Longidorus carniolensis sp. n. (Nematoda, Longidoridae) from vineyard soil in Slovenia

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Abstract
A new needle nematode, Longidorus carniolensis sp. n., recovered from the soil around the roots of grapevine Vitis vinifera L. from Slovenia, is described and illustrated. Longidorus carniolensis is an amphimictic species, characterised by females with a moderately long (L=5.6–8.2 mm) and plump (a=51–72.4, ave. 66.3) body, assuming a spiral to C-shape when heat relaxed. Head region continuous, anteriorly almost flat, lip region 23–25 µm wide; guiding ring situated posteriorly (42–47 µm, 43–50 µm in males), odontostyle long (ave. 146.6 (136–157) µm); pharyngeal glands with normal location, their nuclei of approximately equal size; tail bluntly conoidal to almost hemispherical. Males abundant, spicules slender and long (122–145 µm), ventromedian supplements 13–17, irregularly spaced, preceded by an adanal pair. Four juvenile stages present, the first stage juvenile with bluntly conoidal tail. Codes for identifying the new species when using the key by Chen et al. (1997) are: A 56, B 4, C 4, D 1, E 4, F 35, G 1, H 1, I 2. The new species is morphologically the most similar to L. poessneckensis Altherr, 1974, L. macrosoma Hooper, 1961, L. caespiticola Hooper, 1961, L. helveticus Lamberti et al., 2001, L. macroteromucronatus Altherr, 1974, L. pius Barsi & Lamberti, 2001, L. raskii Lamberti & Agostinelli, 1993, L. kheirii Pedram et al., 2008, L. silvae Roca, 1993, L. ingalandis Roca et al., 1985, L. vinearum Bravo & Roca, 1995 and L. major Roca & d’Erico, 1987, but differs from these species either by the body and odontostyle length,
position of guide ring, head region and tail shape or the shape of the first stage juvenile tail. Sequence data from the D2-D3 region of the 28S rDNA distinguishes this new species from other species of the genus *Longidorus* with known sequences. Relationships of *L. carniolensis* sp. n. with other *Longidorus* species based on analysis of this DNA fragment and morphology are discussed.

**Keywords**

grapevine, morphology, taxonomy, 28S rDNA

**Introduction**

The nematodes of the genus *Longidorus* Micoletzky, 1922 cause damage to many economically important crops by direct feeding on their roots. Additionally, they can cause indirect damage to the host plants by transmitting plant viruses. To date, six *Longidorus* species have been reported from Slovenia (Širca and Urek 2009): *L. elongatus* (de Man, 1876) Micoletzky, 1922, *L. caespiticola* Hooper, 1961, *L. juvenilis* Dalmasso, 1969, *L. helveticus* Lamberti, Kunz, Grunder, Molinari, De Luca, Agostinelli & Radicci, 2001, *L. leptopephalus* Hooper, 1961, *L. moesicus* Lamberti, Choleva & Agostinelli, 1983. The study of relationships between longidorids and Nepoviruses in Slovenia and Bulgaria in the frame of a bilateral project, revealed the presence of a new species described herein as *L. carniolensis* sp. n. The description of the new species is based both on morphological and molecular data, in particular the sequence of D2D3 expansion regions of the large subunit rDNA nuclear gene which proved to be useful in molecular phylogenetic analyses of Longidoridae (Rubtsova et al. 2001, Ye et al. 2004, He et al. 2005). Additionally, sequences of these domains allow species differentiation (Širca and Urek 2009).

**Materials and methods**

Soil samples were collected in July 2008 and October 2009 from the rhizosphere of *Vitis vinifera* L. in Drašiči and Krmačina localities in the southern part of Slovenia. The sampling was performed by digging holes beneath grapevine plants and carefully collecting soil around the roots at 40–50 cm depth. Approximately 500 cm³ of a collected soil sample was gently mixed and two 200 cm³ sub-samples were processed. Nematodes were extracted from the soil using a decanting method followed by the Baermann funnel technique. Longidorid nematodes for morphological study were hand-picked, fixed in TAF (7 ml 40% formalin, 2 ml tri-ethanolamine, and 91 ml distilled water), processed to glycerol (Seinhorst 1959) and mounted on glass microscope slides in anhydrous glycerol.

Drawings and photographs were taken using an Olympus BX51 compound microscope powered with differential interference contrast (DIC). Images were taken with a ColorView IIIu camera and cell^P^ software (Olympus Soft Imaging Solutions GmbH). Measurements were made using an Olympus BX 41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale,
AZ, USA), and Digitrak 1.0f programme (Philip Smith, Scottish Crop Research Institute, Dundee, UK).

**Total DNA extraction and amplification**

Extracted female nematodes for molecular study were transferred into 1.5 ml tube in a 1 μl drop of sterile water. DNA was extracted from a single female nematode from type-locality Drašiči and from Krmačina locality; 10 μl 1M EDTA pH 8 and 50 μl nucleic lysis solution (Promega Wizard DNA purification kit) mixture was added to each tube and homogenised with micropestle. Isolation of DNA was continued according to manufacturer's instructions. Isolated DNA was re-suspended in 10 μl of distilled water of which 2 μl was used in each PCR reaction. A fragment of the D2 and D3 expansion region of the 28S rDNA gene was amplified using the primers D2A (5’-ACA AGT ACC GTG AGG GAA AGT TG-3’) and D3B (5’-TCG GAA GGA ACC AGC TAC TA-3’) (Rubtsova et al. 2001) in a PCR cycler and conditions as described earlier (Širca et al. 2007).

**Analyses of rDNA sequence**

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), the sequences obtained were submitted to the GenBank. Cluster analyses were performed using sequences of several *Longidorus* species from the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) obtained from different phylogenetic studies (Rubtsova et al. 2001, Handoo et al. 2005, He et al. 2005, Lišková 2007, Kumari et al. 2009, Širca and Urek 2009) (Table 1). *Xiphinema index* (AY601628) (He et al. 2005) was used as an out-group. For cluster analyses and tree construction a Neighbour-Joining method was applied using MEGA5 software (Tamura et al. 2011).

**Taxonomy**

*Longidorus carniolensis* sp. n.
urn:lsid:zoobank.org:act:546D321E-CF14-46C1-A623-73A1F76839BD
http://species-id.net/wiki/Longidorus_carniolensis
Figs 1–12

**Measurements.** See Table 2.

**Description.** Female. Body moderately long (L=5.6–8.2 mm) and plump (a=51–72.4), assuming a spiral to C shape when heat relaxed. Cuticle consisting of several layers under light microscope: 11–14 μm thick at guiding ring level; 7–8 μm along the
**Table 1.** Species of fam. Longidoridae used in phylogenetic reconstructions.

| GenBank accession number | Nematode species                  | Origin           | Reference                  |
|--------------------------|-----------------------------------|------------------|----------------------------|
| AY601583                 | *Longidorus africanus* Merny, 1966| California, USA  | He et al. 2005             |
| AY494715                 | *L. americanum* Handoo, Carter & Skantar, 2005 | Georgia, USA | Handoo et al. 2005         |
| AY601571                 | *L. apulus* Lamberti et Bleve-Zacheo, 1977 | Mola di Bari, Italy | He et al. 2005 |
| AY601570                 | *L. arthensis* Brown, Grunder, Hooper, Klingler & Kunz, 1974 | Suter, Switzerland | He et al. 2005 |
| AY601574                 | *L. atreus* Lamberti, Coiro & Agostinelli, 1991 | Italy | He et al. 2005 |
| AY601572                 | *L. attenuatus* Hooper, 1961       | Germany          | He et al. 2005             |
| AY601576                 | *L. breviannulatus* Norton & Hoffmann, 1975 | Nebraska, USA | He et al. 2005             |
| HM447030                 | *L. caespiticola*                  | Brdo, Slovenia   | Širca and Urek 2009        |
| AY601585                 | *L. camelliae* Zheng, Peneva & Brown, 2000 | Hangzhou, China | He et al. 2005             |
| JN631811                 | *L. carniolensis* sp. n.          | Krmčina, Slovenia | This study |
| JN631812                 | *L. carniolensis* sp. n.          | Drašiči, Slovenia | This study |
| AF480072                 | *L. carpathicus* Lišková, Robbins & Brown, 1997 | Germany | Rubtsova 2001 |
| EF654539                 | *L. distinctus* Lamberti, Choleva & Agostinelli, 1983 | Kráľovský Chlmec, Slovakia | Lišková 2007 |
| AY593057                 | *L. dunensis* Brinkman, Loof & Barbez, 1987 |                | Holterman et al. Unpublished |
| AY601575                 | *L. edmundsi* Hunt & Siddiqi      | Caribbean sea beach, Cuba | He et al. 2005 |
| HM447032                 | *L. elongatus*                     | Maribor, Slovenia | Širca and Urek 2009        |
| AY601573                 | *L. euonymus* Mali & Hooper, 1973 | Zabagr, Hungary | He et al. 2005             |
| AY601581                 | *L. goodeyi* Hooper, 1961         | Peebles, Scotland, UK | He et al. 2005 |
| HM447031                 | *L. helveticus*                   | Trška gora, Slovenia | Širca and Urek 2009        |
| AF480074                 | *L. intermedius* Kozlowska & Seinhorst, 1979 | Germany | Rubtsova et al. 2001       |
| DQ364599                 | *L. juvenilis*                    | Svetinje, Slovenia | Širca et al. 2007          |
| AY601568                 | *L. latocephalus* Lamerti, Choleva & Agostinelli, 1983 | Greece | He et al. 2005             |
| DQ364600                 | *L. leptoccephalus*               | Juršinci, Slovenia | Širca et al. 2007          |
| AY601565                 | *L. macrocoma* Hooper, 1961       | Switzerland      | He et al. 2005             |
| HM447029                 | *L. moesicus*                     | Vrhpolje, Slovenia | Širca and Urek 2009        |
| AY601577                 | *L. piceicola* Lišková, Robbins & Brown, 1997 | Branisko, Slovakia | He et al. 2005             |
| EF538750                 | *L. poesneckensis* Altherr, 1974  | Cerne Voderady, Czech Republic | Kumari et al. 2009 |
| AF480073                 | *L. profundiorm* Hooper, 1965     | Germany          | Rubtsova et al. 2001       |
| AF480071                 | *L. sturhani* Rubtsova, Subbotin, Brown & Moens, 2001 | Belgium | Rubtsova et al. 2001       |
| EF538754                 | *L. uroshis* Krnjaic, Lamerti, Krnjaic, Agostinelli & Radicci, 2002 | Velke Pole, Slovakia | Kumari et al. 2009 |
| AY601628                 | *Xiphinema index* Thorne & Allen  | Argentina        | He et al. 2005             |
Table 2. Measurements of females, males and juvenile stages of *Longidorus carniolensis* sp. n., from Slovenia (mean ± standard deviation, with range). All measurements in micrometers.

| Character                  | Holotype | Females  | Males  | J1      | J2      | J3      | J4      |
|----------------------------|----------|----------|--------|---------|---------|---------|---------|
| n                          | n=1      | n=13     | n=14   | n=15    | n=6     | n=11    | n=9     |
| L                          | 7089     | 7447.5±679.0 5653-8226 | 7917.7±753.9 6702-9525 | 1349.9±53.8 1283-1449 | 2584.2±228.0 2329-2872 | 3692.2±238.0 3305-4149 | 5441.2±700.7 4677-6647 |
| a                          | 65.2     | 66.3±6.1 51.0-72.4 | 72.5±4.6 59.6-81.2 | 44.0±2.3 39.3-46.2 | 46.5±4.3 39.8-50.6 | 50.5±4.7 42.8-57.4 | 60.6±3.7 53.2-65.6 |
| b                          | 14.2     | 12.7±0.9 11.6-14.3 | 12.8±0.8 11.8-14.9 | 4.9±0.4 4.3-5.6 | 6.3±0.3 5.8-6.6 | 7.9±0.7 7.0-9.0 | 9.7±1.7 8.4-13.9 |
| c                          | 165.7    | 177.9±35.5 108.1-224.5 | 173.7±27.5 127.6-241.8 | 41.2±2.7 36.8-45.9 | 71.9±4.8 66.4-79.4 | 94.2±9.2 82.5-113.6 | 132.6±17.6 98.2-155.1 |
| c’                         | 0.6      | 0.6±0.1 0.5-1.0 | 0.8±0.1 0.6-1.1 | 1.4±0.1 1.2-1.5 | 0.8±0.4 0.7-0.9 | 0.7±0.1 0.6-0.8 | 0.7±0.04 0.6-0.8 |
| V (%)                      | 49.7     | 49.4±1.4 47.1-51.5 | 49.4±1.4 47.1-51.5 | 49.4±1.4 47.1-51.5 | 49.4±1.4 47.1-51.5 | 49.4±1.4 47.1-51.5 | 49.4±1.4 47.1-51.5 |
| G1 (%)                     | 11.9     | 13.4±2.6 10.6-17.4 | 13.4±2.6 10.6-17.4 | 13.4±2.6 10.6-17.4 | 13.4±2.6 10.6-17.4 | 13.4±2.6 10.6-17.4 | 13.4±2.6 10.6-17.4 |
| G2 (%)                     | 11.8     | 13.3±2.6 9.9-17.3 | 13.3±2.6 9.9-17.3 | 13.3±2.6 9.9-17.3 | 13.3±2.6 9.9-17.3 | 13.3±2.6 9.9-17.3 | 13.3±2.6 9.9-17.3 |
| d                          | 2.0      | 1.8±0.1 1.7-2.0 | 2.1±1.1 1.0-5.8 | 2.0±0.1 1.8-2.2 | 1.9±0.1 1.8-2.1 | 1.9±0.1 1.7-2.1 | 1.7±0.6 0.3-2.0 |
| d’                         | 2.0      | 2.0±0.2 1.8-2.3 | 1.9±0.1 1.8-2.1 | 1.7±0.1 1.6-1.9 | 1.8±0.2 1.7-2.1 | 1.9±0.1 1.7-2.1 | 1.9±0.4 0.8-2.2 |
| Anterior end to guiding ring | 45.4    | 44.6±1.6 42-47 | 46.5±2.3 43-50 | 21±0.7 20-22 | 28.7±1.6 26.5-30.5 | 33.9±1.0 32-35 | 39.8±1.2 38-42 |
| Anterior end to nerve ring  | 267.5   | 258.6±15.4 220-275 | 270.7±9.5 249-289 | 129.6±8.9 114-145 | 182.1±31.0 153-228 | 207.5±24.1 176-273 | 231.1±15.9 213-252 |
| Hemizonid                  | 237.5   | 253.7±18.9 204-270 | 10.4±0.5 10-11 | 10.4±0.5 10-11 | 10.4±0.5 10-11 | 10.4±0.5 10-11 | 10.4±0.5 10-11 |
| Character                  | Holotype | Females | Males | J1     | J2     | J3     | J4     |
|---------------------------|----------|---------|-------|--------|--------|--------|--------|
| n                         | n=1      | n=13    | n=14  | n=15   | n=6    | n=11   | n=9    |
| Odontostyle               | 144      | 147.5±4.7 | 149.1±6.6 | 81.9±3.5 | 88.2±5.0 | 107.7±4.4 | 125.9±3.6 |
|                           |          | 136-157  | 132-159 | 76-88  | 79-94  | 98-114  | 120-131 |
|Replacement odontostyle   |          |         |        |        |        |        |        |
|                           |          | 87.6±2.5 | 85-93  | 104.9±2.4 | 101-108 | 123.7±3.2 | 146.0±3.9 |
|                           |          |          |        | 149.1±6.6 | 119-130 | 119-130 | 142-152 |
| Odontophore               | 92       | 91.4±4.1 | 90.2±6.4 | 49.6±3.9 | 62.4±2.3 | 72.9±4.2 | 85.1±3.4 |
|                           |          | 85-97    | 75-99  | 43-56  | 59-66  | 64-78   | 81-91   |
| Neck length               | 500      | 590.5±59.9 | 618.6±56.6 | 274.1±20.8 | 408.3±33.4 | 472.5±48.7 | 553.6±47.5 |
|                           |          | 462-674  | 510-703 | 237-317 | 370-448 | 409-524 | 478-628 |
| Pharyngeal bulb length    | 139      | 142.7±6.0 | 141.1±6.9 | 72.6±2.3 | 89.6±3.1 | 106.8±6.1 | 123.6±5.6 |
|                           |          | 133-152  | 129-157 | 67-75  | 84-93  | 96-114  | 118-135 |
| Pharyngeal bulb width     | 39       | 40.2±2.8 | 37.9±2.1 | 15.6±0.9 | 22.7±1.4 | 28.1±1.6 | 33.3±1.9 |
|                           |          | 36-44    | 32-41  | 14-17  | 21-24  | 26-31   | 31-37   |
| DO*                       | 11.4     | 11.6±0.3 | 11.0±0.8 | 14.0±2.4 | 10.9±1.3 | 11.3±0.7 | 11.7±1.6 |
|                           |          | 11.2-11.9 | 9.8-11.8 | 11.3-18.1 | 9.1-12.0 | 10.3-11.9 | 9.9-14.8 |
| DN                        | 37.1     | 38.1±2.6 | 37.9±3.0 | 38.6±1.9 | 37.2±1.7 | 37.0±1.0 | 36.8±3.7 |
|                           |          | 32.8-40.2 | 33.2-42.9 | 36.7-42.7 | 35.5-40.3 | 35.6-38.4 | 30.0-42.6 |
| LS1N                      | 53.7     | 56.1±2.6 | 55.0±3.0 | 52.0±2.6 | 50.6±1.7 | 52.9±2.1 | 54.3±2.2 |
|                           |          | 52.3-61.0 | 49.6-62.3 | 47.9-57.3 | 48.6-52.7 | 48.1-55.3 | 52.2-57.7 |
| RS1N                      | 53.7     | 54.6±2.5 | 55.5±2.6 | 51.5±2.7 | 50.9±1.4 | 53.3±2.3 | 53.5±2.5 |
|                           |          | 51.5-58.6 | 52.4-61.2 | 46.3-55.3 | 48.9-52.7 | 48.1-56.3 | 50.7-57.4 |
| S2O                       | 83.4     | 84.9±1.6 | 86.3±5.7 | 84.2±1.0 | 84.8±2.7 | 85.0±1.6 | 82.8±0.9 |
|                           |          | 82.3-87.1 | 81.9-102.5 | 82.7-85.8 | 83.1-90.2 | 81.6-87.1 | 81.6-84.1 |
| Prerectum                 | 347.5    | 419.0±85.9 | 576.8±182.2 | 141.7±60.7 | 183.8±30.1 | 289.1±50.0 | 363.6±91.7 |
|                           |          | 280-550  | 248-832 | 81-290 | 150-224 | 175-363 | 275-538 |
| Rectum                    | 51.5     | 50.3±3.0 | 14.3±1.9 | 12.19  | 22.8±0.8 | 32.6±2.6 | 45.3±2.4 |
|                           |          | 47-57    | 12-19  | 22-24  | 30-38  | 42-49   | 42-49   |
| Tail                      | 42.8     | 43.3±9.2 | 46.4±6.8 | 32.9±1.4 | 35.9±1.6 | 39.4±2.9 | 41.2±3.2 |
|                           |          | 34-69    | 32-61  | 31-35  | 34-38  | 35.5-45 | 38-48   |
| Character                          | Holotype | Females n=1 | Females n=13 | Males n=14 | J1 n=15 | J2 n=6 | J3 n=11 | J4 n=9 |
|-----------------------------------|----------|-------------|--------------|------------|---------|--------|---------|--------|
| Length of hyaline part           | 20.2     | 17.8±0.7    | 16.9±1.2     | 7.9±0.9    | 10.4±0.7| 13.1±0.9| 14.9±0.8| 13-16  |
| Body diameter at:                | 22.9     | 24.2±0.8    | 24.8±1.3     | 10.6±0.4   | 15.0±0.5| 18.4±1.1| 20.9±0.5| 20-21  |
| lip region                       |          | 23-25       | 22-26.5      | 10-11      | 15-16   | 17-20  |         |        |
| guiding ring                     | 46.6     | 48.5±3.3    | 48.0±2.3     | 18.3±0.5   | 26.8±2.2| 35.7±2.7| 43.0±3.9| 38.7-51|
| base of pharynx                  | 95.4     | 93.4±4.5    | 93.8±4.8     | 30.2±1.2   | 50.5±3.4| 63.9±3.8| 76.4±5.6| 69.5-84|
| mid-body/at vulva                | 108.7    | 112.9±9.5   | 109.3±5.4    | 30.7±1.2   | 55.8±4.0| 73.5±5.6| 89.8±10.0| 79-105 |
| anus                             | 68.5     | 68.2±4.3    | 61.2±3.2     | 24.3±1.0   | 44.4±1.7| 55.2±3.6| 63.1±3.6| 59-70  |
| hyaline part                     | 49.5     | 49.0±1.7    | 39.5±2.2     | 16.0±0.9   | 26.8±2.7| 37.6±2.5| 42.9±2.7| 39-47  |

| Spicules                          |          |             |              |            |         |        |         |        |
|                                   |          | 126.9±5.8   | 122-145      |            |         |        |         |        |

* Following Loof and Coomans 1972
body; 13–15 µm on tail posterior to anus. Lateral pores number 10–14 in pharyngeal region: a single pore in front of guide ring, rarely two or none; 3–5 in odontostyle and 1–3 in odontophore regions; 3–4 dorsal pores and 7–10 ventral pores; numerous lateral body pores. Usually the fifth ventral pore (sometimes the fourth) differs in size (Figs 1A, 4F and 6H) compared to the other ventral pores. Lip region continuous, anteriorly almost flat, 7–9 µm high. Labial papillae prominent. Amphid aperture assumed to be a minute pore, difficult to be observed under light microscope. Pouch-like amphidal fovea with convoluted fine dendritic branches (receptors), extending to 1/2 - 2/3 the distance between anterior end and guiding ring, fovea slightly longer (15–18 µm, n=5) than wide (14–16 µm, n=4) with no distinct margins. Fusus (sensillium pouch) at 57±1.9 (55–60) µm from anterior end. Guiding ring 7–9 µm wide. Odontostyle long and very slender, 2 µm wide at the base. Odontophore with weakly developed flanges. In all females a small (2–3 µm long) rudimentary odontostyle tip (vestigium) present, directed forward, and observed in the slender pharynx at 300.5±40.3 (224–350) µm from anterior end; in two specimens the vestigium located in odontophore area. Slender pharynx often coiled in its posterior part. In this region 5–7 glandular bodies are observed in all females. Nerve ring surrounding odontophore base, rarely surrounding mid-odontophore, or just behind it, second nerve ring at a distance of 85.2±6.6 (78–98) µm behind the first one. Hemizonid flat, 10–11 µm long. Pharyngeal bulb about 1/4 of the neck length. Normal arrangement of pharyngeal glands, the nuclei of dorsal and ventrosublateral glands approximately the same size, their diameters 3.4±0.4 (3–4) µm, n=7 and 3.9±0.2 (3.5–4) µm, n=11, respectively. Cardia small, broadly rounded, wider than long, variable in size: 20.1±1.8 (10–23) × 10.1±1.8 (7–12) µm. Reproductive system amphidelphic, varying in dimensions due to the stage of maturity of female. Vagina extending about half body width. *Pars distalis vaginae* with characteristic shape (Fig. 2F, G), 26–28 µm and *pars proximalis vaginae* 32–38 µm long, respectively; muscular walls of the latter almost parallel. Uteri very long, anterior uterus 494.6±52 (430–563) µm long, posterior uterus 510.0±88.7 (357–643) µm long, differentiated, filled with sperm cells in all females examined; well developed sphincter between uterus and *pars dilatata oviductus* also containing numerous sperm cells. Anterior and posterior oviduct of similar size, measured in four specimens: 275–348 µm, and 283–330 µm. Anterior ovidarium 263.4±51.8 (210–347) µm long, n=7, posterior ovidarium 234.3±35.8 (183–309) µm long, n=5; in older mature specimens the length is about 3 times greater (1055–1060 µm for anterior and 1020 µm for posterior ovary). One egg in anterior *pars dilatata oviductus* measuring 227 × 87.5 µm and one uterine egg measuring 225 × 77.5 µm. A weakly developed ovijector present, 112.0±12 (95–125) µm long. In one female a rudimentary adanal pair of supplementary papillae was observed (Fig. 9E). Prerectum variable in length; rectum 0.7±0.1 (0.6–0.8) body width at anus. A short post-intestinal sac present. Tail bluntly conoidal, rounded to almost hemispherical; ventral side straight or slightly convex, the dorsal curvature greater. Two pairs of lateral pores.

**Male.** Body C shaped when heat relaxed, posterior part more strongly coiled ventrally. Similar to females in general morphology except for genital system. Lateral pores
Figure 1. *Longidorus carniolensis* sp. n. Female: A Neck region F Habitus H Pharyngeal bulb Male: G Habitus I Pharyngeal bulb; juveniles: B–E Habitus of first, second, third and forth juvenile stages J–M Pharyngeal bulb of first, second, third and forth juvenile stages. Scale bars: B–G 1 mm; A, H–M 100 µm.
Figure 2. Longidorus carniolensis sp. n. Female. A–D Anterior ends E Vestigium in the walls of the slender part of pharynx F, G Vulval region G Anterior genital branch. Scale bars: A–I 50 µm, J 100 µm.
Figure 3. *Longidorus carniolensis* sp. n. Female: A Anterior region B–D Amphidial fovea E Vestigium F–H Vulval region I Vulval region, uterus and egg J Pharyngeal bulb, dorsal and subventral glands K, L Tail – different optical sections M Sphincter N Prerectum O–Q Variation in tail shape. Scale bars: I, N 200 µm; A–G, H–M, O–Q 50 µm.
Figure 4. *Longidorus carniolensis* sp. n. Male: A–E Anterior end B, D, E in sublateral view F Excretory pore and ventral pores G Partly protracted spicules H–I Tail end. Scale bar: 50 µm.
number 10–15 in pharyngeal region: a single pore in front of guide ring, 3–5 in odontostyle and 1–2 in odontophore regions; 2–5 dorsal pores, mostly 3–4, and 7–10 ventral pores. Cuticle in post-labial region at the guiding ring level 10.5–13.5 µm thick, 6.5–9 µm along body, 9–10 µm in post-cloacal area. Second nerve ring at 80.7±14 (50–100) µm behind the first one (n=14). In all males a small vestigium (2–3 µm, in one specimen 6 µm long), directed forward (in two specimens directed rearward), is observed in the slender pharynx at 300.5±40.3 (224–350) µm from anterior end; in two specimens the vestigium detected in odontophore area. Two to eleven glandular bodies observed in all males in posterior part of the slender pharynx and pharyngeal bulb. In two specimens lens-like hemizonion at a distance of 242 and 271 µm from anterior end observed. Pharyngeal bulb slightly less than 1/4 of neck length (22.9±1.6 (20.9–26.7%). Ventromedian supplements composed of one adanal pair and a row of 13–17 irregularly spaced single ones, the first three appear as double in some specimens. Spicules comparatively slender, of almost equal width along the length, curved to almost at right angle. Lateral guiding piece not bifid, with uneven internal walls. Post-cloacal papilla well developed. Tail short, bluntly conoidal, ventral side almost straight, dorsal side convex. Two or three pairs of lateral caudal pores.

**Juveniles.** Four developmental stages clearly present (Fig. 11) as determined from the position of the replacement odontostyle and the principal morphometric characters of body, odontostyle and replacement odontostyle lengths, and developing gonad (genital primordium) size. The *habitus* of juveniles not changing considerably during successive stages, assuming J or C shape. In first stage juvenile, lip region somewhat different from the next stages, it is rounded with a very weak depression after the second circle of labial papillae, the latter slightly protruding and changing the lip region outline. Amphidial fovea in first two stages has no clearly visible receptors, only small refractive elements discernable. Both the tail and body width at anus is increasing in length and $e’$ ratio is decreasing. Tail shape in J1 is conoidal, ventrally almost straight or slightly concave, dorsally convex, which gives asymmetrical appearance, in successive stages it gradually becomes rounded but always with the dorsal curvature more strongly expressed.

**Differential diagnosis and relationships.** *Longidorus carniolensis* is an amphimictic species, characterized by females with a moderately long (L=5.6–8.2 mm) and plump (a=51–72) body, assuming a spiral to C-shape when heat relaxed; head region continuous, anteriorly almost flat, lip region 23–25 µm wide, guiding ring situated posteriorly (42–47 µm, 43–50 µm in males), long odontostyle (146.6 (136–157) µm), distribution of pharyngeal glands normal, nuclei of approximately equal size, tail bluntly conoid to hemispherical. Males abundant, spicules slender and long (122–145 µm), ventromedian supplements 13–17, irregularly spaced and preceded by an adanal pair. Postembrional development through four juvenile stages.

The codes for identifying the new species when using the polytomous key by Chen et al. (1997) are: A 56, B 4, C 4, D 1, E 4, F 35, G 1, H 1, I 2. The species belongs to the group of species with long odontostyle – over 100 µm and bluntly conoid to hemispherical tail: *L. poessneckensis, L. macrosoma, L. caespiticola, L. helveticus, L.
Figure 5. *Longidorus carniolensis* sp. n. Male: A, B Variation in tail shape. Scale bar: 50 µm.

macroteromucronatus Altherr, 1974, *L. raskii* Lamberti & Agostinelli, 1993, *L. kheirii* Pedram, Niknam, Robbins, Ye & Karegar, 2008, *L. pius* Barsi & Lamberti, 2001, *L. nevesi* Macara, 1986, *L. major* Roca & d’Erigo, 1987, *L. carpathicus*, *L. piceicola*, *L. vinearum* Bravo & Roca, 1995, *L. pauli* Lamberti, Molinari, De Luca, Agostinelli & Di Vito, 1999, *L. arthensis*, *L. iuglandis* Roca, Lamberti & Agostinelli, 1985, *L. piceinus* Roca, Lamberti & Agostinelli, 1985, *Longidorus silvae* Roca, 1993, *L. uroshis*, *L. saginus* Khan, Seshardi, Weissher & Mathen, 1971, *L. orongorongensis* Yeates & Van Etteger, 1992, *L. cretensis* Tzortzakakis, Peneva, Terzakis, Neilson & Brown, 2001, *L. cylindricaudatus* Krnjaic, Roca, Krnjaic & Agostinelli, 2005, *L. fasciatus* Roca & Lamberti, 1981 and *L. litchii*. *Longidorus carniolensis* sp. n. can be differentiated from all these species either by morphometrics or/and quantitative characters. It differs from:

*L. poessneckensis* – by its somewhat longer odontostyle (ave. 147.5 (136–157) vs ave. 133 (122–142), ave. 126 (122–130) and ave. 140.2 (132–148) µm); more posteriorly situated guiding ring (ave. 44.6 (42–47) vs ave. 40 (36–43) and 39 ave. (37–40) µm); tail short conoidal vs elongate conoid in J1 (c’=1.2–1.5 vs 1.8–2.2 and 1.8–2.5); males abundant vs males very rare (Sturhan and Loof 2001, Lišková and Kumari 2010, Kornobis and Peneva 2011);

*L. macrosoma* – by its shorter (5.6–8.2 vs 8.4–11.9 mm) and more plump (a=51–72.4 vs 77–113 and 105–126) body; differently shaped lip region (later-
Figure 6. Longidorus carniolensis sp. n. Male: A Anterior region, B, C Head region, D–G Amphidial fovea, H Vestigium (white arrow), excretory pore (thick arrow) and ventral pores (slender arrows), I Ejaculatory glands (marked by arrows), J Lateral field, K, L Pharyngeal bulb with glandular bodies (marked by arrows), M, N Sperm cells at different stage of development. Scale bars: A 200 µm; B–N 50 µm.
Figure 7. *Longidorus carniolensis* sp. n. Male: A Posterior genital branch B, C, E, F Tail and copulatory apparatus – different optical sections D, G Posterior end H Rectum (marked by arrow), spicules and lateral piece I Partly protracted spicules. Scale bars: A, D, G – 200 µm; B, C, E–F, H, I – 50 µm.
Longidorus carniolensis sp. n. (Nematoda, Longidoridae) from vineyard soil in Slovenia

Figure 8. *Longidorus carniolensis* sp. n. Juveniles: A Neck region of first stage B–D Spear region of second, third and fourth stage. Scale bar: 50 µm.

ally rounded *vs* truncated, flattened); somewhat longer odontostyle (136–157 *vs* 113–148 µm); different tail shape in J1, bluntly conoidal *vs* mucronate; longer spicules in males 122–145 *vs* 105 µm and ave. 116.2 (112–121) µm (Hooper 1961, Lamberti et al. 2001);

*L. caespiticola* – by its longer odontostyle (136–157 *vs* 100–120, 96–109 µm); different numbers of dorsal (2–5 *vs* a single pore) and ventral (7–10 *vs* 5–6) pores; more posteriorly sited guiding ring (42–47 *vs* 30–41, 37, 42.5 µm); longer spicules in males (122–145 *vs* 90, 80–95 µm), smaller c’ value in J1 (1.2–1.5 *vs* almost 2) (Hooper 1961, Širca and Urek 2009);

*L. helveticus* – by different tail shape of J1 being bluntly conoid *vs* mucronated; longer (122–145 *vs* 104–118 µm ) and differently shaped spicules (Lamberti et al. 2001);
L. macroteromucronatus – by having more posteriorly situated guide ring (42–47 vs 38 µm); thicker cuticle along the body (6–7 vs 4 µm) and on tail region (9–10.5 vs 13–15 µm); longer odontostyle (136–157 vs 133 µm) (Altherr 1974);

L. raskii – by its wider lip region (23–25 vs 15–19 and 14–16 µm); more posteriorly situated guiding ring (42–47 vs 33–38 and 33–43 µm); longer odontostyle (136–157 vs 90–103 and 98–100 µm); longer spicules (122–145 vs 82–103 and 79–90 µm) (Lamberti and Agostinelli 1993, Lamberti et al. 2001, Krnjaić et al. 2002);

L. kheirii – by its longer odontostyle (136–157 vs 111–130 µm); different tail shape in J1 (bluntly conoidal vs mucronated); males abundant vs males rare; longer spicules (122–145 vs 80 µm) (Pedram et al. 2008);

L. pius – by its more posterior position of the guiding ring (42–47 vs 35–42 and 37–42.5 µm); different tail shape in J1 (bluntly conoidal vs mucronated); males abundant vs males absent (Barsi and Lamberti 2001a, Barsi and De Luca 2008)

L. nevesi – by having wider lip region (23–25 vs 16–22 µm), different amphidial fovea shape (pouch like, not bilobed vs bilobed); differently shaped and longer spicules in males (122–145 vs 87–100 µm) (Macara 1986);

L. major – by having shorter body (L=5.6–8.2 vs 8.5–12 mm); somewhat narrower lip region (23–25 vs 22–27 µm); different tail shape in J1 (bluntly conoidal vs mucronate) and amphidial fovea (pouch like, not bilobed vs bilobed), males abundant vs males absent (Roca and d’Erico 1987);

L. carpathicus – by its longer body (L=5.6–8.2 mm vs 6.2–6.5 mm); wider (23–25 vs 16–18 µm) and differently shaped lip region; lower c’ value (c’= ave. 0.6 (0.5–1.0) vs c’= 0.8); different shape in J1 (bluntly conoidal vs mucronated with a rather long mucro); males abundant vs males absent (Lišková et al. 1997);

L. piceicola – by having longer body (L=5.6–8.2 vs 4.2–6.5, 4.4–8.0 and 5.2–7.9 mm); wider (23–25 vs 14–17 µm) and differently shaped lip region (continous, almost flat vs broadly rounded); lower c’ value (c’=ave. 0.6 (0.5–1.0) vs c’=0.9–1.3); differently shaped tail in J1 (bluntly conoidal vs elongate conoid) (Lišková et al. 1997, Barsi and Lamberti 2001b);

L. vinearum – by having different lip region shape (abruptly vs gradually tapering), different shape of amphidial fovea (pouch like not bilobed vs irregularly bilobed); longer odontostyle (136–157 vs 105.5–132.5 µm); different tail shape in J1 (bluntly conoidal vs conical, c’=1.2–1.5 vs 1.9–2.8) (Bravo and Roca 1995);

L. pauli – by having different (continous vs slightly offset) and wider (23–25 vs 14–17 µm) lip region, amphidial fovea pouch like, not bilobed vs bilobed; longer odontostyle (136–157 vs 102–118 µm); lower a and c’ values (a=51.0–72.4 vs a=120.3–143.5; c’= ave. 0.6 (0.5–1.0) vs c’=0.9 (0.8–1.0), respectively); more posteriorly situated guiding ring (42–47 vs 27–36 µm); longer spicules (122–145 vs 61–69 µm); different tail shape in J1 (bluntly conoidal vs subdigitate) (Lamberti et al. 1999);

L. arthensis – by its wider (23–25 vs 14–17 µm) lip region, amphidial fovea pouch like not bilobed vs bilobed; longer odontostyle (136–157 vs 102–111 µm); lower c’ values (c’=av 0.6 (0.5–0.1) vs c’=av 0.9 (0.8–1.1); more posteriorly situated guiding ring
Longidorus carniolensis sp. n. (Nematoda, Longidoridae) from vineyard soil in Slovenia

Figure 9. *Longidorus carniolensis* sp. n. Evolution of the tail. A–D Tail of first–fourth juvenile stage
E Tail of female. Scale bar: 100 µm.

(42–47 vs 30–38 µm); longer spicules (122–145 vs 60–66 µm); different tail shape in J1 (bluntly conoidal vs mucronated) (Brown et al. 1994);

*L. iuglandis* – by its wider lip region (23–25 vs 14–16 µm); amphidial pouches not bilobed vs bilobed; longer odontostyle (136–157 vs 112–128 µm); more posterior position of the guiding ring (42–47 vs 31–41 µm); longer tail (34–69 vs 33–41 µm); longer spicules (122–145 vs 93–99 µm); different tail shape in J1 (bluntly conoidal vs mucronated) (Roca et al. 1985);

*L. picenus* - by its wider lip region (23–25 vs 19–22 µm); amphidial fovea not bilobed vs bilobed; more posterior position of the guiding ring (42–47 vs 31–41 µm); longer spicules (122–145 vs 103–112 µm); different tail shape in J1 (bluntly conoidal vs mucronated) (Roca et al. 1985);

*L. silvae* - by its more plump body (*a*=51.0–72.4 vs *a*=87.5–137.5 in Italian population and *a*=87.4–116 in Serbian populations), wider lip region (23–25 vs 14–17 µm); amphidial fovea not bilobed vs bilobed; longer odontostyle (136–157 vs 113.5–133 µm (Italian population) and 108–136 µm (Serbian populations)); different tail shape in J1 (bluntly conoidal vs mucronated); males abundant vs males rare; longer spicules (122–145 vs 77–78 µm) (Roca 1993, Barsi and Lamberti 2004, Barsi et al. 2007);

*L. urohibis* – by having wider (23–25 vs 15–20.5 µm) lip region; lower *a* values (*a*=51.0–72.4 vs *a*=96.9–108.9); different tail shape in J1 (bluntly conoidal vs mucronated); longer spicules (122–145 vs 59–72 µm) (Krnjaic et al. 2000);
Figure 10. *Longidorus carniolensis* sp. n. Juvenile: A–D Anterior region of first, second, third and fourth stages H–K Pharyngeal bulb of first, second, third and forth juvenile stages M, F, G, R genital primordium of first, second, third and forth stages N, S Tail shape of first stage O, T Tail shape of second stage P, U Tail shape of third stage Q, V Tail shape of forth stage Female: E Anterior region L Pharyngeal bulb W Tail shape. Scale bar: 50 µm.
Figure 11. *Longidorus carniolensis* sp. n. Scatter plot of the functional (■) and replacement odontostyle (□) in relation to the body length of the juvenile stages and adults: females (■) and males (□), female with very short odontostyle (■).

*L. saginus* – by having longer body (L=5.6–9.5 vs 4.8–6.4 mm); amphidial fovea pouch shaped not bilobed vs asymmetrically bilobed; longer tail (34–69 vs 21–33 µm) (Khan et al. 1971);

*L. orongorongensis* – by its more anterior position of the guiding ring (42–47 vs 63–73 µm); shorter odontostyle (136–157 vs 152–166 µm); longer spicules (122–145 vs 84–87 µm) (Yeates et al. 1992);

*L. cretensis* – by having normal vs abnormal location of pharyngeal glands; wider lip region (23–25 vs 17–21 µm); longer spicules (122–145 vs 71–91 µm); different tail shape in J1 (bluntly conoidal vs conoid pointed) (Tzortzakakis et al. 2001);

*L. cylindricaudatus* – by having lip region abruptly vs gradually tapering; amphidial fovea not bilobed vs bilobed; shorter odontostyle (136–157 vs 164–178 µm); lower a values (a=51–72.4 vs a=94.4–113.4); males abundant vs males absent (Krnjaić et al. 2005);

*L. fasciatus* – by its wider lip region (23–25 vs 12–14 µm); different amphidial poches (not bilobed vs asymmetrically bilobed); longer odontostyle (136–157 vs 102–119 µm); male abundant vs males absent (Roca and Lamberti 1981);

*L. litchii* – by its somewhat shorter odontostyle (136–157 vs 138–171 mm); different amphidial poches (not bilobed vs bilobed); more anterior position of the guiding
Figure 12. Phylogenetic tree of rDNA D2/D3 expansion region sequences of *Longidorus carniolensis* sp. n. from Slovenia (square mark) and sequences of closely related *Longidorus* species (NCBI GenBank). Sequences were analysed using Neighbour Joining Method. Bootstrap support values higher than 50% are presented.

Ring (42–47 vs 82.5–96.5 µm); different tail shape in J1 (bluntly conoidal vs elongate conoid with long digitate tip, $c' = 1.2–1.5$ vs $c' = 2.7–3.4$); longer spicules (122–145 vs 68.5–71 µm) (Xu and Cheng 1992).

**Type-locality and plant association.** An old vineyard with roots of several *V. vinifera* varieties close to Drašiči village in southern part of Slovenia (45°39’N; 15°23’E), 229 m above sea-level.

Other-locality: a vineyard close to Krmačina village in southern part of Slovenia.

**Distribution notes.** *Longidorus carniolensis* n. sp were detected in 6 out of 10 soil samples from locations of Drašiči and Krmačina. Population density was 4–15 specimens of all developmental stages per 200 cm$^3$ of soil sample.

**Type-material.** Holotype female and 2 female, 5 male and 8 juvenile (3 J1, 1 J2, 2 J3, 2 J4) paratypes deposited in the Nematode Collection of Agricultural Institute of Slovenia, Ljubljana, Slovenia; two female, two male and 10 juvenile (3 J1, 2 J2, 2 J3,
Longidorus carniolensis sp. n. (Nematoda, Longidoridae) from vineyard soil in Slovenia

2 J4) paratypes - in the Wageningen Nematode Collection (WaNeCo), Wageningen, the Netherlands; one female, one male and 6 juvenile (3 J1, 1 J2, 1 J3, 1 J4) paratypes - in the Nematode Collection of The Food and Environment Research Agency, Sand Hutton, UK (former Rothamsted Nematode Collection); one female, three male and 6 juvenile (3 J1, 2 J3, 1 J4) paratypes - in the Nematode Collection of the Zoology Museum, Ghent University, Belgium; one female, three male and two juvenile (1 J3, 1 J4) paratypes - in the Nematode Collection of the Institute of Plant Protection, Bari, Italy; two female, one male and 6 juvenile (3 J1, 1 J2, 1 J3, 1 J4) paratypes - in the Nematode Collection of the University of California at Riverside, USA; one female, one male and two juvenile (1 J3, 1 J4) paratypes - in the USDA Nematode Collection, Beltsville, Maryland, USA; 4 female, 5 male and 8 juvenile (3 J1, 1 J2, 2 J3, 2 J4) paratypes - in the Nematode Collection of the Institute of Biodiversity and Ecosystem Research, BAS, Sofia.

Etymology. The species epithet carniolensis was derived from Carniola which is the Latin name of the Kranjska province, a historical region that comprised parts of what is now Slovenia.

rDNA sequence analysis. Cluster analyses of the D2-D3 expansion regions of the 28S rDNA nuclear gene sequences of L. carniolensis sp. n. and closely related species (Table 1) were performed and a phylogenetic tree was constructed (Fig. 12). The sequences of both populations of L. carniolensis sp. n. from Drašiči and Krmačina were identical. They formed a distinct clade within a cluster of the closely related sequences of L. poessneckensis, L. helveticus, L. macrosoma, L. caespiticola and L. latocephalus. The closest sequence to L. carniolensis sp. n. was the sequence of L. poessneckensis (Acc. No EF538750) with 91.9% of similarity.

Discussion

There are some characteristic morphological features observed in L. carniolensis sp. n. such as the presence of vestigium, hemizonid and hemizonion, and the aberrant ventral pore. The vestigium was present in all specimens (males and females), it was located in the slender pharynx, behind odontophore, in few specimens in the odontophore area. Such a vestigium has been reported also for Longidorus fursti Heyns, Coomans, Hutsebaut & Swart, 1987 from South Africa; two Chinese Longidorus species (Xu and Cheng 1992); it is more frequently observed in Xiphinema spp. (Kruger and Heyns 1987, Swart 1994, Swart and Quénéhervé 1998; Mincheva et al. 2008), reported also for several species of Xiphidorus (Decraemer et al. 1996) and Paraxiphidorus brevistylus (Decraemer et al. 1998).

Hemizonid and hemizonion are not commonly observed structures in dorylaimids (Jairajpuri and Ahmad 1992), the hemizonid was seen both in adults and in the last two juvenile stages of the new species and hemizonion in only two male specimens. Both structures were reported also for L. fursti (Heyns et al. 1987), L. iranicus (Sturhan and Barooti 1983); only hemizonoid – for L. litchii, L. henanus (Xu and Cheng 1992).
L. carpetanensis Arias, Fé Andres & Navas, 1986, L. pawneensis Luc & Coomans, 1988, L. brevis Swart et al., 1996, L. africanaus (Bravo and Roca 1995), L. kheirii (Pedram et al. 2008), L. laevicapitatus Williams, 1959 (Heyns and Luc 1987), in a few specimens of L. fagi Peneva, Choleva & Nedelchev, 1997, for one Xiphidorus and some Paralongidorus species (Siddiqi et al. 1963, Fisher 1964, Luc and Doucet 1984).

The only available data on the excretory system in Longidorus refers to L. macrosoma in which a ventral excretory pore at the level of the nerve ring, leading to a non-canaliculur tissue in its anterior part has been observed together with two nucleated glands embedded in the tissues of a ventrally located ampulla-like structure (Aboul-Eid 1969). In L. carniolensis sp. n. we observed an aberrant ventral pore in all adults, differing in structure from the other ventral pores and also having a longer duct (Figs 1A, 4F and 6H), it probably functions as more specialised part of the excretory system. It was also detected in juvenile stages (Fig. 8B, D).

The data on D2D3 rDNA regions of majority of longidorid species, particularly of those belonging to the genus Longidorus is far to be complete; this does not facilitate the reconstruction of the phylogenetic relationships among the members of this widely distributed group of ectoparasitic nematodes. Despite of this, based on the rDNA results as well as a combination of morphological features the new species is included in a clearly defined group of closely related species (L. poessneckensis (92% similarity), L. macrosoma and L. caespiticola (90%), L. helveticus (89%), sharing some common characters – amphids with pouch-like fovea, not bilobed, amphidial duct well discernable, tapering lip region, which is continuous with the rest of body, normal arrangement of pharyngeal glands, bluntly conoidal to hemispherical tail, much shorter or equal to the anal body width; and the development through 4 juvenile stages. All these species occur in Europe, more frequently in West and Central Europe. The correlation between the amphid structure and clustering of longidorid species has been underlined by Rubtsova et al. (2001) and He et al. (2005) and it is supported by our study.

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