3	53±2.29 50-55.3	69.7±2.55 65-74	87±2.62 82.3-91.2
Tail µm	60.4±1.99 56-61.8	63.7±1.50 61.8-64.7	63.6±2.70 58.8-67.6	58.3±2.91 56-64.7	60.5±1.58 58.8-61.8	65±1.81 61.8-67.6	61.8±2.77 58.8-67.6	59.2±2.89 56-61.8
J (hyaline portion of tail) µm	9.2±0.55 8.8-10	13±0.75 11.8-14	16.8±0.92 14.7-17.6	18.6±1.13 17-20	9.4±0.73 8.8-10.6	12.8±0.45 11.8-13	15.6±0.76 14.7-16.5	17.7±1.31 15.5-19.4
Body diam. at lip region µm	7±0.21 7-7.6	8.3±0.24 8.2-8.8	9±0.31 8.8-9.4	10.8±0.28 10.6-11.2	7.1±0.05 7-7.5	7.9±0.32 7.6-8.2	9.2±0.31 8.8-9.4	11±0.25 10.6-11.2
Body diam. at guide ring µm	15.7±0.36 15.3-16	20±0.45 19.4-20.6	24±1.20 22.3-25.3	28.4±1.03 27-30	16.2±0.64 15.3-17	20.3±0.59 19.4-20.6	23.7±1.06 22.3-24.7	29±1.04 27.6-30.6
Body diam. at base of oesophagus µm	18.4±0.77 17.6-20	23.7±1.45 21.2-25.3	28.7±2.30 25.3-30.6	32±2.55 28.2-37	18.2±0.90 17.6-20	23.2±0.76 21.8-24	27±2.06 24.7-30.6	33.2±1.27 31.2-36
Body diam. at mid-body	19.6±0.77 18.8-21.2	25±1.52 22.3-26.5	30.2±2.25 26.5-32.3	33.7±2.41 30.6-38.2	19.4±0.90 18.8-21.2	24.6±0.66 23.5-25.3	28.4±2.18 26-32.4	34.6±1.34 32.3-37
Body diam. at anus µm	12±0.50 11.2-13	15.8±0.53 14.7-16	19.5±1.26 16.5-20.6	23.6±1.44 22.3-27	12±0.50 11.2-13	16±0.65 14.7-16.5	18.8±1.52 17-21.8	24.7±0.93 23-26.5
Body diam. at beginning of J µm	4.7±0.00 4.7-4.7	5.2±0.64 4.7-6	6.4±0.32 6-7	7.4±0.49 7-8.2	4.8±0.32 4.3-5.3	4.6±0.15 4.3-4.7	6±0.28 5.5-6.5	8±0.42 7.6-8.8

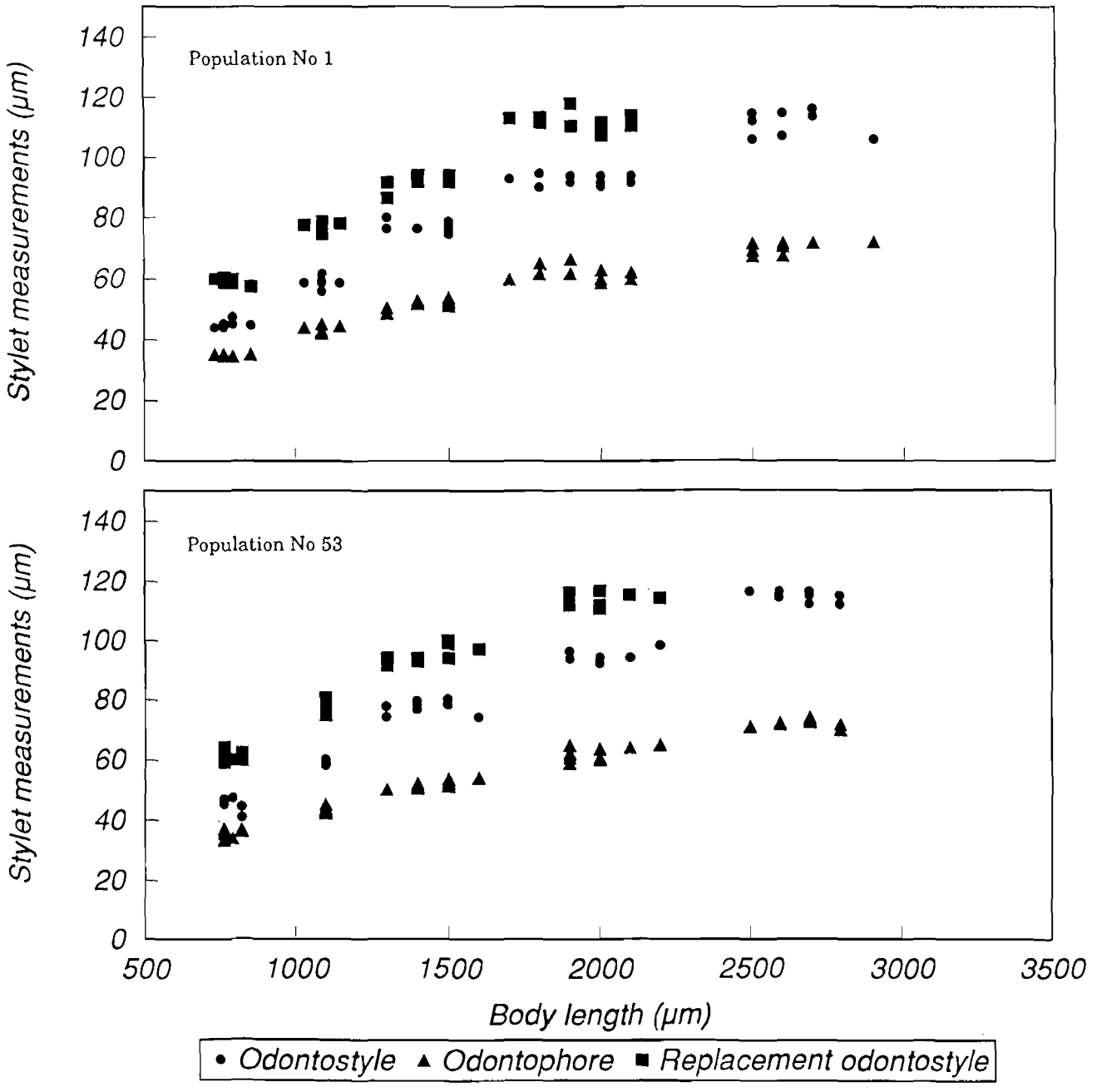

Fig. 1 - Scatter diagrams plotting body and odontostyle length of individual juveniles and females of two populations of *Xiphinema vulgare* from Florida.

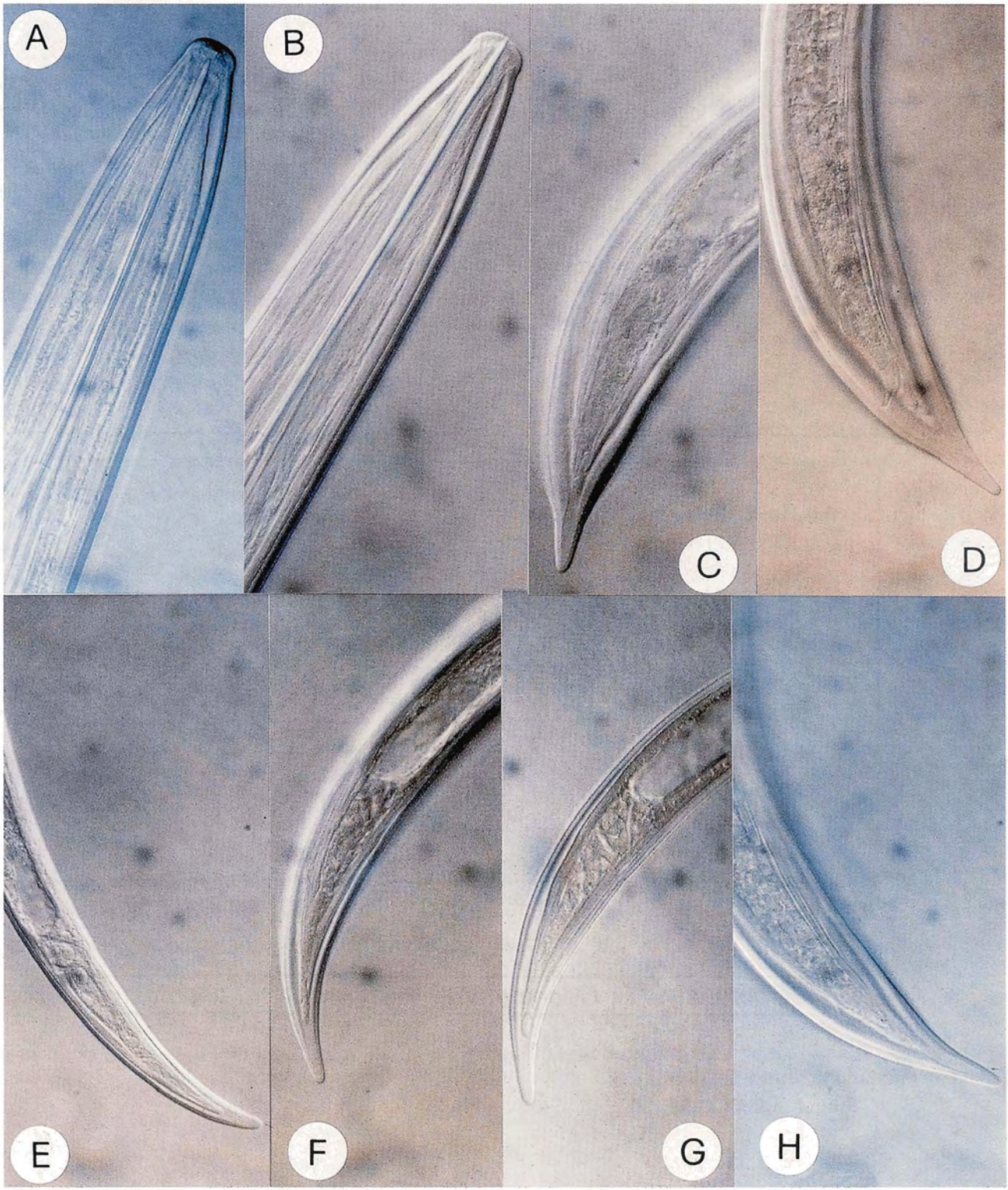

Fig. 2 - Photomicrographs of *X. vulgare* from Florida: A and B, female anterior region; C and D, female tail; E, first stage juvenile tail; F, second stage juvenile tail; G, third stage juvenile tail; H, fourth stage juvenile tail.

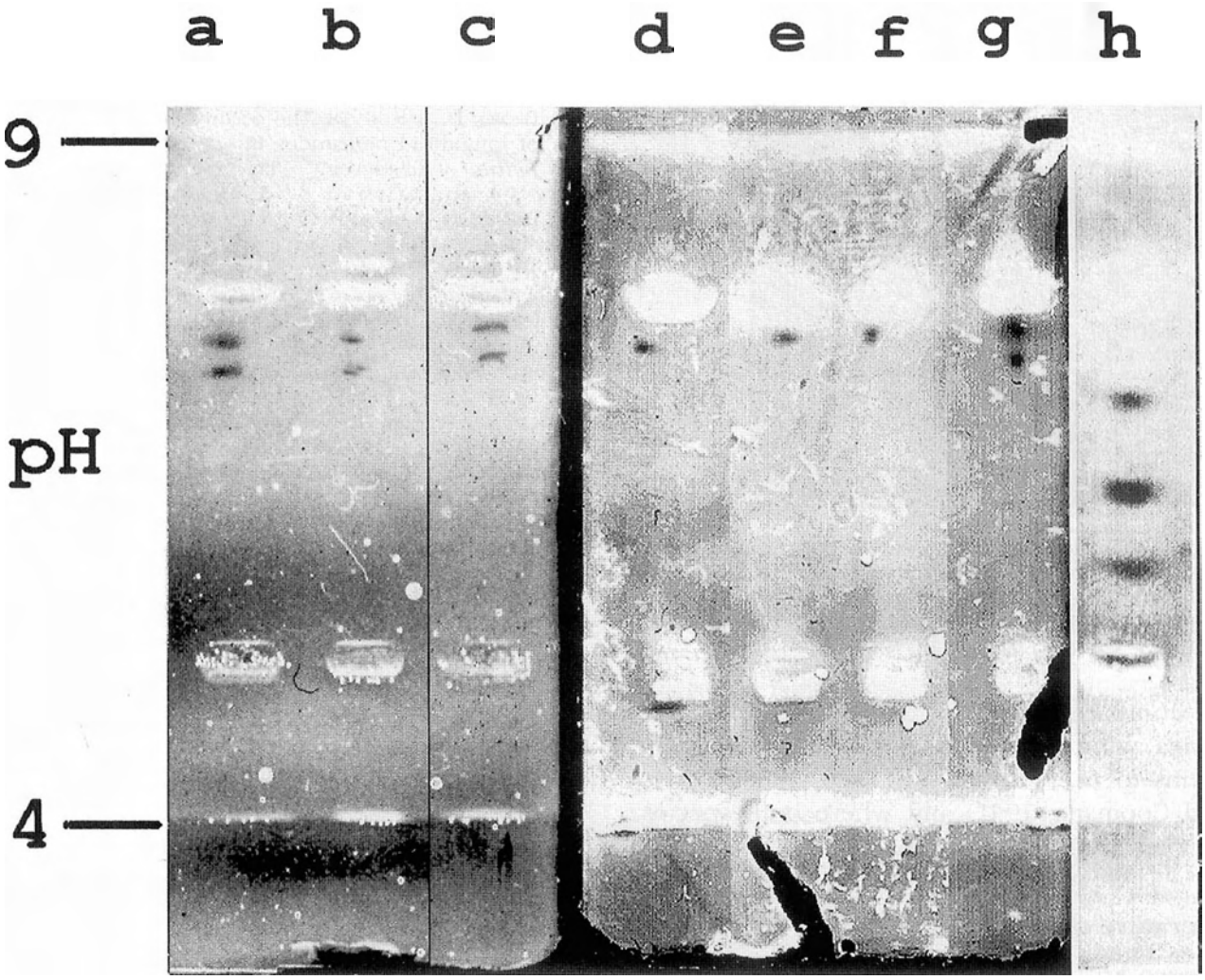

Fig. 3 - Isoelectricfocusing of protein extracts from *X. vulgare* populations stained for SOD. Mini-gels (4x4 cm) were dried and scanned. Computer images were turned into negatives and printed on high quality paper. SOD bands appear black over a grey background. a = pop. n. 179, b = pop. n. 145, c = pop. n. 65, d = pop. n. 53, e = pop. n. 255, f = pop. n. 41,g = pop. n. 1, h = *Xiphinema index*.

TABLE IV - *Estimated restriction fragment sizes (bp) of PCR amplified ITSs of* X. vulgare *from Florida.*

Enzymes	*Band sizes (bp)*
Alu I	880,290,200,170,110
Ban H I	no cut
Dde I	1100,480,150,80
Rsa I	400,280,200,170,90
Xba I	1700, 150
Hinf I	550,350,290,280,90

the resulting fragments were determined (Table IV). All the individuals examined shared the same restriction profile (Fig. 4).

In conclusion, for the characters we studied, the Florida populations of *X. vulgare* are pheno and genotypically homogenous. It would now be interesting to compare them with populations of different geographic origins, such as Puerto Rico (Tarjan, 1964), Java (Tarjan, 1964; Brown *et al.*, 1981) and central and south America (Doucet *et al.*, 1998; Crozzoli *et al.*, 1998). Characteristics of these populations should then

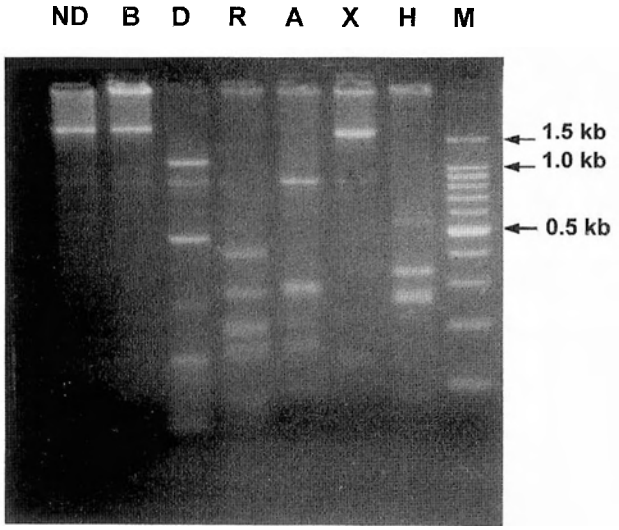

Fig. 4 - Restriction digestion of PCR amplification products of the ITS region of *X. vulgare* from Florida, separated on a 2% agarose gel and stained with ethidium bromide (M = 100 bpDNA ladder; B = Bam He; D = Dde I; R = Rsa I; A = Alu I, X = Xba I; H = Hinf I; ND = not digested.

be compared to those of populations from Africa, which is an areas where *X. setariae* seems to occur frequently (Luc, 1958: Heyns and Coomans, 1991) and where both species are present (Lamberti *et al.*, 1995a).

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