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Xiphinema simile (Nematoda: Longidoridae) in the Czech Republic and a note on other *Xiphinema* species

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Summary

A nematode survey was carried out in South Moravia and Bohemia (Czech Republic) to assess the occurrence of *Xiphinema* in the rhizosphere of fruit orchards. Sixty six orchards in South Moravia and seven in Bohemia were studied during the years 2003 and 2004. Four *Xiphinema* species (*X. diversicaudatum*, *X. pachtaicum, X. simile* and *X. vuittenezi*) were recorded. *X. simile* constitutes a first record for the nematodes fauna of the Czech Republic.

Key words: *Xiphinema diversicaudatum*; *Xiphinema pachtaicum*; *Xiphinema simile*; *Xiphinema vuittenezi*; PCR; Czech Republic

Introduction

Reports on the occurrence of *Xiphinema* in the territory of the Czech Republic date back to 1970s and late 1980s (Mali & Vanek, 1972; Erbenová, 1973, 1975; Pelikán, 1989). After no attention was paid to nematodes of the genus *Xiphinema* and it was until recently (Kumari, 2004; Kumari *et al*., 2005) that problems of *Xiphinema* were put in the foreground again.
X. simile is a localised species in Europe (Lamberti *et al*., 2000) and it was reported from Bulgaria (Lamberti, *et al*., 1983; Peneva & Choleva, 1992), Yugoslovakia (Barsi, 1994) and Slovakia (Lišková & Brown, 1996). Outside Europe the species was recorded from Kenya (Coomans & Heyns, 1997). The present study includes further record of *X. simile* in Europe and first record for the Czech fauna.
Several species of the genus *Xiphinema* include plant-parasitic nematodes and are natural vectors of economically important nepoviruses that cause substantial damage to a wide range of crops (Taylor & Brown, 1997). Thus, accurate identification of those species that have the ability to transmit virus is essential. Problem associated with identification of *Xiphinema* on the bases of morphological and morphometrical characters that many characters overlap. Moreover, substantial morphological and morphometrical variability is apparent between the various species of *Xiphinema*. Recent studies have been demonstrated a great potential for molecular studies in *Xiphinema* species identification (Wang *et al*., 2003; Hübschen *et al.,* 2004; Oliveira *et al*., 2005).
In this study, objectives were to:
(1) survey *Xiphinema* species occurring in the different orchards of South Moravia and Bohemia,
(2) identify different species of *Xiphinema* by morphological and morphometrical characters,
(3) conduct PCR-based methodology to verify the identification of *X. diversicaudatum* and *X. vuittenezi*.

Material and Methods

Morphological and morphometrical study
Nematode survey was carried out in the different regions of South Moravia and Bohemia, Czech Republic. Soil samples were taken at a depth of 0 – 90 cm. Nematodes were recovered from the soil by sieving and decanting technique, heat killed, fixed in TAF, processed in slow glycerin process and mounted in anhydrous glycerin on slides. 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
Total genomic DNA was extracted according to Hübschen *et al.* (2004) and DNA template was amplified by PCR using established primers by Wang *et al.* (2003). PCR reaction and gel electrophoresis condition were same as described by Kumari *et al*. (2005).

Results

Xiphinema simile Lamberti, Choleva *et* Agostinelli, 1983
Fig.1; Table 1, 2
X. simile was recorded at four (Čejkovice – under the rhi-

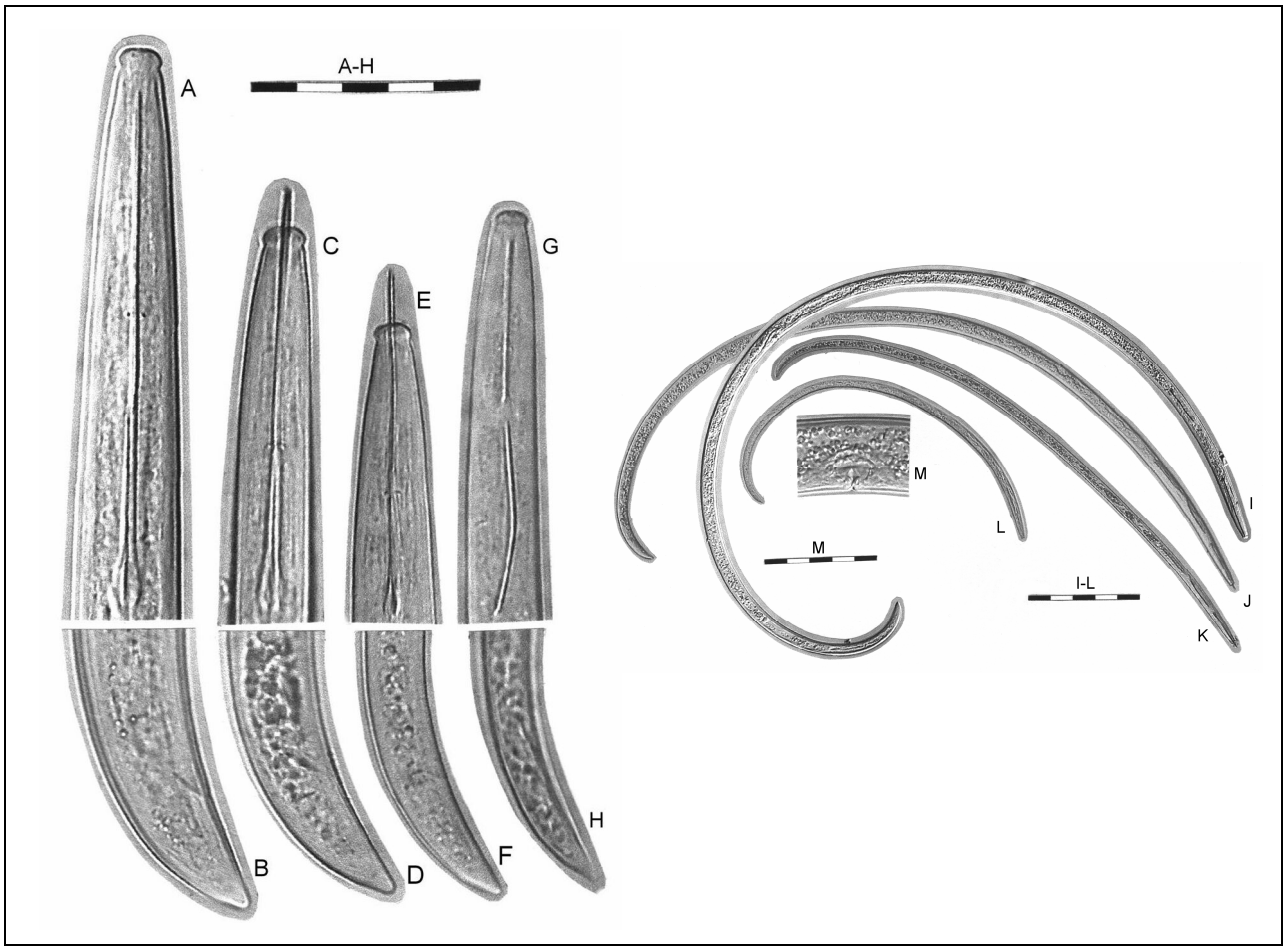

Fig. 1. *Xiphinema simile* Lamberti, Choleva *et* Agostinelli, 1983
A – H. Anterior and posterior regions. A, B: Female; C, D: JIII; E, F: JII; G, H: JI. I – L Entire nematodes. I: Female; J: JIII; K: JII; L: JI. M: Vulva. Scale bars: A – H, M, 50 µm; I – L, 200 µm

zosphere of sour cherry; Dětkovice – under the rhizosphere of sweet cherry; Prušánky – under the rhizosphere of apple; Velké Pavlovice – under the rhizosphere of apricot) of the 73 sampling orchards and occurred with population density of 4 to 25 adult females and 2 to 18 juveniles per 500 g soil. Adult females and juveniles were present at all four orchards, but not a single male was found. When adult females compared with Bulgarian (Peneva & Choleva, 1992), Slovakian (Lišková & Brown, 1996) and Kenyan (Coomans & Heyns, 1997) populations, all morphometrics were closely agree except body length and pharyngeal bulb length. Mean body length of Czech population is similar to Slovakian populations but longer than Bulgarian and as well as Kenyan populations. Pharyngeal bulb length is longer than Kenyan population. Morphometrics of adult females also agree with the paratypes (Lamberti *et al*., 1983) except longer length of oral aperture to guide ring (51 µm *vs.* 60 µm; 58 µm; 60 µm; 59 µm). Beside some morphometrical variability, specimens from four populations appear to be morphologically similar.

According to Coomans and Heyns (1997) and Lamberti *et al*. (2000) the species has only three juvenile development stages. After comparing the body length, functional and replacement odontostyle length, distance of guiding ring from oral aperture and tail shape our data also confirmed the presence of three juvenile stages, with good agreement between the replacement and functional odontostyle length of successive stages. Juveniles were similar to females except for length and the more slender tail which gradually becomes thicker after each moult. JIII have a short conoid tail as in the female, whereas the two other juvenile stages have a longer slender tail. Juveniles of Czech population from Dětkovice and Velké Pavlovice correspond well with juvenile stages described from Kenya (Coomans & Heyns, 1997).

26 orchards and vineyards (Kumari, 2004), 29 vineyards (Kumari *et al*., 2005) and 73 orchards (present study) have been surveyed during the years 2002 to 2004. A total of 128 sites have been surveyed and *X. simile* was recorded only from four sites.

Xiphinema diversicaudatum (Micoletzky, 1927) Thorne, 1939

Xiphinema pachtaicum (Tulaganov, 1938) Kirjanova, 1951

Table 1. Morphometrics of three juvenile stages of *Xiphinema simile* Lamberti, Choleva *et* Agostinelli, 1983. Measurements in µm (in form): mean ± standard deviation (range)

Locality Host	Dětkovice sweet cherry	Dětkovice sweet cherry	Velké Pavlovice apricot	Dětkovice sweet cherry	Velké Pavlovice apricot
Specimens	JI	JII	JII	JIII	JIII
n	3	5	3	7	16
L	864 ± 96 (797 – 974)	1267 ± 100 (1143 – 1420)	1145 ± 48 (1105 – 1199)	1803 ± 174 (1435 – 1975)	1604 ± 84 (1386 – 1711)
a	54.8 ± 5.35 (48.7 – 58.7)	56.1 ± 4.0 (52.4 – 61.7)	53 ± 9.28 (42.5 – 60.0)	66.1 ± 5.5 (58.4 – 74.9)	62.6 ± 4.54 (53.9 – 70.3)
b	—	6.0 ± 0.5 (5.3 – 6.7)	5.2 ± 0.35 (4.8 – 5.4)	6.9 ± 1.0 (5.5 – 8.1)	6.2 ± 0.41 (5.4 – 6.8)
c	—	41.5 ± 4.5 (36.4 – 48.4)	41.4 ± 2.11 (39.0 – 42.8)	54.3 ± 6.4 (49.3 – 68.1)	52.6 ± 5.37 (43.2 – 61.3)
c´	—	2.24 ± 0.23 (2.00 – 2.61)	2.04 ± 0.30 (1.70 – 2.30)	1.93 ± 0.16 (1.70 – 2.23)	1.80 ± 0.15 (1.62 – 2.07)
Replacement odontostyle	44 ± 1.15 (43 – 45)	54 ± 2.61 (50 – 57)	56 ± 2.08 (54 – 58)	67 ± 2.36 (64 – 71)	69 ± 1.78 (65 – 72)
Odontostyle	35 ± 2.08 (33 – 37)	44 ± 1.3 (43 – 46)	45 ± 0.58 (44 – 45)	54 ± 1.07 (52 – 55)	57 ± 1.82 (54 – 60)
Odontophore	29 ± 1.52 (28 – 31)	34 ± 2.86 (30 – 38)	31 ± 3.46 (27 – 33)	36 ± 1.6 (33 – 38)	36 ± 1.61 (33 – 39)
Total stylet length	65 ± 0.58 (64 – 65)	78 ± 3.53 (75 – 84)	76 ± 3.21 (72 – 78)	90 ± 1.95 (86 – 92)	93 ± 2.25 (90 – 98)
Greatest flange width	—	4 ± 0.45 (4 – 5)	4 ± 0.58 (4 – 5)	4 ± 0.97 (3 – 6)	5 ± 0.72 (4 – 7)
Oral aperture to guide ring	30 ± 1.73 (28 – 31)	38 ± 1.3 (37 – 40)	36 ± 1.15 (35 – 37)	47 ± 2.88 (43 – 50)	46 ± 1.76 (44 – 50)
Pharyngeal bulb length	—	60 ± 1.52 (58 – 62)	54 ± 2.00 (52 – 56)	72 ± 6.57 (61 – 83)	65 ± 5.94 (54 – 74)
Pharyngeal bulb diam.	—	11 ± 1.14 (9 – 12)	12 ± 1.00 (11 – 13)	13 ± 1.73 (11 – 16)	13 ± 1.41 (11 – 16)
Tail length	—	31 ± 3.96 (26 – 34)	28 ± 1.53 (26 – 29)	33 ± 3.91 (28 – 38)	31 ± 3.34 (26 – 39)
Length of hyaline tip	5 ± 1.53 (4 – 7)	5 ± 1.09 (4 – 6)	5 ± 0.58 (5 – 6)	6 ± 1.89 (4 – 8)	6 ± 1.02 (3 – 7)
Body diameter at lip region	7 ± 0.58 (7 – 8)	8 ± 0.56 (7 – 8)	8 ± 0.58 (7 – 8)	8 ± 0.58 (7 – 9)	9 ± 0.63 (8 – 10)
at guiding ring	13 ± 0.58 (13 – 14)	15 ± 0.84 (14 – 16)	16 ± 2.64 (14 – 19)	17 ± 0.53 (17 – 18)	17 ± 0.82 (16 – 18)
at base of pharynx	—	20 ± 1.09 (19 – 21)	21 ± 3.21 (19 – 25)	25 ± 2.67 (20 – 28)	23 ± 1.71 (20 – 27)
at mid body	16 ± 3.46 (14 – 20)	23 ± 1.52 (21 – 24)	22 ± 3.46 (20 – 26)	27 ± 3.41 (21 – 31)	26 ± 2.46 (21 – 29)
at anus	—	14 ± 1.64 (12 – 16)	14 ± 1.53 (12 – 15)	17 ± 1.25 (15 – 19)	17 ± 1.41 (14 – 19)
at beginning of hyaline tip	4 ± 1.15 (3 – 5)	6 ± 0.45 (5 – 6)	6 ± 0.58 (5 – 6)	7 ± 1.46 (5 – 9)	7 ± 0.73 (6 – 8)

Xiphinema vuittenezi Luc, Lima, Weischer *et* Flegg, 1964
Figs. 2, 3; Tables 2, 3

Detail description of these three species from the Czech Republic was already given by Kumari (2004) and Kumari *et al*. (2005). Here only additional data is presented.

X. diversicaudatum was recorded in three orchards (peach, plum and sour cherry) at Bílé podolí, Bohemia out of the 73 sampling orchards and occurred with population density of 4 to 34 adult females, 4 to 42 adult males and 8 to 200 juveniles per 500 g soil. Between adult females one female specimen has all morphometrics similar to fourth stage juvenile (Table. 3). *X. diversicaudatum* was not found in South Moravia under rhizosphere of any orchard, but it was recorded in viticulture from South Moravia (Kumari *et*

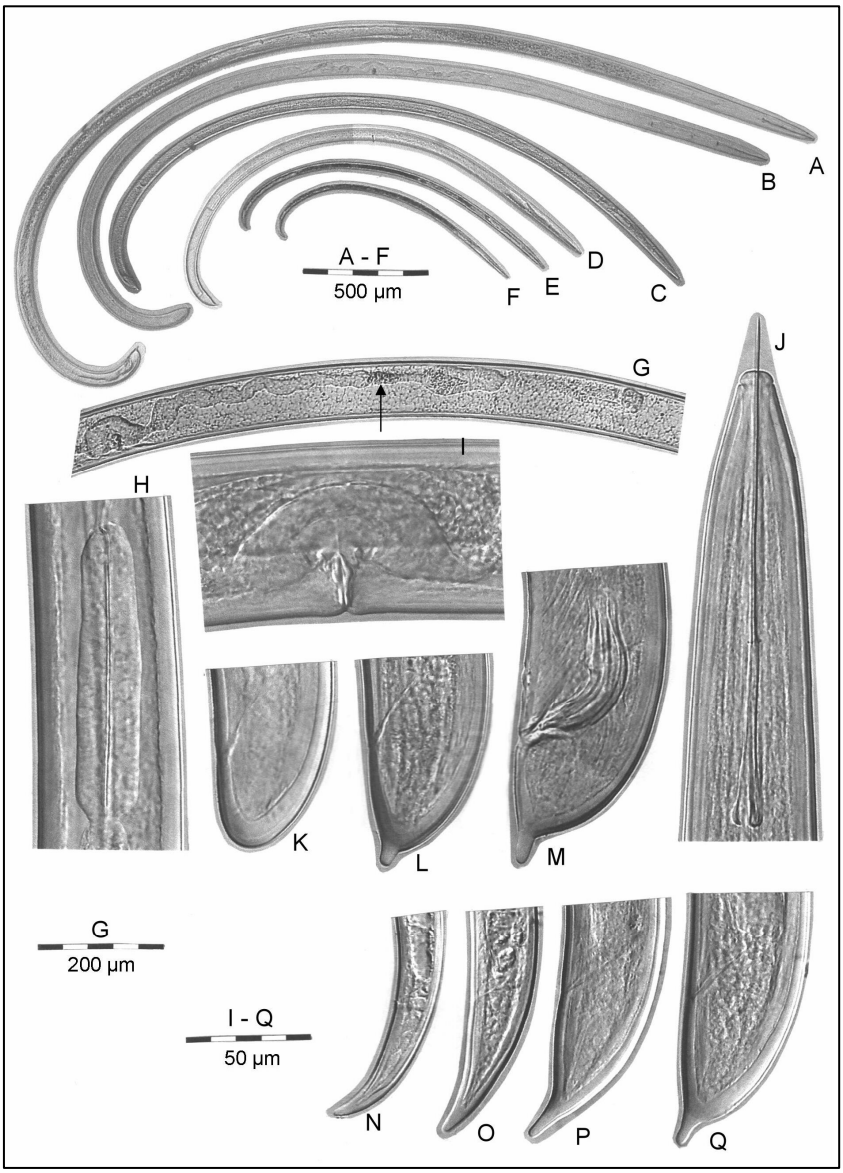

Fig. 2. *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939
A – F Entire nematodes. A: Male; B: Female; C: J4; D: J3; E: J2; F: J1. G: Posterior genital branch (arrow indicating Z-organ); H: Female pharyngeal bulb; I: Vulva; J: Female anterior; K, L: Female tail; M: Male tail; N: J1 tail; O: J2 tail; P: J3 tail: Q: J4 tail

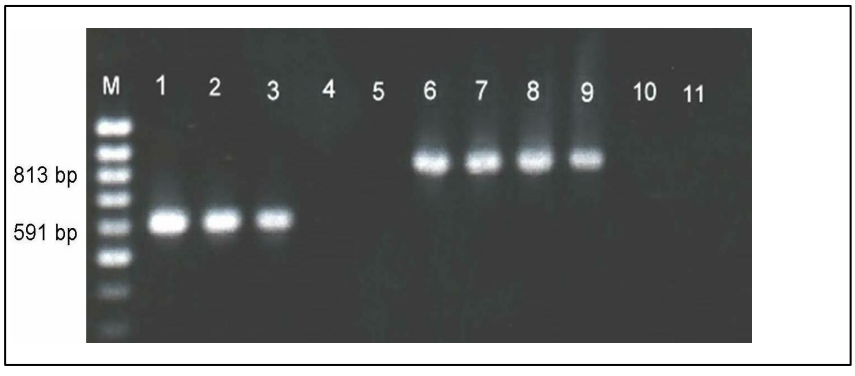

Fig. 3. Electrophoresis of the amplified products from single individual of *Xiphinema vuittenezi* and *X. diversicaudatum*. Lane M: 100 bp DNA ladder; lane 1 to 3 = *X. vuittenezi* (1: Čejkovice; 2: Hodonice; 3: Kobylí); lane 4 negative control without DNA; lane 5: negative control with DNA (*X. diversicaudastum)*; lane 6 to 9 = *X. diversicaudatum* (6: Bílé podolí (peach) for single female; 7: Bílé podolí (plum) for single female; 8 and 9 Bílé podolí (sour cherry) for single female and male; lane 10: negative control without DNA; lane 11: negative control with DNA (*X. vuittenezi*)

Table 2. Morphometrics of adult females of *Xiphinema simile* Lamberti, Choleva *et* Agostinelli, 1983 and *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951. Measurements in µm (in form): mean ± standard deviation (range)

Locality	Čejkovice	Dětkovice	Prušánky		Velké Pavlovice	Mohyla míru
Host	sour cherry	sweet cherry	apple		apricot	apple
Species	*X. simile*					*X. pachtaicum*
n	20	22	2		48	5
L	2175 ± 158 (1899 – 2513)	2293 ± 114 (2035 – 2487)	2243	1974	2210 ± 155 (1838 – 2613)	2081 ± 92 (1988 – 2232)
a	63.0 ± 7.56 (54.0 – 80.5)	72.2 ± 8.16 (59 – 85)	64.1	71.0	72.8 ± 5.51 (62.8 – 82.3)	57.7 ± 5.03 (52.3 – 63.6)
b	7.5 ± 0.47 (6.7 – 8.3)	7.7 ± 0.72 (6.6 – 9.5)	7.8	7.1	7.5 ± 0.95 (5.8 – 10.7)	7.7 ± 0.58 (7.2 – 8.7)
c	77.0 ± 6.80 (65.5 – 95.0)	79 ± 9.43 (62.3 – 99.8)	70.1	70.5	73.7 ± 5.69 (60.8 – 88.9)	72.7 ± 8.73 (63.8 – 86.8)
c´	1.56 ± 0.15 (1.22 – 1.76)	1.65 ± 0.20 (1.35 – 2.19)	1.68	1.65	1.65 ± 0.15 (1.03 – 2.05)	1.74 ± 0.18 (1.50 – 1.94)
V	54 ± 0.91 (52 – 56)	54 ± 1.30 (51 – 57)	54	55	55 ± 1.36 (50 – 58)	54 ± 1.3 (53 – 56)
Odontostyle	67 ± 1.87 (61 – 69)	67 ± 1.91 (62 – 70)	65	67	68 ± 2.33 (62 – 73)	79 ± 2.07 (77 – 82)
Odontophore	42 ± 2.03 (39 – 46)	44 ± 1.82 (41 – 49)	41	40	43 ± 2.07 (38 – 47)	41 ± 2.41 (38 – 44)
Total stylet length	109 ± 2.39 (105 – 113)	111 ± 2.85 (106 – 118)	106	107	111 ± 3.17 (104 – 120)	121 ± 1.48 (119 – 123)
Greatest flange width	5 ± 0.64 (4 – 7)	6 ± 0.74 (4 – 7)	6	7	6 ± 0.71 (4 – 7)	6 ± 0 (6 – 6)
Oral aperture to guide ring	60 ± 2.11 (57 – 65)	58 ± 2.09 (55 – 63)	60	60	59 ± 3.14 (53 – 68)	72 ± 2.59 (69 – 74)
Pharyngeal bulb length	82 ± 3.44 (77 – 90)	86 ± 5.28 (72 – 90)	82	80	80 ± 4.97 (67 – 90)	60 ± 2.88 (56 – 63)
Pharyngeal bulb diam.	14 ± 1.80 (12 – 18)	17 ± 2.20 (11 – 20)	13	13	16 ± 1.75 (13 – 19)	14 ± 1.14 (12 – 15)
Tail length	28 ± 2.64 (24 – 34)	29 ± 3.16 (24 – 37)	32	28	30 ± 2.17 (24 – 35)	29 ± 4.00 (24 – 35)
Length of hyaline tip	4 ± 0.98 (2 – 6)	7 ± 1.27 (4 – 9)	6	7	7 ± 1.27 (5 – 10)	8 ± 2.07 (6 – 11)
Body diameter at lip region	9 ± 0.22 (8 – 9)	9 ± 0.35 (9 – 10)	10	9	9 ± 0.56 (8 – 10)	9 ± 0.55 (8 – 9)
at guiding ring	20 ± 0.94 (18 – 21)	19 ± 1.22 (18 – 22)	19	19	19 ± 0.90 (18 – 22)	22 ± 1.14 (20 – 23)
at base of pharynx	27 ± 2.70 (23 – 31)	28 ± 3.08 (23 – 34)	28	26	26 ± 1.43 (23 – 29)	29 ± 1.92 (26 – 31)
at vulva	35 ± 4.88 (25 – 42)	32 ± 4.54 (25 – 39)	35	28	31 ± 2.88 (26 – 39)	36 ± 2.68 (32 – 39)
at anus	18 ± 1.78 (16 – 22)	18 ± 1.41 (16 – 21)	19	17	18 ± 1.24 (16 – 20)	17 ± 0.89 (16 – 18)
at beginning of hyaline tip	6 ± 0.60 (5 – 7)	8 ± 1.17 (6 – 10)	7	8	9 ± 1.17 (4 – 11)	6 ± 1.10 (5 – 7)

al., 2005), where the species occurred with *X. vuittenezi*. The two species *X. diversicaudatum* and *X. vuittenezi* are so closely related that it was impossible to separate their juvenile stages with certainty. Therefore morphometrical data for juvenile stages was not studied previously from the Czech Republic. Here morphometrical data for all four juvenile stages has been presented from Bílé Podolí under the rhizosphere of sour cherry (Table 3). The four juvenile stages are recognised by the length of the body and of the functional and replacement odontostyle. The length of the replacement odontostyle of one stage corresponds to the length of the functional odontostyle of the next older stage. Only fourth stage juveniles have tails similar to adults, earlier stages have more tapering tails lacking a distinct peg (Fig.2). Morphometrics of juveniles of *X. diversicaudatum* were similar to juveniles from Novi Sad (Barsi &

Table 3. Morphometrics of *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939. Measurements in µm (in form): mean ± standard deviation (range)

Locality				Bílé Podolí			
Host				sour cherry			
Specimens	J1	J2	J3	J4	Females	Female (short)	Males
n	7	6	18	14	22	1	10
L	1010 ± 81 (873 – 1102)	1445 ± 90 (1320 – 1550)	2274 ± 210 (1817 – 2559)	3241 ± 349 (2636 – 3859)	4644 ± 369 (3974 – 5484)	3245	4591 ± 307 (4154 – 5126)
a	42.6 ± 2.80 (36.7 – 45.1)	43.0 ± 2.42 (39.6 – 45.6)	51.9 ± 4.15 (46.5 – 60.5)	59.6 ± 5.07 (52.0 – 71.2)	64.5 ± 3.25 (59.5 – 69.9)	52.3	69.5 ± 3.78 (65.0 – 76.7)
b	4.1 ± 0.40 (3.4 – 4.6)	4.9 ± 0.29 (4.6 – 5.3)	5.9 ± 0.59 (4.9 – 7.1)	7.2 ± 0.76 (6.1 – 8.3)	9.5 ± 0.93 (7.8 – 11.4)	7.6	8.9 ± 0.52 (8.1 – 10.0)
c	18.2 ± 1.14 (16.5 – 20.1)	23.6 ± 1.94 (20.6 – 26.3)	40.0 ± 5.91 (31.7 – 56.3)	61.5 ± 9.00 (48.0 – 78.8)	102.6 ± 8.95 (88.3 – 121.1)	75.5	96.0 ± 9.61 (78.8 – 108.9)
c´	3.64 ± 0.28 (3.36 – 4.07)	2.71 ± 0.32 (2.46 – 3.30)	1.84 ± 0.24 (1.40 – 2.21)	1.31 ± 0.13 (1.17 – 1.65)	0.88 ± 0.08 (0.73 – 1.02)	0.91	0.99 ± 0.06 (0.92 – 1.09)
Replacement odontostyle	67 ± 2.27 (62 – 69)	88 ± 5.05 (81 – 95)	111 ± 5.32 (97 – 118)	136 ± 4.99 (129 – 145)	—	—	—
V/spicule	—	—	—	—	40.0 ± 1.49 (37.7 – 43.2)	42.5	74 ± 5.09 (67 – 85)
Odontostyle	51 ± 1.38 (49 – 52)	64 ± 1.17 (63 – 66)	87 ± 3.12 (81 – 93)	108 ± 4.26 (99 – 113)	132 ± 4.69 (123 – 138)	111	132 ± 4.74 (124 – 140)
Odontophore	37 ± 1.90 (34 – 39)	46 ± 1.67 (44 – 49)	57 ± 2.06 (53 – 61)	69 ± 3.07 (63 – 73)	81 ± 3.11 (76 – 86)	73	80 ± 2.79 (75 – 84)
Total stylet length	87 ± 2.69 (83 – 90)	110 ± 2.32 (107 – 114)	144 ± 6.51 (125 – 154)	178 ± 5.87 (168 – 186)	213 ± 6.36 (200 – 224)	184	212 ± 5.87 (202 – 220)
Greatest flange width	7 ± 0.58 (6 – 8)	9 ± 0.55 (8 – 9)	10 ± 0.99 (8 – 11)	11 ± 0.73 (10 – 13)	12 ± 0.75 (11 – 14)	13	12 ± 1.37 (9 – 14)
Oral aperture to guide ring	41 ± 1.83 (39 – 44)	54 ± 4.13 (50 – 62)	79 ± 5.45 (69 – 93)	94 ± 8.17 (78 – 107)	120 ± 9.31 (104 – 135)	108	120 ± 8.08 (109 – 133)
Pharyngeal bulb length	61 ± 5.19 (51 – 65)	72 ± 4.68 (64 – 76)	85 ± 5.18 (69 – 91)	96 ± 6.59 (83 – 109)	108 ± 6.62 (94 – 118)	102	109 ± 5.59 (102 – 119)
Pharyngeal bulb diam.	14 ± 2.23 (12 – 18)	20 ± 1.83 (18 – 23)	24 ± 2.61 (18 – 28)	27 ± 2.83 (23 – 32)	32 ± 3.59 (26 – 40)	34	31 ± 2.84 (25 – 35)
Tail length	56 ± 4.47 (47 – 60)	62 ± 4.42 (55 – 66)	57 ± 5.07 (42 – 63)	53 ± 4.60 (48 – 61)	45 ± 4.19 (38 – 52)	43	48 ± 5.35 (40 – 57)
Length of hyaline tip	9 ± 1.11 (7 – 10)	15 ± 2.28 (11 – 18)	19 ± 2.32 (14 – 23)	16 ± 1.79 (14 – 19)	16 ± 2.68 (11 – 21)	14	17 ± 2.23 (14 – 21)
Body diam. at lip region	7 ± 0.53 (7 – 8)	8 ± 0.52 (8 – 9)	10 ± 0.84 (8 – 12)	11 ± 0.61 (10 – 12)	13 ± 0.75 (12 – 14)	12	13 ± 0.74 (12 – 14)
at guiding ring	16 ± 0.38 (16 – 17)	23 ± 1.94 (20 – 25)	29 ± 2.43 (26 – 35)	35 ± 3.20 (29 – 40)	45 ± 3.31 (40 – 50)	40	42 ± 4.01 (36 – 47)
at base of pharynx	23 ± 2.21 (20 – 27)	32 ± 3.01 (28 – 36)	41 ± 4.58 (29 – 48)	51 ± 6.96 (37 – 61)	63 ± 4.44 (56 – 70)	56	59 ± 5.38 (50 – 66)
at mid body/at vulva	24 ± 3.24 (20 – 30)	34 ± 2.66 (31 – 38)	44 ± 5.43 (30 – 54)	55 ± 7.27 (37 – 65)	72 ± 4.79 (64 – 83)	62	66 ± 5.07 (58 – 73)
at anus	15 ± 1.38 (13 – 17)	23 ± 1.94 (20 – 26)	31 ± 3.53 (25 – 39)	41 ± 3.46 (32 – 46)	52 ± 5.15 (42 – 63)	47	48 ± 3.62 (42 – 53)
at beginning of hyaline tip	6 ± 0.76 (6 – 8)	8 ± 0.82 (7 – 9)	11 ± 0.98 (9 – 13)	17 ± 1.45 (14 – 19)	24 ± 3.03 (19 – 28)	22	24 ± 4.11 (18 – 32)

Lamberti, 2000), except greater body diameter and mean body length and replacement odontostyle length.

X. pachtaicum was recorded only at one orchard at Mohyla míru (South Moravia) associated with apple. Three samples were collected from the locality but only one sample was positive for *X. pachtaicum* with population density of 5 adult females and 11 juveniles. Male were not found.

X. vuitttenezi recorded at 60 different orchards out of 73 sampling orchards associated with apple, apricot, peach, plum, sour cherry and sweet cherry with population density of 2 to 124 adult females and 1 to 158 juveniles. Males were found at three orchards. At one of the localities (Lednice), within the population of *X. vuittenezi* one specimen was found with posterior vulva (Kumari, 2003).

Molecular study of X. diversicaudatum and X. vuittenezi
Using the established primers (Wang *et al.*, 2003), classical identification of *X. diversicaudatum* and *X. vuittenezi* was verified by PCR. All positive orchards for *X. vuittenezi* were verified by PCR but here data is shown only for three selected localities. PCR with species-specific primers yielded a single fragment of approximately 813 bp for *X. diversicaudatum* and 591 bp for *X. vuittenezi* (Fig.3). No PCR products were obtained in the negative control lacking DNA template and negative control containing DNA template of other *Xiphinema* species.

Discussion

In South Moravia and Bohemia 73 orchards were surveyed, other than previously surveyed orchards (Kumari, 2004). Four *Xiphinema* species *X. diversicaudatum*, *X. pachtaicum*, *X. simile* and *X. vuittenezi* were recorded. Three species of *X. americanum*-lineage (putative and established members) occurring in the Czech Republic are *X. brevicollum*, *X. pachtaicum* and *X. simile*. Two species *X. simile* and *X. pachtaicum* are morphologically similar. *X. simile* and *X. pachtaicum* can be distinguished from each other by their body and odontostyle length, distance of guiding ring from oral aperture and shape of vulva and tail. *X. simile* have a longer and slender body, shorter odontostyle and short distance of oral aperture to guiding ring. *X. simile* tail terminus is more rounded, where *X. pachtaicum* has more pointed tail. The shape of vulva is also different in both species.
As compare to other species of the genus *Xiphinema* (*X. brevicollum*, *X. diversicaudatum*, *X. pachtaicum* and *X. vuittenezi*) described from the Czech Republic, *X. simile* unequivocally has only three juvenile stages. *X. vuittenezi* was the most widespread species, which occurred in 60 out of 73 surveyed orchards.
It has been demonstrated that for routine identification of plant-parasitic nematodes, DNA based diagnostics are quicker than the traditional strategy using morphology and morphometrics (Wang *et al*., 2003; Hübschen *et al.,* 2004; Oliveira *et al*., 2005), but morphological identification is still a prerequisite for DNA based diagnostics. Therefore, combination of molecular and morphological study can be the most reliable approach for the identification of *Xiphinema* species.
In the Czech Republic further comprehensive survey is necessary to study the occurrence and distribution of *Xiphinema* and it will be also important to study virus-vector relationship.

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