The genus *Longidorus* (Nematoda: Longidoridae) from Bohemia and South Moravia in the rhizosphere of fruit orchards and vineyards

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Summary

The occurrence and geographic distribution of the genus *Longidorus* was investigated during a nematological survey of Bohemia and South Moravia between 2003 and 2006. Soil samples were collected from 139 different fruit orchards and vineyards. Three *Longidorus* species (*L. elongatus*, *L. euonymus* and *L. leptcephalus*) were recorded. *L. euonymus* constitutes a new record for the Czech nematofauna.

Key words: *Longidorus elongatus*; *Longidorus euonymus*; *Longidorus leptcephalus*; Czech Republic; PCR; Survey

Introduction

Species of the genus *Longidorus* are considered worldwide as economically important plant parasites, especially since some species are vectors of nepoviruses in a variety of crops (Taylor & Brown, 1997). However, for the Czech Republic little information is available on the presence of the genus *Longidorus* in the rhizosphere of fruit trees and grapevines. In general, compared with other plant pathogens, the study of nematodes has been largely ignored in this country and records of the occurrence and distribution of *Longidorus* are restricted (Erbenová, 1973, 1977; Pelikán, 1989; Bartošová & Háněl, 1994). Moreover, these records represent only lists of species names without additional morphological and biological data. Since the Czech Republic joined the European Union in 2004, it has had to meet the required standards of the EU concerning plant pests. Several species of *Longidorus* are recognized as quarantine pests by both EU and European and Mediterranean Plant Protection regulations. Consequently, it was considered necessary to study the occurrence and distribution of the genus in the Czech Republic more in detail.

A systematic comprehensive survey and a detailed morphological and biological study of these nematodes were initiated in 2002 and results from Central Bohemia were

presented by Kumari (2004).

In the case of perennial crops, plants may often support and compensate for high densities without noticeable loss of vigour. *Longidorus* specimens and their hosts have co-existed for long periods of time although they have not necessarily become more destructive. Over time, however, the cumulative stress of nematode feeding results in lower vigour and yield. Furthermore, with an increase in international trade and open market in the EU, there is greater concern and risk of introducing new species of pests and pathogens. It is clear that *Longidorus* nematodes, and other economically important plant-parasitic nematodes, need more attention in the Czech Republic.

In this study, we present data from nematological surveys from Bohemia and South Moravia conducted between 2003 and 2006 in different fruit orchards and vineyards. The present contribution includes morphological data and morphometrics, data on geographical distribution and complementary identification of *L. elongatus* by species-specific primers.

Materials and Methods

Morphological and morphometrical study

Soil samples were collected from orchards during spring and autumn between 2003 to 2006 at different localities in Bohemia and South Moravia (Czech Republic). A total of 139 sites were sampled, and five to ten cores of soil were taken from the rhizosphere of different orchards (apple, apricot, pear, sour cherry, sweet cherry) and vineyards at a depth of 0 – 100 cm with a 6 cm diameter soil auger. The samples were transported to the laboratory and stored at 4°C in a cold room. Nematodes were extracted within a day from 500 g of soil by using a sieving and decanting method (Brown & Boag, 1988). Final separation was done by adding the suspension containing the nematodes to a 10 cm diameter nylon sieve with 99 µm pore size, partially

submerged in water in a 15 cm diameter Baermann funnel. Nematodes were recovered from the funnels after 24 and 48 hours, and *Longidorus* specimens were hand picked from the resultant suspension into tap water. Females, males and juveniles of *Longidorus* were counted under a stereomicroscope (Olympus SZX-9) and recorded separately, heat killed at 60°C for two minutes, fixed in TAF then processed into glycerin according to the slow glycerin method. Specimens were mounted in anhydrous glycerin on permanent glass slides for identification. Nematode identification was made using a light microscope (Olympus BX-51) 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 imaging software (Olympus DP-soft).

Molecular study

DNA extraction methodology and the relevant PCR and gel electrophoresis conditions are all described by Kumari *et al.* (2005). The following primers were used in the PCR reaction for *L. elongatus*: reverse primer (Lelong1: 5'-TTA TCG TAC GTA TTC CCA GTT CT-3') and forward primer (5'-TTG ATT ACG TCC CTG CCC TTT GT-3') (Hübschen *et al.*, 2004).

Results

Longidorus elongatus (de Man, 1876) Micoletzky, 1922

Fig. 1, 2, 4; Table 1, 2, 3, 4

L. elongatus is a cosmopolitan species associated with different hosts and various soil types. It is also an established vector of *Raspberry ringspot virus* and *Tomato black ring-spot virus* (Taylor & Brown, 1997). *L. elongatus* was recorded from the Czech Republic by Erbenová (1973, 1977), Pelikán (1989) and Bartošová and Háněl (1994) without any additional morphological, morphometrical and ecological data.

In our survey, *L. elongatus* was recorded from 4 orchards, 2 vineyards and at a river bank with population densities of 1 to 4 females, 2 to 6 males and 2 to 10 juveniles per 500 g soil. *L. elongatus* occurred at the following sites (in parenthesis associated host): Bílé podolí (apple, peach); Břeclav (grapevine); Hrušky (grapevine); Kobyli (apple); Lhenice (plum); Velké Němčice (river bank).

Females and juveniles were found at all positive orchards, with males being almost as common as females in some populations but completely absent in other populations. Czech populations are morphologically and morphometrically largely similar to those from other European countries (Table 3) i.e. German populations without males (Sturhan, 1963), German and Dutch populations with males or with males rare/absent (Kozłowska & Seinhorst, 1979), Scottish population with males (Raschké & Boag, 1981), Slovakian population without males (Lišková, 1997), Swiss population without males (Lamberti *et al.*, 2001), Austrian populations without males (Tiefenbrunner & Tiefenbrunner, 2004a,b) and Serbian populations without males (Barsi & De Luca, 2006) but some differences

have been observed between populations from different countries and to a minor extent between populations with males and those without males. For example for females, Czech specimens from Břeclav possess a higher mean value of 'V' (55 vs 45 – 50.9 % for all known populations). The mean odontostyle length of all Czech populations (exc. one specimen from Lhenice) is comparable with the bisexual Dutch population (Kozłowska & Seinhorst, 1979) and parthenogenetic populations from Germany (Sturhan, 1963), Austria (Tiefenbrunner & Tiefenbrunner, 2004a) and Switzerland (Lamberti *et al.*, 2001) but shorter than in all other foreign records, although, all Czech odontostyle values fall within the range known for the species. Further, the odontophore length of the Swiss, Serbian and Slovakian populations is clearly longer than in other populations, but largely overlaps with the high maximum range for the Scottish population.

Kozłowska and Seinhorst (1979) observed for 16 Dutch populations that the six populations with males had an average female odontostyle length up to 86 µm and populations without males over 86 µm (i.e. 87 – 96 µm); similar observations were made between German bisexual and parthenogenic reproducing females (89 vs 96 µm). Czech populations with or without males have similar odontostyle length e.g. Bílé Podolí bisexual populations and Kobyli population with only females. One population with males from Břeclav (Table 1) possesses a shorter odontostyle (mean 77 µm) comparable with the Hrušky population of only females (mean 78 µm). Kozłowska and Seinhorst (1979) also observed that other features such as odontophore length, pharynx length, tail length, body width, and distance of guiding ring from anterior end were on average 10 to 16 % larger in German populations without males; similar observations were made for the Dutch populations. This phenomenon was not observed in any of the Czech populations.

Morphometrics of males from all Czech populations were consistently different from formerly mentioned foreign populations by possessing a shorter mean body length (4.4 – 5.0 vs 5.5 – 5.8 mm), mean spicule length (54 – 55 vs 59.5 µm) and mean odontostyle length (78 – 82 vs 91 – 98 µm). Mean ratio 'c' in Czech populations is also smaller than in German bisexual populations. These geographically induced morphometric differences widen the range of morphometric data as well as the code in the polytomous key of Chen *et al.* (1997), making it less useful for species identification.

Main morphometric characters of juveniles are also clearly different from the morphometrics of the large Scottish population described by Hooper (1961) and also from a population recently described from Novi Sad, Serbia (Barsi & De Luca, 2006) with body length, replacement odontostyle and odontostyle length of all juvenile stages of Czech population being much shorter. Morphometrics of J1 and J2 stages of Czech population are also different from a German population from Hersbruck (Sturhan, 1963) but all four stages are similar to another German population from Bubenreuth (Sturhan, 1963) and also to J3 and J4 stages of

Fig. 1. *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922. A – Entire female; B – Entire male; C – Female anterior region; D – Female pharyngeal bulb; E – Vulva; F – K – Posterior region of J1, J2, J3, J4, female and male respectively. (Scale bar: A – B = 500 μ m; C – K = 50 μ m)

Table 1. Morphometrics of *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922. All measurements in μm except body length in mm [mean $\pm$ standard deviation (range)]

Locality	Břeclav						
Host	Grapevine						
Specimens	J1	J2	J3	J4	Females	Males	
n	11	3	4	2	10	4	
L	1.0 $\pm$ 0.12 (0.8 – 1.2)	1.4 $\pm$ 0.01 (1.42 – 1.44)	2.5 $\pm$ 0.27 (2.2 – 2.7)	3.2	4.0	5.0 $\pm$ 0.48 (4.1 – 5.8)	5.0 $\pm$ 0.32 (4.6 – 5.3)
a	42.0 $\pm$ 5.1 (36.4 – 54.3)	49.8 $\pm$ 2.4 (47.9 – 52.5)	59 $\pm$ 3.5 (54.9 – 63.4)	58.2	73.4	86.7 $\pm$ 10 (68.6 – 104.2)	87.9 $\pm$ 11.6 (73.6 – 98.6)
b	4.9 $\pm$ 1.1 (4.1 – 7.2)	5.7 $\pm$ 0.4 (5.3 – 6.1)	8.9 $\pm$ 1.3 (7.1 – 10.1)	11.5	13.4	12.8 $\pm$ 1.5 (10.0 – 15.8)	13.0 $\pm$ 0.8 (12.3 – 14.2)
c	24.4 $\pm$ 4.2 (18 – 33)	33.9 $\pm$ 1.2 (32.6 – 34.6)	58.5 $\pm$ 2.0 (55.6 – 60.1)	91.5	98.4	118.9 $\pm$ 10.5 (100.4 – 132.7)	120.6 $\pm$ 6.8 (115.1 – 129.9)
c'	2.82 $\pm$ 0.47 (2.43 – 4.00)	2.21 $\pm$ 0.10 (2.09 – 2.27)	1.66 $\pm$ 0.16 (1.52 – 1.88)	1.09	1.20	1.22 $\pm$ 0.12 (1.06 – 1.45)	1.16 $\pm$ 0.08 (1.08 – 1.24)
V/spicule	—	—	—	—	—	55 $\pm$ 4.0 (48.9 – 60.2)	55 $\pm$ 1.5 (54 – 57)
Replacement odontostyle	50.2 $\pm$ 2.7 (46 – 54)	59 $\pm$ 2.9 (57 – 62)	71 $\pm$ 3.1 (69 – 76)	79	81	—	—
Odontostyle	47 $\pm$ 2.8 (43 – 52)	49 $\pm$ 0.6 (49 – 50)	61 $\pm$ 3.8 (57 – 65)	70	74	77 $\pm$ 3.0 (73 – 81)	78 $\pm$ 3.4 (75 – 83)
Odontophore	33 $\pm$ 3.3 (27 – 39)	36 $\pm$ 4.6 (32 – 41)	36 $\pm$ 0.5 (36 – 37)	38	39	44 $\pm$ 3.3 (40 – 48)	44.5 $\pm$ 3.7 (40 – 48)
Total stylet length	80 $\pm$ 4.1 (76 – 87)	85 $\pm$ 4.5 (81 – 90)	97 $\pm$ 3.6 (94 – 101)	108	113	121 $\pm$ 3.9 (117 – 128)	123 $\pm$ 3.4 (118 – 126)
Oral aperture to guide ring	15 $\pm$ 0.7 (14 – 16)	18 $\pm$ 2.3 (17 – 21)	21 $\pm$ 1.0 (20 – 22)	25	29	29 $\pm$ 2.4 (26 – 33)	31 $\pm$ 1.3 (29 – 32)
Pharyngeal bulb length	50 $\pm$ 2.7 (44 – 54)	60 $\pm$ 3.1 (57 – 63)	74 $\pm$ 5.0 (70 – 81)	78	85	97 $\pm$ 4.2 (91 – 105)	94 $\pm$ 6.8 (85 – 101)
Pharyngeal bulb diameter	12 $\pm$ 1.9 (10 – 16)	13 $\pm$ 1.2 (12 – 14)	17 $\pm$ 1.3 (16 – 19)	19	19	21 $\pm$ 2.1 (19 – 26)	20 $\pm$ 1.4 (18 – 21)
Tail length	41 $\pm$ 4.6 (36 – 52)	42 $\pm$ 1.7 (41 – 44)	42 $\pm$ 5.4 (38 – 49)	35	41	42 $\pm$ 3.3 (36 – 48)	41 $\pm$ 2.4 (40 – 45)
Length of hyaline tip	6 $\pm$ 1.1 (5 – 8)	7 $\pm$ 1.0 (6 – 8)	10 $\pm$ 1.7 (8 – 12)	8	10	13 $\pm$ 2.0 (10 – 17)	12 $\pm$ 1.0 (11 – 13)
Number of supplements	—	—	—	—	—	—	12 $\pm$ 1.3 (10 – 13)
Body diam. at lip region	7 $\pm$ 0.6 (6 – 8)	8 $\pm$ 0.6 (8 – 9)	10 $\pm$ 0.5 (10 – 11)	11	11	12 $\pm$ 0.4 (11 – 12)	12 $\pm$ 0.8 (11 – 13)
at guiding ring	13 $\pm$ 0.8 (12 – 14)	14 $\pm$ 0.6 (14 – 15)	15 $\pm$ 0.5 (15 – 16)	18	18	18 $\pm$ 0.7 (17 – 19)	18 $\pm$ 0.6 (18 – 19)
at base of pharynx	23 $\pm$ 2.6 (19 – 28)	29 $\pm$ 1.5 (27 – 30)	36 $\pm$ 1.4 (35 – 38)	38	47	45 $\pm$ 2.8 (41 – 50)	43 $\pm$ 2.1 (41 – 46)
at mid body/at vulva	23 $\pm$ 3.9 (18 – 31)	29 $\pm$ 1.5 (27 – 30)	42 $\pm$ 3.4 (38 – 46)	55	55	58 $\pm$ 4.9 (51 – 65)	57 $\pm$ 4.3 (54 – 63)
at anus	15 $\pm$ 1.3 (13 – 17)	19 $\pm$ 1.7 (18 – 21)	25 $\pm$ 1.3 (24 – 27)	32	34	34 $\pm$ 2.2 (32 – 38)	36 $\pm$ 1.9 (33 – 37)
at beginning of hyaline tip	6 $\pm$ 0.5 (5 – 7)	7 $\pm$ 0.0 (7 – 7)	13 $\pm$ 1.7 (11 – 15)	15	16	19 $\pm$ 1.1 (18 – 21)	15 $\pm$ 0.8 (14 – 16)

Table 2. Morphometrics of male and females of *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922. All measurements in μm except body length in mm [mean $\pm$ standard deviation (range)]

Locality Host Specimens	Bilé podolí apple		Bilé podolí peach		Hrušky grapevine	Kobyli apple	Lhenice plum
	Females	Male	Females	male	Females	Females	Females
n	8	5	4	4	4	33	1
L	5.3 $\pm$ 0.40 (4.7 – 6.1)	5.2 $\pm$ 0.32 (4.8 – 5.6)	5.5 $\pm$ 0.51 (5.0 – 6.1)	4.4 $\pm$ 0.01 (4.3 – 4.5)	5.2 $\pm$ 0.59 (4.4 – 5.8)	5.3 $\pm$ 0.51 (4.2 – 6.2)	4.2
a	87.6 $\pm$ 10.7 (73.2 – 106.7)	81.5 $\pm$ 4.73 (77.4 – 88.0)	77.2 $\pm$ 5.8 (69.0 – 82.5)	80.7 $\pm$ 4.8 (77.4 – 87.8)	94.6 $\pm$ 6.4 (87.2 – 101.1)	94.6 $\pm$ 8.3 (81.2 – 111.4)	71.2
b	13.2 $\pm$ 1.9 (10.9 – 16.5)	12.7 $\pm$ 1.9 (11.4 – 15.9)	12.5 $\pm$ 0.5 (11.9 – 13.0)	11.0 $\pm$ 0.4 (10.4 – 11.3)	11.8 $\pm$ 2.9 (7.6 – 13.7)	13.3 $\pm$ 1.4 (11.1 – 16.3)	11.8
c	137.4 $\pm$ 18.0 (114.2 – 173.2)	120.7 $\pm$ 8.2 (112.6 – 134.1)	122.5 $\pm$ 12.8 (107.1 – 133.4)	107.2 $\pm$ 11.6 (96.7 – 122.5)	129.8 $\pm$ 14.7 (114.0 – 149.3)	134.6 $\pm$ 21.1 (105.5 – 183.8)	97.7
c'	1.00 $\pm$ 0.14 (0.81 – 1.20)	0.99 $\pm$ 0.05 (0.91 – 1.05)	0.97 $\pm$ 0.05 (0.90 – 1.02)	1.10 $\pm$ 0.11 (0.95 – 1.22)	1.15 $\pm$ 0.05 (1.08 – 1.20)	1.04 $\pm$ 0.12 (0.77 – 1.30)	1.19
Vulva/spicule	49 $\pm$ 1.7 (47.3 – 52.9)	54 $\pm$ 3.0 (50 – 58)	48.2 $\pm$ 3.7 (45.0 – 52.0)	54.8 $\pm$ 1.1 (54.0 – 56.0)	50.9 $\pm$ 2.2 (48.2 – 53.4)	49.2 $\pm$ 1.2 (47.0 – 51.4)	48.2
Odontostyle	84 $\pm$ 5.0 (77 – 93)	82 $\pm$ 2.9 (80 – 87)	88 $\pm$ 7.3 (77 – 94)	78 $\pm$ 4.3 (72 – 81)	78 $\pm$ 4.0 (72 – 81)	87 $\pm$ 3.3 (80 – 93)	107
Odontophore	43 $\pm$ 2.5 (39 – 47)	42 $\pm$ 1.2 (41 – 44)	45 $\pm$ 3.0 (42 – 48)	40 $\pm$ 2.1 (38 – 42)	41 $\pm$ 1.2 (40 – 42)	46 $\pm$ 3.2 (40 – 53)	41
Total sylet length	126 $\pm$ 6.0 (119 – 140)	124 $\pm$ 4.1 (121 – 131)	132 $\pm$ 9.8 (119 – 142)	118 $\pm$ 5.5 (110 – 122)	119 $\pm$ 4.8 (112 – 123)	133 $\pm$ 3.8 (125 – 142)	154
Oral aperture to guide ring	30 $\pm$ 1.1 (28 – 31)	30 $\pm$ 1.1 (29 – 32)	31 $\pm$ 2.7 (27 – 33)	29 $\pm$ 1.0 (28 – 30)	27 $\pm$ 1.4 (25 – 28)	29 $\pm$ 0.9 (27 – 31)	33
Pharyngeal bulb length	101 $\pm$ 13.7 (76 – 125)	102 $\pm$ 6.6 (92 – 110)	113 $\pm$ 13.8 (94 – 127)	98 $\pm$ 5.6 (92 – 103)	103 $\pm$ 6.8 (98 – 113)	97 $\pm$ 5.7 (86 – 106)	105
Pharyngeal bulb diam.	24 $\pm$ 3.0 (19 – 28)	27 $\pm$ 2.5 (25 – 31)	31 $\pm$ 2.6 (27 – 33)	23 $\pm$ 2.5 (20 – 26)	22 $\pm$ 2.7 (18 – 24)	21.9 $\pm$ 2.6 (17 – 28)	25
Tail length	39 $\pm$ 5.0 (30 – 47)	43 $\pm$ 0.8 (42 – 44)	45 $\pm$ 4.6 (38 – 48)	41 $\pm$ 3.8 (36 – 44)	40 $\pm$ 1.4 (39 – 42)	40 $\pm$ 4.4 (31 – 51)	43
Length of hyaline tip	12 $\pm$ 1.1 (10 – 13)	11 $\pm$ 1.6 (9 – 13)	13 $\pm$ 1.7 (11 – 15)	12 $\pm$ 1.3 (10 – 13)	12 $\pm$ 1.8 (10 – 14)	11 $\pm$ 1.7 (9 – 16)	12
Number of supplements	—	11 $\pm$ 0.8 (10 – 12)	—	12 $\pm$ 1.3 (10 – 13)	—	—	—
Body diameter at lip region	11 $\pm$ 0.7 (10 – 12)	12 $\pm$ 0.5 (11 – 12)	13 $\pm$ 1.0 (12 – 114)	12 $\pm$ 0.5 (11 – 12)	12 $\pm$ 0.8 (11 – 13)	14 $\pm$ 0.9 (12 – 16)	12
at guiding ring	20 $\pm$ 1.5 (18 – 22)	20 $\pm$ 1.2 (19 – 22)	22 $\pm$ 2.7 (18 – 24)	18 $\pm$ 0.5 (18 – 19)	19 $\pm$ 1.5 (18 – 21)	22 $\pm$ 2.0 (19 – 28)	24
at base of pharynx	51 $\pm$ 5.7 (40 – 59)	54 $\pm$ 2.2 (53 – 58)	60 $\pm$ 3.6 (55 – 63)	48 $\pm$ 3.9 (42 – 50)	45 $\pm$ 4.0 (41 – 50)	46 $\pm$ 5.4 (36 – 60)	50
at mid body/at vulva	62 $\pm$ 6.4 (49 – 69)	63 $\pm$ 2.3 (61 – 67)	71 $\pm$ 5.3 (65 – 77)	55 $\pm$ 3.1 (42 – 50)	55 $\pm$ 3.3 (51 – 59)	56 $\pm$ 5.6 (45 – 66)	59
at anus	39 $\pm$ 3.7 (34 – 44)	43 $\pm$ 1.9 (41 – 46)	46 $\pm$ 2.8 (65 – 77)	38 $\pm$ 1.3 (36 – 39)	35 $\pm$ 1.0 (34 – 36)	39 $\pm$ 4.5 (31 – 50)	36
at beginning of hyaline tip	22 $\pm$ 1.4 (19-23)	18 $\pm$ 1.3 (16-19)	24 $\pm$ 3.0 (20-27)	18 $\pm$ 0.6 (17-18)	20 $\pm$ 1.2 (19-21)	19 $\pm$ 2.4 (14-25)	21

the Hersbruck population. Morphometrics of J2 stage of Scottish population almost overlap those of the J3 stage of German populations from Hersbruck (Table 4). Morphological and morphometrical identification of the

species was also verified by PCR using the ribosomal established primers from single specimens (Hübschen *et al.*, 2004). A single fragment of approximately 847 bp was amplified for all studied individuals (Fig. 2.). No PCR pro-

Fig. 2. Electrophoresis of the amplified products from single females and males of *Longidorus elongatus*: Lane 1 – 100 bp DNA ladder; lane 2 – Bílé podolí (female); lane 3 – Břeclav (male); lane 4 – Hrušky (female); lane 5 – Kobyli (female)

Fig. 3. *Longidorus leptocephalus* Hooper, 1961. A – Male anterior region; B – Male posterior region; C – E – Female posterior region. *Longidorus euonymus*, Mali and Hooper, 1974 F – Female anterior region; G – Female posterior region. (Scale bar: 50 μ m)

ducts were obtained in the negative control lacking DNA template or in the negative control containing DNA of *L. intermedius*, *L. leptocephalus* and *Longidorus* sp. (data not shown).

Longidorus euonymus Mali & Hooper, 1974

Fig. 3, 4

L. euonymus is typically a mainland European species and a putative vector of *Euonymus mosaic necrovirus* (EMV). During current surveys in the Czech Republic, the species was recorded only from one locality, Kojice, 1 female and 4 juvenile specimens being found in association with plum on sandy soil. The juveniles were preserved in 1M NaCl for molecular study. It constitutes a new record for the Czech Republic. A previous record of *L. euonymus* from the rhizosphere of *Euonymus* tree in the Czech Republic (Oro *et al.*, 2005) is in reality from the locality Ivanka pri Dunaji, district Bratislava situated in the Slovak Republic. The morphometrics of the Czech female are: L = 8.1 mm; a = 142; b = 16.87; c = 202; c' = 0.93; V = 51.8; odontostyle = 85 μ m; odontophore = 42 μ m; oral aperture to guide ring = 31 μ m; pharyngeal bulb length = 137 μ m; pharyngeal bulb diameter = 25 μ m; tail length = 40 μ m; length of hyaline tip = 14 μ m; body diameter: at lip region = 17 μ m; at guiding ring = 24 μ m; at base of pharynx = 48 μ m; at vulva = 57 μ m; at anus = 43 μ m; at beginning of hyaline tip = 28 μ m. Compared with the Slovakian population (Mali & Hooper, 1974) and the other more recently recorded European populations: Yugoslavian (Barsi, 1989; Oro *et al.*, 2005), Italian (Roca & Lamberti, 1994), Bulgarian (Lamberti *et al.*, 1997) and Austrian (Tiefenbrunner & Tiefenbrunner, 2004b), the majority of the morphometrics are largely similar to that of the Czech female specimen. The following variations have been observed: the Czech specimen has a shorter tail compared with Slovakian and Austrian females, (40 vs 45 μ m and 51 μ m respectively) i.e. close to the lower range for the species. Its body length is larger compared to Bulgarian population (8.1 vs 5.7 mm). Considerable differences occur in odontophore length between most of the mentioned populations with the greatest variation between the Czech and Austrian (Tiefenbrunner & Tiefenbrunner, 2004b) and Yugoslavian females (Barsi, 1989) (42 vs 65.6 μ m and 63 μ m respectively). In the original species description of *L. euonymus* Mali & Hooper, 1973, *L. closelongatus* and *L. cohnii* were considered to be the most closely related species. *L. euonymus* differs from *L. closelongatus* and *L. cohnii* by a much shorter odontostyle and *L. cohnii* also by a fatter body. *L. euonymus* also resembles *L. vineacola*, *L. elongatus* and *L. attenuatus*. It differs from *L. vineacola* by a more pronounced offset lip region, a shorter odontostyle, fewer body pores in neck region and relatively longer tail; from *L. elongatus* by its more offset lip region and more bluntly rounded tail and from *L. attenuatus* by longer body, more bluntly rounded tail and fewer body pores in neck region.

According to Oro *et al.* (2005) 18S rDNA sequence analysis indicated that *L. euonymus* was more closely related to *L. elongatus* and *L. attenuatus* than to *L. vineacola*. However, they found that distances in the maximum likelihood tree between the species appear to be relatively small. The most closely related species (*L. closelongatus* and *L. cohnii*) were not included in the study of Oro *et al.*

Fig. 4. Distribution of *Longidorus euonymus*, *L. elongatus* and *L. leptocephalus* in vineyards and orchards in Bohemia and South Moravia (Czech Republic)

Table 3. Comparison of morphometrics of females of *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922

Population	Population type	Body length (mm)	Length odontostyle (μm)	Length odontophore (μm)	Tail length (μm)	V (%)
Austrian (n = 11)	parthenogenetic	5.0 (4.4 – 5.6)	86.5 (80 – 90)	56.5 (47.5 – 65.0)	39.3 (35 – 44)	49.9 (47.3–52.4)
Austrian (n = 2)*	parthenogenetic	5.0	88	□	40	47.9
German (n = 15)°	parthenogenetic	4.9 (4.0 – 5.8)	81 (74 – 92)	43 (39 – 51.5)®	50 (38 – 59)®	50.7 (46.8 – 53.1)
German (n = 12)°	parthenogenetic	5.2 (4.3 – 6.0)	90 (79 – 97)	45 (34 – 67)	46 (39 – 51)	(40 (44 – 52)
German (n = 100)°°	gonochoristic	5.8 (5.2 – 6.3)	89 (85 – 92)	45 (42 – 47)	43 (41 – ??)	50 (49 – 51)
German (n = 300)°°	parthenogenetic	5.9 (5.4 – 6.4)	96 (92 – 100)	51 (46 – 57)	50 (47 – 55)	48 (45 – 52)
Dutch (n = 58)	gonochoristic	—	84.5 (72 – 91)	—	42 (35 – 51)	—
Dutch (n = 89)	parthenogenetic	—	94 (83 – 100)	—	46 (40 – 52)	—
Scottish (n = 199)	gonochoristic?	5.8 (4.6 – 7.1)	94.9 (72 – 110)	56.8 (40.5 – 69.5)	42 (36 – 48)	47.6 (42 – 55.5)
Serbian (n = 33)	parthenogenetic	5.7 (5.1 – 6.4)	92 (86 – 98)	61 (53 – 64)	47 (40 – 54)	50.5 (47 – 52.5)
Serbian (n = 10)	parthenogenetic	5.3 (4.7 – 6.5)	90 (86 – 95)	60 (56 – 69)	42 (36 – 51)	50 (48 – 53)
Slovakian (n = 5)	parthenogenetic	5.6 (5.0 – 6.2)	91 (88 – 96)	62 (60 – 64)	45 (41 – 48)	47.5 (46 – 49)
Swiss (n = 10)	parthenogenetic	6.0 (5.1 – 6.5)	87.3 (80 – 94)	65.1 (60.5 – 72)	51.6 (46.5 – 62)	48.6 (47 – 51)
Czech: Břeclav	gonochoristic	5.0 (4.1 – 5.8)	77 (73 – 81)	44 (40 – 48)	42 (36 – 48)	55 (49 – 60)
Bílé Podolí** (n = 8)	gonochoristic	5.3 (4.7 – 6.1)	84 (77 – 93)	43 (39 – 47)	39 (30 – 47)	49 (47.5 – 53)
Bílé Podolí*** (n = 4)	gonochoristic	5.5 (5.0 – 6.1)	88 (77 – 94)	45 (42 – 48)	45 (38 – 48)	48.2 (45 – 52)
Kobyli (n = 33)	parthenogenetic	5.3 (4.2 – 6.2)	87 (80 – 93)	46 (40 – 53)	40 (31 – 51)	49.2 (47 – 51.5)
Lhenice (n = 1)	parthenogenetic	4.2	107	41	43	48.2
Hrušky (n = 4)	parthenogenetic	5.2 (4.4 – 5.8)	78 (72 – 81)	41 (40 – 42)	40 (39 – 42)	50.9 (48.2 – 53.4)
Czech ranges (Lhenice)		4.1 – 6.2 (4.2)	73 – 94 (107)	39 – 53 (41)	30 – 51 (43)	45 – 60 (48.2)

* mean values; ° Sturhan, 1963; ® n 7; °° Kozłowska and Seinhorst, 1974; ?? Error; ? males more rare; ** host-apple; *** host-peach

(2005). *L. piceicola* is morphologically and morphometrically distinct from *L. euonymus* but Oro *et al.* (2005) found it closely related with regard to 18S sequence data.

L. leptocephalus Hooper, 1961

Fig. 3, 4; Table 5

L. leptocephalus is a predominantly pan-European species, widely distributed in central and northern Europe (Alphey and Taylor, 1986; Navas *et al.*, 1993). It is the most common *Longidorus* species recorded in neighbouring Slovakia (Lišková & Brown, 1995, 2003; Lišková *et al.*, 1995). Our survey from South Moravia and Bohemia also showed its frequent occurrence compared to other *Longidorus* species. Recently, morphometrical data for one population of *L. leptocephalus* from the Czech Republic was presented by Kumari (2004). Here morphometrical data for another 5 populations are provided.

Female and juvenile specimens were found in all positive sites but only one male was discovered from a single locality at Bílé podolí associated with sour cherry. It occurred with population densities of 1 to 12 females and 1 to 53 juveniles per 500 g soil. The morphological characteristics and morphometrics of Czech females fit the original description (Hooper, 1961) and a large form of the species (Hooper, 1973), and the population previously recorded in the Czech Republic (Kumari, 2004). Comparison of Czech specimens with populations from neighbouring countries Slovakia (Lišková & Brown, 1995) and Austria (Tiefenbrunner & Tiefenbrunner, 2004b) reveal that their morphometrics are largely similar except for longer body length and tail length. Czech populations are also stouter than those from Slovakia. These differences in morphometrics may be due to morphological plasticity related to environmental factors. This has been previously reported by Lišková and Brown (1995).

Lišková and Brown (1995) compared four Slovakian populations with populations from Scotland and England. Morphometrics of both Slovak populations described are 100 % identical to the morphometrics of the specimens from Scotland and England; suggesting an error in recording of the data.

A single male specimen was recorded from a locality at Bílé podolí under the rhizosphere of sour cherry (Table 4). So far, *L. leptocephalus* males have only been described by Lišková (1996). The Czech male specimen is largely similar to females, apart from male characteristics and a more ventrally curved posterior body. It shows some minor morphometrical variability with the males described by Lišková (1996): shorter odontostyle length of Czech male (59 vs 67 and 63 µm of Slovakian males); shorter spicule length (41 vs 44 and 46 µm); longer tail (49 vs 32 and 41 µm).

L. leptocephalus was found in the following sites (in parenthesis associated hosts): Bílé podolí (sour cherry, plum, apple); Kojice (plum); Lhenice (sweet cherry, plum, apple); Temelín (apple); Třebanice (sweet cherry). *L. leptocephalus* was also found in one nursery at Vysoké Studnice associated with apple and pear. It has not been recorded from vineyards. At Bílé podolí *L. leptocephalus* was

found together with another needle nematode, *L. elongatus*, and at Kojice with *L. euonymus*. *L. leptocephalus* can be differentiated from *L. elongatus* by its shorter style (mean less than 72 vs more than 77 µm) and more pointed vs more bluntly rounded tail.

Table 4. Comparison of morphometrics of juvenile stages *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922

Population	Body length (mm)	Odontostyle (µm)	Replacement odontostyle (µm)
J1			
Czech	0.99	47	50
German°	1.06	49	51
German*	1.01	55	59
Scottish	1.10	55	65
Serbian	1.15	56.4	58.7
J2			
Czech	1.42	49	59
German°	1.41	54	59.5
German*	1.67	58	67
Scottish	1.80	63	73
Serbian	1.86	59.1	70.4
J3			
Czech	2.48	61	71
German°	2.26	67	69
German*	1.81	65	73
Scottish	2.70	73	83
Serbian	2.91	73.4	80.5
J4			
Czech	3.62	72	80
German°	3.61	73	81
German*	3.05	76	87
Scottish	3.90	83	92
Serbian	4.10	81.5	91.8

°Bubenreuth; * Hersbruck

Discussion

The three *Longidorus* species studied, *L. elongatus*, *L. euonymus* and *L. leptocephalus*, are well-known and described species from other European countries, but characteristics of these species from the Czech Republic were previously limited to the species name (Erbenová, 1973, 1977; Pelikán, 1989; Bartošová & Háněl, 1994). Molecular techniques have shown great potential in *Longidorus* identification (Hübschen *et al.*, 2004), but *Longidorus* taxonomy is still largely based on the morpho-species concept. Therefore, the morphometrical data presented here are still important for species identification and understanding of possible variation due to geographical and/or environmental differences. Further, additional data from a larger number of populations appear to widen the range of the diagnostic characters and result in more overlapping between species. They underline the weakness of current polytomous keys (see Chen *et al.*, 1997) based on a few morphological and some more morphometric features. Chen *et al.* (1997) con-

Table 5. Morphometrics of females and male of *Longidorus leptocephalus* Hooper, 1961. All measurements in μm except body length in mm [mean $\pm$ standard deviation (range)]

Locality Host Specimens	Lhenice sweet cherry Females	Vysoké Studnice pear Females	Kojice plum Females	Bílé podolí plum Females	Bílé podolí sour cherry	
	Females	Females	Females	Females	Females	Male
n	5	31	10	4	7	1
L	5.6 $\pm$ 0.57 (5.1 – 6.4)	5.2 $\pm$ 0.37 (4.3 – 6.1)	5.1 $\pm$ 0.42 (4.5 – 5.8)	4.6 $\pm$ 0.34 (4.2 – 5.0)	4.6 $\pm$ 0.32 (4.2 – 5.3)	4.3
a	99.1 $\pm$ 13 (86.4 – 113.6)	91.4 $\pm$ 8.9 (75.6 – 116.7)	91.9 $\pm$ 8.7 (78.1 – 104.4)	88.4 $\pm$ 7.8 (77.5 – 96.2)	93.1 $\pm$ 8.7 (82.9 – 106.9)	95.6
b	14.1 $\pm$ 4.0 (7.5 – 16.8)	13.8 $\pm$ 1.4 (9.4 – 16.4)	13.7 $\pm$ 1.2 (12.5 – 15.9)	14.2 $\pm$ 2.5 (11.6 – 17.5)	13.9 $\pm$ 0.5 (13.2 – 14.5)	12.7
c	119.7 $\pm$ 12.2 (107.4 – 139.5)	110.9 $\pm$ 14.4 (86.6 – 148.0)	117.7 $\pm$ 13.7 (97.4 – 143.4)	116.7 $\pm$ 18.3 (97.3 – 140.0)	111.2 $\pm$ 15.5 (88.5 – 130.6)	87.8
c'	1.31 $\pm$ 0.15 (1.13 – 1.48)	1.25 $\pm$ 0.16 (1.00 – 1.57)	1.23 $\pm$ 0.12 (0.97 – 1.37)	1.31 $\pm$ 0.18 (1.06 – 1.44)	1.33 $\pm$ 0.16 (1.08 – 1.48)	1.69
Vulva/spicule	52.2 $\pm$ 1.0 (51.5 – 53.9)	51.0 $\pm$ 2.8 (46.8 – 60.4)	49.3 $\pm$ 1.1 (47.6 – 50.7)	53.5 $\pm$ 1.0 (52.3 – 54.5)	51.8 $\pm$ 1.7 (49.7 – 54.7)	41
Odontostyle	72 $\pm$ 1.9 (70 – 74)	71 $\pm$ 3.2 (59 – 76)	69 $\pm$ 2.4 (65 – 74)	64 $\pm$ 3.2 (61 – 68)	64 $\pm$ 2.8 (60 – 67)	59
Odontophore	39.4 $\pm$ 3.7 (35 – 44)	41 $\pm$ 3.1 (35 – 48)	39 $\pm$ 1.6 (37 – 43)	41 $\pm$ 2.6 (38 – 44)	39 $\pm$ 3.7 (34 – 44)	34
Total sylet length	113.2 $\pm$ 6.4 (106 – 122)	112 $\pm$ 4.0 (104 – 122)	108 $\pm$ 2.4 (104 – 112)	105 $\pm$ 4.1 (100 – 110)	103 $\pm$ 5.0 (96 – 110)	93
Oral aperture to guide ring	29 $\pm$ 1.6 (27 – 31)	28 $\pm$ 0.9 (27 – 30)	28 $\pm$ 1.3 (26 – 30)	27 $\pm$ 1.3 (26 – 29)	28 $\pm$ 1.9 (25 – 30)	29
Pharyngeal bulb length	101.6 $\pm$ 6.9 (96 – 111)	100 $\pm$ 5.6 (91 – 114)	101 $\pm$ 4.9 (94 – 108)	91 $\pm$ 0.6 (91 – 92)	91 $\pm$ 6.1 (81 – 100)	83
Pharyngeal bulb diam.	18.8 $\pm$ 1.1 (18 – 20)	21 $\pm$ 2.7 (16 – 30)	22 $\pm$ 1.6 (20 – 25)	16 $\pm$ 1.4 (15 – 18)	18 $\pm$ 2.8 (14 – 21)	12
Tail length	47 $\pm$ 7.4 (37 – 55)	47 $\pm$ 5.6 (38 – 61)	44 $\pm$ 4.5 (36 – 50)	40 $\pm$ 4.2 (36 – 46)	42 $\pm$ 6.2 (36 – 52)	49
Length of hyaline tip	14 $\pm$ 3.2 (12 – 20)	14 $\pm$ 1.9 (11 – 17)	13 $\pm$ 2.0 (11 – 18)	12 $\pm$ 1.3 (11 – 14)	13 $\pm$ 1.6 (11 – 16)	17
Number of supplements	—	—	—	—	—	10
Body diameter at lip region	10 $\pm$ 0.5 (10 – 11)	9 $\pm$ 0.6 (9 – 11)	10 $\pm$ 0.5 (10 – 11)	9 $\pm$ 0.6 (8 – 9)	9 $\pm$ 0.4 (9 – 10)	10
at guiding ring	19 $\pm$ 0.8 (18 – 20)	20 $\pm$ 1.4 (18 – 25)	19 $\pm$ 1.0 (18 – 21)	18 $\pm$ 0.00 (18 – 18)	18 $\pm$ 4.3 (17 – 19)	18
at base of pharynx	44 $\pm$ 2.5 (41 – 46)	47 $\pm$ 4.1 (37 – 53)	44 $\pm$ 2.8 (39 – 47)	39 $\pm$ 2.5 (36 – 42)	40 $\pm$ 4.3 (34 – 46)	34
at mid body/at vulva	56 $\pm$ 3.0 (52 – 59)	57 $\pm$ 3.8 (47 – 63)	56 $\pm$ 3.5 (50 – 60)	53 $\pm$ 8.3 (47 – 65)	50 $\pm$ 4.4 (44 – 55)	45
at anus	36 $\pm$ 2.8 (31 – 38)	38 $\pm$ 3.4 (33 – 44)	36 $\pm$ 2.0 (33 – 38)	31 $\pm$ 3.0 (27 – 34)	32 $\pm$ 3.6 (37 – 35)	29
at beginning of hyaline tip	17 $\pm$ 0.7 (16 – 18)	17 $\pm$ 2.6 (13 – 23)	16 $\pm$ 1.5 (15 – 20)	13 $\pm$ 2.1 (11 – 15)	14 $\pm$ 2.4 (10 – 17)	12

sidered, as main characters for their polytomous key, the shape of lip region, amphid base and tail, complemented by numerical characters. When sorting the species in the key on the three main features, different populations of a species do not always group together. Moreover, closely related species i.e. based on similarities may differ from

discussion on relationships in the original species description and based on sequence data (see previously for *L. euonymus*).

In Central Bohemia *Longidorus* species have been recorded only from one orchard out of 26 surveyed orchards and vineyards (Kumari, 2004); in South-East and North-East

Bohemia from 9 orchards out of 34 orchards and in South-Moravia from a single orchard (Kobyli) and two vineyards (Břeclav, Hrušky) out of 76 surveyed orchards and 29 vineyards. The survey data indicate that soil type may be the main limiting factor for the occurrence of *Longidorus* in the Czech Republic as *Longidorus* species in the Czech Republic are only recorded in sandy soil.

L. leptcephalus appeared to have a wider horizontal distribution in the surveyed regions compared to *L. elongatus* and *L. euonymus*. Within the survey presented, only one nursery was included. The nursery was found positive for *L. leptcephalus*. More comprehensive surveys of nurseries might show that the presence of *Longidorus* in nurseries to be one of the reasons which contributes to its wider occurrence in the Czech Republic, but this hypothesis needs to be substantiated.

As compared to other member countries of the European Union, systematic sampling in the Czech Republic is still lacking. From 2002 to 2006, we have systematically surveyed orchards and vineyards in South Moravia and Central, South-East and North-East Bohemia for the presence of longidorid nematodes. But there is still a long way to systematically survey the whole country for the occurrence and distribution of longidorid nematodes in different habitats.

Due to the limitations and constraints of morphological observations, DNA-based methods have recently become a valuable tool for separating *Longidorus* species. DNA base sequences have proved very useful for evaluating genetic distances and for assisting the development of phylogenies (De Luca *et al.*, 2004; He *et al.*, 2005; Lazarova *et al.*, 2006 and others). Due to difficulties obtaining sufficient and quality DNA template from specimens mounted in permanent slides, molecular nematology has until recently been restricted to fresh nematode DNA analysis. However, new DNA extraction technique from glycerin-embedded nematodes such as Rubtsova *et al.* (2005) method has ameliorated this problem to some extent. Therefore, molecular characterization, mainly DNA base sequences of ribosomal and mitochondrial DNA from our permanent slides and also from fresh material, will be the subject of further study.

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