# FIRST REPORT OF A COLD-ACTIVE EPN *STEINERNEMA KRAUSSEI* (RHABDITIDA, STEINERNEMATIDAE) FROM IRAN

**M. Nikdel[1,*] and Gh. Niknam[2]**

[1] *Research Center of Agriculture and Natural Resources of East Azarbaijan, Iran*
[2] *Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Iran*
* *Corresponding author, E-mail: mnikdel1374@gmail.com*

**Summary.** During a survey of entomopathogenic nematodes conducted throughout the Arasbaran area, in the north-west of Iran, an entomopathogenic nematode was isolated by *Galleria* baiting method from soil samples collected from rangelands, near Chichakloo, Varzeghan. It was identified as *Steinernema kraussei* based on morphological, morphometrical, molecular and cross-breeding studies. This is the first record of *S. kraussei* from Iran.

**Keywords:** Entomopathogenic nematode, Iran, molecular characterization, new record.

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* are frequently used as biological control agents of several insect pests (Gaugler and Kaya, 1990). They are obligate pathogens of insects. *Steinernema* harbours a bacterial symbiont, *Xenorhabdus* (Thomas and Poinar, 1979), that kills the insect host and digests tissues, providing suitable conditions for growth and development of the nematode within the cadaver (Forst and Clarke, 2002). Some 67 valid species of the genus *Steinernema* have been described worldwide and these are divided into five groups according to their morphology and molecular characteristics (Nguyen and Hunt, 2007). *Steinernema kraussei* (Steiner) Travassos was the first entomopathogenic nematode described and was isolated from infected sawflies in Germany by Steiner in 1923 (Stock *et al*., 2001).

A survey of entomopathogenic nematodes was conducted in the Arasbaran forests and rangelands, in the north-west of Iran, during 2008. From this survey, a number of species of *Steinernema* were identified. One isolate of the genus was identified as *S. kraussei* based on morphological and molecular studies and is reported for the first time from Iran.

*Steinernema* sp. IRAZ20 was recovered from soil samples collected from rangelands, near Chichakloo, Varzeghan, in the north-west of Iran (from the rhizosphere of a forest habitat dominated by oak tree, *Quercus macrantera* Fish *et* Meyer, longitude E46°45,45, latitude N38°55,20, altitude 2121 m a.s.l., annual average temperature 12 °C, precipitation 415 mm/year) using the *Galleria mellonella* L. baiting method described by Bedding and Akhurst (1975). Infective juveniles (IJ) were collected from *Galleria* cadavers, using the method of White (1927) and stored in aerated water at 7 °C. All nematodes used in this study were reared on *G. mellonella* larvae. Twenty *G. mellonella* larvae were exposed to a suspension of infective juveniles (about 1000 IJ/ml) in a Petri dish lined with two moistened filter papers at laboratory temperature (23 ± 3 ºC). First generation males and females were collected from *Galleria* cadavers (dissected out in Ringer's solution) 4-5 days after inoculation. Infective juveniles (IJs) and second generation adults were collected during the week after their first emergence from *Galleria* cadavers and killed using hot (50-60 °C) Ringer's solution (Nguyen and Smart, 1990). Dead nematodes were fixed in triethanolamine formalin (TAF) and processed to anhydrous glycerin by a slow evaporation method (Woodring and Kaya, 1988) and mounted on microscope slides.

Morphological and morphometrical studies were made using an Olympus BX41 microscope equipped with interference contrast optics and a digital camera. Image tool software (Vilcox *et al*., 2002) was used to obtain quantitative measurements.

Scanning electron microscopy (SEM) observations were made using Nguyen and Smart's (1995) method. First generation adults and infective juveniles were rinsed for 5 minutes in three changes of Ringer's solution. They were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 hours at 8 °C. They were post-fixed with 2% osmium tetroxide ($OsO_4$) solution for 12 hours at 25 °C, dehydrated in a graded ethanol series, mounted on aluminium SEM stubs, coated with gold powder and studied using a LEO 44oi scanning electron microscope.

Total genomic DNA was extracted from single IJs as described by Phan *et al*. (2001), with some modification. Extracted DNA was used in polymerase chain reaction. The forward and reverse primers for amplification of the complete ITS-rDNA region were used in the PCR reaction (Vrain *et al*., 1992). Amplifications were carried out in a 50 µl volume, containing 50 mM KCl, 10 mM Tris (pH 8.4), 1.5 mM $MgCl_2$, 0.1% Triton×100, 0.2 mM of each dNTP, 0.4 mM of each primer, 10 µl of

[1] Corresponding author: mnikdel1374@gmail.com

nematode lysate and 0.25 µl of Taq DNA polymerase (1.25 units). Amplifications were carried out using a Biometra thermocycler with heated lid pre-set at 95 ºC and subjected to the following cycling profile: one cycle of 94 ºC for 5 min followed by 35 cycles of 94 ºC for 1 min, 55 ºC for 1 min and 72 ºC for 1.5 min. A final step of 5 min at 72 °C was also included to ensure that all of the final amplification products were full length. Amplification products were purified with a QIAGEN PCR purification kit. Purified DNA was sequenced in IBMP-CNRS, France. The ITS sequence of the studied isolate IRAZ20 (FJ860038) was aligned using the default option of Clustal X (Thompson *et al*., 1997) with ITS sequences of 22 species of *Steinernema* that have been deposited in GenBank and *Caenorhabditis elegans* (Maupas, 1899) as the outgroup taxon (X03680). Molecular phylogenetic relationships were obtained by equally weighted maximum parsimony (MP) and maximum likelihood (ML) using PAUP 4.0b8 (Swofford, 1998).

The Iranian isolate of *S. kraussei* is characterized by the medium body length of infective juveniles (averaging less than 1000 µm); lateral field with eight ridges of which the central pair is less prominent; head smooth, lacking horn-like structures; secretory-excretory pore located at the level of middle of pharynx. The secretory-excretory pore of adults is situated far in front of the nerve ring. In general, morphological features as observed by optical and scanning electron microscopes (not reported) agree with those reported in the literature for this species. However, the Iranian isolate can be separated from other isolates of *S. kraussei* by having longer (projected) cephalic papillae.

Phylogenetic analysis of rDNA ITS regions showed that the Iranian isolate (FJ860038) is closely related to the *S. kraussei* isolate C46 (EU914856) from Slovenia (Fig. 1). The species in the *S. feltiae* group (*S. cholashanense, S. feltiae, S. texanum, S. kraussei, S. kushidai, S. monticolum* and *S. oregonense*) form a monophyletic group with high bootstrap support (95%), and the Iranian isolate of *S. kraussei* does cluster with this group. Based on the available literature, this is the first record of *S. kraussei* from Iran.

Data on the intraspecific morphological variations of *S. kraussei* are very rare but some molecular studies have recently become available. Yoshida (2003) showed differences in the populations of *S. feltiae* and *S. kraussei* from Japan. He reported that a Japanese isolate of *S. kraussei* had a longer mucron and clear differences in RFLP pat-

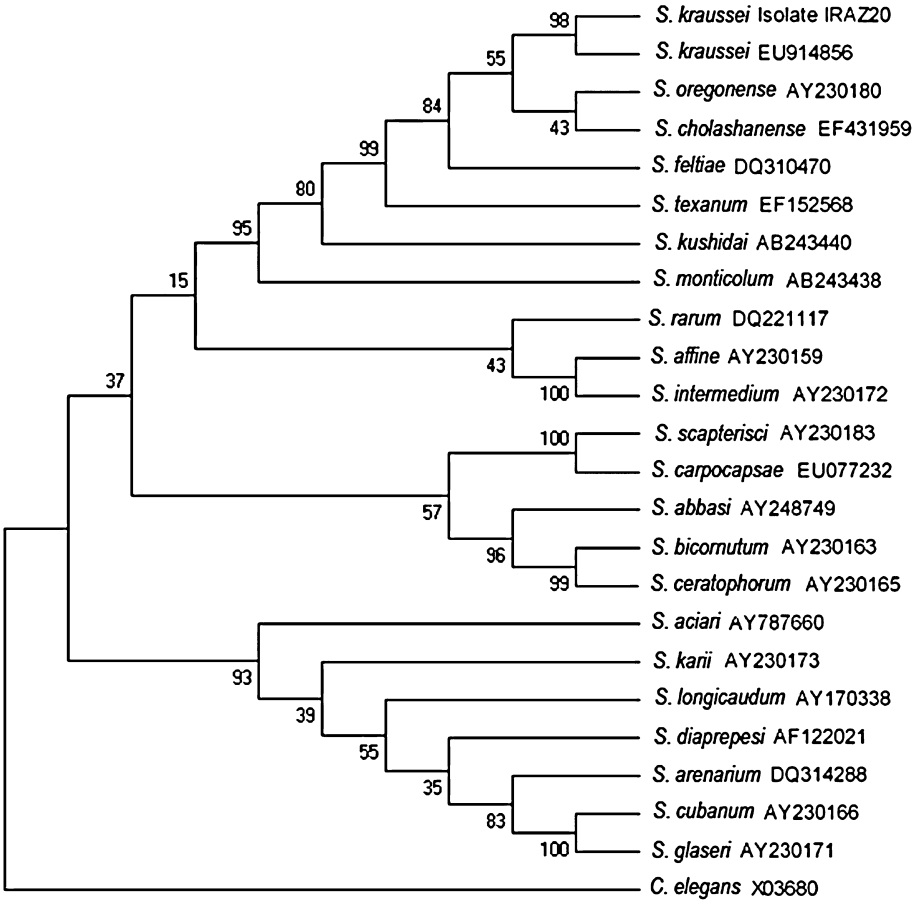


**Fig. 1.** Phylogenetic relationships of *Steinernema kraussei* IRAZ20 (Iranian isolate) with 22 other species of *Steinernema* based on analysis of ITS rDNA regions. The species in the feltiae group form a monophyletic group with high bootstrap support (95%) and *S. kraussei* IRAZ20 does cluster within that group. Numbers at the nodes represent bootstrap values.

terns of ITS rDNA compared with a UK isolate. The Iranian isolate also has a long mucron in the second generation males. Spiridonov *et al.* (2004) indicated that sequence differences between 13 *S. kraussei* isolates from Germany, Russia, UK, Belgium, Iceland, Scotland and Switzerland usually varied between 1 and 11 bp (up to 1%) but reached 21 bp (2.8%) between UK (B2) and Moscow isolates. The sequence divergence of *S. affine* ranged from 0.2-0.6% (2-5 bp) but the difference between sequences of *S. carpocapsae* strains from Europe and the USA was only 3 bp (0.4%). Therefore, the range of the intra-specific variability of *S. kraussei* ITS sequences is more than that of other studied species.

The Iranian isolate of *S. kraussei* was mainly obtained from the rangelands of Arasbaran region. Similarly, and has been reported from alpine grasslands in Switzerland (Steiner, 1996), Scotland (Gwynn and Richardson, 1996), and from alpine meadows in Bulgaria (Shishinova *et al*., 2000). The Iceland isolate of *S. kraussei* was obtained from a swampy, treeless area in a volcanic valley. The distribution of *S. kraussei* is, almost without exception, in woodland habitats in lowland parts of Europe, although this species can also be commonly found outside woodlands at high altitudes and latitudes, where the habitat preference patterns of the species are variable.

## ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. M. Arzanlou of the Department of Plant Protection, Faculty of Agriculture, University of Tabriz, for his valuable suggestions and comments.

## LITERATURE CITED

Bedding R.A. and Akhurst R.J., 1975. A simple technique for detection of insect parasitic rhabditid nematodes in soil. *Nematologica, 21*: 109-110.

Forst S. and Clarke D.J., 2002. Bacteria-nematode symbiosis. Pp. 57-77. In: *Entomopathogenic Nematology* (Gaugler R., ed.). CABI Publishing, New York, USA.

Gaugler R. and Kaya H.K., 1990. *Entomopathogenic nematodes in biological control*. CRC Press, Boca Raton, FL, USA, 365 pp.

Gwynn R.L. and Richardson P.N., 1996. Incidence of entomopathogenic nematodes in soil samples collected from Scotland, England and Wales. *Fundamental and Applied Nematology, 19*: 427-431.

Nguyen K.B. and Smart G.C. Jr., 1990. *Steinernema scapterisci* n. sp. (Steinernematidae: Nematoda). *Journal of Nematology, 22*: 187-199.

Nguyen K.B. and Smart Jr. G.C., 1995. Scanning electron microscope studies of *Steinernema glaseri* (Nematoda: Steinernematidae). *Nematologica, 41*: 183-190.

Nguyen K.B. and Hunt D.J., 2007. *Entomopathogenic Nematodes: systematics, phylogeny and bacterial symbionts*. Nematology Monographs and Perspectives 5 (Hunt D.J. and Perry R.N., series editors). BRILL, Leiden, The Netherlands, 816 pp.

Phan K.L., Nguyen N.C. and Moens M., 2001. *Steinernema sangi* n. sp. (Rhabditida: Steinernematidae) from Vietnam. *Russian Journal of Nematology, 9*: 1-7.

Shishinova M., Budurova L. and Gradinarov D., 2000. Entomopathogenic nematodes from Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in Bulgaria. *IOBC Bulletin, 23*: 75-78.

Spiridonov S.E., Reid A.P., Podrucka K., Subbotin S.A. and Moens M., 2004. Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida) as inferred from analyses of sequences of the ITS1-5.8S-ITS2 region of rDNA and morphological features. *Nematology, 6*: 547-566.

Steiner W.A., 1996. Distribution of entomopathogenic nematodes in the Swiss Alps. *Revue Suisse de Zoologie, 103*: 439-452.

Stock S.P., Campbell J.F. and Nadler S.A., 2001. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *Journal of Parasitology, 87*: 877-889.

Swofford D.L., 1998. PAUP* *Phylogenetic analysis using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts, USA, 128 pp.

Thomas G.M., and Poinar G.O., Jr. 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic nematophilic bacteria of the family Enterobacteriaceae. *International Journal of Systematic Bacteriology, 29*: 352-360.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmough F. and Higgins D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*: 4876-4882.

Vilcox D., Dove B., McDavid D. and Greer D., 2002. *THSC-SA Image Tool for Windows version 3.0*. The University of Texas Health Science Center, San Antonio, USA.

Vrain T.C., Wakarchuk D.A., Levesque A.C. and Hamilton R.I., 1992. Intraspecific rDNA restriction fragment length polymorphisms in the *Xiphinema americanum* group. *Fundamental and Applied Nematology, 15*: 563-574.

White G.F., 1927. A method for obtaining infective nematode larvae from cultures. *Science, 66*: 302-303.

Woodring J.L. and Kaya H.K., 1988. *Steinernematid and heterorhabditid nematodes: A handbook of techniques*. Southern Cooperative Series Bulletin 331, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas, USA, 30 pp.

Yoshida M., 2003. Intraspecific variation in RFLP patterns and morphological studies on *Steinernema feltiae* and *S. kraussei* (Rhabditida: Steinernematidae) from Hokkaido, Japan. *Nematology, 5*: 735-746.

Accepted for publication on 29 March 2010.