

Short note

Characterisation of a new isolate of *Steinernema arenarium* (Steinernematidae, Rhabditida) from Mari El Republic of the Russian Federation

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The entomopathogenic nematode *Steinernema arenarium* (Artyukhovskiy, 1967) Wouts, Mráček, Gerdin & Bedding, 1982 is a member of 'glaseri' species group of the genus *Steinernema* (Spiridonov *et al.*, 2004). This group is characterised by long infective juveniles, which usually exceed 1000 µm. The 'glaseri' group is represented in Europe by several species: *S. arenarium*, *S. glaseri*, *S. apuliae* and *S. boemarei* (Triggiani *et al.*, 2004; Ansari *et al.*, 2005; Lee *et al.*, 2009). *Steinernema arenarium* is found only occasionally and usually from samples taken from sandy soil. The type isolate of this species was collected from the coarse white sand in pinewood near Voronezh, Central part of European Russia (Artyukhovskiy, 1967; Artyukhovskiy *et al.*, 1997). Our recent sampling in similar habitats in Middle Volga revealed the presence of *S. arenarium*, which was found in sandy soil, Urzhum forest, Mari El Republic, vicinity of Cheboksary, on the left coast of Volga river (coordinates N: 56°10'20"; E: 47°28'30"). Examination of infective juveniles and adults of this isolate revealed several distinctive morphological and morphometrical features of *S. arenarium*: juveniles with a mean length = 1026 µm, spicules with characteristic tip thickening, and amoeboid spermatozoa 30-35 µm in diam. from the female uterus.

For molecular characterisation of this nematode we followed the protocols for DNA extraction, PCR of ITS rRNA gene with TW81 and AB28 primers, DNA purification, cloning and sequencing as described by Spiridonov *et al.* (2004). Newly obtained sequences of *S. arenarium* are deposited in the GenBank under the accession numbers KF134911 and KF134912. Several attempts to make a direct sequencing of the PCR product were not successful and chromatograms demonstrated

multiple peaks in many positions. The sequencing of cloned PCR-product obtained from a single infective juvenile revealed the presence of two haplotypes of the ITS rDNA. The difference between two haplotypes was 12 bp (1.6%) for a 789 bp long alignment. The Blast search of both sequences in the GenBank database showed highest similarities with those deposited in the GenBank for *S. arenarium*. Analysis of the multiple alignment with other *S. arenarium* ITS sequences showed that the first clone (No. 1) of the Urzhum isolate was different from the Rjazan isolate (AY230160) in 12 bp and from the Bulgarian isolate [HM160094 and HM160095 (Gradinarov *et al.*, 2011)] in 19 bp. The sequence of the second clone (No. 3) was different from that of the Bulgarian isolate in 18 bp and from Rjazan isolate only in 4 bp. The relationships of these haplotypes with other *S. arenarium* sequences are presented in Fig. 1. The haplotypes of *S. arenarium* from the Urzhum isolate were more closely related to the sequence of the Rjazan isolate than to the Bulgarian isolate.

Bacterial symbionts of the genus *Xenorhabdus* were also isolated from infective juveniles of the Urzhum isolate. The partial sequences of 16S of rRNA and *RecA* genes were obtained with primers proposed by Babic *et al.* (2000) and Sergeant *et al.* (2006), respectively. Newly obtained sequences for both genes were deposited in the GenBank under the accession numbers KF049422 and KF134913. As the BLAST search (Altshul *et al.*, 1990) of these sequences in the GenBank database demonstrated high similarities with those for *Xenorhabdus kozodoii*, the symbiont from the Urzhum isolate was identified as belonging to this species. The 16S of rRNA sequence of the symbiont from the Urzhum isolate differed from that of the symbiont from the

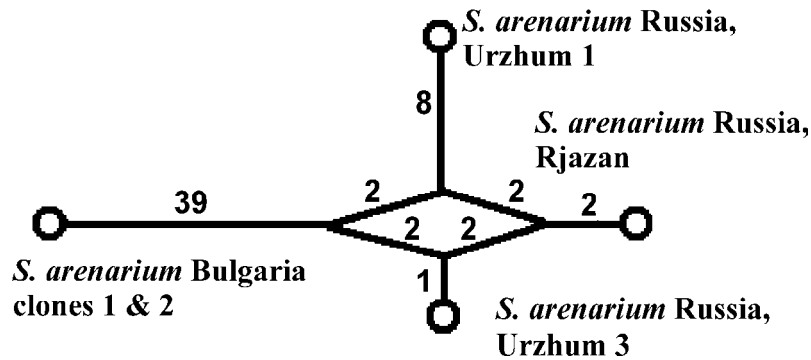


Fig. 1. Parsimonial network of *Steinernema arenarium* ITS rDNA haplotypes. Predicted number of nucleotide changes between haplotypes is presented on the branches. Reduced median network construction by Network 4.6 (Bandelt *et al.*, 1995).

Voronezh isolate of *S. arenarium* in 9 bp and from sequences of the symbionts of *S. boemarei* and *S. apuliae* in 13 and 21 bp, respectively, in a 1380 bp long alignment. The *RecA* gene sequence of the Urzhum isolate was identical with that of *X. kozodoi* from the Voronezh *S. arenarium* isolate (SaV strain – FJ823404).

Thus, our present study revealed that the ITS rRNA gene sequences of *Steinernema arenarium* are quite polymorphic among European isolates and 16S rDNA sequences of *Xenorhabdus kozodoi* strains isolated from different *S. arenarium* isolates are also diverse.

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