Characterisation of *Rotylenchus pumilus* (Perry, 1959) Sher, 1961 (Tylenchida: Hoplolaimidae) from Russia

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Summary. During nematological surveys in Moscow and the Chechen Republic, *Rotylenchus pumilus* was found in these two regions. This species is rare in Russia and has not yet been characterised from this country. The morphological characters and morphometrics of the Moscow and Chechen populations were similar. These populations are characterised by a small female body length of about 450 to 650 µm, a hemispherical lip region with 3-4 annuli and continuous with the body contour, stylet length of 23-26 µm, lateral field areolated only at the pharyngeal region, vulva position at 61-66%, tail tip with thickened cuticle and presence of males. Molecular analysis showed the identity of the sequences of the D2-D3 expansion segments of the 28S rRNA gene of these populations, while the sequences of the *COI* gene were only 97.76% similar to each other.

Key words: morphometric data, nematode, phylogeny, *Rotylenchus*, taxonomy.

Spiral nematode, *Helicotylenchus pumilus* was originally described by Vernon G. Perry (Perry *et al*., 1959) from soil samples collected near the roots of Kentucky bluegrass (*Poa pratensis* L.) in Madison, Wisconsin, USA. Sher (1961) transferred this species to the genus *Rotylenchus* and later (Sher, 1965) redescribed it based on paratypes and topotypes supplied by V.G. Perry. In addition to the type locality, *R. pumilus* has also been reported in Austria, Poland, Hungary, Sweden, England (Sher, 1965), Slovakia (Sabová, 1975), Bulgaria (Katalan-Gateva, 1980), Tajikistan (Kankina & Tebenkova, 1980), Switzerland (Güntzel *et al*., 1987), Germany (Sturhan, 2014), France (Germani & Scotto La Massese, 2002), Spain (Talavera & Navas, 2002), China (Liu, 2004) and Ukraine (Skwiercz *et al*., 2022).

In Russia, *R. pumilus* was reported from the European part by Metlitskaya (1984) and Solovyova (1986); however, the authors did not provide any descriptions. The aim of this research was to conduct the morphometric and molecular characterisation of Russian populations of *R. pumilus*.

MATERIALS AND METHODS

Nematode sampling. In 2023, soil samples with this species were collected near the roots of timothy grass (*Phleum pretense* L.) and Kentucky bluegrass (*Poa pratensis* L.) in the Kolomenskoye Park of Moscow and near the roots of common medlar (*Mespilus germanica* L.) in Koren-Benoi village (Nozhay-Yurtovsky District of the Chechen Republic). The geographical location of the sampling sites were 55°39'19.9"N, 37°40'09.5"E and 42°59'02.2"N, 46°19'45.1"E, respectively. Nematodes were extracted using a modification of the funnel method (Baermann, 1917).

Morphological study. For morphological studies, the nematodes were killed with hot water (60°C for 3 min), fixed in a 5% formalin solution, and mounted in glycerin on slides using the Seinhorst's technique (Seinhorst, 1959). Photomicrographs were taken by the Omax A35140U microscope camera mounted on the Mikmed-6 microscope (LOMO, Russia).

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Molecular and phylogenetic study. Molecular studies were performed using the scientific equipment of the Core Research Facility of the 'Bioengineering' Centre (Moscow, Russia). For this work, nematodes frozen in distilled water were used. Nematode DNA was extracted using the Wizard kit (Promega, USA) according to the manufacturer's instructions with three replicates. Each replicate was a test tube with several nematode specimens. The D2-D3 expansion segments of the 28S rRNA gene were amplified using the forward primer 391F (5'-AGC GGA GGA AAA GAA ACT AA-3') (Nadler & Hudspeth, 1998) and the reverse primer D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (Nunn, 1992). PCR conditions included denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 70 s, annealing at 55°C for 70 s and extension at 72°C for 1.5 min and a final step at 72°C for 7 min. The partial cytochrome c oxidase subunit 1 gene was amplified with the forward primer JB3 (5'-TTT TTT GGG CAT CCT GAG GTT-3') and the reverse primer JB5 (5'-AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG-3') (Derycke *et al*., 2005). PCR conditions included denaturation at 94°C for 5 min, 4 cycles of (94°C for 30 s, 54°C for 30 s and temperature decreasing with 1°C for each cycle, 72°C for 30 s) followed by 36 cycles of (94°C for 30 s, 50°C for 30 s, 72°C for 30 s), and a final extension of 10 min at 72°C. The amplifications were performed in a Tetrad thermal cycler (Bio-Rad, USA). PCR products were purified using the Wizard PCR Preps kit (Promega, USA). The sequencing of the PCR products was carried out with the same primers using the genetic analyser ABI 3730 (Applied Biosystems, USA). Low-quality segments of sequences at the 5' and 3' ends were removed. Newly obtained sequences were submitted to the GenBank database under accession numbers OR899232 (28S rRNA gene) and OR899239 (*COI* gene) for the Moscow population and OR899238 (28S rRNA gene) and OR899240 (*COI* gene) for the Chechen population of *R. pumilus*. New sequences were aligned with other sequences of closely related species of *Rotylenchus* deposited in GenBank NCBI (https://www.ncbi.nlm.nih.gov/genbank/) by Clustal W in MEGA 7 using the default parameters. Phylogenetic trees were inferred using the Maximum Likelihood (ML) method under the GTR + I + G model of evolution using the program MEGA 7 (Kumar *et al*., 2016). To obtain an estimate of the support for each node, a bootstrap analysis using 1000 replicates was performed. Bootstrap support is given on appropriate clades for the ML tree.

RESULTS AND DISCUSSION

Rotylenchus pumilus (Perry, 1959) Sher, 1961 (Fig. 1)

Morphological features of the Moscow and Chechen populations of *R. pumilus* are the same, while morphometric characters differ slightly.

Measurements. See Tables 1 and 2.

Female. Body in spiral shape. Cuticle clearly annulated. Lip region hemispherical or rounded, not set off, with 3-4 annuli and a narrow striated belt bordering rest of body. Lateral field with four lines, areolated only in pharyngeal region. Stylet knobs rounded, about 5 µm across. Dorsal gland orifice 3.5-6 µm posterior from knobs. Median bulb oval. Excretory pore at level of pharyngo-intestinal junction. Hemizonid distinct, located 0-1.5 annuli anterior to excretory pore, extending for *ca* 2-3 body annuli width. Pharyngeal lobe extends for 21-28 µm or 0.8-1.2 body widths posterior to pharyngo-intestinal junction. Phasmid located at anus level. Reproductive system with both genital branches equally developed. Epiptygma single or double, variable in size. Tail conoid, dorsally more curved, with 8 to 14 annuli. Cuticle at tail tip usually thickened and its annulation irregular.

Male. Body C-shaped or 6-shaped after fixation. Lip region hemispherical or rounded, not set off, with 3-4 annuli and a narrow striated belt bordering rest of body. Stylet slightly shorter than that of the female, its knobs about 4 µm across. Median bulb oval. Excretory pore at level of pharyngo-intestinal junction. Hemizonid distinct, located 0-2 annuli anterior to excretory pore, extending for *ca* 2-2.5 body annuli width. Pharyngeal lobe extends for 21-25 µm or about one body width posterior to pharyngo-intestinal junction. Testis single, outstretched. Spicules ventrally arcuate. Gubernaculum thin, with recurved distal portion. Phasmid located at cloacal aperture level.

The matrix code (Castillo & Vovlas, 2005) of the Moscow and Chechen populations of *R. pumilus* is as follows: A2-3, B1-2, C1, D4, E1, F2, G3, H3, I2, J1, K2.

Remark. In the sample from the Chechen Republic, males were almost as common as females (approximate ratio 2:3), while in the sample from Moscow, males were rare (approximate ratio 1:10).

The morphological characteristics of the Moscow and the Chechen population of *R. pumilus* resemble the original description of this species and redescription by Sher (1965).

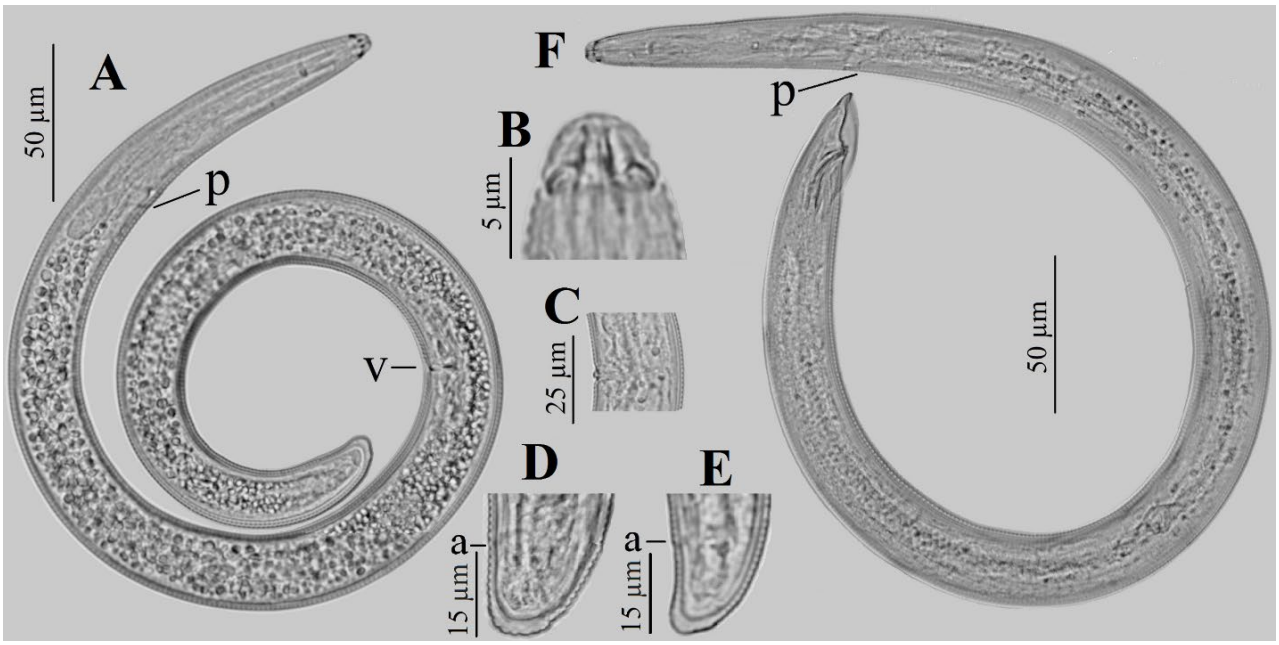

Fig. 1. Light microphotographs of *Rotylenchus pumilus*. A: Entire female, B: Lip region, C: Vulval region with prominent epiptygma, D, E: Posterior end of female, F: Entire male. Abbreviations: a – anus, p – excretory pore, v – vulva.

Table 1. Measurements and ratios of females of *Rotylenchus pumilus* from Russia and their comparison with those from the literature. Measurements are in µm and in the form: mean ± S.D. (range).

Locality / Character	Moscow (This study)	Chechen Republic (This study)	Type locality (Perry *et al.*, 1959)	Type locality (Sher, 1965)	France (Germani & Scotto La Massese, 2002)	California, USA (Cantalapiedra-Navarrete *et al.*, 2013)
n	15	12	10	20	24	6
L	546.15±52.3 (471.7-644)	515.5±45.3 (453-587.7)	610-720	570-740	560±40 (470-620)	825±50.4 (773-906)
a	20.3±3.35 (15.6-25.9)	21±1.95 (17.4-23.9)	20.0-23.7	20-28	23.4±2.2 (19-27)	26.9±1.5 (25.4-28.9)
b	4.9±0.3 (4.3-5.6)	5.3±0.4 (4.5-5.9)	5.0-6.6	5.7-7.5	5.8±0.35 (5.2-6.4)	11.1 ± 0.7 (10.3-12.2)
b'	4.1±0.3 (3.8-4.8)	4.4±0.3 (3.6-5)	-	4.8-5.8	4.9±0.35 (4.4-5.9)	6.5±0.4 (5.9-7.0)
c	35.17±3.5 (30.9-45.4)	34.12±3.87 (31.0-41.1)	33-62	32-59	48.4±4.3 (39.3-59.3)	46.6±2.9 (42.9-50.2)
c'	0.98±0.09 (0.73-1.15)	0.96±0.08 (0.87-1.1)	-	-	0.79±0.07 (0.63-0.93)	0.9±0.1 (0.8-1.0)
V%	64.72±1.14 (62.2-66.4)	63±1.39 (61.2-65.5)	58-64	54-63	62.3±1.8 (58.1-65.2)	56.7±1.8 (54.0-59.0)
Lip region height	4±0.4 (3.5-4.5)	4±0.3 (3.5-4.5)	-	-	-	-
Lip region width	8.0 ± 0.32 (7.3-8.6)	8.16±0.36 (7.6-8.8)	-	-	-	-
Stylet length	24.8±0.76 (23.7-26)	24.1±0.8 (22.9-25.3)	22-28	23-26	27.9±1.1 (26-29)	29.3±1.4 (27.0-31.0)
Dorsal gland opening from stylet base	4.0±0.4 (3.5-4.5)	5.1±0.8 (3.5-6)	-	-	5.1±0.4 (4.5-6)	4.3±0.5 (4.0-5.0)
Anterior end to excretory pore	100.7±8.2 (87.5-114.4)	94.1±6.0 (87.5-107.4)	-	-	94.6±6 (85-104)	105±9.5 (97-117)
Tail length	15.6±1.7 (12.2-18.4)	15.37±1.24 (13.8-16.9)	-	-	11±2.1 (9-13)	17.8±1.5 (16.0-20.0)
Tail annuli number	11.26±1.33 (9-14)	10.1±1.73 (8-13)	-	-	-	10.7±0.8 (10-12)

Table 2. Measurements and ratios of males of *Rotylenchus pumilus* from Russia and their comparison with those from the literature. Measurements are in μm and in the form: mean ± S.D. (range).

Character \ Locality	Moscow (This study)	Chechen Republic (This study)	Type locality (Perry *et al*., 1959)	Type locality (Sher, 1965)	France (Germani & Scotto La Massese, 2002)
n	3	12	10	10	12
L	565.8±21.1 (550.8-589.9)	561.6±45.68 (479.4-635.5)	560-640	480-650	550±50 (450-600)
a	28.2±1.4 (26.9-29.5)	27.33±4.5 (18.9-36.26)	25.2-28.0	24-29	32±1.5 (30-35)
b	5.3±0.2 (5.0-5.5)	5.1±0.4 (4.5-5.9)	4.3-5.8	5.0-6.1	5.9±0.4 (5.4-6.4)
b'	4.4±0.3 (4.2-4.7)	4.5±0.3 (3.8-4.9)	-	4.2-5.1	5±0.3 (4.5-5.7)
c	27.3±2.1 (24.9-28.9)	24.48±2.57 (23-31.5)	25-34	25-30	31.3±2.2 (28.5-34.9)
c'	1.7±0.2 (1.4-1.85)	1.7±0.2 (1.3-2.0)	-	-	1.33±0.1 (1.16-1.56)
Lip region height	4.0±0.2 (3.8-4.2)	4.08±0.43 (3.5-5)	-	-	-
Lip region width	8.0±0.2 (7.8-8.2)	7.7±0.4 (6.7-8.2)	-	-	-
Stylet length	23.2±0.5 (22.6-23.5)	22.7±0.8 (21.1-23.5)	22-26	20-24	24±0.5 (23.5-25.0)
Dorsal gland opening from stylet base	4.7±0.8 (4.0-5.7)	4.7±0.6 (4.0-5.9)	-	-	-
Anterior end to excretory pore	100.9±3.8 (96.6-103.5)	99.07±5.0 (90.5-104.6)	-	-	89.6±6 (87-97)
Tail length	20.8±1.3 (19.7-22.3)	20.33±2.08 (15.2-22.8)	-	-	18±2 (16-21)
Spicules	26.0±0.4 (25.7-26.4)	25.8±1.18 (24-28.6)	-	23-27	-
Gubernaculum	8.7±0.6 (8-9)	9.2 ±0.7 (8-10)	-	8-10	-

A few minor variations were observed, including the shorter body length of females (453-644 *vs* 570-740 μm) and, consequently, lower values of the a ratio (16-25.9 *vs* 20-28), the b ratio (4.3-5.9 *vs* 5.7-7.5) and the b' ratio (3.6-5 *vs* 4.8-5.8). These differences are considered as intraspecific variations. A shorter body length in females than that in the original description can be observed in the description of the species from France (Germani & Scotto La Massese, 2002).

Meanwhile, the morphometric characters of the population from California (Cantalapiedra-Navarrete *et al*., 2013) did not overlap with those of paratypes and topotypes (Sher, 1965), specifically: body length (773-906 *vs* 570-740 μm), b (10.3-12.2 *vs* 5.7-7.5), b' (5.9-7.0 *vs* 4.8-5.8), stylet length (27-31 *vs* 23-26 μm), more anterior position of the excretory pore (excretory pore usually located near the middle of isthmus level *vs* excretory pore at level of pharyngo-intestinal junction), shorter pharyngeal glands and absence of distinct epiptygma *vs* its presence in the redescription of *R. pumilus* by Sher (1965). Based on the above comparison, we suggest that the population from California actually belongs to another species, not to *R. pumilus*.

Molecular characterisation. Despite the wide geographical distribution of *R. pumilis*, there are only two sequences of the 18S rRNA gene and one sequence of the 28S rRNA gene in GenBank. These sequences belong to the population from California, for which we have concluded that it belongs to another species.

The sequences of the D2-D3 expansion segments of the 28S rRNA gene obtained from different individuals of Moscow and Chechen populations in this study were identical to each other. At the same time, the sequences of the *COI* gene were identical within each population, but the sequences of the Moscow population were only 97.76% similar to those of the Chechen population.

The sequences of the D2-D3 expansion segments of the 28S rRNA gene were most similar to the *R. goodeyi* sequence from Belgium (MK114125; Etongwe *et al*., 2020), with 93.48% similarity. The similarity with the population, described as *R. pumilus* from California, USA (JX015423) and considered here as belonging to another species, was only 91.44%.

In the partial 28S rRNA gene phylogenetic tree

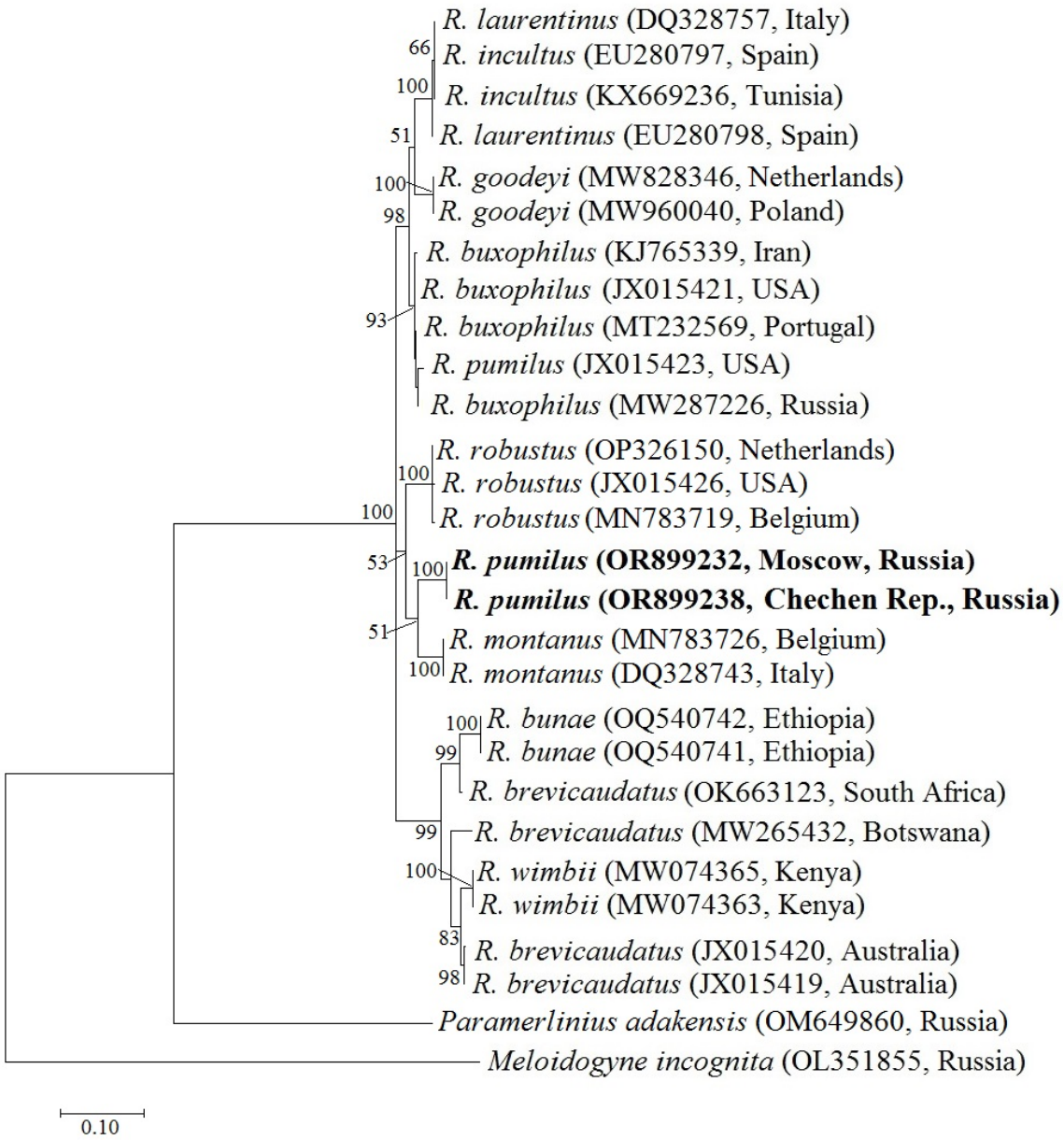

Fig. 2. Phylogenetic relationships of *Rotylenchus pumilus* from Moscow and the Chechen Republic with other *Rotylenchus* species as inferred from the ML method using the D2-D3 expansion segments of the 28S rRNA gene sequences under the GTR + I + G model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The new sequences are indicated in bold.

(Fig. 2), the population of *R. pumilus* from California is close to different populations of *Rotylenchus buxophilus*, while the Russian populations are on a distant branch. Morphometrically similar species such as *R. brevicaudatus*, *R. incultus* and *R. pumilus* are loosely related to each other on the tree.

In the partial *COI* gene phylogenetic tree (Fig. 3), the populations of *R. pumilus* from Moscow and the Chechen Republic are clustered together with *Rotylenchus* sp. from Belgium (MN782382, Etongwe *et al*., 2020). The sequence of the Chechen population shared a 98.06% similarity with the sequence from Belgium, and the sequence of the Moscow population shared a 97.78% similarity with the sequence from Belgium. Some branches inside the tree have a weak bootstrap support. In our opinion, it occurred because only a few sequences of species closely related to *R. pumilus* have been deposited in GenBank.

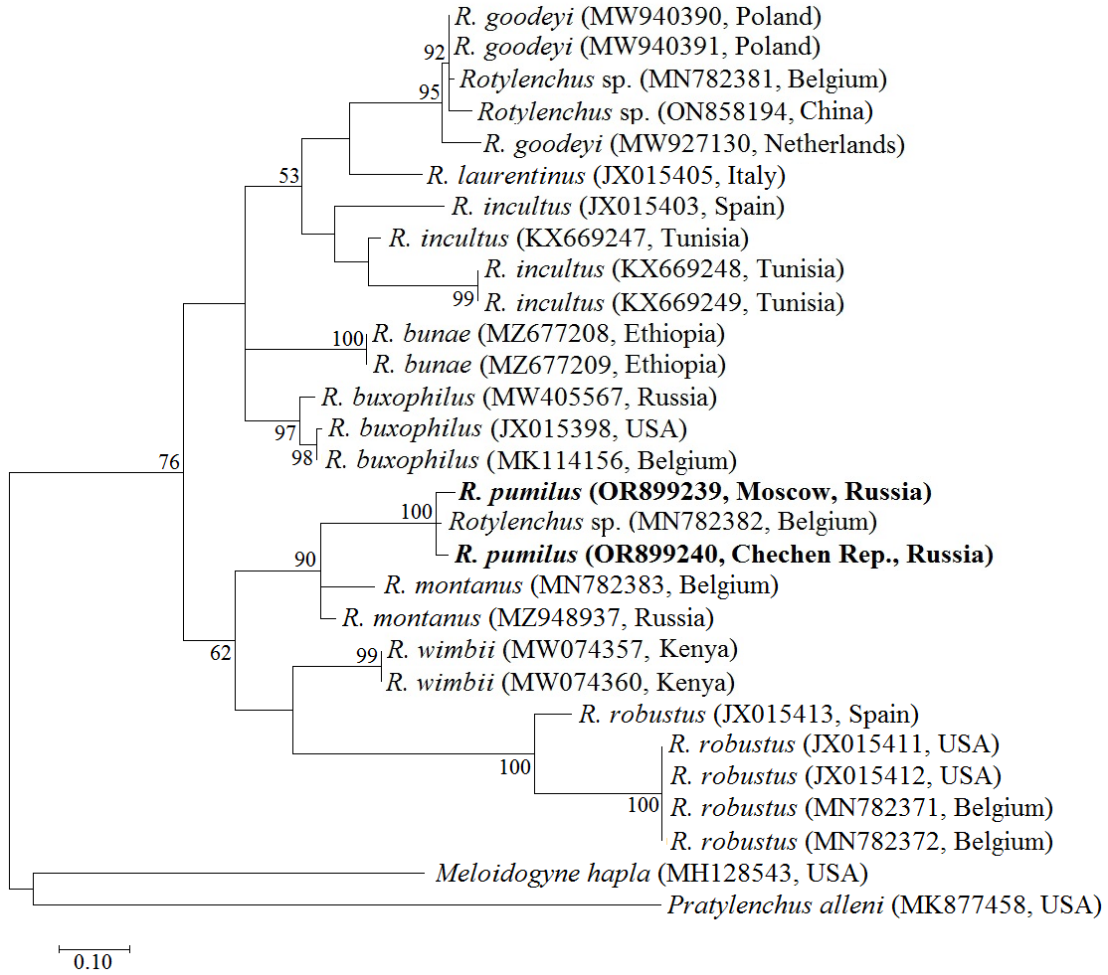

Fig. 3. Phylogenetic relationships of *Rotylenchus pumilus* from Moscow and the Chechen Republic with other *Rotylenchus* species as inferred from the ML method using the *COI* gene sequences under the GTR + I + G model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The new sequences are indicated in bold.

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С.Б. Таболин, С.В. Лычагина и Т.В. Колганова. Характеристика *Rotylenchus pumilus* (Perry, 1959) Sher, 1961 (Tylenchida: Hoplolaimidae) из России.

Резюме. При проведении нематологических исследований в Москве и Чеченской республике обнаружен вид *Rotylenchus pumilus*. В России это редкий вид, ранее его описаний с территории страны не проводилось. Морфологические и морфометрические особенности двух обнаруженных популяций оказались схожи. Для нематод этих популяций были характерны короткая длина тела самок (450-650 мкм), губная область полусферической формы с 3-4 кольцами, слитая с контуром тела, стилет длиной 23-26 мкм, положение вульвы 61-66%, кончик хвоста с утолщённой кутикулой и наличие самцов. Молекулярный анализ показал идентичность последовательностей участка D2-D3 гена 28S рДНК этих популяций, при этом последовательности гена *COI* были схожи друг с другом лишь на 97,76%.