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ORIGINAL ARTICLE

New data on three plant-parasitic nematode species of the genus *Longidorus* (Nematoda: Longidoridae) from Poland

Franciszek Kornobis*

Department of Entomology and Animal Pests, Institute of Plant Protection – National Research Institute, Poznań, Poland

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*Corresponding address:
f.kornobis@iorpib.poznan.pl

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Abstract

More than 4,100 plant-parasitic nematodes species have been described to date, some of which are of significant economic importance since they cause losses in agriculture. This paper presents new data on three species of the genus *Longidorus*: *L. attenuatus*, *L. elongatus* and *L. euonymus* from Poland. The study was based on 1,138 soil samples taken from different regions of the country. A total of 77 populations of *L. elongatus*, 23 of *L. attenuatus* and 7 of *L. euonymus* were found which corresponds with 6.76%, 2.02% and 0.62% of all analyzed samples, respectively. Distribution maps are presented together with data on the morphometrics, molecular markers D2-D3 28S rDNA and data on host plants on which the nematodes were found.

Keywords: *Longidorus attenuatus*, *L. elongatus*, *L. euonymus*, D2-D3 28S rDNA, occurrence

Introduction

More than 4,100 plant-parasitic nematode species have been described to date (Hugot *et al.* 2001). Out of that number 250 species are considered to be of phytosanitary importance (Singh *et al.* 2013). In worldwide agriculture the projected yield loss caused by the plant-parasitic nematodes is 12.3% ($157 billion) (Singh *et al.* 2015). The damage caused by the nematodes is, however, probably underestimated as symptoms on plants are often non-specific and can be confused with other pathogens as well as abiotic stresses such as water or nutrients deficiency (Singh *et al.* 2015). Economic losses in crop value could also be related to a decrease in food quality or visual imperfections associated with infection symptoms (Palomares-Rius *et al.* 2017). One group of plant-parasitic nematodes belongs to the family Longidoridae. This family is further divided into several genera, three of which (*Longidorus*, *Paralongidorus* and *Xiphinema*) have representatives in Polish fauna (Winiszewska *et al.* 2012; Kornobis *et al.* 2015). All members of the family are obligatory ectoparasites of plant roots that live in the soil. They feed by puncturing root cells with a long, needle-like structure called the stylet. Additionally, 19 species from this family are known as vectors of plant nepoviruses (Taylor and Brown 1997). Several of the family species are important pests of agricultural plants, some of which are also listed as EPPO quarantine species on either A1 and A2 lists, for example, *Xiphinema rivesi* Dalmasso, 1969 (www.eppo.int). This paper presents the results of a study of three *Longidorus* species from Poland. The first is *L. attenuatus* Hooper, 1961, a species that parasites many crops, including sugar beet where it is associated with 'docking' disorder (Whitehead and Hooper 1970) and soybean (Kornobis *et al.* 2016). It is also a vector of *Tomato black ring virus* (TBRV) (Harrison 1964). The second species, *L. elongatus* (de Man, 1876) Thorne and Swanger, 1936 also parasites many plant species, causing losses in some crops including sugar beet (Brown and Sykes 1971) and raspberry (Sharma 1965). It is also a vector of TBRV (Harrison *et al.* 1961) and *Raspberry ringspot virus* (RRSV) (Taylor 1962). Compared to these species, there is significantly less data on the harmfulness of the third species, *L. euonymus* Mali and Hooper, 1973. Nevertheless, the fact that this species has been found to be associated with some crops for example, barley and strawberry (Groza *et al.* 2014), suggests that its impact on yield may require further study. All three species were previously recorded from Poland (Witkowska 1958; Brzeski 1968; Szczygieł 1974), however, data on both morphometrics and molecular markers are limited.

In this study, I present new data on localities, the full dataset on morphometrics and molecular marker D2-D3 28S rDNA from Poland.

Materials and Methods

This study was based in part on the results from the PhD thesis of Kornobis (2013) and on results obtained from later research studies. A total of 1,138 soil samples, taken in Poland from both cultivated and wild plants was analyzed. Nematodes were extracted from the soil according to the method of Brown and Boag (1988), using a 100 μm mesh for final extraction. They were fixed in 2% TAF (Courtney *et al.* 1955) then transferred to pure glycerin and mounted on permanent microscope slides according to the method of Seinhorst (1959). The nematodes were identified microscopically and subsequently, six populations were chosen for analysis of the morphometrics and as molecular markers. Their localization and host plants were as follows: *L. attenuatus* populations from Mosina (52.2558N 16.8284E) and Skierniewice (51.962N 20.1424E) were associated with *Pyrus communis* L. and *Populus alba* L., respectively; *L. elongatus* populations from Mosina (52.25N 16.858E) and Ściejowice (49.9996N 19.8133E) were associated with *Acer negundo* L. and *Urtica dioica* L., respectively. Finally, both *L. euonymus* populations were taken close to Mosina (52.24780N 16.85446E and 52.24985N 16.85835E) and were associated with *Juglans regia* L. and *Salix fragilis* L., respectively. The DNA was isolated using DNA DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. The PCR was performed in a 10 μl final volume and using Type-it Microsatellite PCR Master Mix (Qiagen), 4 μl of isolated DNA and 0.25 μM of the primers. The D2D3 28S rDNA was amplified using D2A and D3B primers (Nunn 1992). PCR conditions were as follows: 95°C for 5 min and 35 cycles at 95°C for 30 s, 50°C for 60 s, 72°C for 60 s and a final extension at 72°C for 5 min. The amplicons were sequenced directly using the same primers as for the PCR and subsequently deposited in GenBank under the following accession numbers: *L. attenuatus* population from Mosina MW762996, a population from Skierniewice MW762997; *L. elongatus* a population from Mosina MW762995, a population from Ściejowice MW762994; *L. euonymus* population from Mosina associated with *J. regia* MW762998, population associated with *S. fragilis* MW762999. Some of measurements presented in the tables are expresed as ratios. These ratios are commonly used in nematology, their meaning can be found for example in the work of van Bezooijen (2006).

Results

A total of 77 populations of *L. elongatus*, 23 of *L. attenuatus* and 7 of *L. euonymus* were found which corresponded with 6.76%, 2.02% and 0.62%, respectively, of all analyzed samples. Distribution maps are presented in Figures 1 and 2. Data on morphometry of the analyzed populations is given in Tables 1 and 2. The obtained sequences showed the following similarity with sequences available in GenBank: *L. attenuatus* sequence had 99.28% similarity with both KT755457 and AY601572 sequences; *L. elongatus* sequences had 99.85% and 99.71% with sequences KF242304 and KF242306, respectively; *L. euonymus* had 99.58% similarity with both KF242331 and KF242333, respectively. The nematodes were found to be associated with the following host plants: *L. attenuatus*: alder (*Alnus glutinosa* (L.) Gaertn.), aspen (*Populus tremula* L.), black locust (*Robinia pseudoacacia* L.), box elder (*Acer negundo* L.), hop (*Humulus lupulus* L.), hornbeam (*Carpinus betulus* L.), mirabelle plum (*Prunus domestica* subsp. *syriaca*), common pear (*Pyrus communis* L.), silver poplar (*Populus alba* L.), soybean (*Glycine max* (L.) Merr.), walnut (*Juglans regia* L.) and wild strawberry (*Fragaria vesca* L.); *L. elongatus*: alder, apple tree (*Malus domestica* Borkh.), box elder, buckhorn (*Plantago lanceolata* L.), common mugwort (*Artemisia vulgaris* L.), elder (*Sambucus nigra* L.), field elm (*Ulmus minor* Mill.), nettle (*Urtica dioica* L.), Norway maple (*Acer platanoides* L.), marram grass (*Ammophila arenaria* (L.) Link), pear, rose (*Rosa* sp.); *Rubus* sp., silver poplar, walnut and willow (*Salix* sp.); *L. euonymus*: alder, common pear, crack willow (*Salix fragilis* L.), Norway maple, small-leaved lime (*Tilia cordata* Mill.) and walnut.

Discussion

The results showed that of the three species studied, *L. elongatus* was the most common species, and also the most common longidorid species of the family Longidoridae in Poland (Kornobis 2013, Kornobis unpublished data). Similarly, *L. elongatus* was also the most frequent of all longidorid species in a study conducted

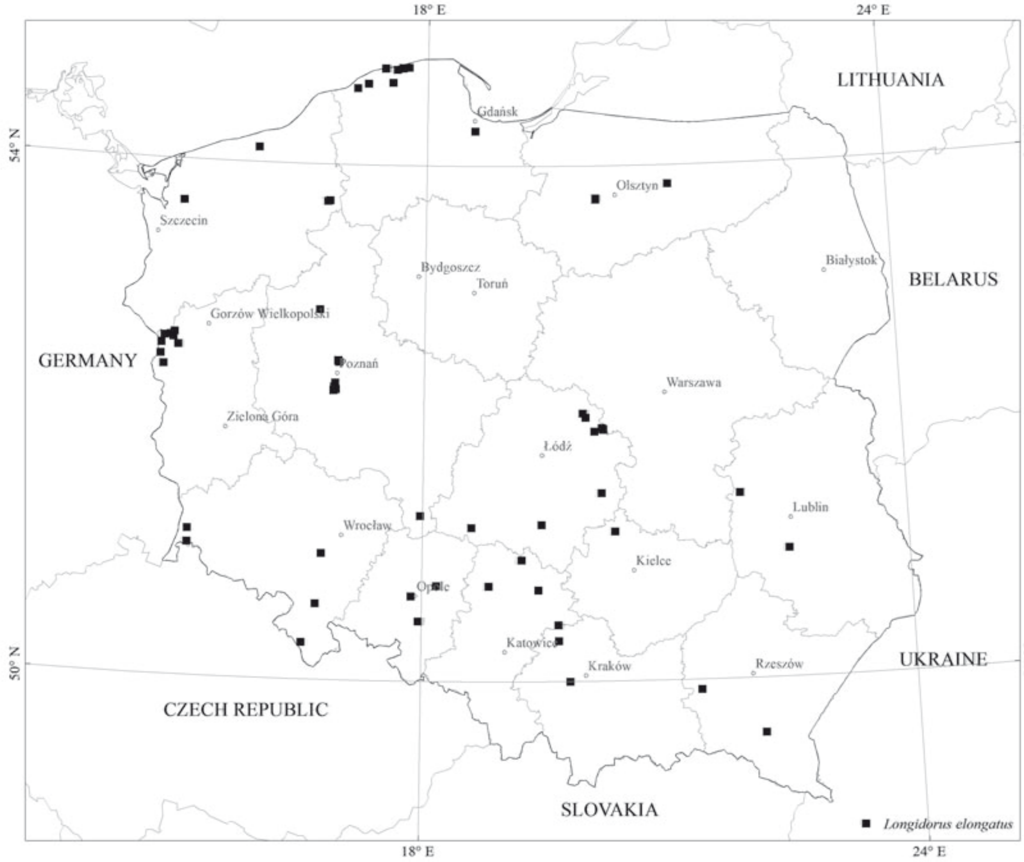

Fig. 1. Distribution map of the *Longidorus elongatus* occurrence in Poland

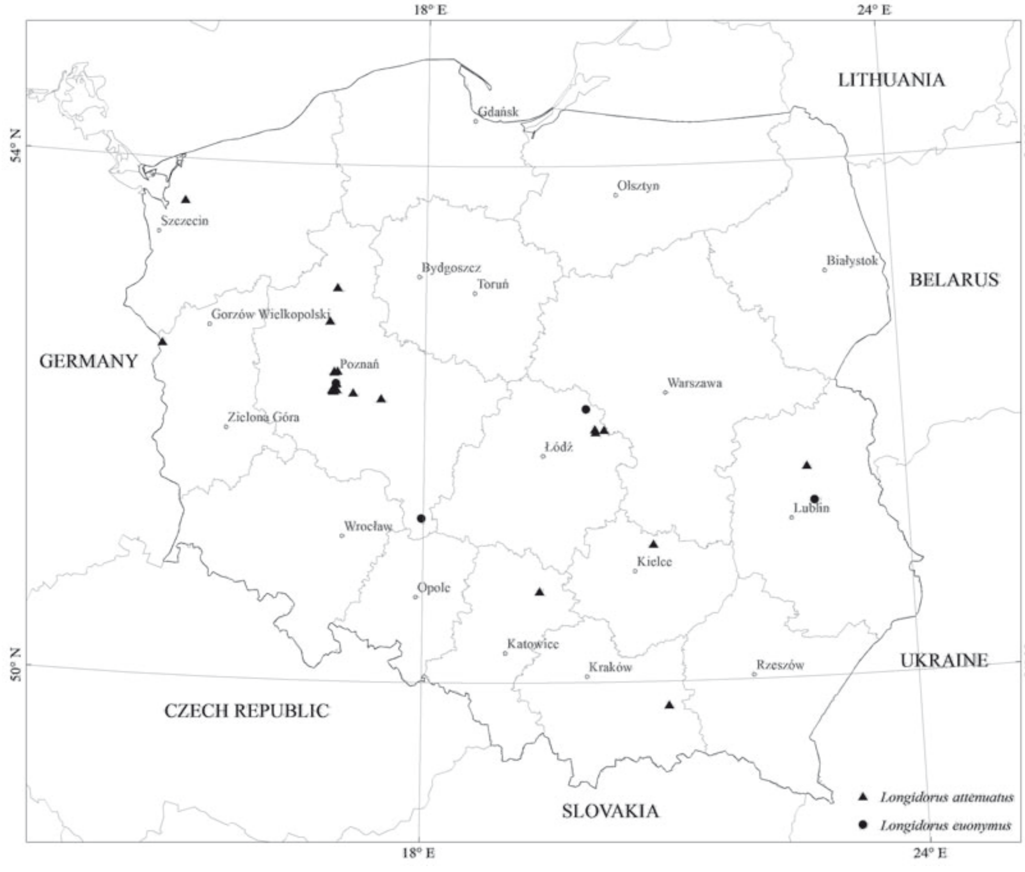

Fig. 2. Distribution map of the *Longidorus attenuatus* and *L. euonymus* occurrence in Poland

Table 1. Morphometrics of female specimens from populations in Poland. All measurements in µm and in format: mean ± standard deviation (range)

Trait	*Longidorus attenuatus*		*Longidorus elongatus*		*Longidorus euonymus*	
	population from Mosina n = 24	population from Skierniewice n = 5	population from Mosina n = 32	population from Ściejowice n = 22	population from Mosina associated with *Juglans regia* n = 22	population from Mosina associated with *Salix fragilis* n = 10
L	5698 ± 493.1 (4970–6722)	6536 ± 548.1 (5958–7411)	5182 ± 452.6 (4401–6240)	5509 ± 259.4 (4991–6049)	6982 ± 485.97 (5887–8085)	7525 ± 709.83 (6097–8226)
a	132.2 ± 8.4 (118–146)	142.1 ± 8.92 (134–154)	92.8 ± 5.84 (83–105)	100.6 ± 5.76 (91–114)	160.4 ± 9.08 (140–180)	170.9 ± 9.88 (154–185)
b	14.2 ± 1.13 (12–17)	18.2 ± 3.15 (14–22)	11.2 ± 0.77 (10–13)	12.1 ± 0.54 (11–13)	15.9 ± 0.92 (13–18)	17.2 ± 1.22 (15–18)
c	121.1 ± 13.69 (103–149)	139.5 ± 21.28 (121–168)	133.2 ± 15.95 (107–167)	116.2 ± 11.32 (100–138)	151.9 ± 14.24 (128–183)	162.5 ± 17.5 (133–186)
c′	1.5 ± 0.11 (1.3–1.7)	1.5 ± 0.16 (1.3–1.7)	1 ± 0.09 (0.9–1.2)	1.2 ± 0.09 (1.1–1.5)	1.3 ± 0.096 (1.1–1.5)	1.3 ± 0.07 (1.2–1.4)
d	2.1 ± 0.1 (1.9–2.4)	2.0 ± 0.04 (2.0–2.1)	2.2 ± 0.1 (2–2.4)	2.2 ± 0.13 (2–2.5)	1.9 ± 0.081 (1.8–2.1)	1.8 ± 0.08 (1.7–1.9)
d′	1.4 ± 0.21 (1.3–2.2)	1.4 ± 0.06 (1.3–1.4)	1.6 ± 0.08 (1.4–1.8)	1.5 ± 0.08 (1.4–1.8)	1.4 ± 0.047 (1.3–1.5)	1.3 ± 0,04 (1.3–1.4)
V	49.1 ± 1.65 (47–55)	47.2 ± 1.26 (45–49)	45.4 ± 0.01 (43–49)	49.4 ± 0.01 (46–52)	53.1 ± 1.6 (50–56)	53.6 ± 0.013 (52–56)
Odontostyle length	80.9 ± 2.59 (75–86)	83.6 ± 1.95 (81–86)	96.2 ± 2.73 (90–103)	89.8 ± 5.6 (77–100)	86.8 ± 2.38 (83–92)	87.4 ± 3.17 (81–92)
Odontophore length	59.1 ± 3.58 (52–67)	63.4 ± 1.52 (61–65)	63 ± 3.8 (58–72)	60.5 ± 3 (56–67)	60.5 ± 4.44 (52–68)	61.8 ± 2.7 (57–65)
Lip region width	14.3 ± 0.56 (13–15)	14.2 ± 0.45 (14–15)	13.2 ± 0.45 (12–14)	14.3 ± 0.78 (12–15)	14.7 ± 0.48 (14–15)	14.8 ± 0.42 (14–15)
Oral aperture to guiding ring	29.3 ± 1.33 (26–32)	28.8 ± 0.84 (28–30)	29.5 ± 0.98 (28–31)	30.8 ± 1.11 (28–32)	28.1 ± 0.73 (27–29)	26.4 ± 1.07 (25–28)
Largest body width	43.1 ± 2.3 (38–47)	46 ± 2.83 (43–49)	55.8 ± 3.54 (49–65)	54.7 ± 2.71 (49–59)	44 ± 2.02 (40–47)	44 ± 2.87 (39–48)
Tail length	47.3 ± 3.87 (41–56)	47.4 ± 5.32 (41–55)	39.1 ± 2.37 (35–46)	47.7 ± 3.14 (42–53)	46.1 ± 3.2 (40–52)	46.4 ± 2.22 (42–49)
Anal width	31.6 ± 1.86 (28–35)	32 ± 1 (31–33)	38.0 ± 2.16 (32–42)	38.4 ± 1.14 (36–40)	34.7 ± 1.28 (31–36)	35.8 ± 1.48 (34–39)

Table 2. Morphometrics of juveniles from populations in Poland. All measurements in µm and in format: mean ± standard deviation (range). Standard deviation was not given when the number of measurements was lower than 5

Trait	*Longidorus attenuatus* population from Mosina				*Longidorus elongatus* population from Mosina				*Longidorus euonymus* population from Mosina associated with *Juglans regia*			
	J1 n = 2	J2 n = 1	J3 n = 7	J4 n = 8	J1 n = 6	J2 n = 1	J3 n = 8	J4 n = 12	J1 n = 8	J2 n = 7	J3 n = 12	J4 n = 6
L	1036.9 (1029–1045)	1806	2762 ± 232.2 (2479–3152)	4218.2 ± 248.5 (3930–4628)	1126 ± 23.73 (1090–1144)	1977	2534.8 ± 100.78 (2396–2707)	3702.5 ± 182.41 (3388–3973)	1589.0 ± 99.13 (1458–1734)	2380.5 ± 367 (1964–2851)	3206.2 ± 330.59 (2514–3769)	4747 ± 147.5 (4484–4900)
a	64.8 (64–65)	75	88.8 ± 5.22 (80–93)	116 ± 6.39 (107–129)	51.5 ± 5.19 (45–59)	53	65.8 ± 2.78 (60–70)	78 ± 4.56 (69–86)	77.0 ± 7.4 (61–85)	98.9 ± 6.03 (92–110)	109.8 ± 7.52 (90–119)	138.8 ± 4.97 (131–145)
b	4.7 (4.5–4.9)	6.6	9.3 ± 1.14 (8.5–11.3)	11.9 ± 0.67 (10.9–12.8)	4.2 ± 0.13 (4–4.4)	7	7.2 ± 0.63 (6.8–8.3)	9.4 ± 0.86 (8.2–10.6)	6.2 ± 0.71 (5.3–7.4)	7.8 ± 1.01 (6.6–9.4)	9.4 ± 0.69 (8.3–10.6)	12.2 ± 0.52 (11.6–13.0)
c	25.6 (25–26)	40	51.3 ± 3.97 (45–55)	76.9 ± 9.66 (69–99)	24.1 ± 1.39 (22–26)	39	56 ± 6.42 (45.9–65.9)	86.1 ± 5.97 (77.8–96.3)	30.9 ± 1.69 (29–35)	45.2 ± 6.61 (37–55)	61.4 ± 8.26 (43–71)	92.4 ± 5.35 (83–100)
c′	3.3 (3–3.5)	2.5	2.3 ± 0.15 (2.1–2.5)	1.9 ± 0.166 (1.6–2.1)	3.2 ± 0.129 (2.9–3.3)	2	1.7 ± 0.137 (1.5–2)	1.2 ± 0.106 (1–1.4)	3.5 ± 0.159 (3.3–3.9)	2.8 ± 0.269 (2.4–3.3)	2.3 ± 0.262 (2–2.6)	1.8 ± 0.98 (1.6–1.9)
Odontosyle length	50 (50–50)	55	63.4 (61–67)	74.1 ± 2,3 (70–80)	61.3 ± 1.86 (58–63)	62	76.5 ± 1.77 (75–80)	86 ± 1.6 (82–88)	53.3 ± 2.05 (50–56)	62.7 ± 2.5 (60–67)	70.8 ± 2.14 (68–75)	76.7 ± 2.94 (72–80)
Replacement odonostyle length	55.5 (54–57)	65	73.7 (72–75)	83.4 ± 1.11 (80–86)	61.5 ± 1.52 (59–63)	78	85.9 ± 2.7 (82–89)	95.6 ± 3.03 (89–98)	61.9 ± 1.64 (59–64)	69.9 ± 4.22 (64–75)	76.8 ± 2.52 (73–80)	87.5 ± 1.05 (86–89)
Anterior end to guiding ring	15.5 (15–16)	20	23.3 (22–26)	25.9 ± 1.38 (25–27)	16.5 ± 0.55 (16–17)	21	24.4 ± 0.74 (23–25)	27.2 ± 1.03 (25–29)	17.9 ± 0.64 (17–19)	20.7 ± 0.95 (20–22)	22.8 ± 1.03 (20–24)	24.7 ± 0.52 (24–25)
Lip region width	8 (8–8)	10	11.6 ± 0.53 (11–12)	12.9 ± 0.35 (12–13)	9.2 ± 0.41 (9–10)	10	11.5 ± 0.53 (11–12)	12.4 ± 0.51 (12–13)	9.6 ± 0.52 (9–10)	11.1 ± 0.90 (10–13)	11.9 ± 0.29 (11–12)	13.3 ± 0.82 (12–14)
Largest body width	16 (16–16)	24	31.1 ± 2.41 (28–34)	36.4 ± 1.51 (34–38)	21.8 ± 1.94 (19–24)	37	38.6 ± 2.97 (35–45)	47.7 ± 4.1 (43–57)	20.8 ± 1.67 (19–24)	24.0 ± 2.71 (20–27)	29.2 ± 1.75 (26–33)	34.0 ± 1.67 (31–36)
Anal width	12.5 (12–13)	18	23.3 ± 1.8 (20–25)	28.5 ± 1.20 (27–31)	14.7 ± 0.82 (14–16)	25	27.4 ± 1.3 (25–29)	36.1 ± 3.7 (33–47)	14.6 ± 0.52 (14–15)	18.9 ± 2.04 (16–22)	23.1 ± 1.16 (22–25)	29.2 ± 1.47 (27–31)

by Szczygieł and Brzeski (1985), which was based on about 2,700 soil samples. This is interesting in the context of the potential impact of climate changes on the distribution and impact of pests on agriculture, which is widely discussed in the literature (Donatelli *et al.* 2017; Pathak *et al.* 2018; Juroszek *et al.* 2020). The results presented here suggest that to date there has been no such impact on *L. elongatus*, since it remains the most frequently occurring species of the family Longidoridae in Poland. However, the harmfulness of this species towards crops, including its ability to transmit viruses under various mean temperatures might require further study. The second most common species in this study was *L. attenuatus*. Populations of this species exhibited some morphometric differences compared to the populations known from the literature. For example, compared to the type population from England (Hooper 1961), the population from Mosina (Table 1) was characterized by a less slender body ['a' index value 132.2 (118–146) vs 158 (120–210)], however, the rest of the morphological traits, as well as D2-D3 28S rDNA confirmed the identification. To the best of my knowledge this nematode has been recorded for the first time from black locust, a tree known to be a host of the TBRV (Jończyk *et al.* 2004a, b). The exact role of this nematode in the circulation of the virus strains present in black locust among trees and possibly other plants requires further study. *Longidorus euonymus* was the least common of the three species. The Polish populations were largely similar to the type population described by Mali and Hooper (1973) from Slovakia. It is worth mentioning the difference which occurs when compared to populations from Italy. Roca *et al.* (1985, 1987, 1988a, 1988b, 1989, 1991) and Roca and Lamberti (1993) have published a series of papers with morphometric data on this species. All the populations presented there are characterized by having a higher c' index compared to Polish populations (means in the range of 1.4–1.6 vs 1.3 in both Polish populations. In contrast, Oro *et al.* (2005) have found lower c' index values in populations from Serbia (mean values in the range of 1.13–1.26).

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