XIPHINEMA PARASIMILE SP. N. FROM SERBIA AND X. SIMILE, FIRST RECORD FROM BOSNIA AND HERZEGOVINA (NEMATODA, DORYLAIMIDA)

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Summary. *Xiphinema parasimile*, a new putative *X. americanum* group species is described from Serbia. *X. parasimile* sp. n. is a monosexual didelphic species with four juvenile stages, and it is characterized by body length of *ca.* 2 mm, lip region set off from body profile, odontostyle length of *ca.* 70 µm, *ca.* 55% V and conical, dorsally convex, ventrally almost straight or slightly concave, tail with pointed terminus. Measurements of populations of *X. simile* Lamberti, Choleva *et* Agostinelli, 1983 from Bosnia and Herzegovina and Serbia are also provided.

Soil samples collected from the rhizosphere of forest trees during 1989-2002 in two localities in Serbia contained many specimens of *Xiphinema* sp. very similar to *Xiphinema simile* Lamberti, Choleva *et* Agostinelli, 1983, but in our opinion representing an undescribed species because of a few but major differences. Such populations are here described and named *Xiphinema parasimile* sp. n.

Populations of *X. simile* were detected in one sample collected at Palié, which constitutes a new record for Serbia, and in one sample collected in June 2003 at Bijeljani, which is the first record of the species for Bosnia and Herzegovina.

Nematodes were extracted by Cobb's wet sieving technique. Specimens were killed by hot FP 4-1 and processed and mounted on permanent slides in dehydrated glycerin. Measurements were made with an eyepiece graticule, except for body length, which was measured with the aid of a drawing tube and map measurer.

XIPHINEMA PARASIMILE **sp. n.** (Tables I and II; Figs. 1-6)

Female *habitus* a single spiral or closed C when killed; body slender, tapering gradually toward the extremities. Labial region 3.4-4.6 µm high, set off from the body profile, from flattened to slightly arcuate frontally, rounded laterally, separated from the rest of the body by a constriction. Amphidial aperture obscure. Odontostyle, odontophore and guiding apparatus typical for the genus. The oesophagus basal bulb 63 ± 2.55 (58-69) µm long and 14 ± 0.87 (12-16) µm wide, occupying about 1/5 to 1/4 of the total oesophagus length. A small "mucro" is sometimes present in the tubular part of the oesophagus. Reproductive system amphidelphic, both branches apparently equally developed. Ovaries with symbionts, oviducts relatively long. Uteri short, anterior branch 26-34 μ m (n = 18), posterior branch 24-37 μ m (n = 16) long. Vulva postequatorial, a transverse slitlike; vagina with short distal part and well-developed proximal part surrounded by sphincter, typical in shape; 13-17 μ m long, or 45-59% of the corresponding body diameter. Prerectum often indistinct, 166-308 μ m long, or 10-20 times the anal body diameter. Rectum 20±2.75 (15-24) μ m long, or 0.9-1.5 times the anal body diameter. Tail conical, dorsally convex, ventrally almost straight or slightly concave with pointed terminus. Rarely dorsally with a tendency towards narrowly subdigitate. Two pairs of caudal pores.

Male not found.

Juveniles clearly separated into four stages (Table III; Figg. 4-5). They are generally similar to adults, except for smaller size and body posture less ventrally curved than adults. Tail conical in all juvenile stages.

Type habitat and locality. Rhizosphere of *Quercus* sp. in the locality Trešnja at Ralja, Serbia (UTM grid DQ63); it also occurred in Matijevica woods at Obrež, Obedska bara (UTM grid DQ15), always in the rhizosphere of *Carpinus betulus* L.

Type material. Holotype, 24 female and 23 juvenile paratypes in the collection of the Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., 70126 Bari, Italy; 47 female and 71 juvenile paratypes, Department of Biology and Ecology, Novi Sad, Serbia; 4 female paratypes, Plant Nematology Laboratory Collection, United States Department of Agriculture, Beltsville, Maryland, U.S.A.

Diagnosis. Xiphinema parasimile sp. n. is a monosexual didelphic species with four juvenile stages. It is characterized by body length of *ca.* 2 mm, lip region set off from body profile, odontostyle length of *ca.* 70 µm, *ca.* 55% V and conical, dorsally convex, ventrally almost straight or slightly concave tail with pointed terminus.

According to the polytomous key of the X. americanum group species (Lamberti et al., 2000) the code for X. parasimile sp. n. should be: A2, B2/3, C1, D3/4, E2/3, F2, G1/2, H2/1, I2/3, J1.

Relationships. Xiphinema parasimile sp. n. resembles X. pachtaicum (Tulaganov, 1938) Kirjanova, 1951, X. opisthohysterum Siddiqi, 1961, X. californicum Lamberti et Bleve-Zacheo, 1979, X. intermedium Lamberti et Bleve-Zacheo, 1979 and X. simile Lamberti, Choleva et Agostinelli, 1983. It differs from X. pachtaicum in having



Fig. 1. Photomicrographs of Xiphinema parasimile sp. n.: A-B, female anterior region; C, vulva region; D, posterior ovary; E, hypodermal cord; F-M, female tail.

a different lip region (set off vs expanded), shorter odontostyle (64.4-73.7 μ m vs 77-90 μ m) and odontophore (38.8-43.8 μ m vs 44-51 μ m), anteriorly located guide ring (59.4-66.3 μ m vs 68-83 μ m) and a different

vagina shape; from X. opisthohysterum because of its different lip region (set off vs expanded), posteriorly located guide ring (59.4-66.3 μ m vs 48-51 μ m) and anterior vulva (52.2-58.7% vs 56-61.3%); from X. californicum



Fig. 2. Photomicrographs of juveniles of *X. parasimile* sp. n.: A-D, anterior region of J1, J2, J3 and J4 stage, respectively; E-I, tail of J1, J2, J3 and J4 stage, respectively.

Locality: Host:	Trešnja at Ralja <i>Quercus</i> sp.						
	Holotype		Paratypes				
n	1 female	53 females	6 J 1	21 J2	15 J3	13 J4	
L (mm)	1.98	$\begin{array}{c} 1.99 \pm 0.13 \\ (1.75 - 2.26) \end{array}$	$\begin{array}{c} 0.62 \pm 0.03 \\ (0.57 - 0.65) \end{array}$	0.86 ± 0.05 (0.74 - 0.95)	$\begin{array}{c} 1.14 \pm 0.08 \\ (0.94 - 1.28) \end{array}$	1.46 ± 0.09 (1.32 - 1.62)	
a	71.5	70.5 ± 3.81 (61.0 - 76.1)	41.3 ± 3.67 (36.1 – 45.6)	48.6 ± 1.90 (44.3 – 51.6)	56.7 ± 2.88 (50.0 - 61.8)	$\begin{array}{c} 64.9 \pm 1.76 \\ (62.9 - 68.8) \end{array}$	
Ь	6.9	7.0 ± 0.49 (6.1 - 8.1)	3.4 ± 0.19 (3.1 - 3.6)	4.3 ± 0.35 (3.7 - 5.1)	5.0 ± 0.42 (4.0 - 5.8)	5.6 ± 0.47 (4.9 – 6.7)	
с	58.4	59.9 ± 4.51 (50.9 – 69.8)	20.2 ± 1.74 (17.4 - 21.9)	27.0 ± 2.31 (23.1 - 32.4)	35.2 ± 1.88 (31.4 - 37.7)	44.1 ± 3.43 (39.3 – 49.2)	
c'	2.04	2.02 ± 0.12 (1.79 - 2.28)	3.07 ± 0.13 (2.89 - 3.25)	2.67 ± 0.18 (2.34 - 3.04)	2.39 ± 0.12 (2.22 - 2.62)	2.16 ± 0.10 (2.01 - 2.35)	
V %	56	55.5 ± 1.38 (52.2 - 58.7)	-	-	_	-	
Odontostyle µm	67.5	69.7 ± 2.22 (64.4 – 73.7)	37.3 ± 0.95 (36.2 - 38.7)	42.4 ± 1.40 (40.0 - 45.6)	50.2 ± 1.66 (47.5 - 53.1)	59.5 ± 0.73 (58.1 – 60.6)	
Odontophore µm	41.3	41.6 ± 1.21 (38.8 - 43.8)	26.2 ± 0.63 (25.0 - 26.9)	29.3 ± 1.29 (27.5 – 31.9)	33.4 ± 1.12 (32.5 - 36.3)	37.1 ± 1.07 (35.0 - 38.8)	
Total stylet μm	108.8	$111.3 \pm 2.61 \\ (105.7 - 116.3)$	$\begin{array}{c} 63.5 \pm 0.96 \\ (62.5 - 65.0) \end{array}$	71.7 ± 2.22 (68.7 – 75.0)	83.6 ± 2.22 (80.0 - 86.9)	96.6 ± 1.33 (93.7 – 98.2)	
Replacement odontostyle µm	-		$\begin{array}{c} 44.0 \pm 0.55 \\ (43.7 - 45.0) \end{array}$	49.8 ± 1.46 (46.9 – 51.9)	59.0 ± 1.82 (55.0 - 61.2)	$\begin{array}{c} 68.0 \pm 1.60 \\ (66.2 - 71.2) \end{array}$	
Oral aperture to basal guide ring µm	60.6	62.6 ± 1.72 (59.4 - 66.3)	31.5 ± 0.65 (30.6 - 32.5)	38.9 ± 1.08 (36.3 - 40.0)	46.1 ± 1.27 (43.8 48.1)	53.5 ± 1.90 (50.0 - 56.3)	
Tail µm	33.9	33.3 ± 1.62 (30.3 - 37.1)	30.7 ± 1.29 (28.9 – 32.5)	31.9 ± 2.30 (28.6 - 36.4)	32.5 ± 1.73 (30.0 - 35.3)	33.2 ± 1.75 (29.6 - 35.3)	
J (hyaline portion of tail) µm	8.1	8.2 ± 0.88 (6.3 - 10.0)	3.7 ± 0.16 (3.4 - 3.8)	5.1 ± 0.54 (4.4 - 6.3)	5.4 ± 0.51 (4.4 - 6.3)	5.8 ± 0.75 (4.6 – 7.5)	
Body diam. at lip region µm	8.9	9.0 ± 0.24 (8.4 – 9.7)	7.1 ± 0.15 (6.9 – 7.2)	7.3 ± 0.15 (7.1 - 7.5)	7.6 ± 0.10 (7.5 – 7.7)	8.3 ± 0.18 (7.9 - 8.4)	
Body diam. at guide ring μm	19.7	19.8 ± 0.47 (18.8 - 21.3)	$\begin{array}{c} 12.7 \pm 0.37 \\ (12.5 - 13.3) \end{array}$	14.1 ± 0.38 (13.4 - 14.7)	15.7 ± 0.20 (15.2 - 15.9)	17.3 ± 0.43 (16.3 - 17.9)	
Body diam. at base of oesophagus μm	25	24.9 ± 0.90 (22.5 - 27.7)	$\begin{array}{c} 14.9 \pm 0.68 \\ (13.8 - 15.9) \end{array}$	17.2 ± 0.76 (15.6 - 18.4)	$\begin{array}{c} 19.4 \pm 0.60 \\ (18.4 - 20.4) \end{array}$	21.5 ± 1.10 (20.0 - 23.1)	
Body diam. at mid – body or vulva μm	27.7	$28.3 \pm 1.26 \\ (24.7 - 30.6)$	15.0 ± 0.80 (13.8 - 16.3)	17.7 ± 0.97 (15.6 - 19.7)	20.2 ± 0.99 (18.8 - 22.5)	$\begin{array}{c} 22.5 \pm 1.38 \\ (20.6 - 24.6) \end{array}$	
Body diam. at anus µm	16.6	16.5 ± 0.65 (15.0 - 17.7)	$\begin{array}{c} 10.0 \pm 0.00 \\ (10.0 - 10.0) \end{array}$	$\begin{array}{c} 12.0 \pm 0.62 \\ (11.3 - 13.1) \end{array}$	13.7 ± 0.68 (12.5 - 15.0)	$\begin{array}{c} 15.3 \pm 0.62 \\ (14.7 - 16.7) \end{array}$	
Body diam. at beginning of J μm	6.9	7.1 ± 0.51 (6.3 - 8.1)	3.3 ± 0.24 (2.8 - 3.4)	4.1 ± 0.44 (3.8 - 5.4)	4.8 ± 0.49 (3.8 - 5.6)	5.4 ± 0.42 (5.0 - 6.3)	

Table I. Morphometric characters of the type population of Xiphinema parasimile sp. n. from Serbia.

because of its different lip region (set off *vs* expanded), shorter odontostyle (64.4-73.7 µm *vs* 83-98 µm) and odontophore (38.8-43.8 µm *vs* 44-53 µm), anteriorly situated guide ring (59.4-66 µm *vs* 66-86 µm) and more slender body (a = 61-76.1 vs a = 52-68); from *X. intermedium* in its shorter odontostyle (64.4-73.7 µm *vs* 68-80 µm) and odontophore (38.8-43.8 µm *vs* 39-50 µm), posterior vulva (52.2-58.7% *vs* 50-57%), higher value of c' (1.79-2.28 *vs* 1.3-1.7) and more slender body (a = 61-76.1 *vs* a = 38-51). *Xiphinema parasimile* sp. n. is most similar to *X. simile.*; in comparison with the type population (Lamberti *et al.*, 1983) it differs in having a different lip region (set off *vs* expanded), posterior guide ring (59.4-66.3 µm vs 49-53 µm) and vulva (52.2-58.7% vs 51-54%), longer and differently shaped (more elongate) tail (30.3-37.1 µm vs 27-30 µm), higher c' value (1.79-2.28 vs 1.6-1.8) and different developmental pattern (four juvenile stages vs three juvenile stages). However, compared to other populations of X. simile (Peneva and Choleva, 1992; Lišková et al., 1993; Barsi, 1994; Lišková and Brown, 1996; Coomans and Heyns, 1997; Lamberti et al., 1999; Barsi and Lamberti, 2002) X. parasimile sp. n. shows considerable overlapping in all morphometrical data, including the data considered above (guide ring position: 59.4-66.3 vs 49-66.6 µm; vulva position: 52.2-58.7% vs 51-62%; tail length: 30.3-37.1 µm vs

	X. parasimile sp. n.		X. simile	
Locality:	Obedska bara,	Obrež, Serbia	Palić, Serbia	Bijeljani, Bosnia and
Host:	Carpinu	s betulus	Zea mays	Herzegovina <i>Cornus</i> sp.
n	7 females	19 females	8 females	9 females
L (mm)	$\begin{array}{c} 1.98 \pm 0.09 \\ (1.79 - 2.05) \end{array}$	$\begin{array}{c} 1.93 \pm 0.08 \\ (1.81 - 2.13) \end{array}$	$\begin{array}{c} 2.19 \pm 0.08 \\ (2.09 - 2.32) \end{array}$	$\begin{array}{c} 2.15 \pm 0.14 \\ (1.90 - 2.35) \end{array}$
a	70.5 ± 1.61	69.3 ± 2.88	82.2 ± 2.27	71.2 ± 4.26
	(68.8 – 72.5)	(62.8 - 75.0)	(79.4 – 85.6)	(66.6 - 78.3)
b	7.0 ± 0.31	6.7 ± 0.31	7.1 ± 0.57	7.2 ± 0.55
	(6.6 - 7.5)	(6.2 - 7.5)	(6.5 - 8.2)	(5.9 - 7.8)
c	62.4 ± 4.63	58.3 ± 4.31	71.8 ± 4.07	73.1 ± 5.34
	(58.2 - 72.3)	(51.0 - 65.8)	(67.4 - 79.0)	(64.2 - 79.9)
c'	2.01 ± 0.15 (1.72 - 2.19)	2.08 ± 0.16 (1.86 - 2.40)	1.84 ± 0.12 (1.59 - 1.97)	$\begin{array}{c} 1.60 \pm 0.10 \\ (1.50 - 1.84) \end{array}$
V	54.5 ± 1.26	55.1 ± 1.43	55.5 ± 0.91	54.8 ± 1.51
	(52.8 - 56.5)	(52.4 - 57.4)	(54.5 – 57.1)	(52.6 - 57.0)
Odontostyle µm	69.4 ± 1.72	69.7 ± 1.55	71.5 ± 2.48	67.5 ± 1.40
	(68.1 - 72.5)	(66.2 – 72.5)	(66.2 - 73.7)	(65.0 - 70.0)
Odontophore µm	40.9 ± 0.95	40.7 ± 1.59	40.4 ± 1.28	40.6 ± 1.53
	(40.0 - 42.5)	(37.5 – 43.8)	(38.8 - 42.5)	(38.8 - 42.5)
Total stylet μm	110.3 ± 1.86	110.4 ± 2.14	112.0 ± 2.69	108.1 ± 1.99
	(108.1 - 113.8)	(106.2 - 114.3)	(107.5 - 116.2)	(106.2 - 111.3)
Oral aperture to basal guide ring µm	63.1 ± 1.17	63.0 ± 1.28	64.1 ± 1.64	60.3 ± 1.03
	(61.3 - 64.4)	(60.6 - 65.0)	(61.9 - 66.9)	(58.8 - 62.5)
Tail µm	31.9 ± 2.74	33.2 ± 2.33	30.6 ± 1.95	29.5 ± 2.26
	(26.8 - 35.0)	(28.9 - 38.2)	(26.4 - 32.8)	(27.1 - 33.9)
J (hyaline portion of tail) μm	8.5 ± 1.33	8.5 ± 0.67	5.6 ± 0.77	6.1 ± 0.93
	(6.9 - 10.9)	(7.5 - 10.0)	(5.0 - 6.9)	(5.0 - 7.5)
Body diam. at lip region µm	9.2 ± 0.23	8.9 ± 0.16	9.8 ± 0.22	9.9 ± 0.16
	(8.9 - 9.6)	(8.8 - 9.4)	(9.4 - 10.0)	(9.7 - 10.0)
Body diam. at guide ring µm	19.6 ± 0.57	19.7 ± 0.51	19.2 ± 0.50	20.4 ± 0.48
	(18.8 - 20.0)	(18.8 - 20.6)	(18.8 - 20.0)	(20.0 - 21.3)
Body diam. at base of oesophagus µm	24.8 ± 0.45	24.5 ± 0.91	24.4 ± 0.56	26.1 ± 0.70
	(23.8 - 25.0)	(22.9 - 27.2)	(23.8 - 25.0)	(25.0 - 27.2)
Body diam. at mid – body or vulva µm	28.1 ± 1.14	27.9 ± 1.36	26.7 ± 0.84	30.2 ± 1.28
	(25.6 - 28.8)	(25.0 - 30.9)	(25.0 - 27.5)	(28.4 - 32.2)
Body diam. at anus µm	15.9 ± 0.69	16.0 ± 0.64	16.7 ± 0.40	18.4 ± 0.83
	(15.0 - 16.7)	(15.0 - 17.5)	(16.3 - 17.2)	(17.5 - 20.0)
Body diam. at beginning of J μm	7.3 ± 0.46 (6.7 - 8.1)	7.2 ± 0.45 (6.3 - 7.8)	$7.1 \pm 0.42 \\ (6.3 - 7.5)$	8.0 ± 0.56 (7.5 - 8.8)

Table II. Morphometric characters of adults of *X. parasimile* sp. n. from Serbia and *X. simile* from Serbia and Bosnia and Herzegovina.

22.5-38.2 μ m; c' value: 1.79-2.28 vs 1.24-2.18) above. Therefore, the use of the biometrics alone might not permit to separate X. parasimile sp. n. from X. simile. Moreover, although an accurate comparison of X. parasimile sp. n. with the type specimens of X. simile has indicated clear differences in the lip and tail regions between the two species, the evaluation of qualitative characters can always give rise to doubts, especially in the presence of few and/or badly preserved specimens.

However, it must also be remembered that the biological pattern of an organism is genetically determined. Thus, the use of essential biological differences may be appropriate in differentiating species. Halbrendt and Brown (1993) proposed the presence of either three or four juvenile stages as a character to be utilized for the separation of species within the *X. americanum* group. Moreover, Luc *et al.* (1998) included the number of juvenile stages in their list of the characters used to define the *X. americanum* group. Such a character can be recommended to separate *X. simile* Lamberti, Choleva *et* Agostinelli, 1983 from *X. parasimile* sp. n. since it has been demonstrated that populations of *X. simile* collected in the same region and from the same habitat as *X. parasimile* possess three juvenile stages (Barsi and Lamberti, 2002).

Xiphinema simile Lamberti, Choleva et Agostinelli,

1983 was found at Palić, Serbia (UTM grid DS00), in the rhizosphere of maize (*Zea mays* L.) and in Dabarsko Polje at Bijeljani, in Bosnia and Herzegovina (UTM grid BN77) in the rhizosphere of *Cornus* sp. Both populations are morphometrically in the range of those previously reported (Lamberti *et al.*, 1983; Peneva and Choleva, 1992; Lišková *et al.*, 1993; Barsi, 1994; Lišková and Brown, 1996; Coomans and Heyns, 1997; Lamberti *et*



Fig. 3. Comparison of selected morphological characters of *X. pachtaicum*, *X. simile* and *X. parasimile* sp. n., respectively: A-C, female anterior region; D-F, oesophagus basal bulb; G-O, vulva region; H-J, L-N, P-R, female tail.

al., 1999; Barsi and Lamberti, 2002). However, to widen its range the biometrics are reported in Table II.

ACKNOWLEDGEMENTS

We thank Dr. Ivan Dulić, NIS Naftagas, Novi Sad for access to an Olympus BX50 photomicroscope. Research



Fig. 4. Intraspecific variability of anterior region, vulva region and posterior region in females of X. *simile* (A-C) and X. *parasimile* sp. n. (D-F).



Fig. 5. Scatter diagram plotting body, odontostyles, odontophore, replacement odontostyle and total stylet length of individual juveniles and females of *X. parasimile* sp. n. from Ralja, Serbia.



Fig. 6. Scatter diagram separating juveniles and females of *X. parasimile* sp. n. and *X. simile* from populations from Serbia (for details see Table III).

Developmental stages and populations	Body length (mm)	Odontostyle (µm)	Odontophore (µm)	Total stylet (μm)	Replacement odontostyle (µm)
Trešnja at Ralja, Serbia ¹	0.62	37.3	26.2	63.5	44.0
Neradin, Serbia ²	0.85	35.2	28.6	63.8	43.2
J2/JII					
Trešnja at Ralja, Serbia	0.86	42.4	29.3	71.7	49.8
Neradin, Serbia	1.19	43.9	33.7	77.6	55.8
J3/JIII					
Trešnja at Ralja, Serbia	1.14	50.2	33.4	83.6	59.0
Neradin, Serbia	1.57	55.3	37.1	92.4	67.0
J4					
Trešnja at Ralja, Serbia	1.46	59.5	37.1	96.6	68.0
Neradin, Serbia	_	-		_	-
Females					
Trešnja at Ralja, Serbia	1.99	69.7	41.6	111.3	-
Neradin, Serbia	2.15	67.9	41.7	109.6	_

Table III. Morphometrics of juvenile stages and females of X. parasimile sp. n. and X. simile from Serbia.

¹ Xiphinema parasimile sp. n., (J1, J2, J3, J4, females) original; ²X. simile, (JI, JII, JIII, females) Barsi and Lamberti, 2002.

by the first author is partly supported by the Ministry of Science, Technologies and Development of the Republic of Serbia, Grant No. 1770.

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Accepted for publication on 22 March 2004.