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Steinernema oregonensis n. sp. (Rhabditida : Steinernematidae) from Oregon, U.S.A.[1]

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Summary – A new species in the genus *Steinernema* was found near Grant's Pass, Oregon. *Steinernema oregonensis* n. sp. can be distinguished from other *Steinernema* species by the length of infective juveniles, the shape of spicules, the absence of a tail mucron in first generation males, and the presence of a tail mucro in second generation males. The length of infective juveniles ranged from 816 to 1112 µm, separating *S. oregonensis* n. sp. from previously described *Steinernema* species except *S. affinis, S. anomali, S. feltiae, S. glaseri, S. neocurtillis,* and *S. puertoricensis.* The spicule shape and the absence of tail mucron in first generation male separate *S. oregonensis* n. sp. from these species. Ratio d and ratio e of the infective juveniles ranged from 0.4 to 0.6 and from 0.9 to 1.1, separating this new species from *S. cubana, S. glaseri, S. neocurtillis,* and *S. puertoricensis.* DNA analysis showed that *S. oregonensis* n. sp. has distinct random amplified polymorphic DNA fragments compared to the other four *Steinernema* species examined. *S. oregonensis* n. sp. did not hybridize with closely related species : *S. anomali, S. feltiae,* or *S. glaseri.*

Résumé – Steinernema oregonensis *n.sp. (Rhabditida : Steinernematidae), originaire de l'Orégon, États-Unis d'Amérique* – Une nouvelle espèce du genre *Steinernema* a été découverte près de Grant's Pass en Orégon. *Steinernema oregonensis* n. sp. se distingue des autres espèces de *Steinernema* par la longueur des juvéniles infestants, la forme des spicules, l'absence de mucron caudal chez les mâles de première génération, et sa présence chez les mâles de seconde génération. La longueur des juvéniles infestants est de 816 à 1112 µm, séparant *S. oregonensis* n. sp. des autres espèces décrites de *Steinernema,* sauf *S. affinis, S. anomali, S. feltiae, S. glaseri, S. neocurtillis,* et *S. puertoricensis.* La forme des spicules et l'absence de mucron caudal chez les mâles de première génération séparent *S. oregonensis* de ces dernières espèces. Les rapports « d » et « e » sont, chez les juvéniles infestants, de 0.4 à 0.6 et 0,9 à 1,1, respectivement, séparant la nouvelle espèce de *S. cubana, S. glaseri, S. neocurtillis,* et *S. puertoricensis.* L'analyse de l'ADN de *S. oregonensis* n. sp. montre un polymorphisme de longueur des fragments amplifiés au hasard différent de ceux de quatre autres espèces de *Steinernema. S. oregonensis* ne s'hybride pas avec les espèces proches *S. anomali, S. feltiae,* ou *S. glaseri.*

Key-words : Entomopathogenic nematodes, *Steinernema oregonensis,* taxonomy.

Nematode species belonging to the genus *Steinernema* are obligate parasites of insects that occur in many parts of the world and are potentially useful biological control agents for numerous agricultural and horticultural insect pests. The present work describes a new *Steinernema* species, found during a survey of entomopathogenic nematodes in Oregon, USA (Liu & Berry, 1995*a*).

Materials and methods

Nematodes were isolated from a soil sample by using greater wax moth, *Galleria mellonella* (L.) larva-baiting technique (Bedding & Akhurst, 1975). For morphological examinations, twenty nematodes were reared on a *G. mellonella* larva. Standard methods (Woodring & Kaya, 1988) were followed to obtain different stages of the nematodes. Adults and infective juveniles were killed, fixed in TAF, and processed to anhydrous glycerin (Poinar, 1975). Measurements were made with an Olympus microscope equipped with differential interference contrast optics. The ratios used in these studies were proposed by Poinar (1986) and Nguyen and Smart (1992). Cross-matings of this new species with *S. anomali, S. feltiae,* and *S. glaseri* were conducted by using the first technique of hanging blood drop method (Nguyen & Smart, 1990). For each combination, the treatment was replicated over 100 times to obtain 30 crosses between two nematode species.

Random amplified polymorphic DNA (RAPD) analysis (Liu & Berry, 1995*b*) was conducted with twelve decamer oligonucleotides, OPA-02, -03, -04, -07, -08, -09, -10, -11, -15, -18, -19, and -20 (Kit A, Operon Technologies, Alameda, CA) for the amplification. Frequency of RAPD polymorphisms (1-*F*) between nematode species was calculated by using the index of genetic distance (Nei & Li, 1979).

Results

Cross-breeding tests between this new species and three previously described species, *S. anomali, S. feltiae,*

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and *S. glaseri* were negative while controls using the same species were positive in 30 crosses.

The RAPD fragments of this new species generated by twelve primers showed strong polymorphisms when compared with other species examined, which allowed easy distinction of these species (Fig. 1). The frequencies of polymorphisms among previously described species ranged from 0.69 to 0.90. The frequencies of polymorphisms among different strains of the same species ranged from 0.16 to 0.53. The frequencies of polymorphisms between this new species and four previously described species ranged from 0.76 to 0.81 (Table 1).

These results, combined with the following morphological studies, indicated that this *Steinernema* nematode found in Oregon is a new species. This species was named *S. oregonensis* after the geographic locality from which it was found, the strain of the new species is called OS21.

Steinernema oregonensis n. sp.

(Figs 1-3)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female, first generation : Cuticle smooth, lateral lines and phasmids indistinct. Head rounded or slightly truncated, not offset. Six lips united at base but distinct at tips, each lip bearing a papilla. Four cepalic papillae further back on the head, located in sub-medial positions as an outer circle surrounding six inner labial papillae. Amphids small, located behind lateral labial papillae. Oral opening triangular, stoma short and wide with non-sclerotized walls and some minute tooth-like structures posterior to the end of stoma. Cheilo-, and telo-rhabdions vestigial. Pharynx muscular, extending nearly to mouth opening, procorpus cylindrical, metacorpus slightly swollen, non-valvated, indistinct isthmus followed by pyriform basal bulb containing valve lined with refractive ridges. Nerve ring surrounding the anterior portion of the basal bulb. Excretory pore opening circular, located anterior to nerve ring. Gonads didelphic, amphidelphic with opposed reflexed ovaries in dorsal position. Vulva a transverse slit, usually protruding from

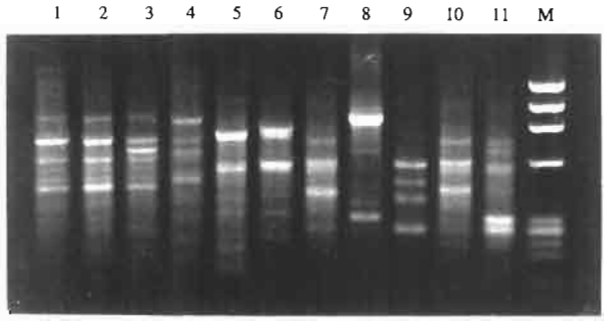

Fig. 1. *RAPD fragments of* Steinernema *species generated by primer OPA-10 (1-3,* S. carpocapsae; *4,* S. anomali; *5-6,* S. glaseri; *7,* S. feltiae; *8-10,* Steinernema *spp. isolated from Oregon; 11,* S. oregonensis *n. sp.; M, DNA size standards).*

Table 1. *Frequency of RAPD polymorphisms (1-F) among eight strains of* Steinernema *species.*

Nematode*	OS21	SFS	SGN	SGK	SAN	SCB	SCA	SCM
OS21	0.00							
SFS	0.79	0.00						
SGN	0.76	0.73	0.00					
SGK	0.79	0.71	0.53	0.00				
SAN	0.81	0.77	0.90	0.82	0.00			
SCB	0.77	0.82	0.77	0.87	0.83	0.00		
SCA	0.78	0.77	0.69	0.85	0.81	0.17	0.00	
SCM	0.81	0.87	0.75	0.90	0.83	0.20	0.16	0.00

* OS21 : *S. oregonensis* n. sp.; SFS : *S. feltiae* SN strain; SGN : *S. glaseri* NJ strain; SGK : *S. glaseri* KG strain; SAN : *S. anomali;* SCB : *S. carpocapsae* British strain; SCA : *S. carpocapsae* Agriotos strain; SCM : *S. carpocapsae* Mexican strain.

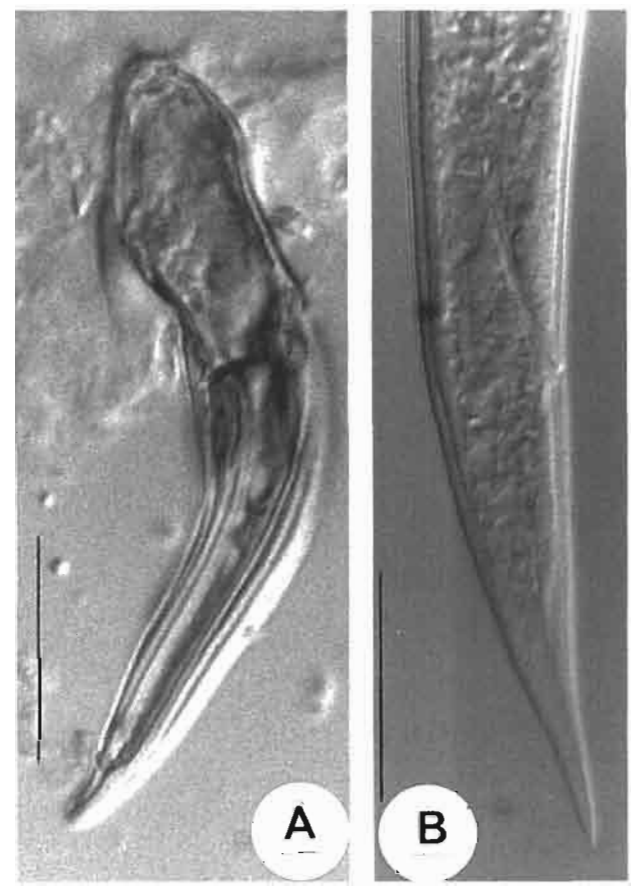

Fig. 2. Steinernema oregonensis *n. sp. Male and infective juvenile : A : Spicule, first generation male; B : Tail, infective juvenile.* (Scale bar : A, B = 20 µm).

Table 2. *Measurements of first and second generation males and females of* Steinernema oregonensis *n. sp.* (all measurements in µm except L in mm).

	Males			Females			Infective Juveniles
	First generation		Second generation	First generation		Second generation	
	Holotype	Paratypes	Paratypes	Allotype	Paratypes	Paratypes	
n	1	20	20	1	20	20	20
L(mm)	1.7	1.68 ± 0.08 (1.56-1.82)	1.29 ± 0.05 (1.22-1.35)	6.2	5.2 ± 0.3 (4.4-6.2)	2.4 ± 0.2 (1.9-2.7)	0.98 ± 0.11 (0.82-1.11)
Greatest diameter	144.9	138 ± 13.4 (104.6-161.0)	77.7 ± 8.4 (64.4-88.6)	265.7	241.5 ± 13.0 (217.4-265.7)	166.2 ± 10.2 (144.9-185.2)	34 ± 3.3 (28-38)
Stoma length	3.0	3.9 ± 0.3 (3.0-4.0)	2.1 ± 0.2 (1.9-2.4)	7.9	6.4 ± 1.0 (3.9-7.9)	3.4 ± 0.5 (2.9-3.9)	
Stoma width	4.0	5.9 ± 0.3 (5.0-6.0)	2.9 ± 0.1 (2.8-3.2)	7.9	7.5 ± 0.8 (5.9-7.9)	5.5 ± 0.6 (4.9-5.9)	
Ant. end to excret. pore (EP)	99.0	111.8 ± 10.7 (95.0-138.6)	85.1 ± 5.8 (75.2-91.1)	112.9	102.6 ± 6.2 (89.1-112.3)	82.1 ± 5.6 (69.3-91.1)	66.0 ± 4.5 (60-72)
Ant. end to nerve ring	102.9	110.6 ± 8.2 (100.9-132.7)	99.5 ± 4.5 (93.1-106.9)	128.7	147.0 ± 8.8 (128.7-162.4)	117.8 ± 5.0 (108.9-126.7)	
Ant. end to pharynx base (Ph. B)	138.6	153.6 ± 11.6 (138.6-182.2)	133.9 ± 4.8 (118.8-140.6)	196.0	209.5 ± 9.6 (186.1-219.8)	168.7 ± 4.9 (158.4-176.2)	132.0 ± 8.9 (116-148)
Testis reflexion length	150.5	297 ± 87.2 (99.0-392.0)	190.8 ± 11.9 (170.3-207.9)				
Tail length	29.7	28.5 ± 3.2 (23.8-31.7)	24.8 ± 2.8 (19.8-29.7)	43.6	36.5 ± 6.2 (27.7-45.5)	47.3 ± 6.3 (39.6-63.4)	70.0 ± 3.7 (64-78)
Anal body with	39.6	46.9 ± 5.2 (37.6-55.4)	35.8 ± 1.9 (31.7-39.6)	59.4	55.8 ± 9.5 (41.6-79.2)	40.6 ± 3.3 (31.7-45.5)	
Spicule length	69.3	71.1 ± 1.9 (65.3-73.3)	58.7 ± 2.2 (53.5-61.4)				
Spicule width	11.8	11.7 ± 0.7 (9.9-11.9)	9.3 ± 0.9 7.9-11.9				
Gubernaculum length	55.4	56.3 ± 3.4 (51.5-59.4)	45.1 ± 3.5 (41.6-51.5)				
Gubernaculum width	7.9	8.2 ± 0.9 (5.9-9.9)	7.3 ± 0.5 6.9-7.9				
Mucron length			4.9 ± 0.9 4.0-6.0				
V				54.4	52.3 ± 3.0 (46.2-56.0)	58.2 ± 3.3 (54.5-60.8)	
Ratio d*	0.71	0.73 ± 0.04 (0.64-0.75)	0.64 ± 0.04 (0.55-0.68)	0.57	0.49 ± 0.03 (0.43-0.57)	0.48 ± 0.03 (0.43-0.54)	0.5 ± 0.04 (0.4-0.6)
Ratio e**	3.3	3.9 ± 0.77 (3.1-6.4)	3.4 ± 0.46 (2.5-3.8)	0.38	2.9 ± 0.6 (2.1-4.0)	1.8 ± 0.2 (1.4-2.1)	1.0 ± 0.1 (0.9-1.1)
Ratio a							30.0 ± 5.2 (24-37)
Ratio b							7.6 ± 0.6 (6-8)
Ratio c							14.0± 1.6 (12-16)

* Ant. end to excret. pore/Ant. end to pharynx base
** Ant. end to excret. pore/Tail length.

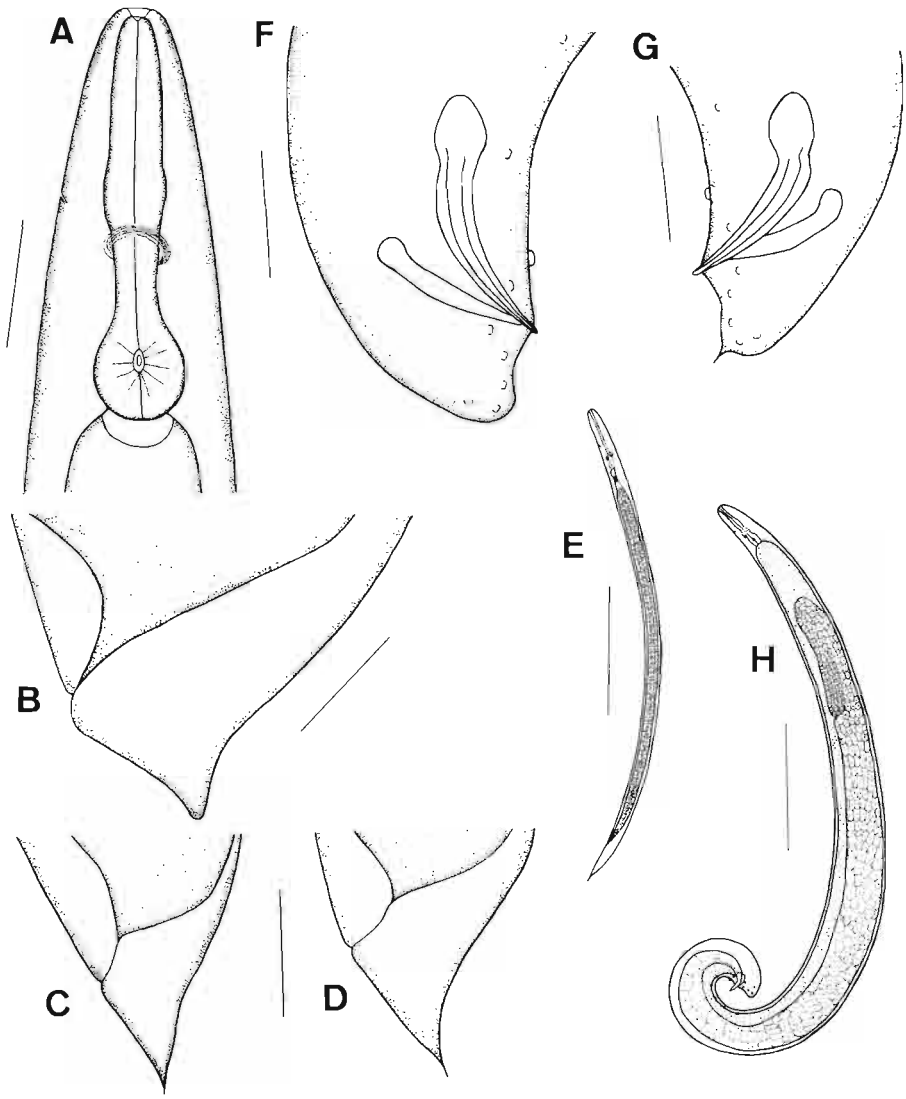

Fig. 3. Steinernema oregonensis *n. sp. Females. A : Pharyngeal region (1rst generation); B : Tail (1rst generation); C, D : Tails (2nd generation). – Infective juvenile. E : Entire body. – Male. F : Tail, lateral view (1rst generation); G : Tail, lateral view (2nd generation); H : Entire body (1rst generation).* (Bar equivalents : A = 65 µm; B, G = 35 µm; C, D = 50 µm; E = 255 µm; F = 40 µm; H = 295 µm).

the body surface. Vagina short, leading into paired uteri. Tail usually wide, shorter than width at anus, with a round wedge-shaped projection on the tip; mucron absent.

Female, second generation : Similar to first generation female but smaller and slender body. Tail straight, longer than width at anus, tapering or pointed bearing a mucro.

Male, first generation : Cuticle, lip, stoma and pharyngeal region similar to first generation female. Body much smaller than female. Testis single, reflexed, and consisting of a germinal growth zone leading into a seminal vesicle. Single *vas deferens* with inconspicuous muscular walls. Spicules paired with ridges and thin velum, usually moderately curved. Capitulum varied in shape, usually broad, slight angular-shaped; lamina bearing two ridges on edges and surface. Distal tip of spicule blunt. Gubernaculum boat-shaped in lateral view, and ventrally curved, with a proximal knob. Twenty-three genital papillae, variable in position, consisting of nine ventrolateral pairs, two lateral pairs and an adanal ventral pair. Six pairs located preanally, one pair in the region of the

gubernaculum, and four pairs located postanally. A single genital papilla located ventrally just anterior to cloaca. Cloacal opening slit-like. Tail conoid, ventral portion usually concaved, tip blunt without mucro. Bursa absent.

Male, second generation : Similar to first generation male but differing by following characters : smaller size, slender body, tail usually bearing a mucro.

Infective juvenile : Often enclosed in the cuticle of the second-stage. Body slender, tapering regularly from base of pharynx to anterior end, and from anus to terminus. Lateral fields distinct with six to eight ridges. Lip region smooth, generally continuous. Mouth and anus closed. Pharynx long and narrow, basal bulb weak and less prominent than in adults. Intestine collapsed. Nerve ring distinct. Hemizonid located at level of basal bulb. Anterior portion of intestine with a pouch containing the symbiotic bacteria. Tail conical with pointed terminus.

Type host and locality

Nematodes were recovered by using *Galleria mellonella* (L.) larvae as bait from a soil sample collected in a grass field near Grants Pass, Oregon, USA. The natural host is unknown.

Type specimens

Holotype (male, first generation) and allotype (female, first generation) deposited in the Nematode Collection at the University of California, Davis, California. Paratypes deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

Diagnosis and relationship

The average length (977 µm), ratio d (0.5) and ratio e (1.0) in infective juveniles, the absence (in first generation) and the presence (in second generation) of a tail mucron and moderately curved spicules in males as well as RAPD fragments and cross-breeding results separate *S. oregonensis* n. sp. from all other previously described species of *Steinernema.*

The length range of infective juveniles of *S. oregonensis* n. sp. is different from those of *S. rara, S. ritteri, S. carpocapsae, S. scapterisci, S. kushidai, S. riobravis, S. intermedia,* and *S. cubana.* The length range of *S. oregonensis* infective juveniles somewhat overlaps with those of *S. affinis, S. anomali, S. feltiae, S. glaseri, S. neocurtillis,* and *S. puertoricensis.* However, the ratio e (anterior end to the excretory pore/tail length) of the infective juveniles of *S. oregonensis* n. sp. does not overlap with those of *S. feltiae, S. neocurtillis* and *S. glaseri.* The ratio d (anterior end to the excretory pore/anterior end to the pharynx base) separates *S. oregonensis* n. sp. from *S. puertoricensis* (Table 3).

Males of *S. oregonensis* n. sp. can be separated from those of other *Steinernema* species by the absence (in first generation) and the presence (in second generation) of a tail mucro (Fig. 4 A, C) and moderately curved spicules (Figs 2 A, 4 A). The absence of a tail mucron in first generation males separates *S. oregonensis* n. sp. from both *S. affinis* and *S. feltiae.* The moderately curved spicules separates *S. oregonensis* n. sp. from *S. intermedia* and *S. riobravis,* in which the spicules are usually strong curved (a line running parallel with the calomus and lamina forms an angle of 70-90°). *S. oregonensis* n. sp. is closely related to *S. anomali, S. cubana,* and *S. glaseri* in general morphological characters. The blunt tip spicules separate *S. oregonensis* n. sp. from notched tip spicules of *S. glaseri* and swollen tip spicules of *S. anomali.* The presence of a tail mucro in second generation males separates *S. oregonensis* n. sp. from *S. cubana.* The males of *S. oregonensis* n. sp. have a ventral concaved tail, which is different from *S. anomali* and *S. glaseri. S. oregonensis* n. sp. is reproductively isolated from *S. anomali, S. feltiae* and *S. glaseri* as indicated by negative results in the cross-breeding tests.

The genetic profiles generated by RAPD fragment analysis showed that *S. oregonensis* n. sp. is distinct. The minimal frequency of RAPD polymorphism of *S. oregonensis* n. sp. to four other *Steinernema* species is 0.76 (Table 1).

Life cycle

S. oregonensis n. sp. has a life cycle comparable to that of other described species of *Steinernema.* The infective juveniles enter the hemocoele of insects and liberate their associated bacteria. At room temperature (*ca* 22 °C), it takes 3-4 days for infective juveniles to reach the adult stage of first generation, and another 2 days to reach the second generation of adults. It usually takes 1 week for the nematodes to complete two generations, and emerge from the insect cadaver as infective juveniles.

Remarks

The morphological characters of entomopathogenic nematodes are difficult to study because there is extensive overlap in morphometric characters and almost no one individual from a population can be reliably assigned to a particular species using only morphological characters. Although morphological characters help in the identification of nematode species, identification becomes more difficult with the increasing number of species and strains in the genus *Steinernema.* This led many taxonomists to investigate alternative methods for nematode diagnosis (Curran *et al.,* 1985).

It is evident that for species descriptions, examination of interspecific variability, hybridization and DNA analysis are needed to confirm distinctness, for a species to be considered new, differences in morphological, genetic profiles, and reproductive isolation should be detectable when compared with other closely related species (Cabanillas *et al.,* 1994). RAPD-PCR is a simple, rapid technique which uses single oligonucleotide primers at

low stringency to generated RAPD fragments (Welsh & McClelland, 1990; Williams *et al.,* 1990). The value of RAPD fragments for the study of inter- and intra- specific variation of nematodes is now being recognized (Caswell-Chen *et al.,* 1992; Gardner *et al.,* 1994; Liu & Berry, 1995*c*). Extreme care must be taken in the extraction, preparation and amplification of DNA for analysis of RAPD fragments. In our studies, this technique was used as a first screen to identify genetic variants which could then be asssigned as new species. Combined with morphological characters, three closely related species were selected to carry out cross-breeding tests with *S. oregonensis* n. sp. For diagnosis of species or strains of nematodes, RAPD fragments also constitute a welcome addition to the sometimes scarce morphological characters. We demonstrated in this study that RAPD-PCR techniques can be used to distinguish *S. oregonensis* n. sp. and *S. anomali.* Both species are very similar in morphological characters. We believe that molecular techniques will become indispensable for distinguishing between closely related species and isolates of *Steinernema* nematodes in the immediate future.

Laboratory evaluation showed this nematode was capable of invading *G. mellonella* larvae within 1 h and killing the host in 48 h at 18 °C (Liu & Berry, unpubl.), which can be considered as a low-temperature infective nematode species.

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