Morphometrics of females, juveniles and a hermaphrodite of *Longidorus distinctus* Lamberti *et al.*, 1983 (Nematoda: Longidoridae) from Slovakia

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Summary

A population of *Longidorus distinctus* Lamberti, Choleva & Agostinelli, 1983 was recovered from soil samples collected from the rhizosphere of plum trees at Kráľovský Chlmec in south-eastern Slovakia. The area is characterized by sandy soil derived from dune sand. The population comprised females, juveniles of four developmental stages and a bisexual specimen. No males were found. It is the first record of this species from Slovakia. Two females were sequenced and the sequence was deposited in GenBank.

Key words: *Longidorus distinctus*; females; hermaphrodite; juvenile developmental stages; plum; sandy soil; Slovak Republic; Ribosomal DNA; 28S gene; PCR

Introduction

Observations of hermaphroditism in longidorid nematodes are rather scarce. This phenomenon was observed in *Longidorus macrosoma* Hooper, 1961 (Aboul-Eid & Coomans, 1966), *L. africanus* Merny, 1966 (Cohn & Mordechai, 1968), *L. elongatus* (deMan, 1876) Thorne & Swanger 1936, including variability between hermaphrodite specimens (Raschké & Boag, 1981), *L. distinctus* Lamberti, Choleva & Agostinelli, 1983 (Krnjaic *et al.*, 1999), *L. helveticus* Lamberti, Kunz, Grunder, Molinari, De Luca, Agostinelli & Radici, 2001 (Barsi & De Luca, 2005) and *Xiphinema ingens* Luc & Dalmasso, 1964 (Lamberti *et al.*, 1975).

During an investigation of occurrence and geographical distribution of plant parasitic nematodes in fruit orchards in south-eastern area of the Slovak Republic, a population of *L. distinctus* was found, and it is the first record of this species from Slovakia. The region is characterized by warm and dry climate with 10 °C annual average temperature, 500 – 600 mm annual average rainfall, an altitude about 100 m and by sandy soils derived from dune-sand.

This population of *L. distinctus*, which consisted of females, juveniles and a hermaphrodite specimen, occurred mixed with *Xiphinema vuittenezi* Luc, Lima Weischer & Flegg, 1964, a longidorid species very frequent in specific drift sand landscape throughout Slovakia. Morphometrics of adults, juveniles and a hermaphrodite specimen are reported here to provide additional information on their natural variability.

Material and Methods

Morphological and morphometrical study

Soil samples were collected from the rhizosphere of plum (*Prunus domestica* L.) at the depth of 20 – 30 cm, from the locality of Kráľovský Chlmec, in the area of Východoslovenská rovina plain. Nematodes (14 females, 47 juveniles and 1 hermaphrodite) were extracted from 500 g of soil by a sieving and decanting method (Brown & Boag, 1988), fixed in FAA and mounted on permanent slides in anhydrous glycerin. Nematode identification was performed by using a high-resolution light microscope with Nomarski differential interference contrast (DIC, Nomarski), photomicrographs were recorded with a digital camera linked to a computer, and measurements were made with the aid of an eyepiece scale. Due to deformation, measurements of some of the juveniles and females are not provided in Table 1.

Molecular study

For the molecular study two female specimens were separately added in 0.5 ml Eppendorf microtubes containing 20 µl of 0.25M NaOH under a binocular microscope and DNA was extracted according to a method described by Stanton *et al.* (1998). The DNA was used for polymerase chain reaction (PCR) directly after extraction. The D2 and D3 expansion regions of the large subunit (28S) of ribosomal DNA were amplified using the primer D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-

TCGGAAGGAACCAGCTACTA-3' (De Ley *et al.*, 1999). The relevant PCR and gel electrophoresis conditions were as described by Kumari *et al.* (2005). The region was sequenced after purification of PCR products from the gel slice with a Qiagen gel purification kit (Qiagen Inc., Valencia, CA). Purified DNA fragments were sequenced directly using forward and reverse primers.

Results and Discussion

Morphology and morphometrics

Longidorus distinctus Lamberti, Choleva & Agostinelli, 1983

Female: body of medium size (4.3 – 5.6 mm), slender,

ventrally curved in an open C, tapering towards, evident glandular structure in hypodermal cords. Cuticle 2.1 – 2.4 μm thick. Lip region laterally rounded, 10.0 – 12.0 μm wide and 4.2 – 4.8 μm high, offset from the rest of body by a slight depression. Amphidial pouches asymmetrically bilobed, reaching to half of anterior end to guide ring distance. Odontostyle, odontophore and guide ring typical of the genus. Oesophageal bulb measuring 98 – 112 x 17 – 21 μm . Cardia small, slightly conoid. Vulva slit like; gonads amphidelphic with anterior branch 264 – 345 μm long and posterior branch 236 – 354 μm long. Ovaries reflexed; anterior reflexed part 120 – 180 μm and posterior 108 – 178 μm long. Uteri consist of two distinct parts, *i.e.* with and without particular characters: 44 – 61 μm and 80 – 108 μm

Table 1. Morphometrics of *Longidorus distinctus* from Slovakia. All measurements in μm except L (in form): mean $\pm$ standard deviation (range).

Locality Host	Kráľovský Chlmec Plum					
Specimens	J1	J2	J3	J4	Females	Intersex
n	1	3	6	6	12	1
L (mm)	1.2	1.5 $\pm$ 0.1 (1.4 – 1.6)	2.2 $\pm$ 0.1 (2.1 – 2.4)	3.6 $\pm$ 0.3 (3.1 – 3.9)	5.1 $\pm$ 0.4 (4.3 – 5.6)	4.3
a	58.6	67.6 $\pm$ 3.0 (64.2 – 69.9)	92.1 $\pm$ 8.8 (83.9 – 99.1)	103.6 $\pm$ 8.8 (88.6 – 111.2)	127.5 $\pm$ 8.8 (108.1 – 136.9)	108.0
b	6.4	7.3 $\pm$ 0.3 (7.1–7.6)	8.6 $\pm$ 1.7 (6.1–9.5)	10.8 $\pm$ 1.5 (8.9–12.5)	13.7 $\pm$ 1.6 (11.3–15.9)	13.2
c	30.6	39.9 $\pm$ 5.8 (35.5 – 46.6)	40.3 $\pm$ 2.5 (36.6 – 43.6)	62.8 $\pm$ 6.6 (53.9 – 70.7)	97.7 $\pm$ 9.9 (77.2 – 108.0)	70.0
c'	3.2	2.7 $\pm$ 0.4 (2.2 – 2.9)	3.0 $\pm$ 0.3 (2.8 – 3.4)	2.3 $\pm$ 0.3 (2.0 – 2.7)	1.9 $\pm$ 0.2 (1.6 – 2.2)	1.9
V	-	-	-	-	47.8 $\pm$ 0.7 (47.2 – 49.5)	47.0
Odontostyle	41.0	48.7 $\pm$ 0.6 (48.0 – 49.0)	61.0 $\pm$ 4.7 (52.0 – 64.0)	73.5 $\pm$ 3.1 (70.0 – 78.0)	82.3 $\pm$ 2.3 (79.8 – 84.0)	80.0
Odontophore	30.0	34.6 $\pm$ 0.6 (34.0 – 35.0)	38.0 $\pm$ 2.5 (32.0 – 44.0)	45.2 $\pm$ 2.2 (43.0 – 48.0)	43.1 $\pm$ 3.2 (40.0 – 48.2)	48.0
Replacement odontostyle	44.0	54.0 $\pm$ 2.0 (52.0 – 56.0)	67.2 $\pm$ 5.2 (58.0 – 72.0)	79.7 $\pm$ 2.1 (76.0 – 82.0)	-	-
Oral aperture to guide ring	18.4	18.7 $\pm$ 0.6 (18.0 – 19.0)	22.0 $\pm$ 1.8 (20.0 – 24.4)	26.0 $\pm$ 1.6 (24.0 – 28.0)	29.5 $\pm$ 1.2 (28.0 – 32.0)	28.0
Tail	38.0	40.0 $\pm$ 4.0 (36.0 – 44.0)	55.8 $\pm$ 2.7 (52.8 – 60.0)	54.9 $\pm$ 2.4 (52.0 – 58.0)	52.0 $\pm$ 2.5 (48.0 – 56.0)	62.0
J (hyaline portion of tail)	2.0	2.7 $\pm$ 1.1 (2.0 – 4.0)	5.8 $\pm$ 1.8 (4.0 – 8.0)	8.2 $\pm$ 2.7 (4.0 – 10.0)	13.5 $\pm$ 2.3 (10.2 – 16.4)	16.0
Body diam. at lip region	6.4	7.7 $\pm$ 0.6 (7.0 – 8.0)	7.8 $\pm$ 0.4 (7.0 – 8.0)	9.3 $\pm$ 1.5 (7.8 – 12.0)	11.2 $\pm$ 0.9 (10.0 – 12.0)	11.6
Body diam. at guide ring	11.9	13.2 $\pm$ 2.4 (11.7 – 16.0)	13.3 $\pm$ 1.1 (11.5 – 14.0)	16.4 $\pm$ 0.9 (15.5 – 18.0)	17.6 $\pm$ 1.1 (16.0 – 19.0)	17.4
Body diam. at base of oesophagus	19.3	19.7 $\pm$ 0.6 (19.0 – 20.0)	24.3 $\pm$ 0.9 (23.6 – 26.0)	30.4 $\pm$ 1.7 (28.0 – 32.0)	34.0 $\pm$ 1.8 (32.0 – 36.0)	38.0
Body diam. at mid body or vulva	19.6	22.3 $\pm$ 0.6 (22.0 – 23.0)	25.1 $\pm$ 1.8 (24.0 – 28.0)	34.4 $\pm$ 1.6 (32.0 – 36.0)	39.8 $\pm$ 2.9 (36.0 – 46.0)	48.0
Body diam. at anus	12.0	15.0 $\pm$ 1.0 (14.0 – 16.0)	18.4 $\pm$ 1.7 (16.0 – 20.0)	23.6 $\pm$ 2.1 (21.0 – 27.2)	27.8 $\pm$ 1.8 (24.0 – 32.0)	35.0
Body diam. at beginning of J	4.0	4.7 $\pm$ 0.5 (4.0 – 5.2)	5.1 $\pm$ 1.4 (4.0 – 7.9)	7.2 $\pm$ 1.6 (5.6 – 10.0)	10.5 $\pm$ 1.6 (10.0 – 12.0)	10.0
Spicules	-	-	-	-	-	44.0
Mucro	3.0	-	-	-	-	-

Fig. 1. *Longidorus distinctus*. A, B, C, E - Intersex specimen: A - Entire nematode; B - Anterior region; C - Posterior region; E - Vulva; D - Posterior region of normal female. Scale bar: A: 500 µm; B-E: 50 µm

in anterior branch and 52 – 77 µm and 81 – 120 µm in posterior branch, respectively. Spermatheca rounded, 17 – 30 µm wide, sometimes covered by reflexed ovary. Tail dorsally convex, conoid, with gently rounded tip, bearing two pairs of caudal pores. Sometimes after beginning of hyaline portion more tapering cca 20 µm from the end. Juveniles: separated into four developmental stages. In comparison to adults they are similar in body shape, which is slender, slightly arcuate; tail in all stages elongated, conoid, dorsally convex, with slightly rounded tip, in J1 with very slightly distinguished 3 µm long mucro. Males: not found.

Intersex: similar to female, 4.3 mm long, ventrally more coiled in the posterior part. Lip region 11.6 µm wide and 5 µm high. Oesophageal bulb measuring 89 x 20 µm. Vulva well developed, in shape related to female vulva. Anterior genital branch 224 µm and posterior branch 264 µm long, respectively. Anterior uterine part 92 µm long; part with particular characters indistinct. Posterior uterine part without particular characters 88 µm long, part with particular characters 43 µm long. Spermatheca and ovary of anterior and posterior genital branches indistinct. The male reproductive system reduced, spicules arcuate, well developed, 44 µm long. The adanal pair of supplements preceded by a row of 11 ventro-median supplements. Tail dorsally convex, conical, tapering, with slightly subdigitate terminus and almost pointed tip. The morphometrics of the Slovak intersex resemble the Serbian one (Krnjaic *et al.*, 1999);

some slight differences are e.g. in „b“ ratio 13.2 vs. 9.8, somewhat longer tail 62.0 vs. 56 µm, a larger body diameter at vulva 48.0 vs. 41.1 µm and at anus 35.0 vs. 28.9 µm. In contrary to the Serbian specimen of intersex, the Slovak specimen has well developed spicules.

Females of *L. distinctus* from Slovakia, in having mostly longer and more slender body and slightly longer odontostyle than females from eastern Serbia (Krnjaic *et al.*, 1999), are more similar to the type population from Bulgaria (Lamberti *et al.*, 1983) and to a population from northern Serbia (Barsi and Lamberti, 2003). Their morphometrics also resemble morphometrics of *Longidorus rubi* Tomilin & Romanenko in Romanenko, 1993 (Romanenko, 1998) and concurrently varied e.g. in having longer and thinner body, longer odontostyle, shorter odontophore, shorter tail and different structure of female reproductive system.

L. distinctus was originally described from Bulgaria from the type locality at Kovachitsa with walnut (*Juglans regia* L.) and quince (*Cydonia oblonga* Mill.) trees, but the species is distributed throughout the country in the rhizosphere of various fruit trees, grapevine, ornamentals, cereals, potato and other plants (Lamberti *et al.*, 1983; 1997) as well as in forest nurseries (Peneva and Choleva, 1992). Besides Bulgaria, the species was found in the rhizosphere of grapevine (Barsi, 1989; Barsi and Lamberti, 2003) and quince in Serbia, where an intersex was also recorded (Krnjaic *et al.*, 1999), as well as in the rhizosphere of raspberry and apple in the Ukraine (Romanenko, 1998).

Molecular study

Two female specimens were sequenced directly from purified PCR products. Identical sequences were obtained for both specimens. The obtained sequence was deposited in NCBI (National Center for Biotechnology Information) with accession number EF654539. A BLAST (Basic Local Alignment Search Tool) search at NCBI was performed using *L. distinctus* as queries to search for similar sequences deposited in GenBank. The BLAST results showed no identical sequences. It showed 94% and 93% homology to *L. juvenilis* (accession number AY601579 and DQ364599 respectively). Morphologically *L. juvenilis* is also close to *L. distinctus*.

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