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The notes on the occurrence of *Longidorus poessneckensis* Altherr, 1974 (Nematoda: Dorylaimida) in the Slovak Republic

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Summary

The record of occurrence of *Longidorus poessneckensis* Altherr, 1974 in forest soils with *Betuleto-Carpineto-Quercetum* in geographically specific conditions of a hill-top contributes to the broadening of ecological knowledge about this species. To date, the species was considered to prefer the habitats along river banks - narrow or large valleys and plains. The ecological, morphometrical and molecular characteristics of recorded *L. poessneckensis* are presented here.

Keywords: *Longidorus poessneckensis*, ecology, morphology, molecular characteristic, Slovak Republic

Introduction

According to current knowledge, the longidorid species *Longidorus poessneckensis* Altherr, 1974 mostly occurs in permanent or seasonal moist soils with various water courses, soils with woodland, mostly associated with *Fraxinetum, Ulmetum* and *Quercetum* or in river banks associated mostly with *Alnetum* and *Salicetum* vegetation types. In the European geographical conditions the occurrence of this species is associated with soils derived from river sediments along the River Danube in Slovakia and March in Austria (Lišková & Sturhan, 2000; Tiefenbrunner & Tiefenbrunner, 2004 resp.), the Rivers Bodrog, Latorica, Tisa and other rivers in Slovakia (Lišková & Sturhan, 2000; Lišková, 2001; Lišková & Čerevková, 2005) and in Germany in soils near or along numerous ri-vers and small brooks (Altherr, 1974; Rau, 1975, 1976; Sturhan, 1995; Sturhan & Loof, 1995). The most recent information is from the Czech Republic (Kumari *et al*., 2009) where the species has been found in the rhizosphere of *Quercus* forest on a plain, probably with higher and fluctuant tailwater level. Surprisingly, this species was found in a soil sample collected from the *Betuleto-Carpineto-Quercetum* forest on the small upland plain on the top of Dariusova hora hill near the city of Košice in eastern Slovakia. The notes on ecology following from the occurrence of the species *L. poessneckensis* in this unusual site on the hilltop and morphometrical and molecular characteristics of this nematode population are presented here.

Material and methods

Characteristic of sampling area

The sampling area is situated in *Betuleto-Carpineto-Quercetum* forest in the vicinity of Ťahanovce village, a suburbs of Košice City (48°44´N, 21°15´E), an orographic unit of Čierna hora mountains on the boundary with the Košická kotlina basin. Being the part of the Košice Forests, it is not only the logging area, but a green belt serving as a rest area with numerous pathways and cycling routes too. This hilly area has an altitude of about 400 m, with an average annual temperature of 8.4°C and annual rainfall of 420 mm. The sampling site was situated in partly moist or wet upland plain on the top of Dáriusova hora hill, with Luvisol or Gleyc Luvisol soil type, clay-loamy soils with sand and gravel admixture of various granularity, soils derived from neogen lacustrine and river sediments. The pH of the soil was 6.0 – 7.0.

Nematological studies

In May 2009, the soil samples were collected from the depth of app. 20 cm from the rhizosphere of forest trees (5 soil samples from the area of 10 x 10 m). Soil was then mixed and the nematodes were extracted from 500g of the mixed soil using a sieving and decanting method (Brown & Boag, 1988), fixed by hot TAF and mounted in anhydrous glycerin on slides for examination. Figures were produced using Camera Lucida.

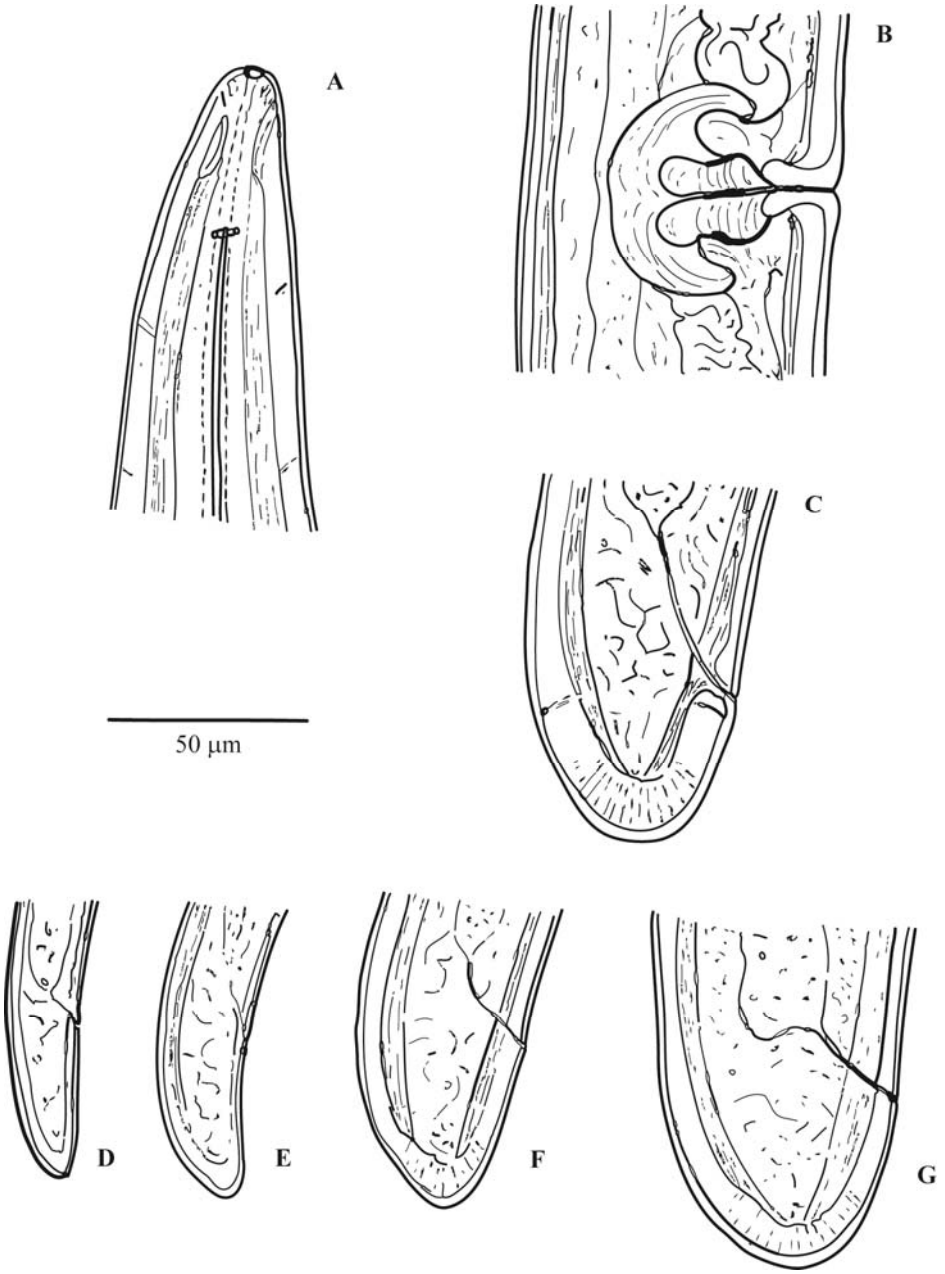

Fig. 1. *Longidorus poessneckensis*. A – C: Female, A - anterior region; B – vulva region; C - tail; D – G: Tails of juveniles D - J1; E – J2; F – J3; G – J4

A few alive specimens were preserved in IM NaCl for sequencing. Temporary mounts of two nematodes were made in a droop of IM NaCl containing glass beads and after taking photomicrographs the slides were dismantled, individual nematodes removed, and added to digest in 0.25M NaOH overnight. Total genomic DNA was extracted from these two single individuals using a rapid technique (Stanton *et al*., 1998). 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 assays were performed in a 25 µl volume with the following master mix: one PCR bead (GE Healthcare, Buckinghamshire, UK), 20.5 µl double distilled sterile water, 2.0 µl each primer (10 pmol/µl) and to this 0.5 µl of DNA was added as a template for PCR. The cycling conditions were: first denaturation for 3 min at 94°C, 40 cycles with 30 s at 94°C, 30 s at 55°C, 30 s at 72°C and a final elongation step was run at 72°C for 10 min. DNA was purified using High Pure Product Purification kit (Roche Diagnostics GmbH, Manheim, 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.

Results and discussion

Notes to ecology

Up to date in Slovakia, the species *L. poessneckensis* occurred exclusively in geographically low-lying sites, i.e. river banks, smaller or larger riverine plains and valleys. The records were mostly from flooded forests, near rivers or streams with vegetation mostly represented by *Fraxinetum, Ulmetum, Quercetum*, *Alnetum* and *Salicetum* vegetation types. Recently, this species was surprisingly found in the soils sample collected at the hilltop with *Betuleto-Carpineto-Quercetum* vegetation. A question has arisen concerning the locality. It is possible to speculate about a linkage between geographical distribution of the nematodes and former geological events during which the area was created by neogeon or river sediments and thus this species has been distributed in this enclave – a hilltop. Weischer and Almeida (1995) have emphasized the importance of geological processes for regional distribution of longidorids. Lišková (2001) has expressed the assumptions for association of some individual longidorid species with specific soil type derived from characteristic mother rock of specific geological origin. Moist soil conditions of the sampling site (small upland plain) also support the aforementioned assumptions. The association of *L. poessneckensis* with this type of soil is in an agreement with the observations of Sturhan and Loof (2001), Tiefenbrunner and Tiefenbrunner (2004) and Lišková and Sturhan, (2000), Lišková (2001), Lišková and Čerevková (2005).

Notes to morphology

(Fig. 1, A – G, Fig. 2, A - D)

The morphometric parameters of females and four larval stages are presented in Table 1. Males were not observed. It is necessary to note that during sampling time in May a surprisingly high portion of L1 was observed (about 30 % out of all specimens). This is especially important as during previous investigations we have had difficulties to collect sufficient amount of L1 for a morphometric study.

Comparing the current data on the population with the previously obtained data from Slovakia (Lišková & Sturhan, 2000), Austria (Tiefenbrunner & Tiefenbrunner, 2004), Germany (Altherr, 1974; Rau, 1975, 1976; Sturhan, 1995; Sturhan & Loof, 2001) and from the Czech Republic (Kumari *et al*., 2009) there are some little variations in several morphometric parameters. For instance the L, a, b, c, the length, body size and odontostyle of females and other parameters differ slightly from an original description given by Altherr (1974), but they agree well with data in „redescription of the species“ given by Sturhan and Loof

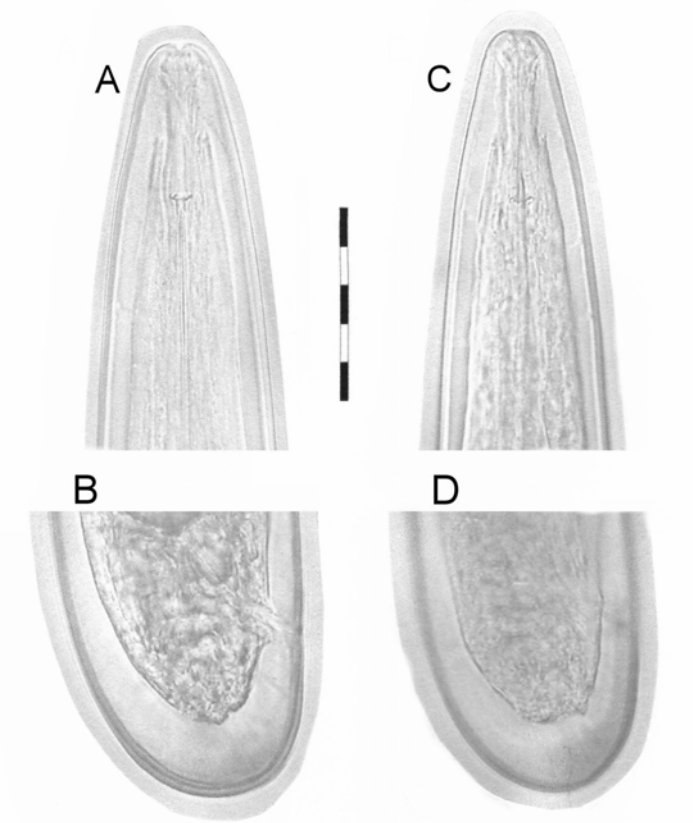

Fig. 2. *Longidorus poessneckensis* - specimens used for sequencing. A, B: Anterior and posterior region of first individual; C, D: Anterior and posterior region of second individual (Scale bar - 50µm)

Table 1. Morphometrics of *Longidorus poessneckensis* (all measurements in µm, except L in mm)

Locality	Ťahanovce near Košice City				
Host	*Betuleto-Carpineto-Quercetum*				
Stage	J1	J2	J3	J4	Females
n	15	11	14	9	9
L	1.49 ± 0.08 (1.39 – 1.70)	2.39 ± 0.32 (2.07 – 3.17)	3.57 ± 0.52 (2.90 – 4.56)	4.82 ± 0.46 (4.38 – 5.78)	6.69 ± 0.46 (6.01 – 7.36)
a	57.6 ± 4.70 (53.3 – 63.3)	61.7 ± 7.40 (52.3 – 72.8)	71.2 ± 6.99 (60.4 – 87.6)	78.3 ± 7.66 (71.6 – 85.0)	90.8 ± 7.24 (75.2 – 99.2)
b	5.2 ± 0.98 (4.0 – 7.6)	6.9 ± 1.18 (5.5 – 9.6)	8.1 ± 0.92 (6.5 – 9.7)	9.9 ± 1.28 (8.8 – 12.6)	11.8 ± 0.94 (10.5 – 13.0)
c	37.8 ± 2.63 (33.5 – 41.7)	54.0 ± 5.40 (47.5 – 66.0)	84.3 ± 16.29 (71.2 – 111.0)	112.8 ± 11.28 (100.5 – 134.4)	158.6 ± 13.12 (142.7 – 186.3)
c′	2.03 ± 0.20 (1.79 – 2.50)	1.41 ± 0.13 (1.20 – 1.69)	1.04 ± 0.21 (0.82 – 1.69)	0.90 ± 0.13 (0.73 – 1.10)	0.78 ± 0.05 (0.69 – 0.85)
V (%)	-	-	-	-	53.1 ± 1.40 (50.4 – 55.0)
Odontostyle	69.3 ± 2.30 (66.0 – 72.0)	82.6 ± 3.15 (72.0 – 88.0)	109.2 ± 7.60 (98.0 – 120.0)	126.6 ± 3.92 (120.0 – 136.0)	140.2 ± 4.47 (132.0 – 148.0)
Odontophore	38.6 ± 1.93 (36.0 – 42.0)	42.0 ± 1.63 (40.0 – 44.0)	48.4 ± 8.83 (36.0 – 57.0)	52.7 ± 8.83 (48.0 – 68.0)	68.3 ± 11.10 (53.0 – 80.0)
Replacement odontostyle	77.8 ± 3.12 (71.0 – 80.0)	103.6 ± 5.82 (96.0 – 116.0)	122.5 ± 4.42 (112.0 – 128.0)	136.0 ± 4.14 (130.0 – 141.0)	-
Oral aperture to guide ring	19.1 ± 0.92 (16.8 – 20.0)	24.4 ± 1.12 (23.0 – 27.6)	30.6 ± 2.21 (28.0 – 33.0)	36.1 ± 1.52 (34.0 – 38.0)	39.6 ± 1.26 (38.0 – 40.0)
Tail length	39.5 ± 2.32 (36.0 – 44.0)	44.2 ± 1.40 (40.0 – 49.0)	43.3 ± 3.27 (36.0 – 48.0)	42.8 ± 1.62 (40.0 – 44.0)	42.3 ± 3.03 (36.0 – 44.0)
Length of hyaline tip J	7.3 ± 0.92 (5.8 – 8.4)	7.2 ± 1.40 (5.0 – 10.0)	10.4 ± 1.63 (8.0 – 12.0)	11.6 ± 2.22 (9.0 – 15.8)	16.1 ± 2.31 (13.0 – 20.0)
Body diameter at lip region	6.9 ± 0.56 (6.5 – 8.0)	7.7 ± 0.70 (7.0 – 8.8)	10.3 ± 1.18 (8.0 – 12.0)	11.6 ± 0.83 (11.0 – 13.0)	14.7 ± 0.82 (14.0 – 16.0)
Body diam. at guid. ring	14.3 ± 1.03 (12.5 – 16.0)	16.7 ± 1.67 (15.5 – 20.0)	22.9 ± 1.77 (20.0 – 26.0)	27.1 ± 1.97 (24.0 – 30.0)	31.1 ± 1.79 (28.0 – 33.2)
Body diam. at base of oesophagus	25.5 ± 2.61 (24.0 – 32.0)	36.7 ± 3.86 (30.4 – 43.0)	45.9 ± 3.17 (42.0 – 52.0)	54.5 ± 5.16 (46.0 – 60.0)	61.2 ± 2.93 (56.0 – 64.8)
Body diam. at mid body/vulva	26.18±3.13 (24.0 – 32.0)	39.1±4.55 (32.0 – 47.0)	50.8±6.39 (38.5 – 64.0)	63.0±5.58 (54.0 – 68.0)	73.8±4.67 (64.0 – 80.0)
Body diam. at anus	19.6 ± 1.45 (16.0 – 22.8)	31.2 ± 1.39 (23.0 – 40.0)	43.6 ± 2.69 (39.5 – 48.0)	53.6 ± 5.46 (48.0 – 65.0)	54.6 ± 3.05 (48.0 – 58.0)
Body diam. at beginning of J	12.1 ± 0.92 (10.0 – 12.2)	17.7±2.31 (14.0 – 20.0)	29.9±3.35 (26.0 – 36.0)	36.9±3.54 (30.0 – 40.0)	41.8±2.52 (36.0 – 44.0)

(2001) and with those from the Czech Republic (Kumari et al., 2009). The most noticeable differences observed in the population from our sampling site (the hilltop of Dáriusova hora) are morphometric parameters of L1. The L1 larvae were of smaller size (some below 1.40 mm) with a shorter odontostyle (66µm) and c´= 2.50 (in comparison to maximum c´ from the Czech Republic 1.91 and from Germany 2.2), meaning that at some populations the L1 can be characterized by a larger and a slightly conical rounded tail (Fig. 1 D).

Notes to molecular analysis

Head and tail shape of the two specimens used for sequencing is presented in Fig. 2. The D2/D3 expansion segments of 28S gene and 18S gene of ribosomal DNA were sequenced from these two single individuals. Identical sequences were obtained for both individuals for each gene. The obtained 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. poessneckensis* accession number EF538750 for D2/D3 and accession number EF538745 for 18S gene.

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