

Original paper

New records of *Longidorus piceicola* Lišková, Robbins & Brown, 1997 (Nematoda: Longidoridae) from Serbia

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Summary. Two populations of *Longidorus piceicola* of different geographical origin from Serbia are reported. Their morphometrics, morphological data and illustrations are presented. Analysis based on published and original data shows that *L. piceicola* as a species exhibits variability through its geographical distribution, which is manifested in differences in body-, odontostyle- and replacement odontostyle lengths, with certain overlapping values between developmental stages in populations of different geographical origin.

Keywords: growth, intraspecific variability, juvenile developmental stages, morphology, morphometrics.

INTRODUCTION

Longidorus piceicola was described from the rhizosphere of *Picea abies* (L.) Karst (Norway spruce) growing in the Muranska Planina Mountains near the village Cervena Skala, Slovakia (Lišková et al. 1997). This species is already known to occur in Serbia (Barsi and Lamberti 2001). Soil samples collected in June 2002 from the rhizosphere of *Picea excelsa* (Lam.) Lk. at Hajdučka česma (43°53'19.09"N, 19°31'40.95"E), Tara Mountain, and in July 2003 from the same host at Kadijevac, loc. Hajdučke vode (43°19'23.22"N, 20°45'42.80"E), Kopaonik Mountain, revealed the presence of populations of *L. piceicola*.

The aim of the present study was to extend knowledge about the geographic distribution, morphology, and intraspecific variability of *L. piceicola*, and to provide detailed illustrations of adult and juvenile developmental stages.

MATERIAL AND METHODS

Nematodes were extracted from soil samples by Cobb's wet sieving technique. Specimens were killed by hot FP 4-1 and transferred to glycerin by a slow evaporation method and mounted on permanent microscope slides. Measurements were made with an ocular micrometer, with the exception of body, pharynx and tail lengths for all developmental stages, or replacement odontostyle length for juvenile stages. These characteristics were drawn using a drawing tube on an Olympus CX31 microscope at the appropriate magnification. Drawings were scanned and measurements were taken from the scanned drawings using Digimizer Version 4.6.1 software for digital measurements. Photographs were taken using a Zeiss Axio Imager A1 compound microscope equipped with an AxioCam MRc 5 digital camera.

Terminology and location of pharyngeal gland nuclei are given according to Andrassy (1998a, 1998b). The location

of the dorsal nucleus (D) is expressed as the percentage of the distance between the anterior end of the body and the posterior end of the pharynx; the positions of the two sub-ventral nuclei (AS_1 , AS_2) are given as the percentage of the distance between the dorsal nucleus and the posterior end of the *cylindrus*. The position of AS_1 as the percentage of the D- AS_2 distance represents K.

RESULTS

Longidorus piceicola Lišková, Robbins & Brown, 1997

(Figs 1-5)

Measurements

See Table 1.

Description

Female. The body has a spiral shape, tapering gradually anteriorly. Cuticle finely transversally striated. Lip region frontally broadly rounded or almost flattened, laterally rounded. Amphidial pouch mostly indistinct, when visible it appears pocket shaped, shallowly symmetrically bilobed at the base. Odontostyle long and very slender, approximately 2-2.4 μm wide at base; odontophore and guiding ring typical of the genus. Pharynx dorylaimoid, 603 ± 34 (521-640) μm long, with basal bulb, 133 ± 3.4 (125-140) \times 26 ± 1.5 (25-31) μm , occupying 21.7 ± 1.08 (19.8-23.5)% of total length and provided with three gland nuclei. Glandularium 90 ± 4.2 (82-96) μm long or 14.9 ± 1.02 (13.8-17.5)% of total pharynx length; D = 85 ± 1.02 (82.5-86)%, $AS_1 = 25.2 \pm 6.1$ (8-35)%, $AS_2 = 31.2 \pm 3.8$ (24-37)%, K = 81.0 ± 18.2 (29-100)% (n = 16). Vulva more or less equatorial, slit like; vagina occupying slightly more than $\frac{1}{2}$ of the corresponding body diameter. Reproductive system amphidelphic with equally developed genital branches; uteri short, anterior uterus 106 ± 16 (89-126) μm long, posterior 100 ± 11.5 (88-120) μm , separated by a sphincter from the oviduct; ovaries opposed, reflexed. Sperm not observed in uteri. Pre-rectum as long as 7 to 16 times the anal body width; rectum as long as 0.6 to 1.2 the anal width. Tail dorsally convex, conoid with rounded terminus, bearing two caudal pores on each side.

Male. Not found.

Juveniles. Clearly separated into four developmental stages (Fig. 4). They resemble adults except for smaller size with some overlap in body length between the first and second juvenile stages. Their posture varies from J to open C. Tail elongate-conoid in the first stage and progressively less conoid in the second, third and fourth stages (Fig. 3).

Individual increase in the length of replacement odontostyle in relation to functional odontostyle in individuals

in four juvenile developmental stages indicate that this increase was 14.1 (9.9-17.6)% in J1, 14.1 (3.2-22)% in J2, 11 (6.9-16.4)% in J3, and 12.3 (4.5-17.7)% in J4 (Fig. 5).

DISCUSSION

According to the published data (Lišková et al. 1997; Barsi and Lamberti 2001; Kornobis and Peneva 2011; Groza et al. 2017) and new data presented in the present study, *L. piceicola* shows some differences in morphometrics between populations of different origin. These differences are reflected mainly in body length and stylet length in all developmental stages, as well as in adults, probably due to intraspecific variability and environmental conditions in which they live (Table 2 and 3).

Using the mean values of body length, odontostyle and replacement odontostyle lengths from J1-J4 stage in six populations and body length and odontostyle length in females from 14 (6 + 8) populations shows that there is a slight overlap between J1 and J2 stages as well as between J3 and stage J4 (Fig. 6). The picture becomes even more interesting when the absolute minimum and maximum values are used for the previously mentioned characteristics in the same populations (Table 2 and 3, Fig. 7). It shows that the absolute minimum values in J2, J3 and J4 stages considerably overlap with the range (minimum-maximum) of the previous stages, i.e. J2 with J1, J3 with J2 and J4 with J3. In females, the absolute minimum value of odontostyle length overlaps with the range in J4.

Based on published and original data, it can be concluded that *L. piceicola* as a species shows variability through its geographical distribution, which is manifested in differences in body-, odontostyle- and replacement odontostyle lengths with certain overlapping values between developmental stages in populations of different geographical origin.

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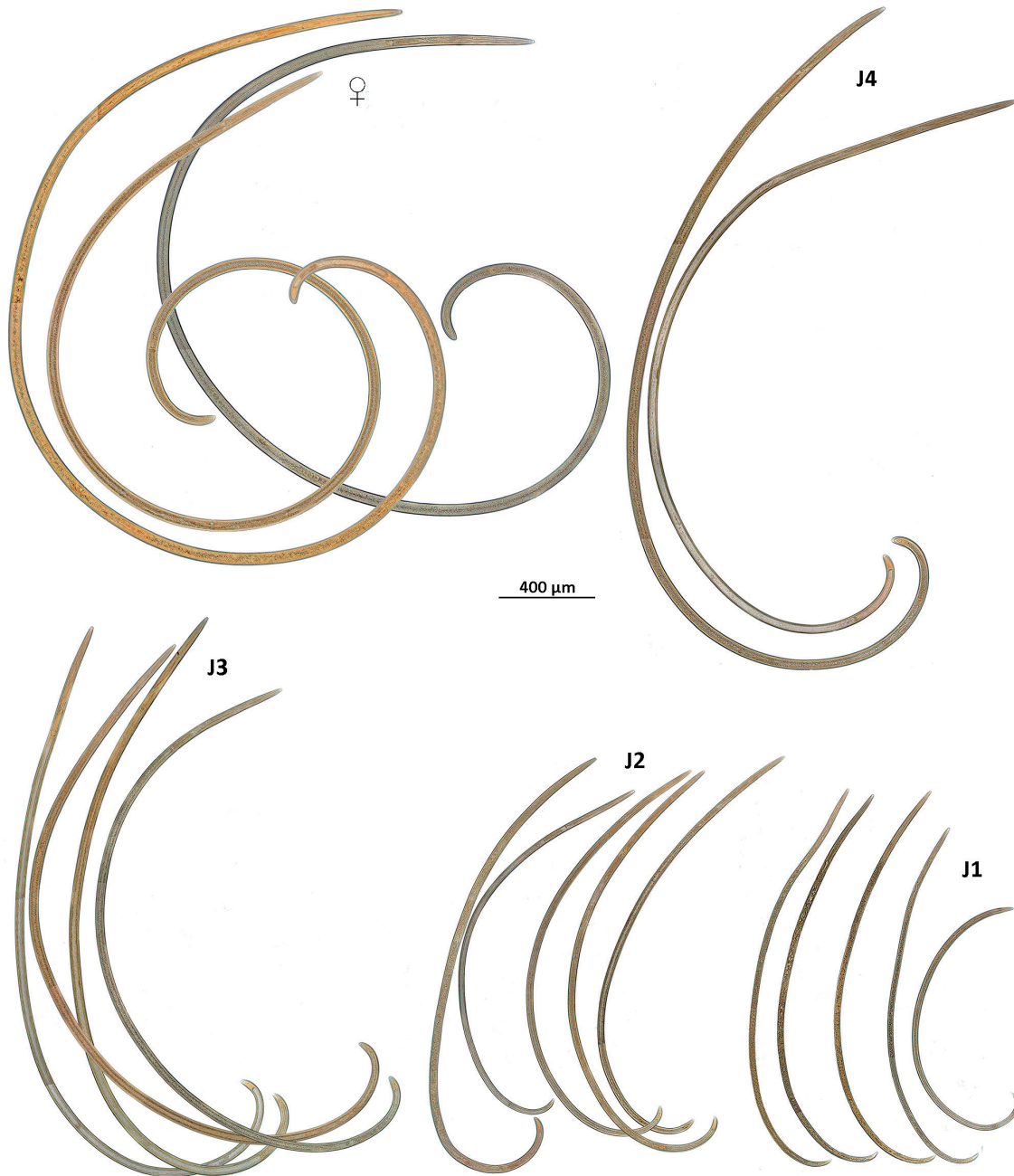


Fig. 1. *Longidorus piceicola* (Kadijevac, loc. Hajdučke vode, Kopaonik Mountain, Serbia). Entire body of female (♀) and four juvenile developmental stages (J1–J4).

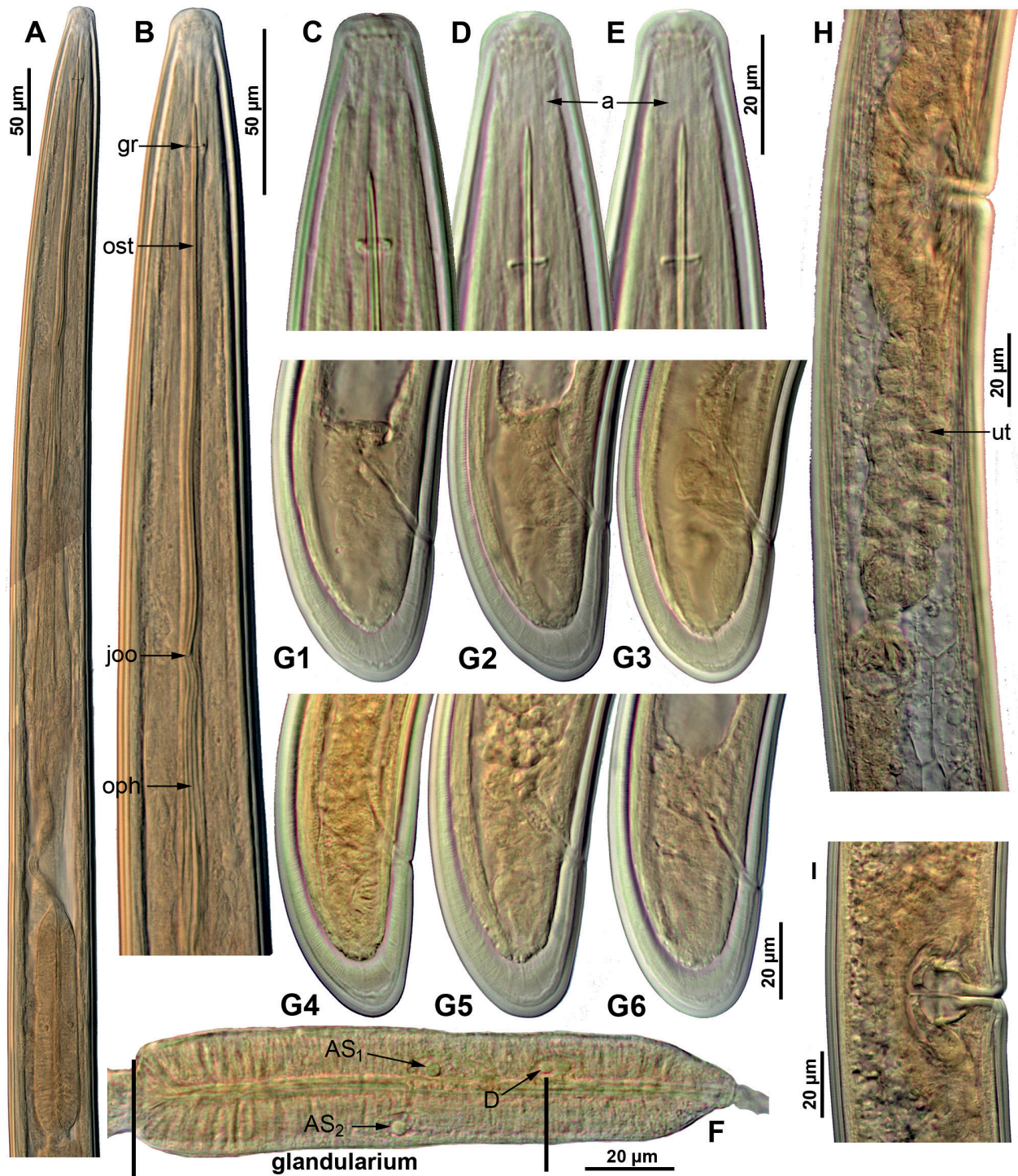


Fig. 2. *Longidorus piceicola* (Kadijevac, loc. Hajdučke vode, Kopaonik Mountain, Serbia). **A-B**, Anterior region of a female in lateral optical view (gr = guide ring; ost = odontostyle; oph = odontophore; joo = junction of the odontostyle with the odontophore); **C-D**, Female lip region; **E**, Female amphidial pouch (a); **F**, Cylindrical basal bulb (*cylindrus*) of pharynx showing nuclei of dorsal and subventral glands (D = dorsal nucleus; glandularium = distance between dorsal nucleus and posterior margin of *cylindrus* – signified by two vertical black lines; AS₁ = first anterior subventral nucleus; AS₂ = second anterior subventral nucleus); **G1-G6**, Female tail region; **H**, Vulval region and posterior uterus (ut); **I**, Vulval region. (Scale bars: A, B = 50 µm; C-E, F, G1-G6, H, I = 20 µm.)

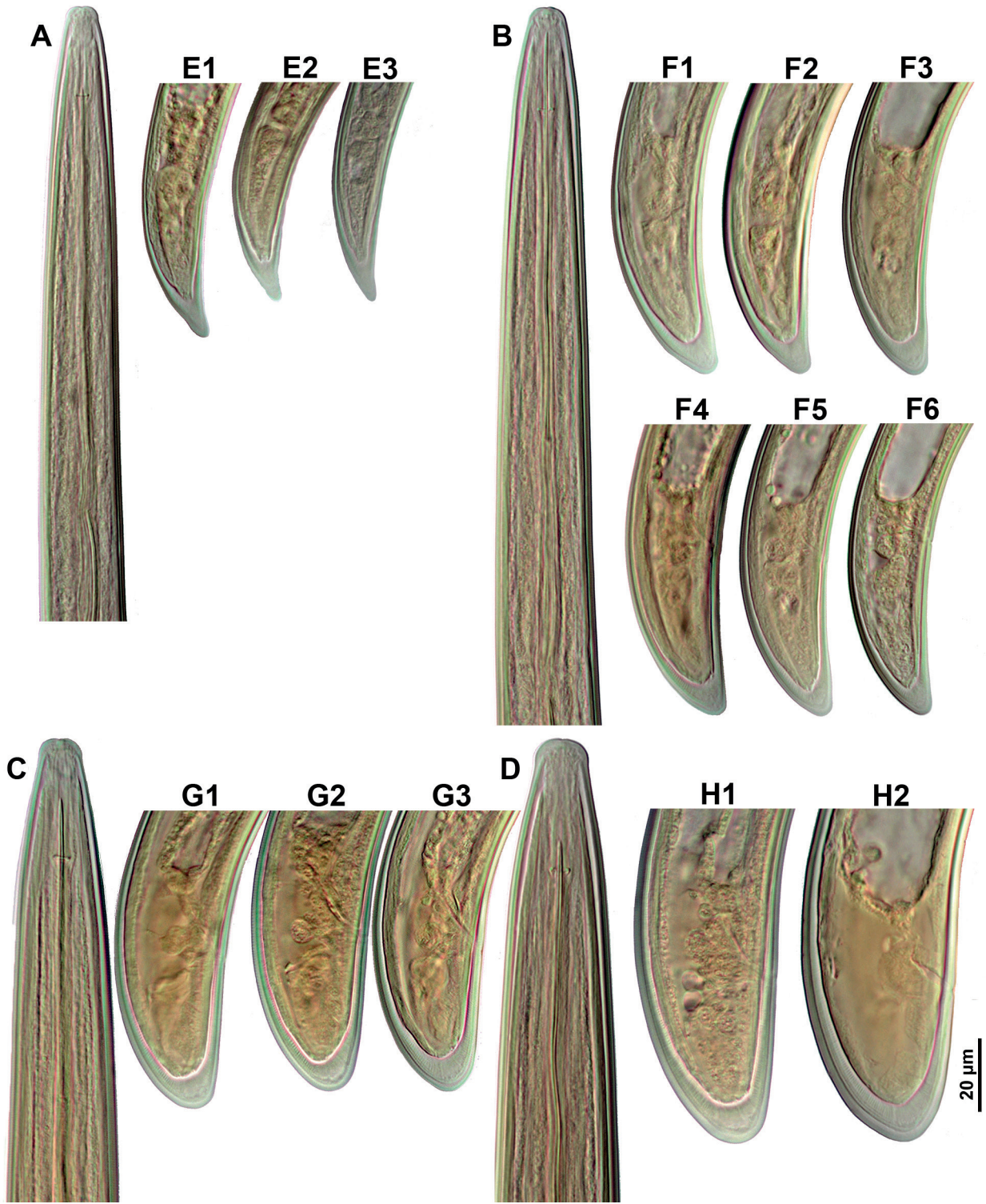


Fig. 3. *Longidorus piceicola* (Kadijevac, loc. Hajdučke vode, Kopaonik Mountain, Serbia), juvenile stages J1-J4. Anterior body region of J1 (A), J2 (B), J3 (C) and J4 (D); Tail region of J1 (E1-E3), J2 (F1-F6), J3 (G1, G2) and J4 (H1, H2). (Scale bar: 20 µm.)

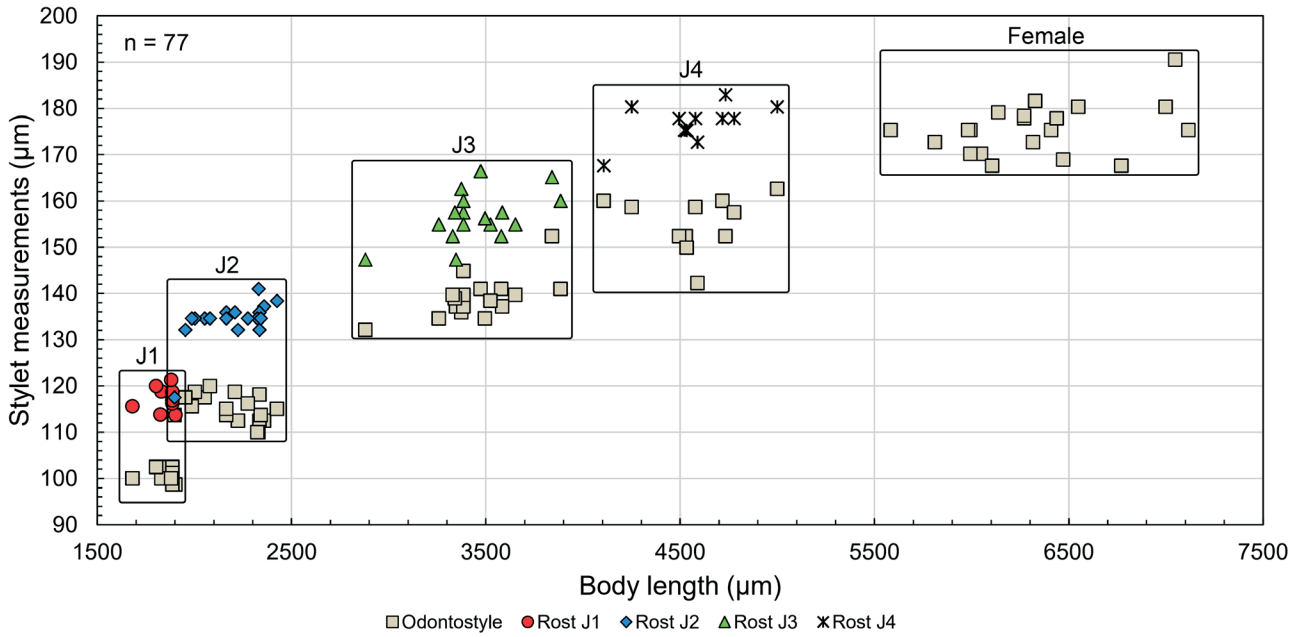


Fig. 4. Scatter diagram plotting the length of stylet measurements vs. body length of individual specimens of the four juvenile stages and adult female of *Longidorus piceicola* (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode, Serbia).

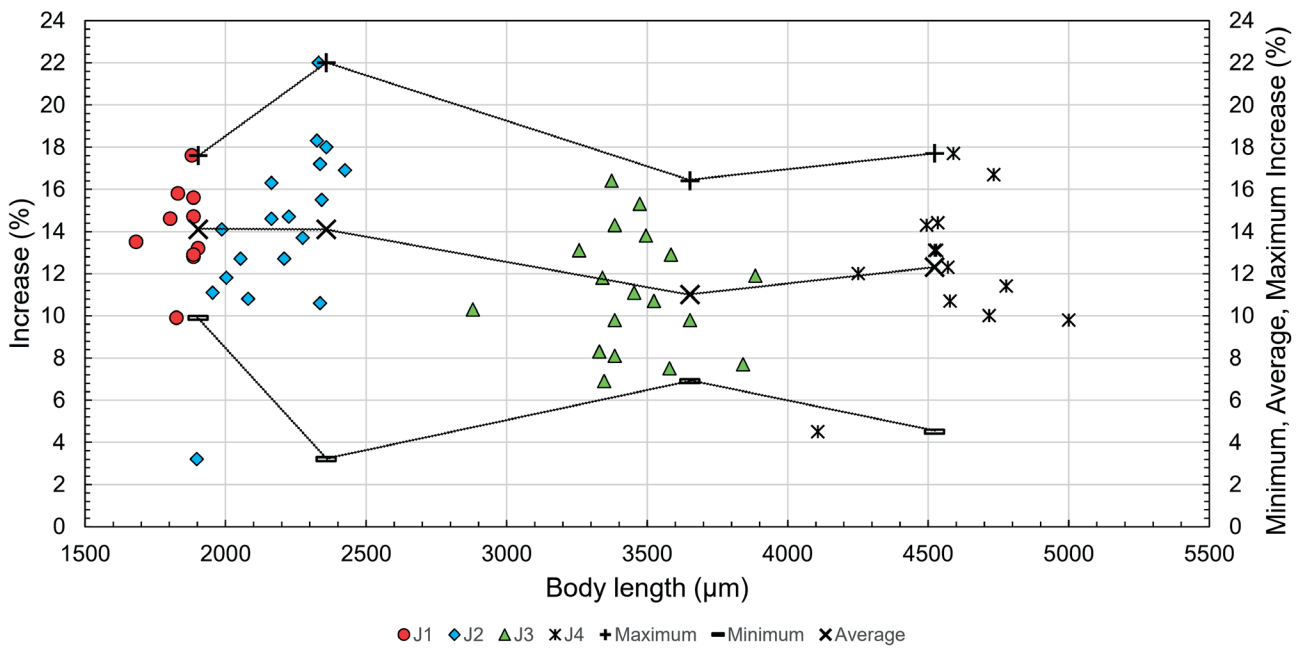


Fig. 5. *Longidorus piceicola* (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode, Serbia). Individual increase in length of replacement odontostyle in relation to functional odontostyle in individuals in 4 juvenile developmental stages; minimum, average and maximum individual increase.

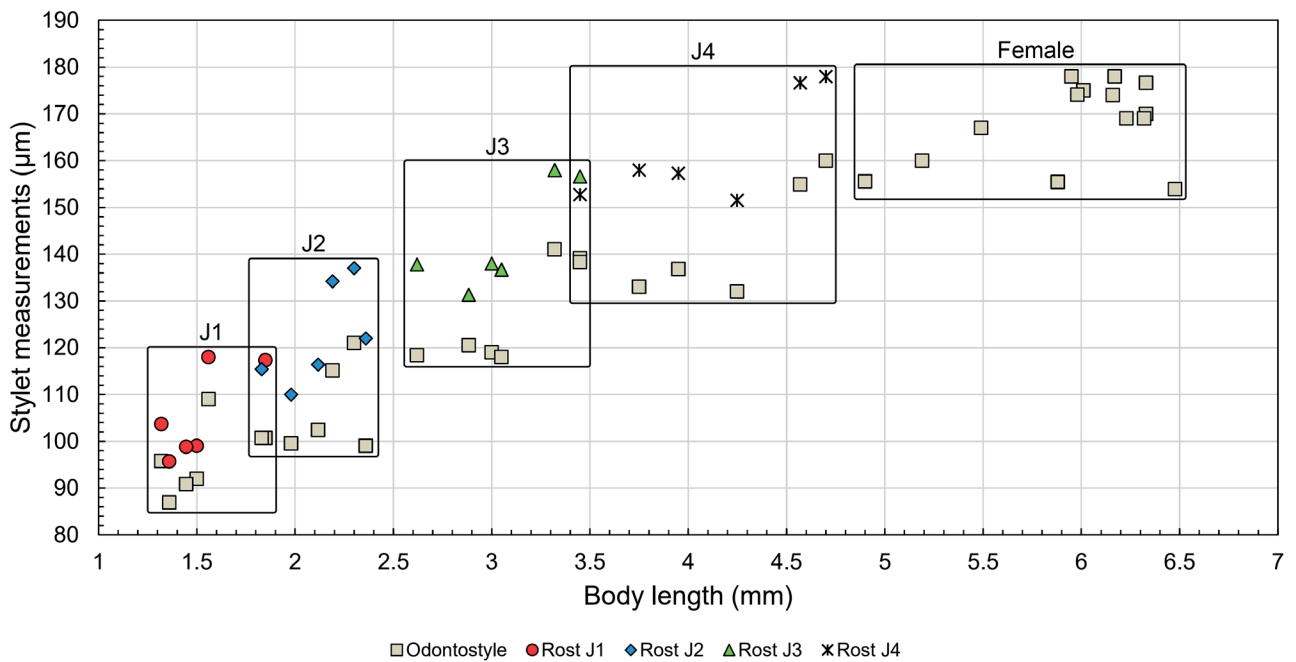


Fig. 6. *Longidorus piceicola*. Scatter diagram of average values of odontostyle length and replacement odontostyle (Rost) length in four juvenile developmental stages and females in relation to the corresponding average values of body length based on published and original data. (Sources: Lišková et al. 1997; Barsi and Lamberti 2001; Kornobis and Peneva 2011; Groza et al. 2017; Original).

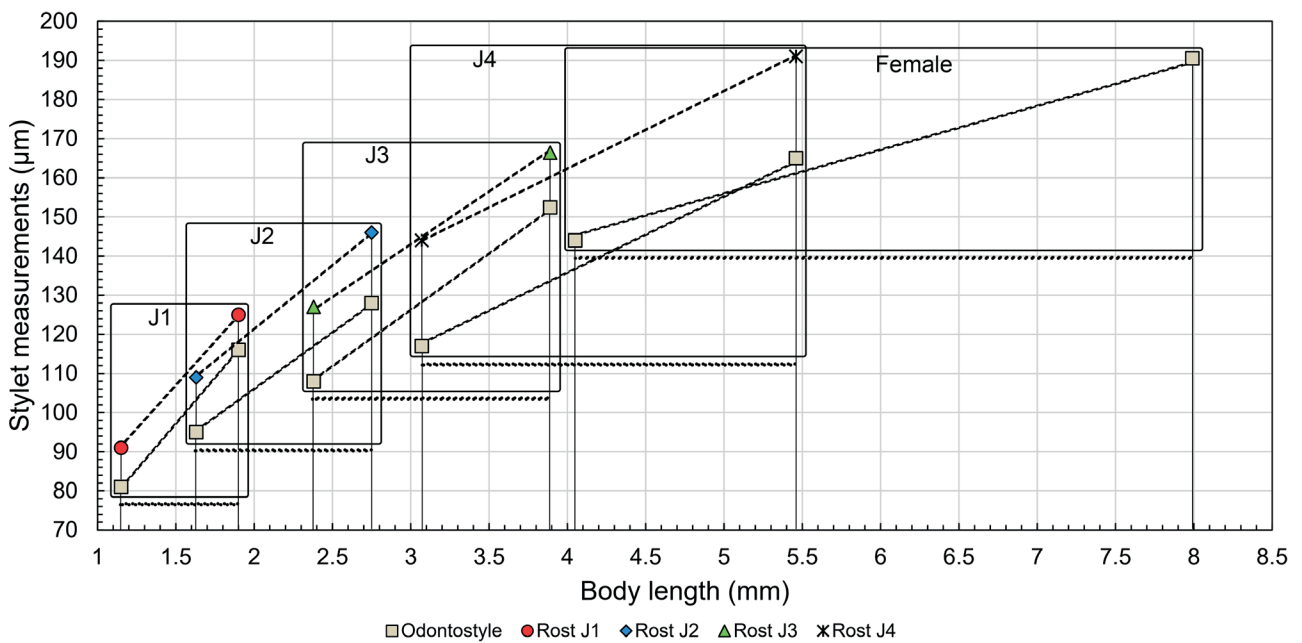


Fig. 7. *Longidorus piceicola*. Scatter diagram of absolute minimum and maximum values of odontostyle length and replacement odontostyle (Rost) length in four juvenile developmental stages and females in relation to the corresponding absolute minimum and maximum values of body length based on published and original data. (Sources: Lišková et al. 1997; Barsi and Lamberti 2001; Kornobis and Peneva 2011; Groza et al. 2017; Original).

Table 1. Morphometric characters of adults and juvenile stages of *L. piceicola* from Serbia.

Locality:	Kopaonik Mountain, Kadijevac, loc. Hajdučke vode				Tara Mountain Hajdučka česma, <i>P. excelsa</i>	
Host:	<i>Picea excelsa</i>					
N	10 J1	18 J2	17 J3	12 J4	20 females	17 females
L (mm)	1.85±0.07 (1.68-1.90)	2.19±0.16 (1.90-2.43)	3.45±0.23 (2.88-3.89)	4.57±0.23 (4.11-5.00)	6.33±0.41 (5.58-7.12)	5.98±0.48 (5.16-6.94)
a	65.4±2.39 (62.9-70.1)	72.3±3.03 (64.3-77.5)	83.7±3.71 (76.1-93.0)	97.9±3.58 (90.7-103.2)	113.0±7.53 (102.6-128.1)	103.4±5.62 (96.0-115.8)
b	5.4±0.66 (4.4-6.8)	5.0±0.47 (4.5-5.8)	6.8±0.61 (5.8-8.1)	7.9±0.56 (6.8-8.8)	10.3±0.74 (9.1-12.1)	10.1±1.40 (8.3-12.5)
c	37.1±2.62 (34.2-43.5)	46.7±3.55 (40.9-51.6)	77.1±6.71 (60.3-87.5)	103.9±9.30 (90.6-120.8)	161.8±15.14 (140.4-191.8)	145.9±15.29 (124.7-190.3)
c'	2.51±0.16 (2.14-2.76)	1.95±0.14 (1.77-2.22)	1.39±0.11 (1.27-1.68)	1.18±0.08 (1.02-1.29)	0.97±0.08 (0.85-1.17)	0.96±0.05 (0.87-1.06)
d	2.8±0.05 (2.7-2.8)	2.7±0.15 (2.3-2.9)	2.7±0.10 (2.5-2.8)	2.6±0.08 (2.5-2.8)	2.6±0.11 (2.3-2.8)	2.7±0.10 (2.5-2.8)
d'	1.8±0.03 (1.7-1.8)	1.7±0.06 (1.6-1.8)	1.8±0.05 (1.6-1.8)	1.8±0.04 (1.7-1.8)	1.8±0.05 (1.7-1.8)	1.8±0.05 (1.8-1.9)
J	1.2±0.13 (1.1-1.5)	0.7±0.05 (0.6-0.8)	0.5±0.04 (0.4-0.6)	0.5±0.04 (0.4-0.5)	0.5±0.05 (0.4-0.6)	0.4±0.03 (0.4-0.5)
V	-	-	-	-	49.6±1.44 (45.9-51.9)	46.8±0.88 (45.0-48.2)
Odontostyle µm	100.7±1.48 (98.7-102.5)	115.1±2.97 (110.0-120.0)	139.1±4.56 (132.1-152.4)	154.9±5.71 (142.2-162.6)	175.6±5.53 (167.6-190.5)	174.1±4.66 (166.4-182.9)
Odontophore µm	52.7±2.52 (48.8-56.3)	60.4±2.42 (56.3-65.0)	69.3±1.98 (66.3-73.8)	77.1±4.14 (68.8-81.3)	82.0±3.43 (76.3-88.8)	80.2±5.12 (70.0-87.5)
Total stylet µm	153.4±3.21 (148.8-157.5)	175.5±3.86 (168.8-183.7)	208.4±5.85 (200.9-226.2)	232.1±4.63 (223.5-240.0)	257.6±5.24 (248.9-273.0)	254.3±7.86 (240.2-269.1)
Replacement odontostyle µm	117.3±2.52 (113.7-121.3)	134.2±4.70 (117.5-141.0)	156.6±5.30 (147.3-166.4)	176.6±3.98 (167.6-182.9)	-	-
Oral aperture to guide ring µm	25.2±0.29 (25.0-25.6)	28.6±1.32 (25.6-30.6)	34.1±1.38 (32.5-36.9)	38.7±0.98 (36.9-40.0)	43.3±2.02 (37.5-46.3)	42.3±1.45 (40.0-45.0)
Tail µm	50.0±3.46 (42.1-53.6)	47.0±2.83 (42.8-52.8)	45.0±3.89 (41.1-55.0)	44.3±3.92 (37.8-50.0)	39.3±2.18 (36.1-42.5)	41.2±2.70 (35.0-46.4)
J (hyaline portion of tail) µm	10.2±0.86 (8.4-11.3)	9.0±1.05 (7.5-11.3)	9.3±0.79 (8.1-10.9)	10.6±1.04 (8.8-12.1)	13.5±0.84 (11.3-15.0)	12.7±1.00 (10.5-13.8)
Body diam. at lip region µm	9.1±0.16 (8.8-9.2)	10.7±0.28 (10.0-11.3)	12.8±0.43 (12.5-13.8)	14.7±0.39 (14.1-15.2)	16.6±0.46 (15.4-17.5)	15.7±0.50 (15.0-16.6)
Body diam. at guide ring µm	16.1±0.21 (15.9-16.3)	18.6±0.62 (17.5-19.7)	22.7±0.52 (22.2-23.8)	25.9±0.63 (25.0-27.2)	29.2±1.07 (26.9-30.3)	28.8±1.00 (27.2-31.3)
Body diam. at base of oesophagus µm	26.9±0.81 (25.6-28.1)	30.0±1.59 (27.2-32.5)	38.9±1.72 (35.0-42.5)	44.2±1.59 (40.0-46.3)	50.5±1.70 (46.6-53.4)	50.0±2.23 (46.7-55.6)
Body diam. at mid- body or vulva µm	28.3±1.45 (26.3-30.0)	30.3±2.19 (26.3-33.4)	41.3±2.66 (35.3-46.3)	46.8±2.26 (42.5-50.6)	56.1±2.68 (51.9-62.5)	57.9±4.15 (53.4-68.4)
Body diam. at anus µm	20.0±0.86 (18.4-21.3)	24.2±1.79 (20.9-26.3)	32.4±1.36 (30.0-35.0)	37.4±1.47 (33.8-39.4)	40.6±2.21 (36.3-45.0)	42.8±2.42 (38.4-48.4)
Body diam. at beginning of J µm	8.5±0.57 (7.5-9.4)	12.7±1.16 (10.9-14.7)	18.7±1.41 (15.9-22.1)	22.9±0.79 (21.3-24.1)	27.8±1.76 (25.4-31.6)	29.5±1.69 (26.3-32.9)

d, anterior to guide-ring/body width at lip region (Brown et al. 1994). d', body width at guide ring/body width at lip region (Brown et al. 1994). J, length of the hyaline region of the tail/hyaline width (Lišková et al. 1997).

Table 2. Morphometrics of juvenile stages and females of the *L. piceicola* populations from Slovakia, Montenegro, Romania, Poland and Serbia in the form mean (range).

Developmental stages and populations	Body length (mm)	Odontostyle (µm)	Replacement odontostyle (µm)
J1			
Slovakia ¹ (Muranska Planina Mountains, Cervena Skala)	1.50 (1.32-1.72)	92.0 (81-99)	99.0 (91-104)
Montenegro ² (Durmitor Mountain, Crno jezero near Žabljak)	1.56 (1.44-1.86)	109.0 (104-116)	118.0 (113-125)
Poland ³ (Rogów)	1.446 (1.368-1.529)	90.8 (86-96)	98.8 (92-105)
Romania ⁴ (Bran, Braşov County)	1.32 (1.15-1.47)	95.8 (82-90.3)	103.7 (99.5-110)
Romania ⁴ (Cernica-Ilfov County)	1.36 (1.21-1.52)	86.9 (82-90)	95.7 (92-102)
Serbia ⁵ (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode)	1.85 (1.68-1.90)	100.7 (98.7-102.5)	117.3 (113.7-121.3)
J2			
Slovakia ¹ (Muranska Planina Mountains, Cervena Skala)	2.36 (1.93-2.73)	99.0 (95-104)	122.0 (118-126)
Montenegro ² (Durmitor Mountain, Crno jezero near Žabljak)	2.30 (1.99-2.75)	121.0 (115-128)	137.0 (126-146)
Poland ³ (Rogów)	2.117 (1.913-2.334)	102.4 (101-104)	116.4 (114-118)
Romania ⁴ (Bran, Braşov County)	1.83 (1.63-2.02)	100.7 (97.5-105)	115.4 (109-123)
Romania ⁴ (Cernica-Ilfov County)	1.98 (1.79-2.16)	99.5 (97-102)	110.0 (109-111)
Serbia ⁵ (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode)	2.19 (1.90-2.43)	115.1 (110.0-120.0)	134.2 (117.5-141.0)
J3			
Slovakia ¹ (Muranska Planina Mountains, Cervena Skala)	3.00 (2.60-3.47)	119.0 (110-128)	138.0 (128-150)
Montenegro ² (Durmitor Mountain, Crno jezero near Žabljak)	3.32 (2.89-3.71)	141.0 (132-151)	158.0 (144-166)
Poland ³ (Rogów)	2.883 (2.714-3.073)	120.5 (118-122)	131.3 (127-140)
Romania ⁴ (Bran, Braşov County)	2.62 (2.38-2.81)	118.4 (115-125)	137.8 (134-143)
Romania ⁴ (Cernica-Ilfov County)	3.05 (2.81-3.29)	118.0 (108-124)	136.7 (132-142)
Serbia ⁵ (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode)	3.45 (2.88-3.89)	139.1 (132.1-152.4)	156.6 (147.3-166.4)
J4			
Slovakia ¹ (Muranska Planina Mountains, Cervena Skala)	3.75 (3.07-4.62)	133.0 (117-144)	158.0 (144-183)
Montenegro ² (Durmitor Mountain, Crno jezero near Žabljak)	4.70 (3.84-5.46)	160.0 (150-165)	178.0 (166-191)
Poland ³ (Rogów)	4.249 (3.956-4.053)	132.0 (127-140)	151.5 (148-160)
Romania ⁴ (Bran, Braşov County)	3.45 (3.21-3.91)	138.3 (130-143)	152.7 (151-154)
Romania ⁴ (Cernica-Ilfov County)	3.95 (3.6-4.07)	136.8 (132-141.5)	157.3 (150-165)
Serbia ⁵ (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode)	4.57 (4.11-5.00)	154.9 (142.2-162.6)	176.6 (167.6-182.9)
Females			
Slovakia ¹ (Muranska Planina Mountains, Cervena Skala)	5.19 (4.22-5.97)	160.0 (151-169)	-
Montenegro ² (Durmitor Mountain, Crno jezero near Žabljak)	5.95 (4.42-7.99)	178.0 (168-188)	-
Poland ³ (Rogów)	6.477 (5.457-7.093)	153.9 (144-158)	-
Romania ⁴ (Bran, Braşov County)	4.90 (4.05-5.64)	155.5 (147-163)	-
Romania ⁴ (Cernica-Ilfov County)	5.88 (5.17-6.54)	155.4 (150-165)	-
Serbia ⁵ (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode)	6.33 (5.58-7.12)	176.6 (167.6-190.5)	-

¹Lišková et al. 1997; ²Barsi and Lamberti 2001; ³Kornobis and Peneva 2011; ⁴Groza et al. 2017; ⁵Original.

Table 3. Morphometrics of females of *L. piceicola* populations from Slovakia, Montenegro, Romania, Poland and Serbia.

Developmental stages and populations	Body length (mm)	Odontostyle (μm)	Total stylet (μm)	Oral aperture to guide ring (μm)
Slovakia ¹ (Muranska Planina Mountains, Cervena Skala)	5.19 (4.22-5.97)	160 (151-169)	241 (226-254)	42 (37-45)
Montenegro ² (Durmitor Mountain, Crno jezero near Žabljak)	5.95 (4.42-7.99)	178 (168-188)	261 (236-280)	41 (34-45)
Montenegro ² (Durmitor Mountain, Zminje jezero)	6.01 (5.07-6.51)	175 (168-183)	264 (254-274)	45 (43-48)
Bosnia and Herzegovina ² (Partizansko Polje)	6.23 (5.21-7.88)	169 (145-183)	254 (236-273)	43 (39-48)
Bosnia and Herzegovina ² (Kupres)	6.33 (5.89-7.32)	170 (165-180)	258 (249-268)	43 (40-48)
Bosnia and Herzegovina ² (Podromanija)	6.32 (5.92-7.38)	169 (164-180)	258 (252-267)	43 (40-48)
Bosnia and Herzegovina ² (Bugojno-Poriće)	6.17 (5.73-6.37)	178 (172-183)	268 (263-274)	42 (40-44)
Serbia ² (Samokovska reka)	5.49 (5.03-5.95)	167 (165-168)	245 (235-250)	44 (43-46)
Serbia ² (Brezovica)	6.16 (5.22-6.88)	174 (165-183)	261 (245-275)	40 (36-43)
Poland ³ (Rogów)	6.477 (5.457-7.093)	153.9 (144-158)	239.6 (221-253)	41.7 (40-44)
Romania ⁴ (Bran, Braşov County)	4.90 (4.05-5.64)	155.5 (147-163)	233.2	38.1 (35-41)
Romania ⁴ (Cernica-Ilfov County)	5.88 (5.17-6.54)	155.4 (150-165)	233.5	42.2 (40-45)
Serbia ⁵ (Kopaonik Mountain, Kadijevac, loc. Hajdučke vode)	6.33 (5.58-7.12)	176.6 (167.6-190.5)	257.6 (248.9-273.0)	43.3 (37.5-46.3)
Serbia ⁵ (Tara Mountain, loc. Hajdučka česma)	5.98 (5.16-6.94)	174.1 (166.4-182.9)	254.3 (240.2-269.1)	42.3 (40.0-45.0)

¹Lišková et al. 1997; ²Barsi and Lamberti 2001; ³Kornobis and Peneva 2011; ⁴Groza et al. 2017; ⁵Original.