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First Reports, Morphological, and Molecular Characterization of *Longidorus caespiticola* and *Longidorus poessneckensis* (Nematoda: Longidoridae) from Ukraine

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Abstract: Seven needle nematode species of the genus *Longidorus* have been reported in Ukraine. Nematological surveys for needle nematodes were carried out in Ukraine between 2016 and 2017 and two nematode species of *Longidorus* (*L. caespiticola* and *L. poessneckensis*) were collected from natural and anthropogenically altered habitats on the territory of Opillia and Zakarpattia in Ukraine. Nematodes were extracted from 500 cm^3 of soil by modified sieving and decanting method. Extracted specimens were processed to glycerol and mounted on permanent slides and subsequently identified morphologically and molecularly. Nematode DNA was extracted from single individuals and PCR assays were conducted as previously described for D2–D3 expansion segments of 28S rRNA. Sequence alignments for D2–D3 from *L. caespiticola* showed 97%–99% similarity to other sequences of *L. caespiticola* deposited in GenBank from Belgium, Bulgaria, Czech Republic, Russia, Slovenia, and Scotland. Similarly, D2–D3 sequence alignments from *L. poessneckensis*, showed 99% to other sequences of *L. poessneckensis* deposited in GenBank from Slovakia and Czech Republic. Morphology, morphometry, and molecular data obtained from these samples were consistent with *L. caespiticola* and *L. poessneckensis* identification. To our knowledge, these are the first reports of *L. caespiticola* and *L. poessneckensis* in Ukraine, extending the geographical distribution of these species.

Key words: 28S rDNA, D2–D3 segments, detection, needle nematodes, taxonomy.

Nematodes of the genus *Longidorus* Micoletzky, 1922 are ectoparasites of vascular plants inhabiting terrestrial biotopes. Some species of this genus are economically important pests of agricultural plants and others are experimentally proved to transmit nepoviruses (Taylor and Brown, 1997). *Longidorus* consists of more than 150 valid species (Archidona-Yuste et al., 2016), but currently only seven have been reported from Ukraine: *L. attenuatus* Hooper, 1961, *L. danuvii* Barsi et al., 2007, *L. distinctus* Lamberti et al., 1983, *L. elongatus* (de Man, 1876) Thorne and Swanger, 1936, *L. holovachovi* Peneva et al., 2009, *L. rubi* Romanenko and Tomilin in Romanenko, 1993, and *L. sylphus* Thorne, 1939 (Peneva et al., 2009; Susulovska et al., 2016). During recent nematode surveys in Ukraine, three needle nematode populations were detected, one resembling *L. caespiticola* Hooper, 1961 and two populations resembling *L. poessneckensis* Altherr, 1974. Both these species were previously reported from several other European countries (Kumari et al., 2009; Kornobis and Peneva, 2011; Kumari, 2014), but not from Ukraine. Therefore, the objective of the present study was to provide an accurate identification of *Longidorus* species detected in Ukraine by an integrative approach of morphological and molecular characterization by using the D2–D3 expansion segments of 28S rRNA.

Materials and Methods

Nematode samples and morphological study: Soil samples containing needle nematodes resembling *L. caespiticola* and *L. poessneckensis* were taken from natural and anthropogenically altered habitats on the territory of Opillia and Zakarpattia in Ukraine. Nematodes were extracted from soil by using a modified sieving and decanting method (Brown and Boag, 1988). Extracted specimens were heat killed, fixed in TAF (triethanolamine [2%] and formalin [8%] in distilled water), processed to glycerol by a slow evaporation method, and mounted on permanent slides (Hooper, 1986). Identification, measurements, and photos were made using Olympus BX 51 microscope with Nomarski differential interference contrast, equipped with a digital camera Olympus DP 72, and computer program Quick PHOTO MICRO 2.3. All other abbreviations used were as defined in Jairajpuri and Ahmad (1992).

Molecular characterization: DNA extraction and PCR assays were conducted on single nematodes as described by Castillo et al. (2003). The D2–D3 expansion segments of 28S rRNA was amplified using the D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) primers (De Ley et al., 1999). PCR products were purified, quantified, and used for direct sequencing as described by Tzortzakakis et al. (2014). The newly obtained sequences were submitted to the GenBank database under accession numbers MF716960-MF716963.

Results and Discussion

Longidorus caespiticola Hooper, 1961 (Fig. 1)

Measurements

See Table 1.

Description

The Ukrainian population was characterized by moderate body length (6.0–7.4 mm in female; and 5.6–6.5 mm in male); C-shaped when killed by gentle heat with slightly greater curvature in the posterior half more pronounced in the case of males. Labial region 6 μm high, smoothly

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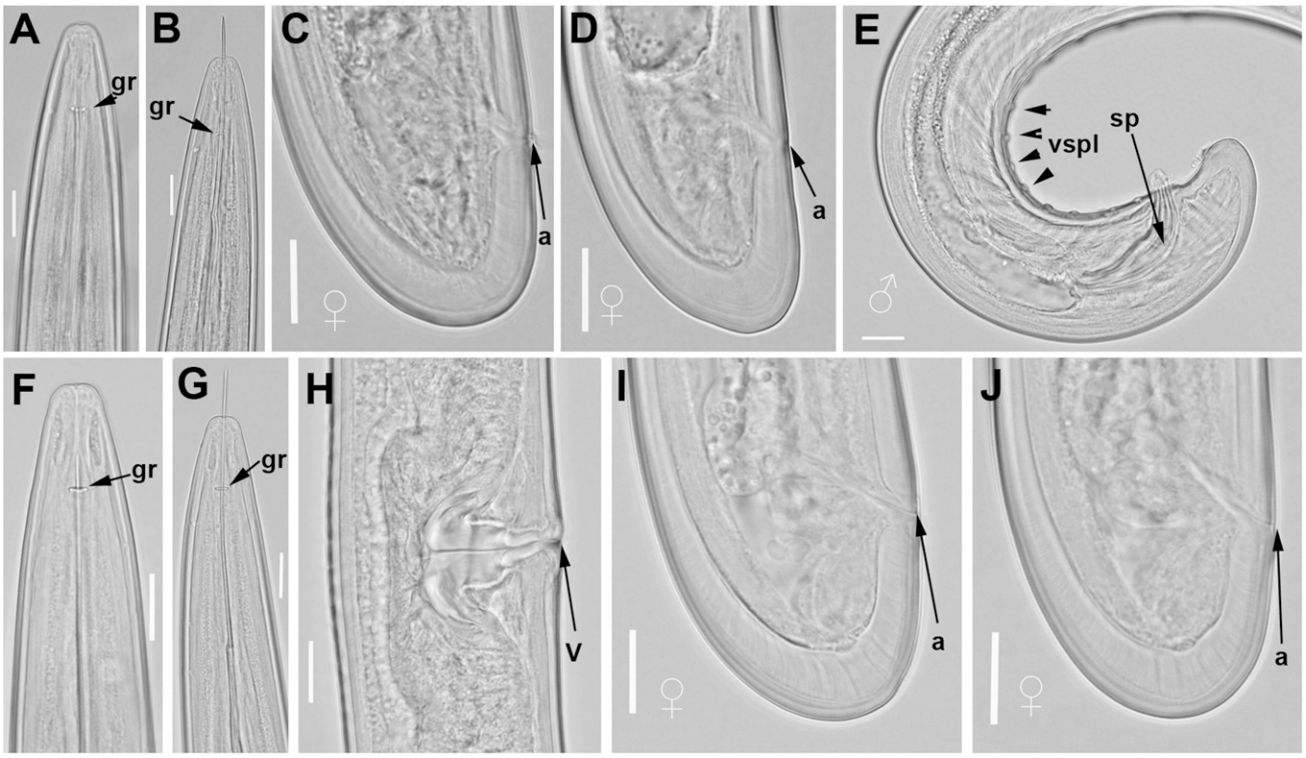

FIG. 1. Light micrographs of *Longidorus caespiticola* Hooper, 1961 (A–E) and *Longidorus poessneckensis* Altherr, 1974 (F–J). A, B, F, G. Female anterior regions. C, D, I, J. Female tail regions. E. Male tail region. H. Details of vagina. Abbreviations: a = anus, gr = guiding ring, sp = spicules, v = vulva, vspl = ventromedian supplements. (Scale bars: A–J = 20 μm).

rounded and continuous with the neck contour. Amphidial pouch elongate, funnel shaped (not lobed). Odontostyle of a medium length and not heavily sclerotized. Vulva located slightly posterior to midbody (52%–56%). Vagina 18.5 (15.0–23.0) μm wide, 41.0 (36.0–47.0) μm long, or 56.4 (49.3–64.3) % of corresponding body width. Genital tract amphidelphic, anterior and posterior genital branches equally developed, 600–679 μm and 589–770 μm long, respectively. Prerectum 411.5 (367–444) μm long, rectum 35.2 (31–41) μm long, or as long as 2/3 of anal body width. Tail dorsally convex hemispherical to bluntly conoid with a bluntly rounded terminus. Male common as female. Mail tail similar to that of the female but more curved. Spicules robust, ventrally curved, and 76–91 μm long. Guiding pieces 17–21 μm long, slightly curved with distinctly bifid distal end. The adanal pair of supplements is preceded by a row of 12–14 ventromedian supplements. According to the polytomous key Chen et al. (1997) and the supplement by Loof and Chen (1999), the Ukrainian population has the following codes (codes in parentheses are exceptions): A3 - B2 - C3 - D1 - E4 - F3(4) - G21 - H1 - I2.

Remarks

The Ukrainian population of *L. caespiticola* was collected from the rhizosphere of *Malus domestica* Borkh. on the bank of Latorytsia river, Zakarpattia region (N 48°26′45.46″; E 22°13′7.65″). Up to our knowledge, this is the first report of this species from Ukraine. Morphometrics of Ukrainian population agree with those of the type population of this species and other previously described populations (Table 1).

The main differences between Ukrainian population of *L. caespiticola* and other populations of this species are slightly shorter odontostyle (♀: 93.9 (89–97) *vs* 110 (109–114) μm (British) (Hooper, 1961); 102.5 (96.1–109.3) μm (Slovenian) (Sirka and Urek, 2009); 96 (90–104) μm (Czech) (Kumari, 2014); ♂: 95.5 (89–101) *vs* 105 (99–111) μm (British); 103.8 (94.5–108.7) μm (Slovenian); 98 (93–103) μm (Czech)); and lower number of supplements (9–13 *vs* 15–20 (British); 12–18 (Czech)).

From type British population (Hooper, 1961) it also differs by the lip region more flattened anteriorly, higher a ratio ♀: 82.4 (74.3–90.3) *vs* 72 (64–80) and ♂: 80.6 (66.8–95.2) *vs* 73 (64–83), higher c ratio ♀: 153.3 (131.8–195.7) *vs* 103 (83–125) and ♂: 139.1 (113.9–171.7) *vs* 104 (76–119). Juveniles of all four developmental stages are very similar to those from type British population. The only differences are shorter odontostyle in J4 and shorter replacement odontostyle in J4 and J3.

Ukrainian specimens of *L. caespiticola* in comparison to Slovenian (Sirka and Urek, 2009) have shorter body length (♀: 6.03 (5.22–7.39) *vs* 7.84 (7.07–8.64) mm; ♂: 5.95 (5.61–6.52) *vs* 7.24 (6.24–8.46) μm) and more slender body; lower a ratio ♀: 82.4 (74.3–90.3) *vs* 96.0 (89.4–103.6); ♂: 80.6 (66.8–95.2) *vs* 101.0 (90.4–109.8) and c ratio ♀: 153.3 (131.8–195.7) *vs* 176.0 (159.5–205.2); ♂: 139.1 (113.9–171.7) *vs* 152.2 (131.6–195.0); more posterior vulva position (V: 53.7% (51.5%–56.1%) *vs* 49.8%

TABLE 1. Morphometrics of *Longidorus caespiticola* Hooper, 1961 from Uzhhorod, Ukraine.

Character[a]	Female	Male	J1	J2	J3	J4
n	12	13	11	13	15	17
L	6.03 ± 0.65 (5.22–7.39)	5.95 ± 0.27 (5.61–6.52)	1.37 ± 0.33 (1.24–1.69)	2.12 ± 0.18 (1.82–2.50)	2.99 ± 0.42 (2.48–3.65)	4.52 ± 0.45 (3.87–5.23)
a	82.4 ± 5.83 (74.3–90.3)	80.6 ± 7.88 (66.8–95.2)	53.0 ± 5.43 (42.7–65.2)	60.5 ± 4.78 (53.5–70.4)	66.1 ± 4.11 (59.1–74.1)	76.3 ± 5.93 (65.8–86.9)
b	13.0 ± 1.12 (11.4–14.8)	12.8 ± 1.00 (10.9–14.4)	4.6 ± 0.67 (3.5–5.6)	6.4 ± 0.71 (5.3–7.7)	7.9 ± 1.34 (6.1–10.7)	11.1 ± 0.68 (9.1–14.8)
c	154.3 ± 17.97 (131.8–195.7)	139.1 ± 16.41 (113.9–171.7)	47.0 ± 3.50 (44.1–56.4)	66.5 ± 10.40 (55.2–92.7)	84.6 ± 5.55 (76.5–96.1)	120.5 ± 15.17 (101.3–149.3)
c′	0.76 ± 0.05 (0.68–0.87)	0.87 ± 0.05 (0.78–0.94)	1.69 ± 0.20 (1.30–2.06)	1.24 ± 0.16 (0.84–1.42)	1.04 ± 0.08 (0.93–1.18)	0.86 ± 0.09(0.68–1.00)
d[b]	2.5 ± 0.15 (2.2–2.7)	2.5 ± 0.29 (2.1–2.9)	2.7 ± 0.21 (2.4–3.0)	2.6 ± 0.29 (2.2–3.1)	2.5 ± 0.18 (2.3–2.8)	2.5 ± 0.21 (2.2–2.9)
d′[c]	2.2 ± 0.12 (2.0–2.4)	2.2 ± 0.18 (1.9–2.5)	1.9 ± 0.18 (1.6–2.2)	2.0 ± 0.16 (1.8–2.3)	2.1 ± 0.14 (1.8–2.3)	2.1 ± 0.12 (1.9–2.3)
V/Spicules length	53.7 ± 1.49 (51.5–56.1)	83.4 ± 4.1 (76–91)	–	–	–	–
Odontostylet length	93.9 ± 2.5 (89–97)	95.5 ± 3.8 (89–101)	56.5 ± 2.5 (52–60)	60.3 ± 1.9 (57–64)	70.1 ± 2.0 (67–74)	81.9 ± 3.3 (77–90)
Odontophore length	70.6 ± 3.4 (66–74)	70.8 ± 3.0 (65–75)	–	47.7 ± 5.1 (39–59)	57.6 ± 5.2 (45–65)	63.9 ± 3.6 (58–72)
Total stylet length	164.9 ± 3.3 (161–171)	166.5 ± 2.5 (164–170)	–	108.4 ± 4.2 (103–118)	127.7 ± 5.9 (117–139)	145.6 ± 4.5 (138–154)
Replacement odontostylet length	–	–	63.0 ± 5.1 (55–75)	72.3 ± 3.5 (65–76)	86.4 ± 3.1 (80–91)	95.7 ± 4.8 (88–105)
Anterior end to guide ring	35.3 ± 2.3 (31–39)	35.5 ± 2.7 (31–40)	19.1 ± 1.2 (17–21)	22.0 ± 1.3 (20–24)	25.6 ± 1.5 (23–28)	30.1 ± 1.8 (27–34)
Pharyngeal bulb length	128.2 ± 6.1 (115–134)	126.2 ± 6.9 (111–140)	73.0 ± 6.4 (64–82)	86.4 ± 4.3 (81–97)	99.1 ± 5.6 (91–107)	117.6 ± 8.2 (98–130)
Pharyngeal bulb width	26.4 ± 2.1 (23–30)	24.8 ± 1.9 (21–28)	12.8 ± 1.7 (11–16)	16.5 ± 1.1 (15–18)	19.5 ± 1.6 (16–22)	22.7 ± 1.4 (20–26)
Tail length	39.2 ± 2.5 (35–42)	43.2 ± 3.7 (38–51)	29.2 ± 1.9 (26–33)	32.2 ± 3.0 (27–36)	36.4 ± 2.8 (31–40)	37.7 ± 3.0 (33–44)
Hyaline part of tail length	14.0 ± 1.5 (11–16)	12.7 ± 1.2 (11–14)	5.5 ± 0.7 (5–7)	7.0 ± 1.4 (4–10)	9.0 ± 1.2 (7–11)	10.5 ± 0.7 (9–12)
Width at level of:						
Lip region	14.0 ± 0.4 (13–15)	14.1 ± 1.0 (13–16)	7.1 ± 0.7 (6–8)	8.4 ± 0.8 (7–10)	10.3 ± 0.5 (10–11)	11.9 ± 0.6 (11–13)
Guide ring	30.8 ± 2.0 (28–34)	31.0 ± 1.2 (30–33)	13.5 ± 0.7 (13–15)	17.0 ± 1.3 (16–21)	21.1 ± 1.1 (19–23)	25.3 ± 1.2 (23–28)
Base of pharynx	59.0 ± 3.4 (55–67)	58.2 ± 1.7 (55–60)	25.2 ± 1.7 (22–29)	33.3 ± 2.1 (29–36)	41.7 ± 3.7 (35–48)	50.8 ± 2.5 (46–56)
Vulva or midbody	73.1 ± 5.0 (67–83)	68.4 ± 6.2 (58–78)	26.0 ± 2.4 (22–31)	35.2 ± 2.5 (29–38)	45.2 ± 5.8 (38–58)	59.3 ± 4.5 (53–70)
Anus	51.7 ± 3.2 (46–57)	49.7 ± 2.8 (45–54)	17.5 ± 2.1 (16–23)	26.2 ± 2.4 (23–32)	35.2 ± 3.5 (31–41)	44.4 ± 3.7 (37–51)

[a] Abbreviations are defined in Jairajpuri and Ahmad (1992).
[b] *d* = anterior to guide ring / body width at lip region (Brown et al., 1994).
[c] *d′* = body width at guide ring / body width at lip region (Brown et al., 1994).

(46.2%–51.9%)); shorter tail ♀: 39.2 (35–42) *vs* 44.6 (39.3–50.3); ♂: 43.2 (38–51) *vs* 48.0 (39.2–56.1) μm and lower c′ ratio in male 0.87 (0.78–0.94) *vs* 1.00 (0.80–1.20).

Finally, it differs from Czech population (Kumari, 2014) in a ratio higher in female (82.4 (74.3–90.3) *vs* 70.1 (63.1–78.3)) but lower in male (80.6 (66.8–95.2) *vs* 87.0 (76.4–100.0) and more slender body at the level of midbody (diam. ♀: 73.1 (67–83) *vs* 92 (83–111); ♂: 68.4 (58–78) *vs* 73 (65–88) μm) and anus (diam. ♀: 51.7 (46–57) *vs* 62 (55–69); 49.7 (45–54) *vs* 55 (52–61) μm).

Consequently, our morphometrics were coincident with previous records, and minor differences detected may be due to the amount of specimens originally studied in those populations or geographical intraspecific variability of them (Archidona-Yuste et al., 2016).

Longidorus poessneckensis Altherr, 1974
(Fig. 1)

Measurements

See Table 2.

Description

Body open C to spiral in shape when killed by gentle heat. Lip region 6 μm high, continuous with the rest of the body. Cuticle with fine transverse cuticular striations present along the entire body. Amphidial fovea pouch-like. Odontostyle long and not heavily sclerotized. Vulva transverse, about median in position (49%–56%). No sperm observed in genital tract. Vagina 20.5 (18.0–23.0) μm wide, 45.1 (40.0–49.0) μm long, occupies more than half of the corresponding body width (63.7% (57.9%–71.0%)). Genital tract amphidelphic, anterior and posterior genital branches equally developed 669 (534–847) μm and 631 (457–757) μm long, respectively. Ovaries equally developed 239 (139–353) μm and 229 (132–340) μm long. Prerectum variable in length 301–525 μm long, rectum 32.7 (28–36) μm or almost as long as 2/3 of anal body width. Tail almost hemispherical to bluntly conoid and shorter than anal body width. Two caudal pores present. According to the polytomous key Chen et al. (1997) and the supplement by Loof and Chen (1999), the Ukrainian populations have the following codes (codes in parentheses are exceptions): A5(4) - B2 - C3(24) - D1 - E1 F3(4) - G2 - H1 - I1.

Remarks

Longidorus poessneckensis populations were collected from the rhizosphere of *Quercus robur* L. in Uzhhorod, Zakarpattia region (N 48°38′39.54″; E 22°18′11.78″), and the rhizosphere of *Populus* sp. in Lviv (N 49°49′30.59″; 24°01′34.78″). Up to our knowledge, this is the first report of this species from Ukraine. Habitats of Ukrainian populations differ from the previously reported populations because this species has been reported from moist soil in riparian forests, river meadows, and on the banks of rivers and springs.

The Ukrainian populations of *L. poessneckensis* morphologically and morphometrically are very similar to each other (Table 2). In comparison to Uzhhorod ones, specimens from Lviv population have longer body 7.62 (6.59–8.70) *vs* 6.55 (5.59–7.54) mm, more posterior position of guiding ring 40.0 (39.0–42.0) *vs* 34.0 (30.0–38.0) μm, higher a ratio 100.0 (94.1–108.8) *vs* 92.4 (82.7–103.8) and c ratio 215.3 (173.3–255.9) *vs* 163.6 (130.2–198.5), and lower c′ ratio 0.65 (0.60–0.72) *vs* 0.73 (0.63–0.94).

Population of *L. poessneckensis* from Uzhhorod (21 females) are the most similar to Czech (Kumari et al., 2009) and Austrian (Tiefenbrunner and Tiefenbrunner, 2004) populations. The main difference is a smaller distance from anterior end to guiding ring 34.2 (30.0–38.0) *vs* 37.0 (32.0–41.0) (Czech) and 38.0 (33.0–41.0) μm (Austrian) (Tiefenbrunner and Tiefenbrunner, 2004; Kumari et al., 2009). From Czech population, it also differs by a slightly higher c′ ratio 0.73 (0.63–0.94) *vs* 0.67 (0.53–0.83) and from Austrian once by a slender body (a ratio 92.4 (82.7–103.8) *vs* 81.87 (68.34–97.26); diameter at vulval level 70 (65–81) *vs* 80 (66–104) μm).

In comparison to type population (Altherr, 1974) and other German populations described by Sturhan and Loof (2001) females from Uzhhorod have shorter body length (6.55 (5.59–7.54) *vs* 8.5–8.9; 8.00 (6.00–9.36); 7.30 (7.15–8.30) mm) and slightly more robust body (a ratio 92.4 (82.7–103.8) *vs* 95–120; 104 (91–124); 110 (92–131)) and more anterior position of guiding ring 34.2 (30–38) *vs* 37–40; 40 (36–43); 39 (37–40) μm.

Uzhhorod population also differs from Polish one (Kornobis and Peneva, 2011) by shorter body 6.55 (5.59–7.54) *vs* 7.93 (6.67–9.74); 7.48 (6.38–8.3); 7.77 (7.01–8.82) mm, lower a ratio 92.4 (82.7–103.8) *vs* 104.3 (93.9–119.5); 97.8 (84–106.1); 97.0 (86.9–114.5) and c ratio 163.6 (130.2–198.5) *vs* 215.8 (179.1–256.1); 180.7 (153.9–237.2); 185.8 (164.2–220.4); slightly shorter odontostyle as in all previously reported populations of this species (129.1 (118–135) *vs* 143.7 (137–151); 142.8 (135–151); 139.0 (136–145)) μm and also a more anterior position of the guiding ring 34.0 (30.0–38.0) *vs* 40.0 (37.0–42.0); 41.5 (36.0–45.0); 36.5 (34.0–39.0) μm (Altherr, 1974; Sturhan and Loof, 2001; Tiefenbrunner and Tiefenbrunner, 2004).

Lviv population (three females) was closer to type population and other German and Polish populations. These specimens have longer body and more typical position of the guiding ring. This population differs from German population described by Sturhan and Loof (2001) by a higher c ratio 215.3 (173.3–255.9) *vs* 104 (91–124) and shorter tail 36.0 (34.0–38.0) *vs* 45.0 (37.0–54.0) μm and from Polish ones by shorter odontostyle 135.0 (133.0–137.0) *vs* 144.0 (137.0–151.0); 143.0 (135.0–151.0); 139.0 (136.0–145.0) μm.

Juveniles of the third and fourth developmental stages of the population from Uzhhorod retain the same differences from the specimens of German and Polish populations as the adult females: shorter body and odontostyle, more anterior position of guiding ring, and shorter

TABLE 2. Morphometrics of *Longidorus poessneckensis* Altherr, 1974 from Ukraine.

Locality	Lviv	Uzhhorod				
Character[a]	Females	Females	J1	J2	J3	J4
n	3	21	10	12	8	8
L	7.62 (6.59–8.70)	6.55 ± 0.57 (5.59–7.54)	1.50 ± 0.82 (1.34–1.61)	2.23 ± 0.12 (2.06–2.47)	3.00 ± 0.27 (2.55–3.29)	4.88 ± 0.56 (4.17–5.70)
a	100.0 (94.1–108.8)	92.4 ± 4.51 (82.7—103.8)	54.8 ± 1.88 (51.3–57.1)	64.1 ± 2.35 (59.1–66.6)	71.2 ± 6.29 (56.6–76.7)	90.7 ± 5.12 (85.1–98.4)
b	13.1 (11.5–15.9)	12.1 ± 1.18 (9.3–13.9)	5.1 ± 0.63 (3.7–6.0)	6.3 ± 0.38 (5.7–6.7)	7.6 ± 0.97 (6.1–8.8)	11.1 ± 1.27 (9.4–13.1)
c	215.3 (173.3–255.9)	163.6 ± 17.6 (130.2–198.5)	41.9 ± 2.3 (38.7–45.3)	57.8 ± 4.12 (50.6–64.1)	79.4 ± 12.20 (62.1–99.9)	118.4 ± 9.79 (101.7–130.4)
c′	0.65 (0.60–0.72)	0.73 ± 0.08 (0.63–0.94)	1.74 ± 0.07 (1.62–1.83)	1.29 ± 0.10 (1.13–1.48)	0.99 ± 0.10 (0.83–1.14)	0.81 ± 0.05 (0.73–0.89)
d[b]	2.4 (2.3–2.6)	2.3 ± 0.19 (1.9–2.7)	2.6 ± 0.22 (2.2–2.9)	2.4 ± 0.14 (2.1–2.6)	2.4 ± 0.19 (2.0–2.6)	2.2 ± 0.20 (1.9–2.6)
d′[c]	2	2.0 ± 0.17 (1.6–2.3)	1.9 ± 0.10 (1.8–2.1)	1.8 ± 0.13 (1.6–2)	1.8 ± 0.09 (1.7–2.0)	1.8 ± 0.09 (1.6–1.9)
V	52.9 (52.7–53.0)	53.4 ± 1.93 (48.8–56.3)	–	–	–	–
Odontostylet length	134.7 (133–137)	129.1 ± 4.4 (118–135)	76.5 ± 2.7 (73–81)	80.0 ± 3.3 (76–88)	93.9 ± 3.8 (89–101)	111.3 ± 3.8 (108–119)
Odontophore length	77.3 (65–85)	78.2 ± 3.7 (70–88)	48.9 ± 5.0 (44–60)	54.7 ± 3.9 (48–60)	61.0 ± 6.1 (52–68)	63.6 ± 5.0 (58–61)
Total stylet length	216 (198–222)	207.3 ± 5.6 (195–216)	125.7 ± 6.5 (120–140)	134.7 ± 6.1 (127–148)	154.9 ± 9.4 (142–169)	174.9 ± 8.2 (166–189)
Replacement odontostylet length	–	–	81.3 ± 3.2 (74–86)	96.1 ± 3.3 (92–102)	107.9 ± 6.3 (98–115)	124.1 ± 5.1 (114–130)
Anterior end to guide ring	40.3 (39–42)	34.2 ± 1.9 (30–38)	20.1 ± 0.3 (20–21)	23.3 ± 1.2 (21–25)	25.9 ± 1.4 (24–27)	30.1 ± 1.5 (27–31)
Pharyngeal bulb length	171.3 (166–180)	144.1 ± 10.6 (127–169)	84.1 ± 6.2 (76–99)	98.3 ± 4.9 (93–107)	111.5 ± 3.4 (108–119)	130.3 ± 5.1 (124–136)
Pharyngeal bulb width	27 (26–28)	25.2 ± 1.1 (23–27)	13.9 ± 0.7 (13–15)	17.0 ± 0.9 (16–18)	19.3 ± 1.0 (18–21)	22.8 ± 1.4 (21–25)
Tail length	35.7 (34–38)	40.2 ± 3.3 (35–47)	35.9 ± 1.9 (33–39)	38.8 ± 2.7 (34–43)	38.3 ± 4.0 (30–42)	41.3 ± 1.2 (36–47)
Hyaline part of tail length	15 (14–17)	14.0 ± 1.7 (11–16)	6.3 ± 0.8 (5–8)	8.9 ± 0.9 (8–11)	10.1 ± 1.6 (8–12)	11.4 ± 1.6 (9–13)
Width at level of:						
Lip region	16.7 (16–17)	15.1 ± 0.9 (14–17)	7.9 ± 0.7 (7–9)	9.9 ± 0.7 (9–11)	11.0 ± 0.5 (10–12)	13.5 ± 0.9 (12–15)
Guide ring	33.3 (32–34)	29.7 ± 1.9 (26–34)	15.1 ± 0.9 (14–17)	17.9 ± 0.9 (17–20)	20.1 ± 0.6 (19–21)	24.6 ± 1.6 (23–27)
Base of pharynx	63 (59–66)	58.4 ± 3.7 (52–65)	26.9 ± 1.0 (26–29)	34.2 ± 1.5 (32–36)	40.4 ± 1.9 (38–43)	50.4 ± 3.1 (46–54)
Vulva or mid-body	76 (70–80)	70.9 ± 4.4 (65–81)	27.4 ± 1.1 (26–29)	34.8 ± 1.7 (32–37)	42.1 ± 2.5 (38–45)	53.8 ± 3.8 (49–58)
Anus	55.3 (53–57)	55.0 ± 4.1 (49–62)	20.6 ± 1.3 (18–22)	30.2 ± 1.6 (29–34)	38.8 ± 2.4 (36–42)	50.8 ± 3.0 (46–55)

[a] Abbreviations are defined in Jairajpuri and Ahmad (1992).
[b] *d* = anterior to guide ring / body width at lip region (Brown et al., 1994).
[c] *d′* = body width at guide ring / body width at lip region (Brown et al., 1994).

replacement odontostyle. Our juveniles also differ from Polish ones by shorter tail in all four developmental stages. In comparison to Czech population, specimens from Uzhhorod have not got any substantial differences.

Molecular characterization of Longidorus spp. from Ukraine: The 785 and 774 bp sequences (MF716960-MF716961) of the 28S D2-D3 for *L. caespiticola* from the rhizosphere of *Malus domestica* Borkh. on the bank of river Latorytsia, Zakarpattia region, were identical. The BLASTsearch of 28S D2-D3 for *L. caespiticola* from Ukraine was 97%–99% similar to other *L. caespiticola* populations deposited in GenBank from Belgium, Bulgaria, Czech Republic, Russia, Slovenia, and Scotland (KJ567472-KJ567473, AF480079-AF480081, KF243344, HM447030, and AY601567), differing in 9–21 bp and 7–9 indels (Rubtsova et al., 2001; He et al., 2005; Sirka and Urek, 2009; Peneva et al., 2013; Kumari, 2014; Subbotin et al., 2014). D2–D3 sequences for *L. poessneckensis* from Lviv and Uzhhorod (MF716962-MF716963) matched well, 99% similar with former sequences from Slovakia and Czech Republic deposited in GenBank (EF538750, EF538751), differing in 3–5 bp and 1–3 indels (Kumari et al., 2009). *Longidorus poessneckensis* from Ukraine showed a high homogeneity for the D2–D3 region (99% similarity, 4 nucleotides and 0 indel) in two sampled populations.

Consequently, the morphology, morphometry, and molecular data obtained from these samples were consistent with *L. caespiticola* and *L. poessneckensis* identification. These reports confirm the occurrence of these two nematodes in Ukraine and extend the geographical distribution of these species. In this sense, the new population of *L. caespiticola* confirms its wide distribution in Europe where it can be found infesting soils from a wide range of ecosystems, both natural and agricultural systems, as well as in a high degree of host plants including crops and forests (Hopper, 1961; Brown and Taylor, 1987; Sirka and Urek, 2009; Kumari, 2014). On the contrary, from the original description of *L. poessneckensis* to date, it has been recorded in several countries always located in the middle part of Europe; however, the new populations from Ukraine make wider the European distribution of this species, extending from Central to Eastern Europe (Altherr, 1974; Tiefenbrunner and Tiefenbrunner, 2004; Kumari et al., 2009; Kornobis and Peneva, 2011).

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