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NEW DATA ON THE MORPHOLOGY AND DISTRIBUTION OF TWO SPECIES OF THE *XIPHINEMA AMERICANUM* GROUP (NEMATODA: DORYLAIMIDA) FROM ROMANIA

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Abstract

Several populations of Xiphinema pachtaicum (Tulaganov, 1938) Kirjanova, 1951 and Xiphinema simile Lamberti, Choleva et Agostinelli, 1983, originating from various localities have been studied during 2011-2012. Soil samples were collected at a depth of 20-40 cm in the rhizosphere of grapevine, cherry and apple trees. The identification of the nematodes was based on morphological and morphometrical characters. Descriptions, including morphometric data and illustrations of adults (females and males) and juveniles from different localities and plant associations are provided. Data of X. pachtaicum and X. simile males are provided for the first time for Romania. Both species occurred together in two locations. Malus pumila Mill and Prunus avium L. are new plant association for these nematodes species for Romania.

Key words: *Longidoridae, Xiphinema pachtaicum, X. simile, morphology.*

INTRODUCTION

Nematodes of genus *Xiphinema* (family *Longidoridae)* are ecomically important plant pest, causing direct and indirect damage to a wide number of crops. The number of species in the genus is some 240 (Decraemer, 2007). In Romania, four species of *Xiphinema americanum* group have been recorded: *Xiphinemaparasimile* Barsi et Lamberti, 2004 in association with grapevine and apricot (Groza et al., 2012); *Xiphinema taylori* Lamberti et al., 1992; *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 and *Xiphinema simile* Lamberti, Choleva et Agostinelli, 1983 in association with grapevine, fruit trees, alfalfa, and peach (Peneva et al., 2006 and Groza et. al., 2012). This work presents a detailed analysis of the morphometrical data of *Xiphinemapachtaicum* and *X. simile* originating from Romania.

MATERIALS AND METHODS

Soil samples were collected from the rhizosphere of grapevine, cherry and apple trees at a depth of 20-40 cm, from different regions of country: Bîrlad, Huşi (Vaslui county), Nazarcea, Ostrov (Constanţa county), Urlaţi (Prahova county), Obrejiţa (Vrancea county).

Nematodes were extracted from 200 cm^3 soil by a sieving and decanting technique. Nematodes were heat killed at 60^oC for two minutes and fixed in a 4% formaldehyde solution. The specimens were processed and mounted on permanent microscopic glass slides (Seinhorst, 1959).

The morphological and morphometrical observations were made using Leica DMLB microscope fitted with Leica FDC 295 camera.

Table 1. Measurements of *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (all in micrometers except for body length)

Locality	Urlati		Nazarcea		Ostrov
Host plant	*Vitis vinifera*		*Vitis vinifera*		*Cerasus avium*
Character	Females	Male	Females	Male	Females
n	10	1	7	1	11
L	2.0±0.88 1.8-2.1	2.1	1.97±0.18 1.77-2.26	1.88	1.89±0.94 1.77-2.11
a	66.4±3.4 62.3-73	70.5	64.0±5.4 56.2-70.9	72.6	63.9±2.5 59.6-68.4
b	6.3±0.6 5.6-7.3	6.4	6.2±0.4 5.7-6.8	6.1	6.1±0.7 4.6-7.1
c	64.0±5.2 56.5-74.1	70.5	62.5±3.6 55.6-67.4	70.1	63.3±4.0 57-71.5
c’	1.8±0.1 1.7-2.1	1.5	1.8±0.1 1.6-1.9	1.4	1.8±0.1 1.7-1.9
V%	57.5±0.7 56.5-58.6	-	57.0±2.8 52.7-59.4	-	57.7±0.6 56.4-58.5
Odontostyle	90.0±1.8 88-92	90	82.6±3.7 79-88	86	87.9±2.5 83-91
Odontophore	50±1.3 48-52	50	49.7±3.0 44-53	47	50.1±1.8 48-54
Oral aperture to guide ring	80.5±3.5 71-83	80	75.0±3.8 70-80	79	78.6±2.9 75-83
Pharynx	318.3±20.7 285-339	326	288.0±15.3 275-320	305	311.5±29.6 269-385
Tail	31.4±2.1 29-35	30	28.4±2.3 25-32	27	29.9±1.4 27-31
Length of hyaline part	8.2±0.6 7-9	8.1	7.8±0.9 6-9	9	9.2±1.1 8-11
Body diameter at: - lip region	9.2±0.3 9-10	10	9.0±0.4 9-10	9	9.0±0.3 8.5-10
- guiding ring	22.5±0.4 22-23	22	21.7±0.9 20-23	21	22.5±1 21-24
- base of pharynx	26.6±1.1 25-28	26.5	26.4±2.6 23-30	25	26.4±1.5 23-30
- mid-body	30.2±1.7 27-34	30	30.8±1.6 29-33	26	29.7±2 28-35
- anus	17.1±0.7	19,6	17.3±0.9 16-19	18	16.7±0.4 16-18
- hyaline part	6.7±0.5 6-7,5	6.5	7.8±0.8 7-9	7	7.9±0.7 7-9
Spicules		40		42	

n=number of specimens; a=body length/greatest body diameter; b=body length/distance from anterior to end of esophageal bulb; c=body length; c´=tail length/anal body diameter; V %distance of vulva from anterior end

RESULTS AND DISCUSSIONS

Xiphinema pachtaicum (Tulaganov, 1938) Kirjanova, 1951 (Tables 1-3; Figures 1A, 2, 3,5)

Female. Habitus in the shape of a single spiral or C when relaxed. Labial region 3-4 µm high, offset from the rest of the body, expanded, frontally flattened, laterally rounded. Genital system with two almost equally developed branches, uteri short. Rectum 23µm long for Ostrov population, 17-21 µm for Urlați, 21-24,5 µm for Bîrlad population.

Male. Body posture as in female, tail end coiled stronger ventrally. Head region as in female, lip region 4 µm high. Ventromedian supplements 1+5 (Urlați specimen) and 1+6 Nazarcea specimen). Spicules slightly curved ventrally.

Tail dorsally convex, with elongated mucro. (Figure 5).

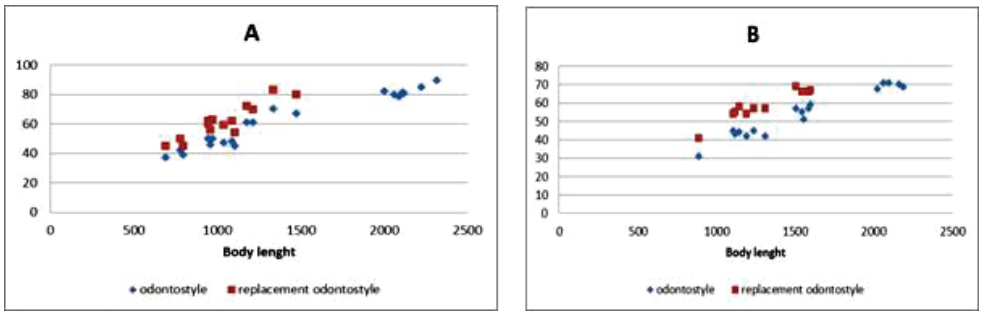

Figure 1. Scatter plot of odontostyle and replacement odontostyle agaist body length of *Xiphinema pachtaicum* (A) and *Xiphinema simile* (B) from Bîrlad populations

Table 2. Measurements of *Xiphinema pachtaicum* (all in micrometers except for body length)

Locality	Birlad-pachtaicum				
Host plant	*Vitisvinifera*				
Character	Females	J1	J2	J3	J4
n	7	3	6	2	2
L	2.13±0.10 2.0-2.3	0.75±0.056 0.69-0.79	1.00±0.07 0.94-1.10	1.17, 1.21	1.33, 1.47
a	69.3±3.3 64.4-74.9	39.8±5.9 33.2-44.7	51.1±5.2 44.2-59.8	51.8, 57.9	61.9, 64.2
b	7.3±1.1 6-9.4	4.6±0.3 4.3-4.9	4.7±0.5 4.3-5.5	4.7	5.2, 5.7
c	49-74.7	24.4±1.5 22.7-25.6	31.7±3.3 26.9-35.1	34.6, 35.3	38.7, 41.4
c’	1.6-2.5	2.6±0.3 2.3-2.9	2.2±1.1 2.2-3	2.3-2.4	2.1, 2.4
V%	56.0±1.2 52.6-57.6	-	-	-	-
Odontostyle	82.4±3.7 79-90	39.8±2.7 37-42	48.2±2.2 45-50	61.61	67.70
Odontophore	51.8±2.6 48-55	31.9±0.1 32-32	35.8±2.4 32-39	37.38	44
Replacement odontostyle	-	47.8±4.5 45-53	59.5±3.7 54-63	70.72	80.83
Oral aperture to guide ring	72.5±2.2 71-76	34.2±1.1 33-35	41.3±3.4 36-45	54-54	41. 55
Pharynx	296.3±31.1 247-345	165.7±6.4 162-173	212.3±10.9 198-223	250-256	235-283
Tail	34.0±3.7 28-41	30.9±0.4 30-31	32.2±2.2 29-35	33.35	32.38
Length of hyaline part	9.5±1.5 8-12	5.0±0.3 5-5	5.1±0.5 4-5	5.6	6.5-8
Body diameter at: - lip region	9.6±0.4 9-10	7.4±0.4 7-8	7.5±0.3 7-8	7.7	8-9
- guiding ring	21.2±0.8 20-22.5	13.7±1.4 13-15	15.4±1.2 15-18	16-17	17-19
- base of pharynx	26.7±1.2 25-28	18.1±3.0 16-21,5	18.4±1.9 16-21	19-21	20-22
- mid-body	30.8±1.9 28-35	19.3±4.1 17-24	19.8±2.4 18-25	21-23	21-23
- anus	18±0.9 17-19	11.9±1.6 11-14	12.8±1.5 12-15	14-14	15-16
- hyaline part	8.6±1 8-10	3.8±0,3 3.5-4	4,9±0,6 4-5	5-6	5.5-7

n=number of specimens; a=body length/greatest body diameter; b=body length/distance from anterior to end of esophageal bulb; c=body length; c´=tail length/anal body diameter; V%=distance of vulva from anterior end

Table 3. Measurements of *Xiphinema pachtaicum* (all in micrometers except for body length)

Locality	Obrejita (Vrancea county)				
Host plant			*Vitis vinifera*		
Character	Females	J1	J2	J3	J4
n	9	4	5	5	4
L	2.03±0.15 1.83-2.23	0.73±0.028	0.93±0.053 0.85-0.98	1.27±0.05 1.22-1.35	1.65±0.07 1.58-1.73
a	69.9±6.1 62.2-78.9	43.7±1.8 41.4-45.6	48±2.1 45.9-50.5	57.3±2.1 53.7-58.8	61.8±3.6 57-65
b	7±0.9 5.4-8.5	4.4±0.4 3.9-4.9	4.9±0.1 4.8-5.1	5.5±0.4 5.0-6.2	6.8±0.3 6.5-7.2
c	70.1±5.6 59.5±76.3	24.8±0.6 24.2-25.3	28.5±1.4 27.5-29.4	40.9±2.8 37.8-43.2	50.3±2.3 47.4
c’	1.7±0.1 1.6-1.9	2.7±0.1 2.6-2.8	2.7±0.3 2.5-2.9	2.1±0.1 2.1-2.3	2.0±0.0 1.9-2.0
V%	54.1±1.2 52-55.6	-	-	-	-
Odontostyle	78.6±1.5 76-80	39.7±0.5 39-40	44.7±2.9 40-47	58.0±1.0 57-59	32.9±1.8 31-35
Odontophore	48.2±2.9 42-53	28.4±2.0 26-30	32.3±2.2 30-35	37.2±1.3 35-38	42±0.8 41-43
Replacement odontostyle	-	45.9±0.7 45-47	40.4±2.5 37-43	68.4±1.5 67-70	78.3±3.6 73-81
Oral aperture to guide ring	70.5±1.7 67-73	34.8±1.0 34-35	40.4±2.5 37-43	51.2±1.1 50-53	59±0.8 58-60
Pharynx	294±26.6 260-342	166.3±11.2 155-177	189.4±7.6 179-198	232.8±13.7 219-253	244.3±10 235-258
Tail	29.1±1.3 28-31	29.3±0.5 29-30	33±0.5 33-33	31.4±1.0 31-33	32.9±1.8 31-35
Length of hyaline part	8.3±0.6 7-9	4.5±0.4 4-5	4.6±0.4 4-5	5.5±0.4 5-6	6.9±0.5 6-7
Body diameter at: - lip region	9.1±0.3 9-10	7.2±0.1 7	7.5±0.3 7-8	8.1±0.4 8-9	8.4±0.3 8-9
- guiding ring	21.5±0.8 20-23	13.3±0.8 12-14	14±0.7 13-15	17.0±0.4 17-17.5	19.1±0.7 18-20
- base of pharynx	25.6±1.5 23-28	16.2±1.0 15-17	17.8±1.2 16-19	20.6±0.5 20-21	23.5±0.1 22-24
- mid-body	29.2±1.6 27-32	16.8±1.0 15-18	19.5±1.4 18-21	22.2±0.8 21-23	26.8±1.5 25-28
- anus	16.7±1.2 15-18	10.8±0.1 11-11	12.4±1.6 11-13,5	14.7±0.5 14-15	16.6±1.1 16-18
- hyaline part	7.3±0.7 6-8	3.6±0.3 3-4	4.4±0.3 4-5	5.2±0.6 5-6	6.4±0.5 6-7

n=number of specimens; a=body length/greatest body diameter; b=body length/distance from anterior to end of esophageal bulb; c=body length; c´=tail length/anal body diameter; V%=distance of vulva from anterior end

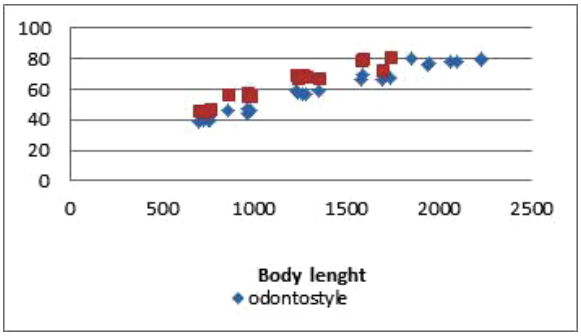

Figure 2. Scatter plot of odontostyle and replacement odontostyle agaist body length of *Xiphinema pachtaicum* from Obrejița population

Table 4. Measurements of *Xiphinema simile* (all in micrometers except for body length)

Locality	Nazarcea		Husi	Bîrlad			
Host plant	*Vitisvinifera*		*Maluspumila*	*Vitisvinifera*			
Character	Females	Male	Femeles	Females	J1	J2	J3
n	3	1	6	5	1	6	5
L	2.16±0.14 2.02-2.30	1.97	2.0±0.1 1.83-2.0	2.05±0.13 1.81-2.18	0.7	1.18±0.077 1.10-1.30	1.55±0.036 1.5-1.59
a	74.2±3.4 70.9-78.3	73.1	72±3 69-78	79±3 76-83	41.6	58.2±5.5 48.0-63.9	73.7±3.9 70.6-80.3
b	7.0±1.0 6.4-7.7	7.2	6.9±0.1 6.8-6.9	7.2±0.7 6.5-8.2	4.3	5.7±0.2 5.4-5.9	6.1±0.4 5.8-6.7
c	70.2±5.4 62.9-76	70.5	66.2±8.8 56.2-79.5	79.9±13 62.9-86.7	22.7	39.6±3.8 35.1-44.5	50.4±3.8 44.1-53.8
c’	1.8±0.1 1.7-1.9	1.3	1.7±0.2 1.4-2	1.7±0.2 1.5-1.9	2.9	2.3±0.2 2.1-2.7	2.1±0.2 1.9-2.5
V%	53.8±0.9 52.6-54.8	-	55.2±0.6 54.4v56	5.,5±1.1 54.6-57.4	-	-	-
Odontostyle	69.5±6.4 66-72	66	68.3±2.3 65-70	69.7±1.3 68-71	37	43.5±1.4 42-45	55.8±3.0 51-59
Odontophore	45.5±5.1 42-50	46	46±1.7 43-48	43.1±2.5 40-46	-	33.0±1.7 31-35	36.8±1.5 35-39
Replacement odontostyle	-	-	-	-	45	55.8±1.7 54-58	66.7±1.4 66-69
Oral aperture to guide ring	61±6.4 56-65	63	60.7±1.0 59-62	59.2±0.8 58-60	33	37.0±1.3 36-39	48.5±1.9 46-51
Pharynx	305.5±17.7 293-318	267	292.3±15 265-304	285.7±21.7 253-306	162	207.9±8.9 200-223	254.7±17.5 231-273
Tail	30.9±1.4 29-32	27,6	30.6±2.8 26-34	27.5±3.7 24-32	30,4	30.1±2.9 26-34	31.1±2.6 28-35
Length of hyaline part	7.1±1.6 5-9	7.1	61±0.7 5-7	6.2±0.6 6-7	5	4.2±0.4 4-5	4.7±0.7 4-6
Body diameter at: - lip region	9.6±0.4 9.1-10	9.8	10±0.4 10-11	9.3±0.4 8.8-10	7.3	7.8±0.3 7-8	8.5±0.1 8-9
- guiding ring	19.4±1.6 19-22	20	19.4±0.6 19-20	18.8±1.1 17-20	12.6	14.0±0.5 14-15	16.8±0.9 15-18
- base of pharynx	25±3.9 22-28	24.7	24.2±0.9 23-26	22.9±2.0 21-26	16	18.2±2.2 16-22	19.9±0.7 19-21
- mid-body	29.2±2.7 26-32	26.6	27.8±1.6 26-30	25.6±1 24-27	16.6	20.6±3.0 18-26	21.2±1.2 19-22
- anus	17.6±1.1 17-19	19.8	18.1±0.9 17-19	16.2±0.4 16-17	10.6	13.2±1.1 12-15	14.6±0.7 14-16
- hyaline part	7.0±0.8 6-7	6.3	7.8±0.6 7-9	7.4±0.8 6-8	4	4.3±0.4 3.8-5	5.5±0.6 5-6
Spicules		38	-	-	-	-	-

n=number of specimens; a=body length/greatest body diameter; b=body length/distance from anterior to end of esophageal bulb; c=body length; c´=tail length/anal body diameter; V%=distance of vulva from anterior end

Juveniles. The scatter plot diagram based on functional and replacement odontostyle and body length reveal the presence of four juveniles stages (Figure 1A, 3)

Xiphinema pachtaicum was found in rhizosphere of *Vitis vinifera* L. (Bîrlad, Urlaţi, Nazarcea, Obrejiţa), and *Prunus avium* L. (Ostrov) (Table 1, 2, 3).

All populations of *X. pachtaicum* studied are similar in most morphological and morphometrical characters. However, Obrejita specimens compared to other have shorter odontostyle (ave.78.6 vs ave. 90-82.4 µm).

This species is wide-spread in Europe and has been shown to occur in several countries such as: Bulgaria (Choleva, 1975, Lamberti et al., 1983, Peneva & Choleva 1992), Croatia (Samota et al. 1994), Czech Republic (Kumari, 2004, Kumari et al. 2005), Macedonia, Montenegro, Serbia (Barsi & Lamberti, 2002). Slovakia (Liskova, 1992, Lamberti et al., 1999), Hungary (Repasi et al., 2008), Spain, Italy (Gutiérrez-Gutiérrez et al., 2011).

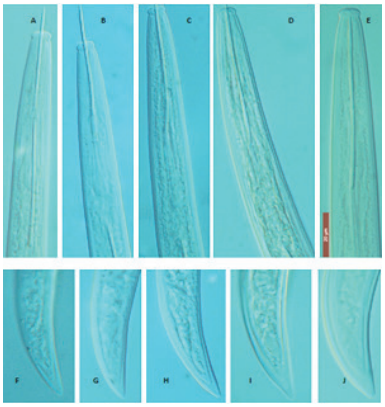

Figure 3. *Xiphinema pachtaicum* A-D, anterior region of first, second, third and fourth stage juveniles; E, female anterior end; F-I, tail of first, second, third and fourth stage juveniles; J, female tail. Scale bar: A-J, 20µm

Xiphinema simile Lamberti, Choleva et Agostinelli, 1983 (Table 4, Figures 1B,4, 5).

Female. Habitus in the form of a single spiral or C when relaxed. Labial region 4 µm high, separated from the rest of the body by a deep constriction. Gonads paired, opposed and reflected. Rectum 20-23 µm long for Huşi population, 16-20 µm for Nazarcea specimens. Tail conoid, dorsally convex, terminus rounded, in some specimens pointed.

Male. Similar to female in habitus, the posterior region more strongly coiled ventral. Anterior testis 75 µm and posterior 77 µm long, filled with sperms. Spicules slightly curved, one adanal pair and 4 ventromedian supplements present (Figure 5).

Juveniles. Three juveniles stages were distinguished on the basis of body, functional and replacement odontostyle lengths (Figure 1B, 4).

The morphometric characters of *X. simile* (Table 4) found in rhisosphere of *Vitis vinifera* L. (Nazarcea, Bîrlad) and in *Maluspumila* Mill. (Husi) are almost identical.

Romanian specimens closely agree in measurement with populations from Bulgaria (Peneva & Choleva, 1992, Lazarova et al., 2008), Slovakia (Liskova & Brawn, 1996: Lamberti et al., 1999), former Yugoslavia (Barsi, 1994; Barsi & Lamberti , 2002, 2004), Czech Republic (Kumari, 2006) and Kenya (Coomans & Heyns, 1997). Body length is shorter than in Hungarian specimens (Repasi et al., 2008).

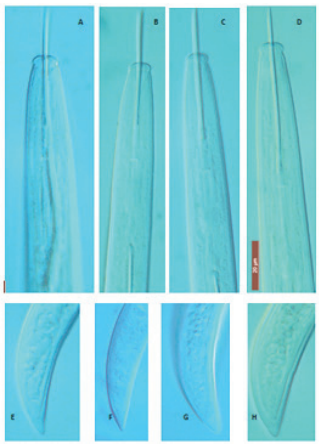

Figure 4. *Xiphinema simile* A-C, anterior region of first, second, and third juvenile stages; D, female anterior end; E-G, tail of first, second and third juvenile stages; H, female tail. Scale bar: A-J, 20µm

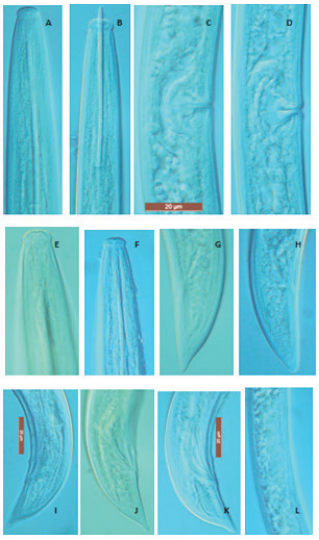

Figure 5. *Xiphinema pachtaicum*, A, anterior region of female; C, vaginal region; E, anterior region of male; G, female tail; I, J,male tail and copulatory apparatus; Xiphinema simile, B, anterior region of female ; D, vaginal region, F, anterior region of male; H, female tail; K, male tail and copulatory apparatus, L, anterior testis. Scale bar: A-J, 20µm

CONCLUSIONS

Morphological data and measurements of juvenile stages and male specimens are presented for the first time for Romanian populations.

Malus pumila and *P. avium* are new host plant associations for these species in our country.

Xiphinema pachtaicum and *X. simile* were found to occur together in Bîrlad and Nazarcea populations in the rhizosphere of grapevines.

All localities are new records for both species.

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