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Steinernema abbasi sp. n. (Nematoda: Steinernematidae) from the Sultanate of Oman

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Summary - Description is given of *Steinernema abbasi* sp. n., isolated from soil in alfalfa fields in the Sultanate of Oman. *S. abbasi* sp. n. was isolated in sub-tropical semi-arid environment where the bollworms, *Helicoverpa armigera* and *Spodoptera littoralis,* are major pests. *S. abbasi* sp. n. could be used as a biological control agent in high temperature situations particularly in the Middle East. Morphological examination, DNA analysis and hybridization have shown that *S. abbasi* sp. n. is distinct from *S. carpocapsae, S. scapterisci* and *S. riobrave.*

Résumé - Steinernema abbasi *sp. n. (Nematoda : Steinernematidae) originaire du Sulatanat d'Oman* - Description est donnée de *Steinernema abbasi* sp. n., extrait du sol de champs de luzerne dans le Sultanat d'Oman. *S. abbasi* sp. n. provient d'environnements subtropicaux semi-arides où les noctuelles *Helicoverpa armigera* et *Spodoptera littoralis* sont des parasites majeurs. *S. abbasi* pourrait être utilisé comme agent de contrôle biologique dans des environnements très chauds, particulièrement au Moyen-Orient. L'observation morphologique, l'analyse de l'ADN et des croisements interspécifiques ont montré que *S. abbasi* sp. n. est une espèces distincte de *S. carpocapsae, S. scapterisci* et *S. riobrave.*

Key-words: alfalfa, biocontrol, entomopathogenic nematodes, *Steinernema abbasi* sp. n., taxonomy.

A steinernematid was isolated from soil on the Agricultural Research Station near Salalah in the south of the Sultanate of Oman in alfalfa fields by Dr. M. A. Hubeis. The semi-arid climate of Oman has light rains 2-4 days per week from late June to August (100% RH). The temperature ranges from 17-23°C (November to February) and is 23-32°C for the remainder of the year. The most important pests of alfalfa are *Helicoverpa armigera* (Hübner) the dominant bollworm pest, and *Spodoptera littoralis* (Boisduval) but the steinernematid was never recovered from a known host. The nematode has been shown to be a new species of *Steinernema, S. abbasi* sp. n., by DNA analysis, hybridization and morpho-metrical study.

Materials and methods

SAMPLING

The nematode was isolated from sandy soils with the Bedding and Akhurst (1975) baiting technique using *Galleria mellonella, H. armigera* and *S. littoralis* as bait insects. The extracted nematodes were sent to Reading University where the infective juveniles (IJs) were reared *in vivo* using *G. mellonella* larvae to test pathogenicity and confirm Koch's postulates.

CROSS HYBRIDIZATION

Crosses between *S. abbasi* sp. n. and *S. riobrave**, *S. carpocapsae, and S. scapterisci* were conducted using an injection technique in *Galleria* larvae (Akhurst & Bedding, 1978). These species were chosen as being the most closely related morphologically to *S. abbasi* sp. n. available in the UK: *S. kushidai* was used in the PCR analysis (see below) but the authors were unable to obtain a culture of *S. kushidai* for the hybridization. IJs of the four species were surface sterilized in 1% hyamine solution for 15 min. Single IJs of two different species were injected into a *Galleria* larva with controls as crosses between IJs of the same species. There were 40 replicates of each cross and the *Galleria* larvae were dissected after 6 days to detect progeny development.

PCR ANALYSIS

Total genomic DNA was isolated as described by Reid and Hominick (1992). DNA purified by this method was used to produce restriction fragment length polymorphism (RFLP) profiles for *S. riobrave, S. carpocapsae* and *S. scapterisci* isolates. The RFLP profiles for *S. kushidai* and *S. abbasi* sp. n. were

* The name *S. riobravis* has been changed to *S. riobrave*, according to Latin grammar, at the COST Workshop, IIP, St Albans, UK, March 1996.

obtained from adult female lysate (Joyce *et al.*, 1994). Primers used in polymerase chain reaction were for the internal transcribed spacer (ITS) as described by Vrain *et al.* (1992). Primers were synthesised by Pharmacia Biotech. Reaction and cycling conditions were identical for both the purified DNA and nematode lysate reactions. Amplifications were carried out in a solution of (100 µl), containing 50 mM KCL, 10 mµ Tris(pH 9.0), 1.5 mM $MgCL_2$, 0.1% Triton X-100, 0.2 mM of each dNTP, 0.5 mM of each primer, 100 ng of purified DNA(or 5 µl of nematode lysate) and eight units of Taq polymerase (Promega Corporation). Amplifications were carried out using Teche PHC-3 thermocycler which was preheated to 95°C and incubated at 94°C for 2 min followed by 40 cycles of 94°C for 30 s, 45°C for 1 min and 72°C for 1.5 min. A final step of 5 min at 72°C was included to ensure all of the final amplification products were full length. Amplified product were digested with a range of restriction endonuclease immediately. Restriction enzymes were purchased from Amersham International or Progmega and used with the buffers supplied by the manufacturers. All digestions were carried out using 4 µl of amplified products at 37°C for a minimum of 2 h. The resulting fragments were separated on 1.5% (w/v) agarose gels in TBE at 5V/cm for 3 h. Fragments were visualized by ethidium bromide staining (Maniatis *et al.*, 1989). DNA from *S. abbasi* sp. n. and four other morphologically similar steinernematid species was amplified by the polymerase chain reaction using primers specific for the ITS (internal transcribed spacer) region. The PCR products for each nematode were then digested with seventeen different restriction enzymes and the fragments separated by agarose gel electrophoresis (Fig. 1). The resulting combination of RFLPs produced by the seventeen enzymes was then compared from species to species for similarities.

Morphological observations

For light microscope study, the infective juveniles, adult males and females were killed using hot water (60°C), fixed in Ringer TAF for 3 days, transferred to 2% glycerol with traces of formalin solution for 8 days and then mounted in anhydrous glycerol on glass slides. Glass wool or small pieces of cover slip were used to prevent the flattening of the specimens. Measurements were made using an ocular micrometer and camera lucida, the drawings were made using a drawing tube mounted on Olympus microscope with Nomarski Interference contrast attachment.

For electron microscopy fresh specimens were fixed in gluteraldehyde for 2 h, washed twice with 0.1M sodium cacodylate, post-fixed in 0.1M osmium tetroxide, after which the specimens were again washed twice with 0.1M sodium cacodylate before dehydration in a graded series of concentrated acetone. The specimens were dried first in 1:1 acetone and hexamethyldislazane for 15 min and finally in pure hexametyldislazane. Then they were mounted on stubs, coated with gold and examined under Jeol JT300 SEM.

Steinernema abbasi* sp. n.

(Figs 1-5).

Measurements

See Table 1.

Description

Males (first generation): Body slender, ventrally curved, J-shaped upon fixation. Cuticle with fine transverse striae. Lateral fields and phasmids inconspicuous. Lip region continuous, with distinct labial

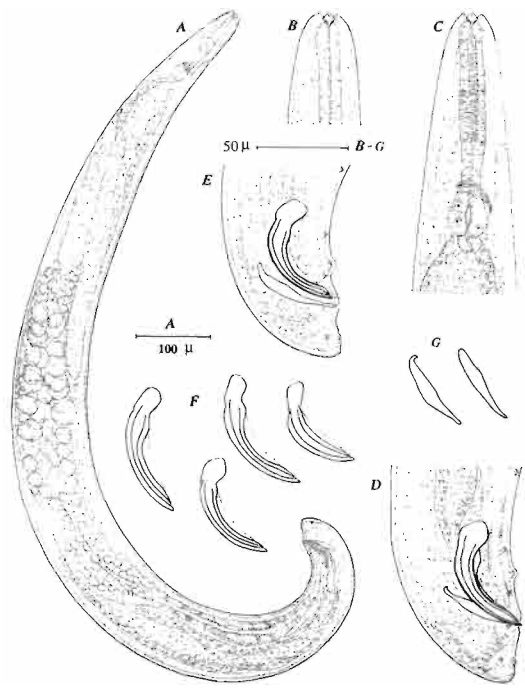

Fig. 1. Steinernema abbasi *sp. n., male. A: Entire body of first generation; B: Head region of second generation; C: Oesophageal region of first generation; D: Tail region of first generation; E: Tail region of second generation; F: Variation in spicule shape of first generation; G: Variation in shape of gubernaculum of first generation.*

* Named after Dr. M.S. Abbas who was one of the people who isolated the nematode in Oman.

Table 1. *Morphometrics of* Steinernema abbasi *sp. n. (all measurements in µm).*

	Third stage juvenile	Males			Females		
		Holotype	First generation	Second generation	Normal form	Giant form	Second generation
n	15	1	15	15	10	10	10
L	541 ± 24 (496-579)	1142	1252 ± 189 (999-1534)	861 ± 121 (606-1035)	3510 ± 638.1 (2453-4477)	10730 ± 2197 (8055-13735)	2609 ± 622 (1897-3917)
Greatest width	29 ± 0.99 (27-30)	80	87 ± 6.7 (82-98)	70 ± 4.6 (64-80)	159 ± 11.5 (143-181)	225 ± 42 (186-303)	130 ± 5 (123-138)
Stoma length		4	4.50 ± 0.64 (4-6)	4 ± 0.26 (4-5)	7.8 ± 0.42 (7-8)	7.5 ± 0.53 (7-8)	6 ± 0.32 (6-7)
Stoma width		7	7.20 ± 0.41 (7-8)	7 ± 0.93 (6-7)	8.6 ± 0.52 (8-9)	9.4 ± 0.52 (9-10)	8 ± 0.42 (8-9)
EP	48 ± 1.5 (46-51)	68	80 ± 7.8 (68-89)	66 ± 5 (62-79)	71 ± 10.9 (58-91)	84 ± 13 (51-99)	66 ± 3.94 (61-73)
EPW		43	45 ± 3.40 (41-51)	39 ± 1.60 (39 ± 1.60			
NR	68 ± 2.4 (64-72)	107	103.20 ± 6.48 (99-123)	99 ± 3.90 (93-106)	125 ± 5.8 (120-137)	149 ± 13.5 (131-182)	114 ± 2.5 (110-118)
ES	89 ± 1.8 (85-92)	134	133 ± 6 (121-144)	121 ± 4.90 (112-130)	165 ± 7.48 (155-176)	193 ± 26 (125-224)	146 ± 6.7 (136-157)
Testis reflexion		259	274 ± 33 (234-319)	241 ± 20 (241 ± 20)			
Anal body width		39	43 ± 4.90 (37-55)	39 ± 1.60 (36-42)	62 ± 6.8 (51-70)	99 ± 16.8 (73-129)	50 ± 2 (47-54)
Tail length	56 ± 3.2 (52-61)	24	26 ± 3 (20-31)	21 ± 1.70 (17-24)	37 ± 3.1 (31-40)	46 ± 4.7 (40-55)	36 ± 2.5 (32-39)
Spicule length		69	65 ± 5.70 (57-74)	61 ± 4.80 (51-69)			
Spicule width		13	12 ± 1.30 (10-14)	10.27 ± 1.62 (8-13)			
Gubernaculum length		50	45 ± 4.30 (33-50)	43 ± 3.30 (35-48)			
Gubernaculum width		8	7 ± 0.10 (6-8.5)	7 ± 0.65 (6-8)			
V					55 ± 2.4 (52-60)	50 ± 5.5 (43-57)	55 ± 2.2 (50-77)
a	18 ± 0.91 (17-20)						
b	6 ± 0.32 (5.5-6.6)						
c	9.8 ± 0.83 (8.1-10.8)						
d (EP/ES)	0.53 ± 0.02 (0.51-0.58)	0.51	0.6 ± 0.05 (0.51-0.68)	0.56 ± 0.07 (0.50-0.70)	0.42 ± 0.05 (0.36-0.48)	0.43 ± 0.03 (0.38-0.47)	0.45 ± 0.01 (0.43-0.47)
e (EP/tail)	0.86 ± 0.05 (0.79-0.94)						
EW		1.58	1.76 ± 0.13 (1.59-2.12)	1.70 ± 0.06 (1.55-2.08)			
SW		1.77	1.56 ± 0.22 (1.07-1.87)	1.59 ± 0.15 (1.28-1.88)			
GS		0.72	0.7 ± 0.07 (0.58-0.85)	0.70 ± 0.07 (0.58-0.81)			

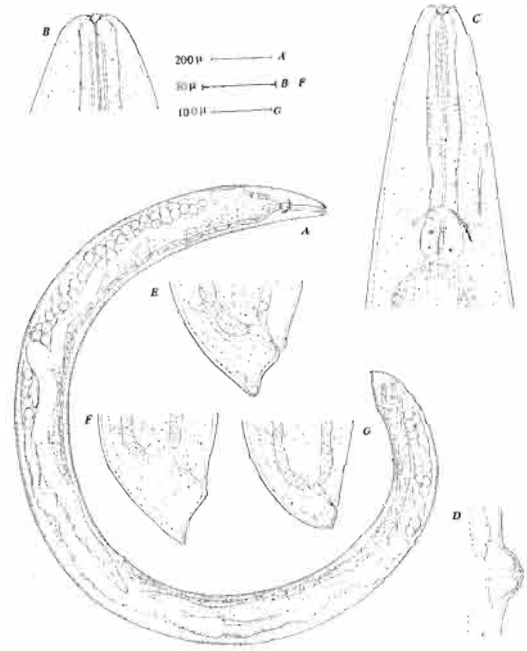

Fig. 2. Steinernema abbasi *sp. n., female. A: Second generation entire body; B: Head of first generation giant form; C: Pharyngeal region of the first generation female normal form; D: Vulva of first generation; E-G: Variation in tail; E: normal first generation tail; F: second generation tail; G: giant female tail.*

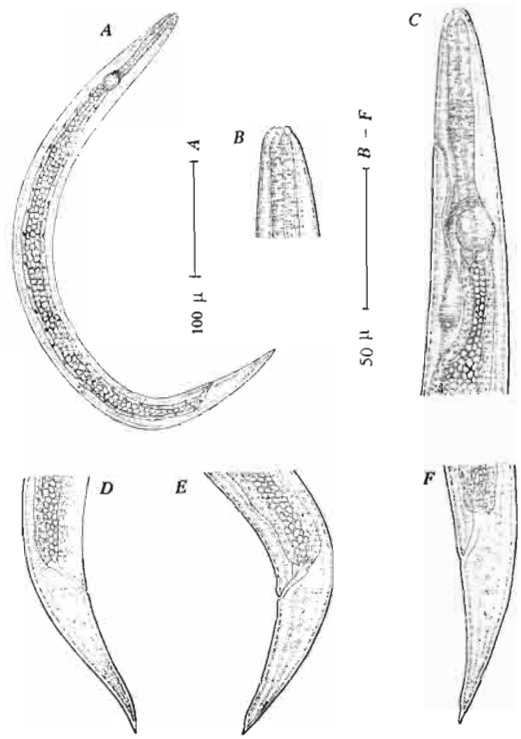

Fig. 3. Steinernema abbasi *sp. n., third stage infective juvenile. A: Entire body; B: Head region; C: Oesophageal region; D: Variation in the shape of the tail.*

papillae. SEM face view with six distinct lips surrounding a cuticular oral aperture with six prominent labial papillae and four cephalic papillae, each with distinct pore. Amphids pore-like located behind lateral papillae. Stoma shallow, partially collapsed and triangular at base. Cheilorhabdions separated by thick ring of sclerotized material. Oesophagus muscular with cylindrical procorpus; almost indistinguishable non-valvate metacorpus; slightly narrow isthmus and round basal bulb with valve plates and three oesopha-geal glands. Nerve ring surrounding isthmus just above the basal bulb. Oesophageal-intestinal valve conoid, bilaterally symmetrical. Excretory pore always above the nerve ring near the base of metacorpus. Distance from the anterior end to the excretory pore always more than body width at excretory pore. Gonads monorchic, testis reflexed. About 60% of males with normal testis and 40% with reduced or collapsed testis, and distance from base of the oesophagus to anterior end of the testis always more than distance from anterior end of nematode to base of oesophagus. Spicules paired, golden dark yellow in colour. Head (manubrium) of spicule 12-15 µm about 20% of spicule length; shaft (calomus) almost absent; blade (lamina) thick, gradually tapering, about three-four times longer than head; blade terminus pointed with a depression on ventral side; head/blade angle ranging between 107-120°; velum present. Each spicule with two internal ribs; shape of spicule, width of head, and the degree of curvature variable. Gubernaculum about 70% of the spicule length, boat-shaped, ventrally curved, slightly swollen in the middle and gradually narrowing distally; proximal end with or without knob or hook. Bursa absent. Twenty-three genital papillae present, with a single large ventral precloacal one, 22 µm from the cloaca; six pairs of ventrosublateral precloacal papillae; a pair of ventrosublateral papillae located almost at the level of the cloaca with four pairs of caudal papillae. Tail short and conoid, about 60% of the anal body width long with rounded terminus; terminal mucron absent.

Males (second generation): Similar to the first generation except smaller and thinner, with collapsed testis, spicules and gubernaculum slightly shorter and thinner. Shape of the spicule and the gubernaculum not

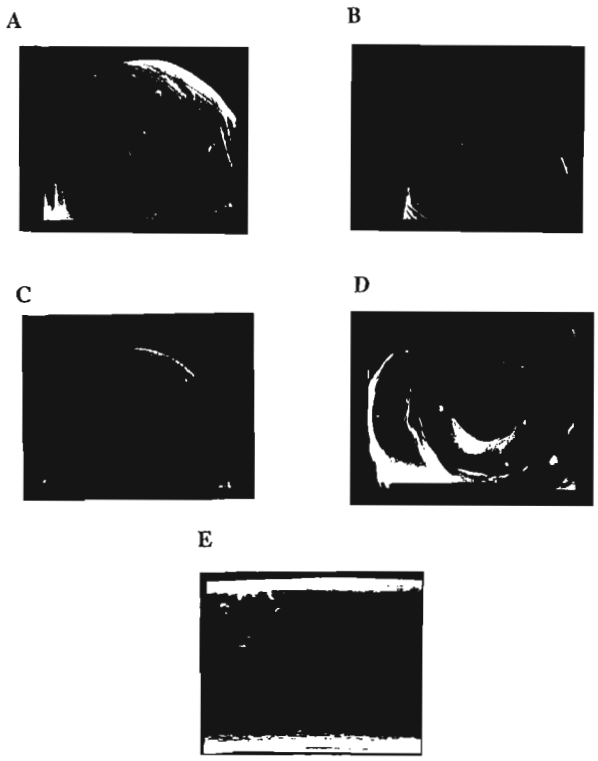

Fig. 4. Steinernema abbasi *sp. n. A: Anterior region of first generation male; B: Anterior region of first generation female; C: Anterior region of giant female; D: Posterior region of first generation male; E: Lateral lines of infective juveniles.*

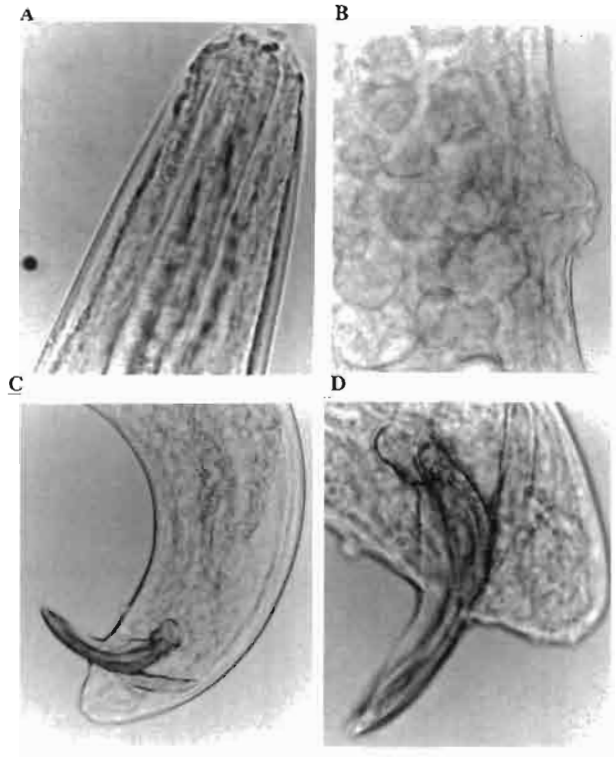

Fig. 5. Steinernema abbasi *sp. n. A: Anterior region of first generation male; B: Vulval region of normal form of first generation female; C, D: Posterior region of first generation male with spicules and gubernaculum.*

different from those of the first generation males, but slightly variable within the individuals of the same generation.

Females (first generation): Body robust, strongly curved, and often C or spiral-shaped. Cuticle with fine striae. Lateral fields and phasmids inconspicuous. Lip region rounded, continuous with the body; pre-oral disc present; SEM face view with six labial and four cephalic papillae; lips amalgamated; amphids small pore-like. Stoma shallow, triangular at base; oral aperture circular. Cheilorhabdions prominent, well sclerotized, oesophagus muscular with cylindrical procorpus, slightly swollen, non-valvate metacorpus, comparatively narrow isthmus and rounded basal bulb with distinct valve plates and oesophageal glands. Cardia short and conical. Nerve ring just above the basal bulb. Excretory pore at the level of metacorpus. Distance of excretory pore from anterior end always more than the body width at excretory pore. Gonads amphidelphic, reflexed, often containing many eggs. Vulva a transverse slit; epiptygma present. Vagina sclerotized, 16 µm deep and about 9% of the corresponding body width. Tail short, conoid, with a pointed tip, about 65% of the anal body width; a ventral postanal swelling always present.

Females (first generation; giant forms): In addition to the normal female of the first generation, a giant form of the female occuring with body spiralled, about three times longer than the normal female; other structures as described for normal females.

Females (second generation): Similar to the first generation female but smaller, length ranging from 50-70% of the first generation females, with three rows of oocytes. Tail more sharply pointed, longer than the first generation and the giant forms; anal body width about 1.5 of the tail length, with ventral post-anal swelling present.

Infective juveniles: Body thin, elongate, sheath (J2 cuticle) not always present. Lip region continuous. Lateral fields with eight incisures. Excretory pore always weak, near the base of metacorpus. Distance from anterior end to excretory pore always more than

Table 2. *The distinguishing characters of the infective juveniles and males of the four species of* Steinernema *most similar to* S. abbasi *sp. n. (all measurements in μm).*

	S. carpocapsae	*S. kushidai*	*S. scapterisci*	*S. riobrave*	*S. abbasi* sp. n.
INFECTIVE JUVENILE					
L	558 (438-650)	589 (564-662)	572 (517-609)	662 (561-701)	541 (496-579)
EP	38 (30-60)	46 (42-50)	39 (36-48)	56 (51-64)	48 (46-51)
d	26 (23-28)	41 (38-44)	31 (27-40)	49 (45-55)	53 (51-58)
e	60 (54-66)	92 (89-108)	73 (60-80)	105 (93-111)	86 (79-94)
MALE					
Mucron	Present	Absent	Present	Absent	Absent
Spicule length	66 (58-77)	63 (48-72)	83 (72-92)	66.9 (62.5-75)	65 (57-74)
Gubernaculum length	47 (39-55)	44 (39-60)	65 (59-75)	51 (47.5-56)	45 (33-50)
d	41 (27-55)	51 (42-59)	38 (32-44)	71 (60-80)	60 (51-68)

body width at the same level. Oesophagus with cylindrical procorpus and slightly swollen median bulb. Nerve ring just above the basal bulb. Tail elongate, attenuated, gradually tapering, dorsally curved at tip with slight ventral depression.

TYPE HOST AND LOCALITY

Type host: unknown but likely to be a bollworm. Type locality: sandy soil in alfalfa fields in the Sultanate of Oman (Lat. 16°N and Long 54°E).

TYPE SPECIMENS

Holotype male, two paratype males, two paratype females and two paratype juveniles deposited at the CABI International Institute of Parasitology at St Albans, UK.

CROSS HYBRIDIZATION

Attempts to cross hybridize *S. abbasi* sp. n. with *S. carpocapsae, S. riobrave* and *S. scapterisci* yielded no progeny, but all intra-specific crossing resulted in offsprings (Table 3).

DNA

It can be seen that the restriction enzyme profiles for *S. abbasi* sp. n. (Fig. 6A) show that for the majority of the enzymes tested the profiles are distinct from those of the four other species. The exceptions to this are those enzymes which do not have recognition sites within the ITS region of these nematodes (*e.g.*, BstOI, Fig. 6A-E, lane 3).

Table 3. *Results of hybridization between* Steinernema abbasi *sp. n. and other related* Steinernema *species.*

Species	*S. carpocapsae*	*S. scapterisci*	*S. riobrave*	*S. abbasi* sp. n.
S. carpocapsae	+	–	–	–
S. scapterisci	–	+	–	–
S. riobrave	–	–	+	–
S. abbasi sp. n.	–	–	–	+

DIAGNOSIS AND RELATIONSHIPS

S. abbasi sp. n. can be separated from *S. carpocapsae* (Weiser), *S. scapterisci* (Nguyen & Smart) and *S. riobrave* (Cabanillas, Poinar & Raulston) by morphological, DNA, and hybridization characters. The detailed differences for infective juveniles and first generation males are compared with the four most similar species (Table 2).

The male of *S. abbasi* sp. n. superficially appears similar to that of *S. riobrave* with golden dark yellow spicules and the absence of a terminal mucro, but it has a shorter body length and less curvature in the head/blade angle of the spicule, ranging from 107-

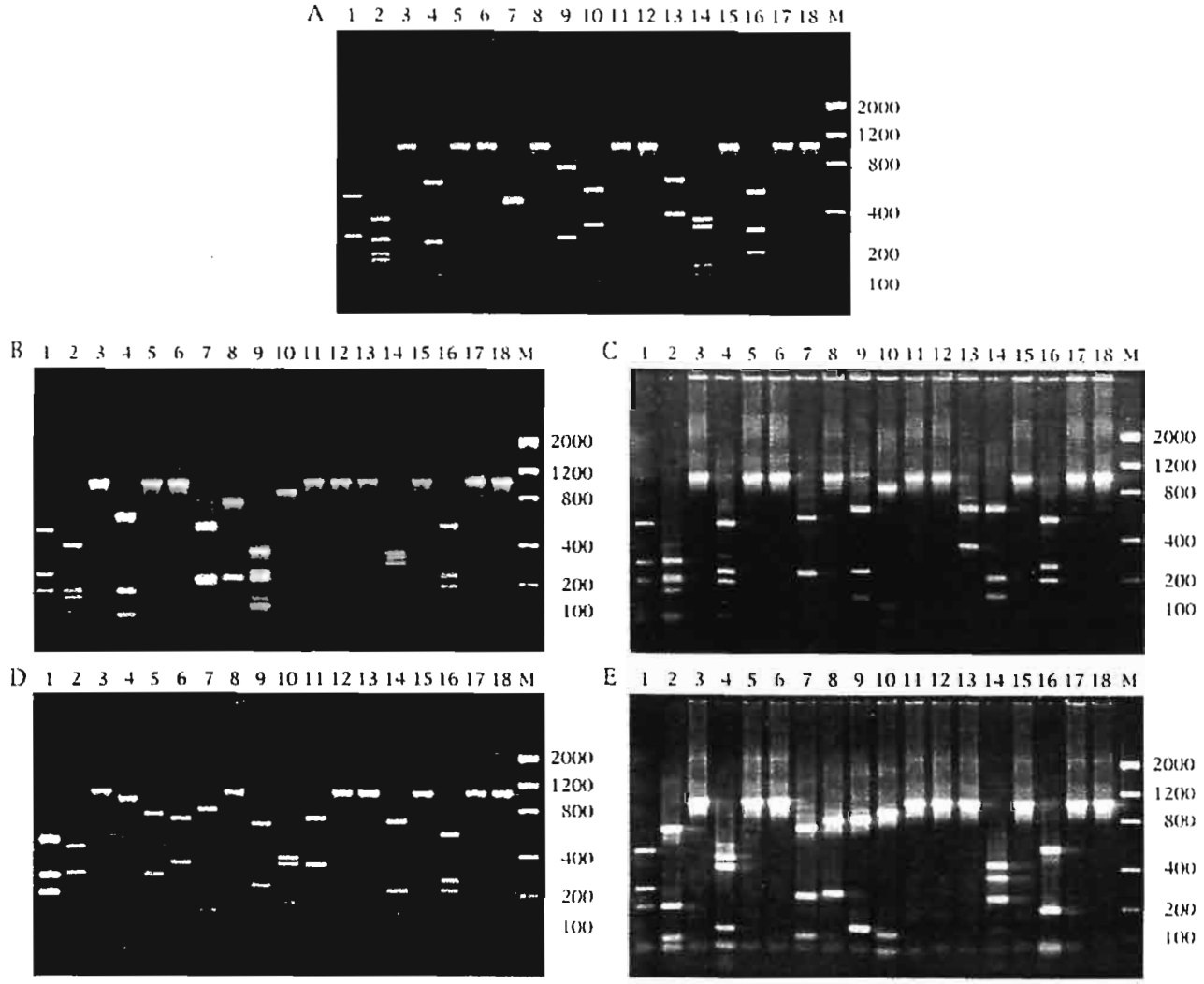

Fig. 6. *PCR Amplified products from the internal transcribed spacer (ITS) region digested with seventeen different restriction enzymes. Fragments were separated on ethidium bromide stained 1.5% (w/v) agarose gels. A: Steinernema abbasi; B: S. carpocapsae; C: S. scapterisci; D: S. kushidai; E: S. riobrave. (In each gel lane 1 is a digest of S. feltiae (UK, site 76) with Alu I. Lanes 2-18 are individual digests of the respective species for that gel with the following restriction enzymes; 2: Alu I; 3: BstO I; 4: Dde I; 5: EcoR I; 6: Hae III; 7: Hha I; 8: Hind III; 9: Hinf I; 10: Hpa II; 11: Kpn I; 12: Pst I; 13: Pvu II; 14: Rsa I; 15: Sal I; 16: Sau3A I; 17: Sau96 I; 18: Xba I; lane M is the molecular weight marker and the band sizes are shown in base pairs.)*

120° for *S. abbbasi* compared to 90-100° for *S. riobrave* (Table 2). The females in the first generation of *S. abbasi* sp. n. are similar to those of *S. riobrave* but the tail shape of the second generation are wider with a rounded wedge-shaped projection on the tip compared to the sharp V-shaped tail in *S. riobrave*. The diagnostic morphological characters of the third-stage infective juveniles and the adults of *S. abbasi* sp. n. do not fit the description of any of the currently recognized species of the genus *Steinernema* (Poinar, 1990; Nguyen & Smart, 1992; Cabanillas *et al.*, 1994).

Comparison of the profiles from *S. abbasi* sp. n. and 33 other steinernematid species/isolates held in a data base at IIP shows it to be a distinct and new species at the molecular level.

S. abbasi sp. n. is reproductively isolated from *S. carpocapsae, S. scapterisci*, and *S. riobrave* indicated by the negative results of cross-breeding tests.

BIOLOGY

The life cycle of *S. abbasi* sp. n. is comparable to existing species of *Steinernema*, including an egg, four juvenile stages and adults. The third-stage infective juvenile enters the haemocoel of insects to deliver the associated bacteria and completes at least two generations before emerging from the cadaver as infective juveniles. In *G. mellonella* adults develop in 48 h at 25°C and in 36 h at 30°C.

S. abbasi sp. n. appears to be more active at higher temperatures than *S. riobrave* as it produces more infective juveniles in *G. mellonella* and *S. littoralis* larvae at 35°C. The LT_{50} for *S. abbasi* sp. n. is slightly superior to that of *S. riobrave* against *G. mellonella* but the temperature profile (thermal niche breadth for establishment) for *S. abbasi* sp. n. is similar to that of *S. riobrave* (Elawad *et al.*, 1996).

S. abbasi sp. n. and *S. riobrave* are clearly nematodes of semi-arid and subtropical regions. *S. riobrave* was recovered from pre-pupae and pupae of *H. zea* and *S. frugiperda* in Texas, USA (Raulston *et al.*, 1992) and has recently been described (Cabanillas *et al.*, 1994). *S. abbasi* sp. n. is associated with bollworms but it has not been actually isolated from a known host: it does however reproduce well in *Spodoptera littoralis* in the laboratory (unpubl.). It is likely that *S. abbasi* sp. n. could be developed to control pre-prupae and pupae of bollworms in the Middle East or elsewhere where bollworms are a major problem, particularly under irrigation.

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Erratum

In the following publication:

ELAWAD, S., AHMAD, W. & REID, A.P. – *Steinernema abbasi* sp. n. (Nematoda: Steinernematidae) from the Sultanate of Oman. *Fundam. appl. Nematol.*, 20 (5): 435-442 (1997),

the figure 4 (p. 439) has been regrettably badly printed.

A correct printing is given below.

With the apologies from the printer.