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Longidorus profundorum Hooper, 1965 (Nematoda: Dorylaimida) in the Slovak Republic

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Summary

The species *Longidorus profundorum* Hooper, 1965 was for the first time recorded in the territory of Slovakia. It was observed in the rhizosphere of *Fraxineto-Quercetum* forest of riverine plain along Moravia River in south-western part of country, close to boundary with Austria. Morphometrics of females, males and four juvenile stages, morfological and molecular characteristic of Slovakian specimens are presented.

Keywords: Nematoda; Longidoridae; *Longidorus profundorum*; morphology; molecular characteristic; ecology; flooded forest; Slovak Republic

Introduction

Longidorus profundorum was for the first time recorded in England, in the rhizosphere of apple and quince stocks, grasses and bushes (Hooper, 1965). The species is occurring in numerous countries of Europe, e. g. in Switzerland (Klingler *et al*., 1983; Lamberti *et al*., 2001), Spain (Andres & Bello, 1984) Belgium, Bulgaria, France, Germany, Great Britain, Northern Ireland and France (Topham & Alphey, 1985; Brown & Taylor, 1987), and Russia (Prikhodko, 1988; Romanenko, 1994). During investigation of longidorid nematodes in floodplain forests in Slovakia a total of five species were identified – *Longidorus elongatus*, *L. euonymus*, *L. intermedius*, *L. poessneckensis* and *X. diversicaudatum* (Lišková & Sturhan, 2000). Most frequent were *L. intermedius*, *L. poessneckensis* (very often in mixture of these two species) and *X. diversicaudatum*, species which can be considered as indicators for flooded forest. Additional study of the longidorids in floodplain forests along Moravia River provides information about occurrence of the species *Longidorus profundorum*, a new species for the territory of Slovakia.

The morphometrical and molecular characteristic and ecology of the Slovak specimens of L. *profundorum* are presented here.

Material and methods

Locality and habitat

In Slovakia the species *Longidorus profundorum* was recorded in the south-western part of the country, orographic unit Borská, or Záhorská nížina plain, near village Gajary, 48º28´N 16º56´E, in soil of regular waterlogging soils along Moravia River with *Fraxineto-Quercetum* forest type with undergrowth of *Rubus caesius*, *Carex* and *Galium* spp. The sampling site is situated at an altitude of about 150 m, it is characteristic by warm climate with an isotherm of 9.5 ºC, isohyet of about 600 mm and clay-loamy soil with lower admixture of sand and gravel, pH 5.0, soil type Fluvisol derived from river sediments.

Nematological studies

In October 2008 soil samples were collected from the rhizosphere of forest trees with undergrowth. The nematodes were extracted from soil using a sieving and decanting technique (Brown & Boag, 1988). They were fixed by 4 % hot formol and mounted in anhydrous glycerin on permanent slides for examination.

Molecular study

Total genomic DNA was extracted from two single individuals with a rapid technique (Stanton *et al*., 1998). In brief, the tubes containing individual nematodes in 20µl of 0.25M NaOH were incubated overnight at room temperature, thereafter heated to 99 ºC for 3 min. Afterwards 10 µl of 0.25 M HCl, and 5 µl each of 0.5 M Tris-HCl (pH 8) and 2 % Triton X-100 were added and the mixture was

incubated again for 3 min at 99 ºC. Finally, the DNA suspension was cooled and the DNA was used directly for PCR. 18S gene of ribosomal DNA was amplified in two overlapping fragments and primer combination was 988F+1912R for the first fragment and 1813F+2646R for the second fragment (Holterman *et al*., 2006). D2/D3 expansion segments of 28S gene were amplified using D2A and D3B primers (De Ley *et al*., 2005). PCR reactions were performed in a 25 µl volume with the following master mix: one PCR bead (GE Healthcare, Buckinghamshire, UK), 20 µl double distilled sterile water, 2.0 µl each primer (10 pmol/µl) and to this 1.0 µl of DNA was added as a template for PCR. The cycling conditions were: first denaturation for 3 min at 94 ºC, 40 cycles with 30 s at 94 ºC, 30 s at 55 ºC, 30 s at 72 ºC and a final elongation step was run at 72 ºC for 10 min. PCR reaction was separated on 1.5 % agarose gel in Tris-Acetate-EDTA (TAE), stained with syber-safe and visualized with UV illumination (312 nm). Amplicons were separated on agarose gels and recovered from the gel by excision and purified with a QIAGEN gel extraction kit (Qiagen, Hilden, Germany) and directly sequenced in both directions (Macrogen, Korea). Sequencher™ 4.8 (Genes Codes. Corp., Ann Arbor, MI, USA) software was used to assemble and view sequences and check for base-calling errors.

Table 1. Morphometrics of *Longidorus profundorum* from the Slovak Republic (measurements given in µm, except for body length)

Locality	Gajary					
Type of vegetation	*Fraxineto - Quercetum*					
Stage	J_1	J_2	J_3	J_4	Females	Males
n	4	4	6	10	8	3
L (mm)	1.42 ± 0.1 (1.31 – 1.57)	2.35 ± 0.1 (2.14 – 2.45)	3.68 ± 0.3 (3.26 – 3.96)	5.09 ± 0.5 (4.01 – 6.09)	6.87 ± 1.9 (6.48 – 7.77)	6.26 ± 2.9 (5.92 – 6.46)
a	63.7 ± 5.9 (56.6 – 72.5)	66.2 ± 7.6 (59.7 – 76.0)	75.0 ± 4.3 (70.9 – 85.8)	90.8 ± 8.1 (75.1 – 103.3)	103.8 ± 10.1 (90.0 – 122.0)	99.9 ± 4.3 (95.6 – 104.1)
b	4.8 ± 0.3 (4.4 – 5.2)	6.9 ± 0.4 (6.6 – 7.4)	9.6 ± 0.9 (8.5 – 11.0)	13.8 ± 2.7 (10.0 – 19.0)	14.3 ± 1.1 (12.9 – 16.1)	14.3 ± 1.5 (12.8 – 15.8)
c	34.4 ± 2.8 (31.0 – 37.8)	57.5 ± 4.1 (53.6 – 61.3)	82.9 ± 6. (75.4 – 90.1)	112.3 ± 10.8 (91.6 – 126.9)	152.7 ± 15.6 (135.0 – 183.3)	143.9 ± 11.2 (134.5 – 156.3)
c´	2.6 ± 0.2 (2.3 – 2.8)	1.5 ± 0.2 (1.3 – 1.7)	1.2 ± 0.04 (1.1 – 1.22)	1.0 ± 0.08 (0.92 – 1.14)	0.9 ± 0.1 (0.8 – 1.0)	0.9 ± 0.05 (0.8 – 0.9)
V (%)	-	-	-	-	52.1 ± 1.5 (50.5 – 55.2)	-
Odontostyle	53.5 ± 1.7 (52.0 – 56.0)	59.5 ± 2.5 (56.0 – 62.0)	70.6 ± 7.9 (60.0 – 80.0)	84.5 ± 3.5 (80.0 – 90.0)	101.3 ± 5.8 (96.0 – 114.0)	92.3 ± 6.4 (85.0 – 96.0)
Odontophore	40.7 ± 0.9 (40.0 – 42.0)	48.5 ± 1.0 (48.0 – 50.0)	48.2 ± 8.9 (40.0 – 60.0)	58.9 ± 5.8 (48.0 – 68.0)	64.8 ± 4.3 (60.0 – 68.0)	70.0 ± 2.0 (68.0 – 72.0)
Replacement odontostyle	59.4 ± 0.8 (58.0 – 60.0)	74.0 ± 4.0 (68.0 – 76.0)	85.0 ± 2.7 (82.0 – 88.0)	94.6 ± 5.9 (85.0 – 102.0)	-	-
Oral aperture to guiding ring	20.1 ± 0.6 (19.6 – 21.0)	22.7 ± 2.2 (22.0 – 24.8)	26.8 ± 1.9 (24.0 – 28.8)	31.1 ± 1.8 (28.0 – 34.0)	36.0 ± 0.0 (36.0 – 36.0)	37.1 ± 1.6 (35.2 – 38.0)
Length of tail	42.0 ± 4.9 (36.0 – 48.0)	41.0 ± 2.0 (40.0 – 44.0)	44.5 ± 2.9 (40.0 – 49.0)	45.1 ± 3.9 (36.0 – 48.8)	45.1 ± 4.3 (36.0 – 48.0)	43.7 ± 3.8 (41.0 – 48.0)
Length of J (hyaline portion of tail)	9.5 ± 0.9 (8.0 – 10.0)	7.0 ± 0.8 (6.0 – 8.0)	7.0 ± 0.9 (6.0 – 8.0)	7.4 ± 0.8 (6.0 – 8.0)	11.4 ± 0.8 (10.1 – 12.0)	12.9 ± 2.7 (10.8 – 16.0)
Body diam.at lip region	6.8 ± 1.3 (5.0 – 8.0)	8.5 ± 1.4 (6.8 – 10.0)	9.2 ± 1.8 (7.9 – 12.0)	10.7 ± 1.5 (8.1 – 12.0)	12.7 ± 1.5 (12.0 – 14.0)	12.7 ± 1.2 (12.0 – 14.0)
Body diam. at guiding ring	13.7 ± 1.03 (12.0 – 14.8)	16.0 ± 3.6 (12.0 – 20.0)	20.0 ± 0.0 (20.0 – 20.0)	24.1 ± 2.5 (21.0 – 29.0)	29.1 ± 1.7 (27.5 – 32.0)	30.3 ± 2.1 (28.0 – 32.0)
Body diam. at base of oesophagus	22.9 ± 0.7 (22.0 – 24.0)	34.5 ± 4.4 (32.0 – 40.0)	41.5 ± 2.8 (39.2 – 46.0)	49.0 ± 5.4 (40.0 – 60.0)	54.8 ± 4.8 (44.0 – 60.0)	56.0 ± 0.0 (56.0 – 56.0)
Body diam. at mid-body or vulva	22.8 ± 1.6 (20.0 – 24.0)	35.9 ± 4.5 (32.0 – 40.0)	48.0 ± 4.1 (44.0 – 55.6)	56.2 ± 5.8 (46.0 – 68.4)	66.4 ± 7.5 (54.0 – 80.2)	62.7 ± 1.2 (62.0 – 64.0)
Body diam. at anus	15.3 ± 1.9 (12.0 – 17.0)	28.1 ± 3.8 (24.4 – 33.0)	37.5 ± 1.5 (36.0 – 40.0)	44.3 ± 3.3 (40.8 – 48.0)	49.3 ± 3.6 (42.0 – 52.8)	50.7 ± 1.2 (50.0 – 52.0)
Body diam. et beginning of J	9.0 ± 1.0 (8.0 – 10.0)	15.8 ± 4.3 (12.0 – 20.0)	21.5 ± 3.3 (18.0 – 27.0)	26.4 ± 1.4 (24.1 – 28.0)	30.4 ± 2.6 (27.0 – 35.0)	21.3 ± 2.2 (20.0 – 23.8)
Spicules	-	-	-	-	-	74.7 ± 4.2 (70.0 – 78.0)

Results and discussion

Morphology (Fig. 1 – 2).
Morphometrics of females, males and four larval stages of *L. profundorum* from the locality Gajary is presented in Table 1. The measurements and morphology agree well with those of original desriptions of the species from England (Hooper, 1965) and with very detailed description from Switzerland (Lamberti *et al*., 2001). The most important characteristic for Slovak specimens are: Average length of females is 6.9 mm, males 6.3 mm, truncate lip region not set off, amphidial pouches pocket-like distinct bilobed, characteristic pouches are distinct already at the first stage juvenile (Fig 1E – F). They are extended till about 60 % of the distance between oral aperture to guide ring. Odontostyle of the length of about 101 μm (females) and 92 μm (males), vulva slightly posterior to mid body, occupying about 65 % of body diameter, gonads paired, opposed and reflexed, anterior branch of the length of about 650 μm, posterior of 550 μm. Tail roundly conoid, short, c´ about 0.9. Male tail with prolongated hemi-elliptical terminus, length of spicules of about 75 μm, number of supplements 13 – 15. The presence of three expressive caudal papillae at one male was observed (Fig. 2). Proportion of males to females was 1 : 3. The first larval stage is characteristic by a subdigitate tail, other three stages juvenile are with conoid rounded tail, shortening from second to fourth stage juvenile, corresponding with index c´= 2.6 at first to c´= 1.0 at forth stage juvenile.
At investigated sampling site the species *L. profundorum* was occurring in a mixture with second one longidorid species *L. helveticus*. The most important morphological

Fig. 1. *Longidorus profundorum*: A – female, anterior region; B – female, posterior region; C – female, vulva region; D – male, posterior region; E – J_1, anterior region; F – J_1, posterior region

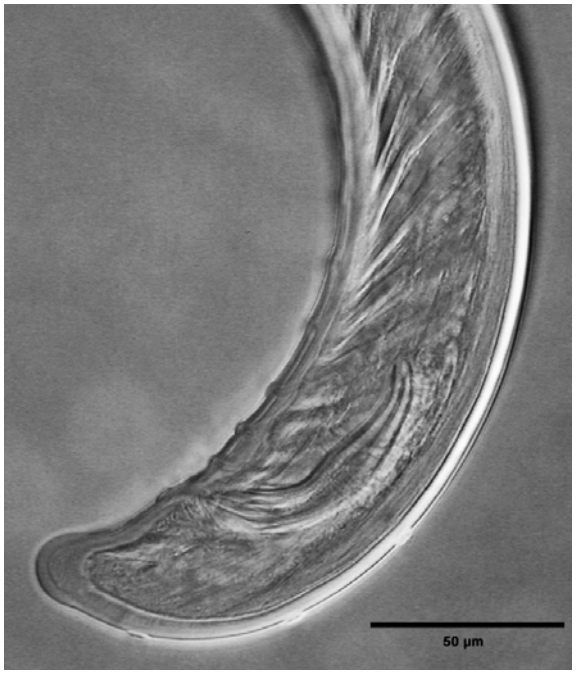

Fig. 2. *Longidorus profundorum*: Mail, posterior region with three distinct caudal papillae

differences in comparison with *L. helveticus*: At *L. helveticus* body is slightly longer, much robust, head more rounded, amphidial pouches not bilobed. Important difference is in the length of odontostyle, at *L. helveticus* it was 144 µm and at females and 139 µm at males *vs*. 101 µm at females and 92 µm at males at *L. profundorum*. Female tail shorter, blant rounded, at males tail not with distict prolongated hemi-elliptical terminus, much longer length of spicules (102 µm *vs*. 75 µm). The tail of first stage juvenile digitate at both species, but at *L. helveticus* with much longer hyaline portion (18.6 µm *vs*. 9.5 µm).

Molecular characteristic

The D2/D3 expansion segments of 28S gene and 18S gene of ribosomal DNA were sequenced from two single individuals. Identical sequences were obtained for both individuals for each gene. The sequences were compared by Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) and the result showed 100 % identity with *L. profundorum* accession number AF480073 (Rubtsova *et al.,* 2001) for D2/D3. Though top fits of sequence of 18S gene (accession number JQ359005) from the same DNA of single individual nematode were to *Longidorus*, but the sequences of this specimen is different from already deposited sequence of *L. profundorum* accession number EU503143 (Pedram *et al.,* 2008). Sequence of D2/D3 expansion segments is not deposited on Genbank because it is 100 % identical to accession number AF480073.

Notices to ecology

During a large nematological study of the occurrence of longidorid nematodes in various types of vegetation (Lišková & Brown, 2003), including investigation in floodplain forests (Lišková & Sturhan, 2000) in Slovakia, the occurrence of *L. profundorum* was not recorded. Recently, the species was observed in additional soil samples from flooded forest along Moravia River, between Slovakia and Austria. After numerous informations throughout the Europe, it seems that the species is preferring cultivated soils with fruit orchards and vineyards, or fruit and grapevine nurseries (Hooper, 1965; Klingler *et al*.; 1983, Bleyer & Rüdel, 1996; Hübschen *et al*., 2005; Romanenko, 1994), but the species was recorded also in arable soil with legumes and cereals (Andres & Bello, 1984) and in rhizosphere of grasses and bushes (Hooper, 1965) and in soil of unspecified natural habitats (Brown & Taylor, 1987). In agreement with Andres and Bello (1984) the species was also occurring in Slovakia in similar more heavy clay-loamy soils. The area of floodplain forests along Moravia River, similar to other rivers, it is characteristic by variability of soil types (depended on origin of river sediments) from light sandy, very often gravelled soils to heavy clay soils. At the same locality Gajary during our previous study of longidorids in flooded forests in light sandy soils we recorded the species *L. poessneckensis* in mixture with *Xiphinema diversicaudatum* (Lišková & Sturhan, 2000). Present observation of *L. profundorum* in floodplain forests indicates variability in occurrence of longidorids in this type of ecosystem.

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