XIPHINEMA CHAMBERSI AND *XIPHINEMA NATURALE* SP.N., TWO MONODELPHIC LONGIDORIDS (NEMATODA, DORYLAIMIDA) FROM FLORIDA

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Summary. Measurements and illustrations of populations of *Xiphinema chambersi* Thorne, 1939 from Florida are provided. *X. naturale* sp.n., also from Florida, is described. Both species are characterized by isoelectrofocusing of superoxide dismutase isoforms profile and by genomic DNA PCR amplification with the ITS primers and digestion of the amplification products with restriction enzymes. *X. chambersi* and *X. naturale* sp.n. are medium size (2 to 3 mm) monodelphic species with anterior vulva (V=23-25%) and elongate, conical tail.

Several populations of *Xiphinema* with monodelphic females were found during a nematode survey in 1996 in Florida, United States of America. They represent a known species, *X. chambersi* Thorne, 1939, which is considered indigenous to North America (Robbins *et al.*, 1996) and, to the best of our knowledge, an undescribed species, which is here named *Xiphinema naturale* sp.n. They were always found in uncultivated land.

Morphometrics of these species are complemented with iso-electricfocusing profiles of superoxide dismutase (SOD) activity and DNA analysis by PCR products of the ITS region.

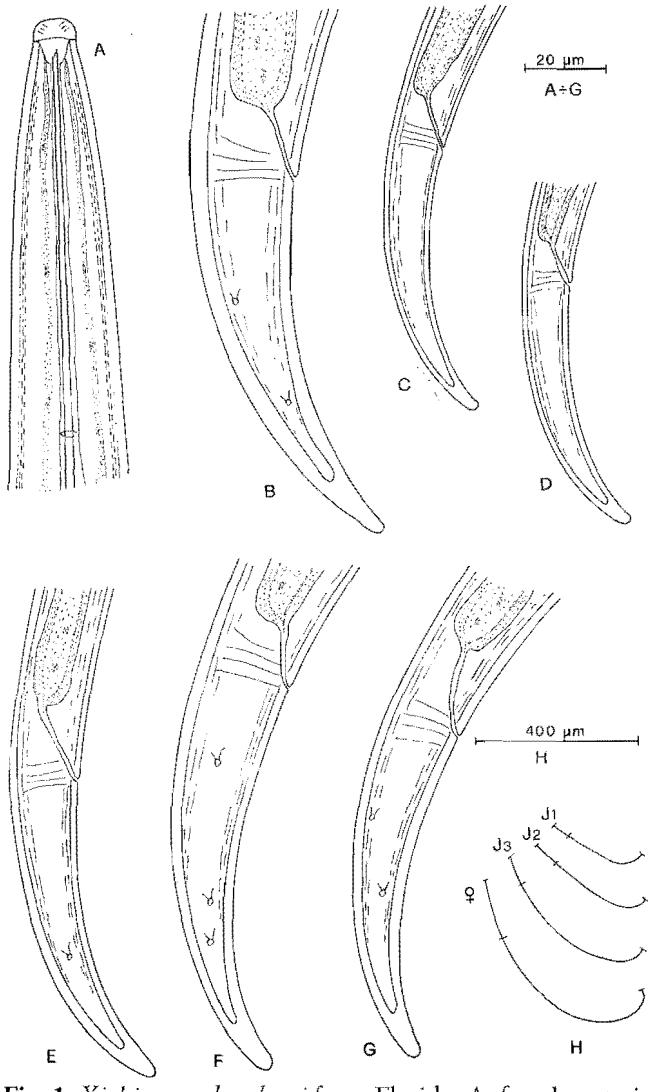

Fig. 1. *Xiphinema chambersi* from Florida: A, female anterior region; B, female posterior region; C and D, first stage juvenile posterior region; E, second stage juvenile posterior region; F and G, third stage juvenile posterior region; H, posture.

MATERIALS AND METHODS

Soil samples were collected during October and November 1996 from the rhizosphere of cultivated plants and in natural habitats. Nematodes were extracted by a wet sieving technique. Specimens for morphometric studies were fixed in 5% boiling formalin and mounted in anhydrous glycerin. Measurements were taken with the aid of a camera lucida.

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

Genomic DNA was isolated and amplified with the ITS primers, as described by Molinari *et al.* (1997). Following PCR, 1/10 of each amplification product was digested with the restriction enzymes *Bam* HI, *Dde* I, *Rsa* I, *Nde* I, *Ava* II, *Alu* I, *Xba* I and *Hinf* I (Lamberti *et al.*, 1999).

DESCRIPTIONS

XIPHINEMA CHAMBERSI Thorne, 1939
(Tables I and III; Figs 1-5)

Xiphinema chambersi was found at Bartow (sample Fl. n. 52) and on Merrit Island (sample Fl. n. 255) in the rhizosphere of live oak, *Quercus virginiana* Mill.

Table I. Morphometrics of *Xiphinema chambersi* from live oak in Florida.

Locality	Bartow				Merrit Island
n	10 ♀♀	15 J_1	15 J_2	15 J_3	8 ♀♀
L (mm)	2.5±0.09	1.0±0.05	1.4±0.11	1.9±0.12	2.5±0.15
	2.4-2.7	0.9-1.1	1.2-1.6	1.8-2.1	2.3-2.7
a	63.7±2.50	45±1.47	50.3±1.57	58.6±2.61	61.4±2.90
	60-68.5	42.5-47.2	48.8-54.6	54.4-61	57.5-66.5
b	6.4±0.39	4±0.27	4.7±0.53	5.4±0.38	6.9±0.19
	5.6-7	3.5-4.4	4.1-5.7	5.1-6.1	6.5-7.1
c	23.6±1.20	14.4±0.63	17±0.78	20.4±0.98	26.5±1.33
	22-25	13.5-15.7	15.5-18.3	19.4-22	25-28.5
c'	4.3±0.20	5±0.17	4.8±0.32	4.4±0.17	3.7±0.23
	4-4.6	4.8-5.2	4.1-5.2	4.2-4.7	3.3-4
V	23±0.79	---	---	---	21±1.06
	22-24				20-23
Odontostyle µm	117±2.70	64.5±1.86	74.3±1.70	94.7±2.06	114.7±1.56
	111.8-122.3	61.8-68.2	70-77.6	92.3-97.6	111.8-116.5
Odontophore µm	65.3±2.00	40.3±1.28	48.8±1.66	57.6±1.46	64±1.55
	63-70	38.2-43	46-52.3	55.3-58.8	61.8-66
Replacement odontostyle µm	---	74.2±1.45	94±2.45	117.7±2.15	---
		71.8-76.5	89.4-97	113-119.4	
Oral aperture to basal guide ring µm	104.3±3.10	52.8±1.86	64.2±3.40	82.2±1.96	102±4.72
	101.2-111.8	50-56	58.8-72.3	79.4-84	92-108.8
Tail µm	105.5±4.40	69±1.90	80.8±2.89	93.2±4.40	95±4.89
	100-111.8	64.7-70.6	76.5-85.3	88.2-100	88.3-100
J (hyaline portion of tail) µm	20.4±2.14	7.8±0.53	8.3±0.58	13±0.85	20.5±2.17
	17.6-23	7-8.8	7.6-8.8	11.8-14	17.6-23.5
Body diam. at lip region µm	11±0.32	7.2±0.30	8.4±0.30	9.6±0.29	10.8±0.28
	10.6-11.2	7-7.6	8.2-8.8	9.4-10	10.6-11.2
Body diam. at guide ring µm	30±1.04	17.3±0.42	21±0.67	25.3±0.71	31.6±1.02
	28.2-31.8	16.5-17.6	20-22.3	24.7-26.5	30.6-33.5
Body diam. at base of oesophagus µm	36.2±1.41	21±1.13	25.6±1.85	31±1.28	37.8±1.30
	33.5-38.2	19.4-23	22.3-28.8	29.4-33.5	36-40
Body diam. at mid-body or vulva µm	39.5±1.62	22.2±1.18	27±1.83	32.5±1.50	40.8±1.53
	37.6-42.3	20.6-24	24.7-30.6	30.6-35.3	38.2-43
Body diam. at anus µm	25.2±1.79	13.7±0.45	17±1.42	21.3±1.53	26±0.95
	23.5-29.4	13-14.7	15.3-20.6	19.4-23.5	24.7-27
Body diam. at beginning of J µm	7.4±0.66	5±0.29	5.7±0.34	6.4±0.38	8.8±0.79
	6.5-8.2	4.7-5.3	5.3-6	6-7	8.2-10

Female dead posture as an open C. Body tapering towards the extremities. Lip region frontally rounded separated from the rest of the body by a slight depression. Amphidial pouches, odontostyle, odontophore, guide tube and oesophagus typical of the genus. Vulva anterior, vagina occupying ca 1/2 of the corresponding body diameter. Anterior genital branch completely lacking; posterior genital branch with short uterus, devoid of any uterine differentiation, with reflexed ovary. Tail elongate, ventrally, arcuate, with three caudal pores on each side.

Male not found.

Juveniles resembling adults, with longer tails, expecially in the two first juvenile stages; they separate into three morphometric groups (Fig. 3).

In the polytomous key of Loof and Luc (1990) these populations have the code: A1, B4, C2, D3, E1, F2/3, G2; H2, I3, J2, K2, L1 which correspond to *X. chambersi.*

Xiphinema chambersi is widespread in the United States of America (Cohn and Sher, 1972; Robbins and Brown, 1991), including Florida (Tarjan, 1974).

In 1983 it was reported in Japan and only three juvenile stages were found (Shishida, 1983). In fact, *X. chambersi* is included among the species of *Xiphinema* determined to have only three juvenile stages (Robbins *et al.*, 1996).

Morphometrically our populations of *X. chambersi* are in the range of the type population (Cohn and Sher, 1972), but differ considerably from the Japanese population in its longer body (1.950 mm in the Japonese population) and shorter tail (121µm in the Japonese population) (Shishida, 1983). However, the female tail of our populations is less ventrally bent with respect to other American and the Japonese populations.

Superoxide dismutase (SOD) isozymes patterns of *X. chambersi* showed one very basic band and one area of activity localized at acidic pHs (Fig. 4a).

The restriction digestions of PCR amplification product of the specimens of the Bartow population of *X. chambersi* amplified ca. 2000 kb fragment upon PCR amplification; the ITS region was digested by all enzymes used (Table III) and yielded two bands (1600 and 360 kb) when cut by *Bam* HI enzyme (Fig. 5).

XIPHINEMA NATURALE sp.n.

(Tables II and III; Figs 4 and 6-9)

Female *habitus* ventrally curved as an open C. Body tapering abruptly towards anterior and more gradually

towards posterior end. Cuticle ca. 2.5 µm thick along body. Lip region separated by a slight depression from the rest of the body, frontally rounded. Amphidial pouches stirrup-shaped with wide slit-like aperture. Odontostyle strongly flanged, odontophore and guide tube typical of the genus. The enlarged basal portion of the oesophagus occupies ca. 1/5 of the oesophagus length and measures 93 (86.5-96) µm long and 20 (18-22.5) µm wide; three nuclei are evident in the basal bulb. Oesophageal-intestinal valve heart-shape. Vulva situated in the anterior fourth of the body, slit-like; vagina occupying ca. 1/2 of the corresponding body diameter. Reproductive system monodelphic completely devoid of the anterior branch; posterior branch with a

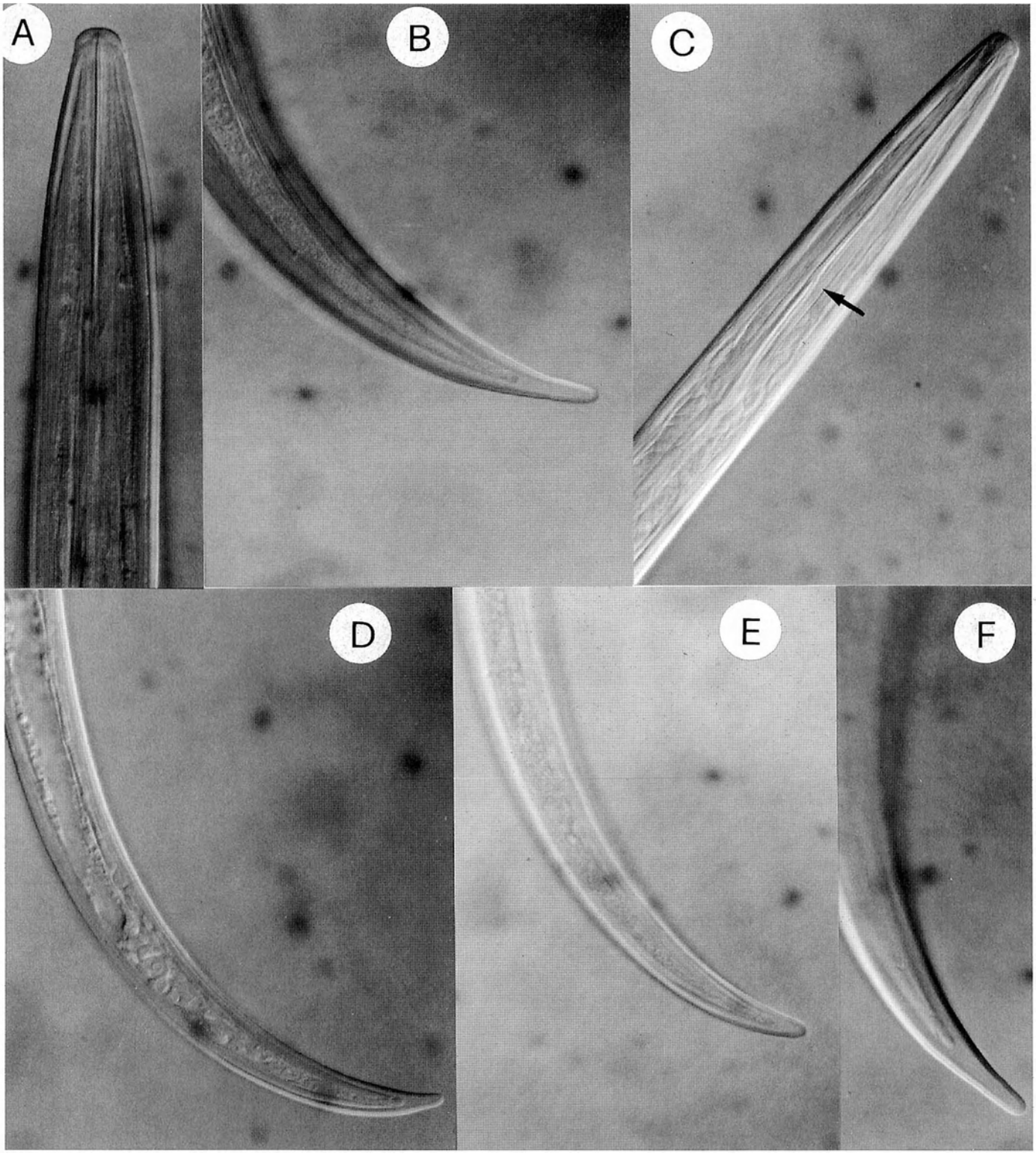

Fig. 2. Photomicrographs of *X. chambersi* from Florida: A, female anterior region; B, female posterior region; C, first juvenile stage anterior region (the arrow indicates the tip of the replacement odontostyle inserted in the odontophore); D, first juvenile stage posterior region; E, second juvenile stage posterior region; F, third juvenile stage posterior region.

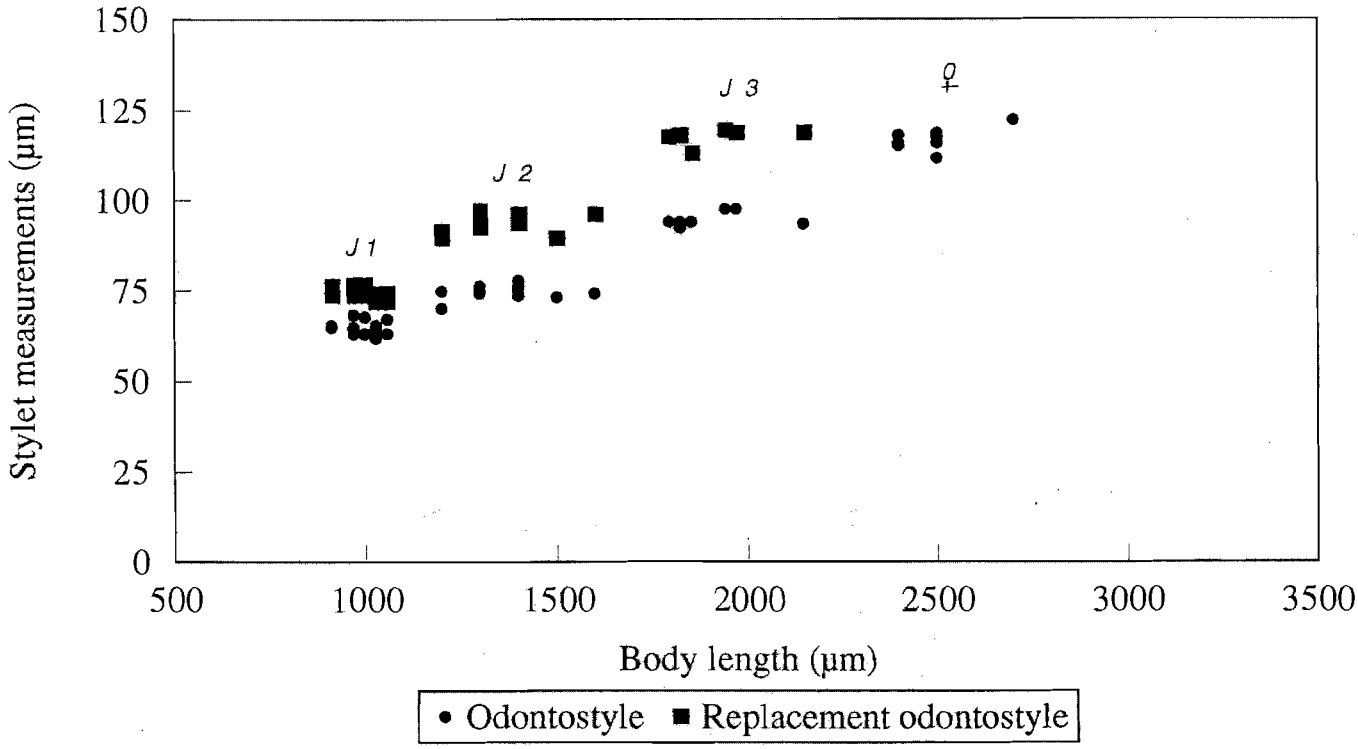

Fig. 3. Scatter diagram plotting body and odontostyle length of individual juveniles and females of *X. chambersi* from Bartow, Florida.

Table II. Morphometrics of *Xiphinema naturale* sp.n. from Florida.

Locality	Green Swamp					Bartow			
	holotype	paratypes							
n	♀	10 ♀♀	1 J_1	1 J_2	8 J_3	10 ♀♀	1 J_1	5 J_2	8 J_3
L (mm)	2.8	2.7±0.08 2.6-2.8	1.1	1.4	2.0±0.15 1.8-2.2	2.8±0.13 2.5-3	1.1	1.4±0.08 1.3-1.5	1.8±0.10 1.7-2
a	61	59.4±2.25 56-63	44	45.2	55.1±2.26 51.7-58	63±1.73 61-67	40.9	49±1.87 47-51	54±1.48 50.8-55.7
b	6.5	6.5±0.43 5.8-7.3	4.3	4.5	5±0.34 4.2-5.2	6.5±0.54 6-7.6	4.3	4.1±0.44 3.6-4.7	4.8±0.45 4.3-5.6
c	37.2	36.6±1.46 34-38	15.4	17.4	25.5±2.03 22.6-27.7	32.4±1.74 30-35.3	13.8	17.2±0.32 16.7-17.5	21±1.80 19.3-24.6
c'	2.7	2.7±0.11 2.5-2.9	4.4	3.8	3.2±0.24 2.7-3.4	3.3±0.2 3-3.5	5.2	4.7±0.19 4.5-5	4±0.28 3.7-4.4
V	23.5	24±0.96 22-25	---	---	---	23±0.84 22-24	---	---	---
Odontostyle µm	135.3	135.3±2.78 132.3-140	69.4	87	111.4±3.30 107-117	133.8±2.18 130-137	68.2	87.5±1.77 85.3-90	107.7±1.79 106-111.2
Odontophore µm	77	76.7±1.70 74.7-79.4	47	56.5	67.4±1.98 65.3-70.6	74.3±2.25 70.6-77.6	43.5	56.4±1.61 54.7-58.8	64.5±1.25 61.8-65.3
Replacement odontostyle µm	---	---	87	107	137.1±4.23 132.3-146	---	84	109.3±3.82 104-114.7	133.7±2.88 128.2-137
Oral aperture to basal guide ring µm	128.2	123.6±4.2 117-132.3	58.2	76	94.5±4.01 88.8-98.8	122±1.78 119-125.3	59.4	75.4±3.07 71.8-78.2	92.6±3.46 86.5-96.5
Tail µm	75.3	75.4±2.85 70.6-79.4	70.6	79.4	78.3±6.07 64.7-85.3	85±3.80 79.4-88.2	76.5	80.6±3.34 76.5-85.3	85.7±4.31 79.4-91.2
J (hyaline portion of tail) µm	21.2	21.6±1.96 19.5-26.5	11.2	14.6	16.9±1.24 15-19.4	21.3±1.41 19-23	10.6	12±1.33 10.6-14.1	16±1.54 14.1-17.6
Body diam. at lip region µm	13	13±0.33 12.4-13.5	8.8	10.6	11.4±0.53 10.6-11.8	12.8±0.34 12.3-13	9.4	9.9±0.50 9.4-10.6	10.6±0.42 10-11.2
Body diam. at guide ring µm	35.9	35.9±0.97 34-37	20	25.3	29.8±0.78 28.8-30.6	34±1.35 31.8-35.3	20.6	23.6±0.42 23-24	28±0.65 27.5-29.4
Body diam. at base of oesophagus µm	41.2	42±2.29 38.2-45.3	23.5	29.4	34.8±2.08 33-38.2	40.7±1.59 38-43	24.7	27±2.16 24.7-30	31.8±1.57 29.4-34
Body diam. at mid-body or vulva µm	45.9	46.3±2.45 41.2-50	24.7	30.6	36.2±2.10 32.1-39.4	44±1.82 40-46	26	28.3±2.13 26-31.2	33.3±2.25 30.6-37.6
Body diam. at anus µm	28.2	28.2±1.43 26-30.6	16	20.6	24.5±0.98 23.5-26	26.1±0.77 25.3-27	14.7	17±0.39 16.5-17.6	21.3±0.68 20.6-22.3
Body diam. at beginning of J µm	10	9.8±0.62 8.8-10.6	5.3	5.9	7.4±0.45 7-8.2	8.5±0.34 8-8.8	4.7	5.5±0.55 4.7-6	6.4±0.32 6-7

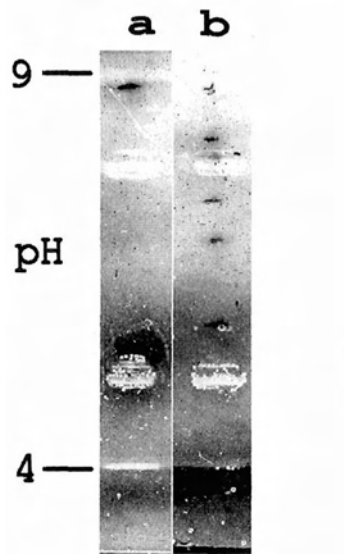

Fig. 4. Isoelectrofocusing of superoxide dismutase (SOD) isozymes of nematode extracts from *X. chambersi* (a) and *Xiphinema naturale* sp.n. (b) from Florida. Black bands indicate enzyme activity over a gray background. Mini-gels (separation area 4x4 cm) were stained for SOD activity, dried,and directly scanned and turned into negative digital images; the images were printed on photo quality paper.

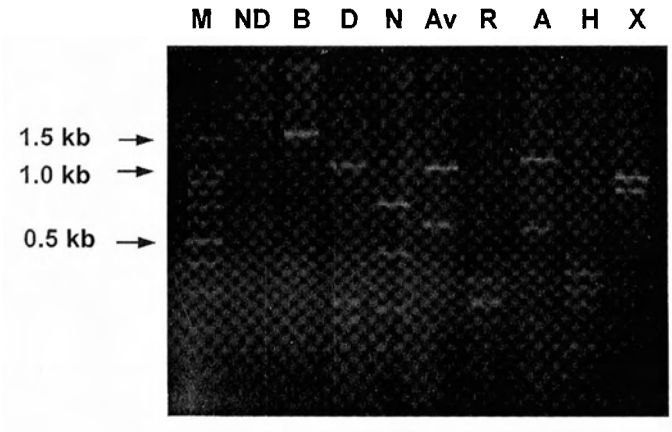

Fig. 5. Restriction digestions of the PCR amplification product of the ITS region of *X. chambersi* from Florida, separated on a 2% agarose gel and stained with ethidium bromide; M=100bp DNA ladder, ND=not digested, B=*Bam* HI, D=*Dde* I, N=*Nde* I, Av=*Ava* II, R=*Rsa* I, A=*Alu* I, H=*Hinf* I, X=*Xba* I.

short uterus without any uterine differentiation; ovary reflexed. Prerectum indistinct; rectum as long as the anal body width. Tail elongate, conical with subdigitate hyaline region, bearing two caudal pores on each side.

Male not found.

Juveniles separated into three stages (Fig. 6), similar to adults, with longer tail; tail clearly ventrally arcuate in the first juvenile stage.

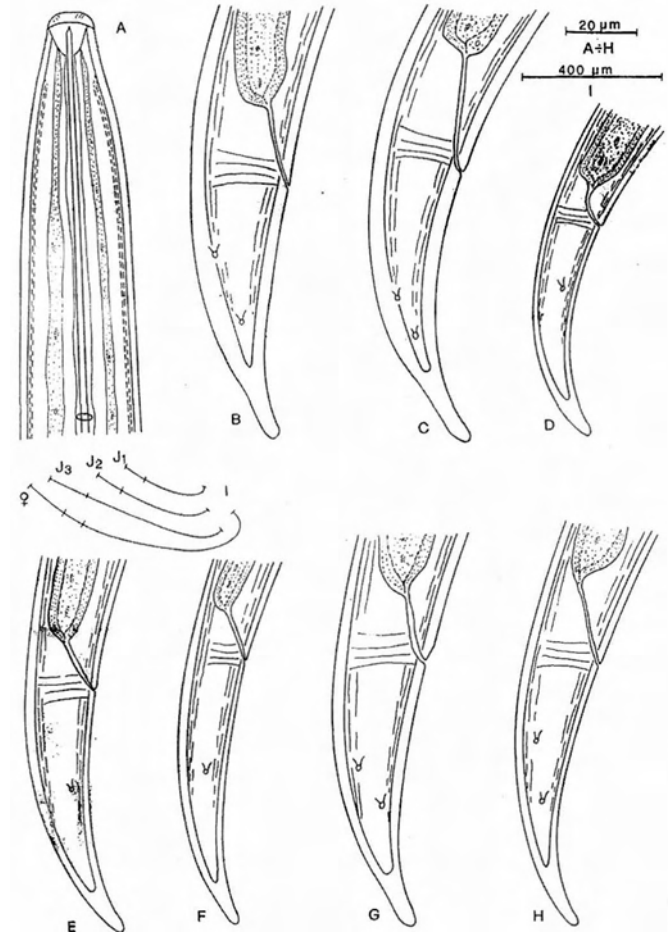

Fig. 6. *Xiphinema naturale* sp.n. from Florida: A, female anterior region; B and C, female posterior region; D, first stage juvenile posterior region; E and F, second stage juvenile posterior region; G and H, third stage juvenile posterior region; I, posture.

Table III. Estimated restriction fragment sizes (bp) of PCR amplified ITS of *Xiphinema* species from Florida.

	X. naturale sp. n.		*X. chambersi*
Enzymes	(Green Swamp pop.)	(Labelle pop.)	(Bartow pop.)
ND	2000	2000	2000
Alu I	1200, 580, 230	1200, 580, 230	1200, 580, 230
Bam HI	uncut	uncut	1600, 360
Dde I	1200, 250, 220, 180	1200, 250, 220, 180	1200, 250, 220, 180
Hinf I	450, 380, 320, 250, 180	450, 380, 320, 250, 180	450, 380, 320, 250, 180
Rsa I	350, 280, 280, 200, 180	350, 280, 280, 200, 180	350, 280, 280, 200, 180
Xba I	1100, 900	1100, 900	1100, 900

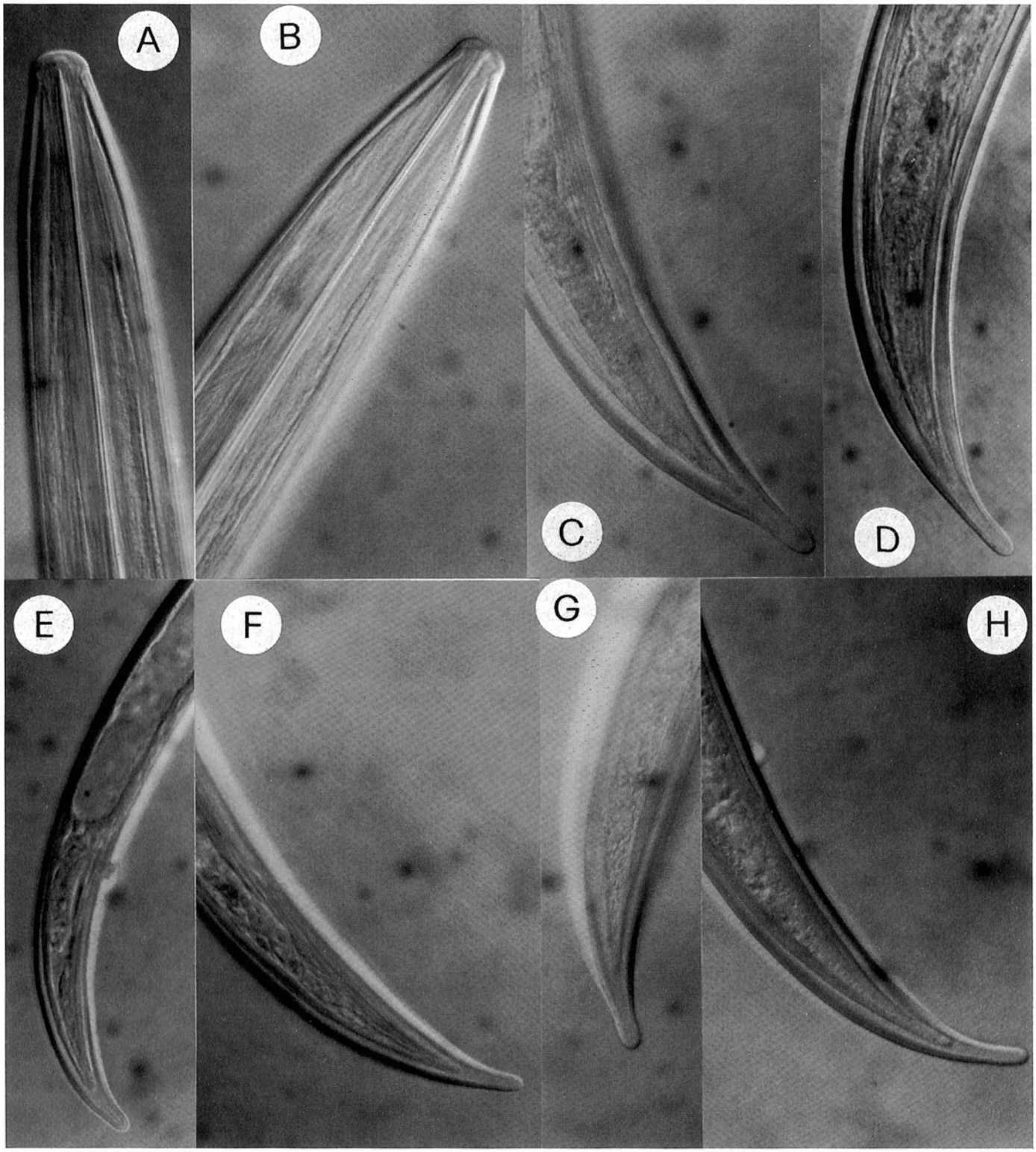

Fig. 7. Photomicrographs of *X. naturale* sp.n. from Florida: A and B, female anterior region; C and D, female posterior region; E, first stage juvenile posterior region; F, second stage juvenile posterior region; G and H, third stage juvenile posterior region.

Type habitat and locality. rhizosphere of long needle pine, *Pinus palustris* Mill., Green Swamp (sample Fl. n. 254), in Florida, United States of America; it also occurred at Bartow (sample Fl. n. 65) and Labelle, Duda (sample Fl. n. 156), always in the rhizosphere of long needle pine.

Type material: holotype female, seven paratype females and paratype juveniles at the Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, Bari, Italy; three paratype females in the Plant Nematology Laboratory Collection,United States Department of Agriculture, Beltsville, Maryland, United States of America.

Diagnosis: *Xiphinema naturale* sp.n. is a monosexual, monodelphic (anterior female genital branch completely

laking) species with three juvenile stages. It is characterized by body length of 2.7-2.8 mm, rounded lip region, separated from the rest of the body by a slight depression, odontostyle length of ca. 135.3 µm, anterior vulva (V= 23-25%) and elongate conical tail with subdigitate terminus.

The code of *X. naturale* in the polytomous key of Loof and Luc (1990) is: A1, B4, C2, D3, E1, F3, G3, H2, I3, J2, K2, L1, which falls in the *Group 1* proposed by its authors.

Relationships. *Xiphinema naturale* sp.n. resembles *X. radicicola* Goodeyi, 1936, *X. chambersi* Thorne, 1939 and *X. monohysterum* Brown, 1968 (see Cohn and Sher, 1972). However, it differs from *X. radicicola* in its longer

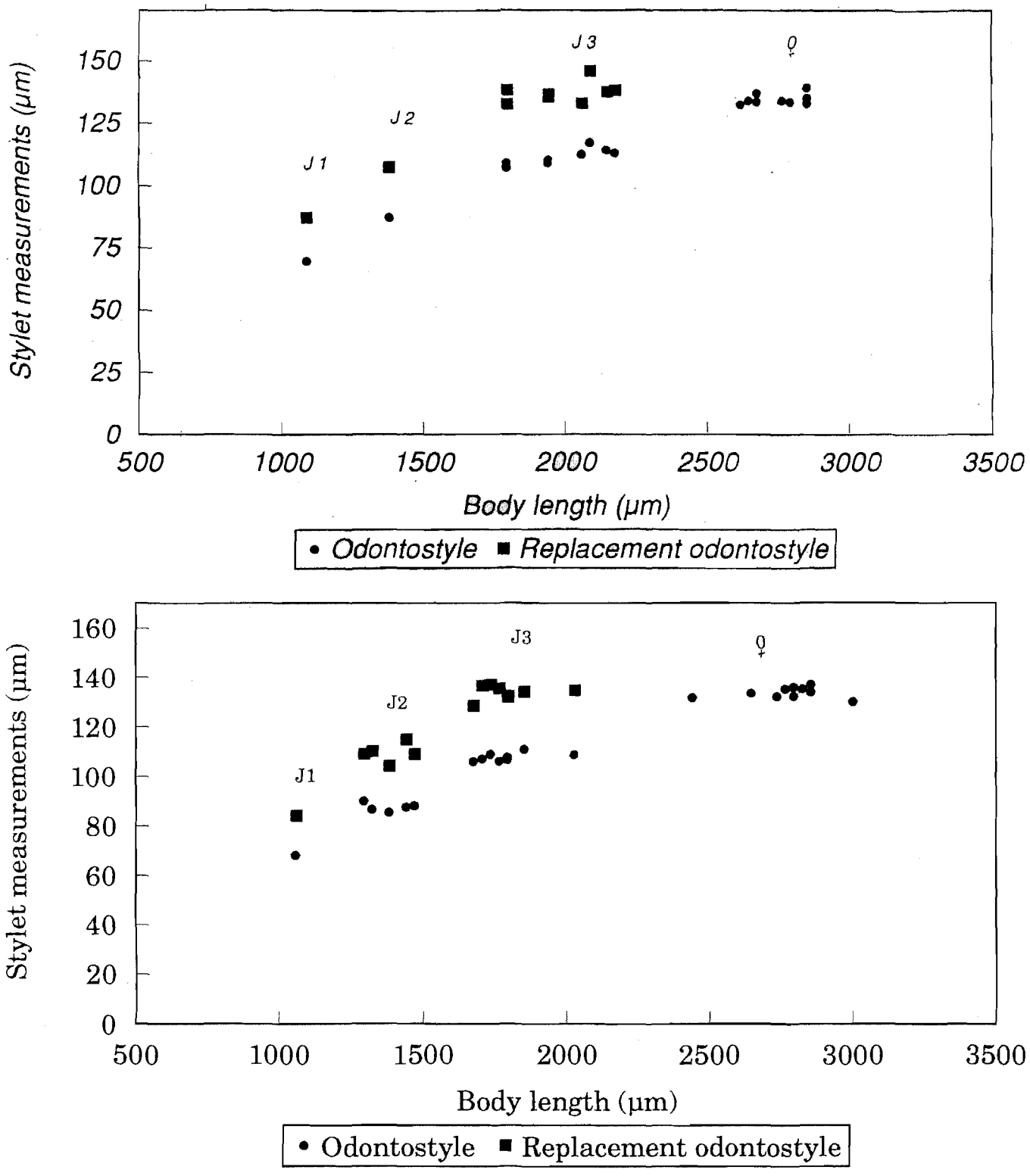

Fig. 8. Scatter diagrams plotting body and odontostyle length of individual juveniles and females of *X. naturale* sp.n. from Florida: top, Green Swamp (paratypes) and bottom, Bartow populations.

body, (L= 2.2 mm in *X. radicicola*), longer total spear length (143 µm in *X. radicicola*), higher value of c' (2.3 in *X. radicicola*), anterior vulva (V= 28% in *X. radicola*) and more elongate tail. It also differs from *X. chambersi* in its longer body (L= 2.4-2.5 in *X. chambersi*), longer odontostyle (114-117 µm in *X. chambersi*) and shorter tail (95-105 µm in *X. chambersi*). Finally, *X. naturale* differs from *X. monohysterum* in its anterior vulva (V= 30% in *X. monohysterum*), longer odontostyle (99 µm in *X. monohysterum*) and odontophore (70 µm in *X. monohysterum*).

Superoxide dismutase isozymes patterns of *X. naturale* sp.n. were clearly distinguishable from those of *X. chambersi* by the presence of numerous bands of activity ranging from very basic to slightly acidic (Fig. 4b).

Specimens of the Green Swamp and the Labelle populations of *X. naturale* sp.n. amplified ca 2000 kb, however, their amplification products were not digested by the enzyme *Bam* HI (Table III; Fig. 9). Enzymes *Nde* I and *Ava* II were not tested with these populations.

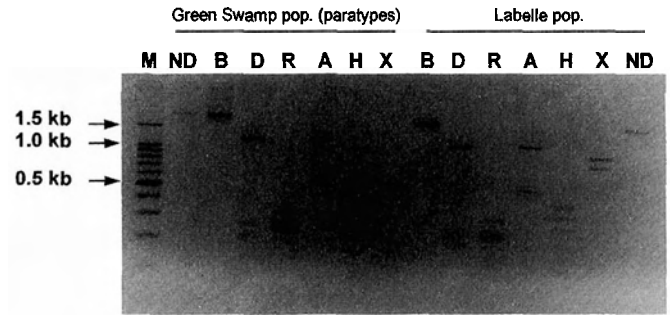

Fig. 9. Restriction digestions of the PCR amplification product of the ITS region of *X. naturale* sp.n. from Florida, separated on a 2% agarose gel and stained with ethidium bromide; M=100 bpDNA ladder, ND=not digested, B=*Bam* HI, D=*Dde* I, R=*Rsa* I, A=*Alu* I, H=*Hinf* I, X= *Xba* I.

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