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Original paper

Xiphinema italiae Meyl, 1953 (Nematoda: Longidoridae) from Montenegro, and comments on the number of juvenile developmental stages in this species

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Summary. In May 2006, a population of *Xiphinema italiae* was found in sandy soil in the rhizosphere of black pine (*Pinus nigra* Arnold) on Velika plaža, a sandy beach in Ulcinj (Montenegro). This population is briefly described, illustrated and the morphometric data of females and four juvenile developmental stages (JDS) are presented, in order to contribute to the knowledge of the intraspecific variability of this species. Based on the available literature data, it was determined that the morphometry of the populations of *X. italiae* shows a wide range of intraspecific variability. Data on the developmental biology of *X. italiae* show the existence of populations with three or four JDS. This discrepancy of data from the literature on the number of JDS in *X. italiae* prompted me to analyze the situation in a little more detail. The results of data analysis regarding the number of juvenile stages in *X. italiae* are presented in this paper.

Keywords: cryptic species, intraspecific variability, juvenile developmental stage, morphology, morphometrics.

INTRODUCTION

Xiphinema spp. are migratory root ectoparasites of various wild and economically important cultivated plants. Some species have been shown to be vectors of nepoviruses (Taylor and Brown 1997).

According to Pajović I and Pajović Lj (2012), eight *Xiphinema* species occur in Montenegro. These are: *X. americanum* Cobb, 1913, *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, *X. illyricum* Barsi & Lamberti, 1999, *X. index* Thorne & Allen, 1950, *X. montenegrinum* Barsi, Lamberti & Agostinelli, 1998, *X. pachtacum* (Tulaganov, 1938) Kirjanova, 1951, *X. simile* Lamberti, Choleva & Agostinelli, 1983, and *X. variurum* Barsi & Lamberti, 1998. Three species such as *X. variurum*, *X. illyricum* and *X. montenegrinum* were first described from Montenegro (Barsi and Lamberti 1998, 1999; Barsi et al. 1998) and a new record of *X. illyricum* was published recently (Barsi 2021). The presence of *X. americanum* in Montenegro, as reported by Krnjaić (1968), is unlikely. According to the present status of *X. americanum* s.s. it is present only in Africa and North America, but nematodes belonging to *X. americanum* s.l. (*X. americanum*-group species) occur in Africa and widely in Asia, Central and South America, Europe and North America, but have been found infrequently in Australasia and Oceania (EPPO 2017). Thus, the report of *X. americanum* s.s. from Montenegro (Krnjaić 1968) should be attributed either to *X. pachtacum* or *X. simile*.

In a sandy soil sample collected on the 10th of May, 2006, in the rhizosphere of a black pine (*Pinus nigra* Arnold) at Velika plaža sandy beach in Ulcinj (Montenegro), a population of *Xiphinema italiae* has been recovered.

Xiphinema italiae has been earlier reported either with

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four (Martelli et al. 1966; Lamberi et al. 1997; Barsi and Lamberti 2003; Groza et al. 2013) or three (Lamberti et al. 1996a; Avgelis and Tzortzakakis 1997) juvenile developmental stages (JDS). This discrepancy of data from the literature regarding the number of JDS in *X. italiae* prompted me to analyze the situation in a little more detail.

The objectives of the present study were: 1) to briefly describe, illustrate and present the morphometric data of females and the four JDS of the population of *X. italiae* from Montenegro, in order to contribute to the knowledge of the intraspecific variability of this species; 2) to present the results of data analysis regarding the number of juvenile stages in *X. italiae*.

MATERIAL AND METHODS

Nematodes were extracted from a soil sample using 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 measurements. Photographs were taken using a Zeiss Axio Imager A1 compound microscope equipped with an AxioCam MRc 5 digital camera.

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 a percentage of the D-AS_2 distance represents K.

Data analysis on the number of juvenile developmental stages in *X. italiae*

Publications of Martelli et al. (1966), Lamberti et al. (1996a, 1997), Barsi and Lamberti (2003), Groza et al. (2013), and original data (presented in this paper) were used as sources of selected morphometrics of six populations of *X. italiae*. For every population the following data were used: mean body length, mean odontostyle and replacement odontostyle lengths and mean midbody diameter, all measured in µm, for every stage. Mean body length (L) and mean midbody diam. (D) were used to calculate the body volume of every stage using the equation of Andrássy (1956): $V = (D^2 \times L)/1.7$. All data were tabulated for each population and used for further calculations. Data sets of *X. italiae* from Montenegro (original data) are presented in Table 1.

A percentage method was used to make the data sets comparable between each other for every population. Absolute data for females (body length, odontostyle length in µm and the calculated body volume in μm^3) were used as 100%. The same data set for every juvenile stage was compared with the data set of the female and expressed as a percentage. The only exception was the replacement odontostyle length. In this case the absolute value of the replacement odontostyle length in the pre-adult stage was used for comparison as 100%.

The percent values for all characters for each of the developmental stages of the six populations of *X. italiae* studied were tabulated (Tables 2 and 3). Also, using percent values for each stage, the relative increase between successive stages was calculated for each population.

Table 1. Working data set for a population of *Xiphinema italiae* from Montenegro (original data). All measurements in µm.

Character/stage	J1	J2	J3	J4	Female
Body length	944	1284	1763	2335	3177
%	**29.7**	**40.4**	**55.5**	**73.5**	**100**
Body diam.	18.8	22.3	25.7	29.5	34.2
Body volume (μm^3)	196263	375600	684967	1195314	2185851
%	**9.0**	**17.2**	**31.3**	**54.7**	**100**
Odontostyle length	48,1	58,6	72,1	84,8	100,7
%	**47.8**	**58.2**	**71.6**	**84.2**	**100**
Replacement odontostyle length	58.0	72.3	85.9	100.2	–
%	**57.9**	**72.1**	**85.7**	**100**	**–**

Only data sets printed in **bold** were used in the further study.

Table 2. Growth patterns in body length, body volume, odontostyle length, replacement odontostyle length and relative increase of body length, body volume, odontostyle length, replacement odontostyle length between successive stages in four populations of *Xiphinema italiae* from Montenegro, Serbia, Bulgaria, and Romania.

	Growth pattern						Relative increase				
	J1	J2	J3	J4	F		J2/J1	J3/J2	J4/J3	F/J4	Sum
						*L µm					
[1]L%	29.7	40.4	55.5	73.5	100	3177	1.36	1.37	1.32	1.36	5.42
[2]L%	29.1	41.8	55.4	73.5	100	2884	1.44	1.33	1.33	1.36	5.45
[3]L%	33.3	48.1	63	81.5	100	2700	1.44	1.31	1.29	1.23	5.27
[4]L%	–	39.2	52.5	72.8	100	3240	–	1.34	1.39	1.37	–
Mean	30.7	42.4	56.6	75.3	100	3000	1.41	1.34	1.33	1.33	5.38
						*Vol mm^3					
[1]Vol%	9	17.2	31.3	54.7	100	0.00219	1.91	1.82	1.75	1.83	7.31
[2]Vol%	6.9	15.3	27.4	48.8	100	0.00178	2.22	1.79	1.78	2.05	7.84
[3]Vol%	9.6	26	35.7	66	100	0.00184	2.71	1.37	1.85	1.52	7.45
[4]Vol%	–	13.0	22.9	45.8	100	0.00218	–	1.76	2.00	2.18	–
Mean	8.5	17.9	29.3	53.8	100	0.00200	2.28	1.69	1.84	1.89	7.53
						*O µm					
[1]O%	47.8	58.2	71.6	84.2	100	100.7	1.22	1.23	1.18	1.19	4.81
[2]O%	46.5	56.3	72.2	86.0	100	95	1.21	1.28	1.19	1.16	4.85
[3]O%	48.3	58.4	71.1	89.2	100	90	1.21	1.22	1.25	1.12	4.80
[4]O%	–	55.1	72.3	87.4	100	92.7	–	1.31	1.21	1.14	–
Mean	47.5	57	71.8	86.7	100	94.6	1.21	1.26	1.21	1.15	4.82
						*R µm					
[1]R%	57.9	72.1	85.7	100	–	100.2	1.25	1.19	1.17	–	3.60
[2]R%	54.5	72	84.8	100	–	95.6	1.32	1.18	1.18	–	3.68
[3]R%	55.3	67.6	82.4	100	–	94	1.22	1.22	1.21	–	3.65
[4]R%	–	70.9	85.9	100	–	93	–	1.21	1.16	–	–
Mean	55.9	70.7	84.7	100	–	95.7	1.26	1.20	1.18		3.64

[1]Montenegro (original); [2]Serbia (Barsi and Lamberti 2003); [3]Bulgaria (Lamberti et al. 1997); [4]Romania (Groza et al. 2013).
Note. Stage J1 was not found in the Romanian population.
L = body length; Vol = body volume; O = odontostyle length; R = replacement odontostyle length; F = female.
*L, *Vol, *O = absolute values for female; *R = absolute value for J4 stage.

RESULTS AND DISCUSSION

Xiphinema italiae from Montenegro

(Figs 1-3)

Measurements

See Table 4.

Female. Habitus most often has the shape of the letter J, and less often with transitional forms to the open letter C after fixation. Body cylindrical, slender, tapering very gradually towards the extremities. Lip region offset, frontally and laterally gently rounded, separated from the rest of the body by a weak depression (shallow constriction). Amphidial fovea stirrup-shaped, with a slit-like aperture, located just anterior to demarcation line. Odontostyle long, 1.6 ± 0.05 (1.4-1.7) times longer than odontophore, and the latter with well-developed flanges 11.5 ± 0.8 (10.0-12.9) µm wide. Pharynx dorylaimoid with basal bulb, 114 ± 4.2 (103-122) × 17 ± 0.6 (16-18) µm, occupying 25.7 ± 0.9 (24.0-27.7)% of total length and provided with three gland nuclei. Glandularium 103 ± 3.6 (93-109) µm long or 23.0 ± 0.6 (21.5-24.3)% of total pharynx length; D = 77 ± 0.62 (75.7-78.5)%, AS_1 = 45.8 ± 1.51 (43.4-50.3)%, AS_2 = 47.0 ± 1.54 (44.6-52.3)%, K = 97.4 ± 1.92 (93.2-100.0)% (n = 37). In most specimens studied a 3.3-5.0 µm long "mucro" is present in anterior region of slender part of pharynx at various distances (44 ±

Table 3. Growth patterns in body length, body volume, odontostyle length, replacement odontostyle length and relative increase of the body length, body volume, odontostyle length, replacement odontostyle length between successive stages in a population of *Xiphinema italiae* from Egypt and Italy.

	Growth pattern					Relative increase			
	JI	JII	JIII	F		JII/JI	JIII/JII	F/JIII	Sum
					*L µm				
[1]L%	43.3	56.7	70	100	3000	1.31	1.23	1.43	3.97
[2]L%	47.2	61	78.4	100	3050	1.29	1.29	1.28	3.85
Mean	45.3	58.9	74.2	100	3025	1.30	1.26	1.35	3.91
					*Vol mm^3				
[1]Vol%	23.9	33.7	51.4	100	0.00216	1.41	1.53	1.95	4.88
[2]Vol%	22.8	41.2	64.6	100	0.00177	1.81	1.57	1.55	4.92
Mean	23.4	37.5	58	100	0.00197	1.61	1.55	1.75	4.90
					*O µm				
[1]O%	55.8	67.4	81.1	100	95	1.21	1.20	1.23	3.64
[2]O%	63.4	72	85.6	100	98	1.14	1.19	1.17	3.49
Mean	59.6	69.7	83.4	100	96.5	1.17	1.20	1.20	3.57
					*R µm				
[1]R%	68.8	81.3	100	–	96	1.18	1.23	–	2.41
[2]R%	75.4	87	100	–	99.1	1.15	1.15	–	2.30
Mean	72.1	84.2	100	–	97.6	1.17	1.19	–	2.36

[1]Egypt (Lamberti et al. 1996a); [2]Italy (Martelli et al. 1966).
L = body length; Vol = body volume; O = odontostyle length; R = replacement odontostyle length; F = female.
*L, *Vol, *O = absolute values for female; *R = absolute value for JIII stage.

11.01 [30-72] µm, n = 32) posterior to odontophore base. Reproductive system didelphic-amphidelphic with equally developed genital branches. Ovaries reflexed, oviduct with a slender part and a *pars dilatata oviductus* separated from the uterus by a conspicuous sphincter muscle. Uterus bipartite, consisting of a wide *pars dilatata uteri* continuing into a narrower, muscular tube-like portion and an ovejector. Vulva pre-equatorial, slit like; vagina extending inwards for 56.4 ± 2.8 (50.7-61.5)% of corresponding body diameter. Prerectum indistinct. Rectum 28.5 ± 1.9 (25-33) µm long and extending more the body width at anus (1.1-1.5). Tail 3.6-4.7 times longer than anal body width; ventrally curved, from elongate bluntly conoid to almost subdigitate, commonly with dorsal or dorsal and ventral constrictions towards the terminus; bearing 2-3 caudal pores on each side.

Male. Not found.

Juvenile stages. Morphologically similar to adult females, but having replacement odontostyle, lacking a developed reproductive system and smaller, clearly separated into four developmental stages. (Fig. 4). Tail in all stages elongate-conoid. Individual increase in length of replacement odontostyle in relation to functional odontostyle in individuals in 4 JDS showed that this increase was 20.6% (16.1-24.8) or 9.9 µm (7.5-11.7) in J1, 23.3% (16.7-32.3) or 13.6 µm (10.0-18.3) in J2, 19.1% (11.9-23.9) or 13.8 µm (9.1-17.0) in J3, and 18.2% (13.4-25.2) or 15.4 µm (11.7-20.6) in J4 (Fig. 5).

J1 – glandularium 59 ± 4.9 (51-71) µm long or 23 ± 1.6 (20.9-26.9)% of total pharynx length; D% = 76.9 ± 1.56 (73.1-79.1), AS_1% = 37.7 ± 1.65 (35.1-40.2), AS_2% = 41.2 ± 2.57 (36.9-46.1), K% = 91.7 ± 6.71 (81.9-99.5) (n =11).

J2 – glandularium 69 ± 3.0 (65-74) µm long or 23.2 ± 1.0 (21.7-24.8)% of total pharynx length; D% = 76.8 ± 1.01 (75.2-78.3), AS_1% = 44.3 ± 2.54 (41.3-50.2), AS_2% = 45.6 ± 2.48 (42.7-50.4), K% = 97.2 ± 2.46 (93.0-100.0) (n = 15).

J3 – glandularium 83 ± 2.9 (79-87) µm long or 23.2 ± 0.9 (21.6-24.7)% of total pharynx length; D% = 77.0 ± 1.12 (75.3-79.2), AS_1% = 44.7 ± 2.76 (39.9-48.3), AS_2% = 46.0 ± 2.03 (42.7-48.6), K% = 97.0 ± 2.57 (92.9-100.0) (n = 12)

J4 – glandularium 92 ± 4.5 (82-99) µm long or 22.7 ± 0.7 (21.1-23.8)% of total pharynx length; D% = 77.3 ± 0.74 (76.2-78.9), AS_1% = 45.0 ± 2.09 (41.9-50.9), AS_2% = 46.4 ± 1.92 (44.0-51.2), K% = 96.9 ± 2.30 (92.8-100.0) (n = 22).

Individual data of glandularium length in relation to pharynx length in four JDS (J1-J4) and females are presented in Fig. 6. Fig. 7 illustrates individual values of D, AS_1, AS_2 and

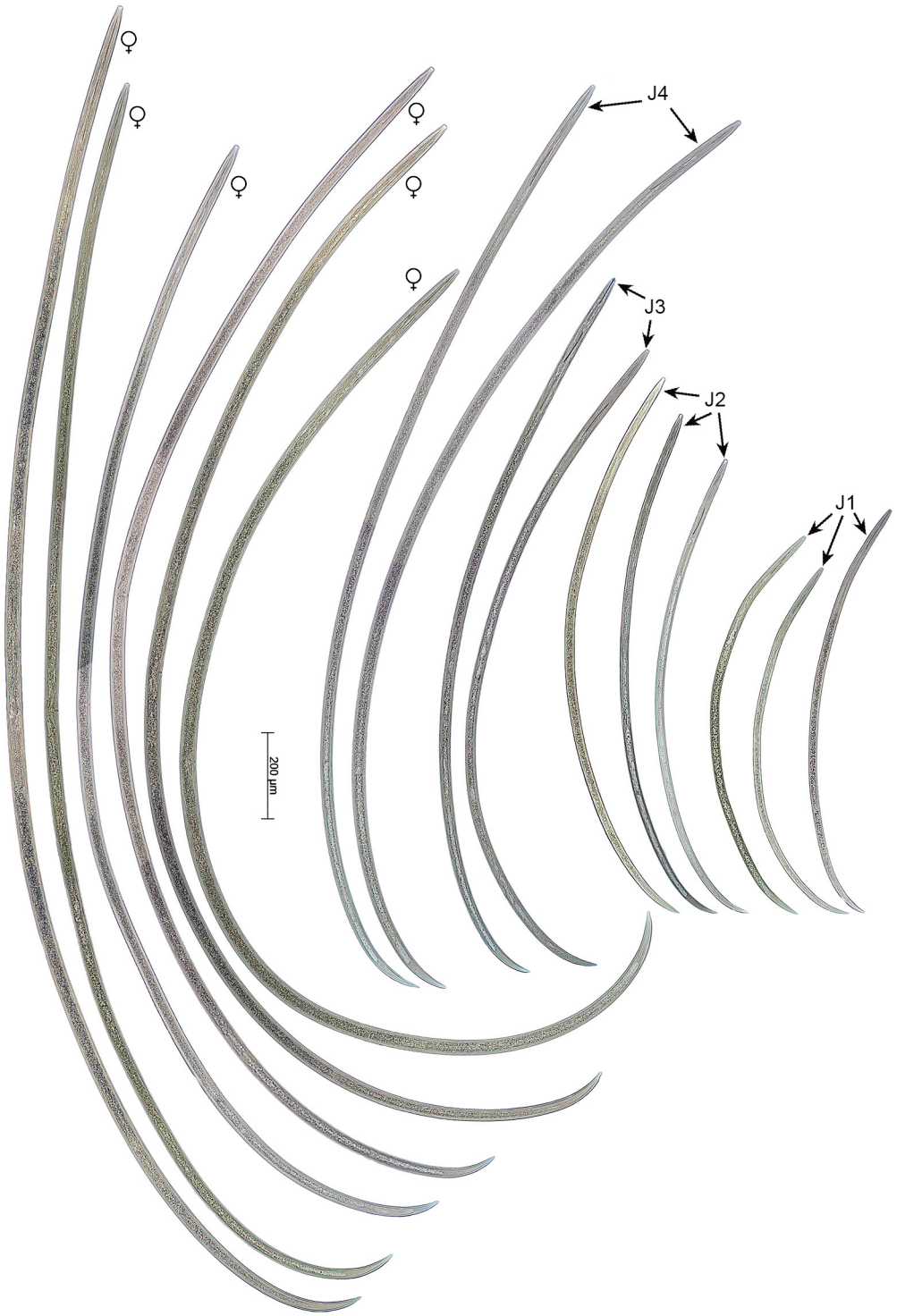


Fig. 1. *Xiphinema italiae* (Ulcinj, Montenegro). Entire body of female (♀), and four juvenile developmental stages (J1-J4).

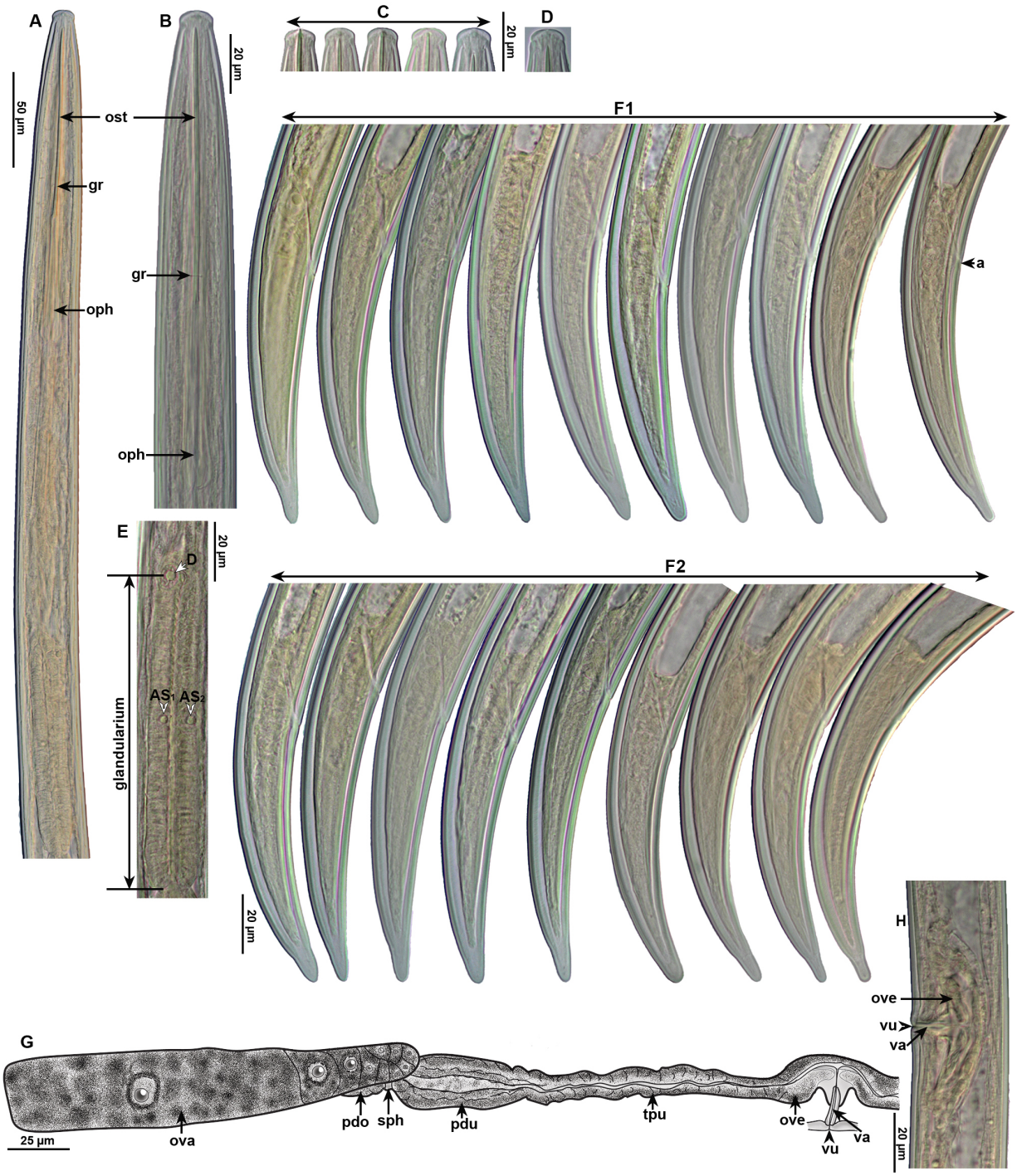


Fig. 2. *Xiphinema italiae*, females (Ulcinj, Montenegro). **A**, Pharyngieal region in lateral optical view; **B**, Anterior region (ost = odontostle, gr = guiding ring, oph = odontophore); **C**, Lip region, lateral view; **D**, Lip region, dorso-ventral view; **E**, Cylindrical basal bulb (cylindrus) of pharynx showing nuclei of dorsal and subventral glands (D = dorsal nucleus; glandularium = distance between dorsal nucleus and posterior margin of *cylindrus* – signified by two horizontal black lines; AS1 = first anterior subventral nucleus; AS2 = second anterior subventral nucleus); **F1-F2**, Variability of tail regions (a = anal opening); **G**, Schematic drawing of the posterior genital branch (ov = ovarium, pdo = *pars dilatata oviductus*, sph = sphincter; pdu = *pars dilatata uteri*, tpu: tube-like portion of uterus, va = vagina, vu = vulva, ove = ovejector); **H**, Vulval region.

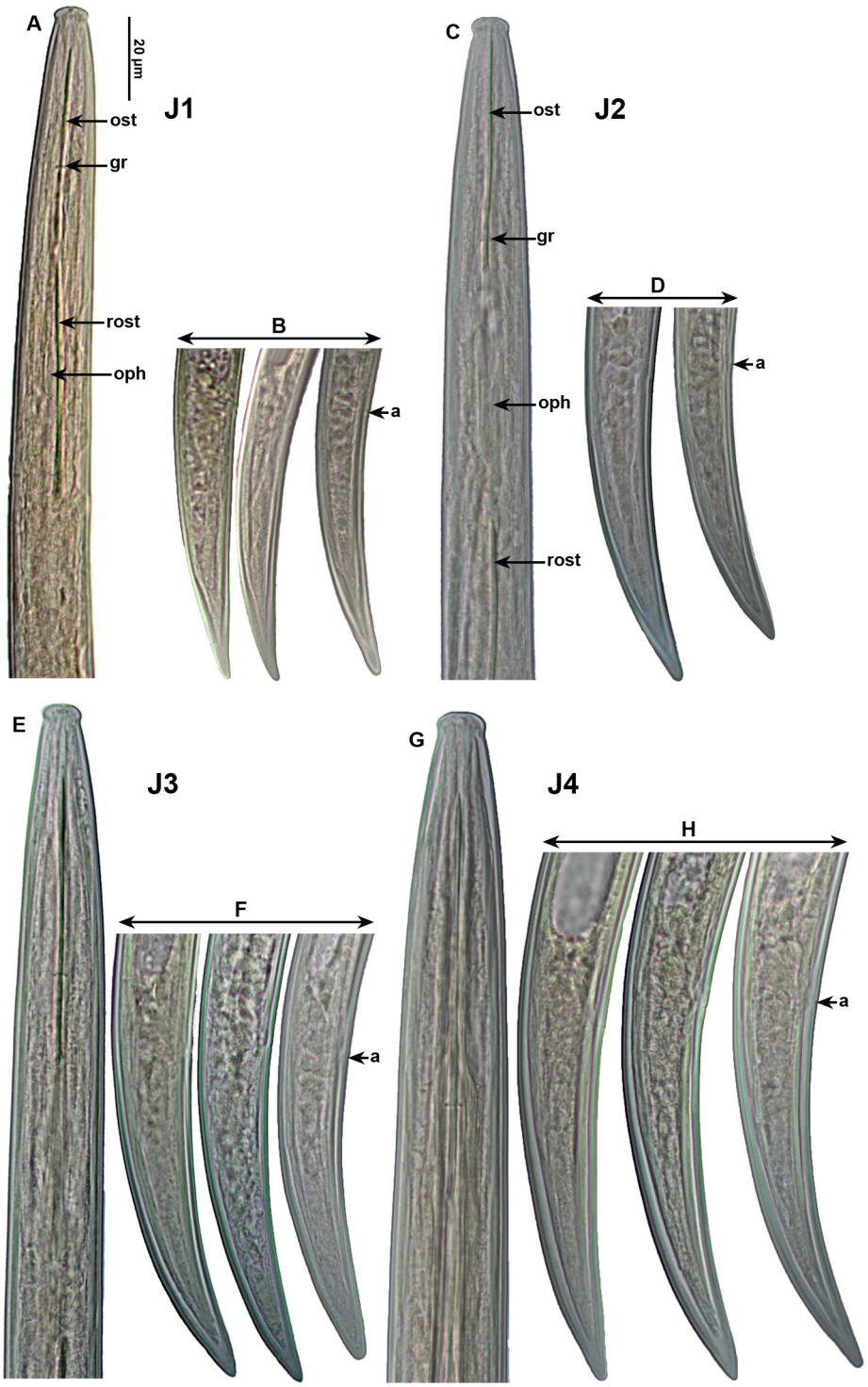


Fig. 3. *Xiphinema italiae*, juvenile developmental stages J1-J4 (Ulcinj, Montenegro). Anterior body region of J1 (**A**), J2 (**C**), J3 (**E**) and J4 (**G**); Tail region of J1 (**B**), J2 (**D**), J3 (**F**) and J4 (**H**). (Abbreviations: ost = odontostyle, gr = guiding ring, rost = replacement odontostyle, oph = odontophore, a = anal opening).

Table 4. Morphometrics of adult and juvenile *Xiphinema italiae* from Montenegro. All measurements in µm (except for L) and in the form: mean ± standard deviation (range).

Locality:	Velika plaža, Ulcinj, Montenegro				
Host:	*Pinus nigra*				
	J1	J2	J3	J4	Female
n	16	20	14	23	37
L (mm)	0.94 ± 0.03 (0.90-1.01)	1.28 ± 0.06 (1.15-1.40)	1.76 ± 0.09 (1.64-1.93)	2.34 ± 0.10 (2.13-2.56)	3.18 ± 0.14 (2.85-3.42)
a	50.4 ± 2.21 (46.1-55.2)	57.8 ± 3.12 (52.4-62.9)	68.6 ± 3.93 (63.8-76.1)	79.4 ± 3.89 (71.9-88.2)	92.9 ± 4.54 (82.9-102.2)
b	3.7 ± 0.17 (3.5-4.1)	4.3 ± 0.22 (4.0-4.7)	4.9 ± 0.24 (4.5-5.4)	5.8 ± 0.35 (5.2-6.4)	7.1 ± 0.35 (6.4-7.9)
c	14.8 ± 0.74 (13.9-16.2)	18.3 ± 1.07 (16.7-20.7)	22.4 ± 0.71 (21.1-23.8)	26.5 ± 1.79 (23.8-30.6)	34.4 ± 1.88 (30.6-38.4)
c’	4.90 ± 0.36 (4.25-5.43)	4.68 ± 0.28 (4.04-5.15)	4.41 ± 0.24 (4.11-4.93)	4.28 ± 0.38 (3.45-5.00)	4.15 ± 0.28 (3.64-4.66))
d	4.8 ± 0.13 (4.6-5.0)	5.8 ± 0.17 (5.5-6.2)	6.7 ± 0.14 (6.4-7.0)	7.1 ± 0.21 (6.8-7.6)	8.0 ± 0.18 (7.6-8.4)
d’	1.8 ± 0.05 (1.7-1.9)	2.0 ± 0.09 (1.7-2.1)	2.1 ± 0.08 (2.0-2.2)	2.2 ± 0.06 (2.1-2.3)	2.3 ± 0.09 (2.1-2.7)
J’	1.8 ± 0.15 (1.4-2.0)	1.6 ± 0.28 (1.1-2.2)	1.5 ± 0.18 (1.2-1.8)	1.6 ± 0.17 (1.2-2.1)	1.6 ± 0.17 (1.3-2.0)
V	–	–	–	–	45.2 ± 0.95 (42.4-47.0)
Odontostyle	48.1 ± 0.89 (46.7-50.0)	58.6 ± 1.43 (56.7-61.7)	72.1 ± 2.30 (69.2-76.7)	84.8 ± 1.85 (81.7-88.3)	100.7 ± 2.01 (95.0-103.4)
Odontophore	36.3 ± 0.76 (35.0-36.7)	43.7 ± 1.03 (41.7-45.0)	50.8 ± 1.54 (48.3-53.3)	56.2 ± 1.17 (54.2-58.3)	64.7 ± 1.42 (60.8-67.5)
Total stylet	84.3 ± 1.30 (81.7-86.7)	102.3 ± 1.78 (98.4-105.0)	123.0 ± 2.70 (117.5-127.5)	141.0 ± 2.14 (136.7-145.0)	165.4 ± 2.53 (158.3-170.0)
Replacement odontostyle	58.0 ± 1.29 (54.2-60.0)	72.3 ± 2.39 (68.8-76.7)	85.9 ± 2.24 (81.7-92.0)	100.2 ± 1.92 (96.7-103.3)	–
Oral aperture to guide ring	39.8 ± 1.04 (38.3-41.7)	53.2 ± 1.82 (50.0-56.7)	67.1 ± 1.43 (64.2-69.6)	77.5 ± 2.10 (73.3-82.5)	95.3 ± 2.28 (90.9-100.0)
Tail	64.0 ± 2.56 (58.0-68.9)	70.3 ± 2.82 (65.7-75.1)	78.6 ± 3.67 (72.4-84.4)	88.3 ± 5.61 (80.3-100.0)	92.6 ± 5.57 (84.4-103.7)
J (hyaline portion of tail)	7.9 ± 0.83 (6.1-9.6)	8.1 ± 1.57 (5.8-11.3)	9.1 ± 1.17 (6.7-10.8)	11.2 ± 0.97 (10.0-13.3)	13.7 ± 1.35 (10.8-16.7)
Body diam. at lip region	8.3 ± 0.07 (8.3-8.6)	9.1 ± 0.19 (8.9-9.4)	10.0 ± 0.11 (9.6-10.0)	10.8 ± 0.16 (10.6-11.3)	11.9 ± 0.15 (11.7-12.2)
Body diam. at guide ring	15.2 ± 0.43 (14.4-15.8)	18.0 ± 0.97 (15.0-19.4)	21.3 ± 0.74 (20.0-22.1)	23.9 ± 0.70 (22.5-25.0)	27.3 ± 1.17 (25.4-32.8)
Body diam. at base of pharynx	18.8 ± 1.13 (16.9-21.3)	21.8 ± 1.29 (20.0-24.2)	25.1 ± 1.20 (23.3-26.7)	28.2 ± 1.11 (26.7-30.0)	32.1 ± 0.96 (30.4-34.4)
Body diam. at mid-body or vulva	18.8 ± 1.28 (16.7-21.7)	22.3 ± 1.54 (20.0-25.4)	25.7 ± 1.58 (23.3-28.3)	29.5 ± 1.86 (26.7-33.3)	34.2 ± 1.34 (31.7-37.5)
Body diam. at anus	13.1 ± 1.02 (11.7-15.3)	15.0 ± 1.00 (13.3-16.9)	17.9 ± 1.00 (16.2-19.6)	20.8 ± 0.98 (18.9-23.3)	22.3 ± 0.60 (21.3-23.6)
Body diam. at beginning of J	4.5 ± 0.31 (3.9-5.0)	5.1 ± 0.30 (4.4-5.6)	6.2 ± 0.77 (5.0-7.1)	7.0 ± 0.49 (6.3-8.3)	8.5± 0.57 (6.9-9.6)

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).

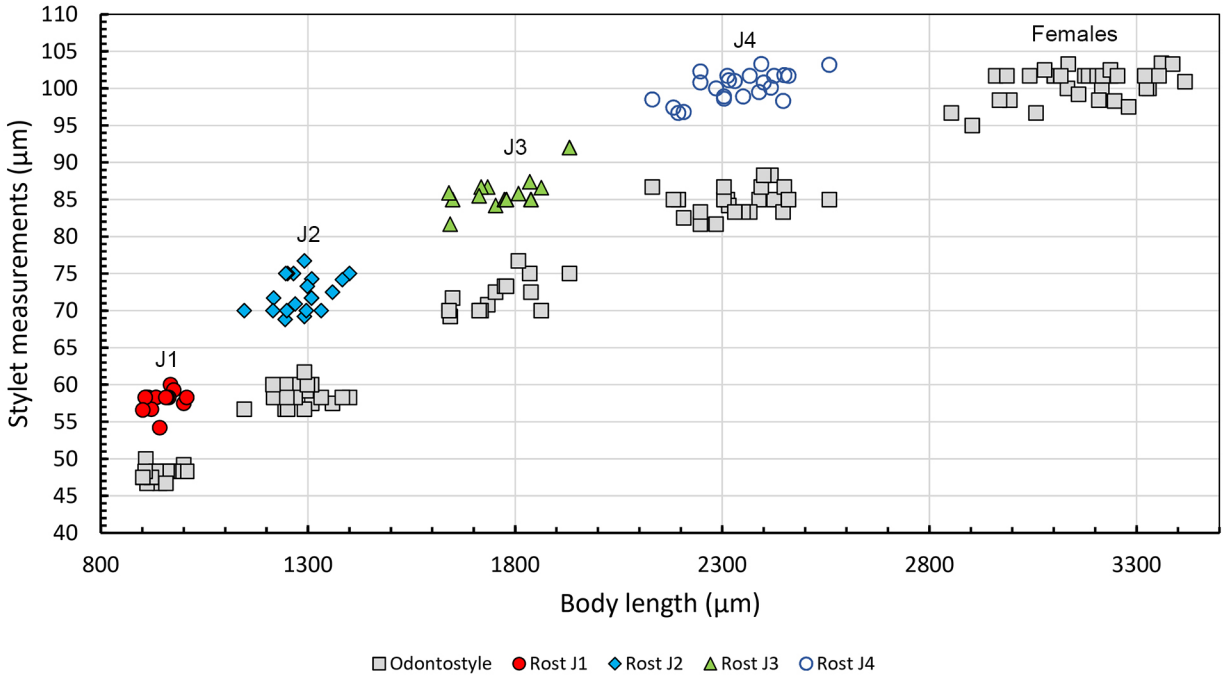


Fig. 4. Scatter diagram separating juveniles and females of *Xiphinema italiae* (Ulcinj, Montenegro). (Rost = replacement odontostyle.)

K in juveniles and adults. In average, position of dorsal nucleus (D) is very similar in all stages. In average, position of AS_1 and AS_2 is very similar in all stages, with exception of J1 stage, where the average values are somewhat lower than in J2, J3, J4 and females. The situation is similar with K values.

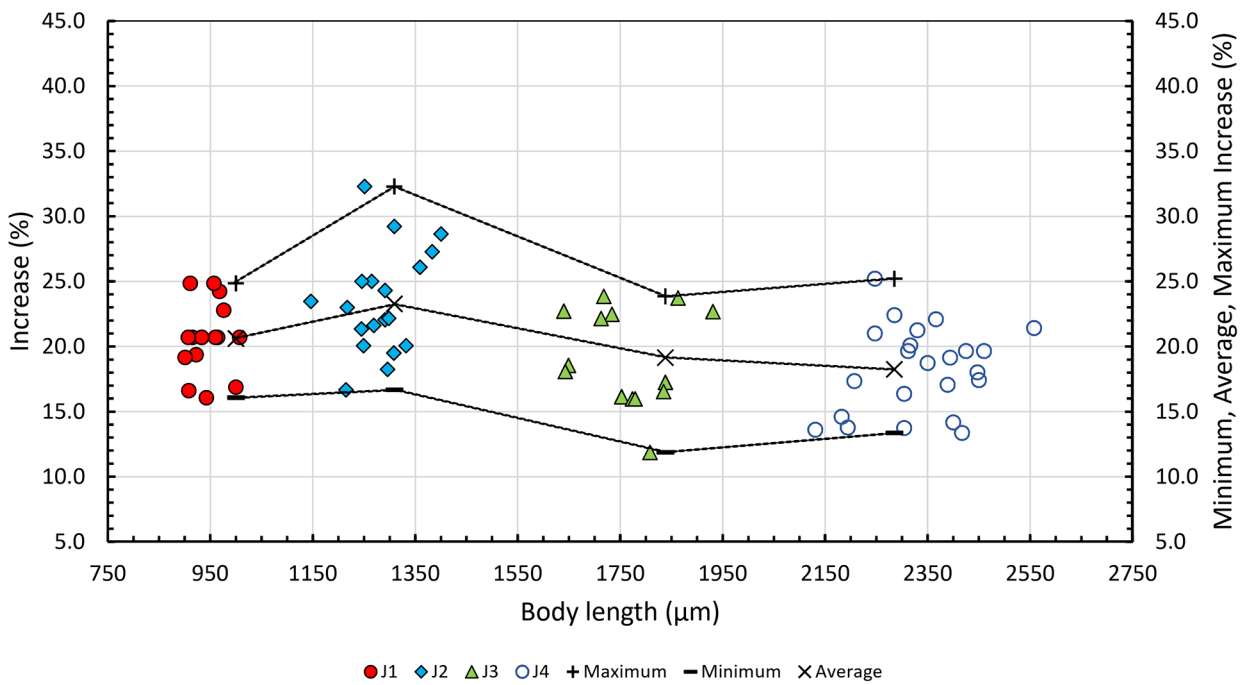


Fig. 5. *Xiphinema italiae* (Ulcinj, Montenegro). Individual increase in length of replacement odontostyle in relation to functional odontostyle in individuals in four juvenile developmental stages (J1-J4); minimum, average and maximum individual increase.

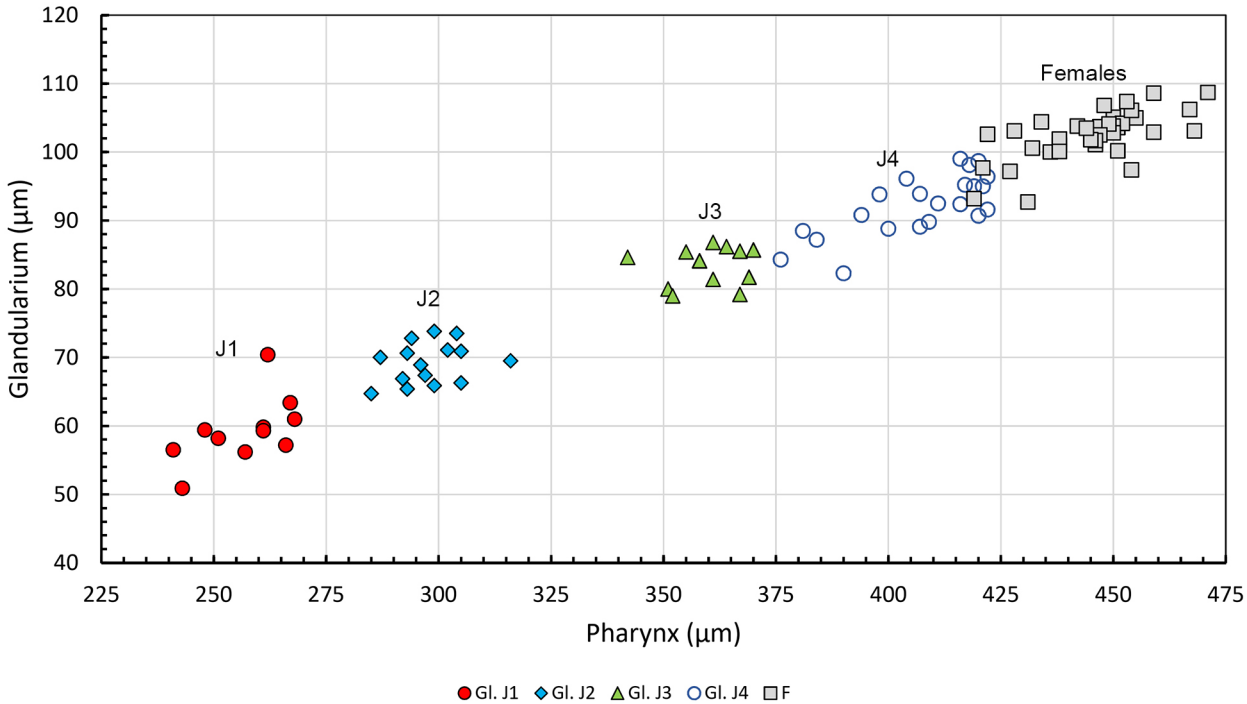


Fig. 6. *Xiphinema italiae* (Ulcinj, Montenegro). Glandularium length in relation to pharynx length in individuals in four juvenile developmental stages (J1-J4) and females. (Gl. = glandularium.)

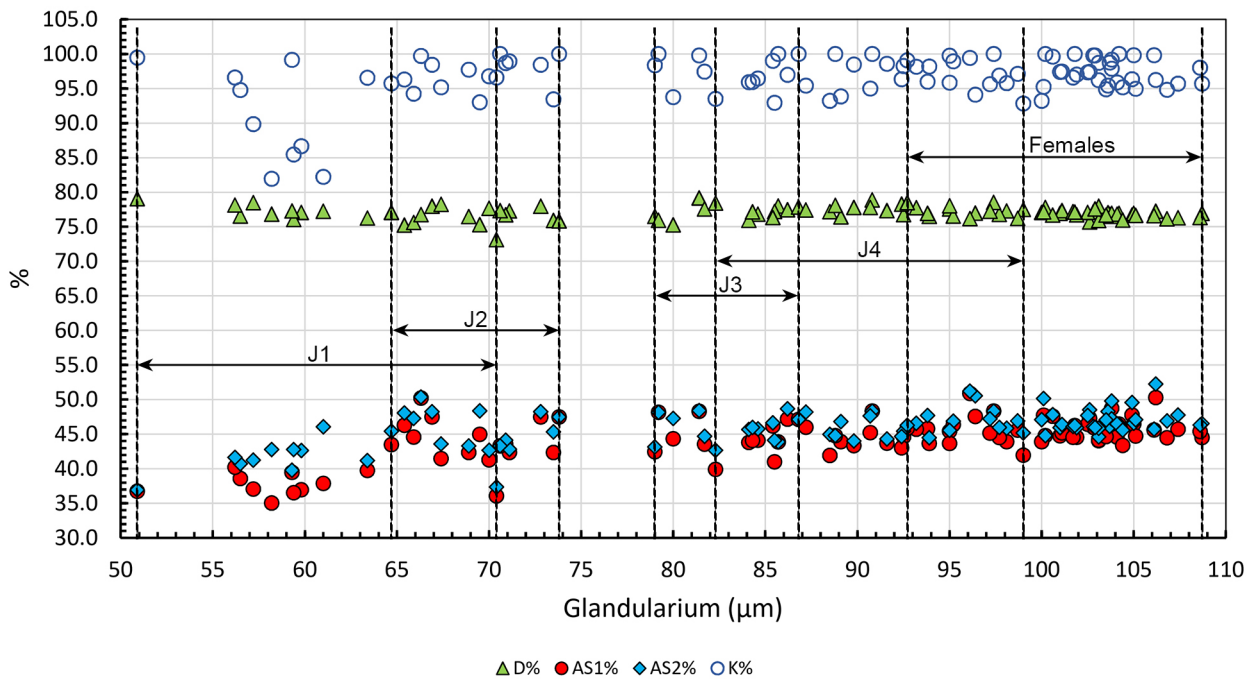


Fig. 7. Individual values of D, AS_1, AS_2, and K in four juvenile developmental stages (J1-J4) and females in a population of *Xiphinema italiae* (Ulcinj, Montenegro). The location of the dorsal nucleus (D) is expressed as a percentage of the distance between the anterior end of the body and the posterior 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 a percentage of the D-AS_2 distance represents K.

On the number of juvenile stages in *Xiphinema italiae*

See Tables 5-7.

Species of the family Longidoridae progress through three or four juvenile developmental stages (JDS) before becoming sexually mature adults (Halbrent and Brown 1992, 1993; Robbins et al. 1995, 1996; Halbrent et al. 1997), and "The number of juvenile stages for a species is a discrete and unambiguous character…" (Halbrendt and Brown 1993). The presence of three or four JDS is a characteristic of the two developmental patterns present in Longidoridae. It is necessary to make a distinction between developmental and growth patterns as two different concepts. 'Developmental pattern' refers to the presence of three (JI, JII, JIII) or four (J1, J2, J3, J4) JDS during the post-embryonic development of a species. 'Growth pattern' refers to a specific pattern of post-embryonic growth, for instance its body length, odontostyle and replacement odontostyle, and body volume.

Table 5. Morphometrics of juvenile stages of the *Xiphinema italiae* populations from Bulgaria, Serbia, Montenegro, and Romania in the form mean (range).

Developmental stages and populations	No. of specimens	Body length (mm)	Odontostyle (µm)	Replacement odontostyle (µm)	Oral aperture to guide ring (µm)	Tail length (µm)
J1						
Bulgaria (Sandanski)[1]	5	0.9 (0.9-1.0)	43.5 (41.7-45)	52 (50.3-54.3)	36.7 (35.4-38.3)	57 (52.6-60)
Serbia (Novi Sad)[2]	14	0.84 (0.79-0.89)	44.2 (41.8-46.2)	52.1 (48.7-53.7)	37.3 (35.6-38.8)	56.2 (50.3-60.7)
Montenegro (Ulcinj)[3]	16	0.94 (0.90-1.01)	48.1 (46.7-50.0)	58.0 (54.2-60.0)	39.8 (38.3-41.7)	64.0 (58.0-68.9)
Romania (Bîrlad)[4]	–[a]	–	–	–	–	–
Sum	35					
J2						
Bulgaria (Sandanski)[1]	1	1.3	52.6	63.5	44.6	65
Serbia (Novi Sad)[2]	13	1.21 (1.12-1.33)	53.5 (51.2-55.0)	68.8 (66.2-73.1)	50.4 (46.9-51.3)	66.5 (61.0-71.0)
Montenegro (Ulcinj)[3]	20	1.28 (1.15-1.40)	58.6 (56.7-61.7)	72.3 (68.8-76.7)	53.2 (50.0-56.7)	70.3 (65.7-75.1)
Romania (Bîrlad)[4]	5	1.27 (1.18-1.40)	51.1 (50-53.5)	65.9 (64-67)	41.9 (37-45)	66.8 (63-70.5)
Sum	39					
J3						
Bulgaria (Sandanski)[1]	7	1.7 (1.6-1.9)	64 (62.3-65.7)	77.5 (75.5-80.6)	55.6 (50.3-58.3)	74.6 (70.5-80)
Serbia (Novi Sad)[2]	14	1.60 (1.45-1.77)	68.6 (66.2-71.2)	81.2 (77.5-83.7)	62.9 (61.3-66.3)	80.5 (73.2-85.0)
Montenegro (Ulcinj)[3]	14	1.76 (1.64-1.93)	72.1 (69.2-76.7)	85.9 (81.7-92.0)	67.1 (64.2-69.6)	78.6 (72.4-84.4)
Romania (Bîrlad)[4]	6	1.70 (1.60-1.92)	67.0 (65-71)	79.9 (78-83)	56.3 (51-64)	82.7 (75-89)
Sum	41					
J4						
Bulgaria (Sandanski)[1]	4	2.2 (2-2.4)	80.3 (77.7-82.3)	94 (92-97)	69.4 (66.9-72.6)	86.3 (77-91.4)
Serbia (Novi Sad)[2]	14	2.12 (1.94-2.39)	81.7 (77.5-86.9)	95.6 (90.0-100.0)	76.8 (73.8-82.5)	91.4 (80.7-98.9)
Montenegro (Ulcinj)[3]	23	2.34 (2.13-2.56)	84.8 (81.7-88.3)	100.2 (96.7-103.3)	77.5 (73.3-82.5)	88.3 (80.3-100.0)
Romania (Bîrlad)[4]	5	2.36 (2.17-2.57)	81.0 (79-84)	93.0 (92-95)	65.6 (63-69)	88.6 (85-92)
Sum	46					

[1]Bulgaria (Lamberti et al. 1997); [2]Serbia (Barsi and Lamberti 2003); [3]Montenegro (original); [4]Romania (Groza et al. 2013)
[a]Stage J1 was not found in the Romanian population.

Table 6. Morphometrics of females of *Xiphinema italiae* populations from Bulgaria, Serbia, Montenegro, and Romania in the form mean (range).

Developmental stages and populations	No. of specimens	Body length (mm)	Odontostyle (µm)	Oral aperture to guide ring (µm)	Tail length (µm)	V (%)
Females						
Bulgaria (Sandanski)[1]	9	2.7 (2.6-2.9)	90 (86.9-95.4)	80.6 (77-85.7)	80.7 (77-94.5)	45 (43-46)
Serbia (Novi Sad)[2]	75	2.88 (2.64-3.18)	95.0 (90.0-101.2)	93.1 (88.7-98.1)	95.3 (78.5-108.5)	44.5 (42.3-47.1)
Montenegro (Ulcinj)[3]	37	3.18 (2.85-3.42)	100.7 (95.0-103.4)	95.3 (90.9-100.0)	92.6 (84.4-103.7)	45.2 (42.4-47.0)
Romania (Bîrlad)[4]	5	3.24 (3.07-3.48)	92.7 (90-94.5)	75.4 (73-78)	93.6 (84-108)	46.2 (44.7-48.1)
Sum	126					

[1]Bulgaria (Lamberti et al. 1997); [2]Serbia (Barsi and Lamberti 2003); [3]Montenegro (original); [4]Romania (Groza et al. 2013)

Xiphinema italiae is reported with either four (Martelli et al. 1966; Lamberti et al. 1997; Barsi and Lamberti 2003; Groza et al. 2013; Barsi in this study) or three JDS (Lamberti et al. 1996a; Avgelis and Tzortzakakis 1997). Halbrendt et al. (1997) listed *X. italiae* with *Xiphinema* species as having only three JDS. They did this on the basis of the Egyptian population of *X. italiae* (Lamberti et al. 1996a) and on the fact that measurements of the functional odontostyles in the

Table 7. Morphometrics of juvenile stages and females of *X. italiae* from Egypt, Italy and Greece.

	JI	JII	JIII	Females
		L (mm)		
Nubaria[1]	[a]1.30	1.7 (1.5-1.8)	2.1 (2.0-2.3)	3.0 (2.8-3.2)
Bari[2]	1.44 (1.35-1.50)	1.86 (1.75-1.95)	*2.39 (2.20-2.60)	3.05 (2.65-3.52)
Island of Samos[3]	–	–	–	2.78
		a		
Nubaria	[a]51	61 (57-62)	70.5 (66-74)	86.5 (82-91)
Bari	66 (64-69)	72 (69-74)	*84 (78-85)	97.0 (83-114)
Island of Samos	–	–	–	–
		b		
Nubaria	[a]5.1	5.9 (5.0-6.9)	6.1 (5.7-6.6)	8.0 (7.5-8.6)
Bari	5.1 (4.9-5.3)	5.5 (5.1-5.9)	*6.2 (5.7-7.1)	8.1 (6.7-9.4)
Island of Samos	–	–	–	–
		c		
Nubaria	[a]23	25 (23-28)	32 (27.5-39)	45 (40-50)
Bari	21 (20-21)	25 (23-26)	*30 (26-33)	44 (38-49)
Island of Samos	–	–	–	–
		c’		
Nubaria	[a]4.1	3.9 (3.4-4.3)	3.4 (3.2-3.7)	3.1 (2.7-3.3)
Bari	4.35 (4.3-4.4)	4.08 (3.9-4.3)	*3.85 (3.7-4.0)	3.3 (2.1-3.6)
Island of Samos	–	–	–	–
		Odontostyle µm		
Nubaria	[a]53	64 (63-65)	77 (75-81)	95 (91-98)
Bari	62.1 (60-64)	70.6 (69-72)	*83.9 (82-87)	98 (92-104)
Island of Samos	**60	**67.5	**80.5	**96.5
		Replacement odontostyle µm		
Nubaria	[a]66	78 (75-80)	96 (94-99)	–
Bari	74.7 (74-76)	86.2 (84-89)	*99.1 (92-104)	–
Island of Samos	**74.2	**84	**97.2	–

[1]Egypt (Lamberti et al. 1996); [2]Italy (Martelli et al. 1966); [3]Greece (Avgelis and Tzortzakakis 1997).
[a]One specimen only.
*Combined data of J3 and J4 from Table III in Martelli et al. (1966).
**Mean values from Table I and II in Avgelis and Tzortzakakis (1997)

third and fourth juvenile stages overlap in a population from Bari, Italy, reported in the species redescription (Table III in Martelli et al. 1966), which suggests that only three JDS may occur in this species. In a population from Bari, the replacement odontostyles in the third juvenile stage are noticeably longer (92-99 µm) than the functional odontostyles in the fourth juvenile stage (82-87 µm) and the replacement odontostyles in the third and fourth juvenile stages form a continuum (92-99 and 99-104 µm), which was overlooked by Cohn (1977) and also by Halbrendt et al. (1997). At the same time, females in this population (Table I in Martelli et al. 1966) have odontostyles that are 92-104 µm long. Speculatively, data presented in Table III of Martelli et al. (1966) make sense only if the morphometrics of the third and fourth juvenile stages are combined (Barsi and Lamberti 2003). So, in that way a new developmental pattern is emerging with three juvenile stages, very similar (Table 3) to the Egyptian population (Lamberti et al. 1996a). Morphometric data of *X. italiae* from the Greek island of Samos, based only on twelve specimens (three females, two first, one second and six third juvenile stages, respectively) presented by Avgelis and Tzortzakakis (1997) also suggest the presence of three JDS (Table 7).

Coomans et al. (2001) in their monograph of the genus *Xiphinema* treated *X. italiae* as having four JDS. Populations from Bulgaria (Lamberti et al. 1997), Serbia (Barsi and Lamberti 2003), Romania (Groza et al. 2013) and Montenegro (this study) have a very similar developmental pattern with four JDS (Table 5 and 6), but populations from Egypt (Lamberti et al. 1996a) and Italy (Martelli et al. 1966) have a different developmental pattern with only three stages (Table 7). The scatter diagram (Fig. 8) with morphometric data of three JDS of the Egyptian and Italian populations and four JDS of the Bulgarian, Serbian, Romanian, and Montenegrin populations, shows that the first, second and third juvenile stages of the Egyptian and Italian populations fit within the second, third and fourth juvenile stages from Bulgaria, Serbia, Romania, and Montenegro. Females show only the usual intraspecific variability present between various populations of the species.

Based on numerous publications (Martelli et al. 1966; Martelli and Lamberti 1967; Prota et al. 1971; Romaşcu 1971; Heyns 1974; Lamberti and D'Errico 1980; Arinç 1982, cited in Mistanoğlu et al. 2015; Lamberti et al. 1983, 1985, 1996a, 1996b, 1997, 1999a, 1999b; Luc and Aubert 1985; Roca et al. 1985, 1987a, 1987b, 1988a, 1988b, 1989, 1990, 1991; Hutsebaut et al. 1987; Barsi 1989; Peneva and Choleva 1992; Lišková et al. 1993; Avgelis and Tzortzakakis 1997; Barsi and Lamberti 2003; Gutiérrez-Gutiérrez et al. 2011; Groza et al. 2013; Mistanoğlu et al. 2015; Feketéné Palkovics et al. 2016;

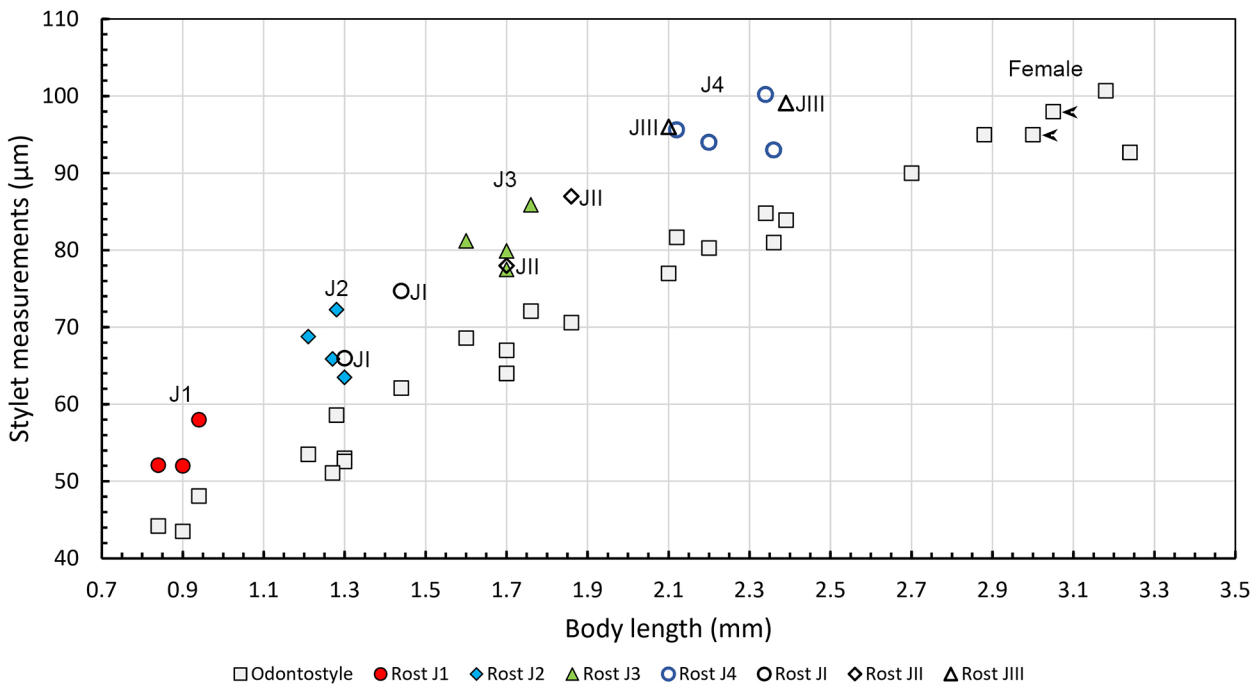


Fig. 8. Scatter diagram separating juveniles and females of six populations of *Xiphinema italiae*. (Rost = replacement odontostyle.) Rost J1-Rost J4 = mean values from four populations with four juvenile stages ([1]Montenegro, [2]Serbia, [3]Bulgaria, [4]Romania); Rost JI-Rost JIII = mean values from two populations with three juvenile stages ([5]Egypt, [6]Italy). Note: stage J1 was not found in the Romanian population. (Arrowhead indicates females from Egypt and Italy.) (Sources: [1]Original, [2]Barsi and Lamberti 2003, [3]Lamberti et al. 1997, [4]Groza et al. 2013, [5]Lamberti et al. 1996a, [6]Martelli et al. 1966.)

Guesmi-Mzoughi et al. 2017; Öztürk et al. 2023) morphometrics of *X. italiae* populations show a wide range of intraspecific variability (min-max) as follows: L = 2.29-3.8 mm, a = 59.9-115.2, b = 6.1-14.5, c = 26-64.9, c' = 1.9-7.4, V = 40-51, odontostyle = 70.2-118.8 µm, odontophore = 40-91.8 µm, oral aperture to basal guide ring = 63-126 µm, tail = 49-108.5 µm, J (hyaline portion of tail) = 7.5-25.5 µm, body diam. at lip region = 8-14 µm, body diam. at guide ring = 18.8-32.8 µm, body diam. at base of oesophagus = 23.5-38 µm, body diam. at vulva = 25.3-42.5 µm, body diam. at anus = 16.5-27 µm, body diam. at beginning of J = 5-12 µm.

Figures 9 and 10 illustrate intra- and inter-populational variability of body, odontostyle, and tail lengths, and V value. In addition to variability in tail length and associated c and c' ratios, tail shape also shows considerable variability, as illustrated in several publications (Martelli et al. 1966; Martelli and Lamberti 1967; Cohn and Sher 1972; Heyns 1974; Hutsebaut et al. 1987; Peneva and Choleva 1992; Lišková et al. 1993; Lamberti et al. 1996a, 1997; Barsi and Lamberti 2003; Gutiérrez-Gutiérrez et al. 2011; Groza et al. 2013; Guesmi-Mzoughi et al. 2017).

Robbins et al. (1996) in their compendium of juvenile stages of *Xiphinema* species wrote: "Determination of the number of JDS (three versus four) is important for understanding the taxonomy and biology of longidorids and has practical significance when distinguishing similar species, especially if one species is a virus vector."

Cohn et al. (1970) reported that *X. italiae* is experimentally a vector of grapevine fanleaf virus (GFLV) in Israel. However, this association was never confirmed (Lamberti and Roca 1987; Catalano et al. 1992; Taylor and Brown 1997). GFLV and arabis mosaic virus (ArMV) are nepoviruses responsible for grapevine degeneration (Belval et al. 2019). The natural spread of GFLV and ArMV is specifically accomplished from grapevine to grapevine by two soil-borne ectoparasitic nematodes species, *Xiphinema index* and *X. diversicaudatum*, respectively (Lamberti and Roca 1987; Taylor and Brown 1997; Demangeat 2007; Villate et al. 2008; Demangeat et al. 2010; Van Ghelder et al. 2015a; Belval et al. 2019). By contrast, *X. vuittenezi*, and *X. italiae*, previously considered to be vectors of GFLV in grapevines (EPPO 2009), have never been confirmed and are now considered to be non-vectors (Demangeat 2007; Van Ghelder et al. 2015a). The three *Xiphinema* species, *X. index*, *X. diversicaudatum* and *X. vuittenezi*, are closely related morphologically. The deep location and often low field densities in the soil (Villate et al. 2008) of these three species plus *X. italiae* in European vineyards, make them difficult to identify by classical diagnostics (Van Ghelder et al. 2015a). Only juvenile stages or a single or few adult individuals may be present, but these are sufficient to transmit the virus to a grapevine plant (Van Ghelder et al. 2015a, b). Therefore, nematode detection and monitoring in contaminated fields require the development of an easy, specific and sensitive technique. Multiplex PCR (Wang et al. 2003) and real-time PCR methods (Van Ghelder et al. 2015a, b) enable the specific detection of single individuals of each of the *X. index*, *X. diversicaudatum*, *X. italiae* and *X. vuittenezi* species, independent of the nematode population.

Together, molecular, morphological and morphometric data for *X. italiae* were published in several papers (Gutiérrez-Gutiérrez et al. 2011; Groza et al. 2013; Feketéné Palkovics et al. 2016; Guesmi-Mzoughi et al. 2017). Only Groza et al. (2013) and Feketéné Palkovics et al. (2016) also contained data on juvenile stages, but with limited value due to a total of only three JDS individuals in the second paper/poster (1 J2, 1 J3 and 1 J4, respectively). Several other papers contain only molecular data (Knoetze et al. 2000; Wang et al. 2003; Kumari and Lišková 2009; Van Ghelder et al. 2015b).

Although Martelli et al. (1966) published data on the number of juvenile stages in *X. italiae* from the Bari population, Robbins et al. (1996) listed this species in their Table 1 as a species for which juvenile stages were not described. Lamberti et al. (1996a) in the same year published a paper where they determined that *X. italiae* from Egypt has three juvenile stages, and the following year in the Bulgarian population of this species they determined the existence of four juvenile stages (Lamberti et al. 1997). Robbins et al. (1996) have described in detail a method for identifying juvenile stages, but also indicate the possible consequences if it is not done correctly. It seems that this is exactly the situation with the data in Martelli et al. (1966).

If we accept that "The number of juvenile stages for a species is a discrete and unambiguous character…" (Halbrendt and Brown 1993), then the presence of two developmental patterns with either three or four JDS in *X. italiae* leads us to the question: do these populations represent the same species or are we dealing with at least two morphologically and morphometrically similar, but genetically different species?

The genus *Xiphinema* has great morphological diversity and is therefore divided into two species groups (Loof and Luc 1990; Lamberti et al. 2000; Coomans et al. 2001; He et al. 2005): (a) the *X. americanum*-group, which contains a complex of over 60 species, and (b) the *X.* non-*americanum*-group, which contains a complex of more than 200 species. Traditional identification of these species by morphological and morphometric studies is very difficult due to their high intraspecific morphological variability, which can lead to a significant overlap of many characteristics and ambiguous interpretations, and cannot distinguish most species that are morphologically similar but genetically different (He

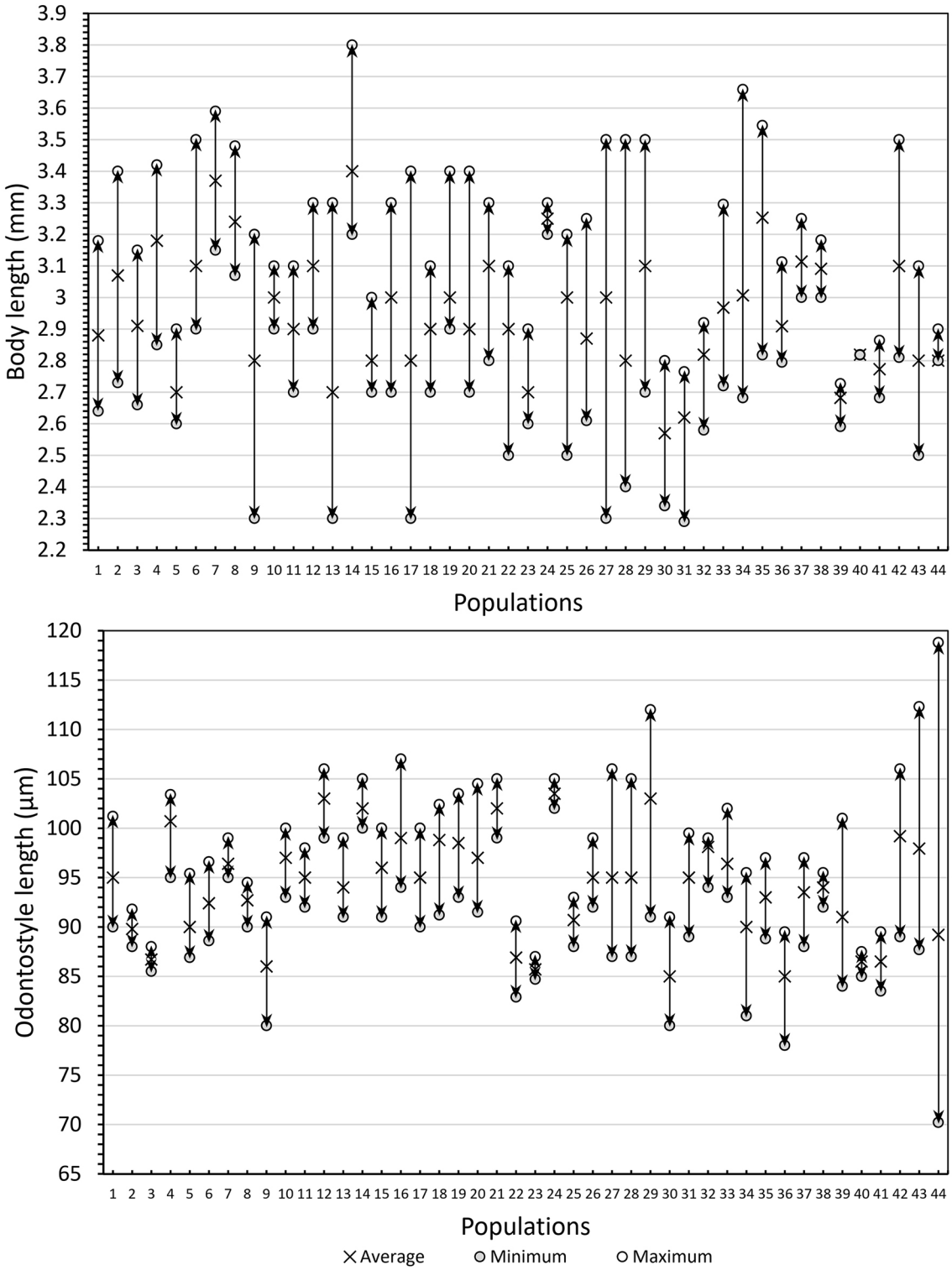


Fig. 9. Intra- and inter-populational variability of body and odontostyle lengths in females in populations of *Xiphinema italiae*.

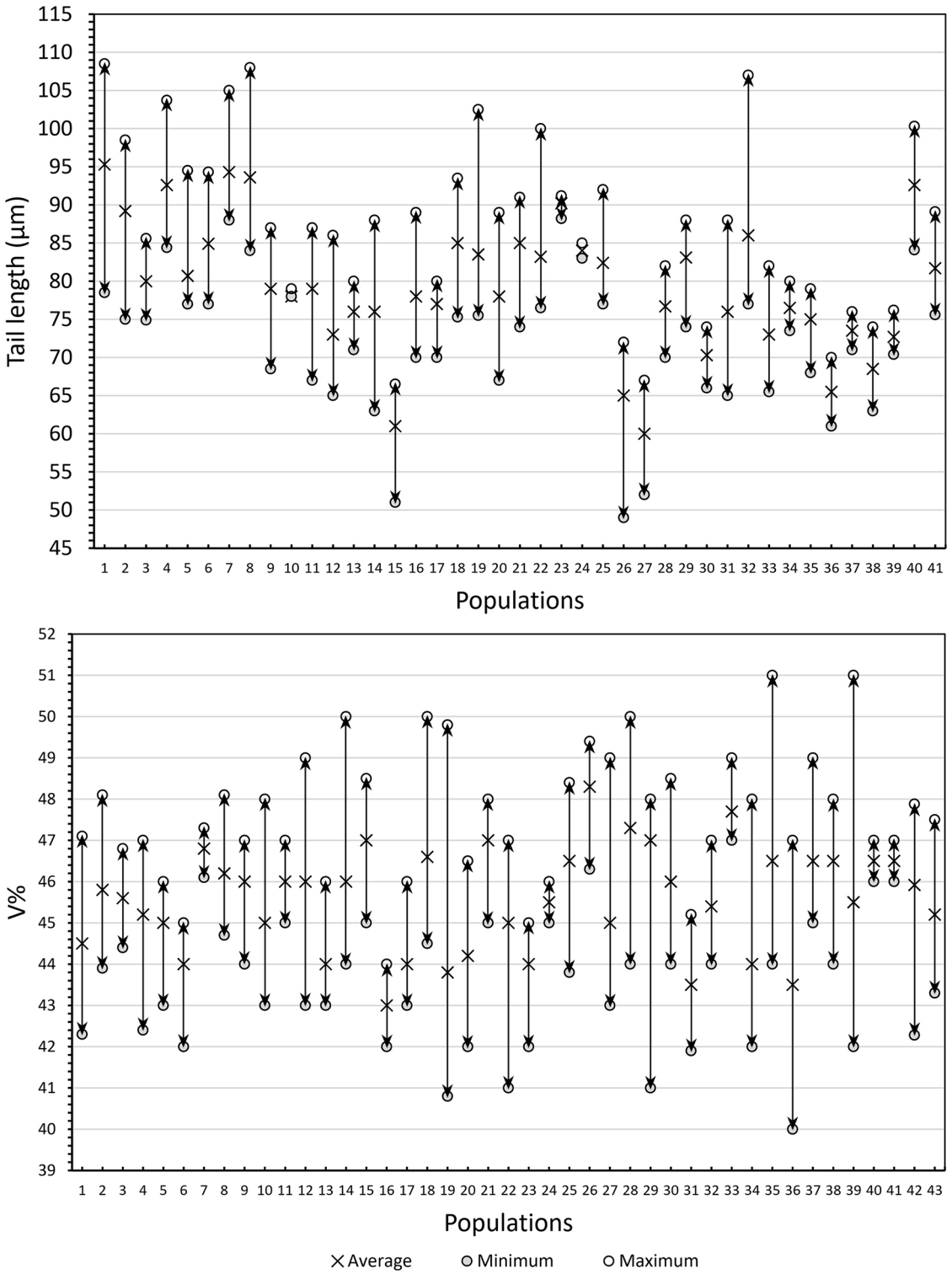


Fig. 10. Intra- and inter-populational variability of tail length and V value in populations of *Xiphinema italiae*.

et al. 2005; Archidona-Yuste et al. 2016a; Nisa et al. 2022). Conserved and basic morphology in Nematoda implies that many species are cryptic to human perception due to the lack of conspicuous differences in both external appearance and internal structure (Palomares-Rius et al. 2014). Correct identification is essential in understanding nematode diversity and designing effective control and management approaches (Wang et al. 2003; Van Ghelder et al. 2015b; Nisa et al. 2022).

The development of molecular technology based on ribosomal DNA provides attractive opportunities for improved detection, identification and systematics of plant nematodes (Hillis and Dixon 1991). The molecular barcode concept extends the phylogenetic groundwork established from classical taxonomic descriptions based on traditional morphological, morphometric and biological information of approximately 25,000 nematode species (Powers 2004). It is clear that cryptic species must be abundant in Nematoda and molecular techniques may be the only practical approach to their recognition (Powers 2004). It is imperative that the specimens used for sequence generation are correctly identified, preferably by a trained taxonomist using morphological and morphometrical characters, before the sequence data is submitted to public databases (Oliveira et al. 2011). In the meantime, classic taxonomy based on morphology has been expanded with molecular, biochemical and other advanced methods and techniques with the aim of improving the identification of nematodes (Abebe et al. 2011; Bogale et al. 2020; Bhat et al. 2022; Nisa et al. 2022; Shao et al. 2023).

Intensive study of morphological and morphometrical characters of *Xiphinema* spp. over more than a decade, coupled with molecular diagnostics/techniques (integrative approach), have revealed several well-established cryptic species or complexes of closely similar species within the genus *Xiphinema* (Gozel et al. 2006; Oliveira et al. 2006, 2011; Barsi and De Luca 2008; Gutiérrez-Gutiérrez et al. 2010, 2011, 2012; Pedram et al. 2012; Palomares-Rius et al. 2014; Archidona-Yuste et al. 2016a, b, c, 2020; Barsi et al. 2017; Jahanshahi Afshar et al. 2019; Lazarova et al. 2019; Cai et al. 2020; Poureskandarian et al. 2023).

In light of the above and considering the controversy concerning *X. italiae*, which is presented in the present study, it can be suspected that *X. italiae* is not a single species. It seems, considering only the number of JDS, that it is possible that we are dealing with at least two similar species. Although there are many published data on *X. italiae*, it seems that this issue will not be resolved soon without the use of an integrative approach, based on a combination of molecular methods, comparative morphology and morphometrics, as well as developmental biology of multiple populations collected in new locations and habitats during a wider geographic survey of different hosts, along with more intensive collection from sites that were previously inadequately sampled. Only by linking and combining these data is it possible to get a clear picture of the taxonomic status of the studied populations.

Ibrahim et al. (2010, 2023) in their papers on the current status of phytoparasitic nematodes and their host plants in Egypt, cite the finding of *X. italiae* in the rhizosphere of grapevines in Nubaria (Lamberti et al. 1996a) as the only finding of the species in that country.

Results of data analysis on the number of juvenile developmental stages in *X. italiae*

The data sets in Tables 2 and 3 were analysed at the intraspecific (four populations with four JDS, and two populations with three JDS) level. The mean values of the selected characters of four populations with four JDS were used for comparison with the mean values of the selected characters of two populations with three JDS. However, it must be strongly emphasised that average values give only an overall picture of the relationship present between the six populations of *X. italiae* on the one hand, and between the populations with three and four JDS on the other hand (Fig. 11).

Designation of the populations. Four populations of *X. italiae* from **M**ontenegro (original), **S**erbia (Barsi and Lamberti 2003), **B**ulgaria (Lamberti et al. 1997), and **R**omania (Groza et al. 2013) were designated as *X. italiae* (**MSBR**), and have four JDS (J1-J4). Two populations of *X. italiae* from **E**gypt (Lamberti et al. 1996a) and **I**taly (Martelli et al. 1966) were designated as *X. italiae* (**EI**), and have three JDS (JI-JIII).

Body length (Tables 2 and 3, Fig. 11)

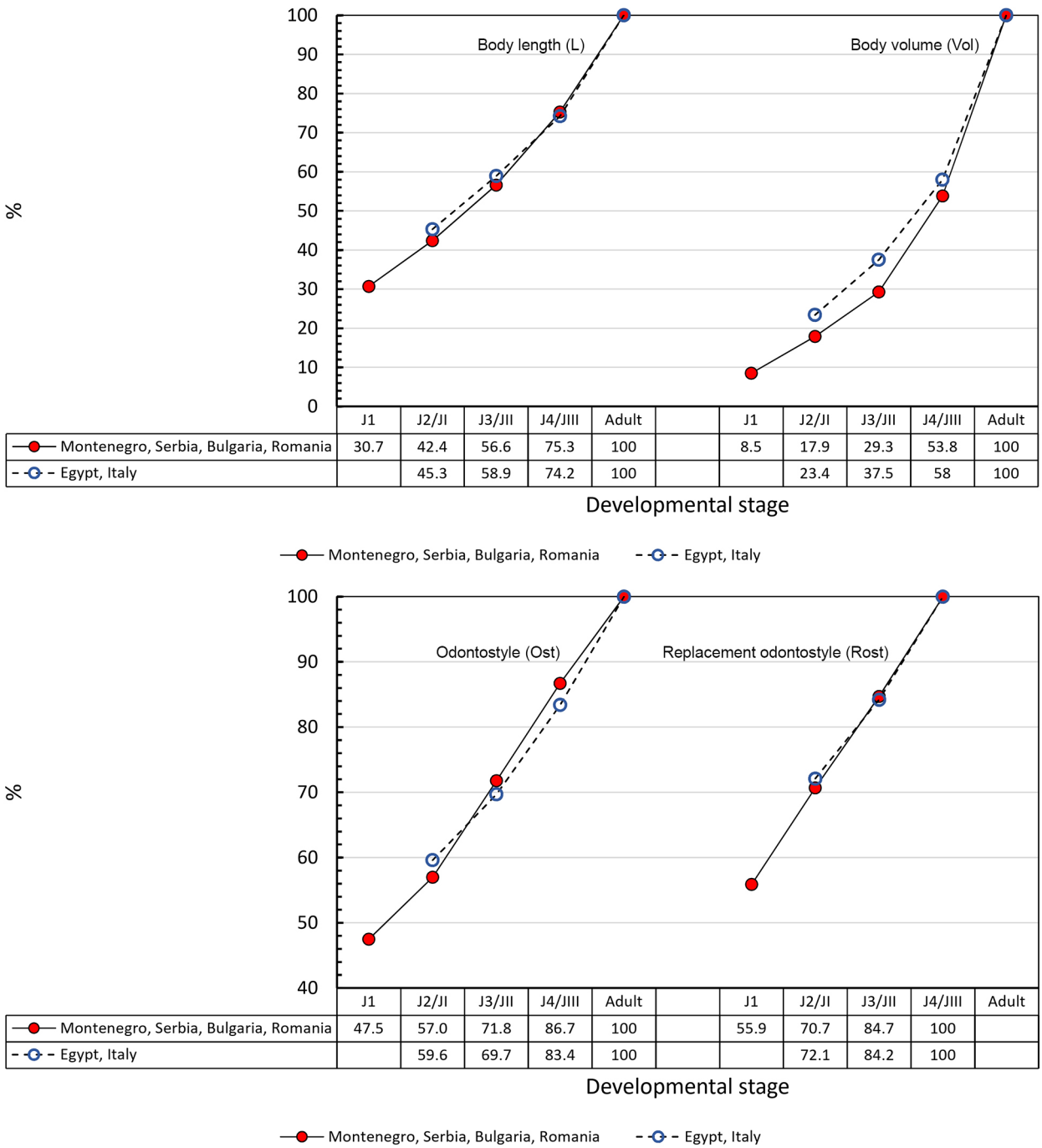
The mean body length of the J1 stage was smaller in comparison to the adult female in *X. italiae* (MSB) than the mean body length of JI stage in comparison to the adult female in *X. italiae* (EI). In *X. italiae* (EI), the mean body length of the JI stage was more similar to that of the J2 stage in *X. italiae* (MSBR) than to the J1 stage of the same species. Generally, the mean body lengths of the first, second and the pre-adult stages in *X. italiae* (EI) (JI = 45.3%, JII = 58.9%, JIII = 74.2%) show similarity with those of the second, third and pre-adult stages (J2 = 42.4%, J3 = 56.6%, J4 = 75.3%) in *X. italiae* (MSBR). In *X. italiae* (MSBR) and *X. italiae* (EI), there was no unique growth pattern of body length from stage to stage applicable for all populations, just a similar trend with more or less similar values.

Using percent body lengths for each stage, the relative increase from stage to stage was calculated for each population. The sum of the relative increases between successive stages was higher in *X. italiae* (MSBR) than in *X. italiae* (EI).

This also supports the fact that in populations of *X. italiae* (EI) (with three JDS) specimens of the JI stage are generally longer in comparison to specimens of the J1 stage in populations of *X. italiae* (MSBR) (with four JDS) (Tables 2 and 3).

Body volume (Tables 2 and 3, Fig. 11)

The mean body volume of the J1 stage was smaller in comparison to the female stage in *X. italiae* (MSB) than the



	J1	J2/JI	J3/JII	J4/JIII	Adult		J1	J2/JI	J3/JII	J4/JIII	Adult
Montenegro, Serbia, Bulgaria, Romania	30.7	42.4	56.6	75.3	100		8.5	17.9	29.3	53.8	100
Egypt, Italy		45.3	58.9	74.2	100			23.4	37.5	58	100

	J1	J2/JI	J3/JII	J4/JIII	Adult		J1	J2/JI	J3/JII	J4/JIII	Adult
Montenegro, Serbia, Bulgaria, Romania	47.5	57.0	71.8	86.7	100		55.9	70.7	84.7	100	
Egypt, Italy		59.6	69.7	83.4	100			72.1	84.2	100	

Fig. 11. Growth patterns of body length (L), body volume (Vol), odontostyle (Ost) and replacement odontostyle (Rost) length in six populations of *Xiphinema italiae*. Mean values from Table 2 of four populations with four developmental juvenile stages ([1]Montenegro, [2]Serbia, [3]Bulgaria, [4]Romania), and from Table 3 of two populations with three juvenile developmental stages ([5]Egypt, [6]Italy). (Sources: [1]Original, [2]Barsi and Lamberti 2003, [3]Lamberti et al. 1997, [4]Groza et al. 2013, [5]Lamberti et al. 1996a, [6]Martelli et al. 1966.)

mean body volume of the JI stage in comparison to the female stage in *X. italiae* (EI). This is in accordance with data on differences in body length between J1 and JI of *X. italiae* (MSB) and *X. italiae* (EI), respectively. Intraspecific (interpopulational) variability of body volume in *X. italiae* (MSBR) and *X. italiae* (EI) was evident and there was no a unique value set for all juvenile stages for all populations studied – just a similar pattern.

Using percent body volumes for each stage, the relative increase from stage to stage was calculated for each species/population. The sum of the relative increases between successive stages was higher in *X. italiae* (MSBR) than in *X. italiae* (EI). This also supports the fact that in *X. italiae* (EI) specimens of the JI stage generally had greater body volumes in comparison with specimens of the same stage in *X. italiae* (MSB).

Odontostyle and replacement odontostyle lengths (Tables 2 and 3, Fig. 11)

The mean odontostyle length in the female stage of *X. italiae* (MSBR) populations (94.6 μm) is similar to that of *X. italiae* (EI) populations (96.5 μm). The first juvenile stage had a longer odontostyle (59.6%) in comparison to the female in *X. italiae* (EI) populations than in *X. italiae* (MSB) populations (47.5%).

Using percent odontostyle lengths for each stage, the relative increase from stage to stage was calculated for each species/population. The sum of the relative increases between successive stages was higher in *X. italiae* (MSBR) populations than in *X. italiae* (EI) populations. Comparison of the relative increase of odontostyle length from stage to stage in *X. italiae* (MSBR) and *X. italiae* (EI) populations, revealed some similarity, but also a clear difference between them. The initially longer odontostyle in the JI stage (59.6%) compared to females in *X. italiae* (EI) enables development to full length (100%) only through three developmental stages. In *X. italiae* (MSBR), the J1 stage initially has a shorter odontostyle (47.5%) compared to females (100%), so it takes four stages to reach full size (100%).

Comparison of the data on replacement odontostyle growth pattern in *X. italiae* (MSBR) and *X. italiae* (EI) with data of the growth pattern of the odontostyle showed that the length of the replacement odontostyle of the first juvenile stage was similar to that of the functional odontostyle of the second stage and substantially larger than the length of the first stage functional odontostyle. This situation also occurred between all other successive developmental stages in both groups of populations. Using percent replacement odontostyle lengths for each stage, the relative increase from stage to stage was calculated for each population. The sum of the relative increases between successive stages was higher in *X. italiae* (MSBR) than in *X. italiae* (EI).

CONCLUSION

Based on the available literature data, it was determined that the morphometrics of *X. italiae* populations show a wide range of intraspecific variability. The length and shape of the tail of this species also shows considerable variability. Relatively scarce data on the developmental biology of *X. italiae* show the existence of populations with three or four juvenile developmental stages. As part of future research, it is necessary to determine the exact number of juvenile stages in the increasing number of *X. italiae* populations of different origins. Since the number of juvenile stages for a species is a discrete and unambiguous character, the question arises in the case of *X. italiae*: does it represent one species or are there at least two morphologically similar, yet genetically different species? This paper cannot answer that question, but the intention of the present study was to point out the existence of the problem. It is necessary to use an integrative approach in the study of *X. italiae* as a species, based on a combination of molecular methods, comparative morphology and morphometrics, as well as the developmental biology of multiple populations collected in different locations and habitats during a wider geographic area of surveys of different hosts. Only by linking and combining these data will it be possible to obtain a clear picture of the taxonomic status of the studied populations.

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