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MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *Longidorus distinctus* (Nematoda: Longidoridae) FROM ROMANIA

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Abstract

Genus Longidorus Micoletzky, 1922 comprises ectoparasitic nematode species feeding on plant roots. Some of them have considerable economic importance directly as plant pests or indirectly as vectors of nepo-viruses. During an extensive study on longidorids in Romania Longidorus distinctus Lamberti, Choleva and Agostinelli, 1983 was recovered from vineyards (Vitis vinifera L.) in Alba county, alfalfa (Medicago sativa L.) in Prahova county and fruiting shrubs in Arges county. The two populations from Prahova and Arges counties were characterised morphometrically. An integrative approach (morphology, morphometrical and DNA sequencing data) was used to identify and characterise the population from Alba county. The phylogenetic position of the species and the D2-D3 28S rDNA sequence dissimilarity with the closest species were discussed. All studied populations have been compared with other European populations; described from Bulgaria, hitherto L. distinctus has been reported from Serbia, Slovakia, Ukraine, Russia and Poland from arable land and natural habitats. It represents a new geographical record for Romania.

Key words*: 18S rDNA, 28S rDNA D2-D3, COI mtDNA.*

INTRODUCTION

Genus *Longidorus* Micoletzky, 1922 includes a great number of ectoparasitic nematode species that have a broad host range of herbaceous and woody plants within agriculture, horticulture and forestry. Over 180 species belonging to the genus *Longidorus* are considered valid (EPPO, 2020). Currently only three species have been reported from Romania: *Longidorus elongatus* (de Man, 1876) (Popovici, 1973); *Longidorus euonymus* Mali & Hooper, 1974 (Groza et al. 2014) from the rhizosphere of barley (*Hordeum vulgare* L.), strawberry (*Fragaria* x *ananasa* Duch), blackberry (*Rubus fruticosus* L.) cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.) and plum (*Prunus domestica* L.); *Longidorus piceicola* Lišková, Robbins & Brown, 1997 (Groza et al., 2017) from the rhizosphere of *Larix decidua* Mill. and deciduous trees (*Quercus* sp., *Tilia* sp., and *Fraxinus* sp.).

Longidorus distinctus Lamberti, Choleva and Agostinelli, 1983 was originally described from Bulgaria (Lamberti et al., 1983) and recorded from other European countries: Serbia (Barsi, 1989, Krnjaic et al., 1999, Barsi & Lamberti, 2003), Poland (Szczygiel & Zepp, 2004, Kornobis & Sobczyńska, 2017), Russia (Subbotin et al., 2014), Slovakia (Lišková, 2007), Ukraine (Romanenko, 1998).

MATERIALS AND METHODS

Soil samples were collected from the rhizosphere of vine (*Vitis vinifera* L.) from Crăciunelul de Jos, Alba county, (46°10'26.46"N, 23°51'0.05"E); alfalfa (*Medicago sativa* L.), Teleajen, Prahova county, 45°56'4.09"N, 27°18'1.30"E and fruiting shrubs from Mărăcineni, Argeș county, (44°54'5.72"N, 24°48'25.52"E).

Nematodes were isolated from soil samples using Oostenbrink elutriator and Baermann

funnels. Recovered specimens were heat killed at 55°C for two minutes, fixed in a 4% formalin/1% glycerol mixture, processed to anhydrous glycerol (Seinhorst, 1959), and mounted on microscope slides. Photographs were taken using an Axio Imager.M2-Carl Zeiss compound microscope with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8). Measurements were made using an Olympus BX41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA), and computer Digitrak 1.0f programme, (Philip Smith, Scottish Crop Research Institute, Dundee, UK).

DNA extraction, amplification and sequencing

DNA extraction of six juvenile specimens from Crăciunelul de Jos population was performed according to the mammalian protocol of the High Pure PCR template preparation kit (Roche, Switzerland). Nematodes were crushed in Tissue Lysis Buffer using a micro-pestle prior to DNA extraction. Three gene regions, the D2-D3 expansion segments of 28S rRNA gene (large ribosomal subunit), partial 18S rRNA gene (small ribosomal subunit) and the partial mitochondrial cytochrome c oxidase subunit 1 gene (COI) were amplified following the nematode section in the European and Mediterranean Plant Protection Organization (EPPO) DNA barcoding standard (EPPO, 2016). PCR products were cycle sequenced in both directions with the BigDye terminator kit 3.1 (Applied Biosystems, United States) and sequenced on a 3500 genetic analyzer (Applied Biosystems, United States). Trace data were analysed using Geneious v6.1 (BioMatters, New Zealand). Consensus sequences were constructed from the sequence reads, and amplification primers and terminal low quality sequence data were trimmed from the consensus sequence. BLASTn similarity search tool was used to compare each gene fragment with those of other nematode species deposited in the GenBank database. The homologous sequences of D2-D3 28S rDNA nearest to those of *L. distinctus* were aligned using MAFFT algorithm at the GUIDANCE2 Server (http://guidance.tau.ac.il/ (Sela et al., 2015) and manually trimmed and edited using Mega 6 (Tamura et al., 2013). Pairwise sequence identities/similarities were computed using the Sequence Manipulation Suite online (http://www.bioinformatics.org/sms2/) (Stothard, 2000). The analysis involved 17 nucleotide sequences with a total of 638 positions in the final dataset including gaps. The sequences have been deposited in GenBank with the following accession numbers: MW701430 (28S rDNA); MW699618 (18S rDNA), and MW699614 (COI).

RESULTS AND DISCUSSIONS

Longidorus distinctus Lamberti, Choleva and Agostinelli, 1983
Figures 1- 3
Morphometric data of specimens are presented in Table 1 and 2.
Description
Female (Crăciunelul de Jos population). Body posture ventrally curved in the shape of a "C" to a spiral shape, tapering strongly toward posterior end. Head region expanded, slightly set off, anteriorly somewhat flattened. Cuticle thin, 2–2.5 µm thick at postlabial region, 2 µm along the body and 3 µm on tail posterior to anus. Amphidial fovea hardly visible, pouch like, asymmetrically bilobed, with code E3 according to Chen et al. (1997) and type 4 according to Decraemer & Coomans (2007), amphidial aperture assumed to be a minute pore. Lateral chord with glandular bodies. Guiding ring 4 µm wide, odontostyle very slender, 1 µm wide at base, odonthophore slightly swolen at base. Nerve ring behind odontophore base, situated at a distance of 161-183 µm, from anterior end. Pharyngo-intestinal valve heart-shaped to bluntly rounded, 7.5-11 µm long and 9-10 µm wide. Normal arrangement of pharyngeal glands: dorsal opening (DO) and dorsal nucleus (DN) situated at 13.3±1.0 (12.0-14.8)% and 26.8±2.6 (24.7-30.5)% of pharyngeal bulb length, respectively (n=5), nuclei of the left and right ventrosublateral glands situated at 51.2±1.3 (48.5-52.7)% and 50.5±1.2 (48.4-52.2)% (n=7), respectively; opening of the ventrosublateral glands at 85.8±1.5 (82.7-87.0)% of the same distance (terminology following Loof and

Coomans, 1972). Nucleus of the dorsal gland slightly smaller than nuclei of the ventrosublateral glands 1.5-2 and 2.5-3 µm diam. respectively. Vagina extending to *ca.* half the corresponding body width. *Pars distalis vaginae* 11-12 µm long; *pars proximalis vaginae* 12-17 µm long. Uteri of almost equal length, bipartite, the distal part narrower and with granular structure; anterior uterus 151.8±6.5 (140-163), posterior uterus 148.2±6.3 (141-161) µm long, respectively;

Table 1. Measurements of *Longidorus distinctus,* Crăciunelul de Jos population (mean ± standard deviation, with range), in micrometers, except body length (in mm)

Character	**Females**	**J1**	**J2**	**J3**	**J4**
N	n=9	n=9	n=2	n=6	n=6
L	4.8±0.34 4.2-5.3	1.0.2±0.072 0.9-1.1	1.5, 1.7	2.4±0.23 2.1-2.7	3.4±0.478 2.9-4.0
A	109.5±5.6 98.6-117.5	56.2±4.4 51.0-65.8	64.8, 61.9	74.8±8.4 62.1-82.8	87.7±7.5 77.2-94.7
B	13.0±1.1 11.6-14.7	4.9±1.0 3.6-6.1	6.1, -	9.6±1.8 7.8-12.6	12.7±3.1 8.9-17.1
C	78.1±9.4 64.6-91.3	22.9±3.4 19.6-29.6	27.7, 32.1	38.5±2.9 35.1-43.9	55.2±6.0 48.9-63.5
c’	2.1±0.2 1.8-2.4	3.6±0.3 2.9-4.0	- , 2.8	2.9±0.1 2.7-3.0	2.5±0.3 2.1-2.9
V (%)	48.9±2.7 44.9-52.9	-	-	-	-
G1 (%)	6.8±0.6 6.1-8.0	-	-	-	-
G2 (%)	6.9±0.9 5.4-8.0	-	-	-	-
D	2.5±0.1 2.3-2.6	2.6±0.2 2.4-3.0	2.3, 2.3	2.4±0.3 2.1-2.7	2.3±0.1 2.2-2.5
d’	1.6±0.1 1.5-1.7	1.8±0.2 1.5-2.1	1.6, 1.6	1.6±0.2 1.4-1.9	1.6±0.1 1.5-1.8
Anterior end to guiding ring	29.0±1.1 27.5-30.5	17.4±0.9 16-19	19, 20	22.1±1.7 20-24	24.6±1.0 23-25
Odontostyle	75.7±2.0 72-79	46.7±1.4 45-48	50.5, 50	59.0±0.7 58-60	65.9±2.0 64-69
Replacement odontostyle	-	49.6±1.7 47.5-53	57, 58	65.3±1.4 64-67	75.3±1.2 74-77
Odontophore	55.7±4.5 50-64	29.6±3.6 24-36	43.5, 41	43.9±2.0 41-46	53.6±7.0 47-63
Pharynx length	370±23.4 327-409	212.9±38.9 167.5-281.5	246, -	253.4±34.6 204-306.5	277.4±39.4 236-335
Tail	61.9±4.1 56-68	45.0±6.0 33-50	54.5, 54	61.9±5.2 54-68	62.3±7.4 54-74
Length of hyaline part	13.6±1.8 11-16	3.8±1.0 3-6	5.5, 6	6.8±1.0 5-8	10.5±1.8 8-12
Body diameter at: - lip region	11.6±0.5 11-12	6.7±0.3 6-7	8, 9	9.4±0.7 8.5-10	10.8±0.3 10-11
- guiding ring	18.5±0.6 17.5-19	11.9±0.6 11-13	13.5, 14	15.3±0.9 14-16.5	17.2±1.1 16-19
- base of pharynx	37.4±2.1 33.5-41	17.4±1.5 14-20	22, 25	28.4±3.1 24-32	32.5±2.6 29-37
- mid-body/at vulva	43.9±2.7 38-47	18.1±1.4 15-20	23, 28	32.3±5.7 25-41	39.5±7.6 31-53
- anus	29.8±1.8 27-32	12.5±1.0 10-13.5	- , 19	21.6±2.1 18-23.5	25.4±2.4 22-29
- hyaline part	10.5±0.6 10-11	4.0±0.5 3-5	5, 5.5	5.6±0.5 5-6	7.3±1.2 6-9

Table 2. Measurements of *Longidorus distinctus* (mean ± standard deviation, with range), in micrometers, except body length (mm)

Localities	**Teleajen**					**Mărăcineni**
Character	**Females**	**J1**	**J2**	**J3**	**J4**	**Females**
N	18	10	10	6	23	3
L	4.3±0.34 3.6-4.8	1.1±0.05 0.9-1.1	1.4±0.11 1.3-1.7	2.06±0.12 1.9-2.2	3.0±0.31 2.5-3.6	4.4, 4.8, 4.1
A	102.2±10 77.5-114.8	60.0±2.5 54.6-63.6	65.3±4.1 59.9-73.1	76.6±3.8 71.7-82.5	90.6±7.5 74.3-103.8	105, 112, -
B	11.4±1.2 9.1-13.4	5.1±1.0 2.3-5.8	6.1±0.5 5.5-7.4	7.3±1.0 6.1-9.1	9.5±1.0 7.3-11.9	11, 12, -
C	80.0±13 64.5-120.5	24.3±1.2 21.7-25.5	30.2±3.0 26.1-36.0	38.9±2.7 34.7-41.8	54.6±6.6 44.2-67.9	74, 92, 79
c’	2.0±0.2 1.4-2.3	3.5±0.2 3.3-3.8	3.0±0.2 2.7-3.3	2.7±0.2 2.5-3.0	2.3±0.2 1.9-2.6	2, 1.7, 1.8
V (%)	45.1±1.6 42.5-47.5	-	-	-	-	48.4, 45.2, 51.8
G1 (%)	6.8±0.8 5.5-8.4	-	-	-	-	7.1, 6.2, 6.8
G2 (%)	6.4±0.7 5.0-8.0	-	-	-	-	7.3, 5.5, 6.6
D	2.6±0.2 2.4-3.0	2.2±1.0 2.8	2.5±0.2 2.2-2.9	2.4±0.1 2.3-2.4	2.1±0.2 1.8-2.5	2.5, 2.5, 2.3
d'	1.7±0.2 1.5-2.4	1.5±0.8 2.1	2.0±0.2 1.6-2.4	2.0±0.3 1.7-2.6	1.9±0.1 1.6-2.2	1.6, 1.5, 1.5
Anterior end to guiding ring	29.9±1.1 28-31	17.3±0.8 16-19	19.1±0.9 18-21	22.5±1.0 21-24	26.4±1.1 24.0-28.5	30, 30, 28
Odontostyle	77.6±5.4 68-86	49.5±3.1 44-54	51.0±2.745- 54.5	62.4±1.8 60-65	69.9±3.4 63-78	83, 84, 81
Replacement odontostyle	-	52.1±2.3 47-55	61.6±3.1 57-67	70.7±2.0 67-72	79.2±2.7 75-86	
Odontophore	52.6±5.5 41-63	32.1±3.9 24-36	37.5±3.7 31-44	44.9±6.9 37-57	48.9±3.4 43-58	56, 60, -
Pharynx length	382.2±25.6 342.8-423	227.2±76.8 184-443	238.6±17.9 209.4-264.3	288.9±35.7 226.5-326	323.4±29.9 279-408	
Tail	54.7±5.6 38-63	44.7±1.3 42-46	48.4±3.2 43-53	53.3±1.1 52-55	55.7±4.6 46-66	60, 52, 53
Length of hyaline part	14.3±1.8 11-18	4.4±0.8 3-5	5.2±0.8 4-7	5.3±0.8 4-7	8.9±2.8 4-15	12, 11, 12
Body diameter at: - lip region	11.4±0.9 10-13	7.1±0.5 7-8	8.6±0.7 8-10	9.5±0.6 9-10	10.4±0.8 9-12	12, 12, 12
- guiding ring	19.3±2.7 18-30	11.9±0.5 11-13	13.8±0.5 13-14	15.2±0.4 15-16	17.4±2.0 16-26	20, 19, 19
- base of pharynx	35.2±2.5 32-41	18.4±0.7 17-19	22.5±1.3 21-25	26.0±1.5 24-28	29.9±2.9 19-34	36, 33
- mid-body/at vulva	42.5±4.2 38-55	18.1±1.1 16-20	22.4±1.8 19.5-26	27.1±2.2 24-30	33.5±3.1 29.5-38.7	42, 43
- anus	27.5±2.2 23-33	13.2±0.8 12-15	16.1±0.8 15-17.5	19.4±0.8 19-21	24.6±1.7 21-28	29, 30, 28

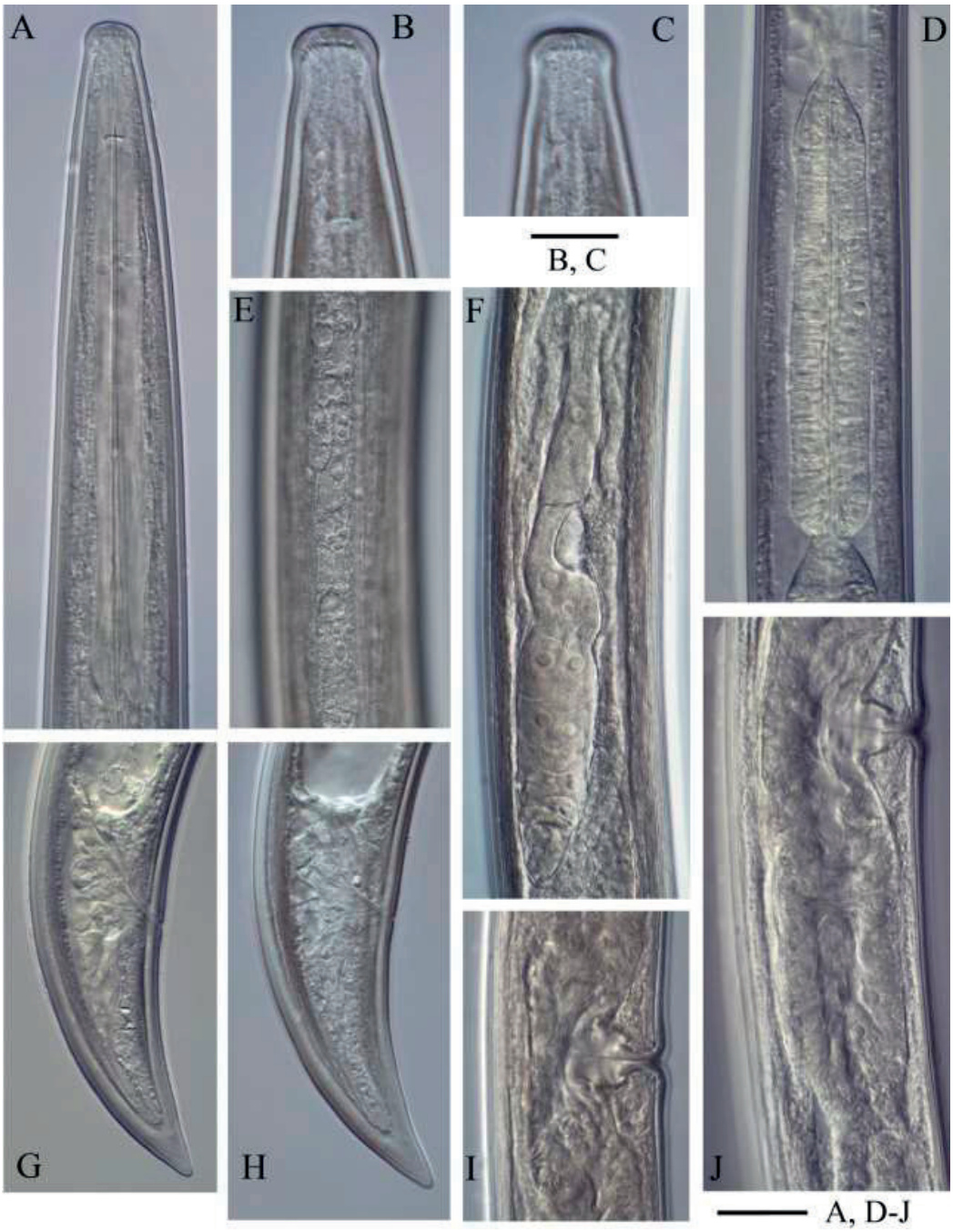

Figure 1. *Longidorus distinctus* Lamberti, Choleva and Agostinelli, 1983, Females: Anterior end (A) Labial region (B), Labial region with amphidial fovea (C) Pharyngial bulb (D) Lateral field (E) Ovary (F) Variations in tail shape, female (G, H) Vagina (I) Vagina and part of posterior reproductive system (J). Scale bars: 20 µm (A, D-J); 12 µm (B, C). (Crăciunelul de Jos population)

Figure 2. *Longidorus distinctus* Lamberti, Choleva and Agostinelli, 1983, Anterior end of first (J1) to fourth (J4) stage juveniles (A–D), Anterior end of female (E) Tail of first to fourth juvenile stages (F–I) Tail of female (J). Scale bars: 20 μm. (Crăciunelul de Jos population)

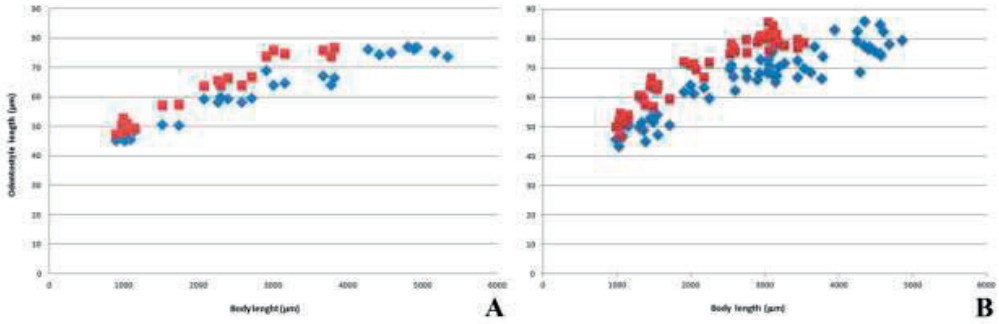

Figure 3. Scatter plot of odontostyle and replacement odontostyle against body length of *Longidorus distinctus,* A) Crăciunelul de Jos population and B) Teleajen population.

well-developed sphincter (8-10 µm) between uterus and weakly developed *pars dilatata oviductus*, *pars dilatata* and uteri not containing sperms. Rectum 26-27 µm or about 0.8-1.0 of body diameter at anus. Tail conical, dorsally convex, ventrally slightly concave, terminus narrowly rounded. Two pairs of lateral pores.

Male. Not found

Juveniles. Morphometrics obtained from juvenile specimens, and of the relationship between the lengths of their functional and replacement odontostyles, and body lengths revealed the presence of four juvenile stages (Figure 3). Habitus does not change significantly during the juvenile development being J shaped to ventrally curved, tail of the first stage juvenile conoid with rounded terminus, often subdigitate; body width at anus level gradually becoming greater and **c'** values becoming progressively lower in subsequent stages.

The codes for identifying *L. distinctus* from Romania when using the polytomous key by Chen et al. (1997) and Peneva et al. (2013) are: A23, B12, C23, D3, E3, F23, G2, H56, I2, J1, K6. Thus, we propose an amendment in the code D originally introduced as D2, since the shape of anterior region of *L. distincus* fits more the code D3 (see Figure 2 I in Chen et al. 1997 as well as the illustrations and descriptions provided by the different authors mention above).

The morphometrics of *L. distinctus* specimens from Romania in general agree with the original (Lamberti et al. 1983) and subsequent descriptions of the species from Bulgaria, Serbia, Slovakia and Poland (Barsi, 1989; Peneva & Choleva, 1992; Krnjaic et al., 1999, Lišková, 2007; Kornobis & Sobczyńska, 2017). Specimens from Slovakia had somewhat longer body compared to the average (av.) body lengths of the type population and our data (av. 5.1 (4.3-5.6) mm *vs* av. 4.6 (3.6-5.3) mm and av.4.8 (4.2-5.3) mm, av. 4.3 (3.6-4.8) mm, while those from Serbia were with a shorter body (L=av. 4.1 (4.09-5.35) mm). Also, the specimens from Slovakia were with highest **a** value when compared with the type population and materials from Romania (av. 127.5 (108-137) *vs* av. 100 (85-127) and av. 109.5 (98.6-117.5), av. 102.2 (75.5-114.8). Studied populations differed slightly in odontostyle length when compared to the type population (av. 75.7 (74-79) and 75.7 (68-86) *vs* av. 80 (71-84) µm; specimens from Kolarovo (Bulgaria) had longer (84-103 µm) while those from Serbia - shorter (av. 74.8 (70-81) odontostyles (Lamberti et al., 1997; Krnjaic et al., 1999). Further, specimens from Romania had long and slender pharyngeal bulb (93-112x16.5-19 µm) *vs* 80-90x20 µm in the type population and materials from Serbia (85-106x18-21) and Poland (85-96x18-20 µm). Some of the populations of *L. distinctus* are characterised with slightly longer tail length (52-68 µm): the type and the population from Petrich, Bulgaria (Lamberti et al., 1997), Crăciunelul de Jos specimens and those from, Serbia (Barsi & Lamberti, 2003) and Poland (Kornobis & Sobczyńska), av. 58 in type population and avs. 59, 61.9, 61.4, 57 and 57.2 µm, respectively. The tail length in specimens from Kolarovo (Bulgaria) (Lamberti et al.,

1997), Serbia (Krnjaic et al., 1999), Slovakia (Lišková) and Telejean (Romania) was somewhat shorter, avs. 52.6, 52.9, 52, 54.7 µm, respectively, and varied from 38 to 65 µm.

Sequence analyses and phylogenetic relationships

The sequencing of the D2-D3 expansion segments of 28S rDNA, the near complete 18S rDNA and the partial COI mtDNA of *L. distinctus* yielded single fragments of 1678, 879 and 393 bp, respectively. The BLASTn search using D2-D3 rDNA sequence revealed 100% identity to sequences of *L. distinctus* from three populations: Slovakia (EF654539, Liskova, 2007), Russia (KF242317, Subbotin et al., 2014) and Poland (KY513282, Kornobis & Sobczyńska, 2017) and 99.86% similarity to another population from Poland (KY513283, Kornobis & Sobczyńska, 2017). In two previous D2-D3 28S rDNA phylogenetic reconstructions (Groza et al., 2017, Cai et al., 2020), the sequence of *L. distinctus* from Slovakia grouped in a well-supported clade (PP=0.9–1.0) with two sequences of *L. juvenilis* Dalmasso, 1969 from Slovakia and Slovenia (AY601579 and DQ364599) and showed close relationships with populations of *L. aetnaeus* Roca, Lamberti, Agostinelli & Vinciguerra, 1986 from Russia and USA and *L. leptocephalus* Hooper, 1961 from Slovenia and UK (Figure 8, Groza et al., 2017). The D2-D3 rDNA sequence dissimilarity between *L. distinctus* and the phylogenetically closest species was 5.3-5.8% (*L. juvenilis*), 5.9-6.5% (*L. aetnaeus*) and 6.21% (*L. leptocephalus*). One nucleotide difference was revealed when comparing 18S rDNA and COI mtDNA sequences of the Romanian population with the corresponding sequences of the Russian population (KF242290, 823 nb length and KY81667, 317 nb length, respectively). The phylogenetic reconstruction based on 18S rRNA gene region showed different phylogenetic relationships of *L. distinctus* (Cai et al., 2020) probably due to the absence of 18S rDNA sequences of *L. juvenilis* and *L. leptocephalus*, and shorter sequence length of *L. aetnaeus*. BLASTn search of COI sequences revealed much lower similarity (≤80%) to sequences with other *Longidorus* species.

CONCLUSIONS

Longidorus distinctus is distributed in Central and south-eastern Europe, reaching Poland to the north, being the most widespread in Bulgaria. It has been recovered more often in agrosystems associated with various crops (tobacco, alfalfa, maize, wheat, grapevine, orchard trees, small fruits, rose, tulip, grapevine, forest nursery seedlings) (Lamberti et al., 1983; Barsi, 1989, Barsi & Lamberti, 2003 etc.) and rarely with natural vegetation (Subbotin et al., 2014; Kornobis & Sobczyńska, 2017).

This species is reported and characterised for the first time from Romania in association with cultivated plants. The studied populations did not differ significantly in morphology and genetically from other materials obtained in different parts of its range. Phylogenetically, *L. distinctus* is close to other three species - *L. juvenilis*, *L. aetnaeus* and *L. leptocephalus*, which are also similar in their morphology. Among them only *L. juvenilis* develops through 3 juvenile stages.

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REFERENCES

Barsi, L. (1989). The Longidoridae (Nematoda: Dorylaimida) in Yugoslavia. I *Nematologia mediterranea, 17.* 97–108.

Barsi, L., Lamberti, F. (2003). Morphometrics of adults and juvenile stages of three longidorid nematodes (Nematoda: Dorylaimida) from Vojvodina province, northern Serbia. *Nematologia mediterranea, 31.* 65–85.

Cai, R., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Palomares-Rius, J.E., Castillo P. (2020). Integrative descriptions and molecular phylogeny of two new needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) from Spain. *European Journal of Plant Pathology*, *156*(1), 67–86.

Chen, Q., Hooper, D.J., Loof P.A.A., Xu J. (1997). A revised polytomous key for the identification of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). *Fundamental and Applied Nematology, 20.* 15–28.

Decraemer, W., Coomans, A. (2007). Revision of some species of the genus *Paralongidorus sensu* Siddiqi *et al.* (1993), with a discussion on the relationships

within the family Longidoridae (Nematoda: Dorylaimida). *Nematology 9*. 643–662. doi: 10.1163/156854107782024776

Dalmasso, A. (1969). Etude anatomique et taxonomique des genres *Xiphinema, Longidorus* et *Paralongidorus* (Nematoda: Dorylaimida). *Mémoires du Muséum National d'Histoire Naturelle. Série A, Zoologie, 61*. 33–82.

EPPO (2020). PM 7/145 (1) *Longidorus diadecturus*. *EPPO Bulletin, 50*. 484–493.

EPPO (2016), PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. *EPPO Bull*, *46.* 501–537.

Groza, M., Lazarova, S., Rosca, I., Peneva, V. (2014). Morphology and distribution of *Longidorus euonymus* (Nematoda) from Romania. *Scientific Papers. Series A. Agronomy, LVII*. 407–414.

Groza, M., Lazarova, S., De Luca, F., Fanelli, E., Elshishka, M., Radoslavov, G., Hristov, P., Coman, M., Peneva, V. (2017). The morphological and molecular identity of *Longidorus piceicola* Lišková, Robbins & Brown, 1997 from Romania (Nematoda, Dorylaimida). *ZooKeys 667*. 1–19. https://doi.org/10.3897/zookeys.667.12011

Hooper, D.J. (1961). A redescription of *Longidorus elongates* (de Man, 1876) Thorne & Swanger, 1936, (Nematoda, Dorylaimidae) and descriptions of five new species of *Longidorus* from Great Britain. *Nematologica*, *6*. 237–257.

Kornobis, F.W., Sobczyńska, U. (2017). Research Note. New data on plant parasitic nematode *Longidorus distinctus* Lamberti, Choleva & Agostinelli, 1983 (Nematoda: Longidoridae) from Poland. *Helminthologia, 54*(2). 179–182.

Krnjaic, D., Krnjaic, S., Lambert, F., Agostinelli, A. (1999). New record of *Longidorus distinctus* Lamberti *et al*., 1983 (Nematoda: Longidoridae) from Serbia. *Nematologia mediterranea*, *27.* 57–61.

Lamberti, F., Choleva, B., Agostinelli, A. (1983). Longidoridae from Bulgaria (Nematoda, Dorylaimida) with description of three new species of *Longidorus* and two new species of *Xiphinema*. *Nematologia mediterranea*, *11.* 49–72.

Lišková, M. (2007). Morphometrics of females, juveniles and a hermaphrodite of *Longidorus distinctus* Lamberti et al., 1983 (Nematoda: Longidoridae) from Slovakia. *Helminthologia, 44*. 210–213. https://doi.org/10.2478/s11687-007-0033-5.

Loof, P.A.A., Coomans, A. (1972). The oesophageal gland nuclei of Longidoridae (Dorylaimida). *Nematologica 18*(2), 213–233. https://doi.org/10.1163/187529272X00458

Mali, V.R., Hooper, D.J. (1974). Observations on *Longidorus euonymus* n. sp. and *Xiphinema vuittenezi* Luc et al., 1964 (Nematoda: Dorylaimida) associated with spindle trees infected with euonymus mosaic virus in Czechoslovakia. *Nematologica, 19*. 459–467.

Peneva, V., Choleva, B. (1992). Nematodes of the family Longidoridae from forest nurseries in Bulgaria. I. The genus *Longidorus* Micoletzky, 1922. *Khelminthologiya*, *32*. 35–45.

Peneva, V., Lazarova, S., De Luca, F., Brown, D.J.F. (2013). Description of *Longidorus cholevae* sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria. *ZooKeys, 330*. 1–26. https://doi.org/10.3897/zookeys.330.5750

Popovici I. (1973). Nematode din sol în fauna României. *St. Cerc. Biol., Seria Zoologie, 25*. 9–15.

Roca, F., Lamberti, F., Agostinelli, A., Vinciguerra, M. T. (1986). *Longidorus aetnaeus*, a new Longidoridae species from Italy. *Nematologia Mediterranea*, *14*. 1–5.

Romanenko, N.D. (1998). Redescription of *Longidorus rubi* Tomilin & Romanenko in Romanenko, 1993 (Nematoda Longidoridae) associated with raspberry in the Poltava region, Ukraine. *Russian Journal of. Nematology, 6*. 185–187.

Sela, I., Ashkenazy, H., Katoh, K., Pupko, T. (2015). GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. *Nucleic Acids Research 43*(Web Server issue), 7–14. https://doi.org/10.1093/nar/gkq443

Stothard, P. (2000). The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. *Biotechniques, 28*. 1102–1104. https://doi.org/10.2144/00286ir01

Subbotin, S.A., Rogozhin, E.A., Chizhov, V.N. (2014). Molecular characterisation and diagnostics of some *Longidorus* species (Nematoda: Longidoridae) from Russia and other countries using rRNA genes. *European Journal of Plant Patholology, 138*. 377–390. https://doi.org/10.1007/s10658-013-0338-9.

Szczygieł, A., Zepp, A. (2004). The association of plant parasitic nematodes with fruit crops in Poland as related to some soil properties. *Fragmenta faunistica*, *47*(1), 7–33.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*(12), 2725–2729. https://doi.org/10.1093/molbev/mst197