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First record of *Longidorus juvenilis* and *L. leptocephalus* (Nematoda: Dorylaimida) in Slovenia and their morphometrical and ribosomal DNA sequence analysis

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Summary. *Longidorus juvenilis* and *L. leptocephalus* are reported from Slovenia for the first time. All developmental stages of these species were recovered from the rhizosphere of *Vitis vinifera* L. growing in the north-eastern part of Slovenia. Nematodes were identified using morphological characters of females and juveniles. All these stages are described. A bivulval female of *L. juvenilis* is also described. In addition, sequences of the D2-D3 expansion region of 28S rRNA gene were analysed. Obtained sequences were compared with sequences of the same and closely related species downloaded from the NCBI database. Cluster analysis of sequences confirmed and supported species identifications. The sequences of *L. juvenilis* and *L. leptocephalus* found in Slovenia and sequences of the same species from databank clustered in two separate clades, respectively. Both clades were supported in bootstrap analyses.

Key words: *Vitis*, rhizosphere, identification, Longidoridae, morphology, 28S rDNA.

The nematodes belonging to the genera *Longidorus* Micoletzky and *Xiphinema* Cobb, commonly referred to as longidorids, can cause damage to many economically important crops by direct feeding on their roots. The interest in these nematodes increased greatly since the discovery that some species of these genera can transmit plant viruses (Hewitt *et al.*, 1958; Sol & Seinhorst, 1961; Lamberti & Roca, 1987; De Waele & Coomans, 1990). An extensive survey of longidorids in Slovenia concentrated mainly on the genus *Xiphinema*, with eleven identified species so far (Širca & Urek, 2004). Only two *Longidorus* species, *L. caespiticola* Hooper and *L. elongatus* (de Man) Thorne & Swanger had been found in Slovenia until recently (Urek *et al.*, 2003). The occurrence and geographical distribution of members of the Longidoridae in Slovenia was comprehensively examined between 2002 and 2004, mostly in vineyards. The occurrence of *L. juvenilis* Dalmasso and *L. leptocephalus* Hooper is recorded for the first time in Slovenia.

Longidorus species are currently identified by using various keys (Lamberti, 1975; Chen *et al.*, 1997; Loof & Chen, 1999). More recently, a cluster analysis approach was proposed by Ye & Robbins (2004) supported by computer software. However, all of the mentioned methods rest on morphometrical data, which are variable in the genus *Longidorus* and can lead to incorrect identifications (Ye & Robbins, 2004). Therefore, additional approaches were developed to aid morphometrical identifications. Sequences of nuclear ribosomal DNA (rDNA) proved to be useful in molecular phylogenetic analyses of Longidoridae. Sequence analyses of D2-D3 expansion region large subunit rRNA nuclear gene was used by Rubtsova *et al.* (2001) and He *et al.* (2005) while Ye *et al.* (2004) used the ITS1 rDNA region to investigate phylogenetic relationships and genetic variation of longidorids.

In our study, species of *L. juvenilis* and *L. leptocephalus* found in Slovenia were identified using morphometrical and molecular methods. Morphometrical characterization displayed intraspecific variation among different populations and, therefore, species identification became unreliable. DNA sequence analysis was applied in

order to confirm morphometric identification. D2-D3 expansion region of 28S rRNA gene of both *Longidorus* species found in Slovenia and sequences of the same and closely related species from the NCBI databank were used for cluster analysis. This approach was more reliable for unequivocal species identification.

MATERIAL AND METHODS

Nematodes. Soil samples were taken from the rhizosphere of *Vitis vinifera* L. near Svetinje, and Juršinci in Slovenske gorice region in the north-eastern part of Slovenia. The samples taken near Svetinje were collected beneath grapevines where *Raspberry bushy dwarf idaeovirus* (RBDV) was previously detected (Mavrič *et al.*, 2003). The sampling was performed by digging holes beneath host plants and carefully collecting soil from around grapevine roots at 40-50 cm depth. Nematodes were extracted from soil samples according to the method of Hržič (1973). Longidorid nematodes were hand picked, killed and fixed in TAF (Courtney *et al.*, 1955), examined and measured under a light microscope connected to an image analysis system, Lucia 5.0 (Laboratory Imaging Ltd, Prague). Species identification, based on female and juvenile average morphometric characters including body length, distance from vulva opening to head end, lip width, odontostyle length, body diameter, tail length and diameter etc., was performed using the polytomous key for *Longidorus* species identifications (Chen *et al.*, 1997). Measurements of the different juvenile stage parameters (body length, length of odontostyle, and odontostyle replacement) and female parameters (body and odontostyle length) were used for the determination of the juvenile stage being examined.

Total DNA extraction and amplification. Singly extracted longidorids were transferred to a 1.5 ml Eppendorf tube in 1 µl of sterile water and stored at -80°C. DNA isolation was performed using the Promega Wizard DNA purification kit according to the manufacturer's instructions. Nematodes were homogenised with a micropestle in an ice cold mixture of 5 µl 1M EDTA (pH 8) and 25 µl nucleic lysis solution. Additional 5 µl of 1M EDTA (pH 8) and 25 µl of nucleic lysis solution were added to each tube and isolation of DNA was continued according to the manufacturer's instructions. Extracted DNA was re-suspended in 10 µl of distilled water. A fragment of the D2-D3 expansion region of 28S rRNA gene was amplified using the primers D2A (5'-ACAAGTACCGT-GAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') described by Rubtsova *et al.* (2001). PCR reactions contained 1 µl of isolated DNA, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 2.5 mM of each of the dNTPs, 1 µM each of the primers, 1U *Taq* DNA Polymerase (Promega) and distilled water up to 25 µl. The amplification was carried out in a thermocycler (A&B gene AMP PCR system 2700) using the following program: initial denaturation at 94°C for 2.5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 s and elongation at 72°C for 1 min; and a final extension at 72°C for 2 min.

rDNA sequence analyses. Obtained PCR products were purified using the JetQuick PCR purification spin kit (Genomed) and sequenced on an ABI PRISM 310 DNA Sequencer using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

Cluster analyses was performed using sequences of *L. juvenilis* and *L. leptocephalus* obtained in this study and sequences of *L. apulus* (AY601571), *L. attenuatus* (AY601572), *L. caespiticola* (AY601567), *L. camelliae* (AY601585), *L. diadecturus* (AY601584), *L. elongatus* (AY601578), *L. juvenilis* (AY601579), *L. latocephalus* (AY601569), *L. leptocephalus* (AY601580), *L. macrosoma* (AY601565) (He *et al.*, 2005) and *L. profundorum* (AY480073) (Rubtsova *et al.*, 2001) from the NCBI GenBank (http://www.ncbi.nlm.nih.gov). Sequence of the same region of *Xiphinema index* (AY601628) was used as the out-group. For cluster analyses and tree construction, a neighbour-joining method was applied using MEGA3 software (Kumar *et al.*, 2004). Sequences of *L. juvenilis* and *L. leptocephalus* from Slovenia were deposited in the GenBank (accession numbers DQ364599 and DQ364600, respectively).

RESULTS

Population density of extracted specimens of both *Longidorus* species was 2 - 5 per 300 g of soil sample.

Morphometrics. *L. juvenilis* from Svetinje was characterised by medium sized (L = 3.52 - 4.71 mm) and slender (a = 89 - 128) body, slightly expanded, anteriorly flat and laterally rounded head (Fig. 1), offset from neck contour by a slight depression, symmetrically bilobed amphidial pouches, medium odontostyle length (61.1 - 67.7 µm) and elongate, conoid tail (47.6 - 56.8 µm; c' = 1.8 - 2.5). The code for identifying *L. juvenilis* when using the identification key of Chen *et al.* (1997) is: A-2, B-12, C-2, D-2, E-2, F-2, G-2, H-6, I-1. Males were not found. A scatter plot of

the morphometrics of odontostyle and replacement odontostyle in relation to body length demonstrates that species has three juvenile stages (Fig. 2). In total, 20 *L. juvenilis* juveniles were measured. The measurements of juvenile stage body length ranged from 1.14 mm to 1.17 mm, from 1.83 mm to 1.98 mm and from 2.53 mm to 3.10 mm for the first-, second- and third- stage juveniles, respectively. The odontostyle of the first-stage juveniles ranged from 40.5 µm to 44.4 µm, of the second-stage from 46.5 µm to 49.6 µm and from 51.4 µm to 58.1 µm for the third-stage juveniles. Measurements of the replacement odontostyle ranged from 46.5 µm to 48.4 µm for the first-stage, from 48.9 µm to 58.1 µm for the second-stage and from 63.4 µm to 67.3 µm for the third-stage juveniles.

In addition, a specimen of the bivulval *L. juvenilis* female was found. The morphometrics of the bivulval female were: L = 4.1 mm; a = 126.2; b = 12.7; c = 74.4; c' = 2.3; V1 = 48.2; V2 = 57.9; odontostyle = 64.4; odontophore = 47.9; oral aperture to guiding ring = 25.4 µm; tail = 55.6 µm; body diameter at lip region = 11.4 µm; body diameter at guiding ring = 16.6 µm; body diameter at base of oesophagus = 29.3 µm; body diameter at vulva = 32.8 µm; body diameter at anus = 23.7 µm. The two vaginas were interconnected and fused with adjacent uteri (Fig. 4). The distal gonads were normally developed. Measurements of *L. juvenilis* from Svetinje are presented in Table 1.

L. leptocephalus from Juršinci was characterised by medium sized (L = 4.00 - 4.90 mm) and slender (a = 99 - 117) body. The lip region was narrow, about half the neck width at the guide ring, very slightly offset from the body contour; amphids somewhat bilobed at the base, medium odontostyle length (61.0 - 70.0 µm). The tail is roundly conoid

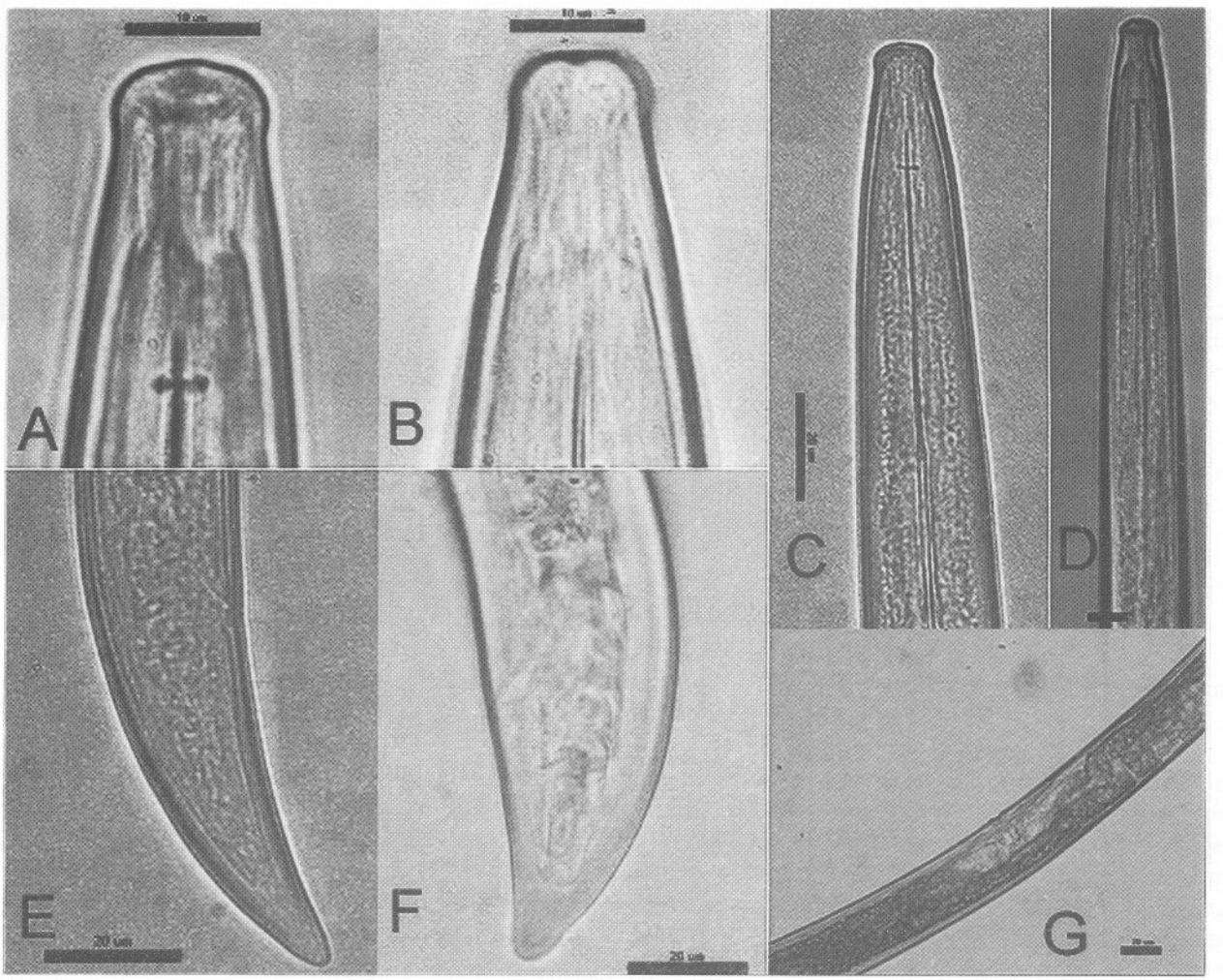

Fig. 1. Head, tail, anterior, and vulva region of *L. juvenilis* Dalmasso (A, C, E & G, respectively) from Svetinje and head, tail, and anterior region of *L. leptocephalus* Hooper (B, F & D, respectively) from Juršinci (Scale bars: A, B & D = 10 µm; C, E, F & G = 20 µm).

Table 1: Morphometric data of *L. leptocephalus* Dalmasso from Juršinci and *L. juvenilis* Hooper from Svetinje, Slovenia.

Species	*L. leptocephalus*	*L. juvenilis*	*L. juvenilis*
Specimes	Female	Female	Bivulval female
n	9	16	1
L	4581.9 ± 283.3 (4029.9 - 4878.4)	3964.2 ± 362.7 (3521.4 - 4718.9)	4138.9
a	109.5 ± 5.4 (98.8 - 117.1)	113.6 ± 11.4 (89.6 - 128.8)	126.2
b	12.4 ± 0.9 (11.0 - 13.8)	12.3 ± 1.1 (11.1 - 14.4)	12.7
c	101.8 ± 8.6 (88.5 - 116.1)	74.5 ± 7.5 (63.2 - 90.1)	74.4
c'	1.54 ± 0.15 (1.35 - 1.90)	2.18 ± 0.19 (1.80 - 2.47)	2.35
V1 [%]	50.7 ± 2.8 (47.8 - 54.6)	48.1 ± 1.0 (46.9 - 51.0)	48.2
V2 [%]	–	–	57.9
Odontostyle	66.2 ± 3.7 (60.9 - 70.9)	64.3 ± 1.7 (61.1 - 67.7)	64.4
Odontofore	39.8 ± 2.3 (36.8 - 44.2)	35.1 ± 3.3 (29.8 - 42.7)	47.9
Total stylet length	107.0 ± 5.0 (100.5 - 117.1)	99.4 ± 4.3 (93.6 - 110.4)	112.3
Oral aperture to guide ring	29.2 ± 1.7 (26.9 - 32.2)	24.8 ± 1.0 (23.4 - 26.9)	25.4
Tail length	45.2 ± 4.0 (39.9 - 54.6)	53.3 ± 2.6 (47.6 - 56.8)	55.6
Body diameter: at lip region	10.2 ± 0.6 (9.8 - 11.6)	11.6 ± 0.4 (11.2 - 12.5)	11.4
at guiding ring level	18.3 ± 0.4 (17.7 - 18.9)	17.0 ± 0.8 (16.0 - 18.7)	16.6
at base of pharynx	34.7 ± 0.9 (33.6 - 36.3)	30.9 ± 1.7 (28.4 - 34.7)	29.3
at vulva level	41.9 ± 1.5 (40.4 - 44.7)	35.1 ± 3.5 (29.8 - 41.4)	32.8
at anus level	29.3 ± 0.8 (28.4 - 31.3)	24.6 ± 1.8 (21.4 - 28.8)	23.7

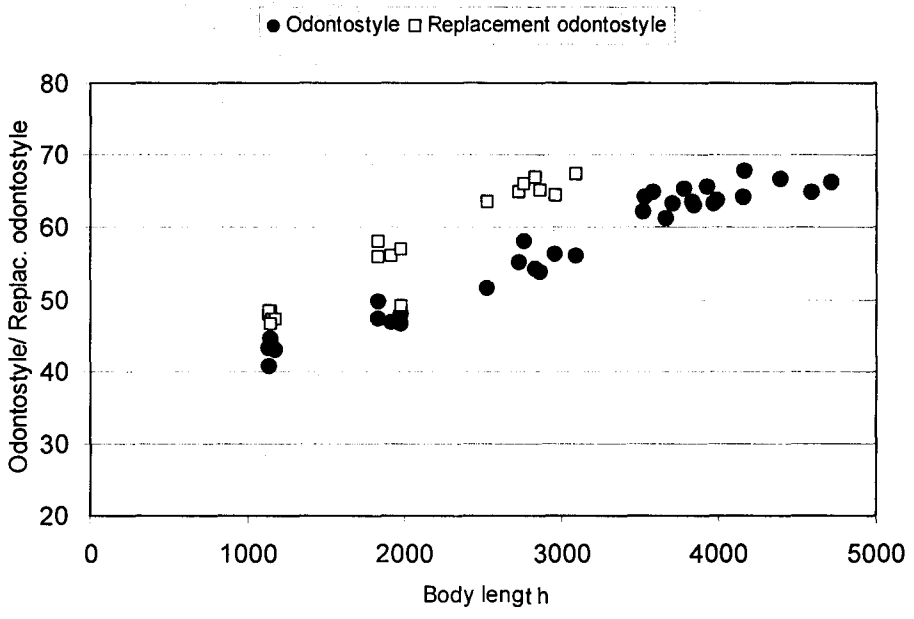

Fig. 2: Scatter plot of odontostyle and replacement odontostyle length against body length separating juveniles from females of *L. juvenilis* Dalmasso from Svetinje.

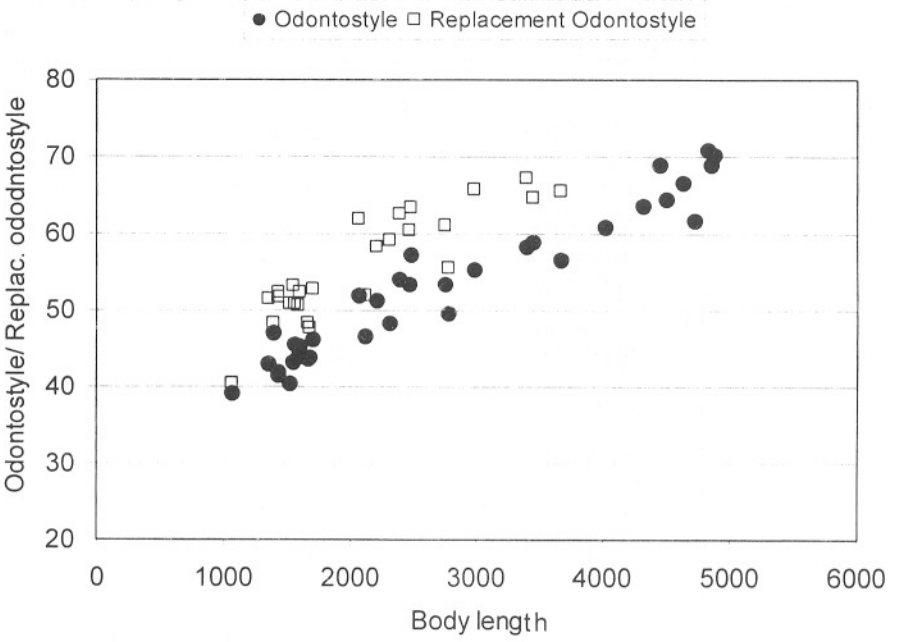

Fig. 3. Scatter plot of odontostyle and replacement odontostyle length against body length separating juveniles from females of *L. leptocephalus* Hooper from Juršinci.

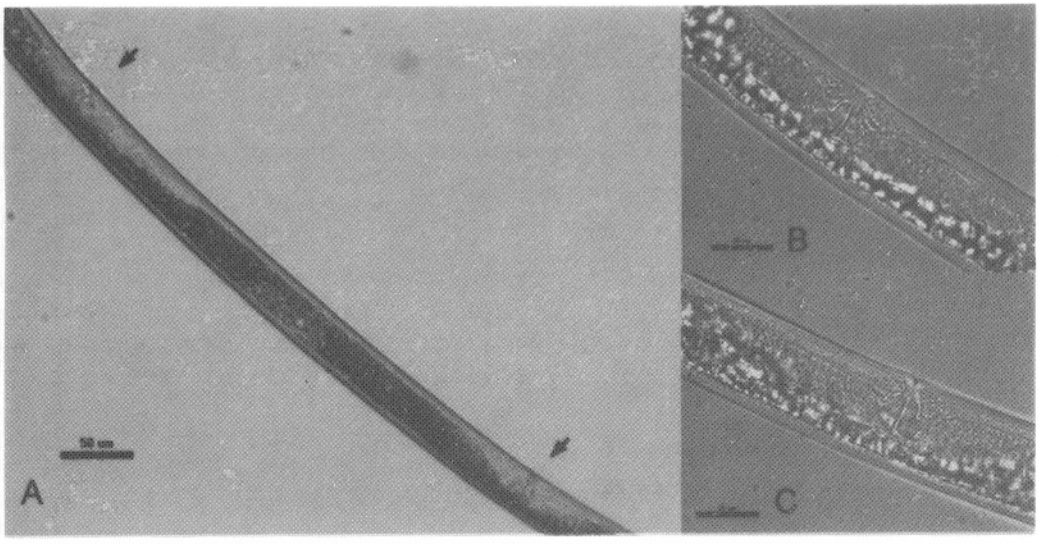

Fig. 4. A bivulval (arrows) female of *L. juvenilis* Dalmasso from Svetinje (A), vulva 1 (B) and vulva 2 (C). (Scale bars: A = 50 µm; B & C = 20 µm).

with a narrow conoid almost digitate terminus (41.7 - 44.9 µm; c' = 1.43 - 1.55). The code for identifying *L. leptocephalus* after the key of Chen *et al.* (1997) is: A-2, B-1, C-23, D-3, E-12, F-2, G-2, H-45, I-1 and the same codes were determined for *L. leptocephalus* from Juršinci. Males were not found. Measurements of *L. leptocephalus* females from Juršinci are presented in Table 1. The species had four juvenile developmental stages (Fig. 3). Among 26 specimens of *L. leptocephalus* juveniles measured, a single first-stage juvenile was detected with 1.07 mm body length, 39.2 µm odontostyle and 40.4 µm odontostyle replacement. Body length ranged between 1.35 and 1.70 mm for the second-stage juveniles, 2.06 and 2.99 mm for the third-stage juveniles, 3.39 and 3.67 mm for the fourth-stage juveniles. Odontostyle measured from 40.4 µm to 47.1 µm for the second-stage juveniles, from 46.5 µm to 57.3 µm for the third-stage juveniles and from 56.7 µm to 59.0 µm for the fourth-stage juveniles. Measurements of the odontostyle

replacement were between 47.6 and 53.1 µm for the second-stage juveniles, between 51.9 and 65.8 µm for the third-stage juveniles and between 64.5 and 67.2 µm for the fourth-stage juveniles.

rDNA sequence analyses. Cluster analyses of the D2-D3 expansion region of 28S rRNA gene sequences of both *Longidorus* species found in Slovenia and sequences of the same and closely related species (He *et al.*, 2005, Rubtsova *et al.*, 2001) were performed. The neighbour-joining method of the bootstrap test was used to construct a phylogenetic tree (Fig. 5). The sequence of *L. juvenilis* from Svetinje clustered together with a sequence of *L. juvenilis* (AY601579) (He *et al.*, 2005). The species clade of *L. juvenilis* was distinct and the branch to that clade was supported by a high bootstrap value (Fig. 5). In the same manner, sequences of *L. leptocephalus* from Juršinci and *L. leptocephalus* (AY601580) (He *et al.*, 2005) formed a separate cluster.

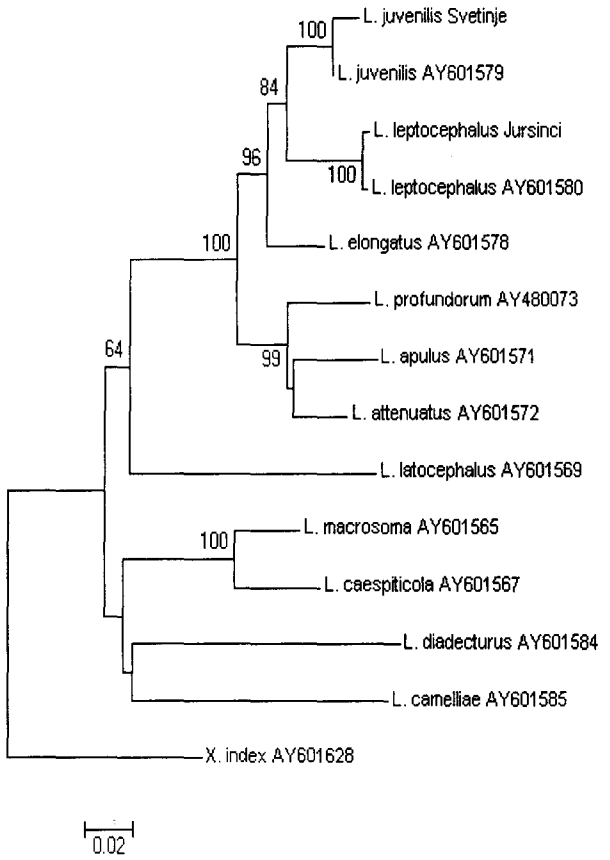

Fig. 5: Neighbour joining tree for D2-D3 expansion region of 28S rRNA gene of *L. juvenilis* and *L. leptocephalus* from Slovenia and sequences of closely related *Longidorus* species (NCBI GenBank). Bootstrap values below 60 % are not given.

DISCUSSION

The genus *Longidorus* currently includes 139 nominal species (Ye & Robbins, 2004). Delimitation of these species is primarily based on morphological data, namely of ranges of lengths and widths of certain parts of the body. Morphometrical data of many *Longidorus* species are variable (Ye & Robbins, 2004), which leads to considerable overlap between species and increases the potential for misidentification. For this reason, species discrimination based entirely on morphology is often difficult and sometimes controversial.

Similar to Dalmasso (1969), we observed three juvenile stages in *L. juvenilis* population from Svetinje. The females analysed here were generally longer than specimens described elsewhere (Dalmasso, 1969; Barsi & Lamberti, 2004). Specimens of the *L. juvenilis* population from Svetinje were about 20% longer than those of the paratypes specimens (Dalmasso, 1969), 3.96 mm (3.52 – 4.71) *vs* 3.32 mm (2.80 – 3.61). There were also differences in parameters a (109.5 *vs* 84.2), b (12.4 *vs* 10.7) and c (101.8 *vs* 64.7), when comparing morphometrical parameters of *L. juvenilis* from Svetinje and the paratype specimens (Dalmasso, 1969). The measurements of *L. juvenilis* body length specimens from Serbia (Barsi & Lamberti, 2004) were somewhere in between *L. juvenilis* from Svetinje and the paratype specimens. Our observations indicate that *L. juvenilis* displays great intraspecific variation in morphometrical data among different populations. This should be considered in the future morphometrical identifications of *L. juvenilis*.

One specimen of a bivulval female of *L. juvenilis* was detected. The female had two vaginas and uteri, with normally developed and functional gonads with clearly visible oocytes in the ovaries. The first vulva was positioned at 48.2% body length, which is characteristic for the species. The second vulva was positioned posteriorly at 57.9% body length. All the other measurements of bivulval female were within the range of the population measurements. The occurrence of a bivulval female in *Longidorus* was reported previously by Lamberti *et al.* (1987) for *L. laevicapitatus* and Barsi (1994) for *L. euonymus*. To our knowledge, this is the first report of a bivulval female of *L. juvenilis*.

Since the population of *L. juvenilis* was found on the same location, where RBDV was also found on grapevine as a first non-*Rubus* natural host (Mavrič *et al.*, 2003), we decided to check for the presence of virus in the nematode. *L. juvenilis*

nematodes were extracted from soil after 4 and 8 months of storage at 4°C. RBDV was detected in *L. juvenilis* specimens using nested RT-PCR. Further investigations to establish the possible role of *L. juvenilis* in RBDV transmission are in progress.

Morphometrical data of *L. leptocephalus* specimens from Juršinci were in the same range as those of *L. leptocephalus* from Slovakia, Scotland and England, including the topotype specimens (Liskova & Brown, 1995). Four juvenile stages for *L. leptocephalus* from Juršinci were found, which is in agreement with the findings of Robbins *et al.* (1995).

The use of rDNA as molecular marker has been used successfully in Longidoridae. In our study cluster analyses of D2-D3 expansion region of 28S rRNA gene sequences were used accurately to define and confirm morphometrical identification of the species. The sequence of *L. juvenilis* from Svetinje clustered together with the sequence of *L. juvenilis* (NCBI database) and formed a separate clade with high bootstrap support. In the same manner, the sequences of *L. leptocephalus* from Juršinci clustered together with the *L. leptocephalus* sequence. Our observations demonstrate that where there is great intraspecific morphometrical variation, the identification of *L. juvenilis* and *L. leptocephalus* can be performed combining morphometrical and molecular methods.

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REFERENCES

BARSI, L. 1994. Bivulval females of *Longidorus euonymus, Xiphinema diversicaudatum* and *X. vuittenezi* (Nematoda: Dorylaimida). *Nematologia Mediterranea* 22: 271-272.

BARSI, L. & LAMBERTI, F. 2004. Morphometrics of *Longidorus juvenilis* and first record of *L. atenuatus* and *L. moesicus* (Nematoda: Dorylaimida) from Serbia. *Nematologia mediterranea* 32: 5-14.

CHEN, Q., HOOPER, D.J., LOOF, P.A.A. & XU, J. 1997. A revised polytomous key for the identification of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). *Fundamental and Applied Nematology* 20: 15-28.

CURTNEY, W.D., POLLEY, D. & MILLER, V.L. 1955. TAF, an improved fixative in nematode technique. *Plant Disease Reporter* 39: 570-571.

DALMASSO, A. 1969. Étude anatomique et taxonomique des genres *Xiphinema, Longidorus* and *Paralongidorus* (Nematoda: Dorylaimidae). *Memoires du Museum National d'Histoire Naturelle, Ser. A. Zoologie* 61: 33-82.

DE WAELE, D. & COOMANS, A. 1990. Occurrence and ecology of longidorid nematodes in Belgium. *Revue Nématologie* 13: 197-202.

HE, Y., SUBBOTIN, S. A., RUBTSOVA, T., LAMBERTI, F., BROWN, D. J. F. & MOENS, M. 2005. A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology* 7: 111-124.

HEWITI, W. B., RASKI, D. J. & GOHEEN. A. C. 1958. Nematode vector of soil-borne fanleaf virus of grapevines. *Phytopathology* 48: 586-595.

HRŽIČ, A. 1973. [Extraction of nematodes from soil with whirling motion]. *Zaštita bilja* 122, 53-60.

KUMAR, S., TAMURA, K. & NEI M. 2004. MEGA3: Integrated Software for Molecular Evolutionary Genetic Analysis and Sequence Alignment. *Briefings in Bioinformatics* 5: 150-163.

LAMBERTI, F. 1975. Taxonomy of *Longidorus* (Micoletzky) Fillipjev and *Paralongidorus* Siddiqi, Hooper & Khan. In: Lamberti, F., Taylor, C. E. & Seinhorst, J. W. eds. *Nematode vectors of plant viruses.* London Plenum Press. pp. 71-90.

LAMBERTI, F. & ROCA, F. 1987. Present status of nematodes vectors of plant viruses. In: Veech, J. A. & Dickson, D. W. (Eds). *Vistas on Nematology.* Florida, De Leon Springs. pp. 321-328.

LAMBERTI, F., AGOSTINELLI, A. & NETO, E. S. S. 1987. A bivulval *Longidorus laevicapitatus* from Sao Tome. *Nematologia mediterranea* 15: 379-381.

LISKOVA, M. & BROWN, D. J. F. 1995. The occurrence and distribution of *Longidorus leptocephalus* (Nematoda: Dorylaimida) in the Slovak Republic with comment on the putative "large" and "small" forms. *Nematologia Mediterranea* 23, 315-320.

LOOF, P. A. A. & CHEN Q.W. 1999. A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). Supplement 1. *Nematology* 1: 55-59.

MAVRIČ, I., VIRŠČEK MARN, M., KORON, D. & ŽEŽLINA, I. 2003. First report of *Raspberry bushy dwarf virus* on red raspberry and grapevine in Slovenia. *Plant disease* 87: 1148.

ROBBINS, R. T., BROWN, D. J. F., HALBRENDT, J. M. & VRAIN, T. C. 1995. Compendium of *Longidorus* juvenile stages with observation on *L. pisi, L. taniwha* and *L. diadecturus* (Nematoda: Longidoridae). *Systematic Parasitology* 32: 33-52.

RUBTSOVA, T. V., SUBBOTIN, S. A., BROWN, D. J. F. & MOENS, M. 2001. Description of *Longidorus sturhani* sp. nov. (Nematoda: Longidoridae) and molecular characterization of several longidorid species from Western Europe. *Russian Journal of Nematology* 9: 127-136.

SOL, H. H. & SEINHORST, J. W. 1961. The transmission of tobacco rade virus by *Trichodorus pachydemus.*

Tijdschrift over Plantenziekten 67: 307-311.

ŠIRCA, S. & UREK, G. 2004. The occurrence of *Xiphinema* species in Slovenia. In: *European Society of Nematologists XXVII International Symposium*: Rome, 14-18 June 2004: programme and abstracts. Rome: European Society of Nematologists, 62 p.

UREK, G., ŠIRCA, S. & KARSSEN, G. 2003. A review of plant-parasitic and soil nematodes in Slovenia. *Nematology* 5: 391-403.

YE, W. M. & ROBBINS, R. T. 2004. Cluster analysis of *Longidorus* species (Nematoda: Longidoridae), a new approach in species identification. *Journal of Nematology* 36: 207-218.

YE, W. M., SZALANSKI, A. L. & ROBBINS, R. T. 2004. Phylogenetic relationships and genetic variation in *Longidorus* and *Xiphinema* species (Nematoda: Longidoridae) using ITS1 sequences of nuclear ribosomal DNA. *Journal of Nematology* 36: 14-19.

Širca S., Stare B. G., Pleško I. M., Virščck-Marn M., Urek G. Первое сообщение о *Longidorus juvenilis* и *L. leptocephalus* (Nematoda: Dorylaimida) из Словении с их морфометрическим и молекулярно-таксономическим анализом.

Резюме. *Longidorus juvenilis* и *L. leptocephalus* впервые обнаружены в Словении. Все стадии развития этих нематод были получены из ризосферы винограда *Vitis vinifera* L., произрастающего в северо-восточной части Словении. Виды были определены по морфологическим признакам самок и личинок. Дано описание всех стадий развития. Приводится описание бивульварной самки *L. juvenilis*. Получены и проанализированы нуклеотидные последовательности D2-D3 сегмента гена 28S рРНК. Проведено сравнение полученных последовательностей с таковыми для других популяций этого и близких видов, депонированным в Генбанке NCBI. Проведенный кластерный анализ подтвердил результаты морфологического определения видов. Последовательности *L. juvenilis* и *L. leptocephalus* из Словении образовывали с последовательностями конспецифичных форм две группы с высокими показателями bootstrap-поддержки.