

# ***Longidorus artemisiae* sp. n. (Nematoda: Longidoridae) from roots of *Artemisia* sp., Rostov region, Russia**

**Tatjana V. Rubtsova\***, **Vladimir N. Chizhov\*\*** and **Sergei A. Subbotin\*\*\***

\*Prospect Mira 184-1, 34, Moscow, 129301, Russia,

\*\*Prospect Mira 184-1, 53, Moscow, 129301, Russia,

\*\*\*Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow, 117071, Russia.

Accepted for publication 27 January 1999

**Summary.** A description of *Longidorus artemisiae* sp. n., a bisexual species associated with *Artemisia* sp. near Starocherkassk, Rostov region, South part of European Russia is provided. The new species is characterised by its medium body length (4.7-6.6 mm), lip region anteriorly flattened, set-off from the rest of the body by a slight depression, pocket-shaped amphidial pouch, odontostyle 84-98  $\mu$ m and tail conical with bluntly rounded tip. Males are present in the population in the ratio 1:1 and have short spicules 39-49  $\mu$ m. Canonical discriminant analysis distinguished this new species from populations of *L. elongatus* and *L. attenuatus*.

**Key words:** *Longidorus artemisiae* sp. n., *L. elongatus*, *L. attenuatus*, Russia, morphometrics, canonical discriminant analysis.

In the summer of 1995 an unknown population of a longidorid species morphologically similar to *Longidorus elongatus* was found near Starocherkassk, Rostov region, southern part of European Russia. Subsequently, morphometric study of this material and multivariate analysis of several morphologically similar longidorids confirmed that it represented a new species (Rubtsova & Chizhov, 1998). A description and comparison of this new species with several populations of *Longidorus elongatus* and *L. attenuatus* are presented here.

## **MATERIAL AND METHODS**

Three populations of *Longidorus elongatus* from the Moscow region, collected from different plant-hosts (*Urtica dioica*, *Trifolium repens* and *Rubus idaeus*, respectively), a population of *L. elongatus* from Dundee, Scotland and a population of *L. attenuatus*, from Norfolk, England, each from grasses and a population of *L. artemisiae* sp. n. from Starocherkassk, Rostov region from *Artemisia* sp., were used in this study. Nematodes were extracted from soil samples by a decanting and sieving method (Flegg, 1967) and fixed in hot 3% TAF, and processed and mounted in glycerin on glass slides by Seinhorst's method (Seinhorst, 1959). The speci-

mens were examined and measured using Jenaval and Olympus light microscopes.

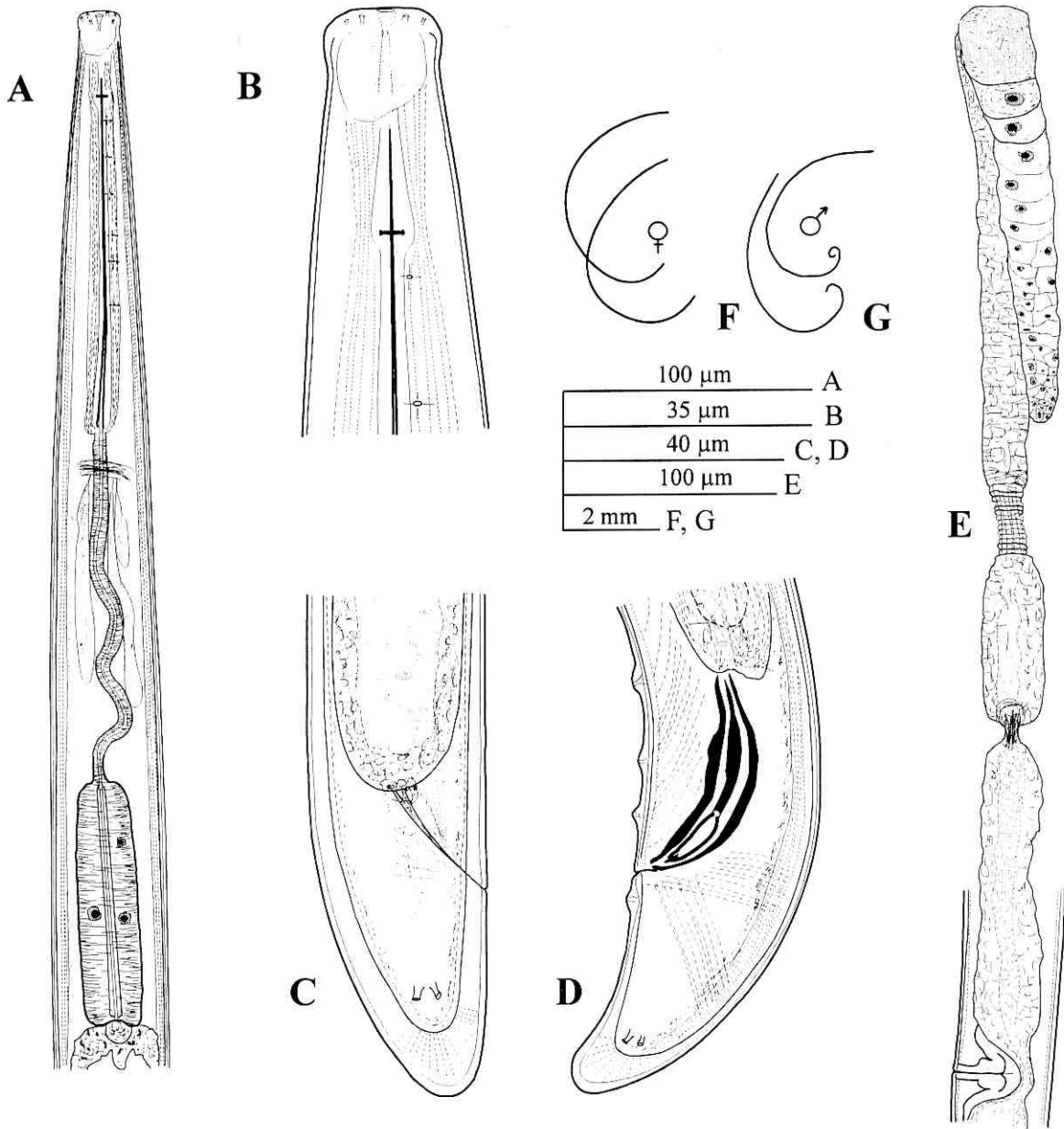
Morphometric data were analysed using the Statistica 4.3 computer package. The factor (principal component) analysis and discriminant analyses were used in the study. Prior selection of morphometric characters was made using the principal component analysis. Cluster analysis with the unweighted pair-group method using arithmetic averages (UPGMA) was applied to prepare a dendrogram showing the clustering of populations at different levels on a scale of dissimilarity.

## **DESCRIPTION**

### ***Longidorus artemisiae* sp. n. (Fig. 1 & Table 1)**

Measurements and other biometrical data of the type population are in Table 1.

**Female.** Body long and narrow, curved ventrally in a C-shape when relaxed by gentle heat. Lip region slightly expanded, anteriorly flattened, set-off from the rest of the body by a slight depression. Labial papillae prominent. Amphid large, more or less pocket-shaped without distinct lobes, extending more than half the distance between stomatal aperture and guide ring. Cuticle with fine transverse striae, 1.4-1.8



**Fig. 1.** *Longidorus artemisiae* sp. n. A: Female oesophageal region; B: Female head region; C: Female tail; D: Male tail; E: Anterior genital branch of female; F: Habitus of females; G: Habitus of males.

μm in the middle of body and thickening visibly towards the tail region. Six to seven lateral body pores in the odontostyle region. Width at guide ring level 23-28 μm. Nerve ring wide, situated at 180-200 μm from anterior end, at 20-25 μm posteriorly from the odontophore base. Odontostyle long and slender, 1.3-1.5 μm wide. Odontophore as parallel tube. Oesophageal bulb about 5 times as long as wide. Location of oesophageal gland nuclei typical for the genus. Dorsal oesophageal gland nucleus and paired

ventrosublateral gland nuclei situated at approximately 22-30% and about middle of the basal bulb, respectively. Vulva not or only slightly elevated as a transverse slit. Vagina perpendicular to body axis with slightly thickened cuticular lining, extending to half the corresponding body width, 24-30 μm long. Reproductive system amphidelphic, ovaries reflexed. Uteri about 140-220 μm long, thick walled, filled with sperm cells in fertile female. Tail conical with a broadly rounded tip, ventrally straight, dorsally

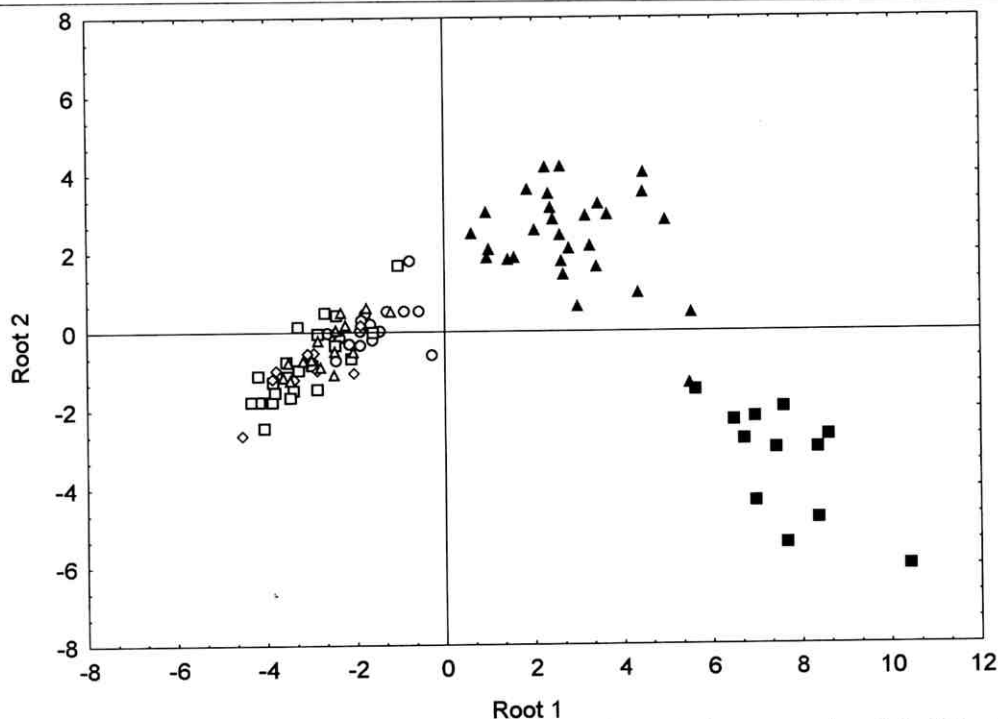


Fig. 2. Distribution of individual females of six *Longidorus* populations relative to axes 1 and 2 of the discriminant analysis using ten characters. ○ - *L. elongatus* (grasses, Dundee); □ - *L. elongatus* (*Urtica dioica*); ◇ - *L. elongatus* (*Rubus idaeus*); △ - *L. elongatus* (*Trifolium repens*); ▲ - *L. artemisiae* sp. n.; ■ - *L. attenuatus* (grasses, Norfolk).

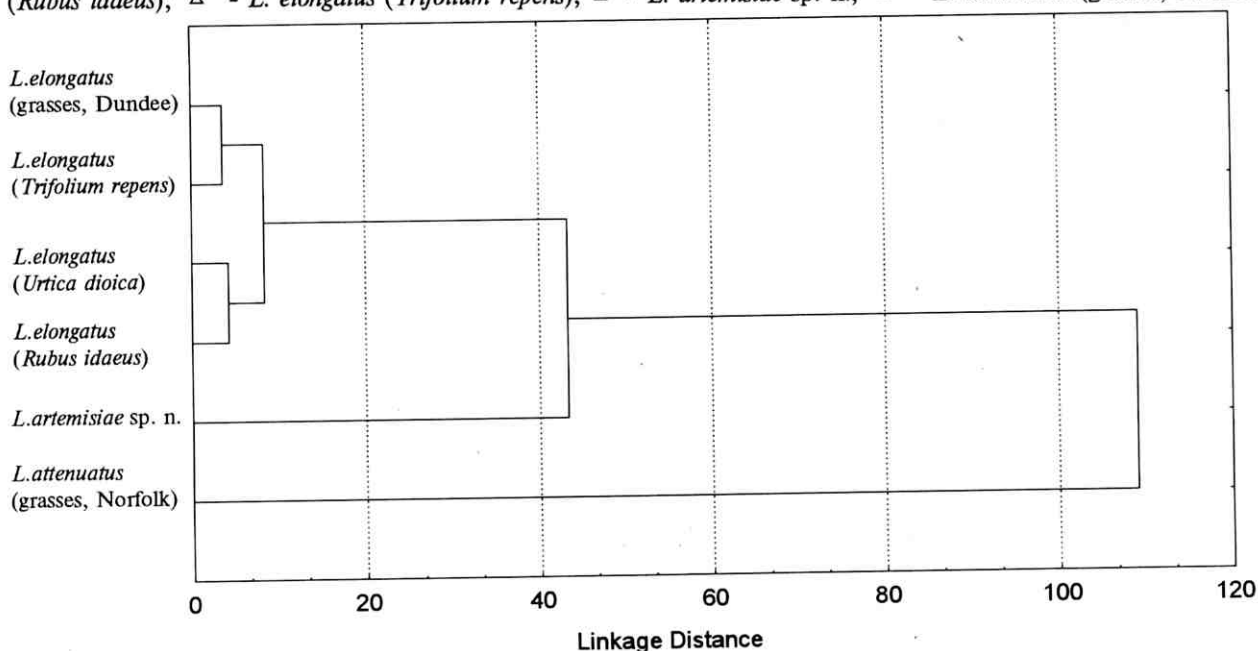


Fig. 3. Dendrogram showing the clustering of six longidorid populations at different levels of dissimilarity as computed by discriminant analysis of ten characters.

convex. Hyaline area of tail 8-12 μm. Two pairs of lateral pores present in the tail region.

A single female with two vulvas was observed in which the vaginas appear normal in length and structure, except that they are connected by an inner-vaginal tube of the otherwise normal uteri.

Distance between vulvas 22.5 μm. Both vulvas slightly protruding. The morphometrics of the abnormal female were similar to those of the normal females (Robbins & Rubtsova, 1996).

**Male.** Morphologically similar to female, more strongly curved in posterior end. Spicules massive.

**Table 1.** Morphometrics of *Longidorus artemisiae* sp. n., *L. elongatus* and *L. attenuatus* from different regions (All measurements in  $\mu\text{m}$  except for L and Ltgo).

Species	<i>L. artemisiae</i> sp. n.			<i>L. attenuatus</i>
Location	Starocherkassk			Norfolk
Plant-host	<i>Artemisia</i> sp.			Grasses
n	Holotype	28 females	26 males	12 females
L (mm)	6.2	5.9±0.1 (5.1-6.5)	5.6±0.1 (4.7-6.6)	6.2±0.1 (5.6-6.5)
a	135	133±1.9 (109-155)	133±1.7 (113-162)	148±1.9 (138-159)
b	15.2	15.1±0.3 (13.0-21.0)	13.9±0.3 (11.8-17.5)	20±0.5 (17-23)
c	158	146±3.9 (120-207)	135±2.0 (105-152)	121±2.6 (107-139)
c'	1.1	1.3±0.03 (1.0-1.6)	1.2±0.02 (1.0-1.6)	1.6±0.04 (1.5-1.8)
V%	49	49±0.3 (46-51)	-	49±0.6 (46-54)
Lip region width	14.0	15.3±0.2 (13.8-16.8)	15.8±0.2 (14.0-16.8)	13.3±0.3 (11.1-16.1)
Odontostyle	93	92±0.7 (84-98)	94±0.6 (90-98)	80±0.9 (75-87)
Odontophore	42	41±0.6 (39-50)	44±0.8 (39-48)	50±1.1 (43-55)
Total stylet	135	135±1.0 (126-140)	137±1.3 (132-146)	130±1.1 (124-136)
Distance to guide ring	27	29±0.3 (27-34)	30±0.3 (28-34)	31±0.5 (29-35)
Oesophagus length	409	412±4.5 (382-464)	416±6.3 (354-452)	321±7.7 (280-364)
Phar. bulbous length	112	110±1.5 (98-119)	111±1.2 (99-116)	-
Phar. bulbous width	22	21±0.4 (19-25)	20±0.3 (17-22)	-
Tail length	39	40±0.9 (31-46)	42±0.7 (36-50)	52±0.9 (45-56)
Prerectum	258	307±4.0 (232-394)	-	297±4.8 (179-370)
Rectum	28	29±0.3 (23-35)	-	25±0.2 (22-30)
Width at mid-body	45	46±0.7 (41-52)	44±1.0 (38-50)	43±0.7 (39-46)
Width at anus	34	32±0.4 (28-35)	35±0.6 (32-41)	32±0.6 (29-34)
Spicules	-	-	44±0.9 (39-49)	-
Ltgo* (mm)	5.7	5.3±0.1 (4.7-6.0)	5.3±0.1 (4.9-6.5)	5.8±0.1 (5.3-6.2)
L/Ltgo	1.08	1.09±0.0 (1.08-1.10)	1.08±0.0 (1.07-1.10)	1.06±0.0 (1.05-1.07)

\*Ltgo = Body length - oesophagus length - tail length.

An adanal pair of supplements followed by a row of eleven to thirteen others. Sperm present in male genital tract. Tail bluntly conoid with a rounded terminus. Presence of males in population in the ratio 1:1.

**Juvenile.** Only two specimens available, each representing the pre-adult stage. Body and lip region shape of similar to those of adults.

**Remark.** The odontophore length reported here for *L. attenuatus*, *L. elongatus* and *L. artemisiae* sp. n. was measured as the distance from the odontostyle/odontophore junction to the posterior end of the ventral sinus. However, the odontophore extends by approximately 20% of its length posteriorly to the end of the ventral sinus (Brown *et al.*, 1994).

**Diagnosis and relationships.** *Longidorus artemisiae* sp. n. is distinguished from other *Longidorus* species by the combination of the following characters: medium body length (4.7-6.6 mm) and odontostyle (84-98  $\mu\text{m}$ ), pocket-shaped amphidial pouch, slightly expanded anteriorly flattened lip region, indexes 'a' (109-162) and 'c' (105-207), short tail

(31-50  $\mu\text{m}$ ), and presence of males with short spicules (39-49  $\mu\text{m}$ ). The code for identifying the new species when using the polytomous key of Chen *et al.* (1997) is: A3-B23-C23-D3-E1-F3-G23-H2-I2.

*Longidorus artemisiae* sp. n. is most similar to *L. elongatus* (de Man, 1876) Micoletzky, 1922, but differs from it by having a relatively slender body [index a = 109-155 vs 71-115 or 76-123 according to our data and Hooper (1961), respectively], smaller mid-body width (41-52  $\mu\text{m}$  vs 49-78  $\mu\text{m}$ ), shorter tail (31-46  $\mu\text{m}$  vs 38-58  $\mu\text{m}$ ) in females, slightly expanded lip region, presence of males with shorter spicule length (39-48  $\mu\text{m}$  vs 55-64 or 58  $\mu\text{m}$ , respectively). The new species is also similar to *L. attenuatus* Hooper, 1961, which has symmetrically bilobed amphids, more elongated tail (c' = 1.5-1.8, tail length 45-56  $\mu\text{m}$ ) and shorter odontostyle length [75-87  $\mu\text{m}$ , 73-84  $\mu\text{m}$  according to our data and Hooper (1961), respectively]; *L. proximus* Sturhan & Argo, 1983 which differs by having a longer body [6.5-8.2 mm, 6.5-9.2 mm according to Sturhan & Argo (1983) and Roca (1986), respectively] and odontostyle (102-112  $\mu\text{m}$ , 103-112  $\mu\text{m}$ ), wider lip

**Table 1 (continued).** Morphometrics of *Longidorus artemisiae* sp. n., *L. elongatus* and *L. attenuatus* from different regions (All measurements in  $\mu\text{m}$  except for L and Ltgo).

Species	<i>L. elongatus</i>				
	Dundee	Moscow region			
		Grasses	<i>Rubus idaeus</i>	<i>Urtica dioica</i>	<i>Trifolium repens</i>
Location	Grasses	<i>Rubus idaeus</i>	<i>Urtica dioica</i>	<i>Trifolium repens</i>	
Plant-host	Grasses	<i>Rubus idaeus</i>	<i>Urtica dioica</i>	<i>Trifolium repens</i>	
n	21 females	22 females	23 females	25 females	13 males
L (mm)	5.2±0.1 (4.5-6.4)	5.4±0.1 (4.8-6.1)	5.6±0.1 (4.9-6.7)	5.2±0.1 (4.5-6.3)	5.4±0.1 (4.4-6.3)
a	91±2.1 (81-105)	86±1.5 (78-95)	82±1.7 (74-104)	83±1.5 (78-98)	95±1.7 (71-115)
b	12.7±0.3 (10.8-15.6)	12.1±0.3 (10.5-13.6)	12.4±0.2 (11.0-15.6)	12.2±0.2 (11.2-14.3)	11.2±0.3 (9.7-12.5)
c	109±2.5 (90-123)	1011.9 (90-110)	1122.6 (97-145)	1092.3 (92-136)	972.3 (87-111)
c'	1.3±0.02 (1.1-1.5)	1.3±0.03 (1.1-1.4)	1.1±0.02 (1.0-1.4)	1.2±0.03 (1.0-1.3)	1.2±0.03 (1.1-1.4)
V%	50±0.3 (47-52)	48±0.4 (46-51)	49±0.4 (47-51)	50±0.5 (42-54)	-
Lip region width	14.2±0.1 (12.9-15.8)	14.2±0.1 (13.7-14.4)	14.0±0.2 (13.0-16.0)	14.1±0.1 (12.5-14.4)	14.7±0.2 (14.5-15.9)
Odontostyle	93±1.0 (86-98)	93±0.4 (92-95)	95±0.7 (90-99)	86±0.9 (75-92)	89±0.6 (84-93)
Odontophore	53±1.2 (46-57)	50±2.0 (37-60)	44±0.8 (38-49)	51±1.3 (34-57)	48±0.8 (43-52)
Total stylet	144±1.4 (135-155)	142±1.9 (129-152)	139±1.1 (130-148)	35±1.4 (121-146)	137±1.0 (130-144)
Distance to guide ring	33±0.3 (32-34)	32±0.3 (30-34)	32±0.3 (29-35)	33±0.3 (32-35)	32±0.4 (29-40)
Oesophagus length	405±5.1 (365-446)	455±7.3 (406-487)	454±5.4 (383-516)	430±3.6 (400-452)	447±9.5 (423-469)
Phar. bulb length	96±1.3 (86-103)	129±1.2 (120-140)	112±1.1 (98-129)	104±0.9 (98-115)	103±0.8 (95-109)
Phar. bulb width	23±0.4 (17-29)	23±0.3 (20-29)	25±0.2 (22-29)	24±0.3 (23-29)	23±0.2 (20-26)
Tail length	49±0.8 (41-58)	54±1.0 (49-58)	49±0.9 (38-58)	49±0.8 (45-55)	47±0.8 (44-52)
Prerectum	345±4.3 (273-418)	420±4.7 (319-551)	368±4.9 (290-464)	327±5.0 (232- 447)	-
Rectum	21±0.2 (17.2-23.0)	15±0.5 (8.6-20)	17±0.4 (14-20)	19.3±0.6 (11.5-25.8)	-
Width at mid-body	56±1.1 (49-61)	63±1.1 (58-70)	67±1.5 (49-78)	59±1.0 (49-66)	60±1.2 (52-70)
Width at anus	39±0.6 (36-42)	41±0.8 (37-46)	43±0.7 (35-47)	42±0.8 (41-46)	44±0.8 (41-46)
Spicules	-	-	-	-	59±0.7 (55-64)
Ltgo (mm)	4.7±0.1 (4.1-5.9)	4.9±0.1 (4.4-5.6)	5.1±0.1 (4.4-6.2)	4.8±0.1 (4.0-5.8)	4.6±0.1 (4.4-4.8)
L/Ltgo	1.10±0.0 (1.08-1.11)	1.10±0.0 (1.09-1.10)	1.10±0.0 (1.08-1.11)	1.10±0.0 (1.08-1.11)	1.11±0.0 (1.10-1.11)

region (17-19  $\mu\text{m}$ , 17-20  $\mu\text{m}$ ), abnormal position of oesophageal gland nuclei, more bluntly conoid or almost hemispherical tail ( $c' = 0.73-0.95$ , 0.5-0.8), longer spicules (59-79  $\mu\text{m}$ ); *L. apulus* Lamberti & Bleve-Zacheo, 1977 has almost symmetrically bilobed amphidial pouches, a longer body (5.3-8.3 mm), odontostyle (91-112  $\mu\text{m}$ ) and odontophore (62-73  $\mu\text{m}$ ), males are very rare (Lamberti & Bleve-Zacheo, 1977). *Longidorus artemisiae* sp. n. is also similar to *L. euonymus* Mali & Hooper, 1974 which has a longer body (6.03-7.63 mm) and tail (37-54  $\mu\text{m}$ ), longer odontophore (45-76  $\mu\text{m}$ ) and absence of males according to the original description by Mali & Hooper, (1973). The new species can be also distinguished from two Bulgarian populations of *L. euonymus*: the Kostinbrod population which have longer bodies (6.0-7.0 mm) and longer odontophore lengths (54.3-56  $\mu\text{m}$ ) and from the Sandanski population which have shorter odontostyles (74.3-81  $\mu\text{m}$ ), shorter distances to the guide ring (24.6-25.7  $\mu\text{m}$ ), longer tails (40-57  $\mu\text{m}$ ) and narrower lip regions (12-13.7  $\mu\text{m}$ ) (Lamberti *et al.*, 1997). A male of *L.*

*euonymus* described by Roca (1991) has a longer odontophore (50.5  $\mu\text{m}$ ), narrower lip region (13.5  $\mu\text{m}$ ) and longer spicules (56.5  $\mu\text{m}$ ), than do the males of *L. artemisiae* sp. n. *Longidorus artemisiae* sp. n. is also similar to *L. dunensis* Brinkman, Loof & Barbez, 1987 which has symmetrically bilobed amphids, a longer stylet (135-165  $\mu\text{m}$ ), wider anal body width (32-38  $\mu\text{m}$ ) and absence of males (Brinkman *et al.*, 1987).

**Type host and locality.** All specimens of *L. artemisiae* sp. n. collected July, 1995 from the rhizosphere of *Artemisia* sp. growing in a sandy soil, 200-300 m from the right bank of the Don river, near bridge, Starocherkassk, Aksai district, Rostov region, southern part of European Russia. The population density was 2-5 specimens per 100 g of soil.

**Type materials.** Holotype female, paratype females and males deposited in the nematode collection of the Institute of Parasitology, Moscow, Russia; four paratype females and one paratype male deposited in the nematode collection of Gent University,

Belgium; one paratype female and two paratype males deposited in the collection at IACR-Rothamsted, England; several paratype females and males deposited in the nematode collection of the University Arkansas, Department of Plant Pathology, USA and the nematode collection of the Zoological Institute, St. Petersburg, Russia.

**Results of the factor and discriminant analysis.** Ten of 21 variables measured or calculated: a, b, c, c', tail length, oesophagus length, widths at mid-body and anus levels, L/Ltgo, body length representing most of the variation between populations were selected for discriminant analysis. Of the 4 canonical variables calculated, the first two accounted for 93.1% and the first three - 97.8% of the variation. Two dimensional scatterplots of individual data of canonical variables used for the first two canonical axes were generated. Figure 2 shows the relative positions and grouping of individual female data of each population on the scatterplots. Three distinct groups of populations were distinguished based on the scatterplots of the first two axes. A dendrogram showing the clustering of five populations was computed from the results of the UPGMA analysis and are presented in Figure 3. The populations of *L. elongatus* clustered to form one group and *L. artemisiae* sp. n. and *L. attenuatus* each formed separate groups. Thus, the result of discriminant analysis supported the separation of *L. artemisiae* sp. n. from two morphologically similar longidorid species.

## ACKNOWLEDGEMENTS

The authors thank Irina Molokanova for help with computing and Derek J.F. Brown for supplying cultures of *L. elongatus* and *L. attenuatus*.

## REFERENCES

- Brinkman, H., Loof, P.A.A. & Barbez, D. 1987. *Longidorus dunensis* n. sp. and *L. kuiperi* n. sp. from the sand dune coastal region of the Netherlands (Nematoda: Longidoridae). *Revue de Nématologie* 10: 299-308.
- Brown, D.J.F., Grunder, J., Hooper, D.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.
- Flegg, J.J. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting sieving technique. *Annals of Applied Biology* 60: 429-437.
- Hooper, D.J. 1961. A redescription of *Longidorus elongatus* (de Man, 1876) Thorne & Swanger, 1936 (Nematoda: Dorylaimidae) and description of five new species of *Longidorus* from Great Britain. *Nematologica* 6: 237-257.
- Lamberti, F. & Bleve-Zacheo, T. 1977. Two new species of *Longidorus* (Nematoda: Longidoridae) from Italy. *Nematologia mediterranea* 5: 73-83.
- Lamberti F., Iovev, T., Choleva B., Brown D.J.F., Agostinelli, A. & Radicci, V. 1997. Morphometric variation and juveniles stages of some longidorid nematodes from Bulgaria with comments on the number of juvenile stages of *Longidorus africanus*, *L. closelongatus* and *Xiphinema santos*. *Nematologia mediterranea* 25: 213-237.
- Mali, V.R. & Hooper, D.J. 1974. Observations on *Longidorus euonymus* n. sp. and *Xiphinema vuittenezi* Luc *et al.*, 1964 (Nematoda: Dorylaimida) associated with spindle trees infected with *Euonymus* mosaic virus in Czechoslovakia. *Nematologica* 19 (1973): 459-467.
- Robbins, R.T. & Rubtsova, T.V. 1996. A specimen of *Longidorus elongatus* with two vulvas from Russia. *Nematropica* 26: 305.
- Roca, F. 1986. A bisexual population of *Longidorus proximus* Sturhan *et* Argo (Nematoda: Dorylaimida) from Greece. *Nematologia mediterranea* 14: 155-158.
- Roca, F. 1991. The undescribed male of *Longidorus euonymus* Mali *et* Hooper (Nematoda: Dorylaimida) from Southern Italy. *Nematologia mediterranea* 19: 129-130.
- Rubtsova, T.V. & Chizhov, V.N. 1998. Morphometric analysis of populations of *Longidorus elongatus* and *Xiphinema diversicaudatum* from the European part of Russia and the Ukraine. *Russian Journal of Nematology* 6: 77.
- Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69.
- Sturhan, D. & Agro, D. 1983. Studies on *Longidorus closelongatus* Stoyanov and *L. cohnii* Heyns, with description of *L. proximus* sp. nov. (Nematoda, Dorylaimida). *Revue de Nématologie* 6: 57-64.

Рубцова Т.В., Чижов В.Н., Субботин С.А. *Longidorus artemisiae* sp. n. (Nematoda: Longidoridae) из корней *Artemisia* sp., Ростовская область, Россия.

**Резюме.** Описывается *Longidorus artemisiae* sp. n. из ризосферы полыни, произрастающей в пойме реки Дон в окрестностях г. Старочеркаска Ростовской области. Новый вид имеет среднюю длину тела (4.7-6.6 мм), плоскую область губ, отделенную от тела, кармановидный амфид.