STEINERNEMA ARASBARANENSE SP. N. (NEMATODA: STEINERNEMATIDAE), A NEW ENTOMOPATHOGENIC NEMATODE FROM ARASBARAN FORESTS, IRAN

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Summary. A new species of entomopathogenic nematode, collected in 2007 from Arasbaran forests, near the Kerengan village, East Azarbaijan Province, Iran, is described herein as *Steinernema arasbaranense* sp. n. The new species can be separated from all the described species in the *affine/intermedium* group by having eight ridges in the lateral fields of infective juveniles, mucron in both generation males (except for the second generation males of *S. sichuanense*) and a hole-like structure at the spicule tip. It differs from *S. affine* by having infective juveniles with longer tail (84 *vs* 66 µm) and absence of the internal tail spine. The new species can be distinguished from *S. intermedium* by the longer tail of the infective juveniles (84 *vs* 64 µm) and shorter spicules of males (75 *vs* 93 µm), and separated from *S. sichuanense* by the shorter pharynx of infective juveniles (112 *vs* 131 µm) and presence of a mucron in the first generation male. DNA sequencing of the rDNA ITS regions revealed a unique sequence in *S. arasbaranense* sp. n., which clustered with species in the *affine/intermedium* group but which clearly differs from these species.

Key words: Description, new species, phylogeny, systematics.

Entomopathogenic nematodes (EPNs) of the family Steinernematidae Filipjev, 1934 are parasites capable of infecting a wide range of insects. Early laboratory experiments and field releases of EPN show that they can be used successfully to control insect pests. Many surveys have been conducted all over the world searching for suitable species for control of economically important insect pests (Hominick, 2002). Finding indigenous EPN populations and species is a critical step towards attaining effective biological control of pests due to their better adaptation to environmental conditions under which such a control is going to be attempted. Currently, some 63 valid species in the genus *Steinernema* Travassos, 1927 have been reported worldwide (Nguyen and Hunt, 2007). The most up-to-date biogeographic account indicates that these nematodes have been isolated from all continents except for Antarctica and almost all regions of the world (Hominick, 2002).

Until now, several species of *Steinernema*, including *S. bicornutum* Tallosi, Peters *et* Ehlers, 1995, *S. carpocapsae* (Weiser, 1955) Wouts, Mraček, Gerdin *et* Bedding,1982, *S. feltiae* (Filipjev, 1934) Wouts, Mraček, Gerdin *et* Bedding, 1982, *S. kraussei* (Steiner, 1923) Travassos, 1927 and *S. glaseri* (Steiner, 1929) Wouts, Mraček, Gerdin *et* Bedding, 1982, have been reported from Iran (Parvizi, 2003; Eivazaian Kary *et al*., 2009; Nikdel *et al*., 2010). In a recent survey on entomopathogenic nematodes, one population of *Steinernema* was obtained and identified as a new species that is described herein as *Steinernema arasbaranense* sp. n. The new species is distinguished from other *Steinernema* species by differences in morphology, morphometrics and ITS-rDNA sequences.

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MATERIALS AND METHODS

Nematode source and culture. The type population was recovered by the *Galleria* trap method (Bedding and Akhurst, 1975) from a soil sample collected in Arasbaran forests and rangelands during a survey. The nematode was subsequently reared on larvae of *Galleria mellonella* L. and established as a laboratory culture at the Nematology Laboratory, University of Tabriz, Tabriz, Iran. The isolate, named as IRAZ21, was used as the type isolate for erection of the new species. The type locality is Arasbaran forests, near the Kerengan village (latitude N 38º 42 36´, longitude E 46º 33 09´, altitude 2160 m a.s.l., annual average temperature 12.5 °C, precipitation 380 mm/year and soil type dark brown sandy clay) in the north of East Azarbaijan Province, Iran.

Light microscopy and morphometry. First generation males and females were collected after 4-5 days from the cadavers of inoculated *Galleria* larvae (dissected out in distilled water). Infective juveniles (IJs) and second generation adults were obtained during the week after their first emergence from *Galleria* cadavers and killed using hot (50-60 °C) Ringer's solution (Stock *et al*., 2004). Dead nematodes were fixed in triethanolamine formalin (TAF) (Courtney *et al*., 1955), processed to anhydrous glycerin by a slow evaporation method (Seinhorst, 1959; Woodring and Kaya, 1988) and mounted on microscope slides. For female specimens, cover slips were supported using pieces of hair to avoid flattening

the nematodes. Morphological studies and morphometric measurements were made using an Olympus BX41 microscope equipped with interference contrast, through a digital DP50 camera and UTHSCSA Image tool software (Vilcox *et al*., 2002).

Scanning electron microscopy. Adults and IJs were processed following protocols described by Nguyen and Smart (1995). For scanning electron microscopy (SEM) examination, the first generation adults and IJs were rinsed three times for 5 min in Ringer's solution. They were then fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 hours at 8 °C, post-fixed with 2% osmium tetroxide (OsO_4) solution for 12 hours at 25 °C, dehydrated in a graded ethanol series (5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 100%), mounted on aluminium SEM stubs, coated with gold powder (200 nm thick) and studied using a LEO 44oi scanning electron microscope.

Molecular characterization. Total genomic extraction from single infective stages and ITS-rDNA amplification were done as described by Phan *et al.* (2001), with some modifications proposed by Eivazian Kary *et al.* (2009). The amplified products were purified using a Qiagen purification kit (Qiagen, Leusden, The Netherlands). Purified DNA was sequenced at the Institut de Biologie Moléculaire des Plantes - Centre National de la Recherche Scientifique (IBMP-CNRS), France. The DNA sequences were edited with Chromas 2.01 and aligned using Clustal X 1.64 (Thompson *et al*., 1997) with the ITS-rDNA sequences of other *Steinernema* species obtained from GenBank.

ITS-rDNA sequences for *S. arasbaranense* sp. n. were deposited in the GenBank under the accession number FJ860039. Molecular phylogenetic relationships were examined by equally weighted maximum parsimony (MP) using PAUP* 4.0b8 (Swofford, 1998). MP was performed with a heuristic search with the following setting: one thousand replicates of random taxon addition (RTA), tree-bisection-reconnection (TBR) branch swapping, multiple trees retained, no steepest descent and accelerated transformation. All data were assumed to be unordered, all characters were treated as equally weighted, and gaps were treated as missing data (Posada and Crandall, 1998). Bootstrap analysis with 1000 replicates was conducted as a measure of support for individual clades in an maximum parsimony tree. This tree was rooted by *Steinernema rarum* Doucet, 1986 (DQ221117). The pairwise distances between closely related species were also calculated using PAUP.

DESCRIPTION

STEINERNEMA ARASBARANENSE **sp. n.**
(Table I; Figs 1-5)

Measurments

Morphometrics of the holotype (infective juvenile), and all other stages of the new nematode species are presented in Table I.

Morphology

First generation males. Body ventrally curved, C-shaped when killed by gentle heat, much smaller and more slender than female. Anterior part similar to females. Cuticle appearing smooth under light microscope, lateral fields not observed. Head rounded and continuous (height as wide as body width at the same region). Six pointed labial and four cephalic papillae visible under SEM (Fig. 2A). Amphidial aperture narrow, slightly rounded. Buccal cavity funnel-shaped, stoma shallow, cheilorhabdions distinct. Pharynx muscular, procorpus cylindrical, metacorpus slightly swollen, non-valvate, isthmus distinct, basal bulb pyriform and valvate. Nerve ring often surrounding anterior portion of basal bulb. Secretory-excretory pore located at middle of metacorpus. Testis monorchic and reflexed. Spicules paired, symmetrical, slightly brownish in color, somewhat curved, length / width ratio = 6.5, head (manubrium) moderately elongate, shaft (calomus) separating manubrium from lamina (Figs 1D and 3C-D), lamina (blade) slightly curved, supported by two internal ribs and almost straight posteriorly, velum present, extending from base of rostrum or beginning of lamina to distal part of lamina, not covering spicule tip. Hole-like structure at the tip of spicules (Figs 3A-B). Gubernaculum *ca* 63% of spicule length, boat-shaped in lateral view, swollen at middle, with slightly ventrally curved knob at proximal end, in ventral view, cuneus short, pointed posteriorly, wing of corpus expanding laterally (Figs 1D and 3A-C). Genital papillae disposed as single mid-ventral pre-cloacal papilla and eleven pairs arranged as follows: six pairs ventral pre-cloacal (three pairs lateral pre-cloacal, three pairs sub-ventral pre-cloacal), two pairs lateral adcloacal, one pair dorsal post-cloacal and two pairs sub-terminal post-cloacal (Figs 2B-D). Tail conoid, terminating in a 8.5 µm long mucron always present (Fig. 1D). Phasmids inconspicuous.

Second generation males. Morphologically similar to the first generation males but more slender and most of the morphometric measurements smaller; for example, body diameter *ca* half that of the first generation males (Table I). Testis flexure extending more posteriorly, mucron more distinct, *ca* 12 µm long.

First generation females. Body robust, habitus C-shaped when killed by gentle heat (Fig. 4A), variable in length. Cuticle appearing smooth under light microscopy, only faintly striated under SEM, lateral fields and phasmids not observed. Head broadly rounded, six pointed labial and four cephalic papillae visible only with SEM. Amphids distinct and located at cephalic papillae level. Buccal cavity funnel-shaped, stoma shallow. Pharynx with cylindrical procorpus, metacorpus

Table I. Morphometrics of *Steinernema arasbaranense* sp. n. Measurements are in micrometers and in the form: mean ± standard deviation (range).

Character	First generation			Second generation		Infective juvenile
	Female	Male		Female	Male	
	Paratypes	Paratypes	Holotype	Paratypes	Paratypes	Paratypes
n	15	22	-	15	20	20
L	3720±1053 (2320-5345)	1265±175 (1057-1645)	1240	2332±249 (2063-2553)	863±37 (824-921)	731±30 (693-795)
a	15±2 (12-18)	10±1 (8-12)	8.5	13±2 (12-16)	13±1 (11-15)	16.4±1 (14-18)
b	19±5 (13-26)	9±1 (8-11)	9.2	14±2 (13-17)	7±0.5 (6-7)	6.5±0.4 (6-7)
c	112±7 (89-128)	37±6 (29-47)	35	51±5 (48-57)	27±1 (26-29)	8.7±0.7 (7-10)
c'	1±0 (1-1)	1±0.1 (0.7-1)	0.9	1±0.3 (0.7-1.3)	1±0 (1-1)	3.6±0.2 (3-5)
V%	56 ±3 (52-60)	-	-	59 ±2 (56-62)	-	-
Body diam.	242±37 (186-314)	125±13 (98-143)	133	170±19 (151-192)	64±6 (56-73)	44±2 (41-50)
Excretory pore (EP)	90±24 (88-149)	65±13 (51-87)	73	67±6 (60-76)	61±5 (55-70)	56±2.5 (53-60)
Nerve ring	125±11 (118-138)	106±11 (98-122)	107	-	-	84±5 (79-88)
Pharynx length (ES)	192±16 (154-215)	133±5 (126-143)	136	152±5 (147-161)	125±6 (116-132)	112±3 (109-118)
Tail length (T)	38±7 (29-46)	32±3 (31-36)	34	45±9 (36-57)	32±2 (29-34)	84±6 (79-101)
Anal body diam. (ABD)	50±4 (45-57)	40±3 (33-44)	41	39±1 (37-42)	34±3 (30-38)	23±2 (20-27)
Mucron length	-	8.5±0.2 (7.5-9)	9	-	12±2 (9-16)	-
Spicule length (SP)	-	75±3 (70-82)	78	-	59±5 (51-67)	-
Spicule width	-	11±1 (11-13)	12	-	9±2 (7-11)	-
Gubernaculum length (G)	-	48±4 (42-53)	49	-	40±3 (35-44)	-
D%	47±8 (38-57)	50±7 (43-59)	52	42±5 (36-47)	49±3 (43-53)	50±3 (45-54)
E%	239±33 (205-284)	187±27 (157-223)	189	149±19 (128-171)	197±16 (168-215)	67±6 (53-75)
SW	-	187±12 (174-213)	184	-	179±23 (149-210)	-
GS	-	63±5 (52-70)	66	-	61±6 (49-72)	-

D% (= EP / ESx100), E% (= EP / Tx100), SW% (= SP / ABDx100), GS% (= G / SPx100).

slightly swollen and non-valvate, isthmus distinct, basal bulb pyriform and valvate. Nerve ring located just anterior to basal bulb. Secretory-excretory pore usually located at mid-pharynx level and secretory-excretory duct cuticularised. Cardia prominent, protruding into intestinal lumen. Genital system didelphic and amphidelphic, reflexed, filled with eggs, vulva posterior to mid-body as a symmetrical and transverse slit, protruding from body, with double flapped epiptygma (Figs 4C, D), vagina short, oblique with muscular walls. Tail dome-shaped, shorter than anal body diameter and with a terminal peg (Fig. 4E).

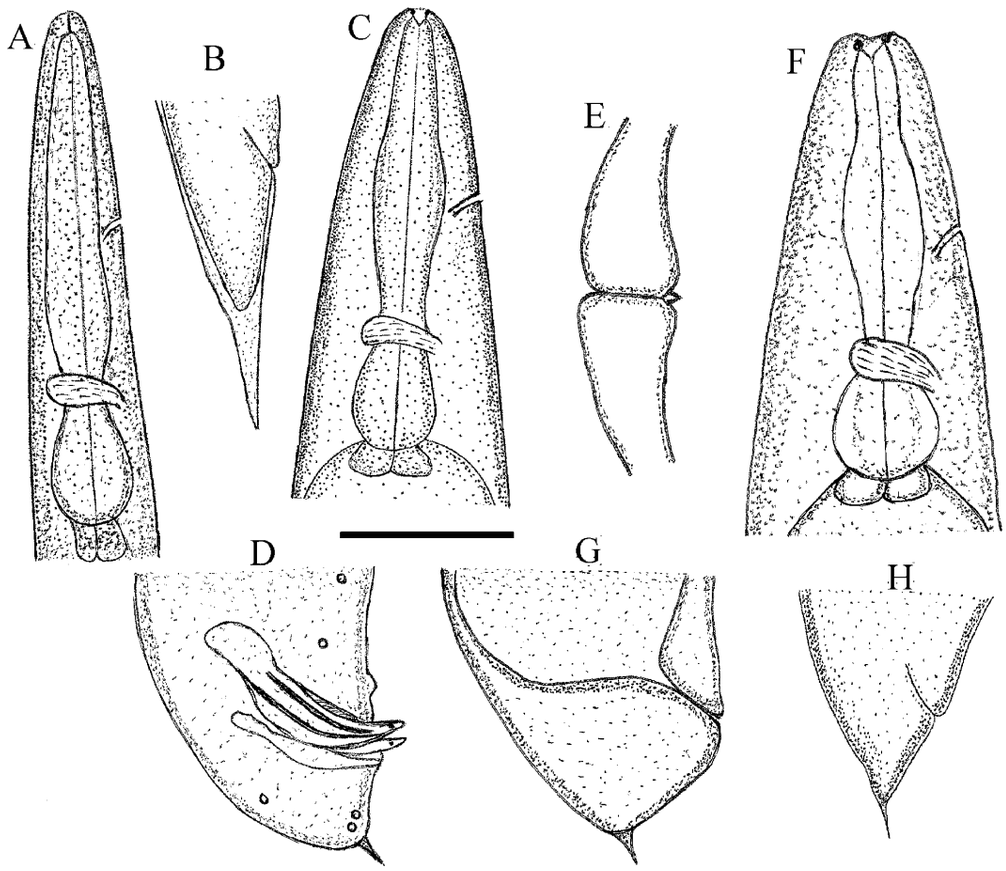

Fig. 1. *Steinernema arasbaranense* sp. n. A and B, anterior and posterior region of infective juvenile; C and D, anterior and posterior region, spicules, gubernaculum and mucron of first generation male; E, vulva; F and G, anterior and posterior region of first generation female; H, posterior region of second generation female. Scale bar (in µm): A, G = 45, B = 50, C = 65, D = 54, E, F = 72, H = 60.

Second generation females. Similar to the first generation in general morphology, but smaller (Table I). Body length and diameter *ca* 75% that of first generation females (Figs 4B, F). Vulval opening behind mid-body. Tail conoid with mucron at its end. Post-anal swelling not developed (Fig. 1H).

Infective third-stage juveniles. Body slender, habitus moderately ventrally curved upon heat-killing, often still enclosed in the second stage juvenile cuticle, tapering regularly from base of pharynx to anterior end and from anus to body terminus. Mouth and anus closed and esophagus and intestine collapsed. Lip region rounded, smooth and labial papillae not seen, but four projected cephalic papillae are visible. Transverse slit-like amphidial aperture situated posterior to labial disc, but at the level of cephalic papillae (Fig. 5A). Pharynx long, narrow, isthmus distinct, surrounded by nerve ring, basal bulb elongate and valvate. Cuticle with distinct transverse striations seen with SEM (Fig. 5A). Secretory-excretory pore located near middle of corpus (Fig. 1A). Hemizonid distinct, positioned anterior to base of terminal bulb. Bacterial vesicle relatively elongate. The lateral fields pattern is 2, 4, 5, 8, 4, 2 and at mid-body with eight equally developed ridges (*i.e.*, nine lines or incisures); numbers 2, 4, 5 and 7 are narrow, prominent and more whitish than the others (Figs 5B, D). Deirids not observed. Tail conical with dorsal depression, no spine like structure was seen on tail tip (Fig. 5C), hyaline portion distinct, *ca* 48% of tail length. Phasmids indistinct.

Molecular characterization

The sequence of the ITS region of *S. arasbaranense* sp. n., including primers TW81 and AB28, can be recognized by its 869 base pairs (bp) length, and its base composition is 25.2% A, 37.3% T, 16.8% C and 20.7% G. Based on a blast search result for the ITS region, *S. arasbaranense* sp. n. has a unique sequence; the closest match is with *S. affine* (AY230159), with identities = 805/837 (97%) and gaps = 11/837 (1%). To examine the interspecific relationships, the distance matrix of the absolute nucleotides of some *Steinernema* species is presented in Table III. Pairwise distances in the 979–total-character multiple alignment show that the new species differs from all the other species, but is much closer to

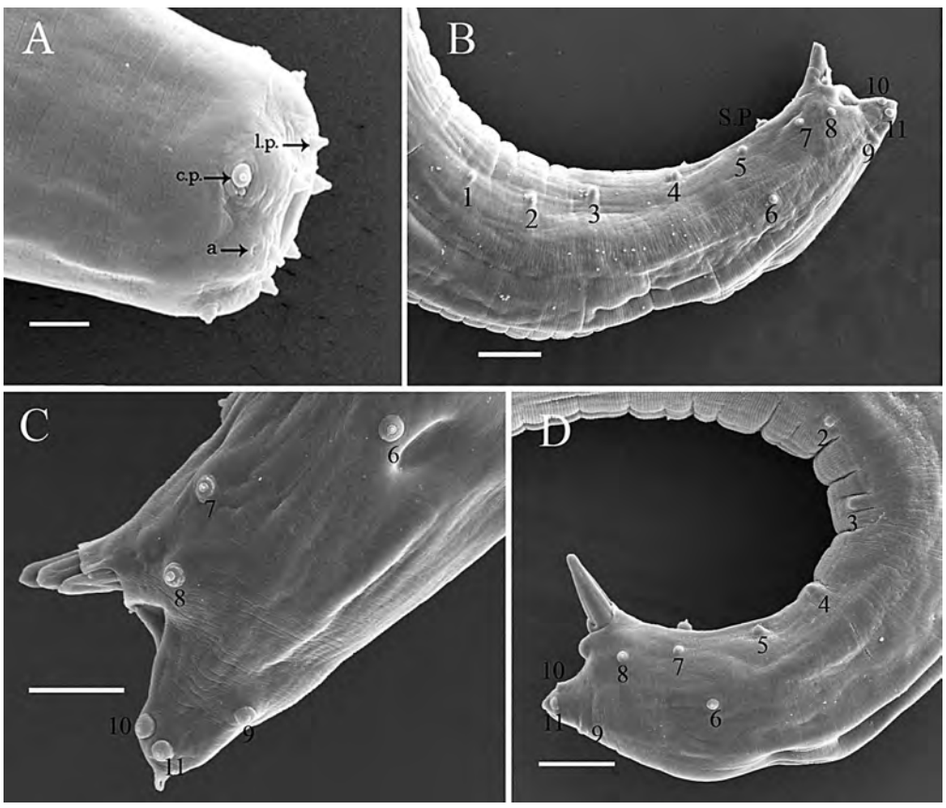

Fig. 2. SEM photographs of first-generation male of *Steinernema arasbaranense* sp. n. A, anterior region showing amphid (a), labial (l.p.) and cephalic (c.p.) papillae; B, C and D, posterior region of male with 11 pairs and a single papilla (s.p), arrangement of genital papillae and spicule. Scale bars (in µm): A = 13, B, D = 28, C = 16.

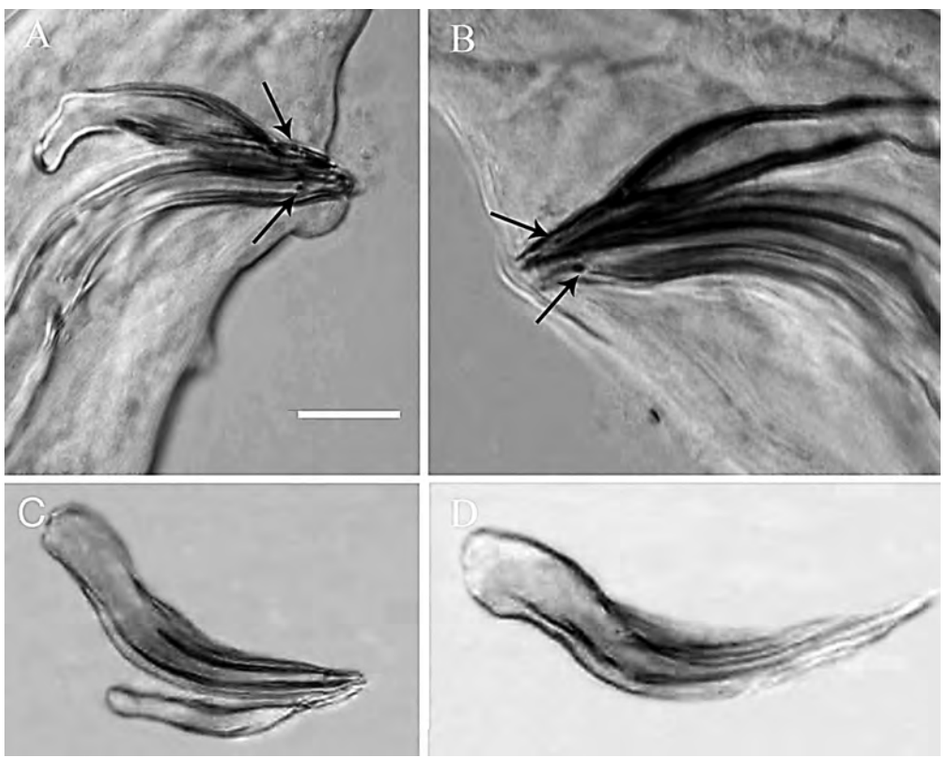

Fig. 3. Light microscope photographs of first generation male spicules and gubernacula of *Steinernema arasbaranense* sp. n. A and B, hole-like structure in the tip of spicules; C and D, spicules and gubernaculum. Scale bar (in µm): A, B, D = 14, C = 21

species in the *affine/intermedium* group (34-61 bp). Among species in the *affine/intermedium* group, the closest is *S. affine* with 34 bp difference; *S. sichuanense, S. intermediumm* and *S. beddingi* have 50, 53 and 61 bp differences, respectively. Comparing with species other than the *affine/intermedium* group, *S. arasbaranense* sp. n. had 229 to 278 bp differences (Table III). Additionally, the multiple sequence alignment (Fig. 7) of this group shows that *S. arasbaranense* sp. n. has some diagnostic character states and differs from its sister taxons.

Phylogenetic analysis

The most closely related *Steinernema* species from the blast search against *S. arasbaranense* sp. n. (FJ860039) in the ITS-rDNA region were selected for phylogenetic analysis. A phylogenetic tree (Fig. 6) revealed that *S. arasbaranense* sp. n. is located in a clade with other species belonging to the *affine/intermedium* group, including *S. affine*, *S. sichuanense, S. intermediumm* and *S. beddingi*, but is closest to its sister taxon, *S. affine*. Although the above-mentioned species form a mono-

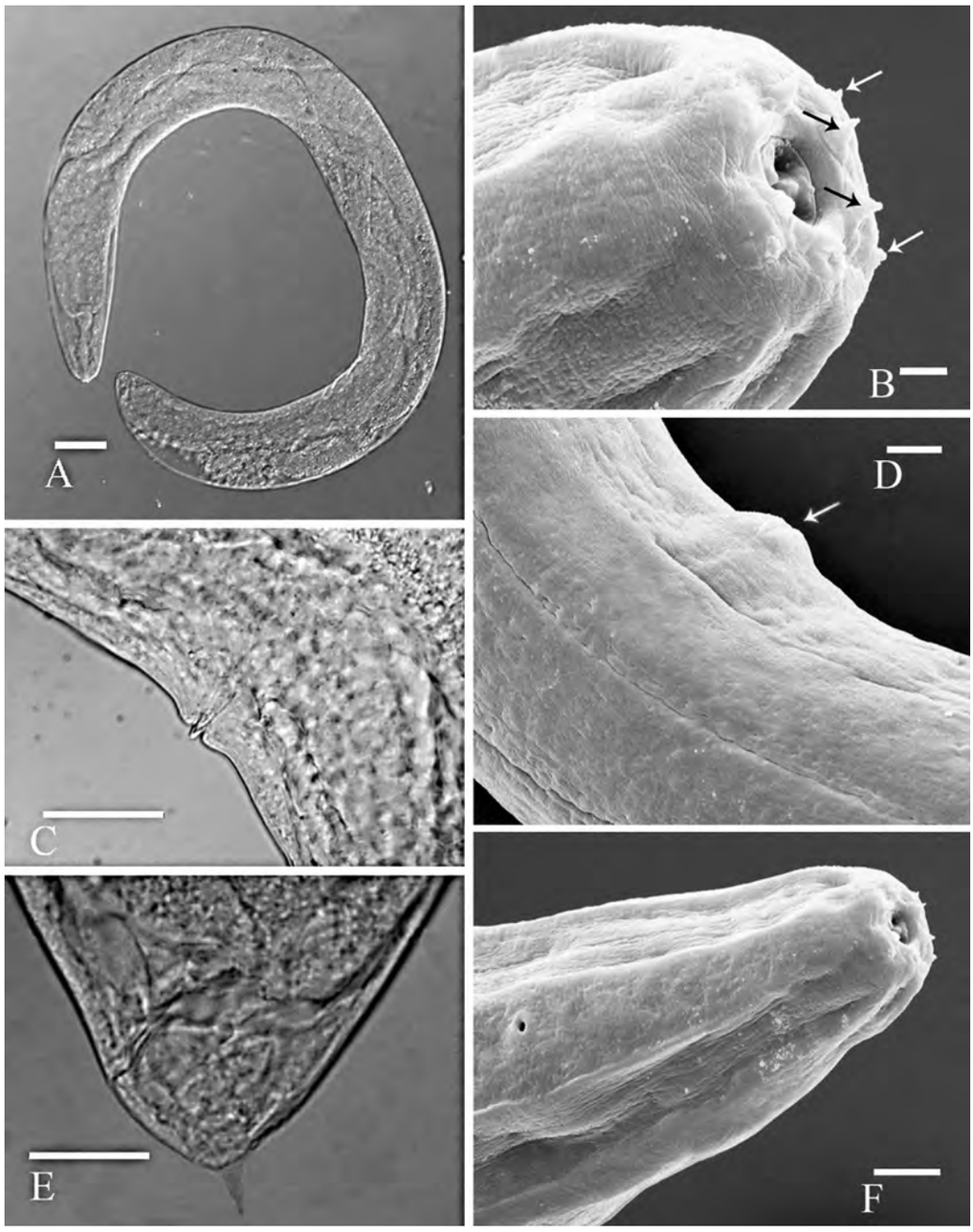

Fig. 4. SEM and light micrographs of first (A-E) and second (F) generation females of *Steinernema arasbaranense* sp. n. A, whole body; B, head showing labial and cephalic papillae; C and D, vulva with double flapped epiptygma and vulval lips; E, posterior region and terminal peg; F, anterior region showing head and secretory-excretory pore. Scale bars (in µm): A = 185, B = 13, C, E = 28, D = 36, F = 11.

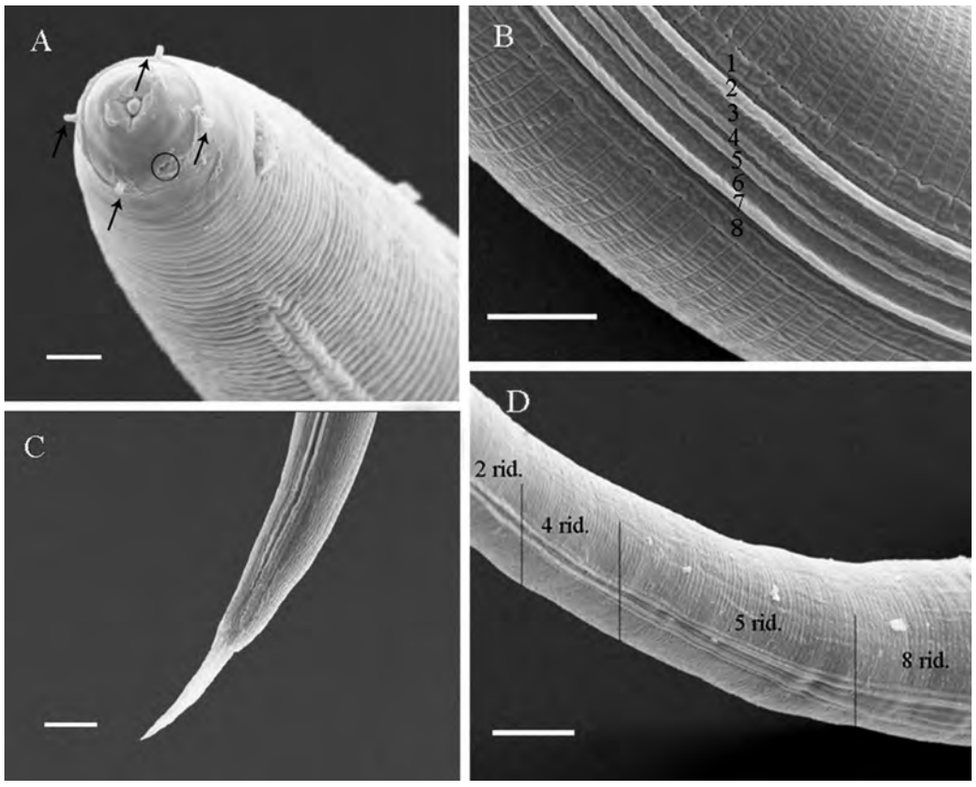

Fig. 5. SEM photographs of infective juveniles of *Steinernema arasbaranense* sp. n. A, anterior region showing closed mouth, four prominent cephalic papillae (arrows), amphid (circle) and lateral field with two starting ridges; B, lateral field in the middle of the body with eight ridges, of which those numbered 2, 4, 5 and 7 are narrow, prominent and brighter than the others; C, posterior part of lateral fields with two ridges and tail; D, lateral field showing the change of pattern regularly from eight to five, four and two ridges. Scale bars (in μm): A = 8, B =10, C = 26, D = 20.

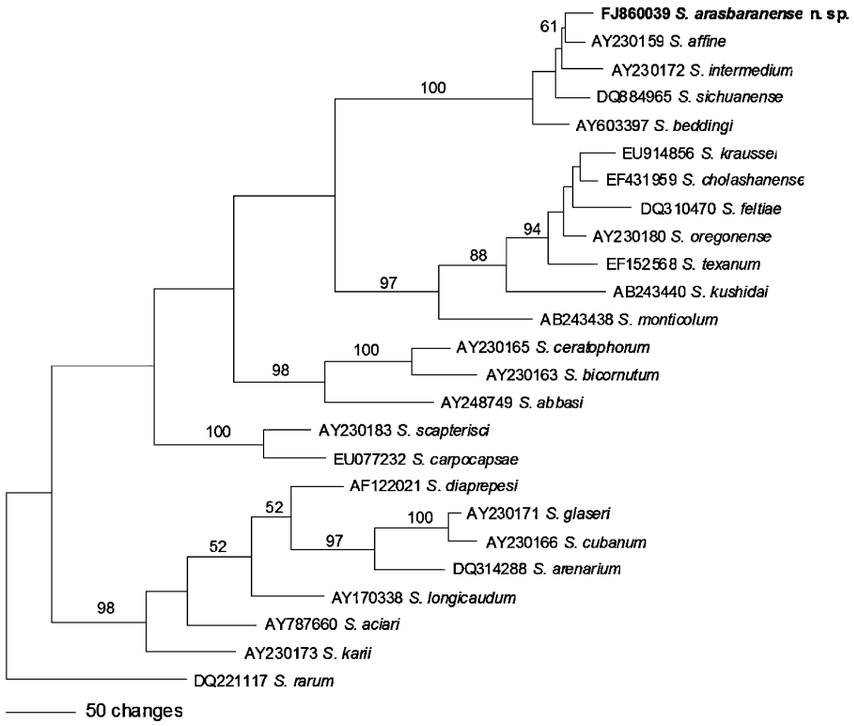

Fig. 6. Phylogenetic relationship of *Steinernema arasbaranense* sp. n. with other *Steinernema* species based on ITS rDNA. The tree is rooted on *Steinernema rarum*. Bootstraps for MP are shown on nodes.

Table II. Comparative morphometrics of *Steinernema arasbaranense* sp. n. and other closely related *Steinernema** species in *affine/intermedium* group (means and range in µm).

Stage/ Character	*S. arasbaranense* n. sp.	*S. affine* (Bovien, 1937)	*S. intermedium* (Poinar, 1985)	*S. beddingi* (Qiu *et al.*, 2005)	*S. sichuanense* (Mračeket *al.*, 2006)
Infective juvenile					
L	731 (693-795)	693 (608-880)	680 (608-800)	743 (700-790)	710 (700-790)
Excretory pore	56 (53-60)	62 (51-69)	65 (61-69)	70 (64-75)	64 (57-68)
Pharynx length	112 (110-118)	126 (115-134)	121 (110-131)	125 (113-130)	131 (121-142)
Tail length	84 (79-101)	66 (64-74)	64 (53-72)	77 (72-83)	72(64-76)
D%	50 (45-54)	49 (43-53)	51 (48-58)	57 (52-64)	49 (46-53)
E%	67 (53-75)	94 (74-108)	96 (89-108)	92 (84-103)	44 (38-51)
Ridges	8	6	6	4	6
1st generation male					
D%	50 (43-59)	61 (60-66)	72 (67-80)	58 (54-63)	51 (45-56)
Spicule length	75 (70-82)	70 (67-86)	93 (80-106)	71 (63-78)	68 (65-72)
Gubernaculum length	48 (42-53)	46 (37-56)	62 (48-96)	43 (38-48)	47 (40-51)
SW%	187 (174-213)	117	124 (103-139)	108 (88-132)	130 (120-140)
GS%	63 (52-70)	66	69 (63-77)	61 (55-66)	78 (60-80)
Mucron	Present	Present[a]	Absent	Absent[b]	Present[c]

D% (= EP / ES×100), E% (= EP / T×100), SW% (= SP / ABD×100), GS% (= G / SP×100)
*Data come from Poinar (1985), Poinar (1988), Qiu *et al.* (2005) and Mračeket *al.* (2006).
[a] According to the re-description given by Poinar in 1988 and Qiu *et al.* (2005), a minute mucron is presented in males of *S. affine* while Mračeket *al.* (2006) claim that it is absent in both generations of males.
[b] In both generations of males.
[c] In the second generation males.

phyletic group with a bootstrap support of 100%, they can be differentiated by pairwise distances (Table III).

Steinernema arasbaranense sp. n. clearly shows morphometrical differences from the other species of the *affine/intermedium* group. Nevertheless, differences in nucleotides (Fig. 7) are small, as also observed between species of other groups (Phan *et al.*, 2006). This suggests that all species of the *affine/intermedium* group could derive from recent speciation.

Type locality and hosts

The type host of this species in nature is unknown as the nematode was recovered from soil by baiting with *G. mellonella* on May 17, 2007. The soil sample was collected from an oak (*Quercus macrantera* L.) forest habitat near the Kerengan village, Kaleibar town, East Azarbaijan Province, Iran.

Etymology

The new species is named after the Arasbaran forests, a mountain area in northwest Iran where the type locality is situated.

Type specimens

Holotype male, and paratype males, females and infective juveniles are deposited in the Nematode Collection, Herbarium and Insect Museum of Natural Resources Research Institute, Tabriz, Iran. Three male, three female and five infective juvenile paratypes on separate microscopic slides are maintained in the Nematode Collection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran. Some paratype specimens will be deposited in the University of California, Davis, Nematode Collection, USA.

Diagnosis

Steinernema arasbaranense sp. n. is characterized by a combination of features of males and IJs. The IJ has average body length of 731 µm, lateral field pattern in a 2, 4, 5, 8, 4, 2 arrangement in which numbers 2, 4, 5 and 7 are narrow, prominent and more whitish than the others, E% of 67 (53-67), four projecting cephalic papillae and tail length of 84 (79-101) µm with a dorsal depression. Males have a hole-like structure at the spicule tip, presence of velum and mucron in both generations, longer mucron in second generation male, high SW ratio, genital papillae disposed as single mid-ventral precloacal papilla and 11 pairs.

Relationships

Both morphometric (Tables I and II) and molecular data (Fig. 6) showed that *S. arasbaranense* sp. n. belongs to the *affine/intermedium* group. The presence of eight ridges in the lateral fields of IJs of the new species, pres-

Table III. Pairwise distance of absolute nucleotides in ITS regions of species in *affine/intermedium* group and some species of *Steinernema* in other groups.

No.	GenBank	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	FJ860039	*S. arasbaranense* n. sp.																									
2	AY230159	*S. affine*	34																								
3	DQ884965	*S. sichuanense*	50	46																							
4	AY230172	*S. intermedium*	53	48	59																						
5	AY603397	*S. beddingi*	61	60	65	68																					
6	EU914856	*S. kraussei*	235	235	235	234	235																				
7	EF431959	*S. cholashanense*	255	252	254	252	256	38																			
8	AY230180	*S. oregonense*	253	251	254	250	256	45	32																		
9	DQ310470	*S. feltiae*	262	264	262	258	267	70	59	60																	
10	EF152568	*S. texanum*	253	255	255	251	260	62	59	62	83																
11	AB243440	*S. kushidai*	281	285	283	278	288	115	104	107	132	118															
12	AB243438	*S. monticolum*	229	229	231	226	234	134	130	135	141	141	156														
13	AF122021	*S. diaprepesi*	279	279	278	279	289	223	221	224	231	225	235	206													
14	AY170338	*S. longicaudum*	254	252	251	257	268	196	197	198	205	205	202	176	93												
15	AY787660	*S. aciari*	264	266	259	268	273	205	199	201	218	206	203	179	128	117											
16	AY230171	*S. glaseri*	275	279	278	281	290	224	228	231	233	228	234	207	128	134	143										
17	AY230166	*S. cubanum*	272	277	276	276	287	227	230	232	237	228	238	211	132	133	144	29									
18	DQ314288	*S. arenarium*	278	277	277	276	290	218	213	215	231	217	219	196	129	139	144	110	120								
19	AY230173	*S. karii*	250	249	250	248	252	193	191	194	190	197	201	167	124	118	127	160	160	147							
20	AY230183	*S. scapterisci*	266	267	266	275	275	211	212	216	224	214	210	194	221	189	210	218	216	225	200						
21	EU077232	*S. carpocapsae*	274	274	276	283	280	218	217	224	222	211	217	200	221	197	216	228	223	220	201	80					
22	AY230165	*S. ceratophorum*	268	266	264	272	270	200	200	198	208	193	214	190	212	185	204	212	217	216	187	215	214				
23	AY230163	*S. bicornutum*	276	272	274	270	279	199	200	198	211	198	212	201	218	201	220	222	225	227	204	212	217	76			
24	AY248749	*S. abbasi*	277	275	272	277	283	232	234	229	235	235	249	214	254	220	238	235	238	251	232	229	236	155	173		
25	DQ221117	*S. rarum*	264	266	272	269	271	208	209	212	222	218	211	208	200	194	204	220	223	214	207	228	233	228	234	254	

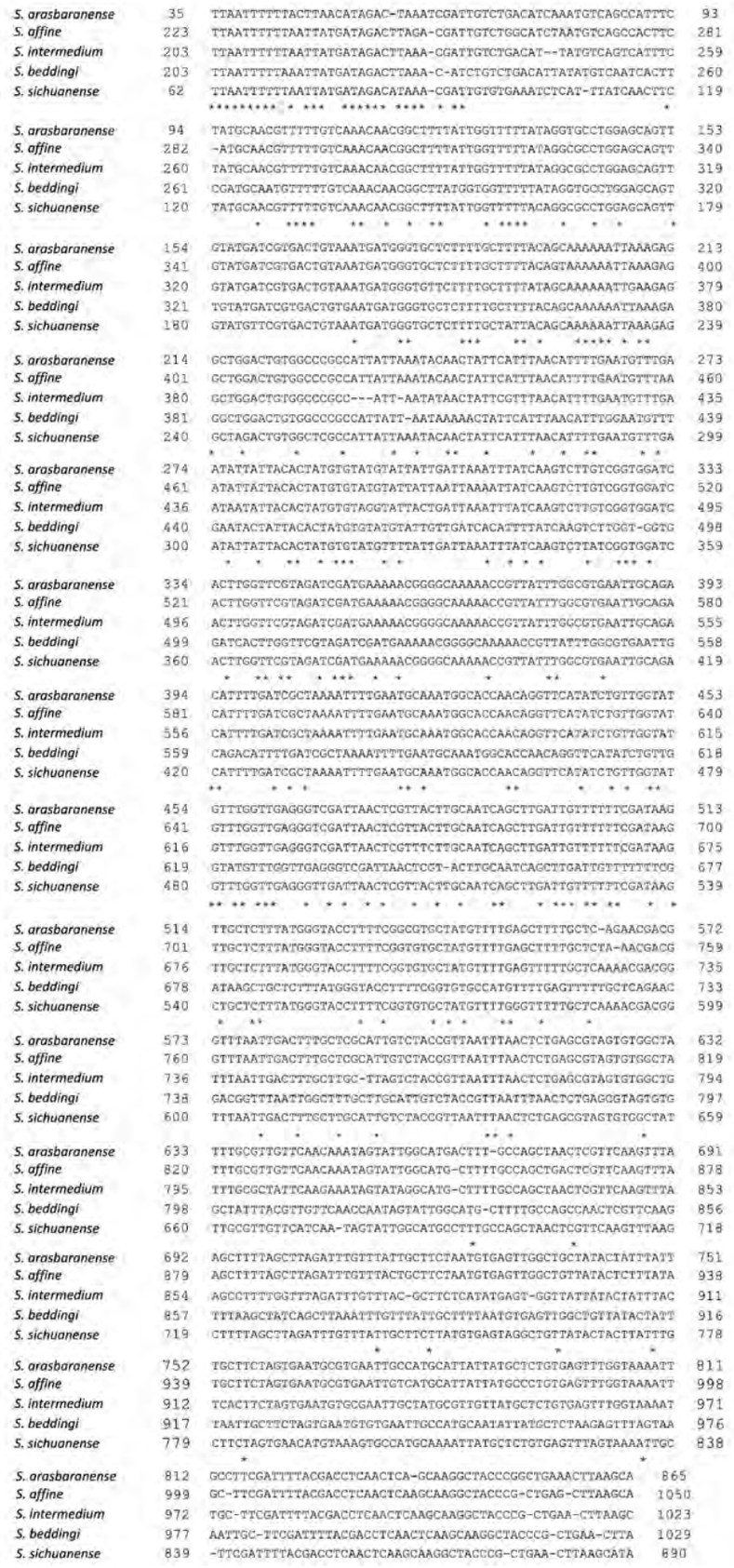

Fig. 7. Multiple sequence alignment of the ITS regions of *Steinernema arasbaranense* sp. n. with the four closely related species, *S. affine*, *S. intermedium*, *S. beddingi* and *S. sichuanense*. Hyphen (-) = gap. Asterisk (*) = No differences found among nematode species.

ence of mucron in both generation males (except second generation male of *S. sichuanense*) and a hole-like structure at the spicule tip are important differences between the new species and all other closely related species in the *affine/intermedium* group. The new species can be distinguished from *S. affine* by its longer tail of 84 (79-101) *vs* 66 (64-74) µm, a lower E% ratio of 67 (53-75) *vs* 94 (74-108) and absence of the internal tail spine in IJs; also by its lower D% ratio of 50 (43-59) *vs* 61 (60-66) in first generation males and presence of a mucron in both generation males (explanatory pointers are given in Tables II, a). *Steinernema arasbaranense* sp. n. differs from *S. intermedium* by its longer tail of 84 (79-101) *vs* 64 (53-72) µm, a shorter distance from the anterior end to the excretory pore of 56 (53-60) *vs* 65 (61-69) µm and lower E% ratio of 67 (53-75) *vs* 96 (89-108) in IJs; shorter spicule of 75 (70-82) *vs* 93 (80-106) µm, shorter gubernaculum of 48 (42-53) *vs* 62 (48-96) µm and lower D% ratio of 50 (43-59) *vs* 72 (67-80) in first generation males. The new species is separated from *S. sichuanense* by having a shorter pharynx of 112 (110-118) *vs* 131 (121-142) µm, a longer tail of 84 (79-101) *vs* 72 (64-76) µm, higher E% value of 67 (53-75) *vs* 44 (38-51) in IJs; a longer spicule of 75 (70-82) *vs* 68 (65-72) µm, a higher SW value of 187 (174-213) *vs* 130 (120-140) and presence of a mucron in the first generation male. *Steinernema arasbaranense* sp. n. differs from *S. beddingi* by a shorter distance from the anterior end to the excretory pore of 56 (53-60) *vs* 70 (64-75) µm, longer tail of 84 (79-101) *vs* 77 (72-83) µm, lower E% ratio of 67 (53-75) *vs* 92 (84-103) in IJs; higher SW% ratio of 187 (174-213) *vs* 108 (88-132) in first generation males. Generally, the morphology of spicules and gubernacula of the four species in the *affine/intermedium* group is different from that of *S. arasbaranense* sp. n. based on SEM photographs.

Comparing with species in other groups, IJs of *S. arasbaranense* sp. n. differ from *S. glaseri* group species (IJ with 950-1250 µm body length) in the much shorter body, and from *S. carpocapsae* group species (IJ body length <600 µm) by the longer body. It can be separated from *S. bicornutum* group species by the absence of horn-like structures on the head. Molecular characterization differentiated the new species from *feltiae-kraussei* group species (steinernematid species that have similar body length and number of ridges in lateral fields). In addition, some other morphological and morphometrical differences, such as lateral fields, projected cephalic papillae of IJs, genital papillae arrangement and hole-like structure in the spicule tip in males are further characters that differ between the new species and *feltiae-kraussei* group species.

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LITERATURE CITED

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

Courtney W.D., Polley D. and Miller V.I., 1955. TAF, an improved fixative in nematode technique. *Plant Disease Reporter, 39*: 570-571.

Eivazian Kary N., Niknam G., Griffn C.T., Mohammadi S.A. and Moghaddam M., 2009. A survey of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) from north-west of Iran. *Nematology, 11*, 107-116.

Hominick W.M., 2002. Biogeography. Pp. 115-143. *In*: Entomopathogenic Nematology (Gaugler, R., ed.). CABI Publishing, Wallingford, UK.

Mraček Z., Nguyen K.B., Tailliez P., Boemare N. and Chen Sh., 2006. *Steinernema sichuanense* n. sp. (Rhabditida, Steinernematidae), a new species of entomopathogenic nematode from the province of Sichuan, East Tibetan Mts., China. *Journal of Invertebrate Pathology, 93*: 157-169.

Nguyen K.B. and Hunt D.J., 2007. *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts*. Nematology Monographs and Perspectives, Vol. 5 (Hunt D.J. and Perry R.N., series editors). Brill, Leiden, The Netherlands, 816 pp.

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

Nikdel M., Niknam G., Griffin C.T. and Eivazian Kary N., 2010. Diversity of entomopathogenic nematodes (Nematoda: Steinernematidae, Heterorhabditidae) from Arasbaran forests and rangelands in the north-west of Iran. *Nematology, 12*: 1-7.

Parvizi R., 2003. An evaluation of the efficacy of the entomopathogenic nematode *Heterorhabditis bacteriophora* and *Steinernema* sp. in controlling immature stages of the apple clearwing, *Synanthedon myopaaeformis*. *Iranian Journal of Agricultural Sciences, 34*, 303-311 [In Persian with English Summary].

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.

Phan L.K., Takemoto S. and Futai K., 2006. *Steinernema ashiuense* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Japan. *Nematology*, *8*: 681-690.

Poinar G.O., 1985. *Neoaplectana intermedia* n. sp. (Steinernematidae: Nematoda) from South Carolina. *Revue de Nématologie, 8*: 321-327.

Poinar G.O., 1988. Redescription of *Neoaplectana affinis* Bovien (Rhabditida: Steinernematidae). *Revue de Nématologie, 11*: 143-147.

Posada D. and Crandall K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics, 14*: 817-818.

Qiu L., Hu X., Zhou Y., Pang Y. and Nguyen K.B., 2005. *Steinernema beddingi* n. sp. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Yunan, China. *Nematology, 7*: 737-749.

Seinhorst J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica, 4*: 67-69.

Stock S.P., Griffin C.T. and Chaerani R., 2004. Morphological and molecular characterisation of *Steinernema hermaphroditum* n. sp. (Nematoda: Steinernematidae), an entomopathogenic nematode from Indonesia, and its phylogenetic relationships with other members of the genus. *Nematology, 6*: 401-412.

Swofford D.L., 1998. PAUP. *Phylogenetic analysis using parsimony. Version 4*. Sunderland, MA, USA, Sinauer, 128 pp.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. and Higgins D.G., 1997. The CLUSTAL-X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research, 25*: 4876-4882.

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

Woodring J.L. and Kaya H.K., 1988. Steinernematid and heterorhabditid nematodes: A handbook of techniques. *Arkansas Agricultural Experimental Station. Southern Cooperative Bulletin*, *331*: 1-30.

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