HELMINTHOLOGIA, 53, 4: 396 - 400, 2016

Research Note

Morphometrical and molecular data on plant parasitic nematodes *Longidorus attenuatus* Hooper, 1961 and *L. danuvii* Barsi et al., 2007 (Nematoda: Longidoridae) reported from Ukraine for the first timeS. SUSULOVSKA¹, A. SUSULOVSKY², F. W. KORNOBIS^{3*}

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Article info

Received January 18, 2016
Accepted September 28, 2016

Summary

Genus *Longidorus* Micoletzky, 1927 consists of obligatory plant root ectoparasites. Some of the genus species are of economic importance as pests of agricultural plants. This paper presents first records of *Longidorus attenuatus* Hooper, 1961 and *L. danuvii* Barsi et al., 2007 from Ukraine. Data on morphometrics and D2-D3 28S rDNA region sequence are provided. Additionally, a D2-D3 28S rDNA sequence from a population described previously from Poland was included. For the first time *L. danuvii* has been found with plants of agricultural importance (*Malus domestica* Borkh., *Pyrus* sp. and *Rubus idaeus* L.). Additionally, this nematode species has been found for the first time in localities with relatively dry conditions (on the tops of the hills), which are distant from banks of rivers – biotopes from which it has been recorded previously.

Keywords: *Longidorus attenuatus*, *Longidorus danuvii*, Ukraine, morphometrics, D2-D3 28S rDNA region

Introduction

Nematode genus *Longidorus* Micoletzky, 1927 consists of about 150 species of obligatory plant root ectoparasites. Some of these species are of economic importance as pests of agricultural plants (Singh *et al.*, 2013 gives general review). This importance is further augmented by the fact that several of genus species are known to be virus vectors. An example of such important species is *Longidorus attenuatus* Hooper, 1961, which is associated with 'docking' disease of sugarbeet (Whitehead & Hooper, 1970). This species is also a vector of Tomato black ring virus (Harrison, 1964), which makes it important to viticulture industry (Hüb-schen *et al.*, 2004). This nematode is rather widely distributed, as it is known from several European countries (e.g. Brown & Taylor, 1987). However, up to date it has not been recorded from Ukraine (Peneva *et al.*, 2009). In comparison with *L. attenunatus*, less is known on *L. danuvii* Barsi, Lamberti and De Luca, 2007, as data

on this species include original description based on populations from Serbia (Barsi *et al.*, 2007) and subsequent description of population from Poland (Kornobis, 2012). Up to date, this nematode was found in association with *Amorpha fruticosa* L. and *Populus* sp. (Barsi *et al.*, 2007) and *Salix alba* L. (Kornobis, 2012). The aim of this Research Note is to present first records of both of the above mentioned nematode species from Ukraine, their morphometrics, D2-D3 rDNA region sequence and data on association of *L. danuvii* with plants of agricultural importance. Additionally, D2-D3 rDNA sequence was obtained from population described previously from Poland (Kornobis, 2012).

Materials and Methods

Soil samples containing *L. attenuatus* and *L. danuvii* were taken from localities and plants given in Table 1. Nematodes were extracted from soil by sieving and decanting method (Brown & Boag,

Table 1. Data on localities, host plants and morphometrics of *L. danuvii* and *L. attenuatus* populations from Lviv, Ukraine.
All measurements are in μm , except for body length (in mm) and in form: mean $\pm$ standard deviation (range)

	<i>L. danuvii</i>		<i>L. attenuatus</i>	
Location and host plant (s)	Citadel park N49.49580°; E24.01222° <i>Acer platanoides</i> L.	St. George's Cathedral garden N49.50184°; E24.00516° <i>Malus domestica</i> Borkh.	Lychakiv school garden N49.50158°; E 24.03499° <i>Malus domestica</i> Borkh., <i>Pyrus</i> sp., <i>Rubus idaeus</i> L.	Lychakiv school garden N49.50158°; E 24.03499° <i>Malus domestica</i> Borkh
Character				
N	14	6	29	9
L	4.80 $\pm$ 0.34 (4.08 – 5.24)	4.16 $\pm$ 0.26 (3.88 – 4.46)	4.32 $\pm$ 0.28 (3.81 – 5.04)	6.34 $\pm$ 0.56 (5.20 – 6.95)
a	113.5 $\pm$ 5.14 (103.9 – 123.9)	102.6 $\pm$ 2.18 (99.8 – 105.0)	100.1 $\pm$ 5.15 (89.5 – 114.4)	139.7 $\pm$ 8.49 (123.8 – 151.1)
b	14.1 $\pm$ 1.45 (11.0 – 17.1)	13.6 $\pm$ 1.49 (12.0 – 16.3)	12.5 $\pm$ 1.96 (9.0 – 16.8)	16.2 $\pm$ 1.53 (13.9 – 18.5)
c	120.4 $\pm$ 12.34 (90.8 – 141.7)	99.6 $\pm$ 6.98 (88.2 – 108.8)	102.9 $\pm$ 7.45 (87.6 – 118.5)	127.9 $\pm$ 10.63 (111.9 – 144.3)
c'	1.45 $\pm$ 0.11 (1.28 – 1.67)	1.59 $\pm$ 0.10 (1.48 – 1.73)	1.52 $\pm$ 0.10 (1.32 – 1.81)	1.53 $\pm$ 0.08 (1.41 – 1.66)
d	2.0 $\pm$ 0.13 (1.8 – 2.3)	2.0 $\pm$ 0.13 (1.8 – 2.2)	2.2 $\pm$ 0.12 (1.9 – 2.4)	2.1 $\pm$ 0.09 (1.9 – 2.2)
d'	1.5 $\pm$ 0.10 (1.3 – 1.6)	1.5 $\pm$ 0.12 (1.4 – 1.7)	1.5 $\pm$ 0.08 (1.3 – 1.7)	1.4 $\pm$ 0.09 (1.3 – 1.6)
V	47.7 $\pm$ 1.64 (43.9 – 49.7)	48.6 $\pm$ 1.99 (45.5 – 51.0)	49.2 $\pm$ 1.32 (46.2 – 52.7)	49.1 $\pm$ 1.45 (47.5 – 52.2)
Odontostylet length	87.1 $\pm$ 2.2 (81 – 90)	90.8 $\pm$ 0.8 (90 – 92)	90.2 $\pm$ 2.12 (86 – 95)	82.7 $\pm$ 2.3 (78 – 85)
Odontophore length	50.9 $\pm$ 2.4 (45 – 54)	54.0 $\pm$ 1.3 (53 – 56)	56.6 $\pm$ 3.57 (49 – 63)	59.4 $\pm$ 2.4 (56 – 63)
Total stylet length	137.9 $\pm$ 3.5 (130 – 143)	144.8 $\pm$ 1.7 (143 – 147)	146.8 $\pm$ 4.45 (136 – 155)	142.1 $\pm$ 3.6 (137 – 147)
Anterior end to guide ring	26.1 $\pm$ 0.9 (24 – 27)	26.3 $\pm$ 1.0 (25 – 28)	27.9 $\pm$ 1.05 (26 – 30)	29.9 $\pm$ 1.2 (28 – 31)
Pharyngeal bulb length	80.0 $\pm$ 3.6 (72 – 86)	81.3 $\pm$ 2.0 (78 – 83)	86.5 $\pm$ 6.71 (73 – 99)	112.2 $\pm$ 4.6 (105 – 121)
Pharyngeal bulb width	16.1 $\pm$ 1.7 (13 – 19)	15.7 $\pm$ 0.8 (15 – 17)	17.5 $\pm$ 1.27 (15 – 20)	18.3 $\pm$ 1.4 (16 – 21)
Tail length	40.1 $\pm$ 2.6 (37 – 45)	41.8 $\pm$ 2.6 (38 – 45)	42.1 $\pm$ 2.71 (37 – 48)	49.7 $\pm$ 3.8 (44 – 56)
Hyaline part of tail length	9.6 $\pm$ 1.2 (8 – 12)	10.5 $\pm$ 1.4 (8 – 12)	10.0 $\pm$ 1.09 (8 – 12)	13.3 $\pm$ 2.0 (9 – 16)
Width at level of:				
lips	12.9 $\pm$ 0.6 (12 – 14)	13.2 $\pm$ 0.8 (12 – 14)	12.9 $\pm$ 0.54 (12 – 14)	14.3 $\pm$ 0.5 (14 – 15)
guide ring	18.9 $\pm$ 1.1 (17 – 21)	19.2 $\pm$ 0.8 (18 – 20)	18.8 $\pm$ 0.62 (18 – 20)	20.2 $\pm$ 1.0 (19 – 22)
base of pharynx	36.7 $\pm$ 1.7 (33 – 39)	34.7 $\pm$ 1.5 (32 – 36)	35.8 $\pm$ 1.36 (32 – 38)	38.9 $\pm$ 1.1 (37 – 40)
vulva	42.4 $\pm$ 3.0 (37 – 47)	40.5 $\pm$ 1.9 (38 – 43)	43.2 $\pm$ 1.97 (40 – 47)	45.3 $\pm$ 1.9 (42 – 48)
anus	27.6 $\pm$ 1.3 (25 – 29)	26.3 $\pm$ 0.8 (25 – 27)	27.7 $\pm$ 1.03 (26 – 30)	32.6 $\pm$ 1.7 (29 – 35)

Table 2. Data on morphometrics of *Longidorus danuvii* juveniles from Citadel Park, Lviv, Ukraine.
All measurements are in μm , except for body length (in mm) and in form: mean $\pm$ standard deviation (range).

Character	J1	J2	J3	J4
n	3	8	16	13
L	1.21 (1.18 – 1.29)	1.66 $\pm$ 0.07 (1.56 – 1.75)	2.09 $\pm$ 0.20 (1.71 – 2.42)	3.28 $\pm$ 0.30 (2.70 – 3.79)
a	54.1 (53.0 – 55.9)	67.4 $\pm$ 4.06 (59.9 – 72.0)	70.7 $\pm$ 3.87 (62.2 – 77.6)	98.9 $\pm$ 6.14 (97.0 – 111.5)
b	6.8 (5.5 – 8.8)	6.1 $\pm$ 0.41 (5.3 – 6.7)	7.6 $\pm$ 1.58 (5.4 – 10.5)	9.9 $\pm$ 0.82 (8.5 – 11.0)
c	32.1 (29.9 – 35.6)	42.7 $\pm$ 2.63 (39.2 – 47.3)	48.2 $\pm$ 6.02 (36.4 – 56.9)	76.9 $\pm$ 8.54 (62.7 – 88.9)
c'	2.43 (2.2 – 2.6)	2.30 $\pm$ 0.28 (2.06 – 2.93)	2.11 $\pm$ 0.27 (1.86 – 2.40)	1.75 $\pm$ 0.15 (1.5 – 2.0)
d	1.9 (1.8 – 2.0)	1.9 $\pm$ 0.10 (1.8 – 2.1)	2.1 $\pm$ 0.15 (1.8 – 2.3)	2.0 $\pm$ 0.12 (1.8 – 2.2)
d'	1.4 (1.3 – 1.6)	1.5 $\pm$ 0.13 (1.3 – 1.7)	1.5 $\pm$ 0.10 (1.4 – 1.8)	1.5 $\pm$ 0.08 (1.3 – 1.6)
Odontostylet length	53.3 (53 – 54)	60.4 $\pm$ 0.9 (59 – 61)	69.3 $\pm$ 2.0 (65 – 72)	76.1 $\pm$ 1.3 (73 – 78)
Odontophore length	–	43.9 $\pm$ 2.6 (39 – 47)	45.7 $\pm$ 2.1 (42 – 49)	49.0 $\pm$ 2.8 (43 – 53)
Total stylet length	–	104.3 $\pm$ 3.0 (98 – 107)	114.9 $\pm$ 2.7 (111 – 121)	125.2 $\pm$ 3.4 (120 – 130)
Replacement odontostylet length	61 (60 – 63)	68.8 $\pm$ 3.0 (62 – 71)	77.9 $\pm$ 2.2 (74 – 82)	84.8 $\pm$ 2.2 (81 – 88)
Anterior end to guide ring	16	18.4 $\pm$ 1.1 (17 – 20)	21.1 $\pm$ 0.7 (20 – 22)	23.2 $\pm$ 0.8 (22 – 24)
Pharyngeal bulb length	48.7 (43 – 52)	57.0 $\pm$ 3.1 (54 – 64)	64.8 $\pm$ 4.8 (57 – 73)	76.2 $\pm$ 4.4 (71 – 83)
Pharyngeal bulb width	10.7 (9 – 12)	12.8 $\pm$ 1.0 (11 – 14)	13.4 $\pm$ 0.8 (13 – 15)	15.5 $\pm$ 1.1 (13 – 17)
Tail length	37.3 (33 – 40)	38.9 $\pm$ 2.2 (37 – 42)	43.6 $\pm$ 2.4 (39 – 48)	42.8 $\pm$ 3.2 (38 – 49)
Hyaline part of tail length	5.3 (5 – 6)	5.9 $\pm$ 0.6 (5 – 7)	7.4 $\pm$ 1.0 (6 – 9)	8.5 $\pm$ 1.1 (7 – 11)
Width at level of:				
lips	8.3 (8 – 9)	9.5 $\pm$ 0.5 (9 – 10)	10.2 $\pm$ 0.8 (9 – 12)	11.6 $\pm$ 0.5 (11 – 12)
guide ring	12 (11 – 13)	14.4 $\pm$ 1.1 (13 – 16)	15.6 $\pm$ 0.6 (14 – 16)	17.2 $\pm$ 1.0 (15 – 18)
base of pharynx	21.3 (21 – 22)	23 $\pm$ 1.1 (22 – 25)	27.4 $\pm$ 1.2 (25 – 29)	31.5 $\pm$ 1.3 (29 – 34)
mid-body	22.3 (22 – 23)	24.6 $\pm$ 1.1 (23 – 26)	29.6 $\pm$ 2.4 (26 – 34)	33.2 $\pm$ 1.3 (31 – 35)
anus	15.3 (15 – 16)	17 $\pm$ 1.3 (14 – 18)	20.7 $\pm$ 0.8 (19 – 22)	24.5 $\pm$ 1.3 (22 – 26)

1988). From each sample a few specimens were hand-picked under Olympus SZX 10 stereomicroscope and fixed in DESS (Yoder *et al.*, 2006). Remaining nematodes were heat killed, fixed in TAF, processed to glycerol by a slow evaporation method and mounted on permanent slides. Identification and measurements were made using Olympus BX 51 microscope with Nomarski differential interference contrast, equipped with a digital camera Olympus

DP 72 and computer program Quick PHOTO MICRO 2.3. DNA was isolated using QIAamp DNA Micro Kit (Qiagen) according to the manufacturer's instruction. Both PCR and sequencing were done using primers D2A and D3B (Nunn 1992). PCR conditions were as described by Kornobis *et al.* (2015). Quality of obtained chromatograms was checked in ChromasLite 2.1.1 (Chromas Lite, Technelysium, Queensland, Australia). Sequences were edited in

Fig. 1. Morphology of *L. danuvii* females from Ukraine:

A – lips and amphidial fovea in lateral view; B – lips and amphidial foveae in dorsal view; C, D – vulva region; E-H – variation in tail shape. Scale bar – 20 μ m.

BioEdit (Hall, 1999). Obtained sequences were deposited in GenBank under KT755457 accession number for *L. attenuatus* and KU507542 and KT755458 for *L. danuvii* from Poland and Ukraine, respectively. Subsequently three alignments were made: in first *L. attenuatus* sequence obtained here was aligned with D2-D3 28S rDNA sequence (GenBank accession: AY601572) provided by He *et al.* (2005). In second *L. danuvii* sequences obtained in this study were compared, in third alignment these sequences were aligned with D3 28S rDNA sequences AM412364-66 provided by Barsi *et al.* (2007) Sequences were aligned using MUSCLE (Edgar, 2004) implemented in MEGA 6 (Tamura *et al.*, 2013).

Results and Discussion

Three populations of *Longidorus danuvii* were collected in the city of Lviv. This is the first record of this species from Ukraine. Lviv populations were found in relatively dry conditions, with two populations on the tops of the hills (300 – 350 m.a.s.l.) in the central part of the city, in biotopes distant from watercourses, unlike Serbian and Polish populations, which were found on the banks of Danube and Strwiąż rivers (Barsi *et al.*, 2007; Kornobis, 2012). Additionally, *L. danuvii* was found in the rhizosphere of plants of agricultural

importance (*Malus domestica* Borkh., *Pyrus* sp., *Rubus idaeus* L.) for the first time. It is possible that this species has a negative impact on these plant species, further studies are however required to evaluate that. *Longidorus attenuatus* population (first record for Ukraine) was collected in the garden in rhizosphere of apple tree, forming mixed population with *L. danuvii*.

Morphometrics of both species are given in Tables 1 and 2. Morphology of *L. danuvii* is illustrated on Fig. 1 A-H. Three Lviv populations of *L. danuvii* are morphologically and morphometrically similar to each other. However, some minor morphometric differences are present between them. These populations differ the most clearly in a (mean 101.5 vs 113.5) and c (mean 99.6 vs 120.4) values. Morphometrical characters of Lviv populations are close to the range of variability of type Serbian population (Barsi *et al.*, 2007). Ukrainian populations differ from Serbian ones mainly in thicker body (mean diameter at vulva 37.4 and 37.8 vs 40.5; 42.4 and 43.2 μ m) which results in lower a index values (means 100.1; 102.6 and 113.5 vs 119.8 and 123.5). Other worth mentioning differences among these populations include dimensions and shape of tail, which is shorter in Ukrainian populations (mean 40.1; 42.1 and 41.8 vs 45.2 and 46.5 μ m), has lower c' index (1.45; 1.52 and 1.59 vs 1.7 and 1.85) and somewhat more rounded terminus in

females (Fig. 1 E-H). In comparison with Polish population (Kornobis, 2012) juveniles from Lviv (Citadel Park) are characterized by shorter odontostylet in J4 (76.1 vs 82.6) and shorter replacement odontostylet in all juvenile stages (J1 – 61.0 vs 64.8; J2 – 68.8 vs 74.3; J3 – 77.9 vs 86.0; J4 – 84.8 vs 92.0). No males were present among collected specimens of *L. danuvii*.

Alignment of D2-D3 rDNA marker in *L. attenuatus* contained 712 positions. In comparison with sequence AY601572 obtained by He *et al.* (2005), sequence from Ukraine differed in one nucleotide. Alignment of D2-D3 *L. danuvii* sequences obtained here contained 698 positions, sequences were identical. Finally, alignment of D3 sequences contained 287 positions. In comparison with sequences provided by Barsi *et al.* (2007) sequences from Ukraine and Poland differed in one nucleotide.

References

- BARSI, L., LAMBERTI, F., DE LUCA, F. (2007): Morphological and molecular characterisation of *Longidorus danuvii* sp. n. and *L. silva Roca*, 1993 (Nematoda: Dorylaimida) from Serbia. *Nematology*, 9(4): 585 – 598. DOI: 10.1163/156854107781487279
- BROWN, D. J. F., BOAG, B. (1988): An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. *Nematol. Mediterr.*, 16(1): 93 – 99
- BROWN, D.J.F., TAYLOR, C.E. (1987): Comments on the occurrence and geographical distribution of longidorid nematodes in Europe and the Mediterranean region. *Nematol. Mediterr.*, 15(2): 333 – 373
- EDGAR, R.C. (2004): MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32(5): 1792 – 1797. DOI: 10.1093/nar/gkh340
- HALL, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.*, 41: 95 – 98
- HARRISON, B.D. (1964): Specific nematode vectors for serologically distinctive forms of raspberry ringspot and tomato black ring viruses. *Virology*, 22(4): 544 – 550. DOI: 10.1016/0042-6822(64)90075-3
- HE, Y., SUBBOTIN, S.A., RUBTSOVA, T.V., LAMBERTI, F., BROWN, D.J., MOENS, M. (2005): A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology*, 7(1): 111 – 124. DOI: 10.1163/1568541054192108
- HÜBSCHEN, J., KLING, L., IPACH, U., ZINKERNAGEL, V., BROWN, D., NEILSON, R. (2004): Development and validation of species-specific primers that provide a molecular diagnostic for virus-vector longidorid nematodes and related species in German viticulture. *Eur. J. Plant Pathol.*, 110(9): 883 – 891. DOI: 10.1007/s10658-004-4841-x
- KORNOBIS, F.W. (2012): First record of *Longidorus danyvii* Barsi, Lamberti and De Luca, 2007 (Nematoda: Longidoridae) from Poland with description of pathologies of the lip region and reproductive system. *Helminthologia*, 49(2): 104 – 107. DOI: 10.2478/s11687-012-0021-2
- KORNOBIS, F.W., SUSULOVSKA, S., SUSULOVSKY, A., SUBBOTIN, S.A. (2015): Morphological and molecular characterisation of *Paralongidorus rex* Andrassy, 1986 (Nematoda: Longidoridae) from Poland and Ukraine. *Eur. J. Plant Pathol.*, 141(2): 385 – 395. DOI: 10.1007/s10658-014-0550-2
- NUNN, G.B. (1992). *Nematode molecular evolution*. Ph.D. Dissertation, University of Nottingham, Nottingham, UK
- PENEVA, V.K., SUSULOVSKY, A., LAZAROVA, S. (2009): Description of *Longidorus holovachovi* sp. n. (Nematoda: Dorylaimida) and *Xiphinema* sp., a member of *Xiphinema americanum* group from Ukraine. *Russ. J. Nematol.*, 17(2): 115 – 126
- SINGH, S.K., HODDA, M., ASH, G.J. (2013): Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses. *EPPO Bulletin*, 43(2): 334 – 374. DOI: 10.1111/epp.12050
- TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., KUMAR, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, 30(12): 2725 – 2729. DOI: 10.1093/molbev/mst197
- WHITEHEAD, A.G., HOOPER, D.J. (1970): Needle nematodes (*Longidorus* spp.) and stubby-root nematodes (*Trichodorus* spp.) harmful to sugar beet and other field crops in England. *Ann. Appl. Biol.*, 65(3): 339 – 350. DOI: 10.1111/j.1744-7348.1970.tb05502.x
- YODER, M., DE LEY, I.T., KING, I.W., MUNDO-OCAMPO, M., MANN, J., BLAXTER, M., POIRAS, L. DE LEY, P. (2006): DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology*, 8(3): 367 – 376. DOI: 10.1163/156854106778493448