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On two closely related species of the *Xiphinema americanum*-group: *X. simile* Lamberti, Choleva et Agostinelli, 1983 and *X. parasimile* Barsi et Lamberti, 2004 (Longidoridae), with a description of the male of *X. parasimile*

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Abstract

Several populations of *Xiphinema simile* Lamberti, Choleva et Agostinelli, 1983 and two of *X. parasimile* Barsi et Lamberti, 2004, originating from various habitats – natural and man-made, have been studied. *Xiphinema simile* was associated both with cultivated and naturally growing plants, while *X. parasimile* was recovered from soil around grapevine. Data on the morphological and biometrical characteristics (including juvenile stages) are presented and variations discussed. Pharyngeal bulbus and glandularium length, vaginal and uterine characteristics were shown to be good differentiating characters. This report of *X. parasimile* is a new record for Bulgaria as well as a new plant association for the species. The description of its male is provided for the first time. The Bulgarian population of *X. parasimile* showed the same pattern as the Serbian population revealed by the RFLP analyses of D1-D2 region.

Keywords

Bulgaria, Longidoridae, male description, morphology, new record, RFLP, D1-D2 region

Introduction

The present study is focused on the morphology and distribution of two closely related species of the *Xiphinema americanum* lineage from Bulgaria – *Xiphinema simile*

Lamberti, Choleva et Agostinelli, 1983 and *X. parasimile* Barsi et Lamberti, 2004. *Xiphinema simile* has been originally described from Bulgaria in association with poplar, grapevine, fruit-trees, black current (Lamberti et al. 1983), and subsequently has been reported from forest nurseries (Peneva and Choleva 1992). In Europe, the species has been found in various habitats: forests and grasslands in former Yugoslavia (Barsi 1994, Barsi and Lamberti 2002), hornbeam tree, maize and field maple in Serbia, and dogwood shrubs in Bosnia and Herzegovina (Barsi and Lamberti 2004, Barsi and De Luca 2008); grapevine, fruit and nut trees, and raspberries in Slovakia (Lišková et al. 1993, Lišková 1995, Lišková and Brown 1996, Lamberti et al. 1999); orchards in Czech Republic (Kumari 2006); various plants in Hungary (fruit trees, grapevine, birch, wild rose and Elsbeere tree) (Repasi et al. 2008). Outside Europe *X. simile* has been reported from Kenya in the rhizosphere of pepper and baobab trees, and riverine forest (Coomans and Heyns 1997).

Xiphinema parasimile was described from forest habitats in Serbia, in association with woody plants (*Quercus* sp. and *Carpinus betulus* L., Barsi and Lamberti 2004). In morphology and morphometrics it showed highest similarity to populations of *X. simile*, with differences in lip region and tail shape, however the authors underlined that both species can be thoroughly differentiated by the number of juvenile stages (four *vs* three).

Materials and methods

Soil samples were collected from different habitats representing various ecosystem types in Bulgaria. Nematodes were extracted from 200 cm^3 soil by a sieving and decanting technique, heat killed at 60°C for two minutes and fixed in a 4% formaldehyde solution. For morphological studies nematodes were processed to anhydrous glycerol and mounted on permanent microscopic glass slides (Seinhorst 1959). Additionally, live specimens were fixed in 1M NaCl for molecular studies. PCR amplification of *X. parasimile* D1-D2 domains of the 26S rDNA was carried out by using the primers FOR and REV as described in De Luca et al. (2004). The RFLP analyses of the D1-D2 region were performed using the following enzymes *Alu* I, *Ava* II, *Dde* I, *Nde* II, *Pst* I and *Rsa* I according to De Luca et al. (2004).

Drawings and photographs were taken using an Olympus BX51 compound microscope powered with differential interference contrast (DIC). Images were taken with a ColorView IIIu camera and cell^P software (Olympus Soft Imaging Solutions Gmbh). Measurements were made using an Olympus BX 41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA), and computer Digitrak 1.0f programme, (Philip Smith, Scottish Crop Research Institute, Dundee, UK).

Results

Xiphinema parasimile represents the first record since its original description (Barsi and Lamberti 2004) and were recovered from two locations: 1) in North-Central (near village of Orlyane (**Or**)) and 2) in South-Central (near village of Vinogradets (**Vi**)) Bulgaria. Both populations were found in the rhizosphere of grapevine which is a new plant association. *Xiphinema simile* was recovered from five locations of North Bulgaria in association with different plants: cherry plum grove (*Prunus cerasifera* Ehrh., Kalimok – Brashlen (**KB**) protected area, along Danube river), abandoned vineyard (*Vitis vinifera* L.*,* near village of Orlyane (**Or**)), around oak trees in a mixed broadleaved forest (*Quercus pedunculiflora* C. Koch, Srebarna Reserve (**SR**) along Danube river) and in the rhizosphere of grasses (Kamen bryag (**Kb**), and Kaliakra areas (**Ka**), Black Sea Coast).

***Xiphinema parasimile* Barsi et Lamberti, 2004**
(Figs 1; 2A, D, F, I-K, N, P, T; 3A-C; 4A-E; 5A-C; 6-8)

Measurements. See Tables 1-3;

Description. *Females.* Body C to spiral shaped. Labial region 4-5 µm high, set-off from the rest of the body. Amphids hardly visible, its opening 4 µm in a paratype specimen (40 % of the corresponding body width); 5-5.5 µm wide in specimens from Vinogradets and Orlyane (50-64 %). Odontostyle with poorly developed basal collar. Pharyngeal characters presented in Table 2. Reproductive system amphidelphic, symbiont bacteria present in the ovaries. Uteri short, ovejector and separate uteri present (Table 3); vagina 41.5 -52.2% of the corresponding body width (averages 48.4±1.4, n=5, paratype material; 46.4±3.4, n=17, **Vi**; 46.8±1.4, n=7, **Or**). Sperm cells observed in some of the females from Vinogradets. Tail conical, dorsally convex, ventrally almost straight or slightly concave with pointed terminus.

Male. One specimen was found in Vinogradets population. Male similar to the female with posterior region more strongly curved. Lip region and tail shape as in females, a difference was observed within anal body width, which reflect the lower **c'** value. Spicules slightly curved, lateral guiding piece 4 µm long. Adanal supplement pair preceded by a row of five irregularly spaced supplements. Tail conoid, ventrally straight, dorsally convex with pointed terminus.

Juveniles. The scatter diagram based on functional and replacement odontostyle, and body length revealed presence of four juvenile stages (Fig. 7).

PCR-RFLP analysis. The amplification of the D1-D2 expansion domains of *X. parasimile* generated a unique band of about 0.8 kb. The restriction fragments of the amplified region of *X. parasimile* population from Vinogradets locality using several enzymes are presented in Fig. 8.

Remarks. Metric data are within the ranges reported in the original description, with slightly lower average values for the female body length (1.78-1.82 *vs* 1.93-1.99

Table 1. Morphometrics of *Xiphinema parasimile* from two localities in Bulgaria. All measurements in micrometres, except body length and ratios, and in form: mean ± standard deviation (range).

Locality Host plant	Orlyane *Vitis vinifera*	Vinogradets *Vitis vinifera*					
	Females	Females	Male	J1	J2	J3	J4
Character	n=11	n=18	n=1	n=4	n=6	n=8	n=10
L (mm)	1.78 ± 0.11 (1.56 - 1.94)	1.82 ± 0.11 (1.66 - 2.04)	1.84	0.68 ± 0.01 (0.67 – 0.70)	0.89 ± 0.09 (0.83 – 1.07)	1.09 ± 0.09 (0.96 – 1.23)	1.45 ± 0.07 (1.35 – 1.54)
a	65.7 ± 2.71 (61.0 - 69.2)	68.8 ± 3.04 (64.0 - 75.9)	71.8	40.8 ± 2.18 (39.0 – 43.7)	47.7 ± 3.08 (45.6 – 53.6)	53.2 ± 2.02 (50.8 – 57.2)	61.9 ± 2.65 (57.8 – 65.5)
b	6.6 ± 0.76 (5.4 - 7.5)	7.3 ± 0.76 (5.3 - 8.3)	6.2	4.5 ± 0.42 (4.1 – 4.9)	5.1 ± 0.48 (4.7 – 5.9)	5.1 ± 0.44 (4.4 – 5.7)	6.4 ± 0.59 (5.1 – 7.0)
c	56.7 ± 2.32 (53.0 - 58.9)	60.5 ± 5.74 (52.3 - 71.2)	64.0	25.3	28.3 ± 1.59 (25.9 – 30.1)	35.7 ± 3.08 (31.9 – 40.4)	46.2 ± 3.31 (40.2 – 52.8)
c’	2.0 ± 0.05 (2.0 - 2.1)	2.0 ± 0.15 (1.7 - 2.3)	1.6	2.54	2.4 ± 0.48 (1.5 – 2.6)	2.4 ± 0.20 (2.1 – 2.6)	2.1 ± 0.11 (2.0 – 2.3)
V (%)	55.4 ± 1.38 (53 - 58)	54.5 ± 1.61 (50.5 – 56)	-	-	-	-	-
Odontostyle	68.9 ± 2.51 (63 - 72)	69.6 ± 1.91 (66 - 74)	72	35.9 ± 0.66 (35 – 37)	42.5 ± 2.47 (40 – 49)	52.2 ± 1.72 (50 – 55)	59.3 ± 1.95 (55 – 62)
Odontophore	38.0 ± 1.31 (36 - 40.5)	37.5 ± 0.87 (36 - 39)	37.5	23.0 ± 1.27 (22 – 24)	30.2 ± 1.52 (28 – 32.5)	31.9 ± 1.39 (30 – 34)	36.2 ± 1.55 (34 – 38.5)
Replacement odontostyle	-	-	-	41.1 ± 2.41 (38.5 – 44)	52.3 ± 1.71 (50 – 55)	60.8 ± 1.42 (59 – 64)	69.2 ± 2.19 (65 – 73)

Locality Host plant	Orlyane *Vitis vinifera*	Vinogradets *Vitis vinifera*					
	Females	Females	Male	J1	J2	J3	J4
Character	n=11	n=18	n=1	n=4	n=6	n=8	n=10
Oral aperture to guide ring	61.6 ± 1.37 (60 - 63.5)	59.8 ± 2.78 (55.5 - 62)	63	30.1 ± 0.47 (30 – 30.5)	35.9 ± 1.75 (33 – 38)	44.1 ± 2.41 (40 – 48)	52.4 ± 1.38 (51 – 55)
Tail	32.2 ± 1.8 (30 – 35	30.3 ± 1.63 (27 – 34)	29	26.7	29.8 ± 1.64 (28 – 33)	30.1 ± 2.03 (28 – 33)	31.4 ± 1.68 (29 – 34)
h (hyaline portion of tail)	7.9 ± 0.46 (7 - 8)	6.9 ± 0.85 (6 - 9)	7	3.2 ± 0.35 (3 – 4)	4.6 ± 0.83 (4 – 6)	4.4 ± 0.50 (4 – 5)	5.3 ± 0.54 (4 – 6)
Body diameter at lip region	8.8 ± 0.35 (8 - 9)	8.7 ± 0.38 (8 – 9)	8.5	6.8 ± 0.25 (6.6 – 7.2)	7.4 ± 0.48 (7 – 8)	7.7 ± 0.37 (7 – 8.5)	8.3 ± 0.21 (8 – 8.5)
Body diameter at guide ring	18.8 ± 0.82 (17 - 20)	19.3 ± 0.64 (18 - 20)	20	12.1 ± 0.97 (11 – 13)	13.9 ± 0.69 (13 – 15	15.1 ± 0.88 (14 – 16)	17.4 ± 0.50 (16 – 18)
Body diameter at pharyngeal base	24.1 ± 1.03 (22 - 26)	23.3 ± 0.80 (22 - 24)	24	15.3 ± 0.30 (15 – 16)	17.3 ± 1.23 (15.5 – 19)	19.0 ± 1.34 (17 – 20.5)	21.7 ± 0.94 (20 – 23)
Body diameter at mid-body	27.1 ± 1.52 (25 – 30)	26.5 ± 1.27 (23 - 29)	26	16.7 ± 1.03 (15.5 – 18)	18.5 ± 0.73 (18 – 20)	20.4 ± 1.56 (18 – 22)	23.4 ± 1.45 (21 – 26)
Body diameter at anus	15.5 ± 0.81 (15 - 17)	15.3 ± 0.63 (14 - 17)	18	10.5	13.2 ± 3.27 (11 – 19)	12.7 ± 0.83 (12 – 14)	14.7 ± 0.80 (13.5 – 16)
Spicules	-	-	30	-	-	-	-

Table 2. Pharyngeal characters of *Xipinema parasimile* and *X. simile* females from different localities.

	Xipinema parasimile				*Xiphinema simile*	
Locality Character	Orlyane	Vinogradets	Ralja, Serbia Paratypes	Srebarna Reserve	Kalimok-Brashlen	Orlyane
	n=6	n=14	n=5	n=8	n=12	n=11
Pharynx length (µm)	266.7±28.9 (245-288)	250.5±21.0 (233-311) n=11	282.2±2.1 (260-310)	303.1±9.6 (293-320)	289.9±10.4 (273-310)	293.9±15.5 (273-316)
Bulbus length (µm)	62.0±2.1 (58-65)	59.8±3.5 (55.5-68)	61.6±4.8 (56-63)	80.2±1.3 (78-82)	83.0±3.4 (78-92)	84.2±5.7 (76-92)
Bulbus width (µm)	11.75±0.4 (11-12)	12.0±0.6 (11-13)	14.4±0.5 (14-15)	13.1±0.4 (12-14)	13.3±1.1 (12-15)	13.1±0.8 (12-14)
Bulbus length/ Pharynx length (%)	23.4±1.9 (20.3-25.6)	24.2±2.3 (19-28) n=11	21.8±0.7 (20.7-22.6)	27.5±1.1 (24.3-28.0)	28.6±1.4 (27.1-31.4)	28.9±1.1 (27.7-30.5)
DN* (%)	11.9, 12.5, 12.2	16.7±3.3 (13.6-18.6) n=8	16.5-17.7	15.6, 13.2, 15.8	15.4±1.9 (11.6-17.7) n=9	16.6±1.6 (13.4-18.4) n=7
DO* (%)		11.1, 13.6	11.6-14.6	?	9.6±1.7 (7.9-11.1), n=5	10.8±1.5 (7.7-12.1), n=7
SVN1* (%)	?	?	55.3-59.7	61.3	61.1	61.8, 59.2, 61.2
SVN2* (%)	?	?	57.3-60.1	64.1	62.2	61.8, 64.1
Glandularium** (µm)	55, 54, 51	49.9±1.4 (48-52) n=11	52.6±2.2 (52-56)	68.2±1.5 (67-71) n=6	71.4±3.0 (68-78)	71.9±4.4 (63-75) n=8
D** (%)	80.9, 78.2, 79.2	80.1±1.7 (77.4-83.6) n=8	81.3± 0.8 (80.0-82.1)	77.7±0.5 (77.2-78.2) n=6	75.5±1.2 (73.4-77.96)	75.5± 0.7 (74.3-76.6) n=7
AS1** (%)	?	?	47.1-51.2, n=4	39.3	53.5, 53.9	52.1, 51.9, 55.3
AS2** (%)	?	?	48.9-53.7, n=4	42.7	54.4, 55.3	52.1, 57.7

Terminology adopted by Loof and Coomans, 1972 * and Andrássy, 1998**

Table 3. Measurements of uteri, ovejector and vaginal parts. All measurements in micrometres presented as mean ± standard deviation (range).

	Characters Locality	Anterior uterus	Posterior uterus	Ovejector	Vagina length	*Pars distalis vaginae*	*Pars proximalis vaginae*
Xiphinema parasimile	Ralja, Trešna Serbia, paratypes	40.0±11.3 (27-46) n=3	-	-	14.5±1.05 (13-16) n=5	7.8±0.8 (7-8.5) n=3	8.75±0.3 (8.5-9) n=4
	Vinogradets	33.1±0.4 (30-38) n=13	31.2±0.7 (24-39) n=13	29.4±4.3 (26-33.5) n=10	14.5±1.7 (13-15) n=17	7.4±0.5 (7-8) n=15	7.4±0.5 (7-9) n=19
	Orlyane	32.3±4.2 (24-43) n=6	30.5±5.7 (24-38) n=6	26.8±2.1 (23-30) n=4	13.9±0.9 (13-15) n=7	7.0±1.4 (6-8) n=6	7.6±1.4 (7-8.5) n=7
Xiphinema simile	Srebarna	18.8±2.8 (14-21) n=6	18.5±2.4 (15-20) n=6	36.3±6.4 (29-41) n=3	14.8±1.3 (13-16) n=5	5.8±0.4 (5.5-6) n=8	9.5±0.9 (8.5-11) n=7
	Kalimok- Brashlen	21.8±1.9 (16.5-24) n=14	21.5±1.8 (19-24) n=14	43.1±3.1 (36.5-48) n=12	16.8±0.8 (15-18) n=15	6.4±0.65 (5.5-7) n=17	8.6±0.5 (8-10) n=17
	Orlyane	21.75±2.2 (17-24) n=7	22.1±2.3 (19-26) n=7	43.8±4.2 (36-50) n=7	16.9±1.1 (15-18) n=8	6.05±0.6 (5.5-7) n=11	9.05±0.8 (8-10) n=10
	Kamen bryag	23.0±4.8 (18-30) n=5	24.2±4.15 (19-30) n=5	47.2±8.9 (37-60) n=5	15.9±1.8 (13-17) n=5	6.4±0.6 (6-7) n=5	9.8±0.8 (9-10) n=5

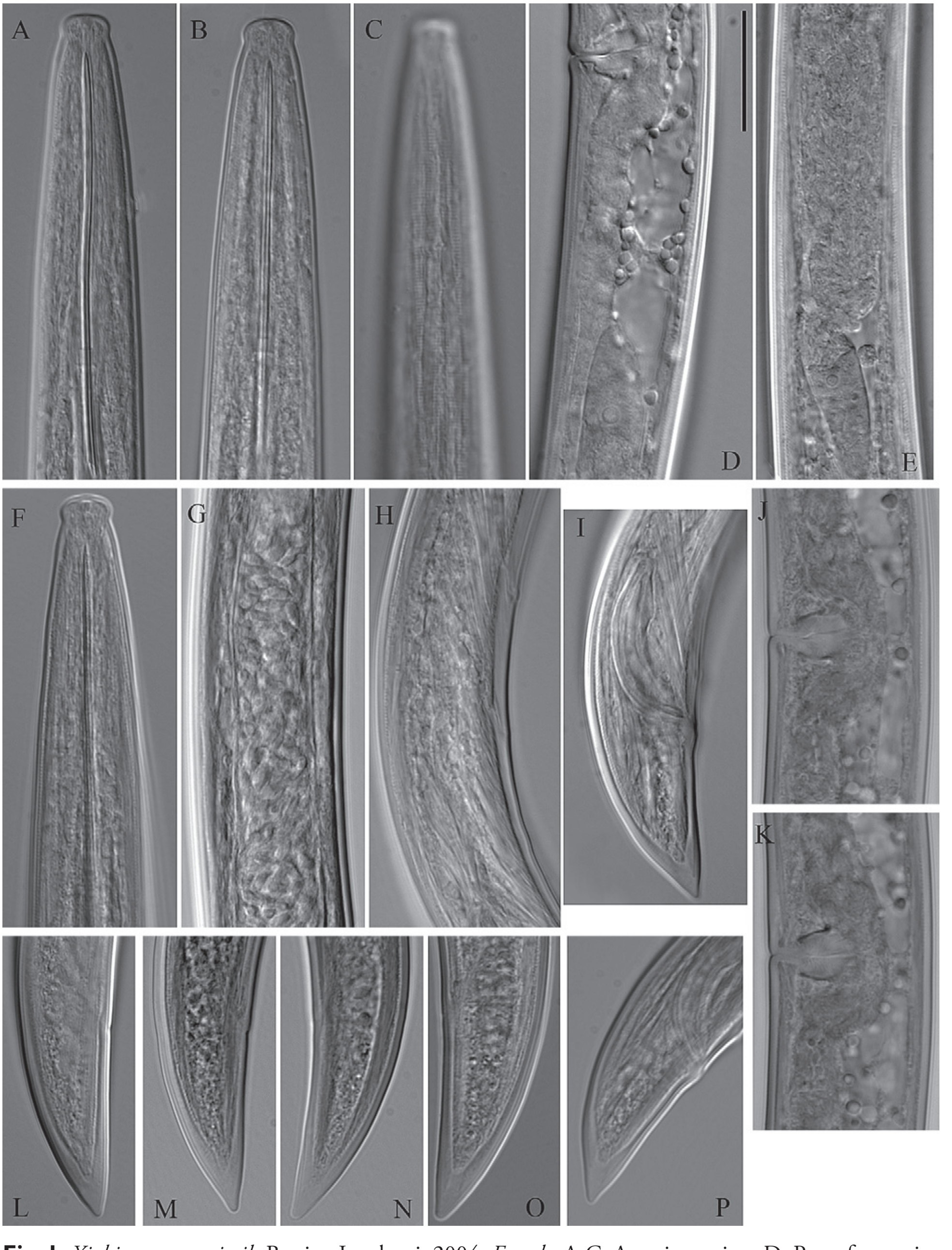

Fig. 1. *Xiphinema parasimile* Barsi et Lamberti, 2004. *Female*: A-C, Anterior region; D, Part of posterior genital branch; E, Posterior ovary; J, K, Vaginal region – different optical sections; L-O, Variation in tail shape; *Male*: F, Anterior end; G, Testis with sperm; H, Posterior end – muscles and supplements; I, Tail and copulatory apparatus; P, Tail. *Scale bar*: 20 µm.

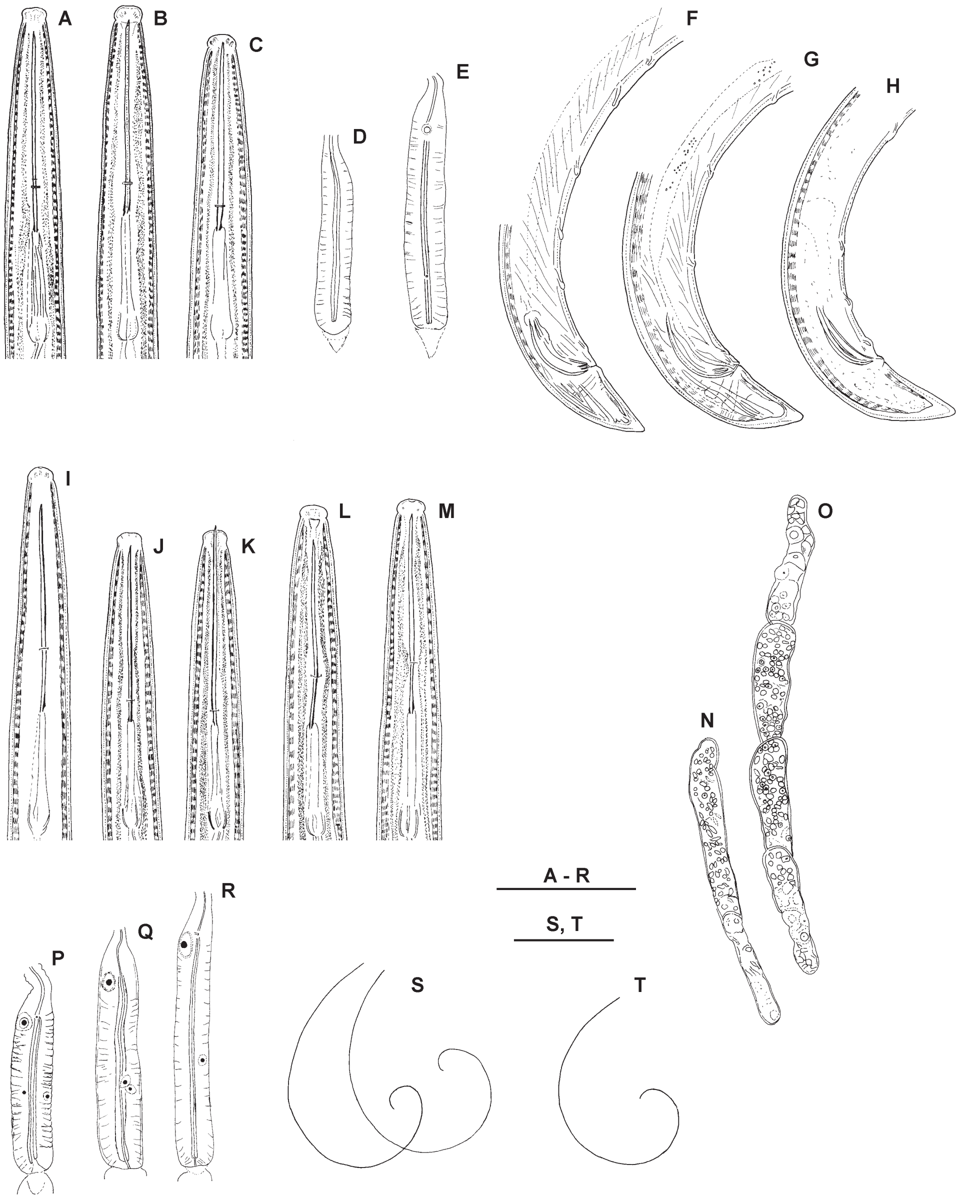

Fig. 2. *Xiphinema parasimile*. A, D, F, N, T: Male. A: Anterior end; D: Pharyngeal bulb; F: Posterior end; N: Posterior testis; T: Habitus; I-K, P: Females. I: Anterior end, paratype specimen; J: Anterior end, **Or**; K: Anterior end, **Vi**; P: Pharyngeal bulb; *Xiphinema simile*; B, C, E, G, H, O, S: Males. B – Anterior end, **SR**; C: Anterior end, **KB**; E: Pharyngeal bulb, **KB**; G: Posterior end, **KB**; H: Posterior end, **SR**; O: Genital system, **KB**; S: Habitus; L, M, Q, R: Females. L: Anterior end, **SR**; M: Anterior end, **KB**; Q, R: Pharyngeal bulb, **SR**; R: Pharyngeal bulb, **KB**. Scale bars: A-R=50 µm; S, T=500 µm.

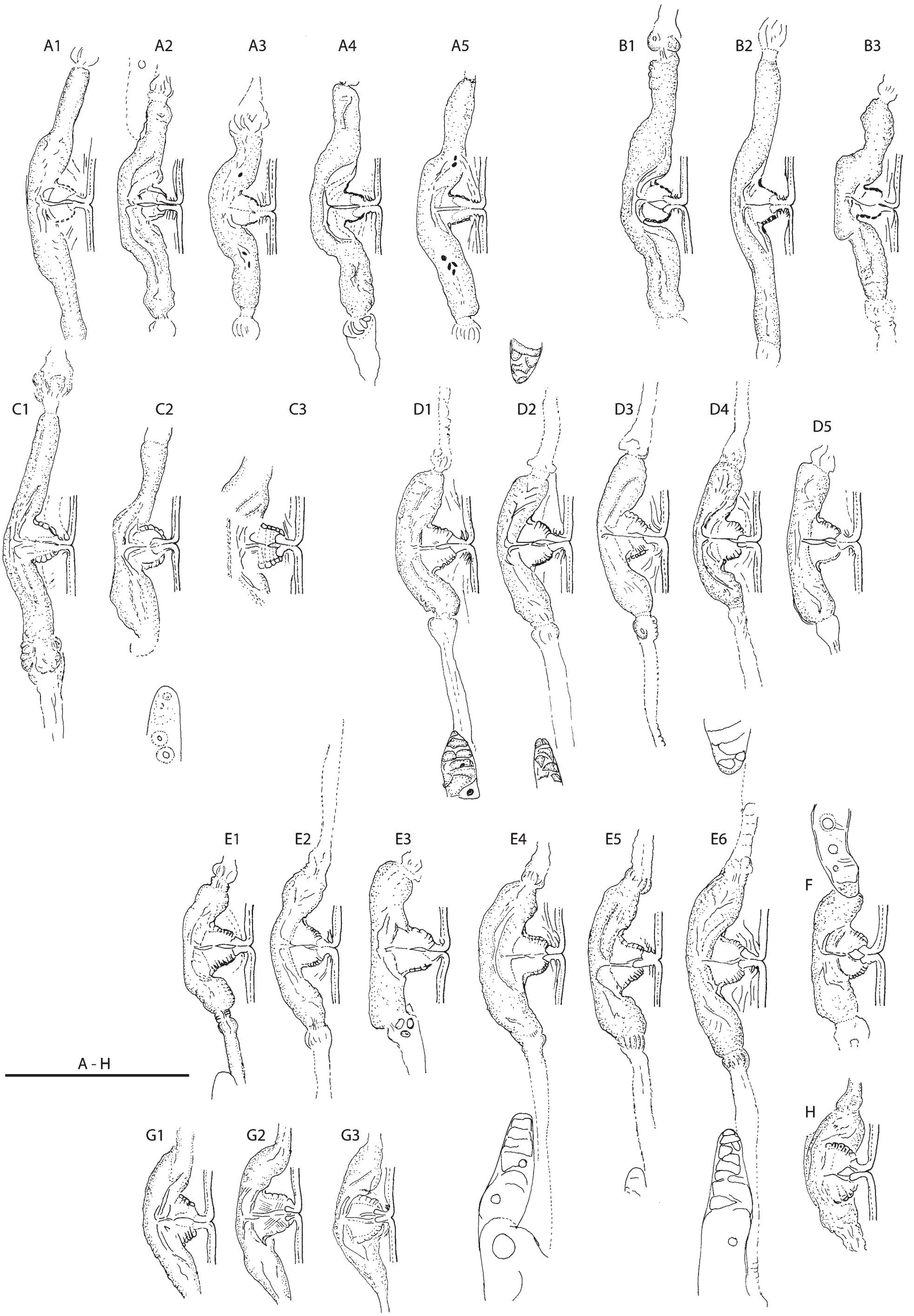

Fig. 3. Variability of vulval region. A-C: *Xiphinema parasimile*, A1-A5: **Vi**; B1-B3: **Or**; C1-C3: paratypes; D-H: *Xiphinema simile*, D1-D5: **KB**; E1-E6: **Or**; F: **Ka**; G1-G3: **SR**; H: **Kb**. Scale bar: A-H=50 μm.

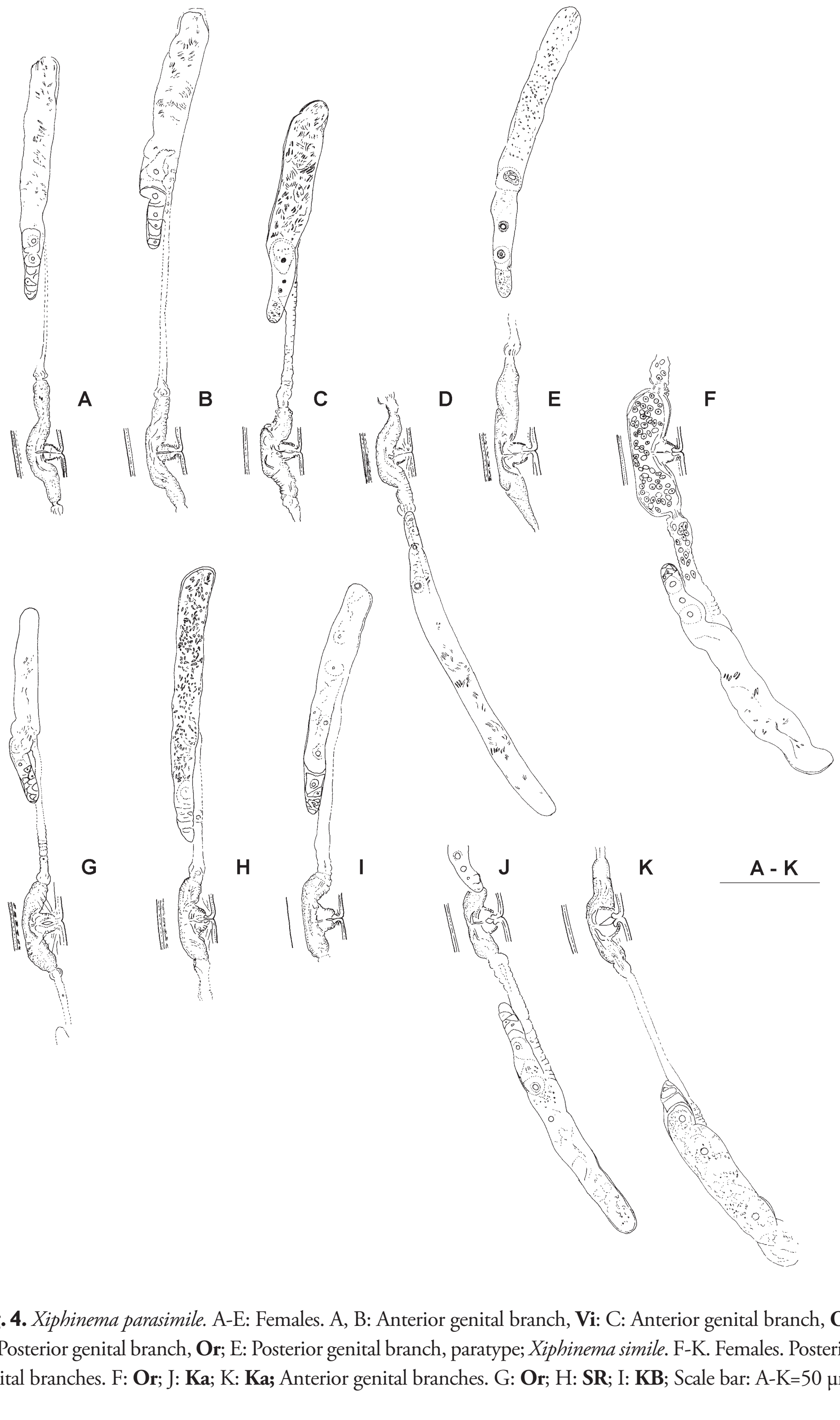

Fig. 4. *Xiphinema parasimile.* A-E: Females. A, B: Anterior genital branch, **Vi**: C: Anterior genital branch, **Or**; D: Posterior genital branch, **Or**; E: Posterior genital branch, paratype; *Xiphinema simile*. F-K. Females. Posterior genital branches. F: **Or**; J: **Ka**; K: **Ka;** Anterior genital branches. G: **Or**; H: **SR**; I: **KB**; Scale bar: A-K=50 µm.

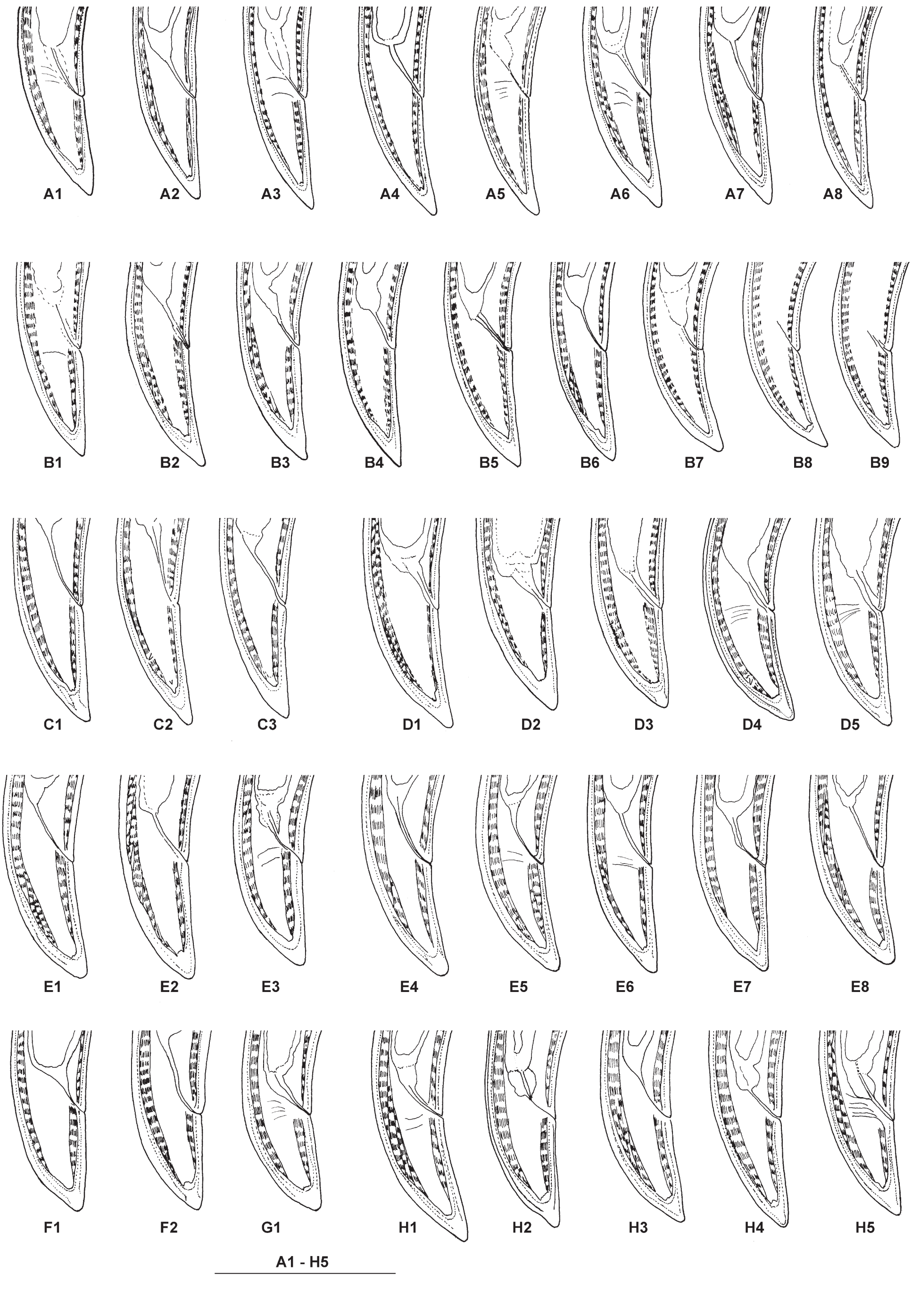

Fig. 5. Variability of female tale shape in studied populations. A-C: *Xiphinema parasimile*, A1-A8: **Vi**; B1-B9: **Or**; C1-C3: paratypes; D-H: *Xiphinema simile*, D1-D5: **SR**; E1-E8: **Or**; F1-F2: **Kb**; G1: **Ka**; H1-H5: **KB**. Scale bar: A-H=50μm.

mm). odontophore length (37.5-38.0 *vs* 40.7-41.6 µm), and body width (26.5-27.1 *vs* 27.9-28.3 µm) for the Bulgarian populations.

Male of *X. parasimile* compared to *X. simile* males from Serbia and Bulgaria had posterior part of the body less curved and a shorter body (1.84 *vs* 1.98-2.13 mm), narrower lip region (8.5 *vs* 10-10.5 µm), longer odontostyle (72 *vs* 63-69 µm) and shorter lateral guiding piece (4 *vs* 6 µm).

The RFLP results showed that the Bulgarian population of *X. parasimile* has the same restriction profiles as that from Serbia (Barsi and De Luca 2008). The enzyme *Ava* II produced in the Bulgarian population two extra bands that were absent in *X. parasimile* from Serbia, suggesting the existence of differences in restriction sites in D1-D2 sequences. Furthermore, the enzyme *Alu* I always showed a band of 0.8 kb, corresponding to the undigested product, along with the expected restriction fragments suggesting microheterogeneity of the D1-D2 region of Bulgarian materials of *X. parasimile* (Powers et al. 1997, Hugall et al. 1999, Hung et al. 1999, Subbotin et al. 2000, Morales-Hojas et al. 2001, Elbadri et al. 2002, Otranto et al. 2003, De Luca et al. 2004).

***Xiphinema simile* Lamberti, Choleva et Agostinelli, 1983**
(Figs 2B, C, E, G, H, L, M, O, Q-S; 3D-H; 4F-K; 5D-H; 9-11)

Measurements. See Tables 2-4

Description. *Females.* Body slender, slightly tapering towards both ends; C- to spiral-shaped. Thickness of the cuticle at postlabial region 1 µm; at dorsal side of the tail cuticle thickness increases gradually from 2 to 3.6 (3-4) µm towards tail end. Lip region expanded, flatly rounded, 4 (4-5) µm high. Amphidial opening 4-5 µm wide, occupying 44-50% of the corresponding body width (n=4), located just below the demarcation line. Odontostyle with moderately developed basal collar, guiding ring not appearing single. Pharyngeal characters presented at Table 2. Genital system with two almost equally developed branches, uteri short (Table 3); vagina 13–16 µm long or 46–56 % of the corresponding body diameter. Sperm cells observed in females from Kalimok and Orlyane populations. Ovaries contain symbiotic bacteria. Rectum 20.1 (18-22) µm long. Tail conoid, dorsally convex, terminus rounded, in some specimens pointed; presence of slight dorsal constriction at the level of hyaline part.

Males. Similar to female apart from body more curved at the posterior end and higher lip region (5-5.5 µm). Spicules slightly curved, one adanal pair and 3 ventromedian supplements present, lateral guiding piece 6 µm long The spicules of the specimen from Srebarna Reserve were not well developed and the testes were not observed while the specimen from Kalimok-Brashlen locality was apperantly functional with well developed testes filled with sperm. Tail longer than in female, especially in the specimen from Srebarna, conoid, dorsally convex with rounded terminus.

Juveniles. The scatter diagram based on functional and replacement odontostyle, and body length reveal presence of three juvenile stages (Figs 11A & B).

Table 3. Morphometrics of *Xiphinema simile* from different localities in Bulgaria. All measurements in micrometres, except body length and ratios, and in form: mean ± standard deviation (range).

Locality	Srebarna Reserve		Kalimok-Brashlen, Protected area		Kamen bryag area	Orlyane
Host plant	*Quercus pedunculiflora*		*Prunus cerasifera*		Poaceae	*Vitis vinifera*
Character	Female	Male	Female	Male	Female	Female
	n=8	n=1	n=10	n=1	n=5	n=13
L (mm)	2.17 ± 0.18 (1.93 - 2.50)	2.13	2.19 ± 0.10 (2.05 - 2.35)	2.10	2.04 ± 0.61 (1.95 - 2.11)	2.16 ± 0.15 (2.03 - 2.50)
a	70.7 ± 4.45 (63.9 - 78.6)	83.6	75.9 ± 4.55 (69.9 - 85.1)	70.8	67.4 ± 3.03 (64.1 - 70.2)	73.2 ± 4.23 (65.0 - 79.2)
b	7.2 ± 0.55 (6.2 - 7.9)	6.7	7.4 ± 0.41 (6.81-8.2)	7.03	6.8, 7.1	7.3 ± 0.35 (6.8 - 8.0)
c	70.2 ± 4.01 (64.1 - 75.7)	59.2	69.3 ± 9.36 (52.5 - 82.5)	67.7	77.0 ± 2.34 (74.4 - 79.6)	69.6 ± 5.44 (58.9 - 76.4)
c’	1.7 ± 0.07 (1.6 - 1.8)	1.8	1.7 ± 0.10 (1.5 - 1.9)	1.53	1.5 ± 0.04 (1.4 - 1.5)	1.7 ± 0.11 (1.6 - 1.9)
V (%)	55.5 ± 0.82 (53.7 - 56.4)	-	55.7 ± 1.22 (53.9 - 57.6)	-	54.8 ± 0.68 (54.2 - 55.9)	56.3 ± 0.79 (55.2 - 58.0)
Odontostyle	68.5 ± 1.61 (66 - 71)	69	69.6 ± 1.89 (66 - 72)	65.8	69.7 ± 1.97 (67 - 72.5)	69.6 ± 1.82 (66 - 72)
Odontophore	40.7 ± 1.87 (38 - 44)	46	42.5 ± 1.32 (40 - 45)	41.0	43.9 ± 1.13 (42 - 45)	41.8 ± 1.59 (40 - 45)

Locality	Srebarna Reserve		Kalimok-Brashlen, Protected area		Kamen bryag area	Orlyane
Host plant	*Quercus pedunculiflora*		*Prunus cerasifera*		Poaceae	*Vitis vinifera*
Character	Female	Male	Female	Male	Female	Female
	n=8	n=1	n=10	n=1	n=5	n=13
Oral aperture to guide ring	60.3 ± 1.82 (56.5 - 62)	62	59.3 ± 3.51 (50 - 61)	60.0	57.0 ± 0.66 (56.5 - 58)	58.1 ± 2.07 (56 - 60)
Tail	31.2 ± 1.29 (29.5 - 33)	36	29.2 ± 1.66 (26 - 31)	31.0	26.5 ± 0.33 (26 - 27)	30.5 ± 2.43 (27 - 35)
h (hyaline portion of tail)	7.2 ± 0.85 (6 - 8)	9	7.9 ± 0.42 (7.5 - 9)	7.9	6.7 ± 083 (6 - 8)	7.0 ± 1.46 (5 - 9)
Body diameter at lip region	10.3 ± 0.49 (10 - 11)	10	9.8 ± 0.29 (9 - 10)	10.1	9.9 ± 0.41 (10 - 10.5)	10.0 ± 0.37 (9 - 10.5)
Body diameter at guide ring	20.3 ± 0.81 (19 - 21.5)	19	20.2 ± 0.70 (19 - 21)	21.7	19.9 ± 0.16 (19.8 - 20.1)	19.5 ± 0.35 (19 - 20)
Body diameter at pharyngeal base	25.9 ± 1.07 (24.5 - 28)	24	26.0 ± 0.82 (25 - 27)	26.7	26.5 ± 0.89 (25 - 27)	25.4 ± 1.20 (23 - 27)
Body diameter at mid-body	30.8 ± 1.52 (28 - 33)	25.5	28.9 ± 1.06 (27 - 30)	29.7	30.3 ± 1.61 (28 - 32)	29.6 ± 1.69 (27 - 32)
Body diameter at anus	18.3 ± 0.35 (18 - 19)	21	17.5 ± 0.72 (16.5 - 18)	20.2	18.2 ± 0.50 (17.5 - 19)	17.5 ± 0.87 (16-19)
Spicules	-	30.5	-	36.0	-	-

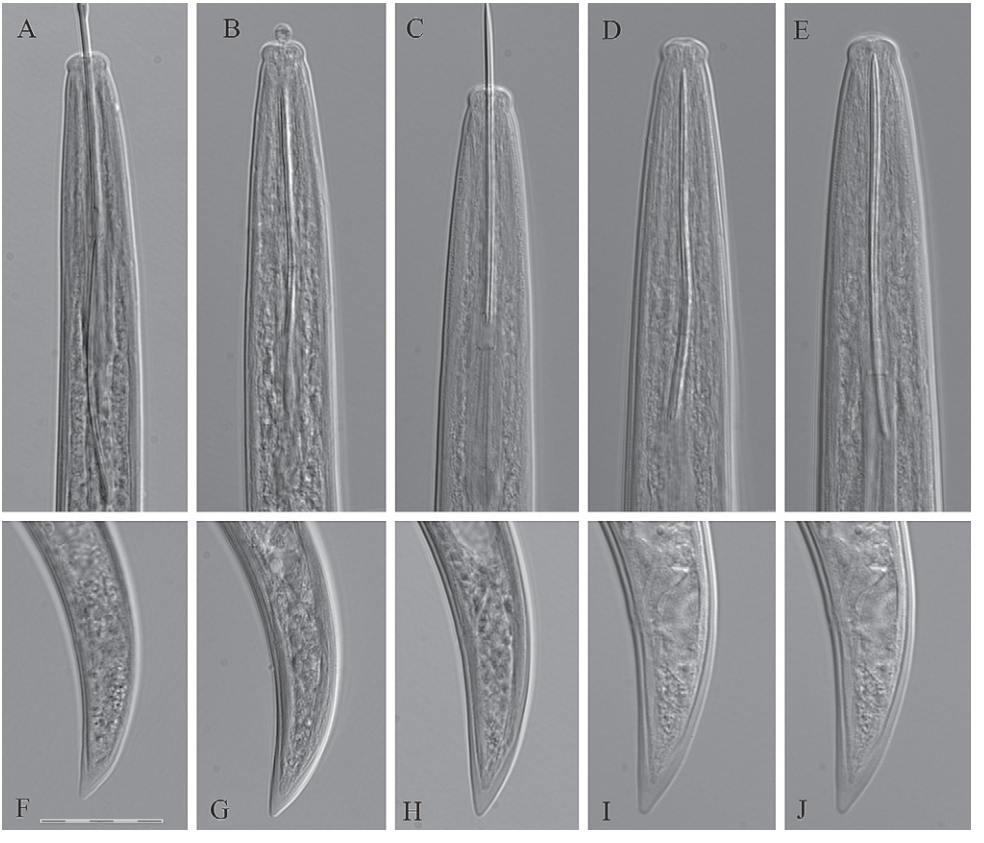

Fig. 6. *Xiphinema parasimile*. A-D, Anterior region of first, second, third and forth juvenile stages; E, Female anterior end; F-I, Tail of first, second, third and forth juvenile stages; J, Female tail. *Scale bar*: A-J, 20 µm.

Remarks. According to Barsi and Lamberti (2002) the populations of *X. simile* found in different localities have shown a broad range of variability in body length with populations with more southern distribution being shorter. This study revealed one population of *X. simile* from Kamen brayg area with lower mean values for body and tail length, **a**- and **c'**-ratios and higher **c**-ratio, as compared to other three populations. The comparisons with populations from different localities, showed that this population has similar body length with other Bulgarian (Lamberti et al. 1983, Peneva and Choleva 1992) and the Kenyan populations (Coomans and Heyns 1997), but still nematodes of this population had shorter tail length, higher **c**-ratios, and smaller **c'**-ratios. The other populations studied were within the range of those reported from northern localities of the range (Barsi 1994, Lišková and Brown 1996, Lamberti et al. 1999, Barsi and Lamberti 2002, Barsi and Lamberti 2004, Kumari 2006, Repasi et al. 2008).

Measurements of juvenile stages and male specimens are presented for the first time for Bulgarian populations. The obtained values were equal or close to those reported by Barsi and Lamberti (2002) and Barsi and De Luca (2008). The frequency distribution graphs of functional and replacement odontostyle lengths represent four

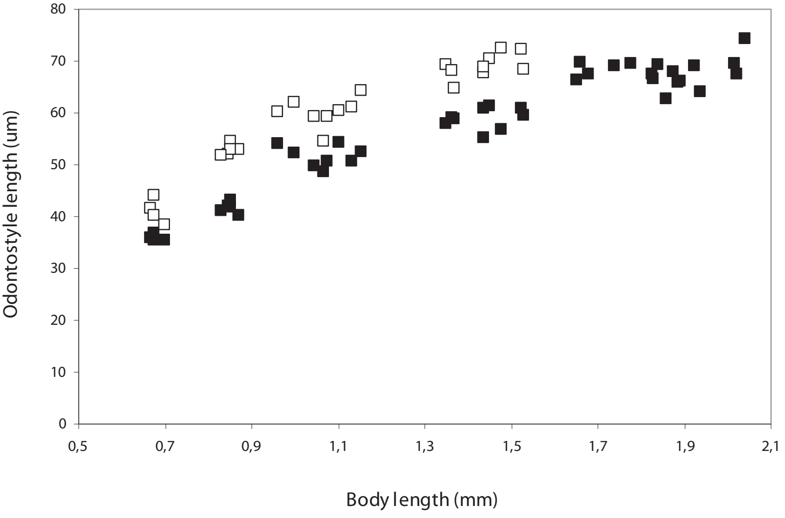

Fig. 7. Scatter plot of odontostyle (■) and replacement odontostyle (□) against body length of *Xiphinema parasimile* juveniles and females from Vinogradets population.

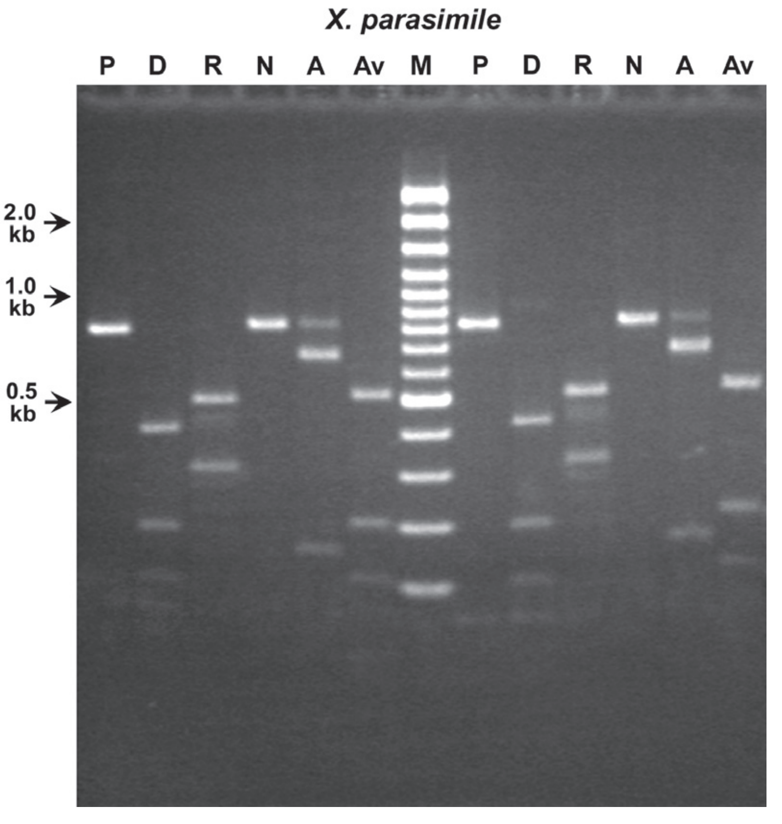

Fig. 8. Restriction fragments of amplified D1-D2 expansion domains of *Xiphinema parasimile.* A: *Alu* I, Av: *Ava* II, D: *Dde* I, N: *Nde* II, P: *Pst* I, R: *Rsa* I and M: 100bp ladder.

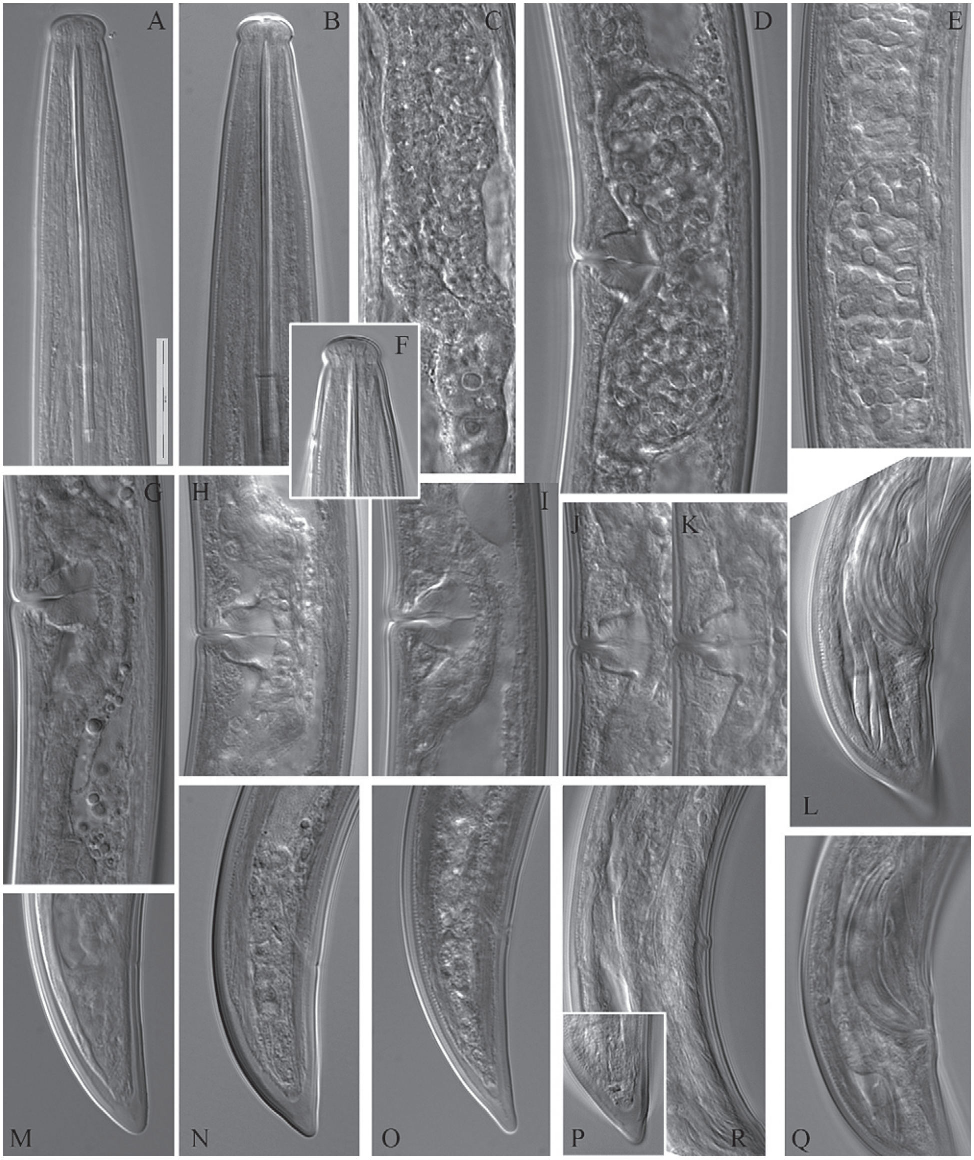

Fig. 9. *Xiphinema simile* Lamberti, Choleva et Agostinelli, 1983. *Female*: A-B, Anterior region; C, Ovarium with endosymbionts; D, Vagina and uteri with sperm; G, Vaginal region and part of the posterior genital branch; H-K, Vaginal region variation; M-O, Variation in tail shape; *Male*: E, Testis with sperm; F, Head end; L, Q, Spicules; P, Tail tip; R, Posterior end – copulatory muscles and midventral precloacal supplements. *Scale bar*: 20 µm.

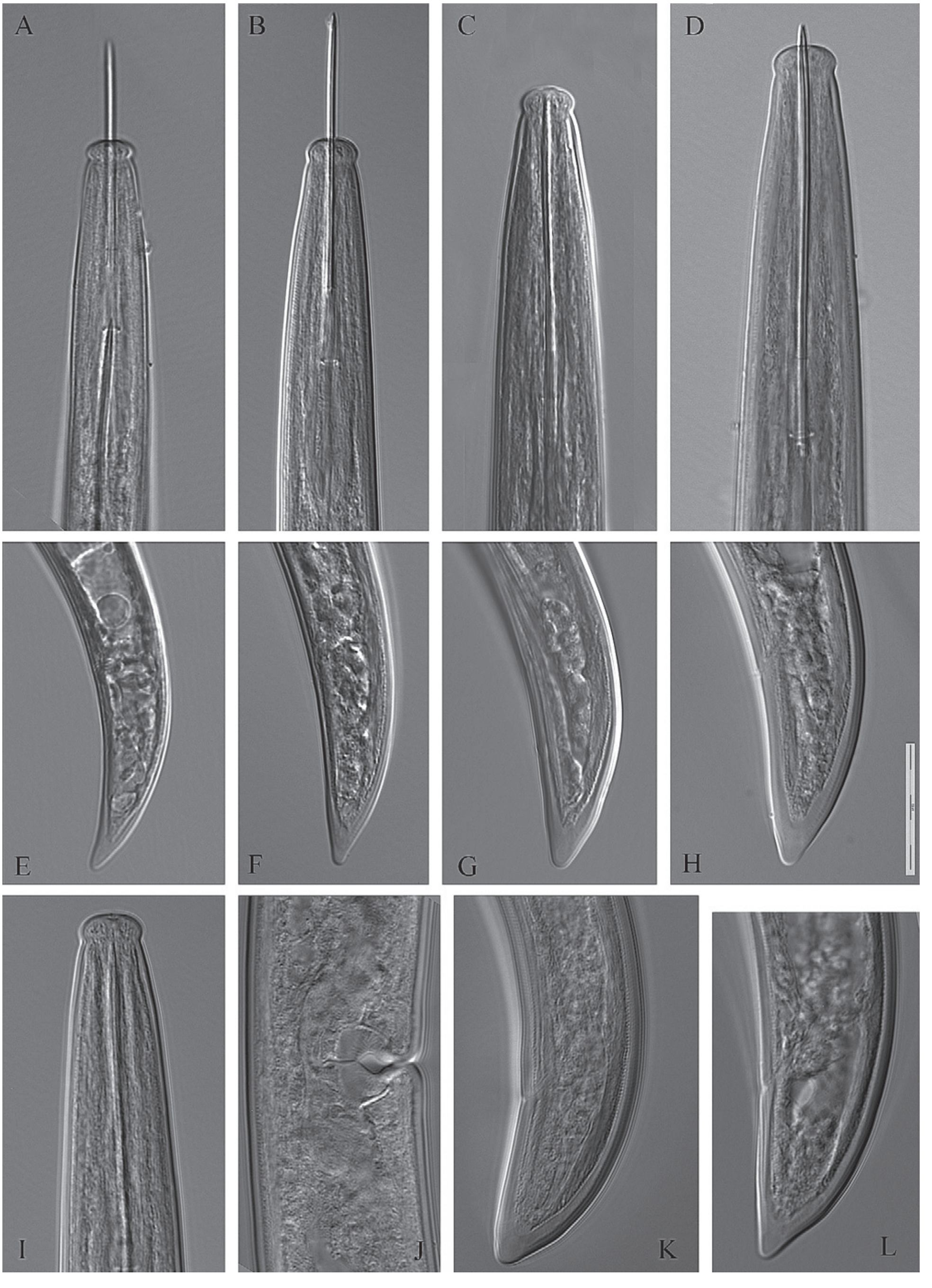

Fig. 10. *Xiphinema simile*. *Juveniles*: A-C, Anterior region of first, second and third juvenile stages; D, Female anterior end; E-G, Tail of first, second and third juvenile stages; H, Female tail. I-L *X. simile*: I, Female anterior end, J, Vaginal region, K, L, Tail. *Scale bar*: 20 µm.

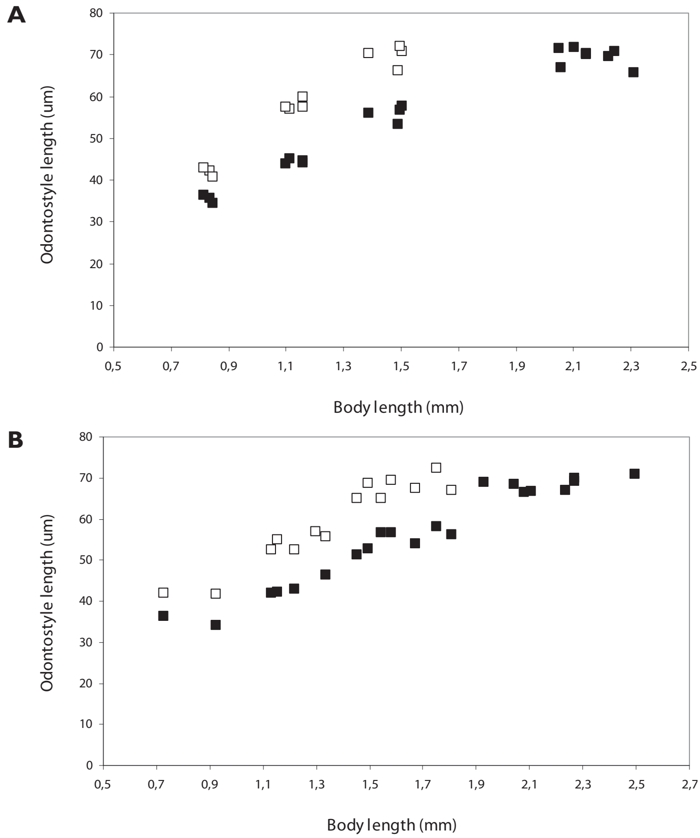

Fig. 11. Scatter plot of odontostyle (■) and replacement odontostyle (□) against body length of *Xiphinema simile* juveniles and females: A, Kalimok-Brashlen and B, Srebarna populations.

groups, corresponding to three juvenile stages and an adult stage and confirm the findings of other authors (Coomans and Heyns 1997, Barsi and Lamberti 2002, Barsi and Lamberti 2004, Kumari 2006) for the developmental pattern of *X. simile*.

Xiphinema simile was found to occur together with *X. parasimile* (Orlyane locality) and *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (Kalimok-Brashlen protected area).

Conclusions

Apparently, *X. simile* has a wider geographical range and has been found in association of numerous plant species in natural habitats and arable lands compared to *X. parasimile* which is so far recorded only from the Balkan Peninsula. The males of both species regarded here, although quite rare, may play an important role in genetic variability and hence contribute to the phenotypic plasticity within and among their populations. The overlapping of the metric characteristics, especially when combining data from many different populations of these species has been widely discussed by Barsi and De Luca (2008). Yet, some qualitative characters can be used to separate *X. simile* and *X. parasimile* such as the shape of lip region and tail (Barsi and Lamberti 2004). We have found that the length of pharyngeal bulbus and glandularium are good characters allowing discrimination of both species, no overlapping of the ranges reported. Comparison between the Bulgarian populations of *X. simile* and *X. parasimile* studied showed that the uteri and the *pars distalis vaginae* of the first species are shorter. Uteri in *X. simile* were represented only by ovejector, while in *X. parasimile* ovejector and separate uteri were observed. Thus, the structure of the uteri can also be used as another differentiating character. Our study confirms the results by Barsi and De Luca (2008) concerning the validity of both species adding new information about their morphology, distribution, plant associations, genetic variability and male characteristics of *X. parasimile*.

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