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Research Note

New data on plant parasitic nematode *Longidorus distinctus* Lamberti, Choleva & Agostinelli, 1983 (Nematoda: Longidoridae) from Poland

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Summary

During a survey on the occurrence of the plant parasitic nematodes of the family Longidoridae in Poland, 925 soil samples were taken. *Longidorus distinctus* was present in 10 (1.08 %) of these samples. In this Research Note we provide: 1) distribution map of these populations, 2) morphometric data, 3) sequence data for D2-D3 28S rDNA and (partial)18S-ITS1 -5.8S(partial) markers and 4) LdistFOR primer (5'-GGCTGTAAAGATATATGCGT-3') effective in obtaining ITS1 sequence for the species. Morphometric similarities and dissimilarities with data on other published populations are discussed.

Keywords: distribution; distribution map; morphometrics; primer; D2-D3 28S rDNA; ITS1; host plants

Introduction

Nematode genus *Longidorus* Micoletzky, 1922 consists of obligatory plant ectoparasites feeding on plant roots. This genus is also rich in species, as for example Peneva *et al.* (2013) listed 158 valid species and several others were described after this paper. Some of these species are of economic importance as pests as well as vectors of plant nepoviruses (Singh *et al.*, 2013).

L. distinctus Lamberti, Choleva and Agostinelli, 1983 was originally described from Bulgaria (Lamberti *et al.*, 1983) and subsequently recorded from some other countries of the Balkan Peninsula (e.g. from Serbia – Barsi, 1989; Krnjaic *et al.*, 1999). Out of the Balkan Peninsula, this species was reported from Poland (Szczygieł & Zepp, 2004), Russia (Subbotin *et al.*, 2014), Slovakia (Lišková, 2007) and Ukraine (Romanenko, 1998). To date, *L. distinctus* was reported from Poland once (Szczygieł & Zepp, 2004). This large and informative work gave data on relation of many plant-parasitic nematode species with fruit crops and soil properties. In particular case of *L. distinctus*, however, only presented information is popu-

lation density in soil. In this Research Note we provide data on D2-D3 28S rDNA and ITS1 molecular markers, morphometric data and primer sequence useful in obtaining ITS1 region sequence.

Materials and Methods

925 soil samples were taken during a survey of the occurrence of Longidoridae in Poland. Specimens were extracted from soil using decanting and sieving method (Brown & Boag, 1988) and several specimens were picked individually and transferred to DESS (Yoder *et al.*, 2006). Remaining specimens were heat killed, TAF fixed (Courtney *et al.*, 1955), transferred to glycerol as described by Seinhorst (1959) and used for a study of morphology. Observations and measurements were made using Zeiss Axioscope II microscope. All populations were identified on the basis of 2 – 3 specimens morphology and subsequently, two populations (Long 672 and Long 812; map on Fig. 1.) were chosen for closer study of morphometrics and DNA markers. DNA isolation and PCR conditions as described by Kornobis *et al.* (2015). PCR was performed

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Fig. 1. Distribution of *Longidorus distinctus* populations. Geographic coordinates of populations: Long 11: 52.3069N, 16.8796E; Long 672: 50.0048 N, 20.9872E; Long 682: 51.9696N, 20.2439E; Long 691: 51.9570N, 20.2574E; Long 692: 51.9506N, 20.2575E; Long 693: 51.9530N, 20.2582E; Long 708: 52.1273N, 20.0178E; Long 711: 52.0798N, 20.0052E; Long 748: 53.8679N, 21.1471E; Long 812: 51.6551N, 22.8700E.

using primers D2A and D3B (Nunn, 1992) for the D2-D3 28S rDNA and rDNA2 (Vrain *et al.*, 1992) and rDNA5.8S primers (Cherry *et al.*, 1997) for (partial) 18S-ITS1-5.8S (partial) region. Sequencing of both D2-D3 and ITS1 amplicons was performed directly from PCR products. In D2-D3 28S rDNA same primers were used as for PCR while ITS1 was sequenced using rDNA2 and LdistFOR: 5'-GGCTGTAAAGATATATGCGT-3' (this study). LdistFOR primer was developed based on previously obtained sequences for *L. distinctus* with the purpose of extending length of the final sequence. Quality of obtained chromatograms was assessed using Chromas Lite 2.1.1 (Technelysium Pty Ltd, South Brisbane, Australia). Subsequently, consensus sequences were obtained in BioEdit (Hall, 1999) and deposited in GenBank under KY513282-3 and KY513284-5 for D2-D3 and ITS1 sequences, respectively. Results of BLAST search refer to the whole D2-D3 28S rDNA region while (partial)18S-ITS1-5.8S(partial) was trimmed before search to include the ITS1 only.

Results and Discussion

Morphometric data of females are presented in Table 1. No males were found. *L. distinctus* from Poland is rather similar to most populations described in the literature (Lamberti *et al.*, 1983; Bar-

si, 1989; Barsi & Lamberti, 2003). Some noteworthy differences are however present compared to data obtained by Krnijaic *et al.* (1999) and Lišková (2007). In comparison with Serbian population (Krnijaic *et al.*, 1999), both populations from Poland are characterised by longer body (4606.9 (4272 – 5400) and 4727.0 (4375 – 5065) vs 4100 (3600 – 4700)) and longer odontostylet 81.7 (79 – 86) and 82.9 (79 – 87) vs 74.8(70 – 81.4). In comparison with Slovakian population (Lišková, 2007), Polish population is characterised by lower 'a' index 106.1 (90 – 118) and 107.4 (97 – 118) vs 127.5 (108.1 – 136.9). Obtained D2-D3 28S rDNA sequences differed by one deletion in sequence from population FK224 compared to sequence FK105. BLAST results indicated 100 % similarity with sequences EF654539 and KF242317 published by Lišková 2007 and Subbotin *et al.* 2014, respectively. Two obtained (partial)18S-ITS1-5.8S(partial) regions were identical. BLAST search of ITS1 sequences revealed similarity to other *Longidorus* sequences, however not a significant one.

L. distinctus was present in 10 out of 925 (1.08 %) taken samples. Map of distribution and geographic coordinates are given at Figure 1. Populations of *L. distinctus* were associated with several different host plants. However, as all nematodes of the family Longidoridae, *L. distinctus* is an active ectoparasite which feeds on plant roots without entering them. In places where more than one plant

Table 1. Morphometrics of *Longidorus distinctus*. All measurements in μm and in form: mean $\pm$ standard deviation (range). Letter 'n' below the measurements indicates number of specimens measured if different than indicated in the heading. Standard deviation was not given if there was less than five measurements.

Feature	Long 672 n= 20	Long 812 n= 7
L	4606.9 $\pm$ 251.9 (4272 – 5400)	4727.0 $\pm$ 270.7 (4375 – 5065)
a	106.1 $\pm$ 7.4 (90 – 118)	107.4 $\pm$ 8.3 (97 – 118)
b	12.6 $\pm$ 1.9 (10.7 – 19.6)	12.1 $\pm$ 0.7 (11.2 – 13.2)
c	81.0 $\pm$ 5.8 (68 – 89)	82.8 $\pm$ 5.7 (74 – 90)
c'	1.93 $\pm$ 0.09 (1.77 – 2.16)	1.91 $\pm$ 0.05 (1.87 – 1.97)
d	2.41 $\pm$ 0.15 (2.15 – 2.75)	2.34 $\pm$ 0.18 (2.10 – 2.58)
d'	1.55 $\pm$ 0.08 (1.42 – 1.75)	1.51 $\pm$ 0.09 (1.38 – 1.62)
V	46.4 $\pm$ 1.3 (45 – 49)	46.8 $\pm$ 1.6 (45 – 49)
Odontostylet	81.7 $\pm$ 2.2 (79 – 86)	82.9 $\pm$ 2.8 (79 – 87)
Odontophore	53.9 $\pm$ 3.4 (46 – 59) n=16	54.1 (52 – 57) n=3
Stylet	135.5 $\pm$ 2.3 (131 – 139)	135.7 $\pm$ 2.5 (133 – 139)
Anterior – stylet guide ring	30.4 $\pm$ 1.4 (27 – 33)	29.7 $\pm$ 1.3 (27 – 31)
Pharyngeal bulb length	91.2 $\pm$ 3.7 (85 – 96) (n=12)	92.5 $\pm$ 3.5 (88 – 96)
Pharyngeal bulb width	19.4 $\pm$ 0.7 (18 – 20) n=12	19.3 $\pm$ 0.8 (18 – 20)
Tail length	57.0 $\pm$ 3.7 (52 – 67)	57.2 $\pm$ 1.8 (55 – 59)
Hyline tip length	12.0 $\pm$ 1.7 (9 – 15)	11.3 $\pm$ 1.3 (10 – 13)
Width at:		
lips	12.6 $\pm$ 0.5 (12 – 14)	12.7 $\pm$ 0.5 (12 – 13)
at guide ring	19.6 $\pm$ 0.9 (18 – 21)	19.2 $\pm$ 1.0 (18 – 21)
base of pharyngeal bulb	36.4 $\pm$ 1.5 (32 – 40)	36.1 $\pm$ 1.9 (33 – 38)
vulva	43.5 $\pm$ 3.0 (39 – 50)	44.1 $\pm$ 3.2 (40 – 48)
anus	29.6 $\pm$ 1.1 (27 – 32)	30.0 $\pm$ 0.6 (29 – 31)

species occur it is not always possible to indicate on which root from a mixture of roots present in a sample did the nematode feed. Only in two sampling points, single plant species with no other plants in vicinity were present. In this points, it was possible to indicate roots of which plant were present in the sample. These were *Carpinus betulus* L. (sample Long 692) and *Alnus glutinosa* Gaertn. (sample Long 693). To our knowledge, both these species were not previously recorded as host plants for *L. distinctus*. Till now *L. distinctus* was mainly reported from southern Europe. However, Szczygieł and Zepp (2004) found this species in 1.1 % of samples taken in orchards. Taken together with results obtained here this lead to the conclusion that this species is widely present in Poland, both in cultivated and non-cultivated areas.

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