Short Note

First report of *Paramerlinius adakensis* (Bernard, 1984) (Nematoda: Tylenchida) from Russia

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Merlinius adakensis was originally described from soil samples collected near the roots of *Elymus mollis* Trin. at Kuluk Bay, Adak Island, Alaska (Bernard, 1984). Brzeski (1991) considered this species to belong to the genus *Geocenamus*. Later, Sturhan (2012) transferred it to the newly erected genus *Paramerlinius*.

Except the type locality, *P. adakensis* has also been reported in Pakistan (Zarina & Maqbool, 1990) and Korea (Choi & Geraert, 1994). Recently this species has been found in a fescue-feather-grass steppe in the Manych Valley, in the Rostov region of Russia. The geographical location of the sampling site is 46°25'38.7"N 42°44'54.0"E. This is the first record of this species in Russia, and the first report of the genus *Paramerlinius* from the European part of the country.

Nematodes were extracted using a modified decanting and sieving method (Flegg, 1967). For morphological studies, the nematodes were killed with hot water, fixed in a 5% formalin solution, and mounted in glycerin on slides using the Seinhorst technique (Seinhorst, 1959). Molecular studies were performed using the scientific equipment of Core Research Facility of the 'Bioengineering' Center (Moscow, Russia). For this work, nematodes frozen in distilled water were used. There were three test tubes containing several specimens of *P. adakensis*. DNA was extracted using the Wizard Kit (Promega, USA), according to the manufacturer's instructions. The forward Nem_18S_F (5'-CGC GAA TRG CTC ATT ACA ACA GC-3') and the reverse Nem_18S_R (5'-GGG CGG TAT CTG ATC GCC-3') primers (Floyd *et al*., 2005) were used to amplify the fragment of the 18S rRNA gene. The D2-D3 expansion segments of the 28S rRNA gene were amplified using the forward D2A (5'-CAA GTA CCG TGA GGG AAA GTT G-3') and the reverse D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') primers (Nunn, 1992). Internal transcribed spacer 1 (ITS1) and a partial sequence of 5.8S ribosomal RNA gene were amplified with the forward primer TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and the reverse primer 5.8SM5 (5'-GGC GCA ATG TGC ATT CGA-3') (Zheng *et al.*, 2000). 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. The newly obtained sequences were submitted to the GenBank database under accession numbers OM649858 (ITS1), OM649859 (18S rRNA gene) and OM649860 (28S rRNA gene).

Description. Female (Fig. 1 A-C, Table 1). Body ventrally arcuate upon fixation. Cuticular annulation distinct, about 1.4 µm wide in mid-body. Lip region bluntly rounded, set off by depression, with 6-7 rings. Cephalic framework heavily sclerotised. Stylet very robust, its cone about equal in length to shaft and knobs directed laterally. Lateral field with six lines, areolated in pharyngeal region. Median bulb oval. Nerve ring usually slightly anterior to middle of isthmus. Excretory pore between nerve ring and pharyngeal-intestinal junction. Hemizonid distinct, 3-4 annuli long, located 1-5 rings anterior to excretory pore. Deirid at level of hemizonid. Pharyngeal-intestinal junction semi-spherical. Rectum about 0.6-0.9 anal widths long. Reproductive system didelphic-amphidelphic, outstretched, spermatheca present. Epiptygma single. Tail subcylindrical with smooth terminus and refractive inner cuticle layer at its end. Phasmid about 1/3 tail length behind anus.

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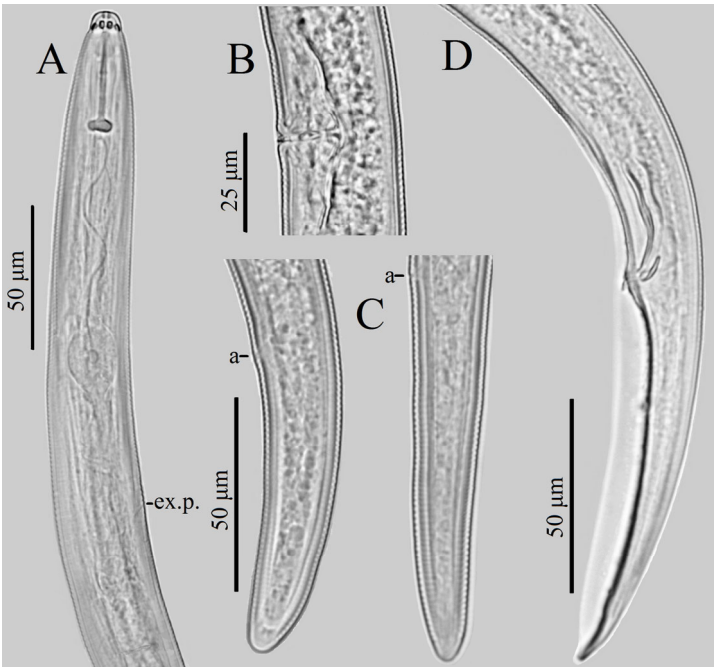

Fig. 1. Light microphotographs of *Paramerlinius adakensis*. A: Anterior end of female, B: Vulva region, C: Female tail variations, D: Posterior end of male. Abbreviations: ex. p. – secretory-excretory pore, a – anus.

Table 1. Morphometric data of 15 females and 15 males of *Paramerlinius adakensis* from the Rostov region of Russia. All measurements are in µm (except for ratio) and in the form: mean ± sd (range).

Character	Females	Males
Body length (L)	1211 ± 76 (1095-1365)	1172.5 ± 79.5 (1100-1410)
a	34.3 ± 2.3 (29.2-37.4)	37.2 ± 2.7 (34.9-43.4)
b	5.9 ± 0.2 (5.6-6.3)	5.8 ± 0.3 (5.5-6.9)
c	13.9 ± 0.9 (12.7-15.9)	11.6 ± 0.8 (10.3-13.0)
c’	3.8 ± 0.3 (3.4-4.6)	4.9 ± 0.5 (4.2-5.5)
V,%	51.7 ± 0.9 (50.3-53.0)	–
Head height	5.9 ± 0.2 (5.5-6.5)	5.7 ± 0.4 (5.0-6.5)
Head width	10.3 ± 0.2 (10.0-11.0)	9.9 ± 0.3 (9.5-10.5)
Stylet length	37.1 ± 1.4 (35.0-40.0)	36.3 ± 1.05 (35.0-37.5)
Stylet knobs	7.2 ± 0.2 (6.5-7.5)	7.0 ± 0.3 (6.5-7.5)
Dorsal gland opening from stylet base	1.7 ± 0.2 (1.5-2.0)	1.95 ± 0.27 (1.5-2.5)
Anterior end to secretory-excretory pore	160 ± 8.9 (145.0-175.0)	158 ± 8.3 (145.0-177.5)
Tail length	87.5 ± 5.9 (77.5-100.0)	101.2 ± 6.3 (92.5-111.0)
Hyaline portion	9.7 ± 1.1 (7.5-12.0)	–
Spicules	–	35.3 ± 1.6 (31.0-37.5)
Gubernaculum	–	9.8 ± 0.6 (9.0-11.0)
Tail annuli	56.6 ± 5.2 (47-64)	–

Male (Fig. 1D, Table 1). Body curved stronger than that of female. General morphology is similar to that of a female. Gonad outstretched. Spicules curved, moderately cephalated, spicular tip weakly notched, the ventral part slightly longer. Gubernaculum arcuate, proximal end bent anteriorly. Hypoptygma prominent. Bursa well developed and extending to tail tip. Phasmid at about 1/3 tail length behind anus.

Remarks. The morphometrical characteristics of the population from the Rostov region resemble those of the original description (Bernard, 1984). A minor variation was observed. The phasmid position in the male is comparable to that of the female in our specimens, and not in the posterior half of the tail as in the males of the original description. This difference is similar to that observed for the South

Korean populations (Choi & Geraert, 1994) and is considered to be an intraspecific variation.

The species *P. falcatus* (Eroshenko, 1981) is close morphologically to *P. adakensis*. However, according to the original description, the S-E pore of *P. falcatus* is located more posteriorly, at the level of the pharyngeal-intestinal junction. *Paramerlinius hexagrammus* (Sturhan, 1966) is also close morphologically to *P. adakensis*. However, according to the original description, this species has a smooth cuticle in the vulva region and lower values of the c ratio.

Molecular characterisation. The sequences of only two species of the genus *Paramerlinius* (*P. hexagrammus* and *P. neohexagrammus*) are available in the GenBank.

In this study, high-quality sequences of the 18S rRNA gene and D2-D3 expansion segments of the 28S rRNA gene were obtained in two replicates and ITS1 only in one replicate. The sequences of the 18S rRNA gene of the studied specimens were most similar to the *P. hexagrammus* sequence from Iran (KX789720), and an unidentified nematode from the USA (KC758237), with 99.07% similarity, and share less similarity (98.95%) with another sequence of *P. hexagrammus* from Iran (KX789719), and Iranian sequences of *Amplimerlinius paraglobigerus* (KX789715), *Merlinius nanus* (KX789709), *M. brevidens* (KX789708) and *Scutylenchus rugosus* (KX789704).

The sequences of the D2-D3 expansion segments of the 28S rRNA gene were most similar to the *P. neohexagrammus* (KJ585423) and *Amplimerlinius globigerus* (KX789691) sequences from Iran, with 97.24% similarity. The similarity with *P. hexagrammus* from Iran (KX789701) was only 96.22%.

The sequences of ITS1 were most similar to *Pratylenchoides leiocauda* from China (MT509390), with 90.95% similarity, and sequences of *P. leiocauda* from South Korea (MZ418133-MZ418135), with the same similarity.

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