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PHENOTYPIC VARIATIONS AND GENETIC CHARACTERIZATION OF *XIPHINEMA* POPULATIONS FROM SLOVAKIA (NEMATODA: DORYLAIMIDA)

by

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Summary. Populations of *X. diversicaudatum*, *X. italiae*, *X. pachtaicum*, *X. simile*, *X. taylori* and *X. vuittenezi* from Slovakia are in the range of specific descriptions and populations of *X. italiae*, *X. taylori* and *X. vuittenezi* show inter and intrapopulation low variability for most of the diagnostic characters. Isoelectrofocusing superoxide dismutase profiles seem appropriate and efficient in separating species. Unequivocable specific identification can also be achieved by ribosomal DNA examination, but amplification does not always occur.

During a nematode survey carried out in 1996-1998 in Slovakia, several species of *Xiphinema* were collected most of which were from the western part of the country, where they commonly occur (Liskova *et al.*, 1995). Selected populations were compared to determine biometric variations and substantial differences from the original descriptions were noted. Analysis of SOD (superoxide dismutase) isozyme profiles and PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) examination of the 5' end of 26 S rDNA (ribosomal) were undertaken to characterize genetically the various *Xiphinema* populations.

Materials and methods

The study involved 15 populations of *Xiphinema vuittenezi* Luc, Lima, Weischer *et* Flegg, 1964; three populations of *X. simile* Lamberti, Choleva *et* Agostinelli, 1983; two populations of *X. italiae* Meyl, 1953 and one population each of *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939; *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 and *X. taylori* Lamberti, Ciancio, Agostinelli *et* Coiro.

Soil samples were collected from the rhizosphere of three perennial plants in cultivated orchards, house gardens, natural habitats or road sides in the localities indicated with the host plant in the tables, placed in plastic bags and extracted next day by the wet sieving technique. Nematodes for biometric studies were fixed in hot 5% formalin, mounted in anhydrous glycerin and measured with the aid of a camera lucida.

SOD isozymes were separated by isoelectric focusing and processed as indicated by Molinari *et al.* (1997).

DNA extraction was carried out as described by Molinari *et al.* (1997).

The 5' end of the 26 S gene which has been used to discriminate different organisms (Gaudet *et al.*, 1989), was amplified from single females with the FOR primer 5'-GCATATCAATAAGCGAGGAAAAG and the REV primer 5'-GGTCCGTGTTTCAAGACG, selected in regions

of the 26 S gene which are known to be highly conserved in many different organisms, so that the same oligonucleotide will bind to corresponding regions in different nematode species (Fig. 1). The PCR amplification was performed with the following cycling parameters: one cycle of 94 °C for 3 min was followed by 35 cycles of denaturation at 94 °C for 1 min and finally at 72 °C for 7 min. Restriction digestions were carried out with 10 µl of the PCR reaction mixture containing the amplified rDNA product using each of six restriction enzymes: *Pst* I, *Dde* I, *Nde* II, *Rsa* I, *Alu* I and *Ava* II. Digestion products were resolved by electrophoresis on 2% agarose gel in IX TBE buffer. DNA was stained with ethidium bromide visualized and photographed under UV transilluminer. A 100 base pair ladder was used as size marker.

The results of RFLP (restriction fragment length polymorphism) were analyzed by the method of the similarity matrix and the genetic similarity between nematode populations was estimated (Nei and Li, 1979). Then a dendrogram was constructed by an unweighted pair group method using arithmetic averages (UPGMA) cluster analysis.

Results and discussion

Ten adult females of each population of *X. vuittenezi* were measured. The population collected from the rhizosphere of plum (*Prunus domestica* L.) at Aleksince (sample n. 191 in the central-western region) is biometrically the most similar to the type population (Luc *et al.*, 1964) from which it differs only in having a shorter odontostyle (131 µm in paratypes), as in all the Slovakian populations, and an anterior basal guide ring (distance of basal guide ring from anterior extremity 113 µm in paratypes).

Among the Slovakian populations of *X. vuittenezi* (Table I), two from Povazany (in the north-western region) were larger than the others in all their biometric parameters. However, in spite of their different hosts: grapevine (*Vitis* sp., sample n. 194) and cherry (*P. avium* L., sample n. 195), they were biometrically identical.

The average population body length within the species, varied from 3.7 mm in the two above mentioned populations to 3.2 mm for two populations collected respectively at Okoc in the central-western region (sample n. 188) from the rhizosphere of poplar, *Populus alba* L. and at Moca, in the south (sample n. 83), near the river Danube, from the rhizosphere of apricot, *P. armeniaca* L., in November 1996. The longest specimen was found at Povazany in the rhizosphere of cherry (4 mm) and the shortest at Moca, in the rhizosphere of apricot in November 1996 (2.6 mm).

The average population value of *a* ranged from 65.3 for the population collected at Moca from apricot in November 1996, to 70.8 for a

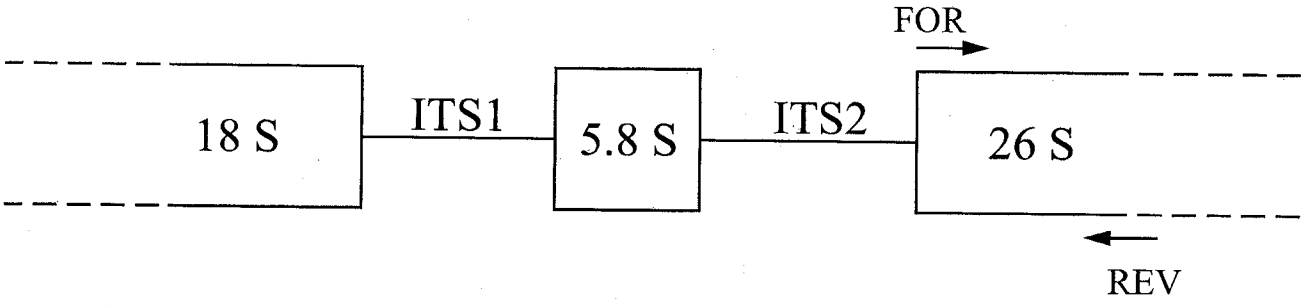

Fig. 1 - Schematic representation of rDNA cistron and location of the primers for PCR used in this study.

TABLE I - *Biometrics of populations of* Xiphinema vuittenezi *from Slovakia.*

Locality:	Aleksince (sample 191)	Hlohovec (sample 192)	Povazany (sample 194)	Povazany (sample 195	Velky Biel (sample 183)	Devinska Nova Ves (sample 179)	Moca (sample 165)	Mana (sample 157)	Zitavce (sample 156)	Moca (sample 162)	Moca (sample 163)	Moca (sample 164)	Moca (sample 83)	Okoc (sample 188)	Hlohovec (sample 193)
Host:	Plum	Grapevine	Grapevine	Cherry	Grapevine	Apple	Grapevine	Walnut	Horse-chestnut	Peach	Apricot	Apricot	Apricot	Poplar	Cherry
n	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀
L (mm)	3.3±0.25 2.7-3.5	3.6±0.22 3.3-3.9	3.7±0.19 3.4-3.9	3.7±0.20 3.4-4	3.4±0.25 3.1-3.9	3.3±0.17 3.1-3.6	3.4±0.26 3.1-3.9	3.3±0.19 3.0-3.6	3.3±0.19 3.1-3.6	3.4±0.14 3.2-3.6	3.3±0.21 3-3.6	3.3±0.17 3-3.6	3.2±0.36 2.6-3.7	3.2±0.18 3-3.6	3.4±0.18 3-3.6
a	65.7±2.60 60.7-69.6	70.7±2.83 64.8-73.9	66.9±2.12 64.3-70.5	68.6±3.43 62.6-74.0	69.4±3.32 65.4-74.8	65.9±2.60 60.3-68.7	70.8±3.36 65.4-75	67.4±2.57 63-70.8	67.6±3.06 63.6-73.8	68.2±2.53 62.2-70.5	68.5±2.91 64.4-73.6	67.1±3.23 62.4-72.4	65.3±3.58 58.4-69.3	68.8±2.90 65.3-73.4	66.7±2.13 63.4-69.6
b	7.1±0.36 6.5-7.6	7.9±0.64 7.3-9.1	7.4±0.52 6.6-8	7.7±0.75 6.8-9	7.5±0.71 6.6-8.8	7.1±0.25 6.8-7.7	7.5±0.52 6.6-8.2	7.2±0.55 6.6-8.6	7.5±0.50 6.9-8.6	7.1±0.17 6.8-7.3	7.5±0.92 6.3-9.7	7.2±0.53 6.7-8.3	7.3±0.98 6-9.1	6.8±0.42 6.2-7.5	7.3±0.25 6.8-7.6
c	96.5±7.52 83.3-106.9	99.9±9.21 85.0-114.4	98.4±8.53 87.6-109.5	100.4±8.07 90.2-111.4	98.0±4.79 90.6-106	93.0±7.24 83.8-110	92.2±7.74 81.7-106.4	101.2±7.04 90-113	94.0±8.88 76.6-110.7	96.2±5.73 87.8-104.5	94.2±5.28 86.4-101.5	92.8±8.77 83.8-113.2	91.9±8.79 80-102	97.5±7.95 87.9-107.8	91.7±4.38 83.6-100.5
c'	0.9±0.05 0.9-1.0	0.95±0.05 0.9-1	0.9±0.05 0.9-1	0.9±0.05 0.9-1	0.99±0.06 0.9-1.1	0.99±0.03 0.9-1	1.0±0.07 0.9-1.1	0.9±0.05 0.9-1.0	0.9±0.05 0.9-1.0	0.9±0.04 0.9-1.0	1.0±0.05 0.9-1.0	1.0±0.07 0.9-1.1	1.0±0.09 0.9-1.1	0.9±0.05 0.9-1.0	1.0±0.03 0.9-1.0
V	50±1.81 47-52	50±0.67 49-51	50±1.86 47-53	50±1.59 49-53	50±1.25 48-52	51±1.79 48-53	51±1.55 49-53	50±1.34 48-53	51±1.57 48-53	51±0.71 49-51	51±1.32 50-53	51±1.56 48-53	50±1.24 48-52	51±1.06 50-53	49±1.37 48-52
Odontostyle µm	120.3±2.20 116.8-124.8	125.1±2.43 121.4-128.3	129.8±3.99 121.2-134	129.3±2.27 125.3-132.3	125.9±2.79 121.4-128.9	124.9±3.80 118.0-129	123.2±2.92 118.5-127.7	123.8±3.71 117.3-128.9	120±4.33 113.0-124.3	122.3±1.95 120.2-125.4	122.9±3.68 120-128.9	120.6±1.40 119-123.7	122.5±2.19 120.2-126.6	122.5±2.89 117.9-128	120.3±3.40 115.6-126.6
Odontophore µm	72.7±2.50 67.6-75.4	73.7±1.18 72.7-75.1	77.6±1.44 75.9-79.4	76.7±2.02 74-81.2	75.5±2.46 72-79	74.2±2.11 70-75.7	72.7±2.26 70-76.9	73.5±2.06 70.5-76.9	73±3.43 65-77.4	72.7±0.87 72-74	72.1±2.16 68.2-75	72.4±1.75 69.4-75	72.8±2.47 67.6-75	73.5±3.03 66.5-76.9	74.0±2.03 70-77
Oral aperture to basal guide ring µm	104.7±4.97 95.9-112.7	114.6±4.18 105.2-118.5	119.0±7.32 101.8-126.5	119.5±2.16 114.7-122.3	114.3±3.27 109.8-120.2	115.5±4.98 107-121.4	110.8±2.89 106.3-115.6	114.8±2.69 109.8-119	110.2±4.30 102.9-116.8	111.5±3.86 105.2-117.3	110.3±3.97 105.2-118.5	108.2±2.77 104-112.7	105±2.88 101-109.2	109.8±5.37 100-118	111.1±3.84 107-120.2
Tail µm	33.8±2.06 31.8-37.6	36.6±2.46 33.5-40.5	37.5±1.80 34.7-40	36.9±3.15 32.9-41.2	35.0±2.39 29.5-38.7	35.6±2.00 31.8-37.6	36.8±2.41 33-41.6	32.6±1.46 30-34.7	33.6±2.62 28.9-38.7	35.3±2.11 31.8-38	34.6±1.04 32.9-36.4	35±2.13 31.8-39.3	35.1±2.13 32.9-39.3	32.7±2.03 30-36.4	36.6±1.33 35-39
J (hyaline portion of tail) µm	10.6±1.15 9.2-12.7	10.8±0.70 9.8-11.6	11.6±1.05 9.4-12.9	11.3±0.73 10.6-12.9	11±0.84 9.5-12	11.0±1.08 10-13	12±1.31 10-13.9	10±0.83 9-11.6	11.4±1.96 9-14.4	10.3±0.89 9-11.6	11.3±0.81 10-12.7	11.7±1.21 10-13.3	11.6±1.24 9.8-13.3	10.6±0.90 9-11.6	11.4±0.89 10.4-13.3
Body diam. at lip region µm	13.4±0.25 13.3-13.9	13.4±0.32 13-13.9	13.9±0.17 13.5-14	13.9±0.35 13.5-14.7	13.2±0.20 12.7-13.3	13.4±0.30 13-13.9	13.5±0.29 13.3-13.9	13.3±0.28 12.7-13.9	13.5±0.29 13.3-13.9	13.6±0.32 13.3-13.9	13.5±0.31 13.3-13.9	13.1±0.30 12.7-13.9	13±0.42 12.7-13.9	13.3±0.49 12.7-13.9	13.5±0.31 13.3-13.9
Body diam. at basal guide ring µm	36.1±1.24 33.5-38	37.7±0.75 37-38.7	39.5±1.56 37.4-42.3	38.8±1.31 37-40.6	36.4±1.15 34.7-38.7	36.6±1.30 34-37.7	35.6±0.90 34.7-37.6	36.8±0.82 34.7-37.6	36.4±0.80 35.8-37.6	36.6±0.89 35.3-38	36.3±0.98 34.7-38	37±2.99 34-42.8	35.7±1.68 33.5-37.6	35.3-±0.61 34.7-36.4	37.1±0.86 35.3-38
Body diam. at base of oesophagus µm	42.4±3.13 37-49	44.6±1.55 41.6-46.2	47.5±1.46 44.7-49.4	45.9±1.91 42.3-48.2	43.2±2.27 40.5-46.2	43.9±1.84 41.6-47.4	41.4±2.12 38.7-46.2	42.3±1.50 39.3-45	43.5±1.70 41.6-46.2	44.1±1.57 41.6-46.2	42.5±1.64 40.5-45.7	43.2±2.56 40.5-49.7	42.9±2.92 39.3-47.4	41.5±1.16 40-42.8	44.4±1.94 40-47.4
Body diam. at mid-body or vulva µm	49.6±3.25 44.5-55.5	51.6±2.01 47.4-53.7	54.9±1.67 52.9-57	53.8±2.16 50-56.5	49.4±3.52 44.5-54.3	50.2±2.19 46.8-54.3	47.9±2.96 43.3-53.2	47.9±3.67 39.3-53.7	49.1±2.24 46.2-52	49.7±1.99 46.8-52	48.2±2.74 45-53.2	48.5±1.87 45.7-51.4	49.4±4.30 43.3-55.5	46.2±2.73 42.2-49	50.2±2.53 45.7-55
Body diam. at anus µm	35.9±1.35 34-38	37.9±0.85 36.4-39.3	39.1±1.35 37.6-41.2	39.6±1.58 38.2-42.3	35.6±1.16 33.5-37	35.6±1.44 32.4-37.6	36.2±1.69 34-39.3	35.3±1.56 32.9-37.6	35.4±1.35 32.9-37.6	37.5±1.72 35.3-40.5	35.8±0.97 34.7-37	35.6±1.46 32.4-37	35.2±2.33 31.8-38.7	35±1.18 33.5-37.6	36.6±1.87 32.9-38.7
Body diam. at beginning of J µm	22.7±1.06 21.4-24.8	22.9±1.85 20.2-25.4	23.6±1.35 21.8-26.5	25.5±1.27 23.5-26.5	23.0±1.13 21.4-24.8	21.4±0.89 20.2-22.5	23.2±0.68 22-24.3	22.7±2.22 18.5-26	23.6±1.80 21.4-26	22.8±1.57 20.2-25.4	23.6±1.80 21.9-26	22.5±1.58 20.8-26	23.4±2.48 20.2-27.2	22.8±1.87 18-24.3	22.5±1.18 20.2-24.3

population collected in the same place, from the rhizosphere of grapevine (sample n. 165) in May 1997. The smallest value of *a* was recorded for a specimen collected at Moca from apricot in November 1996 (58.4) and the largest for a specimen from grapevine at Moca in May 1997 (75).

The average population value of ratio *b* was between 6.8, for the population from poplar at Okoc, and 7.9, for a population from grapevine at Hlohovec (sample n. 192) in the central-western region. The smallest value was observed on a specimen from apricot at Moca, collected in November 1996 (6) and the largest on a specimen collected in the same locality, but from a different apricot grove (sample n. 163) in May 1997 (9.7).

The average population value of *c* was lowest (91.7) for a population collected from the rhizosphere of cherry (sample n. 193) at Hlohveć and highest (101.2) for a population collected from the rhizosphere of walnut (sample n. 157), *Juglans regia* L., at Mana, in the south-western region. The specimen with the lowest value (76.6) occurred at Zitavce (sample n. 156) in the south-west, in the rhizosphere of horse-chestnut, *Aesculus hippocastanum* L. and the one with the highest value at Hlohovec in the rhizosphere of grapevine (114.4). Ratio *c* is the parameter in which the widest variability was noticed.

The ratio *c'* was the least variable of the characters, averaging between 0.9 and 1.0 for all the populations considered.

V was also a stable character, ranging between 50 and 51% with the exception of the population from cherry at Hlohovec, which presented a mean value of 49%. The lowest value observed was 47% on a specimen collected at Aleksince and on a specimen collected at Povazany from grapevine and the highest, 53%, on various specimens distributed at random in the different populations.

With regard to the odontostyle, there were three groups of populations: the two large populations from Povazany with an average length of 129-130 µm, very close to the type population; a group of nine populations with an average length from 122 to 126 µm and four populations, namely Aleksince-plum, Zitavce-horse-chestnut, Moca-apricot (sample n. 164) and Hlohovec-cherry with an average length of 120 µm. The longest odontostyle was observed in a specimen from grapevine at Povazany (134 µm) and the shortest on a specimen from horse-chestnut at Zitavce (113 µm).

The odontophore length was much less variable, ranging from 76 to 78 µm in three populations, including the two large ones at Povazany and a population from the rhizosphere of grapevine (sample n. 183) at Velky Biel in the extreme western region (76 µm). This character ranged between 72 and 74 µm in the remaining populations, being longest on a specimen from Povazany-cherry (81 µm) and shortest on a specimen from Zitavce-horse-chestnut (65 µm).

The distance of the basal guide ring from the anterior extremity was 119-120 µm for the two large populations from Povazany; 114-115 µm for the four populations Hlohovice-grapevine, Velky Biel-grapevine, Devinska Nova Ves (sample n. 179) in the south-western region, from apple, *Malus sylvestris* Mill. and Mana-walnut and 110-111 µm for the six populations Moca-grapevine, Zitavce-horse-chestnut, Moca (sample n. 162) from peach, *Prunus persica* (L.) Batsch, Moca-apricot (sample n. 163), Okoc-poplar and Hlohovec-cherry. This parameter averaged 108 µm for the population Moca-apricot (sample n. 164) and 105 µm for the populations Aleksince-plum and Moca-apricot-November, 1996. The smallest measurement recorded was in a specimen from Aleksince (96 µm) and the largest on a specimen from Povazany-grapevine (126.5 µm).

Average tail length ranged from ca. 33 µm for the Mana and the Okoc populations to the 37.5 µm of the Povazany-grapevine population, being shortest on a specimen from Zitavce (29 µm) and longest on a specimen from Povazany-cherry (41 µm).

The average population length of the hyaline portion of tail ranged from 10 to 12 µm, being shortest (9 µm) on specimens of several populations and longest (14.5 µm) on a specimen from Zitavce.

The average body width at the lip region had the lowest measurement of 12.7 µm on several specimens of different populations and the highest, 14.7 µm, on a specimen from Povazany-cherry.

The body diameter at the basal guide ring ranged from 35 to 40 µm. This parameter was least (33.5 µm) in specimens from Aleksince and Moca-apricot-November and greatest (43 µm) in a specimen from Moca-apricot-May (pop. n. 164).

Average population body diameter at the base of the oesophagus ranged from 41.5 µm of the Moca-grapevine and Okoc-poplar populations to 47.5 µm of the Povazany-grapevine population. This parameter was narrowest (37 µm) in a specimen from Aleksince and widest (50 µm) in a specimen from Moca-apricot (sample n. 164).

At the vulva, the average population body width varied from 46 µm for the Okoc population to 55 µm for the Povazany-grapevine population. This measurement was lowest (39.3 µm) in a specimen from Mana-walnut and highest (57 µm) in a specimen from Povazany-grapevine.

Average population body diameter at the anus was in the range 35-40 µm with the lowest individual value of 32 µm for Moca-apricot-November and the highest individual value of 42.3 µm for the Povazany-cherry population.

Finally, the average population body diameter at the beginning of the hyaline portion of the tail varied from 21.4 in the Devinska Nova Ves-apple population to 25.5 µm in the Povazany-cherry population. The lowest individual value was in a specimen from Okoc-poplar (18 µm) and the highest in a specimen from the Moća-apricot-November population (27.2 µm).

It has already been mentioned that the two populations of *X. vuittenezi* collected from the rhizosphere of grapevine or cherry at Povazany were biometrically identical. Again, populations collected at Hlohovec from the rhizosphere of grapevine and cherry, did not differ consistently from each other; however, the population from grapevine appeared to be larger in some characters, such as body and odontostyle length, compared to the cherry population.

Five populations were collected at Moca: one from the rhizosphere of apricot in November 1996 (pop. n. 83); again from the rhizosphere of the same trees in May 1997 (pop. n. 164) and from the rhizosphere of grapevine in the same place (pop. n. 165) in May 1997; two other populations were collected from other sites in the same locality, from the rhizosphere of either peach (pop. n. 162) or apricot (pop. n. 163), both in May 1997. In spite of the different plants they were associated with and the time at which they were collected, they did not show significant biometric differences.

SOD isozyme analysis was carried out on eleven populations of *X. vuittenezi* (Fig. 2): Hlohovec-grapevine (a), Povazany-grapevine (b), Povazany-cherry (c), Velky Biel-grapevine (d), Devinska Nova Ves-apple (e), Mana-walnut (f), Zitavce-horse-chestnut (g), Moca-apricot-May pop. n. 163 (h), Moca-apricot-May pop. n. 164 (i), Moca-apricot-November (l) and Okoc-poplar (m).

Isoelectrofocusing SOD profile of all the tested populations was characterized by a central low acidic band at pH 6.4; additional fainter bands were observed at pH 7.5 and 4.8 (Fig. 2). Only the population collected from the rhizosphere of apricot in November 1996 at Moca (l) showed a slightly different profile with the central band more basic than the standards. This was the only population, of those tested, that was collected in November; all the others were collected in May. If such timing has any significance, e.g. the November population was entering the winter quiescence as the host plant was defoliated and devoid of new rootlets and the May populations were starting full feeding activ-

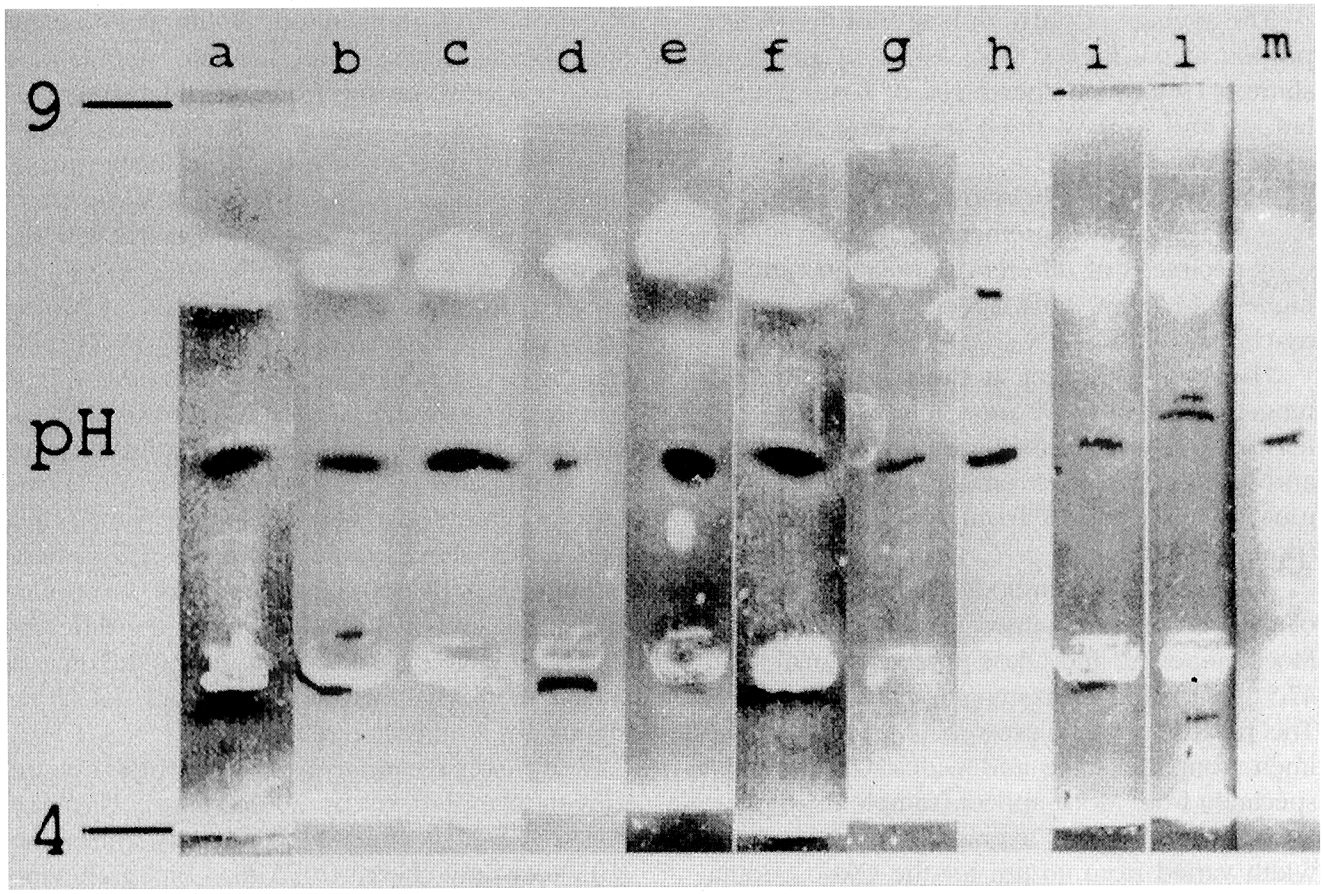

Fig. 2 - IEF SOD profiles of 11 populations of *Xiphinema vuittenezi* from Slovakia, characterized by a central acidic band at pH 6.4. Additional fainter bands are at pH 7.5 and 4.8. Only population l from Moca-apricot-November (sample n. 83) showed a slightly different profile with the central band more basic than the standards.

ity on the new flushed roots, it cannot be established on the base of this information.

RFLP analysis was carried out on seven populations of *X. vuittenezi* (Fig. 3): Aleksinceplum (pop. n. 191), Moca-grapevine (pop. n. 165), Moca-peach (pop. n. 162), Moca-apricot (pop. n. 163), Zitavce-horse-chestnut (pop. n. 156) and Okoc-poplar (pop. n. 188), plus a population collected at Maly in south-eastern Slovakia (Sl 14), from the rhizosphere of grapevine, biometrically in the range of the slovakian population of this species.

Of the several specimens (8-10) individually processed for each population, eleven, representing all the populations tested, amplified the 5' end of the 26 S gene. They all gave a fragment of 850 bp. To detect the sequence variation in the amplified products among the populations of *X. vuittenezi*, RFLP was carried out using six restriction enzymes. The sizes of the resulting fragments are reported in Table II.

There were no restriction sites for *Pst I* in any of the populations studied. The restriction patterns obtained for the seven populations grouped the specimens in four classes: class A which includes seven of the eleven specimens considered (Fig. 3); class B with two specimens (Fig. 4); and classes C (Fig. 5) and D (Fig. 6)

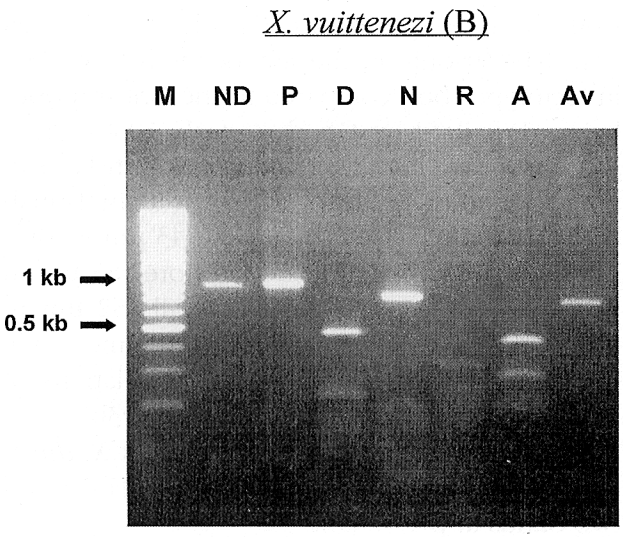

Fig. 4 - *X. vuittenezi* class B agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

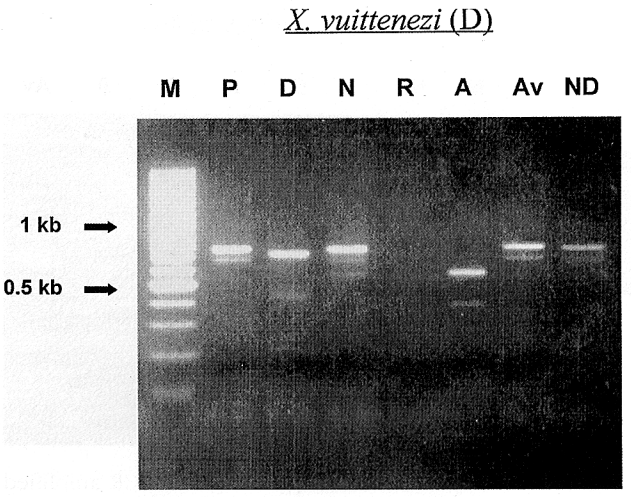

Fig. 6 - *X. vuittenezi* class D agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

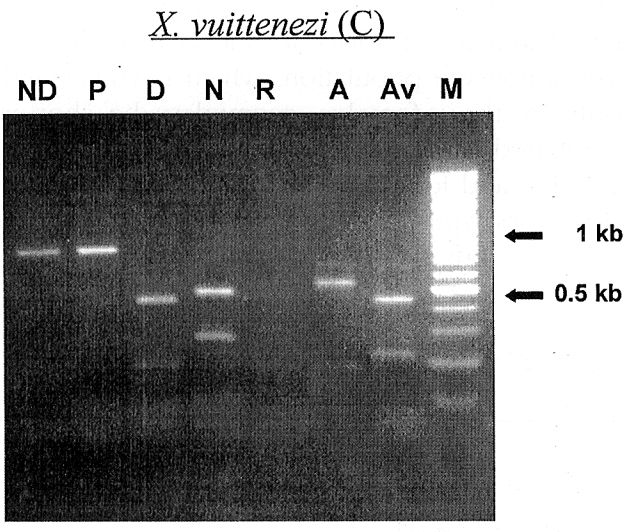

Fig. 5 - *X. vuittenezi* class C agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

Fig. 7 - Relationship of seven populations of *X. vuittenezi* from Slovakia (dendrogram generated by UPGMA cluster analysis from genetic distances).

er odontostyle (91 µm in topotypes). Compared to a Mediterranean population from Crete (Lamberti *et al.*, 1996) *X. italiae* from Slovakia has a shorter body (3.1 mm the Crete specimens) and odontostyle (102 µm the Crete specimens); conversely it is biometrically almost identical to a population of *X. italiae* from Bulgaria (Lamberti *et al.*, 1997).

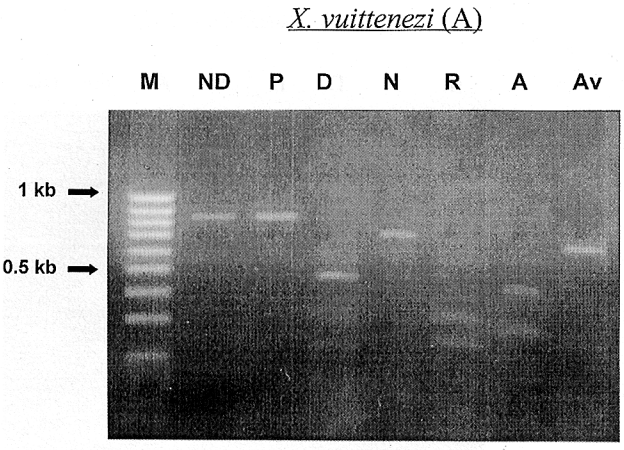

Fig. 3 - *X. vuittenezi* class A agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

with only one specimen each. In the case of populations Moca-peach and Okoc-poplar, different specimens were attributed to different classes, revealing intrapopulation variability. UPGMA analysis revealed on the dendrogram the existence of two different clades (Fig. 7): one containing classes A and B and the other with classes C and D.

The population of *X. diversicaudatum* studied (Table III) was collected from the rhizosphere of plum at Hronsky Benadik (sample n. 150) in the central-western region. It differed from the type specimen of Micoletzky (Goodey *et al.*, 1960) only in the anterior vulva (V=48% in lectotype) but, compared to the British population described by Goodey *et al.* (1960) had a shorter body length (4.9 mm in the British specimens), higher value of ratio *c* (78 in British specimen), shorter odontostyle (143 µm in British specimens), shorter odontophore (85 µm in British specimens) and shorter tail (52 µm in British specimens). However, it is biometrically in the range of other Slovakian populations of *X. diversicaudatum* (Liskova *et al.*, 1993b).

SOD isozyme analysis characterized *X. diversicaudatum* by a very basic band at pH 8.5 and two bands at pH 7.5 and 5.2 (Fig. 8).

The amplification of 5' end of 26 S gene of one specimen of *X. diversicaudatum* from Hronsky Banadik gave a fragment of approximately 850 bp. There was no restriction site for *Pst* I, but the restriction patterns obtained with the other enzymes clearly separated *X. diversicaudatum* from the other species considered (Table II; Fig. 9).

Two populations of *X. italiae* occurred at Moca, near the Danube river, in an apricot orchard and in an adjacent vineyard (Table IV). The grapevine population, which is constituted only by three females, seemed to be shorter compared to that from apricot but with higher *c'* value and longer tail. However, compared to some topotypes (Martelli *et al.*, 1966), they have a longer body (2.5 mm in topotypes) and short-

TABLE II - *Estimated restriction fragment sizes (bp) of the 5' end of 26 S rDNA gene from Slovakian populations of* Xiphinema.

	X. taylori	*X. diversicaudatum*	*X. italiae*	*X. simile*	*X. vuittenezi (A)*	*X. vuittenezi (B)*	*X. vuittenezi (C)*	*X. vuittenezi (D)*
Pst I	900	850	850	850	850	850	850	850
Dde I	470, 230	450, 250	500, 230	380, 250	480, 230	480, 230	480, 230	480, 230
Nde I	510, 230	480, 380	470, 330	480, 260	720	720, 180	550, 300	580
Rsa I	n.d.	n.d.	n.d.	n.d.	370, 270	370, 270	370, 270	370, 270
Alu I	470, 300	700, 200	580, 460, 300	470, 270	420, 280	480, 280	580, 180	560, 370
Ava II	590, 190	700	690	520, 220	700	700	480, 220	850

TABLE III - *Biometrics of a population of* X. diversicaudatum *from Slovakia.*

Locality Host	Hronsky Benadik (sample 150) Plum
n	10 ♀♀
L (mm)	4.1±0.35 3.4-4.5
a	77.1±3.39 68-80.4
b	9.1±0.86 7.6-10.3
c	91.5±11.7 75-109
c'	1.1±0.11 1-1.3
V	43±1.23 41-45
Odontostyle µm	131.2±4.4 121.8-135.9
Odontophore µm	73.9±3.61 66.5-79
Oral aperture to basal guide ring µm	122.8±3.13 119-127.6
Tail µm	45.7±4.09 39.4-52.9
J (hyaline portion of tail) µm	15.7±1.74 13-17.6
Body diam. at lip region µm	13±0.29 12.9-13.5
Body diam. at basal guide ring µm	38.6±1.92 34.7-41.8
Body diam. at base of oesophagus µm	47±3.00 42.3-50
Body diam. at mid-body or vulva µm	53.8±3.06 47.6-56
Body diam. at anus µm	40.7±2.26 37-43.5
Body diam. at beginning of J µm	21.7±1.76 19-24

The SOD profile of the apricot population of *X. italiae* is characterized by two close, almost neutral bands at pH 6.5 and 6.8 (Fig. 8).

The amplification of the 5' end of 26 S gene of one specimen of the apricot population of *X. italiae* gave a fragment of approximately 850 bp. The restriction patterns obtained with the enzymes *Dde* I, *Nde* II, *Alu* I and *Ava* II clearly differentiate *X. italiae* from the other species (Table II; Fig. 10).

A population of *X. pachtaicum* was collected from the rhizosphere of grapevine at Moca (Table V). Biometrically it differs from Italian (Lamberti and Siddiqi, 1977) and Bulgarian (Lamberti *et al.*, 1983) populations in its higher value of ratio *c'* (1.6 in Italian populations and 1.7 in Bulgarian populations) and shorter odontostyle and odontophore (respectively 86 and 46-47 µm for both Italian and Bulgarian populations).

SOD isozyme analysis characterized *X. pachtaicum* by the presence of a central band at pH 6.4 and a highly basic band at pH 8.8 (Fig. 8).

No specimen belonging to this population amplified the 5' end of 26 S gene.

The three populations of *X. simile* found at Velky Lapas, central-western region, in the rhizosphere of poplar, Zitavće, in the rhizosphere of horse-chestnut and Aleksince, in the rhizosphere of plum are biometrically almost identical for most characters (Table VI). However, the Velky Lapas population, compared to the other two, has a higher value of ratio *a*, lower value of ratio *b*, higher value of ratio *c* and shorter tail length. These populations of *X. simile* from Slovakia differ from the original description (Lamberti *et al.*, 1983) in having longer body (L=1.9 mm in the type population) and higher values for all the ratios related to the body length, but *c'*, *V* and odontostyle length, which are identical to the type population. However, body length seems to be much variable within this species, as previous records of *X. simile* from Slovakia report intermediate length (2.1 mm) between these and the type population (Liskova *et al.*, 1993a). Further studies on this species re-

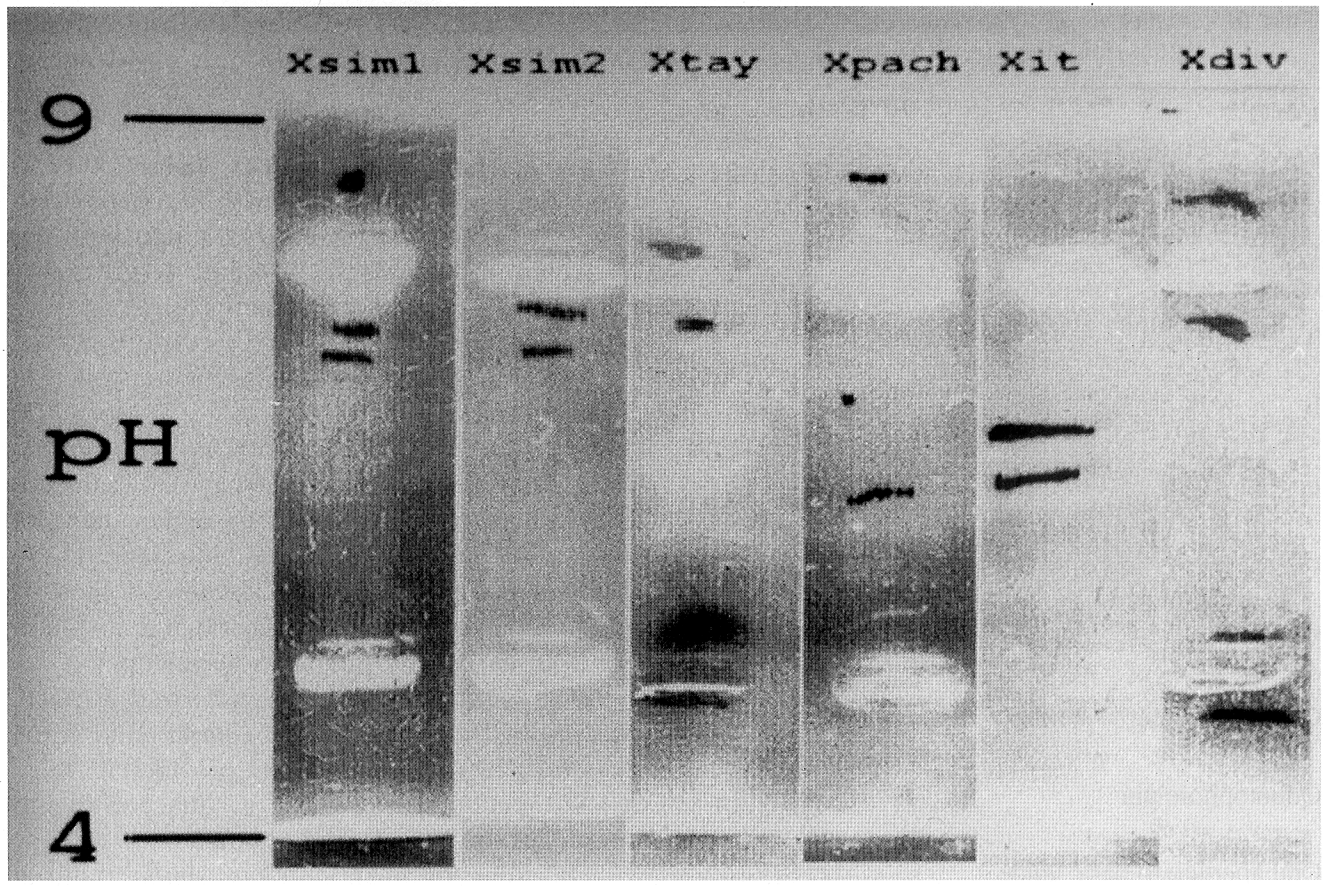

Fig. 8 - IEF SOD profile of *X. simile* (Xsim 1 and 2, characterized by two close basic bands), *X. taylori* (Xtay, characterized by bands at pH 7.5 and 5.2), *X. pachtaicum* (Xpach, characterized by a central band at pH 6.4 and highly basic band at pH 8.8), *X. italiae* (Xit, characterized by two close almost neutral bands at pH 6.5 and 6.8) and *X. diversicaudatum* (Xdiv, which in addition to the bands at pH 7.5 and 5.2 as *X. taylori* shows a very basic band at pH 8.5) from Slovakia.

port average population body length of 2.2-2.3 mm from Slovakia (Liskova and Brown, 1996) and all the range from 1.9 to 2.4 mm from the former territory of Yugoslavia (Barsi, 1994).

SOD profiles characterized the populations from Velky Lapas (X. sim 2) and from Aleksince (X. sim 1) by two close bands at pH 7.3-7.5 (Fig. 8).

The amplification of the 5' end of 26 S gene of one specimen of *X. simile* from Zitavce-horse-chestnut gave a fragment of approximately 850 bp; however, restriction fragments obtained with the enzymes *Dde* I, *Nde* II, *Alu* I and *Ava* II clearly differentiate this species from the others considered (Table II; Fig. 11).

A population of *X. taylori* occurring in the rhizosphere of cherry trees at Zavada (Table VII), in southern-central Slovakia, was biometrically identical with the original description (Lamberti *et al.*, 1992).

SOD profile characterized *X. taylori* from Slovakia by two bands at pH 7.5 and 5.2 respectively (Fig. 8).

In a specimen of *X. taylori* the amplification of the 5' end of the 26 S gene gave one fragment of 900 bp. Digestion of PCR products with the restriction enzymes further differentiated this species from the others considered in this work (Table II; Fig. 12).

TABLE IV - *Biometrics of two populations of* X. italiae *from Slovakia.*

Locality Host	Moca (sample 164) Apricot	Moca (sample 165) Grapevine
n	10 ♀♀	3 ♀♀
L (mm)	2.9±0.16 2.5-3.1	2.7±0.15 2.6-2.9
a	81.7±4.18 74.7-86.7	87.3±7.18 80.5-94.8
b	7.2±0.29 6.8-7.5	7.1±0.31 6.8-7.4
c	34.9±3.59 28.3-39.2	30.3±2.29 28.5-32.9
c'	3.8±0.31 3.4-4.3	4.3±0.40 3.9-4.7
V	45±1.70 41-47	44±1.73 42-45
Odontostyle µm	86.9±2.17 82.9-90.6	85.7±1.19 84.7-87
Odontophore µm	53.4±1.75 50.5-55.9	53.9±0.91 52.9-54.7
Oral aperture to basal guide ring µm	81±1.65 78.2-82.9	77.6±1.15 76.5-78.8
Tail µm	83.2±6.93 76.5-100	90.2±1.73 88.2-91.2
J (hyaline portion of tail) µm	11.2±1.28 9.5-12.9	11.8±0.98 11.2-12.9
Body diam. at lip region µm	10.2±0.42 9.4-10.6	10.4±0.35 10-10.6
Body diam. at basal guide ring µm	23.3±0.54 22.3-24	21.7±0.98 20.6-22.3
Body diam. at base of oesophagus µm	30.4±1.34 28.2-32.4	27.8±0.72 27-28.3
Body diam. at mid-body or vulva µm	35.4±1.97 32.9-38.8	31.4±0.86 30.6-32.3
Body diam. at anus µm	21.9±0.94 20.6-23.5	21.2±1.55 19.4-22.3
Body diam. at beginning of J µm	7.4±0.42 7-8.2	6.9±0.64 6.5-7.6

X. diversicaudatum

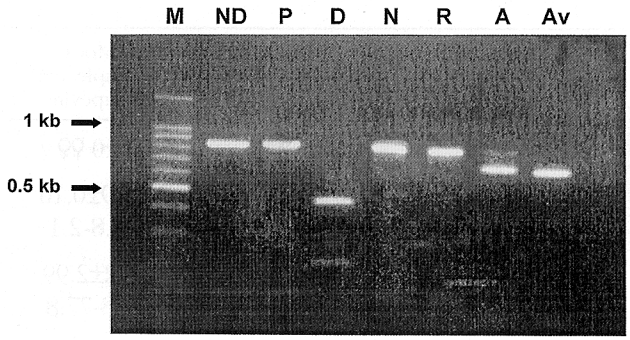

Fig. 9 - *X. diversicaudatum* agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa*I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

X. italiae

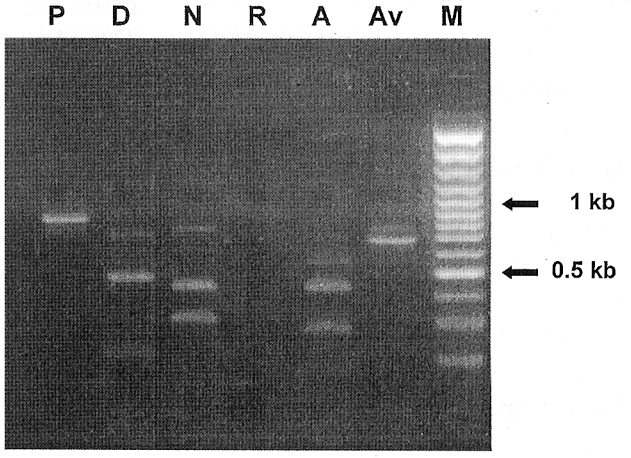

Fig. 10 - *X. italiae* agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

TABLE V - *Biometrics of a population of* X. pachtaicum *from Slovakia.*

Locality Host	Moca (sample 165) Grapevine
n	10 ♀♀
L (mm)	1.9±0.10 1.8-2.1
a	72±2.99 68-77.8
b	7.2±0.39 6.7-7.9
c	65.6±4.78 59-74.5
c'	1.9±0.11 1.7-2
V	55±1.69 53-58
Odontostyle µm	80.2±2.21 76.5-83.5
Odontophore µm	42.5±1.65 39.4-45.3
Oral aperture to basal guide ring µm	71.8±2.20 68.8-75.3
Tail µm	29.9±1.52 27.6-32.3
J (hyaline portion of tail) µm	7.2±0.59 6-8.2
Body diam. at lip region µm	8.4±0.42 8.2-9.4
Body diam. at basal guide ring µm	19.7±0.65 18.2-20.6
Body diam. at base of oesophagus µm	23±0.87 21.8-24.7
Body diam. at mid-body or vulva µm	27±1.15 25-29.4
Body diam. at anus µm	15.5±0.84 14-16.5
Body diam. at beginning of J µm	6.2±0.40 5.3-6.5

X. simile

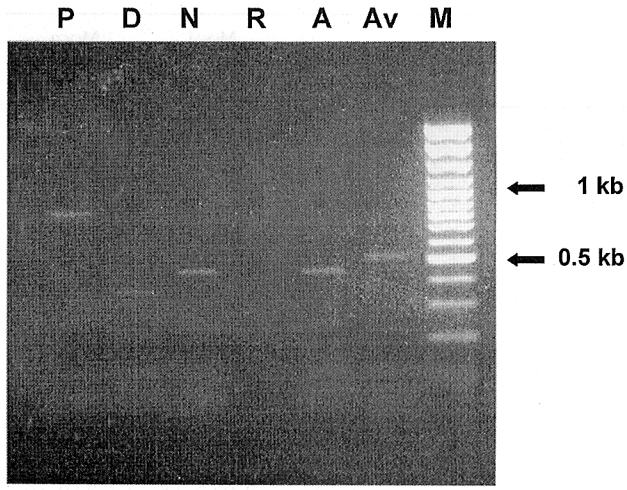

Fig. 11 - *X. simile* agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

X. taylori

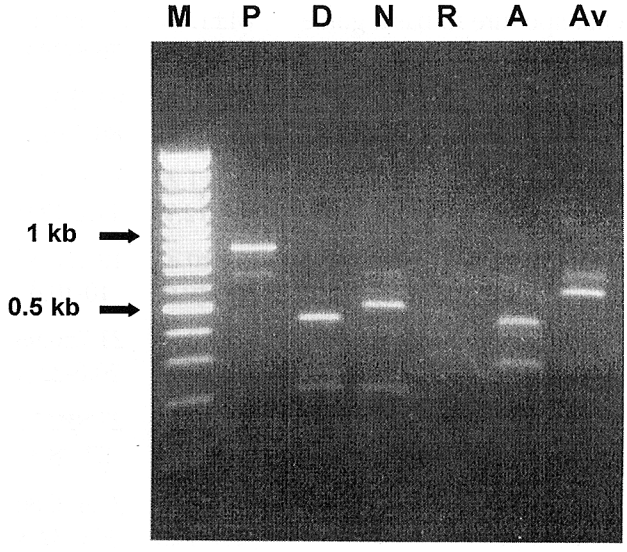

Fig. 12 - *X. taylori* agarose gel of PCR amplified 26 S rDNA digested with *Pst* I (P), *Dde* I (D), *Nde* I (N), *Rsa* I (R), *Alu* I (A) and *Ava* II (Av). The size in base pairs (bp) was estimated from 100 b DNA ladder (M). Small fragments less than 100 bp may be overlooked. Sometimes a non-specific band is amplified and present in all digestions.

TABLE VI - *Biometrics of three populations of* X. simile *from Slovakia.*

Locality Host	Velki Lapas (sample 116) Poplar	Zitavce (sample 156) Horse-chestnut	Aleksince (sample 191) Plum
n	10 ♀♀	8 ♀♀	10 ♀♀
L (mm)	2.4±0.11 2.2-2.5	2.3±0.13 2.2-2.5	2.4±0.09 2.3-2.5
a	86.9±3.56 81.7-94.3	80±5.35 70-85	77.3±5.07 70-86.8
b	7.6±0.35 7-8.2	7.9±0.74 6.8-9.4	8.2±0.91 6.9-9.9
c	82.4±5.66 74.8-94.3	70±5.81 62.5-77.4	74±4.49 70-85.2
c'	1.7±0.12 1.5-1.8	1.8±0.18 1.6-2.1	1.8±0.10 1.7-2
V	54±1.75 52-56	54±1.33 52-56	54±1.86 52-56
Odontostyle µm	69.4±1.44 65.9-70.6	66.6±3.45 59-70	69.4±2.32 65-71.8
Odontophore µm	44.7±1.42 41.8-47	43±2.48 39-45.3	43.5±2.69 38-47
Oral aperture to basal guide ring µm	62.5±2.39 58.8-66.5	59.3±1.34 57-61.2	60.8±1.83 58-64
Tail µm	29.6±2.00 26.5-33.5	33.5±2.66 29.4-38.2	33.1±1.37 30.6-35.3
J (hyaline portion of tail) µm	5.5±0.64 4.7-6.5	6.3±0.60 5.9-7.6	6.2±0.88 5.5-7.6
Body diam. at lip region µm	9.5±0.38 9-10	9.5±0.21 9.4-10	9.6±0.30 9.4-10
Body diam. at basal guide ring µm	18.9±0.57 18.2-20	19.5±0.53 18.8-20.6	19.6±0.73 18.8-20.6
Body diam. at base of oesophagus µm	24.3±0.90 23.5-25.3	24.7±1.28 23-26.5	24.7±0.92 23.5-26.5
Body diam. at mid-body or vulva µm	27.9±1.32 26.5-30.6	29.4±2.26 27-32.9	31.3±2.05 28.8-34.7
Body diam. at anus µm	17.3±0.62 16.5-18.2	18.2±1.16 17-20.6	18.3±0.76 17.6-19.4
Body diam. at beginning of J µm	7.7±0.25 7.6-8.2	7.8±0.40 7.1-8.2	7.6±0.69 6.5-8.2

TABLE VII - *Biometrics of a population of* X. taylori *from Slovakia.*

Locality Host	Zavada (sample 67) Cherry
n	7 ♀♀
L (mm)	2.3±0.04 2.3-2.4
a	51.6±1.83 50-54.4
b	6.9±0.39 6.5-7.7
c	86.8±6.55 78.3-95.4
c'	0.9±0.06 0.8-1
V	50±2.00 48-52
Odontostyle µm	92.2±2.63 89.4-97
Odontophore µm	58.4±2.59 54-61.7
Oral aperture to basal guide ring µm	78.3±1.78 75-80
Tail µm	26.8±1.93 24-29.4
J (hyaline portion of tail) µm	8.2±0.44 7.6-8.8
Body diam. at lip region µm	13.6±0.19 13.5-14
Body diam. at basal guide ring µm	30±1.22 28.2-31.8
Body diam. at base of oesophagus µm	37.9±1.39 36.5-40
Body diam. at mid-body or vulva µm	41.9±1.18 43.5-46
Body diam. at anus µm	29.6±1.24 28.2-31.8
Body diam. at beginning of J µm	15.9±1.12 15-18.2

Conclusions

The populations of *X. diversicaudatum*, *X. italiae*, *X. pachtaicum*, *X. simile*, *X. taylori* and *X. vuittenezi* from Slovakia are biometrically in the range of specific descriptions by other authors and populations of *X. italiae*, *X. taylori* and *X. vuittenezi* show low variability and narrow range for most of the diagnostic characters.

Isoeletricfocusing superoxide dismutase profiles seems appropriate and efficient for separating species; the only deviation from the standards, observed in the population collected from the rhizosphere of apricot at Moca in November 1996, does not affect its identification and perhaps can be considered an intraspecific variation, if not attributable to undetected operational changes. Probably, the limit of this technique is the relatively large numbers of specimens required to run each test: 10-12 for nematodes of the size of *X. vuittenezi* and *X. diversicaudatum* and 15-20 for those like *X. pachtaicum*, *X. simile* etc.

The use of the ribosomal cistron is useful tool for evaluating relationships among known species of nematodes. Also it is an unequivocable method for identification of *Xiphinema* species. However, its main concern is the low yield in the results. In fact, of more than 100 specimens processed among species and populations, only eleven *X. vuittenezi*, one of each of *X. diversicaudatum*, *X. italiae*, *X. simile* and *X. taylori* and no *X. pachtaicum* reacted positively to the treatment. In all the others, for reasons which could be related to several factors (i.e. sample collection, soil contamination or differences in primer sequences), PCR failed.

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