

Longidorus apuloides n. sp. (Nematoda : Longidoridae) from Italy with a hierarchical cluster analysis of the closely related species

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Summary – *Longidorus apuloides* sp. n. is described and illustrated. It was found in the rhizosphere of poplar trees at Formia, province of Latina, central Italy. The new species closely resembles *L. apulus* Lamberti & Bleve-Zacheo, 1977, from which it mainly differs in body length and abundance of males; it is also similar to *L. proximus* Sturhan & Argo, 1983 and *L. vineacola*, Sturhan & Weischer, 1964. A hierarchical cluster analysis of the closely related species was performed.

Résumé – *Longidorus apuloides* sp. n. (Nematoda : Longidoridae) provenant d'Italie et analyse hiérarchique des espèces proches. – *Longidorus apuloides* sp. n. est décrit et figuré. Cette nouvelle espèce a été prélevée dans la rhizosphère de peuplier à Formia, province de Latina, Italie. Elle est proche de *L. apulus* Lamberti & Bleve-Zacheo, 1977 dont elle se distingue par la longueur du corps et la présence de mâles abondants. *L. apuloides* sp. n. est proche aussi de *L. proximus* Sturhan & Argo, 1983 et de *L. vineacola* Sturhan & Weischer, 1964. Ces espèces sont comparées à l'aide d'une analyse des groupes hiérarchiques.

Key-words : *Longidorus apuloides*, nematodes, taxonomy.

Longidorus apulus Lamberti & Bleve-Zacheo, 1977, erroneously identified as *L. attenuatus* Hooper, 1961, is very widespread in the Apulia region (Lamberti *et al.*, 1985), mostly in artichoke fields where it is a natural vector of artichoke italian latent virus (AILV) (Rana & Roca, 1973; Roca *et al.*, 1975; Vovlas & Roca, 1975; Taylor *et al.*, 1976).

In Atlas of Plant Parasitic Nematodes of Italy, Roca and Lamberti (1985) reported *L. apulus* in Latium, central Italy. A population found near Formia was not included in the list of species reported from this region (Roca *et al.*, 1987) because its identification was in doubt. When compared with *L. apulus*, the population from Formia revealed only slight morphological differences, besides the presence of a large number of males. The Formia population differs from *L. apulus* in some biometrical values, previously regarded as intraspecific variations. More detailed observations of morphological characters and a hierarchical cluster analysis of morphometrics of populations of *L. apulus*, *L. proximus* Sturhan & Argo, 1983 and *L. vineacola*, Sturhan & Weischer, 1964 are sufficient to separate, at specific level, the Formia population. The new species is described here under the name *Longidorus apuloides* sp. n., due to its close resemblance to *L. apulus*.

Materials and methods

Nematodes were extracted from soil samples by the Cobb wet sieve technique, killed and fixed in 5 % hot

formaldehyde solution, processed by the glycerol-ethanol method of Seinhorst (1959) and mounted in glycerin on Cobb slides. The specimens were measured with the aid of a camera lucida.

A hierarchical cluster analysis (HCA) was performed on the published morphometric values of six *L. apulus* populations from Apulia region (Lamberti *et al.*, 1985), including the type population and a population from Palese reared in a glass-house (Lamberti & Bleve-Zacheo, 1977). The similarity with *L. proximus* suggested inclusion of the morphometrics of this species in the analysis. Biometrical values of the type population (Sturhan & Argo, 1983) as well as two Italian (Roca *et al.*, 1991; Roca & Lamberti, 1994) and one Greek (Roca, 1986) populations were also included, together with the type population of *L. vineacola* (Sturhan & Weischer, 1964). Table 1 lists the localities of the populations and literature references. Their morphometric measurements (means and ranges), are reported in Table 2. The variables used in the HCA were the mean values of body length, a, c, c' and V ratios, length of odontostyle, odontophore and tail, distance of oral aperture to guide ring and body diameter at lip region and at guiding ring. The dendrogram was obtained using the complete linkage method with a SPSS PC+ program.

Clustering analysis

Fig. 1 shows the dendrogram obtained from the hierarchical cluster analysis. The group of populations G to

Table 1. Populations of *Longidorus* selected for the Hierarchical Cluster Analysis (HCA).

Species	Population	Origin	Reference	Males
<i>L. apulus</i>	A	Type pop., Italy	Lamberti & Bleve-Zacheo, 1977	Absent
<i>L. apulus</i>	B	Palese, Italy	Lamberti & Bleve-Zacheo, 1977	Present
<i>L. apulus</i>	C	Bari, Italy	Lamberti <i>et al.</i> , 1985	Absent
<i>L. apulus</i>	D	Noicattaro, Italy	Lamberti <i>et al.</i> , 1985	Absent
<i>L. apulus</i>	E	Ruvo, Italy	Lamberti <i>et al.</i> , 1985	Absent
<i>L. apulus</i>	F	Squinzano, Italy	Lamberti <i>et al.</i> , 1985	Absent
<i>L. proximus</i>	G	Maratona, Greece	Roca, 1986	Present
<i>L. proximus</i>	H	Terenzano, Italy	Roca & Lamberti, 1994	Absent
<i>L. proximus</i>	I	Pontecagnano, Italy	Roca <i>et al.</i> , 1991	Absent
<i>L. proximus</i>	J	Type pop., Germany	Sturhan & Argo, 1983	Absent
<i>L. apuloides</i> sp. n.	K	Formia, Italy		Present
<i>L. vineacola</i>	L	Type pop., Germany	Sturhan & Weischer, 1964	Present

Table 2. Mean and range of biometrical characters of the populations used for hierarchical cluster analysis of *Longidorus* species (A-L) : see Table 1 (All measurements in μm except L in mm).

Species	<i>L. apulus</i>						<i>L. proximus</i>				<i>L. apuloides</i>	<i>L. vineacola</i>
	A	B	C	D	E	F	G	H	I	J	K	L
n	20	20	14	8	10	4	14	10	6	33	11	18
L	6.7	7.3	6.4	7.3	7.3	7.7	7.9	7.3	7.7	7.3	8.4	8.16
	5.3-8.3	6.4-9.1	5.1-7.3	6.2-7.9	6.4-7.9	7.4-7.9	6.5-9.2	6.7-8.3	7.4-8.2	6.5-8.2	7.4-10.2	6.9-9.2
a	139	142	132	138	152	131	113	119	115	124	155	137
	123-154	123-155	112-144	114-160	135-169	125-142	98-126	107.5-151	100-126	104-138	123-173	120-149
c	170	188	173	187	196	181	237	204.2	216.5	192	192	213
	128-209	164-216	145-193	167-206	165-215	158-202	199-355	177.5-243	199-246.5	165-249	153-230	186-247
c'	1.0	1.0	0.9	0.9	0.9	0.9	0.7	0.8	0.7	0.8	1.0	0.9
	0.9-1.2	0.9-1.1	0.86-1.0	0.8-1.0	0.8-1.0	0.9-1.0	0.5-0.8	0.7-1.0	0.5-0.8	0.7-0.9	0.8-1.3	0.8-0.9
V	52	51	52	52	51	51	51	48.8	47.2	47.6	49.5	52.2
	51-54	49-52	50-54	51-53	50-52	50-53	50-52	46-53	46-49	45-50	46-52	49.7-54.3
Odontostyle	103	104	112	115	110	115	108	111	112.5	107	119	97
	91-112	98-108	102-120	107-122	105-117	110-119	103-112	106-118	109-115	102-112	109-126	90-101
Odontophore	68	67	63	65	67	78	64	55	57	45	43.5	44
	62-75	60-75	53-73	58-70	60-75	71-86	54-71	38.5-68.5	52-63.5	41-51	36.5-50	40-50
Guide ring	31	31	31	31	31	31	37	34.5	35	34	33	32
	27-34	29-34	29-33	29-33	29-33	28-33	34-39	32-39	32.5-38	31.5-37.5	30.5-36.5	29-33
Tail length	40	39	37	39	38	43	38	36	36	38	44.5	21
	31-46	34-44	33-47	37-45	37-43	39-48	21-39	32-46.5	30-41	31-44	36-59	-
Body diam. at lip region	15	15.5	16	16	15.5	17	18	17.5	16.5	18	15.5	28
	14-17	14.5-16.5	15-17	15-17	15-17	17-18	17-20	16.5-19	16-17	17-19	14.5-17.5	-
Body diam at guide ring	22	24	25	24	24	26	27	26	23.5	23	23	-
	19-25	21-25	23-27	23-25	23-25	25-27	25-31	23.5-29	23-25	20-26	20-24	-

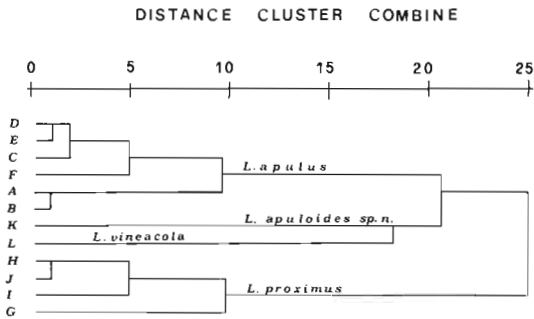


Fig. 1. Dendrogram showing the clustering of populations and related average distances.

J, belonging to *L. proximus*, are separated at the first higher distance level from the populations A to F belonging to *L. apulus*. At the second step, populations K and L are clearly separated from *L. apulus*, and are considered to be intermediate between these two groups. Population L is *L. vineacola* and population K is the population from Formia which is considered here as new, and described under the name *L. apuloides* sp. n.

***Longidorus apuloides* sp. n.**
= *L. apulus* apud Roca & Lamberti, 1985
 (Fig. 2)

MEASUREMENTS

Holotype, females, males and juveniles (see Table 3).

DESCRIPTION

Females: In specimens killed by gentle heat, habitus C shaped, almost straight anteriorly and more curved behind the vulva to single spiral. Cuticle with very fine transverse striations along the body, apparently criss-crossing on the tail region. Cuticle 2-3 μm thick along the body, thicker in the neck region, where it measures 3.5-4 μm at the base of the lip region, and even thicker in the caudal region where it is 6.5-7 μm ventrally and 8.5-9 μm dorsally. Lateral hypodermal cords well visible throughout the length of the body, about a third of the corresponding body diameter wide. Lateral body pores, six in the odontostyle region, one located anterior to guiding ring; three dorsal and five ventral body pores visible in the odontostyle region, one of them located anterior to the level of guiding ring. Lip region broadly rounded frontally and more so laterally, apparently hemispherical, almost continuous or separated from the rest of the body by a very slight constriction, with prominent labial sensilla. Amphidial pouches bilobed reaching half way from anterior end to guide ring, with sinus moderately deep and ventral lobe only slightly longer than the dorsal lobe; amphidial aperture indistinct. Guiding ring 4-4.5 μm wide; odontostyle long and slender, odontophore enlarged at the base. Nerve ring wide, just behind base of odontophore; pharynx dorylaimoid

with pharyngeal bulb cylindrical, containing three gland nuclei; the two subventral ones situated at the same level, near the middle of the bulb or just posterior to it, dorsal nucleus situated at about two third of the distance from the beginning of the bulb to the subventral nuclei. Pharyngo-intestinal valve inconspicuous, heart-shaped, usually surrounded by intestinal tissue. Genital branches equally developed and reflexed. Ovary short, usually with small ovarial sac. *Pars dilatata oviductus* short and separated from the uterus by a strong and muscularized sphincter. Uterus long and wide. Male sperm not observed in the uterus and oviduct. Vulva transverse, slit-like, vagina cuticularized, extending over half or more the corresponding body diameter, ovijector poorly differentiated. Pre-rectum variable in length; rectum smaller than the anal body width. Tail short, bluntly rounded, convex dorsally and less so ventrally with rounded terminus, bearing two caudal pores on each side.

Male: General appearance similar to female with posterior part of the body more curved. Morpho-anatomy similar to female except in the genital apparatus and the somatic structures associated with it; testes well developed; spicules curved, not cephalated; lateral guiding pieces of the gubernaculum well sclerotized, slightly curved, rounded proximally and with distal end bifid. Precloacal pair of papillae at 15 μm from the cloacal aperture, preceded by 11-13, seldom 9, medioventral supplements. Tail different from that of female, rounded conoid, convex dorsally with the ventral profile almost straight, bearing three caudal pores on each side.

Juvenile: Morphologically similar to female but smaller; tail of first stage elongate conoid, slightly curved ventrally, bearing two caudal pores on each side.

TYPE HOST AND LOCALITY

Rhizosphere of poplar (*Populus nigra* L.) at Formia, province of Latina, central Italy.

TYPE SPECIMENS

Holotype, five paratype females, fifteen paratype males and juveniles in the Collection of the Istituto di Nematologia Agraria del Consiglio Nazionale delle Ricerche, Bari, Italy; two paratype females and two paratype males each in the following collections: Muséum National d'Histoire Naturelle, Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Paris, France; Entomology and Nematology Department, Rothamsted Experimental Station, Harpenden, Herts, England; Plant Nematology Laboratory Collection, United States Department of Agriculture, Beltsville, Maryland, USA.

DIAGNOSIS AND RELATIONSHIPS

L. apuloides sp. n. is characterized by a rounded lip region, almost continuous or separated from the rest of the body by a very slight constriction, amphidial pouches bilobed with sinus not very deep, and ventral lobe

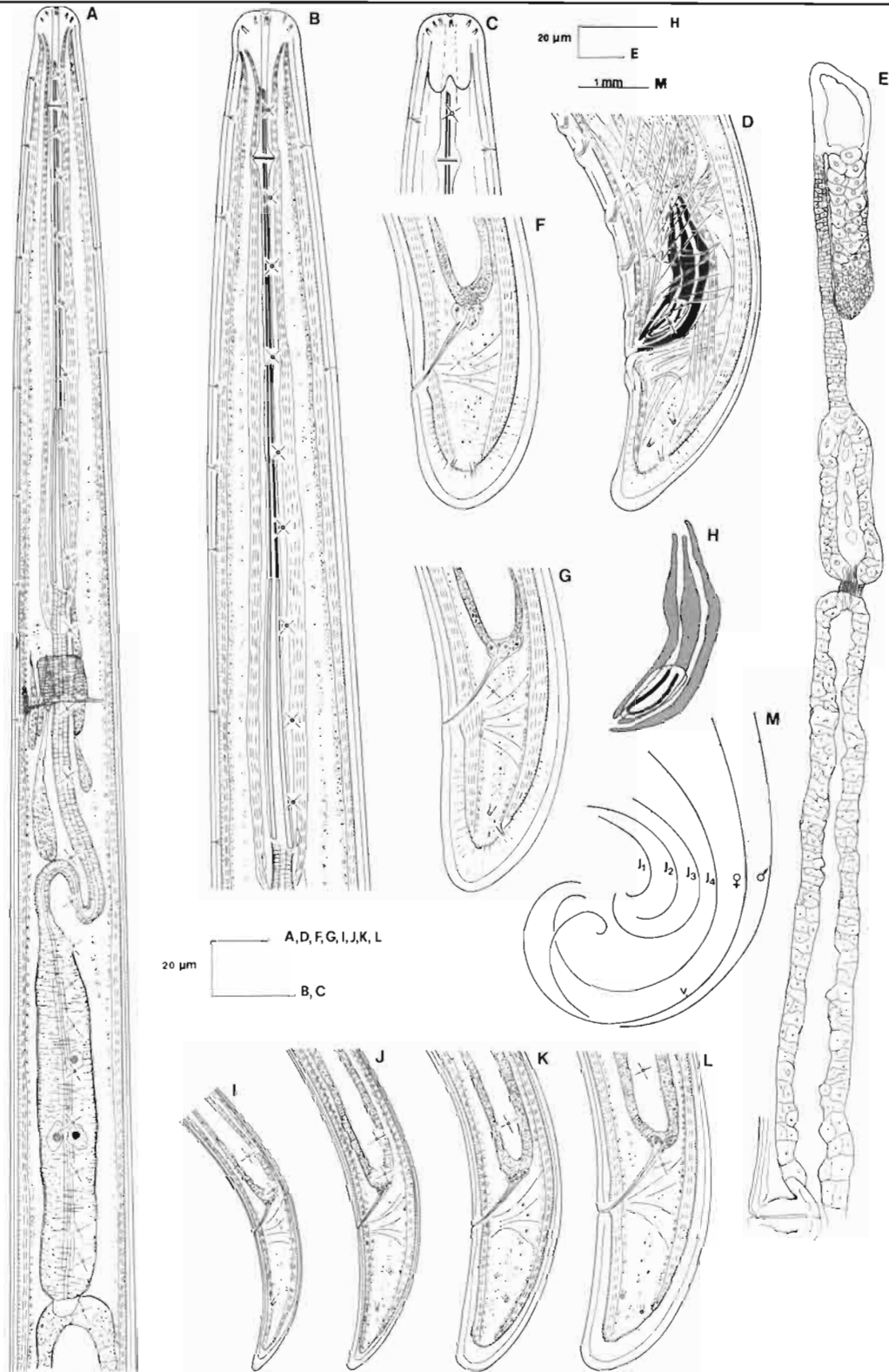


Fig. 2. *Longidorus apuloides* sp. n. A, B : Female anterior region; C : Head end (lateral sub-surface view); D : Male posterior region; E : Anterior branch of the female genital tract; F, G : Female posterior region; H : Spicules; I-L : Posterior region of juveniles, J1-J4, respectively; M : Posture of juveniles and adult stages.

Table 3. Morphometrics of *Longidorus apuloides* sp. n. (All measurements in μm [\pm S.D.] except L).

Stages	Holotype	females	males	J1	J2	J3	J4
n		11	21	3	6	16	22
L (mm)	8.6	8.4 \pm 0.81 (7.4-10.2)	8.3 \pm 0.68 (7.2-9.9)	2.2 \pm 0.50 (1.8-2.7)	2.8 \pm 0.66 (2.2-3.9)	4.0 \pm 0.33 (3.7-4.7)	5.8 \pm 0.63 (4.5-7.0)
a	155.0	155.0 \pm 13.73 (123.0-173.5)	164.5 \pm 10.18 (145.0-185.5)	84.0 \pm 8.84 (78.0-94.0)	94.5 \pm 13.58 (78.5-117.0)	112.5 \pm 8.91 (91.0-129.5)	136.0 \pm 11.94 (104.5-161.0)
b	17.5	21.0 \pm 3.53 (16.5-27.5)	19.5 \pm 2.11 (15.0-23.5)	9.0 \pm 2.06 (7.0-11.0)	10.0 \pm 2.59 (7.0-13.5)	12.0 \pm 1.0 (10.5-14.0)	15.0 \pm 2.46 (9.5-21.5)
c	202.5	192.0 \pm 23.07 (153.0-230.5)	157.5 \pm 19.17 (133.5-196.5)	43.5 \pm 5.57 (37.5-48.5)	53.0 \pm 6.63 (42.5-62.0)	73.0 \pm 8.97 (61.5-92.5)	109.5 \pm 10.23 (85.0-127.5)
c'	0.9	1.0 \pm 0.15 (0.8-1.3)	1.2 \pm 0.11 (1.0-1.5)	2.6 \pm 0.25 (2.4-2.9)	2.3 \pm 0.12 (2.2-2.5)	1.9 \pm 0.22 (1.6-2.3)	1.5 \pm 0.10 (1.3-1.6)
V	50.3	49.5 \pm 1.63 (46.0-52.0)	-	-	-	-	-
Lip region width	15.5	15.5 \pm 1.03 (14.5-17.5)	16.0 \pm 0.85 (13.5-17.5)	10.0 \pm 0.0 (10.0-10.0)	11.5 \pm 0.87 (10.5-13.0)	12.5 \pm 0.55 (11.5-13.5)	13.5 \pm 0.68 (12.5-15.5)
Lip region height	4.5	4.5 \pm 0.62 (3.5-5.5)	4.5 \pm 0.68 (3.0-6.0)	2.5 \pm 0.34 (2.5-3.0)	3.5 \pm 0.24 (3.0-3.5)	4.0 \pm 0.30 (3.5-4.0)	4.0 \pm 0.54 (3.0-4.5)
Odontostyle	123.0	119.0 \pm 4.61 (109.0-126.0)	118.0 \pm 4.34 (109.0-126.0)	66.5 \pm 2.65 (64.0-69.5)	77.0 \pm 4.06 (72.5-83.0)	88.5 \pm 2.74 (84.5-93.5)	104.5 \pm 3.96 (97.5-111.5)
Odontophore	48.0	43.5 \pm 5.13 (36.5-50.0)	45.5 \pm 3.33 (38.0-52.5)	32.0 \pm 5.71 (27.0-38.0)	34.0 \pm 2.64 (31.0-39.0)	41.0 \pm 3.72 (36.0-49.0)	44.5 \pm 4.32 (37.0-54.5)
Total stylet	171.0	162.5 \pm 6.59 (151.5-173.0)	163.5 \pm 6.58 (147.0-176.0)	98.5 \pm 7.83 (93.5-107.5)	111.0 \pm 5.68 (106.5-121.5)	129.5 \pm 4.27 (123.0-134.5)	149.0 \pm 5.31 (137.5-157.0)
Replacement odontostyle	-	-	-	77.5 \pm 3.83 (73.5-81.0)	91.0 \pm 3.89 (86.5-97.5)	103.0 \pm 1.71 (99.0-105.5)	117.5 \pm 4.03 (110.0-124.0)
Guide ring	35.5	33.5 \pm 1.95 (30.5-36.5)	33.5 \pm 1.80 (30.0-36.5)	20.5 \pm 1.76 (19.0-22.5)	23.5 \pm 0.96 (22.5-24.5)	27.0 \pm 1.31 (24.5-29.0)	29.5 \pm 1.87 (26.0-33.0)
Phar. bulb length	143.0	132.5 \pm 8.17 (120.5-142.0)	133.0 \pm 7.79 (121.0-145.0)	71.0 \pm 6.77 (64.0-77.5)	88.0 \pm 7.37 (81.0-100.0)	105.5 \pm 8.46 (90.0-118.0)	124.0 \pm 6.69 (110.5-135.5)
Phar. bulb width	20.5	19.5 \pm 1.86 (17.0-22.0)	19.0 \pm 1.74 (16.5-22.5)	13.0 \pm 1.56 (11.5-14.5)	14.0 \pm 1.31 (12.5-16.0)	16.5 \pm 1.22 (14.0-19.0)	19.0 \pm 1.91 (14.5-23.0)
Ant. genital branch	660.0	617.5 \pm 85.95 (494.0-759.0)	-	-	-	-	-
Post. genital branch	660.0	568.0 \pm 104.70 (453.0-765.0)	-	-	-	-	-
Ant. genital branch %	7.7	7.4 \pm 1.16 (5.9-9.5)	-	-	-	-	-
Post. genital branch %	7.7	6.8 \pm 1.34 (5.2-9.2)	-	-	-	-	-
Body diam. guiding ring	23.5	23.0 \pm 1.21 (20.5-24.5)	22.5 \pm 0.99 (20.5-24.0)	14.5 \pm 1.48 (13.0-16.0)	16.0 \pm 1.23 (14.5-18.0)	18.5 \pm 0.80 (17.0-20.0)	20.5 \pm 1.17 (18.5-23.0)
Body diam. mid-body	55.5	54.5 \pm 3.44 (50.0-60.0)	50.5 \pm 3.04 (44.5-57.0)	26.0 \pm 3.24 (23.0-29.5)	29.5 \pm 3.58 (23.5-33.5)	36.0 \pm 3.27 (30.5-43.0)	43.5 \pm 4.58 (34.0-51.5)
Body diam. anus level	44.5	43.5 \pm 2.09 (40.5-46.5)	42.5 \pm 2.58 (36.0-47.0)	19.5 \pm 3.28 (16.5-23.0)	22.5 \pm 3.23 (18.0-27.5)	28.5 \pm 1.88 (25.5-32.5)	36.5 \pm 2.72 (30.5-40.5)
Rectum	32.5	32.5 \pm 3.39 (27.5-37.5)	31.5 \pm 3.19 (26.5-36.5)	14.0 \pm 2.94 (11.0-17.0)	16.0 \pm 2.73 (11.5-19.5)	20.5 \pm 3.01 (16.5-27.5)	28.0 \pm 3.89 (21.0-35.5)
Tail	42.5	44.5 \pm 6.26 (36.0-59.0)	53.5 \pm 5.67 (42.5-66.5)	50.0 \pm 6.01 (46.0-57.0)	52.0 \pm 7.09 (42.5-63.5)	55.5 \pm 5.12 (44.0-63.0)	54.0 \pm 4.86 (44.0-60.5)
Hyaline tail tip	13.0	12.5 \pm 2.04 (9.5-15.5)	13.0 \pm 2.81 (7.5-17.5)	9.0 \pm 1.80 (7.5-11.0)	7.5 \pm 2.03 (4.5-10.0)	9.3 \pm 1.49 (7.0-11.5)	11.0 \pm 1.35 (9.0-13.5)
Prerectum	582.0	489.5 \pm 65.42 (388.0-564.5)	464.5 \pm 58.34 (354.0-558.5)	160.5 \pm 48.98 (106.0-200.0)	287.0 \pm 80.52 (200.0-417.5)	289.5 \pm 70.89 (211.5-476.5)	404.5 \pm 98.93 (200.0-576.5)
Spicules	-	-	72.5 \pm 6.57 (52.5-86.0)	-	-	-	-
Lat. guid. piece	-	-	17.0 \pm 2.32 (13.0-20.5)	-	-	-	-

slightly longer than dorsal lobe, female tail short, bluntly rounded and males abundant.

The new species is similar to *L. apulus* Lamberti & Bleve-Zacheo, 1977, *L. proximus* Sturhan & Argo, 1983 and *L. vineacola* Sturhan & Weischer, 1964. *L. apuloides* sp. n. differs from *L. apulus* in a longer and more slender body (7.4-10.2 vs 5.3-8.3 mm; "a" value 123-173.5 vs 123-154), longer odontostyle (109-126 vs 91-112 μm), shorter odontophore (36.5-50 vs 62-75 μm), slightly longer distance from oral aperture to guiding ring and tail length (30.5-36.5 vs 27-34 μm and 36-59 vs 31-46 μm , respectively), and vulva slightly more anteriorly situated ($V = 46-52$ vs 51-54), besides the presence of a large number of males; from *L. proximus* in having a more distinctly bilobed amphid pouch, a longer body (7.4-10.2 vs 6.5-8.2 mm), longer odontostyle (109-126 vs 102-112 μm) and longer tail (36-59 vs 31-44 μm), higher "c'" value (0.8-1.3 vs 0.73-0.95) vulva slightly more posteriorly situated ($V = 46-52$ vs 45-50), and abundant males (not observed in the type population of *L. proximus*), first stage juveniles with a longer body (1.8-2.7 vs 1.6-1.8 mm) and longer odontostyle (64-70 vs 56-59 μm); from *L. vineacola* in having slightly longer and more slender body ($L = 7.4-10.2$ vs 6.9-9.2 mm; "a" value 123-173 vs 120-149), lower "c" value (153-230 vs 186-247), higher "c'" value (0.8-1.3 vs 0.8-0.9), vulva slightly more anteriorly situated ($V = 46-52$ vs 49.7-54.3), longer odontostyle (109-126 vs 90-101 μm) and tail (36-59 vs 32-42 μm), amphid pouches in *L. vineacola* reaching well over halfway from anterior end to the guide ring, and spicules appear differently shaped in the male.

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