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## MORPHOMETRIC VARIATION AND JUVENILE STAGES OF SOME LONGIDORID NEMATODES FROM BULGARIA WITH COMMENTS ON THE NUMBER OF JUVENILE STAGES OF *LONGIDORUS AFRICANUS*, *L. CLOSELONGATUS* AND *XIPHINEMA SANTOS*

by

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**Summary.** The morphometric variability of six species of longidorid nematodes occurring in south west Bulgaria is discussed. It is suggested that *Longidorus distinctus*, *Xiphinema italiae* and *X. vuittenezi* may have three or four juvenile stages, *L. euonymus* and *L. latocephalus* have three and *X. pachtaicum* four juvenile stages. *L. closelongatus* from Crete and *L. africanus* from Egypt and *X. santos* from Portugal must be regarded as populations with only three juvenile stages. The evidence of scatter diagrams of body length and stylet measurements for each of the species is discussed in relation to the possibility that in some there may be two juvenile forms with similar stylet measurements but differing in body length.

Morphometric characters of longidorid species occurring in Bulgaria have been reported by various authors (Stoyanov, 1964; Lamberti *et al.*, 1983; Choleva *et al.*, 1991; Peneva and Choleva, 1992a and 1992b; Robbins *et al.*, 1995).

In August-September 1996 soil samples were collected from the rhizosphere of various crops in south west Bulgaria and from the rhizosphere of poplar at Kostinbrod, near Sofia (Central western Bulgaria). The morphometric variability of the species of Longidoridae found is discussed and the occurrence of juvenile stages commented upon.

Nematodes were extracted from 1 l soil aliquots by the Cobb wet sieving technique, fixed in 5% hot formalin and processed to glycerin and mounted as permanent slides. Measurements were made with the aid of a camera lucida.

Three species of *Longidorus*: *L. distinctus* Lamberti, Choleva *et* Agostinelli, *L. euonymus* Mali *et* Hooper and *L. latocephalus* Lamberti, Choleva *et* Agostinelli; and three species of *Xiphinema*: *X. italiae* Meyl, *X. pachtaicum* (Tulaganov) Kirjanova and *X. vuittenezi* Luc, Lima, Weischer *et* Flegg were found.

### Measurements and descriptions

#### *LONGIDORUS DISTINCTUS*

Lamberti, Choleva *et* Agostinelli, 1983

(Table I; Figs 1 A, B-3)

A population comprised of various juvenile stages was found at Kolarovo in the rhizosphere of walnut (*Juglans regia* L.). *Longidorus distinctus*

TABLE I - *Morphometric characters of Longidorus distinctus from Bulgaria.*

Locality	Kolarovo					Petrič
Host	Walnut					Peach
n	3 ♀♀	7 J <sub>1</sub>	10 J <sub>2</sub>	6 J <sub>3</sub>	7 J <sub>4</sub>	7 ♀♀
L (mm)	4.7±0.29 4.5-5.0	1.2±0.05 1.1-1.2	1.8±0.15 1.6-2	2.7±0.16 2.4-2.8	3.5±0.25 3.1-3.9	4.7±0.33 4.1-5.0
a	106.4±6.93 100.9-114.2	57.0±2.47 53.4-60	68.7±3.41 62.2-75	83.5±2.24 80-85.9	93±3.47 90.2-100.2	113.9±8.25 106.6-125
b	11.5±0.70 10.8-12.2	5.4±0.37 5.1-6.2	7.3±0.96 6.4-9.5	9.3±0.80 8-10.2	10.2±1.46 9.2-13.4	12.6±0.71 11.5-13.5
c	88.9±7.35 83.8-97.3	27±1.34 25.6-29.3	33.9±2.55 28.3-38	47±1.14 45-48	60.7±3.87 55-68	79.4±4.33 73.7-88.3
c'	1.9±0.10 1.8-2.0	3.5±0.20 3.2-3.8	3.1±0.14 3-3.4	2.6±0.05 2.6-2.7	2.2±0.12 2-2.3	2.1±0.14 1.9-2.2
V%	45±1.15 44-46	–	–	–	–	47.2±1.30 45-48
Odontostyle µm	90.3±10.97 84-103	54±2.70 49.7-58.3	56.9±1.81 54.3-59.4	66.2±0.83 65-66.9	81.6±6.19 74.9-88.6	85±0.62 84-86
Odontophore µm	52±2.80 48-55	32.2±1.53 30.9-35.4	36.3±1.33 34.3-38.9	44.2±3.15 40-48.6	47.8±2.67 44.6-52.3	51±1.33 49.7-52.6
Replacement odontostyle µm	–	57.7±2.69 52.6-61	63.9±1.68 61.7-65.7	72.5±1.00 71.4-74.3	91.2±5.91 84.6-100	–
Oral aperture to guide ring µm	29.5±2.88 26.9-32.6	17±0.41 16.6-17.7	18.9±0.40 18.3-19.4	22.2±0.66 21.7-23.4	25.4±1.48 22.3-26.9	28.2±1.50 25.7-29.7
Tail µm	52.6±1.15 51.4-53.7	43.2±2.07 41-46.9	51.7±4.52 45.7-58.9	56.3±3.46 51.4-60	57.5±4.02 52.6-64	59±2.07 57-61.7
J (hyaline portion of tail) µm	12.2±2.25 10.9-14.8	3.8±0.42 2.9-4	5.1±0.99 3.9-6.3	6.8±0.84 5.7-8	9.2±1.28 7.2-10.9	12.8±0.74 12-14
Body diam. at lip region µm	11.8±0.35 11.4-12	7.1±0.27 6.9-7.4	8.8±0.21 8.6-9	10.6±1.02 9.7-12.6	10.7±0.29 10.3-10.9	11.4±0.36 11-12
Body diam. at guide ring µm	18.5±1.33 17.7-20	11.8±0.57 11.4-12.6	13.2±0.52 12.6-13.7	15±0.41 14.3-15.4	16.8±0.21 16.6-17	17.2±0.48 16.6-17.7
Body diam. at base of oesophagus µm	35.4±2.52 33.7-38.3	17.9±0.29 17.7-18.3	23±1.89 20.6-26.3	28.4±1.18 26.3-29.7	31.8±1.37 30.3-34.3	34.3±1.31 32.5-36
Body diam. at mid-body or vulva µm	44±4.33 39.4-48	20.3±0.44 20-21	25.5±1.98 22.9-28.6	30±3.69 23.4-33.7	37.5±2.06 34.3-41	41.3±2.97 38.3-45
Body diam. at anus µm	28.2±2.40 26.3-30.9	12.3±0.54 11.4-13	16.7±1.57 14.9-20	21.5±1.23 20-23.4	25.9±1.19 24.6-27.4	28±1.68 25.7-30.3
Body diam. at beginning of J µm	11.1±1.33 10.3-12.6	4.2±0.29 4-4.6	4.7±0.73 3.5-5.7	6±0.33 5.7-6.3	7.7±0.74 6.9-9	11±1.07 9.7-12

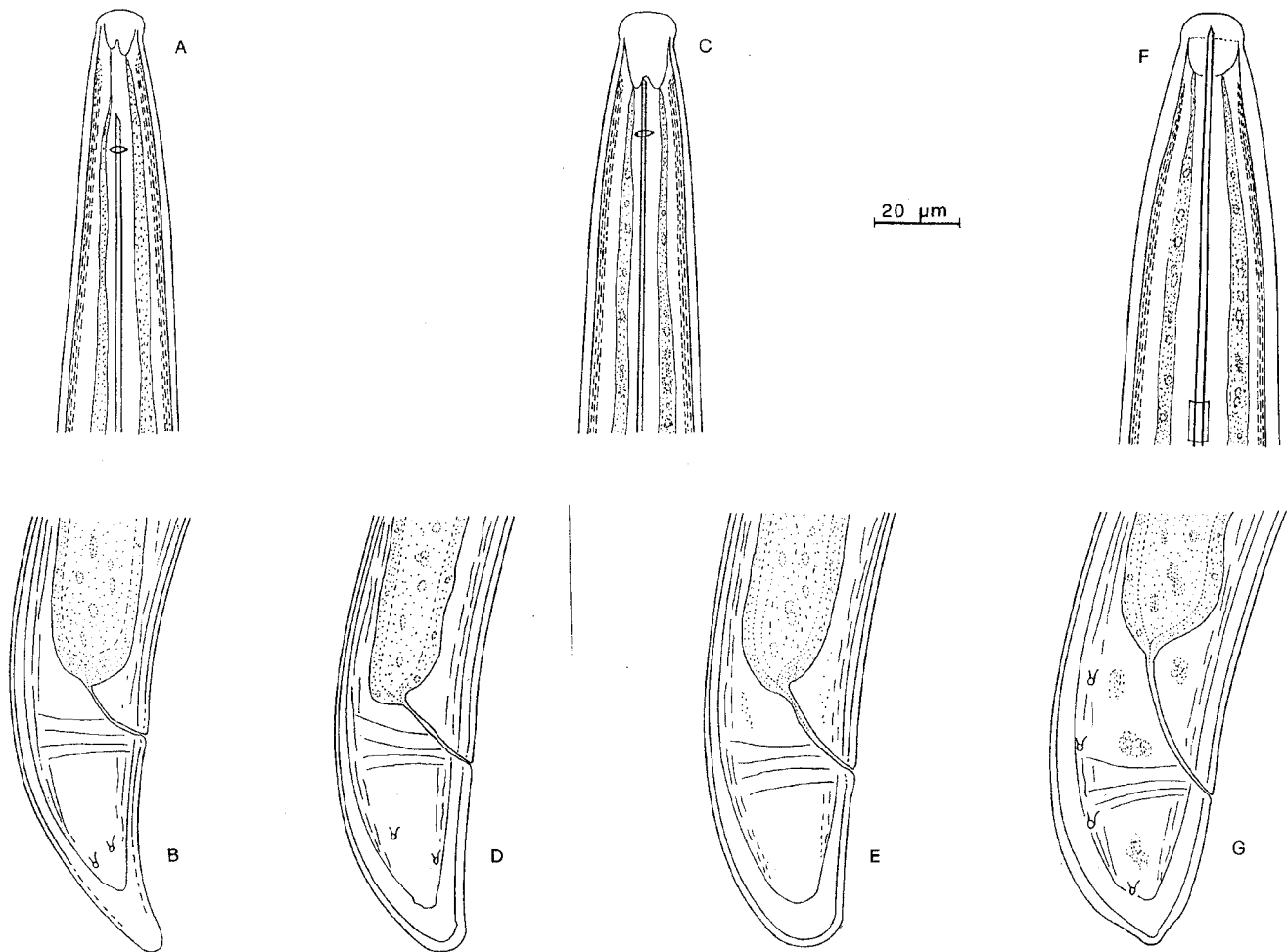


Fig. 1 - Females of longidorids from Bulgaria: A and B, anterior and posterior regions of *Longidorus distinctus*; C and D, anterior and posterior regions of *L. euonymus* (Kolarovo population); E, posterior region of *L. euonymus* (Sandanski population); F and G, anterior and posterior regions of *Xiphinema vuittenezi* (Kostinbrod population).

*tus* also occurred in the rhizosphere of peach [*Prunus persica* (L.) Batsch] at Petrič and in the rhizosphere of grapevine (*Vitis* sp.) and cherry (*Prunus avium* L.) at Pergoli, near Sandanski.

Female: death posture an open C; lip region offset by a depression from the rest of the body, slightly expanded, rounded frontally; amphidial pouches more or less asymmetrically bilobed; genital system didelphic with reflexed ovaries; presence of glandular bodies in the lateral hypodermal cords, more readily visible in the posterior third of the body; rectum about

2/3 of the body diameter of anus; tail conoid, slightly bent ventrally, dorsally convex and ventrally concave (Fig. 1B), sometimes more elongate and gradually tapering (Fig. 2B).

Male: not found.

Juveniles: similar to females with tails tapering more gradually towards the extremity and almost straight or much less bent in the first stages. They can be separated into four groups according to the body length (Table I). However, plotting body length versus odontostyle length the separation between the first two

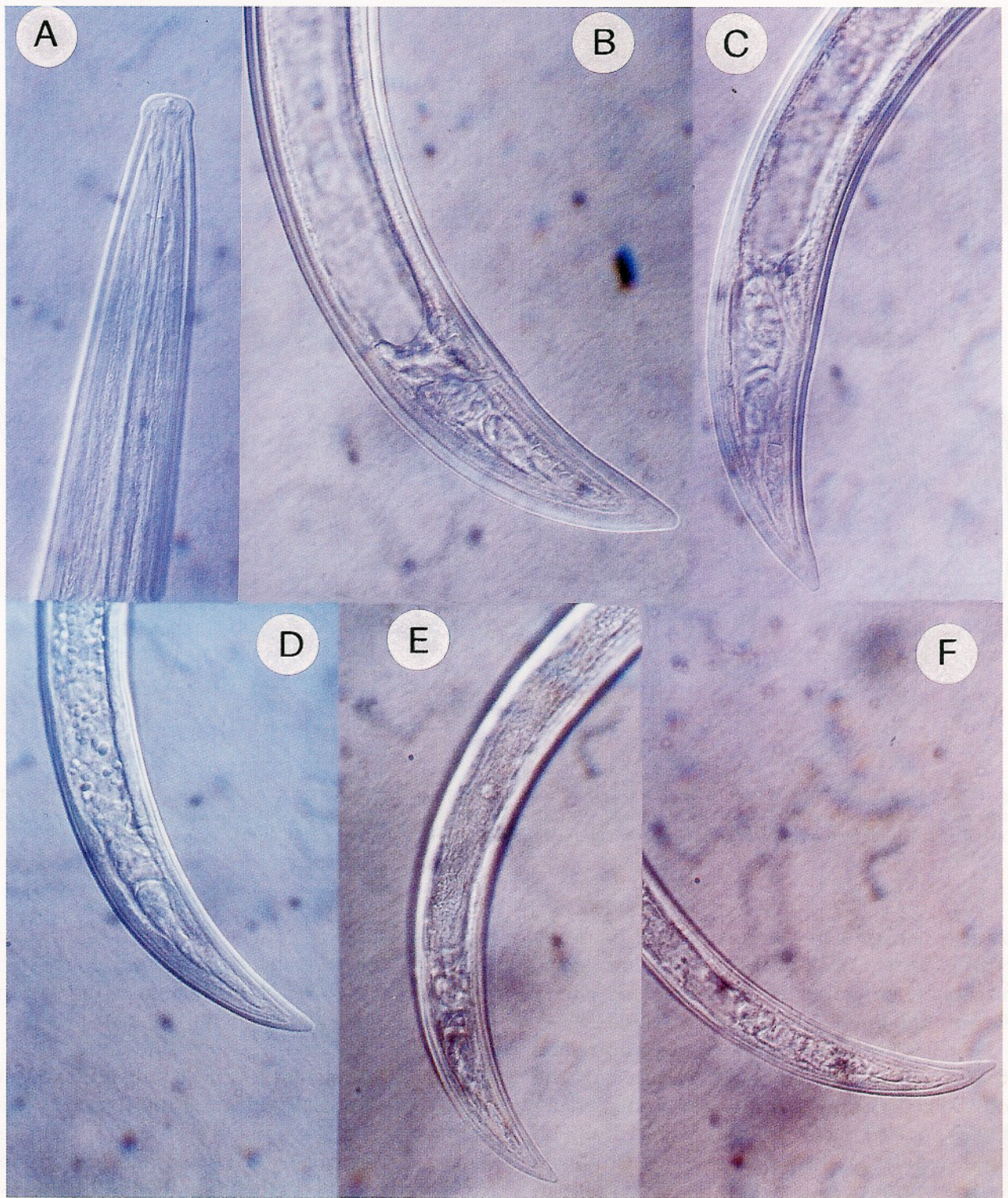


Fig. 2 - Photomicrographs of *L. distinctus*; A, female anterior region; B, female posterior region; posterior regions of fourth (C); third (D), second (E) and first (F) juvenile stages.

groups is not well defined (Fig. 3). In our opinion, the Kolarovo population of *L. distinctus* consists of four juvenile stages, as indicated by Peneva and Choleva (1992a) and the first stage has a subdigidate tail.

Bulgaria and the former Yugoslavia (Barsi, 1989) are the only countries in which *L. distinctus* is reported to occur. The Kolarovo population of *L. distinctus* differs from the type population from north western Bulgaria (Lamberti *et al.*, 1983) mainly by its longer odontostyle (80  $\mu\text{m}$  in paratypes) and shorter tail (58  $\mu\text{m}$  in paratypes). In this respect other populations from Bulgaria (Peneva and Choleva, 1992a) or Vojvodina (Barsi, 1989) seem to be biometrically more similar to the original description of *L. distinctus*, as the Petrič population which differs from the Kolarovo specimens in having larger values for *a* and *c'*, smaller value of *c*, slightly posterior vulva, shorter odontostyle and shorter tail.

*L. distinctus* fits exactly with the code of the polytomous key proposed by Chen *et al.*, 1997 which is:

A 2/3, B 1/2, C 2/3, D 2, E3, F 2/3, G 2/3, H 5/6, I 1/2.

The only proposed amendment is I 1/2 as a few populations without males, have been found.

### ***LONGIDORUS EUONYMUS***

**Mali *et Hooper*, 1974**

(Table II; Figs 1 C-E, 4-6)

Populations with several juveniles occurred at Sandanski in the rhizosphere of olive (*Olea europaea* L.) and at Kostinbrod, in the rhizosphere of poplar (*Populus alba* L.). *Longidorus euonymus* was found also in the rhizosphere of black currant (*Ribes nigrum* L.) at Sandanski and of grapevine at Hotovo.

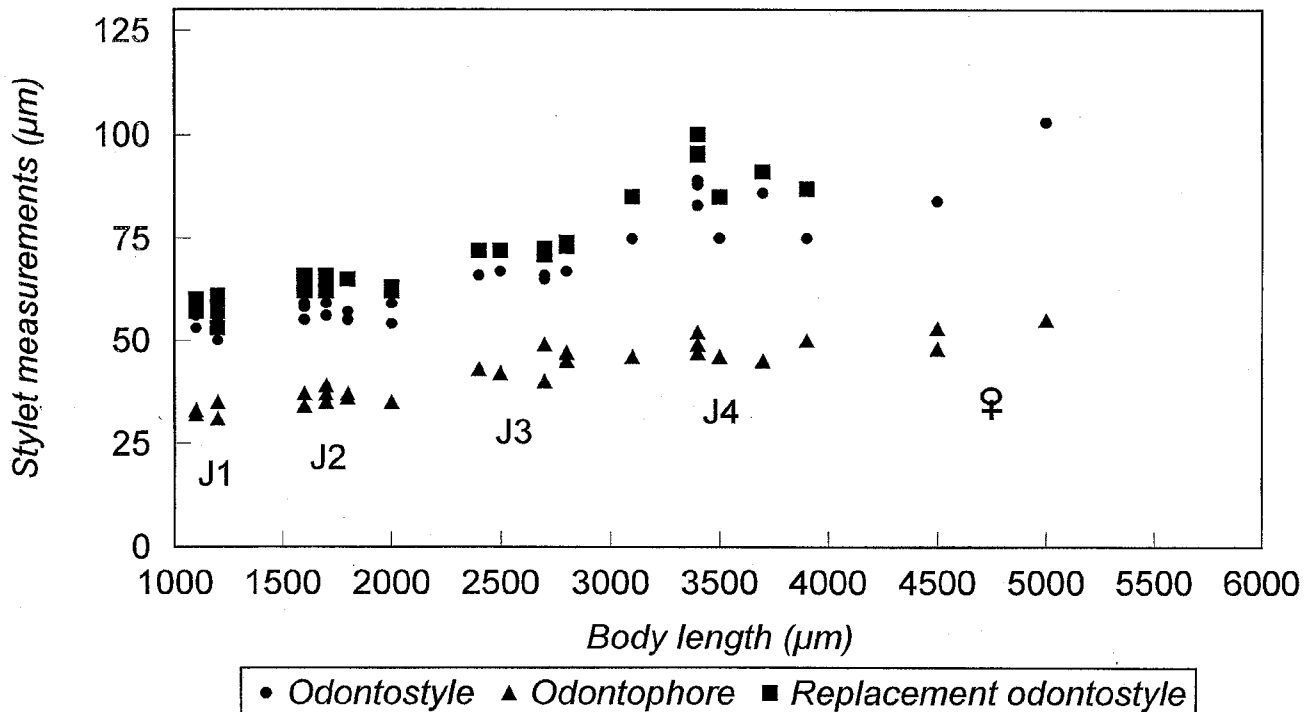


Fig. 3 - Scatter diagram separating juveniles and females of *L. distinctus*.

TABLE II - *Morphometrics of L. euonymus from Bulgaria.*

Locality Host	Kostinbrod							Sandanski				
	Poplar							Olive				
n	2♀♀	12 J <sub>1</sub>	18 J <sub>2</sub>	3 J <sub>3</sub>	2 J <sub>4</sub>	6 ♀♀	1 J <sub>1</sub>	7 J <sub>2</sub>	7 J <sub>3</sub>	14 J <sub>4</sub>		
L (mm)	6.0-7.0	1.4±0.09 1.6-1.6	2.2±0.21 1.9-2.5	3.5±0.06 3.4-3.5	4.5-5.3	5.7±0.68 5-6.8	1.7	2.6±0.28 2.4-3.2	3.5±0.25 3.2-3.9	4.2±0.23 3.9-4.6		
a	130.4-145.8	67±2.84 63-73.7	81.2±3.65 74-85.5	107±0.72 106.2-107.5	131.2-134.5	123±11.01 115.2-144.9	66	87±5.67 80.8-98	106±7.24 96.2-114.6	113.8±8.43 100-127		
b	13.8-15.7	5.6±0.43 4.7-6.4	7.6±0.69 6.3-8.7	10.3±0.66 9.6-10.9	11.2-14.5	15±1.69 12.9-17.5	7.8	9.2±1.20 7.8-11.2	11.7±1.39 10.2-14	12.5±1.27 10.9-15.2		
c	157.9-155.5	31.4-1.90 28.5-34	43.9±2.09 40.5-48.3	63.8±3.31 61.4-67.6	78.9-89.9	121.8±11.25 105.3-137.5	34.9	55.5±6.85 45.6-66.7	80.9±11.69 66-95	89.5±6.86 80.4-100		
c'	1.2-1.3	3.2±0.15 3.1-3.5	2.7±0.11 2.6-2.9	2.5±0.15 2.6-2.6	2.0-1.8	1.4±0.08 1.2-1.4	2.9	2.1±0.13 2-2.4	1.8±0.25 1.5-2.2	1.6±0.14 1.4-1.8		
V%	49-51	-	-	-	-	52±1.63 50-54	-	-	-	-		
Odontostyle µm	83.4-81.7	49.7±1.90 46.9-52.6	55.7±1.52 53-58.9	63.6±0.87 62.9-64.6	73-74.9	78.5±2.51 74.3-81	46.9	61.5±1.29 59.4-62.9	67.8±2.21 64-70.3	70.7±1.72 68-73.7		
Odontophore µm	54.3-56	35.2±1.69 31.4-37	42.8±2.19 38.9-45.7	47±0.61 47-48	50-51	51±1.38 49-53	34.3	39±2.80 37-45	45.6±1.58 42.9-46.9	49±0.89 48-51		
Replacement odontostyle µm	-	56.3±2.05 53-60	65±2.11 61.7-69.7	71.8±1.62 70.9-73.7	82.9-85	-	58.9	69.5±2.11 65.7-72	74.9±1.90 72-77	81.4±3.19 76.6-86.9		
Oral aperture to guide ring µm	27.4-28.6	16.0±0.75 14.3-16.6	19±0.85 17-20.6	22.3±0.98 21.7-23.4	25.7-26.9	25±0.50 24.6-25.7	17.7	20±0.77 18.9-21	21.9±0.95 21-22.9	23±1.02 21-24.6		
Tail µm	38-44.6	43.7±2.19 41-48.6	48.9±4.30 42.9-56	54.2±3.50 50.3-57	57-59	47.3±6.57 40-57	48.6	47.8±3.07 43-52.6	44.2±3.79 40-48.6	47±3.76 40-56		
J (hyaline portion of tail) µm	9.7-9.7	6.3±0.70 5.1-7.4	6.9±0.87 5.7-8	9.2±0.98 8.6-10.3	8-7.4	9.6±1.78 6.9-11.4	6.3	8.1±1.26 6.3-10.3	7.4±1.28 6-8.6	8.3±0.80 7.4-10.3		
Body diam. at lip region µm	13-13.7	8.2±0.27 8-8.6	9.6±0.40 9-10.3	11.2±0.29 10.9-11.4	12-12	12.9±0.67 12-13.7	9.7	10.8±0.50 10.3-11.4	11.4±0.49 10.9-12	12.2±0.61 11.4-13.7		
Body diam. at guide ring µm	18.9-20	12.1±0.35 11.4-12.6	13.7±0.78 12.6-15.4	15.2±0.29 14.9-15.4	17-17	17.8±0.48 17-18.3	13.7	14.8±0.50 14.3-15.4	15.8±0.54 14.9-16.6	16.4±0.55 15.4-17		
Body diam. at base of oesophagus µm	33-35	18.6±0.46 17.7-19.4	23.2±1.83 20-26.3	29.5±0.40 29-29.7	31.4-33.7	35.7±2.56 32-39.4	21.7	27.2±1.04 26.3-29	29.2±1.13 27.4-30.3	32.6±1.94 28.6-35.4		
Body diam. at mid-body or vulva µm	46-48	20.3±0.58 19.4-21.7	26.5±1.63 24-29.5	32.4±0.35 32-32.6	34.3-39.4	46.6±3.81 41-51.4	25.7	30.3±1.32 28.6±32.6	33±2.11 29.7-35.4	37±2.58 34.3-41		
Body diam. at anus µm	32-35	13.5±0.69 13-15.4	17.9±1.35 16.6-20	21.7±0.00 21.7-21.7	29-33	35.4±3.43 31.4-40	17	22.1±1.36 20-24	25.3±1.66 22.3-27	29.7±1.97 26.3-33		
Body diam. at beginning of J µm	17-16.6	5.5±0.40 5.1-6.3	7±0.83 5.7-8	9.3±0.64 8.6-9.7	12-14.3	20.8±1.47 18.3-22.9	5	9.4±1.03 8-11	13±1.69 10.3-15.4	15.2±1.35 13.7-17.7		

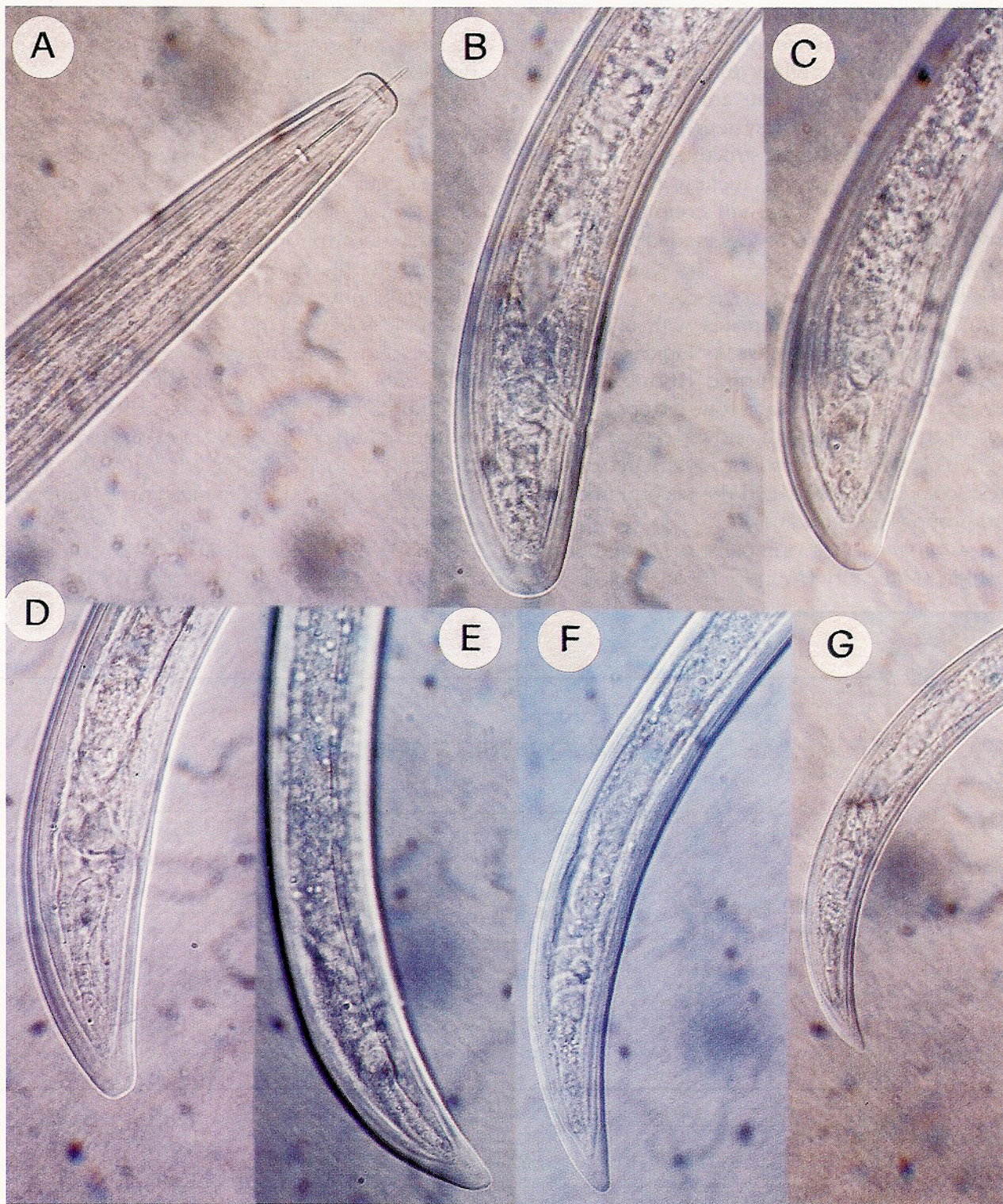


Fig. 4 - Photomicrographs of *L. euonymus*: Sandanski population A, female anterior and B posterior regions; Kostinbrod population, posterior region of C, female; D, fourth stage; E, third stage; F, second stage; G, first stage juveniles.

Female: death posture an open C to single spiral; lip region offset by a depression from the rest of the body, slightly expanded, laterally rounded and frontally flat; amphidial pouches slightly bilobed; genital system didelphic with reflexed ovaries; granular structures occur in the lateral cords along body; rectum about 2/3 of the body width at anus; tail conoid, dorsally convex, tapering more or less gradually towards the rounded extremity.

Male: not found.

Juveniles: similar to females with tails more elongated in the first stages. As indicated in the original description (Mali and Hooper, 1974), they may be separated into four groups, on the basis of their body length. However, the scatter diagrams based on the odontostyle length, indicate a coincidence between the second and the third group (Figs 5 and 6).

*L. euonymus* was originally described from Slovakia (Mali and Hooper, 1974a; Liskova *et*

*al.*, 1995) and subsequently found in Italy (Roca and Lamberti, 1985), Poland (Szczygiel and Brzeski, 1985) and Vojvodina, in former Yugoslavia (Barsi, 1989). The population from Kostinbrod, as with other populations from Bulgaria (Lamberti *et al.*, 1983; Peneva and Choleva, 1992a), Italy (Lamberti *et al.*, 1985) and Vojvodina (Barsi, 1989) fit within the range of the original description (Mali and Hooper, 1974). Conversely, the population from Sandanski has a shorter body and odontostyle, smaller values of ratios a and c and slightly anterior guide ring, as compared to the original one. A male of *L. euonymus*, biometrically very close to the Sandanski population, was described from southern Italy (Roca, 1991). This requires an amendment to the Chen *et al.* (1997) polytomous key, the code of which for *L. euonymus* is now:

A 2/3, B 1/2, C 2/3, D 4, E 2/3, F 3/4, G 2/3/4, H 2/3, I 1/2.

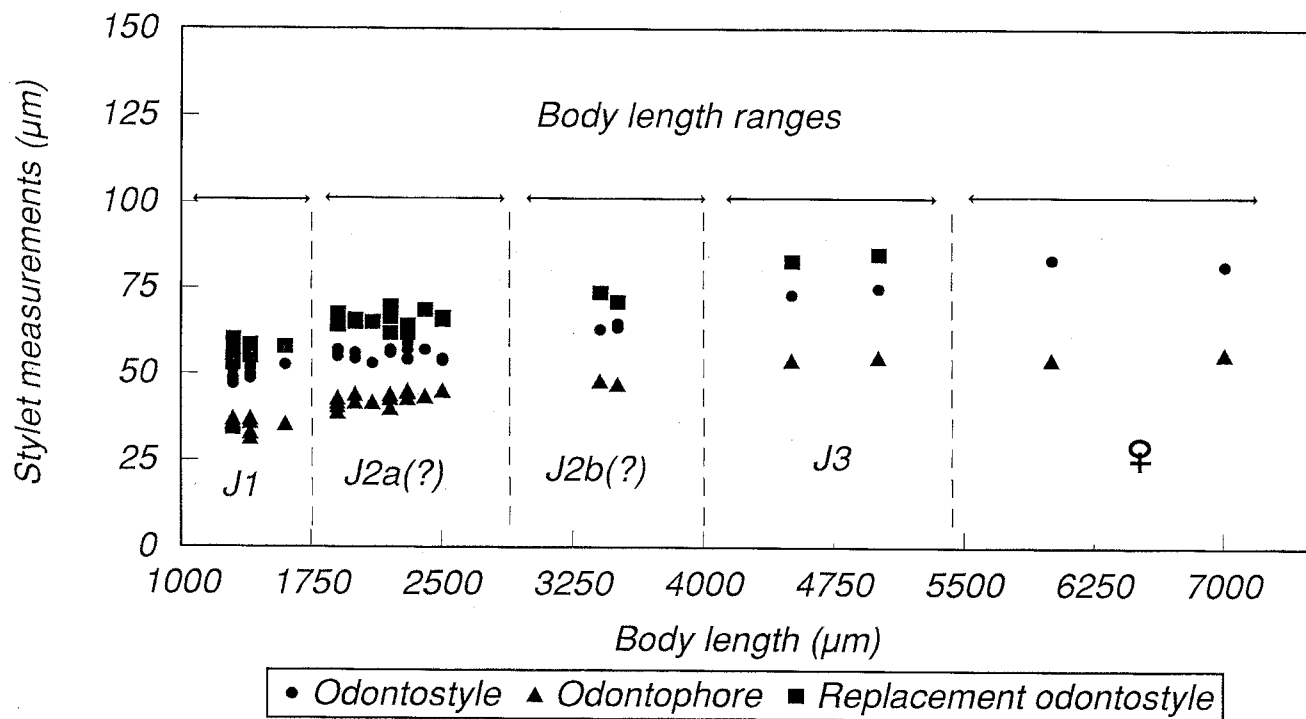


Fig. 5 - Scatter diagram separating juveniles and females of *L. euonymus*, Kostinbrod population.



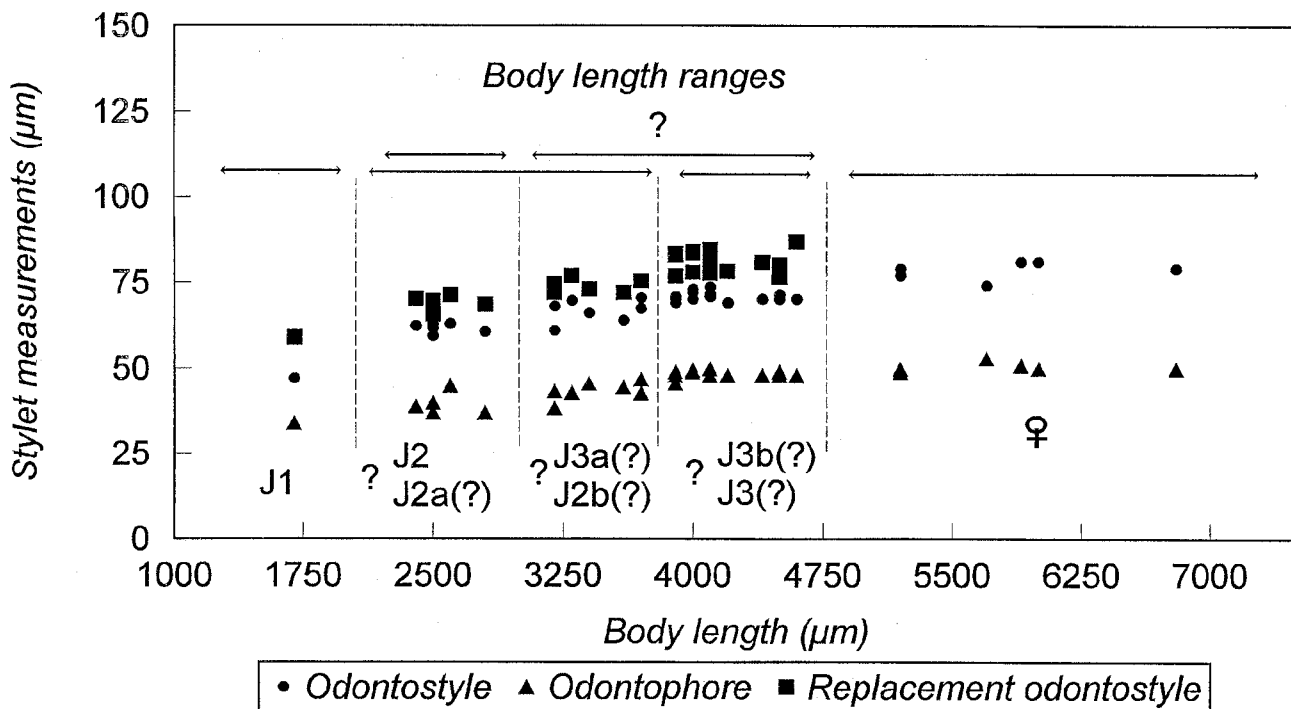


Fig. 6 - Scatter diagram separating juveniles and females of *L. euonymus*, Sandanski population.

### ***LONGIDORUS LATOCEPHALUS***

**Lamberti, Choleva et Agostinelli, 1983**

(Table III, Figs 7 and 8)

*Longidorus latocephalus* seems to be a major pest of tobacco (*Nicotiana tabacum* L.) in the Balcanic region (Melillo *et al.*, 1997). It has been found in the rhizosphere of tobacco at Kolarovo, at Strumica, in the Yugoslavian Macedonia and at Katerini, in the Greek Macedonia. *L. latocephalus* has also been found in the rhizosphere of walnut at Kolarovo and of grapevine at Pergoli and Sandanski, all sites in the neighborhood of the type locality, Petrič.

Female: death posture an open C; lip region well offset from the rest of the body, hemielliptical, expanded; amphids pouch-like, not clearly lobed; genital system didelphic with reflexed ovaries; large globules with granular appearance in the prerectum region; tail conoid, dorsally curved and ventrally flat, with rounded terminus.

Male: four males occurred in the tobacco

population from Kolarovo; they have respectively 5, 7, 8 and 8 ventromedian supplements in addition to the adanal pair.

Junveniles: tail more elongated and more gradually tapering towards the extremity in the first stages. They clearly separate into three groups as indicated by Robbins *et al.* (1995), for *L. pisi*, synonym of *L. latocephalus* (Lamberti *et al.*, 1996) and Lamberti *et al.* (1996a).

The Kolarovo and Pergoli populations of *L. latocephalus* differ from each other only in the latter having a slightly shorter tail.

Compared to the paratype females (Lamberti *et al.*, 1983), the tobacco population from Kolarovo has a larger value of *c* (98 in paratypes), smaller value of *c'* (2 in paratypes), shorter tail (43 µm in paratypes) and shorter hyaline portion of tail J (5 µm in paratypes). Compared to the Egyptian population the tobacco specimens from Kolarovo have a slightly longer odontophore and slightly posterior guide ring.

TABLE III - *Morphometrics of Balkanic populations of L. latocephalus.*

Locality	Kolarovo			Pergoli			Strumica	Katerini
Host	Tobacco			Grapevine			Tobacco	Tobacco
n	10 ♀♀	4 ♂♂	5 J <sub>1</sub>	14 J <sub>2</sub>	9 J <sub>3</sub>	10 ♀♀	5 ♀♀	5 ♀♀
L (mm)	4.1±0.36 3.5-4.7	3.8±0.10 3.7-3.9	1.3±0.10 1.2-1.4	1.9±0.12 1.7-2.1	3.1±0.09 2.9-3.2	4.2±0.21 3.8-4.5	4.3±0.23 3.9-4.5	4.5±0.30 4.1-4.8
a	138±8.60 122.4-152	140.8±3.48 136.4-144.9	68.7±4.06 61.5-71	86±3.25 80.7-92.2	118.2±3.95 111.5-124.5	142.7±6.27 133.9-151.5	122.2±4.16 117.5-127.3	144±9.28 132.7-155
b	13.2±1.33 11.5-15.2	12.4±0.81 11.9-13.6	5.8±0.50 5.4-6.6	7.6±0.68 6.8-8.7	10.8±0.42 10.2-11.5	13±0.60 12.3-14	12.9±0.86 11.9-13.8	14.2±0.87 13-15.2
c	110.7±6.82 99.2-120.8	112±1.81 110.2-113.7	36.7±1.76 35-38.8	48.7±2.60 44.4-53	72.2±3.80 64.6-79.7	102.9±6.65 93.6-110.8	101.5±5.69 97.5-110	108.6±13.3 94.5-127
c'	1.8±0.15 1.65-2.1	1.5±0.05 1.4-1.5	2.9±0.22 2.7-3.2	2.7±0.18 2.5-3	2.2±0.12 2.1-2.5	2±0.12 1.85-2.2	1.9±0.13 1.7-2	2.0±0.17 1.8-2.2
V%	50±0.87 49-51	-	-	-	-	51±0.93 49-53	50±1.30 49-52	51±1.41 49-53
Odontostyle µm	76.9±1.95 74.3-80	77.7±1.37 76.6-79.4	47±1.65 45-49	53.6±1.24 51.4-55.4	64.8±1.94 62.3-68.6	74.8±2.26 71.5-77	76±1.04 74.5-77	76±4.09 70.9-80.6
Odontophore µm	49.6±1.17 48-52	49.3±1.70 47.4-51.4	34±2.64 32.6-38.8	39.8±1.59 36.6-42.9	44.7±1.56 42.3-46.9	50.2±1.30 48.6-52.6	49.2±0.65 48.6-50.3	50±1.11 49-51.4
Replacement odontostyle µm	-	-	53.3±0.98 52-54.3	64.4±1.57 62.9-68.6	77.7±1.47 76-80	-	-	-
Oral aperture to guide ring µm	43.7±1.29 42.3-46.3	43.3±0.52 42.9-44	25.9±2.40 24-30	31.3±1.67 29-35.4	37.5±1.36 36-40	43.4±1.61 41-45	39.8±2.46 37-42.9	44±1.61 42-46.3
Tail µm	37.5±3.53 32.6-43.4	34.2±1.16 32.6-35.4	35.5±4.43 30.9-40	39±2.53 36.6-45	42.6±2.75 41-48	41±2.29 37-43.5	42±1.84 40-44	41.9±3.88 37-44
J (hyaline portion of tail) µm	3.5±0.42 2.9-4	2.7±0.30 2.3-2.9	3±0.22 2.9-3.4	3±0.55 2.3-4.6	3.1±0.26 2.9-3.4	3.6±0.34 3-4	4.4±0.33 4-4.6	4.3±0.31 4-4.6
Body diam. at lip region µm	10.3±0.00 10.3-10.3	10.2±0.30 9.7-10.3	7.6±0.33 7.4-8	8.3±0.31 8-8.6	9.3±0.37 9-9.7	10±0.32 9.7-10.3	10.7±0.33 10.3-10.9	10.4±0.48 9.7-10.9
Body diam. at guide ring µm	17±0.45 16.6-17.7	16.5±0.30 16-16.6	12±1.30 10.9-14.3	13.4±0.83 12.6-16	15.3±0.22 14.9-15.4	17.2±0.92 16.6-19.4	16.4±0.61 15.4-17	16.9±0.18 16.6-17
Body diam. at base of oesophagus µm	25.9±0.96 24.6-27.4	24.9±0.20 24.6-25	17.5±2.14 16-20.6	20.4±0.98 18.9-22.3	23.8±0.42 23.4-24.6	25.5±1.15 24-27	29.6±1.80 28-33	26.6±1.33 25.7-28.6
Body diam. at mid-body or vulva µm	29.9±1.28 28.5-32.6	27.2±0.99 26.3-28.6	19±1.93 17.5-22	22±1.39 20-24.6	26±0.55 25-26.9	29.1±1.00 28-31	34.9±2.66 32-38.3	31.5±1.61 30.3-34.3
Body diam. at anus µm	20.9±1.04 19.4-22.3	23±0.25 22.9-23.4	12.2±2.07 9.7-14.9	14.5±1.41 12.6-17.7	18.8±0.63 17.7-20	20.2±0.60 19.4-21	21.8±1.38 20-23.4	21.3±0.88 20-22.3
Body diam. at beginning of J µm	7.8±0.74 6.9-9.1	6.9±0.45 6.3-7.4	4.1±0.27 4-4.6	4.8±0.64 4-5.7	6±0.88 5-7.4	7.7±0.55 6.9-8.6	8.3±0.46 8-9	8±0.67 7.5-9
Spicules µm		37±0.00 37-37	-	-	-			

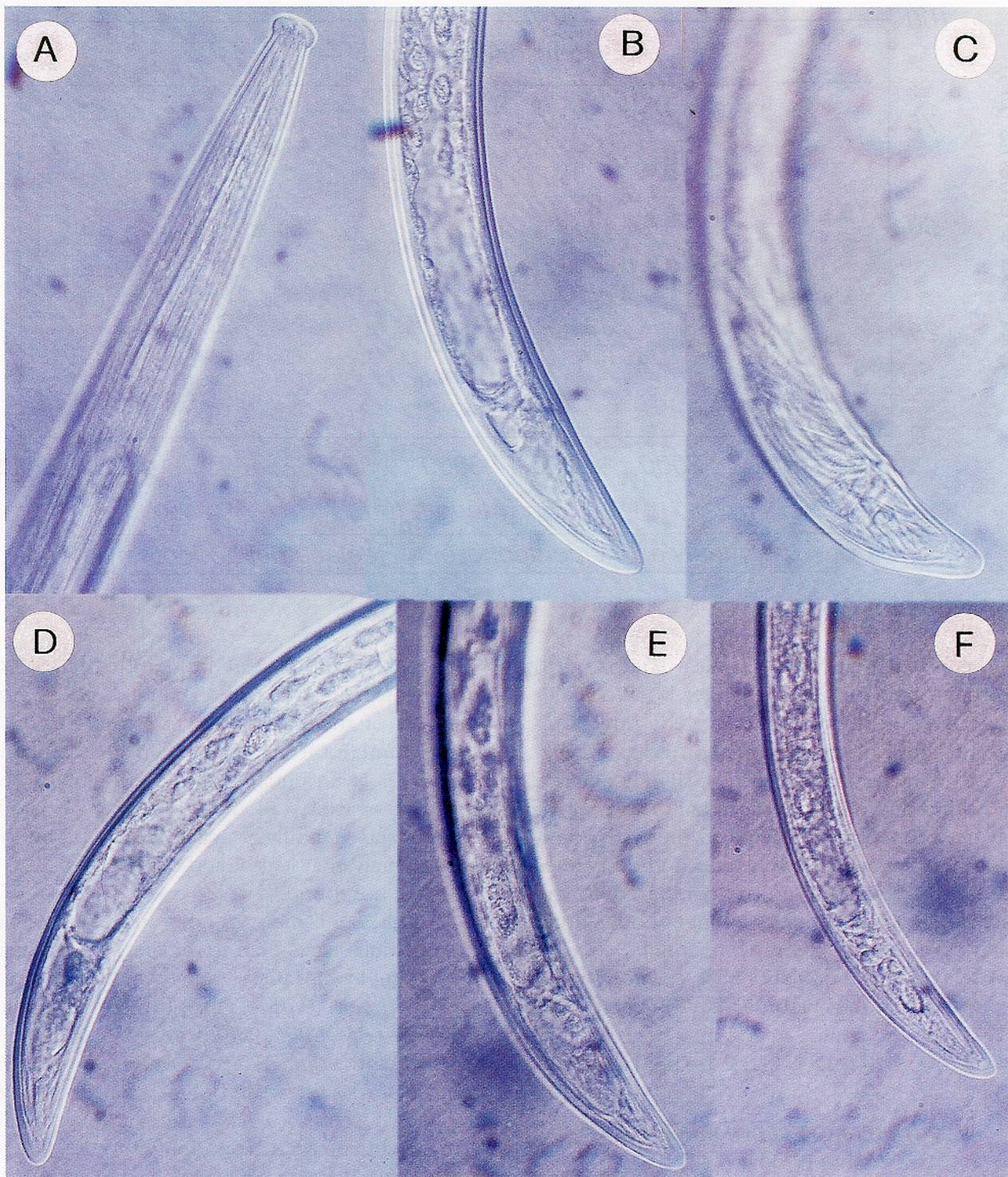


Fig. 7 - Photomicrographs of *L. latocephalus*: A and B, female anterior and posterior region; C, male posterior region; D, preadult (third stage) posterior region; E and F, second and first stage juveniles posterior region.

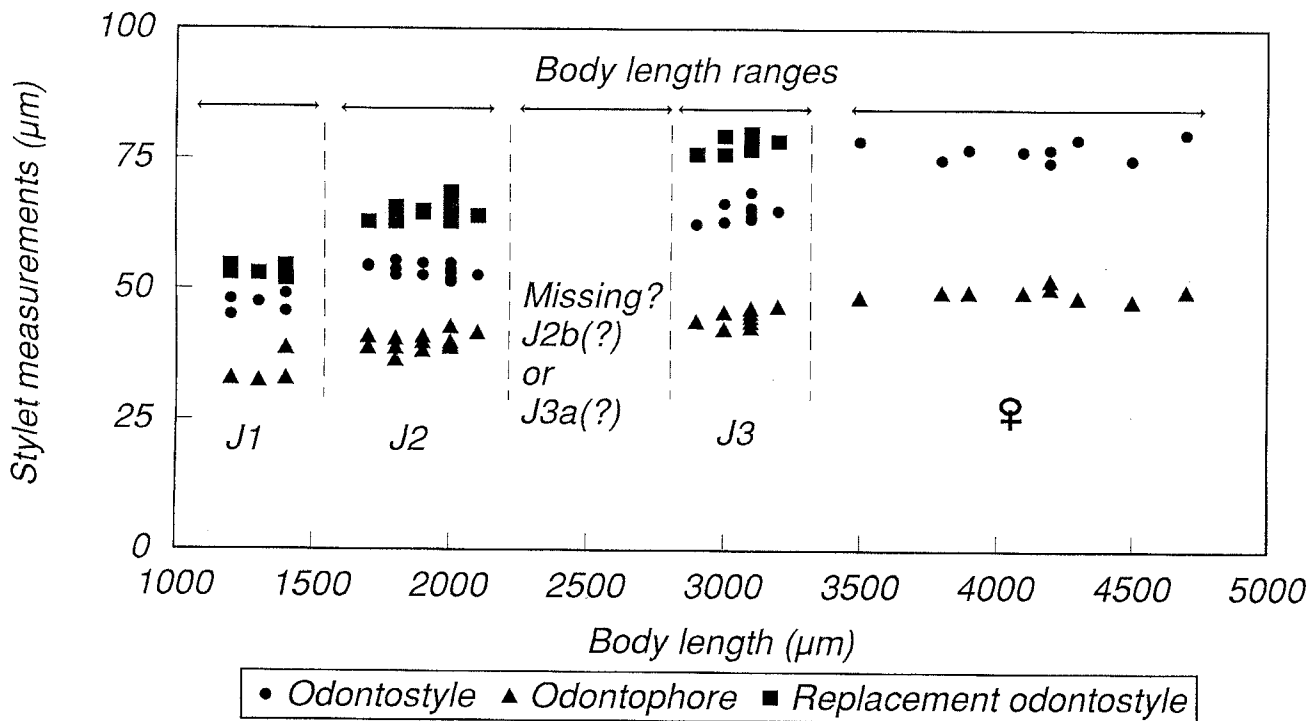


Fig. 8 - Scatter diagram separating juveniles and females of *L. latocephalus*.

The population from Strumica, compared to the type population has a longer body, smaller value of a and slightly anterior guide ring; compared to the Kolarovo population it has a longer body, smaller a and c values, slightly anterior guide ring and slightly longer tail. The population from Katerini, compared to the type population, has a longer body, larger a and c values and slightly anterior vulva; compared to the Kolarovo population it has a longer body, larger a and c' values and slightly longer tail.

The Balkanic populations of *L. latocephalus* fit well with the polytomous key of Chen *et al.* (1997) with the code:

A 2; B 1; C 3/4; D 4; E 2; F 1; G 3; H 5; I 1/2.

### ***XIPHINEMA ITALIAE* Meyl, 1953**

(Table IV; Figs 9-11)

This species occurred in the rhizosphere of black currant at Sandanski and in the rhizosphere of walnut and grapevine at Pergoli.

Female: death posture from a J to an open C; lip region hemi-elliptical, expanded, offset from the rest of the body by a constriction; vulva slightly anterior to mid-body, genital system amphidelphic with equally developed gonads and reflexed ovaries; tail much elongate, dorsally convex and ventrally concave, with a slight dorsal indentation at its pointed extremity.

Male: not found.

Juveniles: tail more slender and elongated in the preadult stages than in females, less gradually tapering toward the extremity in the first stage.

*Xiphinema italiae* is reported with either four (Martelli *et al.*, 1966) or three juvenile stages (Lamberti *et al.*, 1996a). Of the two populations from south west Bulgaria the one from Sandanski seems to have four, although only one specimen could be attributed to the second stage (Fig. 10). Conversely only two groups of juveniles were identified in the population from Pergoli (Fig. 11); they may be attributed to the second and third or third and fourth stages.

TABLE IV - *Morphometrics of Xiphinema italiae from Bulgaria.*

Locality Host	Sandanski						Pergoli	
	Black current						Walnut	
n	9 ♀♀	5 J <sub>1</sub>	1 J <sub>2</sub>	7 J <sub>3</sub>	4 J <sub>4</sub>	17 ♀♀	2 J <sub>3</sub>	11 J <sub>4</sub>
L (mm)	2.7±0.12 2.6-2.9	0.9±0.03 0.4-1.0	1.3	1.7±0.09 1.6-1.9	2.2±0.16 2-2.4	3.1±0.16 2.9-3.5	1.9-1.7	2.4±0.11 2.2-2.5
a	80±4.74 80-8.6	52.2±2.51 49.9-56.5	52	68.2±6.29 62-78.5	72.5±3.97 67.3-78	88.2±5.45 78.7-98.7	69.3-66	79.4±5.43 64.9-83.9
b	7.2±0.48 6.5-8.2	3.9±0.26 3.6-4.2	5.1	5.7±0.48 5.2-6.6	6±0.47 5.5-6.6	7.7±0.42 7.2-8.8	6.0-5.4	6.7±0.22 6.5-7
c	33.7±1.71 30.8-36.4	16.7±1.05 15.2-17.9	20	23.5±1.24 22-25.6	25.6±1.37 23.5-27.3	36±2.32 31.7-39.5	21.4-23.8	27.4±1.02 24.8±28.7
c'	3.7±0.19 3.5-4.1	5±0.23 4.8-5.3	4.1	4.4±0.33 4-4.8	4.3±0.10 4.2-4.4	4.1±0.29 3.5-4.7	5.2-4.6	4.4±0.20 4.1-4.7
V%	45±1.12 43-46	–	–	–	–	44±1.14 42-45	–	–
Odontostyle µm	90±2.65 86.9-95.4	43.5±1.33 41.7-45	52.6	64±1.54 62.3-65.7	80.3±1.69 77.7-82.3	92.4±2.14 88.6-96.6	65-66.9	78.5±1.03 77-80.6
Odontophore µm	54.5±1.20 52.6-56	32.4±1.93 30.3-35.4	37	45.7±2.33 43.5-50.5	50±1.37 48.6-51.5	56.7±1.98 52-58.9	44.6-45	51±1.14 48.6-52.6
Replacement odontostyle µm	–	52±1.49 50.3-54.3	63.5	77.5±2.02 75.5-80.6	94±2.11 92-97	–	75.4-80	91.9±2.00 88-95.4
Oral aperture to basal guide ring µm	80.6±2.72 77-85.7	36.7±1.10 35.4-38.3	44.6	55.6±2.63 50.3-58.3	69.4±2.31 66.9-72.6	83.9±3.19 77-89.7	56-60	68.5±1.75 65-70.9
Tail µm	80.7±5.54 77-94.5	57±3.40 52.6-60	65	74.6±3.22 70.5-80	86.3±5.55 77-91.4	84.9±4.73 77-94.3	88.6-71.4	87.3±3.90 80-91.4
J (hyaline portion of tail) µm	12±1.38 10.3-14.3	6.3±0.81 5.2-6.9	6.9	8.7±1.44 6.9-10.9	10.3±0.69 9.7-11.4	12.6±1.46 9.7-15.4	8.6-9.7	10.5±1.33 8.6-13.7
Body diam. at lip region µm	10.8±0.26 10.3-10.9	7.6±0.33 7.4-8	8.6	8.9±0.26 8.6-9.1	9.9±0.54 9.1-10.3	10.6±0.34 10-10.9	9-9	9.6±0.33 9-10.3
Body diam. at guide ring µm	23.7±0.98 22.3-25.7	13.5±0.83 13-14.9	16	18±1.51 16.3-20	21±0.90 20-22.3	23.8±0.42 23.4-24.6	18.3-17.7	20.8±0.81 20-22.3
Body diam. at base of oesophagus µm	29.6±1.31 28-32.6	16±0.00 16-16	22.5	23±1.50 20.6-25	27±2.03 24-29.7	30.7±1.45 28.6-34.3	25.7-24	27.8±1.32 25-29.7
Body diam. at mid-body or vulva µm	34±1.79 30.9-36.6	18.2±0.27 17.7-18.3	25	25.6±1.70 22.3-27.4	30.6±2.40 26.9-32.6	34.6±2.48 31.4-39.4	27.4-25.7	29.3±1.76 26.3-32.6
Body diam. at anus µm	21.6±0.99 20-22.9	11.3±0.22 10.9-11.5	16	16.7±0.74 15.4-17.7	20±1.00 18.3-20.6	20.9±1.17 18.9-23.4	17-15.4	19.9±1.08 18.3-21.7
Body diam. at beginning of J µm	7.5±0.56 6.9-8.6	4.1±0.27 4-4.6	4	5.5±0.45 5.1-6.3	6.3±0.42 5.7-6.9	7.2±0.59 6.3-8	5-5	5.8±0.26 5.5-6.3

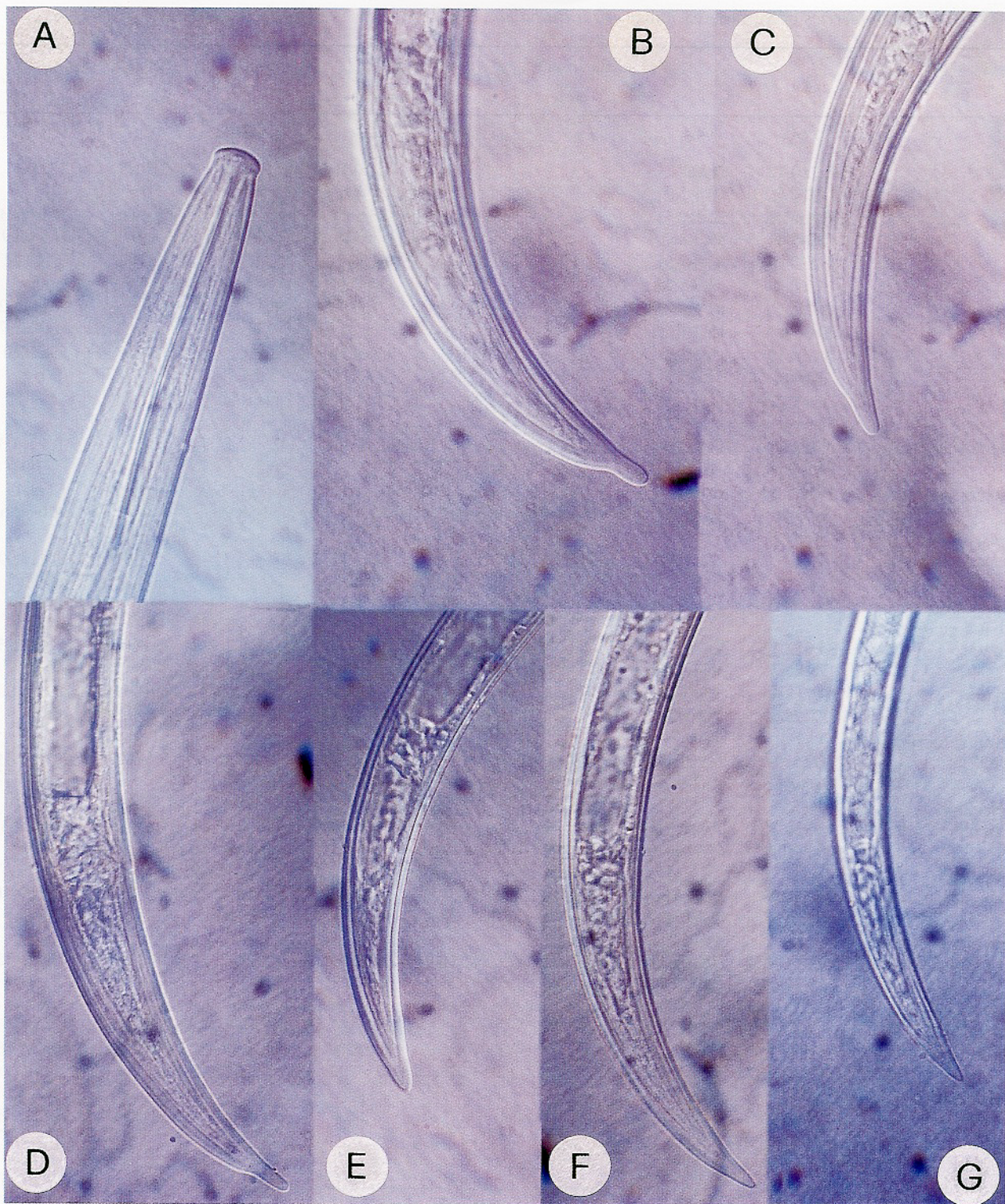


Fig. 9 - Photomicrographs of *X. italiae*: A, female anterior region; B and C, female posterior regions; D, fourth stage; E, third stage; F, second stage; G, first stage juveniles posterior regions.

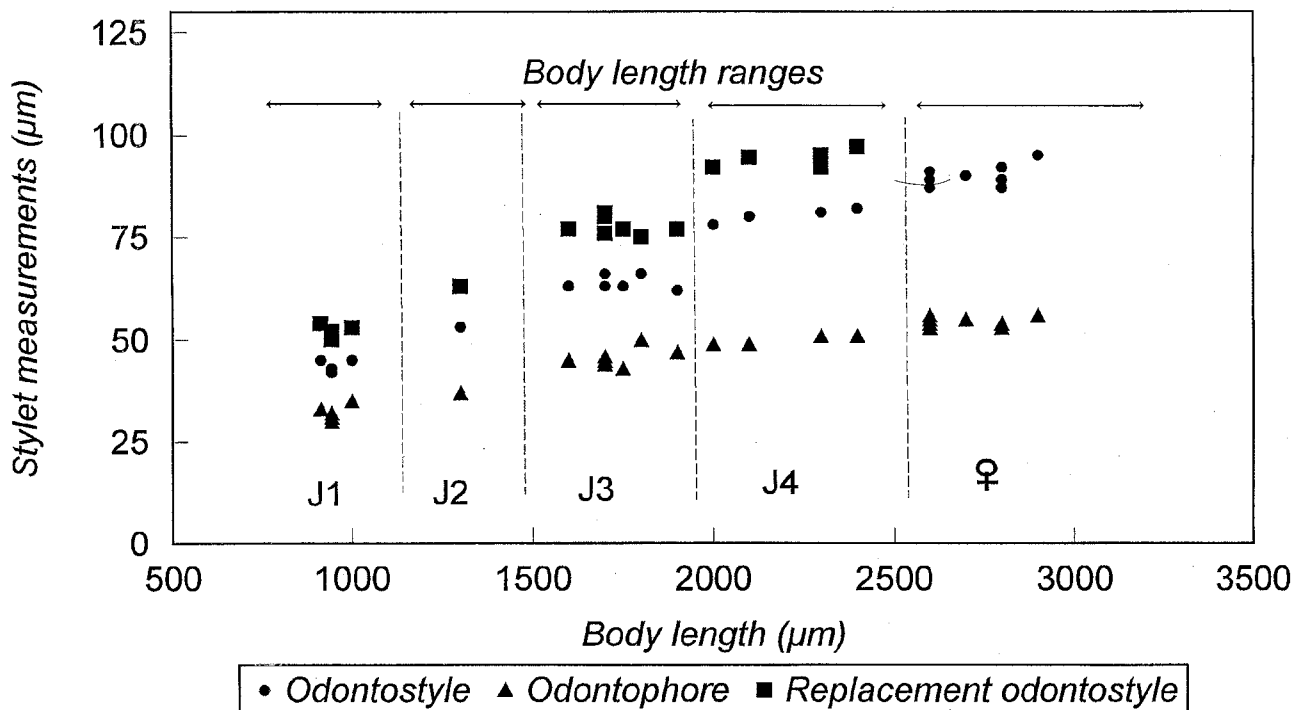


Fig. 10 - Scatter diagram separating juveniles and females *X. italiae*, Sandanski population.

The two populations of *X. italiae* from south west Bulgaria differ from each other mainly in the length of the body and the *c'* value, each larger in the Pergoli population. Compared to other Bulgarian populations, that from Sandanski fits with one female from Varna (Lamberti *et al.*, 1983) and that from Pergoli is much closer to a population from Kalusha (Peneva and Cholova, 1992b). However, they both are larger than the topotypes from the island of Ischia (Martelli *et al.*, 1966) although within the range of other Mediterranean populations.

***XIPHINEMA PACHTAICUM* (Tulaganov, 1938) Kirjanova, 1951**  
(Table V; Figs 12 and 13)

As in the entire country (Lamberti *et al.*, 1983), also in south west Bulgaria *X. pachtaicum* is the most widely distributed and fre-

quently occurring species. It occurred in 18 out of the 29 sites sampled, in the rhizosphere of various plants, such as grapevine, walnut, *Rubus* sp., mulberry (*Morus* sp.), black currant, cherry, olive, laurel (*Laurus nobilis* L.), fig (*Ficus carica* L.) and tobacco.

Female: death posture a closed C to single spiral, lip region hemi-elliptical, slightly expanded, offset by a depression from the rest of the body, vulva slightly posterior to mid-body, genital system didelphic with two equally developed gonads, ovaries reflexed; tail conoid, dorsally convex and ventrally flat, with pointed terminus.

Male: not found.

Juveniles: the tails of the preadult stages are generally similar to those of females, whereas tails of the first and second stages are more elongate and more gradually tapering towards the extremity. They seem to clearly separate into four groups as indicated by Halbrendt and

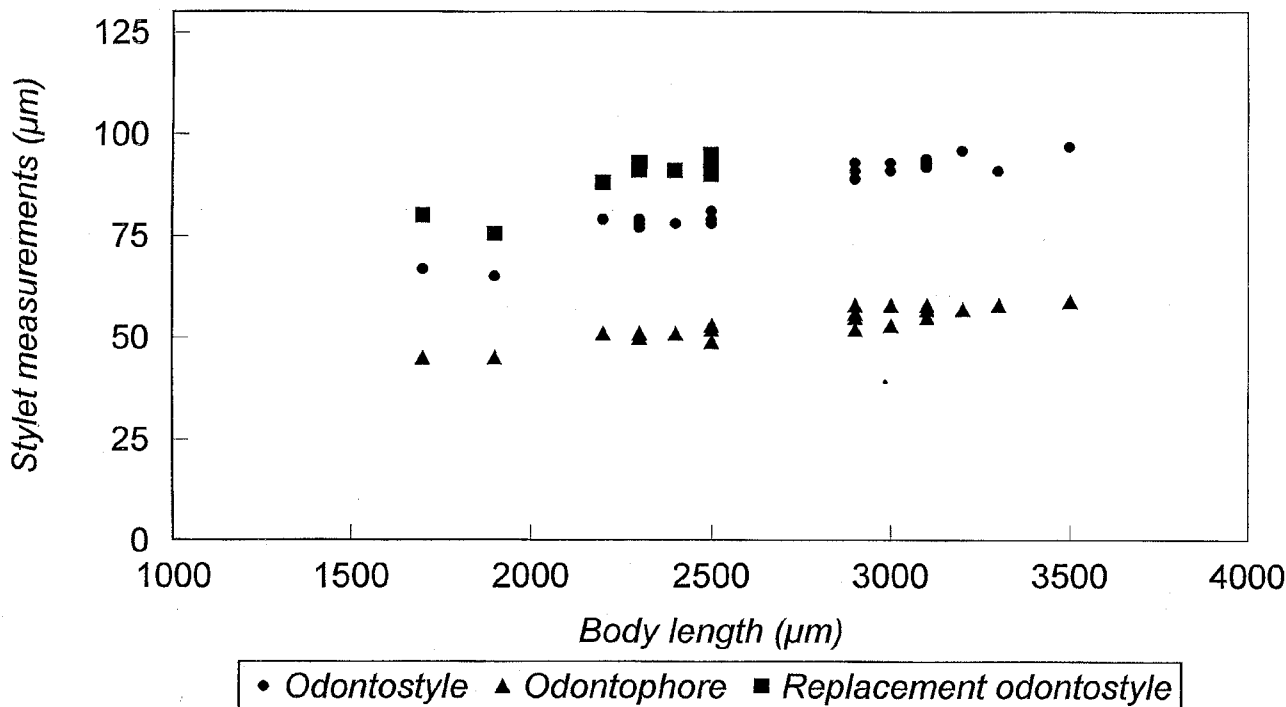


Fig. 11 - Scatter diagram separating juveniles and females of *X. italiae*, Pergoli population.

Brown (1992). In the compendium of juvenile stages of *Xiphinema* species (Robbins *et al.*, 1996), *X. pachtaicum* is included among the species for which males have never been described. However, Lamberti and Martelli (1971) in their description of *X. mediterraneum*, a synonym of *X. pachtaicum*, describe and give measurements of two males. Males of this species have been reported also from other localities (Cohn, 1969; Peneva and Choleva, 1992b; Lamberti *et al.*, 1994).

The morphometrics of *X. pachtaicum* from south west Bulgaria are within the range reported for populations from various geographic origins (Lamberti and Bleve-Zacheo, 1979) including Bulgarian localities (Lamberti *et al.*, 1983; Peneva and Choleva, 1992b). However, as with some Portuguese populations (Lamberti *et al.*, 1994), they have slightly longer bodies and odontostyles, than the average.

***XIPHINEMA VUITTENEZI***  
**Luc, Lima, Weischer *et Flegg*, 1964**  
 (Table VI; Figs 1, 14 and 15)

. A population of *X. vuittenezi* was collected at Kostinbrod, near Sofia, from around the roots of a poplar tree, the same site as indicated by Lamberti *et al.*, (1983).

Female: death posture an open C; lip region anteriorly rounded offset by a slight depression from the rest of the body; vulva at mid-body, genital system didelphic with two equally developed gonads, ovaries reflexed; tail short, conoid broadly rounded ending with a peg which is sometimes absent.

Male: not found.

Juveniles: with tail conoid and subdigitate in the preadult stages, but elongated and very gradually tapering towards the extremity in the first stages. In contrast with the original descrip-



TABLE V - *Morphometrics of Xiphinema pachtaicum from Bulgaria.*

Locality	Pergoli				
Host	Fig				
n	12 ♀♀	7 J <sub>1</sub>	15 J <sub>2</sub>	3 J <sub>3</sub>	12 J <sub>4</sub>
L (mm)	2.1±0.11 1.9-2.2	0.8±0.09 0.7-0.9	1.0±0.06 0.9-1.1	1.3±0.15 1.1-1.4	1.6±0.07 1.5-1.7
a	70.9±4.78 64-79.8	51.4±5.25 41-56.2	50±4.49 43.5-58.8	59±7.84 52.8-67.9	63.7±3.07 58.4-69
b	7.2±0.40 6.4-7.7	4.7±0.41 4.1-5.3	5±0.54 4.1-5.8	5.1±0.86 4.2-5.9	5.7±0.36 5.2-6.3
c	67±4.68 61.2-76.9	28±3.81 21.5-33.4	29.7±2.99 25.4-36.3	40±4.50 35.6-44.6	48.6±2.47 45.2-52.8
c'	1.7±0.13 1.5-1.9	2.8±0.04 2.8-2.9	2.6±0.15 2.5-2.9	2.2±0.20 2-2.4	2±0.09 1.9-2.2
V%	56±1.22 55-58	—	—	—	—
Odontostyle µm	84.8±6.47 72.6-91.4	39.8±1.73 37.7-42.9	48.6±2.15 45.7-51.4	61.6±2.31 58.9-62.9	73.5±1.53 71.4-76
Odontophore µm	46.7±3.92 40.6-51.4	28.2±1.22 26.3-29.7	33±1.41 30.3-35.4	38.4±0.46 38-38.9	42.1±1.63 38.9-44
Replacement odontostyle µm	—	47.8±0.94 46.9-49	61.4±3.35 57-68	73.7±1.51 72.6-75.4	86.8±2.41 83.4-91.4
Oral aperture to basal guide ring µm	73.3±5.86 60.6-78.3	31.6±3.03 27.4-35.4	41.4±3.17 35.4-45.7	50.2±5.54 41.7-56	63±1.84 60.6-66.3
Tail µm	31.5±2.15 28-34.3	27.5±1.49 25.7-30.3	31.6±2.31 28-35.4	31.6±0.87 30.9-32.6	33±1.90 30.3-36.6
J (hyaline portion of tail) µm	8±0.88 6.9-9.7	3.9±0.73 2.9-5	4.8±0.76 4-6.9	5.3±0.64 4.6-5.7	7.8±0.57 6.9-8.6
Body diam. at lip region µm	8.7±0.20 8.6-9	6.9±0.32 6.3-7.4	7±0.26 6.8-7.4	7.8±0.35 7.4-8	8±0.23 8.1-8.6
Body diam. at guide ring µm	21.4±1.46 18.9-23.4	12.3±0.47 11.4-12.6	14.4±0.69 13-16	16.2±0.69 15.4-16.6	19±0.42 18.3-19.4
Body diam. at base of oesophagus µm	26.3±1.97 22.9-28.6	14.5±1.22 13-16.6	17.7±1.49 16-21	19.8±2.33 17.7-22.3	23.3±1.36 21-25.7
Body diam. at mid-body or vulva µm	29.9±2.92 25.7-34.3	15.3±1.35 13.7-17	18.8±1.67 17-22.3	21.5±2.72 19.4-24.6	25±2.09 21.6-28.6
Body diam. at anus µm	18±1.08 16.6-20	9.8±0.56 9-10.9	12±1.07 10.3-14.3	14.4±1.50 13-16	16.3±1.09 15.4-19.4
Body diam. at beginning of J µm	7.5±0.78 6-8.6	3.6±0.43 2.9-4	4.3±0.35 4-5	5.2±0.40 5-5.7	6.7±0.61 5.7-7.4

tion (Luc *et al.*, 1964), they separate into three groups. However, one group, probably the third stage, is missing in the Kostinbrod population, since the gap between the second and eventually the fourth group appear too large.

The morphometric characters of this population of *X. vuittenezi* are in the range known (Luc *et al.*, 1964; Lamberti *et al.*, 1983) for the species.

In the description of *Longidorus closelongatus* Stoyanov, 1964 from Crete (Lamberti *et al.*, 1996) it is stated that the juveniles separate on the basis of body length into four groups. However, considering the scatter diagram (Fig. 16) which is based on the stylet measurements, it clearly appears that the last two groups (J<sub>3</sub> and J<sub>4</sub>) are coincident, and therefore only three juvenile stages should be attributed to this species.

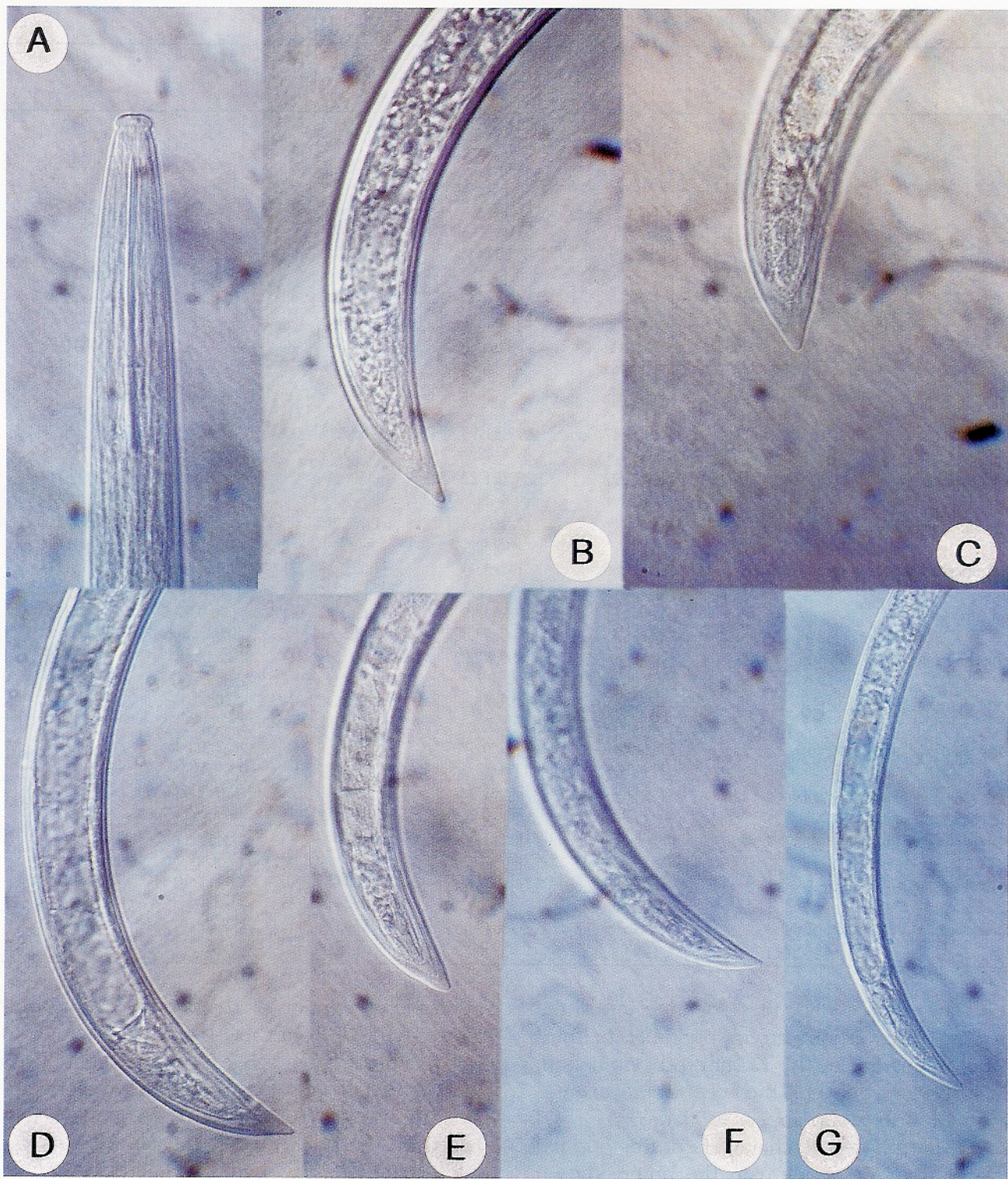


Fig. 12 - Photomicrographs of *X. pachtaicum*; A, female anterior region; B and C female posterior regions; D, fourth stage; E, third stage; F, second stage; G, first stage juveniles posterior regions.

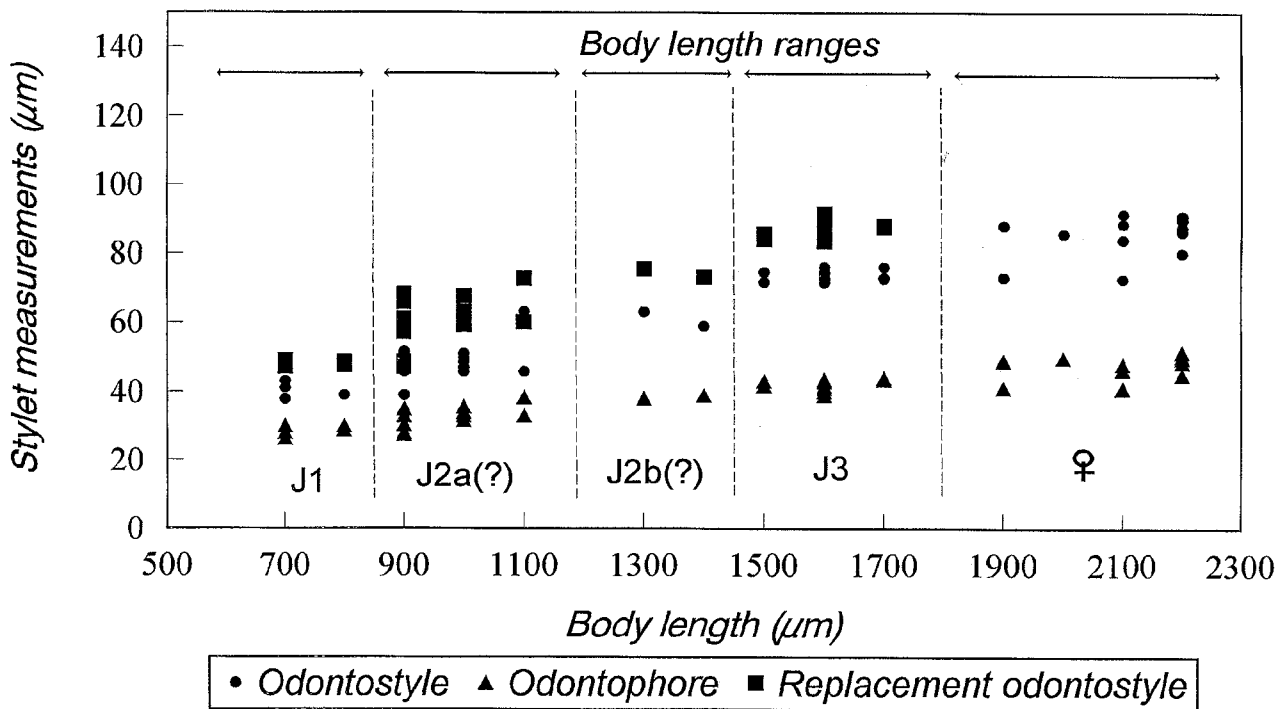


Fig. 13 - Scatter diagram separating juveniles and females of *X. pachtaicum*.

*Longidorus africanus* Merny, 1966 is reported from Egypt with four juvenile stages (Lamberti *et al.*, 1996a). Again looking at the scatter diagram (Fig. 17) it appears that J3 and J4 are coincident. Therefore, this species should also be regarded as one with only three juvenile stages.

Finally *Xiphinema santos* Lamberti, Lemos, Agostinelli *et D'Addabbo*, 1995 is also considered to have four juvenile stages (Lamberti *et al.*, 1996a) but, as apparent in the scatter diagrams (Figs 18 and 19), the second and third stages are coincident in the Portuguese population and the third and fourth stages are coincident in the Egyptian population, therefore separating juveniles in both populations into three groups.

Halbrendt and Brown (1992) reported evidence for the occurrence of three and not four, juvenile developmental stages in populations of several North American *X. americanum*-group species. They explained their results on the ba-

sis of current understanding of stylet development (Coomans and DeConinck, 1963). It is assumed that the replacement odontostyle of each juvenile developmental stage is longer than that of the corresponding functional odontostyle. Halbrendt and Brown (1992) stated that "it is an unlikely possibility that two developmental stages are virtually identical, with the stylet measurements not changing between stages". These authors suggested that "if this phenomenon was possible the same could occur in other members of the Longidoridae reported to have four juvenile developmental stages". However, relatively little research has been done to examine if such a phenomenon occurs in species with four or three juvenile developmental stages.

In the present study four juvenile developmental stages and adult females of populations of *L. distinctus* and *X. italiae* are clearly distinguished when body length and stylet measurements are plotted as scatter diagrams (Figs 3

TABLE VI - *Morphometrics of X. vuittenezi from Bulgaria.*

Locality	Kostinbrod			
Host	Poplar			
n	9 ♀♀	8 J <sub>1</sub>	4 J <sub>2</sub>	9 J <sub>4</sub>
L (mm)	3.2±0.17 2.9-3.5	0.9±0.06 0.8-1.0	1.2±0.10 1.1-1.3	2.3±0.15 2.2-2.6
a	62±3.99 56.4-68.7	41.7±2.21 38.8-45	47.4±2.59 44-49.4	56.7±2.20 53.9-60
b	6.7±0.44 6.3-7.5	3.7±0.20 3.5-3.9	4.2±0.30 3.8-4.5	5.2±0.35 4.6-5.8
c	89.7±6.09 82.5-100.3	19.3±0.79 18.2-20.6	28.7±2.25 25.6-30.4	57.4±4.00 52.8-65
c'	1.0±0.07 0.9-1.1	3.2±0.20 2.9-3.5	2.3±0.10 2.2-2.4	1.3±0.10 1.2-1.5
V%	51±0.85 50-52	—	—	—
Odontostyle µm	124±2.80 120-128	48.9±1.66 45-50.3	62.9±0.45 62.3-63.4	101.5±3.87 96-106.3
Odontophore µm	74.4±1.76 71.4-76.6	36.7±2.37 33.7-40	45.4±0.75 44.6-46.3	65.5±1.72 62.9-68
Replacement odontostyle µm	—	61.8±2.41 57-64.6	77.6±2.91 74.9-81	122.8±3.96 116.6-127.4
Oral aperture to basal guide ring µm	106.6±4.07 100-110.9	39.2±1.77 35.4-41	52.5±1.97 50.9-54.9	89±3.97 82.3-95.4
Tail µm	36±1.91 34.3-40	46±2.39 41.7-48.6	42.7±2.58 39.4-45.7	40.7±2.46 37-44.6
J (hyaline portion of tail) µm	10.3±1.15 9-12	10±0.76 9-11.4	7.9±0.57 7.4-8.6	10±0.86 9-11.4
Body diam. at lip region µm	14±0.42 13.7-14.9	8.5±0.43 8-9	9.2±0.64 8.6-9.7	12±0.61 10.9-12.6
Body diam. at guide ring µm	36.9±0.55 36-37.7	16.4±0.64 16-17.7	20.4±0.49 20-21	30.8±1.23 29-32.6
Body diam. at base of oesophagus µm	46.4±2.42 44.6-52	19.6±1.72 18.3-23.4	24.7±0.47 24-25	37.8±3.22 34.9-42.9
Body diam. at mid-body or vulva µm	52.5±2.23 50-55.4	21.3±1.71 19.4-24.6	25.8±0.62 25-26.3	41.2±3.49 37.7-46.9
Body diam. at anus µm	37.2±1.17 34.3-38.3	14.2±1.00 13.7-16.6	18.7±1.18 17.7-20	32±3.35 28.6-37.7
Body diam. at beginning of J µm	22.8±1.87 19.4-25.7	6.2±0.38 5.7-6.9	7.8±0.50 7-8	14.8±1.27 13.7-17

and 10). The body length ranges for each of the developmental stages in these populations have broadly similar spans. However, in similar scatter diagrams for populations of *L. euonymus* (Figs 5 and 6), *X. pachtaicum* (Fig. 13), *L. africanus* (Fig. 17) and *X. santos* (Fig. 19) the body length spans for the juvenile developmental stages differ. For each of these populations four

clusters of juvenile forms are apparent, but with specimens in two clusters of each population having similar stylet measurements and differing only in body length. For example, with the *L. euonymus* population from Kostinbrod (Fig. 5) the first and pre-adult juvenile developmental stage intermediate between these appears to occur as two forms (J2a and J2b), having similar

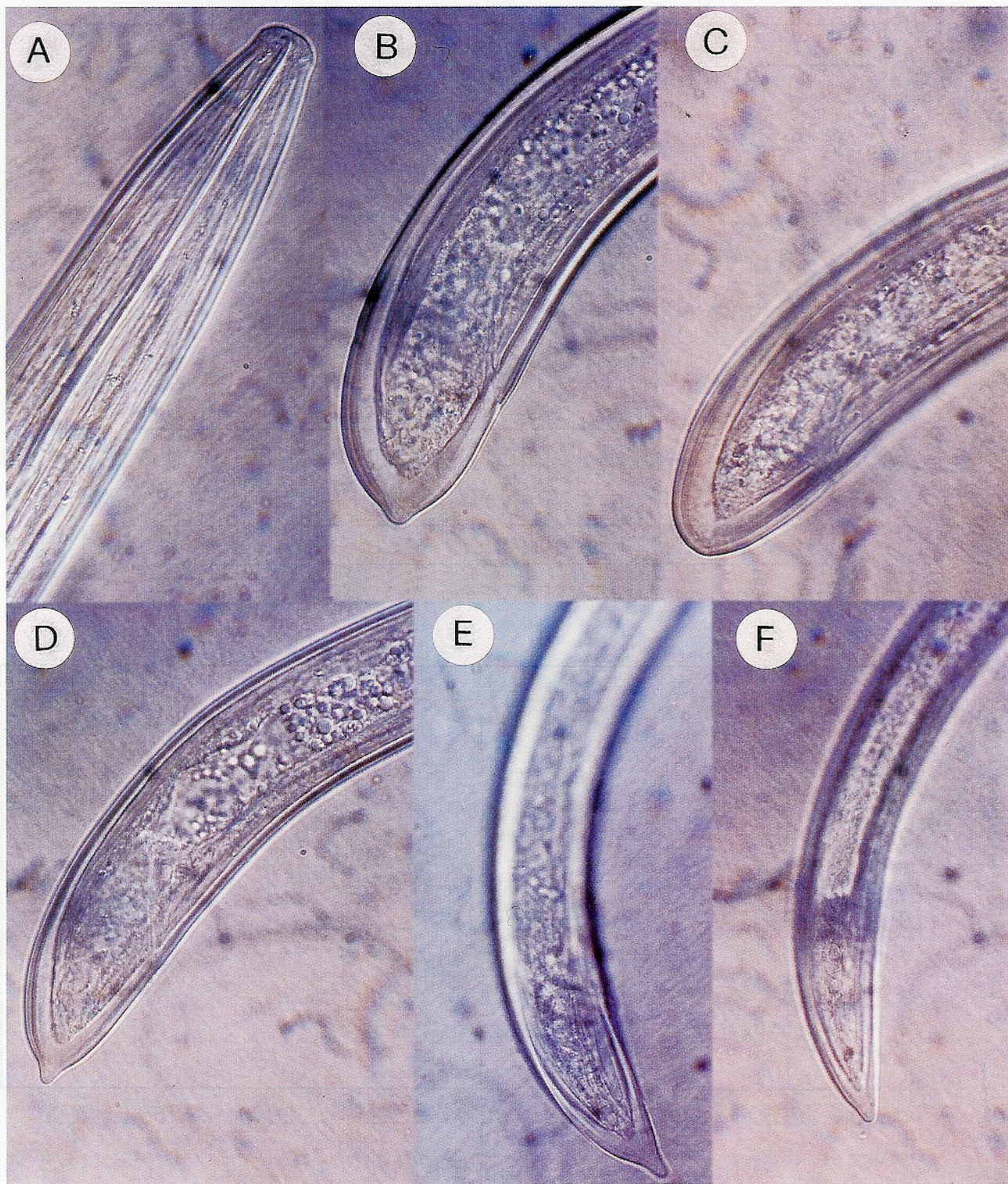


Fig. 14 - Photomicrographs of *X. vuittenezi*; A, female anterior region; B and C, female posterior region; D, third stage; E, second stage; F, first stage juveniles posterior regions.

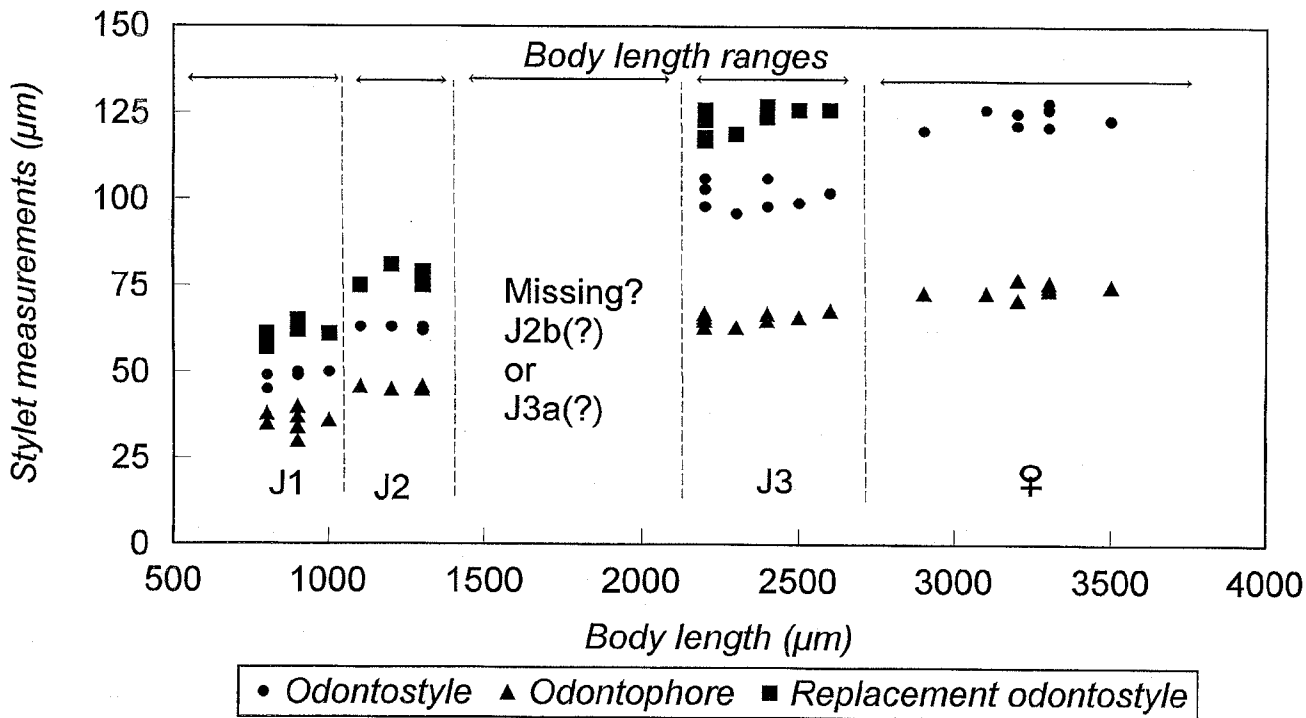


Fig. 15 - Scatter diagram separating juveniles and females of *X. vuittenezi*.

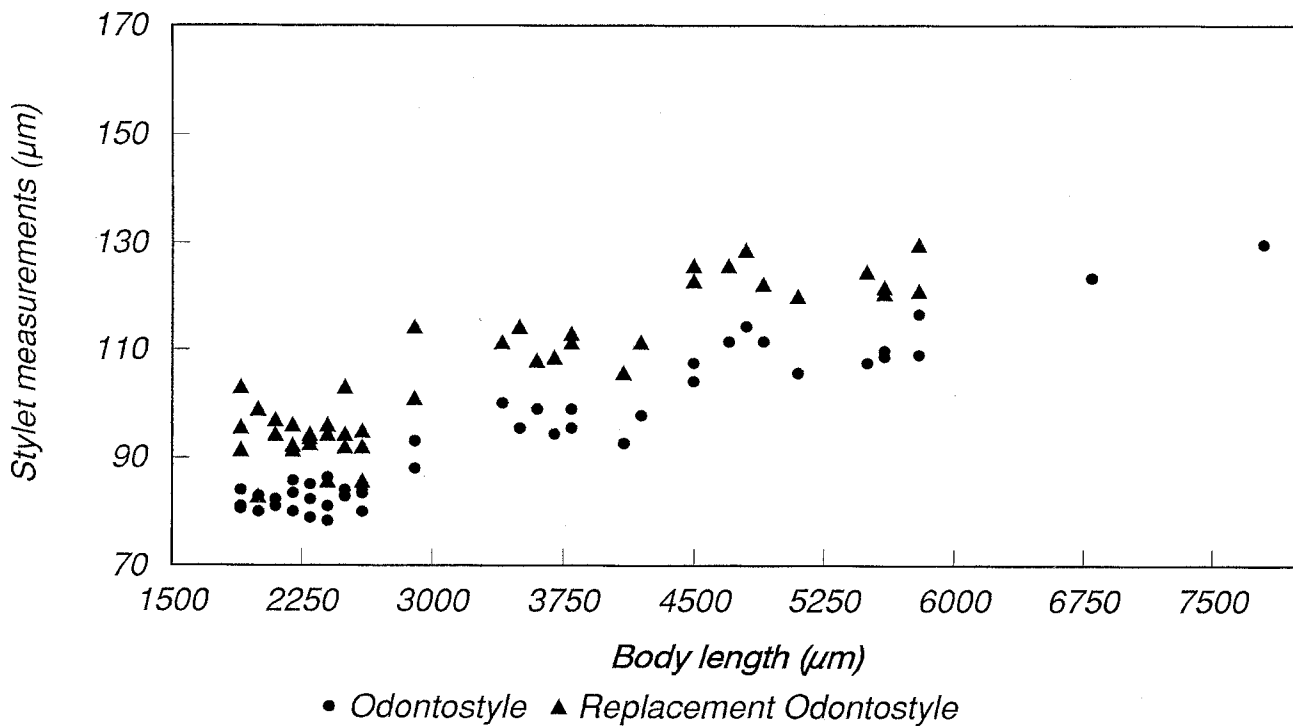


Fig. 16 - Scatter diagram separating juveniles and females of *L. closelongatus*.

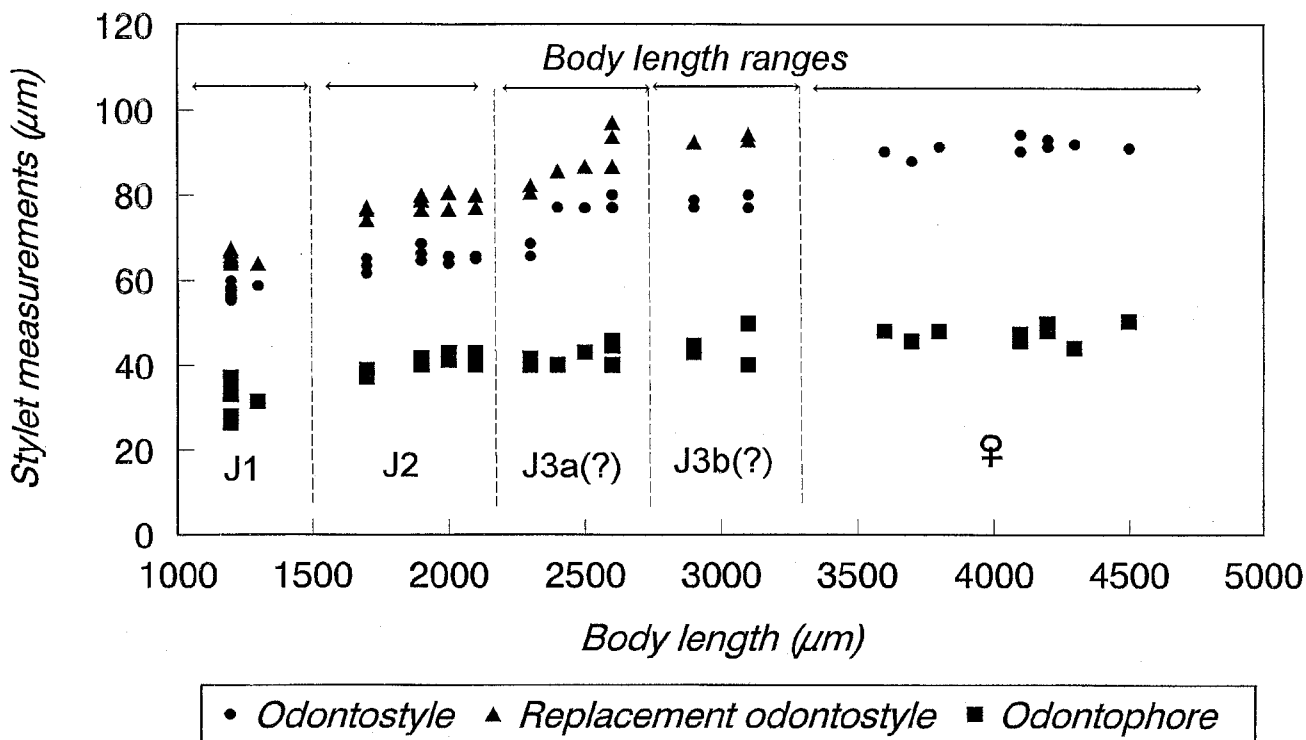


Fig. 17 - Scatter diagram separating juveniles and females of *L. africanus*.

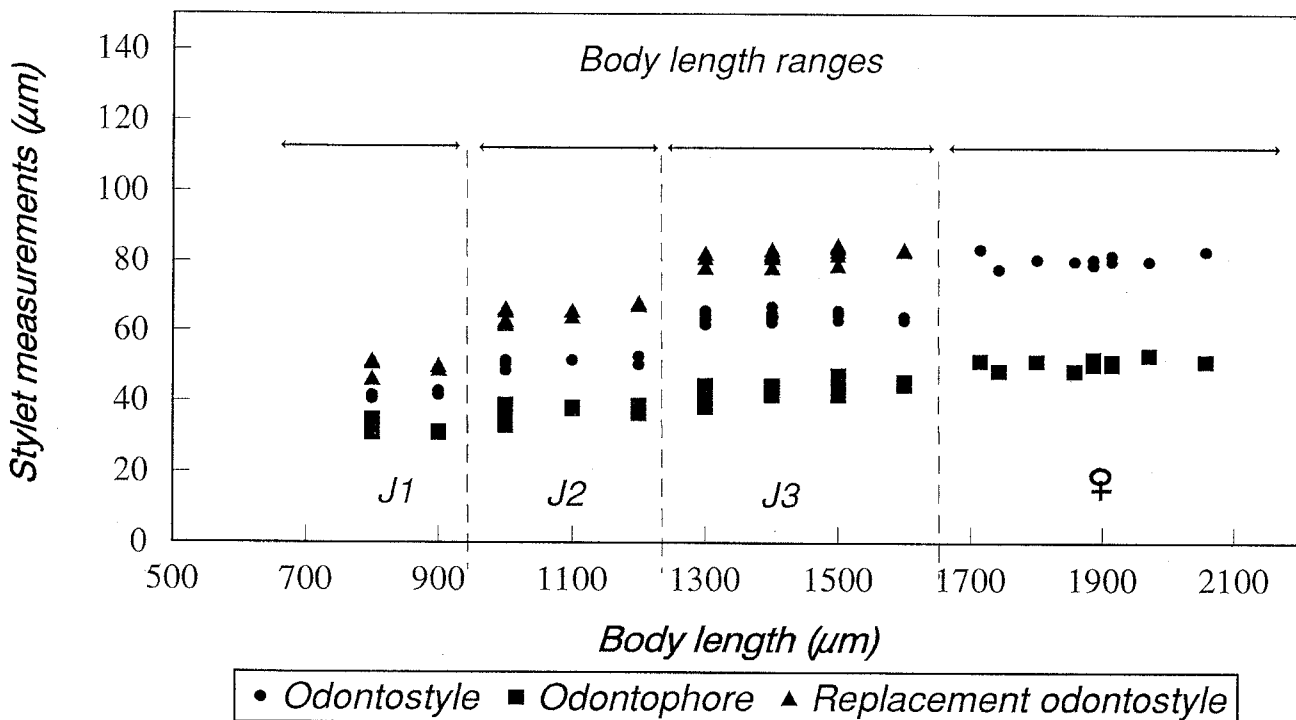


Fig. 18 - Scatter diagram separating juveniles and females of *X. santos* from Portugal.

stylet length measurements but distinguishable by body length. A similar phenomenon is apparent with *L. euonymus* from Sandanski (Fig. 6; J2a and J2b or J3a and J3b); *X. pachtaicum* (Fig. 13; J2a and J2b); *L. africanus* (Fig. 17; J3a and J3b); and *X. santos* (J3a and J3b). Also, discontinuities in body length sizes are apparent with populations of *L. latocephalus* (Fig. 8) and *X. vuittenezi* (Fig. 15). These may represent missing developmental stages with stylet lengths similar to either the J2 or J3 stages, but with different body lengths.

It is conceded that these observations and comments are speculative and are somewhat contrary to current understanding of nematode development. However, evidence that several members of the Longidoridae have only three and not four juvenile developmental stages has only recently gained widespread acceptance. Also, research with *Caenorhabditis elegans* has identified a possible genetic basis for this phenomenon (Ambros and Horvitz, 1984; Robbins

*et al.*, 1995, 1996). The observations reported here indicate a requirement for further research into the possible occurrence of two juvenile developmental forms with similar stylet measurements but differing in body length in members of the Longidoridae reported to have four and three juvenile developmental stages.

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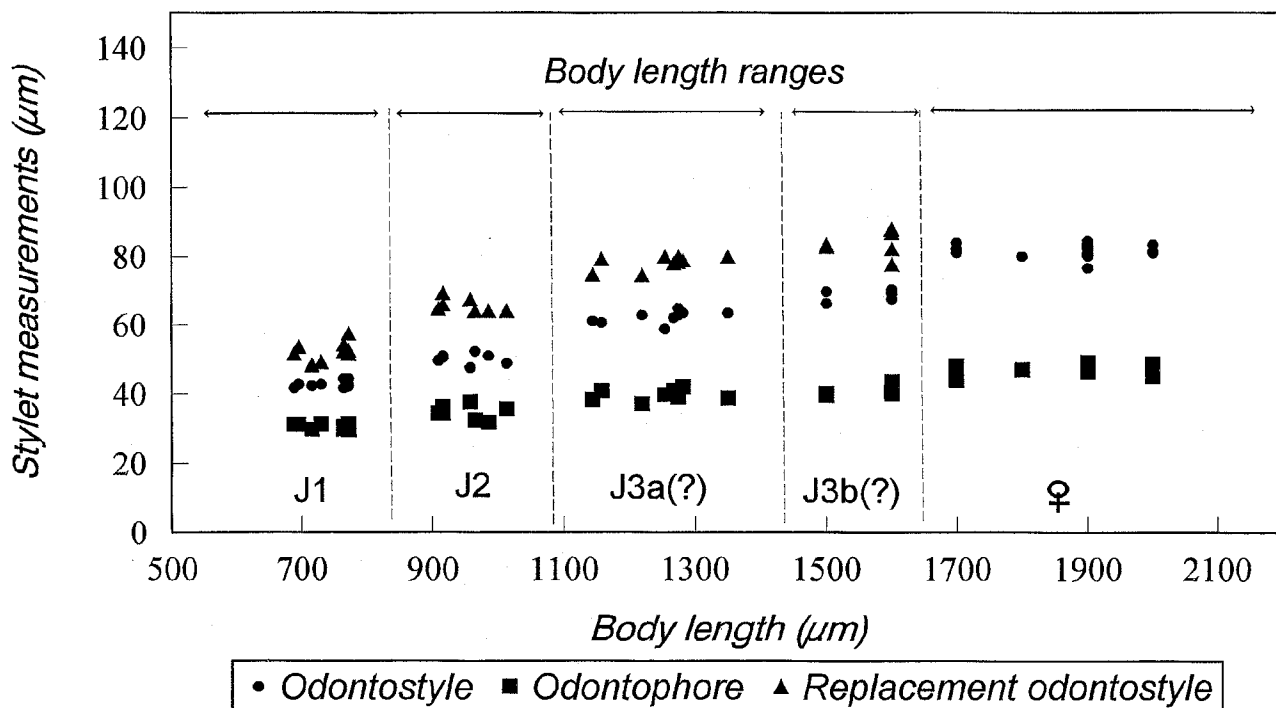


Fig. 19 - Scatter diagram separating juveniles and females of *X. santos* from Egypt.



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