

Morphometric variation and juvenile stages of *Longidorus intermedius* Kozłowska & Seinhorst, 1979 (Nematoda: Dorylaimida) from the territory of the former Yugoslavia

László Barsi* and Franco Lamberti**†

*Faculty of Science, Department of Biology and Ecology, 21000, Novi Sad, Serbia,

**Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., 70126, Bari, Italy.

Accepted for publication 17 March 2004

Summary. *Longidorus intermedius* is reported for the first time from Croatia, Bosnia and Herzegovina, and Serbia and Montenegro. The morphometric variability of females and males are presented. The presence of only three juvenile developmental stages in this species is confirmed.

Key words: *Longidorus intermedius*, first records, morphology, morphometrics, Croatia, Bosnia and Herzegovina, Serbia and Montenegro, variability.

Longidorus intermedius Kozłowska & Seinhorst, 1979 was described from light sandy soil with forest vegetation in the Netherlands and has subsequently been reported from Italy (Roca *et al.*, 1985, 1986, 1987, 1988a, 1988b, 1991a, 1991b; Roca & Lamberti, 1993), Spain (Andrés & Arias, 1987), Slovakia (Lišková & Brown, 1999; Lišková & Sturhan, 2000), and from Bulgaria, Macedonia and Turkey (Peneva *et al.*, 2001).

Soil samples collected in the period 1988-2002 in Croatia, Bosnia and Herzegovina, Serbia and Montenegro contained specimens of *L. intermedius*. The morphology and morphometrics of females, males and three juvenile stages are reported here.

MATERIAL AND METHODS

Soil samples were collected in July 1988 from the rhizosphere of *Urtica dioica* L. in an oak forest at Eminovci (UTM grid YL12) in Croatia; in April 1990 from the rhizosphere of *Corylus avellana* L. at Duvno (UTM grid XJ74) in Bosnia and Herzegovina; in March and June 1989 from the rhizosphere of *Carpinus betulus* L., in May 1990 from the rhizosphere of *Crataegus monogyna* Jacq. and in May 2002 in the rhizosphere of *Quercus robur* L. in an mixed oak forest at Obrež (UTM grid DQ15), in May 2002 in the rhizosphere of *Q. cerris* L. at Rajla (UTM grid DQ63), each in

Serbia; and in August 2001 from the rhizosphere of *Quercus* sp. at Budva-Lazi (UTM grid CM28) in Montenegro.

Nematodes were extracted by Cobb's wet sieving technique, killed and fixed 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, except body length, which was measured with the aid of a drawing tube and map measurer.

DESCRIPTION

Longidorus intermedius Kozłowska & Seinhorst, 1979 (Figs. 1-3)

Measurements: Tables 1-3.

Female. (Obrež, Obedska bara, Serbia, *Q. robur*, n = 33). Body from C-shape to spiral, tapering gradually toward the extremities. Lip region anteriorly flattened or slightly rounded, almost continuous with the rest of the body or very slightly set off by a shallow depression. Amphidial pouches mostly slightly bilobed at the base. The oesophagus basal bulb 107 (90-120) μm long and 22 (20-24) μm wide, occupying about 1/4 to 1/5 of the total oesophagus length. Nuclei

† Prof. Franco Lamberti died August 16, 2004

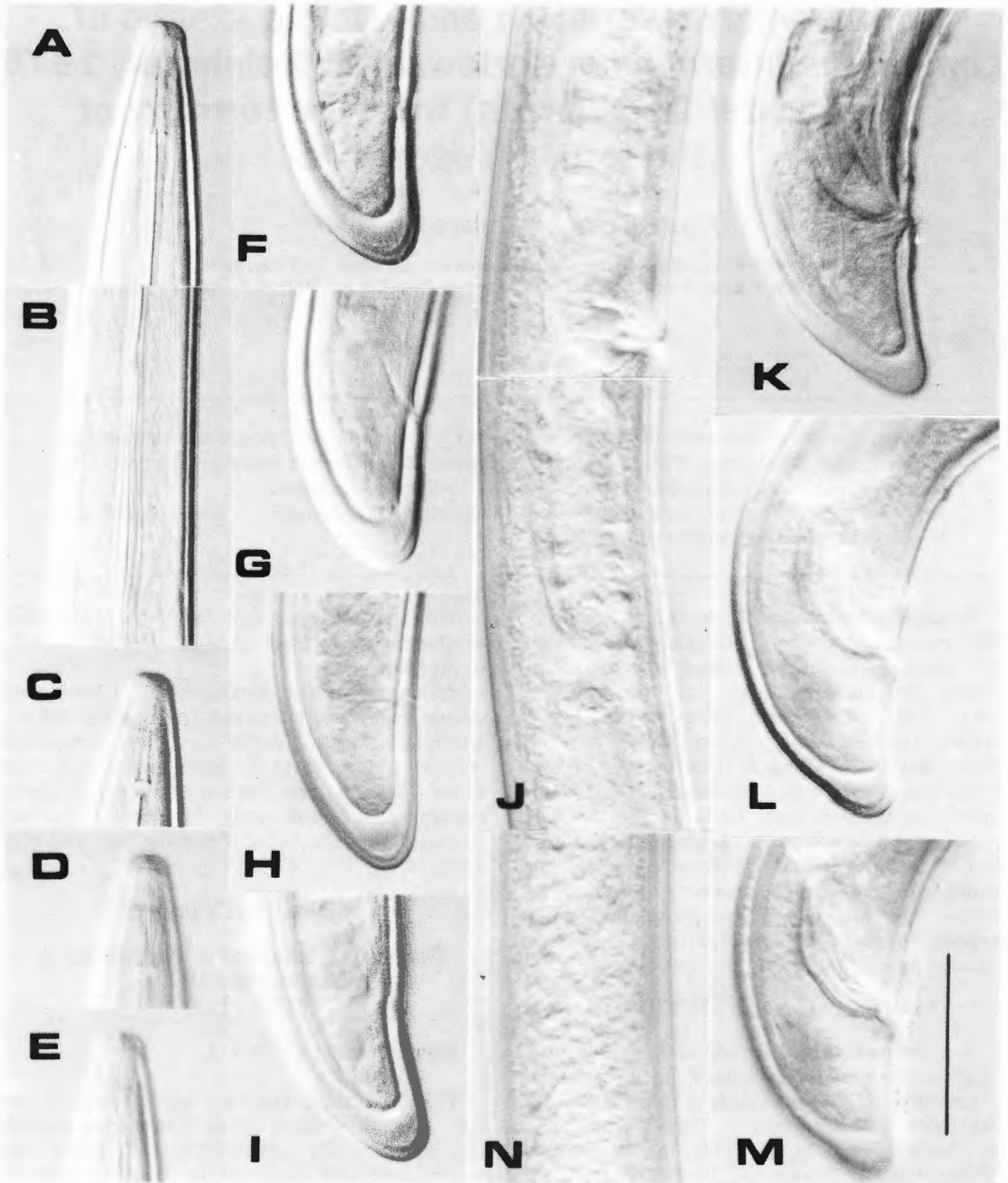


Fig. 1. Photomicrographs of *Longidorus intermedius*. A: Female anterior region; B: Odontophore and posterior end of odontostyle; C-E: Female amphidial pouch; F-I: Female posterior region; J: Vulva region with anterior and posterior uterus; K-M: Male posterior region; N: Testis with sperm. Scale bar - 50 μ m.

Table 1. Morphometric characters of a population of *Longidorus intermedius* from Serbia (all measurements in μm , except for L in mm).

Locality	Obrež, Obedska bara				
Host	<i>Quercus robur</i>				
n	14 JI	20 JII	28 JIII	33 ♀♀	3 ♂♂
L	1.40±0.09 (1.28-1.54)	1.94±0.15 (1.65-2.29)	2.97±0.30 (2.44-3.61)	4.35±0.31 (3.67-5.11)	4.49±0.27 (4.20-4.72)
a	60±3.8 (54-68)	67±3.4 (60-74)	79±4.5 (69-86)	87±7.8 (72-103)	87±5.0 (82-92)
b	4.7±0.3 (4.4-5.5)	5.4±0.4 (4.9-6.1)	7.2±0.6 (5.9-8.3)	9.1±0.5 (8.0-10.3)	9.4±0.7 (8.7-10.0)
c	30±1.5 (27-32)	44±3.0 (39-50)	64±6.5 (51-77)	104±14.0 (86-156)	97±15.8 (81-112)
c'	3.0±0.2 (2.6-3.2)	2.0±0.2 (1.8-2.4)	1.5±0.1 (1.3-1.9)	1.2±0.1 (1.0-1.4)	1.1±0.1 (1.0-1.2)
d	2.6±0.1 (2.4-2.8)	2.6±0.1 (2.5-2.8)	2.6±0.1 (2.5-2.8)	2.5±0.1 (2.3-2.9)	2.5±0.1 (2.4-2.5)
d'	1.7±0.1 (1.6-1.8)	1.8±0.1 (1.7-1.9)	1.8±0.1 (1.7-1.9)	1.8±0.1 (1.7-2.0)	1.9±0.05 (1.9-2.0)
J'	1.3±0.1 (1.2-1.4)	0.7±0.1 (0.6-0.9)	0.6±0.05 (0.4-0.7)	0.5±0.04 (0.4-0.6)	0.6±0.05 (0.5-0.6)
V%	—	—	—	47±1.4 (44-49)	—
Odontostyle	74±3.1 (69-79)	83±2.3 (79-86)	98±3.1 (92-104)	113±3.7 (106-123)	116±4.7 (111-121)
Odontophore	42±1.8 (38-45)	50±3.1 (44-55)	57±3.2 (51-63)	64±1.6 (60-66)	64±2.6 (61-66)
Total stylet	115±4.3 (106-123)	133±3.5 (126-139)	155±5.3 (144-166)	177±3.6 (169-186)	180±7.2 (173-187)
Replacement odontostyle	83±2.0 (79-85)	96±3.3 (91-104)	111±3.8 (104-121)	—	—
Oral aperture to guide ring	20±0.7 (19-21)	24±0.7 (23-25)	27±0.9 (26-29)	30±1.4 (28-34)	32±1.0 (31-33)
Tail	47±2.2 (43-50)	44±2.5 (41-50)	46±3.2 (39-53)	42±3.5 (33-49)	47±5.0 (42-52)
J (hyaline portion of tail)	9.1±0.6 (8.1-10.0)	8.2±1.1 (6.9-10.8)	9.6±1.1 (7.5-12)	12±1.3 (10-14)	11±0.4 (11-11)
Body diam. at lip region	8.0±0.3 (7.5-8.4)	8.9±0.3 (8.4-9.6)	10.3±0.4 (9.7-11)	12±0.5 (11-13)	13±0.4 (13-13)
Body diam. at guide ring	14±0.4 (13-14)	16±0.8 (15-18)	19±0.9 (17-20)	22±0.9 (20-23)	23±1.0 (22-24)
Body diam. at base of oesophagus	23±1.4 (21-25)	28±1.7 (24-32)	35±2.1 (30-39)	42±2.0 (36-48)	44±1.3 (43-45)
Body diam. at mid-body or vulva	23±1.6 (20-26)	29±2.4 (25-34)	38±3.3 (31-46)	50±4.6 (42-60)	51±1.7 (50-53)
Body diam. at anus	16±1.0 (14-18)	22±1.8 (18-25)	30±2.2 (25-34)	36±1.9 (32-40)	42±0.9 (41-43)
Body diam. at beginning of J	7.0±0.4 (6.3-7.5)	11±1.1 (8.8-13)	17±1.1 (15-19)	23±1.7 (19-25)	19±1.3 (18-20)
Spicules	—	—	—	—	49±1.2 (48-50)

d, anterior to guide-ring/body width at lip region (Brown *et al.*, 1994);
d', body width at guiding-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. Morphometric characters of females of *L. intermedius* from Serbia (all measurements in μm , except for L in mm).

Locality Host	Obrež, Obedska bara <i>Carpinus betulus</i>	Obrež, Obedska bara <i>Carpinus betulus</i>	Obrež, Obedska bara <i>Crataegus monogyna</i>	Ralja <i>Quercus cerris</i>
n	9	9	9	9
L	4.16±0.29 (3.78-4.66)	4.38±0.35 (3.46-4.82)	4.45±0.29 (3.89-4.91)	4.43±0.48 (3.56-5.23)
a	79±3.6 (75-86)	83±4.7 (77-90)	83±3.5 (77-89)	88±5.5 (79-95)
b	10.0±0.9 (8.3-11)	10.1±0.6 (8.9-11.1)	9.9±0.9 (8.5-11.4)	9.8±0.9 (8.5-10.9)
c	100±10.1 (90-124)	105±6.6 (90-115)	111±13.3 (91-142)	109±13.2 (97-137)
c'	1.2±0.1 (1.0-1.2)	1.2±0.04 (1.1-1.2)	1.1±0.1 (0.9-1.3)	1.1±0.1 (1.0-1.3)
d	2.7±0.1 (2.6-2.8)	2.8±0.1 (2.7-2.9)	2.7±0.1 (2.5-2.9)	2.6±0.2 (2.4-2.8)
d'	2.0±0.1 (1.8-2.1)	2.0±0.04 (1.9-2.1)	1.9±0.04 (1.8-1.9)	1.9±0.1 (1.7-2.0)
J'	0.5±0.03 (0.5-0.6)	0.5±0.05 (0.4-0.6)	0.5±0.03 (0.5-0.6)	0.5±0.03 (0.4-0.5)
V%	48±1.2 (46-51)	49±1.0 (47-51)	47±1.0 (45-49)	46±1.2 (45-48)
Odontostyle	113±3.5 (105-118)	111±3.1 (107-117)	113±4.2 (108-119)	110±2.8 (105-114)
Odontophore	64±3.2 (59-69)	67±4.0 (60-74)	65±2.9 (59-70)	68±3.4 (65-76)
Total stylet	177±3.2 (173-183)	178±6.5 (169-191)	178±6.4 (167-186)	178±4.4 (171-185)
Oral aperture to guide ring	32±1.4 (30-35)	34±0.9 (33-35)	32±1.8 (30-35)	33±1.9 (31-38)
Tail	42±2.0 (38-44)	42±1.9 (38-44)	41±4.1 (33-48)	41±3.7 (36-49)
J (hyaline portion of tail)	12±0.9 (10-12.5)	12±1.8 (8.8-15)	12±0.9 (10-13)	11±0.9 (10-13)
Body diam. at lip region	12±0.3 (12-12.5)	12±0.4 (11-13)	12±0.4 (11-12.5)	13±0.4 (12-13)
Body diam. at guide ring	24±1.0 (23-26)	24±1.0 (22-25)	23±0.9 (21-24)	24±0.9 (23-26)
Body diam. at base of oesophagus	44±1.7 (41-48)	44±2.5 (39-48)	43±1.7 (41-46)	43±1.3 (41-45)
Body diam. at mid-body or vulva	52±1.9 (50-57)	53±3.7 (45-58)	53±2.9 (49-58)	50±3.5 (45-57)
Body diam. at anus	36±1.5 (34-38)	36±2.1 (31-39)	36±2.6 (31-40)	37±2.2 (32-39)
Body diam. at beginning of J	22±1.6 (19-25)	22±1.5 (20-25)	23±2.2 (20-28)	24±1.4 (21-25)

Table 3. Morphometric characters of adults from populations of *L. intermedius* from Croatia, Bosnia and Herzegovina and Montenegro (all measurements in μm , except for L in mm).

Locality Host	<i>Eminovci</i> <i>Urtica dioica</i>		<i>Duvno</i> <i>Corylus avellana</i>		<i>Budva-Lazi</i> <i>Quercus</i> sp.
	28 ♀♀	1 ♂	18 ♀♀	1 ♂	6 ♀♀
L	4.08±0.24 (3.58-4.48)	3.85	3.95±0.28 (3.25-4.37)	3.35	4.23±0.17 (3.96-4.41)
a	82±4.2 (73-88)	80	80±3.6 (74-87)	75	85±3.7 (80-90)
b	9.9±0.6 (8.6-11.1)	10.4	9.6±0.9 (8.0-12.1)	8.9	9.3±1.4 (7.3-11)
c	107±9.3 (82-127)	84	98±6.8 (88-113)	80	111±11.1 (97-126)
c'	1.1±0.1 (0.9-1.4)	1.2	1.1±0.1 (1.0-1.3)	1.0	1.1±1.0 (1.0-1.2)
d	2.5±0.1 (2.3-2.7)	2.4	2.5±0.1 (2.3-2.7)	2.6	2.8±0.1 (2.7-3.1)
d'	1.8±0.05 (1.7-1.9)	1.7	1.9±0.1 (1.8-2.0)	1.9	1.9±0.1 (1.8-2.1)
J'	0.5±0.1 (0.4-0.7)	0.7	0.5±0.1 (0.4-0.6)	0.6	0.5±0.04 (0.5-0.6)
V%	48±0.9 (46-50)	—	50±1.9 (46.5-52.5)	—	47±0.7 (46-48)
Odontostyle	112±2.4 (108-115)	110	110±3.7 (105-121)	105	112±4.3 (106-119)
Odontophore	61±3.5 (55-68)	63	61±3.6 (56-70)	60	68±2.0 (65-69)
Total stylet	174±4.7 (166-183)	173	171±4.8 (164-179)	165	179±4.4 (175-188)
Oral aperture to guide ring	31±1.4 (29-34)	30	32±1.9 (27-34)	33	34±1.2 (32-36)
Tail	38±3.8 (34-52)	46	40±2.3 (34-44)	42	39±4.9 (34-44)
J (hyaline portion of tail)	12±1.3 (10-16)	13	11±1.7 (8.1-14)	11	12±1.2 (11-14)
Body diam. at lip region	13±0.3 (12-13)	13	12.5±0.6 (11-13)	13	12±0.4 (12-12.5)
Body diam. at guide ring	23±0.9 (21-24)	21	24±1.1 (21-25)	24	23±0.8 (23-25)
Body diam. at base of oesophagus	42±2.1 (38-46)	41	44±2.2 (39-47)	42	45±1.4 (43-47)
Body diam. at mid-body or vulva	49±3.0 (41-56)	48	50±2.8 (44-54)	45	50±1.7 (48-51)
Body diam. at anus	35±2.0 (30-38)	39	37±2.3 (33-40)	43	36±2.1 (33-39)
Body diam. at beginning of J	22±1.3 (20-25)	20	22±1.5 (19-25)	19	23±2.5 (20-25)
Spicules	—	52	—	43	—

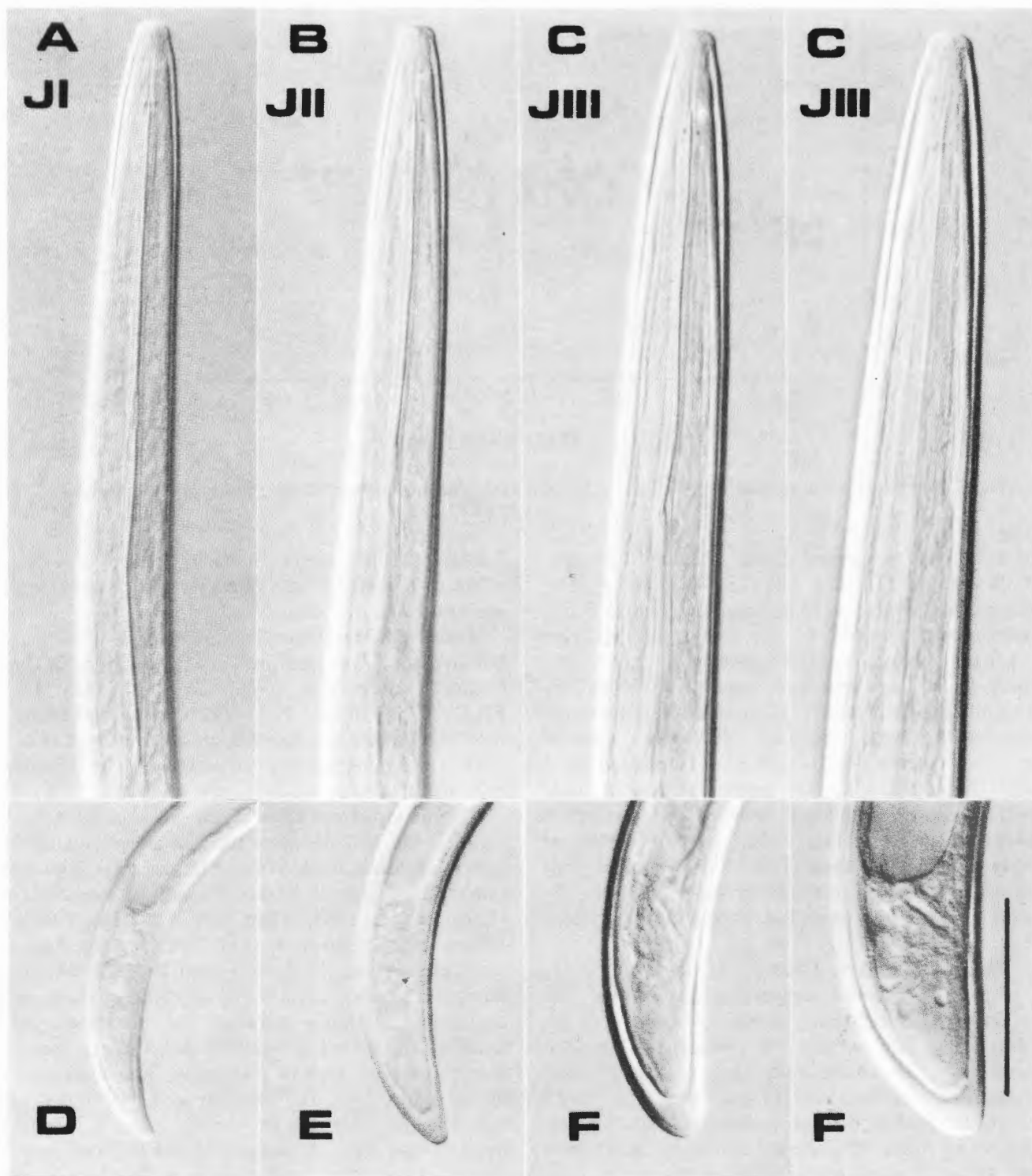


Fig. 2. Photomicrographs of juvenile stages of *L. intermedius*. A-C: Anterior region of JI, JII and JIII stage; D-F: Tail of JI, JII and JIII stage. Scale bar - 50 μ m

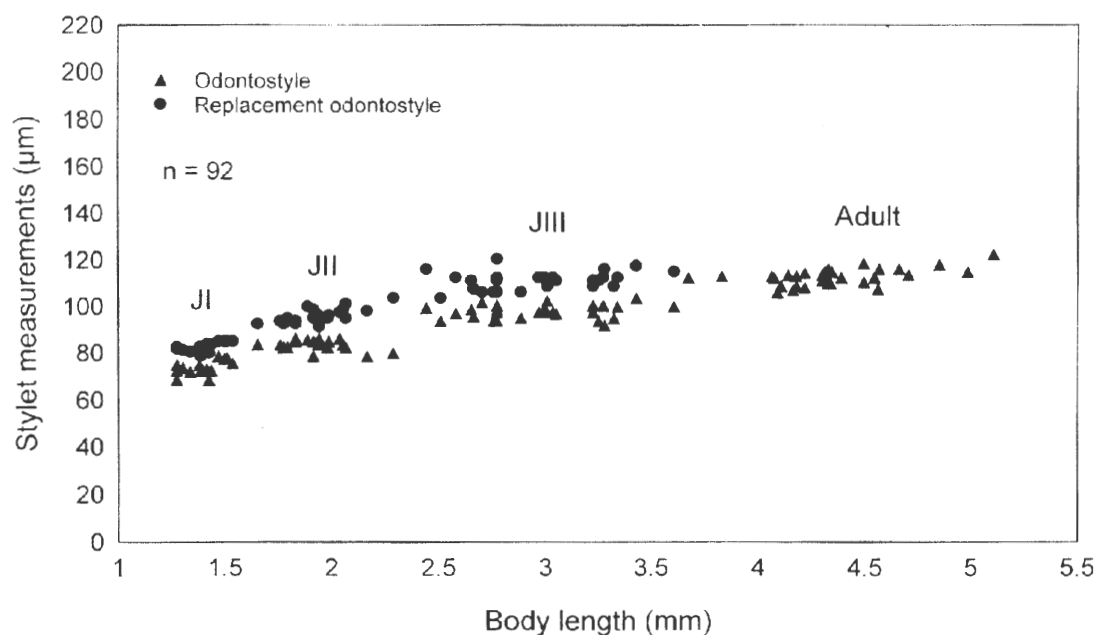


Fig. 3. Scatter diagram separating juveniles and females of *L. intermedius* from Obrež, Obedska bara, Serbia.

of dorsal and subventral glands situated at 28 (24-31)% ($n = 23$) and 52 (45-56)% ($n = 24$), respectively. Vulva a transverse slit, vagina occupying about 44-56% of the corresponding body diameter; *pars distalis vaginae* and moderately thick-walled *pars proximalis vaginae* 13-16 and 8-15 μm long, respectively. Reproductive system amphidelphic with equally developed genital branches; uterus 44-104 μm long, separated by a well developed sphincter from the *pars dilatata oviductus*; ovaries opposed, reflexed. Pre-rectum 6-15 times the anal body width; rectum 68-98% of body diameter at anus. Tail bluntly conical with rounded terminus, dorsally convex, ventrally almost straight, with two caudal pores on each side.

Male. (Eminovci, Croatia, *U. dioica*, $n = 1$; Duvno, Bosnia and Herzegovina, *C. avellana*, $n = 1$; Obrež, Obedska bara, Serbia, *Q. robur*, $n = 3$). Morphologically similar to females, with the posterior region of the body more coiled. The oesophagus basal bulb 88-111 μm long and 21-23 μm wide. Two of the five males with fully developed testes filled with sperm. Spicules slightly curved ventrally, with guiding pieces 14-15 μm long ($n = 3$). The adanal pair of supplements preceded by a row of 4-8 ventromedian supplements. Tail dorsally convex and ventrally slightly concave, conoid with rounded terminus.

Juveniles. (Obrež, Obedska bara, Serbia, *Q. robur*, $n = 62$). Three developmental stages re-

sembling adults except for their smaller size. Tail elongate-conoid in the first stage, and progressively less conoid in the second and third stages.

Remarks. The identification code for the populations of *L. intermedius* from the territory of the former Yugoslavia is: A4(5), B12, C23, D3, E2, F2(3), G12, H(1)2, I12, which is in agreement with the codes given for this species by Chen *et al.* (1997) and subsequently supplemented by Peneva *et al.* (2001).

Populations from Croatia, Bosnia and Herzegovina, Serbia and Montenegro are morphologically and morphometrically similar to the type population and to the populations reported from Italy (Roca *et al.*, 1985, 1986, 1987, 1988a, 1988b, 1991a, 1991b; Roca & Lamberti, 1993), Spain (Andrés & Arias, 1987), and from Bulgaria, Macedonia and Turkey (Peneva *et al.*, 2001). The only consistent difference between the paratypes (10 females) and all other reported populations, including those described in this study, is the distance of the vulva from the anterior end. All females, including the holotype ($n = 318$), have V ranging from 44 and 54%. According to the original description, all of the paratypes ($n = 10$) had V at 43%. This character has been measured in two of three paratype females of *L. intermedius*, deposited in the Istituto per la Protezione delle Piante, Sezione di Bari, Bari, Italy [(formerly, Laboratorio di Nematologia Agraria applicata di Vegetali, Bari, Italy (Kozłowska & Seinhorst, 1979)] and were found to have V at 47 and 48%. Data for all cha-

racters (L, a, b, c, c', odontostyle, guide ring and anterior lateral pores) for 10 paratypes in the original description were given as minimum and maximum values, except V. Consequently it is probable that this single value represents only the minimum value.

Males of *L. intermedius* were described for the first time from Bulgaria (Peneva *et al.*, 2001). The morphology of males found in Croatia, Bosnia and Herzegovina, and Serbia was similar to those from Bulgaria, but they have slightly longer bodies (3.35-4.72 vs 3.163-3.876 mm), slightly longer odontostyles (105-121 vs 104-109 µm), slightly longer tails (42-52 vs 39-49 µm), slightly longer spicules (43-52 vs 41-47 µm) and a wider range in the number of supplements (4-8 vs 6-7).

A study of juvenile *L. intermedius* paratypes and numerous juveniles from a population from Bulgaria revealed that this species has only three juvenile developmental stages (Peneva *et al.*, 2001). Comparison of juvenile developmental stages and adults of *L. intermedius* from Obrež (Serbia) with those from the Netherlands (paratypes) and Bulgaria (Peneva *et al.*, 2001) showed the same developmental pattern.

The presence of low intraspecific variability in *L. intermedius* was considered by Peneva *et al.* (2001) as a probable consequence of the high degree of specificity between the species and its most common host, *Quercus* spp. The similarity of our populations with other populations of *L. intermedius* and their direct or indirect connection with oaks appears to support this opinion.

This is the first record of a *Longidorus* species with only three juvenile developmental stages from Croatia, Bosnia and Herzegovina, and Serbia and Montenegro.

ACKNOWLEDGEMENT

We thank Ivan Dulić, MSc., NIS -Naftagas, Novi Sad for access to an Olympus BX50 photomicroscope. Work of the first author was in part supported by the Ministry of Science, Technologies and Development of the Republic of Serbia, Grant No. 1770.

REFERENCES

- Andrés, M. Fe & Arias, M. 1987. A new species of *Longidorus* (Nematoda: Longidoridae) associated with forest soils and notes on *L. congoensis* Aboul-Eid, 1970 and *L. intermedius* Kozłowska & Seinhorst, 1979, new records for Spain. *Nematologica* 33: 386-392.
- Brown, D.J.F., Grunder, J., Hooper, D.J., Klingler, J. & Kunz, P. 1994. *Longidorus arthensis* sp. n. (Nematoda: Longidoridae) a vector of cherry rosette disease caused by a new nepovirus in cherry trees in Switzerland. *Nematologica* 40: 133-149.
- Chen, Q., Hooper, D.J., Loof, P.A.A. & Xu, J. 1997. A revised polytomous key for identification of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). *Fundamental and Applied Nematology* 20: 15-28.
- Kozłowska, J. & Seinhorst, J.W. 1979. *Longidorus elongatus* and closely related species in The Netherlands and Lower Saxony (Germany), with the description of two new species, *L. cylindricaudatus* and *L. intermedius* (Nematoda: Dorylaimida). *Nematologica* 25: 42-53.
- Lišková, M. & Brown, D.J.F. 1999. The occurrence of Longidoridae (Nematoda) in forests in the Slovak Republic. *Helminthologia* 36: 49-56.
- Lišková, M., Robbins, R.T. & Brown, D.J.F. 1997. Descriptions of Three New *Longidorus* Species from Slovakia (Nematoda: Longidoridae). *Journal of Nematology* 29: 336-348.
- Lišková, M. & Sturhan, D. 2000. Occurrence and ecology of Longidoridae (Nematoda: Dorylaimida) in floodplain forests in the Slovak Republic. *Helminthologia* 37: 113-117.
- Peneva, V., Loof, P.A.A., Penev, L.D. & Brown, D.J.F. 2001. Description of the male and first-stage juvenile of *Longidorus intermedius* Kozłowska & Seinhorst, 1979 (Nematoda: Dorylaimida), and notes on its morphology and distribution. *Systematic Parasitology* 49: 127-137.
- Roca, F. & Lamberti, F. 1993. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. XIII. La Toscana. *Nematologia Mediterranea* 21: 261-272.
- Roca, F., Lamberti, F. & Agostinelli, A. 1985. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. II. Basilicata. *Nematologia Mediterranea* 13: 161-175.
- Roca, F., Lamberti, F. & Agostinelli, A. 1986. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. III. L'Abruzzo e il Molise. *Nematologia Mediterranea* 14: 83-99.
- Roca, F., Lamberti, F. & Agostinelli, A. 1987. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. V. II Lazio. *Nematologia Mediterranea* 15: 71-101.
- Roca, F., Lamberti, F. & Agostinelli, A. 1988a. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. VII. Il Piemonte e La Valle d'Aosta. *Nematologia Mediterranea* 16: 35-51.
- Roca, F., Lamberti, F. & Agostinelli, A. 1988b. I Longidoridae (Nematoda, Dorylaimida) delle regioni Itali-

ane. VIII. L'Emilia-Romagna. *Nematologia Mediterranea* 16: 179-188.

Roca, F., Lamberti, F. & D'Erico, F.P 1991a. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. XI. La Campania. *Nematologia Mediterranea*

19: 139-154.

Roca, F., Lamberti, F. & Elia, F 1991b. I Longidoridae (Nematoda, Dorylaimida) delle regioni Italiane. XII. L'Umbria. *Nematologia Mediterranea* 19: 279-289.

Barsi L., Lamberti F. Морфометрическая изменчивость и описание личиночных стадий *Longidorus intermedius* Kozłowska & Seinhorst, 1979 (Nematoda: Dorylaimida) с территории бывшей Югославии.

Резюме. Впервые для территорий Хорватии, Боснии и Герцеговины, а также Сербии и Черногории, отмечен вид *Longidorus intermedius*. Приводятся данные по морфометрической изменчивости самок и самцов. Подтверждается существование лишь трех личиночных стадий у этого вида.
