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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. Unequivocal 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'-GCATATCAA-TAAGCGAGGAAAAG 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 transilluminator. 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

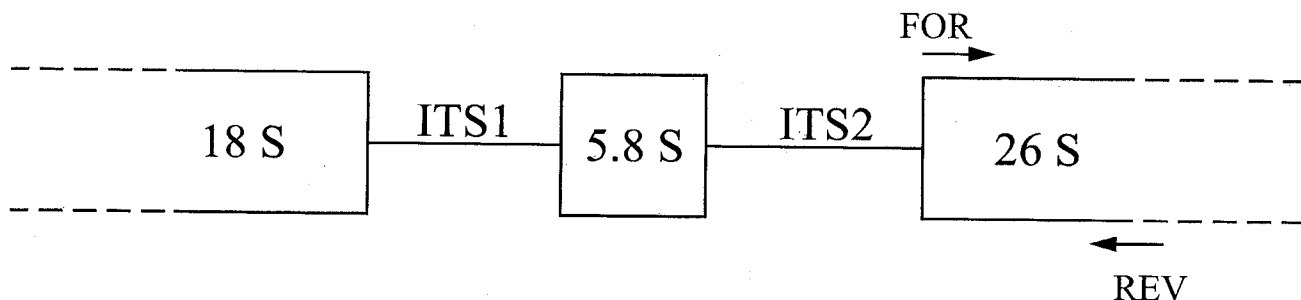


Fig. 1 - Schematic representation of rDNA cistron and location of the primers for PCR used in this study.

TABLE 1 - Biometrics of populations of Xiphinema vuittenezi from Slovakia.

Locality:	Aleksince (sample 191)	Hlohovec (sample 192)	Povazany (sample 194)	Povazany (sample 195)	Veľký Biel (sample 183)	Devínska 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	3.6±0.22	3.7±0.19	3.7±0.20	3.4±0.25	3.3±0.17	3.4±0.26	3.3±0.19	3.3±0.19	3.4±0.14	3.3±0.21	3.3±0.17	3.2±0.36	3.2±0.18	3.4±0.18
a	2.7-3.5	3.3-3.9	3.4-3.9	3.4-4	3.1-3.6	3.1-3.6	3.1-3.9	3.0-3.6	3.1-3.6	3.2-3.6	3-3.6	3-3.6	2.6-3.7	3-3.6	3-3.6
b	65.7±2.60	70.7±2.83	66.9±2.12	68.6±5.43	69.4±3.32	65.9±2.60	70.8±3.36	67.4±2.57	67.6±3.06	68.2±2.53	68.5±2.91	67.1±3.23	65.3±3.58	68.8±2.90	66.7±2.13
c	60.7-69.6	64.8-73.9	64.3-70.5	62.6-74.0	65.4-74.8	60.3-68.7	65.4-75	63-70.8	63.6-73.8	62.2-70.5	64.4-73.6	62.4-72.4	58.4-69.3	65.3-73.4	63.4-69.6
d	7.1±0.36	7.9±0.64	7.4±0.52	7.7±0.75	7.5±0.71	7.1±0.25	7.5±0.52	7.2±0.55	7.5±0.50	7.1±0.17	7.5±0.92	7.2±0.53	7.3±0.98	6.8±0.42	7.3±0.25
e	6.5-7.6	7.3-9.1	6-8	6.8-9	6.6-8.8	6.8-7.7	6.6-8.2	6.6-8.6	6.9-8.6	6.8-7.3	6.3-9.7	6.7-8.3	6-9.1	6.2-7.5	6.8-7.6
f	96.5±7.52	99.9±9.21	98.4±8.53	100.4±8.07	98.0±4.79	93.0±7.24	92.2±7.74	101.2±7.04	94.0±8.88	96.2±5.73	94.2±5.28	92.8±8.77	91.9±8.79	97.5±7.95	91.7±4.38
g	83.3-106.9	85.0-114.4	87.6-109.5	90.2-111.4	90.6-106	83.8-110	81.7-106.4	90-113	76.6-110.7	87.8-104.5	86.4-101.5	83.8-113.2	80-102	87.9-107.8	83.6-100.5
h	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.06	0.9±0.03	0.9±0.07	0.9±0.05	0.9±0.05	0.9±0.04	0.9±0.05	0.9±0.07	1.0±0.09	0.9±0.05	1.0±0.03
i	0.9-1.0	0.9-1	0.9-1	0.9-1	0.9-1.1	0.9-1	0.9-1.1	0.9-1.0	0.9-1.0	0.9-1.0	0.9-1.0	0.9-1.1	0.9-1.1	0.9-1.0	0.9-1.0
j	50±1.81	50±0.67	50±1.86	50±1.59	50±1.25	51±1.79	51±1.55	50±1.34	51±1.57	51±0.71	51±1.32	51±1.56	50±1.24	51±1.06	49±1.37
k	47-52	49-51	47-53	49-53	48-52	48-53	49-53	48-53	48-53	49-51	50-53	48-53	48-52	50-53	48-52
l	120.3±2.20	125.1±2.43	129.8±3.99	129.3±2.27	125.9±2.79	124.9±3.80	123.2±2.92	123.8±3.71	120±4.33	122.3±1.95	122.9±3.68	120.6±1.40	122.5±2.19	122.5±2.89	120.3±3.40
m	116.8-124.8	121.4-128.3	121.2-134	125.3-132.3	121.4-128.9	118.0-129	118.5-127.7	117.3-128.9	113.0-124.3	120.2-125.4	120-128.9	119-123.7	120.2-126.6	117.9-128	115.6-126.6
n	72.7±2.50	73.7±1.18	77.6±1.44	76.7±2.02	75.5±2.46	74.2±2.11	72.7±2.26	73.5±2.06	73±3.43	72.7±0.87	72.1±2.16	72.4±1.75	72.8±2.47	73.5±3.03	74.0±2.03
o	67.6-75.4	72.7-75.1	75.9-79.4	74-81.2	72-79	70-75.7	70-76.9	70.5-76.9	65-77.4	72-74	68-72.5	69-74.5	67-75	66.5-76.9	70-77
p	104.7±9.47	114.6±4.18	119.0±7.32	119.5±2.16	114.3±3.27	115.5±4.98	110.8±2.89	114.8±2.69	110.2±4.30	111.5±5.86	110.3±3.97	108.2±2.77	105±2.88	109.8±5.37	111.1±3.84
q	95.9-112.7	105.2-118.5	101.8-126.5	114.7-122.3	109.8-120.2	107-121.4	106.3-115.6	109.8-119	102.9-116.8	105.2-117.3	105.2-118.5	104-112.7	101-109.2	100-118	107-120.2
r	33.8±2.06	36.6±2.46	37.5±1.80	36.9±3.15	35.0±2.39	35.6±2.00	36.8±2.41	32.6±1.46	33.6±2.62	35.3±2.11	34.6±1.04	35±2.13	35.1±2.13	32.7±2.03	36.6±1.33
s	31.8-37.6	33.5-40.5	34.7-40	32.9-41.2	29.5-38.7	31.8-37.6	33-41.6	30-34.7	28.9-38.7	31.8-38	32.9-36.4	31.8-39.3	32.9-39.3	30-36.4	35-39
t	10.6±1.15	10.8±0.70	11.6±1.05	11.3±0.73	11±0.84	11.0±1.08	12±1.31	10±0.83	11.4±1.96	10.3±0.89	11.3±0.81	11.7±1.21	11.6±1.24	10.6±0.90	11.4±0.89
u	9.2-12.7	9.8-11.6	9.4-12.9	10.6-12.9	9.5-12	10-13	10-13.9	9-11.6	9-14.4	9-11.6	10-12.7	10-13.3	9.8-13.3	9-11.6	10.4-13.3
v	13.4±0.25	13.4±0.32	13.9±0.17	13.9±0.35	13.2±0.20	13.4±0.30	13.5±0.29	13.3±0.28	13.5±0.29	13.6±0.32	13.5±0.31	13.1±0.30	13±0.42	13.3±0.49	13.5±0.31
w	13.3-13.9	13-13.9	13.5-14	13.5-14.7	12.7-13.3	13-13.9	13.3-13.9	12.7-13.9	13.3-13.9	13.3-13.9	13.3-13.9	12.7-13.9	12.7-13.9	12.7-13.9	13.3-13.9
x	36.1±1.24	37.7±0.75	39.5±1.56	38.8±1.31	36.4±1.15	36.6±1.30	35.6±0.90	36.6±0.82	36.4±0.80	36.6±0.89	36.3±0.98	37.2±0.99	35.7±1.68	35.3±0.61	37.1±0.86
y	33.5-38	37-38.7	37.4-42.3	37-40.6	34.7-38.7	34-37.7	34.7-37.6	34.7-37.6	35.8-37.6	35.3-38	34.7-38	34.7-38	33.5-37.6	34.7-36.4	35.3-38
z	42.4±3.13	44.6±1.55	47.5±1.46	45.9±1.91	43.9±1.84	41.4±2.12	42.3±1.50	43.5±1.70	43.5±1.70	44.1±1.57	42.5±1.64	43.2±2.56	42.9±2.92	41.5±1.16	44.4±1.94
aa	37-49	41.6-46.2	44.7-49.4	42.3-48.2	40.5-46.2	43.9-47.4	38.7-46.2	39.3-45	41.6-46.2	41.6-46.2	40.5-45.7	40.5-49.7	39.3-47.4	40-42.8	40-47.4
ab	49.6±3.25	51.6±2.01	54.9±1.67	53.8±2.16	49.4±3.32	50.2±2.19	47.9±2.96	47.9±3.67	49.1±2.24	49.7±1.99	48.2±2.74	48.5±1.87	49.4±4.30	46.2±2.73	50.2±2.53
ac	44.5-55.5	47.4-53.7	52.9-57	50-56.5	44.5-54.3	46.8-54.3	43.3-53.2	39.3-53.7	46.2-52	46.8-52	45-53.2	45.7-51.4	43.3-55.5	42.2-49	45.7-55
ad	35.9±1.35	37.9±0.85	39.1±1.35	39.6±1.58	35.6±1.16	35.6±1.44	36.2±1.69	35.3±1.56	35.4±1.35	37.5±1.72	35.8±0.97	35.6±1.46	35.2±2.33	35±1.18	36.6±1.87
ae	34-38	36.4-39.3	37.6-41.2	38.2-42.3	33.5-37	32.4-37.6	34-39.3	32.9-37.6	32.9-37.6	35.3-40.5	34.7-37	32.4-37	31.8-38.7	33.5-37.6	32.9-38.7
af	22.7±1.06	22.9±1.85	23.6±1.35	25.5±1.27	23.0±1.13	21.4±0.89	23.2±0.68	22.7±2.22	23.6±1.80	22.8±1.57	23.6±1.80	22.5±1.58	23.4±2.48	22.8±1.87	22.5±1.18
ag	21.4-24.8	20.2-25.4	21.8-26.5	23.5-26.5	21.4-24.8	20.2-22.5	22-24.3	18.5-26	21.4-26	20.2-25.4	21.9-26	20.8-26	20.2-27.2	18-24.3	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 Hlohovec 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  $\mu\text{m}$ , very close to the type population; a group of nine populations with an average length from 122 to 126  $\mu\text{m}$  and four populations, namely Aleksince-plum, Zitavce-horsechestnut, Moca-apricot (sample n. 164) and Hlohovec-cherry with an average length of 120  $\mu\text{m}$ . The longest odontostyle was observed in a specimen from grapevine at Povazany (134  $\mu\text{m}$ ) and the shortest on a specimen from horsechestnut at Zitavce (113  $\mu\text{m}$ ).

The odontophore length was much less variable, ranging from 76 to 78  $\mu\text{m}$  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  $\mu\text{m}$ ). This character ranged between 72 and 74  $\mu\text{m}$  in the remaining populations, being longest on a specimen from Povazany-cherry (81  $\mu\text{m}$ ) and shortest on a specimen from Zitavce-horse-chestnut (65  $\mu\text{m}$ ).

The distance of the basal guide ring from the anterior extremity was 119-120  $\mu\text{m}$  for the two large populations from Povazany; 114-115  $\mu\text{m}$  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  $\mu\text{m}$  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  $\mu\text{m}$  for the population Moca-apricot (sample n. 164) and 105  $\mu\text{m}$  for the populations Aleksince-plum and Moca-apricot-November, 1996. The smallest measurement recorded was in a specimen from Aleksince (96  $\mu\text{m}$ ) and the largest on a specimen from Povazany-grapevine (126.5  $\mu\text{m}$ ).

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

The average population length of the hyaline portion of tail ranged from 10 to 12  $\mu\text{m}$ , being shortest (9  $\mu\text{m}$ ) on specimens of several populations and longest (14.5  $\mu\text{m}$ ) on a specimen from Zitavce.

The average body width at the lip region had the lowest measurement of 12.7  $\mu\text{m}$  on several specimens of different populations and the highest, 14.7  $\mu\text{m}$ , on a specimen from Povazany-cherry.

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

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

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

Average population body diameter at the anus was in the range 35-40  $\mu\text{m}$  with the lowest individual value of 32  $\mu\text{m}$  for Moca-apricot-November and the highest individual value of 42.3  $\mu\text{m}$  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  $\mu\text{m}$  in the Povazany-cherry population. The lowest individual value was in a specimen from Okoc-poplar (18  $\mu\text{m}$ ) and the highest in a specimen from the Moča-apricot-November population (27.2  $\mu\text{m}$ ).

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-

