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Original paper

New records of *Xiphinema illyricum* Barsi & Lamberti, 1999 from Montenegro (Nematoda: Dorylaimida)

László BARSI

University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

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Summary. Discovery of *Xiphinema illyricum* in soil samples collected near Donja Seoca in Montenegro (2001, 2003 and 2007) represents the first recording of this species after the original description from the vicinity of Danilovgrad in 1999. A population collected in 2001 is described and illustrated, and morphometric data for females and four juvenile developmental stages are presented. This population is very similar to the type population and minor differences in morphometric data are considered to be intraspecific variability.

Key words: Donja Seoca, geographic distribution, juvenile developmental stages, morphology, morphometrics.

INTRODUCTION

Xiphinema illyricum was described based on a population collected in the rhizosphere of oriental hornbeam (*Carpinus orientalis* Mill.) near Danilovgrad in Montenegro (Barsi and Lamberti 1999). Later, populations of *Xiphinema* sp. very similar to *X. illyricum* were found in soil samples collected in 2001 and 2007 in the rhizosphere of oriental hornbeam, and in 2003 in the rhizosphere of pomegranate (*Punica granatum* L.) in the vicinity of Ivanina špilja cave (42°13′45.02″N, 19°8′6.00″E) approximately 1 km from Donja Seoca (Montenegro). Detailed study of the material on permanent slides confirmed that they were conspecific with *X. illyricum*.

The aim of the present study was to extend our knowledge of the geographic distribution, morphology, and intraspecific variability of *X. illyricum*, and to provide detailed illustrations of adult and juvenile developmental stages.

MATERIAL AND METHODS

Nematodes were extracted by Cobb's wet sieving technique. Specimens were killed by hot FP 4-1 and transferred to glycerin by a slow evaporation method and mounted on permanent microscope slides. Measurements were made with an ocular micrometer: with the exception of body, pharynx and tail lengths for all developmental stages, or replacement odontostyle length for juvenile stages. These characteristics were drawn using a drawing tube on an Olympus CX31 microscope at the appropriate magnification. Drawings were scanned and measurements were taken from the scanned drawings using Digimizer Version 4.6.1 software for digital

E-mail: laslo.barsi@dbe.uns.ac.rs

measurements. Photographs were taken using a Zeiss Axio Imager A1 compound microscope equipped with a digital camera AxioCam MRc 5.

Terminology and location of pharyngeal gland nuclei are given according to Andrássy (1998a, 1998b). The location of the dorsal nucleus (D) is expressed as the percentage of the distance between the anterior end of the body and the posterior end of the pharynx; the positions of the two subventral nuclei (AS_1, AS_2) are given as the percentage of the distance between the dorsal nucleus and the posterior end of the *cylindrus*. The position of AS_1 as the percentage of the D-AS_2 distance represents K.

RESULTS

***Xiphinema illyricum* Barsi & Lamberti, 1999**
(Figs 1-6)

Measurements
See Table 1.

Description

Female. Habitus as open C when killed, body cylindrical, tapering very gradually towards the extremities. Cuticle with very fine transverse striations. Lip region frontally almost flattened and laterally rounded, separated from the rest of the body by a weak depression. Amphidial fovea stirrup-shaped, with conspicuous slit-like aperture, about 70 (52.5-80)% of head diameter, located just anterior to demarcation line. Odontostyle long, 1.7 ± 0.06 (1.6-1.8) times longer than odontophore, and the latter with well-developed flanges 14.9 ± 0.8 (13.3-16.1) µm wide. Guiding sheath variable in length, with guiding ring 5.4 ± 0.5 (5.0-6.7) µm wide. Pharynx dorylaimoid with basal bulb, 97 ± 3.9 (90-107) × 24 ± 1.1 (22-27) µm, occupying 21.0 ± 2.5 (15.4-24.3)% of total length and provided with three gland nuclei. Glandularium 85 ± 4.1 (78-93) µm long or 19.4 ± 0.98 (17.8-22.1)% of total pharynx length; D = 80.5 ± 1.15 (77-82)%, AS_1 = 44.1 ± 2.4 (40-49)%, AS_2 = 46.0 ± 2.7 (41-56.5)%, K = 96.0 ± 3.5 (85-100)% (n = 29). In most specimens studied, a 2.5-4.2 µm long "mucro" is present in the anterior region of the slender part of pharynx at various distances (54 ± 23.4 [13-102] µm, n = 30) posterior to odontophore base. Reproductive system didelphic-amphidelphic with equally developed genital branches (Fig. 2I). Ovaries reflexed, oviduct with a slender part and a *pars dilatata oviductus* separated from the uterus by a conspicuous sphincter muscle. Uterus tripartite, consisting of a wide *pars dilatata uteri* (70 [55-84] µm long anteriorly and 69 [57-86] µm long posteriorly), continuing into a narrower, muscular tube-like portion (146 [126-170] µm long anteriorly and 144 [116-177] µm long posteriorly) and a 74 (61-95) µm wide ovejector. Numerous spiniform structures (spindle shaped spines) are present in the entire lumen of tubular portion. They are irregularly distributed, but in higher concentration just after *pars dilatata* and before the ovejector. Vulva pre-equatorial, slit like; vagina extending inwards for 56.7 ± 3.0 (51.6-62.5)% of the corresponding body diameter. Prerectum 460 ± 78 (324-563) µm long or 12 ± 2.0 (7.9-14.9) times the anal body width. Rectum 39 ± 2.6 (35-45) µm long and extending slightly less or more the body width at anus (0.8-1.2). Tail short conoid, dorsally convex, ventrally slightly convex or almost straight, with blind canal and a 6.1 ± 1.1 (2.9-8.2) µm long mostly ventrally directed conical peg which is sometimes ventrally bended, bearing 2-3 caudal pores on each side.

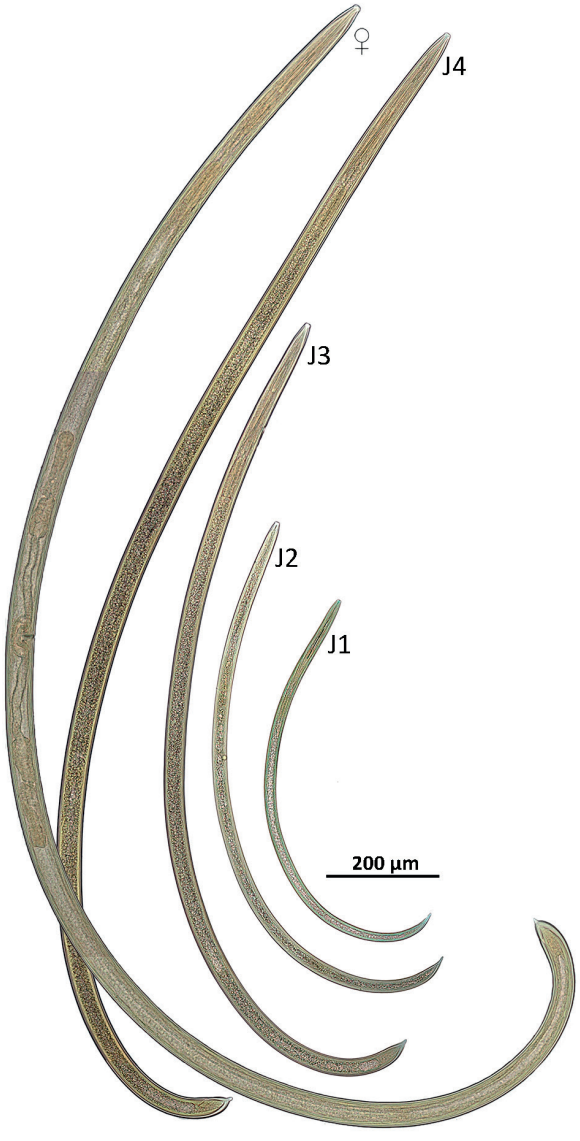

Fig. 1. *Xiphinema illyricum* from Montenegro. Entire body of female (♀) and four juvenile developmental stages (J1-J4).

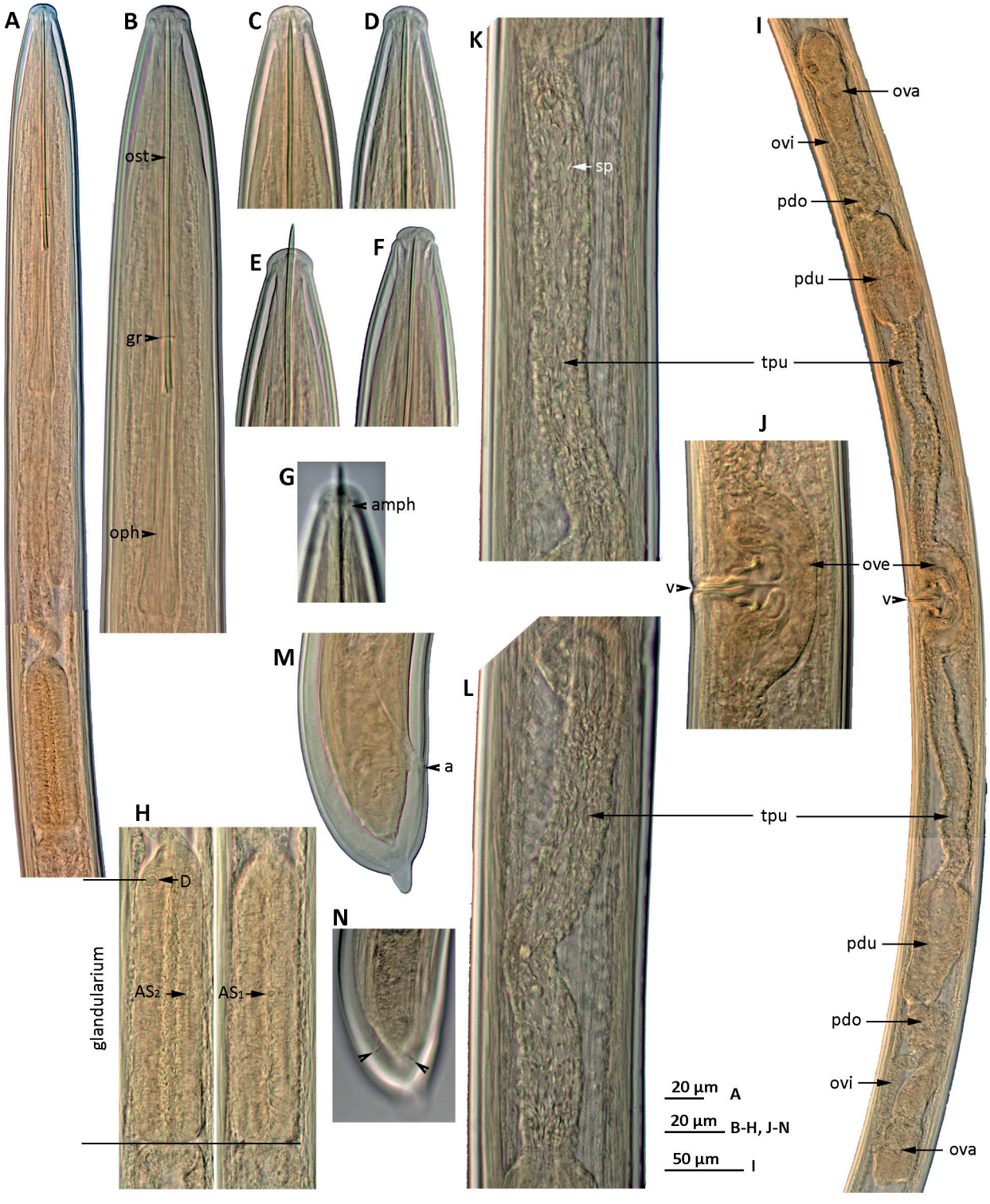

Fig. 2. *Xiphinema illyricum* from Montenegro, females. **A**, pharyngieal region; **B**, anterior region; **C-E**, **G**, lip regions, lateral view; **F**, lip region, dorso-ventral view; **H**, terminal pharyngeal bulb, different focus; **I**, reproductive system; **J**, vulval region; **K-L**, details of female genital tract showing spines; **M**, tail; **N**, caudal pores in lateral optical view. Abbreviations: a = anus; amph = amphidial pouch; AS1 = first anterior subventral nucleus; AS2 = second anterior subventral nucleus; D = dorsal nucleus; ova = ovarium; ove = ovejector; ovi = oviduct; pdo = *pars dilatata oviductus*; pdu = *pars dilatata uteri*; sp = spine; tpu = tubular part of uterus; v = vulva.

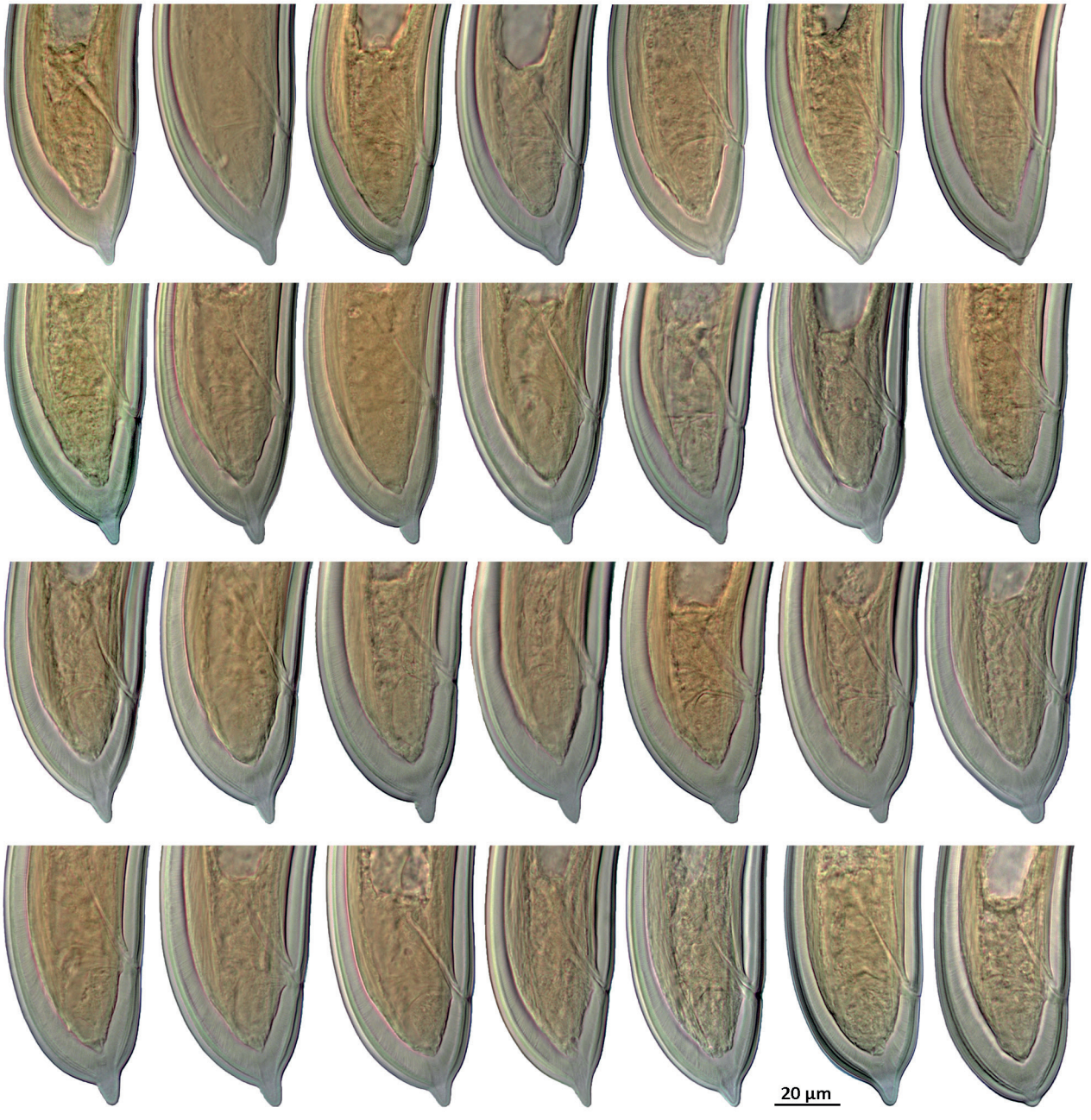

Fig. 3. *Xiphinema illyricum* from Montenegro. Variation of tail shape in females.

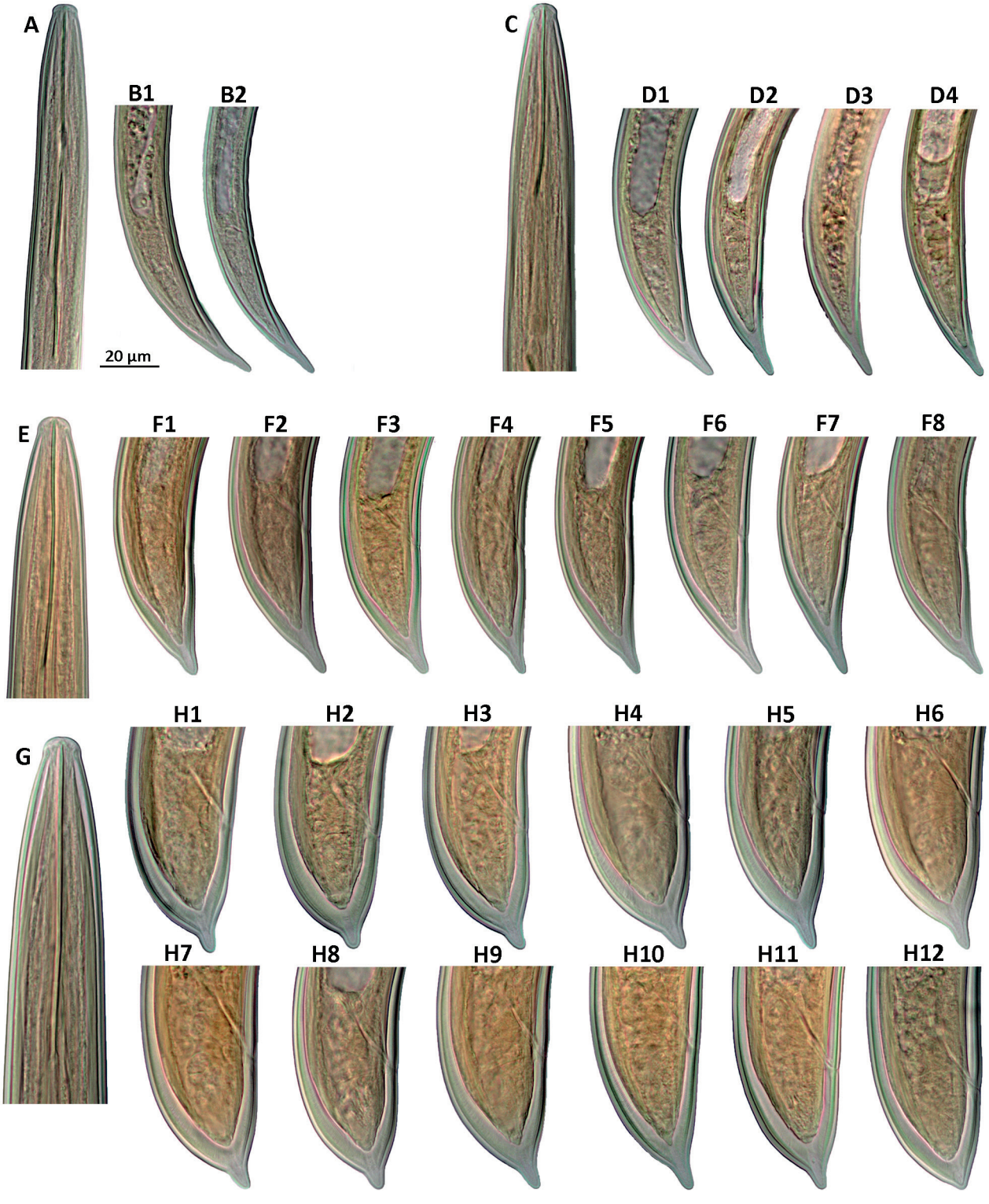

Fig. 4. *Xiphinema illyricum* from Montenegro, juveniles. **A**, **C**, **E**, **G**, anterior region of J1, J2, J3, and J4 developmental stage, respectively; **B1**-**B2**, J1, variation of tail shape; **D1**-**D4**, J2, variation of tail shape; **F1**-**F8**, J3, variation of tail shape; **H1**-**H12**, J4, variation of tail shape.

Male. Not found.

Juvenile stages (J1-J4). Juveniles clearly separated into four stages (Fig. 5) They are morphologically similar to adult females but smaller in size and body posture being less ventrally curved; tail of first and second stage elongate-conoid, and conoid with more or less distinct conical peg in third and fourth stage (Fig. 4).

Individual increase in length of replacement odontostyle in relation to functional odontostyle in individuals in four juvenile developmental stages showed that this increase was 17.9 (13.9-23.8)% in J1, 23.5 (20.0-28.2)% in J2, 19.3 (15.4-23.4)% in J3, and 19.4 (15.9-22.3)% in J4 (Fig. 6). The results suggest that the largest increase is in J2, but due to the small number of individuals available for measurement (n = 5), this may not always be the case for future populations.

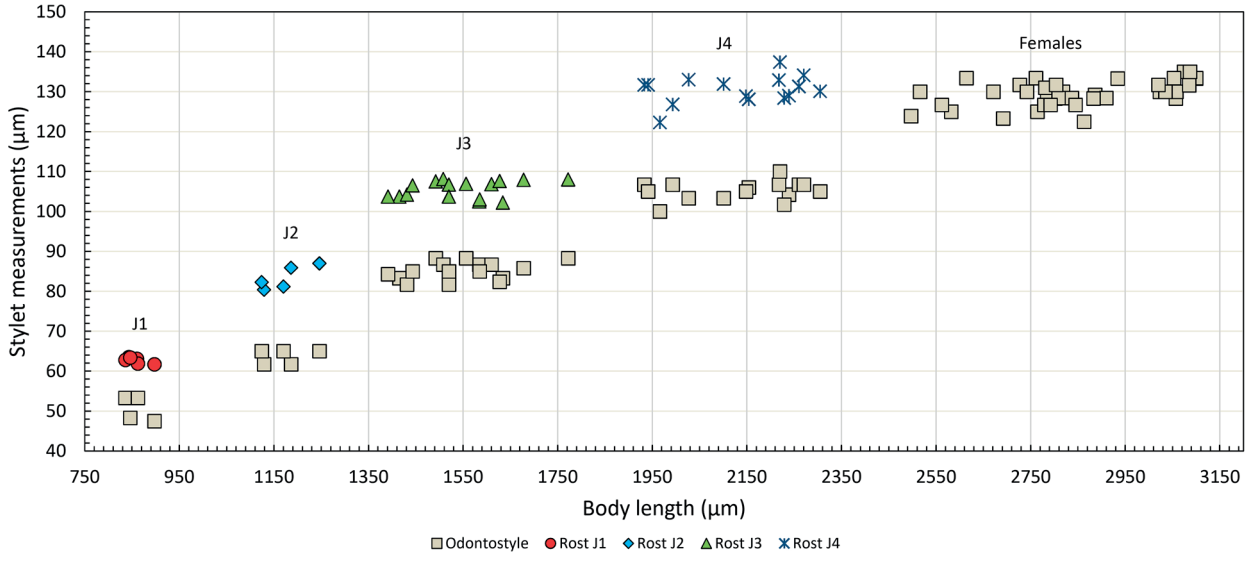

Fig. 5. Scatter diagram plotting the length of stylet measurements vs. body length of individual specimens of the four juvenile stages and adult female of *Xiphinema illyricum* from Montenegro (Replacement odontostyle = Rost).

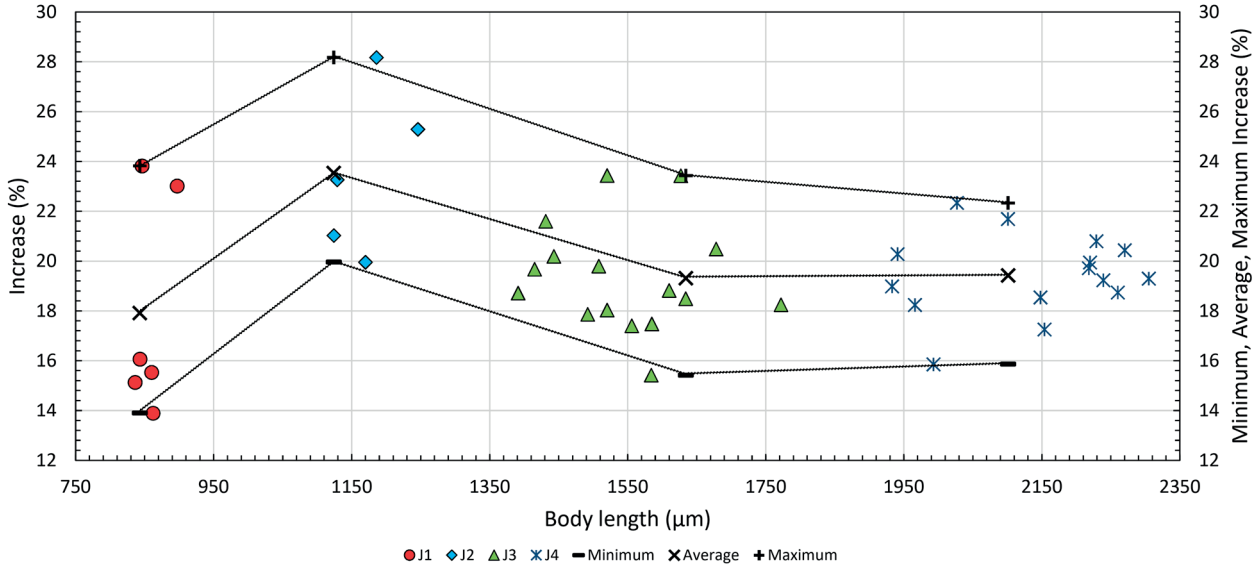

Fig. 6. *Xiphinema illyricum* from Montenegro. Individual increase in length of replacement odontostyle in relation to functional odontostyle in individuals in four juvenile developmental stages (J1-J4); minimum, average and maximum individual increase.

DISCUSSION

The population of *X. illyricum* collected from the area of Donja Seoca is similar to the type population collected from the area of Danilovgrad (Barsi and Lamberti 1999) and shows low intraspecific variability (Fig. 7). Notable differences between these populations are as follows. In females: slightly shorter body length (2.85 [2.50-3.10] vs. 2.92 [2.43-3.42] mm), slightly shorter distance of oral aperture to guide ring (114.7 [108.4-122.5] vs. 121.9 [114.4-130] µm), slightly shorter tail (36.6 [27.9-45.2] vs. 40.1 [31.8-46.4] µm), and slightly higher c value (78.7 [58.3-100.5] vs. 73.0 [61.0-92.2]). In juvenile developmental stages: slightly shorter body length, but with significant overlap of values between the two populations; slightly shorter replacement odontostyle length especially in J1 and J2 stages (62.7 [61.7-63.5], 83.4 [80.4-87.0] vs. 65.1 [63.7-67.5], 87.5 [78.7-92.1] µm); slightly shorter distance from oral aperture to guide ring and slightly shorter tail length in all juvenile stages in comparison to the type population.

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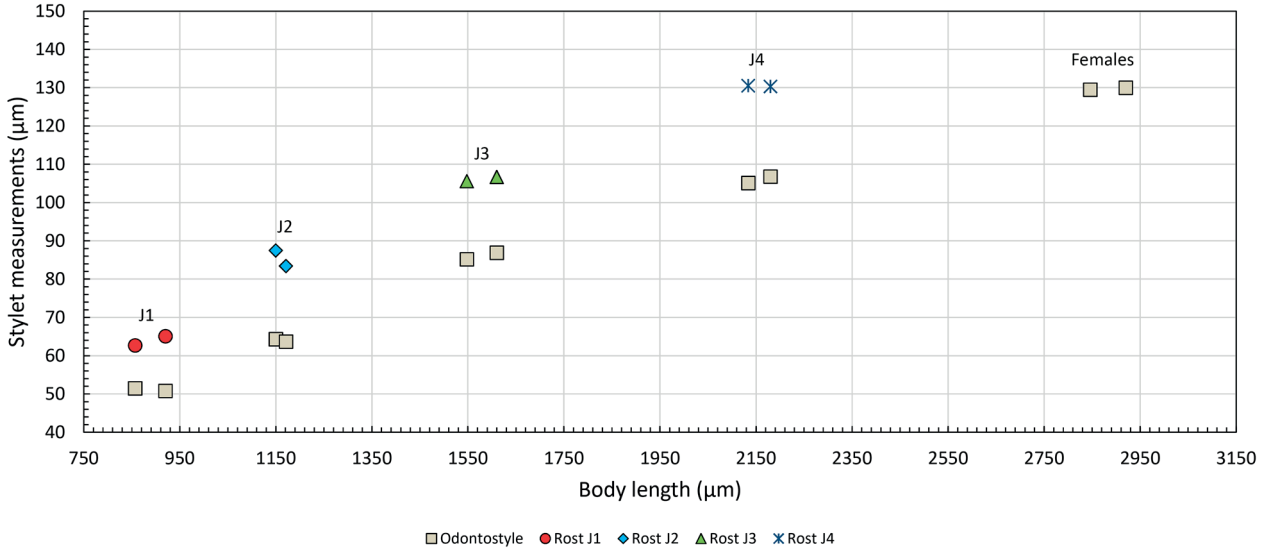

Fig. 7. Scatter diagram separating juveniles (J1-J4) and females of two populations of *Xiphinema illyricum* from Montenegro (Replacement odontostyle = Rost).

Table 1. Morphometrics of adult and juvenile *Xiphinema illyricum*[1] from Montenegro. All measurements in *μm* (except for L) and in the form: mean ± standard deviation (range).

Locality:	vicinity of Ivanina špilja cave, Donja Seoca				
Host:	*Carpinus orientalis*				
	J1	J2	J3	J4	Female
n	6	5	16	15	38
L (mm)	0.86 ± 0.02 (0.84-0.90)	1.17 ± 0.05 (1.12-1.25)	1.55 ± 0.10 (1.39-1.77)	2.13 ± 0.13 (1.93-2.31)	2.85 ± 0.17 (2.50-3.10)
a	39.1 ± 0.63 (38.3-39.9)	40.2 ± 2.79 (36.9-44.5)	45.2 ± 1.82 (41.9-47.9)	50.3 ± 2.50 (45.4-54.7)	55.1 ± 2.2 (49.7-59.0)
b	3.9 ± 0.51 (3.4-4.8)	4.1 ± 0.36 3.5-4.4	4.4 ± 0.40 (3.3-5.0)	5.2 ± 0.30 (4.8-5.7)	6.5 ± 0.4 (5.7-7.7)
c	17.1 ± 1.22 (15.5-18.4)	23.2 ± 0.92 (22.2-24.5)	31.7 ± 1.80 (28.2-34.4)	50.2 ± 3.03 (44.7-56.2)	78.7 ± 9.4 (58.3-100.5)
c’	3.30 ± 0.29 (3.07-3.76)	2.37 ± 0.12 (2.23-2.51)	1.80 ± 0.10 (1.62-1.96)	1.23 ± 0.07 (1.11-1.36)	0.95 ± 0.08 (0.79-1.11)
d	4.6 ± 0.17 (4.7-5.1)	5.5 ± 0.15 (5.3-5.8)	6.4 ± 0.26 (6.0-6.8)	6.9 ± 0.20 (6.6-7.3)	7.6 ± 0.20 (7.2-8.0)
d’	2.1 ± 0.07 (2.0-2.2)	2.4 ± 0.35 (2.2-3.0)	2.4 ± 0.10 (2.2-2.6)	2.5 ± 0.07 (2.4-2.7)	2.6 ± 0.06 (2.5-2.7)
J’	1.8 ± 0.42 (1.2-2.3)	1.8 ± 0.06 (1.8-1.9)	1.3 ± 0.15 (1.1-1.7)	0.8 ± 0.08 (0.7-0.9)	0.6 ± 0.05 (0.5-0.7)
V	–	–	–	–	42.7 ± 1.2 (40.3-45.3)
Odontostyle	51.5 ± 2.80 (47.5-53.3)	63.7 ± 1.81 (61.7-65.0)	85.2 ±2.26 (81.7-88.3)	105.1 ± 2.43 (100.0-110.0)	129.5 ± 3.2 (122.5-135.0)
Odontophore	39.0 ± 2.25 (36.7-42.5)	47.7 ± 1.89 (45.0-50.0)	56.2 ± 1.55 (53.3-58.3)	66.4 ± 2.29 (62.5-+71.7)	74.7 ± 2.2 (71.7-79.2)
Total stylet	90.5 ± 1.71 (88.3-93.3)	111.3 ± 3.58 (106.7-115.0)	141.3 ± 2.95 (136.6-146.6)	171.5 ± 3.89 (164.2-181.7)	204.2 ± 4.1 (195.0-212.5)
Replacement odontostyle	62.7 ± 0.77 (61.7-63.5)	83.4 ± 2.93 (80.4-87.0)	105.6 ± 2.17 (102.2-108.1)	130.5 ± 3.53 (122.3-134)	–
Oral aperture to guide ring	42.4 ± 1.77 (39.2-44.2)	55.0 ± 1.78 (53.3-57.5)	72.6±1.78 (70.0-76.7)	91.7 ± 2.90 (87.5-96.7)	114.7 ± 3.3 (108.4-122.5)
Tail	50.4 ± 3.01 (46.0-54.1)	50.5 ± 0.34 (50.1-50.9)	48.8 ± 1.98 (44.4-51.5)	42.5 ± 2.18 (38.7-46.0)	36.6 ± 4.3 (27.9-45.2)
J (hyaline portion of tail)	11.2 ± 2.07 (8.3-13.3)	14.4 ± 1.07 (13.3-15.6)	15.7 ± 1.56 (13.3-19.2)	15.5 ± 1.58 (12.5-17.9)	16.5 ± 1.9 (11.7-21.7)
Body diam. at lip region	8.7 ± 0.29 (8.3-8.9)	9.9 ± 0.18 (9.6-10.0)	11.3 ± 0.40 (10.8-11.7)	13.2 ± 0.26 (12.5-13.7)	15.2 ± 0.4 (14.2-16.3)
Body diam. at guide ring	18.0 ± 0.22 (17.8-18.3)	23.7 ± 3.56 (21.7-30.0)	27.2 ± 0.87 (25.6-28.9)	33.8 ± 0.90 (32.2-35.0)	40.0 ± 0.9 (38.3-41.7)
Body diam. at base of pharynx	21.7 ± 0.63 (20.8-22.8)	28.5 ± 1.82 (26.1-30.8)	33.4 ± 1.96 (30.6-36.7)	40.8 ± 2.41 (38.3-45.0)	46.8 ± 2.4 (42.5-51.7)
Body diam. at mid-body or vulva	21.9 ± 0.60 (21.2-22.8)	29.2 ± 2.24 (26.3-31.7)	34.3 ± 2.40 (30.8-38.3)	42.5 ± 3.12 (38.9-47.9)	51.8 ± 3.1 (46.1-60.0)
Body diam. at anus	15.3 ± 0.65 (14.4-16.3)	21.3 ± 1.17 (20.0-22.8)	27.1 ± 1.57 (25.0-30.5)	34.6 ± 1.72 (31.9-38.3)	38.6 ± 2.1 (35.0-42.5)
Body diam. at beginning of J	6.3 ± 0.72 (5.0-6.7)	7.8 ± 0.49 (7.2-8.3)	11.7 ± 0.89 (10.2-13.3)	18.9 ±1.03 (16.9-20.8)	25.8 ± 2.5 (18.3-31.7)

[1] Population coded AC609 (rhizosphere of oriental hornbeam, 2001).
d, anterior to guide-ring/body width at lip region (Brown et al. 1994). d’, body width at guide ring/body width at lip region (Brown et al. 1994). J’, length of the hyaline region of the tail/hyaline width (Lišková et al. 1997).